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(54) **Title:** METHODS AND COMPOSITIONS FOR MODULATING IMMUNE RESPONSES AND LYMPHOCYTE ACTIVITY

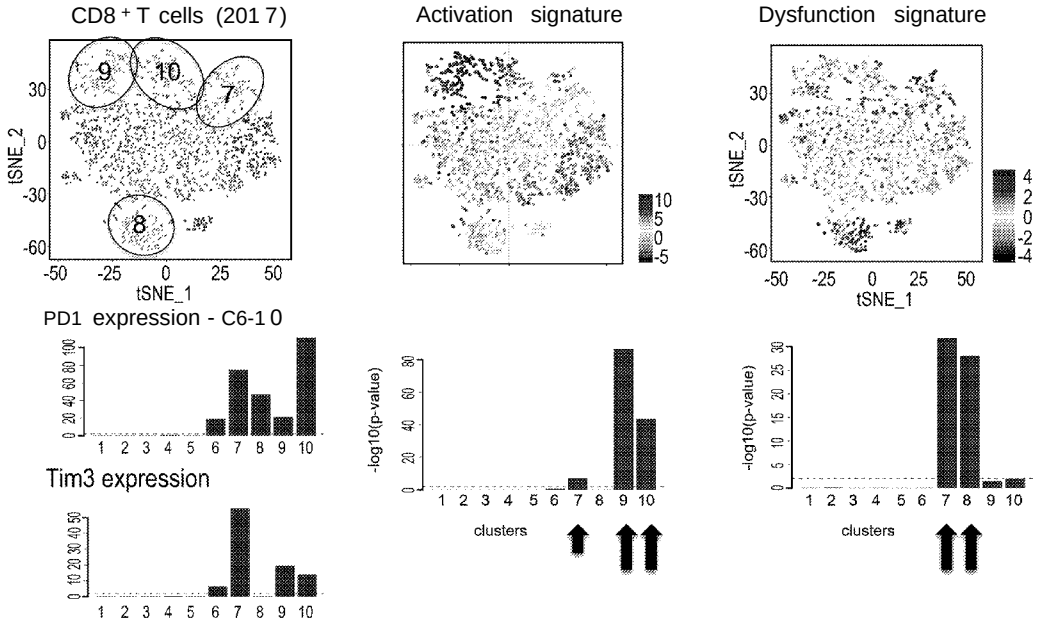

FIG. 31

(57) **Abstract:** The subject matter disclosed herein is generally directed to novel CD8+ and CD4+ T cell subtypes associated with effector, suppressive or regulatory T cell functions. Moreover, the subject matter disclosed herein is generally directed to methods and compositions for use of the subtype. Also, disclosed herein are gene signatures and markers associated with the subtype and use of said signatures and markers. Further disclosed are therapeutic methods of using said gene signatures and immune cell subtype. Further disclosed are pharmaceutical compositions comprising populations of CD4+ and/or CD8+ TILs or populations of immune cells depleted for a specific subtype. Further disclosed are interactions with other T cell subtypes.

[Continued on next page]

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METHODS AND COMPOSITIONS FOR MODULATING IMMUNE RESPONSES AND LYMPHOCYTE ACTIVITY

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 62/588,237, filed November 17, 2017. The entire contents of the above-identified application are hereby fully incorporated herein by reference.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH

[0002] This invention was made with government support under grant numbers MH1 05960, CA1 87975, AI073748 and NS045937 awarded by the National Institutes of Health. The government has certain rights in the invention.

REFERENCE TO AN ELECTRONIC SEQUENCE LISTING

[0003] The contents of the electronic sequence listing (BROD_23l5_ST25.txt"; Size is 16 Kilobytes and it was created on November 14, 2018) is herein incorporated by reference in its entirety.

TECHNICAL FIELD

[0004] The subject matter disclosed herein is generally directed to CD4+ and CD8+ T lymphocyte subtypes and their interactions associated with immune responses in cancer. Moreover, the subject matter disclosed herein is generally directed to detecting, isolating and modulating said subtypes.

BACKGROUND

[0005] Characterizing different T cell subpopulations and their underlying driving mechanisms contributes to our understanding of protective immunity in successful pathogen clearance, T cell regulation during uncontrolled tumor growth and chronic infections, and T cell

regulation during autoimmunity. Recent advances on this front have enabled the development of improved vaccines and novel immune-based therapies for various cancers. Applicants have previously shown that a CD8 T cell dysfunction gene signature can be decoupled from an activation gene signature and have shown that the signatures for each CD8 T cell state is present in distinct single cell populations (see, e.g., WO2017075451A1, WO2017075478A2, WO2017075465A1 and US provisional application number 62/384,557, filed September 7, 2016). Previous studies have characterized subsets of regulatory T cells (Treg) that selectively suppress development of autoantibody formation by inhibiting function of follicular T-helper cells (see, e.g., US20130302276A1; and WO2016196912A1). It is believed that the breadth of the functional potential of CD4+ and CD8+ T cells is far from understood, and that gaining a deeper understanding will lead to further advancements.

[0006] Consequently, there exists a continuous need to provide additional and preferably improved markers, products and methods allowing to determine the functional state of immune cells. Likewise, there exists a continuous need to provide additional and preferably improved molecular targets involved in immune responses, as well as therapeutically useful substances and compositions impinging on such molecular targets to modulate immune responses.

[0007] Citation or identification of any document in this application is not an admission that such document is available as prior art to the present invention.

SUMMARY

[0008] It is an objective of the present invention to identify CD8+ TIL subtypes present in tumor infiltrating lymphocytes (TIL) during tumor growth. It is another objective of the present invention to detect gene signatures and biomarkers specific to the CD8+ and/or CD4+ TIL subtypes, whereby cells may be detected and isolated. It is another objective of the present invention to provide for adoptive cell transfer methods for treatment of a cancer by transferring more functional CD8+ and/or CD4+ TIL populations. It is another objective of the present invention to provide for treatment of a cancer by modulating CD8+ and/or CD4+ T cell populations to be more functional. It is another objective of the present invention to improve immunotherapy treatment.

[0009] In one aspect, the present invention provides for an isolated $CD8^+$ T cell characterized in that the $CD8^+$ T cell comprises expression of a gene signature comprising one or more genes selected from the group consisting of any of tables 1 to 20.

[0010] In certain embodiments, the $CD8^+$ T cell expresses PD-l and TIM3. In certain embodiments, the $CD8^+$ T cell expresses HMMR. In certain embodiments, the $CD8^+$ T cell expresses a gene signature comprising one or more genes selected from Table 20. In certain embodiments, the $CD8^+$ T cell expresses PD-l, TIM3, and KI67 and does not express Helios.

[0011] In certain embodiments, the $CD8^+$ T cell expresses PD-l and does not express TIM3. In certain embodiments, the $CD8^+$ T cell expresses Helios (IKZF2). In certain embodiments, the $CD8^+$ T cell does not express MT1. In certain embodiments, the $CD8^+$ T cell expresses XCL1. In certain embodiments, the $CD8^+$ T cell expresses CCR8. In certain embodiments, the $CD8^+$ T cell expresses a gene signature comprising one or more genes selected from Table 19. In certain embodiments, the $CD8^+$ T cell expresses one or more genes selected from the group consisting of RAMP3, NRGN, SLC16A1 1, MYOlE, FOSB, IL18RAP, OLFR1033, IL2RA, BCL2A1B, CD83, FAM46A, CD74, ENPP2, LAD1, AI836003, DUSP4, ARL14EP, CD81, XDH, KIT, TNFRSF4, RORA, ST6GAL1, ATP2B2, CAPG and PLXDC2.

[0012] In certain embodiments, the $CD8^+$ T cell according to any embodiment herein is a human cell. In certain embodiments, the $CD8^+$ T cell according to any embodiment herein is a CAR T cell. In certain embodiments, the $CD8^+$ T cell according to any embodiment herein is a $CD8^+$ T cell autologous for a subject suffering from cancer. In certain embodiments, the $CD8^+$ T cell according to any embodiment herein expresses an exogenous TCR. In certain embodiments, the $CD8^+$ T cell according to any embodiment herein displays tumor specificity.

[0013] In certain embodiments, the $CD8^+$ T cell expresses an endogenous TCR or CAR specific for a low affinity antigen.

[0014] In another aspect, the present invention provides for a method for detecting or quantifying $CD8^+$ T cells in a biological sample of a subject, or for isolating $CD8^+$ T cells from a biological sample of a subject, the method comprising detecting or quantifying in a biological sample of the subject $CD8^+$ T cells as defined in any embodiment herein, or isolating from the biological sample $CD8^+$ T cells as defined in any embodiment herein. In certain embodiments, $CD8^+$ T cells are detected, quantified or isolated using one or more markers selected from the

group consisting of HMMR, PD-l, TIM3, KI67, Helios, MT1, XCL1 and CCR8. In certain embodiments, the $CD8^+$ T cells are detected, quantified or isolated using a technique comprising flow cytometry, mass cytometry, fluorescence activated cell sorting, fluorescence microscopy, affinity separation, magnetic cell separation, microfluidic separation, or combinations thereof. In certain embodiments, the technique employs one or more agents capable of specifically binding to one or more gene products expressed or not expressed by the $CD8^+$ T cells, preferably on the cell surface of the $CD8^+$ T cells. In certain embodiments, the one or more agents are one or more antibodies. In certain embodiments, the biological sample is a tumor sample obtained from a subject in need thereof and the $CD8^+$ T cells are $CD8^+$ tumor infiltrating lymphocytes (TIL). In certain embodiments, the biological sample comprises *ex vivo* or *in vitro* $CD8^+$ T cells.

[0015] In another aspect, the present invention provides for a population of $CD8^+$ T cells comprising $CD8^+$ T cells as defined in any embodiment herein or isolated according to any embodiment herein.

[0016] In another aspect, the present invention provides for a pharmaceutical composition comprising the $CD8^+$ T cell population as defined in any embodiment herein.

[0017] In another aspect, the present invention provides for a method for treating or preventing cancer comprising administering to a subject in need thereof the pharmaceutical composition according to any embodiment herein.

[0018] In another aspect, the present invention provides for a n isolated $CD8^+$ T cell characterized in that the $CD8^+$ T cell comprises expression of a gene signature comprising one or more genes selected from the group consisting of: GPR56, PDCD1, LAG3, HAVCR2, ENTPD1, 1700017B05RIK, CHN2, 2900026A02RIK, FGL2, SERPINA3H, OSBPL3, S100A4, CCL3, TNFRSF9, UBASH3B, CD244, RGS8, BCL2A1D, CCL4, CIAPIN1, GP49A, CCRL2, IRF8, GRINA, C1QTNF6, CD200R4, FILIP1, THEMIS2, SERPINA3F, LRRK1, ARNT2, MXI1, DAPK2, TWSG1, ADAM8, TRPS1, LAT2, SDCBP2, SLC37A2, MT2, ADAMTS14, GBP10, EPDR1 and DUT; or RGS16, GZMB, SERPINA3G, CXCR6, LITAF, SERPINA3I, TOX, PRF1, EHD1, LILRB4, PLEK, ITGAV, CREM, CDK6, NR4A2, UHRF2, GBP6, IRAK2, PTK2B, OXSR1 and ITGB1BP1; or TIGIT, DGAT1, PLAC8, BHLHE40, GM5069, SAMSN1, RGS1, DENND4A and SIK1.

[0019] In another aspect, the present invention provides for an isolated $CD8^+$ T cell characterized in that the $CD8^+$ T cell comprises expression of a gene signature according to any of tables 1 to 16. The isolated $CD8^+$ T cell may be further characterized in that the $CD8^+$ expresses HMMR. The isolated $CD8^+$ T cell may be further characterized in that the $CD8^+$ expresses PD-l and TIM3. The isolated $CD8^+$ T cell may be further characterized in that the $CD8^+$ expresses PD-l and does not express TIM3. The isolated $CD8^+$ T cell may be further characterized in that the $CD8^+$ expresses PD-l, TIM3, and KI67 and does not express Helios.

[0020] In certain embodiments, the $CD8^+$ T cell is a human cell. In certain embodiments, the cell is a CAR T cell. In certain embodiments, the cell is a $CD8^+$ T cell autologous for a subject suffering from cancer. In certain embodiments, the cell expresses an exogenous CAR or TCR. In certain embodiments, the $CD8^+$ T cell displays tumor specificity.

[0021] In another aspect, the present invention provides for a method for detecting or quantifying $CD8^+$ T cells in a biological sample of a subject, or for isolating $CD8^+$ T cells from a biological sample of a subject, the method comprising detecting or quantifying in a biological sample of the subject $CD8^+$ T cells as defined herein, or isolating from the biological sample $CD8^+$ T cells as defined herein.

[0022] In certain embodiments, $CD8^+$ T cells are detected, quantified or isolated using one or markers selected from the group consisting of HMMR, PD-l, TIM3, KI67 and Helios.

[0023] In certain embodiments, the $CD8^+$ T cells are detected, quantified or isolated using a technique selected from the group consisting of flow cytometry, mass cytometry, fluorescence activated cell sorting, fluorescence microscopy, affinity separation, magnetic cell separation, microfluidic separation, and combinations thereof. The technique may employ one or more agents capable of specifically binding to one or more gene products expressed or not expressed by the $CD8^+$ T cells, preferably on the cell surface of the $CD8^+$ T cells. The one or more agents may be one or more antibodies.

[0024] In certain embodiments, the biological sample is a tumor sample obtained from a subject in need thereof and the $CD8^+$ T cells are $CD8^+$ tumor infiltrating lymphocytes (TIL). The biological sample may comprise *ex vivo* or *in vitro* $CD8^+$ T cells.

[0025] In another aspect, the present invention provides for a population of $CD8^+$ T cells comprising $CD8^+$ T cells as defined in any embodiment herein or isolated according to any embodiment herein.

[0026] In another aspect, the present invention provides for a pharmaceutical composition comprising the $CD8^+$ T cell population as defined herein.

[0027] In another aspect, the present invention provides for a method for treating or preventing cancer comprising administering to a subject in need thereof the pharmaceutical composition according to any embodiment herein.

[0028] In another aspect, the present invention provides for a kit comprising reagents to detect at least one gene or polypeptide as defined herein.

[0029] In another aspect, the present invention provides for an isolated T cell characterized in that the T cell comprises expression of one or more genes selected from the group consisting of TNFRSF9, PRF1, BHLHE40 (DEC1), IRF8, GLDC, STAT3, CST7, IL1R2, EEF2, SLC2A3, SQSTM1, RBPJ, NABP1, ACTN1, TNFRSF4, SERPINB9, FOSL2, CAPG, KLRC1, IL18R1, JUNB, EEF1A1, TNFRSF18, RGS2, NFKB2, RPL5, PEX16, LAT2, KDM5B, HILPDA, GEM, DENND4A, BCL2L1 1, ADAM8, PGLYRP1, IKZF2 (Helios), MT1, KIT, SERPINE2, CCRL2, CSF1, EPAS1, RUNX2 and SPRY2. In another example embodiment, the isolated T cell is characterized in that the T cell does not comprise expression of HMMR and comprises expression of one or more genes selected from TNFRSF9, PRF1, BHLHE40 (DEC1), IRF8, GLDC, STAT3, CST7, IL1R2, EEF2, SLC2A3, SQSTM1, RBPJ, NABP1, ACTN1, TNFRSF4, SERPINB9, FOSL2, CAPG, KLRC1, IL18R1, JUNB, EEF1A1, TNFRSF18, RGS2, NFKB2, RPL5, PEX16, LAT2, KDM5B, HILPDA, GEM, DENND4A, BCL2L1 1, ADAM8, PGLYRP1, IKZF2, MT1, KIT, SERPINE2, CCRL2, CSF1, EPAS1, RUNX2 and SPRY2. In another example embodiment, the isolated T cell is characterized by expression of one or more CD8, TIM3, PD1, MT1, and IKZF2, as well as expression of one or more genes selected from the group consisting of TNFRSF9, PRF1, BHLHE40 (DEC1), IRF8, GLDC, STAT3, CST7, IL1R2, EEF2, SLC2A3, SQSTM1, RBPJ, NABP1, ACTN1, TNFRSF4, SERPINB9, FOSL2, CAPG, KLRC1, IL18R1, JUNB, EEF1A1, TNFRSF18, RGS2, NFKB2, RPL5, PEX16, LAT2, KDM5B, HILPDA, GEM, DENND4A, BCL2L1 1, ADAM8, PGLYRP1, KIT, SERPINE2, CCRL2, CSF1, EPAS1, RUNX2 and SPRY2. In another example embodiment, the isolated T cell may be

characterized by expression of one or more CD8, TIM3, PD1, MT1, and IKZF2, as well as expression of one or more genes selected from the group consisting of TNFRSF9, PRF1, BHLHE40 (DEC1), IRF8, GLDC, STAT3, CST7, IL1R2, EEF2, SLC2A3, SQSTM1, RBPJ, NABP1, ACTN1, TNFRSF4, SERPINB9, FOSL2, CAPG, KLRC1, IL18R1, JUNB, EEF1A1, TNFRSF18, RGS2, NFKB2, RPL5, PEX16, LAT2, KDM5B, HILPDA, GEM, DENND4A, BCL2L1 1, ADAM8, PGLYRP1, KIT, SERPINE2, CCRL2, CSF1, EPAS1, RUNX2 and SPRY2, and does not comprise expression of HMMR. The isolated T cell may be further characterized in that the T cell comprises upregulation of one or more genes selected from the group consisting of TNFRSF9, PRF1, BHLHE40, IRF8, GLDC, STAT3, CST7, IL1R2, EEF2, SLC2A3, SQSTM1, RBPJ, NABP1, ACTN1, TNFRSF4, SERPINB9, FOSL2, CAPG, KLRC1, IL18R1, JUNB, EEF1A1, TNFRSF18, RGS2, NFKB2, RPL5, PEX16, LAT2, KDM5B, HILPDA, GEM, DENND4A, BCL2L1 1, ADAM8, PGLYRP1, IKZF2, KIT, SERPINE2, CCRL2, CSF1, EPAS1, RUNX2 and SPRY2 as compared to all CD8+ TIM3+ PD1+ T cells. The isolated T cell may be further characterized in that the T cell comprises downregulation of a cell cycle signature as compared to all CD8+ TIM3+ PD1+ T cells. The T cell may be further characterized in that the T cell suppresses T cell proliferation. The isolated T cell may be further characterized by a gene signature comprising one or more genes or polypeptides selected from Tables 1 to 5. Tables 1 to 5 list the genes in ranked order (i.e., most specific to the cells described herein). In certain embodiments, the signature may comprise the top 10, 20, 50, 100, 200, 300, 400, or 500 top genes. In preferred embodiments, the signature comprises genes selected from the top 100, 50, 20, or top 10 genes in each ranked list. In other preferred embodiments, T cells are detected, isolated or targeted using cell surface or cytokines (e.g., Table 3). The T cell may be a human cell. The T cell may be autologous for a subject suffering from cancer.

[0030] In another aspect, the present invention provides for a method for detecting or quantifying T cells in a biological sample of a subject, the method comprising detecting or quantifying in a biological sample of the subject T cells as defined in any embodiment herein. The T cells may be detected or quantified using a set of markers comprising: a) TIM3, SERPINE2 and HMMR; or b) SERPINE2 and HMMR; or c)TIM3, KIT and HMMR; or d) TIM3, TNFRSF4 and HMMR; or e) any of (a), (b), (c) or (d) and one or more of CD8, CD45 and PD1; or any of (a), (b), (c), (d) or (e) and one or more of TNFRSF9, PRF1, BHLHE40, IRF8,

GLDC, STAT3, CST7, IL1R2, EEF2, SLC2A3, SQSTM1, RBPJ, NABP1, ACTN1, TNFRSF4, SERPINB9, FOSL2, CAPG, KLRC1, IL18R1, JUNB, EEF1A1, TNFRSF18, RGS2, NFKB2, RPL5, PEX16, LAT2, KDM5B, HILPDA, GEM, DENND4A, BCL2L11, ADAM8, PGLYRP1, IKZF2, KIT, SERPINE2, CCRL2, CSF1, EPAS1, RUNX2 and SPRY2. The T cells may be detected or quantified using a technique selected from the group consisting of RT-PCR, RNA-seq, single cell RNA-seq, flow cytometry, mass cytometry, fluorescence activated cell sorting, fluorescence microscopy, affinity separation, magnetic cell separation, microfluidic separation, and combinations thereof.

[0031] In one embodiment, intact T cells may be detected or quantified using a set of surface markers comprising: a) TIM3, SERPINE2 and HMMR; or b) SERPINE2 and HMMR; or c) TIM3, KIT and HMMR; or d) TIM3, TNFRSF4 and HMMR; or e) any of (a), (b), (c) or (d) and one or more of CD8, CD45 and PD1; or any of (a), (b), (c), (d) or (e) and one or more of TNFRSF9, IL1R2, SLC2A3, TNFRSF4, KLRC1, IL18R1, TNFRSF18, LAT2, ADAM8, KIT and SERPINE2. The intact T cells may be detected or quantified using a technique selected from the group consisting of flow cytometry, fluorescence activated cell sorting, affinity separation, magnetic cell separation, microfluidic separation, and combinations thereof.

[0032] In another aspect, the present invention provides for a method for isolating T cells from a biological sample of a subject, the method comprising isolating from the biological sample T cells as defined in any embodiment herein. The T cells may be isolated using a set of surface markers comprising: a) TIM3, SERPINE2 and HMMR; or b) SERPINE2 and HMMR; or c) TIM3, KIT and HMMR; or d) TIM3, TNFRSF4 and HMMR; or e) any of (a), (b), (c) or (d) and one or more of CD8, CD45 and PD1; or any of (a), (b), (c), (d) or (e) and one or more of TNFRSF9, IL1R2, SLC2A3, TNFRSF4, KLRC1, IL18R1, TNFRSF18, LAT2, ADAM8, KIT and SERPINE2. The T cells may be isolated, using a technique selected from the group consisting of flow cytometry, fluorescence activated cell sorting, affinity separation, magnetic cell separation, microfluidic separation, and combinations thereof.

[0033] In certain embodiments, the technique for detecting, quantitating, or isolating T cells according to any embodiment herein may employ one or more agents capable of specifically binding to one or more gene products expressed or not expressed by the T cells, preferably on the cell surface of the T cells. The one or more agents may be one or more antibodies.

[0034] In certain embodiments, the biological sample may be a tumor sample obtained from a subject in need thereof. In certain embodiments, the biological sample may be a sample obtained from a subject suffering from an autoimmune disease. In certain embodiments, the biological sample may be a sample obtained from a subject suffering from a chronic infection. Not being bound by a theory detecting suppressive T cells in a biological sample may provide information as to the immune state of a subject (e.g., for prognosis, treatment selection). In certain embodiments, the biological sample may comprise *ex vivo* or *in vitro* T cells. Not being bound by a theory, it may be advantageous to detect or quantitate the presence of suppressive T cells in an ex vivo sample of T cells. For example, after the ex vivo T cells are treated with a differentiating agent or immunomodulatory. Not being bound by a theory, it may be advantageous to deplete suppressive T cells from an ex vivo population of T cells.

[0035] In another aspect, the present invention provides for a population of T cells comprising T cells as defined in any embodiment herein. The population of T cells may be depleted for T cells as defined in any embodiment herein by a method of isolation according to any embodiment herein. The population of T cells may comprise chimeric antigen receptor (CAR) T cells or T cells expressing an exogenous T-cell receptor (TCR). The population of T cells may comprise T cells autologous for a subject suffering from cancer. The population of T cells may comprise T cells displaying tumor specificity. Not being bound by a theory, the population of T cells may comprise a heterogeneous population of cells including effector and suppressor T cells. In certain embodiments, it is advantageous to remove the suppressive T cells (e.g., when an enhanced immune response is desired). The population of T cells may be expanded.

[0036] In certain embodiments, the population of T cells may comprise activated T cells. The population of T cells may comprise T cells activated with tumor specific antigens. The tumor specific antigens may be subject specific antigens.

[0037] In another aspect, the present invention provides for a pharmaceutical composition comprising the depleted T cell population as defined in any embodiment herein.

[0038] In another aspect, the present invention provides for a method of treating cancer comprising administering to a subject in need thereof the pharmaceutical composition according to any embodiment herein.

[0039] In another aspect, the present invention provides for a method of treating cancer in a subject in need thereof comprising: depleting T cells as defined in any embodiment herein from a population of T cells obtained from the subject; *in vitro* expanding the population of T cells; and administering the *in vitro* expanded population of T cells to the subject. The T cell population may be administered after ablation therapy or lymphodepletion therapy. Not being bound by a theory, ablation therapy or lymphodepletion therapy will eliminate any endogenous suppressive cells in a subject, whereby the subject and the cells administered may be depleted for suppressive T cells, thus the adoptive cell therapy may result in an enhanced anti-tumor response.

[0040] In another aspect, the present invention provides for a method of treating cancer or chronic infection in a subject in need thereof comprising administering to the subject a therapeutically effective amount of an agent: capable of reducing the activity of a T cell as defined in any embodiment herein; or capable of reducing the activity or expression of one or more genes or polypeptides selected from the group consisting of TNFRSF9, PRF1, BHLHE40, IRF8, GLDC, STAT3, CST7, IL1R2, EEF2, SLC2A3, SQSTM1, RBPJ, NABP1, ACTN1, TNFRSF4, SERPINB9, FOSL2, CAPG, KLRC1, IL18R1, JUNB, EEF1A1, TNFRSF18, RGS2, NFKB2, RPL5, PEX16, LAT2, KDM5B, HILPDA, GEM, DENND4A, BCL2L1 1, ADAM8, PGLYRP1, IKZF2, KIT, SERPINE2, CCRL2, CSF1, EPAS1, RUNX2 and SPRY2; or capable of targeting or binding to one or more cell surface exposed genes or polypeptides on a T cell as defined in any embodiment herein; or capable of targeting or binding to one or more receptors or ligands specific for a cell surface exposed gene or polypeptide on a T cell as defined in any embodiment herein; or capable of targeting or binding to one or more genes or polypeptides secreted from a T cell as defined in any embodiment herein; or capable of targeting or binding to one or more receptors specific for a gene or polypeptide secreted from a T cell as defined in any embodiment herein. The agent may comprise a therapeutic antibody, antibody fragment, antibody-like protein scaffold, aptamer, protein, CRISPR system or small molecule. The therapeutic antibody may be an antibody drug conjugate. The agent capable of targeting or binding to a cell surface exposed gene or polypeptide may comprise a CAR T cell capable of targeting or binding to the cell surface exposed gene or polypeptide.

[0041] In another aspect, the present invention provides for a method of treating an autoimmune disease in a subject in need thereof comprising administering to the subject a

therapeutically effective amount of an agent capable of inducing the activity of a T cell as defined in any embodiment herein.

[0042] In another aspect, the present invention provides for a method of treating an autoimmune disease comprising administering T cells as defined in any embodiment herein to a subject in need thereof. Not being bound by a theory, administering suppressive T cells may reduce an autoimmune response in a subject.

[0043] In another aspect, the present invention provides for a method for identifying an immunomodulant capable of modulating one or more phenotypic aspects of the T cell as defined in any embodiment herein, comprising: applying a candidate immunomodulant to the T cell or T cell population; and detecting modulation of one or more phenotypic aspects of the T cell or T cell population by the candidate immunomodulant, thereby identifying the immunomodulant. The immunomodulant may be capable of modulating suppression of T cell proliferation by the T cell. Thus, in certain embodiments, detecting modulation of one or more phenotypic aspects comprises detecting modulation of a suppressive phenotype. The immunomodulant may comprise a therapeutic antibody, antibody fragment, antibody-like protein scaffold, aptamer, protein or small molecule.

[0044] In another aspect, the present invention provides for a pharmaceutical composition comprising the immunomodulant as defined in any embodiment herein.

[0045] In another aspect, the present invention provides for a method for determining the T cell status of a subject, or for diagnosing, prognosing or monitoring a disease comprising an immune component in a subject, the method comprising detecting or quantifying in a biological sample of the subject T cells as defined in any embodiment herein, wherein an increase as compared to a reference level indicates a suppressed immune response. The disease may be cancer, an autoimmune disease, or chronic infection.

[0046] In another aspect, the present invention provides for a method of preparing cells for use in adoptive cell transfer comprising: obtaining a population of T cells; and depleting suppressive T cells as defined in any embodiment herein from the population of T cells. The method may further comprise expanding the depleted cells. The method may further comprise activating the depleted cells. The population of T cells may comprise CAR T cells. The population of T cells may comprise autologous TILs.

[0047] In another aspect, the present invention provides for a method of screening for genes required for suppression of effector T cells by suppressive CD8+ T cells comprising: introducing a library of sgRNAs specific to a set of target genes to a population of T cells expressing a CRISPR system; culturing the cells in proliferating conditions in the presence of suppressive CD8 T cells according to any embodiment herein; determining sgRNAs that are enriched in proliferating T cells.

[0048] In another aspect, the present invention provides for a method of treating cancer or chronic infection in a subject in need thereof comprising administering to the subject CD8+ T cells modified to be resistant to suppressive CD8+ T cells, wherein the modified CD8+ T cells may be specific for the cancer or chronic infection. In certain embodiments, the CD8+ T cells modified to be resistant to suppressive CD8+ T cells comprise an inducible suicide gene. Not being bound by a theory, the cells may be killed to prevent a pathogenic autoimmune response.

[0049] In another aspect, the present invention provides for a method of treating cancer or chronic infection in a subject in need thereof comprising administering to the subject a therapeutically effective amount of an agent capable of blocking glucocorticoid signaling. The agent may be an antagonist of NR.3C1. The antagonist may be a blocking antibody.

[0050] In another aspect, the present invention provides for a kit comprising reagents to detect at least one gene or polypeptide as defined in any embodiment herein.

[0051] An aspect of the invention provides the immune cell or immune cell population as taught herein for use in immunotherapy, such as adoptive immunotherapy, such as adoptive cell transfer. Also provided is a method of treating a subject in need thereof, particularly in need of immunotherapy, such as adoptive immunotherapy, such as adoptive cell transfer, comprising administering to said subject the immune cell or immune cell population as taught herein. Further provided is use of the immune cell or immune cell population as taught herein for the manufacture of a medicament for immunotherapy, such as adoptive immunotherapy, such as adoptive cell transfer. In certain embodiments, the immune cell is a T-cell, such as a CD8+ T-cell. In certain embodiments, the immunotherapy, adoptive immunotherapy or adoptive cell transfer may be for treating a proliferative disease, such as tumor or cancer, or a chronic infection, such as chronic viral infection.

[0052] In certain embodiments, an immune cell suitable for immunotherapy, such as a CD8+ T-cell, displays tumor specificity, more particularly displays specificity to a tumor antigen. In certain embodiments, an immune cell suitable for immunotherapy, such as a CD8+ T-cell, displays specificity to an antigen of an infectious agent, for example displays viral antigen specificity. In certain embodiments, an immune cell suitable for immunotherapy, such as a CD8+ T-cell, has been isolated from a tumor of a subject, preferably the cell is a tumor infiltrating lymphocyte (TIL). In certain embodiments, an immune cell suitable for immunotherapy, such as a CD8+ T-cell, comprises a chimeric antigen receptor (CAR). Such cell can also be suitably denoted as having been engineered to comprise or to express the CAR. In certain embodiments, the CAR comprises an extracellular antigen-binding element (or portion or domain) configured to specifically bind to a target antigen, a transmembrane domain, and an intracellular signaling domain. In certain embodiments, the intracellular signaling domain comprises a primary signaling domain and/or a costimulatory signaling domain. In certain embodiments, the CAR comprises the antigen-binding element, costimulatory signaling domain and primary signaling domain (such as CD3 zeta portion) in that order. In certain embodiments, the antigen-binding element comprises, consists of or is derived from an antibody, for example, the antigen-binding element is an antibody fragment. In certain embodiments, the antigen-binding element is derived from, for example is a fragment of, a monoclonal antibody, such as a human monoclonal antibody or a humanized monoclonal antibody. In certain embodiments, the antigen-binding element is a single-chain variable fragment (scFv). In certain preferred embodiments, the target antigen is selected from a group consisting of: CD19, BCMA, CLL-l, MAGE A3, MAGE A6, HPV E6, HPV E7, WT1, CD22, CD171, ROR1, MUC16, CD70, and SSX2. In certain preferred embodiments, the target antigen is CD 19. In certain embodiments, the transmembrane domain is derived from the most membrane proximal component of the endodomain. In certain embodiments, the transmembrane domain is not CD3 zeta transmembrane domain. In certain embodiments, the transmembrane domain is a CD8a transmembrane domain or a CD28 transmembrane domain, preferably CD28 transmembrane domain. In certain embodiments, the primary signaling domain comprises a functional signaling domain of a protein selected from the group consisting of CD3 zeta, CD3 gamma, CD3 delta, CD3 epsilon, common FcR gamma (FCERIG), FcR beta (Fc Epsilon Rlb), CD79a, CD79b, Fc gamma Rlla, DAP 10, and DAP12. In

certain preferred embodiments, the primary signaling domain comprises a functional signaling domain of Cü 3ζ or FcRy. In certain preferred embodiments, the primary signaling domain comprises a functional signaling domain of Cü 3ζ. In certain embodiments, the one or more costimulatory signaling domains comprise a functional signaling domain of a protein selected, each independently, from the group consisting of: CD27, CD28, 4-1BB (CD137), 0X40, CD30, CD40, ICOS, lymphocyte function-associated antigen-l (LFA-l), CD2, CD7, LIGHT, NKG2C, B7-H3, a ligand that specifically binds with CD83, CDS, ICAM-l, GITR, BAFFR, HVEM (LIGHTR), SLAMF7, NKp80 (KLRF1), CD160, CD19, CD4, CD8 alpha, CD8 beta, IL2R beta, IL2R gamma, IL7R alpha, ITGA4, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CDl ld, ITGAE, CD103, ITGAL, CDl la, LFA-l, ITGAM, CDl lb, ITGAX, CDl lc, ITGB1, CD29, ITGB2, CD 18, ITGB7, TNFR2, TRANCE/RANKL, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRTAM, Ly9 (CD229), CD 160 (BY55), PSGL1, CD 100 (SEMA4D), CD69, SLAMF6 (NTB-A, Lyl08), SLAM (SLAMF1, CD 150, IPO-3), BLAME (SLAMF8), SELPLG (CD 162), LTBR, LAT, GADS, SLP-76, PAG/Cbp, NKp44, NKp30, NKp46, and NKG2D. In certain preferred embodiments, the one or more costimulatory signaling domains comprise a functional signaling domain of a protein selected, each independently, from the group consisting of: 4-1BB, CD27, and CD28. In certain preferred embodiments, the costimulatory signaling domain comprises a functional signaling domain of CD28. In certain embodiments, the CAR comprises an anti-CDl9 scFv, an intracellular domain of a Cü 3ζ chain, and a signaling domain of CD28. In certain preferred embodiments, the CD28 sequence is as set forth in Genbank identifier NM_006139 (sequence version 1, 2 or 3) starting with the amino acid sequence IEVMYPPPY (SEQ ID NO: 2) and continuing all the way to the carboxy-terminus of the protein. In certain preferred embodiments, the CAR is as included in KTE-C19 (axicabtagene ciloleucel) anti-CD 19 CAR-T therapy product in development by Kite Pharma, Inc. In certain embodiments, an immune cell suitable for immunotherapy, such as a CD8+ T-cell, comprises an exogenous T-cell receptor (TCR). Such cell can also be suitably denoted as having been engineered to comprise or to express the TCR.

[0053] In certain embodiments, an immune cell suitable for immunotherapy, such as a CD8+ T-cell, may be further genetically modified, such as gene edited, i.e., a target locus of interest in the cell may be modified by a suitable gene editing tool or technique, such as without limitation

CRISPR, TALEN or ZFN. An aspect relates to an immune cell obtainable by or obtained by said gene editing method, or progeny thereof, wherein the cell comprises a modification of the target locus not present in a cell not subjected to the method. Another aspect relates to a cell product from said cell or progeny thereof, wherein the product is modified in nature or quantity with respect to a cell product from a cell not subjected to the gene editing method. A further aspect provides an immune cell comprising a gene editing system, such as a CRISPR-Cas system, configured to carry out the modification of the target locus.

[0054] In certain preferred embodiments, the cell may be edited using any CRISPR system and method of use thereof as described herein. In certain preferred embodiments, cells are edited *ex vivo* and transferred to a subject in need thereof.

[0055] Further genetically modifying, such as gene editing, of the cell may be performed for example (1) to insert or knock-in an exogenous gene, such as an exogenous gene encoding a CAR or a TCR, at a preselected locus in the cell; (2) to knock-out or knock-down expression of an endogenous TCR in the cell; (3) to disrupt the target of a chemotherapeutic agent in the cell; (4) to knock-out or knock-down expression of an immune checkpoint protein or receptor in the cell; (5) to knock-out or knock-down expression of other gene or genes in the cell, the reduced expression or lack of expression of which can enhance the efficacy of adoptive therapies using the cell; (6) to knock-out or knock-down expression of an endogenous gene in a cell, said endogenous gene encoding an antigen targeted by an exogenous CAR or TCR; (7) to knock-out or knock-down expression of one or more MHC constituent proteins in the cell; (8) to activate a T cell, and/or increase the differentiation and/or proliferation of functionally exhausted or dysfunctional CD8+ T cells; and/or (9) to modulate CD8+ T cells, such that CD8+ T cells have increased resistance to exhaustion or dysfunction. In certain preferred embodiments, the cell may be edited to produce any one of the following combinations of the modifications set forth above: (1) and (2); (1) and (4); (2) and (4); (1), (2) and (4); (1) and (7); (2) and (7); (4) and (7); (1), (2) and (7); (1), (4) and (7); (1), (2), (4) and (7); optionally adding modification (8) or (9) to any one of the preceding combinations. In certain preferred embodiments, the targeted immune checkpoint protein or receptor is PD-l, PD-L1 and/or CTLA-4. In certain preferred embodiments, the targeted endogenous TCR gene or sequence may be TRBC1, TRBC2 and/or TRAC. In certain preferred embodiments, the targeted MHC constituent protein may be HLA-A,

B and/or C, and/or B2M. In certain embodiments, the cell may thus be multiply edited (multiplex genome editing) to (1) knock-out or knock-down expression of an endogenous TCR (for example, TRBC1, TRBC2 and/or TRAC), (2) knock-out or knock-down expression of an immune checkpoint protein or receptor (for example PD1, PD-L1 and/or CTLA4); and (3) knock-out or knock-down expression of one or more MHC constituent proteins (for example, HLA-A, B and/or C, and/or B2M, preferably B2M).

[0056] These and other aspects, objects, features, and advantages of the example embodiments will become apparent to those having ordinary skill in the art upon consideration of the following detailed description of illustrated example embodiments.

BRIEF DESCRIPTION OF THE DRAWINGS

[0057] **FIG.** 1 - illustrates the study design. Cells were sampled at the indicated time points from a mouse B16 melanoma model. Cells were sorted based on the following markers: 1) CD8+CD45+; 2) CD4+CD45+ (Effector and Regulatory); 3) CD4-CD8-CD45+ (NK cells, dendritic, macrophages); and 4) CD45- (fibroblasts, tumor cells). The cells were sequenced using plate based single cell sequencing.

[0058] **FIG.** 2 - illustrates the number of TILs isolated from the B16 melanoma mouse model, the time points and the number of mice at each time point.

[0059] **FIG.** 3 - illustrates clustering of CD8 T cells (left) and CD4 T cells (right).

[0060] **FIG.** 4 - illustrates tSNE of CD8+ cells. 2592 cells were sequenced and 2017 passed extensive quality control. tSNE and clustering was performed on principal components (PC) 4-9 (PC1 - transcription, PC2, PC3 strongly associated with sequencing batches).

[0061] **FIG.** 5 - illustrates tSNE plots for each of the fifteen CD8+ clusters.

[0062] **FIG.** 6 - illustrates further characterization of clusters 7, 8, 9 and 10 for expression of TIM-3 and PD-l.

[0063] **FIG.** 7 - illustrates a plot showing decoupled dysfunction and activation signatures based on signatures disclosed in Singer et al. 2016.

[0064] FIG. **8** - illustrates that clusters 7 and 9 are distinguished by the decoupling of dysfunction and activation signatures.

[0065] FIG. 9 - illustrates that cluster 7 is Tim3+PDl+ and high for a CD8 Treg signature and cluster 9 does not express a CD8 Treg signature.

[0066] FIG. 10 - illustrates that clusters 7 and 8 express a CD8 Treg signature.

[0067] FIG. 11 - illustrates that cluster 7 expresses MT1 and Helios (IKZF2).

[0068] FIG. 12 - illustrates that MT+ PD-1+ TIM3+ double positive (DP) cells are more suppressive than MT-/- DP.

[0069] FIG. 13 - illustrates that cluster 9 and 10 express different signatures from cluster 7. Cluster 7 is low for a cell cycle and CD8 activation signature.

[0070] FIG. 14 - illustrates transmembrane receptors that can be used to sort cluster 7 cells.

[0071] FIG. 15 - illustrates cytokines/chemokines expressed by cluster 7.

[0072] FIG. 16 - illustrates transcription factors significantly upregulated in cluster 7 as compared to clusters 10 and 9.

[0073] FIG. 17 - illustrates FACS sorting of CD8 T cells for the markers PD1, TIM3, HMMR, Helios and Ki-67.

[0074] FIG. 18 - illustrates FACS sorting of CD8 T cells for the markers PD1, TIM3, cKIT and Helios and Ki-67.

[0075] FIG. 19 - illustrates tSNE of CD4+ cells. 2496 cells were sequenced (26 plates) and 1478 passed extensive quality control. Shown is Foxp3 expression (marker for CD4+ Tregs).

[0076] FIG. 20 - illustrates tSNE plots for each of the fourteen CD4+ clusters.

[0077] FIG. 21 - illustrates major CD4 Treg populations.

[0078] FIG. 22 - illustrates Tim+ expressing Treg populations.

[0079] FIG. 23 - illustrates that clusters 4 and 7 express a Thl signature and cytokine secretion signature.

[0080] FIG. 24 - illustrates that there are positive and negative correlations across the CD8 and CD4 clusters.

[0081] FIG. 25 - illustrates significant correlations between the CD8 and CD4 clusters. Red indicates a negative correlation and blue indicates a positive correlation.

[0082] FIG. 26 - illustrates a heatmap indicating significant correlations between the CD8 and CD4 clusters.

[0083] FIG. 27 - illustrates cell-cell interactions based on expression of receptors and ligands on the CD4 and CD8 clusters

[0084] FIG. 28 - illustrates analysis of single cell TILs.

[0085] FIG. 29 - illustrates the study design. Cells were sampled at 5 time points from 12 B16 melanoma mice. Cells were sorted based on the following markers: CD8+, CD4+ and CD45+. The cells were sequenced using plate based single cell sequencing.

[0086] FIG. 30 - illustrates tSNE clustering of 2,017 CD8 T cells (left) and 1,478 CD4 T cells (right).

[0087] FIG. 31 - illustrates that single-cell RNA-seq identifies activation-like and dysfunction-like populations by clustering CD8 T cells.

[0088] FIG. 32 - illustrates that clusters high for a dysfunction signature are high for a $CD8^+$ T regulatory signature.

[0089] FIG. 33 - illustrates that a suppressive $CD8^+$ population exists in tumors and is weakened by MT KO.

[0090] FIG. 34 - illustrates the identification of CD8 cluster 7 markers by FACS.

[0091] FIG. 35 - illustrates the expression in tSNE plots of CD8 cluster 7 markers.

[0092] FIG. 36 - illustrates that the relative frequency of dysfunctional $CD8^+$ T cells in a tumor is correlated with $CD4^+$ Treg frequency.

[0093] FIG. 37 - illustrates CD4/CD8 cell connections.

[0094] FIG. 38 - illustrates expression in tSNE plots of XCL1 in cluster 8 and XCR1 in cluster 7.

[0095] FIG. 39 - illustrates expression in tSNE plots of CCL1 in cluster 8 and CCR8 in clusters 7 and 8 and in Treg+Tim3+ CD4 cells.

[0096] FIG. 40 - illustrates analysis of single cells from the mouse model time points using the 10X genomics platform. Cell counts taken for cells sorted by day (left) and sorted by size (right) are shown.

[0097] FIG. 41 - illustrates the first step in the 10X analysis. CD3+ cells are selected. The count of all cells and CD3 cells were taken, as well as the percentage of CD3. Shown is time point 11.

[0098] FIG. 42 - illustrates the general statistics for all time points taken.

[0099] **FIG. 43** - illustrates CD8 /CD4 partitioning of the clusters. Shown is time point 9.

[0100] **FIG. 44** - illustrates the fourth step in the 10X analysis. CD8 / CD4 cells are selected and batch corrected across time points.

[0101] **FIG.** 45 - illustrates strict selection of CD8 cells and plots based on mouse, time point / batch, and by clustering.

[0102] **FIG. 46** - illustrates tSNE plots of CD8 cell cluster specific reference genes (CD83 Zfp36ll, Xcll, Bcl6, HMMR, Illr2, Tnfrsf9, Kit and Ikzf2).

[0103] **FIG. 47** - illustrates tSNE plots of **CD8** cell cluster specific reference genes.

[0104] **FIG. 48** - illustrates that the same populations of cells are observed in the plate based and 10X single cell sequencing.

[0105] **Fig. 49A-F - Examples of CD8 TCR clones from B16 mice shown on CD8 T cell tSNE plots** (Light grey- all cells of mouse, Black- cells of mouse with alpha and beta chains detected, Dark grey- cells in a clone). **FIG. 49A** shows clone 108 (TRAV3-3_AGTCAAATCGGACT _TRAJ7 (SEQ ID NO: 21); TRBV5_CAGCCCCCCTGGG(G)CAGAA_TRBJ2-3) (SEQ ID NO: 22). **Fig. 49B** shows clone 137 (TRAV5-1_CAGCAGGGGGTAACT_TRAJ26 (SEQ ID NO: 23); TRBV14_AGCAGCAAGGGACATAGTCA _TRBJ2-4) (SEQ ID NO: 24). **Fig. 49C** shows clone 151 (TRAV10_CAGCAAAAGACTA _TRAJ7 (SEQ ID NO: 25); TRBV5_CAGCCCGACAGGGGGAAACT _TRBJ1-2) (SEQ ID NO: 26). **Fig. 49D** shows clone 246 (TRAV7-2_CAAGCGACTA _TRAJ7 (SEQ ID NO: 27); TRBV16_TTAGAACTGGGGGGGCGCGAACA _TRBJ2-7) (SEQ ID NO: 28). **Fig. 49E** shows clone 164 (TRAV9N-3_CTGTGTATCCGGACT _TRAJ7 (SEQ ID NO: 29); TRBV5_CCAAGTGCTTACGGACAC _TRBJ2-5) (SEQ ID NO: 30). **Fig. 49F** shows clone 153 (TRAV3-3_GTCAGACATAACA_TRAJ27 (SEQ ID NO: 31); TRBV26_AGCAGCCCGATCTGGACAAGTAACT _TRBJ2-1) (SEQ ID NO: 32).

[0106] **Fig. 50 - TCR clones defined do not overlap across mice.** Plot comparing TCR clones across mice (two cells were called in the same clone only if they have an alpha and beta chain in common).

[0107] **Fig. 51 - Clonal expansion in CD8 T cell clusters.** (Left) clone size and (right) relative clonal expansion rate shown on CD8 T cell tSNE plots.

[0108] **Fig. 52 - Clonal expansion in CD8 T cell clusters.** (Left) bar graph showing the number of CD8 T cells in each CD8 cluster (right) violin plots showing the relative clonal expansion in each CD8 cluster.

[0109] **Fig.** 53 - **Clonal expansion in CD8 T cell clusters.** Violin plots showing the relative clonal expansion and clone size in each CD8 cluster. (Top) plots for all cells eligible and (Bottom) plots for one measurement per clone.

[0110] **Fig. 54 - Enrichment of clones in CD8 T cell clusters.** (Left) Plot showing significant clones enriched in clusters. (right) Plot showing specific clones that are significant in more than one cluster.

[0111] **Fig. 55 - SIY vs. OVA antigen signature from a OVA+SIY+ lung cancer mouse model.** Heatmap of differentially expressed genes across SIY binding cells and OVA (OT1) binding cells (tetramer sorted). Tumors were induced in mice and CD4 and CD8 cells were collected during a time course (5 weeks, 8 weeks, 12 weeks and 20 weeks).

[0112] **Fig. 56 - The SIY-signature distinguishes bl6 cluster 8.** B16 CD8 T cell tSNE showing expression of the SIY-up signature.

[0113] **Fig. 57 - The SIY-signature distinguishes bl6 cluster 8.** Violin plots showing expression of the SIY-up signature in B16 CD8 T cell clusters.

[0114] **Fig. 58 - The OVA (SIY-down) signature does not distinguish bl6 clusters.** Violin plots showing expression of the SIY-down signature in B16 CD8 T cell clusters.

[0115] **Fig. 59 - Correlation heatmap of CD8 clusters and gene signatures** (SIY-up, SIY-down, exhaustion, Dysfunction/activation).

[0116] **Fig. 60 - B16 CD8 cluster 8 signature in lung SIY and OT1 specific TILs.** Violin plots showing expression of the cluster 8 signature in SIY+ and OT1+ TILs across the time course.

[0117] **Fig. 61 - B16 CD8 cluster 8 signature compared to SIY-up signature.** Venn diagram showing overlapping genes across the signatures.

[0118] **Fig. 62 - Expression of ZFP36L1 in CD8 T cell clusters.** ZFP36L1 expression shown on the CD8 T cell tSNE plot.

[0119] **Fig. 63 - Differential gene expression across time points.** Heat map showing 630 genes differentially expressed across time points in B16 mice (Day 11, 13, 15, 17 and 18). 15 time clusters are indicated.

[0120] **Fig. 64 - Differential gene expression across time points.** Violin plots showing the expression medians of the 15 time clusters at the indicated time points.

[0121] **Fig. 65 - Differential gene expression across time points.** Heat map showing 630 genes differentially expressed across time points (Day 11, 13, 15, 17 and 18) in individual B16 mice (1-12). 15 time clusters and time point coefficients are indicated. The coefficients per time point indicate the "general value" of expression per gene per time point.

[0122] **Fig. 66 - Connections between time-change clusters (logit) and CD8 T cell clusters (infomap).** Heat map showing enrichment of tSNE clusters and time point clusters for all values.

[0123] **Fig. 67 - Connections between time-change clusters (logit) and CD8 T cell clusters (infomap).** Heat map showing enrichment of tSNE clusters and time point clusters for all values intersecting.

DETAILED DESCRIPTION

[0124] Unless defined otherwise, technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure pertains. Definitions of common terms and techniques in molecular biology may be found in Molecular Cloning: A Laboratory Manual, 2nd edition (1989) (Sambrook, Fritsch, and Maniatis); Molecular Cloning: A Laboratory Manual, 4th edition (2012) (Green and Sambrook); Current Protocols in Molecular Biology (1987) (F.M. Ausubel et al. eds.); the series Methods in Enzymology (Academic Press, Inc.): PCR 2: A Practical Approach (1995) (M.J. MacPherson, B.D. Hames, and G.R. Taylor eds.): Antibodies, A Laboraotry Manual (1988) (Harlow and Lane, eds.): Antibodies A Laboraotry Manual, 2nd edition 2013 (E.A. Greenfield ed.); Animal Cell Culture (1987) (R.I. Freshney, ed.); Benjamin Lewin, Genes IX, published by Jones and Bartlet, 2008 (ISBN 0763752223); Kendrew *et al.* (eds.), The Encyclopedia of Molecular Biology, published by Blackwell Science Ltd., 1994 (ISBN 0632021829); Robert A. Meyers (ed.), Molecular Biology and Biotechnology: a Comprehensive Desk Reference, published by VCH

Publishers, Inc., 1995 (ISBN 9780471 185710); Singleton *et al.,* Dictionary of Microbiology and Molecular Biology 2nd ed., J. Wiley & Sons (New York, N.Y. 1994), March, Advanced Organic Chemistry Reactions, Mechanisms and Structure 4th ed., John Wiley & Sons (New York, N.Y. 1992); and Marten H. Hofker and Jan van Deursen, Transgenic Mouse Methods and Protocols, 2nd edition (201 1).

[0125] As used herein, the singular forms "a", "an", and "the" include both singular and plural referents unless the context clearly dictates otherwise.

[0126] The term "optional" or "optionally" means that the subsequent described event, circumstance or substituent may or may not occur, and that the description includes instances where the event or circumstance occurs and instances where it does not.

[0127] The recitation of numerical ranges by endpoints includes all numbers and fractions subsumed within the respective ranges, as well as the recited endpoints.

[0128] The terms "about" or "approximately" as used herein when referring to a measurable value such as a parameter, an amount, a temporal duration, and the like, are meant to encompass variations of and from the specified value, such as variations of +/-l0% or less, preferably *+1-5%* or less, more preferably +/-l% or less, and still more preferably +/-0.l% or less of and from the specified value, insofar such variations are appropriate to perform in the disclosed invention. It is to be understood that the value to which the modifier "about" or "approximately" refers is itself also specifically, and preferably, disclosed.

[0129] Reference throughout this specification to "one embodiment", "an embodiment" means that a particular feature, structure or characteristic described in connection with the embodiment is included in at least one embodiment of the present invention. Thus, appearances of the phrases "in one embodiment" or "in an embodiment" in various places throughout this specification are not necessarily all referring to the same embodiment, but may. Furthermore, the particular features, structures or characteristics may be combined in any suitable manner, as would be apparent to a person skilled in the art from this disclosure, in one or more embodiments. Furthermore, while some embodiments described herein include some but not other features included in other embodiments, combinations of features of different embodiments are meant to be within the scope of the invention, and form different embodiments, as would be

understood by those in the art. For example, in the appended claims, any of the claimed embodiments can be used in any combination.

[0130] The terms “subject”, “individual” or “patient” are used interchangeably throughout this specification, and typically and preferably denote humans, but may also encompass reference to non-human animals, preferably warm-blooded animals, even more preferably mammals, such as, e.g., non-human primates, rodents, canines, felines, equines, ovines, porcines, and the like. The term “non-human animals” includes all vertebrates, e.g., mammals, such as non-human primates, (particularly higher primates), sheep, dog, rodent (e.g. mouse or rat), guinea pig, goat, pig, cat, rabbits, cows, and non-mammals such as chickens, amphibians, reptiles etc. In one embodiment, the subject is a non-human mammal. In another embodiment, the subject is human. In another embodiment, the subject is an experimental animal or animal substitute as a disease model. The term does not denote a particular age or sex. Thus, adult and newborn subjects, as well as fetuses, whether male or female, are intended to be covered. Examples of subjects include humans, dogs, cats, cows, goats, and mice. The term subject is further intended to include transgenic species.

[0131] The terms “subtype”, “subset” or “subpopulation” are used interchangeably throughout this specification.

[0132] All publications, published patent documents, and patent applications cited herein are hereby incorporated by reference to the same extent as though each individual publication, published patent document, or patent application was specifically and individually indicated as being incorporated by reference.

Overview

[0133] Embodiments disclosed herein relate to cell products, substances, compositions, markers, marker signatures, molecular targets, kits of parts and methods useful in characterizing, evaluating and modulating the immune system and immune responses. The CD8+ and CD4+ T cells of the present invention were discovered by analysis of single immune cells obtained at several time points from a mouse tumor model (B16). The transcriptomes of the CD8+ and CD4+ T cells were analyzed. In certain embodiments, T cells were characterized as a suppressive CD4+ or CD8+ T cell population required to dampen excessive immune responses and prevent autoimmunity (e.g., a subtype of CD8 Tregs). In certain embodiments, T cells were characterized

as an effector CD4+ or CD8+ T cell population (e.g., activated). Applicants identified markers expressed by the CD8+ and CD4+ T cells that can be used to detect and/or quantitate the T cells or specifically target the T cells therapeutically. Furthermore, the surface cell markers can be used to detect, quantitate and isolate the T cells. The identified markers can also be used to distinguish between PD1+ CD8+ T cell subtypes. In certain embodiments, the T cell is characterized by expression of PD-l and TIM3. In certain embodiments, the T cell is characterized by expression of PD-l and lack of expression of TIM3. Moreover, Applicants can confirm the presence of the CD8+ T cells in human samples.

[0134] In certain embodiments, a T cell is a suppressive T cell. In certain embodiments, the T cell is characterized by expression of CD8, TIM3, PD1, MT1, and IKZF2, and low or no expression of HMMR. The T cell may be further characterized by expression of one or more of TNFRSF9, PRF1, BHLHE40 (DEC1), IRF8, GLDC, STAT3, CST7, IL1R2, EEF2, SLC2A3, SQSTM1, RBPJ, NABP1, ACTN1, TNFRSF4, SERPINB9, FOSL2, CAPG, KLRC1, IL18R1, JUNB, EEF1A1, TNFRSF18, RGS2, NFKB2, RPL5, PEX16, LAT2, KDM5B, HILPDA, GEM, DENND4A, BCL2L11, ADAM8, PGLYRP1, KIT, SERPINE2, CCRL2, CSF1, EPAS1, RUNX2, SPRY2 and XCR1, preferably upregulated as compared to all CD8+ TIM3+ PD1+ T cells in a population of cells.

[0135] In certain embodiments, a T cell is an activated T cell. In certain embodiments, the T cell is characterized by expression of PD-l and TIM3. In certain embodiments, the T cell is characterized by expression of PD-l, TIM3 and HMMR. In certain embodiments, the $CD8^+$ T cell expresses PD-l, TIM3, and KI67 and does not express Helios. In certain embodiments, the T cell may be characterized by expression of a gene signature comprising one or more genes selected from one of Table 20.

[0136] In certain embodiments, a T cell is primed to be an activated T cell. In certain embodiments, the T cell is characterized by expression of PD-l and lack of expression of TIM3. In certain embodiments, the T cell is characterized by expression of Helios (IKZF2), XCL1 and/or CCR8. In certain embodiments, the T cell does not express MT1. In certain embodiments, the T cell may be characterized by expression of a gene signature comprising one or more genes selected from one of Table 19.

[0137] In certain embodiments, the T cell may be characterized by expression of a gene signature comprising any gene or combination of genes selected from one of Tables 1 to 20.

[0138] In certain embodiments, depletion of the suppressive T cells may be used in adoptive cell transfer (e.g., TIL therapy, CAR T therapy). In certain embodiments, T cells (e.g., tumor infiltrating lymphocytes or TILs) may obtained from a subject and depleted for the T cells described herein *ex* vivo. The term "ex vivo" is encompassed by the term "in vitro." The term "in vitro" generally denotes outside, or external to, a body, e.g., an animal or human body. In certain embodiments, removing CD8+ Tregs from a population of T cells used in adoptive cell transfer allows an enhanced immune response. In certain embodiments, CD8 Tregs normally prevent the immune system from targeting self-antigens, but in the case of cancer Tregs may prevent immune cells from targeting cancer cells through suppression of effector cells. In certain embodiments, a population of T cells enriched for suppressive T cells may be used in treating an autoimmune disease.

[0139] In certain embodiments, enrichment of activated T cells or T cells primed for activation may be used in adoptive cell transfer (e.g., TIL therapy, CAR T therapy). In certain embodiments, the T cell is selected based on the affinity of the antigen targeted. The antigen may have high affinity for a TCR or MHC molecule or low affinity. In certain embodiments, the subtype may be used in adoptive cell transfer (e.g., TIL therapy, CAR T therapy). In certain embodiments, TILs may be isolated from a tumor and the isolated cells selected for one or more specific subtypes. The one or more specific subtypes may be expanded or may be used to express a CAR or endogenous TCR. In certain embodiments, allogenic CAR T cells may be enriched for one or more specific subtypes.

[0140] Particular advantageous uses include methods for identifying agents capable of inducing or suppressing one or more immune cell subtypes based on the gene signatures, protein signature, and/or other genetic or epigenetic signature as defined herein. In certain example embodiments, detection or quantifying the subtypes may be used to determine responsiveness to various therapeutics.

[0141] Particular advantageous uses include methods for identifying agents capable of modulating the T cells based on their gene signatures, protein signature, and/or other genetic or epigenetic signature as defined herein. In certain example embodiments, detection or quantifying

the T cells may be used to determine responsiveness to various therapeutics (e.g., an increase or decrease in one or more of the T cells may indicate an immunotherapy is effective). Not being bound by a theory, checkpoint blockade therapy may specifically target the T cells of the present invention. In certain embodiments, cytokines or differentiating agents may be used to shift the balance of T cells to be less or more suppressive or more activated.

[0142] In one aspect, the invention relates to a signature or set of biomarkers that distinguish between CD8+ T cells. The signature may be a gene signature, protein signature, and/or other genetic or epigenetic signature of particular tumor cell subpopulations, as defined herein. In certain embodiments, CD8+ T cell subtypes may be detected and isolated by subtype specific signature biomarkers or combinations thereof.

[0143] In certain embodiments, pharmaceutical compositions comprising populations of T cells wherein the T cells of the present invention are depleted or enriched may be used in treating cancer (e.g., adoptive cell transfer). In certain embodiments, populations of cells depleted or enriched for the T cells of the present invention are used in combination with other therapies (e.g., checkpoint blockade therapy, CAR T cell therapy). In certain embodiments, pharmaceutical compositions comprising populations of T cells wherein the suppressive T cells of the present invention are enriched may be used in treating an autoimmune disease.

[0144] The invention further relates to agents capable of inducing or suppressing particular immune cell populations based on the gene signatures, protein signature, and/or other genetic or epigenetic signature as defined herein, as well as their use for modulating, such as inducing or repressing, a particular gene signature, protein signature, and/or other genetic or epigenetic signature. In one embodiment, genes in one population of cells may be activated or suppressed in order to affect the cells of another population (e.g., suppressive T cells may be activated or inactivated to enhance or repress activity of effector T cells). Not being bound by a theory, the CD8+ T cells described herein are effected by other immune cells in the tumor microenvironment (e.g., antigen presenting cells). In related aspects, modulating, such as inducing or repressing, a particular gene signature, protein signature, and/or other genetic or epigenetic signature may modify overall immune cell composition, such as immune cell composition, such as immune cell subpopulation composition or distribution, or functionality.

[0145] In further aspects, the invention relates to a signature or set of biomarkers that may be detected in combination. The signature detected in combination may be a gene signature, protein signature, and/or other genetic or epigenetic signature of a particular tumor cell (sub)population (e.g., tumor cells capable of immune evasion, tumor cells having specific mutations). The invention hereto also further relates to particular tumor cell subpopulations, which may be identified based on the methods according to the invention as discussed herein; as well as methods to target such cell subpopulations, such as in therapeutics (e.g., adoptive cell therapy, CAR T cells, agents capable of modulating T cells as defined herein); and screening methods to identify agents capable of inducing or suppressing particular tumor cell (sub)populations.

[0146] The term "immune cell" as used throughout this specification generally encompasses any cell derived from a hematopoietic stem cell that plays a role in the immune response. The term is intended to encompass immune cells both of the innate or adaptive immune system. The immune cell as referred to herein may be a leukocyte, at any stage of differentiation (e.g., a stem cell, a progenitor cell, a mature cell) or any activation stage. Immune cells include lymphocytes (such as natural killer cells, T-cells (including, e.g., thymocytes, Th or Tc; Thl, Th2, Thl7, Tha.p, CD4+, CD8+, effector Th, memory Th, regulatory Th, CD4+/CD8+ thymocytes, CD4-/CD8- thymocytes, γδ T cells, etc.) or B-cells (including, e.g., pro-B cells, early pro-B cells, late pro-B cells, pre-B cells, large pre-B cells, small pre-B cells, immature or mature B-cells, producing antibodies of any isotype, T1 B-cells, T2, B-cells, naive B-cells, GC B-cells, plasmablasts, memory B-cells, plasma cells, follicular B-cells, marginal zone B-cells, B-l cells, B-2 cells, regulatory B cells, etc.), such as for instance, monocytes (including, e.g., classical, non-classical, or intermediate monocytes), (segmented or banded) neutrophils, eosinophils, basophils, mast cells, histiocytes, microglia, including various subtypes, maturation, differentiation, or activation stages, such as for instance hematopoietic stem cells, myeloid progenitors, lymphoid progenitors, myeloblasts, promyelocytes, myelocytes, metamyelocytes, monoblasts, promonocytes, lymphoblasts, prolymphocytes, small lymphocytes, macrophages (including, e.g., Kupffer cells, stellate macrophages, M1 or M2 macrophages), (myeloid or lymphoid) dendritic cells (including, e.g., Langerhans cells, conventional or myeloid dendritic cells, plasmacytoid dendritic cells, mDC-l, mDC-2, Mo-DC, HP-DC, veiled cells), granulocytes, polymorphonuclear cells, antigen-presenting cells (APC), etc.

[0147] As used throughout this specification, "immune response" refers to a response by a cell of the immune system, such as a B cell, T cell (CD4+ or CD8+), regulatory T cell, antigen-presenting cell, dendritic cell, monocyte, macrophage, NKT cell, NK cell, basophil, eosinophil, or neutrophil, to a stimulus. In some embodiments, the response is specific for a particular antigen (an "antigen-specific response"), and refers to a response by a CD4 T cell, CD8 T cell, or B cell via their antigen-specific receptor. In some embodiments, an immune response is a T cell response, such as a CD4+ response or a CD8+ response. Such responses by these cells can include, for example, cytotoxicity, proliferation, cytokine or chemokine production, trafficking, or phagocytosis, and can be dependent on the nature of the immune cell undergoing the response.

[0148] T cell response refers more specifically to an immune response in which T cells directly or indirectly mediate or otherwise contribute to an immune response in a subject. T cell-mediated response may be associated with cell mediated effects, cytokine mediated effects, and even effects associated with B cells if the B cells are stimulated, for example, by cytokines secreted by T cells. By means of an example but without limitation, effector functions of MHC class I restricted Cytotoxic T lymphocytes (CTLs), may include cytokine and/or cytolytic capabilities, such as lysis of target cells presenting an antigen peptide recognized by the T cell receptor (naturally-occurring TCR or genetically engineered TCR, e.g., chimeric antigen receptor, CAR), secretion of cytokines, preferably IFN gamma, TNF alpha and/or or more immunostimulatory cytokines, such as IL-2, and/or antigen peptide-induced secretion of cytotoxic effector molecules, such as granzymes, perforins or granulysin. By means of example but without limitation, for MHC class II restricted T helper (Th) cells, effector functions may be antigen peptide-induced secretion of cytokines, preferably, IFN gamma, TNF alpha, IL-4, IL5, IL-10, and/or IL-2. By means of example but without limitation, for T regulatory (Treg) cells, effector functions may be antigen peptide-induced secretion of cytokines, preferably, IL-10, IL-35, and/or TGF-beta. B cell response refers more specifically to an immune response in which B cells directly or indirectly mediate or otherwise contribute to an immune response in a subject. Effector functions of B cells may include in particular production and secretion of antigen-specific antibodies by B cells (e.g., polyclonal B cell response to a plurality of the epitopes of an antigen (antigen-specific antibody response)), antigen presentation, and/or cytokine secretion.

[0149] The term “antigen” as used throughout this specification refers to a molecule or a portion of a molecule capable of being bound by an antibody, or by a T cell receptor (TCR) when presented by MHC molecules. At the molecular level, an antigen is characterized by its ability to be bound at the antigen-binding site of an antibody. The specific binding denotes that the antigen will be bound in a highly selective manner by its cognate antibody and not by the multitude of other antibodies which may be evoked by other antigens. An antigen is additionally capable of being recognized by the immune system. In some instances, an antigen is capable of eliciting a humoral immune response in a subject. In some instances, an antigen is capable of eliciting a cellular immune response in a subject, leading to the activation of B- and/or T-lymphocytes. In some instances, an antigen is capable of eliciting a humoral and cellular immune response in a subject. Hence, an antigen may be preferably antigenic and immunogenic. Alternatively, an antigen may be antigenic and not immunogenic. Typically, an antigen may be a peptide, polypeptide, protein, nucleic acid, an oligo- or polysaccharide, or a lipid, or any combination thereof, a glycoprotein, proteoglycan, glycolipid, etc. In certain embodiments, an antigen may be a peptide, polypeptide, or protein. An antigen may have one or more than one epitope. The terms “antigenic determinant” or “epitope” generally refer to the region or part of an antigen that specifically reacts with or is recognized by the immune system, specifically by antibodies, B cells, or T cells.

[0150] An antigen as contemplated throughout this specification may be obtained by any means available to a skilled person, e.g., may be isolated from a naturally-occurring material comprising the antigen, or may be produced recombinantly by a suitable host or host cell expression system and optionally isolated therefrom (e.g., a suitable bacterial, yeast, fungal, plant or animal host or host cell expression system), or may be produced recombinantly by cell-free transcription or translation, or non-biological nucleic acid or peptide synthesis.

[0151] The term “tumor antigen” as used throughout this specification refers to an antigen that is uniquely or differentially expressed by a tumor cell, whether intracellular or on the tumor cell surface (preferably on the tumor cell surface), compared to a normal or non-neoplastic cell. By means of example, a tumor antigen may be present in or on a tumor cell and not typically in or on normal cells or non-neoplastic cells (e.g., only expressed by a restricted number of normal tissues, such as testis and/or placenta), or a tumor antigen may be present in or on a tumor cell in

greater amounts than in or on normal or non-neoplastic cells, or a tumor antigen may be present in or on tumor cells in a different form than that found in or on normal or non-neoplastic cells. The term thus includes tumor-specific antigens (TSA), including tumor-specific membrane antigens, tumor-associated antigens (TAA), including tumor-associated membrane antigens, embryonic antigens on tumors, growth factor receptors, growth factor ligands, etc. The term further includes cancer/testis (CT) antigens. Examples of tumor antigens include, without limitation, β-human chorionic gonadotropin (PHCG), glycoprotein 100 (gplOO/Pmel 17), carcinoembryonic antigen (CEA), tyrosinase, tyrosinase-related protein 1 (gp75/TRPl), tyrosinase-related protein 2 (TRP-2), NY-BR-l, NY-CO-58, NY-ESO-l, MN/gp250, idiotypes, telomerase, synovial sarcoma X breakpoint 2 (SSX2), mucin 1 (MUC-l), antigens of the melanoma-associated antigen (MAGE) family, high molecular weight-melanoma associated antigen (HMW-MAA), melanoma antigen recognized by T cells 1 (MART1), Wilms' tumor gene 1 (WT1), HER2/neu, mesothelin (MSLN), alphafetoprotein (AFP), cancer antigen 125 (CA-125), and abnormal forms of ras or p53 (see also, WO2016187508A2). Tumor antigens may also be subject specific (e.g., subject specific neoantigens; see, e.g., ET.S. patent 9,1 15,402; and international patent application publication numbers W02016100977A1, WO2014168874A2, WO2015085233A1, and W0201509581 1A2).

Biomarkers and Signatures

[0152] The invention further relates to various biomarkers for detecting CD8+ T cell populations. As used herein "marker" and "biomarker" are used interchangeably. In certain example embodiments, suppressive CD8+ T cell populations are present in a population of tumor infiltrating lymphocytes (TIL). The suppressive T cell populations may be detected by detecting one or more biomarkers in a sample. The set of markers may comprise one or more genes or polypeptides, e.g, TIM3, SERPINE2, HMMR, KIT, TNFRSF4, CD8, CD45, PD1, TNFRSF9, PRF1, BITLITE40, IRF8, GLDC, STAT3, CST7, IL1R2, EEF2, SLC2A3, SQSTM1, RBPJ, NABP1, ACTN1, TNFRSF4, SERPINB9, FOSL2, CAPG, KLRC1, IL18R1, JUNB, EEF1A1, TNFRSF18, RGS2, NFKB2, RPL5, PEX16, LAT2, KDM5B, HILPDA, GEM, DENND4A, BCL2L1 1, ADAM8, PGLYRP1, IKZF2, KIT, SERPINE2, CCRL2, CSF1, EPAS1, RUNX2, SPRY2, XCL1, CCR8, MT1 and KI67. In certain embodiments, the markers include the following combinations: a) TIM3, SERPINE2 and ITMMR; or b) SERPINE2 and ITMMR; or c)

TIM3, KIT and HMMR; or d) TIM3, TNFRSF4 and HMMR; or e) any of (a), (b), (c) or (d) and one or more of CD8, CD45 and PD1; or any of (a), (b), (c), (d) or (e) and one or more of TNFRSF9, PRF1, BHLHE40, IRF8, GLDC, STAT3, CST7, IL1R2, EEF2, SLC2A3, SQSTM1, RBPJ, NABP1, ACTN1, TNFRSF4, SERPINB9, FOSL2, CAPG, KLRC1, IL18R1, JUNB, EEF1A1, TNFRSF18, RGS2, NFKB2, RPL5, PEX16, LAT2, KDM5B, HILPDA, GEM, DENND4A, BCL2L1 1, ADAM8, PGLYRP1, IKZF2, KIT, SERPINE2, CCRL2, CSF1, EPAS1, RUNX2, SPRY2, XCL1, CCR8, MT1 and KI67.

[0153] The term “biomarker” is widespread in the art and commonly broadly denotes a biological molecule, more particularly an endogenous biological molecule, and/or a detectable portion thereof, whose qualitative and/or quantitative evaluation in a tested object (e.g., in or on a cell, cell population, tissue, organ, or organism, e.g., in a biological sample of a subject) is predictive or informative with respect to one or more aspects of the tested object’s phenotype and/or genotype. The terms “marker” and “biomarker” may be used interchangeably throughout this specification. Biomarkers as intended herein may be nucleic acid-based or peptide-, polypeptide- and/or protein-based. For example, a marker may be comprised of peptide(s), polypeptide(s) and/or protein(s) encoded by a given gene, or of detectable portions thereof. Further, whereas the term “nucleic acid” generally encompasses DNA, RNA and DNA/RNA hybrid molecules, in the context of markers the term may typically refer to heterogeneous nuclear RNA (hnRNA), pre-mRNA, messenger RNA (mRNA), or complementary DNA (cDNA), or detectable portions thereof. Such nucleic acid species are particularly useful as markers, since they contain qualitative and/or quantitative information about the expression of the gene. Particularly preferably, a nucleic acid-based marker may encompass mRNA of a given gene, or cDNA made of the mRNA, or detectable portions thereof. Any such nucleic acid(s), peptide(s), polypeptide(s) and/or protein(s) encoded by or produced from a given gene are encompassed by the term “gene product(s)”.

[0154] Preferably, markers as intended herein may be extracellular or cell surface markers, as methods to measure extracellular or cell surface marker(s) need not disturb the integrity of the cell membrane and may not require fixation / permeabilization of the cells.

[0155] ETnless otherwise apparent from the context, reference herein to any marker, such as a peptide, polypeptide, protein, or nucleic acid, may generally also encompass modified forms of

said marker, such as bearing post-expression modifications including, for example, phosphorylation, glycosylation, lipidation, methylation, cysteinylation, sulphonation, glutathionylation, acetylation, oxidation of methionine to methionine sulphoxide or methionine sulphone, and the like.

[0156] The term "peptide" as used throughout this specification preferably refers to a polypeptide as used herein consisting essentially of 50 amino acids or less, e.g., 45 amino acids or less, preferably 40 amino acids or less, e.g., 35 amino acids or less, more preferably 30 amino acids or less, e.g., 25 or less, 20 or less, 15 or less, 10 or less or 5 or less amino acids.

[0157] The term "polypeptide" as used throughout this specification generally encompasses polymeric chains of amino acid residues linked by peptide bonds. Hence, insofar a protein is only composed of a single polypeptide chain, the terms "protein" and "polypeptide" may be used interchangeably herein to denote such a protein. The term is not limited to any minimum length of the polypeptide chain. The term may encompass naturally, recombinantly, semi-synthetically or synthetically produced polypeptides. The term also encompasses polypeptides that carry one or more co- or post-expression-type modifications of the polypeptide chain, such as, without limitation, glycosylation, acetylation, phosphorylation, sulfonation, methylation, ubiquitination, signal peptide removal, N-terminal Met removal, conversion of pro-enzymes or pre-hormones into active forms, etc. The term further also includes polypeptide variants or mutants which carry amino acid sequence variations vis-a-vis a corresponding native polypeptide, such as, e.g., amino acid deletions, additions and/or substitutions. The term contemplates both full-length polypeptides and polypeptide parts or fragments, e.g., naturally-occurring polypeptide parts that ensue from processing of such full-length polypeptides.

[0158] The term "protein" as used throughout this specification generally encompasses macromolecules comprising one or more polypeptide chains, i.e., polymeric chains of amino acid residues linked by peptide bonds. The term may encompass naturally, recombinantly, semi-synthetically or synthetically produced proteins. The term also encompasses proteins that carry one or more co- or post-expression-type modifications of the polypeptide chain(s), such as, without limitation, glycosylation, acetylation, phosphorylation, sulfonation, methylation, ubiquitination, signal peptide removal, N-terminal Met removal, conversion of pro-enzymes or pre-hormones into active forms, etc. The term further also includes protein variants or mutants

which carry amino acid sequence variations vis-a-vis a corresponding native protein, such as, e.g., amino acid deletions, additions and/or substitutions. The term contemplates both full-length proteins and protein parts or fragments, e.g., naturally-occurring protein parts that ensue from processing of such full-length proteins.

[0159] The reference to any marker, including any peptide, polypeptide, protein, or nucleic acid, corresponds to the marker commonly known under the respective designations in the art. The terms encompass such markers of any organism where found, and particularly of animals, preferably warm-blooded animals, more preferably vertebrates, yet more preferably mammals, including humans and non-human mammals, still more preferably of humans.

[0160] The terms particularly encompass such markers, including any peptides, polypeptides, proteins, or nucleic acids, with a native sequence, i.e., ones of which the primary sequence is the same as that of the markers found in or derived from nature. A skilled person understands that native sequences may differ between different species due to genetic divergence between such species. Moreover, native sequences may differ between or within different individuals of the same species due to normal genetic diversity (variation) within a given species. Also, native sequences may differ between or even within different individuals of the same species due to somatic mutations, or post-transcriptional or post-translational modifications. Any such variants or isoforms of markers are intended herein. Accordingly, all sequences of markers found in or derived from nature are considered “native”. The terms encompass the markers when forming a part of a living organism, organ, tissue or cell, when forming a part of a biological sample, as well as when at least partly isolated from such sources. The terms also encompass markers when produced by recombinant or synthetic means.

[0161] In certain embodiments, markers, including any peptides, polypeptides, proteins, or nucleic acids, may be human, i.e., their primary sequence may be the same as a corresponding primary sequence of or present in a naturally occurring human markers. Hence, the qualifier “human” in this connection relates to the primary sequence of the respective markers, rather than to their origin or source. For example, such markers may be present in or isolated from samples of human subjects or may be obtained by other means (e.g., by recombinant expression, cell-free transcription or translation, or non-biological nucleic acid or peptide synthesis).

[0162] The reference herein to any marker, including any peptide, polypeptide, protein, or nucleic acid, also encompasses fragments thereof. Hence, the reference herein to measuring (or measuring the quantity of) any one marker may encompass measuring the marker and/or measuring one or more fragments thereof.

[0163] For example, any marker and/or one or more fragments thereof may be measured collectively, such that the measured quantity corresponds to the sum amounts of the collectively measured species. In another example, any marker and/or one or more fragments thereof may be measured each individually. The terms encompass fragments arising by any mechanism, *in vivo* and/or *in vitro*, such as, without limitation, by alternative transcription or translation, exo- and/or endo-proteolysis, exo- and/or endo-nucleolysis, or degradation of the peptide, polypeptide, protein, or nucleic acid, such as, for example, by physical, chemical and/or enzymatic proteolysis or nucleolysis.

[0164] The term "fragment" as used throughout this specification with reference to a peptide, polypeptide, or protein generally denotes a portion of the peptide, polypeptide, or protein, such as typically an N- and/or C-terminally truncated form of the peptide, polypeptide, or protein. Preferably, a fragment may comprise at least about 30%, e.g., at least about 50% or at least about 70%, preferably at least about 80%, e.g., at least about 85%, more preferably at least about 90%, and yet more preferably at least about 95% or even about 99% of the amino acid sequence length of said peptide, polypeptide, or protein. For example, insofar not exceeding the length of the full-length peptide, polypeptide, or protein, a fragment may include a sequence of ≥ 5 consecutive amino acids, or ≥ 10 consecutive amino acids, or ≥ 20 consecutive amino acids, or ≥ 30 consecutive amino acids, e.g., >40 consecutive amino acids, such as for example ≥ 50 consecutive amino acids, e.g., ≥ 60, ≥ 70, ≥ 80, ≥ 90, ≥ 100, ≥ 200, ≥ 300, ≥ 400, ≥ 500 or ≥ 600 consecutive amino acids of the corresponding full-length peptide, polypeptide, or protein.

[0165] The term "fragment" as used throughout this specification with reference to a nucleic acid (polynucleotide) generally denotes a 5'- and/or 3'-truncated form of a nucleic acid. Preferably, a fragment may comprise at least about 30%, e.g., at least about 50% or at least about 70%, preferably at least about 80%, e.g., at least about 85%, more preferably at least about 90%, and yet more preferably at least about 95% or even about 99% of the nucleic acid sequence length of said nucleic acid. For example, insofar not exceeding the length of the full-length

nucleic acid, a fragment may include a sequence of ≥ 5 consecutive nucleotides, or ≥ 10 consecutive nucleotides, or ≥ 20 consecutive nucleotides, or ≥ 30 consecutive nucleotides, e.g., >40 consecutive nucleotides, such as for example ≥ 50 consecutive nucleotides, e.g., ≥ 60, ≥ 70, ≥ 80, ≥ 90, ≥ 100, ≥ 200, ≥ 300, ≥ 400, ≥ 500 or ≥ 600 consecutive nucleotides of the corresponding full-length nucleic acid.

[0166] Cells such as immune cells as disclosed herein may in the context of the present specification be said to "comprise the expression" or conversely to "not express" one or more markers, such as one or more genes or gene products; or be described as "positive" or conversely as "negative" for one or more markers, such as one or more genes or gene products; or be said to "comprise" a defined "gene or gene product signature".

[0167] Such terms are commonplace and well-understood by the skilled person when characterizing cell phenotypes. By means of additional guidance, when a cell is said to be positive for or to express or comprise expression of a given marker, such as a given gene or gene product, a skilled person would conclude the presence or evidence of a distinct signal for the marker when carrying out a measurement capable of detecting or quantifying the marker in or on the cell. Suitably, the presence or evidence of the distinct signal for the marker would be concluded based on a comparison of the measurement result obtained for the cell to a result of the same measurement carried out for a negative control (for example, a cell known to not express the marker) and/or a positive control (for example, a cell known to express the marker). Where the measurement method allows for a quantitative assessment of the marker, a positive cell may generate a signal for the marker that is at least 1.5-fold higher than a signal generated for the marker by a negative control cell or than an average signal generated for the marker by a population of negative control cells, e.g., at least 2-fold, at least 4-fold, at least lO-fold, at least 20-fold, at least 30-fold, at least 40-fold, at least 50-fold higher or even higher. Further, a positive cell may generate a signal for the marker that is 3.0 or more standard deviations, e.g., 3.5 or more, 4.0 or more, 4.5 or more, or 5.0 or more standard deviations, higher than an average signal generated for the marker by a population of negative control cells.

[0168] The present invention is also directed to signatures and uses thereof. As used herein a "signature" may encompass any gene or genes, protein or proteins, or epigenetic element(s) whose expression profile or whose occurrence is associated with a specific cell type, subtype, or

cell state of a specific cell type or subtype within a population of cells (e.g., tumor infiltrating lymphocytes). In certain embodiments, the expression of the signatures (e.g., T cell signature) are dependent on epigenetic modification of the genes or regulatory elements associated with the genes. Thus, in certain embodiments, use of signature genes includes epigenetic modifications that may be detected or modulated. For ease of discussion, when discussing gene expression, any gene or genes, protein or proteins, or epigenetic element(s) may be substituted. Reference to a gene name throughout the specification encompasses the human gene, mouse gene and all other orthologues as known in the art in other organisms. As used herein, the terms "signature", "expression profile", or "expression program" may be used interchangeably. It is to be understood that also when referring to proteins (e.g. differentially expressed proteins), such may fall within the definition of "gene" signature. Levels of expression or activity or prevalence may be compared between different cells in order to characterize or identify for instance signatures specific for cell (sub)populations. Increased or decreased expression or activity of signature genes may be compared between different cells in order to characterize or identify for instance specific cell (sub)populations. The detection of a signature in single cells may be used to identify and quantitate for instance specific cell (sub)populations. A signature may include a gene or genes, protein or proteins, or epigenetic element(s) whose expression or occurrence is specific to a cell (sub)population, such that expression or occurrence is exclusive to the cell (sub)population. A gene signature as used herein, may thus refer to any set of up- and down-regulated genes that are representative of a cell type or subtype. A gene signature as used herein, may also refer to any set of up- and down-regulated genes between different cells or cell (sub)populations derived from a gene-expression profile. For example, a gene signature may comprise a list of genes differentially expressed in a distinction of interest (e.g., a pattern of gene expression).

[0169] The signature as defined herein (being it a gene signature, protein signature or other genetic or epigenetic signature) can be used to indicate the presence of a cell type, a subtype of the cell type, the state of the microenvironment of a population of cells, a particular cell type population or subpopulation, and/or the overall status of the entire cell (sub)population. Furthermore, the signature may be indicative of cells within a population of cells in vivo. The signature may also be used to suggest for instance particular therapies, or to follow up treatment,

or to suggest ways to modulate immune systems. The signatures of the present invention may be discovered by analysis of expression profiles of single-cells within a population of cells from isolated samples (e.g. tumor samples), thus allowing the discovery of novel cell subtypes or cell states that were previously invisible or unrecognized. The presence of subtypes or cell states may be determined by subtype specific or cell state specific signatures. The presence of these specific cell (sub)types or cell states may be determined by applying the signature genes to bulk sequencing data in a sample. Not being bound by a theory the signatures of the present invention may be microenvironment specific, such as their expression in a particular spatio-temporal context. Not being bound by a theory, signatures as discussed herein are specific to a particular pathological context. Not being bound by a theory, a combination of cell subtypes having a particular signature may indicate an outcome. Not being bound by a theory, the signatures can be used to deconvolute the network of cells present in a particular pathological condition. Not being bound by a theory the presence of specific cells and cell subtypes are indicative of a particular response to treatment, such as including increased or decreased susceptibility to treatment. The signature may indicate the presence of one particular cell type.

[0170] The signature according to certain embodiments of the present invention may comprise or consist of one or more genes, proteins and/or epigenetic elements, such as for instance 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more. In certain embodiments, the signature may comprise or consist of two or more genes, proteins and/or epigenetic elements, such as for instance 2, 3, 4, 5, 6, 7, 8, 9, 10 or more. In certain embodiments, the signature may comprise or consist of three or more genes, proteins and/or epigenetic elements, such as for instance 3, 4, 5, 6, 7, 8, 9, 10 or more. In certain embodiments, the signature may comprise or consist of four or more genes, proteins and/or epigenetic elements, such as for instance 4, 5, 6, 7, 8, 9, 10 or more. In certain embodiments, the signature may comprise or consist of five or more genes, proteins and/or epigenetic elements, such as for instance 5, 6, 7, 8, 9, 10 or more. In certain embodiments, the signature may comprise or consist of six or more genes, proteins and/or epigenetic elements, such as for instance 6, 7, 8, 9, 10 or more. In certain embodiments, the signature may comprise or consist of seven or more genes, proteins and/or epigenetic elements, such as for instance 7, 8, 9, 10 or more. In certain embodiments, the signature may comprise or consist of eight or more genes, proteins and/or epigenetic elements, such as for instance 8, 9, 10 or more. In certain

embodiments, the signature may comprise or consist of nine or more genes, proteins and/or epigenetic elements, such as for instance 9, 10 or more. In certain embodiments, the signature may comprise or consist of ten or more genes, proteins and/or epigenetic elements, such as for instance 10, 11, 12, 13, 14, 15, or more. It is to be understood that a signature according to the invention may for instance also include genes or proteins as well as epigenetic elements combined.

[0171] In certain embodiments, a signature is characterized as being specific for a particular immune cell or immune cell (sub)population if it is upregulated or only present, detected or detectable in that particular immune cell or immune cell (sub)population, or alternatively is downregulated or only absent, or undetectable in that particular immune cell or immune cell (sub)population. In this context, a signature consists of one or more differentially expressed genes/proteins or differential epigenetic elements when comparing different cells or cell (sub)populations, including comparing different immune cell or immune cell (sub)populations, as well as comparing immune cell or immune cell (sub)populations with non- immune cell or non-immune cell (sub)populations. It is to be understood that "differentially expressed" genes/proteins include genes/proteins which are up- or down-regulated as well as genes/proteins which are turned on or off. When referring to up-or down-regulation, in certain embodiments, such up- or down-regulation is preferably at least two-fold, such as two-fold, three-fold, four-fold, five-fold, or more, such as for instance at least ten-fold, at least 20-fold, at least 30-fold, at least 40-fold, at least 50-fold, or more. Alternatively, or in addition, differential expression may be determined based on common statistical tests, as is known in the art.

[0172] As discussed herein, differentially expressed genes/proteins, or differential epigenetic elements may be differentially expressed on a single cell level, or may be differentially expressed on a cell population level. Preferably, the differentially expressed genes/ proteins or epigenetic elements as discussed herein, such as constituting the gene signatures as discussed herein, when as to the cell population or subpopulation level, refer to genes that are differentially expressed in all or substantially all cells of the population or subpopulation (such as at least 80%, preferably at least 90%, such as at least 95% of the individual cells). This allows one to define a particular subpopulation of immune cells. As referred to herein, a "subpopulation" of cells preferably refers to a particular subset of cells of a particular cell type which can be distinguished

or are uniquely identifiable and set apart from other cells of this cell type. The cell subpopulation may be phenotypically characterized, and is preferably characterized by the signature as discussed herein. A cell (sub)population as referred to herein may constitute of a (sub)population of cells of a particular cell type characterized by a specific cell state.

[0173] When referring to induction, or alternatively suppression of a particular signature, preferable is meant induction or alternatively suppression (or upregulation or downregulation) of at least one gene/protein and/or epigenetic element of the signature, such as for instance at least two, at least three, at least four, at least five, at least six, or all genes/proteins and/or epigenetic elements of the signature.

[0174] Various aspects and embodiments of the invention may involve analyzing gene signatures, protein signature, and/or other genetic or epigenetic signature based on single cell analyses (e.g. single cell RNA sequencing) or alternatively based on cell population analyses, as is defined herein elsewhere.

[0175] In certain example embodiments, the signature genes may be used to deconvolute the network of cells present in a tumor based on comparing them to data from bulk analysis of a tumor sample. In certain example embodiments, the presence of specific immune cells and immune cell subtypes may be indicative of tumor growth, invasiveness and/or resistance to treatment. In one example embodiment, detection of one or more signature genes may indicate the presence of a particular cell type or cell types. In certain example embodiments, the presence of immune cell types within a tumor may indicate that the tumor will be sensitive to a treatment (e.g., checkpoint blockade therapy). In one embodiment, the signature genes of the present invention are applied to bulk sequencing data from a tumor sample obtained from a subject, such that information relating to disease outcome and personalized treatments is determined. In certain embodiments, the presence of suppressive T cells in a tumor may be determined by deconvolution of bulk tumor sequencing data and the ratio of suppressive T cells compared to clinical outcomes. Not being bound by a theory, a prognosis may be determined based on the immune cell status of a tumor.

Detection, Quantification and Isolation of CD8+ T cells Subtypes

[0176] In one embodiment, the present invention provides for a method comprising detecting or quantifying CD8+ T cells in a biological sample. In preferred embodiments, one or more

PD1+ CD8+ T cells are detected or quantified in the biological sample. The CD8+ T cells may be detected or quantified using a set of markers comprising: one or more genes or polypeptides selected from the group consisting of TIM3, SERPINE2, HMMR, KIT, TNFRSF4, CD8, CD45, PD1, TNFRSF9, PRF1, BHLHE40 (DEC1), IRF8, GLDC, STAT3, CST7, IL1R2, EEF2, SLC2A3, SQSTM1, RBPJ, NABP1, ACTN1, TNFRSF4, SERPINB9, FOSL2, CAPG, KLRC1, IL18R1, JUNB, EEF1A1, TNFRSF18, RGS2, NFKB2, RPL5, PEX16, LAT2, KDM5B, HILPDA, GEM, DENND4A, BCL2L11, ADAM8, PGLYRP1, IKZF2 (HELIOS), KIT, SERPINE2, CCRL2, CSF1, EPAS1, RUNX2, SPRY2, XCL1, CCR8, MT1 and KI67. In certain embodiments, the markers include the following combinations: a) TIM3, SERPINE2 and HMMR; or b) SERPINE2 and HMMR; or c) TIM3, KIT and HMMR; or d) TIM3, TNFRSF4 and HMMR; or e) any of (a), (b), (c) or (d) and one or more of CD8, CD45 and PD1; or any of (a), (b), (c), (d) or (e) and one or more of TNFRSF9, PRF1, BHLHE40, IRF8, GLDC, STAT3, CST7, IL1R2, EEF2, SLC2A3, SQSTM1, RBPJ, NABP1, ACTN1, TNFRSF4, SERPINB9, FOSL2, CAPG, KLRC1, IL18R1, JUNB, EEF1A1, TNFRSF18, RGS2, NFKB2, RPL5, PEX16, LAT2, KDM5B, HILPDA, GEM, DENND4A, BCL2L11, ADAM8, PGLYRP1, IKZF2, KIT, SERPINE2, CCRL2, CSF1, EPAS1, RUNX2, SPRY2, XCL1, CCR8, MT1 and KI67. The T cells may be detected in intact cells by detecting surface markers (e.g., TNFRSF9, IL1R2, SLC2A3, TNFRSF4, KLRC1, IL18R1, TNFRSF18, LAT2, ADAM8, KIT, CCR8 and SERPINE2). The presence of the cells in a sample may also be detected after cells are broken (e.g., lysed, destroyed) or fixed and permeabilized. In an exemplary embodiment, cells are analyzed by single cell RNA sequencing (e.g., scRNA-seq) and the cells are sorted in silico based on gene expression attributed to each single cell. In another exemplary embodiment, fixed and permeabilized cells are analyzed by microscopy (e.g., fluorescent microscopy). In other embodiments, fixed and permeabilized cells may be detected and quantified using FACS. In other embodiments, cells may be detected and quantified using FISH or Flow-FISH. Thus, the specific cells may be detected in a biological sample and cell types quantitated even though the cells have been destroyed. In other embodiments, cells are detected or quantified from a sample without killing the cells, such as by using cell sorting with an affinity reagent specific to a cell surface marker (e.g., FACS).

[0177] In one embodiment, the method comprises isolating CD8+ T cells from a biological sample. In preferred embodiments, one or more PD1+ CD8+ T cells are isolated from the biological sample. In certain embodiments, isolating CD8+ T cells from a biological sample results in depletion of the T cells from the biological sample or enrichment of T cells. The CD8+ T cells may be isolated using a set of markers comprising: one or more surface genes or polypeptides selected from the group consisting of TNFRSF9, IL1R2, SLC2A3, TNFRSF4, KLRC1, IL18R1, TNFRSF18, LAT2, ADAM8, KIT, CCR8 and SERPINE2. In certain embodiments, the markers include the following combinations: a) TIM3, SERPINE2 and HMMR; or b) SERPINE2 and HMMR; or c) TIM3, KIT and HMMR; or d) TIM3, TNFRSF4 and HMMR; or e) any of (a), (b), (c) or (d) and one or more of CD8, CD45 and PD1; or any of (a), (b), (c), (d) or (e) and one or more of TNFRSF9, IL1R2, SLC2A3, TNFRSF4, KLRC1, IL18R1, TNFRSF18, LAT2, ADAM8, KIT, CCR8 and SERPINE2. In certain embodiments, cells are isolated or depleted from a sample by using an affinity reagent specific to a cell surface marker.

[0178] The genes or polypeptides in the group consisting of TNFRSF9, PRF1, BHLHE40, IRF8, GLDC, STAT3, CST7, IL1R2, EEF2, SLC2A3, SQSTM1, RBPJ, NABP1, ACTN1, TNFRSF4, SERPINB9, FOSL2, CAPG, KLRC1, IL18R1, JUNB, EEF1A1, TNFRSF 18, RGS2, NFKB2, RPL5, PEX16, LAT2, KDM5B, HILPDA, GEM, DENND4A, BCL2L1 1, ADAM8, and PGLYRP1 were upregulated in cluster 7 relative to clusters 9 and 10. Thus, the genes may be used to further distinguish between each subtype. Moreover, the overall signatures or subset of the signature genes characteristic of each identified cluster (i.e., CD8+ T cell subtype) may be used to identify each subtype. In further embodiments, surface markers selected from the group of genes may be used to isolate each subtype.

[0179] A marker, for example a gene or gene product, for example a peptide, polypeptide, protein, or nucleic acid, or a group of two or more markers, is “detected” or “measured” in a tested object (e.g., in or on a cell, cell population, tissue, organ, or organism, e.g., in a biological sample of a subject) when the presence or absence and/or quantity of said marker or said group of markers is detected or determined in the tested object, preferably substantially to the exclusion of other molecules and analytes, e.g., other genes or gene products.

[0180] The terms “increased” or “increase” or “upregulated” or “upregulate” as used herein generally mean an increase by a statically significant amount. For avoidance of doubt, “increased” means a statistically significant increase of at least 10% as compared to a reference level, including an increase of at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 100% or more, including, for example at least 2-fold, at least 3-fold, at least 4-fold, at least 5-fold, at least lO-fold increase or greater as compared to a reference level, as that term is defined herein.

[0181] The term “reduced” or “reduce” or “decrease” or “decreased” or “downregulate” or “downregulated” as used herein generally means a decrease by a statistically significant amount relative to a reference. For avoidance of doubt, “reduced” means statistically significant decrease of at least 10% as compared to a reference level, for example a decrease by at least 20%, at least 30%, at least 40%, at least 50%, or at least 60%, or at least 70%, or at least 80%, at least 90% or more, up to and including a 100% decrease (i.e., absent level as compared to a reference sample), or any decrease between 10-100% as compared to a reference level, as that.

[0182] In certain embodiments, the biological sample may be a tumor sample obtained from a subject in need thereof and the CD8+ T cells may be CD8+ tumor infiltrating lymphocytes (TIL). In certain embodiments, the TILs may comprise suppressive and/or activated T cells. In certain embodiments, the biological sample may be a sample obtained from a subject suffering from an autoimmune disease. In certain embodiments, T cells may be isolated from the biological sample.

[0183] In certain embodiments, the biological sample may comprise *ex vivo* or *in vitro* CD8+ T cells. The *ex vivo* or *in vitro* biological sample may be treated with an antigen. The *ex vivo* or *in vitro* biological sample may be treated with a differentiation agent. The differentiating agent may be a cytokine. The *ex vivo* or *in vitro* biological sample may be treated with a test agent. Not being bound by a theory, the *ex vivo* or *in vitro* biological sample may be differentiated to comprise certain types of T cells (e.g., suppressive or effector T cells). The test agent may be any agent predicted to affect the function or gene expression of any of the cells described herein. The agent may affect the ratio of cells in a population of cells (i.e., in the *ex vivo* or *in vitro* biological sample). For example, T cells may be differentiated to the T cells of the present invention. Not being bound by a theory, suppressive T cells differentiated *ex vivo* or *in vitro* may be used to

treat a subject suffering from an autoimmune disease. Not being bound by a theory, suppressive T cells differentiated into effector T cells *ex vivo* or *in vitro* may be used to treat a subject suffering from cancer. The test agent may be a drug candidate. The drug candidate may be used to differentiate or modulate T cell balance *in vivo.* In certain embodiments, the biological sample is assayed to determine the quantity or changes in composition of T cells in the sample after treatment.

[0184] The terms "sample" or "biological sample" as used throughout this specification include any biological specimen obtained from a subject. Particularly useful samples are those known to comprise, or expected or predicted to comprise immune cells as taught herein. Preferably, a sample may be readily obtainable by minimally invasive methods, such as blood collection or tissue biopsy, allowing the removal / isolation / provision of the sample from the subject. Examples of particularly useful samples include without limitation whole blood or a cell-containing fraction of whole blood, such as serum, white blood cells, or peripheral blood mononuclear cells (PBMC), lymph, lymphatic tissue, inflammation fluid, tissue specimens, or tissue biopsies. The term "tissue" as used throughout this specification refers to any animal tissue types including, but not limited to, bone, bone marrow, neural tissue, fibrous connective tissue, cartilage, muscle, vasculature, skin, adipose tissue, blood and glandular tissue or other non-bone tissue. The tissue may be healthy or affected by pathological alterations, e.g., tumor tissue or tissue affected by a disease comprising an immune component. The tissue may be from a living subject or may be cadaveric tissue. The tissue may be autologous tissue or syngeneic tissue or may be allograft or xenograft tissue. A biological sample may also include cells grown in tissue culture, such as cells used for screening drugs or primary cells grown in culture for expansion.

[0185] The terms "quantity", "amount" and "level" are synonymous and generally well-understood in the art. The terms as used throughout this specification may particularly refer to an absolute quantification of a marker in a tested object (e.g., in or on a cell, cell population, tissue, organ, or organism, e.g., in a biological sample of a subject), or to a relative quantification of a marker in a tested object, i.e., relative to another value such as relative to a reference value, or to a range of values indicating a base-line of the marker. Such values or ranges may be obtained as conventionally known.

[0186] An absolute quantity of a marker may be advantageously expressed as weight or as molar amount, or more commonly as a concentration, e.g., weight per volume or mol per volume. A relative quantity of a marker may be advantageously expressed as an increase or decrease or as a fold-increase or fold-decrease relative to said another value, such as relative to a reference value. Performing a relative comparison between first and second variables (e.g., first and second quantities) may but need not require determining first the absolute values of said first and second variables. For example, a measurement method may produce quantifiable readouts (such as, e.g., signal intensities) for said first and second variables, wherein said readouts are a function of the value of said variables, and wherein said readouts may be directly compared to produce a relative value for the first variable vs. the second variable, without the actual need to first convert the readouts to absolute values of the respective variables.

[0187] Reference values may be established according to known procedures previously employed for other cell populations, biomarkers and gene or gene product signatures. For example, a reference value may be established in an individual or a population of individuals characterized by a particular diagnosis, prediction and/or prognosis of said disease or condition (i.e., for whom said diagnosis, prediction and/or prognosis of the disease or condition holds true). Such population may comprise without limitation 2 or more, 10 or more, 100 or more, or even several hundred or more individuals.

[0188] A "deviation" of a first value from a second value may generally encompass any direction (e.g., increase: first value > second value; or decrease: first value < second value) and any extent of alteration.

[0189] For example, a deviation may encompass a decrease in a first value by, without limitation, at least about 10% (about 0.9-fold or less), or by at least about 20% (about 0.8-fold or less), or by at least about 30% (about 0.7-fold or less), or by at least about 40% (about 0.6-fold or less), or by at least about 50% (about 0.5-fold or less), or by at least about 60% (about 0.4-fold or less), or by at least about 70% (about 0.3-fold or less), or by at least about 80% (about 0.2-fold or less), or by at least about 90% (about 0.1-fold or less), relative to a second value with which a comparison is being made.

[0190] For example, a deviation may encompass an increase of a first value by, without limitation, at least about 10% (about 1.1-fold or more), or by at least about 20% (about 1.2-fold

or more), or by at least about 30% (about 1.3-fold or more), or by at least about 40% (about 1.4-fold or more), or by at least about 50% (about 1.5-fold or more), or by at least about 60% (about 1.6-fold or more), or by at least about 70% (about 1.7-fold or more), or by at least about 80% (about 1.8-fold or more), or by at least about 90% (about 1.9-fold or more), or by at least about 100% (about 2-fold or more), or by at least about 150% (about 2.5-fold or more), or by at least about 200% (about 3-fold or more), or by at least about 500% (about 6-fold or more), or by at least about 700% (about 8-fold or more), or like, relative to a second value with which a comparison is being made.

[0191] Preferably, a deviation may refer to a statistically significant observed alteration. For example, a deviation may refer to an observed alteration which falls outside of error margins of reference values in a given population (as expressed, for example, by standard deviation or standard error, or by a predetermined multiple thereof, e.g., ±lxSD or ±2xSD or ±3xSD, or ±lxSE or ±2xSE or ±3xSE). Deviation may also refer to a value falling outside of a reference range defined by values in a given population (for example, outside of a range which comprises >40%, ≥ 50%, >60%, >70%, >75% or >80% or >85% or >90% or >95% or even >100% of values in said population).

[0192] In a further embodiment, a deviation may be concluded if an observed alteration is beyond a given threshold or cut-off. Such threshold or cut-off may be selected as generally known in the art to provide for a chosen sensitivity and/or specificity of the prediction methods, e.g., sensitivity and/or specificity of at least 50%, or at least 60%, or at least 70%, or at least 80%, or at least 85%, or at least 90%, or at least 95%.

[0193] For example, receiver-operating characteristic (ROC) curve analysis can be used to select an optimal cut-off value of the quantity of a given immune cell population, biomarker or gene or gene product signatures, for clinical use of the present diagnostic tests, based on acceptable sensitivity and specificity, or related performance measures which are well-known per se, such as positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (LR+), negative likelihood ratio (LR-), Youden index, or similar.

[0194] The terms “isolating” or “purifying” as used throughout this specification with reference to a particular component of a composition or mixture (e.g., the tested object such as the biological sample) encompass processes or techniques whereby such component is separated

from one or more or (substantially) all other components of the composition or mixture (e.g., the tested object such as the biological sample). The terms do not require absolute purity. Instead, isolating or purifying the component will produce a discrete environment in which the abundance of the component relative to one or more or all other components is greater than in the starting composition or mixture (e.g., the tested object such as the biological sample). A discrete environment may denote a single medium, such as for example a single solution, dispersion, gel, precipitate, etc. Isolating or purifying the specified immune cells from the tested object such as the biological sample may increase the abundance of the specified immune cells relative to all other cells comprised in the tested object such as the biological sample, or relative to other cells of a select subset of the cells comprised in the tested object such as the biological sample, e.g., relative to other white blood cells, peripheral blood mononuclear cells, immune cells, antigen presenting cells, or dendritic cells comprised in the tested object such as the biological sample. By means of example, isolating or purifying the specified immune cells from the tested object such as the biological sample may yield a cell population, in which the specified immune cells constitute at least 40% (by number) of all cells of said cell population, for example, at least 45%, preferably at least 50%, at least 55%, more preferably at least 60%, at least 65%, still more preferably at least 70%, at least 75%, even more preferably at least 80%, at least 85%, and yet more preferably at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or even 100% of all cells of said cell population.

[0195] Any existing, available or conventional separation, detection and/or quantification methods may be used to measure the presence or absence (e.g., readout being present vs. absent; or detectable amount vs. undetectable amount) and/or quantity (e.g., readout being an absolute or relative quantity) of the specified immune cells in, or to isolate the specified immune cells from, a tested object (e.g., a cell population, tissue, organ, organism, or a biological sample of a subject). Such methods allow to detect, quantify or isolate the specified immune cells in or from the tested object (e.g., a cell population, tissue, organ, organism, or a biological sample of a subject) substantially to the exclusion of other cells comprised in the tested object. Such methods may allow to detect, quantify or isolate the specified immune cells with sensitivity of at least 50%, at least 55%, at least 60%, at least 65%, preferably at least 70%, at least 75%, more preferably at least 80%, at least 85%, even more preferably at least 90%, at least 95%, at least

96%, at least 97%, at least 98%, at least 99%, or even 100%, and/or with specificity of at least 50%, at least 55%, at least 60%, at least 65%, preferably at least 70%, at least 75%, more preferably at least 80%, at least 85%, even more preferably at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or even 100%. By means of example, at least 40% (by number), for example at least 45%, preferably at least 50%, at least 55%, more preferably at least 60%, at least 65%, still more preferably at least 70%, at least 75%, even more preferably at least 80%, at least 85%, and yet more preferably at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or even 100% of all cells detected, quantified or isolated by such methods may correspond to the specified immune cells.

Isolated Cells

[0196] In another aspect, the present invention provides for isolated CD8+ T cells as described herein. The isolated CD8+ T cell subtypes may be isolated using any of the markers described herein. The isolated CD8+ T cell subtypes may be isolated from a human subject. The isolated CD8+ T cell may be isolated from an *ex vivo* sample (e.g., CAR T cell, autologous T cells or allogenic T cells grown in culture). In preferred embodiments, the isolated CD8+ T cell may be obtained from a subject suffering from a disease (e.g., cancer, an autoimmune disease, or chronic infection).

[0197] In one aspect, the invention is directed to isolated cell populations (e.g., T cells) comprising the T cells described herein and/or as identified by the signatures defined herein. Accordingly, methods for detecting, quantifying or isolating the specified immune cells may be marker-based or gene or gene product signature-based, i.e., may involve isolation of cells expressing or not expressing marker(s) or combination(s) of markers the expression or lack of expression of which is taught herein as typifying or characterizing the specified immune cells, or may involve detection, quantification or isolation of cells comprising gene or gene product signature(s) taught herein as typifying or characterizing the specified immune cells.

[0198] In another aspect, the present invention provides for a population of CD8+ T cells comprising CD8+ T cells as defined in any embodiment herein or isolated according to a method of any embodiment herein. The isolated population may comprise greater than 30%, 40%, 50%, 60%, 70%, 80%, 90% or 95% of a CD8+ T cell as defined in any embodiment herein. In certain embodiments, the population of cells is less than 30% of any one cell type, such as when cells

are directly isolated from a patient. In certain embodiments, a population of cells isolated from a subject will include a heterogeneous population of cells, such that specific cell subtypes make up less than a majority of the total cells (e.g., less than 30%, 20%, 10%, 5%, or 1%). In certain embodiments, a subtype of cells is expanded or enriched *ex vivo* to obtain a non-naturally occurring cell population enriched for certain cell types. In certain embodiments, T cells according to the present invention are depleted from a population of cells. The isolated population may comprise less than 5%, 1%, 0.1%, 0.01%, or 0.001%, or comprise 0% of a suppressive CD8+ T cell as defined in any embodiment herein. The population of cells depleted for the T cells may be further expanded. Not being bound by a theory suppressive T cells may be depleted from a population of T cells and upon expanding a population enriched for effector T cells may be obtained. Not being bound by a theory an expanded population of T cells may be obtained that does not include suppressive T cells. In certain embodiments, the population of T cells may express a chimeric antigen receptor targeting tumor cell antigens. Not being bound by a theory suppressive T cells may be depleted from a population of CAR T cells.

[0199] The isolated immune cells or immune cell populations as disclosed throughout this specification may be suitably cultured or cultivated *in vitro.* The terms “culturing” or “cell culture” are common in the art and broadly refer to maintenance of cells and potentially expansion (proliferation, propagation) of cells in vitro. Typically, animal cells, such as mammalian cells, such as human cells, are cultured by exposing them to (i.e., contacting them with) a suitable cell culture medium in a vessel or container adequate for the purpose (e.g., a 96-, 24-, or 6-well plate, a T-25, T-75, T-150 or T-225 flask, or a cell factory), at art-known conditions conducive to in vitro cell culture, such as temperature of 37°C, 5% v/v C02 and > 95% humidity.

[0200] The term “medium” as used herein broadly encompasses any cell culture medium conducive to maintenance of cells, preferably conducive to proliferation of cells. Typically, the medium will be a liquid culture medium, which facilitates easy manipulation (e.g., decantation, pipetting, centrifugation, filtration, and such) thereof.

[0201] Typically, the medium will comprise a basal medium formulation as known in the art. Many basal media formulations (available, e.g., from the American Type Culture Collection, ATCC; or from Invitrogen, Carlsbad, California) can be used, including but not limited to

Eagle’s Minimum Essential Medium (MEM), Dulbecco’s Modified Eagle's Medium (DMEM), alpha modified Minimum Essential Medium (alpha-MEM), Basal Medium Essential (BME), Iscove’s Modified Dulbecco's Medium (IMDM), BGJb medium, F-12 Nutrient Mixture (Ham), Liebovitz L-15, DMEM/F-12, Essential Modified Eagle's Medium (EMEM), RPMI-1640, Medium 199, Waymouth's MB 752/1 or Williams Medium E, and modifications and/or combinations thereof. Compositions of basal media are generally known in the art and it is within the skill of one in the art to modify or modulate concentrations of media and/or media supplements as necessary for the cells cultured.

[0202] Such basal media formulations contain ingredients necessary for mammalian cell development, which are known per se. By means of illustration and not limitation, these ingredients may include inorganic salts (in particular salts containing Na, K, Mg, Ca, Cl, P and possibly Cu, Fe, Se and Zn), physiological buffers (e.g., HEPES, bicarbonate), nucleotides, nucleosides and/or nucleic acid bases, ribose, deoxyribose, amino acids, vitamins, antioxidants (e.g., glutathione) and sources of carbon (e.g., glucose, sodium pyruvate, sodium acetate), etc.

[0203] For use in culture, basal media can be supplied with one or more further components. For example, additional supplements can be used to supply the cells with the necessary trace elements and substances for optimal growth and expansion. Furthermore, antioxidant supplements may be added, e.g., β-mercaptoethanol. While many basal media already contain amino acids, some amino acids may be supplemented later, e.g., L-glutamine, which is known to be less stable when in solution. A medium may be further supplied with antibiotic and/or antimycotic compounds, such as, typically, mixtures of penicillin and streptomycin, and/or other compounds, exemplified but not limited to, amphotericin, ampicillin, gentamicin, bleomycin, hygromycin, kanamycin, mitomycin, mycophenolic acid, nalidixic acid, neomycin, nystatin, paromomycin, polymyxin, puromycin, rifampicin, spectinomycin, tetracycline, tylosin, and zeocin.

[0204] Lipids and lipid carriers can also be used to supplement cell culture media. Such lipids and carriers can include, but are not limited to cyclodextrin, cholesterol, linoleic acid conjugated to albumin, linoleic acid and oleic acid conjugated to albumin, unconjugated linoleic acid, linoleic-oleic-arachidonic acid conjugated to albumin, oleic acid unconjugated and

conjugated to albumin, among others. Albumin can similarly be used in fatty-acid free formulations.

[0205] Also contemplated is supplementation of cell culture media with mammalian plasma or sera. Plasma or sera often contain cellular factors and components that facilitate cell viability and expansion. Optionally, plasma or serum may be heat inactivated. Heat inactivation is used in the art mainly to remove the complement. Heat inactivation typically involves incubating the plasma or serum at 56°C for 30 to 60min, e.g., 30min, with steady mixing, after which the plasma or serum is allowed to gradually cool to ambient temperature. A skilled person will be aware of any common modifications and requirements of the above procedure. Optionally, plasma or serum may be sterilized prior to storage or use. Usual means of sterilization may involve, e.g., filtration through one or more filters with pore size smaller than 1pm, preferably smaller than 0.5pm, e.g., smaller than 0.45pm, 0.40pm, 0.35pm, 0.30pm or 0.25pm, more preferably 0.2pm or smaller, e.g., 0.l5pm or smaller, O.lOpm or smaller. Suitable sera or plasmas for use in media as taught herein may include human serum or plasma, or serum or plasma from non-human animals, preferably non-human mammals, such as, e.g., non-human primates (e.g., lemurs, monkeys, apes), fetal or adult bovine, horse, porcine, lamb, goat, dog, rabbit, mouse or rat serum or plasma, etc., or any combination of such. In certain preferred embodiments, a medium as taught herein may comprise bovine serum or plasma, preferably fetal bovine (calf) serum or plasma, more preferably fetal bovine (calf) serum (FCS or FBS). When culturing human cells, media may preferably comprise human serum or plasma, such as autologous or allogeneic human serum or plasma, preferably human serum, such as autologous or allogeneic human serum, more preferably autologous human serum or plasma, even more preferably autologous human serum.

[0206] In certain preferred embodiments, serum or plasma can be substituted in media by serum replacements, such as to provide for serum-free media (i.e., chemically defined media). The provision of serum-free media may be advantageous particularly with view to administration of the media or fraction(s) thereof to subjects, especially to human subjects (e.g., improved bio-safety). By the term “serum replacement” it is broadly meant any a composition that may be used to replace the functions (e.g., cell maintenance and growth supportive function) of animal serum in a cell culture medium. A conventional serum replacement may typically comprise vitamins,

albumin, lipids, amino acids, transferrin, antioxidants, insulin and trace elements. Many commercialized serum replacement additives, such as KnockOut Serum Replacement (KOSR), N2, B27, Insulin-Transferrin-Selenium Supplement (ITS), and G5 are well known and are readily available to those skilled in the art.

[0207] Plasma or serum or serum replacement may be comprised in media as taught herein at a proportion (volume of plasma or serum or serum replacement /volume of medium) between about 0.5% v/v and about 40.0% v/v, preferably between about 5.0% v/v and about 20.0% v/v, e.g., between about 5.0% v/v and about 15.0 % v/v, more preferably between about 8.0% v/v and about 12.0% v/v, e.g., about 10.0% v/v.

Methods of Detection and Isolation of CD8+ T cells using Biomarkers

[0208] In certain embodiments, the CD8+ T cell subtypes may be detected, quantified or isolated using a technique selected from the group consisting of flow cytometry, mass cytometry, fluorescence activated cell sorting (FACS), fluorescence microscopy, affinity separation, magnetic cell separation, microfluidic separation, RNA-seq (e.g., bulk or single cell), quantitative PCR, MERFISH (multiplex (in situ) RNA FISH, Flow-FISH) and combinations thereof. The technique may employ one or more agents capable of specifically binding to one or more gene products expressed or not expressed by the CD8+ T cells, preferably on the cell surface of the CD8+ T cells. The one or more agents may be one or more antibodies. Other methods including absorbance assays and colorimetric assays are known in the art and may be used herein.

[0209] Depending on factors that can be evaluated and decided on by a skilled person, such as, inter alia, the type of a marker (e.g., peptide, polypeptide, protein, or nucleic acid), the type of the tested object (e.g., a cell, cell population, tissue, organ, or organism, e.g., the type of biological sample of a subject, e.g., whole blood, plasma, serum, tissue biopsy), the expected abundance of the marker in the tested object, the type, robustness, sensitivity and/or specificity of the detection method used to detect the marker, etc., the marker may be measured directly in the tested object, or the tested object may be subjected to one or more processing steps aimed at achieving an adequate measurement of the marker.

[0210] In other example embodiments, detection of a marker may include immunological assay methods, wherein the ability of an assay to separate, detect and/or quantify a marker (such

as, preferably, peptide, polypeptide, or protein) is conferred by specific binding between a separable, detectable and/or quantifiable immunological binding agent (antibody) and the marker. Immunological assay methods include without limitation immunohistochemistry, immunocytochemistry, flow cytometry, mass cytometry, fluorescence activated cell sorting (FACS), fluorescence microscopy, fluorescence based cell sorting using microfluidic systems, immunoaffmity adsorption based techniques such as affinity chromatography, magnetic particle separation, magnetic activated cell sorting or bead based cell sorting using microfluidic systems, enzyme-linked immunosorbent assay (ELISA) and ELISPOT based techniques, radioimmunoassay (RIA), Western blot, etc.

[0211] In certain example embodiments, detection of a marker or signature may include biochemical assay methods, including inter alia assays of enzymatic activity, membrane channel activity, substance-binding activity, gene regulatory activity, or cell signaling activity of a marker, e.g., peptide, polypeptide, protein, or nucleic acid.

[0212] In other example embodiments, detection of a marker may include mass spectrometry analysis methods. Generally, any mass spectrometric (MS) techniques that are capable of obtaining precise information on the mass of peptides, and preferably also on fragmentation and/or (partial) amino acid sequence of selected peptides (e.g., in tandem mass spectrometry, MS/MS; or in post source decay, TOF MS), may be useful herein for separation, detection and/or quantification of markers (such as, preferably, peptides, polypeptides, or proteins). Suitable peptide MS and MS/MS techniques and systems are well-known per se (see, e.g., Methods in Molecular Biology, vol. 146: “Mass Spectrometry of Proteins and Peptides”, by Chapman, ed., Humana Press 2000, ISBN 089603609x; Biemann 1990. Methods Enzymol 193: 455-79; or Methods in Enzymology, vol. 402: “Biological Mass Spectrometry”, by Burlingame, ed., Academic Press 2005, ISBN 9780121828073) and may be used herein. MS arrangements, instruments and systems suitable for biomarker peptide analysis may include, without limitation, matrix-assisted laser desorption/ionisation time-of-flight (MALDI-TOF) MS; MALDI-TOF post-source-decay (PSD); MALDI-TOF/TOF; surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF) MS; electrospray ionization mass spectrometry (ESI-MS); ESI-MS/MS; ESI-MS/(MS)n (n is an integer greater than zero); ESI 3D or linear (2D) ion trap MS; ESI triple quadrupole MS; ESI quadrupole orthogonal TOF (Q-TOF); ESI Fourier

transform MS systems; desorption/ionization on silicon (DIOS); secondary ion mass spectrometry (SIMS); atmospheric pressure chemical ionization mass spectrometry (APCI-MS); APCI-MS/MS; APCI- (MS)n; atmospheric pressure photoionization mass spectrometry (APPI-MS); APPI-MS/MS; and APPI- (MS)n. Peptide ion fragmentation in tandem MS (MS/MS) arrangements may be achieved using manners established in the art, such as, e.g., collision induced dissociation (CID). Detection and quantification of markers by mass spectrometry may involve multiple reaction monitoring (MRM), such as described among others by Kuhn et al. 2004 (Proteomics 4: 1175-86). MS peptide analysis methods may be advantageously combined with upstream peptide or protein separation or fractionation methods, such as for example with the chromatographic and other methods.

[0213] In other example embodiments, detection of a marker may include chromatography methods. In a one example embodiment, chromatography refers to a process in which a mixture of substances (analytes) carried by a moving stream of liquid or gas ("mobile phase") is separated into components as a result of differential distribution of the analytes, as they flow around or over a stationary liquid or solid phase ("stationary phase"), between said mobile phase and said stationary phase. The stationary phase may be usually a finely divided solid, a sheet of filter material, or a thin film of a liquid on the surface of a solid, or the like. Chromatography may be columnar. While particulars of chromatography are well known in the art, for further guidance see, e.g., Meyer M., 1998, ISBN: 047198373X, and "Practical HPLC Methodology and Applications", Bidlingmeyer, B. A., John Wiley & Sons Inc., 1993. Exemplary types of chromatography include, without limitation, high-performance liquid chromatography (HPLC), normal phase HPLC (NP-HPLC), reversed phase HPLC (RP-HPLC), ion exchange chromatography (IEC), such as cation or anion exchange chromatography, hydrophilic interaction chromatography (HILIC), hydrophobic interaction chromatography (HIC), size exclusion chromatography (SEC) including gel filtration chromatography or gel permeation chromatography, chromatofocusing, affinity chromatography such as immunoaffmity, immobilized metal affinity chromatography, and the like.

[0214] In certain embodiments, further techniques for separating, detecting and/or quantifying markers may be used in conjunction with any of the above described detection methods. Such methods include, without limitation, chemical extraction partitioning, isoelectric

focusing (IEF) including capillary isoelectric focusing (CIEF), capillary isotachophoresis (CITP), capillary electrochromatography (CEC), and the like, one-dimensional polyacrylamide gel electrophoresis (PAGE), two-dimensional polyacrylamide gel electrophoresis (2D-PAGE), capillary gel electrophoresis (CGE), capillary zone electrophoresis (CZE), micellar electrokinetic chromatography (MEKC), free flow electrophoresis (FFE), etc.

[0215] In certain examples, such methods may include separating, detecting and/or quantifying markers at the nucleic acid level, more particularly RNA level, e.g., at the level of hnRNA, pre-mRNA, mRNA, or cDNA. Standard quantitative RNA or cDNA measurement tools known in the art may be used. Non-limiting examples include hybridization-based analysis, microarray expression analysis, digital gene expression profiling (DGE), RNA-in-situ hybridization (RISH), Northern-blot analysis and the like; PCR, RT-PCR, RT-qPCR, end-point PCR, digital PCR or the like; supported oligonucleotide detection, pyrosequencing, polony cyclic sequencing by synthesis, simultaneous bi-directional sequencing, single-molecule sequencing, single molecule real time sequencing, true single molecule sequencing, hybridization-assisted nanopore sequencing, sequencing by synthesis, single-cell RNA sequencing (sc-RNA seq), or the like.

[0216] In certain embodiments, the invention involves single cell RNA sequencing (see, e.g., Kalisky, T., Blainey, P. & Quake, S. R. Genomic Analysis at the Single-Cell Level. Annual review of genetics 45, 431-445, (201 1); Kalisky, T. & Quake, S. R. Single-cell genomics. Nature Methods 8, 311-314 (201 1); Islam, S. et al. Characterization of the single-cell transcriptional landscape by highly multiplex RNA-seq. Genome Research, (201 1); Tang, F. et al. RNA-Seq analysis to capture the transcriptome landscape of a single cell. Nature Protocols 5, 516-535, (2010); Tang, F. et al. mRNA-Seq whole-transcriptome analysis of a single cell. Nature Methods 6, 377-382, (2009); Ramskold, D. et al. Full-length mRNA-Seq from single-cell levels of RNA and individual circulating tumor cells. Nature Biotechnology 30, 777-782, (2012); and Hashimshony, T., Wagner, F., Sher, N. & Yanai, I. CEL-Seq: Single-Cell RNA-Seq by Multiplexed Linear Amplification. Cell Reports, Cell Reports, Volume 2, Issue 3, p666-673, 2012).

[0217] In certain embodiments, the invention involves plate based single cell RNA sequencing (see, e.g., Picelli, S. et al., 2014, "Full-length RNA-seq from single cells using Smart-seq2" Nature protocols 9, 171-181, doi:l0.l038/nprot.20l4.006).

[0218] In certain embodiments, the invention involves high-throughput single-cell RNA-seq. In this regard reference is made to Macosko et al., 2015, "Highly Parallel Genome-wide Expression Profiling of Individual Cells Using Nanoliter Droplets" Cell 161, 1202-1214; International patent application number PCT/US2015/049178, published as W02016/040476 on March 17, 2016; Klein et al., 2015, "Droplet Barcoding for Single-Cell Transcriptomics Applied to Embryonic Stem Cells" Cell 161, 1187-1201; International patent application number PCT/US20 16/027734, published as WO2016168584A1 on October 20, 2016; Zheng, et al., 2016, "Haplotyping germline and cancer genomes with high-throughput linked-read sequencing" Nature Biotechnology 34, 303-31 1; Zheng, et al., 2017, "Massively parallel digital transcriptional profiling of single cells" Nat. Commun. 8, 14049 doi: l0.l038/ncommsl4049; International patent publication number WO2014210353A2; Zilionis, et al., 2017, "Single-cell barcoding and sequencing using droplet microfluidics" Nat Protoc. Jan;l2(l):44-73; Cao et al., 2017, "Comprehensive single cell transcriptional profiling of a multicellular organism by combinatorial indexing" bioRxiv preprint first posted online Feb. 2, 2017, doi: dx.doi.org/l0.H0l/l04844; Rosenberg et al., 2017, "Scaling single cell transcriptomics through split pool barcoding" bioRxiv preprint first posted online Feb. 2, 2017, doi: dx.doi.org/l0.H0l/l05l63; Rosenberg et al., "Single-cell profiling of the developing mouse brain and spinal cord with split-pool barcoding" Science 15 Mar 2018; Vitak, et al., "Sequencing thousands of single-cell genomes with combinatorial indexing" Nature Methods, l4(3):302-308, 2017; Cao, et al., Comprehensive single-cell transcriptional profiling of a multicellular organism. Science, 357(6352):66l-667, 2017; and Gierahn et al., "Seq-Well: portable, low-cost RNA sequencing of single cells at high throughput" Nature Methods 14, 395-398 (2017), all the contents and disclosure of each of which are herein incorporated by reference in their entirety.

[0219] In certain embodiments, the invention involves single nucleus RNA sequencing. In this regard reference is made to Swiech et al., 2014, "In vivo interrogation of gene function in the mammalian brain using CRISPR-Cas9" Nature Biotechnology Vol. 33, pp. 102-106; Habib et al., 2016, "Div-Seq: Single-nucleus RNA-Seq reveals dynamics of rare adult newborn neurons"

Science, Vol. 353, Issue 6302, pp. 925-928; Habib et al., 2017, "Massively parallel single-nucleus RNA-seq with DroNc-seq" Nat Methods. 2017 Oct;l4(lO):955-958; and International patent application number PCT/US2016/059239, published as WO2017164936 on September 28, 2017, which are herein incorporated by reference in their entirety.

[0220] In one embodiment, immune cells are stained for immune cell subtype specific signature genes. In one embodiment, the cells are fixed. In another embodiment, the cells are formalin fixed and paraffin embedded. In another example embodiment, the immune cell subtypes may be quantitated in a section of a tumor.

[0221] The method may allow to detect or conclude the presence or absence of the specified immune cells in a tested object (e.g., in a cell population, tissue, organ, organism, or in a biological sample of a subject). The method may also allow to quantify the specified immune cells in a tested object (e.g., in a cell population, tissue, organ, organism, or in a biological sample of a subject). The quantity of the specified immune cells in the tested object such as the biological sample may be suitably expressed for example as the number (count) of the specified immune cells per standard unit of volume (e.g., ml, μΐ or nl) or weight (e.g., g or mg or ng) of the tested object such as the biological sample. The quantity of the specified immune cells in the tested object such as the biological sample may also be suitably expressed as a percentage or fraction (by number) of all cells comprised in the tested object such as the biological sample, or as a percentage or fraction (by number) of a select subset of the cells comprised in the tested object such as the biological sample, e.g., as a percentage or fraction (by number) of white blood cells, peripheral blood mononuclear cells, immune cells, antigen presenting cells, or dendritic cells comprised in the tested object such as the biological sample. The quantity of the specified immune cells in the tested object such as the biological sample may also be suitably represented by an absolute or relative quantity of a suitable surrogate analyte, such as a peptide, polypeptide, protein, or nucleic acid expressed or comprised by the specified immune cells.

[0222] Where a marker is detected in or on a cell, the cell may be conventionally denoted as positive (+) or negative (-) for the marker. Semi-quantitative denotations of marker expression in cells are also commonplace in the art, such as particularly in flow cytometry quantifications, for example, "dim" vs. "bright", or "low" vs. "medium" / "intermediate" vs. "high", or "-" vs. "+" vs. "++", commonly controlled in flow cytometry quantifications by setting of the gates. Where a

marker is quantified in or on a cell, absolute quantity of the marker may also be expressed for example as the number of molecules of the marker comprised by the cell.

[0223] Where a marker is detected and/or quantified on a single cell level in a cell population, the quantity of the marker may also be expressed as a percentage or fraction (by number) of cells comprised in said population that are positive for said marker, or as percentages or fractions (by number) of cells comprised in said population that are "dim" or "bright", or that are "low" or "medium" / "intermediate" or "high", or that are "-" or "+" or "++". By means of an example, a sizeable proportion of the tested cells of the cell population may be positive for the marker, e.g., at least about 20%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, or up to 100%.

[0224] In certain embodiments, methods for detecting, quantifying or isolating the specified immune cells may be single-cell-based, *i.e.*, may allow to discretely detect, quantify or isolate the specified immune cells as individual cells. In other embodiments, methods for detecting, quantifying or isolating the specified immune cells may be cell population-based, i.e., may only allow to detect, quantify or isolate the specified immune cells as a group or collection of cells, without providing information on or allowing to isolate individual cells.

[0225] Methods for detecting, quantifying or isolating the specified immune cells may employ any of the above-described techniques for measuring markers, insofar the separation or the qualitative and/or quantitative measurement of the marker(s) can be correlated with or translated into detection, quantification or isolation of the specified immune cells. For example, any of the above-described biochemical assay methods, immunological assay methods, mass spectrometry analysis methods, chromatography methods, or nucleic acid analysis method, or combinations thereof for measuring markers, may be employed for detecting, quantifying or isolating the specified immune cells.

[0226] In certain embodiments, the cells are detected, quantified or isolated using a technique selected from the group consisting of flow cytometry, fluorescence activated cell sorting, mass cytometry, fluorescence microscopy, affinity separation, magnetic cell separation, microfluidic separation, and combinations thereof.

[0227] Flow cytometry encompasses methods by which individual cells of a cell population are analyzed by their optical properties (e.g., light absorbance, light scattering and fluorescence

properties, etc.) as they pass in a narrow stream in single file through a laser beam. Flow cytometry methods include fluorescence activated cell sorting (FACS) methods by which a population of cells having particular optical properties are separated from other cells.

[0228] Elemental mass spectrometry-based flow cytometry, or mass cytometry, offers an approach to analyze cells by replacing fluorochrome-labelled binding reagents with mass tagged binding reagents, i.e., tagged with an element or isotope having a defined mass. In these methods, labeled particles are introduced into a mass cytometer, where they are individually atomized and ionized. The individual particles are then subjected to elemental analysis, which identifies and measures the abundance of the mass tags used. The identities and the amounts of the isotopic elements associated with each particle are then stored and analyzed. Due to the resolution of elemental analysis and the number of elemental isotopes that can be used, it is possible to simultaneously measure up to 100 or more parameters on a single particle.

[0229] Fluorescence microscopy broadly encompasses methods by which individual cells of a cell population are microscopically analyzed by their fluorescence properties. Fluorescence microscopy approaches may be manual or preferably automated.

[0230] Affinity separation also referred to as affinity chromatography broadly encompasses techniques involving specific interactions of cells present in a mobile phase, such as a suitable liquid phase (e.g., cell population in an aqueous suspension) with, and thereby adsorption of the cells to, a stationary phase, such as a suitable solid phase; followed by separation of the stationary phase from the remainder of the mobile phase; and recovery (e.g., elution) of the adsorbed cells from the stationary phase. Affinity separation may be columnar, or alternatively, may entail batch treatment, wherein the stationary phase is collected / separated from the liquid phases by suitable techniques, such as centrifugation or application of magnetic field (e.g., where the stationary phase comprises magnetic substrate, such as magnetic particles or beads). Accordingly, magnetic cell separation is also envisaged herein.

[0231] Microfluidic systems allow for accurate and high throughput cell detection, quantification and/or sorting, exploiting a variety of physical principles. Cell sorting on microchips provides numerous advantages by reducing the size of necessary equipment, eliminating potentially biohazardous aerosols, and simplifying the complex protocols commonly associated with cell sorting. The term “microfluidic system” as used throughout this specification

broadly refers to systems having one or more fluid microchannels. Microchannels denote fluid channels having cross-sectional dimensions the largest of which are typically less than 1 mm, preferably less than 500 pm, more preferably less than 400 pm, more preferably less than 300 pm, more preferably less than 200 pm, e.g., 100 pm or smaller. Such microfluidic systems can be used for manipulating fluid and/or objects such as droplets, bubbles, capsules, particles, cells and the like. Microfluidic systems may allow for example for fluorescent label-based (e.g., employing fluorophore-conjugated binding agent(s), such as fluorophore-conjugated antibody(ies)), bead-based (e.g., bead-conjugated binding agent(s), such as bead-conjugated antibody(ies)), or label-free cell sorting (reviewed in Shields et al., Lab Chip. 2015, vol. 15: 1230-1249).

Use of Specific Binding Agents

[0232] In certain embodiments, the aforementioned methods and techniques may employ agent(s) capable of specifically binding to one or more gene products, e.g., peptides, polypeptides, proteins, or nucleic acids, expressed or not expressed by the immune cells as taught herein. In certain preferred embodiments, such one or more gene products, e.g., peptides, polypeptides, or proteins, may be expressed on the cell surface of the immune cells (i.e., cell surface markers, e.g., transmembrane peptides, polypeptides or proteins, or secreted peptides, polypeptides or proteins which remain associated with the cell surface). Hence, further disclosed are binding agents capable of specifically binding to markers, such as genes or gene products, e.g., peptides, polypeptides, proteins, or nucleic acids as taught herein. Binding agents as intended throughout this specification may include inter alia antibodies, aptamers, spiegelmers (L-aptamers), photoaptamers, protein, peptides, peptidomimetics, nucleic acids such as oligonucleotides (e.g., hybridization probes or amplification or sequencing primers and primer pairs), small molecules, or combinations thereof.

[0233] The term “aptamer” refers to single-stranded or double-stranded oligo-DNA, oligo-RNA or oligo-DNA/RNA or any analogue thereof that specifically binds to a target molecule such as a peptide. Advantageously, aptamers display fairly high specificity and affinity (e.g., K_A in the order $lxlO^9$ M^{-1}) for their targets. Aptamer production is described inter alia in US 5,270,163; Ellington & Szostak 1990 (Nature 346: 818-822); Tuerk & Gold 1990 (Science 249: 505-510); or “The Aptamer Handbook: Functional Oligonucleotides and Their Applications”, by

Klussmann, ed., Wiley-VCH 2006, ISBN 3527310592, incorporated by reference herein. The term "photoaptamer" refers to an aptamer that contains one or more photoreactive functional groups that can covalently bind to or crosslink with a target molecule. The term "spiegelmer" refers to an aptamer which includes L-DNA, L-RNA, or other left-handed nucleotide derivatives or nucleotide-like molecules. Aptamers containing left-handed nucleotides are resistant to degradation by naturally occurring enzymes, which normally act on substrates containing right-handed nucleotides. The term "peptidomimetic" refers to a non-peptide agent that is a topological analogue of a corresponding peptide. Methods of rationally designing peptidomimetics of peptides are known in the art. For example, the rational design of three peptidomimetics based on the sulphated 8-mer peptide CCK26-33, and of two peptidomimetics based on the l l-mer peptide Substance P, and related peptidomimetic design principles, are described in Horwell 1995 (Trends Biotechnol 13: 132-134).

[0234] Binding agents may be in various forms, e.g., lyophilised, free in solution, or immobilised on a solid phase. They may be, e.g., provided in a multi-well plate or as an array or microarray, or they may be packaged separately, individually, or in combination.

[0235] The term "specifically bind" as used throughout this specification means that an agent (denoted herein also as "specific-binding agent") binds to one or more desired molecules or analytes (e.g., peptides, polypeptides, proteins, or nucleic acids) substantially to the exclusion of other molecules which are random or unrelated, and optionally substantially to the exclusion of other molecules that are structurally related. The term "specifically bind" does not necessarily require that an agent binds exclusively to its intended target(s). For example, an agent may be said to specifically bind to target(s) of interest if its affinity for such intended target(s) under the conditions of binding is at least about 2-fold greater, preferably at least about 5-fold greater, more preferably at least about lO-fold greater, yet more preferably at least about 25-fold greater, still more preferably at least about 50-fold greater, and even more preferably at least about 100-fold, or at least about 1000-fold, or at least about 104-fold, or at least about 105-fold, or at least about 106-fold or more greater, than its affinity for a non-target molecule, such as for a suitable control molecule (e.g., bovine serum albumin, casein).

[0236] Preferably, the specific binding agent may bind to its intended target(s) with affinity constant **(KA)** of such binding $K_A \geq 1 \times 10^6\ M^{-1}$, more preferably $K_A \geq 1 \times 10^7\ M^{-1}$, yet more

preferably $K_A \geq 1 \times 10^{8}\ M^{-1}$, even more preferably $K_A \geq 1 \times 10^{9}\ M^{-1}$, and still more preferably $K_A \geq 1 \times 10^{10}\ M^{-1}$ or $K_A \geq 1 \times 10^{11}\ M^{-1}$ or $K_A \geq 1 \times 10^{12}\ M^{-1}$, wherein K_A = [SBA_T]/[SBA][T], SBA denotes the specific-binding agent, T denotes the intended target. Determination of KA can be carried out by methods known in the art, such as for example, using equilibrium dialysis and Scatchard plot analysis.

[0237] In certain embodiments, the one or more binding agents may be one or more antibodies. As used herein, the term "antibody" is used in its broadest sense and generally refers to any immunologic binding agent. The term specifically encompasses intact monoclonal antibodies, polyclonal antibodies, multivalent (e.g., 2-, 3- or more-valent) and/or multi-specific antibodies (e.g., bi- or more-specific antibodies) formed from at least two intact antibodies, and antibody fragments insofar they exhibit the desired biological activity (particularly, ability to specifically bind an antigen of interest, i.e., antigen-binding fragments), as well as multivalent and/or multi-specific composites of such fragments. The term "antibody" is not only inclusive of antibodies generated by methods comprising immunization, but also includes any polypeptide, e.g., a recombinantly expressed polypeptide, which is made to encompass at least one complementarity-determining region (CDR) capable of specifically binding to an epitope on an antigen of interest. Hence, the term applies to such molecules regardless whether they are produced in vitro or in vivo. Antibodies also encompasses chimeric, humanized and fully humanized antibodies.

[0238] An antibody may be any of IgA, IgD, IgE, IgG and IgM classes, and preferably IgG class antibody. An antibody may be a polyclonal antibody, e.g., an antiserum or immunoglobulins purified there from (e.g., affinity-purified). An antibody may be a monoclonal antibody or a mixture of monoclonal antibodies. Monoclonal antibodies can target a particular antigen or a particular epitope within an antigen with greater selectivity and reproducibility. By means of example and not limitation, monoclonal antibodies may be made by the hybridoma method first described by Kohler et al. 1975 (Nature 256: 495), or may be made by recombinant DNA methods (e.g., as in US 4,816,567). Monoclonal antibodies may also be isolated from phage antibody libraries using techniques as described by Clackson et al. 1991 (Nature 352: 624-628) and Marks et al. 1991 (J Mol Biol 222: 581-597), for example.

[0239] Antibody binding agents may be antibody fragments. “Antibody fragments” comprise a portion of an intact antibody, comprising the antigen-binding or variable region thereof. Examples of antibody fragments include Fab, Fab’, F(ab’)2, Fv and scFv fragments, single domain (sd) Fv, such as VH domains, VL domains and VHH domains; diabodies; linear antibodies; single-chain antibody molecules, in particular heavy-chain antibodies; and multivalent and/or multispecific antibodies formed from antibody fragment(s), e.g., dibodies, tribodies, and multibodies. The above designations Fab, Fab’, F(ab’)2, Fv, scFv etc. are intended to have their art-established meaning.

[0240] The term antibody includes antibodies originating from or comprising one or more portions derived from any animal species, preferably vertebrate species, including, e.g., birds and mammals. Without limitation, the antibodies may be chicken, turkey, goose, duck, guinea fowl, quail or pheasant. Also without limitation, the antibodies may be human, murine (e.g., mouse, rat, etc.), donkey, rabbit, goat, sheep, guinea pig, camel (e.g., Camelus bactrianus and Camelus dromaderius), llama (e.g., Lama paccos, Lama glama or Lama vicugna) or horse.

[0241] A skilled person will understand that an antibody can include one or more amino acid deletions, additions and/or substitutions (e.g., conservative substitutions), insofar such alterations preserve its binding of the respective antigen. An antibody may also include one or more native or artificial modifications of its constituent amino acid residues (e.g., glycosylation, etc.).

[0242] Methods of producing polyclonal and monoclonal antibodies as well as fragments thereof are well known in the art, as are methods to produce recombinant antibodies or fragments thereof (see for example, Harlow and Lane, “Antibodies: A Laboratory Manual”, Cold Spring Harbour Laboratory, New York, 1988; Harlow and Lane, “Using Antibodies: A Laboratory Manual”, Cold Spring Harbour Laboratory, New York, 1999, ISBN 0879695447; “Monoclonal Antibodies: A Manual of Techniques”, by Zola, ed., CRC Press 1987, ISBN 0849364760; “Monoclonal Antibodies: A Practical Approach”, by Dean & Shepherd, eds., Oxford University Press 2000, ISBN 0199637229; Methods in Molecular Biology, vol. 248: “Antibody Engineering: Methods and Protocols”, Lo, ed., Humana Press 2004, ISBN 1588290921).

[0243] As used herein, a "blocking" antibody or an antibody "antagonist" is one which inhibits or reduces biological activity of the antigen(s) it binds. In certain embodiments, the

blocking antibodies or antagonist antibodies or portions thereof described herein completely inhibit the biological activity of the antigen(s).

[0244] Antibodies may act as agonists or antagonists of the recognized polypeptides. For example, the present invention includes antibodies which disrupt receptor/ligand interactions either partially or fully. The invention features both receptor-specific antibodies and ligand-specific antibodies. The invention also features receptor-specific antibodies which do not prevent ligand binding but prevent receptor activation. Receptor activation (i.e., signaling) may be determined by techniques described herein or otherwise known in the art. For example, receptor activation can be determined by detecting the phosphorylation (e.g., tyrosine or serine/threonine) of the receptor or of one of its down-stream substrates by immunoprecipitation followed by western blot analysis. In specific embodiments, antibodies are provided that inhibit ligand activity or receptor activity by at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 60%, or at least 50% of the activity in absence of the antibody.

[0245] The invention also features receptor-specific antibodies which both prevent ligand binding and receptor activation as well as antibodies that recognize the receptor-ligand complex. Likewise, encompassed by the invention are neutralizing antibodies which bind the ligand and prevent binding of the ligand to the receptor, as well as antibodies which bind the ligand, thereby preventing receptor activation, but do not prevent the ligand from binding the receptor. Further included in the invention are antibodies which activate the receptor. These antibodies may act as receptor agonists, i.e., potentiate or activate either all or a subset of the biological activities of the ligand-mediated receptor activation, for example, by inducing dimerization of the receptor. The antibodies may be specified as agonists, antagonists or inverse agonists for biological activities comprising the specific biological activities of the peptides disclosed herein. The antibody agonists and antagonists can be made using methods known in the art. See, e.g., PCT publication WO 96/40281; U.S. Pat. No. 5,81 1,097; Deng et al., Blood 92(6): 1981-1988 (1998); Chen et ah, Cancer Res. 58(l6):3668-3678 (1998); Harrop et al., J. Immunol. 161(4):1786-1794 (1998); Zhu et ak, Cancer Res. 58(l5):3209-32l4 (1998); Yoon et ak, J. Immunol. 160(7):3 170-3 179 (1998); Prat et ak, J. Cell. Sci. Ill (Pt2):237-247 (1998); Pitard et ak, J. Immunol. Methods 205(2): 177-190 (1997); Liautard et ak, Cytokine 9(4):233-24l (1997); Carlson et ak, J. Biol. Chem.

272(17): 11295-1 1301 (1997); Taryman et al., Neuron l4(4):755-762 (1995); Muller et al., Structure 6(9): 1153-1 167 (1998); Bartunek et al., Cytokine 8(1): 14-20 (1996).

[0246] The antibodies as defined for the present invention include derivatives that are modified, i.e., by the covalent attachment of any type of molecule to the antibody such that covalent attachment does not prevent the antibody from generating an anti-idiotypic response. For example, but not by way of limitation, the antibody derivatives include antibodies that have been modified, e.g., by glycosylation, acetylation, pegylation, phosphylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. Any of numerous chemical modifications may be carried out by known techniques, including, but not limited to specific chemical cleavage, acetylation, formylation, metabolic synthesis of tunicamycin, etc. Additionally, the derivative may contain one or more non-classical amino acids.

[0247] Simple binding assays can be used to screen for or detect agents that bind to a target protein, or disrupt the interaction between proteins (e.g., a receptor and a ligand). Because certain targets of the present invention are transmembrane proteins, assays that use the soluble forms of these proteins rather than full-length protein can be used, in some embodiments. Soluble forms include, for example, those lacking the transmembrane domain and/or those comprising the IgV domain or fragments thereof which retain their ability to bind their cognate binding partners. Further, agents that inhibit or enhance protein interactions for use in the compositions and methods described herein, can include recombinant peptido-mimetics.

[0248] Detection methods useful in screening assays include antibody-based methods, detection of a reporter moiety, detection of cytokines as described herein, and detection of a gene signature as described herein.

[0249] Another variation of assays to determine binding of a receptor protein to a ligand protein is through the use of affinity biosensor methods. Such methods may be based on the piezoelectric effect, electrochemistry, or optical methods, such as ellipsometry, optical wave guidance, and surface plasmon resonance (SPR).

[0250] The term “antibody-like protein scaffolds” or “engineered protein scaffolds” broadly encompasses proteinaceous non-immunoglobulin specific-binding agents, typically obtained by combinatorial engineering (such as site-directed random mutagenesis in combination with phage

display or other molecular selection techniques). Usually, such scaffolds are derived from robust and small soluble monomeric proteins (such as Kunitz inhibitors or lipocalins) or from a stably folded extra-membrane domain of a cell surface receptor (such as protein A, fibronectin or the ankyrin repeat).

[0251] Such scaffolds have been extensively reviewed in Binz et al. (Engineering novel binding proteins from nonimmunoglobulin domains. Nat Biotechnol 2005, 23:1257-1268), Gebauer and Skerra (Engineered protein scaffolds as next-generation antibody therapeutics. Curr Opin Chem Biol. 2009, 13:245-55), Gill and Damle (Biopharmaceutical drug discovery using novel protein scaffolds. Curr Opin Biotechnol 2006, 17:653-658), Skerra (Engineered protein scaffolds for molecular recognition. J Mol Recognit 2000, 13:167-187), and Skerra (Alternative non-antibody scaffolds for molecular recognition. Curr Opin Biotechnol 2007, 18:295-304), and include without limitation affibodies, based on the Z-domain of staphylococcal protein A, a three-helix bundle of 58 residues providing an interface on two of its alpha-helices (Nygren, Alternative binding proteins: Affibody binding proteins developed from a small three-helix bundle scaffold. FEBS J 2008, 275:2668-2676); engineered Kunitz domains based on a small (ca. 58 residues) and robust, disulphide-crosslinked serine protease inhibitor, typically of human origin (e.g. LACI-D1), which can be engineered for different protease specificities (Nixon and Wood, Engineered protein inhibitors of proteases. Curr Opin Drug Discov Dev 2006, 9:261-268); monobodies or adnectins based on the lOth extracellular domain of human fibronectin III (l0Fn3), which adopts an Ig-like beta-sandwich fold (94 residues) with 2-3 exposed loops, but lacks the central disulphide bridge (Koide and Koide, Monobodies: antibody mimics based on the scaffold of the fibronectin type III domain. Methods Mol Biol 2007, 352:95-109); anticalins derived from the lipocalins, a diverse family of eight-stranded beta-barrel proteins (ca. 180 residues) that naturally form binding sites for small ligands by means of four structurally variable loops at the open end, which are abundant in humans, insects, and many other organisms (Skerra, Alternative binding proteins: Anticalins—harnessing the structural plasticity of the lipocalin ligand pocket to engineer novel binding activities. FEBS J 2008, 275:2677-2683); DARPins, designed ankyrin repeat domains (166 residues), which provide a rigid interface arising from typically three repeated beta-turns (Stumpp et al., DARPins: a new generation of protein therapeutics. Drug Discov Today 2008, 13:695-701); avimers (multimerized LDLR-A module)

(Silverman et al., Multivalent avimer proteins evolved by exon shuffling of a family of human receptor domains. Nat Biotechnol 2005, 23:1556-1561); and cysteine-rich knottin peptides (Kolmar, Alternative binding proteins: biological activity and therapeutic potential of cystine-knot miniproteins. FEBS J 2008, 275:2684-2690).

[0252] Nucleic acid binding agents, such as oligonucleotide binding agents, are typically at least partly antisense to a target nucleic acid of interest. The term "antisense" generally refers to an agent (e.g., an oligonucleotide) configured to specifically anneal with (hybridize to) a given sequence in a target nucleic acid, such as for example in a target DNA, hnRNA, pre-mRNA or mRNA, and typically comprises, consist essentially of or consist of a nucleic acid sequence that is complementary or substantially complementary to said target nucleic acid sequence. Antisense agents suitable for use herein, such as hybridisation probes or amplification or sequencing primers and primer pairs) may typically be capable of annealing with (hybridizing to) the respective target nucleic acid sequences at high stringency conditions, and capable of hybridizing specifically to the target under physiological conditions. The terms "complementary" or "complementarity" as used throughout this specification with reference to nucleic acids, refer to the normal binding of single-stranded nucleic acids under permissive salt (ionic strength) and temperature conditions by base pairing, preferably Watson-Crick base pairing. By means of example, complementary Watson-Crick base pairing occurs between the bases A and T, A and U or G and C. For example, the sequence 5'-A-G-U-3' is complementary to sequence 5'-A-C-U-3'.

[0253] The reference to oligonucleotides may in particular but without limitation include hybridization probes and/or amplification primers and/or sequencing primers, etc., as commonly used in nucleic acid detection technologies.

[0254] Binding agents as discussed herein may suitably comprise a detectable label. The term "label" refers to any atom, molecule, moiety or biomolecule that may be used to provide a detectable and preferably quantifiable read-out or property, and that may be attached to or made part of an entity of interest, such as a binding agent. Labels may be suitably detectable by for example mass spectrometric, spectroscopic, optical, colourimetric, magnetic, photochemical, biochemical, immunochemical or chemical means. Labels include without limitation dyes; radiolabels such as ^{32}P, ^{33}P, ^{35}S, ^{125}I, ^{131}I; electron-dense reagents; enzymes (e.g., horse-radish peroxidase or alkaline phosphatase as commonly used in immunoassays); binding moieties such

as biotin-streptavidin; haptens such as digoxigenin; luminogenic, phosphorescent or fluorogenic moieties; mass tags; and fluorescent dyes alone or in combination with moieties that may suppress or shift emission spectra by fluorescence resonance energy transfer (FRET).

[0255] In some embodiments, binding agents may be provided with a tag that permits detection with another agent (e.g., with a probe binding partner). Such tags may be, for example, biotin, streptavidin, his-tag, myc tag, maltose, maltose binding protein or any other kind of tag known in the art that has a binding partner. Example of associations which may be utilised in the probe:binding partner arrangement may be any, and includes, for example biotin: streptavidin, his-tag:metal ion (e.g., Ni2+), maltose: maltose binding protein, etc.

[0256] The marker-binding agent conjugate may be associated with or attached to a detection agent to facilitate detection. Examples of detection agents include, but are not limited to, luminescent labels; colourimetric labels, such as dyes; fluorescent labels; or chemical labels, such as electroactive agents (e.g., ferrocyanide); enzymes; radioactive labels; or radiofrequency labels. The detection agent may be a particle. Examples of such particles include, but are not limited to, colloidal gold particles; colloidal sulphur particles; colloidal selenium particles; colloidal barium sulfate particles; colloidal iron sulfate particles; metal iodate particles; silver halide particles; silica particles; colloidal metal (hydrous) oxide particles; colloidal metal sulfide particles; colloidal lead selenide particles; colloidal cadmium selenide particles; colloidal metal phosphate particles; colloidal metal ferrite particles; any of the above-mentioned colloidal particles coated with organic or inorganic layers; protein or peptide molecules; liposomes; or organic polymer latex particles, such as polystyrene latex beads. Preferable particles may be colloidal gold particles.

[0257] In certain embodiments, the one or more binding agents are configured for use in a technique selected from the group consisting of flow cytometry, fluorescence activated cell sorting, mass cytometry, fluorescence microscopy, affinity separation, magnetic cell separation, microfluidic separation, and combinations thereof.

Pharmaceutical Compositions using Isolated Cells

[0258] In another aspect, the present invention provides for a pharmaceutical composition comprising the CD8+ T cell or the CD8+ T cell population as defined in any embodiment herein.

In certain embodiments, the CD8+ T cell or the CD8+ T cell population may be formulated into a pharmaceutical composition.

[0259] In certain embodiments, the immune cell or immune cell population is autologous to said subject, i.e., the immune cell or immune cell population is isolated from the same subject as the subject to which / whom the immune cell or immune cell population is to be administered. In certain further embodiments, the immune cell or immune cell population is syngeneic to said subject, i.e., the immune cell or immune cell population is isolated from an identical twin of the subject to which / whom the immune cell or immune cell population is to be administered. In certain further embodiments, the immune cell or immune cell population is allogeneic to said subject, i.e., the immune cell or immune cell population is isolated from a different subject of the same species as the subject to which / whom the immune cell or immune cell population is to be administered. In certain embodiments, the immune cell or immune cell population may even be xenogeneic to said subject, i.e., the immune cell or immune cell population may be isolated from a subject of a different species than the subject to which / whom the immune cell or immune cell population is to be administered.

[0260] Preferably, non-autologous, such as allogeneic cells may be selected such as to maximize the tissue compatibility between the subject and the administered cells, thereby reducing the chance of rejection of the administered cells by patient's immune system or graft-vs.-host reaction. For example, advantageously the cells may be typically selected which have either identical HLA haplotypes (including one or preferably more HLA-A, HLA-B, HLA-C, HLA-D, HLA-DR, HLA-DP and HLA-DQ) to the subject, or which have the most HLA antigen alleles common to the subject and none or the least of HLA antigens to which the subject contains pre-existing anti-HLA antibodies. In certain embodiments, allogenic T cells may be modified to prevent rejection from an allogenic healthy donor (described further herein).

[0261] A "pharmaceutical composition" refers to a composition that usually contains an excipient, such as a pharmaceutically acceptable carrier that is conventional in the art and that is suitable for administration to cells or to a subject.

[0262] The term "pharmaceutically acceptable" as used throughout this specification is consistent with the art and means compatible with the other ingredients of a pharmaceutical composition and not deleterious to the recipient thereof.

[0263] As used herein, “carrier” or “excipient” includes any and all solvents, diluents, buffers (such as, e.g., neutral buffered saline or phosphate buffered saline), solubilizers, colloids, dispersion media, vehicles, fillers, chelating agents (such as, e.g., EDTA or glutathione), amino acids (such as, e.g., glycine), proteins, disintegrants, binders, lubricants, wetting agents, emulsifiers, sweeteners, colorants, flavorings, aromatizers, thickeners, agents for achieving a depot effect, coatings, antifungal agents, preservatives, stabilizers, antioxidants, tonicity controlling agents, absorption delaying agents, and the like. The use of such media and agents for pharmaceutical active components is well known in the art. Such materials should be non-toxic and should not interfere with the activity of the cells or active components.

[0264] The precise nature of the carrier or excipient or other material will depend on the route of administration. For example, the composition may be in the form of a parenterally acceptable aqueous solution, which is pyrogen-free and has suitable pH, isotonicity and stability. For general principles in medicinal formulation, the reader is referred to Cell Therapy: Stem Cell Transplantation, Gene Therapy, and Cellular Immunotherapy, by G. Morstyn & W. Sheridan eds., Cambridge University Press, 1996; and Hematopoietic Stem Cell Therapy, E. D. Ball, J. Lister & P. Law, Churchill Livingstone, 2000.

[0265] The pharmaceutical composition can be applied parenterally, rectally, orally or topically. Preferably, the pharmaceutical composition may be used for intravenous, intramuscular, subcutaneous, peritoneal, peridural, rectal, nasal, pulmonary, mucosal, or oral application. In a preferred embodiment, the pharmaceutical composition according to the invention is intended to be used as an infusion. The skilled person will understand that compositions which are to be administered orally or topically will usually not comprise cells, although it may be envisioned for oral compositions to also comprise cells, for example when gastro-intestinal tract indications are treated. Each of the cells or active components (e.g., immunomodulants) as discussed herein may be administered by the same route or may be administered by a different route. By means of example, and without limitation, cells may be administered parenterally and other active components may be administered orally.

[0266] Liquid pharmaceutical compositions may generally include a liquid carrier such as water or a pharmaceutically acceptable aqueous solution. For example, physiological saline

solution, tissue or cell culture media, dextrose or other saccharide solution or glycols such as ethylene glycol, propylene glycol or polyethylene glycol may be included.

[0267] The composition may include one or more cell protective molecules, cell regenerative molecules, growth factors, anti-apoptotic factors or factors that regulate gene expression in the cells. Such substances may render the cells independent of their environment.

[0268] Such pharmaceutical compositions may contain further components ensuring the viability of the cells therein. For example, the compositions may comprise a suitable buffer system (e.g., phosphate or carbonate buffer system) to achieve desirable pH, more usually near neutral pH, and may comprise sufficient salt to ensure isoosmotic conditions for the cells to prevent osmotic stress. For example, suitable solution for these purposes may be phosphate-buffered saline (PBS), sodium chloride solution, Ringer's Injection or Lactated Ringer's Injection, as known in the art. Further, the composition may comprise a carrier protein, e.g., albumin (e.g., bovine or human albumin), which may increase the viability of the cells.

[0269] Further suitably pharmaceutically acceptable carriers or additives are well known to those skilled in the art and for instance may be selected from proteins such as collagen or gelatine, carbohydrates such as starch, polysaccharides, sugars (dextrose, glucose and sucrose), cellulose derivatives like sodium or calcium carboxymethylcellulose, hydroxypropyl cellulose or hydroxypropylmethyl cellulose, pregeletanized starches, pectin agar, carrageenan, clays, hydrophilic gums (acacia gum, guar gum, arabic gum and xanthan gum), alginic acid, alginates, hyaluronic acid, polyglycolic and polylactic acid, dextran, pectins, synthetic polymers such as water-soluble acrylic polymer or polyvinylpyrrolidone, proteoglycans, calcium phosphate and the like.

[0270] In certain embodiments, a pharmaceutical cell preparation as taught herein may be administered in a form of liquid composition. In embodiments, the cells or pharmaceutical composition comprising such can be administered systemically, topically, within an organ or at a site of organ dysfunction or lesion.

[0271] Preferably, the pharmaceutical compositions may comprise a therapeutically effective amount of the specified immune cells and/or other active components (e.g., immunomodulants). The term “therapeutically effective amount” refers to an amount which can elicit a biological or medicinal response in a tissue, system, animal or human that is being sought by a researcher,

veterinarian, medical doctor or other clinician, and in particular can prevent or alleviate one or more of the local or systemic symptoms or features of a disease or condition being treated.

Activated T Cell Compositions

[0272] A further aspect of the invention relates to a method for preparing a composition comprising activated T cells, the method comprising depleting suppressive T cells from a biological sample of a subject and contacting the remaining T cells in vitro with an immune cell or immune cell population, wherein the immune cell or immune cell population has been loaded with an antigen.

[0273] "Activation" generally refers to the state of a cell, such as preferably T cell, following sufficient cell surface moiety ligation (e.g., interaction between the T cell receptor on the surface of a T cell (such as naturally-occurring TCR or genetically engineered TCR, e.g., chimeric antigen receptor, CAR) and MHC-bound antigen peptide presented on the surface of an antigen presenting cell (e.g., dendritic cell) to induce a noticeable biochemical or morphological change of the cell, such as preferably T cell. In particular, "activation" may refer to the state of a T cell that has been sufficiently stimulated to induce detectable cellular proliferation of the T cell. Activation can also encompass induced cytokine production, and detectable T cell effector functions, e.g., regulatory or cytolytic effector functions. The T cells and antigen presenting cells may be suitably contacted by admixing the T cells and antigen presenting cells in an aqueous composition, e.g., in a culture medium, in sufficient numbers and for a sufficient duration of time to produce the desired T cell activation.

[0274] A further aspect of the invention relates to a method for adoptive immunotherapy in a subject in need thereof comprising administering to said subject a composition comprising activated T cells prepared with the method as taught above.

[0275] In certain embodiments, said T cells are CD8+ T cells, i.e., T cells expressing the CD8+ cell surface marker. More preferably, said T cells may be CD8+ T cells and said subject is suffering from proliferative disease.

[0276] In certain embodiments, the T cell, preferably a CD8+ T cell, may display specificity to a desired antigen, such as specificity to a tumor antigen (tumor antigen specificity). By means of an example, the T cell, preferably a CD8+ T cell, may have been isolated from a tumor of a subject. More preferably, the immune cell may be a tumor infiltrating lymphocyte (TIL).

Generally, “tumor infiltrating lymphocytes” or “TILs” refer to white blood cells that have left the bloodstream and migrated into a tumor. Such T cells typically endogenously express a T cell receptor having specificity to an antigen expressed by the tumor cells (tumor antigen specificity).

[0277] In alternative embodiments, a T cell, preferably a CD8+ T cell, may be engineered to express a T cell receptor having specificity to a desired antigen, such as specificity to a tumor antigen (tumor antigen specificity). For example, the T cell, preferably a CD8+ T cell, may comprise a chimeric antigen receptor (CAR) having specificity to a desired antigen, such as a tumor-specific chimeric antigen receptor (CAR).

Adoptive Cell Transfer

[0278] In certain embodiments, cells as described herein and below may be used for adoptive cell transfer (ACT). As used herein, “ACT”, “adoptive cell therapy” and “adoptive cell transfer” may be used interchangeably. In certain embodiments, the interaction of immune cells is advantageously used, such as modulating and/or transferring one immune cell subtype to cause an effect in another immune cell subtype. The transferred cells may include and be modulated by immune cells or immune cell populations as taught herein. In certain embodiments, the suppressive T cells of the present invention are depleted from cells used in ACT and the depleted cells may be transferred to a subject suffering from a disease (e.g., cancer). In certain embodiments, the cells of the present invention may be transferred to a subject suffering from a disease characteristic of an over reactive immune response (e.g., autoimmune disease). In certain embodiments, adoptive cell transfer may comprise: isolating from a biological sample of the subject a CD4+ and/or $CD8^+$ T cell or CD4+ and/or $CD8^+$ T cell population as described herein; *in vitro* expanding the T cell or T cell population; and administering the *in vitro* expanded T cell or T cell population to the subject. The method may further comprise enriching the expanded T cells for one subtype. In certain embodiments, the method may further comprise formulating the in vitro expanded immune cell or immune cell population into a pharmaceutical composition.

[0279] In certain embodiments, Adoptive cell therapy (ACT) can refer to the transfer of cells to a patient with the goal of transferring the functionality and characteristics into the new host by engraftment of the cells (see, e.g., Mettananda et al., Editing an α-globin enhancer in primary human hematopoietic stem cells as a treatment for β-thalassemia, Nat Commun. 2017 Sep 4;8(l):424). As used herein, the term "engraft" or "engraftment" refers to the process of cell

incorporation into a tissue of interest *in vivo* through contact with existing cells of the tissue. Adoptive cell therapy (ACT) can refer to the transfer of cells, most commonly immune-derived cells, back into the same patient or into a new recipient host with the goal of transferring the immunologic functionality and characteristics into the new host. If possible, use of autologous cells helps the recipient by minimizing GVHD issues. The adoptive transfer of autologous tumor infiltrating lymphocytes (TIL) (Besser et al., (2010) Clin. Cancer Res 16 (9) 2646-55; Dudley et al., (2002) Science 298 (5594): 850-4; and Dudley et al., (2005) Journal of Clinical Oncology 23 (10): 2346-57.) or genetically re-directed peripheral blood mononuclear cells (Johnson et al., (2009) Blood 114 (3): 535-46; and Morgan et al., (2006) Science 314(5796) 126-9) has been used to successfully treat patients with advanced solid tumors, including melanoma and colorectal carcinoma, as well as patients with CD19-expressing hematologic malignancies (Kalos et al., (2011) Science Translational Medicine 3 (95): 95ra73). In certain embodiments, allogenic cells immune cells are transferred (see, e.g., Ren et al., (2017) Clin Cancer Res 23 (9) 2255-2266). As described further herein, allogenic cells can be edited to reduce alloreactivity and prevent graft-versus-host disease. Thus, use of allogenic cells allows for cells to be obtained from healthy donors and prepared for use in patients as opposed to preparing autologous cells from a patient after diagnosis.

[0280] Aspects of the invention involve the adoptive transfer of immune system cells, such as T cells, specific for selected antigens, such as tumor associated antigens or tumor specific neoantigens (see, e.g., Maus et al., 2014, Adoptive Immunotherapy for Cancer or Viruses, Annual Review of Immunology, Vol. 32: 189-225; Rosenberg and Restifo, 2015, Adoptive cell transfer as personalized immunotherapy for human cancer, Science Vol. 348 no. 6230 pp. 62-68; Restifo et al., 2015, Adoptive immunotherapy for cancer: harnessing the T cell response. Nat. Rev. Immunol. 12(4): 269-281; and Jenson and Riddell, 2014, Design and implementation of adoptive therapy with chimeric antigen receptor-modified T cells. Immunol Rev. 257(1): 127-144; and Rajasagi et al., 2014, Systematic identification of personal tumor-specific neoantigens in chronic lymphocytic leukemia. Blood. 2014 Jul 17;124(3):453-62).

[0281] In certain embodiments, an antigen (such as a tumor antigen) to be targeted in adoptive cell therapy (such as particularly CAR or TCR T-cell therapy) of a disease (such as particularly of tumor or cancer) may be selected from a group consisting of: B cell maturation

antigen (BCMA) (see, e.g., Friedman et al., Effective Targeting of Multiple BCMA-Expressing Hematological Malignancies by Anti-BCMA CAR T Cells, Hum Gene Ther. 2018 Mar 8; Berdeja JG, et al. Durable clinical responses in heavily pretreated patients with relapsed/refractory multiple myeloma: updated results from a multicenter study of bb2l2l anti-Bcma CAR T cell therapy. Blood. 2017; 130:740; and Mouhieddine and Ghobrial, Immunotherapy in Multiple Myeloma: The Era of CAR T Cell Therapy, Hematologist, May-June 2018, Volume 15, issue 3); PSA (prostate-specific antigen); prostate-specific membrane antigen (PSMA); PSCA (Prostate stem cell antigen); Tyrosine-protein kinase transmembrane receptor ROR1; fibroblast activation protein (FAP); Tumor-associated glycoprotein 72 (TAG72); Carcinoembryonic antigen (CEA); Epithelial cell adhesion molecule (EPCAM); Mesothelin; Human Epidermal growth factor Receptor 2 (ERBB2 (Her2/neu)); Prostase; Prostatic acid phosphatase (PAP); elongation factor 2 mutant (ELF2M); Insulin-like growth factor 1 receptor (IGF-1R); gplOO; BCR-ABL (breakpoint cluster region-Abelson); tyrosinase; New York esophageal squamous cell carcinoma 1 (NY-ESO-l); κ-light chain, LAGE (L antigen); MAGE (melanoma antigen); Melanoma-associated antigen 1 (MAGE-A1); MAGE A3; MAGE A6; legumain; Human papillomavirus (HPV) E6; HPV E7; prostein; survivin; PCTA1 (Galectin 8); Melan-A/MART-1; Ras mutant; TRP-l (tyrosinase related protein 1, or gp75); Tyrosinase-related Protein 2 (TRP2); TRP-2/INT2 (TRP-2/intron 2); RAGE (renal antigen); receptor for advanced glycation end products 1 (RAGE1); Renal ubiquitous 1, 2 (RU1, RET2); intestinal carboxyl esterase (iCE); Heat shock protein 70-2 (HSP70-2) mutant; thyroid stimulating hormone receptor (TSHR); CD123; CD171; CD19; CD20; CD22; CD26; CD30; CD33; CD44v7/8 (cluster of differentiation 44, exons 7/8); CD53; CD92; CD100; CD148; CD150; CD200; CD261; CD262; CD362; CS-l (CD2 subset 1, CRACC, SLAMF7, CD319, and 19A24); C-type lectin-like molecule-1 (CLL-l); ganglioside GD3 (aNeu5Ac(2-8)aNeu5Ac(2-3)bDGalp(l-4)bDGlcp(l-l)Cer); Tn antigen (Tn Ag); Fms-Like Tyrosine Kinase 3 (FLT3); CD38; CD138; CD44v6; B7H3 (CD276); KIT (CD1 17); Interleukin-13 receptor subunit alpha-2 (IL-l3Ra2); Interleukin 11 receptor alpha (IL-l lRa); prostate stem cell antigen (PSCA); Protease Serine 21 (PRSS21); vascular endothelial growth factor receptor 2 (VEGFR2); Lewis(Y) antigen; CD24; Platelet-derived growth factor receptor beta (PDGFR-beta); stage-specific embryonic antigen-4 (SSEA-4); Mucin 1, cell surface associated (MUC1); mucin 16

(MUC16); epidermal growth factor receptor (EGFR); epidermal growth factor receptor variant III (EGFRvIII); neural cell adhesion molecule (NCAM); carbonic anhydrase IX (CAIX); Proteasome (Prosome, Macropain) Subunit, Beta Type, 9 (LMP2); ephrin type-A receptor 2 (EphA2); Ephrin B2; Fucosyl GM1; sialyl Lewis adhesion molecule (sLe); ganglioside GM3 (aNeu5Ac(2-3)bDGalp(l-4)bDGlcp(l-l)Cer); TGS5; high molecular weight-melanoma-associated antigen (HMWMAA); o-acetyl-GD2 ganglioside (OAcGD2); Folate receptor alpha; Folate receptor beta; tumor endothelial marker 1 (TEM1/CD248); tumor endothelial marker 7-related (TEM7R); claudin 6 (CLDN6); G protein-coupled receptor class C group 5, member D (GPRC5D); chromosome X open reading frame 61 (CXORF61); CD97; CDl79a; anaplastic lymphoma kinase (ALK); Poly sialic acid; placenta-specific 1 (PLAC1); hexasaccharide portion of globoH glycoceramide (GloboH); mammary gland differentiation antigen (NY-BR-l); uroplakin 2 (EIPK2); Hepatitis A virus cellular receptor 1 (HAVCR1); adrenoceptor beta 3 (ADRB3); pannexin 3 (PANX3); G protein-coupled receptor 20 (GPR20); lymphocyte antigen 6 complex, locus K 9 (LY6K); Olfactory receptor 51E2 (OR51E2); TCR Gamma Alternate Reading Frame Protein (TARP); Wilms tumor protein (WT1); ETS translocation-variant gene 6, located on chromosome 12ρ (ETV6-AML); sperm protein 17 (SPA17); X Antigen Family, Member 1A (XAGE1); angiopoietin-binding cell surface receptor 2 (Tie 2); CT (cancer/testis (antigen)); melanoma cancer testis antigen-l (MAD-CT-l); melanoma cancer testis antigen-2 (MAD-CT-2); Fos-related antigen 1; p53; p53 mutant; human Telomerase reverse transcriptase (hTERT); sarcoma translocation breakpoints; melanoma inhibitor of apoptosis (ML-IAP); ERG (transmembrane protease, serine 2 (TMPRSS2) ETS fusion gene); N-Acetyl glucosaminyl-transferase V (NA17); paired box protein Pax-3 (PAX3); Androgen receptor; Cyclin Bl; Cyclin Dl; v-myc avian myelocytomatosis viral oncogene neuroblastoma derived homolog (MYCN); Ras Homolog Family Member C (RhoC); Cytochrome P450 1B1 (CYP1B1); CCCTC-Binding Factor (Zinc Finger Protein)-Like (BORIS); Squamous Cell Carcinoma Antigen Recognized By T Cells-l or 3 (SART1, SART3); Paired box protein Pax-5 (PAX5); proacrosin binding protein sp32 (OY-TES1); lymphocyte-specific protein tyrosine kinase (LCK); A kinase anchor protein 4 (AKAP-4); synovial sarcoma, X breakpoint-l, -2, -3 or -4 (SSX1, SSX2, SSX3, SSX4); CD79a; CD79b; CD72; Leukocyte-associated immunoglobulin-like receptor 1 (LAIR1); Fc fragment of IgA receptor (FCAR); Leukocyte immunoglobulin-like receptor subfamily A member 2

(LILRA2); CD300 molecule-like family member f (CD300LF); C-type lectin domain family 12 member A (CLEC12A); bone marrow stromal cell antigen 2 (BST2); EGF-like module-containing mucin-like hormone receptor-like 2 (EMR2); lymphocyte antigen 75 (LY75); Glypican-3 (GPC3); Fc receptor-like 5 (FCRL5); mouse double minute 2 homolog (MDM2); livin; alphafetoprotein (AFP); transmembrane activator and CAML Interactor (TACI); B-cell activating factor receptor (BAFF-R); V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (KRAS); immunoglobulin lambda-like polypeptide 1 (IGLL1); 707-AP (707 alanine proline); ART-4 (adenocarcinoma antigen recognized by T4 cells); BAGE (B antigen; b-catenin/m, b-catenin/mutated); CAMEL (CTL-recognized antigen on melanoma); CAP1 (carcinoembryonic antigen peptide 1); CASP-8 (caspase-8); CDC27m (cell-division cycle 27 mutated); CDK4/m (cycline-dependent kinase 4 mutated); Cyp-B (cyclophilin B); DAM (differentiation antigen melanoma); EGP-2 (epithelial glycoprotein 2); EGP-40 (epithelial glycoprotein 40); Erbb2, 3, 4 (erythroblastic leukemia viral oncogene homolog-2, -3, 4); FBP (folate binding protein); , fAchR (Fetal acetylcholine receptor); G250 (glycoprotein 250); GAGE (G antigen); GnT-V (N-acetylglucosaminyltransferase V); HAGE (helicose antigen); EILA-A (human leukocyte antigen-A); HST2 (human signet ring tumor 2); KIAA0205; KDR (kinase insert domain receptor); LDLR/FETT (low density lipid receptor/GDP L-fucose: b-D-galactosidase 2-a-L fucosyltransferase); L1CAM (Ll cell adhesion molecule); MC1R (melanocortin 1 receptor); Myosin/m (myosin mutated); MUM-l, -2, -3 (melanoma ubiquitous mutated 1, 2, 3); NA88-A (NA cDNA clone of patient M88); KG2D (Natural killer group 2, member D) ligands; oncofetal antigen (h5T4); pl90 minor bcr-abl (protein of 190KD bcr-abl); Pml/RARa (promyelocytic leukaemia/retinoic acid receptor a); PRAME (preferentially expressed antigen of melanoma); SAGE (sarcoma antigen); TEL/AML1 (translocation Ets-family leukemia/acute myeloid leukemia 1); TPI/m (triosephosphate isomerase mutated); CD70; and any combination thereof.

[0282] In certain embodiments, an antigen to be targeted in adoptive cell therapy (such as particularly CAR or TCR T-cell therapy) of a disease (such as particularly of tumor or cancer) is a tumor-specific antigen (TSA).

[0283] In certain embodiments, an antigen to be targeted in adoptive cell therapy (such as particularly CAR or TCR T-cell therapy) of a disease (such as particularly of tumor or cancer) is a neoantigen.

[0284] In certain embodiments, an antigen to be targeted in adoptive cell therapy (such as particularly CAR or TCR T-cell therapy) of a disease (such as particularly of tumor or cancer) is a tumor-associated antigen (TAA).

[0285] In certain embodiments, an antigen to be targeted in adoptive cell therapy (such as particularly CAR or TCR T-cell therapy) of a disease (such as particularly of tumor or cancer) is a universal tumor antigen. In certain preferred embodiments, the universal tumor antigen is selected from the group consisting of: a human telomerase reverse transcriptase (hTERT), survivin, mouse double minute 2 homolog (MDM2), cytochrome P450 1B 1 (CYP1B), HER2/neu, Wilms' tumor gene 1 (WT1), livin, alphafetoprotein (AFP), carcinoembryonic antigen (CEA), mucin 16 (MUC16), MUC1, prostate-specific membrane antigen (PSMA), p53, cyclin (Dl), and any combinations thereof.

[0286] In certain embodiments, an antigen (such as a tumor antigen) to be targeted in adoptive cell therapy (such as particularly CAR or TCR T-cell therapy) of a disease (such as particularly of tumor or cancer) may be selected from a group consisting of: CD19, BCMA, CD70, CLL-l, MAGE A3, MAGE A6, HPV E6, HPV E7, WT1, CD22, CD171, ROR1, MUC16, and SSX2. In certain preferred embodiments, the antigen may be CD19. For example, CD 19 may be targeted in hematologic malignancies, such as in lymphomas, more particularly in B-cell lymphomas, such as without limitation in diffuse large B-cell lymphoma, primary mediastinal b-cell lymphoma, transformed follicular lymphoma, marginal zone lymphoma, mantle cell lymphoma, acute lymphoblastic leukemia including adult and pediatric ALL, non-Hodgkin lymphoma, indolent non-Hodgkin lymphoma, or chronic lymphocytic leukemia. For example, BCMA may be targeted in multiple myeloma or plasma cell leukemia (see, e.g., 2018 American Association for Cancer Research (AACR) Annual meeting Poster: Allogeneic Chimeric Antigen Receptor T Cells Targeting B Cell Maturation Antigen). For example, CLL1 may be targeted in acute myeloid leukemia. For example, MAGE A3, MAGE A6, SSX2, and/or KRAS may be targeted in solid tumors. For example, HPV E6 and/or HPV E7 may be targeted in cervical cancer or head and neck cancer. For example, WT1 may be targeted in acute myeloid leukemia (AML), myelodysplastic syndromes (MDS), chronic myeloid leukemia (CML), non-small cell lung cancer, breast, pancreatic, ovarian or colorectal cancers, or mesothelioma. For example, CD22 may be targeted in B cell malignancies, including non-Hodgkin lymphoma,

diffuse large B-cell lymphoma, or acute lymphoblastic leukemia. For example, CD171 may be targeted in neuroblastoma, glioblastoma, or lung, pancreatic, or ovarian cancers. For example, ROR1 may be targeted in ROR1+ malignancies, including non-small cell lung cancer, triple negative breast cancer, pancreatic cancer, prostate cancer, ALL, chronic lymphocytic leukemia, or mantle cell lymphoma. For example, MUC16 may be targeted in MUCl6ecto+ epithelial ovarian, fallopian tube or primary peritoneal cancer. For example, CD70 may be targeted in both hematologic malignancies as well as in solid cancers such as renal cell carcinoma (RCC), gliomas (e.g., GBM), and head and neck cancers (HNSCC). CD70 is expressed in both hematologic malignancies as well as in solid cancers, while its expression in normal tissues is restricted to a subset of lymphoid cell types (see, e.g., 2018 American Association for Cancer Research (AACR) Annual meeting Poster: Allogeneic CRISPR Engineered Anti-CD70 CAR-T Cells Demonstrate Potent Preclinical Activity Against Both Solid and Hematological Cancer Cells).

[0287] Various strategies may for example be employed to genetically modify T cells by altering the specificity of the T cell receptor (TCR) for example by introducing new TCR a and β chains with selected peptide specificity (see U.S. Patent No. 8,697,854; PCT Patent Publications: W02003020763, W02004033685, W02004044004, W020051 14215, W02006000830, W02008038002, W02008039818, W02004074322, W020051 13595, WO2006125962, WO2013 166321, WO2013039889, WO2014018863, WO2014083173; U.S. Patent No. 8,088,379).

[0288] As an alternative to, or addition to, TCR modifications, chimeric antigen receptors (CARs) may be used in order to generate immunoresponsive cells, such as T cells, specific for selected targets, such as malignant cells, with a wide variety of receptor chimera constructs having been described (see U.S. Patent Nos. 5,843,728; 5,851,828; 5,912,170; 6,004,81 1; 6,284,240; 6,392,013; 6,410,014; 6,753,162; 8,21 1,422; and, PCT Publication W09215322).

[0289] In general, CARs are comprised of an extracellular domain, a transmembrane domain, and an intracellular domain, wherein the extracellular domain comprises an antigen-binding domain that is specific for a predetermined target. While the antigen-binding domain of a CAR is often an antibody or antibody fragment (e.g., a single chain variable fragment, scFv), the binding domain is not particularly limited so long as it results in specific recognition of a target. For

example, in some embodiments, the antigen-binding domain may comprise a receptor, such that the CAR is capable of binding to the ligand of the receptor. Alternatively, the antigen-binding domain may comprise a ligand, such that the CAR is capable of binding the endogenous receptor of that ligand.

[0290] The antigen-binding domain of a CAR is generally separated from the transmembrane domain by a hinge or spacer. The spacer is also not particularly limited, and it is designed to provide the CAR with flexibility. For example, a spacer domain may comprise a portion of a human Fc domain, including a portion of the CH3 domain, or the hinge region of any immunoglobulin, such as IgA, IgD, IgE, IgG, or IgM, or variants thereof. Furthermore, the hinge region may be modified so as to prevent off-target binding by FcRs or other potential interfering objects. For example, the hinge may comprise an IgG4 Fc domain with or without a S228P, L235E, and/or N297Q mutation (according to Rabat numbering) in order to decrease binding to FcRs. Additional spacers/hinges include, but are not limited to, CD4, CD8, and CD28 hinge regions.

[0291] The transmembrane domain of a CAR may be derived either from a natural or from a synthetic source. Where the source is natural, the domain may be derived from any membrane bound or transmembrane protein. Transmembrane regions of particular use in this disclosure may be derived from CD8, CD28, CD3, CD45, CD4, CD5, CDS, CD9, CD 16, CD22, CD33, CD37, CD64, CD80, CD86, CD 134, CD137, CD 154, TCR. Alternatively, the transmembrane domain may be synthetic, in which case it will comprise predominantly hydrophobic residues such as leucine and valine. Preferably a triplet of phenylalanine, tryptophan and valine will be found at each end of a synthetic transmembrane domain. Optionally, a short oligo- or polypeptide linker, preferably between 2 and 10 amino acids in length may form the linkage between the transmembrane domain and the cytoplasmic signaling domain of the CAR. A glycine-serine doublet provides a particularly suitable linker.

[0292] Alternative CAR constructs may be characterized as belonging to successive generations. First-generation CARs typically consist of a single-chain variable fragment of an antibody specific for an antigen, for example comprising a VL linked to a VH of a specific antibody, linked by a flexible linker, for example by a CD8a hinge domain and a CD8a transmembrane domain, to the transmembrane and intracellular signaling domains of either

Cü 3ζ or FcRγ (scFv-Cü 3ζ or scFv-FcRy; see U.S. Patent No. 7,741,465; U.S. Patent No. 5,912,172; U.S. Patent No. 5,906,936). Second-generation CARs incorporate the intracellular domains of one or more costimulatory molecules, such as CD28, 0X40 (CD134), or 4-1BB (CD137) within the endodomain (for example scFv-CD28/OX40/4-lBB-CC^; see U.S. Patent Nos. 8,91 1,993; 8,916,381; 8,975,071; 9,101,584; 9,102,760; 9,102,761). Third-generation CARs include a combination of costimulatory endodomains, such a CC^-chain, CD97, GDI la-CD18, CD2, ICOS, CD27, CD154, CDS, 0X40, 4-1BB, CD2, CD7, LIGHT, LFA-l, NKG2C, B7-H3, CD30, CD40, PD-l, or CD28 signaling domains (for example scFv-CD28-4-lBB-CD3C or scFv-CD28-OX40-CD3Q see U.S. Patent No. 8,906,682; U.S. Patent No. 8,399,645; U.S. Pat. No. 5,686,281; PCT Publication No. WO2014134165; PCT Publication No. W02012079000). In certain embodiments, the primary signaling domain comprises a functional signaling domain of a protein selected from the group consisting of CD3 zeta, CD3 gamma, CD3 delta, CD3 epsilon, common FcR gamma (FCERIG), FcR beta (Fc Epsilon Rlb), CD79a, CD79b, Fc gamma Rlla, DAP10, and DAP12. In certain preferred embodiments, the primary signaling domain comprises a functional signaling domain of Cü 3ζ or FcRy. In certain embodiments, the one or more costimulatory signaling domains comprise a functional signaling domain of a protein selected, each independently, from the group consisting of: CD27, CD28, 4-1BB (CD137), 0X40, CD30, CD40, PD-l, ICOS, lymphocyte function-associated antigen-l (LFA-l), CD2, CD7, LIGHT, NKG2C, B7-H3, a ligand that specifically binds with CD83, CDS, ICAM-l, GITR, BAFFR, HVEM (LIGHTR), SLAMF7, NKp80 (KLRF1), CD 160, CD 19, CD4, CD8 alpha, CD8 beta, IL2R beta, IL2R gamma, IL7R alpha, ITGA4, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CDl ld, ITGAE, CD103, ITGAL, CDl la, LFA-l, ITGAM, CDl lb, ITGAX, CDl lc, ITGB1, CD29, ITGB2, CD 18, ITGB7, TNFR2, TRANCE/RANKL, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRTAM, Ly9 (CD229), CD 160 (BY55), PSGL1, CD100 (SEMA4D), CD69, SLAMF6 (NTB-A, Lyl08), SLAM (SLAMF1, CD 150, IPO-3), BLAME (SLAMF8), SELPLG (CD 162), LTBR, LAT, GADS, SLP-76, PAG/Cbp, NKp44, NKp30, NKp46, and NKG2D. In certain embodiments, the one or more costimulatory signaling domains comprise a functional signaling domain of a protein selected, each independently, from the group consisting of: 4-1BB, CD27, and CD28. In certain embodiments, a chimeric antigen receptor may have the design as described in U.S. Patent No.

7,446,190, comprising an intracellular domain of Cü 3ζ chain (such as amino acid residues 52-163 of the human CD3 zeta chain, as shown in SEQ ID NO: 14 of US 7,446,190), a signaling region from CD28 and an antigen-binding element (or portion or domain; such as scFv). The CD28 portion, when between the zeta chain portion and the antigen-binding element, may suitably include the transmembrane and signaling domains of CD28 (such as amino acid residues 114-220 of SEQ ID NO: 10, full sequence shown in SEQ ID NO: 6 of US 7,446,190; these can include the following portion of CD28 as set forth in Genbank identifier NM_006139 (sequence version 1, 2 or 3): IEVMYPPP YLDNEK SN GTIIHVKGKHLCP SPLFPGP SKPFWVLVVVGGVLACY SLLVTVA FIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRS)) (SEQ ID No. 1). Alternatively, when the zeta sequence lies between the CD28 sequence and the antigen-binding element, intracellular domain of CD28 can be used alone (such as amino sequence set forth in SEQ ID NO: 9 of US 7,446,190). Hence, certain embodiments employ a CAR comprising (a) a zeta chain portion comprising the intracellular domain of human Cü 3ζ chain, (b) a costimulatory signaling region, and (c) an antigen-binding element (or portion or domain), wherein the costimulatory signaling region comprises the amino acid sequence encoded by SEQ ID NO: 6 of US 7,446,190.

[0293] Alternatively, costimulation may be orchestrated by expressing CARs in antigen-specific T cells, chosen so as to be activated and expanded following engagement of their native a.pTCR, for example by antigen on professional antigen-presenting cells, with attendant costimulation. In addition, additional engineered receptors may be provided on the immunoresponsive cells, for example to improve targeting of a T-cell attack and/or minimize side effects

[0294] By means of an example and without limitation, Kochenderfer et al., (2009) J Immunother. 32 (7): 689-702 described anti-CDl9 chimeric antigen receptors (CAR). FMC63-28Z CAR contained a single chain variable region moiety (scFv) recognizing CD 19 derived from the FMC63 mouse hybridoma (described in Nicholson et al., (1997) Molecular Immunology 34: 1157-1 165), a portion of the human CD28 molecule, and the intracellular component of the human TCR-ζ molecule. FMC63-CD828BBZ CAR contained the FMC63 scFv, the hinge and transmembrane regions of the CD8 molecule, the cytoplasmic portions of CD28 and 4-1BB, and

the cytoplasmic component of the TCR-ζ molecule. The exact sequence of the CD28 molecule included in the FMC63-28Z CAR corresponded to Genbank identifier NM_006139; the sequence included all amino acids starting with the amino acid sequence IEVMYPPPY (SEQ ID No. 2) and continuing all the way to the carboxy-terminus of the protein. To encode the anti-CD19 scFv component of the vector, the authors designed a DNA sequence which was based on a portion of a previously published CAR (Cooper et al., (2003) Blood 101: 1637-1644). This sequence encoded the following components in frame from the 5' end to the 3' end: an Xhol site, the human granulocyte-macrophage colony-stimulating factor (GM-CSF) receptor α-chain signal sequence, the FMC63 light chain variable region (as in Nicholson et al., *supra),* a linker peptide (as in Cooper et al., *supra),* the FMC63 heavy chain variable region (as in Nicholson et al., *supra),* and a Notl site. A plasmid encoding this sequence was digested with Xhol and Noth To form the MSGV-FMC63-28Z retroviral vector, the Xhol and Notl-digested fragment encoding the FMC63 scFv was ligated into a second Xhol and Notl-digested fragment that encoded the MSGV retroviral backbone (as in Hughes et al., (2005) Human Gene Therapy 16: 457-472) as well as part of the extracellular portion of human CD28, the entire transmembrane and cytoplasmic portion of human CD28, and the cytoplasmic portion of the human TCR-ζ molecule (as in Maher et al., 2002) Nature Biotechnology 20: 70-75). The FMC63-28Z CAR is included in the KTE-C19 (axicabtagene ciloleucel) anti-CD19 CAR-T therapy product in development by Kite Pharma, Inc. for the treatment of *inter alia* patients with relapsed/refractory aggressive B-cell non-Hodgkin lymphoma (NHL). Accordingly, in certain embodiments, cells intended for adoptive cell therapies, more particularly immunoresponsive cells such as T cells, may express the FMC63-28Z CAR as described by Kochenderfer et al. *{supra).* Hence, in certain embodiments, cells intended for adoptive cell therapies, more particularly immunoresponsive cells such as T cells, may comprise a CAR comprising an extracellular antigen-binding element (or portion or domain; such as scFv) that specifically binds to an antigen, an intracellular signaling domain comprising an intracellular domain of a Cü 3ζ chain, and a costimulatory signaling region comprising a signaling domain of CD28. Preferably, the CD28 amino acid sequence is as set forth in Genbank identifier NM_006139 (sequence version 1, 2 or 3) starting with the amino acid sequence IEVMYPPPY (SEQ ID NO: 2) and continuing all the way to the carboxy-terminus of the protein. The sequence is reproduced herein:

IEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPLFPGPSKPFWVLVVVGGVLACYSLLVTVA FIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRS (SEQ ID No. 1). Preferably, the antigen is CD19, more preferably the antigen-binding element is an anti-CD19 scFv, even more preferably the anti-CDl9 scFv as described by Kochenderfer et al. *{supra).*

[0295] Additional anti-CDl9 CARs are further described in WO2015187528. More particularly Example 1 and Table 1 of WO2015187528, incorporated by reference herein, demonstrate the generation of anti-CD19 CARs based on a fully human anti-CD19 monoclonal antibody (47G4, as described in US20100104509) and murine anti-CDl9 monoclonal antibody (as described in Nicholson et al. and explained above). Various combinations of a signal sequence (human CD8-alpha or GM-CSF receptor), extracellular and transmembrane regions (human CD8-alpha) and intracellular T-cell signalling domains (Cü 28-Cü 3ζ; 4-1BB-Cü 3ζ; CD27-CD3Q CD28-CD27-CD3C, 4-1BB-CD27-CD3ζ; CD27-4-lBB-CD3Q CD28-CD27-FcsRI gamma chain; or CD28-FcsRI gamma chain) were disclosed. Hence, in certain embodiments, cells intended for adoptive cell therapies, more particularly immunoresponsive cells such as T cells, may comprise a CAR comprising an extracellular antigen-binding element that specifically binds to an antigen, an extracellular and transmembrane region as set forth in Table 1 of WO2015187528 and an intracellular T-cell signalling domain as set forth in Table 1 of WO2015187528. Preferably, the antigen is CD19, more preferably the antigen-binding element is an anti-CD19 scFv, even more preferably the mouse or human anti-CD19 scFv as described in Example 1 of WO2015187528. In certain embodiments, the CAR comprises, consists essentially of or consists of an amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, or SEQ ID NO: 13 as set forth in Table 1 of WO2015187528.

[0296] By means of an example and without limitation, chimeric antigen receptor that recognizes the CD70 antigen is described in W02012058460A2 (see also, Park et al., CD70 as a target for chimeric antigen receptor T cells in head and neck squamous cell carcinoma, Oral Oncol. 2018 Mar;78: 145-150; and Jin et al., CD70, a novel target of CAR T-cell therapy for gliomas, Neuro Oncol. 2018 Jan l0;20(l):55-65). CD70 is expressed by diffuse large B-cell and follicular lymphoma and also by the malignant cells of Hodgkins lymphoma, Waldenstrom's

macroglobulinemia and multiple myeloma, and by HTLV-l- and EBV-associated malignancies. (Agathanggelou et al. Am.J.Pathol. 1995;147: 1152-1 160; Hunter et al., Blood 2004; 104:4881. 26; Lens et al., J Immunol. 2005;174:6212-6219; Baba et al., J Virol. 2008;82:3843-3852.) In addition, CD70 is expressed by non-hematological malignancies such as renal cell carcinoma and glioblastoma. (Junker et al., J Urol. 2005;173:2150-2153; Chahlavi et al., Cancer Res 2005;65:5428-5438) Physiologically, CD70 expression is transient and restricted to a subset of highly activated T, B, and dendritic cells.

[0297] By means of an example and without limitation, chimeric antigen receptor that recognizes BCMA has been described (see, e.g., US20160046724A1; WO2016014789A2; WO201721 1900A1; WO2015158671A1; US20180085444A1; WO2018028647A1; US20 170283 504A1 ; and WO20 13 154760A1).

[0298] In certain embodiments, the immune cell may, in addition to a CAR or exogenous TCR as described herein, further comprise a chimeric inhibitory receptor (inhibitory CAR) that specifically binds to a second target antigen and is capable of inducing an inhibitory or immunosuppressive or repressive signal to the cell upon recognition of the second target antigen. In certain embodiments, the chimeric inhibitory receptor comprises an extracellular antigen-binding element (or portion or domain) configured to specifically bind to a target antigen, a transmembrane domain, and an intracellular immunosuppressive or repressive signaling domain. In certain embodiments, the second target antigen is an antigen that is not expressed on the surface of a cancer cell or infected cell or the expression of which is downregulated on a cancer cell or an infected cell. In certain embodiments, the second target antigen is an MHC-class I molecule. In certain embodiments, the intracellular signaling domain comprises a functional signaling portion of an immune checkpoint molecule, such as for example PD-l or CTLA4. Advantageously, the inclusion of such inhibitory CAR reduces the chance of the engineered immune cells attacking non-target (e.g., non-cancer) tissues.

[0299] Alternatively, T-cells expressing CARs may be further modified to reduce or eliminate expression of endogenous TCRs in order to reduce off-target effects. Reduction or elimination of endogenous TCRs can reduce off-target effects and increase the effectiveness of the T cells (U.S. 9,181,527). T cells stably lacking expression of a functional TCR may be produced using a variety of approaches. T cells internalize, sort, and degrade the entire T cell

receptor as a complex, with a half-life of about 10 hours in resting T cells and 3 hours in stimulated T cells (von Essen, M. et al. 2004. J. Immunol. 173:384-393). Proper functioning of the TCR complex requires the proper stoichiometric ratio of the proteins that compose the TCR complex. TCR function also requires two functioning TCR zeta proteins with ITAM motifs. The activation of the TCR upon engagement of its MHC-peptide ligand requires the engagement of several TCRs on the same T cell, which all must signal properly. Thus, if a TCR complex is destabilized with proteins that do not associate properly or cannot signal optimally, the T cell will not become activated sufficiently to begin a cellular response.

[0300] Accordingly, in some embodiments, TCR expression may eliminated using RNA interference (e.g., shRNA, siRNA, miRNA, etc.), CRISPR, or other methods that target the nucleic acids encoding specific TCRs (e.g., TCR-α and TCR-β) and/or CD3 chains in primary T cells. By blocking expression of one or more of these proteins, the T cell will no longer produce one or more of the key components of the TCR complex, thereby destabilizing the TCR complex and preventing cell surface expression of a functional TCR.

[0301] In some instances, CAR may also comprise a switch mechanism for controlling expression and/or activation of the CAR. For example, a CAR may comprise an extracellular, transmembrane, and intracellular domain, in which the extracellular domain comprises a target-specific binding element that comprises a label, binding domain, or tag that is specific for a molecule other than the target antigen that is expressed on or by a target cell. In such embodiments, the specificity of the CAR is provided by a second construct that comprises a target antigen binding domain (e.g., an scFv or a bispecific antibody that is specific for both the target antigen and the label or tag on the CAR) and a domain that is recognized by or binds to the label, binding domain, or tag on the CAR. See, e.g., WO 2013/044225, WO 2016/000304, WO 2015/057834, WO 2015/057852, WO 2016/070061, US 9,233,125, US 2016/0129109. In this way, a T-cell that expresses the CAR can be administered to a subject, but the CAR cannot bind its target antigen until the second composition comprising an antigen-specific binding domain is administered.

[0302] Alternative switch mechanisms include CARs that require multimerization in order to activate their signaling function (see, e.g., US 2015/0368342, US 2016/0175359, US 2015/0368360) and/or an exogenous signal, such as a small molecule drug (US 2016/0166613,

Yung et al., Science, 2015), in order to elicit a T-cell response. Some CARs may also comprise a "suicide switch" to induce cell death of the CAR T-cells following treatment (Buddee et al., PLoS One, 2013) or to downregulate expression of the CAR following binding to the target antigen (WO 2016/01 1210).

[0303] Alternative techniques may be used to transform target immunoresponsive cells, such as protoplast fusion, lipofection, transfection or electroporation. A wide variety of vectors may be used, such as retroviral vectors, lentiviral vectors, adenoviral vectors, adeno-associated viral vectors, plasmids or transposons, such as a Sleeping Beauty transposon (see U.S. Patent Nos. 6,489,458; 7,148,203; 7,160,682; 7,985,739; 8,227,432), may be used to introduce CARs, for example using 2nd generation antigen-specific CARs signaling through CD3ζ and either CD28 or CD137. Viral vectors may for example include vectors based on HIV, SV40, EBV, HSV or BPV.

[0304] Cells that are targeted for transformation may for example include T cells, Natural Killer (NK) cells, cytotoxic T lymphocytes (CTL), regulatory T cells, human embryonic stem cells, tumor-infiltrating lymphocytes (TIL) or a pluripotent stem cell from which lymphoid cells may be differentiated. T cells expressing a desired CAR may for example be selected through co-culture with γ-irradiated activating and propagating cells (AaPC), which co-express the cancer antigen and co-stimulatory molecules. The engineered CAR T-cells may be expanded, for example by co-culture on AaPC in presence of soluble factors, such as IL-2 and IL-21. This expansion may for example be carried out so as to provide memory CAR+ T cells (which may for example be assayed by non-enzymatic digital array and/or multi-panel flow cytometry). In this way, CAR T cells may be provided that have specific cytotoxic activity against antigen-bearing tumors (optionally in conjunction with production of desired chemokines such as interferon-γ). CAR T cells of this kind may for example be used in animal models, for example to treat tumor xenografts.

[0305] In certain embodiments, ACT includes co-transferring CD4+ Thl cells and CD8+ CTLs to induce a synergistic antitumour response (see, e.g., Li et al., Adoptive cell therapy with CD4+ T helper 1 cells and CD8+ cytotoxic T cells enhances complete rejection of an established tumour, leading to generation of endogenous memory responses to non-targeted tumour epitopes. Clin Transl Immunology. 2017 Oct; 6(10): el60).

[0306] In certain embodiments, Thl7 cells are transferred to a subject in need thereof. Thl7 cells have been reported to directly eradicate melanoma tumors in mice to a greater extent than Thl cells (Muranski P, et al., Tumor-specific Thl7-polarized cells eradicate large established melanoma. Blood. 2008 Jul 15; 112(2):362-73; and Martin-Orozco N, et al., T helper 17 cells promote cytotoxic T cell activation in tumor immunity. Immunity. 2009 Nov 20; 3l(5):787-98). Those studies involved an adoptive T cell transfer (ACT) therapy approach, which takes advantage of $CD4^+$ T cells that express a TCR recognizing tyrosinase tumor antigen. Exploitation of the TCR leads to rapid expansion of Thl7 populations to large numbers ex *vivo* for reinfusion into the autologous tumor-bearing hosts.

[0307] In certain embodiments, ACT may include autologous iPSC-based vaccines, such as irradiated iPSCs in autologous anti-tumor vaccines (see e.g., Kooreman, Nigel G. et al., Autologous iPSC-Based Vaccines Elicit Anti-tumor Responses In Vivo, Cell Stem Cell 22, 1-13, 2018, doi.org/l0.l0l6/j.stem.20l8.0l.0l6).

[0308] ETnlike T-cell receptors (TCRs) that are MHC restricted, CARs can potentially bind any cell surface-expressed antigen and can thus be more universally used to treat patients (see Irving et al., Engineering Chimeric Antigen Receptor T-Cells for Racing in Solid Tumors: Don't Forget the Fuel, Front. Immunol., 03 April 2017, doi.org/l0.3389/fimmu.20l7.00267). In certain embodiments, in the absence of endogenous T-cell infiltrate (e.g., due to aberrant antigen processing and presentation), which precludes the use of TIL therapy and immune checkpoint blockade, the transfer of CAR T-cells may be used to treat patients (see, e.g., Hinrichs CS, Rosenberg SA. Exploiting the curative potential of adoptive T-cell therapy for cancer. Immunol Rev (2014) 257(l):56-7l. doi:l0.l l1l/ imr.l2l32).

[0309] Approaches such as the foregoing may be adapted to provide methods of treating and/or increasing survival of a subject having a disease, such as a neoplasia, for example by administering an effective amount of an immunoresponsive cell comprising an antigen recognizing receptor that binds a selected antigen, wherein the binding activates the immunoresponsive cell, thereby treating or preventing the disease (such as a neoplasia, a pathogen infection, an autoimmune disorder, or an allogeneic transplant reaction).

[0310] In certain embodiments, the treatment can be administered after lymphodepleting pretreatment in the form of chemotherapy (typically a combination of cyclophosphamide and

fludarabine) or radiation therapy. Initial studies in ACT had short lived responses and the transferred cells did not persist in vivo for very long (Houot et al., T-cell-based immunotherapy: adoptive cell transfer and checkpoint inhibition. Cancer Immunol Res (2015) 3(10): 1115-22; and Kamta et al., Advancing Cancer Therapy with Present and Emerging Immuno-Oncology Approaches. Front. Oncol. (2017) 7:64). Immune suppressor cells like Tregs and MDSCs may attenuate the activity of transferred cells by outcompeting them for the necessary cytokines. Not being bound by a theory lymphodepleting pretreatment may eliminate the suppressor cells allowing the TILs to persist.

[0311] In one embodiment, the treatment can be administrated into patients undergoing an immunosuppressive treatment (e.g., glucocorticoid treatment). The cells or population of cells, may be made resistant to at least one immunosuppressive agent due to the inactivation of a gene encoding a receptor for such immunosuppressive agent. In certain embodiments, the immunosuppressive treatment provides for the selection and expansion of the immunoresponsive T cells within the patient.

[0312] In certain embodiments, the treatment can be administered before primary treatment (e.g., surgery or radiation therapy) to shrink a tumor before the primary treatment. In another embodiment, the treatment can be administered after primary treatment to remove any remaining cancer cells.

[0313] In certain embodiments, immunometabolic barriers can be targeted therapeutically prior to and/or during ACT to enhance responses to ACT or CAR T-cell therapy and to support endogenous immunity (see, e.g., Irving et al., Engineering Chimeric Antigen Receptor T-Cells for Racing in Solid Tumors: Don't Forget the Fuel, Front. Immunol., 03 April 2017, doi.org/l0.3389/fimmu.20l7.00267).

[0314] The administration of cells or population of cells, such as immune system cells or cell populations, such as more particularly immunoresponsive cells or cell populations, as disclosed herein may be carried out in any convenient manner, including by aerosol inhalation, injection, ingestion, transfusion, implantation or transplantation. The cells or population of cells may be administered to a patient subcutaneously, intradermally, intratumorally, intranodally, intramedullary, intramuscularly, intrathecally, by intravenous or intralymphatic injection, or intraperitoneally. In some embodiments, the disclosed CARs may be delivered or administered

into a cavity formed by the resection of tumor tissue (i.e. intracavity delivery) or directly into a tumor prior to resection (i.e. intratumoral delivery). In one embodiment, the cell compositions of the present invention are preferably administered by intravenous injection.

[0315] The administration of the cells or population of cells can consist of the administration of 10^4- 10^9 cells per kg body weight, preferably 10^5 to 10^6 cells/kg body weight including all integer values of cell numbers within those ranges. Dosing in CAR T cell therapies may for example involve administration of from 10^6 to 10^9 cells/kg, with or without a course of lymphodepletion, for example with cyclophosphamide. The cells or population of cells can be administrated in one or more doses. In another embodiment, the effective amount of cells are administrated as a single dose. In another embodiment, the effective amount of cells are administrated as more than one dose over a period time. Timing of administration is within the judgment of managing physician and depends on the clinical condition of the patient. The cells or population of cells may be obtained from any source, such as a blood bank or a donor. While individual needs vary, determination of optimal ranges of effective amounts of a given cell type for a particular disease or conditions are within the skill of one in the art. An effective amount means an amount which provides a therapeutic or prophylactic benefit. The dosage administrated will be dependent upon the age, health and weight of the recipient, kind of concurrent treatment, if any, frequency of treatment and the nature of the effect desired.

[0316] In another embodiment, the effective amount of cells or composition comprising those cells are administrated parenterally. The administration can be an intravenous administration. The administration can be directly done by injection within a tumor.

[0317] To guard against possible adverse reactions, engineered immunoresponsive cells may be equipped with a transgenic safety switch, in the form of a transgene that renders the cells vulnerable to exposure to a specific signal. For example, the herpes simplex viral thymidine kinase (TK) gene may be used in this way, for example by introduction into allogeneic T lymphocytes used as donor lymphocyte infusions following stem cell transplantation (Greco, et al., Improving the safety of cell therapy with the TK-suicide gene. Front. Pharmacol. 2015; 6: 95). In such cells, administration of a nucleoside prodrug such as ganciclovir or acyclovir causes cell death. Alternative safety switch constructs include inducible caspase 9, for example triggered by administration of a small-molecule dimerizer that brings together two nonfunctional

icasp9 molecules to form the active enzyme. A wide variety of alternative approaches to implementing cellular proliferation controls have been described (see U.S. Patent Publication No. 20130071414; PCT Patent Publication WO201 1146862; PCT Patent Publication WO20 140 11987; PCT Patent Publication WO20 13040371; Zhou et al. BLOOD, 2014, 123/25:3895 - 3905; Di Stasi et al., The New England Journal of Medicine 201 1; 365:1673-1683; Sadelain M, The New England Journal of Medicine 201 1; 365:1735-173; Ramos et al., Stem Cells 28(6): 1107-15 (2010)).

[0318] In a further refinement of adoptive therapies, genome editing may be used to tailor immunoresponsive cells to alternative implementations, for example providing edited CAR T cells (see Poirot et al., 2015, Multiplex genome edited T-cell manufacturing platform for "off-the-shelf adoptive T-cell immunotherapies, Cancer Res 75 (18): 3853; Ren et al., 2017, Multiplex genome editing to generate universal CAR T cells resistant to PD1 inhibition, Clin Cancer Res. 2017 May l;23(9):2255-2266. doi: 10.1 158/1078-0432.CCR-16-1300. Epub 2016 Nov 4; Qasim et al., 2017, Molecular remission of infant B-ALL after infusion of universal TALEN gene-edited CAR T cells, Sci Transl Med. 2017 Jan 25;9(374); Legut, et al., 2018, CRISPR-mediated TCR replacement generates superior anticancer transgenic T cells. Blood, 131(3), 311-322; and Georgiadis et al., Long Terminal Repeat CRISPR-CAR-Coupled "Universal" T Cells Mediate Potent Anti-leukemic Effects, Molecular Therapy, In Press, Corrected Proof, Available online 6 March 2018). Cells may be edited using any CRISPR system and method of use thereof as described herein. CRISPR systems may be delivered to an immune cell by any method described herein. In preferred embodiments, cells are edited ex vivo and transferred to a subject in need thereof. Immunoresponsive cells, CAR T cells or any cells used for adoptive cell transfer may be edited. Editing may be performed for example to insert or knock-in an exogenous gene, such as an exogenous gene encoding a CAR or a TCR, at a preselected locus in a cell (e.g. TRAC locus); to eliminate potential alloreactive T-cell receptors (TCR) or to prevent inappropriate pairing between endogenous and exogenous TCR chains, such as to knock-out or knock-down expression of an endogenous TCR in a cell; to disrupt the target of a chemotherapeutic agent in a cell; to block an immune checkpoint, such as to knock-out or knock-down expression of an immune checkpoint protein or receptor in a cell; to knock-out or knock-down expression of other gene or genes in a cell, the reduced expression or lack of

expression of which can enhance the efficacy of adoptive therapies using the cell; to knock-out or knock-down expression of an endogenous gene in a cell, said endogenous gene encoding an antigen targeted by an exogenous CAR or TCR; to knock-out or knock-down expression of one or more MHC constituent proteins in a cell; to activate a T cell; to modulate cells such that the cells are resistant to exhaustion or dysfunction; and/or increase the differentiation and/or proliferation of functionally exhausted or dysfunctional CD8+ T-cells (see PCT Patent Publications: WO2013 176915, WO2014059173, WO2014172606, WO2014184744, and WO2014191 128).

[0319] In certain embodiments, editing may result in inactivation of a gene. By inactivating a gene, it is intended that the gene of interest is not expressed in a functional protein form. In a particular embodiment, the CRISPR system specifically catalyzes cleavage in one targeted gene thereby inactivating said targeted gene. The nucleic acid strand breaks caused are commonly repaired through the distinct mechanisms of homologous recombination or non-homologous end joining (NHEJ). However, NHEJ is an imperfect repair process that often results in changes to the DNA sequence at the site of the cleavage. Repair via non-homologous end joining (NHEJ) often results in small insertions or deletions (Indel) and can be used for the creation of specific gene knockouts. Cells in which a cleavage induced mutagenesis event has occurred can be identified and/or selected by well-known methods in the art. In certain embodiments, homology directed repair (HDR) is used to concurrently inactivate a gene (e.g., TRAC) and insert an endogenous TCR or CAR into the inactivated locus.

[0320] Hence, in certain embodiments, editing of cells (such as by CRISPR/Cas), particularly cells intended for adoptive cell therapies, more particularly immunoresponsive cells such as T cells, may be performed to insert or knock-in an exogenous gene, such as an exogenous gene encoding a CAR or a TCR, at a preselected locus in a cell. Conventionally, nucleic acid molecules encoding CARs or TCRs are transfected or transduced to cells using randomly integrating vectors, which, depending on the site of integration, may lead to clonal expansion, oncogenic transformation, variegated transgene expression and/or transcriptional silencing of the transgene. Directing of transgene(s) to a specific locus in a cell can minimize or avoid such risks and advantageously provide for uniform expression of the transgene(s) by the cells. Without limitation, suitable ‘safe harbor’ loci for directed transgene integration include

CCR5 or AAVS1. Homology-directed repair (HDR) strategies are known and described elsewhere in this specification allowing to insert transgenes into desired loci (e.g., TRAC locus).

[0321] Further suitable loci for insertion of transgenes, in particular CAR or exogenous TCR transgenes, include without limitation loci comprising genes coding for constituents of endogenous T-cell receptor, such as T-cell receptor alpha locus (TRA) or T-cell receptor beta locus (TRB), for example T-cell receptor alpha constant (TRAC) locus, T-cell receptor beta constant 1 (TRBC1) locus or T-cell receptor beta constant 2 (TRBC1) locus. Advantageously, insertion of a transgene into such locus can simultaneously achieve expression of the transgene, potentially controlled by the endogenous promoter, and knock-out expression of the endogenous TCR. This approach has been exemplified in Eyquem et al., (2017) Nature 543: 113-1 17, wherein the authors used CRISPR/Cas9 gene editing to knock-in a DNA molecule encoding a CDl9-specific CAR into the TRAC locus downstream of the endogenous promoter; the CAR-T cells obtained by CRISPR were significantly superior in terms of reduced tonic CAR signaling and exhaustion.

[0322] T cell receptors (TCR) are cell surface receptors that participate in the activation of T cells in response to the presentation of antigen. The TCR is generally made from two chains, a and β, which assemble to form a heterodimer and associates with the CD3-transducing subunits to form the T cell receptor complex present on the cell surface. Each a and β chain of the TCR consists of an immunoglobulin-like N-terminal variable (V) and constant (C) region, a hydrophobic transmembrane domain, and a short cytoplasmic region. As for immunoglobulin molecules, the variable region of the a and β chains are generated by V(D)J recombination, creating a large diversity of antigen specificities within the population of T cells. However, in contrast to immunoglobulins that recognize intact antigen, T cells are activated by processed peptide fragments in association with an MHC molecule, introducing an extra dimension to antigen recognition by T cells, known as MHC restriction. Recognition of MHC disparities between the donor and recipient through the T cell receptor leads to T cell proliferation and the potential development of graft versus host disease (GVHD). The inactivation of TCRa or TCRβ can result in the elimination of the TCR from the surface of T cells preventing recognition of alloantigen and thus GVHD. However, TCR disruption generally results in the elimination of the CD3 signaling component and alters the means of further T cell expansion.

[0323] Hence, in certain embodiments, editing of cells (such as by CRISPR/Cas), particularly cells intended for adoptive cell therapies, more particularly immunoresponsive cells such as T cells, may be performed to knock-out or knock-down expression of an endogenous TCR in a cell. For example, NHEJ-based or HDR-based gene editing approaches can be employed to disrupt the endogenous TCR alpha and/or beta chain genes. For example, gene editing system or systems, such as CRISPR/Cas system or systems, can be designed to target a sequence found within the TCR beta chain conserved between the beta 1 and beta 2 constant region genes (TRBC1 and TRBC2) and/or to target the constant region of the TCR alpha chain (TRAC) gene.

[0324] Allogeneic cells are rapidly rejected by the host immune system. It has been demonstrated that, allogeneic leukocytes present in non-irradiated blood products will persist for no more than 5 to 6 days (Boni, Muranski et al. 2008 Blood l;l 12(12):4746-54). Thus, to prevent rejection of allogeneic cells, the host's immune system usually has to be suppressed to some extent. However, in the case of adoptive cell transfer the use of immunosuppressive drugs also have a detrimental effect on the introduced therapeutic T cells. Therefore, to effectively use an adoptive immunotherapy approach in these conditions, the introduced cells would need to be resistant to the immunosuppressive treatment. Thus, in a particular embodiment, the present invention further comprises a step of modifying T cells to make them resistant to an immunosuppressive agent, preferably by inactivating at least one gene encoding a target for an immunosuppressive agent. An immunosuppressive agent is an agent that suppresses immune function by one of several mechanisms of action. An immunosuppressive agent can be, but is not limited to a calcineurin inhibitor, a target of rapamycin, an interleukin-2 receptor a-chain blocker, an inhibitor of inosine monophosphate dehydrogenase, an inhibitor of dihydrofolic acid reductase, a corticosteroid or an immunosuppressive antimetabolite. The present invention allows conferring immunosuppressive resistance to T cells for immunotherapy by inactivating the target of the immunosuppressive agent in T cells. As non-limiting examples, targets for an immunosuppressive agent can be a receptor for an immunosuppressive agent such as: CD52, glucocorticoid receptor (GR), a FKBP family gene member and a cyclophilin family gene member.

[0325] In certain embodiments, editing of cells (such as by CRISPR/Cas), particularly cells intended for adoptive cell therapies, more particularly immunoresponsive cells such as T cells, may be performed to block an immune checkpoint, such as to knock-out or knock-down expression of an immune checkpoint protein or receptor in a cell. Immune checkpoints are inhibitory pathways that slow down or stop immune reactions and prevent excessive tissue damage from uncontrolled activity of immune cells. In certain embodiments, the immune checkpoint targeted is the programmed death-l (PD-l or CD279) gene (PDCD1). In other embodiments, the immune checkpoint targeted is cytotoxic T-lymphocyte-associated antigen (CTLA-4). In additional embodiments, the immune checkpoint targeted is another member of the CD28 and CTLA4 Ig superfamily such as BTLA, LAG3, ICOS, PDL1 or KIR. In further additional embodiments, the immune checkpoint targeted is a member of the TNFR superfamily such as CD40, 0X40, CD137, GITR, CD27 or TIM-3.

[0326] Additional immune checkpoints include Src homology 2 domain-containing protein tyrosine phosphatase 1 (SHP-l) (Watson HA, et al., SHP-l: the next checkpoint target for cancer immunotherapy? Biochem Soc Trans. 2016 Apr l5;44(2):356-62). SHP-l is a widely expressed inhibitory protein tyrosine phosphatase (PTP). In T-cells, it is a negative regulator of antigen-dependent activation and proliferation. It is a cytosolic protein, and therefore not amenable to antibody-mediated therapies, but its role in activation and proliferation makes it an attractive target for genetic manipulation in adoptive transfer strategies, such as chimeric antigen receptor (CAR) T cells. Immune checkpoints may also include T cell immunoreceptor with Ig and ITIM domains (TIGIT/Vstm3/WUCAM/VSIG9) and VISTA (Le Mercier I, et al., (2015) Beyond CTLA-4 and PD-l, the generation Z of negative checkpoint regulators. Front. Immunol. 6:418).

[0327] WO2014172606 relates to the use of MT1 and/or MT2 inhibitors to increase proliferation and/or activity of exhausted CD8+ T-cells and to decrease CD8+ T-cell exhaustion (e.g., decrease functionally exhausted or unresponsive CD8+ immune cells). In certain embodiments, metallothioneins are targeted by gene editing in adoptively transferred T cells.

[0328] In certain embodiments, targets of gene editing may be at least one targeted locus involved in the expression of an immune checkpoint protein. Such targets may include, but are not limited to CTLA4, PPP2CA, PPP2CB, PTPN6, PTPN22, PDCD1, ICOS (CD278), PDL1, KIR, LAG3, HAVCR2, BTLA, CD160, TIGIT, CD96, CRTAM, LAIR1, SIGLEC7, SIGLEC9,

CD244 (2B4), TNFRSF10B, TNFRSF10A, CASP8, CASP10, CASP3, CASP6, CASP7, FADD, FAS, TGFBRII, TGFRBRI, SMAD2, SMAD3, SMAD4, SMAD10, SKI, SKIL, TGIF1, IL10RA, IL10RB, HMOX2, IL6R, IL6ST, EIF2AK4, CSK, PAG1, SIT1, FOXP3, PRDM1, BATF, VISTA, GUCY1A2, GUCY1A3, GUCY1B2, GUCY1B3, MT1, MT2, CD40, 0X40, CD137, GITR, CD27, SHP-l, TIM-3, CEACAM-l, CEACAM-3, or CEACAM-5. In preferred embodiments, the gene locus involved in the expression of PD-l or CTLA-4 genes is targeted. In other preferred embodiments, combinations of genes are targeted, such as but not limited to PD-l and TIGIT.

[0329] By means of an example and without limitation, WO2016196388 concerns an engineered T cell comprising (a) a genetically engineered antigen receptor that specifically binds to an antigen, which receptor may be a CAR; and (b) a disrupted gene encoding a PD-L1, an agent for disruption of a gene encoding a PD- Ll, and/or disruption of a gene encoding PD-L1, wherein the disruption of the gene may be mediated by a gene editing nuclease, a zinc finger nuclease (ZFN), CRISPR/Cas9 and/or TALEN. WO2015142675 relates to immune effector cells comprising a CAR in combination with an agent (such as CRISPR, TALEN or ZFN) that increases the efficacy of the immune effector cells in the treatment of cancer, wherein the agent may inhibit an immune inhibitory molecule, such as PD1, PD-L1, CTLA-4, TIM-3, LAG-3, VISTA, BTLA, TIGIT, LAIR1, CD160, 2B4, TGFR beta, CEACAM-l, CEACAM-3, or CEACAM-5. Ren et al., (2017) Clin Cancer Res 23 (9) 2255-2266 performed lentiviral delivery of CAR and electro-transfer of Cas9 mRNA and gRNAs targeting endogenous TCR, β-2 microglobulin (B2M) and PD1 simultaneously, to generate gene-disrupted allogeneic CAR T cells deficient of TCR, ELLA class I molecule and PD1.

[0330] In certain embodiments, cells may be engineered to express a CAR, wherein expression and/or function of methylcytosine dioxygenase genes (TET1, TET2 and/or TET3) in the cells has been reduced or eliminated, such as by CRISPR, ZNF or TALEN (for example, as described in WO20 17049 16).

[0331] In certain embodiments, editing of cells (such as by CRISPR/Cas), particularly cells intended for adoptive cell therapies, more particularly immunoresponsive cells such as T cells, may be performed to knock-out or knock-down expression of an endogenous gene in a cell, said endogenous gene encoding an antigen targeted by an exogenous CAR or TCR, thereby reducing

the likelihood of targeting of the engineered cells. In certain embodiments, the targeted antigen may be one or more antigen selected from the group consisting of CD38, CD138, CS-l, CD33, CD26, CD30, CD53, CD92, CD100, CD148, CD150, CD200, CD261, CD262, CD362, human telomerase reverse transcriptase (hTERT), survivin, mouse double minute 2 homolog (MDM2), cytochrome P450 1B1 (CYP1B), HER2/neu, Wilms' tumor gene 1 (WT1), livin, alphafetoprotein (AFP), carcinoembryonic antigen (CEA), mucin 16 (MUC16), MUC1, prostate-specific membrane antigen (PSMA), p53, cyclin (Dl), B cell maturation antigen (BCMA), transmembrane activator and CAML Interactor (TACI), and B-cell activating factor receptor (BAFF-R) (for example, as described in W0201601 1210 and WO20 1701 1804).

[0332] In certain embodiments, editing of cells (such as by CRISPR/Cas), particularly cells intended for adoptive cell therapies, more particularly immunoresponsive cells such as T cells, may be performed to knock-out or knock-down expression of one or more MHC constituent proteins, such as one or more HLA proteins and/or beta-2 microglobulin (B2M), in a cell, whereby rejection of non-autologous (e.g., allogeneic) cells by the recipient's immune system can be reduced or avoided. In preferred embodiments, one or more HLA class I proteins, such as HLA-A, B and/or C, and/or B2M may be knocked-out or knocked-down. Preferably, B2M may be knocked-out or knocked-down. By means of an example, Ren et al., (2017) Clin Cancer Res 23 (9) 2255-2266 performed lentiviral delivery of CAR and electro-transfer of Cas9 mRNA and gRNAs targeting endogenous TCR, β-2 microglobulin (B2M) and PD1 simultaneously, to generate gene-disrupted allogeneic CAR T cells deficient of TCR, HLA class I molecule and PD1.

[0333] In other embodiments, at least two genes are edited. Pairs of genes may include, but are not limited to PD1 and TCRa, PD1 and TCRP, CTLA-4 and TCRa, CTLA-4 and TCRP, LAG3 and TCRa, LAG3 and TCRp, Tim3 and TCRa, Tim3 and TCRp, BTLA and TCRa, BTLA and TCRp, BY55 and TCRa, BY55 and TCRp, TIGIT and TCRa, TIGIT and TCRp, B7H5 and TCRa, B7H5 and TCRp, LAIR1 and TCRa, LAIR1 and TCRp, SIGLEC10 and TCRa, SIGLEC10 and TCRp, 2B4 and TCRa, 2B4 and TCRp, B2M and TCRa, B2M and TCRp.

[0334] In certain embodiments, a cell may be multiply edited (multiplex genome editing) as taught herein to (1) knock-out or knock-down expression of an endogenous TCR (for example,

TRBC1, TRBC2 and/or TRAC), (2) knock-out or knock-down expression of an immune checkpoint protein or receptor (for example PD1, PD-L1 and/or CTLA4); and (3) knock-out or knock-down expression of one or more MHC constituent proteins (for example, HLA-A, B and/or C, and/or B2M, preferably B2M).

[0335] Whether prior to or after genetic modification of the T cells, the T cells can be activated and expanded generally using methods as described, for example, in U.S. Patents 6,352,694; 6,534,055; 6,905,680; 5,858,358; 6,887,466; 6,905,681; 7,144,575; 7,232,566; 7,175,843; 5,883,223; 6,905,874; 6,797,514; 6,867,041; and 7,572,631. T cells can be expanded in vitro or in vivo.

[0336] Immune cells may be obtained using any method known in the art. In one embodiment, allogenic T cells may be obtained from healthy subjects. In one embodiment T cells that have infiltrated a tumor are isolated. T cells may be removed during surgery. T cells may be isolated after removal of tumor tissue by biopsy. T cells may be isolated by any means known in the art. In one embodiment, T cells are obtained by apheresis. In one embodiment, the method may comprise obtaining a bulk population of T cells from a tumor sample by any suitable method known in the art. For example, a bulk population of T cells can be obtained from a tumor sample by dissociating the tumor sample into a cell suspension from which specific cell populations can be selected. Suitable methods of obtaining a bulk population of T cells may include, but are not limited to, any one or more of mechanically dissociating (e.g., mincing) the tumor, enzymatically dissociating (e.g., digesting) the tumor, and aspiration (e.g., as with a needle).

[0337] The bulk population of T cells obtained from a tumor sample may comprise any suitable type of T cell. Preferably, the bulk population of T cells obtained from a tumor sample comprises tumor infiltrating lymphocytes (TILs).

[0338] The tumor sample may be obtained from any mammal. Unless stated otherwise, as used herein, the term "mammal" refers to any mammal including, but not limited to, mammals of the order Logomorpha, such as rabbits; the order Carnivora, including Felines (cats) and Canines (dogs); the order Artiodactyla, including Bovines (cows) and Swines (pigs); or of the order Perssodactyla, including Equines (horses). The mammals may be non-human primates, e.g., of the order Primates, Ceboids, or Simoids (monkeys) or of the order Anthropoids (humans and

apes). In some embodiments, the mammal may be a mammal of the order Rodentia, such as mice and hamsters. Preferably, the mammal is a non-human primate or a human. An especially preferred mammal is the human.

[0339] T cells can be obtained from a number of sources, including peripheral blood mononuclear cells (PBMC), bone marrow, lymph node tissue, spleen tissue, and tumors. In certain embodiments of the present invention, T cells can be obtained from a unit of blood collected from a subject using any number of techniques known to the skilled artisan, such as Ficoll separation. In one preferred embodiment, cells from the circulating blood of an individual are obtained by apheresis or leukapheresis. The apheresis product typically contains lymphocytes, including T cells, monocytes, granulocytes, B cells, other nucleated white blood cells, red blood cells, and platelets. In one embodiment, the cells collected by apheresis may be washed to remove the plasma fraction and to place the cells in an appropriate buffer or media for subsequent processing steps. In one embodiment of the invention, the cells are washed with phosphate buffered saline (PBS). In an alternative embodiment, the wash solution lacks calcium and may lack magnesium or may lack many if not all divalent cations. Initial activation steps in the absence of calcium lead to magnified activation. As those of ordinary skill in the art would readily appreciate a washing step may be accomplished by methods known to those in the art, such as by using a semi-automated "flow-through" centrifuge (for example, the Cobe 2991 cell processor) according to the manufacturer's instructions. After washing, the cells may be resuspended in a variety of biocompatible buffers, such as, for example, Ca-free, Mg-free PBS. Alternatively, the undesirable components of the apheresis sample may be removed and the cells directly resuspended in culture media.

[0340] In another embodiment, T cells are isolated from peripheral blood lymphocytes by lysing the red blood cells and depleting the monocytes, for example, by centrifugation through a PERCOLL™ gradient. A specific subpopulation of T cells, such as CD28+, CD4+, CDC, CD45RA+, and CD45RO+ T cells, can be further isolated by positive or negative selection techniques. For example, in one preferred embodiment, T cells are isolated by incubation with anti-CD3/anti-CD28 (i.e., 3><28)-conjugated beads, such as DYNABEADS® M-450 CD3/CD28 T, or XCYTE DYNABEADS™ for a time period sufficient for positive selection of the desired T cells. In one embodiment, the time period is about 30 minutes. In a further embodiment, the

time period ranges from 30 minutes to 36 hours or longer and all integer values there between. In a further embodiment, the time period is at least 1, 2, 3, 4, 5, or 6 hours. In yet another preferred embodiment, the time period is 10 to 24 hours. In one preferred embodiment, the incubation time period is 24 hours. For isolation of T cells from patients with leukemia, use of longer incubation times, such as 24 hours, can increase cell yield. Longer incubation times may be used to isolate T cells in any situation where there are few T cells as compared to other cell types, such in isolating tumor infiltrating lymphocytes (TIL) from tumor tissue or from immunocompromised individuals. Further, use of longer incubation times can increase the efficiency of capture of CD8+ T cells.

[0341] Enrichment of a T cell population by negative selection can be accomplished with a combination of antibodies directed to surface markers unique to the negatively selected cells. A preferred method is cell sorting and/or selection via negative magnetic immunoadherence or flow cytometry that uses a cocktail of monoclonal antibodies directed to cell surface markers present on the cells negatively selected. For example, to enrich for CD4+ cells by negative selection, a monoclonal antibody cocktail typically includes antibodies to CD14, CD20, CDl lb, CD16, HLA-DR, and CD8.

[0342] Further, monocyte populations (i.e., CD14+ cells) may be depleted from blood preparations by a variety of methodologies, including anti-CD14 coated beads or columns, or utilization of the phagocytotic activity of these cells to facilitate removal. Accordingly, in one embodiment, the invention uses paramagnetic particles of a size sufficient to be engulfed by phagocytotic monocytes. In certain embodiments, the paramagnetic particles are commercially available beads, for example, those produced by Life Technologies under the trade name Dynabeads™. In one embodiment, other non-specific cells are removed by coating the paramagnetic particles with “irrelevant” proteins (e.g., serum proteins or antibodies). Irrelevant proteins and antibodies include those proteins and antibodies or fragments thereof that do not specifically target the T cells to be isolated. In certain embodiments, the irrelevant beads include beads coated with sheep anti-mouse antibodies, goat anti-mouse antibodies, and human serum albumin.

[0343] In brief, such depletion of monocytes is performed by preincubating T cells isolated from whole blood, apheresed peripheral blood, or tumors with one or more varieties of irrelevant

or non-antibody coupled paramagnetic particles at any amount that allows for removal of monocytes (approximately a 20:1 beadxell ratio) for about 30 minutes to 2 hours at 22 to 37 degrees C., followed by magnetic removal of cells which have attached to or engulfed the paramagnetic particles. Such separation can be performed using standard methods available in the art. For example, any magnetic separation methodology may be used including a variety of which are commercially available, (e.g., DYNAL® Magnetic Particle Concentrator (DYNAL MPC®)). Assurance of requisite depletion can be monitored by a variety of methodologies known to those of ordinary skill in the art, including flow cytometric analysis of CD14 positive cells, before and after depletion.

[0344] For isolation of a desired population of cells by positive or negative selection, the concentration of cells and surface (e.g., particles such as beads) can be varied. In certain embodiments, it may be desirable to significantly decrease the volume in which beads and cells are mixed together (i.e., increase the concentration of cells), to ensure maximum contact of cells and beads. For example, in one embodiment, a concentration of 2 billion cells/ml is used. In one embodiment, a concentration of 1 billion cells/ml is used. In a further embodiment, greater than 100 million cells/ml is used. In a further embodiment, a concentration of cells of 10, 15, 20, 25, 30, 35, 40, 45, or 50 million cells/ml is used. In yet another embodiment, a concentration of cells from 75, 80, 85, 90, 95, or 100 million cells/ml is used. In further embodiments, concentrations of 125 or 150 million cells/ml can be used. Using high concentrations can result in increased cell yield, cell activation, and cell expansion. Further, use of high cell concentrations allows more efficient capture of cells that may weakly express target antigens of interest, such as CD28-negative T cells, or from samples where there are many tumor cells present (i.e., leukemic blood, tumor tissue, etc). Such populations of cells may have therapeutic value and would be desirable to obtain. For example, using high concentration of cells allows more efficient selection of CD8+ T cells that normally have weaker CD28 expression.

[0345] In a related embodiment, it may be desirable to use lower concentrations of cells. By significantly diluting the mixture of T cells and surface (e.g., particles such as beads), interactions between the particles and cells is minimized. This selects for cells that express high amounts of desired antigens to be bound to the particles. For example, CD4+ T cells express higher levels of CD28 and are more efficiently captured than CD8+ T cells in dilute

concentrations. In one embodiment, the concentration of cells used is 5><l0^6/ml. In other embodiments, the concentration used can be from about l×10^5/ml to lxlOVml, and any integer value in between.

[0346] T cells can also be frozen. Wishing not to be bound by theory, the freeze and subsequent thaw step provides a more uniform product by removing granulocytes and to some extent monocytes in the cell population. After a washing step to remove plasma and platelets, the cells may be suspended in a freezing solution. While many freezing solutions and parameters are known in the art and will be useful in this context, one method involves using PBS containing 20% DMSO and 8% human serum albumin, or other suitable cell freezing media, the cells then are frozen to -80° C at a rate of 1° per minute and stored in the vapor phase of a liquid nitrogen storage tank. Other methods of controlled freezing may be used as well as uncontrolled freezing immediately at -20° C. or in liquid nitrogen.

[0347] T cells for use in the present invention may also be antigen-specific T cells. For example, tumor-specific T cells can be used. In certain embodiments, antigen-specific T cells can be isolated from a patient of interest, such as a patient afflicted with a cancer or an infectious disease. In one embodiment, neoepitopes are determined for a subject and T cells specific to these antigens are isolated. Antigen-specific cells for use in expansion may also be generated in vitro using any number of methods known in the art, for example, as described in U.S. Patent Publication No. US 20040224402 entitled, Generation and Isolation of Antigen-Specific T Cells, or in U.S. Pat. Nos. 6,040,177. Antigen-specific cells for use in the present invention may also be generated using any number of methods known in the art, for example, as described in Current Protocols in Immunology, or Current Protocols in Cell Biology, both published by John Wiley & Sons, Inc., Boston, Mass.

[0348] In a related embodiment, it may be desirable to sort or otherwise positively select (e.g. via magnetic selection) the antigen specific cells prior to or following one or two rounds of expansion. Sorting or positively selecting antigen-specific cells can be carried out using peptide-MHC tetramers (Altman, et al., Science. 1996 Oct. 4; 274(5284):94-6). In another embodiment, the adaptable tetramer technology approach is used (Andersen et al., 2012 Nat Protoc. 7:891-902). Tetramers are limited by the need to utilize predicted binding peptides based on prior hypotheses, and the restriction to specific HLAs. Peptide-MHC tetramers can be generated using

techniques known in the art and can be made with any MHC molecule of interest and any antigen of interest as described herein. Specific epitopes to be used in this context can be identified using numerous assays known in the art. For example, the ability of a polypeptide to bind to MHC class I may be evaluated indirectly by monitoring the ability to promote incorporation of ^{125}I labeled p2-microglobulin ($\beta 2m$) into MHC class I/p2m/peptide heterotrimeric complexes (see Parker et al., J. Immunol. 152:163, 1994).

[0349] In one embodiment cells are directly labeled with an epitope-specific reagent for isolation by flow cytometry followed by characterization of phenotype and TCRs. In one embodiment, T cells are isolated by contacting with T cell specific antibodies. Sorting of antigen-specific T cells, or generally any cells of the present invention, can be carried out using any of a variety of commercially available cell sorters, including, but not limited to, MoFlo sorter (DakoCytomation, Fort Collins, Colo.), FACSAria™, FACSArray™, FACSVantage™, BD™ LSR II, and FACSCalibur™ (BD Biosciences, San Jose, Calif.).

[0350] In a preferred embodiment, the method comprises selecting cells that also express CD3. The method may comprise specifically selecting the cells in any suitable manner. Preferably, the selecting is carried out using flow cytometry. The flow cytometry may be carried out using any suitable method known in the art. The flow cytometry may employ any suitable antibodies and stains. Preferably, the antibody is chosen such that it specifically recognizes and binds to the particular biomarker being selected. For example, the specific selection of CD3, CD8, TIM-3, LAG-3, 4-1BB, or PD-l may be carried out using anti-CD3, anti-CD8, anti-TIM-3, anti-LAG-3, anti-4-lBB, or anti-PD-l antibodies, respectively. The antibody or antibodies may be conjugated to a bead (e.g., a magnetic bead) or to a fluorochrome. Preferably, the flow cytometry is fluorescence-activated cell sorting (FACS). TCRs expressed on T cells can be selected based on reactivity to autologous tumors. Additionally, T cells that are reactive to tumors can be selected for based on markers using the methods described in patent publication Nos. WO2014133567 and WO2014133568, herein incorporated by reference in their entirety. Additionally, activated T cells can be selected for based on surface expression of CD 107a.

[0351] In one embodiment of the invention, the method further comprises expanding the numbers of T cells in the enriched cell population. Such methods are described in U.S. Patent No. 8,637,307 and is herein incorporated by reference in its entirety. The numbers of T cells may

be increased at least about 3-fold (or 4-, 5-, 6-, 7-, 8-, or 9-fold), more preferably at least about lO-fold (or 20-, 30-, 40-, 50-, 60-, 70-, 80-, or 90-fold), more preferably at least about lOO-fold, more preferably at least about 1,000 fold, or most preferably at least about 100,000-fold. The numbers of T cells may be expanded using any suitable method known in the art. Exemplary methods of expanding the numbers of cells are described in patent publication No. WO 2003057171, U.S. Patent No. 8,034,334, and U.S. Patent Application Publication No. 2012/0244133, each of which is incorporated herein by reference.

[0352] In one embodiment, *ex vivo* T cell expansion can be performed by isolation of T cells and subsequent stimulation or activation followed by further expansion. In one embodiment of the invention, the T cells may be stimulated or activated by a single agent. In another embodiment, T cells are stimulated or activated with two agents, one that induces a primary signal and a second that is a co-stimulatory signal. Ligands useful for stimulating a single signal or stimulating a primary signal and an accessory molecule that stimulates a second signal may be used in soluble form. Ligands may be attached to the surface of a cell, to an Engineered Multivalent Signaling Platform (EMSP), or immobilized on a surface. In a preferred embodiment both primary and secondary agents are co-immobilized on a surface, for example a bead or a cell. In one embodiment, the molecule providing the primary activation signal may be a CD3 ligand, and the co-stimulatory molecule may be a CD28 ligand or 4-1BB ligand.

[0353] In certain embodiments, T cells comprising a CAR or an exogenous TCR, may be manufactured as described in W02015120096, by a method comprising: enriching a population of lymphocytes obtained from a donor subject; stimulating the population of lymphocytes with one or more T-cell stimulating agents to produce a population of activated T cells, wherein the stimulation is performed in a closed system using serum-free culture medium; transducing the population of activated T cells with a viral vector comprising a nucleic acid molecule which encodes the CAR or TCR, using a single cycle transduction to produce a population of transduced T cells, wherein the transduction is performed in a closed system using serum-free culture medium; and expanding the population of transduced T cells for a predetermined time to produce a population of engineered T cells, wherein the expansion is performed in a closed system using serum-free culture medium. In certain embodiments, T cells comprising a CAR or an exogenous TCR, may be manufactured as described in W02015120096, by a method

comprising: obtaining a population of lymphocytes; stimulating the population of lymphocytes with one or more stimulating agents to produce a population of activated T cells, wherein the stimulation is performed in a closed system using serum-free culture medium; transducing the population of activated T cells with a viral vector comprising a nucleic acid molecule which encodes the CAR or TCR, using at least one cycle transduction to produce a population of transduced T cells, wherein the transduction is performed in a closed system using serum-free culture medium; and expanding the population of transduced T cells to produce a population of engineered T cells, wherein the expansion is performed in a closed system using serum-free culture medium. The predetermined time for expanding the population of transduced T cells may be 3 days. The time from enriching the population of lymphocytes to producing the engineered T cells may be 6 days. The closed system may be a closed bag system. Further provided is population of T cells comprising a CAR or an exogenous TCR obtainable or obtained by said method, and a pharmaceutical composition comprising such cells.

[0354] In certain embodiments, T cell maturation or differentiation in vitro may be delayed or inhibited by the method as described in W02017070395, comprising contacting one or more T cells from a subject in need of a T cell therapy with an ART inhibitor (such as, e.g., one or a combination of two or more AKT inhibitors disclosed in claim 8 of W02017070395) and at least one of exogenous Interleukin-7 (IL-7) and exogenous Interleukin-15 (IL-15), wherein the resulting T cells exhibit delayed maturation or differentiation, and/or wherein the resulting T cells exhibit improved T cell function (such as, e.g., increased T cell proliferation; increased cytokine production; and/or increased cytolytic activity) relative to a T cell function of a T cell cultured in the absence of an AKT inhibitor.

[0355] In certain embodiments, a patient in need of a T cell therapy may be conditioned by a method as described in WO2016191756 comprising administering to the patient a dose of cyclophosphamide between 200 mg/m2/day and 2000 mg/m2/day and a dose of fludarabine between 20 mg/m2/day and 900 mg/m^2/day.

[0356] In one embodiment, adoptive cell transfer may comprise: depleting T cells as defined herein from a population of T cells obtained from the subject; *in vitro* expanding the T cell population; and administering the *in vitro* expanded T cell population to the subject. In one embodiment, adoptive cell transfer may comprise: enriching T cells as defined herein from a

population of T cells obtained from the subject; *in vitro* expanding the enriched T cell population; and administering the *in vitro* expanded T cell population to the subject. In certain embodiments, the method may further comprise formulating the in vitro expanded immune cell or immune cell population into a pharmaceutical composition.

[0357] In certain embodiments, suppressive CD8+ T cells are administered in combination with an autoimmune drug. Non-limiting examples of such drugs include methotrexate, cyclophosphamide, Imuran (azathioprine), cyclosporin, and steroid compounds such as prednisone and methylprednisolone.

Cancer

[0358] In certain example embodiments, the pharmaceutical compositions and adoptive cell transfer strategies may be used to treat various forms of cancer. Examples of cancer include but are not limited to carcinoma, lymphoma, blastoma, sarcoma, and leukemia or lymphoid malignancies. More particular examples of such cancers include without limitation: squamous cell cancer (e.g., epithelial squamous cell cancer), lung cancer including small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, squamous carcinoma of the lung and large cell carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastric or stomach cancer including gastrointestinal cancer, pancreatic cancer, glioma, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, rectal cancer, colorectal cancer, endometrial cancer or uterine carcinoma, salivary gland carcinoma, kidney or renal cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, anal carcinoma, penile carcinoma, as well as CNS cancer, melanoma, head and neck cancer, bone cancer, bone marrow cancer, duodenum cancer, oesophageal cancer, thyroid cancer, or hematological cancer.

[0359] Other non-limiting examples of cancers or malignancies include, but are not limited to: Acute Childhood Lymphoblastic Leukemia, Acute Lymphoblastic Leukemia, Acute Lymphocytic Leukemia, Acute Myeloid Leukemia, Adrenocortical Carcinoma, Adult (Primary) Hepatocellular Cancer, Adult (Primary) Liver Cancer, Adult Acute Lymphocytic Leukemia, Adult Acute Myeloid Leukemia, Adult Hodgkin's Disease, Adult Hodgkin's Lymphoma, Adult Lymphocytic Leukemia, Adult Non-Hodgkin's Lymphoma, Adult Primary Liver Cancer, Adult Soft Tissue Sarcoma, AIDS-Related Lymphoma, AIDS-Related Malignancies, Anal Cancer,

Astrocytoma, Bile Duct Cancer, Bladder Cancer, Bone Cancer, Brain Stem Glioma, Brain Tumours, Breast Cancer, Cancer of the Renal Pelvis and Urethra, Central Nervous System (Primary) Lymphoma, Central Nervous System Lymphoma, Cerebellar Astrocytoma, Cerebral Astrocytoma, Cervical Cancer, Childhood (Primary) Hepatocellular Cancer, Childhood (Primary) Liver Cancer, Childhood Acute Lymphoblastic Leukemia, Childhood Acute Myeloid Leukemia, Childhood Brain Stem Glioma, Glioblastoma, Childhood Cerebellar Astrocytoma, Childhood Cerebral Astrocytoma, Childhood Extracranial Germ Cell Tumours, Childhood Hodgkin's Disease, Childhood Hodgkin's Lymphoma, Childhood Hypothalamic and Visual Pathway Glioma, Childhood Lymphoblastic Leukemia, Childhood Medulloblastoma, Childhood Non-Hodgkin's Lymphoma, Childhood Pineal and Supratentorial Primitive Neuroectodermal Tumours, Childhood Primary Liver Cancer, Childhood Rhabdomyosarcoma, Childhood Soft Tissue Sarcoma, Childhood Visual Pathway and Hypothalamic Glioma, Chronic Lymphocytic Leukemia, Chronic Myelogenous Leukemia, Colon Cancer, Cutaneous T-Cell Lymphoma, Endocrine Pancreas Islet Cell Carcinoma, Endometrial Cancer, Ependymoma, Epithelial Cancer, Esophageal Cancer, Ewing's Sarcoma and Related Tumours, Exocrine Pancreatic Cancer, Extracranial Germ Cell Tumour, Extragonadal Germ Cell Tumour, Extrahepatic Bile Duct Cancer, Eye Cancer, Female Breast Cancer, Gaucher's Disease, Gallbladder Cancer, Gastric Cancer, Gastrointestinal Carcinoid Tumour, Gastrointestinal Tumours, Germ Cell Tumours, Gestational Trophoblastic Tumour, Hairy Cell Leukemia, Head and Neck Cancer, Hepatocellular Cancer, Hodgkin's Disease, Hodgkin's Lymphoma, Hypergammaglobulinemia, Hypopharyngeal Cancer, Intestinal Cancers, Intraocular Melanoma, Islet Cell Carcinoma, Islet Cell Pancreatic Cancer, Kaposi's Sarcoma, Kidney Cancer, Laryngeal Cancer, Lip and Oral Cavity Cancer, Liver Cancer, Lung Cancer, Lymphoproliferative Disorders, Macroglobulinemia, Male Breast Cancer, Malignant Mesothelioma, Malignant Thymoma, Medulloblastoma, Melanoma, Mesothelioma, Metastatic Occult Primary Squamous Neck Cancer, Metastatic Primary Squamous Neck Cancer, Metastatic Squamous Neck Cancer, Multiple Myeloma, Multiple Myeloma/Plasma Cell Neoplasm, Myelodysplastic Syndrome, Myelogenous Leukemia, Myeloid Leukemia, Myeloproliferative Disorders, Nasal Cavity and Paranasal Sinus Cancer, Nasopharyngeal Cancer, Neuroblastoma, Non-Hodgkin's Lymphoma During Pregnancy, Nonmelanoma Skin Cancer, Non-Small Cell Lung Cancer, Occult Primary Metastatic Squamous Neck Cancer,

Oropharyngeal Cancer, Osteo-/Malignant Fibrous Sarcoma, Osteosarcoma/Malignant Fibrous Histiocytoma, Osteosarcoma/Malignant Fibrous Histiocytoma of Bone, Ovarian Epithelial Cancer, Ovarian Germ Cell Tumour, Ovarian Low Malignant Potential Tumour, Pancreatic Cancer, Paraproteinemias, Purpura, Parathyroid Cancer,Penile Cancer, Pheochromocytoma, Pituitary Tumour, Plasma Cell Neoplasm/Multiple Myeloma, Primary Central Nervous System Lymphoma, Primary Liver Cancer, Prostate Cancer, Rectal Cancer, Renal Cell Cancer, Renal Pelvis and Urethra Cancer, Retinoblastoma, Rhabdomyosarcoma, Salivary Gland Cancer, Sarcoidosis Sarcomas, Sezary Syndrome, Skin Cancer, Small Cell Lung Cancer, Small Intestine Cancer, Soft Tissue Sarcoma, Squamous Neck Cancer, Stomach Cancer, Supratentorial Primitive Neuroectodermal and Pineal Tumours, T-Cell Lymphoma, Testicular Cancer, Thymoma, Thyroid Cancer, Transitional Cell Cancer of the Renal Pelvis and Urethra, Transitional Renal Pelvis and Urethra Cancer, Trophoblastic Tumours, Urethra and Renal Pelvis Cell Cancer, Urethral Cancer, Uterine Cancer, Uterine Sarcoma, Vaginal Cancer, Visual Pathway and Hypothalamic Glioma, Vulvar Cancer, Waldenstrom's Macroglobulinemia, or Wilms' Tumour.

[0360] In further examples, any combinations of methods such as discussed herein may be employed.

Autoimmune Diseases

[0361] In certain example embodiments, the pharmaceutical compositions and adoptive cell transfer strategies may be used to treat various autoimmune diseases. As used throughout the present specification, the terms "autoimmune disease" or "autoimmune disorder" used interchangeably refer to a diseases or disorders caused by an immune response against a self-tissue or tissue component (self-antigen) and include a self-antibody response and/or cell-mediated response. The terms encompass organ-specific autoimmune diseases, in which an autoimmune response is directed against a single tissue, as well as non-organ specific autoimmune diseases, in which an autoimmune response is directed against a component present in two or more, several or many organs throughout the body.

[0362] Non-limiting examples of autoimmune diseases include but are not limited to acute disseminated encephalomyelitis (ADEM); Addison's disease; ankylosing spondylitis; antiphospholipid antibody syndrome (APS); aplastic anemia; autoimmune gastritis; autoimmune hepatitis; autoimmune thrombocytopenia; Behget's disease; coeliac disease; dermatomyositis;

diabetes mellitus type I; Goodpasture's syndrome; Graves' disease; Guillain-Barre syndrome (GBS); Hashimoto's disease; idiopathic thrombocytopenic purpura; inflammatory bowel disease (IBD) including Crohn's disease and ulcerative colitis; mixed connective tissue disease; multiple sclerosis (MS); myasthenia gravis; opsoclonus myoclonus syndrome (OMS); optic neuritis; Ord's thyroiditis; pemphigus; pernicious anaemia; polyarteritis nodosa; polymyositis; primary biliary cirrhosis; primary myoxedema; psoriasis; rheumatic fever; rheumatoid arthritis; Reiter's syndrome; scleroderma; Sjogren's syndrome; systemic lupus erythematosus; Takayasu's arteritis; temporal arteritis; vitiligo; warm autoimmune hemolytic anemia; or Wegener's granulomatosis.

Identifying Immunomodulators

[0363] A further aspect of the invention relates to a method for identifying an immunomodulant capable of modulating one or more phenotypic aspects of an immune cell or immune cell population as disclosed herein, comprising: a) applying a candidate immunomodulant to the immune cell or immune cell population; b) detecting modulation of one or more phenotypic aspects of the immune cell or immune cell population by the candidate immunomodulant, thereby identifying the immunomodulant.

[0364] The term "modulate" broadly denotes a qualitative and/or quantitative alteration, change or variation in that which is being modulated. Where modulation can be assessed quantitatively - for example, where modulation comprises or consists of a change in a quantifiable variable such as a quantifiable property of a cell or where a quantifiable variable provides a suitable surrogate for the modulation - modulation specifically encompasses both increase (e.g., activation) or decrease (e.g., inhibition) in the measured variable. The term encompasses any extent of such modulation, e.g., any extent of such increase or decrease, and may more particularly refer to statistically significant increase or decrease in the measured variable. By means of example, modulation may encompass an increase in the value of the measured variable by at least about 10%, e.g., by at least about 20%, preferably by at least about 30%, e.g., by at least about 40%, more preferably by at least about 50%, e.g., by at least about 75%, even more preferably by at least about 100%, e.g., by at least about 150%, 200%, 250%, 300%, 400% or by at least about 500%, compared to a reference situation without said modulation; or modulation may encompass a decrease or reduction in the value of the measured

variable by at least about 10%, e.g., by at least about 20%, by at least about 30%, e.g., by at least about 40%, by at least about 50%, e.g., by at least about 60%, by at least about 70%, e.g., by at least about 80%, by at least about 90%, e.g., by at least about 95%, such as by at least about 96%, 97%, 98%, 99% or even by 100%, compared to a reference situation without said modulation. Preferably, modulation may be specific or selective, hence, one or more desired phenotypic aspects of an immune cell or immune cell population may be modulated without substantially altering other (unintended, undesired) phenotypic aspect(s).

[0365] The term "immunomodulant" broadly encompasses any condition, substance or agent capable of modulating one or more phenotypic aspects of an immune cell or immune cell population as disclosed herein. Such conditions, substances or agents may be of physical, chemical, biochemical and/or biological nature. The term "candidate immunomodulant" refers to any condition, substance or agent that is being examined for the ability to modulate one or more phenotypic aspects of an immune cell or immune cell population as disclosed herein in a method comprising applying the candidate immunomodulant to the immune cell or immune cell population (e.g., exposing the immune cell or immune cell population to the candidate immunomodulant or contacting the immune cell or immune cell population with the candidate immunomodulant) and observing whether the desired modulation takes place.

[0366] Immunomodulants may include any potential class of biologically active conditions, substances or agents, such as for instance antibodies, proteins, peptides, nucleic acids, oligonucleotides, small molecules, or combinations thereof.

[0367] By means of example but without limitation, immunomodulants can include low molecular weight compounds, but may also be larger compounds, or any organic or inorganic molecule effective in the given situation, including modified and unmodified nucleic acids such as antisense nucleic acids, RNAi, such as siRNA or shRNA, CRISPR/Cas systems, peptides, peptidomimetics, receptors, ligands, and antibodies, aptamers, polypeptides, nucleic acid analogues or variants thereof. Examples include an oligomer of nucleic acids, amino acids, or carbohydrates including without limitation proteins, oligonucleotides, ribozymes, DNAzymes, glycoproteins, siRNAs, lipoproteins, aptamers, and modifications and combinations thereof. Agents can be selected from a group comprising: chemicals; small molecules; nucleic acid sequences; nucleic acid analogues; proteins; peptides; aptamers; antibodies; or fragments thereof.

A nucleic acid sequence can be RNA or DNA, and can be single or double stranded, and can be selected from a group comprising; nucleic acid encoding a protein of interest, oligonucleotides, nucleic acid analogues, for example peptide - nucleic acid (PNA), pseudo-complementary PNA (pc-PNA), locked nucleic acid (LNA), modified RNA (mod-RNA), single guide RNA etc. Such nucleic acid sequences include, for example, but are not limited to, nucleic acid sequence encoding proteins, for example that act as transcriptional repressors, antisense molecules, ribozymes, small inhibitory nucleic acid sequences, for example but are not limited to RNAi, shRNAi, siRNA, micro RNAi (mRNAi), antisense oligonucleotides, CRISPR guide RNA, for example that target a CRISPR enzyme to a specific DNA target sequence etc. A protein and/or peptide or fragment thereof can be any protein of interest, for example, but are not limited to: mutated proteins; therapeutic proteins and truncated proteins, wherein the protein is normally absent or expressed at lower levels in the cell. Proteins can also be selected from a group comprising; mutated proteins, genetically engineered proteins, peptides, synthetic peptides, recombinant proteins, chimeric proteins, antibodies, midibodies, minibodies, triabodies, humanized proteins, humanized antibodies, chimeric antibodies, modified proteins and fragments thereof. Alternatively, the agent can be intracellular within the cell as a result of introduction of a nucleic acid sequence into the cell and its transcription resulting in the production of the nucleic acid and/or protein modulator of a gene within the cell. In some embodiments, the agent is any chemical, entity or moiety, including without limitation synthetic and naturally-occurring non-proteinaceous entities. In certain embodiments, the agent is a small molecule having a chemical moiety. Agents can be known to have a desired activity and/or property, or can be selected from a library of diverse compounds.

[0368] In certain embodiments, an immunomodulant may be a hormone, a cytokine, a lymphokine, a growth factor, a chemokine, a cell surface receptor ligand such as a cell surface receptor agonist or antagonist, or a mitogen.

[0369] Non-limiting examples of hormones include growth hormone (GH), adrenocorticotropic hormone (ACTH), dehydroepiandrosterone (DHEA), cortisol, epinephrine, thyroid hormone, estrogen, progesterone, testosterone, or combinations thereof.

[0370] Non-limiting examples of cytokines include lymphokines (e.g., interferon-γ, IL-2, IL-3, IL-4, IL-6, granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon-γ,

leukocyte migration inhibitory factors (T-LIF, B-LIF), lymphotoxin-alpha, macrophage-activating factor (MAF), macrophage migration-inhibitory factor (MIF), neuroleukin, immunologic suppressor factors, transfer factors, or combinations thereof), monokines (e.g., IL-1, TNF-alpha, interferon-a, interferon-β, colony stimulating factors, e.g., CSF2, CSF3, macrophage CSF or GM-CSF, or combinations thereof), chemokines (e.g., beta-thromboglobulin, C chemokines, CC chemokines, CXC chemokines, CX3C chemokines, macrophage inflammatory protein (MIP), or combinations thereof), interleukins (e.g., IL-l, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-l l, IL-12, IL-13, IL-14, IL-15, IL-17, IL-18, IL-l 9, IL-20, IL-21, IL-22, IL-23, IL-24, IL-25, IL-26, IL-27, IL-28, IL-29, IL-30, IL-31, IL-32, IL-33, IL-34, IL-35, IL-36, or combinations thereof), and several related signalling molecules, such as tumour necrosis factor (TNF) and interferons (e.g., interferon-a, interferon-β, interferon-γ, interferon-λ, or combinations thereof).

[0371] Non-limiting examples of growth factors include those of fibroblast growth factor (FGF) family, bone morphogenic protein (BMP) family, platelet derived growth factor (PDGF) family, transforming growth factor beta (TGFbeta) family, nerve growth factor (NGF) family, epidermal growth factor (EGF) family, insulin related growth factor (IGF) family, hepatocyte growth factor (HGF) family, hematopoietic growth factors (HeGFs), platelet-derived endothelial cell growth factor (PD-ECGF), angiopoietin, vascular endothelial growth factor (VEGF) family, glucocorticoids, or combinations thereof.

[0372] Non-limiting examples of mitogens include phytohaemagglutinin (PHA), concanavalin A (conA), lipopolysaccharide (LPS), pokeweed mitogen (PWM), phorbol ester such as phorbol myristate acetate (PMA) with or without ionomycin, or combinations thereof.

[0373] Non-limiting examples of cell surface receptors the ligands of which may act as immunomodulants include Toll-like receptors (TLRs) (e.g., TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLR10, TLR1 1, TLR12 or TLR13), CD80, CD86, CD40, CCR7, or C-type lectin receptors.

Altering Expression Using Immunomodulants

[0374] In certain embodiments, an immunomodulant may alter expression and/or activity of one or more endogenous genes of the CD8+ T cells. The term “altered expression” denotes that the modification of the immune cell alters, i.e., changes or modulates, the expression of the

recited gene(s) or polypeptides(s). The term “altered expression” encompasses any direction and any extent of said alteration. Hence, “altered expression” may reflect qualitative and/or quantitative change(s) of expression, and specifically encompasses both increase (e.g., activation or stimulation) or decrease (e.g., inhibition) of expression.

[0375] In certain embodiments, the present invention provides for gene signature screening. The concept of signature screening was introduced by Stegmaier et al. (Gene expression-based high-throughput screening (GE-HTS) and application to leukemia differentiation. Nature Genet. 36, 257-263 (2004)), who realized that if a gene-expression signature was the proxy for a phenotype of interest, it could be used to find small molecules that effect that phenotype without knowledge of a validated drug target. The signatures of the present may be used to screen for drugs that induce or reduce the signature in immune cells as described herein. The signature may be used for GE-HTS. In certain embodiments, pharmacological screens may be used to identify drugs that selectively reduce or increase activity of immune cells. In certain embodiments, drugs that selectively activate or repress suppressive or activated T cells are used for treatment of a cancer patient or a patient suffering from an autoimmune disease.

[0376] In certain embodiments, cmap can be used to screen for small molecules capable of modulating a signature of the present invention in silico. The Connectivity Map (cmap) is a collection of genome-wide transcriptional expression data from cultured human cells treated with bioactive small molecules and simple pattern-matching algorithms that together enable the discovery of functional connections between drugs, genes and diseases through the transitory feature of common gene-expression changes (see, Lamb et al., The Connectivity Map: ETsing Gene-Expression Signatures to Connect Small Molecules, Genes, and Disease. Science 29 Sep 2006: Vol. 313, Issue 5795, pp. 1929-1935, DOI: 10. H26/science. 1132939; and Lamb, J., The Connectivity Map: a new tool for biomedical research. Nature Reviews Cancer January 2007: Vol. 7, pp. 54-60).

[0377] Any one or more of the several successive molecular mechanisms involved in the expression of a given gene or polypeptide may be targeted by the immune cell modification as intended herein. Without limitation, these may include targeting the gene sequence (e.g., targeting the polypeptide-encoding, non-coding and/or regulatory portions of the gene sequence), the transcription of the gene into RNA, the polyadenylation and where applicable splicing and/or

other post-transcriptional modifications of the RNA into mRNA, the localization of the mRNA into cell cytoplasm, where applicable other post-transcriptional modifications of the mRNA, the translation of the mRNA into a polypeptide chain, where applicable post-translational modifications of the polypeptide, and/or folding of the polypeptide chain into the mature conformation of the polypeptide. For compartmentalized polypeptides, such as secreted polypeptides and transmembrane polypeptides, this may further include targeting trafficking of the polypeptides, i.e., the cellular mechanism by which polypeptides are transported to the appropriate sub-cellular compartment or organelle, membrane, e.g. the plasma membrane, or outside the cell.

[0378] Hence, “altered expression” may particularly denote altered production of the recited gene products by the modified immune cell. As used herein, the term “gene product(s)” includes RNA transcribed from a gene (e.g., mRNA), or a polypeptide encoded by a gene or translated from RNA.

[0379] Also, “altered expression” as intended herein may encompass modulating the activity of one or more endogenous gene products. Accordingly, “altered expression”, “altering expression”, “modulating expression”, or “detecting expression” or similar may be used interchangeably with respectively “altered expression or activity”, “altering expression or activity”, “modulating expression or activity”, or “detecting expression or activity” or similar. As used herein, “modulating” or “to modulate” generally means either reducing or inhibiting the activity of a target or antigen, or alternatively increasing the activity of the target or antigen, as measured using a suitable in vitro, cellular or in vivo assay. In particular, “modulating” or “to modulate” can mean either reducing or inhibiting the (relevant or intended) activity of, or alternatively increasing the (relevant or intended) biological activity of the target or antigen, as measured using a suitable in vitro, cellular or in vivo assay (which will usually depend on the target or antigen involved), by at least 5%, at least 10%, at least 25%, at least 50%, at least 60%, at least 70%, at least 80%, or 90% or more, compared to activity of the target or antigen in the same assay under the same conditions but without the presence of the inhibitor/antagonist agents or activator/agonist agents described herein.

[0380] As will be clear to the skilled person, “modulating” can also involve effecting a change (which can either be an increase or a decrease) in affinity, avidity, specificity and/or

selectivity of a target or antigen, for one or more of its targets compared to the same conditions but without the presence of a modulating agent. Again, this can be determined in any suitable manner and/or using any suitable assay known per se, depending on the target. In particular, an action as an inhibitor/antagonist or activator/agonist can be such that an intended biological or physiological activity is increased or decreased, respectively, by at least 5%, at least 10%, at least 25%, at least 50%, at least 60%, at least 70%, at least 80%, or 90% or more, compared to the biological or physiological activity in the same assay under the same conditions but without the presence of the inhibitor/antagonist agent or activator/agonist agent. Modulating can also involve activating the target or antigen or the mechanism or pathway in which it is involved.

[0381] In certain embodiments, an immunomodulant may be or may result in a genetic modification (e.g., mutation, editing, transgenesis, or combinations thereof) of an immune cell, for example, a genetic perturbation, such as a knock-out (i.e., resulting in a complete absence of expression and/or activity) of one or more endogenous genes / gene products, or a knock-down (i.e., resulting in a partial absence of expression and/or activity) of one or more endogenous genes / gene products, or another type of genetic modification modulating the expression and/or activity of one or more endogenous genes / gene products, or for example, introduction of one or more transgenes, such as one or more transgenes encoding one or more gene products. Such transgene may be suitably operably linked to suitable regulatory sequences, e.g., may be comprised in an expression cassette or an expression vector comprising suitable regulatory sequences, or may be configured to become operably linked to suitable regulatory sequences once inserted into the genetic material (e.g., genome) of the immune cell.

[0382] Any types of mutations achieving the intended effects are contemplated herein. For example, suitable mutations may include deletions, insertions, and/or substitutions. The term “deletion” refers to a mutation wherein one or more nucleotides, typically consecutive nucleotides, of a nucleic acid are removed, i.e., deleted, from the nucleic acid. The term “insertion” refers to a mutation wherein one or more nucleotides, typically consecutive nucleotides, are added, i.e., inserted, into a nucleic acid. The term “substitution” refers to a mutation wherein one or more nucleotides of a nucleic acid are each independently replaced, i.e., substituted, by another nucleotide.

[0383] In certain embodiments, a mutation may introduce a premature in-frame stop codon into the open reading frame (ORF) encoding a gene product. Such premature stop codon may lead to production of a C-terminally truncated form of said polypeptide (this may preferably affect, such as diminish or abolish, some or all biological function(s) of the polypeptide) or, especially when the stop codon is introduced close to (e.g., about 20 or less, or about 10 or less amino acids downstream of) the translation initiation codon of the ORF, the stop codon may effectively abolish the production of the polypeptide. Various ways of introducing a premature in-frame stop codon are apparent to a skilled person. For example but without limitation, a suitable insertion, deletion or substitution of one or more nucleotides in the ORF may introduce the premature in-frame stop codon.

[0384] In other embodiments, a mutation may introduce a frame shift (e.g., +**1** or +2 frame shift) in the ORF encoding a gene product. Typically, such frame shift may lead to a previously out-of-frame stop codon downstream of the mutation becoming an in-frame stop codon. Hence, such frame shift may lead to production of a form of the polypeptide having an alternative C-terminal portion and/or a C-terminally truncated form of said polypeptide (this may preferably affect, such as diminish or abolish, some or all biological function(s) of the polypeptide) or, especially when the mutation is introduced close to (e.g., about 20 or less, or about 10 or less amino acids downstream of) the translation initiation codon of the ORF, the frame shift may effectively abolish the production of the polypeptide. Various ways of introducing a frame shift are apparent to a skilled person. For example but without limitation, a suitable insertion or deletion of one or more (not multiple of 3) nucleotides in the ORF may lead to a frame shift.

[0385] In further embodiments, a mutation may delete at least a portion of the ORF encoding a gene product. Such deletion may lead to production of an N-terminally truncated form, a C-terminally truncated form and/or an internally deleted form of said polypeptide (this may preferably affect, such as diminish or abolish, some or all biological function(s) of the polypeptide). Preferably, the deletion may remove about 20% or more, or about 50% or more of the ORF's nucleotides. Especially when the deletion removes a sizeable portion of the ORF (e.g., about 50% or more, preferably about 60% or more, more preferably about 70% or more, even more preferably about 80% or more, still more preferably about 90% or more of the ORF's

nucleotides) or when the deletion removes the entire ORF, the deletion may effectively abolish the production of the polypeptide. The skilled person can readily introduce such deletions.

[0386] In further embodiments, a mutation may delete at least a portion of a gene promoter, leading to impaired transcription of the gene product.

[0387] In certain other embodiments, a mutation may be a substitution of one or more nucleotides in the ORF encoding a gene product resulting in substitution of one or more amino acids of the polypeptide. Such mutation may typically preserve the production of the polypeptide, and may preferably affect, such as diminish or abolish, some or all biological function(s) of the polypeptide. The skilled person can readily introduce such substitutions.

[0388] In certain preferred embodiments, a mutation may abolish native splicing of a pre-mRNA encoding a gene product. In the absence of native splicing, the pre-mRNA may be degraded, or the pre-mRNA may be alternatively spliced, or the pre-mRNA may be spliced improperly employing latent splice site(s) if available. Hence, such mutation may typically effectively abolish the production of the polypeptide's mRNA and thus the production of the polypeptide. Various ways of interfering with proper splicing are available to a skilled person, such as for example but without limitation, mutations which alter the sequence of one or more sequence elements required for splicing to render them inoperable, or mutations which comprise or consist of a deletion of one or more sequence elements required for splicing. The terms "splicing", "splicing of a gene", "splicing of a pre-mRNA" and similar as used herein are synonymous and have their art-established meaning. By means of additional explanation, splicing denotes the process and means of removing intervening sequences (introns) from pre-mRNA in the process of producing mature mRNA. The reference to splicing particularly aims at native splicing such as occurs under normal physiological conditions. The terms "pre-mRNA" and "transcript" are used herein to denote RNA species that precede mature mRNA, such as in particular a primary RNA transcript and any partially processed forms thereof. Sequence elements required for splicing refer particularly to cis elements in the sequence of pre-mRNA which direct the cellular splicing machinery (spliceosome) towards correct and precise removal of introns from the pre-mRNA. Sequence elements involved in splicing are generally known per se and can be further determined by known techniques including inter alia mutation or deletion analysis. By means of further explanation, "splice donor site" or "5' splice site" generally refer to

a conserved sequence immediately adjacent to an exon-intron boundary at the 5' end of an intron. Commonly, a splice donor site may contain a dinucleotide GU, and may involve a consensus sequence of about 8 bases at about positions +2 to -6. "Splice acceptor site" or "3' splice site" generally refers to a conserved sequence immediately adjacent to an intron-exon boundary at the 3' end of an intron. Commonly, a splice acceptor site may contain a dinucleotide AG, and may involve a consensus sequence of about 16 bases at about positions -14 to +2.

Small Molecules

[0389] In certain embodiments, the one or more modulating agents may be a small molecule. The term "small molecule" refers to compounds, preferably organic compounds, with a size comparable to those organic molecules generally used in pharmaceuticals. The term excludes biological macromolecules (e.g., proteins, peptides, nucleic acids, etc.). Preferred small organic molecules range in size up to about 5000 Da, e.g., up to about 4000, preferably up to 3000 Da, more preferably up to 2000 Da, even more preferably up to about 1000 Da, e.g., up to about 900, 800, 700, 600 or up to about 500 Da. In certain embodiments, the small molecule may act as an antagonist or agonist (e.g., blocking an enzyme active site or activating a receptor by binding to a ligand binding site).

[0390] One type of small molecule applicable to the present invention is a degrader molecule. Proteolysis Targeting Chimera (PROTAC) technology is a rapidly emerging alternative therapeutic strategy with the potential to address many of the challenges currently faced in modern drug development programs. PROTAC technology employs small molecules that recruit target proteins for ubiquitination and removal by the proteasome (see, e.g., Bondeson and Crews, Targeted Protein Degradation by Small Molecules, Annu Rev Pharmacol Toxicol. 2017 Jan 6; 57: 107-123; and Lai et al., Modular PROTAC Design for the Degradation of Oncogenic BCR-ABL Angew Chem Int Ed Engl. 2016 Jan 11; 55(2): 807-810).

Genetic Modifying Agents

[0391] In certain embodiments, the one or more modulating agents may be a genetic modifying agent. The genetic modifying agent may comprise a CRISPR system, a zinc finger nuclease system, a TALE system, a meganuclease or RNAi system.

[0392] In general, a CRISPR-Cas or CRISPR system as used in herein and in documents, such as WO 2014/093622 (PCT/US2013/074667), refers collectively to transcripts and other

elements involved in the expression of or directing the activity of CRISPR-associated ("Cas") genes, including sequences encoding a Cas gene, a tracr (trans-activating CRISPR) sequence (e.g. tracrRNA or an active partial tracrRNA), a tracr-mate sequence (encompassing a "direct repeat" and a tracrRNA-processed partial direct repeat in the context of an endogenous CRISPR system), a guide sequence (also referred to as a "spacer" in the context of an endogenous CRISPR system), or "RNA(s)" as that term is herein used (e.g., RNA(s) to guide Cas, such as Cas9, e.g. CRISPR RNA and transactivating (tracr) RNA or a single guide RNA (sgRNA) (chimeric RNA)) or other sequences and transcripts from a CRISPR locus. In general, a CRISPR system is characterized by elements that promote the formation of a CRISPR complex at the site of a target sequence (also referred to as a protospacer in the context of an endogenous CRISPR system). See, e.g, Shmakov et al. (2015) "Discovery and Functional Characterization of Diverse Class 2 CRISPR-Cas Systems", Molecular Cell, DOI: dx.doi.org/l0.l0l6/j.molcel. 2015. 10.008.

[0393] In certain embodiments, a protospacer adjacent motif (PAM) or PAM-like motif directs binding of the effector protein complex as disclosed herein to the target locus of interest. In some embodiments, the PAM may be a 5' PAM (i.e., located upstream of the 5' end of the protospacer). In other embodiments, the PAM may be a 3' PAM (i.e., located downstream of the 5' end of the protospacer). The term "PAM" may be used interchangeably with the term "PFS" or "protospacer flanking site" or "protospacer flanking sequence".

[0394] In a preferred embodiment, the CRISPR effector protein may recognize a 3' PAM. In certain embodiments, the CRISPR effector protein may recognize a 3' PAM which is 5'H, wherein H is A, C or U.

[0395] In the context of formation of a CRISPR complex, "target sequence" refers to a sequence to which a guide sequence is designed to have complementarity, where hybridization between a target sequence and a guide sequence promotes the formation of a CRISPR complex. A target sequence may comprise RNA polynucleotides. The term "target RNA" refers to a RNA polynucleotide being or comprising the target sequence. In other words, the target RNA may be a RNA polynucleotide or a part of a RNA polynucleotide to which a part of the gRNA, i.e. the guide sequence, is designed to have complementarity and to which the effector function mediated by the complex comprising CRISPR effector protein and a gRNA is to be directed. In some embodiments, a target sequence is located in the nucleus or cytoplasm of a cell.

[0396] In certain example embodiments, the CRISPR effector protein may be delivered using a nucleic acid molecule encoding the CRISPR effector protein. The nucleic acid molecule encoding a CRISPR effector protein, may advantageously be a codon optimized CRISPR effector protein. An example of a codon optimized sequence, is in this instance a sequence optimized for expression in eukaryote, e.g., humans (i.e. being optimized for expression in humans), or for another eukaryote, animal or mammal as herein discussed; see, e.g., SaCas9 human codon optimized sequence in WO 2014/093622 (PCT/US2013/074667). Whilst this is preferred, it will be appreciated that other examples are possible and codon optimization for a host species other than human, or for codon optimization for specific organs is known. In some embodiments, an enzyme coding sequence encoding a CRISPR effector protein is a codon optimized for expression in particular cells, such as eukaryotic cells. The eukaryotic cells may be those of or derived from a particular organism, such as a plant or a mammal, including but not limited to human, or non-human eukaryote or animal or mammal as herein discussed, e.g., mouse, rat, rabbit, dog, livestock, or non-human mammal or primate. In some embodiments, processes for modifying the germ line genetic identity of human beings and/or processes for modifying the genetic identity of animals which are likely to cause them suffering without any substantial medical benefit to man or animal, and also animals resulting from such processes, may be excluded. In general, codon optimization refers to a process of modifying a nucleic acid sequence for enhanced expression in the host cells of interest by replacing at least one codon (e.g. about or more than about 1, 2, 3, 4, 5, 10, 15, 20, 25, 50, or more codons) of the native sequence with codons that are more frequently or most frequently used in the genes of that host cell while maintaining the native amino acid sequence. Various species exhibit particular bias for certain codons of a particular amino acid. Codon bias (differences in codon usage between organisms) often correlates with the efficiency of translation of messenger RNA (mRNA), which is in turn believed to be dependent on, among other things, the properties of the codons being translated and the availability of particular transfer RNA (tRNA) molecules. The predominance of selected tRNAs in a cell is generally a reflection of the codons used most frequently in peptide synthesis. Accordingly, genes can be tailored for optimal gene expression in a given organism based on codon optimization. Codon usage tables are readily available, for example, at the "Codon Usage Database" available at kazusa.orjp/codon/ and these tables can be adapted in a

number of ways. See Nakamura, Y., et al. “Codon usage tabulated from the international DNA sequence databases: status for the year 2000” Nucl. Acids Res. 28:292 (2000). Computer algorithms for codon optimizing a particular sequence for expression in a particular host cell are also available, such as Gene Forge (Aptagen; Jacobus, PA), are also available. In some embodiments, one or more codons (e.g. 1, 2, 3, 4, 5, 10, 15, 20, 25, 50, or more, or all codons) in a sequence encoding a Cas correspond to the most frequently used codon for a particular amino acid.

[0397] In certain embodiments, the methods as described herein may comprise providing a Cas transgenic cell in which one or more nucleic acids encoding one or more guide RNAs are provided or introduced operably connected in the cell with a regulatory element comprising a promoter of one or more gene of interest. As used herein, the term “Cas transgenic cell” refers to a cell, such as a eukaryotic cell, in which a Cas gene has been genomically integrated. The nature, type, or origin of the cell are not particularly limiting according to the present invention. Also the way the Cas transgene is introduced in the cell may vary and can be any method as is known in the art. In certain embodiments, the Cas transgenic cell is obtained by introducing the Cas transgene in an isolated cell. In certain other embodiments, the Cas transgenic cell is obtained by isolating cells from a Cas transgenic organism. By means of example, and without limitation, the Cas transgenic cell as referred to herein may be derived from a Cas transgenic eukaryote, such as a Cas knock-in eukaryote. Reference is made to WO 2014/093622 (PCT/US13/74667), incorporated herein by reference. Methods of US Patent Publication Nos. 20120017290 and 201 10265198 assigned to Sangamo BioSciences, Inc. directed to targeting the Rosa locus may be modified to utilize the CRISPR Cas system of the present invention. Methods of US Patent Publication No. 20130236946 assigned to Cellectis directed to targeting the Rosa locus may also be modified to utilize the CRISPR Cas system of the present invention. By means of further example reference is made to Platt et. al. (Cell; 159(2):440-455 (2014)), describing a Cas9 knock-in mouse, which is incorporated herein by reference. The Cas transgene can further comprise a Lox-Stop-polyA-Lox(LSL) cassette thereby rendering Cas expression inducible by Cre recombinase. Alternatively, the Cas transgenic cell may be obtained by introducing the Cas transgene in an isolated cell. Delivery systems for transgenes are well known in the art. By means of example, the Cas transgene may be delivered in for instance eukaryotic cell by means

of vector (e.g., AAV, adenovirus, lentivirus) and/or particle and/or nanoparticle delivery, as also described herein elsewhere.

[0398] It will be understood by the skilled person that the cell, such as the Cas transgenic cell, as referred to herein may comprise further genomic alterations besides having an integrated Cas gene or the mutations arising from the sequence specific action of Cas when complexed with RNA capable of guiding Cas to a target locus, such as for instance one or more oncogenic mutations, as for instance and without limitation described in Platt et al. (2014), Chen et al., (2014) or Kumar et al. (2009).

[0399] In some embodiments, the Cas sequence is fused to one or more nuclear localization sequences (NLSs), such as about or more than about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more NLSs. In some embodiments, the Cas comprises about or more than about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more NLSs at or near the amino-terminus, about or more than about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more NLSs at or near the carboxy-terminus, or a combination of these (e.g. zero or at least one or more NLS at the amino-terminus and zero or at one or more NLS at the carboxy terminus). When more than one NLS is present, each may be selected independently of the others, such that a single NLS may be present in more than one copy and/or in combination with one or more other NLSs present in one or more copies. In a preferred embodiment of the invention, the Cas comprises at most 6 NLSs. In some embodiments, an NLS is considered near the N- or C-terminus when the nearest amino acid of the NLS is within about 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 40, 50, or more amino acids along the polypeptide chain from the N- or C-terminus. Non-limiting examples of NLSs include an NLS sequence derived from: the NLS of the SV40 virus large T-antigen, having the amino acid sequence PKKKRKV(SEQ ID NO: 5); the NLS from nucleoplasmin (e.g. the nucleoplasmin bipartite NLS with the sequence KRPAATKKAGQAKKKK) (SEQ ID NO: 6); the c-myc NLS having the amino acid sequence PAAKRVKLD (SEQ ID NO: 7) or RQRRNELKRSP(SEQ ID NO: 8); the hRNPAl M9 NLS having the sequence NQSSNFGPMKGGNF GGRS SGPYGGGGQYFAKPRNQGGY (SEQ ID NO: 9); the sequence RMRIZFKNKGKDT AELRRRRVE VSVELRK AKKDEQILKRRNV (SEQ ID NO: 10) of the IBB domain from importin-alpha; the sequences VSRKRPRP (SEQ ID NO: 11) and PPKKARED (SEQ ID NO: 12) of the myoma T protein; the sequence POPKKKPL (SEQ ID NO: 13) of human p53; the sequence SALIKKKKKMAP (SEQ ID NO: 14) of mouse

c-abl IV; the sequences DRLRR (SEQ ID NO: 15) and PKQKKRK (SEQ ID NO: 16) of the influenza virus NS1; the sequence RKLKKKIKKL (SEQ ID NO: 17) of the Hepatitis virus delta antigen; the sequence REKKKFLKRR (SEQ ID NO: 18) of the mouse Mxl protein; the sequence KRKGDEVDGVDEVAKKKSKK (SEQ ID NO: 19) of the human poly(ADP-ribose) polymerase; and the sequence RKCLQAGMNLEARKTKK (SEQ ID NO: 20) of the steroid hormone receptors (human) glucocorticoid. In general, the one or more NLSs are of sufficient strength to drive accumulation of the Cas in a detectable amount in the nucleus of a eukaryotic cell. In general, strength of nuclear localization activity may derive from the number of NLSs in the Cas, the particular NLS(s) used, or a combination of these factors. Detection of accumulation in the nucleus may be performed by any suitable technique. For example, a detectable marker may be fused to the Cas, such that location within a cell may be visualized, such as in combination with a means for detecting the location of the nucleus (e.g. a stain specific for the nucleus such as DAPI). Cell nuclei may also be isolated from cells, the contents of which may then be analyzed by any suitable process for detecting protein, such as immunohistochemistry, Western blot, or enzyme activity assay. Accumulation in the nucleus may also be determined indirectly, such as by an assay for the effect of CRISPR complex formation (e.g. assay for DNA cleavage or mutation at the target sequence, or assay for altered gene expression activity affected by CRISPR complex formation and/or Cas enzyme activity), as compared to a control no exposed to the Cas or complex, or exposed to a Cas lacking the one or more NLSs.

[0400] In certain aspects, the invention involves vectors, e.g. for delivering or introducing in a cell Cas and/or RNA capable of guiding Cas to a target locus (i.e. guide RNA), but also for propagating these components (e.g. in prokaryotic cells). A used herein, a “vector” is a tool that allows or facilitates the transfer of an entity from one environment to another. It is a replicon, such as a plasmid, phage, or cosmid, into which another DNA segment may be inserted so as to bring about the replication of the inserted segment. Generally, a vector is capable of replication when associated with the proper control elements. In general, the term “vector” refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. Vectors include, but are not limited to, nucleic acid molecules that are single-stranded, double-stranded, or partially double-stranded; nucleic acid molecules that comprise one or more free ends, no free ends (e.g. circular); nucleic acid molecules that comprise DNA, RNA, or both; and

other varieties of polynucleotides known in the art. One type of vector is a “plasmid,” which refers to a circular double stranded DNA loop into which additional DNA segments can be inserted, such as by standard molecular cloning techniques. Another type of vector is a viral vector, wherein virally-derived DNA or RNA sequences are present in the vector for packaging into a virus (e.g. retroviruses, replication defective retroviruses, adenoviruses, replication defective adenoviruses, and adeno-associated viruses (AAVs)). Viral vectors also include polynucleotides carried by a virus for transfection into a host cell. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g. bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively-linked. Such vectors are referred to herein as “expression vectors.” Common expression vectors of utility in recombinant DNA techniques are often in the form of plasmids.

[0401] Recombinant expression vectors can comprise a nucleic acid of the invention in a form suitable for expression of the nucleic acid in a host cell, which means that the recombinant expression vectors include one or more regulatory elements, which may be selected on the basis of the host cells to be used for expression, that is operatively-linked to the nucleic acid sequence to be expressed. Within a recombinant expression vector, “operably linked” is intended to mean that the nucleotide sequence of interest is linked to the regulatory element(s) in a manner that allows for expression of the nucleotide sequence (e.g. in an in vitro transcription/translation system or in a host cell when the vector is introduced into the host cell). With regards to recombination and cloning methods, mention is made of U.S. patent application 10/815,730, published September 2, 2004 as US 2004-0171 156 Al, the contents of which are herein incorporated by reference in their entirety. Thus, the embodiments disclosed herein may also comprise transgenic cells comprising the CRISPR effector system. In certain example embodiments, the transgenic cell may function as an individual discrete volume. In other words samples comprising a masking construct may be delivered to a cell, for example in a suitable delivery vesicle and if the target is present in the delivery vesicle the CRISPR effector is activated and a detectable signal generated.

[0402] The vector(s) can include the regulatory element(s), e.g., promoter(s). The vector(s) can comprise Cas encoding sequences, and/or a single, but possibly also can comprise at least 3 or 8 or 16 or 32 or 48 or 50 guide RNA(s) (e.g., sgRNAs) encoding sequences, such as 1-2, 1-3, 1-4 1-5, 3-6, 3-7, 3-8, 3-9, 3-10, 3-8, 3-16, 3-30, 3-32, 3-48, 3-50 RNA(s) (e.g., sgRNAs). In a single vector there can be a promoter for each RNA (e.g., sgRNA), advantageously when there are up to about 16 RNA(s); and, when a single vector provides for more than 16 RNA(s), one or more promoter(s) can drive expression of more than one of the RNA(s), e.g., when there are 32 RNA(s), each promoter can drive expression of two RNA(s), and when there are 48 RNA(s), each promoter can drive expression of three RNA(s). By simple arithmetic and well established cloning protocols and the teachings in this disclosure one skilled in the art can readily practice the invention as to the RNA(s) for a suitable exemplary vector such as AAV, and a suitable promoter such as the U6 promoter. For example, the packaging limit of AAV is ~4.7 kb. The length of a single U6-gRNA (plus restriction sites for cloning) is 361 bp. Therefore, the skilled person can readily fit about 12-16, e.g., 13 U6-gRNA cassettes in a single vector. This can be assembled by any suitable means, such as a golden gate strategy used for TALE assembly (genome-engineering.org/taleffectors/). The skilled person can also use a tandem guide strategy to increase the number of U6-gRNAs by approximately 1.5 times, e.g., to increase from 12-16, e.g., 13 to approximately 18-24, e.g., about 19 U6-gRNAs. Therefore, one skilled in the art can readily reach approximately 18-24, e.g., about 19 promoter-RNAs, e.g., U6-gRNAs in a single vector, e.g., an AAV vector. A further means for increasing the number of promoters and RNAs in a vector is to use a single promoter (e.g., U6) to express an array of RNAs separated by cleavable sequences. And an even further means for increasing the number of promoter-RNAs in a vector, is to express an array of promoter-RNAs separated by cleavable sequences in the intron of a coding sequence or gene; and, in this instance it is advantageous to use a polymerase II promoter, which can have increased expression and enable the transcription of long RNA in a tissue specific manner. (see, e.g., nar.oxfordjournals.org/content/34/7/e53. short and nature.com/mt/journal/vl6/n9/abs/mt2008l44a.html). In an advantageous embodiment, AAV may package U6 tandem gRNA targeting up to about 50 genes. Accordingly, from the knowledge in the art and the teachings in this disclosure the skilled person can readily make and use vector(s), e.g., a single vector, expressing multiple RNAs or guides under the control or

operatively or functionally linked to one or more promoters—especially as to the numbers of RNAs or guides discussed herein, without any undue experimentation.

[0403] The guide RNA(s) encoding sequences and/or Cas encoding sequences, can be functionally or operatively linked to regulatory element(s) and hence the regulatory element(s) drive expression. The promoter(s) can be constitutive promoter(s) and/or conditional promoter(s) and/or inducible promoter(s) and/or tissue specific promoter(s). The promoter can be selected from the group consisting of RNA polymerases, pol I, pol II, pol III, T7, U6, Hl, retroviral Rous sarcoma virus (RSV) LTR promoter, the cytomegalovirus (CMV) promoter, the SV40 promoter, the dihydrofolate reductase promoter, the β-actin promoter, the phosphoglycerol kinase (PGK) promoter, and the EFla promoter. An advantageous promoter is the promoter is U6.

[0404] Additional effectors for use according to the invention can be identified by their proximity to casl genes, for example, though not limited to, within the region 20 kb from the start of the casl gene and 20 kb from the end of the casl gene. In certain embodiments, the effector protein comprises at least one HEPN domain and at least 500 amino acids, and wherein the C2c2 effector protein is naturally present in a prokaryotic genome within 20 kb upstream or downstream of a Cas gene or a CRISPR array. Non-limiting examples of Cas proteins include Casl, CaslB, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8, Cas9 (also known as Csnl and Csxl2), CaslO, Csyl, Csy2, Csy3, Csel, Cse2, Cscl, Csc2, Csa5, Csn2, Csm2, Csm3, Csm4, Csm5, Csm6, Cmrl, Cmr3, Cmr4, Cmr5, Cmr6, Csbl, Csb2, Csb3, Csxl7, Csxl4, CsxlO, Csxl6, CsaX, Csx3, Csxl, Csxl5, Csfl, Csf2, Csf3, Csf4, homologues thereof, or modified versions thereof. In certain example embodiments, the C2c2 effector protein is naturally present in a prokaryotic genome within 20kb upstream or downstream of a Cas 1 gene. The terms "orthologue" (also referred to as "ortholog" herein) and "homologue" (also referred to as "homolog" herein) are well known in the art. By means of further guidance, a "homologue" of a protein as used herein is a protein of the same species which performs the same or a similar function as the protein it is a homologue of. Homologous proteins may but need not be structurally related, or are only partially structurally related. An "orthologue" of a protein as used herein is a protein of a different species which performs the same or a similar function as the protein it is an orthologue of. Orthologous proteins may but need not be structurally related, or are only partially structurally related.

Guide Molecules

[0405] The methods described herein may be used to screen inhibition of CRISPR systems employing different types of guide molecules. As used herein, the term "guide sequence" and "guide molecule" in the context of a CRISPR-Cas system, comprises any polynucleotide sequence having sufficient complementarity with a target nucleic acid sequence to hybridize with the target nucleic acid sequence and direct sequence-specific binding of a nucleic acid-targeting complex to the target nucleic acid sequence. The guide sequences made using the methods disclosed herein may be a full-length guide sequence, a truncated guide sequence, a full-length sgRNA sequence, a truncated sgRNA sequence, or an E+F sgRNA sequence. In some embodiments, the degree of complementarity of the guide sequence to a given target sequence, when optimally aligned using a suitable alignment algorithm, is about or more than about 50%, 60%, 75%, 80%, 85%, 90%, 95%, 97.5%, 99%, or more. In certain example embodiments, the guide molecule comprises a guide sequence that may be designed to have at least one mismatch with the target sequence, such that a RNA duplex formed between the guide sequence and the target sequence. Accordingly, the degree of complementarity is preferably less than 99%. For instance, where the guide sequence consists of 24 nucleotides, the degree of complementarity is more particularly about 96% or less. In particular embodiments, the guide sequence is designed to have a stretch of two or more adjacent mismatching nucleotides, such that the degree of complementarity over the entire guide sequence is further reduced. For instance, where the guide sequence consists of 24 nucleotides, the degree of complementarity is more particularly about 96% or less, more particularly, about 92% or less, more particularly about 88% or less, more particularly about 84% or less, more particularly about 80% or less, more particularly about 76% or less, more particularly about 72% or less, depending on whether the stretch of two or more mismatching nucleotides encompasses 2, 3, 4, 5, 6 or 7 nucleotides, etc. In some embodiments, aside from the stretch of one or more mismatching nucleotides, the degree of complementarity, when optimally aligned using a suitable alignment algorithm, is about or more than about 50%, 60%, 75%, 80%, 85%, 90%, 95%, 97.5%, 99%, or more. Optimal alignment may be determined with the use of any suitable algorithm for aligning sequences, non-limiting example of which include the Smith-Waterman algorithm, the Needleman-Wunsch algorithm, algorithms based on the Burrows-Wheeler Transform (e.g., the Burrows Wheeler Aligner), ClustalW, Clustal X,

BLAT, Novoalign (Novocraft Technologies; available at www.novocraft.com), ELAND (Illumina, San Diego, CA), SOAP (available at soap.genomics.org.cn), and Maq (available at maq.sourceforge.net). The ability of a guide sequence (within a nucleic acid-targeting guide RNA) to direct sequence-specific binding of a nucleic acid -targeting complex to a target nucleic acid sequence may be assessed by any suitable assay. For example, the components of a nucleic acid-targeting CRISPR system sufficient to form a nucleic acid-targeting complex, including the guide sequence to be tested, may be provided to a host cell having the corresponding target nucleic acid sequence, such as by transfection with vectors encoding the components of the nucleic acid-targeting complex, followed by an assessment of preferential targeting (e.g., cleavage) within the target nucleic acid sequence, such as by Surveyor assay as described herein. Similarly, cleavage of a target nucleic acid sequence (or a sequence in the vicinity thereof) may be evaluated in a test tube by providing the target nucleic acid sequence, components of a nucleic acid-targeting complex, including the guide sequence to be tested and a control guide sequence different from the test guide sequence, and comparing binding or rate of cleavage at or in the vicinity of the target sequence between the test and control guide sequence reactions. Other assays are possible, and will occur to those skilled in the art. A guide sequence, and hence a nucleic acid-targeting guide RNA may be selected to target any target nucleic acid sequence.

[0406] In certain embodiments, the guide sequence or spacer length of the guide molecules is from 15 to 50 nt. In certain embodiments, the spacer length of the guide RNA is at least 15 nucleotides. In certain embodiments, the spacer length is from 15 to 17 nt, e.g., 15, 16, or 17 nt, from 17 to 20 nt, e.g., 17, 18, 19, or 20 nt, from 20 to 24 nt, e.g., 20, 21, 22, 23, or 24 nt, from 23 to 25 nt, e.g., 23, 24, or 25 nt, from 24 to 27 nt, e.g., 24, 25, 26, or 27 nt, from 27-30 nt, e.g., 27, 28, 29, or 30 nt, from 30-35 nt, e.g., 30, 31, 32, 33, 34, or 35 nt, or 35 nt or longer. In certain example embodiment, the guide sequence is 15, 16, 17,18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39 40, 41, 42, 43, 44, 45, 46, 47 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100 nt.

[0407] In some embodiments, the guide sequence is an RNA sequence of between 10 to 50 nt in length, but more particularly of about 20-30 nt advantageously about 20 nt, 23-25 nt or 24 nt. The guide sequence is selected so as to ensure that it hybridizes to the target sequence. This is

described more in detail below. Selection can encompass further steps which increase efficacy and specificity.

[0408] In some embodiments, the guide sequence has a canonical length (e.g., about 15-30 nt) is used to hybridize with the target RNA or DNA. In some embodiments, a guide molecule is longer than the canonical length (e.g., >30 nt) is used to hybridize with the target RNA or DNA, such that a region of the guide sequence hybridizes with a region of the RNA or DNA strand outside of the Cas-guide target complex. This can be of interest where additional modifications, such deamination of nucleotides is of interest. In alternative embodiments, it is of interest to maintain the limitation of the canonical guide sequence length.

[0409] In some embodiments, the sequence of the guide molecule (direct repeat and/or spacer) is selected to reduce the degree secondary structure within the guide molecule. In some embodiments, about or less than about 75%, 50%, 40%, 30%, 25%, 20%, 15%, 10%, 5%, 1%, or fewer of the nucleotides of the nucleic acid-targeting guide RNA participate in self-complementary base pairing when optimally folded. Optimal folding may be determined by any suitable polynucleotide folding algorithm. Some programs are based on calculating the minimal Gibbs free energy. An example of one such algorithm is rnFold, as described by Zuker and Stiegler (Nucleic Acids Res. 9 (1981), 133-148). Another example folding algorithm is the online Webserver RNAfold, developed at Institute for Theoretical Chemistry at the University of Vienna, using the centroid structure prediction algorithm (see e.g., A.R. Gruber et al., 2008, Cell 106(1): 23-24; and PA Carr and GM Church, 2009, Nature Biotechnology 27(12): 1151-62).

[0410] In some embodiments, it is of interest to reduce the susceptibility of the guide molecule to RNA cleavage, such as to cleavage by Casl3. Accordingly, in particular embodiments, the guide molecule is adjusted to avoide cleavage by Casl3 or other RNA-cleaving enzymes.

[0411] In certain embodiments, the guide molecule comprises non-naturally occurring nucleic acids and/or non-naturally occurring nucleotides and/or nucleotide analogs, and/or chemically modifications. Preferably, these non-naturally occurring nucleic acids and non-naturally occurring nucleotides are located outside the guide sequence. Non-naturally occurring nucleic acids can include, for example, mixtures of naturally and non-naturally occurring nucleotides. Non-naturally occurring nucleotides and/or nucleotide analogs may be modified at

the ribose, phosphate, and/or base moiety. In an embodiment of the invention, a guide nucleic acid comprises ribonucleotides and non-ribonucleotides. In one such embodiment, a guide comprises one or more ribonucleotides and one or more deoxyribonucleotides. In an embodiment of the invention, the guide comprises one or more non-naturally occurring nucleotide or nucleotide analog such as a nucleotide with phosphorothioate linkage, a locked nucleic acid (LNA) nucleotides comprising a methylene bridge between the 2d/or non-naturally occurring nucleotides and/or nucleotide analogs, and/or chemically modifications. Preferably, these non-naturally occurring nucleic acids and nouoro analogs. Further examples of modified bases include, but are not limited to, 2-aminopurine, 5-bromo-uridine, pseudouridine, inosine, 7-methylguanosine. Examples of guide RNA chemical modifications include, without limitation, incorporation of 2ccurrinhyl (M), 2'-0-methyl 3'phosphorothioate (MS), k-con strained ethyl(cEt), or 2ained ethyl(cEtxamples of guide RNA chemical modifications include, without limitation, incorporation of 2ccurrinhyl (M), 2(M), 2rinhyl (M), 2orothioate (MS), r chemically modifications. Preferably, these non-naturally occurring nucleic acids and no. (See, Hendel, 2015, Nat Biotechnol. 33(9):985-9, doi: l0.l038/nbt.3290, published online 29 June 2015 Ragdarm et al., 0215, *PNAS,* E71 10-E71 11; Allerson et al., *J Med. Chem.* 2005, 48:901-904; Bramsen et al., *Front. Genet.,* 2012, 3:154; Deng et al., *PNAS,* 2015, 112:1 1870-1 1875; Sharma et al., *MedChemComm.,* 2014, 5:1454-1471; Hendel et al., *Nat. Biotechnol.* (2015) 33(9): 985-989; Li et al., *Nature Biomedical Engineering,* 2017, 1, 0066 D01:l0.l038/s4l55l-0l7-0066).

In some embodiments, the 5' and/or 3' end of a guide RNA is modified by a variety of functional moieties including fluorescent dyes, polyethylene glycol, cholesterol, proteins, or detection tags. (See Kelly et al., 2016, *J. Biotech.* 233:74-83). In certain embodiments, a guide comprises ribonucleotides in a region that binds to a target RNA and one or more deoxyribonucletides and/or nucleotide analogs in a region that binds to Casl3. In an embodiment of the invention, deoxyribonucleotides and/or nucleotide analogs are incorporated in engineered guide structures, such as, without limitation, stem-loop regions, and the seed region. For Casl3 guide, in certain embodiments, the modification is not in the 5'-handle of the stem-loop regions. Chemical modification in the 5'-handle of the stem-loop region of a guide may abolish its function (see Li, et al., *Nature Biomedical Engineering,* 2017, 1:0066). In certain embodiments, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35,

40, 45, 50, or 75 nucleotides of a guide is chemically modified. In some embodiments, 3-5 nucleotides at either the 3' or the 5' end of a guide is chemically modified. In some embodiments, only minor modifications are introduced in the seed region, such as 2'-F modifications. In some embodiments, 2'-F modification is introduced at the 3' end of a guide. In certain embodiments, three to five nucleotides at the 5' and/or the 3' end of the guide are chemically modified with 2'-0-methyl (M), 2'-0-methyl 3' phosphorothioate (MS), *S*-constrained ethyl(cEt), or 2'-0-methyl 3' thioPACE (MSP). Such modification can enhance genome editing efficiency (see Hendel et al., *Nat. Biotechnol.* (2015) 33(9): 985-989). In certain embodiments, all of the phosphodiester bonds of a guide are substituted with phosphorothioates (PS) for enhancing levels of gene disruption. In certain embodiments, more than five nucleotides at the 5' and/or the 3' end of the guide are chemically modified with 2'-0-Me, 2'-F or *S*-constrained ethyl(cEt). Such chemically modified guide can mediate enhanced levels of gene disruption (see Ragdarm et al., 0215, *PNAS*, E71 10-E71 11). In an embodiment of the invention, a guide is modified to comprise a chemical moiety at its 3' and/or 5' end. Such moieties include, but are not limited to amine, azide, alkyne, thio, dibenzocyclooctyne (DBCO), or Rhodamine. In certain embodiment, the chemical moiety is conjugated to the guide by a linker, such as an alkyl chain. In certain embodiments, the chemical moiety of the modified guide can be used to attach the guide to another molecule, such as DNA, RNA, protein, or nanoparticles. Such chemically modified guide can be used to identify or enrich cells generically edited by a CRISPR system (see Lee et al., *eLtfe,* 2017, 6:e253l2, DOI: 10.7554).

[0412] In some embodiments, the modification to the guide is a chemical modification, an insertion, a deletion or a split. In some embodiments, the chemical modification includes, but is not limited to, incorporation of 2'-0-methyl (M) analogs, 2'-deoxy analogs, 2-thiouridine analogs, N6-methyladenosine analogs, 2'-fluoro analogs, 2-aminopurine, 5-bromo-uridine, pseudouridine (Ψ), Nl-methylpseudouridine (ιηεΙΨ), 5-methoxyuridine(5moEi), inosine, 7-methylguanosine, 2'-0-methyl 3'phosphorothioate (MS), S-constrained ethyl(cEt), phosphorothioate (PS), or 2'-0-methyl 3'thioPACE (MSP). In some embodiments, the guide comprises one or more of phosphorothioate modifications. In certain embodiments, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or 25 nucleotides of the guide are chemically modified. In certain embodiments, one or more nucleotides in the seed region are

chemically modified. In certain embodiments, one or more nucleotides in the 3'-terminus are chemically modified. In certain embodiments, none of the nucleotides in the 5'-handle is chemically modified. In some embodiments, the chemical modification in the seed region is a minor modification, such as incorporation of a 2'-fluoro analog. In a specific embodiment, one nucleotide of the seed region is replaced with a 2'-fluoro analog. In some embodiments, 5 to 10 nucleotides in the 3'-terminus are chemically modified. Such chemical modifications at the 3'-terminus of the Casl3 CrRNA may improve Casl3 activity. In a specific embodiment, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 nucleotides in the 3'-terminus are replaced with 2'-fluoro analogues. In a specific embodiment, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 nucleotides in the 3'-terminus are replaced with 2'- O-methyl (M) analogs.

[0413] In some embodiments, the loop of the 5'-handle of the guide is modified. In some embodiments, the loop of the 5'-handle of the guide is modified to have a deletion, an insertion, a split, or chemical modifications. In certain embodiments, the modified loop comprises 3, 4, or 5 nucleotides. In certain embodiments, the loop comprises the sequence of UCUU, UUUU, UAUU, or UGUU.

[0414] In some embodiments, the guide molecule forms a stemloop with a separate non-covalently linked sequence, which can be DNA or RNA. In particular embodiments, the sequences forming the guide are first synthesized using the standard phosphoramidite synthetic protocol (Herdewijn, P., ed., Methods in Molecular Biology Col 288, Oligonucleotide Synthesis: Methods and Applications, Humana Press, New Jersey (2012)). In some embodiments, these sequences can be functionalized to contain an appropriate functional group for ligation using the standard protocol known in the art (Hermanson, G. T., Bioconjugate Techniques, Academic Press (2013)). Examples of functional groups include, but are not limited to, hydroxyl, amine, carboxylic acid, carboxylic acid halide, carboxylic acid active ester, aldehyde, carbonyl, chlorocarbonyl, imidazolylcarbonyl, hydrozide, semicarbazide, thio semicarbazide, thiol, maleimide, haloalkyl, sufonyl, ally, propargyl, diene, alkyne, and azide. Once this sequence is functionalized, a covalent chemical bond or linkage can be formed between this sequence and the direct repeat sequence. Examples of chemical bonds include, but are not limited to, those based on carbamates, ethers, esters, amides, imines, amidines, aminotrizines, hydrozone, disulfides, thioethers, thioesters, phosphorothioates, phosphorodithioates, sulfonamides,

sulfonates, fulfones, sulfoxides, ureas, thioureas, hydrazide, oxime, triazole, photolabile linkages, C-C bond forming groups such as Diels-Alder cyclo-addition pairs or ring-closing metathesis pairs, and Michael reaction pairs.

[0415] In some embodiments, these stem-loop forming sequences can be chemically synthesized. In some embodiments, the chemical synthesis uses automated, solid-phase oligonucleotide synthesis machines with 2'-acetoxyethyl orthoester (2'-ACE) (Scaringe et al., J. Am. Chem. Soc. (1998) 120: 11820-1 1821; Scaringe, Methods Enzymol. (2000) 317: 3-18) or 2'-thionocarbamate (2'-TC) chemistry (Dellinger et al., J. Am. Chem. Soc. (201 1) 133: 11540-11546; Hendel et al., Nat. Biotechnol. (2015) 33:985-989).

[0416] In certain embodiments, the guide molecule comprises (1) a guide sequence capable of hybridizing to a target locus and (2) a tracr mate or direct repeat sequence whereby the direct repeat sequence is located upstream (i.e., 5') from the guide sequence. In a particular embodiment the seed sequence (i.e. the sequence essential critical for recognition and/or hybridization to the sequence at the target locus) of th guide sequence is approximately within the first 10 nucleotides of the guide sequence.

[0417] In a particular embodiment the guide molecule comprises a guide sequence linked to a direct repeat sequence, wherein the direct repeat sequence comprises one or more stem loops or optimized secondary structures. In particular embodiments, the direct repeat has a minimum length of 16 nts and a single stem loop. In further embodiments the direct repeat has a length longer than 16 nts, preferably more than 17 nts, and has more than one stem loops or optimized secondary structures. In particular embodiments the guide molecule comprises or consists of the guide sequence linked to all or part of the natural direct repeat sequence. A typical Type V or Type VI CRISPR-cas guide molecule comprises (in 3' to 5' direction or in 5' to 3' direction): a guide sequence a first complimentary stretch (the "repeat"), a loop (which is typically 4 or 5 nucleotides long), a second complimentary stretch (the "anti-repeat" being complimentary to the repeat), and a poly A (often poly E T in RNA) tail (terminator). In certain embodiments, the direct repeat sequence retains its natural architecture and forms a single stem loop. In particular embodiments, certain aspects of the guide architecture can be modified, for example by addition, subtraction, or substitution of features, whereas certain other aspects of guide architecture are maintained. Preferred locations for engineered guide molecule modifications,

including but not limited to insertions, deletions, and substitutions include guide termini and regions of the guide molecule that are exposed when complexed with the CRISPR-Cas protein and/or target, for example the stemloop of the direct repeat sequence.

[0418] In particular embodiments, the stem comprises at least about 4bp comprising complementary X and Y sequences, although stems of more, e.g., 5, 6, 7, 8, 9, 10, 11 or 12 or fewer, e.g., 3, 2, base pairs are also contemplated. Thus, for example X2-10 and Y2-10 (wherein X and Y represent any complementary set of nucleotides) may be contemplated. In one aspect, the stem made of the X and Y nucleotides, together with the loop will form a complete hairpin in the overall secondary structure; and, this may be advantageous and the amount of base pairs can be any amount that forms a complete hairpin. In one aspect, any complementary X:Y basepairing sequence (e.g., as to length) is tolerated, so long as the secondary structure of the entire guide molecule is preserved. In one aspect, the loop that connects the stem made of X:Y basepairs can be any sequence of the same length (e.g., 4 or 5 nucleotides) or longer that does not interrupt the overall secondary structure of the guide molecule. In one aspect, the stemloop can further comprise, e.g. an MS2 aptamer. In one aspect, the stem comprises about 5-7bp comprising complementary X and Y sequences, although stems of more or fewer basepairs are also contemplated. In one aspect, non-Watson Crick basepairing is contemplated, where such pairing otherwise generally preserves the architecture of the stemloop at that position.

[0419] In particular embodiments the natural hairpin or stemloop structure of the guide molecule is extended or replaced by an extended stemloop. It has been demonstrated that extension of the stem can enhance the assembly of the guide molecule with the CRISPR-Cas proten (Chen et al. Cell. (2013); 155(7): 1479-1491). In particular embodiments the stem of the stemloop is extended by at least 1, 2, 3, 4, 5 or more complementary basepairs (i.e. corresponding to the addition of 2,4, 6, 8, 10 or more nucleotides in the guide molecule). In particular embodiments these are located at the end of the stem, adjacent to the loop of the stemloop.

[0420] In particular embodiments, the susceptibility of the guide molecule to RNAses or to decreased expression can be reduced by slight modifications of the sequence of the guide molecule which do not affect its function. For instance, in particular embodiments, premature termination of transcription, such as premature transcription of U6 Pol-III, can be removed by

modifying a putative Pol-III terminator (4 consecutive U's) in the guide molecules sequence. Where such sequence modification is required in the stemloop of the guide molecule, it is preferably ensured by a basepair flip.

[0421] In a particular embodiment, the direct repeat may be modified to comprise one or more protein-binding RNA aptamers. In a particular embodiment, one or more aptamers may be included such as part of optimized secondary structure. Such aptamers may be capable of binding a bacteriophage coat protein as detailed further herein.

[0422] In some embodiments, the guide molecule forms a duplex with a target RNA comprising at least one target cytosine residue to be edited. Upon hybridization of the guide RNA molecule to the target RNA, the cytidine deaminase binds to the single strand RNA in the duplex made accessible by the mismatch in the guide sequence and catalyzes deamination of one or more target cytosine residues comprised within the stretch of mismatching nucleotides.

[0423] A guide sequence, and hence a nucleic acid-targeting guide RNA may be selected to target any target nucleic acid sequence. The target sequence may be mRNA.

[0424] In certain embodiments, the target sequence should be associated with a PAM (protospacer adjacent motif) or PFS (protospacer flanking sequence or site); that is, a short sequence recognized by the CRISPR complex. Depending on the nature of the CRISPR-Cas protein, the target sequence should be selected such that its complementary sequence in the DNA duplex (also referred to herein as the non-target sequence) is upstream or downstream of the PAM. In the embodiments of the present invention where the CRISPR-Cas protein is a Casl3 protein, the compelementary sequence of the target sequence is downstream or 3' of the PAM or upstream or 5' of the PAM. The precise sequence and length requirements for the PAM differ depending on the Casl3 protein used, but PAMs are typically 2-5 base pair sequences adjacent the protospacer (that is, the target sequence). Examples of the natural PAM sequences for different Casl3 orthologues are provided herein below and the skilled person will be able to identify further PAM sequences for use with a given Casl3 protein.

[0425] Further, engineering of the PAM Interacting (PI) domain may allow programing of PAM specificity, improve target site recognition fidelity, and increase the versatility of the CRISPR-Cas protein, for example as described for Cas9 in Kleinstiver BP et al. Engineered CRISPR-Cas9 nucleases with altered PAM specificities. Nature. 2015 Jul 23;523(756l):48l-5.

doi: l0.l038/naturel4592. As further detailed herein, the skilled person will understand that Casl3 proteins may be modified analogously.

[0426] In particular embodiment, the guide is an escorted guide. By "escorted" is meant that the CRISPR-Cas system or complex or guide is delivered to a selected time or place within a cell, so that activity of the CRISPR-Cas system or complex or guide is spatially or temporally controlled. For example, the activity and destination of the 3 CRISPR-Cas system or complex or guide may be controlled by an escort RNA aptamer sequence that has binding affinity for an aptamer ligand, such as a cell surface protein or other localized cellular component. Alternatively, the escort aptamer may for example be responsive to an aptamer effector on or in the cell, such as a transient effector, such as an external energy source that is applied to the cell at a particular time.

[0427] The escorted CRISPR-Cas systems or complexes have a guide molecule with a functional structure designed to improve guide molecule structure, architecture, stability, genetic expression, or any combination thereof. Such a structure can include an aptamer.

[0428] Aptamers are biomolecules that can be designed or selected to bind tightly to other ligands, for example using a technique called systematic evolution of ligands by exponential enrichment (SELEX; Tuerk C, Gold L: "Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase." Science 1990, 249:505-510). Nucleic acid aptamers can for example be selected from pools of random-sequence oligonucleotides, with high binding affinities and specificities for a wide range of biomedically relevant targets, suggesting a wide range of therapeutic utilities for aptamers (Keefe, Anthony D., Supriya Pai, and Andrew Ellington. "Aptamers as therapeutics." Nature Reviews Drug Discovery 9.7 (2010): 537-550). These characteristics also suggest a wide range of uses for aptamers as drug delivery vehicles (Levy-Nissenbaum, Etgar, et al. "Nanotechnology and aptamers: applications in drug delivery." Trends in biotechnology 26.8 (2008): 442-449; and, Hicke BJ, Stephens AW. "Escort aptamers: a delivery service for diagnosis and therapy." J Clin Invest 2000, 106:923-928.). Aptamers may also be constructed that function as molecular switches, responding to a que by changing properties, such as RNA aptamers that bind fluorophores to mimic the activity of green flourescent protein (Paige, Jeremy S., Karen Y. Wu, and Sarnie R. Jaffrey. "RNA mimics of green fluorescent protein." Science 333.6042 (201 1):

642-646). It has also been suggested that aptamers may be used as components of targeted siRNA therapeutic delivery systems, for example targeting cell surface proteins (Zhou, Jiehua, and John J. Rossi. "Aptamer-targeted cell-specific RNA interference." Silence 1.1 (2010): 4).

[0429] Accordingly, in particular embodiments, the guide molecule is modified, e.g., by one or more aptamer(s) designed to improve guide molecule delivery, including delivery across the cellular membrane, to intracellular compartments, or into the nucleus. Such a structure can include, either in addition to the one or more aptamer(s) or without such one or more aptamer(s), moiety(ies) so as to render the guide molecule deliverable, inducible or responsive to a selected effector. The invention accordingly comprehends an guide molecule that responds to normal or pathological physiological conditions, including without limitation pH, hypoxia, 0_2 concentration, temperature, protein concentration, enzymatic concentration, lipid structure, light exposure, mechanical disruption (e.g. ultrasound waves), magnetic fields, electric fields, or electromagnetic radiation.

[0430] Light responsiveness of an inducible system may be achieved via the activation and binding of cryptochrome-2 and CIB1. Blue light stimulation induces an activating conformational change in cryptochrome-2, resulting in recruitment of its binding partner CIB1. This binding is fast and reversible, achieving saturation in <15 sec following pulsed stimulation and returning to baseline <15 min after the end of stimulation. These rapid binding kinetics result in a system temporally bound only by the speed of transcription/translation and transcript/protein degradation, rather than uptake and clearance of inducing agents. Crytochrome-2 activation is also highly sensitive, allowing for the use of low light intensity stimulation and mitigating the risks of phototoxicity. Further, in a context such as the intact mammalian brain, variable light intensity may be used to control the size of a stimulated region, allowing for greater precision than vector delivery alone may offer.

[0431] The invention contemplates energy sources such as electromagnetic radiation, sound energy or thermal energy to induce the guide. Advantageously, the electromagnetic radiation is a component of visible light. In a preferred embodiment, the light is a blue light with a wavelength of about 450 to about 495 nm. In an especially preferred embodiment, the wavelength is about 488 nm. In another preferred embodiment, the light stimulation is via

pulses. The light power may range from about 0-9 mW/cm^2. In a preferred embodiment, a stimulation paradigm of as low as 0.25 sec every 15 sec should result in maximal activation.

[0432] The chemical or energy sensitive guide may undergo a conformational change upon induction by the binding of a chemical source or by the energy allowing it act as a guide and have the Casl3 CRISPR-Cas system or complex function. The invention can involve applying the chemical source or energy so as to have the guide function and the Casl3 CRISPR-Cas system or complex function; and optionally further determining that the expression of the genomic locus is altered.

[0433] There are several different designs of this chemical inducible system: 1. ABI-PYL based system inducible by Abscisic Acid (ABA) (see, e.g., stke.sciencemag.org/cgi/content/abstract/sigtrans;4/l64/rs2), 2. FKBP-FRB based system inducible by rapamycin (or related chemicals based on rapamycin) (see, e.g., www.nature.com/nmeth/journal/v2/n6/full/nmeth763.html), 3. GID1-GAI based system inducible by Gibberellin (GA) (see, e.g., www.nature.com/nchembio/journal/v8/n5/full/nchembio.922.html).

[0434] A chemical inducible system can be an estrogen receptor (ER) based system inducible by 4-hydroxytamoxifen (40HT) (see, e.g., www.pnas.org/content/l04/3/l027. abstract). A mutated ligand-binding domain of the estrogen receptor called ERT2 translocates into the nucleus of cells upon binding of 4-hydroxytamoxifen. In further embodiments of the invention any naturally occurring or engineered derivative of any nuclear receptor, thyroid hormone receptor, retinoic acid receptor, estrogren receptor, estrogen-related receptor, glucocorticoid receptor, progesterone receptor, androgen receptor may be used in inducible systems analogous to the ER based inducible system.

[0435] Another inducible system is based on the design using Transient receptor potential (TRP) ion channel based system inducible by energy, heat or radio-wave (see, e.g., www.sciencemag.org/content/336/608l/604). These TRP family proteins respond to different stimuli, including light and heat. When this protein is activated by light or heat, the ion channel will open and allow the entering of ions such as calcium into the plasma membrane. This influx of ions will bind to intracellular ion interacting partners linked to a polypeptide including the guide and the other components of the Casl3 CRISPR-Cas complex or system, and the binding

will induce the change of sub-cellular localization of the polypeptide, leading to the entire polypeptide entering the nucleus of cells. Once inside the nucleus, the guide protein and the other components of the Casl3 CRISPR-Cas complex will be active and modulating target gene expression in cells.

[0436] While light activation may be an advantageous embodiment, sometimes it may be disadvantageous especially for in vivo applications in which the light may not penetrate the skin or other organs. In this instance, other methods of energy activation are contemplated, in particular, electric field energy and/or ultrasound which have a similar effect.

[0437] Electric field energy is preferably administered substantially as described in the art, using one or more electric pulses of from about 1 Volt/cm to about 10 kVolts/cm under *in vivo* conditions. Instead of or in addition to the pulses, the electric field may be delivered in a continuous manner. The electric pulse may be applied for between 1 μs and 500 milliseconds, preferably between 1 ps and 100 milliseconds. The electric field may be applied continuously or in a pulsed manner for 5 about minutes.

[0438] As used herein, 'electric field energy' is the electrical energy to which a cell is exposed. Preferably the electric field has a strength of from about 1 Volt/cm to about 10 kVolts/cm or more under *in vivo* conditions (see WO97/49450).

[0439] As used herein, the term "electric field" includes one or more pulses at variable capacitance and voltage and including exponential and/or square wave and/or modulated wave and/or modulated square wave forms. References to electric fields and electricity should be taken to include reference the presence of an electric potential difference in the environment of a cell. Such an environment may be set up by way of static electricity, alternating current (AC), direct current (DC), etc, as known in the art. The electric field may be uniform, non-uniform or otherwise, and may vary in strength and/or direction in a time dependent manner.

[0440] Single or multiple applications of electric field, as well as single or multiple applications of ultrasound are also possible, in any order and in any combination. The ultrasound and/or the electric field may be delivered as single or multiple continuous applications, or as pulses (pulsatile delivery).

[0441] Electroporation has been used in both *in vitro* and *in vivo* procedures to introduce foreign material into living cells. With *in vitro* applications, a sample of live cells is first mixed

with the agent of interest and placed between electrodes such as parallel plates. Then, the electrodes apply an electrical field to the cell/implant mixture. Examples of systems that perform *in vitro* electroporation include the Electro Cell Manipulator ECM600 product, and the Electro Square Porator T820, both made by the BTX Division of Genetronics, Inc (see ET.S. Pat. No 5,869,326).

[0442] The known electroporation techniques (both *in vitro* and *in vivo*) function by applying a brief high voltage pulse to electrodes positioned around the treatment region. The electric field generated between the electrodes causes the cell membranes to temporarily become porous, whereupon molecules of the agent of interest enter the cells. In known electroporation applications, this electric field comprises a single square wave pulse on the order of 1000 V/cm, of about 100 .mu.s duration. Such a pulse may be generated, for example, in known applications of the Electro Square Porator T820.

[0443] Preferably, the electric field has a strength of from about 1 V/cm to about 10 kV/cm under *in vitro* conditions. Thus, the electric field may have a strength of 1 V/cm, 2 V/cm, 3 V/cm, 4 V/cm, 5 V/cm, 6 V/cm, 7 V/cm, 8 V/cm, 9 V/cm, 10 V/cm, 20 V/cm, 50 V/cm, 100 V/cm, 200 V/cm, 300 V/cm, 400 V/cm, 500 V/cm, 600 V/cm, 700 V/cm, 800 V/cm, 900 V/cm, 1 kV/cm, 2 kV/cm, 5 kV/cm, 10 kV/cm, 20 kV/cm, 50 kV/cm or more. More preferably from about 0.5 kV/cm to about 4.0 kV/cm under *in vitro* conditions. Preferably the electric field has a strength of from about 1 V/cm to about 10 kV/cm under *in vivo* conditions. However, the electric field strengths may be lowered where the number of pulses delivered to the target site are increased. Thus, pulsatile delivery of electric fields at lower field strengths is envisaged.

[0444] Preferably the application of the electric field is in the form of multiple pulses such as double pulses of the same strength and capacitance or sequential pulses of varying strength and/or capacitance. As used herein, the term “pulse” includes one or more electric pulses at variable capacitance and voltage and including exponential and/or square wave and/or modulated wave/square wave forms.

[0445] Preferably the electric pulse is delivered as a waveform selected from an exponential wave form, a square wave form, a modulated wave form and a modulated square wave form.

[0446] A preferred embodiment employs direct current at low voltage. Thus, Applicants disclose the use of an electric field which is applied to the cell, tissue or tissue mass at a field

strength of between lV/cm and 20V/cm, for a period of 100 milliseconds or more, preferably 15 minutes or more.

[0447] Ultrasound is advantageously administered at a power level of from about 0.05 W/cm2 to about 100 W/cm2. Diagnostic or therapeutic ultrasound may be used, or combinations thereof.

[0448] As used herein, the term “ultrasound” refers to a form of energy which consists of mechanical vibrations the frequencies of which are so high they are above the range of human hearing. Lower frequency limit of the ultrasonic spectrum may generally be taken as about 20 kHz. Most diagnostic applications of ultrasound employ frequencies in the range 1 and 15 MHz' (From Ultrasonics in Clinical Diagnosis, P. N. T. Wells, ed., 2nd. Edition, Publ. Churchill Livingstone [Edinburgh, London & NY, 1977]).

[0449] Ultrasound has been used in both diagnostic and therapeutic applications. When used as a diagnostic tool ("diagnostic ultrasound"), ultrasound is typically used in an energy density range of up to about 100 mW/cm2 (FDA recommendation), although energy densities of up to 750 mW/cm2 have been used. In physiotherapy, ultrasound is typically used as an energy source in a range up to about 3 to 4 W/cm2 (WHO recommendation). In other therapeutic applications, higher intensities of ultrasound may be employed, for example, HIFU at 100 W/cm up to 1 kW/cm2 (or even higher) for short periods of time. The term "ultrasound" as used in this specification is intended to encompass diagnostic, therapeutic and focused ultrasound.

[0450] Focused ultrasound (FUS) allows thermal energy to be delivered without an invasive probe (see Morocz et al 1998 Journal of Magnetic Resonance Imaging Vol.8, No. 1, pp. 136-142. Another form of focused ultrasound is high intensity focused ultrasound (HIFU) which is reviewed by Moussatov et al in Ultrasonics (1998) Vol.36, No. 8, pp. 893-900 and TranHuuHue et al in Acustica (1997) Vol.83, No. 6, pp. 1103-1 106.

[0451] Preferably, a combination of diagnostic ultrasound and a therapeutic ultrasound is employed. This combination is not intended to be limiting, however, and the skilled reader will appreciate that any variety of combinations of ultrasound may be used. Additionally, the energy density, frequency of ultrasound, and period of exposure may be varied.

[0452] Preferably the exposure to an ultrasound energy source is at a power density of from about 0.05 to about 100 Wcm-2. Even more preferably, the exposure to an ultrasound energy source is at a power density of from about 1 to about 15 Wcm-2.

[0453] Preferably the exposure to an ultrasound energy source is at a frequency of from about 0.015 to about 10.0 MHz. More preferably the exposure to an ultrasound energy source is at a frequency of from about 0.02 to about 5.0 MHz or about 6.0 MHz. Most preferably, the ultrasound is applied at a frequency of 3 MHz.

[0454] Preferably the exposure is for periods of from about 10 milliseconds to about 60 minutes. Preferably the exposure is for periods of from about 1 second to about 5 minutes. More preferably, the ultrasound is applied for about 2 minutes. Depending on the particular target cell to be disrupted, however, the exposure may be for a longer duration, for example, for 15 minutes.

[0455] Advantageously, the target tissue is exposed to an ultrasound energy source at an acoustic power density of from about 0.05 Wcm-2 to about 10 Wcm-2 with a frequency ranging from about 0.015 to about 10 MHz (see WO 98/52609). However, alternatives are also possible, for example, exposure to an ultrasound energy source at an acoustic power density of above 100 Wcm-2, but for reduced periods of time, for example, 1000 Wcm-2 for periods in the millisecond range or less.

[0456] Preferably the application of the ultrasound is in the form of multiple pulses; thus, both continuous wave and pulsed wave (pulsatile delivery of ultrasound) may be employed in any combination. For example, continuous wave ultrasound may be applied, followed by pulsed wave ultrasound, or vice versa. This may be repeated any number of times, in any order and combination. The pulsed wave ultrasound may be applied against a background of continuous wave ultrasound, and any number of pulses may be used in any number of groups.

[0457] Preferably, the ultrasound may comprise pulsed wave ultrasound. In a highly preferred embodiment, the ultrasound is applied at a power density of 0.7 Wcm-2 or 1.25 Wcm-2 as a continuous wave. Higher power densities may be employed if pulsed wave ultrasound is used.

[0458] Use of ultrasound is advantageous as, like light, it may be focused accurately on a target. Moreover, ultrasound is advantageous as it may be focused more deeply into tissues

unlike light. It is therefore better suited to whole-tissue penetration (such as but not limited to a lobe of the liver) or whole organ (such as but not limited to the entire liver or an entire muscle, such as the heart) therapy. Another important advantage is that ultrasound is a non-invasive stimulus which is used in a wide variety of diagnostic and therapeutic applications. By way of example, ultrasound is well known in medical imaging techniques and, additionally, in orthopedic therapy. Furthermore, instruments suitable for the application of ultrasound to a subject vertebrate are widely available and their use is well known in the art.

[0459] In particular embodiments, the guide molecule is modified by a secondary structure to increase the specificity of the CRISPR-Cas system and the secondary structure can protect against exonuclease activity and allow for 5' additions to the guide sequence also referred to herein as a protected guide molecule.

[0460] In one aspect, the invention provides for hybridizing a "protector RNA" to a sequence of the guide molecule, wherein the "protector RNA" is an RNA strand complementary to the 3' end of the guide molecule to thereby generate a partially double-stranded guide RNA. In an embodiment of the invention, protecting mismatched bases (i.e. the bases of the guide molecule which do not form part of the guide sequence) with a perfectly complementary protector sequence decreases the likelihood of target RNA binding to the mismatched basepairs at the 3' end. In particular embodiments of the invention, additional sequences comprising an extented length may also be present within the guide molecule such that the guide comprises a protector sequence within the guide molecule. This "protector sequence" ensures that the guide molecule comprises a "protected sequence" in addition to an "exposed sequence" (comprising the part of the guide sequence hybridizing to the target sequence). In particular embodiments, the guide molecule is modified by the presence of the protector guide to comprise a secondary structure such as a hairpin. Advantageously there are three or four to thirty or more, e.g., about 10 or more, contiguous base pairs having complementarity to the protected sequence, the guide sequence or both. It is advantageous that the protected portion does not impede thermodynamics of the CRISPR-Cas system interacting with its target. By providing such an extension including a partially double stranded guide moleucle, the guide molecule is considered protected and results in improved specific binding of the CRISPR-Cas complex, while maintaining specific activity.

[0461] In particular embodiments, use is made of a truncated guide (tru-guide), i.e. a guide molecule which comprises a guide sequence which is truncated in length with respect to the canonical guide sequence length. As described by Nowak et al. (Nucleic Acids Res (2016) 44 (20): 9555-9564), such guides may allow catalytically active CRISPR-Cas enzyme to bind its target without cleaving the target RNA. In particular embodiments, a truncated guide is used which allows the binding of the target but retains only nickase activity of the CRISPR-Cas enzyme.

CRISPR RNA-Targeting Effector Proteins

[0462] In one example embodiment, the CRISPR system effector protein is an RNA-targeting effector protein. In certain embodiments, the CRISPR system effector protein is a Type VI CRISPR system targeting RNA (e.g., Casl3a, Casl3b, Casl3c or Casl3d). Example RNA-targeting effector proteins include Casl3b and C2c2 (now known as Casl3a). It will be understood that the term "C2c2" herein is used interchangeably with "Casl3a". "C2c2" is now referred to as "Casl3a", and the terms are used interchangeably herein unless indicated otherwise. As used herein, the term "Casl3" refers to any Type VI CRISPR system targeting RNA (e.g., Casl3a, Casl3b, Casl3c or Casl3d). When the CRISPR protein is a C2c2 protein, a tracrRNA is not required. C2c2 has been described in Abudayyeh et al. (2016) "C2c2 is a single-component programmable RNA-guided RNA-targeting CRISPR effector"; Science; DOI: l0.H26/science.aaf5573; and Shmakov et al. (2015) "Discovery and Functional Characterization of Diverse Class 2 CRISPR-Cas Systems", Molecular Cell, DOI: dx.doi.org/l0.l0l6/j.molcel. 2015. 10.008; which are incorporated herein in their entirety by reference. Casl3b has been described in Smargon et al. (2017) "Casl3b Is a Type VI-B CRISPR-Associated RNA-Guided RNases Differentially Regulated by Accessory Proteins Csx27 and Csx28," Molecular Cell. 65, 1-13; dx.doi.org/l0.l0l6/j.molcel. 2016. 12.023., which is incorporated herein in its entirety by reference.

[0463] In some embodiments, one or more elements of a nucleic acid-targeting system is derived from a particular organism comprising an endogenous CRISPR RNA-targeting system. In certain example embodiments, the effector protein CRISPR RNA-targeting system comprises at least one HEPN domain, including but not limited to the HEPN domains described herein, HEPN domains known in the art, and domains recognized to be HEPN domains by comparison

to consensus sequence motifs. Several such domains are provided herein. In one non-limiting example, a consensus sequence can be derived from the sequences of C2c2 or Casl3b orthologs provided herein. In certain example embodiments, the effector protein comprises a single HEPN domain. In certain other example embodiments, the effector protein comprises two HEPN domains.

[0464] In one example embodiment, the effector protein comprise one or more HEPN domains comprising a RxxxxH motif sequence. The RxxxxH motif sequence can be, without limitation, from a HEPN domain described herein or a HEPN domain known in the art. RxxxxH motif sequences further include motif sequences created by combining portions of two or more HEPN domains. As noted, consensus sequences can be derived from the sequences of the orthologs disclosed in Ei.S. Provisional Patent Application 62/432,240 entitled "Novel CRISPR Enzymes and Systems," Ei.S. Provisional Patent Application 62/471,710 entitled "Novel Type VI CRISPR Orthologs and Systems" filed on March 15, 2017, and U.S. Provisional Patent Application entitled "Novel Type VI CRISPR Orthologs and Systems," labeled as attorney docket number 47627-05-2133 and filed on April 12, 2017.

[0465] In certain other example embodiments, the CRISPR system effector protein is a C2c2 nuclease. The activity of C2c2 may depend on the presence of two HEPN domains. These have been shown to be RNase domains, *i.e.* nuclease (in particular an endonuclease) cutting RNA. C2c2 HEPN may also target DNA, or potentially DNA and/or RNA. On the basis that the HEPN domains of C2c2 are at least capable of binding to and, in their wild-type form, cutting RNA, then it is preferred that the C2c2 effector protein has RNase function. Regarding C2c2 CRISPR systems, reference is made to U.S. Provisional 62/351,662 filed on June 17, 2016 and U.S. Provisional 62/376,377 filed on August 17, 2016. Reference is also made to U.S. Provisional 62/351,803 filed on June 17, 2016. Reference is also made to U.S. Provisional entitled "Novel Crispr Enzymes and Systems" filed December 8, 2016 bearing Broad Institute No. 10035.PA4 and Attorney Docket No. 47627.03.2133. Reference is further made to East-Seletsky *et al.* "Two distinct RNase activities of CRISPR-C2c2 enable guide-RNA processing and RNA detection" Nature doi: 10/1038/nature 19802 and Abudayyeh *et al.* "C2c2 is a single-component programmable RNA-guided RNA targeting CRISPR effector" bioRxiv doi: 10. 1101/054742.

[0466] In certain embodiments, the C2c2 effector protein is from an organism of a genus selected from the group consisting of: Leptotrichia, Listeria, Corynebacter, Sutterella, Legionella, Treponema, Filifactor, Eubacterium, Streptococcus, Lactobacillus, Mycoplasma, Bacteroides, Flaviivola, Flavobacterium, Sphaerochaeta, Azospirillum, Gluconacetobacter, Neisseria, Roseburia, Parvibaculum, Staphylococcus, Nitratifractor, Mycoplasma, Campylobacter, and Lachnospira, or the C2c2 effector protein is an organism selected from the group consisting of: Leptotrichia shahii, Leptotrichia. wadei, Listeria seeligeri, Clostridium aminophilum, Carnobacterium gallinarum, Paludibacter propionicigenes, Listeria weihenstephanensis, or the C2c2 effector protein is a L. wadei F0279 or L. wadei F0279 (Lw2) C2C2 effector protein. In another embodiment, the one or more guide RNAs are designed to detect a single nucleotide polymorphism, splice variant of a transcript, or a frameshift mutation in a target RNA or DNA.

[0467] In certain example embodiments, the RNA-targeting effector protein is a Type VI-B effector protein, such as Casl3b and Group 29 or Group 30 proteins. In certain example embodiments, the RNA-targeting effector protein comprises one or more HEPN domains. In certain example embodiments, the RNA-targeting effector protein comprises a C-terminal HEPN domain, a N-terminal HEPN domain, or both. Regarding example Type VI-B effector proteins that may be used in the context of this invention, reference is made to US Application No. 15/331,792 entitled "Novel CRISPR Enzymes and Systems" and filed October 21, 2016, International Patent Application No. PCT/US2016/058302 entitled "Novel CRISPR Enzymes and Systems", and filed October 21, 2016, and Smargon *et al.* "Casl3b is a Type VI-B CRISPR-associated RNA-Guided RNase differentially regulated by accessory proteins Csx27 and Csx28" Molecular Cell, 65, 1-13 (2017); dx.doi.org/l0.l0l6/j.molcel. 2016. 12.023, and U.S. Provisional Application No. to be assigned, entitled "Novel Casl3b Orthologues CRISPR Enzymes and System" filed March 15, 2017. In particular embodiments, the Casl3b enzyme is derived from *Bergeyella zoohelcum.*

[0468] In certain example embodiments, the RNA-targeting effector protein is a Casl3c effector protein as disclosed in U.S. Provisional Patent Application No. 62/525,165 filed June 26, 2017, and PCT Application No. US 2017/047193 filed August 16, 2017.

[0469] In some embodiments, one or more elements of a nucleic acid-targeting system is derived from a particular organism comprising an endogenous CRISPR RNA-targeting system. In certain embodiments, the CRISPR RNA-targeting system is found in *Eubacterium* and *Ruminococcus.* In certain embodiments, the effector protein comprises targeted and collateral ssRNA cleavage activity. In certain embodiments, the effector protein comprises dual HEPN domains. In certain embodiments, the effector protein lacks a counterpart to the Helical-1 domain of Casl3a. In certain embodiments, the effector protein is smaller than previously characterized class 2 CRISPR effectors, with a median size of 928 aa. This median size is 190 aa (17%) less than that of Casl3c, more than 200 aa (18%) less than that of Casl3b, and more than 300 aa ***(26%)*** less than that of Casl3a. In certain embodiments, the effector protein has no requirement for a flanking sequence (e.g., PFS, PAM).

[0470] In certain embodiments, the effector protein locus structures include a WYL domain containing accessory protein (so denoted after three amino acids that were conserved in the originally identified group of these domains; see, e.g., WYL domain IPR026881). In certain embodiments, the WYL domain accessory protein comprises at least one helix-turn-helix (HTH) or ribbon-helix-helix (RHH) DNA-binding domain. In certain embodiments, the WYL domain containing accessory protein increases both the targeted and the collateral ssRNA cleavage activity of the RNA-targeting effector protein. In certain embodiments, the WYL domain containing accessory protein comprises an N-terminal RHH domain, as well as a pattern of primarily hydrophobic conserved residues, including an invariant tyrosine-leucine doublet corresponding to the original WYL motif. In certain embodiments, the WYL domain containing accessory protein is WYL1. WYL1 is a single WYL-domain protein associated primarily with *Ruminococcus.*

[0471] In other example embodiments, the Type VI RNA-targeting Cas enzyme is Casl3d. In certain embodiments, Casl3d is *Eubacterium siraeum* DSM 15702 (EsCasl3d) or *Ruminococcus* sp. N15.MGS-57 (RspCasl3d) (see, e.g., Yan et al., Casl3d Is a Compact RNA-Targeting Type VI CRISPR Effector Positively Modulated by a WYL-Domain-Containing Accessory Protein, Molecular Cell (2018), doi.org/l0.l0l6/j.molcel.20l8.02.028). RspCasl3d and EsCasl3d have no flanking sequence requirements (e.g., PFS, PAM).

Cas13 RNA Editing

[0472] In one aspect, the invention provides a method of modifying or editing a target transcript in a eukaryotic cell. In some embodiments, the method comprises allowing a CRISPR-Cas effector module complex to bind to the target polynucleotide to effect RNA base editing, wherein the CRISPR-Cas effector module complex comprises a Cas effector module complexed with a guide sequence hybridized to a target sequence within said target polynucleotide, wherein said guide sequence is linked to a direct repeat sequence. In some embodiments, the Cas effector module comprises a catalytically inactive CRISPR-Cas protein. In some embodiments, the guide sequence is designed to introduce one or more mismatches to the RNA/RNA duplex formed between the target sequence and the guide sequence. In particular embodiments, the mismatch is an A-C mismatch. In some embodiments, the Cas effector may associate with one or more functional domains (e.g. via fusion protein or suitable linkers). In some embodiments, the effector domain comprises one or more cytindine or adenosine deaminases that mediate endogenous editing of via hydrolytic deamination. In particular embodiments, the effector domain comprises the adenosine deaminase acting on RNA (ADAR) family of enzymes. In particular embodiments, the adenosine deaminase protein or catalytic domain thereof capable of deaminating adenosine or cytidine in RNA or is an RNA specific adenosine deaminase and/or is a bacterial, human, cephalopod, or Drosophila adenosine deaminase protein or catalytic domain thereof, preferably TadA, more preferably ADAR, optionally huADAR, optionally (hu)ADARl or (hu)ADAR2, preferably huADAR2 or catalytic domain thereof.

[0473] The present application relates to modifying a target RNA sequence of interest (see, e.g, Cox et al., Science. 2017 Nov 24;358(6366): 1019-1027). Using RNA-targeting rather than DNA targeting offers several advantages relevant for therapeutic development. First, there are substantial safety benefits to targeting RNA: there will be fewer off-target events because the available sequence space in the transcriptome is significantly smaller than the genome, and if an off-target event does occur, it will be transient and less likely to induce negative side effects. Second, RNA-targeting therapeutics will be more efficient because they are cell-type independent and not have to enter the nucleus, making them easier to deliver.

[0474] A further aspect of the invention relates to the method and composition as envisaged herein for use in prophylactic or therapeutic treatment, preferably wherein said target locus of interest is within a human or animal and to methods of modifying an Adenine or Cytidine in a

target RNA sequence of interest, comprising delivering to said target RNA, the composition as described herein. In particular embodiments, the CRISPR system and the adenonsine deaminase, or catalytic domain thereof, are delivered as one or more polynucleotide molecules, as a ribonucleoprotein complex, optionally via particles, vesicles, or one or more viral vectors. In particular embodiments, the invention thus comprises compositions for use in therapy. This implies that the methods can be performed in vivo, ex vivo or in vitro. In particular embodiments, when the target is a human or animal target, the method is carried out ex vivo or in vitro.

[0475] A further aspect of the invention relates to the method as envisaged herein for use in prophylactic or therapeutic treatment, preferably wherein said target of interest is within a human or animal and to methods of modifying an Adenine or Cytidine in a target RNA sequence of interest, comprising delivering to said target RNA, the composition as described herein. In particular embodiments, the CRISPR system and the adenonsine deaminase, or catalytic domain thereof, are delivered as one or more polynucleotide molecules, as a ribonucleoprotein complex, optionally via particles, vesicles, or one or more viral vectors.

[0476] In one aspect, the invention provides a method of generating a eukaryotic cell comprising a modified or edited gene. In some embodiments, the method comprises (a) introducing one or more vectors into a eukaryotic cell, wherein the one or more vectors drive expression of one or more of: Cas effector module, and a guide sequence linked to a direct repeat sequence, wherein the Cas effector module associate one or more effector domains that mediate base editing, and (b) allowing a CRISPR-Cas effector module complex to bind to a target polynucleotide to effect base editing of the target polynucleotide within said disease gene, wherein the CRISPR-Cas effector module complex comprises a Cas effector module complexed with the guide sequence that is hybridized to the target sequence within the target polynucleotide, wherein the guide sequence may be designed to introduce one or more mismatches between the RNA/RNA duplex formed between the guide sequence and the target sequence. In particular embodiments, the mismatch is an A-C mismatch. In some embodiments, the Cas effector may associate with one or more functional domains (e.g. via fusion protein or suitable linkers). In some embodiments, the effector domain comprises one or more cytidine or adenosine deaminases that mediate endogenous editing of via hydrolytic deamination. In

particular embodiments, the effector domain comprises the adenosine deaminase acting on RNA (ADAR) family of enzymes. In particular embodiments, the adenosine deaminase protein or catalytic domain thereof capable of deaminating adenosine or cytidine in RNA or is an RNA specific adenosine deaminase and/or is a bacterial, human, cephalopod, or Drosophila adenosine deaminase protein or catalytic domain thereof, preferably TadA, more preferably ADAR, optionally huADAR, optionally (hu)ADARl or (hu)ADAR2, preferably huADAR2 or catalytic domain thereof.

[0477] The present invention may also use a Casl2 CRISPR enzyme. Casl2 enzymes include Casl2a (Cpfl), Casl2b (C2cl), and Casl2c (C2c3), described further herein.

[0478] A further aspect relates to an isolated cell obtained or obtainable from the methods described herein comprising the composition described herein or progeny of said modified cell, preferably wherein said cell comprises a hypoxanthine or a guanine in replace of said Adenine in said target RNA of interest compared to a corresponding cell not subjected to the method. In particular embodiments, the cell is a eukaryotic cell, preferably a human or non-human animal cell, optionally a therapeutic T cell or an antibody-producing B-cell.

[0479] In some embodiments, the modified cell is a therapeutic T cell, such as a T cell suitable for adoptive cell transfer therapies (e.g., CAR-T therapies). The modification may result in one or more desirable traits in the therapeutic T cell, as described further herein.

[0480] The invention further relates to a method for cell therapy, comprising administering to a patient in need thereof the modified cell described herein, wherein the presence of the modified cell remedies a disease in the patient.

[0481] The present invention may be further illustrated and extended based on aspects of CRISPR-Cas development and use as set forth in the following articles and particularly as relates to delivery of a CRISPR protein complex and uses of an RNA guided endonuclease in cells and organisms:

- Multiplex genome engineering using CRISPR-Cas systems. Cong, L., Ran, F.A., Cox, D., Lin, S., Barretto, R., Habib, N., Hsu, P.D., Wu, X., Jiang, W., Marraffmi, L.A., & Zhang, F. Science Feb 15;339(6121):819-23 (2013);
- RNA-guided editing of bacterial genomes using CRISPR-Cas systems. Jiang W., Bikard D., Cox D., Zhang F, Marraffmi LA. Nat Biotechnol Mar;3l(3):233-9 (2013);

- One-Step Generation of Mice Carrying Mutations in Multiple Genes by CRISPR-Cas-Mediated Genome Engineering. Wang EL, Yang EL, Shivalila CS., Dawlaty MM., Cheng AW., Zhang F., Jaenisch R. Cell May 9;153(4):910-8 (2013);
- Optical control of mammalian endogenous transcription and epigenetic states. Konermann S, Brigham MD, Trevino AE, Hsu PD, Heidenreich M, Cong L, Platt RJ, Scott DA, Church GM, Zhang F. Nature. Aug 22;500(7463):472-6. doi: l0.l038/Naturel2466. Epub 2013 Aug 23 (2013);
- Double Nicking by RNA-Guided CRISPR Cas9 for Enhanced Genome Editing Specificity. Ran, FA., Hsu, PD., Lin, CY., Gootenberg, JS., Konermann, S., Trevino, AE., Scott, DA., Inoue, A., Matoba, S., Zhang, Y., & Zhang, F. Cell Aug 28. pii: S0092-8674(13)01015-5 (2013-A);
- DNA targeting specificity of RNA-guided Cas9 nucleases. Hsu, P., Scott, D., Weinstein, J., Ran, FA., Konermann, S., Agarwala, V., Li, Y., Fine, E., Wu, X., Shalem, O., Cradick, TT, Marraffmi, LA., Bao, G., & Zhang, F. Nat Biotechnol doi:l0.l038/nbt.2647 (2013);
- Genome engineering using the CRISPR-Cas9 system. Ran, FA., Hsu, PD., Wright, J., Agarwala, V., Scott, DA., Zhang, F. Nature Protocols Nov;8(l l):228l-308 (2013-B);
- Genome-Scale CRISPR-Cas9 Knockout Screening in Human Cells. Shalem, O., Sanjana, NE., Hartenian, E., Shi, X., Scott, DA., Mikkelson, T., Heckl, D., Ebert, BL., Root, DE., Doench, JG., Zhang, F. Science Dec 12. (2013);
- Crystal structure of cas9 in complex with guide RNA and target DNA. Nishimasu, H , Ran, FA., Hsu, PD., Konermann, S., Shehata, ST, Dohmae, N., Ishitani, R., Zhang, F., Nureki, O. Cell Feb 27, l56(5):935-49 (2014);
- Genome-wide binding of the CRISPR endonuclease Cas9 in mammalian cells. Wu X., Scott DA., Kriz AT, Chiu AC., Hsu PD., Dadon DB., Cheng AW., Trevino AE., Konermann S., Chen S., Jaenisch R., Zhang F., Sharp PA. Nat Biotechnol. Apr 20. doi: l0.l038/nbt.2889 (2014);
- CRISPR-Cas9 Knockin Mice for Genome Editing and Cancer Modeling. Platt RJ, Chen S, Zhou Y, Yim MJ, Swiech L, Kempton HR, Dahlman JE, Parnas O, Eisenhaure TM, Jovanovic M, Graham DB, Jhunjhunwala S, Heidenreich M, Xavier RJ, Langer R,

Anderson DG, Hacohen N, Regev A, Feng G, Sharp PA, Zhang F. Cell 159(2): 440-455 DOI: 10. l0l6/j. cell. 2014. 09. 014(2014);

- Development and Applications of CRISPR-Cas9 for Genome Engineering, Hsu PD, Lander ES, Zhang F., Cell. Jun 5;l57(6):l262-78 (2014).
- Genetic screens in human cells using the CRISPR-Cas9 system, Wang T, Wei JJ, Sabatini DM, Lander ES., Science. January 3; 343(6166): 80-84. doi: 10. 1126/science. 1246981 (2014);
- Rational design of highly active sgRNAs for CRISPR-Cas9-mediated gene inactivation, Doench JG, Hartenian E, Graham DB, Tothova Z, Hegde M, Smith I, Sullender M, Ebert BL, Xavier RJ, Root DE., (published online 3 September 2014) Nat Biotechnol. Dec;32(l2): 1262-7 (2014);
- *In vivo* interrogation of gene function in the mammalian brain using CRISPR-Cas9, Swiech L, Heidenreich M, Banerjee A, Habib N, Li Y, Trombetta J, Sur M, Zhang F., (published online 19 October 2014) Nat Biotechnol. Jan;33(l): 102-6 (2015);
- Genome-scale transcriptional activation by an engineered CRISPR-Cas9 complex, Konermann S, Brigham MD, Trevino AE, Joung J, Abudayyeh OO, Barcena C, Hsu PD, Habib N, Gootenberg JS, Nishimasu H, Nureki O, Zhang F., Nature. Jan 29;5 17(7536): 583-8 (2015).
- A split-Cas9 architecture for inducible genome editing and transcription modulation, Zetsche B, Volz SE, Zhang F., (published online 02 February 2015) Nat Biotechnol. Feb;33(2): 139-42 (2015);
- Genome-wide CRISPR Screen in a Mouse Model of Tumor Growth and Metastasis, Chen S, Sanjana NE, Zheng K, Shalem O, Lee K, Shi X, Scott DA, Song J, Pan JQ, Weissleder R, Lee H, Zhang F, Sharp PA. Cell 160, 1246-1260, March 12, 2015 (multiplex screen in mouse), and
- In *vivo* genome editing using *Staphylococcus aureus* Cas9, Ran FA, Cong L, Yan WX, Scott DA, Gootenberg JS, Kriz AJ, Zetsche B, Shalem O, Wu X, Makarova KS, Koonin EV, Sharp PA, Zhang F., (published online 0 1 April 2015), Nature. Apr 9;520(7546): 186-91 (2015).

- Shalem et al., "High-throughput functional genomics using CRISPR-Cas9," Nature Reviews Genetics 16, 299-31 1 (May 2015).
- Xu et al., "Sequence determinants of improved CRISPR sgRNA design," Genome Research 25, 1147-1 157 (August 2015).
- Parnas et al., "A Genome-wide CRISPR Screen in Primary Immune Cells to Dissect Regulatory Networks," Cell 162, 675-686 (July 30, 2015).
- Ramanan et al., CRISPR-Cas9 cleavage of viral DNA efficiently suppresses hepatitis B virus," Scientific Reports 5:10833. doi: l0.l038/srepl0833 (June 2, 2015)
- Nishimasu et al., Crystal Structure of Staphylococcus aureus Cas9," Cell 162, 1113-1 126 (Aug. 27, 2015)
- BCL1 1A enhancer dissection by Cas9-mediated in situ saturating mutagenesis, Canver et al., Nature 527(7577): 192-7 (Nov. 12, 2015) doi: l0.l038/naturel552l. Epub 2015 Sep 16.
- *Cpfl Is a Single RNA-Guided Endonuclease of a Class 2 CRISPR-Cas System*, Zetsche et al., Cell 163, 759-71 (Sep 25, 2015).
- *Discovery and Functional Characterization of Diverse Class 2 CRISPR-Cas Systems,* Shmakov et al., Molecular Cell, 60(3), 385-397 doi: 10. l0l6/j.molcel. 2015. 10.008 Epub October 22, 2015.
- *Rationally engineered Cas9 nucleases with improved specificity*, Slaymaker et al., Science 2016 Jan 1 351(6268): 84-88 doi: l0.H26/science.aad5227. Epub 2015 Dec 1.
- Gao *et al*, "Engineered Cpfl Enzymes with Altered PAM Specificities," bioRxiv 09161 1; doi: http://dx.doi.org/l0.H0l/09l6l l (Dec. 4, 2016).
- Cox et al., "RNA editing with CRISPR-Cas 13," Science. 2017 Nov 24;358(6366):1019-1027. doi: l0.H26/science.aaq0l80. Epub 2017 Oct 25.
- Gaudelli et al. "Programmable base editing of A-T to G-C in genomic DNA without DNA cleavage" Nature 464(551); 464-471 (2017).

each of which is incorporated herein by reference, may be considered in the practice of the instant invention, and discussed briefly below:

- Cong *et al.* engineered type II CRISPR-Cas systems for use in eukaryotic cells based on both *Streptococcus thermophilus* Cas9 and also *Streptococcus pyogenes* Cas9 and

demonstrated that Cas9 nucleases can be directed by short RNAs to induce precise cleavage of DNA in human and mouse cells. Their study further showed that Cas9 as converted into a nicking enzyme can be used to facilitate homology-directed repair in eukaryotic cells with minimal mutagenic activity. Additionally, their study demonstrated that multiple guide sequences can be encoded into a single CRISPR array to enable simultaneous editing of several at endogenous genomic loci sites within the mammalian genome, demonstrating easy programmability and wide applicability of the RNA-guided nuclease technology. This ability to use RNA to program sequence specific DNA cleavage in cells defined a new class of genome engineering tools. These studies further showed that other CRISPR loci are likely to be transplantable into mammalian cells and can also mediate mammalian genome cleavage. Importantly, it can be envisaged that several aspects of the CRISPR-Cas system can be further improved to increase its efficiency and versatility.

- Jiang *el al.* used the clustered, regularly interspaced, short palindromic repeats (CRISPR)-associated Cas9 endonuclease complexed with dual-RNAs to introduce precise mutations in the genomes of *Streptococcus pneumoniae* and *Escherichia coli.* The approach relied on dual-RNA:Cas9-directed cleavage at the targeted genomic site to kill unmutated cells and circumvents the need for selectable markers or counter-selection systems. The study reported reprogramming dual-RNA:Cas9 specificity by changing the sequence of short CRISPR RNA (crRNA) to make single- and multinucleotide changes carried on editing templates. The study showed that simultaneous use of two crRNAs enabled multiplex mutagenesis. Furthermore, when the approach was used in combination with recombineering, in *S. pneumoniae* , nearly 100% of cells that were recovered using the described approach contained the desired mutation, and in *E. coli,* 65% that were recovered contained the mutation.
- Wang *et al.* (2013) used the CRISPR-Cas system for the one-step generation of mice carrying mutations in multiple genes which were traditionally generated in multiple steps by sequential recombination in embryonic stem cells and/or time-consuming intercrossing of mice with a single mutation. The CRISPR-Cas system will greatly

accelerate the *in vivo* study of functionally redundant genes and of epistatic gene interactions.

- Konermann *el al.* (2013) addressed the need in the art for versatile and robust technologies that enable optical and chemical modulation of DNA-binding domains based CRISPR Cas9 enzyme and also Transcriptional Activator Like Effectors
- Ran *el al.* (2013-A) described an approach that combined a Cas9 nickase mutant with paired guide RNAs to introduce targeted double-strand breaks. This addresses the issue of the Cas9 nuclease from the microbial CRISPR-Cas system being targeted to specific genomic loci by a guide sequence, which can tolerate certain mismatches to the DNA target and thereby promote undesired off-target mutagenesis. Because individual nicks in the genome are repaired with high fidelity, simultaneous nicking *via* appropriately offset guide RNAs is required for double-stranded breaks and extends the number of specifically recognized bases for target cleavage. The authors demonstrated that using paired nicking can reduce off-target activity by 50- to 1,500-fold in cell lines and to facilitate gene knockout in mouse zygotes without sacrificing on-target cleavage efficiency. This versatile strategy enables a wide variety of genome editing applications that require high specificity.
- Hsu *et al.* (2013) characterized SpCas9 targeting specificity in human cells to inform the selection of target sites and avoid off-target effects. The study evaluated >700 guide RNA variants and SpCas9-induced indel mutation levels at >100 predicted genomic off-target loci in 293T and 293FT cells. The authors that SpCas9 tolerates mismatches between guide RNA and target DNA at different positions in a sequence-dependent manner, sensitive to the number, position and distribution of mismatches. The authors further showed that SpCas9-mediated cleavage is unaffected by DNA methylation and that the dosage of SpCas9 and guide RNA can be titrated to minimize off-target modification. Additionally, to facilitate mammalian genome engineering applications, the authors reported providing a web-based software tool to guide the selection and validation of target sequences as well as off-target analyses.
- Ran *et al.* (2013-B) described a set of tools for Cas9-mediated genome editing *via* non-homologous end joining (NHEJ) or homology-directed repair (HDR) in mammalian cells,

as well as generation of modified cell lines for downstream functional studies. To minimize off-target cleavage, the authors further described a double-nicking strategy using the Cas9 nickase mutant with paired guide RNAs. The protocol provided by the authors experimentally derived guidelines for the selection of target sites, evaluation of cleavage efficiency and analysis of off-target activity. The studies showed that beginning with target design, gene modifications can be achieved within as little as 1-2 weeks, and modified clonal cell lines can be derived within 2-3 weeks.

- Shalem *el al.* described a new way to interrogate gene function on a genome-wide scale. Their studies showed that delivery of a genome-scale CRISPR-Cas9 knockout (GeCKO) library targeted 18,080 genes with 64,751 unique guide sequences enabled both negative and positive selection screening in human cells. First, the authors showed use of the GeCKO library to identify genes essential for cell viability in cancer and pluripotent stem cells. Next, in a melanoma model, the authors screened for genes whose loss is involved in resistance to vemurafenib, a therapeutic that inhibits mutant protein kinase BRAF. Their studies showed that the highest-ranking candidates included previously validated genes NF1 and MED12 as well as novel hits NF2, CUL3, TADA2B, and TADA1. The authors observed a high level of consistency between independent guide RNAs targeting the same gene and a high rate of hit confirmation, and thus demonstrated the promise of genome-scale screening with Cas9.
- Nishimasu *et al.* reported the crystal structure of *Streptococcus pyogenes* Cas9 in complex with sgRNA and its target DNA at 2.5 A° resolution. The structure revealed a bilobed architecture composed of target recognition and nuclease lobes, accommodating the sgRNA:DNA heteroduplex in a positively charged groove at their interface. Whereas the recognition lobe is essential for binding sgRNA and DNA, the nuclease lobe contains the HNH and RuvC nuclease domains, which are properly positioned for cleavage of the complementary and non-complementary strands of the target DNA, respectively. The nuclease lobe also contains a carboxyl-terminal domain responsible for the interaction with the protospacer adjacent motif (PAM). This high-resolution structure and accompanying functional analyses have revealed the molecular mechanism of RNA-

guided DNA targeting by Cas9, thus paving the way for the rational design of new, versatile genome-editing technologies.

- Wu *et al.* mapped genome-wide binding sites of a catalytically inactive Cas9 (dCas9) from *Streptococcus pyogenes* loaded with single guide RNAs (sgRNAs) in mouse embryonic stem cells (mESCs). The authors showed that each of the four sgRNAs tested targets dCas9 to between tens and thousands of genomic sites, frequently characterized by a 5-nucleotide seed region in the sgRNA and an NGG protospacer adjacent motif (PAM). Chromatin inaccessibility decreases dCas9 binding to other sites with matching seed sequences; thus 70% of off-target sites are associated with genes. The authors showed that targeted sequencing of 295 dCas9 binding sites in mESCs transfected with catalytically active Cas9 identified only one site mutated above background levels. The authors proposed a two-state model for Cas9 binding and cleavage, in which a seed match triggers binding but extensive pairing with target DNA is required for cleavage.
- Platt *et al.* established a Cre-dependent Cas9 knockin mouse. The authors demonstrated *in vivo* as well as *ex vivo* genome editing using adeno-associated virus (AAV)-, lentivirus-, or particle-mediated delivery of guide RNA in neurons, immune cells, and endothelial cells.
- Hsu *et al.* (2014) is a review article that discusses generally CRISPR-Cas9 history from yogurt to genome editing, including genetic screening of cells.
- Wang *et al.* (2014) relates to a pooled, loss-of-function genetic screening approach suitable for both positive and negative selection that uses a genome-scale lentiviral single guide RNA (sgRNA) library.
- Doench *et al.* created a pool of sgRNAs, tiling across all possible target sites of a panel of six endogenous mouse and three endogenous human genes and quantitatively assessed their ability to produce null alleles of their target gene by antibody staining and flow cytometry. The authors showed that optimization of the PAM improved activity and also provided an on-line tool for designing sgRNAs.
- Swiech *et al.* demonstrate that AAV-mediated SpCas9 genome editing can enable reverse genetic studies of gene function in the brain.

- Konermann *et al.* (2015) discusses the ability to attach multiple effector domains, e.g., transcriptional activator, functional and epigenomic regulators at appropriate positions on the guide such as stem or tetraloop with and without linkers.
- Zetsche *et al.* demonstrates that the Cas9 enzyme can be split into two and hence the assembly of Cas9 for activation can be controlled.
- Chen *et al.* relates to multiplex screening by demonstrating that a genome-wide *in vivo* CRISPR-Cas9 screen in mice reveals genes regulating lung metastasis.
- Ran *et al.* (2015) relates to SaCas9 and its ability to edit genomes and demonstrates that one cannot extrapolate from biochemical assays.
- Shalem *et al.* (2015) described ways in which catalytically inactive Cas9 (dCas9) fusions are used to synthetically repress (CRISPRi) or activate (CRISPRa) expression, showing. advances using Cas9 for genome-scale screens, including arrayed and pooled screens, knockout approaches that inactivate genomic loci and strategies that modulate transcriptional activity.
- Xu *et al.* (2015) assessed the DNA sequence features that contribute to single guide RNA (sgRNA) efficiency in CRISPR-based screens. The authors explored efficiency of CRISPR-Cas9 knockout and nucleotide preference at the cleavage site. The authors also found that the sequence preference for CRISPRi/a is substantially different from that for CRISPR-Cas9 knockout.
- Parnas *et al.* (2015) introduced genome-wide pooled CRISPR-Cas9 libraries into dendritic cells (DCs) to identify genes that control the induction of tumor necrosis factor (Tnf) by bacterial lipopolysaccharide (LPS). Known regulators of Tlr4 signaling and previously unknown candidates were identified and classified into three functional modules with distinct effects on the canonical responses to LPS.
- Ramanan *et al* (2015) demonstrated cleavage of viral episomal DNA (cccDNA) in infected cells. The HBV genome exists in the nuclei of infected hepatocytes as a 3.2kb double-stranded episomal DNA species called covalently closed circular DNA (cccDNA), which is a key component in the HBV life cycle whose replication is not inhibited by current therapies. The authors showed that sgRNAs specifically targeting

highly conserved regions of HBV robustly suppresses viral replication and depleted cccDNA.

- Nishimasu *el al.* (2015) reported the crystal structures of SaCas9 in complex with a single guide RNA (sgRNA) and its double-stranded DNA targets, containing the 5'-TTGAAT-3' PAM and the 5'-TTGGGT-3' PAM. A structural comparison of SaCas9 with SpCas9 highlighted both structural conservation and divergence, explaining their distinct PAM specificities and orthologous sgRNA recognition.
- Canver *el al.* (2015) demonstrated a CRISPR-Cas9-based functional investigation of non-coding genomic elements. The authors we developed pooled CRISPR-Cas9 guide RNA libraries to perform *in situ* saturating mutagenesis of the human and mouse BCL1 1A enhancers which revealed critical features of the enhancers.
- Zetsche et al. (2015) reported characterization of Cpfl, a class 2 CRISPR nuclease from Francisella novicida U 112 having features distinct from Cas9. Cpfl is a single RNA-guided endonuclease lacking tracrRNA, utilizes a T-rich protospacer-adjacent motif, and cleaves DNA via a staggered DNA double-stranded break.
- Shmakov et al. (2015) reported three distinct Class 2 CRISPR-Cas systems. Two system CRISPR enzymes (C2cl and C2c3) contain RuvC-like endonuclease domains distantly related to Cpfl. Unlike Cpfl, C2cl depends on both crRNA and tracrRNA for DNA cleavage. The third enzyme (C2c2) contains two predicted HEPN RNase domains and is tracrRNA independent.
- Slaymaker et al (2016) reported the use of structure-guided protein engineering to improve the specificity of Streptococcus pyogenes Cas9 (SpCas9). The authors developed "enhanced specificity" SpCas9 (eSpCas9) variants which maintained robust on-target cleavage with reduced off-target effects.
- Cox et al., (2017) reported the use of catalytically inactive Casl3 (dCasl3) to direct adenosine-to-inosine deaminase activity by ADAR2 (adenosine deaminase acting on RNA type 2) to transcripts in mammalian cells. The system, referred to as RNA Editing for Programmable A to I Replacement (REPAIR), has no strict sequence constraints and can be used to edit full-length transcripts. The authors further engineered the system to create a high-specificity variant and minimized the system to facilitate viral delivery.

[0482] The methods and tools provided herein are may be designed for use with or Casl3, a type II nuclease that does not make use of tracrRNA. Orthologs of Casl3 have been identified in different bacterial species as described herein. Further type II nucleases with similar properties can be identified using methods described in the art (Shmakov et al. 2015, 60:385-397; Abudayeh et al. 2016, Science, 5;353(6299)). In particular embodiments, such methods for identifying novel CRISPR effector proteins may comprise the steps of selecting sequences from the database encoding a seed which identifies the presence of a CRISPR Cas locus, identifying loci located within 10 kb of the seed comprising Open Reading Frames (ORFs) in the selected sequences, selecting therefrom loci comprising ORFs of which only a single ORF encodes a novel CRISPR effector having greater than 700 amino acids and no more than 90% homology to a known CRISPR effector. In particular embodiments, the seed is a protein that is common to the CRISPR-Cas system, such as Casl. In further embodiments, the CRISPR array is used as a seed to identify new effector proteins.

[0483] Also, "Dimeric CRISPR RNA-guided Fokl nucleases for highly specific genome editing", Shengdar Q. Tsai, Nicolas Wyvekens, Cyd Khayter, Jennifer A. Foden, Vishal Thapar, Deepak Reyon, Mathew J. Goodwin, Martin J. Aryee, J. Keith Joung Nature Biotechnology 32(6): 569-77 (2014), relates to dimeric RNA-guided Fokl Nucleases that recognize extended sequences and can edit endogenous genes with high efficiencies in human cells.

[0484] Also, Harrington et al. "Programmed DNA destruction by miniature CRISPR-Casl4 enzymes" Science 2018 doi:l0/H26/science.aav4293, relates to Casl4.

[0485] With respect to general information on CRISPR/Cas Systems, components thereof, and delivery of such components, including methods, materials, delivery vehicles, vectors, particles, and making and using thereof, including as to amounts and formulations, as well as CRISPR-Cas-expressing eukaryotic cells, CRISPR-Cas expressing eukaryotes, such as a mouse, reference is made to: US Patents Nos. 8,999,641, 8,993,233, 8,697,359, 8,771,945, 8,795,965, 8,865,406, 8,871,445, 8,889,356, 8,889,418, 8,895,308, 8,906,616, 8,932,814, and 8,945,839; US Patent Publications US 2014-0310830 (US App. Ser. No. 14/105,031), US 2014-0287938 Al (U.S. App. Ser. No. 14/213,991), US 2014-0273234 Al (U.S. App. Ser. No. 14/293,674), US2014-0273232 Al (U.S. App. Ser. No. 14/290,575), US 2014-0273231 (U.S. App. Ser. No. 14/259,420), US 2014-0256046 Al (U.S. App. Ser. No. 14/226,274), US 2014-0248702 Al

(U.S. App. Ser. No. 14/258,458), US 2014-0242700 A1 (U.S. App. Ser. No. 14/222,930), US 2014-0242699 A1 (U.S. App. Ser. No. 14/183,512), US 2014-0242664 A1 (U.S. App. Ser. No. 14/104,990), US 2014-0234972 A1 (U.S. App. Ser. No. 14/183,471), US 2014-0227787 A1 (U.S. App. Ser. No. 14/256,912), US 2014-0189896 A1 (U.S. App. Ser. No. 14/105,035), US 2014-0186958 (U.S. App. Ser. No. 14/105,017), US 2014-0186919 A1 (U.S. App. Ser. No. 14/104,977), US 2014-0186843 A1 (U.S. App. Ser. No. 14/104,900), US 2014-0179770 A1 (U.S. App. Ser. No. 14/104,837) and US 2014-0179006 A1 (U.S. App. Ser. No. 14/183,486), US 2014-0170753 (US App Ser No 14/183,429); US 2015-0184139 (U.S. App. Ser. No. 14/324,960); 14/054,414 European Patent Applications EP 2 771 468 (EP13818570.7), EP 2 764 103 (EP13824232.6), and EP 2 784 162 (EP 14 170383. 5); and PCT Patent Publications WO20 14/093 661 (PCT/US20 13/074743), WO20 14/093 694 (PCT/US20 13/074790), WO2014/093595 (PCT/US20 13/0746 11), WO20 14/0937 18 (PCT/US20 13/074825), WO20 14/093 709 (PCT/US20 13/0748 12), WO20 14/093 622 (PCT/US20 13/074667), WO2014/093635 (PCT/US20 13/074691), WO2014/093655 (PCT/US20 13/074736), WO20 14/0937 12 (PCT/US20 13/0748 19), WO20 14/093 701 (PCT/US20 13/074800), WO20 14/0 18423 (PCT/US2013/051418), WO20 14/204723 (PCT/US20 14/04 1790), WO20 14/204724 (PCT/US20 14/04 1800), WO20 14/204725 (PCT/US20 14/04 1803), WO20 14/204726 (PCT/US20 14/04 1804), WO20 14/204727 (PCT/US20 14/04 1806), WO20 14/204728 (PCT/US20 14/04 1808), WO20 14/204729 (PCT/US20 14/04 1809), WO20 15/0893 51 (PCT/US20 14/069897), WO20 15/0893 54 (PCT/US20 14/069902), WO2015/089364 (PCT/US20 14/069925), WO20 15/089427 (PCT/US20 14/070068), WO20 15/089462 (PCT/US20 14/070 127), WO20 15/0894 19 (PCT/US20 14/070057), WO20 15/089465 (PCT/US2014/070135), WO20 15/089486 (PCT/US20 14/070 175), W02015/058052 (PCT/US20 14/06 1077), WO20 15/070083 (PCT/US20 14/064663), WO20 15/0893 54 (PCT/US20 14/069902), WO20 15/0893 51 (PCT/US20 14/069897), WO2015/089364 (PCT/US20 14/069925), WO20 15/089427 (PCT/US20 14/070068), WO20 15/089473 (PCT/US20 14/070 152), WO20 15/089486 (PCT/US20 14/070 175), WO20 16/049258 (PCT/US20 15/05 1830), WO20 16/094867 (PCT/US20 15/0653 85), WO20 16/094872 (PCT/US20 15/065393), WO20 16/094874 (PCT/US20 15/065396), WO20 16/106244 (PCT/US20 15/067 177).

[0486] Mention is also made of US application 62/180,709, l7-Jun-l5, PROTECTED GUIDE RNAS (PGRNAS); US application 62/091,455, filed, l2-Dec-l4, PROTECTED GUIDE RNAS (PGRNAS); US application 62/096,708, 24-Dec-l4, PROTECTED GUIDE RNAS (PGRNAS); US applications 62/091,462, l2-Dec-l4, 62/096,324, 23-Dec-l4, 62/180,681, 17-Jun-20l5, and 62/237,496, 5-Oct-20l5, DEAD GUIDES FOR CRISPR TRANSCRIPTION FACTORS; US application 62/091,456, l2-Dec-l4 and 62/180,692, l7-Jun-20l5, ESCORTED AND FUNCTIONALIZED GUIDES FOR CRISPR-CAS SYSTEMS; US application 62/091,461, 12-Dec-14, DELIVERY, USE AND THERAPEUTIC APPLICATIONS OF THE CRISPR-CAS SYSTEMS AND COMPOSITIONS FOR GENOME EDITING AS TO HEMATOPOETIC STEM CELLS (HSCs); US application 62/094,903, l9-Dec-l4, UNBIASED IDENTIFICATION OF DOUBLE-STRAND BREAKS AND GENOMIC REARRANGEMENT BY GENOME-WISE INSERT CAPTURE SEQUENCING; US application 62/096,761, 24-Dec-14, ENGINEERING OF SYSTEMS, METHODS AND OPTIMIZED ENZYME AND GUIDE SCAFFOLDS FOR SEQUENCE MANIPULATION; US application 62/098,059, 30-Dec-l4, 62/181,641, l8-Jun-20l5, and 62/181,667, l8-Jun-20l5, RNA-TARGETING SYSTEM; US application 62/096,656, 24-Dec-l4 and 62/181,151, l7-Jun-20l5, CRISPR HAVING OR ASSOCIATED WITH DESTABILIZATION DOMAINS; US application 62/096,697, 24-Dec-14, CRISPR HAVING OR ASSOCIATED WITH AAV; US application 62/098,158, 30-Dec-l4, ENGINEERED CRISPR COMPLEX INSERTIONAL TARGETING SYSTEMS; US application 62/151,052, 22-Apr-l5, CELLULAR TARGETING FOR EXTRACELLULAR EXOSOMAL REPORTING; US application 62/054,490, 24-Sep-l4, DELIVERY, USE AND THERAPEUTIC APPLICATIONS OF THE CRISPR-CAS SYSTEMS AND COMPOSITIONS FOR TARGETING DISORDERS AND DISEASES USING PARTICLE DELIVERY COMPONENTS; US application 61/939,154, 12-F EB-14, SYSTEMS, METHODS AND COMPOSITIONS FOR SEQUENCE MANIPULATION WITH OPTIMIZED FUNCTIONAL CRISPR-CAS SYSTEMS; US application 62/055,484, 25-Sep-l4, SYSTEMS, METHODS AND COMPOSITIONS FOR SEQUENCE MANIPULATION WITH OPTIMIZED FUNCTIONAL CRISPR-CAS SYSTEMS; US application 62/087,537, 4-Dec-l4, SYSTEMS, METHODS AND COMPOSITIONS FOR SEQUENCE MANIPULATION WITH OPTIMIZED FUNCTIONAL CRISPR-CAS SYSTEMS; US application 62/054,651, 24-Sep-l4, DELIVERY,

USE AND THERAPEUTIC APPLICATIONS OF THE CRISPR-CAS SYSTEMS AND COMPOSITIONS FOR MODELING COMPETITION OF MULTIPLE CANCER MUTATIONS IN VIVO; US application 62/067,886, 23-Oct-l4, DELIVERY, USE AND THERAPEUTIC APPLICATIONS OF THE CRISPR-CAS SYSTEMS AND COMPOSITIONS FOR MODELING COMPETITION OF MULTIPLE CANCER MUTATIONS IN VIVO; US applications 62/054,675, 24-Sep-l4 and 62/181,002, l7-Jun-20l5, DELIVERY, USE AND THERAPEUTIC APPLICATIONS OF THE CRISPR-CAS SYSTEMS AND COMPOSITIONS IN NEURONAL CELLS/TISSUES; US application 62/054,528, 24-Sep-l4, DELIVERY, USE AND THERAPEUTIC APPLICATIONS OF THE CRISPR-CAS SYSTEMS AND COMPOSITIONS IN IMMUNE DISEASES OR DISORDERS; US application 62/055,454, 25-Sep-l4, DELIVERY, USE AND THERAPEUTIC APPLICATIONS OF THE CRISPR-CAS SYSTEMS AND COMPOSITIONS FOR TARGETING DISORDERS AND DISEASES USING CELL PENETRATION PEPTIDES (CPP); US application 62/055,460, 25-Sep-l4, MULTIFUNCTIONAL-CRISPR COMPLEXES AND/OR OPTIMIZED ENZYME LINKED FUNCTIONAL-CRISPR COMPLEXES; US application 62/087,475, 4-Dec-l4 and 62/181,690, l8-Jun-20l5, FUNCTIONAL SCREENING WITH OPTIMIZED FUNCTIONAL CRISPR-CAS SYSTEMS; US application 62/055,487, 25-Sep-l4, FUNCTIONAL SCREENING WITH OPTIMIZED FUNCTIONAL CRISPR-CAS SYSTEMS; US application 62/087,546, 4-Dec-l4 and 62/181,687, l8-Jun-20l5, MULTIFUNCTIONAL CRISPR COMPLEXES AND/OR OPTIMIZED ENZYME LINKED FUNCTIONAL-CRISPR COMPLEXES; and US application 62/098,285, 30-Dec-l4, CRISPR MEDIATED IN VIVO MODELING AND GENETIC SCREENING OF TUMOR GROWTH AND METASTASIS.

[0487] Mention is made of US applications 62/181,659, l8-Jun-20l5 and 62/207,318, 19-Aug-20l5, ENGINEERING AND OPTIMIZATION OF SYSTEMS, METHODS, ENZYME AND GUIDE SCAFFOLDS OF CAS9 ORTHOLOGS AND VARIANTS FOR SEQUENCE MANIPULATION. Mention is made of US applications 62/181,663, l8-Jun-20l5 and 62/245,264, 22-Oct-20l5, NOVEL CRISPR ENZYMES AND SYSTEMS, US applications 62/181,675, l8-Jun-20l5, 62/285,349, 22-Oct-20l5, 62/296,522, l7-Feb-20l6, and 62/320,231, 8-Apr-20l6, NOVEL CRISPR ENZYMES AND SYSTEMS, US application 62/232,067, 24-Sep-20l5, US Application 14/975,085, !8-Dec-20l5, European application No. 16150428.7, US

application 62/205,733, l6-Aug-20l5, US application 62/201,542, 5-Aug-20l5, US application 62/193,507, 16-M-2015, and US application 62/181,739, l8-Jun-20l5, each entitled NOVEL CRISPR ENZYMES AND SYSTEMS and of US application 62/245,270, 22-Oct-20l5, NOVEL CRISPR ENZYMES AND SYSTEMS. Mention is also made of US application 61/939,256, 12-Feb-20l4, and WO 2015/089473 (PCT/US20 14/070 152), l2-Dec-20l4, each entitled ENGINEERING OF SYSTEMS, METHODS AND OPTIMIZED GUIDE COMPOSITIONS WITH NEW ARCHITECTURES FOR SEQUENCE MANIPULATION. Mention is also made of PCT/US20 15/045504, l5-Aug-20l5, US application 62/180,699, l7-Jun-20l5, and US application 62/038,358, l7-Aug-20l4, each entitled GENOME EDITING USING CAS9 NICKASES.

[0488] Each of these patents, patent publications, and applications, and all documents cited therein or during their prosecution ("appln cited documents") and all documents cited or referenced in the appln cited documents, together with any instructions, descriptions, product specifications, and product sheets for any products mentioned therein or in any document therein and incorporated by reference herein, are hereby incorporated herein by reference, and may be employed in the practice of the invention. All documents (e.g., these patents, patent publications and applications and the appln cited documents) are incorporated herein by reference to the same extent as if each individual document was specifically and individually indicated to be incorporated by reference.

[0489] In particular embodiments, pre-complexed guide RNA and CRISPR effector protein, (optionally, adenosine deaminase fused to a CRISPR protein or an adaptor) are delivered as a ribonucleoprotein (RNP). RNPs have the advantage that they lead to rapid editing effects even more so than the RNA method because this process avoids the need for transcription. An important advantage is that both RNP delivery is transient, reducing off-target effects and toxicity issues. Efficient genome editing in different cell types has been observed by Kim et al. (2014, Genome Res. 24(6): 1012-9), Paix et al. (2015, Genetics 204(l):47-54), Chu et al. (2016, BMC Biotechnol. 16:4), and Wang et al. (2013, Cell. 9;153(4):910-8).

[0490] In particular embodiments, the ribonucleoprotein is delivered by way of a polypeptide-based shuttle agent as described in WO2016161516. WO2016161516 describes efficient transduction of polypeptide cargos using synthetic peptides comprising an endosome

leakage domain (ELD) operably linked to a cell penetrating domain (CPD), to a histidine-rich domain and a CPD. Similarly these polypeptides can be used for the delivery of CRISPR-effector based RNPs in eukaryotic cells.

Tale Systems

[0491] As disclosed herein editing can be made by way of the transcription activator-like effector nucleases (TALENs) system. Transcription activator-like effectors (TALEs) can be engineered to bind practically any desired DNA sequence. Exemplary methods of genome editing using the TALEN system can be found for example in Cermak T. Doyle EL. Christian M. Wang L. Zhang Y. Schmidt C, et al. Efficient design and assembly of custom TALEN and other TAL effector-based constructs for DNA targeting. Nucleic Acids Res. 20l l;39:e82; Zhang F. Cong L. Lodato S. Kosuri S. Church GM. Arlotta P Efficient construction of sequence-specific TAL effectors for modulating mammalian transcription. Nat Biotechnol. 201 1;29:149-153 and US Patent Nos. 8,450,471, 8,440,431 and 8,440,432, all of which are specifically incorporated by reference.

[0492] In advantageous embodiments of the invention, the methods provided herein use isolated, non-naturally occurring, recombinant or engineered DNA binding proteins that comprise TALE monomers as a part of their organizational structure that enable the targeting of nucleic acid sequences with improved efficiency and expanded specificity.

[0493] Naturally occurring TALEs or "wild type TALEs" are nucleic acid binding proteins secreted by numerous species of proteobacteria. TALE polypeptides contain a nucleic acid binding domain composed of tandem repeats of highly conserved monomer polypeptides that are predominantly 33, 34 or 35 amino acids in length and that differ from each other mainly in amino acid positions 12 and 13. In advantageous embodiments the nucleic acid is DNA. As used herein, the term "polypeptide monomers", or "TALE monomers" will be used to refer to the highly conserved repetitive polypeptide sequences within the TALE nucleic acid binding domain and the term "repeat variable di-residues" or "RVD" will be used to refer to the highly variable amino acids at positions 12 and 13 of the polypeptide monomers. As provided throughout the disclosure, the amino acid residues of the RVD are depicted using the IUPAC single letter code for amino acids. A general representation of a TALE monomer which is comprised within the DNA binding domain is Xl-l 1-(X12X13)-X14-33 or 34 or 35, where the subscript indicates the

amino acid position and X represents any amino acid. X12X13 indicate the RVDs. In some polypeptide monomers, the variable amino acid at position 13 is missing or absent and in such polypeptide monomers, the RVD consists of a single amino acid. In such cases the RVD may be alternatively represented as X*, where X represents X12 and (*) indicates that X13 is absent. The DNA binding domain comprises several repeats of TALE monomers and this may be represented as (X1-11-(X12X13)-X14-33 or 34 or 35)z, where in an advantageous embodiment, z is at least 5 to 40. In a further advantageous embodiment, z is at least 10 to 26.

[0494] The TALE monomers have a nucleotide binding affinity that is determined by the identity of the amino acids in its RVD. For example, polypeptide monomers with an RVD of NI preferentially bind to adenine (A), polypeptide monomers with an RVD of NG preferentially bind to thymine (T), polypeptide monomers with an RVD of HD preferentially bind to cytosine (C) and polypeptide monomers with an RVD of NN preferentially bind to both adenine (A) and guanine (G). In yet another embodiment of the invention, polypeptide monomers with an RVD of IG preferentially bind to T. Thus, the number and order of the polypeptide monomer repeats in the nucleic acid binding domain of a TALE determines its nucleic acid target specificity. In still further embodiments of the invention, polypeptide monomers with an RVD of NS recognize all four base pairs and may bind to A, T, G or C. The structure and function of TALEs is further described in, for example, Moscou et al., Science 326:1501 (2009); Boch et al., Science 326:1509-1512 (2009); and Zhang et al., Nature Biotechnology 29:149-153 (2011), each of which is incorporated by reference in its entirety.

[0495] The TALE polypeptides used in methods of the invention are isolated, non-naturally occurring, recombinant or engineered nucleic acid-binding proteins that have nucleic acid or DNA binding regions containing polypeptide monomer repeats that are designed to target specific nucleic acid sequences.

[0496] As described herein, polypeptide monomers having an RVD of HN or NH preferentially bind to guanine and thereby allow the generation of TALE polypeptides with high binding specificity for guanine containing target nucleic acid sequences. In a preferred embodiment of the invention, polypeptide monomers having RVDs RN, NN, NK, SN, NH, KN, HN, NQ, HH, RG, KH, RH and SS preferentially bind to guanine. In a much more advantageous embodiment of the invention, polypeptide monomers having RVDs RN, NK, NQ, HH, KH, RH,

SS and SN preferentially bind to guanine and thereby allow the generation of TALE polypeptides with high binding specificity for guanine containing target nucleic acid sequences. In an even more advantageous embodiment of the invention, polypeptide monomers having RVDs HH, KH, NH, NK, NQ, RH, RN and SS preferentially bind to guanine and thereby allow the generation of TALE polypeptides with high binding specificity for guanine containing target nucleic acid sequences. In a further advantageous embodiment, the RVDs that have high binding specificity for guanine are RN, NH RH and KH. Furthermore, polypeptide monomers having an RVD of NV preferentially bind to adenine and guanine. In more preferred embodiments of the invention, polypeptide monomers having RVDs of H*, HA, KA, N*, NA, NC, NS, RA, and S* bind to adenine, guanine, cytosine and thymine with comparable affinity.

[0497] The predetermined N-terminal to C-terminal order of the one or more polypeptide monomers of the nucleic acid or DNA binding domain determines the corresponding predetermined target nucleic acid sequence to which the TALE polypeptides will bind. As used herein the polypeptide monomers and at least one or more half polypeptide monomers are "specifically ordered to target" the genomic locus or gene of interest. In plant genomes, the natural TALE-binding sites always begin with a thymine (T), which may be specified by a cryptic signal within the non-repetitive N-terminus of the TALE polypeptide; in some cases this region may be referred to as repeat 0. In animal genomes, TALE binding sites do not necessarily have to begin with a thymine (T) and TALE polypeptides may target DNA sequences that begin with T, A, G or C. The tandem repeat of TALE monomers always ends with a half-length repeat or a stretch of sequence that may share identity with only the first 20 amino acids of a repetitive full length TALE monomer and this half repeat may be referred to as a half-monomer (FIG. 8), which is included in the term "TALE monomer". Therefore, it follows that the length of the nucleic acid or DNA being targeted is equal to the number of full polypeptide monomers plus two.

[0498] As described in Zhang et al., Nature Biotechnology 29:149-153 (2011), TALE polypeptide binding efficiency may be increased by including amino acid sequences from the "capping regions" that are directly N-terminal or C-terminal of the DNA binding region of naturally occurring TALEs into the engineered TALEs at positions N-terminal or C-terminal of the engineered TALE DNA binding region. Thus, in certain embodiments, the TALE

polypeptides described herein further comprise an N-terminal capping region and/or a C-terminal capping region.

An exemplary amino acid sequence of a N-terminal capping region is:

M D P I R S R T P S P A R E L L S G P Q P D G V Q P T A D R G V S P

P A G G P L D G L P A R R T M S R T R L P S P P A P S P A F S A D S

F S D L L R Q F D P S L F N T S L F D S L P P F G A H H T E AAT G

E W D E V Q S G F R AAD A P P P T M R VAV TAA R P P R A K P A

P R R R A A Q P S D A S P A A Q V D F R T F G Y S Q Q Q Q E K I K P

K V R S T V A Q H H E A F V G H G F T H A H I V A F S Q H P A A F G

T VAVKY Q D M I A A L P E A T H E A I V G V G K Q W S G A R A L

E A F F T V A G E F R G P P F Q F D T G Q F F K I A K R G G V T A V

E A V H A W R N A F T G A P F N (SEQ ID No. 3)

An exemplary amino acid sequence of a C-terminal capping region is:

R P A F E S I V A Q F S R P D P A F AAF T N D H F VAF A C F G

G R P A L D AVK K G L P H A P A L I K R T N R R I P E R T S H R

V A D H A Q V V R V F G F F Q C H S H P A Q A F D D A M T Q F G M

S R H G F F Q F F R R V G V T E F E A R S G T F P P A S Q R W D R

I F Q A S G M K R A K P S P T S T Q T P D Q A S F H A F A D S F E

R D F D A P S P M H E G D Q T R A S (SEQ ID No. 4)

[0499] As used herein the predetermined "N-terminus" to "C terminus" orientation of the N-terminal capping region, the DNA binding domain comprising the repeat TALE monomers and the C-terminal capping region provide structural basis for the organization of different domains in the d-TALEs or polypeptides of the invention.

[0500] The entire N-terminal and/or C-terminal capping regions are not necessary to enhance the binding activity of the DNA binding region. Therefore, in certain embodiments, fragments of the N-terminal and/or C-terminal capping regions are included in the TALE polypeptides described herein.

[0501] In certain embodiments, the TALE polypeptides described herein contain a N-terminal capping region fragment that included at least 10, 20, 30, 40, 50, 54, 60, 70, 80, 87, 90, 94, 100, 102, 110, 117, 120, 130, 140, 147, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260 or 270 amino acids of an N-terminal capping region. In certain embodiments, the N-terminal capping region fragment amino acids are of the C-terminus (the DNA-binding region proximal end) of an N-terminal capping region. As described in Zhang et al., Nature Biotechnology 29:149-153 (2011), N-terminal capping region fragments that include the C-terminal 240 amino acids enhance binding activity equal to the full length capping region, while fragments that include the C-terminal 147 amino acids retain greater than 80% of the efficacy of the full length capping region, and fragments that include the C-terminal 117 amino acids retain greater than 50% of the activity of the full-length capping region.

[0502] In some embodiments, the TALE polypeptides described herein contain a C-terminal capping region fragment that included at least 6, 10, 20, 30, 37, 40, 50, 60, 68, 70, 80, 90, 100, 110, 120, 127, 130, 140, 150, 155, 160, 170, 180 amino acids of a C-terminal capping region. In certain embodiments, the C-terminal capping region fragment amino acids are of the N-terminus (the DNA-binding region proximal end) of a C-terminal capping region. As described in Zhang et al., Nature Biotechnology 29:149-153 (2011), C-terminal capping region fragments that include the C-terminal 68 amino acids enhance binding activity equal to the full length capping region, while fragments that include the C-terminal 20 amino acids retain greater than 50% of the efficacy of the full length capping region.

[0503] In certain embodiments, the capping regions of the TALE polypeptides described herein do not need to have identical sequences to the capping region sequences provided herein. Thus, in some embodiments, the capping region of the TALE polypeptides described herein have sequences that are at least 50%, 60%, 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical or share identity to the capping region amino acid sequences provided herein. Sequence identity is related to sequence homology. Homology comparisons may be conducted by eye, or more usually, with the aid of readily available sequence comparison programs. These commercially available computer programs may calculate percent (%) homology between two or more sequences and may also calculate the sequence identity shared by two or more amino acid or nucleic acid sequences. In some preferred embodiments, the

capping region of the TALE polypeptides described herein have sequences that are at least 95% identical or share identity to the capping region amino acid sequences provided herein.

[0504] Sequence homologies may be generated by any of a number of computer programs known in the art, which include but are not limited to BLAST or FASTA. Suitable computer program for carrying out alignments like the GCG Wisconsin Bestfit package may also be used. Once the software has produced an optimal alignment, it is possible to calculate % homology, preferably % sequence identity. The software typically does this as part of the sequence comparison and generates a numerical result.

[0505] In advantageous embodiments described herein, the TALE polypeptides of the invention include a nucleic acid binding domain linked to the one or more effector domains. The terms “effector domain” or “regulatory and functional domain” refer to a polypeptide sequence that has an activity other than binding to the nucleic acid sequence recognized by the nucleic acid binding domain. By combining a nucleic acid binding domain with one or more effector domains, the polypeptides of the invention may be used to target the one or more functions or activities mediated by the effector domain to a particular target DNA sequence to which the nucleic acid binding domain specifically binds.

[0506] In some embodiments of the TALE polypeptides described herein, the activity mediated by the effector domain is a biological activity. For example, in some embodiments the effector domain is a transcriptional inhibitor (i.e., a repressor domain), such as an mSin interaction domain (SID). SID4X domain or a Kriippel-associated box (KRAB) or fragments of the KRAB domain. In some embodiments the effector domain is an enhancer of transcription (i.e. an activation domain), such as the VP16, VP64 or p65 activation domain. In some embodiments, the nucleic acid binding is linked, for example, with an effector domain that includes but is not limited to a transposase, integrase, recombinase, resolvase, invertase, protease, DNA methyltransferase, DNA demethylase, histone acetylase, histone deacetylase, nuclease, transcriptional repressor, transcriptional activator, transcription factor recruiting, protein nuclear-localization signal or cellular uptake signal.

[0507] In some embodiments, the effector domain is a protein domain which exhibits activities which include but are not limited to transposase activity, integrase activity, recombinase activity, resolvase activity, invertase activity, protease activity, DNA

methyltransferase activity, DNA demethylase activity, histone acetylase activity, histone deacetylase activity, nuclease activity, nuclear-localization signaling activity, transcriptional repressor activity, transcriptional activator activity, transcription factor recruiting activity, or cellular uptake signaling activity. Other preferred embodiments of the invention may include any combination the activities described herein.

ZN-Finger Nucleases

[0508] Other preferred tools for genome editing for use in the context of this invention include zinc finger systems. One type of programmable DNA-binding domain is provided by artificial zinc-finger (ZF) technology, which involves arrays of ZF modules to target new DNA-binding sites in the genome. Each finger module in a ZF array targets three DNA bases. A customized array of individual zinc finger domains is assembled into a ZF protein (ZFP).

[0509] ZFPs can comprise a functional domain. The first synthetic zinc finger nucleases (ZFNs) were developed by fusing a ZF protein to the catalytic domain of the Type IIS restriction enzyme Fokl. (Kim, Y. G. et al., 1994, Chimeric restriction endonuclease, Proc. Natl. Acad. Sci. U.S.A. 91, 883-887; Kim, Y. G. et al., 1996, Hybrid restriction enzymes: zinc finger fusions to Fok I cleavage domain. Proc. Natl. Acad. Sci. U.S.A. 93, 1156-1 160). Increased cleavage specificity can be attained with decreased off target activity by use of paired ZFN heterodimers, each targeting different nucleotide sequences separated by a short spacer. (Doyon, Y. et ak, 201 1, Enhancing zinc-fmger-nuclease activity with improved obligate heterodimeric architectures. Nat. Methods 8, 74-79). ZFPs can also be designed as transcription activators and repressors and have been used to target many genes in a wide variety of organisms. Exemplary methods of genome editing using ZFNs can be found for example in U.S. Patent Nos. 6,534,261, 6,607,882, 6,746,838, 6,794,136, 6,824,978, 6,866,997, 6,933,1 13, 6,979,539, 7,013,219, 7,030,215, 7,220,719, 7,241,573, 7,241,574, 7,585,849, 7,595,376, 6,903,185, and 6,479,626, all of which are specifically incorporated by reference.

Meganucleases

[0510] As disclosed herein editing can be made by way of meganucleases, which are endodeoxyribonucleases characterized by a large recognition site (double-stranded DNA sequences of 12 to 40 base pairs). Exemplary method for using meganucleases can be found in

US Patent Nos: 8,163,514; 8,133,697; 8,021,867; 8,119,361; 8,119,381; 8,124,369; and 8,129,134, which are specifically incorporated by reference.

RNAi

[0511] In certain embodiments, the genetic modifying agent is RNAi (e.g., shRNA). As used herein, "gene silencing" or "gene silenced" in reference to an activity of an RNAi molecule, for example a siRNA or miRNA refers to a decrease in the mRNA level in a cell for a target gene by at least about 5%, about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 95%, about 99%, about 100% of the mRNA level found in the cell without the presence of the miRNA or RNA interference molecule. In one preferred embodiment, the mRNA levels are decreased by at least about 70%, about 80%, about 90%, about 95%, about 99%, about 100%.

[0512] As used herein, the term "RNAi" refers to any type of interfering RNA, including but not limited to, siRNAi, shRNAi, endogenous microRNA and artificial microRNA. For instance, it includes sequences previously identified as siRNA, regardless of the mechanism of down-stream processing of the RNA (i.e. although siRNAs are believed to have a specific method of in vivo processing resulting in the cleavage of mRNA, such sequences can be incorporated into the vectors in the context of the flanking sequences described herein). The term "RNAi" can include both gene silencing RNAi molecules, and also RNAi effector molecules which activate the expression of a gene.

[0513] As used herein, a "siRNA" refers to a nucleic acid that forms a double stranded RNA, which double stranded RNA has the ability to reduce or inhibit expression of a gene or target gene when the siRNA is present or expressed in the same cell as the target gene. The double stranded RNA siRNA can be formed by the complementary strands. In one embodiment, a siRNA refers to a nucleic acid that can form a double stranded siRNA. The sequence of the siRNA can correspond to the full-length target gene, or a subsequence thereof. Typically, the siRNA is at least about 15-50 nucleotides in length (e.g., each complementary sequence of the double stranded siRNA is about 15-50 nucleotides in length, and the double stranded siRNA is about 15-50 base pairs in length, preferably about 19-30 base nucleotides, preferably about 20-25 nucleotides in length, e.g., 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides in length).

[0514] As used herein “shRNA” or “small hairpin RNA” (also called stem loop) is a type of siRNA. In one embodiment, these shRNAs are composed of a short, e.g. about 19 to about 25 nucleotide, antisense strand, followed by a nucleotide loop of about 5 to about 9 nucleotides, and the analogous sense strand. Alternatively, the sense strand can precede the nucleotide loop structure and the antisense strand can follow.

[0515] The terms “microRNA” or “miRNA” are used interchangeably herein are endogenous RNAs, some of which are known to regulate the expression of protein-coding genes at the posttranscri phonal level. Endogenous microRNAs are small RNAs naturally present in the genome that are capable of modulating the productive utilization of mRNA. The term artificial microRNA includes any type of RNA sequence, other than endogenous microRNA, which is capable of modulating the productive utilization of mRNA. MicroRNA sequences have been described in publications such as Lim, et al., Genes & Development, 17, p. 991 - 1008 (2003), Lim et al Science 299, 1540 (2003), Lee and Ambros Science, 294, 862 (2001), Lau et al., Science 294, 858-861 (2001), Lagos-Quintana et al, Current Biology, 12, 735-739 (2002), Lagos Quintana et al, Science 294, 853- 857 (2001), and Lagos-Quintana et al, RNA, 9, 175- 179 (2003), which are incorporated by reference. Multiple microRNAs can also be incorporated into a precursor molecule. Furthermore, miRNA-like stem-loops can be expressed in cells as a vehicle to deliver artificial miRNAs and short interfering RNAs (siRNAs) for the purpose of modulating the expression of endogenous genes through the miRNA and or RNAi pathways.

[0516] As used herein, “double stranded RNA” or “dsRNA” refers to RNA molecules that are comprised of two strands. Double-stranded molecules include those comprised of a single RNA molecule that doubles back on itself to form a two-stranded structure. For example, the stem loop structure of the progenitor molecules from which the single-stranded miRNA is derived, called the pre-miRNA (Bartel et al. 2004. Cell 1 16:281 -297), comprises a dsRNA molecule.

Transcriptional Activation/Repression

[0517] In certain embodiments, an immunomodulant may comprise (i) a DNA-binding portion configured to specifically bind to the endogenous gene and (ii) an effector domain mediating a biological activity.

[0518] In certain embodiments, the DNA-binding portion may comprise a zinc finger protein or DNA-binding domain thereof, a transcription activator-like effector (TALE) protein or DNA-binding domain thereof, or an RNA-guided protein or DNA-binding domain thereof.

[0519] In certain embodiments, the DNA-binding portion may comprise (i) Cas9 or Cpfl or any Cas protein described herein modified to eliminate its nuclease activity, or (ii) DNA-binding domain of Cas9 or Cpfl or any Cas protein described herein.

[0520] In some embodiments, the effector domain may be a transcriptional inhibitor (i.e., a repressor domain), such as an mSin interaction domain (SID). SID4X domain or a Kriippel-associated box (KRAB) or fragments of the KRAB domain. In some embodiments, the effector domain may be an enhancer of transcription (i.e. an activation domain), such as the VP16, VP64 or p65 activation domain. In some embodiments, the nucleic acid binding portion may be linked, for example, with an effector domain that includes but is not limited to a transposase, integrase, recombinase, resolvase, invertase, protease, DNA methyltransferase, DNA demethylase, histone acetylase, histone deacetylase, nuclease, transcriptional repressor, transcriptional activator, transcription factor recruiting, protein nuclear-localization signal or cellular uptake signal. In some embodiments, the effector domain may be a protein domain which exhibits activities which include but are not limited to transposase activity, integrase activity, recombinase activity, resolvase activity, invertase activity, protease activity, DNA methyltransferase activity, DNA demethylase activity, histone acetylase activity, histone deacetylase activity, nuclease activity, nuclear-localization signaling activity, transcriptional repressor activity, transcriptional activator activity, transcription factor recruiting activity, or cellular uptake signaling activity. Other preferred embodiments of the invention may include any combination the activities described herein.

Antibody Drug Conjugate

[0521] In certain embodiments, the agent capable of specifically binding to a gene product expressed on the cell surface of the immune cell is an antibody.

[0522] By means of an example, an agent, such as an antibody, capable of specifically binding to a gene product expressed on the cell surface of the immune cells may be conjugated with a therapeutic or effector agent for targeted delivery of the therapeutic or effector agent to the immune cells.

[0523] Examples of such therapeutic or effector agents include immunomodulatory classes as discussed herein, such as without limitation a toxin, drug, radionuclide, cytokine, lymphokine, chemokine, growth factor, tumor necrosis factor, hormone, hormone antagonist, enzyme, oligonucleotide, siRNA, RNAi, photoactive therapeutic agent, anti-angiogenic agent and pro-apoptotic agent.

[0524] Example toxins include ricin, abrin, alpha toxin, saporin, ribonuclease (RNase), DNase I, Staphylococcal enterotoxin-A, pokeweed antiviral protein, gelonin, diphtheria toxin, Pseudomonas exotoxin, or Pseudomonas endotoxin.

[0525] Example radionuclides include ^{103m}Rh, ^{103}Ru, ^{105}Rh, ^{105}Ru, ^{107}Hg, ^{109}Pd, ^{109}Pt, ^{111}Ag, ^{111}In, ^{113m}In ^{119}Sb, ^{11}C, ^{121m}Te, ^{122m}Te, ^{125}I, ^{125m}Te, ^{126}I, ^{131}I, ^{133}I, ^{13}N, ^{142}Pr, ^{143}Pr, ^{149}Pm, ^{152}Dy, ^{153}Sm, ^{15}0 , ^{161}Ho, ^{161}Tb, ^{165}Tm, ^{166}Dy, ^{166}Ho, ^{167}Tm, ^{168}Tm, ^{169}Er, ^{169}Yb, ^{177}Lu, ^{186}Re, ^{188}Re, ^{189m}Os, ^{189}Re, ^{192}Ir, ^{194}Ir, ^{197}Pt, ^{198}Au, ^{199}Au, ^{201}Tl, ^{203}Hg, ^{211}At, ^{211}Bi, ^{211}Pb, ^{212}Bi, ^{212}Pb, ^{213}Bi, ^{215}Po, ^{217}At, ^{219}Rn, ^{221}Fr, ^{223}Ra, ^{224}Ac, ^{225}Ac, ^{225}Fm, ^{32}P, ^{33}P, ^{47}Sc, ^{51}Cr, ^{57}Co, ^{58}Co, ^{59}Fe, ^{62}Cu, ^{67}Cu, ^{67}Ga, ^{75}Br, ^{75}Se, ^{76}Br, ^{77}As, ^{77}Br, ^{80m}Br, ^{89}Sr, ^{90}Y, ^{95}Ru, ^{97}Ru, ^{99}Mo or ^{99m}Tc. Preferably, the radionuclide may be an alpha-particle-emitting radionuclide.

[0526] Example enzymes include malate dehydrogenase, staphylococcal nuclease, delta-V-steroid isomerase, yeast alcohol dehydrogenase, alpha-glycerophosphate dehydrogenase, triose phosphate isomerase, horseradish peroxidase, alkaline phosphatase, asparaginase, glucose oxidase, beta-galactosidase, ribonuclease, urease, catalase, glucose-6-phosphate dehydrogenase, glucoamylase or acetylcholinesterase. Such enzymes may be used, for example, in combination with prodrugs that are administered in relatively non-toxic form and converted at the target site by the enzyme into a cytotoxic agent. In other alternatives, a drug may be converted into less toxic form by endogenous enzymes in the subject but may be reconverted into a cytotoxic form by the therapeutic enzyme.

[0527] By means of an example, an agent, such as a bi-specific antibody, capable of specifically binding to a gene product expressed on the cell surface of suppressive or activated immune cells and another cell may be used for targeting suppressive or activated immune cells away from or towards TILs and/or a tumor.

Targeting T cell subtypes

[0528] In another aspect, detecting or quantifying CD8+ T cells may be used to select a treatment for a subject in need thereof. In certain embodiments, subjects comprising suppressive T cells as described herein are treated with an immunotherapy (e.g., checkpoint blockade therapy). In certain embodiments, the suppressive T cells express coinhibitory receptors (e.g., checkpoint proteins) that can be specifically targeted. The checkpoint blockade therapy may be an inhibitor of any check point protein described herein. The checkpoint blockade therapy may comprise anti-TIM3, anti-CTLA4, anti-PD-Ll, anti-PDl, anti-TIGIT, anti-LAG3, or combinations thereof. Specific check point inhibitors include, but are not limited to anti-CTLA4 antibodies (e.g., Ipilimumab), anti-PD-l antibodies (e.g., Nivolumab, Pembrolizumab), and anti-PD-Ll antibodies (e.g., Atezolizumab).

[0529] The treatment may involve transferring CAR T cells to a patient. The CAR T cells may be modified such that they are resistant to suppression by the CD8+ T cells of the present invention.

[0530] Bhlhe40 is also known as BHLHB2, Clast5, DEC1, HLHB2, SHARP-2, SHARP2, STRA13 and Stral4. As used herein Bhlhe40 refers to the human gene, mouse gene and all other orthologues. Bhlhe40 may refer to the gene identified by accession number NM_003670.2. DEC1 is a basic helix-loop-helix transcription factor that is known to be highly induced in a CD28-dependent manner upon T cell activation (Martinez-Llordella et al. "CD28-inducible transcription factor DEC1 is required for efficient autoreactive CD4+ T cell response." J Exp Med. 2013 Jul 29;2l0(8): 1603-19. doi: l0.l084/jem.20l22387. Epub 2013 Jul 22). DEC1 is required for the development of experimental autoimmune encephalomyelitis and plays a critical role in the production of the proinflammatory cytokines GM-CSF, IFNy, and IL-2 (Martinez-Llordella, 2013). Applicants previously demonstrated that DEC1 has a role in promoting pathogenic Thl7 differentiation (see, WO2015130968A2). Applicants have discovered that Bhlhe40 is upregulated in suppressive T cells and may therefore be targeted for downregulation in order to enhance an immune response.

[0531] IKZF2 is also known as ANF1A2, HELIOS, ZNF1A2, ZNFN1A2. As used herein Helios refers to the human gene, mouse gene and all other orthologues. Helios may refer to the gene identified by accession numbers NM_0 16260.2, NM_00 1079526.1 and NM_0 11770.4. Helios is a T cell-specific zinc finger transcription factor that is encoded by the Ikzf2 gene. It

belongs to the Ikaros family of zinc finger proteins, which also includes Ikaros (Ikzf1), Aiolos (Ikzf3), Eos (Ikzf4), and Pegasus (Ikzf5). Helios, along with other Ikaros proteins, regulate lymphocyte development and differentiation. Helios has been shown to have specific roles in Tregs (Nakagawa et ah, Instability of Helios-deficient Tregs is associated with conversion to a T-effector phenotype and enhanced antitumor immunity, Proc Natl Acad Sci U S A. 2016 May 31;1 13(22):6248-53, and Kim et ah, Stable inhibitory activity of regulatory T cells requires the transcription factor Helios, Science. 2015 Oct 16;350(6258):334-9). Applicants have shown a role for Helios in a specific suppressive T cell population (i.e., cluster 7). Not being bound by a theory, targeting Helios in specific T cells can enhance treatment and avoid unwanted side effects caused by targeting all Helios expressing T cells.

Diagnosis and Treatment Selection

[0532] In a further embodiment, the present invention provides for a method for determining the CD8+ T cell status of a subject, or for diagnosing, prognosing or monitoring a disease comprising an immune component in a subject by detecting or quantifying CD8+ T cells as defined in any embodiment herein in a biological sample of the subject. The CD8+ T cell status of the subject may be determined before and after therapy, whereby the efficacy of the therapy is determined or monitored. The therapy may be an immunotherapy (e.g., checkpoint blockade therapy). Not being bound by a theory, an immunotherapy is effective if after treatment the suppressive CD8+ T cells decrease or activated T cells increase. Not being bound by a theory, a subject suffering from cancer having less suppressive CD8+ T cells has a better prognosis than a subject having more suppressive CD8+ T cells.

[0533] The terms “diagnosis” and “monitoring” are commonplace and well-understood in medical practice. By means of further explanation and without limitation the term “diagnosis” generally refers to the process or act of recognizing, deciding on or concluding on a disease or condition in a subject on the basis of symptoms and signs and/or from results of various diagnostic procedures (such as, for example, from knowing the presence, absence and/or quantity of one or more biomarkers characteristic of the diagnosed disease or condition).

[0534] The term “monitoring” generally refers to the follow-up of a disease or a condition in a subject for any changes which may occur over time.

[0535] The terms “prognosing” or “prognosis” generally refer to an anticipation on the progression of a disease or condition and the prospect (e.g., the probability, duration, and/or extent) of recovery. A good prognosis of the diseases or conditions taught herein may generally encompass anticipation of a satisfactory partial or complete recovery from the diseases or conditions, preferably within an acceptable time period. A good prognosis of such may more commonly encompass anticipation of not further worsening or aggravating of such, preferably within a given time period. A poor prognosis of the diseases or conditions as taught herein may generally encompass anticipation of a substandard recovery and/or unsatisfactorily slow recovery, or to substantially no recovery or even further worsening of such.

[0536] The terms also encompass prediction of a disease. The terms “predicting” or “prediction” generally refer to an advance declaration, indication or foretelling of a disease or condition in a subject not (yet) having said disease or condition. For example, a prediction of a disease or condition in a subject may indicate a probability, chance or risk that the subject will develop said disease or condition, for example within a certain time period or by a certain age. Said probability, chance or risk may be indicated inter alia as an absolute value, range or statistics, or may be indicated relative to a suitable control subject or subject population (such as, e.g., relative to a general, normal or healthy subject or subject population). Hence, the probability, chance or risk that a subject will develop a disease or condition may be advantageously indicated as increased or decreased, or as fold-increased or fold-decreased relative to a suitable control subject or subject population. As used herein, the term “prediction” of the conditions or diseases as taught herein in a subject may also particularly mean that the subject has a 'positive' prediction of such, i.e., that the subject is at risk of having such (e.g., the risk is significantly increased vis-a-vis a control subject or subject population). The term “prediction of no” diseases or conditions as taught herein as described herein in a subject may particularly mean that the subject has a 'negative' prediction of such, i.e., that the subject’s risk of having such is not significantly increased vis-a-vis a control subject or subject population.

Kits

[0537] In another aspect, the invention is directed to kit and kit of parts. The terms “kit of parts” and “kit” as used throughout this specification refer to a product containing components necessary for carrying out the specified methods (e.g., methods for detecting, quantifying or

isolating immune cells as taught herein), packed so as to allow their transport and storage. Materials suitable for packing the components comprised in a kit include crystal, plastic (e.g., polyethylene, polypropylene, polycarbonate), bottles, flasks, vials, ampules, paper, envelopes, or other types of containers, carriers or supports. Where a kit comprises a plurality of components, at least a subset of the components (e.g., two or more of the plurality of components) or all of the components may be physically separated, e.g., comprised in or on separate containers, carriers or supports. The components comprised in a kit may be sufficient or may not be sufficient for carrying out the specified methods, such that external reagents or substances may not be necessary or may be necessary for performing the methods, respectively. Typically, kits are employed in conjunction with standard laboratory equipment, such as liquid handling equipment, environment (e.g., temperature) controlling equipment, analytical instruments, etc. In addition to the recited binding agents(s) as taught herein, such as for example, antibodies, hybridization probes, amplification and/or sequencing primers, optionally provided on arrays or microarrays, the present kits may also include some or all of solvents, buffers (such as for example but without limitation histidine-buffers, citrate-buffers, succinate-buffers, acetate-buffers, phosphate-buffers, formate buffers, benzoate buffers, TRIS (Tris(hydroxymethyl)-aminomethan) buffers or maleate buffers, or mixtures thereof), enzymes (such as for example but without limitation thermostable DNA polymerase), detectable labels, detection reagents, and control formulations (positive and/or negative), useful in the specified methods. Typically, the kits may also include instructions for use thereof, such as on a printed insert or on a computer readable medium. The terms may be used interchangeably with the term “article of manufacture”, which broadly encompasses any man-made tangible structural product, when used in the present context.

[0538] The invention is further described in the following examples, which do not limit the scope of the invention described in the claims.

EXAMPLES

Example 1 - Identification of novel tumor infiltrating CD8+ T cells populations

[0539] Applicants identified novel CD8 and CD4 populations using the B16 melanoma mouse model. For single-cell RNA-Seq experiments, TILs from B16 melanomas were collected in 96-well plates. Applicants performed SMART-seq2 following the published protocol (Picelli

et al., 2013 Nat Methods 10, 1096-1098) with minor modifications. Standard Illumina sequencing was performed. Cells in tumors tend to be high for inhibitory receptors (e.g., PD1, Tim3, TIGIT, LAG3). Therapies that block these receptors work in tumor therapy. Therefore, Applicants studied populations of TILs to elucidate the complexity of subpopulations that express these co-inhibitory receptors. Further, Applicants studied how these cell types interact with other cell types in the tumor.

[0540] **Figures 1 and 2** illustrate the study design. Cells were sampled at each time point indicated after tumor cell implantation. Tumor size was measured in two dimensions by caliper and is expressed as the product of two perpendicular diameters. Cells were sorted based on cell markers. CD8 T cells were obtained by sorting for CD8+CD45+ cells. CD4 T cells (both Effector and Regulatory) were obtained by sorting for CD4+CD45+ cells. NK cells, dendritic cells, and macrophages were obtained by sorting for $CD4^{-}CD8^{-}CD45+$ cells. $CD45^{-}$ cells included fibroblasts and tumor cells. **Figures 3** illustrates clustering of the CD8 and CD4 T cells. DP refers to double positive for TIM3 and PD-l. DN refers to double negative for TIM3 and PD-1. SP refers to single positive for TIM3 and PD-l.

[0541] **Figure 4** illustrates dimension reduction analysis of the cells sequenced for CD8 T cells. Applicants sequenced 2592 cells (27 plates). 2313 cells passed the basic QC (89%) and 2017 cells passed the extensive QC (78%). Principal component (PC) analysis was performed using gene expression measured in the single cells. PC1 was associated with transcription and PC2 and PC3 were strongly associated with sequencing batches. tSNE and clustering was performed on PCs 4-9 **(Fig. 4).** All of the CD8 cells were pooled together on a normalized tSNE. The CD8 cells clustered into 15 clusters. **Figure 5** illustrates each cluster individually. **Figure 6** illustrates 4 populations that stand out based on expression of the co-inhibitory receptors PD1 and TIM3. Clusters 9, 10 and 7 are PDl+Tim3+ (C9, C10, C7). Cluster 8 is PDl+Tim3- (C8). Applicants determined that the clusters are transcriptionally different. Not being bound by a theory the clusters are functionally different. Applicants provide data herein suggesting that the cells are functionally different.

[0542] **Figure 7** illustrates decoupled dysfunction and activation scores based on previous work by Applicants (Singer et al., 2016). **Figure 8** illustrates that Clusters 7 and 9 are distinguished by the decoupling of dysfunction and activation scores. **Figures 9 and 10** illustrate

that cluster 7 is high for a CD8 Treg signature (Kim et al., 2015 Science 350(6258):334-339) despite also expressing the co-inhibitory receptors PD-l and TIM-3. The CD8 Treg signature of Kim et al. includes 343 genes that are upregulated and 153 genes that are downregulated. Cluster 7 signature genes that overlap ($p< 10^{-4}$) with the Treg upregulated gene signature are ADAM8, CCL3, HAVCR2, IRF8, LAT2, MUO10 and SLC37A2. None of the downregulated Treg genes are expressed in cluster 7. Thus, Cluster 7 is enriched for genes upregulated in CD8 Tregs. Not being bound by a theory the cluster 7 CD8 T cells represent a novel suppressive CD8 T cell with gene expression signatures similar to CD8 Tregs. Additionally, cluster 8 overlaps with ($p<0.0l$) with the Treg upregulated gene signature. The overlapping cluster 8 genes are CD74, CD81, CD83, KLRK1, SDC4 and SPRY2. None of the cluster 9 or cluster 10 CD8 signature genes overlap with the CD8 Treg signature. Cluster 8, 9 and 10 express genes downregulated in the Treg downregulated gene signature. Cluster 8 includes expression of LRIG1, NRN1, NRP1 and PTPRK ($\rho=0.012$). Thus, cluster 8 is enriched for genes either up or down in CD8 Tregs. Cluster 9 expresses ASPM, BUB1, CCNA2, CCNB2, CDCA8, CDKN3, CENPE, HMMR, KIF1 1, KIF4, MELK, NEK2, SPAG5 and TPX2 ($\rho<10^{-13}$) (i.e., genes downregulated in Treg signature). Thus, cluster 9 may express a signature anti-correlated to the Treg signature. Cluster 10 expresses POLA1 and RRM2.

[0543] **Figure 11** illustrates that cluster 7 is high for MT1 (left). MT1 is significantly upregulated in cluster 7. **Figure 11** also illustrates that clusters 7 and 8 are marked by expression of the transcription factor Helios (IKZF2) (Kim et al., 2015) (right). Helios expression was found to be significantly upregulated in cluster 7 as compared to cluster 9 and cluster 10. Thus, Applicants have identified for the first time at least two Helios expressing subpopulations of CD8 T cells expressing PD1 and distinguished by at least expression of TIM3.

[0544] **Figure 12** illustrates that PD1+TIM3+ (DP) MT (WT) expressing cells are the most suppressive in a CFSE assay for T cell proliferation. Greater suppression leads to a few cells with a higher concentration of CFSE (proliferating cells divide and CFSE is diluted among daughter cells). ETpon knockout of MT the PD1+TIM3+ (DP) MT-/- cells are less suppressive. Cluster 7 represents the population of CD8 cells that are both PD1+TIM3+ and have high MT1 expression. Thus, Applicants have shown for the first time that the cluster 7 population of cells may be suppressive to T cell proliferation. Based on cell type specific markers, cluster 7 T cells

may be specifically targeted for therapeutic purposes (e.g., cancer, autoimmune diseases, chronic infection).

[0545] **Figure 13** illustrates further characterization of CD8 cell populations. Cluster 9 is high for cell cycle genes and a CD8 activation (effector) signature. Cluster 7 is low for both signatures. Cluster 9 is also high for an exhaustion signature (Wherry and Kurachi, 2015, Nature reviews Immunology 15, 486-499). Clusters 9, 10, 7 and 8 express a decoupled dysfunction signature determined by bulk expression data from populations of T cells (Singer et al., 2016).

[0546] **Figure 14** illustrates transmembrane receptors expressed or not expressed by the cluster 7 population. Sorting CD8 cell populations can use these markers. For example, cluster 7 can be sorted out using a combination of SERPINE2 + HMMR-; KIT + Tim3+ HMMR-; or TNFRSF4 + Tim3+ HMMR-. **Figures 17 and 18** show sorting of CD8 T cells. PD1+TIM3+ and PD1+TIM3- are further sorted by HMMR, cKIT and Helios, as well as the proliferation marker Ki-67.

[0547] **Figure 15** illustrates cytokines/chemokines expressed by the cluster 7 cell population. IL1 is a proinflammatory cytokine and IL1R2 is a decoy receptor that dampens the proinflammatory response by removing IL1 from the system. Cluster 7 cells express IL1R2. Not being bound by a theory blocking IL1R2 or modulating its expression either through drug or genetic mechanisms (e.g., CRISPR) on cluster 7 cells can inhibit cluster 7 suppressive function. **Figure 16** illustrates transcription factors expressed by the cluster 7 cell population. All of these transcription factors are significantly upregulated in cluster 7 as compared to clusters 9 and 10. IKZF2 (Helios) may be involved in the regulation of Tregs and the STAT5 pathway. EPAS1 regulates VEGF. Further, EPAS1 is specific to cluster 7. RUNX2 is involved in CD8 memory differentiation. RBPJ is involved in Notch signaling. Thus, these transcription factors may be targeted to inhibit the suppressive function of cluster 7 cells.

[0548] Applicants hypothesize that cluster 7 is sensitive to steroid signaling. Specifically, cluster 7 may be sensitive to glucocorticoid signaling (see, e.g., Oakley and Cidlowsk J Allergy Clin Immunol. 2013 Nov; 132(5): 1033-1044). Glucocorticoid signaling turns on expression of MT's and correlates to TIM3/PD1 expression. NR3C1 is the glucocorticoid receptor and is differentially expressed on cluster 7 cells (see, e.g., **Tables 1-5).** Targeting glucocorticoid sensing may be a target for inhibiting the suppressive function of cluster 7 cells. Glucocorticoid

inhibiting drugs have previously been described (see, e.g., Clark, Curr Top Med Chem. 2008;8(9):8l3-38) and may be used in combination with checkpoint blockade therapy as described herein.

[0549] Cluster 7 can be further characterized by expression of genes markers. **Tables 1 to 5** lists ranked genes differentially expressed in cluster 7. **Table 1** lists the top 500 ranked genes. **Tables 2 and 3** list transcription factors and cell surface/cytokines. **Table 4** lists genes differentially expressed in cluster 7 as compared to all 15 CD8 clusters. **Table 5** lists a cluster 7 signature. Cluster 8, 9 and 10 signature genes are listed in ranked order in **Tables 6-17. Tables 18-20** list ranked signatures for clusters 7, 8 and 9/10 determined using modified statistical analysis. In certain embodiments, **Tables 18-20** were determined using a more statistically accurate analysis of the CD8 clusters. In certain embodiments, **Tables 18-20** represent gene signatures based on analyzing more single cells.

Table 1. Ranked top 500 genes differentially expressed in cluster 7

Gene	TP	TN	thresh_mhg	hyper_pval	hyper_qval	gen_qval	rank_hyper_qval	rank_gen_qval	mean_rank
GLDC	0.719457014	0.847995546	0.705	1.75E-67	3.63E-64	-85.741	2	1	1.5
TNFRSF9	0.873303167	0.744988864	8.537	2.48E-73	1.03E-69	-74.603	1	2	1.5
PRF1	0.936651584	0.628619154	7.253	8.69E-64	1.20E-60	-70.08	3	3	3
IRF8	0.886877828	0.663697105	1.501	4.18E-58	3.46E-55	-60.58	5	4	4.5
CCRL2	0.7239819	0.791759465	1.05	1.60E-52	9.50E-50	-57.892	7	5	6
PCYT1A	0.656108597	0.83518931	0.575	4.90E-51	2.54E-48	-57.84	8	7	7.5
HAVCR2	0.873303167	0.683184855	2.154	5.96E-59	6.17E-56	-51.383	4	11	7.5
LAT2	0.647058824	0.83518931	0.903	2.19E-49	6.50E-47	-57.892	14	6	10
2900026A02RIK	0.687782805	0.807906459	0.926	9.38E-50	3.24E-47	-53.112	12	10	11
CSF1	0.556561086	0.885300668	0.911	1.02E-47	2.63E-45	-56.864	16	8	12
ADAM8	0.787330317	0.726057906	0.864	7.94E-50	2.99E-47	-50.88	10	14	12
ITGAV	0.85520362	0.657572383	0.084	7.25E-50	2.99E-47	-50.642	11	15	13
TMPRSS6	0.466063348	0.91481069	0.546	1.18E-41	1.87E-39	-53.736	26	9	17.5
ADAMTS14	0.619909502	0.834632517	0.506	1.83E-44	4.00E-42	-49.686	19	16	17.5
C1QTNF6	0.538461538	0.877505568	0.379	3.24E-42	5.59E-40	-51.184	24	12	18
RGS16	0.977375566	0.510022272	2.506	1.88E-54	1.30E-51	-39.659	6	31	18.5
SERPINE2	0.542986425	0.873051225	3.895	1.05E-41	1.73E-39	-51.019	25	13	19
LITAF	0.936651584	0.551224944	5.893	1.35E-49	4.31E-47	-41.548	13	25	19
RBPJ	0.873303167	0.632516704	2.763	4.56E-49	1.26E-46	-42.315	15	24	19.5
TNFRSF4	0.773755656	0.723830735	3.144	8.39E-47	2.05E-44	-42.493	17	23	20
GPR56	0.647058824	0.806792873	0.696	2.30E-42	4.15E-40	-44.618	23	18	20.5
PGLYRP1	0.923076923	0.53674833	4.954	5.99E-44	1.18E-41	-43.178	21	21	21
HILPDA	0.764705882	0.711024499	5.185	1.14E-42	2.16E-40	-41.529	22	26	24
ANXA2	0.923076923	0.520601336	10.29	1.88E-41	2.78E-39	-43.087	28	22	25
PLEK	0.914027149	0.566815145	1.395	1.02E-46	2.36E-44	-39.004	18	32	25
LAG3	0.963800905	0.513919822	4.793	7.81E-51	3.60E-48	-36.69	9	42	25.5
RGS8	0.538461538	0.865812918	0.275	5.40E-39	5.60E-37	-47.201	40	17	28.5
NABP1	0.828054299	0.63363029	2.705	2.53E-40	3.09E-38	-38.264	34	35	34.5
GPD2	0.714932127	0.732182628	1.007	3.13E-38	3.09E-36	-40.178	42	29	35.5
SLC37A2	0.502262443	0.878062361	0.546	1.80E-36	1.43E-34	-43.31	52	20	36
IKZF2	0.719457014	0.737193764	0.084	6.49E-40	7.47E-38	-37.446	36	37	36.5
AA467197	0.466063348	0.896993318	2.233	5.25E-36	3.96E-34	-43.648	55	19	37
UBASH3B	0.660633484	0.787861915	5.56	1.95E-40	2.58E-38	-35.97	32	44	38
EPAS1	0.529411765	0.863585746	0.986	5.63E-37	4.77E-35	-38.289	49	34	41.5
SERPINB9	0.850678733	0.593541203	3.333	4.94E-38	4.65E-36	-36.781	44	40	42
GAPDH	0.895927602	0.54844098	12.436	7.42E-40	8.31E-38	-35.534	37	47	42
CCNG1	0.873303167	0.587973274	2.284	1.79E-41	2.75E-39	-32.322	27	58	42.5
ACOT7	0.895927602	0.555122494	3.285	6.97E-41	9.63E-39	-32.602	30	56	43

Gene	TP	TN	thresh_mhg	hyper_pval	hyper_qval	gen_qval	rank_hyper_qval	rank_gen_qval	mean_rank
BHLHE40	0.977375566	0.422048998	6.796	6.69E-41	9.57E-39	-32.471	29	57	43
TPI1	0.954751131	0.462138085	5.874	2.28E-40	2.86E-38	-32.629	33	55	44
RGS2	0.900452489	0.541202673	1.411	1.09E-39	1.19E-37	-34.658	38	51	44.5
CDK6	0.696832579	0.73830735	1.978	1.79E-36	1.43E-34	-36.931	51	39	45
CXCR6	0.986425339	0.423719376	5.506	4.32E-44	8.96E-42	-29.649	20	71	45.5
MNDA	0.470588235	0.889755011	4.02	1.12E-34	7.15E-33	-40.982	65	27	46
GEM	0.719457014	0.716592428	5.361	3.84E-36	2.95E-34	-36.705	54	41	47.5
GM5177	0.909502262	0.517817372	3.82	4.31E-38	4.16E-36	-33.888	43	53	48
CST7	0.963800905	0.445991091	7.082	1.99E-40	2.58E-38	-30.448	31	68	49.5
SLC2A3	0.656108597	0.768374165	5.663	8.80E-36	6.40E-34	-36.69	57	43	50
KIT	0.466063348	0.88752784	0.516	2.23E-33	1.27E-31	-40.679	73	28	50.5
GZMB	0.850678733	0.579064588	2.384	7.84E-36	5.80E-34	-35.205	56	50	53
S100A11	0.968325792	0.418708241	8.456	8.11E-38	7.31E-36	-32.158	46	60	53
IL1R2	0.407239819	0.915924276	3.396	2.28E-32	1.20E-30	-40.178	79	30	54.5
DSCAM	0.479638009	0.876948775	0.189	1.06E-32	5.69E-31	-38.384	77	33	55
CCL3	0.642533937	0.773942094	3.904	8.83E-35	5.72E-33	-35.708	64	46	55
FAM3C	0.832579186	0.605790646	0.287	1.12E-36	9.25E-35	-31.563	50	63	56.5
CASP3	0.895927602	0.549554566	3.644	5.01E-40	5.94E-38	-28.856	35	80	57.5
NR4A2	0.914027149	0.498886414	0.595	2.62E-36	2.05E-34	-31.351	53	64	58.5
CD244	0.466063348	0.883073497	3.545	3.23E-32	1.67E-30	-37.285	80	38	59
SLC16A11	0.429864253	0.901447661	1.029	1.21E-31	6.02E-30	-38.005	83	36	59.5
DUSP4	0.755656109	0.677616927	0.444	2.87E-35	2.02E-33	-31.906	59	61	60
CAPG	0.864253394	0.558463252	4.057	3.05E-35	2.11E-33	-31.567	60	62	61
SAMSN1	0.941176471	0.473830735	1.029	1.01E-38	1.02E-36	-27.927	41	87	64
FAM110A	0.683257919	0.7344098	0.731	1.08E-33	6.37E-32	-32.314	70	59	64.5
CIAPIN1	0.859728507	0.581291759	1.669	8.11E-38	7.31E-36	-27.949	45	86	65.5
NRGN	0.484162896	0.865812918	0.604	1.10E-30	5.06E-29	-35.762	90	45	67.5
PLAC8	0.43438914	0.896436526	10.661	5.77E-31	2.75E-29	-35.511	87	48	67.5
IMPA2	0.714932127	0.707683742	0.832	6.52E-34	3.92E-32	-30.742	69	66	67.5
SRGAP3	0.529411765	0.840757238	0.39	1.24E-31	6.14E-30	-34.118	84	52	68
FOXRED2	0.425339367	0.900890869	1.731	8.24E-31	3.88E-29	-35.48	88	49	68.5
NRP1	0.751131222	0.670935412	0.163	1.76E-33	1.03E-31	-30.537	71	67	69
ARL14EP	0.7239819	0.703786192	2.084	1.20E-34	7.55E-33	-29.195	66	75	70.5
EHD1	0.832579186	0.594654788	3.266	5.47E-35	3.60E-33	-28.975	63	78	70.5
LGALS1	0.923076923	0.508351893	10.112	1.29E-39	1.37E-37	-25.523	39	105	72
MT1	0.556561086	0.814587973	2.173	3.92E-30	1.78E-28	-33.823	91	54	72.5
ERGIC1	0.71040724	0.699888641	0.333	5.94E-32	3.00E-30	-29.048	82	76	79
OSBPL3	0.800904977	0.615256125	0.176	8.51E-33	4.64E-31	-28.093	76	85	80.5
SMIM3	0.497737557	0.857461024	6.263	9.72E-31	4.53E-29	-29.22	89	74	81.5
SERPINA3G	0.877828054	0.540089087	4.066	4.39E-35	2.94E-33	-25.966	62	101	81.5
TOX	0.904977376	0.520044543	3.455	1.76E-37	1.55E-35	-23.972	47	122	84.5
PKM	0.805429864	0.583518931	9.882	5.15E-29	2.09E-27	-29.958	102	69	85.5
CX3CR1	0.511312217	0.843541203	1.646	1.21E-29	5.27E-28	-28.883	95	79	87
ID2	0.972850679	0.341314031	4.705	6.02E-29	2.42E-27	-29	103	77	90
PEX16	0.624434389	0.759465479	2.725	1.51E-29	6.38E-28	-28.699	98	82	90
GPR65	0.760180995	0.652561247	2.585	5.08E-32	2.60E-30	-26.057	81	100	90.5
SEPT11	0.837104072	0.582405345	0.88	5.90E-34	3.60E-32	-24.768	68	117	92.5
NFKB2	0.846153846	0.561804009	2.359	1.52E-32	8.07E-31	-25.366	78	108	93
FDX1	0.574660633	0.79454343	1.77	7.17E-29	2.80E-27	-28.187	106	84	95
ENTPD1	0.701357466	0.692093541	0.202	2.00E-29	8.39E-28	-26.13	99	99	99
BCL2A1D	0.959276018	0.403674833	2.198	1.91E-33	1.10E-31	-23.195	72	127	99.5
DNMT3A	0.660633484	0.729398664	2.63	1.40E-29	6.04E-28	-25.499	96	106	101
ZMIZ1	0.751131222	0.655345212	0.214	4.47E-31	2.16E-29	-24.896	86	116	101
NRN1	0.538461538	0.815144766	3.643	9.38E-28	3.14E-26	-28.795	124	81	102.5
STAT3	0.909502262	0.43596882	6.143	3.70E-27	1.13E-25	-29.78	136	70	103
CLIC4	0.619909502	0.758351893	1.202	9.73E-29	3.74E-27	-26.3	108	98	103
GDPD5	0.438914027	0.878062361	2.606	4.27E-27	1.28E-25	-29.436	138	72	105
CCR8	0.443438914	0.874164811	5.401	7.64E-27	2.23E-25	-29.292	142	73	107.5
NEDD9	0.665158371	0.714922049	5.851	6.40E-28	2.17E-26	-26.928	122	93	107.5
GSTO1	0.624434389	0.751670379	5.507	3.04E-28	1.08E-26	-25.935	117	102	109.5
PGK1	0.936651584	0.402561247	7.541	2.92E-28	1.04E-26	-25.564	116	104	110
PDCD1	0.968325792	0.415367483	5.101	2.34E-37	2.02E-35	-19.612	48	172	110
UHRF2	0.542986425	0.809576837	0.971	2.48E-27	7.85E-26	-27.41	131	91	111
PLSCR1	0.696832579	0.688752784	2.785	2.73E-28	1.00E-26	-25.165	113	111	112
TIGIT	0.981900452	0.354120267	4.895	4.50E-33	2.49E-31	-21.13	75	151	113
ALDOA	0.954751131	0.360801782	9.477	5.16E-27	1.53E-25	-27.783	140	88	114
LILRB4	0.597285068	0.767817372	5.621	2.81E-27	8.68E-26	-26.812	134	95	114.5
KLRC1	0.841628959	0.546213808	4.198	1.16E-29	5.11E-28	-22.062	94	135	114.5
TFF1	0.384615385	0.904788419	5.97	6.36E-26	1.60E-24	-30.862	165	65	115
HNRNPA1	0.78280543	0.589643653	9.38	1.65E-26	4.52E-25	-28.44	151	83	117
PTPRS	0.606334842	0.763919822	1.989	7.79E-28	2.62E-26	-25.035	123	113	118
1700017B05RIK	0.71040724	0.675946548	1.618	2.83E-28	1.03E-26	-23.832	114	124	119
PTPLAD1	0.805429864	0.580734967	0.748	1.22E-28	4.65E-27	-22.786	109	129	119
VAMP8	0.923076923	0.462138085	2.807	4.06E-33	2.27E-31	-20.364	74	164	119

Gene	TP	TN	thresh_mhg	hyper_pval	hyper_qval	gen_qval	rank_hyper_qval	rank_gen_qval	mean_rank
ESD	0.886877828	0.528396437	4.135	4.08E-35	2.77E-33	-19.013	61	183	122
GM14295	0.755656109	0.631959911	3.009	2.37E-28	8.78E-27	-22.272	112	134	123
NUCB1	0.895927602	0.478841871	0.444	4.44E-30	2.00E-28	-21.027	92	154	123
TUBB6	0.429864253	0.879175947	4.374	4.18E-26	1.06E-24	-27.679	163	89	126
SH2D2A	0.837104072	0.541759465	8.666	2.34E-28	8.76E-27	-21.518	111	142	126.5
RCN1	0.574660633	0.778953229	0.949	3.65E-26	9.34E-25	-27.183	162	92	127
TRPS1	0.511312217	0.829064588	0.986	9.50E-27	2.75E-25	-25.165	143	112	127.5
RPS27L	0.742081448	0.646993318	4.99	1.54E-28	5.82E-27	-21.356	110	147	128.5
SH3BGRL	0.828054299	0.559020045	0.546	3.27E-29	1.34E-27	-20.969	101	156	128.5
FKBP1A	0.972850679	0.393652561	4.911	1.17E-35	8.36E-34	-18.268	58	200	129
AFG3L2	0.674208145	0.697104677	0.411	1.82E-26	4.89E-25	-24.111	154	119	136.5
KDELR2	0.805429864	0.573496659	2.934	1.12E-27	3.68E-26	-21.276	126	148	137
IL2RB	0.561085973	0.782293987	9.964	5.64E-25	1.26E-23	-27.616	185	90	137.5
SLC25A4	0.950226244	0.405902004	7.096	1.34E-31	6.55E-30	-18.569	85	190	137.5
LYRM4	0.574660633	0.773942094	0.39	2.42E-25	5.71E-24	-25.643	175	103	139
BCL2L11	0.660633484	0.708240535	1.748	2.60E-26	6.81E-25	-24.04	158	120	139
DUT	0.814479638	0.609131403	2.151	4.25E-34	2.63E-32	-17.989	67	211	139
SERPINB6A	0.656108597	0.712694878	3.637	2.23E-26	5.93E-25	-23.705	156	125	140.5
RFK	0.714932127	0.660356347	0.595	1.16E-26	3.28E-25	-22.062	146	136	141
EEA1	0.547511312	0.795100223	0.189	2.24E-25	5.36E-24	-25.035	173	114	143.5
GALK1	0.56561086	0.787861915	3.819	1.74E-26	4.71E-25	-21.626	153	140	146.5
KLRC2	0.778280543	0.597995546	1.766	5.80E-27	1.71E-25	-20.64	141	161	151
TMBIM4	0.597285068	0.757238307	1.876	1.44E-25	3.55E-24	-21.991	168	137	152.5
PKP4	0.633484163	0.723273942	0.31	5.06E-25	1.15E-23	-22.686	183	130	156.5
RPS26	0.950226244	0.370824053	11.127	3.10E-27	9.52E-26	-19.14	135	181	158
LRRK1	0.50678733	0.819042316	0.322	2.49E-24	5.09E-23	-24.904	203	115	159
GLIPR1	0.755656109	0.613585746	5.081	7.31E-26	1.83E-24	-21.055	166	153	159.5
STK39	0.502262443	0.820155902	0.263	5.90E-24	1.15E-22	-25.214	211	109	160
SERPINA3H	0.714932127	0.646993318	0.251	7.63E-25	1.65E-23	-22.871	192	128	160
SLC52A3	0.325791855	0.927616927	3.605	1.15E-23	2.12E-22	-26.78	225	96	160.5
GM5069	0.728506787	0.623608018	2.694	1.45E-23	2.63E-22	-26.822	228	94	161
CCDC50	0.65158371	0.708240535	0.367	3.85E-25	8.92E-24	-21.472	179	144	161.5
ACTG1	0.945701357	0.349665924	11.98	7.96E-24	1.51E-22	-25.203	218	110	164
SLA2	0.823529412	0.550668151	0.39	2.03E-27	6.46E-26	-18.315	130	199	164.5
IL10RA	0.837104072	0.538975501	0.214	5.38E-28	1.86E-26	-18.023	120	209	164.5
CENPA	0.773755656	0.61247216	2.791	2.92E-28	1.04E-26	-17.723	115	217	166
RUNX2	0.78280543	0.582962138	1.546	1.20E-25	2.97E-24	-20.044	167	167	167
NEK6	0.339366516	0.918708241	1.144	3.51E-23	6.06E-22	-26.34	240	97	168.5
TXN1	0.959276018	0.367483296	3.623	7.60E-29	2.95E-27	-17.362	107	231	169
RPN1	0.891402715	0.457126949	0.356	1.45E-26	4.07E-25	-18.422	148	195	171.5
STARD3NL	0.647058824	0.720489978	2.227	2.34E-26	6.17E-25	-18.589	157	189	173
KDM2B	0.678733032	0.677616927	0.88	2.60E-24	5.29E-23	-21.433	204	145	174.5
MPHOSPH6	0.601809955	0.745545657	2.59	2.42E-24	4.96E-23	-21.241	202	150	176
IL18RAP	0.733031674	0.626948775	0.66	1.40E-24	2.93E-23	-21.027	199	155	177
CLTC	0.828054299	0.525612472	0.176	5.84E-25	1.30E-23	-19.944	187	169	178
DEGS1	0.769230769	0.605790646	5.83	1.03E-26	2.96E-25	-17.807	144	214	179
0610007P14RIK	0.63800905	0.718262806	3.406	7.29E-25	1.58E-23	-19.986	191	168	179.5
TNFRSF18	0.895927602	0.459910913	4.331	1.10E-27	3.65E-26	-16.98	125	240	182.5
TIPRL	0.773755656	0.600222717	3.18	1.29E-26	3.64E-25	-17.608	147	220	183.5
ATXN10	0.778280543	0.595768374	1.131	1.14E-26	3.26E-25	-17.466	145	227	186
SERPINB6B	0.787330317	0.561247216	3.908	1.41E-23	2.57E-22	-21.418	227	146	186.5
ISY1	0.886877828	0.482182628	1.975	6.73E-29	2.66E-27	-16.471	105	268	186.5
CMTM7	0.864253394	0.512249443	1.761	6.60E-29	2.63E-27	-16.368	104	273	188.5
SLC16A3	0.479638009	0.827394209	0.496	2.07E-22	3.16E-21	-25.499	271	107	189
ARSB	0.79638009	0.585746102	0.251	5.85E-28	2.00E-26	-16.597	121	258	189.5
DDIT4	0.601809955	0.735523385	0.356	7.08E-23	1.16E-21	-22.58	253	131	192
PRELID1	0.977375566	0.298997773	7.483	4.65E-25	1.07E-23	-18.071	181	206	193.5
RBL2	0.832579186	0.511135857	0.239	6.99E-24	1.35E-22	-19.557	215	173	194
HSP90B1	0.823529412	0.521714922	7.442	7.64E-24	1.46E-22	-19.492	217	174	195.5
HMGCR	0.65158371	0.694877506	2.791	2.84E-23	4.97E-22	-20.939	237	158	197.5
CETN2	0.705882353	0.658129176	0.669	3.71E-25	8.65E-24	-17.656	178	219	198.5
TWSG1	0.466063348	0.835746102	0.367	3.34E-22	4.90E-21	-23.987	282	121	201.5
COPS4	0.764705882	0.609131403	3.651	1.59E-26	4.40E-25	-16.618	150	256	203
TMEM123	0.891402715	0.464922049	4.878	1.61E-27	5.23E-26	-16.349	128	278	203
PREP	0.56561086	0.767817372	2.782	2.97E-23	5.18E-22	-19.715	238	170	204
VPS52	0.642533937	0.700445434	0.782	6.35E-23	1.05E-21	-20.519	251	163	207
NCOR2	0.656108597	0.688752784	0.239	5.32E-23	8.90E-22	-19.689	247	171	209
S100A4	0.769230769	0.596325167	6.264	1.76E-25	4.32E-24	-16.811	169	250	209.5
CALR	0.923076923	0.384187082	2.844	1.64E-23	2.98E-22	-18.422	229	196	212.5
RABGAP1L	0.787330317	0.551781737	2.223	1.87E-22	2.90E-21	-20.528	268	162	215
UAP1	0.461538462	0.83908686	2.275	3.08E-22	4.54E-21	-21.07	281	152	216.5
PGAM1	0.918552036	0.421492205	5.865	4.67E-27	1.39E-25	-15.989	139	295	217
SERPINA3I	0.687782805	0.659242762	0.918	5.24E-23	8.84E-22	-18.496	246	191	218.5
PTGER2	0.479638009	0.824053452	0.227	7.64E-22	1.03E-20	-22.388	308	132	220

Gene	TP	TN	thresh_mhg	hyper_pval	hyper_qval	gen_qval	rank_hyper_qval	rank_gen_qval	mean_rank
COX17	0.868778281	0.493875278	5.638	2.54E-27	7.99E-26	-15.776	132	308	220
BCL2A1B	0.954751131	0.369153675	1.937	5.07E-28	1.77E-26	-15.472	119	322	220.5
NAP1L1	0.968325792	0.359131403	4.392	5.38E-30	2.40E-28	-14.871	93	348	220.5
PIGS	0.78280543	0.587416481	4.183	3.21E-26	8.25E-25	-16.223	161	284	222.5
SIK1	0.864253394	0.478285078	1.257	1.04E-24	2.21E-23	-16.799	195	252	223.5
FLNB	0.515837104	0.79844098	0.163	5.22E-22	7.35E-21	-20.969	294	157	225.5
SEMA6D	0.380090498	0.888084633	0.367	1.74E-21	2.16E-20	-24.639	334	118	226
MRPS21	0.701357466	0.658129176	3.124	1.43E-24	2.97E-23	-16.703	200	255	227.5
MAP2K3	0.742081448	0.623608018	4.48	2.42E-25	5.71E-24	-16.349	176	279	227.5
ENO3	0.470588235	0.829064588	3.527	1.34E-21	1.69E-20	-22.303	328	133	230.5
SMARCB1	0.65158371	0.698218263	4.752	9.93E-24	1.84E-22	-17.095	224	238	231
ATXN1	0.841628959	0.489420935	0.138	1.17E-22	1.85E-21	-18.207	263	202	232.5
CDV3	0.787330317	0.580178174	0.585	6.30E-26	1.59E-24	-15.902	164	301	232.5
SMPDL3B	0.470588235	0.828507795	0.832	1.66E-21	2.07E-20	-21.964	332	138	235
AI662270	0.809954751	0.550111359	1.195	2.36E-25	5.63E-24	-15.982	174	296	235
SERPINA3F	0.42081448	0.86247216	1.384	1.67E-21	2.08E-20	-21.93	333	139	236
PNKD	0.606334842	0.727728285	0.623	2.55E-22	3.83E-21	-18.402	276	197	236.5
CISD1	0.592760181	0.744432071	3.873	4.57E-23	7.80E-22	-17.332	243	232	237.5
NCF4	0.805429864	0.546213808	1.791	3.08E-24	6.21E-23	-16.455	206	270	238
PTPN7	0.764705882	0.599109131	3.651	3.21E-25	7.52E-24	-15.868	177	304	240.5
IL12RB2	0.447963801	0.841870824	0.66	4.40E-21	5.09E-20	-23.202	359	126	242.5
PADI2	0.647058824	0.702672606	2.709	8.75E-24	1.64E-22	-16.48	221	266	243.5
ETFB	0.828054299	0.542873051	3.503	4.17E-27	1.26E-25	-14.849	137	350	243.5
MED11	0.597285068	0.737750557	2.676	1.19E-22	1.87E-21	-17.51	264	224	244
RAB27A	0.769230769	0.602449889	2.16	2.83E-26	7.33E-25	-15.278	160	331	245.5
TYK2	0.683257919	0.66091314	0.604	1.15E-22	1.82E-21	-17.137	262	237	249.5
GABARAPL1	0.597285068	0.733853007	0.595	4.23E-22	6.05E-21	-17.973	290	212	251
CTSC	0.764705882	0.587416481	2.689	9.44E-24	1.75E-22	-16.298	223	281	252
AW112010	0.923076923	0.382516704	10.134	2.54E-23	4.48E-22	-16.372	234	272	253
ARL1	0.737556561	0.616926503	3.406	6.93E-24	1.34E-22	-16.038	214	293	253.5
PRDX2	0.864253394	0.480512249	4.836	5.65E-25	1.26E-23	-15.473	186	321	253.5
GNPNAT1	0.552036199	0.767260579	1.651	1.50E-21	1.89E-20	-18.958	330	184	257
SLC39A1	0.819004525	0.509465479	0.546	8.38E-22	1.12E-20	-18.183	311	204	257.5
GM14440	0.665158371	0.673719376	0.401	4.02E-22	5.79E-21	-17.437	288	228	258
CYB5B	0.674208145	0.669821826	2.29	1.03E-22	1.65E-21	-16.529	259	262	260.5
ERO1L	0.497737557	0.806792873	1.373	3.47E-21	4.11E-20	-19.368	350	175	262.5
NDFIP2	0.610859729	0.721046771	0.84	6.18E-22	8.42E-21	-17.516	302	223	262.5
PGLS	0.846153846	0.528396437	4.531	4.70E-28	1.65E-26	-13.831	118	407	262.5
ACSL4	0.755656109	0.577394209	1.541	2.15E-21	2.62E-20	-18.853	340	186	263
FUCA2	0.50678733	0.800111359	1.803	3.35E-21	3.98E-20	-18.882	349	185	267
CD200	0.34841629	0.901447661	1.614	2.61E-20	2.71E-19	-21.601	400	141	270.5
XPNPEP1	0.647058824	0.689309577	0.345	5.53E-22	7.72E-21	-16.861	297	245	271
PLP2	0.832579186	0.54064588	1.064	1.64E-27	5.28E-26	-13.715	129	418	273.5
MT2	0.325791855	0.913140312	1.646	5.06E-20	4.93E-19	-23.836	426	123	274.5
LPIN2	0.393665158	0.873051225	1.245	3.48E-20	3.53E-19	-21.488	408	143	275.5
3830406C13RIK	0.520361991	0.787861915	2.733	6.52E-21	7.28E-20	-19.078	371	182	276.5
SSR2	0.859728507	0.498886414	3.64	1.72E-26	4.70E-25	-13.864	152	403	277.5
NDUFS2	0.819004525	0.557349666	4.721	1.34E-27	4.37E-26	-13.502	127	431	279
2700060E02RIK	0.923076923	0.432628062	5.072	2.72E-29	1.13E-27	-13.17	100	459	279.5
MTHFD1L	0.597285068	0.729955457	0.918	1.47E-21	1.85E-20	-17.233	329	235	282
HIP1	0.647058824	0.682628062	0.163	4.02E-21	4.67E-20	-18.054	357	208	282.5
DYNLT3	0.429864253	0.849665924	0.774	2.78E-20	2.87E-19	-20.33	402	165	283.5
EFHD2	0.79638009	0.548997773	0.263	2.49E-23	4.43E-22	-15.078	233	339	286
TNFSF4	0.384615385	0.878619154	3.874	3.78E-20	3.79E-19	-20.871	414	159	286.5
FARS2	0.556561086	0.755011136	0.604	2.51E-20	2.61E-19	-19.261	398	177	287.5
CST3	0.791855204	0.551781737	1.7	4.71E-23	8.01E-22	-15.102	244	337	290.5
NOL7	0.809954751	0.532293987	0.832	3.34E-23	5.80E-22	-14.987	239	343	291
OXSR1	0.524886878	0.779510022	0.595	3.35E-20	3.41E-19	-19.212	407	179	293
DUSP6	0.57918552	0.740534521	1.475	6.50E-21	7.28E-20	-17.756	370	216	293
SEPT2	0.954751131	0.329064588	0.536	2.54E-23	4.48E-22	-14.651	235	357	296
UTF1	0.330316742	0.909242762	4.976	1.01E-19	9.36E-19	-21.255	447	149	298
ENO1	0.895927602	0.393652561	9.733	4.25E-20	4.22E-19	-19.242	418	178	298
MTMR1	0.56561086	0.756681514	0.202	1.32E-21	1.68E-20	-16.458	327	269	298
DCTN5	0.714932127	0.631959911	2.932	6.66E-23	1.10E-21	-14.969	252	344	298
PDCL3	0.647058824	0.687639198	3.007	9.13E-22	1.20E-20	-16.187	315	286	300.5
DDB1	0.764705882	0.58908686	4.051	5.87E-24	1.15E-22	-13.99	210	392	301
HDAC1	0.837104072	0.505011136	2.214	8.21E-24	1.55E-22	-14.043	220	386	303
SREBF2	0.733031674	0.597438753	0.138	5.92E-21	6.69E-20	-16.921	367	241	304
COMMD3	0.733031674	0.60467706	3.134	8.29E-22	1.11E-20	-15.961	310	298	304
GM9855	0.619909502	0.703786192	0.202	1.07E-20	1.15E-19	-17.276	385	233	309
CTSB	0.959276018	0.346325167	2.31	2.72E-26	7.09E-25	-13.17	159	460	309.5
SIVA1	0.57918552	0.739977728	2.032	7.74E-21	8.61E-20	-16.842	373	248	310.5
COX7B	0.877828054	0.466035635	3.203	2.15E-25	5.18E-24	-13.303	172	449	310.5
BEND4	0.705882353	0.630846325	0.356	1.18E-21	1.52E-20	-15.651	321	312	316.5

Gene	TP	TN	thresh_mhg	hyper_pval	hyper_qval	gen_qval	rank_hyper_qval	rank_gen_qval	mean_rank
CBLB	0.981900452	0.267260579	1.609	1.33E-22	2.08E-21	-14.327	265	368	316.5
ANKRD39	0.466063348	0.821269488	2.245	8.50E-20	7.96E-19	-18.44	443	193	318
KARS	0.873303167	0.465478842	0.299	1.31E-24	2.74E-23	-13.425	198	438	318
LXN	0.50678733	0.791202673	1.373	7.43E-20	7.05E-19	-18.233	437	201	319
D16ERTD472E	0.841628959	0.496659243	0.926	1.73E-23	3.11E-22	-13.828	230	409	319.5
SPCS3	0.714932127	0.61636971	3.074	5.30E-21	6.05E-20	-16.349	363	280	321.5
TPM4	0.941176471	0.361358575	4.167	2.84E-24	5.75E-23	-13.413	205	440	322.5
CHST12	0.619909502	0.703229399	1.599	1.26E-20	1.35E-19	-16.596	387	259	323
ACOT9	0.692307692	0.64532294	1.305	8.52E-22	1.13E-20	-15.211	312	335	323.5
METAP2	0.832579186	0.508908686	2.057	1.28E-23	2.34E-22	-13.618	226	424	325
LAP3	0.429864253	0.845211581	0.88	1.60E-19	1.43E-18	-18.741	465	187	326
FUBP1	0.733031674	0.605233853	0.31	7.11E-22	9.61E-21	-14.855	307	349	328
TANK	0.647058824	0.674276169	1.35	4.42E-20	4.38E-19	-17.063	419	239	329
MNF1	0.610859729	0.707683742	2.362	3.61E-20	3.64E-19	-16.808	411	251	331
GM12669	0.769230769	0.569599109	2.278	3.39E-22	4.96E-21	-14.119	283	380	331.5
ST14	0.438914027	0.837416481	0.556	2.83E-19	2.40E-18	-19.188	489	180	334.5
IPO7	0.547511312	0.755567929	1.131	2.17E-19	1.88E-18	-18.429	478	194	336
TARS	0.63800905	0.691536748	0.465	3.30E-21	3.93E-20	-15.468	348	324	336
SLC25A17	0.597285068	0.722717149	3.434	1.38E-20	1.47E-19	-16.218	389	285	337
PFKL	0.615384615	0.71325167	2.284	2.05E-21	2.50E-20	-15.05	339	340	339.5
TMBIM1	0.330316742	0.90701559	2.462	3.10E-19	2.61E-18	-18.339	492	198	345
CCT3	0.891402715	0.46325167	0.848	2.59E-27	8.08E-26	-12.015	133	559	346
OS9	0.823529412	0.514476615	0.411	5.39E-23	8.97E-22	-13.34	249	444	346.5
CALM3	0.85520362	0.491648107	1.163	6.53E-25	1.43E-23	-12.592	189	504	346.5
DAPK2	0.43438914	0.841314031	2.31	2.15E-19	1.87E-18	-17.678	477	218	347.5
SIL1	0.407239819	0.856904232	0.807	6.91E-19	5.45E-18	-19.348	526	176	351
GTF2E2	0.533936652	0.768930958	1.546	1.02E-19	9.46E-19	-16.707	448	254	351
CANX	0.950226244	0.325167038	0.401	5.66E-22	7.87E-21	-13.839	298	406	352
NDUFA11	0.733031674	0.60857461	0.422	2.82E-22	4.21E-21	-13.536	277	427	352
UBE2N	0.850678733	0.472160356	0.31	5.39E-22	7.56E-21	-13.808	296	410	353
BAX	0.846153846	0.489420935	4.082	2.63E-23	4.63E-22	-13.004	236	473	354.5
IFRD1	0.764705882	0.565701559	0.669	3.58E-21	4.21E-20	-14.579	352	361	356.5
SDCBP2	0.352941176	0.890311804	0.971	1.38E-18	1.03E-17	-20.683	555	160	357.5
BIRC2	0.57918552	0.730512249	0.444	1.42E-19	1.29E-18	-16.549	456	260	358
MARC2	0.696832579	0.654231626	3.455	1.75E-23	3.15E-22	-12.853	231	485	358
RABGGTB	0.615384615	0.703786192	1.111	3.50E-20	3.54E-19	-15.695	410	311	360.5
QDPR	0.606334842	0.717706013	2.618	5.86E-21	6.64E-20	-14.657	366	356	361
LAMTOR4	0.656108597	0.675389755	2.776	2.91E-21	3.50E-20	-14.154	345	378	361.5
USMG5	0.868778281	0.466592428	2.884	4.90E-24	9.76E-23	-12.516	208	515	361.5
CUEDC2	0.57918552	0.732739421	4.581	7.26E-20	6.91E-19	-16.101	435	289	362
TSSC1	0.588235294	0.725501114	0.848	6.27E-20	6.03E-19	-16.02	431	294	362.5
GNB1	0.963800905	0.29844098	1.521	8.26E-22	1.11E-20	-13.733	309	416	362.5
TMEM254B	0.719457014	0.621380846	0.807	3.74E-22	5.45E-21	-13.326	284	445	364.5
CTLA4	0.936651584	0.413140312	2.685	1.42E-29	6.07E-28	-11.36	97	632	364.5
RILPL2	0.760180995	0.576837416	2.284	6.77E-22	9.20E-21	-13.577	305	426	365.5
WDR61	0.592760181	0.718819599	0.816	1.43E-19	1.30E-18	-16.362	458	274	366
SPRY2	0.533936652	0.762806236	0.872	7.08E-19	5.56E-18	-18.137	528	205	366.5
XPOT	0.466063348	0.815701559	0.411	6.17E-19	4.92E-18	-17.777	520	215	367.5
INF2	0.443438914	0.830734967	0.189	1.01E-18	7.72E-18	-18.451	544	192	368
GLUD1	0.814479638	0.511135857	0.379	2.19E-21	2.65E-20	-13.988	342	394	368
HCCS	0.461538462	0.819599109	1.753	5.04E-19	4.08E-18	-17.495	511	226	368.5
ACTR10	0.719457014	0.635300668	2.895	6.71E-24	1.31E-22	-12.403	213	524	368.5
ITGB1BP1	0.606334842	0.714922049	0.526	1.36E-20	1.46E-19	-14.84	388	351	369.5
BSG	0.932126697	0.357461024	0.575	3.86E-22	5.57E-21	-13.243	286	453	369.5
LIMS1	0.751131222	0.589643653	0.401	2.86E-22	4.25E-21	-13.109	278	465	371.5
BCAP29	0.615384615	0.69766147	3.131	2.06E-19	1.80E-18	-16.406	473	271	372
FARP1	0.488687783	0.798997773	0.299	5.70E-19	4.55E-18	-17.401	519	230	374.5
DGAT1	0.755656109	0.565144766	0.345	5.29E-20	5.13E-19	-15.472	427	323	375
MMD	0.687782805	0.635300668	0.485	4.70E-20	4.62E-19	-15.295	422	330	376
SSR3	0.764705882	0.556792873	4.001	3.62E-20	3.64E-19	-15.008	412	342	377
RHOF	0.705882353	0.630846325	2.272	1.18E-21	1.52E-20	-13.472	323	433	378
ZBTB32	0.43438914	0.83518931	1.084	2.09E-18	1.52E-17	-18.611	570	188	379
VDAC3	0.733031674	0.605790646	1.774	6.10E-22	8.42E-21	-13.233	304	454	379
SMS	0.733031674	0.595768374	1.333	9.26E-21	1.01E-19	-14.095	380	381	380.5
AKR1A1	0.918552036	0.384187082	7.662	1.02E-22	1.63E-21	-12.587	258	505	381.5
ACTN1	0.574660633	0.731069042	0.506	3.79E-19	3.16E-18	-16.472	498	267	382.5
ATP6V0B	0.628959276	0.693207127	3.939	2.22E-20	2.32E-19	-14.244	397	371	384
PTK2B	0.809954751	0.505567929	1.438	3.48E-20	3.53E-19	-14.567	409	362	385.5
REEP5	0.904977376	0.436525612	1.753	2.03E-26	5.42E-25	-11.423	155	618	386.5
CREM	0.678733032	0.658129176	2.531	9.14E-22	1.20E-20	-13.17	314	461	387.5
HK1	0.687782805	0.628062361	0.322	3.24E-19	2.72E-18	-16.255	495	283	389
EIF1AX	0.674208145	0.654788419	2.68	8.11E-21	8.92E-20	-13.883	377	401	389
RAP1A	0.769230769	0.56013363	1.864	4.22E-21	4.89E-20	-13.689	358	420	389
SEC61G	0.850678733	0.482182628	2.353	3.95E-23	6.77E-22	-12.302	242	536	389

Gene	TP	TN	thresh_mhg	hyper_pval	hyper_qval	gen_qval	rank_hyper_qval	rank_gen_qval	mean_rank
SAR1B	0.674208145	0.64922049	1.705	3.81E-20	3.81E-19	-14.438	415	365	390
RNH1	0.751131222	0.58518931	1.077	9.65E-22	1.27E-20	-13.101	316	467	391.5
BMYC	0.43438914	0.834632517	1.098	2.55E-18	1.82E-17	-18.196	581	203	392
TMEM256	0.601809955	0.70935412	2.104	2.27E-19	1.95E-18	-15.827	481	306	393.5
NHP2	0.692307692	0.644766147	6.673	9.98E-22	1.30E-20	-13.062	318	471	394.5
TMEM135	0.57918552	0.723830735	0.251	1.02E-18	7.74E-18	-16.86	545	246	395.5
OTUB1	0.823529412	0.506124722	3.671	4.88E-22	6.90E-21	-12.63	293	499	396
MEA1	0.683257919	0.651447661	5.398	1.80E-21	2.22E-20	-13.176	336	458	397
SSBP1	0.755656109	0.570155902	1.281	1.45E-20	1.54E-19	-13.843	390	405	397.5
CYP51	0.538461538	0.758351893	0.687	9.01E-19	6.93E-18	-16.602	539	257	398
DCTN2	0.79638009	0.528953229	0.687	5.10E-21	5.84E-20	-13.445	362	436	399
TXNDC17	0.714932127	0.624164811	1.669	6.13E-22	8.42E-21	-12.67	303	496	399.5
PHB	0.787330317	0.542873051	2.43	1.99E-21	2.44E-20	-13.17	338	462	400
CISD3	0.538461538	0.760022272	2.973	5.38E-19	4.33E-18	-16.105	515	288	401.5
SDF4	0.923076923	0.371937639	4.852	3.85E-22	5.57E-21	-12.513	287	516	401.5
ETOHI1	0.78280543	0.520044543	0.202	2.19E-18	1.59E-17	-17.157	572	236	404
LDHA	0.63800905	0.662026726	11.313	1.19E-17	7.69E-17	-20.115	643	166	404.5
UQCR11	0.656108597	0.664253898	2.007	6.89E-20	6.60E-19	-14.167	433	376	404.5
MVP	0.452488688	0.824053452	1.993	1.07E-18	8.11E-18	-16.541	549	261	405
DENND4A	0.886877828	0.396436526	0.124	4.72E-19	3.85E-18	-15.873	509	303	406
DNAJC1	0.592760181	0.716592428	0.766	2.76E-19	2.35E-18	-15.39	487	325	406
RAB8B	0.819004525	0.501113586	1.05	7.14E-21	7.96E-20	-13.365	372	442	407
ABHD4	0.343891403	0.894766147	5.112	2.25E-18	1.62E-17	-16.901	575	242	408.5
PLK2	0.380090498	0.870267261	3.374	4.47E-18	3.09E-17	-17.608	600	221	410.5
MIF	0.941176471	0.315701559	6.412	2.87E-19	2.43E-18	-15.258	490	332	411
FBXW11	0.615384615	0.691536748	0.151	1.15E-18	8.63E-18	-16.351	552	277	414.5
SLC25A3	0.764705882	0.546213808	10.008	5.17E-19	4.17E-18	-15.605	514	315	414.5
XDH	0.533936652	0.759465479	0.411	1.98E-18	1.45E-17	-16.526	567	263	415
MLF2	0.719457014	0.610244989	3.862	8.04E-21	8.87E-20	-13.226	375	456	415.5
MRPL40	0.65158371	0.682628062	3.936	1.18E-21	1.52E-20	-12.54	322	514	418
PDIA4	0.63800905	0.678173719	2.128	1.54E-19	1.38E-18	-14.183	464	375	419.5
CD200R1	0.384615385	0.865812918	1.454	8.03E-18	5.31E-17	-17.97	627	213	420
GUK1	0.583710407	0.724387528	2.563	2.78E-19	2.36E-18	-14.688	488	354	421
OSTF1	0.755656109	0.547884187	9.286	3.86E-18	2.69E-17	-16.818	594	249	421.5
9530068E07RIK	0.737556561	0.594654788	0.496	3.54E-21	4.18E-20	-12.556	351	507	429
RAC1	0.918552036	0.378619154	0.872	4.21E-22	6.05E-21	-11.928	289	570	429.5
PLEKHB2	0.719457014	0.628062361	4.253	5.57E-23	9.24E-22	-11.492	250	609	429.5
DNAJB4	0.330316742	0.899777283	2.183	9.69E-18	6.30E-17	-17.561	638	222	430
IL21R	0.841628959	0.457126949	0.993	3.88E-19	3.22E-18	-14.583	500	360	430
SSR4	0.859728507	0.499443207	5.195	1.47E-26	4.09E-25	-10.771	149	712	430.5
SMYD5	0.352941176	0.884743875	1.157	1.54E-17	9.81E-17	-18.019	652	210	431
IQSEC1	0.764705882	0.542316258	0.422	1.34E-18	1.01E-17	-15.713	554	310	432
STX11	0.515837104	0.770044543	0.556	6.80E-18	4.55E-17	-16.858	620	247	433.5
GGH	0.443438914	0.827951002	2.856	2.74E-18	1.95E-17	-16.107	583	287	435
ATPIF1	0.678733032	0.652561247	4.168	4.49E-21	5.17E-20	-12.554	360	510	435
IDI1	0.475113122	0.79844098	0.367	2.03E-17	1.27E-16	-18.065	665	207	436
CNIH	0.660633484	0.653674833	3.426	3.86E-19	3.20E-18	-14.217	499	373	436
NAPSA	0.389140271	0.861358575	0.949	1.40E-17	8.99E-17	-17.416	646	229	437.5
BCL2A1C	0.642533937	0.669265033	1.036	5.67E-19	4.54E-18	-14.621	518	359	438.5
COMT	0.529411765	0.79064588	3.849	2.09E-22	3.18E-21	-11.528	272	605	438.5
APRT	0.787330317	0.547327394	1.189	6.16E-22	8.42E-21	-11.831	300	578	439
ATP10A	0.502262443	0.780066815	0.322	8.62E-18	5.68E-17	-16.799	629	253	441
ECH1	0.886877828	0.436525612	2.348	2.12E-23	3.79E-22	-11.212	232	650	441
SEPHS2	0.610859729	0.694877506	2.293	1.41E-18	1.05E-17	-15.315	556	328	442
GLRX3	0.755656109	0.571826281	4.684	9.36E-21	1.02E-19	-12.555	381	508	444.5
FDPS	0.570135747	0.731625835	3.992	1.00E-18	7.64E-18	-14.722	543	353	448
RNPEP	0.606334842	0.703786192	0.816	3.57E-19	2.98E-18	-13.896	496	400	448
TFG	0.601809955	0.706570156	0.74	5.04E-19	4.08E-18	-14.035	512	387	449.5
TNFRSF1B	0.877828054	0.453786192	4.715	5.90E-24	1.15E-22	-10.995	212	687	449.5
YWHAE	0.968325792	0.285634744	1.07	2.19E-21	2.65E-20	-12.026	343	558	450.5
GNG2	0.719457014	0.597438753	0.287	2.35E-19	2.02E-18	-13.659	482	422	452
TRPV2	0.701357466	0.615256125	4.401	2.71E-19	2.31E-18	-13.709	486	419	452.5
HSP90AB1	0.936651584	0.31013363	9.834	6.45E-18	4.33E-17	-16.101	618	290	454
XBP1	0.619909502	0.691536748	0.401	3.69E-19	3.08E-18	-13.808	497	411	454
MRPS36	0.619909502	0.690979955	1.454	4.31E-19	3.54E-18	-13.853	505	404	454.5
STRAP	0.79638009	0.526726058	1.674	9.02E-21	9.87E-20	-12.345	379	530	454.5
EIF4E	0.696832579	0.624721604	4.205	7.35E-20	6.99E-19	-12.983	436	474	455
MXI1	0.371040724	0.871937639	0.669	2.46E-17	1.50E-16	-17.254	679	234	456.5
ECE1	0.429864253	0.832962138	2.101	1.46E-17	9.30E-17	-16.498	648	265	456.5
GM17745	0.42081448	0.837416481	1.305	2.95E-17	1.77E-16	-17.498	689	225	457
CHCHD10	0.556561086	0.739977728	4.486	2.50E-18	1.79E-17	-15.187	580	336	458
BIN1	0.78280543	0.552895323	3.067	5.40E-22	7.56E-21	-11.382	295	623	459
TDG	0.411764706	0.845768374	3.288	1.45E-17	9.30E-17	-16.362	649	275	462
TMEM30A	0.701357466	0.60467706	0.356	4.04E-18	2.81E-17	-15.306	596	329	462.5

Gene	TP	TN	thresh_mhg	hyper_pval	hyper_qval	gen_qval	rank_hyper_qval	rank_gen_qval	mean_rank
ACP1	0.633484163	0.680400891	4.013	2.64E-19	2.25E-18	-13.396	485	441	463
PFDN1	0.619909502	0.694877506	3.848	1.43E-19	1.30E-18	-13.078	457	469	463
SEPT9	0.85520362	0.458797327	4.236	3.69E-21	4.31E-20	-11.881	355	575	465
PSMB4	0.868778281	0.474944321	6.441	5.11E-25	1.15E-23	-10.579	184	746	465
PA2G4	0.787330317	0.526726058	2.995	1.22E-19	1.12E-18	-12.943	454	477	465.5
P4HB	0.936651584	0.365256125	0.526	7.55E-24	1.45E-22	-10.729	216	718	467
CCDC6	0.642533937	0.665367483	0.124	1.62E-18	1.20E-17	-14.167	561	377	469
BC004004	0.660633484	0.658685969	0.888	9.88E-20	9.19E-19	-12.722	446	493	469.5
COPS6	0.850678733	0.475501114	1.47	2.27E-22	3.44E-21	-11.122	274	665	469.5
SLC25A11	0.7239819	0.60857461	4.59	3.62E-21	4.26E-20	-11.666	353	588	470.5
IGBP1	0.742081448	0.594654788	4.527	9.84E-22	1.29E-20	-11.378	317	626	471.5
SSR1	0.895927602	0.404231626	1.007	3.13E-21	3.75E-20	-11.611	346	598	472
TRAF4	0.429864253	0.832962138	2.223	1.46E-17	9.30E-17	-15.968	650	297	473.5
HSD17B12	0.552036199	0.739420935	0.444	8.88E-18	5.84E-17	-15.594	631	317	474
COX6C	0.936651584	0.374164811	6.362	7.14E-25	1.56E-23	-10.522	190	758	474
RIOK1	0.619909502	0.685412027	1.17	2.01E-18	1.46E-17	-14.086	569	383	476
RNASEK	0.805429864	0.530066815	1.379	2.49E-22	3.75E-21	-11.025	275	680	477.5
SYTL2	0.502262443	0.776726058	0.345	2.41E-17	1.48E-16	-16.283	674	282	478
ORMDL1	0.606334842	0.69766147	0.444	2.00E-18	1.46E-17	-14.009	568	388	478
FXR1	0.56561086	0.729955457	2.401	4.96E-18	3.40E-17	-14.748	605	352	478.5
UGP2	0.497737557	0.781737194	1.642	1.55E-17	9.85E-17	-15.839	653	305	479
PTMS	0.524886878	0.763919822	1.208	4.77E-18	3.28E-17	-14.634	602	358	480
LAMP2	0.547511312	0.744432071	1.982	6.23E-18	4.19E-17	-14.908	617	347	482
HSPD1	0.846153846	0.458797327	1.585	6.50E-20	6.23E-19	-12.229	432	538	485
HSBP1	0.746606335	0.582962138	0.856	6.34E-21	7.12E-20	-11.562	368	602	485
PDIA3	0.945701357	0.331291759	6.479	9.01E-22	1.19E-20	-11.136	313	660	486.5
ITFG1	0.556561086	0.736636971	1.807	6.60E-18	4.42E-17	-14.66	619	355	487
PERP	0.371040724	0.869153675	1.195	7.32E-17	4.13E-16	-16.896	735	244	489.5
EXT2	0.542986425	0.751113586	1.844	2.62E-18	1.87E-17	-13.946	582	397	489.5
TRAF1	0.859728507	0.444877506	4.098	2.73E-20	2.82E-19	-11.745	401	581	491
NDUFB8	0.805429864	0.527839644	1.922	4.48E-22	6.36E-21	-10.949	292	691	491.5
TCP1	0.900452489	0.426503341	4.705	1.85E-24	3.82E-23	-10.352	201	782	491.5
NFKBIE	0.470588235	0.804008909	0.911	1.02E-17	6.64E-17	-14.917	639	346	492.5
HIF1A	0.800904977	0.521158129	4.03	9.86E-21	1.06E-19	-11.55	384	603	493.5
ATP5G1	0.837104072	0.488864143	4.903	5.86E-22	8.13E-21	-10.995	299	688	493.5
PLEKHO2	0.57918552	0.712694878	0.367	2.34E-17	1.45E-16	-15.604	672	316	494
SIN3B	0.814479638	0.518930958	1.422	2.87E-22	4.25E-21	-10.781	280	710	495
EDF1	0.936651584	0.36636971	6.946	5.63E-24	1.12E-22	-10.298	209	787	498
PRDX1	0.977375566	0.29064588	5.303	4.29E-24	8.60E-23	-10.269	207	790	498.5
VPS35	0.615384615	0.68986637	0.705	1.82E-18	1.34E-17	-13.455	565	434	499.5
RFC2	0.733031674	0.593541203	0.766	1.67E-20	1.75E-19	-11.507	395	606	500.5
EIF2S3X	0.819004525	0.494988864	0.367	3.32E-20	3.39E-19	-11.607	405	599	502
MRPS33	0.669683258	0.650334076	4.597	9.21E-20	8.60E-19	-11.993	444	562	503
CNOT3	0.583710407	0.708797327	0.39	2.30E-17	1.43E-16	-15.029	669	341	505
ELK3	0.592760181	0.706013363	0.263	5.55E-18	3.76E-17	-13.924	612	398	505
JAK3	0.914027149	0.376948775	0.651	3.66E-21	4.28E-20	-11.151	354	657	505.5
IRF5	0.34841629	0.883073497	3.694	1.01E-16	5.61E-16	-16.505	750	264	507
NFS1	0.470588235	0.800111359	0.864	3.60E-17	2.14E-16	-15.516	698	320	509
STK24	0.656108597	0.652004454	1.803	1.89E-18	1.38E-17	-13.25	566	452	509
AGPAT4	0.56561086	0.722717149	0.263	3.80E-17	2.25E-16	-15.531	701	319	510
FAM162A	0.63800905	0.671492205	2.198	9.69E-19	7.44E-18	-12.924	540	480	510
NDUFA9	0.71040724	0.621380846	5.242	4.67E-21	5.36E-20	-11.14	361	659	510
NDUFB7	0.868778281	0.478285078	2.186	2.04E-25	4.95E-24	-10.011	171	854	512.5
BRI3BP	0.520361991	0.777839644	2.924	1.87E-19	1.65E-18	-12.044	472	556	514
DYNLT1C	0.619909502	0.681514477	3.331	5.76E-18	3.90E-17	-13.725	613	417	515
TMEM173	0.692307692	0.625835189	6.206	1.80E-19	1.59E-18	-11.951	469	566	517.5
DPYSL2	0.923076923	0.35467706	0.526	2.84E-20	2.92E-19	-11.329	403	635	519
LCP1	0.932126697	0.328507795	9.251	4.84E-19	3.93E-18	-12.345	510	531	520.5
TIMM8B	0.466063348	0.804565702	3.967	2.60E-17	1.58E-16	-14.459	682	364	523
ARHGAP18	0.416289593	0.836859688	0.496	1.10E-16	6.03E-16	-16.043	756	292	524
KLRK1	0.760180995	0.561247216	1.064	4.09E-20	4.07E-19	-11.367	417	631	524
RAB14	0.728506787	0.58908686	1.824	1.82E-19	1.60E-18	-11.771	470	579	524.5
PIGP	0.479638009	0.793986637	1.257	2.77E-17	1.67E-16	-14.466	687	363	525
SERINC1	0.701357466	0.606904232	2.578	2.31E-18	1.66E-17	-13.057	578	472	525
UQCRC2	0.79638009	0.543429844	6.406	1.13E-22	1.79E-21	-10.287	261	789	525
CYFIP1	0.393665158	0.853006682	1.036	1.00E-16	5.55E-16	-15.898	749	302	525.5
MFSD1	0.597285068	0.70935412	1.029	7.10E-19	5.56E-18	-12.419	529	522	525.5
PPIP5K2	0.475113122	0.793429844	0.111	9.81E-17	5.45E-16	-15.815	747	307	527
GATAD1	0.547511312	0.739977728	0.485	2.25E-17	1.40E-16	-13.999	667	390	528.5
ILK	0.800904977	0.53674833	5.389	1.69E-22	2.63E-21	-10.267	266	791	528.5
SNRPA	0.714932127	0.59298441	1.506	2.36E-18	1.69E-17	-12.937	579	479	529
PHPT1	0.470588235	0.79844098	1.709	6.10E-17	3.47E-16	-15.224	728	334	531
GLRX	0.755656109	0.588530067	3.046	1.04E-22	1.65E-21	-10.201	260	803	531.5
CSTB	0.65158371	0.660356347	1.628	6.39E-19	5.08E-18	-12.185	522	542	532

Gene	TP	TN	thresh_mhg	hyper_pval	hyper_qval	gen_qval	rank_hyper_qval	rank_gen_qval	mean_rank
MRPS2	0.307692308	0.905345212	4.43	3.63E-16	1.82E-15	-16.9	824	243	533.5
RSL24D1	0.71040724	0.596325167	0.986	3.27E-18	2.30E-17	-12.943	589	478	533.5
ADAM17	0.533936652	0.752783964	2.124	1.46E-17	9.30E-17	-13.689	647	421	534
ODC1	0.755656109	0.548997773	1.93	2.95E-18	2.09E-17	-12.707	584	494	539
DUSP14	0.330316742	0.892538976	5.323	2.30E-16	1.20E-15	-16.063	791	291	541
MTDH	0.65158371	0.659242762	3.239	8.62E-19	6.64E-18	-12.105	538	550	544
MRPL33	0.751131222	0.574610245	2.738	1.61E-20	1.70E-19	-10.843	392	701	546.5
PSMA4	0.828054299	0.523385301	3.329	1.09E-24	2.30E-23	-9.744	196	897	546.5
GPI1	0.954751131	0.295657016	7.555	1.15E-19	1.05E-18	-11.256	451	645	548
NSUN2	0.556561086	0.731625835	1.876	2.75E-17	1.66E-16	-13.79	686	412	549
SEC11C	0.900452489	0.408685969	5.759	1.95E-22	3.00E-21	-10.105	270	830	550
YWHAH	0.886877828	0.451002227	4.974	4.45E-25	1.02E-23	-9.634	180	921	550.5
REXO2	0.628959276	0.677616927	2.642	1.77E-18	1.30E-17	-12.204	562	541	551.5
LMAN1	0.411764706	0.839643653	1.22	1.28E-16	6.98E-16	-14.937	761	345	553
LAMTOR5	0.683257919	0.629732739	4.233	6.71E-19	5.30E-18	-11.701	525	584	554.5
ANXA4	0.393665158	0.850222717	0.546	2.72E-16	1.41E-15	-15.638	797	313	555
MRPL17	0.542986425	0.748329621	1.709	5.99E-18	4.04E-17	-12.621	615	500	557.5
CERS6	0.334841629	0.888641425	1.287	3.55E-16	1.79E-15	-15.916	822	300	561

Table 2. Ranked top transcription factors differentially expressed in cluster 7

Gene	TP	TN	thresh_mhg	hyper_pval	hyper_qval	gen_qval	rank_hyper_qval	rank_gen_qval	mean_rank
IRF8	0.886877828	0.663697105	1.501	4.18E-58	1.63E-55	-60.58	1	1	1
RBPJ	0.873303167	0.632516704	2.763	4.56E-49	5.93E-47	-42.315	3	2	2.5
LITAF	0.936651584	0.551224944	5.893	1.35E-49	2.64E-47	-41.548	2	3	2.5
IKZF2	0.719457014	0.737193764	0.084	6.49E-40	5.06E-38	-37.446	5	6	5.5
BHLHE40	0.977375566	0.422048998	6.796	6.69E-41	6.53E-39	-32.471	4	7	5.5
EPAS1	0.529411765	0.863585746	0.986	5.63E-37	3.14E-35	-38.289	7	5	6
MNDA	0.470588235	0.889755011	4.02	1.12E-34	4.86E-33	-40.982	9	4	6.5
NR4A2	0.914027149	0.498886414	0.595	2.62E-36	1.28E-34	-31.351	8	8	8
TOX	0.904977376	0.520044543	3.455	1.76E-37	1.14E-35	-23.972	6	15	10.5
ID2	0.972850679	0.341314031	4.705	6.02E-29	1.96E-27	-29	12	10	11
NFKB2	0.846153846	0.561804009	2.359	1.52E-32	5.92E-31	-25.366	10	12	11
STAT3	0.909502262	0.43596882	6.143	3.70E-27	1.03E-25	-29.78	14	9	11.5
UHRF2	0.542986425	0.809576837	0.971	2.48E-27	7.44E-26	-27.41	13	11	12
ZMIZ1	0.751131222	0.655345212	0.214	4.47E-31	1.58E-29	-24.896	11	14	12.5
TRPS1	0.511312217	0.829064588	0.986	9.50E-27	2.47E-25	-25.165	15	13	14
KDM2B	0.678733032	0.677616927	0.88	2.60E-24	5.97E-23	-21.433	17	16	16.5
RUNX2	0.78280543	0.582962138	1.546	1.20E-25	2.92E-24	-20.044	16	18	17
RBL2	0.832579186	0.511135857	0.239	6.99E-24	1.43E-22	-19.557	19	20	19.5
NCOR2	0.656108597	0.688752784	0.239	5.32E-23	9.02E-22	-19.689	23	19	21
CALR	0.923076923	0.384187082	2.844	1.64E-23	2.91E-22	-18.422	22	22	22
SMARCB1	0.65158371	0.698218263	4.752	9.93E-24	1.84E-22	-17.095	21	26	23.5
UTF1	0.330316742	0.909242762	4.976	1.01E-19	1.17E-18	-21.255	34	17	25.5
SREBF2	0.733031674	0.597438753	0.138	5.92E-21	7.97E-20	-16.921	29	27	28
COMMD3	0.733031674	0.60467706	3.134	8.29E-22	1.24E-20	-15.961	26	30	28
HDAC1	0.837104072	0.505011136	2.214	8.21E-24	1.60E-22	-14.043	20	36	28
FUBP1	0.733031674	0.605233853	0.31	7.11E-22	1.11E-20	-14.855	25	35	30
GTF2E2	0.533936652	0.768930958	1.546	1.02E-19	1.17E-18	-16.707	33	28	30.5
SPRY2	0.533936652	0.762806236	0.872	7.08E-19	6.91E-18	-18.137	40	23	31.5
ZBTB32	0.43438914	0.83518931	1.084	2.09E-18	1.89E-17	-18.611	43	21	32
DENND4A	0.886877828	0.396436526	0.124	4.72E-19	4.72E-18	-15.873	39	31	35
CREM	0.678733032	0.658129176	2.531	9.14E-22	1.32E-20	-13.17	27	44	35.5
SMYD5	0.352941176	0.884743875	1.157	1.54E-17	1.25E-16	-18.019	48	24	36
PHB	0.787330317	0.542873051	2.43	1.99E-21	2.77E-20	-13.17	28	45	36.5
MXI1	0.371040724	0.871937639	0.669	2.46E-17	1.88E-16	-17.254	51	25	38
XBP1	0.619909502	0.691536748	0.401	3.69E-19	3.78E-18	-13.808	38	39	38.5
NFKBIE	0.470588235	0.804008909	0.911	1.02E-17	8.49E-17	-14.917	47	34	40.5
CNOT3	0.583710407	0.708797327	0.39	2.30E-17	1.79E-16	-15.029	50	33	41.5
PFDN1	0.619909502	0.694877506	3.848	1.43E-19	1.55E-18	-13.078	36	47	41.5
PA2G4	0.787330317	0.526726058	2.995	1.22E-19	1.36E-18	-12.943	35	48	41.5
ELK3	0.592760181	0.706013363	0.263	5.55E-18	4.71E-17	-13.924	46	38	42
IRF5	0.34841629	0.883073497	3.694	1.01E-16	7.06E-16	-16.505	56	29	42.5
GATAD1	0.547511312	0.739977728	0.485	2.25E-17	1.79E-16	-13.999	49	37	43
EDF1	0.936651584	0.36636971	6.946	5.63E-24	1.22E-22	-10.298	18	71	44.5
HSBP1	0.746606335	0.582962138	0.856	6.34E-21	8.24E-20	-11.562	30	60	45
HIF1A	0.800904977	0.521158129	4.03	9.86E-21	1.24E-19	-11.55	31	61	46
HIVEP1	0.561085973	0.723830735	0.151	8.15E-17	5.78E-16	-13.609	54	41	47.5
SMYD2	0.393665158	0.847995546	0.623	5.95E-16	3.57E-15	-15.744	65	32	48.5
HTATIP2	0.533936652	0.743875278	0.748	1.86E-16	1.25E-15	-13.747	58	40	49
MORF4L2	0.656108597	0.648106904	2.157	5.22E-18	4.53E-17	-12.013	45	57	51
TBX21	0.628959276	0.66481069	5.5	5.13E-17	3.85E-16	-12.289	52	52	52

Gene	TP	TN	thresh_mhg	hyper_pval	hyper_qval	gen_qval	rank_hyper_qval	rank_gen_qval	mean_rank
MED7	0.457013575	0.802895323	1.536	3.88E-16	2.40E-15	-13.313	63	43	53
C1D	0.601809955	0.688195991	0.516	7.59E-17	5.58E-16	-12.182	53	54	53.5
TMF1	0.701357466	0.592427617	0.202	8.04E-17	5.78E-16	-12.152	55	55	55
CSDA	0.570135747	0.707126949	0.926	8.49E-16	4.94E-15	-12.863	67	49	58
MED14	0.50678733	0.753340757	0.151	6.71E-15	3.44E-14	-13.341	76	42	59
NFAT5	0.592760181	0.683184855	0.124	2.17E-15	1.17E-14	-12.415	72	51	61.5
IKZF3	0.846153846	0.448218263	0.287	8.29E-19	7.89E-18	-8.956	41	84	62.5
GNPTAB	0.479638009	0.772828508	0.275	1.39E-14	6.79E-14	-13.104	80	46	63
NFIL3	0.398190045	0.83908686	3.536	4.07E-15	2.17E-14	-12.098	73	56	64.5
MYEF2	0.547511312	0.718262806	0.454	7.72E-15	3.91E-14	-12.214	77	53	65
PHB2	0.819004525	0.486636971	2.284	2.58E-19	2.72E-18	-8.162	37	93	65
ZRANB2	0.497737557	0.761135857	1.227	6.12E-15	3.18E-14	-11.704	75	58	66.5
NT5C	0.819004525	0.494988864	4.353	3.32E-20	4.05E-19	-7.857	32	101	66.5
ZMAT2	0.647058824	0.640868597	3.444	2.83E-16	1.84E-15	-9.591	60	79	69.5
RNF14	0.502262443	0.754454343	0.465	1.35E-14	6.68E-14	-11.308	79	65	72
ZC3H15	0.755656109	0.528953229	1.208	3.20E-16	2.05E-15	-9.211	61	83	72
COPS2	0.50678733	0.748329621	0.918	2.52E-14	1.20E-13	-11.369	82	64	73
NFKBIB	0.7239819	0.559576837	1	7.08E-16	4.19E-15	-9.252	66	82	74
PPIE	0.552036199	0.722717149	4.715	8.90E-16	5.10E-15	-9.543	68	81	74.5
TFDP1	0.502262443	0.751113586	1.401	3.25E-14	1.51E-13	-11.08	84	67	75.5
NR4A3	0.583710407	0.683184855	0.678	1.59E-14	7.67E-14	-10.379	81	70	75.5
YAF2	0.334841629	0.871937639	4.538	1.85E-13	7.68E-13	-11.498	94	62	78
FUBP3	0.398190045	0.820712695	0.604	1.08E-12	3.94E-12	-12.6	107	50	78.5
AEBP2	0.56561086	0.695434298	0.111	4.19E-14	1.83E-13	-10.681	89	68	78.5
TSG101	0.642533937	0.625835189	0.766	2.63E-14	1.24E-13	-10.099	83	74	78.5
GTF2E1	0.334841629	0.868596882	2.88	5.70E-13	2.18E-12	-11.665	102	59	80.5
PHF15	0.475113122	0.765033408	0.444	2.90E-13	1.17E-12	-11.133	97	66	81.5
SND1	0.597285068	0.667037862	3.206	3.82E-14	1.73E-13	-9.617	86	78	82
RBX1	0.805429864	0.496659243	4.931	1.13E-18	1.05E-17	-6.842	42	122	82
TCERG1	0.583710407	0.668151448	0.287	5.07E-13	1.98E-12	-10.298	100	72	86
MYBBP1A	0.63800905	0.623051225	0.791	1.28E-13	5.44E-13	-9.591	92	80	86
FOSL2	0.647058824	0.620267261	3.109	3.42E-14	1.57E-13	-8.551	85	88	86.5
SKIL	0.864253394	0.391982183	0.202	1.37E-15	7.65E-15	-7.736	70	104	87
VAMP7	0.411764706	0.807349666	1.531	2.45E-12	8.37E-12	-11.4	114	63	88.5
CAND1	0.475113122	0.757238307	0.189	2.03E-12	7.00E-12	-10.627	113	69	91
NDUFA13	0.972850679	0.288975501	4.019	8.55E-23	1.39E-21	-5.73	24	160	92
SSRP1	0.737556561	0.526726058	1.632	3.85E-14	1.73E-13	-7.942	87	99	93
STAT4	0.823529412	0.45545657	4.008	1.04E-16	7.09E-16	-6.4	57	132	94.5
SARNP	0.773755656	0.506124722	3.952	5.80E-16	3.53E-15	-6.629	64	126	95
KEAP1	0.457013575	0.771158129	0.356	2.58E-12	8.68E-12	-9.681	116	77	96.5
FLI1	0.791855204	0.448775056	0.275	1.15E-12	4.15E-12	-8.951	108	85	96.5
BTF3	0.941176471	0.279510022	9.346	1.26E-15	7.10E-15	-6.717	69	124	96.5
GTF2H5	0.619909502	0.635300668	1.459	3.95E-13	1.56E-12	-8.07	99	96	97.5
RUVBL2	0.497737557	0.739420935	2.441	1.53E-12	5.42E-12	-8.425	110	91	100.5
PRDM1	0.34841629	0.849109131	2.993	1.22E-11	3.74E-11	-10.067	127	75	101
DDX54	0.610859729	0.63752784	0.379	1.55E-12	5.44E-12	-8.125	111	94	102.5
RUNX3	0.791855204	0.457126949	0.926	2.21E-13	8.99E-13	-7.477	96	109	102.5
CCNT2	0.470588235	0.756124722	1.903	6.54E-12	2.13E-11	-8.809	120	87	103.5
FLII	0.669683258	0.58518931	2.915	5.12E-13	1.98E-12	-7.567	101	107	104
SMARCC2	0.642533937	0.609131403	0.214	9.69E-13	3.56E-12	-7.738	106	103	104.5
HCLS1	0.904977376	0.36247216	4.471	3.12E-18	2.76E-17	-5.668	44	165	104.5
ARNT	0.447963801	0.766703786	0.31	4.45E-11	1.27E-10	-9.974	136	76	106
MLX	0.50678733	0.724387528	0.722	8.18E-12	2.57E-11	-8.452	124	89	106.5
MKI67IP	0.466063348	0.755011136	0.566	2.05E-11	6.20E-11	-8.911	129	86	107.5
ERH	0.873303167	0.388641425	5.282	2.03E-16	1.34E-15	-5.735	59	157	108
ZBTB1	0.402714932	0.799554566	0.367	1.09E-10	2.94E-10	-10.1	144	73	108.5
MED28	0.778280543	0.486636971	0.333	1.29E-14	6.45E-14	-6.088	78	139	108.5
HMGB3	0.457013575	0.760022272	0.444	3.68E-11	1.09E-10	-8.432	132	90	111
RARA	0.438914027	0.776169265	0.566	2.76E-11	8.27E-11	-8.247	130	92	111
RUVBL1	0.520361991	0.704899777	0.485	4.26E-11	1.23E-10	-8.11	135	95	115
KDM5C	0.574660633	0.65701559	0.444	3.14E-11	9.34E-11	-7.773	131	102	116.5
IRF2	0.678733032	0.573496659	0.356	8.58E-13	3.19E-12	-6.591	105	128	116.5
YBX1	0.760180995	0.463808463	0.029	6.17E-11	1.72E-10	-7.488	140	108	124
MAX	0.515837104	0.706570156	1.138	6.96E-11	1.93E-10	-7.405	141	110	125.5
POLR1E	0.2760181	0.889198218	5.21	3.11E-10	7.78E-10	-8.022	156	97	126.5
BOLA2	0.520361991	0.703786192	2.444	5.38E-11	1.52E-10	-7.054	138	116	127
AES	0.683257919	0.545100223	0.422	8.39E-11	2.29E-10	-7.207	143	114	128.5
GTF3A	0.592760181	0.646993318	0.848	7.65E-12	2.43E-11	-6.216	122	135	128.5
GTF2F1	0.642533937	0.599665924	3.216	6.63E-12	2.14E-11	-6.206	121	136	128.5
LRRFIP1	0.895927602	0.330734967	1.632	5.98E-14	2.59E-13	-5.435	90	170	130
RPL7L1	0.529411765	0.704899777	3.505	7.60E-12	2.43E-11	-5.987	123	140	131.5
RNF166	0.601809955	0.630289532	3.454	3.90E-11	1.14E-10	-6.417	133	131	132
CBX3	0.986425339	0.185412027	1.07	5.34E-15	2.81E-14	-4.911	74	191	132.5
GTF2B	0.588235294	0.642538976	1.86	4.44E-11	1.27E-10	-6.586	137	129	133

Gene	TP	TN	thresh_mhg	hyper_pval	hyper_qval	gen_qval	rank_hyper_qval	rank_gen_qval	mean_rank
ZBTB17	0.343891403	0.841314031	4.305	2.64E-10	6.70E-10	-7.265	154	113	133.5
PLAGL2	0.380090498	0.80623608	0.506	1.54E-09	3.56E-09	-7.878	169	100	134.5
MED17	0.384615385	0.808463252	2.128	3.96E-10	9.78E-10	-7.307	158	111	134.5
TBPL1	0.511312217	0.706013363	0.401	1.79E-10	4.71E-10	-6.725	148	123	135.5
REL	0.574660633	0.645879733	0.506	2.77E-10	6.97E-10	-7.026	155	117	136
MORF4L1	0.850678733	0.393652561	3.668	4.01E-14	1.78E-13	-5.141	88	184	136
ARID5B	0.484162896	0.718819599	0.367	1.67E-09	3.83E-09	-7.671	170	106	138
DR1	0.461538462	0.742761693	0.864	6.95E-10	1.64E-09	-7.307	165	112	138.5
HCFC1	0.683257919	0.563474388	0.731	2.53E-12	8.59E-12	-5.699	115	163	139
RNPS1	0.778280543	0.471046771	0.748	3.16E-13	1.26E-12	-5.239	98	180	139
MED24	0.411764706	0.776726058	0.31	3.60E-09	7.83E-09	-7.686	179	105	142
TOX4	0.574660633	0.659799555	2.004	1.78E-11	5.44E-11	-5.76	128	156	142
FUS	0.737556561	0.503340757	1.556	4.21E-12	1.39E-11	-5.641	118	166	142
SMARCA5	0.42081448	0.772828508	0.444	1.68E-09	3.83E-09	-7.181	171	115	143
CNOT8	0.561085973	0.662583519	0.322	1.27E-10	3.38E-10	-5.879	147	145	146
DEK	0.823529412	0.420935412	0.422	1.58E-13	6.64E-13	-4.677	93	202	147.5
LZTR1	0.371040724	0.801224944	0.496	2.27E-08	4.39E-08	-7.993	202	98	150
BAZ1A	0.497737557	0.702672606	0.379	3.69E-09	7.97E-09	-6.864	180	120	150
VGLL4	0.633484163	0.589643653	1.454	2.47E-10	6.34E-10	-5.836	152	149	150.5
PURB	0.547511312	0.654231626	0.176	6.32E-09	1.33E-08	-6.933	186	119	152.5
SUB1	0.972850679	0.198775056	4.258	2.15E-13	8.83E-13	-4.325	95	210	152.5
SQSTM1	0.932126697	0.275055679	7.008	8.09E-14	3.47E-13	-4.191	91	218	154.5
FOXN3	0.484162896	0.714365256	0.367	3.95E-09	8.46E-09	-6.397	182	133	157.5
PTTG1	0.669683258	0.580734967	4.849	1.28E-12	4.56E-12	-4.408	109	209	159
DEDD	0.416289593	0.763919822	0.356	2.24E-08	4.35E-08	-7.023	201	118	159.5
TARDBP	0.63800905	0.594654788	0.556	4.20E-11	1.22E-10	-5.107	134	185	159.5
CDC5L	0.520361991	0.684855234	3.622	2.28E-09	5.09E-09	-5.844	175	147	161
SMARCE1	0.719457014	0.5077951	0.832	7.11E-11	1.95E-10	-5.182	142	182	162
NOTCH2	0.714932127	0.500556793	0.202	6.02E-10	1.44E-09	-5.706	163	162	162.5
UBTF	0.57918552	0.638084633	1.526	5.36E-10	1.30E-09	-5.679	161	164	162.5
CNOT7	0.479638009	0.719933185	1.891	2.91E-09	6.45E-09	-5.83	176	150	163
RELB	0.497737557	0.699888641	0.444	6.19E-09	1.31E-08	-5.947	185	142	163.5
RLIM	0.696832579	0.522828508	0.731	3.57E-10	8.86E-10	-5.428	157	171	164
GTF3C1	0.533936652	0.669821826	0.098	3.70E-09	7.97E-09	-5.839	181	148	164.5
DTX3	0.416289593	0.761135857	0.345	3.83E-08	7.29E-08	-6.681	205	125	165
PSMC3	0.841628959	0.427616927	1.655	3.41E-16	2.14E-15	-3.255	62	268	165
CCNH	0.50678733	0.69766147	1.151	2.06E-09	4.61E-09	-5.732	174	158	166
STAT5A	0.606334842	0.60467706	0.014	1.99E-09	4.49E-09	-5.721	173	161	167
GTF2F2	0.461538462	0.719376392	0.993	6.16E-08	1.14E-07	-6.629	210	127	168.5
HMG20B	0.479638009	0.703786192	0.687	5.63E-08	1.05E-07	-6.421	208	130	169
BCLAF1	0.678733032	0.535634744	0.379	1.03E-09	2.42E-09	-5.391	166	173	169.5
CNOT1	0.624434389	0.599665924	0.151	2.03E-10	5.30E-10	-4.943	149	190	169.5
NFATC1	0.85520362	0.373051225	0.475	7.80E-13	2.93E-12	-3.762	104	235	169.5
VPS72	0.511312217	0.687082405	1.269	6.87E-09	1.42E-08	-5.822	189	151	170
MTA2	0.841628959	0.386414254	0.651	1.69E-12	5.89E-12	-3.939	112	229	170.5
ECD	0.488687783	0.703786192	0.151	1.34E-08	2.63E-08	-5.915	198	144	171
MBD1	0.352941176	0.804565702	0.367	2.25E-07	3.96E-07	-6.864	222	121	171.5
NMI	0.65158371	0.575723831	0.864	1.15E-10	3.10E-10	-4.687	145	201	173
KAT5	0.375565611	0.790089087	0.379	1.02E-07	1.87E-07	-6.288	213	134	173.5
E2F4	0.429864253	0.747772829	0.465	5.35E-08	1.01E-07	-5.982	207	141	174
COPS5	0.502262443	0.702115813	3.004	1.90E-09	4.31E-09	-5.288	172	178	175
CBX4	0.407239819	0.764476615	0.138	8.67E-08	1.60E-07	-5.935	212	143	177.5
TCEA1	0.787330317	0.424832962	0.623	2.51E-10	6.41E-10	-4.522	153	206	179.5
TSC22D4	0.647058824	0.576280624	2.632	2.39E-10	6.17E-10	-4.423	151	208	179.5
TARBP2	0.357466063	0.803452116	0.941	1.36E-07	2.47E-07	-5.877	215	146	180.5
NFKBIA	0.923076923	0.30623608	5.781	2.11E-15	1.16E-14	-2.701	71	292	181.5
RNF4	0.705882353	0.516146993	3.663	2.14E-10	5.57E-10	-4.246	150	215	182.5
GTF2H2	0.343891403	0.81013363	0.848	3.19E-07	5.46E-07	-6.141	228	138	183
RBBP4	0.79638009	0.432071269	0.202	1.01E-11	3.14E-11	-3.694	125	241	183
UBXN4	0.56561086	0.649777283	3.402	6.58E-10	1.57E-09	-4.587	164	204	184
DPF2	0.583710407	0.619710468	0.782	6.55E-09	1.37E-08	-5.145	187	183	185
EGR1	0.597285068	0.609131403	1.485	4.23E-09	9.02E-09	-4.95	183	189	186
ZFPL1	0.321266968	0.830734967	1.043	1.92E-07	3.41E-07	-5.793	219	154	186.5
SERTAD2	0.470588235	0.704899777	0.202	1.85E-07	3.31E-07	-5.773	218	155	186.5
UBE2K	0.601809955	0.605790646	0.566	3.53E-09	7.73E-09	-4.757	178	198	188
HDAC3	0.524886878	0.662026726	0.475	6.10E-08	1.14E-07	-5.502	209	168	188.5
ATF6B	0.443438914	0.727728285	0.299	2.30E-07	4.03E-07	-5.731	223	159	191
ANAPC11	0.556561086	0.644766147	1.795	7.50E-09	1.54E-08	-4.786	190	195	192.5
EYA3	0.343891403	0.807906459	0.275	4.91E-07	8.21E-07	-5.802	233	153	193
UTP6	0.34841629	0.797884187	0.202	1.57E-06	2.44E-06	-6.145	251	137	194
ZHX1	0.343891403	0.80623608	0.322	6.73E-07	1.10E-06	-5.822	239	152	195.5
SCAP	0.357466063	0.799554566	0.151	2.91E-07	5.01E-07	-5.584	226	167	196.5
MED27	0.393665158	0.772271715	0.757	1.71E-07	3.08E-07	-5.315	217	177	197
TCF25	0.850678733	0.343541203	0.39	5.43E-10	1.31E-09	-3.774	162	233	197.5

Gene	TP	TN	thresh_mhg	hyper_pval	hyper_qval	gen_qval	rank_hyper_qval	rank_gen_qval	mean_rank
CCNT1	0.457013575	0.712138085	0.556	4.17E-07	7.07E-07	-5.456	230	169	199.5
MED15	0.570135747	0.628619154	2.667	1.31E-08	2.60E-08	-4.651	197	203	200
NPM1	0.932126697	0.232182628	9.342	4.25E-10	1.04E-09	-3.692	160	242	201
NR1H2	0.642533937	0.560690423	1.475	7.76E-09	1.59E-08	-4.264	191	212	201.5
PHRF1	0.488687783	0.68596882	0.263	2.81E-07	4.87E-07	-5.248	225	179	202
EIF3H	0.986425339	0.152004454	7.886	1.07E-11	3.30E-11	-2.924	126	280	203
TFAM	0.384615385	0.774498886	0.227	4.58E-07	7.70E-07	-5.33	232	176	204
AIP	0.674208145	0.545657016	1.501	4.08E-10	1.00E-09	-3.528	159	250	204.5
ATF1	0.429864253	0.739420935	0.39	2.38E-07	4.15E-07	-5.067	224	187	205.5
GLRX2	0.402714932	0.778953229	3.191	1.07E-08	2.14E-08	-4.243	195	216	205.5
NR3C1	0.452488688	0.719933185	0.138	2.23E-07	3.93E-07	-4.898	221	192	206.5
CHD4	0.746606335	0.450445434	0.202	7.90E-09	1.61E-08	-4.171	192	222	207
KAT2A	0.321266968	0.824053452	0.506	7.40E-07	1.20E-06	-5.388	241	174	207.5
TBC1D2B	0.380090498	0.776726058	0.239	6.08E-07	1.00E-06	-5.218	237	181	209
TBL1XR1	0.461538462	0.720489978	1.373	5.06E-08	9.57E-08	-4.261	206	213	209.5
SMARCA4	0.65158371	0.55233853	0.202	6.78E-09	1.41E-08	-3.872	188	231	209.5
RELA	0.592760181	0.609131403	4.227	8.94E-09	1.81E-08	-3.943	193	228	210.5
TWISTNB	0.429864253	0.734966592	0.465	5.08E-07	8.47E-07	-5.026	234	188	211
KDM6A	0.407239819	0.747772829	0.227	1.60E-06	2.47E-06	-5.406	252	172	212
PHF5A	0.556561086	0.643095768	4.788	1.00E-08	2.01E-08	-3.898	194	230	212
YEATS4	0.466063348	0.706013363	0.895	3.01E-07	5.18E-07	-4.713	227	199	213
NONO	0.923076923	0.270044543	1.417	4.07E-12	1.36E-11	-2.279	117	310	213.5
GABPA	0.42081448	0.740534521	0.227	7.51E-07	1.21E-06	-5.096	242	186	214
SNW1	0.63800905	0.557906459	0.687	2.55E-08	4.89E-08	-4.059	203	225	214
PBXIP1	0.683257919	0.530066815	0.189	1.20E-09	2.79E-09	-3.361	167	261	214
CREB3	0.343891403	0.800111359	0.39	2.06E-06	3.12E-06	-5.382	257	175	216
RNF125	0.570135747	0.618596882	1.911	6.79E-08	1.26E-07	-4.176	211	221	216
ZBTB7A	0.50678733	0.661469933	0.163	9.46E-07	1.51E-06	-4.85	244	193	218.5
HES6	0.307692308	0.832405345	0.66	1.20E-06	1.91E-06	-4.822	245	194	219.5
SBDS	0.561085973	0.635300668	1.084	1.82E-08	3.55E-08	-3.682	200	244	222
HMGB1	0.977375566	0.175946548	3.997	4.24E-12	1.39E-11	-1.798	119	327	223
WHSC1	0.452488688	0.70935412	0.214	1.24E-06	1.96E-06	-4.706	247	200	223.5
BLOC1S1	0.452488688	0.714365256	0.941	5.60E-07	9.25E-07	-4.185	236	219	227.5
BAZ2A	0.416289593	0.737750557	0.151	2.24E-06	3.36E-06	-4.773	260	197	228.5
RNF19A	0.49321267	0.670935412	0.239	1.51E-06	2.36E-06	-4.511	250	207	228.5
PFDN5	0.932126697	0.2655902	4.42	5.83E-13	2.21E-12	-0.818	103	356	229.5
XAB2	0.475113122	0.694320713	0.696	5.32E-07	8.83E-07	-4.025	235	226	230.5
PQBP1	0.520361991	0.655345212	0.941	3.49E-07	5.95E-07	-3.726	229	237	233
LIMD1	0.488687783	0.673162584	0.176	2.02E-06	3.07E-06	-4.297	256	211	233.5
GTF2A2	0.466063348	0.703786192	0.669	4.33E-07	7.31E-07	-3.707	231	240	235.5
RBM38	0.7239819	0.485523385	3.483	1.47E-09	3.42E-09	-2.402	168	303	235.5
ILF3	0.714932127	0.481069042	0.411	1.50E-08	2.93E-08	-3.092	199	274	236.5
MAZ	0.447963801	0.70155902	0.401	7.33E-06	1.03E-05	-4.783	278	196	237
SMAD2	0.371040724	0.771158129	0.176	5.70E-06	8.14E-06	-4.524	273	205	239
CNBP	1	0.107461024	6.679	5.57E-11	1.56E-10	-1.214	139	341	240
PHF20L1	0.497737557	0.660356347	0.151	3.80E-06	5.55E-06	-4.183	267	220	243.5
GABPB1	0.407239819	0.742761693	0.848	3.55E-06	5.23E-06	-4.109	265	224	244.5
CDK7	0.65158371	0.505567929	0.014	6.19E-06	8.74E-06	-4.248	276	214	245
HNRNPD	0.547511312	0.625835189	0.138	6.25E-07	1.02E-06	-3.476	238	254	246
DNMT1	0.606334842	0.577951002	0.275	1.54E-07	2.79E-07	-2.951	216	277	246.5
BAZ1B	0.479638009	0.673719376	0.163	6.18E-06	8.74E-06	-4.166	275	223	249
DTX3L	0.438914027	0.721603563	3.113	1.22E-06	1.93E-06	-3.521	246	252	249
NACA	0.950226244	0.185412027	9.038	1.25E-08	2.49E-08	-2.421	196	302	249
KDM5A	0.642533937	0.525055679	0.239	1.62E-06	2.50E-06	-3.528	253	251	252
ATF4	0.864253394	0.334632517	1.239	1.19E-10	3.17E-10	-0.639	146	360	253
ATF2	0.42081448	0.71714922	0.263	2.57E-05	3.43E-05	-4.226	292	217	254.5
UHRF1	0.375565611	0.770044543	0.379	3.65E-06	5.35E-06	-3.683	266	243	254.5
CIZ1	0.43438914	0.718819599	0.239	3.50E-06	5.17E-06	-3.681	264	245	254.5
THRAP3	0.71040724	0.473273942	2.441	1.04E-07	1.89E-07	-2.599	214	295	254.5
UIMC1	0.447963801	0.704899777	0.516	4.52E-06	6.50E-06	-3.709	271	239	255
EED	0.375565611	0.763919822	0.585	9.63E-06	1.32E-05	-3.997	284	227	255.5
TRIM27	0.325791855	0.816815145	2.077	1.47E-06	2.30E-06	-3.29	249	267	258
CCNL1	0.719457014	0.470489978	2.428	3.62E-08	6.93E-08	-2.261	204	312	258
MED8	0.366515837	0.771158129	0.774	1.05E-05	1.43E-05	-3.77	286	234	260
RNF44	0.656108597	0.510579065	0.227	1.74E-06	2.67E-06	-3.238	254	270	262
RNF5	0.398190045	0.744988864	1.091	8.53E-06	1.19E-05	-3.661	280	246	263
CHURC1	0.43438914	0.717706013	6.17	4.14E-06	6.00E-06	-3.374	269	259	264
MED12	0.547511312	0.614142539	0.124	3.31E-06	4.91E-06	-3.326	263	265	264
PWP1	0.375565611	0.757238307	0.251	2.61E-05	3.47E-05	-3.737	293	236	264.5
MAF1	0.800904977	0.393652561	2.403	3.34E-09	7.36E-09	-0.873	177	352	264.5
EOMES	0.352941176	0.773942094	0.176	4.02E-05	5.24E-05	-3.778	299	232	265.5
PREB	0.520361991	0.638084633	0.214	4.36E-06	6.30E-06	-3.347	270	262	266
MED1	0.57918552	0.566258352	0.227	2.91E-05	3.86E-05	-3.711	295	238	266.5
MYSM1	0.457013575	0.69766147	2.856	3.92E-06	5.70E-06	-3.307	268	266	267

Gene	TP	TN	thresh_mhg	hyper_pval	hyper_qval	gen_qval	rank_hyper_qval	rank_gen_qval	mean_rank
TBP	0.34841629	0.783407572	0.642	1.74E-05	2.35E-05	-3.606	289	247	268
TGIF1	0.633484163	0.533407572	0.585	1.83E-06	2.79E-06	-2.887	255	282	268.5
TRIP12	0.56561086	0.58908686	0.084	8.95E-06	1.24E-05	-3.337	282	264	273
MMS19	0.34841629	0.777839644	0.356	4.03E-05	5.24E-05	-3.557	300	248	274
BUD31	0.588235294	0.577394209	1.449	2.20E-06	3.32E-06	-2.816	259	290	274.5
MLLT6	0.511312217	0.64142539	0.098	8.87E-06	1.23E-05	-3.208	281	271	276
SMYD3	0.398190045	0.736080178	0.163	3.09E-05	4.07E-05	-3.385	296	258	277
SREBF1	0.447963801	0.687639198	0.227	4.82E-05	6.24E-05	-3.42	301	256	278.5
BATF	0.601809955	0.571269488	1.098	7.94E-07	1.27E-06	-2.155	243	315	279
IKZF1	0.828054299	0.334632517	0.39	2.13E-07	3.77E-07	-1.485	220	338	279
SCAND1	0.334841629	0.786191537	1.036	6.54E-05	8.31E-05	-3.512	307	253	280
CTNNB1	0.321266968	0.795100223	0.422	9.70E-05	0.000120418	-3.534	313	249	281
MKL1	0.561085973	0.59688196	0.176	5.76E-06	8.19E-06	-2.851	274	288	281
HMGB2	0.868778281	0.30623608	1.967	5.95E-09	1.26E-08	0	184	379	281.5
PER1	0.669683258	0.497772829	0.824	1.46E-06	2.29E-06	-2.116	248	316	282
AATF	0.343891403	0.777839644	0.687	7.07E-05	8.96E-05	-3.391	308	257	282.5
TCF20	0.674208145	0.482739421	0.084	5.65E-06	8.10E-06	-2.571	272	298	285
E4F1	0.330316742	0.793429844	0.496	3.98E-05	5.21E-05	-3.193	298	273	285.5
ING3	0.42081448	0.70935412	0.322	7.21E-05	9.10E-05	-3.347	309	263	286
CXXC1	0.479638009	0.669265033	0.506	1.14E-05	1.55E-05	-2.876	288	285	286.5
CNOT2	0.529411765	0.623051225	0.651	1.03E-05	1.41E-05	-2.822	285	289	287
PNN	0.574660633	0.56403118	0.287	6.43E-05	8.20E-05	-3.239	306	269	287.5
GTF2A1	0.407239819	0.71714922	0.176	0.000127921	0.000156392	-3.367	319	260	289.5
REXO4	0.371040724	0.756124722	0.614	5.36E-05	6.90E-05	-2.964	303	276	289.5
SF1	0.800904977	0.349665924	0.422	2.43E-06	3.62E-06	-2.069	262	317	289.5
MLXIP	0.529411765	0.600222717	0.202	0.00016273	0.000195276	-3.441	325	255	290
ATRX	0.547511312	0.59298441	0.07	4.94E-05	6.38E-05	-2.951	302	278	290
ABT1	0.791855204	0.340757238	0.111	2.92E-05	3.86E-05	-2.876	294	286	290
CDCA4	0.466063348	0.673162584	0.422	3.60E-05	4.73E-05	-2.718	297	291	294
SP3	0.411764706	0.71325167	0.263	0.000124537	0.000152734	-3.195	318	272	295
MTF2	0.34841629	0.770601336	0.322	0.000111835	0.000138024	-3.09	316	275	295.5
STAT6	0.633484163	0.519487751	0.163	1.11E-05	1.51E-05	-2.377	287	304	295.5
PNRC1	0.597285068	0.559576837	0.526	7.11E-06	1.00E-05	-2.217	277	314	295.5
ING4	0.452488688	0.682071269	0.731	5.74E-05	7.37E-05	-2.615	304	294	299
RORA	0.366515837	0.755011136	0.74	0.000107207	0.000132733	-2.881	315	284	299.5
TRIM28	0.375565611	0.747772829	0.31	9.67E-05	0.000120418	-2.853	314	287	300.5
SP110	0.814479638	0.342427617	0.214	7.28E-07	1.18E-06	-0.59	240	361	300.5
NFYC	0.461538462	0.673162584	0.433	6.12E-05	7.83E-05	-2.468	305	301	303
RNF114	0.701357466	0.461024499	0.299	2.27E-06	3.39E-06	-1.109	261	345	303
PNRC2	0.520361991	0.614142539	0.496	9.06E-05	0.000113305	-2.581	312	296	304
IFI35	0.529411765	0.624721604	0.766	8.28E-06	1.16E-05	-1.773	279	329	304
CIR1	0.380090498	0.736080178	0.322	0.000255489	0.000301029	-2.932	331	279	305
CAMTA2	0.36199095	0.75389755	0.239	0.000208539	0.000247959	-2.887	328	283	305.5
NCOA4	0.43438914	0.690423163	0.275	0.000160385	0.000193056	-2.656	324	293	308.5
JARID2	0.380090498	0.729955457	0.138	0.000527461	0.000606814	-2.894	339	281	310
MLL5	0.742081448	0.388084633	0.163	7.78E-05	9.75E-05	-2.262	311	311	311
HSF1	0.425339367	0.70155902	0.536	0.000114139	0.000140423	-2.376	317	306	311.5
DNM2	0.701357466	0.444877506	0.275	1.76E-05	2.36E-05	-1.605	290	335	312.5
RPL7	0.914027149	0.208797327	11.198	2.12E-06	3.20E-06	-0.328	258	369	313.5
PMF1	0.402714932	0.718262806	1.007	0.000185052	0.000220704	-2.377	327	305	316
PLRG1	0.321266968	0.784521158	0.848	0.000406362	0.00047167	-2.58	336	297	316.5
CEBPZ	0.389140271	0.723830735	0.401	0.00041407	0.000479191	-2.495	337	299	318
TLE3	0.511312217	0.615256125	0.176	0.000214079	0.000253771	-2.313	329	309	319
BRD8	0.610859729	0.511135857	0.07	0.000385821	0.000449165	-2.37	335	307	321
PTMA	0.995475113	0.066258352	7.631	9.55E-06	1.32E-05	-0.646	283	359	321
MED30	0.497737557	0.632516704	1.064	0.000133075	0.00016168	-1.936	321	323	322
PHF6	0.398190045	0.70545657	0.214	0.001251094	0.001402088	-2.481	348	300	324
ZNRD1	0.398190045	0.722717149	1.406	0.000177338	0.000212153	-1.93	326	324	325
TAF1B	0.36199095	0.741648107	0.632	0.000898856	0.001016098	-2.338	345	308	326.5
SMAD7	0.479638009	0.643095768	0.239	0.000281171	0.000330291	-1.953	332	322	327
ILF2	0.447963801	0.668708241	0.299	0.000450103	0.000519349	-2.044	338	319	328.5
MYC	0.429864253	0.683184855	0.333	0.000587319	0.000671713	-2.057	341	318	329.5
SPOP	0.552036199	0.578507795	0.379	0.000155797	0.000188114	-1.526	323	336	329.5
CREBBP	0.479638009	0.642538976	0.163	0.000298881	0.000348993	-1.873	334	326	330
STAT1	0.837104072	0.290089087	0.124	2.21E-05	2.96E-05	-0.239	291	371	331
NFX1	0.470588235	0.631959911	0.176	0.00208768	0.002299986	-2.258	354	313	333.5
NCOR1	0.746606335	0.378619154	0.832	0.000128543	0.000156662	-1.081	320	347	333.5
NFYB	0.425339367	0.683184855	0.485	0.000918983	0.001035848	-1.885	346	325	335.5
THOC2	0.43438914	0.665924276	0.138	0.002194353	0.002410698	-1.958	355	321	338
VAV1	0.805429864	0.29844098	0.07	0.000602489	0.000687049	-1.636	342	334	338
MBNL1	0.904977376	0.195991091	5.051	7.55E-05	9.50E-05	-0.362	310	366	338
GABPB2	0.57918552	0.517260579	0.029	0.004168801	0.004554152	-1.987	357	320	338.5
GTF3C2	0.470588235	0.634187082	0.239	0.00169389	0.0018821	-1.775	351	328	339.5
GON4L	0.42081448	0.684298441	0.057	0.001265881	0.001412898	-1.767	349	330	339.5

Gene	TP	TN	thresh_mhg	hyper_pval	hyper_qval	gen_qval	rank_hyper_qval	rank_gen_qval	mean_rank
NCOA2	0.447963801	0.661469933	0.084	0.000963269	0.001082637	-1.68	347	333	340
HDAC7	0.628959276	0.484966592	0.263	0.000823589	0.00093372	-1.495	344	337	340.5
MIER1	0.615384615	0.5	0.287	0.000736355	0.000837254	-1.158	343	343	343
STAT5B	0.502262443	0.599665924	0.07	0.002431195	0.002663388	-1.733	356	331	343.5
RNF7	0.678733032	0.44376392	1.05	0.000292294	0.000342326	-0.696	333	357	345
PML	0.36199095	0.726057906	0.163	0.004461983	0.00484728	-1.725	359	332	345.5
HBP1	0.479638009	0.615256125	0.227	0.004253197	0.004633371	-1.436	358	339	348.5
RPL6	0.995475113	0.052895323	10.212	0.000146579	0.000177533	-0.022	322	377	349.5
NFKB1	0.411764706	0.674832962	0.151	0.006755416	0.007257885	-1.283	363	340	351.5
ELF1	0.787330317	0.319599109	0.872	0.000559335	0.00064159	-0.41	340	365	352.5
IRF3	0.43438914	0.650890869	0.696	0.008241059	0.008829706	-1.122	364	344	354
MXD1	0.479638009	0.610801782	0.189	0.006176842	0.006673043	-1.081	361	348	354.5
JUNB	0.963800905	0.106347439	8.485	0.000222969	0.000263509	0	330	380	355
SMARCA2	0.366515837	0.704899777	0.275	0.01882142	0.019785321	-1.209	371	342	356.5
GATA3	0.511312217	0.582962138	0.379	0.004795813	0.005195464	-0.857	360	354	357
NRF1	0.380090498	0.696547884	0.322	0.0132967	0.014053423	-1.107	369	346	357.5
DAXX	0.375565611	0.704342984	0.546	0.00996954	0.01062328	-1.033	366	349	357.5
CCNL2	0.687782805	0.415367483	0.496	0.001805368	0.001994599	-0.426	353	364	358.5
NCOA3	0.619909502	0.461581292	0.176	0.012714663	0.013474779	-1.007	368	350	359
NFATC3	0.511312217	0.574053452	0.111	0.009750684	0.010418539	-0.868	365	353	359
NSD1	0.597285068	0.493318486	0.084	0.006586043	0.007095461	-0.678	362	358	360
KLF6	0.800904977	0.296213808	0.333	0.001267985	0.001412898	-0.093	350	375	362.5
PIAS1	0.343891403	0.716035635	0.322	0.039478525	0.041057666	-0.945	375	351	363
ELF4	0.366515837	0.697104677	0.251	0.033149145	0.034659964	-0.857	373	355	364
KLF13	0.923076923	0.147550111	0.903	0.001765897	0.001956534	0	352	381	366.5
ARID1A	0.696832579	0.375835189	0.124	0.019757865	0.02071389	-0.589	372	362	367
NR4A1	0.665158371	0.413697105	0.275	0.014002034	0.014758901	-0.341	370	368	369
JUN	0.371040724	0.668708241	0.367	0.134780588	0.138327445	-0.494	380	363	371.5
BTG2	0.71040724	0.370824053	0.214	0.010060004	0.010690468	-0.02	367	378	372.5
ATF7IP	0.552036199	0.508351893	0.275	0.052223876	0.054024699	-0.239	377	372	374.5
MAML2	0.416289593	0.617483296	0.098	0.183886522	0.187247372	-0.343	383	367	375
RNF138	0.610859729	0.454342984	0.401	0.038087039	0.039716431	-0.06	374	376	375
LDB1	0.398190045	0.634743875	0.516	0.18815501	0.191094932	-0.24	384	370	377
MTA3	0.429864253	0.60467706	0.444	0.179506517	0.183265816	-0.22	382	373	377.5
NOTCH1	0.524886878	0.512249443	0.07	0.165838562	0.169756009	-0.165	381	374	377.5
SP100	0.814479638	0.238864143	0.475	0.043616752	0.04524078	0	376	382	379
GTF2I	0.751131222	0.299554566	0.263	0.067640587	0.069787907	0	378	383	380.5
WHSC1L1	0.746606335	0.298997773	0.111	0.091267343	0.093916263	0	379	384	381.5
ARID5A	0.57918552	0.437082405	0.111	0.349663504	0.354204588	0	385	385	385
ZFP36L1	0.574660633	0.415367483	0.333	0.640096896	0.646730025	0	386	386	386
IRF1	0.56561086	0.415367483	0.642	0.730789354	0.736454387	0	387	387	387
PYHIN1	0.357466063	0.595211581	0.333	0.924007685	0.928770611	0	388	388	388
ZFP36L2	0.628959276	0.323496659	0.189	0.931648747	0.934043731	0	389	389	389
FOS	0.461538462	0.423162584	0.31	0.999549404	0.999549404	0	390	390	390

Table 3. Ranked top surface cytokines differentially expressed in cluster 7

Gene	TP	TN	thresh_mhg	hyper_pval	hyper_qval	gen_qval	rank_hyper_qval	rank_gen_qval	mean_rank
TNFRSF9	0.873303167	0.744988864	8.537	2.48E-73	5.27E-71	-74.603	1	1	1
CCRL2	0.7239819	0.791759465	1.05	1.60E-52	1.13E-50	-57.892	3	2	2.5
HAVCR2	0.873303167	0.683184855	2.154	5.96E-59	6.31E-57	-51.383	2	4	3
CSF1	0.556561086	0.885300668	0.911	1.02E-47	3.08E-46	-56.864	7	3	5
ADAM8	0.787330317	0.726057906	0.864	7.94E-50	2.80E-48	-50.88	5	6	5.5
ITGAV	0.85520362	0.657572383	0.084	7.25E-50	2.80E-48	-50.642	6	7	6.5
SERPINE2	0.542986425	0.873051225	3.895	1.05E-41	1.85E-40	-51.019	12	5	8.5
TNFRSF4	0.773755656	0.723830735	3.144	8.39E-47	2.22E-45	-42.493	8	10	9
LAG3	0.963800905	0.513919822	4.793	7.81E-51	4.14E-49	-36.69	4	14	9
GPR56	0.647058824	0.806792873	0.696	2.30E-42	4.44E-41	-44.618	11	8	9.5
PGLYRP1	0.923076923	0.53674833	4.954	5.99E-44	1.27E-42	-43.178	10	9	9.5
CXCR6	0.986425339	0.423719376	5.506	4.32E-44	1.02E-42	-29.649	9	19	14
KIT	0.466063348	0.88752784	0.516	2.23E-33	2.63E-32	-40.679	18	11	14.5
CCL3	0.642533937	0.773942094	3.904	8.83E-35	1.17E-33	-35.708	16	15	15.5
NR4A2	0.914027149	0.498886414	0.595	2.62E-36	3.71E-35	-31.351	15	16	15.5
IL1R2	0.407239819	0.915924276	3.396	2.28E-32	2.42E-31	-40.178	20	12	16
CD244	0.466063348	0.883073497	3.545	3.23E-32	3.26E-31	-37.285	21	13	17
NRP1	0.751131222	0.670935412	0.163	1.76E-33	2.20E-32	-30.537	17	18	17.5
LGALS1	0.923076923	0.508351893	10.112	1.29E-39	2.10E-38	-25.523	13	27	20
CX3CR1	0.511312217	0.843541203	1.646	1.21E-29	1.07E-28	-28.883	23	21	22
GPR65	0.760180995	0.652561247	2.585	5.08E-32	4.89E-31	-26.057	22	26	24
ENTPD1	0.701357466	0.692093541	0.202	2.00E-29	1.63E-28	-26.13	26	25	25.5
TIGIT	0.981900452	0.354120267	4.895	4.50E-33	5.02E-32	-21.13	19	32	25.5
PDCD1	0.968325792	0.415367483	5.101	2.34E-37	3.54E-36	-19.612	14	37	25.5

Gene	TP	TN	thresh_mhg	hyper_pval	hyper_qval	gen_qval	rank_hyper_qval	rank_gen_qval	mean_rank
CLIC4	0.619909502	0.758351893	1.202	9.73E-29	7.37E-28	-26.3	28	24	26
TFF1	0.384615385	0.904788419	5.97	6.36E-26	3.74E-25	-30.862	36	17	26.5
CCR8	0.443438914	0.874164811	5.401	7.64E-27	4.76E-26	-29.292	34	20	27
KLRC1	0.841628959	0.546213808	4.198	1.16E-29	1.07E-28	-22.062	24	30	27
LILRB4	0.597285068	0.767817372	5.621	2.81E-27	1.86E-26	-26.812	32	23	27.5
IL2RB	0.561085973	0.782293987	9.964	5.64E-25	3.23E-24	-27.616	37	22	29.5
KLRC2	0.778280543	0.597995546	1.766	5.80E-27	3.73E-26	-20.64	33	35	34
IL10RA	0.837104072	0.538975501	0.214	5.38E-28	3.93E-27	-18.023	29	40	34.5
IL18RAP	0.733031674	0.626948775	0.66	1.40E-24	7.83E-24	-21.027	38	33	35.5
TNFRSF18	0.895927602	0.459910913	4.331	1.10E-27	7.78E-27	-16.98	30	44	37
CMTM7	0.864253394	0.512249443	1.761	6.60E-29	5.18E-28	-16.368	27	47	37
PTGER2	0.479638009	0.824053452	0.227	7.64E-22	3.52E-21	-22.388	46	29	37.5
IL12RB2	0.447963801	0.841870824	0.66	4.40E-21	1.90E-20	-23.202	50	28	39
NCOR2	0.656108597	0.688752784	0.239	5.32E-23	2.68E-22	-19.689	42	36	39
CALR	0.923076923	0.384187082	2.844	1.64E-23	8.71E-23	-18.422	40	39	39.5
TMEM123	0.891402715	0.464922049	4.878	1.61E-27	1.10E-26	-16.349	31	49	40
CD200	0.34841629	0.901447661	1.614	2.61E-20	1.04E-19	-21.601	53	31	42
GABARAPL1	0.597285068	0.733853007	0.595	4.23E-22	1.99E-21	-17.973	44	41	42.5
TNFSF4	0.384615385	0.878619154	3.874	3.78E-20	1.49E-19	-20.871	54	34	44
SEPT2	0.954751131	0.329064588	0.536	2.54E-23	1.31E-22	-14.651	41	53	47
SIVA1	0.57918552	0.739977728	2.032	7.74E-21	3.17E-20	-16.842	52	45	48.5
CTSB	0.959276018	0.346325167	2.31	2.72E-26	1.65E-25	-13.17	35	62	48.5
LAP3	0.429864253	0.845211581	0.88	1.60E-19	5.67E-19	-18.741	60	38	49
CTLA4	0.936651584	0.413140312	2.685	1.42E-29	1.20E-28	-11.36	25	75	50
BSG	0.932126697	0.357461024	0.575	3.86E-22	1.90E-21	-13.243	43	61	52
XPOT	0.466063348	0.815701559	0.411	6.17E-19	2.04E-18	-17.777	64	43	53.5
CD200R1	0.384615385	0.865812918	1.454	8.03E-18	2.40E-17	-17.97	71	42	56.5
MIF	0.941176471	0.315701559	6.412	2.87E-19	9.80E-19	-15.258	62	51	56.5
RAC1	0.918552036	0.378619154	0.872	4.21E-22	1.99E-21	-11.928	45	69	57
PDIA4	0.63800905	0.678173719	2.128	1.54E-19	5.54E-19	-14.183	59	56	57.5
ATPIF1	0.678733032	0.652561247	4.168	4.49E-21	1.90E-20	-12.554	49	66	57.5
IL21R	0.841628959	0.457126949	0.993	3.88E-19	1.30E-18	-14.583	63	54	58.5
HSP90AB1	0.936651584	0.31013363	9.834	6.45E-18	1.98E-17	-16.101	69	50	59.5
TRPV2	0.701357466	0.615256125	4.401	2.71E-19	9.43E-19	-13.709	61	58	59.5
LAMP2	0.547511312	0.744432071	1.982	6.23E-18	1.94E-17	-14.908	68	52	60
ECE1	0.429864253	0.832962138	2.101	1.46E-17	4.12E-17	-16.498	75	46	60.5
P4HB	0.936651584	0.365256125	0.526	7.55E-24	4.10E-23	-10.729	39	83	61
HSPD1	0.846153846	0.458797327	1.585	6.50E-20	2.46E-19	-12.229	56	68	62
PDIA3	0.945701357	0.331291759	6.479	9.01E-22	4.07E-21	-11.136	47	77	62
KLRK1	0.760180995	0.561247216	1.064	4.09E-20	1.58E-19	-11.367	55	74	64.5
ADAM17	0.533936652	0.752783964	2.124	1.46E-17	4.12E-17	-13.689	74	59	66.5
GPI1	0.954751131	0.295657016	7.555	1.15E-19	4.21E-19	-11.256	58	76	67
CD82	0.941176471	0.334632517	6.884	2.75E-21	1.21E-20	-9.818	48	89	68.5
CTSD	0.850678733	0.418151448	9.5	2.16E-16	5.52E-16	-14.202	83	55	69
KLRE1	0.330316742	0.886414254	2.583	2.75E-15	6.28E-15	-16.362	93	48	70.5
TFRC	0.49321267	0.771158129	2.356	1.05E-15	2.49E-15	-13.761	88	57	72.5
CCL4	0.619909502	0.667594655	5.124	2.13E-16	5.52E-16	-12.771	82	65	73.5
M6PR	0.891402715	0.40701559	4.087	7.77E-21	3.17E-20	-8.074	51	99	75
IRAK2	0.542986425	0.737750557	1.536	1.23E-16	3.22E-16	-11.616	81	72	76.5
KLRD1	0.78280543	0.493318486	5.966	1.01E-15	2.46E-15	-11.474	87	73	80
IL2RA	0.303167421	0.902561247	2.452	3.98E-15	8.78E-15	-12.37	96	67	81.5
AIMP1	0.683257919	0.618040089	0.986	1.32E-17	3.84E-17	-9.402	73	90	81.5
CD44	0.805429864	0.478285078	0.367	8.14E-17	2.18E-16	-10.21	79	85	82
HSPA9	0.701357466	0.594097996	3.417	5.39E-17	1.47E-16	-10.168	78	86	82
CD8A	0.945701357	0.273942094	8.096	7.90E-16	1.95E-15	-10.988	86	79	82.5
ERP44	0.787330317	0.513363029	1.899	3.16E-18	1.01E-17	-7.981	65	100	82.5
ITGB3	0.389140271	0.83518931	0.124	1.12E-13	2.23E-13	-13.483	106	60	83
TMX3	0.502262443	0.75	0.227	4.34E-14	8.93E-14	-12.816	103	64	83.5
USP14	0.497737557	0.761135857	2.091	6.12E-15	1.34E-14	-11.619	97	71	84
CD27	0.904977376	0.378062361	4.648	7.70E-20	2.86E-19	-7.169	57	111	84
C1QBP	0.696832579	0.599665924	4.777	4.27E-17	1.17E-16	-9.227	77	92	84.5
FERMT3	0.932126697	0.316258352	3.5	8.68E-18	2.56E-17	-8.337	72	97	84.5
PEBP1	0.85520362	0.430957684	1.637	3.13E-18	1.01E-17	-7.934	66	103	84.5
GPR160	0.357466063	0.85467706	0.766	3.12E-13	6.06E-13	-12.901	109	63	86
IL18R1	0.597285068	0.668708241	3.714	2.59E-14	5.38E-14	-11.684	102	70	86
ANXA5	0.696832579	0.58518931	3.898	1.28E-15	3.01E-15	-10.769	90	82	86
IDE	0.737556561	0.537861915	0.651	3.54E-15	7.90E-15	-9.306	95	91	93
LYST	0.624434389	0.645879733	0.669	1.43E-14	3.03E-14	-10.055	100	87	93.5
CD2BP2	0.678733032	0.606347439	0.345	6.58E-16	1.64E-15	-7.848	85	105	95
SCARB2	0.371040724	0.84298441	3.57	5.76E-13	1.07E-12	-11.036	114	78	96
LY75	0.457013575	0.777282851	0.39	5.47E-13	1.04E-12	-10.847	112	80	96
IFNG	0.488687783	0.750556793	4.6	6.54E-13	1.21E-12	-10.788	115	81	98
SEMA4D	0.895927602	0.341314031	2.077	6.49E-15	1.40E-14	-8.169	98	98	98
ITGB2	0.959276018	0.253340757	6.382	3.58E-16	9.05E-16	-7.109	84	112	98

Gene	TP	TN	thresh_mhg	hyper_pval	hyper_qval	gen_qval	rank_hyper_qval	rank_gen_qval	mean_rank
FLOT2	0.443438914	0.786191537	0.888	9.14E-13	1.67E-12	-10.567	116	84	100
CD96	0.692307692	0.579621381	3.331	1.25E-14	2.68E-14	-7.454	99	107	103
GRN	0.339366516	0.855790646	3.126	1.17E-11	2.01E-11	-9.864	123	88	105.5
H13	0.909502262	0.353563474	4.489	5.21E-18	1.65E-17	-4.232	67	144	105.5
ATP5B	0.990950226	0.146993318	6.427	2.81E-12	5.01E-12	-9.158	119	93	106
PDLIM2	0.520361991	0.724944321	4.378	4.78E-13	9.13E-13	-7.959	111	101	106
HNRNPU	0.787330317	0.485523385	0.516	1.77E-15	4.13E-15	-6.105	91	122	106.5
NCKAP1L	0.764705882	0.492761693	0.856	8.83E-14	1.78E-13	-7.248	105	110	107.5
PGRMC1	0.561085973	0.679844098	0.614	3.65E-12	6.44E-12	-8.598	120	96	108
CD226	0.687782805	0.572383073	1.05	1.60E-13	3.14E-13	-7.374	108	108	108
LY6A	0.683257919	0.596325167	5.895	2.33E-15	5.37E-15	-5.969	92	125	108.5
GDI2	0.959276018	0.263919822	4.652	3.10E-17	8.64E-17	-4.262	76	143	109.5
SMPD1	0.398190045	0.810690423	5.212	1.62E-11	2.73E-11	-8.841	126	94	110
AAMP	0.760180995	0.513363029	3.018	3.33E-15	7.52E-15	-5.882	94	128	111
CD9	0.43438914	0.782293987	5.837	1.53E-11	2.61E-11	-7.854	124	104	114
TNIP1	0.619909502	0.620824053	1.111	8.05E-12	1.40E-11	-7.367	122	109	115.5
ADAM10	0.737556561	0.492761693	0.214	3.07E-11	5.08E-11	-7.667	128	106	117
CD38	0.429864253	0.777282851	2.091	1.20E-10	1.91E-10	-7.94	133	102	117.5
CD74	0.312217195	0.86247216	1.683	4.65E-10	6.81E-10	-8.642	145	95	120
FASL	0.656108597	0.60467706	3.863	1.47E-13	2.92E-13	-5.108	107	133	120
PSTPIP1	0.778280543	0.479398664	3.501	5.82E-14	1.19E-13	-4.954	104	137	120.5
CD3E	0.900452489	0.287861915	6.221	7.79E-11	1.25E-10	-6.868	132	113	122.5
F2R	0.520361991	0.706013363	2.744	3.37E-11	5.53E-11	-6.63	129	117	123
ATP6AP2	0.547511312	0.685412027	2.956	1.54E-11	2.61E-11	-6.121	125	121	123
LSM1	0.497737557	0.723273942	0.546	5.85E-11	9.47E-11	-6.477	131	119	125
TLN1	0.936651584	0.287861915	0.926	1.03E-15	2.49E-15	-2.194	89	164	126.5
PTPRCAP	0.968325792	0.2422049	7.465	8.60E-17	2.28E-16	-1.214	80	175	127.5
ERP29	0.592760181	0.630846325	0.956	1.85E-10	2.84E-10	-6.503	138	118	128
CAP1	0.737556561	0.479398664	0.824	3.34E-10	4.95E-10	-6.815	143	114	128.5
CCR5	0.511312217	0.703229399	3.733	3.14E-10	4.73E-10	-6.744	141	116	128.5
CR1L	0.606334842	0.618596882	2.31	1.59E-10	2.48E-10	-6.103	136	123	129.5
CCL5	0.977375566	0.233853007	3.234	7.29E-18	2.21E-17	-0.345	70	189	129.5
H2-M3	0.529411765	0.688752784	2.766	2.22E-10	3.38E-10	-5.642	139	130	134.5
IL27RA	0.665158371	0.56013363	1.227	1.61E-10	2.49E-10	-5.354	137	132	134.5
SLC3A2	0.846153846	0.368596882	4.681	1.68E-11	2.80E-11	-4.424	127	142	134.5
CD48	0.864253394	0.364699332	5.154	3.94E-13	7.59E-13	-2.79	110	161	135.5
CAST	0.705882353	0.518930958	1.064	1.30E-10	2.05E-10	-4.945	135	138	136.5
TNFSF10	0.343891403	0.814587973	2.926	1.31E-07	1.75E-07	-6.763	159	115	137
EZR	0.895927602	0.319599109	0.632	5.78E-13	1.07E-12	-2.638	113	162	137.5
NOTCH2	0.714932127	0.500556793	0.202	6.02E-10	8.69E-10	-5.706	147	129	138
ITGAL	0.932126697	0.28285078	4.104	1.54E-14	3.22E-14	-1.055	101	177	139
THY1	0.868778281	0.33518931	5.619	3.62E-11	5.90E-11	-3.591	130	149	139.5
CLPTM1	0.402714932	0.770044543	0.299	6.24E-08	8.47E-08	-6.014	156	124	140
IGF2R	0.552036199	0.629732739	0.111	1.80E-07	2.37E-07	-6.348	161	120	140.5
CD160	0.488687783	0.714365256	0.895	1.83E-09	2.57E-09	-5.379	151	131	141
CD47	0.959276018	0.216035635	6.062	1.34E-12	2.42E-12	-2.122	117	165	141
LRPAP1	0.443438914	0.7344098	0.333	7.17E-08	9.68E-08	-5.951	157	126	141.5
CD164	0.918552036	0.282293987	4.285	1.38E-12	2.48E-12	-1.546	118	172	145
HMGB1	0.977375566	0.175946548	3.997	4.24E-12	7.43E-12	-1.798	121	170	145.5
CD55	0.325791855	0.821269488	0.299	6.28E-07	8.02E-07	-5.938	166	127	146.5
TRAF3	0.371040724	0.785634744	0.163	4.80E-07	6.21E-07	-5.108	164	134	149
CMTM6	0.574660633	0.634187082	3.522	2.37E-09	3.31E-09	-3.97	152	147	149.5
CD3G	0.981900452	0.140311804	8.624	1.13E-09	1.60E-09	-3.555	149	150	149.5
CD6	0.823529412	0.380289532	2.757	3.24E-10	4.84E-10	-3.009	142	157	149.5
ITGA4	0.886877828	0.299554566	0.556	2.88E-10	4.37E-10	-2.818	140	159	149.5
NR3C1	0.452488688	0.719933185	0.138	2.23E-07	2.92E-07	-4.898	162	139	150.5
SBDS	0.561085973	0.635300668	1.084	1.82E-08	2.50E-08	-3.682	154	148	151
TGFBR2	0.769230769	0.438752784	1.646	8.22E-10	1.18E-09	-3.089	148	155	151.5
RPS6KB1	0.502262443	0.670378619	0.251	4.61E-07	5.99E-07	-4.584	163	141	152
IL12RB1	0.321266968	0.820155902	0.465	1.56E-06	1.92E-06	-5.039	172	135	153.5
RALA	0.371040724	0.781737194	0.401	9.65E-07	1.21E-06	-4.625	169	140	154.5
TSPAN32	0.321266968	0.816258352	0.824	3.20E-06	3.89E-06	-4.996	174	136	155
SPN	0.737556561	0.444320713	0.251	9.53E-08	1.28E-07	-3.122	158	154	156
HSPA5	0.923076923	0.222160356	6.319	2.99E-08	4.08E-08	-2.998	155	158	156.5
HSP90AA1	0.823529412	0.378062361	2.521	4.77E-10	6.93E-10	-2.037	146	167	156.5
CD52	0.941176471	0.224387528	9.42	1.24E-10	1.97E-10	-0.714	134	180	157
CD5	0.57918552	0.59688196	5.913	4.91E-07	6.31E-07	-3.346	165	152	158.5
ROCK1	0.56561086	0.601336303	0.111	1.72E-06	2.11E-06	-4.092	173	145	159
PEAR1	0.371040724	0.7655902	0.287	1.36E-05	1.63E-05	-4.013	177	146	161.5
CD37	0.882352941	0.304008909	3.294	3.90E-10	5.74E-10	-0.561	144	182	163
IL2RG	1	0.08908686	5.342	3.79E-09	5.25E-09	-1.145	153	176	164.5
LTB	0.891402715	0.283964365	6.104	1.54E-09	2.17E-09	-0.806	150	179	164.5
BST2	0.502262443	0.663697105	1.345	1.27E-06	1.59E-06	-2.813	170	160	165
ICAM1	0.515837104	0.631403118	0.251	1.88E-05	2.22E-05	-3.054	179	156	167.5

Gene	TP	TN	thresh_mhg	hyper_pval	hyper_qval	gen_qval	rank_hyper_qval	rank_gen_qval	mean_rank
STX4A	0.316742081	0.800111359	0.816	8.20E-05	9.40E-05	-3.347	185	151	168
CD97	0.79638009	0.375278396	0.546	1.36E-07	1.80E-07	-0.861	160	178	169
SLAMF1	0.325791855	0.79064588	0.444	0.00010523	0.000119298	-3.3	187	153	170
IFNAR1	0.714932127	0.439309577	3.279	5.74E-06	6.95E-06	-1.854	175	169	172
B4GALT1	0.882352941	0.253340757	2.046	1.52E-06	1.88E-06	-0.618	171	181	176
CORO1A	0.914027149	0.214922049	10.504	8.20E-07	1.04E-06	-0.466	168	187	177.5
GPR174	0.334841629	0.760022272	0.043	0.001761276	0.001914823	-2.295	195	163	179
FLT3L	0.389140271	0.715478842	0.918	0.001048641	0.001145938	-2.047	194	166	180
ICOS	0.678733032	0.449331849	0.39	0.000162874	0.00017984	-1.965	192	168	180
SYNJ2BP	0.859728507	0.248886414	0.585	0.000123973	0.000138328	-1.673	190	171	180.5
CCND2	0.737556561	0.405902004	0.111	1.72E-05	2.05E-05	-0.541	178	184	181
B2M	0.995475113	0.061247216	11.727	2.69E-05	3.13E-05	-0.551	182	183	182.5
PSEN1	0.452488688	0.647550111	0.401	0.00245109	0.002651179	-1.501	196	173	184.5
CD53	0.950226244	0.162583519	5.988	7.25E-07	9.20E-07	0	167	202	184.5
NUP85	0.36199095	0.716035635	0.526	0.01085838	0.011567721	-1.259	199	174	186.5
STK10	0.742081448	0.384187082	0.239	0.000120222	0.000134852	-0.54	189	185	187
CD3D	0.986425339	0.087416481	6.219	7.12E-06	8.57E-06	-0.029	176	199	187.5
HCST	0.819004525	0.301781737	2.546	7.28E-05	8.39E-05	-0.043	184	197	190.5
MSN	0.950226244	0.140868597	5.287	2.53E-05	2.97E-05	-0.028	181	200	190.5
PTPRC	0.986425339	0.081848552	3.674	2.06E-05	2.42E-05	0	180	203	191.5
ITGB1	0.656108597	0.44376392	0.239	0.002730536	0.002938445	-0.385	197	188	192.5
HSPA8	0.986425339	0.063474388	10.474	0.000590012	0.000648096	-0.119	193	194	193.5
CD8B1	0.968325792	0.106347439	8.815	7.23E-05	8.38E-05	0	183	204	193.5
MYO9B	0.389140271	0.652004454	0.163	0.128803746	0.133854873	-0.495	204	186	195
CD28	0.687782805	0.39142539	0.251	0.012756924	0.013522339	-0.298	200	190	195
LY6E	0.954751131	0.126391982	6.449	8.36E-05	9.53E-05	0	186	205	195.5
IL4RA	0.488687783	0.571269488	0.287	0.052465723	0.054791789	-0.253	203	191	197
RPS19	0.963800905	0.110801782	9.967	0.000111218	0.000125416	0	188	206	197
NOTCH1	0.524886878	0.512249443	0.07	0.165838562	0.171501343	-0.165	205	193	199
CNP	0.592760181	0.472717149	0.496	0.038042717	0.03992602	-0.048	202	196	199
SELPLG	0.995475113	0.052895323	0.678	0.000146579	0.000162694	0	191	207	199
CD247	0.832579186	0.246659243	0.766	0.004665044	0.004994896	-0.019	198	201	199.5
DPP4	0.325791855	0.691536748	0.239	0.324787096	0.331033002	-0.171	208	192	200
PDE4B	0.475113122	0.546770601	0.31	0.292572864	0.299639841	-0.081	207	195	201
CD84	0.520361991	0.513363029	0.275	0.190762572	0.196318763	-0.038	206	198	202
CD2	0.868778281	0.188195991	0.888	0.021107237	0.022262359	0	201	208	204.5
IL16	0.457013575	0.549554566	0.287	0.454103629	0.460621863	0	209	209	209
IL17RA	0.411764706	0.572383073	0.163	0.698347268	0.704998194	0	210	210	210
CCR7	0.325791855	0.587416481	0.322	0.995063869	0.999581424	0	211	211	211
CD69	0.484162896	0.400334076	0.516	0.999581424	0.999581424	0	212	212	212

Table 4. Ranked top 100 differentially expressed genes in cluster 7 as compared to all 15 CD8 T cell clusters

	adj.pval.cluster 1	adj.pval.cluster 2	adj.pval.cluster 3	adj.pval.cluster 4	adj.pval.cluster 5	adj.pval.cluster 6	adj.pval.cluster 7	adj.pval.cluster 8	adj.pval.cluster 9	adj.pval.cluster 10	adj.pval.cluster 11	adj.pval.cluster 12	adj.pval.cluster 13	adj.pval.cluster 14	adj.pval.cluster 15
GLDC	0	0	0	0	0	0	-85.741	-0.349	-8.74	-5.177	-0.001	-0.109	0	0	-1.259
TNFRSF9	0	0	0	0	0	0	-74.603	-5.868	-9.018	-6.921	-0.27	-15.96	0	0	-0.619
PRF1	0	0	0	0	0	-1.31	-70.08	0	-8.936	-6.49	-0.001	-1.811	0	0	-0.032
IRF8	0	0	0	0	0	0	-60.58	-8.638	-11.165	-5.51	-0.001	-13.999	0	0	-0.83
CCRL2	0	0	0	0	0	0	-57.892	-0.224	-14.77	-4.152	-0.001	0	0	0	-0.091
LAT2	0	0	0	0	0	0	-57.892	-0.572	-10.136	-2.469	-0.001	0	0	0	-0.049
PCYT	0	0	0	0	0	0	-	-	-	-	-	-	0	0	-

	adj.pval.cluster 1	adj.pval.cluster 2	adj.pval.cluster 3	adj.pval.cluster 4	adj.pval.cluster 5	adj.pval.cluster 6	adj.pval.cluster 7	adj.pval.cluster 8	adj.pval.cluster 9	adj.pval.cluster 10	adj.pval.cluster 11	adj.pval.cluster 12	adj.pval.cluster 13	adj.pval.cluster 14	adj.pval.cluster 15
1A							57.84	0.662	11.248	2.084	0.001	0.683			0.336
CSF1	0	0	0	0	0	0	-56.864	-5.975	-6.433	-2.708	-0.001	0	0	0	-0.198
MYO10	0	0	0	0	0	0	-54.348	-1.064	-1.523	-1.588	-0.001	0	0	0	-0.063
TMPRSS6	0	0	0	0	0	0	-53.736	0	-3.033	-4.619	-0.001	0	0	0	-0.008
2900026A02RIK	0	0	0	0	0	-0.094	-53.112	-0.364	-7.417	-8.528	-0.001	0	0	0	-0.051
HAVCR2	0	0	0	0	0	-5.269	-51.383	0	-16.568	-11.845	-0.001	-3.738	0	0	0
C1QTNF6	0	0	0	0	0	0	-51.184	-0.046	-5.849	-8.317	-0.001	0	0	0	-0.048
SERPINE2	0	0	0	0	0	0	-51.019	-1.886	-10.282	-1.85	-0.001	0	0	0	-1.4
ADAM8	0	0	0	0	0	-0.568	-50.88	0	-13.585	-3.536	-0.001	-0.079	0	-1.071	-0.132
ITGAV	0	0	0	0	0	0	-50.642	-5.945	-8.788	-6.619	-0.001	-5.295	0	0	-0.93
ADAMTS14	0	0	0	0	0	0	-49.686	-0.611	-5.926	-9.936	-0.001	-0.273	-0.141	0	-0.294
RGS8	0	0	0	0	0	0	-47.201	-0.241	-5.434	-8.407	-0.001	0	-0.141	0	-0.032
GPR56	0	0	0	0	0	-11.808	-44.618	0	-7.91	-8.024	-0.001	-0.171	0	0	-0.011
AA467197	0	0	0	0	0	0	-43.648	-0.129	-3.303	-2.284	-0.001	0	0	-0.152	-0.006
SLC37A2	0	0	0	0	0	0	-43.31	-0.479	-1.185	-5.219	-0.001	0	0	0	-0.225
PGLYRP1	0	0	0	0	0	0	-43.178	-5.804	-13.116	-7.444	-0.001	0	0	0	-0.597
ANXA2	0	0	0	0	0	-4.007	-43.087	-7.039	-17.945	-7.336	-0.252	-0.485	0	-0.065	-1.032
TNFRSF4	0	0	0	0	0	0	-42.493	-37.64	-6.475	-1.862	-0.093	-5.661	0	0	-4.988
RBPJ	0	0	0	0	0	0	-42.315	-9.234	-5.337	-3.259	-0.001	-5.328	0	0	-1.932
LITAF	0	0	0	0	0	-3.89	-41.5	-3.66	-13.0	-7.349	-0.001	-0.137	0	0	-1.067

	adj.pval.cluster 1	adj.pval.cluster 2	adj.pval.cluster 3	adj.pval.cluster 4	adj.pval.cluster 5	adj.pval.cluster 6	adj.pval.cluster 7	adj.pval.cluster 8	adj.pval.cluster 9	adj.pval.cluster 10	adj.pval.cluster 11	adj.pval.cluster 12	adj.pval.cluster 13	adj.pval.cluster 14	adj.pval.cluster 15
						9	48		46						
HILPDA	0	0	0	-2.032	0	0	-41.529	-0.028	-4.275	-6.589	-0.001	-0.247	0	0	-0.493
MNDA	0	0	0	0	0	0	-40.982	-10.506	-8.14	-0.138	-0.001	-0.022	0	0	-1.517
KIT	0	0	0	0	0	0	-40.679	-22.015	-0.725	-3.094	-0.001	-0.022	0	0	-0.121
GPD2	0	0	0	0	0	0	-40.178	-0.519	-13.917	-7.064	-0.001	-1.482	0	0	-0.483
IL1R2	0	0	0	0	0	0	-40.178	-9.473	-0.35	0	-0.001	-0.517	0	-0.697	-5.067
RGS16	0	0	0	0	0	-9.843	-39.659	-3.736	-22.439	-12.941	-0.095	-2.418	-0.595	0	-0.986
PLEK	0	0	0	0	0	-1.487	-39.004	-2.892	-10.591	-8.925	-0.001	-7.355	0	0	-0.548
DSCAM	0	0	0	0	0	0	-38.384	-2.139	-8.24	-5.086	-0.001	0	-0.438	0	-0.461
EPAS1	0	0	0	0	0	0	-38.289	-0.12	-4.994	-2.887	-0.001	-0.023	-0.184	0	-0.233
NABP1	0	0	0	0	0	0	-38.264	-0.256	-4.082	-3.117	-0.001	-0.877	0	0	-0.04
SLC16A11	0	0	0	0	0	0	-38.005	-14.163	-4.89	-1.423	-0.001	0	0	0	-1.047
GZMF	0	0	0	0	0	0	-37.709	0	-1.555	-0.551	-0.001	-0.002	0	0	-0.052
IKZF2	0	0	0	0	0	0	-37.446	-10.474	-4.472	-3.435	-0.001	-0.15	0	0	-1.383
CD244	0	0	0	0	0	0	-37.285	0	-10.792	-12.777	-0.001	0	0	0	-0.012
GZMC	0	0	0	0	0	0	-37.022	-0.047	-3.473	-0.97	-0.001	0	0	-0.326	-0.088
CDK6	0	0	0	0	0	-0.679	-36.931	0	-3.291	-11.204	-0.001	-3.568	0	0	-0.434
SERPINB9	0	0	0	0	0	0	-36.781	-0.198	-6.539	-2.276	-0.001	-1.839	0	0	-0.411
GEM	0	0	0	0	0	0	-36.705	-2.772	-7.375	-1.815	-0.001	0	0	0	-0.647
LAG3	0	0	0	0	0	-10.838	-36.69	-24.857	-4.285	-9.648	-0.108	-4.982	0	0	-1.917
SLC2	0	0	0	0	0	0	-	-	-	-	-	-0.46	0	0	-

	adj.pval.cluster 1	adj.pval.cluster 2	adj.pval.cluster 3	adj.pval.cluster 4	adj.pval.cluster 5	adj.pval.cluster 6	adj.pval.cluster 7	adj.pval.cluster 8	adj.pval.cluster 9	adj.pval.cluster 10	adj.pval.cluster 11	adj.pval.cluster 12	adj.pval.cluster 13	adj.pval.cluster 14	adj.pval.cluster 15
A3							36.69	0.858	5.126	0.392	0.001				0.668
UBASH3B	0	0	0	0	0	-1.011	-35.97	-1.123	-9.111	-2.621	-0.001	-3.389	0	0	-0.022
NRGN	0	0	0	0	0	0	-35.762	-16.934	-6.835	-1.884	-0.001	0	-0.069	0	-3.422
CCL3	0	0	0	0	0	-1.341	-35.708	0	-7.774	-2.31	-0.001	-10.724	0	0	-0.093
GAPDH	0	0	0	0	0	-3.878	-35.534	-1.651	-28.733	-14.377	-0.417	-4.935	0	0	-1.463
PLAC8	0	0	0	0	0	-0.15	-35.511	0	-12.622	-3.435	-0.001	0	0	0	0
FOXRED2	0	0	0	0	0	0	-35.48	-0.706	-4.364	-10.171	-0.001	-0.4	0	0	-0.252
GZMB	0	0	0	0	0	-17.027	-35.205	0	-17.517	-9.369	-0.001	-0.426	0	0	0
FILIP1	0	0	0	0	0	0	-34.687	0	-5.945	-2.659	-0.001	-0.039	0	0	-0.068
RGS2	0	0	0	-0.462	0	0	-34.658	-4.62	-6.204	-2.36	-0.001	0	0	0	-0.672
EXPH5	0	0	0	0	0	0	-34.452	-3.942	-1.893	-0.23	-0.001	-0.184	-0.485	0	-1.176
SRGAP3	0	0	0	0	0	0	-34.118	-0.864	-6.231	-5.259	-0.001	-0.701	0	0	-0.018
GM5177	0	0	0	0	0	-1.85	-33.888	-2.021	-26.362	-13.092	-0.432	-4.98	0	0	-1.502
MT1	0	0	0	0	0	0	-33.823	0	-12.657	-10.364	-0.001	-0.183	0	0	-0.232
TPI1	0	0	0	0	0	0	-32.629	-2.816	-21.536	-12.198	-0.09	-3.506	0	0	-2.568
ACOT7	0	0	0	0	0	0	-32.602	-9.357	-6.331	-9.956	-0.001	-0.436	0	-2.29	-2.183
BHLHE40	0	0	0	0	0	-0.301	-32.471	-34.275	-4.262	-0.569	-0.125	-4.679	0	0	-1.518
CCNG1	0	0	0	0	0	0	-32.322	-0.599	-10.943	-5.354	-0.001	0	0	0	-0.919
FAM110A	0	0	0	0	0	0	-32.314	-3.177	-12.491	-6.369	-0.001	-2.034	0	0	-1.327
S100A11	0	0	0	0	0	-2.849	-32.158	-2.028	-10.953	-5.371	-0.001	0	0	0	-0.729

	adj.pval.cluster 1	adj.pval.cluster 2	adj.pval.cluster 3	adj.pval.cluster 4	adj.pval.cluster 5	adj.pval.cluster 6	adj.pval.cluster 7	adj.pval.cluster 8	adj.pval.cluster 9	adj.pval.cluster 10	adj.pval.cluster 11	adj.pval.cluster 12	adj.pval.cluster 13	adj.pval.cluster 14	adj.pval.cluster 15
DUSP4	0	0	0	0	0	0	-31.906	-22.596	-2.854	-4.287	-0.001	-9.348	0	0	-2.344
CAPG	0	0	0	0	0	0	-31.567	-16.812	-3.977	-1.984	-0.001	0	0	-0.408	-2.146
FAM3C	0	0	0	0	0	0	-31.563	-2.316	-9.625	-8.321	-0.001	-0.472	0	0	-0.726
NR4A2	0	0	0	0	0	-1.781	-31.351	-10.637	-8.09	-7.402	-0.001	-1.067	-0.279	0	-0.99
TFF1	0	0	0	0	0	0	-30.862	-4.387	-2.848	-4.229	-0.001	0	0	-0.185	-0.85
IMPA2	0	0	0	0	0	0	-30.742	-2.661	-20.435	-11.402	-0.001	0	0	-0.066	-1.337
NRP1	0	0	0	0	0	0	-30.537	-30.902	-2.005	-4.848	0	-2.099	0	-3.231	-3.686
CST7	0	0	0	0	0	0	-30.448	-8.982	-1.397	-2.186	-0.001	-1.396	0	0	-0.093
PLXND1	0	0	0	0	0	0	-30.229	-0.273	-5.416	-2.422	-0.001	0	0	0	-0.068
PKM	0	0	0	0	0	0	-29.958	-0.613	-12.041	-9.718	-0.001	-6.583	0	0	-1.77
STAT3	0	0	0	0	0	0	-29.78	-4.825	-1.639	-1.529	-0.496	-10.55	-1.172	0	-0.553
CXCR6	0	0	0	-0.53	0	-17.027	-29.649	0	-2.242	-10.395	-0.001	-0.018	0	0	0
GDPD5	0	0	0	0	0	0	-29.436	-4.136	-4.718	-1.394	-0.001	-0.882	0	-2.974	-2.711
CCR8	0	0	0	0	0	0	-29.292	-36.056	-1.86	-0.433	-0.001	-1.64	0	0	-2.288
SMIM3	0	0	0	0	0	0	-29.22	-6.398	-14.507	-0.831	-0.001	-0.51	0	-1.908	-1.071
ARL14EP	0	0	0	0	0	0	-29.195	-9.693	-12.507	-5.873	-0.001	-0.244	0	0	-3.948
ERGIC1	0	0	0	0	0	0	-29.048	-4.429	-5.476	-8.829	-0.022	-7.328	0	0	-1.126
ID2	0	0	0	0	0	-3.382	-29	0	-4.316	-3.54	-0.001	-0.416	0	0	-0.061
EHD1	0	0	0	0	0	-0.727	-28.975	-1.406	-6.07	-2.538	-0.001	-4.721	0	0	-0.262
CX3CR1	0	0	0	0	0	0	-28.8	-4.26	-11.0	-1.089	-0.001	-0.17	0	0	-0.165

	adj.pval.cluster 1	adj.pval.cluster 2	adj.pval.cluster 3	adj.pval.cluster 4	adj.pval.cluster 5	adj.pval.cluster 6	adj.pval.cluster 7	adj.pval.cluster 8	adj.pval.cluster 9	adj.pval.cluster 10	adj.pval.cluster 11	adj.pval.cluster 12	adj.pval.cluster 13	adj.pval.cluster 14	adj.pval.cluster 15
							83	2	74						
CASP3	0	0	0	0	0	-8.408	-28.856	-0.755	-13.285	-15.872	-0.001	-1.659	0	0	-2.174
NRN1	0	0	0	0	0	0	-28.795	-39.128	-4.33	-1.816	-0.001	-0.014	0	0	-5.958
PEX16	0	0	0	0	0	0	-28.699	-2.777	-2.117	-1.075	-0.001	0	0	-1.498	-0.78
HNRNPA1	0	0	0	0	0	0	-28.44	-1.621	-11.938	-8.143	-0.055	-7.899	0	0	-2.267
FDX1	0	0	0	0	0	0	-28.187	-1.805	-14.381	-4.935	-0.001	0	0	0	-1.121
OSBPL3	0	0	0	0	0	-5.169	-28.093	-0.769	-16.399	-10.473	-0.001	-0.386	0	0	-0.401
GZME	0	0	0	0	0	0	-28.084	0	-1.257	-0.075	-0.001	0	0	-0.472	-0.068
CIAPIN1	0	0	0	0	0	-0.55	-27.949	-3.79	-6.224	-8.771	-0.001	-1.959	0	0	-0.719
SAMSN1	0	0	0	0	0	-0.812	-27.927	-5.485	-9.544	-4.631	-0.001	-2.181	0	-0.061	-0.308
ALDOA	0	0	0	0	0	-5.221	-27.783	-0.04	-3.877	-2.09	-0.001	-2.078	0	0	-0.055
TUBB6	0	0	0	0	0	0	-27.679	0	-27.279	-5.756	-0.001	0	0	-9.47	-4.64
IL2RB	0	0	0	0	0	-5.313	-27.616	-0.082	-2.376	-5.386	-0.001	0	0	0	-0.001
GZMD	0	0	0	0	0	0	-27.52	0	-1.044	-0.241	-0.001	0	0	0	-0.072
UHRF2	0	0	0	0	0	0	-27.41	-1.698	-7.039	-3.537	-0.001	-0.063	0	0	-1.752

Table 5. Cluster 7 Specific Gene Signature

	0	8	9	10	rank_0	rank_8	rank_9	rank_10	mean_rank
TNFRSF9	-91.791	-14.331	-14.793	-13.779	2	6	1	1	2.5
PRF1	-79.24	-29.216	-13.275	-11.21	6	1	2	2	2.75
GLDC	-113.856	-14.208	-7.744	-8.202	1	7	5	5	4.5
IRF8	-83.708	-4.672	-7.942	-9.831	4	44	4	3	13.75
ADAM8	-63.45	-16.254	-3.094	-6.172	18	4	36	9	16.75
SERPINB9	-43.259	-11.006	-4.253	-6.666	36	15	18	6	18.75
LAT2	-78.786	-8.281	-3.519	-5.725	8	24	30	13	18.75
CCRL2	-79.074	-12.487	-2.528	-6.172	7	12	48	11	19.5

	0	8	9	10	rank_0	rank_8	rank_9	rank_10	mean_rank
NABP1	-45.027	-9.903	-6.648	-5.353	34	19	7	19	19.75
HILPDA	-50.028	-12.93	-6.973	-3.258	27	10	6	38	20.25
SLC2A3	-42.969	-7.103	-4.913	-8.887	38	28	13	4	20.75
PCYT1A	-79.651	-8.38	-2.581	-5.38	5	23	45	18	22.75
2900026A02RIK	-71.856	-10.685	-3.96	-2.875	11	16	23	41	22.75
TMPRSS6	-67.607	-10.618	-4.739	-2.384	15	17	15	55	25.5
MYO10	-70.953	-5.129	-4.707	-3.772	14	40	16	33	25.75
ID2	-32.463	-12.505	-5.358	-3.609	47	11	12	36	26.5
RBPJ	-57.177	-1.82	-5.411	-6.536	21	67	11	8	26.75
ITGAV	-71.527	-5.709	-3.643	-3.728	13	35	26	34	27
STAT3	-34.747	-2.777	-8.191	-6.615	45	55	3	7	27.5
LITAF	-55.012	-6.153	-2.705	-6.172	26	33	44	10	28.25
SERPINE2	-73.535	-5.105	-2.535	-5.436	9	42	47	16	28.5
PGLYRP1	-59.204	-4.039	-3.643	-5.014	19	47	27	21	28.5
ALDOA	-31.109	-12.007	-3.074	-5.422	48	13	37	17	28.75
CSF1	-87.97	-2.774	-3.364	-4.438	3	56	32	25	29
GEM	-47.97	-4.672	-3.388	-5.711	28	45	31	14	29.5
HAVCR2	-64.475	-28.612	-2.425	-2.598	17	2	50	49	29.5
IL2RB	-30.885	-11.694	-5.935	-2.605	49	14	9	47	29.75
EPAS1	-47.895	-9.44	-3.336	-3.315	29	21	33	37	30
AA467197	-55.604	-7.01	-3.64	-3	24	29	28	40	30.25
RGS2	-45.144	-3.008	-4.105	-5.039	33	53	19	20	31.25
LILRB4	-30.192	-8.596	-5.464	-2.766	50	22	10	43	31.25
SLC37A2	-55.738	-6.098	-5.942	-2.076	23	34	8	61	31.5
UBASH3B	-46.981	-6.695	-2.92	-4.125	30	31	41	29	32.75
SH2D2A	-22.905	-13.497	-3.954	-3.078	62	9	24	39	33.5
CCL3	-42.757	-13.952	-2.096	-3.705	40	8	54	35	34.25
PLAC8	-42.778	-15.592	-1.435	-4.693	39	5	72	22	34.5
ANXA2	-57.193	-5.113	-1.633	-6.172	20	41	66	12	34.75
GPR56	-55.013	-20.844	-2.995	-1.571	25	3	40	73	35.25
ADAMTS14	-71.564	-7.445	-3.954	-1.436	12	27	25	78	35.5
BCL2L11	-27.381	-4.72	-4.051	-4.63	56	43	21	24	36
PEX16	-36.099	-2.394	-4.791	-4.42	44	60	14	26	36
C1QTNF6	-71.908	-10.129	-2.852	-1.567	10	18	42	74	36
EHD1	-36.304	-5.416	-3.159	-3.847	43	36	35	31	36.25
RGS8	-67.32	-7.49	-3.585	-1.429	16	25	29	79	37.25
S100A11	-40.349	-6.891	-2.113	-3.933	42	30	53	30	38.75
GPD2	-56.588	-9.512	-1.734	-1.998	22	20	62	62	41.5
GZMC	-46.719	-5.383	-1.956	-2.723	32	37	56	44	42.25
DENND4A	-17.686	-2.592	-4.01	-4.635	70	57	22	23	43
EXPH5	-46.88	-1.602	-2.757	-4.261	31	71	43	28	43.25
CBLB	-15.239	-6.307	-4.266	-2.107	72	32	17	59	45
GZMF	-42.15	-5.285	-2.132	-2.474	41	38	52	51	45.5
CCNG1	-43.191	-7.49	-1.353	-2.419	37	26	79	53	48.75
SERPINB6B	-27.486	-1.621	-1.918	-5.66	55	70	57	15	49.25
IL12RB2	-29.107	-2.489	-1.779	-4.264	52	58	61	27	49.5
GDPD5	-43.403	-1.575	-1.85	-2.683	35	72	59	45	52.75
RPS2	-11.518	-2.415	-2.998	-2.774	75	59	39	42	53.75

	0	8	9	10	rank_0	rank_8	rank_9	rank_10	mean_rank
SLC25A4	-22.912	-2.934	-4.07	-1.377	61	54	20	80	53.75
GIPC2	-34.711	-1.384	-2.169	-2.605	46	75	51	48	55
LPIN2	-26.773	-3.391	-1.581	-2.621	57	50	69	46	55.5
GZME	-28.851	-3.604	-1.388	-2.41	54	48	76	54	58
MAP3K1	-11.979	-2.207	-3.043	-2.217	74	63	38	58	58.25
DGAT1	-18.408	-4.261	-1.786	-1.994	69	46	60	63	59.5
RARA	-7.991	-2.277	-3.295	-1.733	78	61	34	68	60.25
RIOK1	-15.921	-5.188	-1.96	-1.523	71	39	55	77	60.5
AI662270	-19.804	-3.455	-1.376	-2.526	67	49	77	50	60.75
NPNT	-28.907	-1.748	-1.677	-2.32	53	68	65	57	60.75
GZMD	-29.386	-3.315	-1.399	-1.823	51	52	73	67	60.75
ADCK3	-21.125	-1.311	-2.528	-2.436	64	80	49	52	61.25
SDCBP2	-26.342	-3.357	-1.395	-1.836	58	51	74	66	62.25
MVP	-20.821	-1.34	-1.393	-3.779	65	78	75	32	62.5
TRAF4	-19.908	-1.884	-1.454	-2.337	66	65	70	56	64.25
CALR	-24.033	-2.21	-1.376	-2.091	60	62	78	60	65
SKIL	-8.274	-2.069	-2.539	-1.532	77	64	46	76	65.75
FUZ	-21.768	-1.404	-1.86	-1.588	63	74	58	70	66.25
OSR2	-18.773	-1.73	-1.697	-1.588	68	69	64	71	68
ZC3H12C	-26.294	-1.322	-1.436	-1.855	59	79	71	65	68.5
FNDC3A	-9.517	-1.378	-1.701	-1.957	76	77	63	64	70
ZFP296	-12.662	-1.822	-1.62	-1.545	73	66	67	75	70.25
FXYD5	-3.349	-1.494	-1.614	-1.58	80	73	68	72	73.25
CD3E	-7.741	-1.383	-1.336	-1.615	79	76	80	69	76

Table 6. Cluster 8 Specific Gene Signature

	0	7	9	10	rank_0	rank_7	rank_9	rank_10	mean_rank
XCL1	-78.965	-29.841	-24.436	-20.16	2	1	1	3	1.75
CD83	-88.362	-15.592	-20.823	-22.072	1	7	3	2	3.25
CRTAM	-33.776	-14.059	-12.701	-15.302	19	8	11	6	11
CCR7	-18.368	-21.529	-23.19	-28.251	41	2	2	1	11.5
PLXDC2	-71.452	-9.421	-11.795	-10.375	3	17	12	19	12.75
TNFSF8	-24.19	-15.693	-13.869	-11.662	29	6	7	13	13.75
ITGB1	-24.198	-15.992	-10.416	-12.554	28	5	16	10	14.75
LAD1	-55.63	-6.096	-9.452	-12.126	8	33	20	11	18
BACE2	-69.074	-8.157	-9.459	-7.48	4	21	19	34	19.5
DAPL1	-13.482	-18.487	-13.608	-11.095	52	4	9	17	20.5
NFKBIA	-16.006	-7.19	-10.896	-15.26	44	27	14	8	23.25
RAMP3	-34.608	-8.003	-8.408	-9.242	18	23	27	26	23.5
BHLHE40	-42.042	-2.784	-13.721	-18.354	14	70	8	4	24
ZFP36L1	-6.392	-12.969	-14.084	-15.601	81	10	6	5	25.5
MS4A4C	-11.437	-13.198	-8.726	-10.831	58	9	25	18	27.5
CD82	-23.206	-3.998	-15.113	-9.885	32	51	5	22	27.5
GPM6B	-57.374	-3.421	-9.528	-8.458	7	58	18	29	28
TNFSF11	-36.834	-9.097	-10.238	-5.111	17	18	17	67	29.75
TNFRSF18	-30.082	-2.855	-10.896	-11.284	23	68	15	16	30.5
BCL6	-21.408	-10.089	-7.222	-8.015	36	14	40	32	30.5
DUSP1	-14.369	-8.166	-9.038	-7.455	49	20	22	35	31.5
CD81	-49.127	-7.93	-5.697	-7.028	11	24	54	43	33
CD74	-51.295	-4.662	-8.008	-6.573	9	47	32	47	33.75
TBC1D4	-25.573	-6.179	-6.977	-7.4	26	32	45	37	35
TNFRSF4	-60.701	-1.854	-8.088	-11.56	6	97	30	14	36.75

	0	7	9	10	rank_0	rank_7	rank_9	rank_10	mean_rank
SAT1	-12.105	-5.073	-8.132	-10.182	57	41	28	21	36.75
SLAMF6	-8.565	-18.545	-6.896	-8.584	71	3	48	28	37.5
ZC3H12D	-19.725	-5.207	-5.383	-11.679	40	39	61	12	38
TRAF1	-20.148	-2.371	-7.982	-15.302	38	75	33	7	38.25
TNFSF14	-12.7	-10.057	-7.055	-6.816	55	15	43	45	39.5
NRN1	-65.513	-2.043	-7.482	-7.788	5	87	37	33	40.5
SYNPO	-33.308	-6.868	-6.115	-5.708	20	28	52	62	40.5
CD160	-25.982	-5.073	-8.529	-4.497	25	40	26	75	41.5
KLRK1	-22.898	-2.81	-6.977	-9.685	33	69	46	23	42.75
GRAMD1B	-23.425	-7.52	-3.543	-7.442	30	25	80	36	42.75
JUNB	-1.558	-4.775	-17.825	-13.964	116	45	4	9	43.5
ARAP2	-15.032	-6.868	-7.826	-5.523	48	29	34	65	44
REL	-24.382	-3.171	-5.587	-7.202	27	63	56	39	46.25
SPRY2	-45.566	-1.864	-6.899	-8.152	13	96	47	31	46.75
CPNE8	-1.962	-5.508	-8.096	-11.307	110	37	29	15	47.75
NDFIP1	-17.485	-1.877	-7.288	-9.401	43	94	39	25	50.25
BACH2	-6.88	-5.282	-7.776	-6.197	77	38	35	51	50.25
RPL34-PS1	-2.54	-4.801	-11.304	-7.216	107	44	13	38	50.5
RAB37	-9.84	-11.336	-3.886	-6.153	63	13	74	52	50.5
SLC2A6	-38.243	-4.75	-3.903	-5.078	16	46	72	68	50.5
NRP1	-47.337	-1.333	-9.082	-6.03	12	116	21	54	50.75
SDF4	-21.778	-1.66	-7.185	-8.659	35	104	41	27	51.75
CXXC5	-20.248	-6.361	-5.486	-4.019	37	31	60	81	52.25
1700019D03RIK	-50.655	-1.905	-4.792	-7.012	10	91	66	44	52.75
2310001H17RIK	-15.603	-8.427	-3.388	-5.691	46	19	84	63	53
RPL7	-3.694	-3.536	-13.608	-6.234	100	56	10	49	53.75
SAMD3	-8.112	-11.786	-3.879	-5.736	72	12	75	59	54.5
CTSW	-4.513	-9.713	-7.546	-4.974	98	16	36	70	55
FAM53B	-7.148	-6.806	-5.644	-4.785	75	30	55	73	58.25
TESPA1	-3.511	-12.574	-3.721	-6.637	101	11	77	46	58.75
PFDN5	-6.67	-3.872	-6.46	-5.736	78	52	50	58	59.5
BTLA	-9.134	-6.064	-4.655	-4.888	67	34	67	71	59.75
SESN3	-7.815	-4.637	-5.566	-5.718	73	48	58	61	60
TGIF1	-9.583	-2.202	-4.807	-8.312	64	82	65	30	60.25
JUN	-8.569	-3.411	-5.012	-6.202	70	59	63	50	60.5
CD37	-4.975	-3.728	-7.175	-5.924	92	53	42	55	60.5
FAS	-5.692	-8.037	-3.888	-5.523	84	22	73	66	61.25
ST6GAL1	-3.123	-5.516	-6.458	-5.769	105	36	51	56	62
LTA	-15.725	-2.871	-5.57	-4.304	45	67	57	79	62
CALCOCO1	-13.449	-1.752	-5.799	-6.305	53	98	53	48	63
TNFAIP3	-1.882	-2.684	-7.055	-9.626	113	72	44	24	63.25
RPS15A-PS6	-5.402	-2.319	-8.778	-5.006	86	78	24	69	64.25
CD9	-26.185	-2.184	-1.607	-7.173	24	83	111	40	64.5
GUCY1A3	-31.31	-3.185	-3.772	-2.615	21	62	76	101	65
RPL29	-4.605	-3.279	-6.896	-5.736	95	61	49	57	65.5
SDC4	-30.372	-1.453	-4.837	-4.814	22	109	64	72	66.75
PENK	-38.625	-1.936	-3.608	-3.791	15	89	79	85	67
NFKBIZ	-2.299	-4.07	-2.907	-10.277	108	50	95	20	68.25
RPS15A	-5.036	-1.752	-9.013	-5.639	90	99	23	64	69
RPS15A-PS4	-5.192	-2.072	-8.063	-4.42	87	84	31	77	69.75
LRIG1	-7.284	-4.928	-4.075	-3.25	74	42	71	93	70
SSH1	-22.485	-1.425	-2.485	-7.106	34	110	99	41	71
PAIP2	-6.132	-2.346	-3.453	-7.105	83	76	83	42	71
SLA	-9.49	-5.667	-2.994	-3.335	65	35	94	91	71.25
ASAP1	-5.046	-7.354	-3.363	-3.469	88	26	85	88	71.75
EEF1B2	-4.603	-2.394	-7.394	-3.964	96	74	38	82	72.5
RAF1	-13.043	-3.641	-2.426	-3.964	54	55	100	83	73
CPNE3	-6.527	-3.394	-3.713	-4.447	79	60	78	76	73.25
ABCA3	-9.907	-3.43	-3.055	-3.883	62	57	91	84	73.5
TSPAN32	-23.34	-2.212	-1.847	-4.762	31	80	110	74	73.75

	0	7	9	10	rank_0	rank_7	rank_9	rank_10	mean_rank
B4GALNT1	-10.134	-1.571	-3.252	-6.072	61	106	89	53	77.25
SIGIRR	-8.915	-2.065	-1.966	-5.736	68	86	107	60	80.25
RORA	-12.639	-1.721	-4.402	-2.504	56	100	69	104	82.25
EGR2	-10.901	-2.319	-1.38	-4.384	60	77	117	78	83
AFF3	-1.421	-4.9	-3.291	-3.708	117	43	87	87	83.5
CXCR5	-3.297	-4.07	-3.128	-2.941	102	49	90	95	84
H2-OA	-14.122	-1.566	-3.314	-3.25	50	107	86	94	84.25
RNF19A	-15.401	-1.669	-2.679	-3.285	47	103	96	92	84.5
KLRI2	-13.58	-2.21	-3.033	-1.385	51	81	93	116	85.25
LANCL1	-19.914	-1.91	-1.567	-2.257	39	90	113	106	87
CAR2	-11.17	-2.931	-1.937	-1.545	59	66	109	114	87
PRNP	-5.582	-2.768	-2.659	-2.677	85	71	97	98	87.75
UQCRH	-6.526	-1.385	-3.491	-4.15	80	113	82	80	88.75
PTPRK	-17.906	-1.408	-1.579	-3.383	42	112	112	89	88.75
RPL35A	-1.571	-2.593	-4.636	-2.558	114	73	68	102	89.25
SLC25A42	-3.975	-3.654	-1.996	-2.536	99	54	105	103	90.25
ZHX2	-3.151	-2.256	-4.242	-2.069	104	79	70	108	90.25
PER1	-7.043	-1.381	-3.543	-3.335	76	115	81	90	90.5
RPS25	-3.088	-1.625	-5.538	-2.792	106	105	59	96	91.5
MGAT5	-8.877	-1.708	-3.054	-2.082	69	101	92	107	92.25
NT5E	-1.976	-3.026	-3.278	-1.934	109	64	88	111	93
RPSA	-1.904	-1.882	-5.333	-1.952	112	93	62	109	94
ZFP467	-6.381	-2.981	-1.522	-1.522	82	65	114	115	94
NFKB1	-9.258	-1.954	-1.455	-1.379	66	88	115	117	96.5
NDUFA6	-4.952	-1.425	-2.376	-3.786	93	111	101	86	97.75
2010015L04RIK	-3.282	-2.072	-2.271	-1.92	103	85	102	112	100.5
EPHX1	-5.021	-1.479	-1.977	-2.627	91	108	106	100	101.25
CD3D	-1.945	-1.87	-2.066	-2.688	111	95	104	97	101.75
DHRS3	-5.037	-1.901	-1.391	-1.744	89	92	116	113	102.5
GM10548	-4.635	-1.31	-2.09	-2.635	94	117	103	99	103.25
SCP2	-4.523	-1.385	-2.581	-2.345	97	114	98	105	103.5
UTRN	-1.565	-1.693	-1.947	-1.946	115	102	108	110	108.75

Table 7. Ranked top transcription factors differentially expressed in cluster 8

Gene	TP	TN	thresh_mhg	hyper_pval	hyper_qval	gen_qval	rank_hyper_qval	rank_gen_qval	mean_rank
BHLHE40	0.937062937	0.525613661	8.143	5.56E-31	1.37E-28	-34.275	1	1	1
SPRY2	0.671328671	0.766275347	0.986	3.37E-26	4.15E-24	-30.895	2	2	2
BCL6	0.363636364	0.92529349	2.409	3.05E-20	2.50E-18	-24.943	3	3	3
REL	0.692307692	0.688367129	2.398	3.25E-19	2.00E-17	-19.474	4	4	4
NFKBIA	0.636363636	0.715581644	10.017	5.68E-17	2.33E-15	-16.891	6	5	5.5
NFAT5	0.573426573	0.782283885	1.417	1.35E-18	6.63E-17	-16.854	5	6	5.5
NR4A3	0.664335664	0.677694771	0.604	9.07E-16	3.19E-14	-14.001	7	7	7
CALCOCO1	0.349650335	0.893276414	1.975	2.22E-13	4.97E-12	-12.968	11	8	9.5
KDM2B	0.622377622	0.696905016	1.934	3.97E-14	1.09E-12	-10.571	9	12	10.5
HIF1A	0.846153846	0.44076841	1.014	1.32E-12	2.50E-11	-12.65	13	9	11
RNF19A	0.601398601	0.703308431	1.687	4.25E-13	8.72E-12	-11.762	12	10	11
ZFP36L1	0.818181818	0.5	2.578	2.23E-14	6.85E-13	-10.353	8	16	12

Gene	TP	TN	thresh_mhg	hyper_pval	hyper_qval	gen_qval	rank_hyper_qval	rank_gen_qval	mean_rank
NR4A2	0.811188811	0.473852721	0.595	5.87E-12	1.03E-10	-10.637	14	11	12.5
RORA	0.51048951	0.763073639	0.903	1.13E-11	1.64E-10	-10.539	17	13	15
IKZF2	0.573426573	0.707043757	0.084	2.00E-11	2.74E-10	-10.474	18	15	16.5
BACH2	0.370629371	0.880469584	1.293	2.15E-13	4.97E-12	-8.19	10	23	16.5
JUN	0.573426573	0.712913554	2.154	6.92E-12	1.14E-10	-9.04	15	20	17.5
MNDA	0.20979021	0.950373533	7.663	2.85E-10	3.05E-09	-10.506	23	14	18.5
NFKB2	0.727272727	0.563500534	4.05	1.06E-11	1.62E-10	-8.281	16	22	19
TGIF1	0.713286713	0.566168623	2.82	6.71E-11	7.50E-10	-9.342	22	18	20
RBPJ	0.734265734	0.544290288	0.227	6.51E-11	7.50E-10	-9.234	21	19	20
IRF5	0.307692308	0.893276414	5.865	4.48E-10	4.59E-09	-9.75	24	17	20.5
IRF8	0.636363636	0.644610459	5.574	4.77E-11	5.87E-10	-8.638	20	21	20.5
SQSTM1	0.832167832	0.437566702	8.455	3.04E-11	3.94E-10	-7.861	19	24	21.5
NFKB1	0.559440559	0.696371398	0.642	9.86E-10	8.98E-09	-7.433	27	25	26
PFDN5	0.769230769	0.488794023	8.598	7.83E-10	7.40E-09	-7.298	26	27	26.5
RELB	0.433566434	0.795624333	6.262	2.66E-09	2.26E-08	-6.795	29	29	29
FUBP3	0.405594406	0.808964781	0.367	1.24E-08	8.69E-08	-7.384	35	26	30.5
ELK3	0.34965035	0.851654216	3.559	1.00E-08	7.26E-08	-7.039	34	28	31
DTX3	0.447552448	0.779615795	1.496	6.44E-09	4.95E-08	-6.601	32	30	31
PER1	0.594405594	0.652614728	4.371	6.05E-09	4.80E-08	-6.384	31	31	31
TOX	0.804195804	0.451974386	0.536	4.86E-10	4.78E-09	-4.979	25	40	32.5
RPL7	0.608391608	0.640875133	11.985	4.94E-09	4.05E-08	-5.287	30	36	33
TCF25	0.755244755	0.486659552	6.649	8.57E-09	6.39E-08	-5.271	33	37	35
RUNX2	0.72027972	0.513340448	0.124	3.70E-08	2.39E-07	-5.793	38	33	35.5
STAT5A	0.51048951	0.719850587	3.797	2.06E-08	1.37E-07	-5.447	37	34	35.5
TRPS1	0.426573427	0.778014941	0.214	1.39E-07	8.13E-07	-5.965	42	32	37
JUNB	0.804195804	0.442902882	10.527	1.69E-09	1.49E-08	-3.803	28	47	37.5
STAT3	0.818181818	0.409284952	6.094	1.56E-08	1.07E-07	-4.825	36	41	38.5
NFKBIB	0.657342657	0.573105656	3.442	7.39E-08	4.54E-07	-5	40	39	39.5
FOSB	0.454545455	0.749199573	1.93	3.22E-07	1.76E-06	-5.363	45	35	40
ZC3H15	0.650349665	0.580576307	5.379	6.97E-08	4.40E-07	-4.217	39	45	42
EIF3H	0.9720279	0.1606189	8.3	6.93E-07	3.62E-06	-4.647	47	42	44.5

Gene	TP	TN	thresh_mhg	hyper_pval	hyper_qval	gen_qval	rank_hyper_qval	rank_gen_qval	mean_rank
	72	97							
LITAF	0.769230769	0.452508004	1.104	8.79E-08	5.27E-07	-3.66	41	48	44.5
NFE2L1	0.265734266	0.884738527	4.66	1.93E-06	8.94E-06	-5.245	53	38	45.5
HIVEP1	0.489510491	0.708644661	0.227	1.31E-06	6.63E-06	-4.628	48	43	45.5
NSD1	0.692307692	0.528815368	0.454	2.09E-07	1.20E-06	-3.636	43	49	46
STAT4	0.790209791	0.415154749	3.141	4.24E-07	2.27E-06	-3.552	46	51	48.5
PLRG1	0.272727273	0.889541089	7.45	2.72E-07	1.52E-06	-3.384	44	53	48.5
BATF	0.622377622	0.565635005	1.098	1.01E-05	4.35E-05	-4.344	57	44	50.5
MLLT3	0.538461538	0.654215582	0.345	4.39E-06	1.96E-05	-4.2	55	46	50.5
NACA	0.335664336	0.835645678	10.873	1.32E-06	6.63E-06	-3.506	49	52	50.5
FOSL2	0.748251748	0.441835646	0.214	4.23E-06	1.93E-05	-3.379	54	54	54
HMG20B	0.251748252	0.884738527	5.986	1.20E-05	5.01E-05	-3.553	59	50	54.5
NR4A1	0.587412587	0.607257204	7.138	4.62E-06	2.03E-05	-3.299	56	55	55.5
ATRX	0.594405594	0.592849532	0.189	1.05E-05	4.47E-05	-3.294	58	56	57
SPOP	0.461538462	0.731590181	6.173	1.60E-06	7.82E-06	-2.646	51	63	57
VGLL4	0.608391608	0.595517609	2.254	1.74E-06	8.22E-06	-2.56	52	64	58
CHD4	0.706293706	0.475453575	0.926	1.45E-05	5.94E-05	-3.143	60	57	58.5
ZMIZ1	0.482517483	0.690501601	2.007	2.41E-05	9.14E-05	-2.945	65	59	62
CAND1	0.377622378	0.783351121	1.899	2.01E-05	7.73E-05	-2.707	63	61	62
MED27	0.349650350	0.802027748	4.335	3.55E-05	0.000128279	-2.886	67	60	63.5
CSDA	0.475524476	0.685699039	0.696	7.91E-05	0.000266655	-3.114	72	58	65
NFKBIE	0.237762238	0.892209178	6.61	1.87E-05	7.43E-05	-2.308	62	68	65
MORF4L1	0.846153846	0.308964781	2.242	2.88E-05	0.000107482	-2.521	66	65	65.5
ARID5B	0.265734266	0.8660619	5.459	4.74E-05	0.000169149	-2.35	69	67	68
BTF3	0.979020979	0.123265742	7.6	1.74E-05	7.02E-05	-2.072	61	75	68
GATA3	0.405594406	0.748132337	5.593	7.84E-05	0.000266655	-2.425	73	66	69.5
SMARCE1	0.643356643	0.537886873	3.002	1.98E-05	7.73E-05	-2.036	64	77	70.5
MED24	0.363636364	0.777481323	1.144	0.000165835	0.000503646	-2.65	81	62	71.5
RPL6	0.951048951	0.164887914	10.87	3.51E-05	0.000128279	-1.971	68	80	74
MYSM1	0.559440559	0.602988226	0.585	0.00011387	0.000359127	-2.169	78	71	74.5
LZTR1	0.139860114	0.948772679	7.504	0.000109278	0.000351326	-2.131	76	73	74.5

Gene	TP	TN	thresh_mhg	hyper_pval	hyper_qval	gen_qval	rank_hyper_qval	rank_gen_qval	mean_rank
PBXIP1	0.594405594	0.576307364	3.812	5.59E-05	0.000196549	-1.866	70	83	76.5
MYEF2	0.433566434	0.69850587	0.454	0.000897689	0.002349272	-2.241	94	70	82
HDAC3	0.482517483	0.651013874	0.475	0.001066063	0.002731785	-2.292	96	69	82.5
TLE3	0.531468531	0.611526147	0.176	0.000587785	0.001624665	-2.062	89	76	82.5
EDF1	0.867132867	0.309498399	2.635	1.62E-06	7.82E-06	-1.2	50	115	82.5
HCLS1	0.846153846	0.294557097	0.496	0.000117041	0.000364458	-1.623	79	89	84
JARID2	0.398601399	0.72678762	0.138	0.001215883	0.003083579	-2.075	97	74	85.5
ANAPC11	0.566433566	0.570971185	0.202	0.000996094	0.00257936	-1.985	95	78	86.5
GATAD1	0.034965035	0.996798292	8.116	0.000545003	0.001523606	-1.802	88	85	86.5
ILF3	0.657342657	0.466915688	0.227	0.002461267	0.005821855	-2.156	104	72	88
DPF2	0.426573427	0.721451441	5.761	0.000187098	0.000561294	-1.451	82	94	88
MED15	0.433566434	0.702241195	5.027	0.000643695	0.001759432	-1.72	90	87	88.5
TCF7	0.531468531	0.630202775	0.287	0.000109968	0.000351326	-1.372	77	100	88.5
NFATC1	0.783216783	0.353788687	0.287	0.000425336	0.001202675	-1.508	87	91	89
SMARCB1	0.496503497	0.631803629	0.585	0.001717899	0.004226031	-1.981	100	79	89.5
NT5C	0.692307692	0.45624333	0.918	0.000330403	0.000967609	-1.451	84	95	89.5
SCAND1	0.335664336	0.781216649	1.036	0.001351791	0.003393272	-1.862	98	84	91
HSBP1	0.573426573	0.556029883	0.856	0.001842416	0.004443475	-1.939	102	81	91.5
BTG2	0.699300699	0.463180363	4.946	9.47E-05	0.000314926	-1.269	74	110	92
CALR	0.783216783	0.333511206	0.632	0.002126523	0.005078881	-1.887	103	82	92.5
HDAC1	0.671328671	0.46905016	1.043	0.000701578	0.001875957	-1.45	92	96	94
COPS5	0.335664336	0.788153682	7.241	0.000680003	0.00183825	-1.389	91	99	95
SNW1	0.34965035	0.781216649	6.926	0.000403447	0.001154113	-1.356	86	104	95
PQBP1	0.475524476	0.644610459	0.941	0.002966167	0.006883746	-1.76	106	86	96
KDM5C	0.503496503	0.625400213	0.189	0.001681029	0.004177101	-1.43	99	97	98
GTF2A1	0.398601399	0.7113127	0.176	0.004355241	0.009829259	-1.612	109	90	99.5
HMGB3	0.363636364	0.743863394	0.444	0.004098352	0.009422379	-1.502	107	92	99.5
GTF2E2	0.363636364	0.739060832	1.029	0.005977103	0.013128228	-1.667	112	88	100
PHB2	0.692307692	0.455176094	1.17	0.000361132	0.001045159	-1.132	85	123	104
CAMTA2	0.34965035	0.748132337	0.239	0.007846193	0.016639334	-1.497	116	93	104.5
TOX4	0.2097902	0.8826040	7.013	0.0017912	0.0043628	-1.259	101	111	106

Gene	TP	TN	thresh_mhg	hyper_pval	hyper_qval	gen_qval	rank_hyper_qval	rank_gen_qval	mean_rank
	1	55		34	07				
MAX	0.426573427	0.680896478	0.506	0.006075808	0.013226981	-1.362	113	102	107.5
NDUFA13	0.839160839	0.308431163	5.877	7.02E-05	0.000243334	-0.823	71	145	108
NCOA3	0.643356643	0.459978655	0.176	0.010131389	0.020769347	-1.42	120	98	109
GTF2H5	0.496503497	0.612593383	0.88	0.006888713	0.014735857	-1.294	115	109	112
BLOC1S1	0.391608392	0.702774813	0.941	0.012737582	0.024868612	-1.372	126	101	113.5
HDAC7	0.622377622	0.479722519	0.263	0.011272481	0.022729756	-1.34	122	105	113.5
GLRX2	0.258741259	0.850053362	4.508	0.000843321	0.002230719	-0.981	93	134	113.5
COMMD3	0.545454545	0.567235859	1.411	0.005869327	0.013007699	-1.172	111	117	114
CIR1	0.363636364	0.729989328	0.322	0.011602863	0.023018583	-1.332	123	107	115
STAT6	0.601398601	0.510672359	0.163	0.006160575	0.013293872	-1.186	114	116	115
NR1H2	0.580419581	0.536286019	0.575	0.004562854	0.0102042	-1.138	110	122	116
CREM	0.496503497	0.601921025	0.731	0.013604679	0.026352369	-1.327	127	108	117.5
PPIE	0.419580419	0.678762006	1.144	0.011047485	0.022460177	-1.21	121	114	117.5
RELA	0.573426573	0.544290288	0.401	0.004255991	0.009694201	-1.023	108	129	118.5
UBE2K	0.524475524	0.580042689	0.202	0.009728043	0.020280497	-1.15	118	121	119.5
GTF3C1	0.433566434	0.653681964	0.098	0.023036811	0.041365337	-1.361	137	103	120
ING4	0.419580419	0.673959445	0.731	0.015070881	0.028739832	-1.238	129	112	120.5
HSF1	0.391608392	0.693703308	0.536	0.022662307	0.040992113	-1.337	136	106	121
PHF6	0.391608392	0.700640342	0.214	0.014659258	0.028173304	-1.165	128	119	123.5
GTF2F1	0.538461538	0.563500534	0.651	0.011562838	0.023018583	-1.072	124	126	125
RBX1	0.692307692	0.430096051	0.566	0.002500625	0.005858607	-0.814	105	146	125.5
CREBBP	0.468531469	0.6366061 9	0.163	0.008338674	0.017532597	-0.979	117	135	126
CIZ1	0.377622378	0.708110993	0.239	0.020908084	0.038099176	-1.167	135	118	126.5
MED8	0.321678322	0.762006403	0.774	0.017872006	0.033307021	-1.123	132	124	128
LRRFIP1	0.797202797	0.292422625	0.401	0.012424909	0.024452222	-0.993	125	131	128
NONO	0.853146853	0.284951974	4.189	0.000129207	0.000397311	-0.536	80	178	129
PHF20L1	0.433566434	0.648879402	0.151	0.030262913	0.052799125	-1.163	141	120	130.5
PFDN1	0.426573427	0.653148346	1.07	0.034580757	0.057127802	-1.233	149	113	131
YBX1	0.643356643	0.445570971	0.029	0.023294215	0.041524471	-1.116	138	125	131.5
AIP	0.580419581	0.514941302	0.411	0.017250076	0.032393272	-0.979	131	136	133.5

Gene	TP	TN	thresh_mhg	hyper_pval	hyper_qval	gen_qval	rank_hyper_qval	rank_gen_qval	mean_rank
STAT5B	0.48951049	0.594450374	0.07	0.030759407	0.053287423	-1.027	142	128	135
NPM1	0.98601398	0.096051227	7.479	0.000105358	0.000345573	-0.355	75	196	135.5
KDM5A	0.59440559	0.502134472	0.084	0.016031586	0.030336694	-0.859	130	142	136
SMARCC2	0.50349650	0.588046958	0.214	0.020605234	0.037827519	-0.92	134	139	136.5
ATF2	0.37062937	0.707577375	0.263	0.031987067	0.054308374	-0.979	144	137	140.5
ZBTB7A	0.42657342	0.648345784	0.163	0.044589518	0.070018891	-1.047	157	127	142
GTF3C2	0.44755244	0.628068303	0.239	0.044686853	0.070018891	-0.991	154	132	143
GTF2A2	0.38461538	0.690501601	0.669	0.039834119	0.064047113	-0.983	153	133	143
UBXN4	0.59440559	0.494130203	0.084	0.025034559	0.044305766	-0.662	139	156	147.5
SND1	0.53146853	0.545891142	0.227	0.044564418	0.070018891	-0.875	155	141	148
CNOT8	0.41958042	0.642475987	0.322	0.081913949	0.118288494	-1.001	170	130	150
SREBF2	0.51048951	0.566702241	0.138	0.044430666	0.070018891	-0.842	156	144	150
YEATS4	0.38461538	0.692636073	0.895	0.035396521	0.058050295	-0.791	150	151	150.5
PML	0.34265734	0.720917823	0.163	0.064815807	0.096634476	-0.973	165	138	151.5
RBL2	0.60839160	0.479722519	0.239	0.025361876	0.044564404	-0.623	140	163	151.5
CDCA4	0.40559440	0.662753469	0.422	0.059530039	0.090397467	-0.813	162	147	154.5
GON4L	0.38461538	0.677161153	0.057	0.078251103	0.114581972	-0.858	168	143	155.5
XBP1	0.40559440	0.662219851	0.401	0.06110584	0.092221084	-0.797	163	148	155.5
LIMD1	0.39860139	0.659551761	0.176	0.094429449	0.130503621	-0.881	178	140	159
C1D	0.40559440	0.661152615	0.516	0.064352513	0.096528769	-0.649	164	157	160.5
MORF4L2	0.48251748	0.597652081	0.651	0.037221533	0.060240108	-0.577	152	170	161
HMGB2	0.83916083	0.295090715	1.84	0.000242843	0.000719752	0	83	242	162.5
UBTF	0.46853146	0.592849552	0.275	0.089063751	0.126645566	-0.723	173	153	163
PNN	0.51048951	0.553361793	0.287	0.082224929	0.118288494	-0.677	171	155	163
HBP1	0.46153846	0.609925293	0.227	0.055986197	0.086620154	-0.605	159	167	163
SSRP1	0.60139860	0.482390608	0.287	0.032011034	0.054308374	-0.519	145	181	163
DR1	0.33566433	0.719850587	0.345	0.094270969	0.130503621	-0.794	177	150	163.5
MTA2	0.71328671	0.364461046	0.345	0.036213597	0.058996985	-0.541	151	176	163.5
PTTG1	0.55244755	0.532017076	0.322	0.031297292	0.053840096	-0.457	143	184	163.5
BUD31	0.50349650	0.564034152	1.449	0.069722873	0.103324258	-0.626	166	162	164
PURB	0.4335664	0.6360725	0.138	0.0587630	0.0897869	-0.592	161	169	165

Gene	TP	TN	thresh_mhg	hyper_pval	hyper_qval	gen_qval	rank_hyper_qval	rank_gen_qval	mean_rank
	34	72		3	9				
IRF2	0.531468531	0.551760939	0.356	0.033143696	0.055844858	-0.426	146	187	166.5
CNOT3	0.377622378	0.680896478	0.39	0.089691999	0.126805929	-0.638	174	160	167
MLX	0.356643357	0.703308431	0.722	0.080361563	0.116976004	-0.621	169	165	167
CEBPZ	0.342657343	0.715581644	0.401	0.084334111	0.120617391	-0.622	172	164	168
AES	0.531468531	0.524012807	0.422	0.116424164	0.151536213	-0.795	189	149	169
MKL1	0.496503497	0.585378869	0.176	0.034601799	0.057127802	-0.358	148	194	171
NCOA2	0.405594406	0.653681964	0.084	0.090828883	0.127679459	-0.564	175	174	174.5
AEBP2	0.377622378	0.670224112	0.111	0.140771452	0.180363423	-0.645	192	158	175
GABPB1	0.321678322	0.729989328	0.848	0.108856919	0.144296015	-0.614	184	166	175
BRD8	0.545454545	0.501067236	0.07	0.162139267	0.200433466	-0.783	199	152	175.5
NCOA4	0.370629371	0.680362886	0.275	0.122812686	0.159010109	-0.634	190	161	175.5
TBX21	0.489510499	0.557097118	0.433	0.160233183	0.19907759	-0.712	198	154	176
CCNT2	0.342657343	0.702241195	0.31	0.150742274	0.189196935	-0.642	196	159	177.5
ZRANB2	0.321678322	0.732123799	0.757	0.098695522	0.132672669	-0.568	183	172	177.5
TMF1	0.496503497	0.562966916	0.138	0.098236071	0.132672669	-0.538	182	177	179.5
PHB	0.573426573	0.498399146	0.642	0.058110224	0.08934447	-0.347	160	199	179.5
PA2G4	0.622377622	0.459978655	0.31	0.033847539	0.056642821	-0.194	147	214	180.5
HTATIP2	0.328671329	0.716648879	0.748	0.144962238	0.182875438	-0.599	194	168	181
CCNH	0.377622378	0.673425827	0.506	0.123782177	0.159426259	-0.572	191	171	181
HMGB1	0.944055944	0.120064034	0.356	0.010017874	0.020709218	0	119	243	181
NFYC	0.391608392	0.662219851	0.433	0.112496833	0.147706182	-0.552	188	175	181.5
FUBP1	0.496503497	0.570437567	0.151	0.071287384	0.105010159	-0.353	167	197	182
TCF20	0.587412587	0.469583778	0.084	0.109101865	0.144296015	-0.521	186	179	182.5
SARNP	0.601398601	0.455709712	0.956	0.108011214	0.144296015	-0.503	185	183	184
LDB1	0.419580402	0.635005336	0.516	0.112881147	0.147706182	-0.443	187	185	186
ZMAT2	0.496503497	0.563500534	0.367	0.096095714	0.132064501	-0.35	179	198	188.5
ERH	0.748251748	0.339381003	1.176	0.018546483	0.034304022	0	133	244	188.5
VPS72	0.377622378	0.658484525	0.444	0.215413336	0.25354874	-0.566	209	173	191
CNOT7	0.356643357	0.685165422	0.454	0.172842905	0.211539078	-0.519	200	182	191
SP110	0.741258741	0.330309498	0.214	0.045752596	0.071235054	-0.138	158	224	191

Gene	TP	TN	thresh_mhg	hyper_pval	hyper_qval	gen_qval	rank_hyper_qval	rank_gen_qval	mean_rank
NFX1	0.433566434	0.624866596	0.176	0.097646028	0.132672669	-0.257	181	204	192.5
TET3	0.34965035	0.691568837	0.124	0.175211299	0.2127228	-0.398	202	188	195
SMARCA4	0.503496503	0.532550694	0.202	0.227808254	0.26434354	-0.521	212	180	196
CCNL2	0.65034965	0.408217716	0.496	0.098343384	0.132672669	-0.192	180	215	197.5
PNRC2	0.433566434	0.601921025	0.496	0.227133778	0.26434354	-0.427	211	186	198.5
CXXC1	0.384615385	0.655816435	0.506	0.18685466	0.223137119	-0.369	206	192	199
NFATC3	0.475524476	0.567769477	0.111	0.178923239	0.215760376	-0.358	204	195	199.5
TSG101	0.440559441	0.599252935	0.766	0.198155958	0.235489689	-0.364	207	193	200
ZNRD1	0.321678322	0.711846318	1.406	0.223503921	0.261818879	-0.379	210	191	200.5
PHF5A	0.461538462	0.582710779	0.74	0.172132209	0.211539078	-0.299	201	201	201
DDX54	0.41958042	0.612593383	0.379	0.250025471	0.287412458	-0.396	214	189	201.5
TRIP12	0.468531469	0.575240128	0.084	0.175539547	0.2127228	-0.311	203	200	201.5
MED12	0.447552448	0.599786553	0.124	0.153010283	0.191068679	-0.244	197	206	201.5
RNF7	0.615384615	0.433831377	1.05	0.144881668	0.182875438	-0.232	195	208	201.5
ECD	0.342657343	0.684631804	0.151	0.27796789	0.316574541	-0.396	216	190	203
MBNL1	0.958041958	0.07470651	0.189	0.092503138	0.129294218	-0.031	176	233	204.5
MIER1	0.552447552	0.490394877	0.287	0.1838009	0.22056108	-0.229	205	209	207
SBDS	0.426573427	0.611526147	0.722	0.208171758	0.246203137	-0.216	208	211	209.5
MAZ	0.335664336	0.686766275	0.401	0.318864273	0.356548232	-0.294	220	202	211
TCEA1	0.657342657	0.391675556	0.239	0.142010537	0.181008249	-0.05	193	230	211.5
ILF2	0.370629371	0.657950907	0.299	0.271875058	0.311075648	-0.227	215	210	212.5
XAB2	0.342657343	0.677161153	0.696	0.343455099	0.382307485	-0.243	221	207	214
TCERG1	0.384615385	0.642475987	0.287	0.285973116	0.324190771	-0.21	217	212	214.5
NFYB	0.342657343	0.672358591	0.485	0.388068091	0.4224104	-0.247	226	205	215.5
GTF2B	0.412587413	0.613660619	0.669	0.296079986	0.332582998	-0.204	219	213	216
SREBF1	0.335664336	0.673425827	0.227	0.444408803	0.475324198	-0.285	230	203	216.5
BOLA2	0.335664336	0.68036286	2.444	0.377759743	0.414861146	-0.173	224	217	220.5
MYC	0.342657343	0.671824973	0.333	0.393116468	0.42602049	-0.185	227	216	221.5
RNF166	0.440559441	0.567235859	0.356	0.461339079	0.489178506	-0.17	232	218	225
CNOT1	0.433566434	0.575773746	0.151	0.446822803	0.475837271	-0.165	231	219	225
CDC5L	0.3846153	0.6254002	0.475	0.4383996	0.4709446	-0.156	229	221	225

Gene	TP	TN	thresh_mhg	hyper_pval	hyper_qval	gen_qval	rank_hyper_qval	rank_gen_qval	mean_rank
	85	13		85	4				
RBBP4	0.608391608	0.408217716	0.202	0.383247493	0.419017259	-0.131	225	226	225.5
RNF114	0.573426573	0.444503735	0.299	0.372201809	0.410590337	-0.069	222	229	225.5
MED1	0.461538462	0.551227321	0.227	0.416482261	0.449362439	-0.138	228	225	226.5
PSMC3	0.636363636	0.390608324	0.696	0.293008681	0.330642823	-0.025	218	235	226.5
RNPS1	0.587412587	0.446104589	0.748	0.245525482	0.283564642	-0.014	213	240	226.5
SMAD7	0.370629371	0.629669157	0.239	0.530296375	0.555118758	-0.16	235	220	227.5
RUVBL1	0.321678322	0.68036286	0.485	0.513132931	0.539447441	-0.156	234	222	228
RNF44	0.524475524	0.493596585	0.227	0.370922777	0.410590337	-0.031	223	234	228.5
MLXIP	0.398601399	0.584845251	0.202	0.68149627	0.701456412	-0.149	239	223	231
GTF3A	0.377622378	0.620597652	0.848	0.549945672	0.573248455	-0.085	236	228	232
RPL7L1	0.671328671	0.317502668	0.251	0.647171388	0.668925048	-0.119	238	227	232.5
KLF6	0.720279720 2	0.286019211	0.333	0.478913334	0.505633821	-0.024	233	236	234.5
MLL5	0.608391608	0.372465315	0.163	0.708870887	0.723577735	-0.048	241	231	236
MYBBP1A	0.398601399	0.581109925	0.299	0.711963723	0.723731718	-0.046	242	232	237
TSC22D4	0.503496503	0.485058698	0.356	0.637279135	0.661479609	-0.02	237	238	237.5
TARDBP	0.419580420 2	0.561366062	0.176	0.701153823	0.718682668	-0.018	240	239	239.5
MED30	0.356643357	0.616328709	1.064	0.766554961	0.776018603	-0.024	243	237	240
IFI35	0.356643357	0.605122732	0.766	0.839261146	0.846140335	-0.004	244	241	242.5
IKZF3	0.545454545	0.412486666	0.263	0.857972288	0.861474216	0	245	245	245
EGR1	0.363636364	0.555496265	0.214	0.976020285	0.976020285	0	246	246	246

Table 8. Ranked top surface cytokines differentially expressed in cluster 8

Gene	TP	TN	thresh_mhg	hyper_pval	hyper_qval	gen_qval	rank_hyper_qval	rank_gen_qval	mean_rank
XCL1	0.741258741	0.892209178	5.031	2.31E-62	3.55E-60	-85.872	1	1	1
CD83	0.664335664	0.918890075	6.743	9.79E-59	7.54E-57	-80.672	2	2	2
BACE2	0.314685315	0.985058698	5.661	3.92E-36	2.01E-34	-64.954	3	3	3
CD81	0.349650335	0.970117396	7.464	1.28E-32	3.95E-31	-41.599	4	4	4
TNFRSF4	0.622377622	0.842582711	8.793	1.07E-32	3.95E-31	-37.64	5	6	5.5
CD74	0.489510489	0.913020277	3.975	9.03E-32	2.32E-30	-37.012	6	7	6.5
CCR8	0.566433566	0.857524013	4.547	6.52E-29	1.43E-27	-36.056	7	8	7.5

Gene	TP	TN	thresh_mhg	hyper_pval	hyper_qval	gen_qval	rank_hyper_qval	rank_gen_qval	mean_rank
TNFSF11	0.300699301	0.967982924	4.371	4.59E-25	5.44E-24	-40.55	13	5	9
TNFSF8	0.398601399	0.930629669	0.872	2.22E-25	3.42E-24	-34.906	10	9	9.5
NRP1	0.692307692	0.758804696	4.885	1.78E-27	3.04E-26	-30.902	9	10	9.5
LAG3	0.951048951	0.471718225	1.401	9.29E-28	1.79E-26	-24.857	8	13	10.5
CCR7	0.769230769	0.670757737	6.844	4.40E-25	5.44E-24	-26.11	12	11	11.5
ITGB1	0.650349655	0.775346852	7.52	3.42E-25	4.79E-24	-24.519	11	15	13
TNFRSF18	0.930069930	0.464781217	5.798	7.20E-24	7.92E-23	-24.642	14	14	14
CD200	0.440559441	0.886872999	0.632	9.74E-21	8.82E-20	-25.027	17	12	14.5
TNFSF4	0.461538462	0.870330843	3.183	4.63E-20	3.75E-19	-24.486	19	16	17.5
CD160	0.664335664	0.723052295	1.674	3.04E-20	2.61E-19	-21.394	18	18	18
PDCD1	0.965034965	0.398078975	5.024	3.15E-23	3.24E-22	-16.441	15	23	19
KIT	0.454545455	0.869797225	0.379	2.64E-19	1.84E-18	-22.015	22	17	19.5
CD82	0.657342657	0.722518677	9.763	1.55E-19	1.19E-18	-21.225	20	19	19.5
KLRK1	0.818181818	0.562966916	1.956	1.69E-19	1.24E-18	-18.913	21	20	20.5
TIGIT	0.972027972	0.381003202	7.438	6.24E-23	6.01E-22	-14.863	16	26	21
CD9	0.475524476	0.853255069	7.807	8.10E-19	5.43E-18	-18.364	23	21	22
TSPAN32	0.363636364	0.916755603	7.665	1.67E-18	1.07E-17	-16.662	24	22	23
KLRC1	0.832167832	0.515474992	1.828	7.29E-17	4.32E-16	-15.365	26	25	25.5
KLRD1	0.832167832	0.517609392	6.666	4.90E-17	3.02E-16	-12.835	25	29	27
IL2RA	0.391608392	0.879935966	1.664	4.36E-15	2.17E-14	-16.072	31	24	27.5
GABARAPL1	0.517482517	0.798292423	4.788	1.28E-15	6.77E-15	-14.714	29	27	28
IL18R1	0.699300699	0.645677695	2.803	7.51E-16	4.13E-15	-12.642	28	30	29
AXL	0.349650349	0.887940235	0.465	1.16E-12	5.41E-12	-13.839	33	28	30.5
IL18RAP	0.594405594	0.732657417	5.275	3.63E-15	1.86E-14	-11.837	30	31	30.5
KLRC2	0.713286713	0.612059765	2.356	3.24E-14	1.56E-13	-10.905	32	32	32
CTLA4	0.860139860	0.476520811	6.115	1.59E-16	9.08E-16	-8.491	27	37	32
NR4A2	0.811188811	0.473852721	0.595	5.87E-12	2.66E-11	-10.637	34	33	33.5
CD8A	0.853146853	0.394877268	8.687	3.19E-10	1.29E-09	-8.998	38	36	37
ECE1	0.468531469	0.776414088	0.322	5.61E-10	2.06E-09	-9.736	41	34	37.5
GDI2	0.867132867	0.375133404	7.442	3.98E-10	1.53E-09	-9.626	40	35	37.5
FAS	0.3216783	0.8820704	2.534	7.86E-10	2.82E-09	-7.925	43	38	40.5

Gene	TP	TN	thresh_mhg	hyper_pval	hyper_qval	gen_qval	rank_hyper_qval	rank_gen_qval	mean_rank
	22	38							
TNFRSF9	0.734265734	0.55923159	2.104	6.46E-12	2.84E-11	-5.868	35	49	42
CD52	0.657342657	0.601921025	10.995	1.48E-09	4.96E-09	-7.664	46	39	42.5
TNFSF10	0.405594406	0.821237994	3.084	1.18E-09	4.04E-09	-6.28	45	43	44
ITGAV	0.643356643	0.622198506	0.151	5.59E-10	2.06E-09	-5.945	42	46	44
ICOS	0.811188811	0.462646745	0.714	3.18E-11	1.32E-10	-5.503	37	52	44.5
PEBP1	0.853146853	0.419423693	1.664	8.95E-12	3.83E-11	-5.136	36	55	45.5
GYPC	0.307692308	0.878335112	5.377	1.75E-08	5.18E-08	-6.795	52	41	46.5
PRKCA	0.307692308	0.876200604	2.903	2.81E-08	8.01E-08	-7.019	54	40	47
CD37	0.923076923	0.284951974	1.501	1.95E-09	6.40E-09	-5.937	47	47	47
CD96	0.664335664	0.575773746	3.936	2.10E-08	6.11E-08	-5.973	53	45	49
PTGER2	0.391608392	0.808964781	0.678	8.37E-08	2.26E-07	-6.744	57	42	49.5
PGLYRP1	0.741258741	0.498399146	4.224	1.38E-08	4.33E-08	-5.804	49	50	49.5
ANXA5	0.531468531	0.706510139	7.636	9.66E-09	3.10E-08	-5.387	48	53	50.5
ATPIF1	0.573426573	0.665421558	5.814	1.58E-08	4.76E-08	-5.684	51	51	51
CSF1	0.293706294	0.868729989	2.678	9.30E-07	2.27E-06	-5.975	63	44	53.5
GRN	0.363636364	0.820704376	0.536	4.48E-07	1.15E-06	-5.908	60	48	54
LY6E	0.804195804	0.418890075	9.168	3.82E-08	1.05E-07	-4.603	56	56	56
CTSB	0.909090909	0.309498399	0.214	1.16E-09	4.04E-09	-2.478	44	68	56
PTPN11	0.377622378	0.808964781	0.632	5.12E-07	1.27E-06	-5.357	62	54	58
ITGB3	0.279720278	0.884738527	3.894	2.74E-07	7.15E-07	-4.517	59	57	58
CD3G	0.671328671	0.554962647	10.492	1.21E-07	3.22E-07	-3.89	58	59	58.5
BSG	0.839160839	0.376734258	5.705	3.70E-08	1.04E-07	-3.198	55	64	59.5
TMEM123	0.818181818	0.381003202	1.214	4.83E-07	1.22E-06	-3.639	61	61	61
LTB	0.902097902	0.249733191	5.194	7.27E-06	1.62E-05	-3.642	69	60	64.5
CX3CR1	0.391608392	0.775880477	0.287	1.17E-05	2.50E-05	-4.262	72	58	65
CD6	0.776223776	0.406616862	5.365	6.29E-06	1.46E-05	-2.782	66	66	66
SLAMF7	0.314685315	0.843116329	4.72	5.24E-06	1.24E-05	-2.753	65	67	66
LAMP1	0.300699301	0.849519744	4.044	1.02E-05	2.25E-05	-3.446	70	63	66.5
CD27	0.713286713	0.484525088	7.773	2.59E-06	6.24E-06	-2.34	64	70	67
TNIP1	0.531468531	0.651013874	2.457	1.31E-05	2.76E-05	-3.065	73	65	69

Gene	TP	TN	thresh_mhg	hyper_pval	hyper_qval	gen_qval	rank_hyper_qval	rank_gen_qval	mean_rank
LGALS1	0.972027972	0.21398079	3.829	3.32E-10	1.31E-09	-1.081	39	102	70.5
HSP90AB1	1	0.123265742	8.641	1.41E-08	4.33E-08	-1.368	50	92	71
CD44	0.713286713	0.458911419	0.356	3.51E-05	6.67E-05	-3.624	81	62	71.5
ADAM10	0.685314685	0.49733191	0.66	1.50E-05	3.12E-05	-2.415	74	69	71.5
2-Sep	0.783216783	0.386872999	3.131	2.07E-05	4.14E-05	-1.893	77	75	76
CD97	0.692307692	0.479722519	5.654	4.07E-05	7.64E-05	-1.939	82	74	78
CD3D	0.867132867	0.27054429	8.86	9.74E-05	0.000170432	-2.242	88	71	79.5
IFNAR1	0.643356643	0.521878335	4.367	9.15E-05	0.000162009	-2.225	87	72	79.5
PDIA3	0.902097902	0.242796158	0.333	1.56E-05	3.20E-05	-1.498	75	87	81
ITGAL	0.727272727	0.439167556	6.641	5.43E-05	9.84E-05	-1.78	85	80	82.5
TRAF3	0.286713287	0.837780149	3.392	0.000245285	0.000401849	-2.188	94	73	83.5
CD84	0.545454545	0.624332978	2.722	5.32E-05	9.75E-05	-1.585	84	83	83.5
GPR174	0.27972028	0.848452508	4.085	0.000126254	0.00021366	-1.857	91	77	84
FERMT3	0.797202797	0.371931697	6.194	1.84E-05	3.73E-05	-1.202	76	96	86
CD48	0.832167832	0.340448239	3.865	6.56E-06	1.49E-05	-0.956	68	105	86.5
P4HB	0.734265734	0.436499466	6.456	3.40E-05	6.55E-05	-1.201	80	97	88.5
AMICA1	0.307692308	0.80416222	0.465	0.001498757	0.002285233	-1.827	101	78	89.5
CD53	0.888111888	0.241728922	7.389	0.000119166	0.000203906	-1.402	90	89	89.5
M6PR	0.741258741	0.4226254	6.386	6.10E-05	0.000109315	-1.329	86	93	89.5
CALR	0.783216783	0.333511206	0.632	0.002126523	0.00314889	-1.887	104	76	90
CD226	0.58041958	0.570971185	2.108	0.000319444	0.000501983	-1.587	98	82	90
CD28	0.741258741	0.392209178	0.251	0.000830814	0.001279454	-1.636	100	81	90.5
IL2RG	0.804195804	0.334044824	9.555	0.000295753	0.000474437	-1.516	96	85	90.5
CD69	0.608391608	0.564034152	7.249	4.80E-05	8.91E-05	-1.13	83	99	91
ITGB2	0.93006993	0.2113127	5.017	6.34E-06	1.46E-05	-0.64	67	120	93.5
ERP29	0.496503497	0.614194237	0.956	0.006186193	0.008582647	-1.792	111	79	95
TMX3	0.384615385	0.730522946	0.227	0.002599987	0.00377734	-1.508	106	86	96
SPN	0.692307692	0.433297759	0.251	0.001985611	0.002997884	-1.327	102	94	98
IGF2R	0.489510489	0.617395945	0.111	0.007838293	0.010682275	-1.577	113	84	98.5
PEAR1	0.342657343	0.75773746	0.287	0.006082512	0.008515517	-1.38	110	90	100
RPS19	0.8391608	0.3201707	10.803	2.21E-05	4.37E-05	-0.499	78	124	101

Gene	TP	TN	thresh_mhg	hyper_pval	hyper_qval	gen_qval	rank_hyper_qval	rank_gen_qval	mean_rank
	39	58							
CLPTM1	0.335664336	0.75773746	0.299	0.009829981	0.013050148	-1.487	116	88	102
IL27RA	0.587412587	0.533084312	0.444	0.003517435	0.005062477	-1.121	107	100	103.5
CD8B1	0.958041958	0.132870864	9.067	0.000387061	0.000602095	-0.836	99	110	104.5
ERP44	0.636363636	0.476520811	0.475	0.005603821	0.007917324	-1.03	109	104	106.5
NAMPT	0.314685315	0.767342583	0.189	0.019141924	0.023773035	-1.374	124	91	107.5
CLIC4	0.384615385	0.712913554	0.263	0.009931626	0.013072397	-1.175	117	98	107.5
SYNJ2BP	0.293706294	0.836179296	4.12	0.000147864	0.000247512	-0.441	92	125	108.5
ROCK1	0.503496503	0.589647812	0.111	0.018799573	0.023537677	-1.224	123	95	109
IL2RB	0.979020979	0.127001067	2.976	1.07E-05	2.31E-05	-0.082	71	148	109.5
IL10RA	0.601398601	0.505336179	0.214	0.008657115	0.011693943	-0.944	114	106	110
RAC1	0.762237762	0.348986126	0.485	0.003749065	0.005345889	-0.739	108	113	110.5
CD3E	0.986013986	0.072572038	0.748	0.002042816	0.003054307	-0.65	103	118	110.5
ATP5B	0.965034965	0.133404482	6.142	0.000102936	0.000178114	-0.246	89	135	112
GPI1	0.909090909	0.228922092	6.698	2.49E-05	4.86E-05	-0.088	79	147	113
PSEN1	0.454545455	0.643543223	0.401	0.012639807	0.016221086	-0.873	120	109	114.5
NCKAP1L	0.650349655	0.455176094	0.322	0.008732449	0.011693943	-0.701	115	114	114.5
AIMP1	0.489510489	0.590715048	0.986	0.037419439	0.044671268	-1.048	129	103	116
TRPV2	0.559440559	0.537353255	0.444	0.015893899	0.020062791	-0.819	122	111	116.5
MIF	0.860139860	0.267876201	1.891	0.000283486	0.000459545	-0.187	95	138	116.5
MS4A6B	0.804195804	0.309498399	7.085	0.002176457	0.003192137	-0.36	105	129	117
IL21R	0.643356643	0.411419424	0.367	0.115265771	0.131488361	-1.119	135	101	118
GPR65	0.489510489	0.596051227	0.642	0.028218632	0.033950542	-0.9	128	108	118
PTPRCAP	0.923076923	0.188367129	1.036	0.000232134	0.000384394	-0.141	93	143	118
CD5	0.601398601	0.491462113	0.566	0.019663834	0.024225843	-0.765	125	112	118.5
CTSD	0.944055944	0.155816435	4.385	0.000310803	0.000493444	-0.158	97	140	118.5
C1QBP	0.538461538	0.560298826	0.956	0.014024554	0.017849415	-0.654	121	117	119
PDIA4	0.440559441	0.631270011	0.356	0.053404352	0.061836618	-0.923	133	107	120
AAMP	0.643356643	0.447171825	0.465	0.021336414	0.026077839	-0.668	126	116	121
ITGA4	0.853146853	0.22945571	0.084	0.012018357	0.015553168	-0.547	119	123	121
CD2	0.895104895	0.187833511	0.888	0.006478663	0.008908162	-0.339	112	130	121

Gene	TP	TN	thresh_mhg	hyper_pval	hyper_qval	gen_qval	rank_hyper_qval	rank_gen_qval	mean_rank
HNRNPU	0.629370629	0.458377801	0.356	0.025313734	0.030695394	-0.625	127	121	124
ADAM17	0.356643357	0.693703308	0.251	0.123421792	0.13975703	-0.692	136	115	125.5
ATP6AP2	0.419580402	0.644076841	0.526	0.076181691	0.087552092	-0.65	134	119	126.5
PGRMC1	0.412587413	0.658484525	0.614	0.052731009	0.061519604	-0.58	132	122	127
CAST	0.587412587	0.491995731	0.401	0.040257455	0.0476896	-0.303	130	132	131
SIVA1	0.335664336	0.708110993	2.032	0.15594075	0.174020837	-0.379	138	126	132
IFNG	0.328671329	0.699573106	0.949	0.267352682	0.289470869	-0.371	142	127	134.5
HMGB1	0.944055944	0.120064034	0.356	0.010017874	0.013074174	0	118	151	134.5
IDE	0.545454545	0.503735326	0.251	0.147487788	0.165789193	-0.225	137	136	136.5
CD2BP2	0.447552448	0.576840982	0.345	0.314275824	0.331496417	-0.364	146	128	137
LY9	0.307692308	0.719850587	0.566	0.268794378	0.289470869	-0.286	143	133	138
SBDS	0.426573427	0.611526147	0.722	0.208171758	0.228988934	-0.216	140	137	138.5
HSPD1	0.657342657	0.419423693	0.623	0.042833945	0.050354409	-0.089	131	146	138.5
CR1L	0.440559441	0.582177161	0.714	0.327701742	0.340986948	-0.323	148	131	139.5
LSM1	0.321678322	0.700640342	0.546	0.317551735	0.332673247	-0.266	147	134	140.5
CXCR3	0.342657343	0.689434365	0.614	0.239110071	0.261155681	-0.155	141	142	141.5
RPS6KB1	0.370629371	0.653148346	0.251	0.312065574	0.331435161	-0.169	145	139	142
CAP1	0.573426573	0.45357524	0.401	0.295867542	0.316413899	-0.156	144	141	142.5
EZR	0.741258741	0.296691569	0.465	0.194332008	0.215303088	0	139	152	145.5
HSPA9	0.482517483	0.530416222	0.411	0.415633226	0.426716779	-0.108	150	144	147
ICAM1	0.384615385	0.615261473	0.251	0.534335901	0.544951846	-0.098	151	145	148
PDE4B	0.447552448	0.54375667	0.31	0.612589334	0.620649972	-0.08	152	149	150.5
CXCR6	0.664335664	0.351654216	0.714	0.38664039	0.3996149	0	149	153	151
H2-M3	0.335664336	0.646211313	0.678	0.699273421	0.703843835	-0.067	153	150	151.5
LILRB4	0.335664336	0.608858058	0.632	0.920475822	0.920475822	0	154	154	154

Table 9. Ranked top 100 differentially expressed genes in cluster 8 as compared to all 15 CD8 T cell clusters

	adj.pval.cluster 1	adj.pval.cluster 2	adj.pval.cluster 3	adj.pval.cluster 4	adj.pval.cluster 5	adj.pval.cluster 6	adj.pval.cluster 7	adj.pval.cluster 8	adj.pval.cluster 9	adj.pval.cluster 10	adj.pval.cluster 11	adj.pval.cluster 12	adj.pval.cluster 13	adj.pval.cluster 14	adj.pval.cluster 15
XCL1	0	0	0	0	0	0	-	-	-	-	-	-	0	0	-

	adj.pval.cluster 1	adj.pval.cluster 2	adj.pval.cluster 3	adj.pval.cluster 4	adj.pval.cluster 5	adj.pval.cluster 6	adj.pval.cluster 7	adj.pval.cluster 8	adj.pval.cluster 9	adj.pval.cluster 10	adj.pval.cluster 11	adj.pval.cluster 12	adj.pval.cluster 13	adj.pval.cluster 14	adj.pval.cluster 15
							0.001	85.872	0.006	0.093	0.001	15.948			13.484
CD83	0	0	0	0	0	0	-6.859	-80.672	-0.137	0	-0.001	-1.561	0	-11.426	-16.922
PLXDC2	0	0	0	0	0	0	-2.094	-68.219	-0.023	-0.022	-0.001	-0.224	0	-0.534	-13.298
BACE2	0	0	0	0	0	0	-1.489	-64.954	-0.031	-0.124	-0.001	0	0	0	-4.874
LAD1	0	0	0	0	0	0	-8.069	-44.851	-0.738	0	-0.001	-3.17	0	-0.181	-8.383
GPM6B	0	0	0	0	0	0	-9.613	-43.038	-0.164	-0.174	-0.001	-2.795	0	0	-3.772
CD81	0	0	0	0	0	0	-1.064	-41.599	-1.361	-0.194	-0.001	0	0	0	-12.714
AI836003	0	0	0	0	0	0	-23.667	-41.348	-1.667	-0.126	-0.001	-0.044	0	0	-3.175
SYNPO	0	0	0	0	0	0	-0.059	-41.343	-0.007	0	-0.001	-0.4	-0.09	0	-1.027
TNFSF11	0	0	0	0	0	0	-0.109	-40.55	0	-0.42	-0.001	-1.526	0	-0.711	-21.519
NRN1	0	0	0	0	0	0	-28.795	-39.128	-4.33	-1.816	-0.001	-0.014	0	0	-5.958
TNFRSF4	0	0	0	0	0	0	-42.493	-37.64	-6.475	-1.862	-0.093	-5.661	0	0	-4.988
CD74	0	0	0	0	0	0	-8.642	-37.012	-1.217	-1.089	-0.001	0	0	-17.193	-7.211
CCR8	0	0	0	0	0	0	-29.292	-36.056	-1.86	-0.433	-0.001	-1.64	0	0	-2.288
RAMP3	0	0	0	0	0	0	-0.562	-36.056	-0.069	0	-0.001	0	0	0	-1.282
CRTAM	0	0	0	0	0	0	-0.457	-35.199	-0.223	0	-0.001	-2.397	0	0	-9.187
SLC2A6	0	0	0	0	0	0	-0.86	-35.046	-0.559	-0.021	-0.001	0	0	-0.157	-4.533
TNFSF8	-1.234	0	-2.538	0	0	0	0	-34.906	0	0	-0.001	0	0	0	-7.213
BHLHE40	0	0	0	0	0	-0.301	-32.471	-34.275	-4.262	-0.569	-0.125	-4.679	0	0	-1.518
1700019D03RI	0	0	0	0	0	0	-13.97	-32.226	-2.363	-0.185	-0.001	-0.788	0	0	-9.608

	adj.pval.cluster 1	adj.pval.cluster 2	adj.pval.cluster 3	adj.pval.cluster 4	adj.pval.cluster 5	adj.pval.cluster 6	adj.pval.cluster 7	adj.pval.cluster 8	adj.pval.cluster 9	adj.pval.cluster 10	adj.pval.cluster 11	adj.pval.cluster 12	adj.pval.cluster 13	adj.pval.cluster 14	adj.pval.cluster 15
K															
NRP1	0	0	0	0	0	0	-30.537	-30.902	-2.005	-4.848	0	-2.099	0	-3.231	-3.686
SPRY2	0	0	0	0	0	0	-18.137	-30.895	-2.979	-0.819	-0.001	-0.701	0	0	-3.881
GUCY1A3	0	0	0	0	0	0	-0.733	-29.136	-0.078	-0.249	-0.001	-0.203	0	0	-4.883
CXXC5	0	0	0	0	0	0	0	-26.956	0	-0.013	-0.001	0	0	0	-2.097
CCR7	-54.429	0	-33.406	0	0	0	0	-26.11	0	0	-0.001	-0.051	0	-3.52	-3.252
TBC1D4	0	0	0	0	0	0	-0.726	-25.601	-0.096	-0.012	-0.001	0	0	-18.345	-8.954
CD200	0	0	0	0	0	0	-21.601	-25.027	-0.568	-1.085	-0.001	-1.573	0	0	-7.798
PENK	0	0	0	0	0	0	-7.181	-24.963	-1.51	-0.82	-0.001	-0.206	0	0	-0.432
BCL6	0	0	0	0	0	0	0	-24.943	-0.049	0	-0.001	-0.107	0	-18.827	-4.745
SDC4	0	0	0	0	0	0	-5.069	-24.939	-0.048	-0.007	-0.001	0	0	-0.044	-2.261
LAG3	0	0	0	0	0	-10.838	-36.69	-24.857	-4.285	-9.648	-0.108	-4.982	0	0	-1.917
TNFRSF18	0	0	0	0	0	0	-16.98	-24.642	-1.505	-0.839	-0.001	-3.163	0	-0.591	-1.756
ITGB1	0	-2.378	0	0	-16.568	0	-0.385	-24.519	-2.107	-0.221	-0.001	0	0	-0.06	-5.316
TNFSF4	0	0	0	0	0	0	-20.871	-24.486	-3.686	-0.181	-0.001	-0.319	0	0	-6.935
CCL1	0	0	0	0	0	0	-11.492	-23.03	-0.853	-0.077	-0.001	-0.877	0	0	-1.692
DUSP4	0	0	0	0	0	0	-31.906	-22.596	-2.854	-4.287	-0.001	-9.348	0	0	-2.344
DAPL1	-8.031	0	-8.574	0	0	0	0	-22.015	0	0	-0.001	0	0	-5.26	-10.394
KIT	0	0	0	0	0	0	-40.679	-22.015	-0.725	-3.094	-0.001	-0.022	0	0	-0.121
FAM178B	0	0	0	0	0	0	-6.313	-21.751	-0.037	0	-0.001	0	0	0	-13.298
CD16	0	0	0	0	0	0	-	-	-0.39	-	-	-	0	0	-

	adj.pval.cluster 1	adj.pval.cluster 2	adj.pval.cluster 3	adj.pval.cluster 4	adj.pval.cluster 5	adj.pval.cluster 6	adj.pval.cluster 7	adj.pval.cluster 8	adj.pval.cluster 9	adj.pval.cluster 10	adj.pval.cluster 11	adj.pval.cluster 12	adj.pval.cluster 13	adj.pval.cluster 14	adj.pval.cluster 15
0							5.379	21.394		1.583	0.923	1.312			1.156
CD82	0	0	0	0	0	-1.578	-9.818	-21.225	-0.062	-0.582	-0.137	-8.247	0	0	-0.86
GRAMD1B	0	0	0	0	0	0	-0.624	-20.691	-3.117	-0.1	-0.114	-4.058	0	0	-3.445
FAM46A	0	0	0	0	0	0	-10.576	-19.874	-2.797	-0.944	-0.001	-1.282	0	0	-2.034
REL	0	0	0	0	0	0	-7.026	-19.474	-1.999	-0.32	-0.001	-8.761	0	0	-2.751
KLRK1	0	-2.058	0	0	-0.691	0	-11.367	-18.913	-1.273	-0.232	-0.001	-0.49	0	-1.307	-3.329
PLK2	0	0	0	0	0	0	-17.608	-18.471	-3.47	-0.019	-0.001	0	0	0	-0.214
CD9	0	0	0	0	0	0	-7.854	-18.364	-8.001	-0.13	-0.001	-0.732	0	-0.374	-6.112
TNFSF14	0	-0.387	0	0	0	0	0	-17.998	0	0	-0.001	-15.183	0	0	-3.254
TRAF1	0	0	0	0	0	0	-11.745	-17.992	-1.584	0	-0.246	-0.741	0	-0.181	-1.331
ZC3H12D	0	0	0	0	0	0	-3.017	-17.992	-2.226	0	-0.001	-1.307	0	-1.216	-3.317
SDF4	0	0	0	0	0	0	-12.513	-17.847	-1.981	-0.432	-0.001	-1.699	0	0	-1.163
PTPRS	0	0	0	0	0	0	-25.035	-17.249	-3.841	-1.861	-0.001	-13.526	0	-1.475	-5.062
SH3BGRL	0	0	0	0	0	0	-20.969	-17.143	-11.59	-6.456	-0.001	-0.25	0	0	-2.387
SSH1	0	0	0	0	0	0	-6.551	-16.979	-2.032	0	-0.001	-0.067	0	0	-1.857
NRGN	0	0	0	0	0	0	-35.762	-16.934	-6.835	-1.884	-0.001	0	-0.069	0	-3.422
NFKBIA	0	0	0	0	0	0	-2.701	-16.891	-0.07	0	-0.346	-11.345	0	-0.623	-2.116
NFAT5	0	0	0	0	0	0	-12.415	-16.854	-2.562	-1.357	-0.001	-1.66	-0.528	0	-2.986
CAPG	0	0	0	0	0	0	-31.567	-16.812	-3.977	-1.984	-0.001	0	0	-0.408	-2.146
TSPAN32	0	0	0	0	0	0	-4.996	-16.662	-4.315	-0.328	-0.001	0	0	0	-6.298

	adj.pval.cluster 1	adj.pval.cluster 2	adj.pval.cluster 3	adj.pval.cluster 4	adj.pval.cluster 5	adj.pval.cluster 6	adj.pval.cluster 7	adj.pval.cluster 8	adj.pval.cluster 9	adj.pval.cluster 10	adj.pval.cluster 11	adj.pval.cluster 12	adj.pval.cluster 13	adj.pval.cluster 14	adj.pval.cluster 15
DCLK1	0	0	0	0	0	0	-7.663	-16.465	-0.155	-0.109	-0.001	0	0	0	-4.026
PDCD1	0	0	0	0	0	-12.311	-19.612	-16.441	-4.73	-10.914	-0.108	-0.747	0	0	-2.191
CD70	0	0	0	0	0	0	-11.464	-16.414	-0.688	-0.21	-0.001	-0.472	0	0	-8.022
IL2RA	0	0	0	0	0	0	-12.37	-16.072	-0.42	0	-0.001	-18.953	0	-4.167	-3.093
SLC17A6	0	0	0	0	0	0	-3.633	-15.953	-0.111	0	-0.046	-1.909	0	0	-4.662
LTA	0	0	0	0	0	0	-1.15	-15.777	-0.004	-0.024	-0.001	-0.611	0	0	-2.925
2310001H17RIK	0	0	0	0	0	0	-0.006	-15.775	-1.097	-0.04	-0.127	-0.203	0	0	-8.022
ARAP2	0	0	-0.024	0	0	0	-0.177	-15.632	-0.022	-0.108	-0.001	-4.204	0	0	-0.485
KLRC1	0	0	0	0	-0.71	0	-22.062	-15.365	-0.951	-1.67	-0.001	-2.988	0	-0.825	-0.612
NDFIP1	0	0	0	0	0	-6.306	-9.414	-15.255	-1.111	-0.182	-0.363	-8.984	0	0	-0.4
TMEM173	0	0	0	0	0	0	-11.951	-15.191	-4.45	-0.62	-0.108	-0.26	0	-2.827	-1.27
TMEM180	0	0	0	0	0	0	-14.371	-15.057	-1.516	-0.671	-0.001	-0.012	0	0	-2.691
TIGIT	0	0	0	-0.592	0	-12.815	-21.13	-14.863	-2.042	-6.684	-0.001	-2.558	0	0	-1.039
MRPS6	0	0	0	0	0	0	-10.613	-14.806	-8.826	-4.719	-0.494	-4.025	0	0	-5.964
MS4A4C	0	0	-2.75	0	-2.782	0	0	-14.793	0	0	-0.142	0	0	-4.832	-2.582
GABARAPL1	0	0	0	0	0	0	-17.973	-14.714	-6.467	-3.276	-0.001	-0.237	0	0	-4.419
DUSP1	0	-1.807	0	0	0	0	-0.715	-14.698	-0.091	-0.257	-0.001	0	0	-0.46	-0.704
RABGAP1L	0	0	0	0	0	0	-20.528	-14.584	-6.418	-3.152	-0.001	-0.426	0	-0.038	-1.191
BCL2A1D	0	0	0	0	0	-0.653	-23.195	-14.247	-6.435	-3.423	-0.159	-10.26	0	-0.06	-2.176
SLC1	0	0	0	0	0	0	-	-	-4.89	-	-	0	0	0	-

	adj.pval.cluster 1	adj.pval.cluster 2	adj.pval.cluster 3	adj.pval.cluster 4	adj.pval.cluster 5	adj.pval.cluster 6	adj.pval.cluster 7	adj.pval.cluster 8	adj.pval.cluster 9	adj.pval.cluster 10	adj.pval.cluster 11	adj.pval.cluster 12	adj.pval.cluster 13	adj.pval.cluster 14	adj.pval.cluster 15
6A11							38.005	14.163		1.423	0.001				1.047
PTPRK	0	0	0	0	0	0	-2.189	-14.03	-1.059	-0.027	-0.001	-0.734	0	-0.269	-0.989
NR4A3	0	0	0	0	0	0	-10.379	-14.001	-1.716	-0.936	-0.001	-1.897	0	0	-1.576
AXL	0	0	0	0	0	0	-5.289	-13.839	-1.299	-0.194	-0.001	-0.029	0	0	-2.65
DUSP14	0	0	0	0	0	0	-16.063	-13.49	-1.658	-2.67	-0.001	-0.159	0	0	-4.806
FAM162A	0	0	0	0	0	0	-12.924	-13.483	-9.985	-6.418	-0.159	-4.136	0	-0.059	-1.398
LANCL1	0	0	0	0	0	0	-5.749	-13.483	-4.907	-2.058	-0.001	0	0	0	-2.346
SCYL2	0	0	0	0	0	0	-8.356	-13.271	-4.874	-0.362	-0.001	0	0	0	-0.504
OSTF1	0	0	0	0	0	0	-16.818	-13.223	-2.754	-0.26	-0.001	0	0	0	-1.322
PLSCR1	0	0	0	0	0	0	-25.165	-13.217	-8.918	-1.544	-0.08	-3.263	0	0	-2.864
EEA1	0	0	0	0	0	0	-25.035	-13.021	-4.131	-2.609	-0.001	-6.371	0	0	-1.547
CALCOCO1	0	0	0	0	0	0	-2.727	-12.968	-0.014	0	-0.001	0	0	0	-1.929
KLRD1	0	0	0	0	0	0	-11.474	-12.835	-0.521	-0.002	-0.001	0	0	-1.221	-0.417
SEMA4C	0	0	0	0	0	0	-12.592	-12.74	-1.81	-0.087	-0.001	-2.399	0	0	-1.003
GM17745	0	0	0	0	0	0	-17.498	-12.714	-8.41	-0.208	-0.001	0	0	0	-2.315
HIF1A	0	0	0	0	0	0	-11.55	-12.65	-5.468	-3.819	-0.001	-3.236	0	0	-1.165
ASNSD1	0	0	0	0	0	0	-10.228	-12.642	-8.015	-3.68	-0.001	-1.692	0	0	-2.151
IL18R1	0	0	0	0	-0.23	0	-11.684	-12.642	-0.356	0	-0.001	-0.216	0	-0.458	-2.815
SAT1	0	0	0	0	0	-0.246	-2.947	-12.499	-0.328	0	-0.123	0	0	-0.577	-1.062
RAB37	0	0	0	0	-9.038	0	0	-12.41	-0.254	0	-0.001	0	0	0	-0.829
SLAMF6	-0.287	0	-2.279	0	-5.378	0	0	-12.355	-0.024	0	-0.001	0	0	-2.727	-5.477

Table 10. Cluster 9 Specific Gene Signature

	0	7	8	10	rank_0	rank_7	rank_8	rank_10	mean_rank
CCNB2	-179.56	-51.664	-44.528	-34.919	1	6	3	2	3
CDCA8	-158.625	-55.88	-45.215	-23.277	6	1	1	9	4.25
CDC20	-175.23	-51.349	-42.164	-29.065	3	8	6	3	5
CDCA3	-178.094	-52.286	-38.435	-26.01	2	5	11	6	6
KIF20A	-154.631	-48.789	-39.127	-18.853	8	11	10	13	10.5
CKS1B	-144.648	-43.681	-40.952	-23.893	17	16	7	8	12
CCNA2	-173.875	-54.064	-42.24	-12.412	4	3	5	36	12
PLK1	-166.042	-47.328	-33.924	-18.523	5	14	22	15	14
TACC3	-141.435	-47.916	-37.916	-18.853	19	13	12	14	14.5
MKI67	-147.761	-51.664	-39.364	-13.34	12	7	8	32	14.75
BIRC5	-95.888	-53.595	-44.432	-28.504	53	4	4	5	16.5
HMGB2	-83.804	-54.823	-45.213	-24.98	64	2	2	7	18.75
TPX2	-100.118	-50.085	-39.209	-18.489	44	9	9	16	19.5
UBE2C	-103.176	-43.227	-37.295	-29.065	43	18	14	4	19.75
KIF22	-148.115	-48.136	-32.069	-13.443	11	12	27	31	20.25
FAM64A	-154.576	-41.671	-32.052	-15.867	9	22	28	23	20.5
NEK2	-157.543	-39.398	-31.625	-16.275	7	29	29	22	21.75
CCNB1	-140.302	-39.431	-31.289	-19.096	20	28	30	12	22.5
CEP55	-144.648	-42.701	-30.625	-14.675	16	19	33	25	23.25
CENPA	-98.818	-37.574	-35.626	-36.54	48	34	20	1	25.75
BUB1B	-147.538	-36.302	-32.525	-13.874	13	39	25	29	26.5
RACGAP1	-71.392	-48.815	-37.5	-18.362	75	10	13	17	28.75
KNSTRN	-122.541	-35.023	-31.096	-16.976	28	43	31	20	30.5
NUSAP1	-146.985	-41.727	-31.013	-9.861	15	21	32	54	30.5
TUBB4B	-70.217	-39.693	-35.915	-22.291	77	26	19	10	33
CDKN3	-147.353	-30.413	-25.753	-16.819	14	56	47	21	34.5
HMGN2	-71.595	-41.762	-35.977	-14.236	74	20	17	27	34.5
KIF23	-121.029	-38.856	-28.209	-12.459	30	33	40	35	34.5
CKAP2L	-148.887	-39.005	-28.955	-8.454	10	31	37	61	34.75
CENPE	-129.908	-36.68	-25.386	-12.227	23	37	48	37	36.25
KIF2C	-131.562	-35.795	-26.003	-11.967	22	41	45	39	36.75
CKS2	-76.204	-39.652	-27.946	-17.346	68	27	41	19	38.75
CKAP2	-123.937	-29.319	-27.238	-13.808	26	62	42	30	40
BUB1	-142.772	-40.064	-28.797	-5.397	18	25	38	86	41.75
H2AFZ	-68.778	-43.399	-28.257	-11.967	79	17	39	38	43.25
TUBA1C	-63.159	-34.361	-30.271	-20.656	90	46	34	11	45.25
KIF4	-128.784	-34.735	-24.757	-8.059	24	45	52	64	46.25
TUBA1B	-64.686	-40.286	-37.163	-8.299	85	24	16	63	47
TUBB5	-53.949	-44.478	-35.92	-10.101	107	15	18	49	47.25
NCAPD2	-110.146	-34.779	-34.085	-5.006	36	44	21	92	48.25
SAPCD2	-120.02	-28.864	-23.363	-10.797	31	63	55	46	48.75
AURKB	-135.847	-38.959	-26.877	-3.947	21	32	44	108	51.25
AURKA	-99.39	-30.476	-20.601	-11.219	46	55	67	42	52.5
REEP4	-67.915	-33.507	-25.248	-12.858	80	48	51	34	53.25
GTSE1	-123.444	-31.905	-26.001	-5.322	27	51	46	89	53.25
ECT2	-105.291	-29.705	-23.123	-7.607	40	59	58	67	56
ASPM	-121.198	-26.672	-19.798	-8.457	29	67	75	60	57.75
SKA1	-114.202	-30.339	-24.663	-5.397	34	57	53	87	57.75
CDK1	-105.176	-36.452	-29.529	-3.686	41	38	36	118	58.25
PARPBP	-105.457	-28.232	-20.388	-8.597	39	65	71	59	58.5
MAD2L1	-115.359	-37.046	-32.437	-3.258	32	36	26	142	59
CDC25C	-99.915	-25.925	-19.798	-10.315	45	69	76	48	59.5
H2AFV	-48.503	-37.385	-20.591	-18.004	118	35	69	18	60
DEPDC1A	-124.747	-29.772	-22.719	-4.302	25	58	61	102	61.5
MELK	-112.089	-30.911	-23.29	-4.146	35	52	56	104	61.75
CENPF	-56.405	-28.447	-25.284	-13.081	103	64	50	33	62.5

	0	7	8	10	rank_0	rank_7	rank_8	rank_10	mean_rank
TROAP	-108.467	-25.697	-17.046	-10.079	38	70	90	52	62.5
KIF11	-74.302	-33.049	-27.196	-5.089	70	50	43	90	63.25
SHCBP1	-109.76	-29.345	-22.73	-4.815	37	61	60	98	64
CDC25B	-50.288	-33.801	-20.482	-13.883	112	47	70	28	64.25
NDC80	-90.692	-33.228	-25.29	-4.104	59	49	49	105	65.5
NUF2	-73.529	-35.179	-21.721	-4.98	71	42	62	94	67.25
SPAG5	-104.263	-26.243	-21.481	-4.847	42	68	64	97	67.75
ARHGAP19	-97.172	-23.112	-17.625	-9.41	51	79	86	56	68
HMGB1	-58.399	-36.091	-37.295	-3.463	99	40	15	127	70.25
1190002F15RIK	-94.177	-22.019	-19.592	-6.909	54	81	79	68	70.5
H2AFX	-59.392	-29.394	-20.373	-9.136	95	60	72	57	71
SPC25	-114.751	-30.777	-23.961	-3.109	33	54	54	146	71.75
PIF1	-98.927	-20.048	-14.463	-9.6	47	86	102	55	72.5
FOXM1	-91.42	-25.188	-17.754	-5.6	57	72	85	81	73.75
SPC24	-65.249	-39.398	-33.678	-2.755	83	30	23	167	75.75
HMMR	-39.329	-24.63	-19.969	-14.675	130	75	74	26	76.25
DLGAP5	-51.509	-24.614	-23.264	-6.857	111	76	57	69	78.25
SGOL1	-98.207	-30.824	-21.481	-2.931	50	53	63	159	81.25
KPNA2	-63.979	-18.154	-20.6	-6.219	88	94	68	76	81.5
C330027C09RIK	-90.854	-23.374	-19.705	-3.778	58	78	78	115	82.25
SKA2	-85.065	-24.456	-19.798	-3.895	63	77	77	113	82.5
PRC1	-58.811	-25.407	-20.996	-4.613	98	71	66	99	83.5
ESPL1	-86.403	-22.165	-17.536	-4.088	62	80	87	106	83.75
ARHGAP11A	-87.862	-24.762	-21.088	-3.381	61	74	65	135	83.75
CKAP5	-54.057	-16.44	-17.31	-11.099	106	104	88	45	85.75
HMGB3	-96.663	-15.565	-14.816	-5.439	52	109	99	84	86
MIS18BP1	-91.908	-21.308	-16.521	-3.636	56	83	92	120	87.75
INCENP	-75.685	-21.311	-19.586	-3.405	69	82	80	130	90.25
HIST1H2AO	-92.506	-40.899	-33.536	-1.579	55	23	24	273	93.75
CEP89	-59.75	-13.438	-11.499	-11.197	93	119	121	43	94
MXD3	-83.12	-17.893	-13.103	-4.373	65	96	114	101	94
2700094K13RIK	-52.648	-15.868	-18.948	-5.532	109	108	82	83	95.5
CENPW	-90.096	-17.24	-20.059	-2.968	60	99	73	155	96.75
RAD21	-52.375	-15.188	-15.669	-6.326	110	110	95	75	97.5
GPSM2	-59.261	-16.018	-12.996	-5.798	96	106	116	79	99.25
TUBA1A	-25.055	-18.888	-13.555	-15.531	182	90	109	24	101.25
ODF2	-55.174	-11.337	-10.685	-11.651	104	133	133	41	102.75
FAM83D	-81.828	-19.462	-15.053	-2.777	66	87	97	165	103.75
TMPO	-62.994	-17.441	-10.3	-4.408	91	98	134	100	105.75
NUCKS1	-44.767	-18.158	-12.544	-4.858	123	93	118	96	107.5
KIF20B	-57.473	-16.967	-11.644	-3.688	101	101	119	117	109.5
ANP32E	-40.347	-20.145	-17.306	-3.36	129	85	89	136	109.75
HN1	-23.019	-25.105	-10.937	-11.189	196	73	129	44	110.5
NEIL3	-98.245	-27.947	-19.277	-1.789	49	66	81	246	110.5
FZR1	-57.498	-10.914	-8.354	-10.101	100	137	155	51	110.75
ARL6IP1	-27.494	-12.621	-14.564	-9.973	170	124	100	53	111.75
SPDL1	-64.603	-14.996	-9.935	-3.94	86	111	141	109	111.75
NDE1	-42.082	-12.351	-11.332	-6.364	128	126	124	72	112.5
POC1A	-73.514	-14.316	-13.474	-3.009	72	114	110	154	112.5
CALM3	-45.058	-9.314	-11.208	-8.454	121	148	128	62	114.75
CLIC1	-25.633	-19.151	-11.311	-6.364	179	89	126	73	116.75
ZWILCH	-69.398	-21.092	-16.514	-2.025	78	84	93	213	117
KIF14	-48.505	-13.826	-9.062	-3.988	117	116	149	107	122.25
DDX39	-31.017	-10.898	-18.359	-3.702	151	138	84	116	122.25
LSM5	-47.57	-14.966	-11.481	-3.314	119	112	122	137	122.5
BUB3	-33.012	-14.96	-13.952	-3.538	147	113	105	126	122.75
HIST1H2BC	-58.831	-7.843	-6.778	-8	97	159	175	65	124
2610318N02RIK	-43.8	-9.318	-8.442	-6.506	127	147	154	71	124.75
C920025E04RIK	-35.554	-6.841	-9.916	-10.101	141	169	142	50	125.5
1500009L16RIK	-44.509	-7.953	-13.883	-3.581	124	158	107	124	128.25

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BORA	-48.966	-11.156	-11.397	-3.294	114	136	123	140	128.25
PLK4	-80.305	-16.181	-17	-1.78	67	105	91	250	128.25
CIT	-71.676	-18.38	-15.037	-1.725	73	92	98	255	129.5
MIIP	-24.876	-13.14	-6.786	-11.742	185	121	173	40	129.75
SEC11C	-30.2	-6.343	-14.501	-5.388	156	179	101	88	131
CCNF	-64.549	-18.524	-14.342	-1.741	87	91	104	254	134
PRR11	-20.159	-11.311	-11.292	-7.85	213	135	127	66	135.25
HIST1H2AB	-61.483	-17.079	-13.438	-1.821	92	100	112	238	135.5
HJURP	-56.727	-16.886	-13.441	-1.9	102	102	111	228	135.75
RANGAP1	-33.852	-11.828	-11.318	-3.057	143	127	125	150	136.25
RDM1	-48.712	-7.55	-5.33	-6.1	116	162	201	77	139
CALM2	-20.848	-14.029	-8.044	-5.803	209	115	156	78	139.5
GEN1	-65.12	-17.772	-13.072	-1.597	84	97	115	271	141.75
PFN1	-25.71	-10.358	-30.222	-1.998	178	141	35	216	142.5
HIST1H1C	-31.197	-5.199	-9.801	-5.727	150	198	144	80	143
DBF4	-27.525	-11.682	-13.936	-2.707	169	128	106	169	143
CENPN	-71.122	-19.191	-10.712	-1.543	76	88	132	283	144.75
CENPL	-30.883	-13.439	-8.635	-2.919	152	118	152	160	145.5
TTK	-66.79	-15.961	-14.463	-1.475	81	107	103	295	146.5
DSN1	-65.912	-17.963	-16.044	-1.359	82	95	94	319	147.5
CENPP	-59.459	-12.482	-12.595	-1.677	94	125	117	259	148.75
SMTN	-44.39	-6.511	-6.524	-3.611	125	175	180	122	150.5
DIAP3	-63.174	-13.019	-13.797	-1.497	89	122	108	293	153
EMP3	-25.041	-10.005	-7.301	-3.583	183	143	166	123	153.75
HIST1H2AG	-46.006	-11.439	-8.498	-2.025	120	130	153	214	154.25
ARHGEF39	-27.823	-8.673	-5.839	-3.94	167	154	189	110	155
HP1BP3	-20.499	-12.715	-4.367	-8.72	212	123	229	58	155.5
UBE2S	-43.857	-9.929	-15.508	-1.635	126	144	96	265	157.75
CMTM7	-38.507	-5.649	-22.958	-1.744	131	190	59	253	158.25
H2-T22	-22.185	-7.37	-10.002	-3.405	201	164	138	131	158.5
CENPT	-36.817	-6.544	-9.492	-2.316	136	174	145	183	159.5
CDKN2D	-14.34	-13.43	-4.766	-10.722	258	120	215	47	160
TMEM97	-52.986	-6.193	-10.182	-1.932	108	180	135	224	161.75
NUDCD2	-37.492	-3.326	-7.934	-3.87	135	243	158	114	162.5
IQGAP3	-36.412	-9.13	-5.307	-2.875	137	150	202	161	162.5
PIH1D1	-34.796	-6.563	-8.927	-2.164	142	173	151	193	164.75
LBR	-14.413	-16.766	-18.92	-1.996	256	103	83	217	164.75
CEP72	-36.083	-10.604	-6.784	-2.04	139	139	174	210	165.5
UEVLD	-32.049	-4.592	-6.423	-3.425	149	209	183	128	167.25
AP1M1	-15.611	-9.63	-7.941	-3.62	248	145	157	121	167.75
TRAIP	-49.163	-13.754	-10.937	-1.329	113	117	130	321	170.25
SGOL2	-15.199	-11.435	-10.889	-2.536	252	132	131	174	172.25
SHC1	-25.063	-6.15	-6.577	-3.064	181	181	179	149	172.5
TNFAIP8L1	-17.116	-8.092	-5.008	-5.074	236	157	208	91	173
CARHSP1	-28.724	-8.146	-6.969	-2.094	164	156	169	203	173
OAT	-35.699	-6.088	-9.138	-1.96	140	182	147	223	173
G2E3	-27.898	-7.416	-6.027	-2.482	166	163	188	176	173.25
1700097N02RIK	-33.179	-5.269	-4.818	-3.313	146	197	214	138	173.75
HIST1H1B	-44.804	-11.316	-9.967	-1.412	122	134	140	304	175
STK38	-9.11	-9.367	-6.383	-6.353	296	146	185	74	175.25
MNS1	-36.257	-10.296	-6.589	-1.789	138	142	178	247	176.25
KIFC1	-54.086	-11.438	-7.707	-1.383	105	131	161	312	177.25
CEP70	-37.856	-5.782	-4.427	-2.816	134	188	225	163	177.5
ACSL5	-30.6	-5.184	-6.033	-2.564	155	199	187	173	178.5
KIFC5B	-33.355	-9.146	-6.928	-1.648	145	149	170	261	181.25
MTMR14	-22.063	-4.296	-5.167	-3.903	202	211	204	111	182
HMGN5	-48.906	-4.671	-7.466	-1.794	115	204	165	245	182.25
ANAPC5	-24.624	-8.999	-13.349	-1.548	186	152	113	279	182.5
0610010K14RIK	-24.245	-7.769	-3.998	-3.226	187	160	241	143	182.75
MAPRE1	-19.935	-6.597	-10.009	-2.032	218	172	137	211	184.5

	0	7	8	10	rank_0	rank_7	rank_8	rank_10	mean_rank
MED30	-28.753	-8.421	-9.889	-1.548	162	155	143	280	185
HIST1H1E	-28.537	-10.481	-7.905	-1.532	165	140	159	284	187
LDLR	-38.487	-1.54	-6.833	-3.296	132	308	172	139	187.75
PIK3CG	-27.733	-2.481	-10.178	-2.333	168	266	136	181	187.75
BRD8	-20.853	-5.71	-4.252	-3.675	208	189	236	119	188
PSRC1	-30.878	-3.622	-5.154	-2.624	153	229	205	170	189.25
POC5	-21.709	-3.955	-5.458	-3.405	205	223	198	133	189.75
MRPL27	-24.168	-2.932	-7.629	-2.793	188	250	163	164	191.25
EFCAB11	-27.196	-6.407	-5.62	-1.93	173	178	196	226	193.25
PPP2R5D	-29.558	-4.634	-4.404	-2.278	158	206	226	186	194
CCDC61	-24.093	-4.112	-4.349	-3.088	189	216	231	147	195.75
TRIM59	-23.902	-6.429	-5.836	-1.904	190	176	190	227	195.75
SNRPG	-16.103	-8.946	-4.697	-2.618	246	153	217	171	196.75
TCEB2	-22.411	-6.642	-9.42	-1.597	200	171	146	272	197.25
SUN2	-8.493	-4.974	-5.52	-4.985	299	202	197	93	197.75
NUP37	-38.119	-4.61	-3.031	-2.26	133	208	264	189	198.5
SC5D	-28.748	-1.802	-9.982	-2.124	163	293	139	199	198.5
PPP1CA	-14.446	-6.904	-11.628	-1.722	255	168	120	256	199.75
C230052I12RIK	-30.674	-2.911	-7.739	-1.808	154	252	160	241	201.75
CENPC1	-32.875	-5.415	-7.115	-1.45	148	194	167	298	201.75
NRF1	-19.141	-6.029	-9.129	-1.65	223	183	148	260	203.5
EVI2B	-11.021	-3.529	-5.343	-4.302	281	233	200	103	204.25
PAGR1A	-29.15	-5.944	-4.999	-1.643	161	185	209	263	204.5
KCTD20	-21.121	-2.065	-6.611	-2.934	207	280	177	158	205.5
TRIOBP	-27.306	-3.49	-3.429	-2.76	171	235	253	166	206.25
ELOF1	-29.677	-4.003	-5.354	-1.787	157	221	199	248	206.25
APOBEC3	-19.039	-5.984	-2.162	-3.574	224	184	299	125	208
TRAF7	-19.947	-3.886	-4.326	-2.949	217	225	233	157	208
GNB2	-20.578	-2.483	-4.846	-3.19	211	265	213	144	208.25
RNF5	-26.006	-3.955	-7.662	-1.566	175	222	162	275	208.5
HIST2H4	-22.883	-7.738	-4.368	-1.78	197	161	228	251	209.25
CDC23	-27.167	-5.535	-6.407	-1.525	174	193	184	289	210
CDK2AP2	-4.208	-11.623	-1.586	-5.581	316	129	314	82	210.25
ADPRH	-18.119	-3.339	-2.376	-5.425	228	239	291	85	210.75
H2-Q4	-2.515	-6.423	-2.652	-6.545	319	177	283	70	212.25
H3F3B	-4.435	-7.047	-7.006	-2.117	314	167	168	200	212.25
PPP2R5C	-9.913	-5.639	-4.129	-3.405	289	191	238	134	213
RHNO1	-14.395	-5.568	-4.867	-2.144	257	192	212	196	214.25
PSMC1	-21.901	-4.119	-4.976	-1.834	203	215	210	236	216
DAP	-18.089	-2.2	-2.99	-4.865	229	275	268	95	216.75
CNIH4	-27.26	-2.4	-3.709	-2.406	172	270	247	179	217
SETD8	-33.467	-3.886	-2.885	-1.894	144	224	274	229	217.75
CCDC77	-24.941	-5.408	-3.197	-1.852	184	195	258	234	217.75
PSMB9	-18.089	-7.202	-2.509	-2.274	230	166	289	187	218
HIST1H3B	-25.75	-6.838	-4.27	-1.507	177	170	235	291	218.25
SDCBP	-5.81	-4.291	-7.543	-2.157	309	212	164	194	219.75
HIST3H2A	-10.108	-2.439	-5.774	-3.405	287	268	193	132	220
ATL2	-12.253	-4.452	-6.425	-1.93	272	210	182	225	222.25
CCDC163	-21.293	-2.88	-3.116	-2.613	206	253	261	172	223
IFT46	-22.614	-2.264	-1.608	-3.903	198	273	313	112	224
TMEM194	-20.035	-7.283	-5.202	-1.377	216	165	203	313	224.25
GM14005	-5.611	-4.101	-5.775	-2.397	310	218	192	180	225
CNEP1R1	-23.411	-2.568	-3.017	-2.283	193	261	266	185	226.25
MTMR12	-13.416	-3.328	-3.349	-3.151	264	242	257	145	227
DERL2	-25.844	-3.556	-6.445	-1.313	176	231	181	323	227.75
SSNA1	-25.53	-3.331	-6.755	-1.365	180	240	176	317	228.25
RSPH3A	-23.253	-3.328	-1.393	-2.847	194	241	320	162	229.25
DYNLL1	-19.811	-2.761	-4.14	-2.084	219	256	237	205	229.25
NGDN	-9.815	-2.665	-8.993	-1.986	291	258	150	220	229.75
H2-T10	-9.785	-5.871	-4.605	-1.963	292	187	220	221	230

	0	7	8	10	rank_0	rank_7	rank_8	rank_10	mean_rank
TAP1	-10.041	-4.205	-4.587	-2.117	288	214	222	201	231.25
ALDH16A1	-23.685	-3.738	-4.078	-1.602	192	226	240	269	231.75
ANAPC16	-16.673	-3.018	-2.293	-3.08	239	249	293	148	232.25
ING1	-19.615	-4.614	-5.781	-1.372	221	207	191	315	233.5
CNTROB	-16.429	-2.773	-6.902	-1.572	241	255	171	274	235.25
2210039B01RIK	-2.28	-9.036	-1.363	-3.057	320	151	321	151	235.75
STAT2	-9.41	-3.649	-3.855	-2.431	294	228	244	178	236
HIST1H2BK	-29.32	-3.373	-3.946	-1.4	160	237	242	305	236
GM2382	-16.148	-3.622	-3.417	-1.961	245	230	255	222	238
SH3BP2	-23.159	-1.423	-1.557	-3.414	195	315	315	129	238.5
MND1	-17.959	-3.37	-3.695	-1.829	231	238	248	237	238.5
CDK19	-11.233	-5.355	-4.905	-1.598	278	196	211	270	238.75
BBIP1	-11.041	-3.679	-3.722	-2.047	280	227	246	209	240.5
ERCC1	-16.89	-1.322	-4.72	-2.251	237	321	216	190	241
CYBASC3	-14.311	-1.442	-5.026	-2.196	259	312	207	191	242.25
CHKB	-12.206	-4.237	-2.603	-2.142	273	213	287	197	242.5
DDX52	-20.098	-1.39	-4.326	-2.091	214	318	234	204	242.5
SERINC3	-17.435	-3.224	-5.71	-1.462	235	245	194	296	242.5
VBP1	-20.79	-1.859	-3.533	-1.996	210	291	252	218	242.75
INO80C	-19.809	-2.532	-2.052	-2.266	220	264	301	188	243.25
CEP57L1	-15.391	-4.073	-2.889	-1.869	250	219	273	232	243.5
2310036O22RIK	-17.772	-1.828	-6.341	-1.648	234	292	186	262	243.5
MDM1	-22.593	-5.9	-2.837	-1.377	199	186	275	314	243.5
GMPR2	-17.82	-1.979	-2.78	-2.441	232	287	279	177	243.75
C2CD5	-23.822	-3.535	-3.361	-1.44	191	232	256	299	244.5
CARS	-21.836	-1.56	-4.36	-1.803	204	304	230	243	245.25
NDUFA2	-15.15	-2.063	-2.213	-3.055	253	281	296	152	245.5
GM6682	-12.649	-5.151	-2.105	-2.017	267	201	300	215	245.75
COG6	-18.949	-1.704	-2.541	-2.535	225	297	288	175	246.25
STAP1	-29.431	-1.441	-2.049	-2.026	159	313	303	212	246.75
OPA3	-12.353	-2.241	-5.133	-1.819	271	274	206	239	247.5
DNAHC8	-3.074	-3.437	-2.186	-3.286	318	236	297	141	248
CPT1A	-16.502	-3.294	-3.779	-1.614	240	244	245	267	249
NLRC3	-10.326	-2.004	-2.802	-3.021	286	286	277	153	250.5
HYLS1	-9.636	-4.103	-2.614	-2.049	293	217	285	208	250.75
IRF9	-3.594	-2.652	-3.182	-2.755	317	259	260	168	251
EMC9	-8.181	-3.116	-2.89	-2.302	300	248	272	184	251
TMUB1	-10.895	-2.313	-3.422	-2.149	284	272	254	195	251.25
FAM126A	-12.79	-1.517	-4.471	-2.052	266	310	224	207	251.75
CEP19	-18.743	-1.433	-1.434	-2.962	226	314	317	156	253.25
FBXL8	-12.094	-3.516	-2.92	-1.808	275	234	271	242	255.5
NFYC	-14.802	-2.562	-2.83	-1.882	254	262	276	231	255.75
PAPOLA	-16.383	-1.628	-4.593	-1.682	242	302	221	258	255.75
2810428I15RIK	-17.78	-2.146	-4.328	-1.548	233	278	232	281	256
PLEKHG3	-12.062	-4.638	-2.987	-1.532	276	205	269	287	259.25
MPP1	-13.843	-3.197	-2.608	-1.797	262	246	286	244	259.5
BUD31	-16.752	-2.78	-3.029	-1.532	238	254	265	286	260.75
PEA15A	-7.438	-1.54	-3.549	-2.319	302	309	251	182	261
SLC25A38	-12.994	-1.306	-3.593	-2.072	265	324	249	206	261
OAZ1-PS	-8.51	-4.004	-4.496	-1.394	298	220	223	310	262.75
PEX7	-20.088	-1.621	-4.08	-1.397	215	303	239	307	266
STK19	-16.312	-1.93	-2.051	-1.863	243	289	302	233	266.75
ATG4D	-12.381	-1.693	-2.996	-1.835	270	298	267	235	267.5
CD48	-11.349	-2.453	-2.791	-1.778	277	267	278	252	268.5
PNRC2	-13.929	-1.751	-2.673	-1.819	261	295	282	240	269.5
SAP130	-12.496	-2.912	-3.55	-1.396	269	251	250	309	269.75
MVD	-14.252	-1.309	-5.641	-1.414	260	323	195	303	270.25
4921524J17RIK	-18.695	-2.313	-2.177	-1.532	227	271	298	288	271
EHMT2	-16.296	-1.472	-4.651	-1.392	244	311	219	311	271.25
2610044O15RIK8	-6.656	-2.152	-4.376	-1.557	306	277	227	277	271.75

	0	7	8	10	rank_0	rank_7	rank_8	rank_10	mean_rank
SEPT7	-15.362	-1.708	-4.669	-1.319	251	296	218	322	271.75
USF1	-10.99	-1.553	-1.876	-2.178	282	306	308	192	272
PXMP4	-6.837	-1.316	-2.684	-2.129	304	322	281	198	276.25
RPPH1	-13.43	-2.046	-2.24	-1.635	263	282	295	266	276.5
ANKRD50	-11.228	-2.734	-1.357	-1.787	279	257	322	249	276.75
GM5860	-1.615	-4.941	-1.878	-1.553	323	203	307	278	277.75
ARID3B	-1.333	-2.583	-3.082	-1.614	324	260	262	268	278.5
PPP1R10	-15.738	-1.56	-2.953	-1.505	247	305	270	292	278.5
R3HCC1L	-8.716	-2.026	-1.548	-1.996	297	283	316	219	278.75
PRR14	-10.956	-2.56	-1.417	-1.717	283	263	318	257	280.25
ZFP414	-19.25	-1.421	-1.402	-1.639	222	316	319	264	280.25
EAPP	-12.114	-1.657	-3.057	-1.532	274	301	263	285	280.75
CTDSP2	-5.463	-5.159	-1.356	-1.525	311	200	323	290	281
UGDH	-12.59	-1.769	-2.389	-1.559	268	294	290	276	282
IGTP	-1.624	-2.007	-1.336	-2.101	322	285	324	202	283.25
SPSB3	-9.113	-3.123	-2.259	-1.459	295	247	294	297	283.25
STARD3	-15.429	-2.152	-1.799	-1.362	249	276	309	318	288
VRK3	-4.857	-2.084	-2.708	-1.548	312	279	280	282	288.25
RBBP6	-9.841	-1.667	-3.193	-1.4	290	300	259	306	288.75
RGS14	-6.176	-1.546	-1.688	-1.884	307	307	312	230	289
CHTOP	-6.782	-1.406	-3.913	-1.371	305	317	243	316	295.25
ATF7IP	-4.24	-2.434	-1.984	-1.415	315	269	305	302	297.75
ARRDC3	-6.058	-1.685	-2.322	-1.476	308	299	292	294	298.25
ZFP748	-7.427	-1.932	-1.713	-1.44	303	288	311	300	300.5
SERTAD3	-7.733	-1.906	-1.736	-1.427	301	290	310	301	300.5
PCIF1	-10.478	-1.326	-2.637	-1.336	285	320	284	320	302.25
TAGAP1	-4.681	-2.007	-1.903	-1.397	313	284	306	308	302.75
CRLF3	-2.123	-1.36	-2.035	-1.306	321	319	304	324	317

Table 11. Ranked top transcription factors differentially expressed in cluster 9

Gene	TP	TN	thresh_mhg	hyper_pval	hyper_qval	gen_qval	rank_hyper_qval	rank_gen_qval	mean_rank
HMGB2	0.921568627	0.926502146	9.084	6.23E-122	2.69E-119	-83.608	1	3	2
FOXM1	0.477124183	0.969420601	2.57	1.94E-54	2.09E-52	-96.119	4	1	2.5
HMGB3	0.849673203	0.792381974	2.087	1.15E-58	2.48E-56	-67.08	2	4	3
MXD3	0.366013072	0.986051502	4.405	1.75E-47	1.26E-45	-84.852	6	2	4
HMGB1	0.888888889	0.749463519	9.01	4.20E-57	6.03E-55	-55.462	3	5	4
PMF1	0.843137255	0.763948498	2.878	1.36E-51	1.17E-49	-55.018	5	6	5.5
RAD54B	0.450980392	0.931330472	0.227	8.98E-34	4.30E-32	-51.036	9	7	8
PTMA	0.816993464	0.755901288	11.115	9.50E-46	5.85E-44	-42.495	7	9	8
TRIP13	0.437908497	0.932939914	0.556	1.61E-32	6.94E-31	-48.63	10	8	9
UHRF1	0.686274501	0.803648069	2.42	1.41E-35	7.59E-34	-35.788	8	10	9
WHSC1	0.705882353	0.724248927	0.214	5.75E-26	1.91E-24	-26.048	13	11	12
MED30	0.810457516	0.675429185	2.718	2.41E-32	9.45E-31	-23.986	11	13	12
ILF2	0.764705882	0.690450644	0.299	1.76E-28	6.33E-27	-22.543	12	14	13
TCF19	0.4640522	0.875	0.757	2.86E-22	4.74E-21	-24.957	26	12	19

Gene	TP	TN	thresh_mhg	hyper_pval	hyper_qval	gen_qval	rank_hyper_qval	rank_gen_qval	mean_rank
	88								
NFYB	0.673202614	0.738197425	2.763	3.55E-24	8.06E-23	-19.398	19	20	19.5
RNF5	0.633986928	0.759120172	1.091	9.84E-23	1.70E-21	-20.356	25	17	21
PHF5A	0.816993464	0.614806867	1.043	7.77E-26	2.39E-24	-17.113	14	29	21.5
DEK	0.908496732	0.474785408	3.866	3.39E-23	6.36E-22	-19.216	23	21	22
CDCA4	0.686274511	0.720493562	4.167	2.25E-23	4.41E-22	-18.414	22	23	22.5
GTF2F1	0.830065359	0.593347639	1.623	4.36E-25	1.25E-23	-16.908	15	30	22.5
GTF2H5	0.751633987	0.657188841	3.38	4.70E-23	8.44E-22	-17.751	24	25	24.5
HCFC1	0.862745098	0.549356223	0.111	1.55E-24	3.93E-23	-15.818	17	34	25.5
RBBP8	0.535947712	0.807403433	0.287	2.66E-19	3.02E-18	-20.872	38	15	26.5
BRD8	0.607843137	0.774141631	2.969	5.28E-22	8.43E-21	-17.417	27	26	26.5
HDAC1	0.856209155	0.558476395	6.419	1.19E-24	3.22E-23	-15.235	16	38	27
EZH2	0.549019608	0.799356223	0.299	1.68E-19	1.95E-18	-19.409	37	19	28
ERH	0.973856209	0.376609442	3.926	2.66E-24	6.38E-23	-14.092	18	44	31
COMMD3	0.823529412	0.591201717	1.485	4.44E-24	9.58E-23	-14.081	20	45	32.5
DNMT1	0.732026144	0.660407725	3.834	2.42E-21	3.48E-20	-15.63	30	37	33.5
CBX3	0.973856209	0.316523605	3.941	5.57E-19	5.71E-18	-17.185	42	27	34.5
ANAPC11	0.803921569	0.591201717	0.202	7.56E-22	1.16E-20	-14.436	28	41	34.5
RBL1	0.457516344	0.851931333	1.722	5.76E-18	4.51E-17	-20.453	55	16	35.5
TERF1	0.496732026	0.828326188	0.566	2.24E-18	1.97E-17	-18.837	49	22	35.5
NRF1	0.647058824	0.715665236	0.322	6.09E-19	6.11E-18	-17.123	43	28	35.5
LITAF	0.901960784	0.487660944	3.522	1.54E-23	3.16E-22	-13.046	21	50	35.5
CHAF1A	0.424836601	0.872854077	0.526	5.74E-18	4.51E-17	-20.331	54	18	36
ING1	0.503267974	0.822961373	2.832	2.84E-18	2.45E-17	-16.453	50	31	40.5
POLE3	0.470588235	0.840128755	2.797	1.52E-17	1.13E-16	-18.041	58	24	41
PFDN1	0.705882353	0.677038627	1.098	1.80E-20	2.28E-19	-13.57	34	48	41
BUD31	0.797385621	0.590665236	1.748	4.37E-21	6.08E-20	-12.817	31	51	41
MAZ	0.640522876	0.711909871	0.401	6.01E-18	4.62E-17	-15.683	56	36	46
SSRP1	0.849673203	0.509656652	0.848	3.23E-19	3.57E-18	-12.57	39	54	46.5
RUVBL2	0.627450981	0.731223176	1.014	8.35E-19	8.12E-18	-13.378	45	49	47
YAF2	0.450980392	0.848175966	0.614	7.66E-17	5.08E-16	-16.148	65	32	48.5

Gene	TP	TN	thresh_mhg	hyper_pval	hyper_qval	gen_qval	rank_hyper_qval	rank_gen_qval	mean_rank
RUVBL1	0.633986928	0.728540773	4.359	4.06E-19	4.37E-18	-12.325	40	57	48.5
HSBP1	0.797385621	0.59388412	2.943	2.11E-21	3.13E-20	-11.512	29	69	49
AIP	0.843137255	0.537017167	0.411	5.56E-21	7.48E-20	-11.643	32	67	49.5
MYEF2	0.620915033	0.714592275	0.454	1.72E-16	1.09E-15	-15.858	68	33	50.5
TFDP1	0.588235294	0.748927039	1.401	3.47E-17	2.34E-16	-14.454	64	40	52
IRF8	0.751633987	0.625536481	0.367	8.92E-20	1.10E-18	-11.165	35	73	54
ELK3	0.640522876	0.700107296	0.31	9.24E-17	6.04E-16	-14.175	66	43	54.5
LZTR1	0.490196078	0.818133047	1.696	1.75E-16	1.10E-15	-13.717	69	47	58
COPS5	0.633986928	0.710300429	3.206	3.36E-17	2.30E-16	-12.421	62	56	59
CTCF	0.535947712	0.780579399	0.227	4.27E-16	2.52E-15	-13.872	73	46	59.5
PNRC2	0.732026144	0.635193133	1.118	8.48E-19	8.12E-18	-10.78	44	77	60.5
PSMC3	0.895424837	0.455472103	4.378	9.68E-20	1.16E-18	-9.774	36	88	62
CDC5L	0.673202614	0.675965665	2.759	2.71E-17	1.95E-16	-11.644	60	66	63
ZNHIT3	0.379084967	0.881437768	3.543	5.18E-15	2.55E-14	-14.798	88	39	63.5
MTA1	0.437908497	0.839055794	0.356	1.55E-14	7.36E-14	-14.27	91	42	66.5
CBY1	0.307189542	0.915236052	1.526	1.12E-13	4.77E-13	-15.751	101	35	68
NFYC	0.647058824	0.686158798	0.807	5.49E-16	3.20E-15	-11.683	74	65	69.5
C1D	0.653594771	0.688304721	1.664	9.40E-17	6.04E-16	-11.201	67	72	69.5
CCNH	0.620915033	0.707081545	1.963	9.26E-16	5.11E-15	-11.828	78	63	70.5
UBE2K	0.758169935	0.599785408	0.202	5.54E-18	4.51E-17	-9.673	53	90	71.5
SMARCB1	0.692810458	0.64860515	0.585	1.83E-16	1.13E-15	-11.076	70	74	72
E2F4	0.549019608	0.751072961	0.465	3.94E-14	1.77E-13	-12.771	95	52	73.5
RBBP4	0.882352941	0.433476395	0.367	2.19E-16	1.33E-15	-10.972	71	76	73.5
YBX1	0.712418301	0.638412017	3.768	2.83E-17	2.00E-16	-9.802	61	87	74
PTTG1	0.803921569	0.556866953	1.828	1.34E-18	1.23E-17	-9.135	47	102	74.5
GABPA	0.555555556	0.745708155	0.227	3.92E-14	1.77E-13	-12.452	96	55	75.5
TOX	0.849673203	0.507510733	3.831	5.00E-19	5.26E-18	-8.559	41	111	76
SMARCE1	0.823529412	0.503218884	0.465	5.60E-16	3.22E-15	-10.63	75	79	77
KEAP1	0.516339869	0.778969957	1.714	2.66E-14	1.23E-13	-11.749	93	64	78.5
RBX1	0.875816993	0.462982833	2.485	4.37E-18	3.62E-17	-8.985	52	105	78.5
CBFB	0.4967320	0.7913090	0.516	5.24E-14	2.30E-13	-12.07	98	60	79

Gene	TP	TN	thresh_mhg	hyper_pval	hyper_qval	gen_qval	rank_hyper_qval	rank_gen_qval	mean_rank
	26	13							
YEATS4	0.575163399	0.737124464	4.142	7.07E-15	3.42E-14	-11.348	89	71	80
ILF3	0.803921569	0.518240343	1.316	2.46E-15	1.26E-14	-10.497	83	81	82
GTF3C5	0.411764706	0.845493562	0.678	3.78E-13	1.51E-12	-12.296	108	58	83
ASXL1	0.411764706	0.845493562	3.118	3.78E-13	1.51E-12	-12.184	107	59	83
PQBP1	0.666666667	0.669527897	2.805	4.09E-16	2.45E-15	-9.361	72	95	83.5
GTF3C2	0.660130719	0.665236052	1.322	3.55E-15	1.80E-14	-10.33	85	84	84.5
MED21	0.418300654	0.837982833	1.104	8.16E-13	2.96E-12	-12.576	119	53	86
CTNNB1	0.464052288	0.80472103	0.623	7.88E-13	2.90E-12	-11.935	117	61	89
TARDBP	0.745098039	0.587982833	0.176	9.49E-16	5.18E-15	-9.188	79	100	89.5
METTL14	0.450980392	0.814377682	0.475	8.08E-13	2.95E-12	-11.878	118	62	90
MED4	0.39869281	0.853004292	4.53	5.64E-13	2.15E-12	-11.537	113	68	90.5
ATF1	0.470588235	0.807403433	4.658	1.28E-13	5.40E-13	-10.525	102	80	91
CNOT8	0.647058824	0.684012876	3.377	8.70E-16	4.87E-15	-8.976	77	106	91.5
CCNC	0.444444444	0.820278937	1.546	6.20E-13	2.32E-12	-11.51	115	70	92.5
NONO	0.993464052	0.290236052	3.702	1.07E-20	1.40E-19	-6.768	33	153	93
NRBF2	0.437908497	0.826180258	3.438	4.66E-13	1.79E-12	-10.992	112	75	93.5
CHD4	0.882352941	0.454399142	0.202	4.20E-18	3.55E-17	-7.391	51	136	93.5
GABPB2	0.535947712	0.760193133	1.433	5.53E-14	2.41E-13	-9.745	99	89	94
GABPB1	0.535947712	0.748390558	0.888	7.16E-13	2.66E-12	-10.664	116	78	97
AEBP2	0.607843137	0.689377682	0.111	3.99E-13	1.57E-12	-10.148	109	85	97
VPS72	0.633986928	0.681866953	0.791	1.62E-14	7.60E-14	-8.934	92	107	99.5
RNPS1	0.843137255	0.478004292	1.245	7.14E-16	4.05E-15	-8.096	76	124	100
BATF	0.712418301	0.596566524	4.673	1.06E-13	4.57E-13	-9.042	100	103	101.5
NAB2	0.366013072	0.869635193	2.573	2.45E-12	8.30E-12	-10.417	127	83	105
SREBF2	0.712418301	0.583690987	0.138	1.04E-12	3.72E-12	-9.627	120	92	106
MED7	0.444444444	0.814377682	3.82	2.56E-12	8.62E-12	-9.832	128	86	107
ZNRD1	0.555555556	0.734442006	1.941	4.34E-13	1.68E-12	-9.04	111	104	107.5
CIR1	0.529411765	0.748927039	0.856	1.96E-12	6.77E-12	-9.291	125	98	111.5
FLII	0.758169935	0.573497854	2.257	1.08E-15	5.81E-15	-6.924	80	148	114
MORF4L2	0.614379085	0.683476395	5.566	4.00E-13	1.57E-12	-8.242	110	119	114.5

Gene	TP	TN	thresh_mhg	hyper_pval	hyper_qval	gen_qval	rank_hyper_qval	rank_gen_qval	mean_rank
MSL3	0.437908497	0.805257511	0.791	5.98E-11	1.70E-10	-10.452	152	82	117
MTA2	0.91503268	0.411480687	2.58	2.10E-18	1.88E-17	-5.789	48	186	117
TBX21	0.745098039	0.580472103	0.642	4.00E-15	2.00E-14	-6.857	86	149	117.5
TBP	0.464052288	0.788090129	0.642	3.15E-11	9.42E-11	-9.437	144	93	118.5
SARNP	0.849673203	0.488733906	2.753	2.05E-17	1.50E-16	-6.082	59	178	118.5
THRAP3	0.869281046	0.4222103	0.872	3.66E-14	1.68E-13	-6.984	94	144	119
KDM2B	0.640522876	0.637875536	0.287	1.89E-11	5.85E-11	-9.179	139	101	120
ELF4	0.568627451	0.711373391	0.251	4.58E-12	1.52E-11	-8.536	130	112	121
EOMES	0.464052288	0.787017167	0.888	3.93E-11	1.16E-10	-9.255	146	99	122.5
TBL1XR1	0.614379085	0.667918455	0.454	7.20E-12	2.37E-11	-8.352	131	115	123
SUZ12	0.392156863	0.837446352	1.05	8.87E-11	2.45E-10	-9.66	156	91	123.5
SPOP	0.718954248	0.609978541	2.248	2.45E-15	1.26E-14	-6.408	84	163	123.5
PWP1	0.450980392	0.804184549	4.676	8.58E-12	2.80E-11	-8.347	132	116	124
MLX	0.562091503	0.720493562	0.722	2.34E-12	8.02E-12	-8.13	126	122	124
NR4A2	0.738562092	0.55472103	3.667	1.37E-12	4.83E-12	-8.09	122	126	124
NFKBIB	0.771241833	0.550965665	0.642	5.20E-15	2.55E-14	-6.475	87	161	124
TSG101	0.686274511	0.619635193	0.766	1.87E-13	7.76E-13	-6.927	104	146	125
MED8	0.477124183	0.781115858	1.918	1.58E-11	4.97E-11	-8.363	137	114	125.5
GTF2F2	0.549019608	0.719957082	0.993	2.24E-11	6.88E-11	-8.467	140	113	126.5
CENPB	0.385620915	0.841201717	0.556	1.10E-10	2.97E-10	-9.332	160	96	128
ZC3H15	0.732026144	0.572424893	4.849	2.13E-13	8.73E-13	-6.789	105	152	128.5
HDAC3	0.562091503	0.712446352	3.541	1.10E-11	3.50E-11	-7.989	135	127	131
PA2G4	0.823529412	0.495708155	2.114	2.23E-15	1.17E-14	-5.901	82	182	132
ZMIZ1	0.601307189	0.674892704	1.485	1.76E-11	5.49E-11	-7.885	138	128	133
RNF4	0.777777778	0.525214592	3.988	1.42E-13	5.93E-13	-6.338	103	165	134
NMI	0.725490196	0.573497854	0.864	5.82E-13	2.20E-12	-6.612	114	156	135
USF1	0.352941176	0.863733906	3.491	1.21E-10	3.21E-10	-8.917	162	109	135.5
COPS2	0.535947712	0.730686695	0.299	2.44E-11	7.45E-11	-7.668	141	131	136
ZFYVE19	0.333333333	0.870171674	1.293	6.77E-10	1.66E-09	-9.316	176	97	136.5
BAZ1B	0.594771242	0.677575107	0.163	3.13E-11	9.42E-11	-7.735	143	130	136.5
STAT2	0.3529411	0.8551502	0.411	9.50E-10	2.26E-09	-9.432	181	94	137.5

Gene	TP	TN	thresh_mhg	hyper_pval	hyper_qval	gen_qval	rank_hyper_qval	rank_gen_qval	mean_rank
	76	15							
ZFP91	0.437908497	0.799892704	0.214	1.85E-10	4.82E-10	-8.842	165	110	137.5
HNRNPD	0.653594771	0.629291845	0.189	9.58E-12	3.08E-11	-7.114	133	142	137.5
PREB	0.620915033	0.671673832	2.345	1.21E-12	4.31E-12	-6.63	121	155	138
TAF11	0.385620915	0.835836901	0.714	3.74E-10	9.54E-10	-8.933	169	108	138.5
EED	0.431372549	0.807403433	3.645	1.10E-10	2.97E-10	-8.254	159	118	138.5
HDAC7	0.777777778	0.493025751	0.263	2.75E-11	8.36E-11	-7.4	142	135	138.5
GTF2E2	0.496732026	0.75751073	2.025	7.57E-11	2.12E-10	-8.096	154	125	139.5
SF1	0.732026144	0.549892704	4.441	9.58E-12	3.08E-11	-6.964	134	145	139.5
HIF1A	0.843137255	0.464592275	2.198	7.99E-15	3.82E-14	-5.468	90	196	143
CALR	0.915032688	0.375536481	3.187	1.65E-15	8.79E-15	-5.326	81	205	143
SMARCD2	0.437908497	0.801502146	1.157	1.32E-10	3.50E-10	-7.852	163	129	146
PML	0.522875817	0.736051502	0.163	7.13E-11	2.01E-10	-7.14	153	141	147
EDF1	0.967320261	0.317060086	2.496	6.36E-18	4.81E-17	-4.552	57	238	147.5
SREBF1	0.568627451	0.692596567	0.227	1.38E-10	3.63E-10	-7.438	164	134	149
CCDC71	0.326797386	0.872317597	1.731	1.19E-09	2.79E-09	-8.289	184	117	150.5
GTF2B	0.627450980	0.651287554	3.622	1.58E-11	4.97E-11	-6.284	136	169	152.5
MNDA	0.339869281	0.862660944	2.674	1.39E-09	3.24E-09	-8.14	185	121	153
PKNOX1	0.405228758	0.814914163	0.333	1.43E-09	3.29E-09	-8.163	187	120	153.5
RNF14	0.503267974	0.745171674	0.465	2.78E-10	7.17E-10	-7.199	167	140	153.5
GATA3	0.679738562	0.593347639	0.379	5.20E-11	1.51E-10	-6.599	149	158	153.5
PRDM1	0.424836601	0.799892704	0.214	1.41E-09	3.26E-09	-8.122	186	123	154.5
BHLHE40	0.954248366	0.35139485	4.789	9.84E-19	9.22E-18	-4.262	46	263	154.5
HTATIP2	0.490196078	0.75751073	4.108	2.11E-10	5.47E-10	-6.926	166	147	156.5
GTF2A1	0.522875817	0.722103004	0.176	8.64E-10	2.09E-09	-7.247	178	139	158.5
ID2	0.973856209	0.295064378	3.079	3.31E-17	2.30E-16	-4.316	63	255	159
FUBP1	0.686274501	0.586373391	0.151	5.54E-11	1.58E-10	-6.3	151	168	159.5
AES	0.705882353	0.582081545	2.263	4.26E-12	1.42E-11	-5.718	129	191	160
RBM38	0.823529412	0.456545064	0.911	1.84E-12	6.40E-12	-5.384	124	200	162
HDAC5	0.326797386	0.869098712	1.852	2.57E-09	5.75E-09	-7.652	193	132	162.5
PBRM1	0.470588235	0.761802575	0.138	1.85E-09	4.20E-09	-7.354	190	137	163.5

Gene	TP	TN	thresh_mhg	hyper_pval	hyper_qval	gen_qval	rank_hyper_qval	rank_gen_qval	mean_rank
XBP1	0.575163399	0.692060086	2.278	5.48E-11	1.58E-10	-5.972	150	180	165
CIZ1	0.522875817	0.720493562	0.239	1.14E-09	2.68E-09	-6.807	183	150	166.5
TARBP2	0.411764706	0.802038627	0.941	6.58E-09	1.40E-08	-7.528	203	133	168
CTBP1	0.483660131	0.747854077	0.506	3.19E-09	6.92E-09	-7.317	199	138	168.5
BLOC1S1	0.529411765	0.719420601	2.138	5.18E-10	1.31E-09	-6.211	171	173	172
TCERG1	0.594771242	0.659871245	0.287	6.11E-10	1.52E-09	-6.224	173	172	172.5
PLRG1	0.431372549	0.789699571	0.848	3.69E-09	7.95E-09	-6.805	200	151	175.5
TCF3	0.437908497	0.785407725	1.628	3.12E-09	6.79E-09	-6.759	198	154	176
SNW1	0.679738562	0.595493562	3.37	3.68E-11	1.09E-10	-5.09	145	212	178.5
UHRF2	0.444444444	0.771995708	0.379	1.33E-08	2.61E-08	-7.039	220	143	181.5
RBL2	0.745098039	0.520386266	1	1.08E-10	2.95E-10	-5.267	158	208	183
CNOT7	0.529411765	0.71083691	1.379	2.21E-09	4.99E-09	-6.101	191	176	183.5
CNOT1	0.614379085	0.630901288	0.722	3.11E-09	6.79E-09	-6.152	197	174	185.5
ECD	0.490196078	0.749463519	2.333	9.22E-10	2.22E-09	-5.608	179	192	185.5
ATF2	0.418300654	0.794527897	2.993	1.02E-08	2.05E-08	-6.509	214	159	186.5
GATAD1	0.503267974	0.725858369	0.485	7.78E-09	1.62E-08	-6.336	207	166	186.5
TRIM28	0.437908497	0.783261803	3.336	4.62E-09	9.92E-09	-6.102	201	175	188
CBX4	0.457516344	0.762339056	0.138	1.11E-08	2.21E-08	-6.426	216	162	189
REXO4	0.418300654	0.795064378	4.815	9.25E-09	1.89E-08	-6.262	211	170	190.5
ATF6B	0.503267974	0.726394845	0.299	7.13E-09	1.49E-08	-6.023	206	179	192.5
MEN1	0.392156863	0.811158798	0.526	2.06E-08	3.88E-08	-6.605	229	157	193
RFC1	0.392156863	0.811695279	2.251	1.87E-08	3.56E-08	-6.503	226	160	193
NFKB2	0.764705882	0.501072961	0.275	7.64E-11	2.12E-10	-4.63	155	234	194.5
RBPJ	0.666666667	0.583690987	2.154	1.73E-09	3.93E-09	-5.337	189	204	196.5
GTF3A	0.601307191	0.653969957	2.86	5.93E-10	1.49E-09	-4.904	172	224	198
DEAF1	0.333333333	0.853004292	1.551	3.22E-08	5.90E-08	-6.408	235	164	199.5
NDUFA13	0.947712418	0.283798283	4.374	1.38E-12	4.84E-12	-3.99	123	276	199.5
DAXX	0.516339869	0.712982833	0.546	9.92E-09	2.01E-08	-5.724	213	190	201.5
NT5C	0.790849673	0.468347639	1.852	1.20E-10	3.21E-10	-4.522	161	242	201.5
VEZF1	0.411764706	0.798283262	0.832	1.32E-08	2.59E-08	-5.798	219	185	202
NOTCH1	0.7320261	0.5278969	0.07	2.92E-10	7.50E-10	-4.574	168	237	202.5

Gene	TP	TN	thresh_mhg	hyper_pval	hyper_qval	gen_qval	rank_hyper_qval	rank_gen_qval	mean_rank
	44	96							
HTATSF1	0.37254902	0.826716738	3.346	1.86E-08	3.56E-08	-5.735	225	188	206.5
PHF6	0.418300654	0.802038627	3.586	2.49E-09	5.60E-09	-4.94	192	222	207
CREB1	0.444444444	0.763412017	0.124	5.61E-08	1.01E-07	-6.1	239	177	208
HLTF	0.359477124	0.837982833	2.362	1.39E-08	2.69E-08	-5.558	222	194	208
TSC22D4	0.699346405	0.5472103	1.967	2.69E-09	5.94E-09	-4.971	195	221	208
SUB1	0.967320261	0.22639485	5.41	4.98E-11	1.45E-10	-4.099	148	271	209.5
MED28	0.790849673	0.505364807	2.807	3.36E-13	1.37E-12	-3.125	106	315	210.5
SMARCA5	0.437908497	0.762339056	0.275	1.58E-07	2.64E-07	-6.257	258	171	214.5
HMG20B	0.516339869	0.700107296	0.687	7.05E-08	1.25E-07	-5.775	243	187	215
TWISTNB	0.450980392	0.764484979	3.623	1.92E-08	3.65E-08	-5.347	227	203	215
MED24	0.405228758	0.7972103	2.611	4.02E-08	7.34E-08	-5.476	236	195	215.5
TAF1B	0.457516344	0.757510733	1.362	2.49E-08	4.61E-08	-5.415	233	198	215.5
BOLA2	0.516339869	0.709763948	4.932	1.64E-08	3.18E-08	-5.26	223	209	216
ZRANB2	0.450980392	0.766630901	3.018	1.34E-08	2.61E-08	-5.078	221	214	217.5
GTF2A2	0.483660131	0.739806867	5.343	1.23E-08	2.44E-08	-5.036	218	217	217.5
HES6	0.352941176	0.831008584	0.66	1.36E-07	2.31E-07	-5.822	254	183	218.5
PIAS4	0.333333333	0.839592275	0.687	4.25E-07	6.61E-07	-6.325	277	167	222
MTA3	0.535947712	0.69527897	4.553	1.05E-08	2.11E-08	-4.665	215	231	223
MED27	0.411764706	0.785944206	2.521	1.12E-07	1.94E-07	-5.42	250	197	223.5
FUS	0.777777778	0.447961373	0.566	1.74E-08	3.35E-08	-4.915	224	223	223.5
RELA	0.692810458	0.563841202	1.39	6.39E-10	1.57E-09	-4.087	175	272	223.5
MTF2	0.424836601	0.772532189	0.322	1.75E-07	2.91E-07	-5.588	259	193	226
BRD1	0.470588235	0.731223176	0.299	2.63E-07	4.25E-07	-5.734	266	189	227.5
SND1	0.673202614	0.565987124	0.475	8.43E-09	1.74E-08	-4.416	209	248	228.5
RLIM	0.758169935	0.490343348	0.138	1.08E-09	2.57E-09	-3.978	182	277	229.5
AIM2	0.633986928	0.575643777	0.516	4.24E-07	6.61E-07	-5.809	276	184	230
TOX4	0.686274501	0.546137339	0.043	2.03E-08	3.85E-08	-4.63	228	235	231.5
ATF7IP	0.718954248	0.519849785	0.275	6.84E-09	1.44E-08	-4.278	204	260	232
PNN	0.679738562	0.567596567	0.287	2.62E-09	5.81E-09	-4.14	194	270	232
PLAGL2	0.346405229	0.833154506	2.82	2.27E-07	3.72E-07	-5.348	263	202	232.5

Gene	TP	TN	thresh_mhg	hyper_pval	hyper_qval	gen_qval	rank_hyper_qval	rank_gen_qval	mean_rank
RELB	0.477124183	0.735515021	3.689	5.92E-08	1.06E-07	-4.901	241	225	233
AGGF1	0.307189542	0.862660944	3.372	2.10E-07	3.46E-07	-5.271	261	207	234
PHRF1	0.529411765	0.688841202	0.485	6.62E-08	1.18E-07	-4.817	242	226	234
L3MBTL2	0.346405229	0.825643777	0.356	8.41E-07	1.25E-06	-5.943	291	181	236
TCEA1	0.725490196	0.528433476	3.658	7.58E-10	1.85E-09	-3.551	177	297	237
SMARCC2	0.575163399	0.64055794	1.328	1.55E-07	2.60E-07	-5.02	257	218	237.5
TBPL1	0.470588235	0.739270386	2.687	7.86E-08	1.38E-07	-4.667	245	230	237.5
CREM	0.594771242	0.642703863	2.753	8.54E-09	1.75E-08	-4.217	210	265	237.5
GTF3C1	0.549019608	0.663626609	0.098	1.85E-07	3.07E-07	-5.042	260	216	238
IRF3	0.562091503	0.658261803	0.696	7.32E-08	1.29E-07	-4.663	244	232	238
ING4	0.522875817	0.702253219	2.57	2.15E-08	4.02E-08	-4.453	230	246	238
RUNX2	0.705882353	0.550429185	1.118	6.27E-10	1.55E-09	-3.467	174	303	238.5
IRF4	0.424836601	0.769313305	0.299	2.89E-07	4.67E-07	-5.053	267	215	241
THOC2	0.535947712	0.670600858	0.138	3.66E-07	5.78E-07	-5.244	273	210	241.5
ZMAT2	0.653594771	0.576716738	0.367	2.93E-08	5.39E-08	-4.382	234	250	242
NFATC1	0.830065359	0.393776824	1.59	6.20E-09	1.32E-08	-3.773	202	282	242
STAT6	0.712418301	0.545064378	1.618	5.04E-10	1.28E-09	-3.188	170	314	242
OVCA2	0.359477124	0.817596567	0.516	5.87E-07	8.97E-07	-5.309	282	206	244
NCOR2	0.555555556	0.660944206	0.124	1.17E-07	1.99E-07	-4.587	253	236	244.5
TRIM27	0.359477124	0.8277897	3.365	9.86E-08	1.71E-07	-4.533	248	241	244.5
HCLS1	0.947712418	0.303648069	0.496	4.88E-14	2.17E-13	-1.096	97	393	245
CNOT2	0.601307189	0.623390558	0.651	5.47E-08	9.91E-08	-4.32	238	253	245.5
LRRFIP1	0.882352941	0.359978541	3.089	4.88E-11	1.43E-10	-2.185	147	349	248
SBDS	0.588235294	0.625	0.722	2.35E-07	3.83E-07	-4.634	264	233	248.5
CSDA	0.509803922	0.689377682	0.696	7.14E-07	1.07E-06	-5.197	288	211	249.5
CNOT3	0.509803922	0.692060086	0.39	4.98E-07	7.67E-07	-4.985	280	219	249.5
GTF2H1	0.359477124	0.820815451	1.996	3.40E-07	5.41E-07	-4.793	271	228	249.5
EYA3	0.366013072	0.804184549	0.275	2.19E-06	3.11E-06	-5.393	302	199	250.5
PHB2	0.758169935	0.506974249	6.564	9.39E-11	2.58E-10	-2.142	157	351	254
SMARCA4	0.633986928	0.579935622	1.208	2.48E-07	4.04E-07	-4.511	265	244	254.5
FLI1	0.7908496	0.4399141	0.275	6.82E-09	1.44E-08	-3.426	205	307	256

Gene	TP	TN	thresh_mhg	hyper_pval	hyper_qval	gen_qval	rank_hyper_qval	rank_gen_qval	mean_rank
	73	63							
ZFPL1	0.333333333	0.831008584	2.305	1.86E-06	2.66E-06	-5.09	301	213	257
IKZF3	0.705882353	0.525214592	3.372	2.16E-08	4.03E-08	-3.773	231	283	257
GON4L	0.516339869	0.688304721	0.057	3.73E-07	5.87E-07	-4.512	274	243	258.5
MORF4L1	0.758169935	0.483369099	5.498	2.89E-09	6.36E-09	-2.892	196	322	259
TRIP12	0.522875817	0.681330472	1.531	4.30E-07	6.65E-07	-4.534	279	240	259.5
UTP6	0.37254902	0.794527897	0.202	4.19E-06	5.65E-06	-5.354	320	201	260.5
IFI35	0.60130719	0.634120172	1.761	1.21E-08	2.40E-08	-3.457	217	305	261
PURB	0.555555556	0.656115888	0.422	2.25E-07	3.71E-07	-4.21	262	267	264.5
BAZ1A	0.496732026	0.689377682	0.202	3.27E-06	4.48E-06	-4.974	313	220	266.5
DDX54	0.549019608	0.667918455	3.557	1.03E-07	1.77E-07	-3.737	249	285	267
NFX1	0.457516344	0.736051502	3.637	6.64E-07	1.00E-06	-4.317	286	254	270
NCOA2	0.535947712	0.671673882	0.31	3.17E-07	5.08E-07	-4.049	269	273	271
SIN3A	0.450980392	0.738733906	0.202	1.01E-06	1.47E-06	-4.44	296	247	271.5
PIAS1	0.470588235	0.724248927	0.322	7.09E-07	1.06E-06	-4.288	287	257	272
NAB1	0.385620915	0.785407725	0.202	3.32E-06	4.53E-06	-4.73	316	229	272.5
IRF9	0.444444444	0.747854077	2.536	6.10E-07	9.25E-07	-4.273	284	262	273
ATF4	0.895424837	0.321888412	0.526	9.39E-10	2.25E-09	-1.756	180	368	274
GNPTAB	0.418300654	0.758583691	0.275	3.09E-06	4.27E-06	-4.551	312	239	275.5
JUND	0.516339869	0.677038627	0.227	1.64E-06	2.36E-06	-4.342	299	252	275.5
ARNT	0.418300654	0.754828326	0.239	5.15E-06	6.81E-06	-4.803	326	227	276.5
UBXN4	0.581699346	0.642167382	3.297	5.36E-08	9.76E-08	-3.023	237	318	277.5
IKZF2	0.483660131	0.701180258	0.084	3.26E-06	4.48E-06	-4.472	314	245	279.5
TFAM	0.366013072	0.804184549	3.072	2.19E-06	3.11E-06	-4.287	303	258	280.5
SP3	0.437908497	0.751072961	1.417	8.55E-07	1.26E-06	-4.159	292	269	280.5
STAT3	0.941176471	0.25	0.31	1.52E-09	3.48E-09	-1.639	188	373	280.5
STAT5A	0.607843137	0.598712446	0.322	5.95E-07	9.06E-07	-3.78	283	281	282
BPTF	0.575163399	0.625536481	0.084	1.07E-06	1.55E-06	-4.183	297	268	282.5
MIER1	0.712418301	0.503755365	0.287	1.40E-07	2.37E-07	-3.226	255	313	284
EGR1	0.614379085	0.597103004	1.227	3.27E-07	5.22E-07	-3.484	270	301	285.5
CCNT1	0.424836601	0.761802575	3.671	8.95E-07	1.32E-06	-3.844	293	280	286.5

Gene	TP	TN	thresh_mhg	hyper_pval	hyper_qval	gen_qval	rank_hyper_qval	rank_gen_qval	mean_rank
NR1H2	0.679738562	0.545064378	0.575	5.73E-08	1.03E-07	-2.644	240	333	286.5
XRCC6	0.346405229	0.814377682	0.604	5.06E-06	6.71E-06	-4.402	325	249	287
NFIL3	0.326797386	0.830472103	4.118	4.61E-06	6.17E-06	-4.311	322	256	289
CHURC1	0.522875817	0.693133047	5.643	8.31E-08	1.46E-07	-2.647	246	332	289
MLLT6	0.503267974	0.691523605	0.748	1.17E-06	1.69E-06	-3.731	298	286	292
VAV1	0.725490196	0.503755365	2.639	2.32E-08	4.31E-08	-2.11	232	354	293
ELF1	0.843137255	0.374463519	1.941	9.39E-09	1.91E-08	-1.628	212	374	293
PHB	0.705882353	0.532725322	4.066	7.82E-09	1.62E-08	-1.5	208	378	293
NCOA4	0.496732026	0.700643777	1.275	7.60E-07	1.13E-06	-3.541	289	298	293.5
CAND1	0.392156863	0.774678112	1.35	6.99E-06	9.16E-06	-4.276	329	261	295
MED14	0.424836601	0.743562232	0.379	1.07E-05	1.39E-05	-4.284	334	259	296.5
MED1	0.62745098	0.564914163	0.227	3.27E-06	4.48E-06	-3.978	315	278	296.5
IRF2	0.633986928	0.560622318	0.356	2.53E-06	3.53E-06	-3.742	309	284	296.5
ZBTB32	0.339869281	0.816523605	0.941	8.02E-06	1.05E-05	-4.226	330	264	297
MED17	0.385620915	0.771995708	0.163	2.07E-05	2.57E-05	-4.36	347	251	299
TMF1	0.614379085	0.580472103	0.263	2.44E-06	3.42E-06	-3.65	308	291	299.5
VAMP7	0.385620915	0.782188841	0.444	5.25E-06	6.92E-06	-4.036	327	275	301
TRPS1	0.379084967	0.788090129	0.454	4.90E-06	6.51E-06	-3.934	324	279	301.5
NFRKB	0.37254902	0.784871245	0.299	1.61E-05	2.04E-05	-4.214	340	266	303
NR4A1	0.732026144	0.467811159	4.142	8.31E-07	1.24E-06	-3.002	290	319	304.5
MMS19	0.385620915	0.776287554	0.356	1.18E-05	1.51E-05	-4.042	336	274	305
IKZF1	0.732026144	0.485515021	4.707	9.77E-08	1.70E-07	-1.98	247	363	305
DR1	0.411764706	0.761266094	3.046	4.58E-06	6.14E-06	-3.671	321	290	305.5
NFATC2	0.424836601	0.747317597	0.214	6.63E-06	8.71E-06	-3.644	328	292	310
PPIE	0.503267974	0.686158798	1.144	2.33E-06	3.29E-06	-3.065	305	316	310.5
CAMTA2	0.411764706	0.753755365	0.239	1.23E-05	1.58E-05	-3.675	337	289	313
MLXIP	0.581699346	0.599785408	0.202	1.03E-05	1.33E-05	-3.638	333	293	313
NCOA3	0.718954248	0.490343348	0.433	3.08E-07	4.95E-07	-2.073	268	358	313
RPL7L1	0.490196078	0.704935622	4.299	9.35E-07	1.37E-06	-2.584	294	335	314.5
KAT5	0.37254902	0.783798283	0.379	1.86E-05	2.34E-05	-3.695	343	288	315.5
EP300	0.4836601	0.6920600	0.098	9.99E-06	1.30E-05	-3.513	332	299	315.5

Gene	TP	TN	thresh_mhg	hyper_pval	hyper_qval	gen_qval	rank_hyper_qval	rank_gen_qval	mean_rank
	31	86							
XAB2	0.496732026	0.689914163	0.696	3.06E-06	4.24E-06	-2.996	311	320	315.5
FOXN3	0.418300654	0.74195279	2.053	2.65E-05	3.27E-05	-3.702	349	287	318
CBFA2T2	0.339869281	0.832081545	1.651	6.63E-07	1.00E-06	-2.132	285	352	318.5
TCF20	0.712418301	0.480150215	0.084	2.29E-06	3.24E-06	-2.642	304	334	319
DPF2	0.594771242	0.607296137	0.299	9.89E-07	1.45E-06	-2.36	295	345	320
RNF2	0.37254902	0.787553648	2.488	1.12E-05	1.44E-05	-3.313	335	309	322
PHF20L1	0.516339869	0.65611588	0.151	1.97E-05	2.46E-05	-3.511	345	300	322.5
CEBPZ	0.444444444	0.724248927	0.401	1.41E-05	1.79E-05	-3.452	339	306	322.5
CDK7	0.392156863	0.778433476	2.299	4.15E-06	5.61E-06	-2.753	319	328	323.5
SCAP	0.352941176	0.793454936	0.151	4.45E-05	5.42E-05	-3.628	354	294	324
LIMD1	0.503267974	0.668454936	0.176	1.92E-05	2.41E-05	-3.328	344	308	326
BCLAF1	0.660130719	0.521995708	0.189	9.52E-06	1.24E-05	-2.833	331	324	327.5
ZFX	0.339869281	0.810085837	2.667	2.03E-05	2.52E-05	-3.313	346	310	328
UIMC1	0.45751634	0.700107296	0.516	5.92E-05	7.07E-05	-3.6	361	296	328.5
TBC1D2B	0.379084967	0.770922747	0.239	4.75E-05	5.77E-05	-3.48	355	302	328.5
MAF1	0.823529412	0.379291845	1.718	1.16E-07	1.98E-07	-0.705	252	406	329
HIVEP1	0.450980392	0.704399142	0.151	7.06E-05	8.36E-05	-3.612	364	295	329.5
ING3	0.450980392	0.707081545	0.322	5.26E-05	6.36E-05	-3.466	357	304	330.5
TCF25	0.758169935	0.446351931	6.119	3.46E-07	5.48E-07	-1.135	272	390	331
STAT1	0.758169935	0.44527897	4.683	3.93E-07	6.17E-07	-1.189	275	389	332
RNF166	0.607843137	0.581008584	0.356	4.80E-06	6.41E-06	-2.168	323	350	336.5
PFDN5	0.928104575	0.239806867	2.205	1.15E-07	1.98E-07	-0.068	251	424	337.5
RNF114	0.712418301	0.493025751	2.947	5.17E-07	7.94E-07	-1.06	281	395	338
EIF3H	0.973856209	0.154506438	8.085	4.27E-07	6.63E-07	-0.973	278	398	338
UBN1	0.411764706	0.738197425	0.098	8.01E-05	9.45E-05	-3.249	365	312	338.5
PHF14	0.31372549	0.827253219	2.021	3.50E-05	4.28E-05	-2.776	352	327	339.5
PHF15	0.405228758	0.740343348	0.151	0.000119637	0.000139361	-3.297	370	311	340.5
BTF3	0.980392157	0.149141631	8.357	1.45E-07	2.44E-07	-0.021	256	425	340.5
DMTF1	0.359477124	0.786480687	1.275	5.39E-05	6.47E-05	-2.858	359	323	341
CCNL2	0.751633987	0.439377682	1.465	1.82E-06	2.61E-06	-1.259	300	386	343

Gene	TP	TN	thresh_mhg	hyper_pval	hyper_qval	gen_qval	rank_hyper_qval	rank_gen_qval	mean_rank
MYC	0.483660131	0.683476395	0.333	2.69E-05	3.31E-05	-2.364	350	344	347
GTF2H2	0.326797386	0.803111588	0.848	0.000200549	0.000228065	-3.026	379	317	348
KDM5C	0.522875817	0.627682403	0.189	0.000193485	0.000221787	-2.924	376	321	348.5
SERTAD1	0.418300654	0.731759657	0.791	8.64E-05	0.000101409	-2.693	367	330	348.5
CXXC1	0.496732026	0.665236052	0.506	5.28E-05	6.36E-05	-2.458	356	341	348.5
MBD2	0.424836601	0.724248927	0.824	0.000104235	0.000121749	-2.688	369	331	350
NSD1	0.640522876	0.533261803	0.556	2.38E-05	2.95E-05	-2.129	348	353	350.5
IRF1	0.745098039	0.444206009	1.884	2.38E-06	3.36E-06	-0.922	306	399	352.5
RNF19A	0.496732026	0.665236052	0.239	5.28E-05	6.36E-05	-2.268	358	348	353
RUNX3	0.692810458	0.495708155	2.269	4.11E-06	5.57E-06	-1.195	318	388	353
RNF44	0.660130719	0.504828326	0.227	5.44E-05	6.51E-05	-2.316	360	347	353.5
CCNL1	0.751633987	0.432403433	0.475	3.94E-06	5.36E-06	-1.103	317	392	354.5
ZBTB1	0.339869281	0.787017167	0.367	0.000358696	0.000401553	-2.808	385	325	355
STAT5B	0.555555556	0.600321888	0.07	0.000131324	0.000151339	-2.564	374	336	355
TLE3	0.542483666	0.613197425	0.176	0.000128277	0.000148223	-2.501	373	338	355.5
ZBTB17	0.346405229	0.782725322	0.516	0.00031086	0.000349819	-2.741	383	329	356
HIVEP2	0.392156863	0.737124464	0.111	0.000560128	0.000620604	-2.787	389	326	357.5
MED15	0.581699346	0.588519313	1.59	3.39E-05	4.16E-05	-1.855	351	365	358
MLLT3	0.503267974	0.646995708	0.163	0.000180445	0.000207397	-2.381	375	342	358.5
DNM2	0.732026144	0.442060086	0.275	1.40E-05	1.79E-05	-1.493	338	379	358.5
ABT1	0.254901961	0.860515021	4.262	0.000222909	0.000252825	-2.496	380	339	359.5
RNF125	0.568627451	0.599785408	0.585	3.95E-05	4.82E-05	-1.844	353	366	359.5
MKI67IP	0.398692841	0.741416309	0.566	0.000198924	0.000226816	-2.375	378	343	360.5
CREBBP	0.483660131	0.672746781	0.74	8.55E-05	0.000100724	-2.104	366	356	361
SP100	0.895424837	0.262339056	1.664	2.41E-06	3.38E-06	-0.386	307	416	361.5
NFAT5	0.483660131	0.657188841	0.057	0.000393066	0.000437755	-2.562	387	337	362
REL	0.522875817	0.631974249	0.367	0.000128003	0.000148223	-1.999	372	362	367
NFKBIE	0.339869281	0.774678112	0.506	0.001295403	0.001411775	-2.471	396	340	368
MAX	0.457516354	0.684012876	0.506	0.000309565	0.000349274	-2.109	382	355	368.5
NACA	1	0.083154506	7.483	2.93E-06	4.07E-06	-0.001	310	427	368.5
CREB3	0.3202614	0.7929184	0.39	0.0011428	0.0012534	-2.325	392	346	369

Gene	TP	TN	thresh_mhg	hyper_pval	hyper_qval	gen_qval	rank_hyper_qval	rank_gen_qval	mean_rank
	38	55		98	07				
ARID5B	0.424836601	0.705472103	0.214	0.000691156	0.000763816	-2.098	390	357	373.5
KDM5A	0.496732026	0.645922747	2.014	0.0003574	0.000401144	-1.727	384	369	376.5
SCAND1	0.333333333	0.781652361	1.036	0.001140785	0.001253407	-2.006	393	361	377
ARID1A	0.718954248	0.43776824	1.091	8.67E-05	0.000101559	-1.245	368	387	377.5
HSF1	0.424836601	0.696888412	0.536	0.001491896	0.001615596	-2.054	398	359	378.5
ATRX	0.555555556	0.588519313	0.07	0.0003882	0.000433479	-1.644	386	372	379
ZBTB7A	0.470588235	0.652360515	0.163	0.001743754	0.001874209	-2.01	401	360	380.5
VGLL4	0.575163399	0.572961373	0.705	0.000279845	0.00031657	-1.462	381	380	380.5
FOSB	0.444444444	0.680793991	0.214	0.001252232	0.001369827	-1.657	394	371	382.5
SERTAD2	0.424836601	0.694742489	0.202	0.001792375	0.001916907	-1.914	403	364	383.5
BCL11B	0.470588235	0.652360515	0.111	0.001743754	0.001874209	-1.777	400	367	383.5
STAT4	0.771241833	0.38304721	1.803	6.56E-05	7.79E-05	-0.734	363	404	383.5
MKL1	0.542483666	0.589592275	0.176	0.001048303	0.001155546	-1.503	391	377	384
MLL5	0.751633987	0.405579399	0.516	6.18E-05	7.36E-05	-0.666	362	407	384.5
NPM1	0.980392157	0.116416309	8.269	1.61E-05	2.04E-05	0	341	428	384.5
SQSTM1	0.941176471	0.181330472	3.383	1.64E-05	2.06E-05	0	342	429	385.5
NR3C1	0.405228758	0.709763948	0.138	0.002309675	0.002457951	-1.615	405	375	390
NCOR1	0.640522876	0.511266094	2.467	0.000196195	0.000224297	-0.744	377	403	390
NR4A3	0.437908497	0.660944206	0.604	0.009325689	0.009732135	-1.716	413	370	391.5
SMYD3	0.372549022	0.729077253	0.163	0.005458298	0.005751899	-1.605	409	376	392.5
IRF7	0.346405229	0.753755365	0.356	0.00503697	0.005320917	-1.424	408	381	394.5
MYSM1	0.614379085	0.498390558	0.043	0.004550735	0.004819083	-1.297	407	383	395
KLF6	0.803921569	0.339055794	2.618	0.000123318	0.000143261	-0.074	371	423	397
DENND4A	0.640522876	0.488733906	2.425	0.00129713	0.001411775	-0.866	395	400	397.5
NFKB1	0.424836601	0.672746781	0.151	0.009688986	0.010062537	-1.304	415	382	398.5
MED12	0.496732026	0.604077253	0.124	0.009598852	0.009993008	-1.279	414	385	399.5
PNRC1	0.575163399	0.552038627	0.526	0.001606225	0.001735045	-0.726	399	405	402
CCNT2	0.372549022	0.704935622	0.31	0.02915911	0.029780987	-1.296	422	384	403
NFATC3	0.535947712	0.572961373	0.111	0.005918713	0.006206728	-1.035	411	396	403.5
CHD2	0.477124183	0.620708155	0.07	0.0111921	0.011567854	-1.108	417	391	404

Gene	TP	TN	thresh_mhg	hyper_pval	hyper_qval	gen_qval	rank_hyper_qval	rank_gen_qval	mean_rank
UBTF	0.516339869	0.597103004	0.275	0.004173597	0.004430592	-0.745	406	402	404
RNF7	0.620915033	0.501609442	5.687	0.002240673	0.002390421	-0.521	404	408	406
ZFP36L2	0.660130719	0.466738197	2.348	0.001483667	0.001610735	-0.395	397	415	406
LDB1	0.450980392	0.637875536	0.516	0.018555368	0.019041342	-1.075	420	394	407
CHD3	0.464052288	0.626609442	0.176	0.017116817	0.017607036	-1.007	419	397	408
MXD1	0.490196078	0.608369099	0.189	0.010973178	0.011368847	-0.756	416	401	408.5
ETS1	0.941176471	0.153433476	5.962	0.000406242	0.000451263	0	388	430	409
BTG2	0.745098039	0.373927039	0.595	0.001782086	0.001910645	-0.002	402	426	414
SKIL	0.732026144	0.371781116	0.202	0.005842934	0.006142213	-0.298	410	420	415
PER1	0.640522876	0.461909871	0.227	0.008718086	0.009120133	-0.335	412	419	415.5
SMAD7	0.444444444	0.635729614	0.239	0.030500087	0.031076921	-0.459	423	410	416.5
NOTCH2	0.588235294	0.475321888	0.138	0.076060047	0.077133836	-0.509	425	409	417
MYBBP1A	0.496732026	0.589055794	0.299	0.024162196	0.024736132	-0.421	421	413	417
TGIF1	0.575163399	0.522532189	0.585	0.012493321	0.012881755	-0.373	418	417	417.5
DTX3L	0.607843137	0.438304721	0.163	0.153324771	0.154039511	-0.437	429	411	420
HBP1	0.437908497	0.608369099	0.227	0.149341221	0.150388005	-0.436	428	412	420
JMJD1C	0.424836601	0.630901288	0.151	0.100304434	0.101481716	-0.42	426	414	420
PYHIN1	0.450980392	0.604613734	0.333	0.103601863	0.104572372	-0.369	427	418	422.5
PBXIP1	0.555555556	0.511802575	0.189	0.064451529	0.065515587	-0.121	424	422	423
MAML2	0.398692841	0.614806867	0.098	0.401887503	0.402822125	-0.169	430	421	425.5
SP110	0.673202614	0.325107296	0.214	0.556195696	0.556195696	0	431	431	431

Table 12. Ranked top surface cytokines differentially expressed in cluster 9

Gene	TP	TN	thresh_mhg	hyper_pval	hyper_qval	gen_qval	rank_hyper_qval	rank_gen_qval	mean_rank
REEP4	0.784313725	0.833690987	4.746	1.02E-56	6.73E-55	-69.638	3	1	2
HMGB1	0.888888889	0.749463519	9.01	4.20E-57	4.14E-55	-55.462	2	2	2
HMMR	0.614379085	0.969420601	3.732	9.80E-80	1.93E-77	-39.839	1	3	2
CMTM7	0.888888889	0.586909871	7.429	2.70E-32	1.33E-30	-30.936	4	4	4
ATPIF1	0.803921569	0.658261803	4.569	2.70E-29	7.60E-28	-22.452	6	6	6
ENTPD1	0.777777778	0.684549356	0.263	2.51E-29	7.60E-28	-21.068	7	7	7
LGALS1	1	0.3894849	9.413	7.01E-32	2.76E-30	-19.396	5	9	7

Gene	TP	TN	thresh_mhg	hyper_pval	hyper_qval	gen_qval	rank_hyper_qval	rank_gen_qval	mean_rank
		79							
LDLR	0.352941176	0.93776824	3.8	3.33E-23	5.96E-22	-25.975	11	5	8
CLIC4	0.653594771	0.735515021	0.263	8.13E-22	1.23E-20	-20.97	13	8	10.5
HAVCR2	0.771241833	0.641094421	0.322	1.95E-23	3.84E-22	-16.568	10	11	10.5
PGLYRP1	0.908496732	0.519313305	4.99	1.20E-27	2.95E-26	-13.116	8	20	14
HNRNPU	0.869281046	0.482296137	0.516	5.29E-19	5.79E-18	-13.845	18	16	17
CCRL2	0.568627451	0.768776824	3.306	1.24E-17	1.11E-16	-14.77	22	13	17.5
ADAM8	0.660130719	0.706008584	1.722	3.90E-19	4.52E-18	-13.585	17	18	17.5
PGP	0.385620915	0.886802575	1.485	2.06E-16	1.45E-15	-18.956	28	10	19
CD2BP2	0.745098039	0.629828326	2.842	1.51E-19	1.86E-18	-11.644	16	23	19.5
PGRMC1	0.653594771	0.704399142	3.269	2.32E-18	2.29E-17	-12.887	20	21	20.5
TFRC	0.562091503	0.766630901	2.325	8.10E-17	6.14E-16	-13.808	26	17	21.5
GDI2	0.993464052	0.307939914	6.483	3.01E-22	4.94E-21	-10.08	12	33	22.5
CD48	0.973856209	0.384656652	6.166	4.69E-25	1.03E-23	-9.295	9	36	22.5
CD200R1	0.405228758	0.869635193	2.832	1.13E-15	6.52E-15	-14.696	34	14	24
NUP85	0.555555556	0.765021459	2.82	4.41E-16	2.90E-15	-13.391	30	19	24.5
SMPD1	0.522875817	0.785407725	0.632	1.62E-15	9.04E-15	-14.049	36	15	25.5
SIVA1	0.601307189	0.730150215	2.032	2.29E-16	1.56E-15	-11.903	29	22	25.5
ULBP1	0.385620915	0.878218884	0.926	3.90E-15	1.92E-14	-16.339	41	12	26.5
CAST	0.810457516	0.553648069	2.934	5.58E-19	5.79E-18	-9.419	19	35	27
GPR65	0.725490196	0.625536481	1.66	2.79E-17	2.20E-16	-10.338	25	30	27.5
IFNG	0.594771242	0.723175966	1.345	3.93E-15	1.92E-14	-10.799	40	27	33.5
TNFRSF9	0.784313725	0.548819742	0.454	5.19E-16	3.20E-15	-9.018	32	38	35
BSG	0.954248366	0.377145923	5.178	6.04E-21	8.50E-20	-6.558	14	59	36.5
H2-M3	0.647058824	0.671673862	0.678	1.13E-14	4.84E-14	-10.397	46	29	37.5
CKLF	0.405228758	0.851394855	3.815	2.59E-13	9.09E-13	-11.349	56	24	40
CX3CR1	0.418300654	0.843884112	4.682	1.72E-13	6.18E-13	-11.074	55	25	40
USP14	0.568627451	0.737124464	0.84	2.37E-14	9.72E-14	-10.133	48	32	40
MIF	0.947712418	0.311695279	6.893	1.21E-14	5.08E-14	-9.936	47	34	40.5
EZR	0.934640523	0.356759657	4.227	1.94E-16	1.41E-15	-6.892	27	56	41.5
CD96	0.745098039	0.591201717	4.496	5.07E-16	3.20E-15	-7.582	31	54	42.5

Gene	TP	TN	thresh_mhg	hyper_pval	hyper_qval	gen_qval	rank_hyper_qval	rank_gen_qval	mean_rank
ERP29	0.666666667	0.653433476	3.668	1.11E-14	4.84E-14	-8.397	45	43	44
PDIA3	0.973856209	0.305257511	5.897	4.84E-18	4.54E-17	-6.206	21	68	44.5
SPN	0.816993464	0.501609442	1.705	3.14E-15	1.63E-14	-7.667	38	52	45
CORO1A	0.973856209	0.251609442	10.66	8.36E-14	3.17E-13	-8.653	52	41	46.5
P4HB	0.901960784	0.400751073	4.749	5.68E-16	3.39E-15	-6.485	33	62	47.5
PDLIM2	0.588235294	0.708690987	2.918	2.72E-13	9.39E-13	-8.398	57	42	49.5
RPS6KB1	0.601307119	0.693669528	1.428	5.40E-13	1.73E-12	-8.895	62	39	50.5
PDIA4	0.607843137	0.699034335	4.39	5.85E-14	2.26E-13	-7.88	51	50	50.5
GOLPH3	0.418300654	0.8277897	0.401	1.01E-11	2.56E-11	-11.004	78	26	52
PSEN1	0.614379085	0.682939914	1.566	4.43E-13	1.48E-12	-8.171	59	45	52
ERP44	0.823529412	0.49248927	0.475	3.99E-15	1.92E-14	-6.424	39	65	52
PDCD1	0.928104575	0.379291845	0.669	2.08E-17	1.71E-16	-4.73	24	82	53
CAP1	0.823529412	0.474248927	0.401	9.75E-14	3.56E-13	-7.64	54	53	53.5
CD244	0.392156863	0.844420601	0.444	1.68E-11	4.05E-11	-10.792	81	28	54.5
CALR	0.915032678	0.375536481	3.187	1.65E-15	9.04E-15	-5.326	35	75	55
LAG3	0.882352941	0.478540773	2.746	3.39E-20	4.45E-19	-4.285	15	95	55
SERPINE2	0.405228758	0.835300429	1.131	1.60E-11	3.95E-11	-10.282	80	31	55.5
NR4A2	0.738562092	0.554721013	3.667	1.37E-12	4.02E-12	-8.09	67	47	57
PSTPIP1	0.856209155	0.449570815	1.696	6.09E-15	2.82E-14	-5.554	42	72	57
CCR5	0.607843137	0.68776824	2.575	5.45E-13	1.73E-12	-7.453	60	55	57.5
CR1L	0.673202614	0.621781116	2.748	1.28E-12	3.83E-12	-6.612	66	57	61.5
ITGAV	0.620915033	0.654506438	0.678	2.61E-11	6.12E-11	-8.788	84	40	62
TMX3	0.509803922	0.741416309	0.227	2.03E-10	4.39E-10	-9.051	91	37	64
LAMP2	0.549019608	0.716738197	0.585	4.06E-11	9.27E-11	-8.157	87	46	66.5
CD9	0.509803922	0.752145923	1.48	2.65E-11	6.15E-11	-8.001	85	48	66.5
ATP5B	0.934640523	0.2972103	9.253	5.31E-12	1.39E-11	-6.592	75	58	66.5
PTPRCAP	0.954248366	0.295064378	8.586	2.96E-14	1.17E-13	-4.66	50	86	68
CCL3	0.516339869	0.743562232	3.02	4.88E-11	1.09E-10	-7.774	88	51	69.5
IL10RA	0.751633987	0.538626609	1.305	1.92E-12	5.30E-12	-6.242	72	67	69.5
SEPT2	0.934640523	0.337446352	1.257	6.15E-15	2.82E-14	-4.275	43	96	69.5
CTSB	0.9411764	0.3583690	4.926	1.95E-17	1.67E-16	-2.912	23	117	70

Gene	TP	TN	thresh_mhg	hyper_pval	hyper_qval	gen_qval	rank_hyper_qval	rank_gen_qval	mean_rank
	71	99							
ANXA5	0.725490196	0.568133047	2.568	1.46E-12	4.23E-12	-5.509	68	74	71
RAC1	0.849673203	0.434012876	3.898	3.64E-13	1.24E-12	-4.661	58	85	71.5
GPR56	0.470588235	0.772532189	0.433	2.52E-10	5.23E-10	-7.91	95	49	72
HSP90AA1	0.888888889	0.377682403	2.609	7.27E-13	2.24E-12	-4.743	64	80	72
TRPV2	0.732026144	0.553648069	0.585	5.19E-12	1.38E-11	-5.671	74	71	72.5
FLOT2	0.457516344	0.773068677	0.475	1.66E-09	3.15E-09	-8.368	104	44	74
NCKAP1L	0.810457516	0.472639485	0.506	1.72E-12	4.92E-12	-4.73	69	83	76
CTLA4	0.888888889	0.390557944	2.101	8.59E-14	3.19E-13	-3.965	53	102	77.5
TLN1	0.960784314	0.290236052	1.202	8.55E-15	3.83E-14	-3.502	44	111	77.5
TNFRSF4	0.562091503	0.695815451	4.457	2.16E-10	4.59E-10	-6.475	93	63	78
PDE4D	0.588235294	0.667381974	0.239	4.81E-10	9.67E-10	-6.555	98	60	79
CD44	0.758169935	0.53111588	2.687	2.05E-12	5.53E-12	-4.415	73	92	82.5
CLPTM1	0.444444444	0.775751073	0.848	6.92E-09	1.24E-08	-6.519	110	61	85.5
PEBP1	0.856209150	0.418991416	0.956	1.08E-12	3.28E-12	-3.796	65	106	85.5
GABARAPL1	0.490196078	0.738197425	1.74	6.44E-09	1.16E-08	-6.467	109	64	86.5
TIGIT	0.934640523	0.344420601	5.588	1.79E-15	9.55E-15	-2.042	37	137	87
AIMP1	0.660130719	0.614270386	2.362	4.09E-11	9.27E-11	-4.412	86	93	89.5
CXCR6	0.888888889	0.396995708	4.511	2.88E-14	1.16E-13	-2.242	49	131	90
HSPD1	0.836601307	0.447424893	1.74	5.97E-13	1.87E-12	-2.737	63	121	92
CCL4	0.594771242	0.647532189	4.132	4.16E-09	7.73E-09	-4.809	106	79	92.5
ADAM17	0.516339869	0.707081545	0.251	2.48E-08	4.15E-08	-5.713	118	69	93.5
SLAMF1	0.405228758	0.792918455	0.444	8.43E-08	1.36E-07	-6.327	122	66	94
NOTCH1	0.732026144	0.527896996	0.07	2.92E-10	5.94E-10	-4.574	97	91	94
THY1	0.947712418	0.282188841	4.979	1.80E-12	5.07E-12	-2.795	70	120	95
LY6E	0.980392157	0.206545064	7.954	1.68E-11	4.05E-11	-3.592	82	109	95.5
AAMP	0.803921569	0.463519313	0.824	2.47E-11	5.87E-11	-3.319	83	112	97.5
CTSD	0.993464052	0.195278897	7.378	5.39E-13	1.73E-12	-2.064	61	136	98.5
FLT3L	0.437908497	0.769313305	3.761	5.17E-08	8.42E-08	-4.876	121	78	99.5
PTPN11	0.379084967	0.800965665	0.151	7.02E-07	1.05E-06	-5.679	132	70	101
PTGER2	0.372549022	0.809549356	0.782	4.05E-07	6.18E-07	-5.531	129	73	101

Gene	TP	TN	thresh_mhg	hyper_pval	hyper_qval	gen_qval	rank_hyper_qval	rank_gen_qval	mean_rank
LY75	0.424836601	0.769849785	0.475	2.66E-07	4.13E-07	-4.981	127	77	102
F2R	0.575163399	0.658261803	0.444	1.29E-08	2.20E-08	-4.588	115	89	102
RALA	0.372549002	0.809012876	3.905	4.43E-07	6.71E-07	-5.317	130	76	103
M6PR	0.862745098	0.365343348	0.566	1.03E-09	2.00E-09	-3.788	102	107	104.5
NCOR2	0.555555556	0.660944206	0.124	1.17E-07	1.87E-07	-4.587	123	90	106.5
SBDS	0.588235294	0.625	0.722	2.35E-07	3.67E-07	-4.634	126	88	107
SEMA4D	0.875816993	0.358905579	3.009	2.15E-10	4.59E-10	-2.452	92	125	108.5
GPI1	0.960784314	0.229077253	6.568	2.24E-10	4.68E-10	-2.529	94	124	109
ITGB2	0.960784314	0.248390558	6.447	1.03E-11	2.56E-11	-1.824	79	141	110
LY6A	0.633986928	0.59388412	6.556	4.02E-08	6.59E-08	-3.987	120	101	110.5
IL12RB1	0.339869281	0.825643777	1.623	1.95E-06	2.70E-06	-4.738	142	81	111.5
H13	0.928104575	0.313841202	3.084	1.94E-12	5.30E-12	-1.119	71	155	113
CD55	0.300653595	0.854613734	4.114	2.32E-06	3.19E-06	-4.711	143	84	113.5
IL2RB	0.960784314	0.222103004	7.189	6.60E-10	1.31E-09	-2.376	99	128	113.5
CD8A	0.993464052	0.136802575	6.459	1.00E-08	1.76E-08	-2.857	112	119	115.5
SCARB2	0.346405229	0.814377682	0.506	5.06E-06	6.83E-06	-4.636	146	87	116.5
ICAM1	0.529411765	0.671673882	4.07	7.01E-07	1.05E-06	-3.925	131	103	117
FERMT3	0.915032688	0.323497854	4.461	1.02E-11	2.56E-11	-1.02	77	158	117.5
HSPA5	0.960784314	0.220493562	6.325	8.45E-10	1.65E-09	-2.081	101	135	118
CMTM6	0.620915033	0.607296137	1.967	3.70E-08	6.13E-08	-2.895	119	118	118.5
ITGB1	0.790849673	0.4527897	0.31	1.15E-09	2.20E-09	-2.107	103	134	118.5
LSM1	0.477124183	0.716201717	0.748	9.74E-07	1.44E-06	-3.859	133	105	119
GPR174	0.398692841	0.778433476	0.782	1.91E-06	2.68E-06	-4.163	141	98	119.5
IDE	0.699346405	0.516630901	0.251	1.58E-07	2.50E-07	-2.991	125	115	120
TMEM123	0.849673203	0.369098712	0.367	6.36E-09	1.16E-08	-2.232	108	133	120.5
TSPAN32	0.339869281	0.81276824	0.824	1.39E-05	1.82E-05	-4.315	150	94	122
CD38	0.392156863	0.783798283	3.77	1.92E-06	2.68E-06	-3.868	140	104	122
ROCK1	0.601307189	0.598712446	0.176	1.29E-06	1.85E-06	-3.617	138	108	123
STX4A	0.359477124	0.799356223	0.816	9.71E-06	1.29E-05	-4.147	148	99	123.5
ATP6AP2	0.535947712	0.662553648	1.438	1.05E-06	1.53E-06	-3.163	134	113	123.5
GRN	0.3267973	0.8175965	0.422	3.11E-05	4.05E-05	-4.271	151	97	124

Gene	TP	TN	thresh_mhg	hyper_pval	hyper_qval	gen_qval	rank_hyper_qval	rank_gen_qval	mean_rank
	86	67							
CD97	0.784313725	0.442596567	4.364	1.31E-08	2.23E-08	-2.237	116	132	124
CD47	0.980392157	0.194742489	5.119	1.17E-10	2.57E-10	-0.957	90	160	125
ITGA4	0.843137255	0.376609442	1.799	7.03E-09	1.25E-08	-1.947	111	140	125.5
TNFRSF18	0.803921569	0.431866953	3.39	2.52E-09	4.74E-09	-1.505	105	146	125.5
CD5	0.68627451	0.531115888	3.165	1.43E-07	2.28E-07	-2.265	124	130	127
CD2	0.941176471	0.240879828	6.025	5.78E-09	1.06E-08	-1.363	107	149	128
XPOT	0.352941176	0.791845494	0.111	5.47E-05	6.86E-05	-4.105	157	100	128.5
IL21R	0.771241833	0.4222103	0.367	1.04E-06	1.53E-06	-2.625	135	122	128.5
CD52	1	0.145922747	8.417	9.16E-11	2.03E-10	-0.696	89	168	128.5
CD82	0.947712418	0.274678112	4.369	6.13E-12	1.59E-11	-0.062	76	186	131
LY9	0.437908497	0.730686695	0.566	1.29E-05	1.71E-05	-3.114	149	114	131.5
FASL	0.653594771	0.5472103	0.526	1.20E-06	1.74E-06	-2.448	136	127	131.5
CD247	0.895424837	0.304721033	4.586	1.05E-08	1.83E-08	-1.127	113	154	133.5
ITGAL	0.954248366	0.233369099	1.753	6.89E-10	1.36E-09	-0.606	100	169	134.5
NAMPT	0.372549002	0.772532189	0.189	7.67E-05	9.39E-05	-3.543	161	110	135.5
CD164	0.888888889	0.311695279	5.856	1.39E-08	2.34E-08	-1.118	117	156	136.5
ADAM10	0.660130719	0.52360515	1.316	8.02E-06	1.07E-05	-2.289	147	129	138
HSPA9	0.633986928	0.566523605	2.316	1.28E-06	1.83E-06	-1.713	137	142	139.5
PEAR1	0.372549002	0.760729614	0.287	0.000292242	0.000340661	-2.925	169	116	142.5
CD27	0.849673203	0.336909871	2.606	4.00E-07	6.16E-07	-1.021	128	157	142.5
HSP90AB1	0.928104575	0.212446352	9.441	3.90E-06	5.34E-06	-1.534	144	145	144.5
CCL5	0.960784314	0.227467811	4.626	2.87E-10	5.90E-10	0	96	193	144.5
IGF2R	0.529411765	0.621244635	0.111	0.000197024	0.000233818	-2.452	166	126	146
MYO9B	0.483660131	0.658261803	0.163	0.000355806	0.000409905	-2.536	171	123	147
CD3G	0.993464052	0.135729614	8.608	1.19E-08	2.05E-08	-0.323	114	180	147
NRP1	0.509803922	0.635729614	0.163	0.000292112	0.000340661	-2.005	168	138	153
TNFSF10	0.313725549	0.813304721	3.029	0.000217591	0.000256679	-2.003	167	139	153
KLRK1	0.594771242	0.571888412	3.883	4.96E-05	6.30E-05	-1.273	155	151	153
IL18RAP	0.529411765	0.594420601	0.411	0.001968858	0.002179017	-1.686	178	143	160.5
NR3C1	0.405228758	0.709763948	0.138	0.002309675	0.002527811	-1.615	180	144	162

Gene	TP	TN	thresh_mhg	hyper_pval	hyper_qval	gen_qval	rank_hyper_qval	rank_gen_qval	mean_rank
CD28	0.738562092	0.423819742	1.433	4.27E-05	5.50E-05	-0.399	153	173	163
TNIP1	0.535947712	0.586373391	0.433	0.002239312	0.002464494	-1.394	179	148	163.5
KLRC2	0.568627451	0.559012876	1.202	0.0015465 8	0.001731116	-1.189	176	152	164
B4GALT1	0.908496732	0.238733906	1.7	4.75E-06	6.45E-06	-0.137	145	183	164
LRPAP1	0.379084967	0.722639485	0.333	0.005659077	0.006125484	-1.42	182	147	164.5
CD3E	0.836601307	0.301502146	6.523	0.000112033	0.000134576	-0.726	164	167	165.5
IL2RG	1	0.086373391	5.406	1.75E-06	2.48E-06	0	139	194	166.5
CD84	0.614379085	0.519849785	0.275	0.000912275	0.001026961	-0.958	175	159	167
IL16	0.581699346	0.559549356	0.287	0.000513618	0.000588272	-0.919	172	162	167
LILRB4	0.477124183	0.620171674	0.632	0.011588922	0.012340636	-1.288	185	150	167.5
C1QBP	0.581699346	0.564377682	0.956	0.000337964	0.000391 64	-0.727	170	166	168
KLRC1	0.627450981	0.498390558	1.339	0.001729156	0.001924541	-0.951	177	161	169
IRAK2	0.503267974	0.591738197	0.239	0.014063724	0.014780977	-1.132	187	153	170
LTB	0.882352941	0.245708155	4.932	0.000102855	0.000124 31	-0.384	163	177	170
SELPLG	0.986928105	0.094957082	6.566	5.45E-05	6.86E-05	-0.104	156	185	170.5
CD3D	1	0.065450644	2.26	4.83E-05	6.18E-05	-0.027	154	189	171.5
CD37	0.856209150	0.287553648	2.43	4.12E-05	5.34E-05	-0.003	152	192	172
B2M	1	0.064377682	11.746	5.72E-05	7.13E-05	-0.041	158	187	172.5
SLC3A2	0.816993464	0.324570815	3.578	0.000114552	0.000136768	-0.311	165	181	173
IL27RA	0.575163399	0.532725322	0.444	0.006484301	0.006942431	-0.753	184	165	174.5
CNP	0.660130719	0.475858369	0.496	0.000720175	0.000815371	-0.398	174	175	174.5
CD226	0.549019608	0.547746781	0.705	0.013177153	0.013956447	-0.83	186	164	175
IFNGR1	0.928104575	0.186158798	7.705	8.56E-05	0.000104151	-0.034	162	188	175
MSN	0.973856209	0.116952 79	4.455	7.35E-05	9.05E-05	-0.011	160	190	175
CD8B1	1	0.063304721	7.859	6.76E-05	8.38E-05	-0.008	159	191	175
LYST	0.509803922	0.585300429	0.111	0.014105704	0.014780977	-0.896	188	163	175.5
TGFBR2	0.712418301	0.391630901	0.299	0.006312132	0.006795028	-0.399	183	174	178.5
CD6	0.732026144	0.401287554	5.207	0.000623793	0.000710331	-0.122	173	184	178.5
PDE4B	0.542483666	0.551502146	0.31	0.015560524	0.016219171	-0.578	189	170	179.5
ICOS	0.673202614	0.444206009	0.39	0.002885539	0.003140614	-0.363	181	178	179.5
STK10	0.7058823	0.3766094	0.239	0.0246782	0.0255874	-0.432	190	172	181

Gene	TP	TN	thresh_mhg	hyper_pval	hyper_qval	gen_qval	rank_hyper_qval	rank_gen_qval	mean_rank
	53	42		19	17				
NOTCH2	0.588235294	0.475321888	0.138	0.076060047	0.078040778	-0.509	192	171	181.5
CD160	0.359477124	0.696351931	0.895	0.090137346	0.092005477	-0.39	193	176	184.5
IL18R1	0.522875817	0.549356223	0.151	0.050731163	0.052324812	-0.356	191	179	185
BST2	0.39869281	0.649141631	1.345	0.135699441	0.137797886	-0.214	194	182	188
ITGB7	0.339869281	0.616416309	0.401	0.876904402	0.885898293	0	195	195	195
CCND2	0.562091503	0.386266094	0.111	0.910102194	0.914745572	0	196	196	196
IL4RA	0.366013072	0.559012876	0.287	0.970844302	0.970844302	0	197	197	197

Table 13. Ranked top 100 differentially expressed genes in cluster 9 as compared to all 15 CD8 T cell clusters

	adj.pval.cluster 1	adj.pval.cluster 2	adj.pval.cluster 3	adj.pval.cluster 4	adj.pval.cluster 5	adj.pval.cluster 6	adj.pval.cluster 7	adj.pval.cluster 8	adj.pval.cluster 9	adj.pval.cluster 10	adj.pval.cluster 11	adj.pval.cluster 12	adj.pval.cluster 13	adj.pval.cluster 14	adj.pval.cluster 15
CDC20	0	0	0	0	0	0	0	0	-184.362	-2.344	-0.001	0	0	0	-19.657
PLK1	0	0	0	0	0	0	0	0	-175.867	-4.542	-0.001	0	0	0	-13.485
CDCA3	0	0	0	0	0	0	-0.024	0	-167.524	-10.223	-0.001	0	0	0	-15.116
CCNB2	0	0	0	0	0	0	-1.05	0	-165.839	-8.768	-0.001	0	0	0	-15.116
FAM64A	0	0	0	0	0	0	0	0	-165.547	-3.612	-0.001	0	0	0	-11.262
NEK2	0	0	0	0	0	0	0	0	-163.602	-5.146	-0.001	0	0	0	-7.58
CCNA2	0	0	0	0	0	0	0	0	-158.623	-27.377	-0.001	0	0	0	-13.106
KIF20A	0	0	0	0	0	0	0	0	-155.825	-8.605	0	0	0	0	-13.485
CEP55	0	0	0	0	0	0	0	0	-153.213	-4.218	-0.001	0	0	0	-13.685
CDCA8	0	0	0	0	0	0	0	0	-150.419	-20.638	-0.001	0	0	-1.963	-11.541
CDKN3	0	0	0	0	0	0	-0.139	0	-148.364	-1.429	-0.001	0	0	0	-10.472
KIF2C	0	0	0	0	0	0	0	0	-144.555	-3.85	-0.001	0	0	0	-16.857
CKAP	0	0	0	0	0	0	0	0	-	-	-	0	0	0	-

	adj.pval.cluster 1	adj.pval.cluster 2	adj.pval.cluster 3	adj.pval.cluster 4	adj.pval.cluster 5	adj.pval.cluster 6	adj.pval.cluster 7	adj.pval.cluster 8	adj.pval.cluster 9	adj.pval.cluster 10	adj.pval.cluster 11	adj.pval.cluster 12	adj.pval.cluster 13	adj.pval.cluster 14	adj.pval.cluster 15
2L									142.355	13.708	0.001				7.314
KIF22	0	0	0	0	0	0	0	0	-140.192	-16.462	-0.001	0	0	0	-6.208
CENPE	0	0	0	0	0	0	0	0	-136.216	-3.348	-0.001	0	0	0	-8.748
BUB1	0	0	0	0	0	0	0	0	-134.146	-20.446	-0.001	0	0	0	-11.29
BUB1B	0	0	0	0	0	0	-0.206	0	-134.119	-8.876	-0.001	-0.045	0	0	-10.758
CCNB1	0	0	0	0	0	0	-0.191	0	-133.892	-4.436	-0.001	0	0	0	-16.09
MKI67	0	0	0	0	0	0	0	0	-133.503	-25.436	0	0	-0.086	0	-9.865
NUSAP1	0	0	0	0	0	0	0	0	-129.805	-32.937	-0.001	0	0	0	-7.459
KIF4	0	0	0	0	0	0	0	0	-129.036	-8.502	-0.001	0	0	0	-11.574
TACC3	0	0	0	0	0	0	-0.179	0	-127.27	-16.479	0	0	0	0	-10.348
TROAP	0	0	0	0	0	0	0	0	-126.503	-1.124	-0.001	0	0	0	-8.15
ASPM	0	0	0	0	0	0	0	0	-125.186	-4.045	-0.001	0	0	0	-3.083
CKS1B	0	0	0	0	0	0	-2.318	0	-123.685	-23.331	-0.001	0	0	-0.721	-11.29
SAPCD2	0	0	0	0	0	0	0	0	-123.685	-3.581	-0.001	0	0	0	-7.515
KIF23	0	0	0	0	0	0	0	0	-123.218	-5.376	-0.001	0	0	0	-10.472
CKAP2	0	0	0	0	0	0	-0.29	0	-121.254	-3.232	-0.001	0	0	0	-10.687
PIF1	0	0	0	0	0	0	0	0	-120.466	-0.217	-0.001	0	0	0	-0.702
GTSE1	0	0	0	0	0	0	0	0	-115.338	-13.376	-0.001	0	0	0	-4.787
PARPBP	0	0	0	0	0	0	0	0	-115.188	-3.439	-0.001	0	0	0	-9.09
AURKB	0	0	0	0	0	0	0	0	-115.142	-33.175	-0.001	0	0	0	-7.378

	adj.pval.cluster 1	adj.pval.cluster 2	adj.pval.cluster 3	adj.pval.cluster 4	adj.pval.cluster 5	adj.pval.cluster 6	adj.pval.cluster 7	adj.pval.cluster 8	adj.pval.cluster 9	adj.pval.cluster 10	adj.pval.cluster 11	adj.pval.cluster 12	adj.pval.cluster 13	adj.pval.cluster 14	adj.pval.cluster 15
DEPDC1A	0	0	0	0	0	0	0	0	-115.01	-15.849	-0.001	0	0	0	-6.805
CDC25C	0	0	0	0	0	0	0	0	-114.908	-1.332	-0.001	0	0	0	-15.116
KNSTRN	0	0	0	0	0	0	-0.391	0	-114.296	-4.993	-0.001	0	0	0	-11.96
SKA1	0	0	0	0	0	0	0	0	-112.855	-11.739	-0.001	0	0	0	-4.976
AURKA	0	0	0	0	0	0	0	0	-111.39	-1.655	-0.001	0	0	0	-11.464
ECT2	0	0	0	0	0	0	0	0	-106.144	-7.388	-0.001	0	0	0	-8.951
SHCBP1	0	0	0	0	0	0	0	0	-104.967	-11.892	-0.001	0	0	0	-5.066
SPAG5	0	0	0	0	0	0	0	0	-104.142	-8.895	-0.001	0	0	0	-10.147
MELK	0	0	0	0	0	0	0	0	-103.052	-15.26	-0.001	0	0	0	-6.238
ARHGAP19	0	0	0	0	0	0	0	0	-103.014	-1.382	-0.001	0	0	0	-7.167
UBE2C	0	0	0	0	0	0	-0.472	0	-102.684	-2.539	-0.001	0	0	0	-7.412
SPC25	0	0	0	0	0	0	0	0	-100.821	-24.336	-0.001	0	0	0	-5.838
TPX2	0	0	0	0	0	0	0	0	-100.808	-10.384	-0.001	0	0	0	-10.249
NCAPG	0	0	0	0	0	0	0	0	-99.319	-40.113	-0.001	0	0	0	-5.526
SGOL1	0	0	0	0	0	0	0	0	-98.56	-15.25	-0.001	0	0	0	-5.511
STMN1	0	0	0	0	0	0	-0.635	0	-98.221	-68.25	-0.001	0	0	0	-8.858
FOXM1	0	0	0	0	0	0	0	0	-96.119	-4.817	-0.001	0	0	0	-2.686
BIRC5	0	0	0	0	0	0	-0.026	0	-94.968	-14.204	-0.001	0	0	0	-7.921
MAD2L1	0	0	0	0	0	0	-0.454	0	-94.515	-33.55	-0.001	0	0	0	-7.613
2810417H13RI	0	0	0	0	0	0	-0.877	0	-94.353	-61.938	-0.001	0	0	0	-7.68

	adj.pval.cluster 1	adj.pval.cluster 2	adj.pval.cluster 3	adj.pval.cluster 4	adj.pval.cluster 5	adj.pval.cluster 6	adj.pval.cluster 7	adj.pval.cluster 8	adj.pval.cluster 9	adj.pval.cluster 10	adj.pval.cluster 11	adj.pval.cluster 12	adj.pval.cluster 13	adj.pval.cluster 14	adj.pval.cluster 15
K															
NEIL3	0	0	0	0	0	0	0	0	-91.051	-21.987	-0.001	0	0	0	-6.154
NCAPD2	0	0	0	0	0	0	-0.408	0	-90.985	-35.029	0	0	0	0	-10.266
CDK1	0	0	0	0	0	0	0	0	-89.58	-36.898	-0.001	0	0	0	-5.968
CENPA	0	0	0	0	0	0	-17.723	-1.237	-88.504	-1.854	-0.001	0	0	0	-8.377
NDC80	0	0	0	0	0	0	0	0	-87.237	-16.831	-0.001	0	0	0	-3.648
ESPL1	0	0	0	0	0	0	0	0	-87.003	-8.242	-0.001	0	0	0	-7.975
MIS18BP1	0	0	0	0	0	0	0	0	-86.091	-8.204	-0.001	0	0	0	-3.528
MXD3	0	0	0	0	0	0	0	0	-84.852	-4.135	-0.001	0	0	0	-4.433
C330027C09RIK	0	0	0	0	0	0	0	0	-84.459	-10.643	-0.001	0	0	0	-9.216
HMGB2	0	0	0	0	0	0	0	0	-83.608	-27.111	-0.001	0	0	0	-7.061
CDCA2	0	0	0	0	0	0	-0.02	0	-82.191	-36.305	-0.001	0	0	0	-5.418
ARHGAP11A	0	0	0	0	0	0	0	0	-79.407	-14.392	-0.001	0	0	-0.499	-7.496
1190002F15RIK	0	0	0	0	0	0	-0.771	-0.022	-79.269	-7.813	-0.001	0	0	0	-12.234
HIST1H2AO	0	0	0	0	0	0	-0.199	0	-77.944	-37.764	-0.001	0	-0.147	0	-5.609
SKA2	0	0	0	0	0	0	-0.013	0	-76.368	-12.484	-0.001	0	0	0	-4.225
RACGAP1	0	0	0	0	0	0	0	0	-76.052	-8.624	0	0	0	0	-7.106
CDCA5	0	0	0	0	0	0	0	0	-75.623	-38.383	-0.001	0	0	0	-3.513
ASF1B	0	0	0	0	0	0	-0.123	0	-73.726	-56.208	-0.001	0	0	-4.035	-3.737
NCAPH	0	0	0	0	0	0	-0.439	0	-72.57	-38.07	-0.001	-0.609	0	0	-2.757

	adj.pval.cluster 1	adj.pval.cluster 2	adj.pval.cluster 3	adj.pval.cluster 4	adj.pval.cluster 5	adj.pval.cluster 6	adj.pval.cluster 7	adj.pval.cluster 8	adj.pval.cluster 9	adj.pval.cluster 10	adj.pval.cluster 11	adj.pval.cluster 12	adj.pval.cluster 13	adj.pval.cluster 14	adj.pval.cluster 15
									4	6					
CKS2	0	0	0	0	0	0	-0.558	-0.086	-72.359	-6.677	-0.001	0	0	-0.039	-5.626
NUF2	0	0	0	0	0	0	0	0	-72.288	-9.096	0	0	0	0	-4.518
CASC5	0	0	0	0	0	0	0	0	-70.176	-16.007	-0.001	0	0	0	-1.245
TOP2A	0	0	0	0	0	0	-1.539	-0.04	-69.996	-49.278	-0.001	0	0	0	-3.78
REEP4	0	0	0	0	0	0	0	0	-69.638	-3.201	-0.001	0	0	0	-7.177
TUBB4B	0	0	0	0	0	0	-1.175	0	-69.61	-5.712	-0.001	0	0	0	-3.285
KIF11	0	0	0	0	0	0	0	0	-69.213	-16.195	-0.001	0	0	0	-4.304
HIST2H3C2	0	0	0	0	0	0	0	0	-68.991	-31.983	-0.001	0	0	0	-6.158
FAM83D	0	0	0	0	0	0	-0.064	0	-68.714	-13.104	-0.001	0	0	0	-1.971
HMGN2	0	0	0	0	0	0	-0.795	0	-68.584	-21.667	-0.001	0	0	0	-5.602
SMC2	0	0	0	0	0	0	-0.203	0	-68.225	-39.346	-0.001	0	0	0	-3.648
CENPW	0	0	0	0	0	0	-3.078	-0.012	-67.606	-17.172	-0.001	0	0	0	-5.443
ZWILCH	0	0	0	0	0	0	0	0	-67.361	-9.87	-0.001	0	0	0	-7.311
HMGB3	0	0	0	0	0	0	-8.432	-1.502	-67.08	-12.654	-0.001	0	0	0	-9.633
CIT	0	0	0	0	0	0	0	0	-66.701	-12.005	-0.001	0	0	0	-3.906
RRM2	0	0	0	0	0	0	-0.044	0	-66.459	-72.59	-0.001	0	0	0	-3.577
H2AFZ	0	0	0	0	0	0	-0.317	-0.763	-65.313	-19.273	-0.001	0	0	0	-7.39
CEP89	0	0	0	0	0	0	-0.285	0	-63.892	-0.003	-0.001	0	0	0	-8.453
TUBA1C	0	0	0	0	0	0	-1.248	0	-63.107	-1.775	-0.001	-1.171	0	-0.241	-1.841
PLK4	0	0	0	0	0	0	-0.876	0	-62.50	-16.71	-0.001	0	0	0	-2.986

	adj.pval.cluster 1	adj.pval.cluster 2	adj.pval.cluster 3	adj.pval.cluster 4	adj.pval.cluster 5	adj.pval.cluster 6	adj.pval.cluster 7	adj.pval.cluster 8	adj.pval.cluster 9	adj.pval.cluster 10	adj.pval.cluster 11	adj.pval.cluster 12	adj.pval.cluster 13	adj.pval.cluster 14	adj.pval.cluster 15
									4	1					
POC1A	0	0	0	0	0	0	-0.437	0	-62.189	-7.108	-0.001	0	0	0	-9.259
TUBA1B	0	0	0	0	0	0	-0.341	0	-62.089	-32	-0.001	-0.81	0	0	-4.026
GPSM2	0	0	0	0	0	0	-0.012	0	-61.281	-1.655	-0.001	0	0	0	-2.287
CENPN	0	0	0	0	0	0	0	-0.069	-61.278	-12.165	-0.001	0	0	0	-6.532
TTK	0	0	0	0	0	0	0	0	-61.232	-11.957	-0.001	0	0	0	-12.143
GEN1	0	0	0	0	0	0	0	0	-61.077	-12.717	-0.001	0	0	0	-3.094
HIST2H3B	0	0	0	0	0	0	0	0	-60.755	-33.065	-0.001	0	0	0	-5.492
PBK	0	0	0	0	0	0	0	0	-60.6	-36.206	-0.001	0	0	0	-4.177

Table 14. Cluster 10 Specific Gene Signature

	0	7	8	9	rank_0	rank_7	rank_8	rank_9	mean_rank
MCM5	-90.123	-32.703	-34.411	-14.846	3	3	1	6	3.25
MCM7	-83.194	-33.793	-32.988	-18.085	8	1	2	4	3.75
LIG1	-93.006	-28.89	-31.839	-11.559	2	4	3	8	4.25
MCM3	-81.522	-25.562	-30.682	-18.786	12	7	4	2	6.25
MCM2	-68.448	-23.944	-29.597	-22.092	20	10	6	1	9.25
TIPIN	-75.17	-25.275	-25.529	-11.425	15	9	9	9	10.5
PRIM1	-85.258	-22.464	-25.424	-6.265	6	12	11	25	13.5
MCM6	-53.377	-21.391	-25.799	-18.381	31	16	7	3	14.25
CDC6	-89.823	-19.414	-20.77	-8.491	4	20	23	15	15.5
POLA1	-80.75	-22.871	-22.23	-6.85	13	11	19	23	16.5
FEN1	-81.94	-27.864	-24.896	-3.926	9	5	12	45	17.75
DHFR	-75.759	-17.755	-20.45	-6.927	14	21	26	22	20.75
HELLS	-84.063	-14.995	-20.765	-6.689	7	30	24	24	21.25
SLBP	-43.549	-21.883	-24.035	-10.174	49	13	14	10	21.5
MCM4	-48.681	-20.669	-22.398	-9.103	37	18	18	13	21.5
RFC3	-81.642	-21.693	-16.201	-6.136	11	14	35	26	21.5
UHRF1	-87.385	-21.415	-25.465	-3.552	5	15	10	56	21.5
DUT	-61.556	-16.56	-23.6	-7.124	25	25	16	21	21.75
DTL	-81.642	-12.956	-20.973	-8.365	10	40	22	17	22.25
POLD1	-64.464	-20.796	-24.315	-4.562	22	17	13	38	22.5
CCNE1	-62.539	-15.342	-17.384	-8.704	23	29	31	14	24.25
PCNA	-45.092	-15.554	-23.972	-8.416	43	28	15	16	25.5
DNMT1	-45.002	-20.339	-25.599	-5.229	44	19	8	32	25.75
CHAF1B	-50.588	-14.572	-16.878	-7.345	34	31	32	20	29.25
DNAJC9	-33.663	-27.523	-22.908	-5.381	68	6	17	29	30
TCF19	-70.03	-25.562	-18.913	-3.012	18	8	27	68	30.25
CDK2	-56.916	-15.972	-14.41	-4.101	26	26	41	41	33.5
CHEK1	-61.86	-14.505	-14.205	-4.779	24	33	43	35	33.75

	0	7	8	9	rank_0	rank_7	rank_8	rank_9	mean_rank
DCK	-43.927	-17.41	-15.557	-5.357	48	22	37	30	34.25
GINS2	-69.56	-15.692	-17.414	-3.214	19	27	30	62	34.5
CDCA7	-54.772	-8.493	-14.713	-13.381	28	65	40	7	35
CDCA7L	-46.809	-11.956	-13.162	-9.167	41	47	46	12	36.5
RRM2	-96.054	-33.678	-29.597	-1.388	1	2	5	140	37
RANBP1	-34.581	-12.464	-20.765	-5.674	66	44	25	28	40.75
CCNE2	-54.01	-10.64	-11.3	-5.789	30	50	57	27	41
POLE	-70.119	-13.094	-16.346	-2.524	17	38	34	78	41.75
UNG	-51.405	-9.422	-9.92	-18.085	32	57	74	5	42
RPA2	-36.145	-12.413	-21.529	-3.959	61	45	20	43	42.25
ORC6	-56.235	-13.409	-21.479	-2.338	27	35	21	86	42.25
POLD2	-39.416	-14.115	-16.193	-3.679	54	34	36	54	44.5
TYMS	-38.405	-17.245	-18.75	-2.444	57	23	28	83	47.75
SYCE2	-46.6	-8.869	-12.167	-4.542	42	61	53	39	48.75
WDHD1	-49.543	-8.473	-13.05	-3.793	36	66	48	48	49.5
RFC2	-42.522	-9.381	-13.835	-3.74	51	58	44	49	50.5
CHAF1A	-67.019	-12.956	-16.531	-1.761	21	41	33	115	52.5
SIVA1	-54.441	-5.14	-11.562	-4.243	29	94	56	40	54.75
CTPS	-38.876	-5.774	-10.471	-8.365	55	86	69	18	57
MCM10	-71.959	-16.96	-13.732	-1.352	16	24	45	145	57.5
PAICS	-32.263	-9.302	-8.927	-7.918	70	60	82	19	57.75
RPA1	-25.501	-11.755	-14.822	-3.448	90	48	39	57	58.5
CAD	-31.972	-6.842	-11.038	-4.859	71	76	59	33	59.75
POLD3	-34.711	-9.428	-11.3	-3.288	65	56	58	61	60
E2F1	-34.143	-7.228	-8.388	-9.253	67	74	90	11	60.5
PPIL1	-50.14	-10.663	-12.721	-1.932	35	49	51	107	60.5
IPO5	-37.104	-6.707	-9.883	-5.337	60	77	75	31	60.75
WDR76	-44.185	-9.37	-14.309	-2.067	47	59	42	96	61
MYBL2	-47.404	-12.618	-9.979	-2.08	39	43	73	94	62.25
SHMT1	-39.536	-6.17	-10.813	-3.711	53	83	62	52	62.5
ATAD5	-47.253	-11.973	-8.325	-2.493	40	46	92	79	64.25
PASK	-47.422	-13.083	-8.796	-2.067	38	39	85	97	64.75
CDK4	-25.426	-13.124	-8.704	-3.926	91	37	87	46	65.25
CDT1	-18.754	-14.535	-18.171	-2.247	111	32	29	90	65.5
TFDP1	-44.751	-5.669	-12.984	-2.448	45	87	49	82	65.75
HAT1	-37.445	-10.189	-11.737	-2.027	59	53	55	101	67
TIMELESS	-42.896	-10.424	-13.162	-1.621	50	52	47	119	67
ZFP367	-51.286	-13.232	-9.689	-1.574	33	36	77	125	67.75
4930422G04RIK	-44.633	-9.677	-7.637	-2.929	46	54	102	70	68
ATAD2	-22.651	-8.638	-10.471	-3.73	99	63	68	50	70
SMC6	-22.559	-8.762	-8.82	-4.644	100	62	83	37	70.5
HNRNPD	-28.669	-7.888	-10.506	-3.17	83	72	66	65	71.5
PARP1	-31.808	-5.203	-10.51	-3.37	72	92	65	58	71.75
SLC29A1	-38.605	-8.588	-8.092	-2.717	56	64	94	75	72.25
POLA2	-28.277	-7.476	-10.921	-2.799	85	73	60	72	72.5
NASP	-31.654	-12.642	-15.514	-1.379	74	42	38	142	74
MTHFD1	-30.483	-3.588	-10.343	-4.087	77	111	71	42	75.25
PAQR4	-31.745	-5.803	-8.82	-3.008	73	85	84	69	77.75
NAP1L1	-28.365	-4.112	-10.613	-3.21	84	102	64	63	78.25
NUP85	-32.877	-10.626	-12.489	-1.362	69	51	52	143	78.75
MSH2	-23.769	-5.83	-8.069	-3.956	97	84	95	44	80
DSCC1	-41.202	-8.301	-10.354	-1.52	52	70	70	131	80.75
MTBP	-35.475	-8.339	-7.861	-1.964	63	69	98	103	83.25
NT5C3L	-26.158	-6.851	-9.16	-2.136	88	75	80	93	84
SLC43A3	-35.982	-8.363	-9.27	-1.438	62	67	78	136	85.75
USP37	-25.276	-3.546	-7.032	-4.779	93	112	104	36	86.25
RBBP7	-26.131	-8.341	-9.817	-1.556	89	68	76	129	90.5
MCMBP	-14.129	-6.679	-4.822	-4.791	127	78	125	34	91
EXOSC7	-24.542	-5.076	-5.504	-3.634	95	96	118	55	91
DTYMK	-29.657	-6.609	-8.723	-1.618	80	79	86	120	91.25

	0	7	8	9	rank_0	rank_7	rank_8	rank_9	mean_rank
CISD1	-37.888	-1.972	-10.823	-1.766	58	133	61	114	91.5
TKT	-22.08	-3.706	-8.115	-3.032	102	108	93	66	92.25
DNA2	-35.236	-5.076	-6.534	-1.796	64	95	106	112	94.25
UMPS	-30.1	-6.37	-7.688	-1.736	78	81	101	118	94.5
PRDX1	-22.428	-3.916	-12.859	-1.594	101	104	50	123	94.5
PFAS	-21.576	-4.045	-4.517	-3.895	103	103	126	47	94.75
NOP56	-9.608	-4.819	-12.159	-2.324	139	98	54	88	94.75
VDAC3	-27.822	-2.584	-10.118	-1.829	86	122	72	111	97.75
HSPA8	-7.171	-5.407	-8.702	-2.76	143	89	88	73	98.25
D430020J02RIK	-31.014	-3.695	-3.997	-2.472	76	110	129	81	99
FXN	-14.452	-8.072	-8.054	-1.961	126	71	96	105	99.5
MB21D1	-21.521	-5.158	-6.34	-2.08	104	93	108	95	100
PPAT	-30.083	-3.353	-5.093	-2.336	79	114	121	87	100.25
TRMT2A	-19.903	-3.235	-7.745	-2.472	107	115	100	80	100.5
DDB2	-10.851	-9.528	-9.113	-1.53	136	55	81	130	100.5
SRSF7	-10.641	-5.473	-10.799	-1.761	137	88	63	116	101
MEAF6	-29.64	-6.356	-6.161	-1.509	81	82	112	133	102
TIMM23	-16.88	-2.544	-5.536	-3.7	117	124	117	53	102.75
IDE	-18.63	-1.829	-6.401	-3.303	112	134	107	60	103.25
MAD2L2	-29.167	-1.521	-5.301	-2.8	82	144	119	71	104
TMEM109	-26.971	-4.731	-5.989	-1.757	87	99	114	117	104.25
LRDD	-23.864	-3.707	-9.249	-1.452	96	107	79	135	104.25
SHMT2	-19.307	-1.636	-7.904	-2.74	108	140	97	74	104.75
NCL	-8.413	-3.369	-6.743	-3.185	142	113	105	64	106
INTS7	-18.073	-1.45	-8.365	-2.565	113	145	91	76	106.25
NHP2L1	-15.754	-3.812	-7.845	-1.964	121	105	99	104	107.25
NAA38	-17.88	-6.51	-7.218	-1.504	114	80	103	134	107.75
FH1	-31.651	-3.228	-3.723	-1.915	75	116	133	108	108
SLC25A5	-13.401	-2.525	-8.646	-2.148	128	125	89	92	108.5
SNX5	-16.108	-2.054	-4.93	-3.338	120	132	124	59	108.75
MRPL49	-23.216	-4.622	-5.75	-1.618	98	100	116	121	108.75
NAA50	-24.697	-2.572	-5.853	-1.957	94	123	115	106	109.5
GM5141	-25.378	-5.054	-4.943	-1.569	92	97	123	126	109.5
POLE4	-16.116	-2.844	-3.438	-3.03	119	118	137	67	110.25
GART	-18.883	-2.096	-6.061	-2.286	110	131	113	89	110.75
ADAM19	-8.578	-2.481	-10.484	-1.888	141	126	67	109	110.75
2310022A10RIK	-21.145	-2.737	-3.497	-2.386	106	121	135	85	111.75
TONSL	-21.5	-5.292	-6.29	-1.359	105	91	109	144	112.25
ZMYND19	-17.551	-2.787	-4.948	-2.039	116	119	122	99	114
SET	-12.486	-1.562	-3.662	-3.712	134	143	134	51	115.5
SCARB1	-12.553	-4.609	-3.493	-2.067	132	101	136	98	116.75
9130206I24RIK	-17.736	-3.792	-3.303	-1.836	115	106	139	110	117.5
PPRC1	-15.17	-2.156	-5.239	-2.006	123	129	120	102	118.5
MBOAT1	-12.7	-2.32	-2.953	-2.439	130	128	140	84	120.5
PMM1	-11.704	-2.781	-6.276	-1.6	135	120	110	122	121.75
CBX2	-12.558	-5.308	-2.473	-1.594	131	90	142	124	121.75
ARL6IP6	-15.73	-3.703	-1.941	-1.796	122	109	145	113	122.25
NEFH	-14.673	-1.777	-3.801	-2.032	125	137	132	100	123.5
PACS1	-3.545	-1.613	-3.383	-2.565	146	141	138	77	125.5
SFMBT1	-16.43	-3.018	-1.942	-1.566	118	117	144	128	126.75
PPID	-14.792	-2.116	-4.242	-1.569	124	130	128	127	127.25
DPYSL2	-19.228	-1.602	-3.828	-1.514	109	142	131	132	128.5
DHX9	-12.773	-1.768	-6.219	-1.391	129	138	111	139	129.25
AKAP8	-5.348	-1.438	-2.754	-2.189	145	146	141	91	130.75
TSN	-12.538	-1.804	-4.324	-1.381	133	135	127	141	134
FKBP3	-8.617	-2.432	-2.426	-1.392	140	127	143	138	137
SLC38A1	-10.5	-1.788	-1.436	-1.414	138	136	146	137	139.25
ACPL2	-6.848	-1.733	-3.942	-1.313	144	139	130	146	139.75

Table 15. Ranked top transcription factors differentially expressed in cluster 10

Gene	TP	TN	thresh_mhg	hyper_pval	hyper_qval	gen_qval	rank_hyper_qval	rank_gen_qval	mean_rank
UHRF1	0.838461538	0.803921569	1.816	2.68E-51	1.02E-48	-63.748	1	1	1
TCF19	0.646153846	0.889772125	5.024	5.89E-43	1.13E-40	-59.212	2	2	2
CCNE1	0.607692308	0.8945416	0.526	4.03E-39	3.08E-37	-56.691	5	3	4
CHAF1A	0.6	0.883942766	0.856	1.19E-35	6.48E-34	-48.964	7	4	5.5
CHAF1B	0.607692308	0.875993641	5.16	7.63E-35	3.64E-33	-45.683	8	5	6.5
DNMT1	0.792307692	0.767355591	5.755	3.98E-38	2.53E-36	-40.123	6	8	7
PMF1	0.807692308	0.762056174	3.684	2.43E-39	2.32E-37	-39.271	4	10	7
PTMA	0.761538462	0.80445151	11.266	2.83E-40	3.61E-38	-34.989	3	12	7.5
BRCA1	0.384615385	0.951775305	0.546	1.01E-27	2.98E-26	-42.986	13	6	9.5
WDHD1	0.561538462	0.878113408	0.214	6.32E-30	2.41E-28	-39.503	10	9	9.5
MYBL2	0.369230769	0.951245363	0.651	9.79E-26	2.34E-24	-41.707	16	7	11.5
HMGB2	0.938461538	0.562798092	7.028	1.84E-32	7.82E-31	-27.111	9	17	13
E2F1	0.376923077	0.955484897	2.642	5.09E-28	1.77E-26	-30.829	11	16	13.5
TIMELESS	0.507692308	0.892951775	0.88	5.58E-27	1.42E-25	-34.828	15	13	14
E2F8	0.369230769	0.948065713	0.275	8.68E-25	1.84E-23	-38.663	18	11	14.5
TFDP1	0.692307692	0.772655008	4.093	4.12E-27	1.13E-25	-31.03	14	15	14.5
WHSC1	0.730769231	0.746687864	1.233	8.22E-28	2.62E-26	-26.17	12	18	15
RAD54B	0.392307692	0.930047695	0.604	1.14E-22	2.18E-21	-31.811	20	14	17
HNRNPD	0.815384615	0.645468998	0.39	2.55E-25	5.72E-24	-23.382	17	19	18
HMGB1	0.661538462	0.763645999	9.144	7.18E-23	1.44E-21	-22.967	19	20	19.5
DEK	0.830769231	0.582935877	6.123	6.64E-21	1.10E-19	-22.005	23	21	22
RBL1	0.530769231	0.835188129	0.31	7.92E-20	1.26E-18	-20.873	24	24	24
E2F3	0.415384615	0.893481717	0.575	6.19E-18	8.16E-17	-21.687	29	22	25.5
NMRAL1	0.407692308	0.895071542	3.065	1.70E-17	2.03E-16	-21.391	32	23	27.5
ERH	0.907692308	0.454689984	7.808	2.04E-18	2.89E-17	-17.295	27	28	27.5
SSRP1	0.876923077	0.51245363	1.251	1.13E-19	1.72E-18	-16.688	25	30	27.5
TOX	0.9	0.498145204	3.281	4.67E-21	8.11E-20	-14.785	22	34	28
RBBP4	0.815384615	0.581875994	3.707	2.75E-19	4.05E-18	-15.892	26	32	29
CBX3	0.915384615	0.425013249	5.158	5.12E-17	5.93E-16	-17.551	33	27	30
BAZ1B	0.6846153	0.6989931	0.888	4.53E-18	6.19E-17	-14.523	28	35	31.5

Gene	TP	TN	thresh_mhg	hyper_pval	hyper_qval	gen_qval	rank_hyper_qval	rank_gen_qval	mean_rank
	85	11							
EZH2	0.546153846	0.797562268	0.678	1.31E-16	1.43E-15	-16.795	35	29	32
ANAPC11	0.792307692	0.587705352	0.433	1.24E-17	1.53E-16	-14.892	31	33	32
E2F2	0.369230769	0.899841017	0.322	6.93E-15	6.78E-14	-18.005	39	26	32.5
RFC1	0.561538462	0.786963434	0.189	1.04E-16	1.17E-15	-16.019	34	31	32.5
PA2G4	0.853846154	0.518812931	3.128	7.96E-18	1.01E-16	-13.825	30	36	33
TRIP13	0.323076923	0.922098569	2.359	2.04E-14	1.73E-13	-18.924	45	25	35
CDCA4	0.623076923	0.72972973	5.025	6.24E-16	6.63E-15	-11.574	36	45	40.5
XRCC6	0.4	0.875993641	5.96	3.90E-14	3.24E-13	-13.106	46	38	42
RBBP8	0.476923077	0.821409645	1.864	8.80E-14	6.59E-13	-13.798	51	37	44
MAZ	0.630769231	0.706942236	0.401	1.69E-14	1.54E-13	-11.557	42	46	44
RUVBL2	0.569230769	0.760466349	6.094	1.15E-14	1.07E-13	-11.503	41	47	44
POLE3	0.446153846	0.843137255	4.173	8.28E-14	6.33E-13	-12.77	50	41	45.5
PHF5A	0.715384615	0.641229465	4.608	1.55E-15	1.60E-14	-9.926	37	54	45.5
SUZ12	0.469230769	0.822469528	0.475	2.53E-13	1.82E-12	-12.873	53	39	46
HMGB3	0.561538462	0.756756757	0.444	9.23E-14	6.78E-13	-12.654	52	42	47
GTF3A	0.676923077	0.6645469	4.167	1.89E-14	1.64E-13	-10.318	44	51	47.5
HDAC1	0.792307692	0.540540541	6.094	5.10E-14	4.15E-13	-10.352	47	50	48.5
TARDBP	0.684615385	0.650768415	3.32	6.15E-14	4.89E-13	-10.033	48	53	50.5
SMYD2	0.407692308	0.857445681	3.791	1.58E-12	9.76E-12	-12.772	62	40	51
LITAF	0.938461538	0.458929518	0.774	1.47E-22	2.68E-21	-7.349	21	81	51
TCERG1	0.592307692	0.725490196	2.272	2.89E-13	2.04E-12	-10.741	54	49	51.5
UBTF	0.692307692	0.650238474	2.485	1.80E-14	1.60E-13	-9.196	43	60	51.5
PPRC1	0.4	0.862215156	0.214	1.72E-12	1.04E-11	-12.568	63	43	53
GTF2F2	0.507692308	0.789613143	5.486	6.71E-13	4.34E-12	-11.27	59	48	53.5
TFDP2	0.307692308	0.916269201	1.876	3.15E-12	1.80E-11	-11.858	67	44	55.5
RBL2	0.753846154	0.568627451	2.406	4.92E-13	3.35E-12	-9.537	56	56	56
AES	0.776923077	0.542660307	0.934	5.69E-13	3.75E-12	-9.525	58	57	57.5
MSL3	0.461538462	0.812930578	2.029	7.53E-12	4.11E-11	-10.245	70	52	61
CBX6	0.453846154	0.819289878	0.748	6.44E-12	3.62E-11	-9.482	68	58	63
TFAM	0.484615385	0.785373609	1.084	5.76E-11	2.86E-10	-9.718	77	55	66

Gene	TP	TN	thresh_mhg	hyper_pval	hyper_qval	gen_qval	rank_hyper_qval	rank_gen_qval	mean_rank
COMMD3	0.738461538	0.595654478	4.241	8.22E-14	6.33E-13	-7.277	49	86	67.5
GTF2H5	0.676923077	0.638049815	2.511	1.94E-12	1.16E-11	-7.617	64	73	68.5
FUBP1	0.707692308	0.589295178	0.345	3.43E-11	1.72E-10	-8.408	76	62	69
NR4A2	0.823076923	0.485426603	1	1.08E-12	6.85E-12	-7.402	60	78	69
SUB1	0.823076923	0.466878643	6.946	1.66E-11	8.66E-11	-7.991	73	66	69.5
EDF1	0.953846154	0.341282459	6.46	1.79E-15	1.80E-14	-6.142	38	103	70.5
TCEA1	0.8	0.519342872	3.287	3.75E-13	2.60E-12	-7.214	55	87	71
MED7	0.476923077	0.783253842	0.516	2.65E-10	1.20E-09	-9.255	84	59	71.5
ILF3	0.815384615	0.479597244	0.465	9.98E-12	5.37E-11	-7.587	71	74	72.5
NONO	0.953846154	0.295177753	4.81	2.32E-12	1.34E-11	-7.38	66	79	72.5
RBX1	0.876923077	0.446740859	1.406	1.11E-14	1.06E-13	-5.969	40	107	73.5
HDAC3	0.623076923	0.659777424	0.475	2.03E-10	9.32E-10	-8.03	83	65	74
OVCA2	0.392307692	0.843137255	3.874	5.03E-10	2.19E-09	-8.536	88	61	74.5
YBX1	0.730769231	0.560148384	1.926	7.41E-11	3.54E-10	-7.541	80	75	77.5
RNPS1	0.746153846	0.560148384	5.837	6.63E-12	3.67E-11	-7.167	69	89	79
VAMP7	0.423076923	0.815050344	3.342	1.56E-09	6.19E-09	-8.053	96	64	80
GATAD1	0.515384615	0.750927398	1.57	3.38E-10	1.50E-09	-7.498	86	77	81.5
HCFC1	0.753846154	0.536830949	0.111	6.10E-11	2.99E-10	-7.181	78	88	83
CBFB	0.438461538	0.800211977	1.485	2.77E-09	1.04E-08	-7.873	101	68	84.5
CENPB	0.353846154	0.865924748	4.012	1.16E-09	4.75E-09	-7.499	93	76	84.5
MED28	0.792307692	0.520932697	3.759	1.14E-12	7.15E-12	-5.891	61	110	85.5
SMARCC2	0.623076923	0.643879173	1.362	2.11E-09	8.08E-09	-7.634	100	72	86
MTF2	0.469230769	0.775304716	0.526	3.15E-09	1.17E-08	-7.685	103	71	87
PARN	0.4	0.834128246	4.282	1.04E-09	4.30E-09	-7.329	92	83	87.5
HMGXB4	0.3	0.892421834	2.029	9.13E-09	3.17E-08	-7.978	110	67	88.5
CASP8AP2	0.361538462	0.847906673	0.345	1.61E-08	5.25E-08	-8.375	117	63	90
NCOR2	0.607692308	0.665076842	0.189	7.81E-10	3.32E-09	-7.15	90	90	90
UBE2K	0.692307692	0.590885003	0.202	2.67E-10	1.20E-09	-6.775	85	95	90
BOLA2	0.576923077	0.696873344	2.444	4.25E-10	1.87E-09	-7.021	87	94	90.5
ZNHIT3	0.323076923	0.875993641	3.996	1.21E-08	4.06E-08	-7.782	114	69	91.5
TRIM28	0.4846153	0.7615262	1.556	3.93E-09	1.44E-08	-7.351	104	80	92

Gene	TP	TN	thresh_mhg	hyper_pval	hyper_qval	gen_qval	rank_hyper_qval	rank_gen_qval	mean_rank
	85	32							
HCFC2	0.4	0.819289878	0.696	1.72E-08	5.56E-08	-7.758	118	70	94
SCAP	0.384615385	0.836248013	3.975	6.14E-09	2.21E-08	-7.349	106	82	94
IKZF3	0.846153846	0.435082141	0.345	2.67E-11	1.36E-10	-5.683	75	115	95
BDP1	0.469230769	0.771065183	0.227	6.38E-09	2.28E-08	-7.286	107	85	96
PNN	0.730769231	0.570747218	0.465	1.55E-11	8.25E-11	-5.518	72	122	97
PHB2	0.838461538	0.471648119	2.039	5.09E-13	3.41E-12	-4.957	57	137	97
CDC5L	0.576923077	0.689984102	4.233	1.23E-09	5.01E-09	-6.283	94	101	97.5
IRF8	0.684615385	0.616322205	0.367	1.89E-11	9.76E-11	-5.51	74	123	98.5
CCNH	0.461538462	0.784843667	5.84	1.74E-09	6.80E-09	-6.314	98	100	99
GTF2A2	0.546153846	0.704292528	1.384	8.52E-09	2.98E-08	-7.124	109	91	100
SMARCB1	0.592307692	0.673555909	4.507	1.80E-09	6.94E-09	-6.278	99	102	100.5
AIP	0.753846154	0.533651298	1.163	9.59E-11	4.52E-10	-5.548	81	121	101
GTF3C5	0.361538462	0.845257022	0.941	2.67E-08	8.29E-08	-7.308	122	84	103
SARNP	0.807692308	0.474827769	1.911	7.18E-11	3.47E-10	-5.346	79	127	103
PRDM1	0.4	0.820349762	1.77	1.42E-08	4.68E-08	-7.027	116	93	104.5
ILF2	0.576923077	0.674085851	0.963	1.24E-08	4.13E-08	-6.724	115	96	105.5
MED30	0.584615385	0.688394277	4.883	5.48E-10	2.35E-09	-5.504	89	125	107
MYBBP1A	0.615384615	0.649178559	3.079	2.78E-09	1.04E-08	-5.75	102	113	107.5
TBC1D2B	0.453846154	0.774244833	0.239	2.83E-08	8.71E-08	-7.068	124	92	108
MKI67IP	0.476923077	0.760996229	3.216	1.18E-08	3.98E-08	-6.1	113	105	109
PHF6	0.446153846	0.779014308	2.531	3.54E-08	1.05E-07	-6.376	129	98	113.5
MED14	0.461538462	0.768415474	1.021	2.65E-08	8.29E-08	-6.061	123	106	114.5
PLRG1	0.430769231	0.786963434	0.848	7.05E-08	2.01E-07	-6.341	134	99	116.5
SAP18	0.738461538	0.496555379	0.287	9.91E-08	2.74E-07	-6.475	138	97	117.5
GTF2E2	0.469230769	0.760466349	3.469	3.42E-08	1.02E-07	-5.969	127	108	117.5
GNPTAB	0.361538462	0.838367778	3.399	9.41E-08	2.64E-07	-6.14	136	104	120
RNF4	0.792307692	0.471648119	2.011	1.25E-09	5.01E-09	-4.74	95	145	120
GTF3C1	0.546153846	0.695283519	0.766	3.08E-08	9.41E-08	-5.679	125	116	120.5
ZC3H15	0.746153846	0.506094333	0.651	1.14E-08	3.90E-08	-5.268	112	129	120.5
GTF3C2	0.615384615	0.642819229	0.536	6.77E-09	2.39E-08	-5.031	108	134	121

Gene	TP	TN	thresh_mhg	hyper_pval	hyper_qval	gen_qval	rank_hyper_qval	rank_gen_qval	mean_rank
SMARCA5	0.4	0.810280869	1.911	8.03E-08	2.27E-07	-5.795	135	111	123
FUS	0.553846154	0.679915209	4.226	9.67E-08	2.70E-07	-5.754	137	112	124.5
SF1	0.807692308	0.430312666	2.18	2.32E-08	7.31E-08	-5.336	121	128	124.5
TCF3	0.430769231	0.783783784	1.683	1.16E-07	3.15E-07	-5.613	140	117	128.5
PFDN1	0.569230769	0.674085851	3.429	3.26E-08	9.88E-08	-5.208	126	132	129
ATF1	0.461538462	0.758346582	1.546	1.21E-07	3.27E-07	-5.583	141	120	130.5
RUVBL1	0.507692308	0.728139905	5.51	3.42E-08	1.02E-07	-5.173	128	133	130.5
CSDA	0.538461538	0.688924218	0.696	1.83E-07	4.75E-07	-5.591	147	119	133
UTP6	0.384615385	0.819289878	0.903	1.27E-07	3.38E-07	-5.472	144	126	135
ZRANB2	0.461538462	0.75145734	1.828	3.24E-07	8.04E-07	-5.604	154	118	136
HCLS1	0.869230769	0.42236354	6.652	2.29E-12	1.34E-11	-2.841	65	208	136.5
PPIE	0.530769231	0.699523052	3.717	1.12E-07	3.07E-07	-5.003	139	135	137
L3MBTL2	0.3	0.879173291	5.453	1.63E-07	4.26E-07	-5.212	146	131	138.5
HMGA1	0.361538462	0.82617912	1.949	7.24E-07	1.68E-06	-5.722	165	114	139.5
FOXN2	0.492307692	0.722840488	0.263	4.45E-07	1.08E-06	-5.509	157	124	140.5
GTF2B	0.592307692	0.657127716	5.004	1.83E-08	5.87E-08	-4.155	119	164	141.5
RPL7L1	0.584615385	0.674615792	2.98	4.27E-09	1.55E-08	-3.73	105	178	141.5
TERF1	0.369230769	0.815580286	0.566	1.48E-06	3.24E-06	-5.953	175	109	142
SND1	0.553846154	0.668786433	3.945	4.00E-07	9.85E-07	-5.245	155	130	142.5
E2F4	0.353846154	0.845786963	5.891	6.81E-08	1.96E-07	-4.583	133	152	142.5
SREBF1	0.469230769	0.759936407	3.198	3.71E-08	1.09E-07	-4.288	130	157	143.5
SIN3A	0.430769231	0.774774775	1.58	4.40E-07	1.08E-06	-4.929	156	138	147
BAZ1A	0.461538462	0.752517223	1.637	2.79E-07	7.02E-07	-4.798	152	143	147.5
COPS5	0.484615385	0.734499205	5.938	2.26E-07	5.77E-07	-4.708	150	147	148.5
NFYB	0.530769231	0.685214626	0.485	7.02E-07	1.64E-06	-4.819	163	142	152.5
MORF4L2	0.6	0.644409115	3.343	3.82E-08	1.11E-07	-3.784	131	176	153.5
RAI1	0.361538462	0.827239004	1.47	6.12E-07	1.46E-06	-4.658	160	149	154.5
CNOT1	0.615384615	0.607843137	0.444	5.62E-07	1.35E-06	-4.627	159	151	155
SMARCA4	0.676923077	0.547429783	0.263	4.99E-07	1.21E-06	-4.43	158	155	156.5
PHB	0.723076923	0.532591415	4.168	9.31E-09	3.21E-08	-3.115	111	202	156.5
LRRFIP1	0.8384615	0.4313725	5.275	1.69E-10	7.87E-10	-2.399	82	231	156.5

Gene	TP	TN	thresh_mhg	hyper_pval	hyper_qval	gen_qval	rank_hyper_qval	rank_gen_qval	mean_rank
	38	49							
CNOT3	0.507692308	0.699523052	0.696	1.53E-06	3.29E-06	-5.002	178	136	157
HIF1A	0.807692308	0.41600424	0.299	1.24E-07	3.32E-07	-3.819	143	172	157.5
TBP	0.4	0.793322734	1.541	1.09E-06	2.44E-06	-4.78	172	144	158
GTF2F1	0.630769231	0.59936407	4.07	2.62E-07	6.62E-07	-4.001	151	168	159.5
CAND1	0.453846154	0.744568098	0.189	1.94E-06	4.07E-06	-4.861	182	140	161
SREBF2	0.638461538	0.578696343	0.333	1.16E-06	2.56E-06	-4.646	174	150	162
HTATSF1	0.384615385	0.802331744	1.091	1.75E-06	3.74E-06	-4.689	179	148	163.5
PWP1	0.376923077	0.808161102	5.403	1.82E-06	3.86E-06	-4.477	180	153	166.5
ID2	0.923076923	0.259671436	0.88	2.19E-07	5.61E-07	-3.54	149	185	167
PSMC3	0.776923077	0.467408585	5.738	1.96E-08	6.25E-08	-2.725	120	214	167
MED23	0.353846154	0.821409645	1.195	3.68E-06	7.43E-06	-4.731	189	146	167.5
BCLAF1	0.6	0.616852146	3.619	1.10E-06	2.44E-06	-4.039	171	166	168.5
UIMC1	0.5	0.706412295	0.807	1.52E-06	3.29E-06	-4.205	177	161	169
MTA2	0.807692308	0.414414414	3.71	1.49E-07	3.92E-07	-3.261	145	193	169
METTL14	0.369230769	0.804981452	0.433	6.72E-06	1.28E-05	-4.871	200	139	169.5
NFX1	0.569230769	0.643349232	0.566	1.51E-06	3.27E-06	-4.159	176	163	169.5
THRAP3	0.784615385	0.42554319	1.257	8.56E-07	1.95E-06	-3.843	168	171	169.5
TAF1B	0.407692308	0.787493376	3.977	1.04E-06	2.34E-06	-3.897	170	170	170
PHF20	0.323076923	0.845786963	1.401	3.23E-06	6.59E-06	-4.46	187	154	170.5
RBPJ	0.669230769	0.562798092	1.189	2.11E-07	5.45E-07	-3.259	148	194	171
ZMAT2	0.638461538	0.58399576	1.118	6.44E-07	1.52E-06	-3.646	162	181	171.5
AATF	0.376923077	0.803921569	3.087	3.33E-06	6.77E-06	-4.346	188	156	172
TSG101	0.561538462	0.656597774	4.563	7.41E-07	1.71E-06	-3.724	166	179	172.5
YAF2	0.323076923	0.835718071	0.614	1.45E-05	2.67E-05	-4.858	207	141	174
ING3	0.484615385	0.717541077	0.651	2.05E-06	4.28E-06	-4.133	183	165	174
PREB	0.569230769	0.641229465	0.546	1.92E-06	4.05E-06	-3.922	181	169	175
RBM38	0.769230769	0.465818776	2.66	6.70E-08	1.94E-07	-2.6	132	218	175
MLX	0.484615385	0.711711712	0.722	4.13E-06	8.22E-06	-4.223	192	160	176
DPF2	0.576923077	0.649178579	3.629	3.22E-07	8.04E-07	-3.161	153	199	176
NDUFA13	0.930769231	0.291467939	5.001	8.88E-10	3.73E-09	-1.892	91	264	177.5

Gene	TP	TN	thresh_mhg	hyper_pval	hyper_qval	gen_qval	rank_hyper_qval	rank_gen_qval	mean_rank
SMARCE1	0.723076923	0.492315845	0.465	1.03E-06	2.32E-06	-3.513	169	187	178
EED	0.346153846	0.827239004	5.106	3.75E-06	7.54E-06	-4.032	190	167	178.5
CTBP1	0.415384615	0.767885533	1.757	6.18E-06	1.19E-05	-4.233	199	159	179
C1D	0.515384615	0.697933227	3.825	8.03E-07	1.84E-06	-3.317	167	191	179
CXXC1	0.538461538	0.668256492	0.766	2.30E-06	4.77E-06	-3.795	184	175	179.5
CREM	0.607692308	0.608903021	0.731	1.17E-06	2.56E-06	-3.479	173	188	180.5
CBFA2T2	0.323076923	0.836248013	1.949	1.34E-05	2.52E-05	-4.254	204	158	181
CALR	0.869230769	0.339692634	0.911	1.21E-07	3.27E-07	-2.538	142	223	182.5
MYEF2	0.484615385	0.701112878	0.454	1.38E-05	2.58E-05	-4.163	205	162	183.5
CIZ1	0.476923077	0.718071012	0.911	4.34E-06	8.59E-06	-3.813	193	174	183.5
CNOT8	0.546153846	0.659247483	1.718	2.86E-06	5.87E-06	-3.558	186	184	185
ECD	0.384615385	0.794912516	5.027	4.92E-06	9.64E-06	-3.745	195	177	186
NR3C1	0.461538462	0.730259671	1.077	5.03E-06	9.81E-06	-3.622	196	182	189
IFI35	0.576923077	0.623741388	1.245	5.87E-06	1.13E-05	-3.586	198	183	190.5
BUD31	0.6	0.621621622	5.295	6.36E-07	1.51E-06	-2.549	161	222	191.5
ZMIZ1	0.569230769	0.625331214	0.275	1.07E-05	2.03E-05	-3.229	202	195	198.5
MLLT6	0.423076923	0.754107048	2.621	1.51E-05	2.77E-05	-3.293	208	192	200
FLII	0.661538462	0.534181214	0.595	1.06E-05	2.01E-05	-2.892	201	207	204
KAT2A	0.323076923	0.817170111	0.506	0.000158092	0.000255895	-3.814	236	173	204.5
UHRF2	0.392307692	0.765765766	0.379	7.89E-05	0.000134623	-3.537	224	186	205
RNF5	0.423076923	0.739798622	1.091	7.46E-05	0.000128378	-3.435	222	189	205.5
TCF20	0.607692308	0.576046635	1.864	3.48E-05	6.15E-05	-3.214	216	196	206
MAX	0.446153846	0.727080021	3.294	3.36E-05	5.99E-05	-3.186	214	198	206
IKZF2	0.469230769	0.697933227	0.084	8.20E-05	0.000139287	-3.435	225	190	207.5
PURB	0.523076923	0.658187599	0.595	3.15E-05	5.66E-05	-3.09	213	203	208
NPM1	0.992307692	0.100158983	7.766	2.57E-05	4.65E-05	-2.919	211	206	208.5
ELF2	0.323076923	0.816110228	0.275	0.000178828	0.000285825	-3.686	239	180	209.5
MLLT3	0.523076923	0.651828299	0.345	5.91E-05	0.000102562	-3.141	220	200	210
FLI1	0.692307692	0.495495495	1.727	2.02E-05	3.68E-05	-2.753	210	210	210
TBX21	0.638461538	0.567037626	0.433	4.07E-06	8.13E-06	-2.377	191	232	211.5
ABT1	0.2846153	0.8532061	4.143	7.77E-05	0.0001330	-3.123	223	201	212

Gene	TP	TN	thresh_mhg	hyper_pval	hyper_qval	gen_qval	rank_hyper_qval	rank_gen_qval	mean_rank
	85	47			87				
THOC2	0.507692308	0.672496025	0.345	3.12E-05	5.63E-05	-2.716	212	215	213.5
PLAGL2	0.353846154	0.790673026	0.124	0.00017998	0.000286468	-3.194	240	197	218.5
BHLHE40	0.915384615	0.31054584	0.731	1.59E-09	6.26E-09	-0.569	97	341	219
RUNX3	0.753846154	0.449920509	1.091	2.56E-06	5.28E-06	-2.085	185	254	219.5
PML	0.430769231	0.726550079	0.163	0.00014519	0.000236018	-2.726	235	213	224
STAT3	0.861538462	0.333863275	4.622	7.19E-07	1.68E-06	-1.529	164	285	224.5
RNF2	0.4	0.748277689	0.299	0.00024526	0.000382017	-2.984	246	204	225
FOXJ3	0.376923077	0.766825649	0.239	0.00028253	0.000431707	-2.956	250	205	227.5
SMYD3	0.423076923	0.731319555	0.163	0.00017743	0.000285577	-2.635	238	217	227.5
CNBP	0.907692308	0.234234234	8.625	3.96E-05	6.95E-05	-2.303	218	238	228
NMI	0.546153846	0.643349232	5.658	1.59E-05	2.91E-05	-2.16	209	250	229.5
GABPB1	0.415384615	0.736089033	0.848	0.00021616969	0.000341226	-2.593	242	219	230.5
YEATS4	0.453846154	0.696873344	0.895	0.00034011 2	0.000515566	-2.746	252	211	231.5
CHURC1	0.492307692	0.671436142	5.333	0.00013847	0.000226049	-2.414	234	230	232
HMG20B	0.461538462	0.693693694	0.687	0.00024009 7	0.00037589	-2.58	244	221	232.5
IRF2	0.6	0.570217276	0.888	0.00011831 1	0.000196498	-2.311	230	236	233
PHRF1	0.476923077	0.676735559	0.263	0.00031148 4	0.000474051	-2.7	251	216	233.5
MEN1	0.323076923	0.803921569	0.526	0.00067216 5	0.000976301	-2.79	263	209	236
RELA	0.623076923	0.546899841	0.401	0.00012126 4	0.000200532	-2.237	231	241	236
CTCF	0.369230769	0.765235824	0.227	0.00063258	0.000922311	-2.742	262	212	237
ATRX	0.576923077	0.588235294	0.07	0.00017792 5	0.000285577	-2.304	237	237	237
CCNT2	0.438461538	0.708532061	0.31	0.00041750 2	0.000625435	-2.585	255	220	237.5
REXO4	0.392307692	0.751457374	0.614	0.00034655 2	0.000523252	-2.538	253	224	238.5
NFATC1	0.815384615	0.361950185	0.632	1.41E-05	2.62E-05	-1.791	206	271	238.5
XAB2	0.469230769	0.685744568	0.696	0.00026148 6	0.000404404	-2.359	247	233	240
TCF25	0.830769231	0.353471118	1.787	5.25E-06	1.02E-05	-1.468	197	287	242
TSC22D4	0.669230769	0.501854796	0.604	0.00010085 4	0.000169718	-1.968	227	260	243.5
HSBP1	0.584615385	0.598834128	4.583	3.43E-05	6.09E-05	-1.745	215	275	245
MLL5	0.615384615	0.558028617	3.902	8.99E-05	0.000151901	-1.887	226	265	245.5
DDX54	0.538461538	0.620561738	0.379	0.00027047 7	0.000414948	-2.221	249	243	246

Gene	TP	TN	thresh_mhg	hyper_pval	hyper_qval	gen_qval	rank_hyper_qval	rank_gen_qval	mean_rank
CHD4	0.692307692	0.488606253	1.195	3.83E-05	6.74E-05	-1.73	217	277	247
GTF2H2	0.323076923	0.801271816	0.848	0.000877194	0.001245618	-2.486	269	227	248
TWISTNB	0.407692308	0.725490196	0.465	0.001070377	0.001502316	-2.516	273	225	249
GABPA	0.4	0.731319555	0.227	0.001171536	0.001627337	-2.509	275	226	250.5
TBL1XR1	0.530769231	0.636459989	0.287	0.000126733	0.000207776	-1.796	233	270	251.5
EP300	0.453846154	0.687864335	0.098	0.000746758	0.001076459	-2.286	265	240	252.5
MED4	0.3	0.816110228	0.872	0.001369096	0.001881276	-2.485	278	228	253
LDB1	0.507692308	0.640699523	0.516	0.000581964	0.000861668	-2.161	258	249	253.5
TMF1	0.569230769	0.567567568	0.138	0.001662961	0.002260608	-2.429	281	229	255
NOTCH1	0.646153846	0.524642289	0.263	0.000109848	0.000183239	-1.53	229	284	256.5
ING1	0.330769231	0.788553259	0.422	0.001580999	0.002156935	-2.343	280	234	257
HTATIP2	0.415384615	0.722310546	0.748	0.000775953	0.001114338	-2.177	266	248	257
DR1	0.415384615	0.724960254	0.345	0.000613734	0.000905198	-2.015	259	257	258
SP110	0.761538462	0.408055114	2.501	6.19E-05	0.000106945	-1.29	221	297	259
NCOA4	0.453846154	0.685744568	0.275	0.000891511	0.001261321	-2.128	270	251	260.5
TRIM27	0.315384615	0.820879703	3.219	0.000212144	0.000336261	-1.655	241	280	260.5
RNF44	0.653846154	0.502384738	0.227	0.000362585	0.000545305	-1.843	254	268	261
EIF3H	0.984615385	0.129835718	6.915	4.81E-06	9.47E-06	-0.817	194	328	261
SP3	0.423076923	0.708002112	0.263	0.001445503	0.001979148	-2.215	279	244	261.5
NRF1	0.430769231	0.696343402	0.322	0.002090047	0.002762623	-2.319	289	235	262
AEBP2	0.453846154	0.675145734	0.111	0.002070131	0.002755365	-2.226	286	242	264
MTA3	0.538461538	0.610492846	0.444	0.000623021	0.000915362	-1.79	260	272	266
PQBP1	0.492307692	0.644939057	0.941	0.001323084	0.001824614	-2.006	277	258	267.5
MED17	0.346153846	0.767355591	0.163	0.003123932	0.003991111	-2.298	299	239	269
CNOT7	0.438461538	0.690514043	0.454	0.001897181	0.002542888	-2.087	285	253	269
BLOC1S1	0.430769231	0.704822477	0.941	0.001055242	0.001487463	-1.822	271	269	270
MORF4L1	0.661538462	0.499735029	5.916	0.000236223	0.000371358	-1.194	243	300	271.5
DNM2	0.676923077	0.491255962	2.87	0.000125926	0.000207344	-1.045	232	311	271.5
SNW1	0.615384615	0.545310016	0.411	0.000266795	0.000410951	-1.3	248	296	272
IKZF1	0.769230769	0.393216746	2.698	0.000106491	0.000178422	-0.926	228	318	273
CBX4	0.3615384	0.7530471	0.138	0.0033093	0.0041859	-2.191	302	245	273.5

Gene	TP	TN	thresh_mhg	hyper_pval	hyper_qval	gen_qval	rank_hyper_qval	rank_gen_qval	mean_rank
	62	65		26	69				
CEBPZ	0.4	0.719130896	0.401	0.003148239	0.004008758	-2.181	300	247	273.5
NRBF2	0.307692308	0.801801802	0.595	0.002844803	0.003658972	-2.088	297	252	274.5
GON4L	0.453846154	0.681505034	0.057	0.001259528	0.001743259	-1.739	276	276	276
GABPB2	0.607692308	0.514573397	0.029	0.004489896	0.005586776	-2.191	307	246	276.5
KDM2B	0.507692308	0.624271333	0.263	0.002081507	0.002760887	-1.866	288	266	277
ZNRD1	0.407692308	0.71754107	1.406	0.002065194	0.002755365	-1.857	287	267	277
FOSB	0.446153846	0.679385268	0.214	0.002578397	0.003338806	-1.966	295	261	278
RUNX2	0.630769231	0.505564388	0.124	0.0016932	0.002293667	-1.757	282	274	278
KEAP1	0.361538462	0.753577107	0.356	0.0031715	0.004024961	-2.027	301	256	278.5
NCOA2	0.469230769	0.657657658	0.084	0.002610066	0.003368397	-1.927	296	262	279
STAT1	0.746153846	0.401695813	3.65	0.000440334	0.000657061	-1.161	256	303	279.5
MED24	0.346153846	0.763116057	0.31	0.004397886	0.005490172	-2.044	306	255	280.5
MED1	0.576923077	0.5590885	0.227	0.001770331	0.002389634	-1.706	283	278	280.5
BTF3	0.984615385	0.121886592	7.506	1.31E-05	2.47E-05	-0.271	203	359	281
CAMTA2	0.361538462	0.748277689	0.239	0.004805109	0.005959584	-2.004	308	259	283.5
NFKB2	0.669230769	0.491255962	0.275	0.000246011	0.000382017	-0.882	245	322	283.5
TARBP2	0.315384615	0.792792793	0.941	0.003506398	0.004391622	-1.906	305	263	284
MED8	0.353846154	0.763645999	0.774	0.002422937	0.003158915	-1.704	293	279	286
MED15	0.576923077	0.55590885	0.214	0.002216947	0.002910222	-1.634	291	281	286
PTTG1	0.607692308	0.535241123	0.322	0.001073645	0.001502316	-1.165	272	302	287
CNOT2	0.515384615	0.614732379	0.651	0.002432379	0.003160438	-1.442	294	288	291
CHD3	0.515384615	0.629040806	0.176	0.000827506	0.0011805	-0.981	268	314	291
BATF	0.584615385	0.561738209	1.098	0.000828204	0.0011805	-0.915	267	319	293
RNF7	0.661538462	0.485426603	5.049	0.000729503	0.001055568	-0.882	264	323	293.5
COPS2	0.392307692	0.71754107	0.299	0.005951618	0.007357664	-1.611	309	282	295.5
PNRC2	0.523076923	0.607843137	0.496	0.002342715	0.003064784	-1.206	292	299	295.5
TRIP12	0.530769231	0.579226285	0.084	0.009416967	0.011276745	-1.771	319	273	296
SBDS	0.5	0.616322205	0.722	0.005997032	0.007389891	-1.603	310	283	296.5
PFDN5	0.876923077	0.271860095	5.965	5.41E-05	9.44E-05	-0.049	219	375	297
NT5C	0.692307692	0.455219926	0.918	0.000625642	0.000915691	-0.781	261	334	297.5

Gene	TP	TN	thresh_mhg	hyper_pval	hyper_qval	gen_qval	rank_hyper_qval	rank_gen_qval	mean_rank
RLIM	0.646153846	0.479597244	0.138	0.003398602	0.004270612	-1.323	304	294	299
STAT5A	0.538461538	0.589825119	0.014	0.002910944	0.003731478	-1.168	298	301	299.5
VAV1	0.592307692	0.549549555	4.64	0.001164709	0.001623791	-0.841	274	326	300
NACA	0.976923077	0.105988341	8.157	0.000474445	0.000705206	-0.541	257	343	300
BRD8	0.607692308	0.505034446	0.07	0.008136744	0.009898842	-1.394	314	289	301.5
CCNT1	0.407692308	0.700582936	0.556	0.007246944	0.008844508	-1.367	313	292	302.5
MIER1	0.630769231	0.495495495	0.287	0.003373312	0.004252822	-1.156	303	304	303.5
MLXIP	0.515384615	0.593004769	0.202	0.010157615	0.012125653	-1.326	320	293	306.5
HDAC4	0.384615385	0.715951245	0.239	0.010789167	0.012839445	-1.303	321	295	308
ARID1A	0.746153846	0.382617912	0.287	0.001833786	0.002466571	-0.787	284	332	308
ELF4	0.392307692	0.695813461	0.251	0.024082657	0.027793278	-1.484	331	286	308.5
HIVEP1	0.392307692	0.698463169	0.151	0.020633997	0.024178487	-1.386	326	291	308.5
SERTAD1	0.376923077	0.727080021	0.791	0.008177881	0.009917303	-1.148	315	305	310
EGR1	0.546153846	0.568627451	0.214	0.007139558	0.008741382	-1.076	312	309	310.5
ATF6B	0.369230769	0.714361432	0.299	0.028613822	0.032628298	-1.392	335	290	312.5
CNOT4	0.315384615	0.767885533	0.299	0.022358071	0.025959827	-1.247	329	298	313.5
NFATC3	0.538461538	0.571807101	0.111	0.0093423	0.011241079	-1.067	317	310	313.5
NFYC	0.438461538	0.665076842	0.433	0.011271759	0.013372087	-1.138	322	307	314.5
NR1H2	0.576923077	0.535241123	0.575	0.008507946	0.010284923	-1.01	316	313	314.5
SCAND1	0.3	0.777954425	1.036	0.028500335	0.032596191	-1.14	334	306	320
LIMD1	0.430769231	0.661367225	0.176	0.021686374	0.025256692	-1.016	328	312	320
VGLL4	0.546153846	0.569157393	0.705	0.006907501	0.008484455	-0.794	311	330	320.5
PHF20L1	0.438461538	0.648648649	0.151	0.029163857	0.033156528	-1.077	336	308	322
ATF4	0.8	0.320084791	1.22	0.002184212	0.002877135	-0.28	290	357	323.5
NR4A3	0.430769231	0.659247483	0.604	0.024422379	0.028100448	-0.936	332	316	324
DAXX	0.384615385	0.701112878	0.546	0.026852701	0.030804	-0.894	333	320	326.5
ARID5B	0.376923077	0.700582936	0.214	0.041029908	0.046098309	-0.98	340	315	327.5
HDAC7	0.615384615	0.478537361	0.263	0.023208899	0.026866059	-0.849	330	325	327.5
TET3	0.384615385	0.693693694	0.124	0.040264914	0.045372262	-0.932	339	317	328
TOX4	0.546153846	0.533651298	0.043	0.047337129	0.052873635	-0.893	342	321	331.5
CCNL2	0.6846153	0.4101748	0.496	0.0197639	0.0232302	-0.622	325	338	331.5

Gene	TP	TN	thresh_mhg	hyper_pval	hyper_qval	gen_qval	rank_hyper_qval	rank_gen_qval	mean_rank
	85	81		79	77				
MED12	0.484615385	0.602013778	0.124	0.032517067	0.036859108	-0.83	337	327	332
RNF166	0.523076923	0.572866985	0.356	0.020862813	0.024371849	-0.654	327	337	332
NR4A1	0.692307692	0.411764706	0.275	0.011525026	0.013630217	-0.361	323	349	336
DENND4A	0.723076923	0.371489136	0.124	0.017803265	0.020990269	-0.36	324	350	337
NFKBIB	0.576923077	0.533651298	0.642	0.009357756	0.011241079	-0.282	318	356	337
BCL11B	0.423076923	0.647588765	0.111	0.063888514	0.071152806	-0.785	343	333	338
NFKB1	0.392307692	0.669316375	0.151	0.090500848	0.099058234	-0.81	349	329	339
RNF19A	0.407692308	0.657127716	0.239	0.080864207	0.089277824	-0.716	346	335	340.5
HSF1	0.361538462	0.691043985	0.536	0.124988239	0.135256395	-0.794	353	331	342
CDK7	0.553846154	0.491255962	0.014	0.182958536	0.193066742	-0.873	362	324	343
VPS72	0.4	0.659777424	0.444	0.099009723	0.108062041	-0.667	350	336	343
ING4	0.392307692	0.671436142	0.731	0.082559966	0.090887334	-0.56	347	342	344.5
NCOA3	0.623076923	0.457869634	0.176	0.043719541	0.048976143	-0.397	341	348	344.5
CREBBP	0.430769231	0.633280339	0.163	0.086408678	0.094850905	-0.476	348	344	346
RNF14	0.315384615	0.729199788	0.465	0.158320707	0.168934386	-0.588	358	339	348.5
NOTCH2	0.584615385	0.474297827	0.138	0.112776801	0.12238846	-0.472	352	345	348.5
GATA3	0.484615385	0.576576577	0.379	0.101937767	0.110940818	-0.402	351	347	349
KDM5A	0.584615385	0.500794913	0.084	0.036243786	0.040961912	-0.269	338	362	350
TLE3	0.446153846	0.604663487	0.176	0.146499903	0.157199334	-0.426	356	346	351
DTX3L	0.630769231	0.439321675	0.163	0.07039306	0.077942461	-0.27	345	360	352.5
SMAD7	0.415384615	0.632750397	0.239	0.157350387	0.168369321	-0.305	357	354	355.5
REL	0.423076923	0.623211447	0.367	0.168793051	0.179108182	-0.32	360	352	356
MYSM1	0.546153846	0.492315845	0.043	0.224355068	0.236098171	-0.329	363	351	357
NSD1	0.561538462	0.486486486	0.084	0.166267058	0.176919265	-0.296	359	355	357
RNF114	0.623076923	0.447800742	0.299	0.06852014	0.076089225	-0.091	344	371	357.5
STAT6	0.546153846	0.506094333	0.163	0.144163849	0.155128424	-0.27	355	361	358
GLRX2	0.7	0.293587705	0.111	0.604781803	0.609568994	-0.58	378	340	359
MYC	0.369230769	0.673555909	0.333	0.181584894	0.192148004	-0.276	361	358	359.5
TBPL1	0.338461538	0.683624801	0.401	0.332148047	0.34384974	-0.317	369	353	361
IRF3	0.392307692	0.643879173	0.696	0.22933455	0.24067527	-0.241	364	364	364

Gene	TP	TN	thresh_mhg	hyper_pval	hyper_qval	gen_qval	rank_hyper_qval	rank_gen_qval	mean_rank
CCNL1	0.607692308	0.420243773	0.475	0.298309917	0.312203803	-0.152	365	367	366
KLF6	0.761538462	0.28881823	0.333	0.128582033	0.138752363	0	354	379	366.5
RNF125	0.438461538	0.588765236	0.585	0.301200842	0.313511503	-0.152	366	368	367
ATF7IP	0.515384615	0.502914679	0.275	0.377191966	0.387331535	-0.16	372	366	369
ATF2	0.307692308	0.702702703	0.263	0.435327885	0.442274606	-0.248	376	363	369.5
SPOP	0.461538462	0.565977742	0.379	0.300549638	0.313511503	-0.088	367	372	369.5
MKL1	0.415384615	0.579226285	0.176	0.582407766	0.590132007	-0.167	377	365	371
XBP1	0.353846154	0.658187599	0.401	0.423690283	0.432753177	-0.122	374	369	371.5
PER1	0.561538462	0.455219926	0.227	0.390605456	0.400030252	-0.119	373	370	371.5
PNRC1	0.476923077	0.543720191	0.526	0.356539543	0.367110797	-0.064	371	373	372
FOSL2	0.592307692	0.429782724	0.214	0.345579917	0.356787914	-0.016	370	376	373
MAF1	0.669230769	0.354531002	0.585	0.327521118	0.33998116	0	368	380	374
UBXN4	0.523076923	0.488606253	0.084	0.433942783	0.442043048	-0.051	375	374	374.5
PYHIN1	0.392307692	0.599894012	0.333	0.603709408	0.609568994	-0.016	379	377	378
MAML2	0.338461538	0.610492846	0.098	0.894958451	0.899668759	-0.016	380	378	379
PBXIP1	0.415384615	0.501324854	0.189	0.973312343	0.973312343	0	381	381	381
TGIF1	0.407692308	0.509803922	0.585	0.972336961	0.973312343	0	382	382	382

Table 16. Ranked top surface cytokines differentially expressed in cluster 10

Genes	TP	TN	thresh_mhg	hyper_pval	hyper_qval	gen_qval	rank_hyper_qval	rank_gen_qval	mean_rank
SIVA1	0.753846154	0.761526232	6.445	1.91E-32	3.26E-30	-35.654	1	1	1
NUP85	0.653846154	0.789083201	4.43	2.40E-25	1.37E-23	-26.704	3	3	3
PAQR4	0.423076923	0.905140435	0.632	1.17E-20	4.02E-19	-26.737	5	2	3.5
HMGB1	0.661538462	0.763645999	9.144	7.18E-23	3.07E-21	-22.967	4	4	4
LGALS1	0.953846154	0.407525172	9.608	1.90E-20	5.42E-19	-14.204	6	8	7
PDCD1	1	0.411234764	6.848	2.41E-29	2.06E-27	-10.914	2	13	7.5
ATPIF1	0.753846154	0.641229465	4.118	8.73E-19	1.49E-17	-15.563	10	6	8
ULBP1	0.384615385	0.910439852	3.345	5.93E-18	8.45E-17	-21.514	12	5	8.5
IDE	0.830769231	0.526232114	0.485	3.12E-16	3.60E-15	-14.337	15	7	11
HAVCR2	0.746153846	0.644409115	1.761	2.20E-18	3.41E-17	-11.845	11	11	11
CXCR6	0.9615384	0.3826179	1.996	1.40E-19	3.00E-18	-10.395	8	16	12

Genes	TP	TN	thresh_mhg	hyper_pval	hyper_qval	gen_qval	rank_hyper_qval	rank_gen_qval	mean_rank
	62	12							
LAG3	0.915384615	0.464758877	0.774	4.17E-20	1.02E-18	-9.648	7	18	12.5
CMTM7	0.823076923	0.535241123	6.084	3.16E-16	3.60E-15	-11.642	14	12	13
TFRC	0.592307692	0.731319555	0.444	9.31E-14	6.92E-13	-13.128	23	9	16
HSPD1	0.853846154	0.496555379	5.669	4.06E-16	4.08E-15	-9.938	17	17	17
CD244	0.392307692	0.872284049	4.52	4.44E-13	2.92E-12	-12.777	26	10	18
MIF	0.961538462	0.319024907	7.358	6.74E-15	6.06E-14	-9.141	19	20	19.5
ENTPD1	0.646153846	0.672496025	0.575	7.65E-13	4.67E-12	-10.653	28	14	21
HNRNPU	0.784615385	0.549549555	2.834	4.77E-14	3.88E-13	-9.082	21	21	21
PGLYRP1	0.823076923	0.534181224	6.746	3.81E-16	4.07E-15	-7.444	16	27	21.5
TIGIT	0.984615385	0.325384208	3.669	7.83E-19	1.49E-17	-6.684	9	37	23
TNFRSF9	0.769230769	0.572337043	4.358	1.76E-14	1.50E-13	-6.921	20	32	26
HSPA9	0.769230769	0.551669316	0.575	5.21E-13	3.30E-12	-7.58	27	26	26.5
LAP3	0.376923077	0.861685215	4.92	8.61E-11	3.51E-10	-10.474	42	15	28.5
USP14	0.492307692	0.781664017	4.578	3.71E-11	1.81E-10	-8.614	35	22	28.5
NR4A2	0.823076923	0.485426603	1	1.08E-12	6.35E-12	-7.402	29	28	28.5
HSPA8	0.907692308	0.348171701	11.569	4.53E-11	2.09E-10	-7.744	37	25	31
M6PR	0.907692308	0.420243773	6.122	7.70E-16	7.32E-15	-5.78	18	45	31.5
2-Sep	0.923076923	0.346051934	1.618	2.24E-12	1.24E-11	-6.85	31	34	32.5
CTLA4	0.930769231	0.391626921	2.257	2.65E-16	3.49E-15	-5.182	13	54	33.5
IFNG	0.561538462	0.718071012	1.47	1.21E-10	4.72E-10	-7.877	44	24	34
XPOT	0.453846154	0.797032326	0.111	5.70E-10	1.95E-09	-9.176	50	19	34.5
ADAM10	0.8	0.485956545	0.214	5.33E-11	2.28E-10	-7.117	40	30	35
C1QBP	0.723076923	0.574986751	1.705	2.74E-11	1.38E-10	-6.748	34	36	35
PEBP1	0.838461538	0.480127186	7.427	1.37E-13	9.77E-13	-5.371	24	51	37.5
IL10RA	0.792307692	0.518282989	0.251	1.71E-12	9.77E-12	-5.555	30	47	38.5
P4HB	0.861538462	0.427133015	6.085	4.98E-12	2.66E-11	-5.755	32	46	39
CTSB	0.946153846	0.322204557	1.064	2.97E-13	2.03E-12	-5.196	25	53	39
GPR56	0.476923077	0.771595125	0.604	2.07E-09	6.33E-09	-8.024	56	23	39.5
NCOR2	0.607692308	0.665076842	0.189	7.81E-10	2.52E-09	-7.15	53	29	41
HSP90AB1	0.984615385	0.195018548	9.312	7.18E-10	2.36E-09	-7.088	51	31	41

Genes	TP	TN	thresh_mhg	hyper_pval	hyper_qval	gen_qval	rank_hyper_qval	rank_gen_qval	mean_rank
PDIA3	0.938461538	0.289348172	5.431	2.23E-10	7.96E-10	-6.798	48	35	41.5
PGRMC1	0.553846154	0.724960254	5.442	1.16E-10	4.60E-10	-6.256	43	41	42
HSP90AA1	0.869230769	0.401165872	4.724	4.76E-11	2.14E-10	-5.526	38	48	43
IL2RB	1	0.164811871	5.599	1.59E-10	6.02E-10	-5.386	45	50	47.5
SEMA4D	0.923076923	0.28881823	0.189	5.95E-09	1.73E-08	-6.308	59	40	49.5
ITGAV	0.638461538	0.617912003	0.084	9.93E-09	2.74E-08	-6.619	62	38	50
IL12RB1	0.392307692	0.818229995	0.465	5.69E-08	1.41E-07	-6.877	69	33	51
LYST	0.576923077	0.667196608	1.401	3.19E-08	8.15E-08	-6.418	67	39	53
ERP29	0.630769231	0.643349232	3.343	8.02E-10	2.54E-09	-5.341	54	52	53
CD38	0.453846154	0.775834658	2.92	2.20E-08	5.69E-08	-5.903	66	43	54.5
IGF2R	0.6	0.635400106	0.275	1.21E-07	2.83E-07	-6.171	73	42	57.5
ISG20	0.369230769	0.836777954	5.339	4.54E-08	1.14E-07	-5.493	68	49	58.5
FLOT2	0.438461538	0.775304716	0.895	1.63E-07	3.66E-07	-5.816	76	44	60
NRP1	0.607692308	0.644939057	0.422	1.36E-08	3.64E-08	-4.848	64	57	60.5
ERP44	0.769230769	0.500264971	2.227	9.11E-10	2.83E-09	-3.984	55	66	60.5
FERMT3	0.938461538	0.296237414	3.077	8.59E-11	3.51E-10	-3.067	41	81	61
IL21R	0.807692308	0.432432432	0.84	1.79E-08	4.72E-08	-4.477	65	60	62.5
CTSD	0.984615385	0.162162162	5.25	6.91E-08	1.69E-07	-4.731	70	58	64
CLIC4	0.484615385	0.738738739	1.669	1.26E-07	2.90E-07	-5.052	74	55	64.5
RAC1	0.838461538	0.405405405	3.048	5.01E-09	1.50E-08	-3.585	57	72	64.5
GDI2	0.961538462	0.260201378	5.029	5.01E-11	2.20E-10	-2.658	39	90	64.5
FASL	0.561538462	0.674085851	6.847	8.30E-08	2.00E-07	-4.257	71	61	66
ATP5B	0.953846154	0.233704293	8.83	1.24E-08	3.36E-08	-3.718	63	70	66.5
GPI1	0.953846154	0.287758347	7.6	6.92E-12	3.59E-11	-2.297	33	100	66.5
LY6A	0.661538462	0.595654478	6.686	9.45E-09	2.65E-08	-3.074	61	80	70.5
LRPAP1	0.423076923	0.779014308	4.695	5.88E-07	1.24E-06	-4.182	81	63	72
CCR5	0.561538462	0.655537891	0.546	8.41E-07	1.73E-06	-4.208	83	62	72.5
ITGB2	0.930769231	0.300476948	7.403	2.62E-10	9.16E-10	-2.383	49	97	73
PDLIM2	0.507692308	0.707472178	3.87	5.70E-07	1.22E-06	-3.972	80	67	73.5
LSM1	0.484615385	0.717011129	0.895	2.19E-06	4.20E-06	-4.546	89	59	74
EZR	0.8923076	0.3593004	4.859	2.01E-10	7.31E-10	-2.293	47	101	74

Genes	TP	TN	thresh_mhg	hyper_pval	hyper_qval	gen_qval	rank_hyper_qval	rank_gen_qval	mean_rank
	92	77							
CCRL2	0.453846154	0.746687864	0.632	1.47E-06	2.93E-06	-4.152	86	64	75
TLN1	0.915384615	0.326974033	3.176	1.76E-10	6.55E-10	-2.174	46	104	75
SCARB2	0.361538462	0.813460519	0.506	4.84E-06	8.63E-06	-5.039	96	56	76
MYO9B	0.523076923	0.703232644	1.214	1.69E-07	3.75E-07	-3.28	77	75	76
CCL5	0.984615385	0.258611553	7.976	5.11E-14	3.97E-13	-0.865	22	132	77
CLPTM1	0.423076923	0.767885533	0.678	2.71E-06	5.08E-06	-4.014	91	65	78
BSG	0.907692308	0.348171701	2.239	4.53E-11	2.09E-10	-1.568	36	121	78.5
PDIA4	0.553846154	0.647588765	0.978	4.71E-06	8.57E-06	-3.785	94	69	81.5
CD2BP2	0.623076923	0.588765236	0.345	2.05E-06	3.98E-06	-3.246	88	77	82.5
CALR	0.869230769	0.339692634	0.911	1.21E-07	2.83E-07	-2.538	72	93	82.5
GRN	0.323076923	0.840487546	2.31	7.26E-06	1.27E-05	-3.919	98	68	83
NR3C1	0.461538462	0.730259671	1.077	5.03E-06	8.87E-06	-3.622	97	71	84
PTPRCAP	0.984615385	0.195018548	1.811	7.18E-10	2.36E-09	-1.636	52	116	84
H13	0.9	0.323794383	3.905	5.12E-09	1.51E-08	-1.702	58	113	85.5
ADAM8	0.515384615	0.678325384	0.345	7.98E-06	1.38E-05	-3.536	99	73	86
LILRB4	0.530769231	0.668786433	2.882	4.82E-06	8.63E-06	-3.139	95	79	87
CR1L	0.530769231	0.6709062	5.969	3.80E-06	7.06E-06	-2.958	92	84	88
CD52	0.984615385	0.141494436	8.378	1.07E-06	2.18E-06	-2.52	84	94	89
REEP4	0.353846154	0.812400636	5.512	1.29E-05	2.19E-05	-3.201	101	78	89.5
GPR65	0.6	0.604663487	0.949	4.20E-06	7.72E-06	-2.846	93	86	89.5
NAMPT	0.392307692	0.772125066	0.189	3.85E-05	6.10E-05	-3.307	108	74	91
GABARAPL1	0.469230769	0.709062003	0.595	2.60E-05	4.19E-05	-3.276	106	76	91
H2-M3	0.515384615	0.672496025	2.296	1.50E-05	2.49E-05	-3.027	103	82	92.5
CD8A	1	0.137784844	6.569	8.48E-09	2.42E-08	-1.481	60	125	92.5
CD3G	0.992307692	0.118706942	8.288	2.26E-06	4.29E-06	-2.319	90	98	94
PSTPIP1	0.746153846	0.461579226	3.269	1.85E-06	3.64E-06	-1.969	87	107	97
KLRC1	0.715384615	0.510863805	3.234	3.31E-07	7.16E-07	-1.67	79	115	97
BST2	0.507692308	0.684684685	4.919	8.56E-06	1.46E-05	-2.474	100	96	98
CCL4	0.553846154	0.626391097	1.257	4.18E-05	6.49E-05	-2.82	110	87	98.5
NCKAP1L	0.692307692	0.494435612	2.118	2.23E-05	3.64E-05	-2.589	105	92	98.5

Genes	TP	TN	thresh_mhg	hyper_pval	hyper_qval	gen_qval	rank_hyper_qval	rank_gen_qval	mean_rank
CD48	0.853846154	0.358770535	5.697	1.32E-07	3.02E-07	-1.496	75	124	99.5
PSEN1	0.515384615	0.647058824	0.401	0.000180441	0.000252913	-3.022	122	83	102.5
CORO1A	0.930769231	0.211976683	10.514	1.33E-05	2.23E-05	-2.149	102	105	103.5
SPN	0.7	0.463169051	1.257	0.000172896	0.000248447	-2.775	119	89	104
THY1	0.907692308	0.271860095	4.919	7.11E-07	1.48E-06	-1.247	82	127	104.5
LAMP2	0.446153846	0.706412295	0.585	0.000268583	0.000367422	-2.916	125	85	105
SLC3A2	0.861538462	0.3290938	3.721	1.21E-06	2.43E-06	-1.331	85	126	105.5
TNFRSF18	0.784615385	0.439321675	4.927	1.88E-07	4.12E-07	-0.839	78	133	105.5
CD96	0.623076923	0.543190249	0.367	0.000166732	0.00024162	-2.507	118	95	106.5
PEAR1	0.376923077	0.759406465	0.287	0.000583902	0.00077401	-2.815	129	88	108.5
TNFRSF4	0.507692308	0.669846317	1.07	4.09E-05	6.41E-05	-1.862	109	109	109
RPS6KB1	0.484615385	0.660837308	0.251	0.000661106	0.000869608	-2.611	130	91	110.5
CAP1	0.723076923	0.463698993	0.401	1.83E-05	3.01E-05	-1.569	104	120	112
KLRC2	0.623076923	0.561208267	1.202	3.33E-05	5.32E-05	-1.553	107	122	114.5
CCL3	0.407692308	0.726550079	1.379	0.000977161	0.001246974	-2.31	134	99	116.5
AIMP1	0.569230769	0.595654478	0.986	0.000179206	0.000252913	-1.709	121	112	116.5
ECE1	0.361538462	0.764175941	0.227	0.0012909	0.00163514	-2.269	135	102	118.5
CD44	0.692307692	0.456279809	0.356	0.000576875	0.000770669	-1.758	128	110	119
ROCK1	0.546153846	0.591944886	0.111	0.001463222	0.001839787	-2.26	136	103	119.5
NOTCH1	0.646153846	0.524642289	0.263	0.000109848	0.000162337	-1.53	116	123	119.5
AAMP	0.653846154	0.524112348	5.699	5.74E-05	8.62E-05	-1.174	114	128	121
PDE4D	0.476923077	0.655537891	0.151	0.001782987	0.002209354	-2.074	138	106	122
F2R	0.484615385	0.64917859	0.444	0.001667431	0.002081247	-1.743	137	111	124
CD164	0.884615385	0.262851086	3.392	4.94E-05	7.54E-05	-0.671	112	136	124
HCST	0.823076923	0.338102809	5.272	5.61E-05	8.49E-05	-0.665	113	137	125
CD82	0.907692308	0.232644409	1.091	4.64E-05	7.16E-05	-0.582	111	141	126
ADAM17	0.407692308	0.696873344	0.251	0.009299564	0.010891955	-1.872	146	108	127
STK10	0.730769231	0.428192899	1.202	0.00020202	0.000280858	-0.812	123	134	128.5
IRAK2	0.523076923	0.591944886	0.239	0.006827407	0.008107545	-1.697	144	114	129
CD160	0.415384615	0.699523052	0.895	0.004746152	0.005755972	-1.583	141	118	129.5
SBDS	0.5	0.6163222	0.722	0.0059970	0.0071712	-1.603	143	117	130

Genes	TP	TN	thresh_mhg	hyper_pval	hyper_qval	gen_qval	rank_hyper_qval	rank_gen_qval	mean_rank
		05		32	76				
CD3E	0.807692308	0.345521993	7.041	0.00014529	0.000212347	-0.411	117	145	131
SMPD1	0.330769231	0.768415474	0.632	0.008326184	0.009819155	-1.575	145	119	132
ITGAL	0.884615385	0.254372019	3.415	0.000110123	0.000162337	-0.332	115	150	132.5
B4GALT1	0.907692308	0.218865925	1.339	0.00017627	0.000251184	-0.384	120	147	133.5
ANXA5	0.592307692	0.541070482	0.367	0.002142195	0.002635363	-1.01	139	130	134.5
TMEM123	0.776923077	0.361420244	0.367	0.000704655	0.000919817	-0.497	131	142	136.5
TRPV2	0.569230769	0.53736089	0.444	0.011812575	0.013648313	-1.036	148	129	138.5
CD6	0.776923077	0.376258612	3.457	0.000214698	0.000296076	-0.21	124	156	140
ATP6AP2	0.446153846	0.645468998	0.526	0.023239485	0.026493013	-0.959	150	131	140.5
CAST	0.615384615	0.493375729	0.401	0.010226771	0.011896448	-0.787	147	135	141
CD97	0.769230769	0.368309486	0.687	0.000804983	0.001034978	-0.303	133	151	142
HSPA5	0.9	0.224165342	6.751	0.000278772	0.000378333	-0.086	126	159	142.5
CNP	0.646153846	0.473237944	0.496	0.005127118	0.006174206	-0.387	142	146	144
IL27RA	0.553846154	0.529941706	0.444	0.03930102	0.044213648	-0.643	152	139	145.5
FLT3L	0.353846154	0.70800212	0.918	0.083001879	0.091569815	-0.648	155	138	146.5
CD47	0.907692308	0.202437732	6.03	0.000784417	0.001016177	-0.059	132	161	146.5
CD3D	0.992307692	0.080021198	5.681	0.000327523	0.000440996	0	127	166	146.5
ICOS	0.646153846	0.4409115	0.39	0.031709005	0.035908874	-0.366	151	148	149.5
IL18RAP	0.461538462	0.588235294	0.411	0.153414613	0.163961868	-0.643	160	140	150
CMTM6	0.492307692	0.573396926	0.39	0.085468407	0.093236223	-0.493	157	143	150
CD27	0.807692308	0.31054584	0.696	0.002306039	0.002816662	-0.079	140	160	150
NOTCH2	0.584615385	0.474297827	0.138	0.112776801	0.121288257	-0.472	159	144	151.5
CD226	0.515384615	0.544250132	0.705	0.109734793	0.118763605	-0.359	158	149	153.5
KLRK1	0.553846154	0.523582406	0.31	0.052818796	0.059032772	-0.232	153	154	153.5
ITGB1	0.638461538	0.437731849	0.239	0.053520057	0.059428115	-0.221	154	155	154.5
CD37	0.815384615	0.276099629	1.501	0.012826956	0.014720869	-0.011	149	163	156
CD5	0.576923077	0.489136195	0.566	0.085602848	0.093236223	-0.178	156	157	156.5
ICAM1	0.423076923	0.6179120 3	0.251	0.201104231	0.212276688	-0.238	162	152	157
IL16	0.492307692	0.551669316	0.287	0.188351528	0.200050381	-0.237	161	153	157
TNIP1	0.430769231	0.57763646	0.433	0.460212051	0.479855248	-0.139	164	158	161

Genes	TP	TN	thresh_mhg	hyper_pval	hyper_qval	gen_qval	rank_hyper_qval	rank_gen_qval	mean_rank
CCND2	0.623076923	0.391096979	0.111	0.412262776	0.432496532	-0.006	163	164	163.5
CXCR3	0.3	0.68627451	0.614	0.661022409	0.680932723	-0.026	166	162	164
CD28	0.615384615	0.382617912	0.251	0.557553199	0.577827886	0	165	167	166
KLRD1	0.553846154	0.40063593	1.189	0.867473835	0.882964439	-0.002	168	165	166.5
IL4RA	0.415384615	0.563328034	0.287	0.713069164	0.730148665	0	167	168	167.5
CD84	0.438461538	0.50609433	0.275	0.905698529	0.916416855	0	169	169	169
PDE4B	0.392307692	0.540010599	0.31	0.944621169	0.950177764	0	170	170	170
IL18R1	0.346153846	0.536301007	0.151	0.996721649	0.996721649	0	171	171	171

Table 17. Ranked top 100 differentially expressed genes in cluster 10 as compared to all 15 CD8 T cell clusters

	adj.pval.cluster 1	adj.pval.cluster 2	adj.pval.cluster 3	adj.pval.cluster 4	adj.pval.cluster 5	adj.pval.cluster 6	adj.pval.cluster 7	adj.pval.cluster 8	adj.pval.cluster 9	adj.pval.cluster 10	adj.pval.cluster 11	adj.pval.cluster 12	adj.pval.cluster 13	adj.pval.cluster 14	adj.pval.cluster 15
CDC6	0	0	0	0	0	0	-0.375	0	-5.963	-80.932	-0.001	0	0	0	-0.692
LIG1	0	0	0	0	0	0	-2.952	0	-29.581	-75.296	-0.001	0	0	-0.587	-3.286
MCM5	0	0	0	0	0	0	-3.082	0	-28.644	-75.296	-0.001	-0.056	0	0	-3.117
MCM7	0	0	0	0	0	0	-2.487	0	-23.605	-72.59	-0.001	0	0	0	-3.339
RRM2	0	0	0	0	0	0	-0.044	0	-66.459	-72.59	-0.001	0	0	0	-3.577
PRIM1	0	0	0	0	0	0	-1.408	0	-19.24	-68.79	-0.001	-0.142	0	0	-1.436
RAD51	0	0	0	0	0	0	-0.085	0	-52.488	-68.583	-0.001	0	0	0	-4.518
MCM3	0	0	0	0	0	0	-5.735	0	-17.252	-68.528	-0.001	0	0	0	-1.39
STMN1	0	0	0	0	0	0	-0.635	0	-98.221	-68.25	-0.001	0	0	0	-8.858
CDC45	0	0	0	0	0	0	-0.079	0	-47.1	-67.854	-0.001	0	0	0	-3.684
POLA1	0	0	0	0	0	0	-0.896	0	-15.48	-65.955	-0.001	-0.045	0	0	-1.104
DHFR	0	0	0	0	0	0	-0.791	0	-9.228	-64.125	-0.001	-0.042	0	0	-0.949
UHR	0	0	0	0	0	0	-	0	-	-	-	0	0	0	-

	adj.pval.cluster 1	adj.pval.cluster 2	adj.pval.cluster 3	adj.pval.cluster 4	adj.pval.cluster 5	adj.pval.cluster 6	adj.pval.cluster 7	adj.pval.cluster 8	adj.pval.cluster 9	adj.pval.cluster 10	adj.pval.cluster 11	adj.pval.cluster 12	adj.pval.cluster 13	adj.pval.cluster 14	adj.pval.cluster 15
F1							3.683		35.788	63.748	0.001				5.496
FEN1	0	0	0	0	0	0	-0.895	0	-34.054	-63.6	-0.001	-0.303	0	0	-2.709
NCAPG2	0	0	0	0	0	0	-0.015	0	-58.374	-63.475	-0.001	0	0	0	-2.562
HELLS	0	0	0	0	0	0	-4.143	0	-15.141	-63.122	-0.001	0	0	0	-0.671
RFC3	0	0	0	0	0	0	-0.713	-0.208	-16.602	-62.998	-0.001	-0.006	0	0	-1.728
TK1	0	0	0	0	0	0	-3.788	-0.031	-58.393	-62.819	-0.001	0	0	0	-5.968
DTL	0	0	0	0	0	0	-5.276	0	-10.026	-62.775	-0.001	-0.031	0	0	-0.278
28104 17H13RIK	0	0	0	0	0	0	-0.877	0	-94.353	-61.938	-0.001	0	0	0	-7.68
MCM2	0	0	0	0	0	0	-4.813	0	-7.004	-61.904	-0.001	-0.208	0	0	-0.528
TIPIN	0	0	0	0	0	0	-4.244	-0.074	-20.86	-60.518	-0.001	0	0	0	-1.756
TCF19	0	0	0	0	0	0	0	0	-24.957	-59.212	-0.001	0	0	0	-2.241
RAD51AP1	0	0	0	0	0	0	0	0	-32.834	-57.189	-0.001	0	0	0	-0.739
CCNE1	0	0	0	0	0	0	-1.07	0	-2.819	-56.691	-0.001	0	0	0	-1.985
ASF1B	0	0	0	0	0	0	-0.123	0	-73.726	-56.208	-0.001	0	0	-4.035	-3.737
MCM10	0	0	0	0	0	0	-0.009	0	-22.236	-55.388	-0.001	0	0	0	-2.856
GINS2	0	0	0	0	0	0	-0.865	0	-18.673	-53.678	-0.001	0	0	0	-1.156
POLD1	0	0	0	0	0	0	-0.966	0	-21.264	-53.491	-0.001	-0.052	0	0	-2.884
CHEK1	0	0	0	0	0	0	-0.307	0	-6.859	-53.253	-0.001	-0.674	0	0	-2.143
RRM1	0	0	0	0	0	0	-3.401	0	-47.743	-52.264	-0.001	0	0	-1.259	-4.578
POLE	0	0	0	0	0	0	-1.436	0	-17.43	-52.10	-0.001	0	0	0	-1.845

	adj.pval.cluster 1	adj.pval.cluster 2	adj.pval.cluster 3	adj.pval.cluster 4	adj.pval.cluster 5	adj.pval.cluster 6	adj.pval.cluster 7	adj.pval.cluster 8	adj.pval.cluster 9	adj.pval.cluster 10	adj.pval.cluster 11	adj.pval.cluster 12	adj.pval.cluster 13	adj.pval.cluster 14	adj.pval.cluster 15
									5	2					
GMNN	0	0	0	0	0	0	-0.53	0	-41.762	-51.69	-0.001	-0.138	0	0	-1.572
CLSPN	0	0	0	0	0	0	0	0	-33.627	-49.936	-0.001	0	0	0	-0.956
TOP2A	0	0	0	0	0	0	-1.539	-0.04	-69.996	-49.278	-0.001	0	0	0	-3.78
CENPH	0	0	0	0	0	0	-0.171	0	-52.651	-49.136	-0.001	0	0	0	-2.809
CHAF1A	0	0	0	0	0	0	-1.454	0	-20.331	-48.964	-0.001	-0.001	0	0	-1.211
FIGNL1	0	0	0	0	0	0	-0.599	0	-42.968	-48.643	-0.001	0	0	0	-4.277
MCM6	0	0	0	0	0	0	-7.123	-0.004	-9.263	-48.616	-0.001	-1.281	0	-0.497	-1.868
CDCA7	0	0	0	0	0	0	-5.71	0	-0.193	-47.71	-0.001	-4.091	0	0	-1.131
DUT	0	0	0	0	0	0	-17.989	-0.155	-22.209	-47.471	-0.001	-2.552	0	0	-1.356
UNG	0	0	0	0	0	0	-3.748	-0.435	0	-47.352	-0.001	-0.293	0	-0.301	-0.182
CHAF1B	0	0	0	0	0	0	-1.148	0	-4.203	-45.683	-0.045	0	0	0	-0.552
CDK2	0	0	0	0	0	0	-0.863	-0.068	-12.311	-45.448	-0.001	-0.09	0	-0.088	-1.209
RFC4	0	0	0	0	0	0	-1.406	0	-33.508	-44.901	-0.001	0	0	0	-5.641
ORC6	0	0	0	0	0	0	-3.025	0	-16.032	-44.275	-0.001	-0.008	0	0	-1.409
CHTF18	0	0	0	0	0	0	-0.003	0	-29.359	-44.221	-0.001	0	0	0	-5.619
CCNE2	0	0	0	0	0	0	-1.961	-0.069	-3.802	-43.691	-0.001	0	0	0	-0.378
MCM4	0	0	0	0	0	0	-2.143	0	-14.929	-43.425	-0.001	0	0	-0.35	-0.86
PASK	0	0	0	0	0	0	0	0	-6.489	-43.132	-0.001	0	0	0	-0.148
BRCA1	0	0	0	0	0	0	0	0	-16.998	-42.986	-0.001	-0.006	0	0	-0.579
RFC5	0	0	0	0	0	0	-1.127	-0.067	-46.27	-42.44	-0.001	0	0	0	-2.609

	adj.pval.cluster 1	adj.pval.cluster 2	adj.pval.cluster 3	adj.pval.cluster 4	adj.pval.cluster 5	adj.pval.cluster 6	adj.pval.cluster 7	adj.pval.cluster 8	adj.pval.cluster 9	adj.pval.cluster 10	adj.pval.cluster 11	adj.pval.cluster 12	adj.pval.cluster 13	adj.pval.cluster 14	adj.pval.cluster 15
									8	9					
MYBL2	0	0	0	0	0	0	-0.017	0	-7.383	-41.707	-0.001	0	0	0	-0.636
STIL	0	0	0	0	0	0	0	0	-36.048	-41.461	-0.001	0	0	0	-5.602
E2F7	0	0	0	0	0	0	0	0	-25.101	-40.974	-0.001	0	0	0	-0.125
SLBP	0	0	0	0	0	0	-2.511	0	-8.783	-40.456	-0.001	0	0	0	-1.242
SYCE2	0	0	0	0	0	0	-1.447	0	-3.05	-40.147	-0.001	-0.051	0	0	-0.962
DNMT1	0	0	0	0	0	0	-2.951	0	-15.63	-40.123	-0.001	0	0	-2.411	-0.78
NCAPG	0	0	0	0	0	0	0	0	-99.319	-40.113	-0.001	0	0	0	-5.526
RAD54L	0	0	0	0	0	0	-0.05	0	-26.147	-40.015	-0.001	0	0	0	-4.47
WDHD1	0	0	0	0	0	0	-2.686	0	-5.165	-39.503	-0.001	-0.302	0	0	-0.76
ZFP367	0	0	0	0	0	0	-0.228	-0.15	-12.738	-39.379	-0.001	0	0	0	-1.099
SMC2	0	0	0	0	0	0	-0.203	0	-68.225	-39.346	-0.001	0	0	0	-3.648
PMF1	0	0	0	0	0	0	-2.377	0	-55.018	-39.271	-0.001	0	0	-3.218	-4.469
PCNA	0	0	0	0	0	0	-10.539	0	-11.735	-39.204	-0.001	-0.357	0	0	-0.536
PKMYT1	0	0	0	0	0	0	0	0	-25.968	-38.888	-0.001	0	0	0	-2.823
ATAD5	0	0	0	0	0	0	-0.175	-0.094	-6.27	-38.839	-0.001	0	0	0	-1.576
CDCA7L	0	0	0	0	0	0	-4.42	-0.146	-3.625	-38.797	-0.001	-0.391	0	0	-0.51
E2F8	0	0	0	0	0	0	0	0	-25.071	-38.663	-0.001	0	0	0	-1.624
DCK	0	0	0	0	0	0	-0.666	0	-9.393	-38.53	-0.001	0	0	-4.515	-0.928
CDCA5	0	0	0	0	0	0	0	0	-75.623	-38.383	-0.001	0	0	0	-3.513
NCAPH	0	0	0	0	0	0	-0.439	0	-72.57	-38.07	-0.001	-0.609	0	0	-2.757

	adj.pval.cluster 1	adj.pval.cluster 2	adj.pval.cluster 3	adj.pval.cluster 4	adj.pval.cluster 5	adj.pval.cluster 6	adj.pval.cluster 7	adj.pval.cluster 8	adj.pval.cluster 9	adj.pval.cluster 10	adj.pval.cluster 11	adj.pval.cluster 12	adj.pval.cluster 13	adj.pval.cluster 14	adj.pval.cluster 15
									4	6					
CDC7	0	0	0	0	0	0	-0.777	0	-24.66	-37.902	-0.001	-0.094	0	0	-1.439
HIST1H2AO	0	0	0	0	0	0	-0.199	0	-77.944	-37.764	-0.001	0	-0.147	0	-5.609
RNASEH2B	0	0	0	0	0	0	-8.308	0	-30.417	-37.634	-0.054	0	0	0	-1.948
FANCI	0	0	0	0	0	0	0	0	-12.873	-37.117	-0.001	0	0	0	-0.44
4930422G04RIK	0	0	0	0	0	0	-0.342	-0.121	-3.941	-36.923	-0.001	-0.04	0	0	-0.48
CDK1	0	0	0	0	0	0	0	0	-89.58	-36.898	-0.001	0	0	0	-5.968
CDCA2	0	0	0	0	0	0	-0.02	0	-82.191	-36.305	-0.001	0	0	0	-5.418
PBK	0	0	0	0	0	0	0	0	-60.6	-36.206	-0.001	0	0	0	-4.177
TICRR	0	0	0	0	0	0	0	0	-25.958	-35.716	-0.001	0	0	0	-1.981
SIVA1	0	0	0	0	0	0	-16.842	-0.379	-11.903	-35.654	-0.001	-0.768	0	0	-3.203
FBXO5	0	0	0	0	0	0	0	0	-50.949	-35.498	-0.001	0	0	0	-2.173
PPIL1	0	0	0	0	0	0	-6.372	-0.284	-19.117	-35.338	-0.001	-0.195	0	0	-2.917
NCAPD2	0	0	0	0	0	0	-0.408	0	-90.985	-35.029	0	0	0	0	-10.266
PTMA	0	0	0	0	0	0	-0.646	0	-42.495	-34.989	-0.269	-8.647	0	0	-1.945
TIMELESS	0	0	0	0	0	0	-0.681	0	-11.07	-34.828	-0.001	-0.25	0	0	-0.341
WDR76	0	0	0	0	0	0	-1.801	0	-11.48	-34.787	0	0	0	0	-1.739
DSCC1	0	0	0	0	0	0	-0.433	0	-7.576	-34.079	-0.001	0	0	0	-3.431
MAD2L1	0	0	0	0	0	0	-0.454	0	-94.515	-33.55	-0.001	0	0	0	-7.613
KNTC1	0	0	0	0	0	0	0	0	-22.085	-33.52	-0.001	0	0	0	-2.562

	adj.pval.cluster 1	adj.pval.cluster 2	adj.pval.cluster 3	adj.pval.cluster 4	adj.pval.cluster 5	adj.pval.cluster 6	adj.pval.cluster 7	adj.pval.cluster 8	adj.pval.cluster 9	adj.pval.cluster 10	adj.pval.cluster 11	adj.pval.cluster 12	adj.pval.cluster 13	adj.pval.cluster 14	adj.pval.cluster 15
POLD2	0	0	0	0	0	0	-2.099	0	-9.613	-33.433	-0.125	-1.255	0	0	-0.872
TYMS	0	0	0	0	0	0	-2.408	-0.016	-16.524	-33.365	-0.001	0	0	0	-2.73
DNAJC9	0	0	0	0	0	-2.61	-0.078	0	-13.66	-33.305	-0.001	0	0	-0.716	-1.62
AURKB	0	0	0	0	0	0	0	0	-115.142	-33.175	-0.001	0	0	0	-7.378
NUP62	0	0	0	0	0	0	-2.183	-0.036	-24.712	-33.144	-0.001	-0.297	0	-3.122	-2.342
HIST2H3B	0	0	0	0	0	0	0	0	-60.755	-33.065	-0.001	0	0	0	-5.492
NUSAP1	0	0	0	0	0	0	0	0	-129.805	-32.937	-0.001	0	0	0	-7.459
RFWD3	0	0	0	0	0	0	-1.287	-0.255	-17.015	-32.598	-0.001	0	0	-1.951	-3.148

Table 18. Cluster 7 Specific Gene Signature

CD8_cluster7				
Genes 1-25	**Genes 26-50**	**Genes 51-75**	**Genes 76-100**	**Genes 101-124**
PRF1	ADAMTS14	GZMD	SLC16A3	STK24
GLDC	RGS8	SERPINE2	GPR65	FKBP1A
LAT2	CCNG1	SLC25A4	FCRL6	DSCAM
ADAM8	CDK6	PADI2	GM14295	STK39
TNFRSF9	GPR56	PPP1R3B	ITGB1BP1	ISY1
HILPDA	GPD2	MYO10	SRGAP3	MRC2
TMPRSS6	PLAC8	SLC52A3	FOXRED2	NUDT18
CCRL2	HAVCR2	ASB2	NAGPA	SIL1
ID2	GZMF	LRRK1	RCN1	ENO1
NABP1	CBLB	AFG3L2	GBP10	LPIN2
LILRB4	EHD1	PTK2B	SLA2	GP49A
2900026A02RIK	FILIP1	ACOXL	RHOC	ACOT7
AA467197	SLC2A3	NEK6	STAT3	RGS1
SERPINB9	GZME	NEDD9	SYNGR3	SLC27A1
UBASH3B	BCL2L11	ANXA2	PLXND1	CST7
CXCR6	INSRR	DGAT1	SLC24A1	TIPRL
PCYT1A	PLEK	IRAK2	ERO1L	CTSD
IL2RB	RGS2	MT2	RLN3	CIAPIN1
CCL3	GZMC	THEMIS2	EPDR1	PTPN5

CD8_cluster7				
Genes 1-25	**Genes 26-50**	**Genes 51-75**	**Genes 76-100**	**Genes 101-124**
LITAF	GEM	RGS16	IRF8	IL10RA
EPAS1	GZMB	PGLYRP1	MT1	GSTO1
C1QTNF6	SDCBP2	FXYD5	TMEM135	GBP6
ALDOA	ITGAV	SLC35D3	SLCO2A1	SERINC1
S100A11	PKM	CCL4	TOMM40L	RAB19
SLC37A2	SH2D2A	DGKH	D16ERTD472E	

Table 19. Cluster 8 Specific Gene Signature

CD8_cluster8					
Genes 1-33	**Genes 33-66**	**Genes 67-99**	**Genes 100-132**	**Genes 133-165**	**Genes 166-198**
XCL1	REL	LANCL1	CPNE8	EEF1B2	CSRNP1
CD83	DUSP1	ETV6	SPRY1	SH3BGRL	KLRK1
CCR7	SLC25A42	PLK2	FAS	RNF40	NFKBIZ
LAD1	SAT1	MRPS6	TRAF1	AHCYL2	B4GALNT1
CRTAM	FAM46A	PER1	CALCOCO1	NMRK1	PRKCA
TNFSF8	RAF1	ZFP467	ARC	ASNSD1	SYNJ2
CD81	LTA	KLRB1F	RPL34-PS1	NEK7	CDON
ITGB1	TESPA1	2610301B20RIK	ICOS	PTPRS	NPTN
PLXDC2	GPM6B	IMMP2L	CPNE3	PDCD1LG2	IGSF3
BACE2	SESN3	IER3	SH3RF1	FOSB	ATP6V1G2
TNFSF11	GM12505	PRNP	RNF19A	DHRS3	CCR8
RAMP3	CD9	CCL1	FBXO11	NR4A3	PXMP2
BCL6	ABCA3	TGFB1	ITPR1	SLC17A6	PAIP2
GRAMD1B	B3GNT2	PENK	SPRY2	PARP3	TLCD2
CD74	REEP3	TSPAN32	HIF1A	ANKRD46	CD68
DAPL1	GUCY1A3	LRIG1	AI836003	TNFRSF18	PKP3
ARAP2	1700019D03RIK	FAM178B	AKAP8L	SDF4	NDUFA6
TBC1D4	IL2RA	AXL	SYT11	GABARAPL1	GTF2IRD1
SLC2A6	BTLA	MS4A4B	PACSIN1	PPAP2A	DGKZ
SYNPO	SSH1	TNFSF4	MPC1	SDC4	GALM
ZFP36L1	ASAP1	TIAM1	FAM195B	CD70	CXCR5
BACH2	SIGIRR	GM12942	DUSP4	ALCAM	HGSNAT
SLAMF6	SLA	BHLHE40	ARL3	JAK2	TNFRSF4
ZC3H12D	JUNB	LRRC8D	TMEM173	RELB	RPL41
CXXC5	CTSW	SCYL2	TGIF1	NRN1	FAM162A
2310001H17RIK	FAM53B	RASGEF1A	NT5E	EPHX1	H2-OA
MS4A4C	MGAT5	GYPC	ST6GAL1	SPOCK2	RPL7
RAB37	GALNT9	RORA	NFAT5	CTSZ	VWA5A
SAMD3	HEG1	NFKB1	GADD45B	ORAI2	ITM2A
JUN	DCLK1	KLRI2	RIPK2	CD200	IER5

CD8_cluster8					
Genes 1-33	**Genes 33-66**	**Genes 67-99**	**Genes 100-132**	**Genes 133-165**	**Genes 166-198**
TNFSF14	PTPRK	2010015L04RIK	FAM168A	GM10548	0610011F06RIK
CD160	EGR2	PIKFYVE	B9D2	RCCD1	TG
NFKBIA	CAR2	CD82	TMEM243	EFHA2	GDI2

Table 20. Cluster 9/10 Specific Gene Signature

CD8_cluster9/10					
Genes 1-75	**Genes 76-150**	**Genes 151-225**	**Genes 226-300**	**Genes 301-375**	**Genes 376-451**
2810417H13RIK	CLSPN	NCAPH2	WHSC1	BRIP1	EIF1AD
STMN1	RFC5	MCM4	POLE	PFN1	TMEM97
HMGB2	FEN1	POLA1	RFC2	KIFC1	CENPO
BIRC5	KIF23	HAUS4	FANCA	CEP89	SYCE2
CCNA2	FBXO5	UBE2S	TIMELESS	SF3B5	PIH1D1
SPC24	SMC2	CCNF	PKMYT1	RFWD3	HMGN5
CDCA8	1190002F15RIK	RFC4	RAD21	ARL6IP1	CDCA4
HIST1H2AO	TCF19	H2AFX	2700099C18RIK	TXN1	1600002H07RIK
TK1	PTMA	AURKA	LRR1	ANAPC5	MMS22L
TPX2	TUBB5	PRIM1	CRIP1	AGFG2	HAT1
HMGN2	MELK	PLK4	TCEB2	CENPL	GM12504
MKI67	KNSTRN	RFC3	CMTM7	TUBE1	4930422G04RIK
CDK1	CENPE	RPA2	1500009L16RIK	LNP	WDR90
RRM2	GMNN	CDC25B	CALM3	U2AF1	IPO9
NUSAP1	MXD3	PPIL1	NSL1	BC055324	PHF11B
ASF1B	PPIA	CHTF18	PRR11	TUBA1A	DDB2
KIF20A	KIF4	SLBP	PASK	POLA2	ERCC6L
CCNB2	RAN	ANP32E	SMC4	FDFT1	NDE1
CDC20	PARPBP	MIS18A	KIF20B	NUCKS1	DNAAF2
CKS1B	ARHGAP11A	KIF18A	ARSB	PUF60	HIST1H1C
NUF2	TIPIN	TUBA1C	HAUS5	TRP53I13	EHD4
KIF11	LMNB1	POC1A	LSM5	TMEM107	RANBP1
MAD2L1	SHCBP1	SUV39H1	SNRPD1	DDX39	NT5C3L
NEK2	GZMK	ZWILCH	RCBTB2	HAUS3	MCM8
NCAPD2	HIST2H3B	BRCA1	ORC6	SLC43A3	HNRNPAB
TOP2A	SKA2	RAD54L	RPA1	HIST1H2BJ	GINS4
UBE2C	HIST2H3C2	PCNA	TERF1	RQCD1	PLEKHF1
NCAPG	PBK	YWHAH	HIST1H1B	ZBTBD6	MUTYH
TACC3	ESPL1	SGOL2	PPP1CA	BLM	RBBP4
TUBA1B	RAD54B	CDC6	GLTP	CDK4	POLD2
CDCA3	CENPW	CENPK	MCM6	MED30	CALM2
H2AFZ	HMMR	CEP57	RANGAP1	NUP107	CENPC1
FAM64A	FOXM1	POLD1	BUB3	GIMAP7	SLFN3

CD8_cluster9/10					
Genes 1-75	**Genes 76-150**	**Genes 151-225**	**Genes 226-300**	**Genes 301-375**	**Genes 376-451**
MCM7	UHRF1	ANLN	DBI	EME1	RNF5
MCM5	BANF1	CENPI	RNF26	UCHL5	NUTF2-PS1
HMGB1	BC030867	CENPM	PHGDH	CENPT	CCDC18
CDCA2	ASPM	CENPP	TRAIP	CKAP5	EXOSC7
PLK1	HELLS	FXN	ARHGAP33	GPAA1	2700029M09RIK
KIF22	ANKLE1	WDR62	ULBP1	ATP5J	E2F1
BUB1	CENPA	DEK	HIST1H4I	EXOSC8	TFDP1
CKAP2L	RNASEH2B	SNRPB	6430706D22RIK	HN1	NUP205
RAD51	STIL	GPSM2	BARD1	RECQL4	HIST1H2BC
PMF1	ECT2	HMGB3	ZFP367	CDK2	SHC1
PRC1	MIS18BP1	CBX3	EMP3	HIST1H2BG	PARD6A
CDCA5	CDC25C	FANCI	GINS1	A730008H23RIK	RAD9A
CDC45	FIGNL1	LSM2	OAZ1-PS	HIST1H2AG	HNRNPD
AURKB	C330027C09RIK	FKBP2	SPDL1	MTBP	SMC3
RACGAP1	PIF1	LSM3	DTL	NDUFA4	1700097N02RIK
SPC25	DUT	1810037I17RIK	PRIM2	CDCA7L	WDHD1
CKAP2	TTK	NUP62	CCNE1	PSMB9	PRDX1
SAPCD2	E2F8	KNTC1	DCK	NELFE	ENKD1
KIF2C	DNAJC9	TOPBP1	MASTL	GINS3	TAP1
SKA1	TROAP	RPA3	NASP	SLC29A1	CEP70
BUB1B	CIT	TRIP13	MIS12	VIM	FANCG
NCAPG2	ESCO2	MCM2	FAM111A	MB21D1	CD48
NCAPH	4930579G24RIK	TMSB4X	KIFC5B	APITD1	STUB1
TUBB4B	RAD51AP1	TICRR	PSAT1	TSEN15	HIST1H3B
DEPDC1A	CASC5	CHAF1A	LRRC40	NDUFB7	TAF12
LIG1	CLIC1	POLQ	SMTN	MAPRE1	TUBG1
DLGAP5	E2F7	CBX5	CCNE2	TMPO	DSCC1
TYMS	NRM	4930427A07RIK	ATP5O	GAS2L3	SRSF10
SPAG5	2700094K13RIK	FANCD2	H2-T22	NLRP1A	SUZ12
NEIL3	CENPN	HIST1H2AB	KIF14	SEC11C	MNS1
CENPH	INCENP	UBE2T	CCDC34	SLMO2	PAGR1A
CEP55	GEN1	DHFR	MAZ	HIST1H2AE	HELB
GTSE1	ARHGAP19	GINS2	SEPHS1	ATAD5	0610010K14RIK
CKS2	H2AFV	RBL1	HIST1H2AI	CASP7	POLD3
CDKN3	KPNA2	HJURP	WDR76	UEVLD	ELOF1
SKA3	MCM10	DNMT1	HIST1H2AK	CEP72	NFYB
RRM1	DSN1	NCAPD3	HIST1H4D	NGFRAP1	CSE1L
NDC80	REEP4	4632434I11RIK	ARHGEF39	TINF2	GM5141
CENPF	CMC2	E030024N20RIK	LBR	MYBL2	CARHSP1
MCM3	CDC7	CHEK1	PSMC3IP	EFCAB11	OAT
CCNB1	FAM83D	ERH	BORA	GLRX	ERI2

CD8_cluster9/10					
Genes 1-75	Genes 76-150	Genes 151-225	Genes 226-300	Genes 301-375	Genes 376-451
SGOL1	DIAP3	CTC1	RBBP7	SH3BGRL3	PLP2
					PSRC1

Example 2 - Identification of novel tumor infiltrating CD4+ T cells populations

[0550] CD4 cells were analyzed from the mouse tumor model at time points as discussed in Example 1 herein. CD4 T cells (both Effector and Regulatory) were obtained by sorting for CD4+CD45+ cells. NK cells, dendritic cells, and macrophages were obtained by sorting for CD4- CD8-CD45+ cells. CD45- cells included fibroblasts and tumor cells.

[0551] **Figure 19** illustrates dimension reduction analysis of the cells sequenced for CD4 T cells. Applicants sequenced 2496 cells (26 plates). 2114 cells passed the basic QC (85%) and 1478 cells passed the extensive QC (59%). Principal component (PC) analysis was performed using gene expression measured in the single cells. PC1 was associated with transcription and PC2 and PC3 were strongly associated with sequencing batches. tSNE and clustering was performed on PCs 4-6. All of the CD4 cells were pooled together on a normalized tSNE. The CD4 cells clustered into 14 clusters. **Figure 20** illustrates each cluster individually. **Figure 21** illustrates 4 populations that stand out based on expression of the CD4 Treg marker Foxp3 and a Treg signature. **Figure 22** illustrates 5 populations that stand out based on expression of the coinhibitory receptor Tim3. Tim3+CD4 Tregs are the most repressive in the tumor environment (Sakuishi et al., TIM3+FOXP3+ regulatory T cells are tissue-specific promoters of T-cell dysfunction in cancer. Oncoimmunology. 2013 Apr l;2(4):e23849). The clusters also express Tbet which has been described in the context of Tregs that suppress Thl responses (Levine et al., Stability and function of regulatory T cells expressing the transcription factor T-bet. Nature. 2017 Jun l5;546(7658):42l-425). **Figure 23** illustrates that CD4 clusters 4 and 7 have high expression of a Thl and cytokine secretion signature. The Thl signature includes Tbet, IFNy, 112, TNFa, STAT4, CXCR6, CCR5 AND CXCR3 . The cytokine signature includes GZMB, GZMK, PRF1, GZMA, GZMF, GZMC, GZMM, IFNG, TNF, GZMD, GZME and IL2.

Example 3 - Identification of Cell-Cell Interactions in CD8+ and CD4+ T cell populations

[0552] **Figures 24-26 and 36** illustrate that the different CD4 and CD8 T cell subtypes identified positively and negatively correlate with each other. Positive correlation relates to the

situation wherein high expression of one cell subtype correlates to high expression of another cell type. Negative correlation relates to the situation wherein high expression of one cell subtype correlates to low expression of another cell type. For example, CD4 clusters 8 and 10 expression correlates to expression of CD8 cluster 7 **(Fig. 26).** Thus, DP suppressive and dysfunctional CD8 T cells (cluster 7) correlate with CD4 Tim3+ Tregs and CD4 Helios[10] iTregs. **Figure 36** shows that the relative frequency of dysfunctional $CD8^+$ T cells in a tumor is correlated with $CD4^+$ Treg frequency.

[0553] **Figure 27** shows a heatmap plotting a signature for ligands and a signature for receptors. Thus, clusters of T cells can be analyzed for expression of receptor/ligand pairs. The clusters expressing the receptor/ligand pairs may functionally interact. For example, CD8 cluster 7 expresses a receptor for the ligand expressed by CD4 cluster 1.

[0554] **Figure** 28 shows that cells analyzed by single cell RNA-seq provide results consistent with bulk sequencing.

[0555] **Figure 37** shows interactions between CD8 PD1+ populations and CD4 populations (Th1-like and Treg). Connections were specifically made based on chemokine / chemokine receptor pairs (with CD45+). Applicants analyzed the interactions between Cluster 8 and cluster 7 (i.e., dysfunctional clusters).

[0556] **Figure 38** shows that XCL1 is expressed strongly in cluster 8 and XCR1 is expressed in cluster 7. Previous studies described the role of XCL1, a chemokine associated with immune suppression and allergy, on CD4(+)CD25(high)CDl27(low/-) regulatory T cell (Treg) function in allergic asthma (Nguyen et al., J Immunol. 2008 Oct 15; 181(8): 5386-95). Several studies suggest that during early tumor response NK cells secrete XCL-l thereby recruiting XCR1+ cDCl, which have been shown to be crucial to cross-present antigens to CD8 T cells (e.g PMID: 22566900 and 29429633). This cross-presentation is crucial to differentiate cytotoxic CD8 T cells. Thus, a widespread blockade of this molecule during early tumor responses could potentially hinder rather than enhance immune response. Nevertheless, some reports show that XCR-l is expressed in Tregs and that its ligand XCL-l increases suppressive activity in models of allergic asthma (PMID: 18832695). Moreover, it is now better recognized that chemokine-chemoreceptors axis can be exploited by Tregs to directly suppress T conventional cells cytotoxic activity (PMID: 26854929). Applicants hypothesize that C7 CD8 cells are recruited to

the tumor via this axis and/or Tregs are recruited through secretion of C8 CD8-derived XCL-l. Applicants hypothesize that the XCL1+CD8+ : XCR1+CD8+ axis may enhance regulatory activity of CD8+TILs. Thus, targeting XCL1 and/or XCR1 in CD8 clusters 8 and 7 may be used to enhance or inhibit T cell suppression.

[0557] **Figure 39** shows that CCL1 is expressed in cluster 8 and CCR8 is expressed in cluster 8 and cluster 7. CCR8 is also expressed in Treg+Tim3+ CD4 cells. Applicants hypothesize that CCL1+CD8+ cells (cluster 8) have a regulatory interaction with dysfunctional CD8 (cluster 7) and CD4+Tregs. Previous studies confirm the importance of this axis in recruiting Tregs to lymphoid tissues and inflammatory sites and sustaining their inflammatory phenotype (PMID: 11560999, 23798714). Additionally, blockade of CCL1 has been suggested to enhance tumor immunity (Hoelzinger et al., Blockade of CCL1 Inhibits T Regulatory Cell Suppressive Function Enhancing Tumor Immunity without Affecting T Effector Responses, J Immunol. 2010 Jun 15;184(12):6833-42).

[0558] In certain embodiments, cluster 8 CD8 T cells are primed for activation.

Example 4 - Identification of CD8+ T cells populations using 10X genomics platform

[0559] Applicants performed single cell RNA sequencing on the B16 mouse model as described in Example 1 using the 10X genomics platform (lOx Genomics, Inc., Pleasanton, CA, www.lOxgenomics.com/solutions/single-cell/). Applicants validated the previous results in that the 10X time course data revealed the same populations as in the CD8 plates, including cluster 7. **Figure 40** shows cell counts taken for cells sorted by day (left) and sorted by size (right). The first step performed was to select for CD3+ cells **(Figure 41). Figure 42** shows the general statistics for all time points taken. **Figure 43** shows CD8 /CD4 partitioning of the clusters. The portioned cells could be classified as strict CD4, Strict CD8, weak CD8. Cluster 3 and cluster 0 are CD8 clusters. Clusters 2 and 5 are CD4 clusters. **Figure 44** shows the number of cells after selecting for CD8/CD4 cells and batch correction across the time points. **Figure 45** shows plots of strict CD8 cells based on mouse, time point / batch, and by clustering. The single cells did not cluster by mouse or by time point, but clustered according to cell type (e.g., gene expression, tSNE). Applicants measured cluster specific gene expression in the strict CD8 cells **(Figures 46-47). Figure 48** shows a comparison of the plate based clusters and the lOx clusters. For example,

a cluster corresponding to clusters 7, 8, 9 and 10 in the plate based analysis were also present in single cells analyzed by the 10x platform.

Example 5- Clonal expansion in CD8 T cell clusters

[0560] Applicants measured clonal expansion in CD8 T cell clusters (B16). Applicants identified clonal identity of the CD8 T cells at single cell resolution. In certain embodiments, alpha and beta TCR chains were called and predicted to be functional using TRACER (see, e.g., Stubbington et al, (2016) T cell fate and clonality inference from single-cell transcriptomes. Nature Methods, 13 329-332). Applicants defined a clone, such that two cells share a clone if they have a reconstructed alpha and beta chain that are identical. This definition is stricter than the TRACER default. **Figure 49** shows examples of CD8 TCR clones identified. Applicants analyzed a total of 2017 CD8 cells. There were 1130 cells with both chains and these were considered "eligible cells." There were 104 clones with greater than or equal to 2 cells. There were 708 cells in clones with greater than or equal to 2 cells. Finally, there were 66 clones with greater than or equal to 4 cells.

[0561] Overlap of clones identified were detected across plates used in single cell RNA sequencing. There were no overlaps across plates from different mice **(Figure 50).** Applicants calculated clonal expansion in the CD8 clusters. The "relative expansion" score per clone was computed by #cells_in_clone / #eligible_cells_in_mouse. **Figures 51-53** show that clonal expansion is highest in the PD1+ clusters, but is lower in cluster 8 (PD1+TIM3-). Cluster 8 is PD1+ and shows significantly lower clonal expansion than the other PD1+ clusters (6, 7, 9, 10). Cluster 8 shows significantly higher clonal expansion compared to the PD1- clusters (1, 3, 5). Applicants observed that different clusters are enriched for different clones **(Figure 54).** Clusters 7 and 8 showed enrichment of multiple clones. Clusters 7, 9 and 10 (TIM3+, PD1+) shared clone 108 and 9 and 7 shared clone 185, suggesting a potential connection between the clusters. This is evidence suggesting two separate trajectories from naive T cells to either clusters 7, 9 and 10 or to cluster 8. The types of TCR clone for each cluster may also differ for function.

[0562] Applicants used a OVA+SIY+ lung cancer mouse model to induce tumors in mice. In this model SIY is a low affinity antigen and OVA is a high affinity antigen. In certain embodiments, a low affinity antigen binds a TCR weakly (>10 μM in a range of about 1-100 pM) or binds MHC weakly as compared to a high affinity antigen. In certain embodiments,

affinity is defined as the probability of initial TCR:pMHC bond formation (see, e.g., Martinez and Evavold, 2015, Lower Affinity T Cells are Critical Components and Active Participants of the Immune Response, Front Immunol. 2015; 6: 468). Low affinity T cells efficiently propagate the signaling cascade as low-affinity T cells do expand, and differentiate during the immune response (see, e.g., Martinez and Evavold, 2015).

[0563] Applicants collected CD8 and CD4 T cells across a time course (5 weeks, 8 weeks, 12 weeks, and 20 weeks). The cells were tetramer sorted for SIY binding cells and OVA (OT1) binding cells and genes differentially expressed across the cells were annotated **(Figure 55).** SIY high genes (SIY-up) were upregulated in SIY binding cells and downregulated in OVA binding cells. SIY low genes (SIY-down) were downregulated in SIY binding cells and upregulated in OVA binding cells (OVA-high or SIY-down). Applicants compared the differentially expressed genes in lung cancer mice to the B16 clusters. The top markers for B16 cluster 8 (CD83, CD81) are highly ranked in the "lower-affinity" differential expression list (SIY-high). The SIY-signature distinguishes bl6 cluster 8 as shown by violin plots and tSNE plots shaded by expression of the SIY-up signature **(Figure 56 and 57).** The OVA (SIY-down) signature does not distinguish bl6 clusters **(Figure 58).** The OVA-signature (vs. SIY) doesn't discriminate between clusters, but does align with the dysfunctional CD8Treg cluster 7 signature **(Figure 59).** **Figure 60** shows the reciprocal view in that the cluster 8 signature (bl6 melanoma) is higher in lung SIY-specific TILs at weeks 5 and 8 post tumor initiation. **Figure 61** shows that the B16 cluster 8 signature highly overlaps with the SIY-up signature, specifically, CD83 and Zfp36ll. Zfp36ll is highly expressed in cluster 8 **(Figure 62).** Thus, Zfp36ll may be a marker for cluster 8 and low-affinity antigen T cells and may be a key gene required for the function of T cells. Other overlapping genes between the cluster 8 and SIY-up signatures include CD81, CCR7, CD83, Zfp36Ll, TBC1D4, BCL6, CRTAM, GPM6B, ZC3H12D, NFKBIA, TRAF1 and TNFRS4.

Example 6 - Differentially expressed genes across time points

[0564] Applicants clustered genes differentially expressed across time points to determine clusters of genes that change or are co-regulated over time **(Figures 63-65).** Applicants identified 15 time-change clusters (Logit). Cluster 1 has 16 genes and the top 5 genes are ANAX2, GPR18, TMA7, PRKCH and LIME1. Cluster 2 has 69 genes and the top 5 genes are PDCD4, ERDR1,

ARGLU1, NDFIP1 and IER2. Cluster 3 has 55 genes and the top 5 genes are RHOX8, RN4.5S, ALKBH5, USP28 and IER3. Cluster 4 has 34 genes and the top 5 genes are GZMK, IL10RA, CHSY1, GIMAP7 and CCR5. Cluster 5 has 49 genes and the top 5 genes are H2-EB1, H2-AA, A430107P09RIK, H2-AB1 and TMPR. Cluster 6 has 52 genes and the top 5 genes are CCR7, EMB, GM12505, TCF7 and WDR92. Cluster 7 has 30 genes and the top 5 genes are DAPL1, ATP1B1, SH3BP5, DPP4 and GM5424. Cluster 8 has 136 genes and the top 5 genes are GPR56, PDCD1, LAG3, HAVCR2 and OSGIN1. Cluster 9 has 46 genes and the top 5 genes are RAMP3, NRGN, SLC16A1 1, MYOlE and HILPDA. Cluster 10 has 19 genes and the top 5 genes are CCL5, TIGIT, DGAT1, PLAC8 and BHLHE40. Cluster 11 has 53 genes and the top 5 genes are RGS16, GZMB, ARSB, SERPINA3G and CXCR6. Cluster 12 has 2 1 genes and the top 4 genes are PPP1R16B, MAP3K1, KDM6B andVMN2R-PSl29. Cluster 13 has 10 genes and the top 5 genes are LY6C2, IL18R1, KLRK1, MAFK and ITGB1. Cluster 14 has 15 genes and the top 5 genes are ZSWIM5, RNF187, PPP4C, PISD-PS3 and SOCS1. Cluster 15 has 25 genes and the top 5 genes are 5430440P10RIK, IL7R, SEPP1, IGFBP4 and RGCC.

[0565] Applicants observed connections between the time-change clusters and infomap clusters (B16 CD8 T cells) **(Figures 66-67).** The overlapping genes indicate genes that both characterize cluster specific CD8 T cell subtypes and that change over time during tumorigenesis. Thus, the overlapping genes are targets for modulating immune responses during tumorigenesis. The overlapping genes are also markers for the specific T cell subtypes. tSNE-cluster 8 is enriched for a different gene set than cluster 7. Clusters 9 and 10 are not enriched for any time-related clusters. Clusters 7 and 6 go up drastically after day 11 and then down slightly. This is possibly due to an anti-tumor immune response followed by a suppressive immune response. Thus, targeting these genes may enhance an anti-tumor response.

[0566] The genes that overlap infomap cluster 7 and logit 8 include GPR56, PDCD1, LAG3, HAVCR2, ENTPD1, 1700017B05RIK, CHN2, 2900026A02RIK, FGL2, SERPINA3H, OSBPL3, S100A4, CCL3, TNFRSF9, UBASH3B, CD244, RGS8, BCL2A1D, CCL4, CIAPIN1, GP49A, CCRL2, IRF8, GRINA, C1QTNF6, CD200R4, FILIP 1, THEMIS2, SERPINA3F, LRRK1, ARNT2, MXI1, DAPK2, TWSG1, ADAM8, TRPS1, LAT2, SDCBP2, SLC37A2, MT2, ADAMTS14, GBP 10, EPDR1 and DUT

[0567] The genes that overlap infomap cluster 7 and logit 11 include RGS16, GZMB, SERPINA3G, CXCR6, LITAF, SERPINA3I, TOX, PRF1, EHD1, LILRB4, PLEK, ITGAV, CREM, CDK6, NR4A2, UHRF2, GBP6, IRAK2, PTK2B, OXSR1 and ITGB1BP1.

[0568] The genes that overlap infomap cluster 7 and logit 10 include TIGIT, DGAT1, PLAC8, BHLHE40, GM5069, SAMSN1, RGS1, DENND4A and SIK1.

[0569] The genes that overlap infomap cluster 8 and logit 9 include RAMP3, NRGN, SLC16A11, MYOlE, FOSB, IL18RAP, OLFR1033, IL2RA, BCL2A1B, CD83, FAM46A, CD74, ENPP2, LAD1, AI836003, DUSP4, ARL14EP, CD81, XDH, KIT, TNFRSF4, RORA, ST6GAL1, ATP2B2, CAPG and PLXDC2.

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[0570] Various modifications and variations of the described methods, pharmaceutical compositions, and kits of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific embodiments, it will be understood that it is capable of further modifications and that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention that are obvious to those skilled in the art are intended to be within the scope of the invention. This application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure come within known customary practice within the art to which the invention pertains and may be applied to the essential features herein before set forth.

CLAIMS

What is claimed is:

1. An isolated $CD8^+$ T cell characterized in that the $CD8^+$ T cell comprises expression of a gene signature comprising one or more genes selected from the group consisting of any of tables 1 to 20.

2. The isolated $CD8^+$ T cell according to claim 1, wherein the $CD8^+$ T cell expresses PD-l and TIM3.

3. The isolated $CD8^+$ T cell according to claim 2, wherein the $CD8^+$ T cell expresses HMMR.

4. The isolated $CD8^+$ T cell according to claim 2, wherein the $CD8^+$ T cell expresses a gene signature comprising one or more genes selected from Table 20.

5. The isolated $CD8^+$ T cell according to claim 1, wherein the $CD8^+$ T cell expresses PD-l, TIM3, and KI67 and does not express Helios.

6. The isolated $CD8^+$ T cell according to claim 1, wherein the $CD8^+$ T cell expresses PD-l and does not express TIM3.

7. The isolated $CD8^+$ T cell according to claim 6, wherein the $CD8^+$ T cell expresses Helios (IKZF2).

8. The isolated $CD8^+$ T cell according to claim 6, wherein the $CD8^+$ T cell does not express MT1.

9. The isolated $CD8^+$ T cell according to claim 6, wherein the $CD8^+$ T cell expresses XCL1.

10. The isolated $CD8^+$ T cell according to claim 6, wherein the $CD8^+$ T cell expresses CCR8.

11. The isolated $CD8^+$ T cell according to claim 6, wherein the $CD8^+$ T cell expresses

a gene signature comprising one or more genes selected from Table 19.

12. The isolated $CD8^+$ T cell according to claim 6, wherein the $CD8^+$ T cell expresses one or more genes selected from the group consisting of RAMP3, NRGN, SLC16A1 1, MYOlE, FOSB, IL18RAP, OLFR1033, IL2RA, BCL2A1B, CD83, FAM46A, CD74, ENPP2, LAD1, AI836003, DUSP4, ARL14EP, CD81, XDH, KIT, TNFRSF4, RORA, ST6GAL1, ATP2B2, CAPG and PLXDC2.

13. The isolated $CD8^+$ T cell according to any of claims 1 to 12, wherein the $CD8^+$ T cell is a human cell.

14. The isolated $CD8^+$ T cell according to any of claims 1 to 13, wherein the $CD8^+$ T cell is a CAR T cell.

15. The isolated $CD8^+$ T cell according to any of claims 1 to 14, wherein the $CD8^+$ T cell is a $CD8^+$ T cell autologous for a subject suffering from cancer.

16. The isolated $CD8^+$ T cell according to any of claims 1 to 15, wherein the cell expresses an exogenous TCR.

17. The isolated $CD8^+$ T cell according to any of claims 1 to 16, wherein the $CD8^+$ T cell displays tumor specificity.

18. The isolated $CD8^+$ T cell according to any of claims 6 to 17, wherein the $CD8^+$ T cell expresses an endogenous TCR or CAR specific for a low affinity antigen.

19. A method for detecting or quantifying $CD8^+$ T cells in a biological sample of a subject, or for isolating $CD8^+$ T cells from a biological sample of a subject, the method comprising detecting or quantifying in a biological sample of the subject $CD8^+$ T cells as defined in any one of claims 1 to 12, or isolating from the biological sample $CD8^+$ T cells as defined in any one of claims 1 to 12.

20. The method according to claim 19, wherein $CD8^+$ T cells are detected, quantified or isolated using one or more markers selected from the group consisting of HMMR, PD-l,

TIM3, KI67, Helios, MT1, XCL1 and CCR8.

21. The method according to claim 19 or 20, wherein the $CD8^+$ T cells are detected, quantified or isolated using a technique comprising flow cytometry, mass cytometry, fluorescence activated cell sorting, fluorescence microscopy, affinity separation, magnetic cell separation, microfluidic separation, or combinations thereof.

22. The method according to claim 21, wherein the technique employs one or more agents capable of specifically binding to one or more gene products expressed or not expressed by the $CD8^+$ T cells, preferably on the cell surface of the $CD8^+$ T cells.

23. The method according to claim 22, wherein the one or more agents are one or more antibodies.

24. The method according to any of claims 19 to 23, wherein the biological sample is a tumor sample obtained from a subject in need thereof and the $CD8^+$ T cells are $CD8^+$ tumor infiltrating lymphocytes (TIL).

25. The method according to any of claims 19 to 24, wherein the biological sample comprises *ex vivo* or *in vitro* $CD8^+$ T cells.

26. A population of $CD8^+$ T cells comprising $CD8^+$ T cells as defined in any one of claims 1 to 12 or isolated according to any one of claims 19 to 25.

27. A pharmaceutical composition comprising the $CD8^+$ T cell population as defined in claim 26.

28. A method for treating or preventing cancer comprising administering to a subject in need thereof the pharmaceutical composition according to claim 27.

29. A kit comprising reagents to detect at least one gene or polypeptide as defined in any of claims 1 to 12.

30. An isolated $CD8^+$ T cell characterized in that the $CD8^+$ T cell comprises expression of a gene signature comprising one or more genes selected from the group consisting

of:

a. GPR56, PDCD1, LAG3, HAVCR2, ENTPD1, 1700017B05RIK, CHN2, 2900026A02RIK, FGL2, SERPINA3H, OSBPL3, S100A4, CCL3, TNFRSF9, UBASH3B, CD244, RGS8, BCL2A1D, CCL4, CIAPIN1, GP49A, CCRL2, IRF8, GRINA, C1QTNF6, CD200R4, FILIP1, THEMIS2, SERPINA3F, LRRK1, ARNT2, MXI1, DAPK2, TWSG1, ADAM8, TRPS1, LAT2, SDCBP2, SLC37A2, MT2, ADAMTS14, GBP10, EPDR1 and DUT; or

b. RGS16, GZMB, SERPINA3G, CXCR6, LITAF, SERPINA3I, TOX, PRF1, EHD1, LILRB4, PLEK, ITGAV, CREM, CDK6, NR4A2, UHRF2, GBP6, IRAK2, PTK2B, OXSR1 and ITGB1BP1; or

c. TIGIT, DGAT1, PLAC8, BHLHE40, GM5069, SAMSN1, RGS1, DENND4A and SIK1.

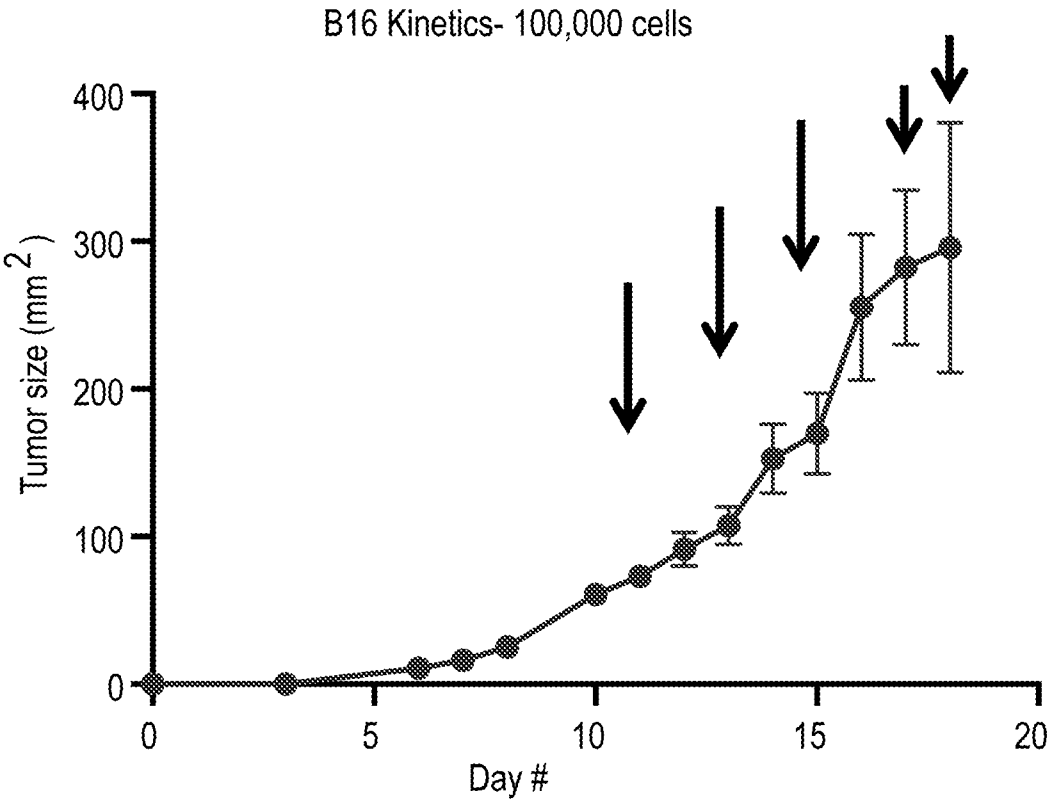

B16 Kinetics- 100,000 cells
Tumor size (mm 2)
400
300
200
100
0
0
5
10
15
20
Day #

FIG. 1

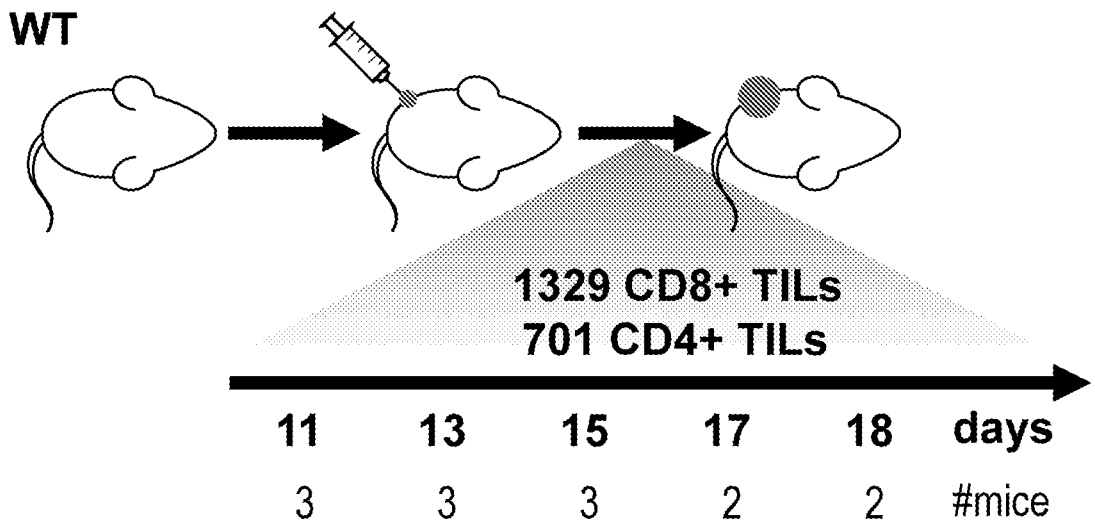
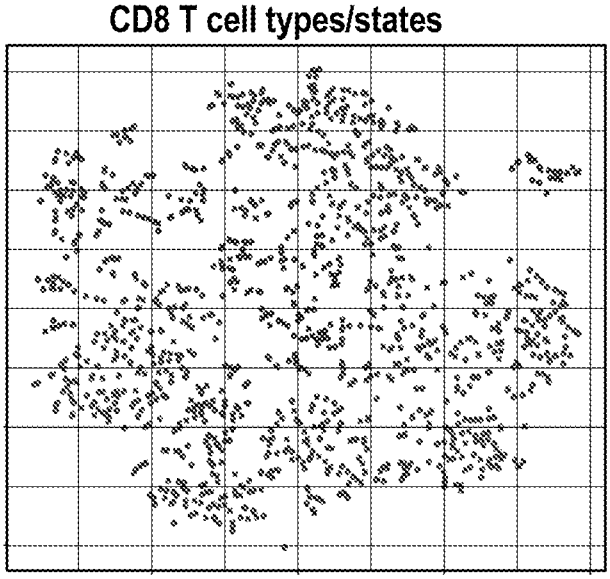


FIG. 2



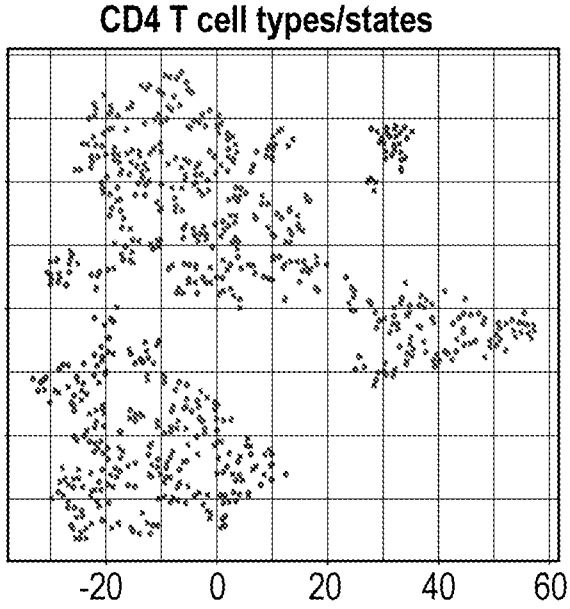
DP, DYS/ACT (Ki67, GZMB ,low IL2, low IFNg)

DP, DYS

DP, "arrested"?

SP, expanded, Tcf7-Bcl6-Cxcr5 (R Ahmed)

DN, Naive (Tcf7), no expansion, d18 cells



Tregs(proliferating. FoxP3, Helios, Tim3)

iTreg? (Helios^{lo})

Memory like? (Ccr7+)

Th1, effector-like?

FIG. 3

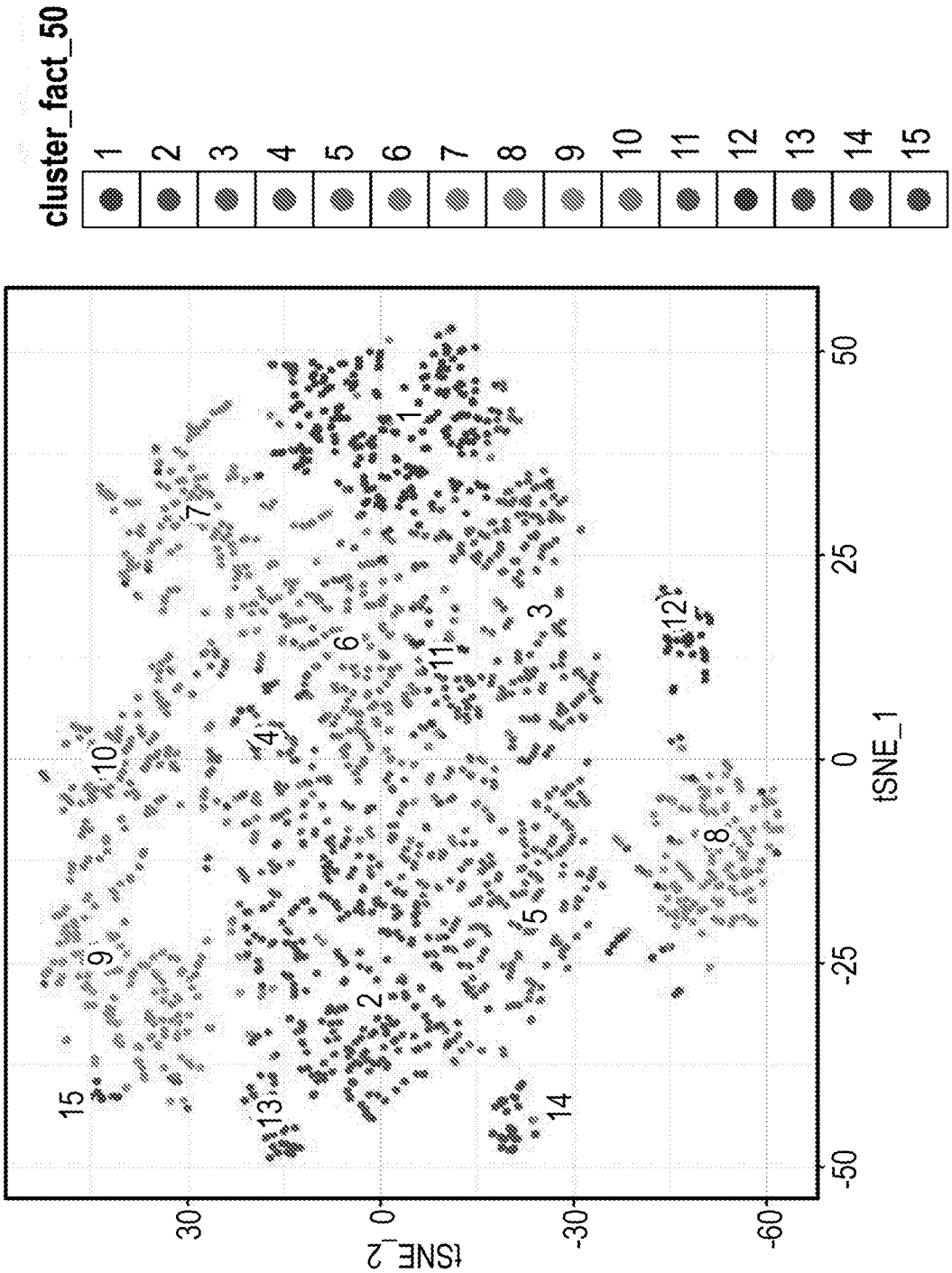

cluster_fact_50
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
tSNE_2
30
0
-30
-60
tSNE_1
-50
-25
0
25
50

FIG. 4

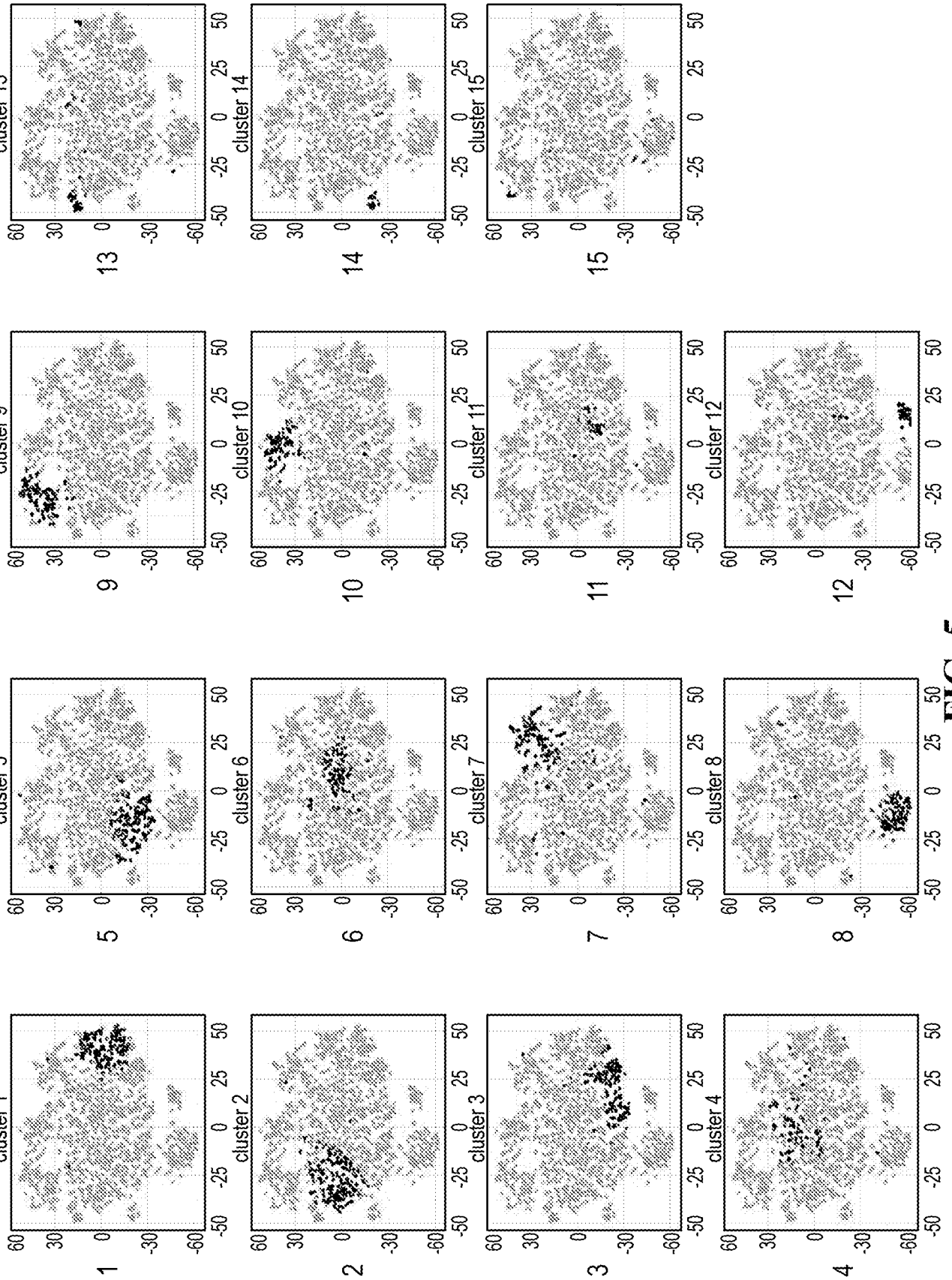

cluster 1
cluster 2
cluster 3
cluster 4
cluster 5
cluster 6
cluster 7
cluster 8
cluster 9
cluster 10
cluster 11
cluster 12
cluster 13
cluster 14
cluster 15

FIG. 5

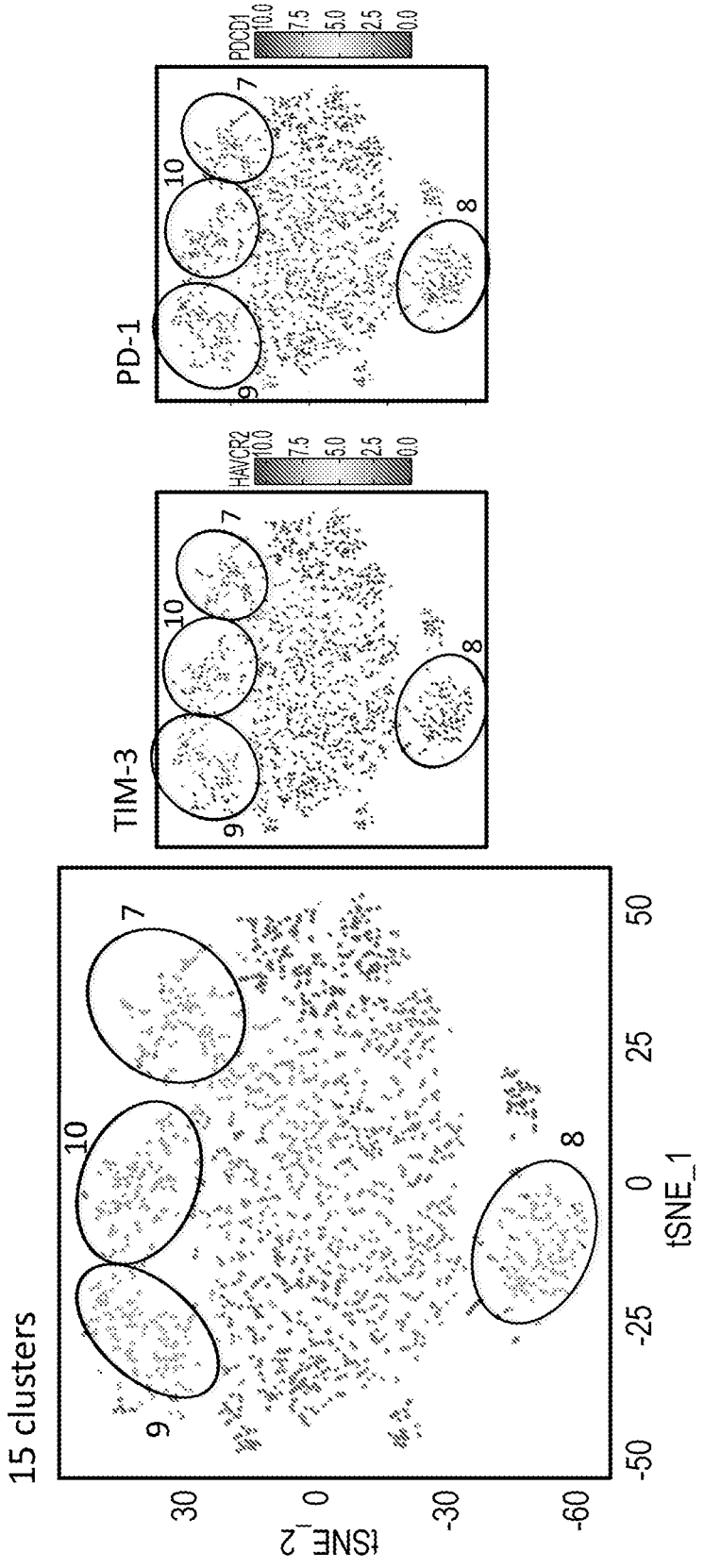

15 clusters
TIM-3
HAVCR2
10.0
7.5
5.0
2.5
0.0
PD-1
PDCD1
10.0
7.5
5.0
2.5
0.0
tSNE_2
tSNE_1
30
0
-30
-60
-50
-25
0
25
50
7
10
9
8

FIG. 6

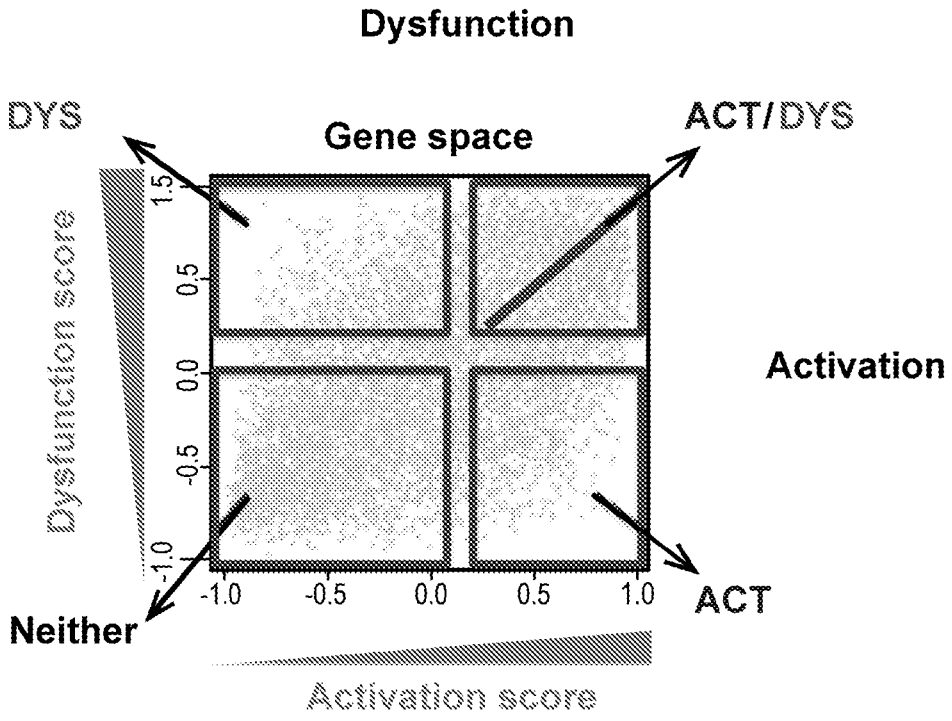

Dysfunction
DYS
Gene space
ACT/DYS
Activation
ACT
Neither
Dysfunction score
Activation score
1.5
0.5
0.0
-0.5
-1.0
-1.0
-0.5
0.0
0.5
1.0

FIG. 7

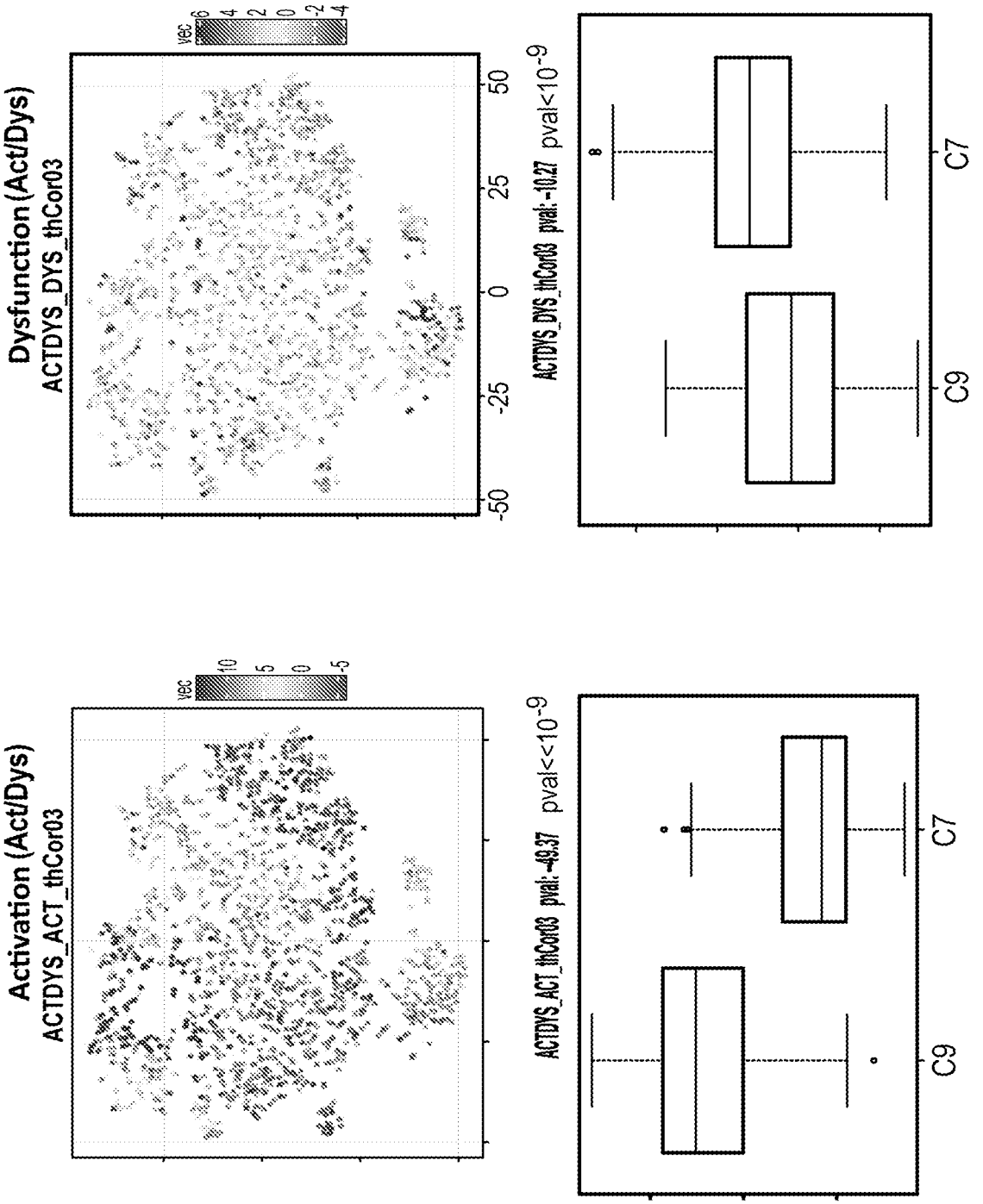

Activation (Act/Dys)
ACTDYS_ACT_thCor03
vec
10
5
0
-5
ACTDYS_ACT_thCor03 pval: -49.37 pval<<10-9
C9
C7
Dysfunction (Act/Dys)
ACTDYS_DYS_thCor03
vec
6
4
2
0
-2
-4
50
25
0
-25
-50
ACTDYS_DYS_thCor03 pval: -10.27 pval<10-9
C9
C7

FIG. 8

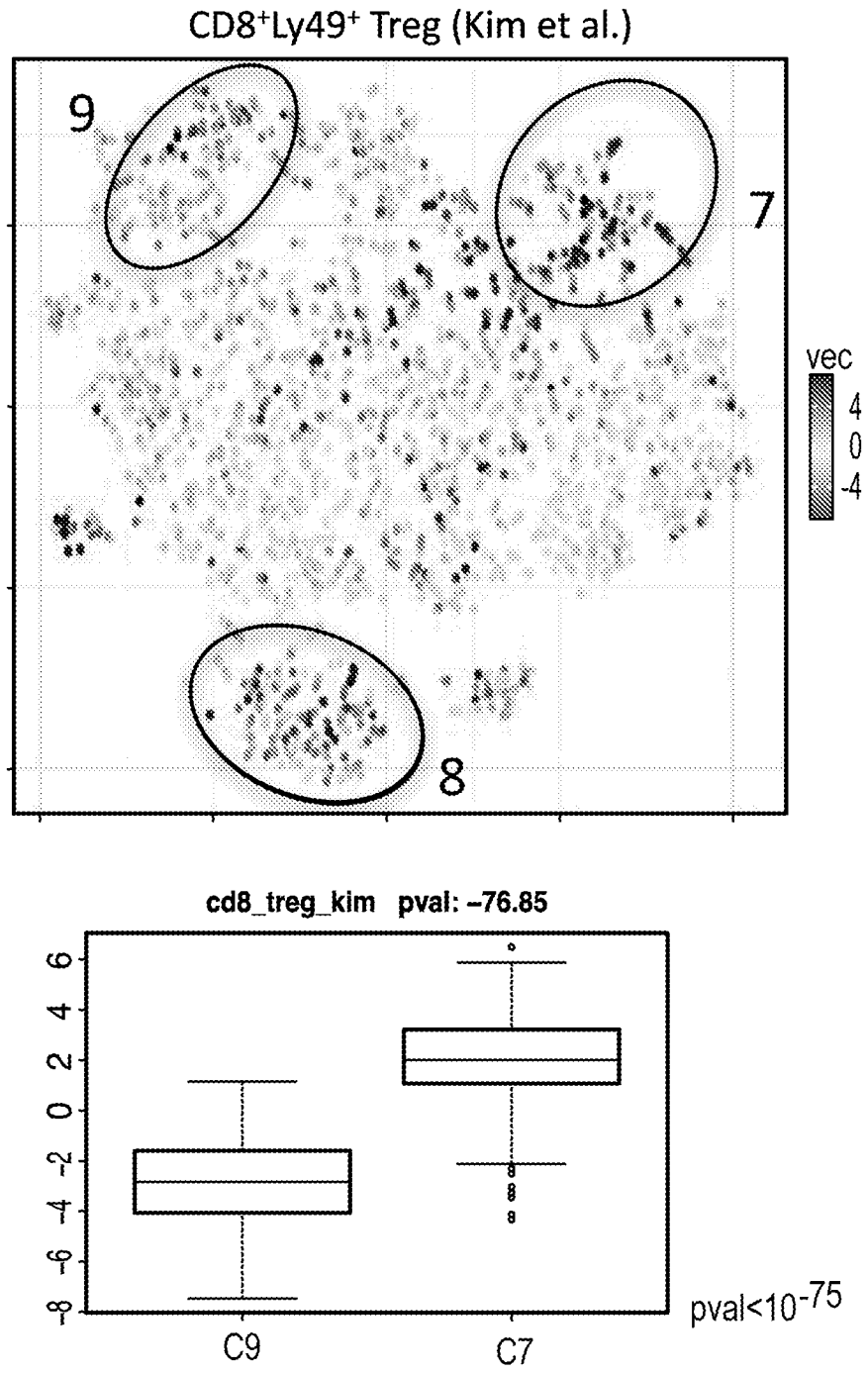

CD8+Ly49+ Treg (Kim et al.)
9
7
8
vec
4
0
-4
cd8_treg_kim pval: −76.85
6
4
2
0
-2
-4
-6
-8
C9
C7
pval<10-75

FIG. 9

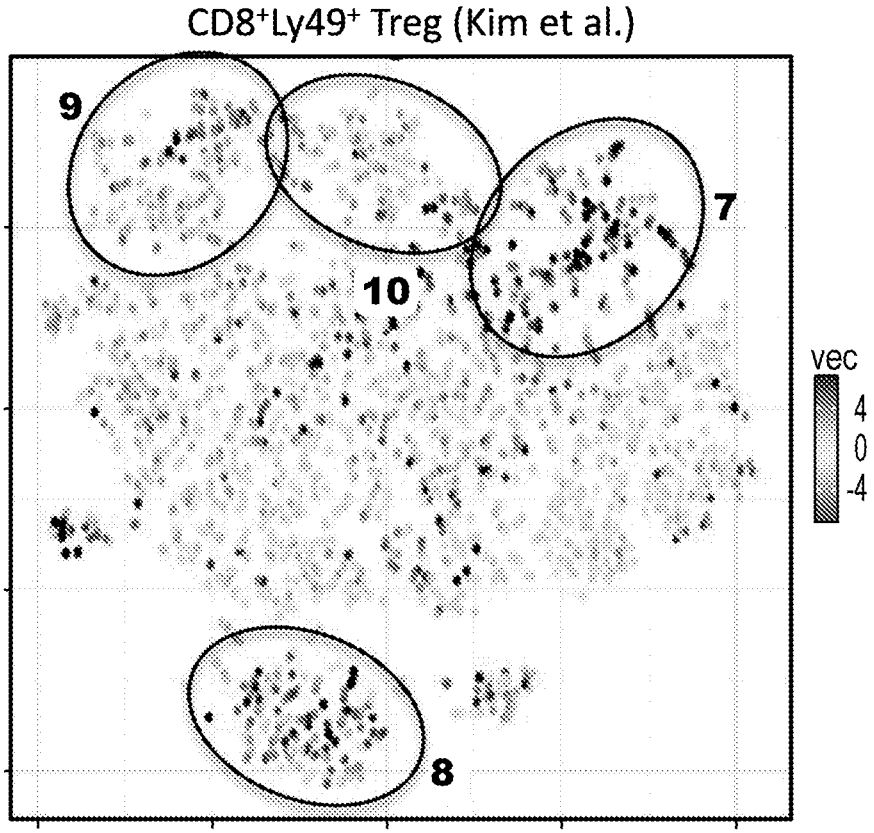


FIG. 10

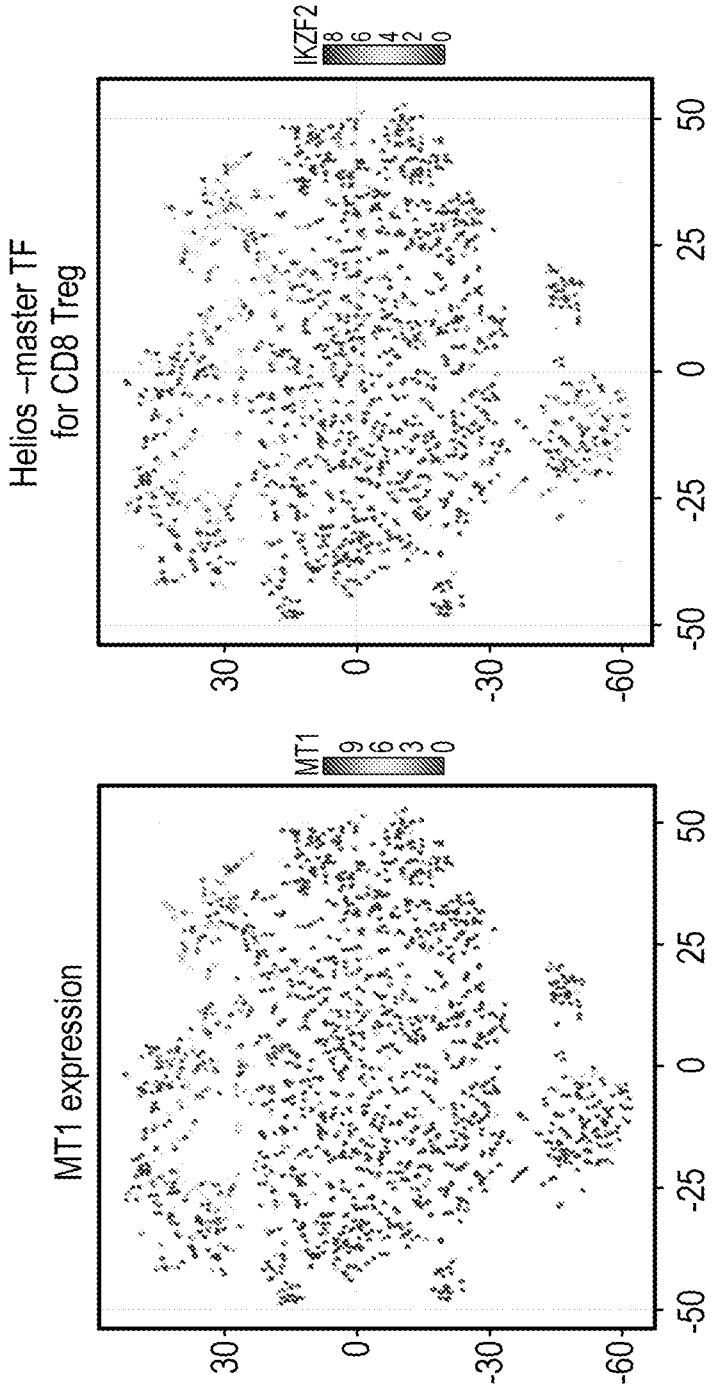

Helios –master TF
for CD8 Treg
IKZF2
8
6
4
2
0
30
0
-30
-60
-50
-25
0
25
50
MT1 expression
MT1
9
6
3
0

FIG. 11

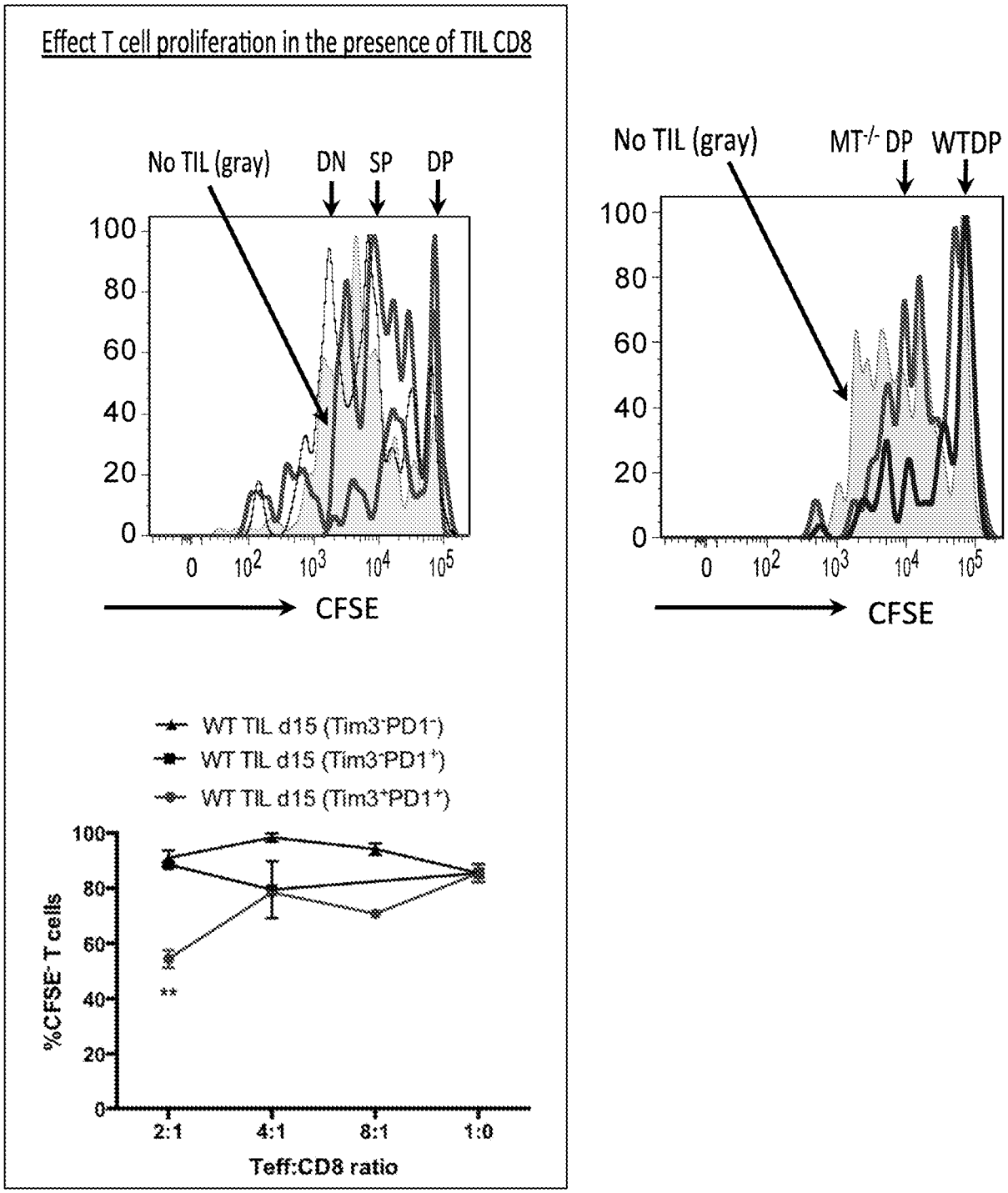

Effect T cell proliferation in the presence of TIL CD8
No TIL (gray)
DN
SP
DP
100
80
60
40
20
0
0
10^2
10^3
10^4
10^5
CFSE
No TIL (gray)
MT-/- DP
WTDP
100
80
60
40
20
0
0
10^2
10^3
10^4
10^5
CFSE
WT TIL d15 (Tim3-PD1-)
WT TIL d15 (Tim3-PD1+)
WT TIL d15 (Tim3+PD1+)
%CFSE- T cells
100
80
60
40
20
0
**
2:1
4:1
8:1
1:0
Teff:CD8 ratio

FIG. 12

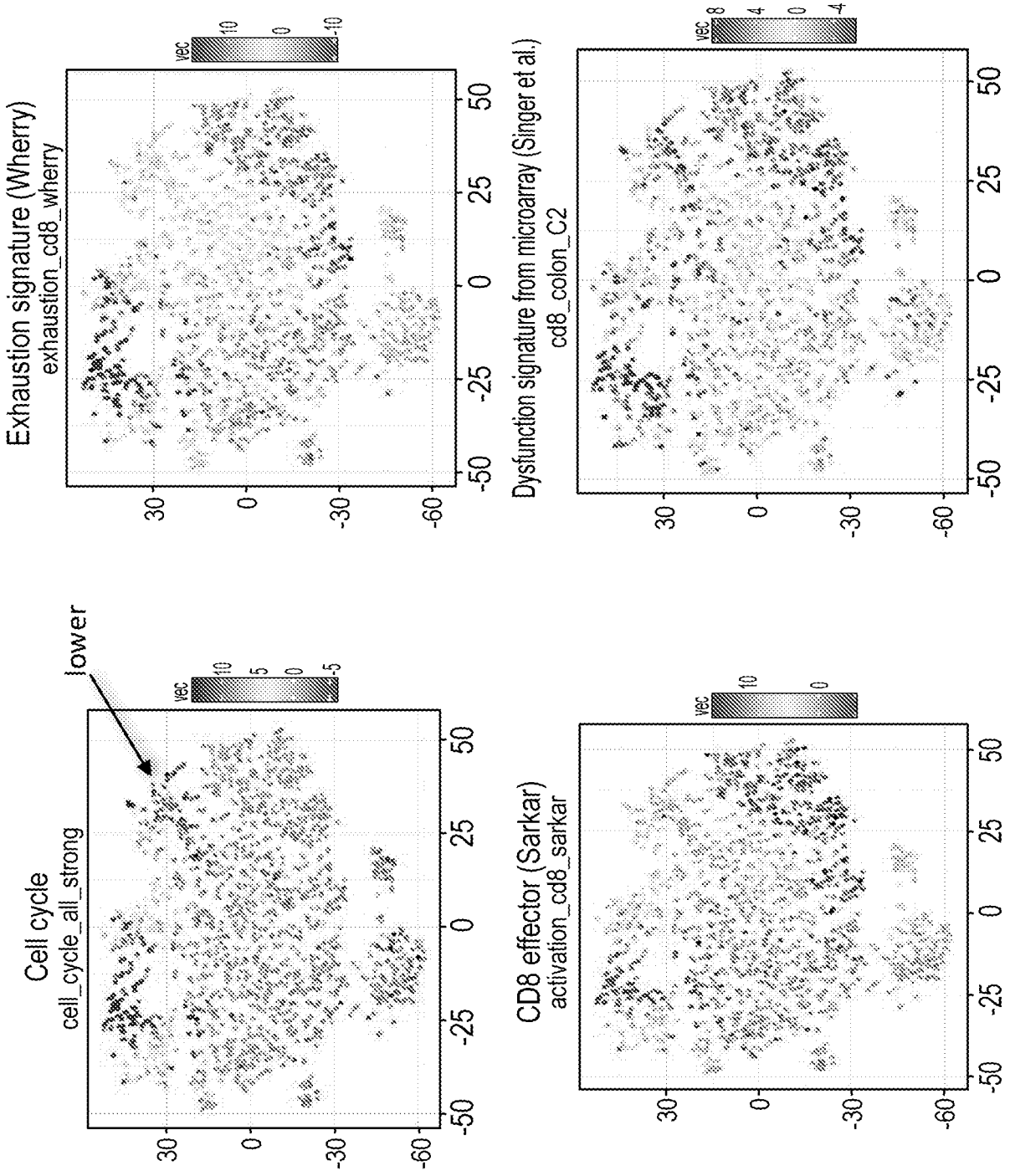

Exhaustion signature (Wherry)
exhaustion_cd8_wherry
Dysfunction signature from microarray (Singer et al.)
cd8_colon_C2
Cell cycle
cell_cycle_all_strong
lower
CD8 effector (Sarkar)
activation_cd8_sarkar
vec

FIG. 13

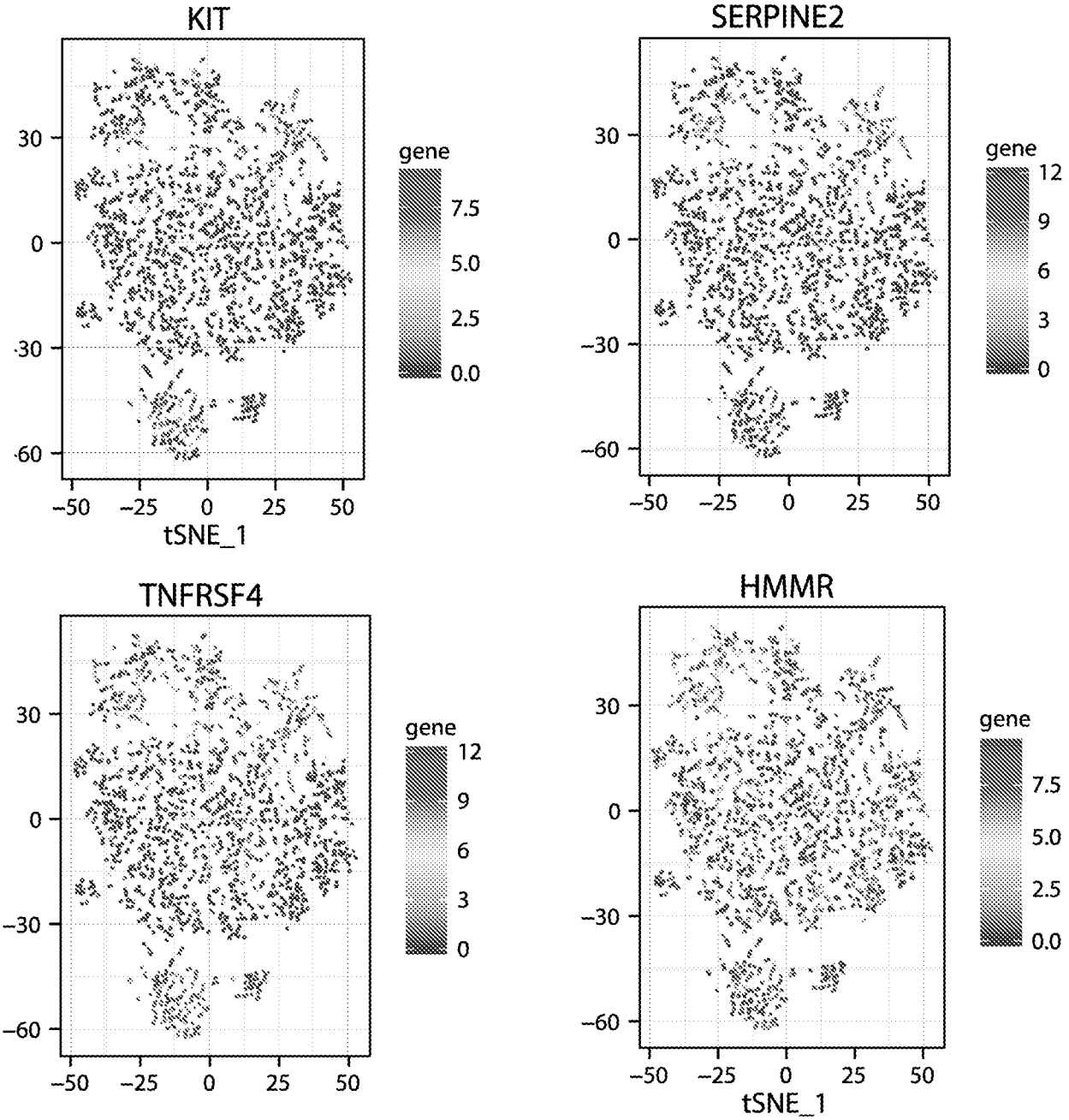

KIT
SERPINE2
TNFRSF4
HMMR
gene
7.5
5.0
2.5
0.0
12
9
6
3
0
30
0
-30
-60
-50
-25
0
25
50
tSNE_1

FIG. 14

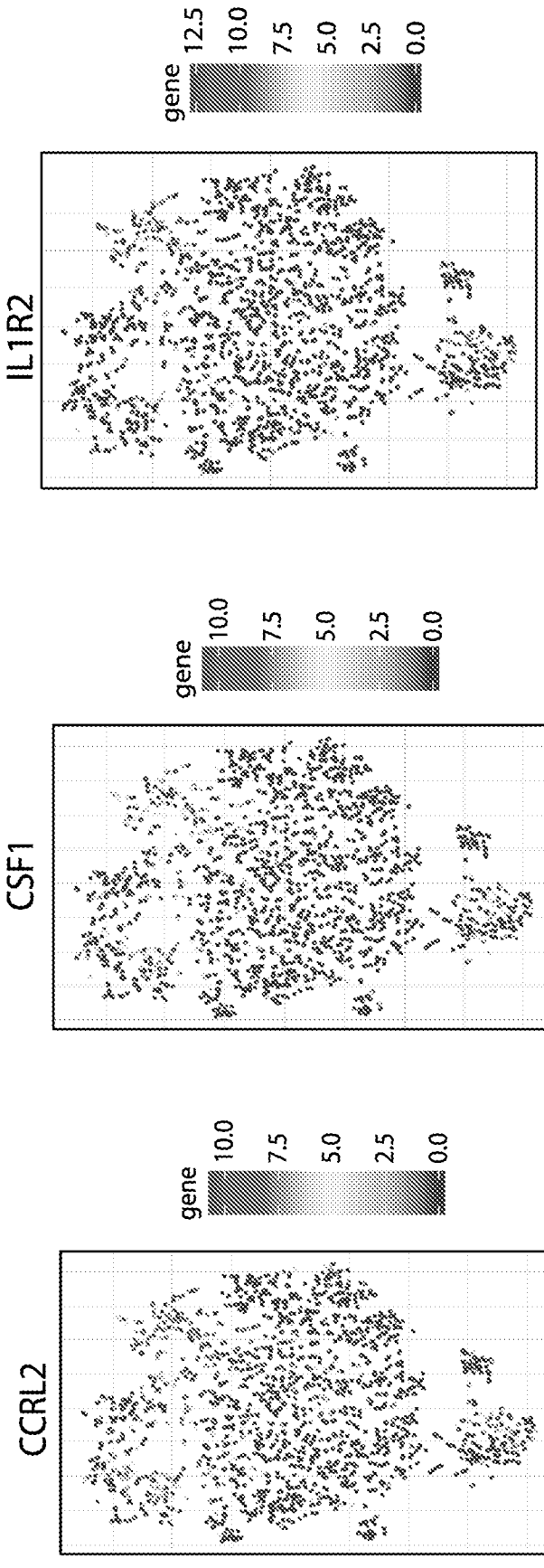

IL1R2
gene
12.5
10.0
7.5
5.0
2.5
0.0
CSF1
gene
10.0
7.5
5.0
2.5
0.0
CCRL2
gene
10.0
7.5
5.0
2.5
0.0

FIG. 15

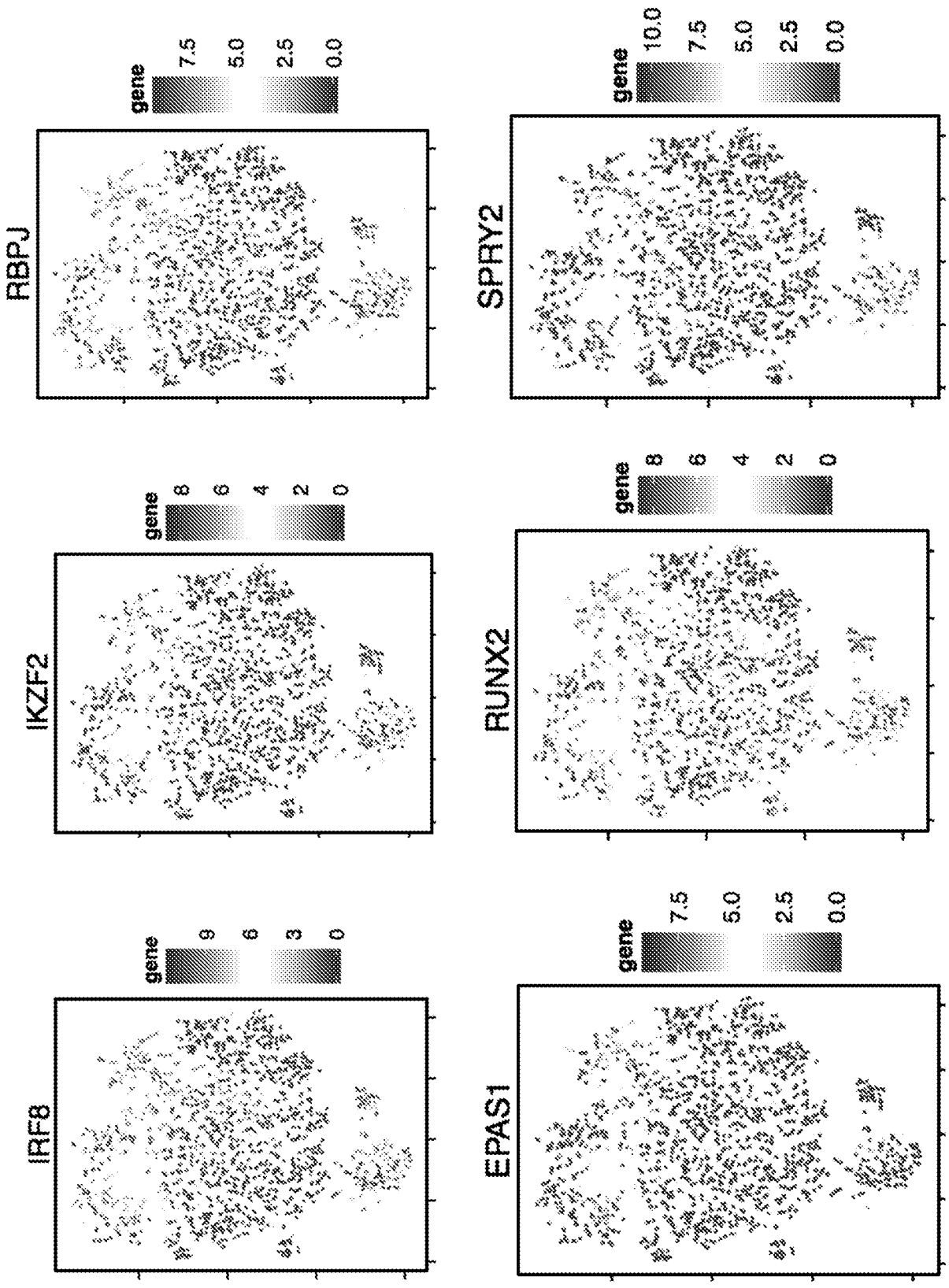

RBPJ
gene
7.5
5.0
2.5
0.0
SPRY2
gene
10.0
7.5
5.0
2.5
0.0
IKZF2
gene
8
6
4
2
0
RUNX2
gene
8
6
4
2
0
IRF8
gene
9
6
3
0
EPAS1
gene
7.5
5.0
2.5
0.0

FIG. 16

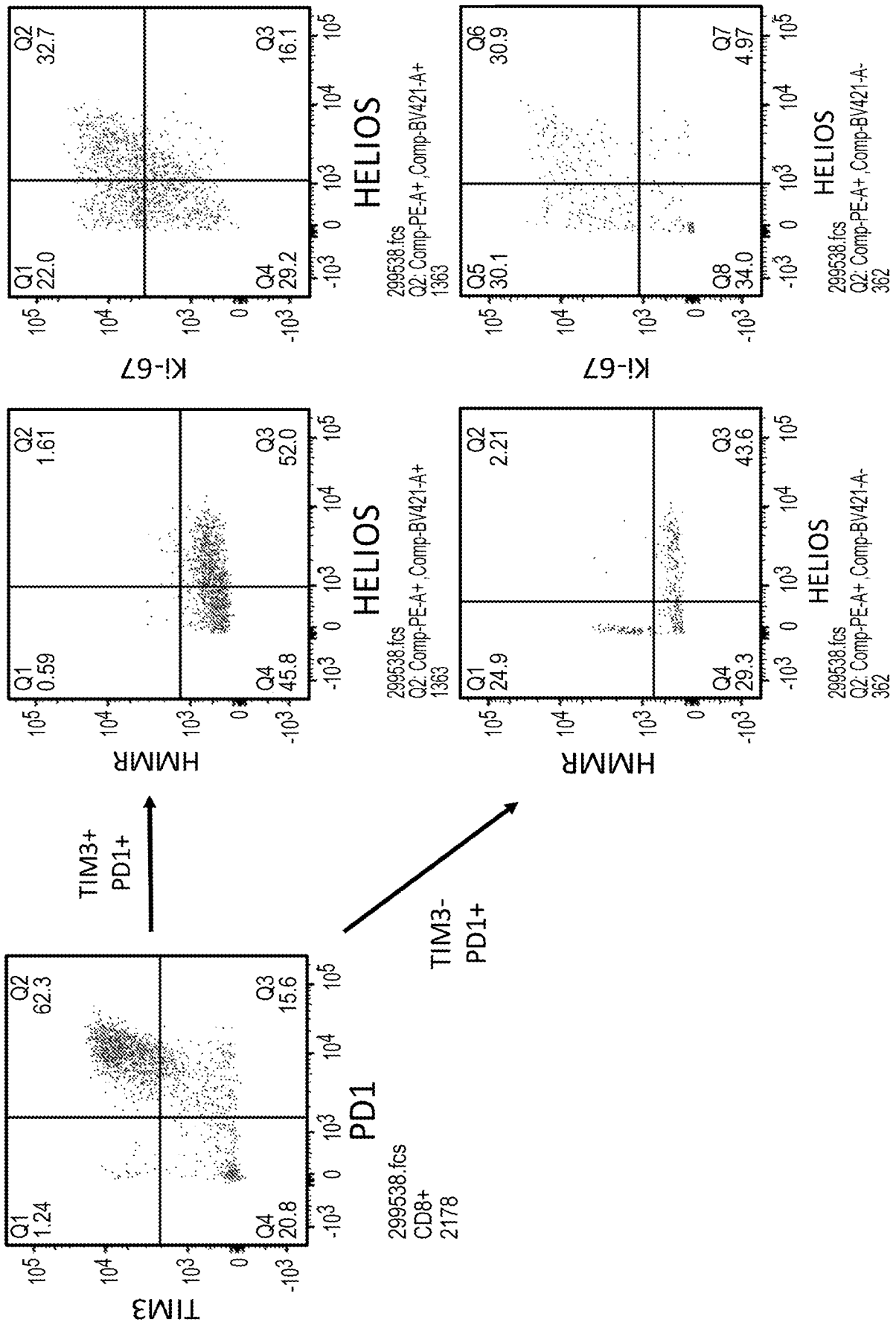

TIM3
PD1
299538.fcs
CD8+
2178
Q1 1.24
Q2 62.3
Q3 15.6
Q4 20.8
TIM3+ PD1+
TIM3- PD1+
HMMR
HELIOS
299538.fcs
Q2: Comp-PE-A+ ,Comp-BV421-A+
1363
Q1 0.59
Q2 1.61
Q3 52.0
Q4 45.8
Q1 24.9
Q2 2.21
Q3 43.6
Q4 29.3
Ki-67
HELIOS
299538.fcs
Q2: Comp-PE-A+ ,Comp-BV421-A+
1363
Q1 22.0
Q2 32.7
Q3 16.1
Q4 29.2
Q5 30.1
Q6 30.9
Q7 4.97
Q8 34.0
299538.fcs
Q2: Comp-PE-A+ ,Comp-BV421-A-
362

FIG. 17

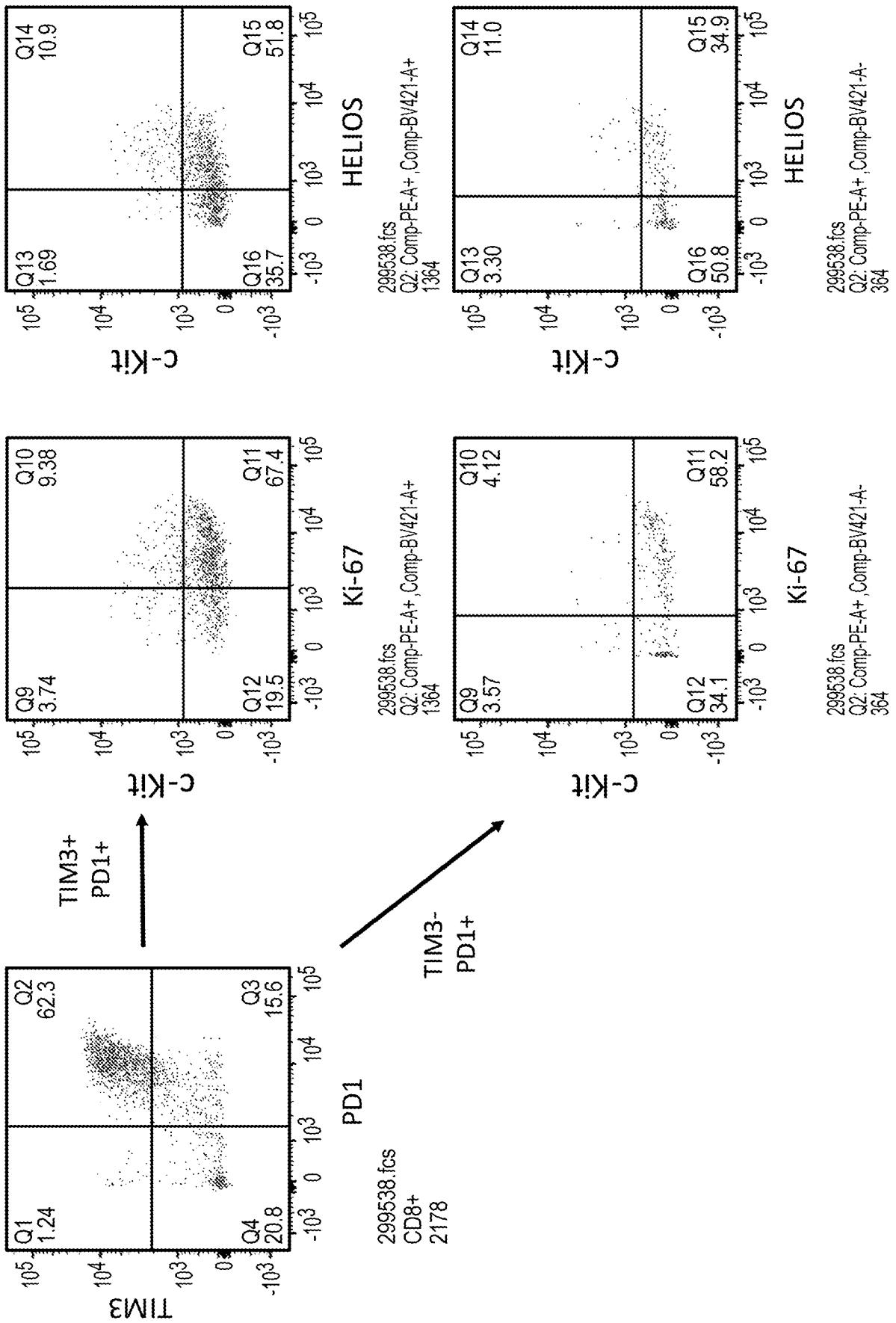

TIM3
PD1
299538.fcs
CD8+
2178
Q1
1.24
Q2
62.3
Q3
15.6
Q4
20.8
TIM3+
PD1+
TIM3-
PD1+
c-Kit
Ki-67
Q9
3.74
Q10
9.38
Q11
67.4
Q12
19.5
299538.fcs
Q2: Comp-PE-A+, Comp-BV421-A+
1364
Q9
3.57
Q10
4.12
Q11
58.2
Q12
34.1
299538.fcs
Q2: Comp-PE-A+, Comp-BV421-A-
364
HELIOS
Q13
1.69
Q14
10.9
Q15
51.8
Q16
35.7
Q13
3.30
Q14
11.0
Q15
34.9
Q16
50.8

FIG. 18

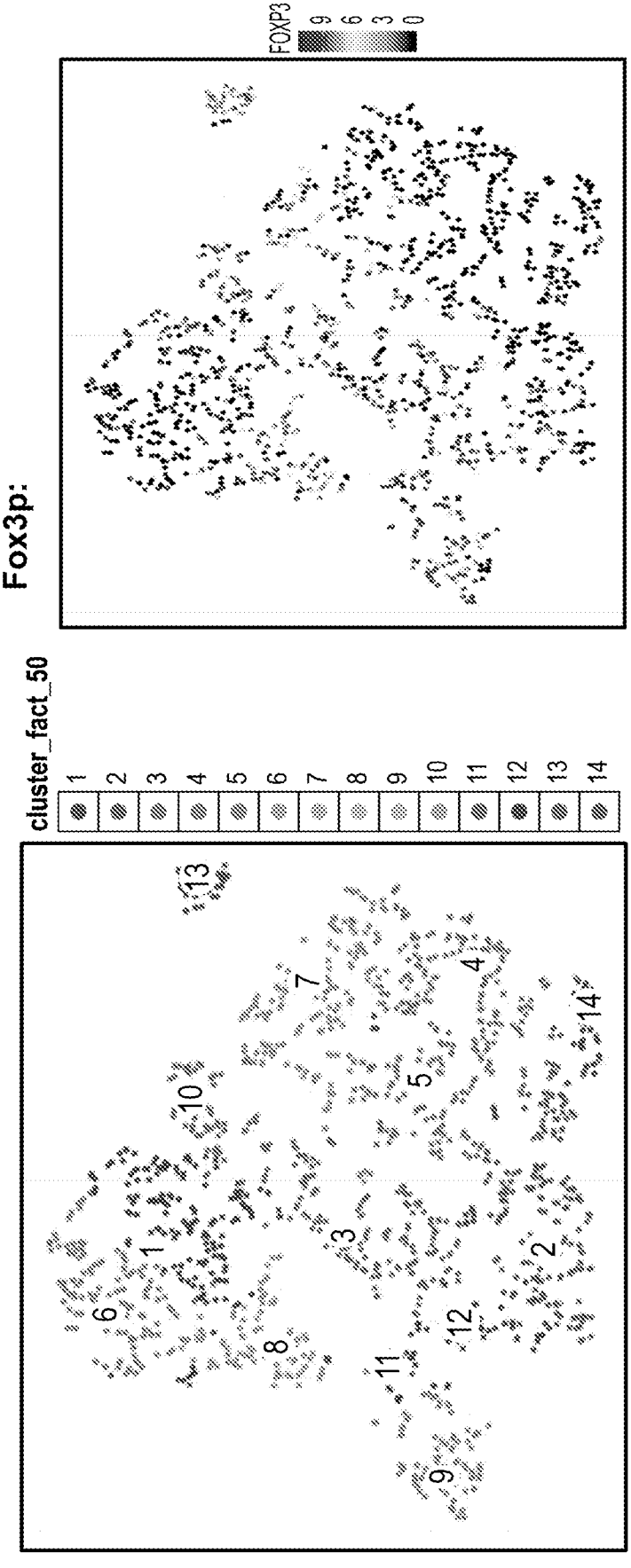


FIG. 19

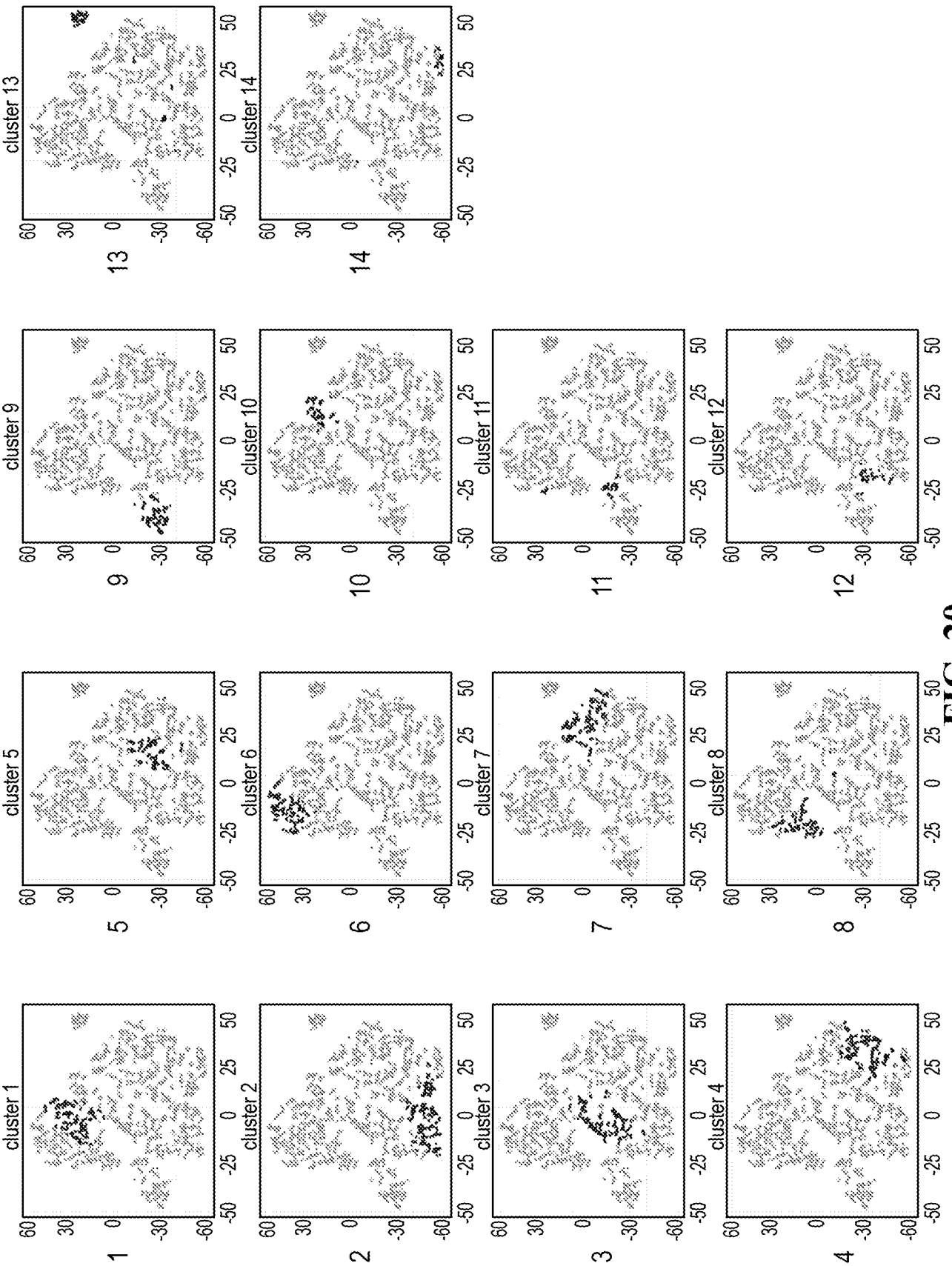

cluster 1
cluster 2
cluster 3
cluster 4
cluster 5
cluster 6
cluster 7
cluster 8
cluster 9
cluster 10
cluster 11
cluster 12
cluster 13
cluster 14

FIG. 20

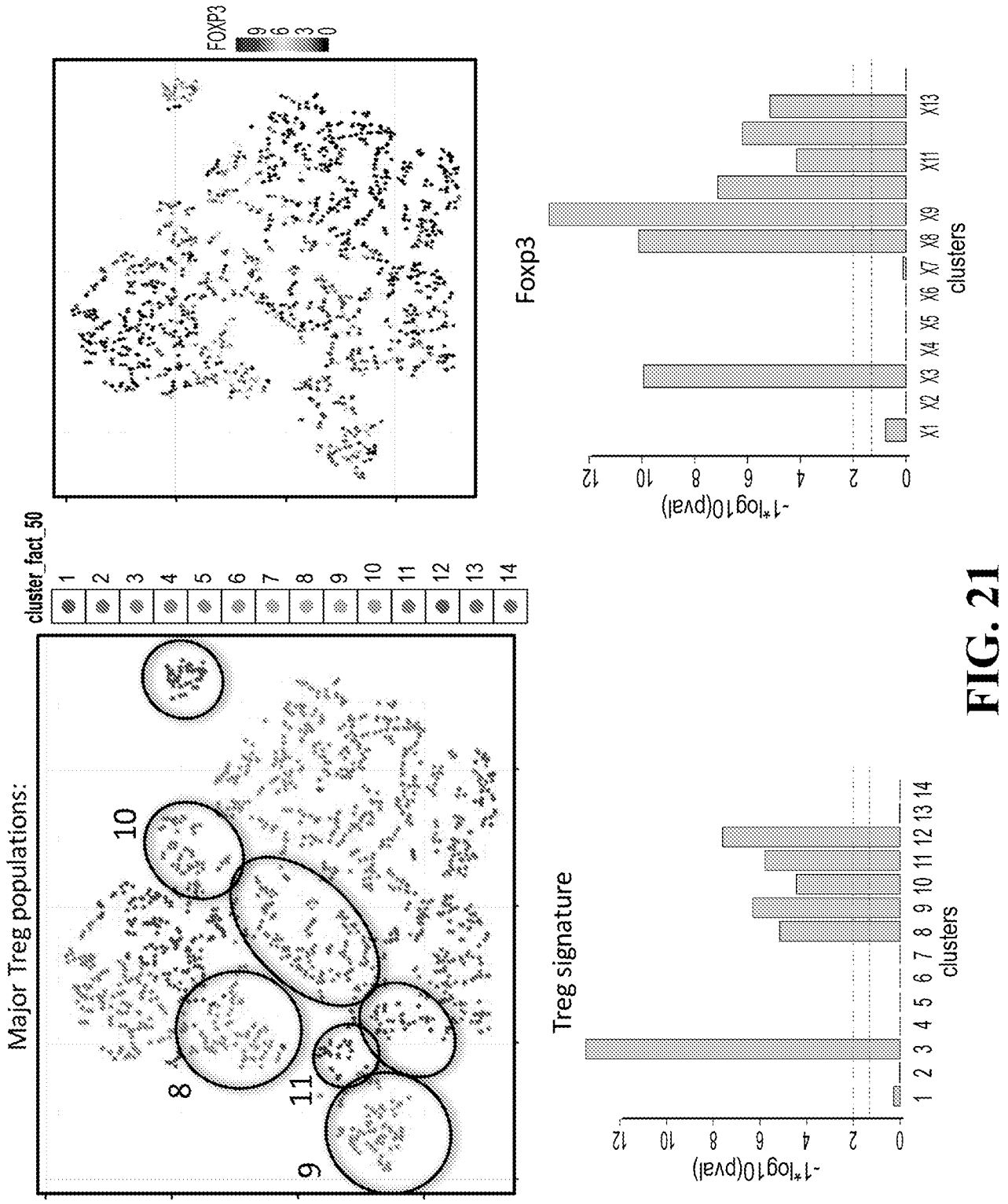

Major Treg populations:
cluster_fact_50
1
2
3
4
5
6
7
8
9
10
11
12
13
14
FOXP3
9
6
3
0
Foxp3
-1*log10(pval)
0
2
4
6
8
10
12
X1 X2 X3 X4 X5 X6 X7 X8 X9 X11 X13
clusters
Treg signature
-1*log10(pval)
0
2
4
6
8
10
12
1 2 3 4 5 6 7 8 9 10 11 12 13 14
clusters

FIG. 21

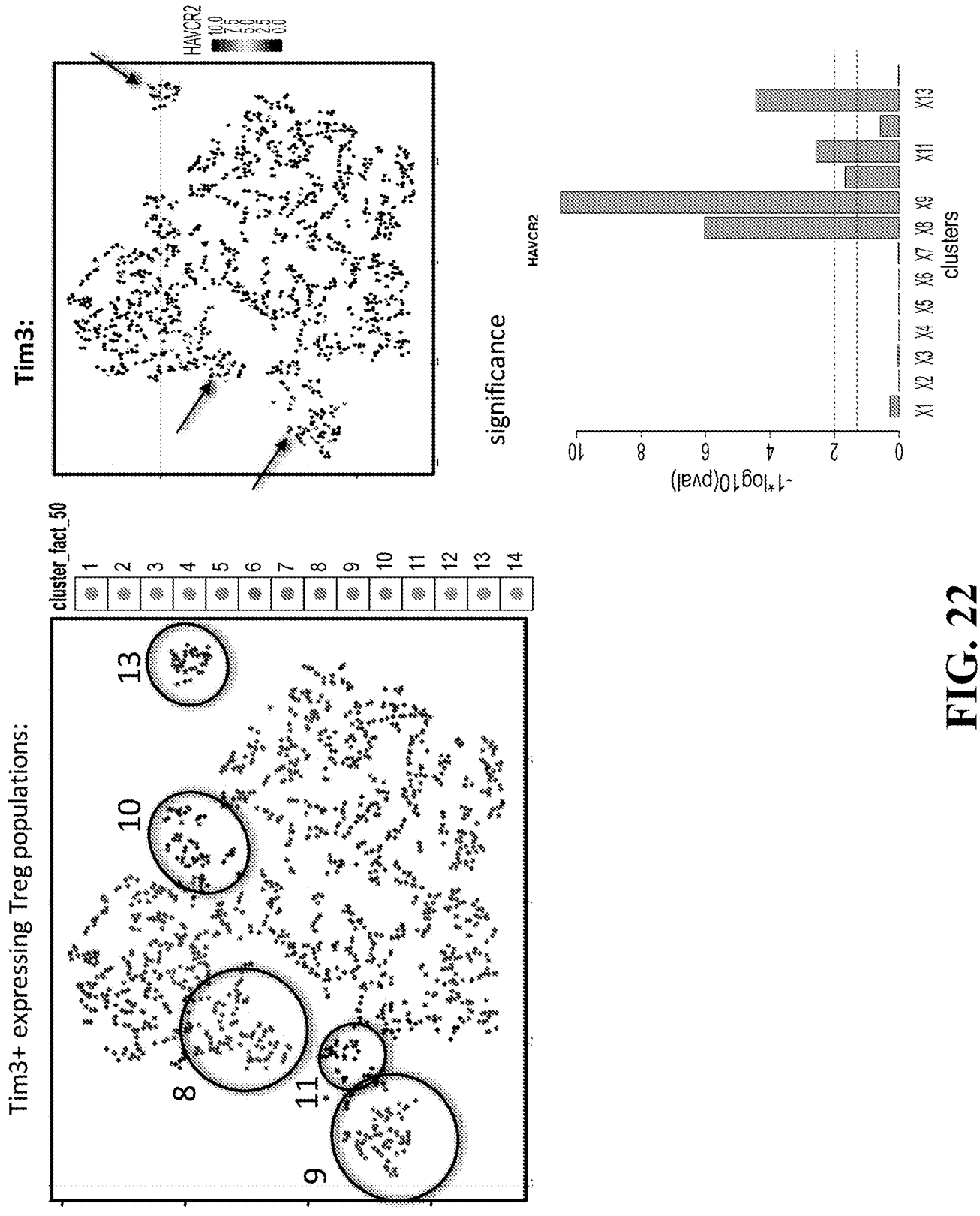

Tim3+ expressing Treg populations:
cluster_fact_50
1
2
3
4
5
6
7
8
9
10
11
12
13
14
13
10
8
11
9
Tim3:
HAVCR2
10.0
7.5
5.0
2.5
0.0
significance
HAVCR2
-1*log10(pval)
0
2
4
6
8
10
X1
X2
X3
X4
X5
X6
X7
X8
X9
X11
X13
clusters

FIG. 22

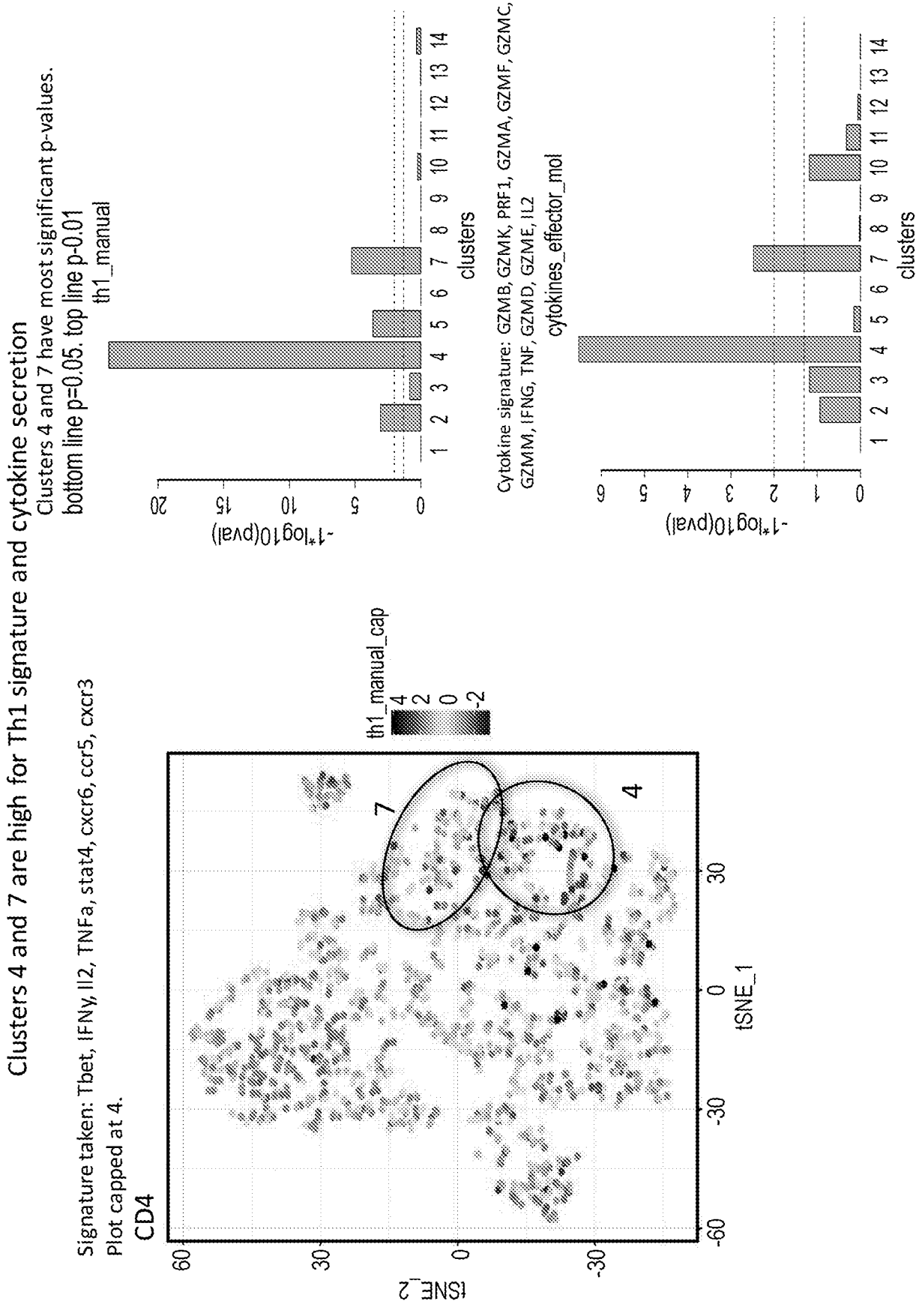

Clusters 4 and 7 are high for Th1 signature and cytokine secretion
Signature taken: Tbet, IFNγ, Il2, TNFa, stat4, cxcr6, ccr5, cxcr3
Plot capped at 4.
CD4
th1_manual_cap
4
2
0
-2
tSNE_2
tSNE_1
7
4
Clusters 4 and 7 have most significant p-values.
bottom line p=0.05. top line p-0.01
th1_manual
-1*log10(pval)
clusters
Cytokine signature: GZMB, GZMK, PRF1, GZMA, GZMF, GZMC, GZMM, IFNG, TNF, GZMD, GZME, IL2
cytokines_effector_mol
-1*log10(pval)
clusters

FIG. 23

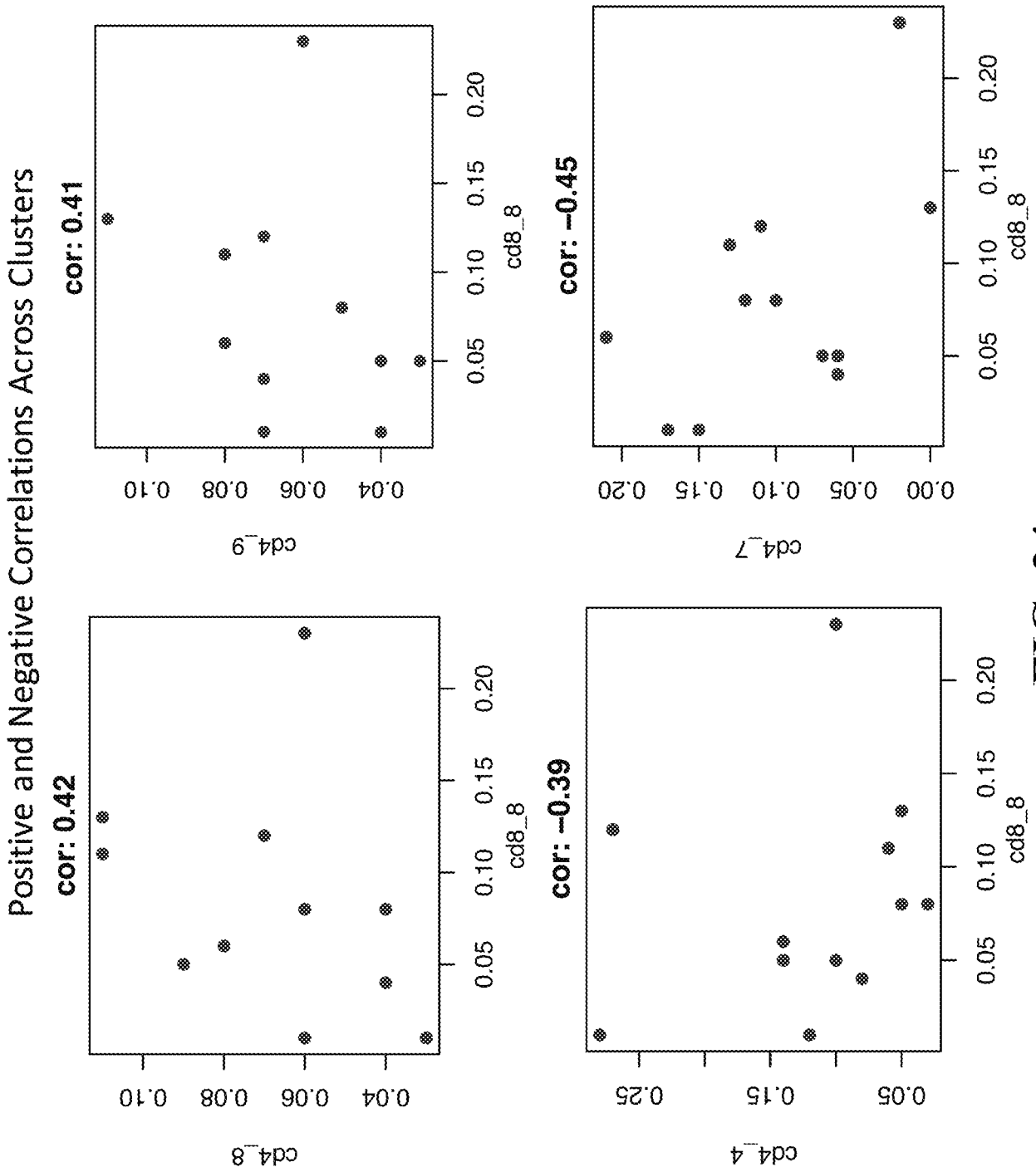

Positive and Negative Correlations Across Clusters
cor: 0.41
cor: 0.42
cor: −0.45
cor: −0.39
cd4_9
cd4_8
cd4_7
cd4_4
cd8_8
0.04
0.06
0.08
0.10
0.05
0.15
0.20
0.00
0.25

FIG. 24

Significant correlations of the CD8 population, a "complementary" Structure is revealed between the "Treg-CD8" and "activated-tim3-CD8" with TregCD4 and Th1-like CD4.

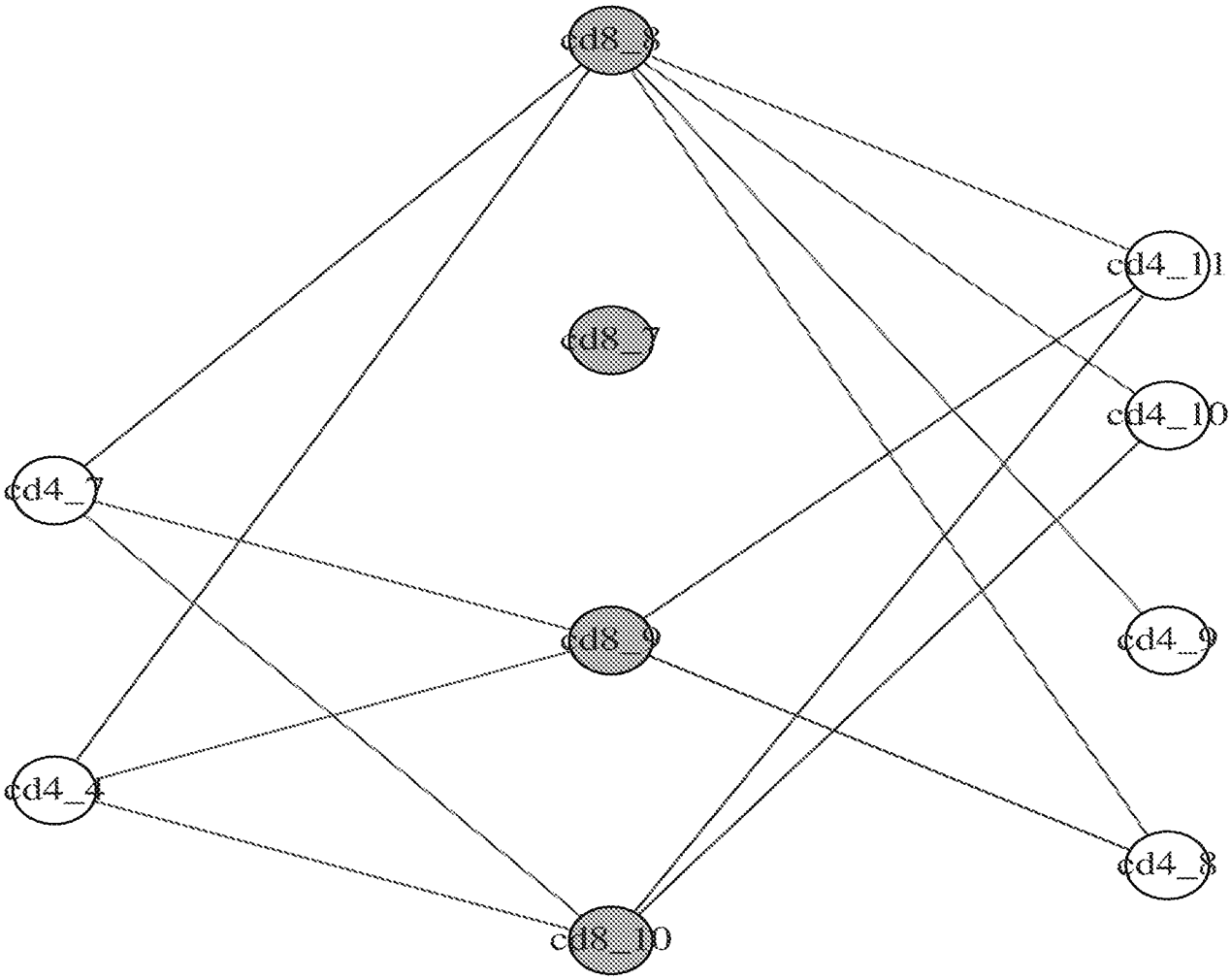


FIG. 25

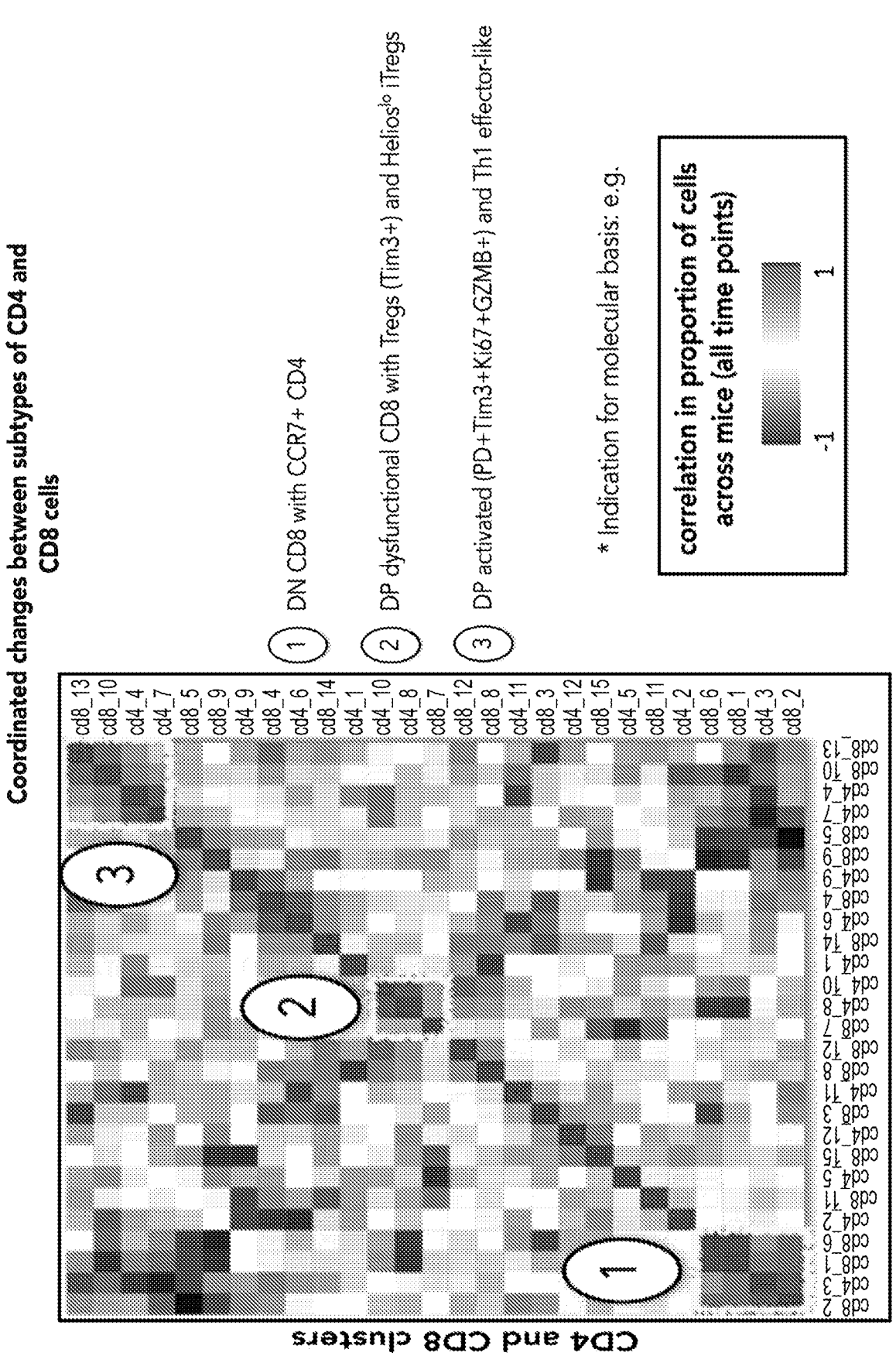

Coordinated changes between subtypes of CD4 and CD8 cells
1 DN CD8 with CCR7+ CD4
2 DP dysfunctional CD8 with Tregs (Tim3+) and Helios^lo iTregs
3 DP activated (PD+Tim3+Ki67+GZMB+) and Th1 effector-like
* Indication for molecular basis: e.g.
correlation in proportion of cells across mice (all time points)
-1
1
CD4 and CD8 clusters
CD4 and CD8 clusters
cd8_13
cd8_10
cd4_4
cd4_7
cd8_5
cd8_9
cd4_9
cd8_4
cd4_6
cd8_14
cd4_1
cd4_10
cd4_8
cd8_7
cd8_12
cd8_8
cd4_11
cd8_3
cd4_12
cd8_15
cd4_5
cd8_11
cd4_2
cd8_6
cd8_1
cd4_3
cd8_2

FIG. 26

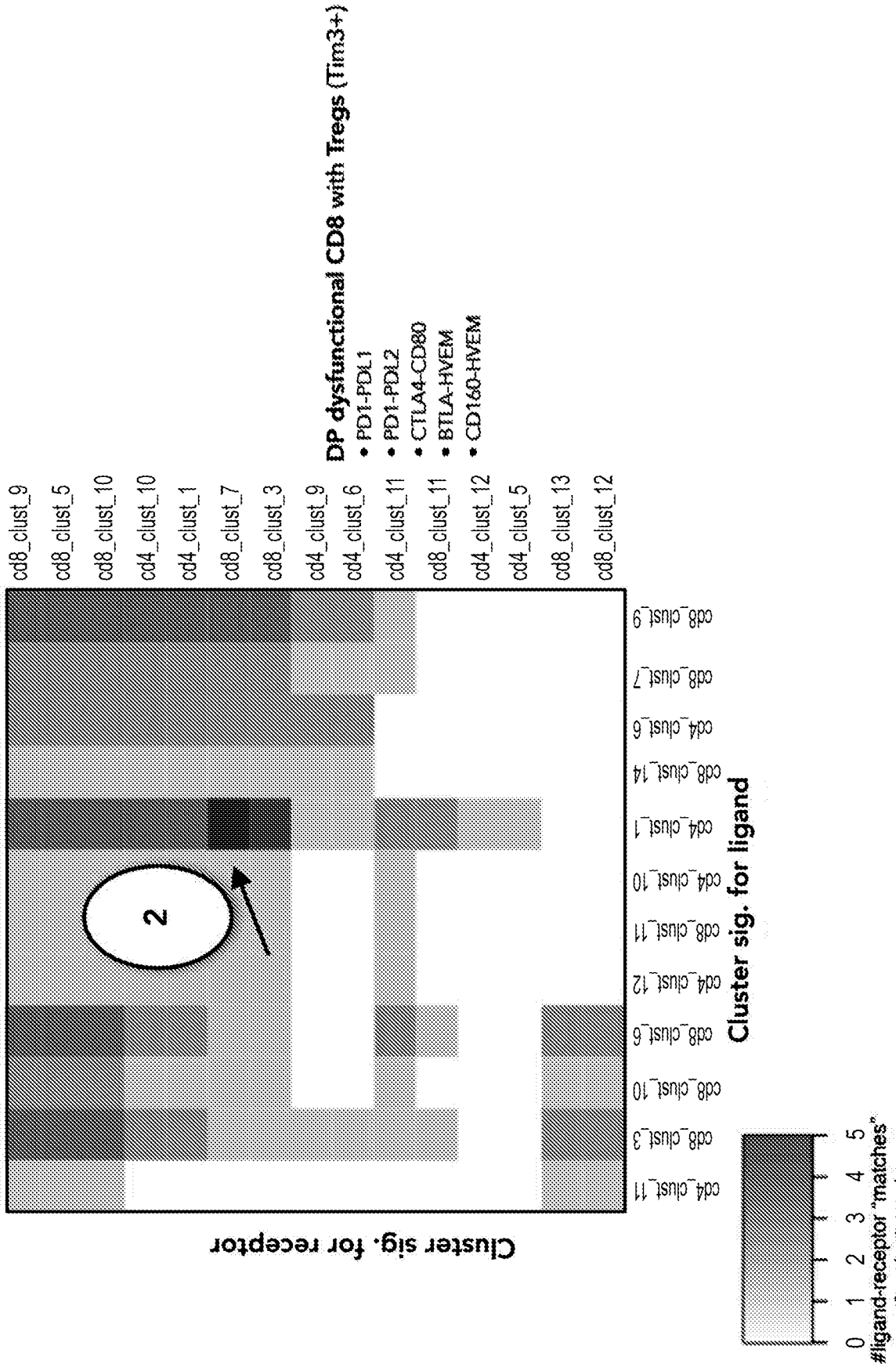

DP dysfunctional CD8 with Tregs (Tim3+)
• PD1-PDL1
• PD1-PDL2
• CTLA4-CD80
• BTLA-HVEM
• CD160-HVEM
cd8_clust_9
cd8_clust_5
cd8_clust_10
cd4_clust_10
cd4_clust_1
cd8_clust_7
cd8_clust_3
cd4_clust_9
cd4_clust_6
cd4_clust_11
cd8_clust_11
cd4_clust_12
cd4_clust_5
cd8_clust_13
cd8_clust_12
2
Cluster sig. for receptor
cd4_clust_11
cd8_clust_3
cd8_clust_10
cd8_clust_6
cd4_clust_12
cd8_clust_11
cd4_clust_10
cd4_clust_1
cd8_clust_14
cd4_clust_6
cd8_clust_7
cd8_clust_9
Cluster sig. for ligand
0 1 2 3 4 5
#ligand-receptor "matches" for cluster pair

FIG. 27

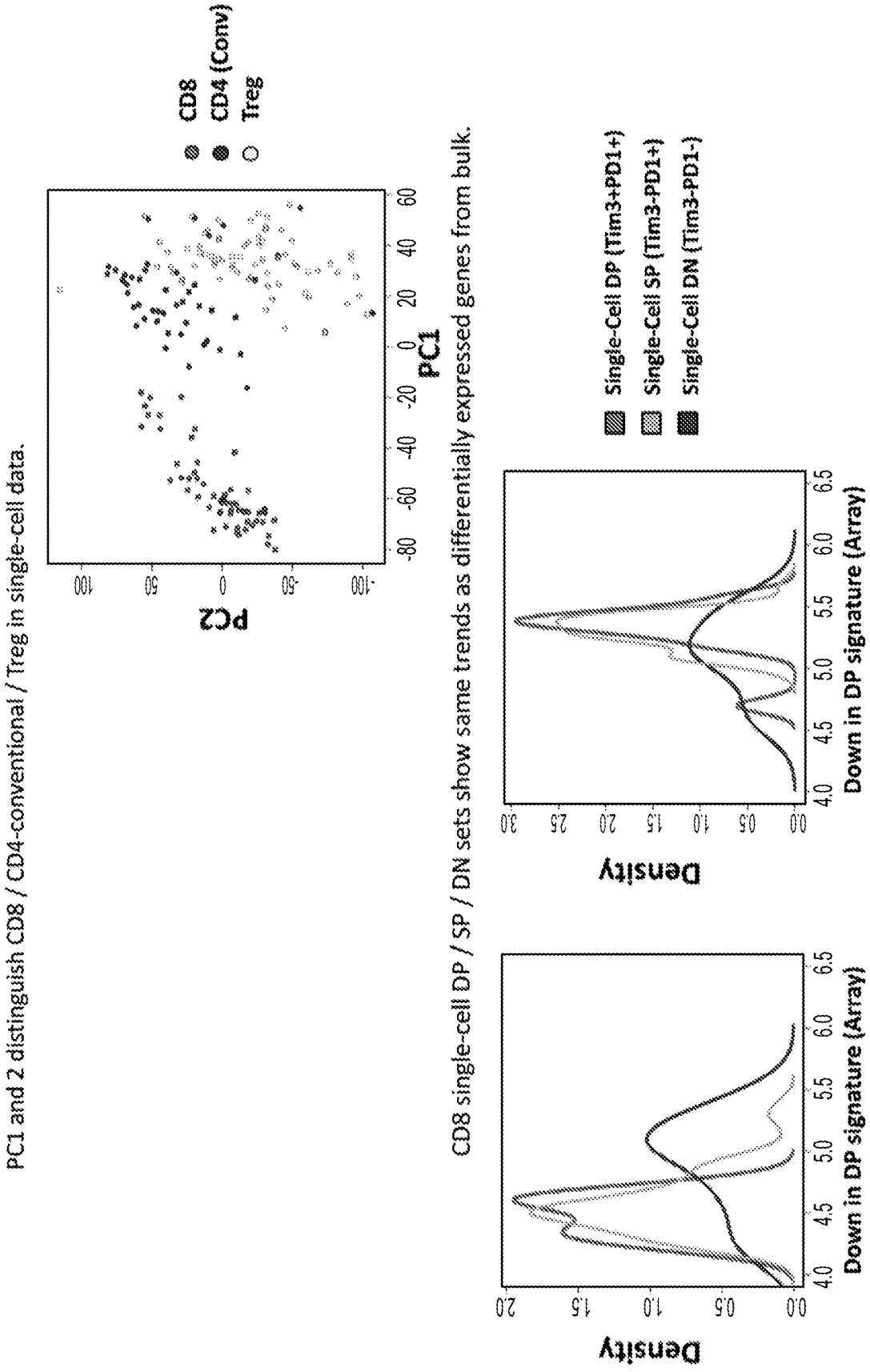

PC1 and 2 distinguish CD8 / CD4-conventional / Treg in single-cell data.
CD8
CD4 (Conv)
Treg
PC2
PC1
CD8 single-cell DP / SP / DN sets show same trends as differentially expressed genes from bulk.
Single-Cell DP (Tim3+PD1+)
Single-Cell SP (Tim3-PD1+)
Single-Cell DN (Tim3-PD1-)
Density
Down in DP signature (Array)

FIG. 28

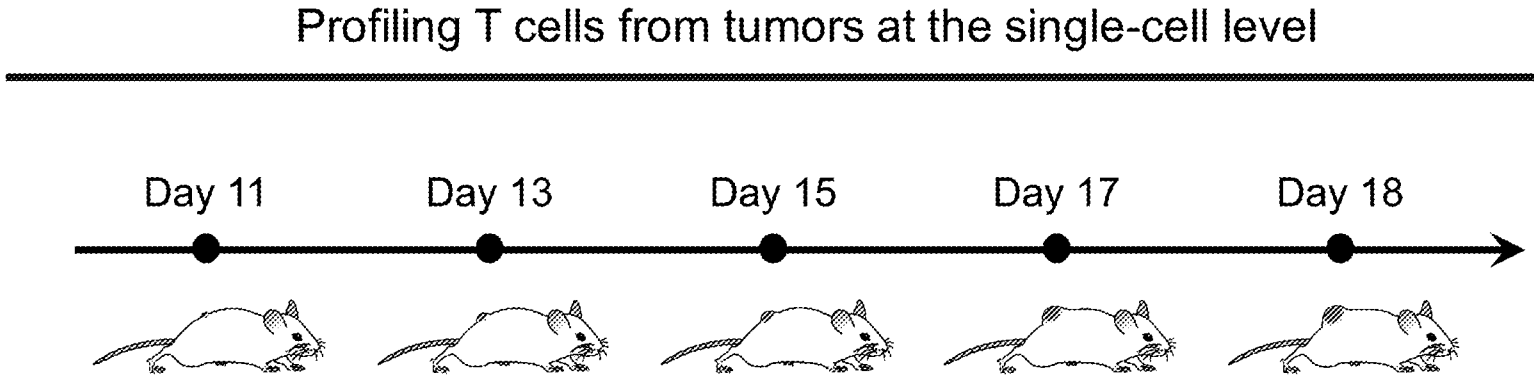

Profiling T cells from tumors at the single-cell level
Day 11
Day 13
Day 15
Day 17
Day 18
Model: B16F10 Melanoma, 5 time points, 12 mice.
Profiling: CD8+, CD4+, CD45+
Method: Plate-based single-cell RNA-seq

FIG. 29

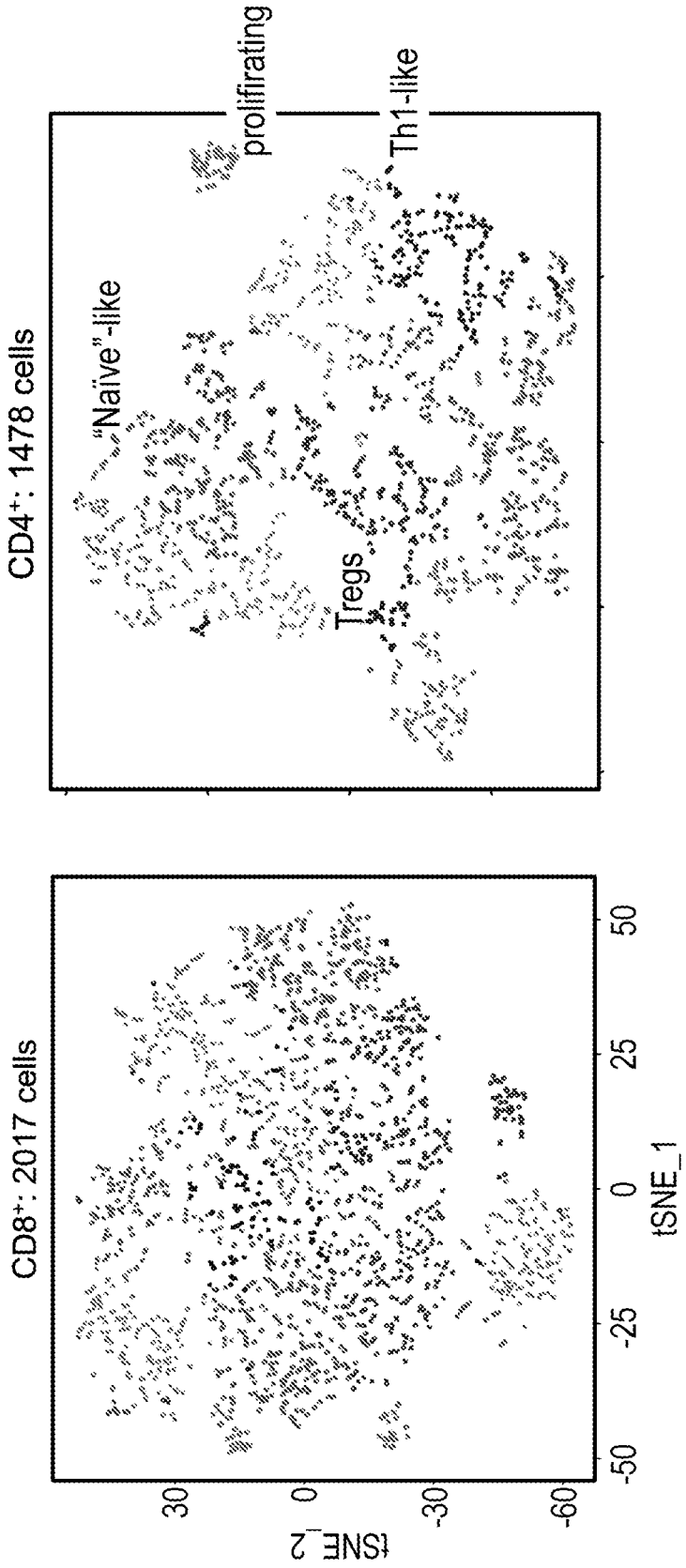

CD4+: 1478 cells
"Naïve"-like
prolifirating
Th1-like
Tregs
CD8+: 2017 cells
tSNE_2
tSNE_1
30
0
-30
-60
50
25
0
-25
-50

FIG. 30

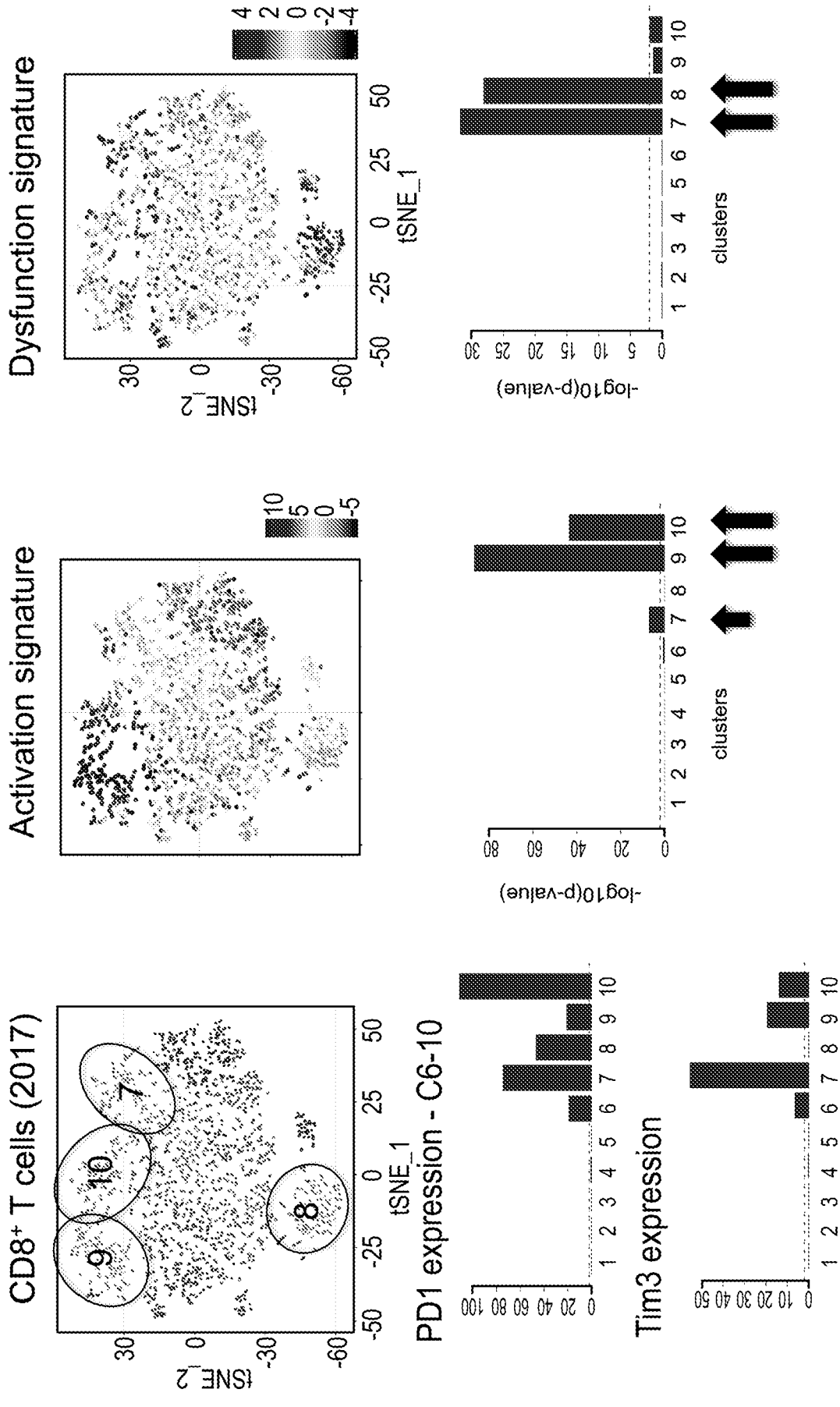

Dysfunction signature
Activation signature
CD8+ T cells (2017)
tSNE_1
tSNE_2
clusters
-log10(p-value)
PD1 expression - C6-10
Tim3 expression

FIG. 31

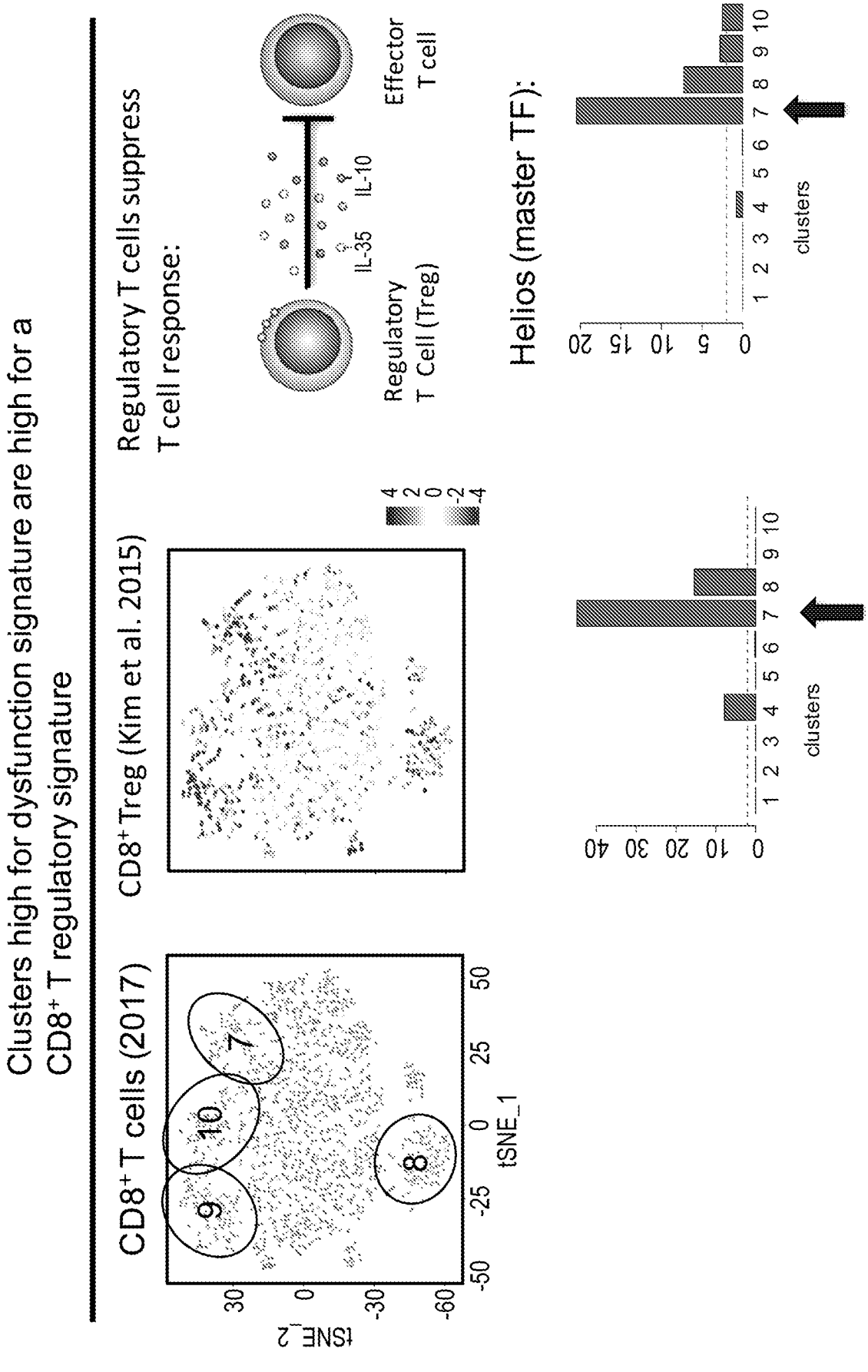
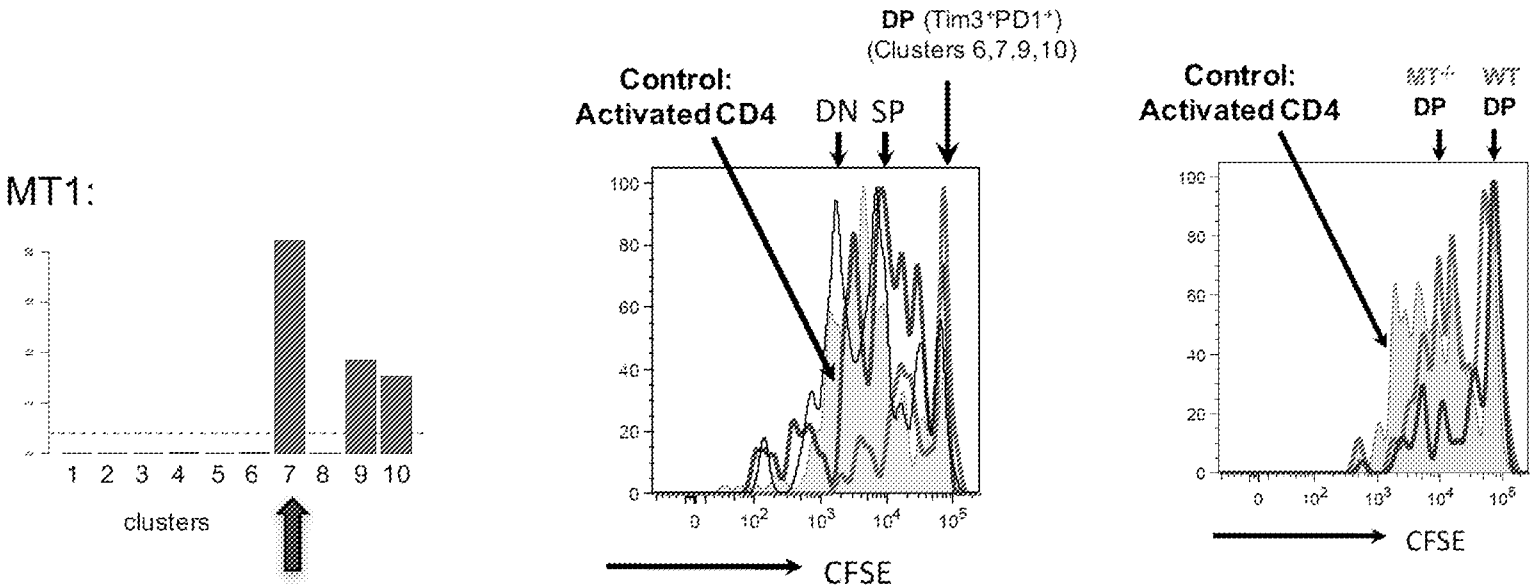

Clusters high for dysfunction signature are high for a CD8+ T regulatory signature
CD8+ T cells (2017)
CD8+ Treg (Kim et al. 2015)
Regulatory T cells suppress T cell response:
IL-35
IL-10
Regulatory T Cell (Treg)
Effector T cell
Helios (master TF):
tSNE_1
tSNE_2
clusters
clusters

FIG. 32

A suppressive CD8$^+$ population exists in tumor and is weakened by MT KO



Dysfunctional CD8 T cells restrict T cell proliferation and this is weakened in MT KO CD8 T cells.

FIG. 33

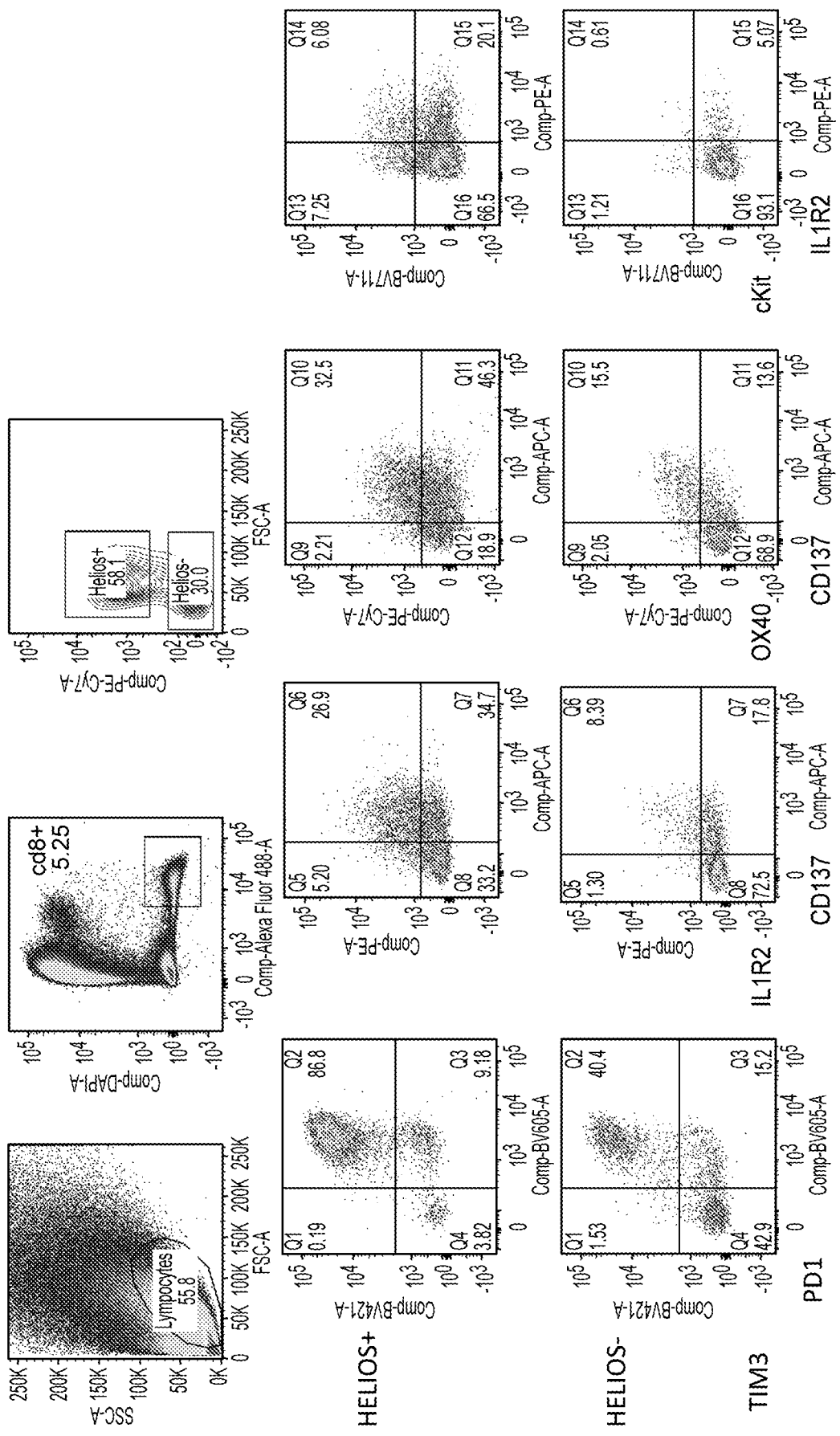

SSC-A
FSC-A
Lymphocytes
55.8
Comp-DAPI-A
Comp-Alexa Fluor 488-A
cd8+
5.25
Comp-PE-Cy7-A
Helios+
58.1
Helios-
30.0
HELIOS+
HELIOS-
TIM3
PD1
IL1R2
CD137
OX40
cKit
Comp-BV421-A
Comp-BV605-A
Comp-APC-A
Comp-PE-A
Comp-BV711-A
Q1 0.19
Q2 86.8
Q3 9.18
Q4 3.82
Q5 5.20
Q6 26.9
Q7 34.7
Q8 33.2
Q9 2.21
Q10 32.5
Q11 46.3
Q12 18.9
Q13 7.25
Q14 6.08
Q15 20.1
Q16 66.5
Q1 1.53
Q2 40.4
Q3 15.2
Q4 42.9
Q5 1.30
Q6 8.39
Q7 17.8
Q8 72.5
Q9 2.05
Q10 15.5
Q11 13.6
Q12 68.9
Q13 1.21
Q14 0.61
Q15 5.07
Q16 93.1

FIG. 34

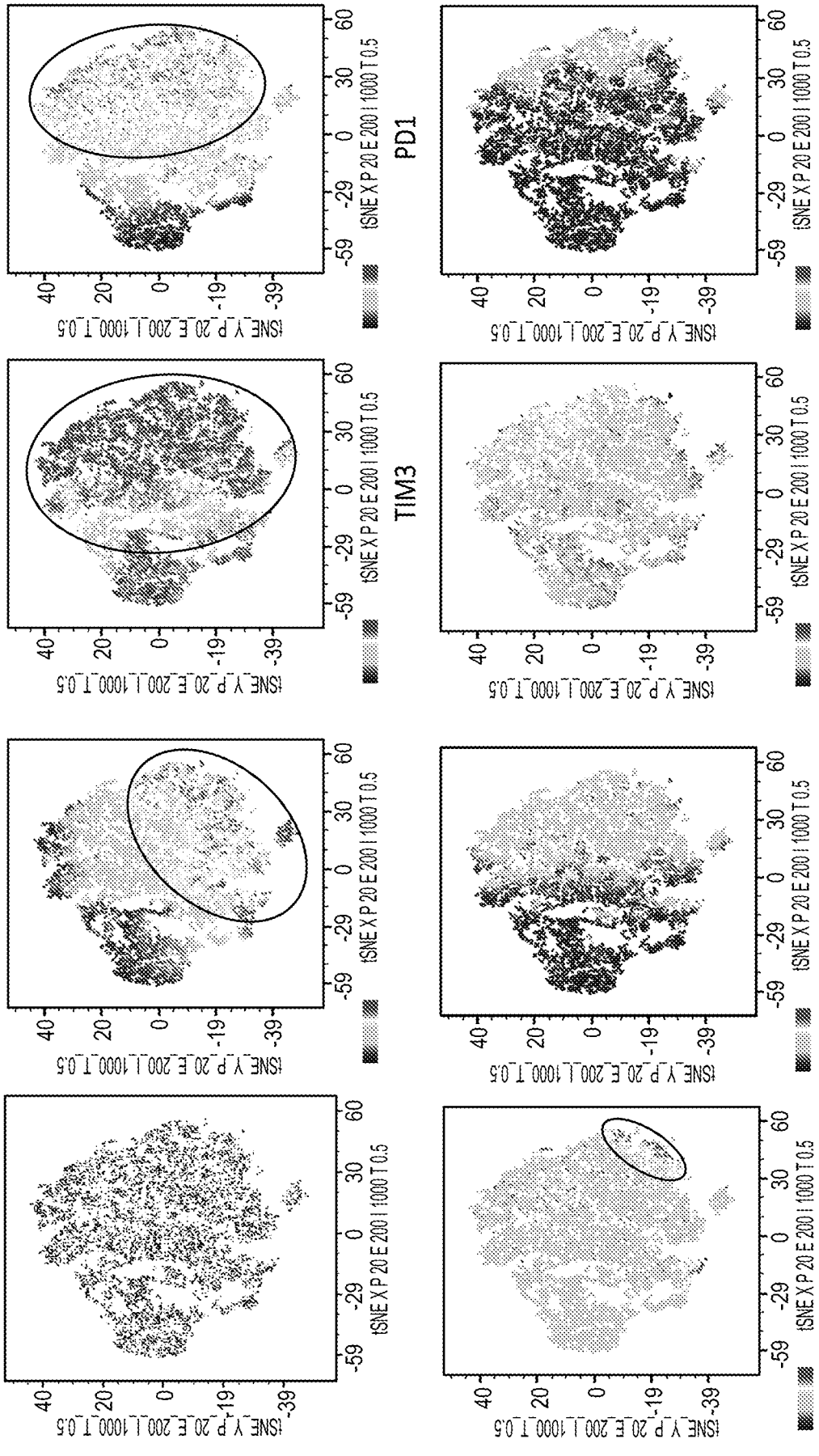

cKit
CD137
OX40
IL1R2
TIM3
PD1
tSNE_Y_P_20_E_200_I_1000_T_0.5
tSNE X P 20 E 200 I 1000 T 0.5

FIG. 35

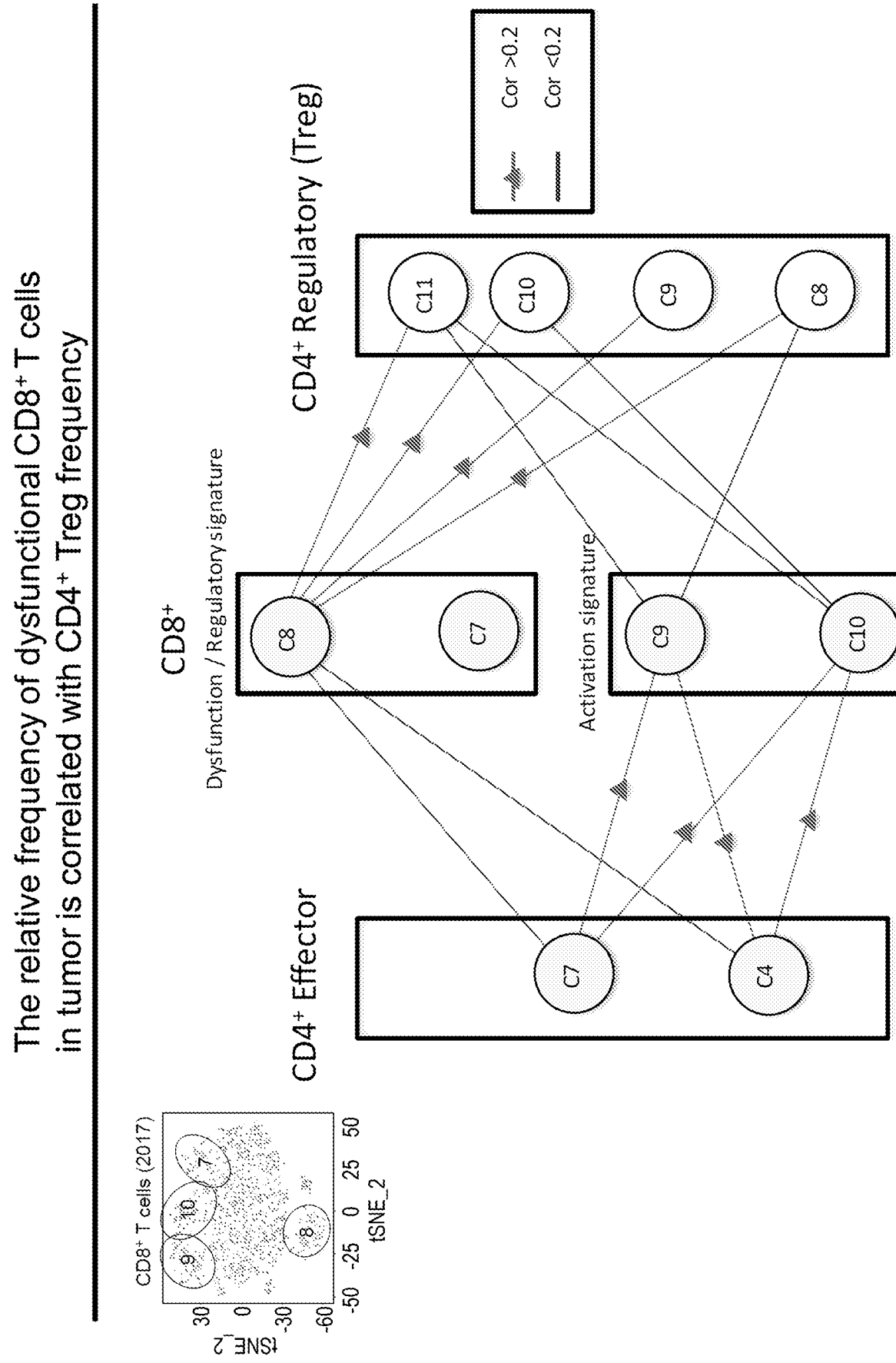

The relative frequency of dysfunctional CD8+ T cells in tumor is correlated with CD4+ Treg frequency
CD8+ T cells (2017)
tSNE_2
tSNE_1
CD8+
Dysfunction / Regulatory signature
CD4+ Regulatory (Treg)
CD4+ Effector
Activation signature
Cor >0.2
Cor <0.2
C11
C10
C9
C8
C7
C4

FIG. 36

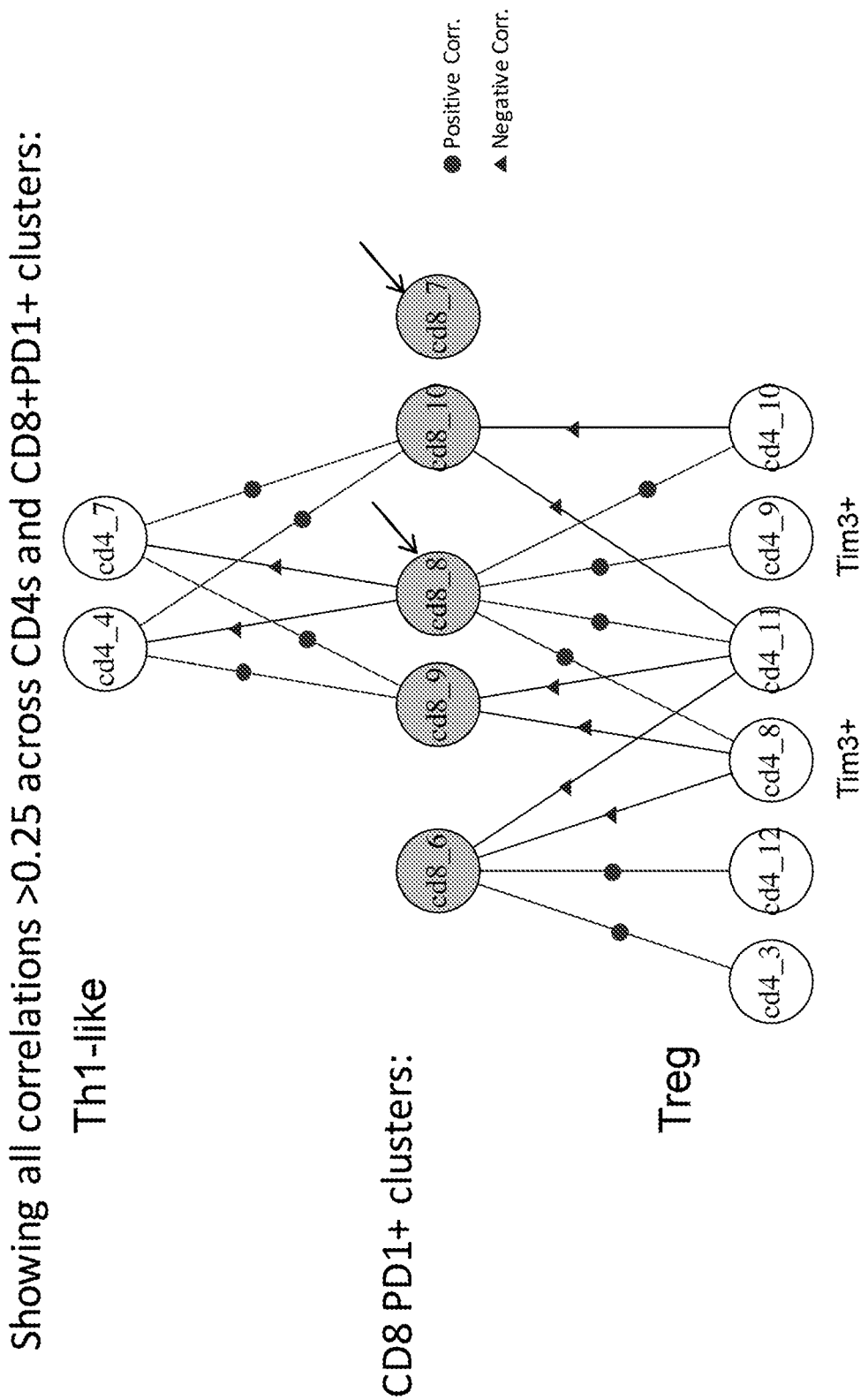

Showing all correlations >0.25 across CD4s and CD8+PD1+ clusters:
Th1-like
cd4_4
cd4_7
CD8 PD1+ clusters:
cd8_6
cd8_9
cd8_8
cd8_10
cd8_7
Treg
cd4_3
cd4_12
cd4_8
cd4_11
cd4_9
cd4_10
Tim3+
Tim3+
Positive Corr.
Negative Corr.

FIG. 37

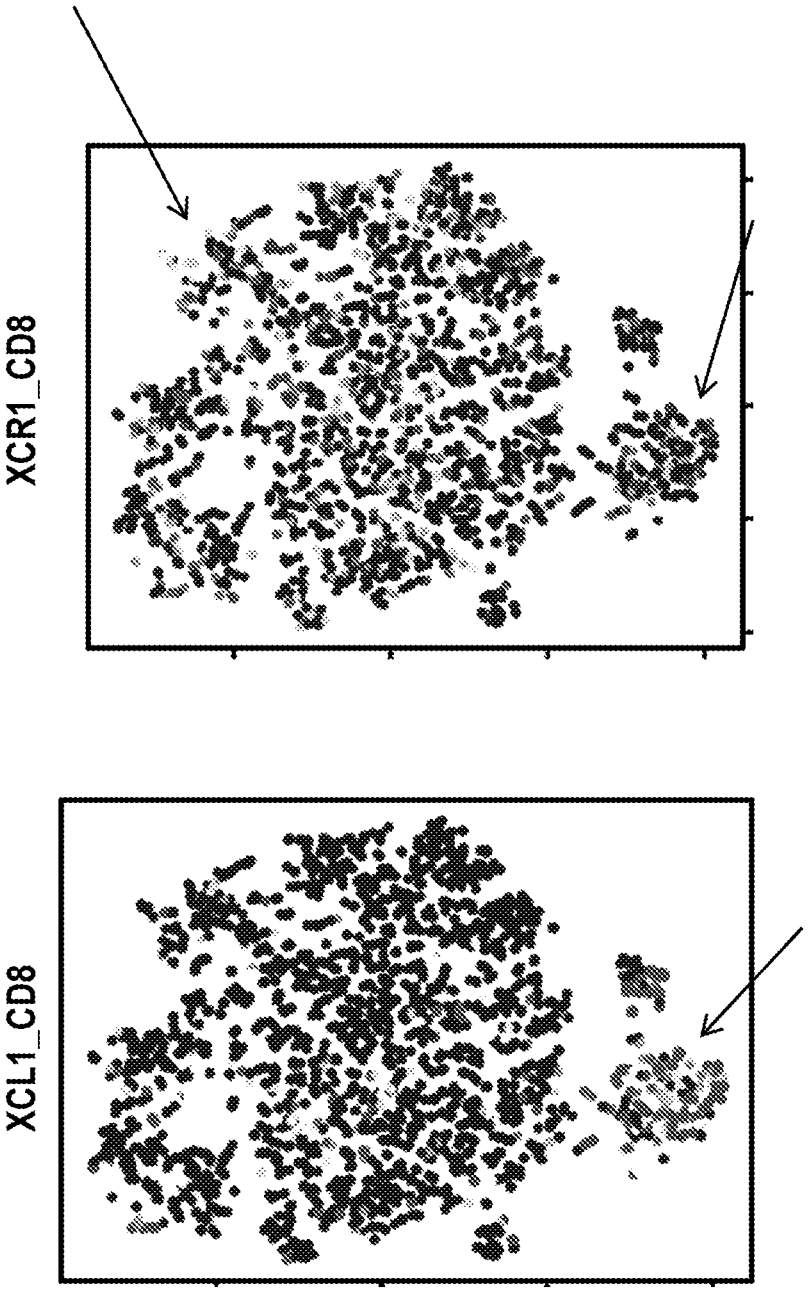


FIG. 38

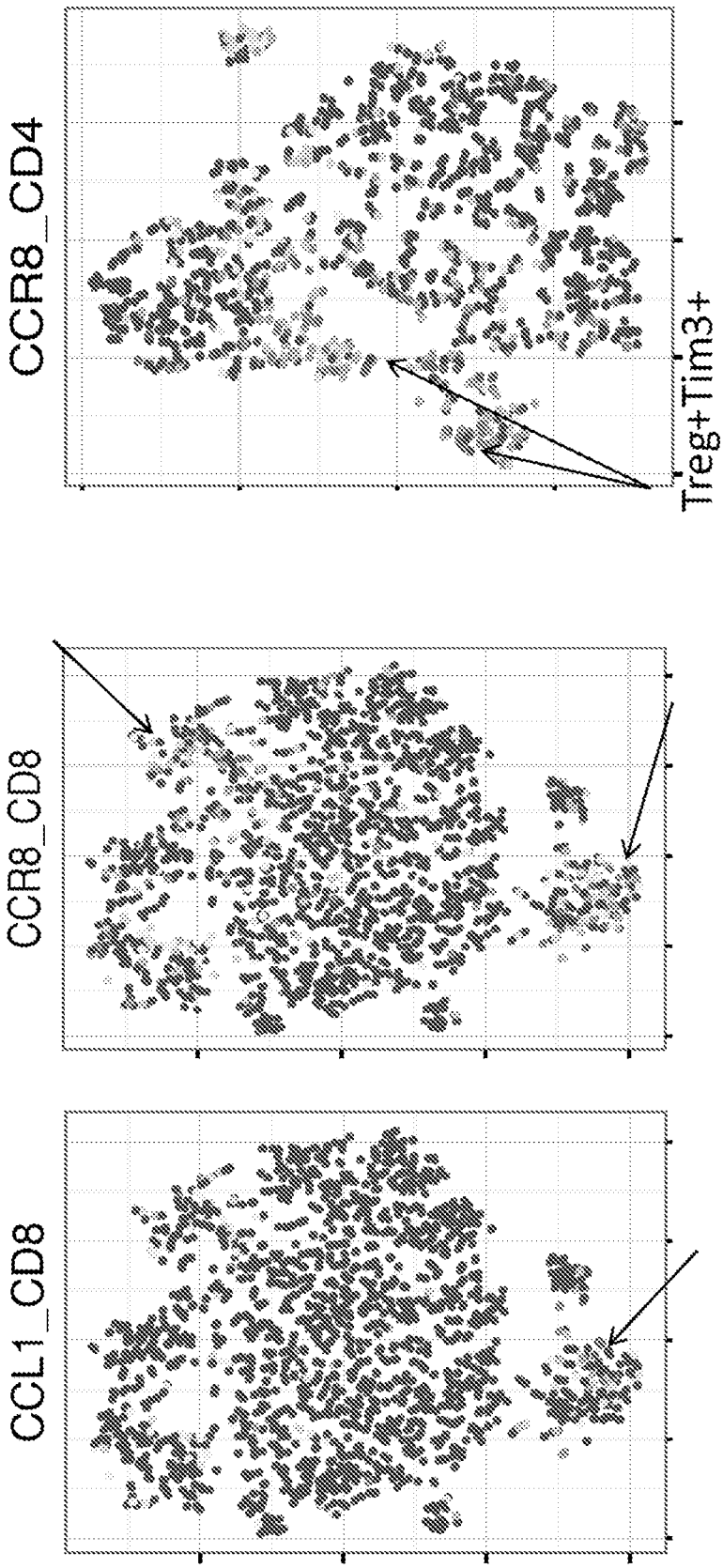


FIG. 39

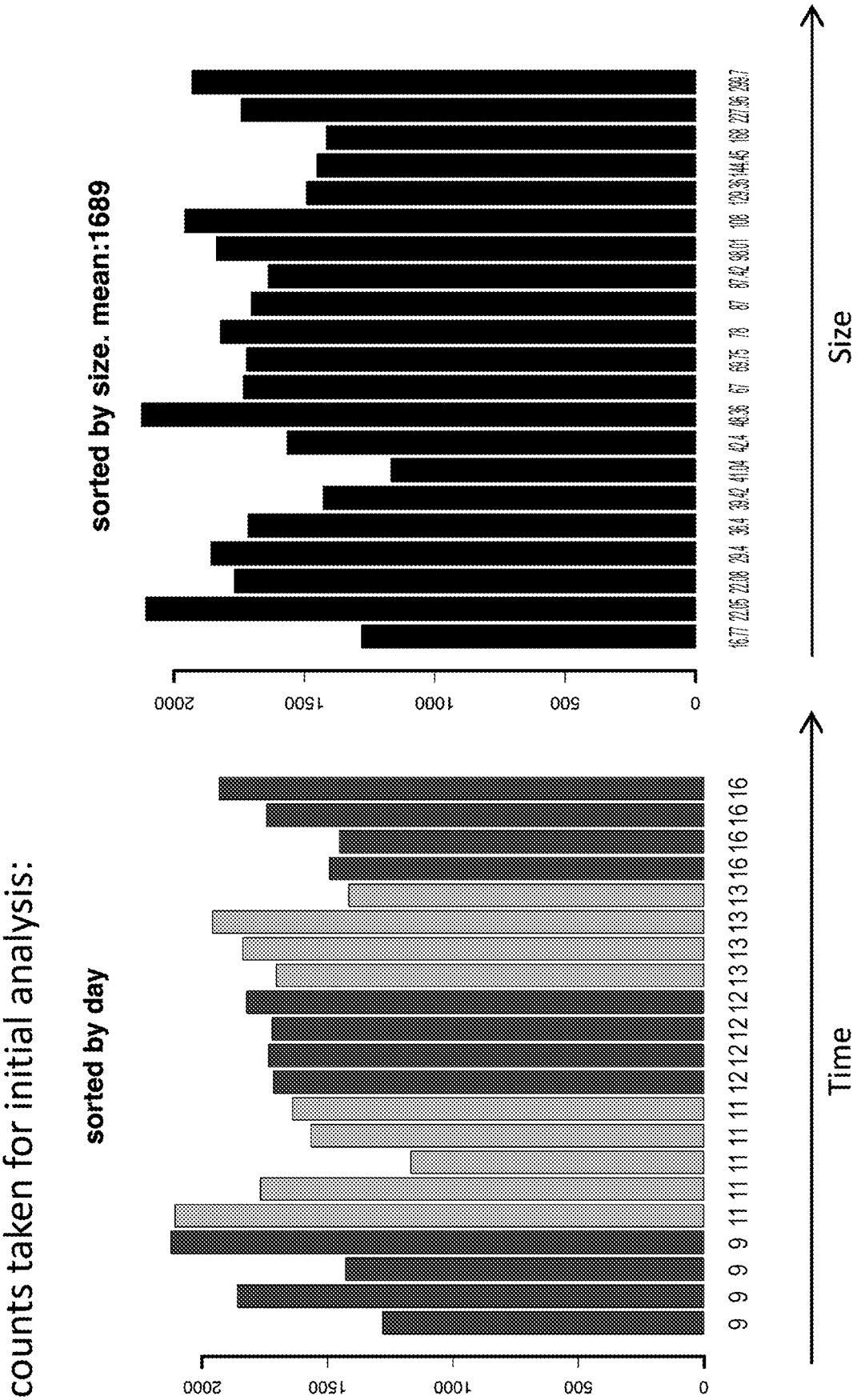

counts taken for initial analysis:
sorted by day
0
500
1000
1500
2000
9 9 9 11 11 11 11 12 12 12 12 13 13 13 13 16 16 16 16
Time
sorted by size. mean:1689
0
500
1000
1500
2000
16.77 22.05 22.08 29.4 38.4 39.42 41.04 42.4 48.36 67 69.75 78 87 87.42 98.01 108 120.36 144.45 182 227.95 286.7
Size

FIG. 40

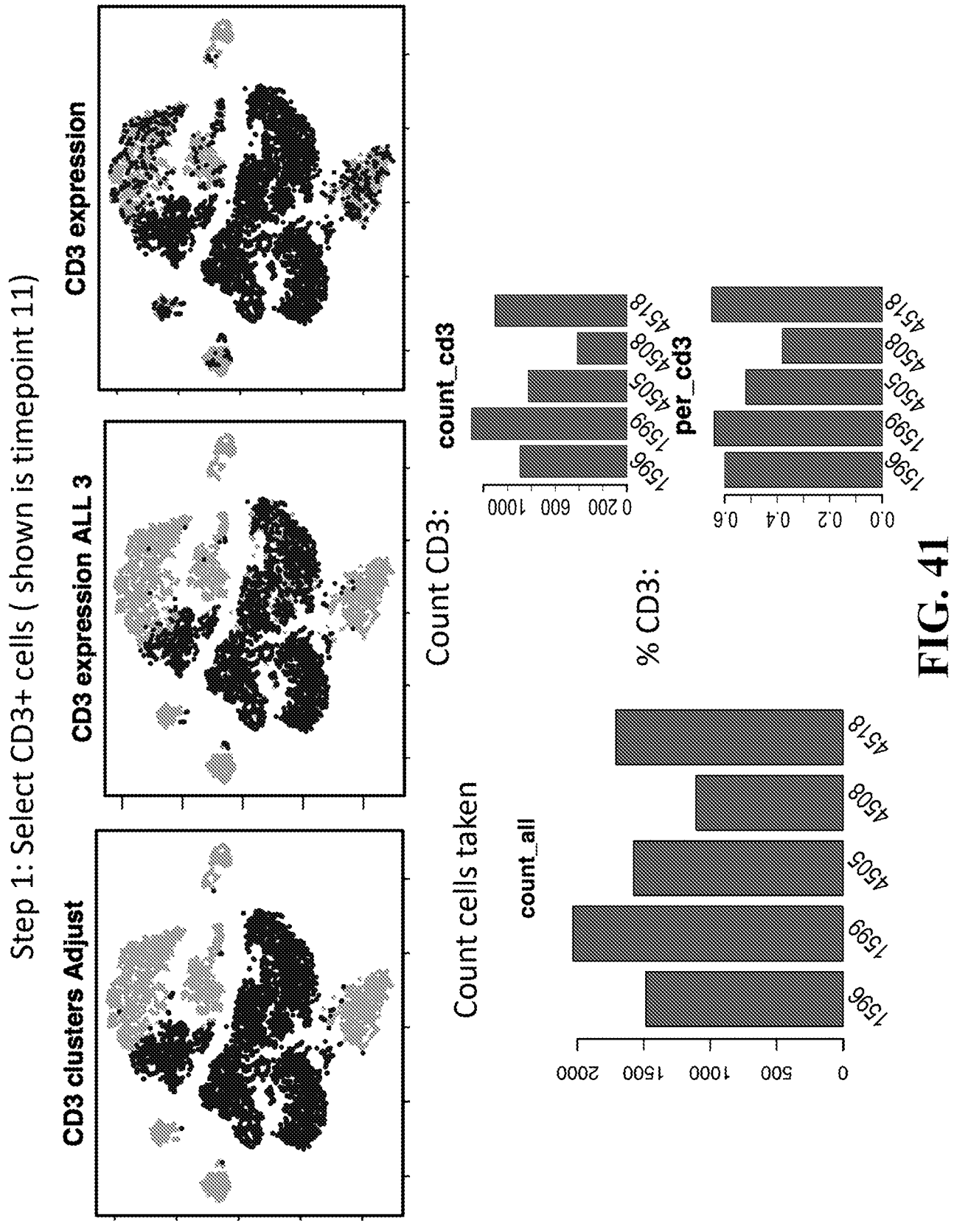

Step 1: Select CD3+ cells (shown is timepoint 11)
CD3 clusters Adjust
CD3 expression ALL 3
CD3 expression
Count cells taken
count_all
0
500
1000
1500
2000
1596
1599
4505
4508
4518
Count CD3:
count_cd3
0
200
600
1000
% CD3:
per_cd3
0.0
0.2
0.4
0.6

FIG. 41

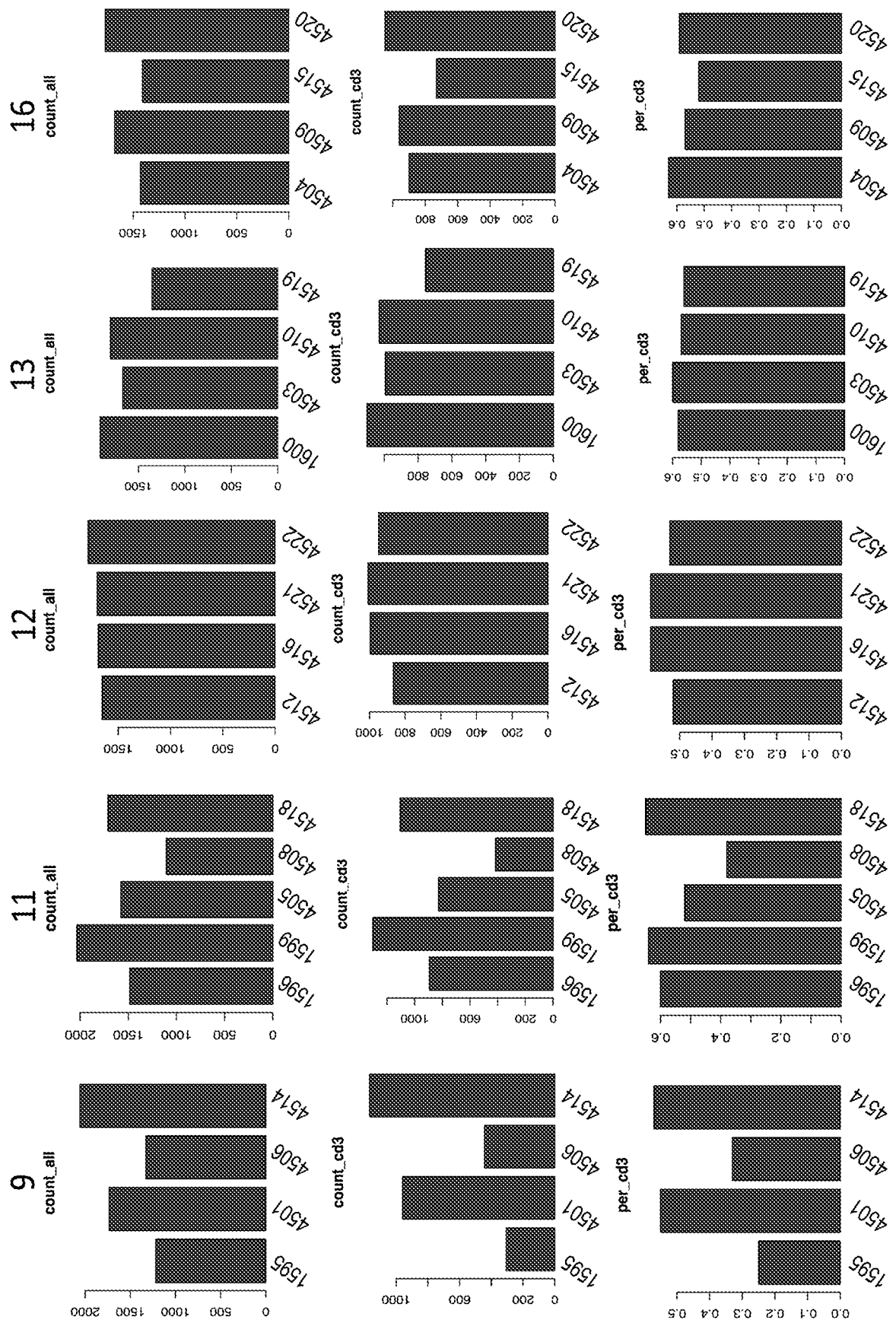

9
count_all
1595
4501
4506
4514
0 500 1000 1500 2000
count_cd3
1595
4501
4506
4514
0 200 600 1000
per_cd3
1595
4501
4506
4514
0.0 0.1 0.2 0.3 0.4 0.5
11
count_all
1596
1599
4505
4508
4518
0 500 1000 1500 2000
count_cd3
1596
1599
4505
4508
4518
0 200 600 1000
per_cd3
1596
1599
4505
4508
4518
0.0 0.2 0.4 0.6
12
count_all
4512
4516
4521
4522
0 500 1000 1500
count_cd3
4512
4516
4521
4522
0 200 400 600 800 1000
per_cd3
4512
4516
4521
4522
0.0 0.1 0.2 0.3 0.4 0.5
13
count_all
1600
4503
4510
4519
0 500 1000 1500
count_cd3
1600
4503
4510
4519
0 200 400 600 800
per_cd3
1600
4503
4510
4519
0.0 0.1 0.2 0.3 0.4 0.5 0.6
16
count_all
4504
4509
4515
4520
0 500 1000 1500
count_cd3
4504
4509
4515
4520
0 200 400 600 800
per_cd3
4504
4509
4515
4520
0.0 0.1 0.2 0.3 0.4 0.5 0.6

FIG. 42

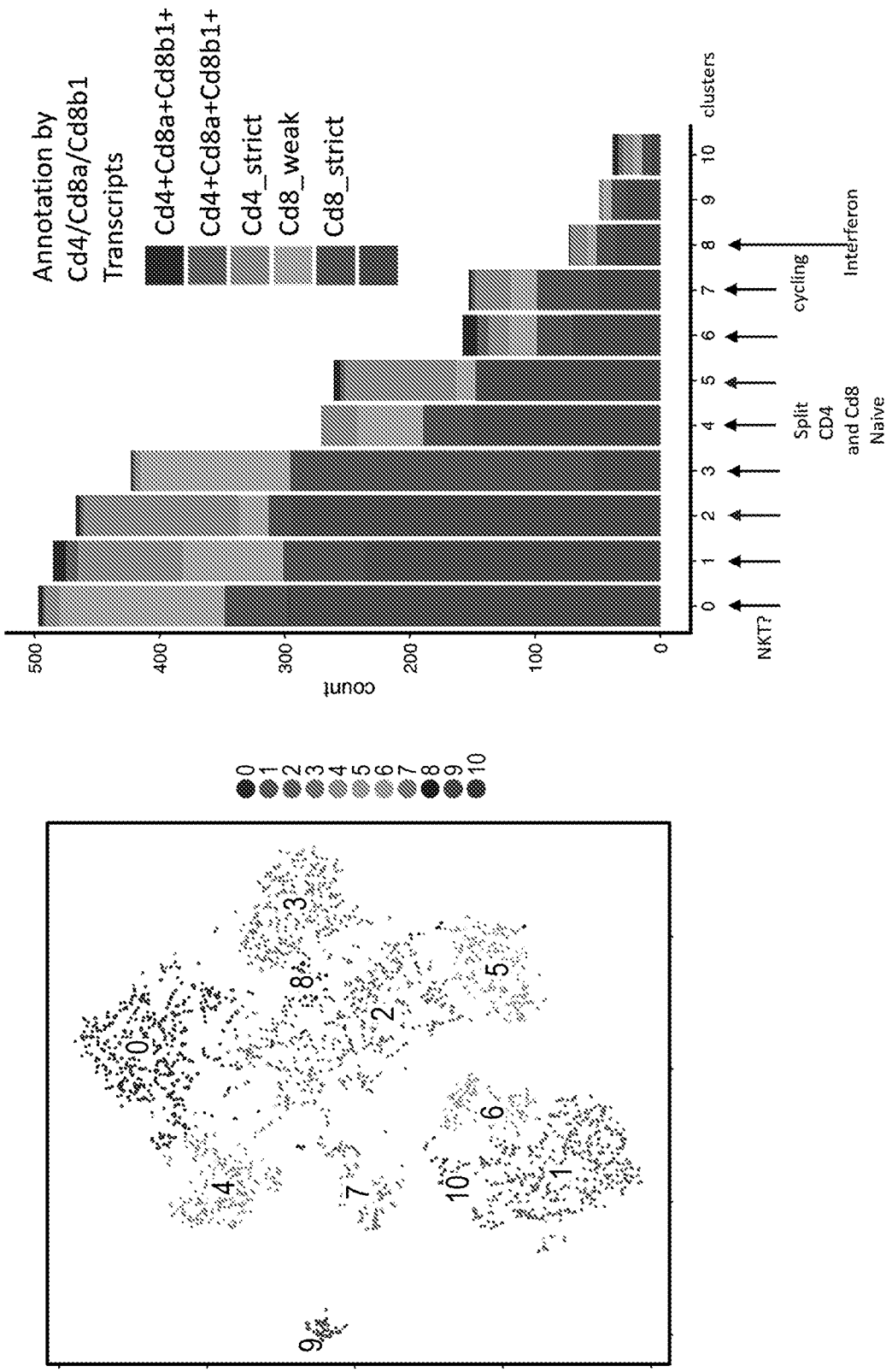

Annotation by
Cd4/Cd8a/Cd8b1
Transcripts
Cd4+Cd8a+Cd8b1+
Cd4+Cd8a+Cd8b1+
Cd4_strict
Cd8_weak
Cd8_strict
count
500
400
300
200
100
0
clusters
0 1 2 3 4 5 6 7 8 9 10
NKT?
Split CD4 and Cd8 Naive
cycling
Interferon
0 1 2 3 4 5 6 7 8 9 10

FIG. 43

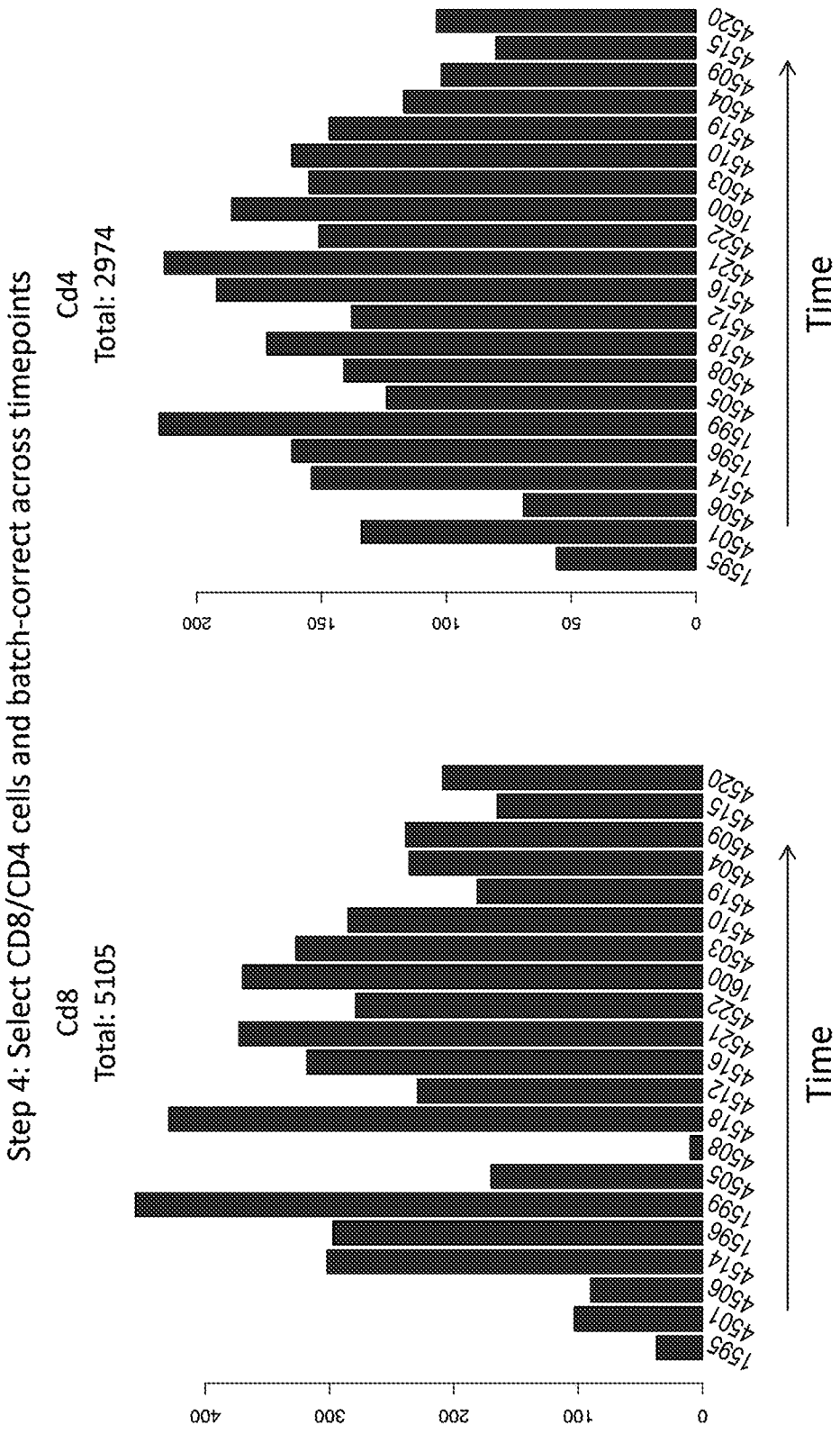

Step 4: Select CD8/CD4 cells and batch-correct across timepoints
Cd8
Total: 5105
Cd4
Total: 2974
Time
Time
0
100
200
300
400
0
50
100
150
200
1595
4501
4506
4514
1596
1599
4505
4508
4518
4512
4516
4521
4522
1600
4503
4510
4519
4504
4509
4515
4520

FIG. 44

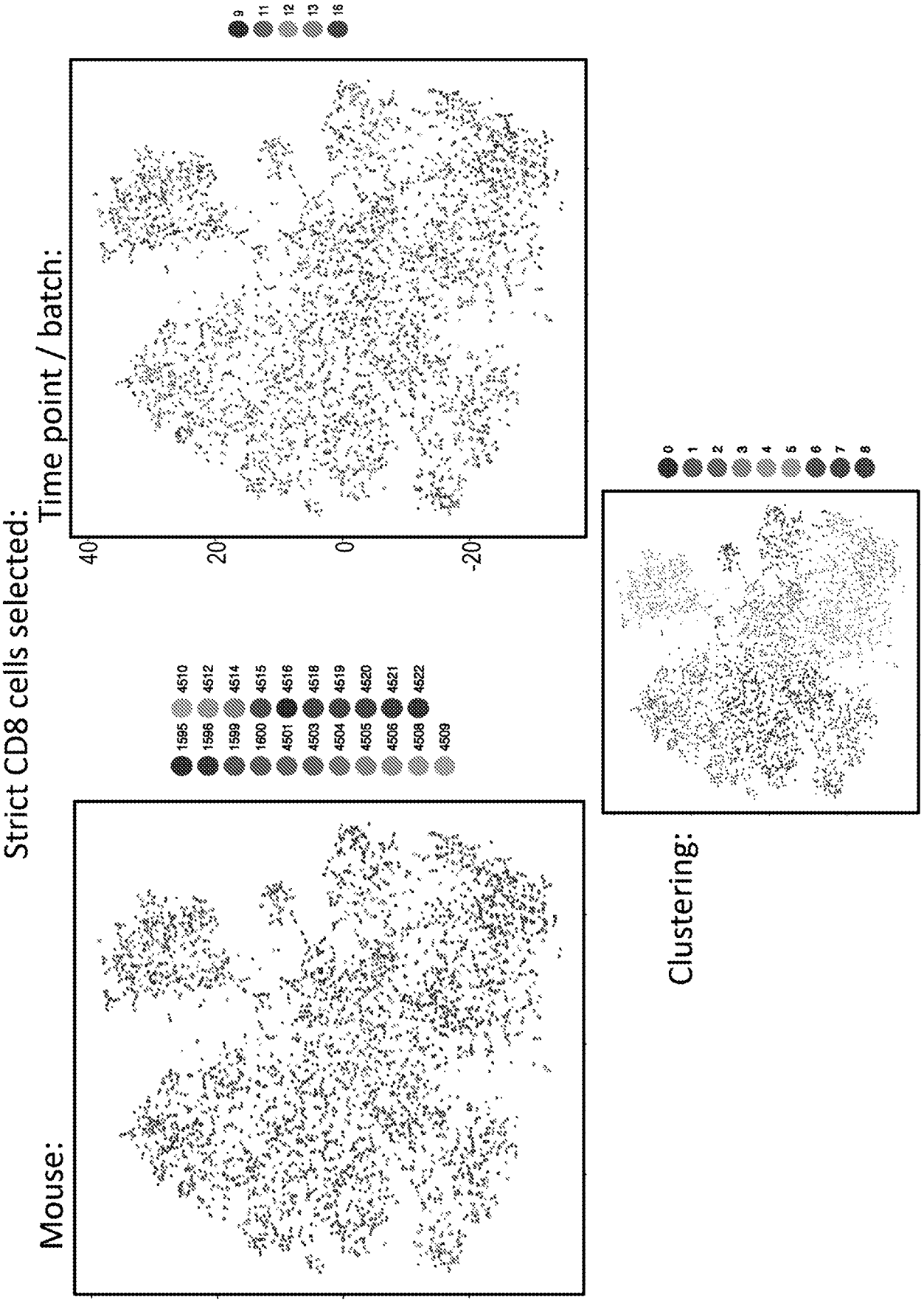

Strict CD8 cells selected:
Mouse:
Time point / batch:
9
11
12
13
16
40
20
0
-20
1595
1596
1599
1600
4501
4503
4504
4505
4506
4508
4509
4510
4512
4514
4515
4516
4518
4519
4520
4521
4522
Clustering:
0
1
2
3
4
5
6
7
8

FIG. 45

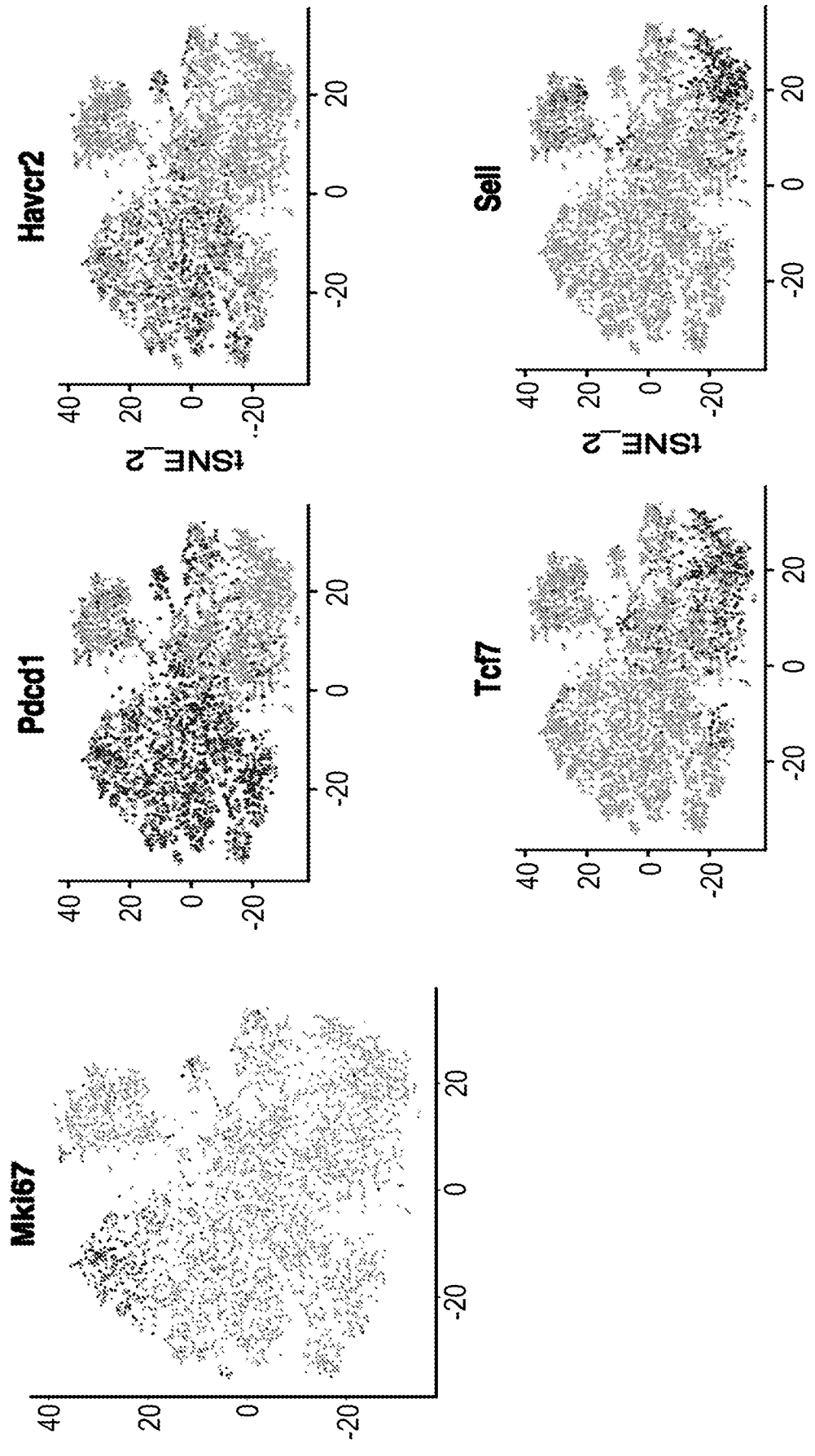


FIG. 46

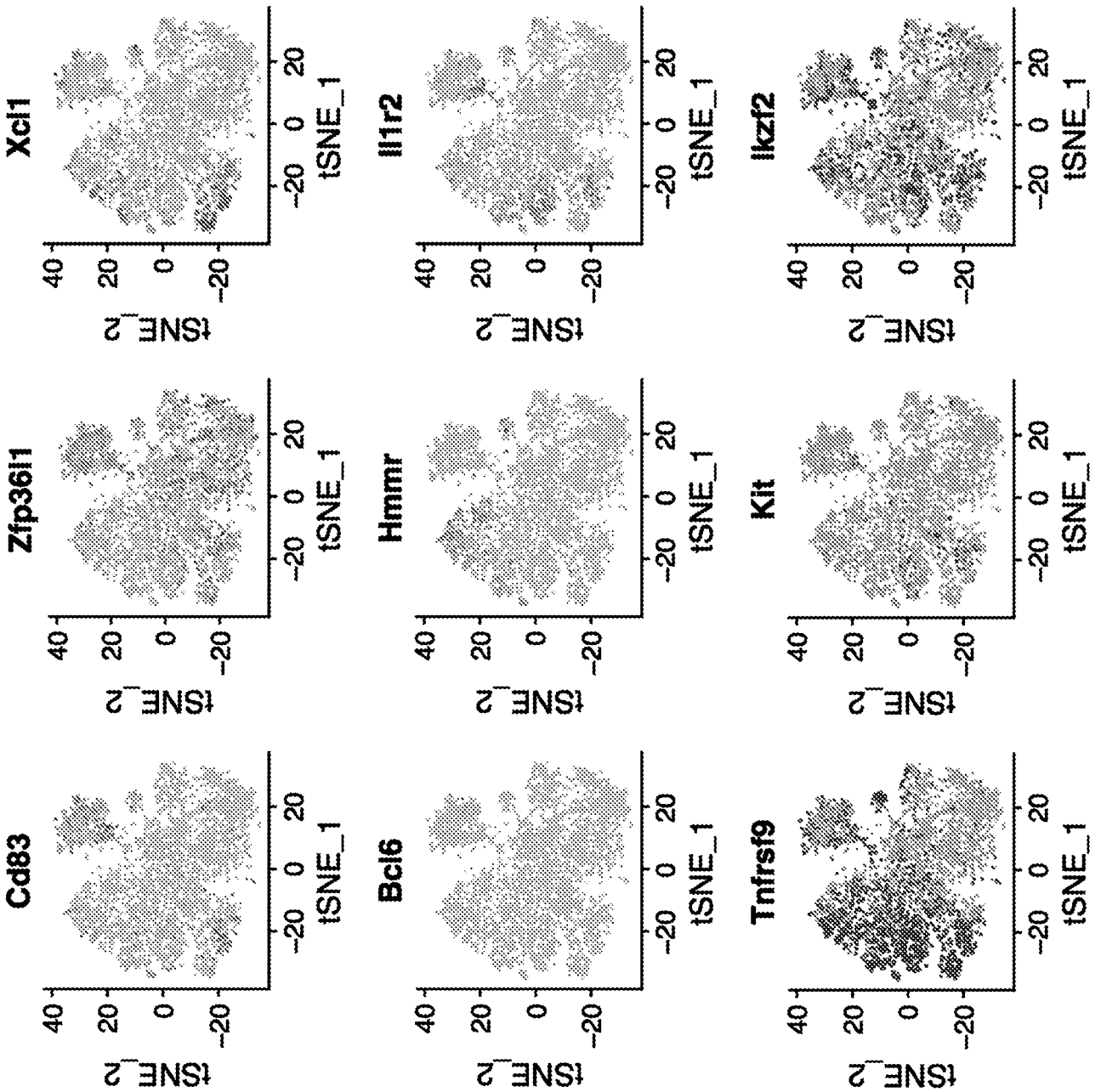

Xcl1
Il1r2
Ikzf2
Zfp36l1
Hmmr
Kit
Cd83
Bcl6
Tnfrsf9
tSNE_1
tSNE_2
40
20
0
−20
20
0
−20

FIG. 47

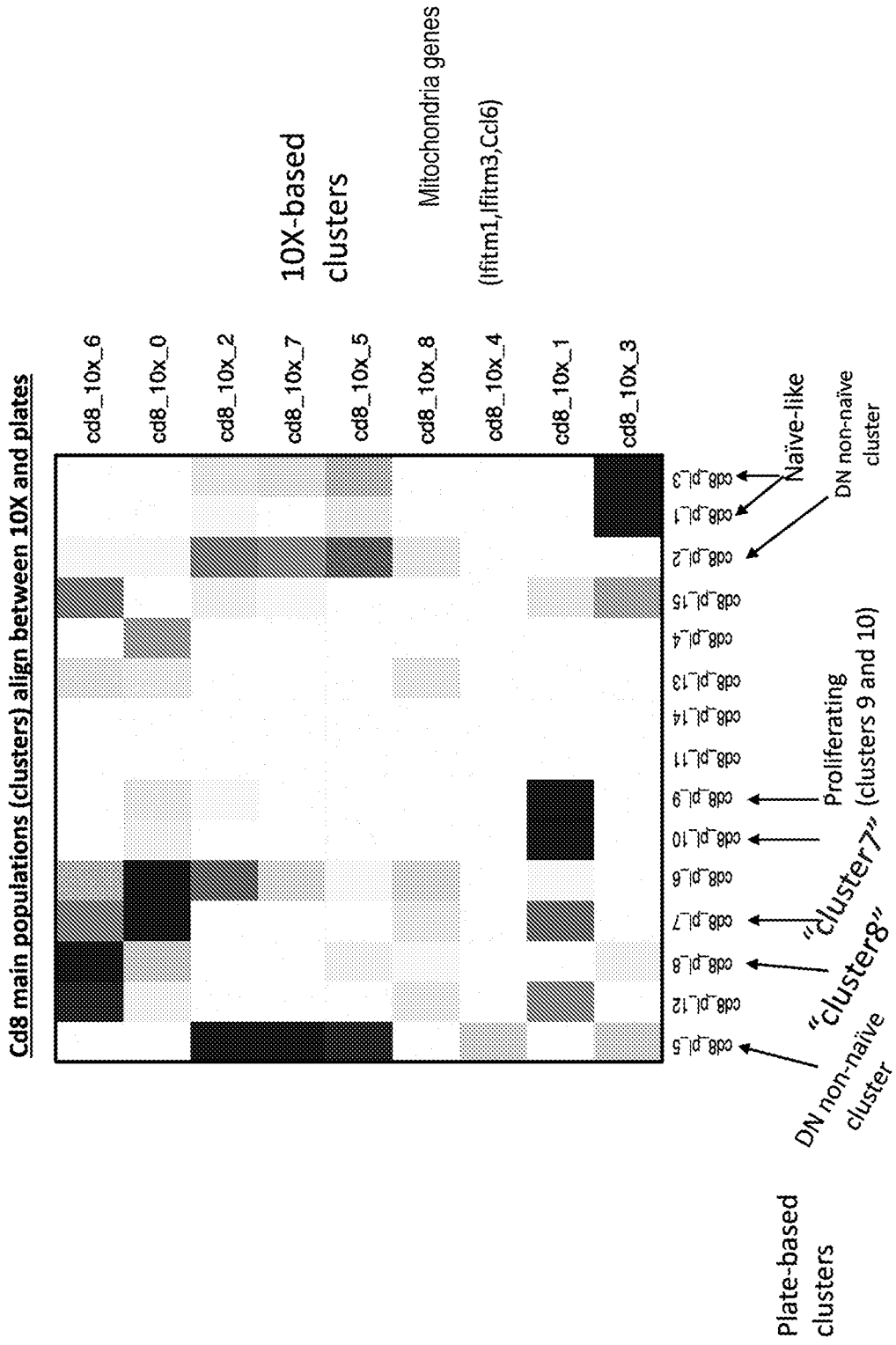

Cd8 main populations (clusters) align between 10X and plates
cd8_10x_6
cd8_10x_0
cd8_10x_2
cd8_10x_7
cd8_10x_5
cd8_10x_8
cd8_10x_4
cd8_10x_1
cd8_10x_3
10X-based clusters
Mitochondria genes
(Ifitm1,Ifitm3,Ccl6)
cd8_pl_5
cd8_pl_12
cd8_pl_8
cd8_pl_7
cd8_pl_6
cd8_pl_10
cd8_pl_9
cd8_pl_11
cd8_pl_14
cd8_pl_13
cd8_pl_4
cd8_pl_15
cd8_pl_2
cd8_pl_1
cd8_pl_3
Plate-based clusters
DN non-naïve cluster
"cluster8"
"cluster7"
Proliferating (clusters 9 and 10)
DN non-naïve cluster
Naïve-like

FIG. 48

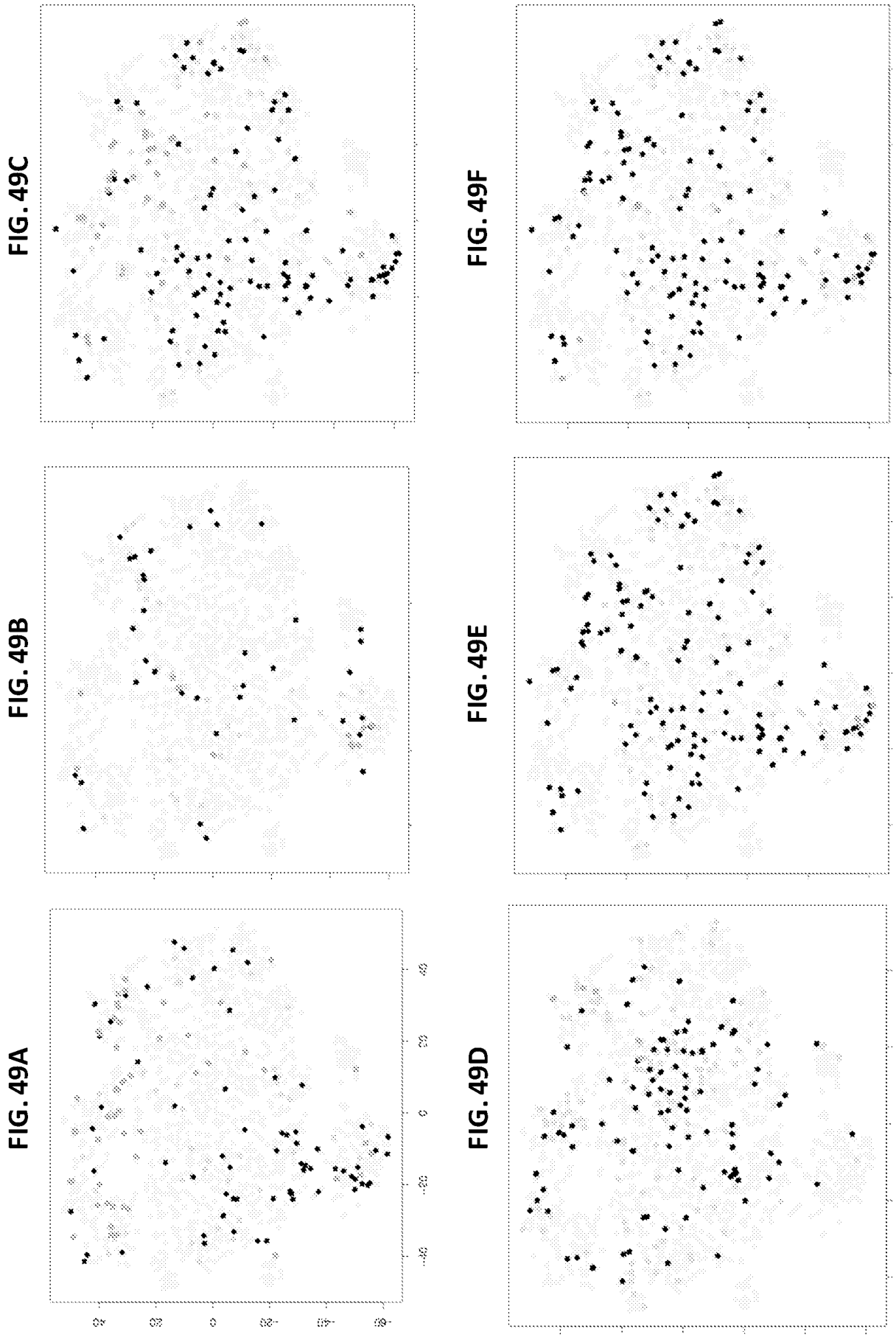


FIG. 49

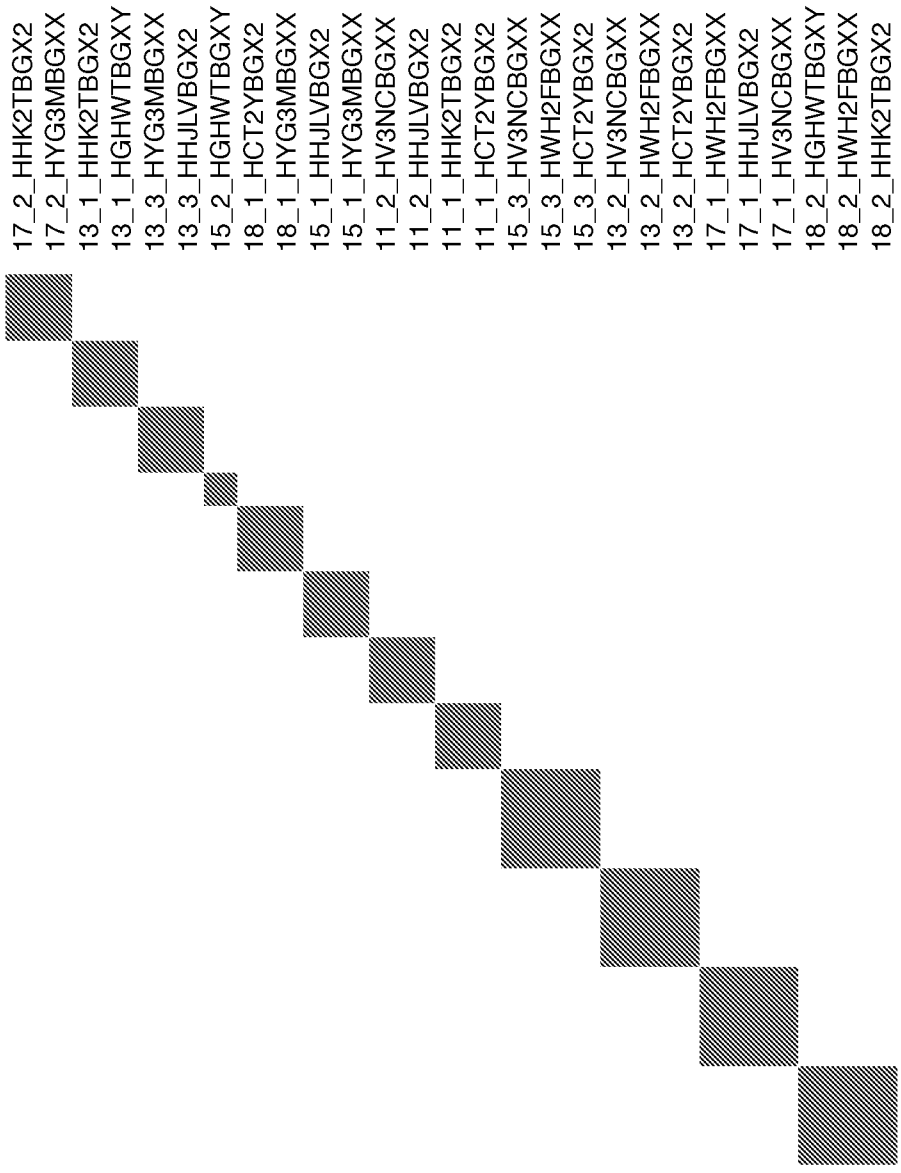

17_2_HHK2TBGX2
17_2_HYG3MBGXX
13_1_HHK2TBGX2
13_1_HGHWTBGXY
13_3_HYG3MBGXX
13_3_HHJLVBGX2
15_2_HGHWTBGXY
18_1_HCT2YBGX2
18_1_HYG3MBGXX
15_1_HHJLVBGX2
15_1_HYG3MBGXX
11_2_HV3NCBGXX
11_2_HHJLVBGX2
11_1_HHK2TBGX2
11_1_HCT2YBGX2
15_3_HV3NCBGXX
15_3_HWH2FBGXX
15_2_HCT2YBGX2
13_2_HV3NCBGXX
13_2_HWH2FBGXX
13_2_HCT2YBGX2
17_1_HWH2FBGXX
17_1_HHJLVBGX2
17_1_HV3NCBGXX
18_2_HGHWTBGXY
18_2_HWH2FBGXX
18_2_HHK2TBGX2
18_2_HHK2TBGX2
18_2_HWH2FBGXX
18_2_HGHWTBGXY
17_1_HV3NCBGXX
17_1_HHJLVBGX2
17_1_HWH2FBGXX
13_2_HCT2YBGX2
13_2_HWH2FBGXX
13_2_HV3NCBGXX
15_3_HCT2YBGX2
15_3_HWH2FBGXX
15_3_HV3NCBGXX
11_1_HCT2YBGX2
11_1_HHK2TBGX2
11_2_HHJLVBGX2
11_2_HV3NCBGXX
15_1_HYG3MBGXX
15_1_HHJLVBGX2
18_1_HYG3MBGXX
18_1_HCT2YBGX2
15_2_HGHWTBGXY
13_3_HHJLVBGX2
13_3_HYG3MBGXX
13_1_HGHWTBGXY
13_1_HHK2TBGX2
17_2_HYG3MBGXX
17_2_HHK2TBGX2

FIG. 50

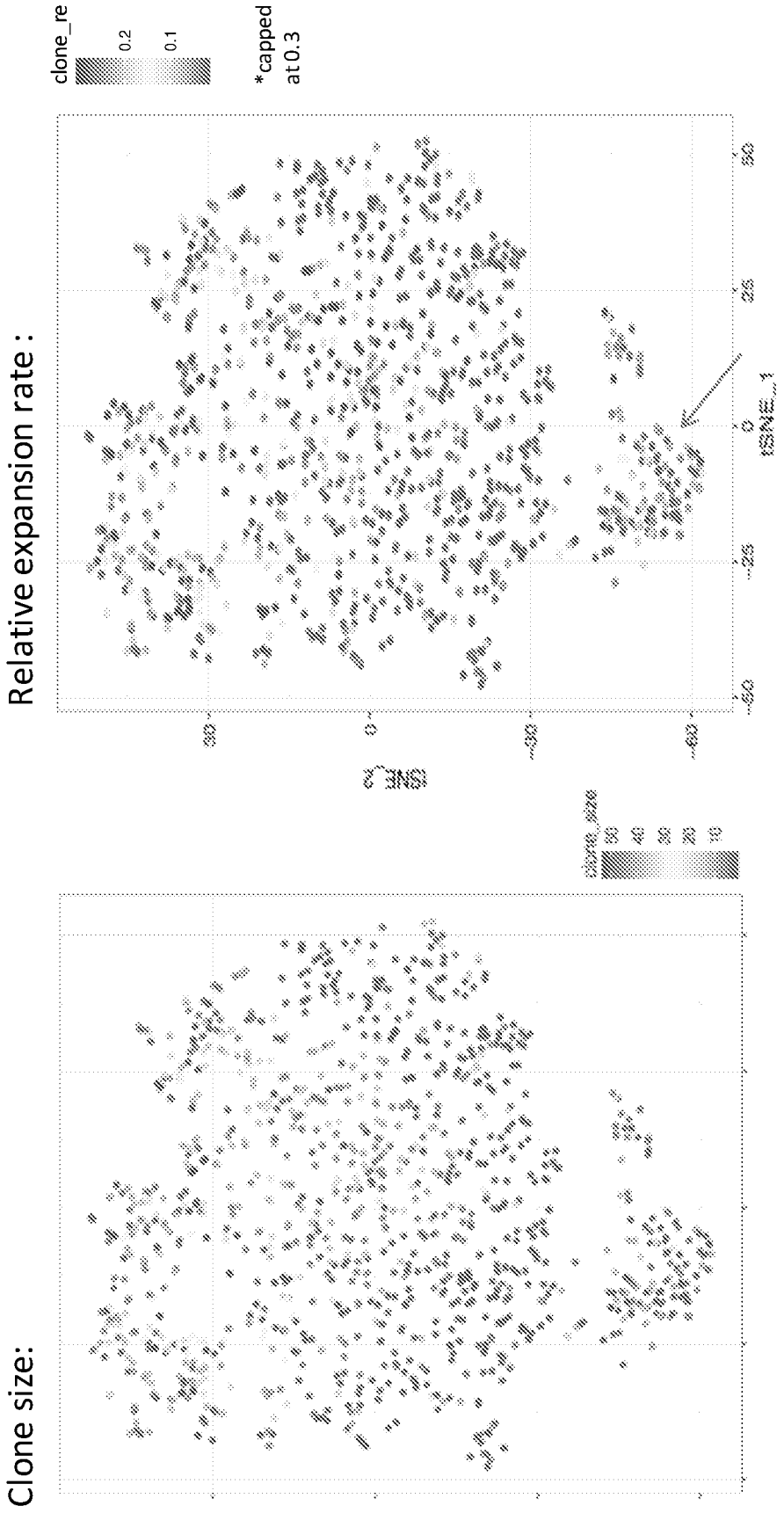

Relative expansion rate :
clone_re
0.2
0.1
*capped at 0.3
tSNE_2
tSNE_1
50
0
-50
-50
-25
0
25
50
Clone size:
clone_size
60
40
30
20
10

FIG. 51

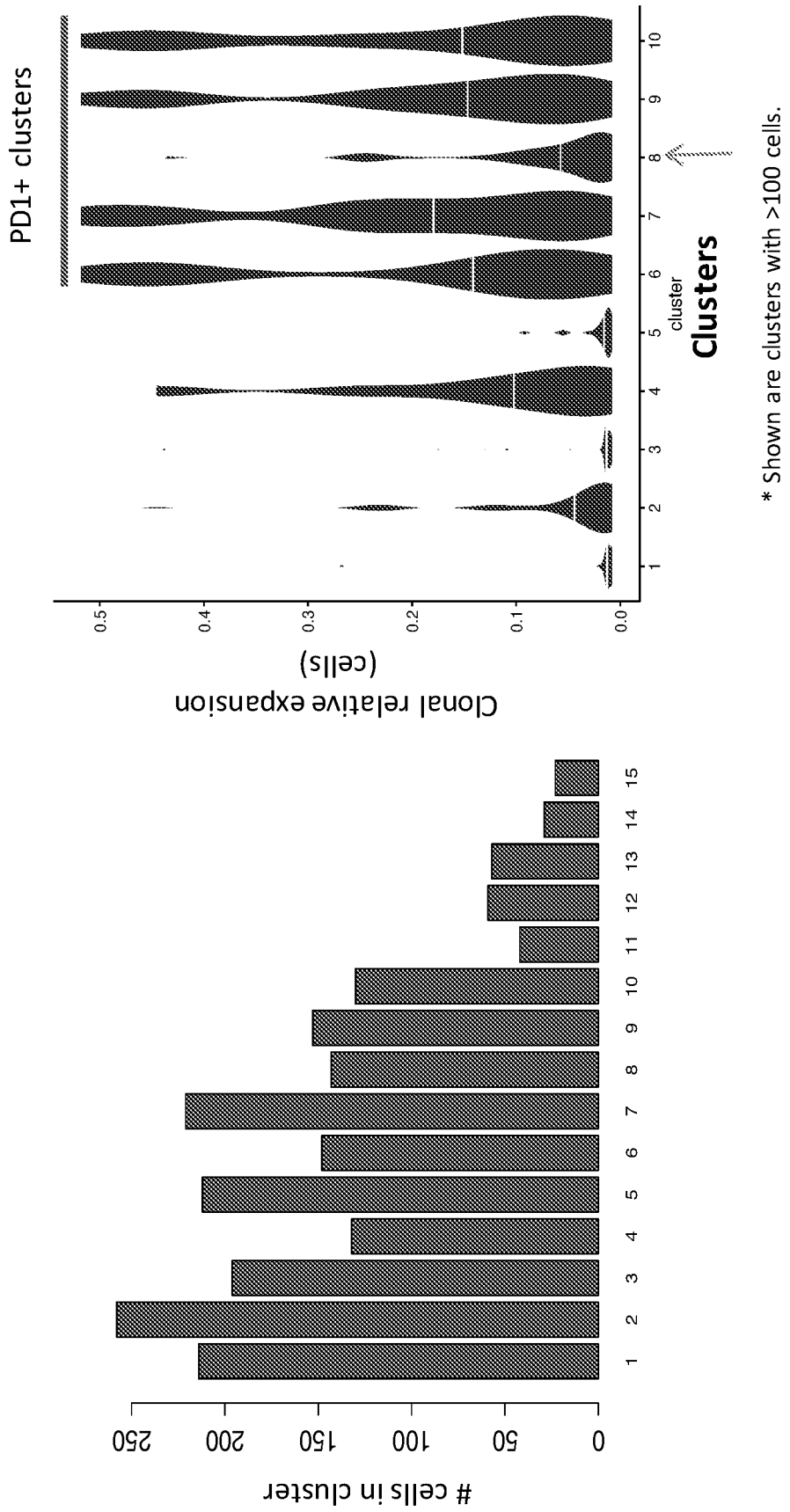

PD1+ clusters
Clonal relative expansion
(cells)
0.0
0.1
0.2
0.3
0.4
0.5
1 2 3 4 5 6 7 8 9 10
cluster
Clusters
* Shown are clusters with >100 cells.
# cells in cluster
0
50
100
150
200
250
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

FIG. 52

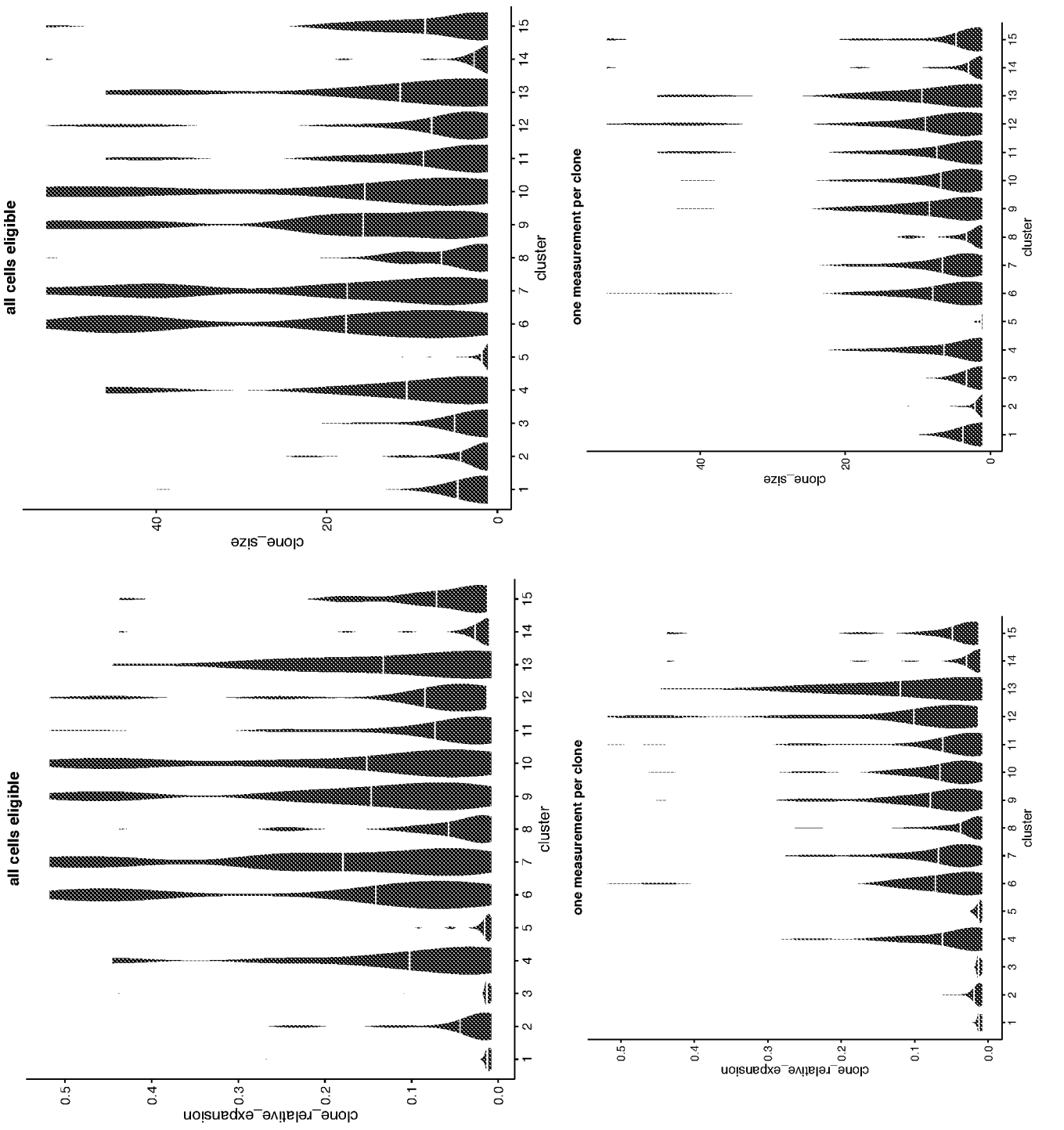
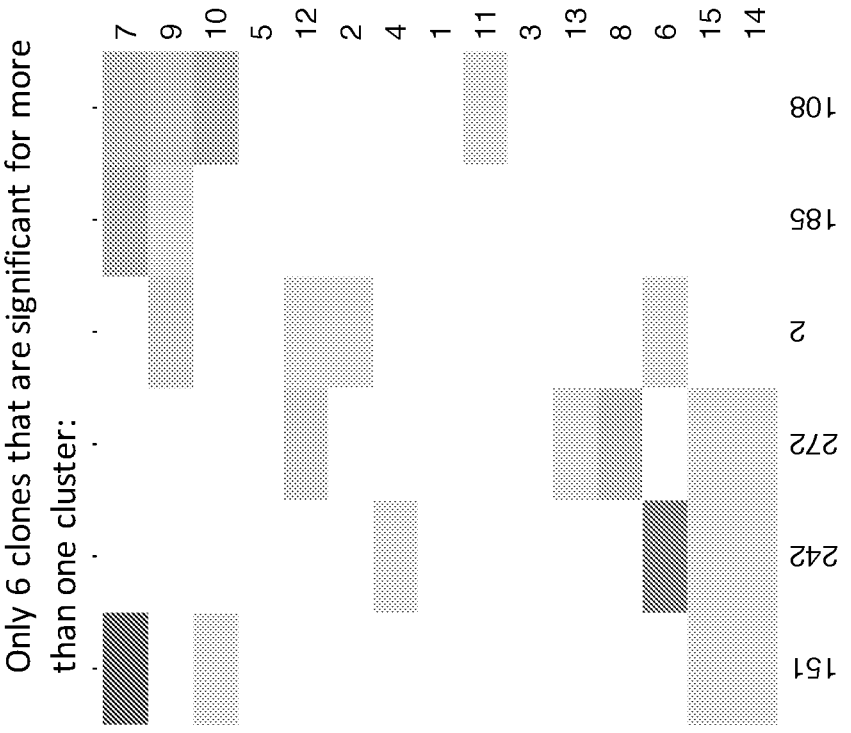

all cells eligible
one measurement per clone
clone_size
clone_relative_expansion
cluster
0
20
40
0.0
0.1
0.2
0.3
0.4
0.5
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15

FIG. 53


Only 6 clones that are significant for more than one cluster:
7
9
10
5
12
2
4
1
11
3
13
8
6
15
14
151
242
272
2
185
108

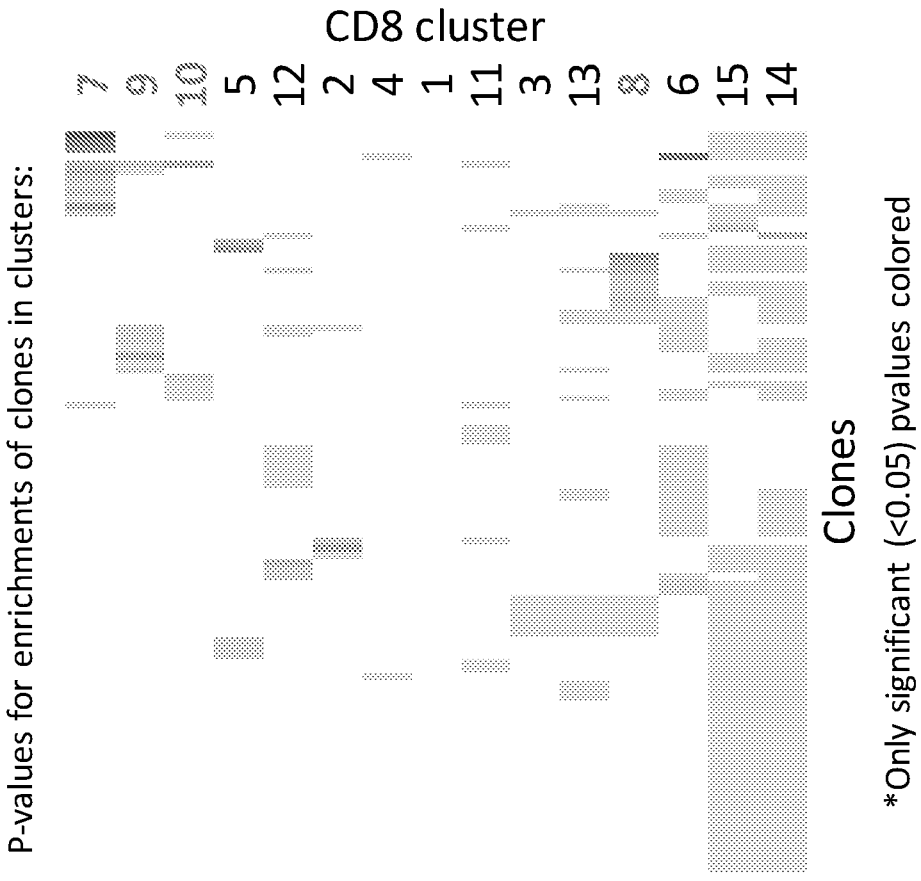

P-values for enrichments of clones in clusters:
CD8 cluster
7
9
10
5
12
2
4
1
11
3
13
8
6
15
14
Clones
*Only significant (<0.05) pvalues colored

FIG. 54

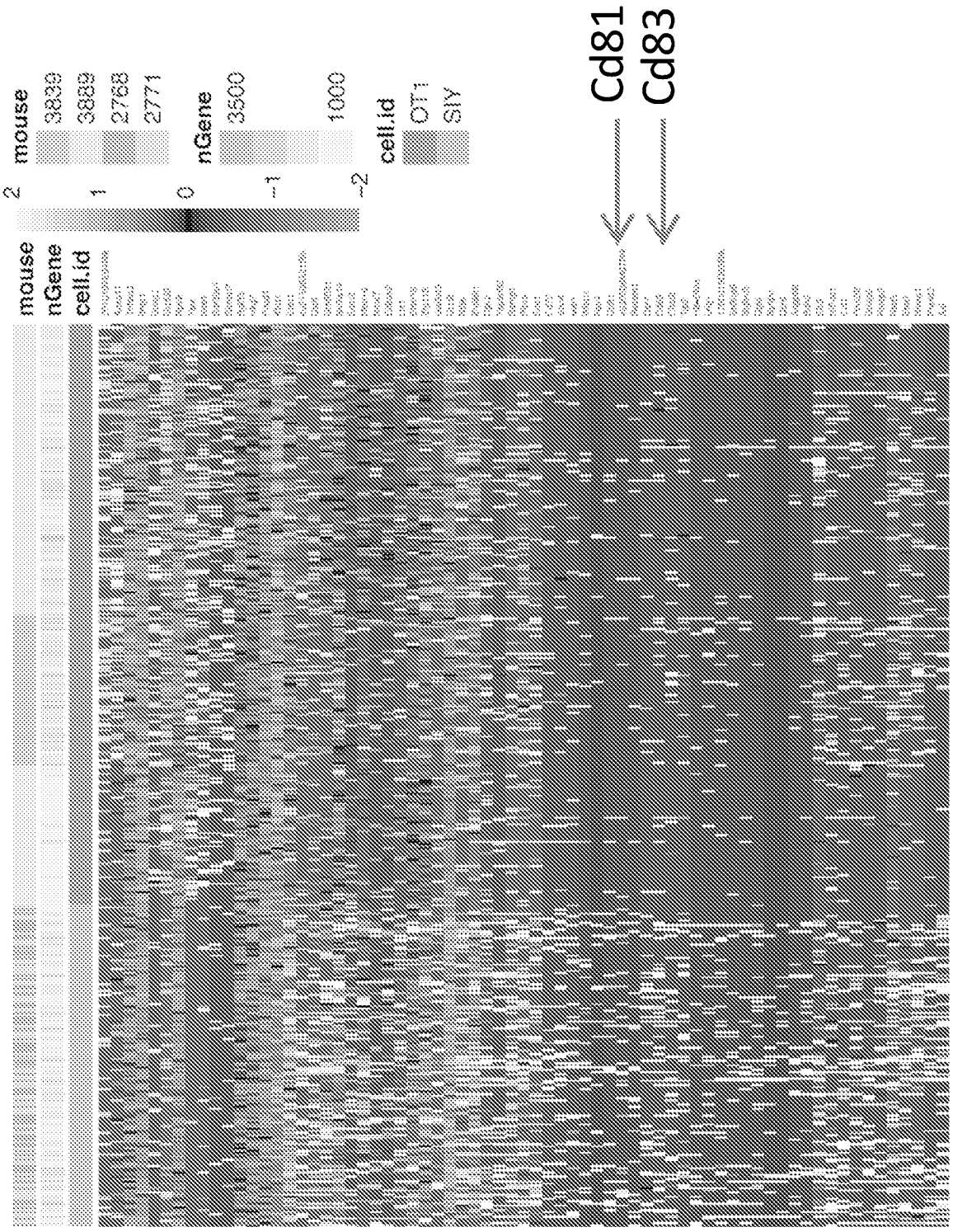

mouse
3839
3889
2768
2771
nGene
3500
1000
cell.id
OT1
SIY
2
1
0
−1
−2
mouse
nGene
cell.id
Cd81
Cd83
SIY-high genes
OVA-high genes

FIG. 55

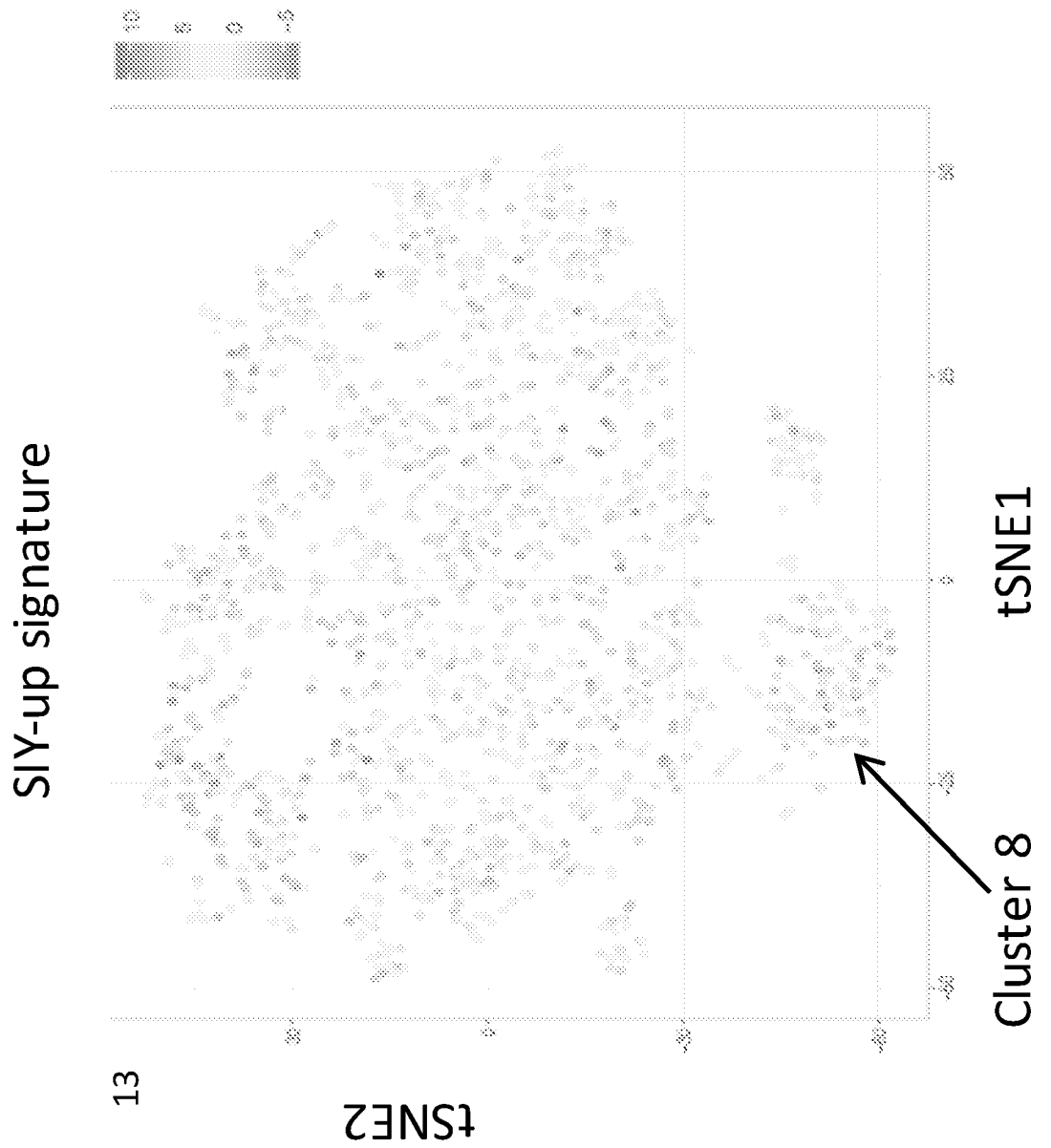
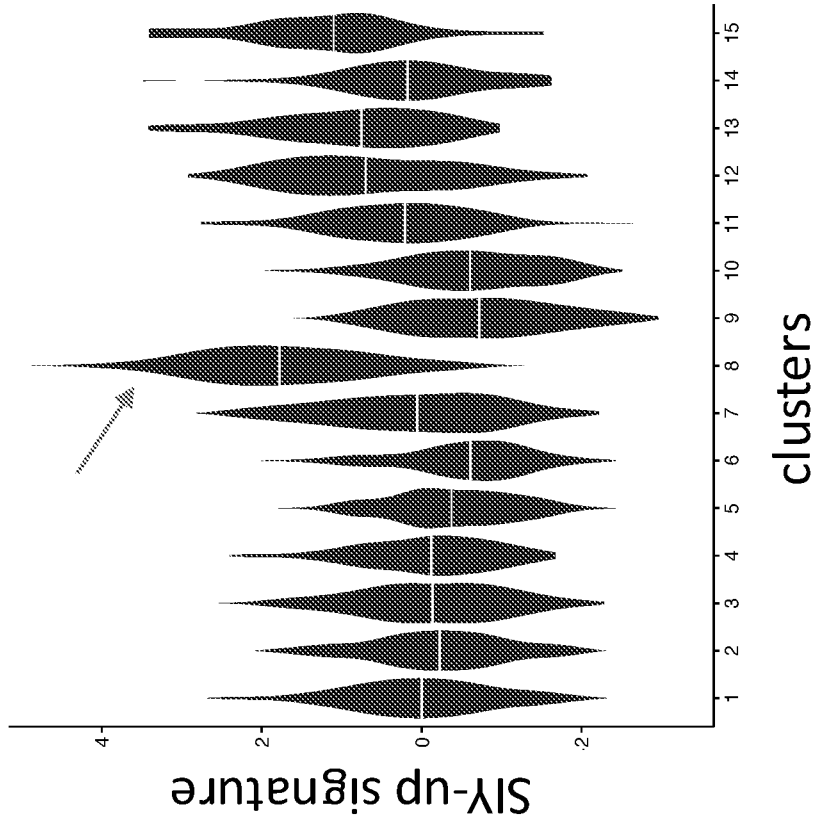


FIG. 56


SIY-up signature
4
2
0
-2
clusters
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15

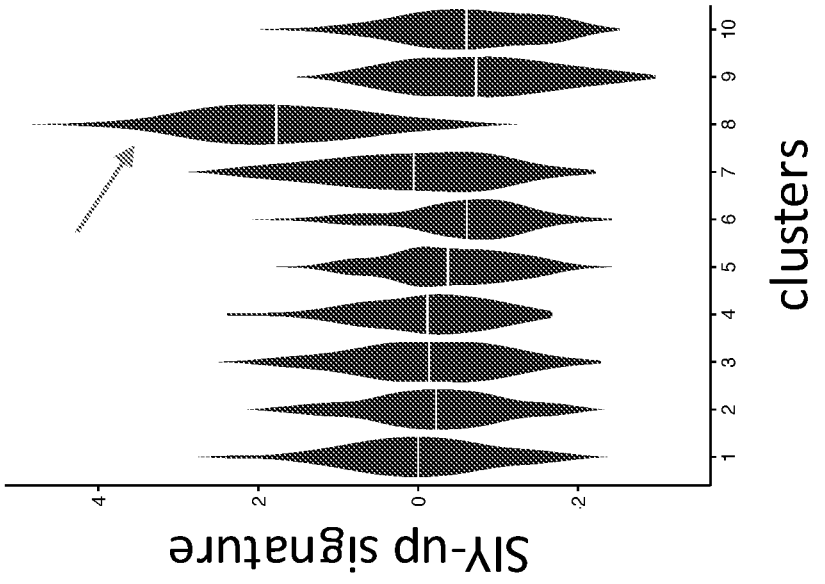

SIY-up signature
4
2
0
-2
clusters
1
2
3
4
5
6
7
8
9
10

FIG. 57

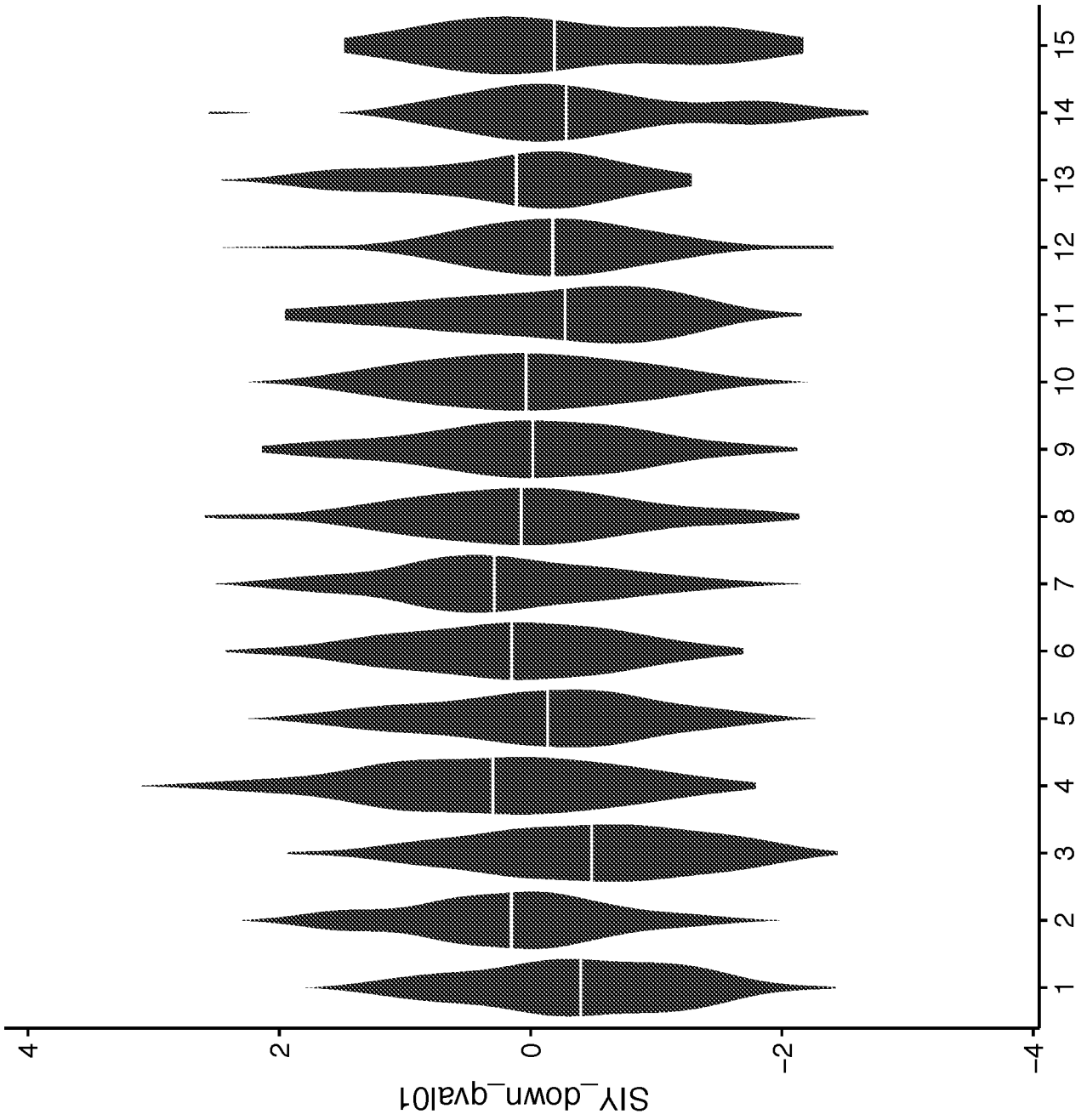


FIG. 58

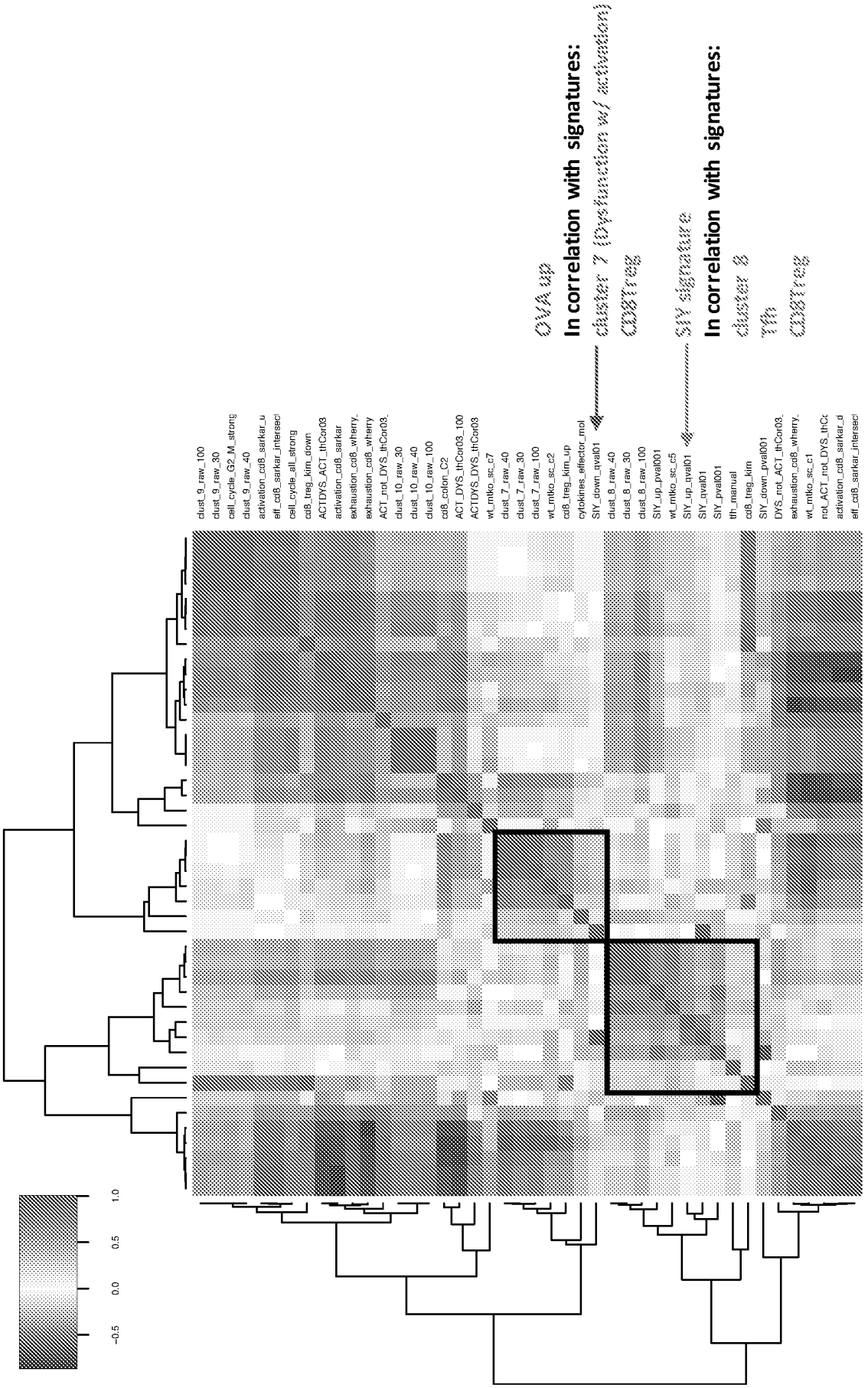

OVA up
In correlation with signatures:
cluster 7 (Dysfunction w/ activation)
CD8Treg
SIV signature
In correlation with signatures:
cluster 8
Tfh
CD8Treg
clust_9_raw_100
clust_9_raw_30
cell_cycle_G2_M_strong
clust_9_raw_40
activation_cd8_sarkar_u
eff_cd8_sarkar_intersect
cell_cycle_all_strong
cd8_treg_kim_down
ACTDYS_ACT_thCor03
activation_cd8_sarkar
exhaustion_cd8_wherry_
exhaustion_cd8_wherry
ACT_not_DYS_thCor03_
clust_10_raw_30
clust_10_raw_40
clust_10_raw_100
cd8_colon_C2
ACT_DYS_thCor03_100
ACTDYS_DYS_thCor03
wt_mko_sc_c7
clust_7_raw_40
clust_7_raw_30
clust_7_raw_100
wt_mko_sc_c2
cd8_treg_kim_up
cytokines_effector_mol
SIV_down_qval01
clust_8_raw_40
clust_8_raw_30
clust_8_raw_100
SIV_up_pval001
wt_mko_sc_c5
SIV_up_qval01
SIV_qval01
SIV_pval001
tfh_manual
cd8_treg_kim
SIV_down_pval001
DYS_not_ACT_thCor03_
exhaustion_cd8_wherry_
wt_mko_sc_c1
not_ACT_not_DYS_thCo
activation_cd8_sarkar_d
eff_cd8_sarkar_intersect
sarkar_intersect_DOWN
vation_cd8_sarkar_down
_not_DYS_thCor03_100
wt_mko_sc_c1
stion_cd8_wherry_down
_not_ACT_thCor03_100
SIV_down_pval001
cd8_treg_kim
tfh_manual
SIV_pval001
SIV_qval01
SIV_up_qval01
wt_mko_sc_c5
SIV_up_pval001
clust_8_raw_100
clust_8_raw_30
clust_8_raw_40
SIV_down_qval01
cytokines_effector_mol
cd8_treg_kim_up
wt_mko_sc_c2
clust_7_raw_100
clust_7_raw_30
clust_7_raw_40
wt_mko_sc_c7
ACTDYS_DYS_thCor03
ACT_DYS_thCor03_100
cd8_colon_C2
clust_10_raw_100
clust_10_raw_40
clust_10_raw_30
_not_DYS_thCor03_100
exhaustion_cd8_wherry
ıaustion_cd8_wherry_up
activation_cd8_sarkar
ACTDYS_ACT_thCor03
cd8_treg_kim_down
cell_cycle_all_strong
db_sarkar_intersect_UP
ctivation_cd8_sarkar_up
clust_9_raw_40
ell_cycle_G2_M_strong
clust_9_raw_30
clust_9_raw_100
1.0
0.5
0.0
−0.5

FIG. 59

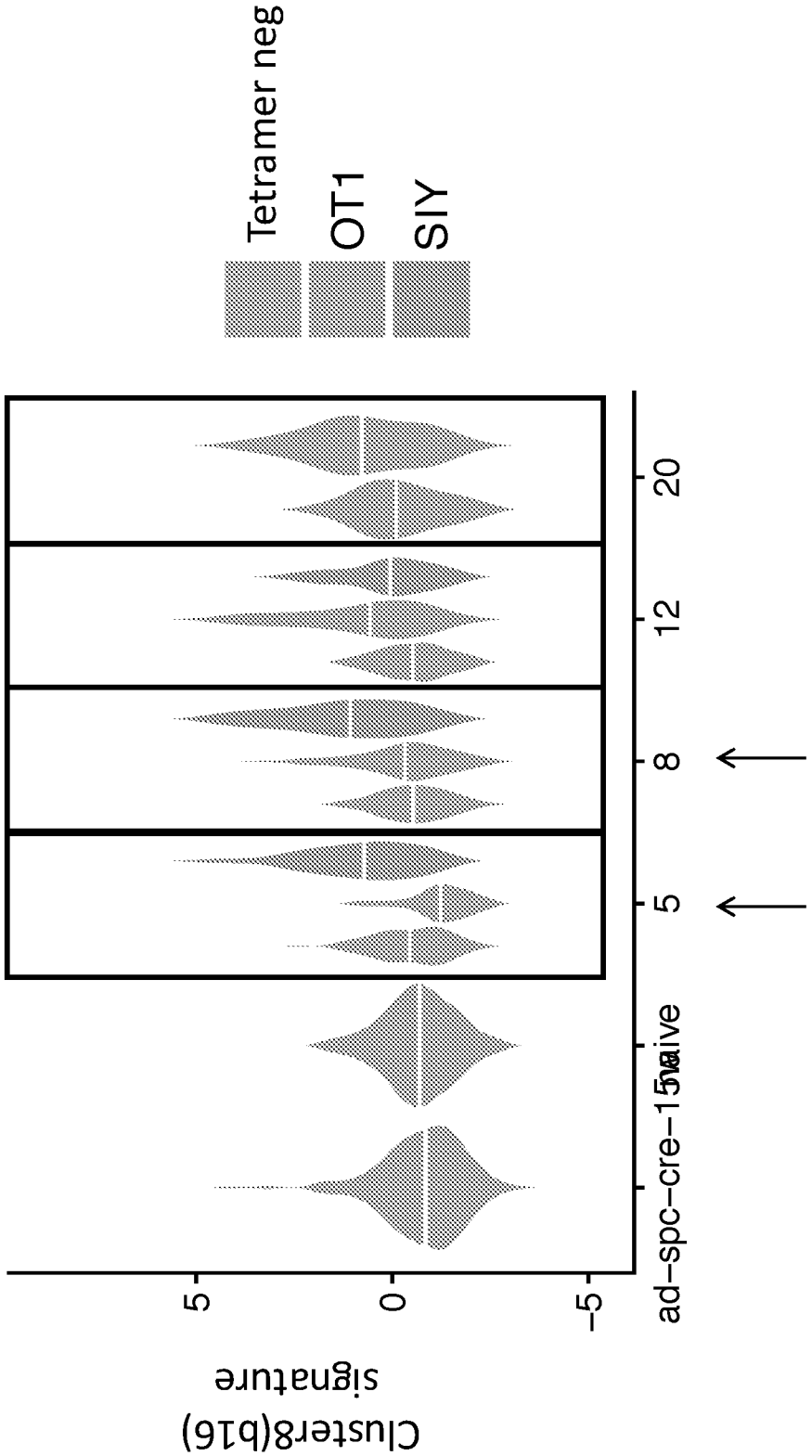

Tetramer neg
OT1
SIY
Cluster8(b16)
signature
5
0
−5
ad−spc−cre−15wk
naive
5
8
12
20

FIG. 60

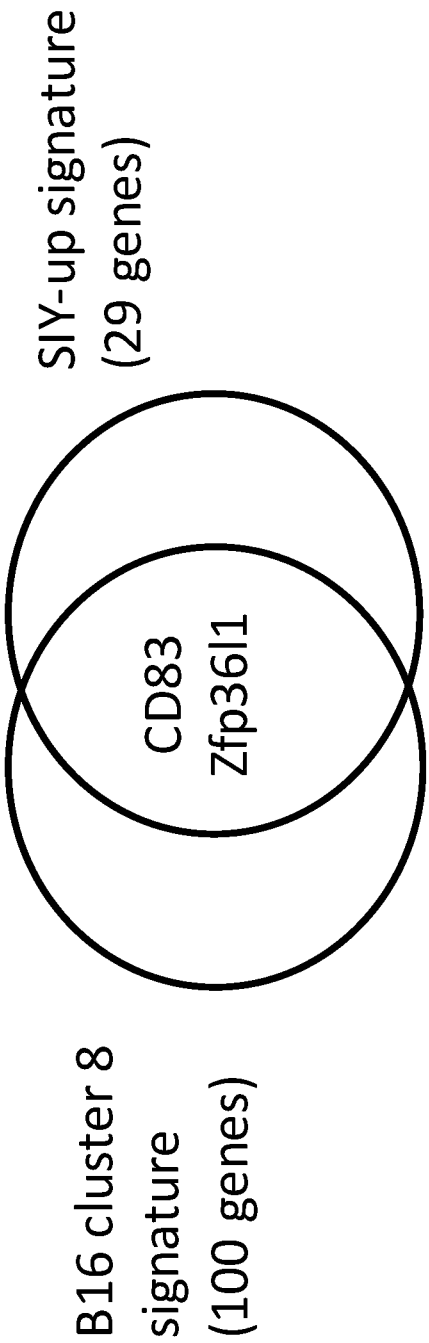

SIY-up signature
(29 genes)
CD83
Zfp36l1
B16 cluster 8
signature
(100 genes)

FIG. 61

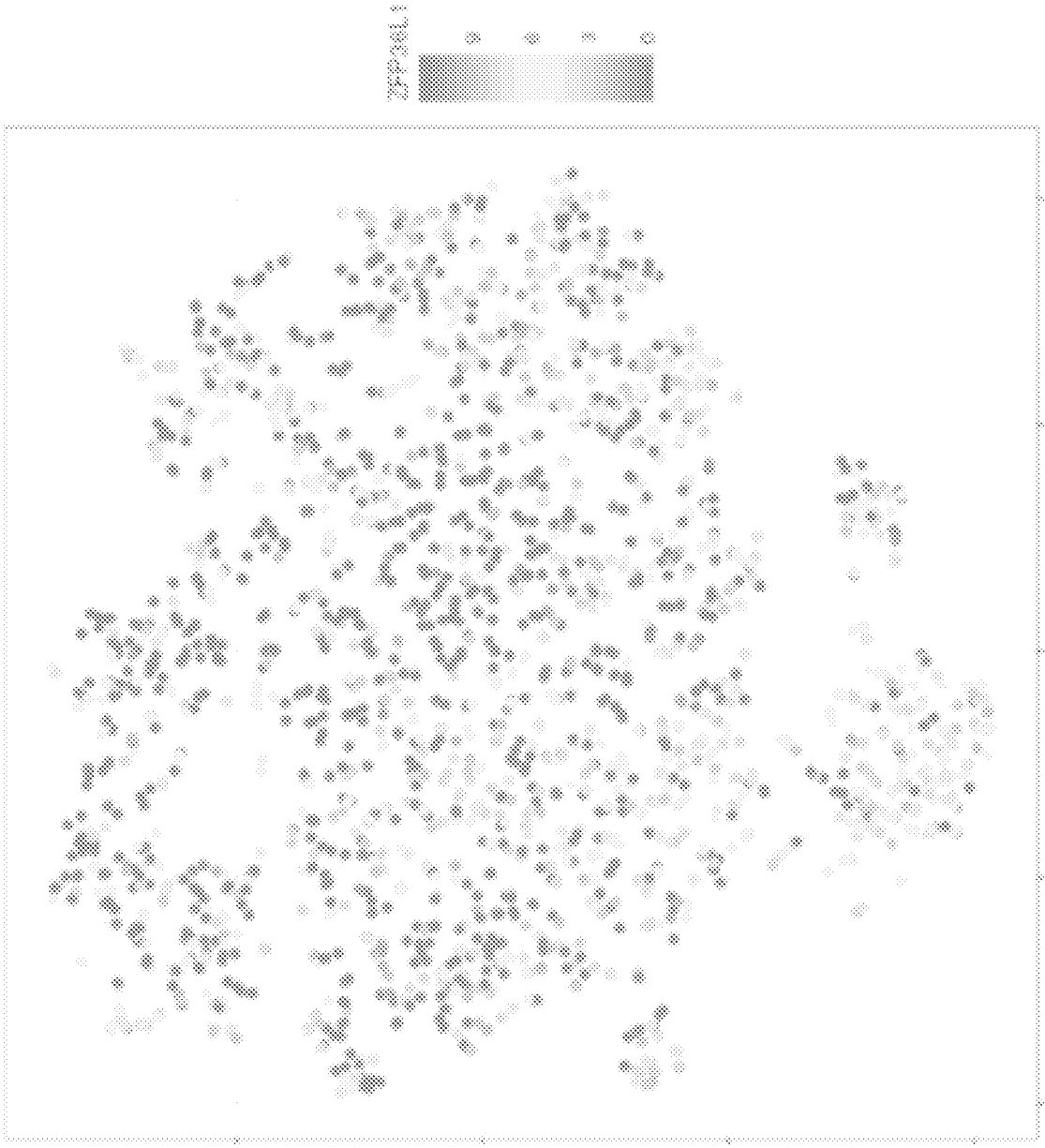


FIG. 62

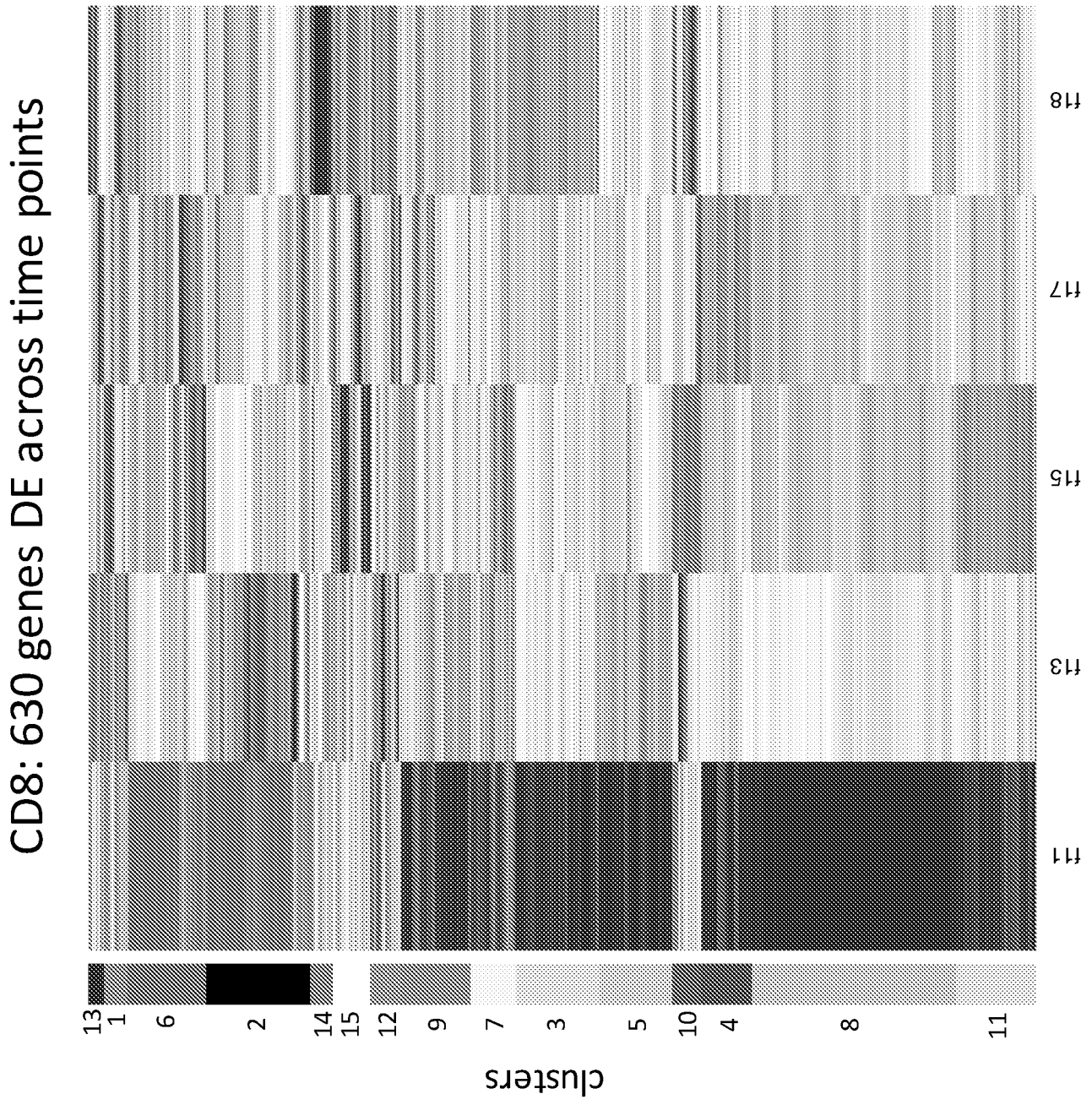

CD8: 630 genes DE across time points
clusters
13
1
6
2
14
15
12
9
7
3
5
10
4
8
11
t1
t3
t5
t7
t8

FIG. 63

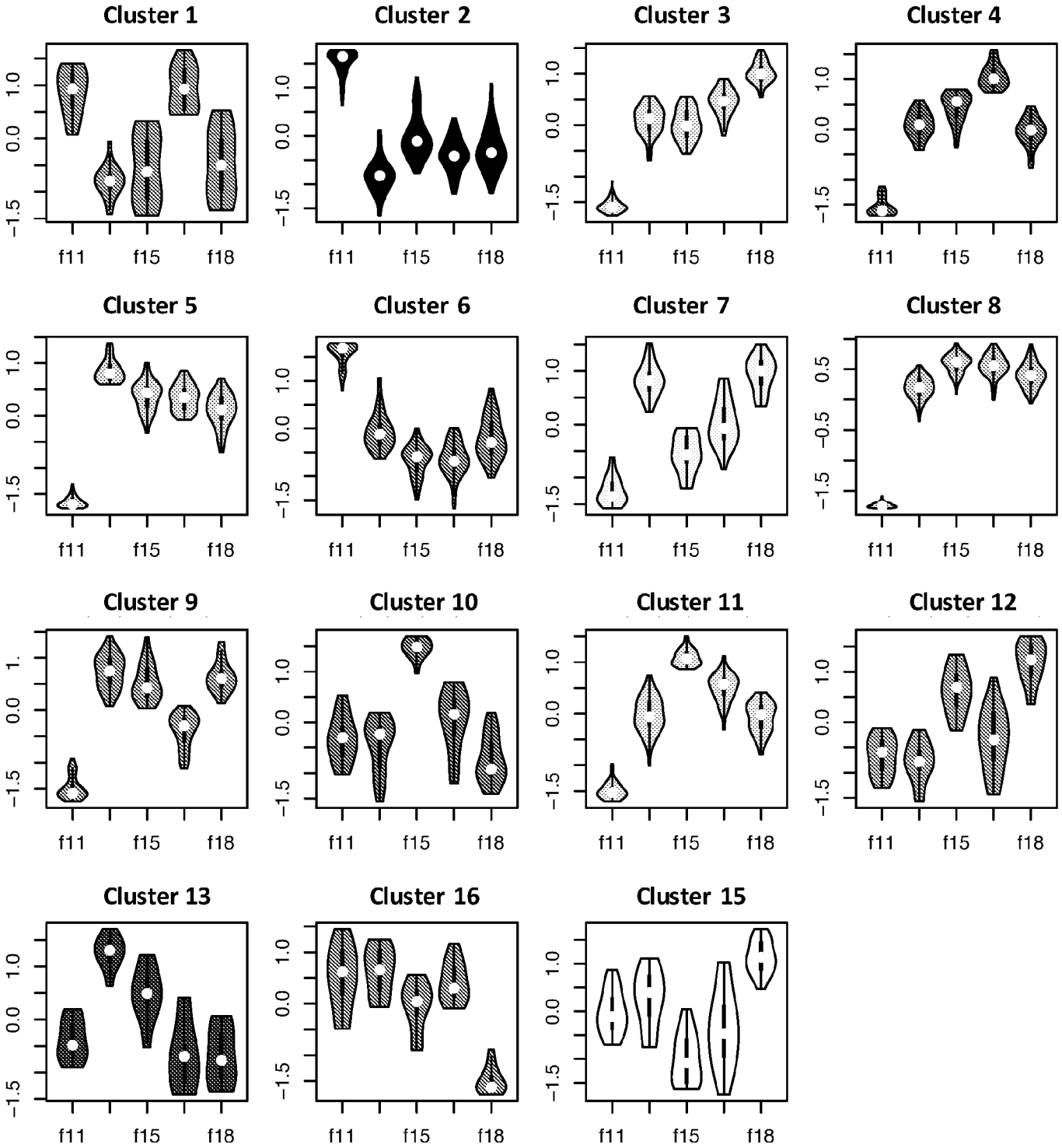

Cluster 1
Cluster 2
Cluster 3
Cluster 4
Cluster 5
Cluster 6
Cluster 7
Cluster 8
Cluster 9
Cluster 10
Cluster 11
Cluster 12
Cluster 13
Cluster 16
Cluster 15
f11
f15
f18

FIG. 64

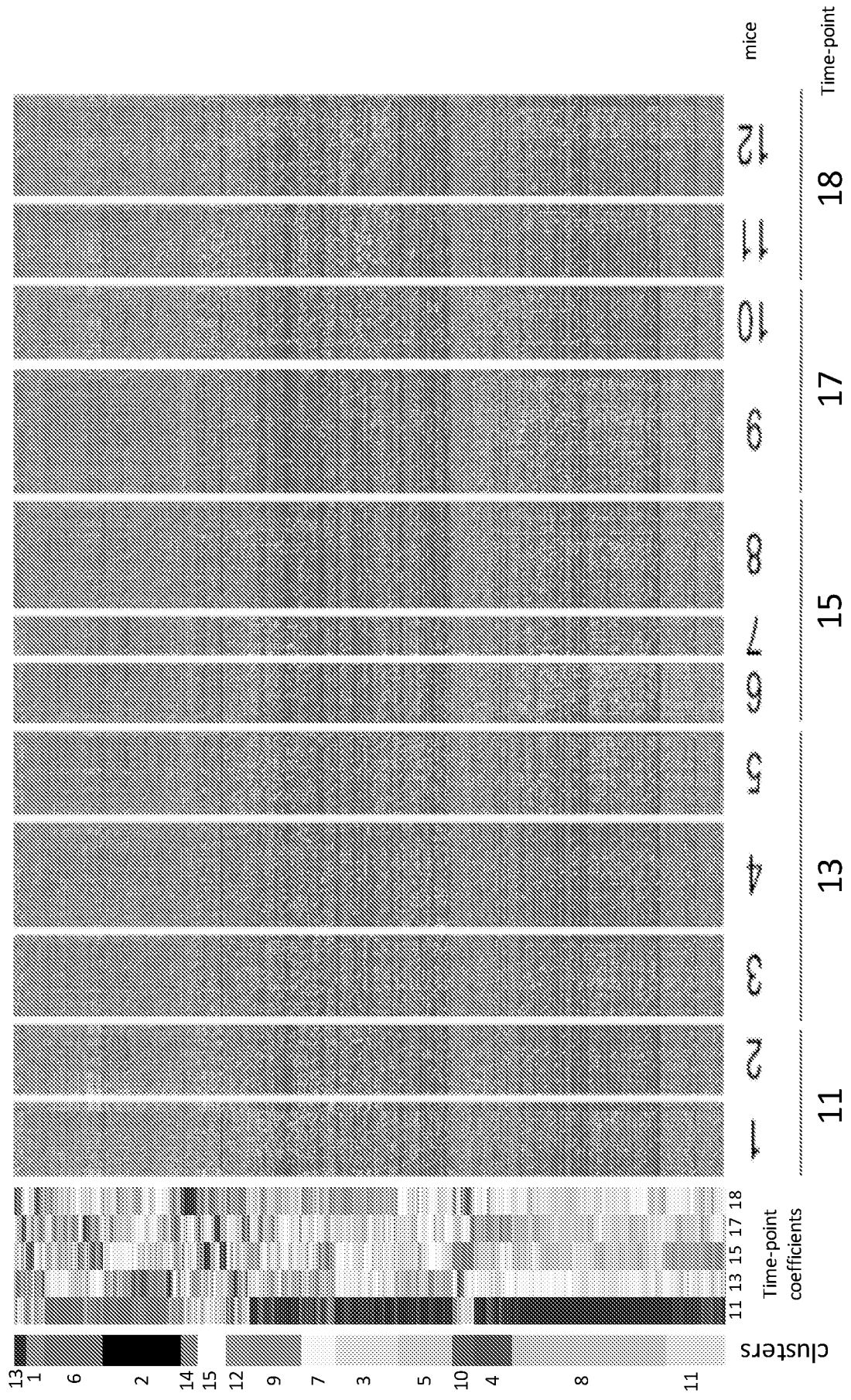

clusters
13
1
6
2
14
15
12
9
7
3
5
10
4
8
11
Time-point coefficients
11 13 15 17 18
mice
1
2
3
4
5
6
7
8
9
10
11
12
Time-point
11
13
15
17
18

FIG. 65

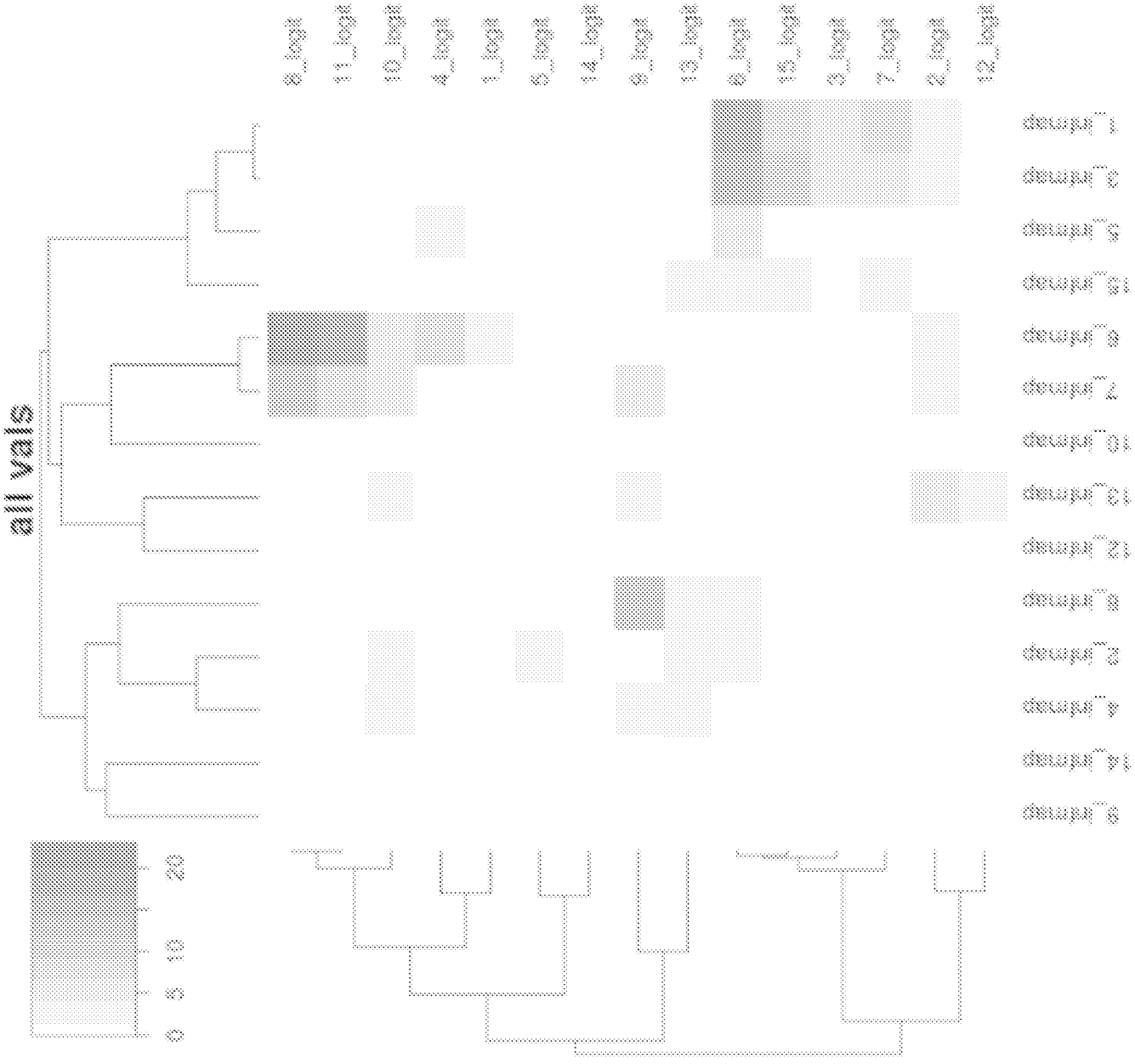

all vals
8_bpt
11_bpt
10_bpt
4_bpt
1_bpt
5_bpt
14_bpt
9_bpt
13_bpt
0_bpt
15_bpt
3_bpt
7_bpt
2_bpt
12_bpt
deup_1
deup_3
deup_5
deup_15
deup_6
deup_7
deup_10
deup_13
deup_12
deup_11
deup_2
deup_4
deup_14
deup_9
0
5
10
20

FIG. 66

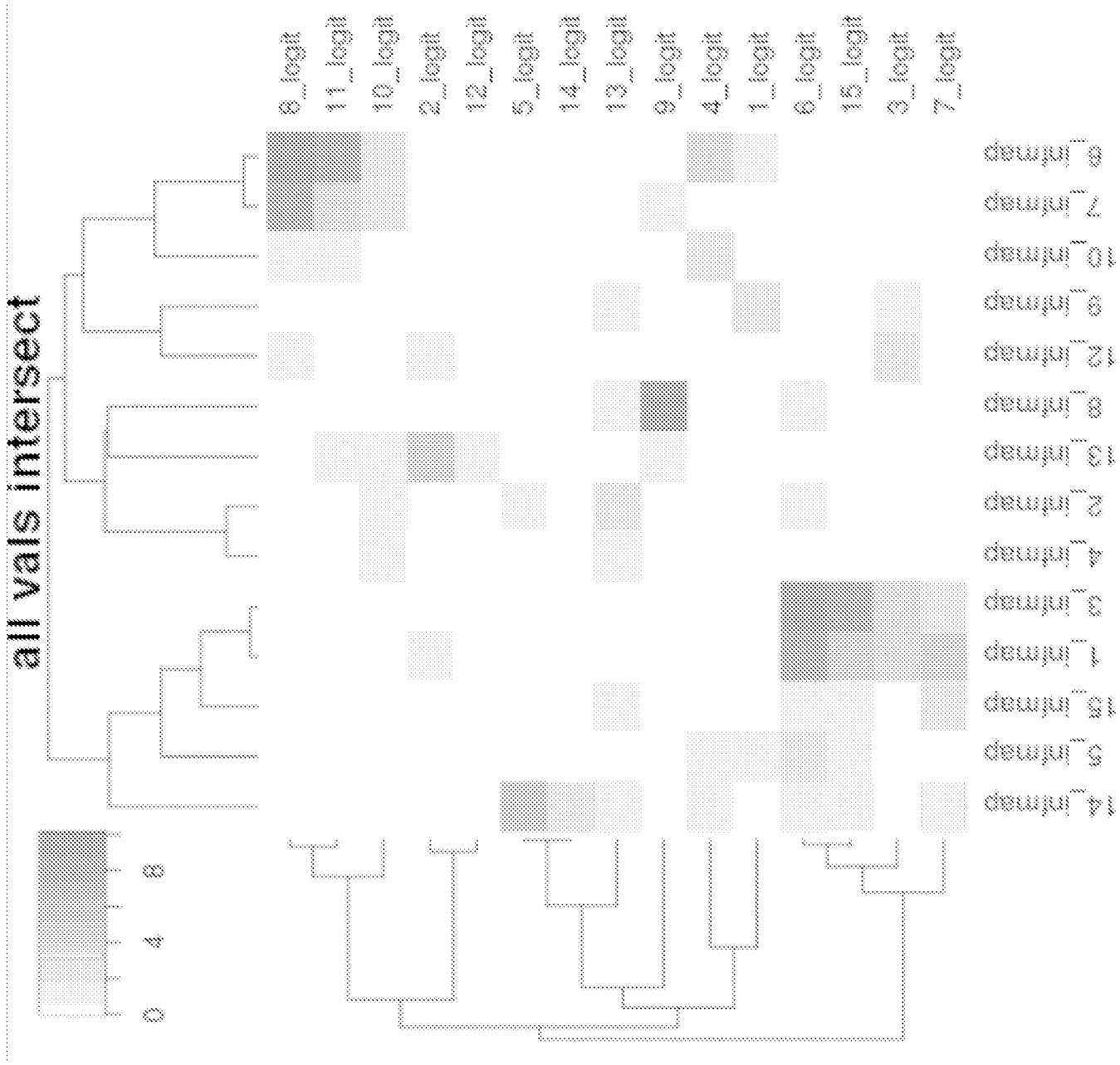

all vals intersect
0
4
8
0_logit
11_logit
10_logit
2_logit
12_logit
5_logit
14_logit
13_logit
9_logit
4_logit
1_logit
6_logit
15_logit
3_logit
7_logit
6_inmap
7_inmap
10_inmap
9_inmap
12_inmap
8_inmap
13_inmap
2_inmap
4_inmap
3_inmap
1_inmap
15_inmap
5_inmap
14_inmap

FIG. 67

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US18/61812

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. ☒ forming part of the international application as filed:

      ☒ in the form of an Annex C/ST.25 text file.

      ☐ on paper or in the form of an image file.

   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. ☐ furnished subsequent to the international filing date for the purposes of international search only:

      ☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US18/61812

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 14-18, 21-28
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
-***-Please See within the next Supplemental Page-***-

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
Group 1+ Claims 1, 13, 19, 29; and GLDC (gene)

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US18/61812

A. CLASSIFICATION OF SUBJECT MATTER

IPC - C12N 5/0783, 15/09; C12Q 1/6809 (2019.01)

CPC - C12N 5/0783, 15/09; C12Q 1/6809

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

See Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2015/0309048 A1 (INSERM et al.) 29 October, 2015; abstract; paragraph [0007]	1, 13/1, 19/1, 29/1
A	WO 2017/075478 A2 (THE BROAD INSTITUTE INC. et al.) 04 May, 2017; paragraph [00157]	1, 13/1, 19/1, 29/1
A	WO 2017/147196 A1 (MASSACHUSETTS INSTITUTE OF TECHNOLOGY et al.) 31 August, 2017; paragraph [00271]; table 34	1, 13/1, 19/1, 29/1
A	(KIM, SK et al.) Differential Expression of Enzymes Associated with Serine/Glycine Metabolism in Different Breast Cancer Subtypes. PLoS One. 30 June, 2014; pages 1-14; abstract; figures 1, 3; page 6, column 1, paragraph 4 – column 2, paragraph 1; DOI: 10.1371/journal.pone.0101004	1, 13/1, 19/1, 29/1

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
25 February 2019 (25.02.2019)	14 MAR 2019

Name and mailing address of the ISA/	Authorized officer
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/US18/61812

-***-Continued from Box III Observations where unity of invention is lacking -***-

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Groups I+, Claims 1-13, 19, 20, 29, 30; and GLDC (gene) are directed toward a method for detecting or quantifying CD8+ T cells in a biological sample of a subject; isolated CD8+ T cells; and a kit therefor.

The isolated CD8+ T cells, method and kit will be searched to the extent they encompass GLDC (gene). Applicant is invited to elect additional gene(s) to be searched. Additional gene(s) will be searched upon the payment of additional fees. It is believed that claims 1 (in-part), 13 (in-part), 19 (in-part), and 29 (in-part) encompass this first named invention and thus these claims will be searched without fee to the extent that they encompass GLDC (gene). Applicants must specify the claims that encompass any additionally elected gene (s). Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined. An exemplary election would be TNFRSF9 (gene).

No technical features are shared between the genes of Groups I+ and, accordingly, these groups lack unity a priori.

Additionally, even if Groups I+ were considered to share the technical features including: an isolated CD8+ T cell characterized in that the CD8+ T cell comprises expression of a gene signature comprising one or more genes; a method for detecting or quantifying CD8+ T cells in a biological sample of a subject, or for isolating CD8+ T cells from a biological sample of a subject, the method comprising detecting or quantifying in a biological sample of the subject CD8+ T cells, or isolating from the biological sample CD8+ T cells; and a kit comprising reagents to detect at least one gene or polypeptide; these shared technical features are previously disclosed by US 2015/0309048 A1 to INSERM et al. (hereinafter 'INSERM').

INSERM discloses an isolated CD8+ T cell (an isolated CD8+ T cell; abstract, paragraph [0007]), characterized in that the CD8+ T cell comprises expression of a gene signature comprising one or more genes (characterized in that the CD8+ T cell comprises expression of a gene signature comprising one or more genes; abstract, paragraph [0007]); a method for detecting or quantifying CD8+ T cells in a biological sample of a subject (a method for detecting or quantifying CD8+ T cells in a biological sample of a subject; paragraph [0007]), the method comprising detecting or quantifying in a biological sample of the subject CD8+ T cells, or isolating from the biological sample CD8+ T cells (the method comprising detecting or quantifying in a biological sample of the subject CD8+ T cells, or isolating from the biological sample CD8+ T cells; paragraph [0007]); and a kit comprising reagents to detect at least one gene or polypeptide (a kit comprising reagents to detect at least one gene or polypeptide; paragraph [0050]).

Since none of the special technical features of the Groups I+ inventions is found in more than one of the inventions, and since all of the shared technical features are previously disclosed by the INSERM reference, unity of invention is lacking.