

The gut has a complex microbial community (microbiome)

Dominant gut phyla:

Bacteroidetes, Firmicutes, Actinobacteria, Proteobacteria, Verrucomicrobia

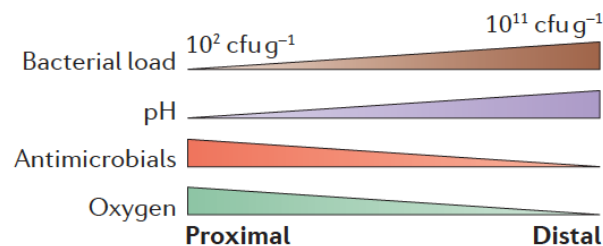
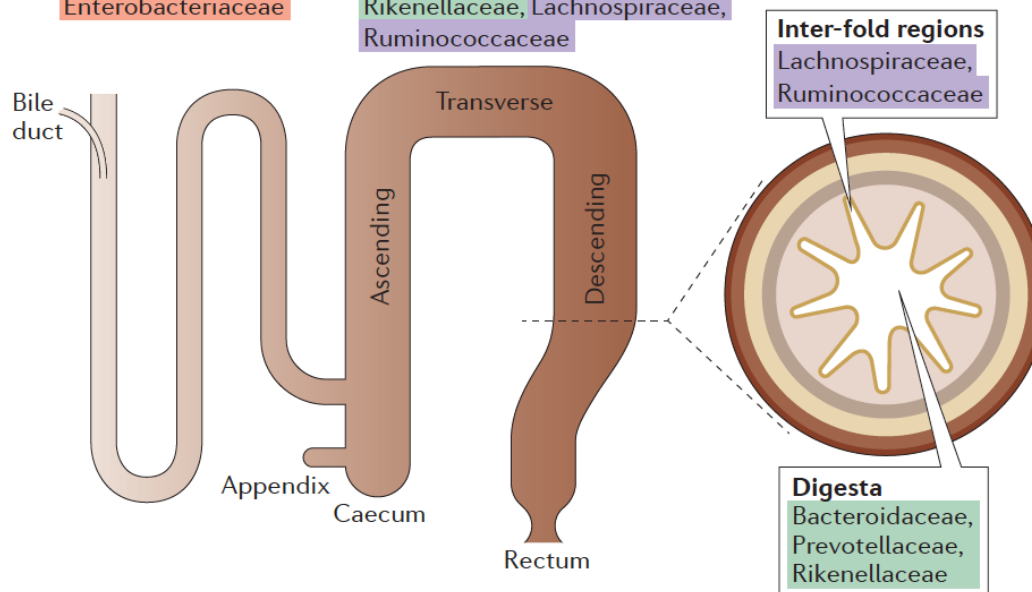
Predominant families in the:

Small intestine

Lactobacillaceae,
Enterobacteriaceae

Colon

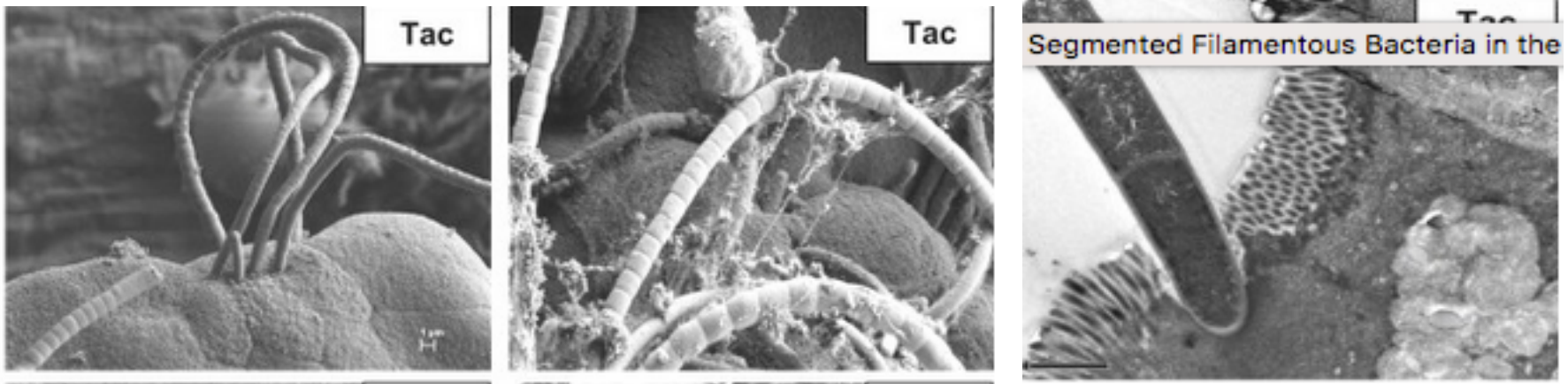
Bacteroidaceae, Prevotellaceae,
Rikenellaceae, Lachnospiraceae,
Ruminococcaceae



The gut has a complex microbial community (microbiome)



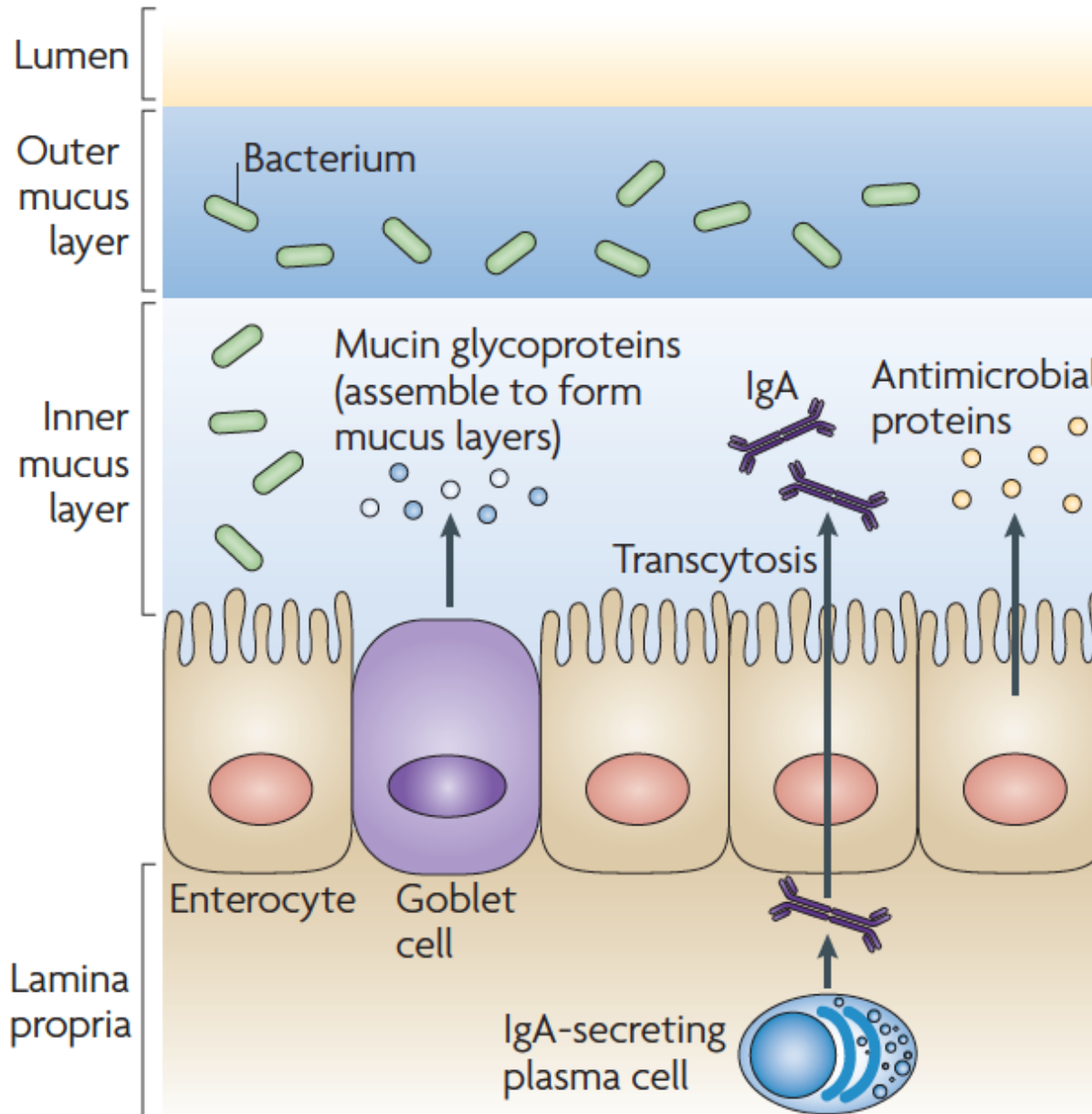
The gut has a complex microbial community (microbiome)



Mice from Taconic but not mice from Jackson have Segmented Filamentous Bacterium (SFB), which attaches itself to the epithelium in the small intestines and grows as a chain.

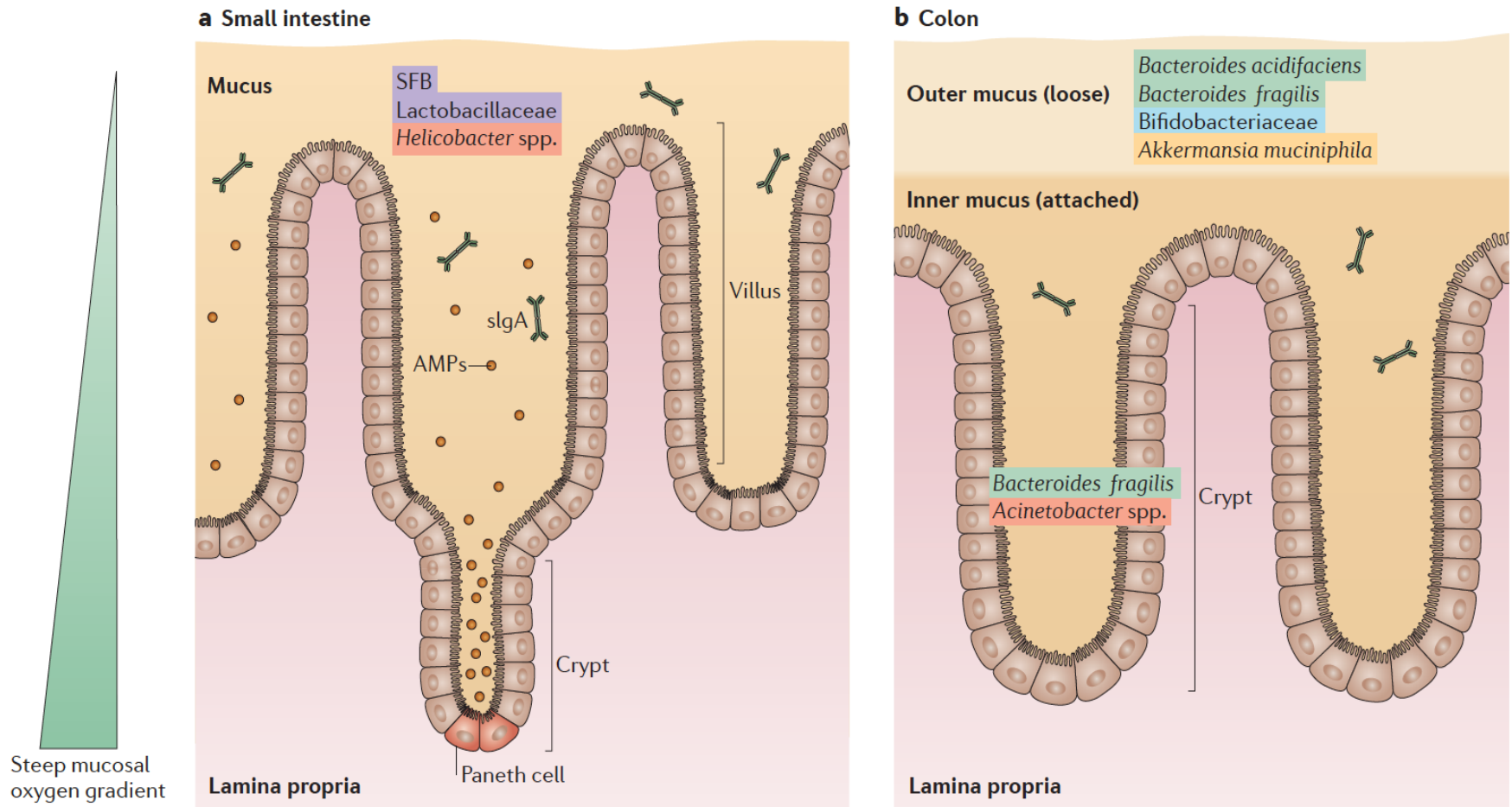
Ivanov et al, Cell 139: 485, 2009.

Immune control of gut microbes



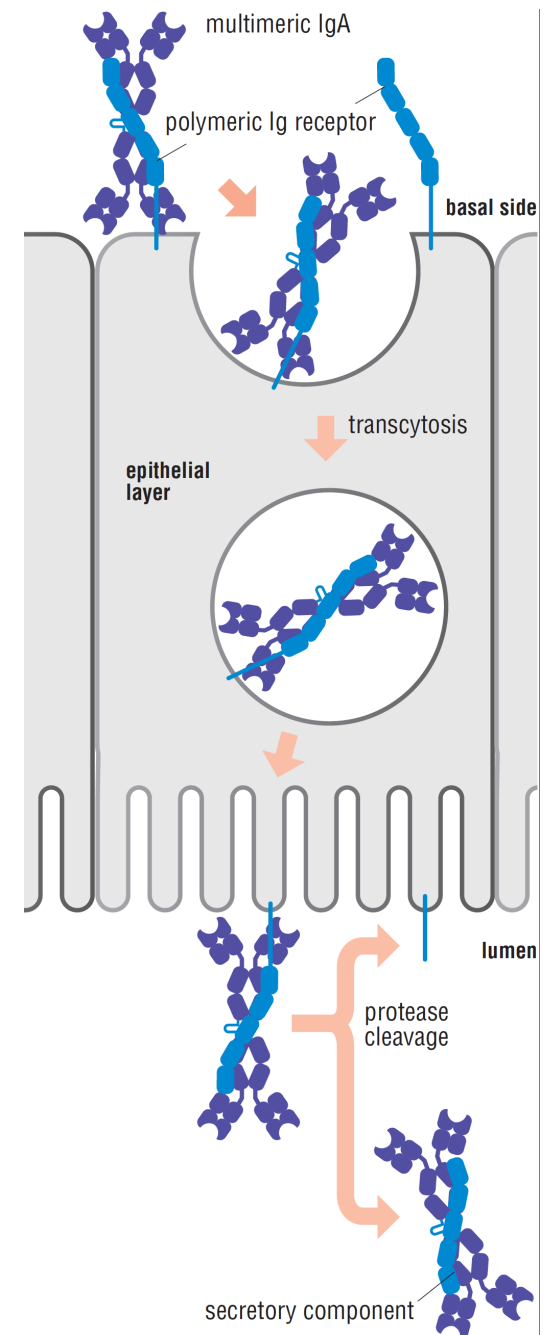
Hooper and MacPherson,
Nature Rev
Immunol. 10:
159, 2010

Immune control of gut microbes

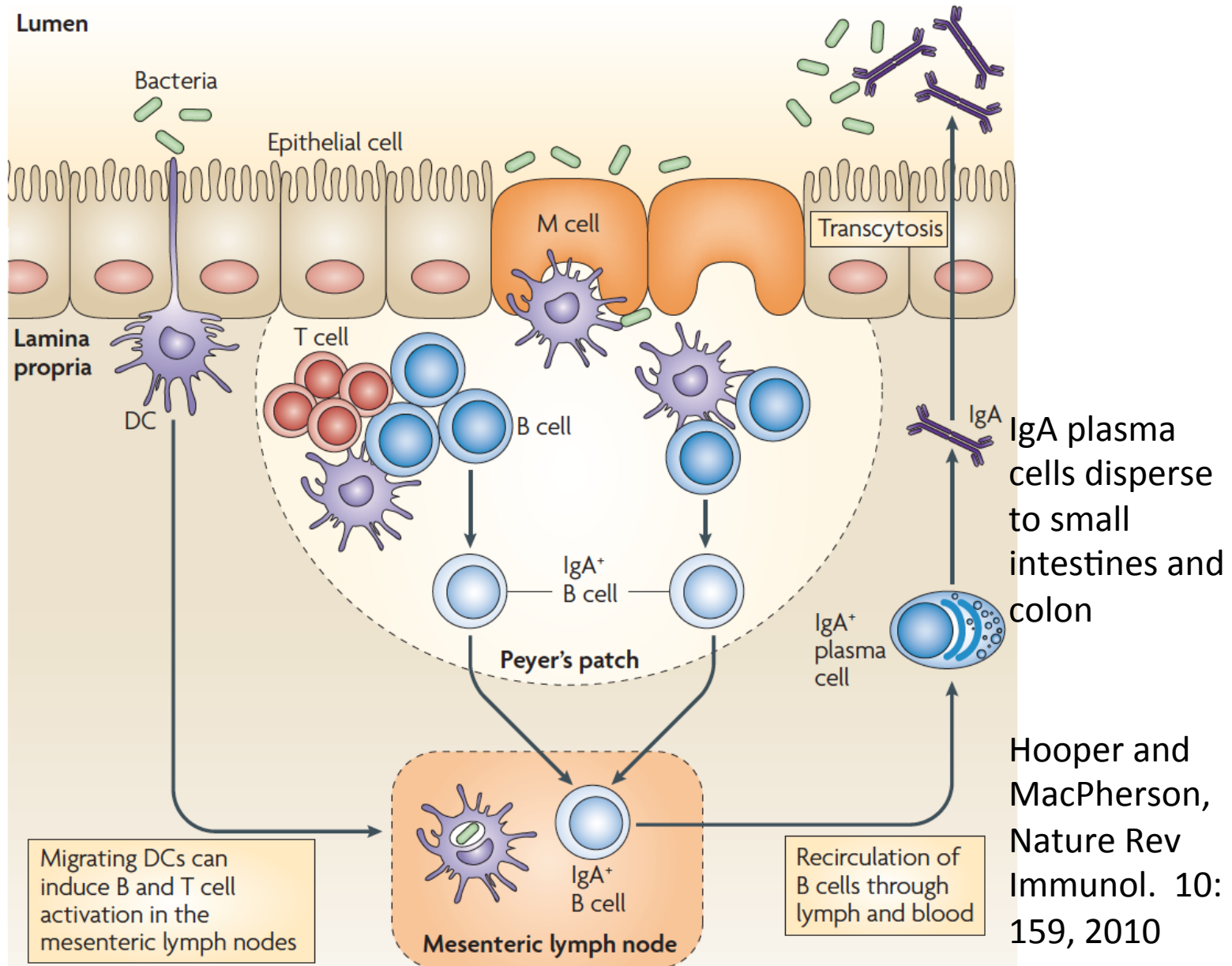


IgA Background

- IgA is secreted in mucosal tissue and is transported across mucosal epithelial barriers by the poly-Ig receptor
- Poly-Ig receptor is cleaved; the part that stays bound to IgA is “secretory component”



IgA in small intestines

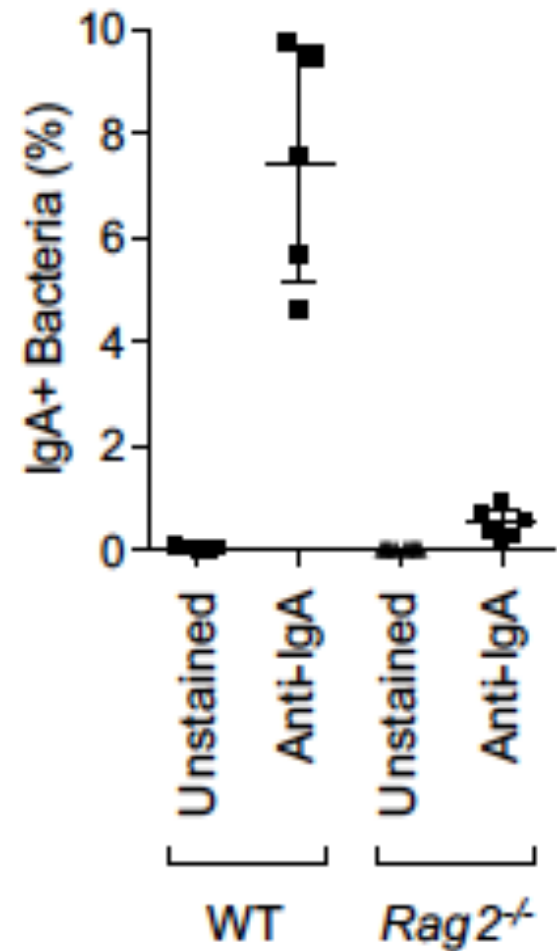
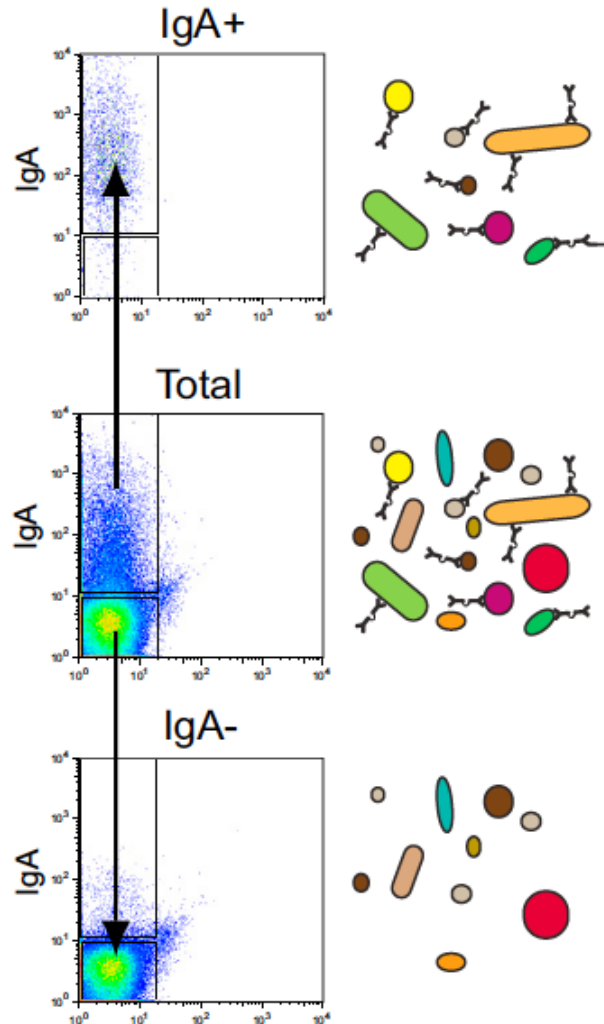
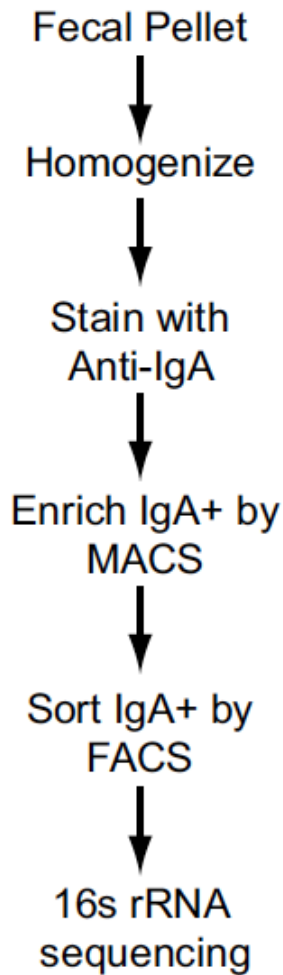


IgA Background

- Some IgA is T cell-dependent; some IgA is T cell-independent
- Some IgA is produced in germinal center responses and exhibits somatic mutation
- Deep sequencing of IgA reveals a diverse response that includes a few specificities produced abundantly
- Gut bacteria that have IgA on them can be revealed by staining with a fluorescent anti-IgA and using flow cytometry and cell sorting followed by sequencing of 16S rRNA

Sorting IgA-coated bacteria

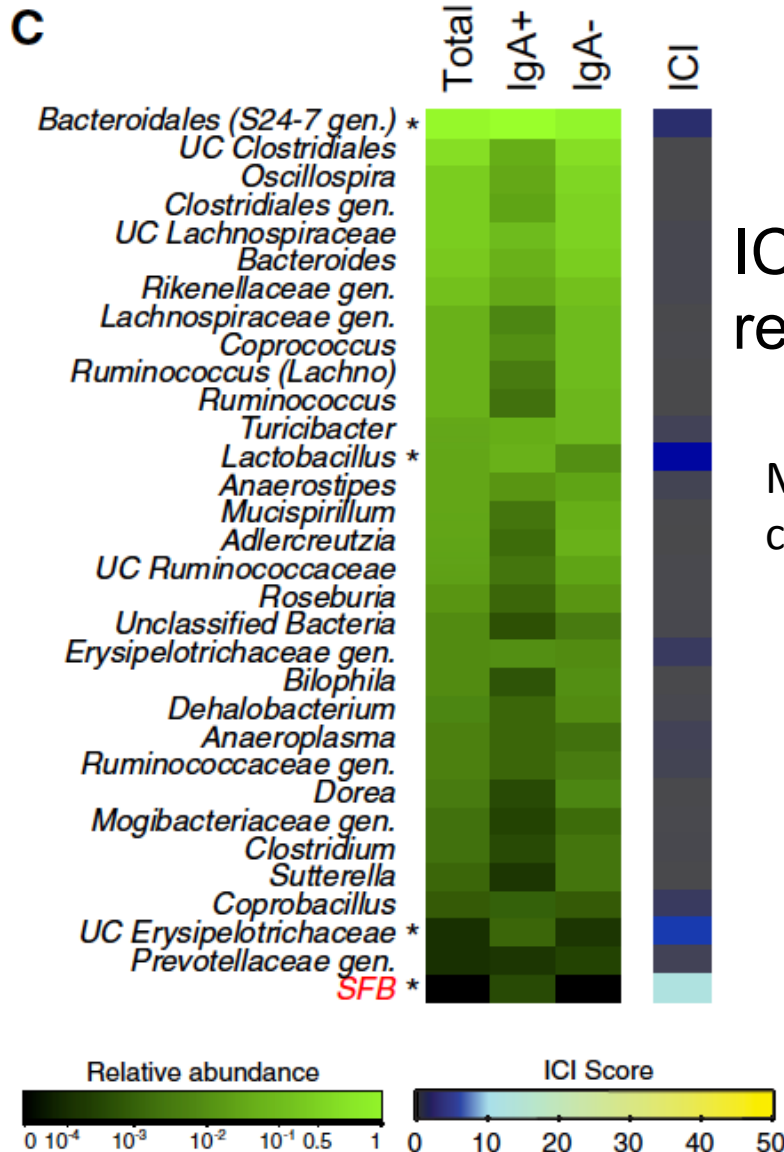
B



C

MICE

Question 1: What about gut bacteria induces an IgA response?



ICI: relative abundance IgA+/
relative abundance IgA-

Mice: 4 taxons enriched in IgA+
coated; 22 taxons enriched in IgA-

Palm et al. Cell
158: 1000-10, 2014

Conclusion: bugs differ in what
proportion is coated with IgA or
not coated

IgA Question 1: What about gut bacteria induces an IgA response?

Formulate **two or three** hypotheses and come up with **one** experimental approach to address one or more of these hypotheses

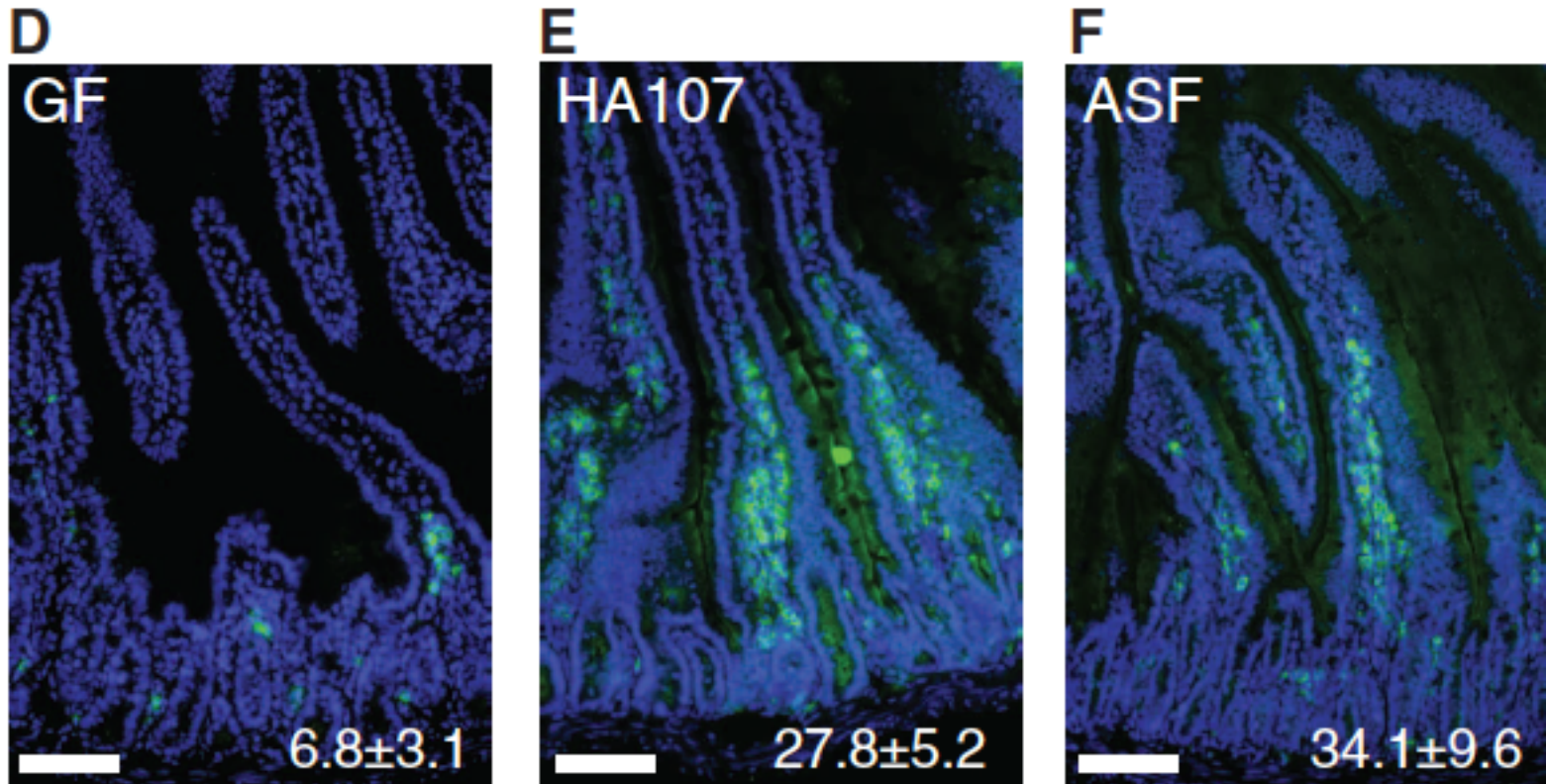
Tools to consider:

- Flow cytometry to identify bugs that are coated with IgA or not coated (and relative proportions)
- Introduction of individual species or sets of species into germ free mice
- Mice or people with diseases, immunodeficiencies, and/or different diets

Question 1: What about gut bacteria induces an IgA response?

Hypothesis one: amount of antigen available to the immune system.

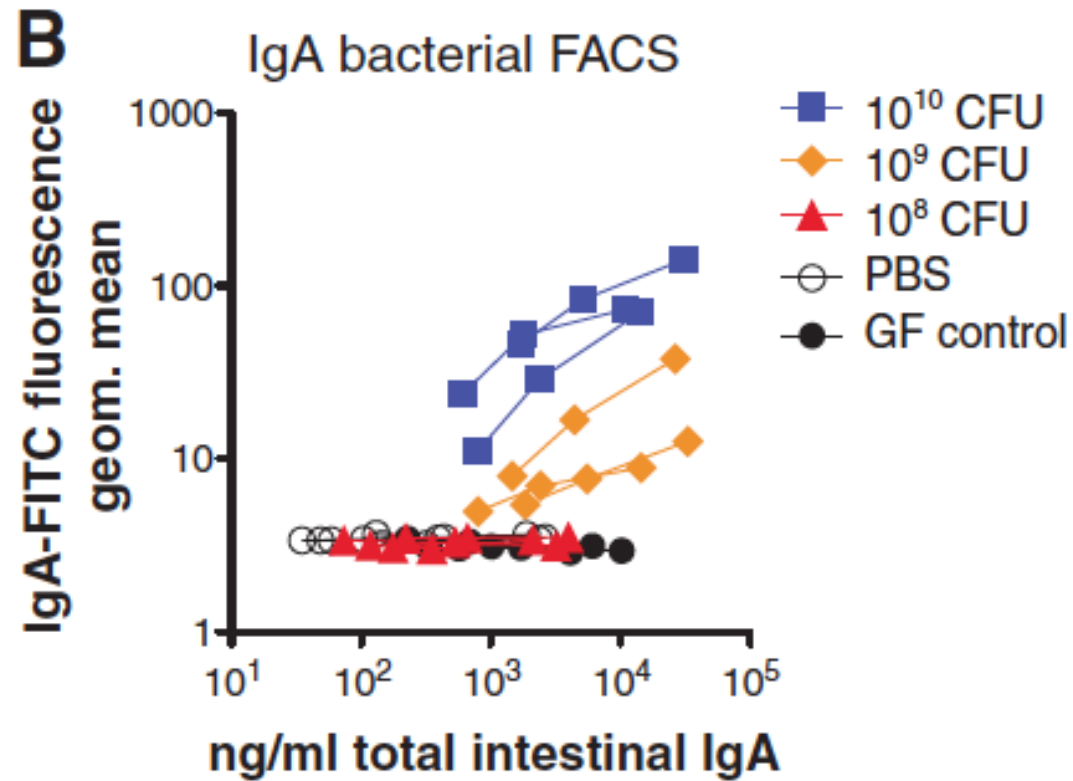
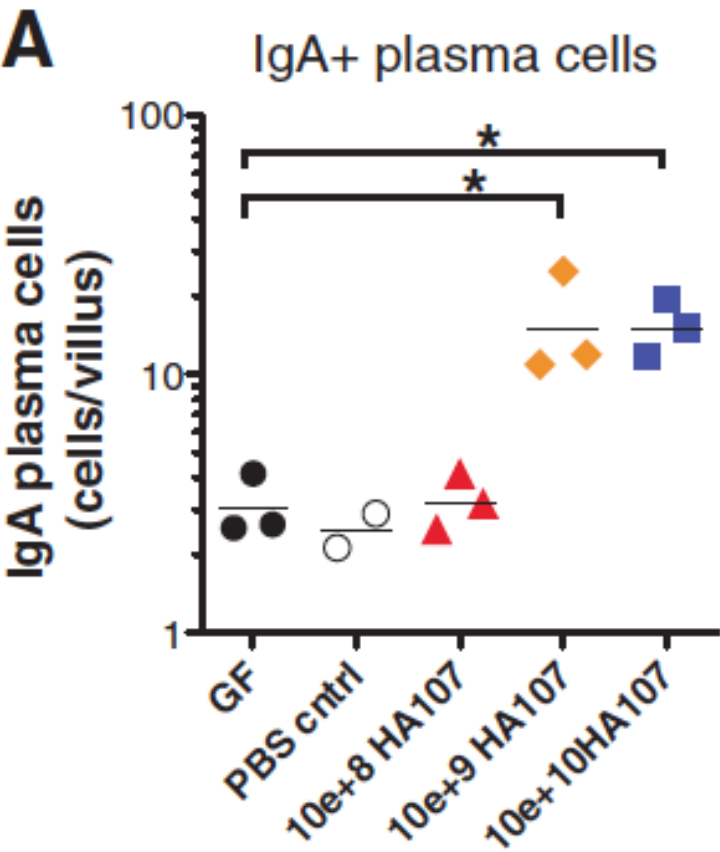
Most IgA plasma cells in gut are dependent on gut bacteria (B6 mice)



Hapfelmeier et al. Science 328: 1705-9, 2010

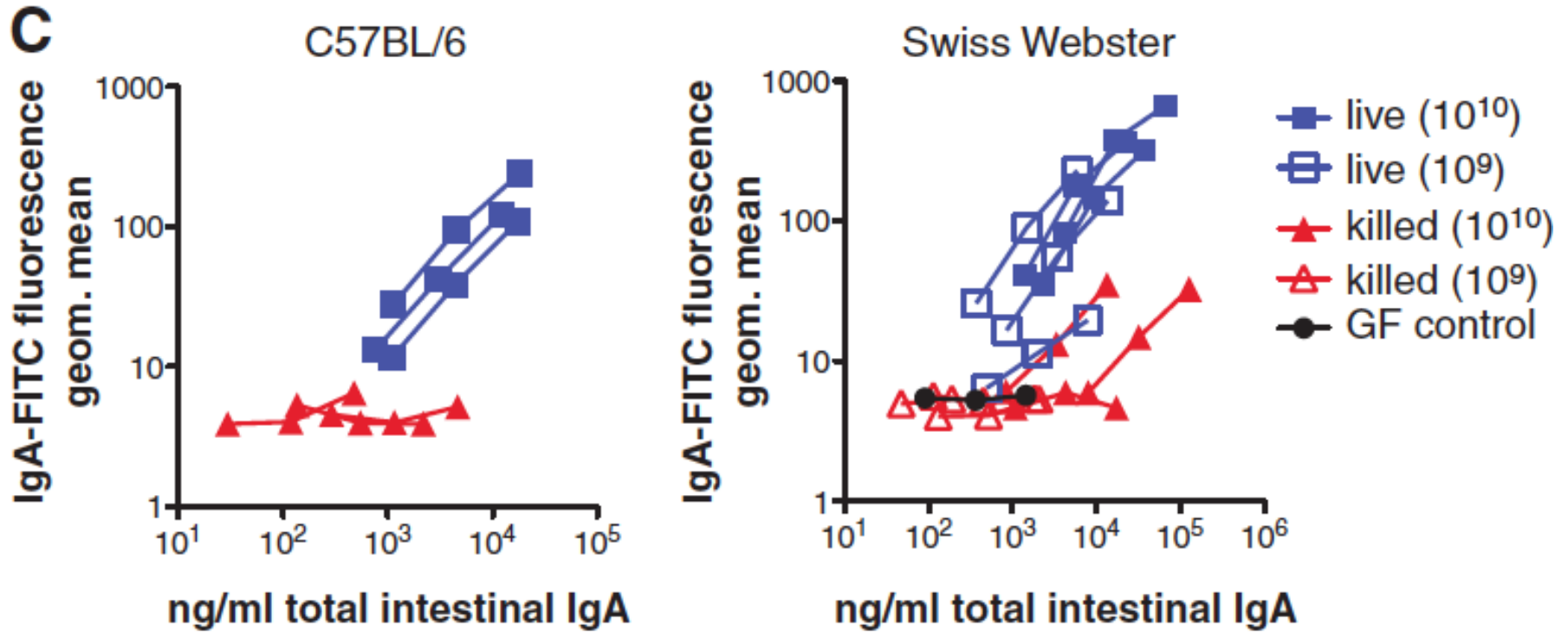
HA107 gavaged 6 times over 14 days; IgA plasma cells (green) evaluated after 4 weeks total (blue: DAPI staining of nuclei) (ASF: altered Schaedler flora, a mixture of 8 mouse gut bacteria that creates a relatively stable community)

Threshold for IgA response to E coli K-12



Hapfelmeier et al.
Science 328: 1705-9, 2010

Question 1: What about gut bacteria induces an IgA response?



Hapfelmeier et al. Science 328: 1705-9, 2010

Live bugs induce IgA >10X better than heat-killed bugs

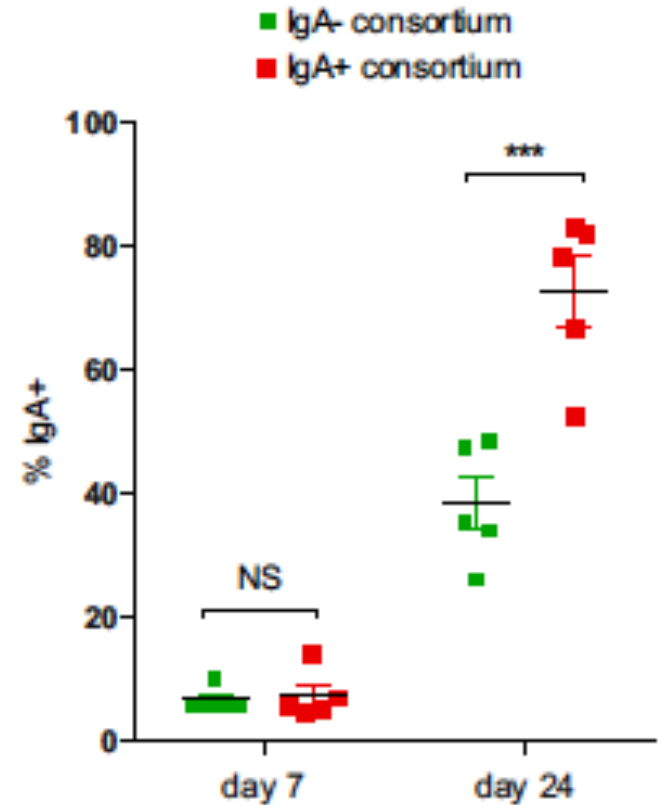
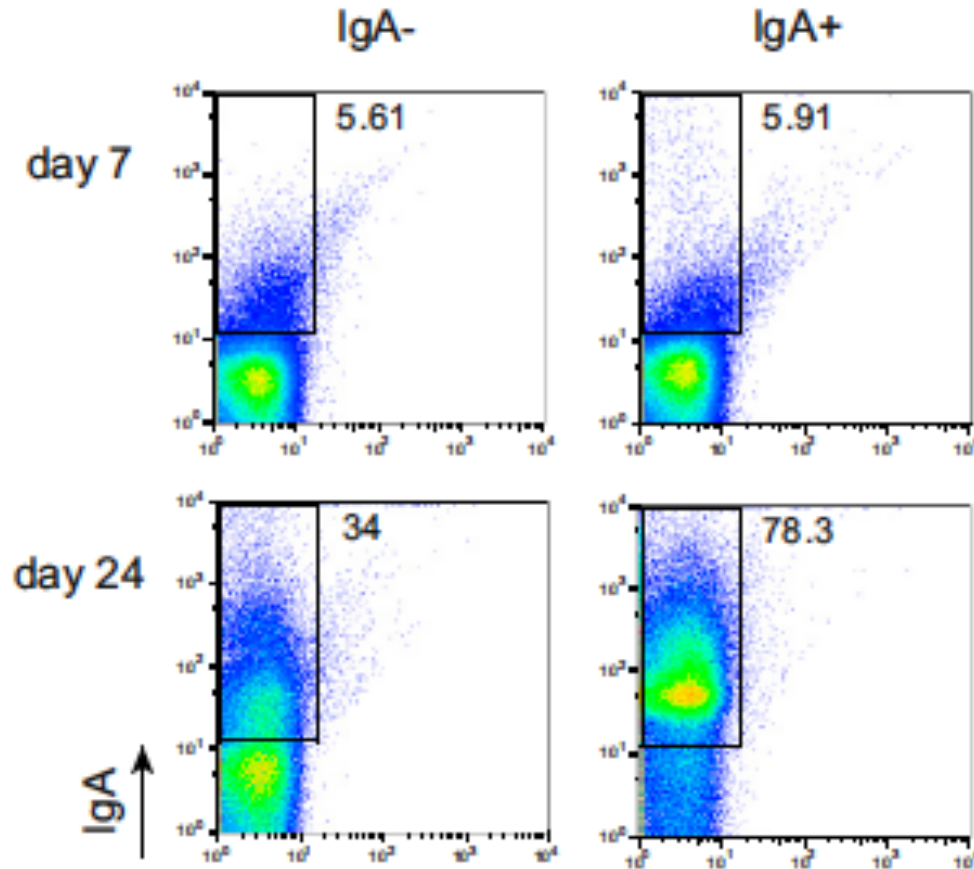
Question 1: What about gut bacteria induces an IgA response?

live E. coli are 10x better stimulus than heat killed E. coli

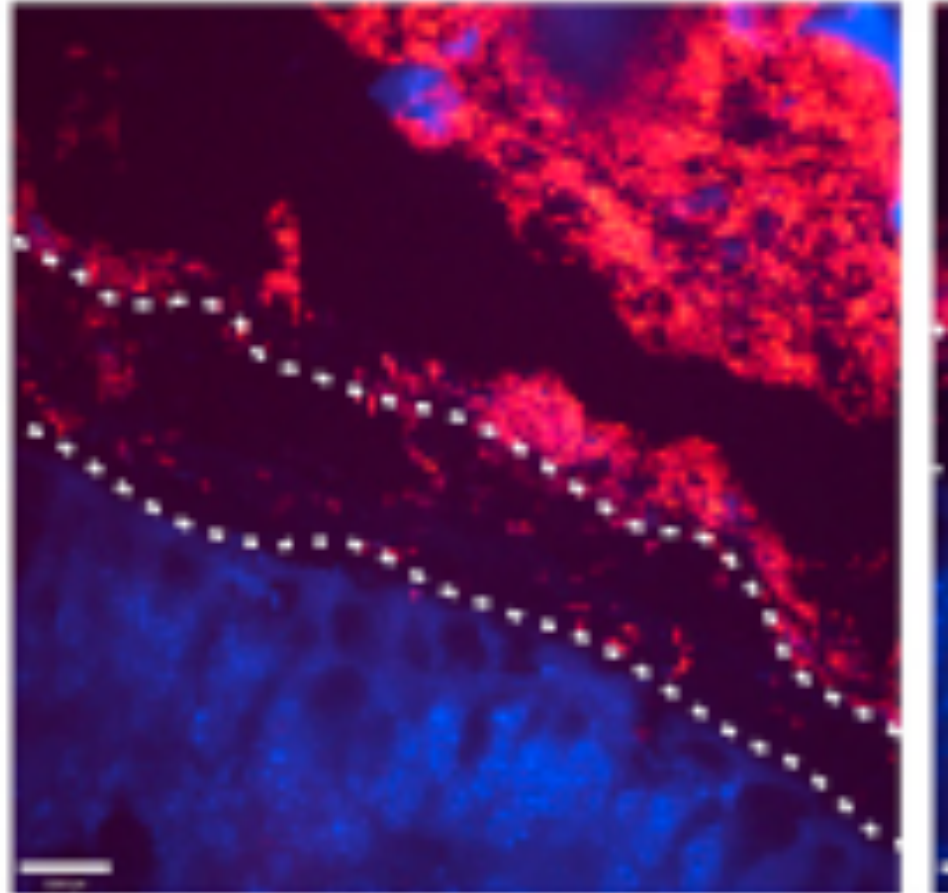
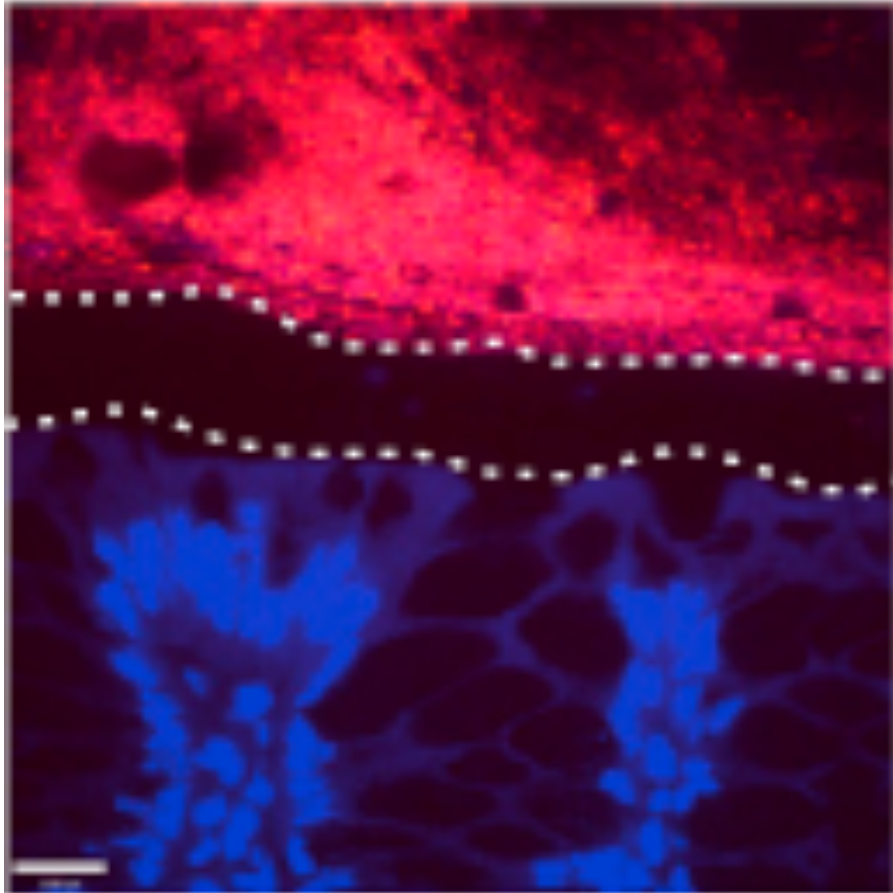
Hypothesis two: location of bugs relative to gut epithelium is important (bug must penetrate mucus layer and/or invade tissue to induce IgA response)

Consortia of IgA+ vs. IgA- microbes: different degrees of IgA induction

D



Consortia of IgA+ vs. IgA- microbes: invasion of the mucus layer by the IgA+ bugs



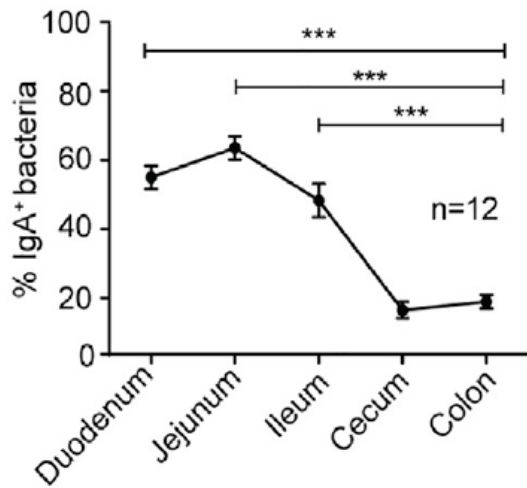
Palm et al. Cell 158: 1000-10, 2014

16S rRNA universal probe DAPI

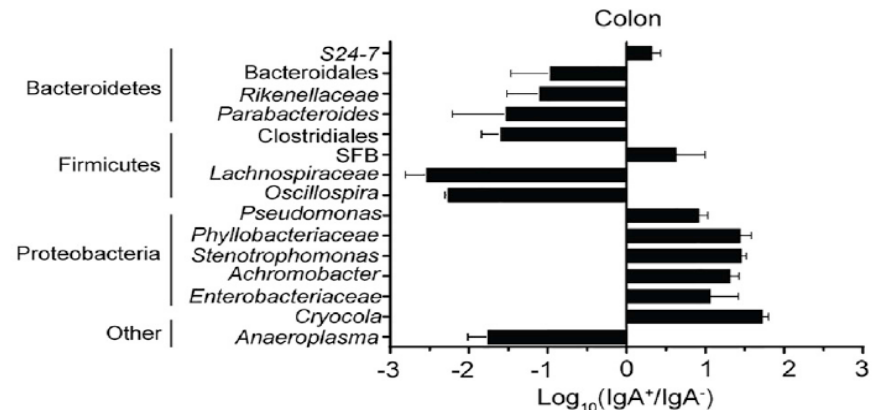
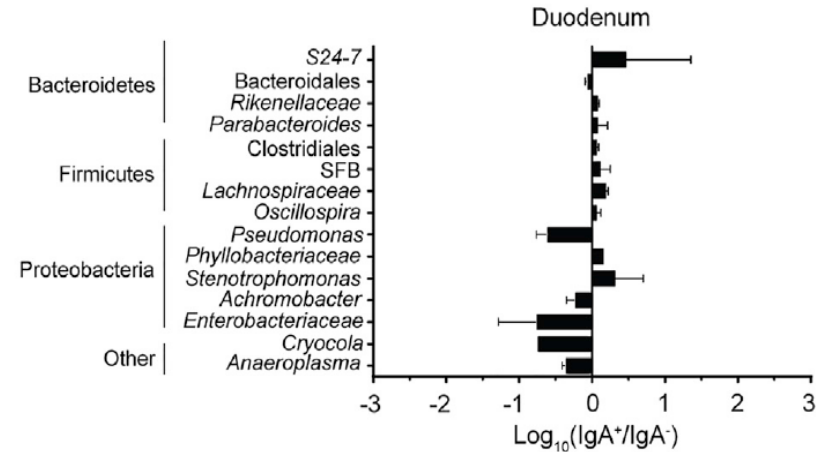
Question 1: What about gut bacteria induces an IgA response?

Hypothesis three: bugs found in lumen of colon AND not in small intestines do not induce much IgA

Question 1: What about gut bacteria induces an IgA response?



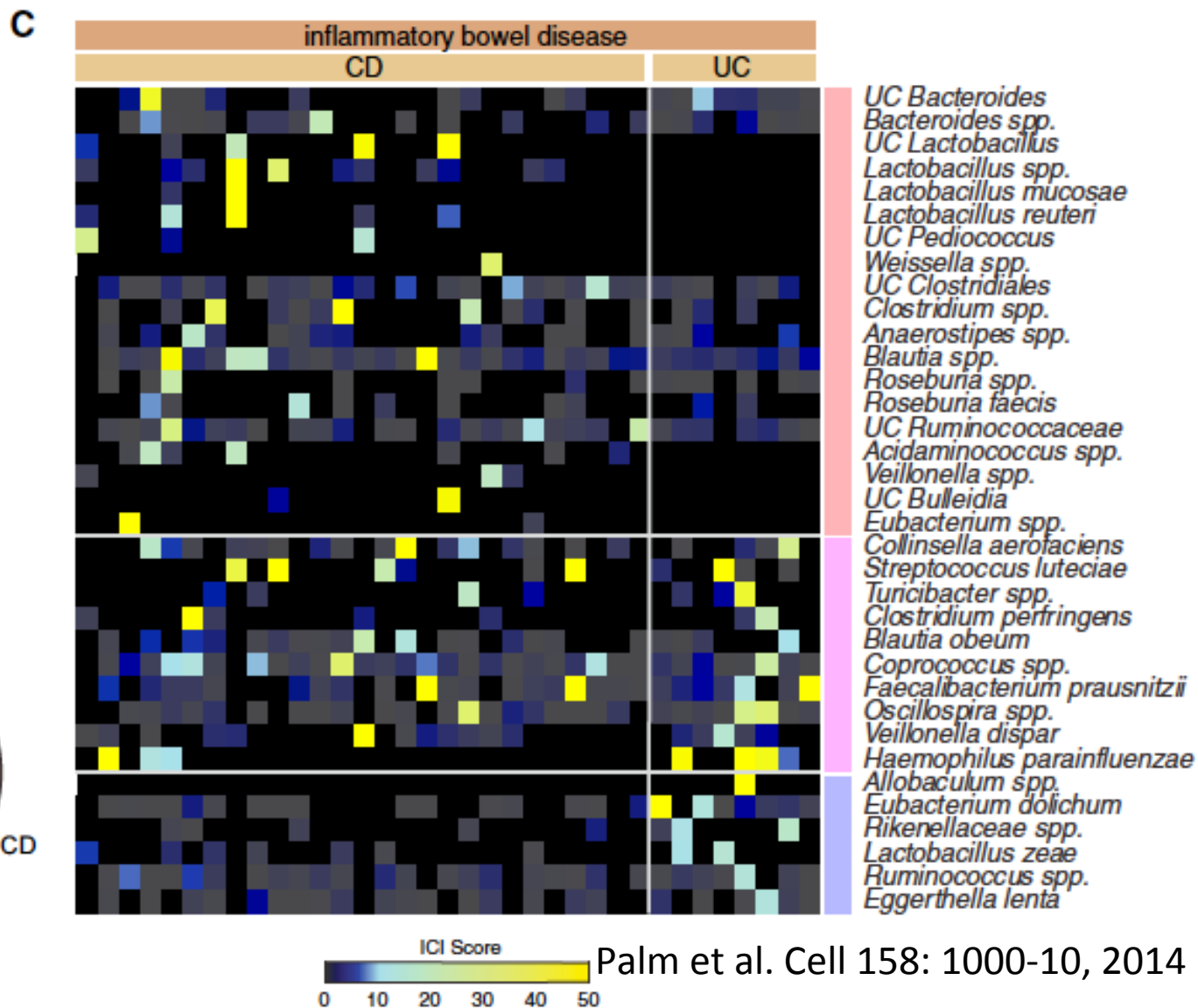
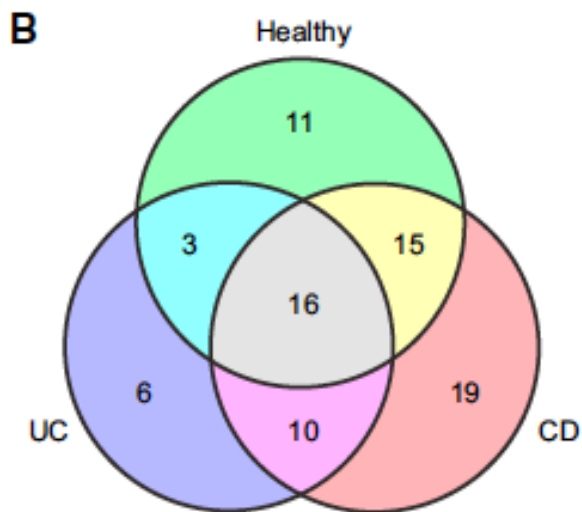
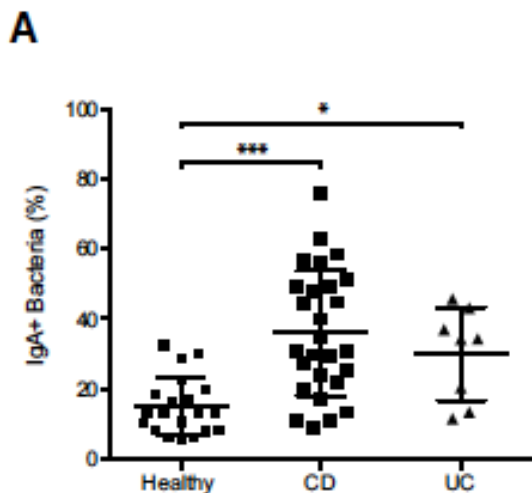
Conclusion: most bugs in small intestines induce specific IgA; most bugs in colon may not induce IgA



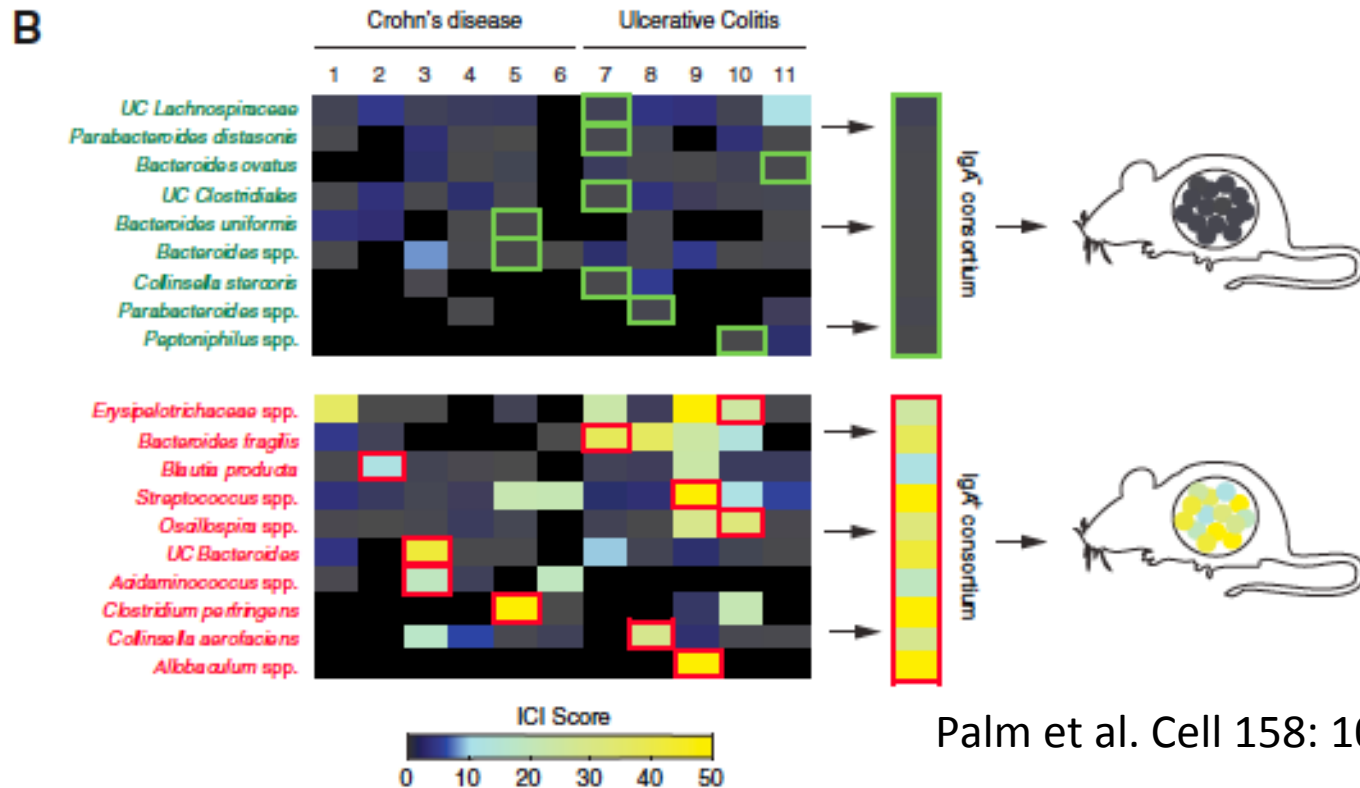
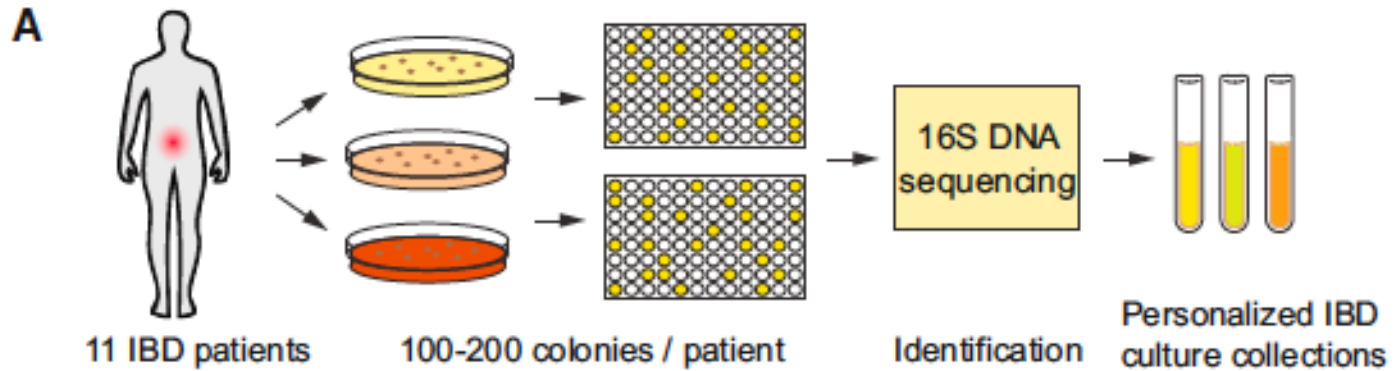
Question 1: What about gut bacteria induces an IgA response?

Hypothesis four: more “pathogenic” bugs preferentially induce IgA

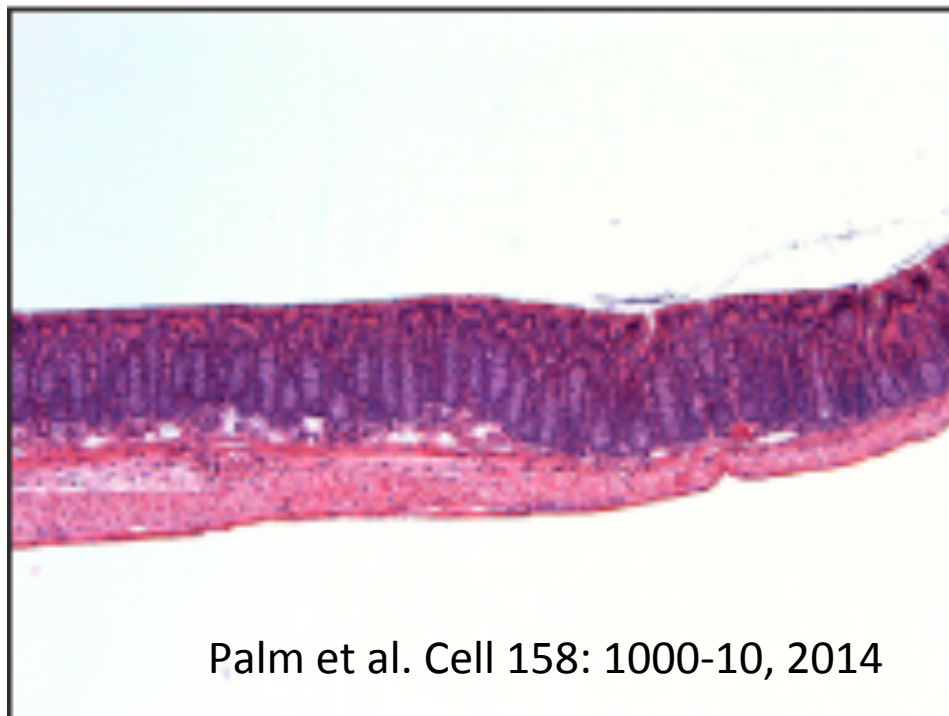
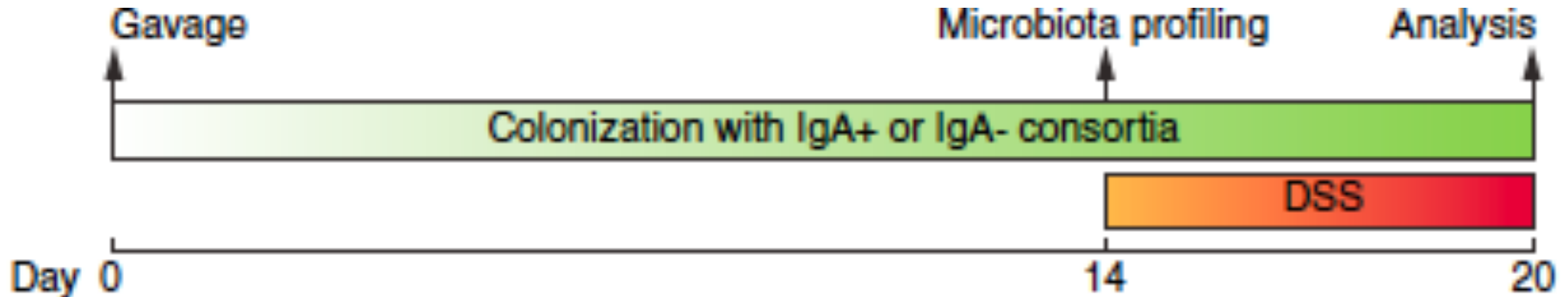
Sorting IgA-coated bacteria from people with Inflammatory Bowel Disease



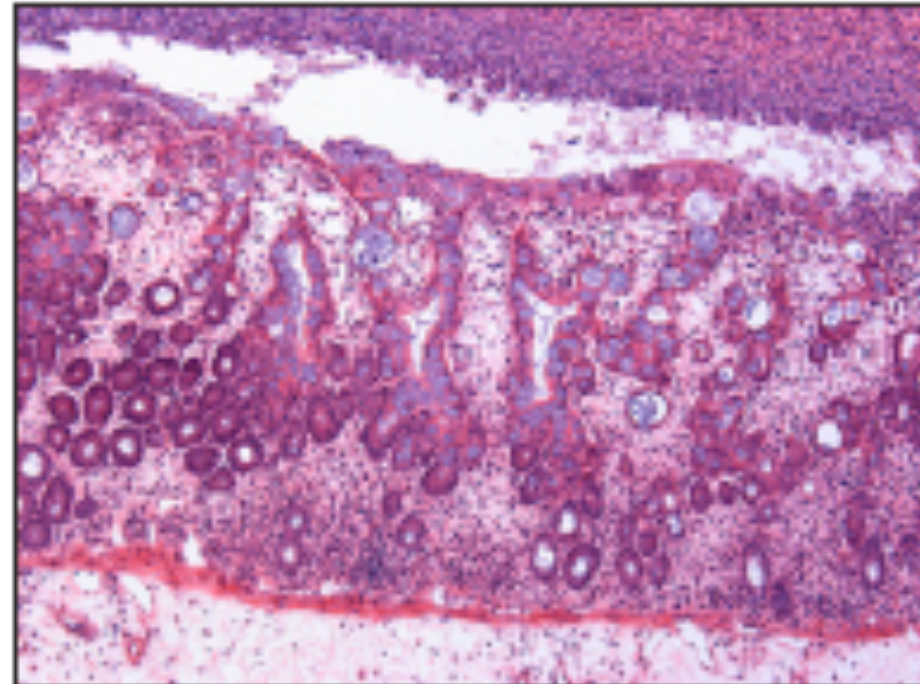
Consortia of IgA+ vs. IgA- microbes



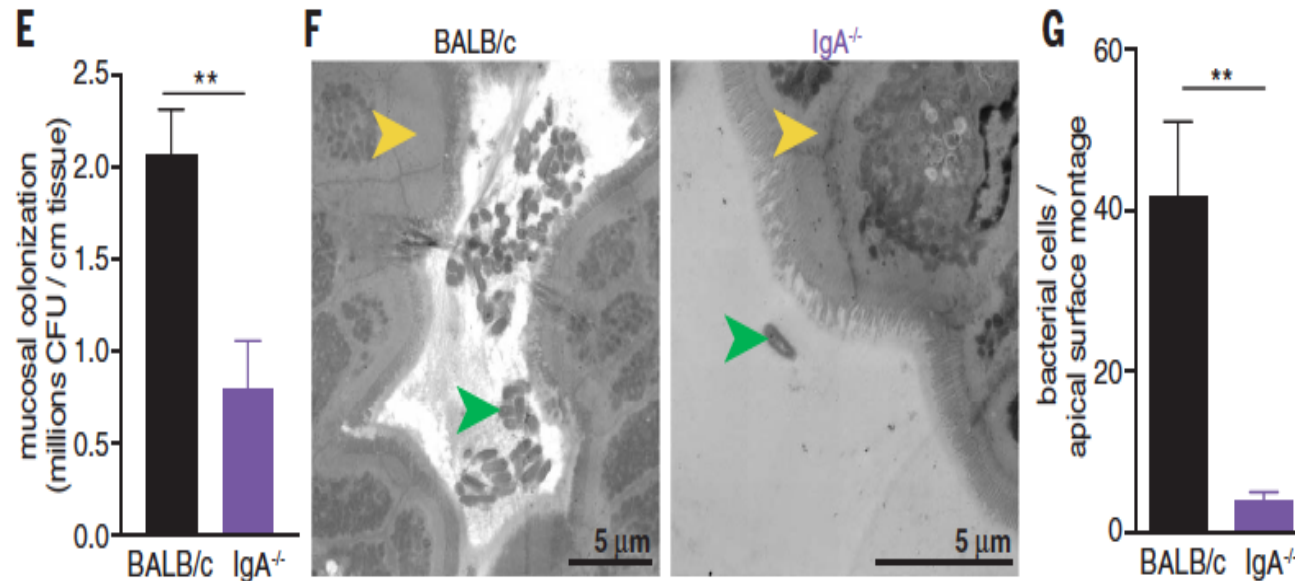
Consortia of IgA+ vs. IgA- microbes: inflammatory action



Palm et al. Cell 158: 1000-10, 2014



A commensal gut microbe uses IgA to help it colonize the mucus layer



Bacteriodes fragilis, an abundant commensal bacterium (which is naturally coated with IgA), requires IgA to colonize the mucus layer well (green arrow: bacteria; yellow arrow: epithelium; in the gut it induces expression of a capsular polysaccharide that induces strong IgA production)

Microbiota: an important variable in animal research

Accounting for reciprocal host– microbiome interactions in experimental science

Thaddeus S. Stappenbeck¹ & Herbert W. Virgin¹

9 JUNE 2016 | VOL 534 | NATURE | 191

Sorting IgA-coated bacteria: role of location in IgA induction

