Special Series on Laboratory Animal Science

Recent development in microbiome research: effect of diet and microbiota composition on the development of diseases

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Microbiota - Microbiome

Microbiota

- an "ecological community of commensal, symbiotic and pathogenic microorganisms“ *Lederberg and McCray (2001) Scientist. 15: 8. Nih Hmp Working, Group; Peterson, J; Garges, S; et al. (2009). Genome Res. 19 (12): 2317–2323.*
- found in and on all multicellular organisms (e.g. plants, humans and non-human animals such as amphibians, mice, insects)
- includes bacteria, archaea, protists, fungi and viruses
- crucial for immunologic, hormonal and metabolic homeostasis of the host.

Microbiome

- A synonymous term which describes either the collective genomes of the microorganisms that reside in an environmental niche or the microorganisms themselves. *Backhed et al. (2005). Science. 307 (5717): 1915–1920. Turnbaugh et al. (2007). Nature. 449 (7164): 804–810. Ley et al. (2006). Cell. 124 (4): 837–848.*

Types of microbiota – host relationship

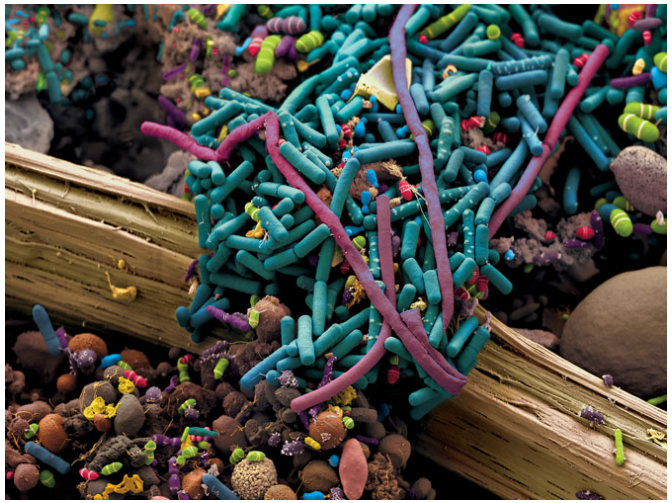
Commensalism: non-harmful coexistence / or the unaffected host benefits the microbiota

Mutualistic/Symbiotic: microbiota which are useful for the host / or both have a benefit

Parasitic: microbiota which are disadvantageous to or harmful for the host.

Dysbiosis*: Deviation or imbalance in gut microbial community membership and physiology.*
Correlates with several negative health outcomes (like pathogen susceptibility, inflammatory bowel disease, colon cancer, etc.)

Gut microbiota – Intestinal microbiome



Photograph by Martin Oeggerli, with support from School of Life Sciences, FHNW

The **“forgotten organ”** (O’Hara and Shanahan, 2006).

- comprises trillions of bacteria
- characterized by approximately 1000 species
- outnumber the genes present in the human genome (Ley et al., 2006; Qin et al., 2010)
- dominated by two bacterial phylotypes: Firmicutes and Bacteroidetes
- core gut microbiota and microbiome are shared in human besides interindividual variability (Qin et al., 2010).

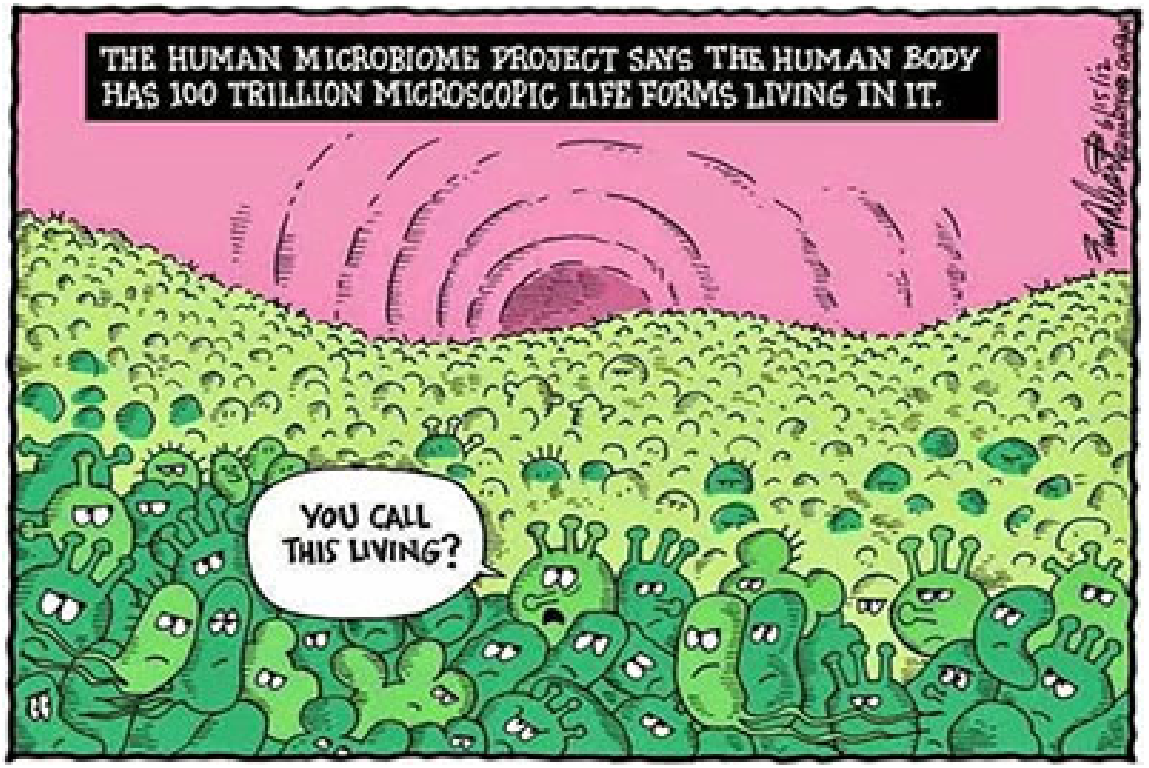
Gut microbiota – Intestinal microbiome

Production of fundamental molecules for many host biological processes

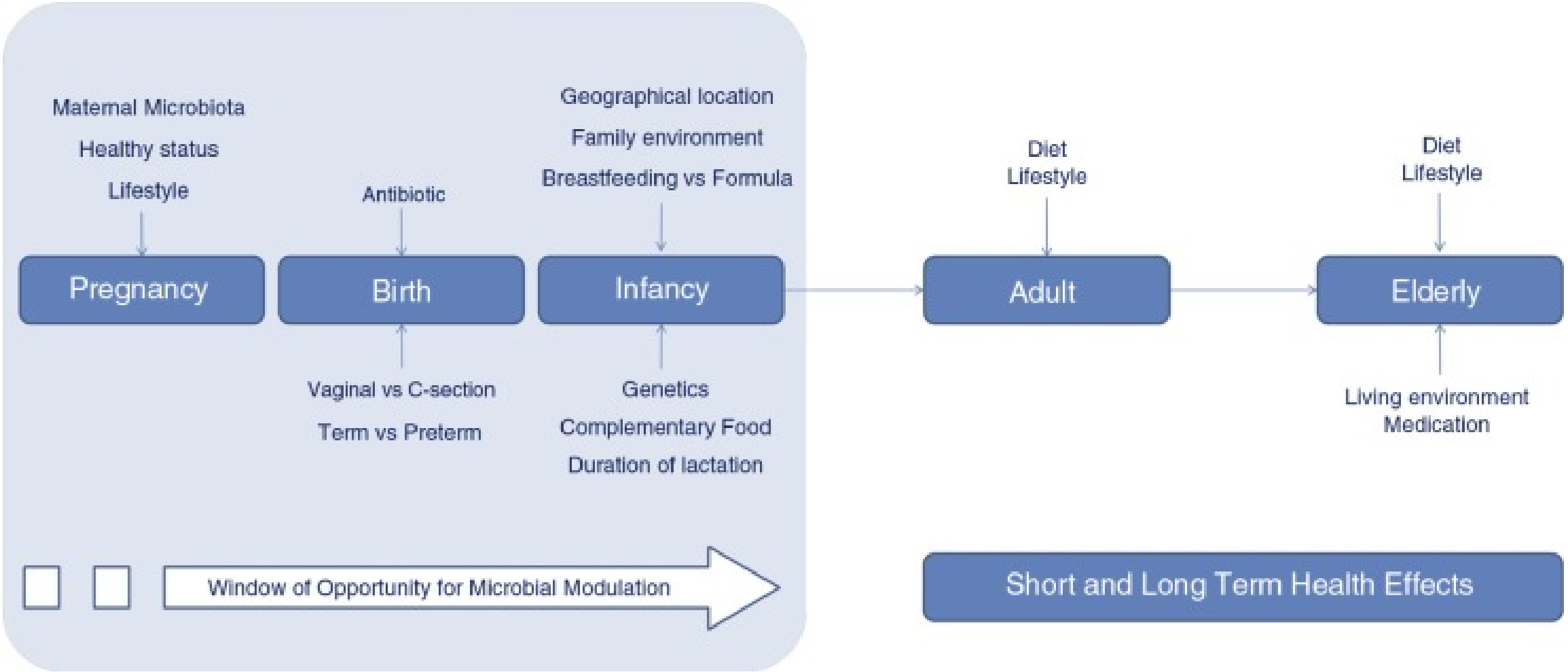
- vitamins,
- short chain fatty acids (SCFA: propionate, acetate and butyrate),
- amino acids and their derivatives,
- neurotransmitters.

Metabolism of

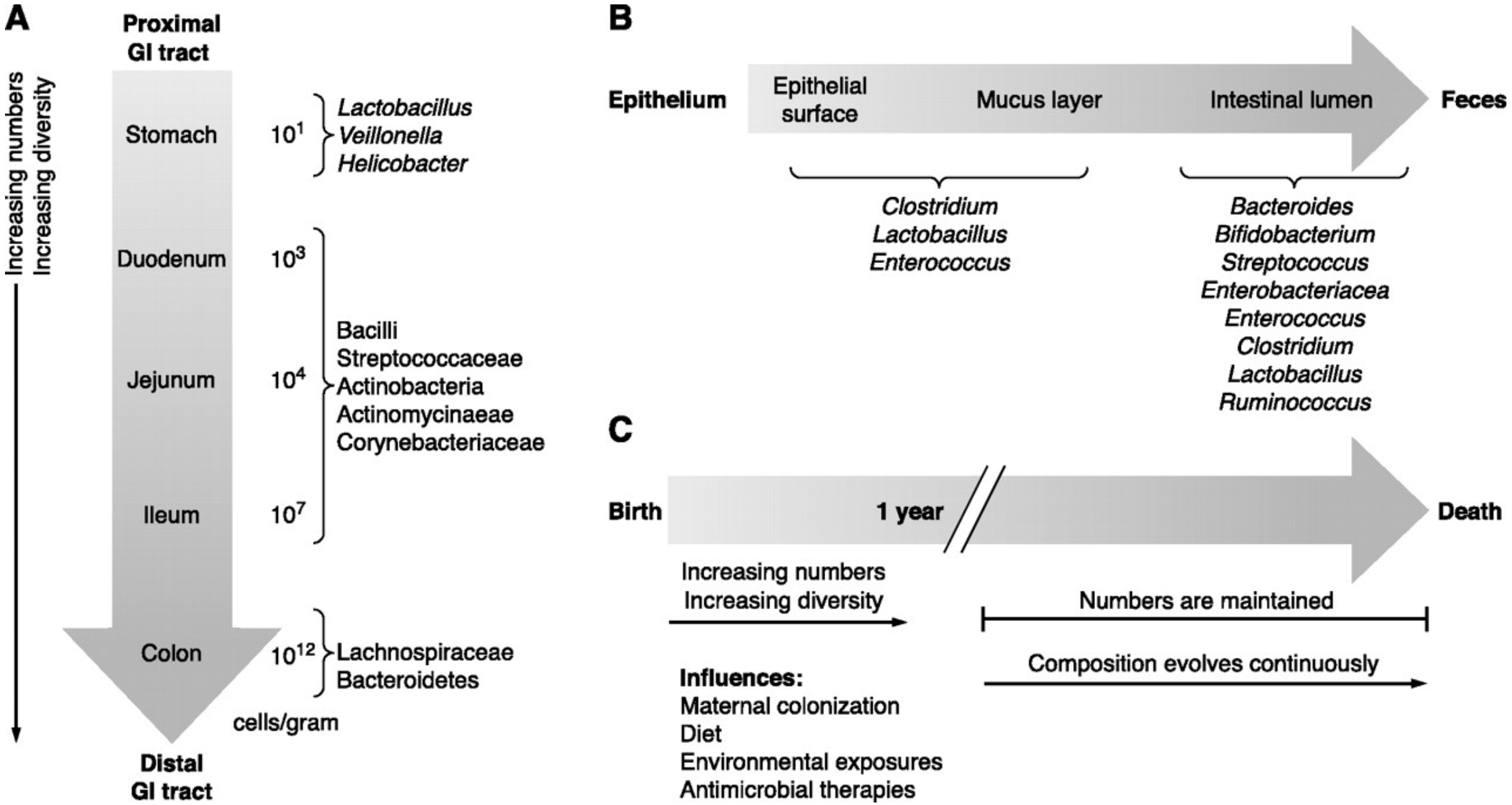
- choline,
- bile acids,
- polyamine,
- various lipids (Nicholson et al., 2012).



Impact on GIT - Colonization

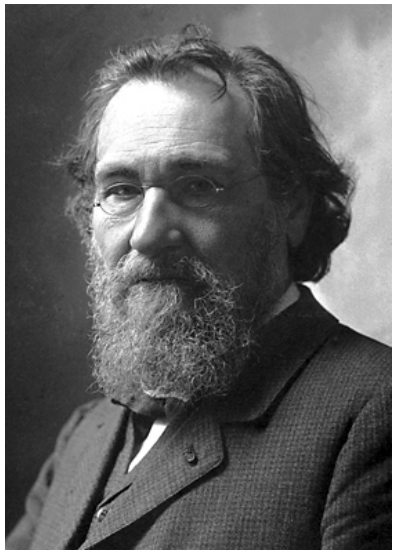


Rodriguez et al. Microb Ecol Health Dis. 2015; 26: 10.3402/mehd.v26.26050.



Sekirov et al. Physiological Reviews 2010 Vol. 90 no. 3, 859-904

Gut microbiota - Concept of pro-/prebiotics



Élie (Ilya Ilyich) Metchnikoff (1845 –1916)

introduction of the concept of “pro-/prebiotics”

1907: "the dependence of the intestinal microbes on the food makes it possible to adopt measures to modify the flora in our bodies and to replace the harmful microbes by useful microbes".

Probiotic: live microorganisms that are believed to provide health benefits when consumed

Prebiotics: food ingredients that induce the growth or activity of beneficial microorganisms (e.g., bacteria and fungi) → e.g. alteration of gut microbiome composition using non-digestible fiber compounds (pass undigested through the upper part of the gastrointestinal tract and stimulate the growth or activity of advantageous bacteria that colonize the large bowel by acting as substrate for them) As a functional food component, prebiotics, like probiotics, are conceptually intermediate between foods and drugs.

Élie [Ilya Ilyich] Metchnikoff, 2004 [1907], ***The prolongation of life: Optimistic studies****, p. 116. Springer Classics in Longevity and Aging, New York, NY:Springer, ISBN 0826118771, reprint of 1908 English edition by É.M., same title (P. Chalmers Mitchell, Ed.), New York, NY:Putnam, ISBN 0826118763, itself a translation of 1907 French edition by I.I.M., Essais optimistes, Paris:Heinemann, Retrieved 12 November 2015.*

Dietary fiber

- Recommended daily range of fiber intake: 28-35 g (adults)
- Direct effects: e.g. fecal bulking and laxation, substrate for microbiota
- Accepted health benefits of consuming dietary fiber
- Little knowledge about impact on gut microbiota and alteration of disease risk
- Decreased fiber intake in industrialized nations
  → linked to several diseases
  → linked to thinner colonic mucus

Microbiome studies - Mice

Germ free (GF) mice = completely sterile animals that have never had contact with any microorganism free of ALL microorganisms, including those that are typically found in the gut

Gnotobiotic mice = inoculated with a cocktail of one or more non-pathogenic microorganisms, all of which are known. = “defined flora” mice

Specific pathogen free (SPF) mice = animals raised with a normal gut flora, free of a specific list of pathogens by routine testing

Cell

A Dietary Fiber-Deprived Gut Microbiota Degrades the Colonic Mucus Barrier and Enhances Pathogen Susceptibility

Desai et al., 2016, Cell *167*, 1339–1353
November 17, 2016
http://dx.doi.org/10.1016/j.cell.2016.10.043

Study

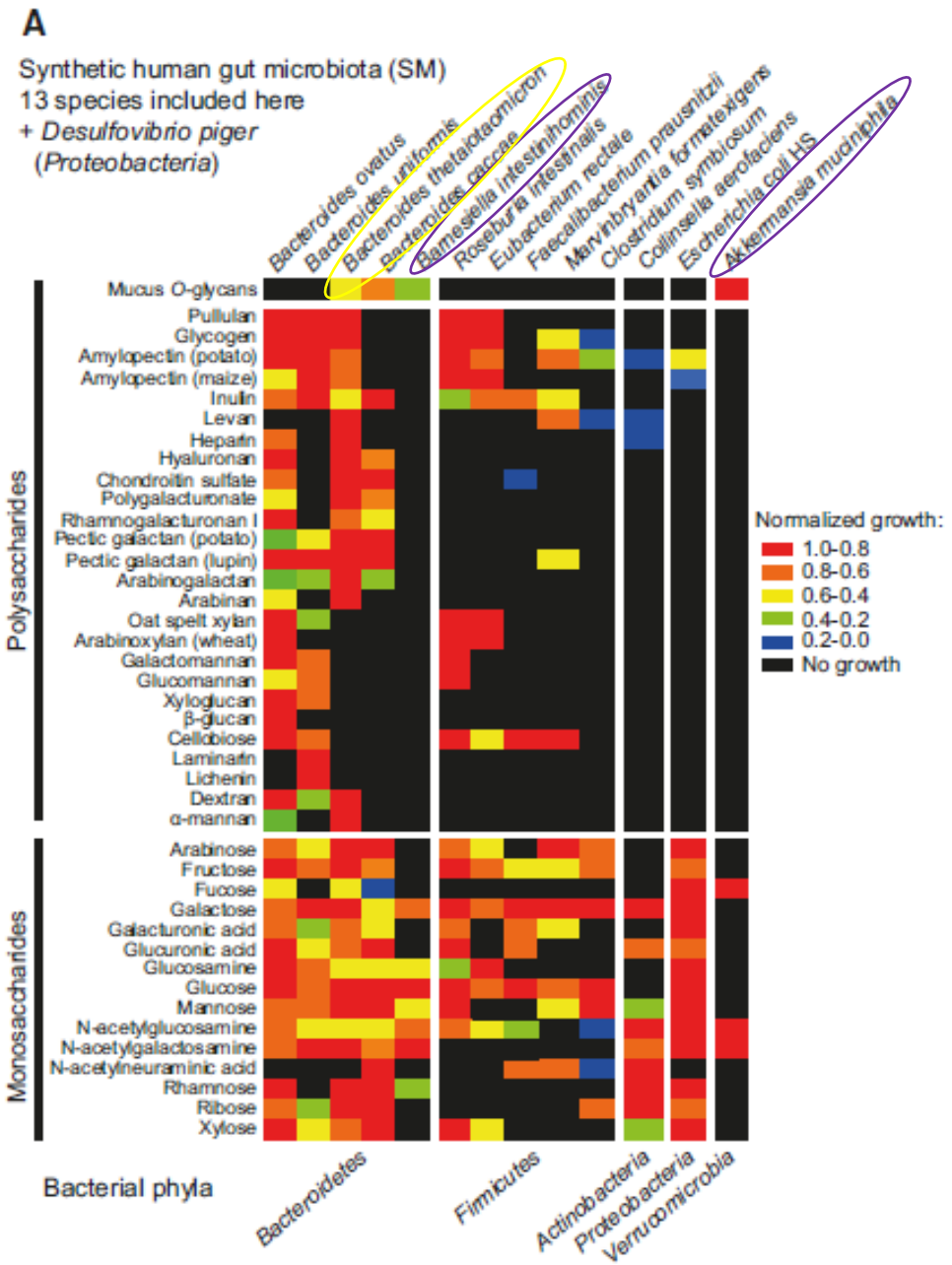
- *Hypothesis: Specific members within a fiber-deprived gut microbiota cause damage by increasingly foraging for nutrients in the protective mucus layer*

- mechanistic connections between chronic or intermittent dietary fiber deprivation on microbiota composition and physiology

- resulting effects on the mucus barrier

- Gnotobiotic mice with Synthetic gut microbiota (SM) from fully sequenced commensal human gut bacteria (14 species) chosen to represent five dominant phyla and collectively possess important core metabolic capacities

Figure 1. Carbohydrate Utilization by the Synthetic Human Gut Microbiota Members and Gnotobiotic Mouse Treatments



- Heatmap showing normalized *in vitro* growth values of 13/14 synthetic human gut microbiota (SM) members on a panel of 42 plant- and animal-derived mono- and polysaccharides (including mucin O-glycans (MOGs) as sole carbon sources)

- All major groups of dietary fiber and host mucopolysaccharides could be used by one or more SM species
- Identification which bacteria could target each glycan
- → Mucin specialists
- → Mucin generalists

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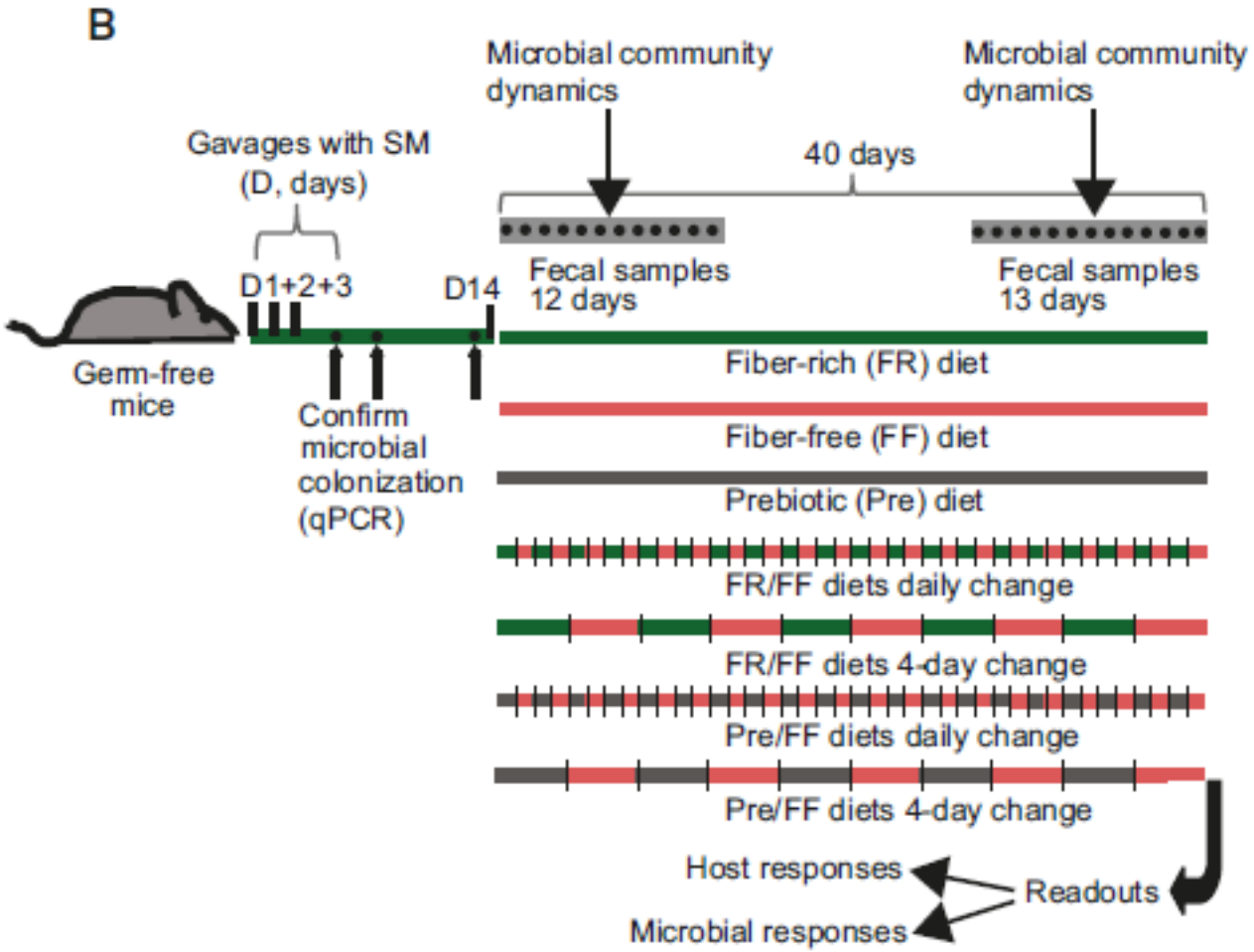
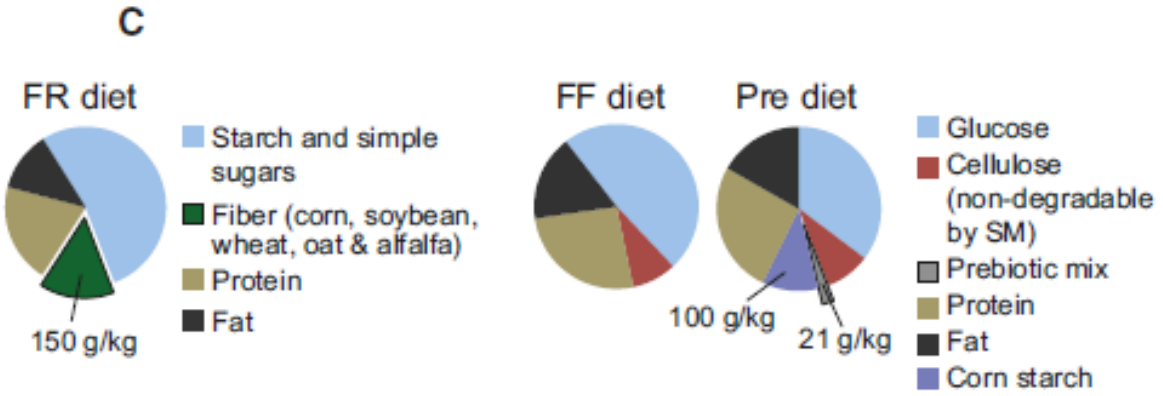




Timeline of colonization, feeding strategies and fecal sampling in the gnotobiotic mouse model

Diets

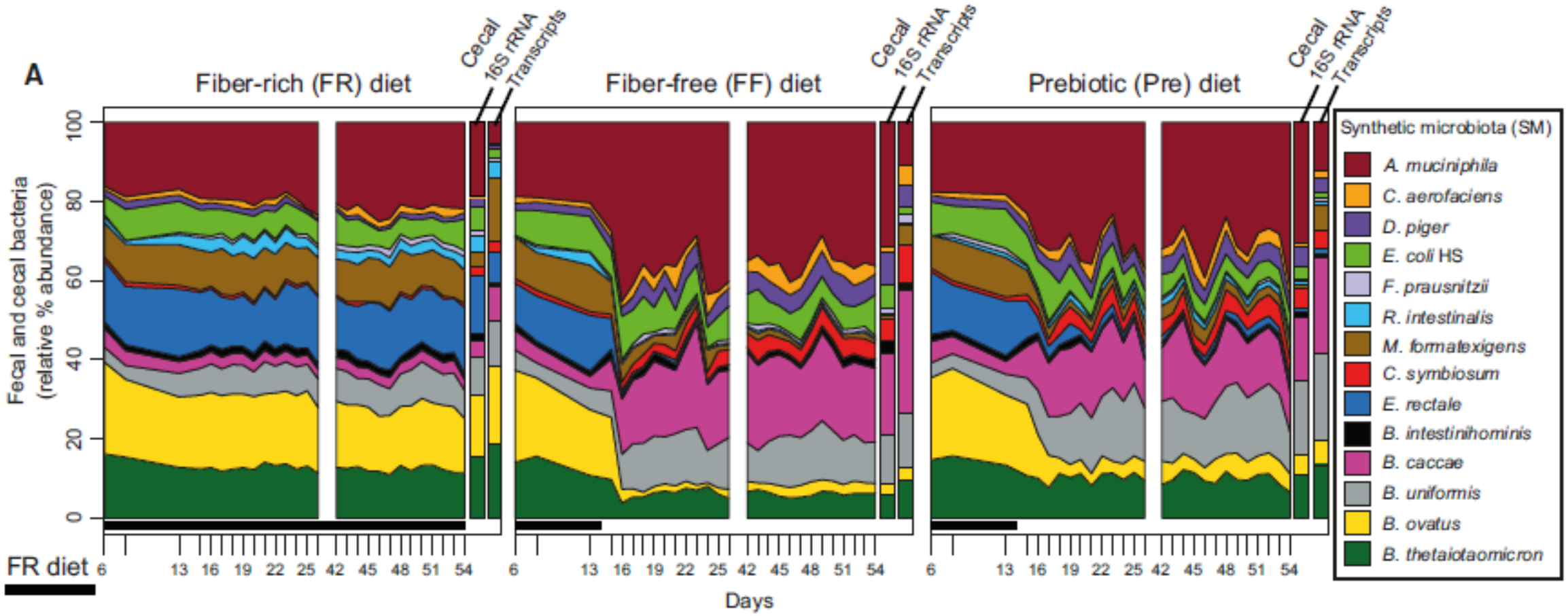
FR: fiber rich = ~15% dietary fiber

FF: fiber free

Pre: Prebiotics mix = equal proportions of 14 host indigestible polysaccharides

Simulation of fluctuating amounts of fibers from meal-to-meal (humans):
FR/FF diets daily/4-day change
Pre/FF diets daily/4-day change

Figure 2. Complex Dietary Fiber Deficiency Leads to Proliferation of Mucus-Degrading Bacteria



Stream plot:

Fecal and cecal microbial community dynamics and average abundance of total species-specific transcripts

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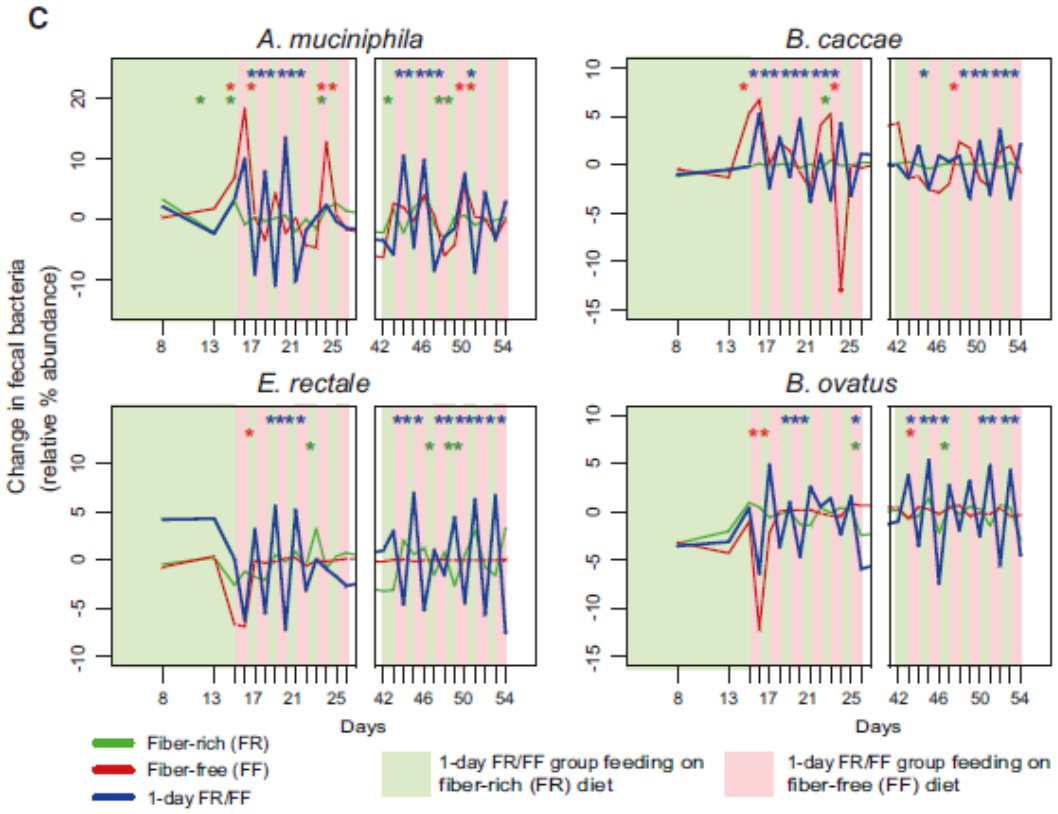
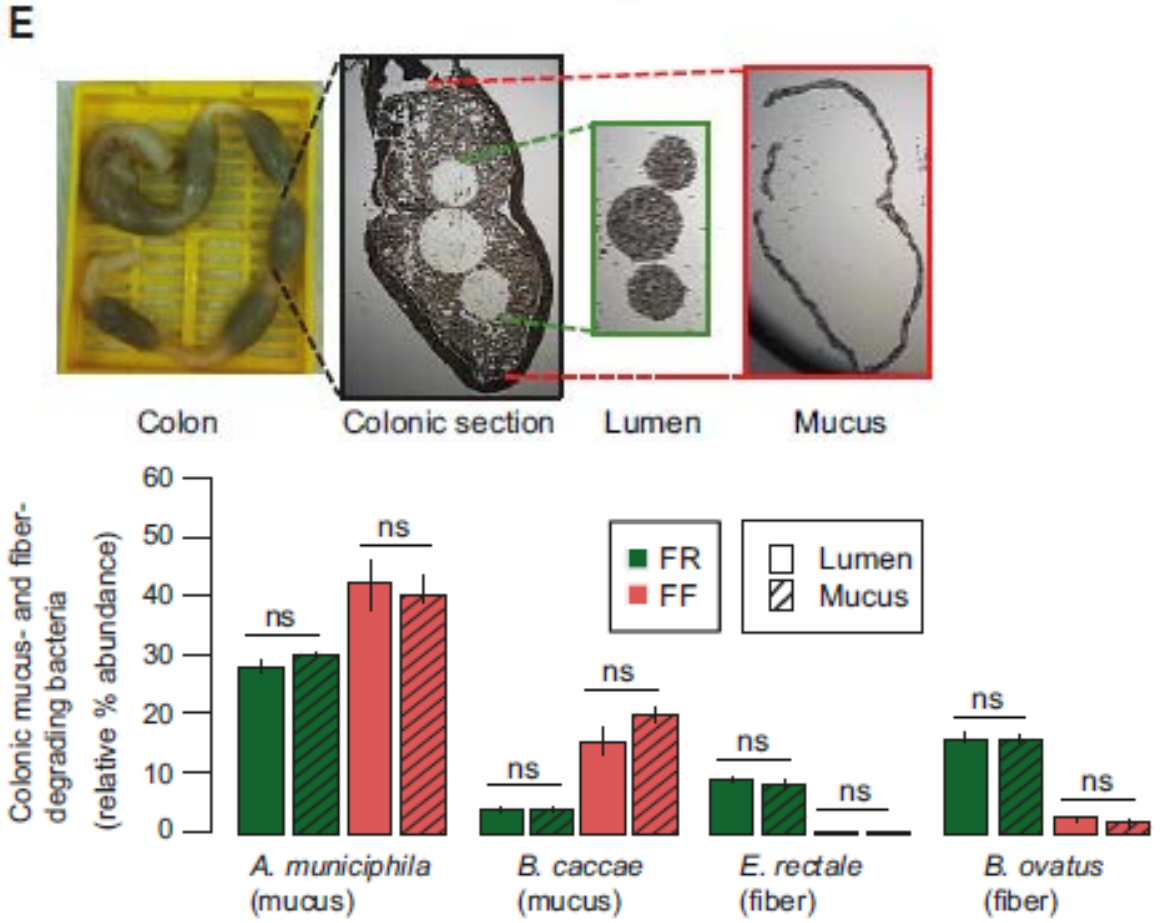
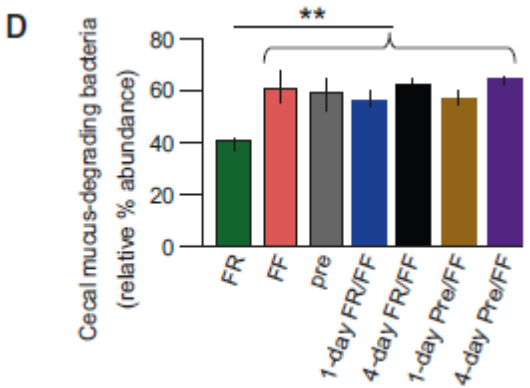


Rapid fluctuation on a daily basis when FR and FF diets are oscillated

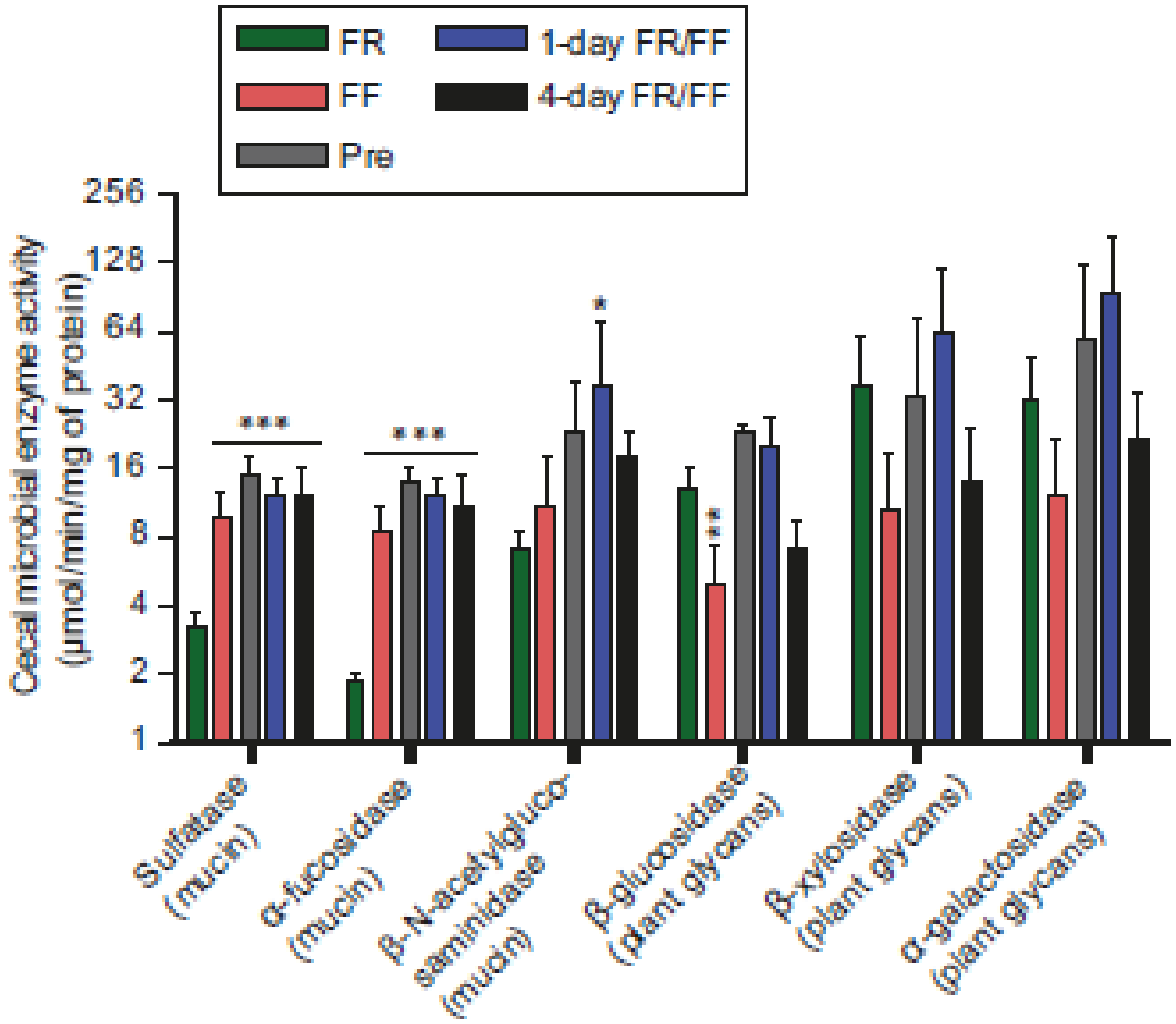




Abundances of four mucus-degrading bacteria

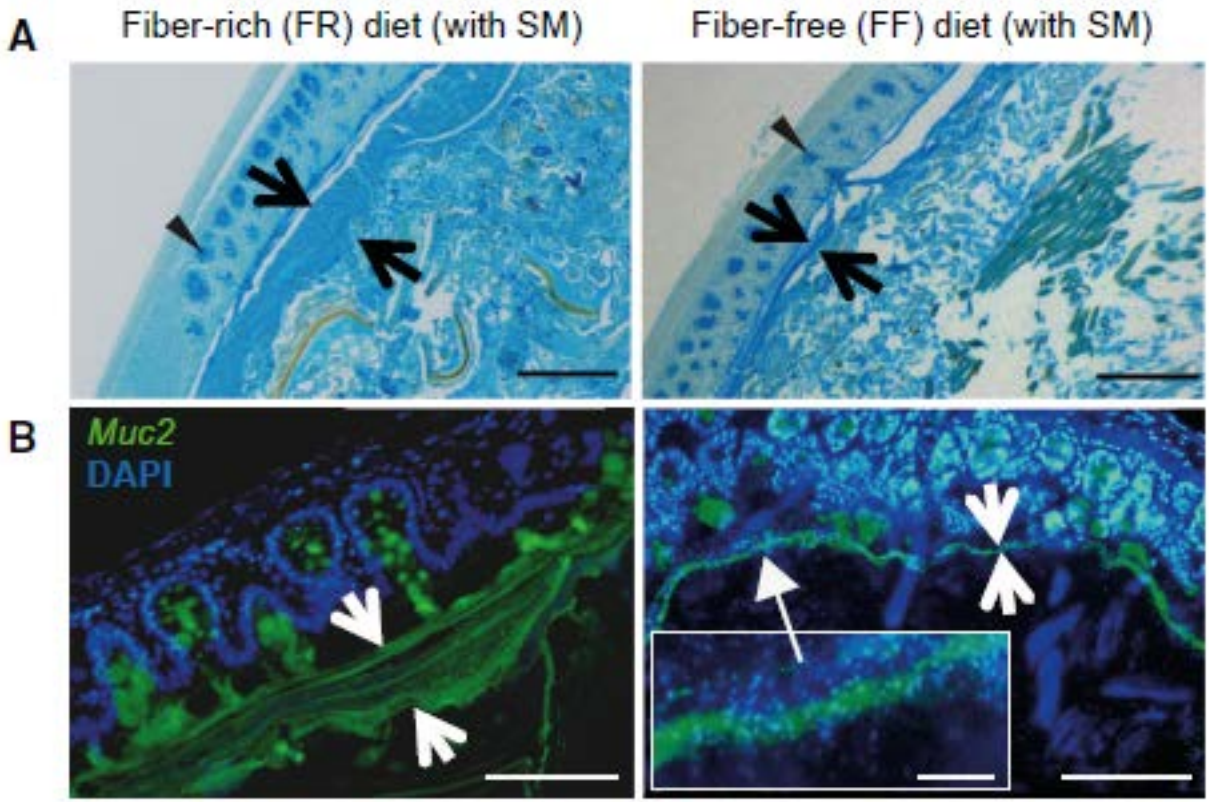
Relative abundance in laser capture microdissected colonic lumen and mucus samples (n=3/group)

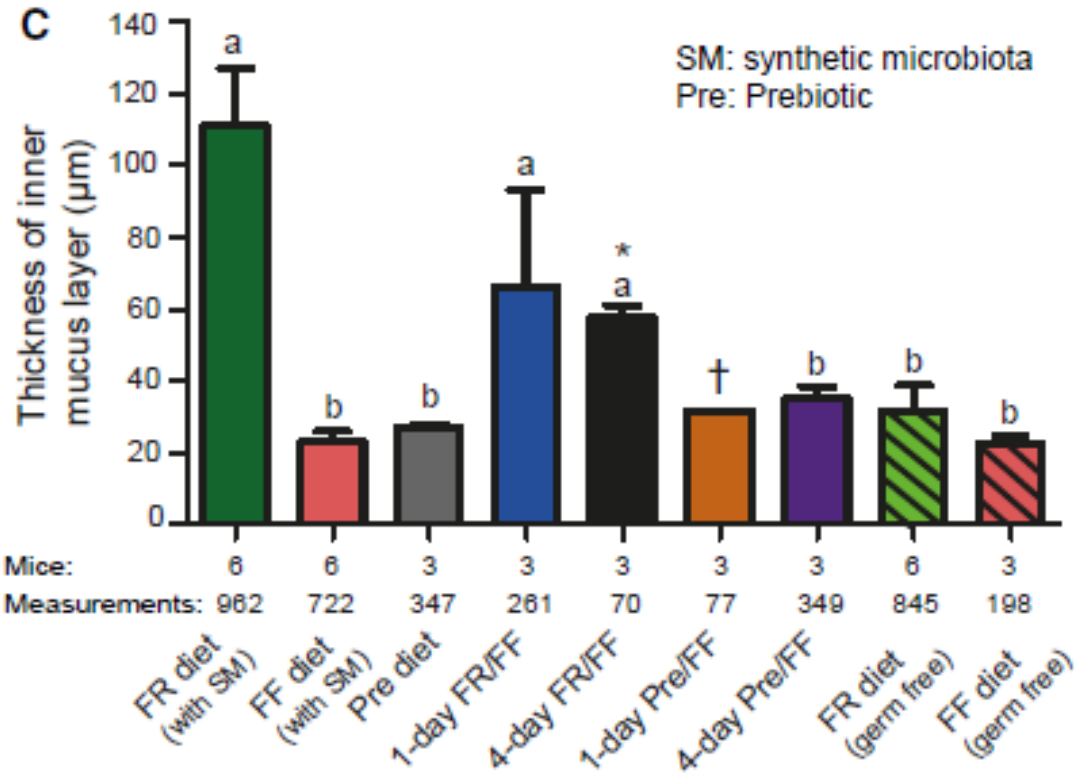
Figure 3. Diet-Specific Changes in Carbohydrate Active Enzyme Expression Reveal a Community Shift from Fiber to Mucus Degradation



- Increased bacterial enzymes targeting mucin linkages (sulfatase and alpha-fucosidase) in FF diet (chronic and intermittent)
- Reduced enzymes targeting linkages in fiber polysccharides (beta-glucosidase) in FF diet

Figure 4. Microbiota-Mediated Erosion of the Colonic Mucus Barrier and Host Responses





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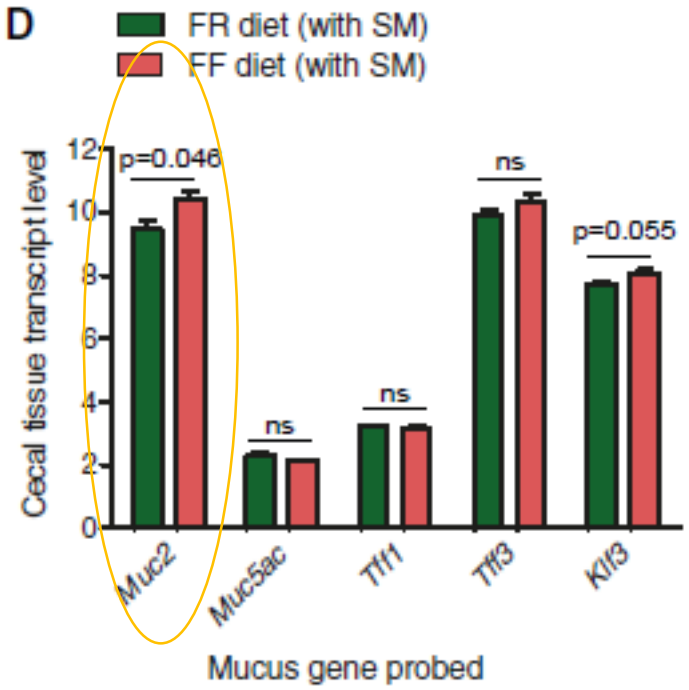
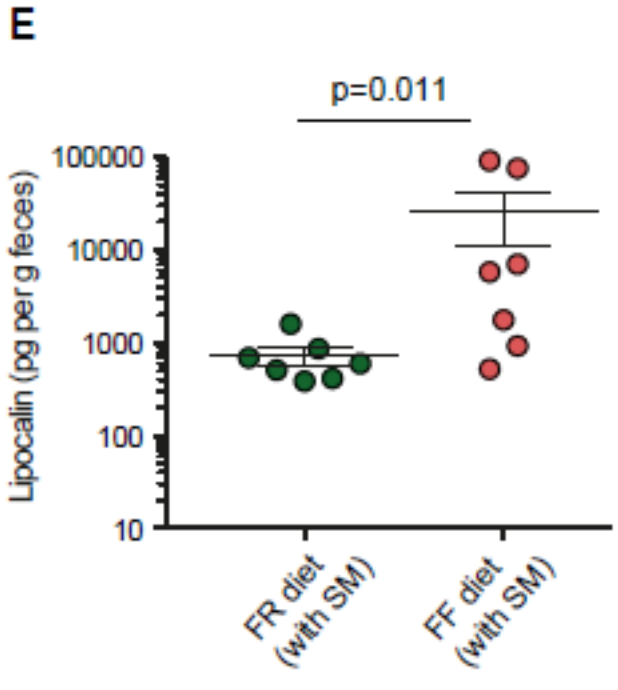
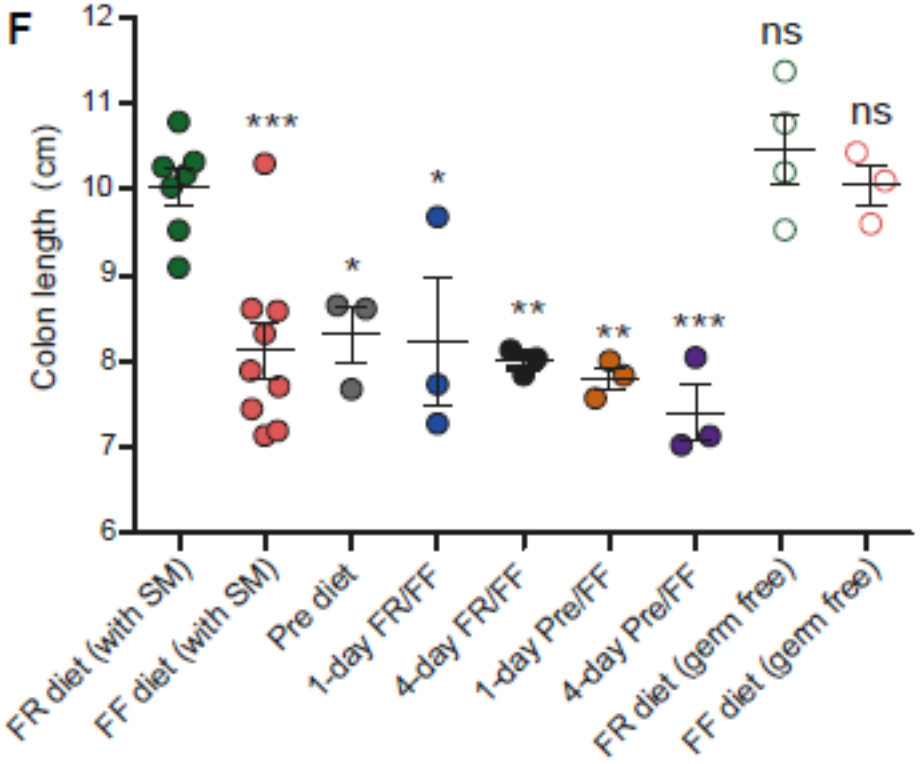


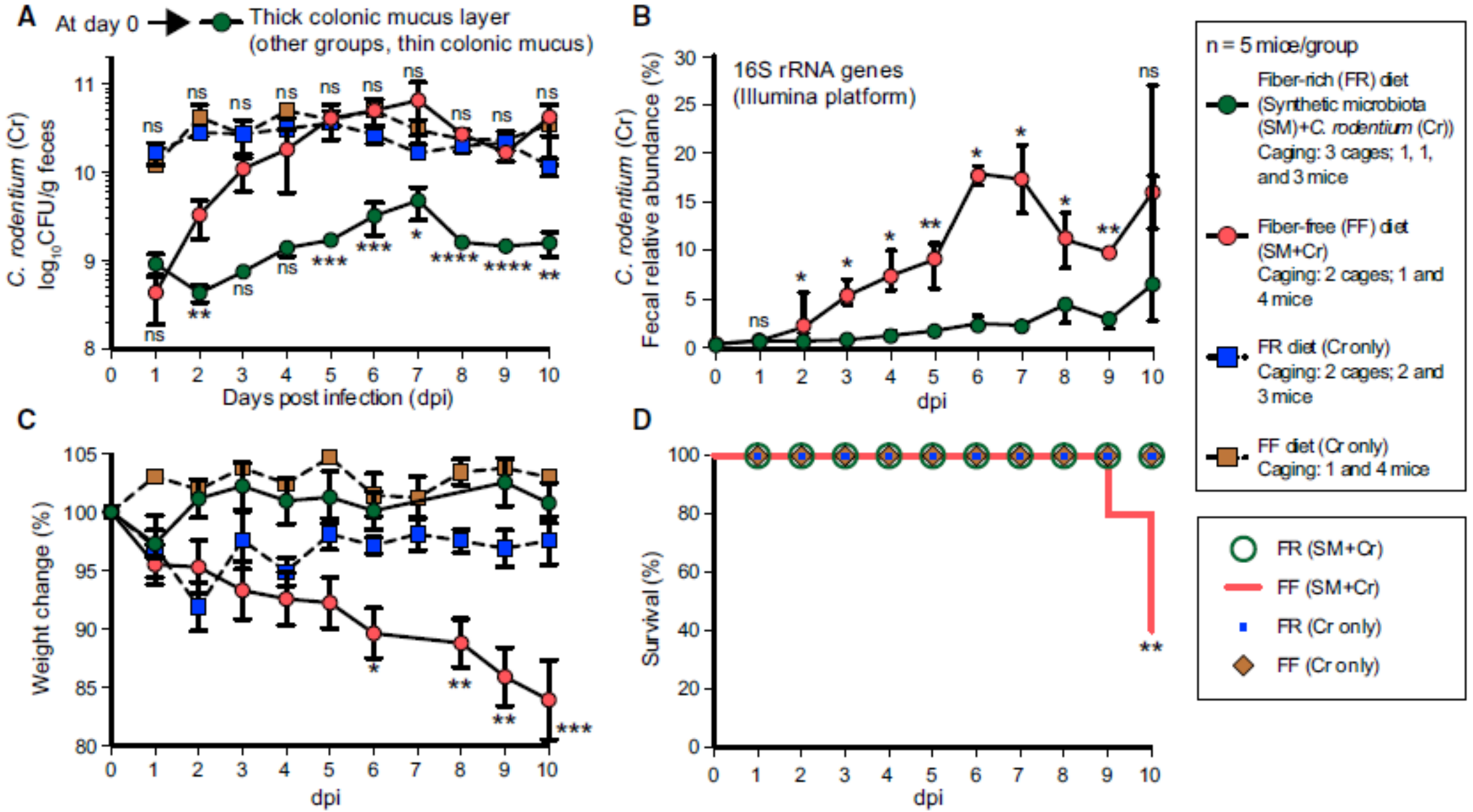




Key proteins for building and regulating the mucus barrier

Altered host response but no overt signs of disease

Figure 5. Fiber-Deprived Gut Microbiota Contributes to Lethal Colitis by *Citrobacter rodentium*



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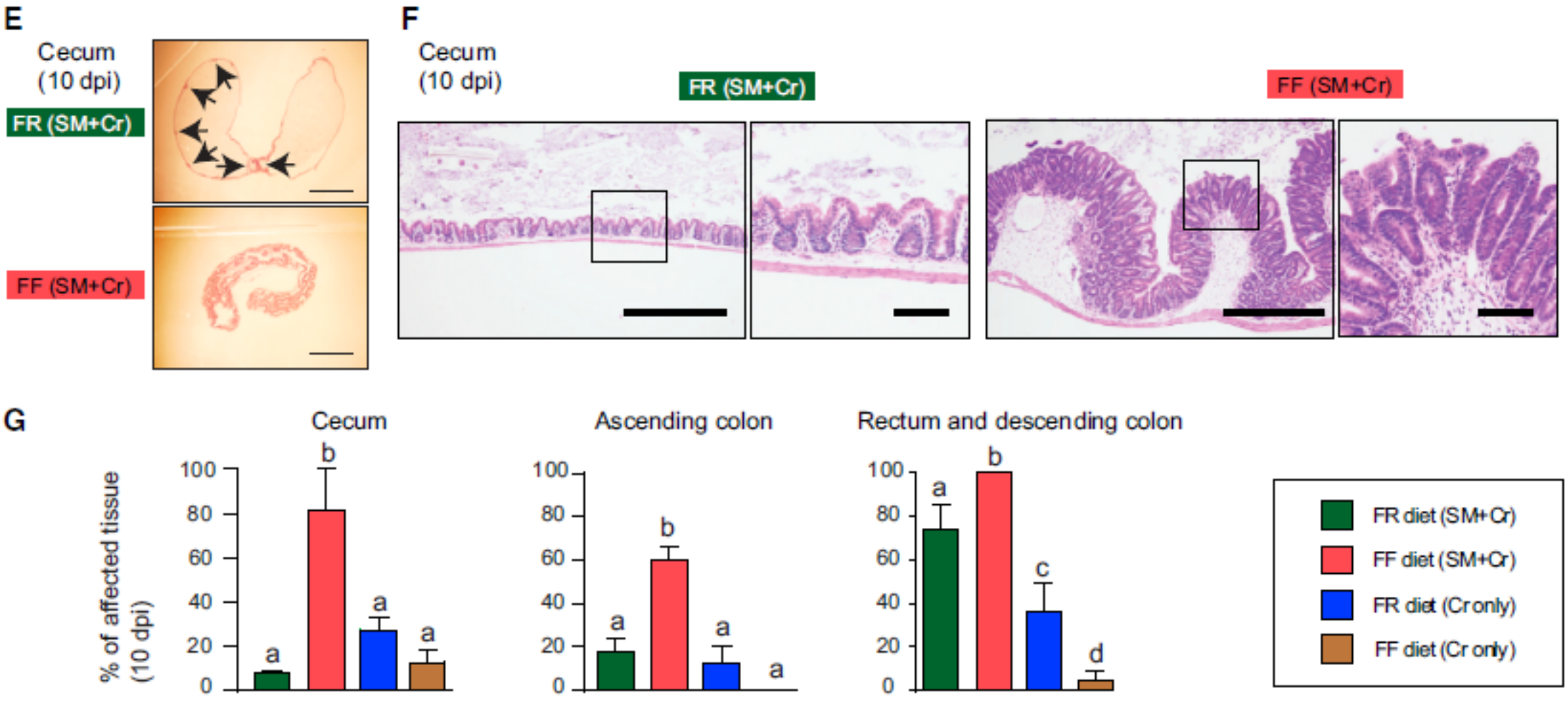


Figure 6. Fiber-Deprived Gut Microbiota Promotes Faster *C. rodentium* Access to the Colonic Epithelium

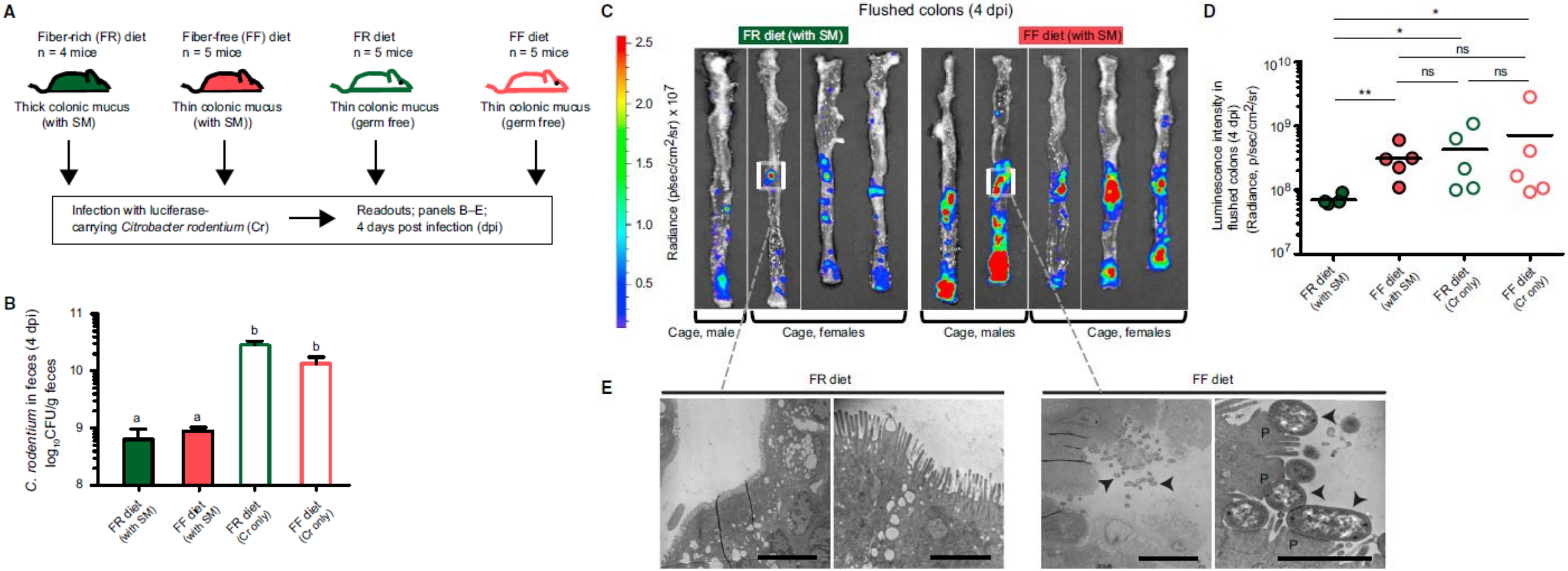
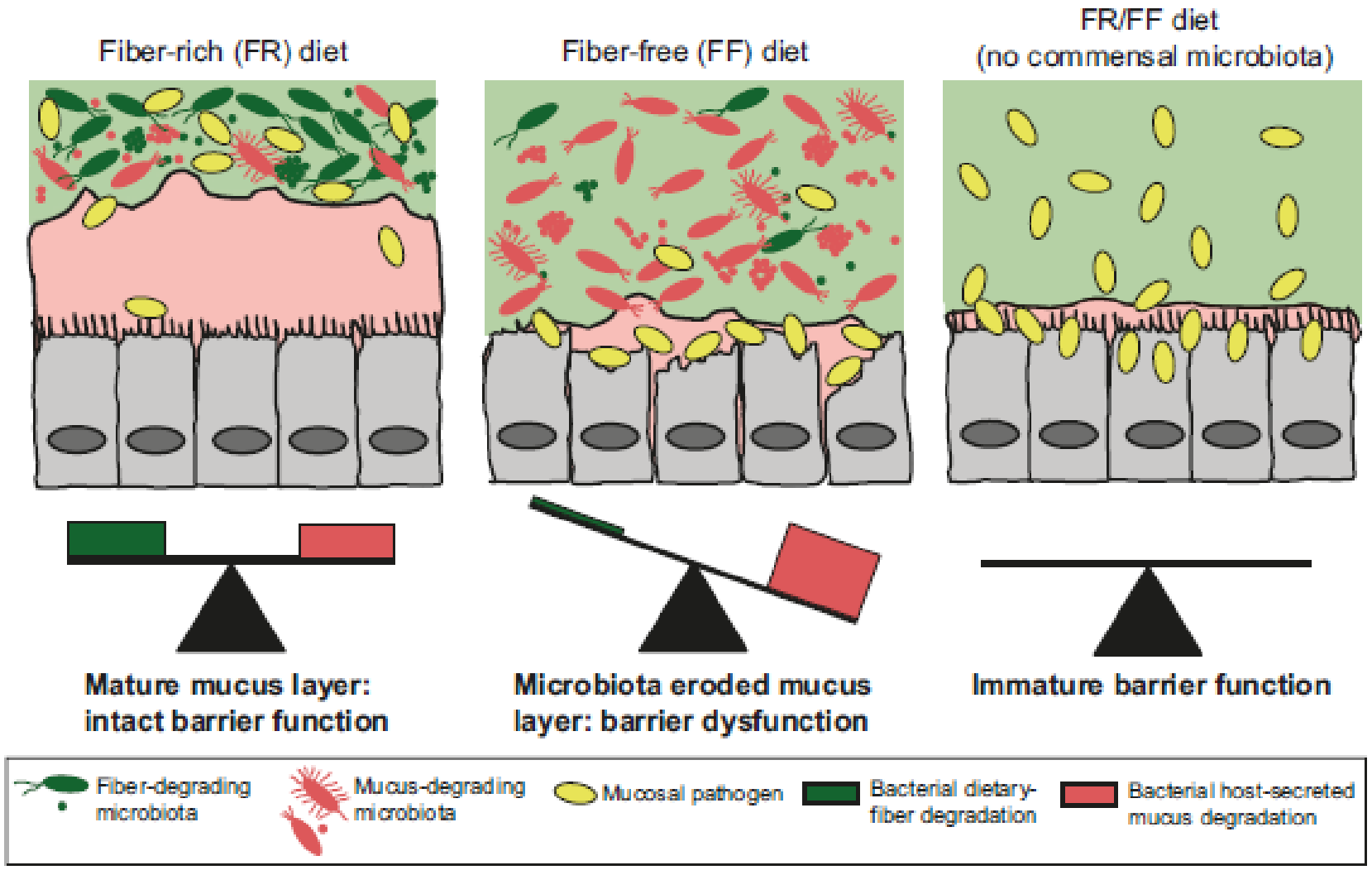


Figure 7. Model of How a Fiber-Deprived Gut Microbiota Mediates Degradation of the Colonic Mucus Barrier and Heighted Pathogen Susceptibility



Cell

Gut Microbiota Regulate Motor Deficits and Neuroinflammation in a Model of Parkinson's Disease

Sampson et al., 2016, Cell *167*, 1469–1480
December 1, 2016
http://dx.doi.org/10.1016/j.cell.2016.11.018

Gut-Brain Axis

- Proposed bidirectional communication between the gut and the brain
- Interactions between enteric microbiota, central (CNS) and enteric nervous systems (ENS)
- GF mice and Antibiotics-treated SPF mice show altered hippocampal neurogenesis (spatial and object recognition)
- GF mice show altered cortical myelination and impaired BBB function
- Gut bacteria control differentiation and function of immune cells in intestine, periphery and brain
- Microbiota regulate expression of 5-HT_{1A} receptor, BDNF and NR2A

PD (Synucleinopathy)

- fecal and mucosa-associated gut microbes are different compared to healthy controls
- PD subjects show intestinal inflammation and GIT abnormalities (constipation often precedes motor deficits)
- alphaSyn inclusions appear early in the ENS and glossopharyngeal and vagal nerves (vagotomized individuals are at reduced risk for PD)

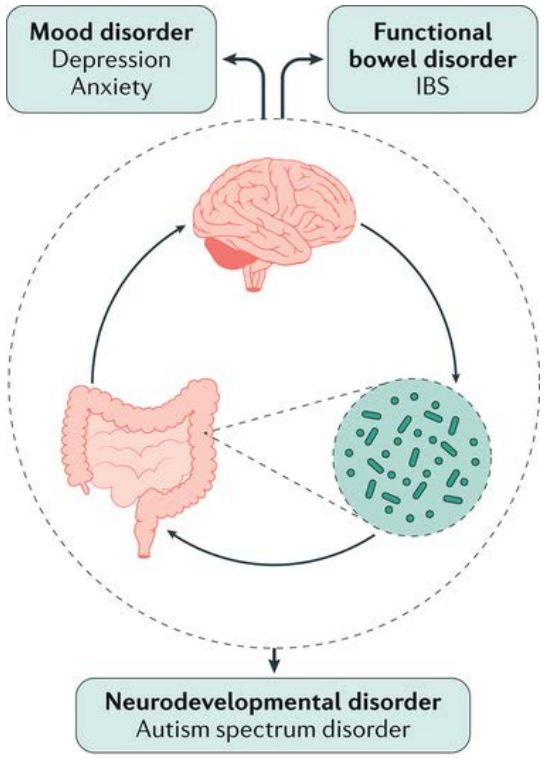
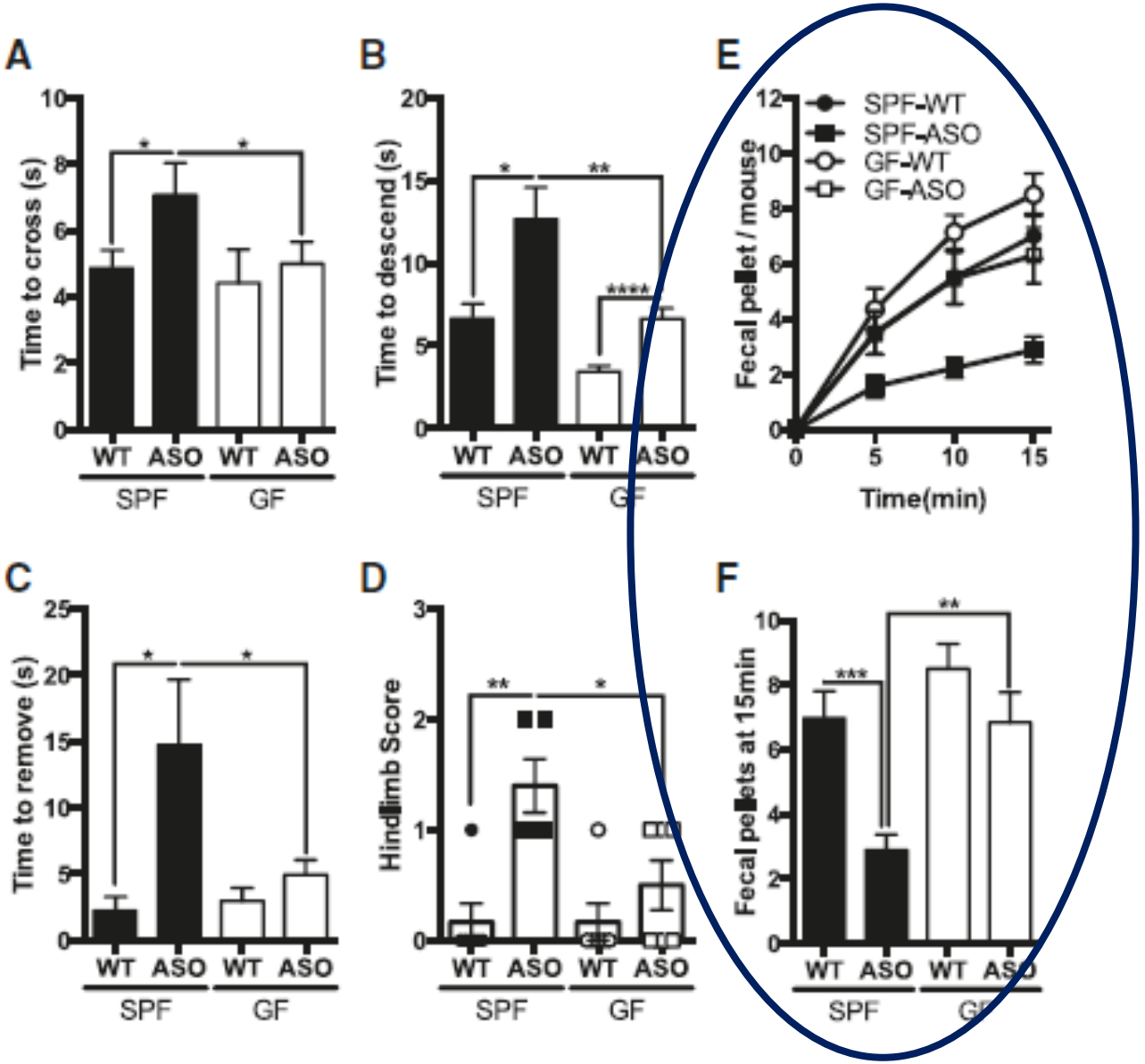


Figure 1. Gut Microbes Promote Motor and Gastrointestinal Dysfunction

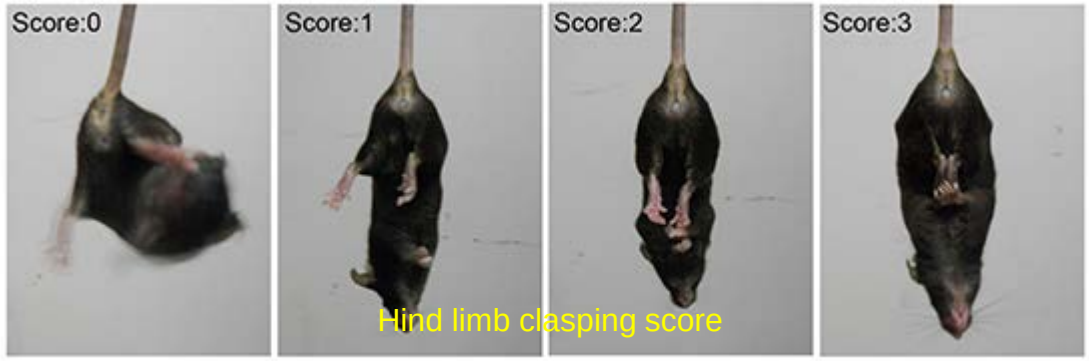


- WT and Thy1-aSyn overexpressing (ASO) mice
- SPF and GF
- Age 12-13 weeks

Traverse beam apparatus

Pole descend

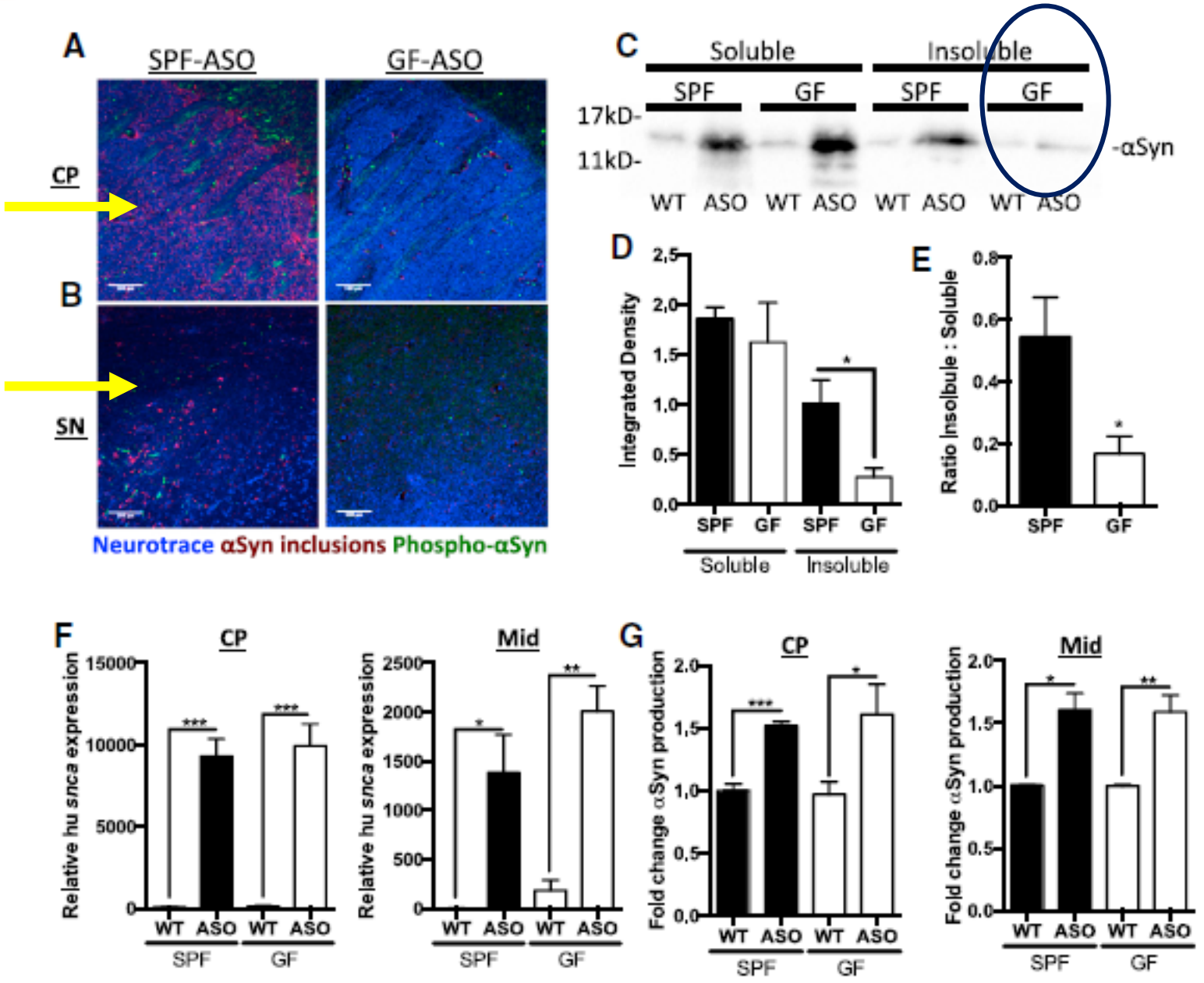
Adhesive tape removal



Zhu et al. Front. Neurosci., 16 February 2016
https://doi.org/10.3389/fnins.2016.00033

http://qpsneuro.com/in-vivo-services/behavioral-tests/motor-ability

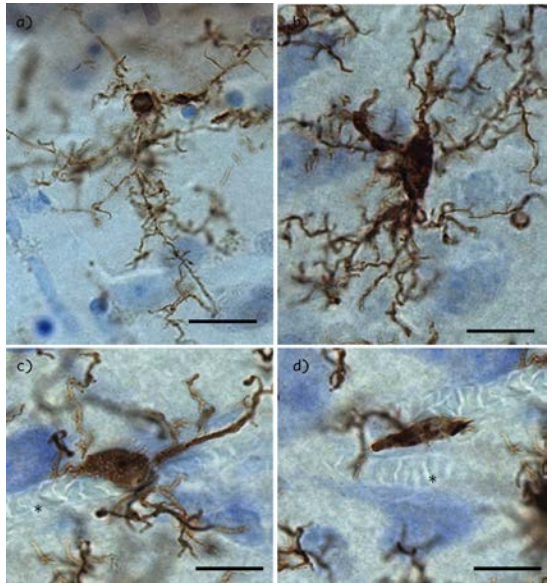
Figure 2. αSyn Pathology Is Increased in Mice Harboring a Gut Microbiota



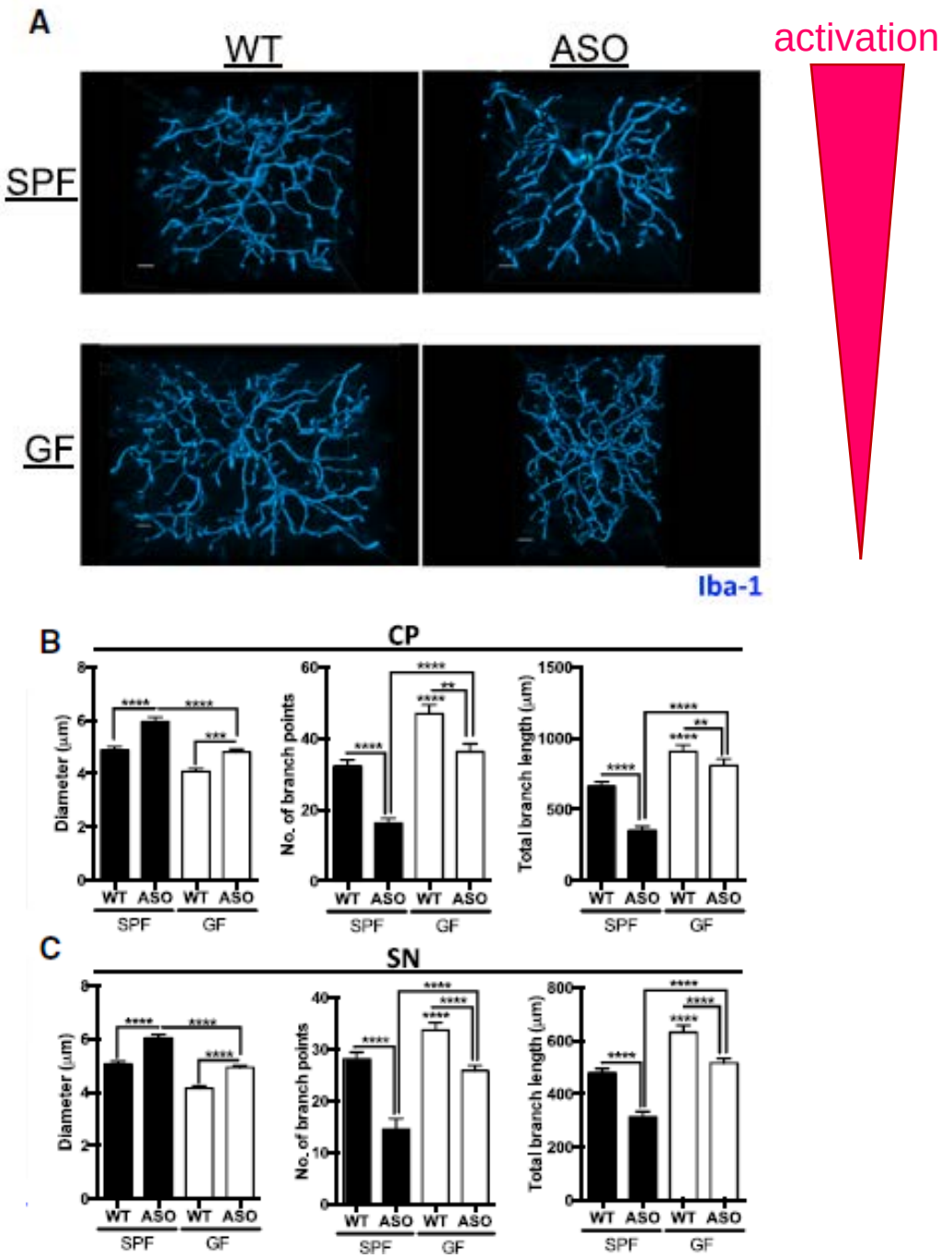
transgene expression: similar levels of aSyn transcript and protein between SPF- and GF-ASO mice

Figure 3. αSyn-Dependent Microglia Activation by the Microbiota

- Microbiota modulates immune development in the cns
- aSyn aggregates activate immune cells like microglia



Torres-Platas et al.
Journal of Neuroinflammation 2014; **11**:12



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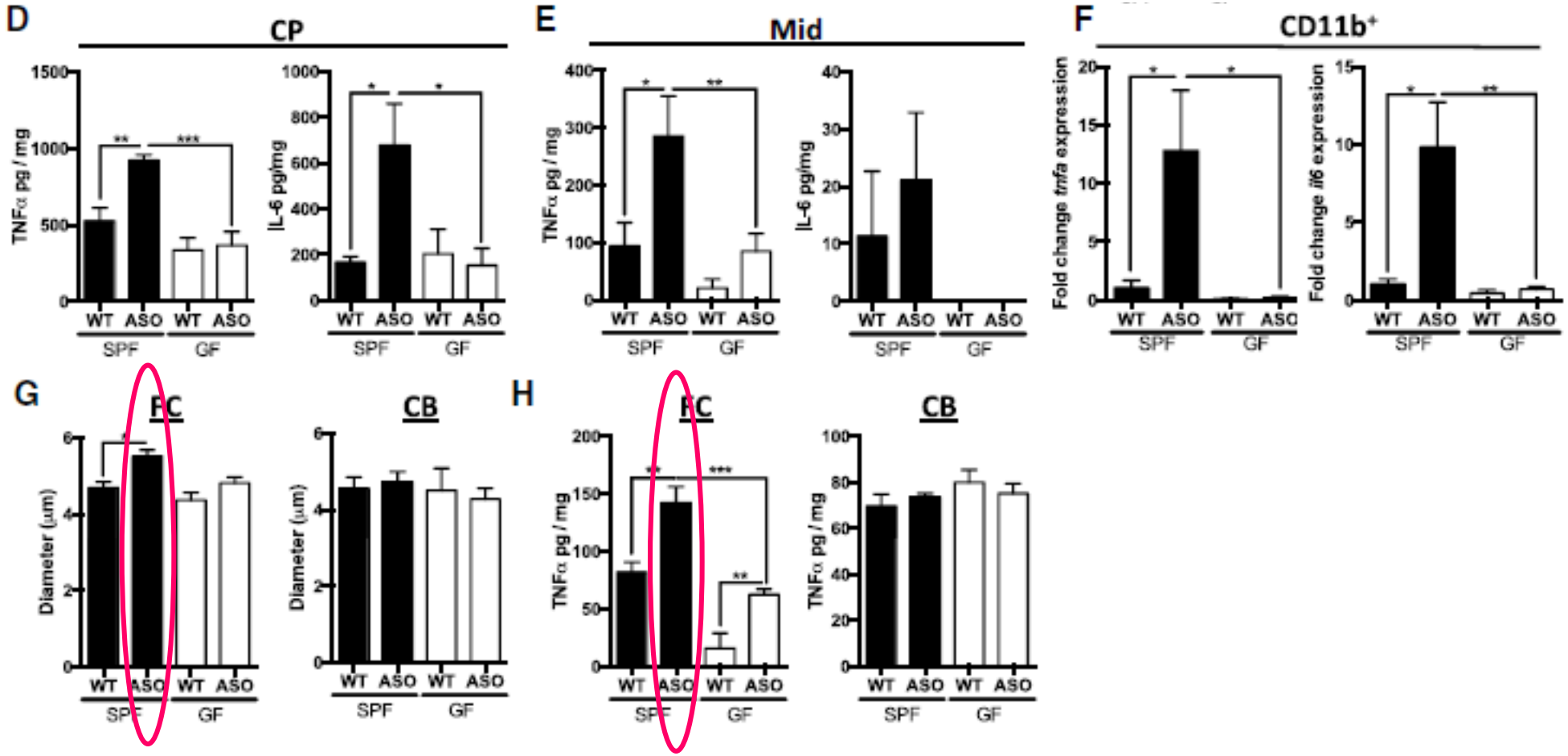
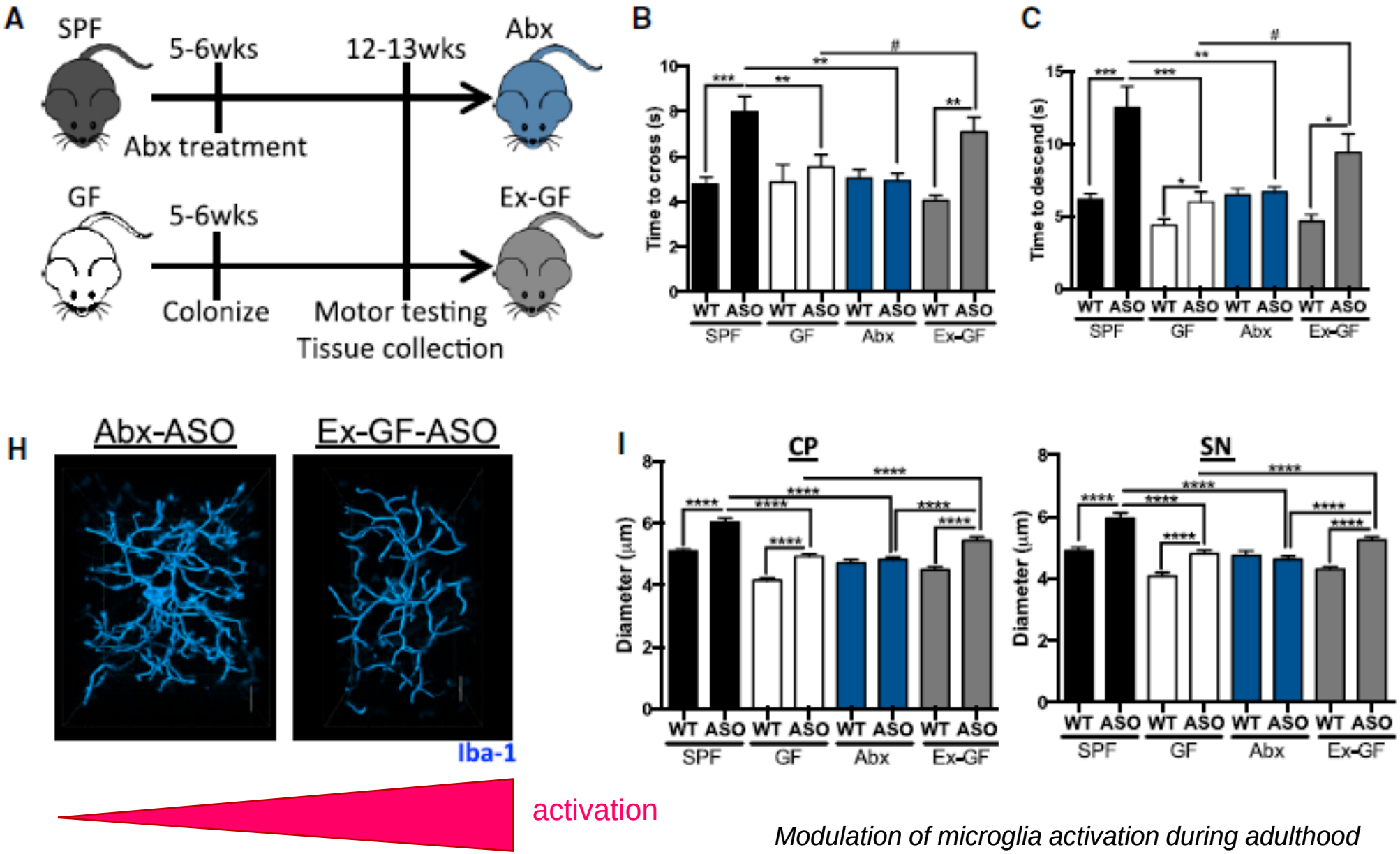


Neuroinflammatory response is region specific!

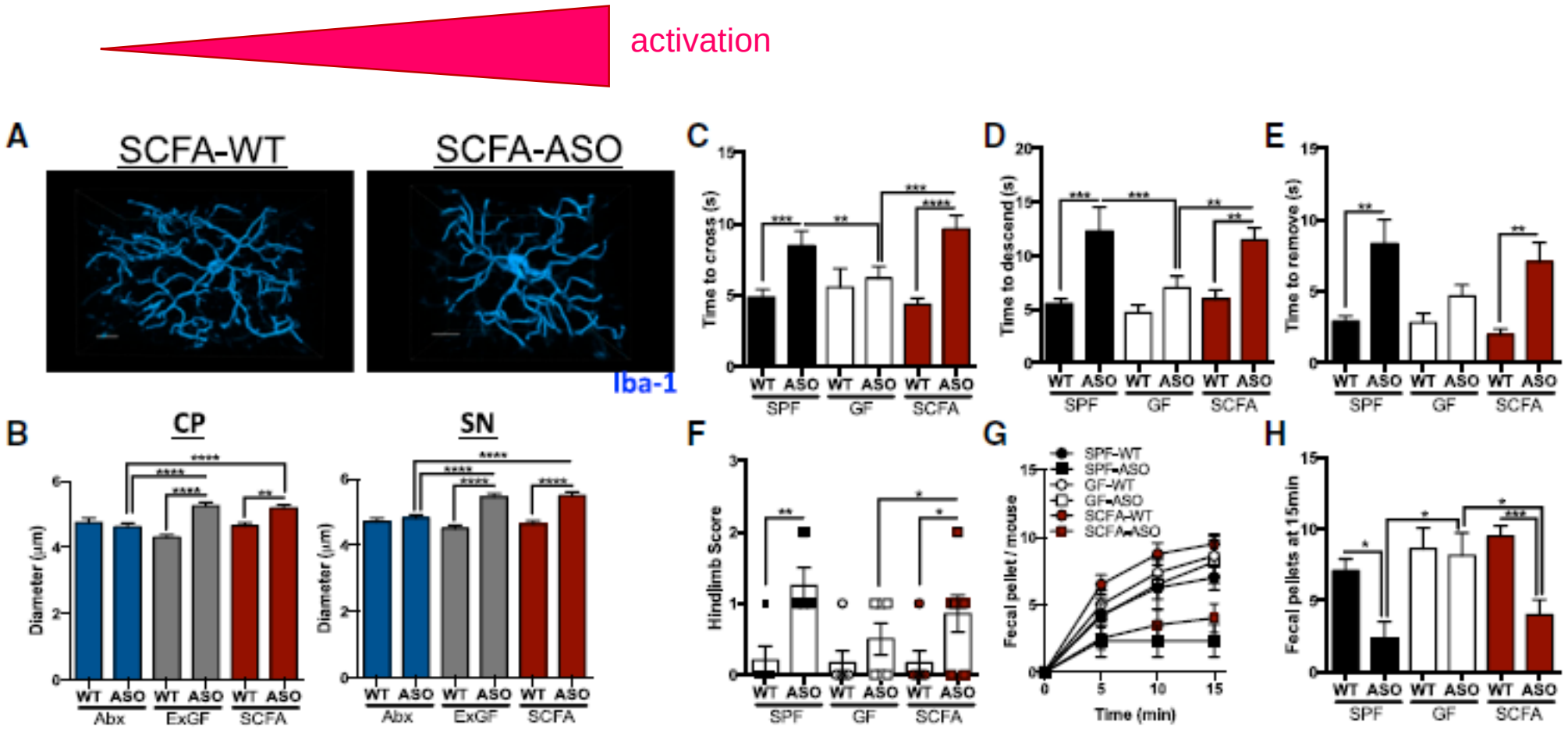
Activation in Frontal Cx

Figure 4. Postnatal Microbial Signals Promote Motor and Gastrointestinal Dysfunction



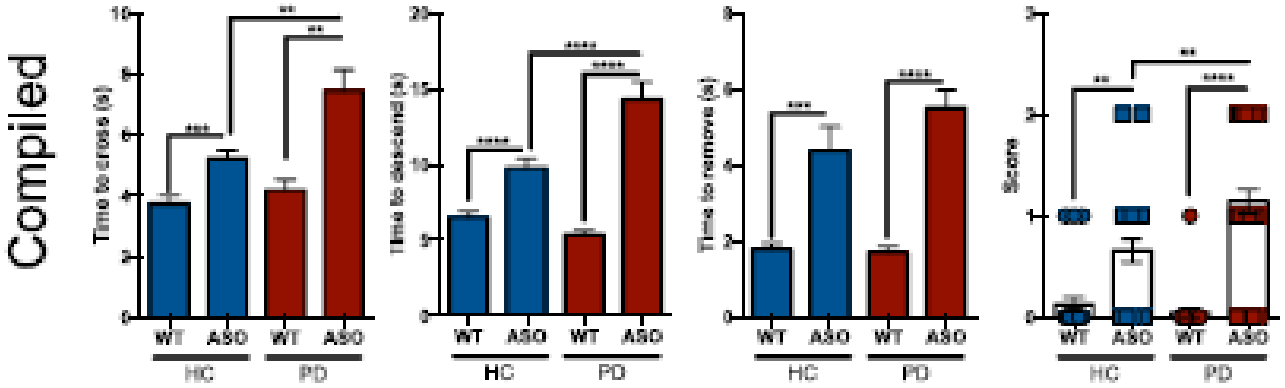
Modulation of microglia activation during adulthood contributes to aSyn-mediated motor dysfunction.

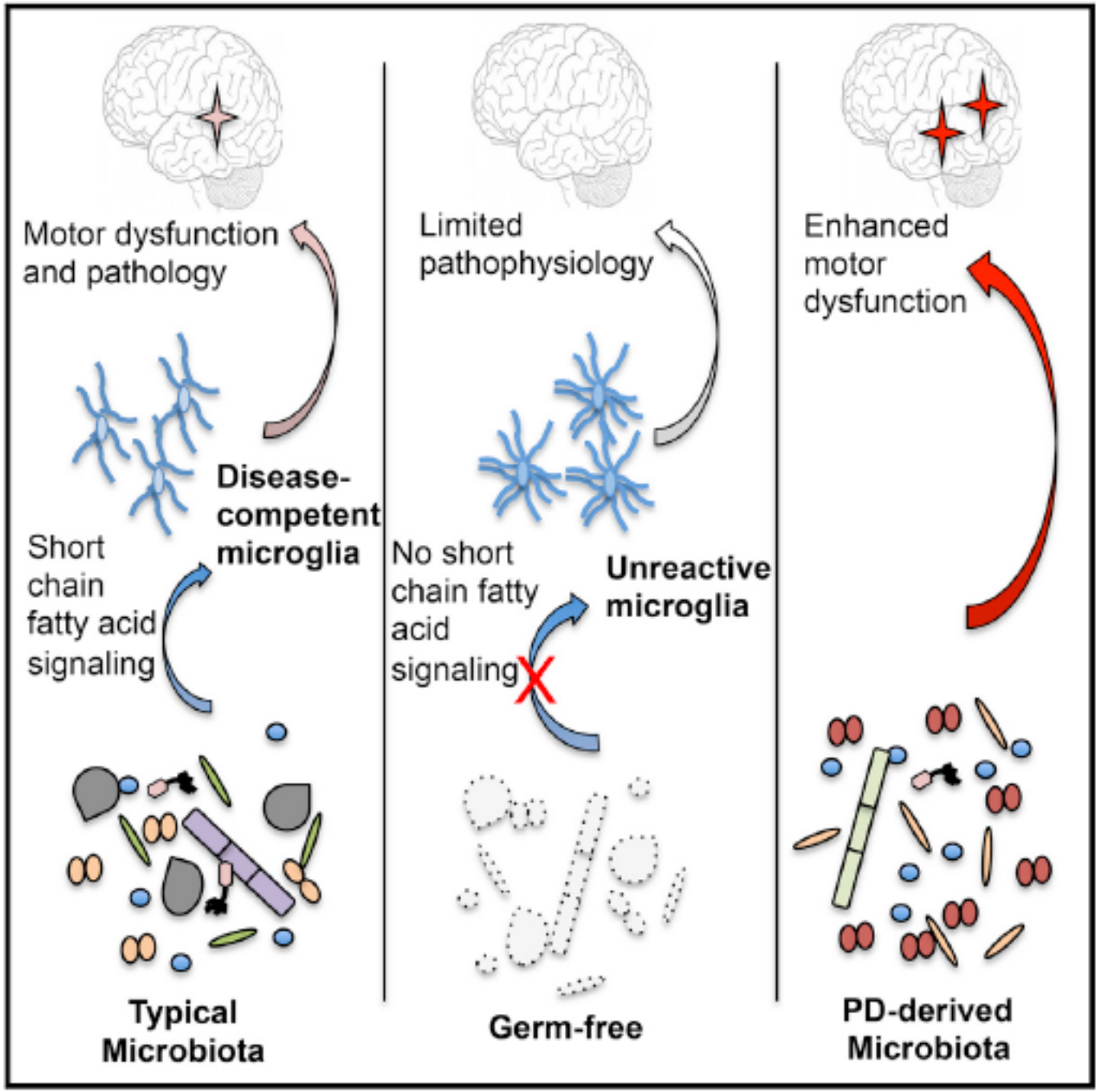
Figure 5. SCFAs Promote αSyn-Stimulated Microglia Activation and Motor Dysfunction



SCFA = Short-chain fatty acids (produced by microbiota → modulate microglia activation)

Figure 7. Microbiota from PD Patients Induce Increased αSyn-Mediated Motor Deficits





- Gut microbes promote α-synuclein-mediated motor deficits and brain pathology
- Depletion of gut bacteria reduces microglia activation
- SCFAs modulate microglia and enhance PD pathophysiology
- Human gut microbiota from PD patients induce enhanced motor dysfunction in mice

Pros and Cons - both studies

Overall:

Small animal numbers per experiment but extremely high efforts/cost intensive (housing/care taking of animals/analyses)

Mix of female and male mice during different experiments

Germ free (GF) mice

+ simplified experimental system in which diseases or specific members of the gut microbiota can be studied in isolation

- altered gut microbiota is crucial for the proper development of the host

- responses exhibited by GF animals might not reflect what actually occurs in the natural setting.

- Challenging to transfer results obtained in a GF system to the same series of events occurring in a conventional host.

Pros and Cons - both studies

Gnotobiotic mice

+ Mono-associated animal models provide information about that particular microbe's ecological niche, what it provides the host with, and how the host responds to this microbe in the absence of competition from other microbes

+ bi-associated animals can reveal how two microbes interact with one another and with the host, and whether cocolonization changes the functional roles they establish in the gut ecosystem as a result of competition for space and nutrients.

+ mono-associated and bi-associated microbiota models are powerful tools that enable us to observe how members of the microbiota change their metabolic needs in response to co-colonization.

- bacteria respond to cocolonization in species- and sequence-dependent manners.

- Interactions following artificial colonization of GF animals might not reflect the natural state.

Links

The Human Microbiome Project

http://commonfund.nih.gov/hmp/initiatives#resources

The international human microbiome consortium

http://www.human-microbiome.org/

The Earth Microbiome Project (EMP)

http://www.earthmicrobiome.org/

https://www.nestlenutrition-institute.org/resources/videos/details/gut-brain-axis-and-behavior

Further Questions?

Thank you!