NASDC

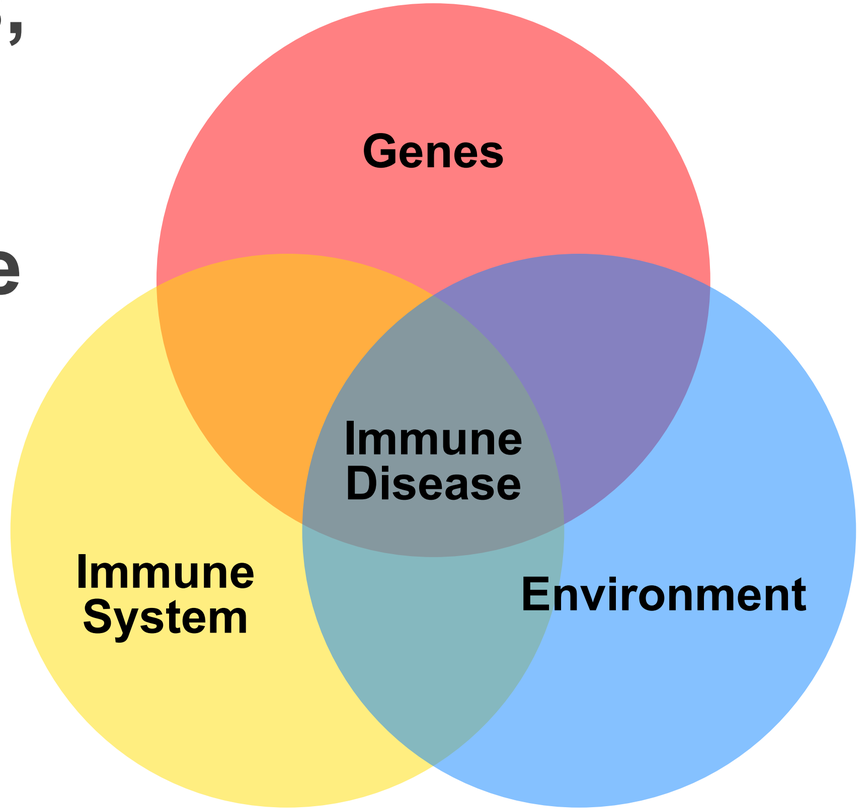
NCI Awardee Skills Development Consortium

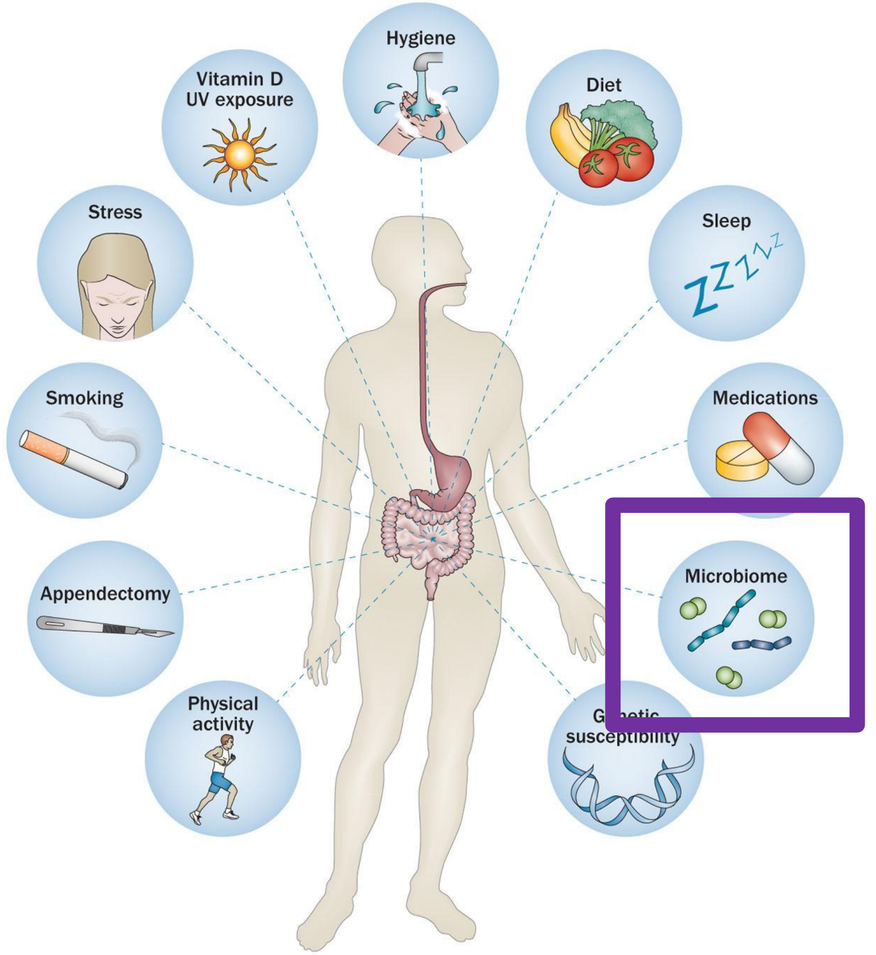
Microbiota and Human Health: A Role in Cancer

Gretchen Diehl, PhD

Memorial Sloan Kettering Cancer Center

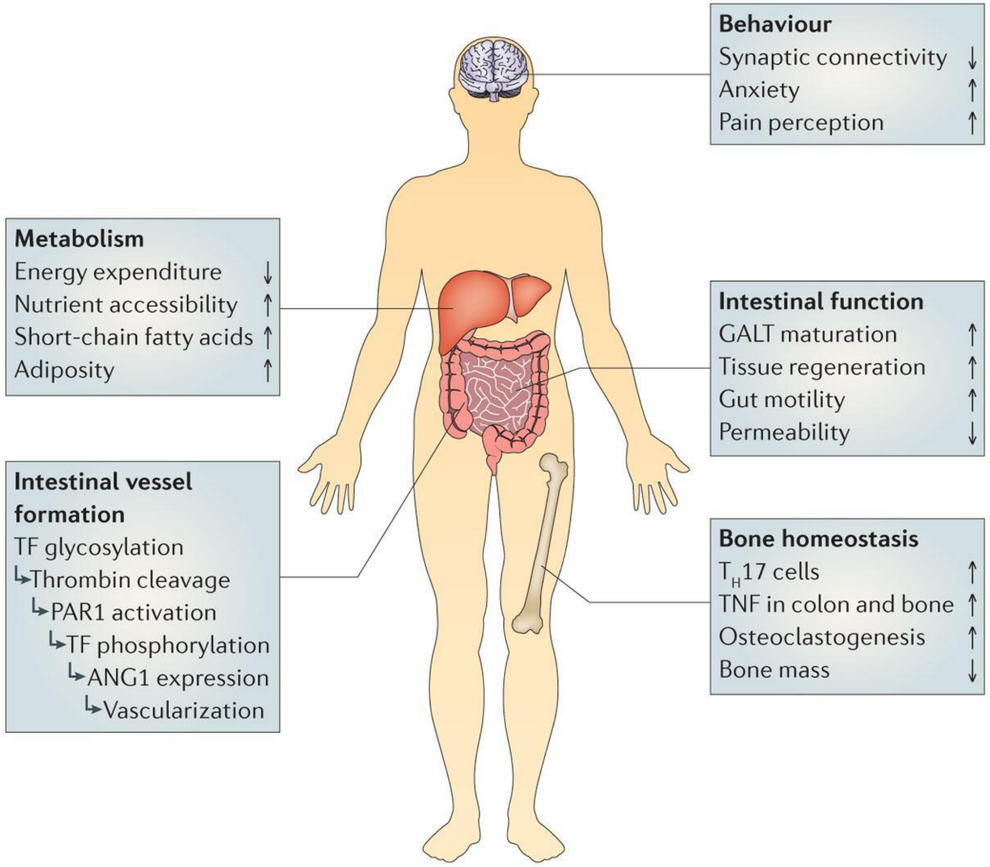
Interaction between genetics, immune system and environment underlies multiple diseases

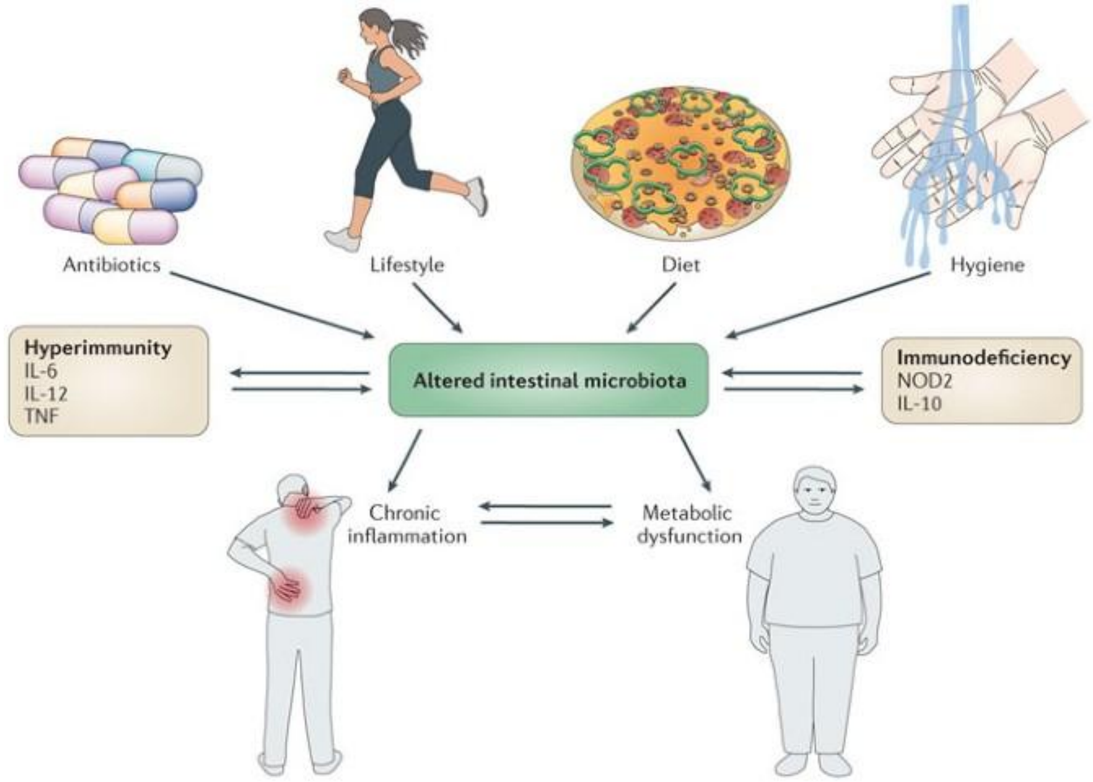




Ananthakrishnan, A. N. (2015) Epidemiology and risk factors for IBD Nat. Rev. Gastroenterol. Hepatol. doi:10.1038/nrgastro.2015.34

Microbiota (all the microbes)/Microbiome (all the genomes) are implicated in human disease





Sommer & Bäckhed (2013) The gut microbiota — masters of host development and physiology, Nature Reviews Microbiology PMID: 23435359

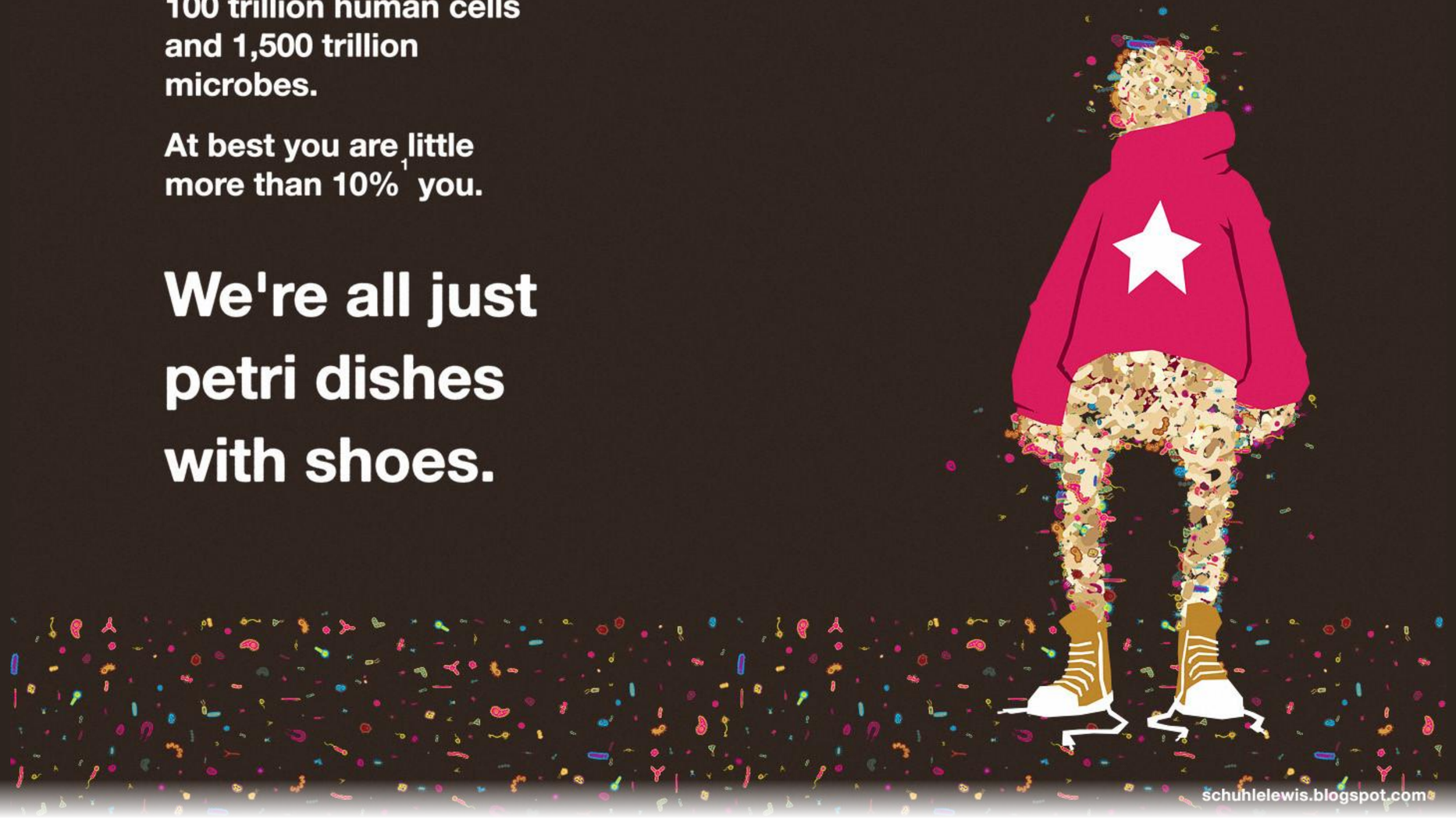
Microbiota, Health, and Cancer

- How to measure the microbiota
- Describe key examples of microbiota regulation of health: colonization resistance, immune system development, hematopoiesis, vaccines, barrier repair, breakdown of dietary components and drugs
- Describe microbial metabolites (eg SCFA) that regulate host functions
- Describe contribution to disease states: alteration in community abundance in inflammatory disease
- Microbiota and tumors: drivers, dysbiosis, therapies, outcomes
- Keeping the microbiota in mind (more work to be done)

In the average adult are 100 trillion human cells and 1,500 trillion microbes.

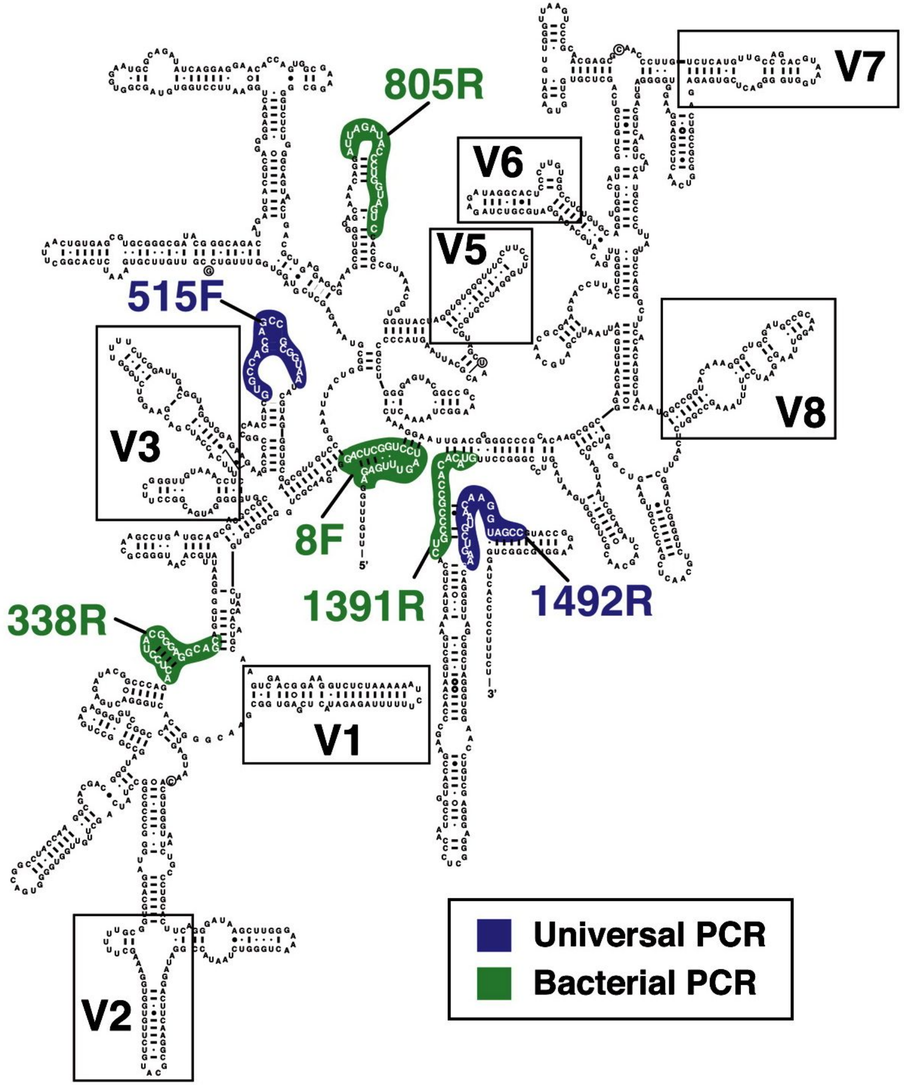
At best you are little more than 10%[1] you.

We're all just petri dishes with shoes.



Defining the Microbiota

- Culture-independent system based on 16S rRNA to organize bacteria species
- Metagenomics: composite genomes



Memorial Sloan Kettering Cancer Center

Comparing the Microbiota: alpha and beta diversity

***Alpha diversity* ≈** species diversity WITHIN community (sample)

- Richness: the total number of species in a community. May underestimate as limited by sequencing depth

- Phylogenetic diversity: phylogenetic relationships between species in a community (sample).

- Evenness: species abundances (diversity normalized to richness)

- Dominance: negatively correlated with diversity. High dominance: one or few species are the majority

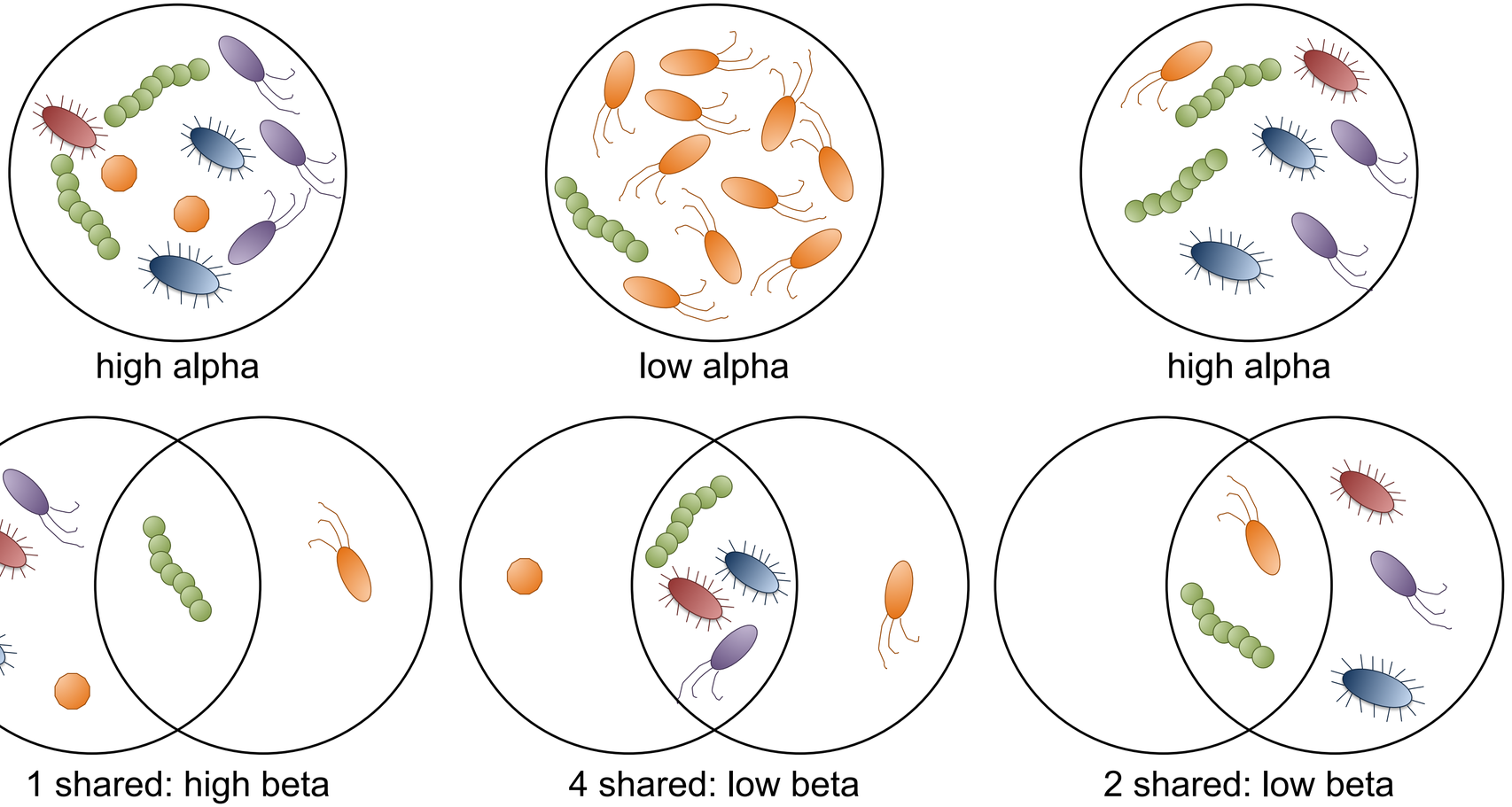
Diversity increases with increased richness and evenness.

***Beta diversity* ≈** (dis-)similarities BETWEEN communities (samples)

- High alpha diversity : high number of equally abundant species
- High beta diversity : two communities share few species
- Low beta diversity : two communities share most species

https://microbiome.github.io/OMA/microbiome-diversity.html

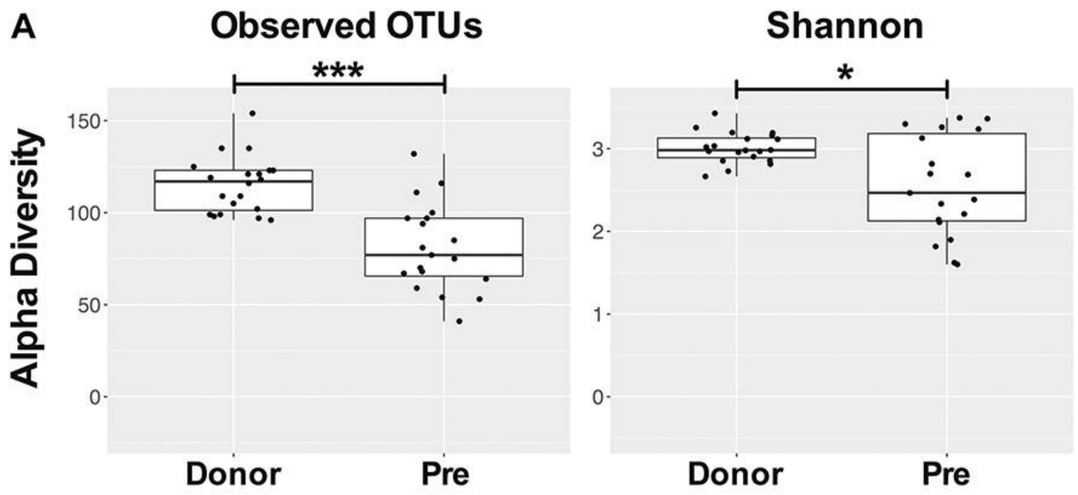
Comparing the Microbiota: alpha and beta diversity



Adapted from
Finotello 2018
PMID: 28025179

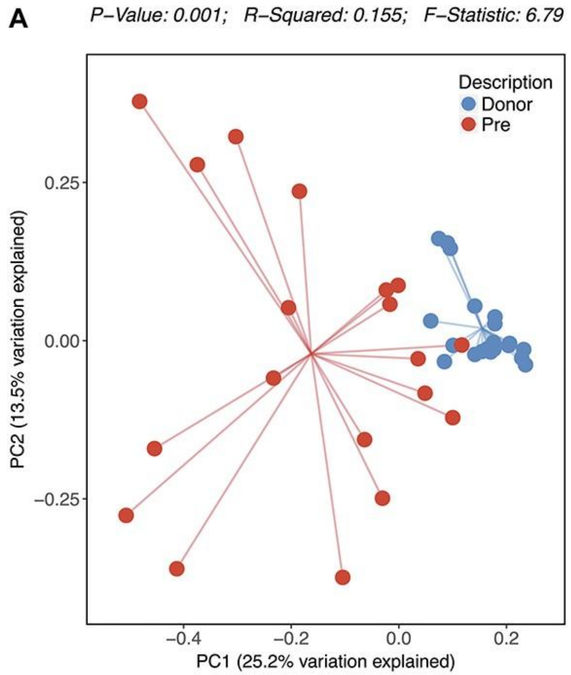
Comparing the Microbiota: alpha and beta diversity

Alpha



Jacob 2017 PMCID: PMC6159890

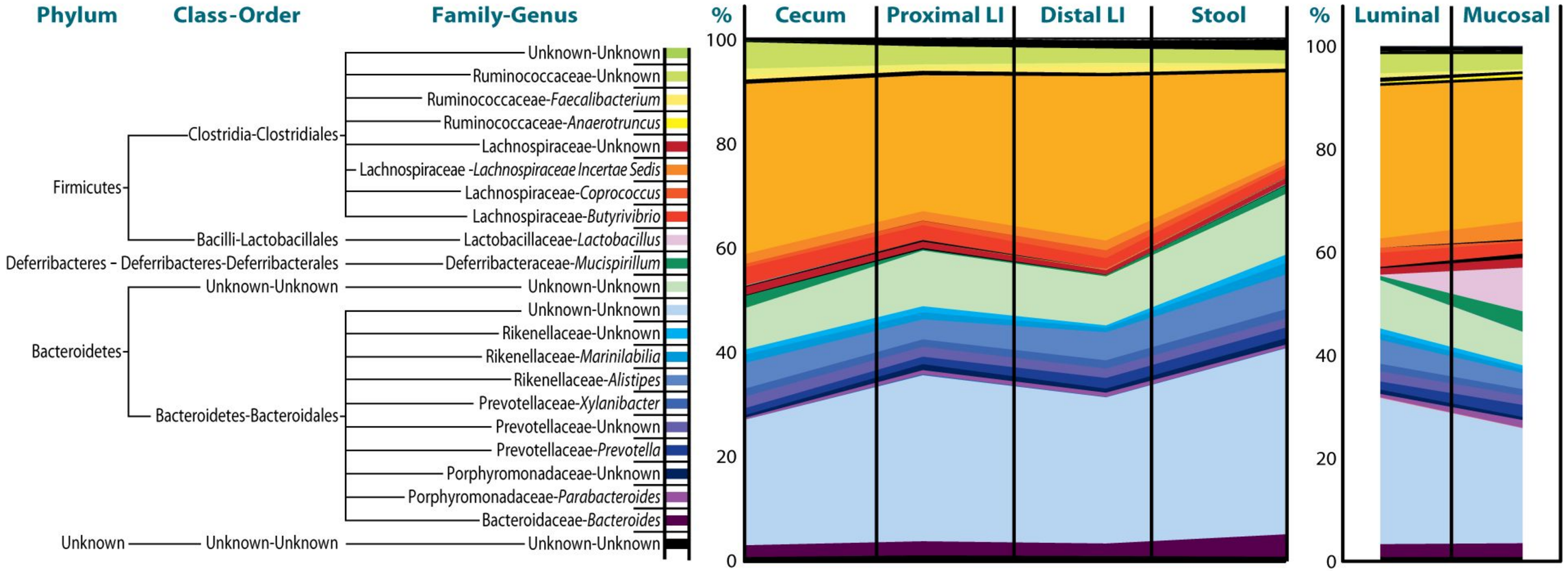
Beta



Principal coordinate analysis plot

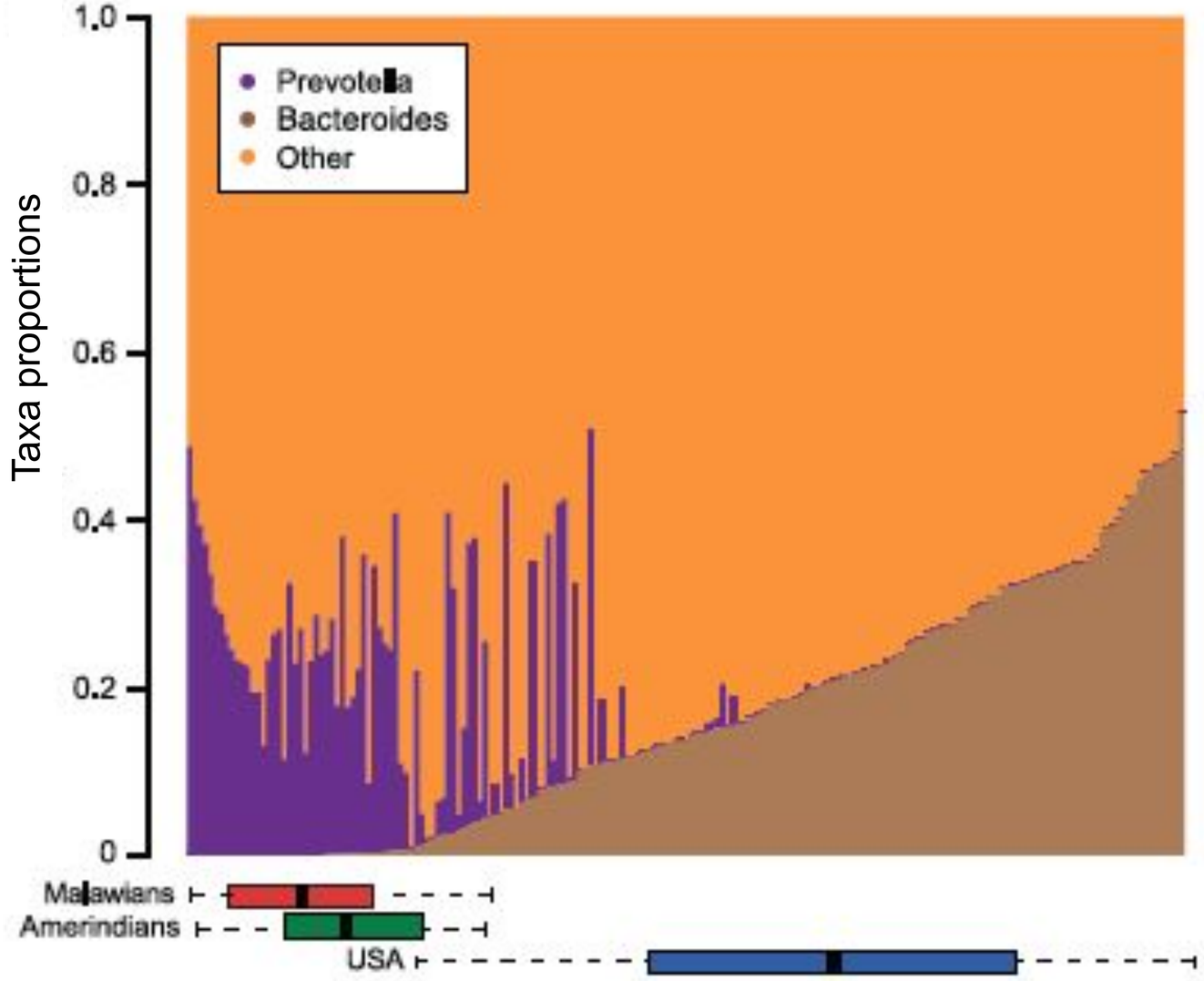
Boxplot to display the top differential abundant bacteria (genus, family, etc)

Bacteroidetes and Firmicutes Dominate Intestinal Microbiome



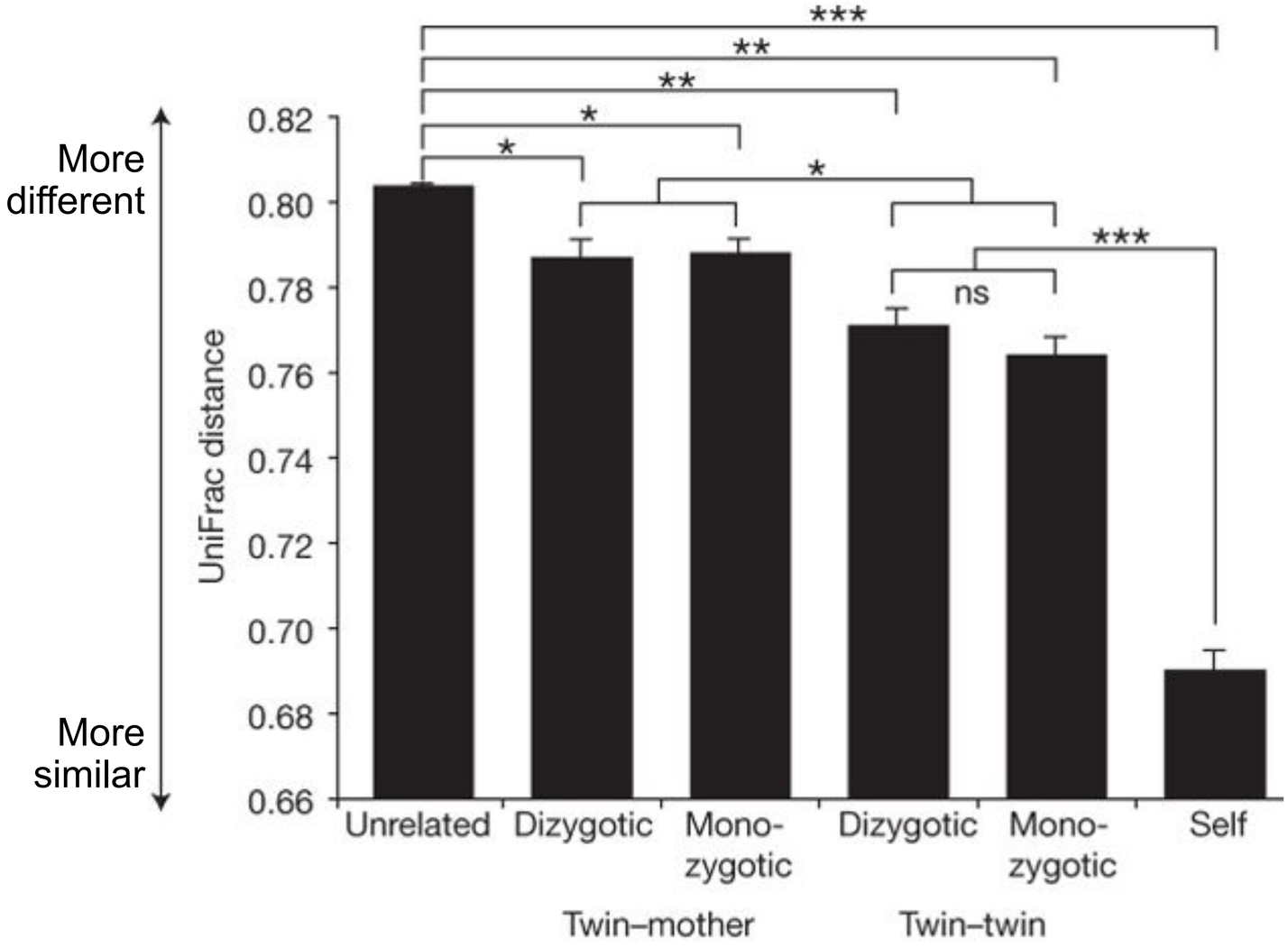
Hill DA, Artis D. 2010. Annu. Rev. Immune. 28:623–67

Cultural/Geographic Region Affects Microbiome



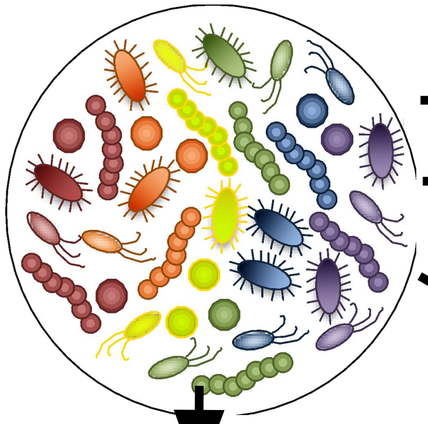
Yatsunenko, Nature 2012 PMCID: PMC3376388

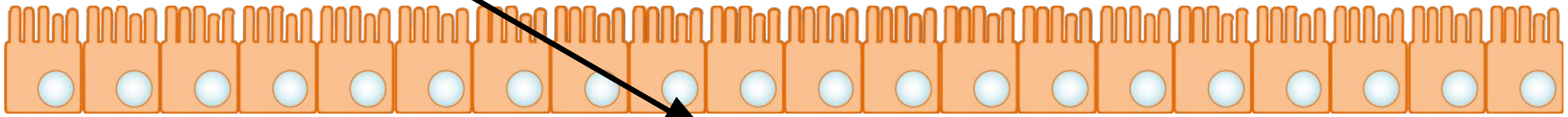
Significant Interpersonal Variation in Microbiota



Turnbaugh Nature 2009 PMCID: PMC2677729

Microbiota



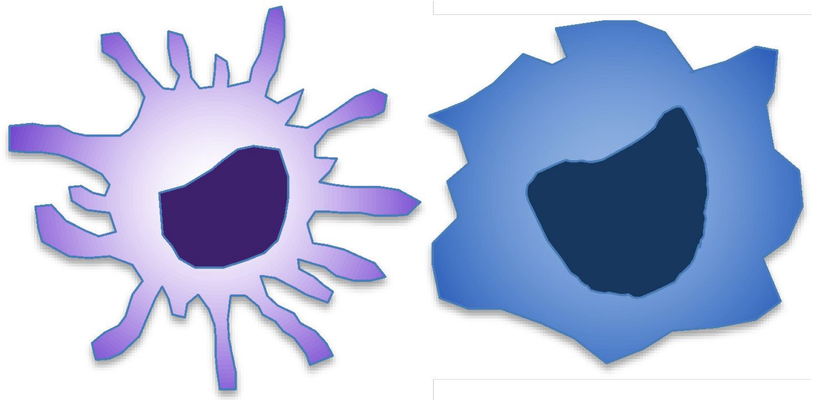


Barrier function

- Epithelial cell differentiation and proliferation
- Intestinal repair

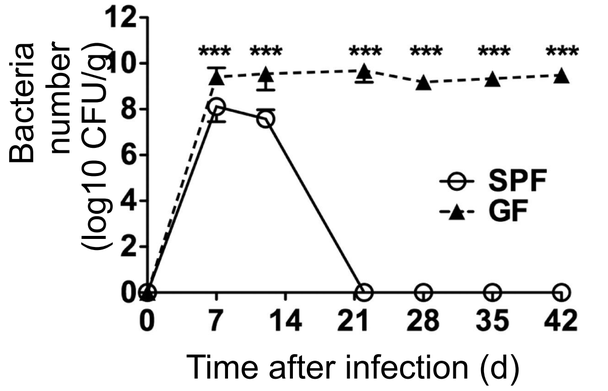
Anti-microbial function

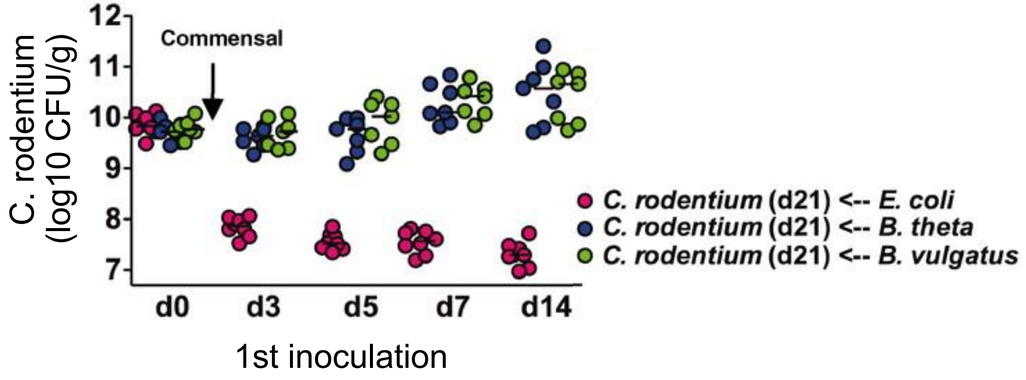
- Induction of anti microbial peptides
- Mucus

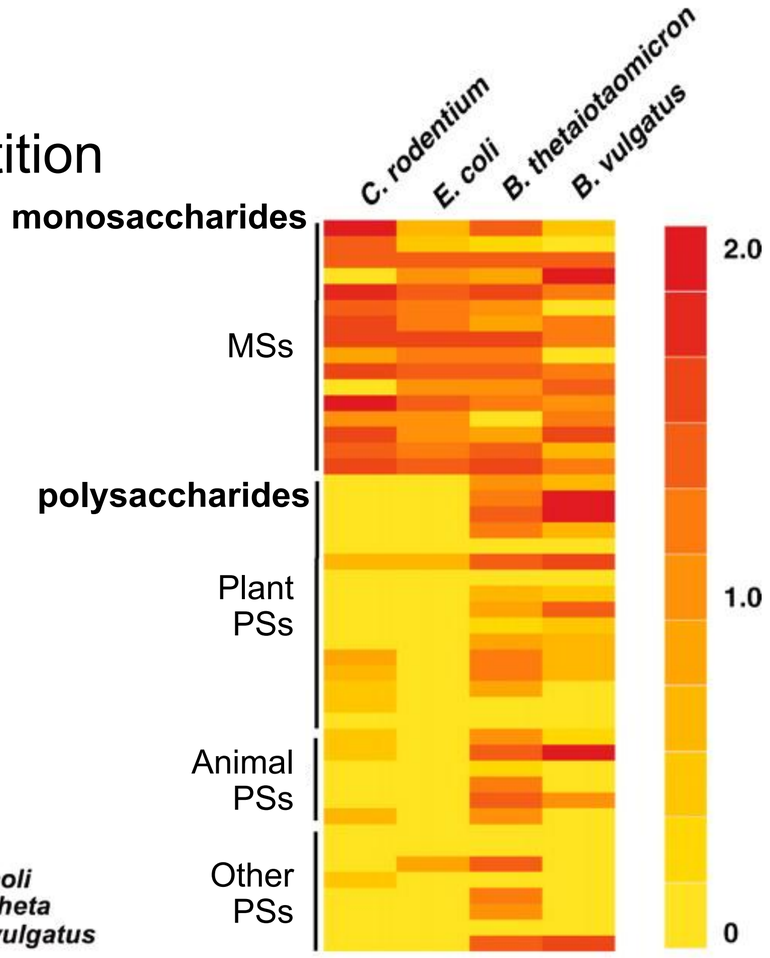


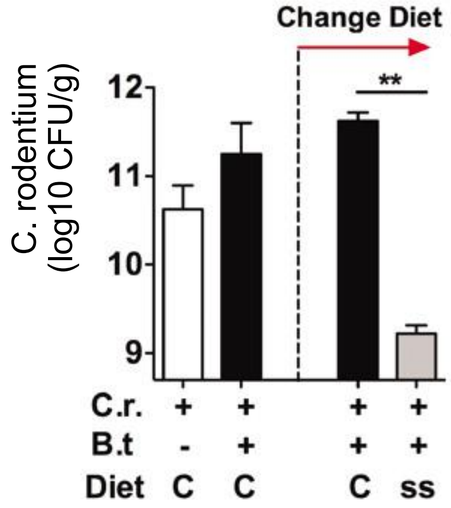
Colonization Resistance

Microbiota protect through nutrient competition

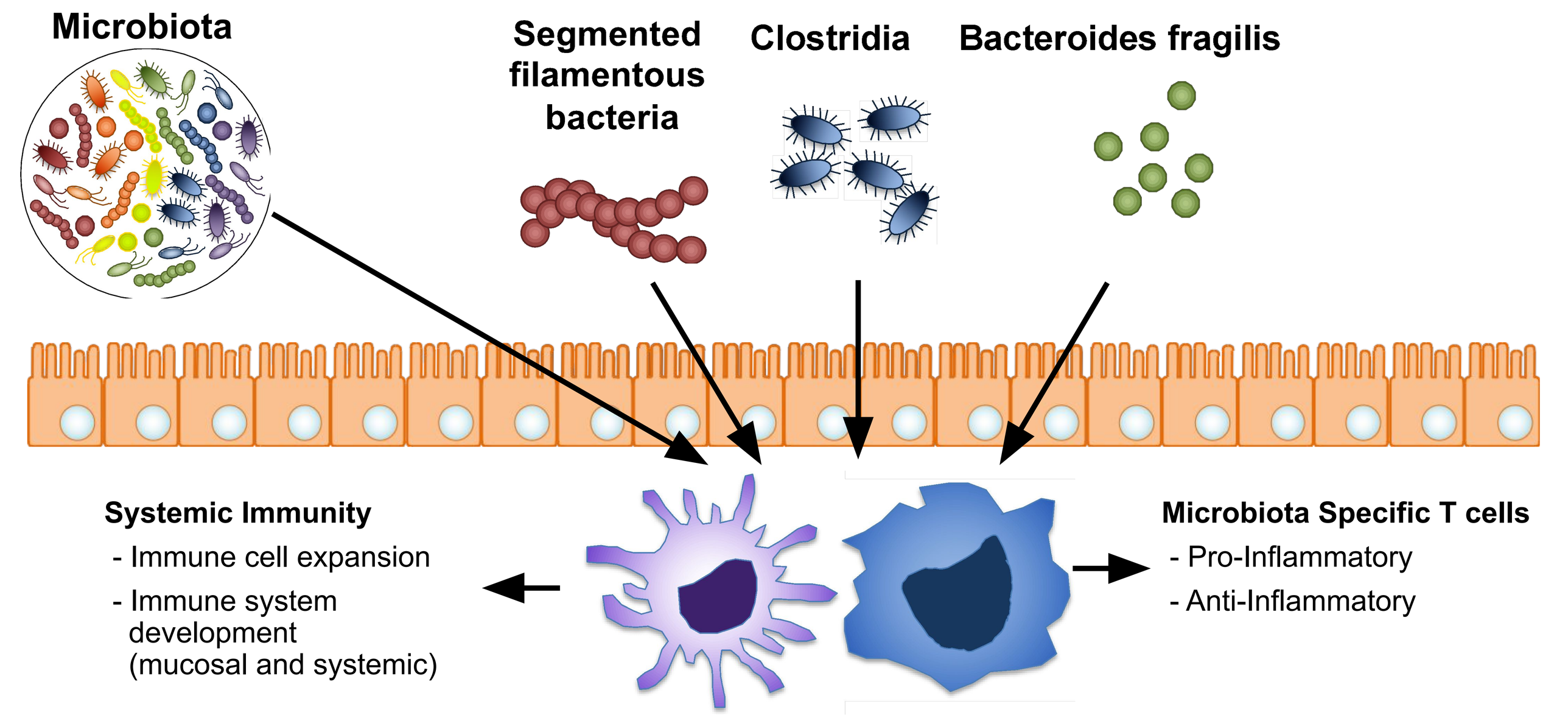








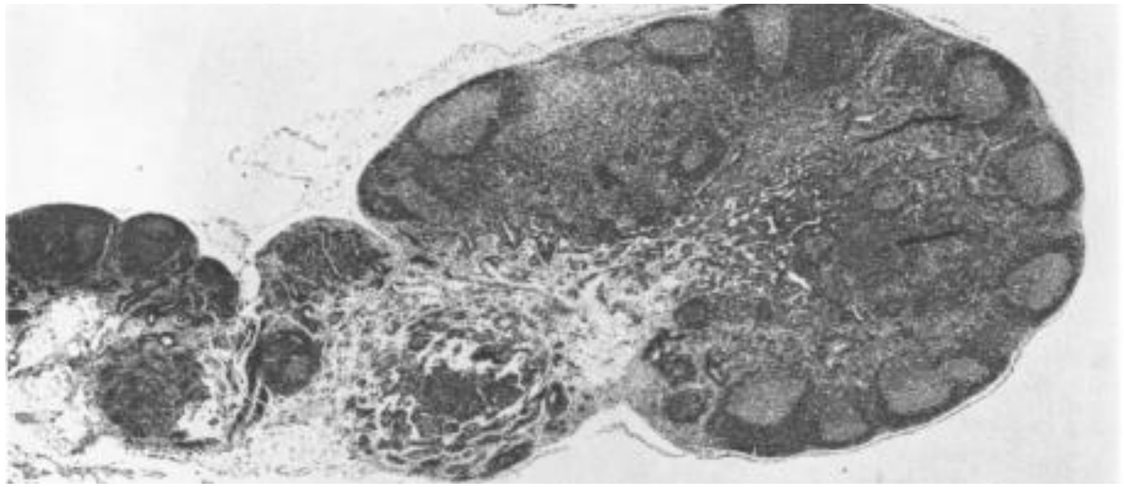
Kamada et al. Science 2012 PMCID: PMC3439148


Microbiota
Segmented filamentous bacteria
Clostridia
Bacteroides fragilis
Systemic Immunity
- Immune cell expansion
- Immune system development (mucosal and systemic)
Microbiota Specific T cells
- Pro-Inflammatory
- Anti-Inflammatory

Microbiota Regulation of Immune System Development and Function

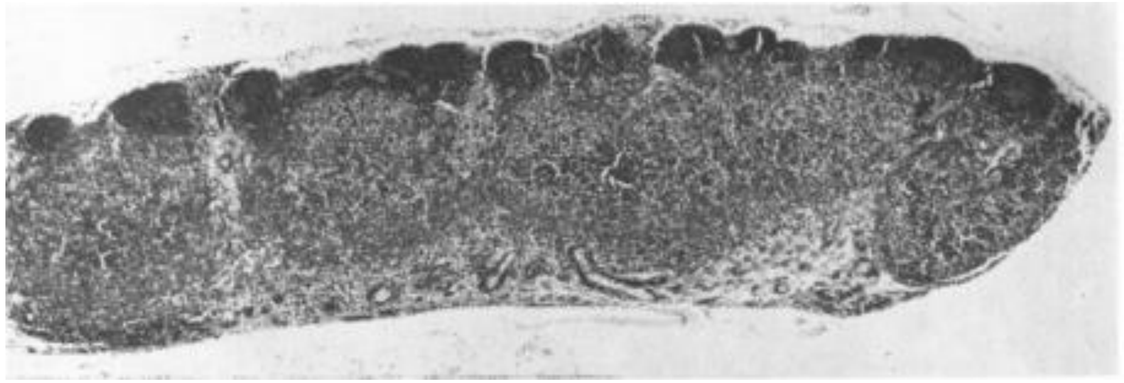
Mesenteric lymph node in conventional mice:

Numerous B cell follicles



Mesenteric lymph node in germ free mice:

No B cell follicles



Bauer, Horowitz, Levenson, Popper. 1963.The response of the lymphatic tissue to the microbial flora. Studies on germfree mice. Am J Pathol. 42:471-83. PMCID: PMC1949649

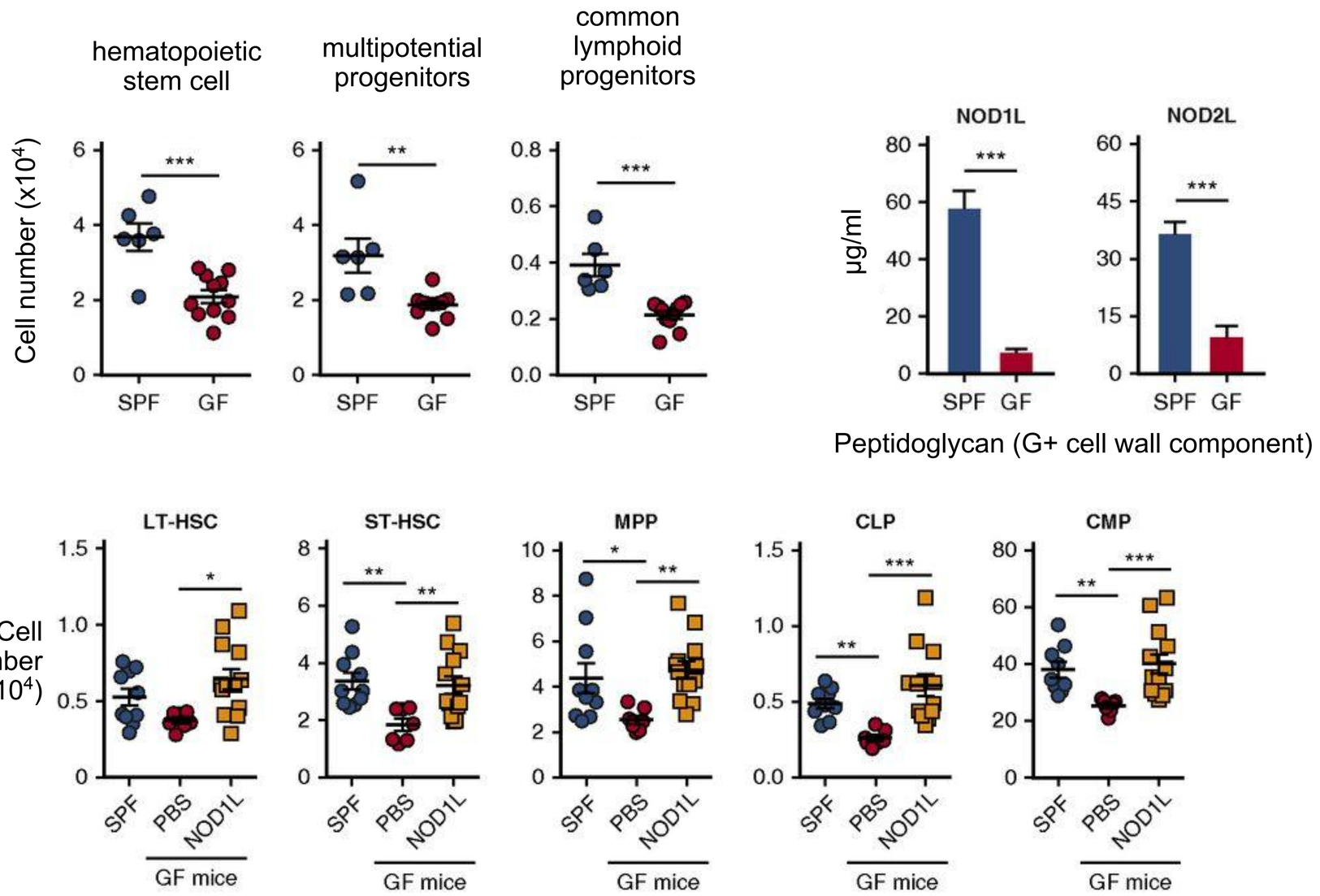
Immune Defects in germfree Animals

Immunological defect	Site	Phenotype in germ-free mice compared with conventionally housed mice
Development of small intestine	Peyer's patches	Fewer and less cellular
	Lamina propria	Thinner and less cellular
	Germinal centres	Fewer plasma cells
	Isolated lymphoid follicles	Smaller and less cellular
Development of mesenteric lymph nodes	Germinal centres	Smaller, less cellular and with fewer plasma cells
$CD8^+$ T cells	Intestinal epithelial lymphocytes	Fewer cells and with reduced cytotoxicity
$CD4^+$ T cells	Lamina propria	Fewer cells; decreased T_H17 cells in the small intestine but increased T_H17 cells in the colon
$CD4^+CD25^+$ T cells	Mesenteric lymph nodes	Reduced expression of FOXP3 and reduced suppressive capacity
Expression of angiogenin 4	Paneth cells	Reduced
Expression of REG3γ	Paneth cells	Reduced
Production of secretory IgA	B cells	Reduced
Levels of ATP	Intestine	Reduced
Expression of MHC class II molecules	Intestinal epithelial cells	Reduced
Expression of TLR9	Intestinal epithelial cells	Reduced
Levels of IL-25	Intestinal epithelial cells	Reduced

FOXP3, forkhead box P3; IL-25, interleukin 25; REG3γ, regenerating islet-derived 3γ; T_H17, T helper 17; TLR9, Toll-like receptor 9.

Round & Mazmanian. 2009
doi:10.1038/nri2515

Microbiota Regulation of Hematopoiesis



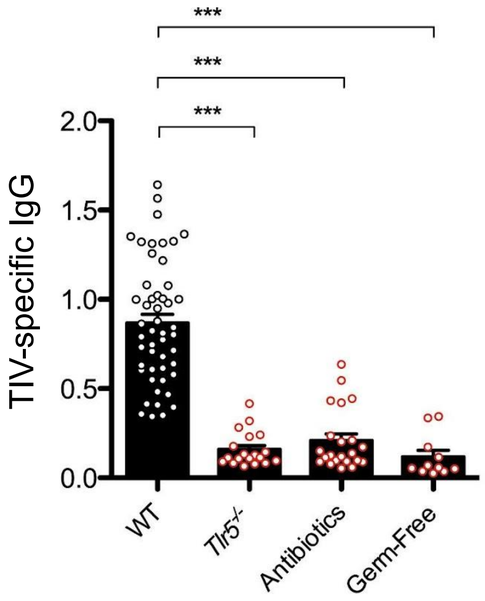
Iwamura (2017) Blood,
PMCID: PMC5234217

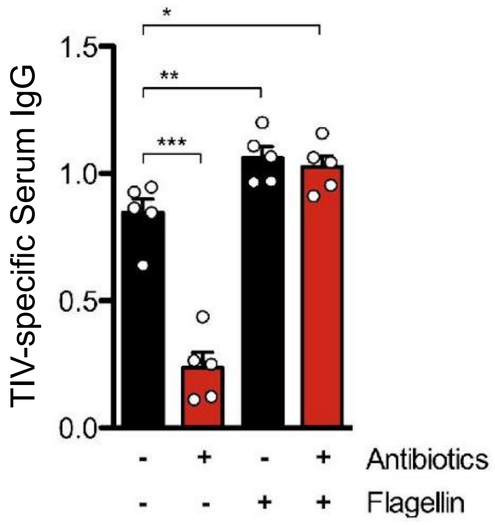
Microbiota as a Vaccine Adjuvant

- Trivalent inactivated influenza vaccine (TIV): subunit vaccine, HA from 3 flu strains, unadjuvanted

- Earlier work: in humans found correlation between higher TLR5 expression and flu vaccine responsiveness

- Loss of responses in antibiotic treated in inactivated polio but not adjuvanted vaccines or live-attenuated yellow fever



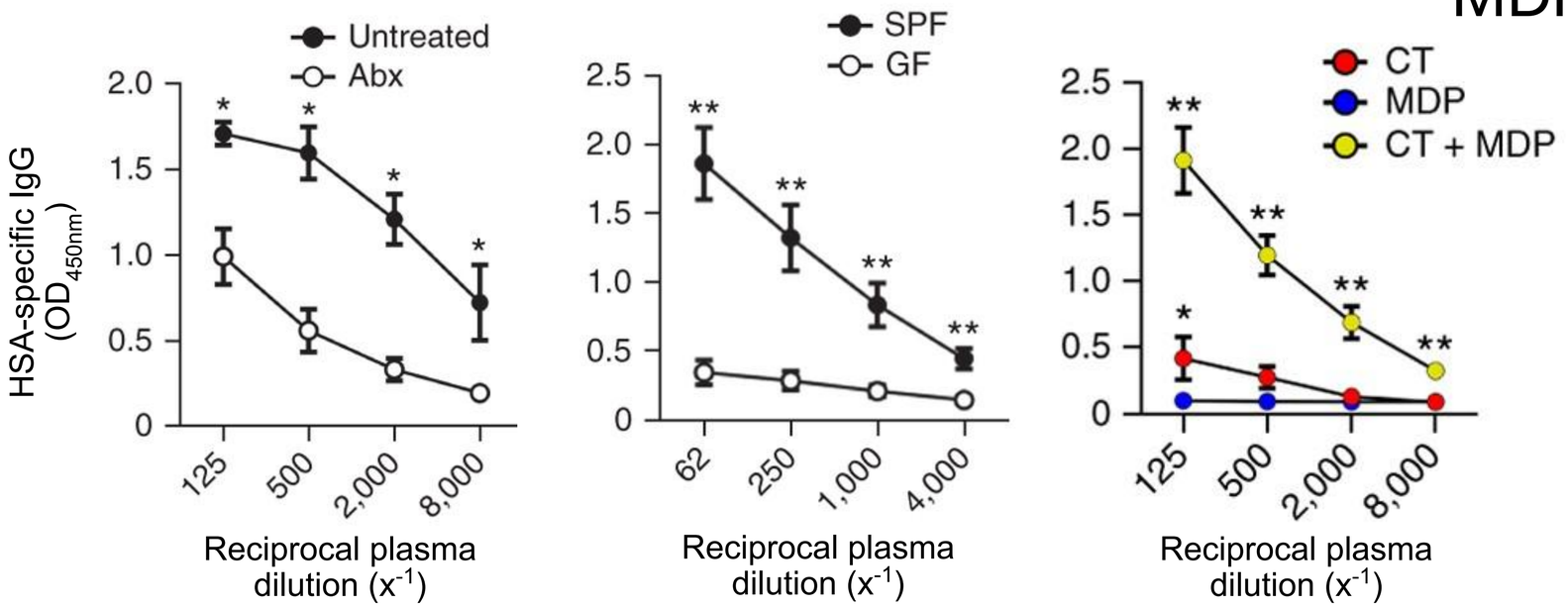


Oh. 2014. Immunity PMID: 25220212

Microbiota as a Vaccine Adjuvant

- Cholera toxin: mucosal adjuvant (responsible for diarrhea associated with cholera)

MDP=NOD ligand

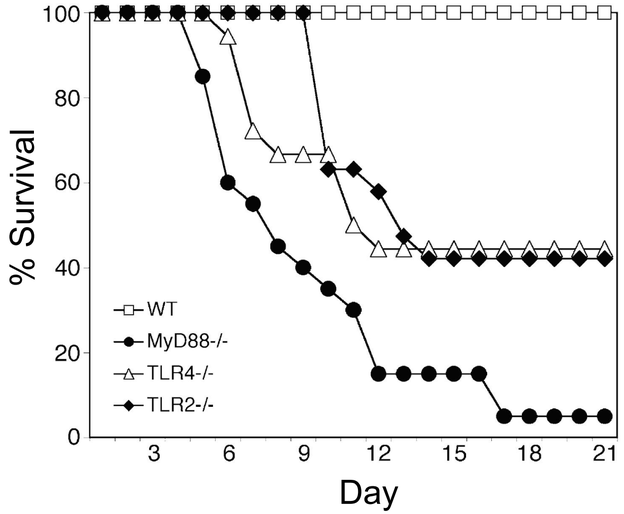


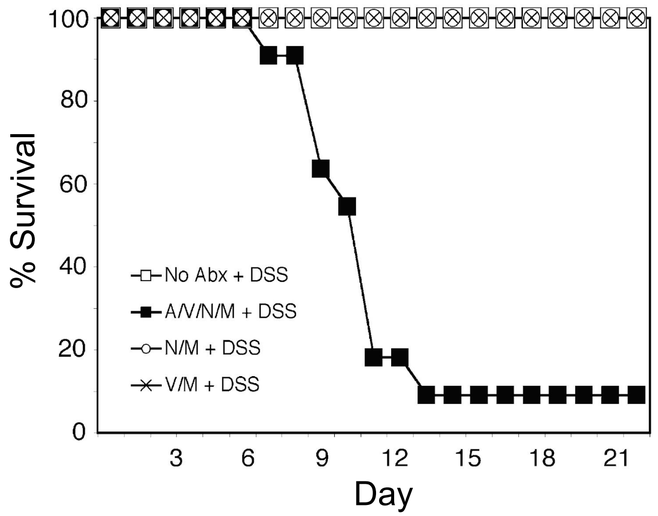
Kim. 2016. Nature Medicine PMID: 27064448

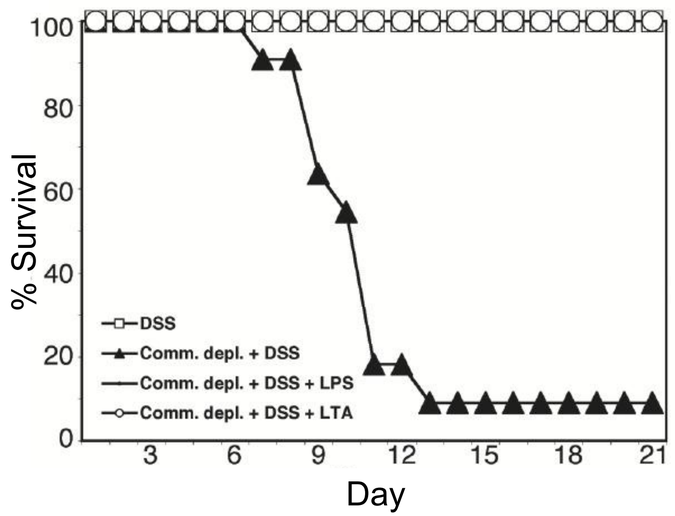
Microbiota Improves Barrier Repair After Intestinal Injury

Dextran sodium sulfate (DSS):

- chemical model of colitis
- causes epithelial damage







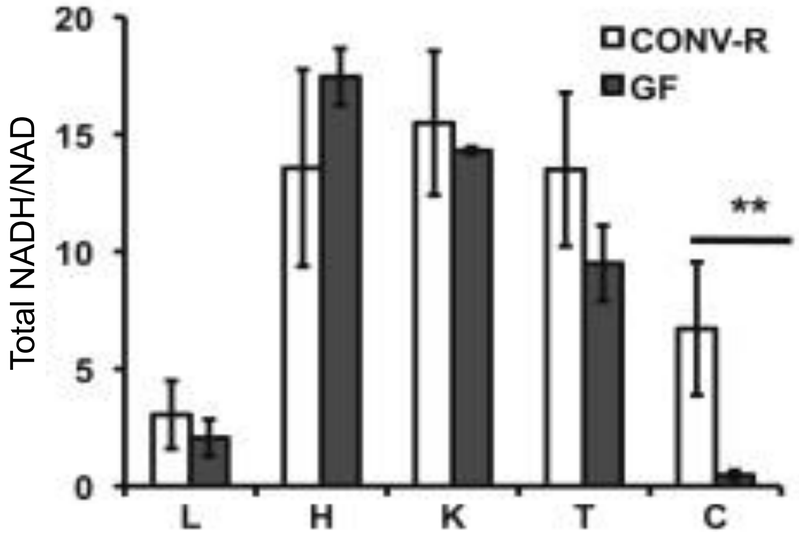
Rakoff-Nahoum (2004)
Cell PMID: 15260992

Microbiota Directly Impacts Health of Epithelial Cells

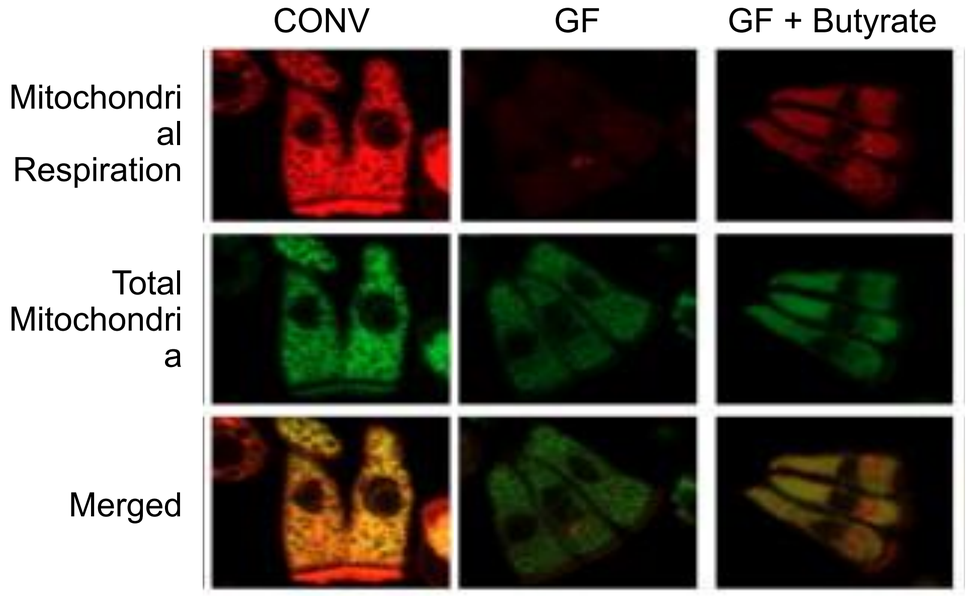
- Short-chain fatty acids (SCFAs), primarily acetate, propionate and butyrate:
  product of bacterial fermentation by obligate anaerobes (Firmicutes, Bacteroidetes, and Clostridium)
- Energy salvage: breakdown of undigested dietary carbohydrates (fiber) (primarily), mucus, and sloughed epithelial cells.
  contribute 5–15% of human total caloric requirements
- Energy source
  Butyrate: epithelial cells,
  Acetate: muscle and adipose tissue
-Signal through receptors: GPR109 (butyrate), GPR43 (acetate/proprionate)
  Butyrate can activate transcription as a HDAC inhibitor

*Multiple beneficial effects: anti-inflammatory, epithelial barrier, Tregs, macrophage microbial killing, satiety (regulates GLP-1), and oxidative stress**

SCFA Butyrate is an Energy Source for Colonic Epithelial Cells



- Specific metabolic defect in defect in colon from GF mice

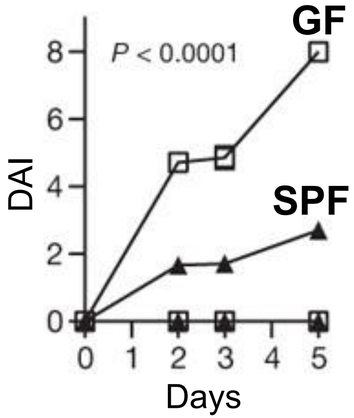


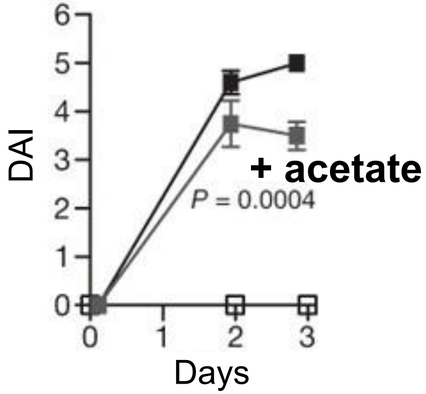
- Butyrate rescues (partially) mitochondria respiration

Donohoe et al. (2011) Cell Metabolism. PMID: 21531334

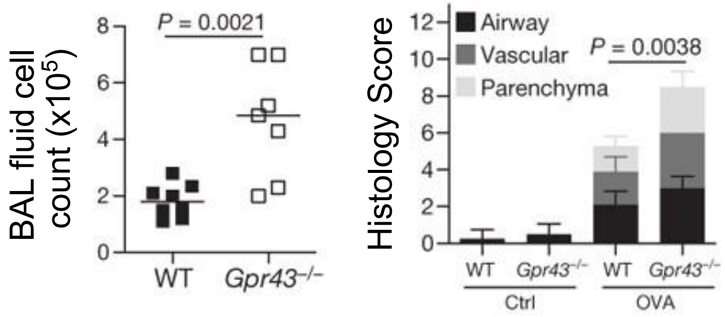
SCFA Protect from Inflammatory Disease

Colitis



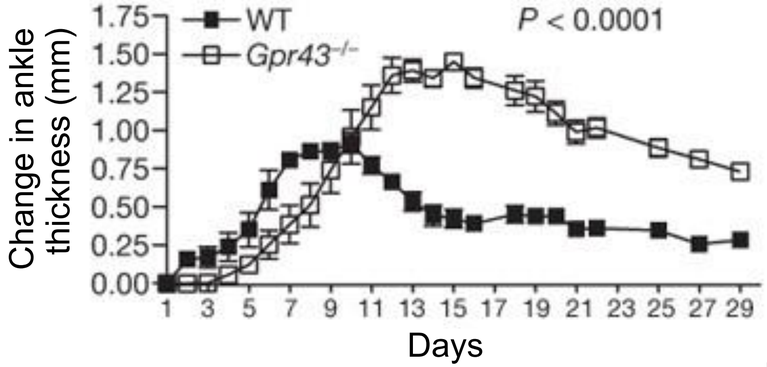


Asthma



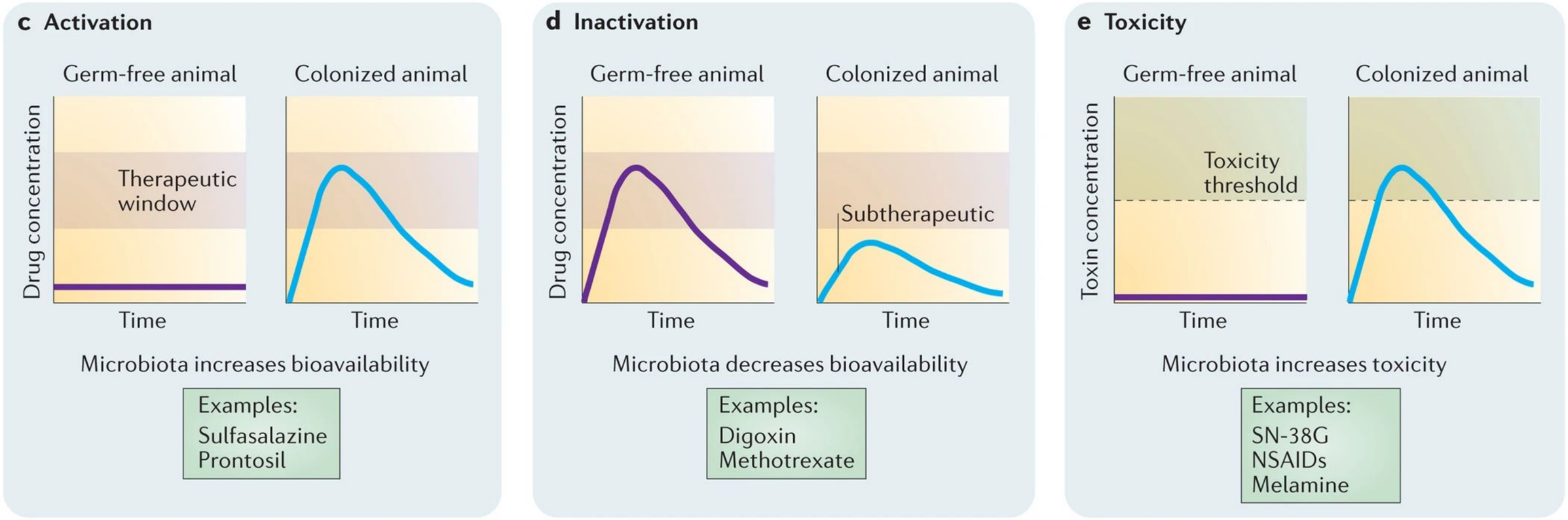
acetate/propionate receptor

Arthritis



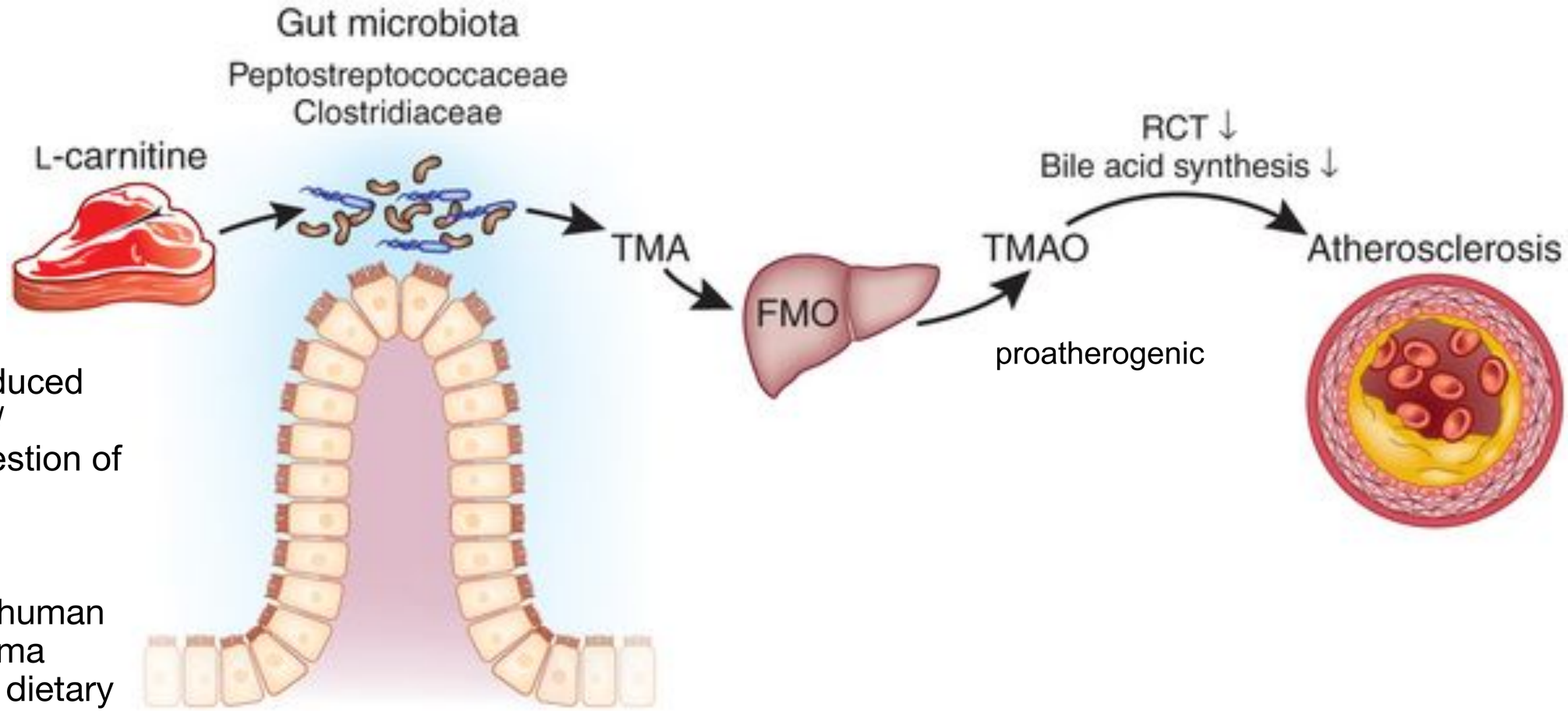
Maslowski (2009) Nature PMID: 19865172

Microbiota Metabolize Xenobiotics



Koppel (2017) Science. PMID: 28642381

Microbiota Metabolize Xenobiotics: Meat-metabolizing bacteria in atherosclerosis

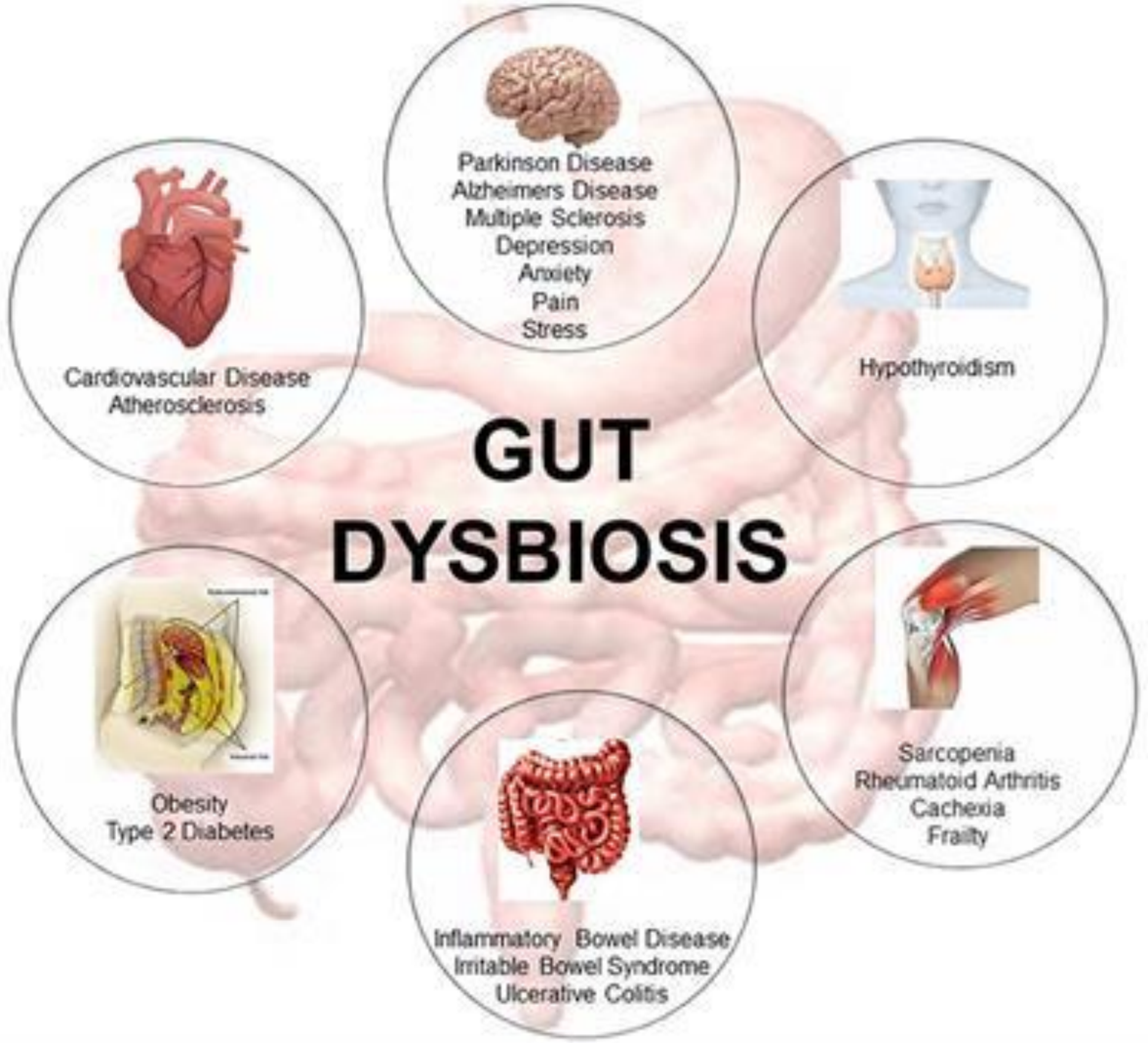


Omnivorous humans produced more TMAO than vegans/ vegetarians following ingestion of L-carnitine

Specific bacterial taxa in human feces associate with plasma TMAO concentration and dietary status.

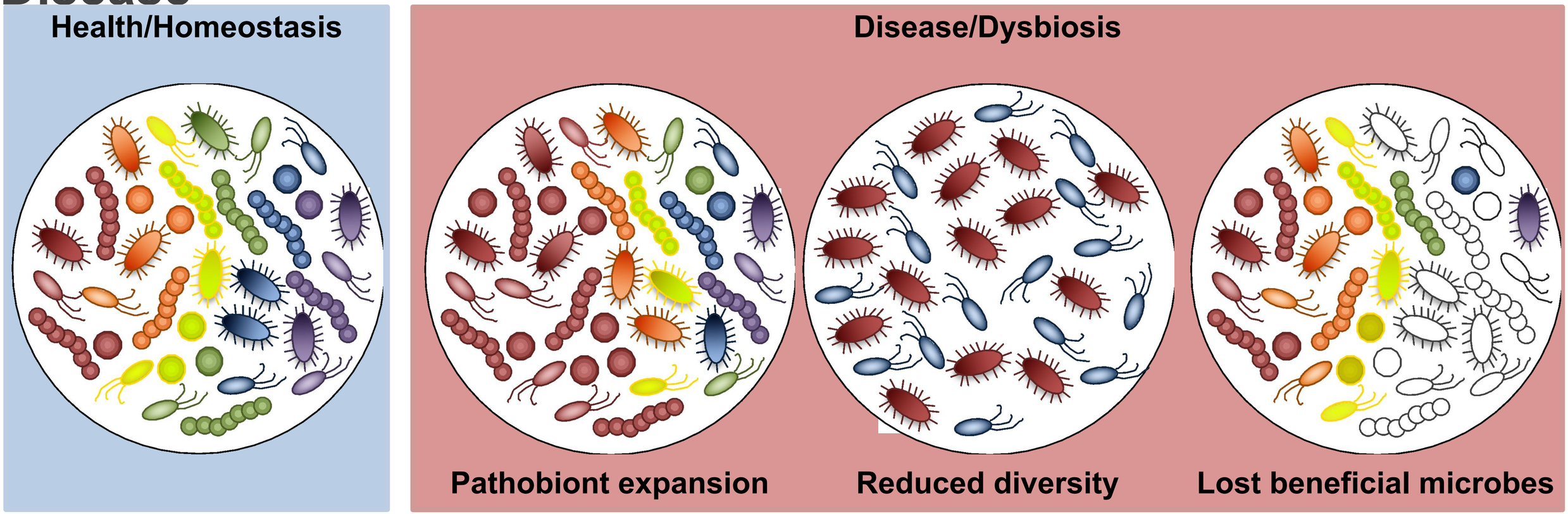
Koeth, Nature Med (2013) PMID: 23563705

Changes in Microbiota Composition are Associated with Human disease



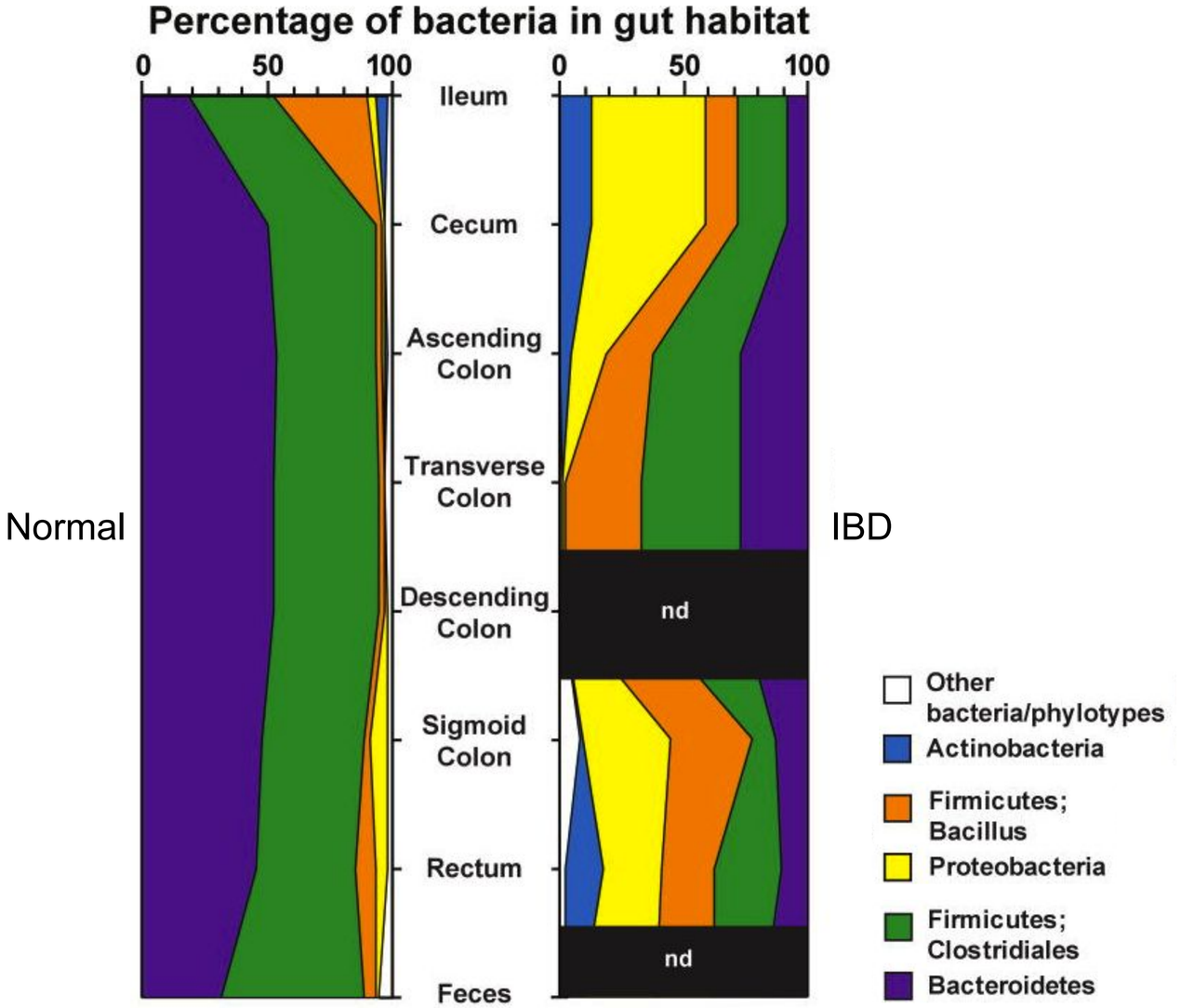
Baptista et al (2020) Frontiers in Nutrition. https://doi.org/10.3389/fnut.2020.00017

Changes in Microbiota Composition are Associated with Disease



Adapted from Peterson & Round 2014. Cell Microbiol.

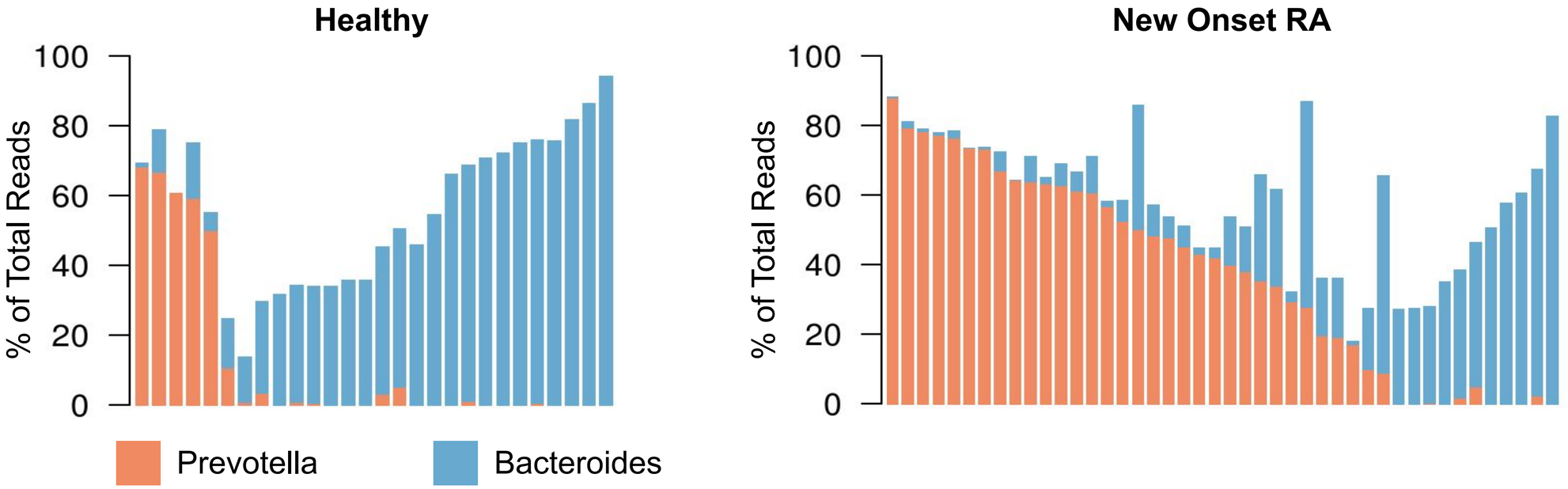
Dysbiosis is Associated With Human Disease: IBD



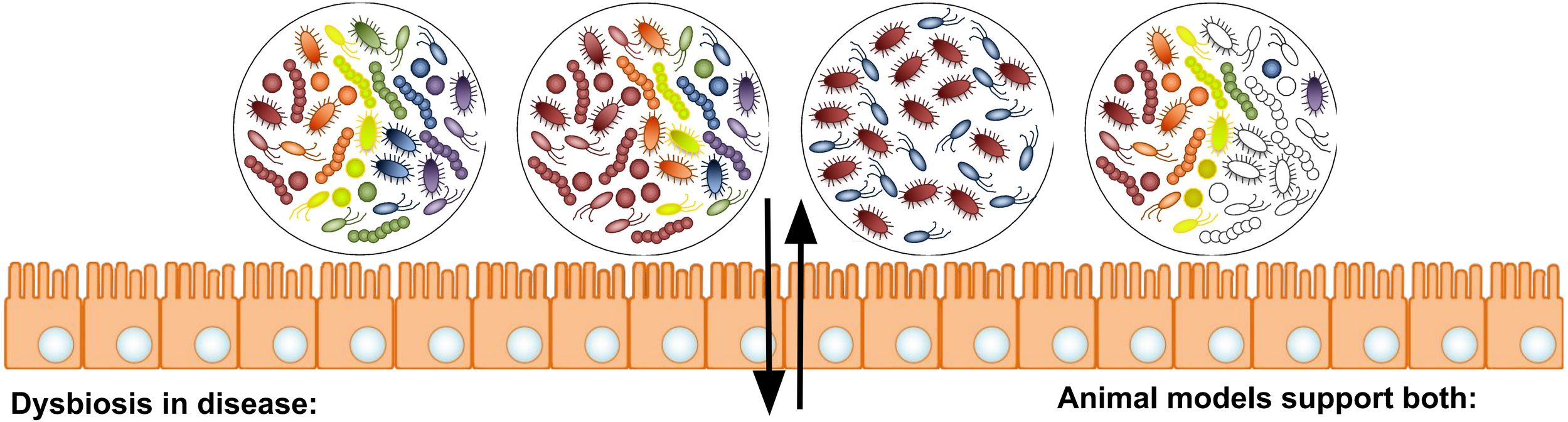
Peterson Cell Host Microbe
2008 PMCID: PMC2872787

Dysbiosis is Associated With Human Disease: RA

Prevotella dominates the intestinal microbiota in Rheumatoid Arthritis (RA)



Scher*, Sczesnak*, Longman* et al
eLife 2013 PMCID: PMC3816614



Dysbiosis in disease:

- Increased mucosal-associated bacteria
- Outgrowth of pathobionts eg proteobacteria
- Loss of anti-inflammatory microbes (Bacteroidetes, Lachnospiraceae and Faecalibacterium prausnitzii)

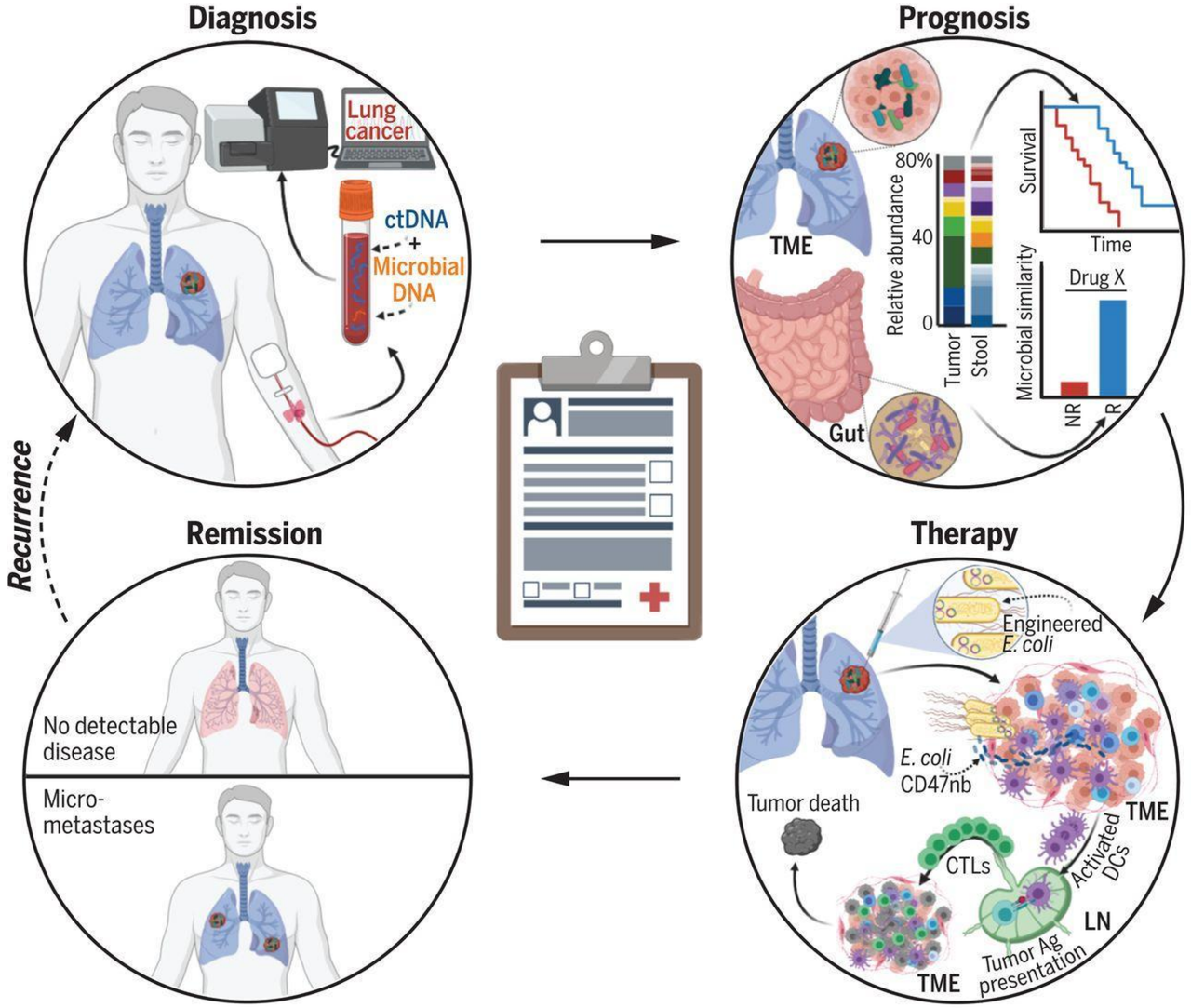
Does dysbiosis support disease or does disease support dysbiosis?

Animal models support both:

Inflammatory environment supports microbiota shift

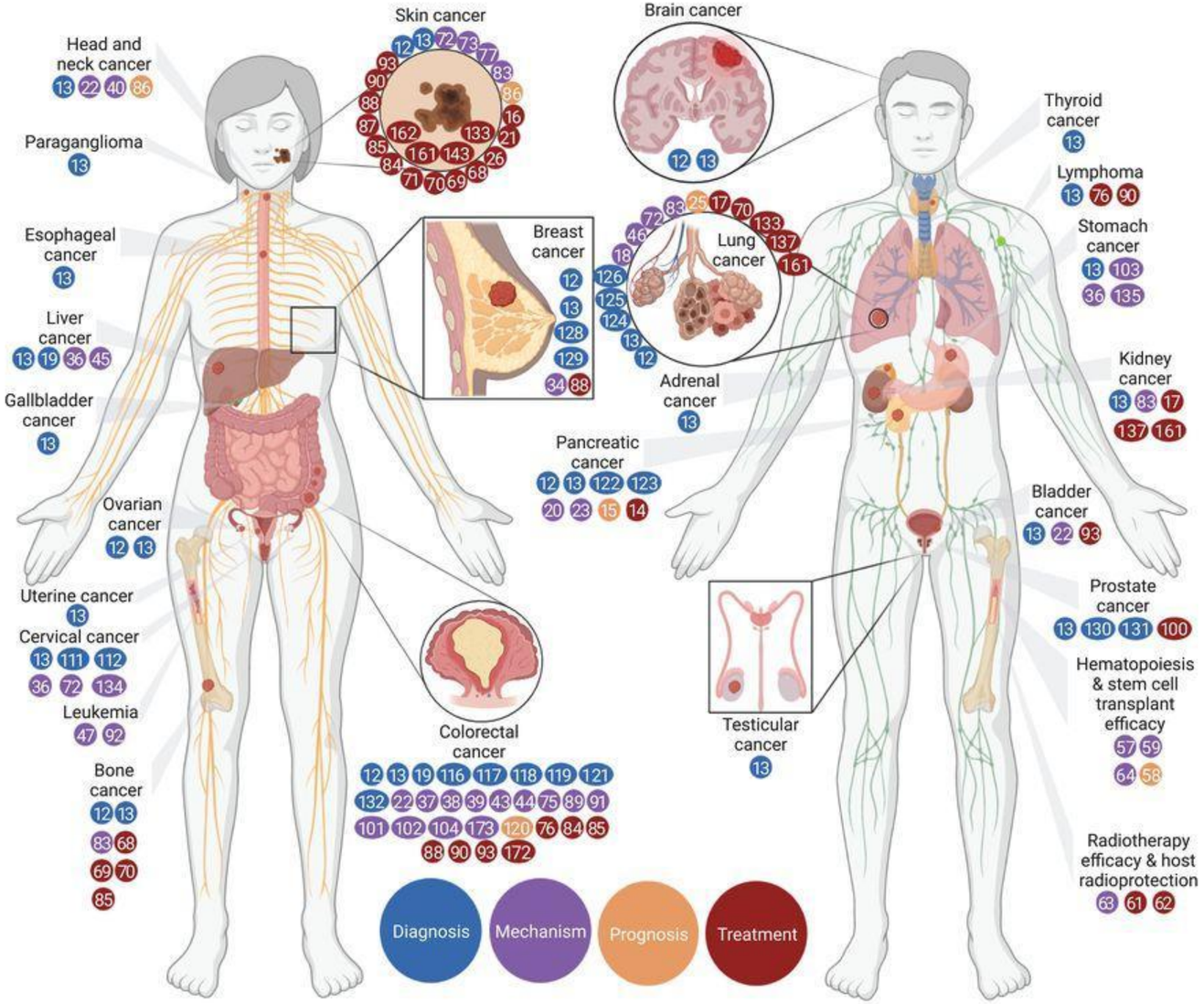
Microbial shifts can cause disease

What About Cancer?



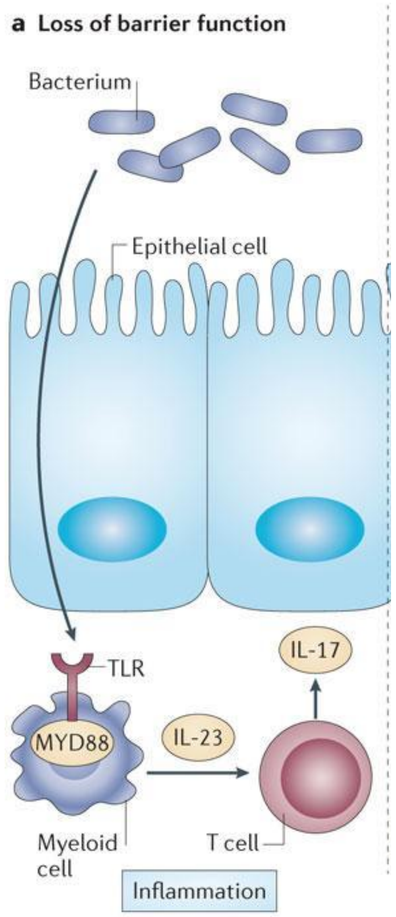
Sepich-Poore (2121)
Science. PMID: 33766858

Microbiota Association with Multiple Types of Human Cancers



Sepich-Poore (2121)
Science. PMID: 33766858

Microbiota Associated with Colorectal Cancer



Elinav (2013) Nat Rev Cancer
PMID: 24154716

Microbial Drivers of Cancer

11 microbes are identified by the International Association for Cancer Registries as human carcinogens

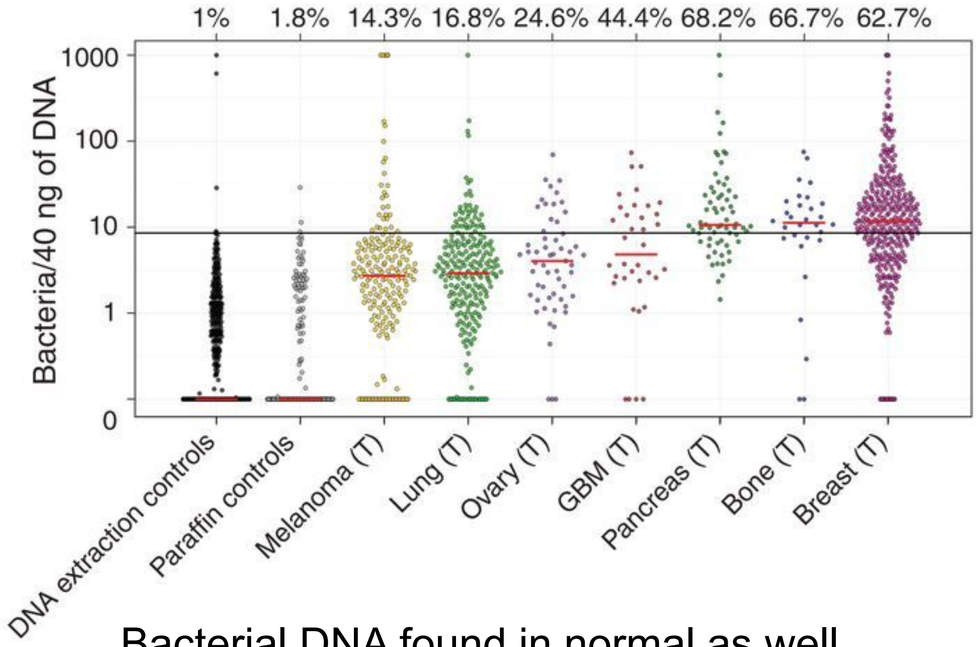
- Epstein Barr virus
- Hepatitis B virus
- Hepatitis C virus
- Kaposi Sarcoma herpesvirus
- HIV-1
- Human Papillomaviruses
- Human T-cell Lymphotrophic virus type 1
- Opisthorchis viverrini and Clonorchis sinensis (flatworms)
- Schistosoma haematobium
- Helicobacter pylori

These account for ~13% of global cancer cases

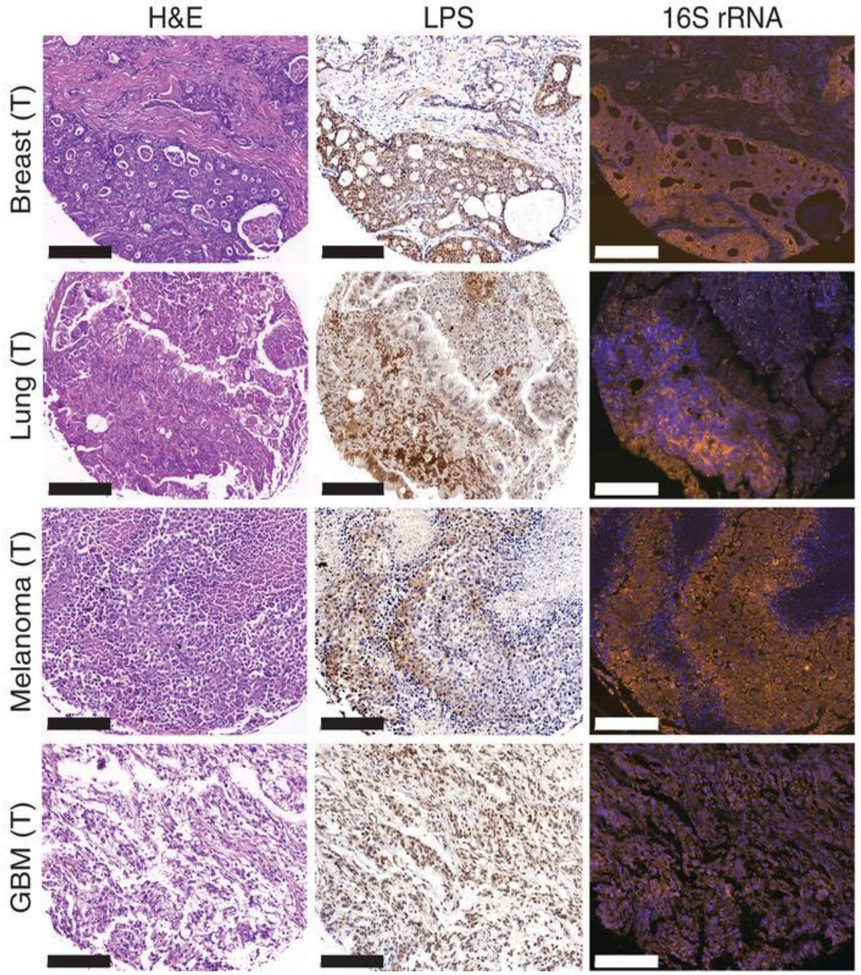
IARC Monogr Eval Carcinog Risks Hum. (2012) PMID: 23189750

Intra-Tumoral Microbes

Tissue	Normal: # samples (# centers)	Tumor: # samples (# centers)
Breast	256 (3)	355 (3)
Lung	231(3)	245 (3)
Melanoma	—	206 (3)
Pancreas	—	67 (2)
Ovary	29 (2)	58 (2)
Bone	—	39 (2)
GBM	—	40 (2)
Total	1526	
DNA extraction controls	437	
16S 5R PCR controls	206	
Paraffin Controls	168 (4)	

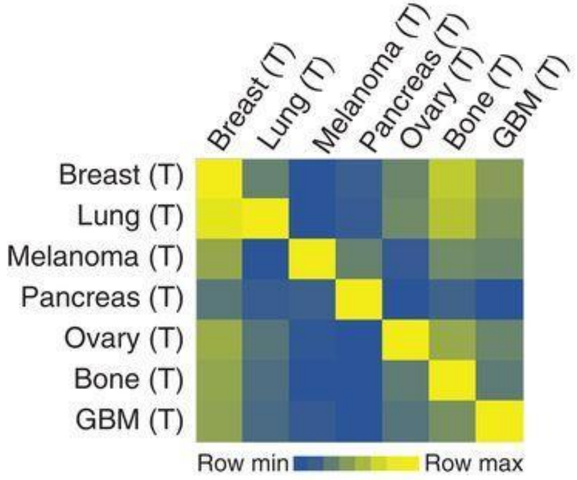


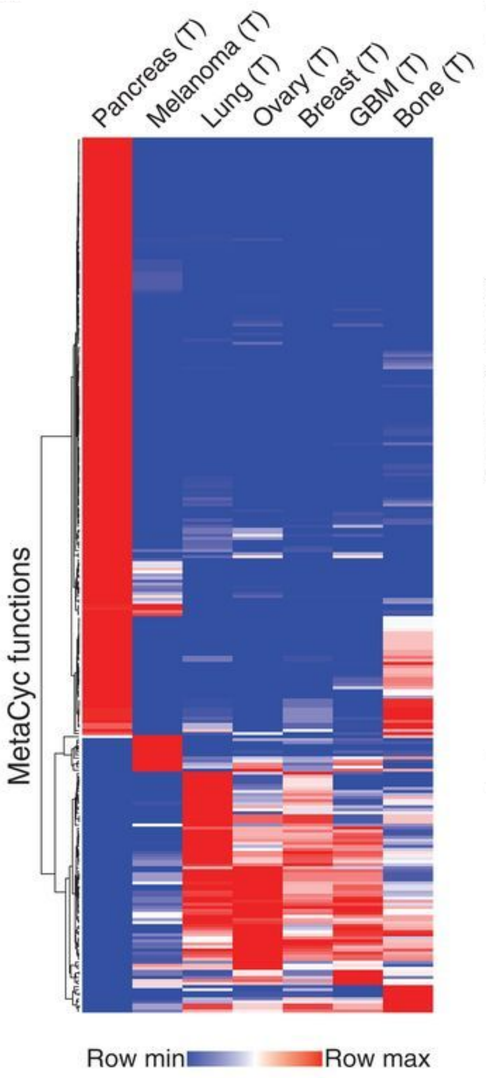
Bacterial DNA found in normal as well

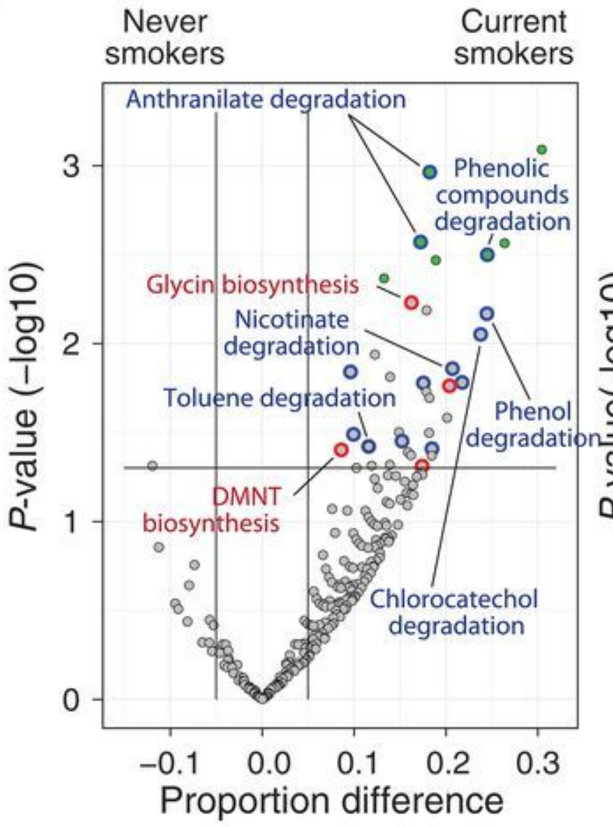


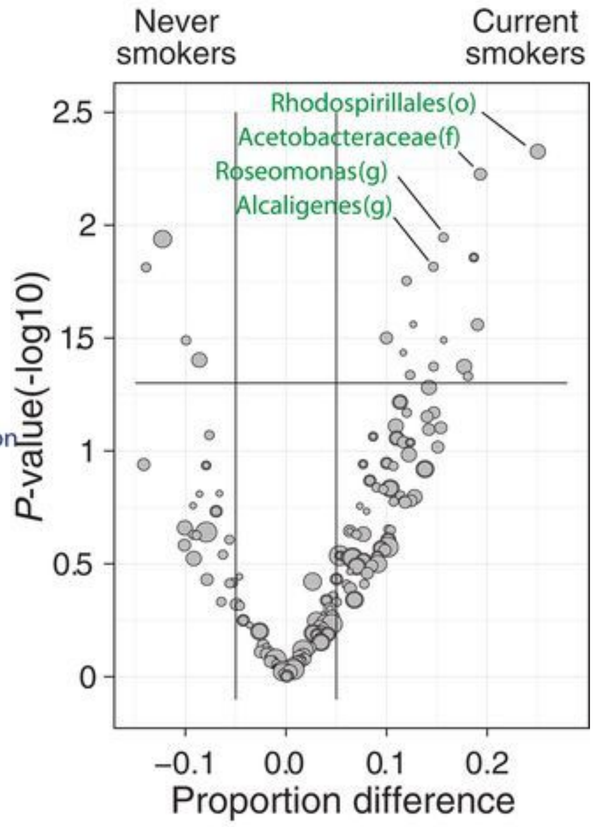
Nejman (2020) Science PMID: 32467386

Intra-Tumoral Microbes



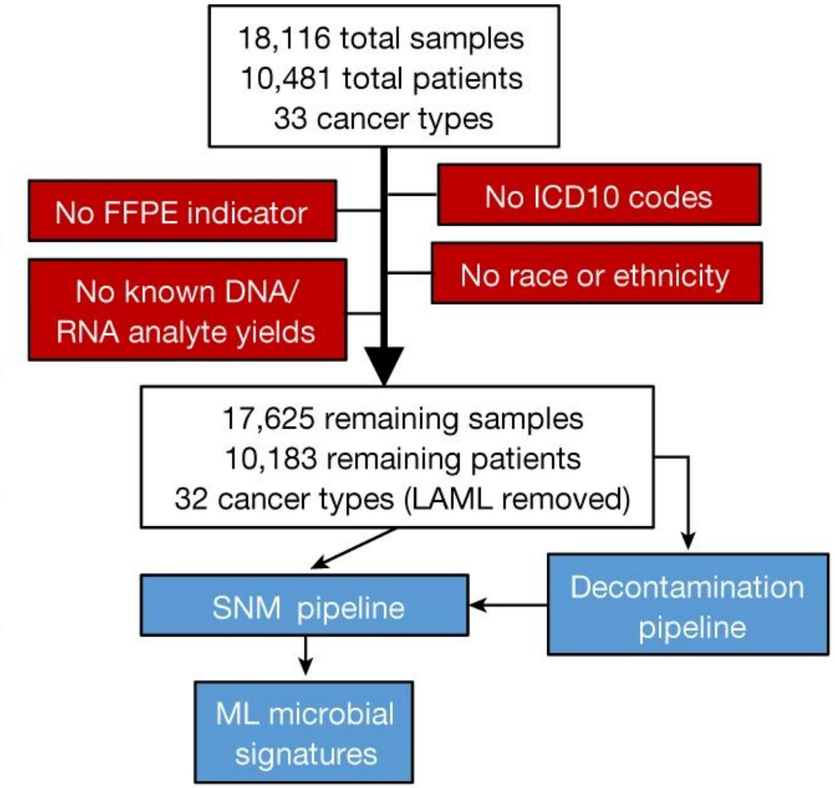


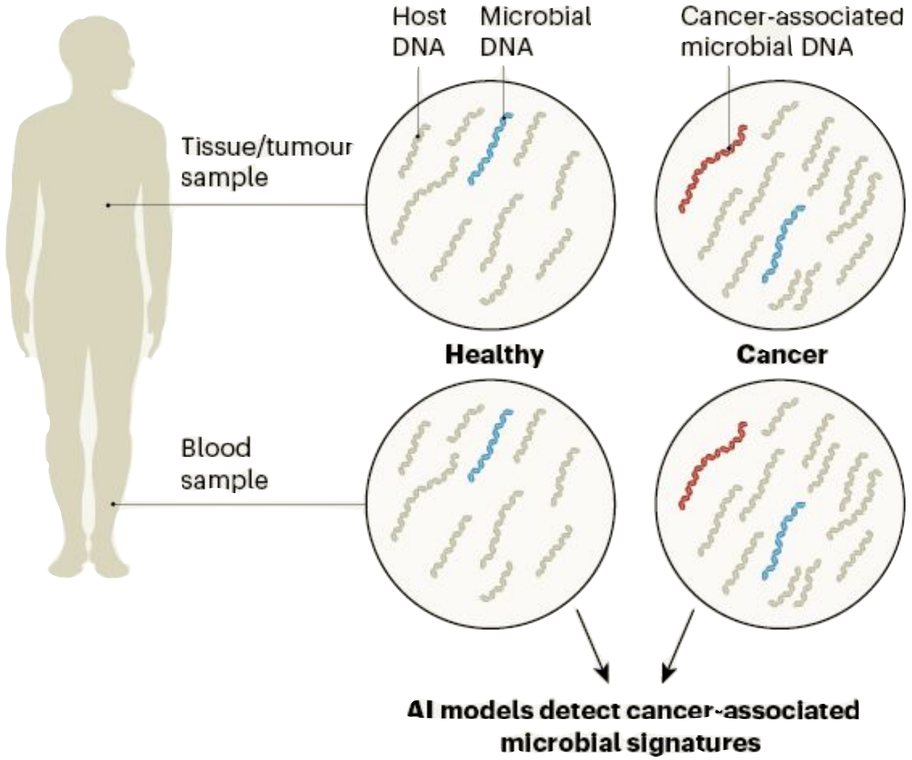




Nejman (2020) Science PMID: 32467386

Microbial Signatures in Blood May Reveal Cancer Presence





Poore (2020). Nature. PMID: 32214244

Ajami (2020) Nature. PMID: 32161344

Intratumoral Microbes

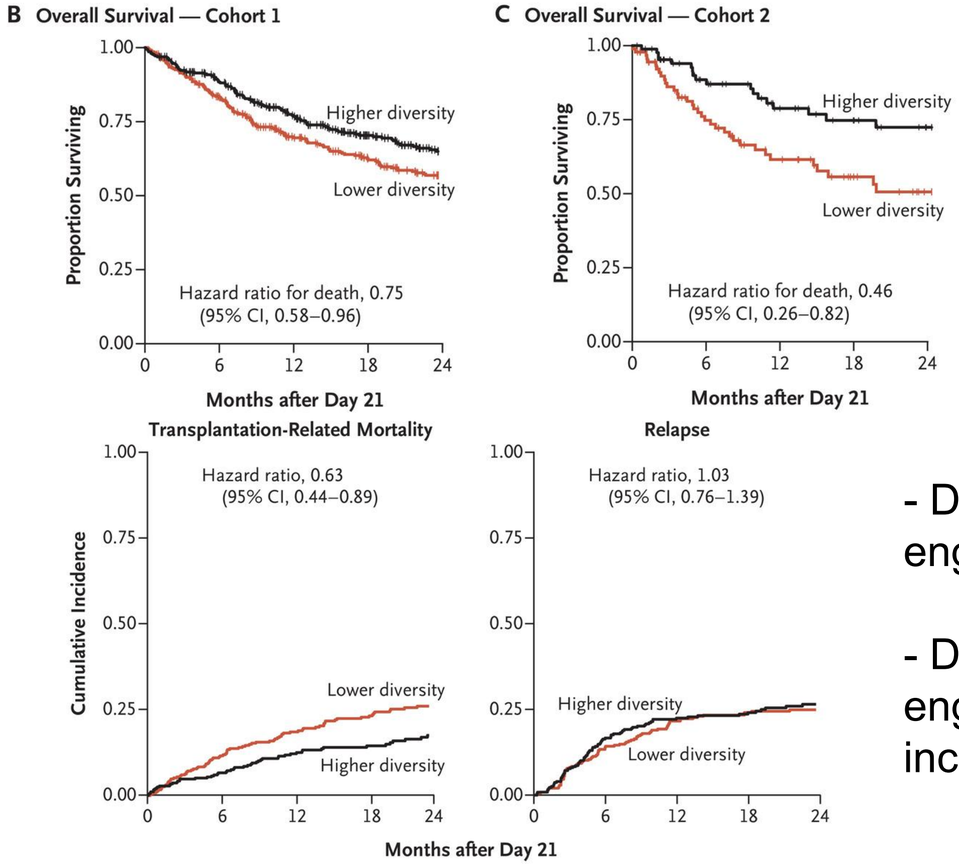
- Genotoxins increase mutations
- Activate pro-tumor survival or proliferation
- Suppress anti-tumor immunity
- Drug resistance through microbial metabolism
- As therapies:

  - Can we selectively target intratumor specific microbes?

  - If there are specific tumor homing microbes, can we target them to tumors to deliver drugs (anti-cd47) to avoid systemic toxicity?

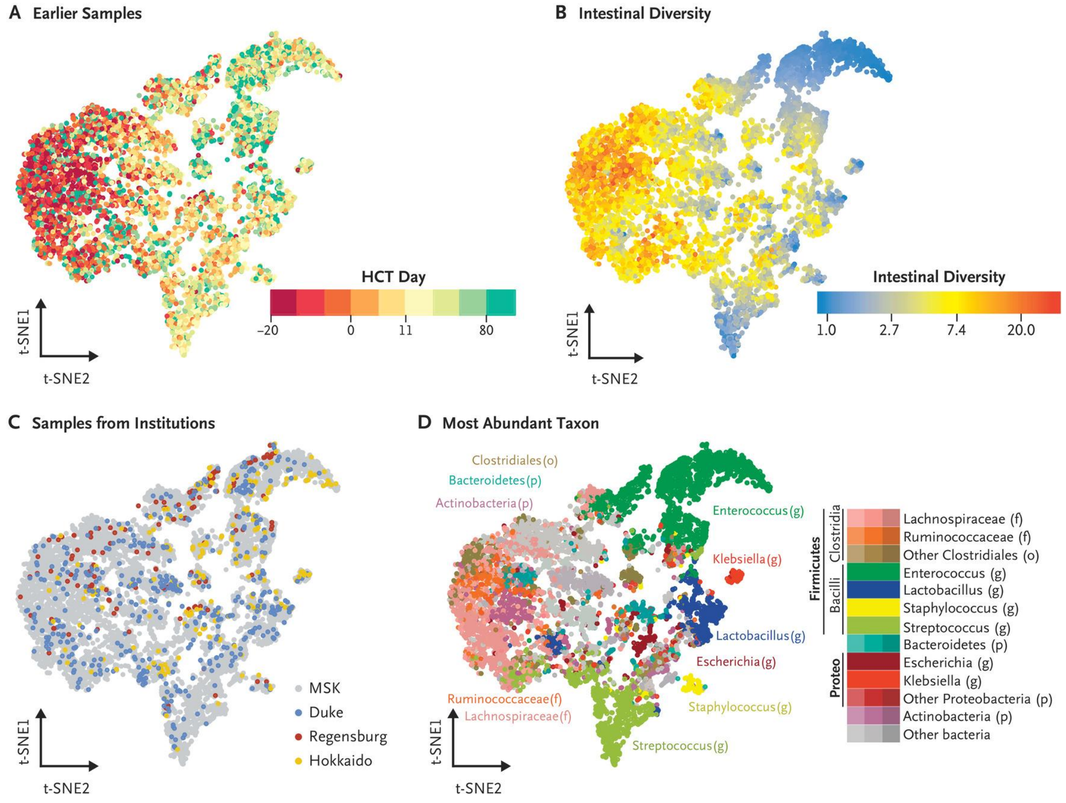
Better Outcomes: Microbiota Diversity Supports HSCT

8767 fecal samples obtained from 1362 patients, 4 sites



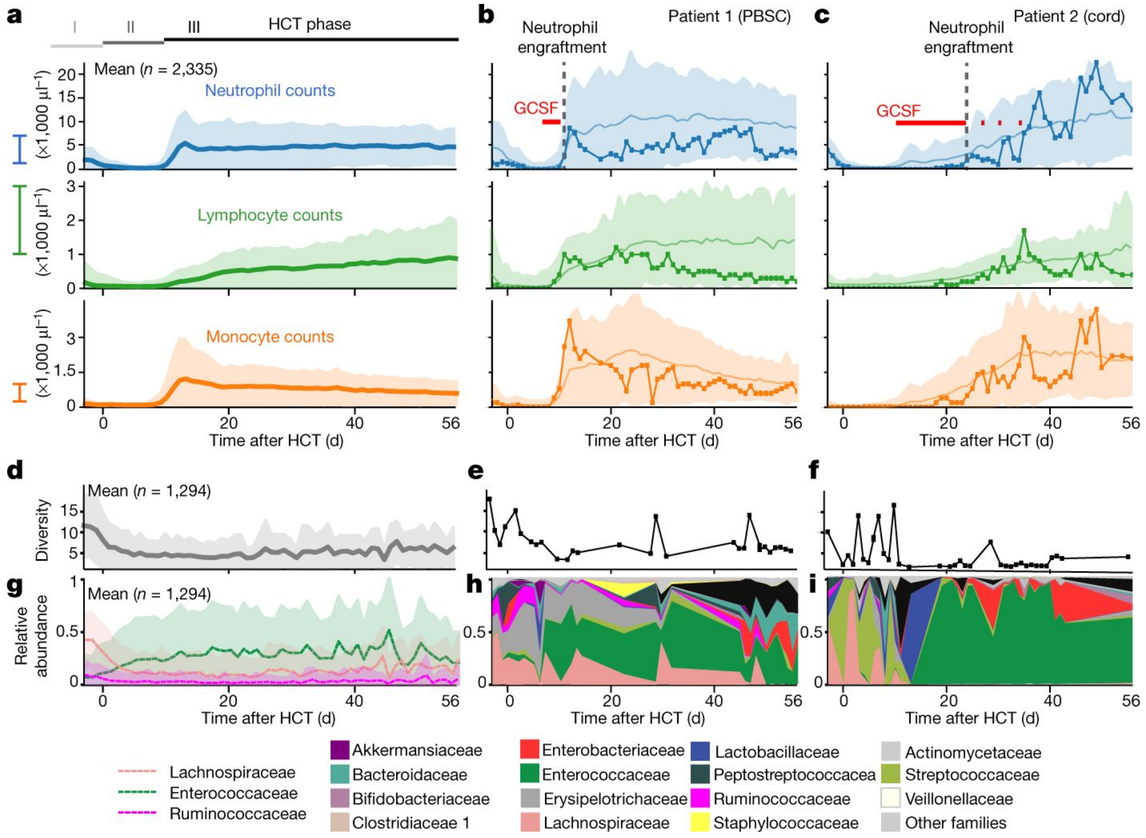
- Diversity decreases before engraftment

- Diversity at time of engraftment corresponds to increased survival

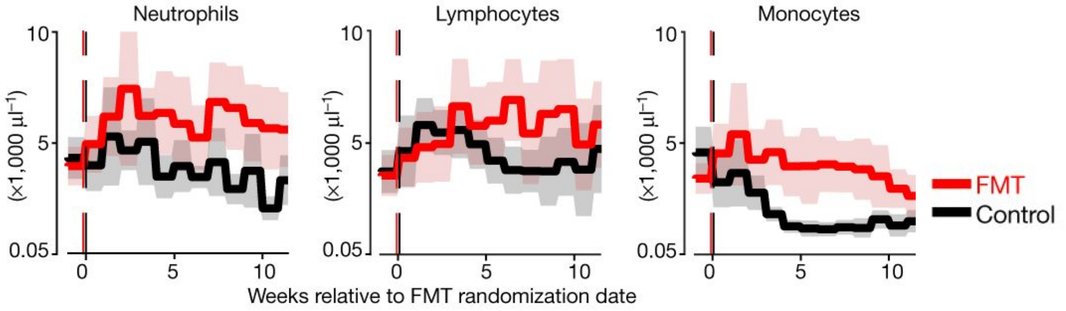


Peled 2020 NEJM PMID: 32101664

Better Outcomes: Microbiota Diversity Supports HSCT



- Diversity is good
-Allo-Fecal microbiota transplant (FMT) improves engraftment

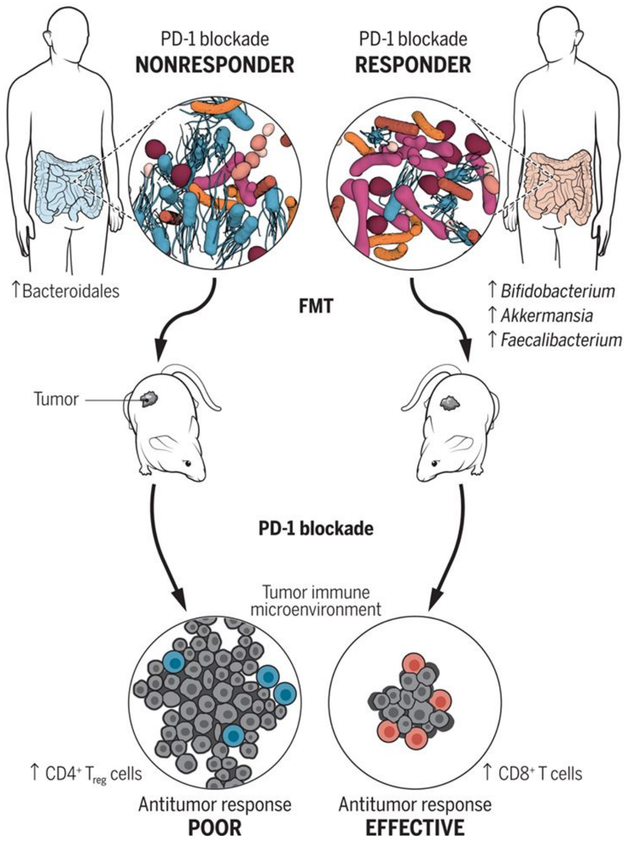


How does a diverse microbiota help?

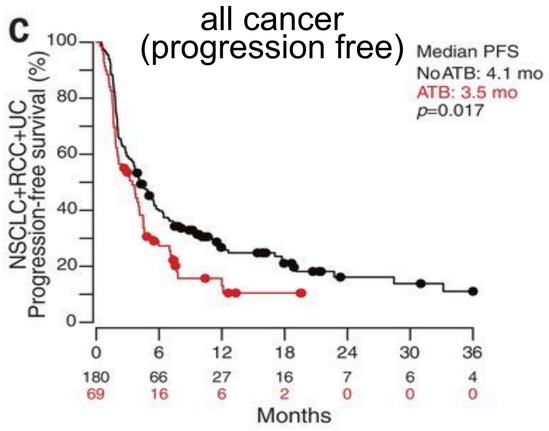
- Immune stimulatory (including mylo/lymphopoiesis)
- Nutritional benefits
- Radiation resistance
- etc...

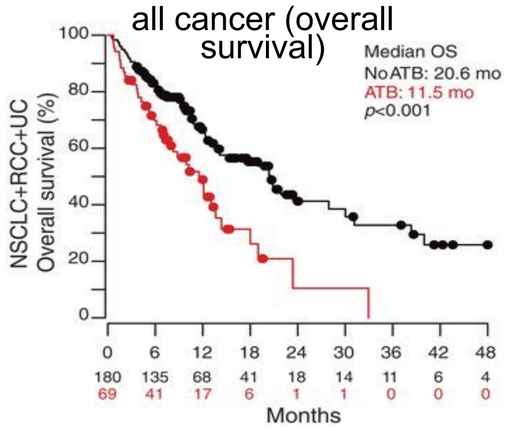
Schluter (2020) Nature
PMID: 33239790

Predicting Outcomes: Microbiota and Checkpoint Therapy

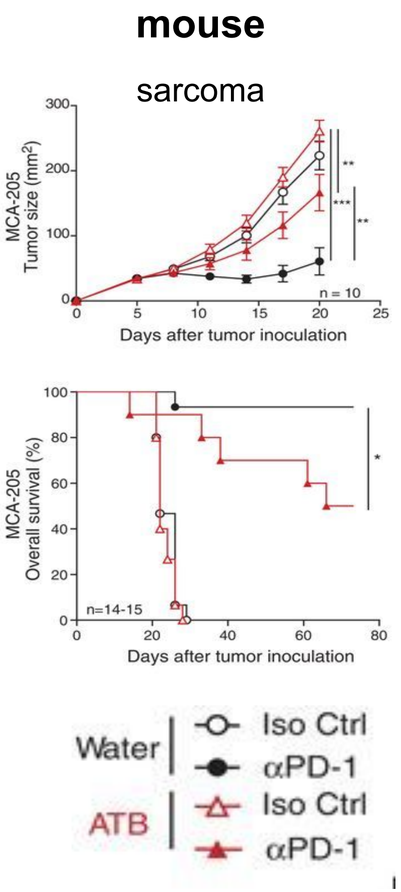


Jobin (2018) Science, PMID: 29302001



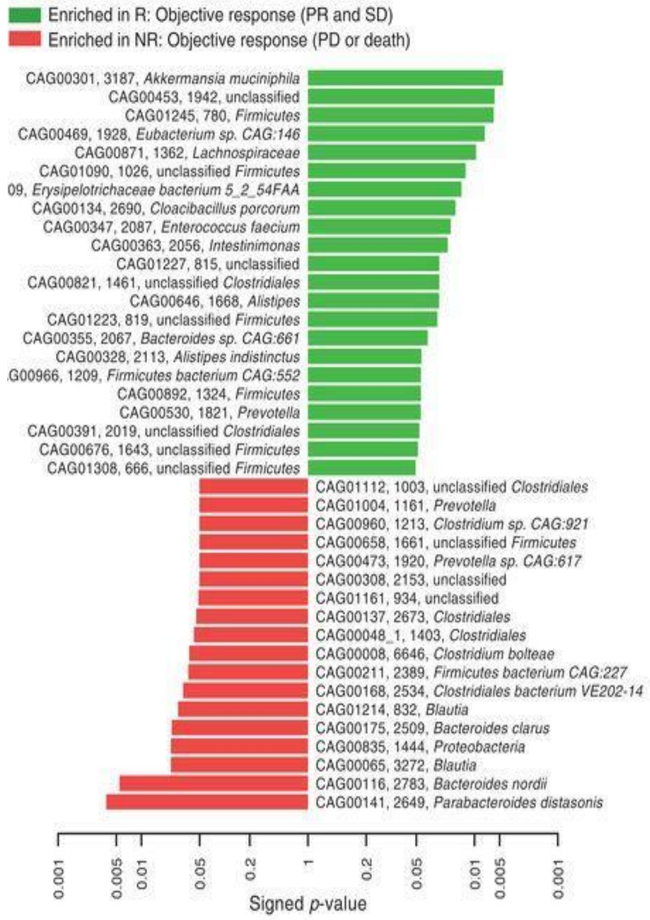


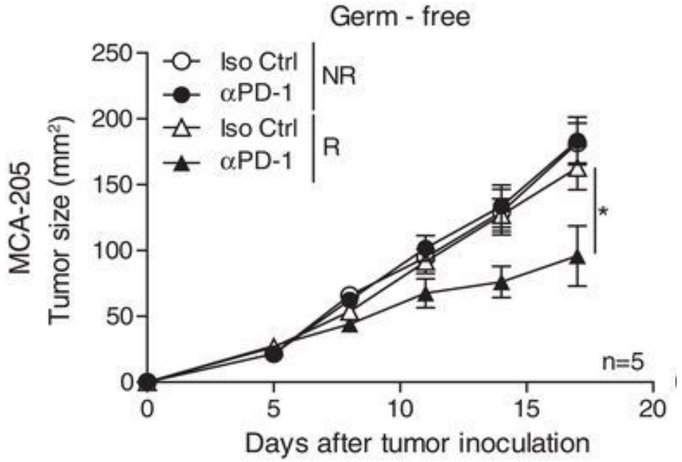
No ABX No ABX

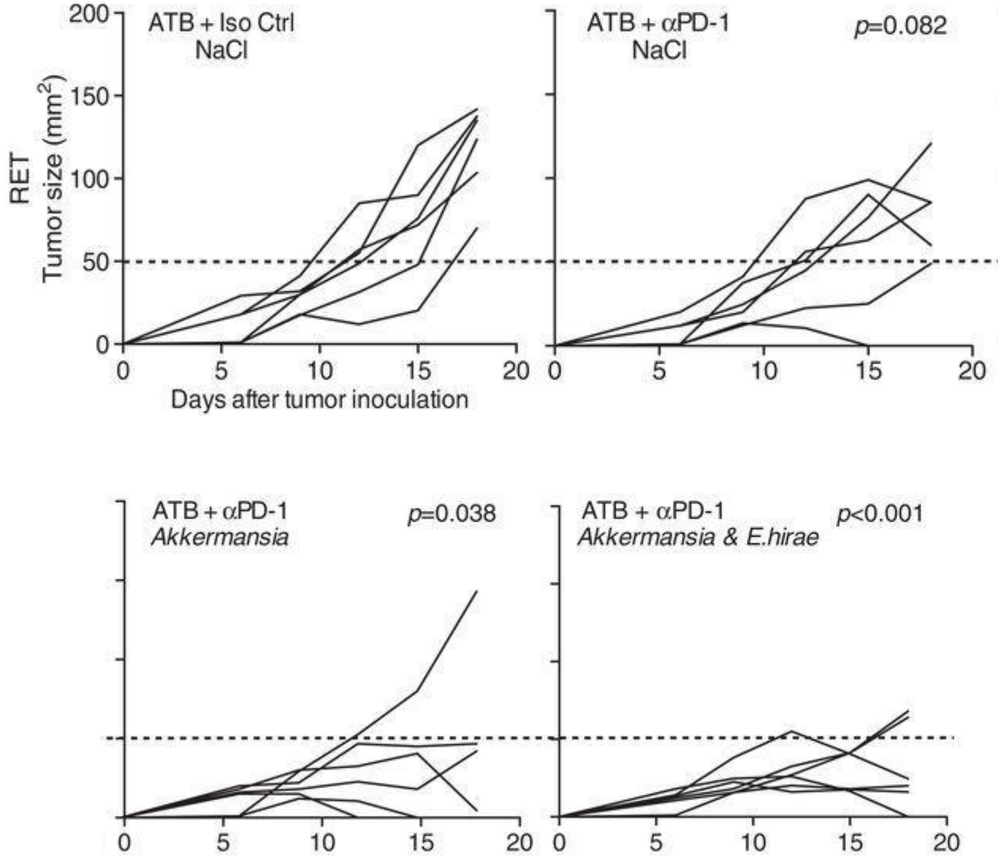


Routy 2018 Science PMID: 29097494

Predicting Outcomes: Microbiota and Checkpoint Therapy

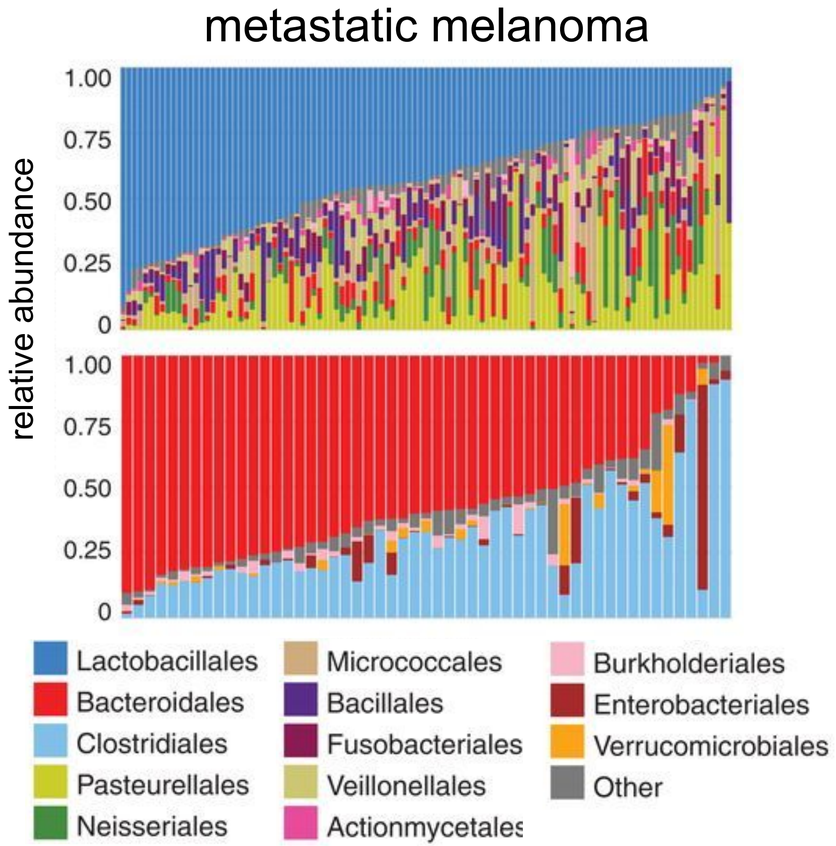


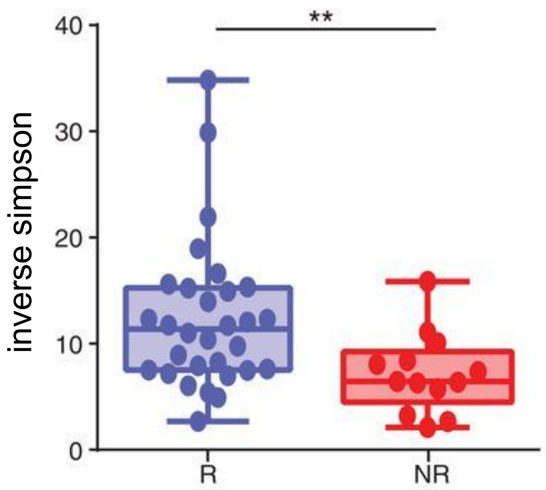


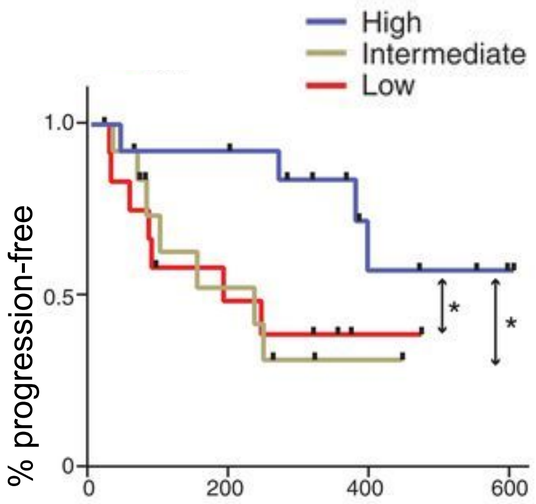


Routy 2018 Science PMID: 29097494

Predicting Outcomes: Microbiota and Checkpoint Therapy

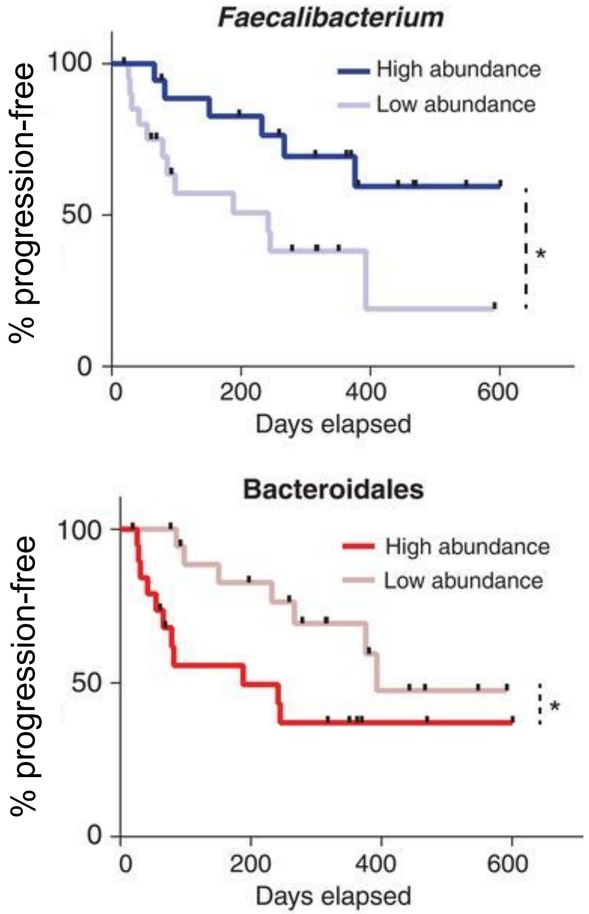


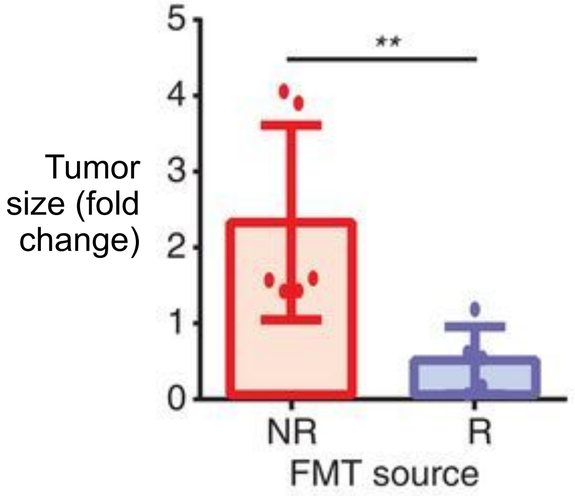


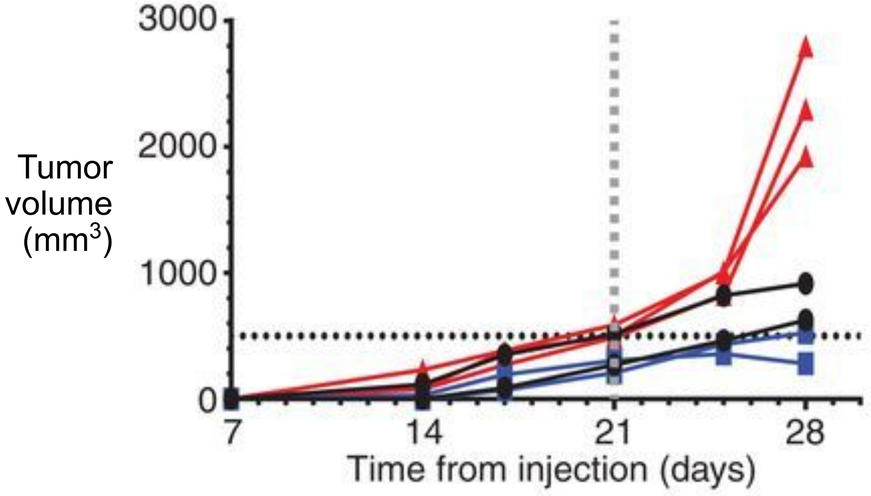


Gopalakrishnan (2018) Science PMID: 29097493

Predicting Outcomes: Microbiota and Checkpoint Therapy

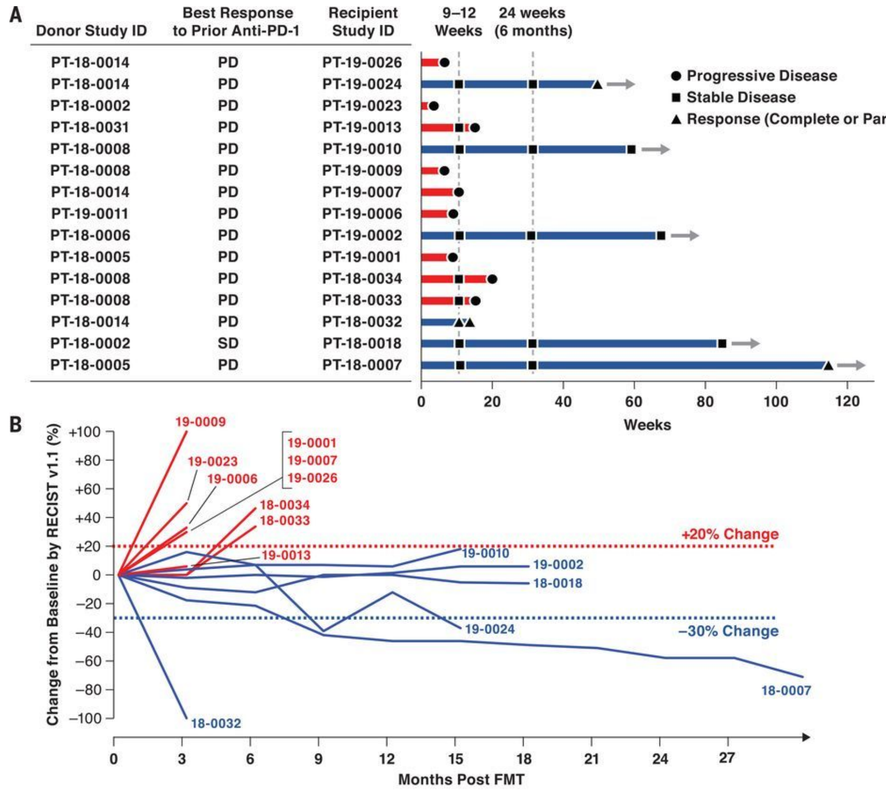


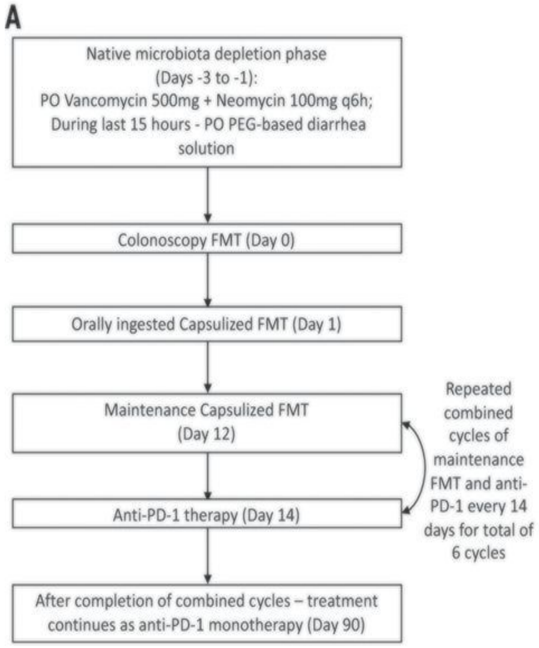


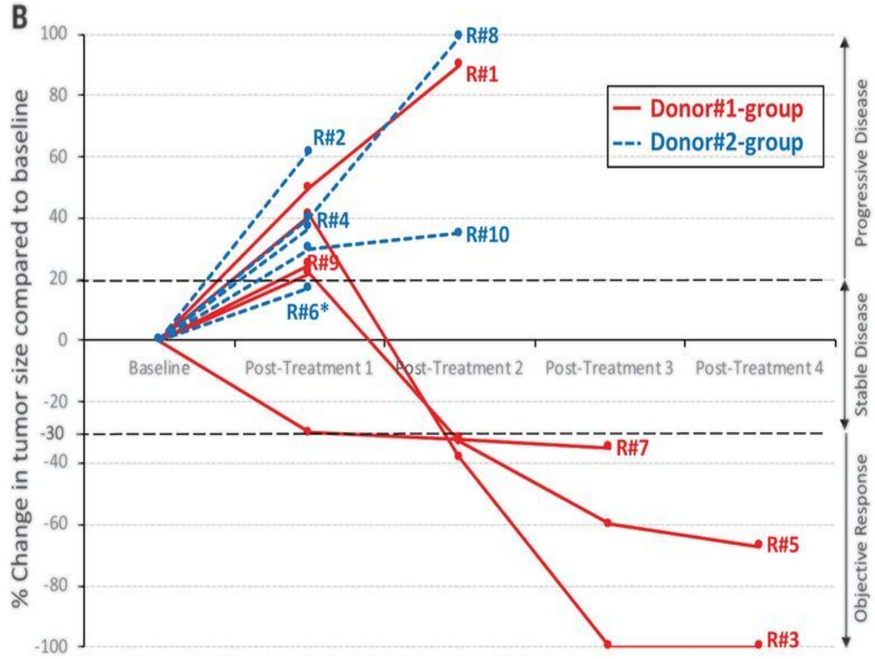


Gopalakrishnan (2018) Science PMID: 29097493

Fecal Microbiota Transplant Improves Outcomes in Anti-PD1 Resistant Melanoma Patients



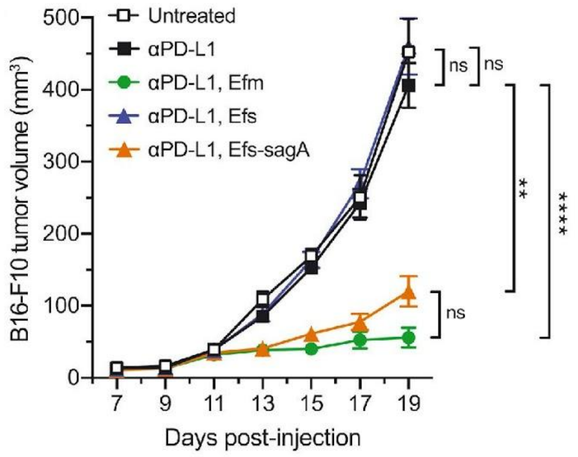


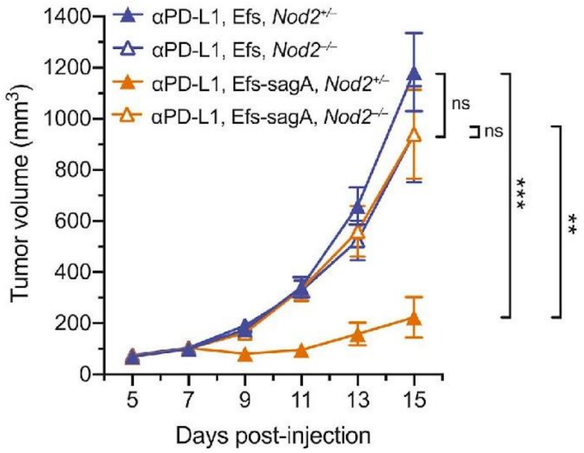


Davar, Dzutsev (2021) Science PMID: 33542131

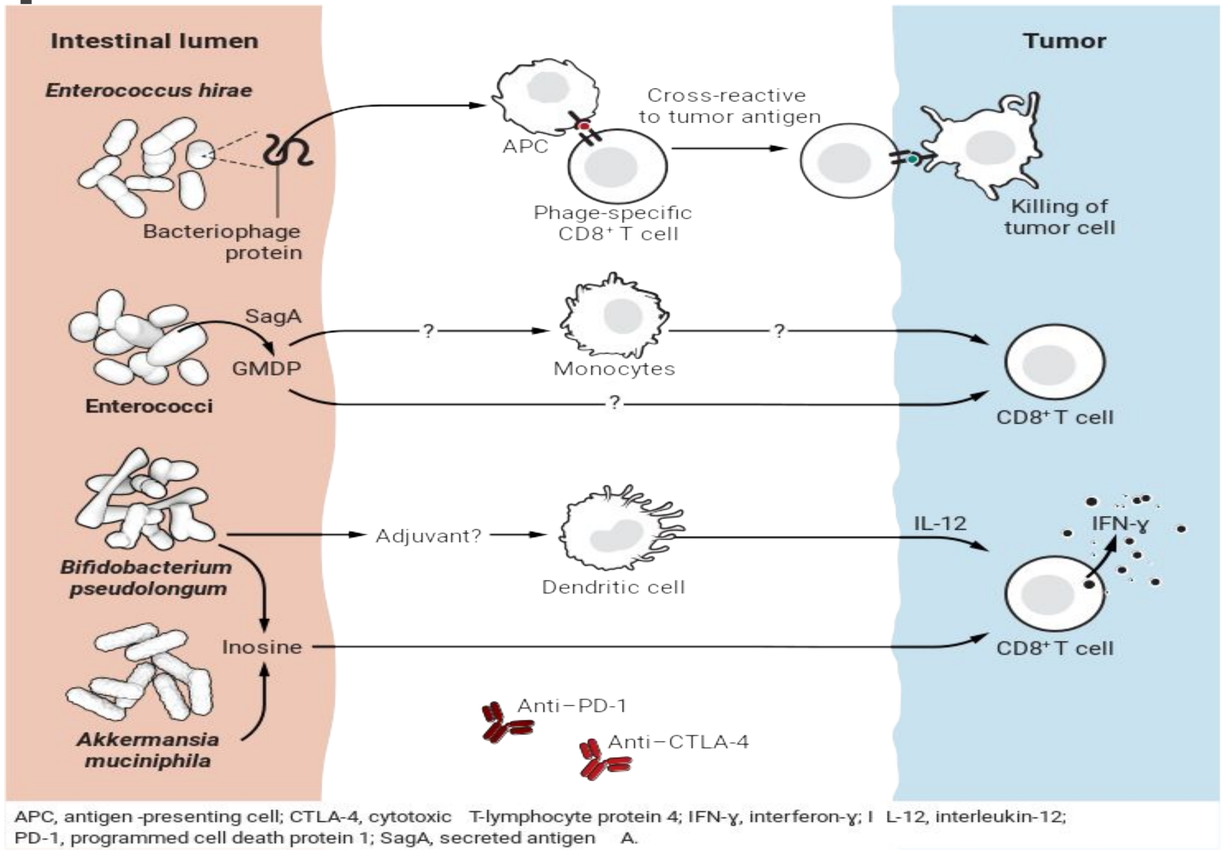
Baruch (2021) Science PMID: 33303685

How Do Microbes Improve Checkpoint Blockade?





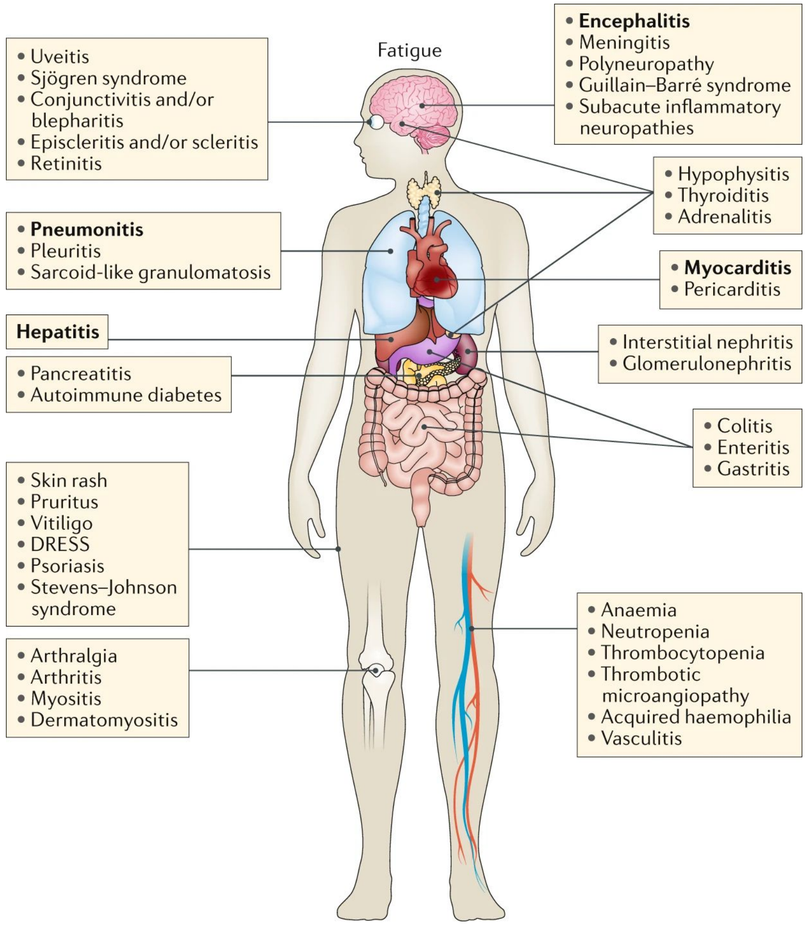
- Identified protective and non-protective enterocci (mice)
- Protective have distinct peptidoglycan with many small non-crosslinked fragments
- Identified conserved hydrolase (SagA)
- Overexpress SagA, bacteria now improves outcome (nod2/MDP)

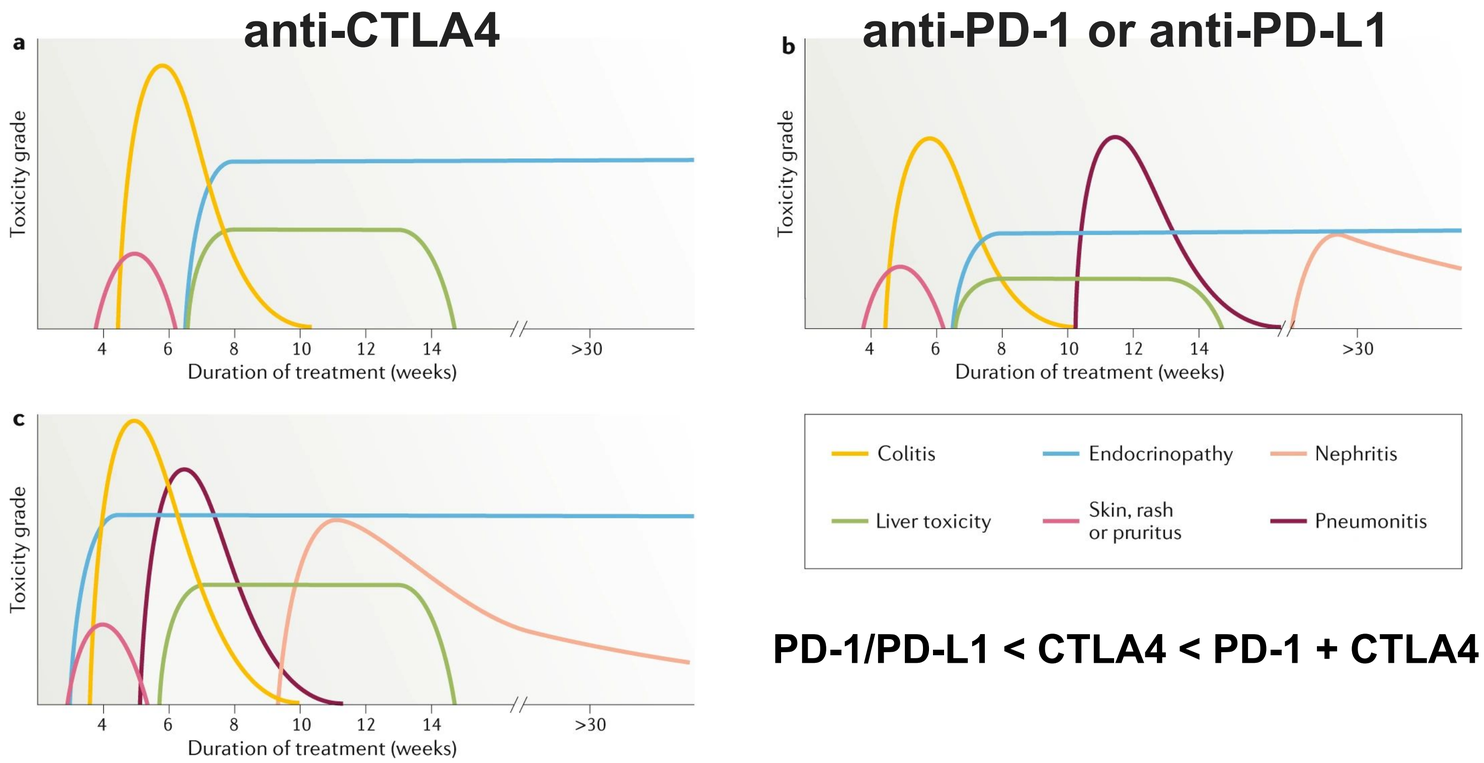


Griffin 2021 Science PMID: 34446607

Ansaldo 2021. Science DOI: (10.1126/science.abl3656)

Immune related adverse events





anti-PD-1 plus anti-CTLA4

PD-1/PD-L1 < CTLA4 < PD-1 + CTLA4

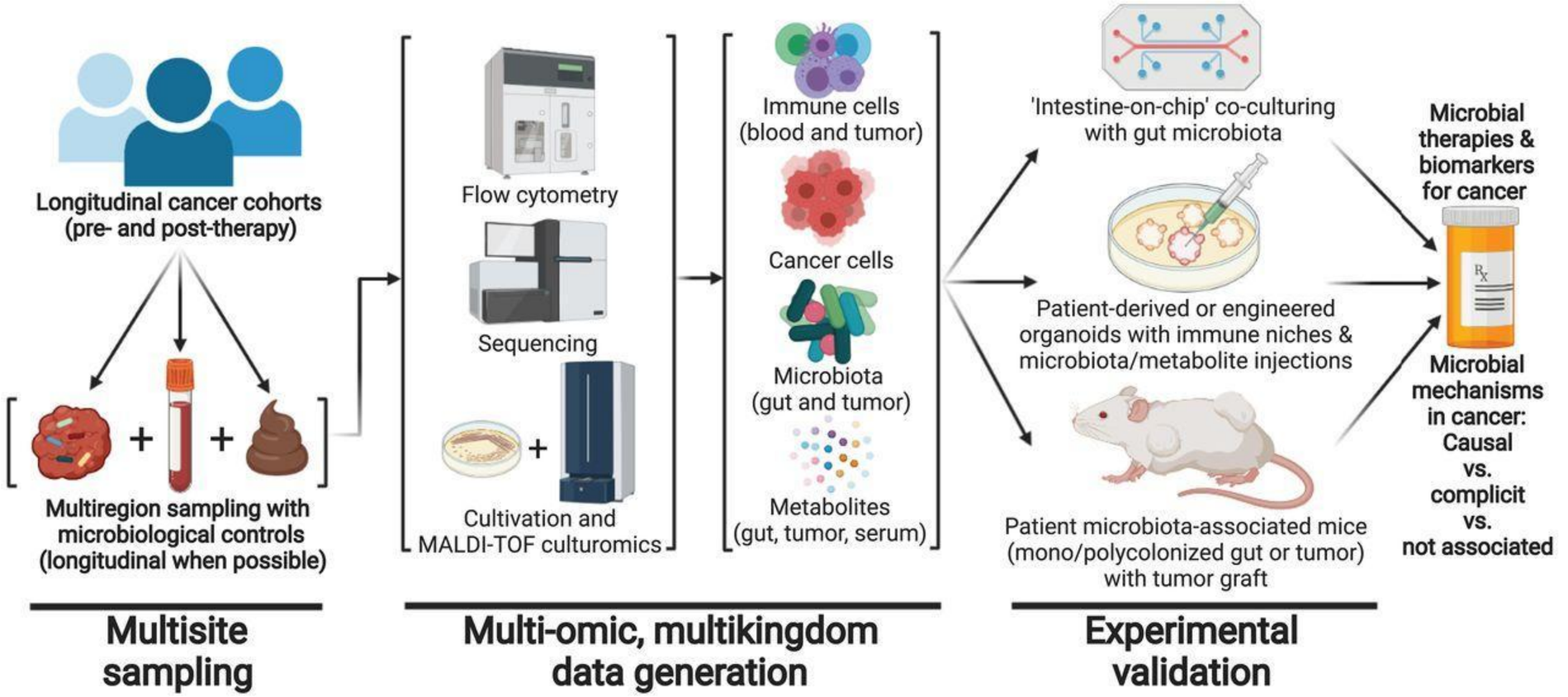
Martins. 2019. Nature Reviews Clinical Oncology PMID: 31092901

Immune related adverse events: microbiota

- Colitis is best studied for role of microbiota
- Antibiotics leads to increased incidence of adverse events
- FMT has promising results
- Changes in specific microbes found in people with and without adverse events

Wang JEM 2023 PMID: 36622383

So How Do We Use the Microbiota to Improve Outcomes?



Sepich-Poore
(2121) Science.
PMID: 33766858

NASDC

NCI Awardee Skills Development Consortium

Microbiota and Human Health: A Role in Cancer

Questions