

A dense, colorful field of various rod-shaped bacteria, representing the human microbiota. The bacteria are in shades of blue, green, yellow, orange, red, and pink, set against a dark background.

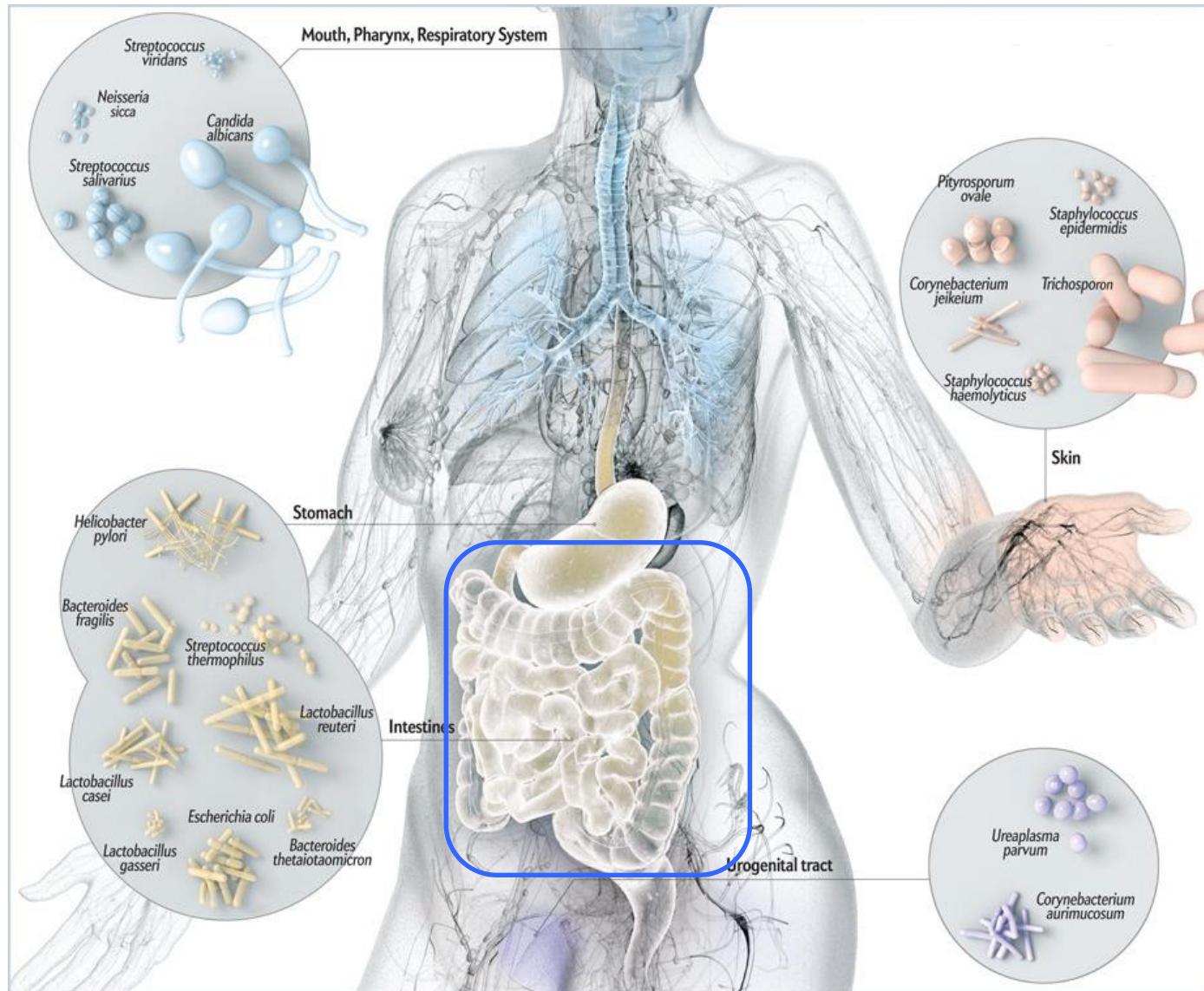
Il Microbiota

Chiara Pozzi

Humanitas Clinical and Research Center

The microbiota

“The entire microbial community living in symbiosis with our body”

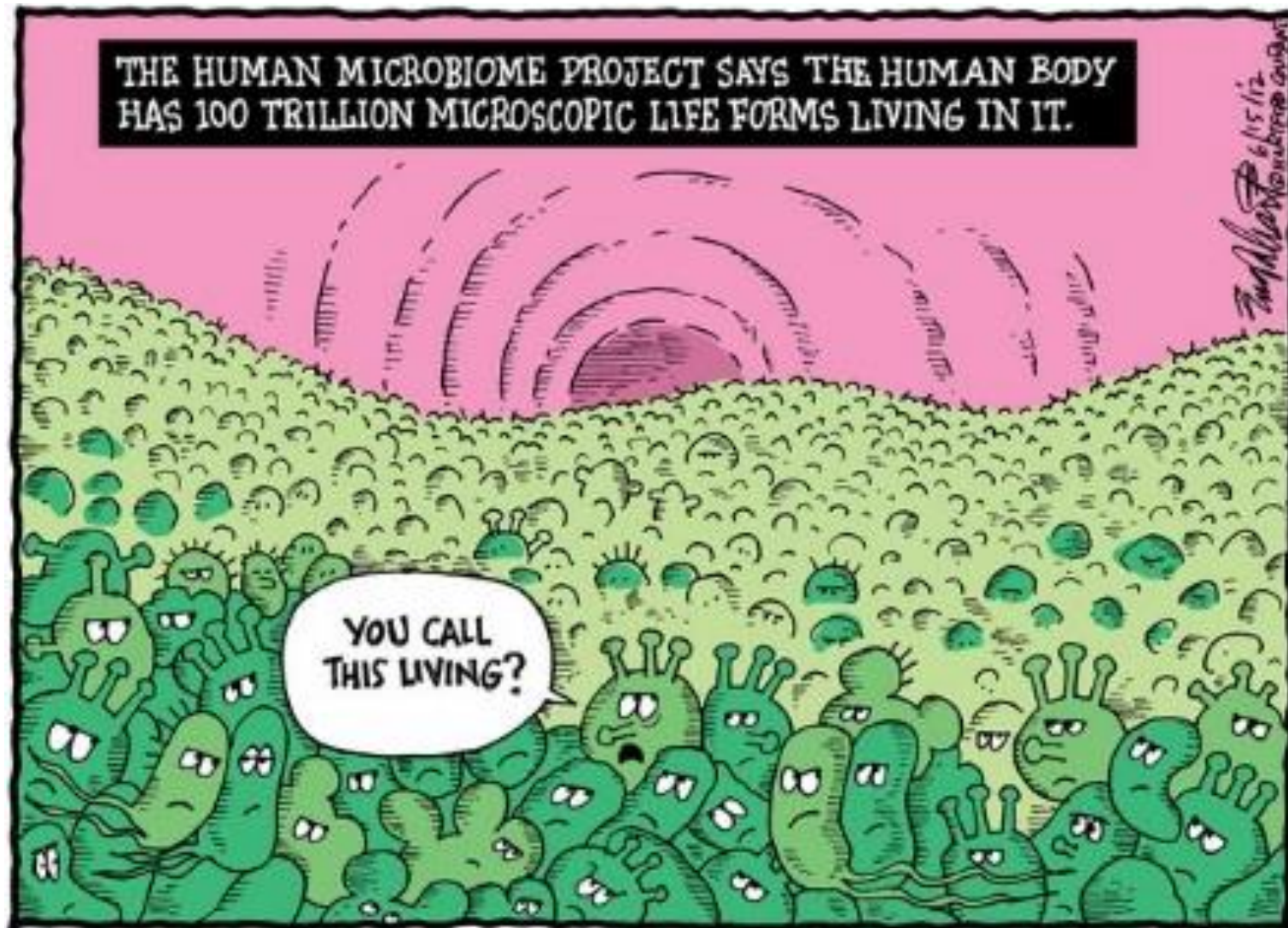


Bacteria (97.6%),
Archea (2.2%),
Viruses (0.2%),
Eukaryotes (<0.01%)

- skin,
- mammary glands,
- placenta,
- seminal fluid,
- uterus,
- ovarian follicles,
- lung,
- saliva,
- oral mucosa,
- conjunctiva,
- gastrointestinal tract

The microbiota

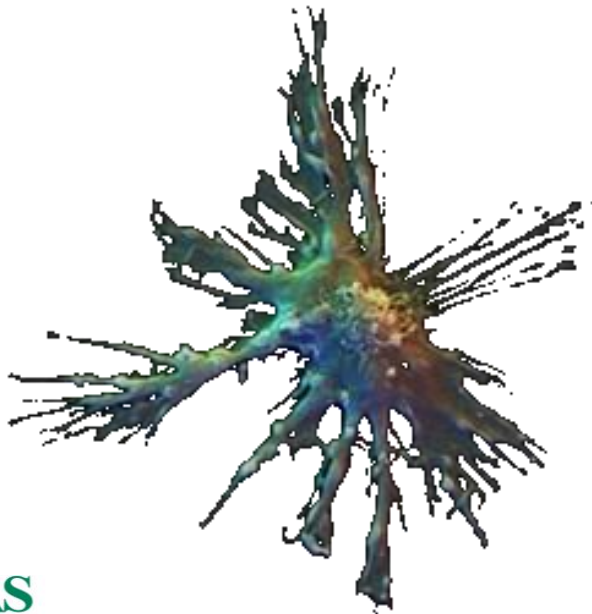
- ✓ 10^{14} bacteria vs. 10^{13} mammalian cells
- ✓ 1000 bacterial species (7000-36000 strain)



The microbial genes

✓ 2×10^6 microbial genes (100 times more than human genes - 2×10^4)

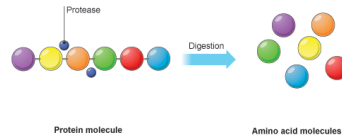
1:100



THE MICROBIOTA



Functions of the intestinal microbiota

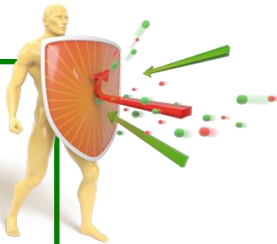


Food
breakdown

Vitamin
metabolism



Immune
system
maturation



**Intestinal
microbiota**

Brain
development



Defense
against
pathogens

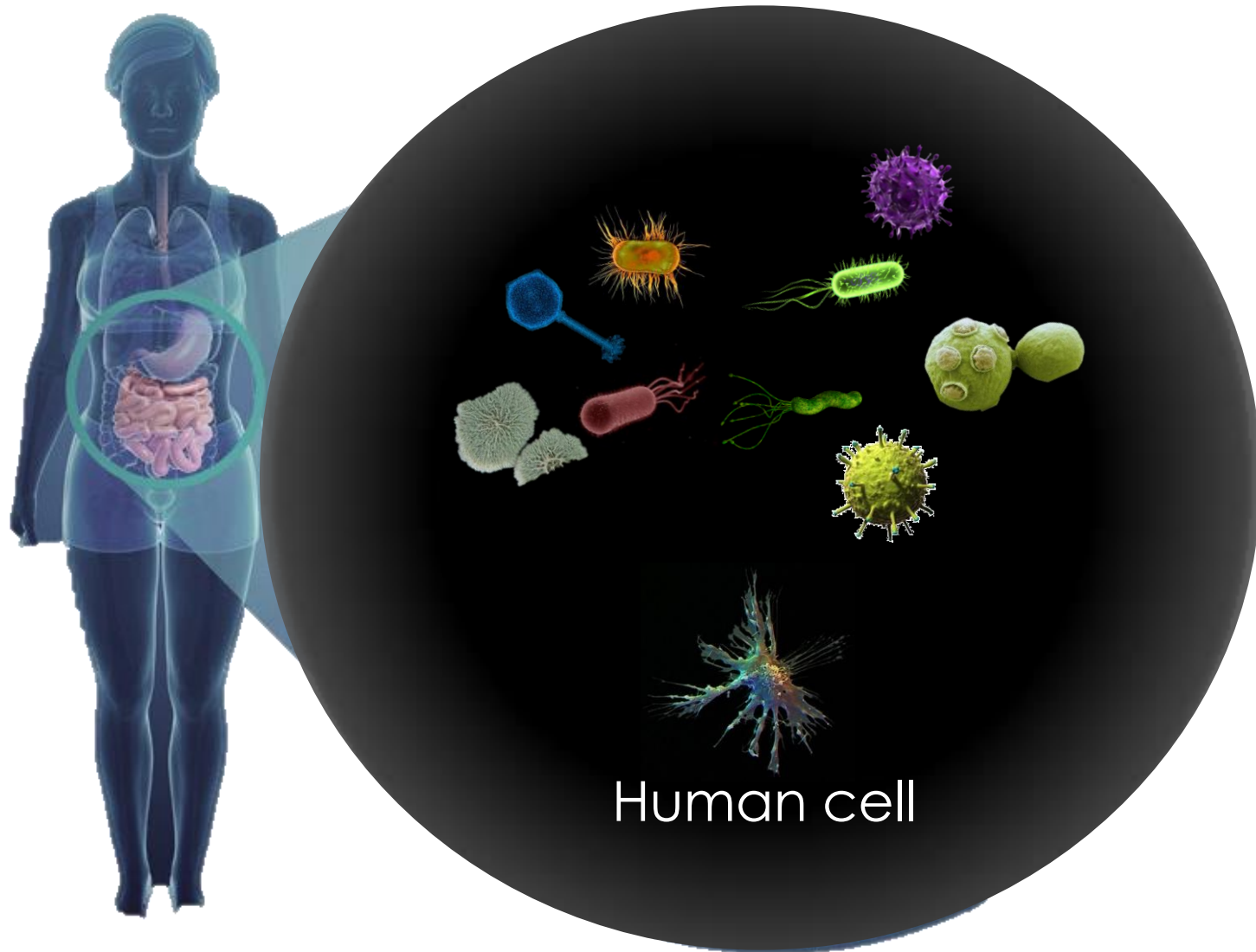


Xenobiotic
metabolism

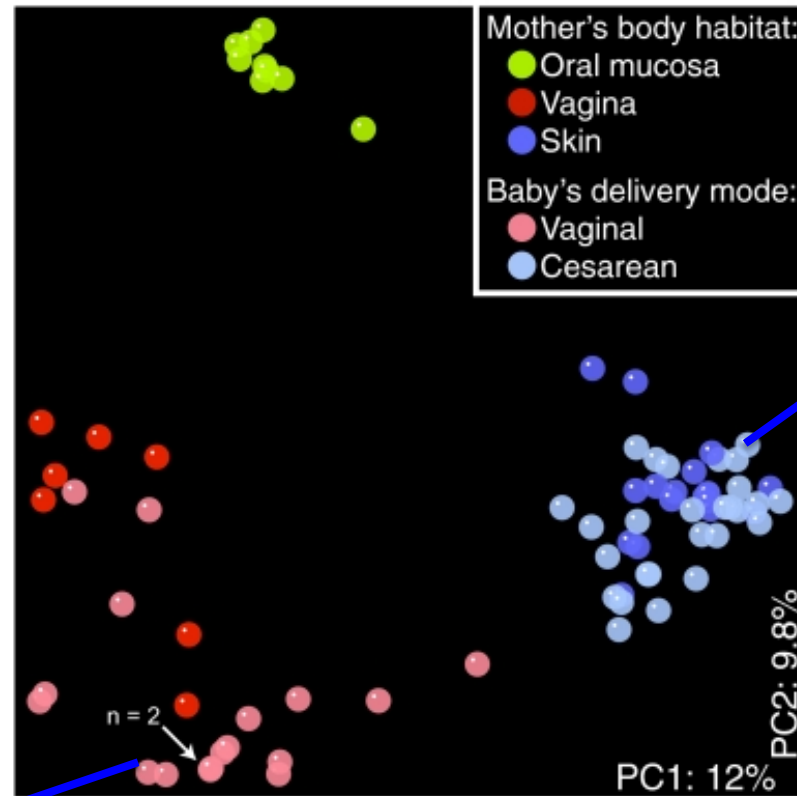


Eberl, Mucosal Immunology. 2010

“Eubiosis”



Microbiota is acquired at birth and during lactation

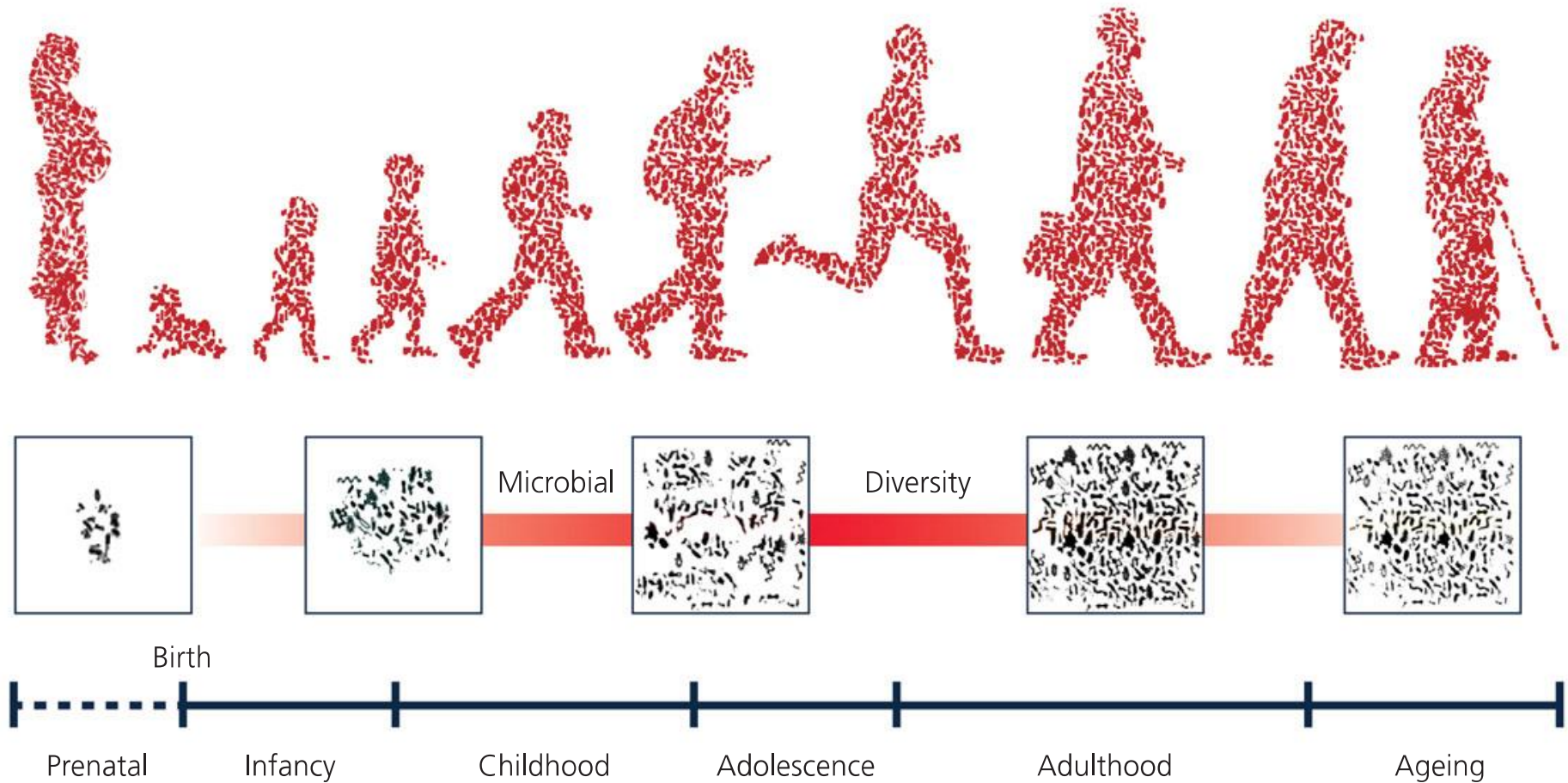


C-section: typical skin taxa
(*Staphylococcus* spp.)

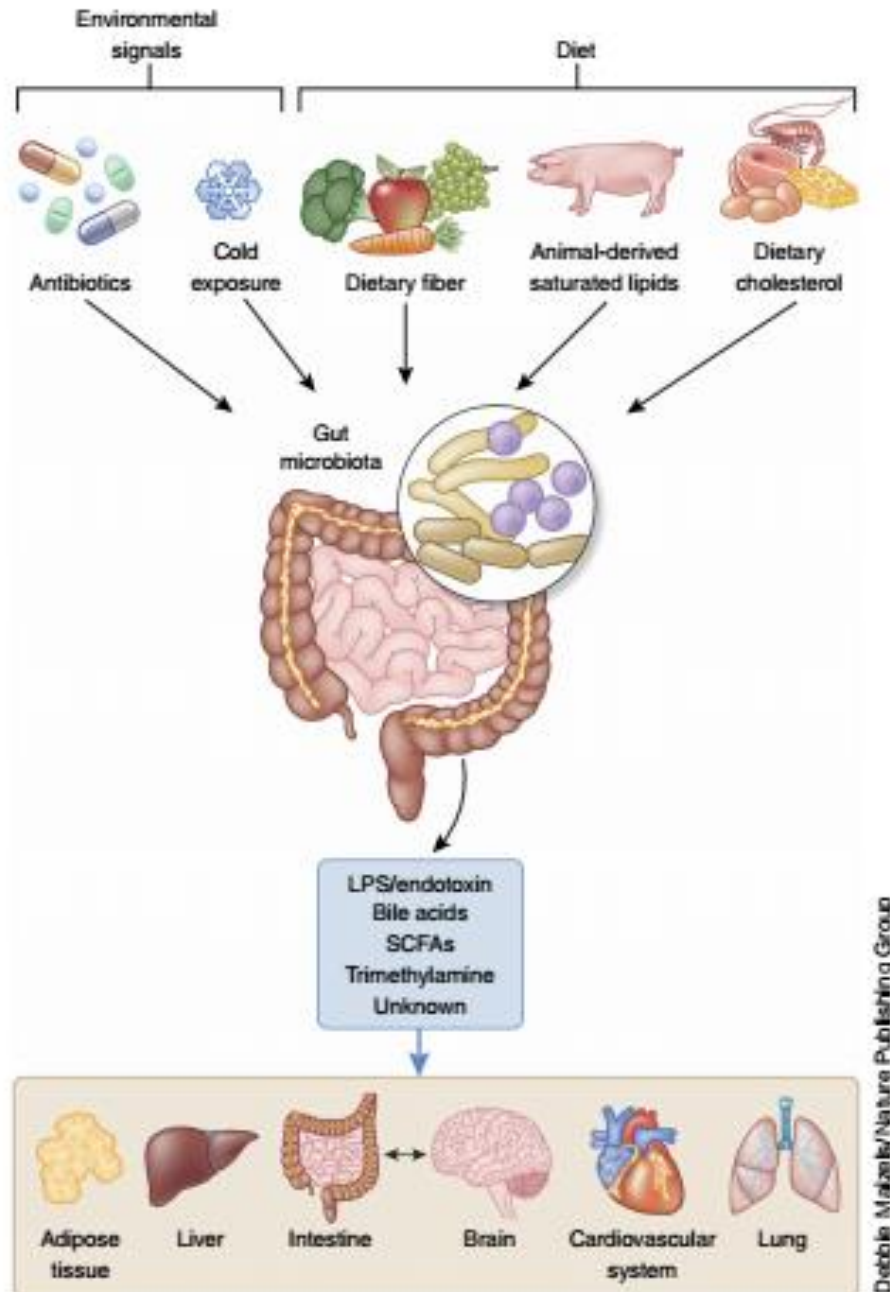
Natural delivery

(*Lactobacillus*, *Prevotella*,
Atopobium, or *Sneathia* spp.)

The microbiota dynamically changes across the lifespan



Factors that can alter the composition of the gut microbiota

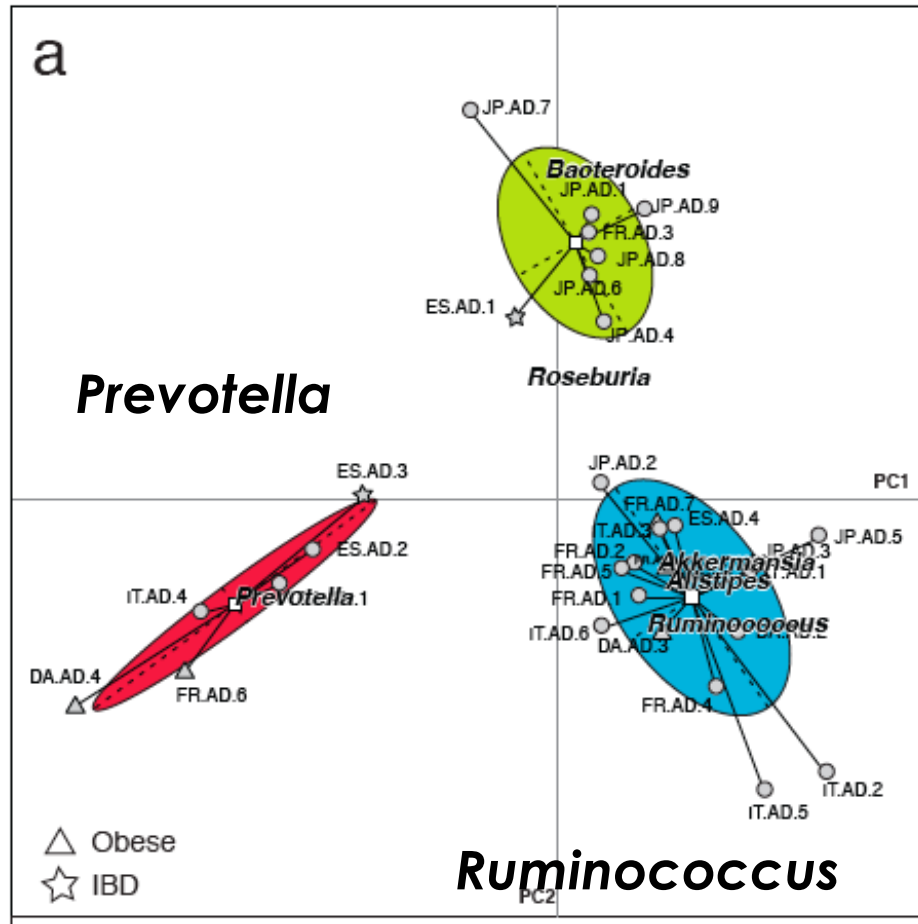


Gut microbiota convert environmental signals and dietary molecules into signaling metabolites to communicate with different organs and tissues in the host.

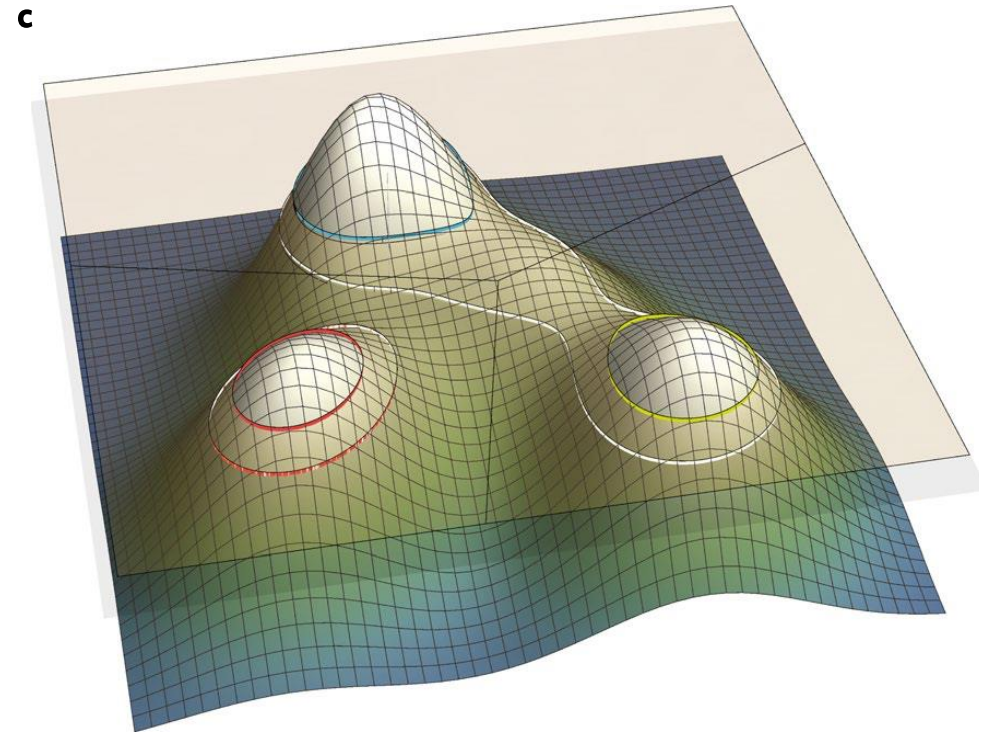
Three different enterotypes characterize the human population

39 individuals including
Europeans, American
Japanese

Bacteroides

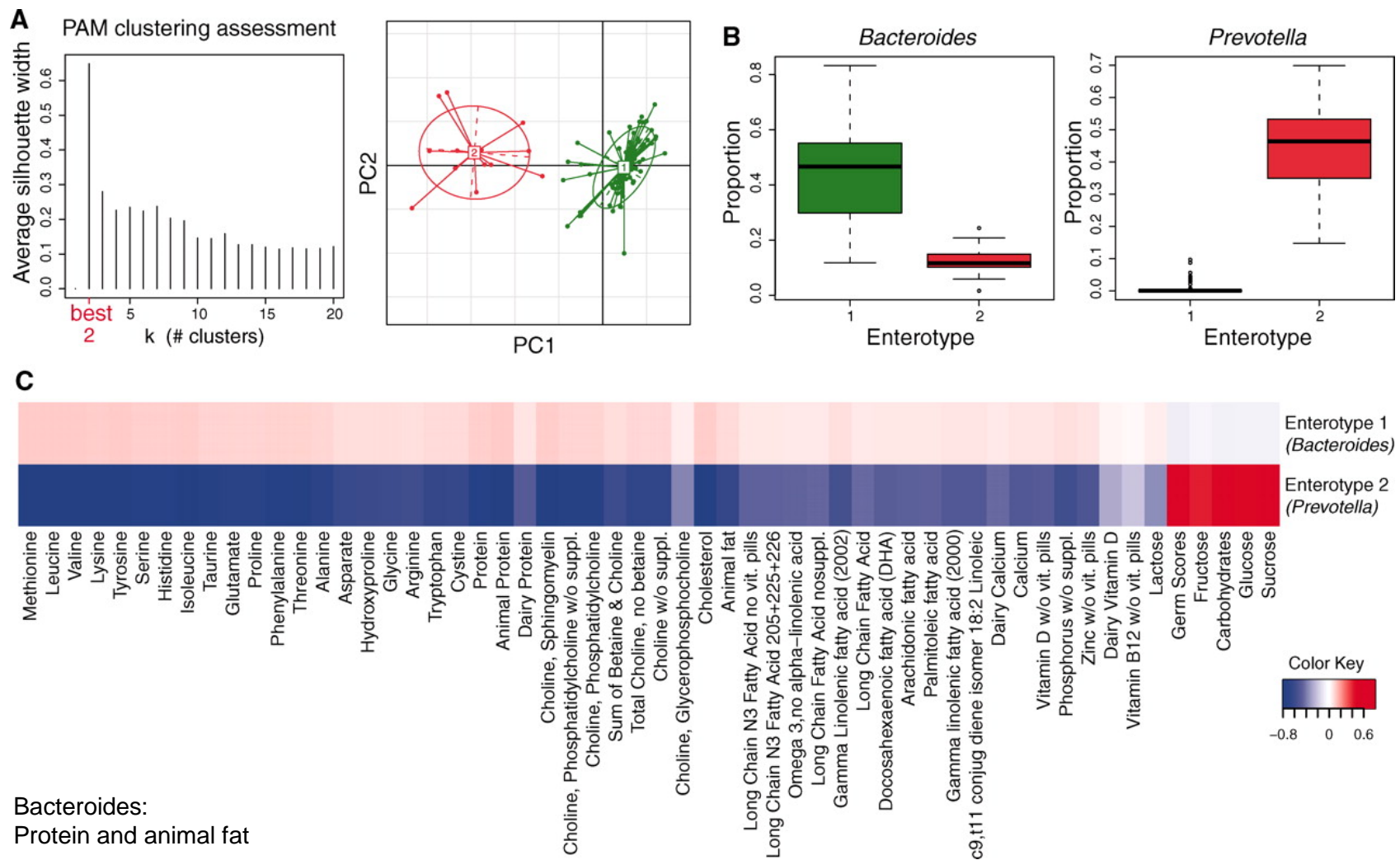


Stratification of the microbial composition landscape of the human gut microbiome



Enterotypes of the human gut microbiome. Arumugam M. et al. Nature April 2011 (Metahit consortium)

Diet can affect the microbiota

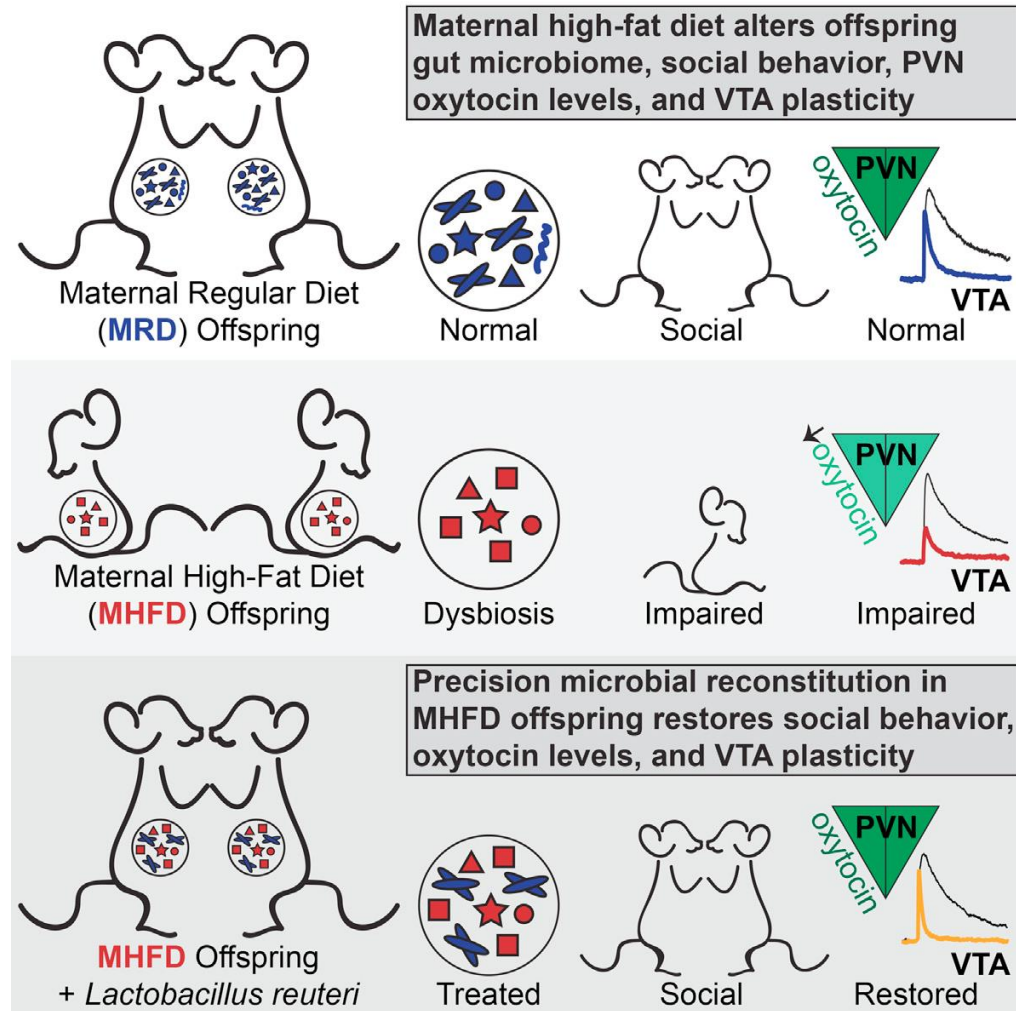


Bacteroides:
Protein and animal fat

Prevotella:
Carbohydrates

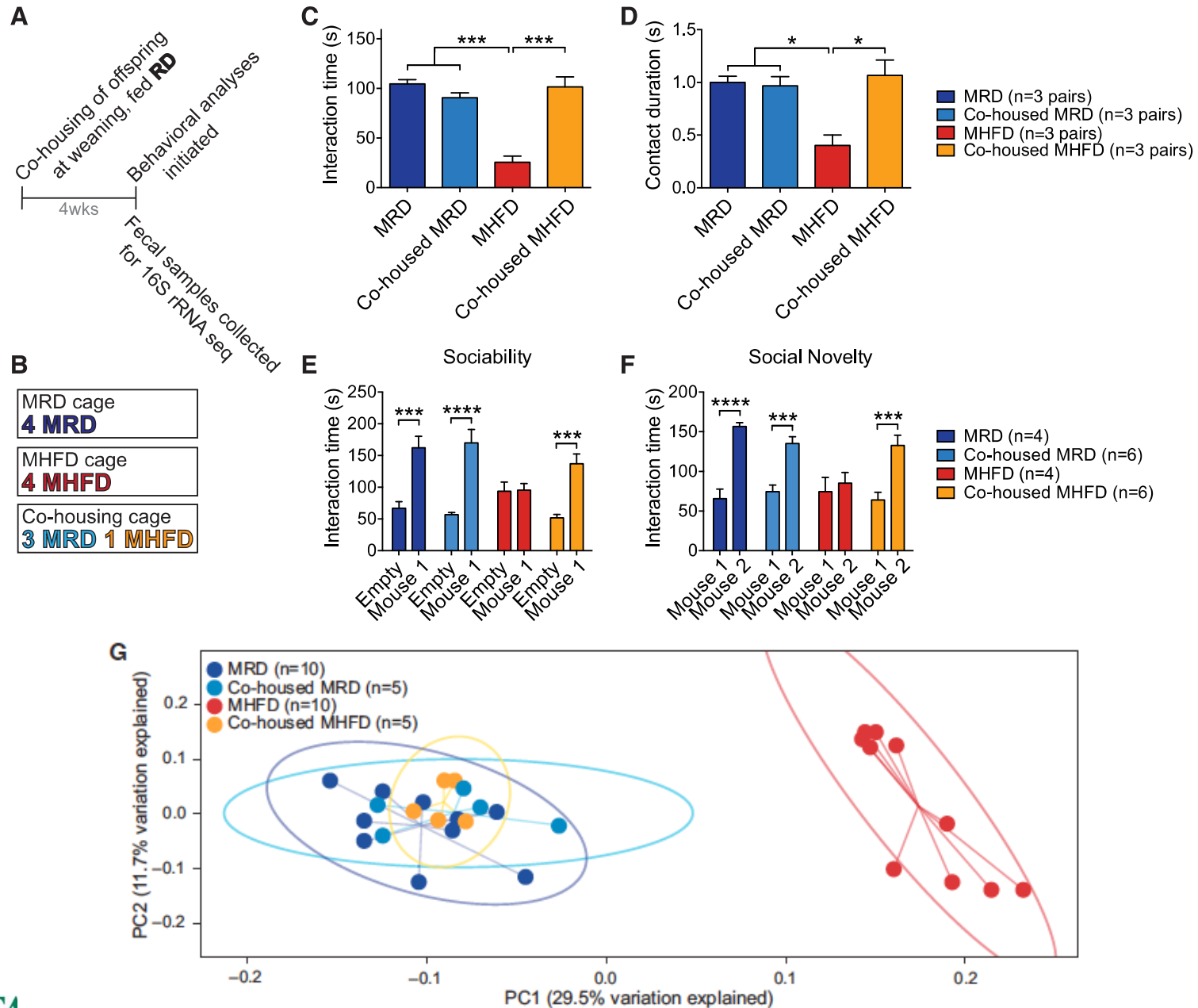
Wu et al *Science* 7 October 2011:

Diet controls social behaviour via the microbiota



Cell 165, 1762–1775, June 16, 2016 1763

Diet controls social behaviour via the microbiota



The Gastro-Intestinal System

➤ Intestinal Epithelial barrier (IEB)

- Epithelial cells
- Paneth cells
- Enterochromaffin cells

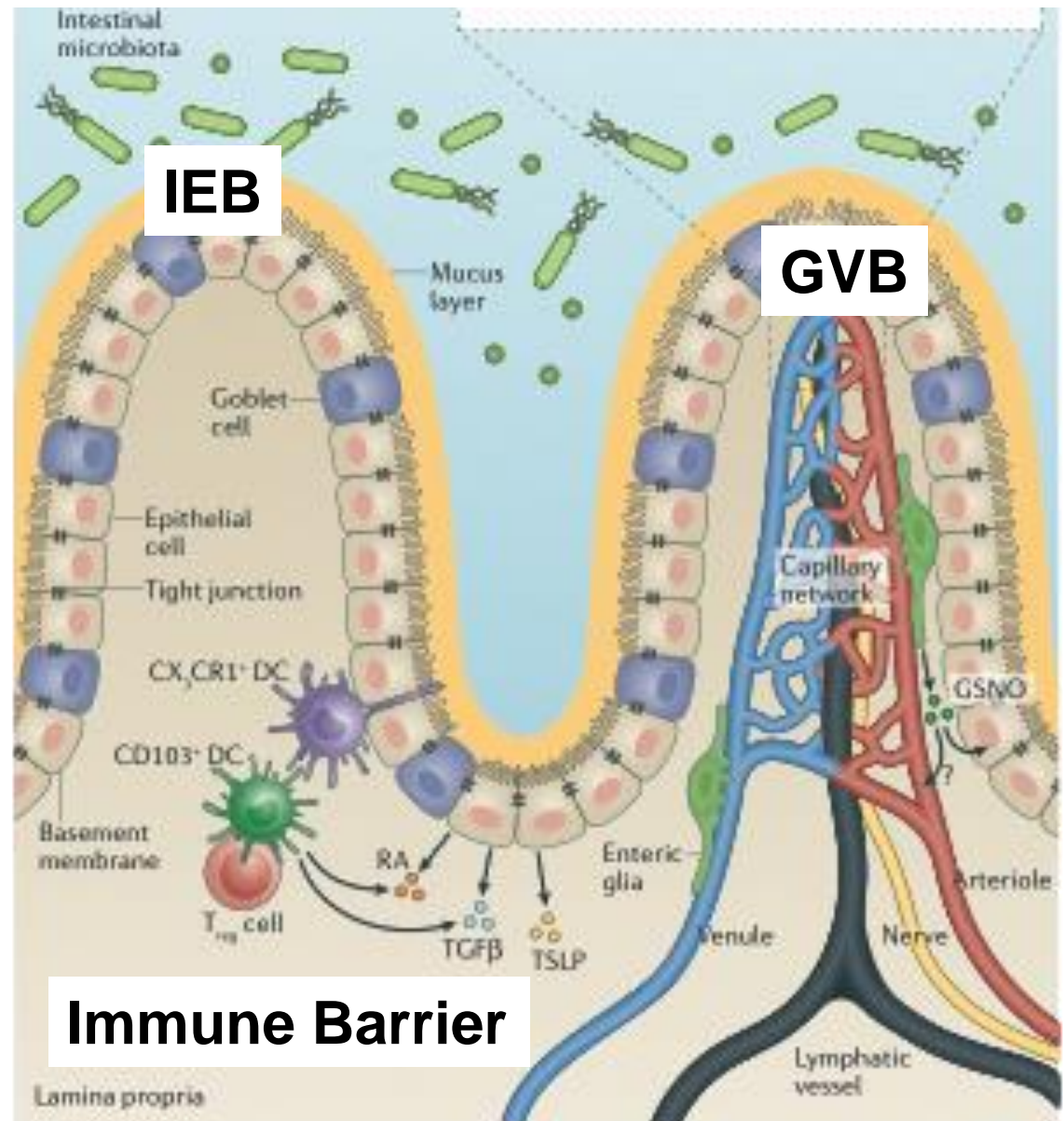
➤ Immune barrier

- Intraepithelial lymphocytes
- IgA-producing plasma cells
- Dendritic cells
- Macrophages

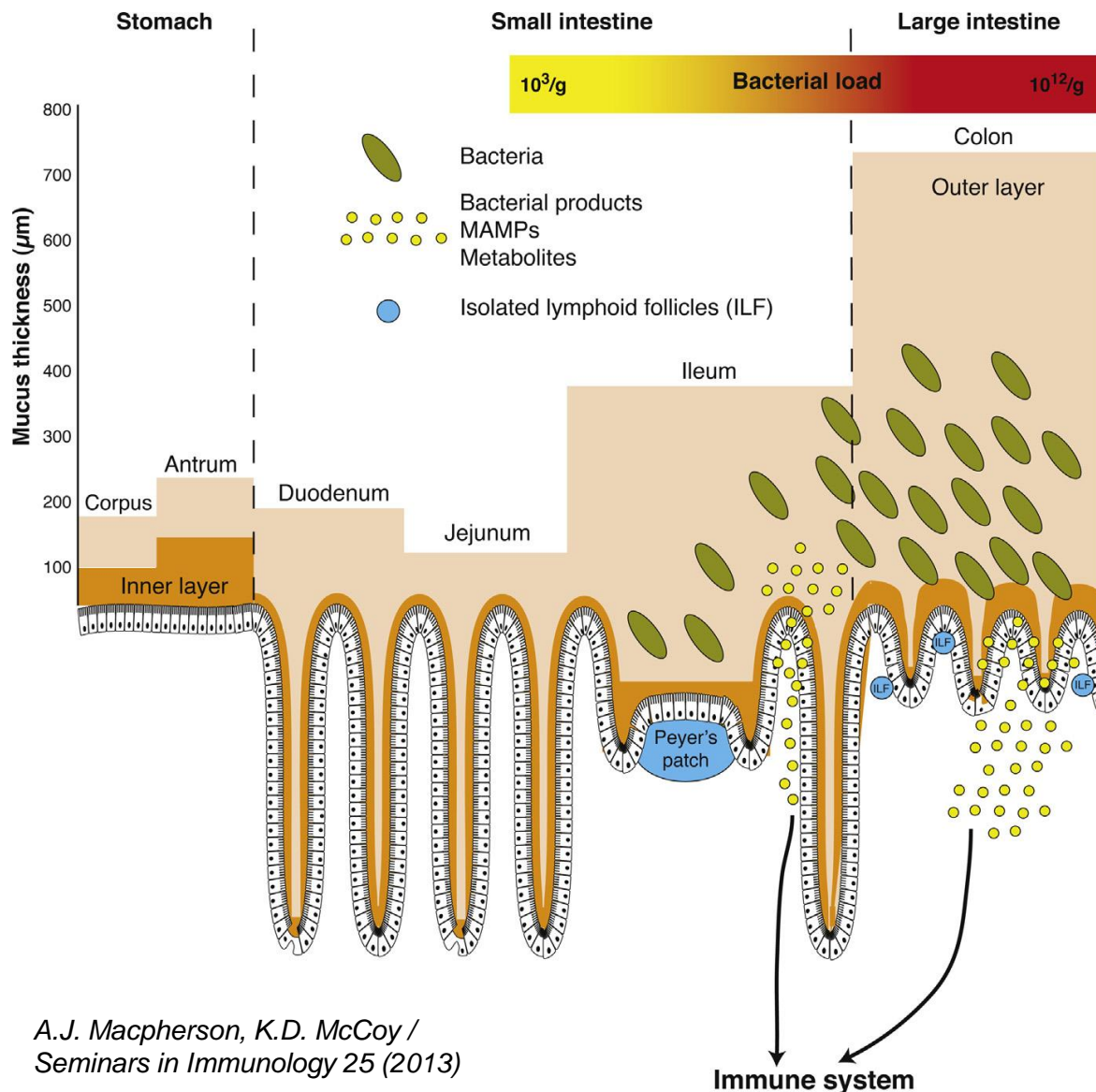
➤ Gut vascular barrier (GVB)

- Enteric endothelial cells (lack PLVAP, have TJs & AJs)
- Enteric glial cells
- Pericytes

(Spadoni et al, Science, 2015)

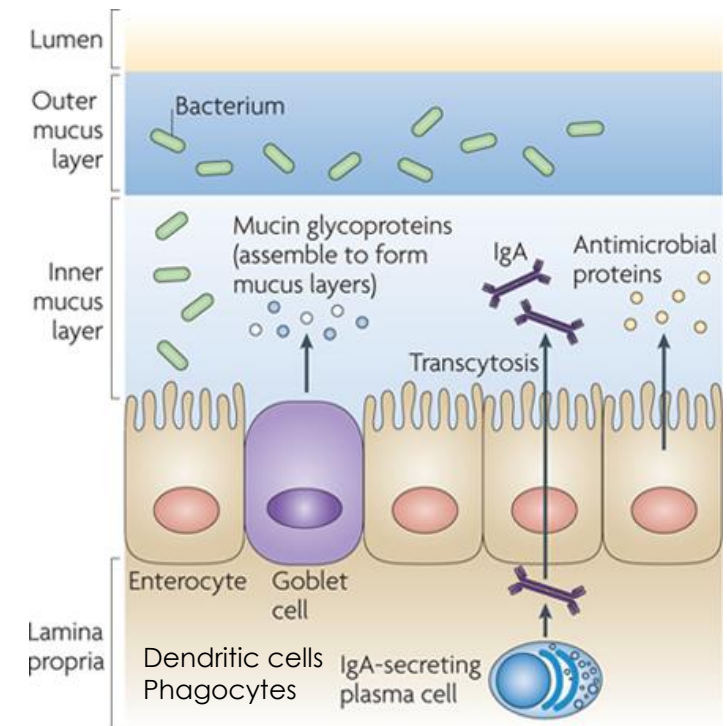
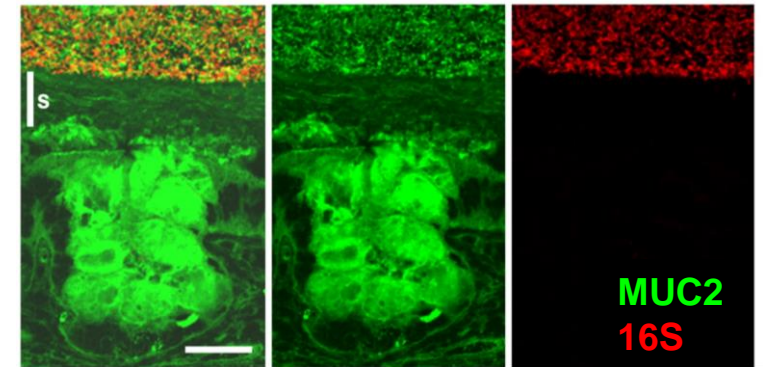


The intestinal microbiota is separated from the epithelium by the mucus

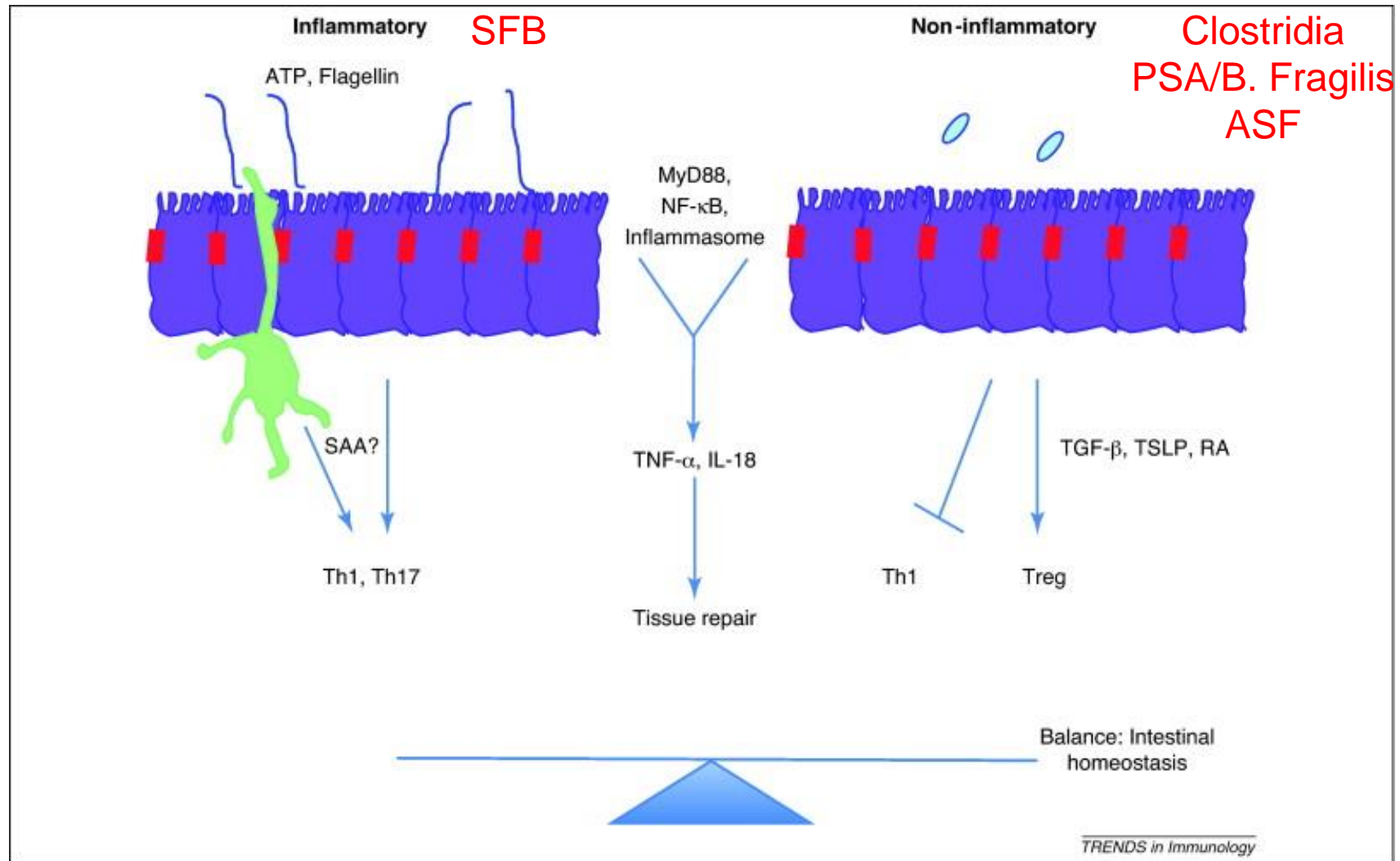


A.J. Macpherson, K.D. McCoy /
Seminars in Immunology 25 (2013)

Mucus (goblet cells)

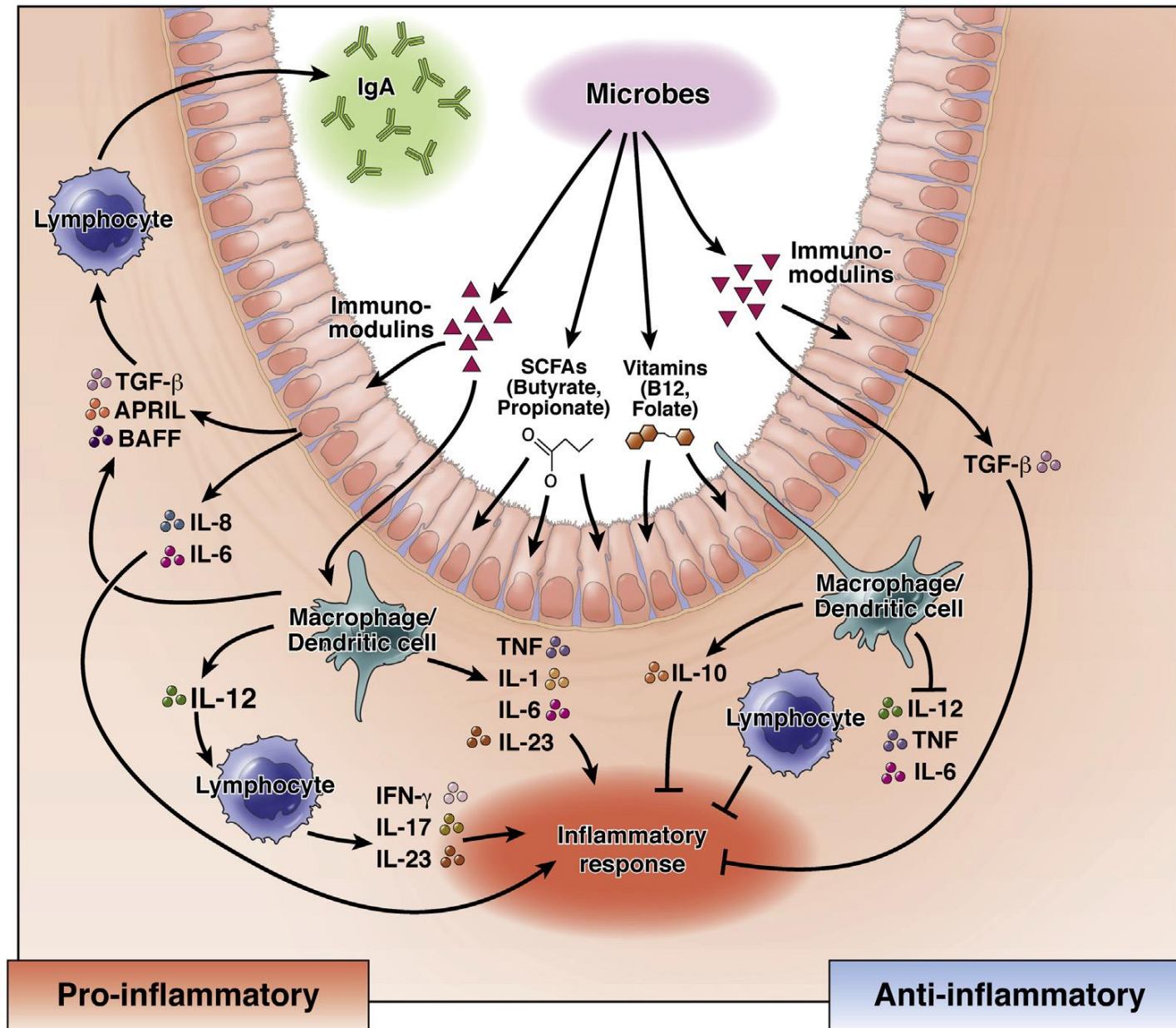


Inflammatory/tolerogenic bacteria control intestinal immune homeostasis

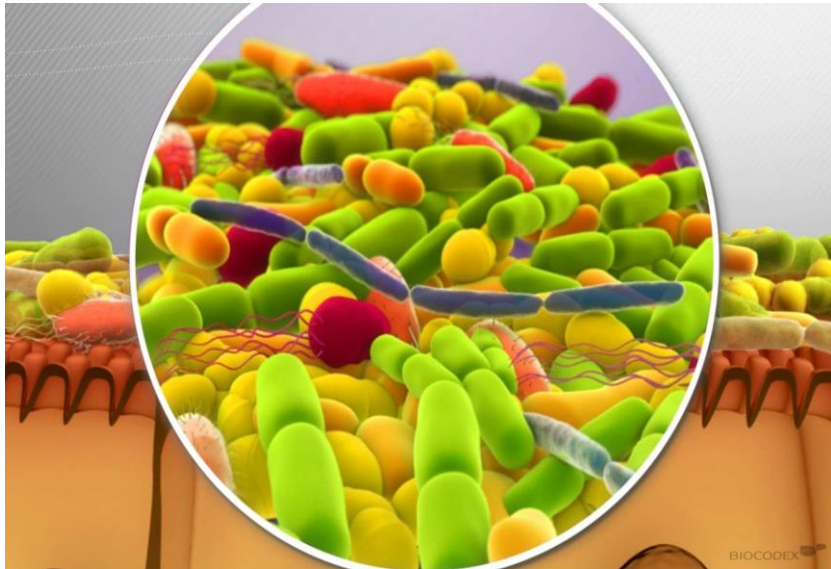


Rescigno Trends Immunol 2011, 32: 256–264

Most of the homeostatic activities are dependent on metabolites (Postbiotics)



PRE-, PRO- and POST-BIOTICS



PREBIOTIC



PROBIOTIC



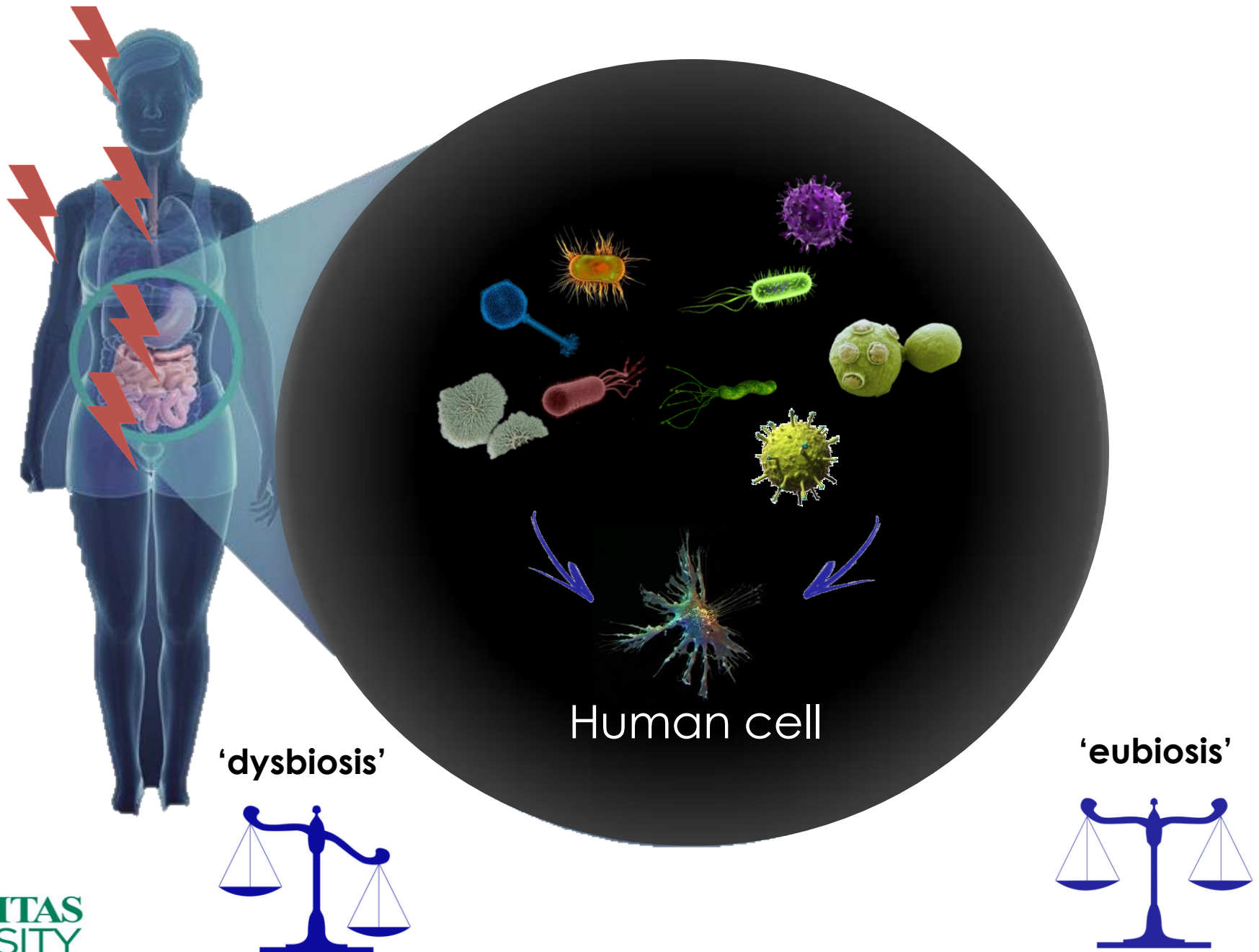
POSTBIOTIC

Prebiotics are substances (non-digestible fiber) that induce the growth or activity of microorganisms that contribute to the well-being of their host.

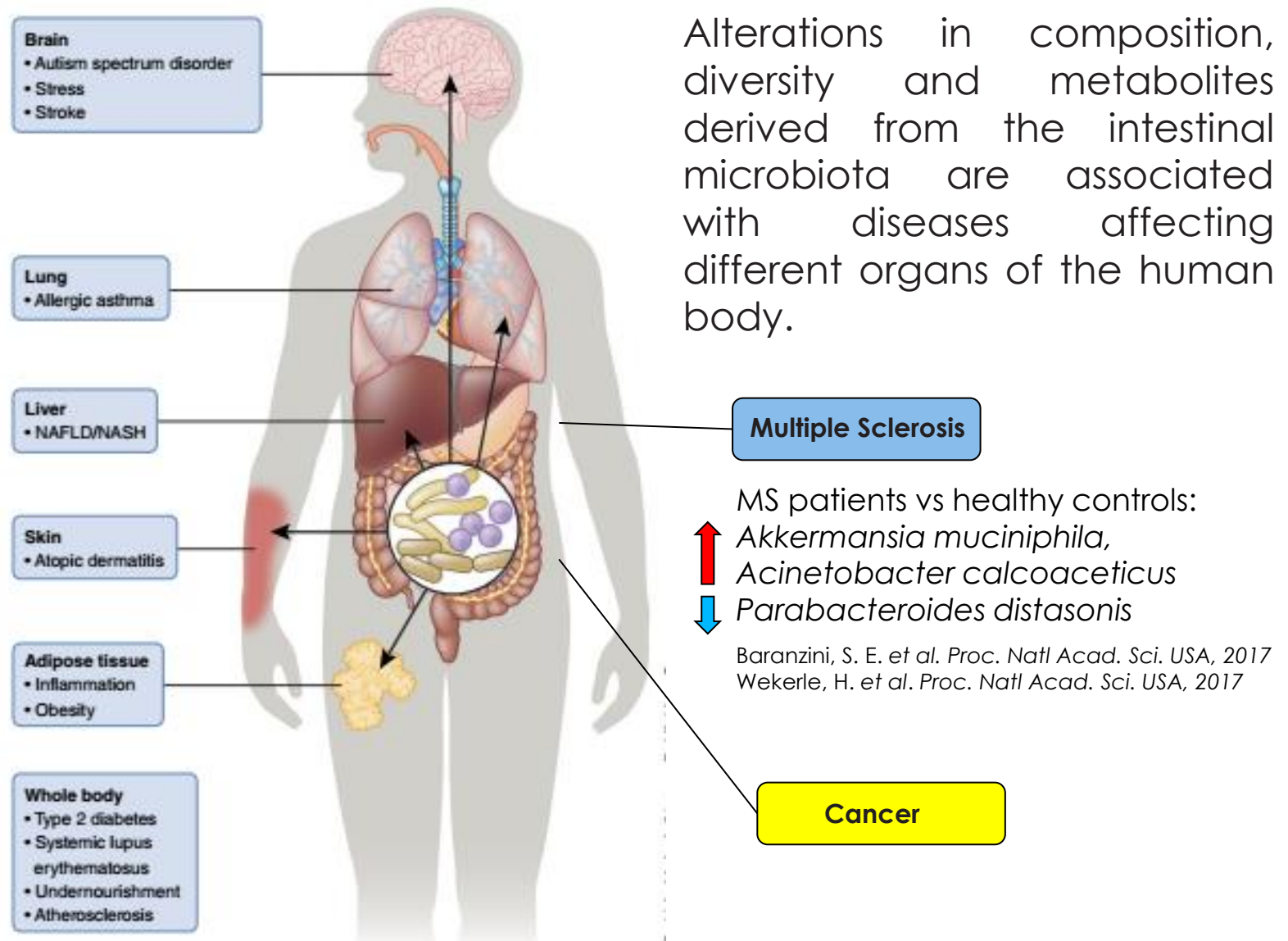
Probiotics are "live" microorganisms which, when administered in adequate amounts, confer a health benefit on the host"
FAO/WHO

Postbiotics are substances generated by probiotics metabolism, key factors in maintaining long-term health benefit

Dysbiosis



The intestinal microbiota is associated with various diseases in humans



Microbial pathogens drive certain cancers

- Microbial pathogens are the etiologic agents for 15% to 20% of cancers.

Microbes Designated as Class 1 (Carcinogens)

MICROBE	SITE OF CANCER
<i>Helicobacter pylori</i>	Stomach
Hepatitis B virus (HBV)	Liver
Hepatitis C virus (HCV)	
<i>Opisthorchis viverrini</i>	
<i>Clonorchis sinensis</i>	
Human papillomavirus (HPV)	Cervix Vagina Vulva Anus Penis Oropharynx
Epstein-Barr virus (EBV)	Nasopharynx Non-Hodgkin lymphoma Hodgkin lymphoma
Kaposi sarcoma-associated herpesvirus (KSHV or HHV8)	Kaposi sarcoma Primary effusion lymphoma
Human T-cell lymphotropic virus type 1 (HTLV-1)	Adult T-cell lymphoma
<i>Schistosoma haematobium</i>	Bladder

Commensal bacteria and cancer incidence

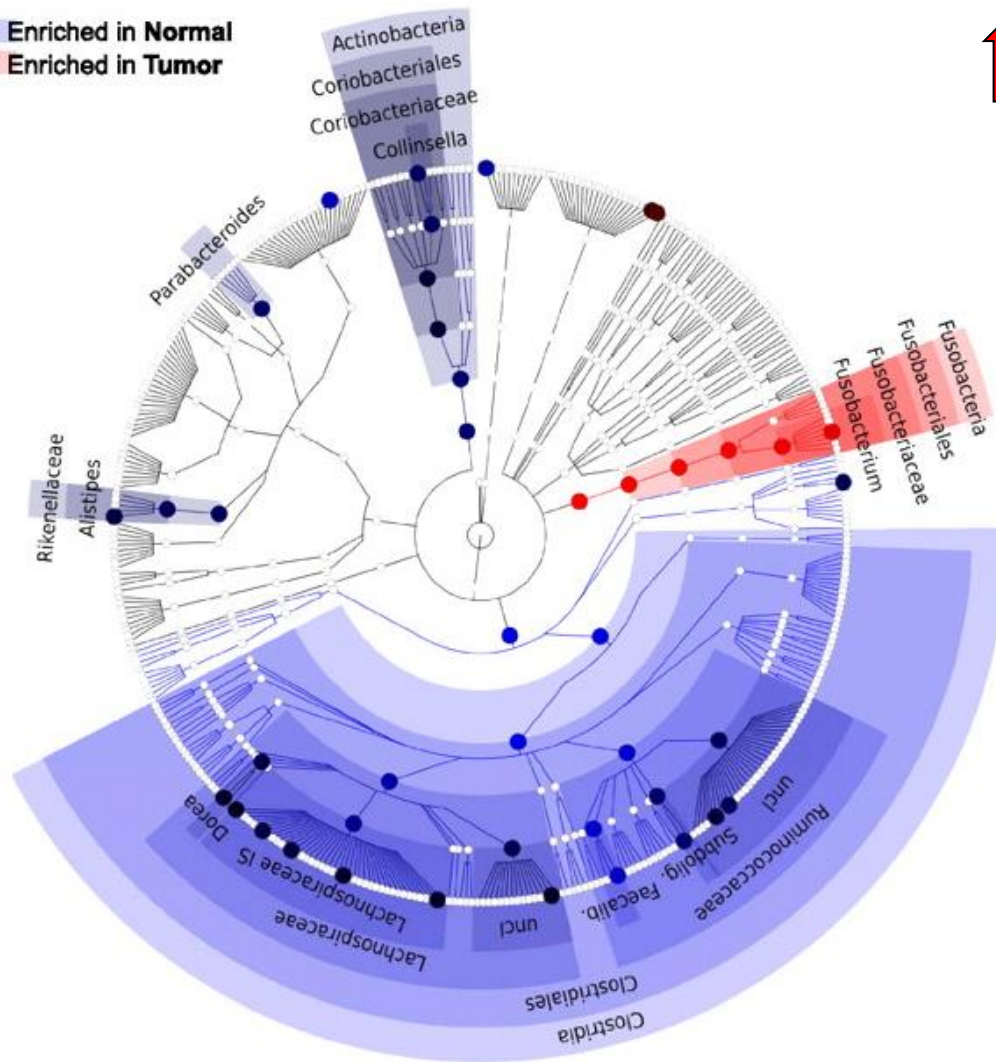
- Metagenomic sequencing studies have detected significant **differences in the composition of microbial communities in numerous human cancer cases** compared with controls.
- Commensal microbiota have a more widespread influence on the initiation and progression of tumorigenesis.

hCRC microbiome

✓ Microbial dysbiosis in CRC:

↓ *Bacteroidetes* and *Firmicutes*

↑ *Fusobacterium* sp (invasive anaerobe)



✓ No clear picture on a group of microorganisms associated with CRC.

Correlations between microbiota and CRC

- ✓ **Apc^{Min/+} mice in GF conditions: drop in tumor incidence.**

Li et al Carcinogenesis 2012

- ✓ **Colonization of APCMin mice with the human commensal *Enterotoxigenic Bacteroides fragilis* (ETBF) results in increased colon carcinogenesis, by increasing Th17.**

Wu et al. Nature Med 2009

- ✓ **ETBF found in 38% of CRC and 12% controls.**

Toprak et al. Clin. Microb. Infect. 2006

- ✓ ***E. coli* induces DNA DS breaks via the action of polyketide kinase (pks) and tumorigenesis in IL-10-/- mice.**

Arthur et al. Science 2012

- ✓ ***Fusobacterium nucleatum* promotes CRC by modulating E-cadherin/b-catenin signaling.**

Rubinstein MR, et al. Cell Host Microbe. 2013

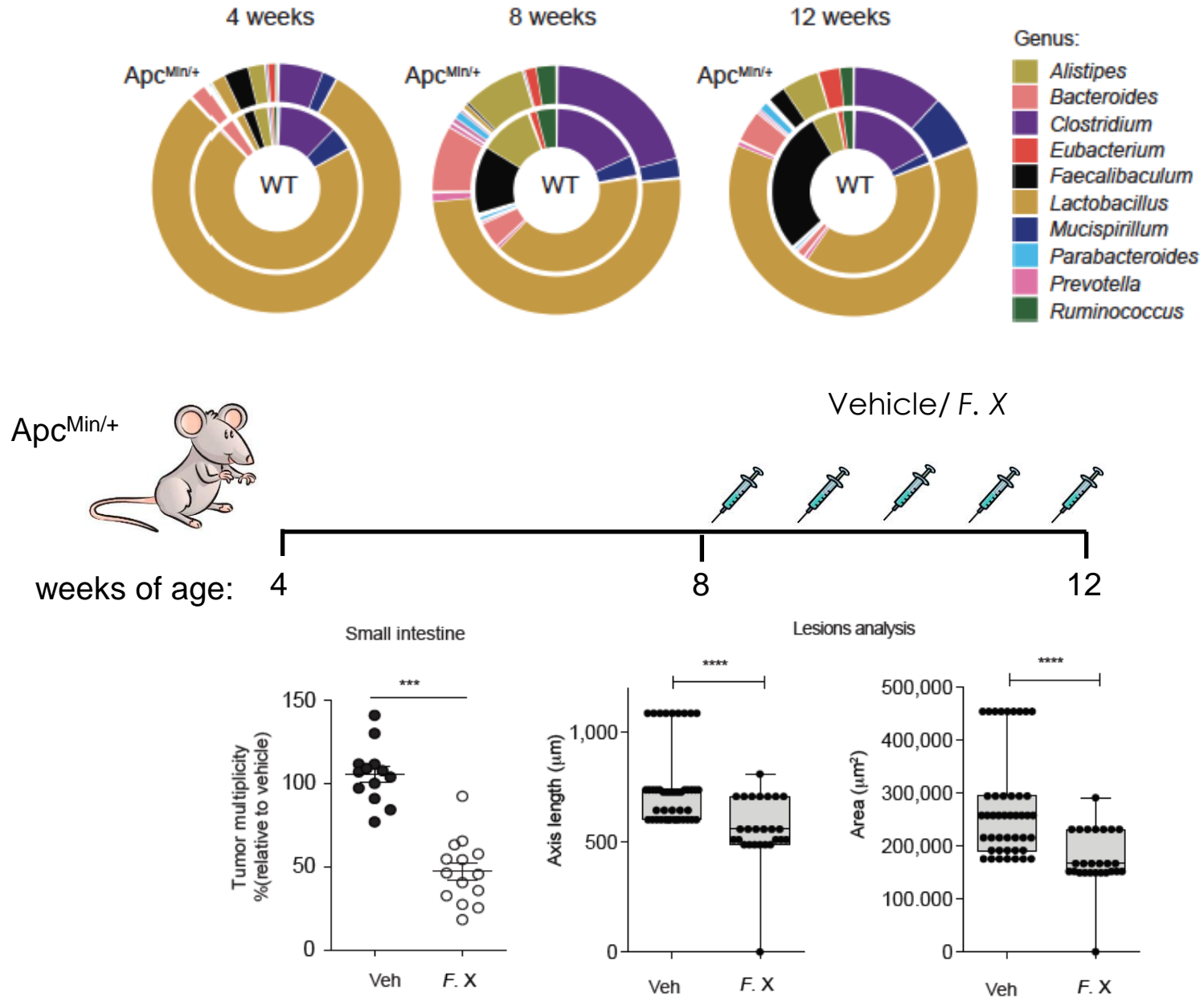
- ✓ **Enterococcal infections (endocarditis) are associated with occult malignant disease (*Streptococcus gallolyticus*: growth advantage in the presence of tumor cells).**

McCoy et al 1951J Med Assoc state Ala ; Boleij et al. Lancet Inf dis 2013

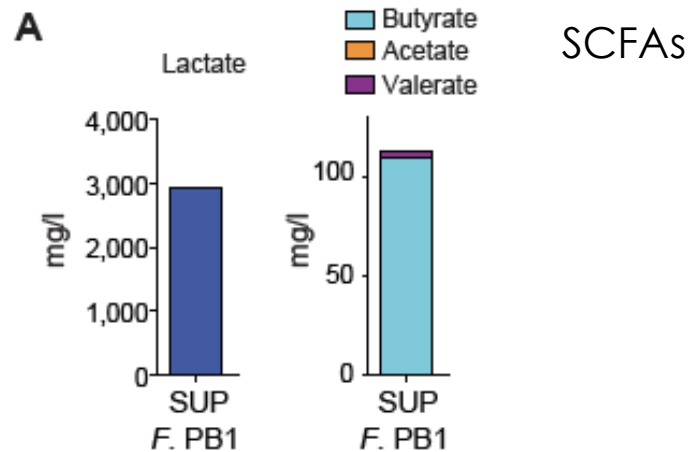
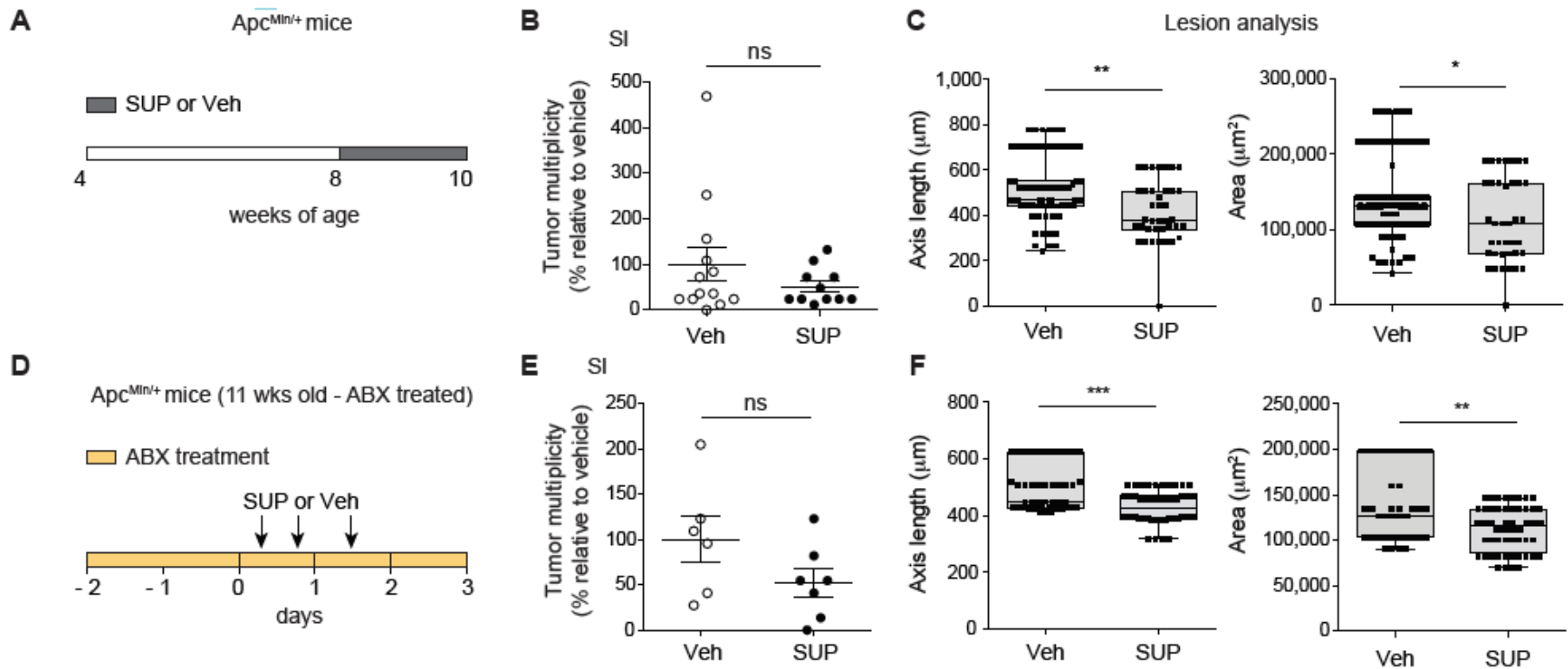
CRC tumorigenesis and microbiota

1. Increased penetrance of protumorigenic bacteria
2. Contraction of anti-tumorigenic bacteria

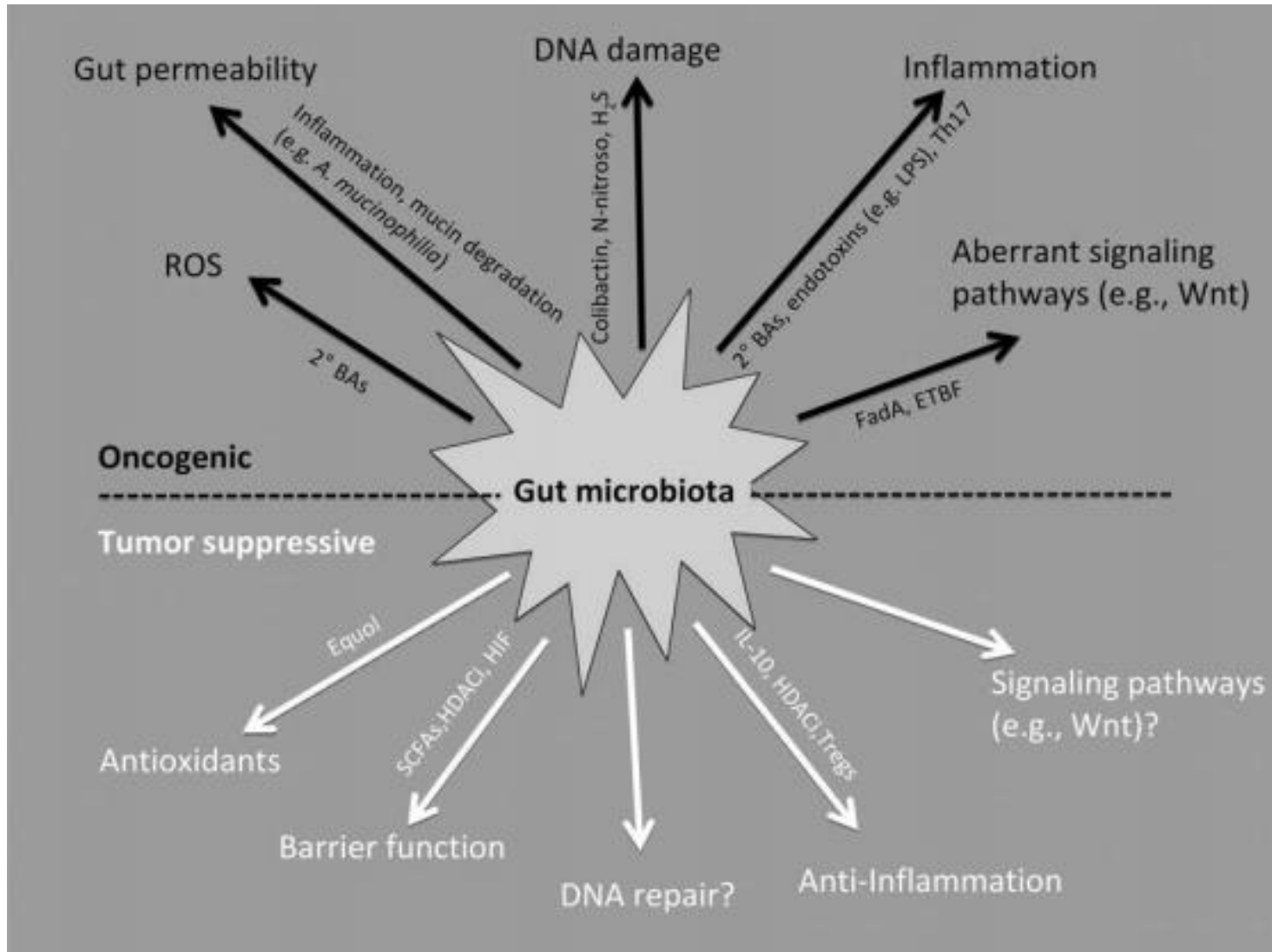
F. X reduces tumor multiplicity in $Apc^{Min/+}$ mice



F. X supernatant controls tumor growth *in vivo*

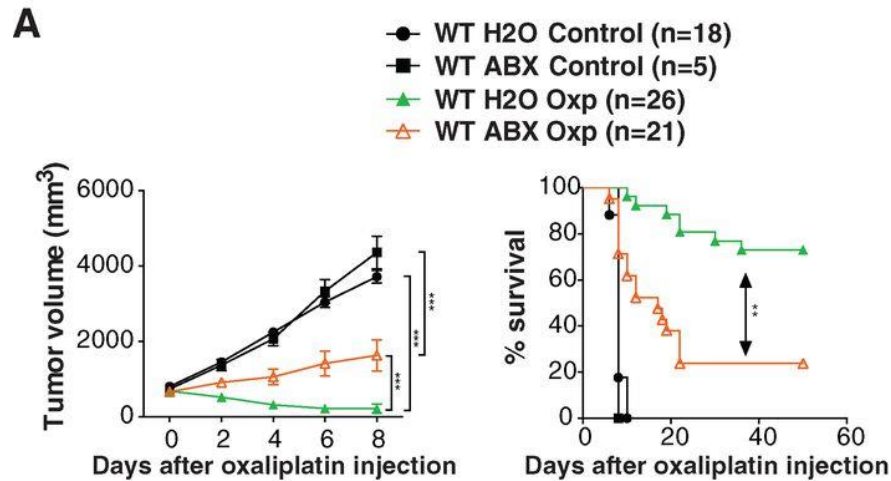


Microbial mechanisms of oncogenesis and tumor suppression



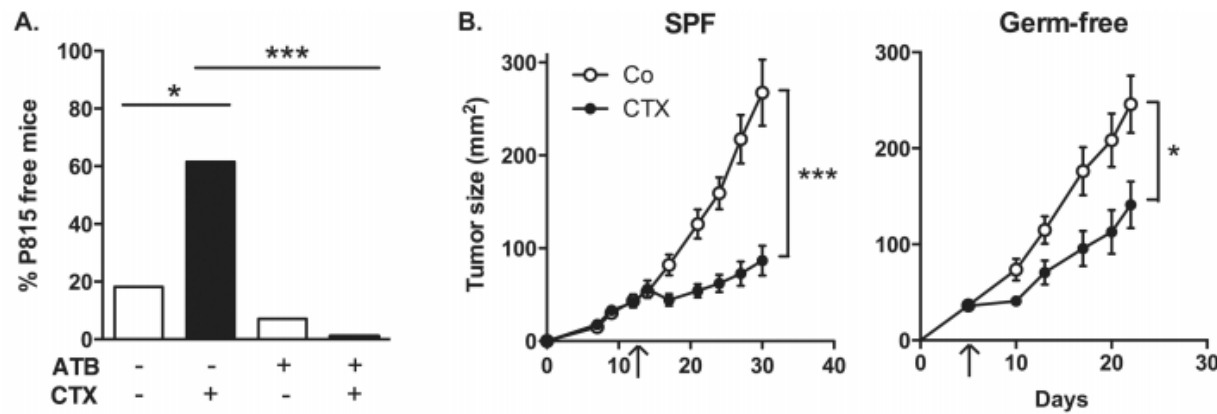
Microbiota influences chemotherapy

- Commensal bacteria control cancer response to therapy by modulating the tumor microenvironment.



Commensal bacteria increase the effect of **oxaliplatin**

N lida et al. Science 2013;342:967-970

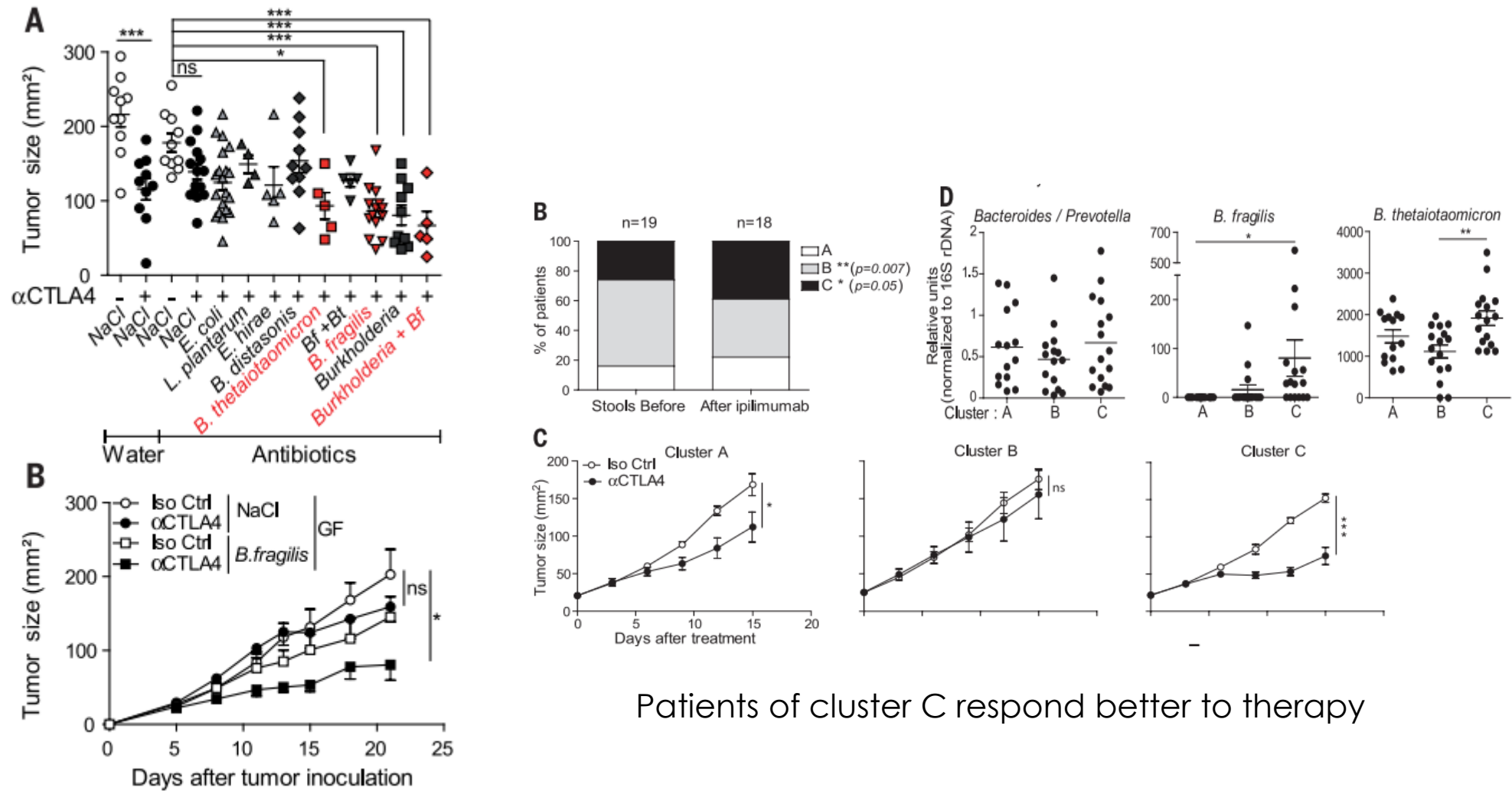


Commensal bacteria increase the effect of **cyclophosphamide** (CTX)

S Viaud et al. Science 2013;342:971-976

Microbiota influences ICB-immunotherapy

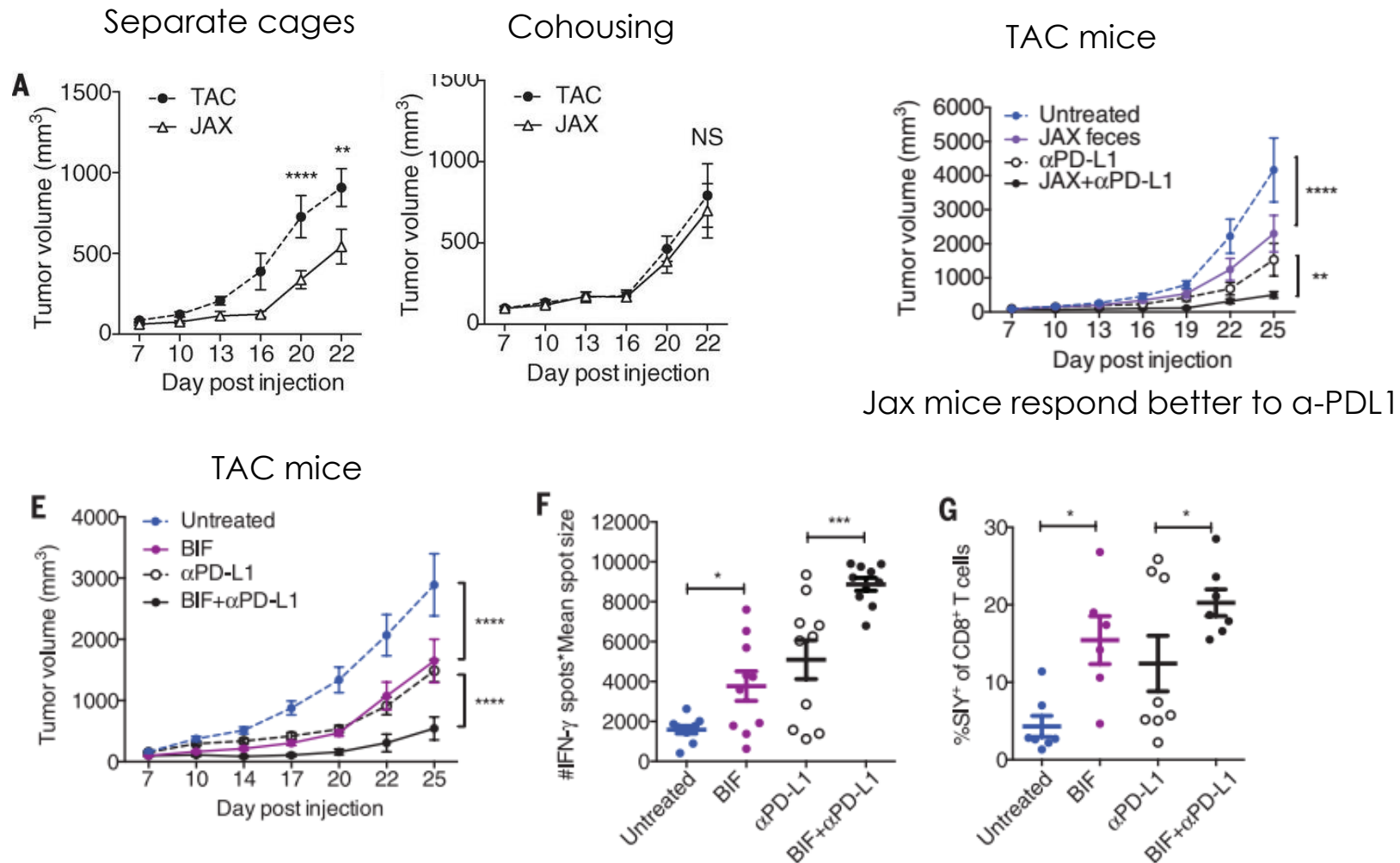
Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota



Patients of cluster C respond better to therapy

Microbiota influences ICB-immunotherapy

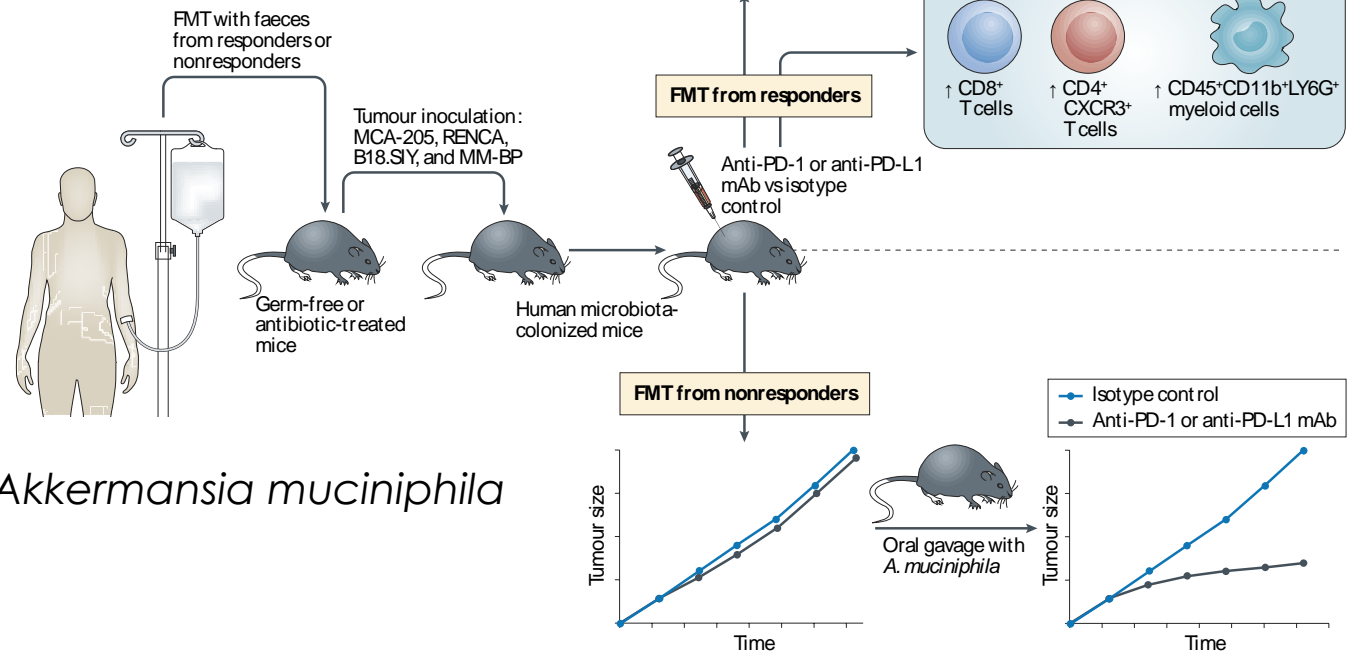
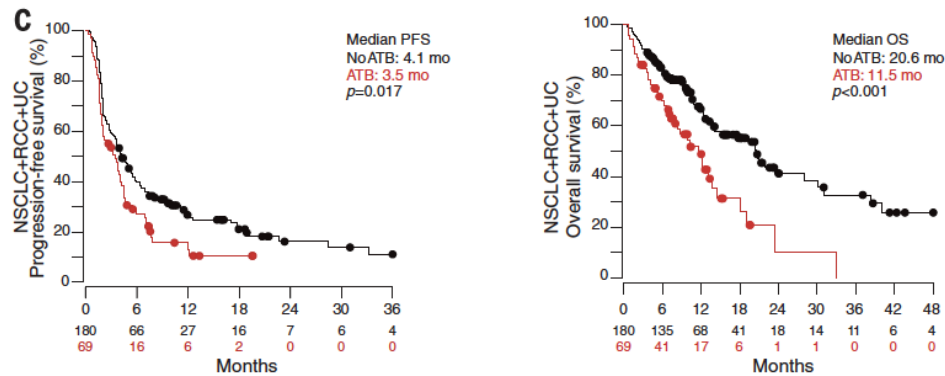
Commensal *Bifidobacterium* promotes antitumor immunity and facilitates anti-PD-L1 efficacy



Microbiota influences ICB-immunotherapy

Gut microbiome influences efficacy of PD-1-based immunotherapy against epithelial tumors

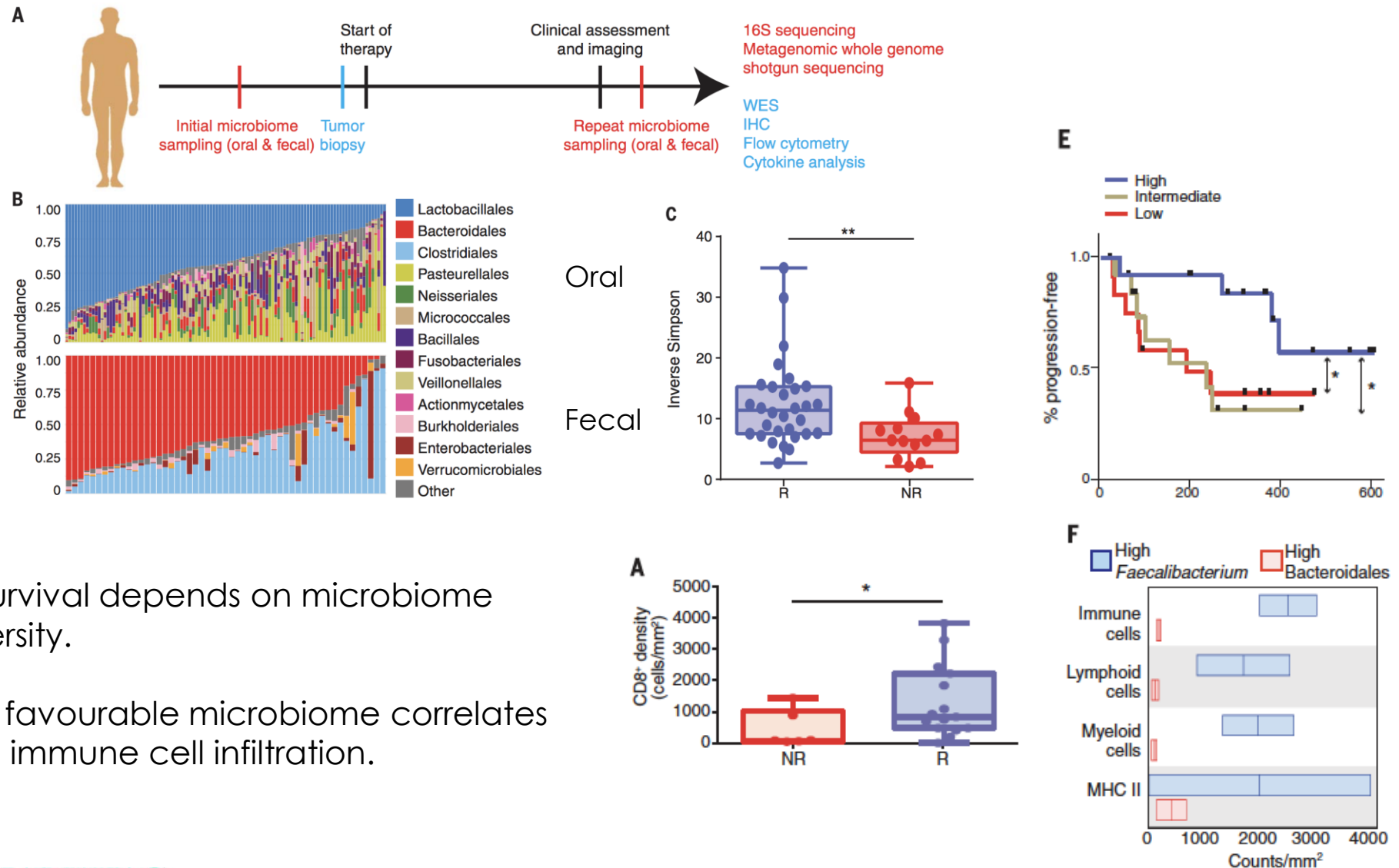
✓ Antibiotics affect ICB therapy (PD-1/PD-L1) in RCC, NSCLC and UC



✓ Efficacy depends primarily on *Akkermansia muciniphila*

Microbiota influences ICB-immunotherapy

Gut microbiome modulates response to anti-PD-1 immunotherapy in melanoma patients.



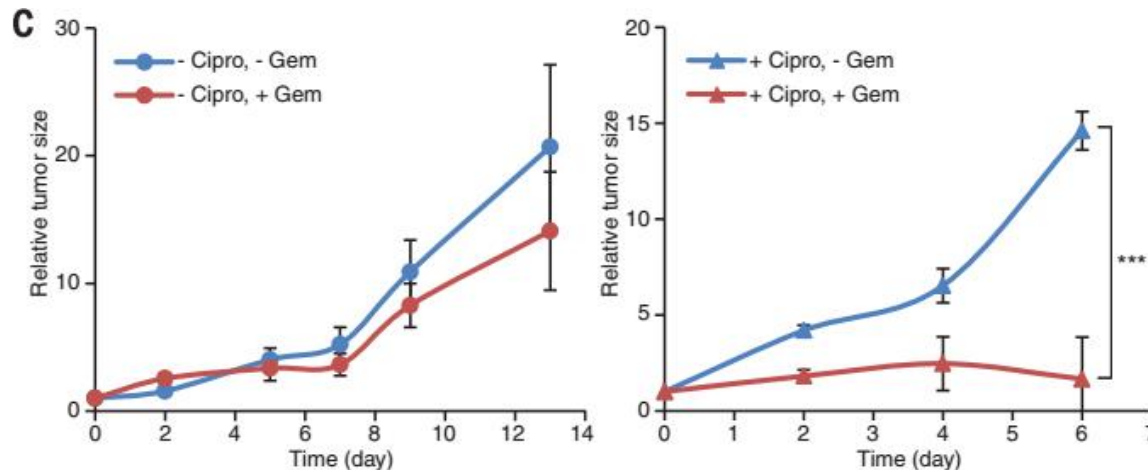
✓ Survival depends on microbiome diversity.

✓ A favourable microbiome correlates with immune cell infiltration.

Microbiota contributes to drug resistance

Bacteria can metabolize **gemcitabine** into its **inactive form** through the bacterial enzyme cytidine deaminase (CDDL), seen in *Gammaproteobacteria*.

CRC mouse model



Gemcitabine resistance was induced by intratumor *Gammaproteobacteria*, and abrogated by cotreatment with the antibiotic ciprofloxacin.

Gemcitabine used to treat pancreatic ductal adenocarcinoma (PDAC):
intratumor bacteria might contribute to drug resistance of these tumors → 113 human PDACs that were tested, 86 (76%) were positive for bacteria, mainly *Gammaproteobacteria*.

Conclusions

✓ How can we intervene?

Combine standard therapy or immunotherapy with:

Controlled diet supplementation:

- Prebiotics (Fibers favoring the growth of certain bacteria)
- Probiotics (Lactobacilli, bifidobacteria, Clostridia)
- **Postbiotics** (SCFAs...)

✓ Should we use antibiotics?

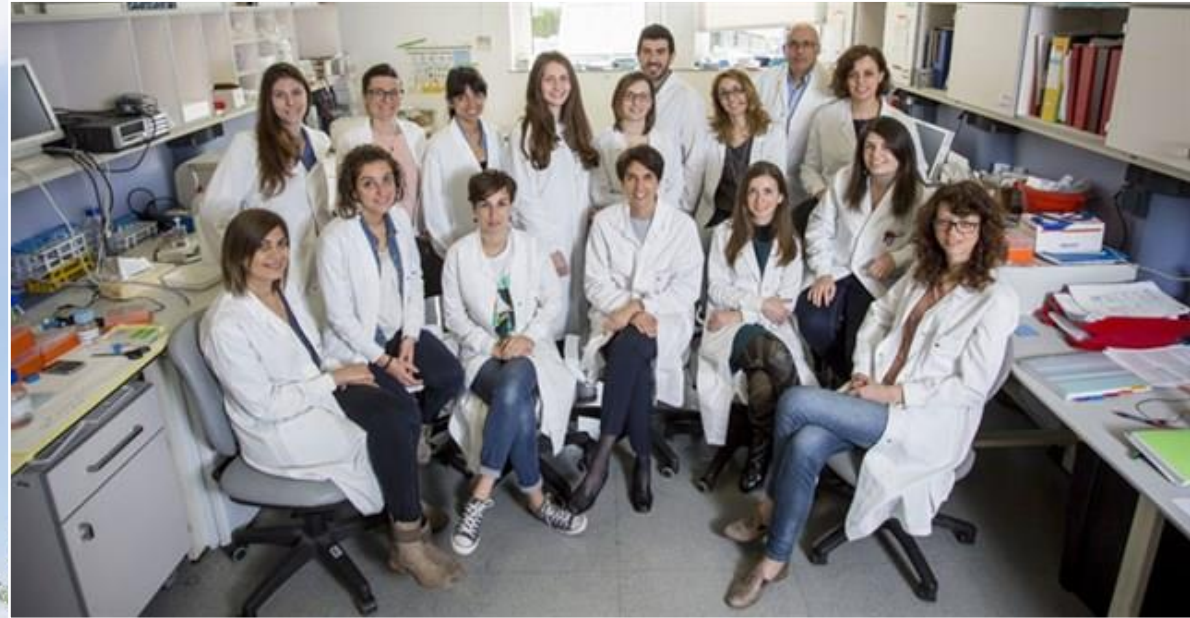
✓ Which is the best probiotic mixture? Should we focus on their metabolic output rather than species?

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