The gut-brain axis

The gut microbiome in MS

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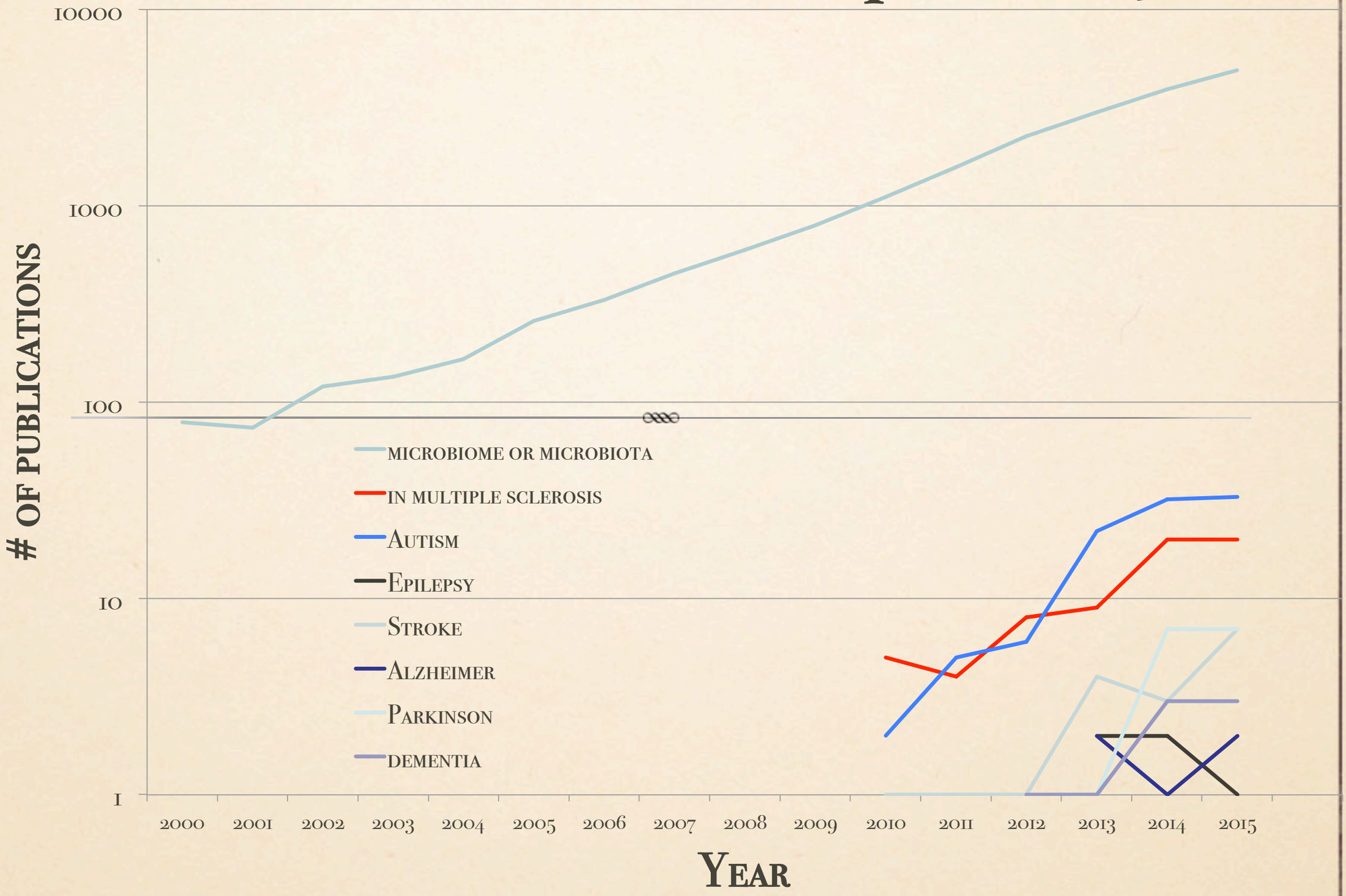
AN EMERGING FIELD OF RESEARCH

Literature review (up to 2015)



A FEW DEFINITIONS

- Microbiota: A collection of microbial communities living in a specific niche
- Microbiome: The collective set of genes from a given microbiota
- Probiotic bacteria: Live microorganisms which when administered in adequate amounts confer a health benefit on the host
- Prebiotics: Selectively fermented food ingredients that allow specific changes, both in the composition and/or activity in the gastrointestinal microbiota that confer benefits upon host health

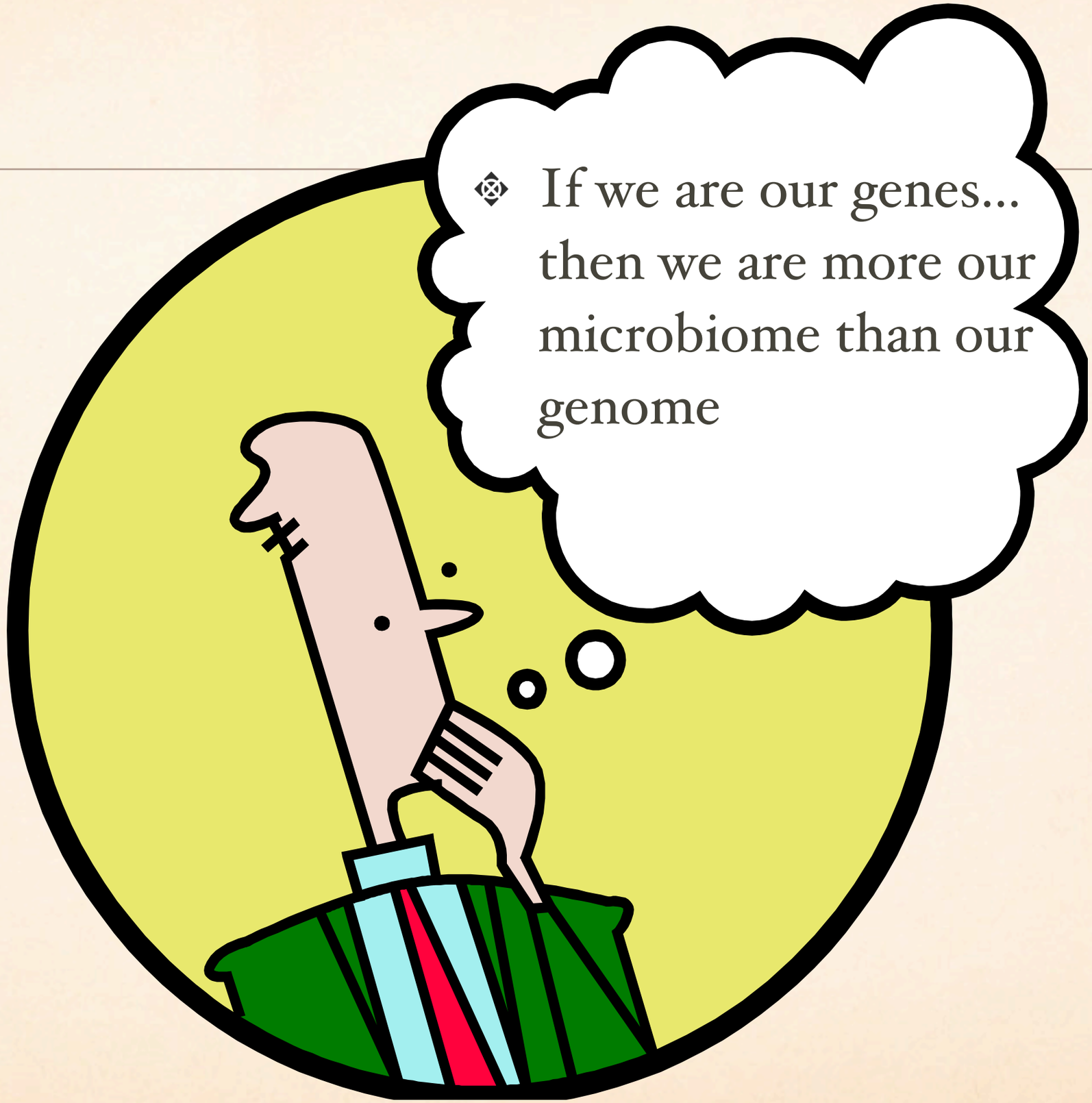
THE ENTERIC NERVOUS SYSTEM

- "Second brain"
- 400 million neurons (=spinal cord)
- Extends from esophagus to anus
- Controls digestive system

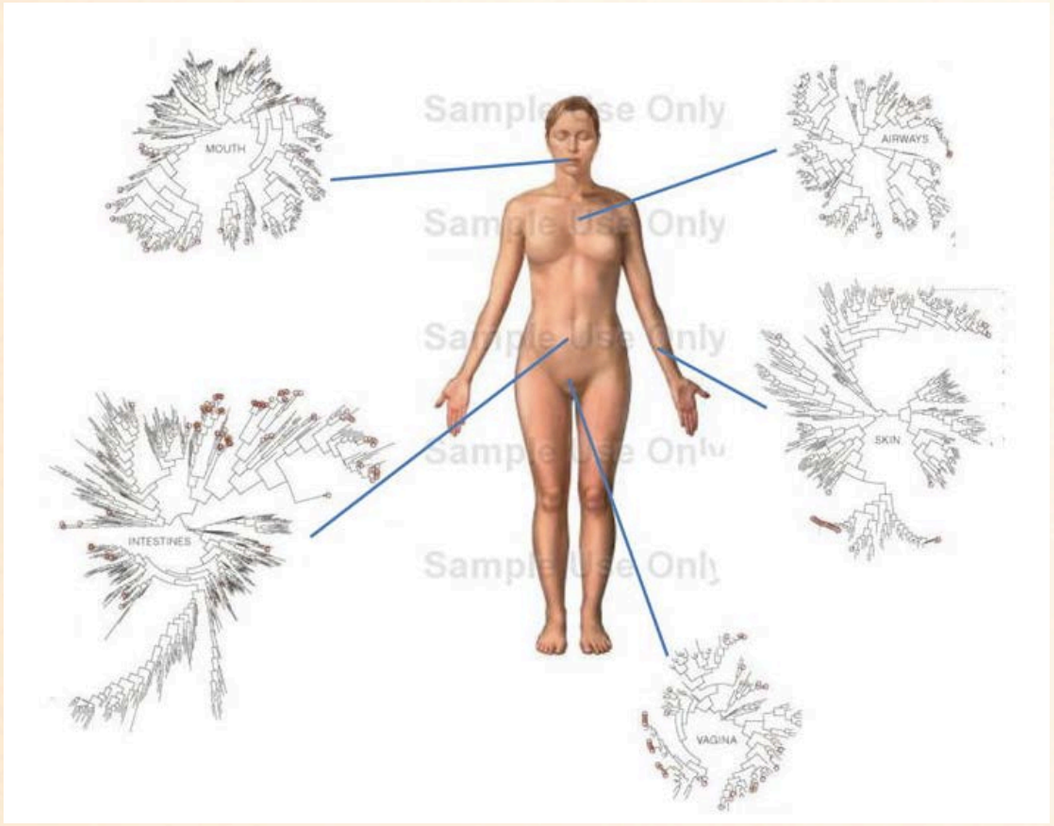
COMPOSITION OF THE HUMAN GUT MICROBIOTA

- 10^{13}-10^{14} microorganisms
- collective microbiome = 100X human genome
- humans are super-organisms whose metabolisms represents an amalgamation of microbial and human attributes

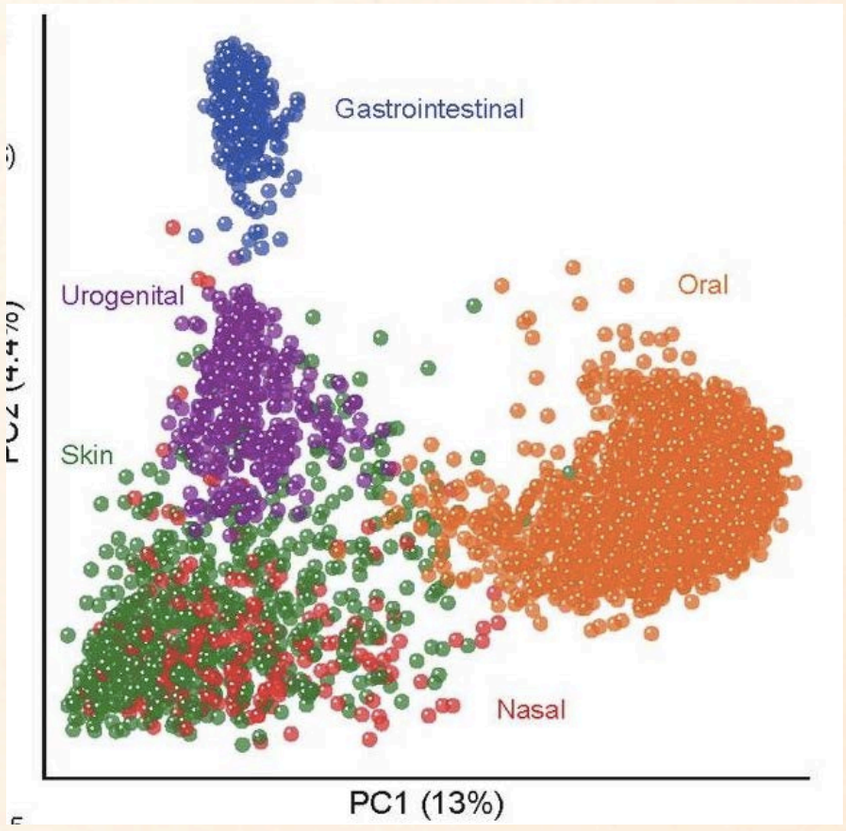
Gill et al. Science 2006


If we are our genes... then we are more our microbiome than our genome

MICROBIOME VARIES ACCORDING TO ANATOMICAL LOCATION OF THE HUMAN BODY

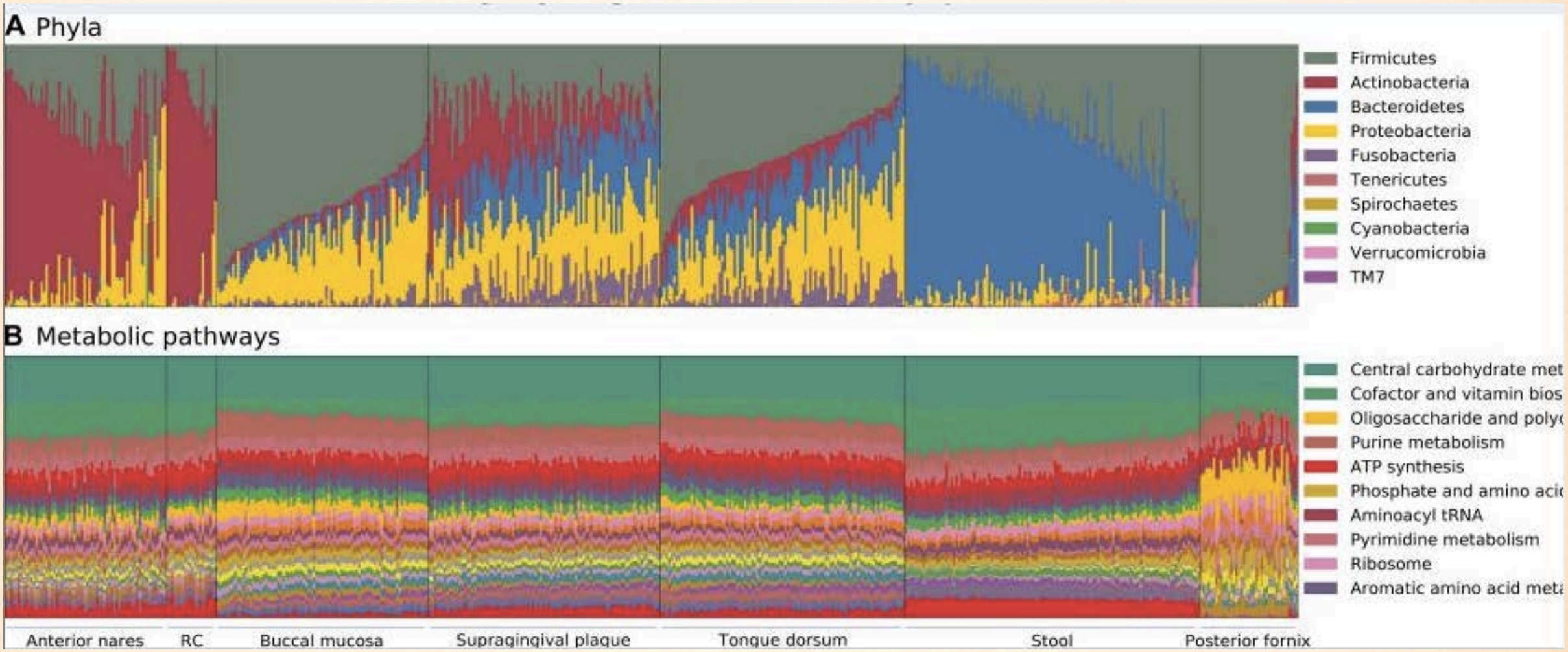


Lee et al. Science 2010



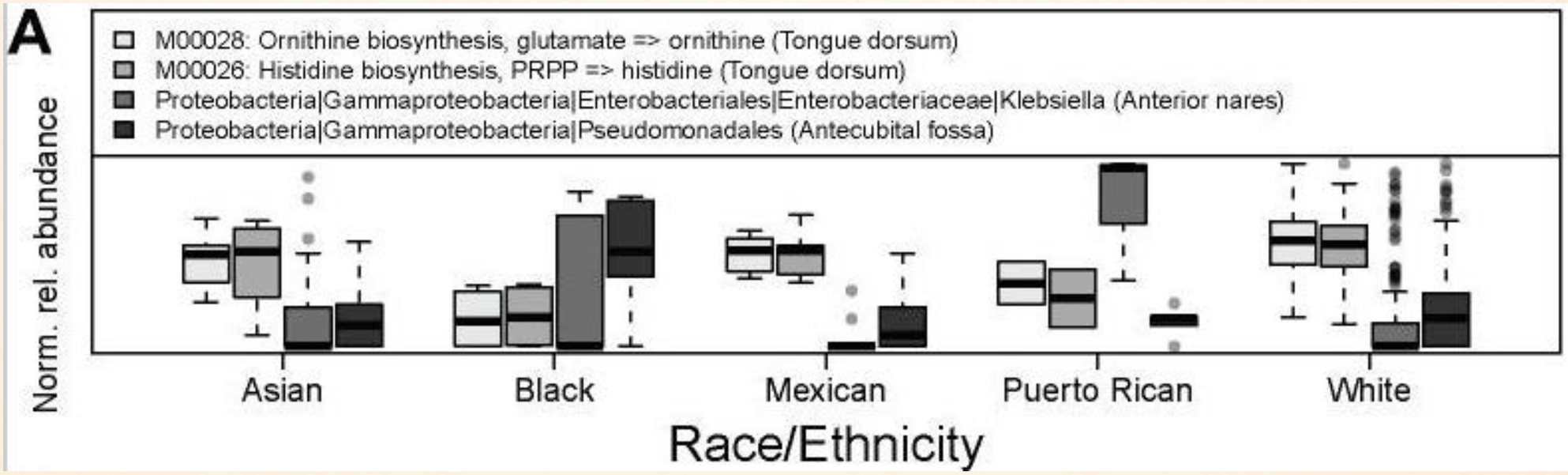
The human Microbiome Project Consortium. Nature 2012

WHILE DIFFERENCES IN MICROBIAL TAXA EXIST, METABOLIC PATHWAYS REMAIN CONSTANT



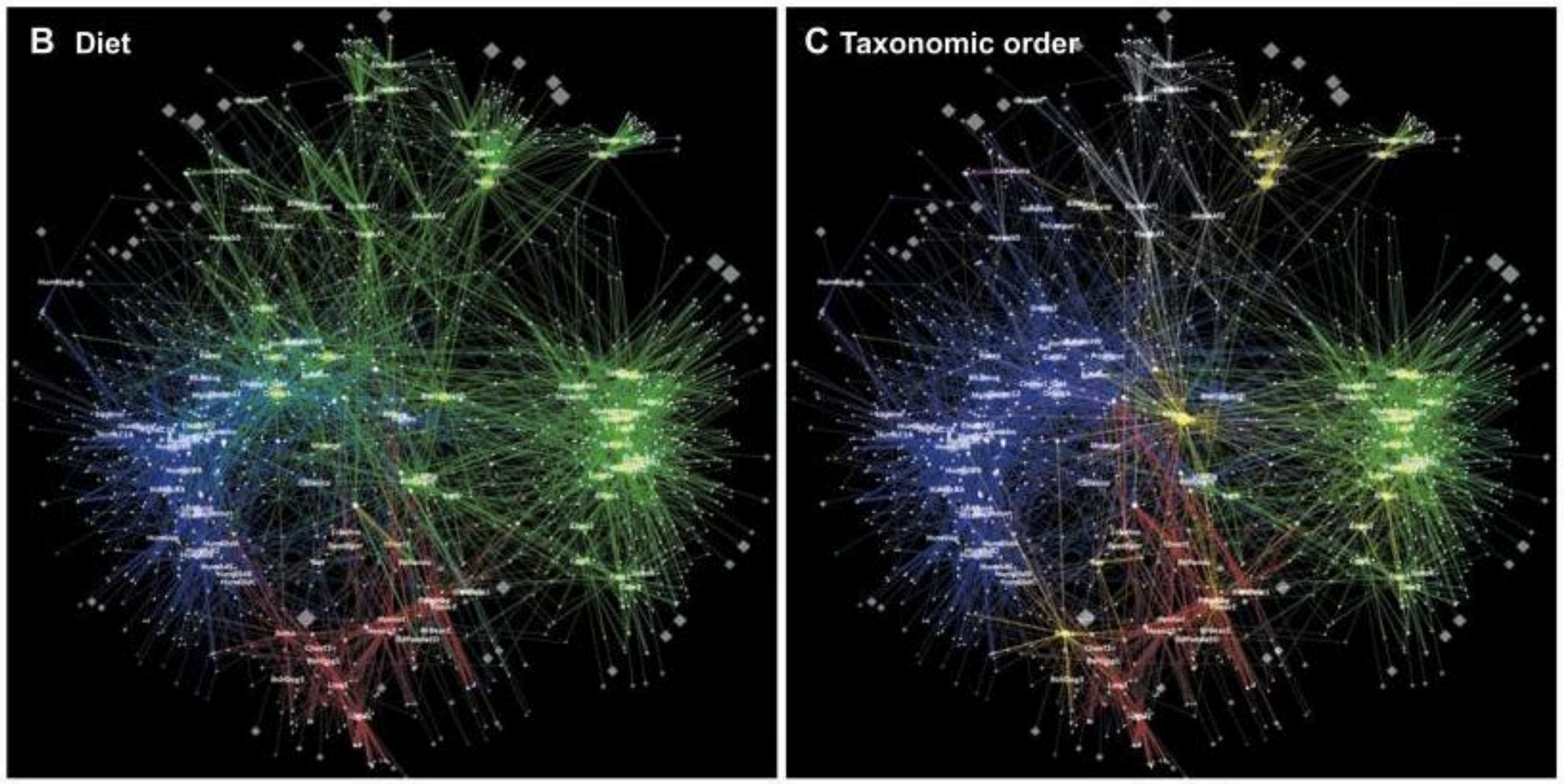
The human Microbiome Project Consortium. Nature 2012

MICROBIAL COMMUNITIES AND THEIR PATHWAYS ARE ASSOCIATED WITH ETHNICITY



The human Microbiome Project Consortium. Nature 2012

MICROBIOME CORRELATES WITH DIET AND TAXONOMY



- Herbivores
- Carnivores
- Omnivores

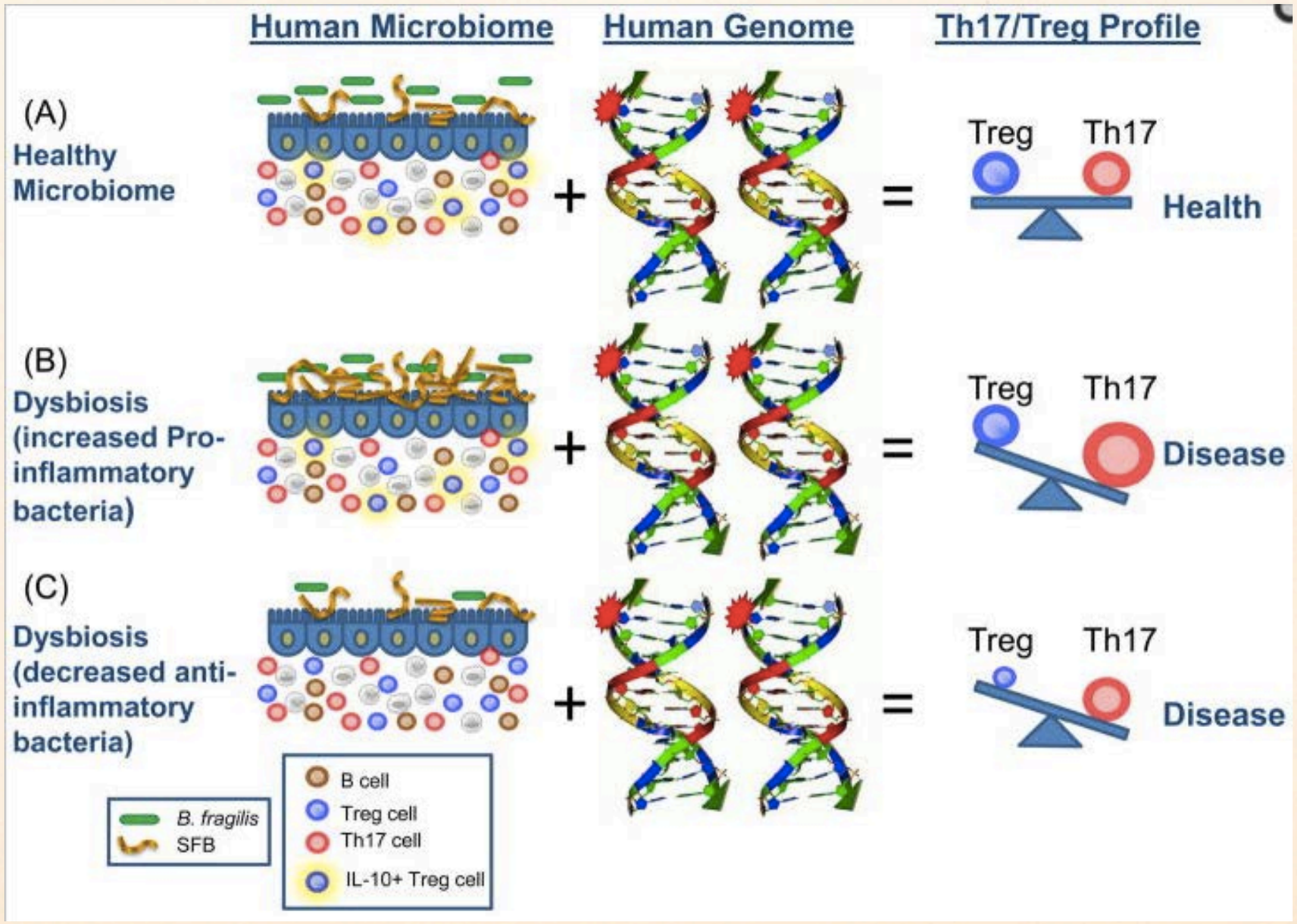
Ley et al. Science 2008

THE HUMAN-MICROBE RELATIONSHIP

- microbes are human symbionts more than pathogens
- gut microbes shape immune responses through the interaction of their metabolism with that of humans
- B. fragilis activates TLR2 through PSA and stimulates Treg to enable its colonization
- short-chain fatty acids regulate size and function of colonic Treg population and protect against colitis

Lee and Mazmanian. Science 2010
Round et al. Science 2011
Smith et al. Science 2013

THE HUMAN MICROBIOME HELPS BALANCE IMMUNE RESPONSES



Lee and Mazmanian. Science 2010

MICROBIOTA AND OBESITY

- Microbiota from twins discordant for obesity can transmit phenotype when transferred into germ-free mice

- When co-housed, mice recipient of the lean microbiota were dominant.

- Obesity is associated with reduced bacterial diversity and altered representation of bacterial genes and metabolic pathways

Ridaura et al. Science 2013

Turnbaugh et al. Nature 2009

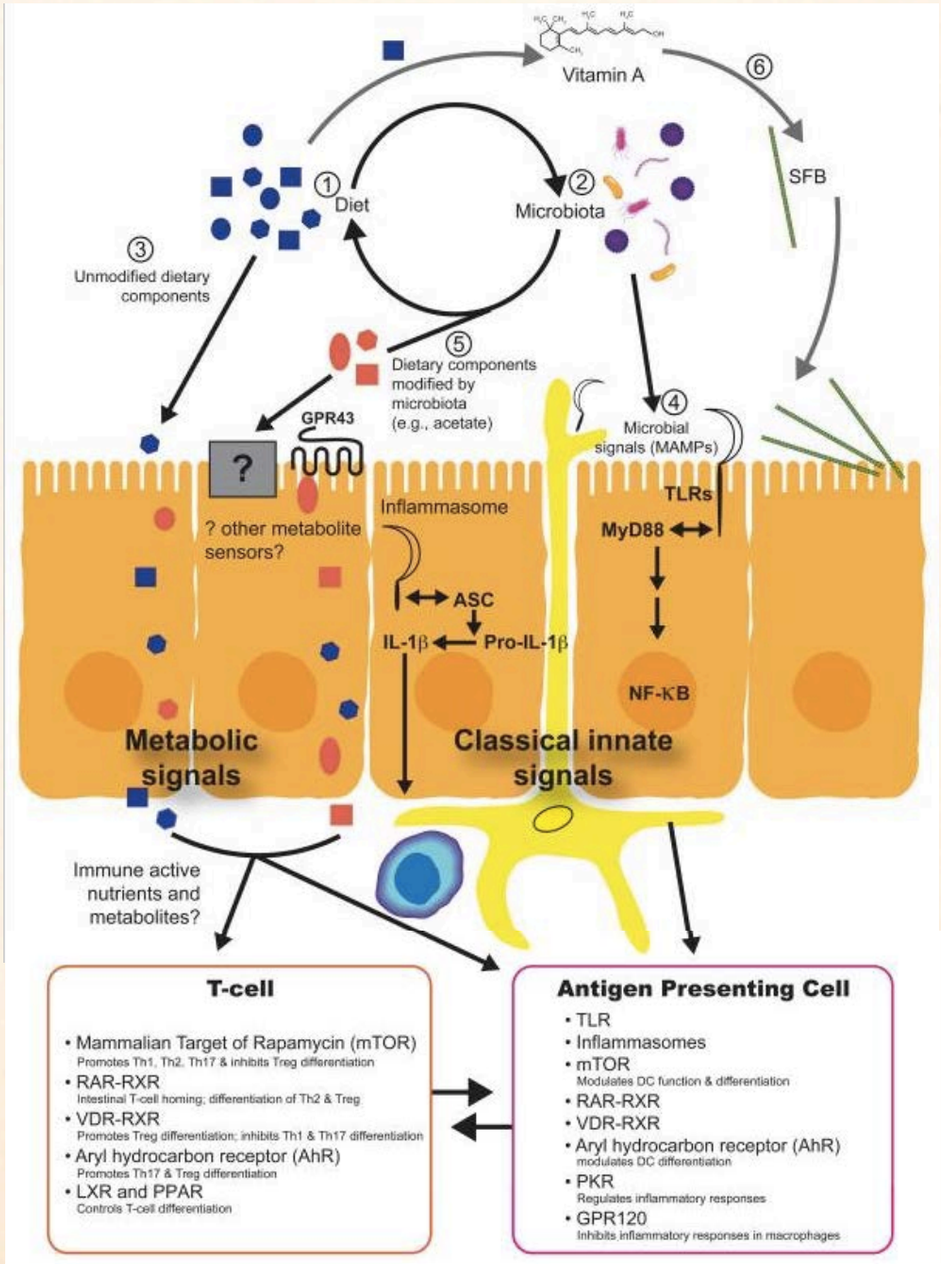
MICROBIOTA AND CANCER THERAPY

- Elimination of the microbiota impairs the response of subcutaneous tumors to CpG-oligonucleotide immunotherapy and platinum chemotherapy

- elimination of microbiota results in resistance to cyclophosphamide in tumor-bearing mice

Iida et al. Science 2013
Viaud et al. Science 2013

CROSSTALK BETWEEN MICROBIOTA AND THE IMMUNE SYSTEM



NORMAL GUT MICROBIOTA MODULATES BRAIN DEVELOPMENT AND BEHAVIOR

- GF mice display increased motor activity and reduced anxiety, compared with SPF
- behavior is related to expression of genes involved in second messenger and LTP
- GF co-housed with SPF mice adopted the behavior of SPF mice

Diaz-Heijtz et al. PNAS 2011

INGESTION OF LACTOBACILLUS MODULATES EMOTIONAL BEHAVIOR VIA THE VAGUS NERVE

- L. rhamnosus induces changes in expression of GABABɪb gene in cortex, hippocampus, and amygdala
- L. rhamnosus reduced corticosterone levels and anxiety- and depression-related behavior
- These effects were abrogated by vagotomy

Bravo et al. PNAS 2011

MICROBIOTA AND AUTISM

- children with ASD have GI disturbances
- severity of GI dysbiosis is associated with severity of autism
- ASD microbiome =! heathy microbiome (↑bacteroidetes, ↓firmicutes)
- Toxins produced by Clostridia affect behavior in ASD
- Urinary metabolites (from gut bacteria) characterize ASD subjects from healthy siblings
- Oral treatment of MIA offspring with B. fragilis corrects gut permeability, alters microbial composition, and ameliorates defects in communicative, stereotypic, anxiety-like and sensorimotor behaviors

Hsiao et al. Cell 2013

LACTATION PROMOTES DEVELOPMENT OF HEALTHY MICROBIOTA

- Lactating mice develop a different microbiota than non-lactating mice

- milk contains SIgA, which prevents translocation of aerobic bacteria from the gut to draining lymph nodes

- Maternal IgA ameliorated colonic damage after dextran sulfate administration

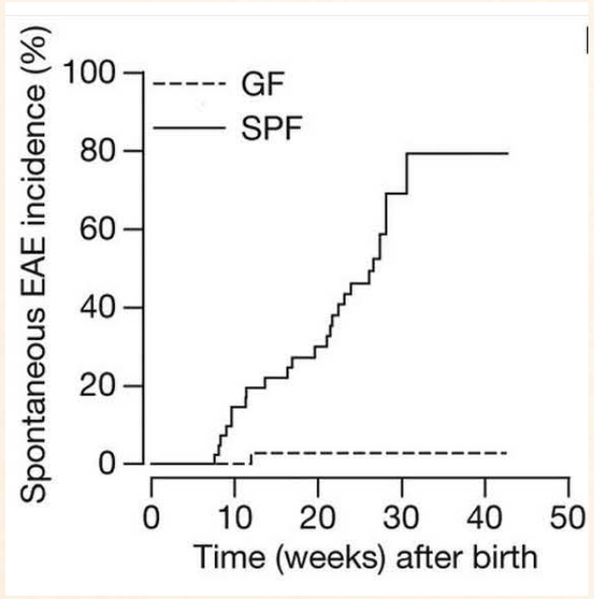
Rogier et al. PNAS 2014

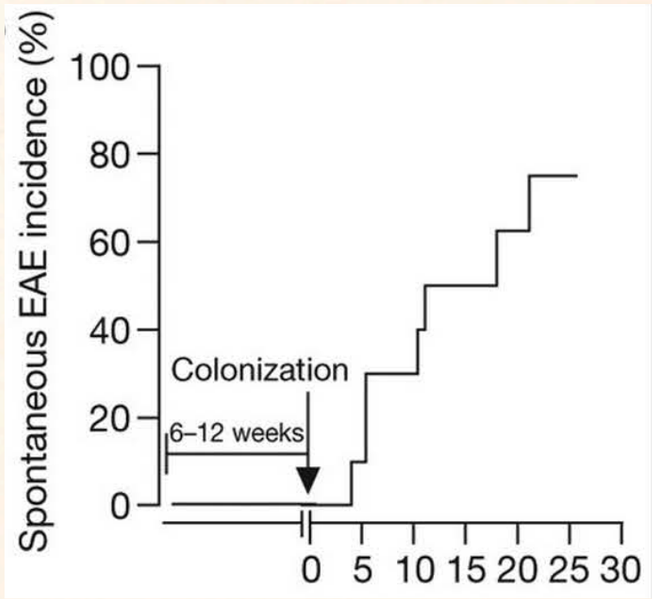
THE EFFECT OF GUT MICROFLORA IN AUTOIMMUNE DEMYELINATION

- Mice treated with wide-spectrum antibiotics are resistant to EAE
- Effect associated to ↓inflammatory cytokines, ↑ IL-10 and IL13 and ↑Treg population.
- Also, ↑CD5+ B cells (regulatory role)
- Oral administration of PSA (from B.fragilis) protects mice from EAE in an IL-10 dependent fashion.
- Exposure of DCs to PSA, promotes Treg
- Monocolonization with SFB restored Th17 responses and EAE susceptibility in GF mice.

Ochoa-Reparaz et al. J Immunol. 2009
Ochoa-Reparaz et al. Gut Microbes. 2010
Ochoa-Reparaz et al. Mucosal Immunol. 2010
Lee et al. PNAS. 2011

GUT MICROBIOTA IS REQUIRED FOR SPONTANEOUS EAE MODEL





- GF TCR/BCR TG mice do not develop spontaneous EAE
- This effect is mediated by impaired Th17 differentiation
- Also B cell recruitment is impaired

Berer et al. Nature. 2011

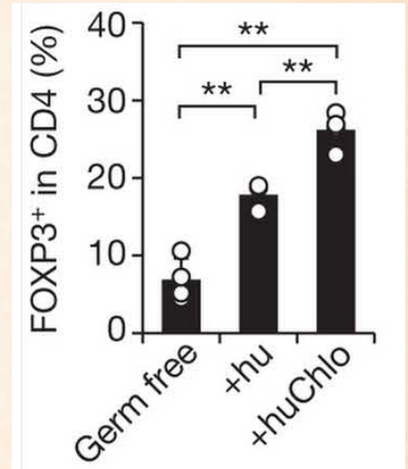
THE CLOSTRIDIUM CONNECTION

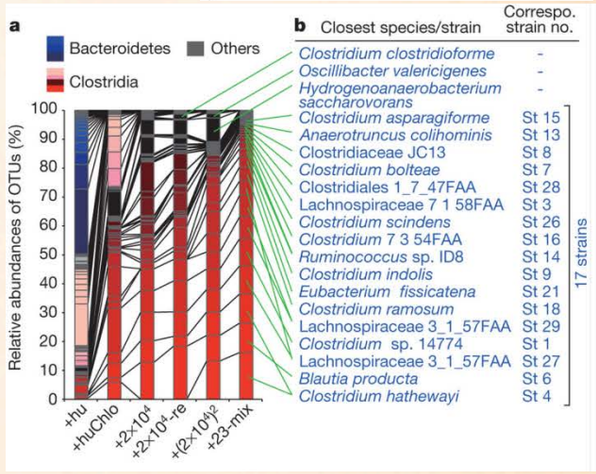
- T cells from NMO patients recognize an AQP4 epitope with high homology to C. perfringens ABC transporter permease.
- C. perfringens type B was isolated from a CIS patient.
- ε toxin from C. perfringens (ETX) cross BBB and binds OL
- C. perfringens type A is less prevalent in MS, while reactivity against ETX is 10x higher in MS

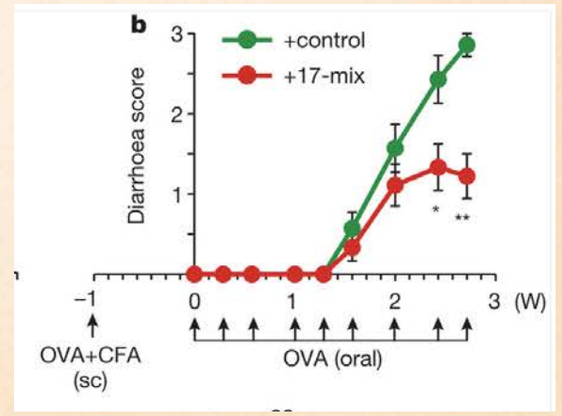
Varrin-Doyer et al. Ann Neurol. 2012
Rumah, et al. PLoS One. 2013

THE CLOSTRIDIUM CONNECTION

- Stool sample from healthy human was treated with Chloroform and transferred to GF mice
- Mouse stool showed enrichment in Clostridia
- T_{reg} cell induction by human intestinal bacteria is transmitted horizontally and vertically
- Treatment with 17-mix suppresses experimental colitis





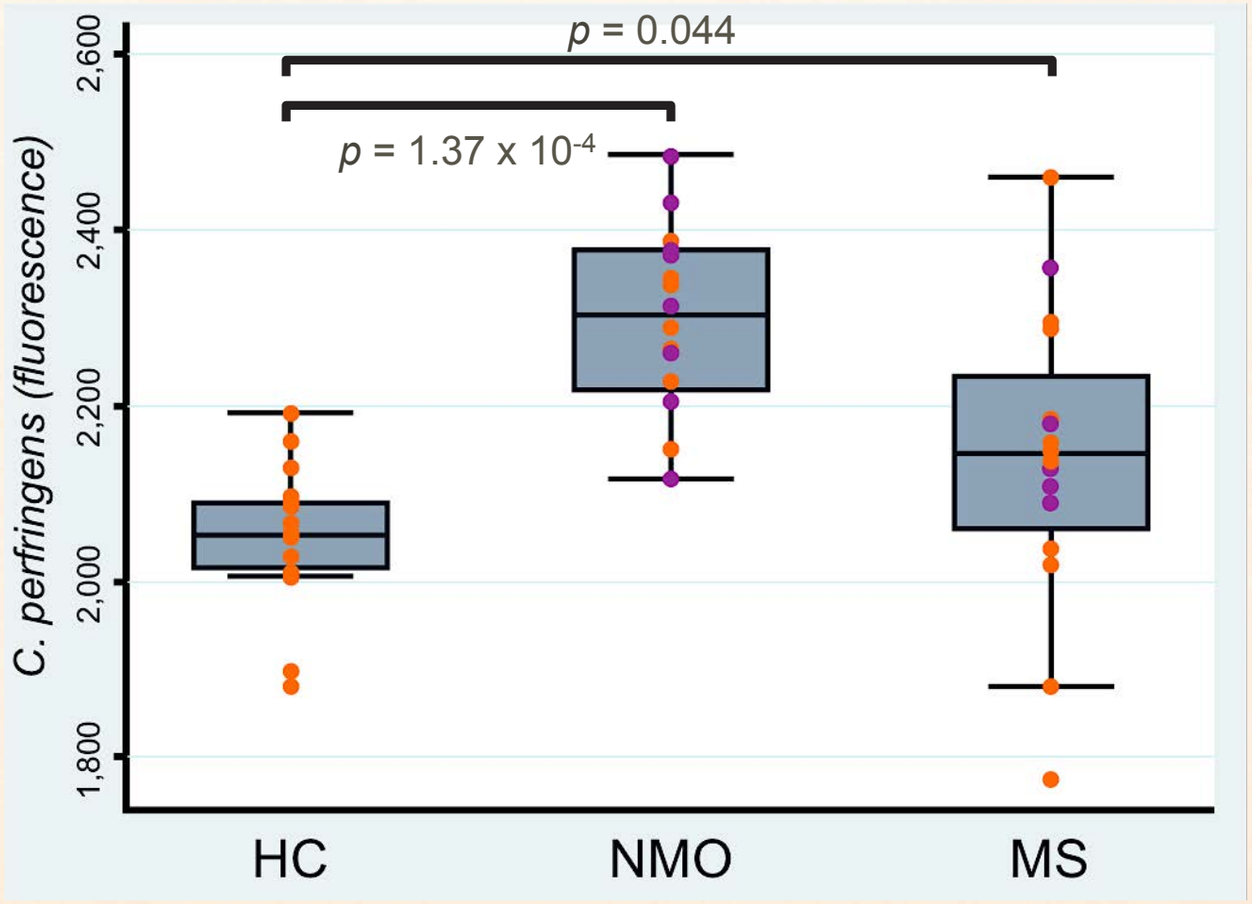


Atarashi, et al. Nature. 2013

THE CLOSTRIDIUM CONNECTION continued

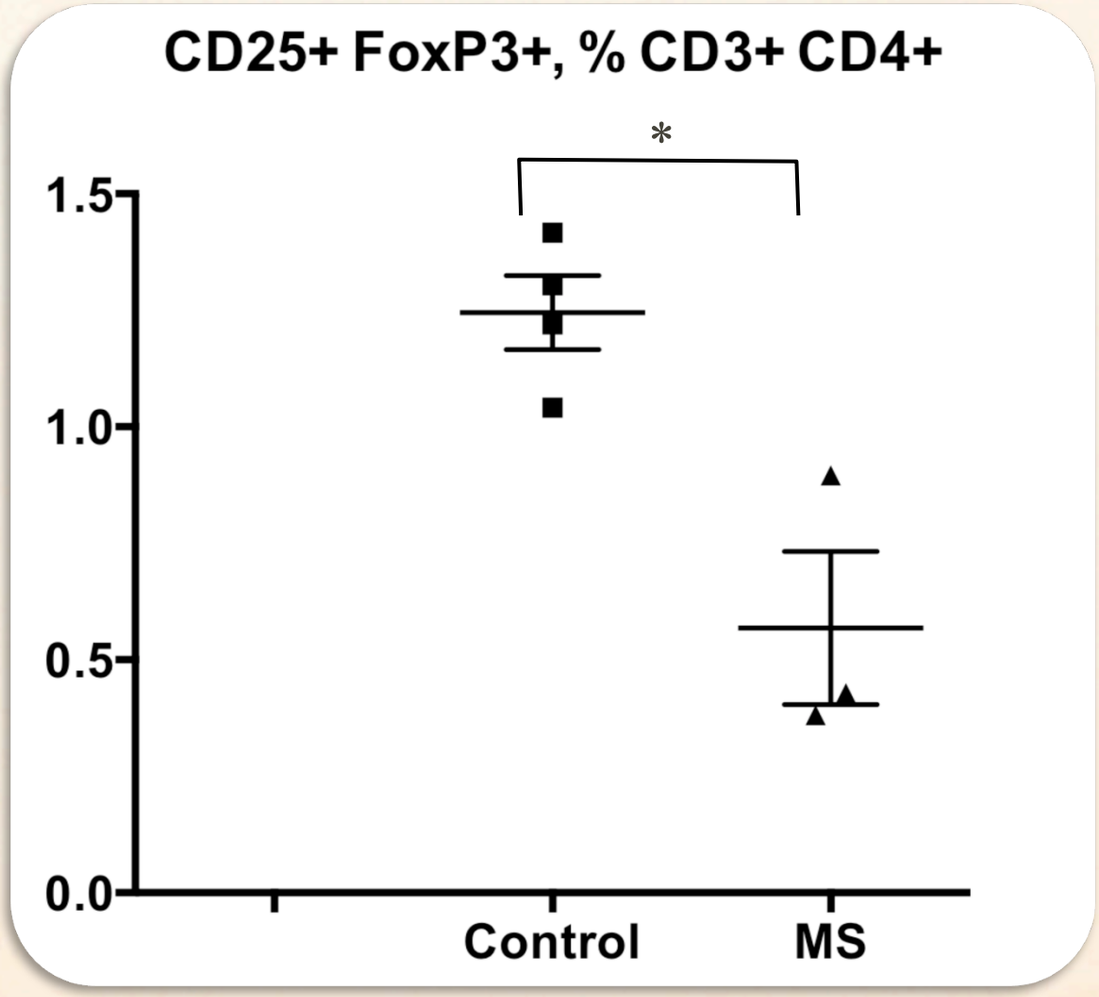
- T cells from NMO patients recognize an AQP4 epitope with high homology to C. perfringens ABC transporter permease.

- PhyloChip analysis of NMO (n=16), MS (n=16) and CTRLS (n=15) revealed enrichment of C. perfringens in demyelinating disorders



Varrin-Doyer et al. Ann Neurol. 2012
Cree BA, et al. Ann. Neurol. 2016

Autologous stimulation of PMBC with MS microbiota results in impaired Treg differentiation



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Microbiota transfer into germ-free mice

- Microbiota from 3 pairs of subjects (MS:CTRL) was transferred to germ-free mice (n=8 per group)

- Mice were immunized with MOG_{35-55} or CFA

- Disease scores were recorded over 30 days

Population-based microbiome analysis

- ~150 MS patients (50% untreated) and household controls
- Collected stool via overnight mail
- Purified DNA
- Sequenced 16S rRNA gene

No global differences in microbiota between MS and CTRLs

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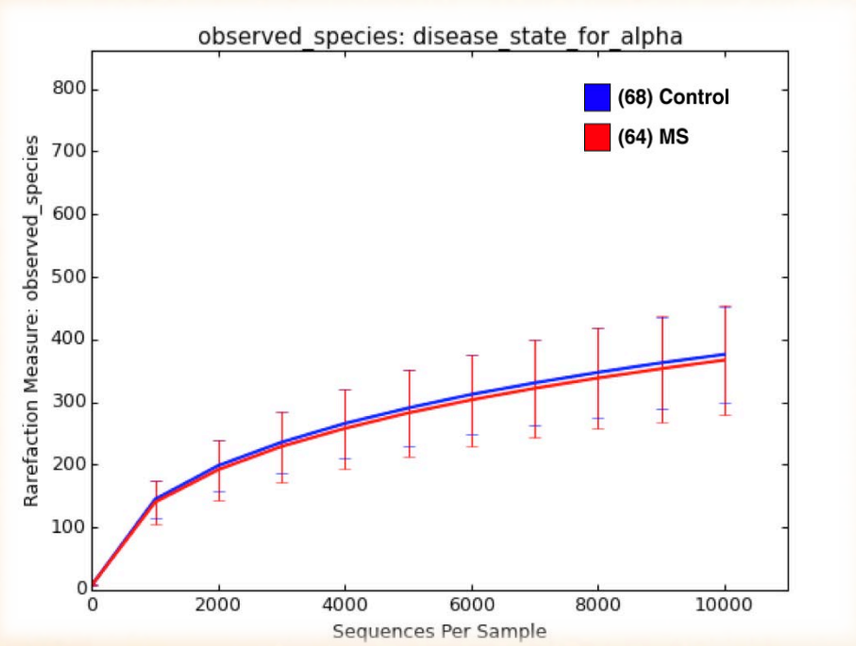

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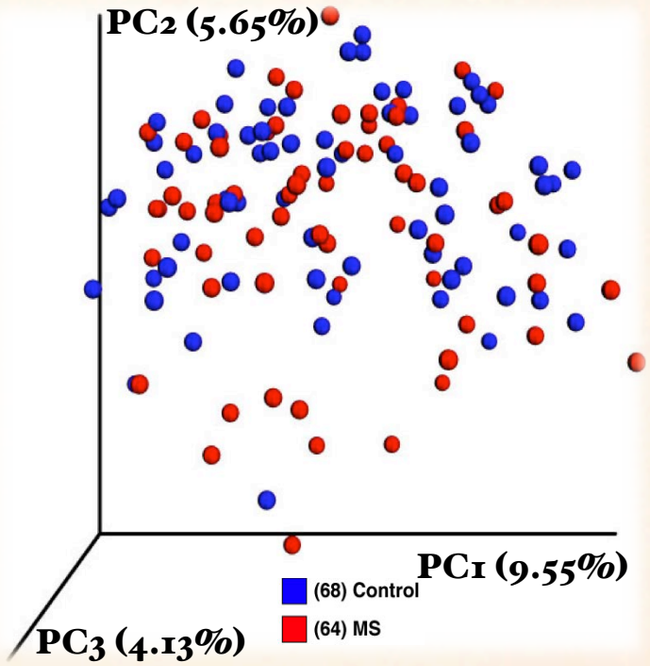

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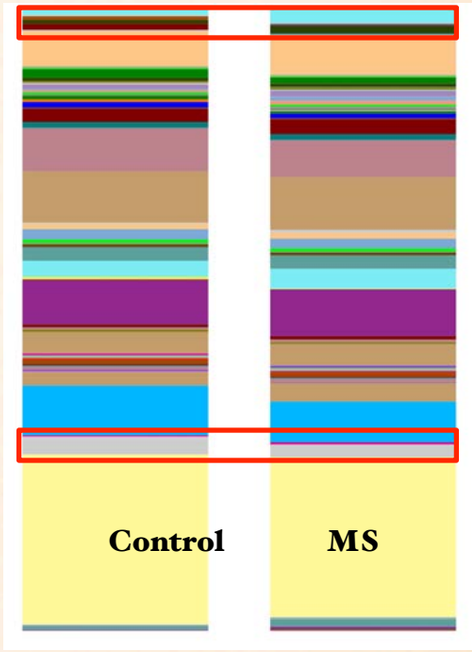
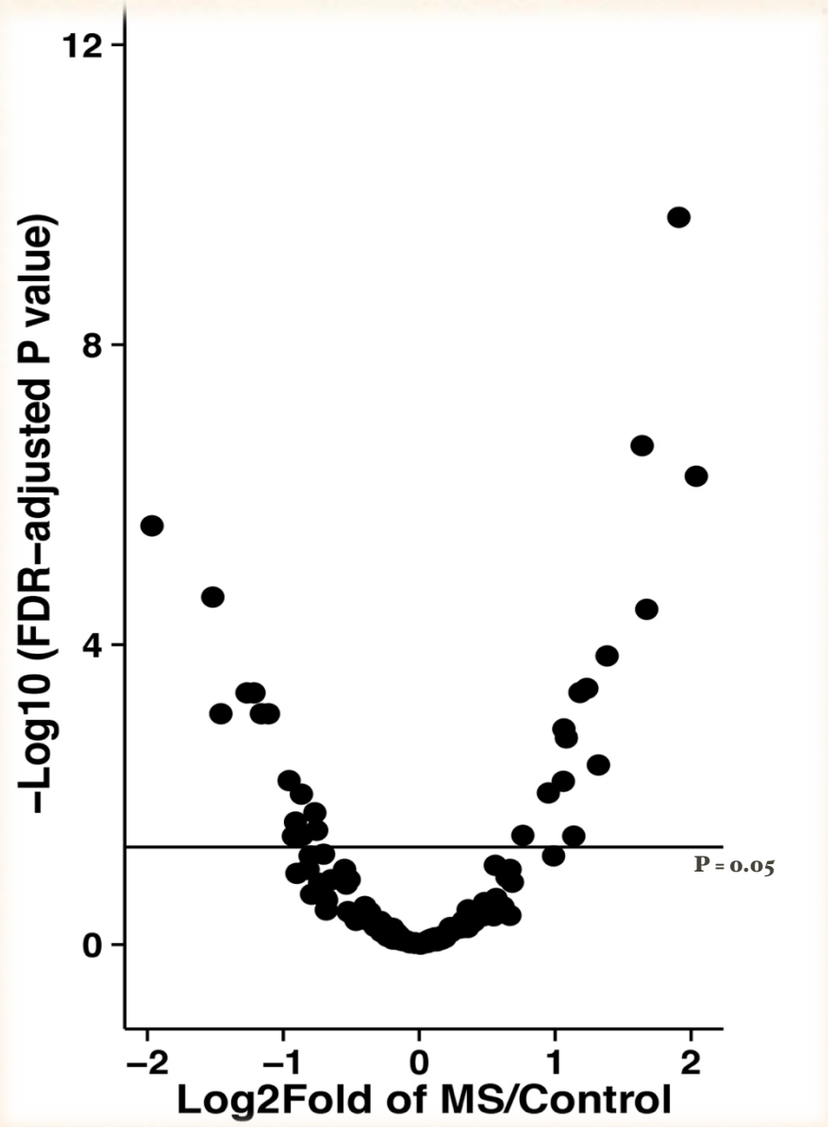


Significant differences in relative abundance of several OTUs



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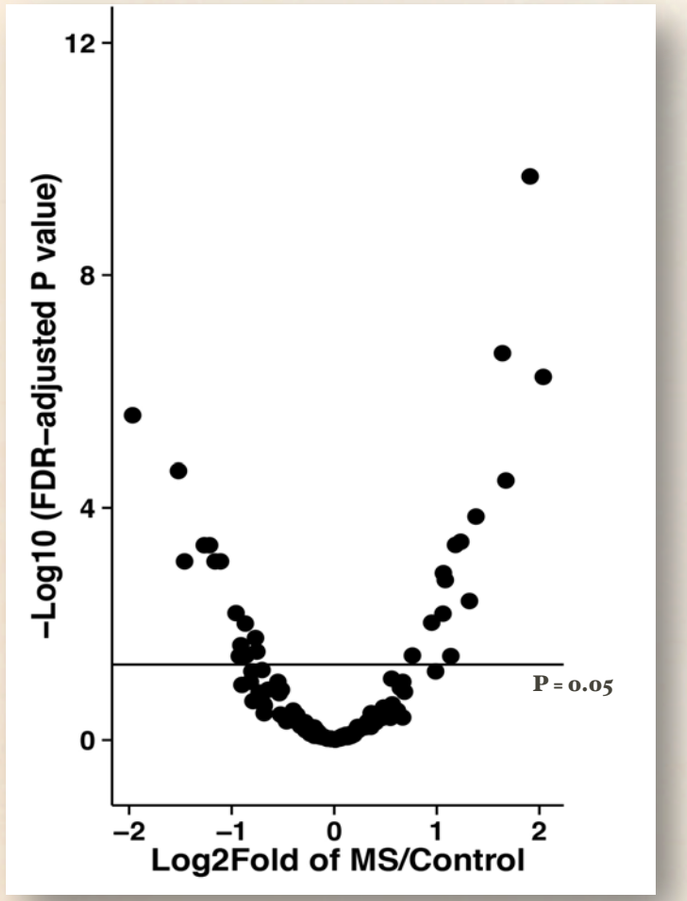

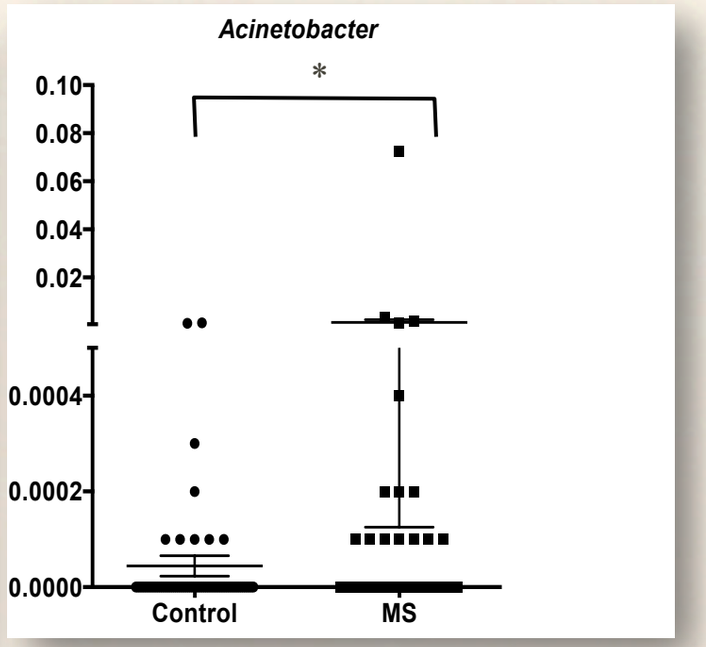

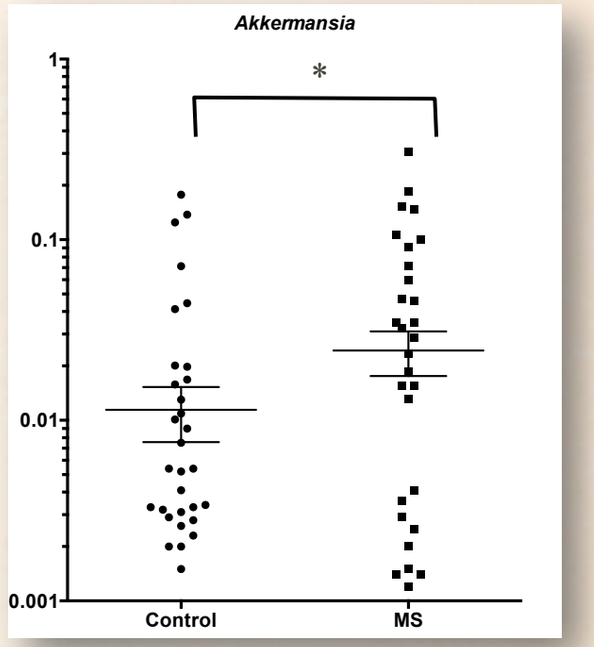

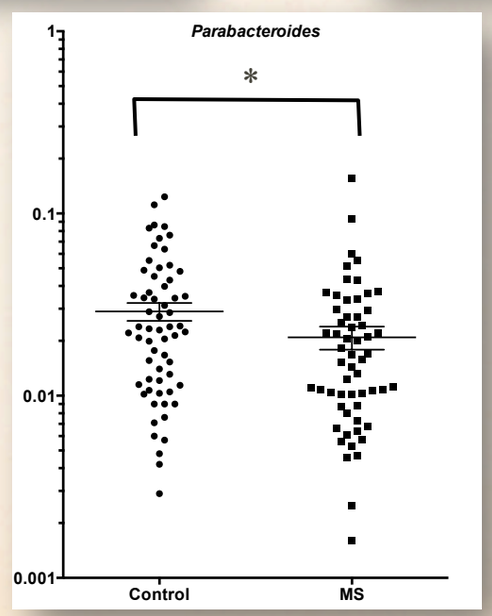

A. calcoaceticus inhibits Treg differentiation in vitro

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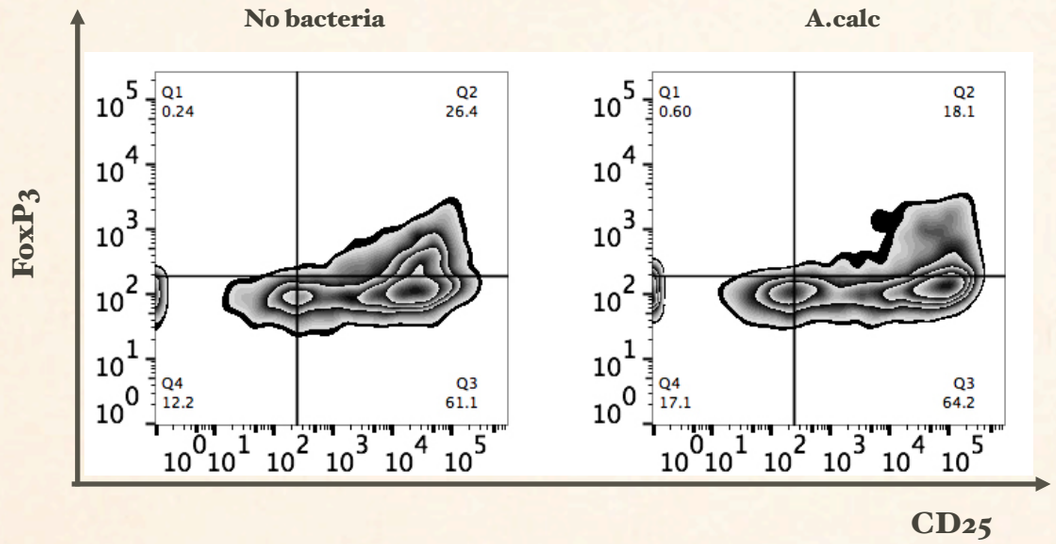

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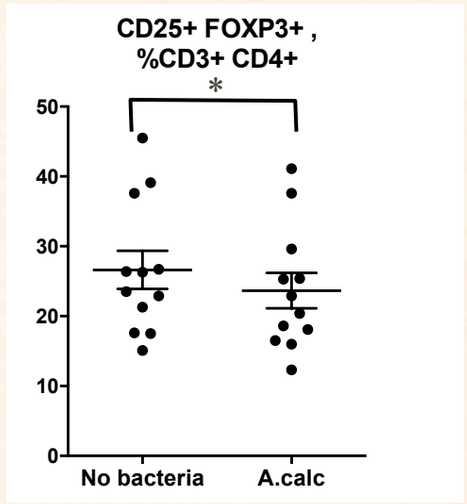

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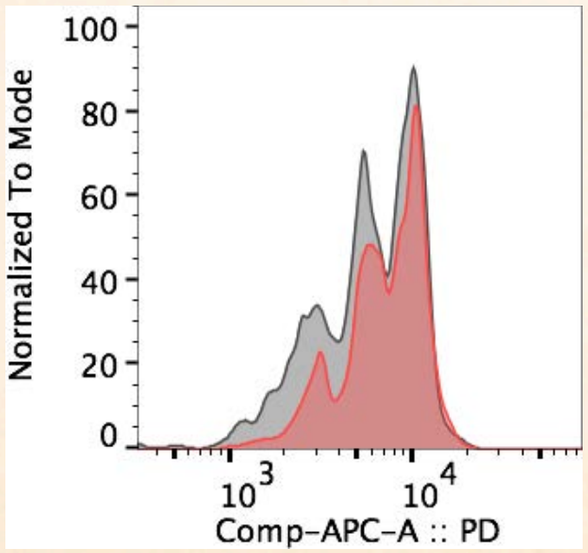

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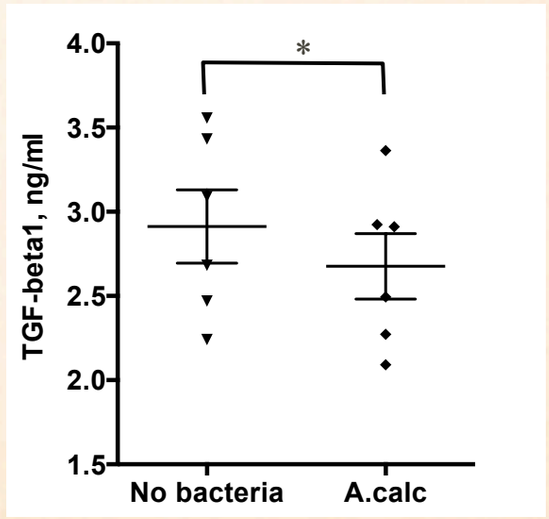
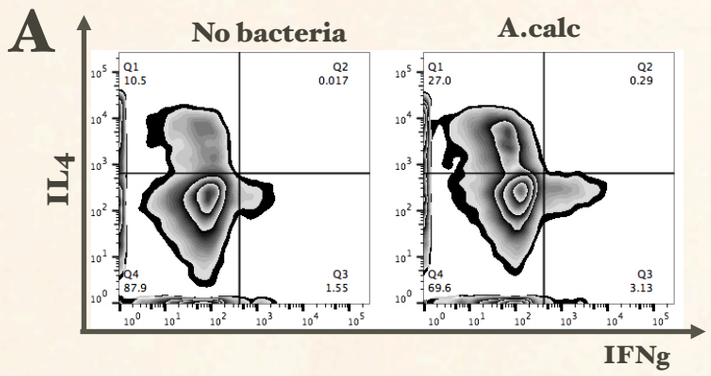
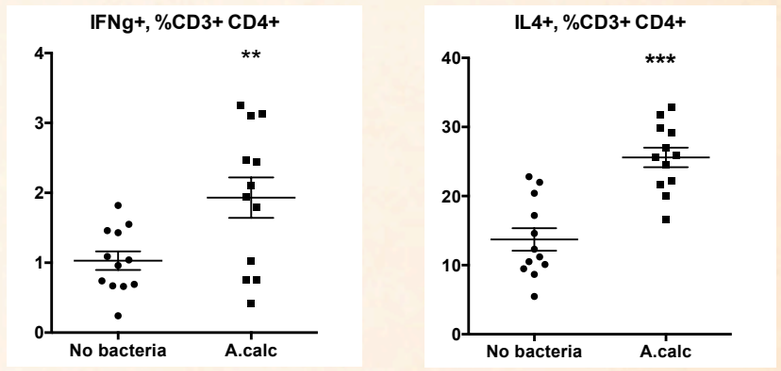
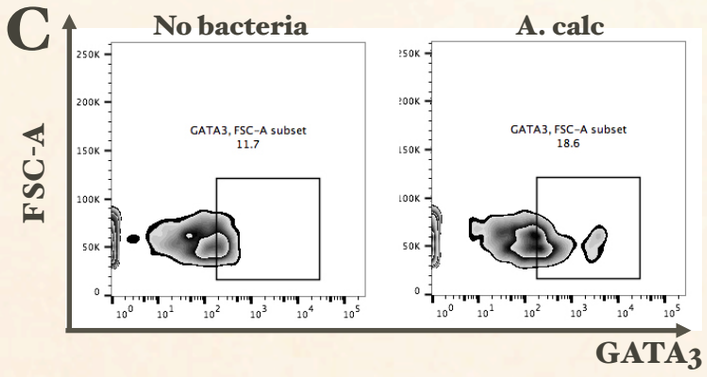
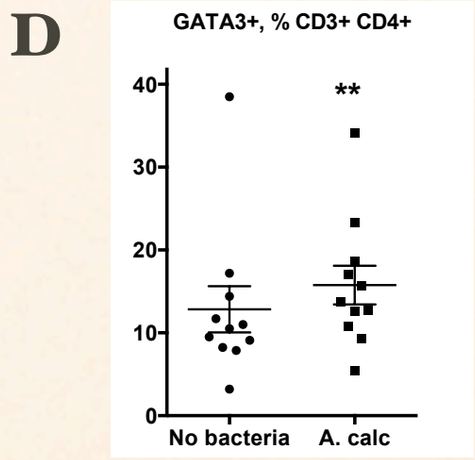


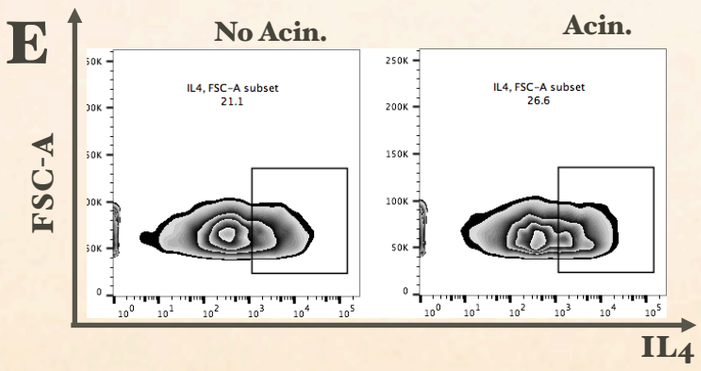
A. calcoaceticus stimulates Th1 and Th2 differentiation in vitro

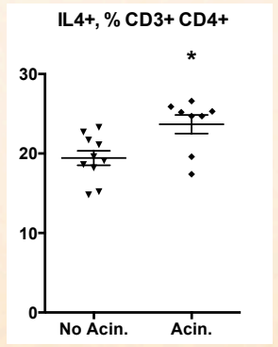












A. muciniphila stimulates Th1 differentiation in vitro

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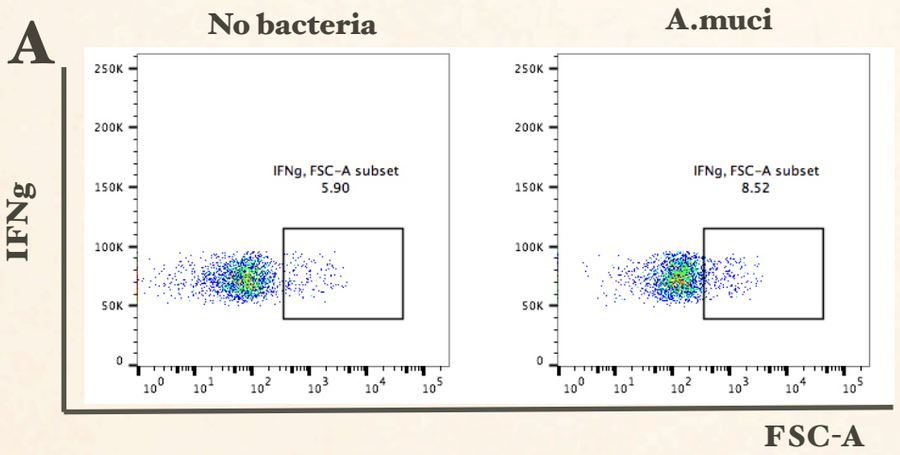

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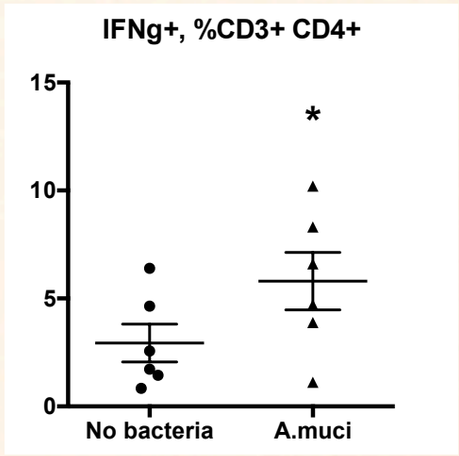

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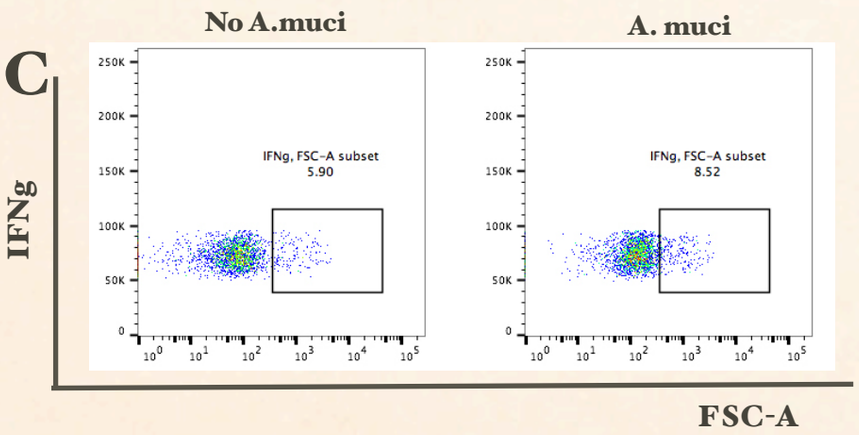

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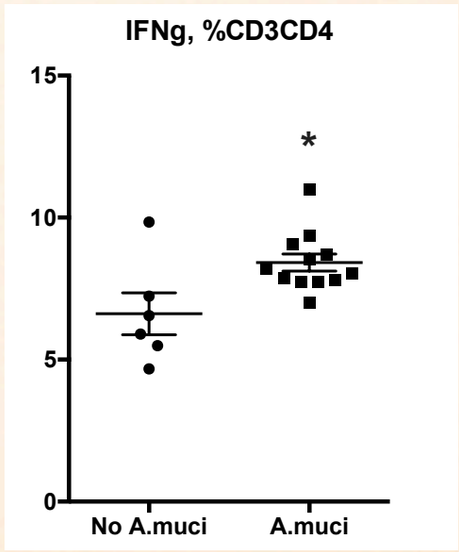

P. distasonis promotes IL-10+ Treg differentiation in vitro

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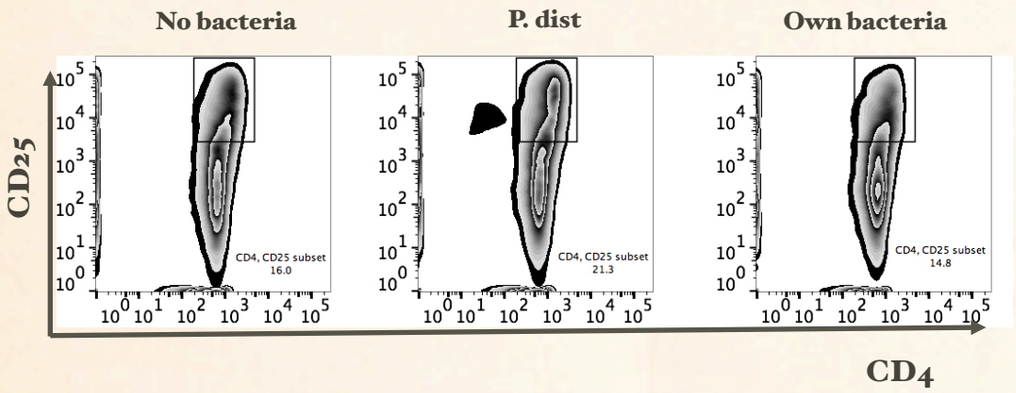

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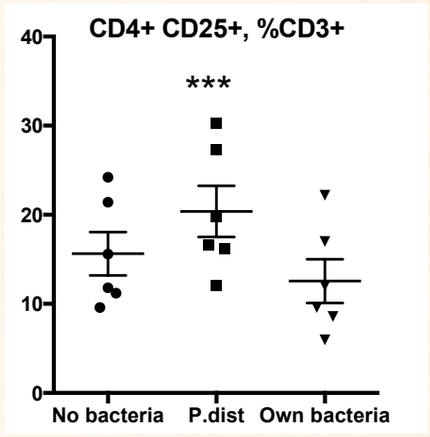

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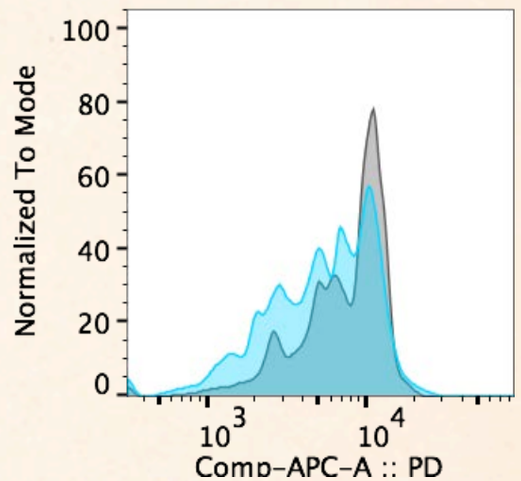

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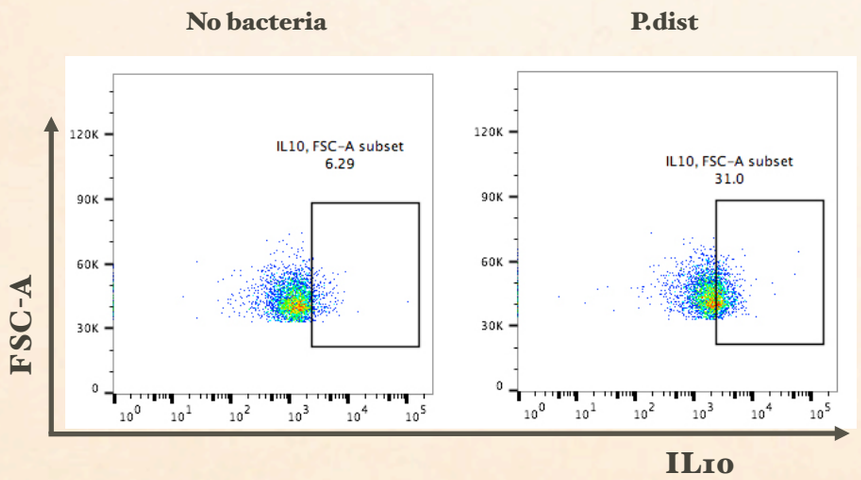

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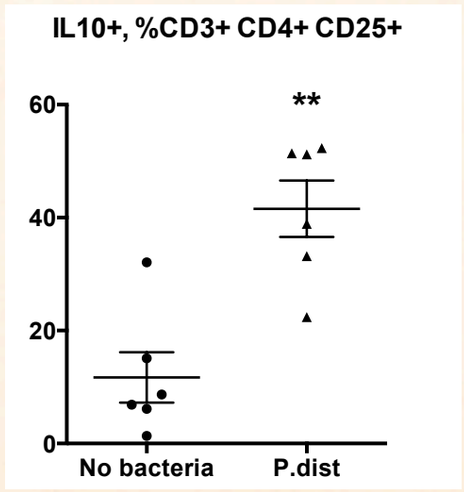

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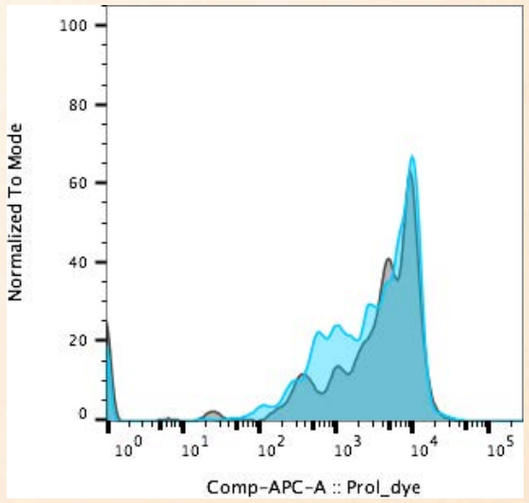
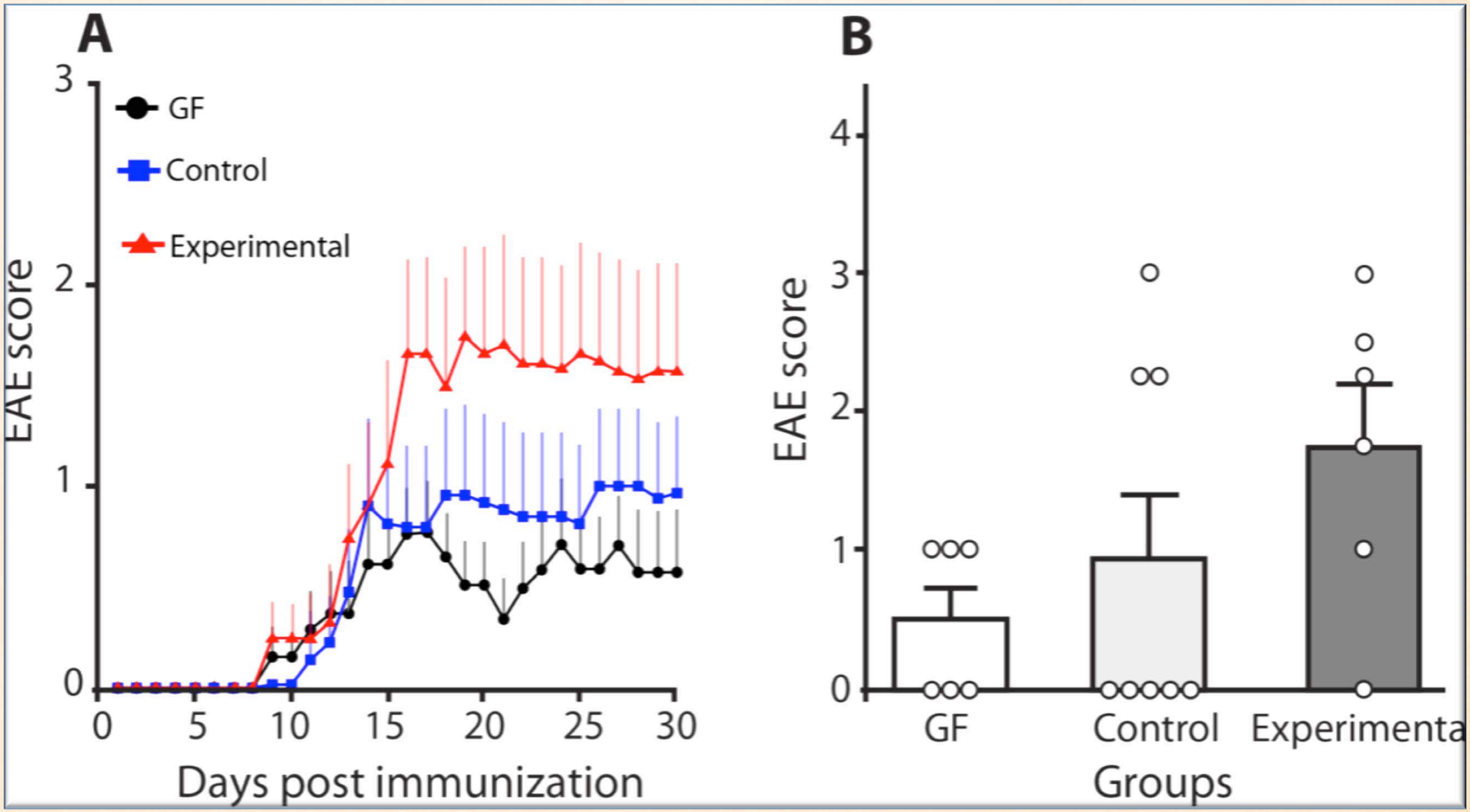


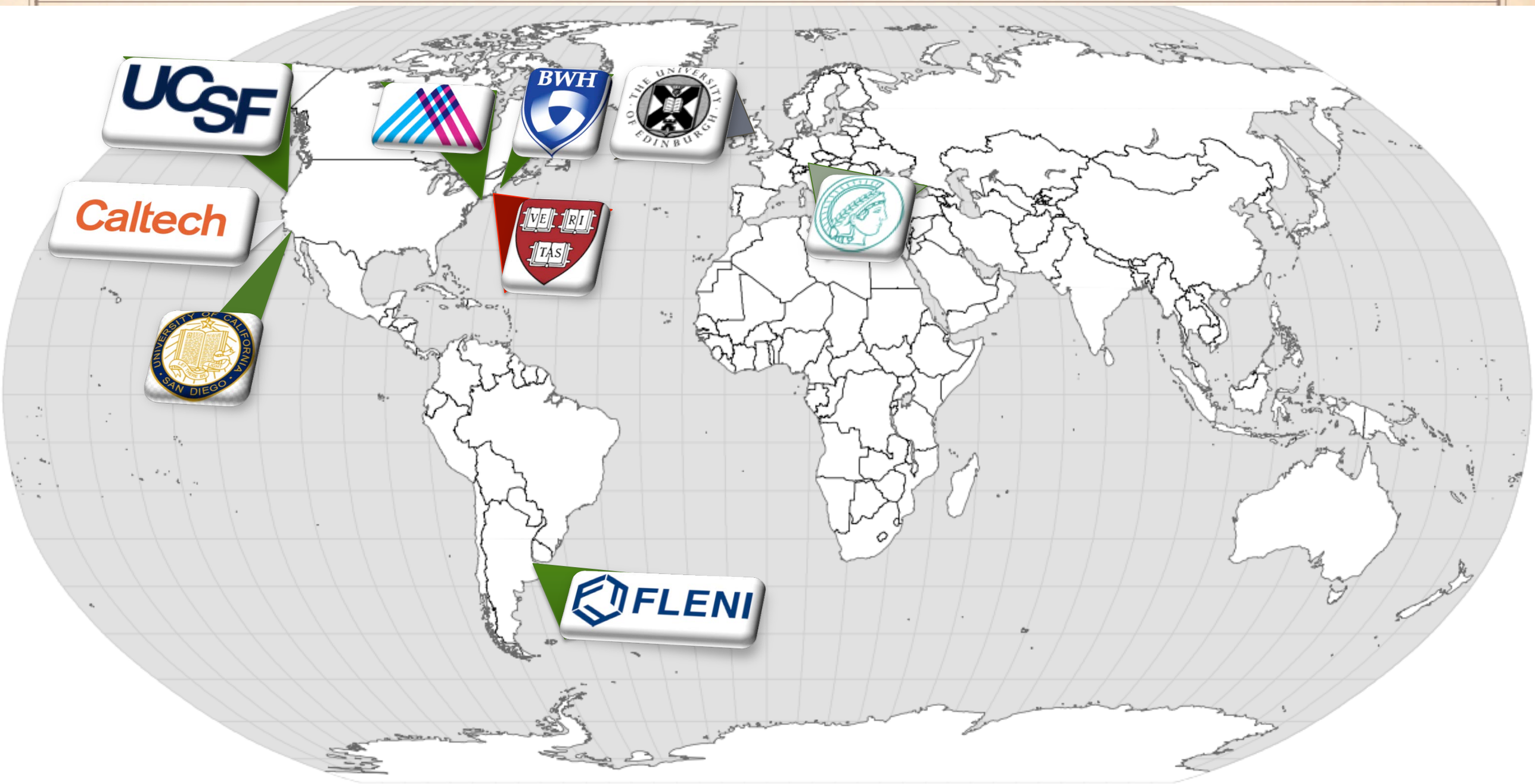
Mice respond differently to MS gut bacteria



Sarkis Mazmanian (Caltech)

The iMSMS

An International Collaboration



The goal

To identify gut microbes associated with MS

How?

- Humans
  - Sequence bacterial DNA to characterize over/under represented communities in MS

- Germ-free mice
  - Test causality of MS associated microbes

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INTERNATIONAL PROGRESSIVE MS ALLIANCE
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