Antibiotics, the microbiome, and chronic disease

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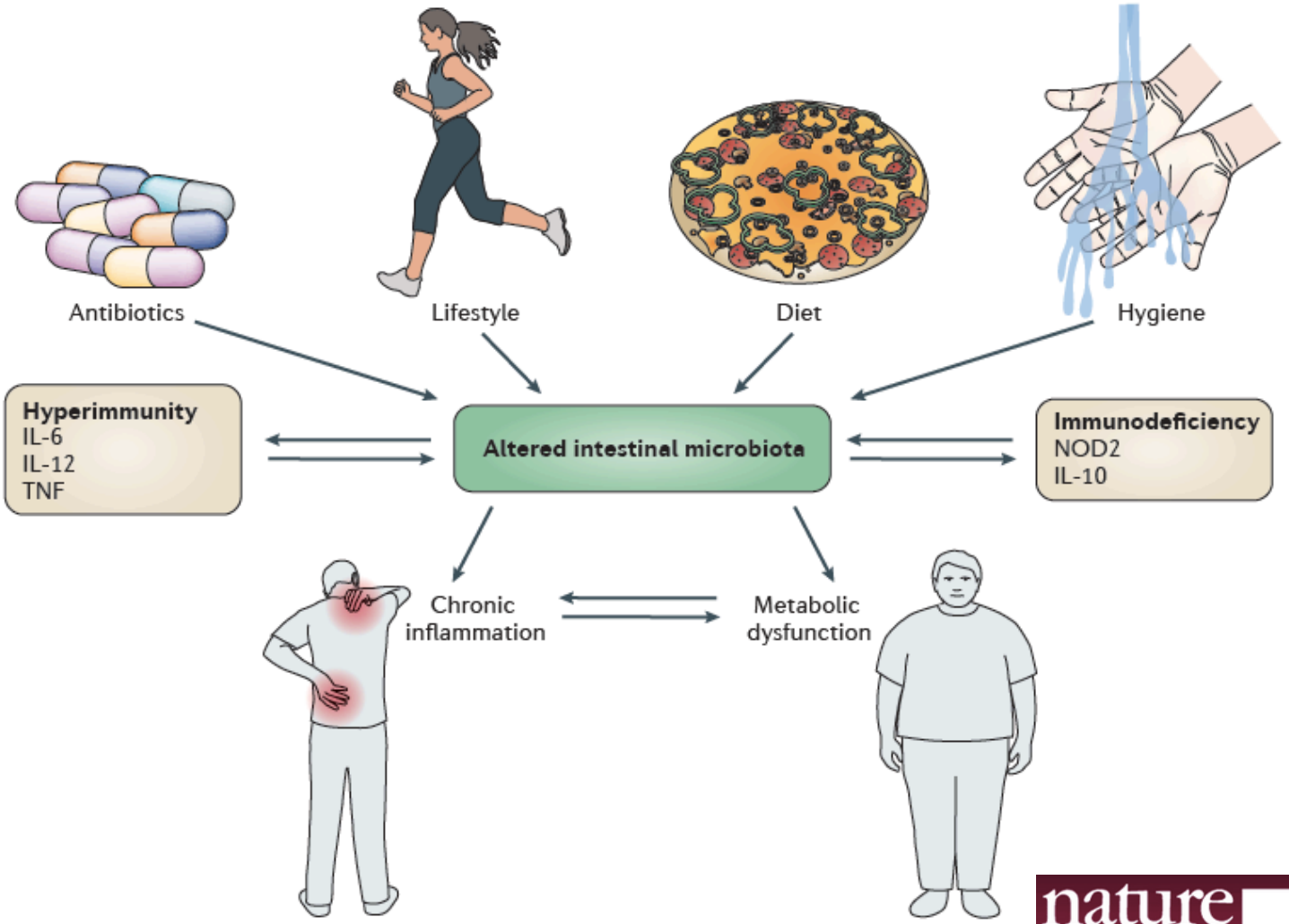
The gut microbiota — masters of host development and physiology

Felix Sommer[1,2] and Fredrik Bäckhed[1,2,3]



nature REVIEWS MICROBIOLOGY

The gut microbiota — masters of host development and physiology

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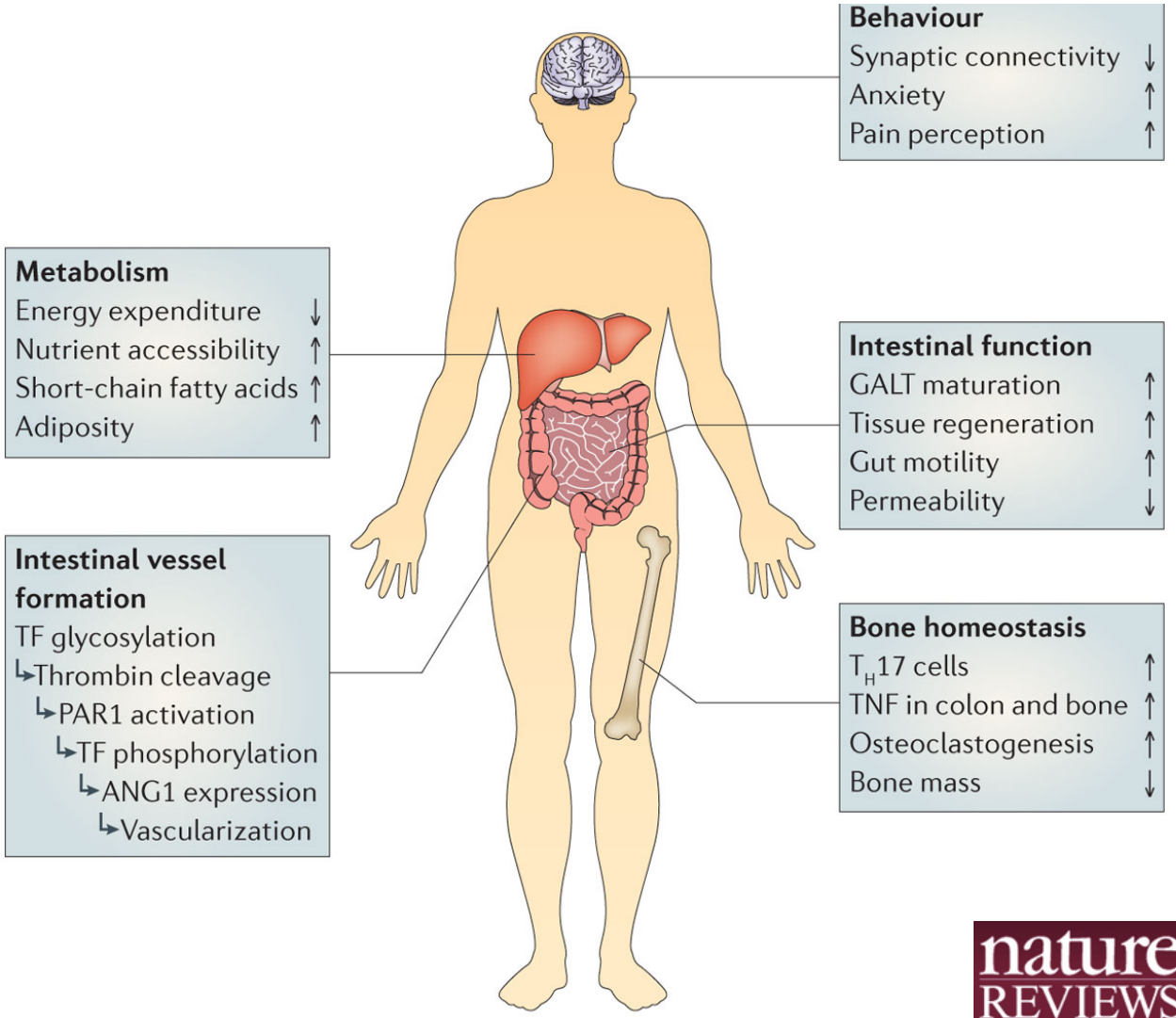
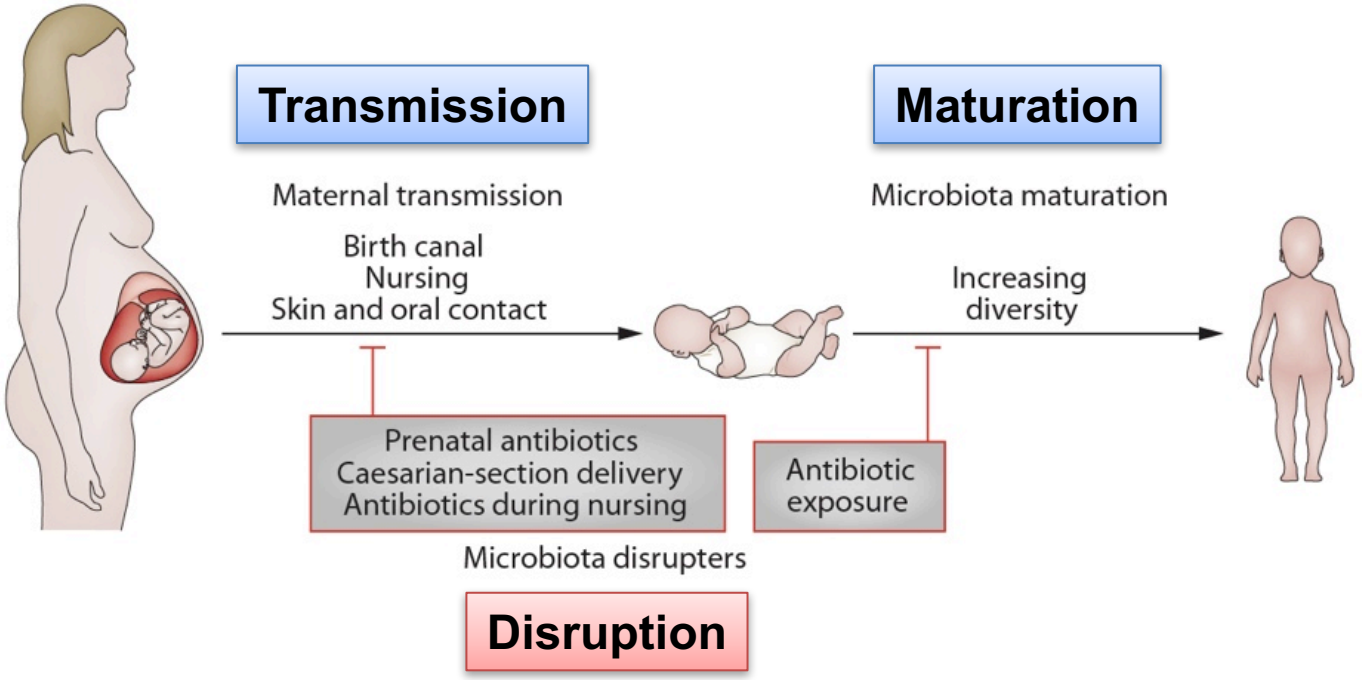


nature REVIEWS MICROBIOLOGY

Microbiota transmission and maturation

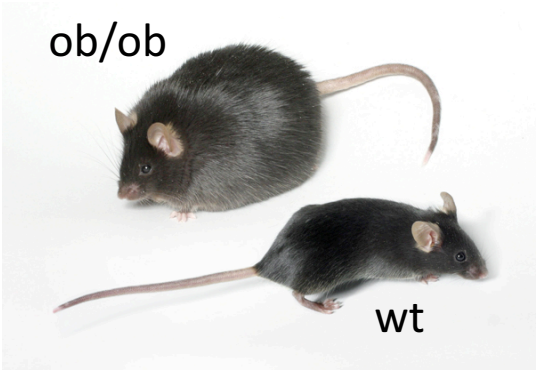


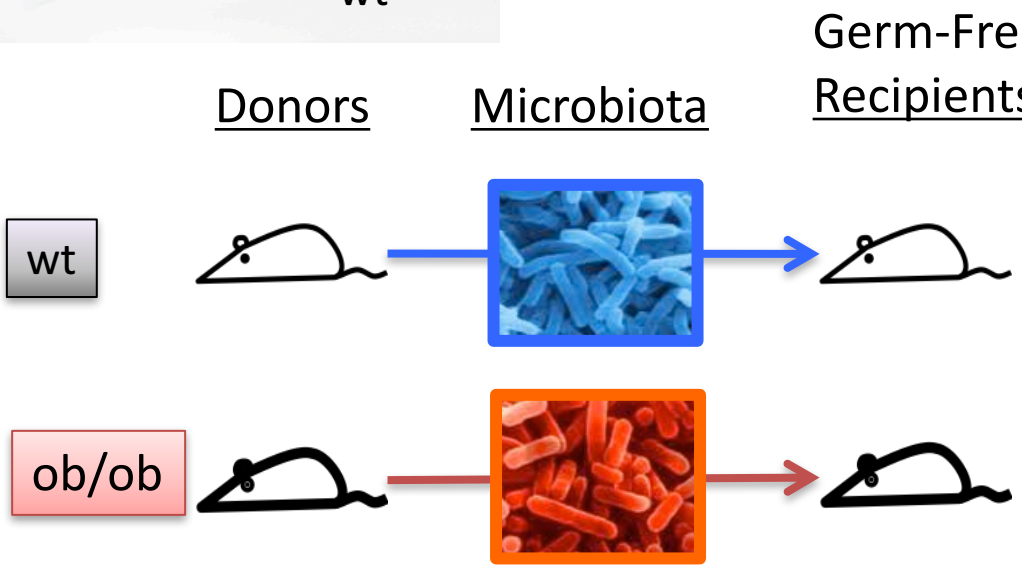
Early-life microbiota disruption can alter:

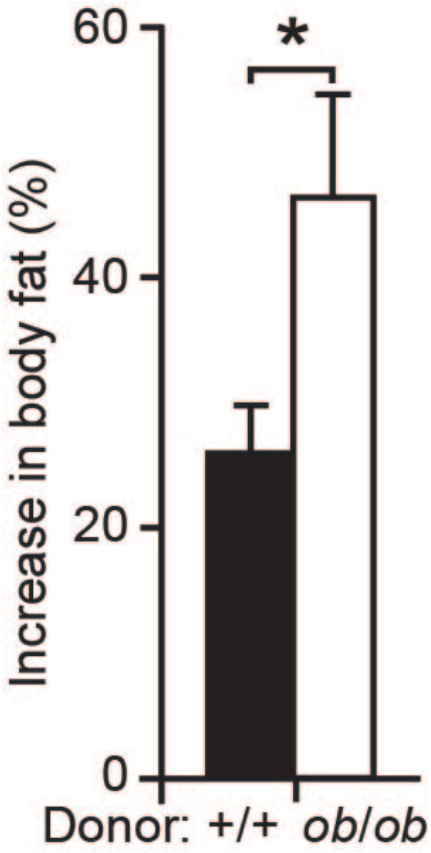
- Immunologic development
- Behavior and neurobiology
- Growth and metabolism

Cox and Blaser, Nature Reviews Endocrinology, 2014

Altered-microbiota can shape body composition



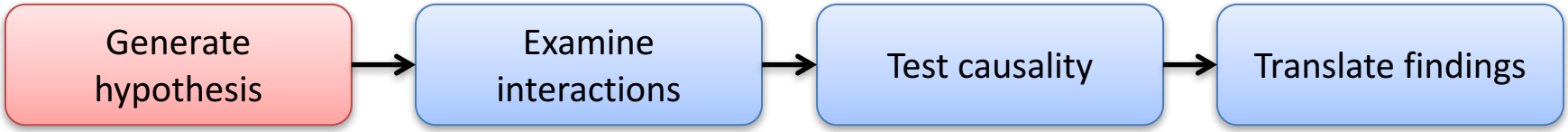




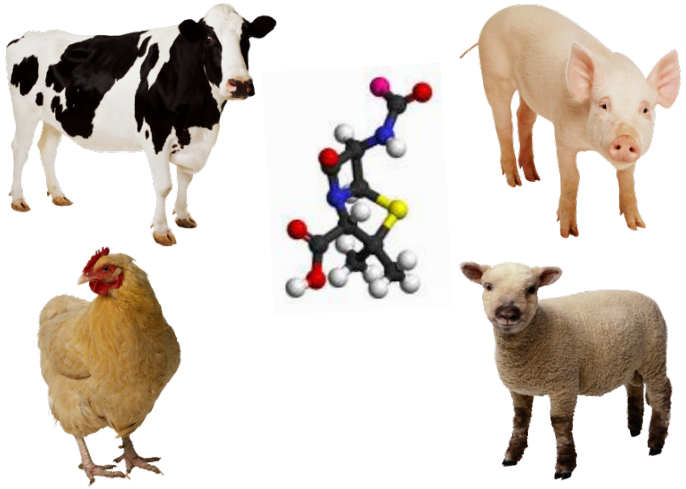
* $p < 0.05$, t-test

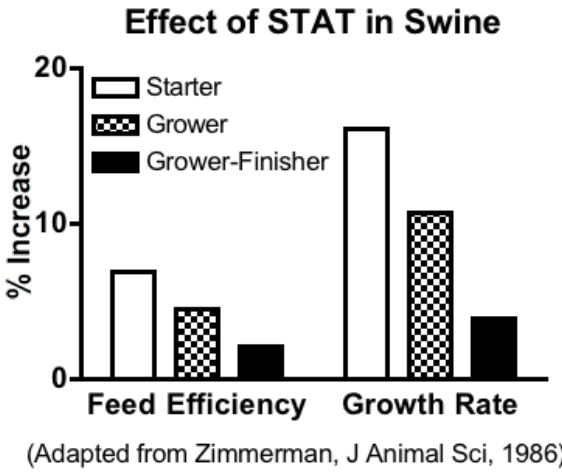
(Turnbaugh, Nature 2006)

Developing a model of microbe-induced obesity



Sub-therapeutic antibiotic treatment promotes growth

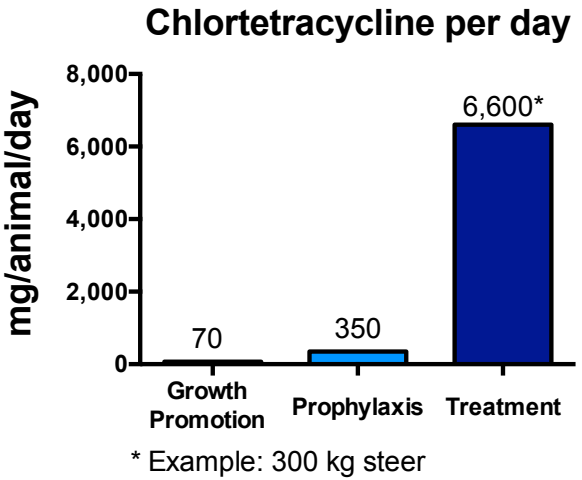


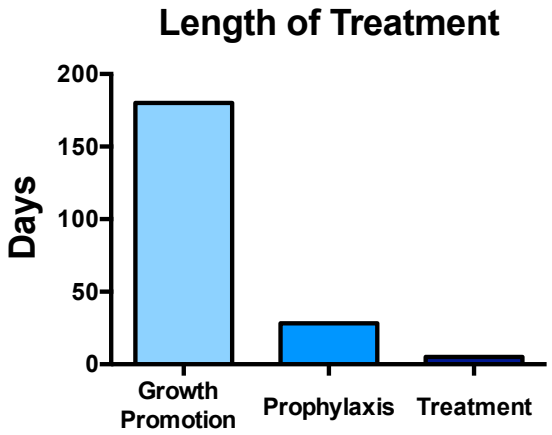


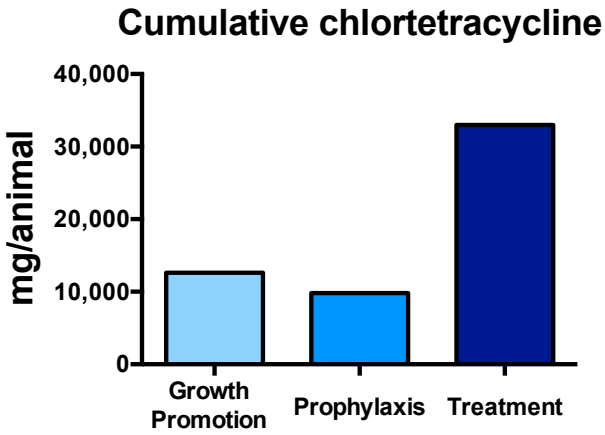
(Adapted from Zimmerman, J Animal Sci, 1986)

Antibiotic	Class	Spectrum	Target
Bambermycin	Glycolipid	Gram +	Cell wall
Virginiamycin	Streptogrammin	Gram +	Protein synthesis
Chlortetracycline	Tetracycline	Broad	Protein synthesis
Bacitracin	Cyclic peptide	Gram +	Cell wall synthesis
Penicillin	Beta-lactam	Gram +	Cell wall synthesis
Sulfathiazole	Sulfonamides	Broad	Metabolic inhibition
Carbadox	Quinoxaline	Broad	DNA Synthesis

Comparison of antimicrobial dosing levels







* Example: 300 kg steer

Usage	Dosage, duration, purpose
Growth promotion	70 mg/head/day throughout feedlot period for growth promotion (FDA, 1996)
Disease control	350 mg/head/day for 28 days to prevent shipping fever (Gadberry, 2012)
Disease treatment	22 mg/kg bw/day for 5 days to treat bacterial enteritis or pneumonia (FDA, 2002)

Adapted from: Cazer, Foodborne Pathogens and Disease, 2014

Acknowledgements:

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Arlin Rogers

Bioinformatics
Alex Alekseyenko

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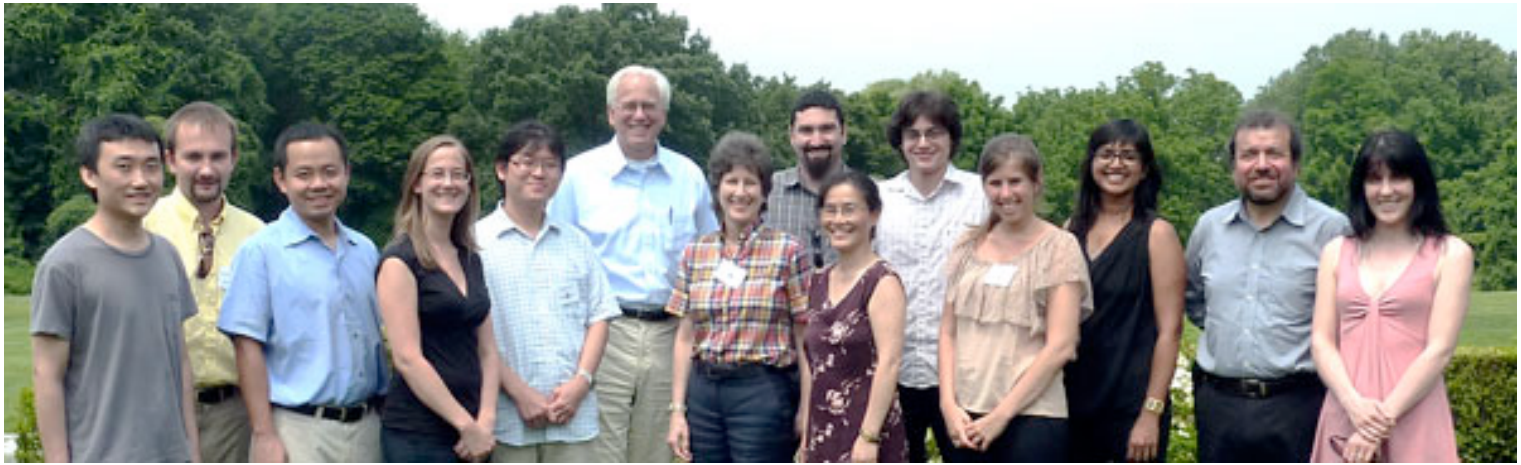
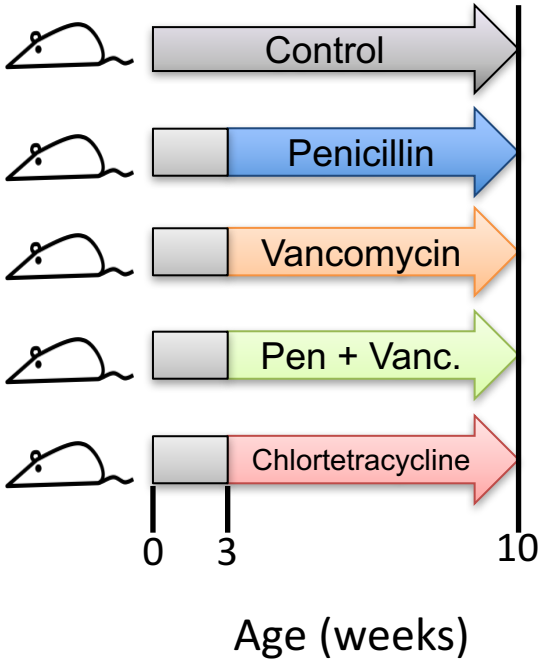
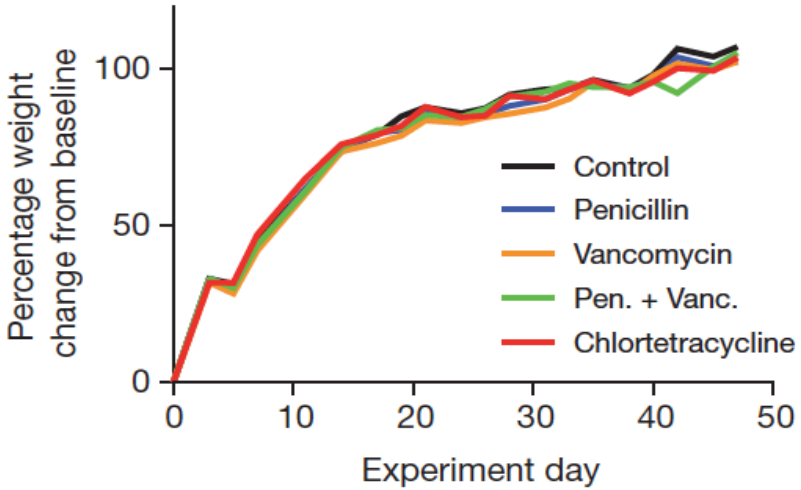
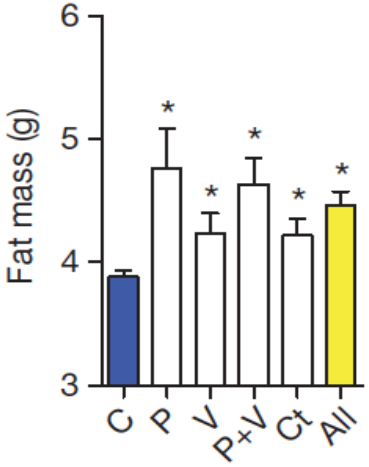
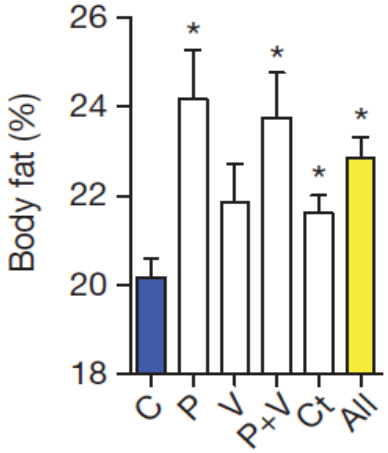


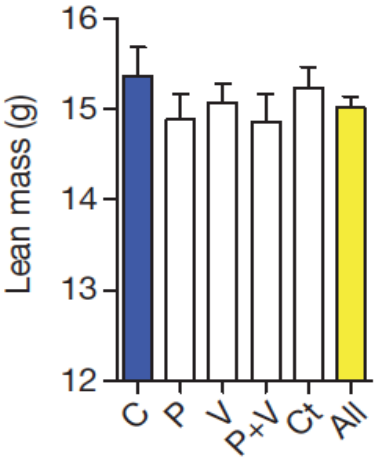
The effect of sub-therapeutic antibiotic treatment on body composition





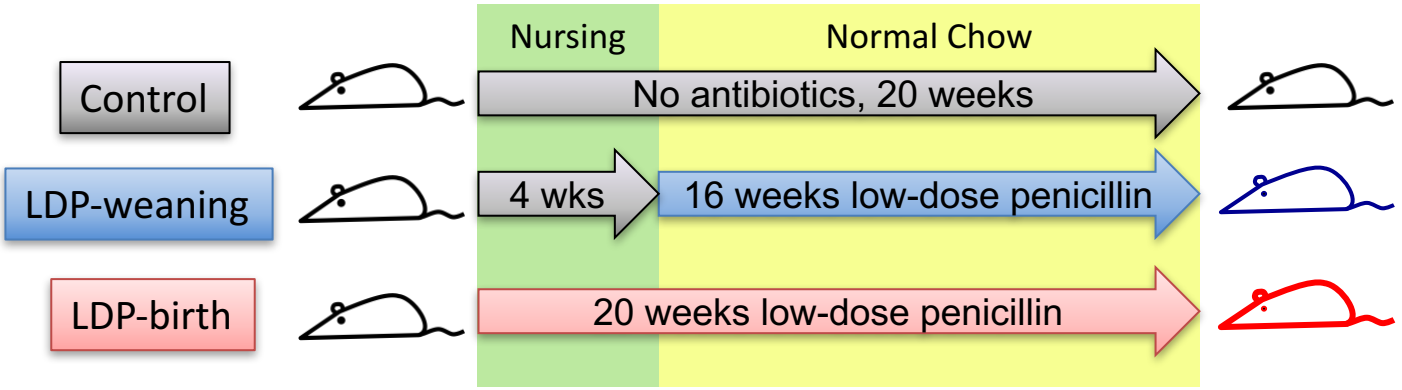




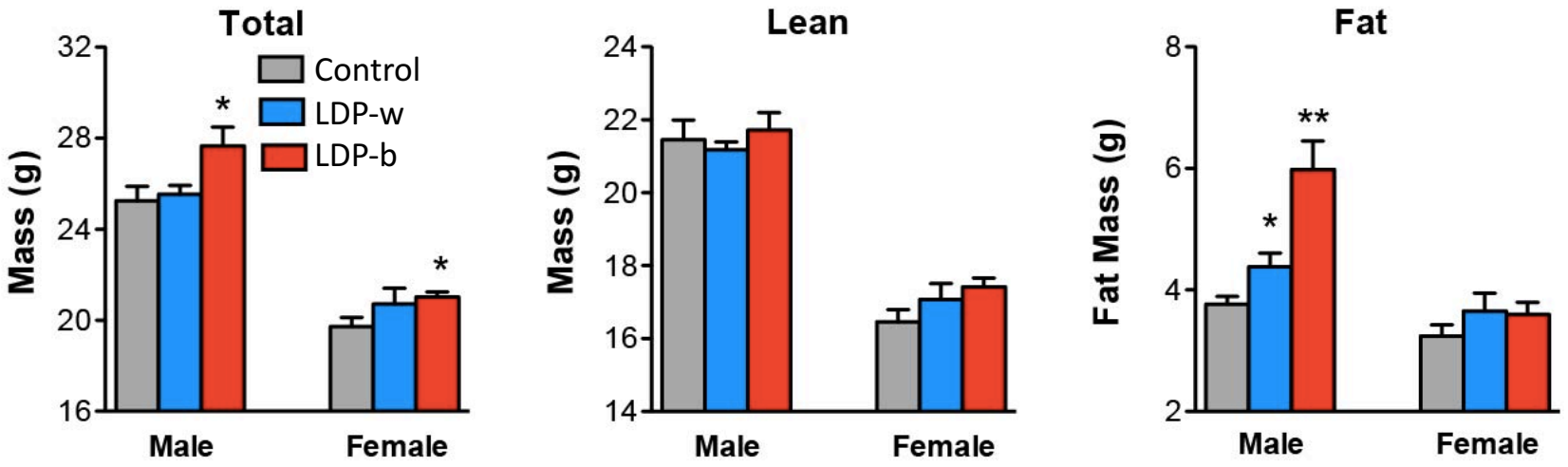


Cho, Nature, 2012

The effect of pre- or post-weaning antibiotic exposure



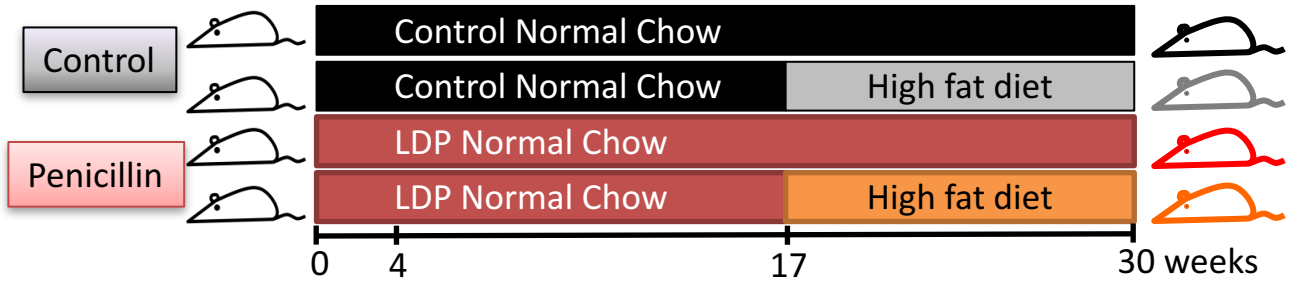
Body Composition at 20 weeks by DEXA scanning



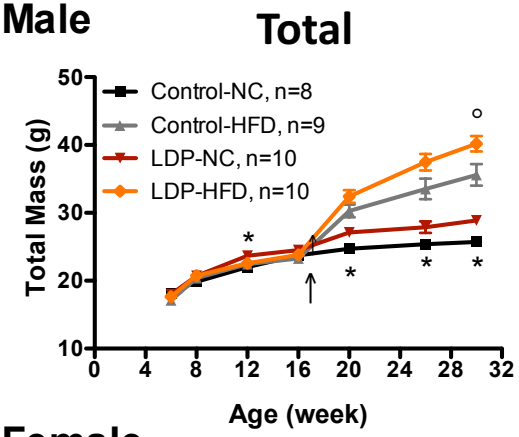
Cox et al., Cell, 2014

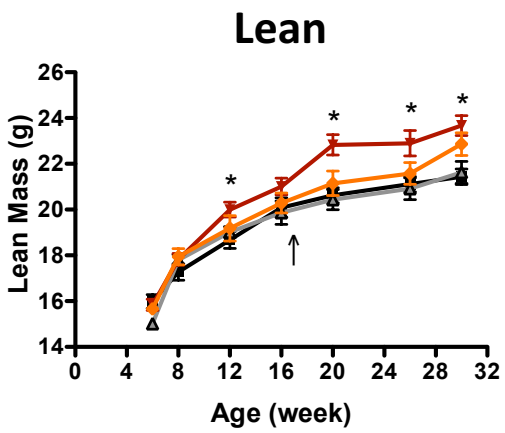
* P < 0.05, ** p < 0.01, *** p <0.001

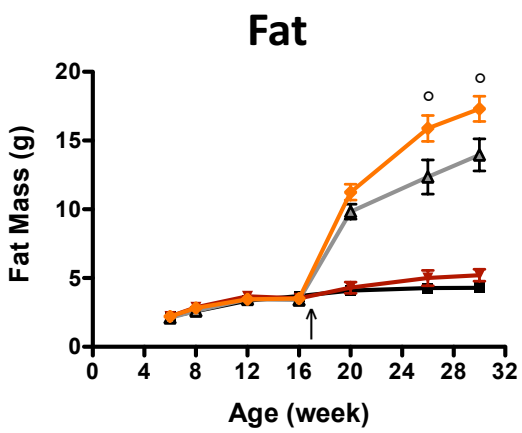
The effect of HFD and LDP on body composition



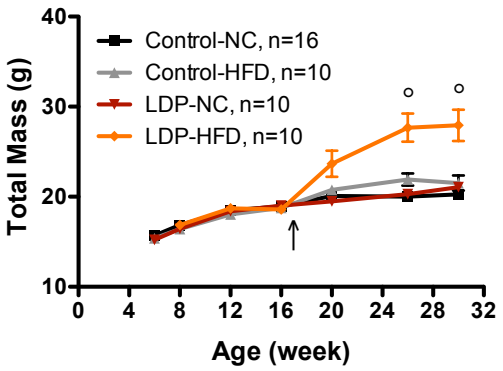
Male

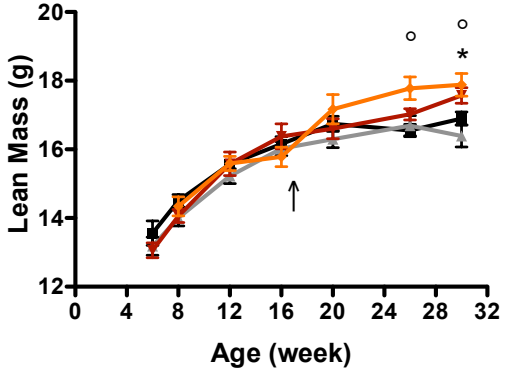


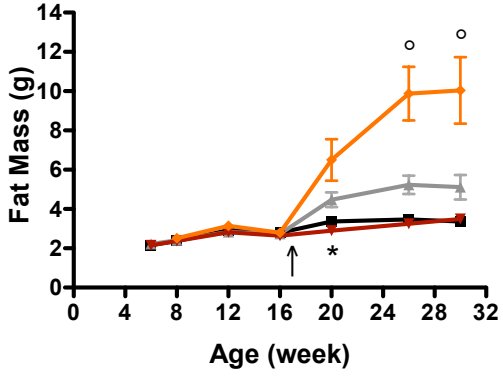




Female







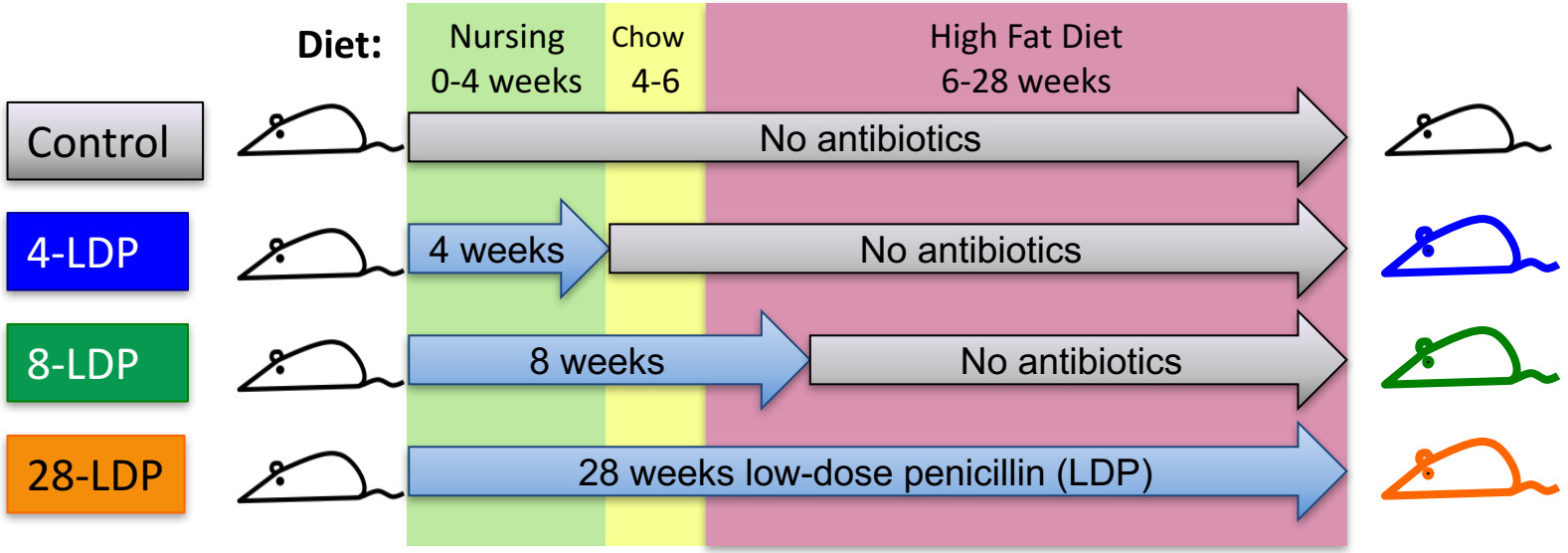
Cox et al., Cell, 2014

↑ High fat diet introduced

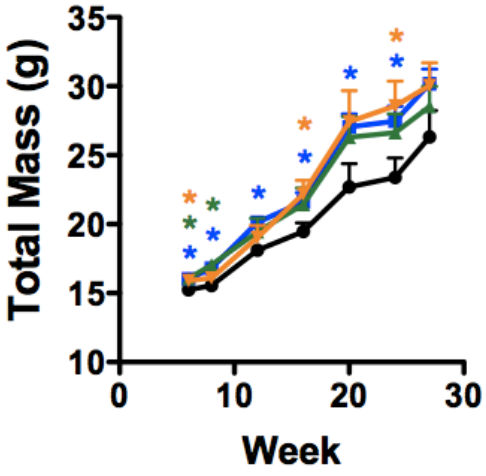
* $p < 0.05$ NC

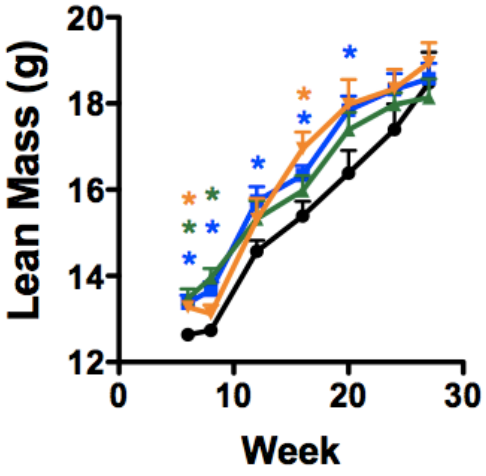
° $p < 0.05$ HFD

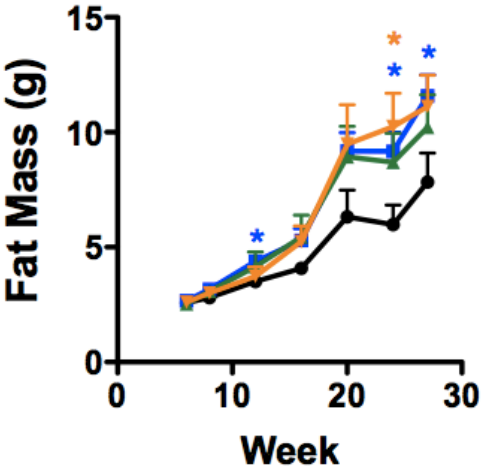
Is adiposity durable with limited antibiotic exposure?



Control n = 13
4-LDP n = 9
8-LDP n = 12
28-LDP n = 8



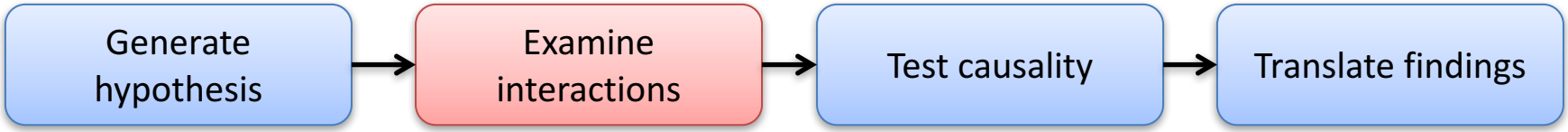




* P < 0.05, t-test

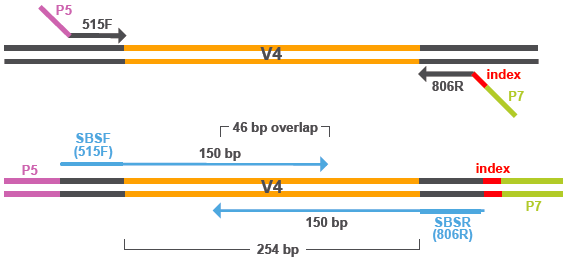
Microbiota Analysis:

How does the microbiota respond to and recover from low-dose penicillin over time?



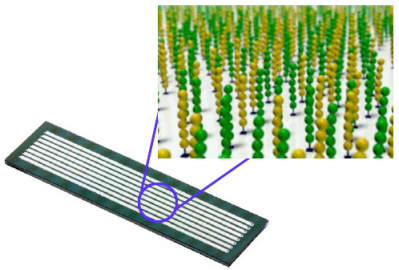
Microbiome Sequencing Strategy

1. Amplify the V4 region of microbial 16S rRNA gene with hundreds of barcoded reverse primers

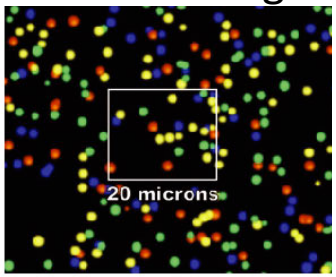


2. Sequence on Illumina MiSeq platform

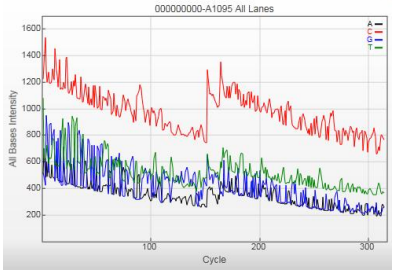
Flow Cell



Flow Cell Image



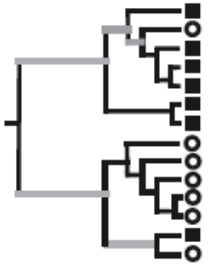
Signal Intensity



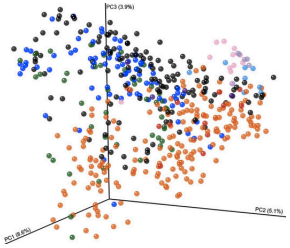
3. Assemble paired end reads with EA-utils, quality filter, demultiplex, assign taxonomy, construct a phylogenetic tree, calculate diversity metrics and relative abundance with QIIME

Quantitative Insights Into Microbial Ecology

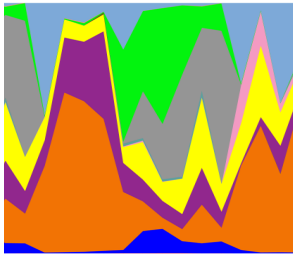
Tree



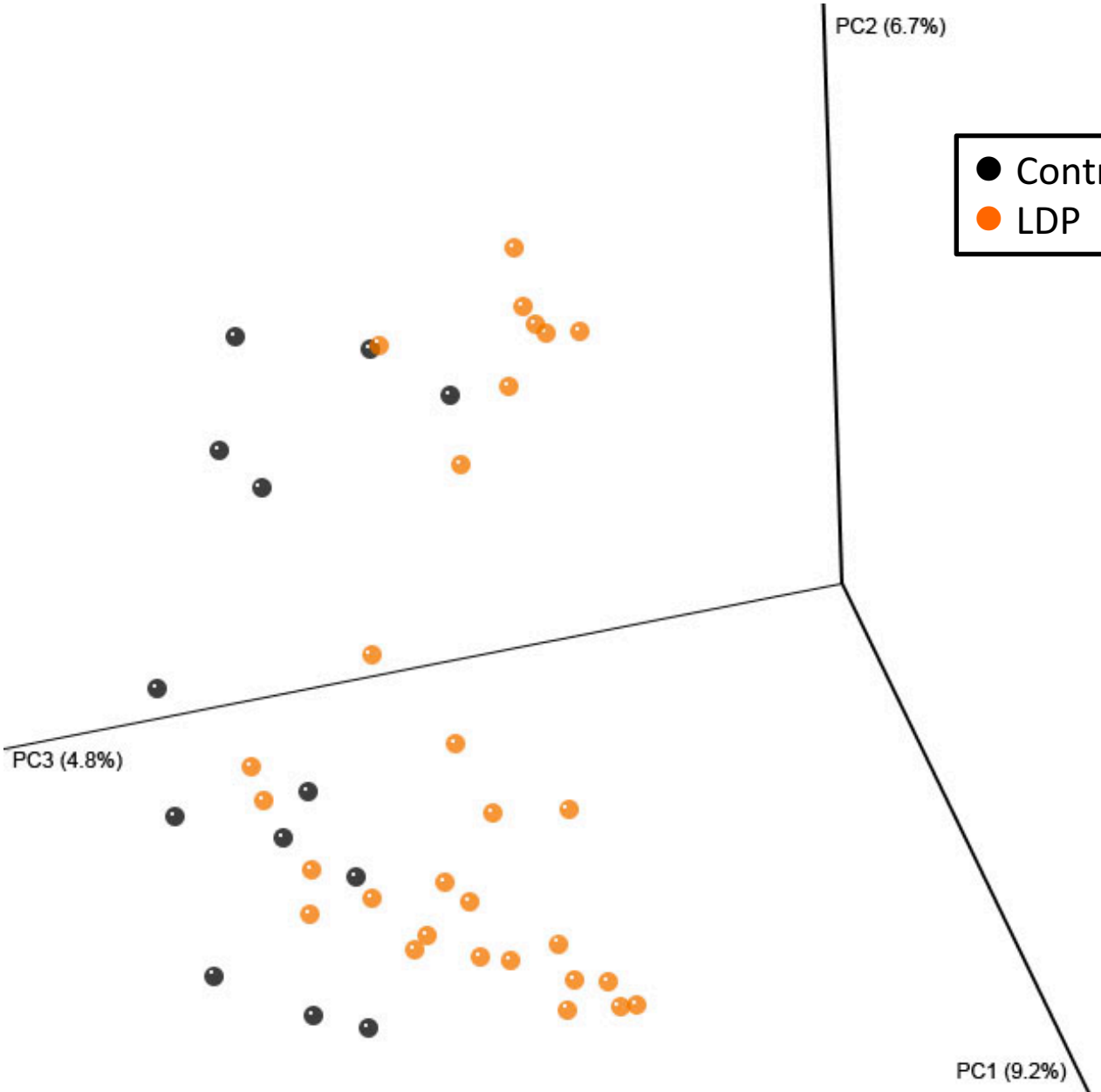
β-diversity



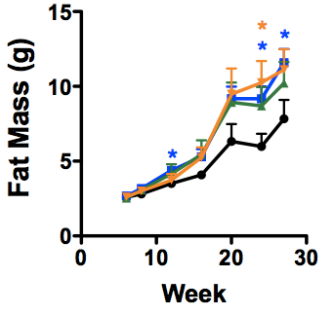
Composition

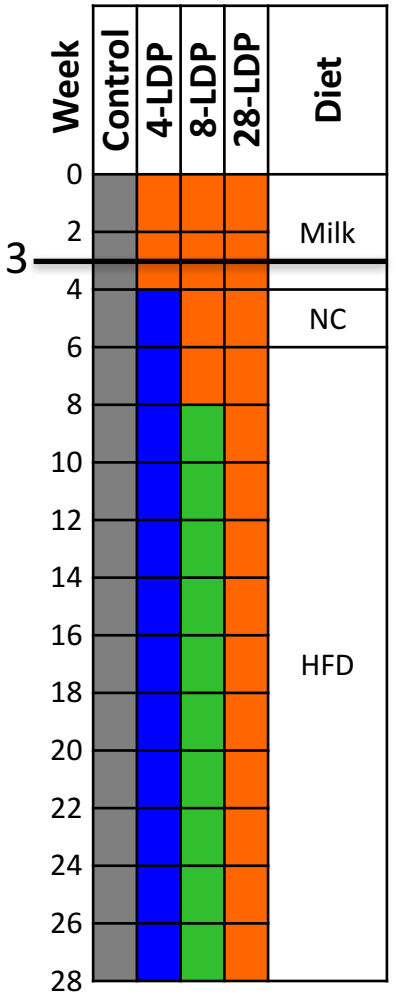


3-week fecal community structure

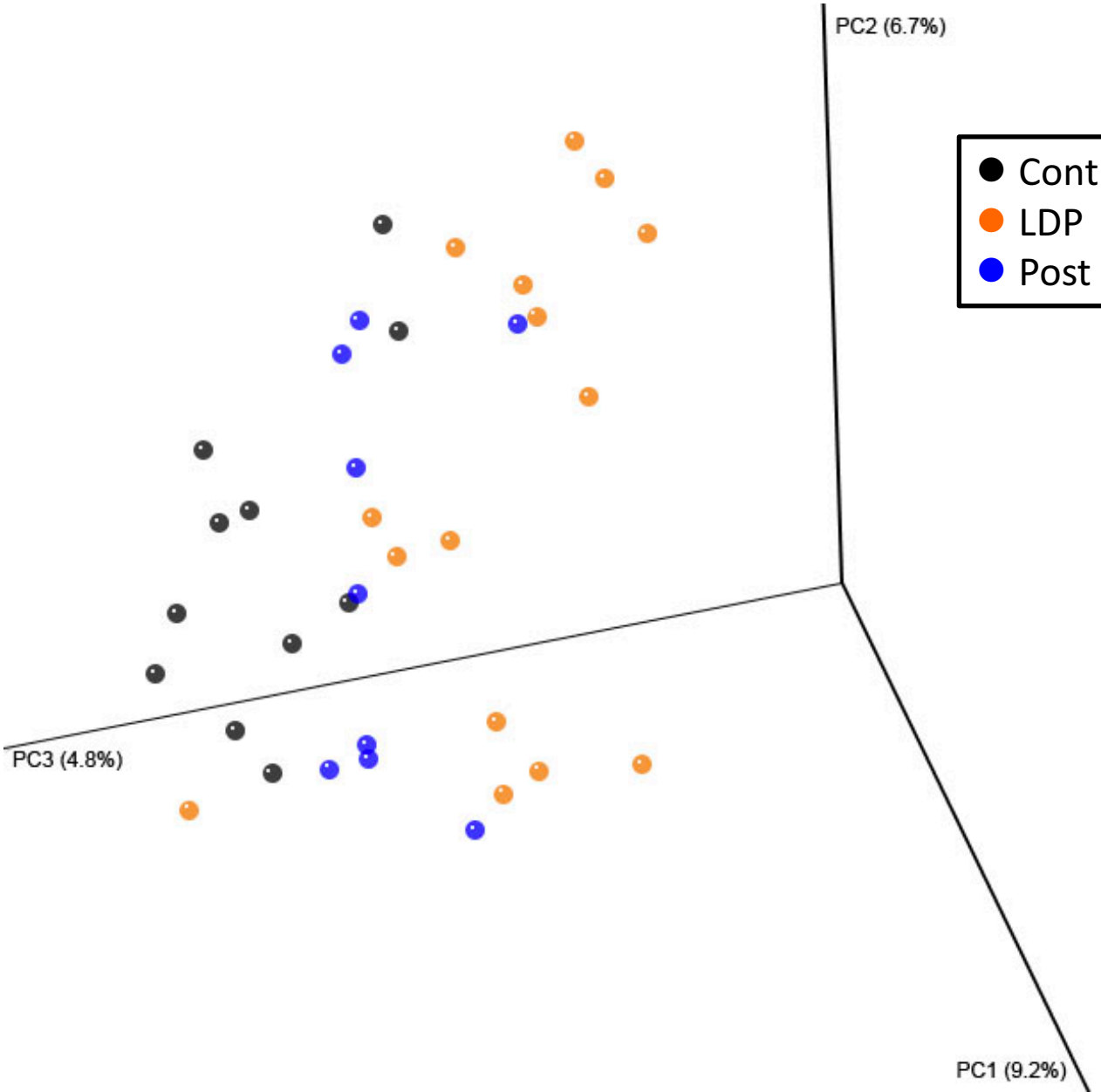


Control
LDP

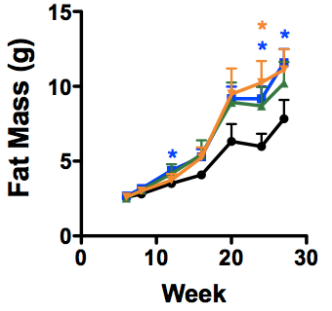


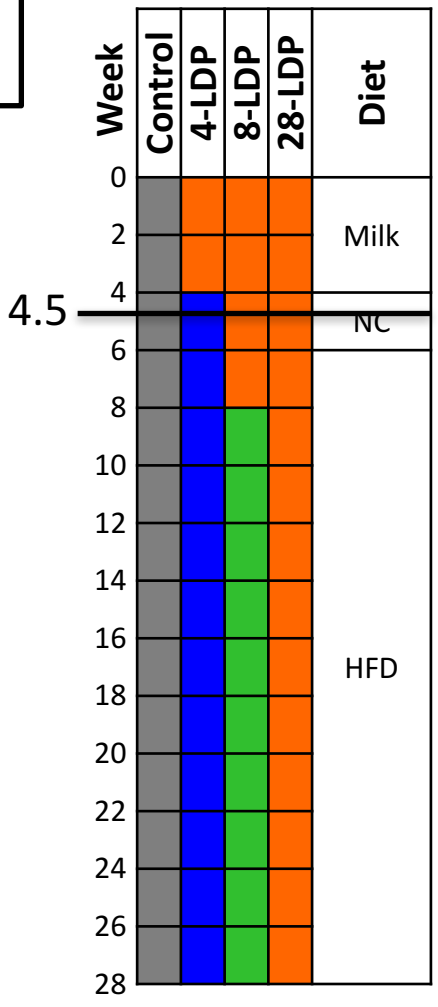


4.5-week fecal community structure

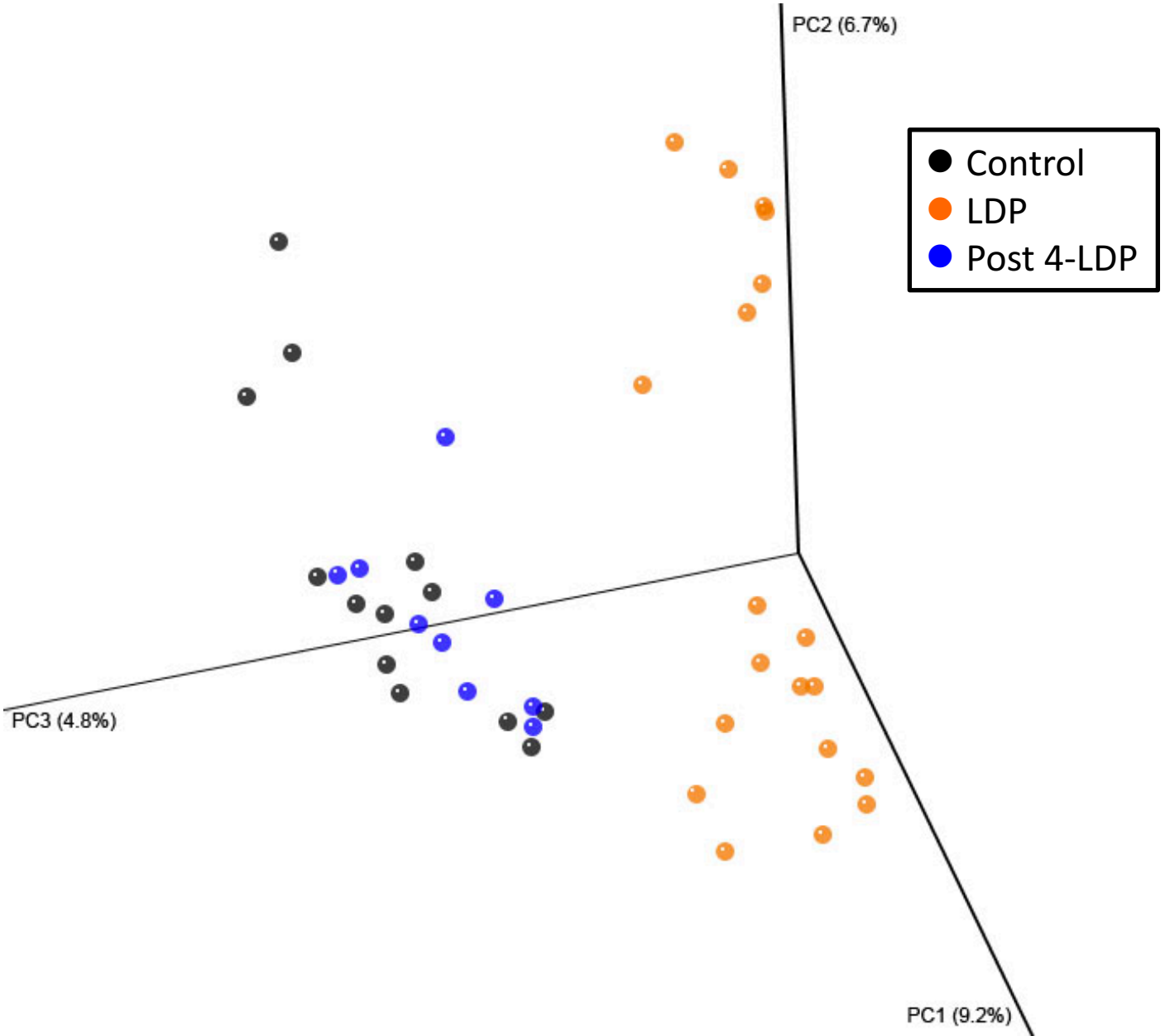


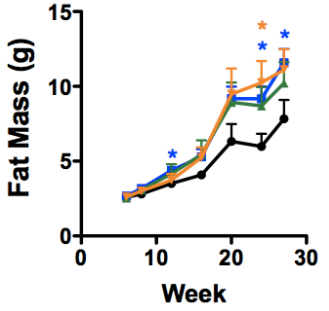
Control
LDP
Post 4-LDP

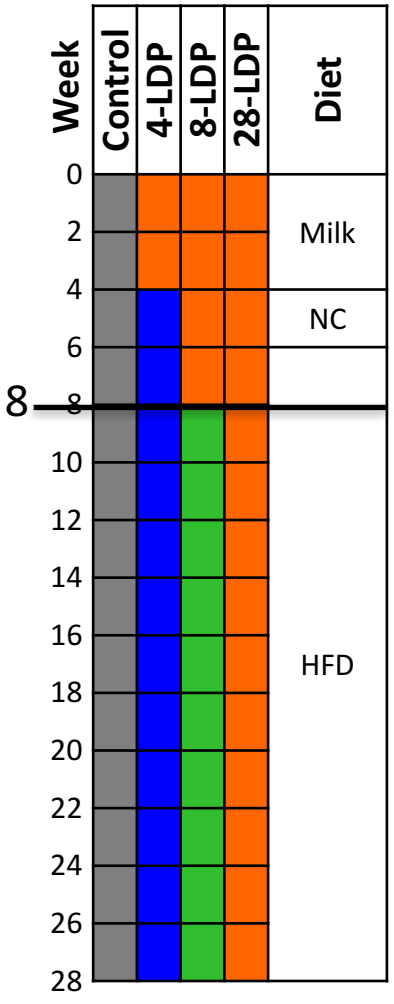




8-week fecal community structure

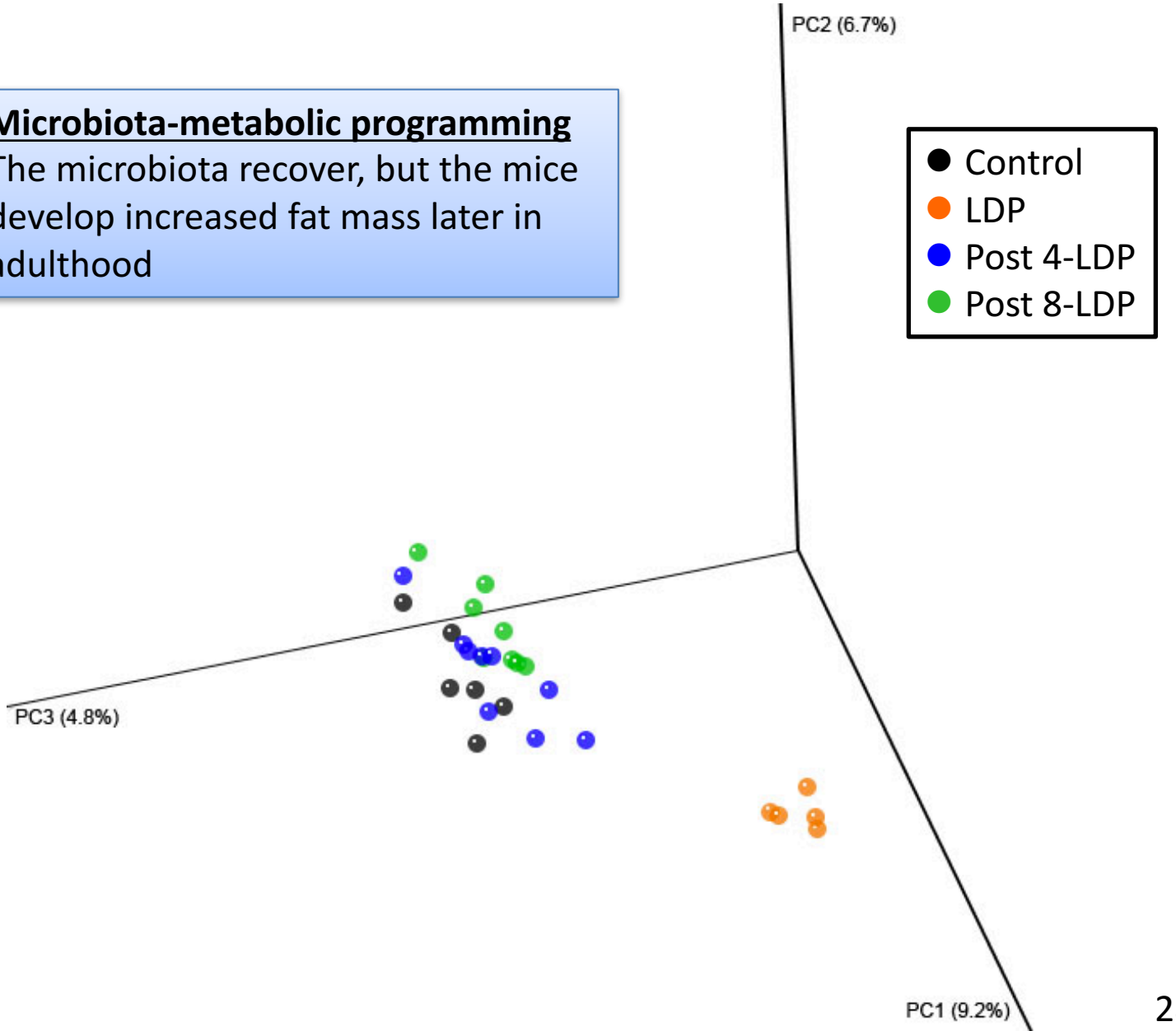


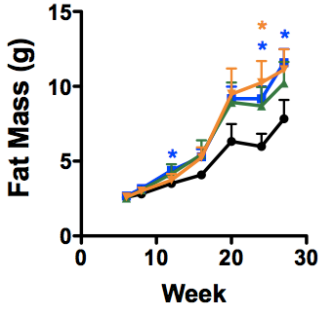


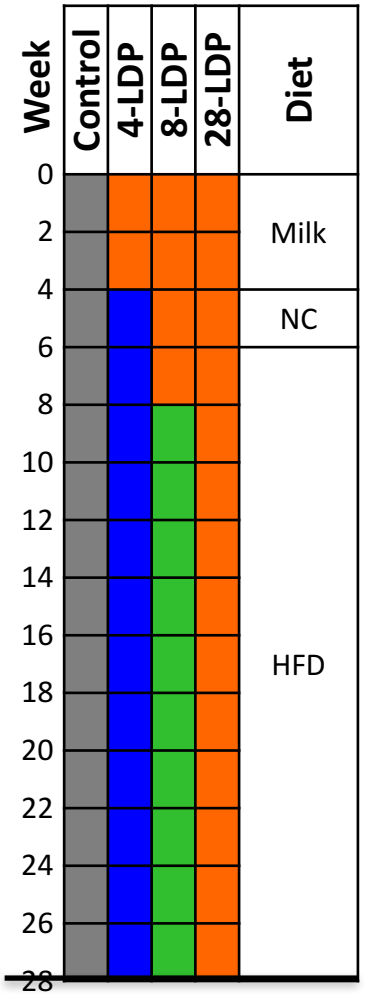


28-week fecal community structure

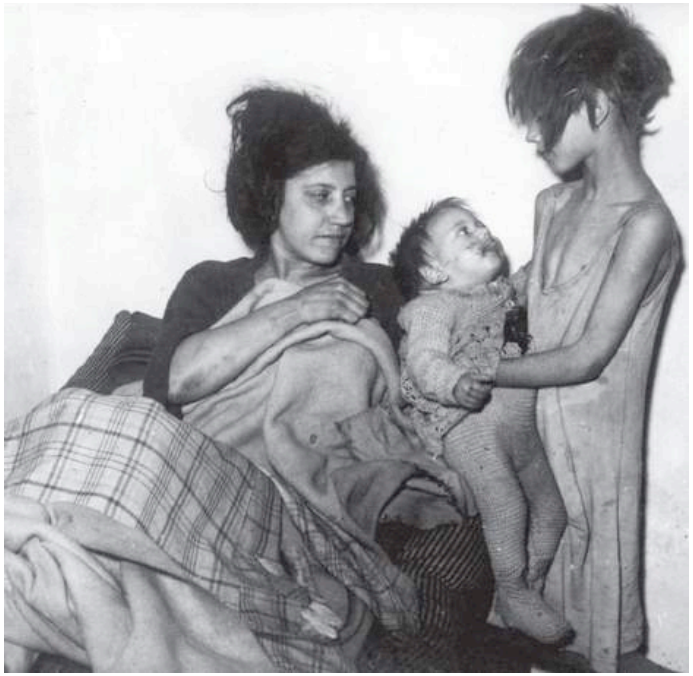
Microbiota-metabolic programming
The microbiota recover, but the mice develop increased fat mass later in adulthood







Metabolic programming during the Dutch hunger winter



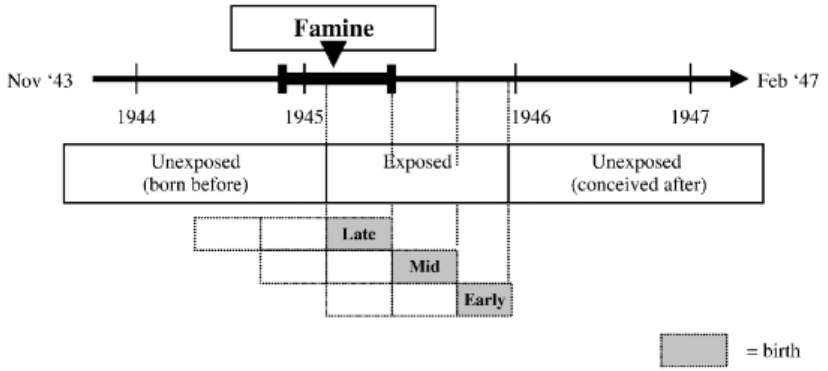


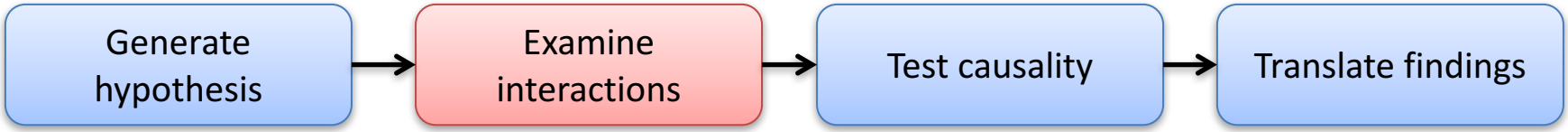
Figure 1 The Dutch famine birth cohort: famine exposure and birth in relation to the timing of the Dutch famine.

(Roseboom et al., Early Child Development, 2006)

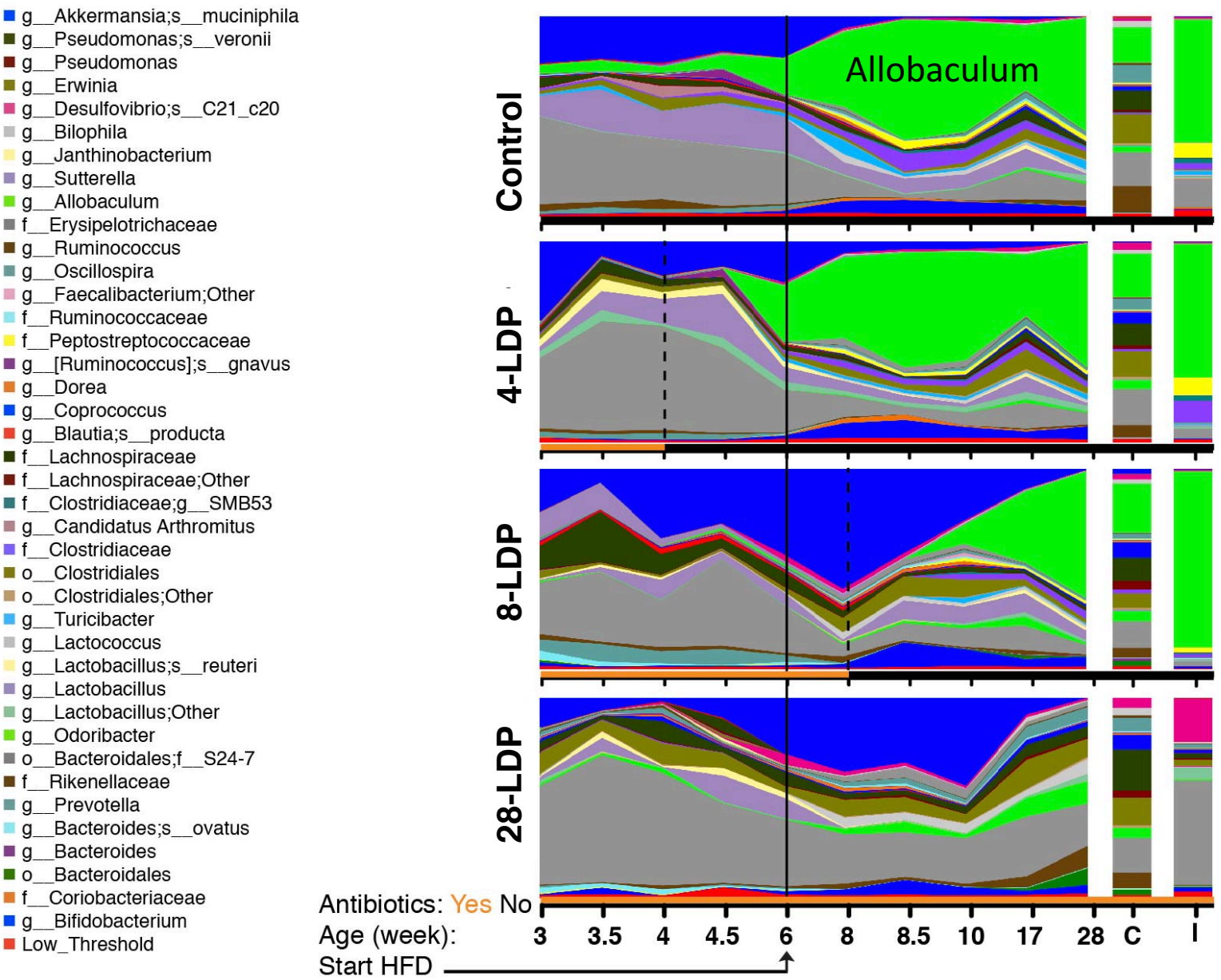
- A massive famine affected the Netherlands during world war II
- Pregnant women were rationed additional food when it was possible
- During the worst times, all citizens were rationed between 400-800 calories per day
- Children exposed to low calories during the early part of gestation, followed by a rapid catch up had higher rates of cardiovascular disease, dislipidemia, and obesity

Bacterial Composition:

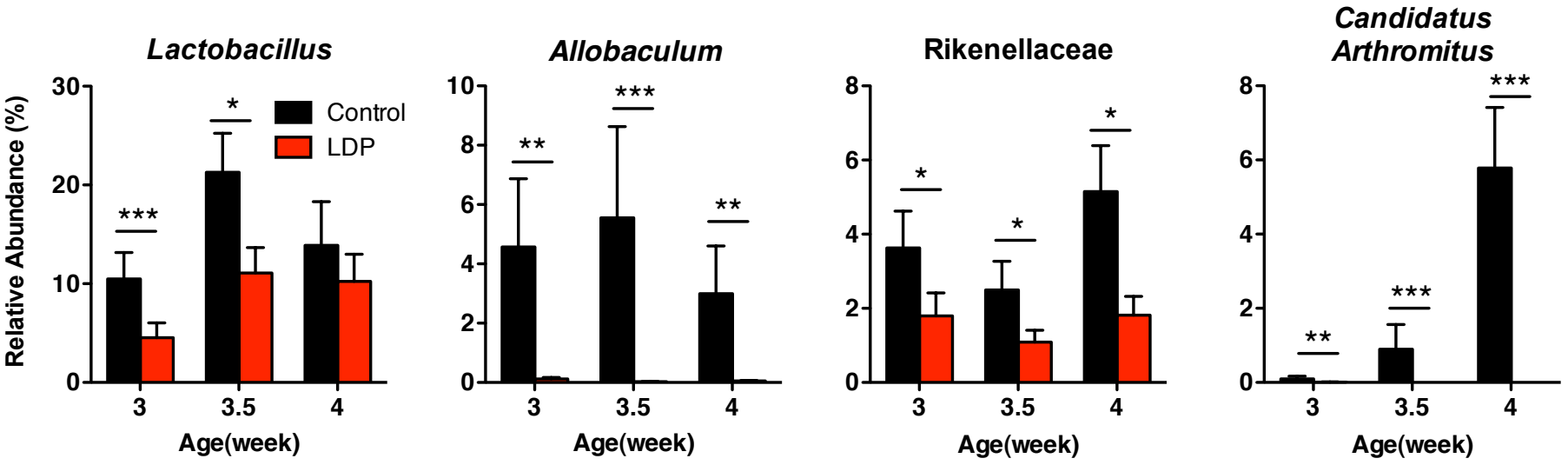
Find consistent changes



Changes in microbiome composition with limited LDP



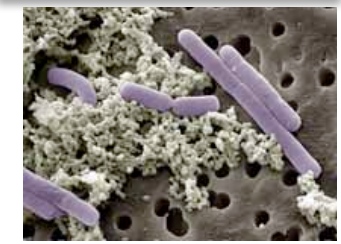
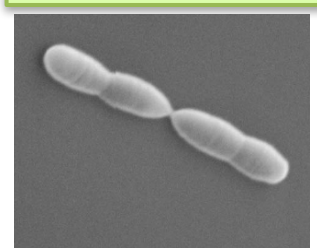
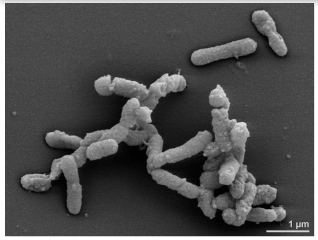
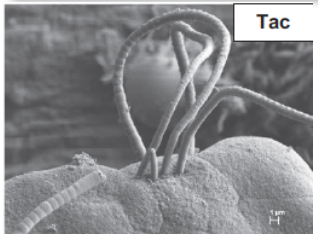
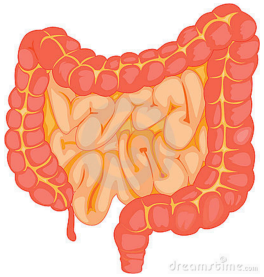
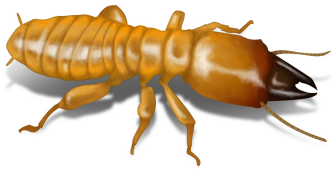
Early-life microbiota disrupted by penicillin



3/3 independent experiments

2/3 independent experiments

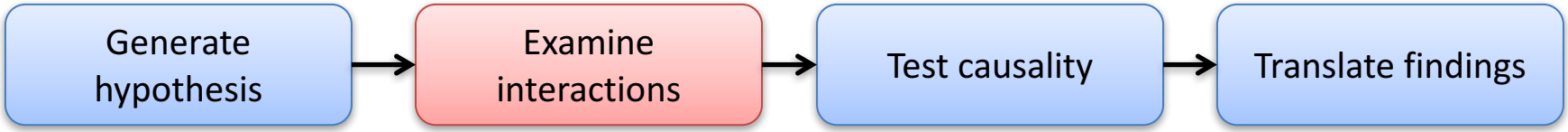
Summary of candidate protective organisms

	Lactobacillus	***Allobaculum***	**Rikenellaceae**	***"Arthromitus"***
Electron photo-micrograph	 bioweb.usu.edu/microscopy	 Greetham, Anaerobe, 2004	 Mavromatis, Stand Genom Sci, 2013	 Ivanov & Littman, et al. , Cell 2009
Original source				

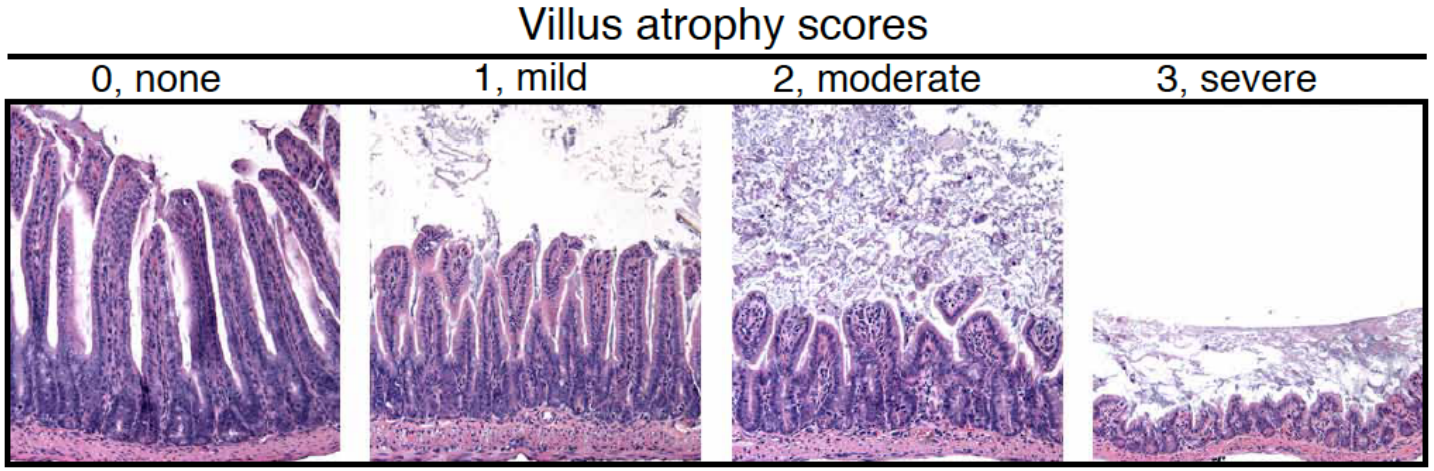
Organism	***Lactobacillus***	***Allobaculum***	**Rikenellaceae**	***"Arthromitus"***
Class	Bacilli	Erysipelotrichi	Bacteroidia	Clostridia
Discovered	Pre-1905 from yogurt, early studies Metchnikoff	2004, Labrador, Reading, UK	Members isolated 1937 Family named in 2003	1849, termite gut, Joseph Leidy
Pubmed ref's	30,736	22	102	209 (SFB)
Special characteristics	Major lactic acid producer	Highly responsive to dietary changes	Adherent to epithelial cells in leech intestine	Induces Th17 Cells

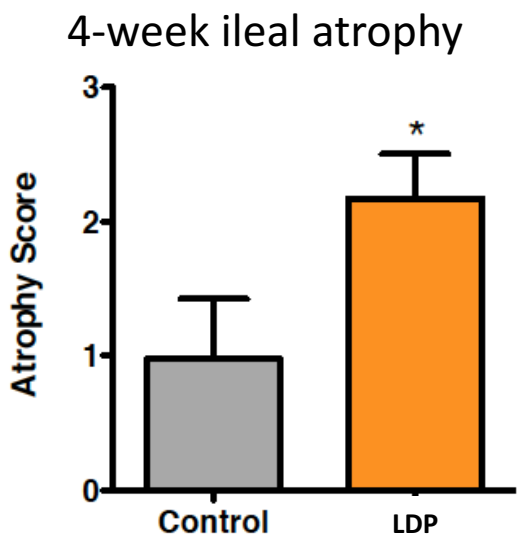
Host defenses:

Find altered states



Early life changes in ileal architecture





* P < 0.05 Mann-Whitney

Cox et al., Cell, 2014

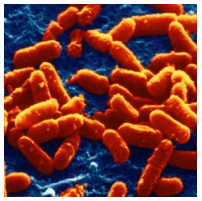
(Arlin Rogers, Tufts University)

“Good fences make good neighbors”

-old English proverb
-Robert Frost, “The Mending Wall”

Archer & Buchanan

Modulation at the intestinal interface



Antigen

Antigen Presenting cell

Cytokines

Naïve T-cell

MHCII Antigen presentation

Differentiation

T-helper cell

Cytokines

Enterocyte
Paneth cell
Goblet cell

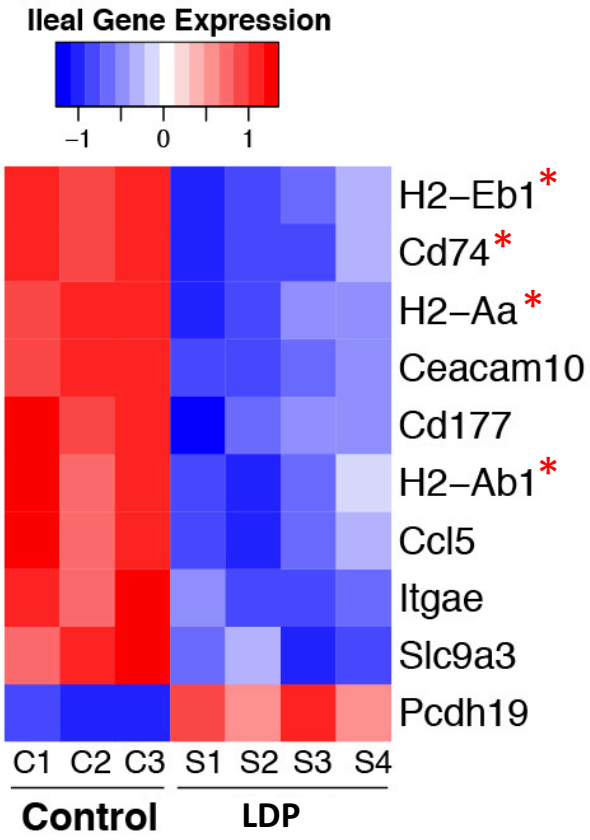
Antimicrobial peptide

The effect of LDP on ileal expression

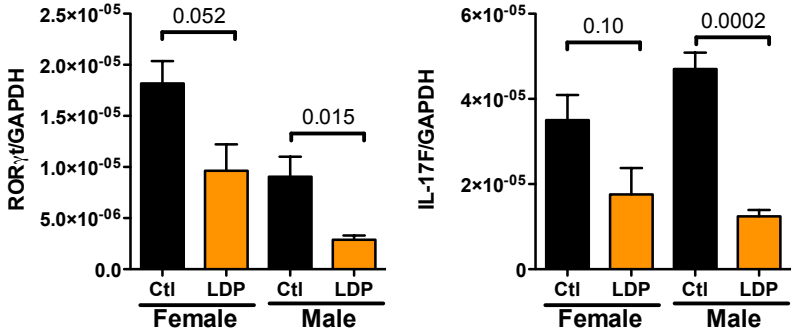
Microarray

qPCR

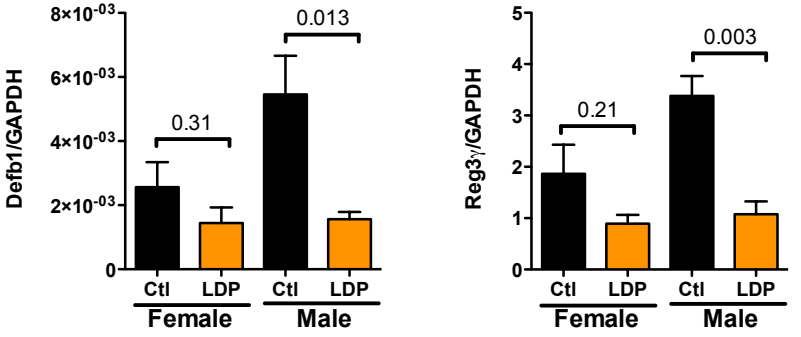
*Antigen Presentation



Th17

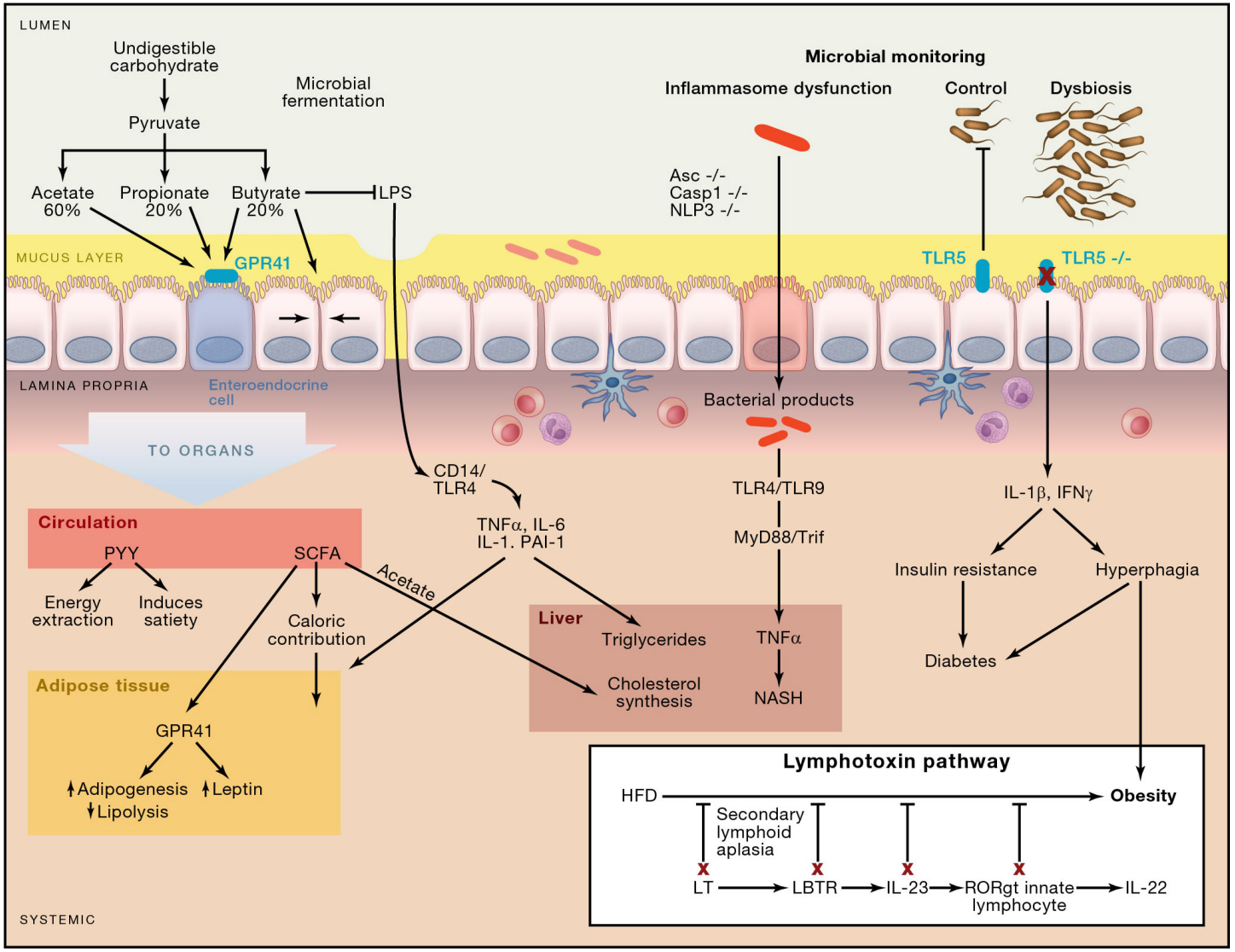


Antimicrobial peptides



(Shingo Yamanishi, Jiho Sohn)

Interactions between microbes, immunity, and metabolism



Disruption at the ileal interface

Translocation of bacterial products

Altered metabolic and inflammatory signaling

Obesity

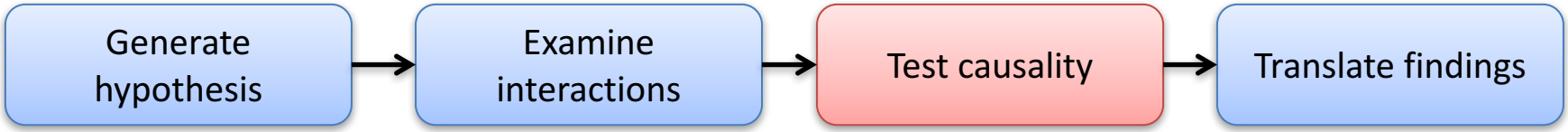
NASH

Diabetes

LM Cox & MJ Blaser. Cell Metabolism 2013

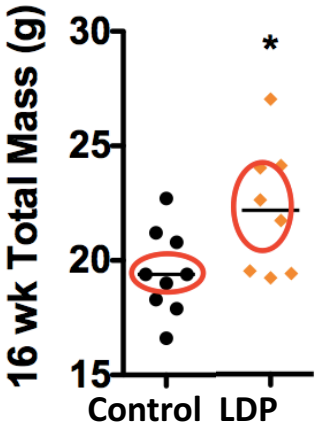
Testing Causation:

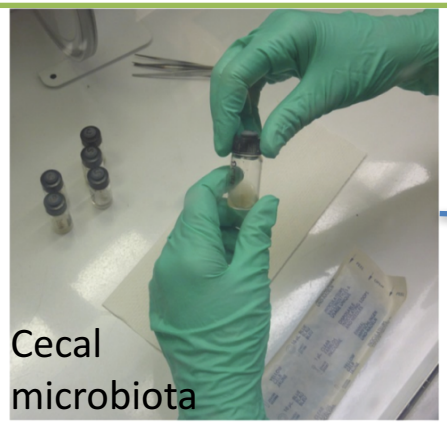
Microbiota transfer to germ-free mice



Is the LDP-adiposity phenotype transferable?

Donors 18-week female C57B/L6J



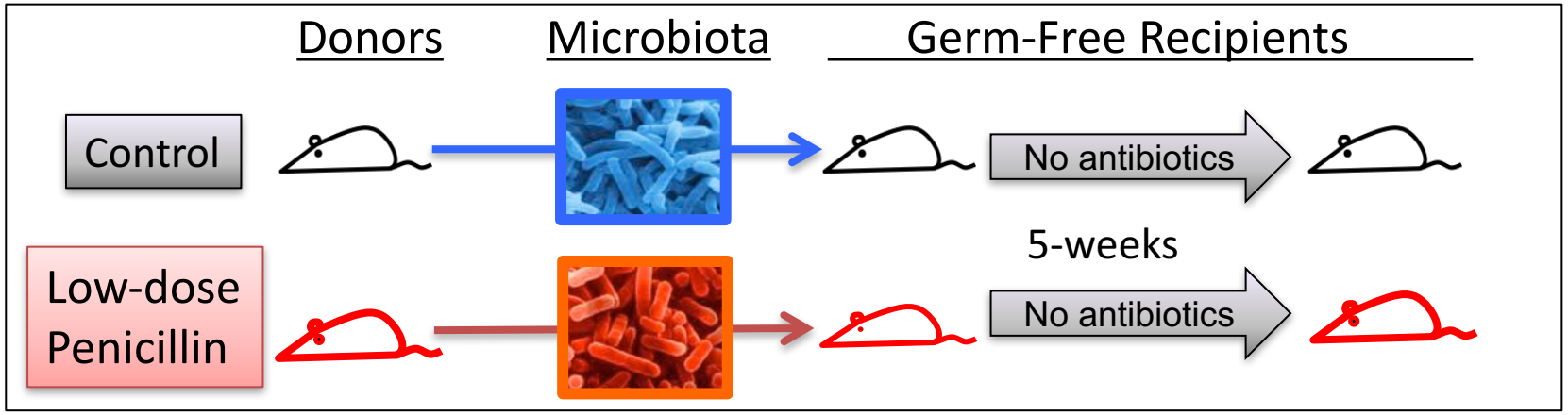


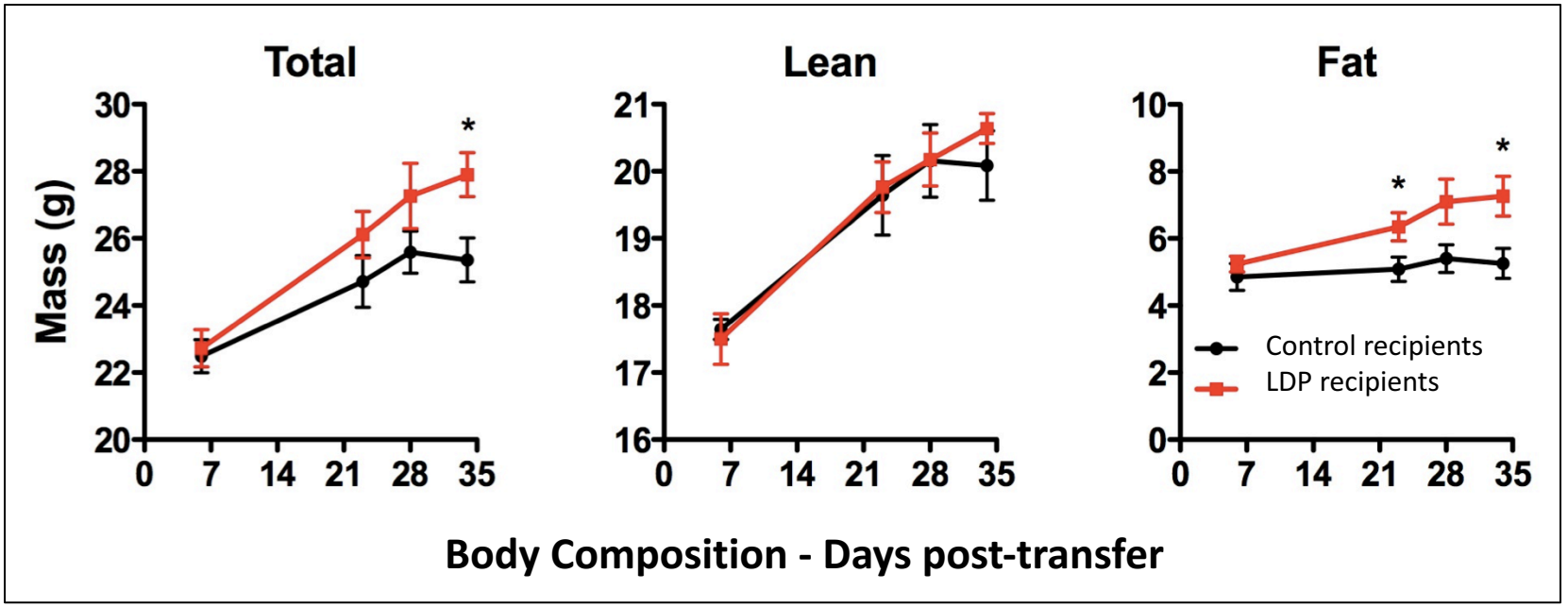
Recipient 3-week female germ-free Swiss-Webster

Oral Gavage

Specific-pathogen free conditions

Penicillin-altered microbiota confer the obesogenic phenotype



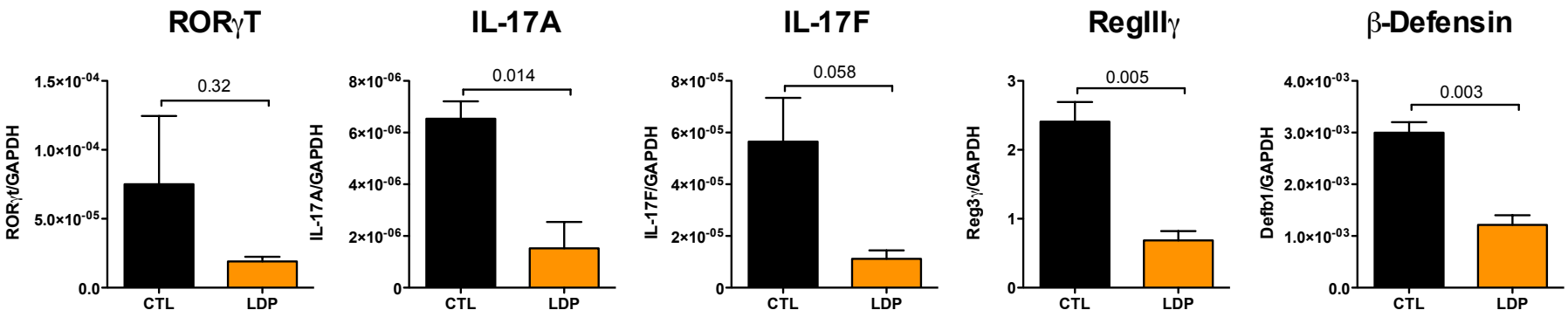


Expression of genes involved in ileal defense in microbiota donor and recipient mice

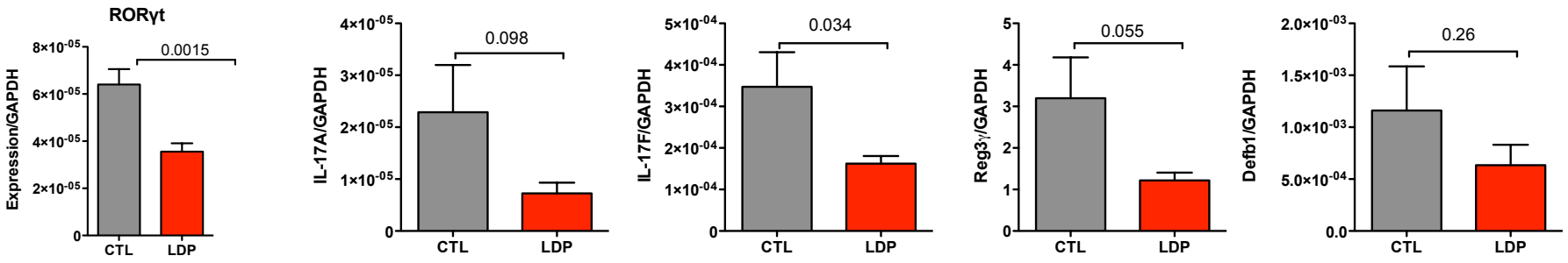
Donors

Th17

Antimicrobial peptides

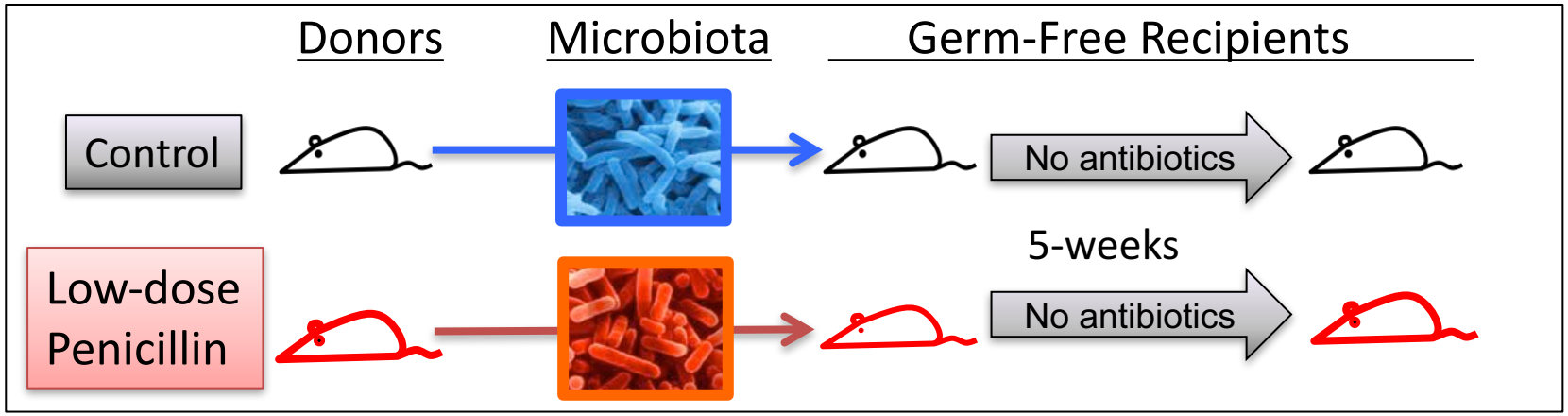


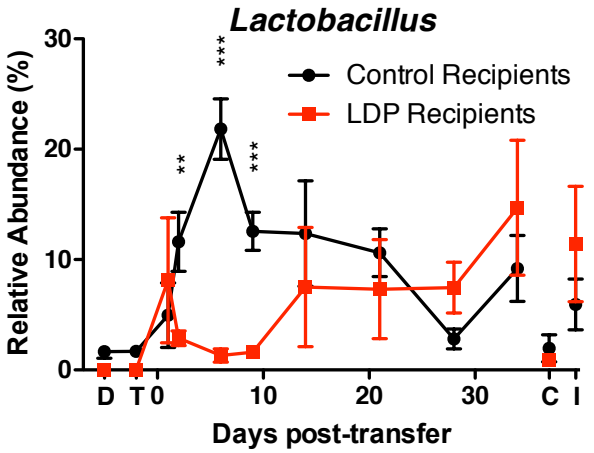
Recipients

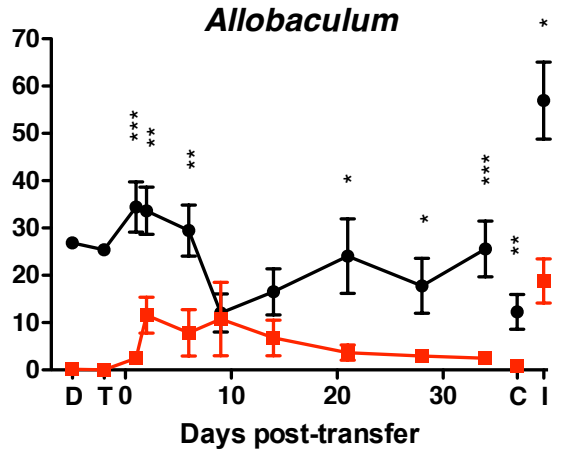


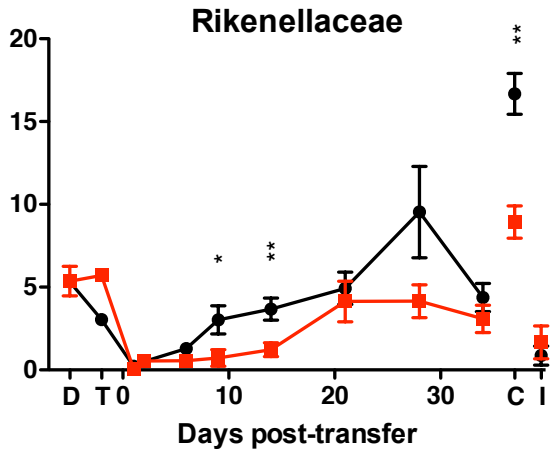
p-values t-test

Consistent reductions in LDP-microbiota recipients









4/4 independent experiments

3/4 independent experiments

Predicted functional profiling with PICRUSt

16S rRNA abundance

↓

Known genomes + inferred ancestral genomes

↓

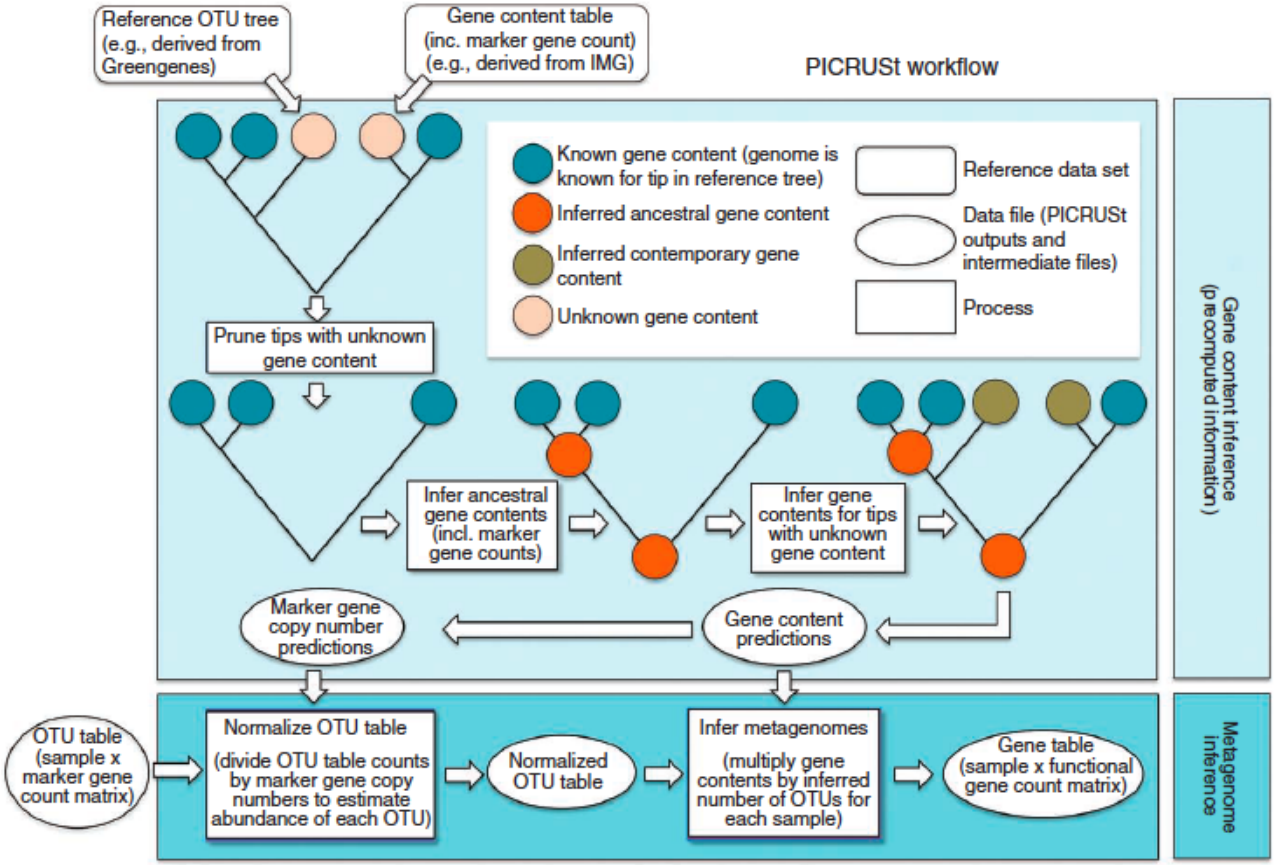
Functional gene abundance

↓

Categorize by KEGG

↓

Test with LEfSe



(Langille et. al., Nature Biotechnol, 2013
Segata et. al., Genome Biol. 2001)

Huttenhower Lab

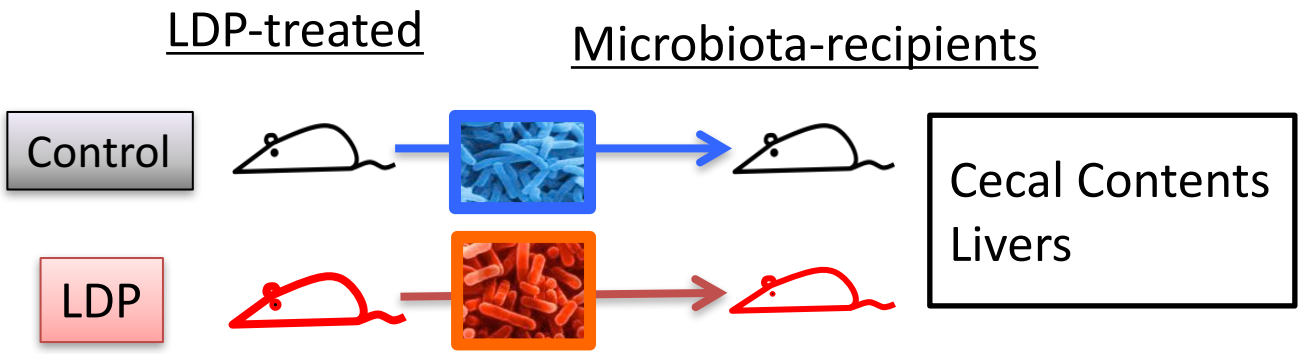
Altered predicted microbiota functions

KEGG Pathway (L3)		Low-dose pencillin	LDP-microbiota recipients
Down	Amino sugar and nucleotide sugar metabolism	2*	3
	Fructose and mannose metabolism	4	3
	General function prediction only	2	4
	Glycolysis/Gluconeogenesis	2	2
	Peptidoglycan biosynthesis	2	2
	Terpenoid backbone biosynthesis	2	2
Up	Arginine and proline metabolism	2	3
	C5-Branched dibasic acid metabolism	4	3
	Fatty acid biosynthesis	2	3
	Glycine, serine and threonine metabolism	2	2
	Lipid biosynthesis proteins	2	3
	Lysine biosynthesis	2	2
	Pantothenate and CoA biosynthesis	3	3
	Phenylalanine, tyrosine and tryptophan biosynthesis	2	4
	Protein kinases	2	2
	Sphingolipid metabolism	3	2
	Two-component system	2	2
	Valine, leucine and isoleucine biosynthesis	2	3

*Number of significant hits out of 5 early-life time points surveyed by LEfSe

Metabolomics workflow

Samples



Measurement

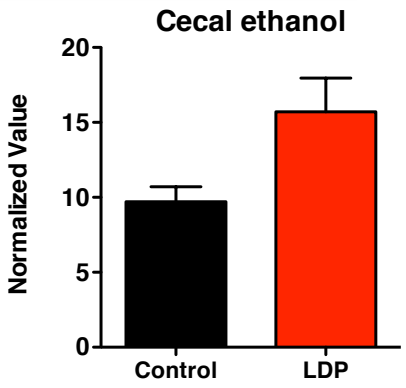
NMR spectra obtained on a Bruker Avance III 700 MHz NMR spectrometer

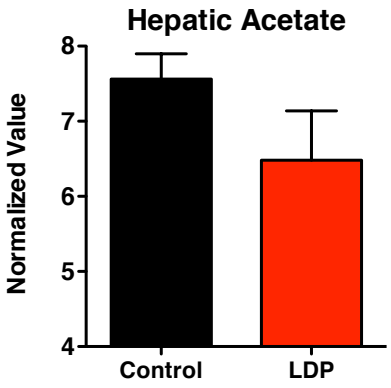
Analysis

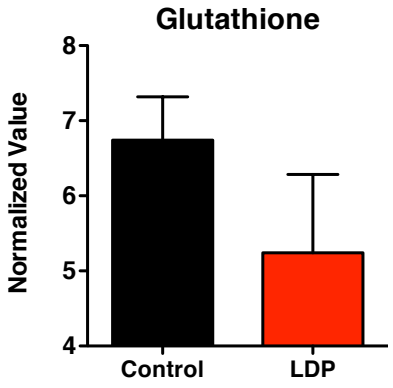
Processing: Spectra binned and normalized
Statistics: Orthogonal Partial Least Squares Projections to Latent Structures Discriminant Analysis (OPLS-DA) with Variable Importance in the Projection (VIP)
Identification: Chenomx NMR Suite 7.7 Professional

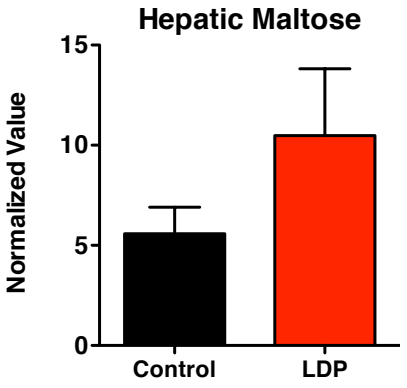
Consistently altered metabolites

LDP-treated mice

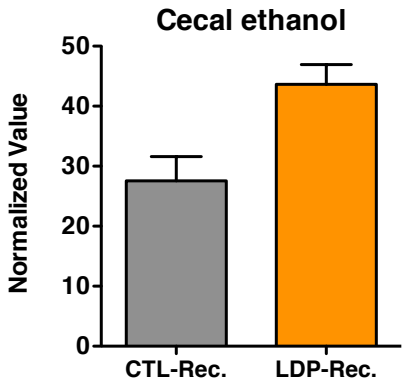


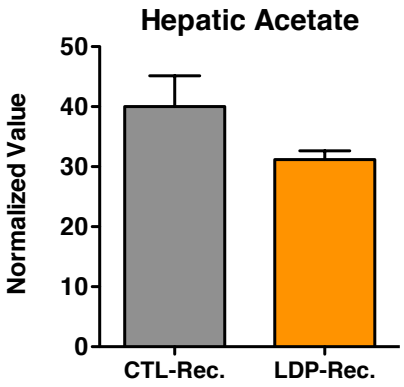


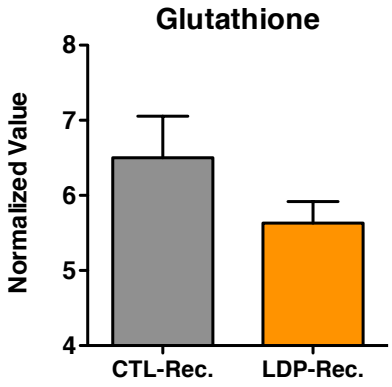


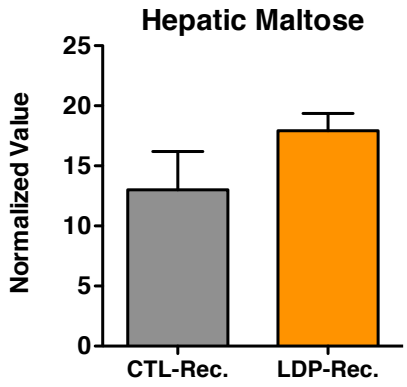


Microbiota-transfer



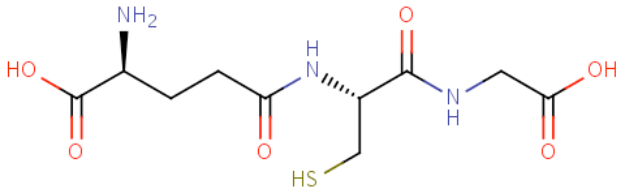




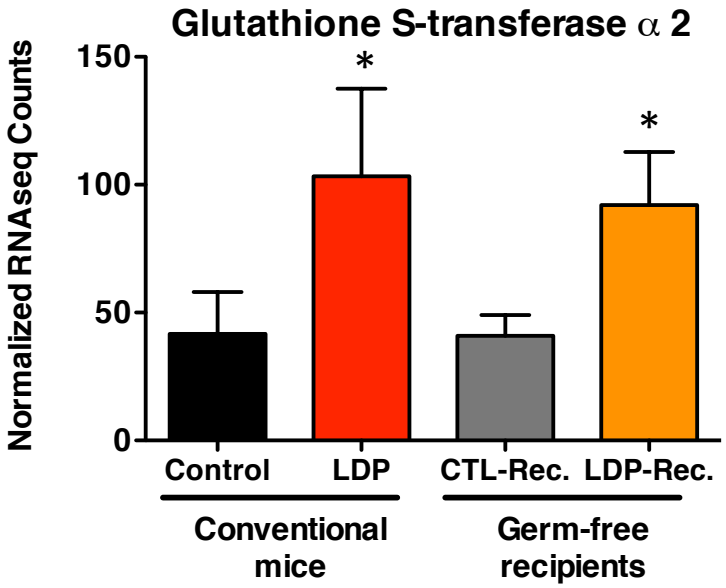


Variable importance projection >1

Hepatic glutathione utilization

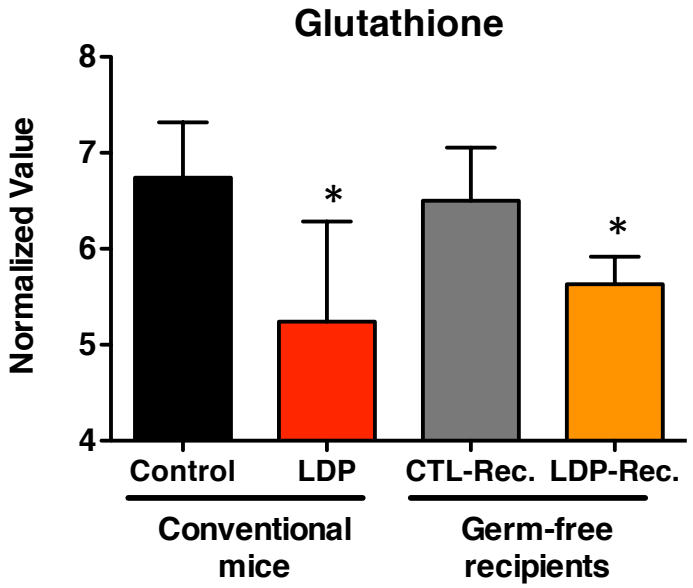


RNAseq



* FDR p < 0.05 by DeSeq

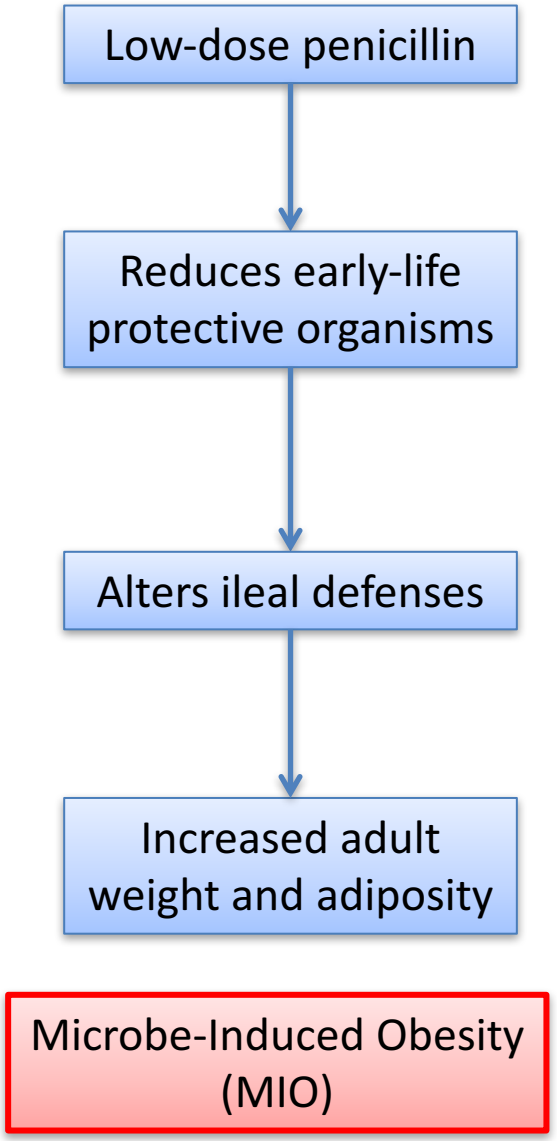
NMR



* Variable Importance Projection >1

Key aspects of a model of microbe-induced obesity

- Infancy is a critical window of host-microbe metabolic interaction.
- Low-dose penicillin adds to high-fat diet metabolic alterations.
- Altered microbiota reduces markers of ileal defense and alters hepatic metabolites.
- The antibiotic-perturbed microbiota plays a causal role.
- There is a consistent reduction in particular bacteria.



Modeling Pediatric Antibiotic Exposures

ARTICLE

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Metabolic and metagenomic outcomes from early-life pulsed antibiotic treatment

Yael R. Nobel[1,*], Laura M. Cox[1,2,*], Francis F. Kirigin[1], Nicholas A. Bokulich[1], Shingo Yamanishi[1], Isabel Teitler[1], Jennifer Chung[1], Jiho Sohn[1], Cecily M. Barber[1], David S. Goldfarb[1,3], Kartik Raju[1], Sahar Abubucker[4], Yanjiao Zhou[4,5,9], Victoria E. Ruiz[1], Huilin Li[6], Makedonka Mitreva[4,7], Alexander V. Alekseyenko[1,8], George M. Weinstock[4,9], Erica Sodergren[4,9] & Martin J. Blaser[1,2,3]

Generate hypothesis → Examine interactions → Test causality → Translate findings

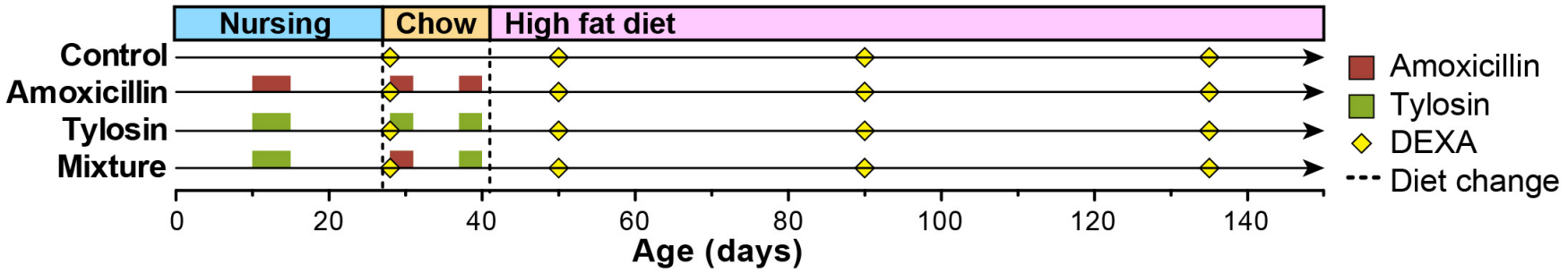
Outpatient antibiotic use, by age, 2010

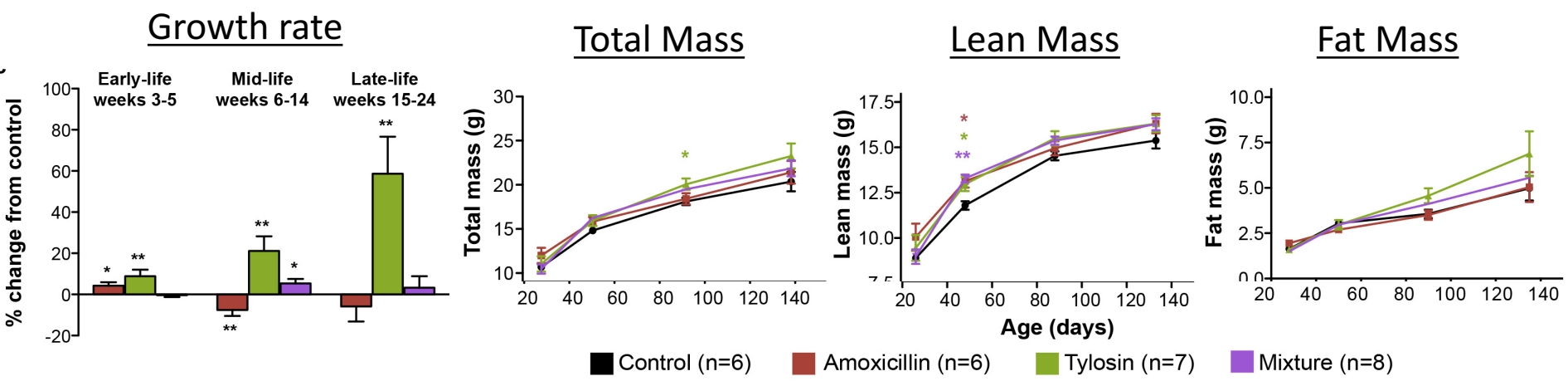
Patient age group (years)	Number of prescriptions (millions)	Prescriptions /1000 people
0 - 1	16.6	1365
2 - 9	29.0	1021
10 - 19	28.9	677
20 - 39	55.4	669
40 - 64	81.6	797
≥ 65	41.1	1020
Total	258.0	833

Most common classes:
Beta-lactam
Macrolide

Source: L Hicks, TH Taylor, RJ Hunkler. NEJM 2013, 368:1461.

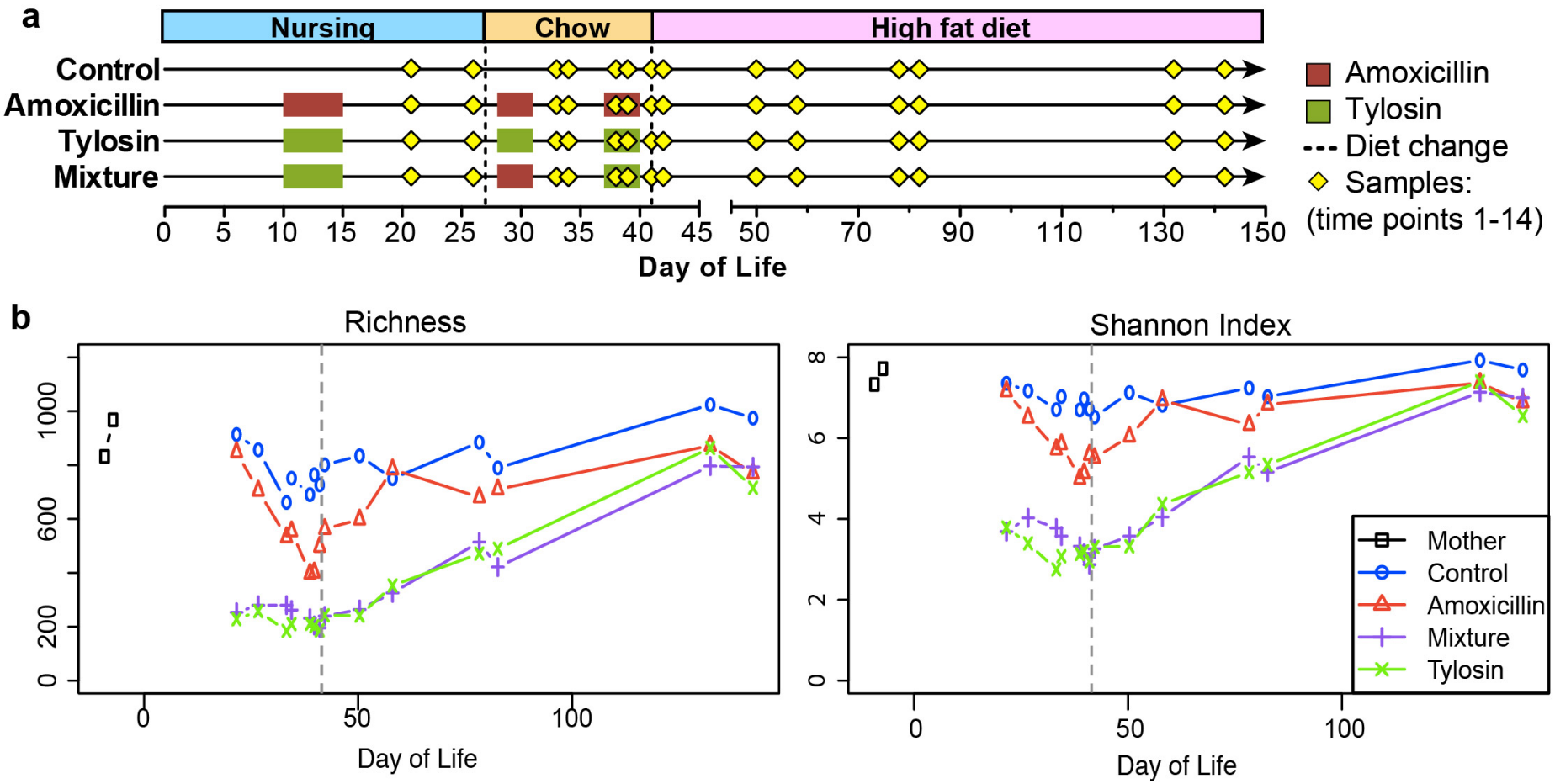
The effect of pulsed-antibiotic therapy (PAT) on body composition





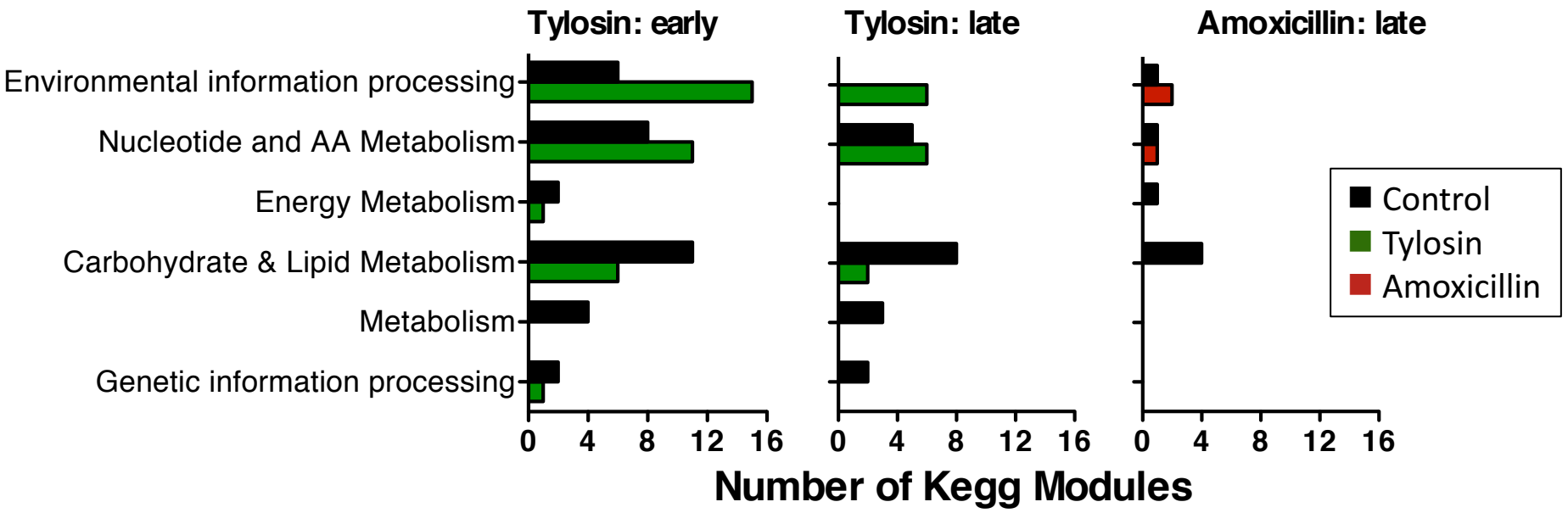
(Nobel et. al, Nature Communications, 2015)

The effect of PAT on alpha diversity



(Nobel et. al, Nature Communications, 2015)

PAT alters the microbial metagenomic KEGG modules



Category/sub-category	KEGG Module	Tylosin Early	Tylosin Late	Amoxicillin late
Central carbohydrate metabolism	Glycolysis (Ebden Meyer pathway)	Down	Down	Down
	Entner-Doudoroff pathway	Up	Up	
	Pyruvate oxidation			Up
LPS synthesis	ADP-L-glycero-D-manno-heptose biosynthesis	Up	Up	Up
Metabolism	Aminoacyl-tRNA synthesis	Down	Down	Down
Environmental processing	Dipeptide transport system	Up	Up	Up

Early-life microbiota and Type 1 Diabetes

nature microbiology

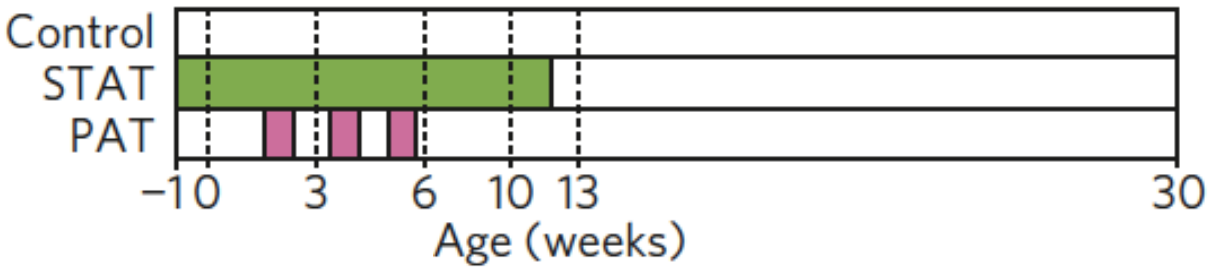
ARTICLES

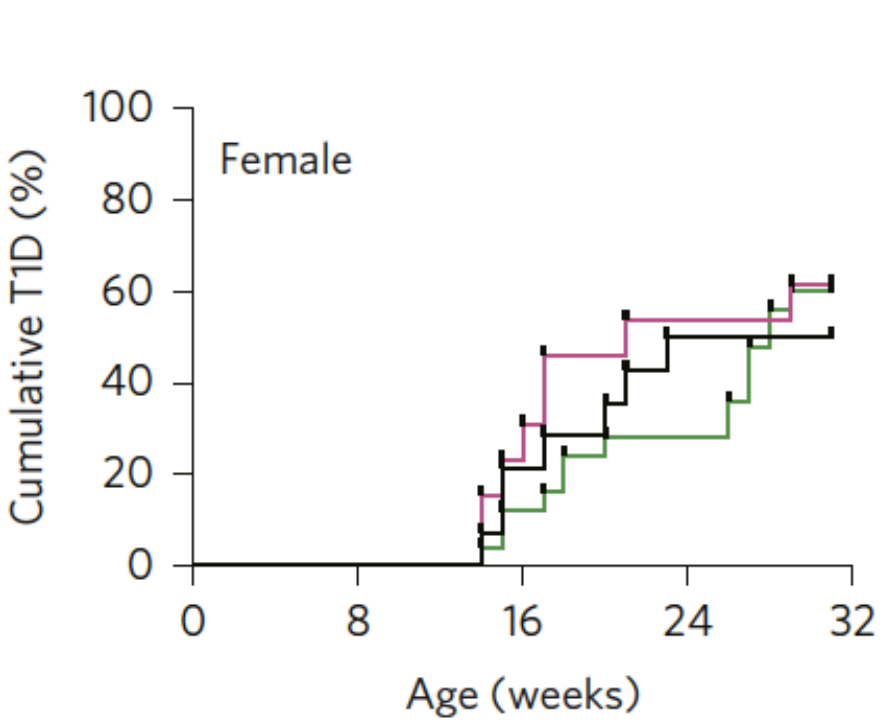
PUBLISHED: 22 AUGUST 2016 | ARTICLE NUMBER: 16140 | DOI: 10.1038/NMICROBIOL.2016.140

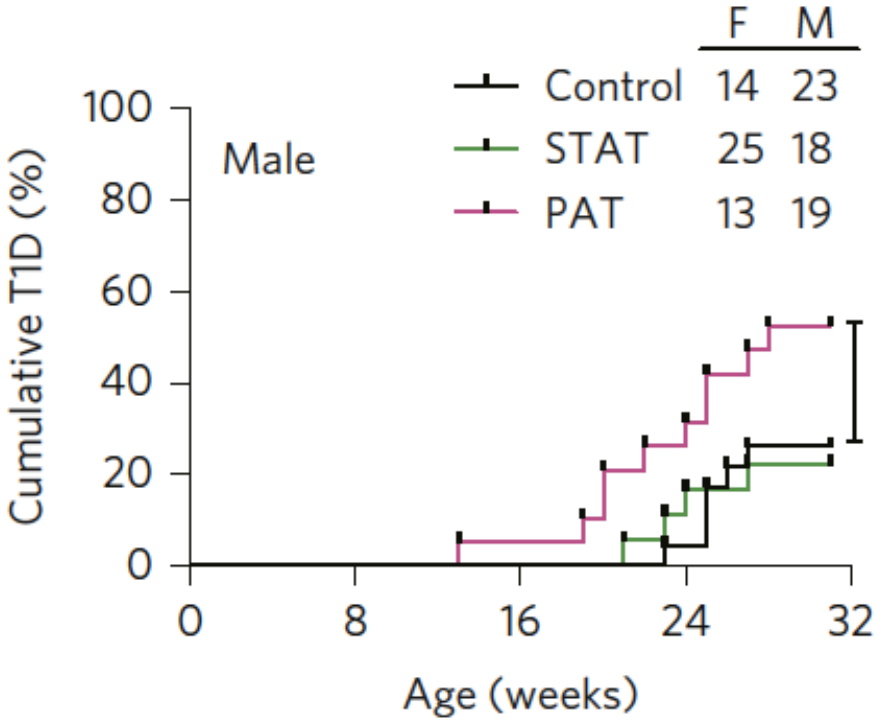
Antibiotic-mediated gut microbiome perturbation accelerates development of type 1 diabetes in mice

Alexandra E. Livanos[1], Thomas U. Greiner[2], Pajau Vangay[3], Wimal Pathmasiri[4], Delisha Stewart[4], Susan McRitchie[4], Huilin Li[5], Jennifer Chung[1], Jiho Sohn[1], Sara Kim[1], Zhan Gao[1], Cecily Barber[1], Joanne Kim[1], Sandy Ng[1], Arlin B. Rogers[6], Susan Sumner[4], Xue-Song Zhang[1], Ken Cadwell[7,8], Dan Knights[9,10], Alexander Alekseyenko[1,11], Fredrik Bäckhed[2,12] and Martin J. Blaser[1,13]*

PAT alters diabetes incidence in NOD mice



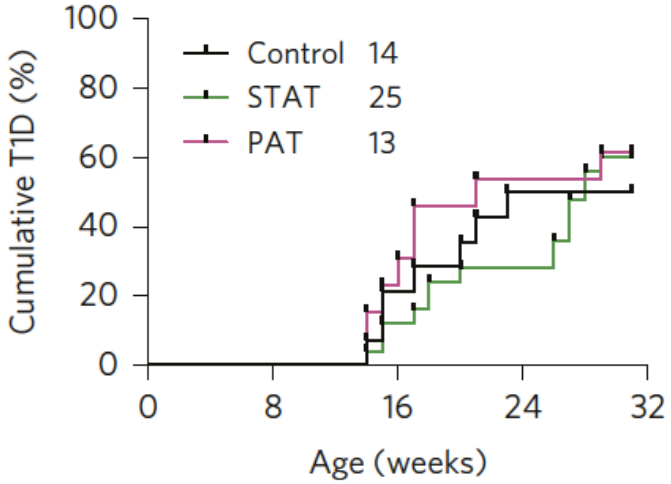




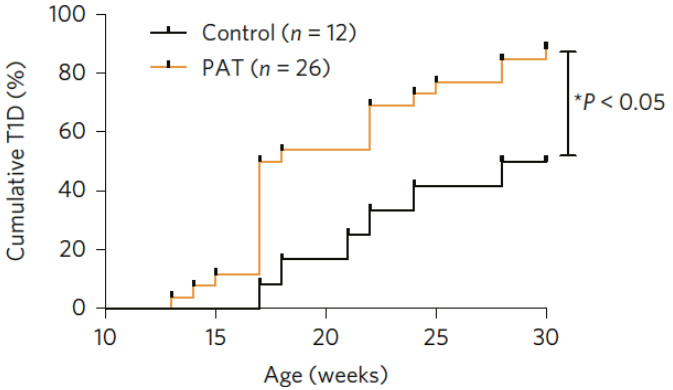
Livanos, Nature Microbiology, 2016

Environment alters antibiotic response in female NOD mice

Animal Facility 1

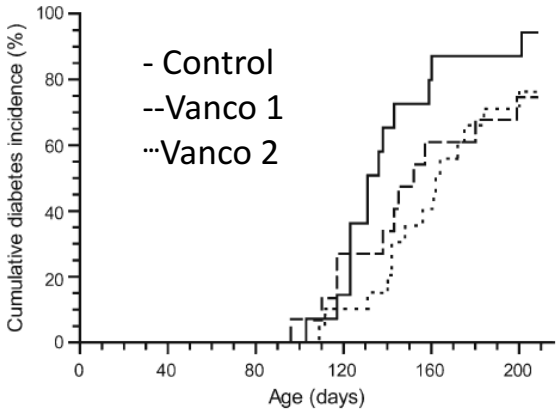


Animal Facility 2



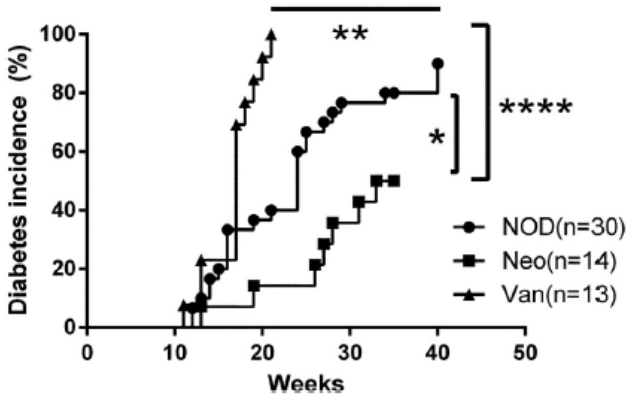
Livanos, Nature Microbiol, 2016

Animal Facility 3



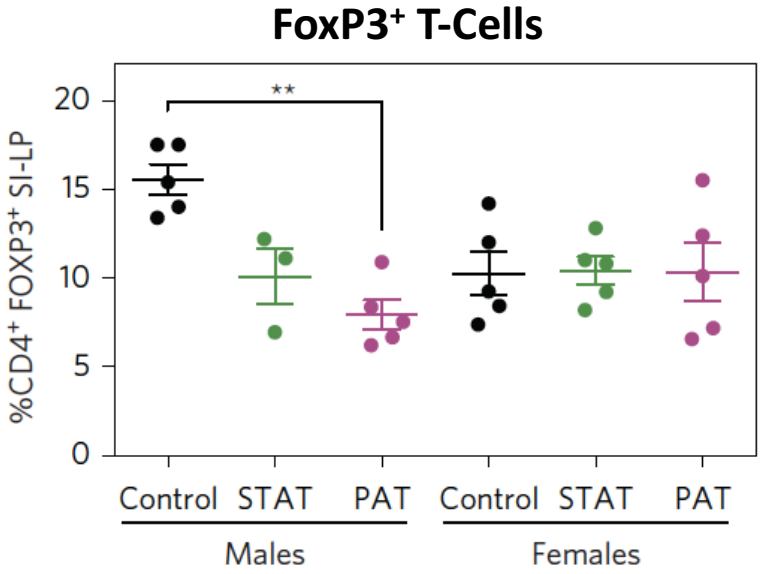
Hansen, Diabetologia, 2012

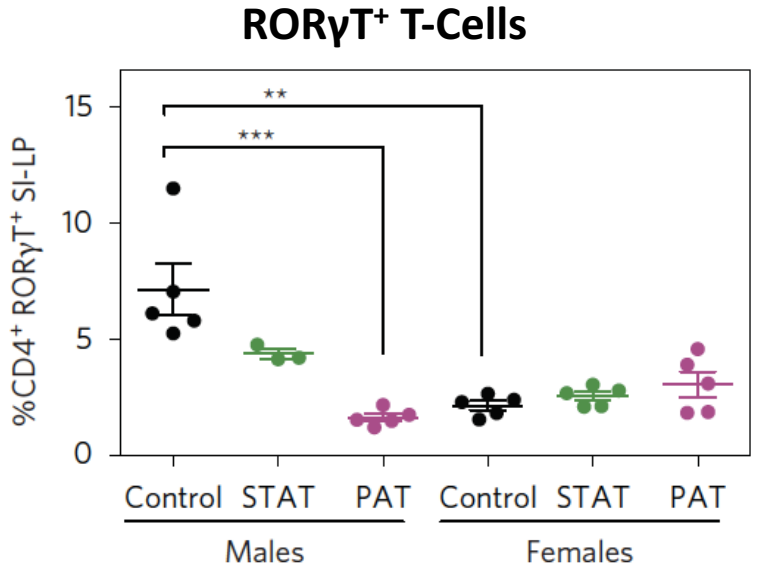
Animal Facility 4



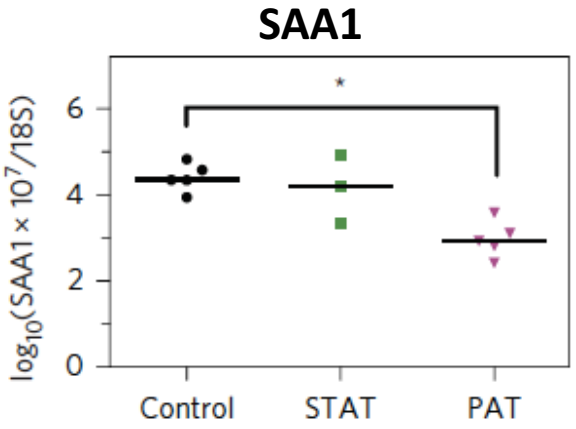
Hu, J Autoimmunity, 2016

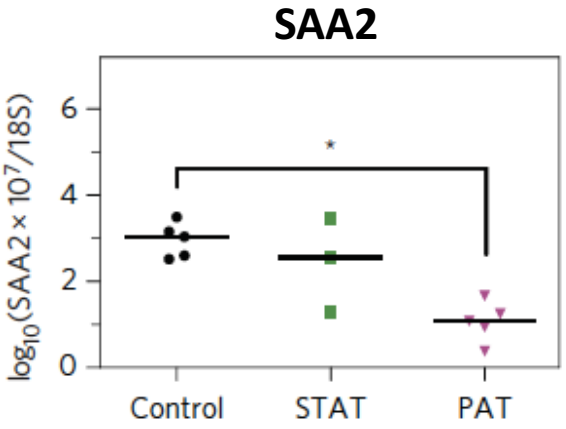
The effect of PAT on intestinal immunity





RNAseq top hit:
Serum amyloid-alpha





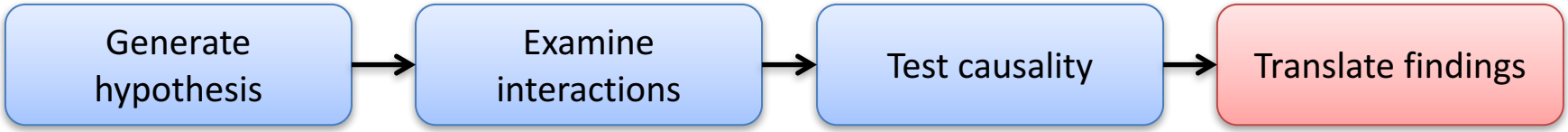
Livanos, Nature Microbiology, 2016

Key aspects of pulsed antibiotic treatment (PAT)

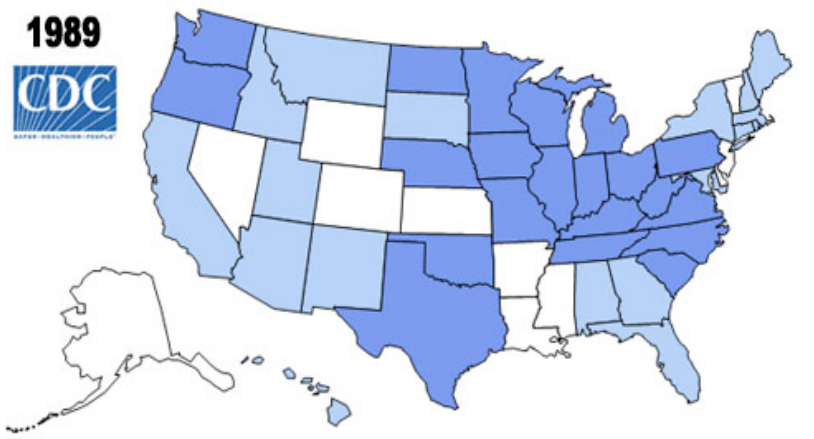
- Both macrolide and beta-lactam exposure can increase growth rate and lean mass, but to a lesser extent than sub-therapeutic antibiotic treatment (STAT)
- PAT produces lasting changes in microbial diversity that do not recover 100 days after antibiotic cessation.
- The macrolide antibiotic more severely impacts microbiota diversity than the beta-lactam.
- PAT, but not STAT, increases diabetes incidence in NOD mice, with variable effects in different facilities
- PAT decreases markers of intestinal immunity

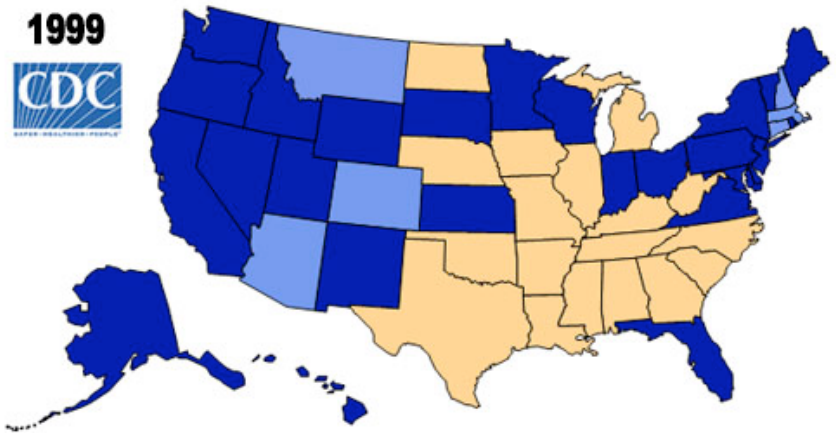
Translation to humans:

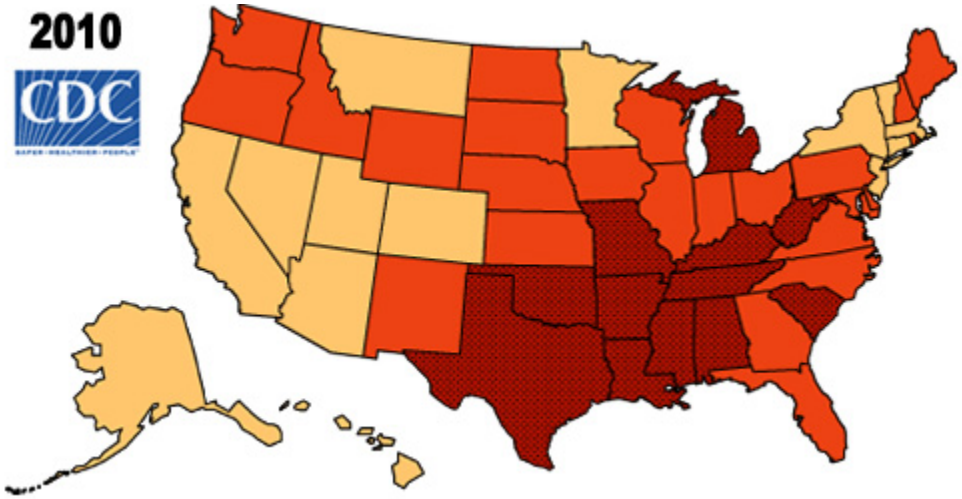
Population studies

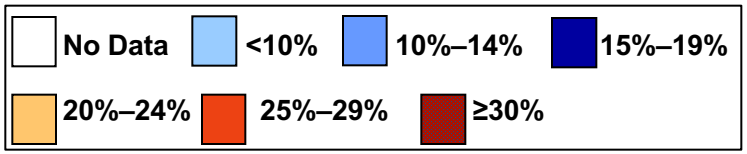


Obesity trends in US adults: changing physiology



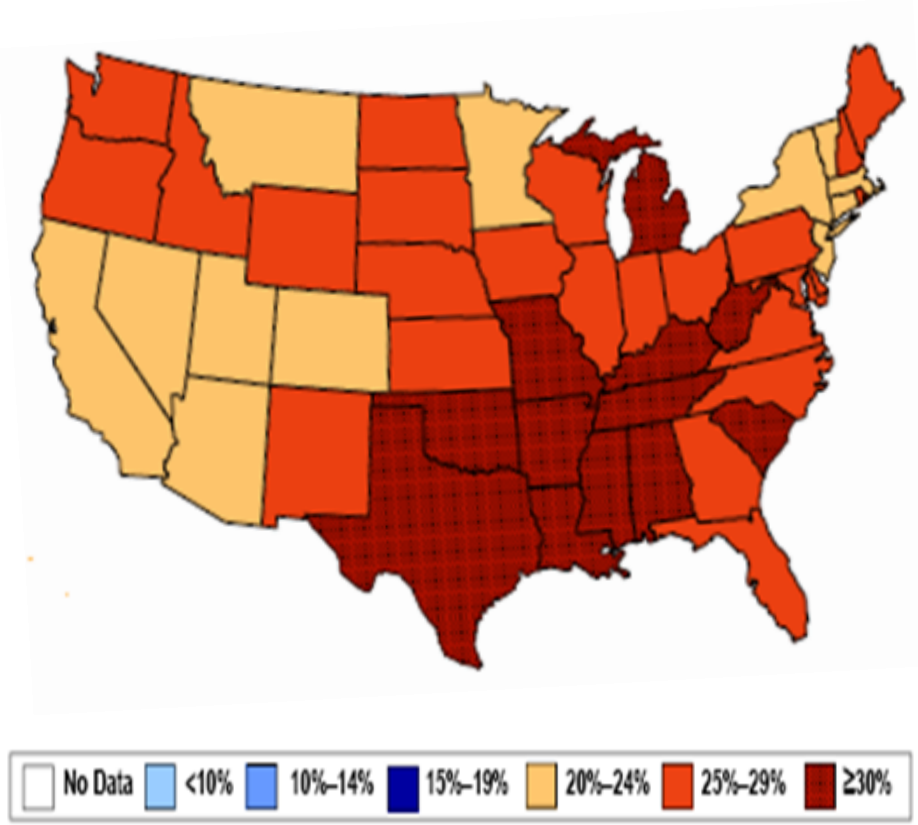


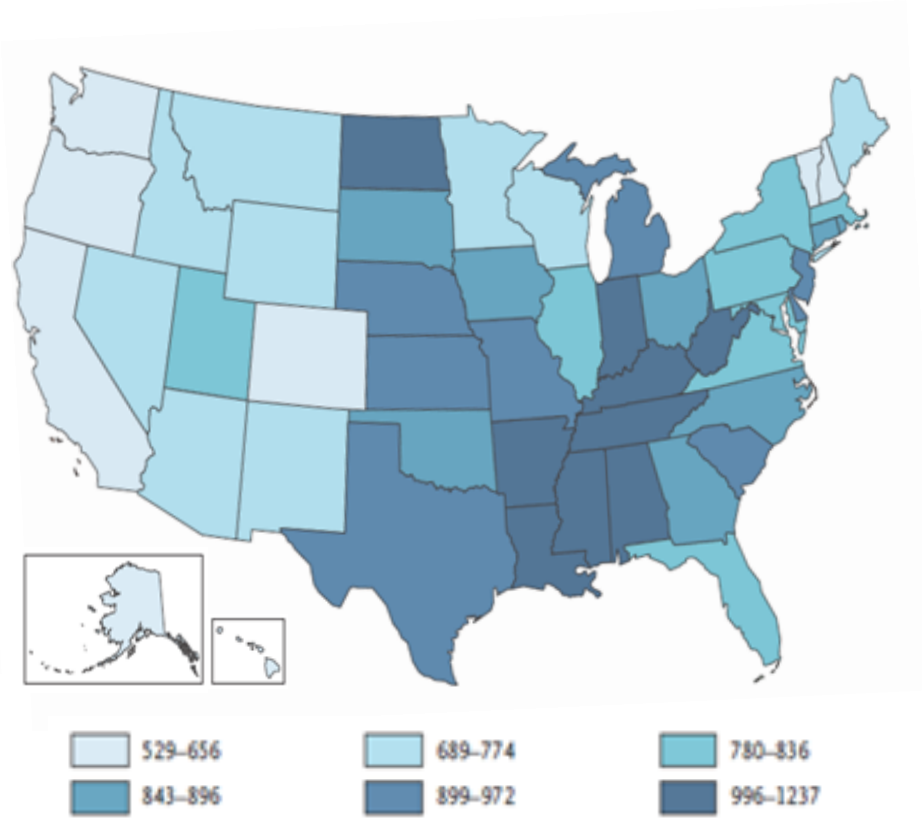




Source: CDC Behavioral Risk Factor Surveillance System.

Comparisons between the geography of obesity and antibiotic use, 2010





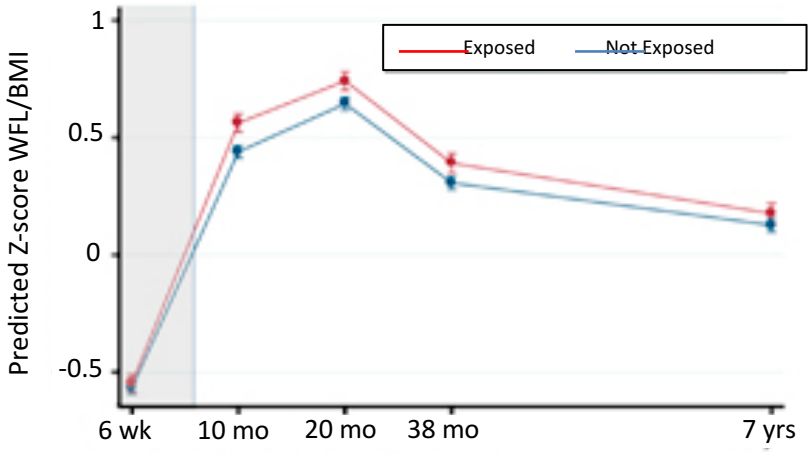
Antibiotic prescriptions per 1000 persons, 2010

Source: L Hicks, TH Taylor, RJ Hunkler. NEJM 2013, 368:1461.

Early life microbiome disruption is associated with weight gain in humans

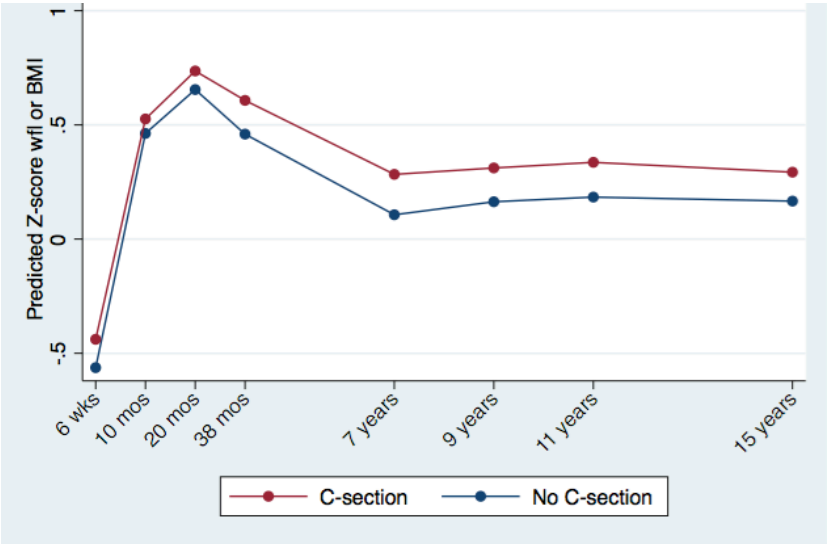
Avon Longitudinal Study of Parents and Children: > 10,000 Children

Antibiotic exposure in first 6 months



Trasande et al., International Journal of Obesity, 2012

Delivery by Cesarean-section

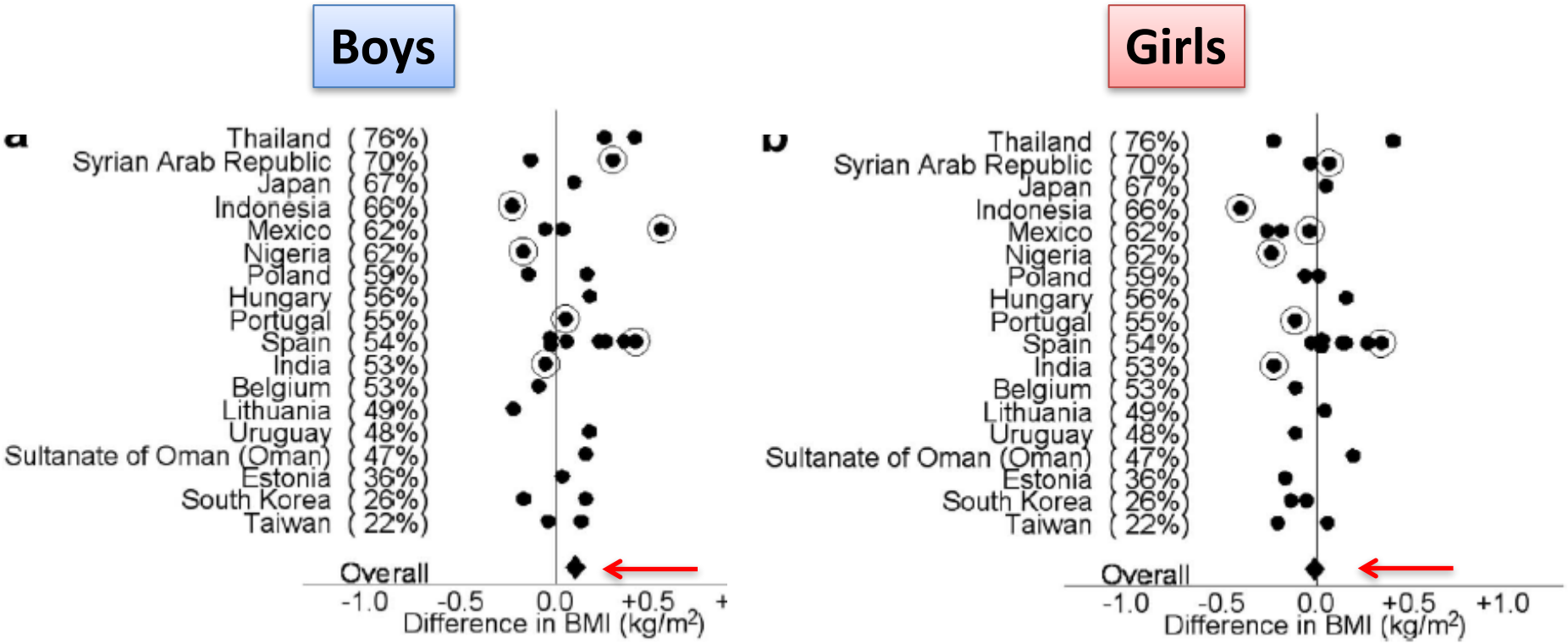


Blustein et al., International Journal of Obesity, 2012

Early-life antibiotics and weight

Study	# Subjects	Association	Reference
Danish National Birth Cohort	>28,000	Antibiotics in the first 6 months increased overweight risk at 7 years	Ajslev, Int J Obes, 2011
Avon Longitudinal Study of Parents and Children	>10,000	Antibiotics in the first 6 months associated with higher weight at 38 months	Trasande, Int J Obes, 2012
		Delivery by C-section increased risk of over weight	Blustein, Int J Obes, 2013
Canadian longitudinal birth cohort	>400	Antibiotics increased risk of overweight at 12 years in boys	Azad, Int J Obes, 2014
Children's Hospital of Philadelphia	>64,000	Early broad-spectrum antibiotics associated with obesity	Bailey, JAMA Pediatr, 2014
International cross-sectional study	>74,000	Early-antibiotics associated with higher weight in boys	Murphy, Int J Obes, 2013

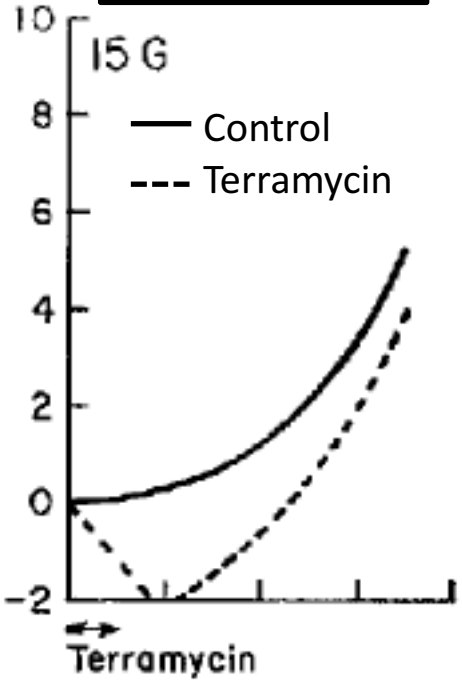
Antibiotic treatment during infancy and increased body mass index in boys: an international cross-sectional study



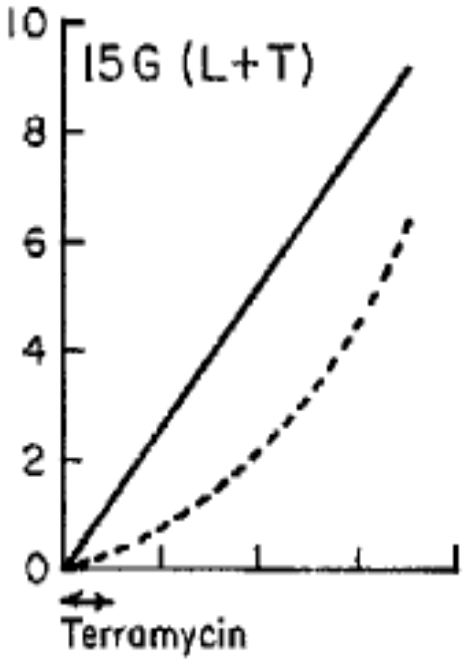
R Murphy, et. al, International Journal of Obesity, 2013

Early studies on antibiotics and weight

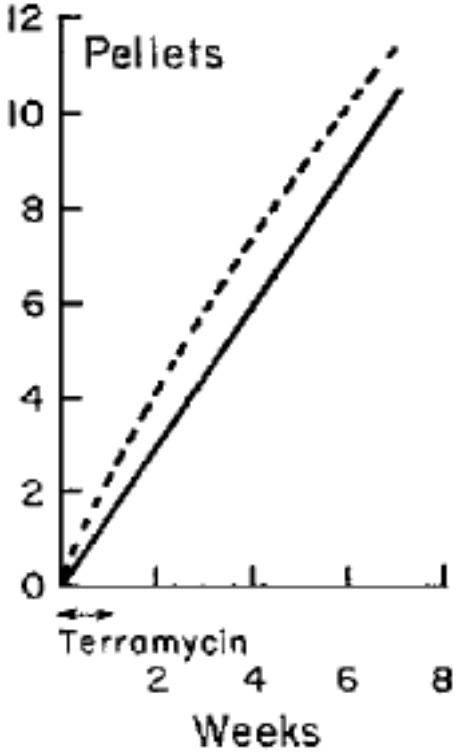
Gluten sole protein source



Gluten + lysine and threonine



Complex protein mixture

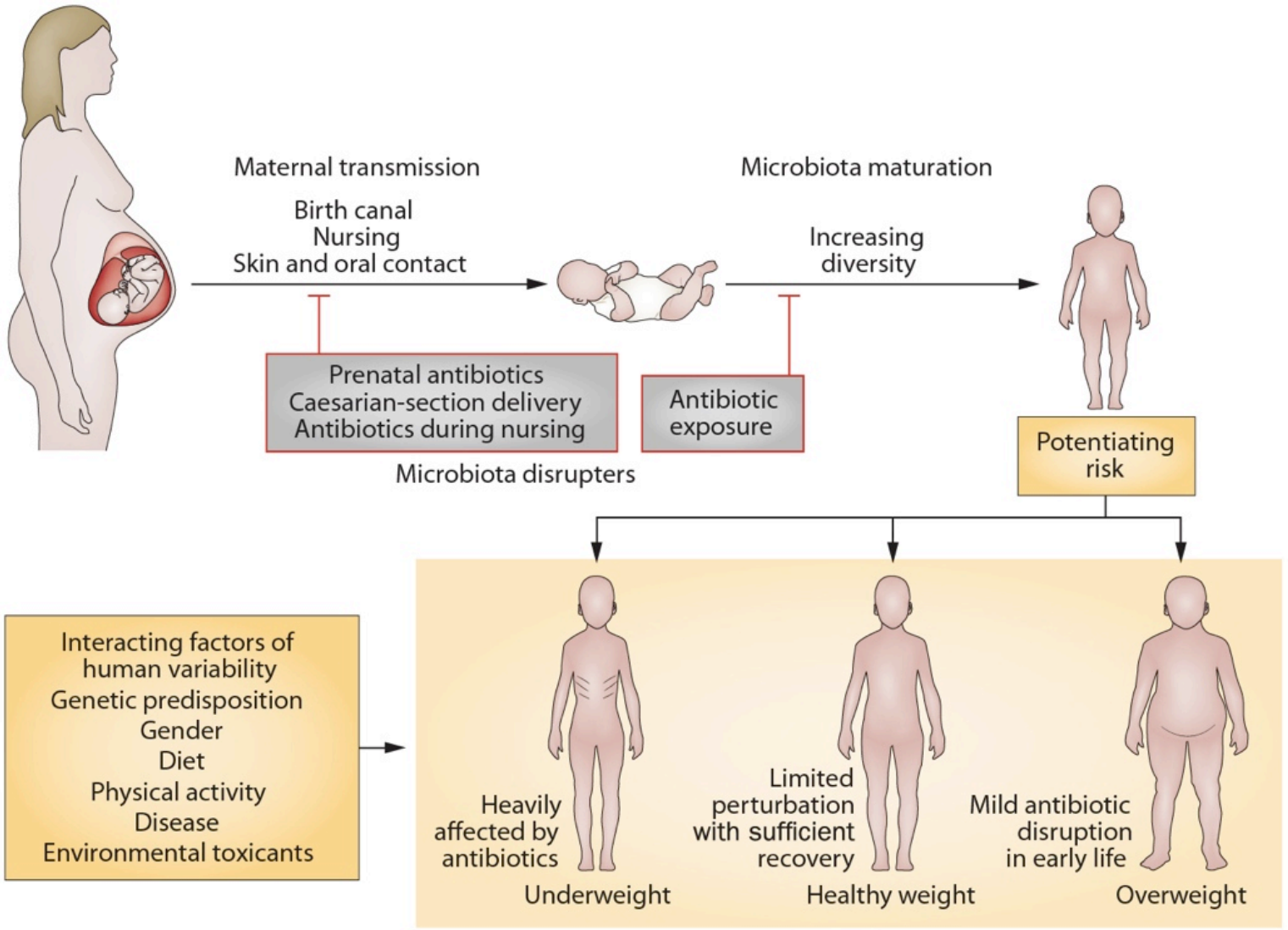


<u>Weight gain or loss dependent on</u>:

- Antibiotic dose
- Mouse strain
- Mouse diet

Dubos et al., J Exp Med, 1963

Antibiotics in early life and obesity



Cox and Blaser, Nature Reviews Endocrinology, 2014

Early-life microbiota and diabetes

Study Population	# Subjects	Association	Reference
Meta-analysis of 20 studies	9,938 cases	20% increased risk of T1D in children delivered by C-section	Cardwell, Diabetologia 2008
Denmark case-control	1,578 cases	Increased risk of T1D with 5 courses of antibiotics, especially linked to broad spectrum antibiotics	Mikkelsen, Diabet Med, 2016
Denmark	5.6 million subjects	Increased risk of T2D with past antibiotic exposure, greater association with multiple courses	Mikkelsen, J Endocrinol Metab 2015
Danish Nationwide cohort	858,201 children, 1503 cases	Broad-spectrum antibiotics in the first two years of life increased the risk of T1D in children delivered by C-section (suggests a double-hit increases risk)	Clausen, PlosOne 2016

Translational challenges and opportunities:

Prevention

- Focus on antibiotic stewardship:
  - Immune system is under developed, thus infants are more susceptible to serious bacterial infections, but early-life microbiota disruption can have lasting effects
- Encourage the use of narrow spectrum antibiotics

Diagnosis

- Develop diagnostic tools to screen the microbiome to predict patients at risk for developing a microbiome related disease.
- Identify bacteria in the human population that can shape metabolism.

Treatment

- Timing is critical for post-antibiotic restoration strategies
- Options: prebiotics, probiotics, microbiota transfer, antibiotics
- Create evidence-based clinical guidelines and evaluate microbiome therapeutic efficacy
- Weigh the severity of infection/dysbiosis with the risk of the therapeutic intervention

Partial restoration of the microbiota of Cesarean-born infants via vaginal microbiota transfer

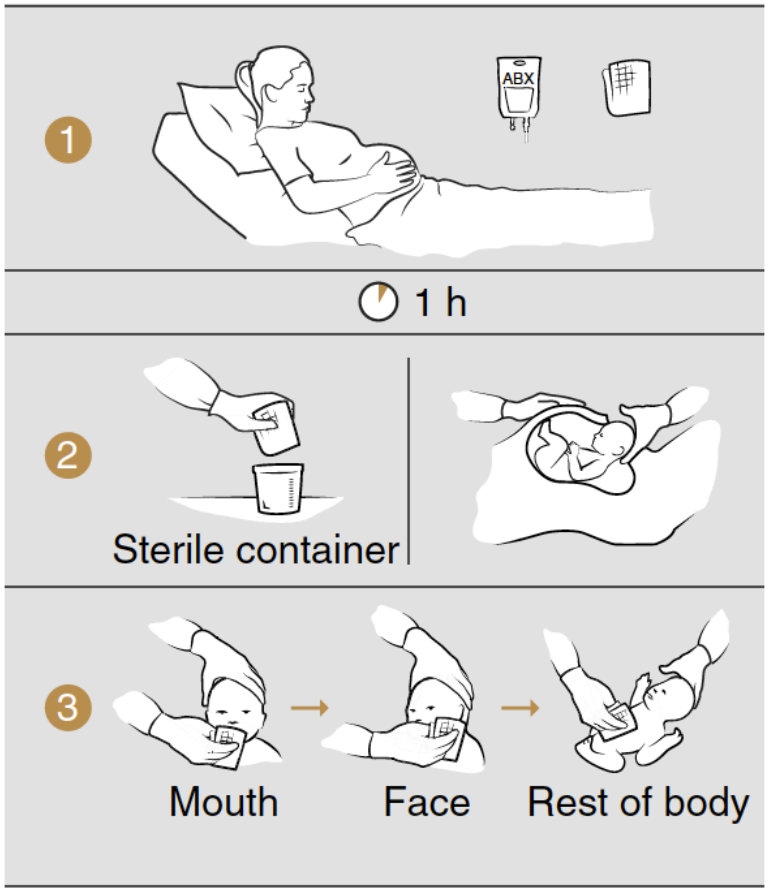
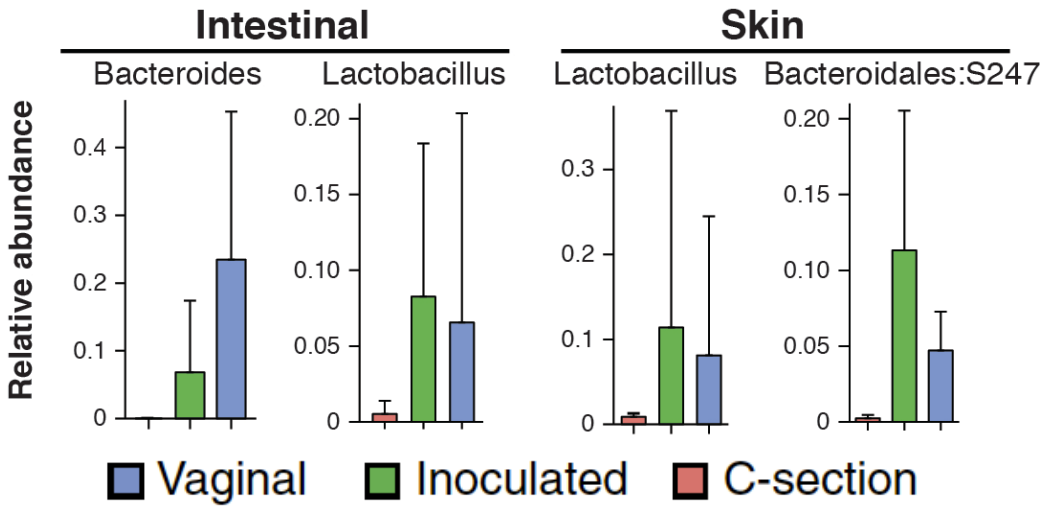


Image: M.J. Schoen



Important Considerations:

- Screen mothers for known neonatal pathogens
- Long-term effects are unknown

Dominguez-Bello, Nature Medicine, 2016

Acknowledgements:

Martin Blaser

Ilseung Cho
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Zhan Gao

Phenotype Characterization
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Sungheon Kim
Arlin Rogers

Bioinformatics
Alex Alekseyenko

Sequencing Library Prep
Sarah Owens
Jacqueline Leung
P'ng Loke

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Nicholas Robine
Soren Germer

Genome Technology Center
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Elisa Venturini

Metagenomics
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Erica Sodergren

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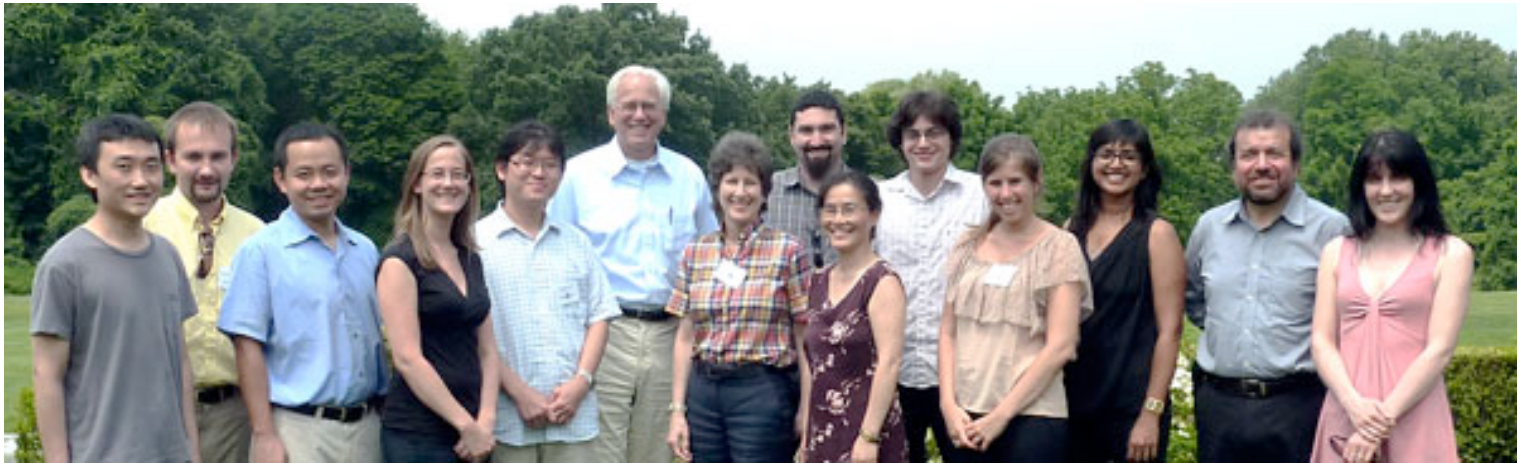
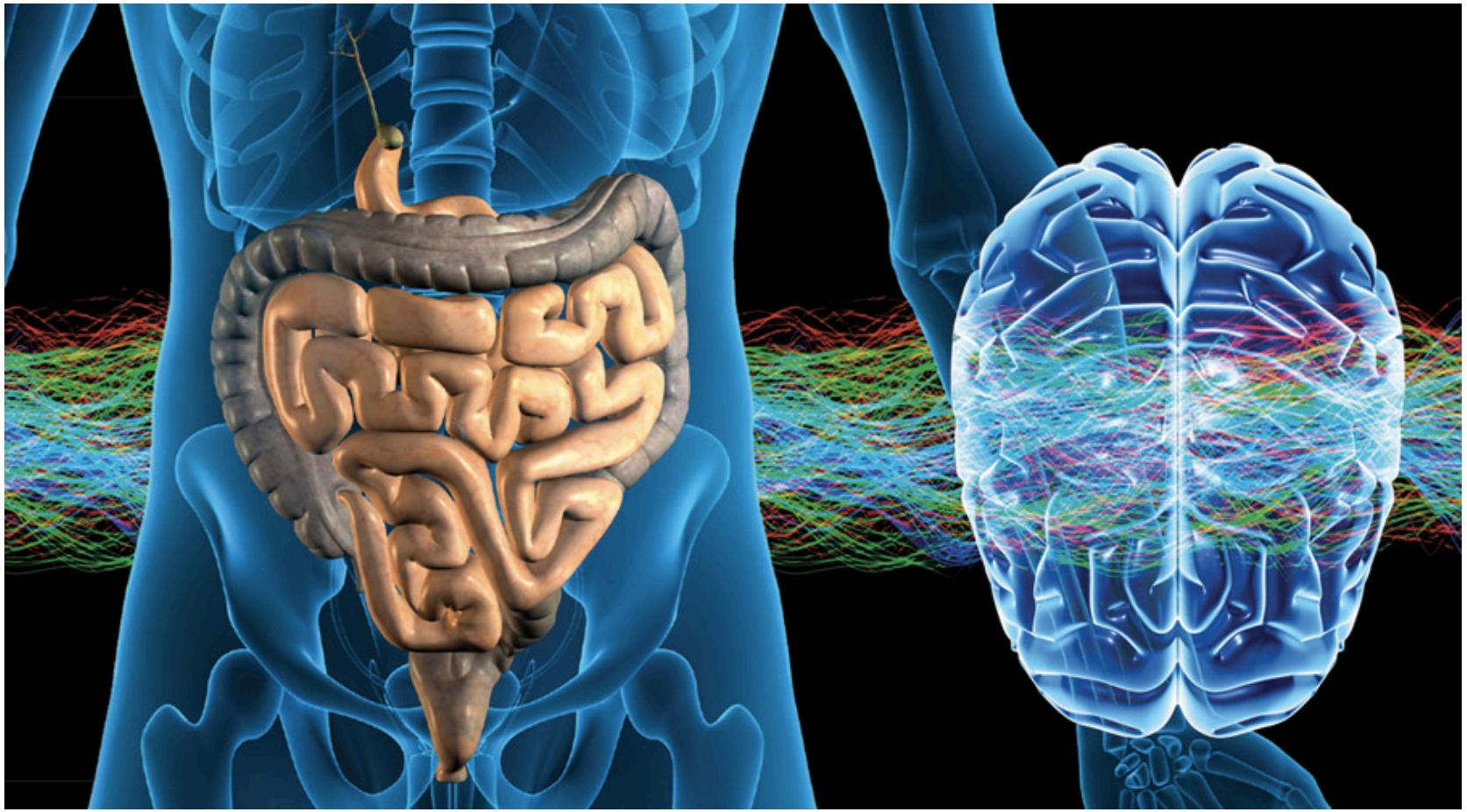
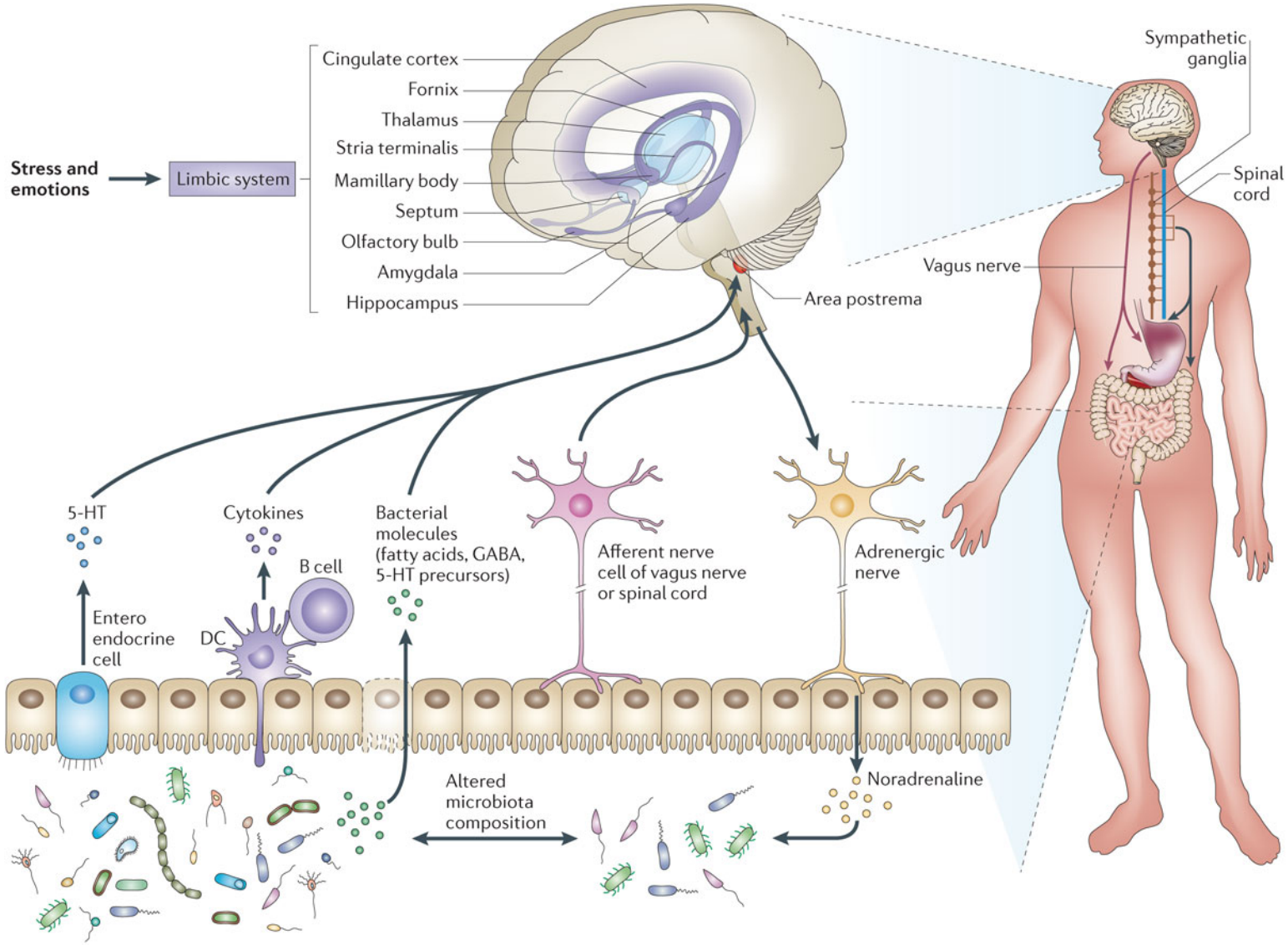




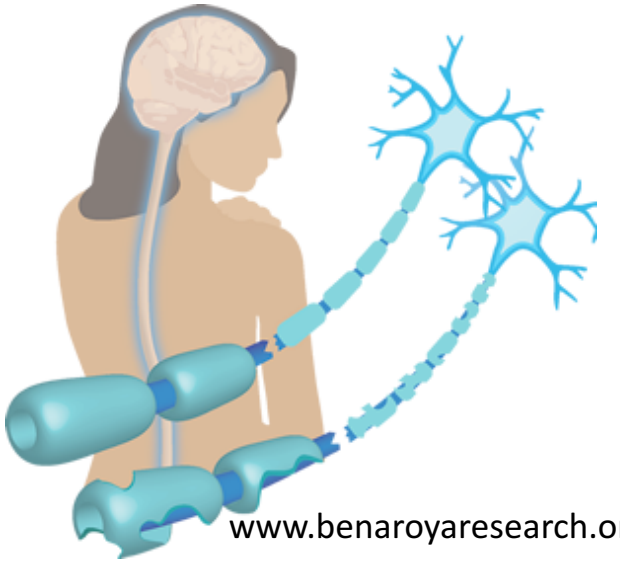
Microbiota-Gut Brain Interactions



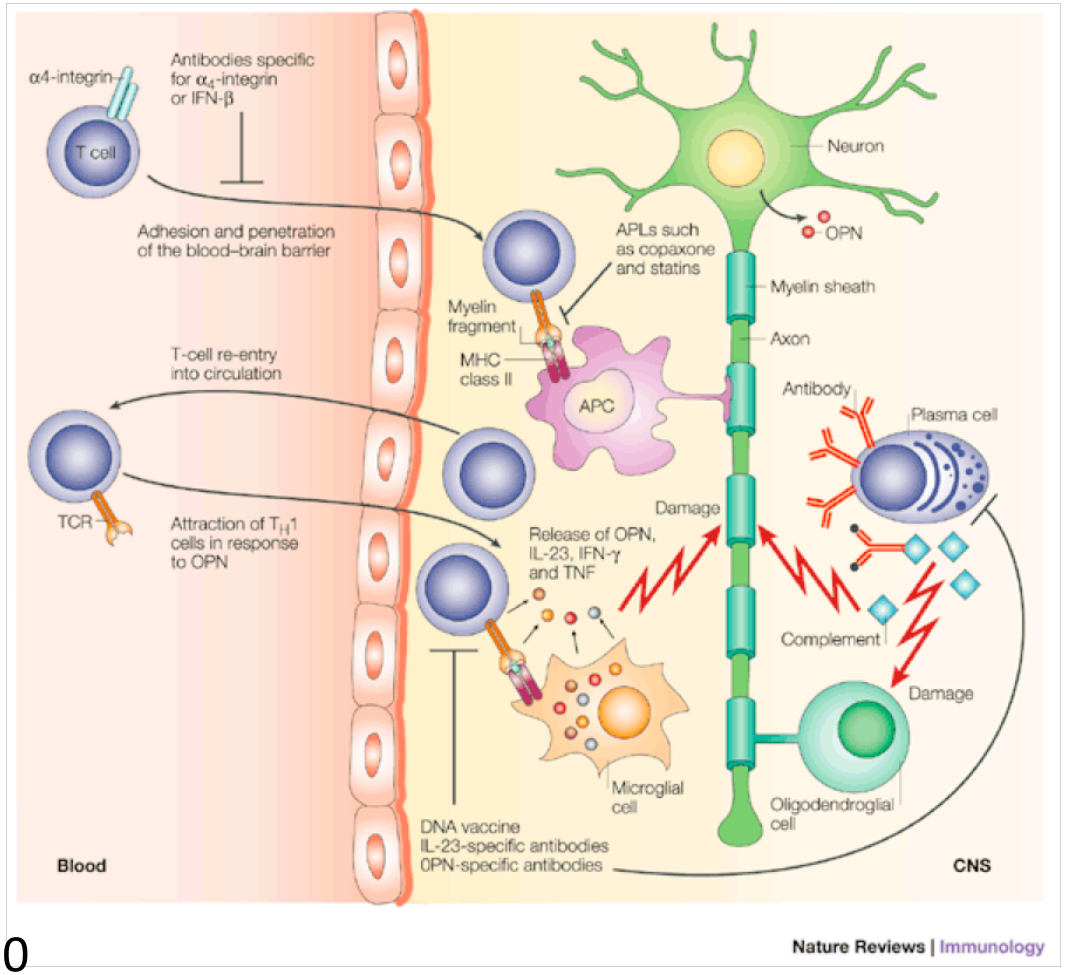
Collins et al., Nat Rev Microbiol, 2012

Multiple Sclerosis

An immune-mediated demyelinating disease of the central nervous system



www.benaroyaresearch.org/



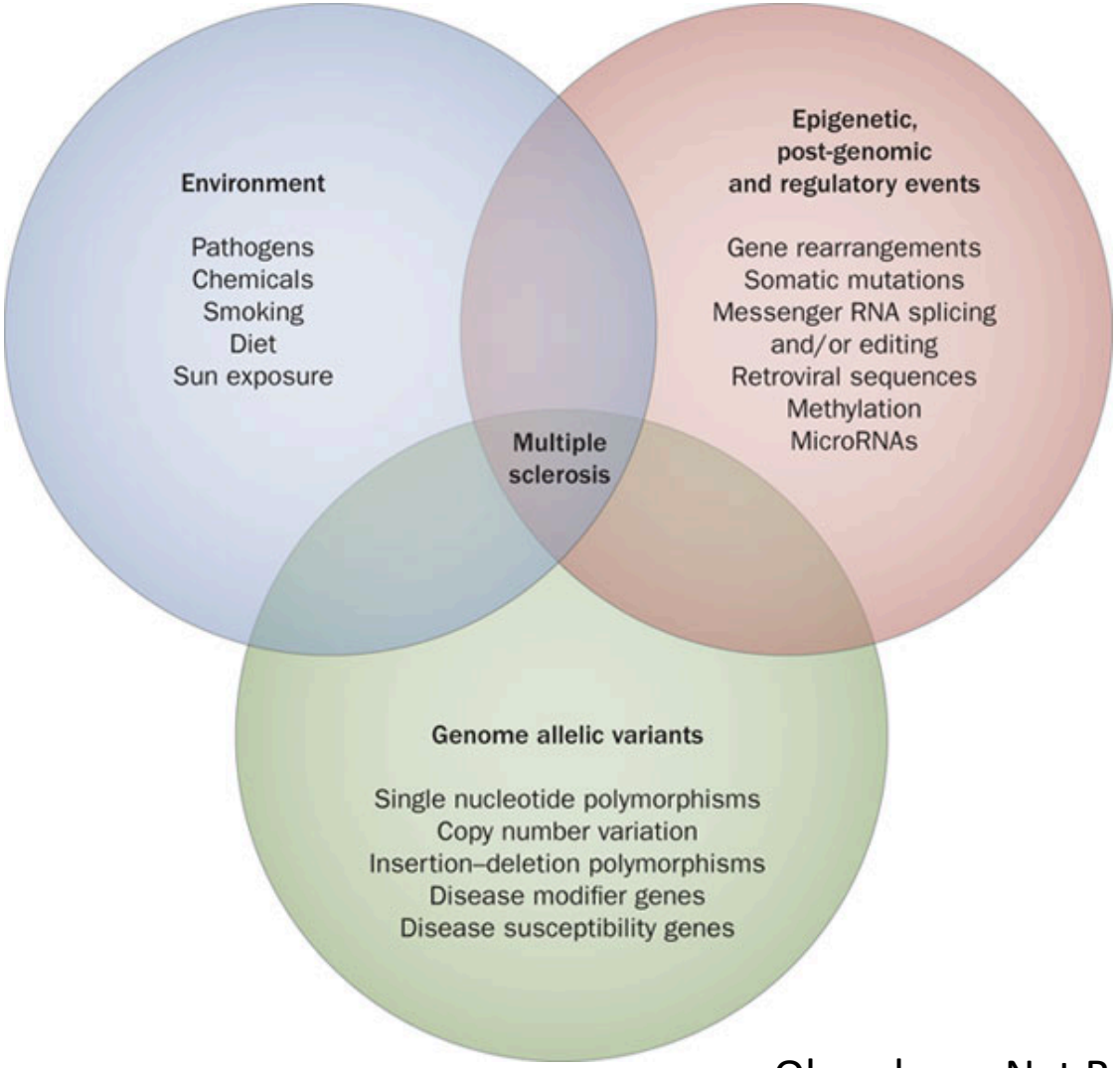
Pathogenesis

- Th1 → IFN-ϒ
- Th17 → IL-17

Recovery

- Foxp3⁺ Treg → IL-10
- LAP⁺ Treg → TGF-β

Environmental, Genetic, and Epigenetic Risk Factors for Multiple Sclerosis

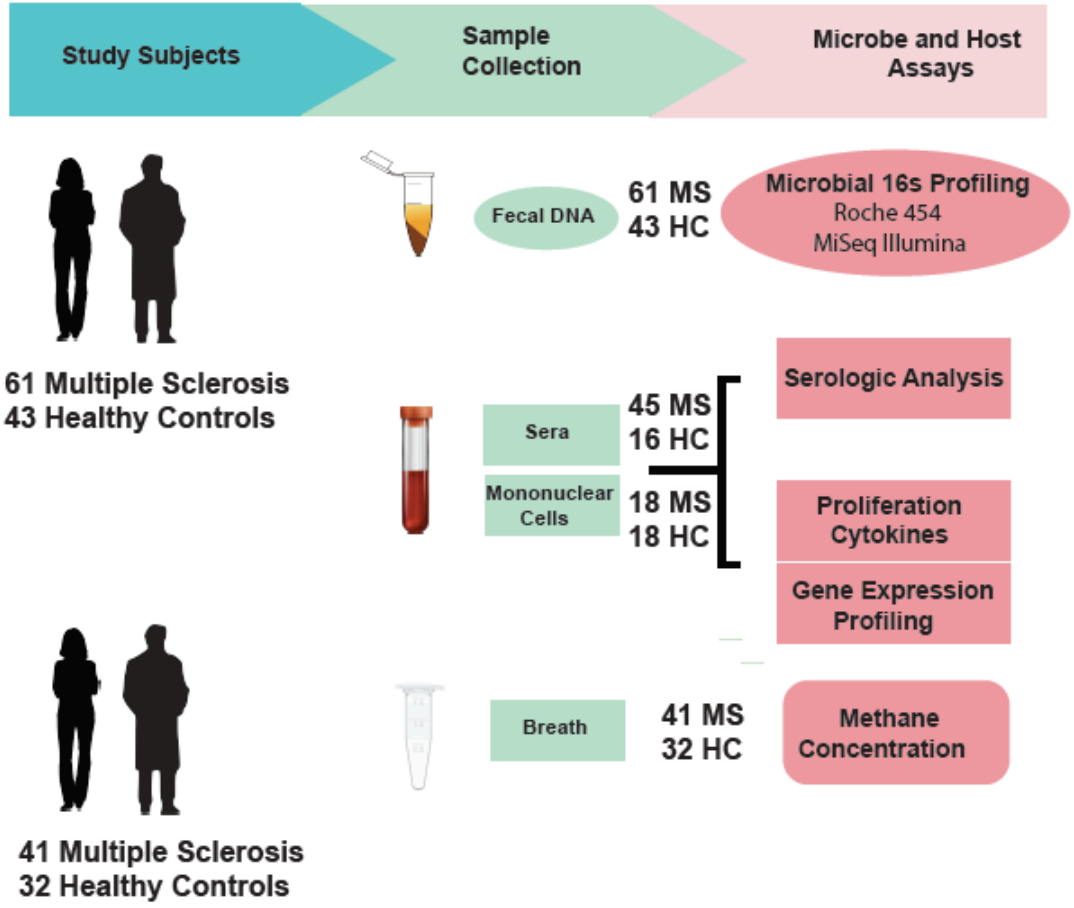


Oksenberg, Nat Rev Neurology, 2010

nature COMMUNICATIONS

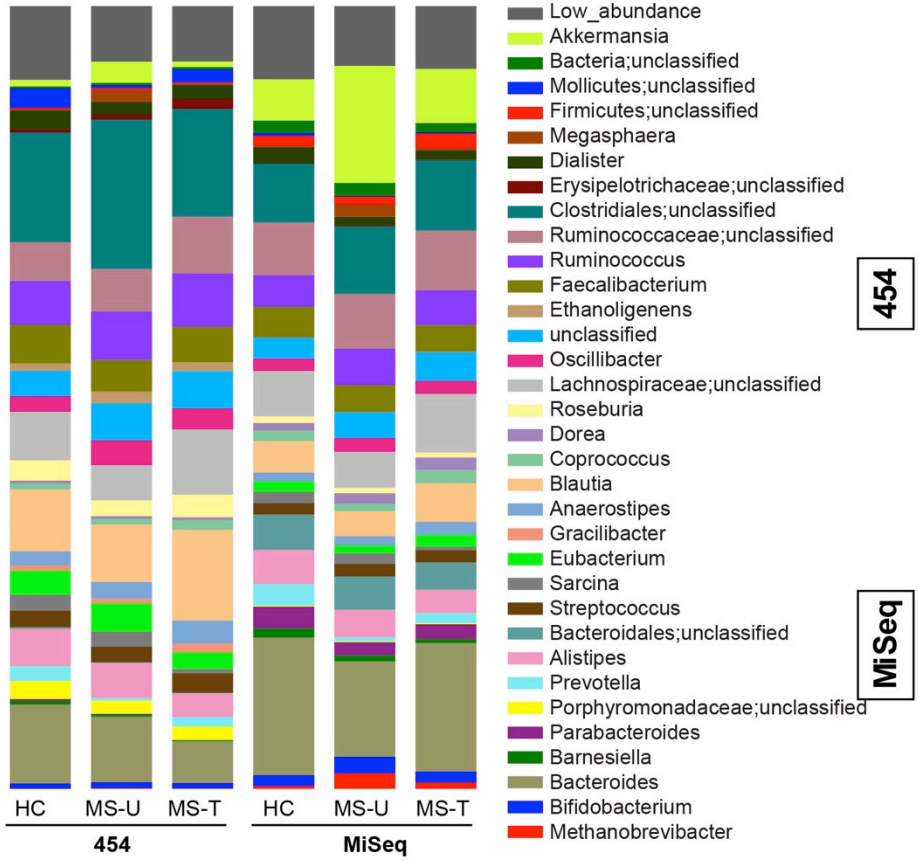
Alterations of the human gut microbiome in multiple sclerosis

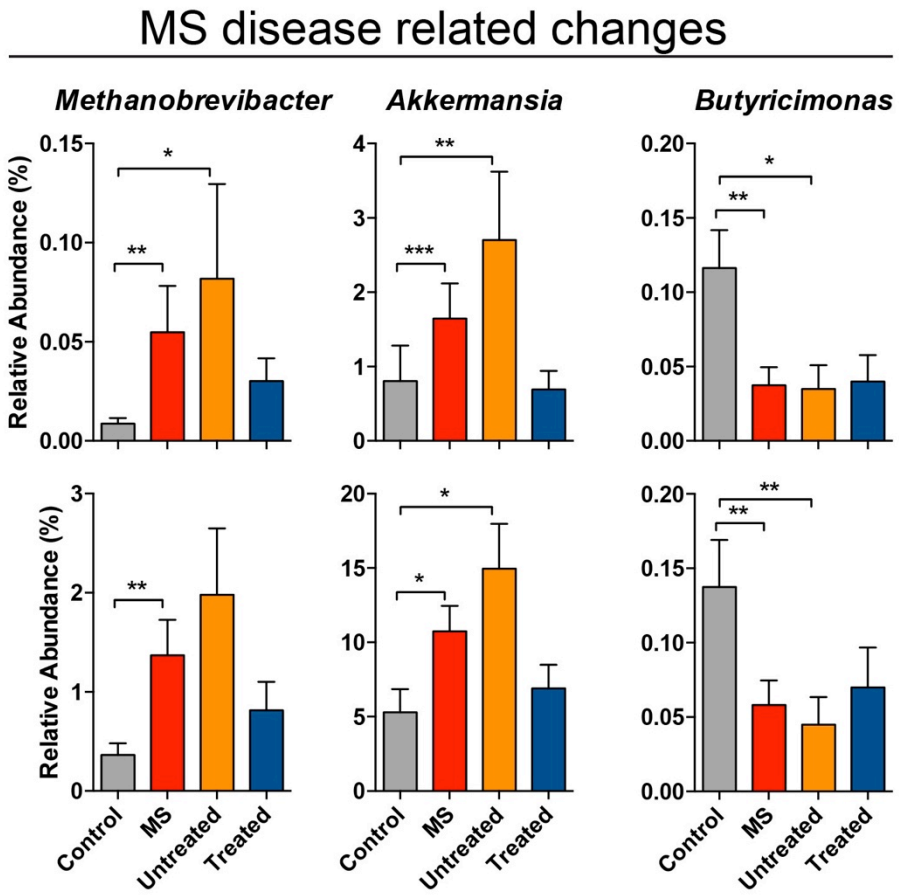
Sushrut Jangi, Roopali Gandhi, Laura M. Cox, Ning Li, Felipe von Glehn, Raymond Yan, Bonny Patel, Maria Antonietta Mazzola, Shirong Liu, Bonnie L. Glanz, Sandra Cook, Stephanie Tankou, Fiona Stuart, Kirsy Melo, Parham Nejad, Kathleen Smith, Begüm D. Topçuolu, James Holden, Pia Kivisäkk, Tanuja Chitnis, Philip L. De Jager, Francisco J. Quintana, Georg K. Gerber, Lynn Bry & Howard L. Weiner



(S. Jangi et. al., Nat. Comm, 2016)

Genus level changes

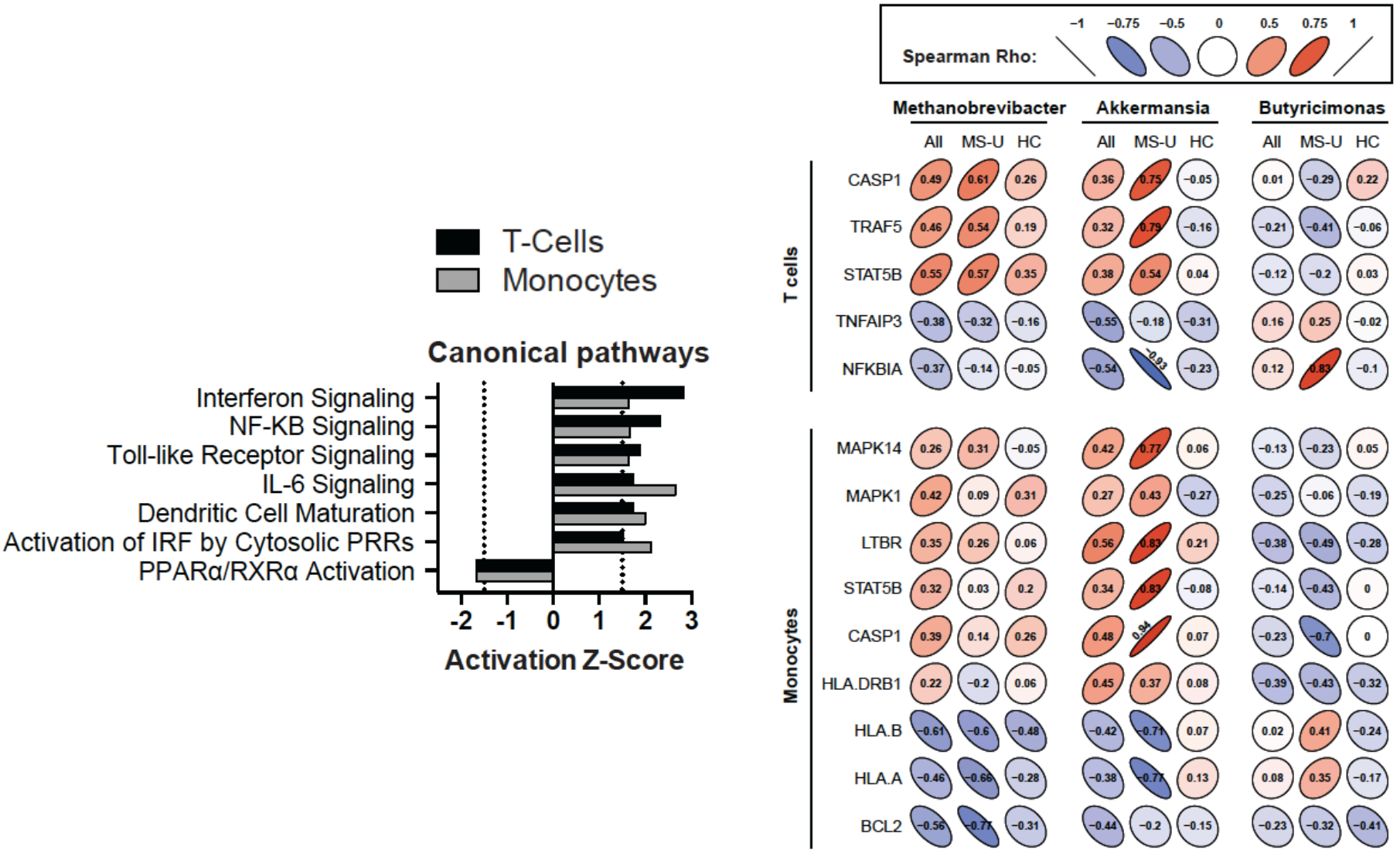




(S. Jangi et. al., Nat. Comm, 2016)

P < 0.05, ** p < 0.01, *** p < 0.001,
BH-adjusted DESeq

Microbiome-Immune Associations

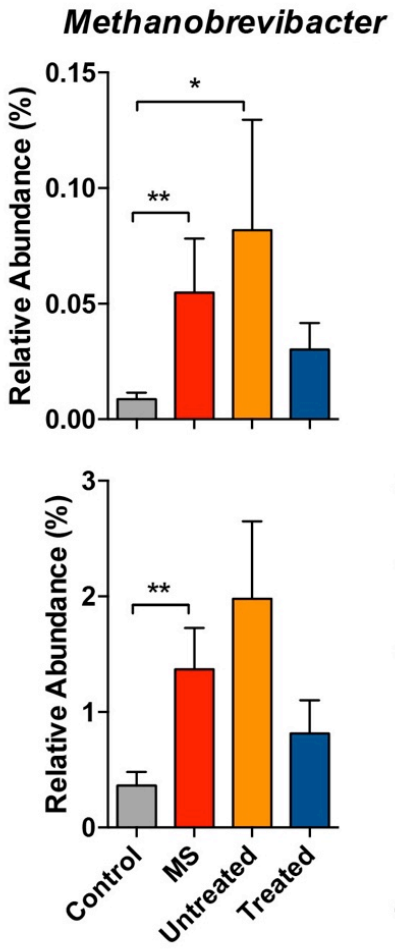


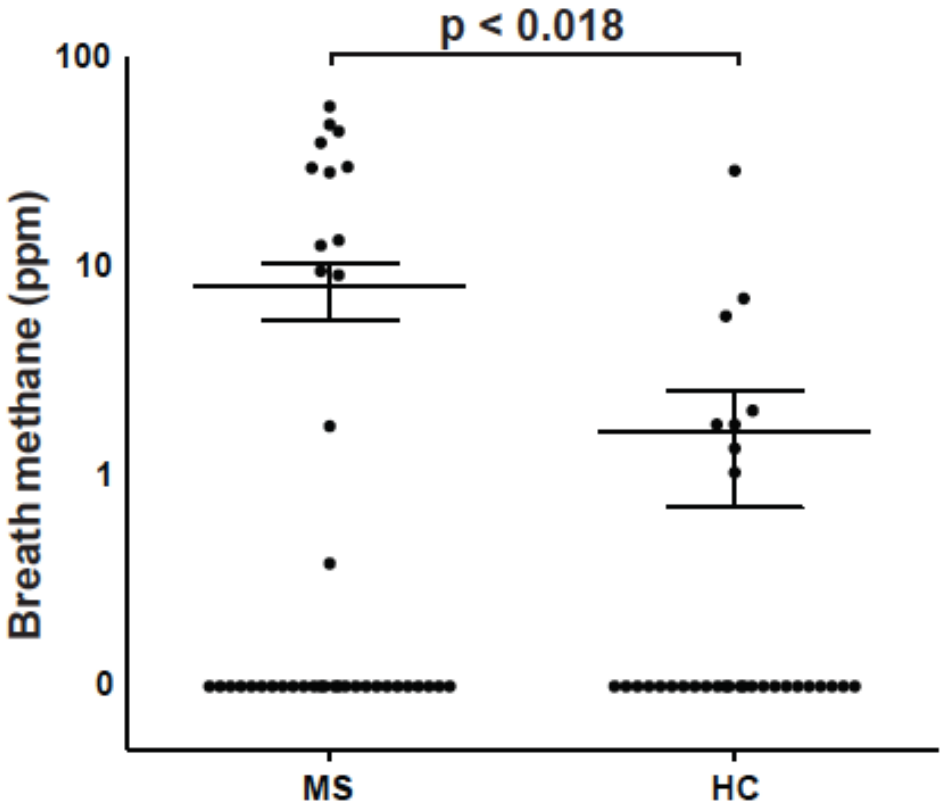
(S. Jangi et. al., Nat. Comm, 2016)

Methane Breath Test

454

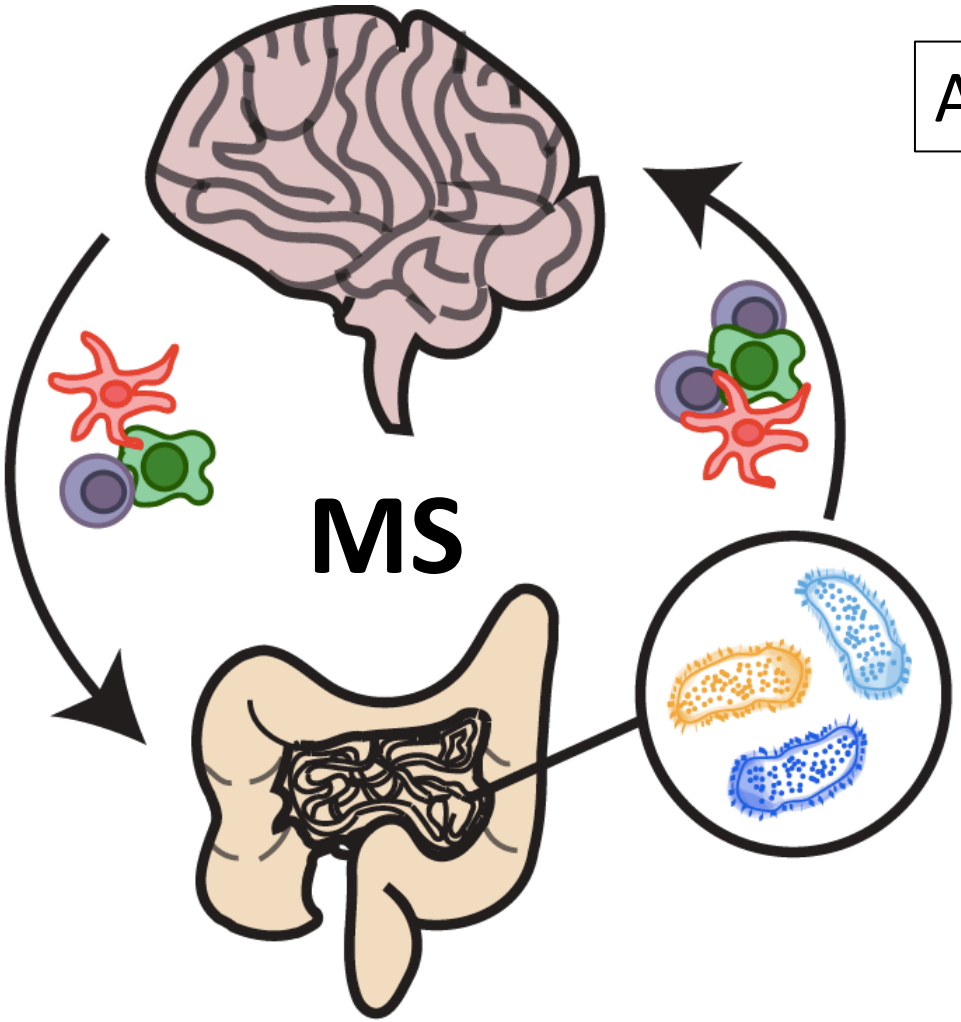
MiSeq





(S. Jangi et. al., Nat. Comm, 2016)

MS Microbiome and Peripheral Immune Response



Altered Immune Responses

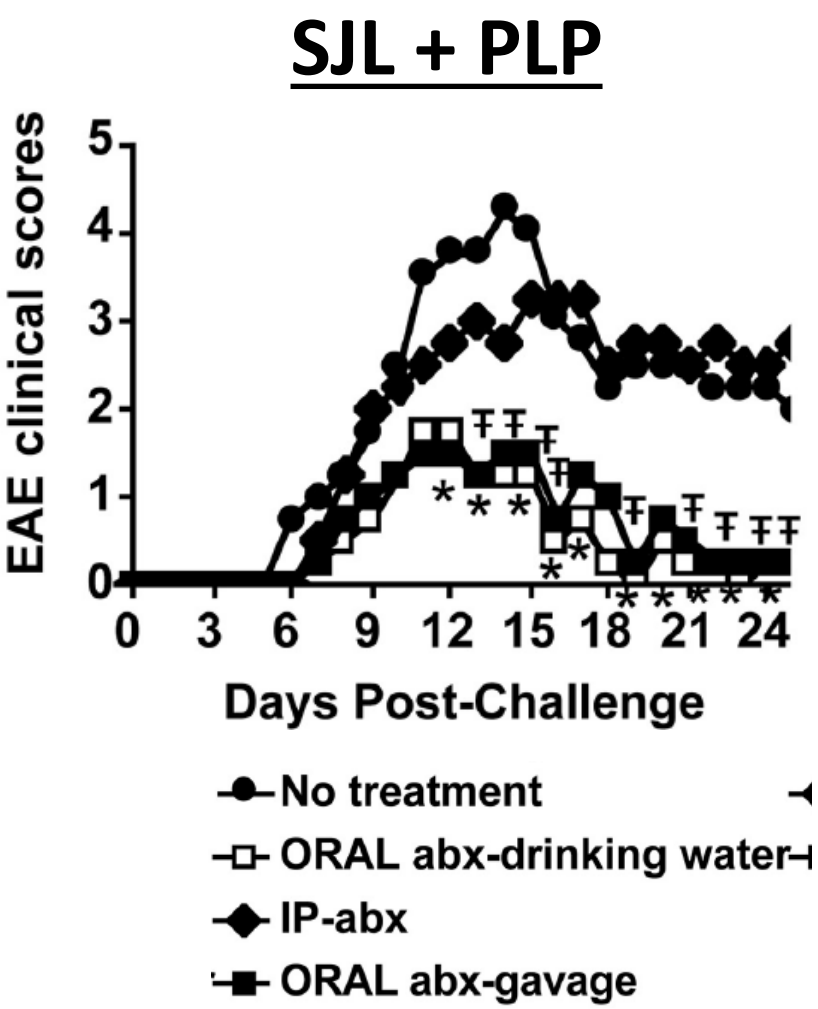
TNF, IL-6, NF-kB signaling increased in peripheral T-cells and monocytes

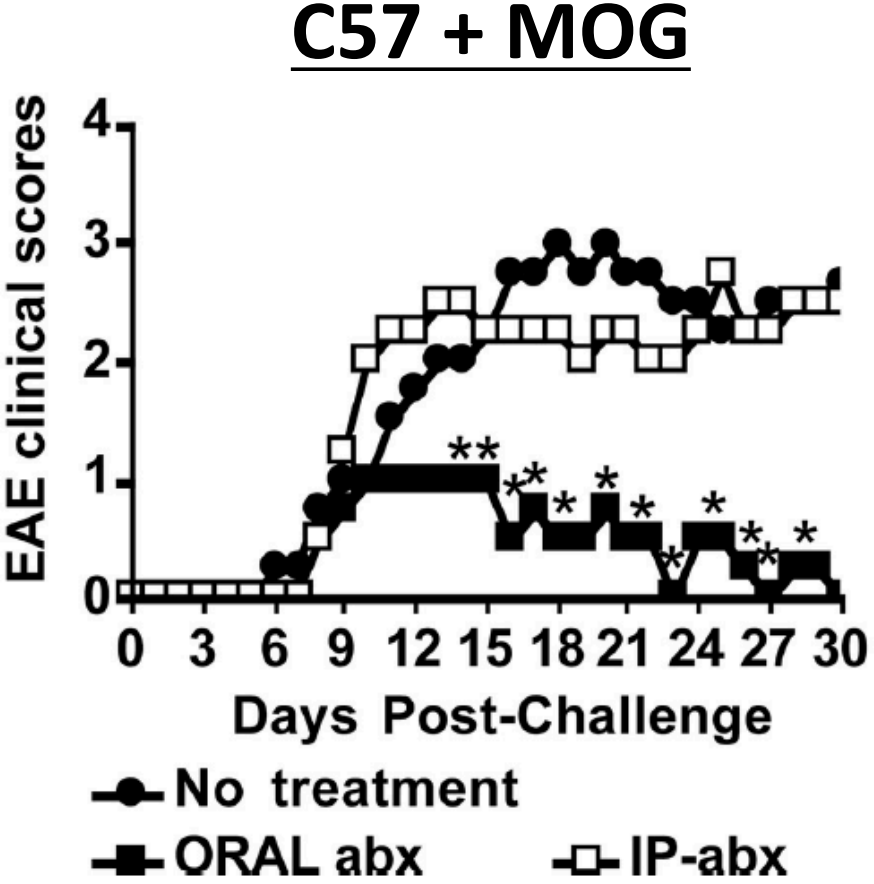
Altered Gut Microbiome

↑ Methanobrevibacter
Akkermansia

↓ Butyricimonas

High-dose antibiotics ameliorate EAE

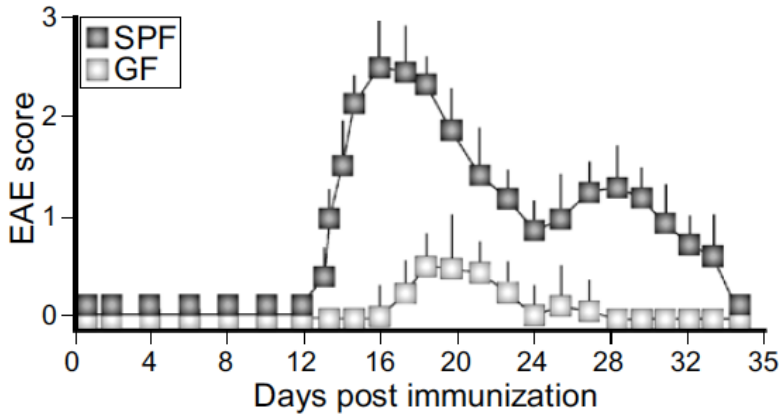


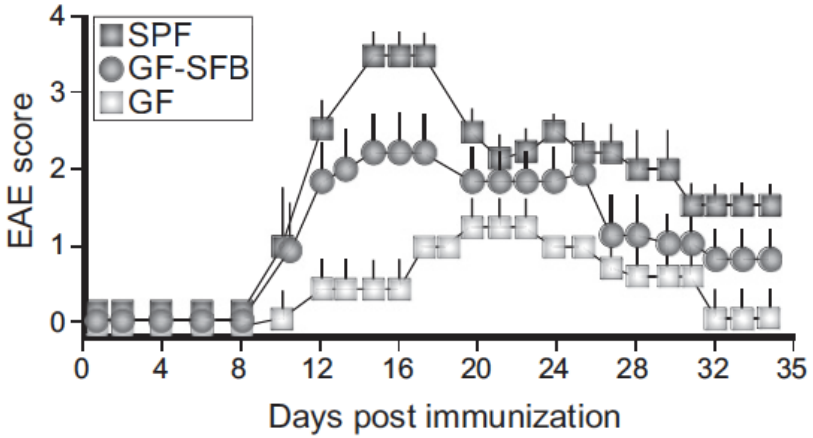


Ochoa-Reparaz, J Immunol

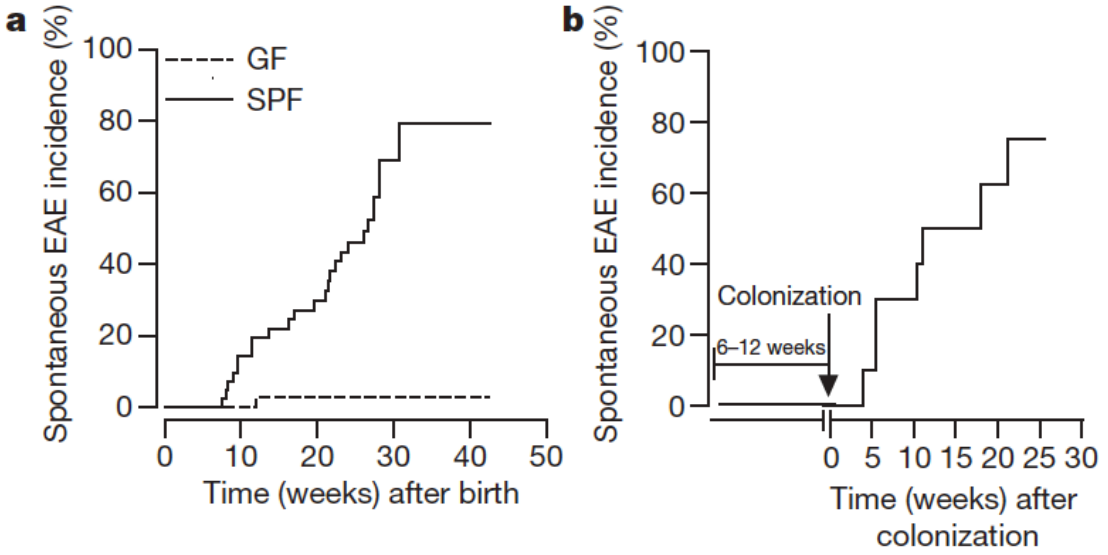
Germ-free mice resist experimental autoimmune enchephalomyelitis (EAE)

Induced, C57BL6 +MOG/CFA, Lee et al, PNAS, 2010





Spontaneous – SJL/J TCR MOG92-106, Berer et al, Nature 2011



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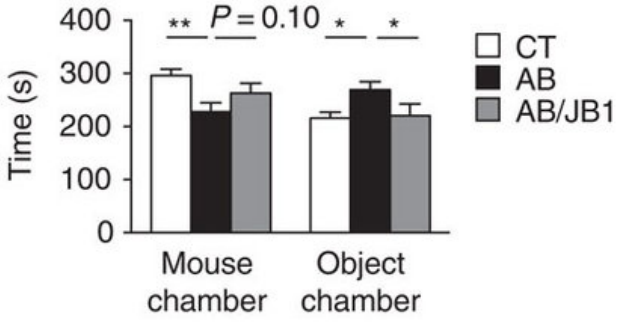
nature COMMUNICATIONS

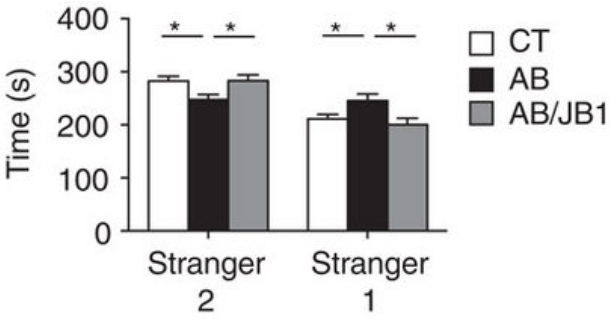
Low-dose penicillin in early life induces long-term changes in murine gut microbiota, brain cytokines and behavior

Sophie Leclercq[1,2], Firoz M. Mian[1], Andrew M. Stanisz[1], Laure B. Bindels[3], Emmanuel Cambier[4], Hila Ben-Amram[5], Omry Koren[5], Paul Forsythe[1,6] & John Bienenstock[1,2]

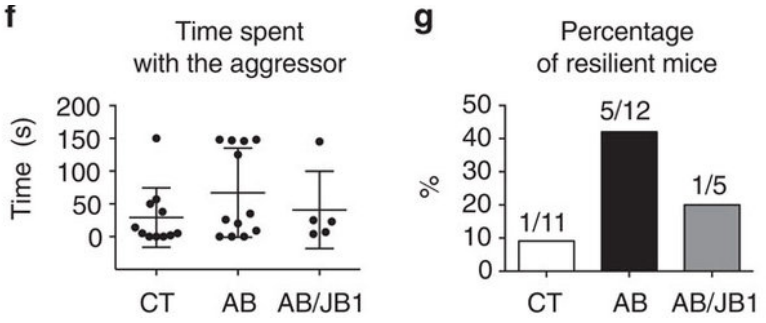
Social Behavior



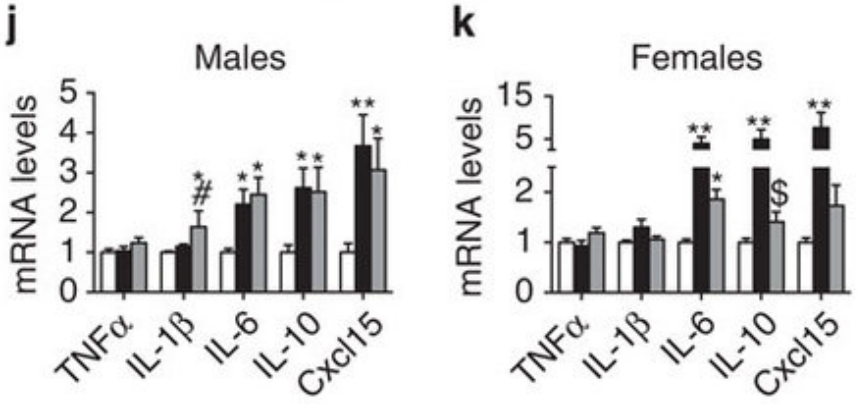
Social Novelty



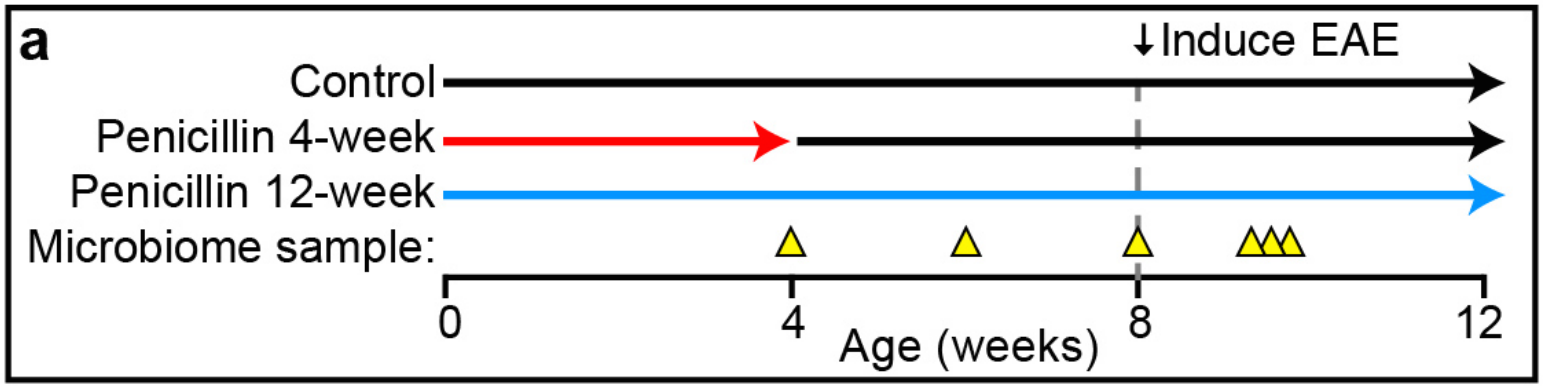
Social Avoidance following microdefeat

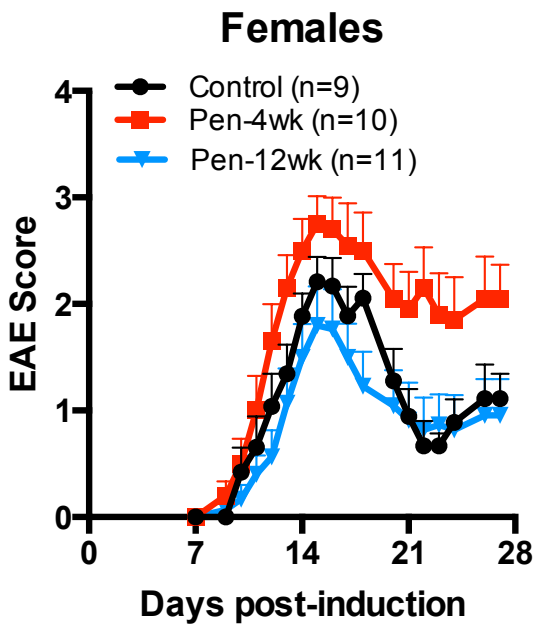


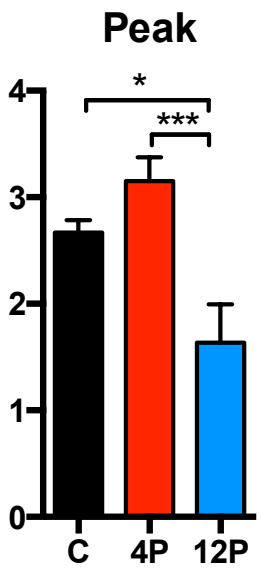
Cytokines: Frontal Cortex

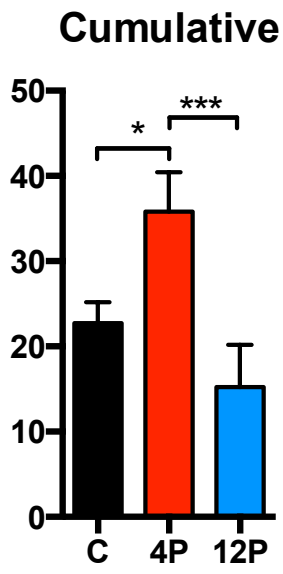


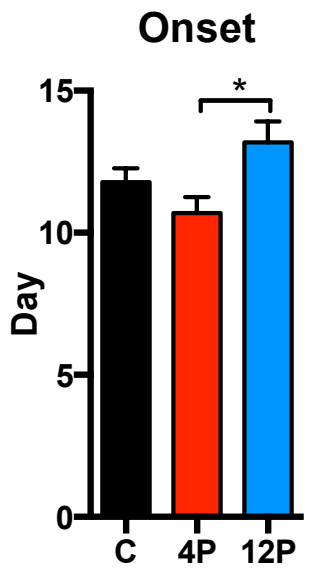
Effect of early-life antibiotics on EAE





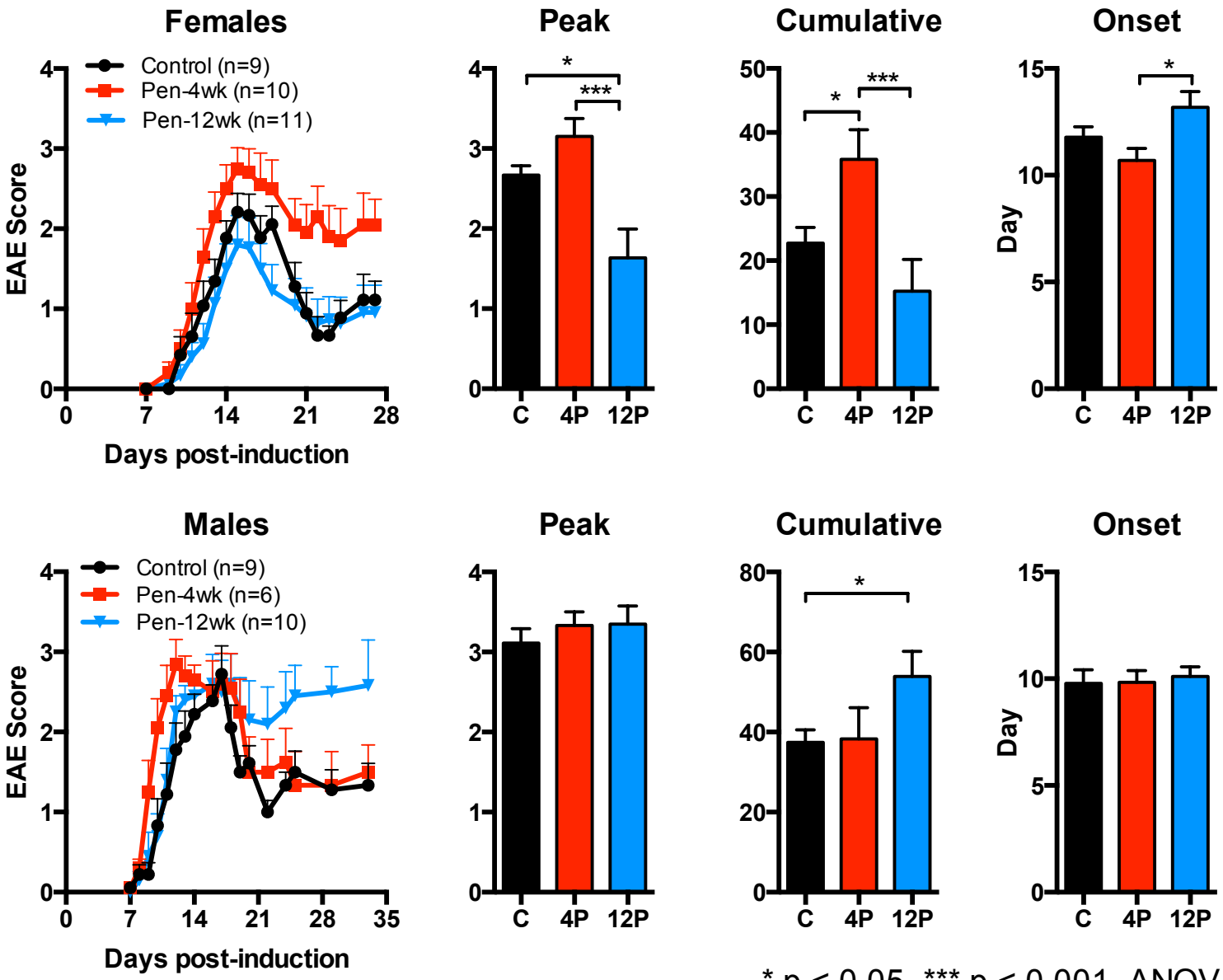






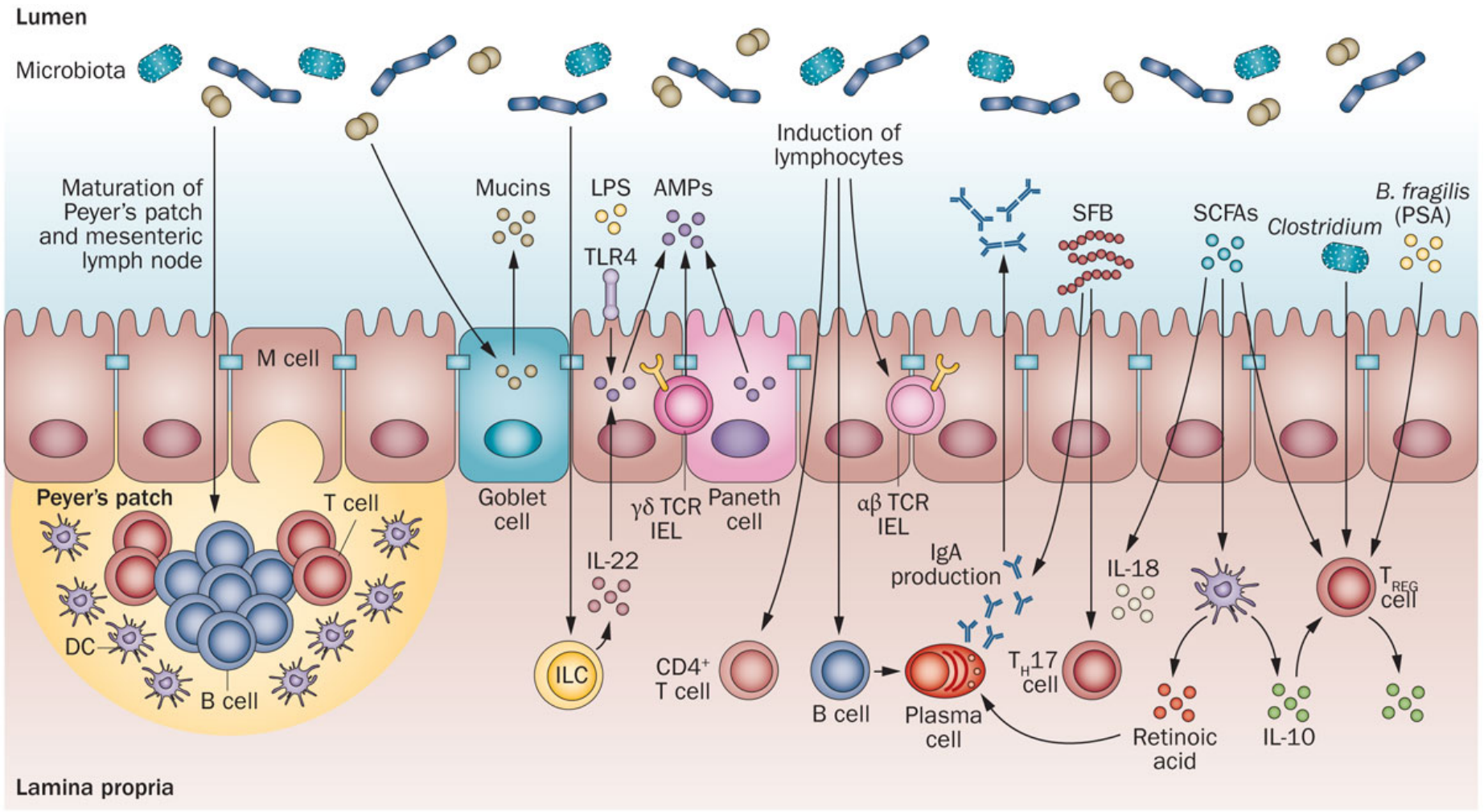
* P < 0.05, *** p < 0.001, ANOVA with Tukey's posttest

Gender-dependent differences in EAE severity



* p < 0.05, *** p < 0.001, ANOVA

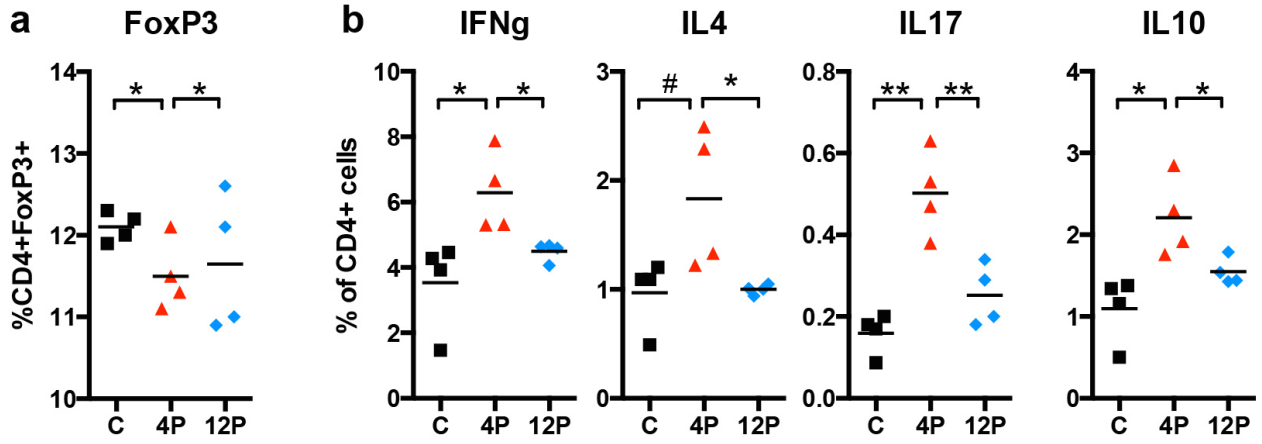
Gut microbiota shapes host immunity



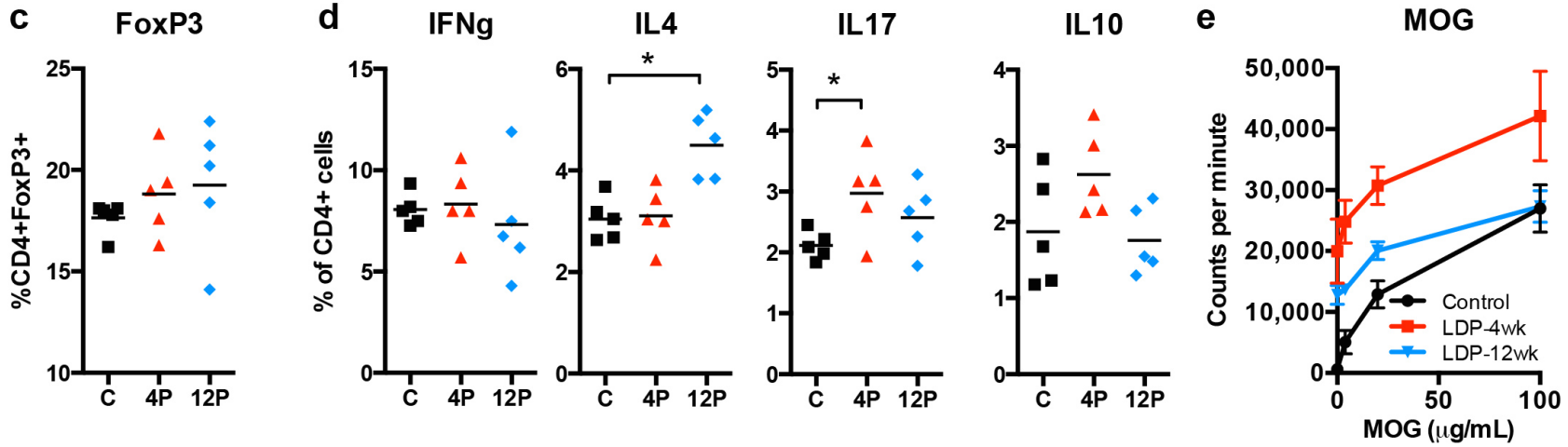
Verdu, E. F. *et al.* (2015) ***Nat. Rev. Gastroenterol. Hepatol.***

Splenic immune responses

Pre-induction, 8-weeks of age

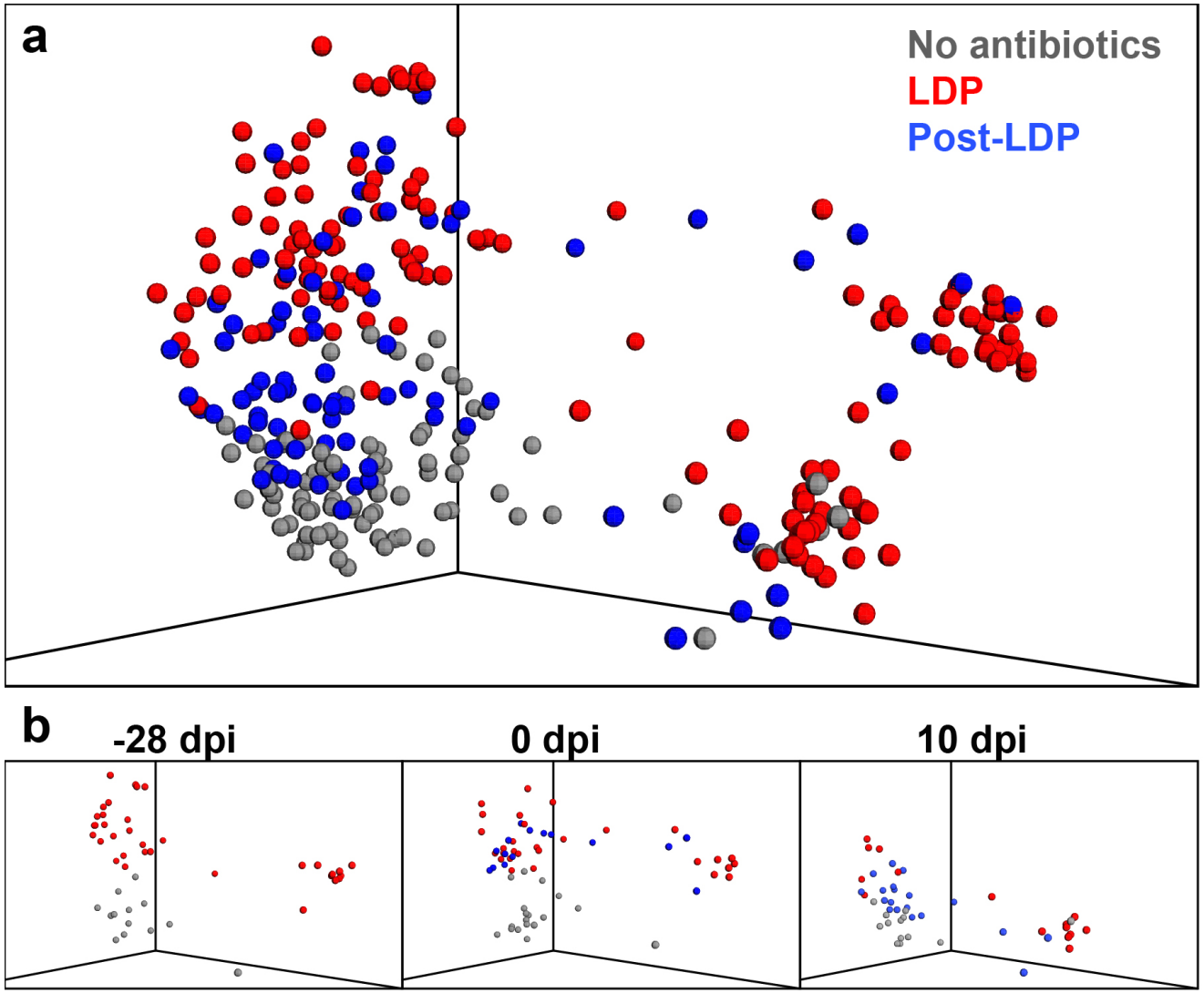


Post-induction, 12-weeks of age



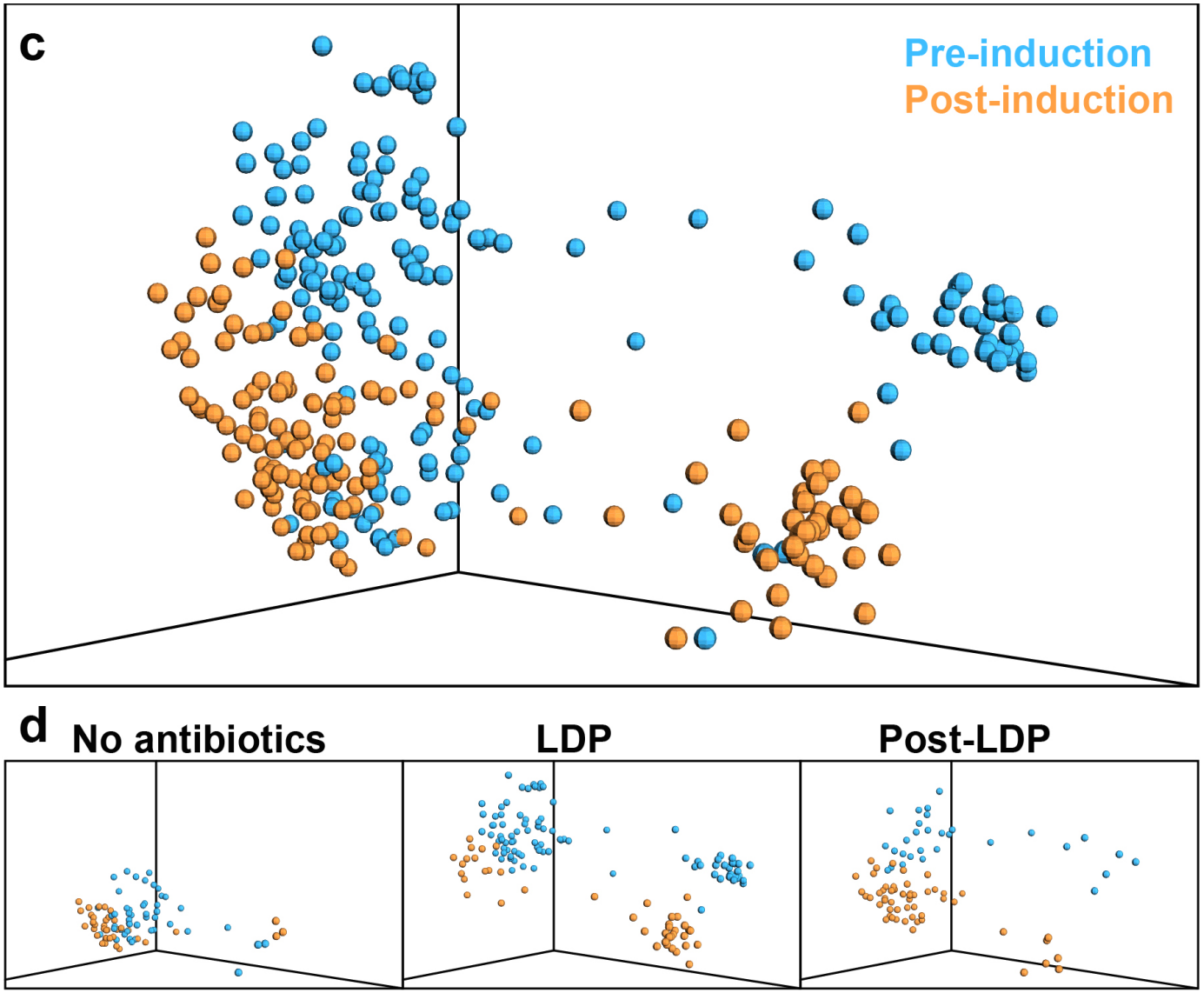
* P < 0.05, # p < 0.1, ANOVA with Tukey's posttest

Microbiome responses to antibiotics



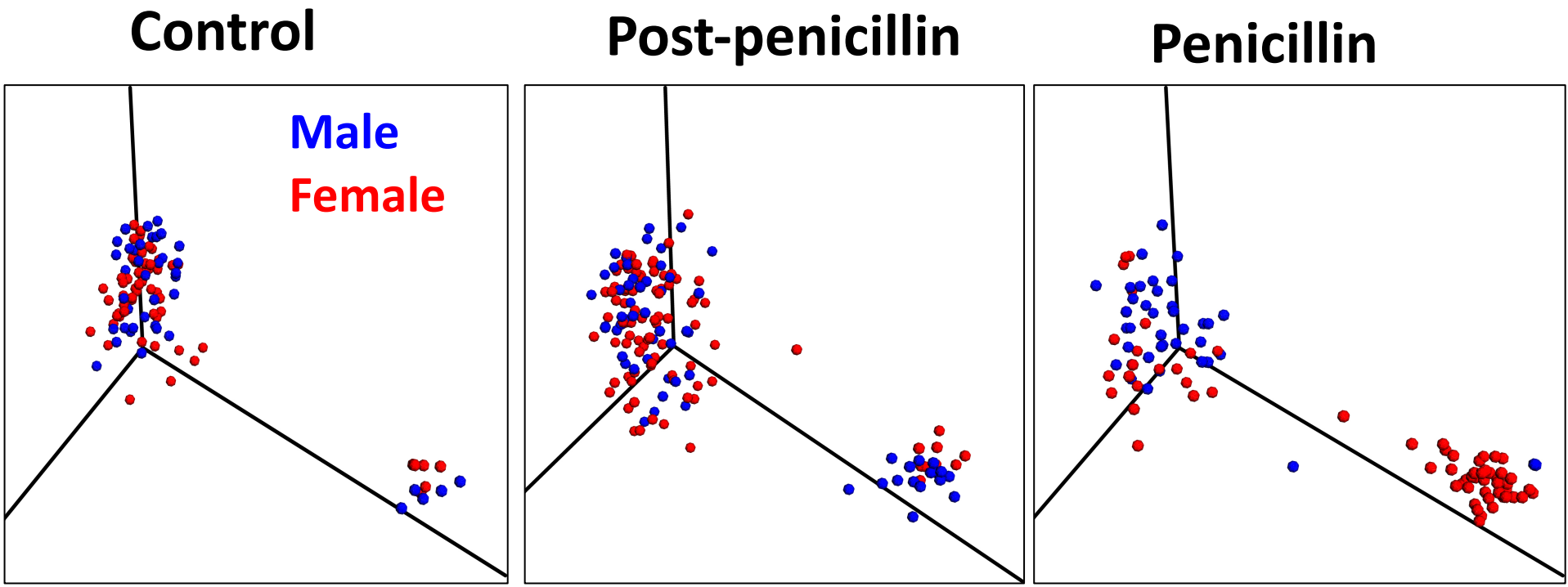
DPI = days post induction

Microbiota response to immunization



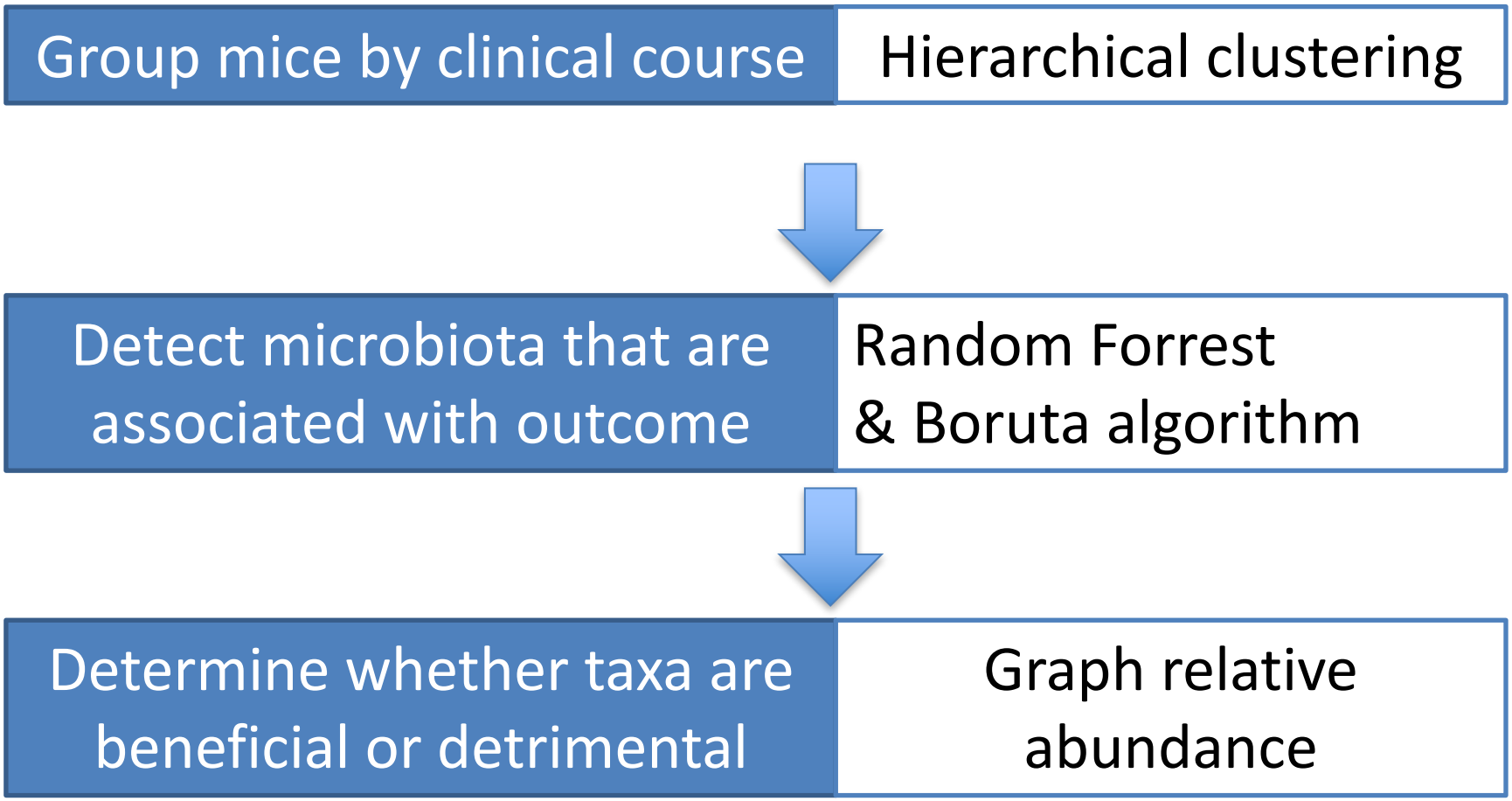
(L Cox et al, in preparation)

Gender-dependent shifts in microbiota

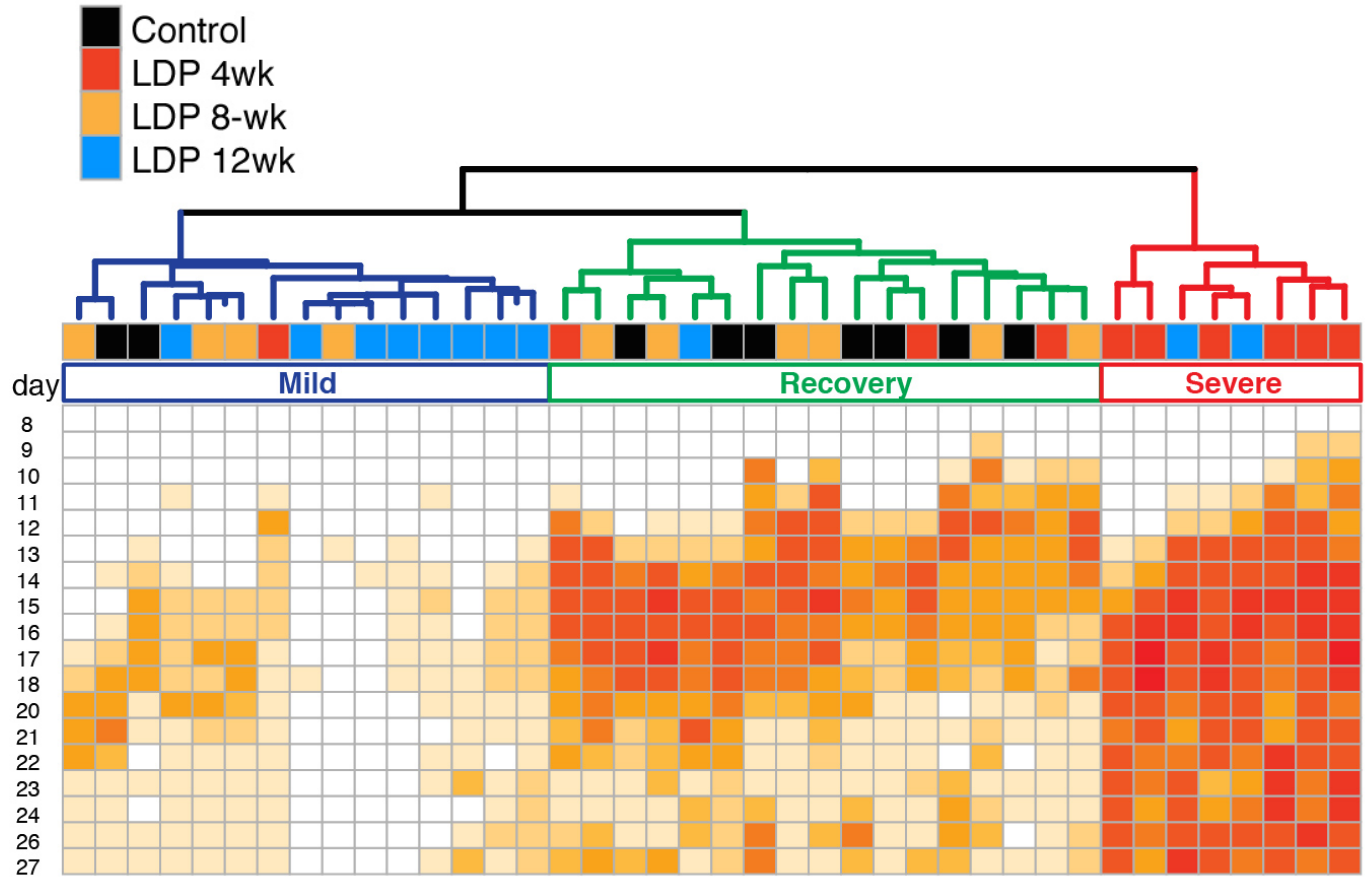


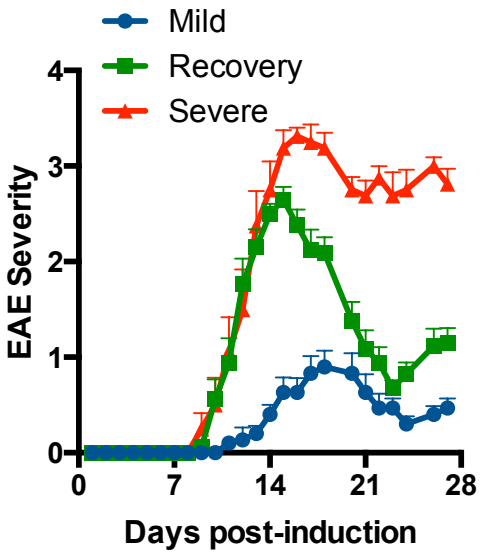
Fecal microbiota after immunization

Associating microbiota with disease

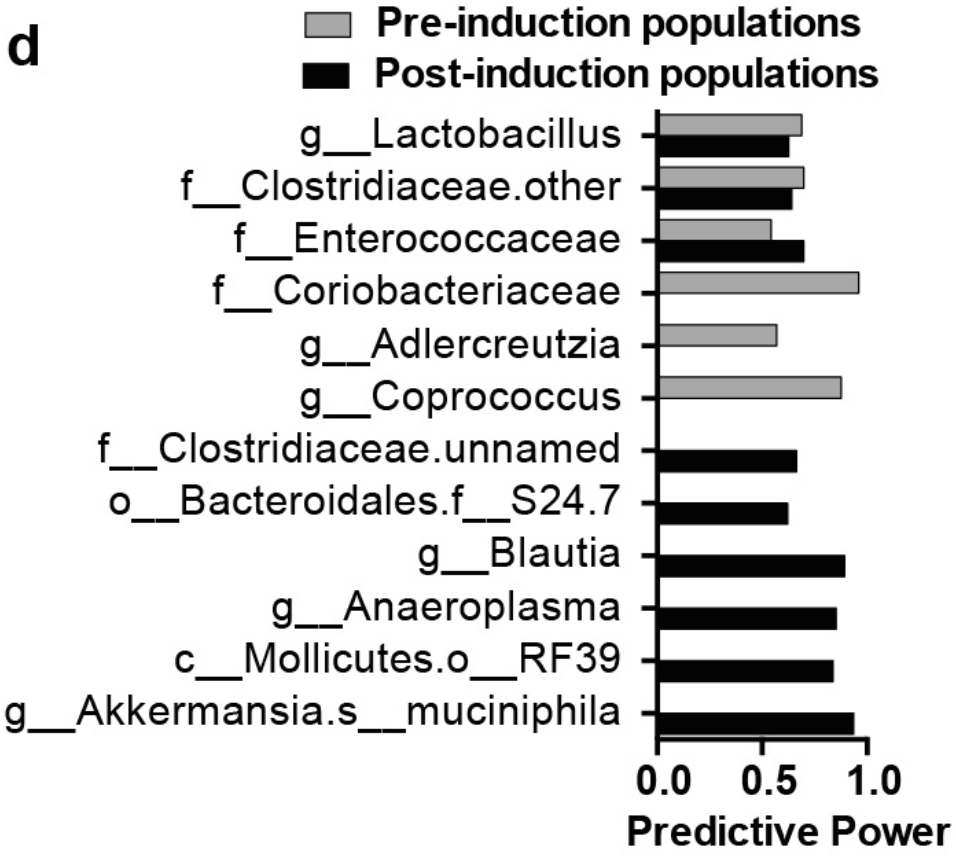


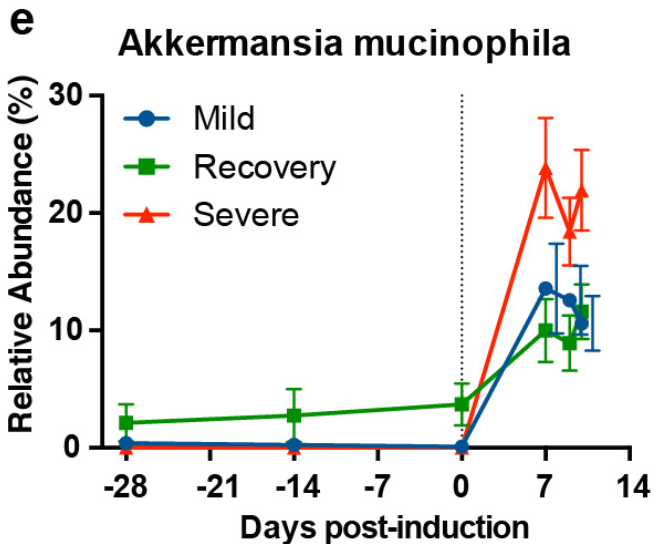
Hierarchical clustering of EAE scores in female mice

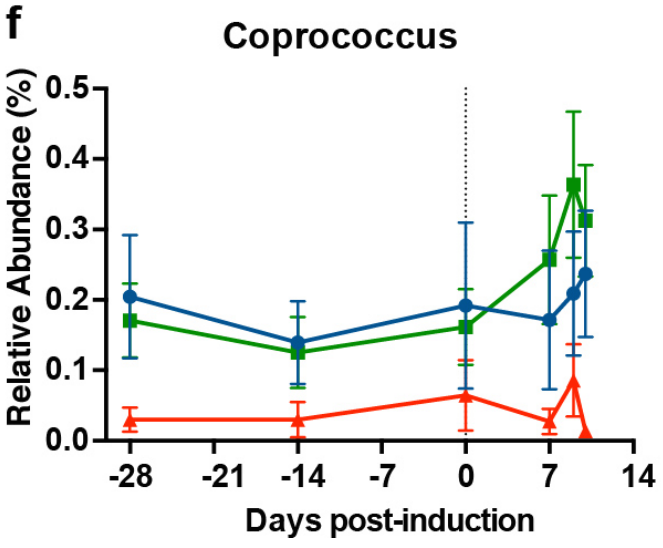




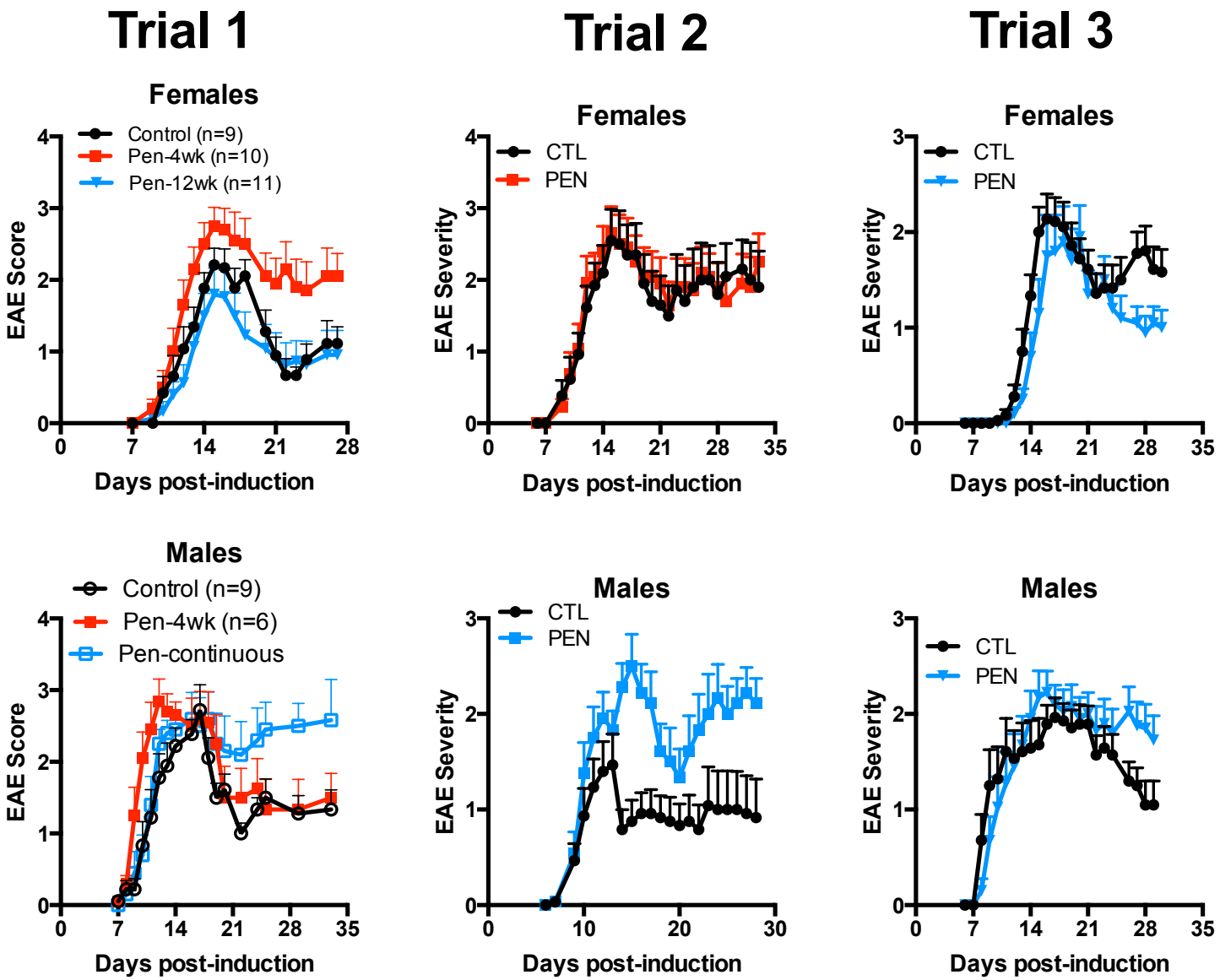
Bacteria associated with disease severity



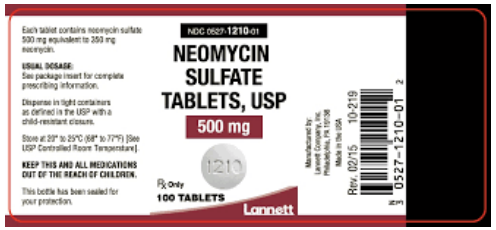


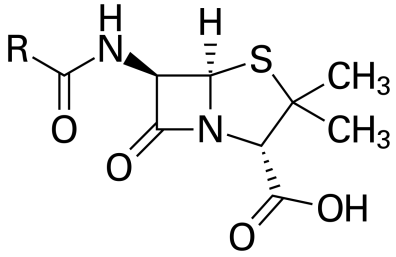


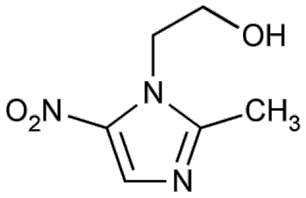
Sex-dependent responses to penicillin

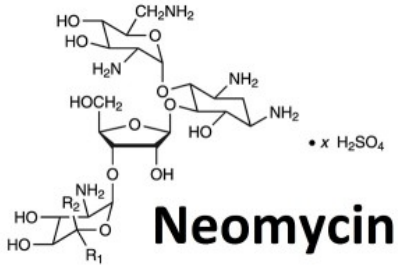


Select antibiotic spectrum of activity and mechanism of action



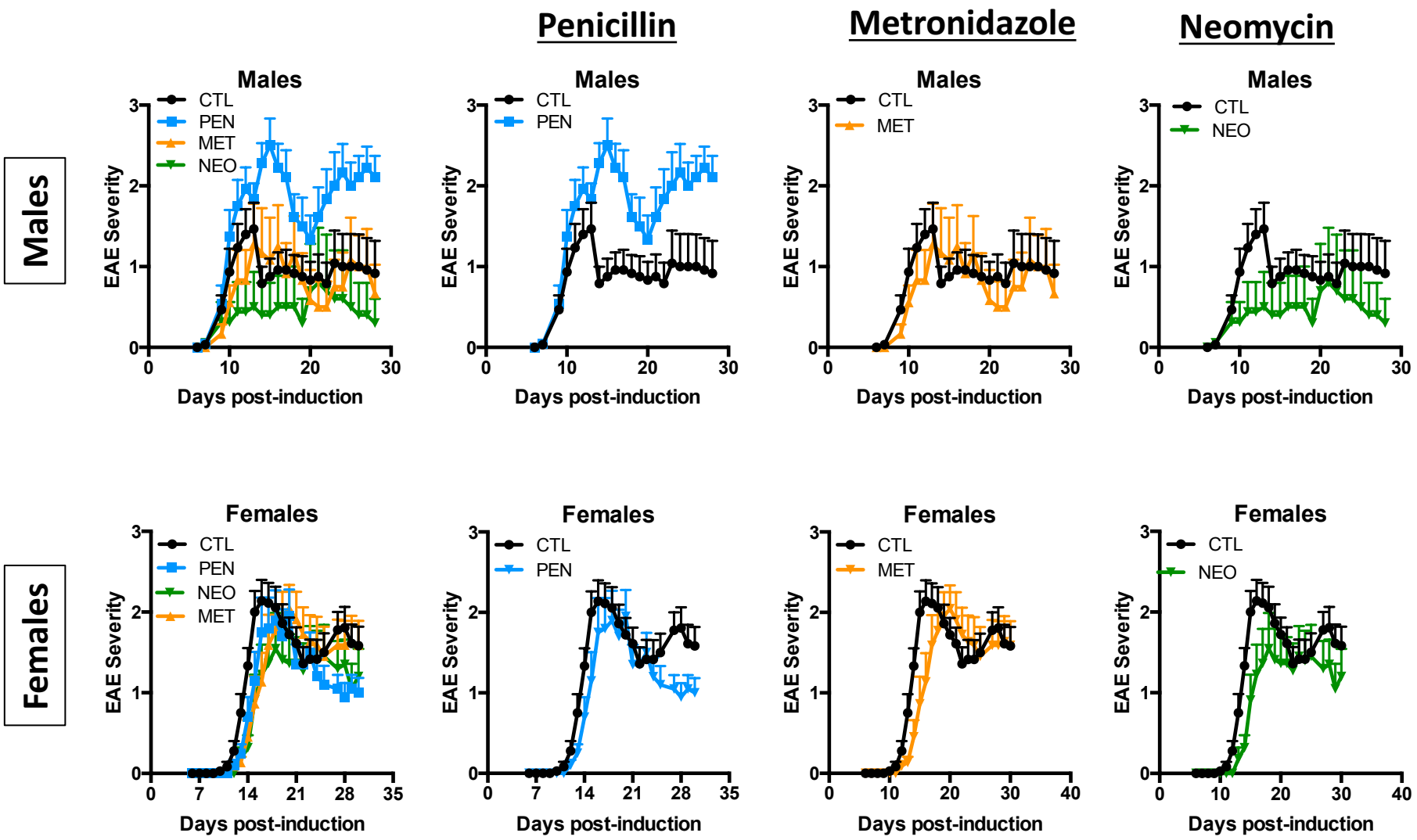






	Penicillin	Metronidazole	Neomycin
Class	Beta-lactam	Nitroimidazole	Aminoglycoside
Spectrum of activity	Gram positive bacteria	Anaerobic bacteria Protozoa	Aerobic bacteria
Mode of Action	Blocks peptidoglycan cell wall biosynthesis	Inhibits DNA synthesis under anaerobic conditions	Binds to the 30S rRNA subunit, blocks translation

Effect of antibiotic class on EAE in males



Conclusions

- Microbiota disruption can alter EAE severity, and depends on gender, class of antibiotic, and timing of exposure.
- Increased severity in 4-week treated females is associated with elevated systemic proinflammatory cytokines and lowered regulatory T cells
- Antibiotics, immunization, and gender shape the microbiota composition
- *Akkermansia* is associated with worse disease and *Coprococcus* is associated with less severe EAE in Female mice, which are also detected in the human population

Antibiotics and multiple sclerosis in the human population

American Journal of Epidemiology

Antibiotic Use and Risk of Multiple Sclerosis

Alvaro Alonso[1], Susan S. Jick[2], Hershel Jick[2], and Miguel A. Hernán[1]

- 163 MS cases, up to 10 controls matched per patient in Great Britain
- Penicillin **reduced** the risk of multiple sclerosis
- Was not affected by sex or age

American Journal of Epidemiology

Use of Penicillin and Other Antibiotics and Risk of Multiple Sclerosis: A Population-based Case-Control Study

Mette Nørgaard*, Rikke Beck Nielsen, Jacob Bonde Jacobsen, Jaimie L. Gradus, Egon Stenager, Nils Koch-Henriksen, Timothy L. Lash, and Henrik Toft Sørensen

- >3,000 cases vs >30,000 controls in the Danish population
- Penicillin and other antibiotics **increased the** risk of MS

Allergies, antibiotics use, and multiple sclerosis

CURRENT MEDICAL RESEARCH AND OPINION, 2017
Jinma Ren[a], Huijuan Ni[b], Minchul Kim[a], Kimberly L. Cooley[c,d], Reuben M. Valenzuela[d] and Carl V. Asche[a,e]

- 829 MS patients, 2441 controls in the US population
- **No risk association** with antibiotics

Translational implications and future challenges

Microbiome involvement in multiple sclerosis

- More studies are need to assess whether antibiotics can modify the symptoms of MS
- Clear evidence from animal models shows that manipulating the microbiome with antibiotics can alter the clinical course of EAE.

Treatment:

- Can we improve the disease by eliminating proinflammatory microbes?
- Can we improve the disease by administering microbes to beneficially tune the immune system?
- Do we need to consider gender-specific approaches to microbiome therapeutics?

Target validation in large cohort studies:

- Systems: 2000 patients in a multi-omics study at Partners MS Center
- iMSMS: 1000 patients recruited from 7 international locations

SysteMS
A partnership between your MS Center, Biogen Idec and Google Life Sciences

iMSMS
THE INTERNATIONAL MS MICROBIOME STUDY

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Lynn Bry
Georg Gerber

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Harvard NeuroDiscovery Center
COLLABORATING TO CURE NEUROLOGIC AND PSYCHIATRIC DISEASE

THE WOMEN'S ALZHEIMER'S MOVEMENT

NIH National Institutes of Health

WEINERLAB

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Sequencing:

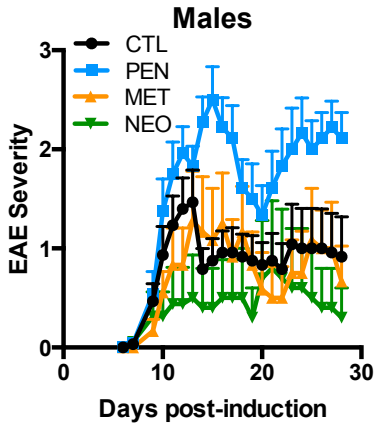
Biopolymers facility

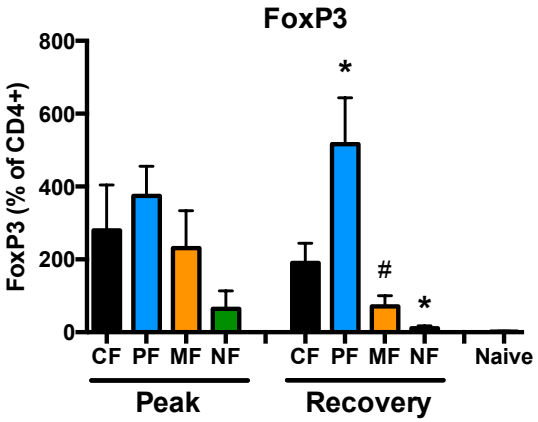
Funding:

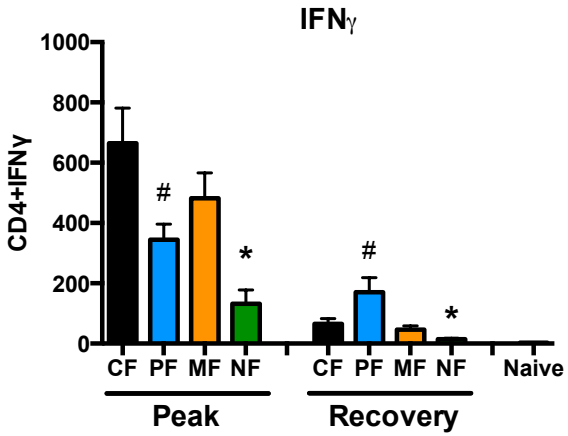
Harvard NeuroDiscovery Center Pilot Award

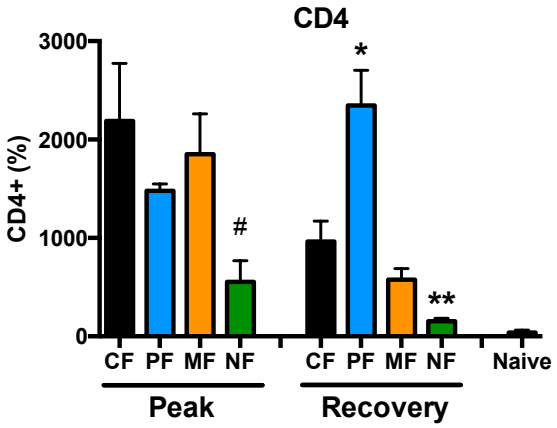
Susan Furbacher Fellowship

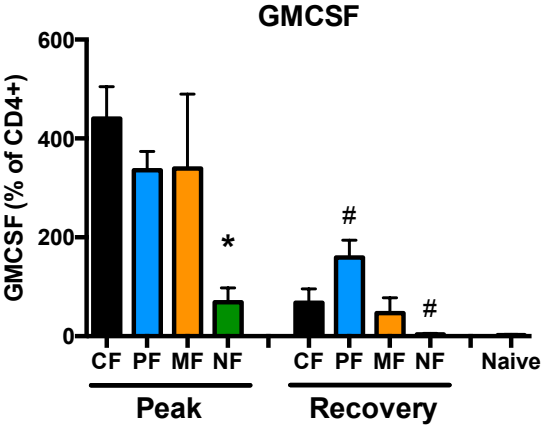
Effect of antibiotics on CNS cytokines during EAE in male mice

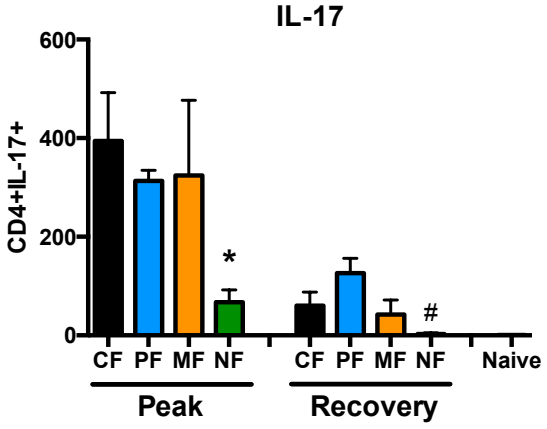






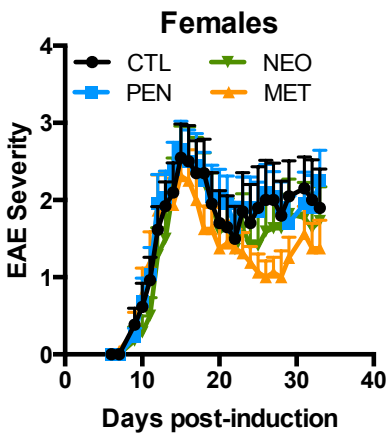


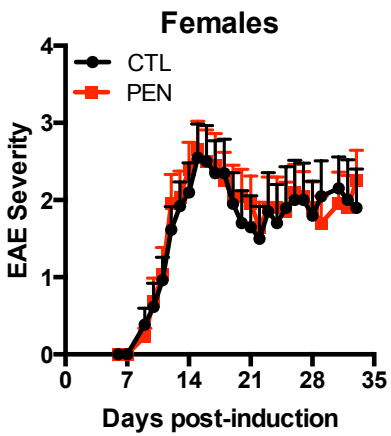


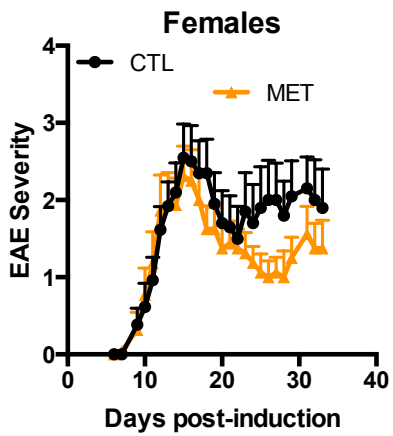


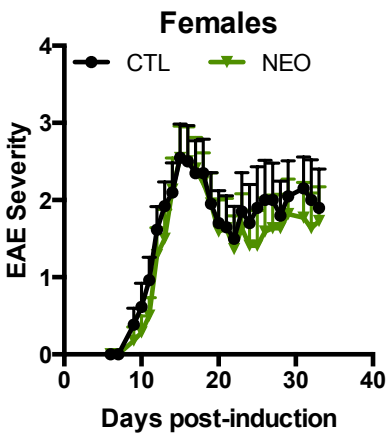
Effect of antibiotic class on EAE in females

Limited, early-life

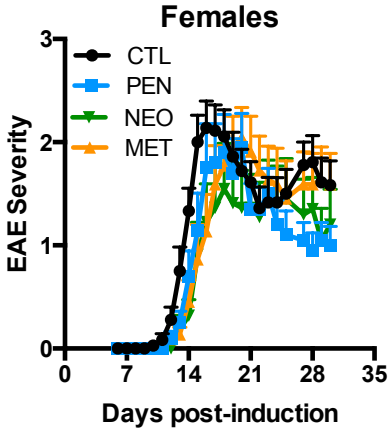


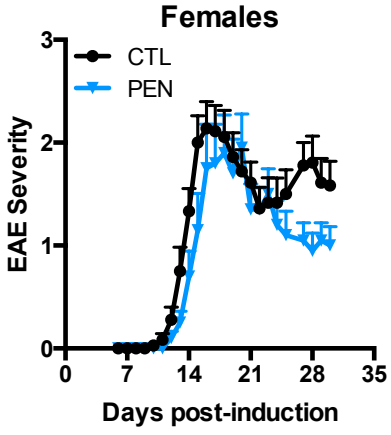


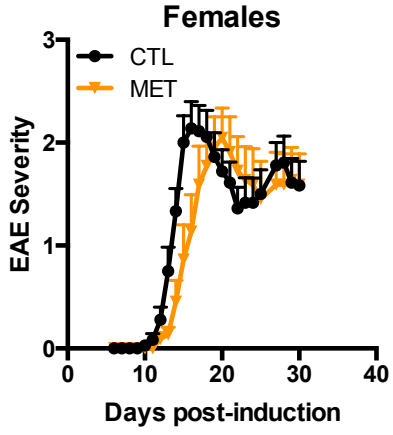


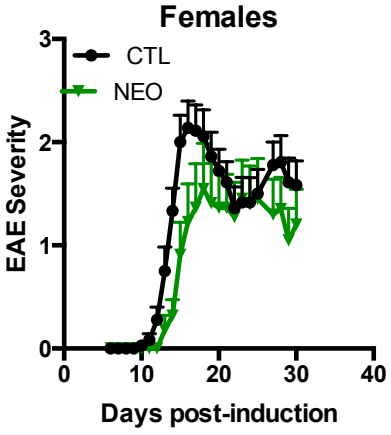


Continuous treatment

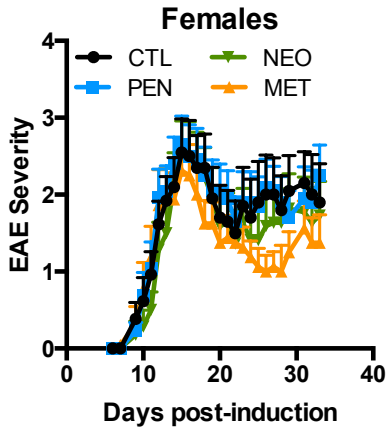


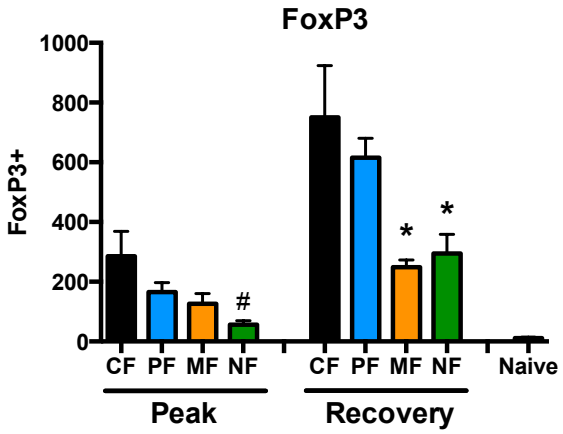


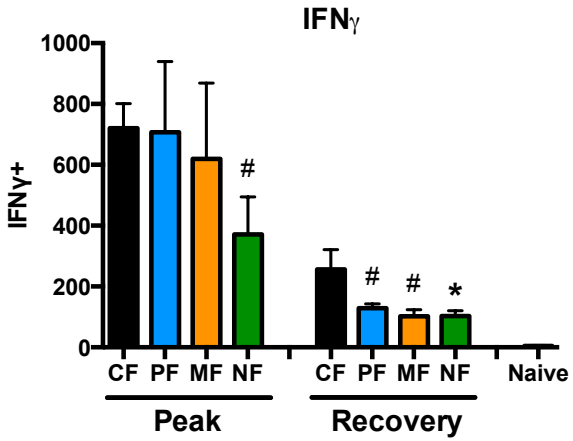


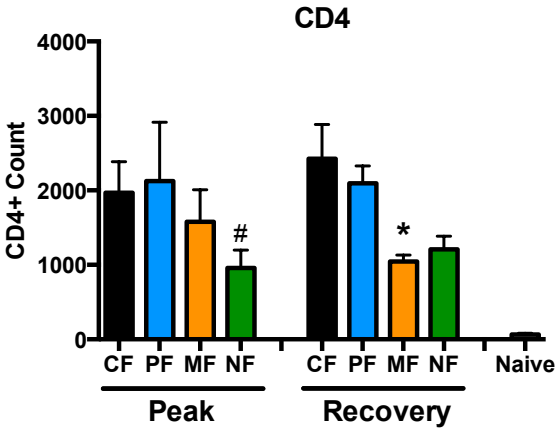


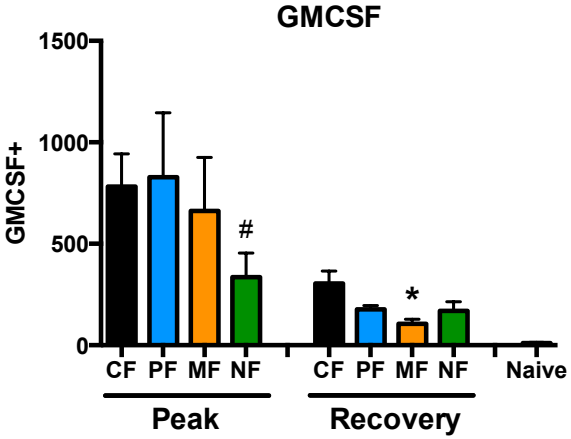
Effect of antibiotics on CNS cytokines during EAE in female mice

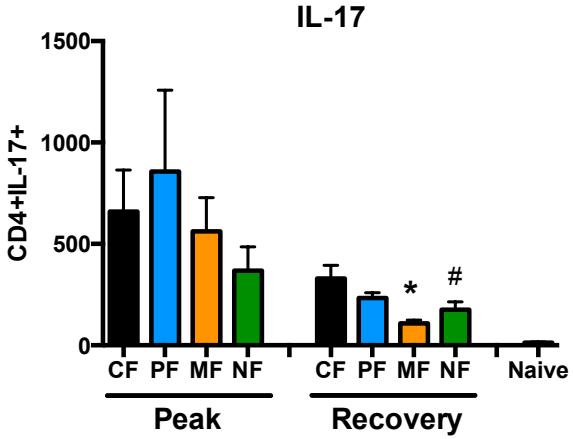




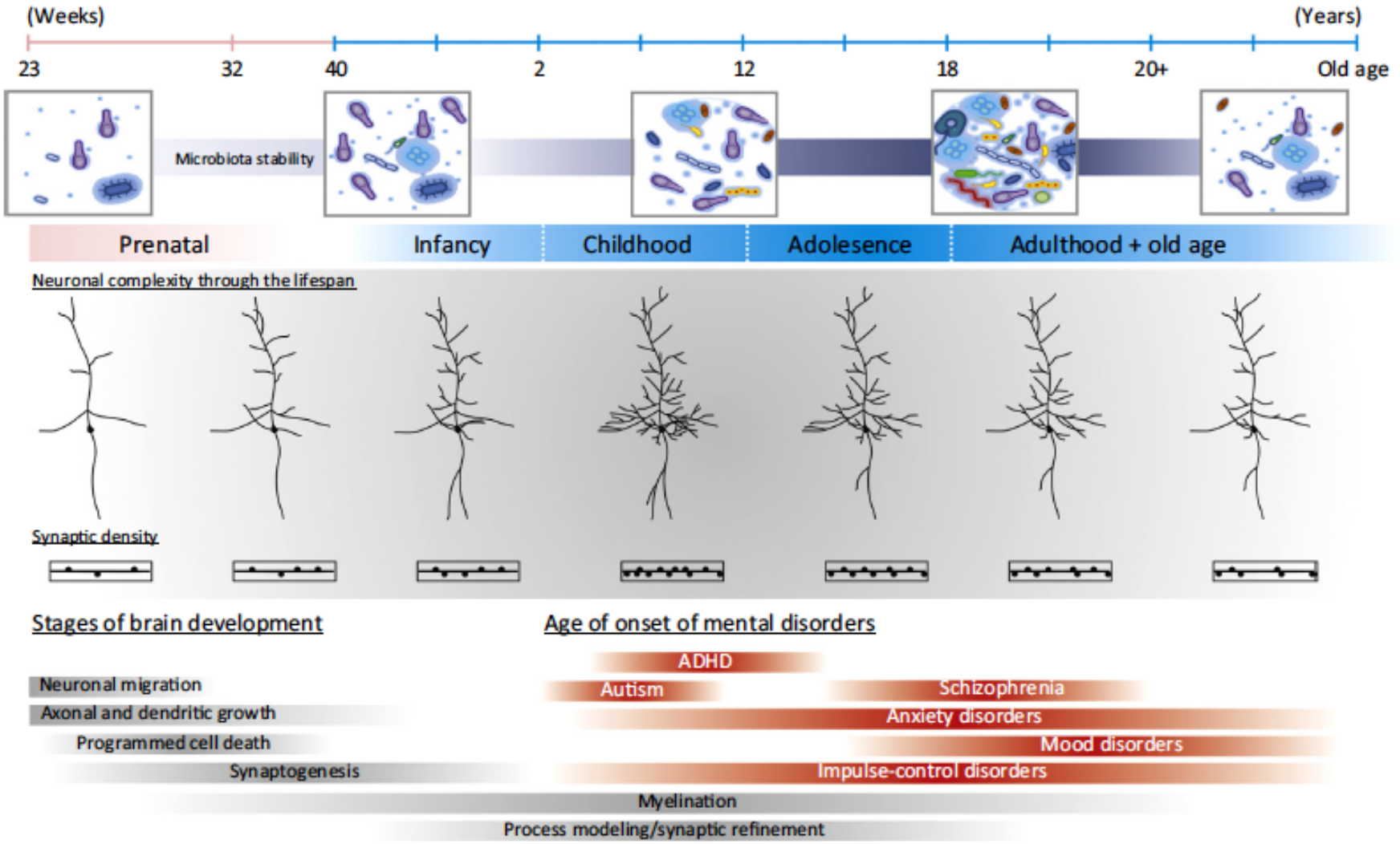








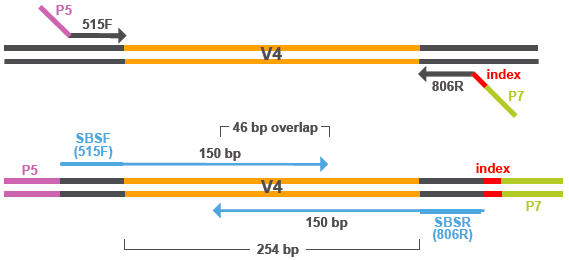
Microbiota stability, brain development, and mental disorder onset throughout life



(Borre et al., Trends Molecular Medicine, 2014)

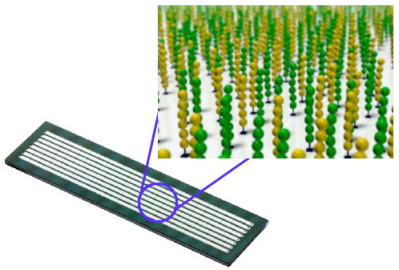
Microbiome Sequencing Strategy

1. Amplify the V4 region of microbial 16S rRNA gene with hundreds of barcoded reverse primers

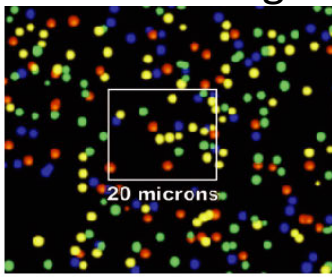


2. Sequence on Illumina MiSeq platform

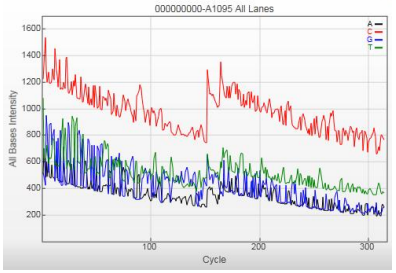
Flow Cell



Flow Cell Image



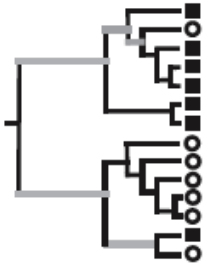
Signal Intensity



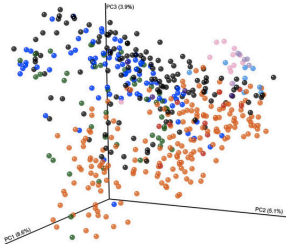
3. Assemble paired end reads with EA-utils, quality filter, demultiplex, assign taxonomy, construct a phylogenetic tree, calculate diversity metrics and relative abundance with QIIME

Quantitative Insights Into Microbial Ecology

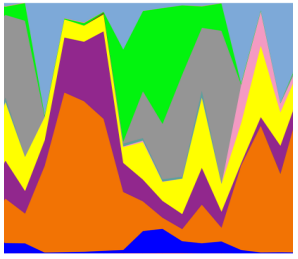
Tree



β-diversity

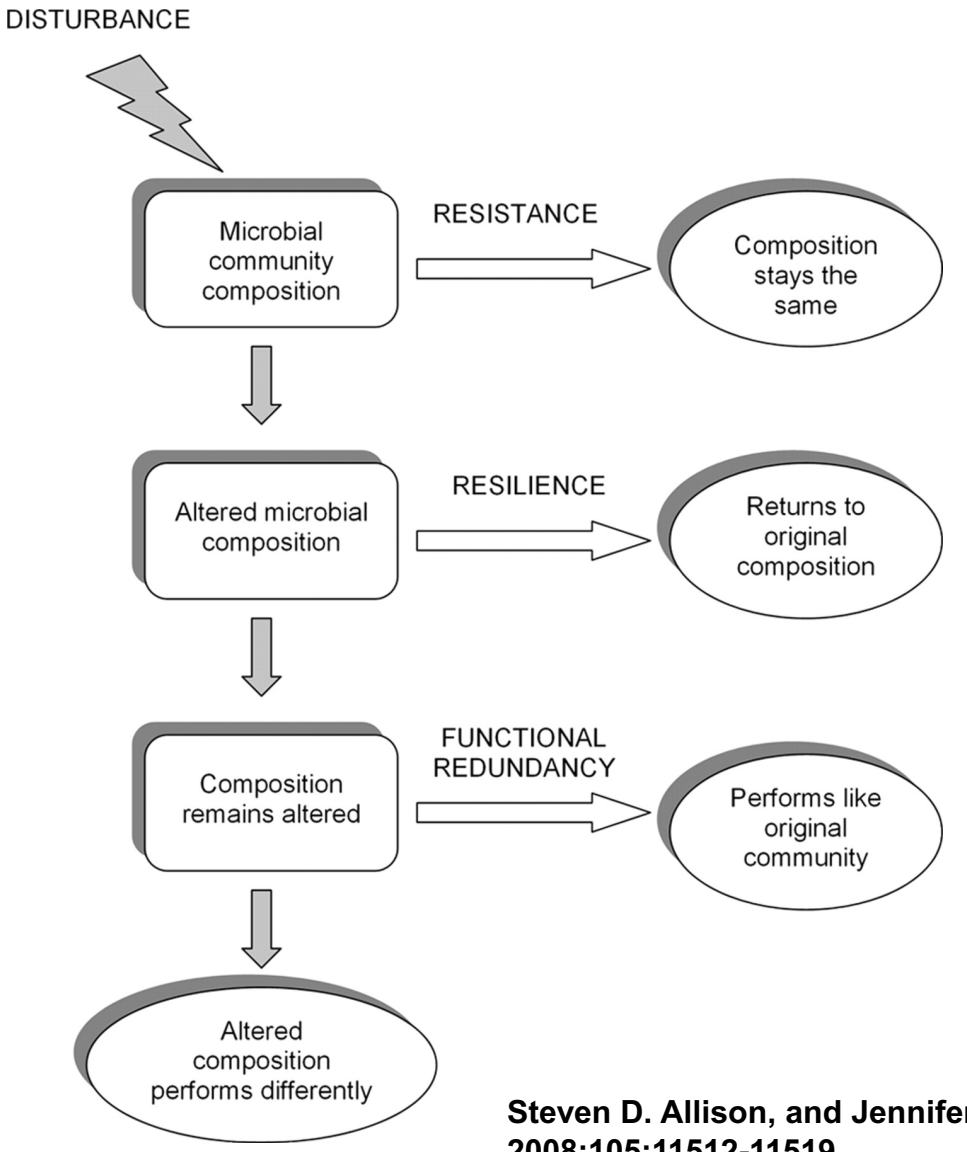


Composition



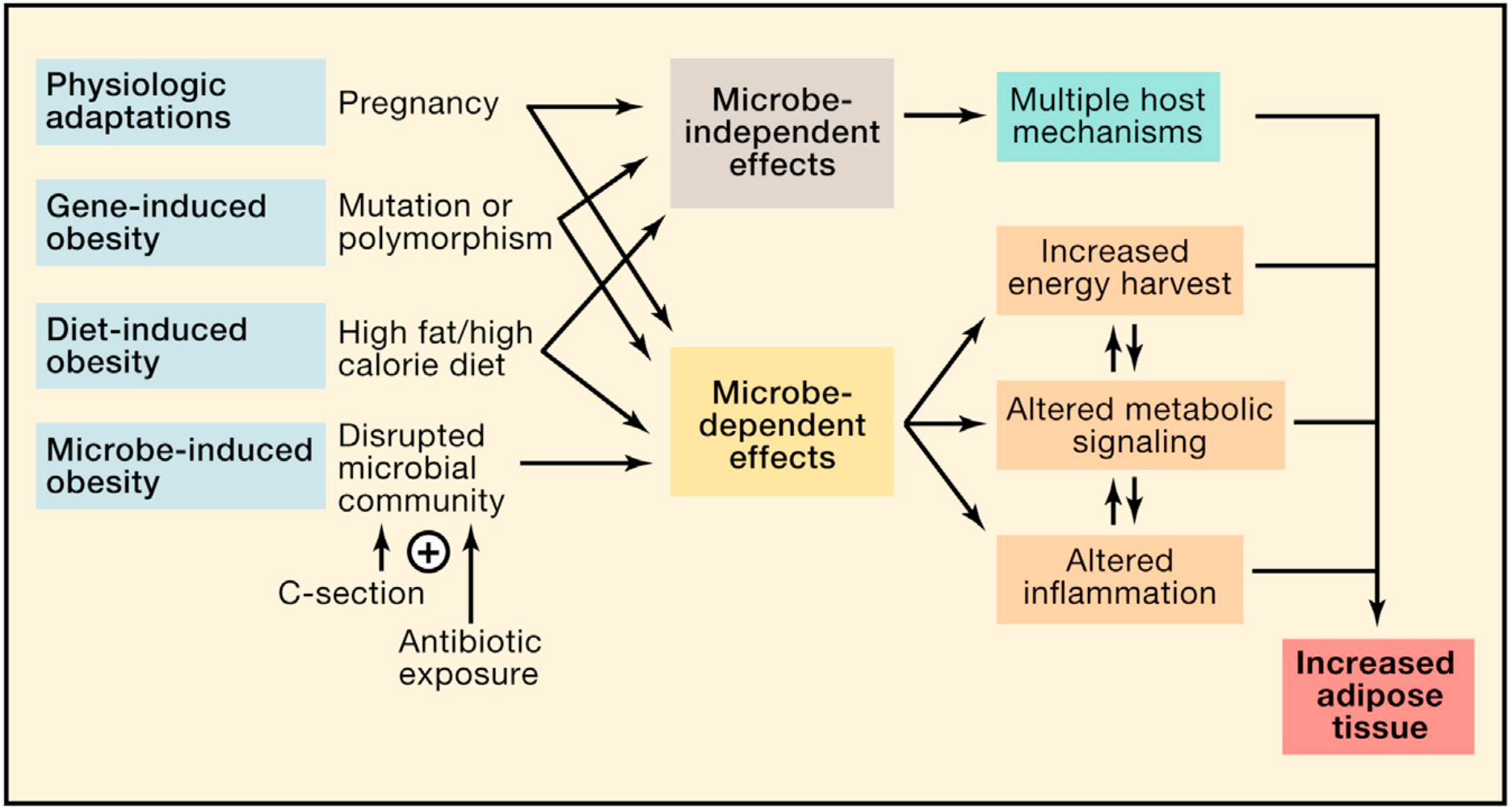
Resistance, Resilience, and Redundancy

Disturbance:
Antibiotics
Immunization

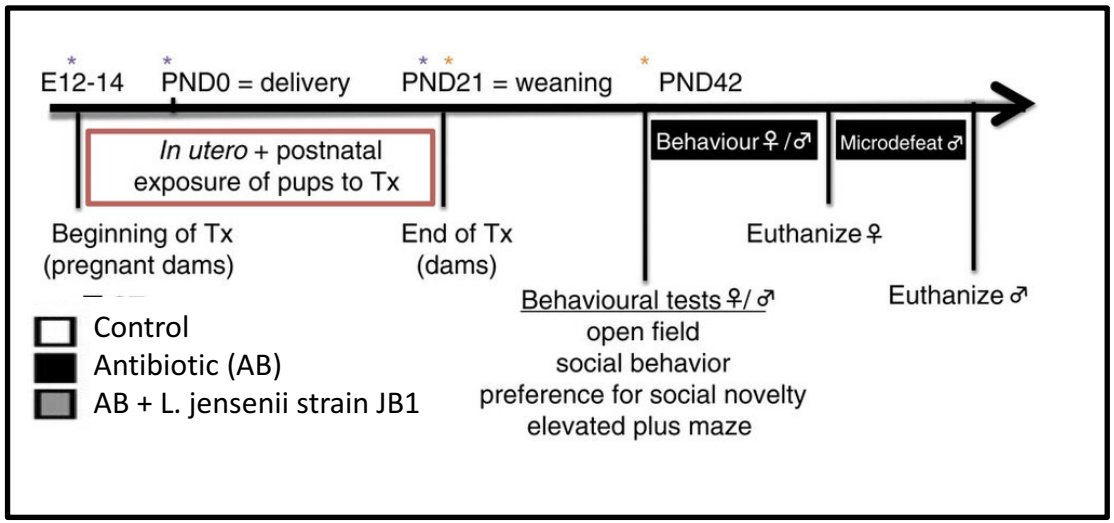


Steven D. Allison, and Jennifer B. H. Martiny PNAS 2008;105:11512-11519

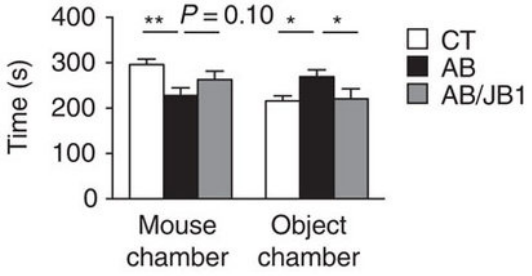
Models of microbe-induced obesity



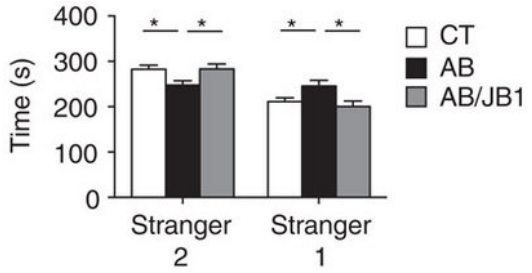
Low dose penicillin alters behavior and cytokines



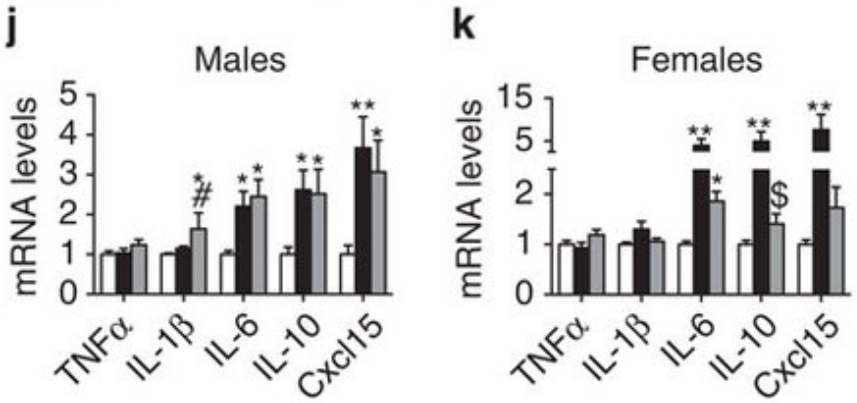
Social Behavior



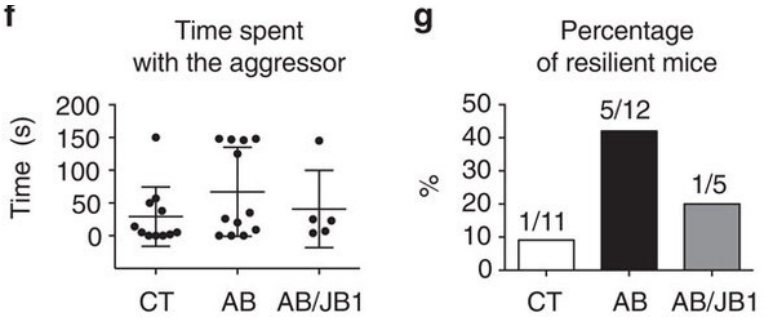
Social Novelty



Cytokines: Frontal Cortex



Social Avoidance following microdefeat



Leclercq, Nature Communications, 2017

nature COMMUNICATIONS

Microbiome Manipulations

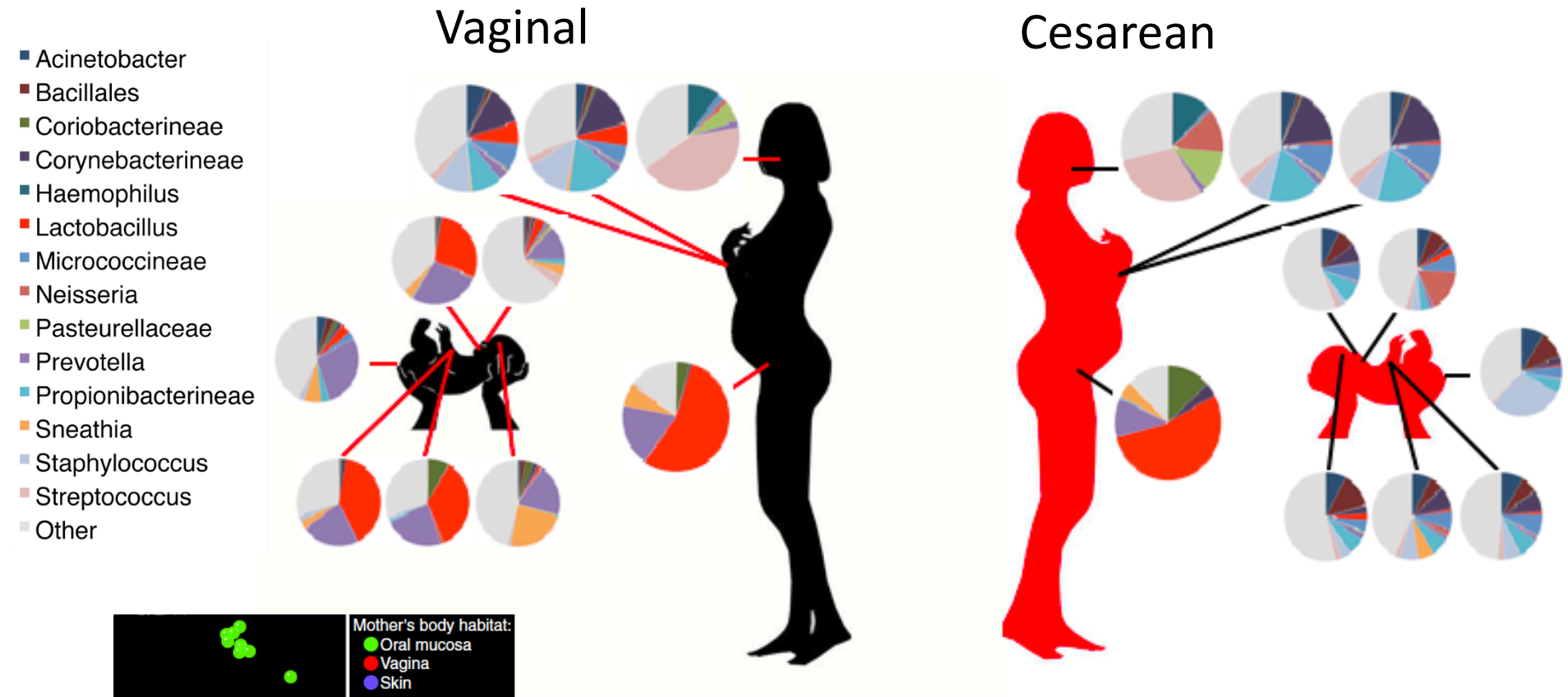
Deplete

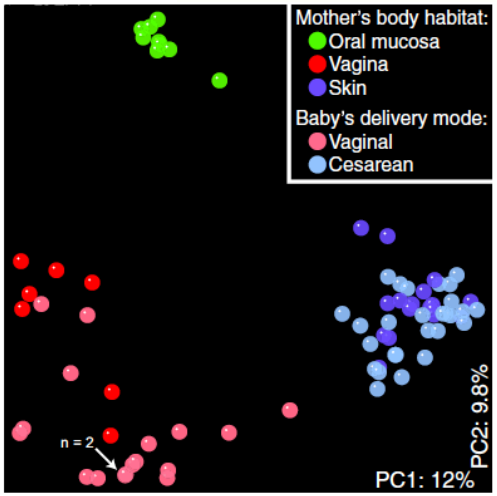
- Germ-free (axenic)
- High-dose, broad-spectrum antibiotics
- Single antibiotics, varies by dose, duration, and spectrum of activity
- Narrow-spectrum bacteriocidal proteins
- Phage therapy

Augment

- Single organism
- Defined collection of organisms
- Whole community
  - Fecal/vaginal microbiota transfer
  - Cohousing (mice)
  - Shared bedding (mice)

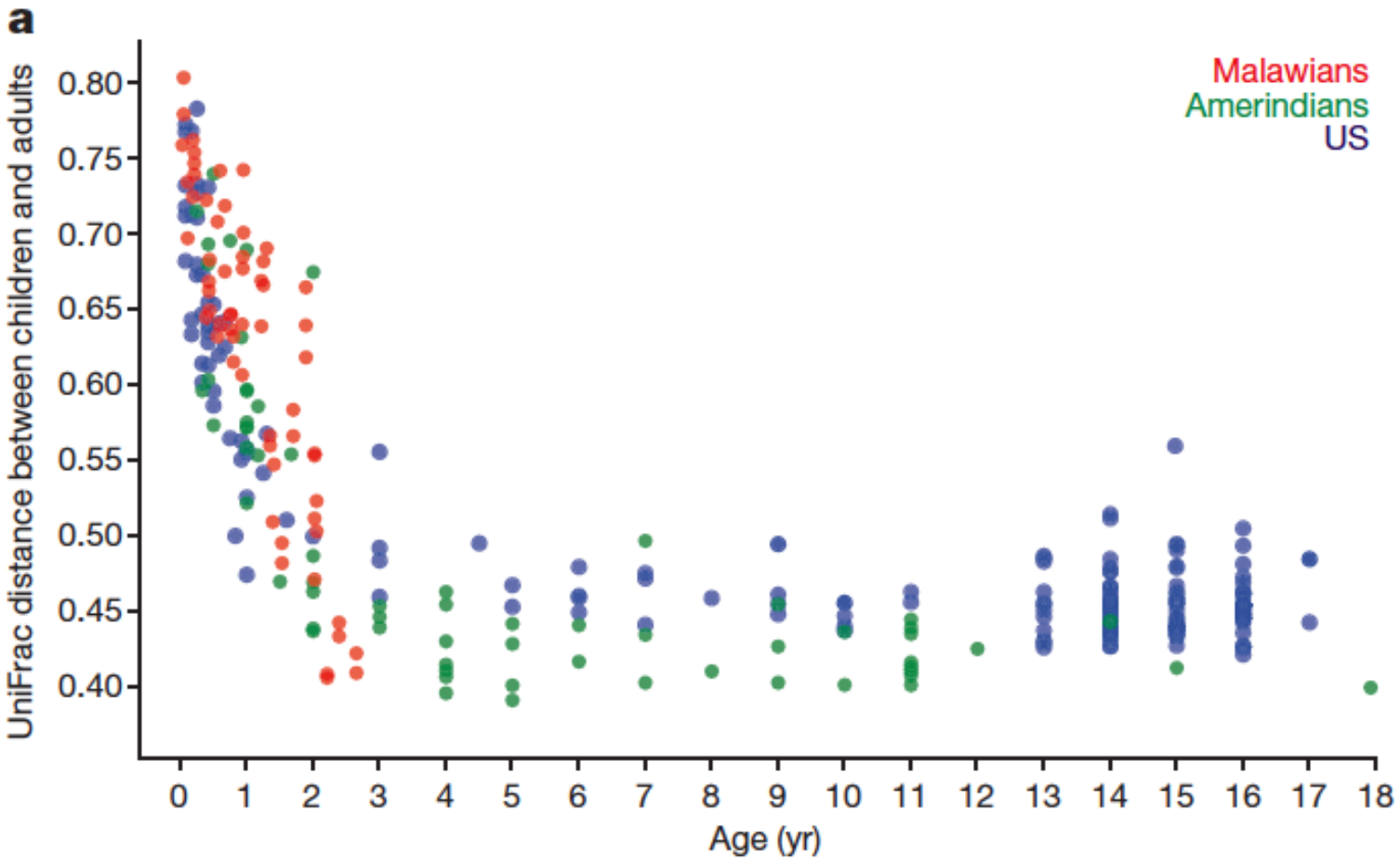
Delivery mode shapes infant microbiota





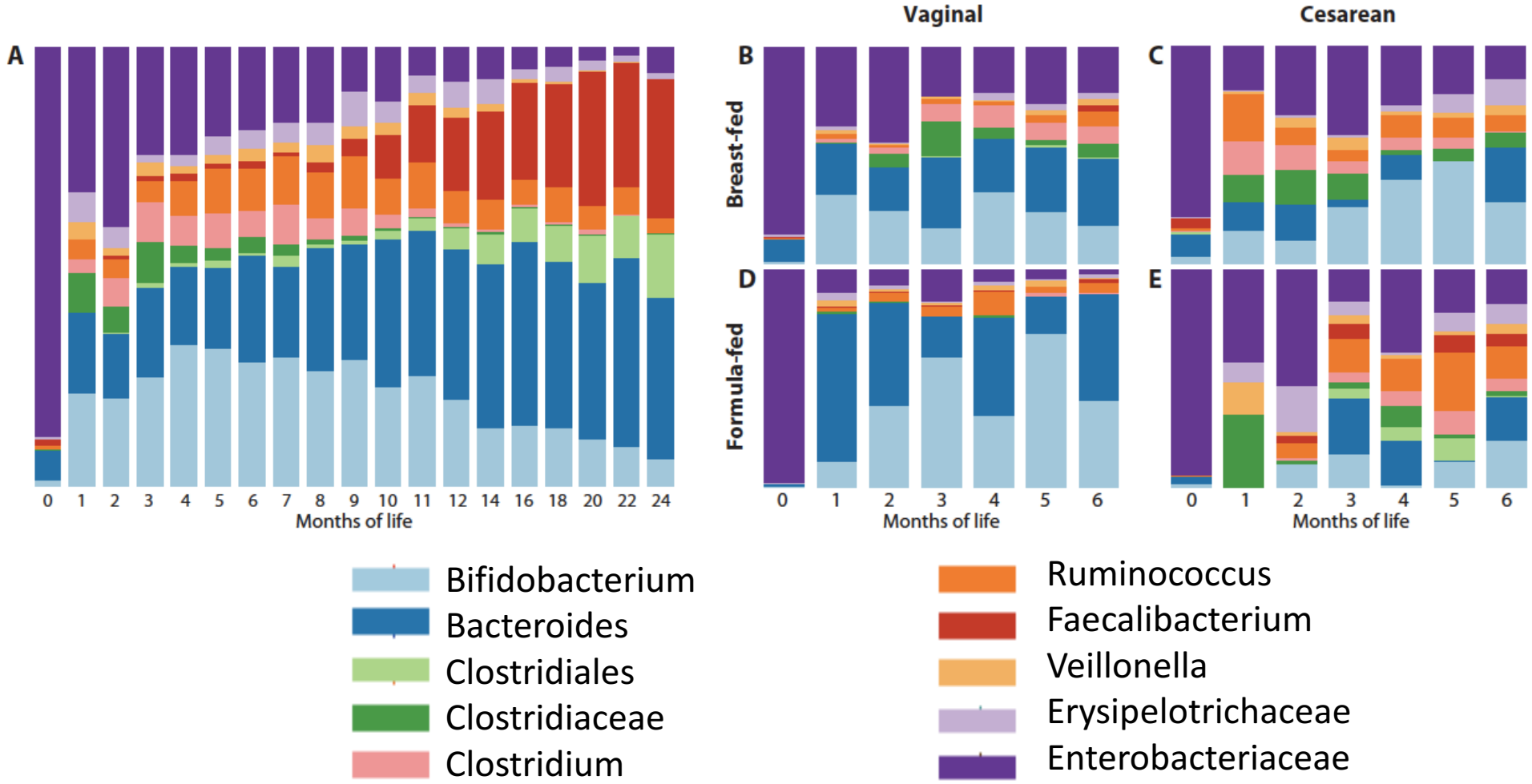
MG Dominguez Bello, E Costello, et al. PNAS 2010

Similarity of adult and child microbiota



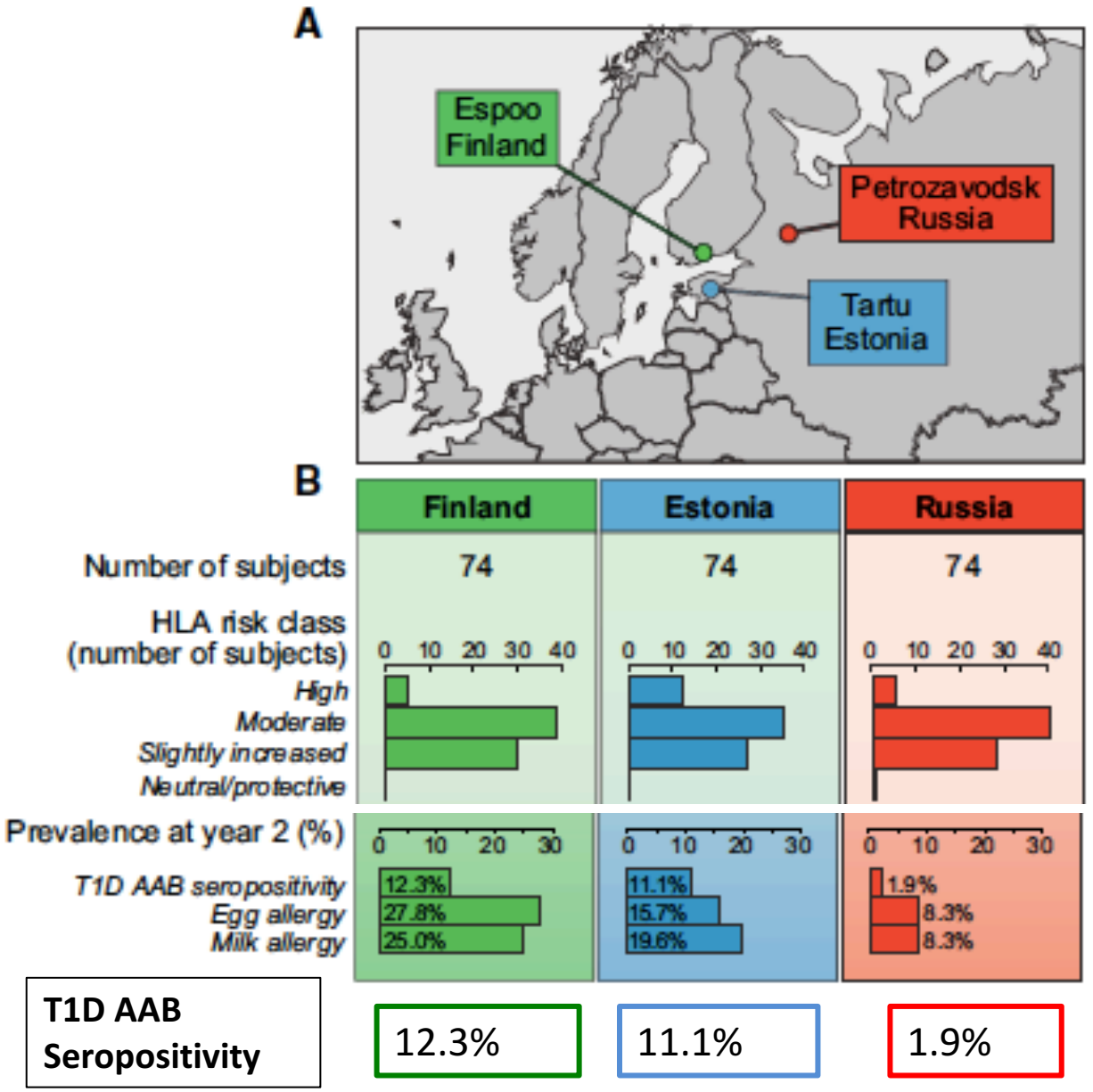
Yatsunenko, Nature 2012

Intestinal microbiota composition over the first two years of life



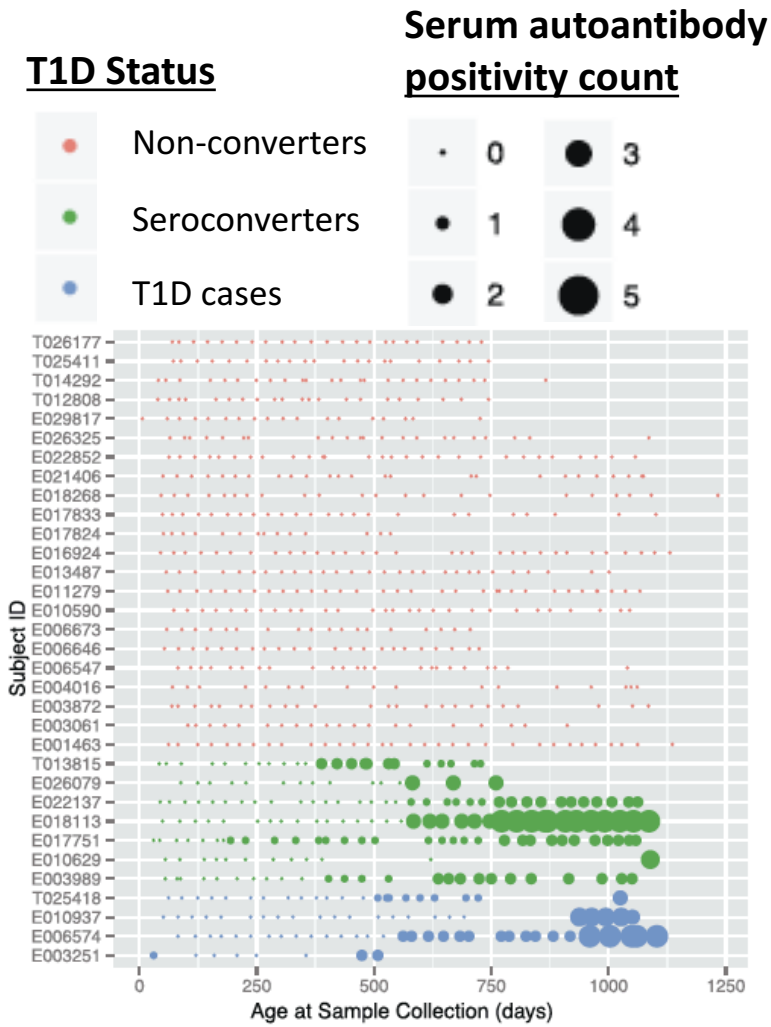
Bokulich et al, Science Translational Medicine, 2016

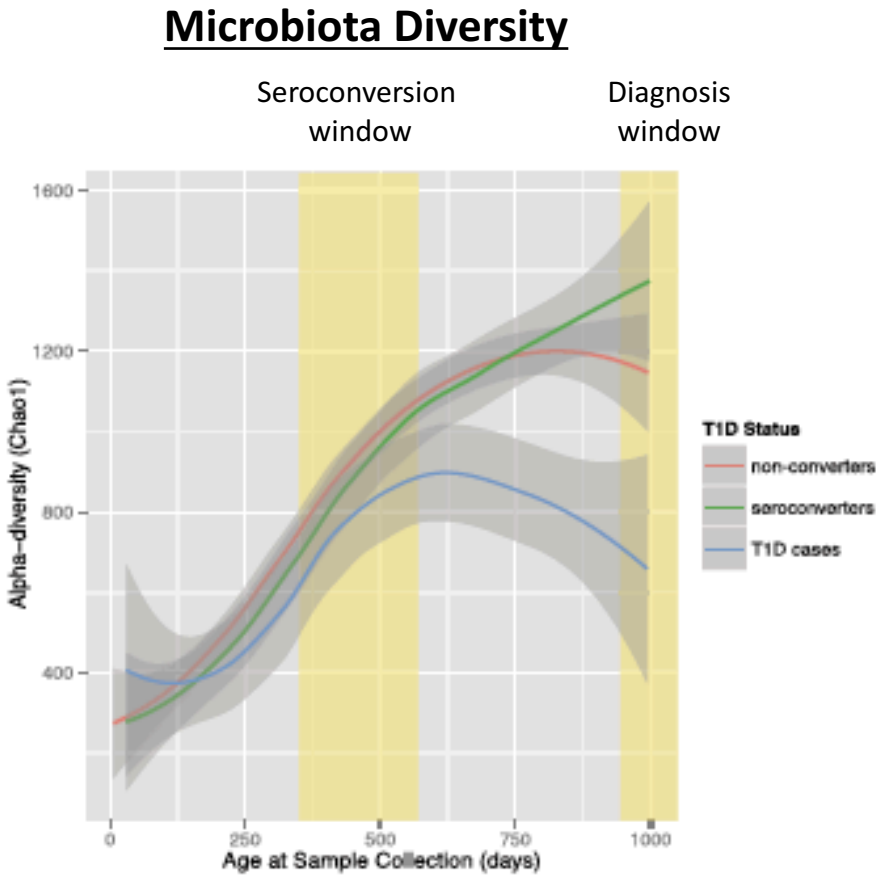
Regional variation in T1D autoantibodies



Vantanen et al, Cell, 2016

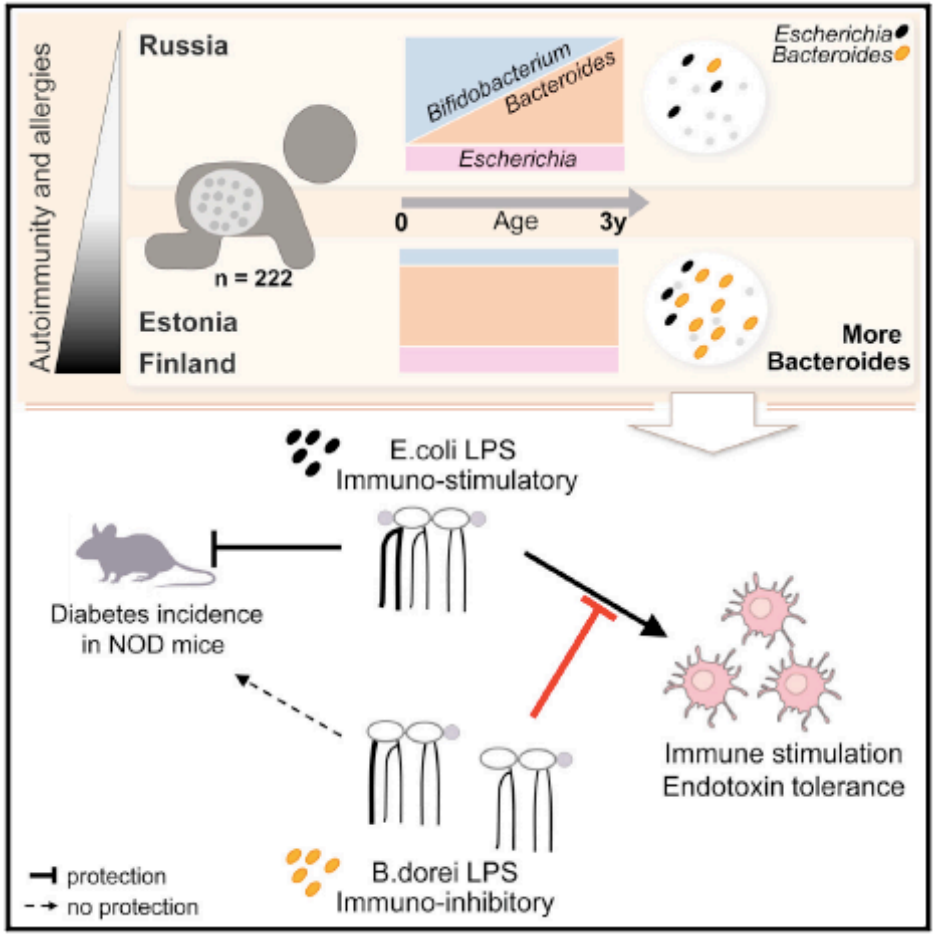
Early-life microbiota and T1D development in 33 at-risk infants from Finland and Estonia

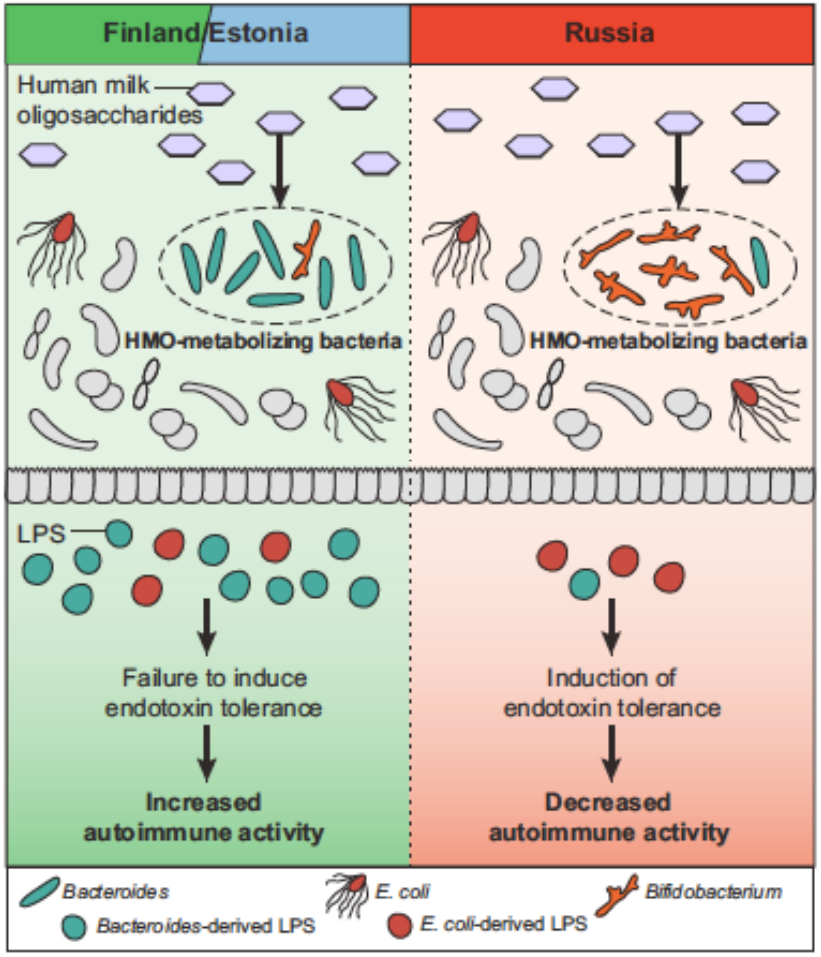




Kostic et al, Cell Host and Microbe, 2015

Region-specific early-life microbiota may protect against autoimmune disease





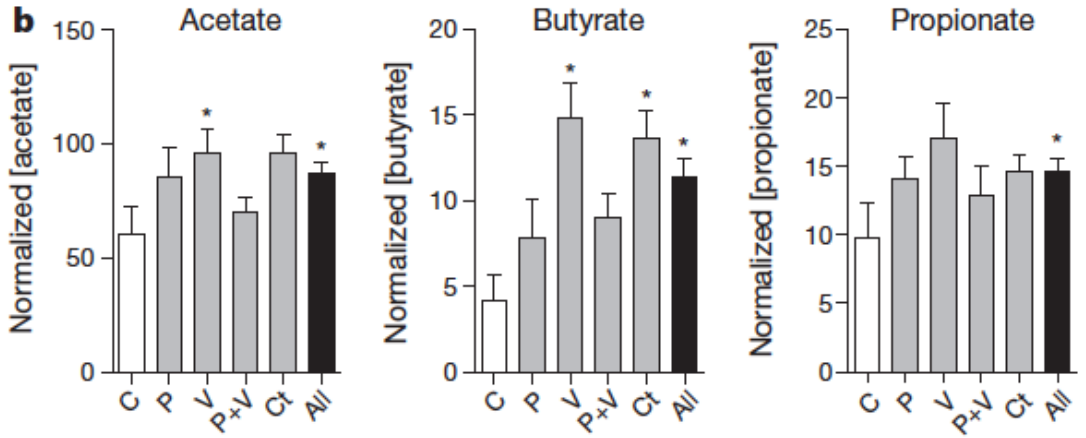
Potential Mechanisms

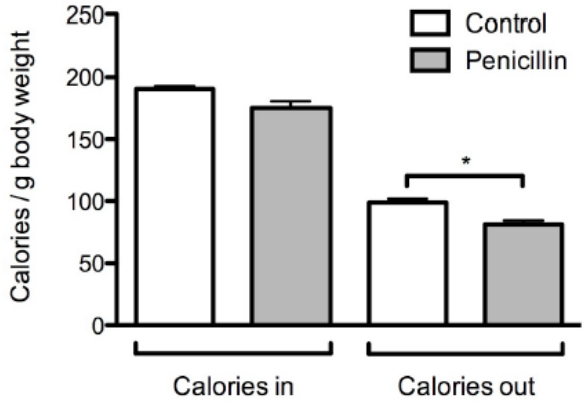
1. Altered milk-derived metabolites
2. Systemic immune tolerance induction

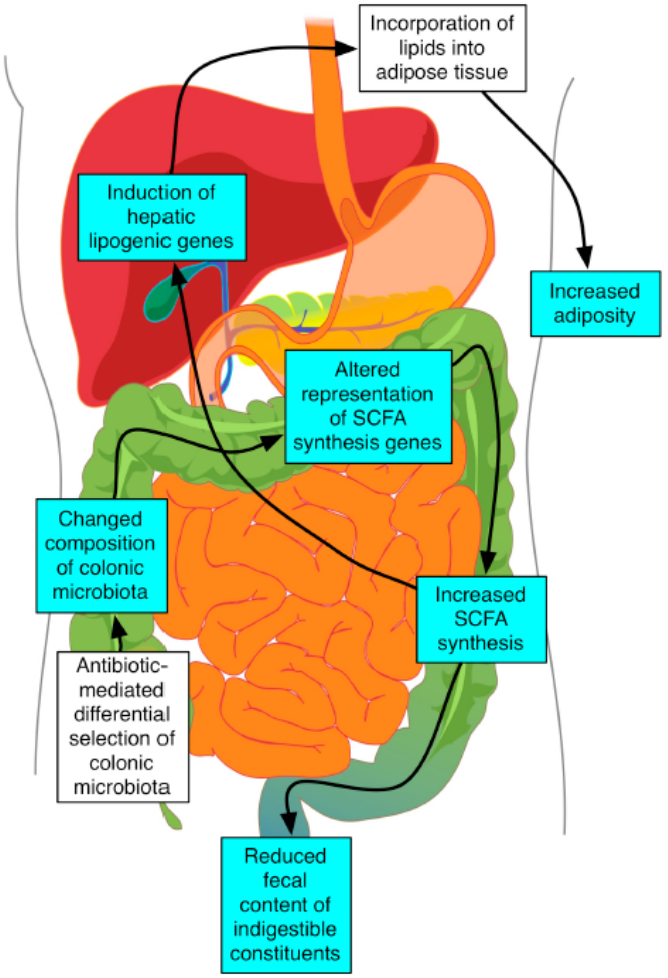
Vantanen et al, Cell, 2016

Systemic effects of sub-therapeutic antibiotic treatment

Increased cecal short-chain fatty acids



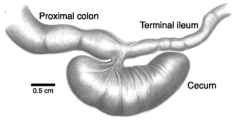




Cho, Nature, 2012

Isolating *Allobaculum*

4 Microbiota Samples

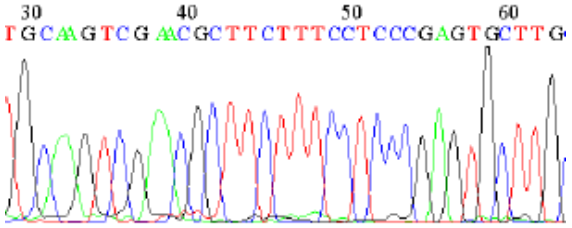


- Frozen mouse cecum
- Microbiota transfer inoculum
- Fresh mouse small intestine
- Fresh mouse cecal contents

Cultivation

Isolated 188 bacteria in anaerobic conditions

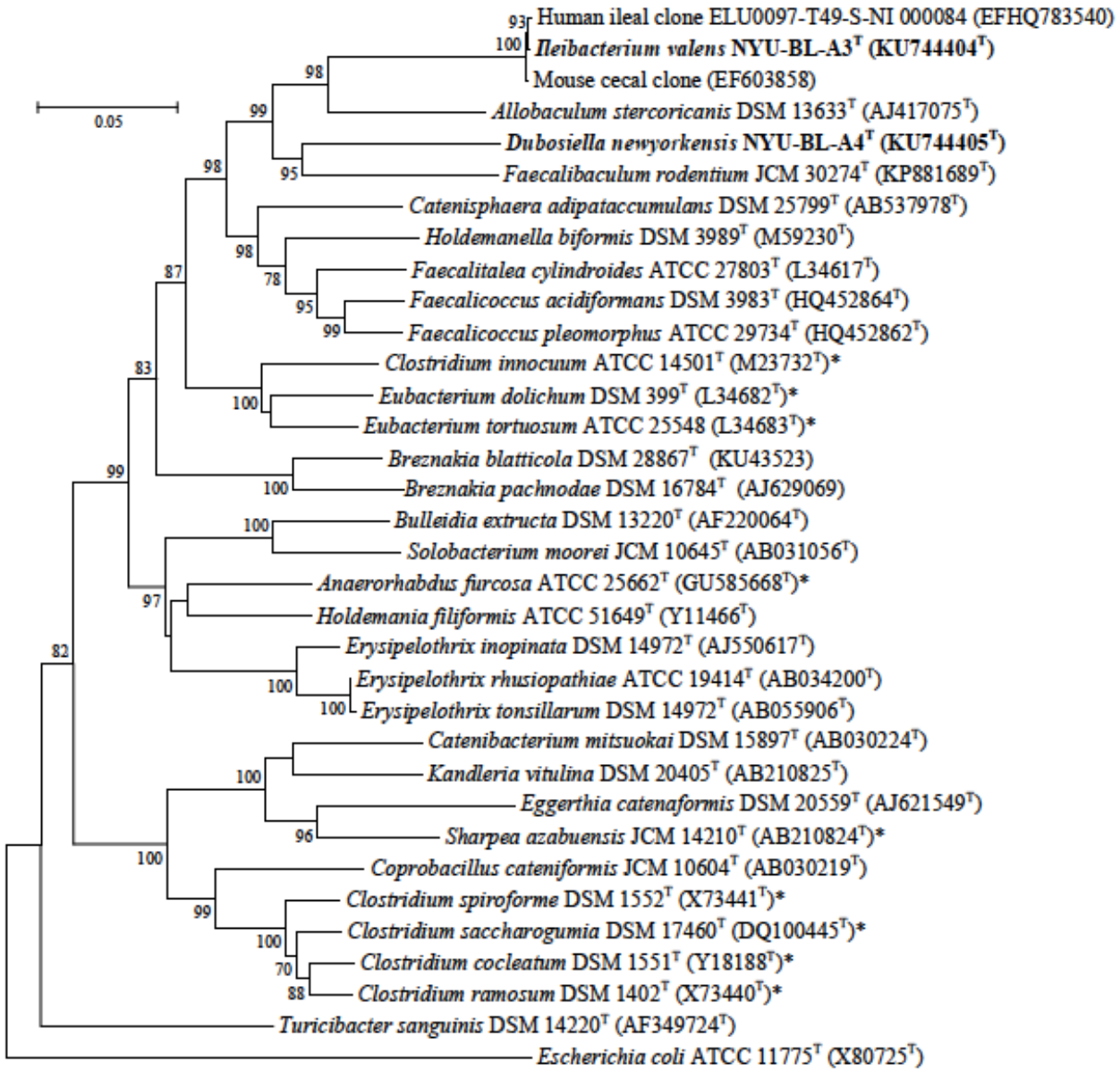
Identification



Sequenced near full length microbial 16S rRNA

Recovered 111 Allobaculum-like isolates

Phylogeny of *Allobaculum*-like isolates



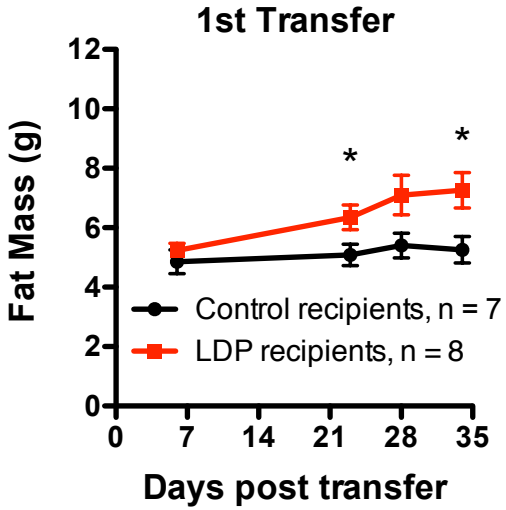
Ileibacterium valens
83 isolates recovered

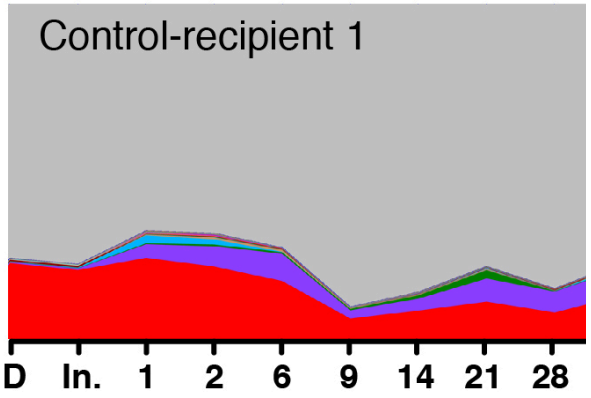
Dubosiella newyorkensis
17 isolates recovered

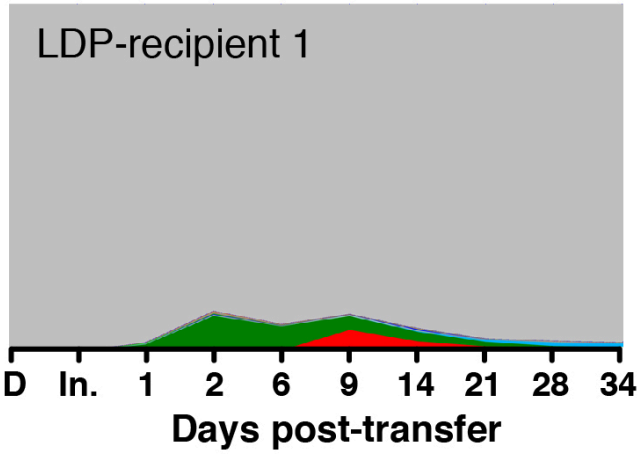
Faecalibaculum rodentium
11 isolates recovered

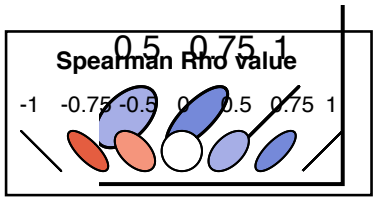
Cox et al, IJSEM, 2017

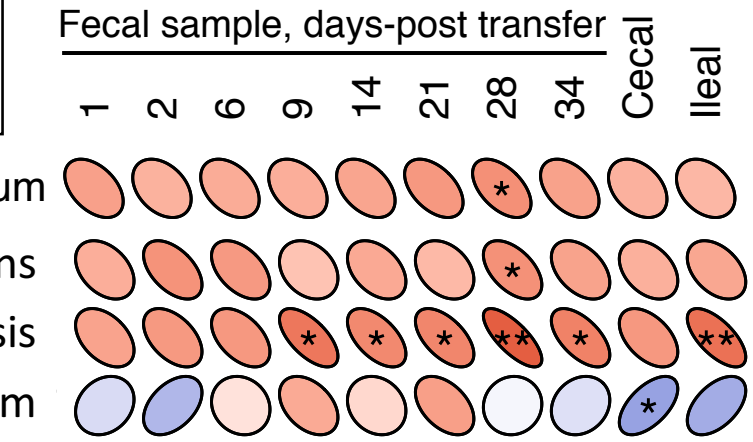
Strain specific correlations

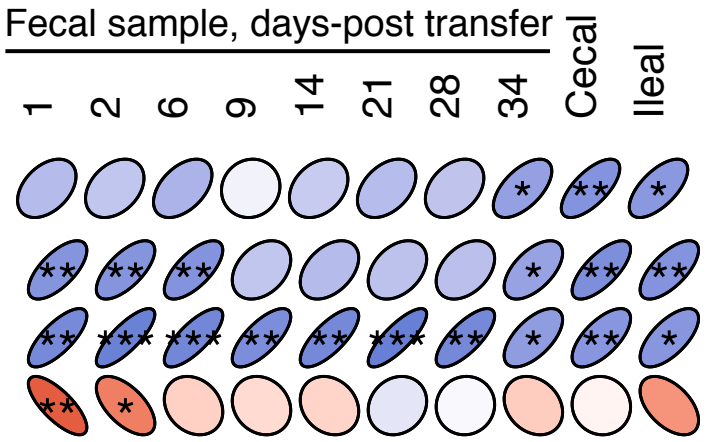




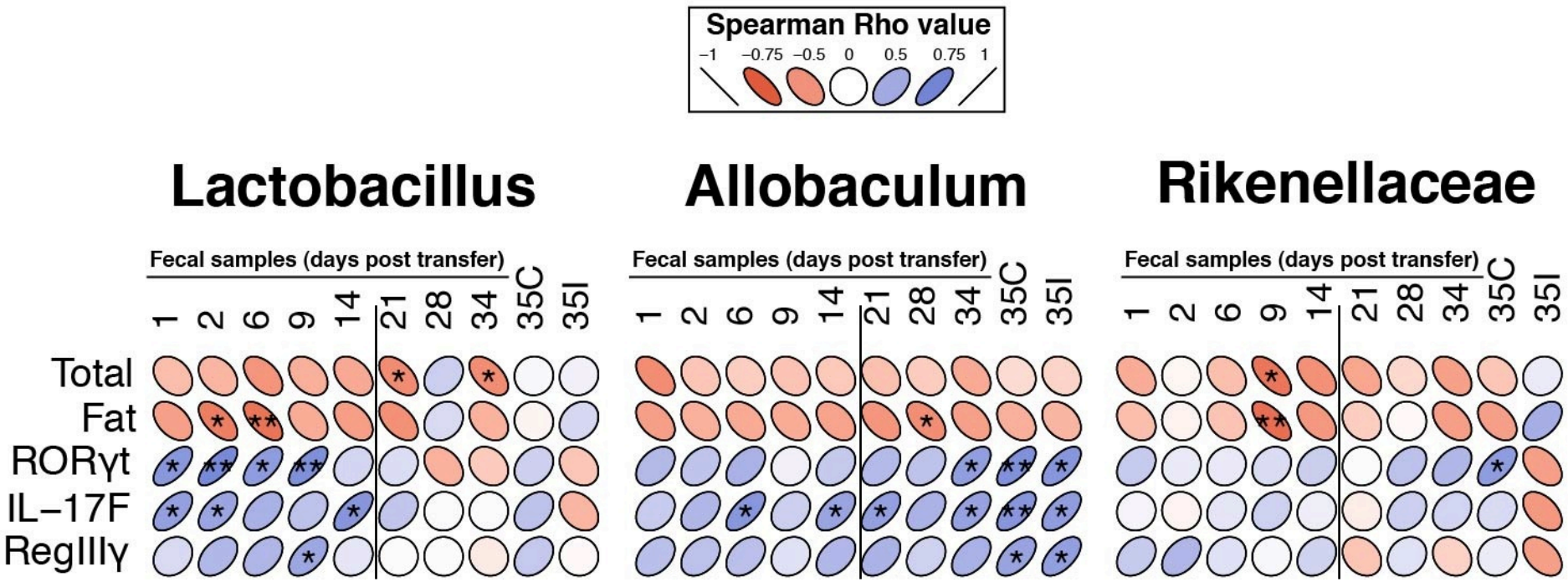






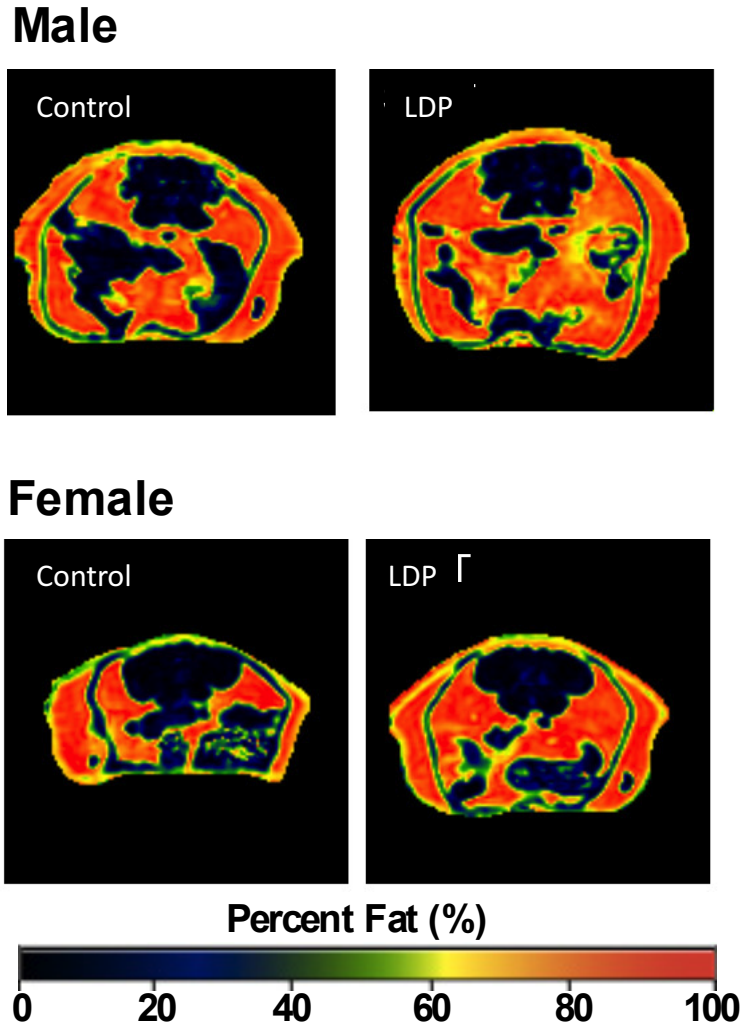


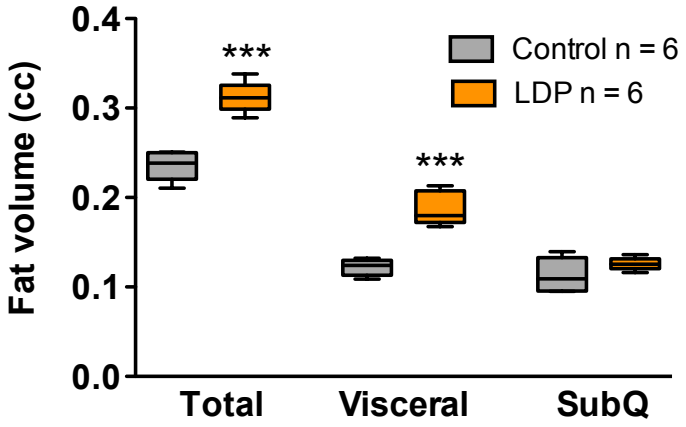
Microbe-host correlations

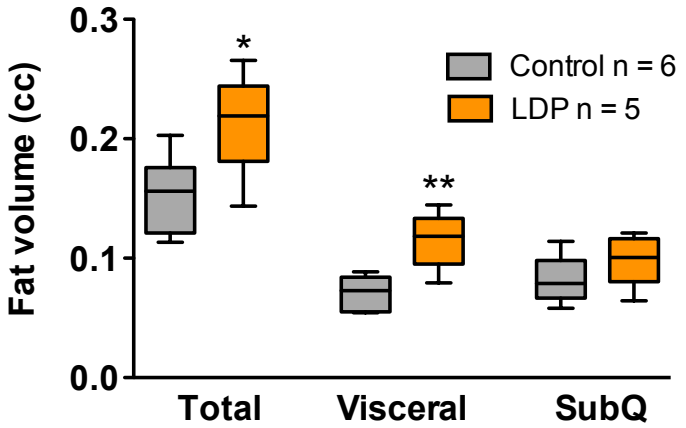


* P < 0.05. ** p < 0.01 Spearman correlation

The effect of LDP on body fat distribution







Cox et al., Cell, 2014

26-weeks of age, HFD
* $p < 0.05$, ** $p < 0.01$ T-test

Predicted effect of altered-microbiota on ileal immunologic function

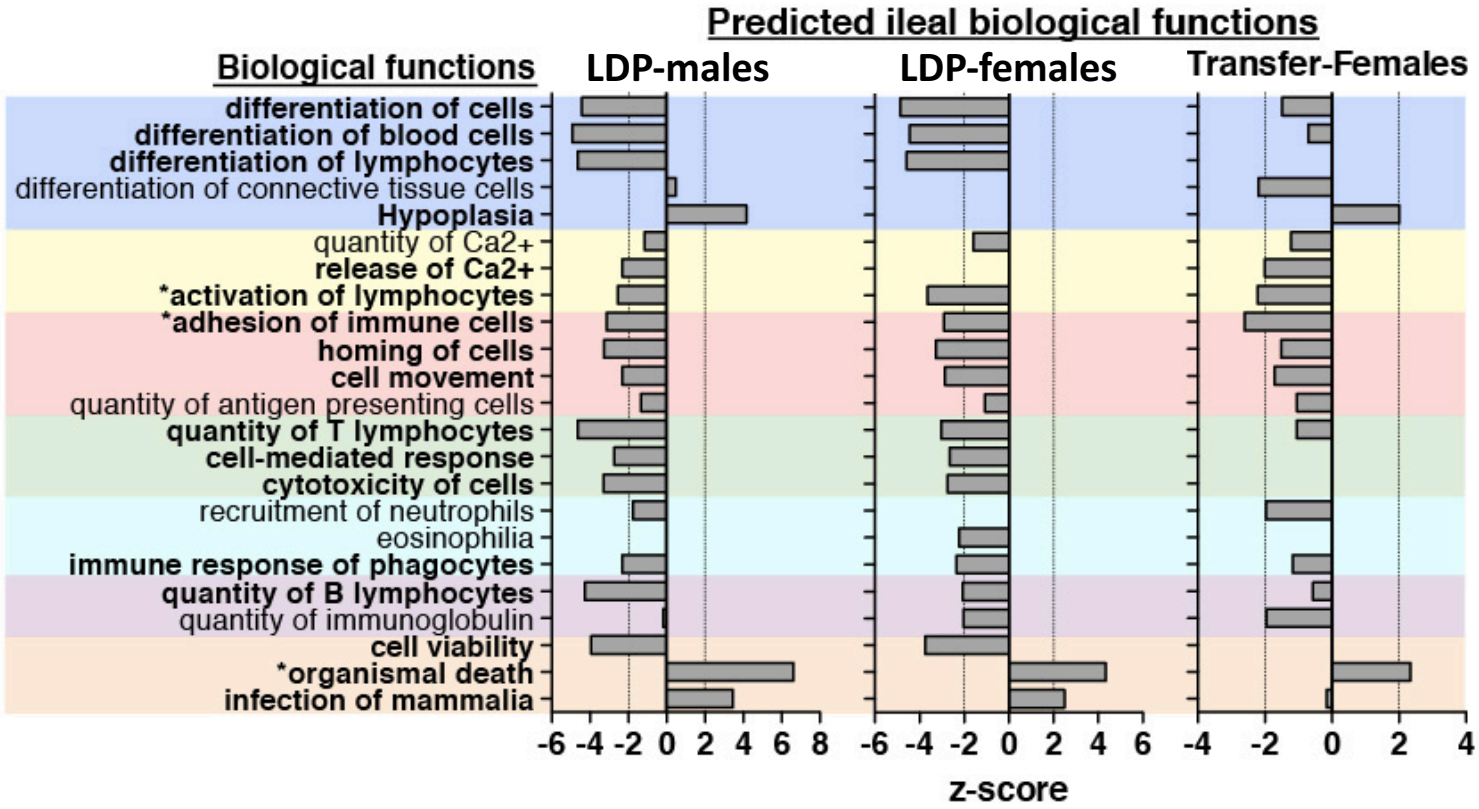
Extract ileal RNA from 8-week old control and STAT mice and microbiota recipients → **Quantify** expression of 547 immunology genes → **Detect** changes at the gene level → **Predict** changes in biological function

Control vs. LDP male
Control vs. LDP female
Control vs. LDP microbiota recipients

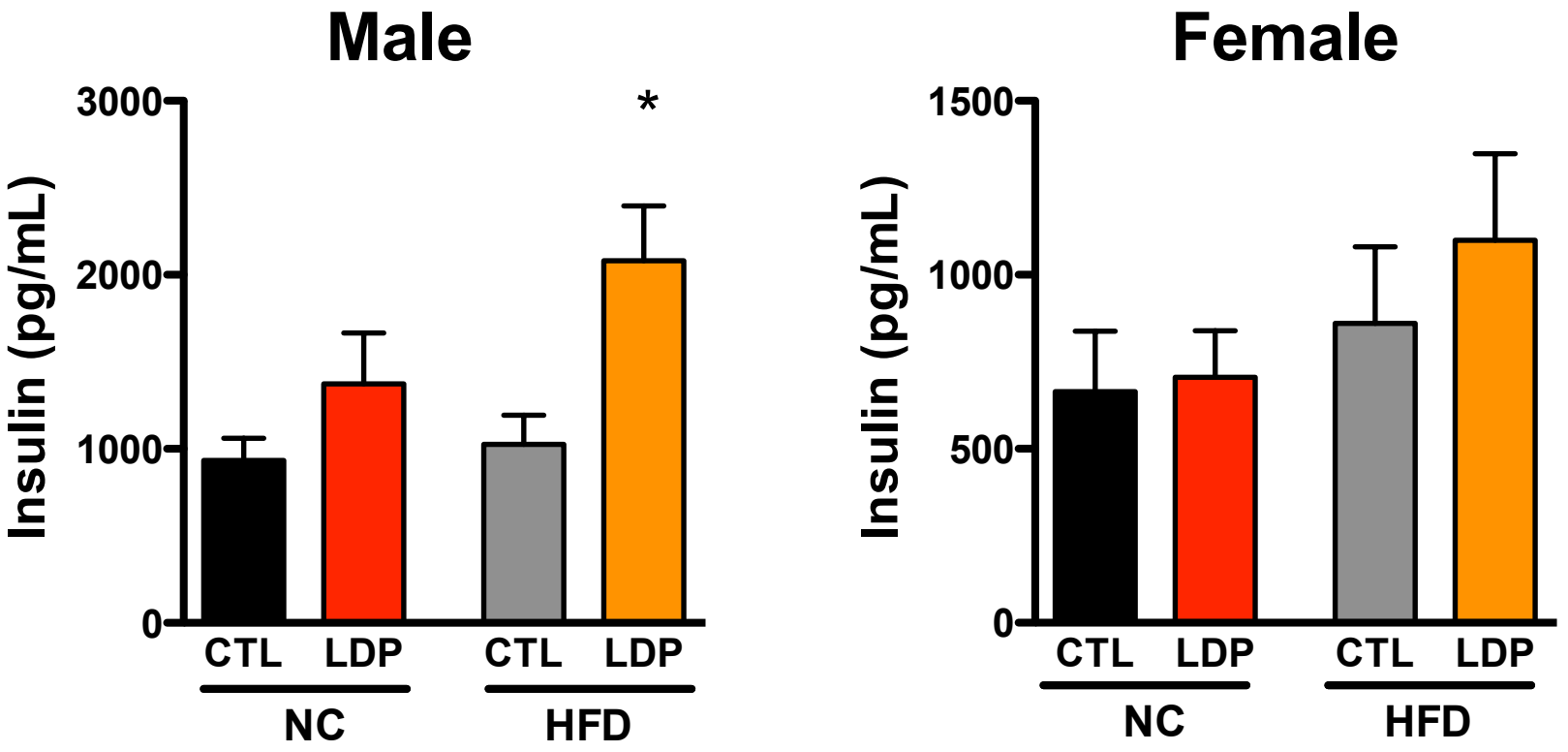
nanoString TECHNOLOGIES

	Up	Down
LDP-Male	1	111
LDP-Female	7	74
LDP-Female	5	16

INGENUITY PATHWAY ANALYSIS

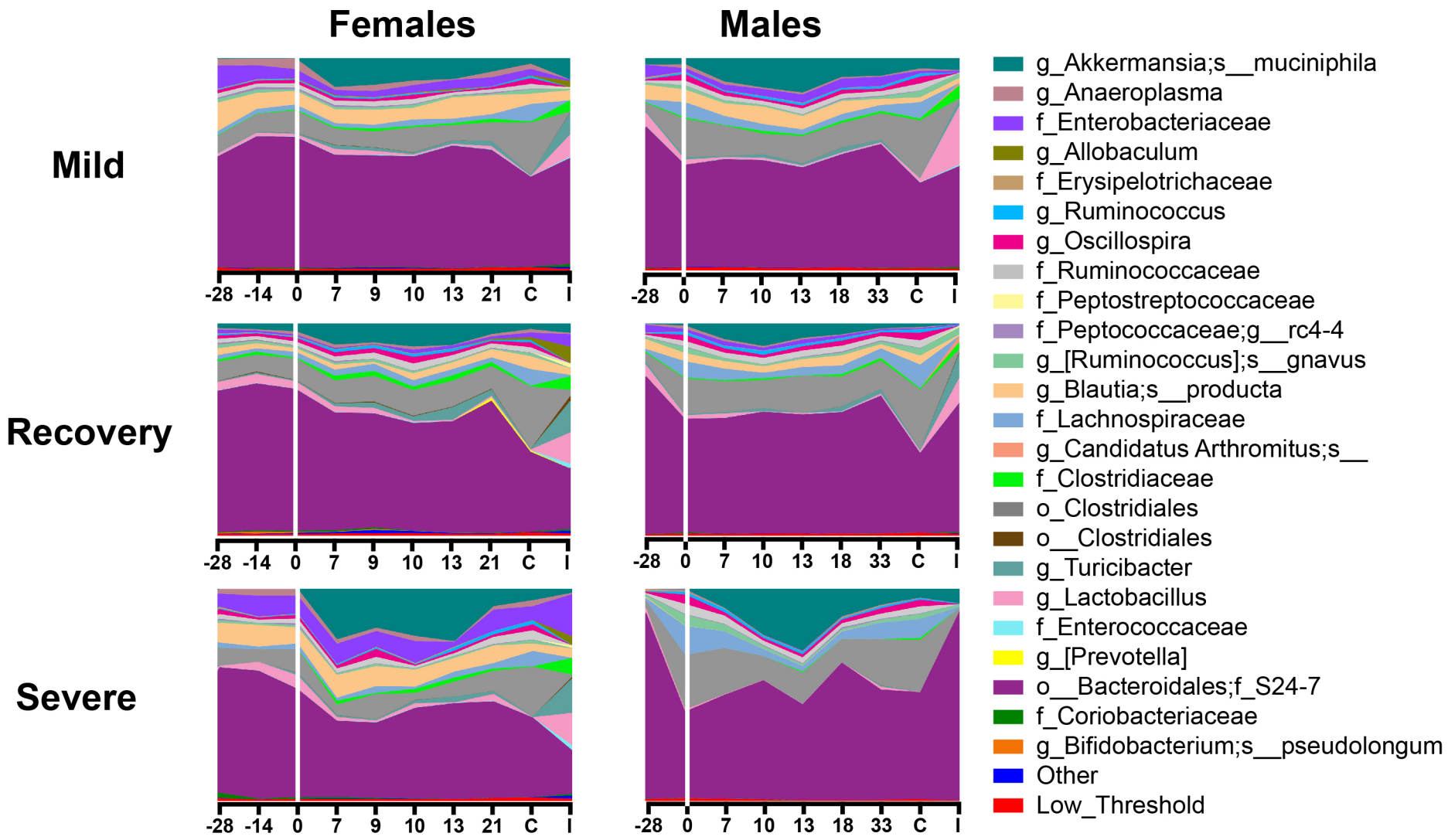


Effect of LDP and HFD on fasting insulin

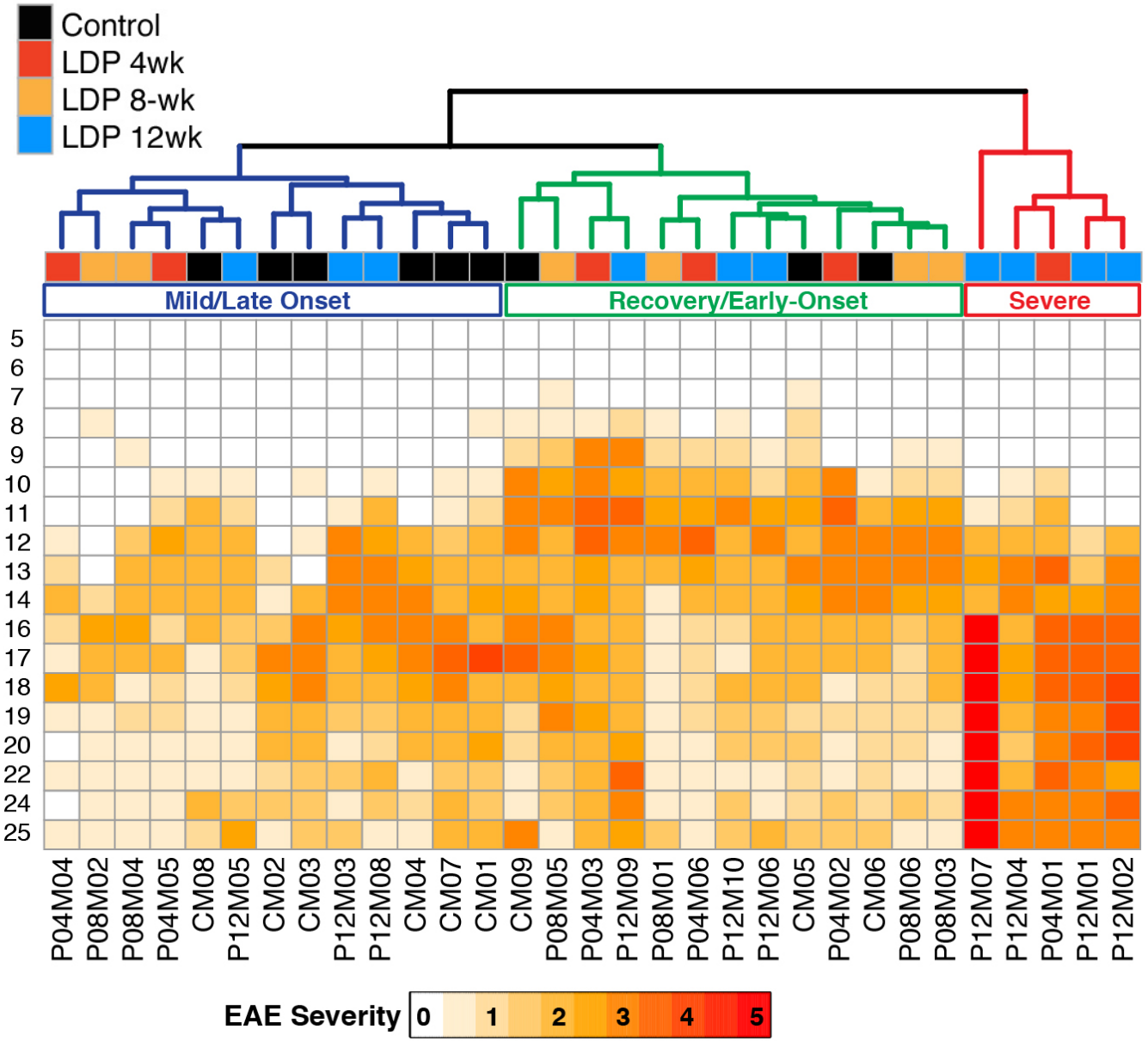


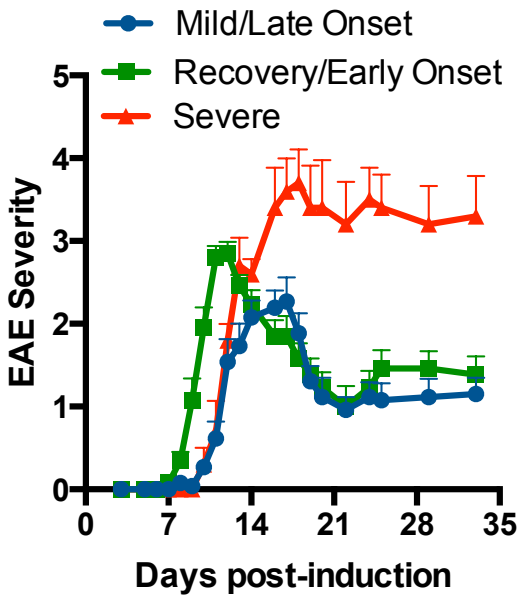
* $p < 0.05$ Mann-Whitney U test, n = 8-10 per group

Microbiota composition by clinical outcomes



Hierarchical clustering of EAE scores in male mice





REPORTS

Sex Differences in the Gut Microbiome Drive Hormone-Dependent Regulation of Autoimmunity

Janet G. M. Markle,[1,2] Daniel N. Frank,[3] Steven Mortin-Toth,[1] Charles E. Robertson,[4] Leah M. Feazel,[3] Ulrike Rolle-Kampczyk,[5] Martin von Bergen,[5,6,7] Kathy D. McCoy,[8] Andrew J. Macpherson,[8] Jayne S. Danska[1,2,9*]

- Male NOD mice are have a lower incidence of type I diabetes
- Male microbiota can protect female mice from from TID
- Associated with increased levels of testosterone in female mice colonized with male microbiota