The Harvard T.H. Chan Center for the Microbiome in Public Health

Human microbiome science is at a unique point in history: a solid foundation of basic biology and translational infrastructure has been created by the research community, and it remains to apply in therapeutic and prognostic settings.

The microbiome has been strongly associated with health phenotypes from autism to cancer, but taking advantage of these associations to develop live cell therapies, microbially-derived bioactives, or ecological biomarkers of outcome or treatment response requires population-scale validation.

Much as human genetic epidemiology has, thanks to public health research, begun to make the leap from academic research to commercial applications, microbiome epidemiology is approaching the same opportunity.

The Harvard T.H. Chan Center for the Microbiome in Public Health (HCMPH) was thus created as an environment for academic-industry partnerships in this space – a resource for the entire life sciences ecosystem to realize these opportunities.

The Center's scientific foundation is unparalleled, comprised of:

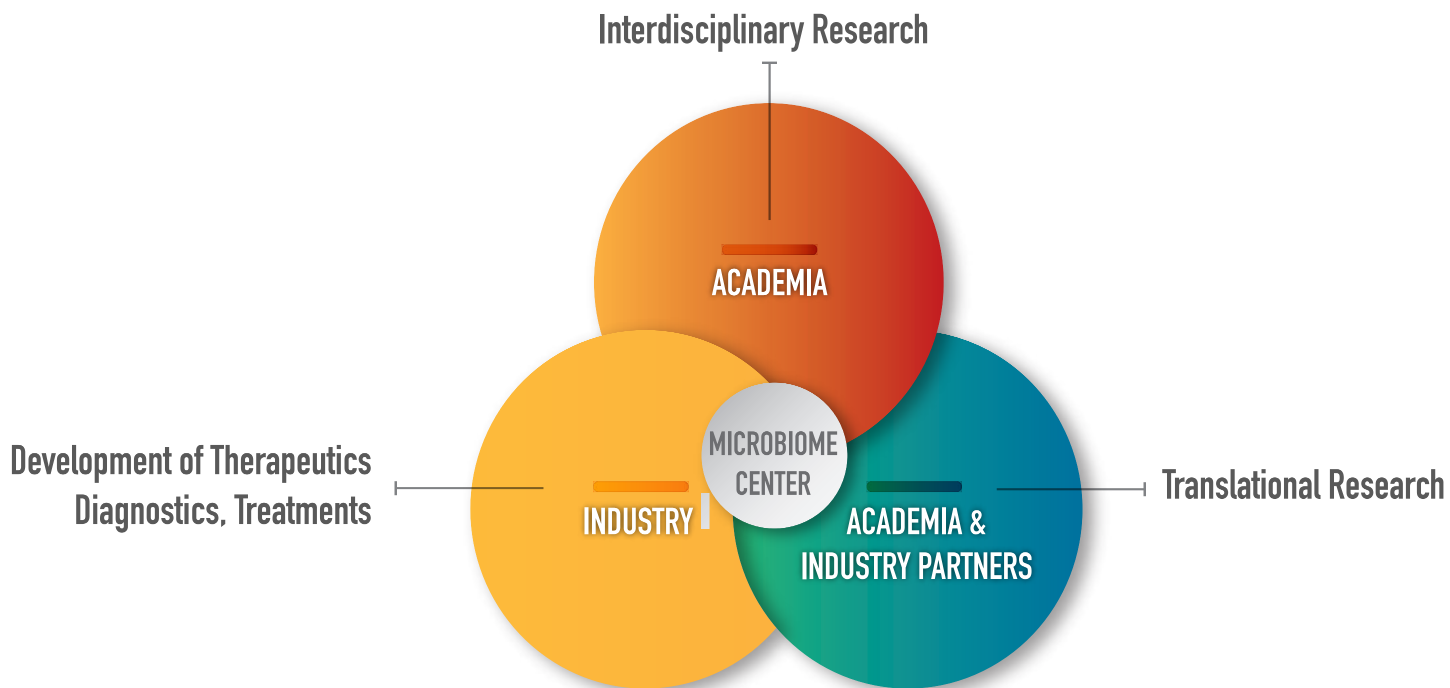
- *Pioneering research by Harvard Chan faculty around the interplay of human and microbial systems.*
- *Robust research platforms, including high-throughput microbiome sampling, multi'omic data generation, immunoprofiling, a gnotobiotic facility, and the computational Microbiome Analysis Core.*
- *Harvard's Longitudinal Cohort Studies, a unique epidemiologic resource with biennial participant data collected from more than 200,000 participants over 30+ years, including measures of lifestyle, behavior, and characterization of over 60 diseases. These studies have generated more than 3.5 million biospecimens, and make it possible to study links between lifestyle, metabolism, genetic susceptibility, and disease.*

The HCMPH is home to the BIOM-Mass platform for population-scale microbiome studies, which has developed a standardized oral and gut microbiome sampling kit, dietary and environmental surveys to detail microbiome samples, and cost-effective means of collecting molecular and microbiological culture data from home-collected samples.

The platform's ongoing flagship collection comprises 25,000 stool and oral microbiome samples from a subset of cohort participants, with capacity to collect additional samples from targeted populations/phenotypes.

To facilitate a substantive, symbiotic relationship between academia and industry and to ensure that the School's microbiome research platform is valuable across sectors, HCMPH is establishing the HCMPH Microbiome Consortium.

Select partner companies will be invited to join the Consortium for a one-time membership fee of $1 million, funds that will fast-track the collection's evolution into a scientific resource with maximum utility to academia and industry.



Benefits of Consortium membership

Access to longitudinal metagenomic, metatranscriptomic, and metabolomic data (both raw and pre-processed) with exclusivity period

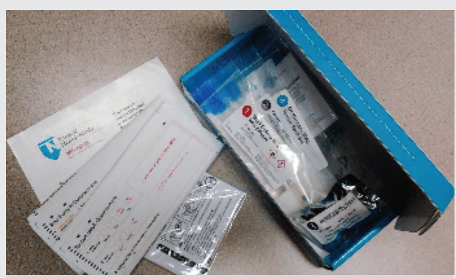
Input on the prioritization of sample profiling, creation of targeted collections, subcohorts, research inquiries, and other projects

Collaborative data sharing, discovery advancement and project planning with Harvard faculty

Unique partnership with the Harvard Chan School to advance research and development in the life sciences

The HCMPH Microbiome Consortium will:

1 Fast-track sample sequencing and further development of the microbiome platform.



Nurses' Health Study: Micro N Kit

User instruction trifold brochure, stool sample questionnaire, tongue swab questionnaire, toilet accessory, stool accessory pack barcode labels, bio specimen bags with absorbent pads, stool collection kit with 95% ethanol,OMNIgene.-GUT stool collection kit, anaerobic stool collection kit, OMNIgene.ORAL tongue swab kit and air cushion

- Shotgun metagenomic and metatranscriptomic data generation for samples from subject phenotypes of interest to Consortium members.
- Metabolomic profiling of a subset of samples, chosen based on disease phenotypes, environmental factors (e.g. diet), or (after receipt of initial metagenomic data) microbiome configurations.
- Follow-up metagenomic/metabolomic profiling of additional samples, cross-sectionally or longitudinally.
- Computational and biostatistical analysis of the data above, including data informatics, quality control, biological feature extraction (microbial taxonomic, functional, strain profiling, and assembly), and collaborative statistical epidemiology associating the microbiome with disease outcomes, cohort covariates (e.g. medical history, medications, demographics, biometrics, diet), and existing molecular data (e.g. genetics and serum metabolites).

2 Leverage the microbiome's full research potential by connecting expertise from academia and the life sciences industry.

The Consortium model will provide a framework for data sharing, discovery, and project planning. Together, member companies and Harvard Chan faculty will identify ways in which the BIOM-Mass platform and Harvard cohorts can be leveraged to better understand disease predisposition, development, and progression, and suggest promising – and practical – research lines and projects. Through these exchanges, new opportunities will emerge for industry R&D, collaborative research projects, additional/more detailed collection from subcohorts, and novel basic research.

3 **Provide HCMPH with industry expertise and a translational perspective, inform priority setting and platform development, and ensure that the data generated is valuable, actionable, and comprehensive.**

Consortium members will have the opportunity to help Harvard Chan prioritize the microbiome profiling process so that data generated are as scientifically valuable and immediately actionable as possible. Member companies can also offer valuable insights on further investments in infrastructure, how and to whom samples and sample data are accessible, and how to ensure the HCMPH is a user-friendly, robust scientific resource

4 **Facilitate the development of therapeutics and diagnostics by connecting bench and bedside from the outset.**

Carried out collaboratively, microbiome research will open the door to innovation in disease prevention, diagnostics, early detection, therapeutics and chemoprevention, and precision medicine. Real-world application of these innovations will happen more quickly, more effectively, and more consistently when we forge close ties between academia and industry. To that end, we will seek input from consortium members on:

- Intervention trials of pharmaceuticals, dietary compounds/supplements, or lifestyle modifications, with microbiome samples collected and analysis conducted before, during, and/or after, spanning up to several months each.
- Microbiological isolation, genetics, and functional characterization of individual strains, microbial gene products, or small molecule metabolites implicated in chronic disease.
- Murine validation of microbiome-associated traits, including the humanization of gnotobiotic mice based on specific human donors; dietary, small molecular, or microbial strain interventions; and host immune and molecular profiling.

ENDPOINTS	FULL N=116,429**	WITH BLOOD SAMPLES**	WITH CHEEK SWABS**
Colon polyps	9042	3649	3106
Diabetes	7896	2001	2147
MS cases	533	185	199
Breast cancer	3203	964	1159
Melanoma	609	202	200
IBD UC	535	161	202
RA disease	519	169	177
Ovarian cancer	269	78	95
Colon/rectal cancer	292	92	97

**Alive by the end of 2015

NHS2 CANCER INCIDENCE

CANCER SITE	1989-2021***
Breast	6397
Ovarian	805
Cervical	79
Endometrial	1020
Melanoma	828
Thyroid	584
Colorectal	672
Lung	554
Kidney	286
Brain	173
Connective Tissue	128
Pancreatic	154
Bladder	157
Leukemias/Lymphomas	788

***Projected Total Through 2021

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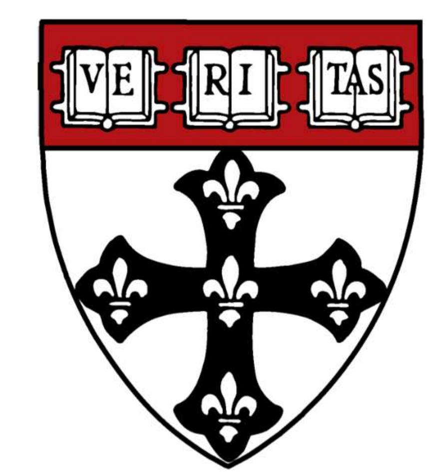
Distinct Actions of the Fermented Beverage Kefir on Host Behaviour, Immunity and Microbiome Gut-Brain Modules in the Mouse

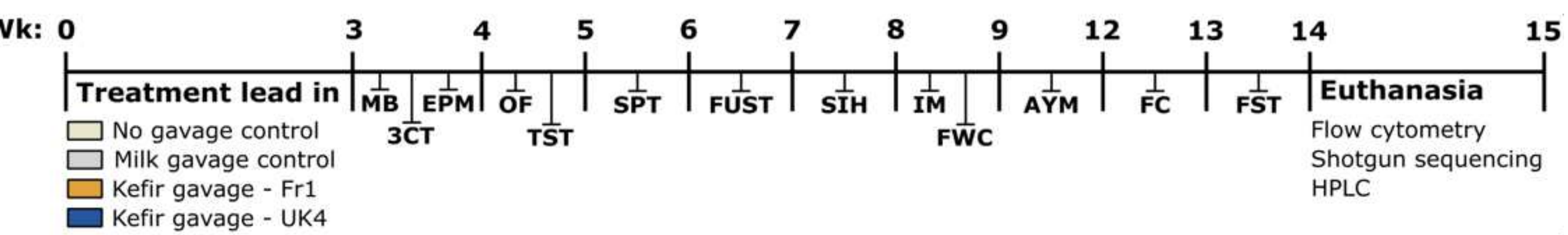


Aaron M. Walsh[1,2,3,4,5], Marcel van de Wouw[4,5], Fiona Crispie[3,4], Lucas van Leuven[4], Joshua M. Lyte[4], Marcus Boehme[4], Gerard Clarke[4,5], Timothy G. Dinan[4,5], John F. Cryan[4,5], Paul D. Cotter[3,4]

[1]Broad Institute of MIT and Harvard [2]Harvard T.H. Chan School of Public Health
[3]Moorepark Teagasc Food Research Centre [4]APC Microbiome Ireland [5]University College Cork

Mounting evidence suggests a role for the gut microbiota in modulating brain physiology and behaviour through bi-directional communication along the gut-brain axis. As such, the gut microbiota represents a potential therapeutic target for influencing centrally-mediated events and host behaviour. It is thus notable that the fermented milk beverage kefir has recently been shown to modulate the composition of the gut microbiota in mice. It is unclear whether kefirs have differential effects on microbiota-gut-brain axis and whether they can modulate host behaviour. To address this, two distinct kefirs (Fr1 and UK4) or unfermented milk control were administered to mice that underwent a battery of tests to characterise their behavioural phenotype. In addition, shotgun metagenomic sequencing of ileal, cecal and faecal matter was performed, as was faecal metabolome analysis. Fr1 ameliorated reward-seeking behaviour, while UK4 decreased repetitive behaviour. In the peripheral immune system, Fr1 reduced neutrophil levels, while UK4 increased IL-10 levels. Analysis of the gut microbiota revealed that both kefirs significantly changed the composition and functional capacity of the host microbiota. Furthermore, both kefirs increased the capacity of the gut microbiota to produce GABA, which was linked to an increased prevalence in *Lactobacillus reuteri*. Altogether, these data show that kefir can signal through the microbiota-gut-immune-brain axis and modulate host behaviour. In addition, different kefirs may direct the microbiota toward distinct immunological and behavioural modulatory effects. These results indicate that kefir can positively modulate specific aspects of the microbiota-gut-brain axis.

Study design and methodology

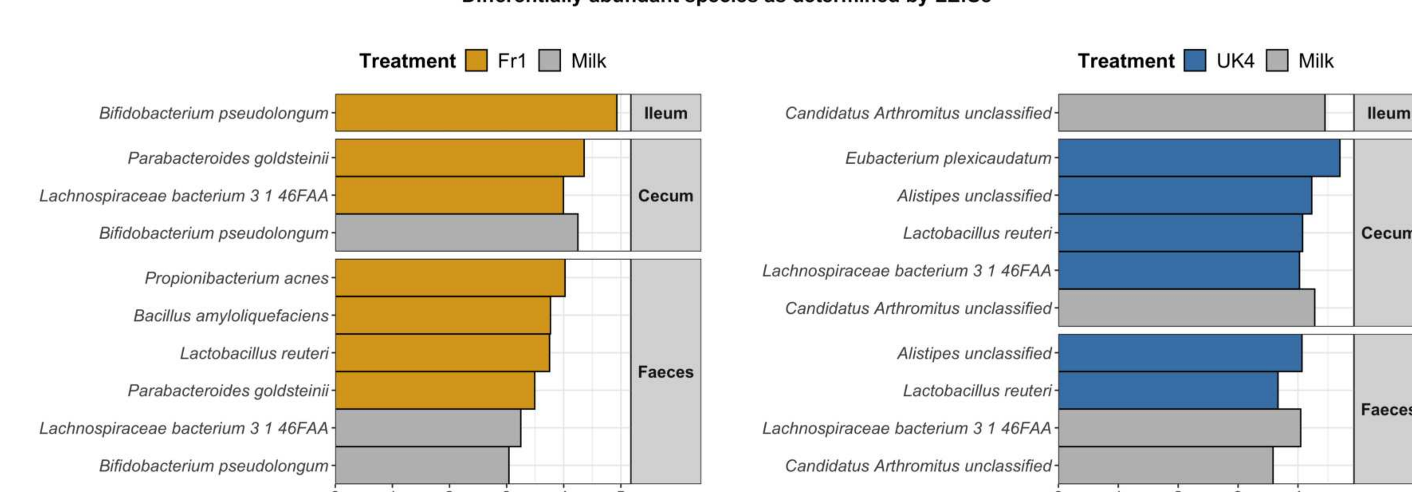


12 mice per group: (i) no gavage control, (ii) milk gavage control, (iii) Fr1, and (iv) and UK4.

Behavioural tests: marble burying test (MB), 3-chamber social interaction test (3CT), elevate plus maze (EPM), open field test (OF), tail suspension test (TST), saccharin preference test (SPT), female urine sniffing test (FUST), stress-induced hyperthermia test (SIH), intestinal motility test (IM), faecal water content assessment (FWC), appetitive Y-maze, fear conditioning (FC), and forced swim test (FST).

Postmortem analyses: flow cytometry, shotgun metagenomics, and metabolomics.

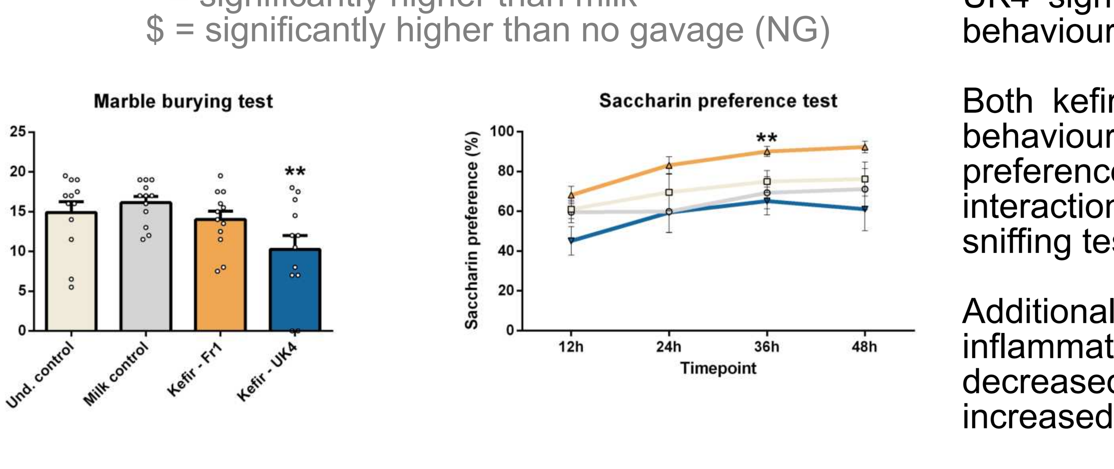
Kefir altered the composition of the mouse gut microbiota

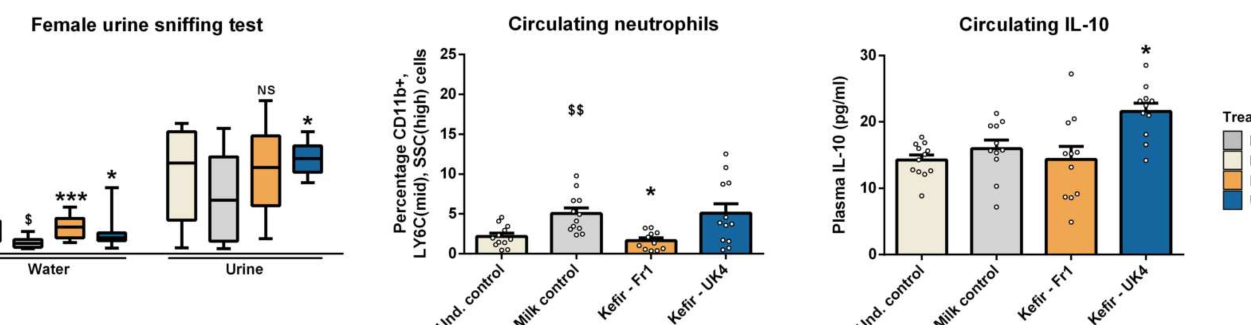


Both kefirs increased *B. pseudolongum*, *E. plexicaudatum*, and *L. reuteri*, while they decreased *B. amyloliquifaciens*, *Lachnospiraceae*, and *P. acnes*.

Kefir induced behavioural and immunological responses in mice

* = significantly higher than milk
$ = significantly higher than no gavage (NG)



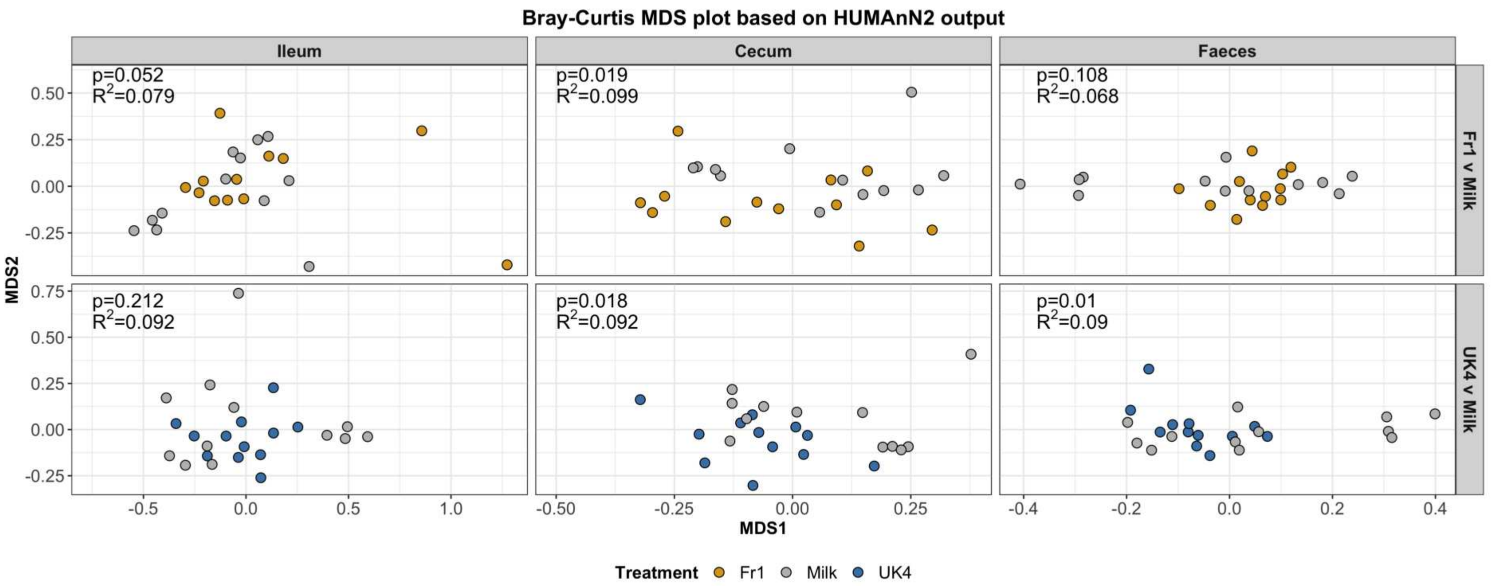


UK4 significantly decreased repetitive behaviour in the marble burying test.

Both kefirs increased reward seeking behaviours: Fr1 increased saccharin preference and UK4 increased interaction time in the female urine sniffing test.

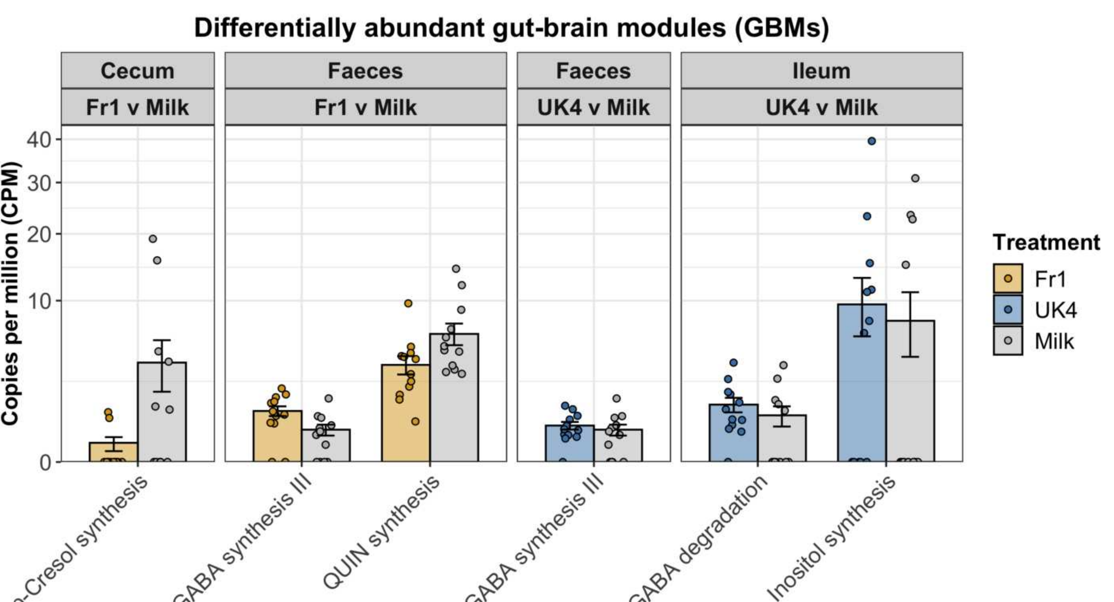
Additionally, both kefirs elicited anti-inflammatory responses: Fr1 decreased neutrophil levels and UK4 increased IL-10.

Kefir modulated the functional potential of the gut microbiome



PERMANOVA of pathway abundances (as measured by HUMAnN2) revealed that the dissimilarity between kefir- and milk-fed mice was significant.

Kefir enhanced the capacity of the microbiome to produce GABA



Gut-brain modules are microbial pathways that produce neurochemicals.

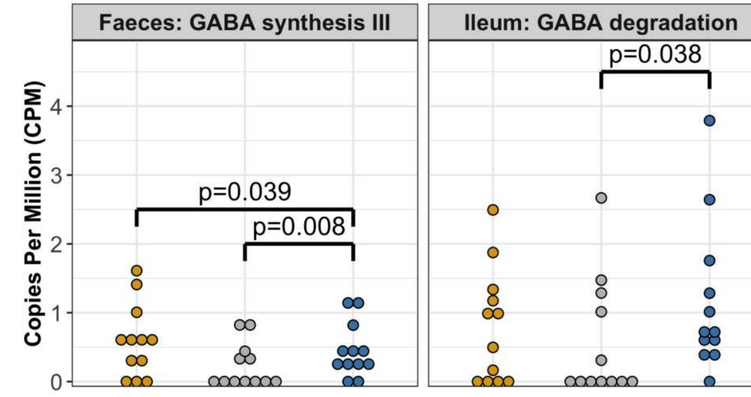
The abundance of GBMs was measured using Omixer-RPM.

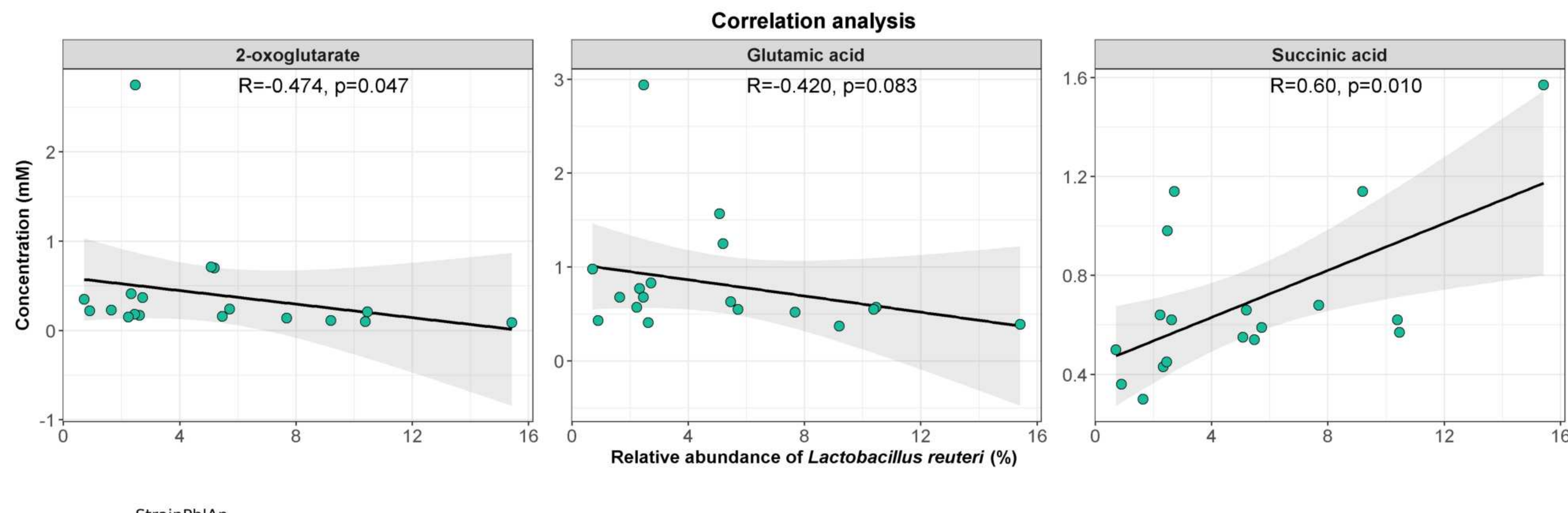
LEfSe indicated that both kefirs significantly increased GABA synthesis III.

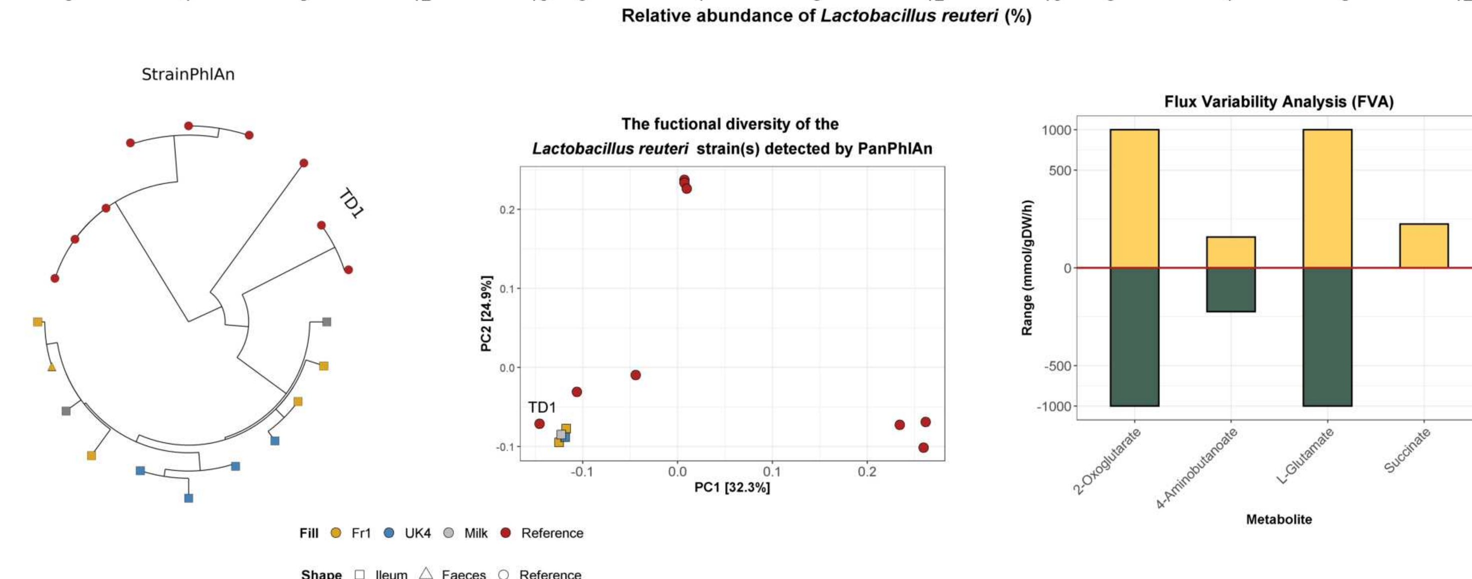
Kefir increased the abundance of a *Lactobacillus reuteri* strain with the potential to produce GABA

GABA synthesis III from *L. reuteri* was significantly increased in the kefir groups

The abundance of *L. reuteri* correlated with the concentrations of GABA-associated compounds in the gut.







Strain-level metagenomic analysis of *L. reuteri* was performed using StrainPhlAn (**left**) and PanPhlAn (**middle**), both of which indicated that the *L. reuteri* strain present in the gut of Fr1-, UK4-, and Milk-fed mice was most closely related to *L. reuteri* TD1.

Assembly-based metagenomic analysis of *L. reuteri* was performed as follows: (i) the metagenome was co-assembled with MEGAHIT; (ii) contigs were binned with MetaBAT 2; (iii) the quality of bins was determined with CheckM; (iv) bins were classified with Kaiju; (v) a metabolic model of the the recovered *L. reuteri* genome was built with CarveMe; and (vi) Flux Variability Analysis (FVA) was performed with COBRApy to simulate the metabolism of *L. reuteri* at 95% growth. The ranges of rates of consumption/secretion of metabolites is shown here (**right**). FVA indicated that the *L. reuteri* strain was capable of secreting GABA.

Acknowledgments

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http://huttenhower.sph.harvard.edu

Prioritization and annotation of novel bioactive small molecules from the microbiome

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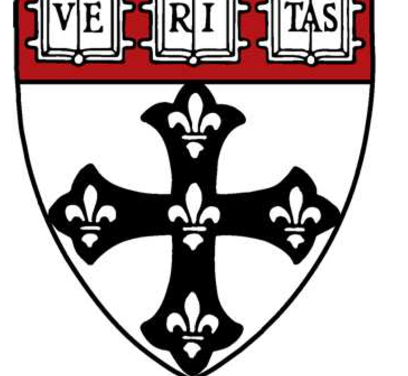
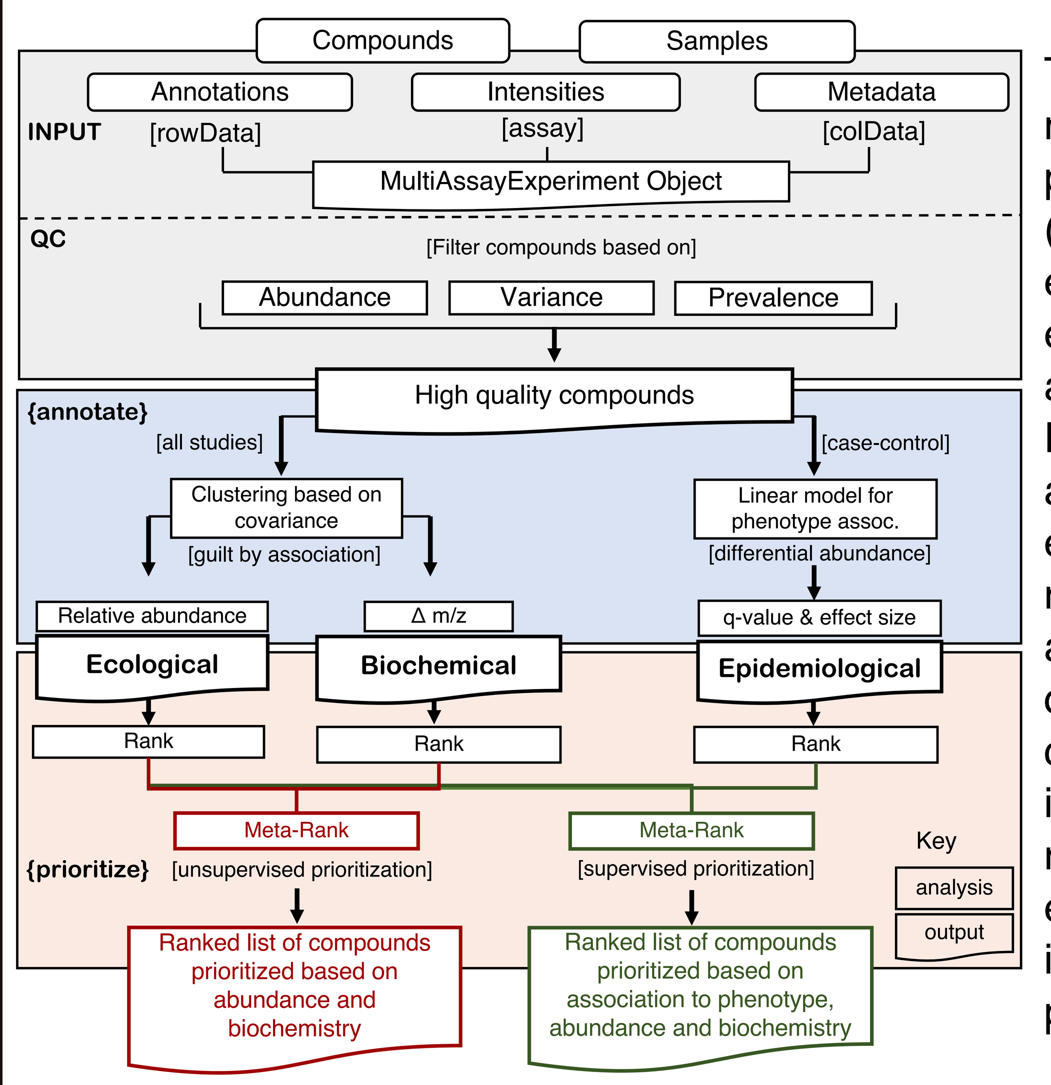


Bioactive microbiome metabolites

Thousands of metabolites have been assayed from microbial communities, the human gut microbiome in particular, but as yet with minimal biochemical characterization or knowledge of their therapeutic potential. Here, we developed a new approach, **MACARRoN** (**M**etabolome **A**nalysis and **C**ombined **A**nnotation **R**anks for p**R**ediction of **N**ovel **B**ioactives), for identifying potential bioactives by integrating knowledge of known/standard compounds with phenotypic or environmental indicators of bioactivity to annotate and prioritize the unknown metabolites. We have applied this approach to identify novel bioactives from the inflammatory bowel disease (IBD) metabolomes in the Integrative Human Microbiome Project (HMP2) metabolomes.

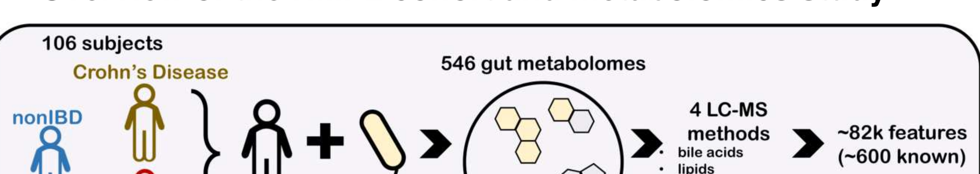
MACARRoN ranks features based on multiple quantitative annotations



The workflow contains modules for (a) data preprocessing and QC, (b) quantitative estimation of ecological, biochemical and epidemiological properties and (c) prioritization. For each feature, abundance and m/z are examined w.r.t a (known) reference with similar abundance pattern, and differential abundance q-value against a phenotype is determined from a linear regression model. Ranks from each of these properties are integrated into a single prioritization 'meta-rank'.

MACARRoN is used to identify inflammation-associated compounds

Overview of the HMP2 cohort and metabolomics study

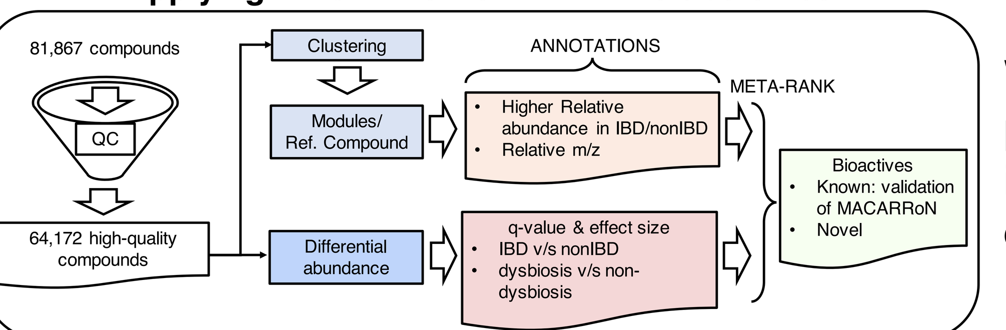


We applied the workflow to prioritize compounds from the metabolomes of IBD patients and nonIBD controls in the HMP2 (Lloyd-Price, 2019.

Bray-Curtis ordination using metabolite abundances

Based on metabolite abundances, IBD and nonIBD, and dysbiotic and non-dysbiotic metabolomes can be distinguised.

Applying MACARRoN on HMP2 metabolomes

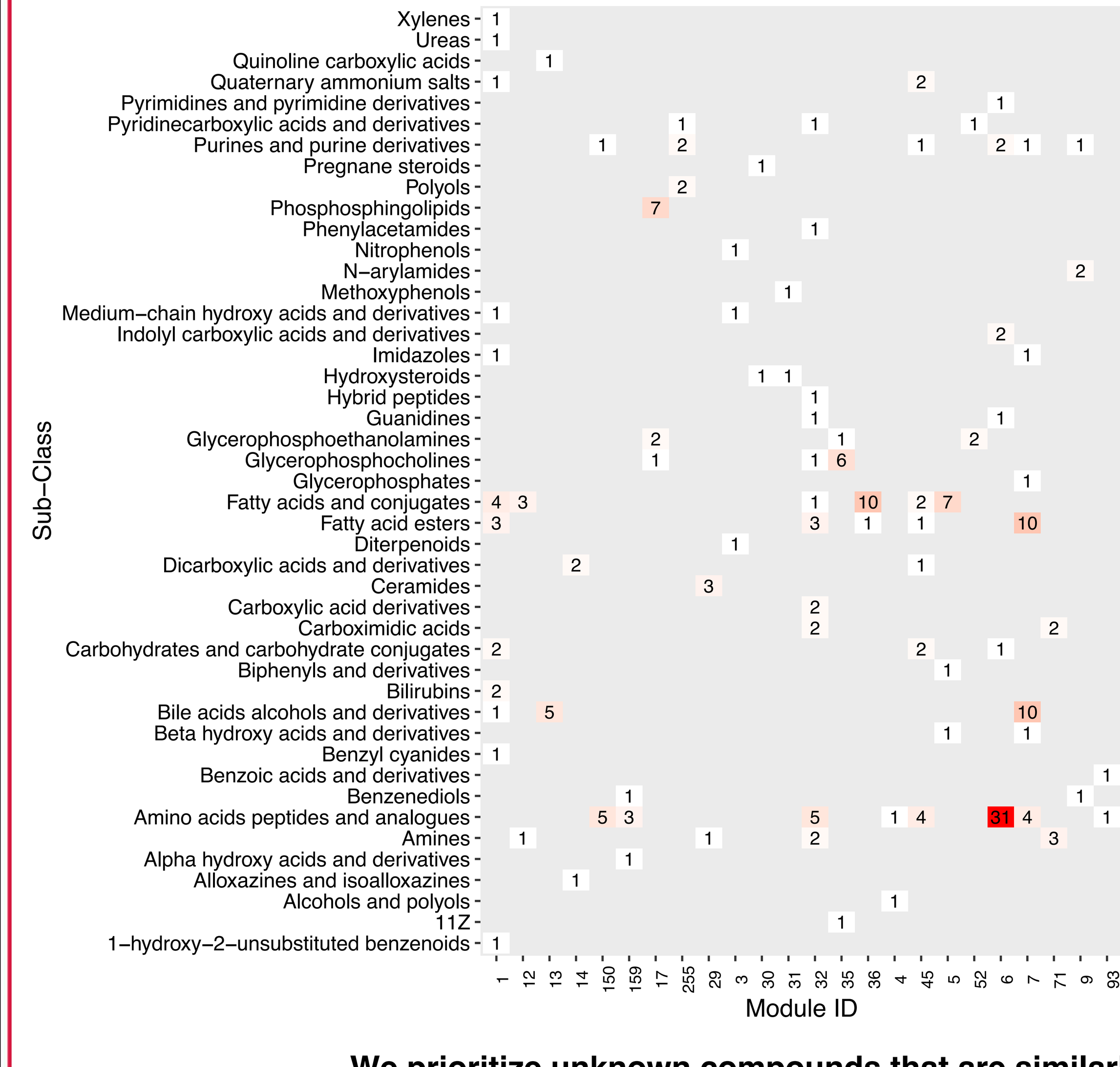


We adopted the supervised prioritization approach to identify bioactives linked to dysbiosis and inflammation.

Quantitative metabolite annotations

Guilt-by-association for Ecological and Biochemical properties

To associate unknown compounds with known metabolites, we clustered features based on co-varying abundances: 47,913 features were distributed into 269 modules of varying sizes and 16,259 features were observed as singletons. 83 (~31%) of the 269 modules had at least one known metabolite.

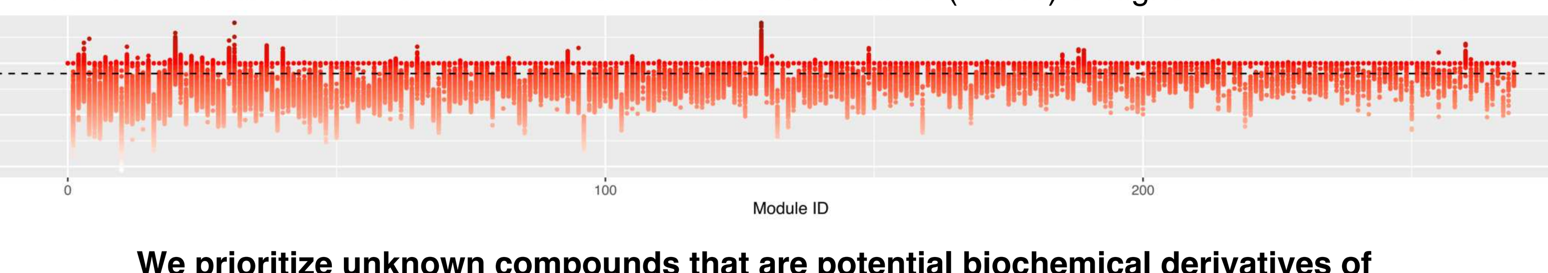


Of the 83, most modules had features belonfing to the same chemical sub-class. Modules with compounds belonging to >1 sub-class capture sub-classes that co-vary as a result of co-occurrence in the bug/ biochemical pathway.

Picking a reference in each module: Compound (known metabolite if present) with the highest mean abundance in all samples was chosen as the reference.

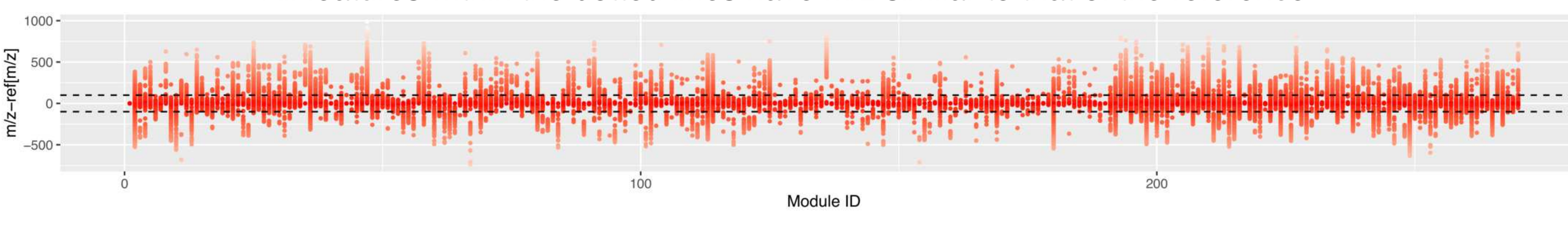
We prioritize unknown compounds that are similarly abundant as compared to their co-clustered reference

All features above the dotted line have abundances similar to (> x0.1) or higher than the reference



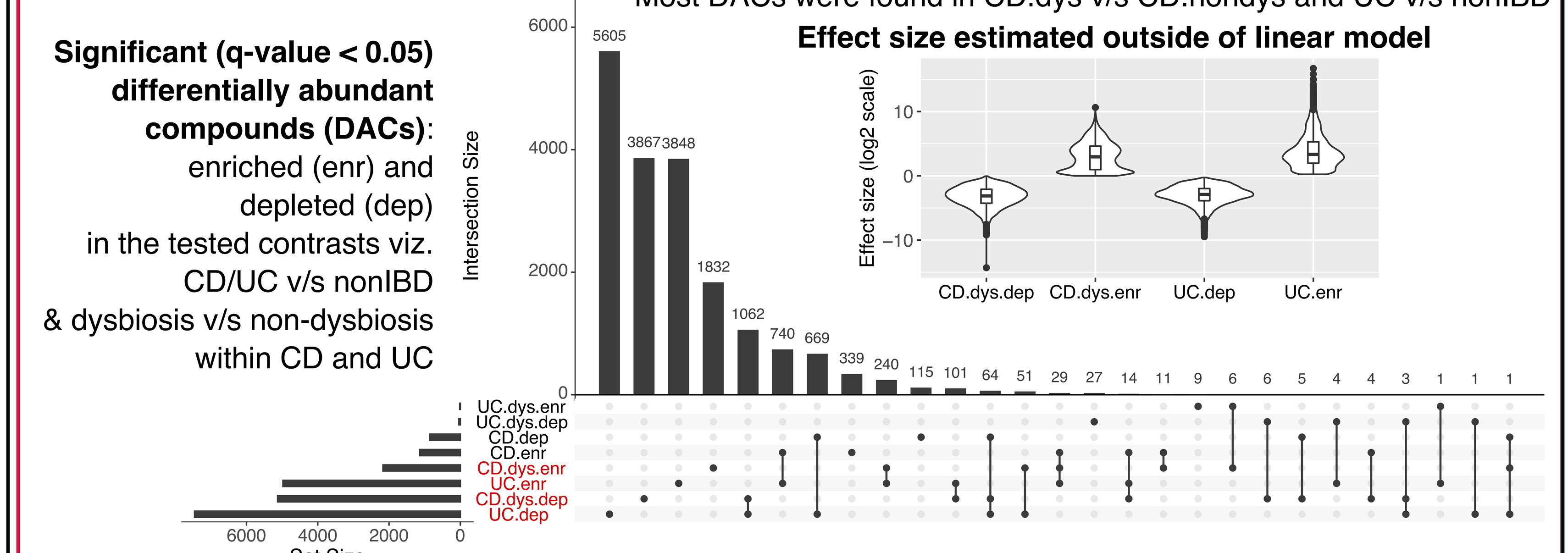
We prioritize unknown compounds that are potential biochemical derivatives of their co-clustered reference

All features within the dotted lines have m/z similar to that of the reference



A mixed effect linear model is used for estimating phenotype association

Significant (q-value < 0.05) differentially abundant compounds (DACs): enriched (enr) and depleted (dep) in the tested contrasts viz. CD/UC v/s nonIBD & dysbiosis v/s non-dysbiosis within CD and UC



Prioritized metabolites

Metabolites highly prioritized by MACARRoN include classes previously implicated in IBD as well as novel potential bioactives

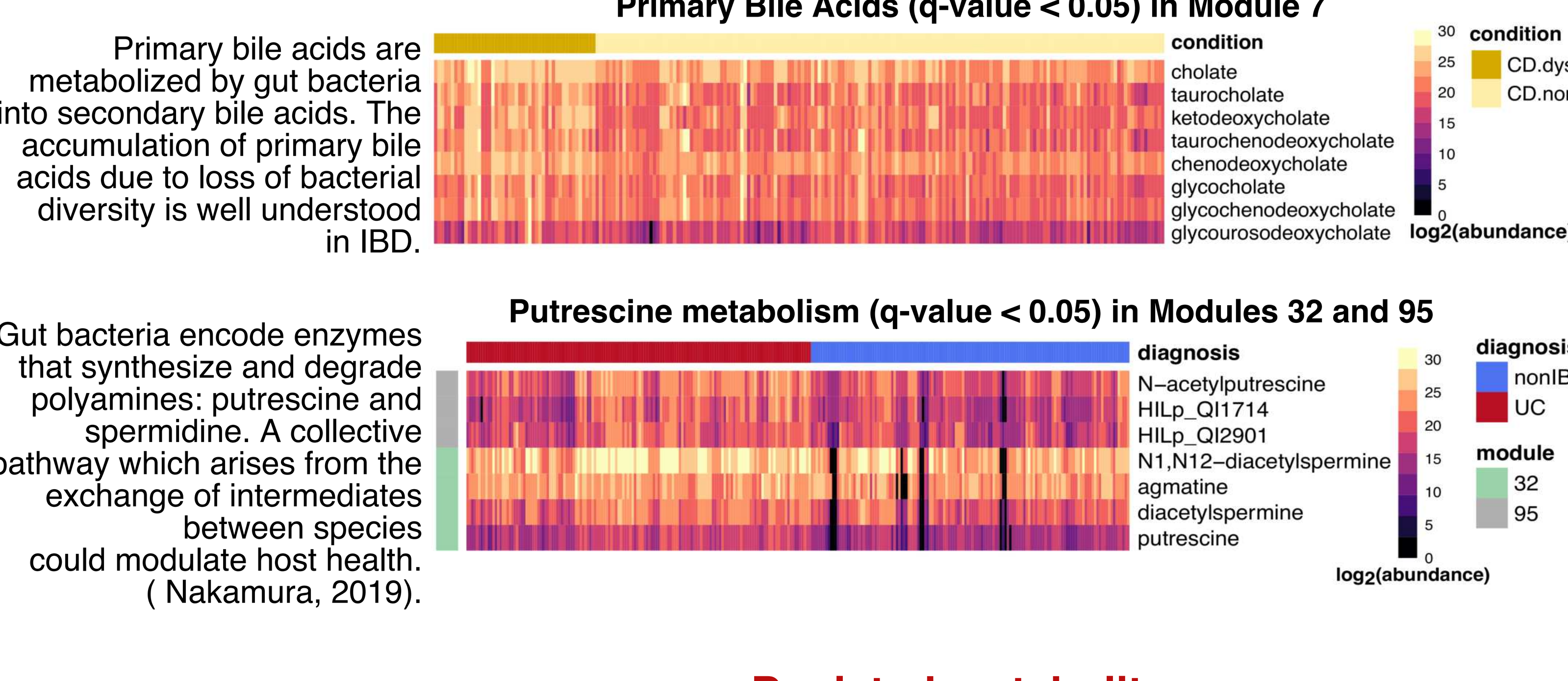
Enriched metabolites

Primary Bile Acids (q-value < 0.05) in Module 7

Primary bile acids are metabolized by gut bacteria into secondary bile acids. The accumulation of primary bile acids due to loss of bacterial diversity is well understood in IBD.

Putrescine metabolism (q-value < 0.05) in Modules 32 and 95

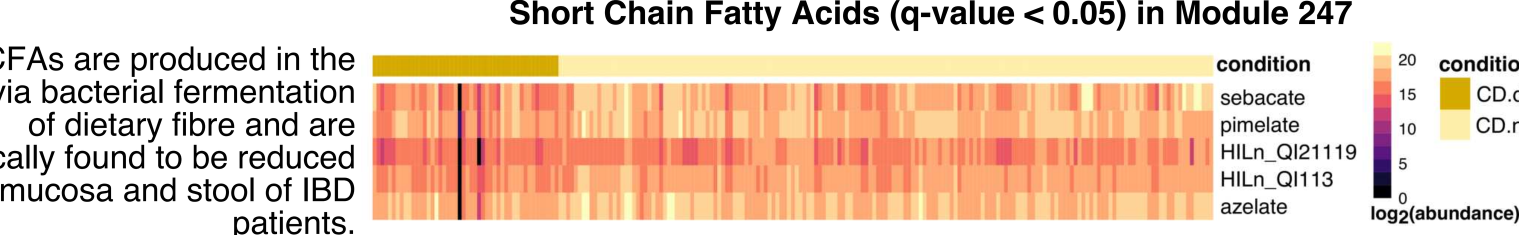
Gut bacteria encode enzymes that synthesize and degrade polyamines: putrescine and spermidine. A collective pathway which arises from the exchange of intermediates between species could modulate host health. (Nakamura, 2019).



Depleted metabolites

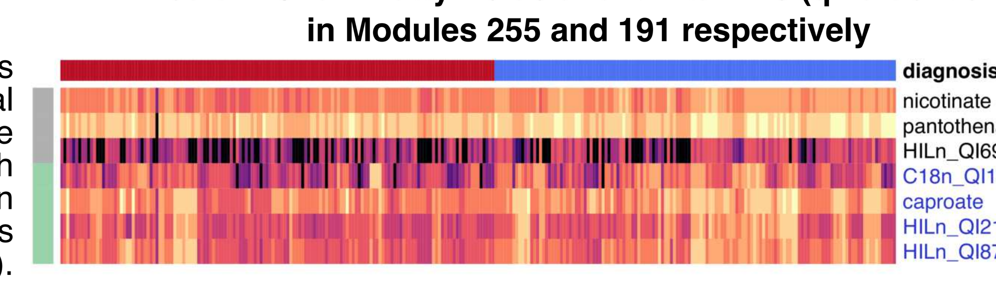
Short Chain Fatty Acids (q-value < 0.05) in Module 247

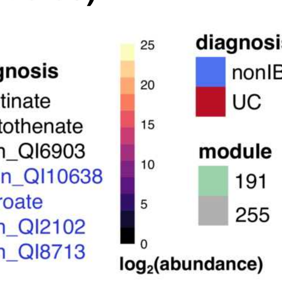
SCFAs are produced in the gut via bacterial fermentation of dietary fibre and are typically found to be reduced in mucosa and stool of IBD patients.



Medium Chain Fatty Acids and B vitamins (q-value < 0.05) in Modules 255 and 191 respectively

Production of MCFAs has been attributed to bacterial thioesterases. MCFAs activate GPR40 have been which co-localizes with insulin producing beta-cells (Briscoe, 2003).





Conclusions and future work

MACARRoN integrates ecological, biochemical and epidemiological annotations to prioritize metabolites in the microbiome. In the HMP2 metabolomes, prioritization of classes such as bile acids and SCFA previously implicated in IBD validates the workflow. Novel highly prioritized compounds covary with known metabolites, have a high relative abundance and are significantly differentially abundant in the phenotype of interest. Metabolites with lesser known roles in IBD such as putrescine metabolites, medium-chain fatty acids and B vitamins were among the highly prioritized ones. MACARRoN is generalizable to other microbial communities ans is being developed as an open-source R package.

Acknowledgements

This work has been funded by NIH NIDDK grant R24DK110499

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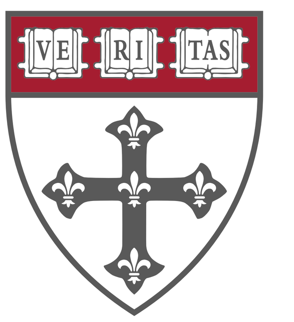
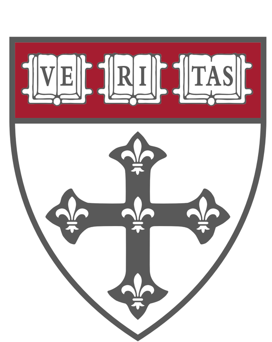




Modulation of the intestinal immune cell compartment by *Fusobacterium nucleatum* in mice with a minimal complexity microbiota

Caitlin A. Brennan, Sydney L. Lavoie, Jessica K. Lang, Kathryn G. Rosinski, Sena Bae, Slater L. Clay and Wendy S. Garrett

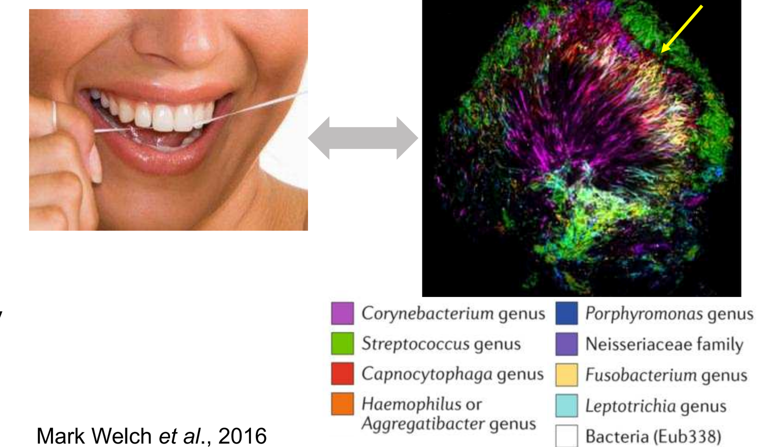
Harvard T.H. Chan School of Public Health, Cancer Research UK OPTIMISTICC Team

Abstract:

Colorectal cancer (CRC) is a multifaceted disease, influenced by host genetic and environmental factors. Growing evidence suggests that specific members of the microbiota mediate CRC development, growth and spread. One such microbe is *Fusobacterium nucleatum,* a normal constituent of the human oral cavity, that has been largely studied for its role in shaping dental biofilms. *Fusobacterium* spp., while rare in the gut microbiota of healthy individuals, are enriched in human colorectal adenomas and adenocarcinomas, compared to normal colonic tissues, and specifically associated with certain epidemiological subtypes of colorectal cancer. Further experimental evidence has suggested that *F. nucleatum* can potentiate tumorigenesis in mouse models, influence immune-mediated killing of tumor cells, and promote resistance to chemotherapy drugs. Taken together, this research supports that a greater understanding of the biology underlying *F. nucleatum* in the gastrointestinal tract—both before and during tumorigenesis—may provide insights into improving CRC diagnosis and treatment. To that end, we seek to understand how *F. nucleatum* modulates the intestinal immune cell environment. In previous works, *F. nucleatum* has been shown to influence myeloid cell and T cell frequency in murine and human tumors, respectively. However, we do not yet understand how this oncomicrobe may shape different immune cell populations prior to tumorigenesis, potentially influencing the conversion of healthy intestinal tissue into a pro-tumorigenic microenvironment. As *F. nucleatum* is a bacterium evolved to live in the oral cavity, we are leveraging gnotobiotic mouse models—in which *F. nucleatum* can become a stable member of the intestinal microbiota—along with bacterial genetics and immunological approaches to disentangle the interactions at play among *F. nucleatum*, the colonic epithelium, and the immune system.

Fusobacterium nucleatum — symbiont, opportunist and oncobacterium

- Anaerobic, Gram-negative fusiform rod
- A member of the oral microbiota where it plays a role in oral biofilms important in periodontal and gingival health and disease
- Associated with myriad extra-oral diseases, including adverse pregnancy outcomes, inflammatory bowel disease, atherosclerosis, and, recently, colorectal cancer
- A distinct bacterial phylum, suggesting the potential for novel biology but limiting the application of tools developed for better studied organisms



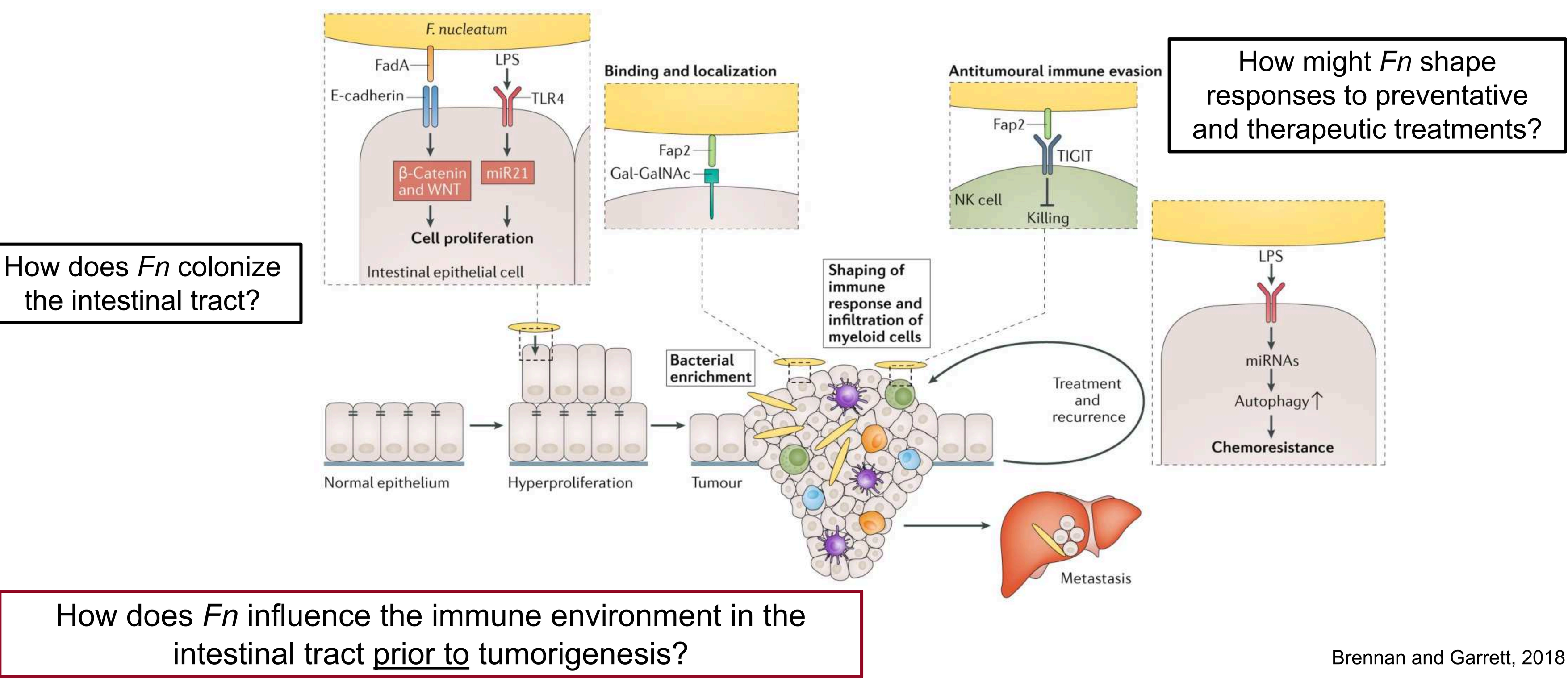
Mark Welch *et al.*, 2016

Identifying an association between CRC and *F. nucleatum*

- Specific enrichment of *Fn* in CRC tissues identified by:
  - Multiple techniques: mining cancer 'omics data for microbial signatures (PathSeq), 16S rRNA and metagenomic surveys, RNAseq-based approaches, fluorescent *in situ* hybridization, strain isolation (directly and from xenografts)
  - Independent studies and researchers investigating both distinct patient populations and different stages of CRC
- Epidemiological associations link *Fn* load to certain CRC subtypes (e.g. MSI-high tumors) and poorer patient prognosis
- Growing experimental evidence in cell culture and mouse models suggests a causal role for *Fn* in CRC

Normal
Tumor

Mechanisms by which *Fn* may contribute to CRC



How does *Fn* colonize the intestinal tract?

How might *Fn* shape responses to preventative and therapeutic treatments?

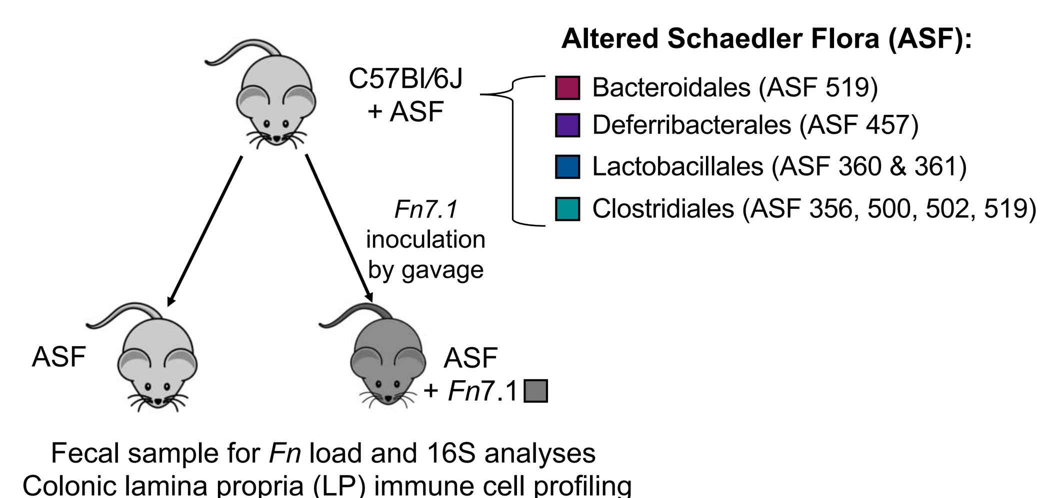
How does *Fn* influence the immune environment in the intestinal tract prior to tumorigenesis?

Brennan and Garrett, 2018

Leveraging gnotobiotics to study *Fn* in the intestinal tract

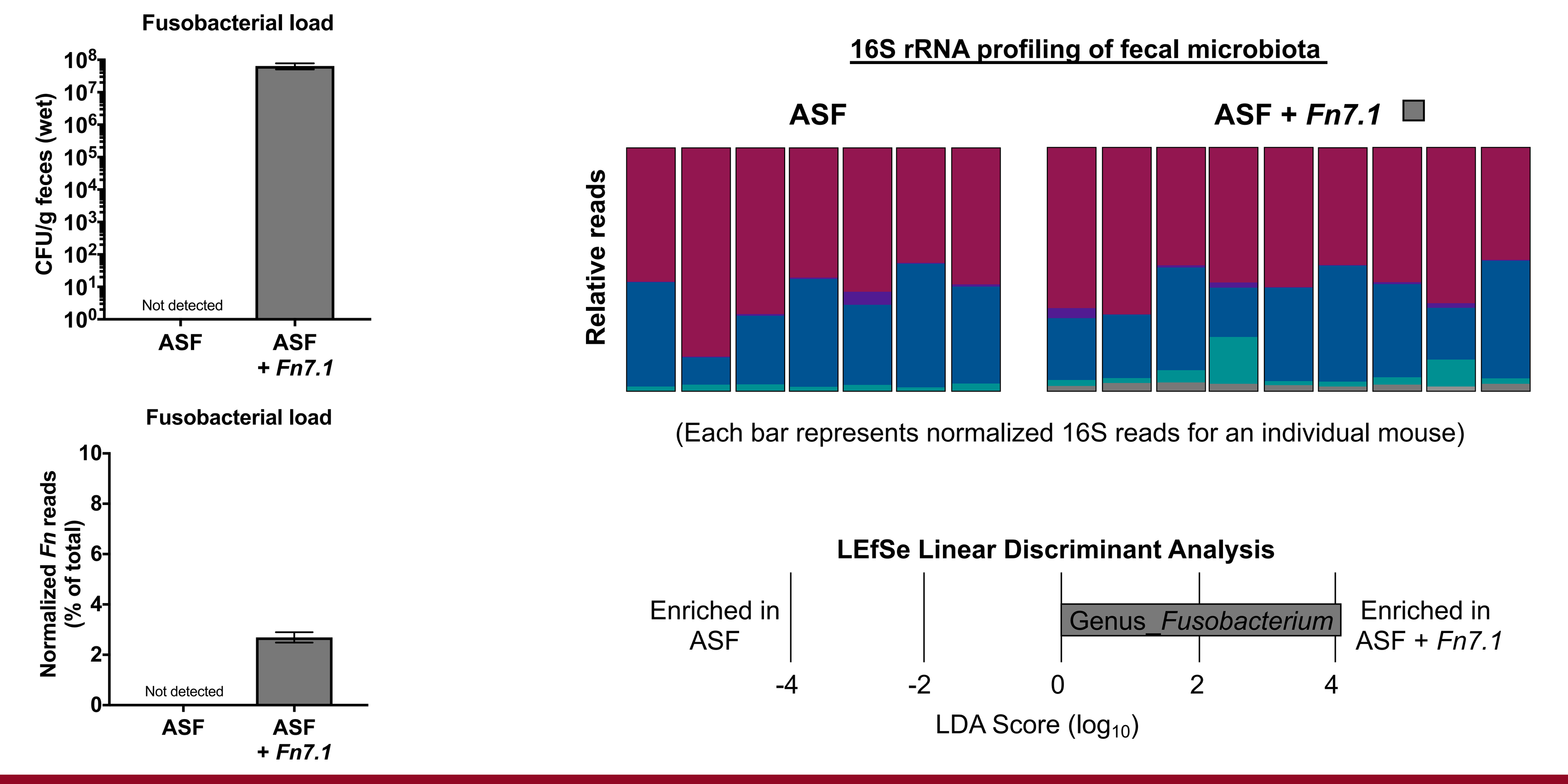
Why use mice colonized with a defined microbial community?

- Allows the reproducibility of gnotobiotic research while more adequately resembling specific pathogen-free mice than germ-free mice in terms of cecum size, immune cell development, reproduction, *etc.*
- Circumvents technical limitations to study important biological interactions: some microbes (like *Fn*) that cannot stably colonize SPF mice are able to maintain a niche in mice with a less complex microbiota



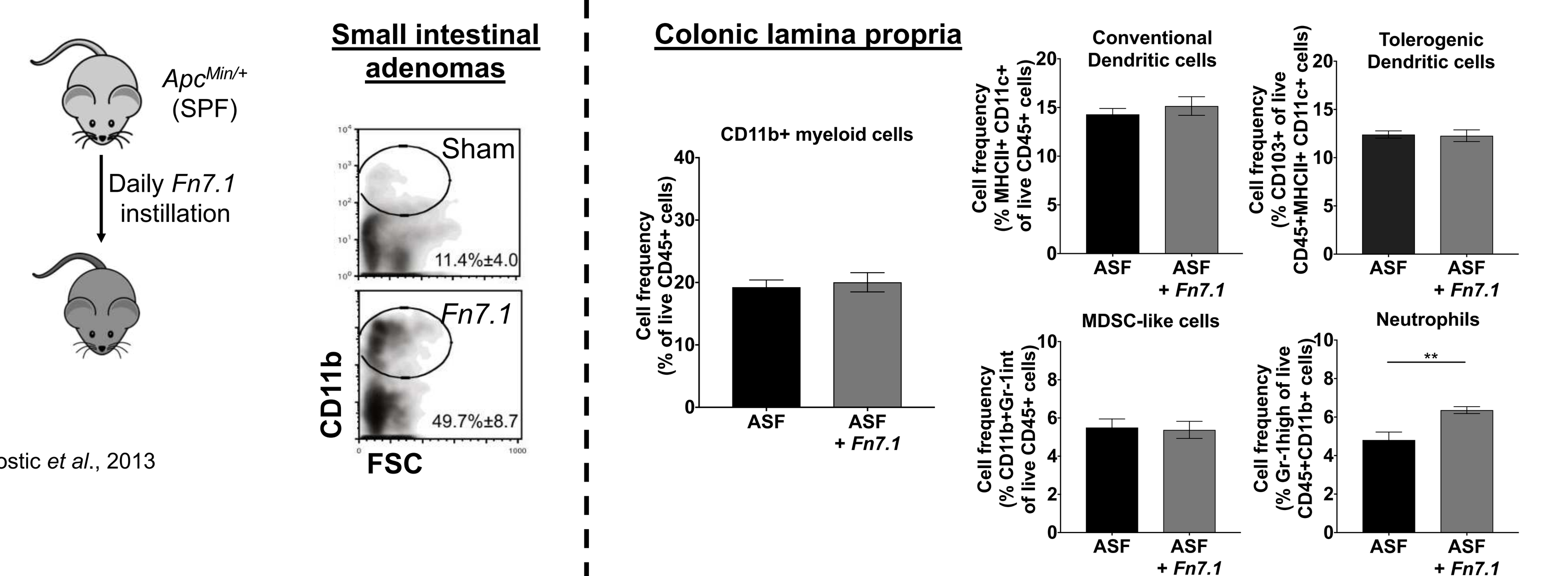
Fecal sample for *Fn* load and 16S analyses
Colonic lamina propria (LP) immune cell profiling by flow cytometry (d14)

_Fn7.1_ colonizes ASF mice to high abundance without affecting the proportion of other ASF members:

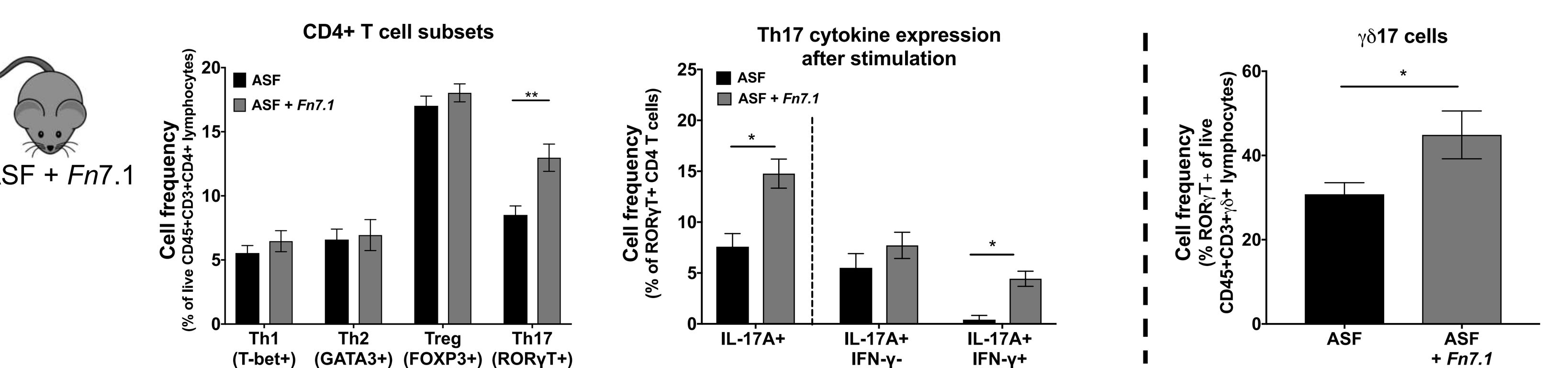


Fn influences colonic immune cell populations in ASF mice

***Fn7.1* has only mild effects on myeloid cell subsets in the colonic LP, unlike in adenomas:**

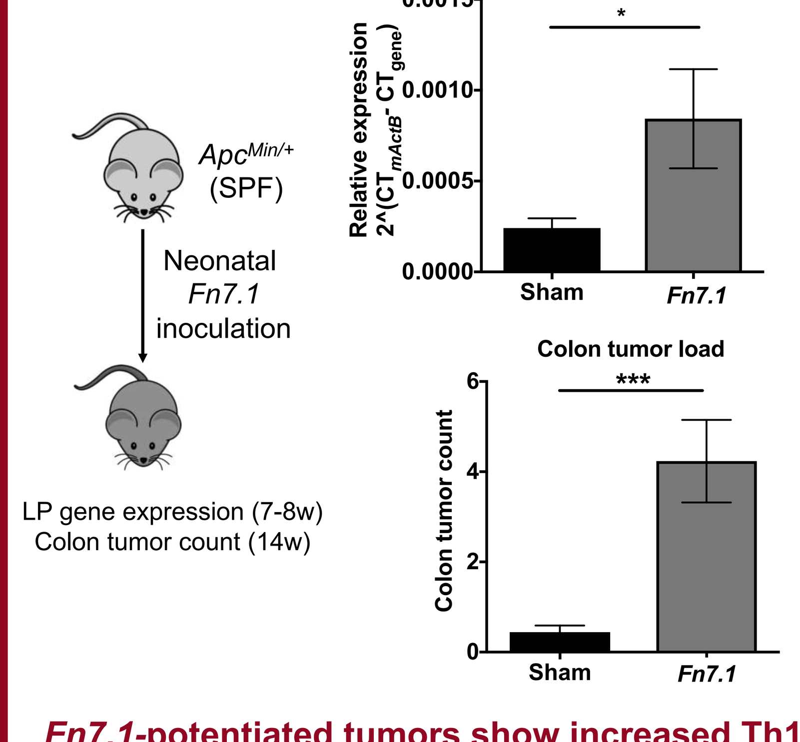


***Fn7.1* drives Th17 and γδ17 responses in the colonic lamina propria of healthy ASF mice:**

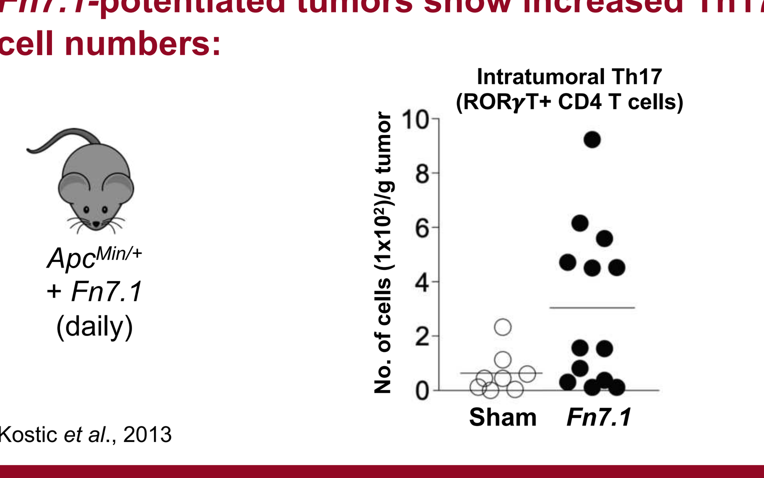


Fn is associated with a Th17-like signature in models of intestinal tumorigenesis

***Fn7.1* induces *Il17a* expression prior to tumor formation in a neonatal model of intestinal tumorigenesis:**

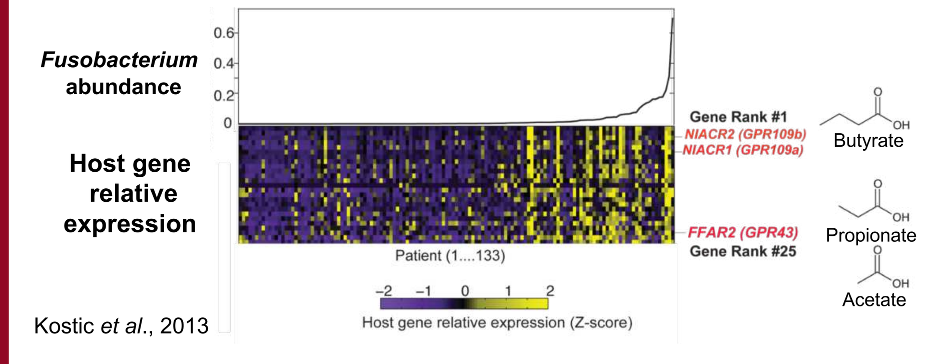


***Fn7.1*-potentiated tumors show increased Th17 cell numbers:**

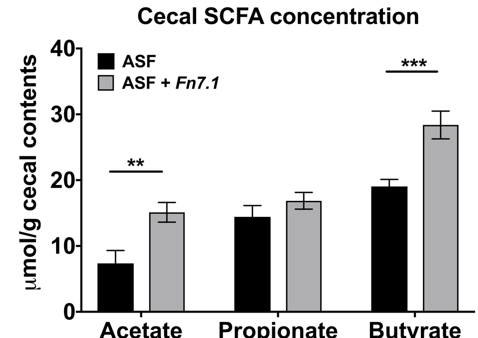


Short-chain fatty acids (SCFA) may mediate *Fn* immune cell modulation

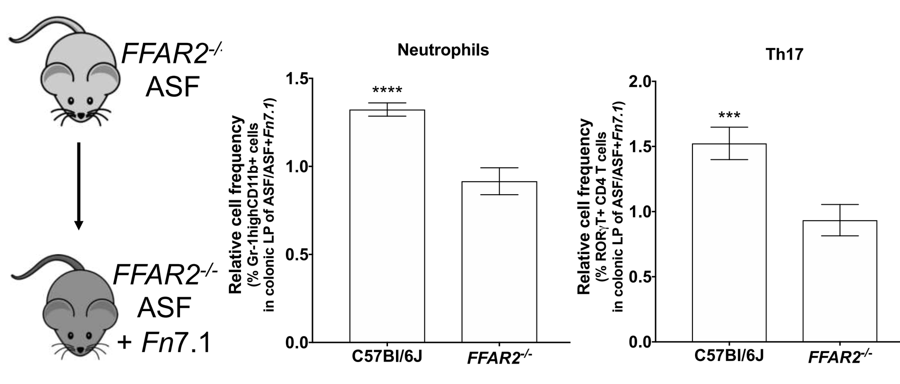
Fusobacterial load is correlated with SCFA receptor expression in human tumors:



***Fn7.1* colonization increases SCFA concentrations in the ceca of ASF mice:**



Loss of the SCFA receptor FFAR2 abrogates *Fn*-associated immune cell responses:



Conclusions and future directions

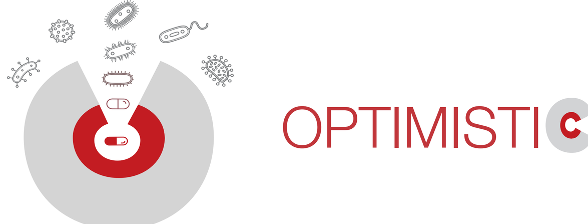
- Gnotobiotic mice serve as an important tool for probing the role of *Fn* in the intestinal environment
- *Fn* modulates the intestinal and intratumoral environment by mediating a Th17-type immune response
  - Convergent mechanism to promote tumorigenesis as another oncobacterium, enterotoxigenic *Bacteroides fragilis*?
- The SCFA receptor FFAR2 is important for *Fn*-potentiated immune cell responses in the ASF model
- Next steps:
  - Further delineate the immune cell changes in response to *Fn*
  - Interrogate known immune and signaling pathways that may mediate the response to *Fn*, including defining the role of FFAR2
  - Consider how characterizing and manipulating this interaction may inform CRC diagnosis and treatment

How might *Fn* influence these immune cell populations?

F. nucleatum
Effector proteins?
Metabolites? (SCFA)
FFAR2
APC
SAAs?
MAMPs?
γδ17
Th17
Naive
Neutrophil

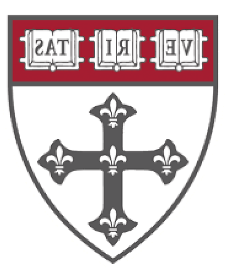
Acknowledgements:

The authors thank members of the Garrett lab for their technical assistance and overall contributions to the scientific direction of this project. Funding for this project was provided by Cancer Research UK (to the OPTIMISTICC group), the National Institutes of Health (R01CA154426 to WSG). CAB was the Dennis and Marsha Dammerman Fellow of the Damon Runyon Cancer Research Foundation (DRG-2205-14) and a postdoctoral trainee of the DFCI Training Grant in Cancer Immunology (T32CA207021).



THE HARVARD CHAN MICROBIOME IN PUBLIC HEALTH CENTER

HARVARD CHAN MICROBIOME IN PUBLIC HEALTH CENTER (HCMPH) SYMPOSIUM



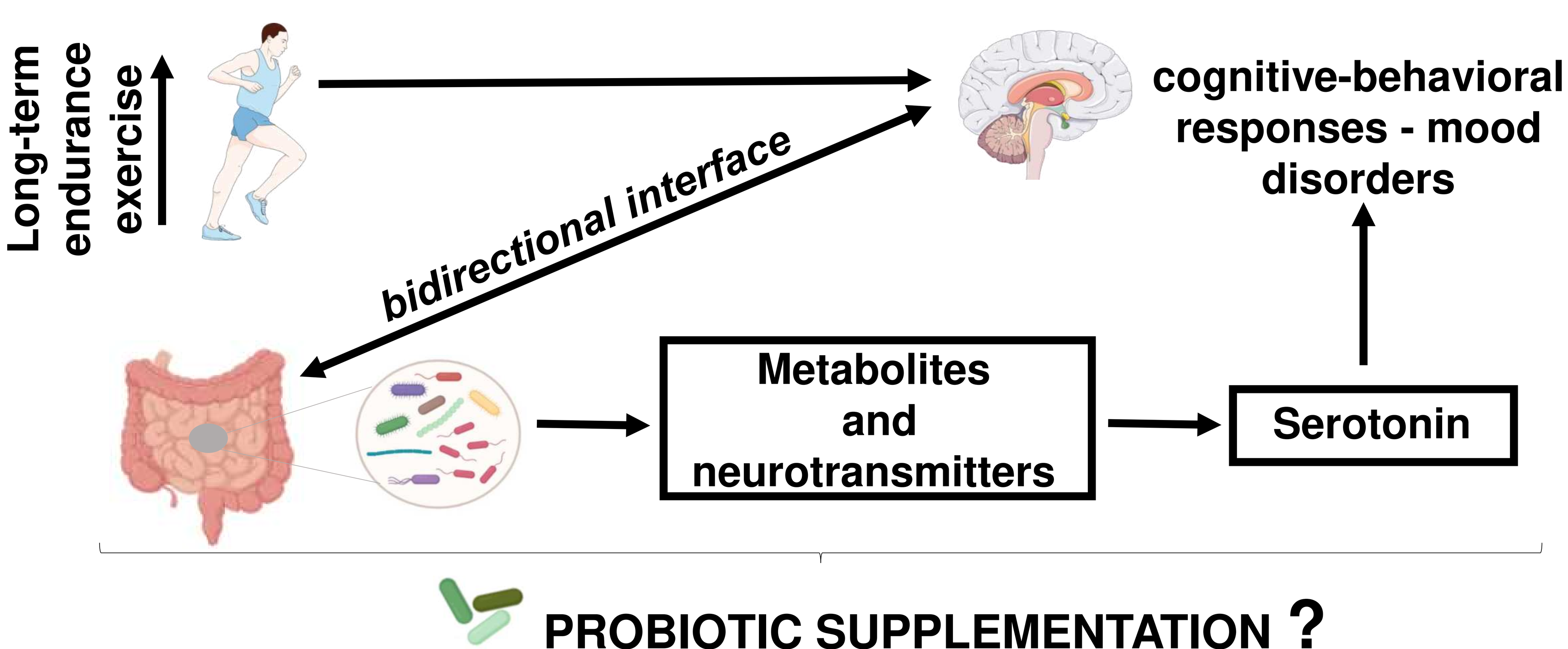
Effects of endurance exercise on mood state and serotonin levels after thirty days of probiotic supplementation: a pilot study

Marques CG[2]; Tavares-Silva E[1,2]; Tonelli L.[4]; Lemos VA[1]; Caris AV[2]; Santos SA[1]; Ravacci GR[3]; Thometieli-Santos RV[1,2]

[1]Department of Bioscience, Federal University of São Paulo, Brazil; [2]Department of Psychobiology Federal University of São Paulo, Brazil; [3]Department of Gastroenterology of Medicine Faculty, University of Sao Paulo, Brazil; [4]Department of Psychological Sciences and Biomedical Engineering, University of Connecticut, United States of America

BACKGROUND

Long-term or short high intensity exercise induces cognitive-behavioral responses. Thus, during training/competition, athletes generally presented mood disorders. Although the concept of the microbiota-gut-brain axis is relatively new, it is suggested that the gut microbiota influences psychological and behavioral aspects. Gut microbiota is able to secretes serotonin, mainly in response to physical and emotional stress. Preliminary data showed that probiotics and prebiotics intake can affect the hypothalamic-pituitary-adrenal (HPA) axis and other pathways in athletes. However, this crosstalk between the gut and brain is not completed study in long-term endurance exercise, such as marathon .

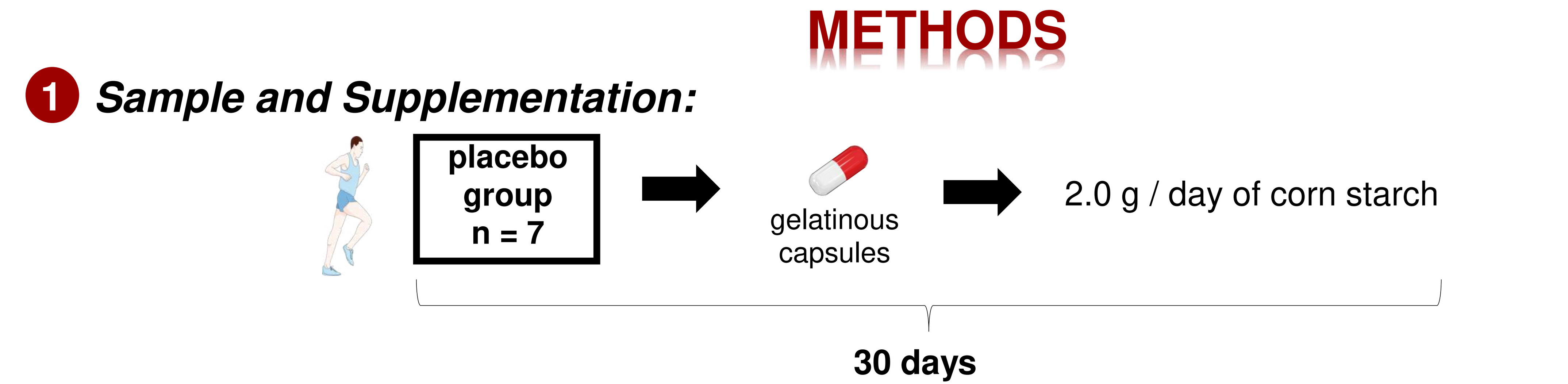


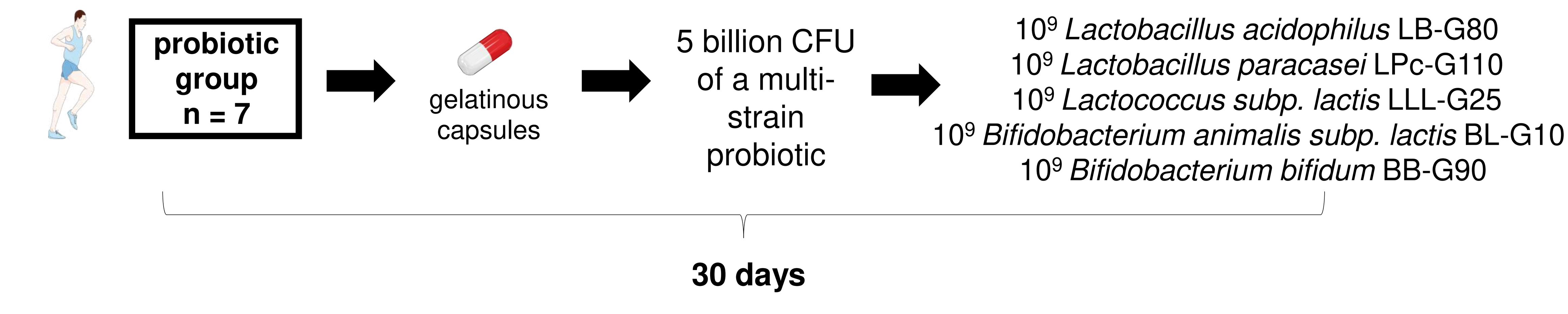
PURPOSE

The present study aimed to evaluate the effect of marathon running on mood aspects and plasma serotonin levels after probiotic supplementation in athletes.

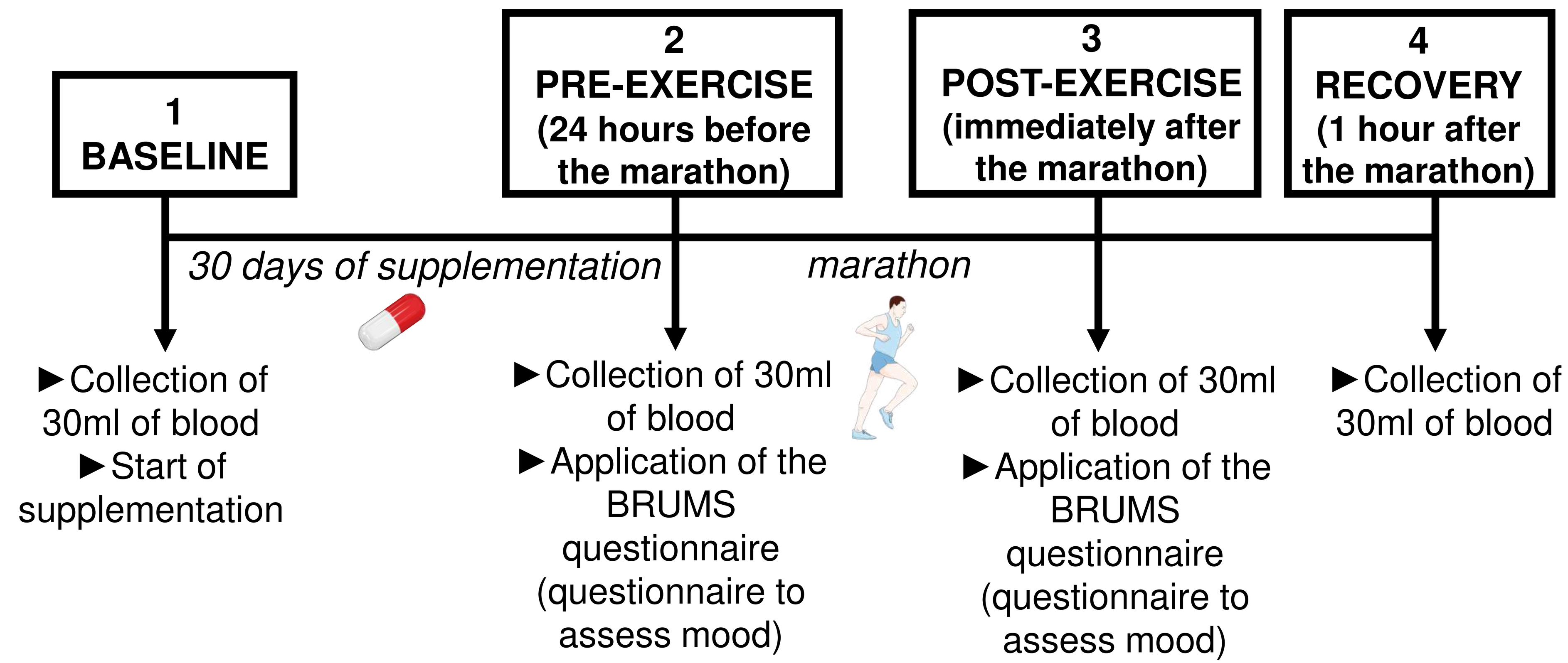
METHODS

1 Sample and Supplementation:





2 Exercise protocol and experimental design:



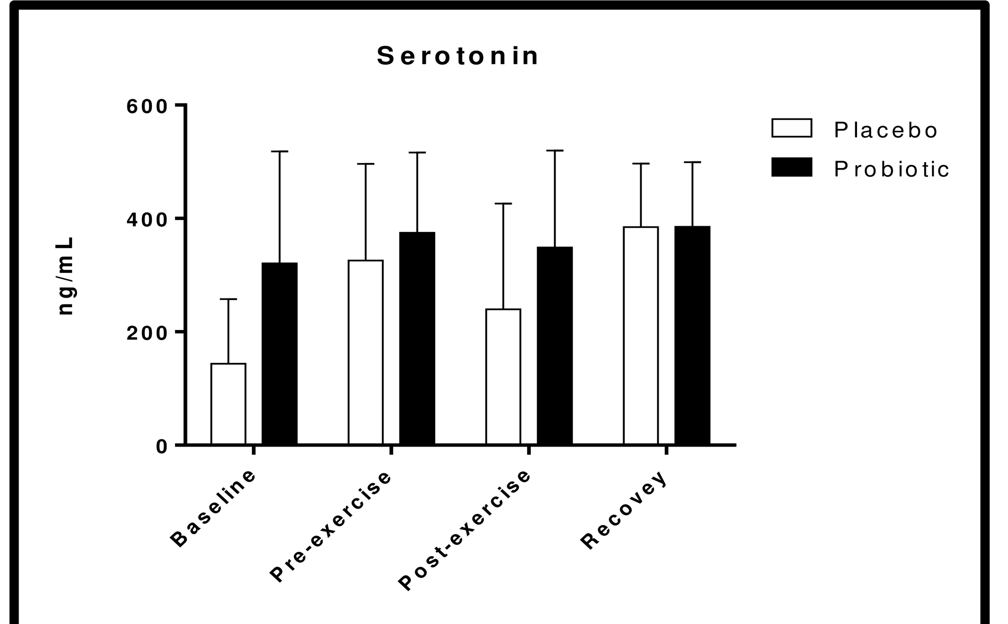
3 Statistical analysis:

To verify differences between group and time, the ANOVA two-way with Tukey Post-hoc was performed being considered $p < 0.05$.

RESULTS

After the marathon, fatigue and mental confusion increased and vigor reduced (without significant difference between the groups; $p > 0.05$).

Moreover, no significant differences were found in serotonin concentrations at different times evaluated and between groups.



CONCLUSION

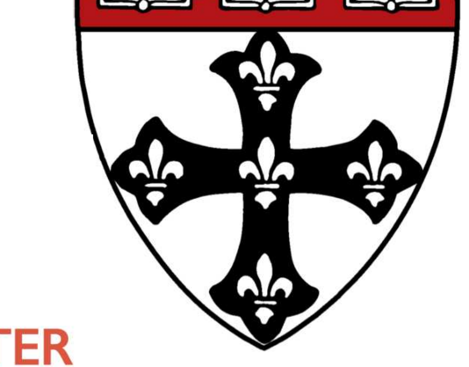
Probiotic supplementation for 30 days was not effective in altering mood parameters and plasma serotonin levels in this protocol. Thus, further studies are needed to understand the possible effects of probiotic supplementation on psychobiological parameters in endurance long-term endurance exercises.

The Harvard T.H. Chan School of Public Health Microbiome Collection Core

Chengchen Li[1], Jeremy E. Wilkinson[1], Curtis Huttenhower[1,2,3]

[1]Department of Biostatistics, Harvard T.H. Chan School of Public Health [2]Broad Institute of MIT and Harvard

[3]Department of Immunology and Infectious Diseases, Harvard T.H. Chan School of Public Health



The Microbiome Collection Core at the Harvard T.H. Chan School of Public Health (HCMCC) was established in response to a strong demand among the research community for validated microbiome sample collection kit configurations and easy usability for in-home sampling. Under the umbrella of the Harvard Chan Microbiome in Public Health Center (HCMPH), HCMCC aims to support population-scale microbiome sample collection and expand our understanding of the microbiome to improve population health. The HCMCC has developed a multi carrier-compatible home stool and oral sample collection kit that permits cost-effective multi'omic microbiome studies, leveraging the intellectual and infrastructure foundation laid by the HMP2 (the 2nd phase of the NIH Human Microbiome Project) and the MLSC (Massachusetts Life Sciences Center)-funded MICRO-N (MICRObiome Among Nurses) collection. By providing this customizable microbiome collection kit, we enable researchers to perform multiple different molecular assays, as well as to tailor the kit configuration and collection plan to study-specific needs.

HCMCC services

Consultation on microbiome sample collection plan development
- Collection kit configuration
- Sample transport plan
- Sample processing and storage plan

Kit ordering & shipment
- Customization and implementation of the study-specific collection kit
- Kit shipment through the preferred carrier to selected clinical sites or participants

Kit tracking
- Returning samples via pre-paid shipment to the BIOM-Mass platform for automated aliquoting
- Tracking samples throughout the collection, return, storage process via barcodes

Streamlined post-collection assistance
- Short and long term -80°C storage offered by the BiOS Freezer core
- Fast sample retrieval and shipment to sequencing labs for meta'omics and metabolomic profiling

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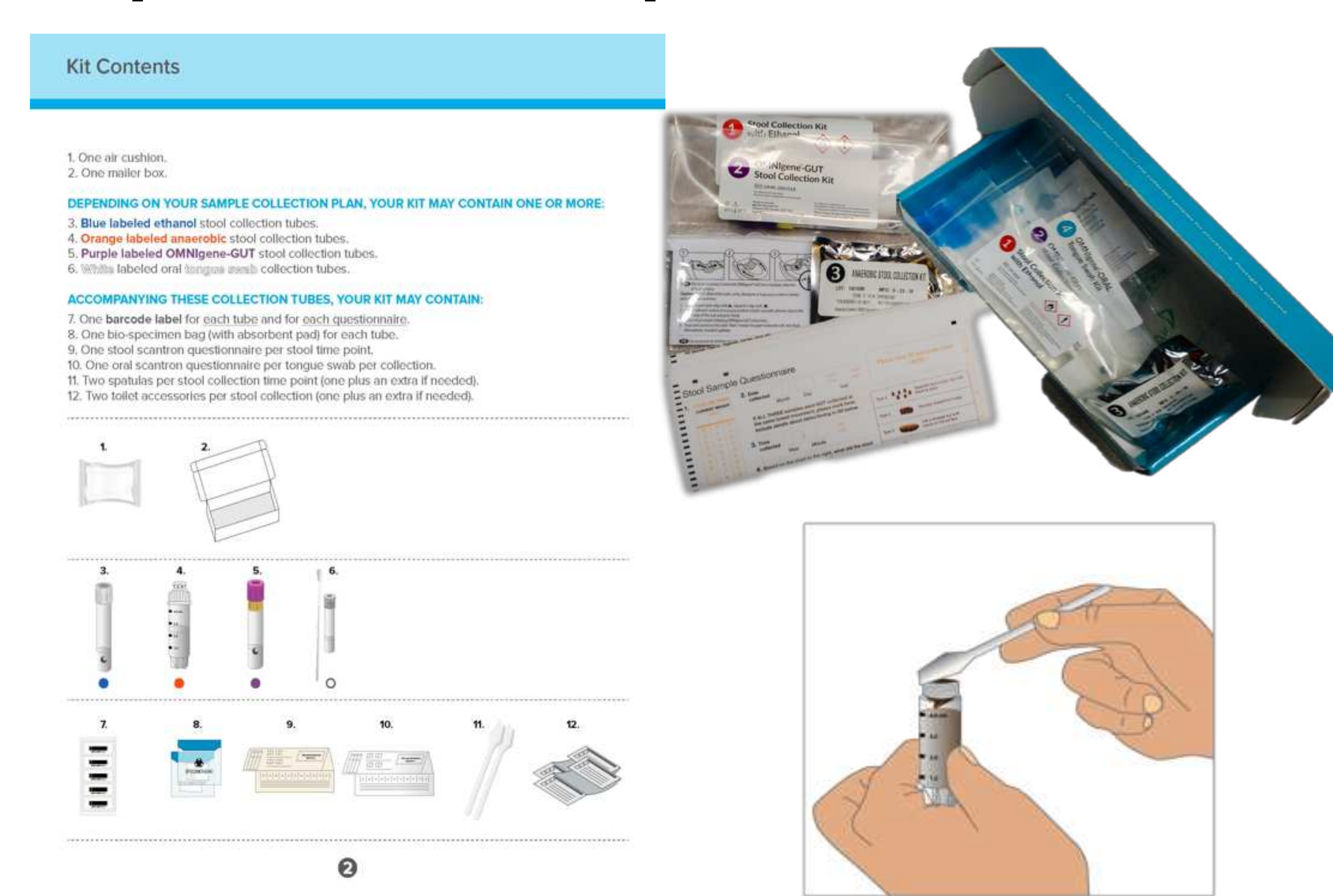
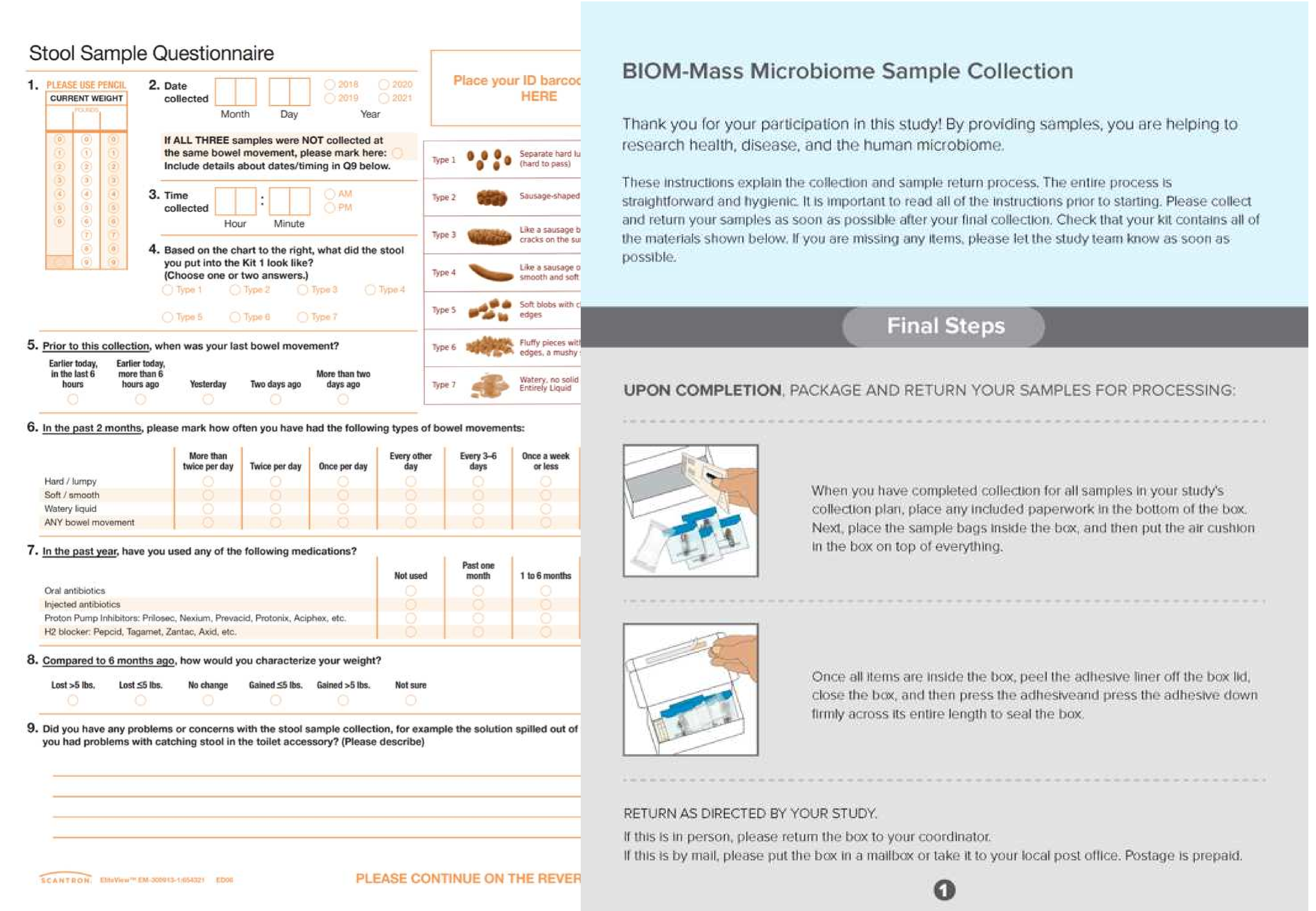
A scalable gut and oral microbiome sample collection platform

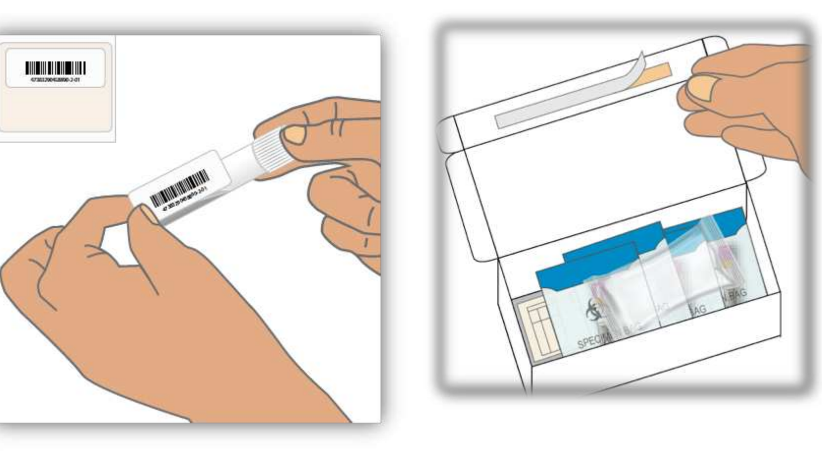


This customizable microbiome sample collection kit avoids the need for expensive, bulky, and inconvenient ice packs by providing several different room temperature storage media that are also compatible with multiple different molecular assays including **any combination of amplicon (16S), metagenomic, metatranscriptomic sequencing, metabolomics, and other molecular assays**. This kit further includes a collection method that uses anaerobic transport media that **yields live microbes for culture or gnotobiotic research**.



In addition to storage media, this sample collection kit includes **user-friendly instructions** and toilet accessories to maximumly facilitate and smooth the in-home stool sample collection experience. **Standardized questionnaires**, as companions to collected samples, are included to capture **recent medications, diet, anthropometric measurements, and gastrointestinal health status measured by the Bristol Stool Scale**. The modularity of this kit allows researchers to tailor kit components to study-specific needs and conduct cost-effective microbiome research ranging from **pilot studies to large-scale studies involving 10,000s of participants.**

HCMCC-supported study activities within the BIOM-Mass platform



Pre-collection
- Enrollment
- Kit ordering
- Kit shipment

Collection
- Self-collection
- Kit return through pre-paid shipment

Post-collection
- Sample aliquoting via Hamilton STAR automated liquid handler
- -80°C storage via BiOS Freezer Core
- Data generation
- Data analysis via Microbiome Analysis Core

Microbiome population health research opportunities

- Accessible microbiome population studies' data on BIOM-Mass Data Portal
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https://hcmph.sph.harvard.edu/hcmcc
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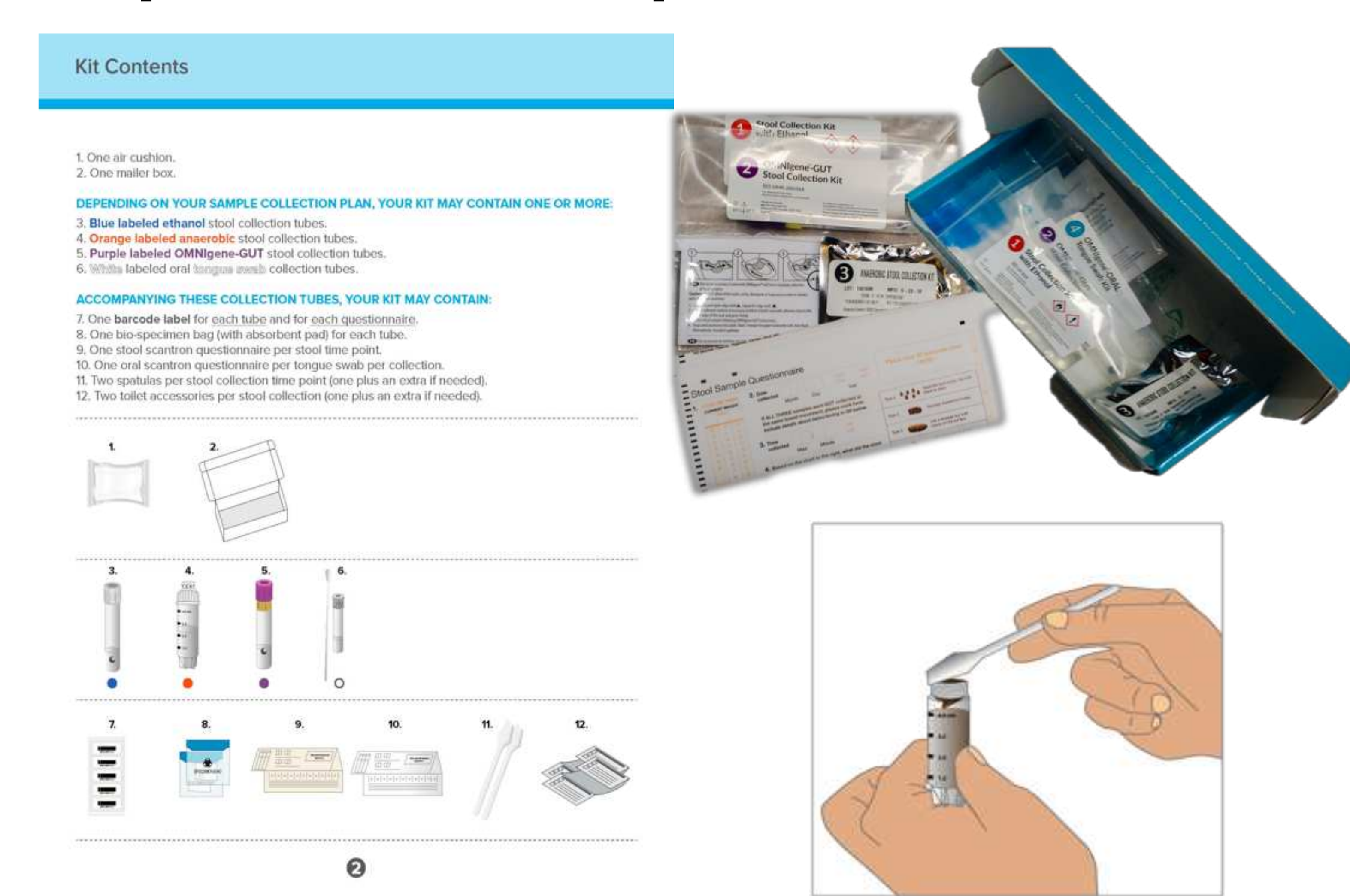

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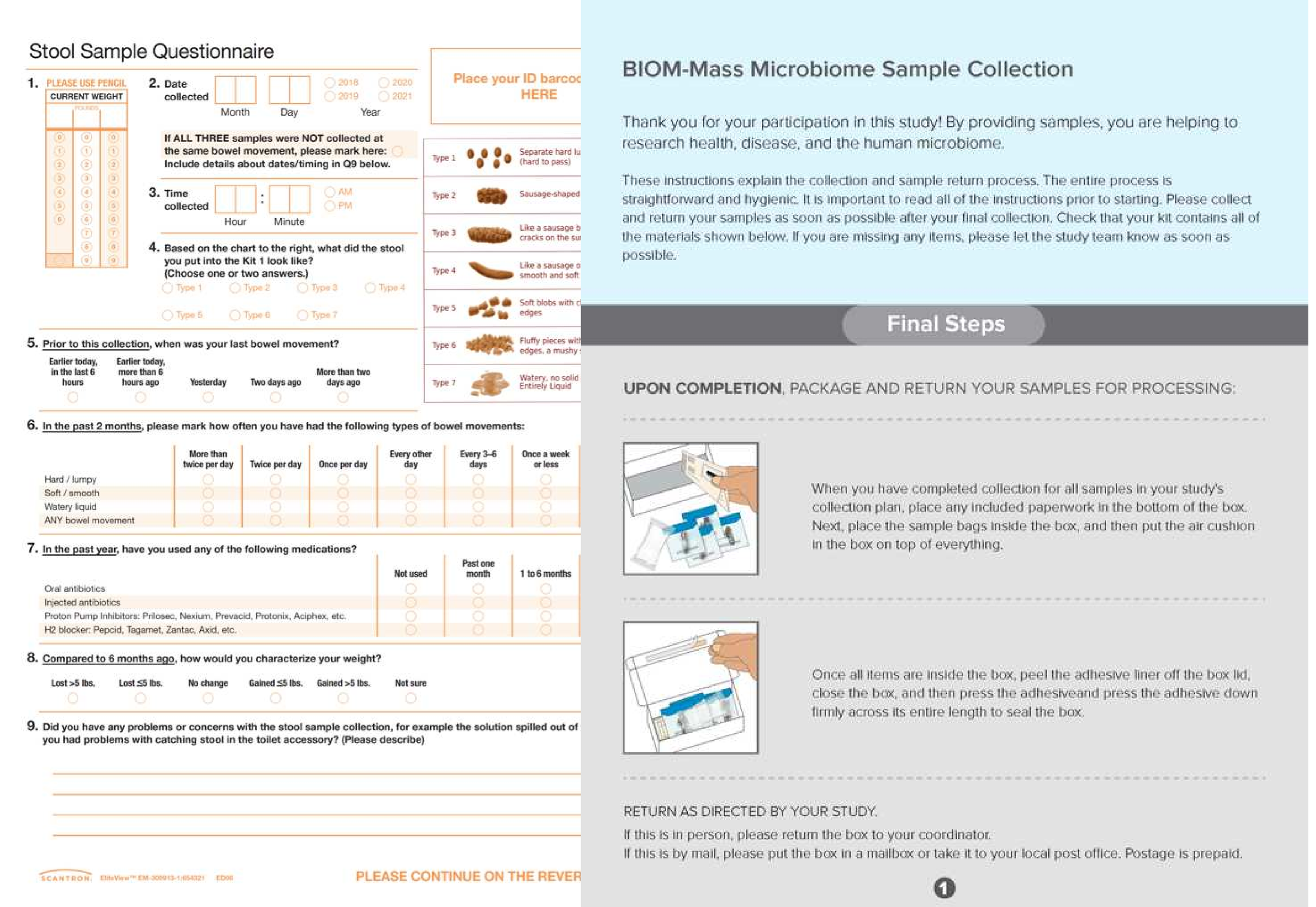

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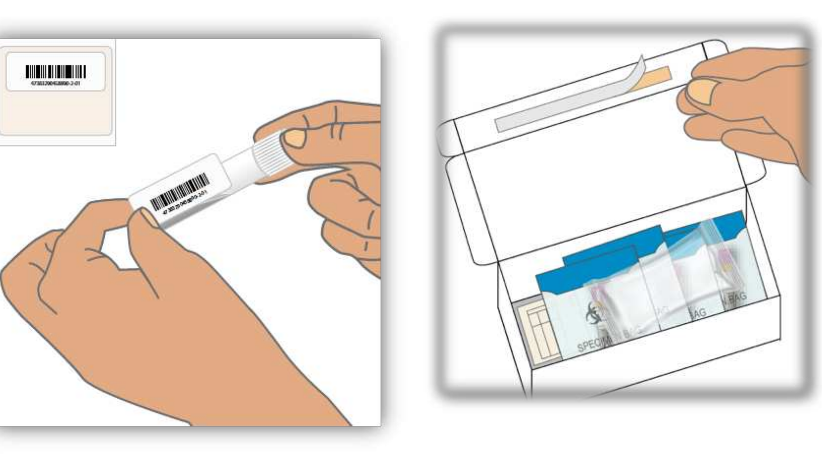

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DENITRIFICATION-PWY: nitrate reduction I (denitrification)

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Effects of 30 days Probiotic Supplementation on Monocytes function after a Marathon Race. A randomized, double-blind-placebo study.

HARVARD T.H. CHAN SCHOOL OF PUBLIC HEALTH
Department of Biostatistics

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2- Department of Biodynamics of the Movement of the Human Body, School of Physical Education and Sports, University of São Paulo, Brazil.
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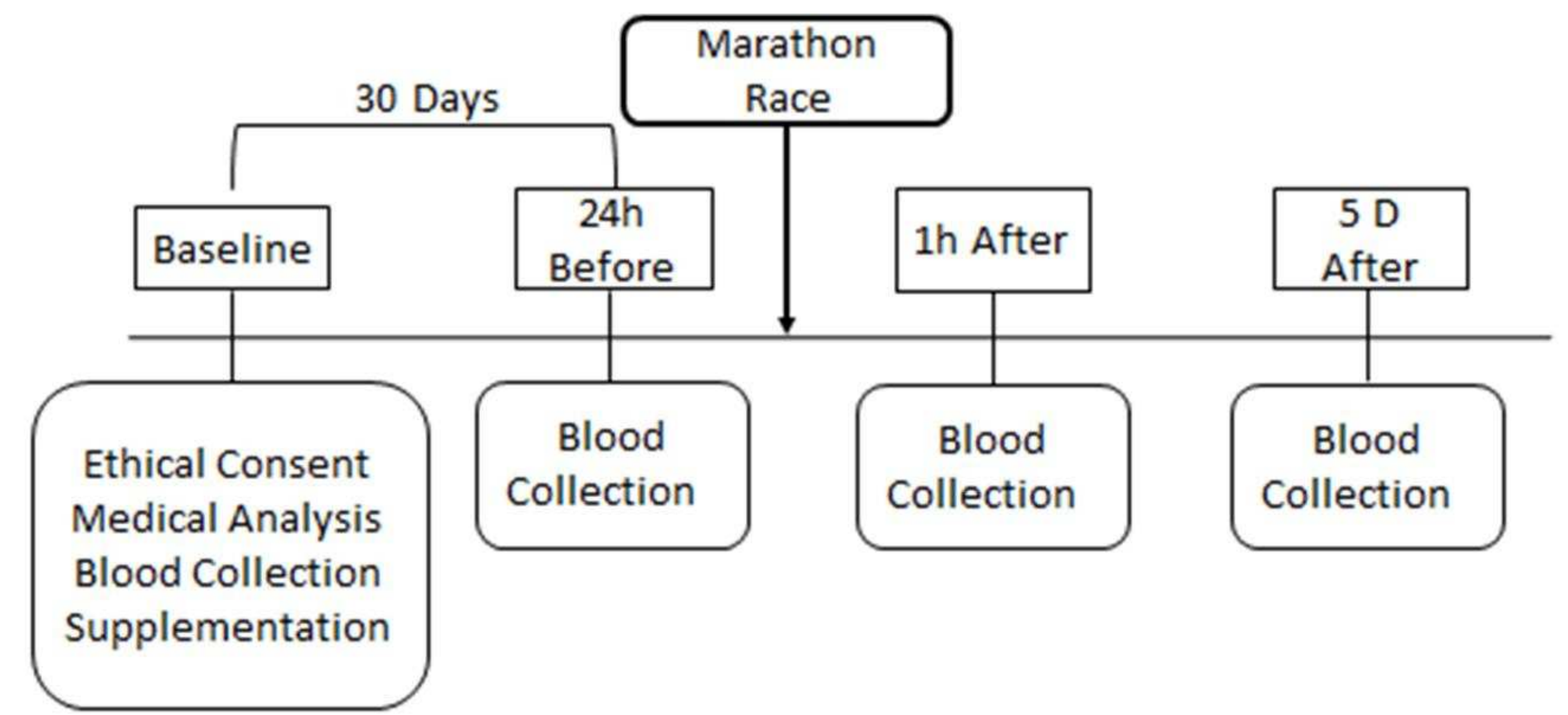
Background

Marathon runs transiently harm the immune and gastrointestinal systems. Nutritional strategies are adopted to mitigate the damage caused by strenuous exercises. This research investigates the possible protective effect of chronic probiotics supplementation on monocytes functions before and after marathon race.

Materials and Methods

Thirty runners were supplemented for 30 days, double-blind, with probiotics 10x10^9 CFU of *Lactobacillus Acidophilus* LA-G80 and 10x10^9 CFU of *Bifidobacterium animalis subsp. Lactis* BL-G101 or placebo (maltodextrin 5g). Before the supplementation period (Baseline), 24 hours before the race (Pre), one hour after (Post), and five days after the marathon (Recovery), blood was collected for the following analysis: Cellular functionality, verified through cellular exposure to Lipopolysaccharide - LPS and opsonized Zymozan. Cytokine analysis, hydrogen peroxide production, and phagocytic capacity of cells. Immunophenotyping was also performed to analyze the monocytes populations. The data normality was verified using the Shapiro-Wilk test, and the Anova Two-Way applied with a significance level of p ≤ 5%.

Experimental Design



Results

Phagocytic Capacity; Hydrogen Peroxide Production; IL-1; IL-6; IL-8; IL-10; IL-15; TNF-alpha (Placebo / Probiotic; Baseline, 24h Before, 1h After, 5D After)

Monocytes functions at baseline, 24h before, 1h after and 5D after a marathon race. a.different from baseline. b. different from 24h before. c. different from 1h after. p ≤ 5%.

Conclusions

We conclude that 10x10^9 CFU supplementation of *Lactobacillus Acidophilus* LA-G80 and 10x10^9 CFU of *Bifidobacterium animalis subsp. Lactis* BL-G101 was able to modify the cellular functionality of monocytes, concerning phagocytic percentage after 30 days of supplementation. However, these differences cannot be observed between the Placebo and Probiotic groups in the other parameters evaluated. The monocytes population was equal, with no statistical differences verified by the immunophenotyping. Several studies observed benefits with the supplementation of these bacteria. Because of that, it is necessary to verify the time and dose offered for this specific population of marathon runners.

Acknowledgements: Financial Support: FAPESP #2016/25821-5 and we declare that there is no conflict of interest in research.

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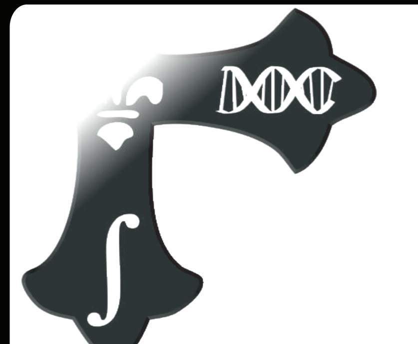

Determinants of *S. aureus* carriage in the developing infant nasal microbiome

Emma Accorsi[1], Eric A. Franzosa[1,2], Tiffany Hsu[1,2], Regina J. Cordy[3], Ayala Maayan-Metzger[4,5], Hanaa Jaber[5], Aylana Reiss-Mandel[5], Casey DuLong[1], Marc Lipsitch[1], Gili Regev-Yochay[4,5], Curtis Huttenhower[1,2]

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Staphylococcus aureus is a leading cause of healthcare- and community-associated infections and can be difficult to treat due to antimicrobial resistance. About 30% of individuals carry *S. aureus* asymptomatically in their nares, a risk factor for later infection, and interactions with other species in the nasal microbiome likely modulate its carriage. It is thus important to identify ecological or functional genetic elements within the maternal or infant nasal microbiomes that influence *S. aureus* acquisition and retention in early life. We recruited 36 mother-infant pairs and profiled a subset of monthly longitudinal nasal samples from the first year after birth (n=284) using shotgun metagenomic sequencing. The infant nasal microbiome was highly variable, particularly within the first 1-2 months. It was weakly influenced by maternal nasal microbiome composition, but primarily shaped by developmental and external factors (e.g. daycare). Infants displayed distinctive patterns of *S. aureus* carriage, positively associated with *Acinetobacter* species, *Streptococcus parasanguinis*, *Streptococcus salivarius*, and *Veillonella* species and inversely associated with maternal *Dolosigranulum pigrum*. Furthermore, we identified a gene family, likely acting as a taxonomic marker for an unclassified species, that was significantly anticorrelated with *S. aureus* in infants and mothers. In gene-content based strain profiling, infant *S. aureus* strains were more similar to maternal strains. This improved understanding of *S. aureus* colonization is an important first step toward development of novel, ecological therapies for controlling *S. aureus* carriage.

Infants display striking patterns of *S. aureus* carriage

(A) 36 mother-infant pairs gave nasal swabs monthly over the first year after birth. Culture testing for *S. aureus* was performed on all samples and a subset (*n*=284) were profiled with shotgun metagenomic sequencing.

(B) The percent of positive time points after *S. aureus* acquisition was not significantly different between early and late acquirers, likely due to the small sample size (*n*=28).

(C) Identification of *S. aureus* by culture and sequencing showed strong, although not complete, concordance.

(A)

Early

Late

Never

Mother−infant pairs

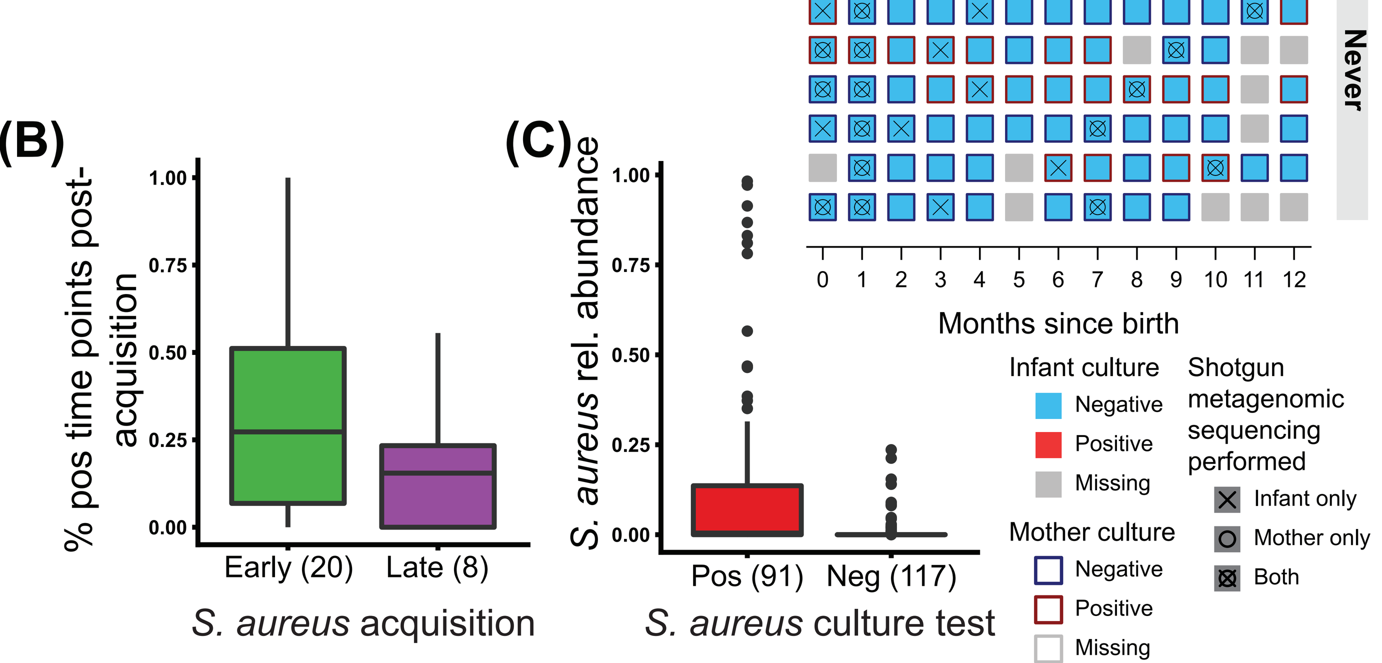
0 1 2 3 4 5 6 7 8 9 10 11 12

Months since birth

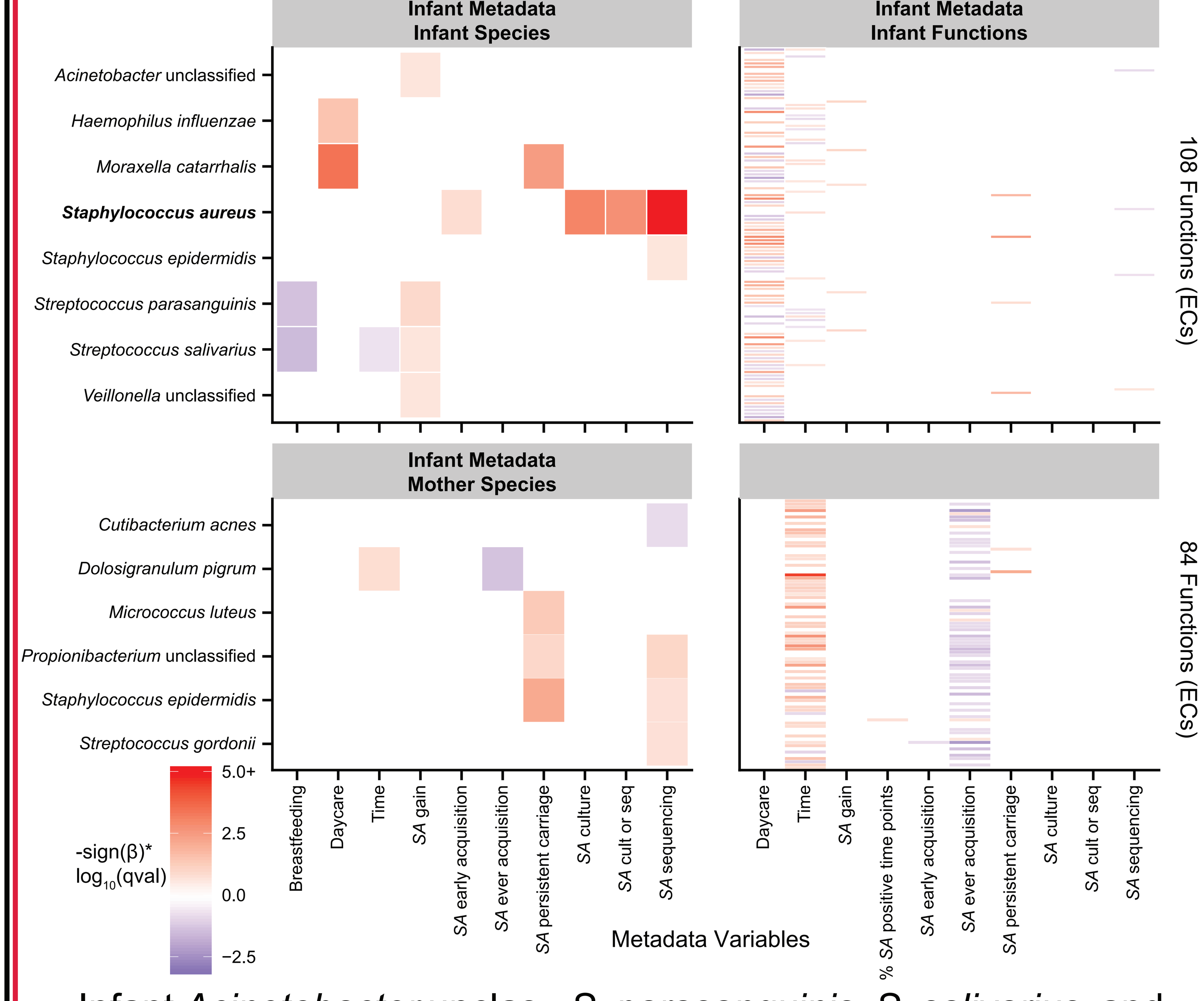
Infant culture: Negative, Positive, Missing

Mother culture: Negative, Positive, Missing

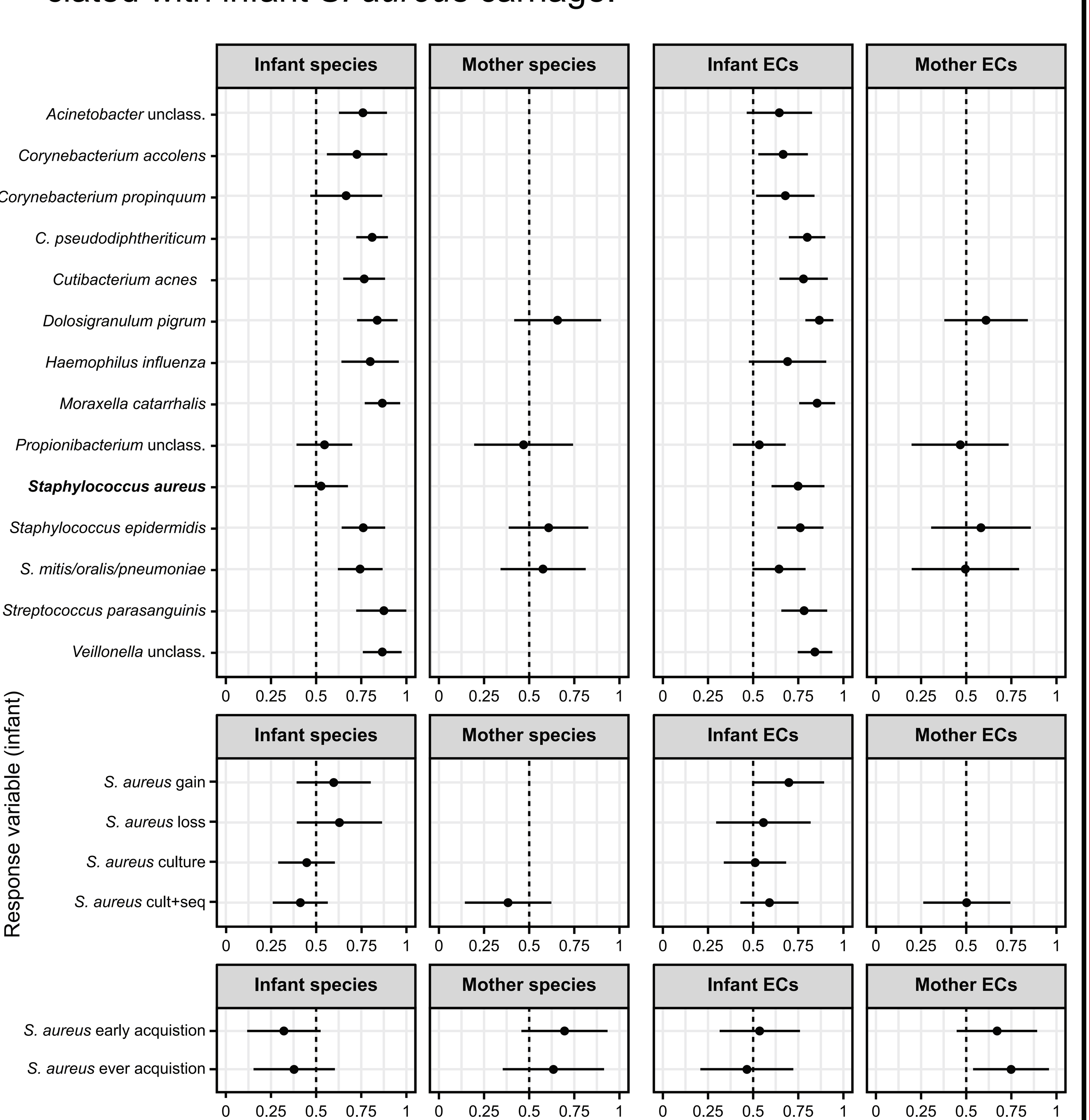
Shotgun metagenomic sequencing performed: Infant only, Mother only, Both



Microbiome drivers of infant *S. aureus* phenotypes



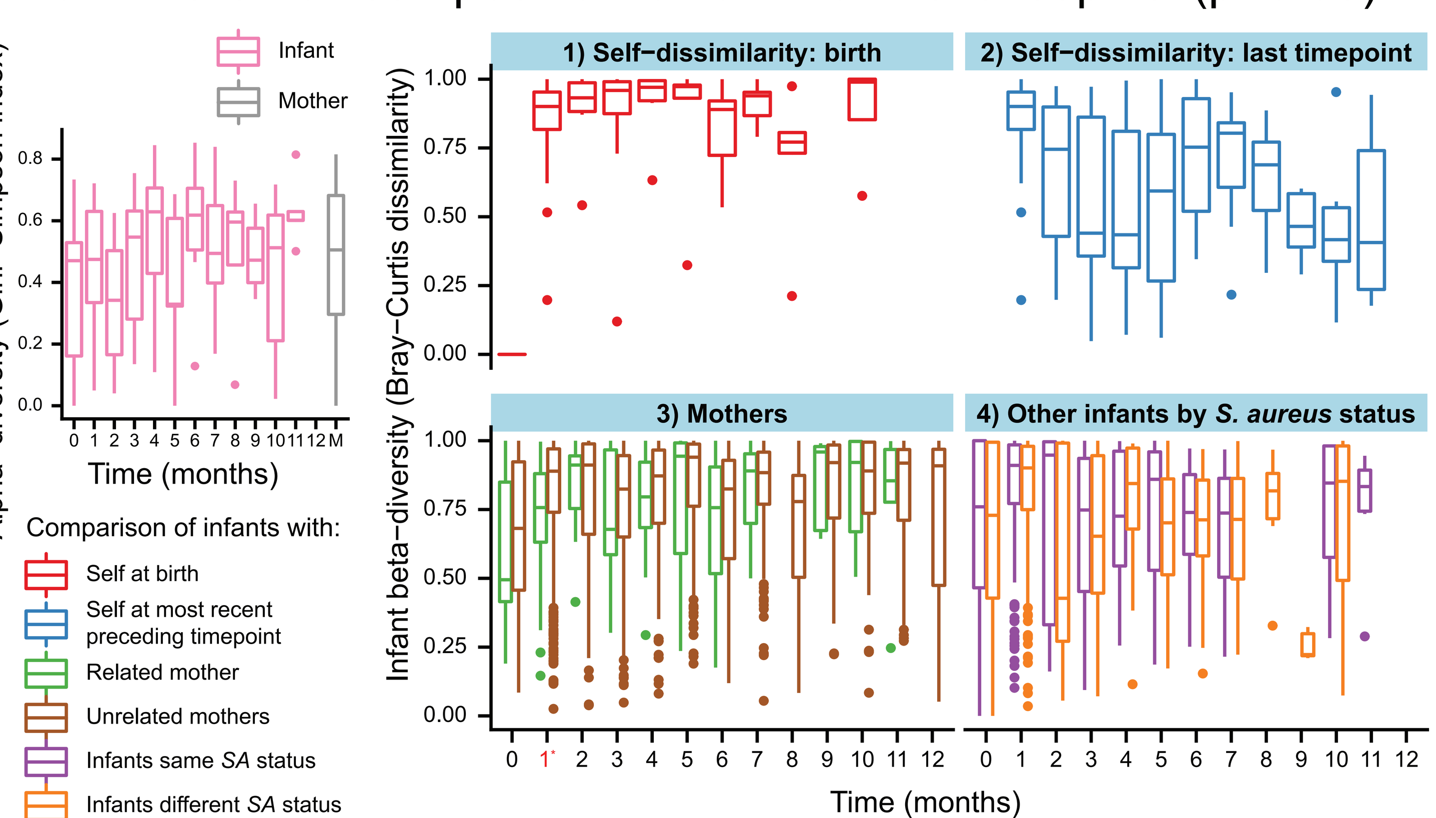
Infant *Acinetobacter* unclas., *S. parasanguinis*, *S. salivarius*, and *Veillonella* unclas. and maternal *D. pigrum* were positively associated with infant *S. aureus* carriage.



Many species in the infant microbiome were predictable (11/13 using species, 9/13 using ECs), but *S. aureus* was consistently difficult to predict, although performance improved using ECs.

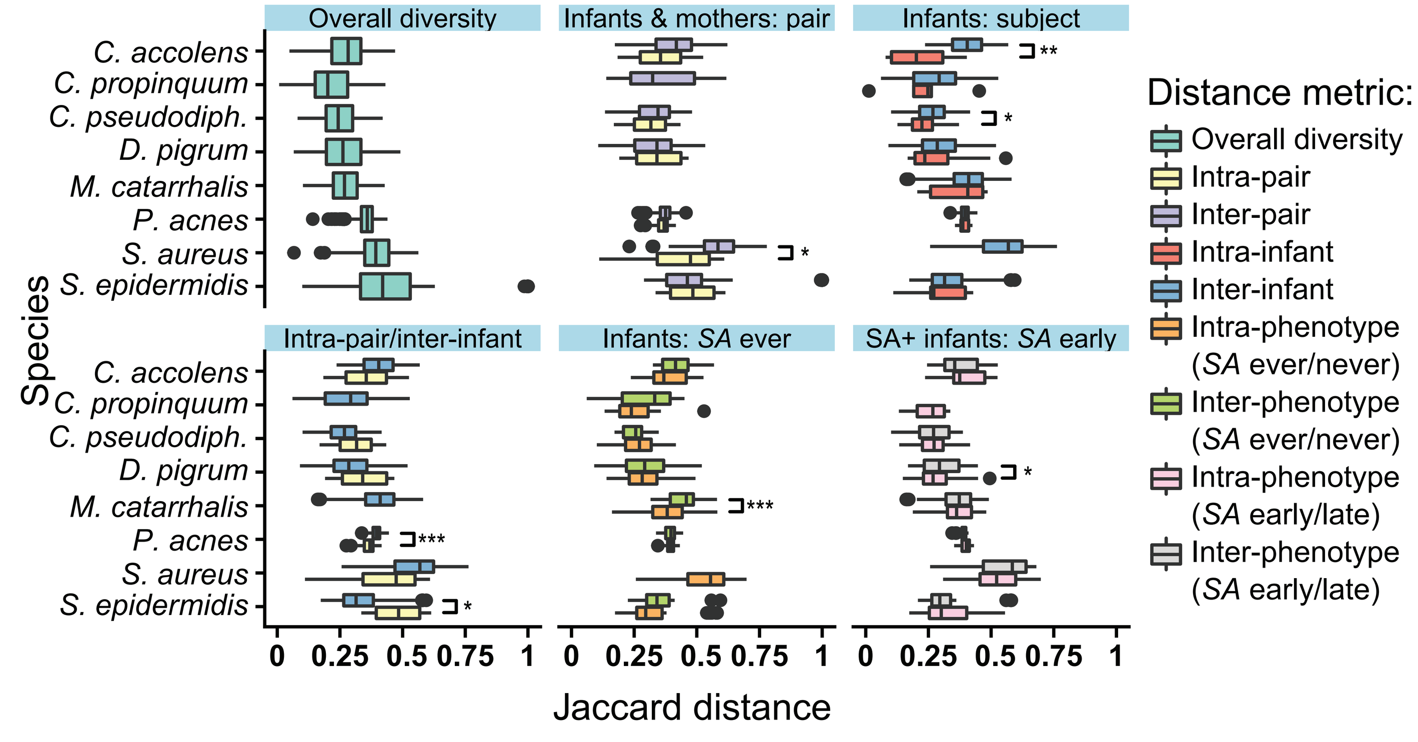
Infant nasal microbiomes mature over the first year, but remain distinct from mothers

Infants had a weak upward trend in alpha diversity over time. They rapidly diverged from their species composition at birth, but the rate of change slowed over time indicating stabilization toward a more mature microbiome. Infants were more similar to their own mother than to unrelated mothers at month 1 (PERMANOVA, p=0.005), although infant composition was distinct from maternal composition at all months except 8 (p<0.05).



Strain genotypes show similarity in mother & infant *S. aureus*

Infant *S. aureus* strains were more similar to those of their own mothers, compared to unrelated mothers or other infants.



Acknowledgements

The authors express their gratitude to all participants for generously providing their time and samples, and to the NIAID of the NIH (grants R21AI112991 to CH and T32AI007535 to EA), the Chief Scientist, Ministry of Health, Israel (grant 3-00000-5622 to GRY), and the Israel Science Foundation (grants 1590/09, and 1658/15 to GRY) for funding.

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The landscape of novel lateral gene transfer events in the human microbiome

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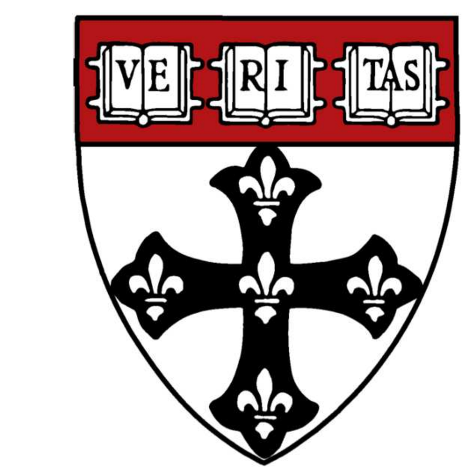
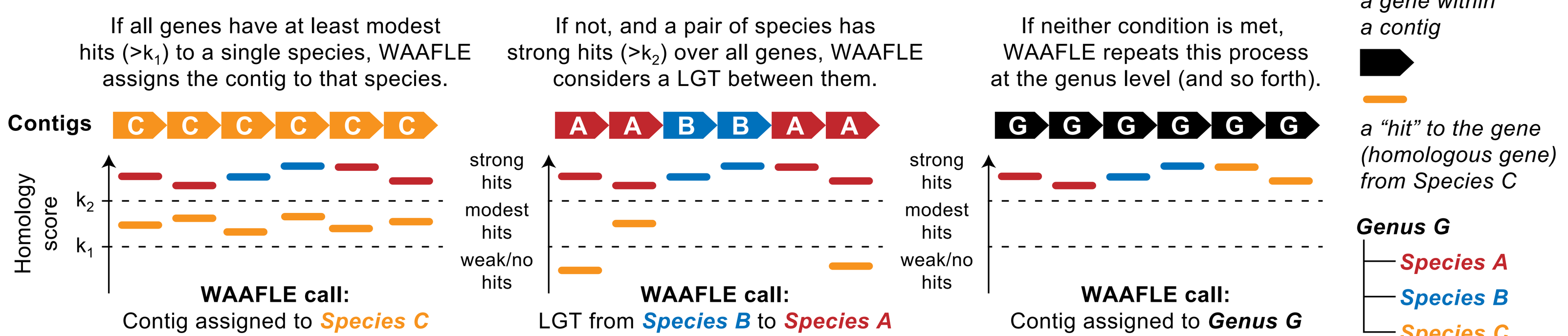


Finding novel LGT in metagenomes

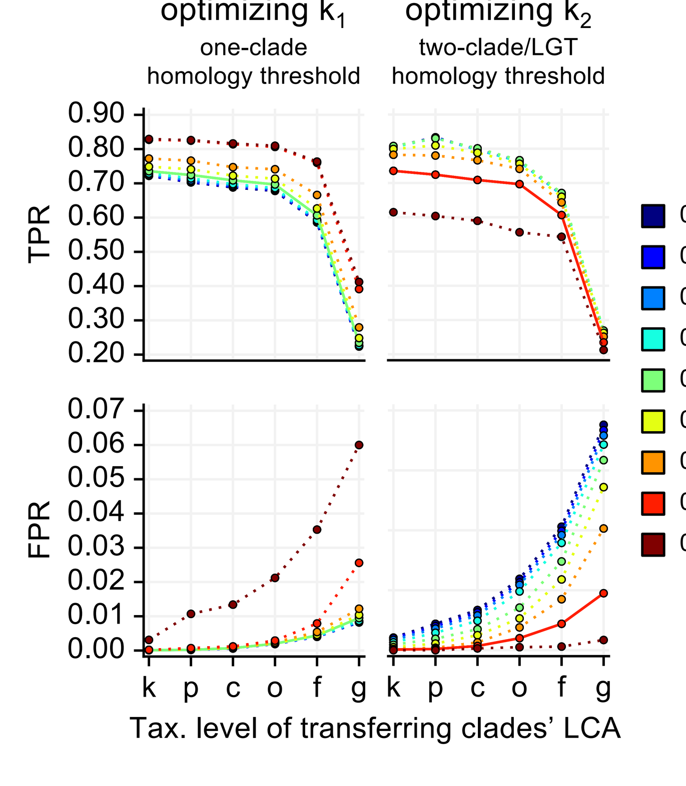
Lateral gene transfer (LGT) is an important mechanism for genome diversification in microbial communities, including the human microbiome. While previous efforts have cataloged LGT in human-associated microbial isolate genomes, directly identifying novel (and potentially recent) LGT events in human microbiomes is an open challenge. To address this, we developed a computational method (WAAFLE) to identify novel LGT events from assembled metagenomes.

Overview of the WAAFLE algorithm

WAAFLE uses homology-based search to identify metagenomic contigs that *can't* be reasonably assigned to any single taxon, but which *can* be confidently assigned to a pair of taxa (a putative LGT). Downstream filters exclude alternative explanations, e.g. gene deletion and misassembly.

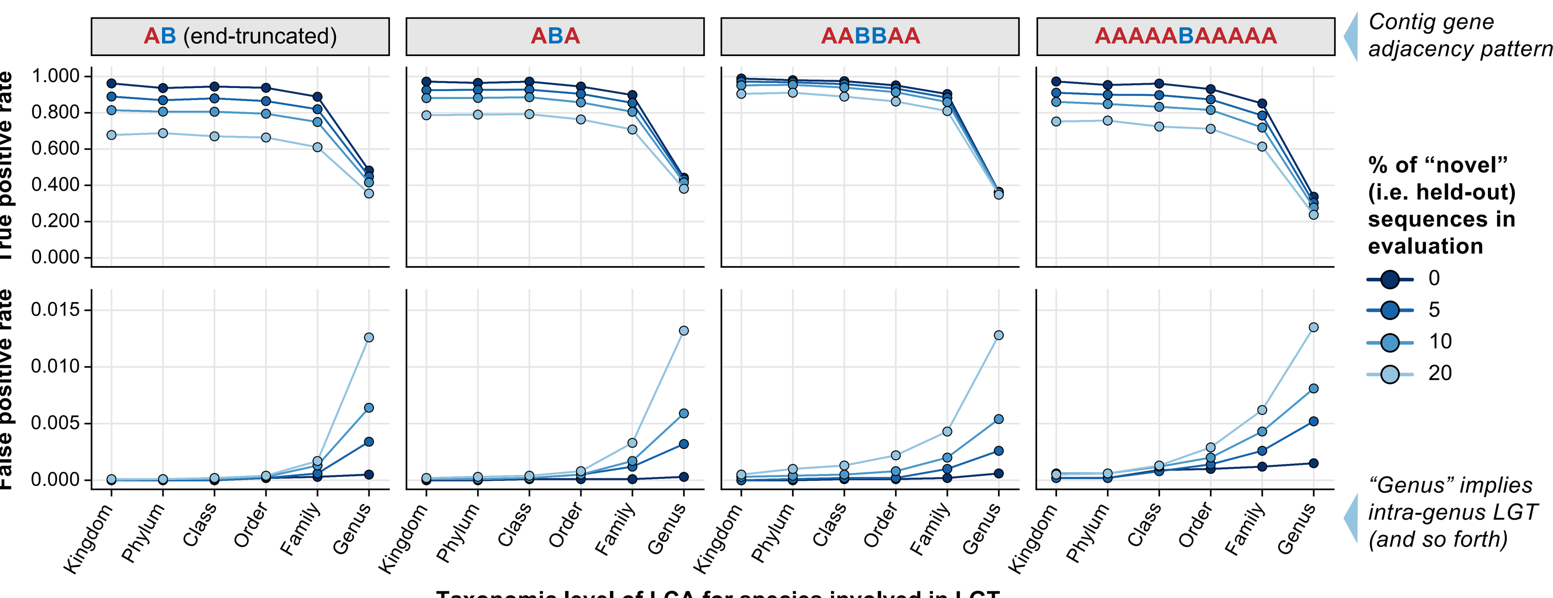


WAAFLE is sensitive, specific, and robust to novel genes



We trained and evaluated WAAFLE using synthetic contigs built from single species (non-LGT controls) and from pairs of increasingly diverged species (mock LGTs). These contigs further varied in gene adjacency and novel sequence content.

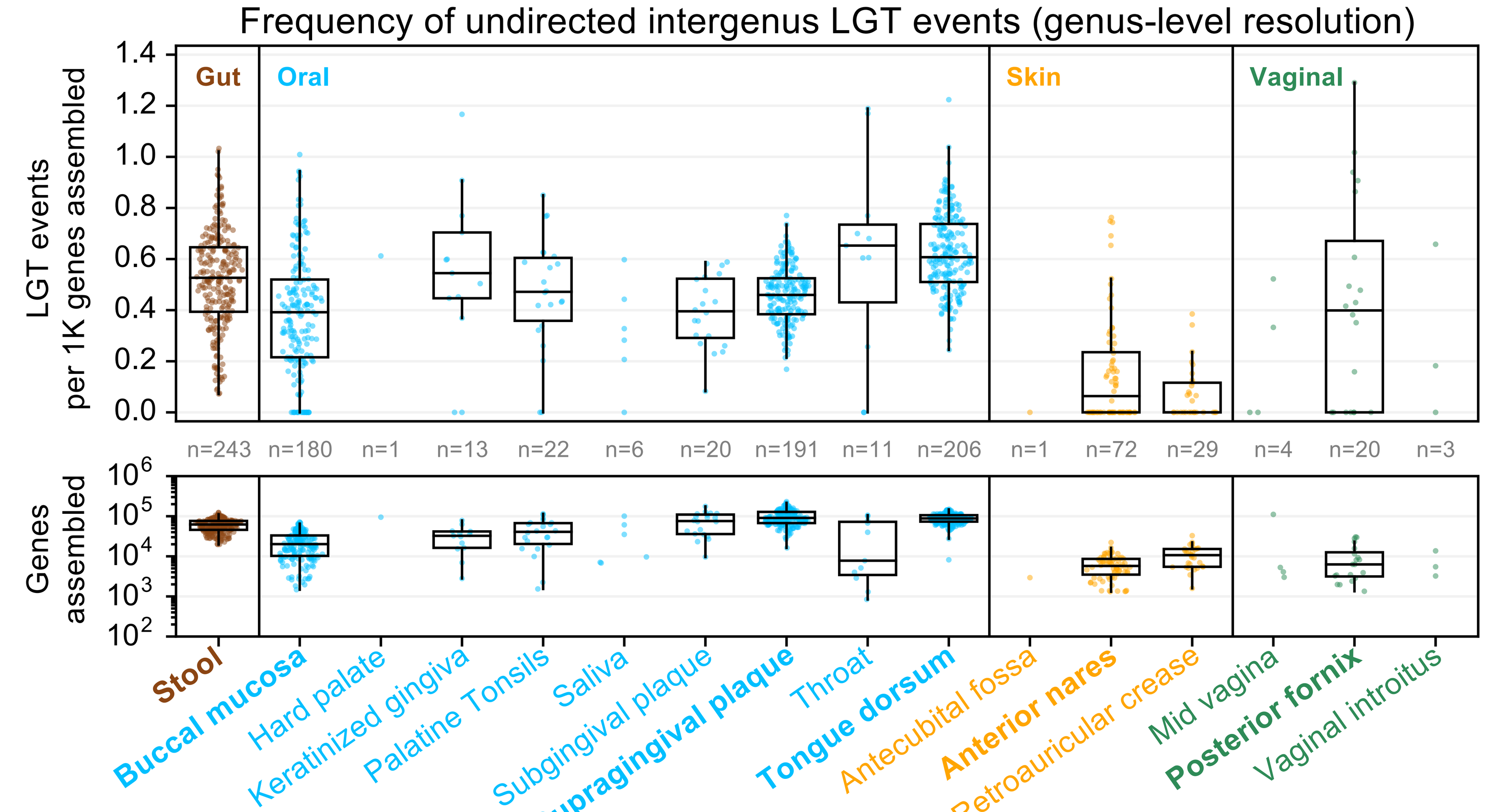
WAAFLE performed best when it was fairly lenient about assigning a contig to a single clade (k_1 = 0.5) and fairly stringent about assigning a contig to a pair of clades (k_2 = 0.8).



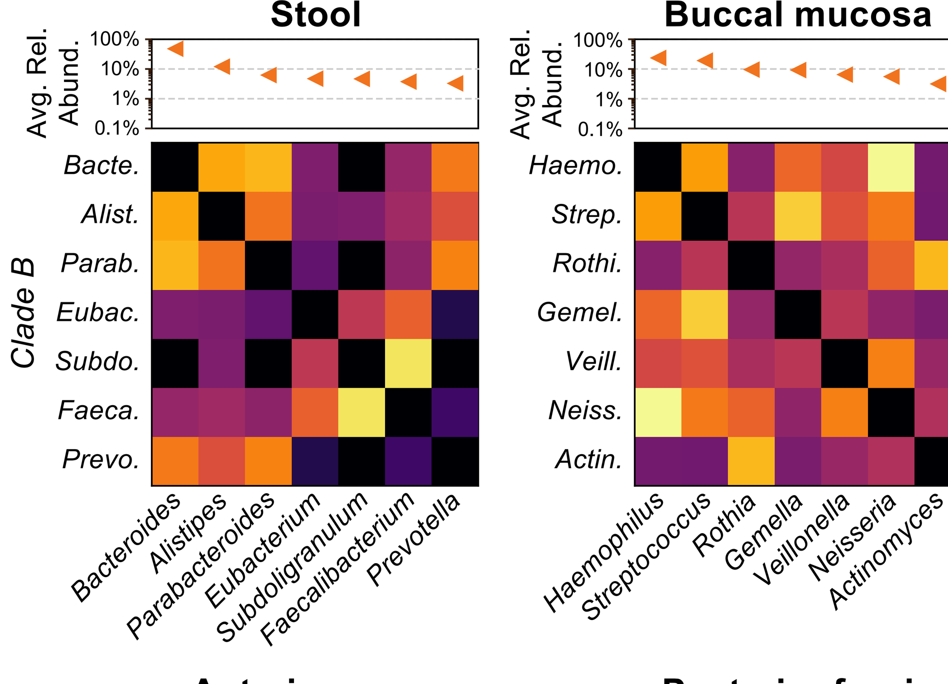
WAAFLE is highly sensitive to *inter*genus LGT and moderately sensitive to *intra*genus LGT. WAAFLE was additionally highly specific, even in the presence of a large fraction of novel sequences. False positives occurred mostly in the form of spurious intragenus LGT calls.

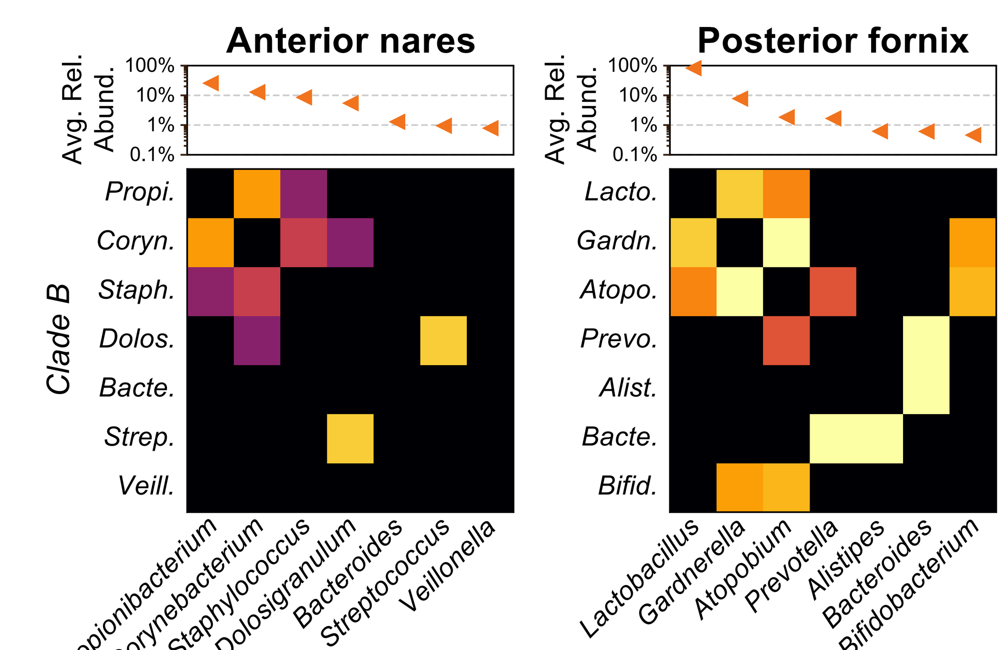
Novel LGT in the human microbiome

We applied WAAFLE to >2K diverse, assembled human metagenomes (HMP1-II), identifying >100K high-confidence, novel LGT events. Novel intergenus LGT was observed roughly once per 2K assembled genes.

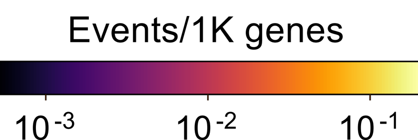


Determinants of LGT rate among human microbiome clades





At the left, heatmaps detail the rates of LGT between major genera at selected human body sites (rates are normalized to total joint assembly size).



LGT rates were higher for clade pairs in which 1) the clades were more phylogenetically similar and 2) the donor clade was more ecologically abundant.

Such rate matrices can be additionally realized as LGT networks. These networks tended to exhibit "small world" behavior, with abundant donor "hub" clades transferring preferentially with phylogenetic neighbors.

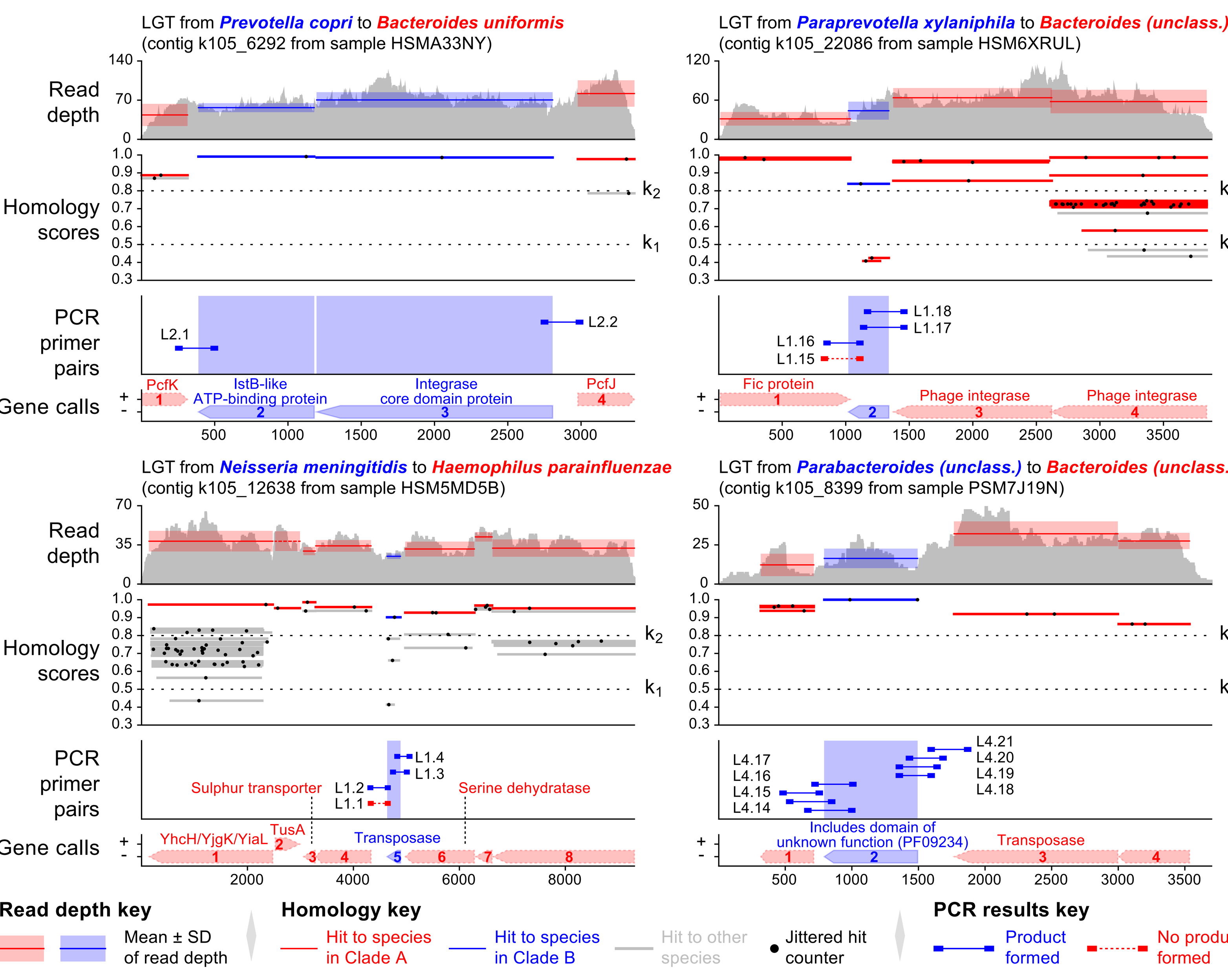
Actinomyces, Actinobaculum, Corynebacterium, Capnocytophaga, Neisseria, Rothia, Haemophilus, Veillonella, Streptococcus, Porphyromonas, Lautropia

Supragingival plaque

Firmicutes   Actinobacteria   Bacteroidetes   Proteobacteria

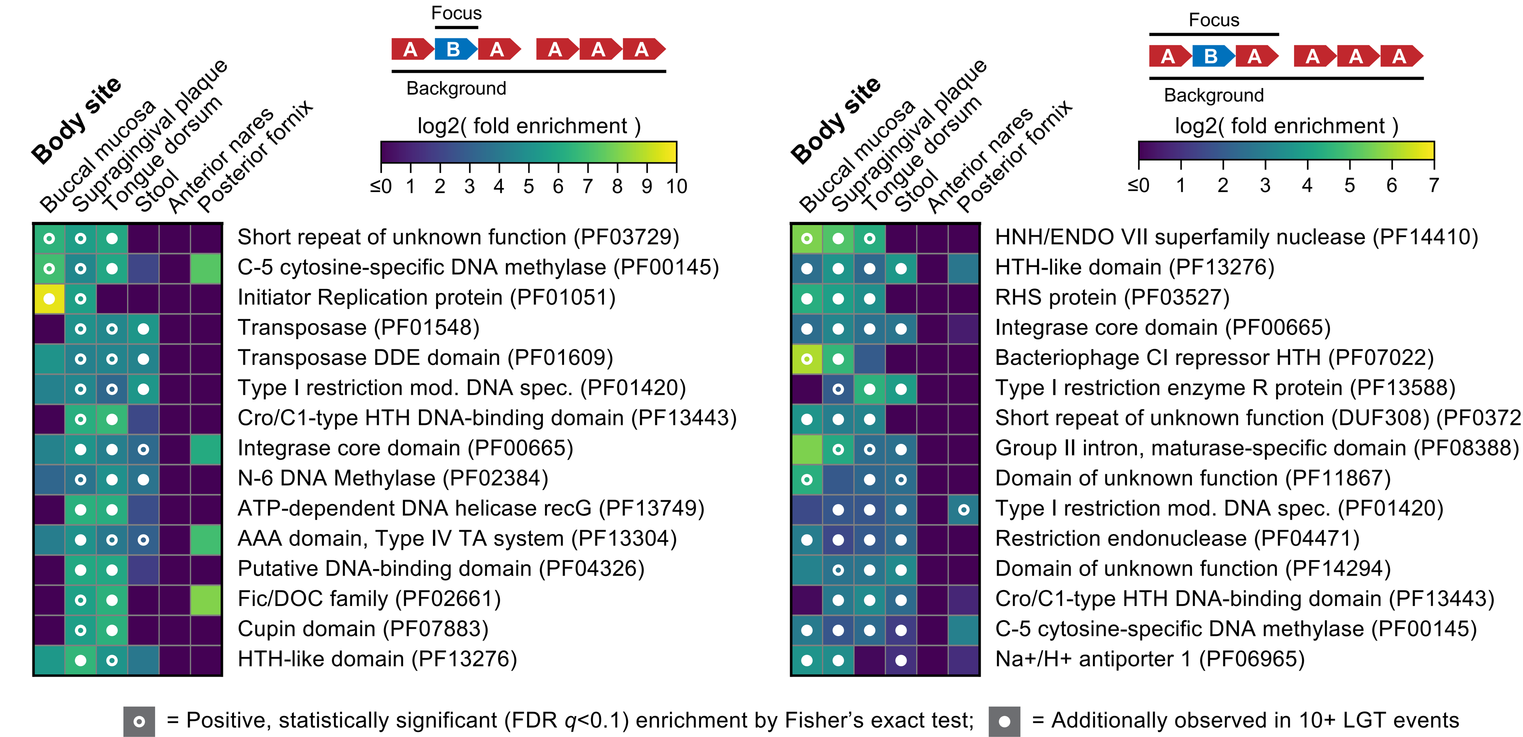
Validating predicted LGT events

We validated 21 additional LGT events identified from healthy human gut metagenomes (HMP2) by PCR. 18 of these (86%) were supported by PCR amplification of one or both LGT junctions (see examples below).



Functional enrichments among LGT-containing contigs

Novel LGTs were highly enriched for mobile elements (as expected), along with methylation, transport, and uncharacterized Pfam domains.



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The Anti-Diabetic Effects of Palmitic Acid Hydroxy Stearic Acid (PAHSA) Lipids are Transmissible by Fecal Microbiota Transplantation (FMT) in Mice

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A MAJOR TEACHING HOSPITAL OF HARVARD MEDICAL SCHOOL

INTRODUCTION

OBJECTIVE: A newer class of lipids called PAHSAs have anti-diabetic effects in high-fat diet (HFD)-fed mice and circulating PAHSA levels strongly associate with insulin sensitivity in humans[1]. Whether the gut microbiome contributes to the beneficial effects of PAHSAs on improving glucose homeostasis[2] is unknown. **We aimed to determine** whether the insulin-sensitizing properties of PAHSAs are transmissible by fecal microbiota transplantation (FMT) in mice.

METHODS: Fecal pellets collected from male mice treated with insulin-sensitizing PAHSAs or vehicle (50% PEG400, 0.5% Tween80 in water) for 21 days were used for FMT into recipient germ-free (GF) HFD-fed male mice. Donor feces from PAHSA or vehicle-treated mice were Dounce homogenized and resuspended in 100μL PBS under anaerobic conditions. Recipient GF-HFD mice were conventionalized with two oral doses of donor feces at days 0 and 28 and metabolically phenotyped. Cecal contents from the same mice were used for metagenome sequencing and metabolomics analyses.

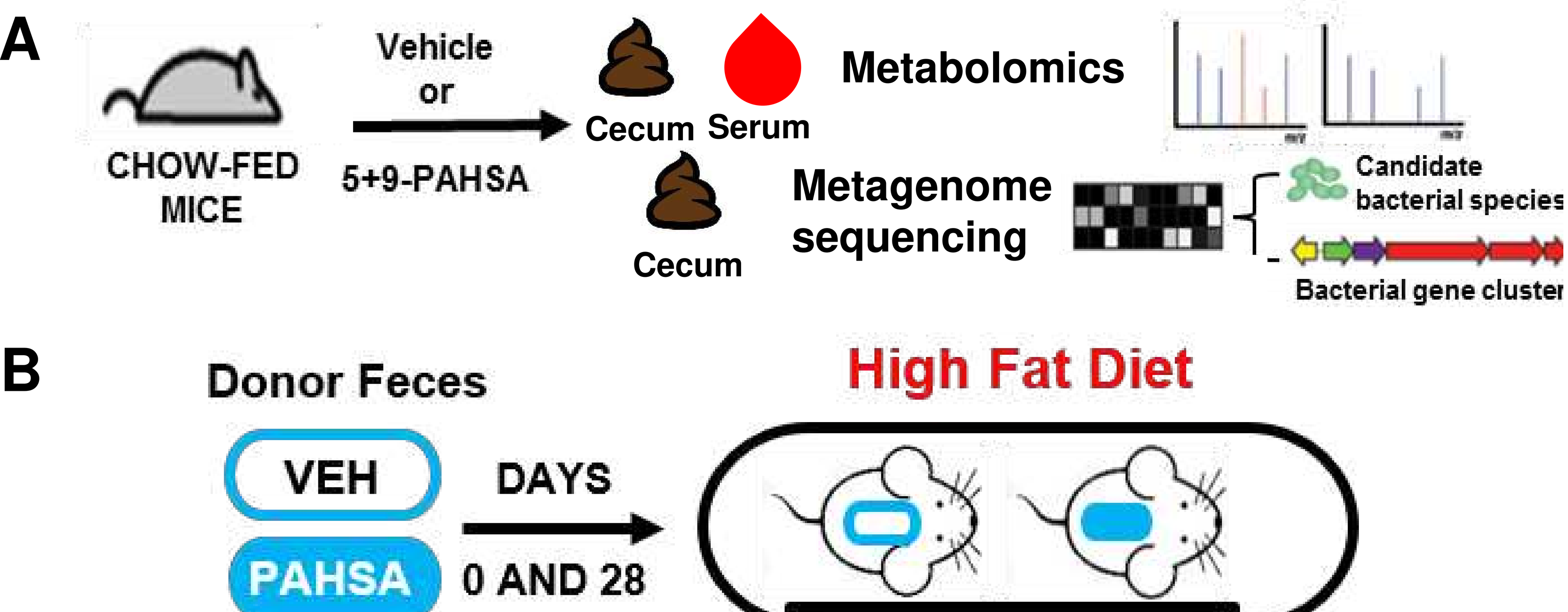

Figure 1. Experimental Workflow. (A) 6-week old male C57bl6 chow-fed mice were treated once daily with oral vehicle or 5- and 9-PAHSAs (15mg/kg of each PAHSA) for 21 days. Cecal contents were collected for 16S rRNA and metagenome sequencing. Terminal fecal pellets were used for FMT studies. **(B)** Feces collected from the same mice in (A) were used as FMT inoculum to conventionalize HFD-fed GF male mice.

Chronic oral PAHSA treatment improves insulin sensitivity in chow-fed mice

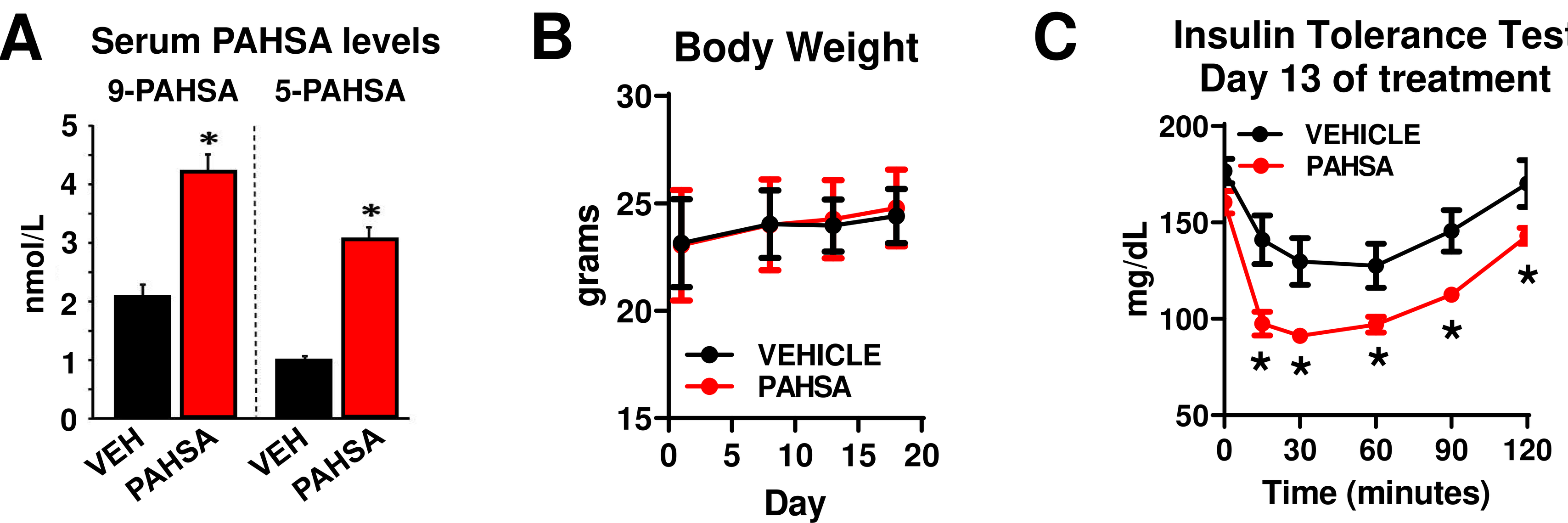

Figure 2. Daily Oral PAHSA Treatment Improves Insulin Sensitivity in Chow-fed Mice. Male C57bl6 mice treated with vehicle or 5- and 9-PAHSAs **(A)** have elevated serum PAHSA levels and **(B)** no body weight change after 21 days of treatment. **(C)** Mice treated with PAHSAs have improved insulin sensitivity after 13 days of treatment. *p<0.05 PAHSA vs. vehicle. n=12/group.

PAHSAs have beneficial effects on the gut microbiome

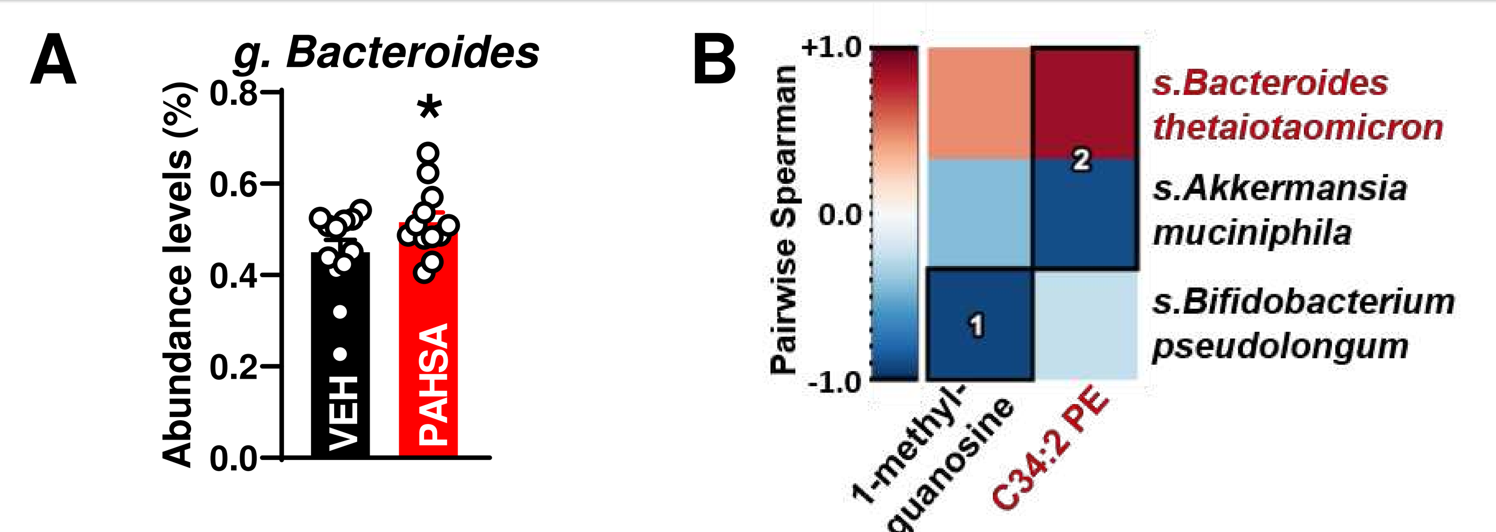

Figure 3. PAHSA Effects on the Gut Microbiome. (A) *Bacteroides* (genus) levels are elevated in male C57bl6 mice treated once daily with PAHSAs for 17 days. **(B)** HUMAnN2 functional profiling of cecal contents identifies *Bacteroides thetaiotaomicron* to be most strongly associated with the insulin-sensitizing effects of PAHSA lipids in mice. *p<0.05 PAHSA vs. VEH. n=12/group.

The Anti-Diabetic Effects of PAHSAs are Transmissible by Fecal Microbiota Transplantation (FMT)

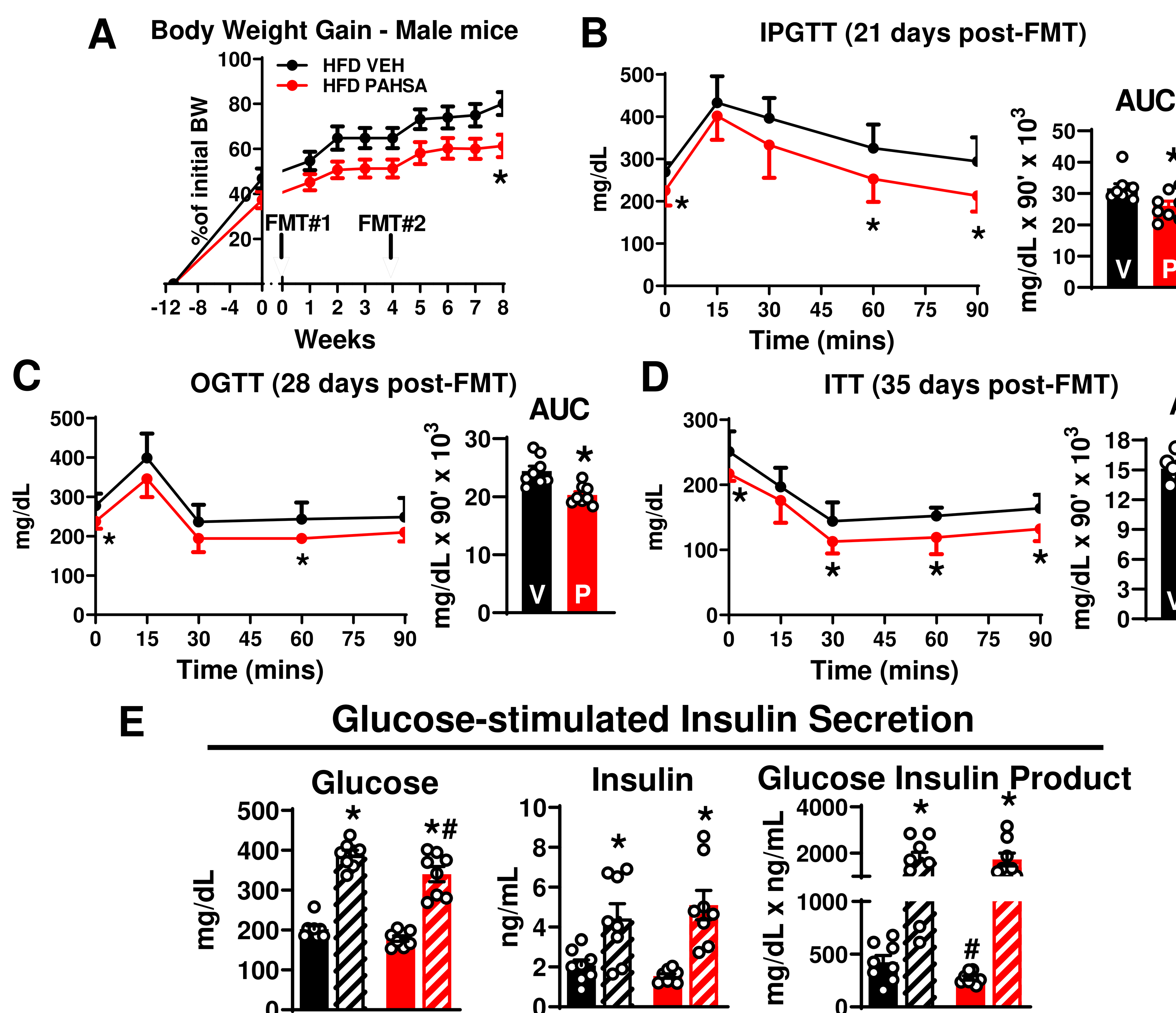

Figure 4. Beneficial Effects of FMT in HFD-GF Mice Conventionalized with Donor Feces From PAHSA-treated Insulin Sensitive Mice. HFD-GF PAHSA-treated mice **(A)** gain less body weight, **(B-C)** are more glucose tolerant, and **(D)** are more insulin sensitive compared to control HFD-GF mice conventionalized with vehicle-treated donor feces. These PAHSA-mediated FMT effects are independent of **(E)** insulin secretion. *p<0.05 HFD-GF PAHSA-treated mice vs HFD-GF VEH-treated control mice. #p<0.05 vs. HFD-GF VEH mice for the same time point. Statistics analyzed by repeated-measures 2-way ANOVA or t-test. n=8/group.

SUMMARY

- Once daily PAHSA treatment improves insulin sensitivity in chow-fed C57bl6 male mice as early as 13 days of treatment; this effect is independent of body weight.
- Abundance of *Bacteroides* (genus) is increased in PAHSA-treated chow-fed mice and *Bacteroides thetaiotaomicron* (species) is associated with improved insulin sensitivity.
- Compared to control mice, HFD-fed germ-free mice conventionalized with PAHSA donor feces:
  - gain less weight
  - are more glucose tolerant and insulin sensitive
  - have reduced glycemia 5-hours after food removal

CONCLUSIONS

- PAHSAs have beneficial effects on the gut microbiome.
- The insulin-sensitizing effects of PAHSAs can be conferred by FMT to improve glucose homeostasis in recipient HFD-fed mice.
- Identifying PAHSA-mediated microbes and their metabolites that improve host metabolism may lead to novel therapeutic strategies to treat diet-induced obesity and insulin resistance.

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BADERC
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THE JPB FOUNDATION

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Harvard T.H. Chan School of Public Health Microbiome Analysis Core

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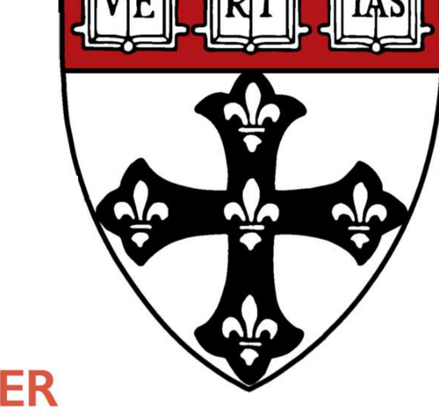

The Microbiome Analysis Core at the Harvard T.H. Chan School of Public Health was established in response to the rapidly emerging field of microbiome research and its potential to affect studies across the biomedical sciences. The Core's goal is to aid researchers with microbiome study design and interpretation, reducing the gap between primary data and translatable biology. The Microbiome Analysis Core provides end-to-end support for microbial community and human microbiome research, from experimental design through data generation, bioinformatics, and statistics. This includes general consulting, power calculations, selection of data generation options, and analysis of data from amplicon (16S/18S/ITS), shotgun metagenomic sequencing, metatranscriptomics, metabolomics, and other molecular assays. The Microbiome Analysis Core has extensive experience with microbiome profiles in diverse populations, including taxonomic and functional profiles from large cohorts, qualitative ecology, multi'omics and meta-analysis, and microbial systems and human epidemiological analysis. By integrating microbial community profiles with host clinical and environmental information, we enable researchers to interpret molecular activities of the microbiota and assess its impact on human health.

Core services

Consultation for microbiome project development.
This includes consultation on experimental design, sample collection and sequencing, grant proposal development, study power estimation, bioinformatics, and statistical data analysis.

Validated end-to-end meta'omic analysis of microbial community data.
Using open-source analytical methods developed in the Huttenhower laboratory and by other leaders in the field, we provide cutting-edge microbiome informatics and analysis.

Support fully-collaborative grant-funded investigations.
Includes preliminary data development, hypothesis formulation, grant narrative development, data analysis and inference, custom software development, and co-authored dissemination of findings.

Study Design
- Consultation
- Grant assistance
- Power analysis
- Collection methods
- Wet lab
- Dry lab

Analysis
- Bioinformatics (raw data processing, taxonomic and functional profiling)
- Downstream analysis and statistics

Interpretation
- Results
- Discussion
- Manuscript writing/editing
- Response to reviewers

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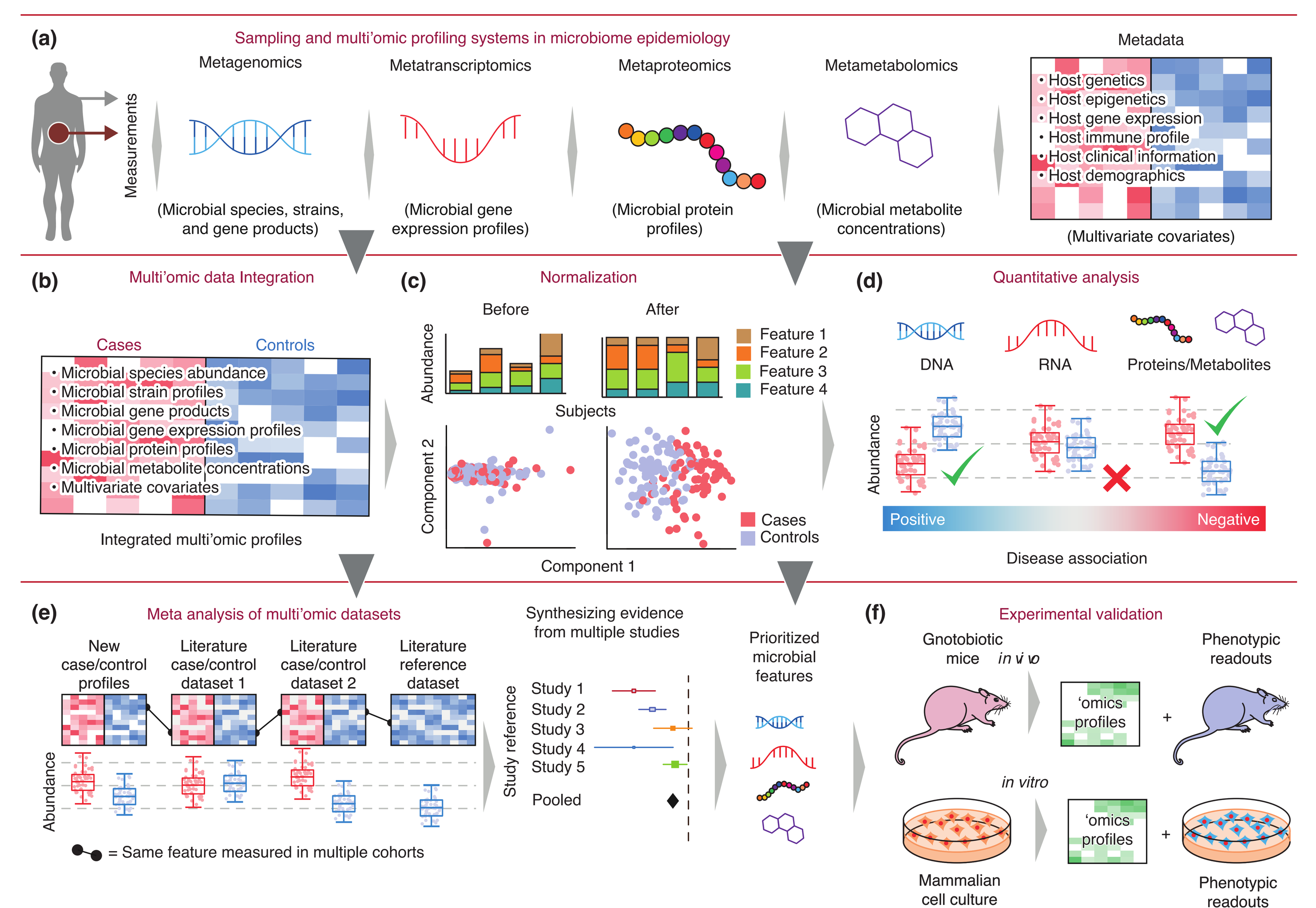
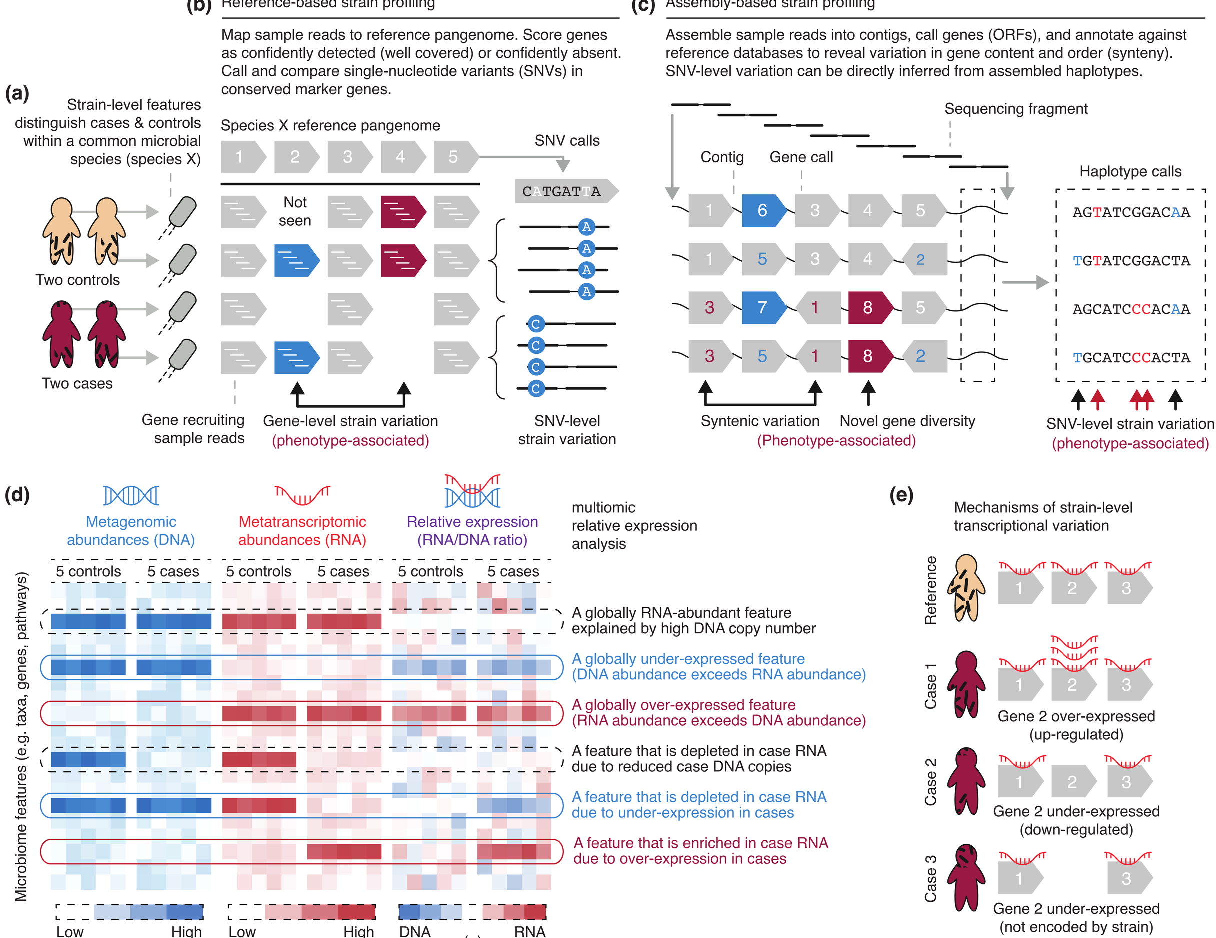
Microbial multi'omics



The Harvard Chan Microbiome Analysis Core supports microbiome analysis for a variety of molecular data types in human populations or in model systems. Typical analysis workflow steps include **a)** molecular data generation of a variety of types, including but not limited to sequencing, which are **b)** bioinformatically processed into biologically interpretable features and **c)** quality controlled per dataset. This permits **d)** microbiome-tailored statistical methods to associate molecular features with covariates and outcomes, and optionally **e)** meta-analysis of multiple data types per project or across multiple projects. Finally, **f)** the Core can assist with study design for downstream evaluation of statistical associations in in vivo or in vitro model systems.



Shotgun metagenomic and metatranscriptomic sequence data are particularly amenable to detailed computational analysis, including multiple complementary methods for **a)** strain tracking or differential microbial expression. **b)** Reference-based methods can identify strains using either single nucleotide or structural (genomic) variants, and **c)** can be used in tandem with assembly-based methods for novel microbial discovery. **d)** Whole-community microbial differential expression can additionally be detected either in tandem with or in addition to metagenomic copy number changes, and **e)** analyzed per gene, pathway, microbe, or human individual.

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Microbial community profiling

The first step in microbiome molecular data analysis is quality control (KneadData) and profiling to transform raw data into biologically interpretable features using a reproducible workflow (AnADAMA2). This includes identifying microbial species (MetaPhlAn2) and strains (PanPhlAn/StrainPhlAn), characterizing their functional potential or activity (HUMAnN2, ShortBRED), and integrating metagenomics with other data types (PICRUSt, MelonnPan), among others.

KneadData – Perform quality control of meta'omic reads, including host read removal

MetaPhlAn – Profile microbial clades and their abundances

StrainPhlAn – Identify and track microbial strains using SNPs in conserved, unique marker genes

HUMAnN – Profile microbial genes and pathways; stratify by contributing species

PICRUSt – Predict metagenome functional content from marker genes

ShortBRED – Profile proteins of interest with very high specificity

WAAFLE – Identify novel LGT events in assembled metagenomes

MelonnPan – Predict metabolome content from microbial enzyme abundances

PhyloPhlAn – Reconstruct microbial phylogenetic trees

PPANINI – Prioritize microbial genes based on their meta'omic properties

AnADAMA – Perform and document automated scientific workflows

Downstream analysis and statistics

Once profiled, microbial communities are amenable to downstream statistics and visualization much like other molecular epidemiology such as human genetic or transcriptional profiles. Like these other data types, microbial communities often require tailored statistics for environmental, exposure, or phenotype association (LEfSe, MaAsLin) or for ecological interaction discovery (BAnOCC). The Harvard Chan Microbiome Analysis Core also provides a variety of tools for bioinformaticians working in the microbiome space.

SparseDOSSA – A hierarchical model of microbial ecological population structure

ARepA – Extract and normalize 'omics data from online repositories

MMUPHin – Correct batch effects, meta-analyze microbes, genes, and pathways across multiple studies

GraPhlAn – Generate cladograms and decorate with metadata

LEfSe – Associate up to two metadata with microbiome features

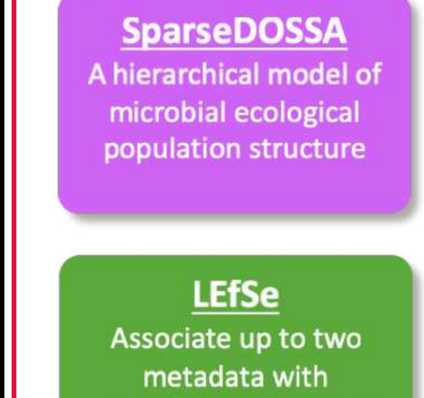
MaAsLin – Associate arbitrarily complex metadata with microbiome features

CCREPE – Assess the significance of similarity measures in compositional data

HAllA – Perform well-powered comparisons of paired, high-dimensional datasets

BAnOCC – Bayesian assessment of association in compositional data

MicroPITA – Select samples for follow-up analysis in two-stage tiered studies



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Research Project Manager and Data Analyst: Chengchen (Cherry) Li
Scientific Director: Curtis Huttenhower

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The gut microbiome in patients with inflammatory arthritis

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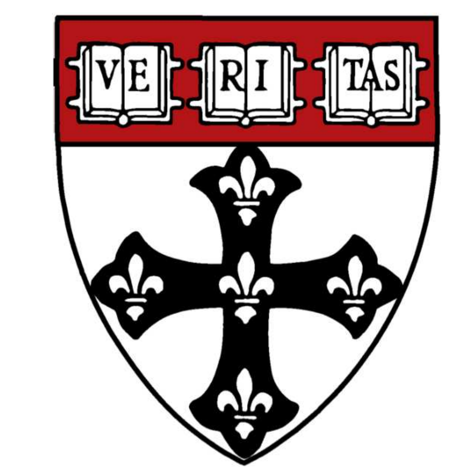
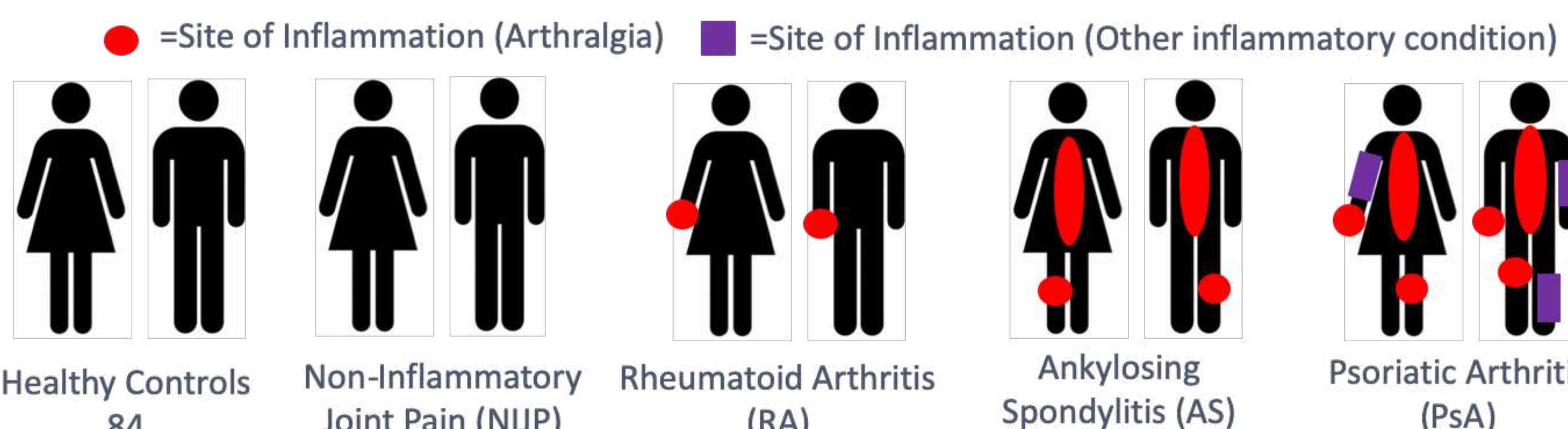
BROAD INSTITUTE

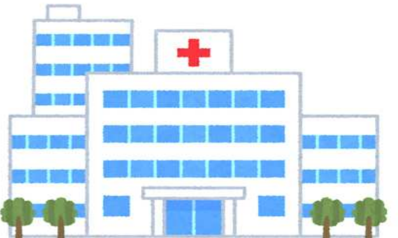


Around 350 million people worldwide are thought to suffer from arthritis. While the etiology of arthritis is largely unknown, the gut microbiome is often implicated in autoimmune conditions. To investigate the role of the gut microbiome in arthritis, we used metagenomic shotgun sequencing of 327 adults diagnosed with various forms of arthritis, as well as 69 control individuals. Approximately 2.9% of the variability in gut microbial taxonomic profiles was explained by patient diagnosis. Similar amounts (2.7%) were explained by diagnosis in functional profiles, indicating a reasonably close link between perturbed microbial community structure and function in the disease. We further identified increases in taxa normally characteristic of the oral cavity among patients with inflammatory arthritis, including several species from the genus *Streptococcus*. This strikingly mirrors similar findings regarding oral microbial enrichment in the gut during conditions such as IBD. Together, this represents the first in-depth study of the gut microbiome across different etiologies of arthritis using culture-independent techniques.

Experimental design for microbiome profiling in inflammatory arthritis

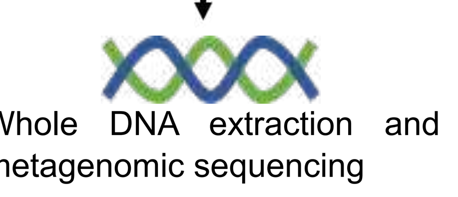


Study methodology



Diagnosis, collection of a blood sample and assement for clinical covariates

Stool collection and diet survey collected



Whole DNA extraction and metagenomic sequencing

Taxonomic and functional assignment of metagenomes and downstream statistics and visualizations

Patient demographics

Patient Diagnosis: AS, HC, NIJP, PsA, RA

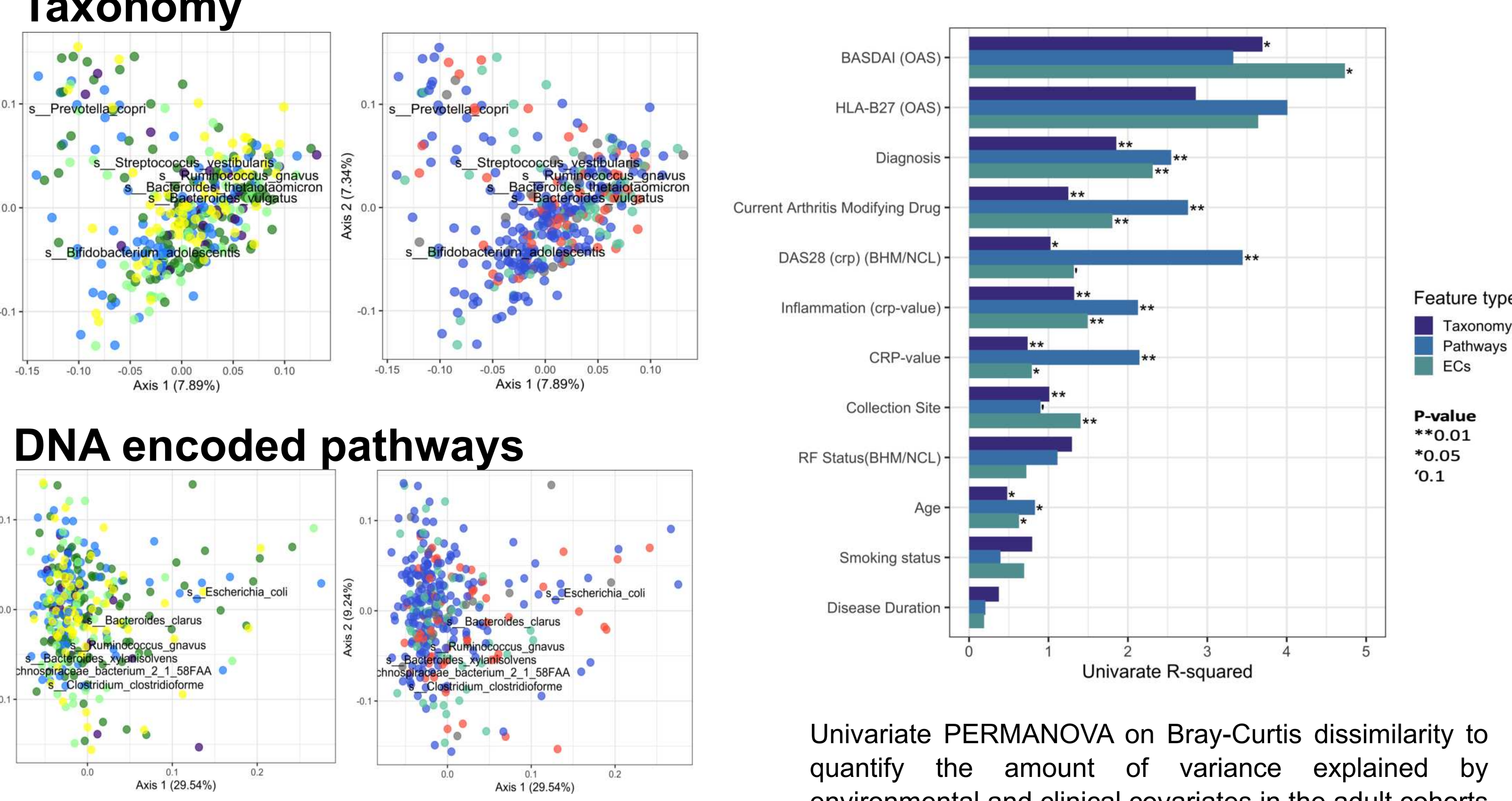
Age (years)

Inflammation Status: Not Inflammed, Some Inflammation, Inflammation, Not recorded

HLA-B27 Status

RF Status

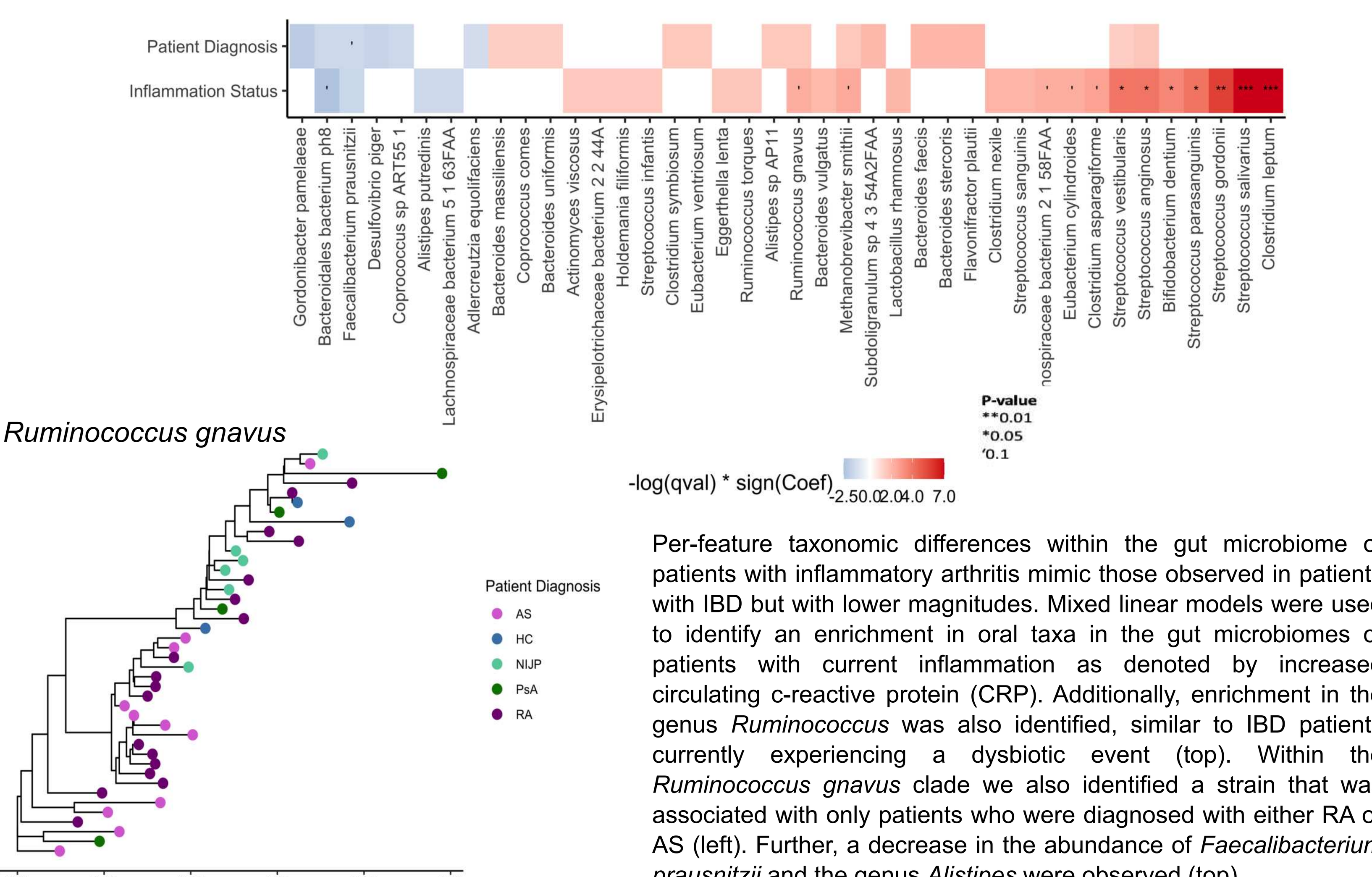
The human gut microbiome is altered in inflammatory arthritis



Principal coordinates analysis of Bray-Curtis dissimilarity on filtered community features (taxonomy, DNA pathways and enzymes (ECs)) at 0.01% abundance and 10% prevalence. Displayed by covariates of patient diagnosis (left) and Inflammation status (right) as defined by quartiles of crp (1st quartile = no inflammation, 2nd and 3rd quartiles = some inflammation, 4th quartile = inflammed).

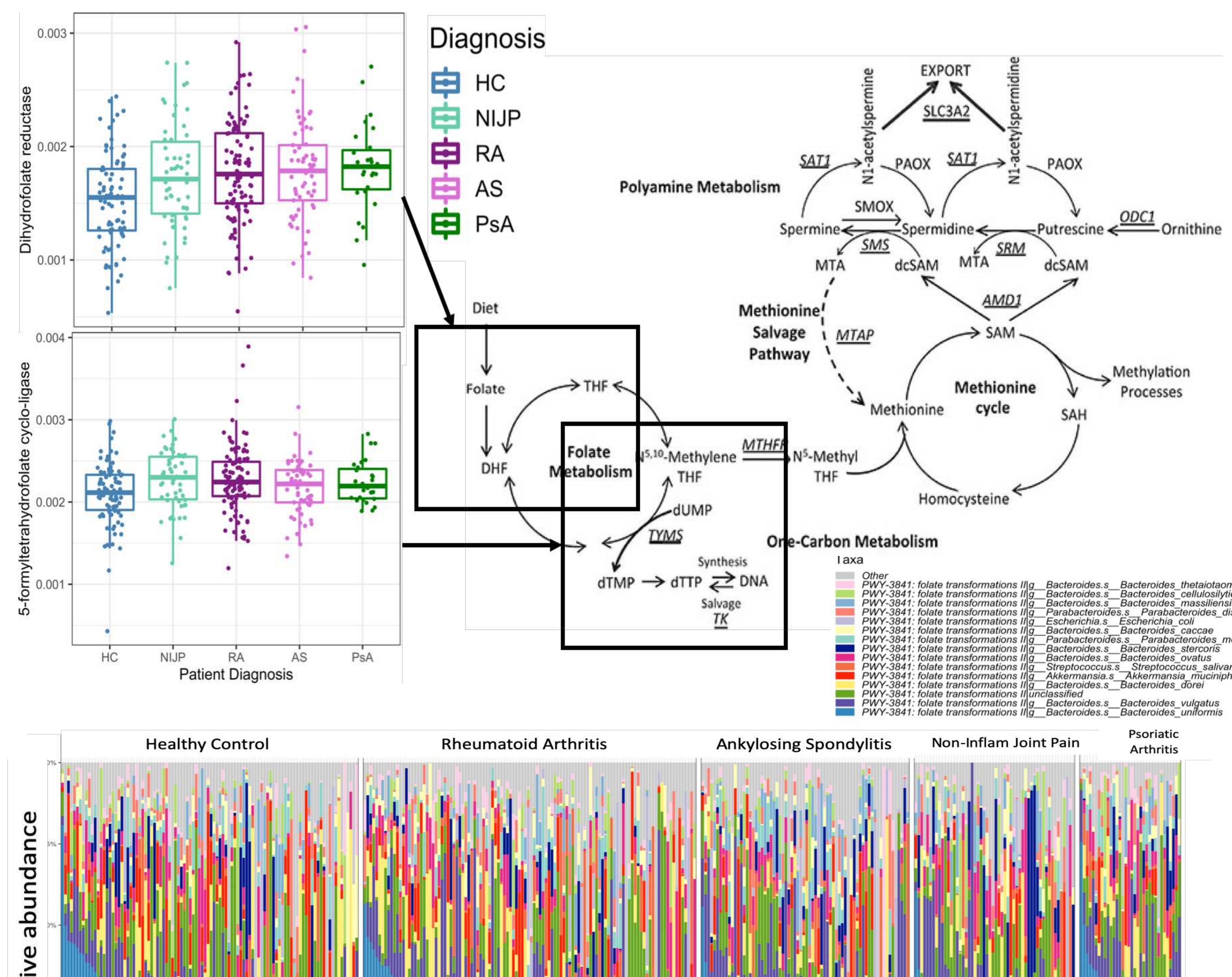
Univariate PERMANOVA on Bray-Curtis dissimilarity to quantify the amount of variance explained by environmental and clinical covariates in the adult cohorts with arthritis. Each covariate is corrected by sequencing batch (i.e. Bray-Curtis ~ batch + covariate). Where denoted, either by a BHM (RA cohort) or a OAS (AS cohort), only the patient samples from that cohort were used in the calculation. Many of the disease related covariates explain significant amounts of variation within the gut microbiome's structure and functional potential.

Patients with inflammatory arthritis exhibit consistent taxonomic differences

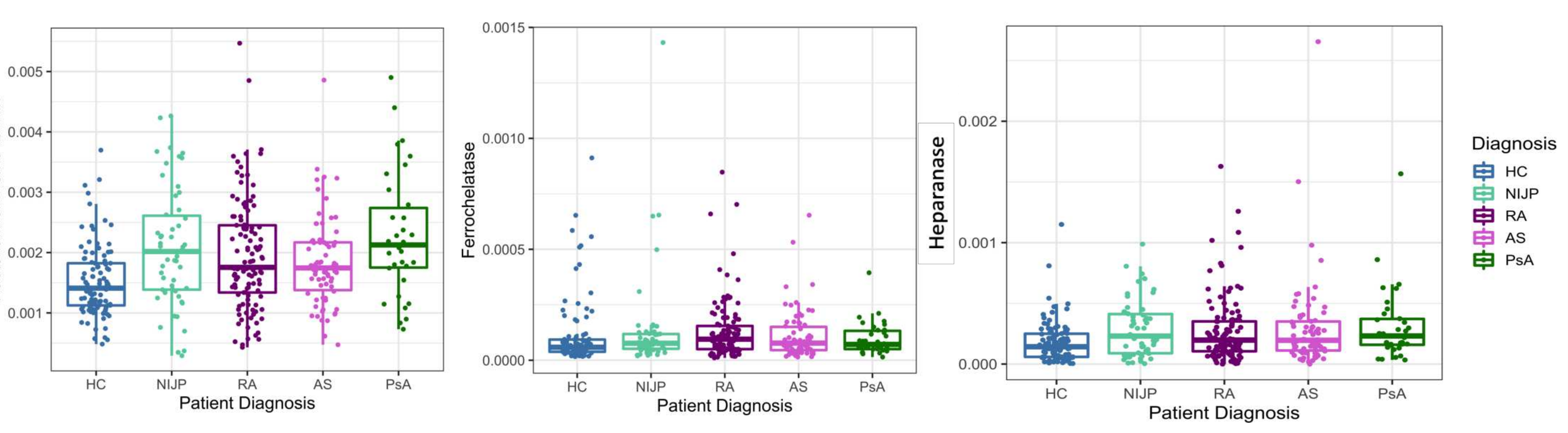


Per-feature taxonomic differences within the gut microbiome of patients with inflammatory arthritis mimic those observed in patients with IBD but with lower magnitudes. Mixed linear models were used to identify an enrichment in oral taxa in the gut microbiomes of patients with current inflammation as denoted by increased circulating c-reactive protein (CRP). Additionally, enrichment in the genus *Ruminococcus* was also identified, similar to IBD patients currently experiencing a dysbiotic event (top). Within the *Ruminococcus gnavus* clade we also identified a strain that was associated with only patients who were diagnosed with either RA or AS (left). Further, a decrease in the abundance of *Faecalibacterium prausnitzii* and the genus *Alistipes* were observed (top).

Increased nutrient uptake in the arthritis gut microbiome



Functional profiles are more conversed across the human population than taxonomic profiles. However, we did find several pathways and enzymes that have increased encoding across the gut microbiome of patients diagnosed with arthritis. Many of these functions were associated with nutrient acquisition and processing within the gut ecosystem. Here we enumerate two of these trends in folic acid metabolism (top) and iron savaging (bottom). Independent of methotrexate usage, a well-known competitive inhibitor of folic acid metabolism, we identified a significant increase in the encoding of several pathways (top) and enzymes within the folic acid metabolism pathway. This pathway lies upstream of DNA synthesis, production of choline, and epigenetic methylation to name a few processes dependent on correct folic acid metabolism. Additionally, many enzymes associated with the scavenging of iron were identified to have increased encoding in patients with inflammatory arthritis. Patients with inflammatory arthritis often are concurrently diagnosed with anemia and previous work has found decreased concentrations of folic acid, vitamin B, and iron in the serum of these patients. We have yet to elucidate if the increased encoding of these pathways and enzymes within the gut communities are causal or in response to the changes within the host.



Acknowledgments

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VERSUS ARTHRITIS

Characterizing microbial community viability using propidium monoazide

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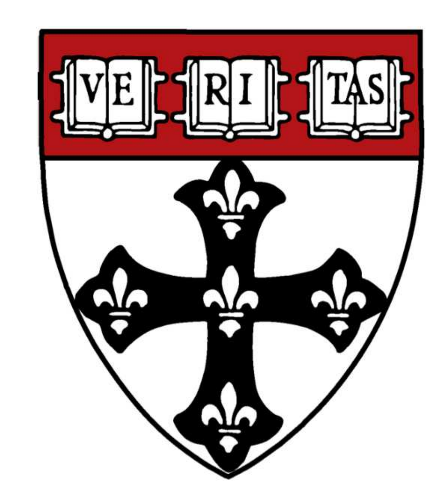
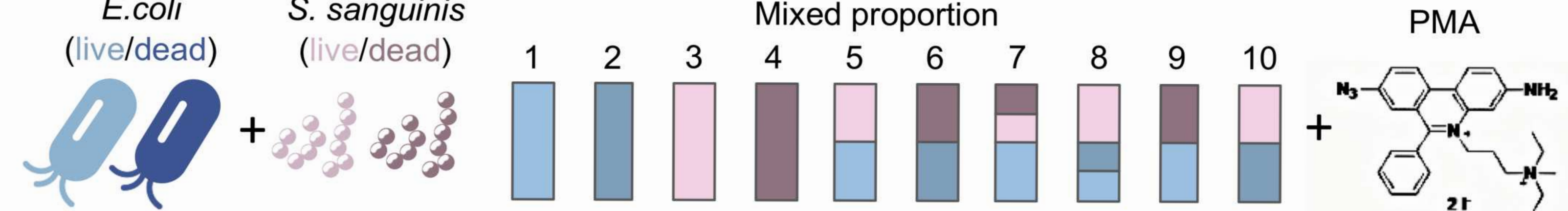


Characterization of built environment (BE) microbiomes is of great importance given the associations between microbial exposure and human health in indoor settings. Although many studies have explored the taxonomic composition of BE microbiomes using DNA sequencing, this method on its own suffers from an inability to discern viability. Here, we present our work to rigorously benchmark "PMA-seq" (propidium monoazide treatment followed by 16S rRNA amplicon sequencing) as a screen for microbial viability in both synthetic and environmental microbial communities. Our validation started with synthetic mixtures of live and heat-killed E.coli and S.sanguinis in known proportions. PMA-seq successfully reconstruct the communities of simple mixed culture. We next evaluated the effects of community background on PMA-seq in various community samples spiked with known concentrations of viable and heat-killed E.coli. Against a realistically complex communities, viability was no longer accurately assessed, with the results largely affected by initial biomass and compositional diversity. Finally, we applied PMA-seq to swabs from the Boston subway system. Not all samples respond consistently to PMA treatment. Overall, we revealed that PMA-seq was effective in simple synthetic communities, but may be premature for viability assessment in realistically complex community samples.

Propidium monoazide (PMA) treatment for viability assessment

Propidium monoazide is a DNA-intercalating dye that is membrane-excluded by viable cells, but can be photoactivated to deplete unprotected DNA. To evaluate its performance in microbial communities, we first constructed ten synthetic communities with live and heat-killed *E.coli* and *S.sanguinis* mixed in different proportion.



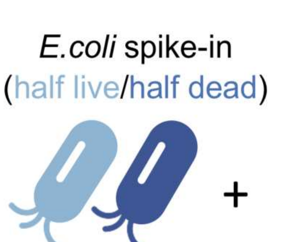
We next evaluated the effects of microbial community background on the performance PMA-seq in diverse natural communities from computer screens and mice, soil and human saliva. Swabs were collected in four biological replicates and spiked with known concentrations of live and heat-killed E.coli culture.

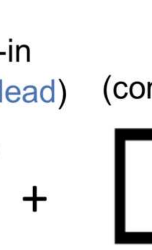
PMA efficacy was calculated using the absolute amount of E.coli with and without PMA treatment.

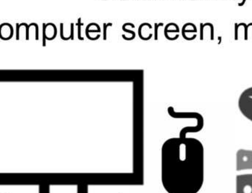
E.coli spike-in (half live/half dead)

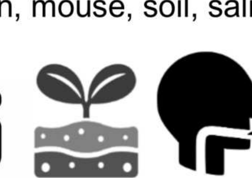
Community samples (computer screen, mouse, soil, saliva)

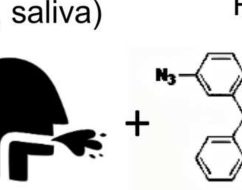
PMA

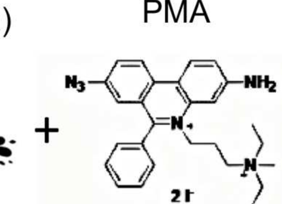




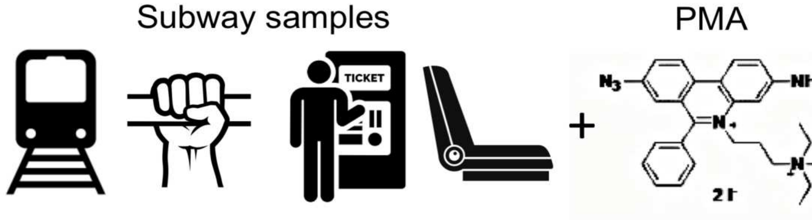








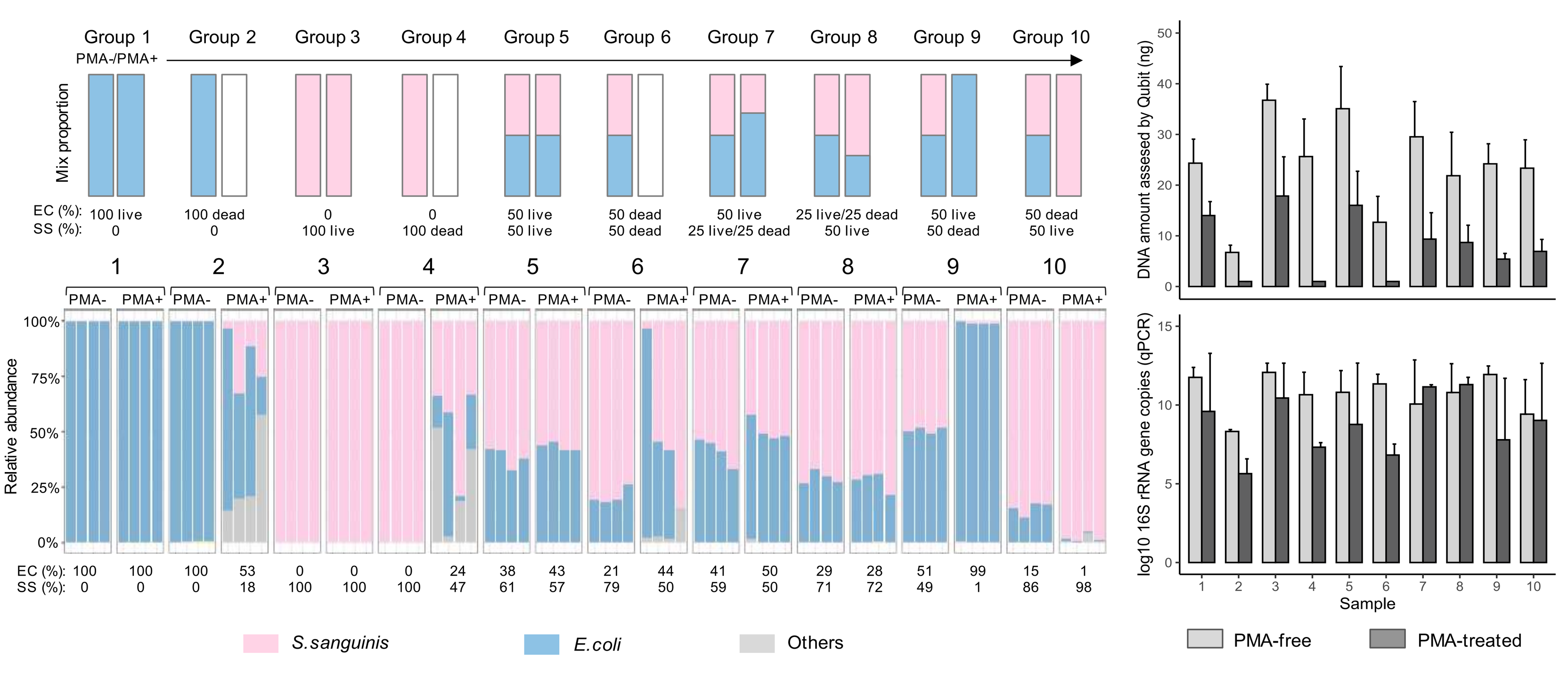
Built environment microbiome were collected from Boston subway systems. Surfaces were swabbed from three seats, three walls, four touchscreens and four grips on the Green Line E branch and from Park Street Station.



For this study, we had a total of 190 16S samples, comprising of those from pure cultures, E.coli spike in experiments, the subway samples and various experimental and technical controls

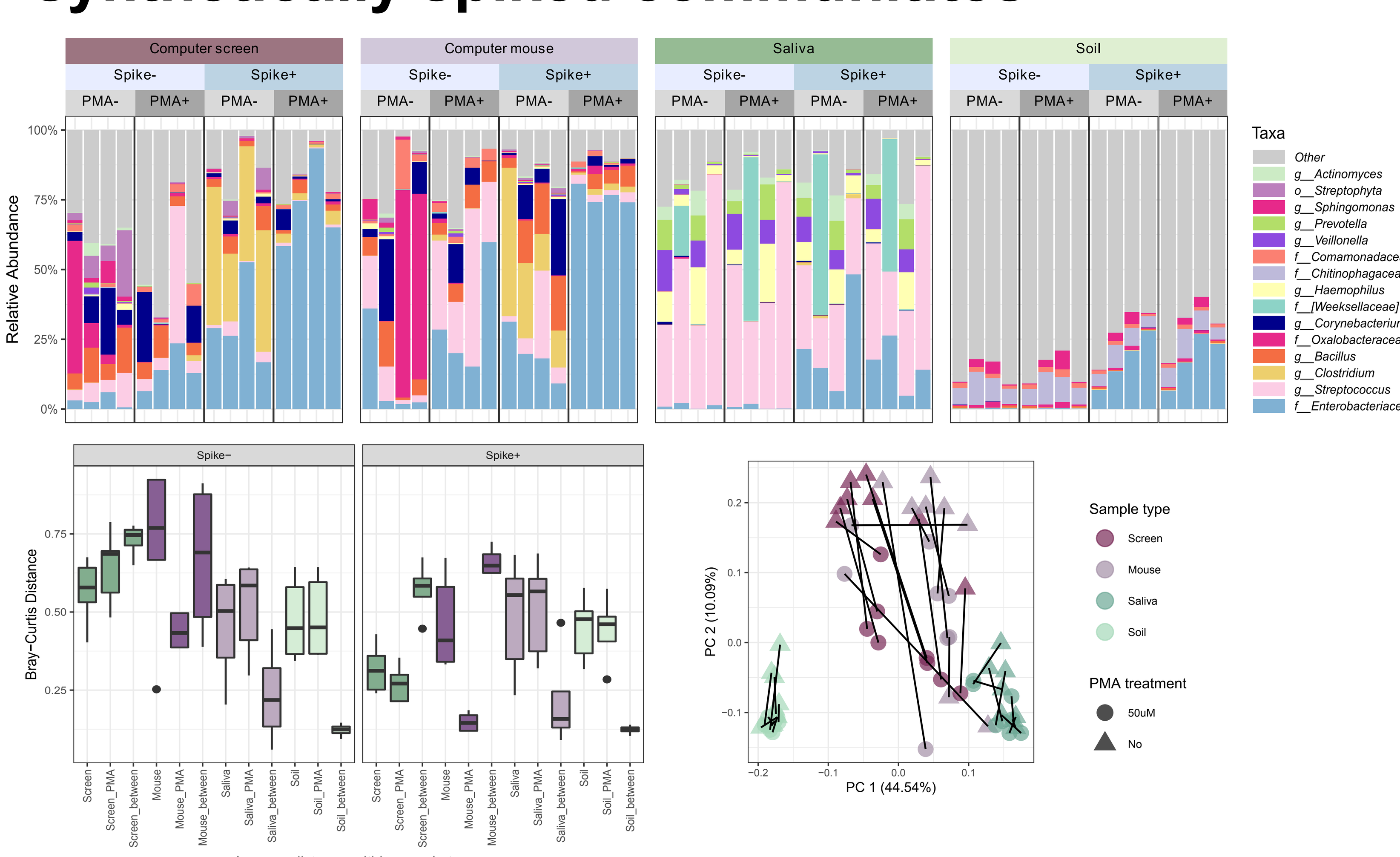
16S rRNA qPCR was performed on the samples of synthetic cultures and spiked natural samples to determine bacterial mass.

PMA treatment successfully depletes relic DNA in synthetic communities



- PMA treatment depletes relic DNA completely in groups containing pure live or dead bacterial culture (Group 2, 4 and 6);
- In mixed cultures (Group 5, 7 and 8), the average abundances of two microorganisms change in consistent trends with the mixed proportion.
- These results agree with previous studies in pure cultures/simple microbial communities, e.g. Nocker et al. 2007, Chen et al. 2011, and Kim and Ko 2012.

Viability is not accurately assessed by PMA in synthetically spiked communitites



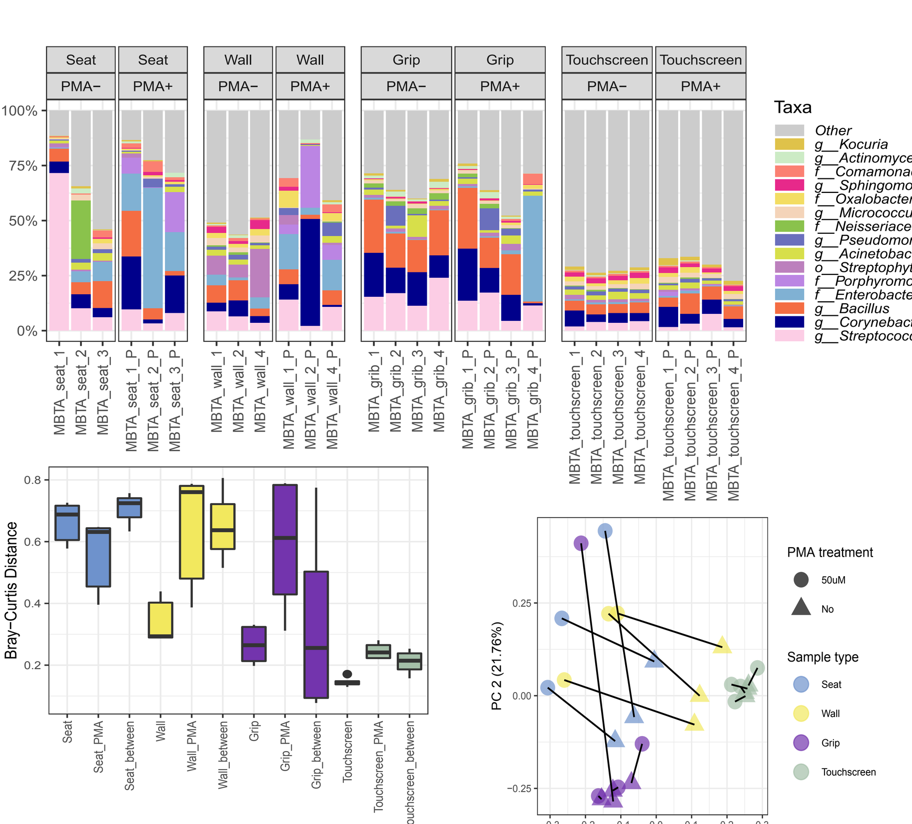
PMA-seq does not accurately assess the viability of spiked community samples.

- Low biomass samples from the built environment had larger changes in microbial compositions after PMA treatment, while fewer were observed in the higher biomass samples;
- Samples were clusted by source material in all sample types and by PMA treatment in low biomass samples from indoor office;
- Calculating from the absolute amount of spiked *E.coli*, PMA treatment partially depleted viable cells in computer screens, mice and soil samples, while incompletely removed relic DNA from dead cells in saliva samples.

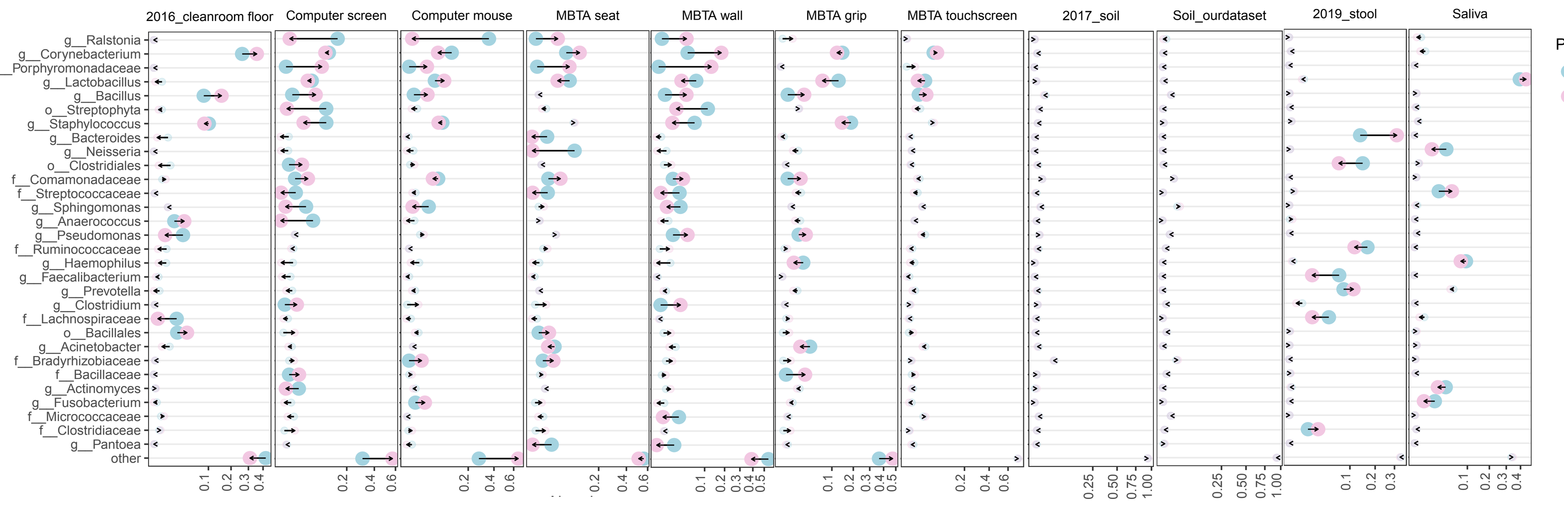
The effect of PMA-treatment varied by different surface types in the built environment

Samples from Boston's subway system did not respond consistently to PMA treatment.

- All surface types were dominated by human commensals;
- Seats and walls had larger compositional differences after PMA treatment;
- Samples clustered by Source material for all samples and by PMA treatment for the seats and walls.



Similar samples among different studies respond consistently to PMA treatment



Microbial taxa with apparent abundance changes (>= 0.01) after PMA treatment are sometimes replicated in similar sources among different studies.

- *Corynebacterium*, *Bacillus* and *Staphylococcus* have apparent abundance changes after PMA treatment in microbial communities from clean room floors, office built environment and Boston subway systems. By comparison, soil communities are relatively stable to PMA-treatment.
- The "PMA-reactive microbes" in the BE samples are mostly commensals from human skin or oral cavity, suggesting that the abundant, human-derived microbes are present in non-viable forms

Conclusions

Overall, we revealed that PMA-seq was effective in simple synthetic communities, but may be premature for viability assessment in realistically complex community samples. In the next step, we will compare PMA-seq to RNA-based high-throughput sequencing in determination of viability in BE microbiome.

Acknowledgments

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http://huttenhower.sph.harvard.edu

The role of the microbiota in neutralizing trypsin activity in a mouse model of Type 1 diabetes

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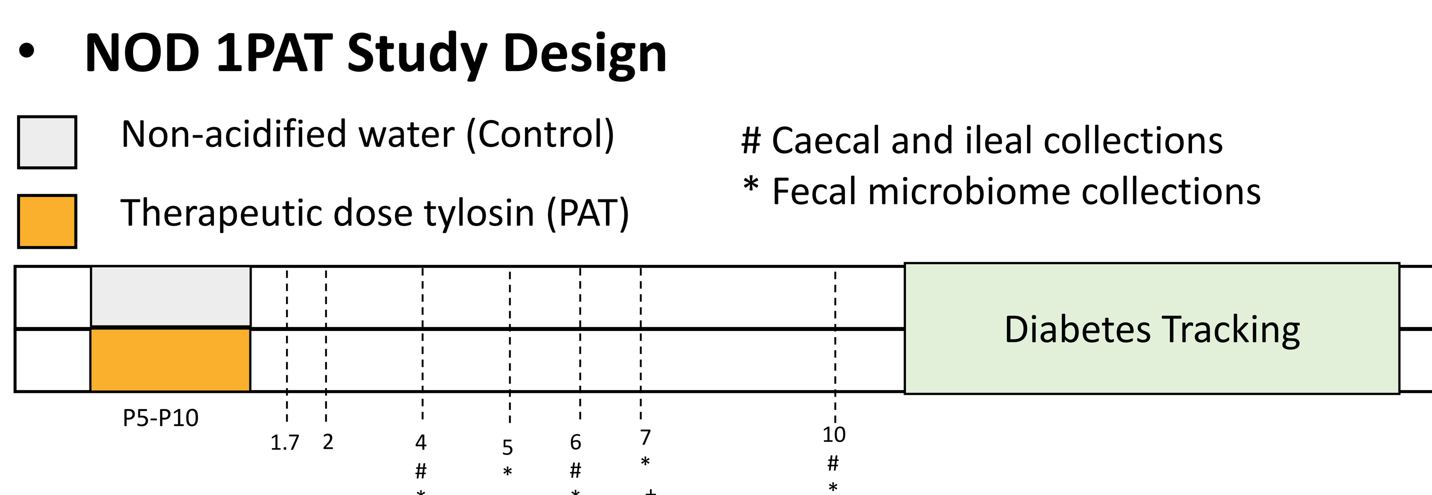
Abstract

The gastrointestinal tract contains high levels of proteases, one of the most abundant of which is trypsin, which is synthesized and secreted by pancreatic acinar cells. In addition to its primary function in digestion, proteolytic activity is also believed to play a role in mucus consistency and mucosal antigen processing. Accumulated evidence indicates that dysregulated proteolysis plays a pivotal role in the pathophysiology of several disorders centered on the colon. In our recent studies to investigate the effects of early-life gut microbiota on T1D onset in the non-obese diabetic (NOD) mouse model, we observed that the perturbed early life gut microbiota may dysregulate mucosal physiology through mucin genes muc2 and muc4. Therefore, in this study, we aimed to evaluate intestinal trypsin activities in cecal contents in germ-free and conventional C57BL/6 mice, as well as in the single pulsed antibiotic (1PAT)-exposed NOD mice. Using an enzymatic approach, as expected, we found significantly higher trypsin activity in the cecum of germ-free compared with conventional mice. We also found that mice with antibiotic-perturbed microbiota had increased fecal trypsin levels at the end of the antibiotic treatment; however, the differences became reduced over time. By tracking the occurrence and development of T1D, we observed that the fecal trypsin levels in mice that developed T1D were higher than the mice that did not develop T1D. These results suggest that gut microbiota perturbation may lead to increased distal gut trypsin activity, which may have downstream effects on colonic luminal and cell-surface proteins, affecting T1D pathogenesis.

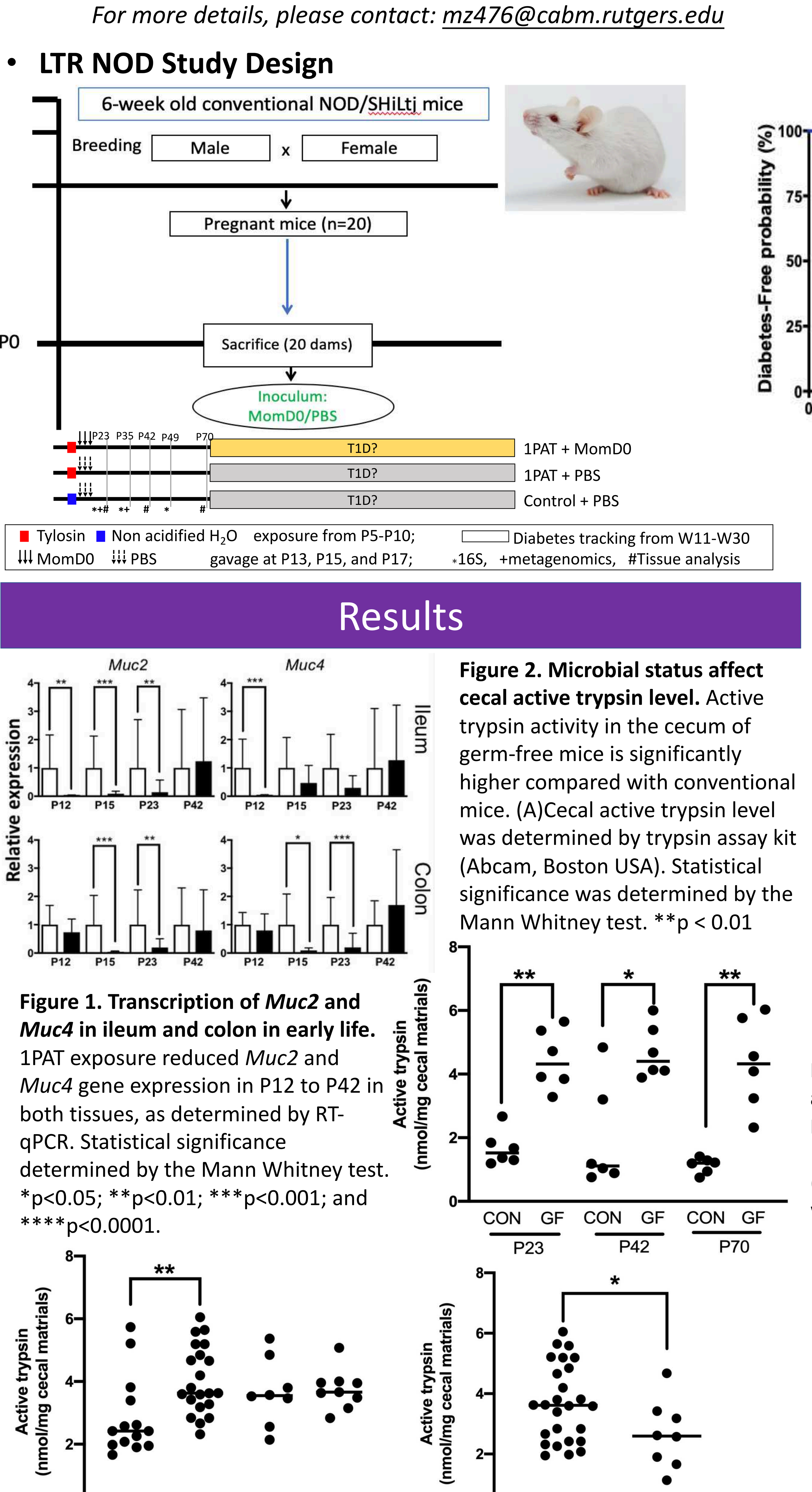
Study Design

- **NOD 1PAT Study Design**



Methods

- p-NA concentration was measured as an indicator of active trypsin concentration in each samples. Absorbances was determined at OD=405nm using microplate reader.



Results

Figure 1. Transcription of *Muc2* and *Muc4* in ileum and colon in early life. 1PAT exposure reduced *Muc2* and *Muc4* gene expression in P12 to P42 in both tissues, as determined by RT-qPCR. Statistical significance determined by the Mann Whitney test. *p<0.05; **p<0.01; ***p<0.001; and ****p<0.0001.

Figure 2. Microbial status affect cecal active trypsin level. Active trypsin activity in the cecum of germ-free mice is significantly higher compared with conventional mice. (A)Cecal active trypsin level was determined by trypsin assay kit (Abcam, Boston USA). Statistical significance was determined by the Mann Whitney test. **p < 0.01

Figure 3. (A) Fecal active trypsin level in single pulse antibiotic (1PAT) - exposed NOD mice and control mice. Antibiotic-perturbed microbiota increase the fecal trypsin level in the mice at the end of antibiotic treatment, but the change is transient. (B) Fecal trypsin levels after antibiotic and its relationship to T1D development. Active fecal trypsin levels were detected in 4-week old mice using the colorimetric assay. All mice were monitored for diabetes by weekly measurement of tail blood glucose. Statistical significance was determined by the Mann-Whitney test. *p < 0.05.

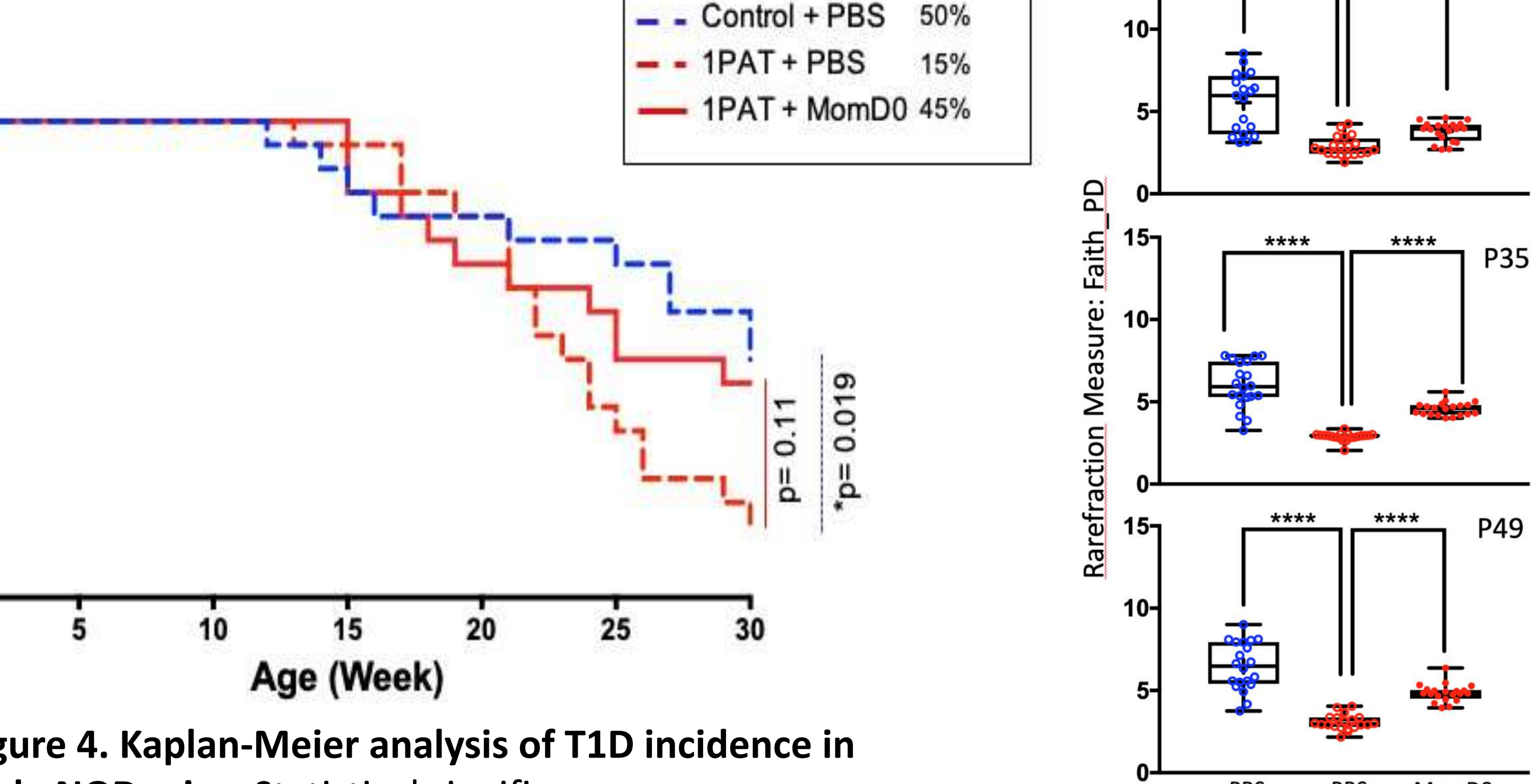


Figure 4. Kaplan-Meier analysis of T1D incidence in male NOD mice. Statistical significance was determined by the log-rank test. *p=0.019.

Figure 5. Transfer of MomD0 restored 1PAT-induced gut microbiome structure. (A) Alpha-diversity (phylogenic diversity (PD)) of fecal microbiota in control-PBS, 1PAT-PBS and 1PAT-MomD0 over time. Significance was determined by one-way-ANOVA ****p<0.0001. (B) Beta-diversity, as determined by unweighted UniFrac analysis of control-PBS, 1PAT-PBS and 1PAT-MomD0 fecal microbiota over time, analyzed by QIIME2. Inter-group UniFrac distances were all significant (*p<0.002), determined by one-way-ANOVA with Tukey correction for multiples comparisons

Axis 1 (20%)
Axis 2 (16%)
Axis 3 (10%)

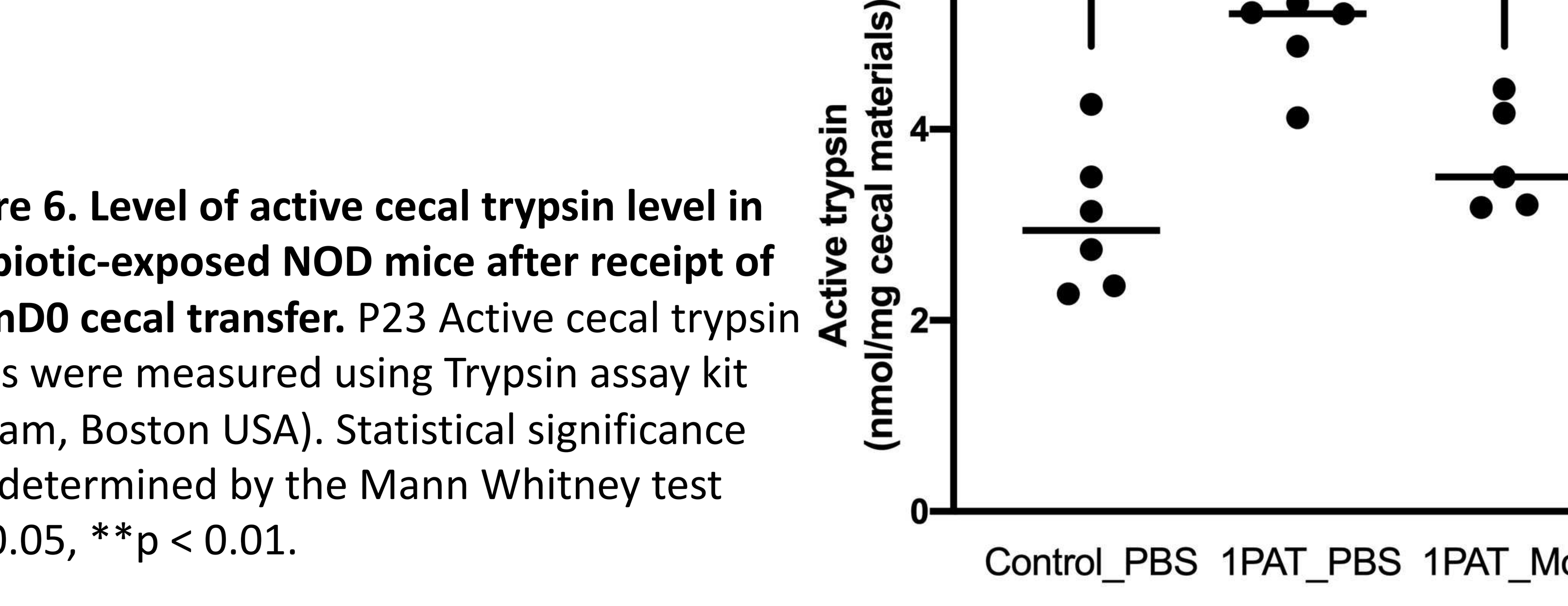


Figure 6. Level of active cecal trypsin level in antibiotic-exposed NOD mice after receipt of MomD0 cecal transfer. P23 Active cecal trypsin levels were measured using Trypsin assay kit (Abcam, Boston USA). Statistical significance was determined by the Mann Whitney test *p<0.05, **p < 0.01.

Conclusion

- Active trypsin in cecal contents was significantly increased in Germ-free mice compared to control mice, thus microbiota neutralize trypsin activity.
- Mice with an antibiotic-perturbed microbiota had increased fecal trypsin levels at the end of the antibiotic treatment, with changes lasting for at least 4 weeks (but not 10).
- Fecal trypsin levels in week4 mice that developed T1D later are higher than in the mice without T1D, suggesting a possible pathogenic role.
- After receiving maternal cecal materials, active cecal trypsin activity reverted to normal.-thus transfer restored microbiome function.

Changes in Intestinal Gene Expression in Antibiotic-Treated NOD Mice

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Background

Over the past decade, the incidence of Type 1 Diabetes (T1D) has been globally on the rise[1]. Not only is the incidence increasing, it is also developing earlier in life. Therefore, we need to better understand childhood exposures[1]. Studies have been done on this topic with non-obese diabetic (NOD) mice which are a strain of mice that spontaneously develop T1D. That research has demonstrated that NOD mice exposed to a single course of antibiotics showed accelerated onset of T1D[2]. Treatment with antibiotics changed the gut microbiome profile of these mice[2].

With this information, we sought to analyze how these changes impact gene expression of proteins that play a role in epithelial barrier function and innate immunity. The three genes I focused on were REG3-γ, F2RL1, and zonulin. REG3-γ is a gene that encodes an antimicrobial peptide that plays a role in innate immunity[3]. F2RL1 is a G-protein coupled receptor involved in inflammation[4]. Zonulin is a haptoglobin precursor that functions in tight junction disassembly via an epidermal growth factor-like motif[5].

Methods

RT-qPCR:

Ileum and colon samples were taken from control mice, NOD mice treated with PAT (a single course of the macrolide antibiotic, Tylosin) and NOD mice treated with PAT that were given a gavage of their mother's intestinal microbiome. The intestinal samples from the pups were taken at postnatal days 23 and 42. In order to analyze changes in gene expression, RNA was extracted, converted to cDNA, and expression was analyzed using RT-qPCR using primers specifically designed for each gene. Expression was normalized to the housekeeping gene, GAPDH.

Protein Assay and ELISA:

A total protein assay and ELISA were performed to analyze levels of zonulin gene expression. The protein assay was done on ileal and colonic samples obtained from mice at day 23. Samples were from the control, antibiotic-treatment, and restoration groups. The protein assay was performed using the Pierce BCA Protein Assay Kit and protocol. The ELISA was done on the same samples using a MyBioSource Mouse Zonulin ELISA kit and protocol.

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Results

REG3-γ:

There was a significant decrease in gene expression between the control and the treatment groups and between the restoration and treatment groups at day 23. The difference between the control and treatment group remained significant at day 42. There were also significant increases from P23 to P42 in gene expression within each group (Figure 1). No significance was found for the colon samples (Figure 2).

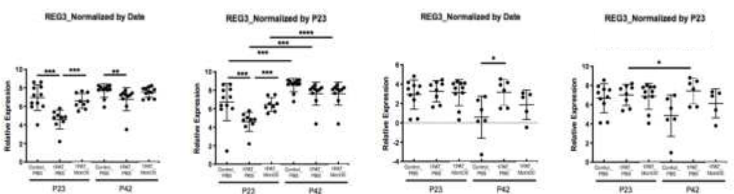
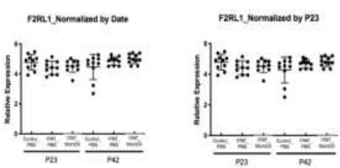


Figure 1: Ileal REG3-γ data normalized by each date's control (left) and by P23 controls (right)

Figure 2: Colon REG3-γ data normalized by each date's control (left) and by P23 controls (right)

F2RL1:

No significant differences were found for the ileal samples (Figure 3). However, the colonic samples showed a significant decrease between the treatment group and the control at day 23. This remained at day 42, as well as a significant difference between the control and restoration (Figure 4).



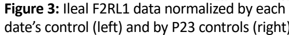


Figure 3: Ileal F2RL1 data normalized by each date's control (left) and by P23 controls (right)

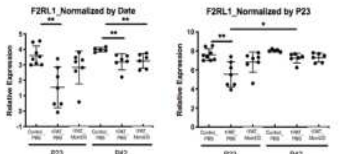


Figure 4: Colon F2RL1 data normalized by each date's control (left) and by P23 controls (right)

Zonulin:

There was no significant change in expression within either the ileal or colon samples.

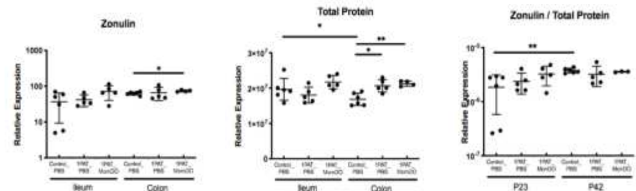


Figure 5: From left to right: data from ELISA on ileal and colon, total protein assay data, comparison of zonulin to total protein.

Discussion

REG3-γ:

REG3-γ may have an impact on the earlier onset of T1D since it was significantly impacted by the antibiotic treatment. The restoration treatment was successful since there was no significant difference between the control and restoration groups. The colon does not seem to be affected by antibiotic treatment.

F2RL1:

F2RL1 expression does not seem to be affected by antibiotic treatment in the ileum. F2RL1 in the colon may play a role in the earlier onset of T1D since the expression was significantly decreased in the treatment group compared to the control. The restoration treatment was also successful.

Zonulin:

Zonulin gene expression may be too low to analyze via RT-qPCR. According to the assay and ELISA results, zonulin does not seem to have an impact on early T1D onset.

Future Direction

REG3-γ and F2RL1:

In the future, it will be beneficial to research the regulation of REG3-γ and F2RL1. Researching how these genes are regulated will help us understand what is being disturbed following antibiotic treatment. It would also be useful to test intestinal epithelial cells using RT-qPCR to analyze gene expression. This would help us understand gene expression in epithelial cells specifically rather than the tissue as a whole.

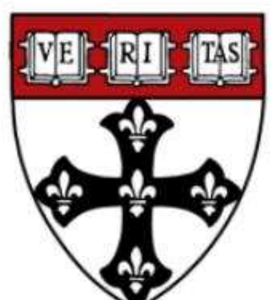
Zonulin:

I would like to test this gene again using a more sensitive protein assay. It would also be beneficial to examine other similar tight junction related genes such as zonula occuldens-1 (ZO-1). Additionally, I am interested in zonulin expression in control mice compared to the NOD mice since studies have shown that relatives of patients with T1D also have higher levels of zonulin expression[5]. The question this raises is whether there is a difference in expression due to antibiotic-treatment or is the difference present beforehand?

Acknowledgements

I would like to thank Dr. Xue-Song Zhang, Dr.Martin Blaser and the rest of the Blaser lab for all their support and guidance this summer.

Discovery of new bioactive microbial metabolites in Inflammatory Bowel Disease



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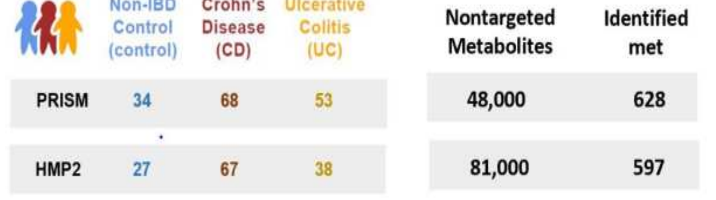
Abstract

The gut microbiota and associated bioactive compounds have been implicated as causal and as protective factors in gastrointestinal disorders, including the inflammatory bowel diseases (IBD). Both host immune interactions with gut microbes and microbial small molecule products are likely responsible for these bioactivities. Several gut microbial metabolites, e.g. short-chain fatty acids and a subset of omega-3 fatty acids depleted in GI inflammation, have demonstrated therapeutic potential in IBD by attenuating gut inflammation. However, discovery of new bioactive compounds from the gut microbiome relevant to IBD or inflammation is challenging due to the vast numbers of uncharacterized metabolites produced by the microbiome.

To address this challenge, we investigated two IBD cohorts with integrated metagenomic and metabolomic profiles of the gut microbiome: PRISM, the Prospective Registry in IBD Study at MGH, and the Integrative Human Microbiome Project (HMP2). Putrescine and a potentially novel family of metabolites microbially derived from it were among the ~10,000 metabolites differentially abundant (PRISM n=8,792 and HMP2 n=9,444) during gut inflammation, of which only ~100 were characterized (PRISM n=157 and HMP2 n=99). We validated the dependence of these putrescine derivatives on the gut microbiome and their bioactivity *in vivo* by treating germ-free, gnotobiotic and conventional mice with dietary putrescine, which induced changes in immune system activity in a microbial community-dependent manner. This included that putrescine selectively affects host colonic and ileum M2 macrophage cell populations only in conventional mice. These results underscore the power of combined computational and experimental approaches for identifying microbially derived metabolites with general immunomodulatory activity and specific relevance for IBD patient care.

Introduction

Although there are highly effective IBD therapies that directly target the immune system, many IBD patients do not achieve durable remission, lose responsiveness to treatment over time, or suffer from the broad immuno-suppressive effects of such treatments. Despite the strong association of gut microbiome configurations with IBD and advances in taxonomical profiling of the gut microbiome, the effective translation of specific mechanisms of host-microbiota signaling and microbial metabolites for IBD clinical care remains largely elusive.



	Non-IBD Control (control)	Crohn's Disease (CD)	Ulcerative Colitis (UC)	Nontargeted Metabolites	Identified met
PRISM	34	68	53	48,000	628
HMP2	27	67	38	81,000	597

Table 1. IBD metabolomic datasets used for this project and IBD cohorts. Number of identified and unidentified metabolites in each data set.

Human fecal metabolomics, using untargeted high-resolution liquid chromatography-mass spectrometry (LC-MS), can provide comprehensive functional readouts of gut microbial activity and host-microbial interactions. Untargeted LC-MS techniques profile tens of thousands of metabolites in individual human stool samples; however, our understanding of their bioactivity is limited to ~<1% (Table 1). Thus, an *in silico* technique to prioritize these metabolites is a critical unmet medical need for realizing the potential of microbial metabolites for IBD treatment. We identified new IBD-associated uncharacterized metabolites using two publicly available IBD metabolomic datasets, PRISM and IBD, by MACARRoN and tested its biological function *in vivo*.

Validation of MACARRoN

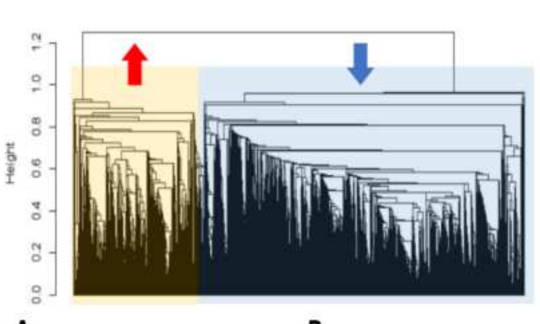


Figure 1. Hierarchical clustering of differentially abundant metabolomic associations (n=8,792) from the PRISM metabolomics dataset. The yellow box represents groups of metabolites that are enriched (n=6,400) in IBD patients and the blue box represents depleted groups of metabolites (n=2,392) that are classified into the same group using MACARRoN.

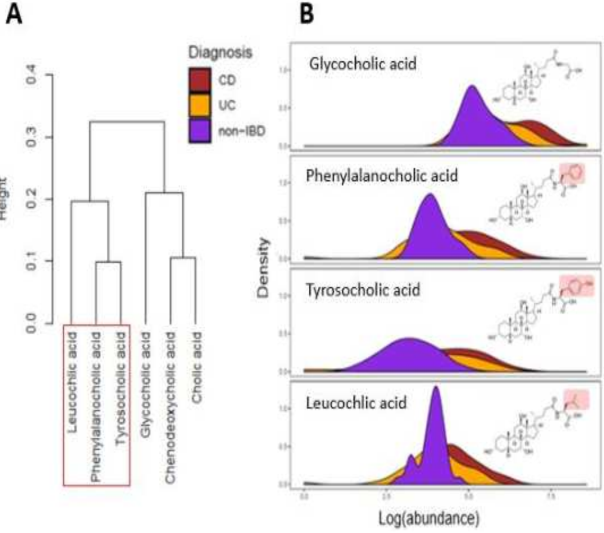


Figure 2. Preliminary validation of our computational pipeline, MACARRoN. (A) A dendrogram depicting hierarchical clustering result of differentially abundant glycocholic acid cluster. The newly identified microbially-associated bile acid conjugate metabolites (inside of red box), phenylalanocholic, tyrosocholic and leucocholic acid, were clustered with the known bile acid conjugate metabolite using MACARRoN. (B) Abundance of the four bile acid conjugate metabolites in CD, UC, and non-IBD individuals. The chemical structure of the 4 metabolites and chemical modifications are shown in red).

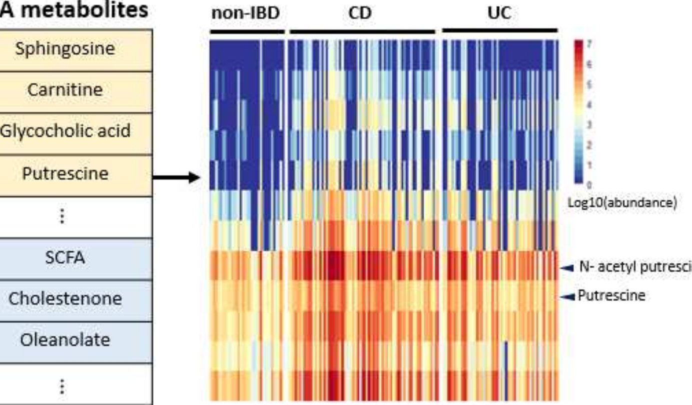


Figure 3. Partial list of differentially abundant (DA) known metabolites used to assign uncharacterized metabolites to chemical classes by hierarchical clustering. The yellow box represents of enriched known metabolites that are enriched and the blue box represents of depleted metabolites in IBD patients.

Heatmap representing abundance of prioritized IBD-associated metabolites, putrescine group, that enriched in IBD patients with known metabolites in the same group between CD, UC and non-IBD individuals. Each column represents abundance of metabolites from individual.

Screen high-priority metabolites *in vivo*

Objective : (1) to demonstrate that the uncharacterized metabolites could be generated *in vivo* from a chemical precursor in a gut microbiota dependent process and (2) to evaluate a change in the host immune system in response to the precursor in a gut microbiota dependent manner.

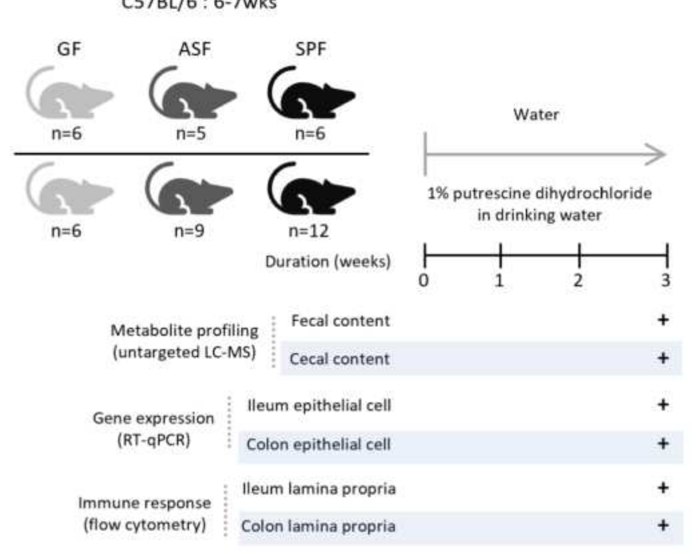
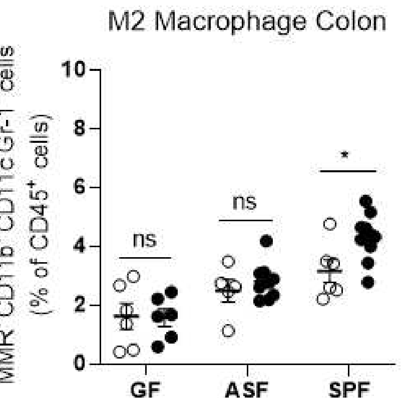


Figure 4. Schematic of the experimental design.

We employed mice with distinct gut microbiota communities, germ free (GF), Altered Schaedler Flora (ASF, a minimal microbiota of 8 species), and SPF C57BL/6J mice, in the presence or absence of putrescine and profile their microbial activities, host gut barrier function, and immune cell phenotypes.



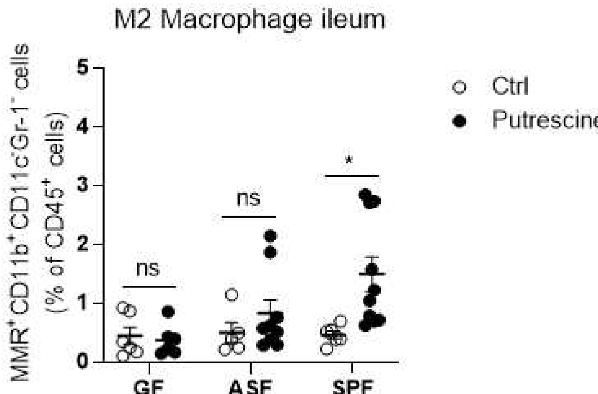


Figure 5. Lamina propria flow cytometry of colonic and ileal M2 macrophage cells, $MMR^{+}CD11b^{+}CD11c^{-}Gr\text{-}1^{-}$ cells out of $CD45^{+}$ cells, from GF, ASF, and SPF mice fed putrescine or control. Data shown as the mean ± SEM *p <0.05, two-tailed t-test.

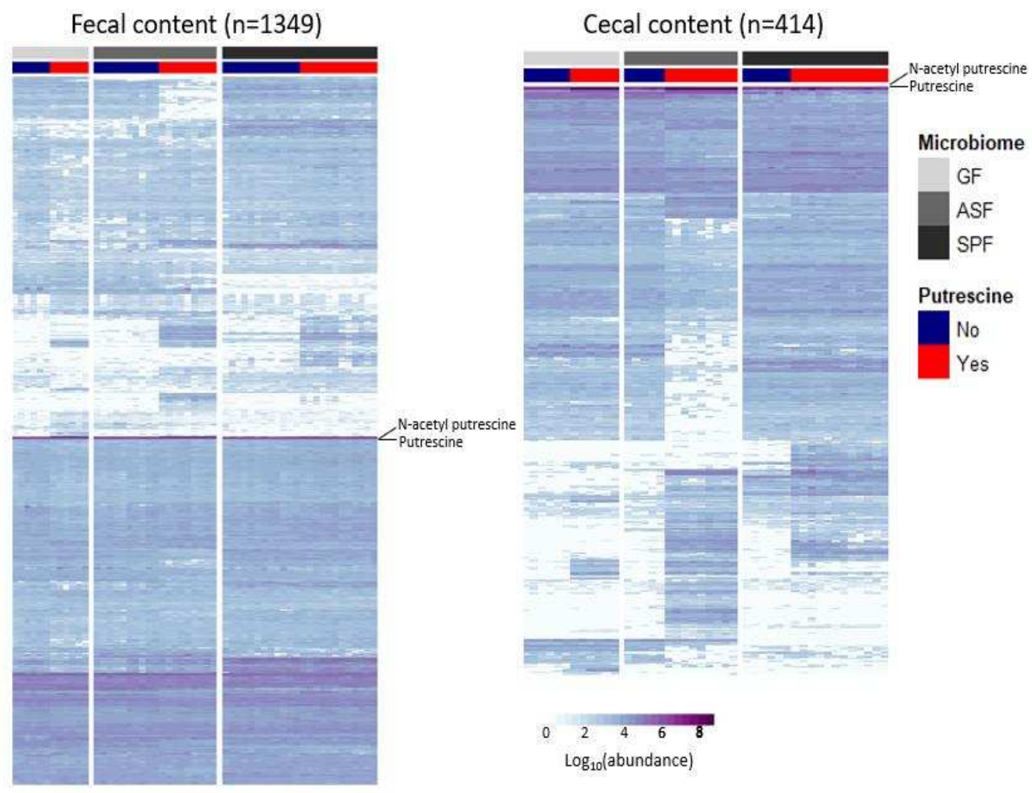


Figure 6. Heatmap representing DA metabolites from fecal and cecal untargeted LC-MS metabolomics in response to putrescine treatement. The rows display metabolites that are differentially abundant metabolites in respect to putrescine treatment and the column represents individual sample.

Future Directions

- Characterize the chemical structure of the microbially-associated new bioactive metabolites followed by metabolite synthesis.
- Determine the efficacy of the bioactive metabolites in IBD preclinical mouse models.
- Identify bacterial species that generates the bioactive metabolites.

Conclusion

- Putrescine selectively affects host colonic and ileum M2 macrophage cell populations in a gut microbiota- dependent manner.
- The bioinformatically-prioritized uncharacterized metabolites of the putrescine group from the IBD cohort data are also differentially abundant features in the mouse metabolomes in the presence of a gut microbiota.

References & Funding

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2. Lloyd-Price. *et al.* Multi-omics of the gut microbial ecosystem in inflammatory bowel diseases. *Nature* (2019)
3. Quinn, R.A. *et al.* Global chemical effects of the microbiome include new bile-acid conjugations. *Nature*, (2020)

4. NIH NIDDK grant R24DK110499

A Hierarchical Model for Microbial Community Structures

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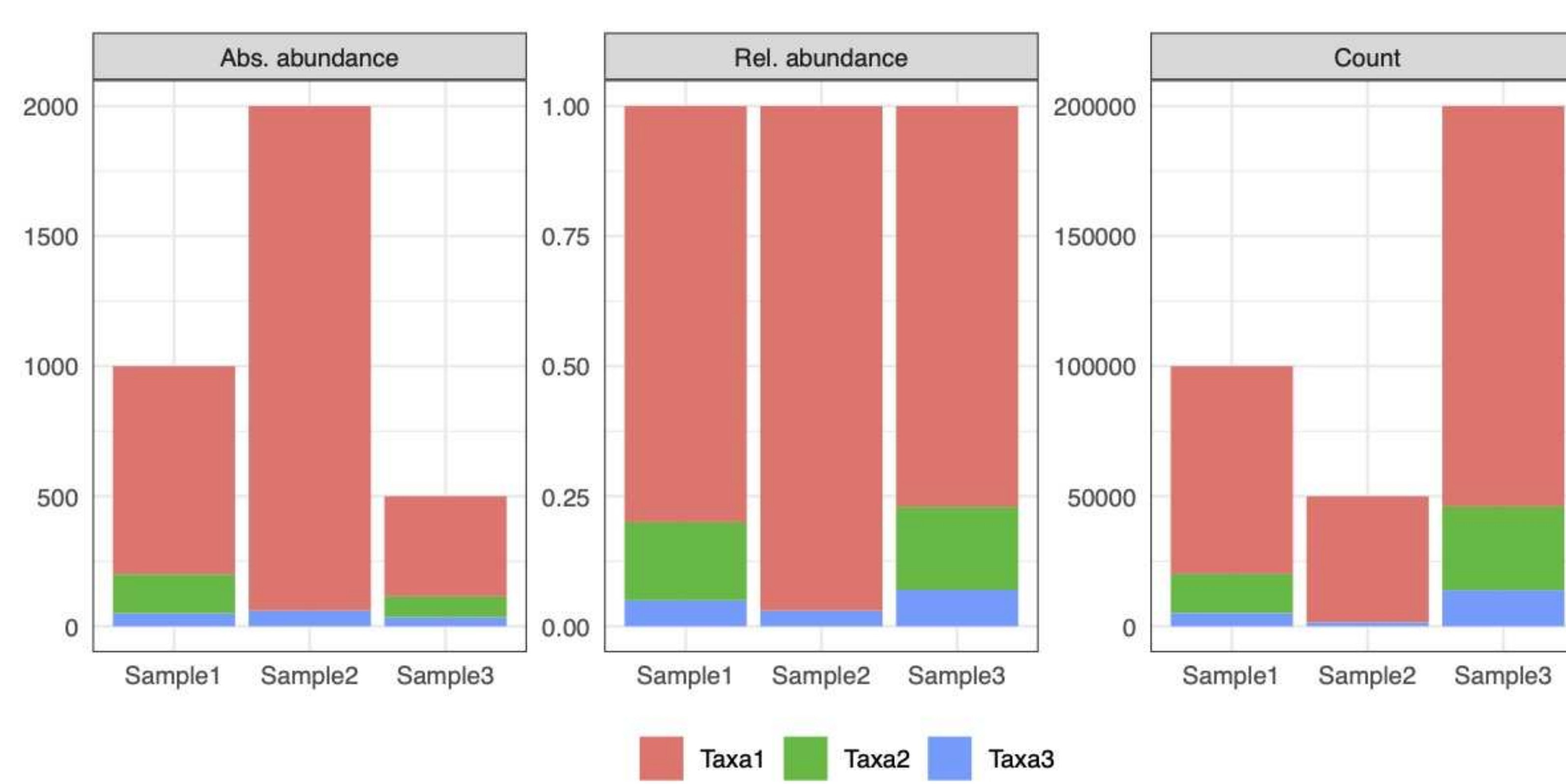
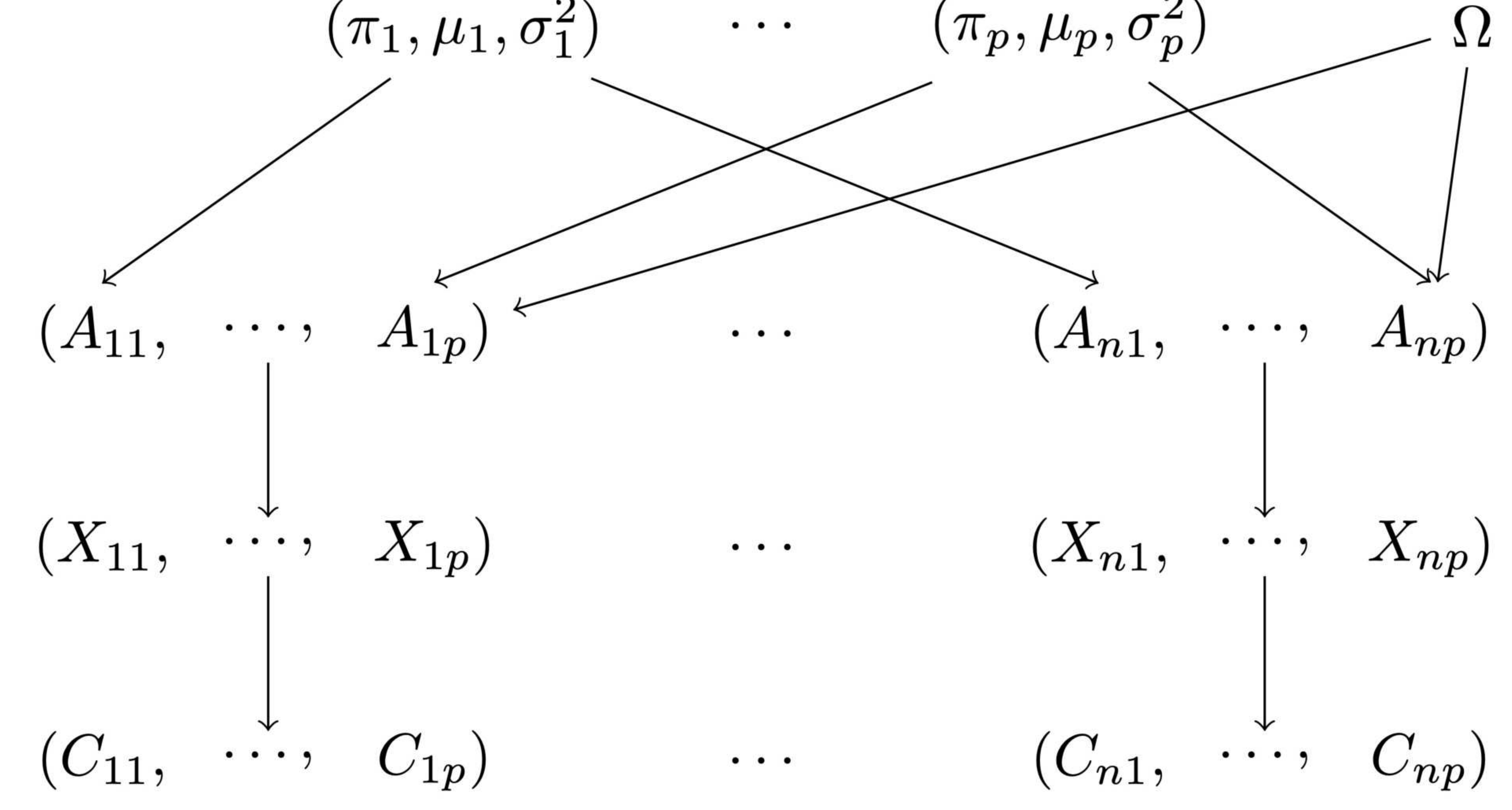
We present SparseDOSSA2, a hierarchical model of microbial community count observations, suitable for simulation of such data at population scale. Our model has specialized components targeting characteristics unique to microbiome data, including sparsity, joint effects of biological and sequencing variation, and ecological feature dependencies, and is capable of simulating mock microbial counts that recapitulate the population structures in training template communities. We hope that these methods and findings will be of broad applicability in human transcriptional and microbial epidemiology, and will inform future population study designs and analysis practices.

The SparseDOSSA2 model for microbial abundance observations



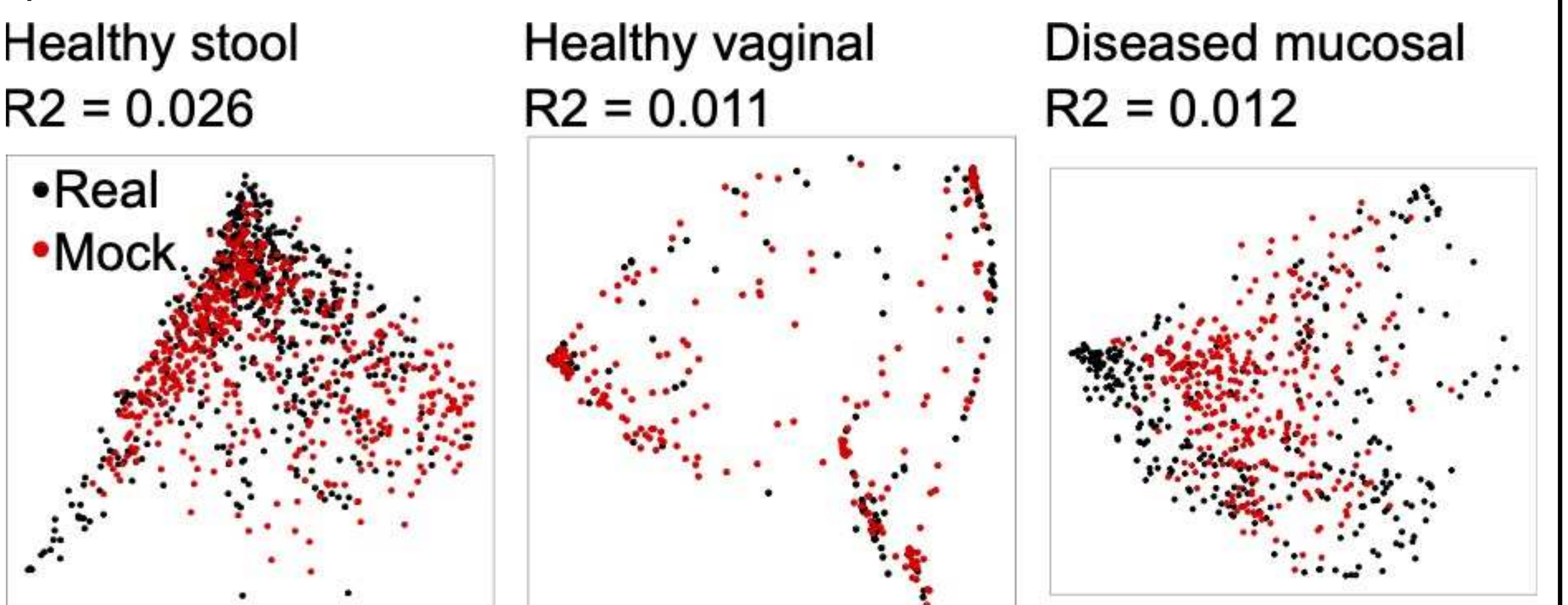
The SparseDOSSA2 model hierarchy is motivated by the data generation mechanism of microbial sequencing count measurements. Absolute abundance of microbial taxonomic and functional features, unmeasured with sequencing technology only, give rise to compositional relative abundances. Sequencing count measurements accurately approximate such values with enough sequencing depth.



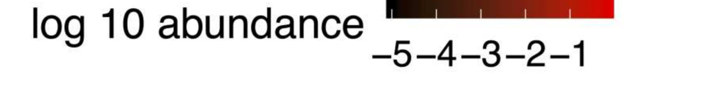
The hierarchical model is setup according to the same mechanism. Unobserved absolute abundances (*A*) are generated according to zero-inflated log normal distributions (parameters *pi*, *mu*, *sigma*, and *Omega*). They then give rise to the relative abundances (*X*) and sequencing counts (*C*).

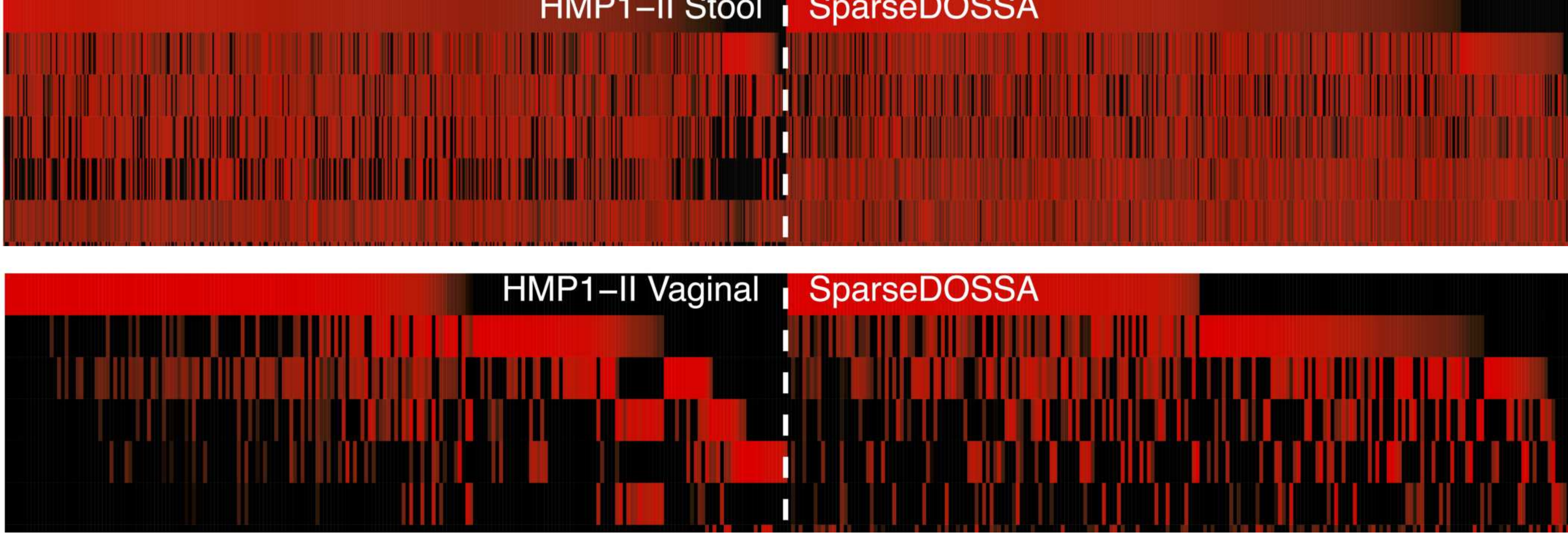
SparseDOSSA2 recapitulates microbial community structures

We evaluated SparseDOSSA2's fitting performance on datasets with different microbial community population structures. In both continuous (healthy and inflammatory bowel disease stool gut mucosal) and discrete (heathy human vaginal) communities, "mock" samples generated from SparseDOSSA2 closely follows the distribution of the original real samples, as evidenced by ordination visualization as well as PERMANOVA quantitative evaluation.

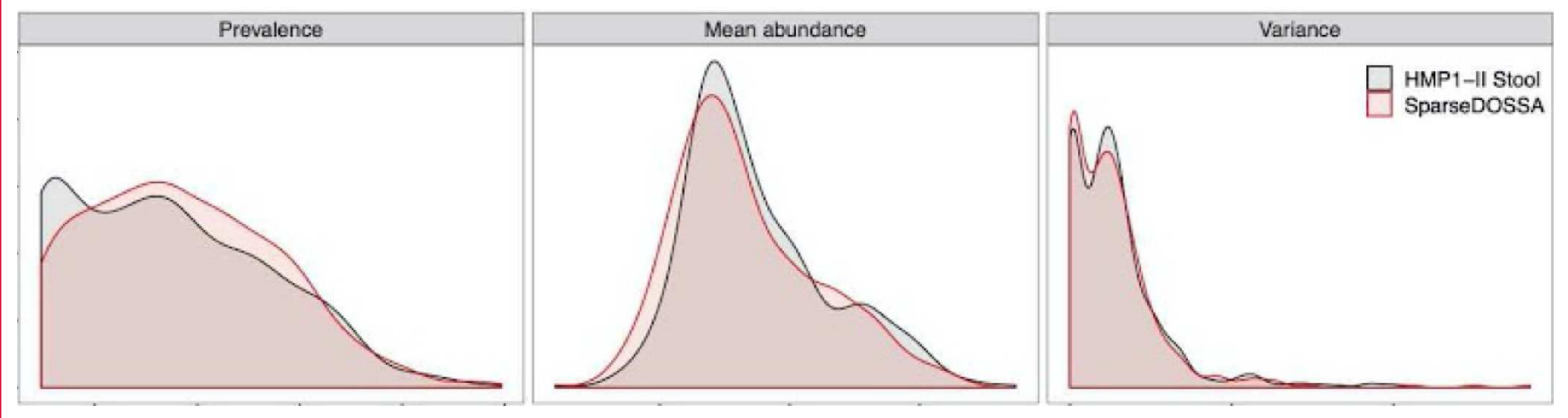


Per-feature relative abundances also have similar distributions between original datasets and SparseDOSSA2 simulation samples. Agreeing with overall population structure, the stool samples display gradients of relative abundances, whereas vaginal samples are often characterized by the dominating taxa.





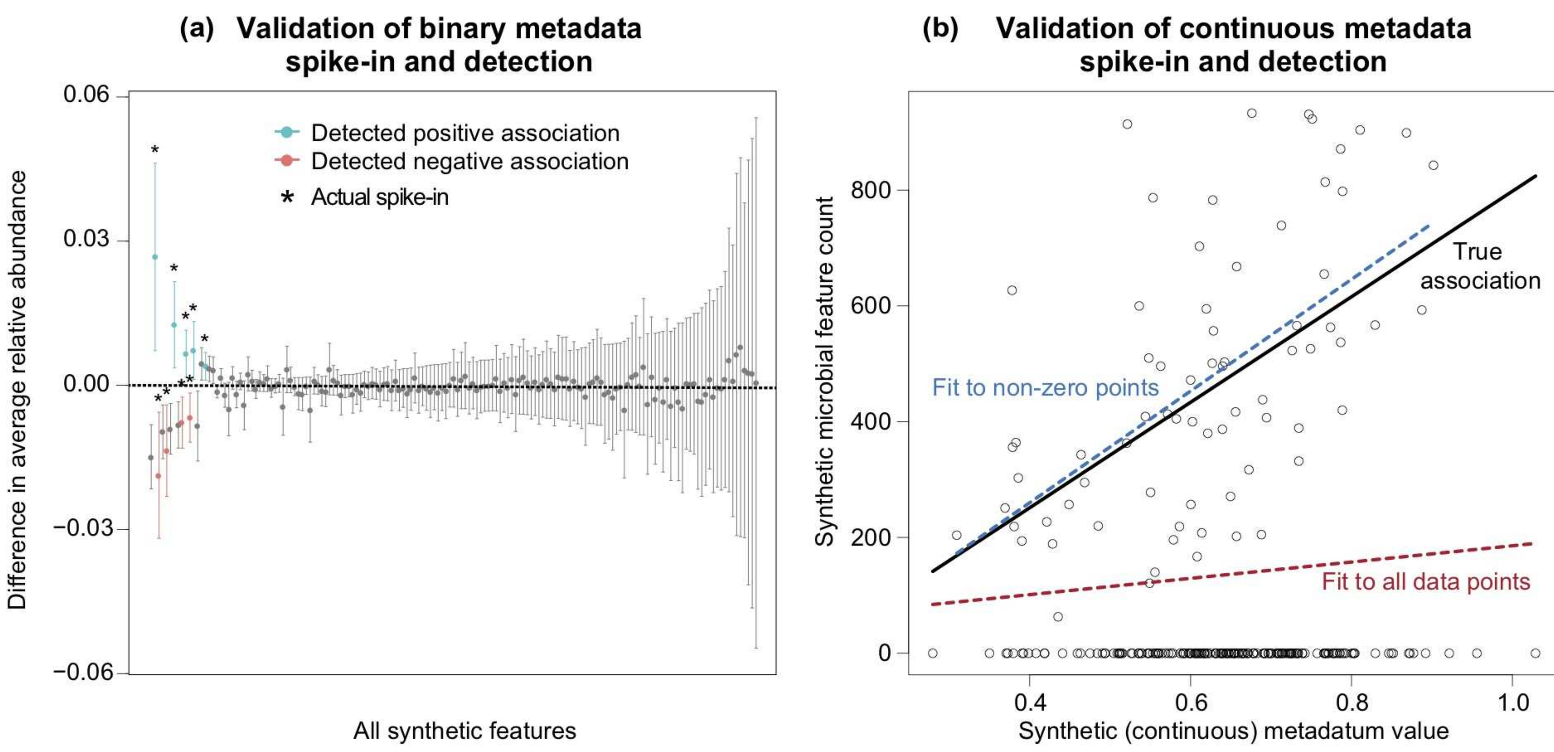
SparseDOSSA2 generates new microbial features similar to originals



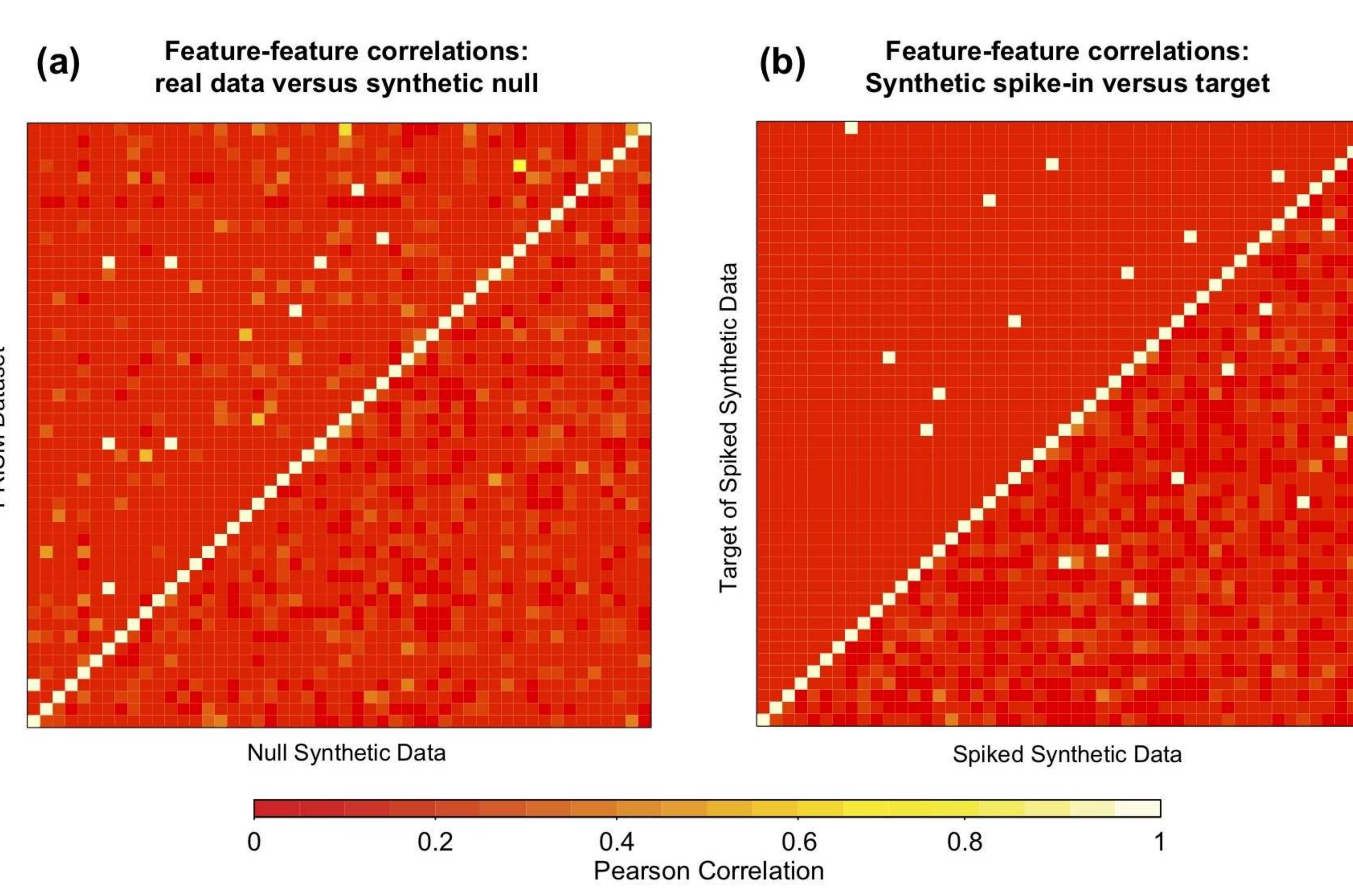
Per-feature prevalence, mean, and variance parameters are estimated in the SparseDOSSA2 model, and then used to generate new microbial features that follow similar distributions as the original dataset.

Metadata and feature-feature association spike-in

SparseDOSSA2 can simulate spiked-in associations with metadata for, e.g., benchmarking purposes. Differences in mean relative abundances between two classes of a simulated binary sample property (metadatum) along with the empirical inter-quartile range of all features as contrasted between metadatum levels (a). For continuous data, correlation of one feature into which an association to a sample metadatum has been spiked with that metadatum's value (b).



SparseDOSSA2 can also simulate associations between microbial features. The pairwise (absolute) Pearson correlations based on raw counts between microbial features in the PRISM cohort (above the diagonal) and in the SparseDOSSA2 fit to this dataset (below the diagonal) are similar (a). Pairs of features that are targeted to be correlated with each other (above the diagonal) and pairwise Pearson correlations in the resulting modified dataset (below the diagonal) also agree (b).



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Diet-linked dynamics of metagenomes and metatranscriptomes

BROAD INSTITUTE

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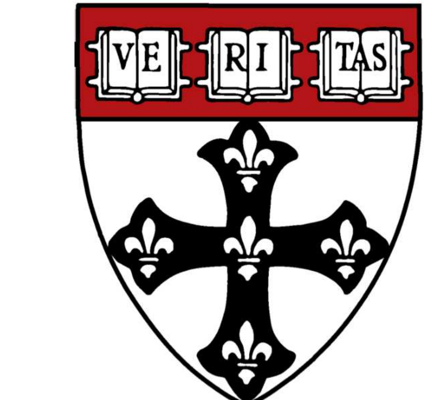
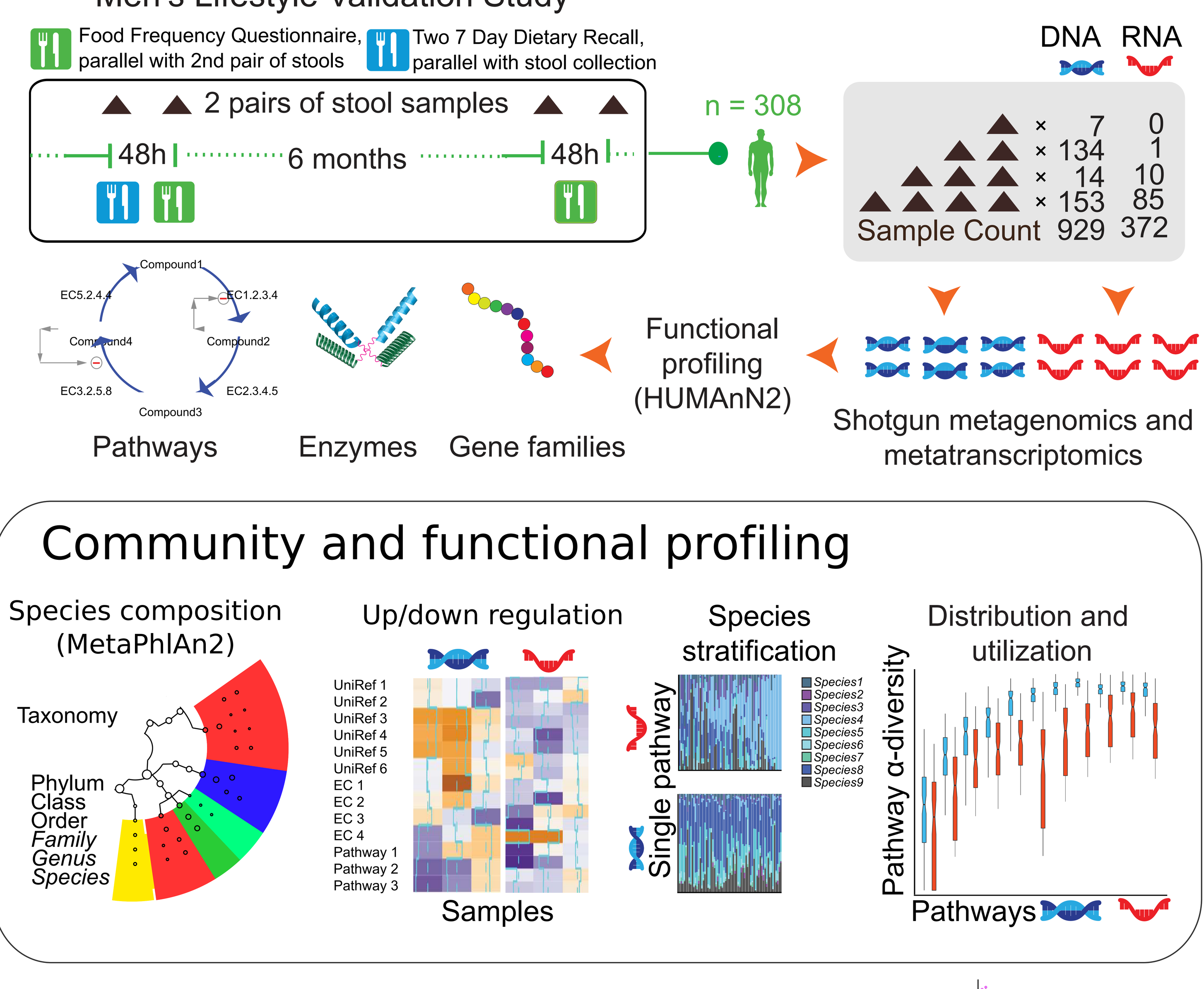
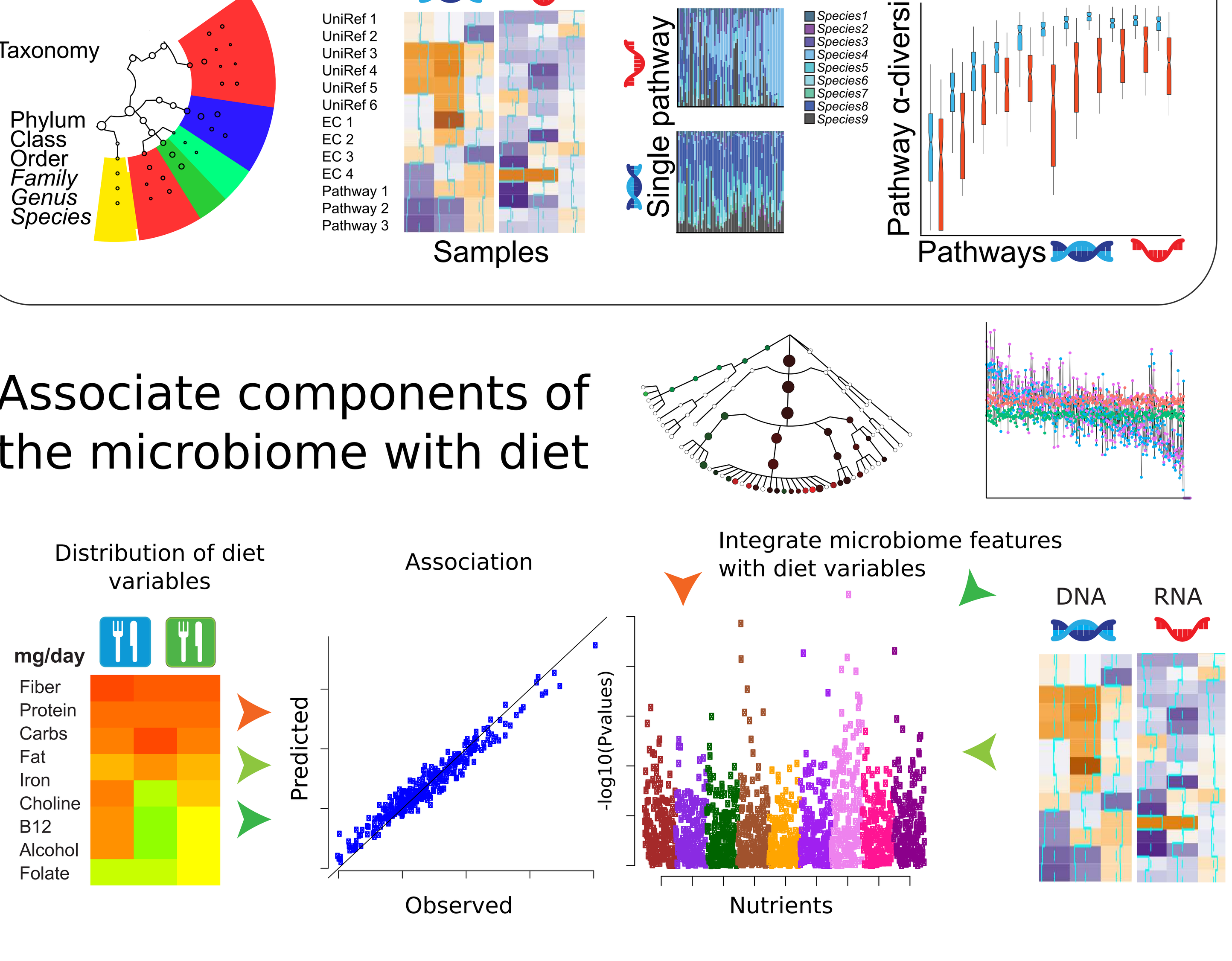


The microbiome plays a role in metabolic health and is modulated by host diet. The specific interactions between microbial enzymes and dietary compounds are not yet known. To understand this mechanism, we assess the relationship between dietary compounds and metabolic pathways that are carried and transcribed by individual species. Here, we report on the gut microbiome of 307 participants from the Health Professionals Follow-Up Study, a prospective cohort designed to relate nutrition to health outcomes. The gut metagenome of each participant was surveyed at four timepoints with short (1-3 days) and long (6 month) time intervals. Metatranscriptomes were also generated for 96 participants. These data were complemented with seven-day dietary recalls and long-term dietary histories from food frequency questionnaires. Taxonomic profiling and metabolic reconstruction were performed using MetaPhlAn2 and HUMAnN2, respectively. We integrated the profiled taxonomy and metabolic pathways with dietary compounds using a multivariate linear model (MaAsLin2). We revlealed a "core" set of pathways encoded by many species, and a variably transcribed set that consists of specialized pathways. Dietary fiber was associated with metagenomic pathways such as nucleotide and amino acid biosynthesis, in addition to the carbohydrate fermenter, Collinsella aerofacians. A Gaussian process model is applied to assess the longtudinal relationship between microbiome features and dietary variables. Our findings could help us understand the direct mechanism by which bioavailability impacts microbial metabolism.

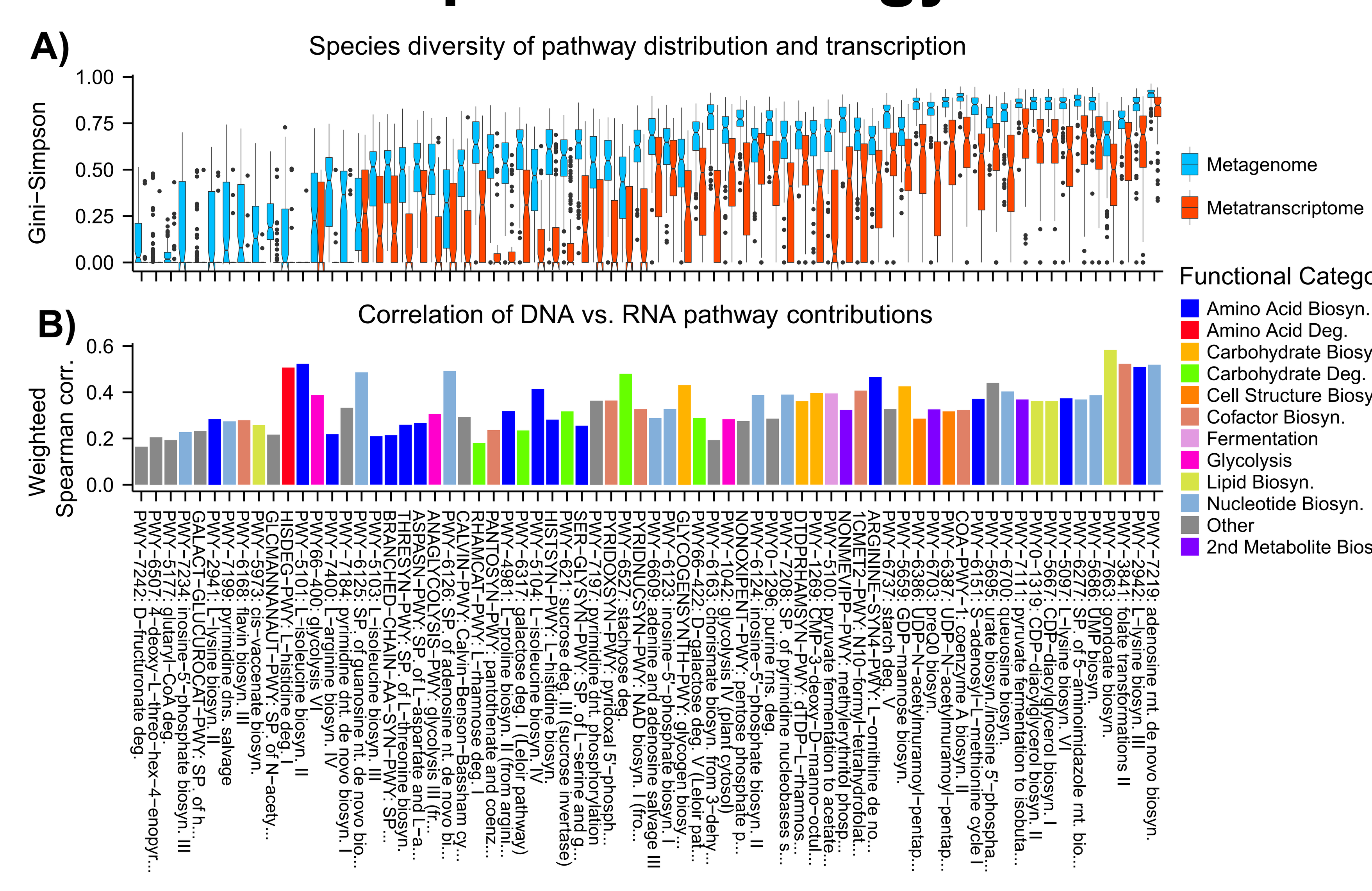
Cohort microbiome sampling and meta'omics

Stool self-collection, data generation and profiling



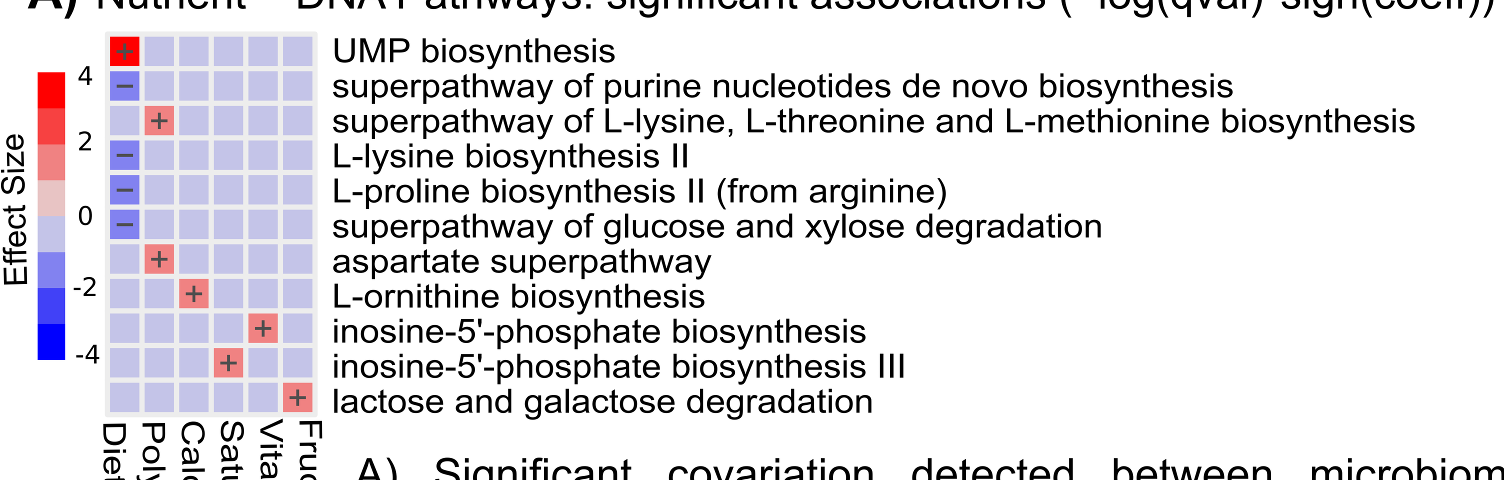


Metatranscriptional ecology

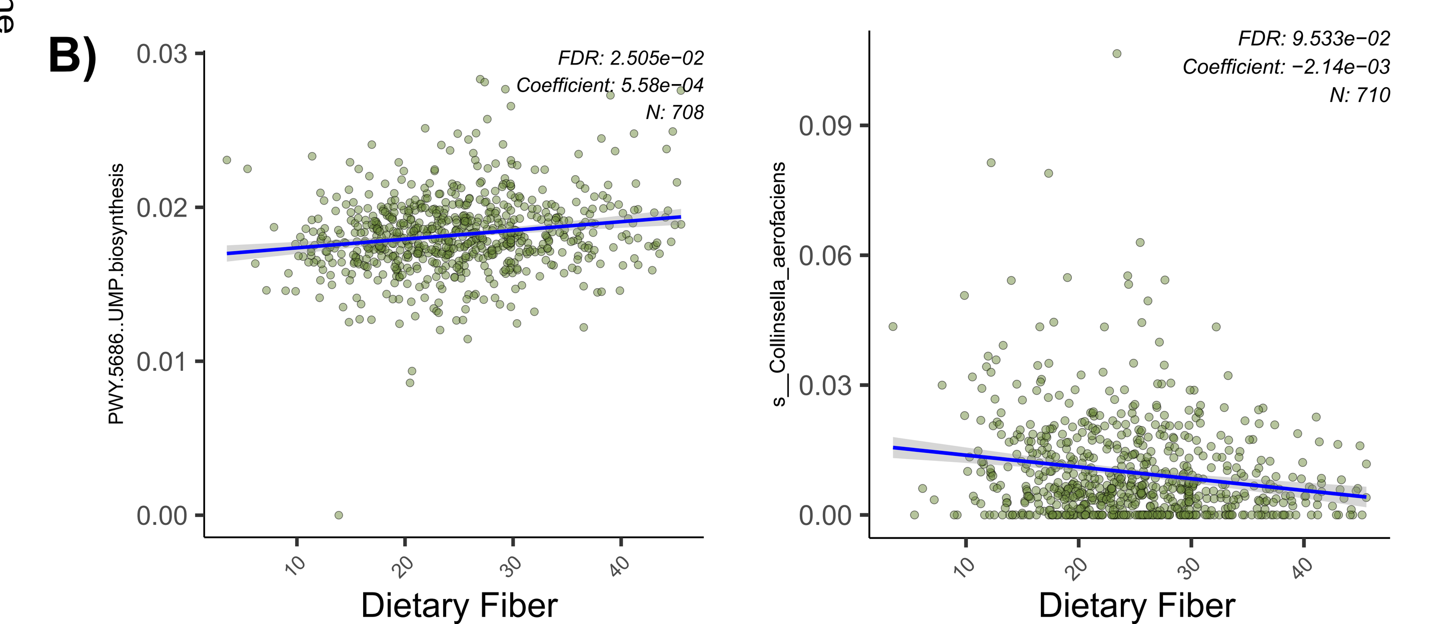


A) Comparison of pathways encoded vs. transcribed in the gut microbiome. These include a continuum from housekeeping processes near-equally transcribed by all encoding microbes, to niche metabolic modules rarely expressed by the few organisms that carry them. B) Similarly, housekeeping processes are rarely differentially expressed, whereas context-specific pathways are differentially regulated among human hosts.

Associations between specific nutrients and microbiome features



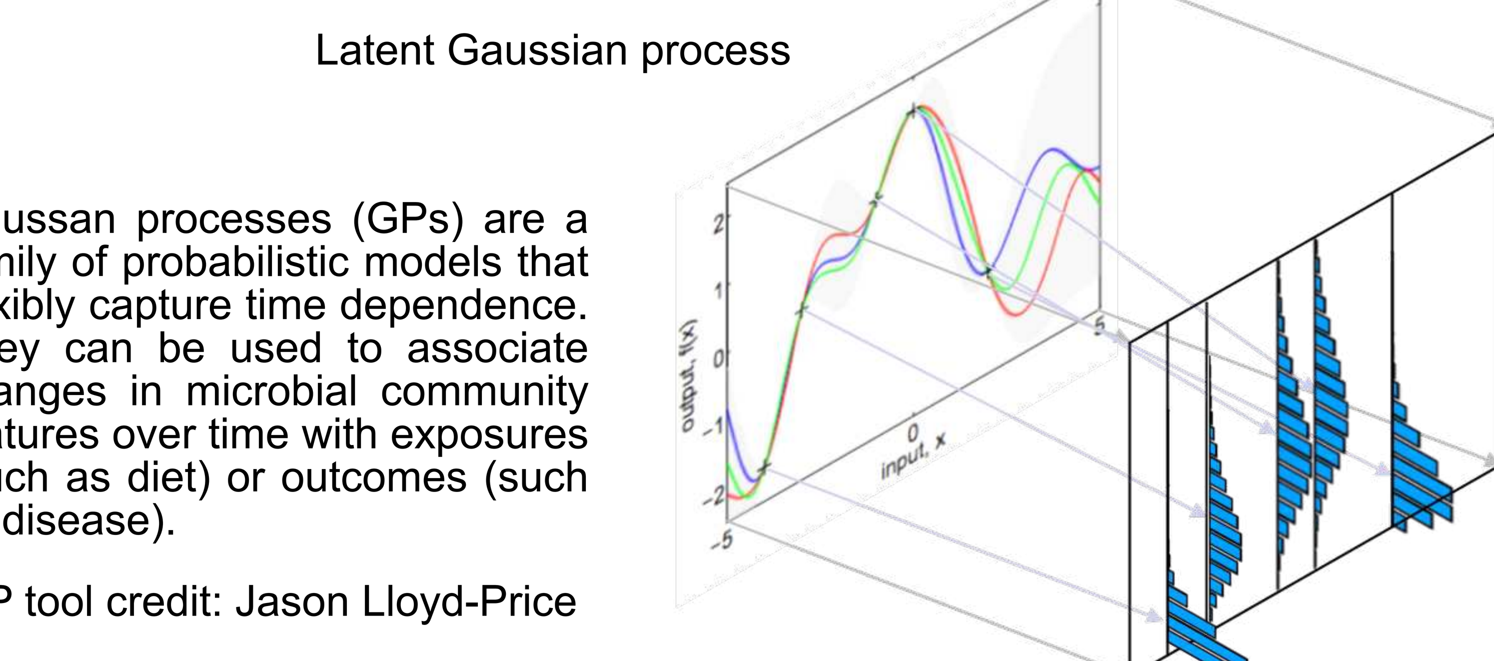
A) Significant covariation detected between microbiome functional profiles (pathways) and dietary components by a genealized linear model implemented in MaAsLin2. B) Left: Nucleotide biosynthesis, specifically uridine monophosphate, is positively associated with dietary fiber (q<0.25). Right: The abundance of *Collinsella aerofaciens*, carbohydrate fermenter, decreases with increased dietary fiber intake (q<0.25).



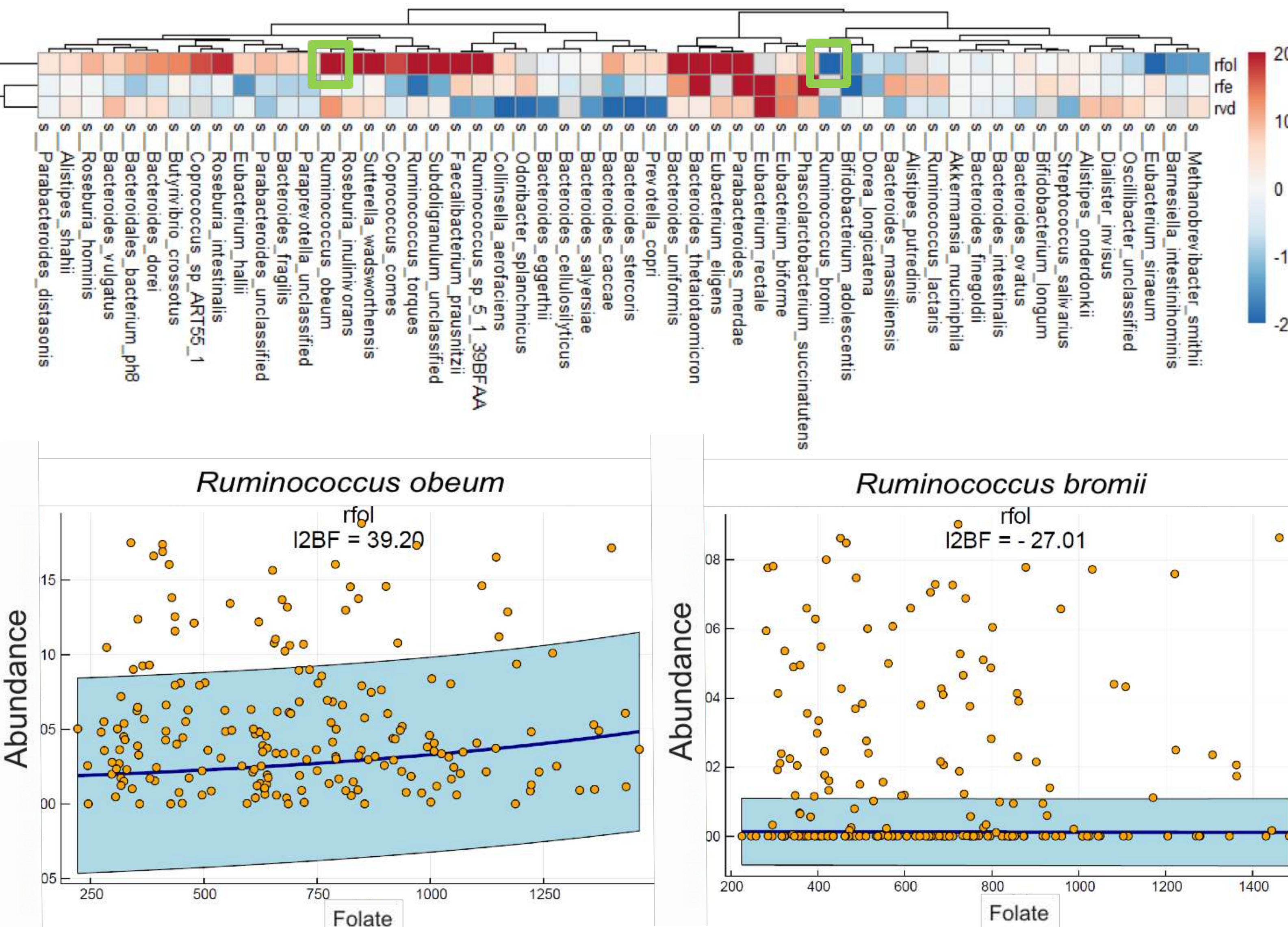
Gaussian process models for time-series microbiome analysis

Gaussan processes (GPs) are a family of probabilistic models that flexibly capture time dependence. They can be used to associate changes in microbial community features over time with exposures (such as diet) or outcomes (such as disease).

GP tool credit: Jason Lloyd-Price



GPs more specifically link dietary nutrients to microbial responses



GPs associate folate to *Ruminococcus obeum*, but folate is not associated with *Ruminococcus bromii*. A positive log2 Bayes Factor (l2BF) indicates favorable goodness of fit and a negative l2BF indicates poor fit. The GP model that contains a folate prediction term is preferred to the model that excludes folate when predicting *R. obeum*. Inclusion of folate in the model predicting *R. bromii* is less fit than the null model.

Acknowledgments

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Dietary Fiber Intake, the Gut Microbiome, and Chronic Systemic Inflammation

Wenjie Ma[1], Long H. Nguyen[1], Mingyang Song[1,2,3], Dong D. Wang[3], Eric Franzosa[4], Yin Cao[6], Amit Joshi[1], David A. Drew[1], Raaj Mehta[1], Kerry Ivey[3], Jacques Izard[7], Wendy Garrett[5], Eric B. Rimm[2,3,8], Curtis Huttenhower[4,5,9]* & Andrew T. Chan[1,5,8,9]*

[1]Clinical and Translational Epidemiology Unit and Division of Gastroenterology, Massachusetts General Hospital and Harvard Medical School; Departments of Epidemiology[2], Nutrition[3], Biostatistics[4], and Immunology and Infectious Diseases[5], Harvard T.H. Chan School of Public Health; [6]Washington University in St. Louis; [7]Department of Food Science & Technology, University of Nebraska-Lincoln; [8]Channing Division of Network Medicine, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School; [9]Broad Institute of MIT and Harvard (* equal contribution)

Higher intake of dietary fiber is associated with a decreased risk of inflammatory diseases such as diverticulitis and inflammatory bowel disease. Dietary fiber may abrogate the chronic systemic inflammation induced by factors including dysbiotic gut communities. Data regarding the detailed influences of long-term and recent intake of differing fiber sources in human populations are lacking. This study analyzed gut microbiome data profiled by shotgun metagenomic sequencing, long- and recent dietary fiber intake, and plasma levels of C-reactive protein (CRP). A greater intake of dietary fiber was associated with shifts in gut microbiome composition in particular Clostridiales and functions in carbohydrate utilization such as polysaccharide degradation. The microbial influences of fiber were varying according to food sources. In addition, circulating CRP was associated with differences in microbial composition. Last, gut microbial composition, primarily via *Prevotella copri*, modifies the association between dietary fiber and CRP. Our findings offer human population evidence supporting a fiber-gut microbiota interaction relevant to chronic systemic inflammation and related diseases.

Study design

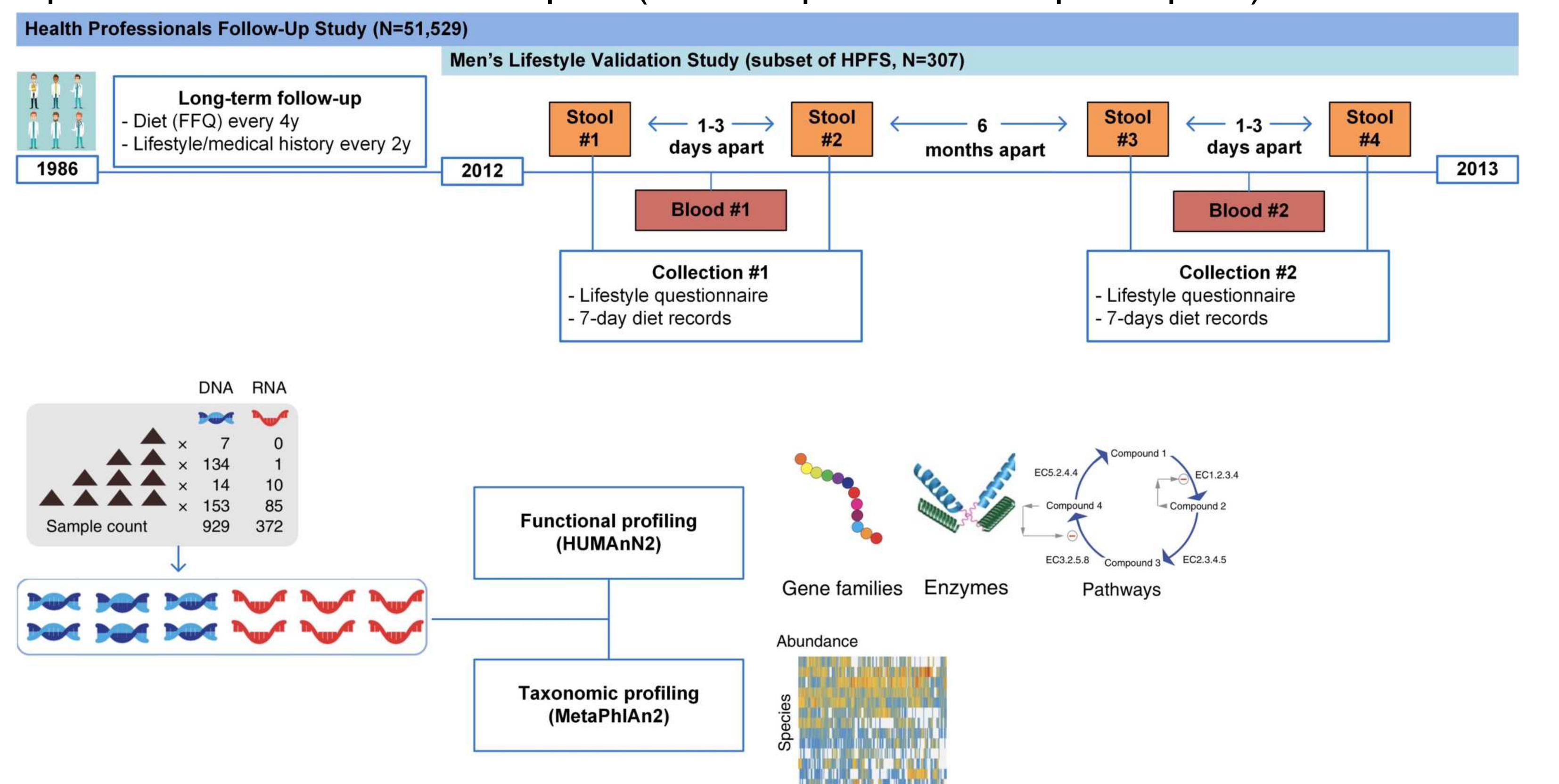
Study population: 307 participants in the Men's Lifestyle Validation Study

Dietary fiber intake:

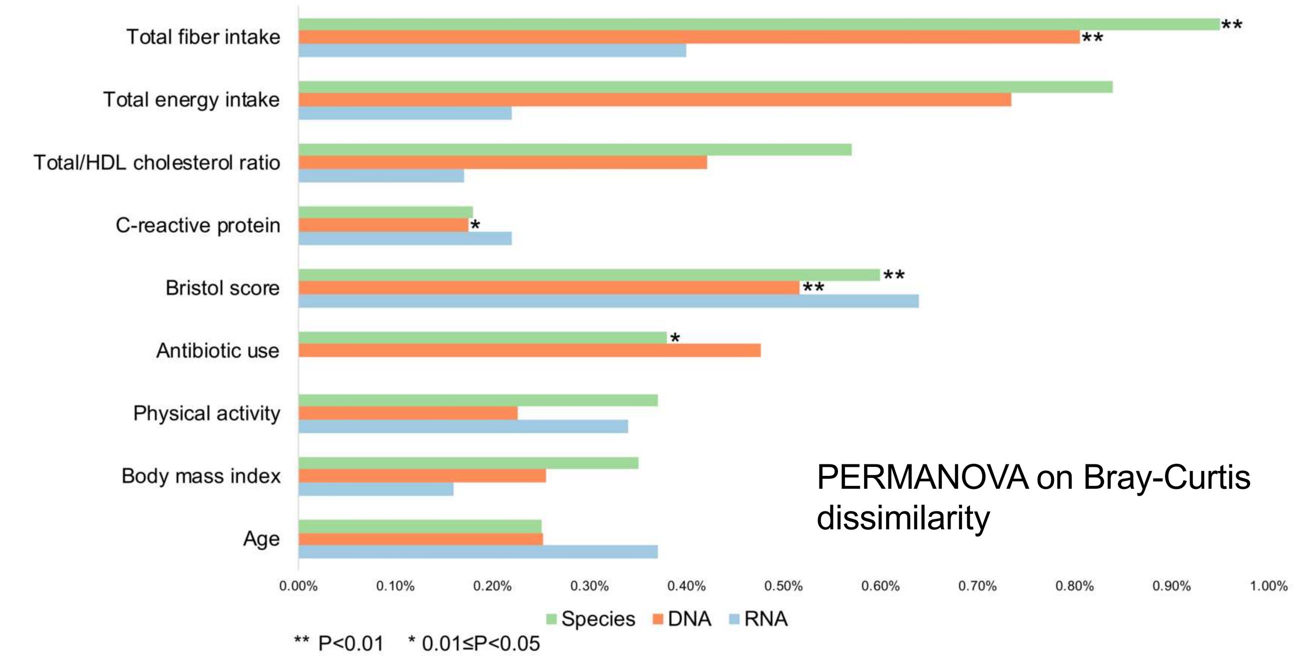
- Recent: 7-day dietary records
- Long-term: Validated semi-quantitative food frequency questionnaires every 4 years since 1986

Circulating inflammatory biomarker: High-sensitivity CRP

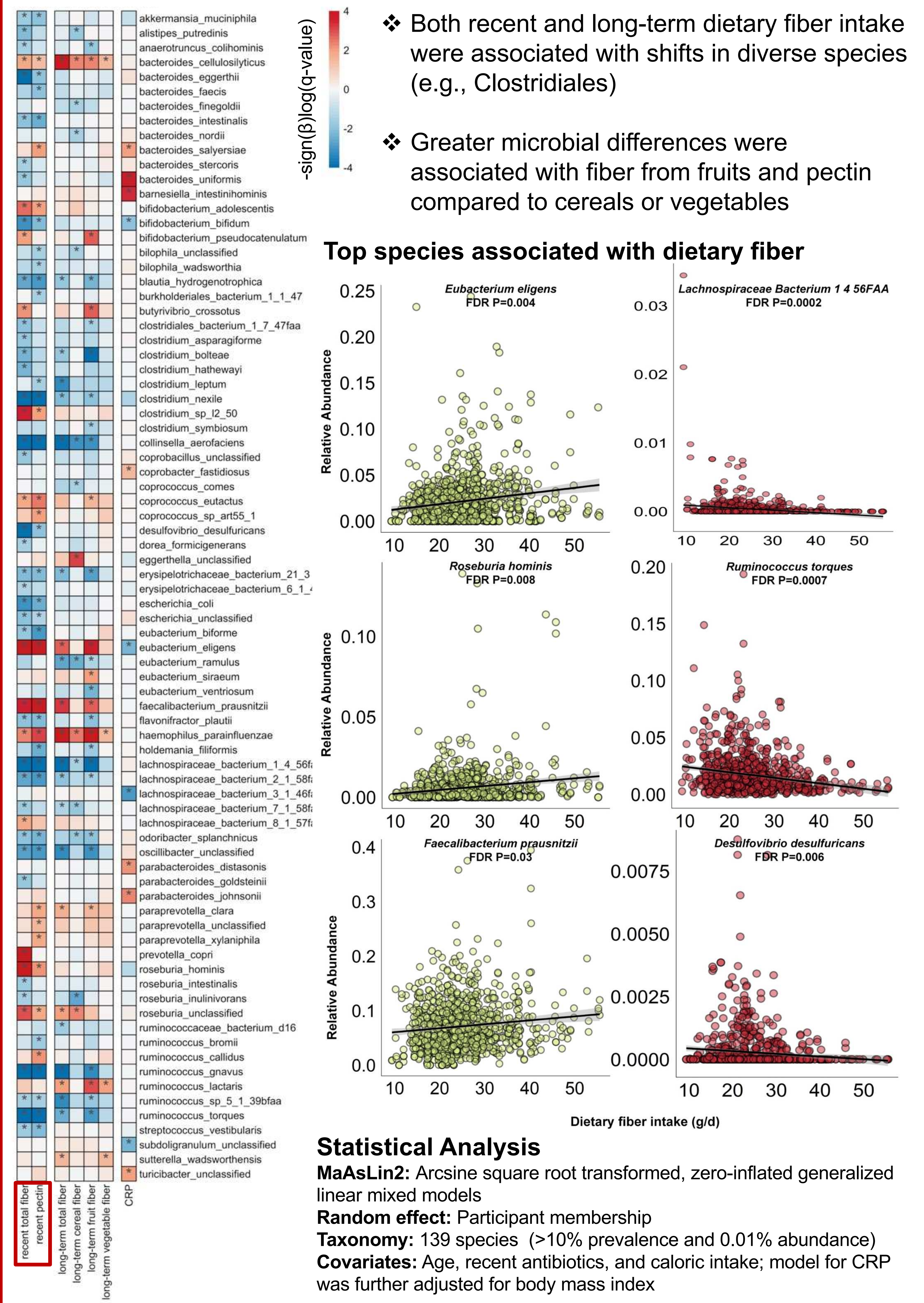
Gut microbiome: Shotgun metagenomic and metatranscriptomic sequencing on repeated collected fecal samples (1-4 samples for each participant)



Fiber and Bristol score are associated with overall microbiome structure



Individual microbial members are associated with fiber intake and CRP



- Both recent and long-term dietary fiber intake were associated with shifts in diverse species (e.g., Clostridiales)
- Greater microbial differences were associated with fiber from fruits and pectin compared to cereals or vegetables

Statistical Analysis

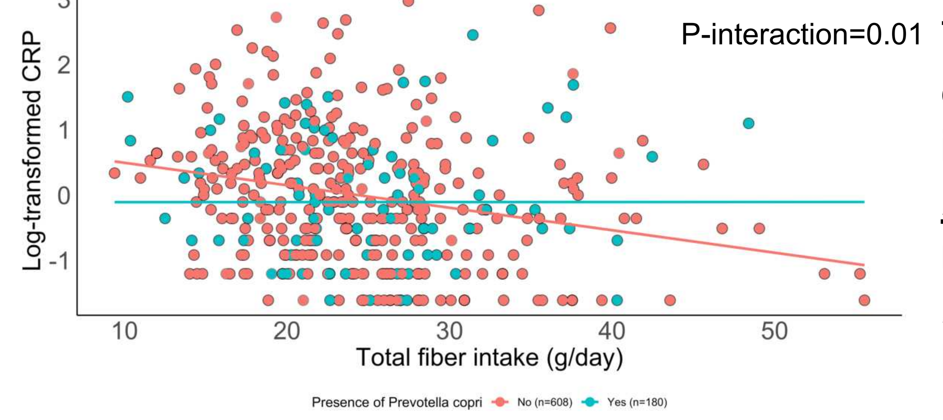
MaAsLin2: Arcsine square root transformed, zero-inflated generalized linear mixed models

Random effect: Participant membership

Taxonomy: 139 species (>10% prevalence and 0.01% abundance)

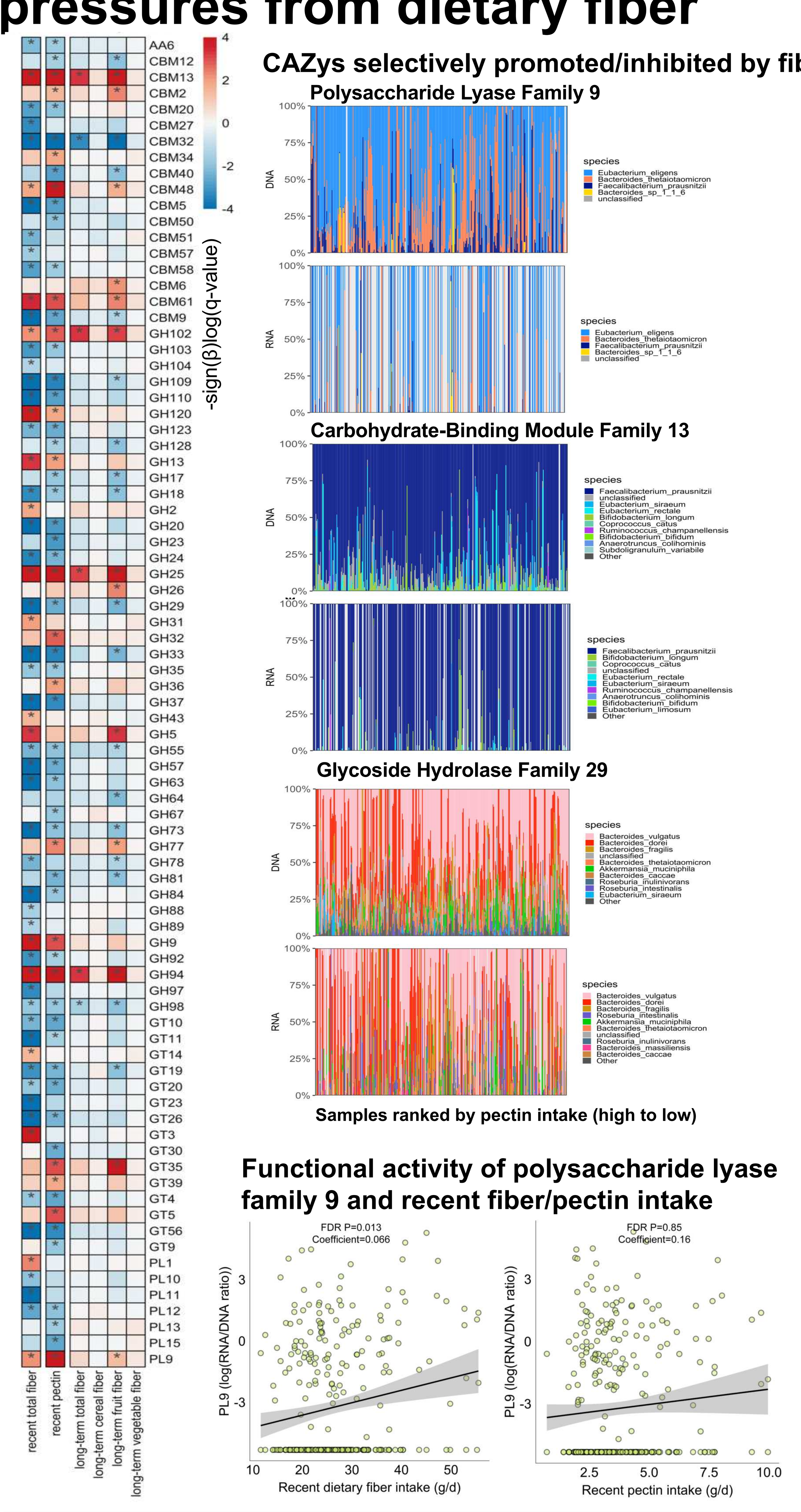
Covariates: Age, recent antibiotics, and caloric intake; model for CRP was further adjusted for body mass index

Gut microbial composition modifies the fiber-CRP relationship



The inverse association between dietary fiber and CRP was stronger in participants who did not have ***Prevotella copri***, suggesting that microbiome structure, primarily via *P copri* carriage, is a mediator between fiber intake and CRP.

Potential biochemical contributors to microbe-specific selection pressures from dietary fiber



Conclusion

Our findings support a fiber-gut microbiota interaction on chronic inflammation. Findings regarding *P. copri* need further investigations and may inform personalized, microbiome-based dietary intervention.

Acknowledgements & Funding sources: We thank the participants in this research. This work was supported by MGH ECOR Tosteson and Fund for Medical Discovery Postdoctoral Fellowship Award, NIH grants U54DE023798, UM1CA167552, U01CA152904, R01HL35464, R01CA202704, R01DK101495 and K24DK098311, and by the Starr Center Consortium.

Microbiome Differential Abundance Analysis by Quantile-Based Method

Wodan Ling, Michael C. Wu

Fred Hutchinson Cancer Research Center

Background

- Recurring objective of microbiome studies is to identify differentially abundant taxa
- Normalization affects the performance of existing methods
- Microbiome data is complex: heavy-tailed, heterogeneous and high-dimensional
- Quantile regression:
  - Distribution-free: robust for any normalization, flexible to address hundreds of various taxa
  - Detects higher order associations: over the entire distribution of abundance

Objective

To improve the power to detect differentially abundant taxa while controlling Type I error, regardless of the normalization method

Two-Part Quantile Regression Model

- $logit\{P(Y>0|\boldsymbol{X}) = \boldsymbol{Z}^\top\boldsymbol{\zeta} + \gamma C$
- $Q_Y(\tau|\boldsymbol{X}, Y>0) = \boldsymbol{Z}^\top\boldsymbol{\alpha}(\tau) + \beta(\tau)C$

Where

- Y: the transformed abundance by any method
- C: the key condition (e.g. health/disease status),
- Z: the remaining covariates including the intercept
- If Y is a count variable:

$$Q_W(\tau|\boldsymbol{X}, Y>0) = \boldsymbol{Z}^\top\boldsymbol{\alpha}(\tau) + \beta(\tau)C$$

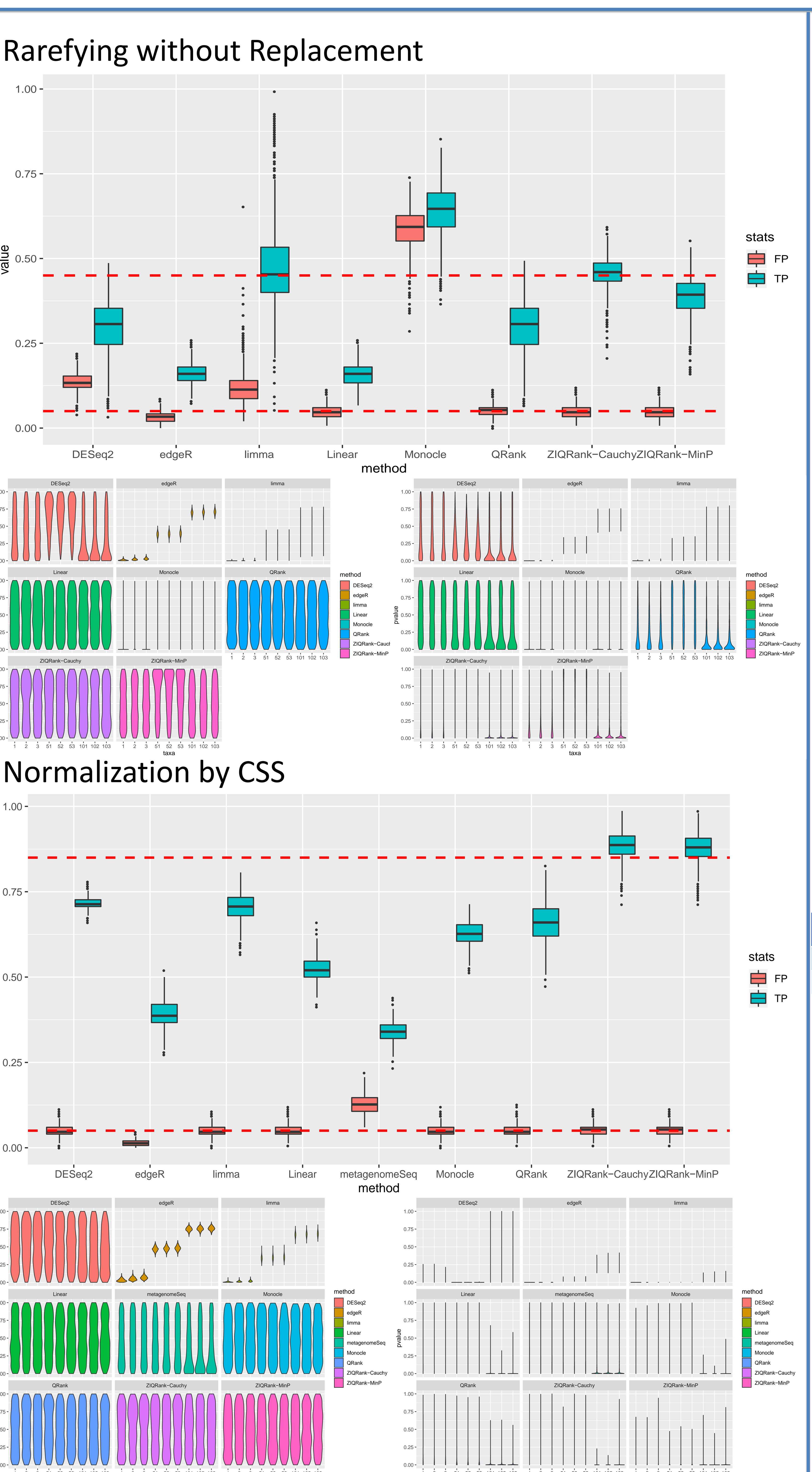
where W $= Y+U, U \sim U(0,1)$

Zero-Inflated Quantile Rank-Score Based Test

- Hypotheses testing:
  - $H_0: \gamma = \beta(\tau) = 0, \tau \in [\epsilon, 1-\epsilon]$
  - $H_1: otherwise$
- To test $\gamma = 0$: any valid test in logistic regression
- To test $\beta(\tau) = 0, \tau \in [\epsilon, 1-\epsilon]$:
  - A grid of quantile levels: $\epsilon \le \tau_1 < \cdots < \tau_K \le 1 - \epsilon$
  - Quantile rank-score test: on positive abundance
  - Addressing undersampling biases: correct zero-inflation in rank-score's covariance matrix
- Combine p-values by MinP / Cauchy procedures

Type I Error and Power in Simulation

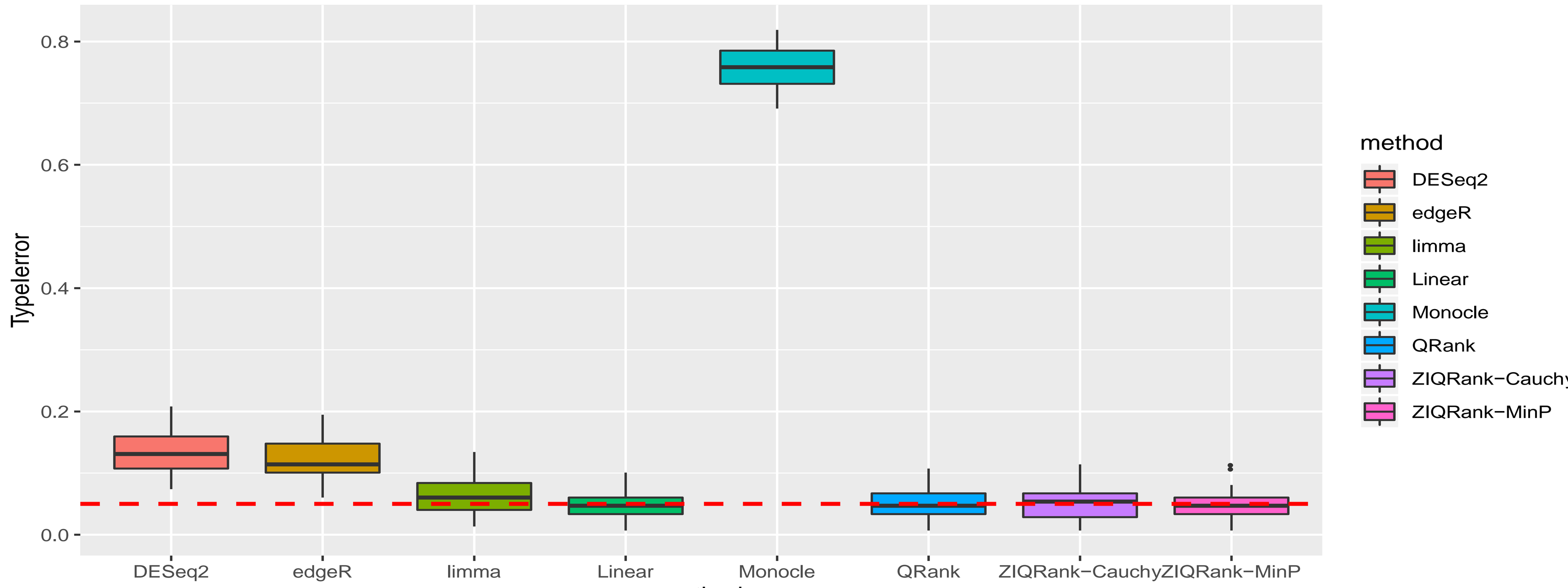
- Based on a real genus-level gut microbiota dataset
- 150 taxa, 531 samples each, repeat 1000 times
- Adjusted analysis with clinical condition: HBP (0=No, 1=Yes)



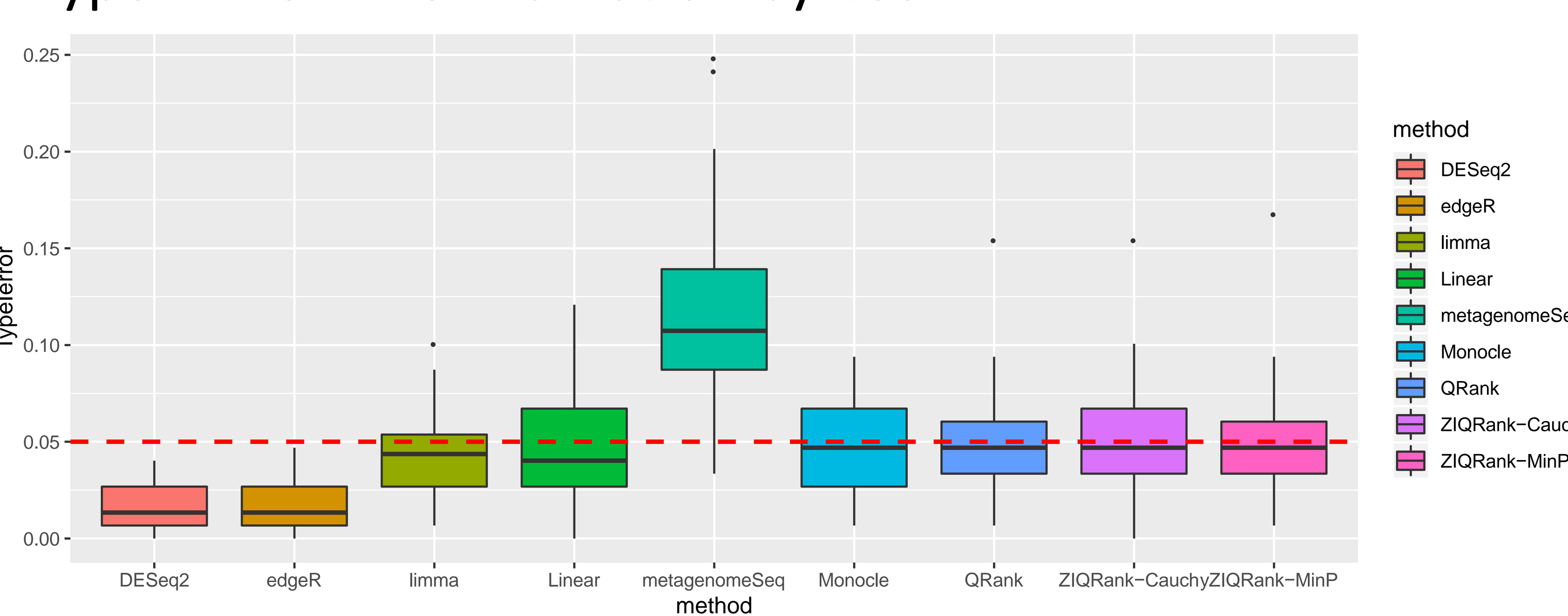
Real Data Analysis

- A gut microbiota dataset: 149 genus-level taxa, from 531 samples
- Key clinical condition: HBP (0=No, 1=Yes), adjusted by age, physical activity and dietary quality score
- Outcome: read counts with zero-inflation rate 0 – 74.6%

Type I Error: Rarefying without Replacement



Type I Error: Normalization by CSS

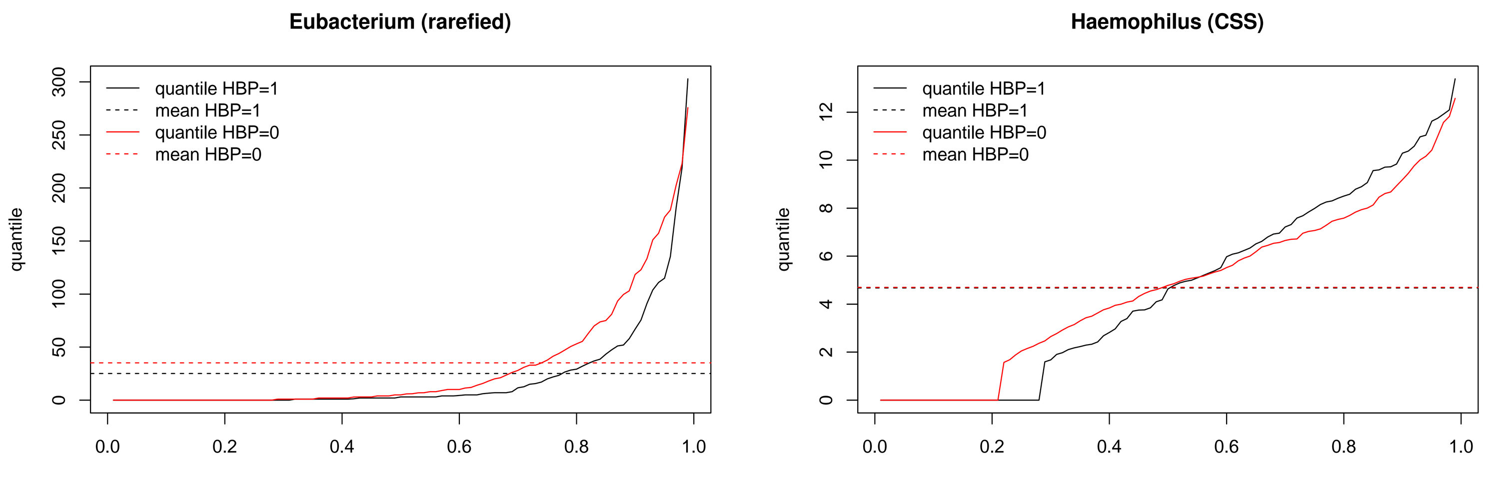


Numbers of Detected Differentially Abundant Taxa

	DESeq2	edgeR	limma	linear	metagenome Seq	Monocle	QRank	ZIQRank-Cauchy	ZIQRank-MinP
Rarefraction	34	33	24	5	--	121	13	49	48
CSS	13	37	20	25	40	20	12	41	37

Characteristics of Taxa Detected by ZIQRank Only

- Spindle-shaped / Crossing



Conclusion

- Quantile-based method increases the power by detecting higher order associations in addition to mean effect
- Approach is robust for any normalization method
- Some fine tuning of the grid of quantile levels may be necessary

Acknowledgement

- Grants: R01 GM129512, R01 MD011504, and The Hope Foundation

Identifying strain-specific functional genes in colorectal cancer

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[1]Harvard T.H. Chan School of Public Health, [2]Broad Institute of MIT and Harvard, [3]CIBIO Department, University of Trento, Trento, Italy, [4]Clinical and Translational Epidemiology Unit, [5]Division of Gastroenterology, Massachusetts General Hospital and Harvard Medical School
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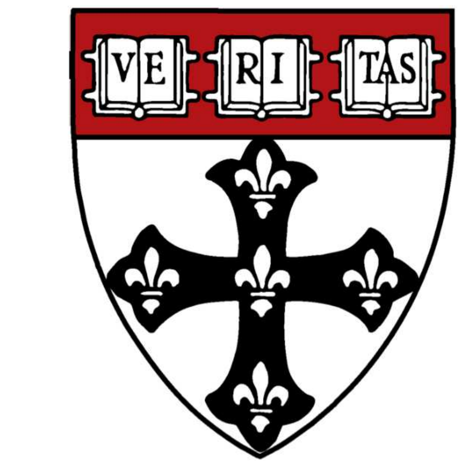
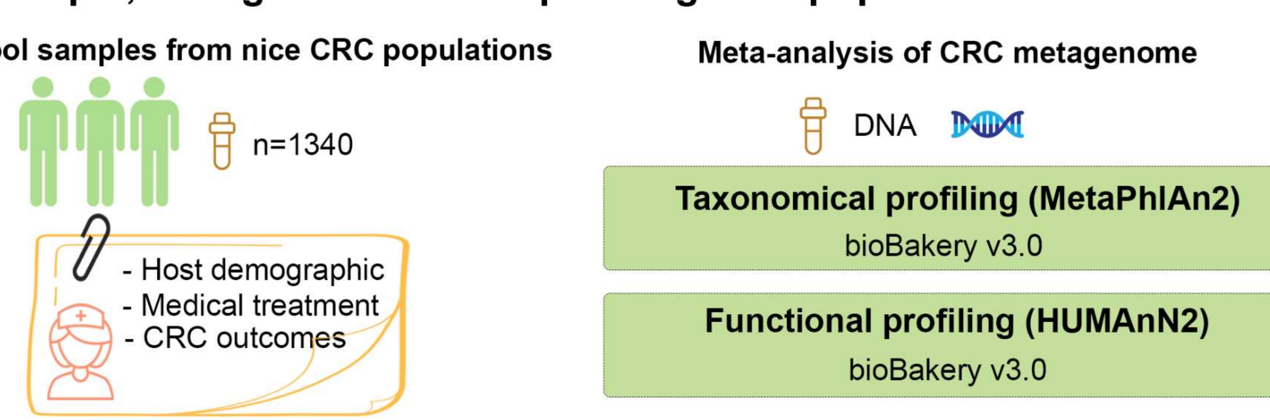
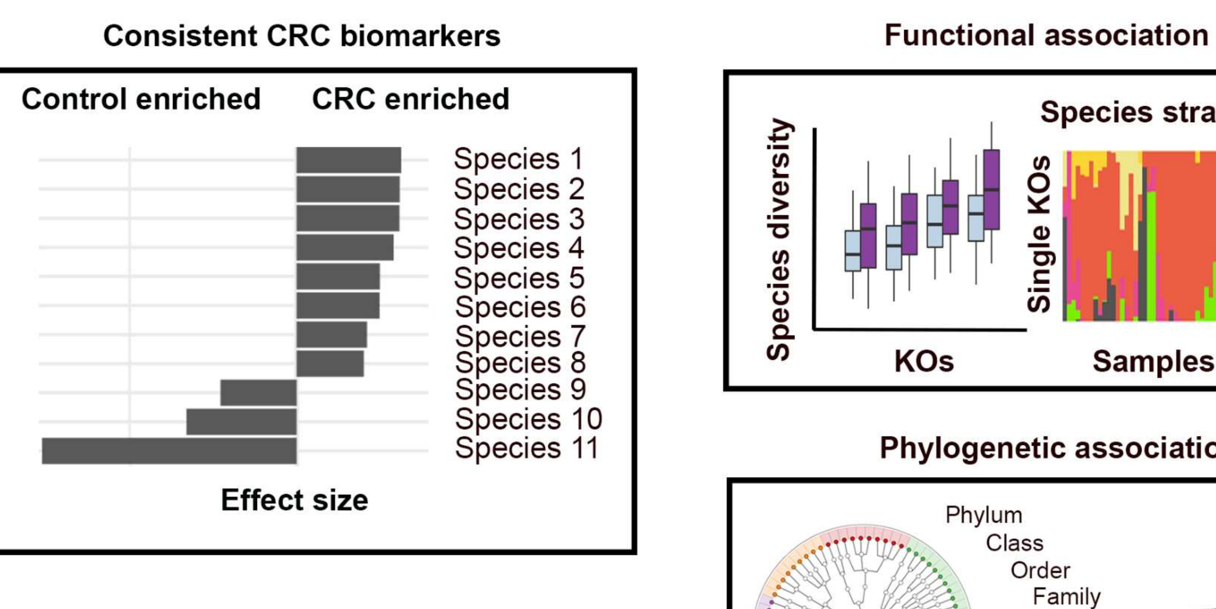


Colorectal cancer (CRC) most often occurs sporadically (as compared to genetic forms of the disease) and is one of the leading causes of cancer-related death worldwide. Environmental factors contribute substantially to CRC risk and development, particularly the intestinal microbiota. Recent meta-analyses of gut microbial profiles in CRC have identified multiple taxa (including Fusobacterium) reproducibly associated with late-stage cancers across populations. However, neither the causal mechanisms nor corresponding microbial strains and small molecule products have been pinpointed for CRC, particularly among subsets of non-Fusobacterium clades newly associated with the disease. We leveraged stool metagenomic profiles from 352 CRC patients, 143 with pre-cancerous adenomas, and 312 healthy controls from seven recent CRC microbiome studies in combination with our integrated metagenomic and metatranscriptomic data from the Integrative Human Microbiome Project, Nurses' Health Study, and Health Professionals Follow-Up Study. Within CRC-associated species, we assessed strain-specific gene carriage and sub-species phylogenetic enrichments via gene- and variant-based culture-independent profiling. The former identified gene families carried significantly more or less frequently by individual strains during disease, and the latter called out subclades with significant phylogenetic associations with carcinogenesis. In some cases, these genes and nucleotide variants also corresponded with transcriptional changes. This study adds further evidence to the hypothesis that strain-level genomic variation in gut microbes may be a major driver in the initiation or development of colorectal cancer.

a. Stool sample, data generation and profiling CRC populations

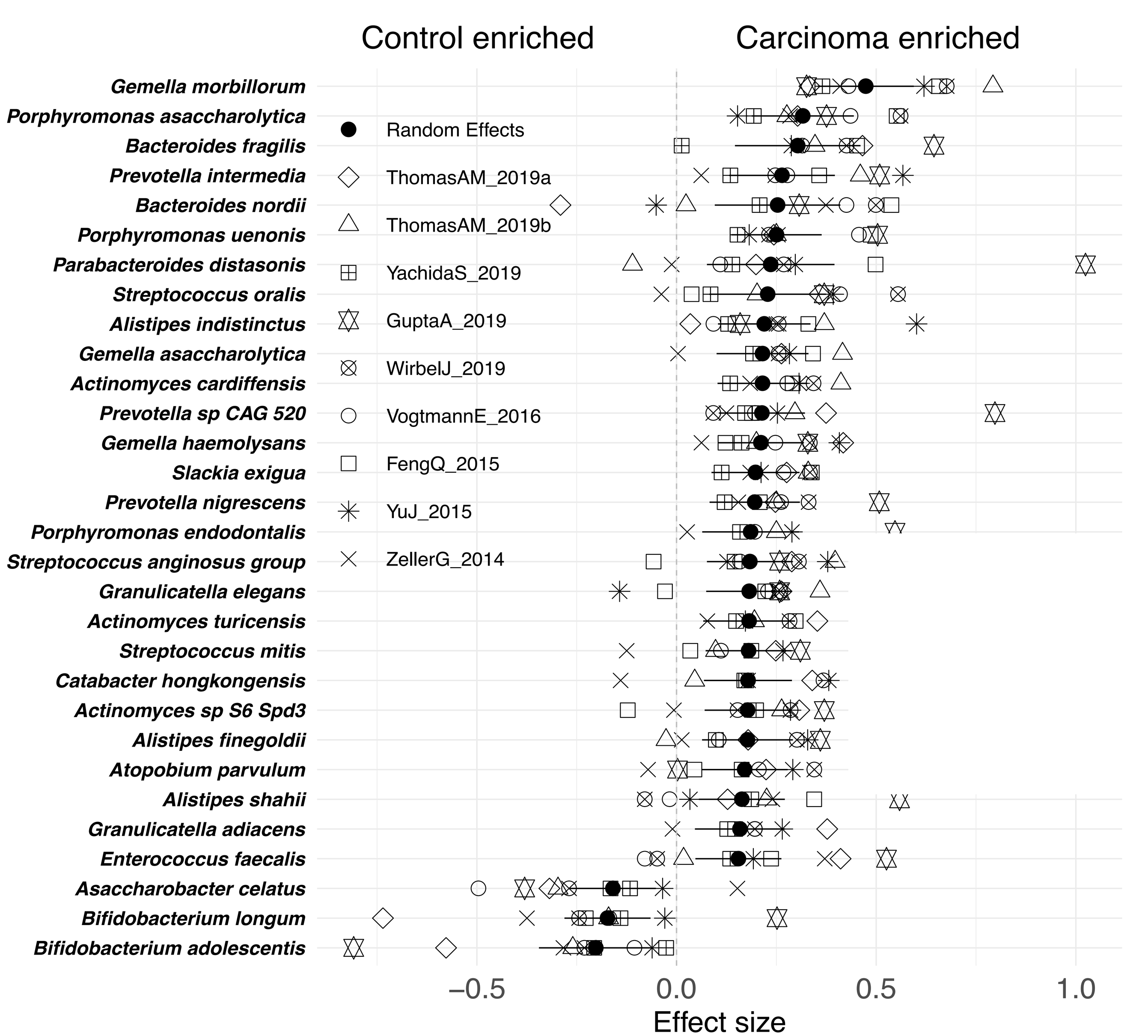


b. Data analysis



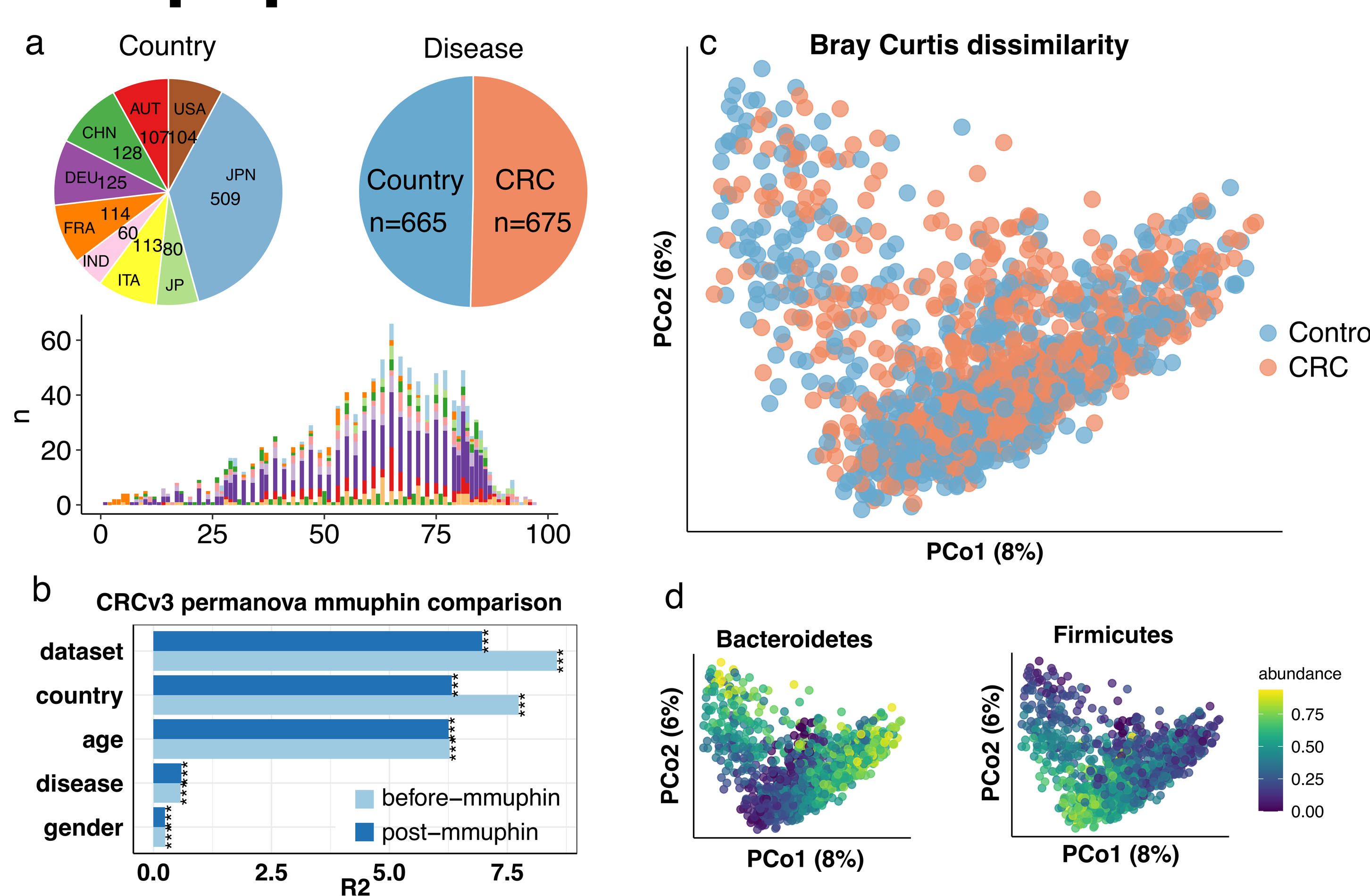
Metagenomics of the stool microbiome in CRC populations (n=1340). a) Samples were metagenomically shotgun sequenced to yield taxonomic and functional profiles. b) Features of the microbiome were associated with CRC outcomes using meta-analysis modeling, in addition to phylogenomic association with CRC outcomes.

Consistent taxonomic microbioal biomarkers of CRC



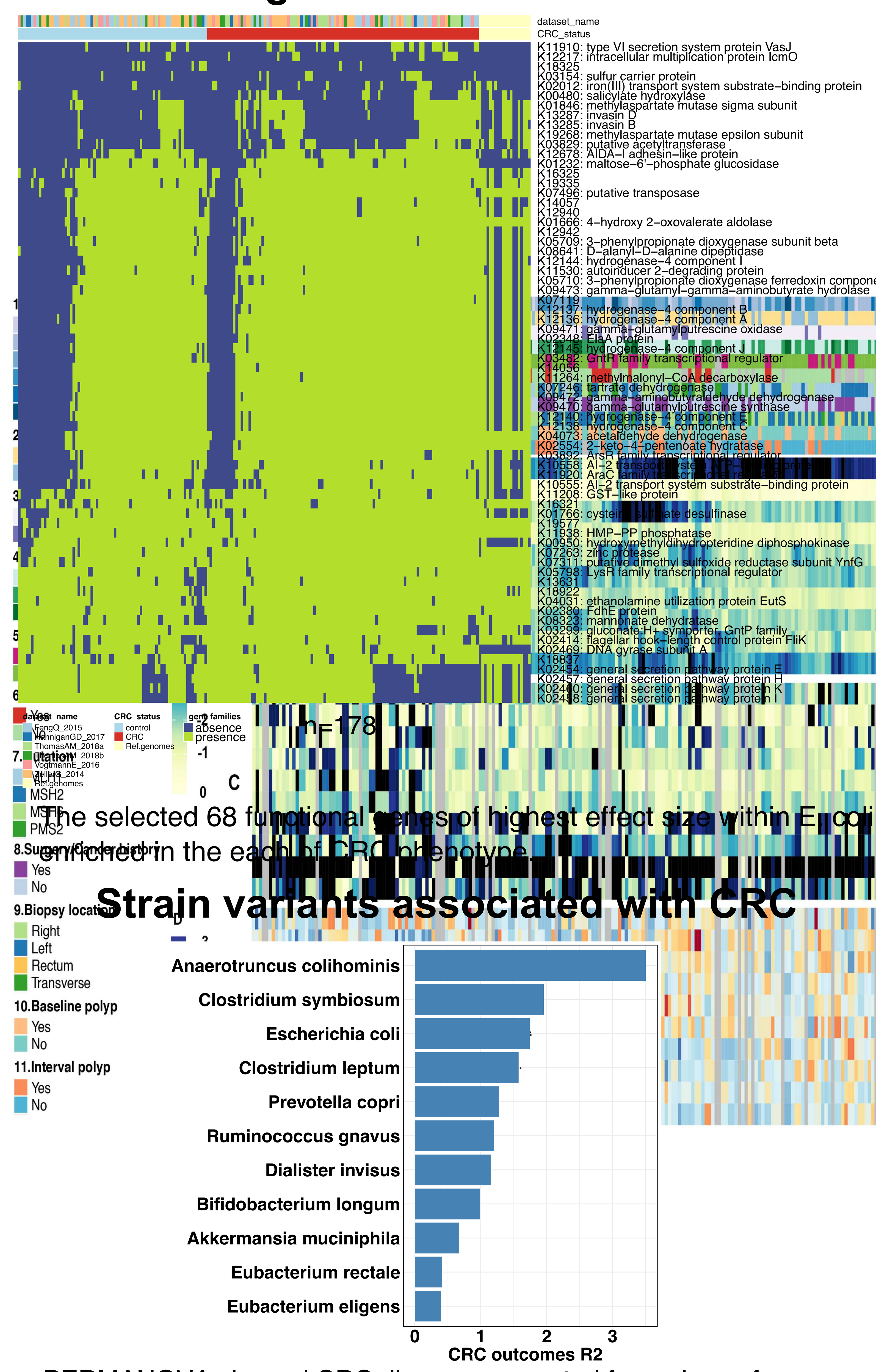
Pooled effect sizes for the 30 significant features with the largest effect size, calculated using a random effects model on expanded MetaPhlAn2 species abundance.

1340 CRC shotgun metagenomes from nine populations



Metagenomics of the stool microbiome in CRC populations. a) size and characteristics of the large scale CRC metagenomic datasets. b) Performing batch (study) effect adjustment in CRC microbial features.c) Principal coordinate analysis (PCoA) of stool metagenomic species. d) Typical of western populations, gradients of Bacteriodetes and Firmicutes dominance are seen across populations.

E.coli strain genomes associated with CRC



The selected 68 functional genes of highest effect size within E.coli enriched in the each of CRC phenotype.

Strain variants associated with CRC

PERMANOVA showed CRC disease accounted for variance for some of the CRC-associated species.

Acknowledgements

This work was funded by STARR Cancer Consortium Award #I7-A714, in part by Cancer Research UK Grand Challenge Initiative C10674/A27140 (Wendy S. Garrett) and by NIH NIDDK R24DK110499 (CH). Methods used for analysis are available in the bioBakery at:

BROAD INSTITUTE

Identifying novel bioactive microbial gene products in inflammatory bowel disease

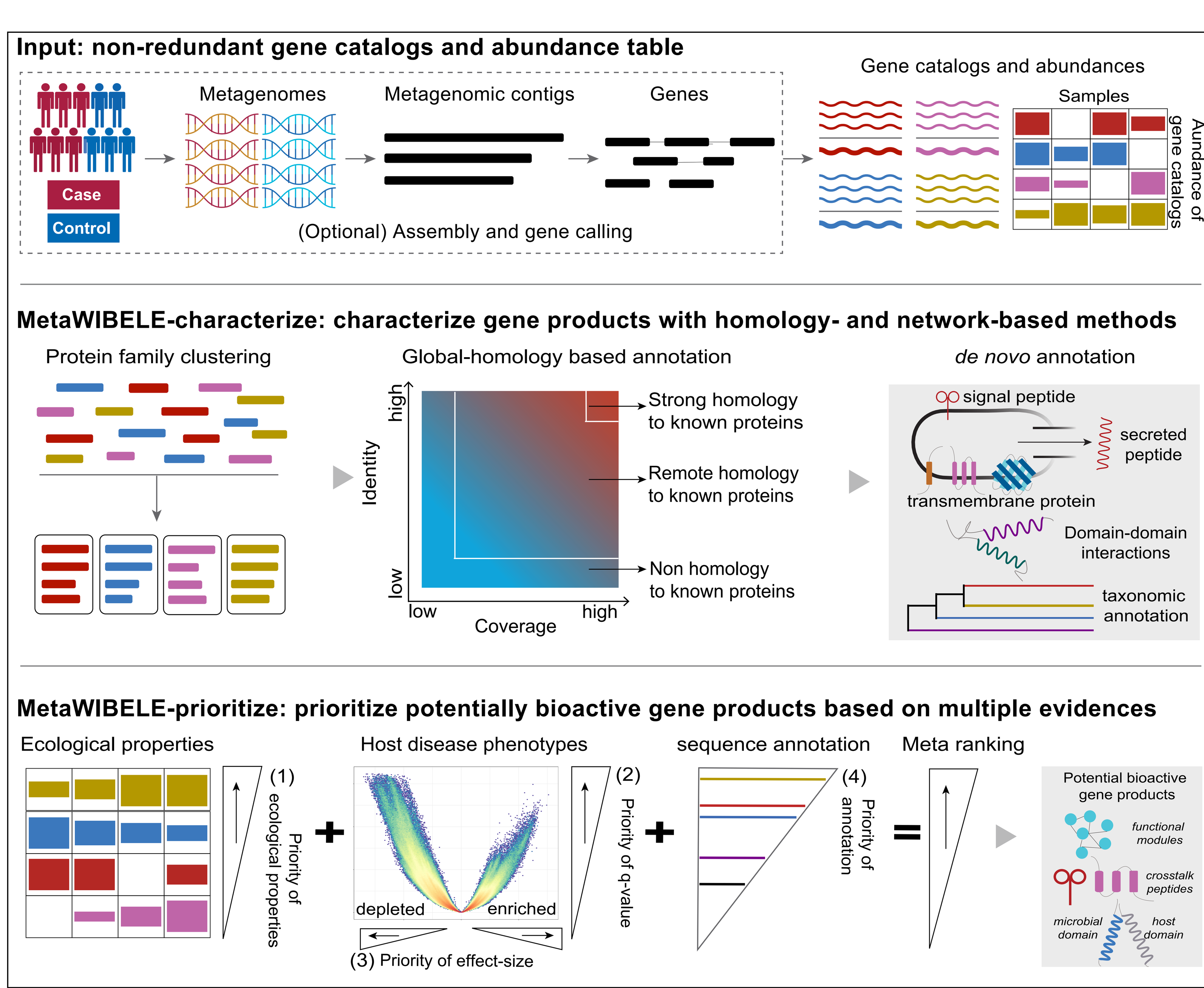
VE RI TAS

Yancong Zhang[1,2], Eric A. Franzosa[1,2], Sena Bae[2], Lauren J. McIver[2], Gholamali Rahnavard[1,2], Cesar Arze[2], Damian R. Plichita[1], Ayshwarya Subramanian[1,2], Wendy S. Garrett[2], Andy Krueger[3], Hera Vlamakis[1], Ramnik J. Xavier[1,4,5], Curtis Huttenhower[1,2,*]

[1]Broad Institute of Harvard and MIT, [2]Harvard T. H. Chan School of Public Health, [3]Takeda Pharmaceutical Company Ltd, [4]Massachusetts General Hospital, [5]Center for Microbiome Informatics and Therapeutics, Massachusetts Institute of Technology

The gut microbiome and associated bioactive compounds are often disrupted in gastrointestinal conditions such as the inflammatory bowel diseases (IBD). Since more than one-third of all proteins in the gut microbiome are uncharacterized, we prioritized potentially bioactive novel proteins from the metagenomes in the Integrative Human Microbiome Project (HMP2). Remarkably, >340,000 protein families are specifically prioritized as potentially bioactive by integrating criteria based on ecological properties and host disease phenotypes. Strikingly, ~23% of them were novel proteins, 36% of which expanded the pangenomes of common gut taxa and >90% of the remainder were assigned at least one putative biochemical annotation. Our analysis methods are generalizable to other microbial communities and human disease phenotypes, and we provide an open source implementation as MetaWIBELE (**W**orkflow to **I**dentify novel **B**ioactive **Ele**ments in the microbiome). The prioritized results provide thousands of new microbial genes likely to interact with host immunity in IBD and gut inflammation, expanding our understanding of bioactive gene products in chronic disease states.

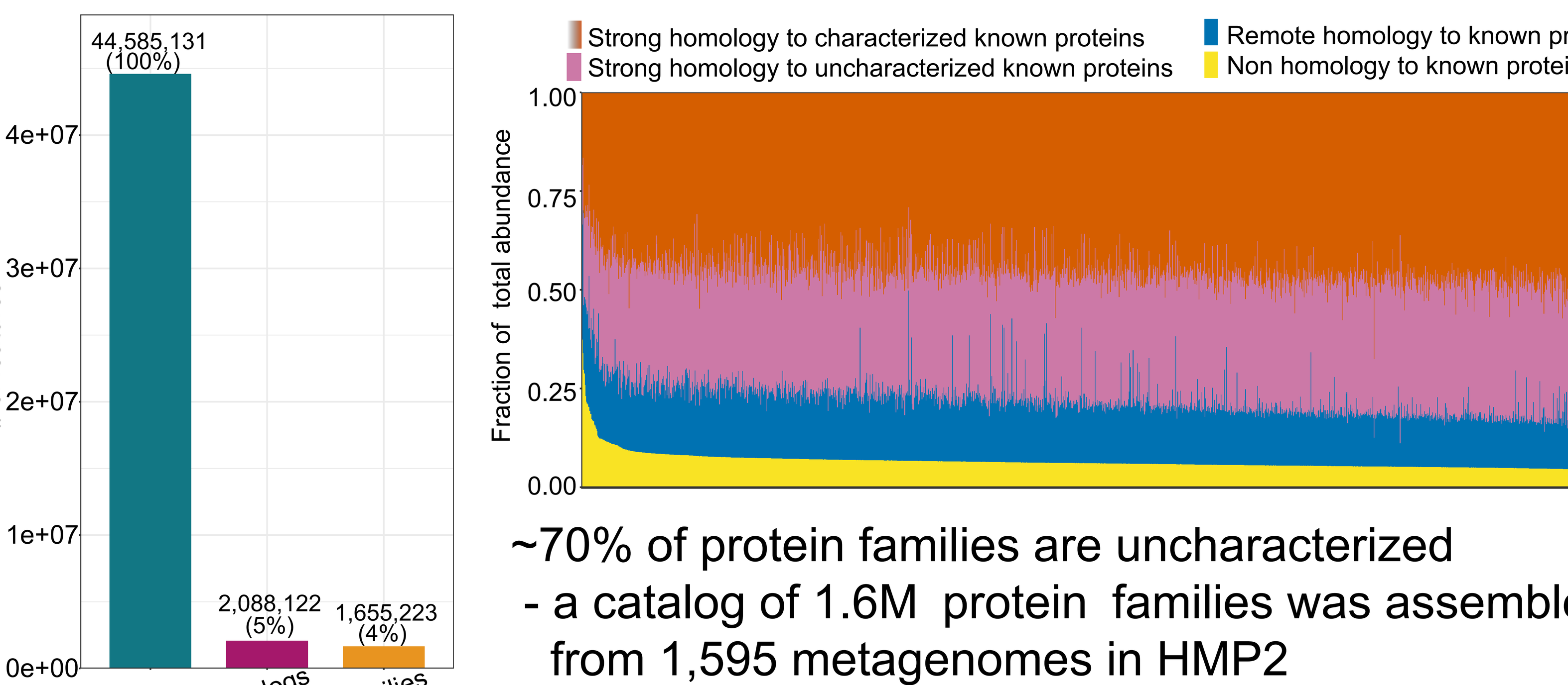
Computational prioritization workflow



MetaWIBELE: assembly-based workflow to prioritize potentially bioactive microbial gene products

- identify characterized and uncharacterized protein families
- assign functional and taxonomic annotations to protein families
- prioritize protein families by combining evidences from abundance-based and sequence-based annotations

Many protein families are uncharacterized, but can be assigned new annotations

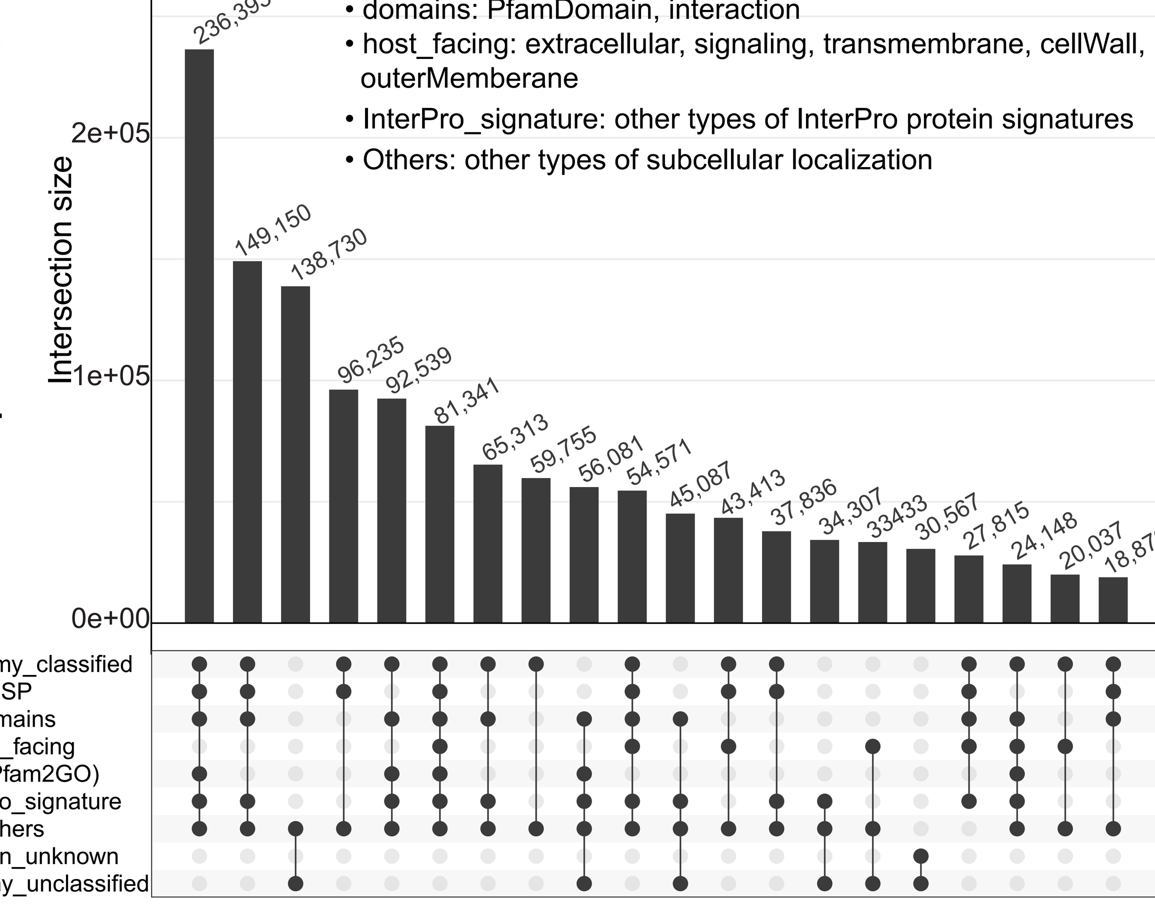


~70% of protein families are uncharacterized

- a catalog of 1.6M protein families was assembled from 1,595 metagenomes in HMP2
- uncharacterized proteins were identified with global homolog search

Uncharacterized proteins are assigned new annotations

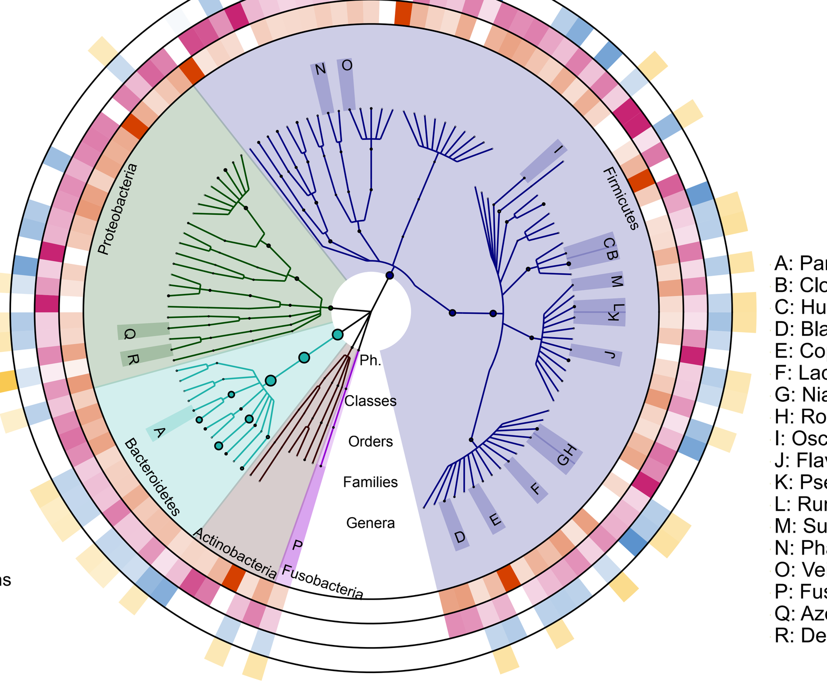
- biocheminal annotations were predicted by MetaWIBELE
- only ~3% were still functionally and taxonomically unknown



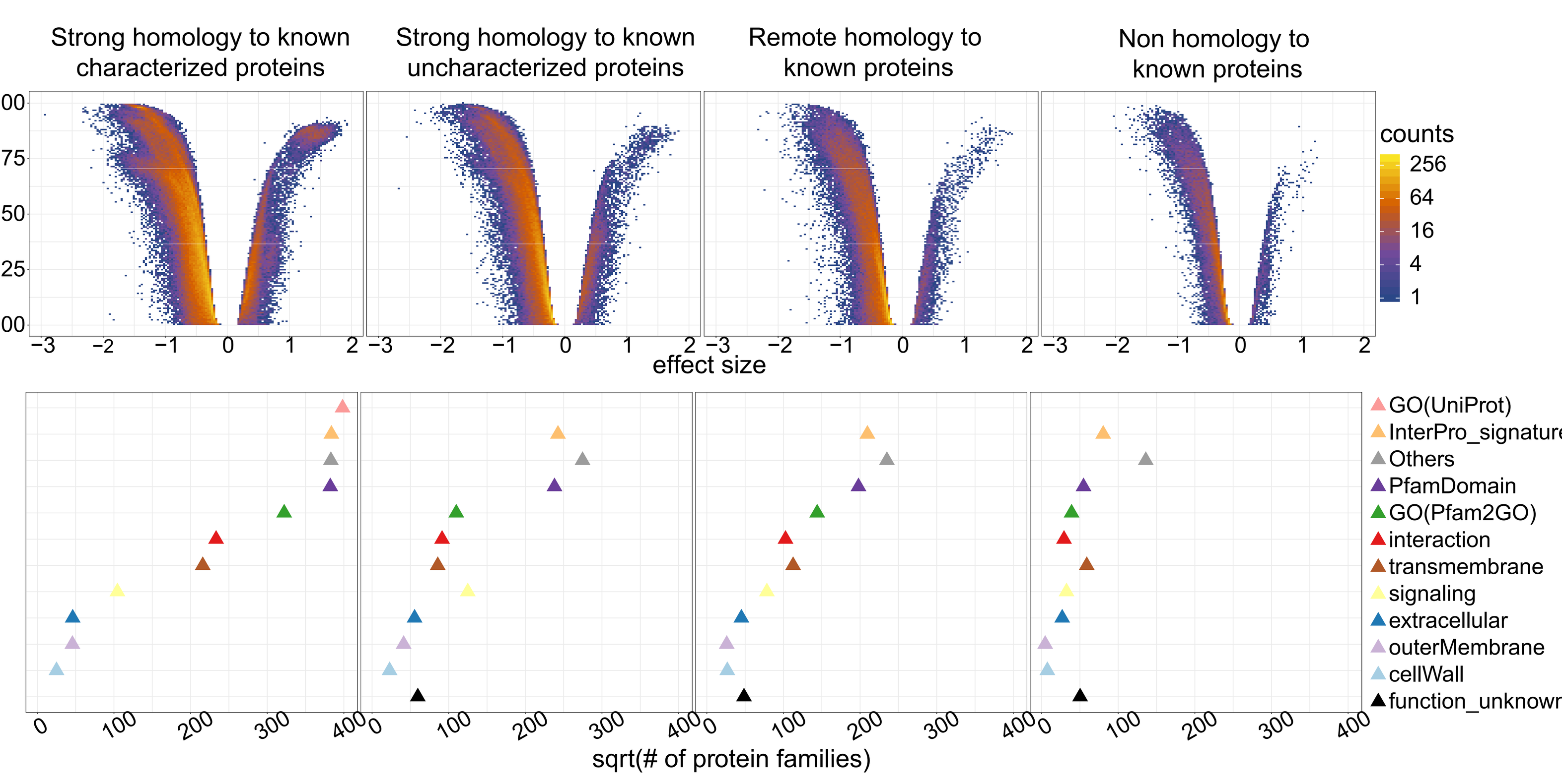
Uncharacterized proteins expanded the pangenomes of common gut taxa

Uncharacterized proteins are classified into phylogenetic clades

- uncharacterized proteins covered common gut taxa
- some novel proteins dominated in some clades



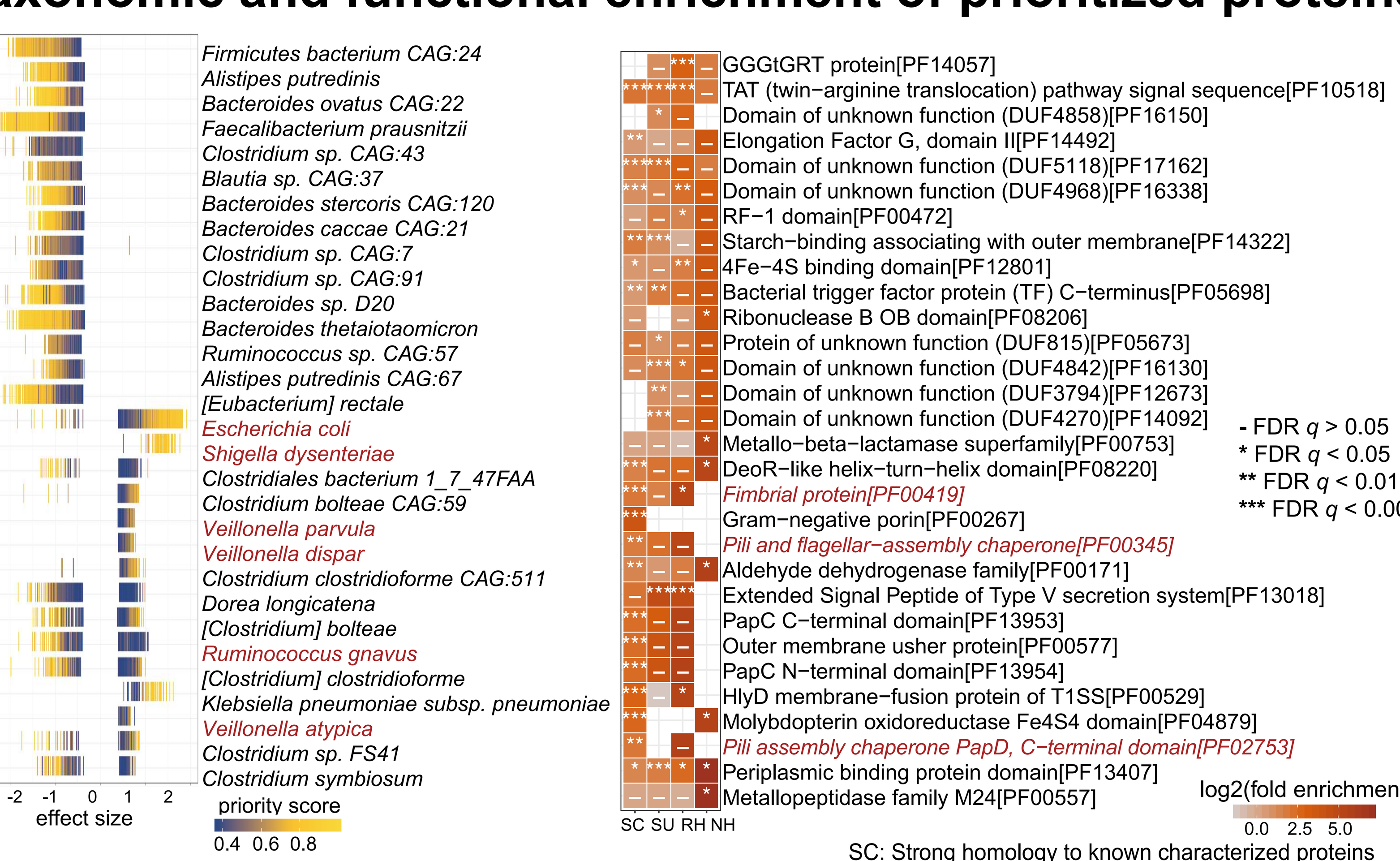
Uncharacterized proteins implicated in bioactivity are prioritized



Many uncharacterized proteins are prioritized

- 90% of prioritized proteins were depleted in disease-active state
- half of prioritized proteins were uncharacterized with 23% novel proteins
  - 39% of them expanded the pangenomes of common gut taxa
  - 90% of the remainder were assigned at least one putative annotation

Taxonomic and functional enrichment of prioritized proteins



Highly prioritized proteins are potentially bioactive related to gut inflammation during IBD

- prioritized protein families showed taxonomic agreement with reported IBD-associated species
- enriched pilin proteins could interact with the human epithelial surface

Acknowledgements

This work has been supported by NIH NIDDK grant R24DK110499.

MetaWIBELE is coming out soon and will be available at https://huttenhower.sph.harvard.edu/biobakery/

http://huttenhower.sph.harvard.edu

Antibiotic-induced perturbations of the gut microbiota alter ileal microRNA expression profiles in non-obese diabetic mice

RUTGERS

Yue (Sandra) Yin, Xue-Song Zhang, Martin J. Blaser
Center for Advanced Biotechnology and Medicine, Rutgers University, Piscataway, NJ 08854

Abstract

Background: Disruptions to the intestinal microbiota in early life increase the risk for autoimmune diseases, such as type 1 diabetes (T1D). A single course of antibiotic treatment (1PAT) from 5-10 days of life accelerated T1D development in male non-obese diabetic (NOD) mice, inducing substantial changes in gut microbial composition and ileal gene expression. MicroRNAs (miRNAs) are important post-transcriptional regulators of gene expression, and recent findings suggest an association of particular miRNAs with T1D pathogenesis. Here we investigated the role of ileal miRNAs in microbiota-mediated regulation of host protein-encoding genes.

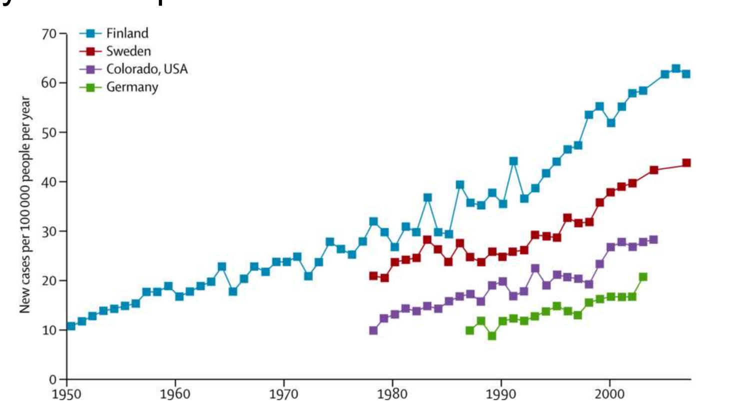
Methods: We treated NOD mice of 5-10 days of age with 1PAT only or with no antibiotics (Control), and a group of 1PAT-mice were given cecal microbiota from healthy donors, as a restorative (Restore). Ileal mRNA and miRNA gene expression were evaluated by RNA-Seq and NanoString, respectively, with further quantitation using RT-qPCR. We then employed a computational approach to predict the interactions between differentially expressed mRNAs and miRNAs.

Results: Receiving cecal microbiota transfer rescued the antibiotic-induced acceleration of T1D in NOD mice. Unsupervised hierarchical clustering of mRNA and miRNA expression showed restorative effects of the cecal microbiota transfer at a global level. Among 599 miRNAs measured, 59 had significantly differential expression between treatment groups, including six major miRNAs that responded to both antibiotic exposure and microbiota restoration. These six miRNAs were predicted to target 432 significantly differential mRNAs, many related to host defenses and inflammation. Particular miRNAs also were identified to regulate critical antimicrobial genes via multiple signaling pathways.

Conclusion: These findings provide evidence that perturbations of the gut microbiota alter ileal miRNA expression profiles which further impact mRNA gene expression. Signaling from both the 1PAT-perturbed and the cecally transplanted restored microbiota involve specific miRNA expression differences to affect ileal mRNA expression. Further investigations of the identified miRNAs and their targeted mRNA genes will deepen insights into the role of miRNAs in mediating microbiota-host interactions and T1D development.

Introduction

Type 1 diabetes is a chronic condition in which the body produces little or no insulin. The incidence of T1D has sharply risen globally in the past few decades. Genetic changes alone cannot explain this dramatic change – environmental and lifestyle changes leading to altered microbiome have played an important role.

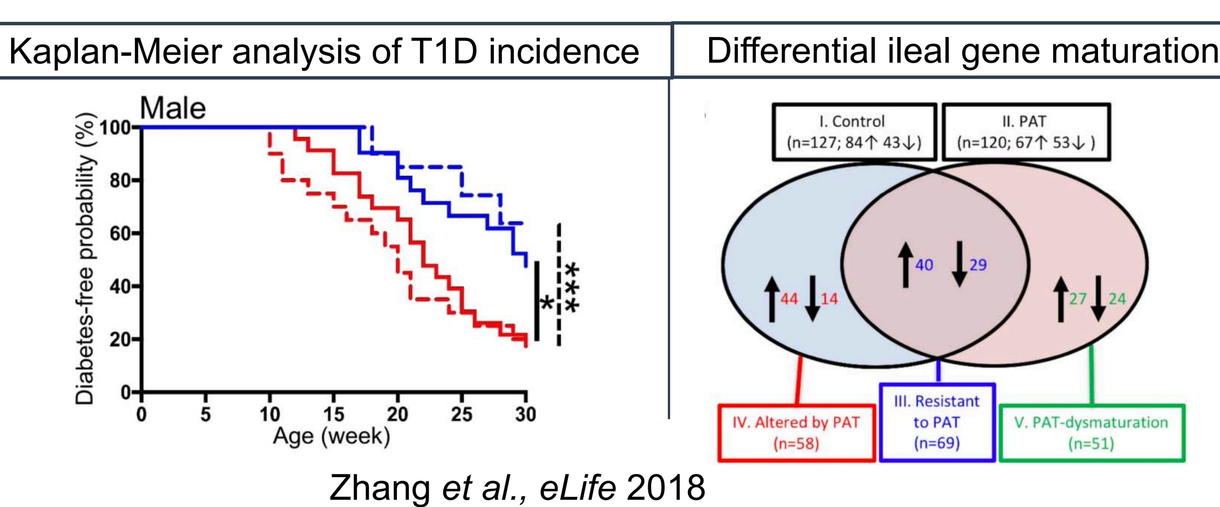


Incidence of T1D in children over time

Atkinson *et al., The Lancet* 2014

Exposing male NOD mice to 1PAT early in life induced gut microbiota perturbations and ileal gene expression alterations, which interfered with the development of host immunity and significantly accelerated T1D onset.

Kaplan-Meier analysis of T1D incidence | Differential ileal gene maturation



Zhang *et al., eLife* 2018

Methods

Schematic of long-term restoration (LTR) experiment

T1D? 1PAT + MomD0 (TM)
T1D? 1PAT + PBS (TP)
T1D? Control + PBS (CP)

Tylosin; Non acidified H_2O; exposure from P5-P10; Diabetes tracking from W12-W30; MomD0; PBS; gavage at P13, P15, and P17; *16S; + metagenomics; #Tissue analysis

A Full list: NanoString (miRNA) N 599; RNAseq (mRNA) N 52460
IPA edited & mapped: 526; 19654

Venn diagrams: Log_2-fold difference ≥ |0.5| and p < 0.05

B Significantly differential miRNAs (n = 526) — CP < TP 10, 11, TP > TM 15; CP > TP 1, 4, TP < TM 90 (n = 131)

C Significantly differential mRNAs (n = 19654) — CP < TP 1619, 45, TP > TM 99; CP > TP 1181, 25, TP < TM 107

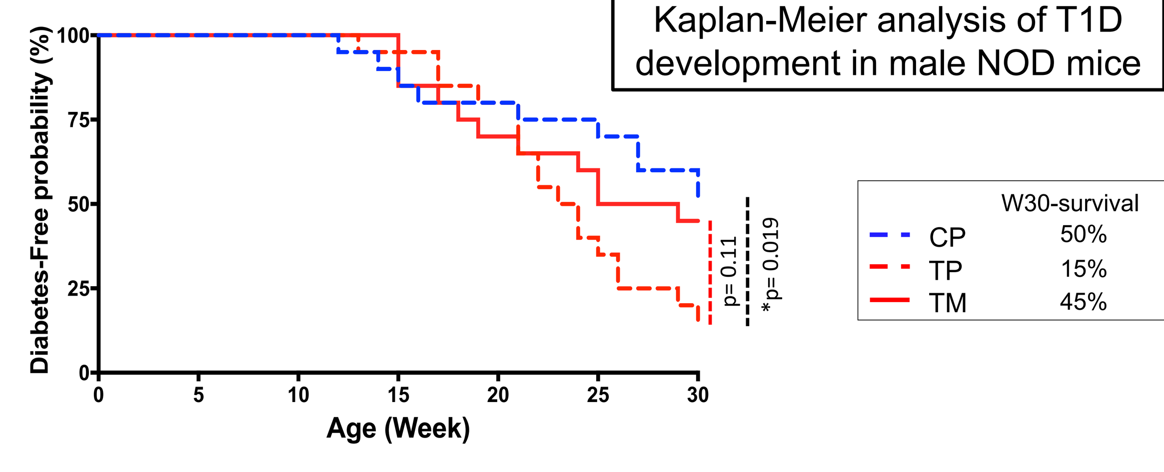
D miRNAs filtered by miRNA-mRNA interaction (n=63) — CP < TP n = 4, n = 5, TP > TM n = 15; CP > TP n = 0, n = 1, TP < TM n = 43

E mRNAs targeted by 6 major miRNAs (n = 3076) — CP < TP n = 236, n = 6, TP > TM n = 7; CP > TP n = 173, n = 1, TP < TM n = 9

Ingenuity Pathway Analysis workflow for the analysis of miRNA and target mRNA interactions

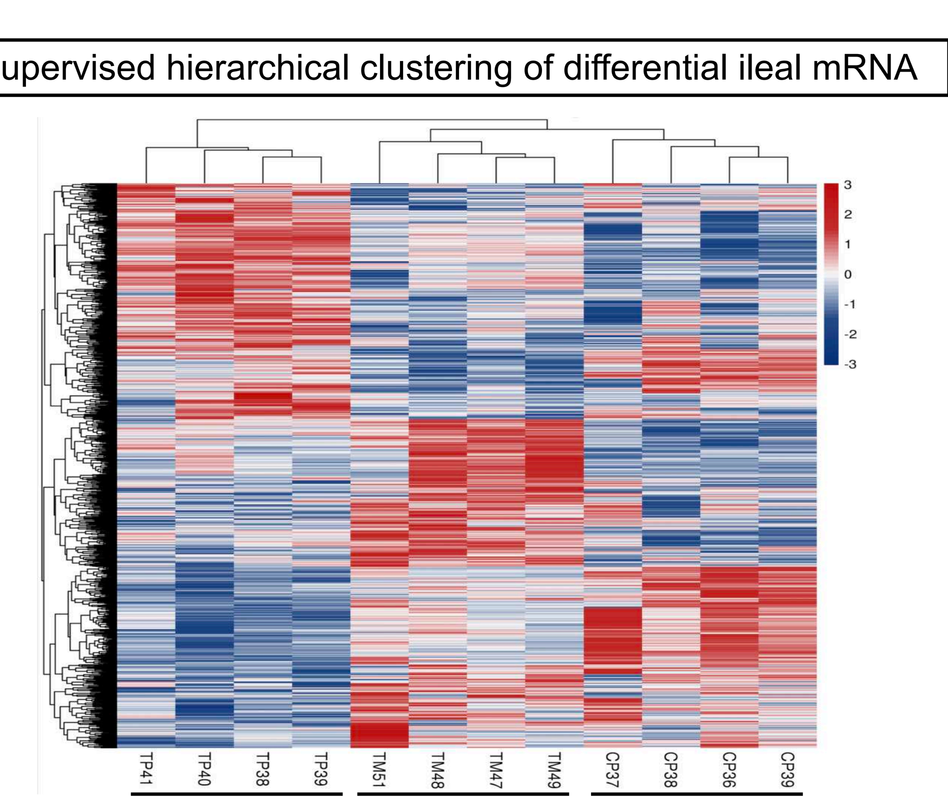
Results

Receiving cecal microbiota transfer from MomD0 increased 1PAT mice survival rate at W30 and tended to rescue the 1PAT accelerated T1D development. It restored the 1PAT-induced alteration of ileal mRNA and miRNA gene expression, which now closely resemble the expression profiles in control mice.

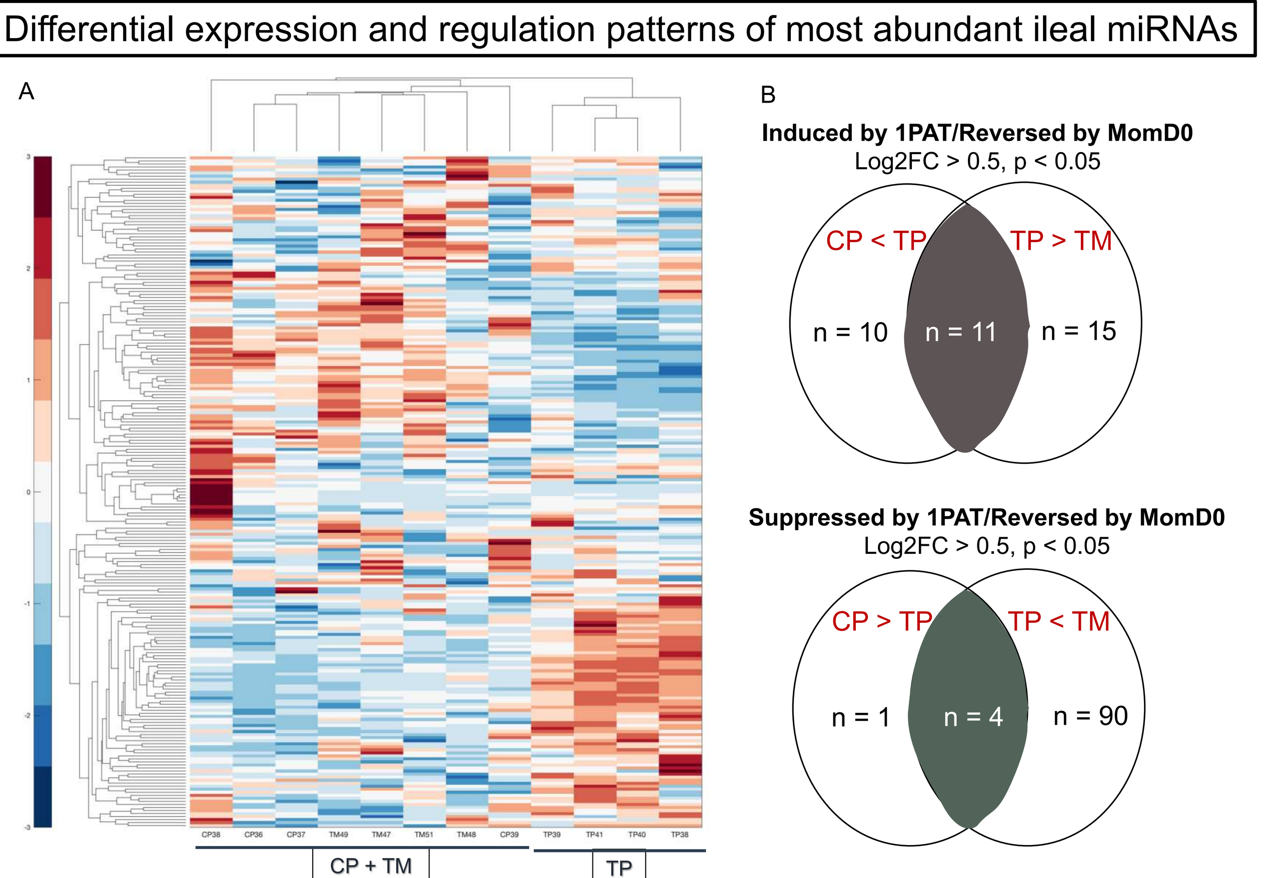


Kaplan-Meier analysis of T1D development in male NOD mice

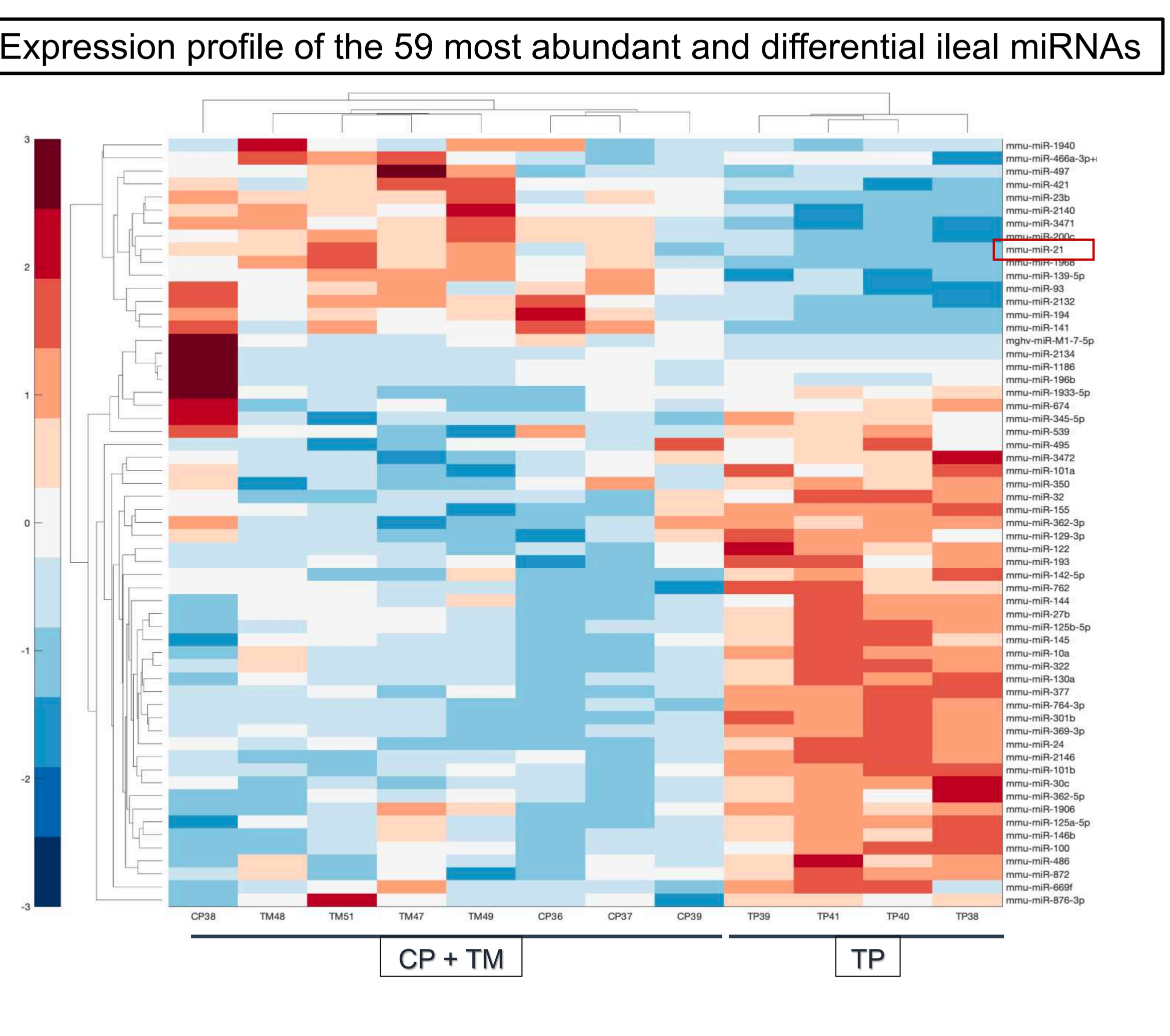
Unsupervised hierarchical clustering of differential ileal mRNA

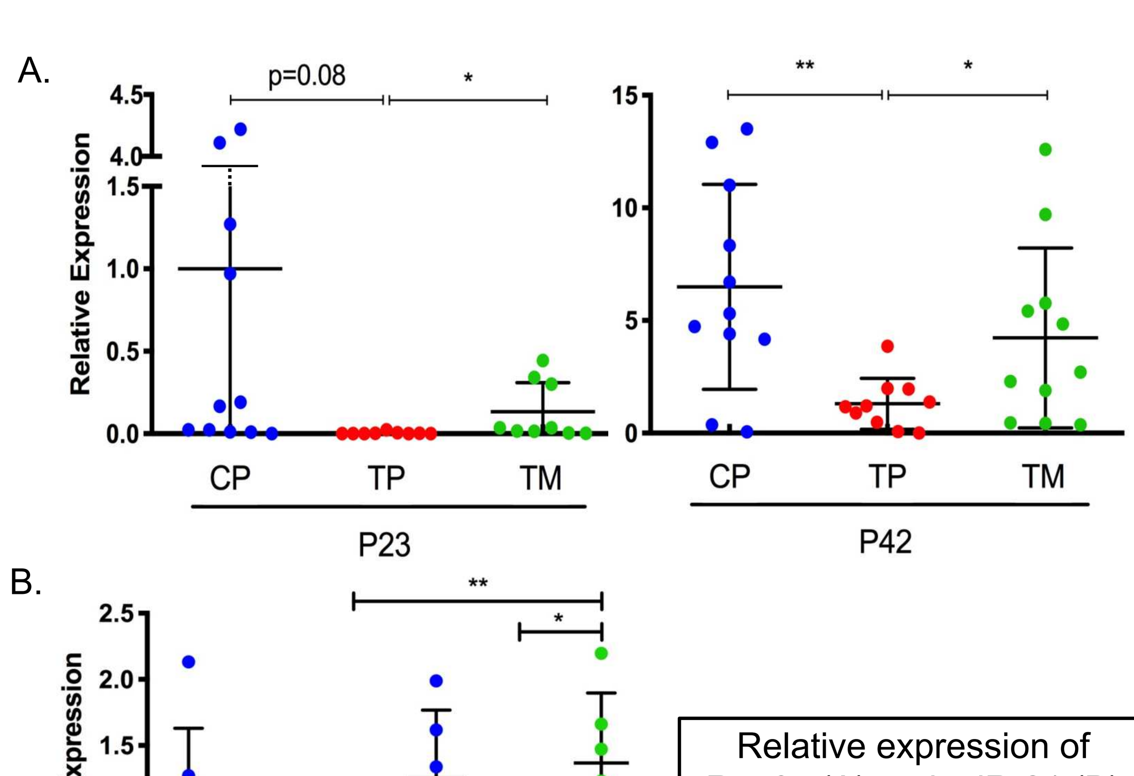


Differential expression and regulation patterns of most abundant ileal miRNAs

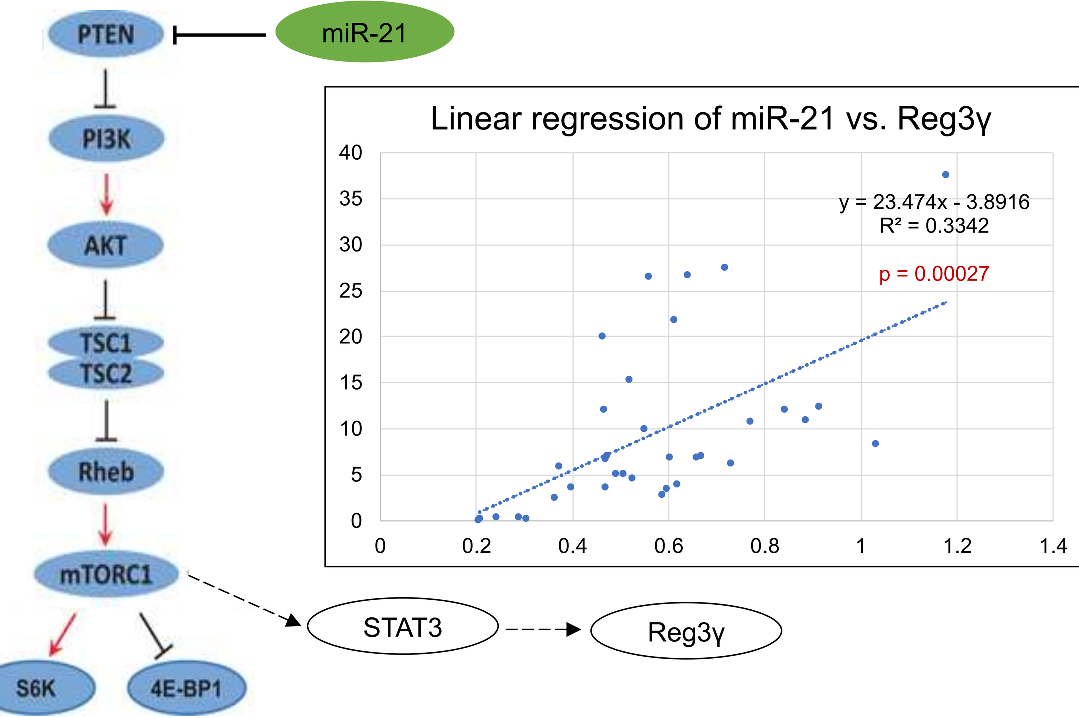


Expression profile of the 59 most abundant and differential ileal miRNAs

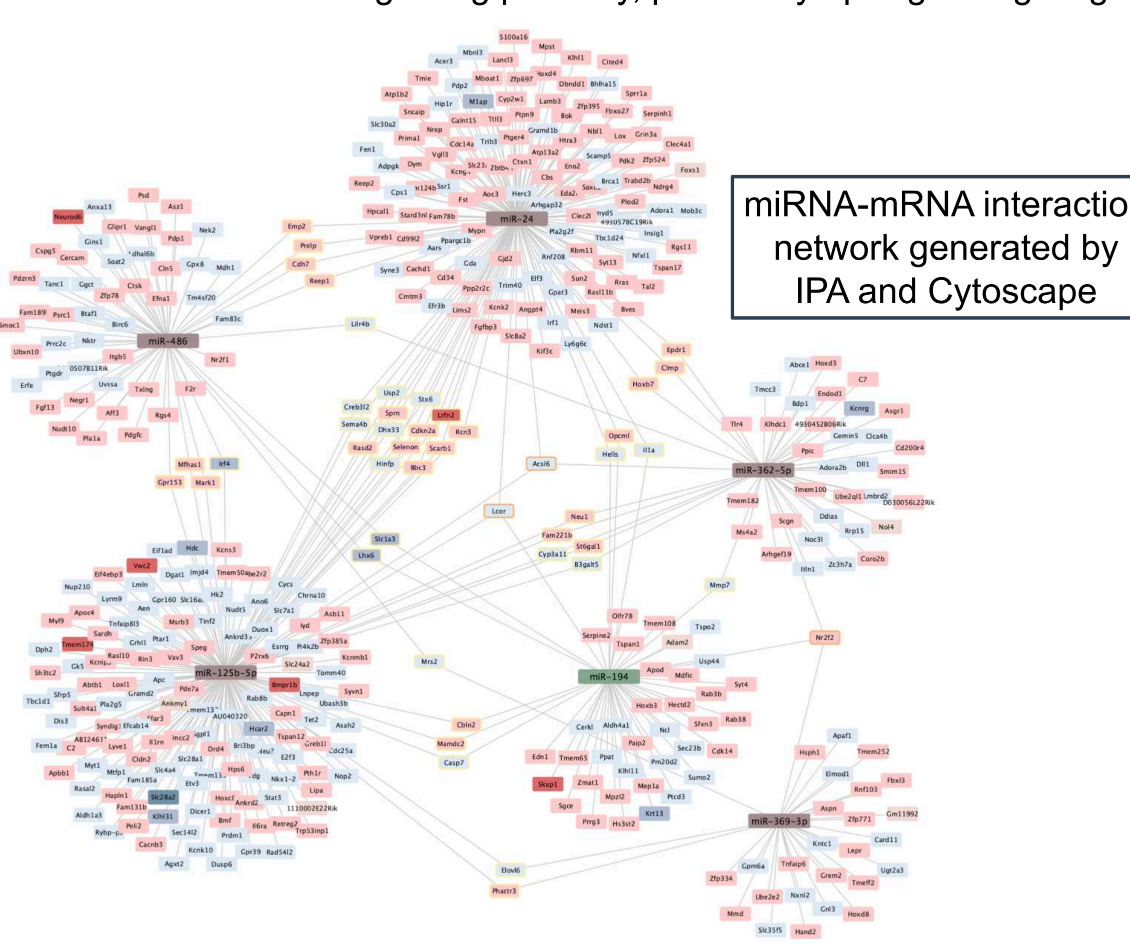




Relative expression of Reg3γ (A) and miR-21 (B)



A linear relationship was observed between miR-21 and Reg3γ. miR-21 activates the mTOR signaling pathway, potentially up-regulating Reg3γ.



miRNA-mRNA interaction network generated by IPA and Cytoscape

Conclusion

Differential analyses of mRNA and miRNA gene expression provide evidence that antibiotic-induced perturbations of the gut microbiota alter ileal miRNA expression which further impact mRNA gene expression.

The close resemblance of miRNA and mRNA gene expression profiles between control mice and mice receiving microbiota transfer suggest that receiving cecal materials from healthy dams early in life can potentially restore 1PAT-accelerated T1D development and reshape immunity.

A key miRNA miR-21 was identified to regulate important intestinal gene Reg3γ via the mTOR signaling pathway. Additional six major miRNAs were found through the miRNA−mRNA interaction network, targeting 432 significantly differential mRNAs.

Further investigations of the identified miRNAs and their targeted mRNA genes will provide new insights into the role of miRNAs in mediating microbiota-host interactions and T1D development.

Acknowledgements

I would like to thank Dr. Martin J. Blaser and Dr. Xue-Song Zhang for their guidance and unwavering support.

Science for a
Healthy Life

Effect of the Moro and Pera orange juice intake on gut microbiota composition in obese individuals

SANTANA[1], A.A.; SANTOS[1,2], K.G.; KOVACS[2], C.; MAGNONI[2], C.D.; THOMATIELLI-SANTOS[3], R.V; HASSIMOTTO[1], N.M.A; LAJOLO[1], F.

[1]Food Research Center (FoRC), School of Pharmaceutical Science, University of São Paulo, São Paulo-SP/Brazil aline_alves@usp.br; [2]Amb. Nutr., Dante Pazzanese Institute of Cardiology, São Paulo, Brazil; [3] Departament of Bioscience, Federal University of São Paulo (UNIFESP), Santos, Brazil.

INTRODUCTION

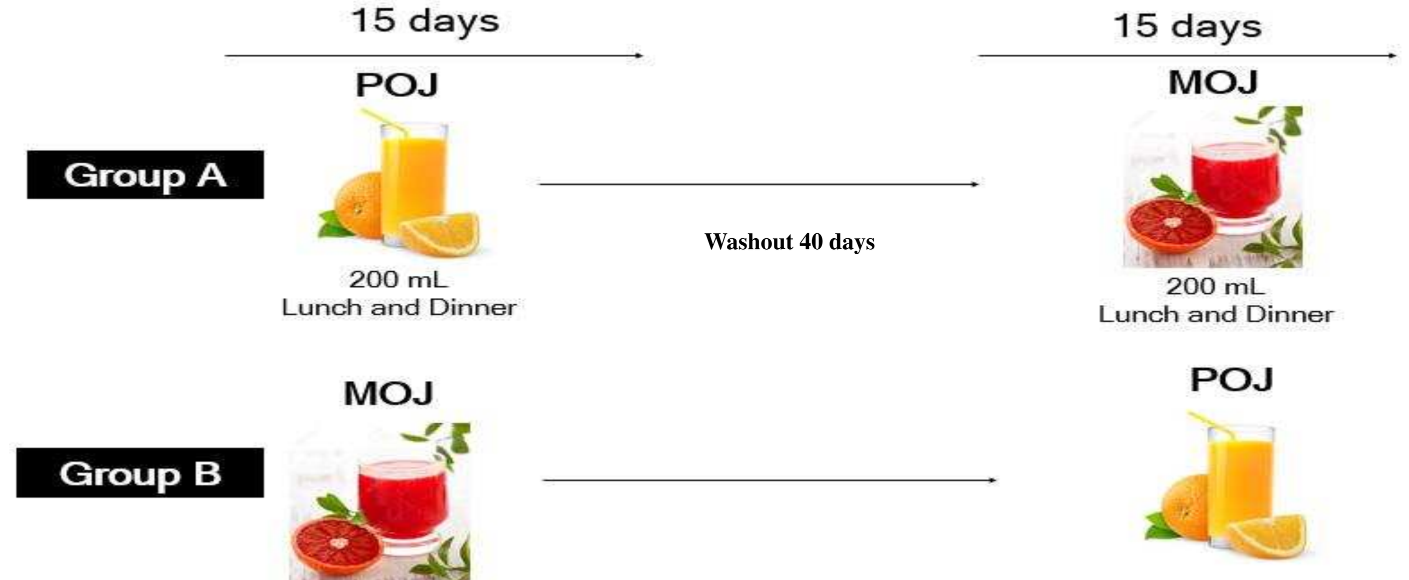
Citrus juices contain greater quantities of bioactive compounds as flavones, flavonols, anthocyanins, and flavanones. The blood oranges are a pigmented sweet orange and the most common types of blood orange are varieties *Moro*, *Tarocco*, and *Sanguinello*. In recent years, there was an increase of interest in blood orange varieties because of their higher content of anthocyanins compared with non-pigmented variants (*Pera*, *Navel*, *Valencia*, and *Ovale*). Many studies have been described blood orange juice, especially variety Moro with antioxidant, antimutagenic, and anti-obesity properties. These are all bioactive compounds in juice, and it is health benefits may be mediated by the synergic effects of its compounds. It has been demonstrated that obese individuals have a disrupted gut microbiota. This altered gut microbiota is related to the metabolic disorders contributing to the development of type 2 diabetes, metabolic syndrome, cardiovascular diseases, and certain cancers.

OBJECTIVE

The objective of this study was to evaluate the effect of orange juice intake and the order of treatment in the modulation of gut microbiota of obese individuals with insulin resistance.

METHODS

A crossover clinical trial was conducted with 22 adults between 40 and 60 years old classified as obese according to BMI and insulin resistance by HOMA-IR. Microbiome analysis: New generation sequencing techniques of 16S rRNA genes. Statistical analysis: data are expressed as means ± SEM. The data were analyzed using a Mann-Whitney t-test for comparison between two groups. A value of $P < 0.05$ was considered statistically significant.



RESULTS AND DISCUSSION

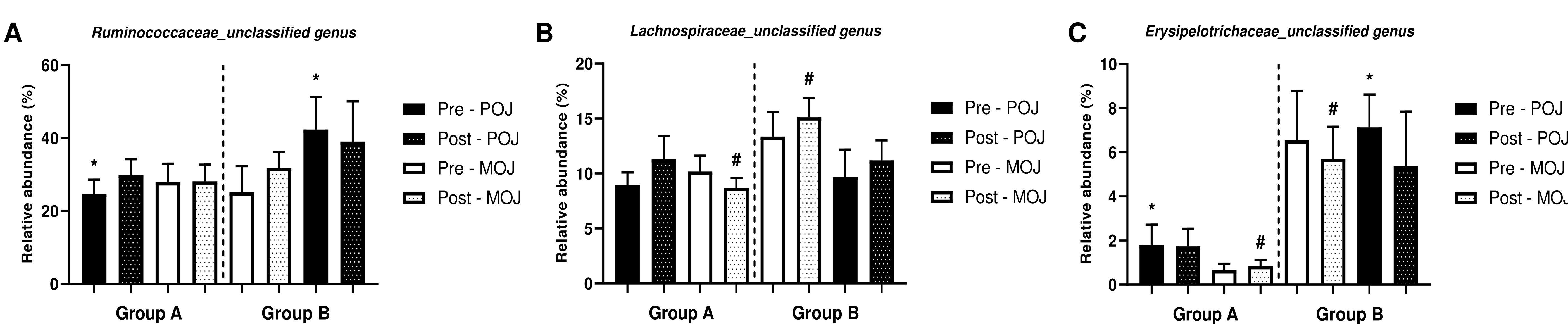
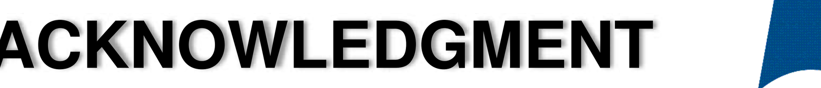


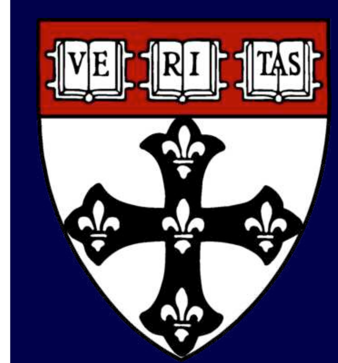
Figure 1. Relative abundance of *Ruminococcaceae* (A), *Lachnospiraceae* (B) and *Erysipelotrichaceae* (C) unclassified genus by time and order the treatment. Significant difference between group A and B, Pre-POJ (*); post-MOJ (#).

Our results suggests that phenolic compounds found on MOJ could directly stimulate the growth of a specific bacteria. This difference can be explained by the phenolic compounds found in both juice types associated with synergistic effects that are dependents on the order of the treatment. The *Ruminoccocaceae* and *Lachnospiraceae* families have been linked to the production of short-chain fat acid, an important energy source for colonic epithelial. In addition, the *Lachnospiraceae* family has been associated with improved insulin resistance, lipid metabolism, reduction of body weight, and antioxidant effects. *Erysipelotrichaceae*, as well as *Lachnospiraceae* family, were associated with inflammation reduction and improved insulin sensitivity in mice.

CONCLUSION

Our data suggest that MOJ followed by POJ intake may improve the dysbiosis associated with obesity through the stimulus of bacterial growth - especially the bacteria that: (a) metabolize bioactive compounds and (b) enhance the synthesis of beneficial metabolic products protecting gut homeostasis.





Metabolite-sensing receptor Ffar2 regulates colonic group 3 innate lymphoid cells and gut homeostasis

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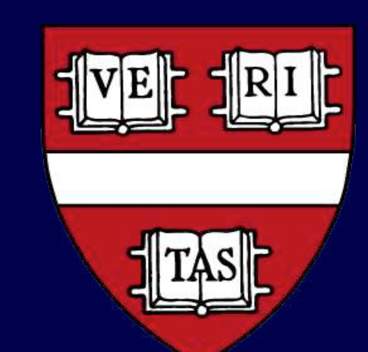
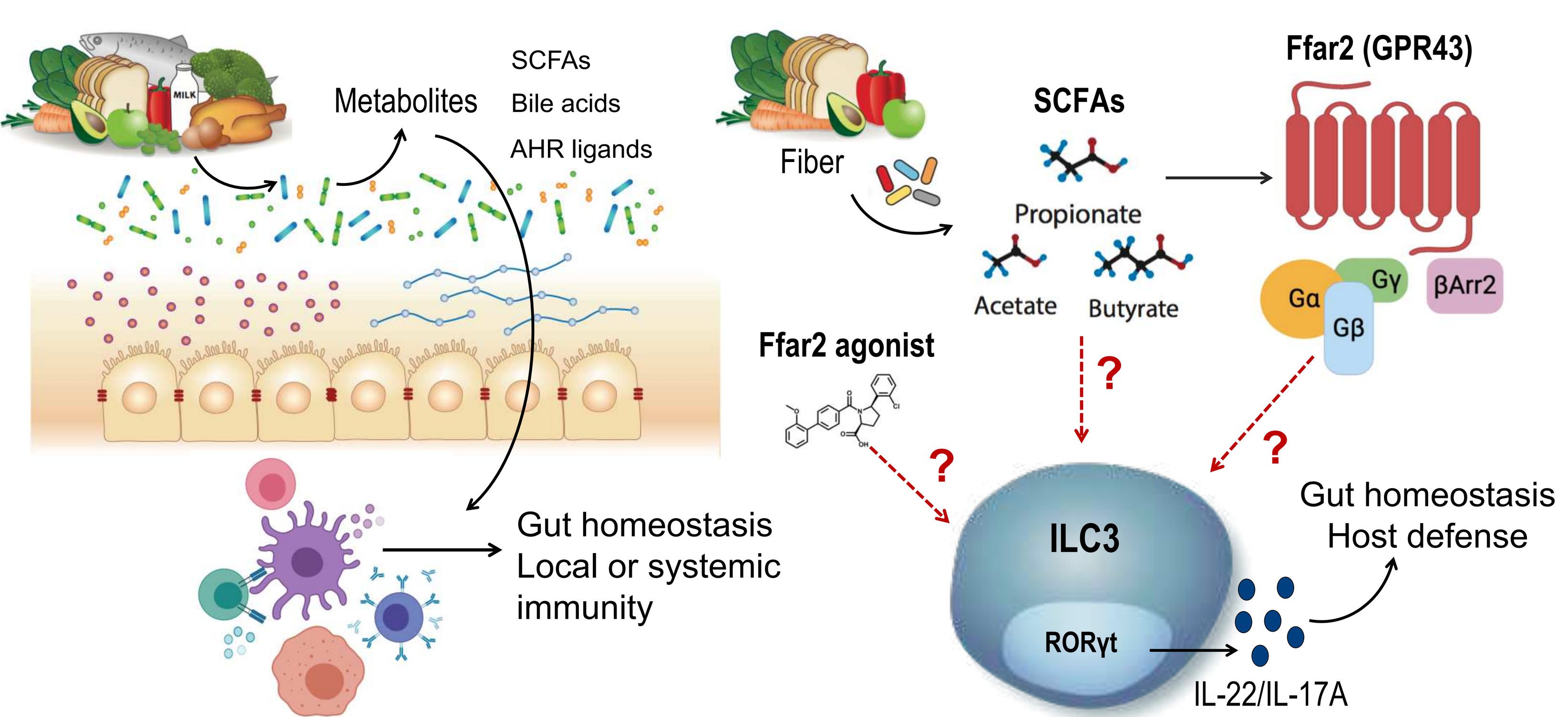


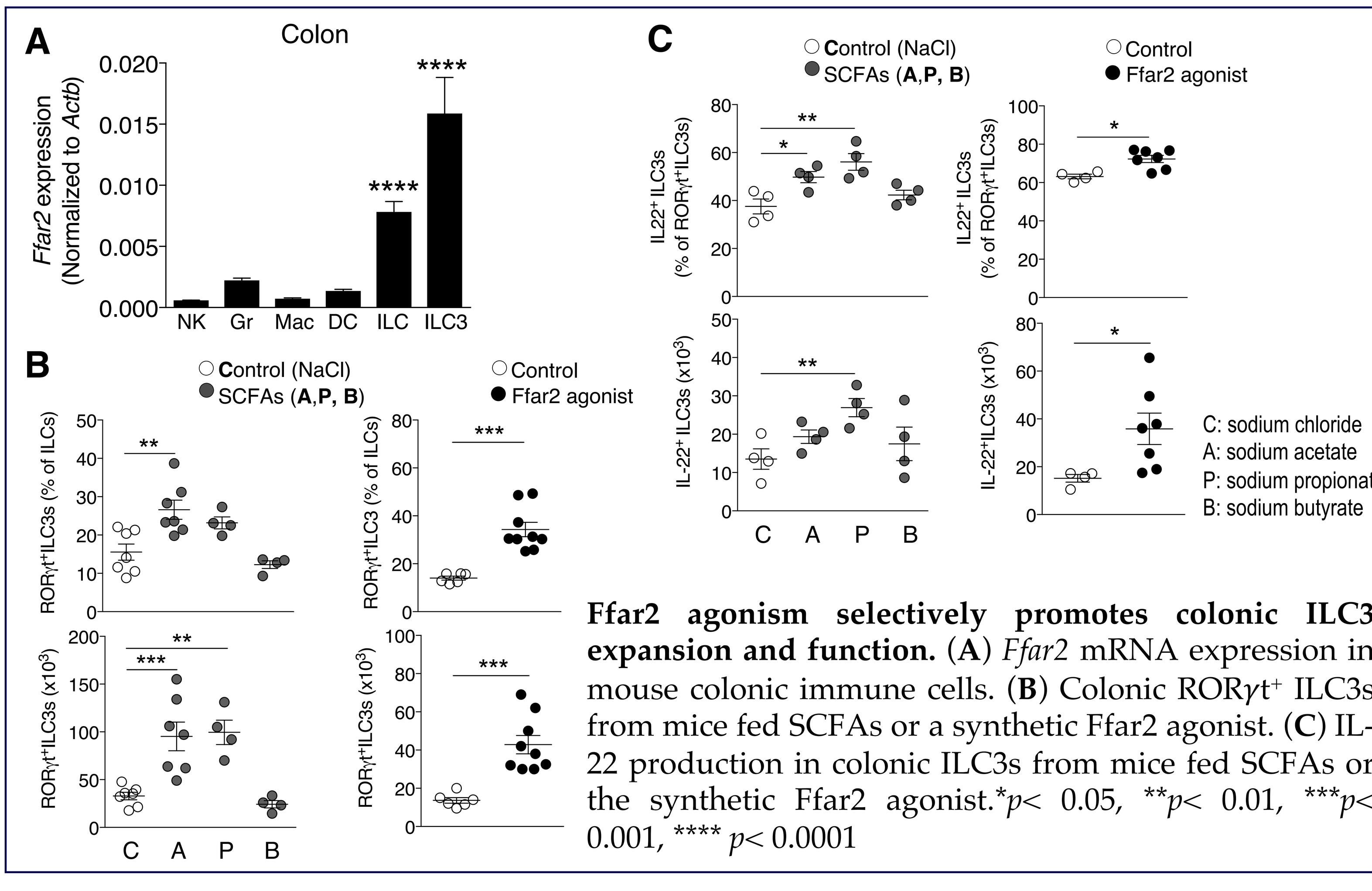
Abstract

Group 3 innate lymphoid cells (ILC3s) sense environmental signals that are critical for gut homeostasis and host defense. However, the metabolite-sensing G-protein-coupled receptors that regulate colonic ILC3s remain poorly understood. We found that colonic ILC3s expressed Ffar2, a microbial metabolite-sensing receptor, and that Ffar2 agonism promoted ILC3 expansion and function. Deficiency of Ffar2 in ILC3s decreased their *in situ* proliferation and ILC3-derived interleukin-22 (IL-22) production. This led to impaired gut epithelial function characterized by altered mucus-associated proteins and anti-microbial peptides and increased susceptibility to colonic injury and bacterial infection. Ffar2 increased IL-22⁺CCR6⁺ILC3s and influenced ILC3 abundance in colonic lymphoid tissues. Ffar2 agonism differentially activated AKT or ERK signaling and increased ILC3-derived IL-22 via an AKT and STAT3 axis. Our findings suggest that Ffar2 regulates colonic ILC3 proliferation and function, and they identify an ILC3-receptor signaling pathway modulating gut homeostasis and pathogen defense.

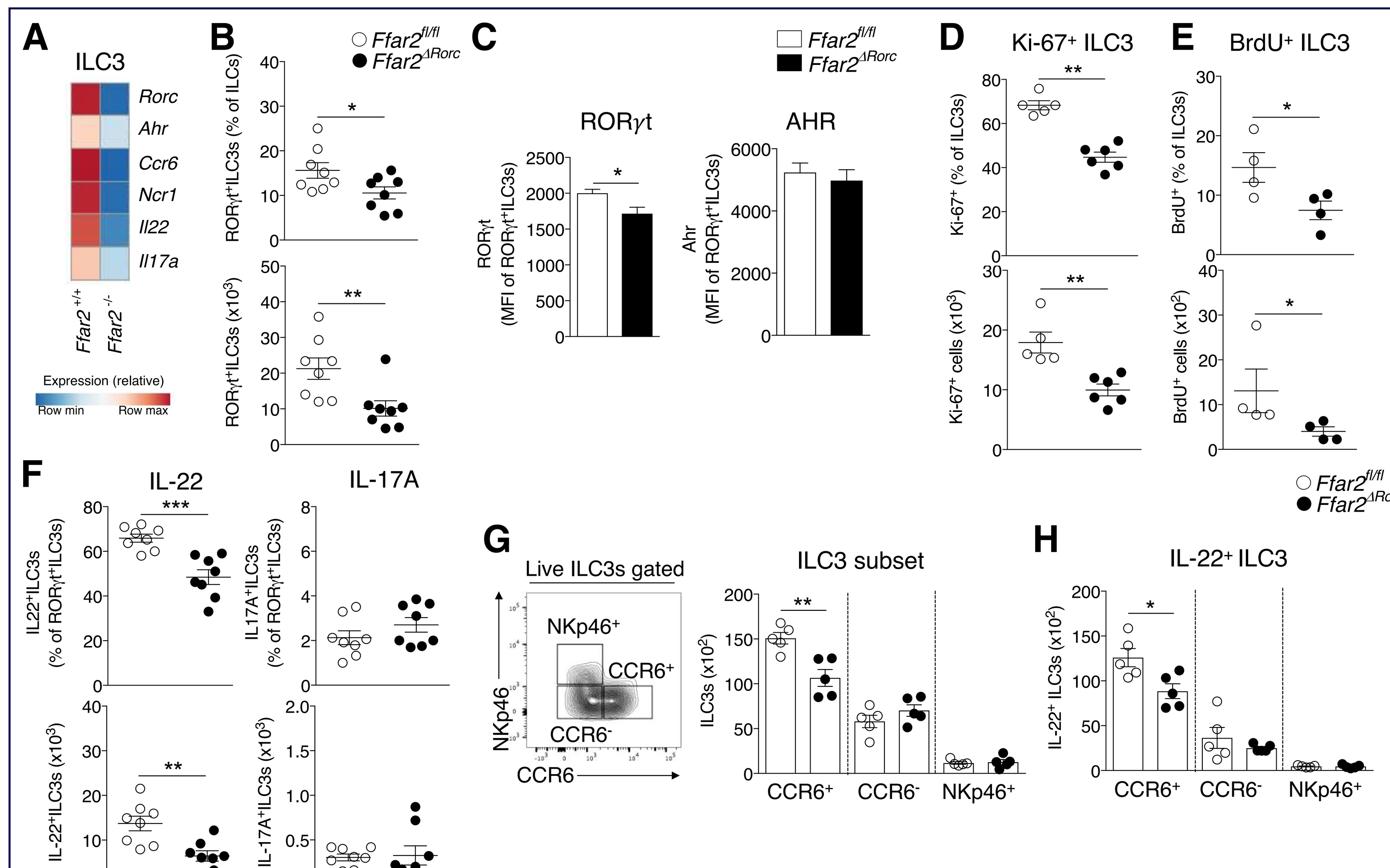
Introduction



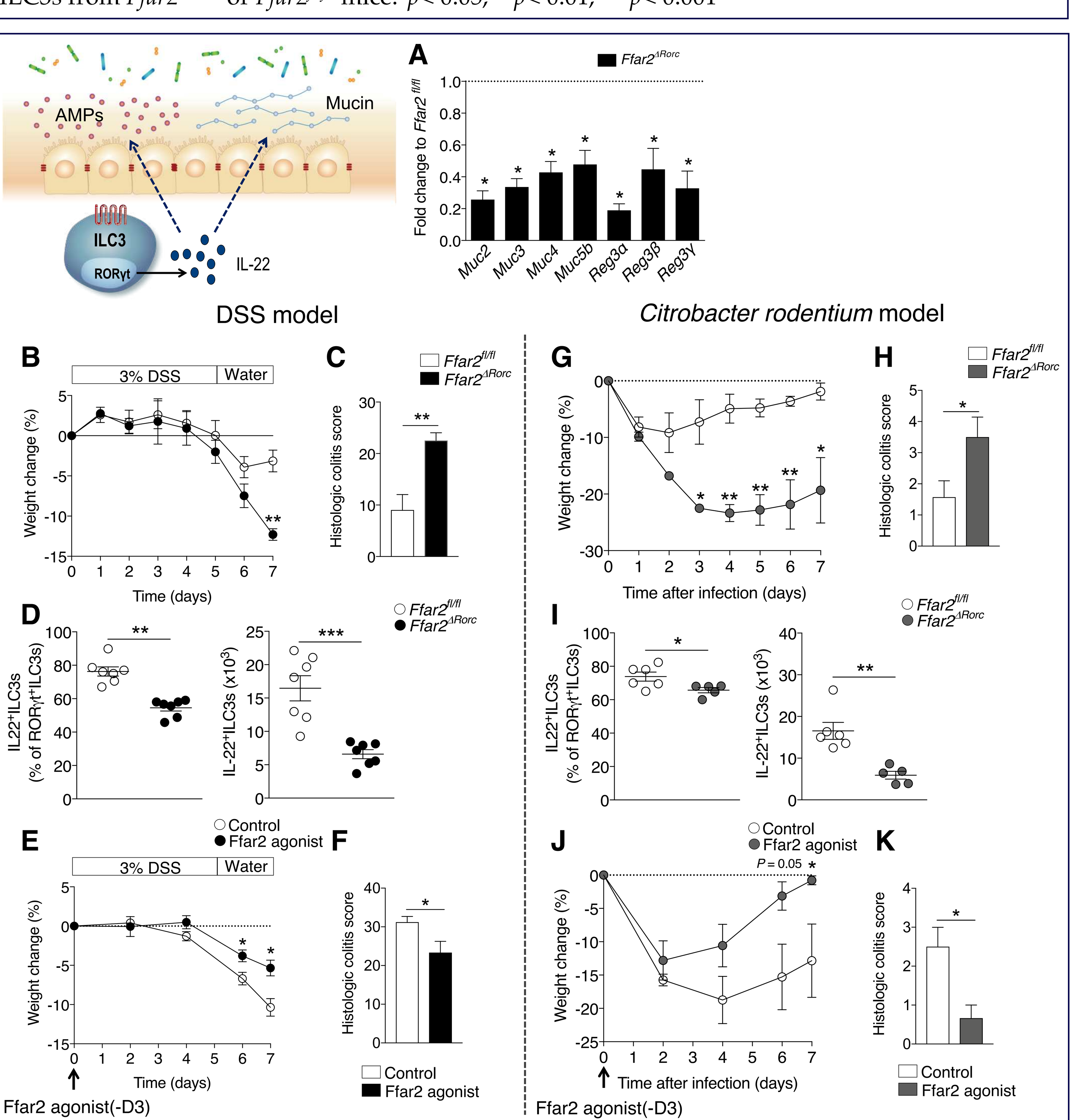
Results



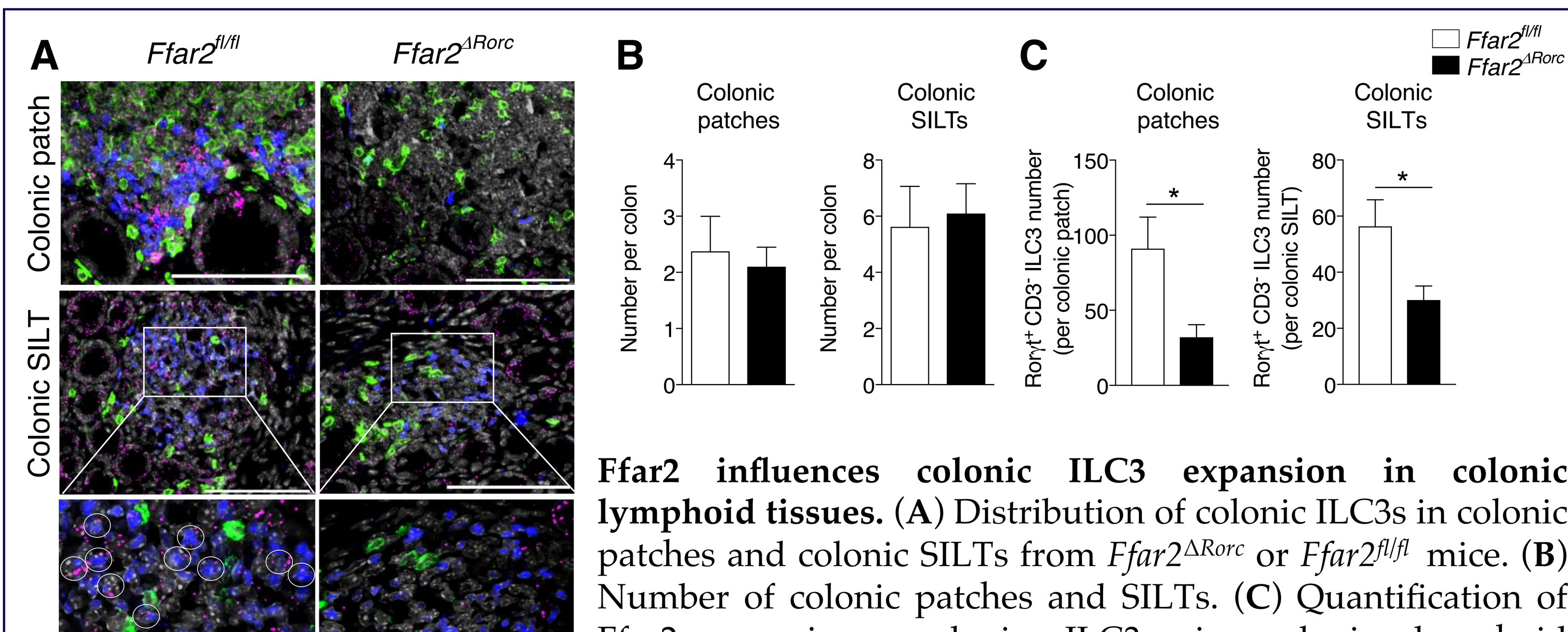
Ffar2 agonism selectively promotes colonic ILC3 expansion and function. (A) *Ffar2* mRNA expression in mouse colonic immune cells. (B) Colonic RORγt⁺ ILC3s from mice fed SCFAs or a synthetic Ffar2 agonist. (C) IL-22 production in colonic ILC3s from mice fed SCFAs or the synthetic Ffar2 agonist.*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, **** $p < 0.0001$



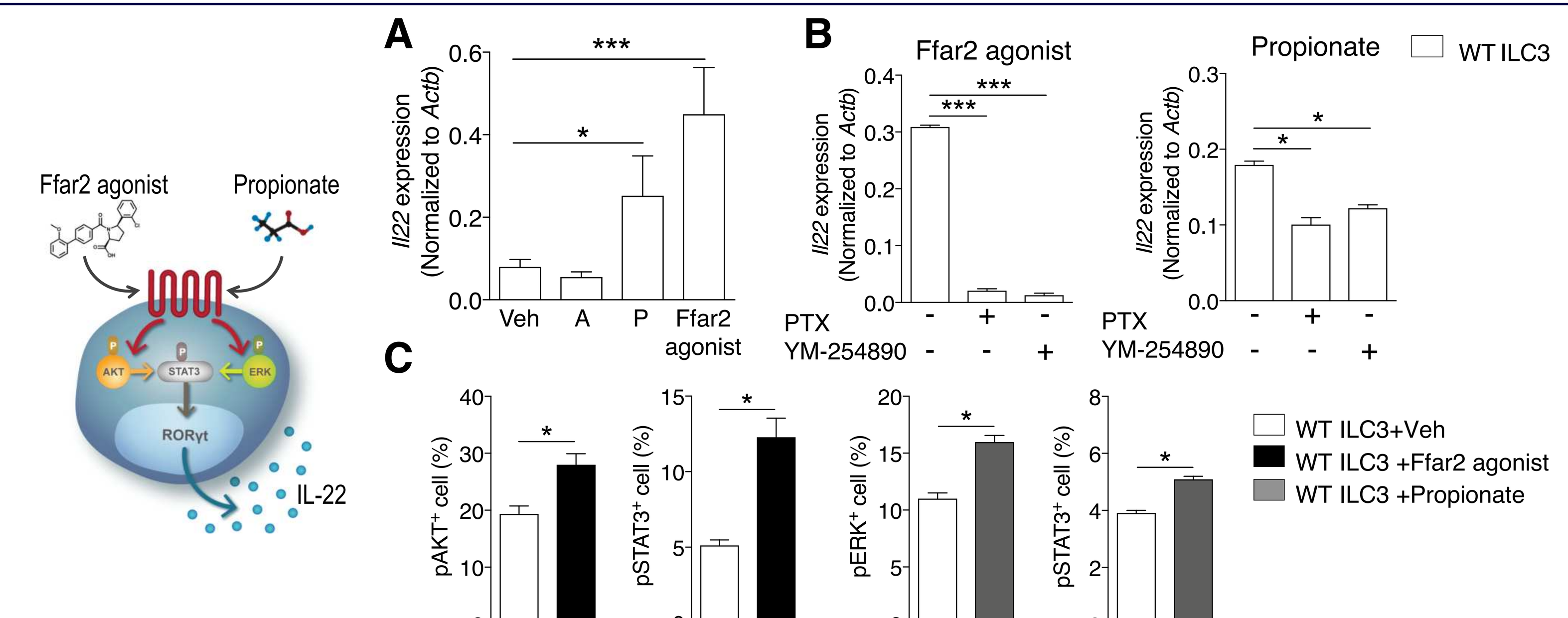
Ffar2 regulates colonic ILC3 proliferation and ILC3-derived IL-22. (A) Heatmap represents relative expression of select ILC3 signature genes. (B) Colonic RORγt⁺ ILC3s from *Rorc-cre Ffar2fl/fl* ($Ffar2^{\Delta Rorc}$) or control $Ffar2^{fl/fl}$ mice. (C) RORγt and AHR expression in colonic ILC3s from $Ffar2^{\Delta Rorc}$ or $Ffar2^{fl/fl}$ mice. (D) Ki-67 expression in colonic ILC3s from $Ffar2^{\Delta Rorc}$ or $Ffar2^{fl/fl}$ mice. (E) BrdU⁺ colonic ILC3s from $Ffar2^{\Delta Rorc}$ or $Ffar2^{fl/fl}$ mice. (F) IL-22⁺ and IL-17A⁺ colonic ILC3s from $Ffar2^{\Delta Rorc}$ or $Ffar2^{fl/fl}$ mice. (G) Colonic ILC3 subsets in $Ffar2^{\Delta Rorc}$ or $Ffar2^{fl/fl}$ mice. (H) IL-22⁺ CCR6⁺ ILC3s from $Ffar2^{\Delta Rorc}$ or $Ffar2^{fl/fl}$ mice.*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$



Ffar2-expressing ILC3s contribute to protection against colonic inflammation and bacterial infection. (A) Gene expression in epithelial cells from $Ffar2^{\Delta Rorc}$ or $Ffar2^{fl/fl}$ mice. (B-F) DSS model in $Ffar2^{\Delta Rorc}$ or $Ffar2^{fl/fl}$ mice. (B) Body weight changes. (C) Colitis score. (D) Colonic IL-22⁺ ILC3s. (E-F) DSS model in WT mice treated with Ffar2 agonist. (E) Body weight changes. (F) Colitis score. (G-K) *Citrobacter rodentium* model in $Ffar2^{\Delta Rorc}$ or $Ffar2^{fl/fl}$ mice. (G) Body weight changes. (H) Colitis score. (I) Colonic IL-22⁺ ILC3s. (J-K) *Citrobacter rodentium* model in WT mice treated with Ffar2 agonist. (J) Body weight changes. (K) Colitis score. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

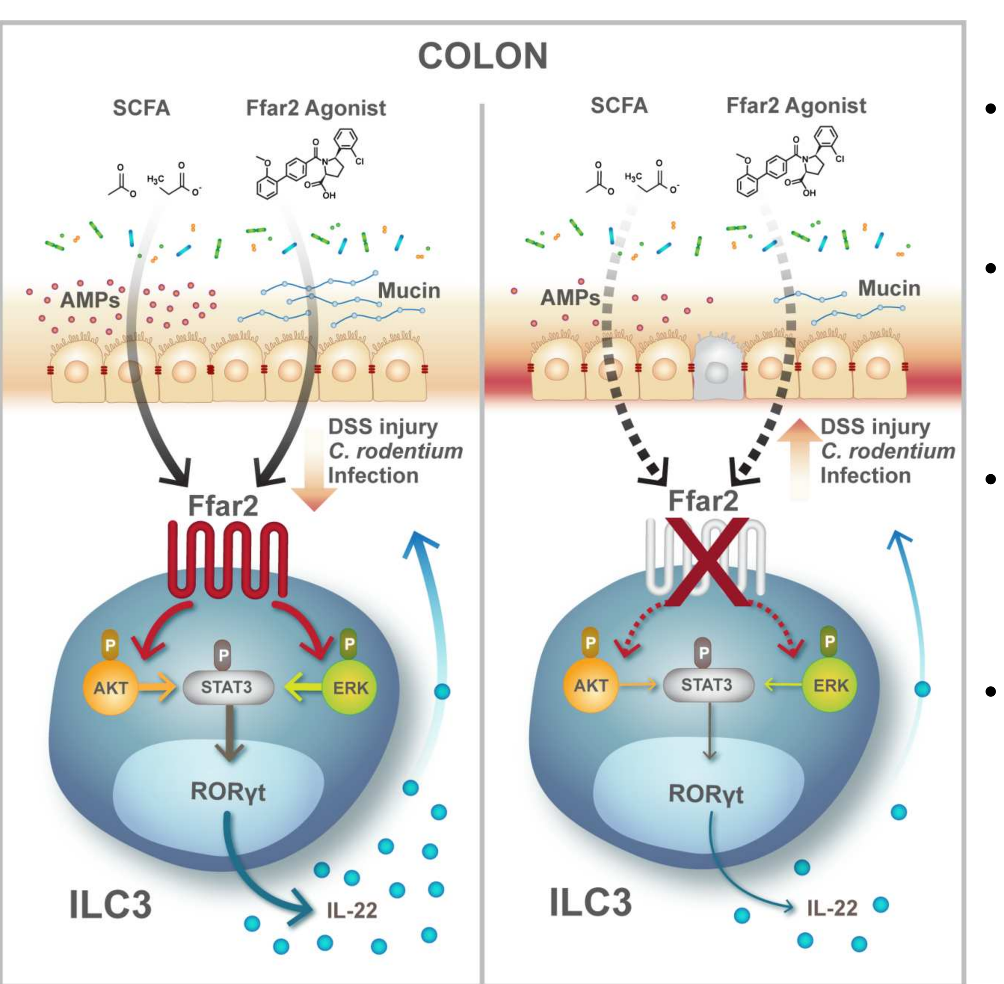


Ffar2 influences colonic ILC3 expansion in colonic lymphoid tissues. (A) Distribution of colonic ILC3s in colonic patches and colonic SILTs from $Ffar2^{\Delta Rorc}$ or $Ffar2^{fl/fl}$ mice. (B) Number of colonic patches and SILTs. (C) Quantification of Ffar2-expressing colonic ILC3s in colonic lymphoid tissues.*$p < 0.05$, **$p < 0.01$



Ffar2 regulates colonic ILC3-derived IL-22 via AKT and STAT3 activation or partially ERK and STAT3 activation. (A) *Il22* mRNA expression in sorted ILC3s cultured with acetate, propionate or Ffar2 agonist. (B) *Il22* mRNA expression in sorted ILC3s cultured with $G_{i/o}$ inhibitor (PTX) or G_q inhibitor (YM-254890) under the Ffar2 agonist or propionate activation. (C) AKT, ERK, or STAT3 activation in sorted ILC3s cultured with the Ffar2 agonist or propionate.*$p < 0.05$, ***$p < 0.001$

Conclusion



- Ffar2 agonism regulates colonic ILC3 expansion and function (IL-22 production)
- Ffar2 regulates colonic ILC3 proliferation and ILC3-derived IL-22 in a cell-intrinsic manner.
- Ffar2-deficient ILC3s enhance susceptibility to colonic inflammation and infection.
- Ffar2 agonism controls ILC3-derived IL-22 via AKT and STAT3 activation, or partially ERK and STAT3 activation.

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