BioSciences

Genetically diverse mouse population-based approaches to advance personalized medicine

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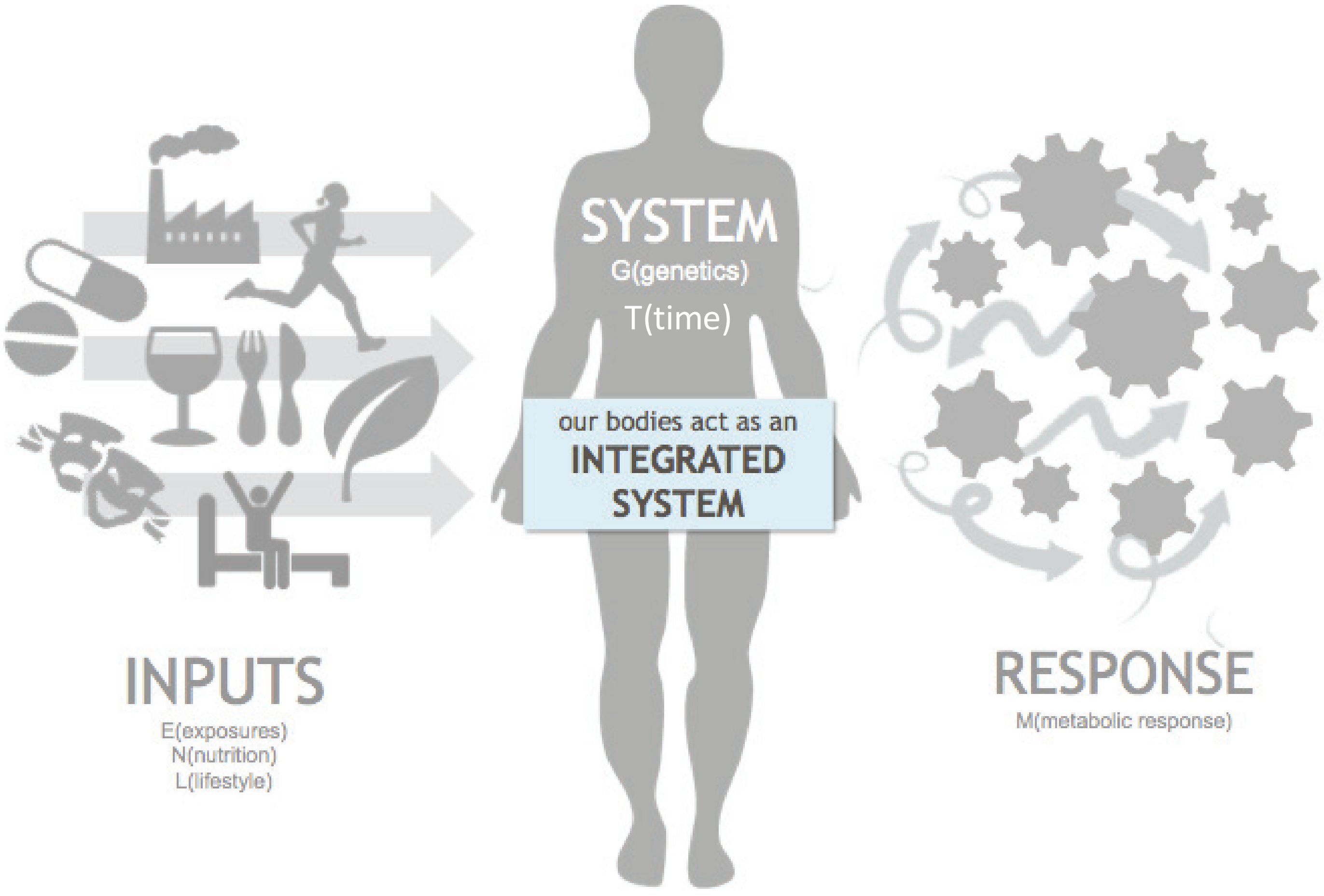
Outline

- Introduction to population-based mouse models
- Spontaneous cancer development
- Influence of early life environment and genetics on gut microbiome
- Influence of microbiome on memory and anxiety

Interaction of Genetics and Environment determine the response to external stimuli



INPUTS
E(exposures)
N(nutrition)
L(lifestyle)

SYSTEM
G(genetics)
T(time)

our bodies act as an
INTEGRATED SYSTEM

RESPONSE
M(metabolic response)

$$f_G(E,N,L)= M$$

Model systems are needed to elucidate causal relationships between exposures and phenotypes

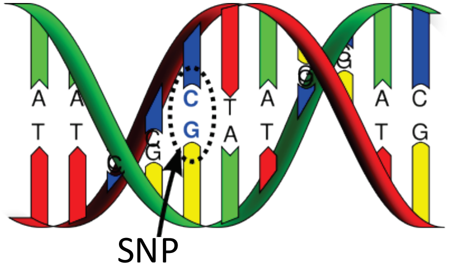
Model systems allow for:

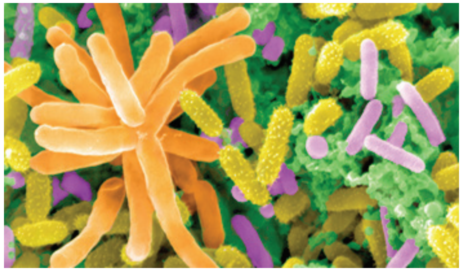
- precise exposures
- well-designed populations for a specific question
- standardized husbandry to control environmental components of risk
- comprehensive analysis of phenotypes

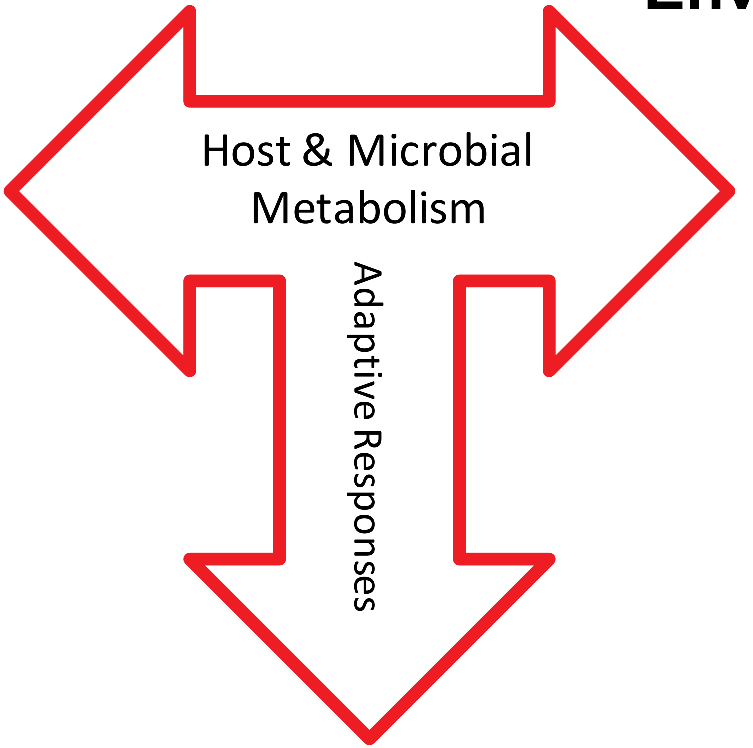
Must incorporate spectrum of genetic & epigenetic variation

Contextualizing exposure models

Host & Microbial Interactions



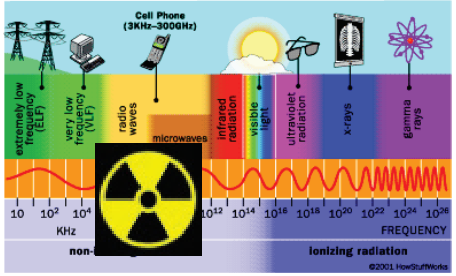




Host & Microbial Metabolism

Adaptive Responses

Environmental factors



Factors that influence individual robustness and susceptibility

Similarities between mouse and human:

Mouse's life cycle is man's life cycle in miniature

1. **Mice and humans share ~98% of their genes**
2. **Mice and humans share common inherited diseases**
3. **Similarity in microbial communities**

Single strain studies can be poor predictors of human responses

All models are wrong, but some can be useful.

Use the right model for a specific question.

Optimal Characteristics of a Model for *in vivo* Systems Biology

Genome-wide variation so that all components of the system are interrogated

Randomization of genetic variation so causative relationships can be identified

Large to power detection of modest to weak interactions

Infinitely reproducible to support data integration and reproduction

The Collaborative Cross mouse population based model system

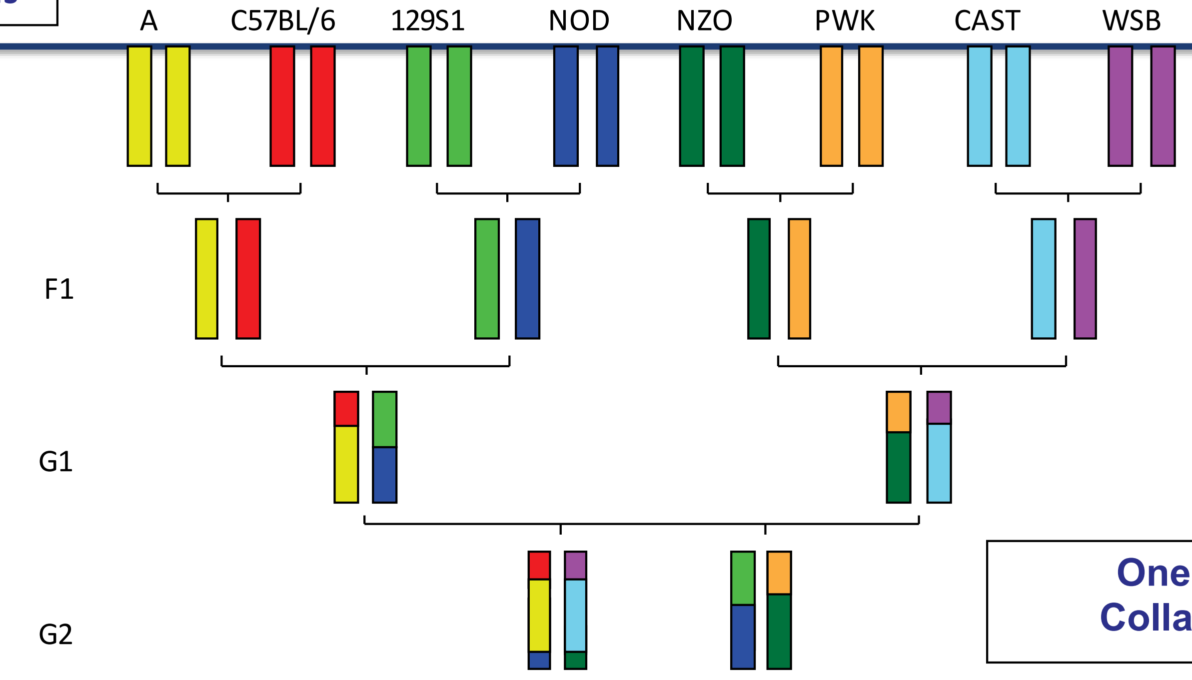
Collaborative Cross mice

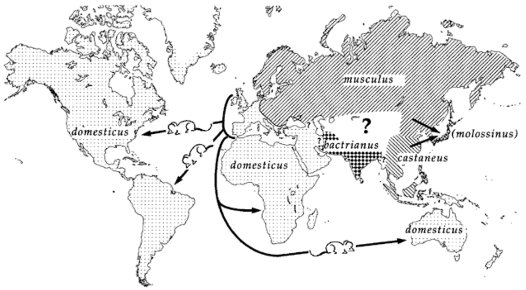
≈

A mouse resource that mimics the variable genetic backgrounds found in the human population.

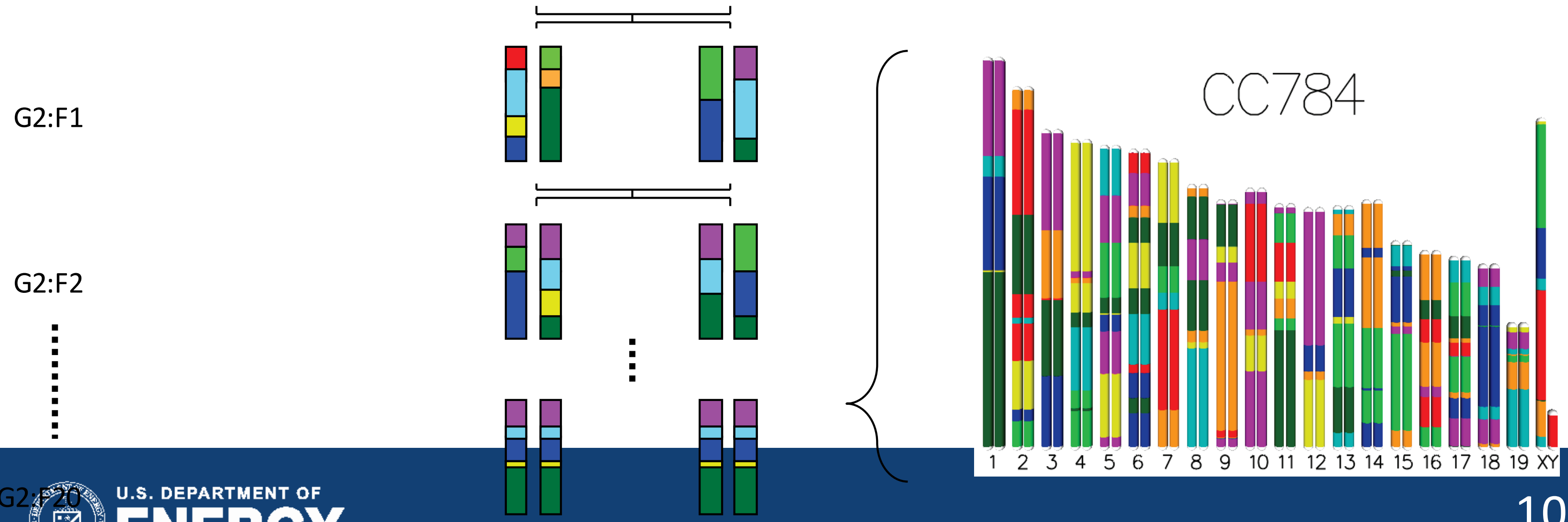
Parental Inbred Strains

One Representative Chromosome

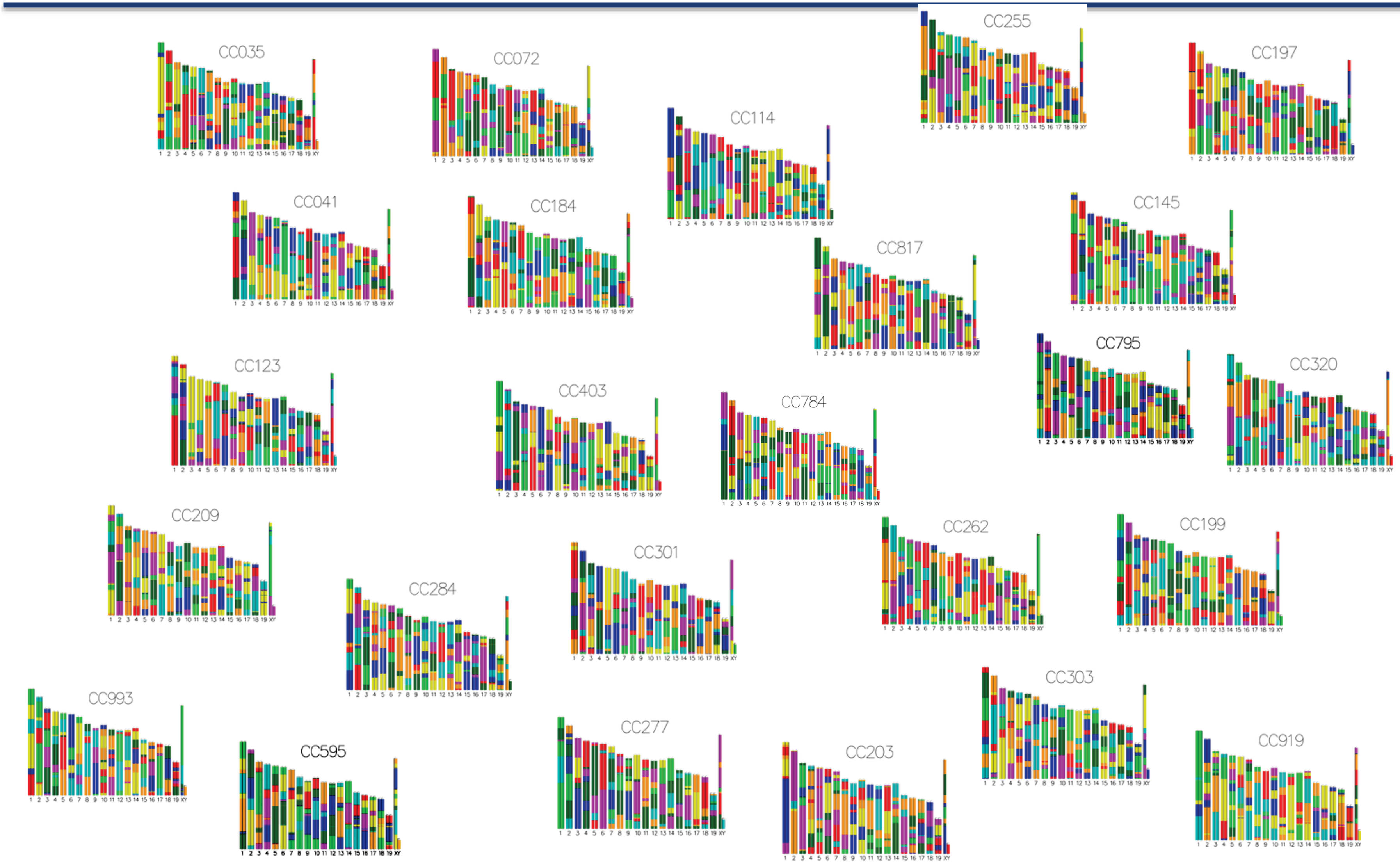




One of 1,000 Independent Collaborative Cross Strains



Power in Numbers!!



Genome Wide Diversity in Collaborative Cross Founders

A/J C57BL6/J 129S1/SvIm NOD/Lt NZO/HlLt PWK/PhJ CAST/EiJ WSB/EiJ

Captures 90% of variation

The variation is randomly distributed across the genome (there are no blind spots)

> 2X the amount of variation present in humans

Mammalian Genome, 2007

Collaborative Cross is an Optimal Model for *in vivo* Systems Biology

Predictive?

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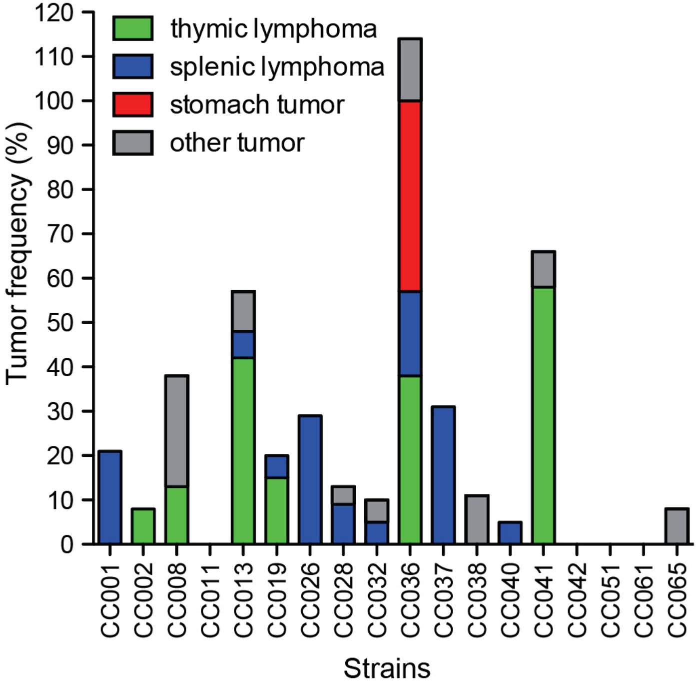
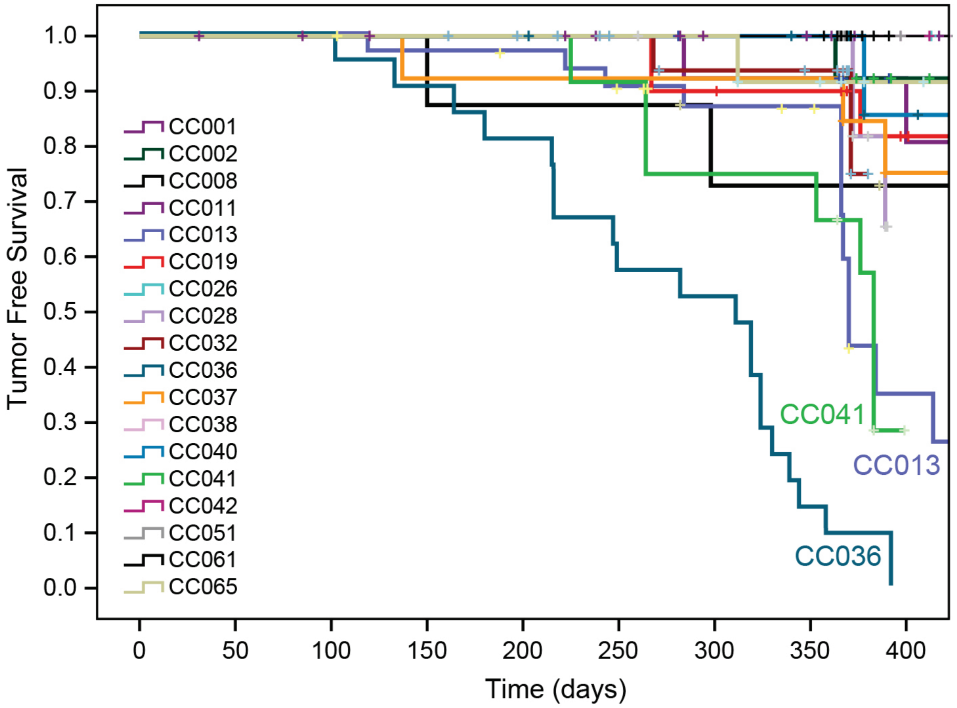
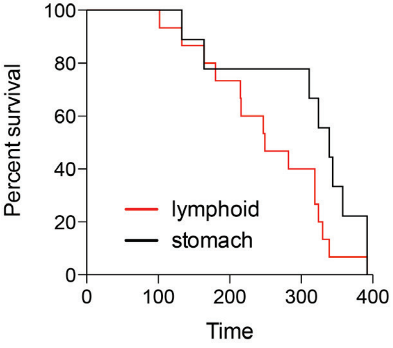
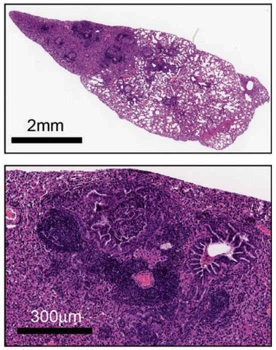
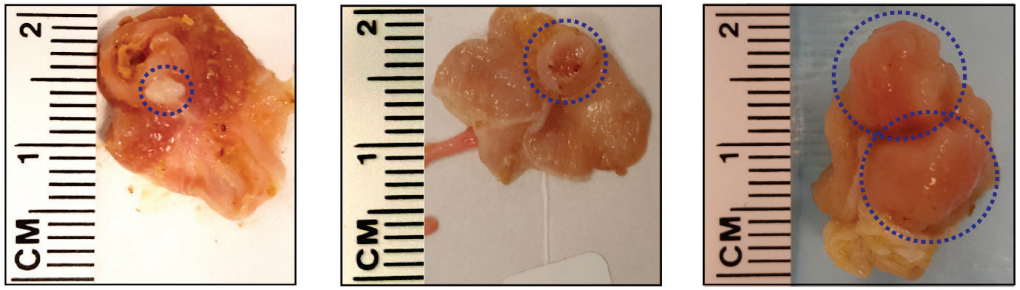
Huge variation in tumor incidence across Collaborative Cross mice





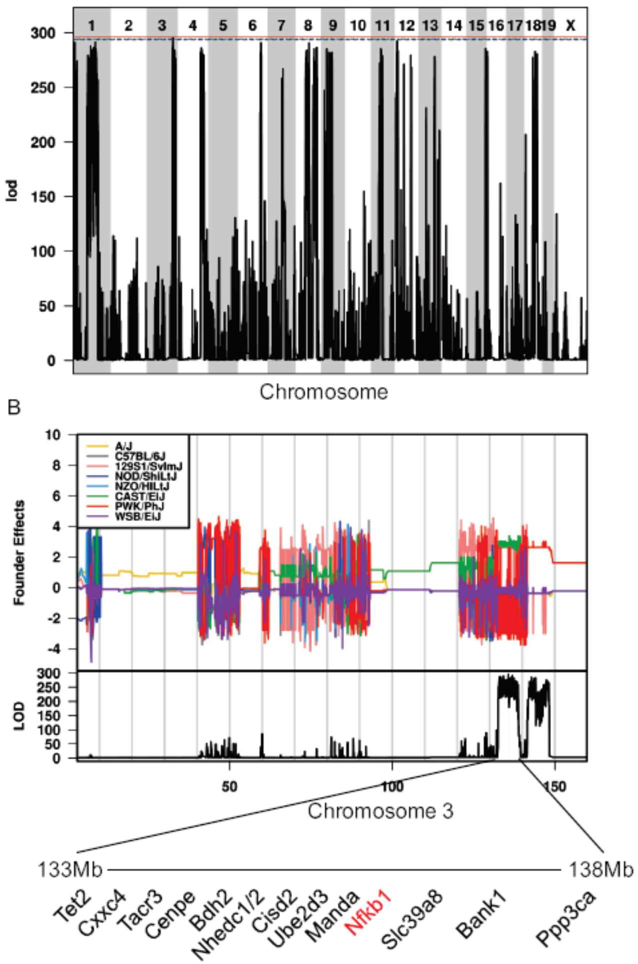




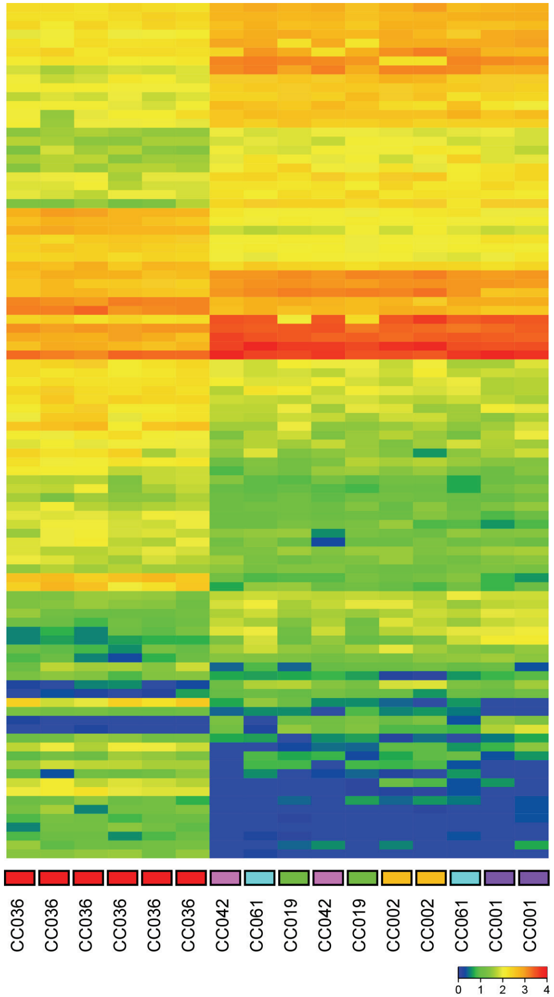


Wang et al. Gut 2019

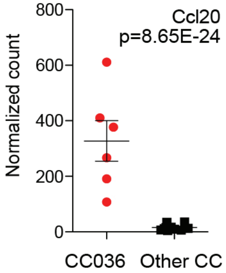
Identification of genetic loci associated with gastric cancer susceptibility in CC036.

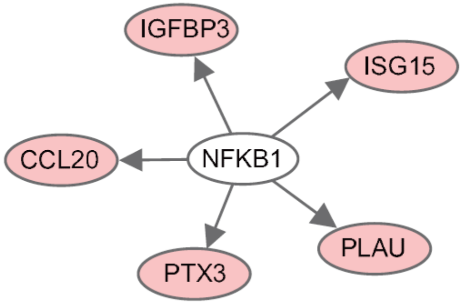


RNA-sequencing of stomach samples to identify the underlying mechanism of tumor susceptibility

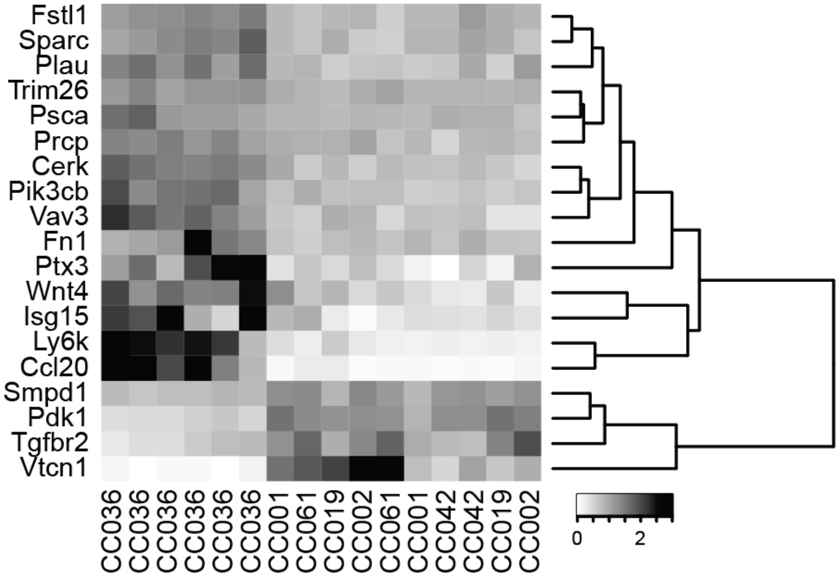


Upregulation of CCL20 and Recruitment of CCR6+ Gastric Infiltrating Lymphocytes in Helicobacter pylori Gastritis

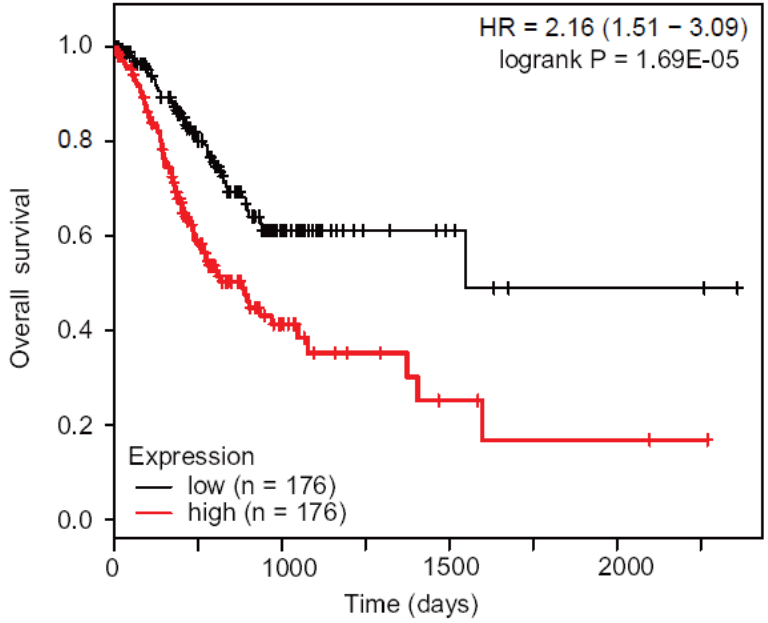


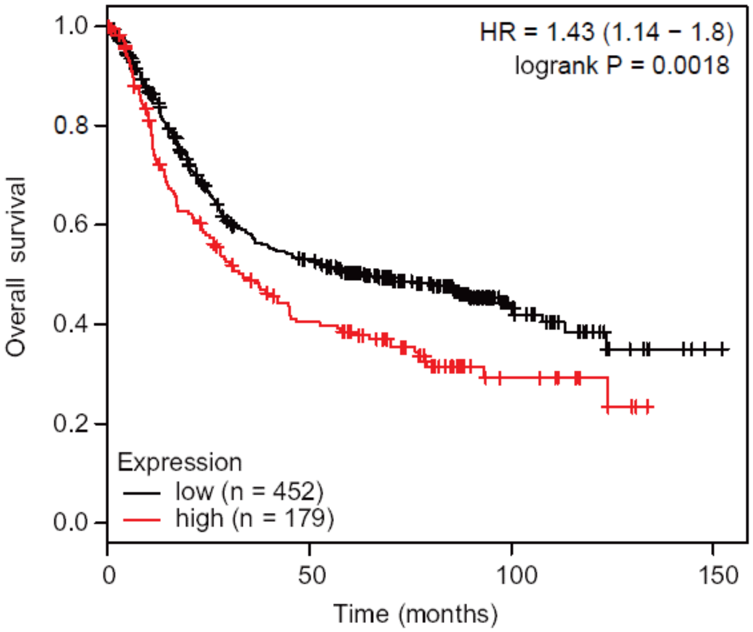


Enrichment of Inflammatory Response genes

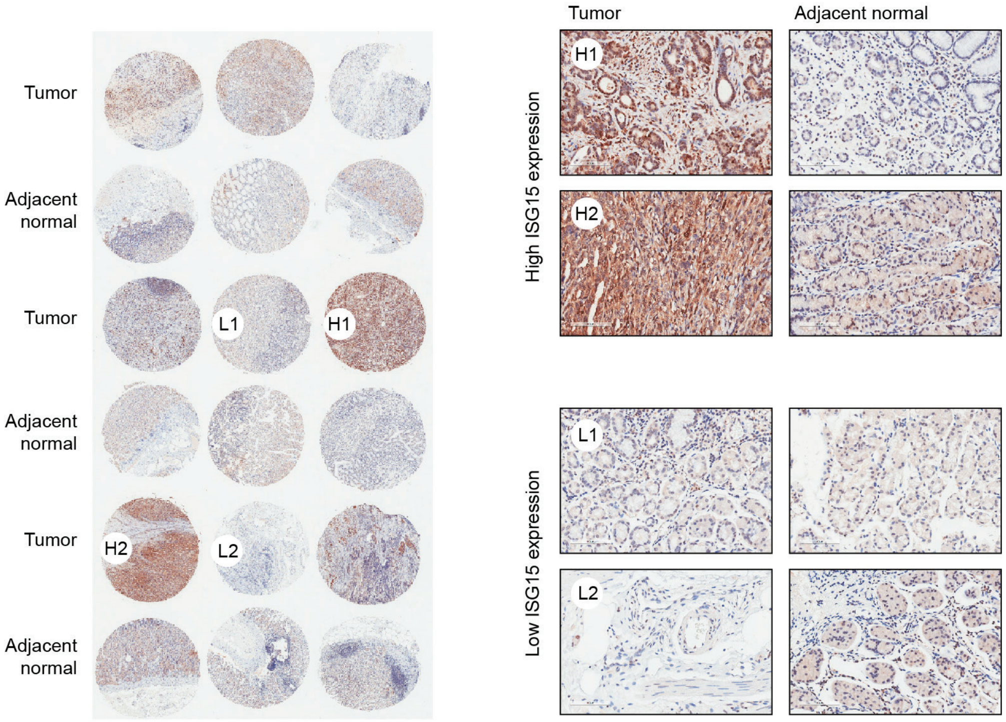


Inflammatory gene signature predicts overall survival in stomach cancer patients.



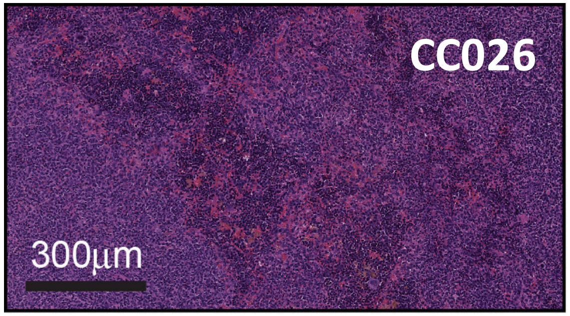


ISG15 protein expression is increased in stomach adenocarcinoma and associated with poor prognosis.

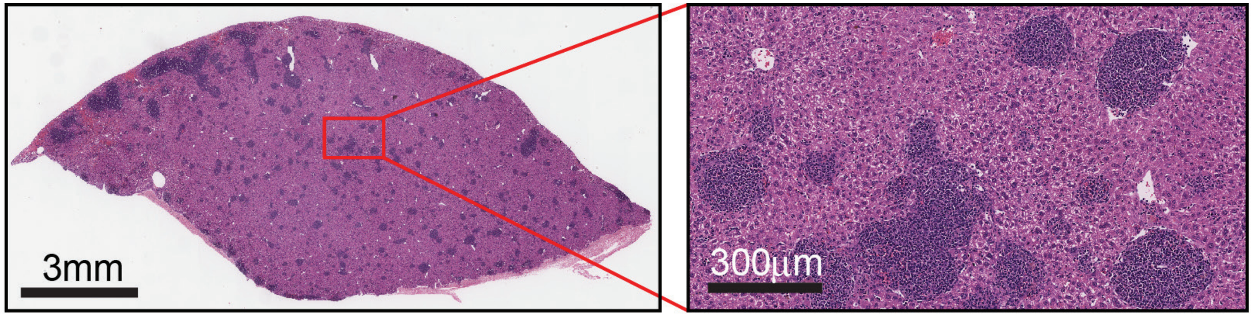


Broad range pathologies in CC mice

Spleen - lymphoma



Liver - metastases



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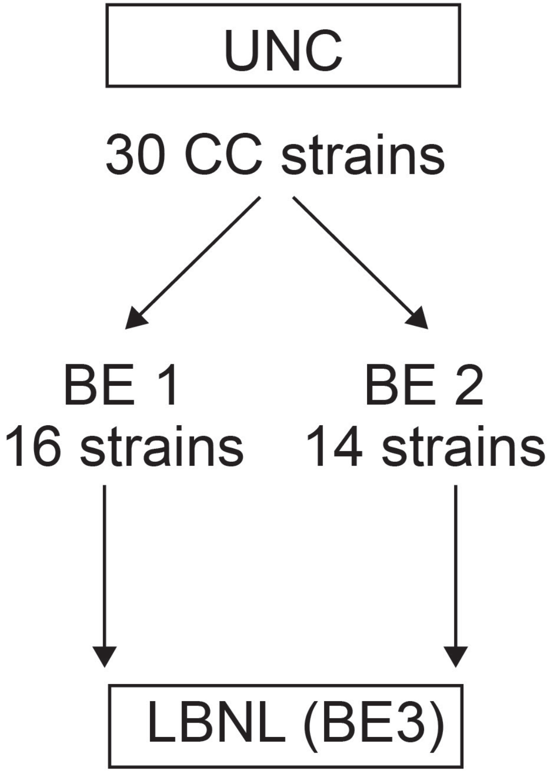
Early life environment determines microbiome structure



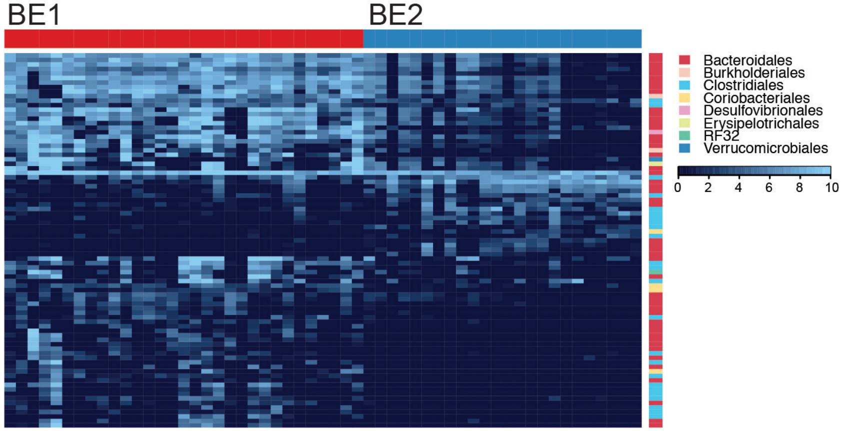
UNC

30 CC strains

BE 1
16 strains

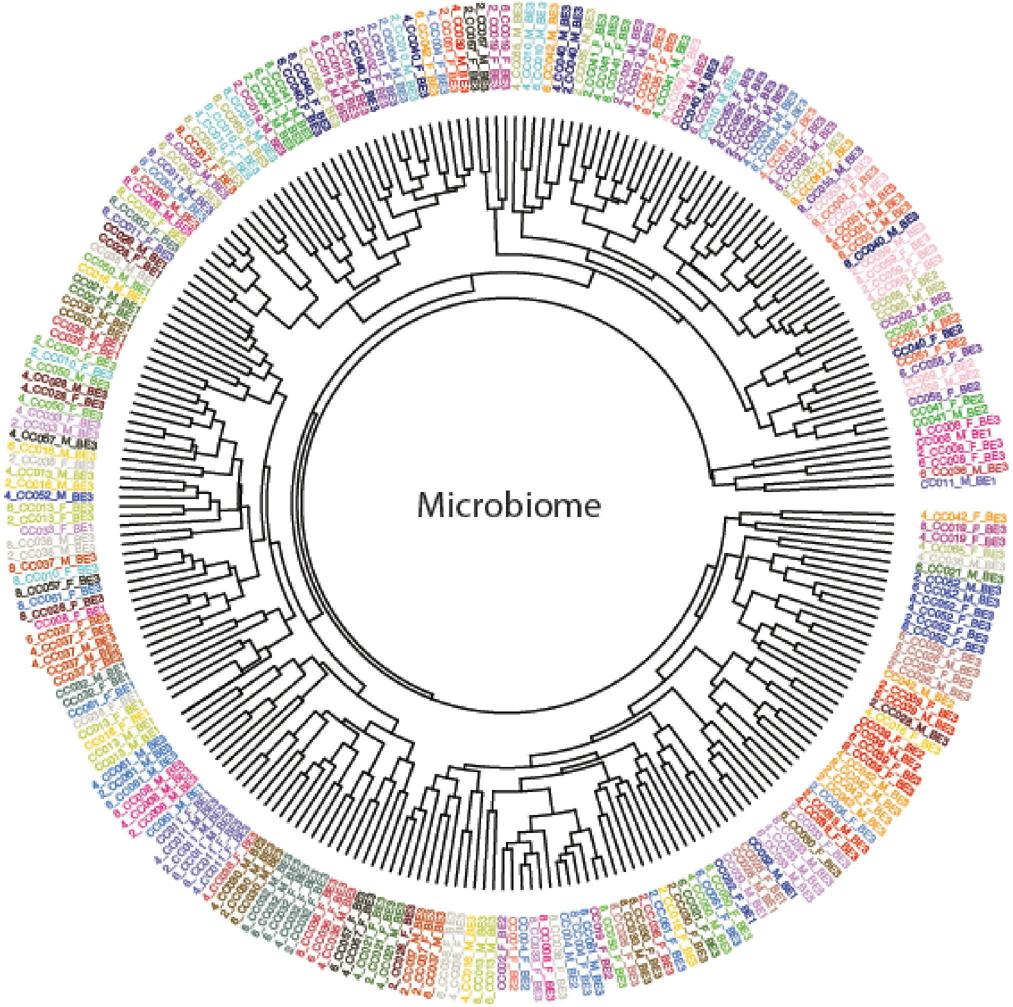
BE 2
14 strains

LBNL (BE3)



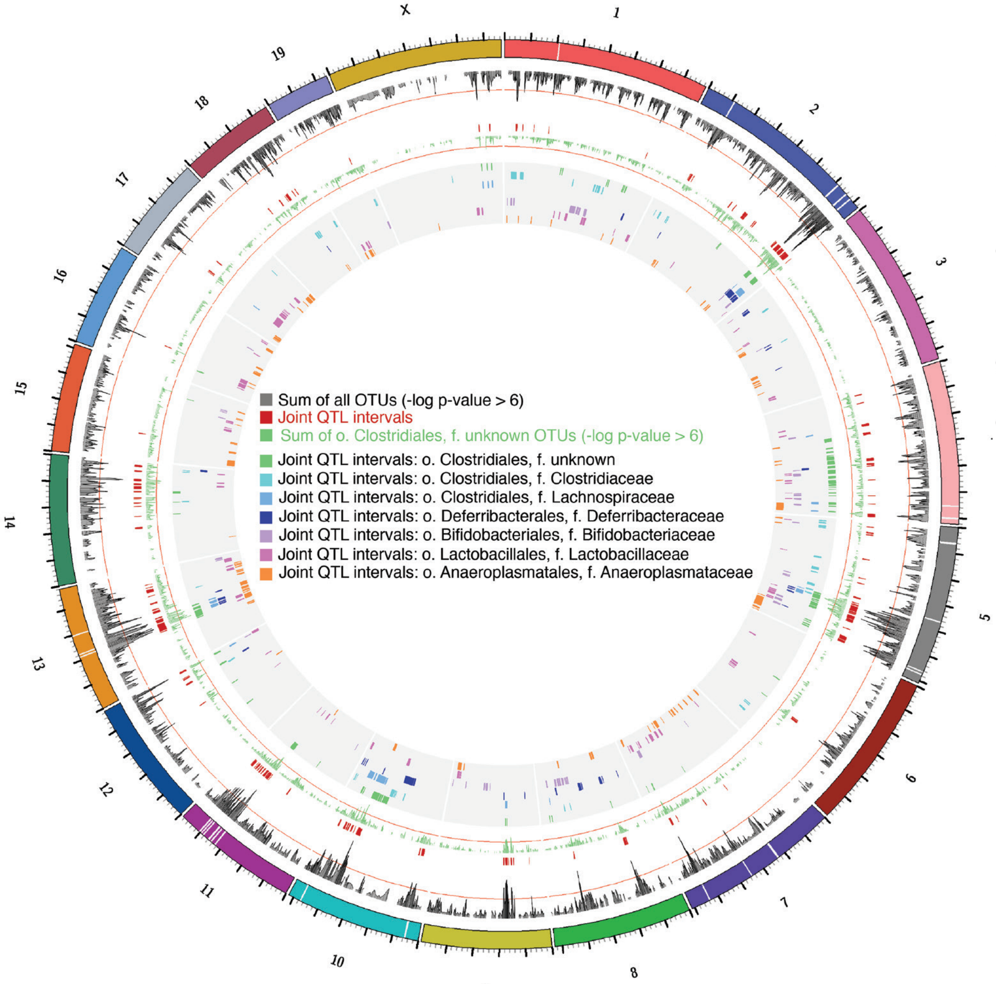
The early life built environment plays a persistent role in determining the composition of the gut microbiome

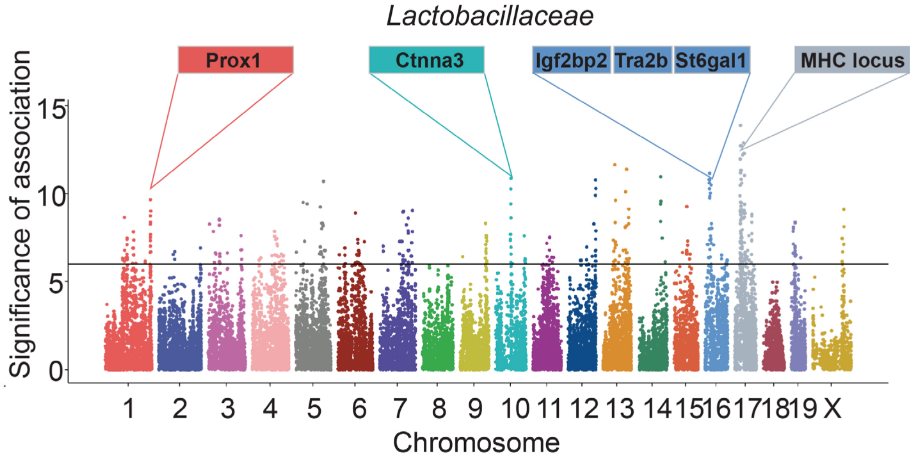
Hierarchical clustering of OTUs revealed that samples collected from the same strains of mice at different time points clustered together

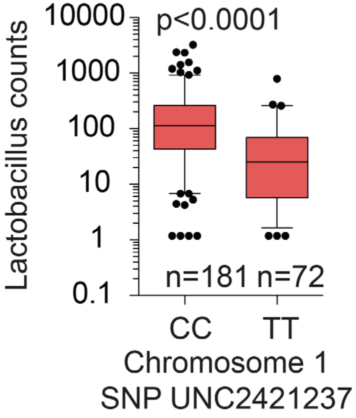


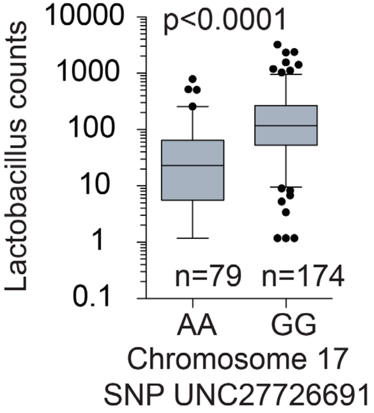
This suggested that host genetics was also an important factor in determining the gut microbiome

Linkage analysis identified host genetic influence on the gut microbiome



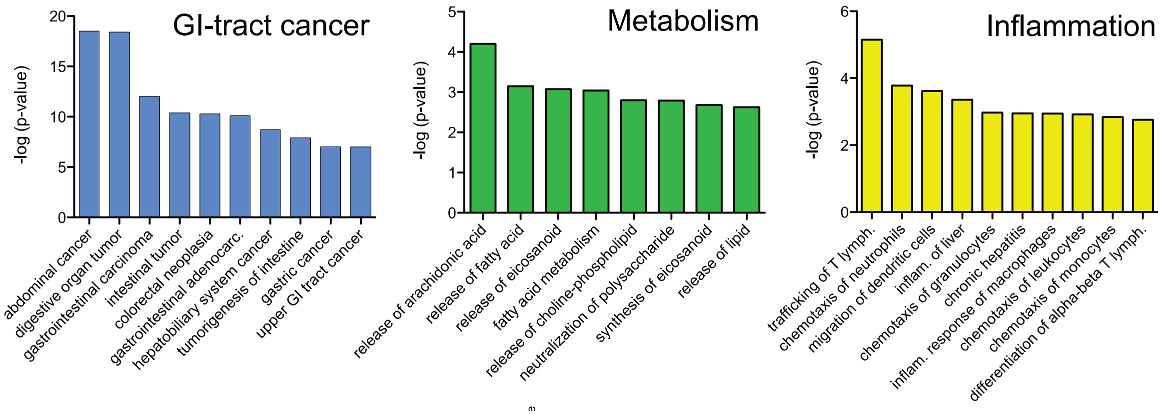






Snijders AM, Langley SA, Kim YM, Brislawn CJ, Noecker C, Zink EM, Fansler SJ, Casey CP, Miller DR, Huang H, Karpen GH, Celniker SE, Brown JB, Borenstein E, **Jansson JK, Metz TO, Mao JH**. Nature Microbiology, 2016. Nov 28;2:16221

Human relevance of mouse genomic loci associated with microbiome structure.



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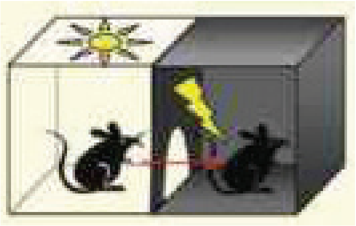
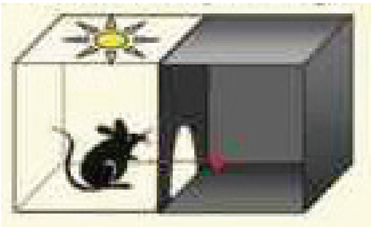
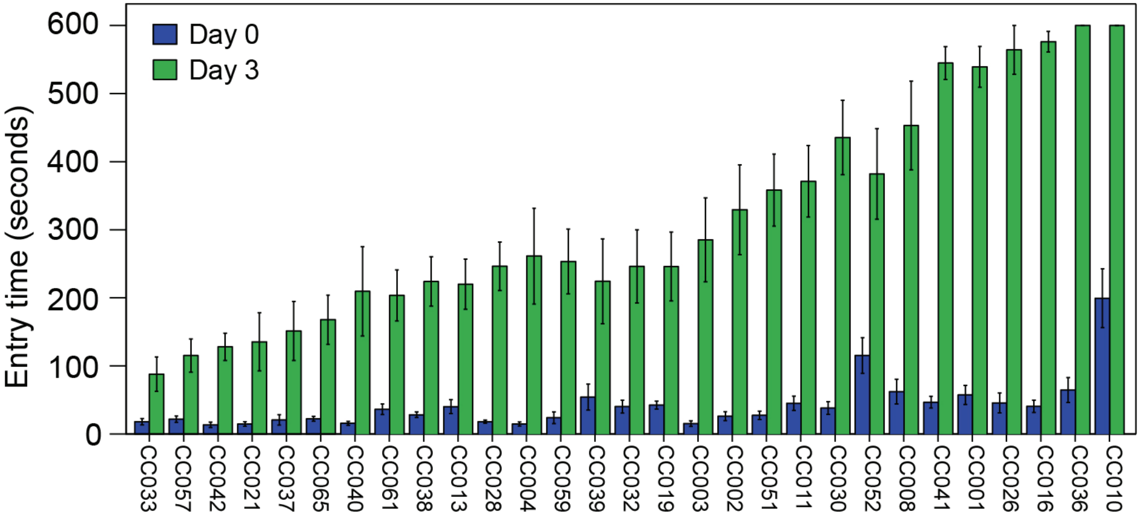
Variation in memory potential across CC mice

Training: 0.3 mA 5 sec



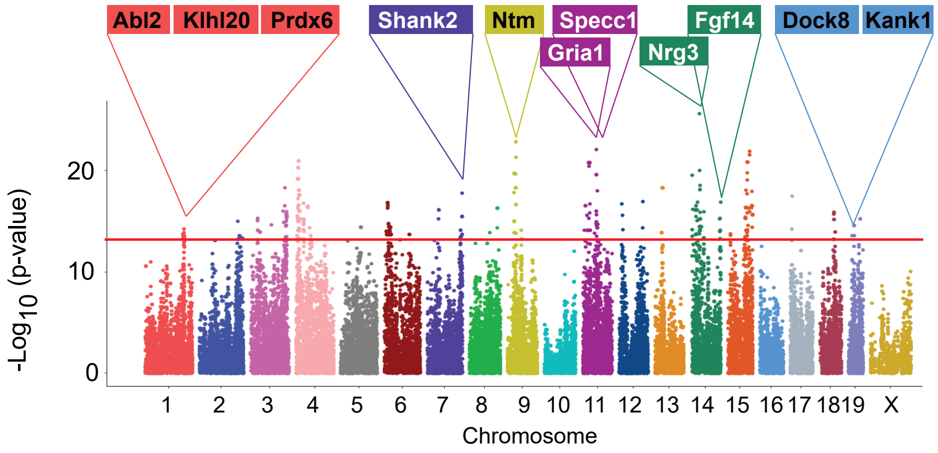
Testing: latency to enter

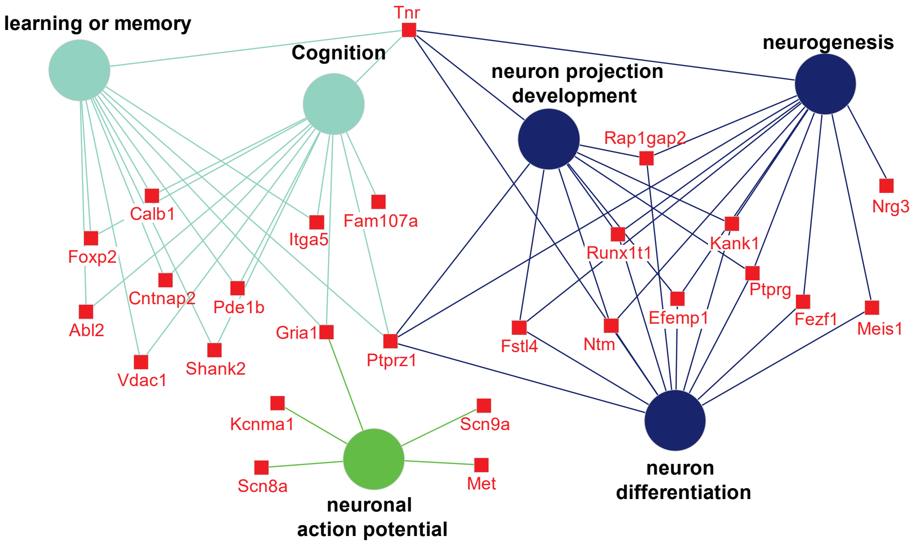




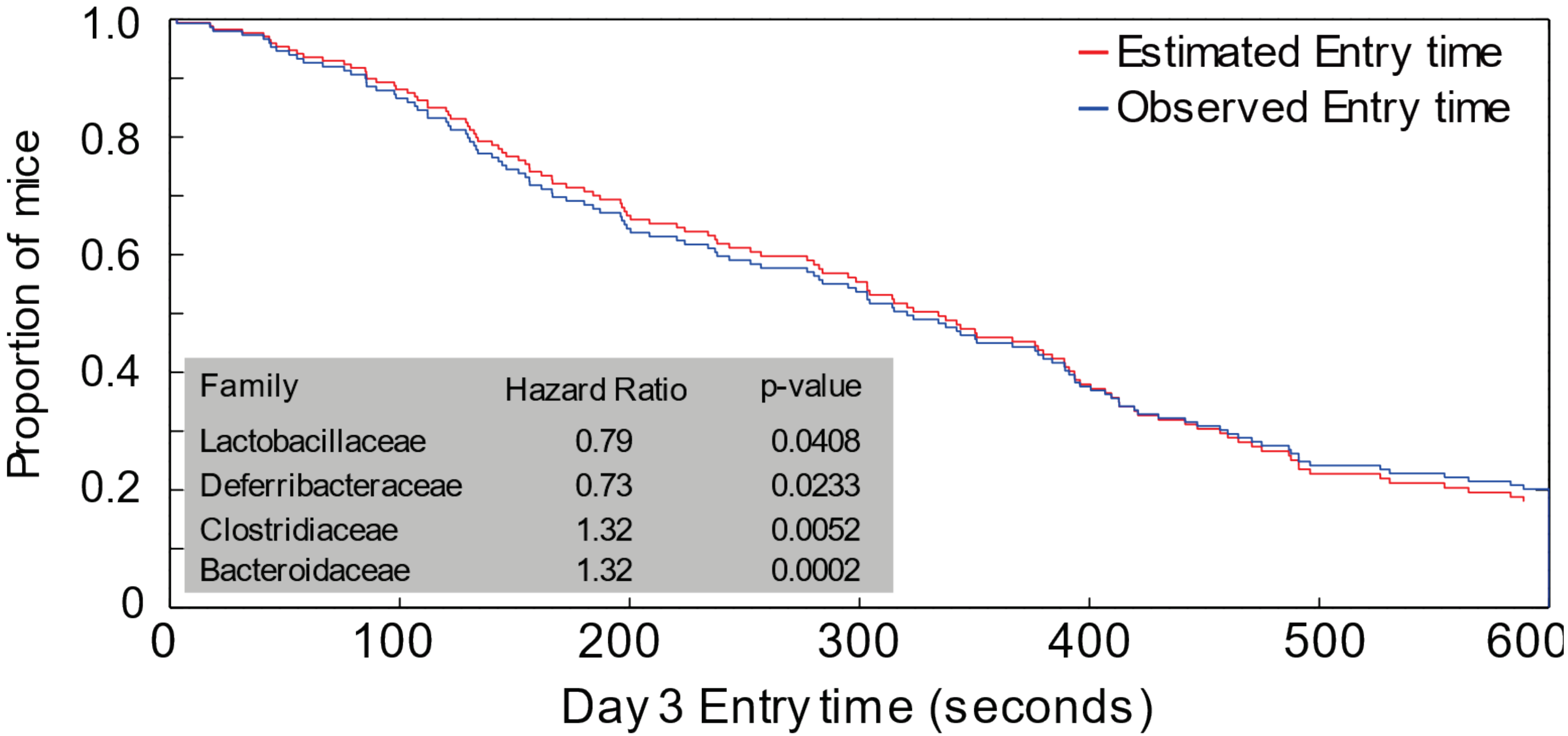
Mao et al. Microbiome. 2020 Apr 17;8(1):53. doi: 10.1186/s40168-020-

Genetic variations and candidate genes associated with memory in CC mice



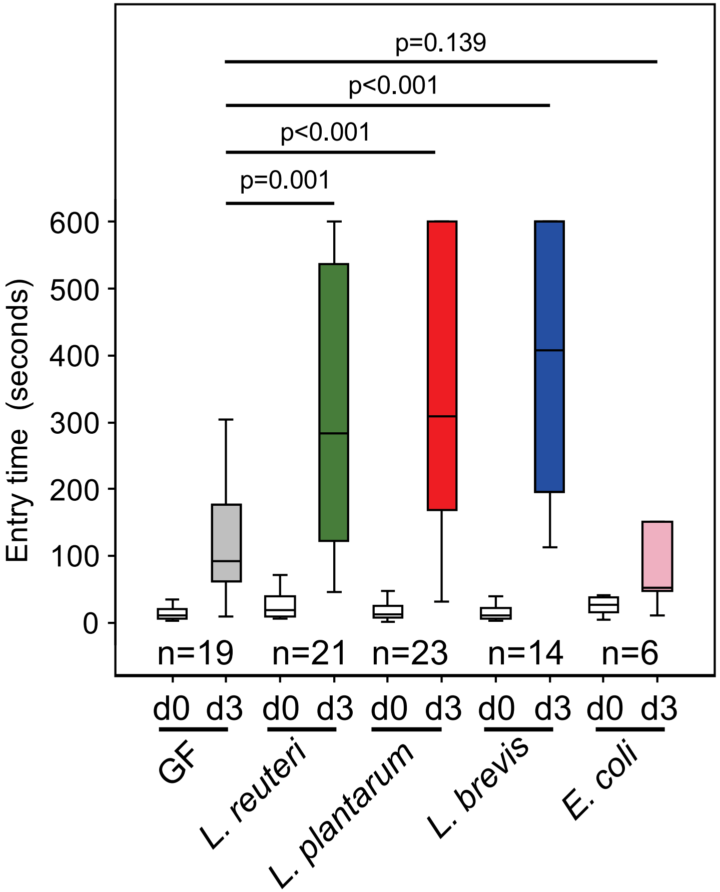


Candidate microbes associated with memory in CC mice

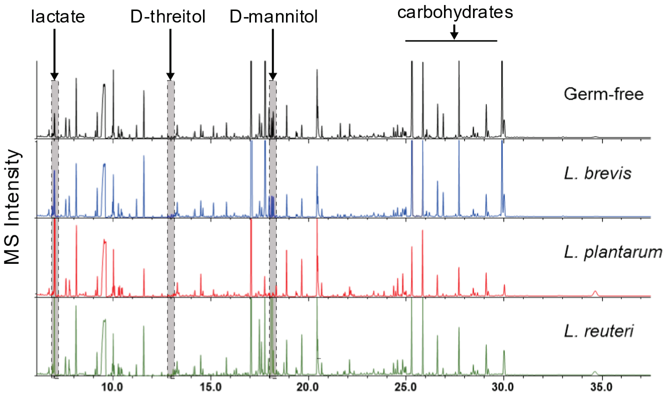


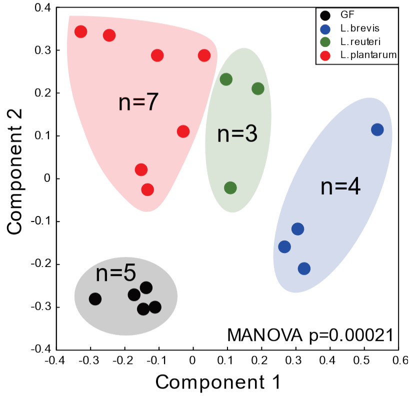
Family	Hazard Ratio	p-value
Lactobacillaceae	0.79	0.0408
Deferribacteraceae	0.73	0.0233
Clostridiaceae	1.32	0.0052
Bacteroidaceae	1.32	0.0002

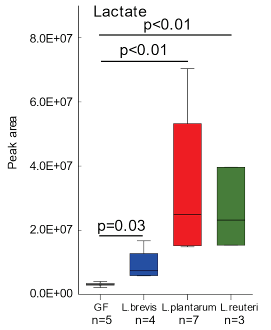
The impact of *Lactobacillus* on memory

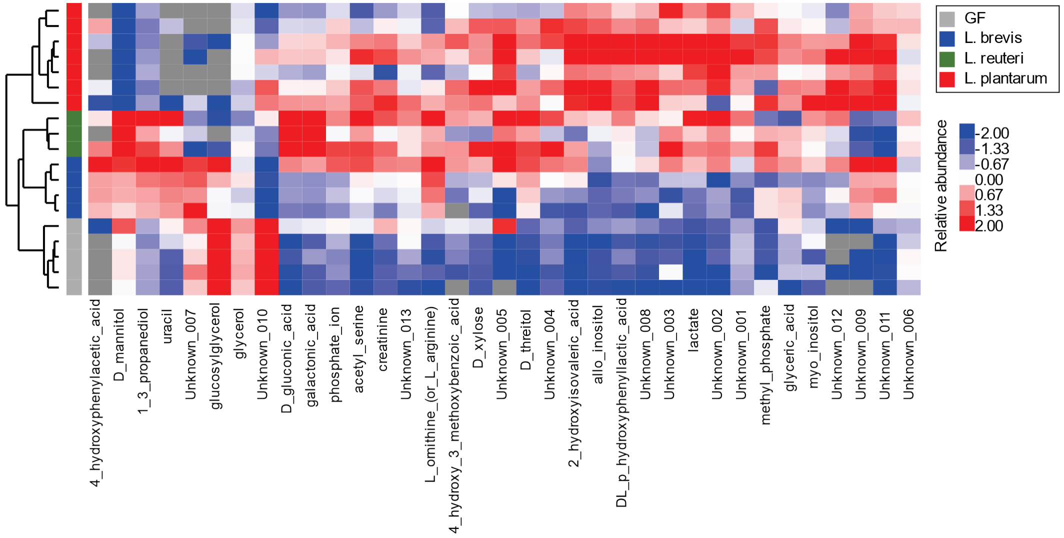


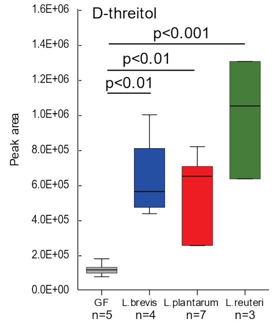
Metabolomics analysis of fecal samples from *Lactobacillus*-colonized and germ-free mice

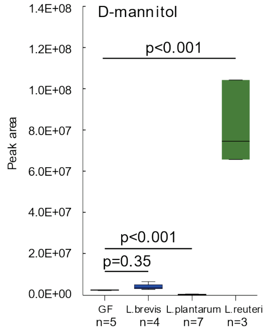




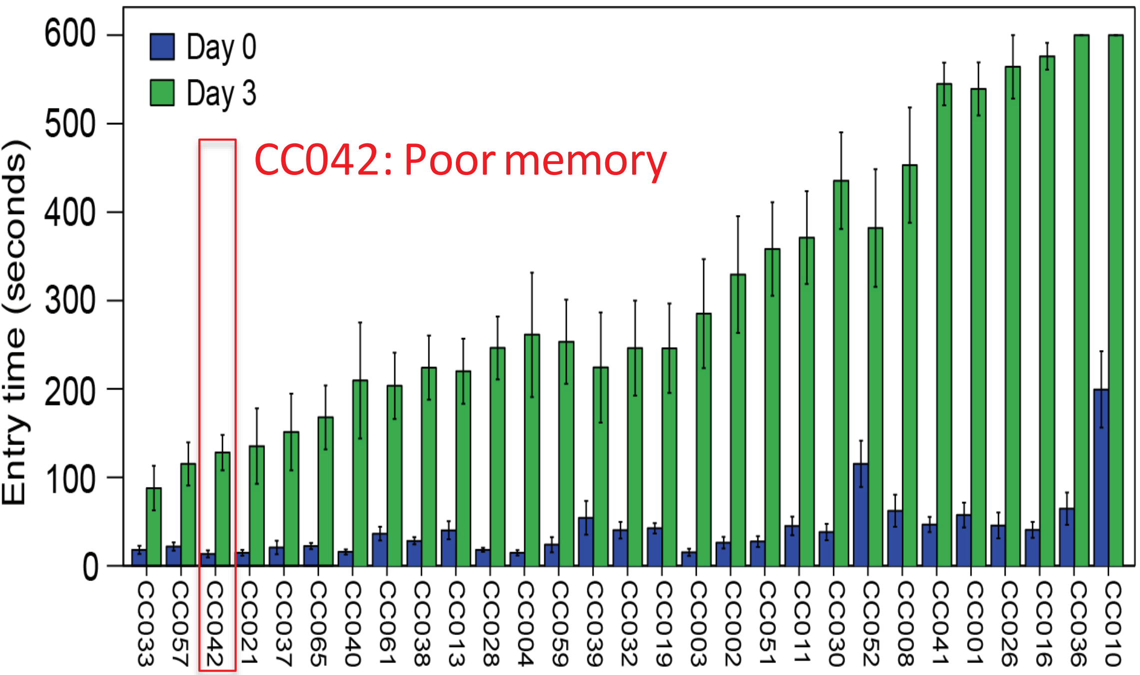


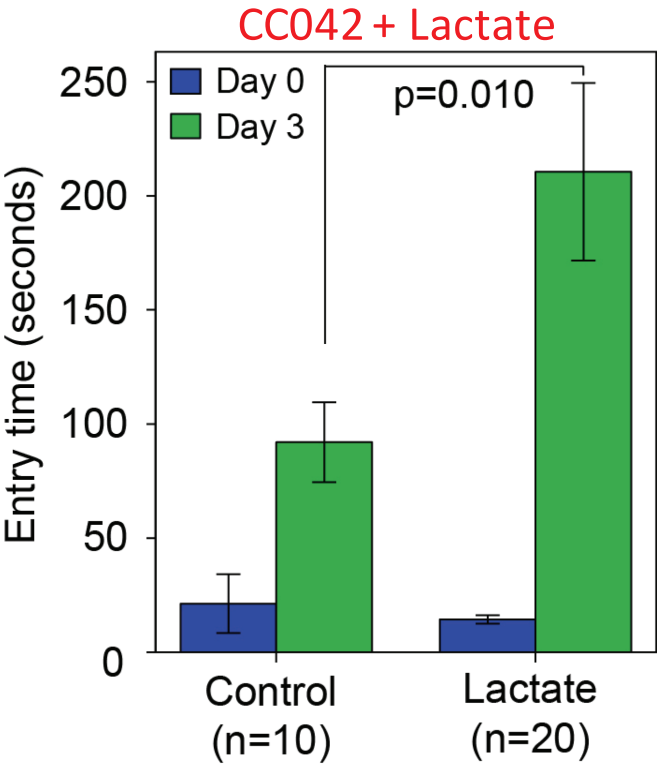




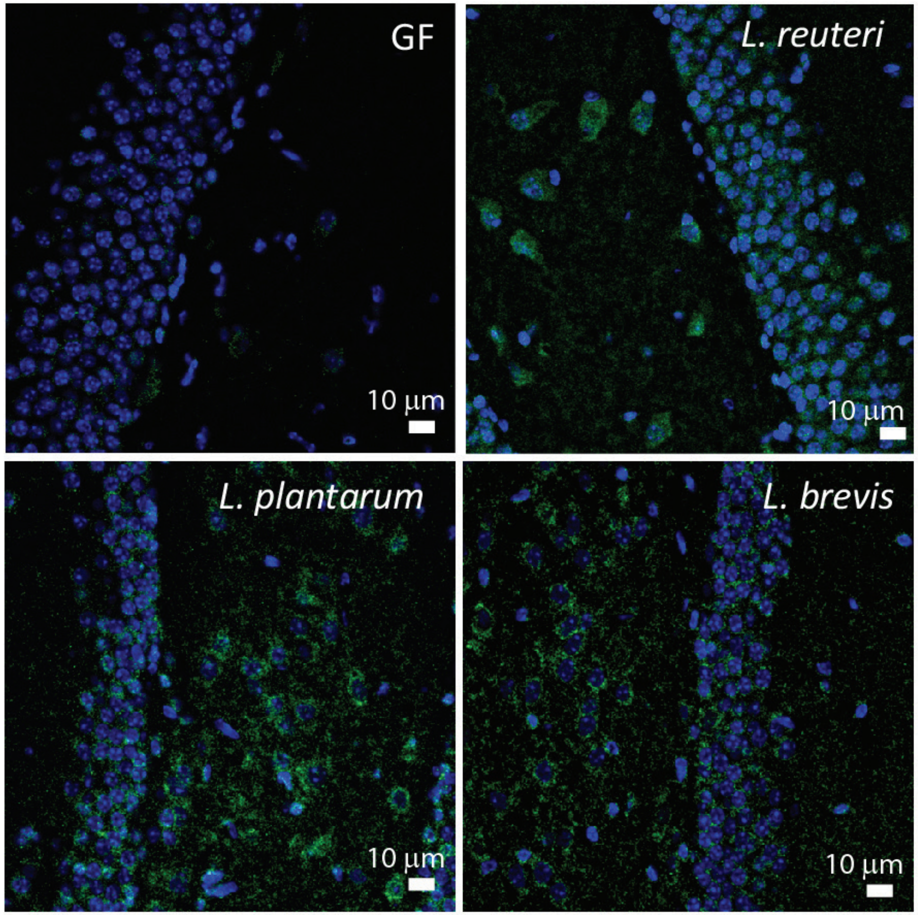


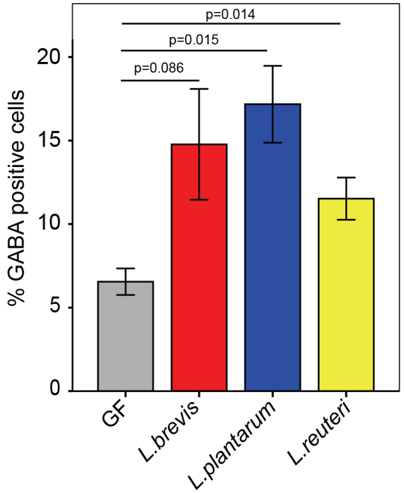
Lactate treatment improves memory potential

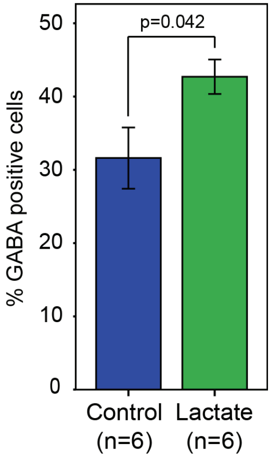




Influence of *Lactobacillus* or lactate treatment on GABA in the hippocampus







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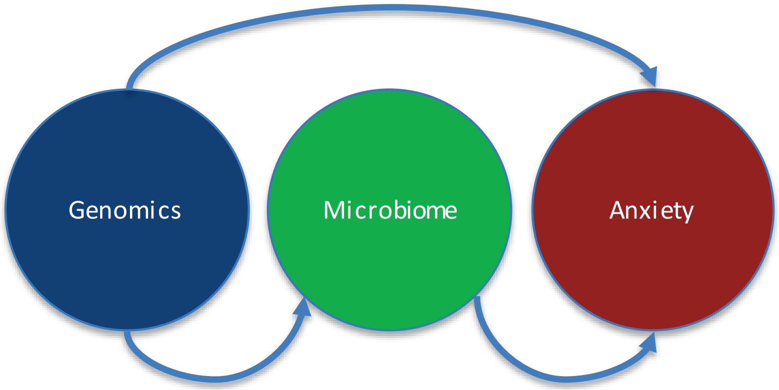
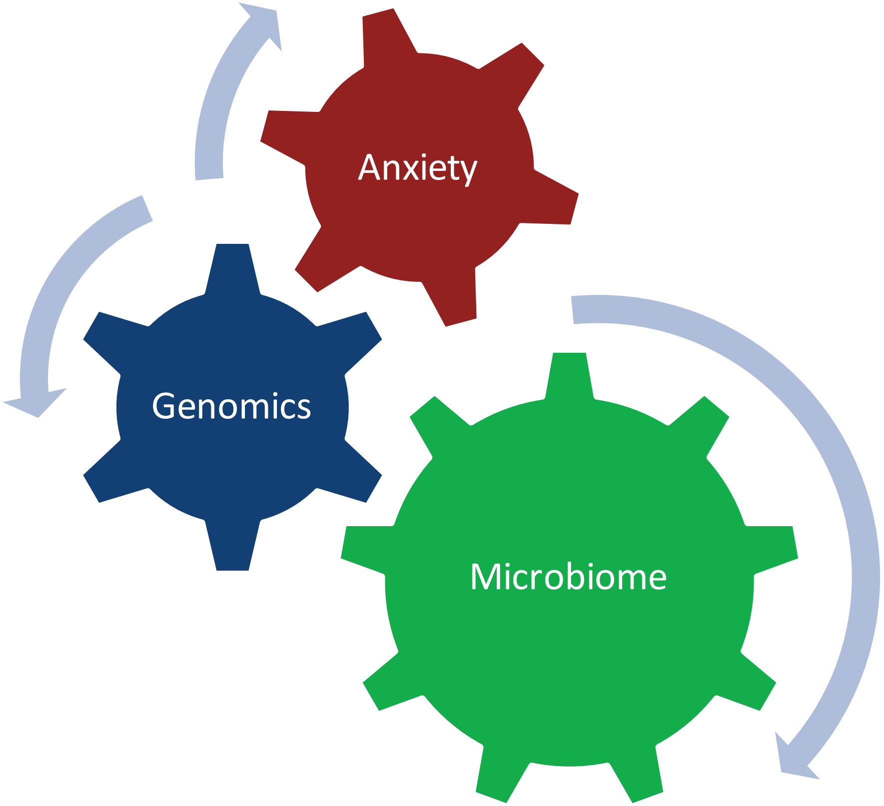
Background and Motivation

Background



1. Goodrich, et al. Human genetics shape the gut microbiome. Cell 159, 789–799 (2014).
2. Gilbert, J. A. et al. Current understanding of the human microbiome. Nat Med 24, 392-400, doi:10.1038/nm.4517 (2018).
3. Meier S M, et al. Genetic variants associated with anxiety and stress-related disorders: a genome-wide association study and mouse-model study[J]. JAMA psychiatry, 2019.

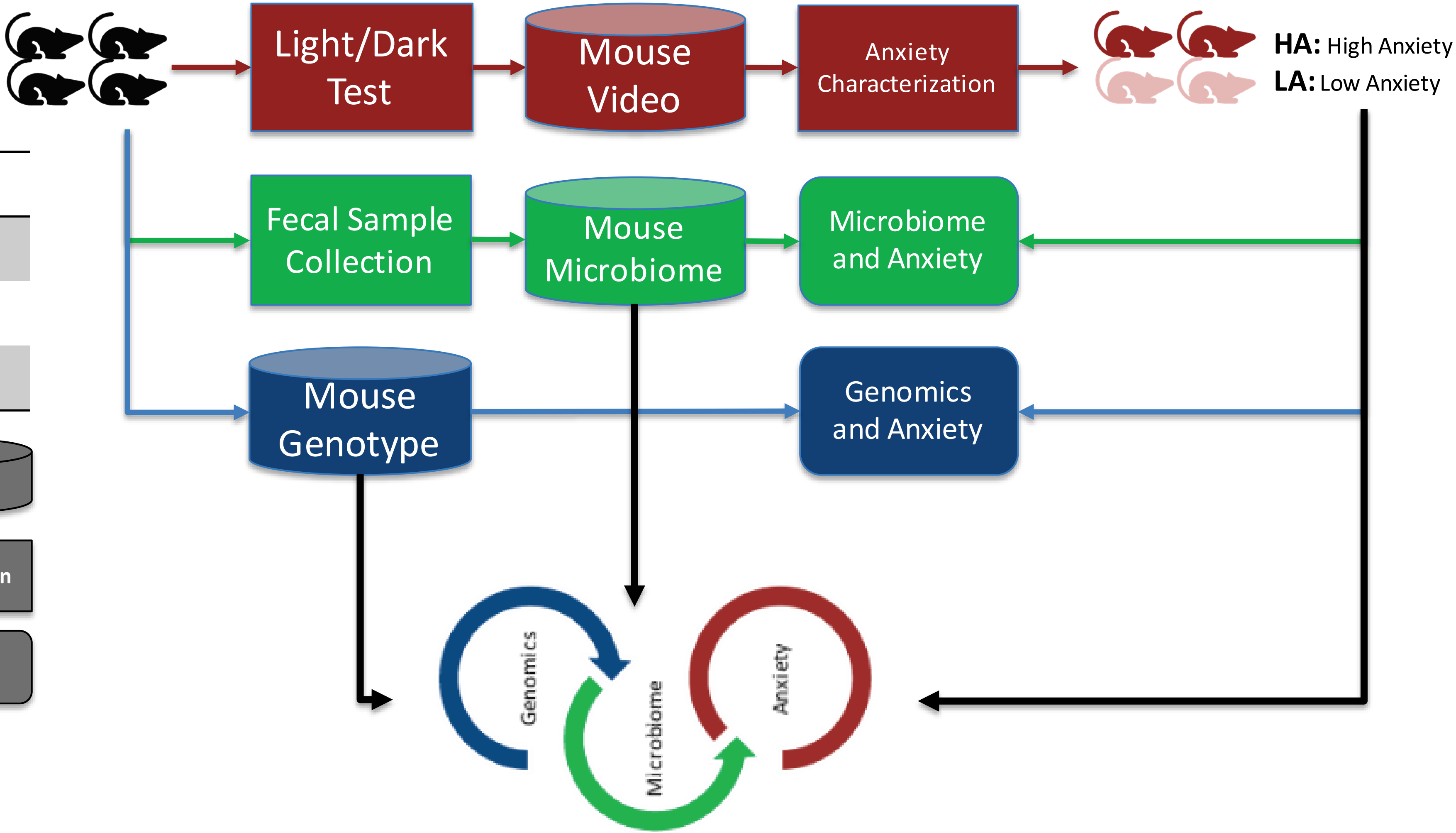
Motivation



To identify host genetics and gut microbiome factors associated with anxiety

Jin et al. Sci Rep. 2021 Jan 11;11(1):270.
doi: 10.1038/s41598-020-79538-x.

Study Design – Overall Flowchart



Light/Dark Test

Mouse Video

Anxiety Characterization

HA: High Anxiety

LA: Low Anxiety

Fecal Sample Collection

Mouse Microbiome

Microbiome and Anxiety

Mouse Genotype

Genomics and Anxiety

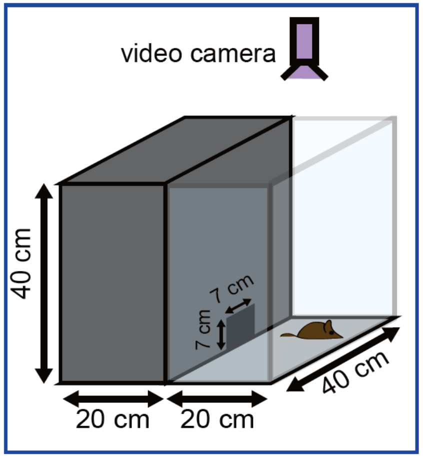
Mice#
445
Strain#
30

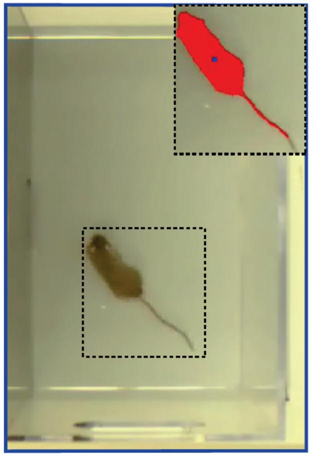
Data

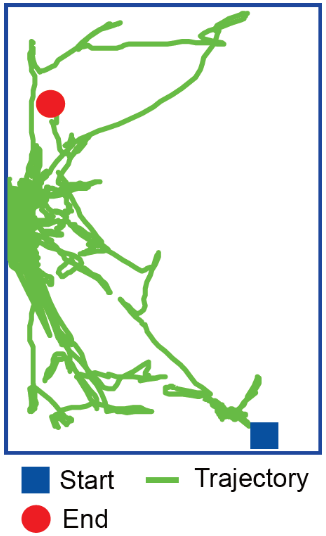
Operation

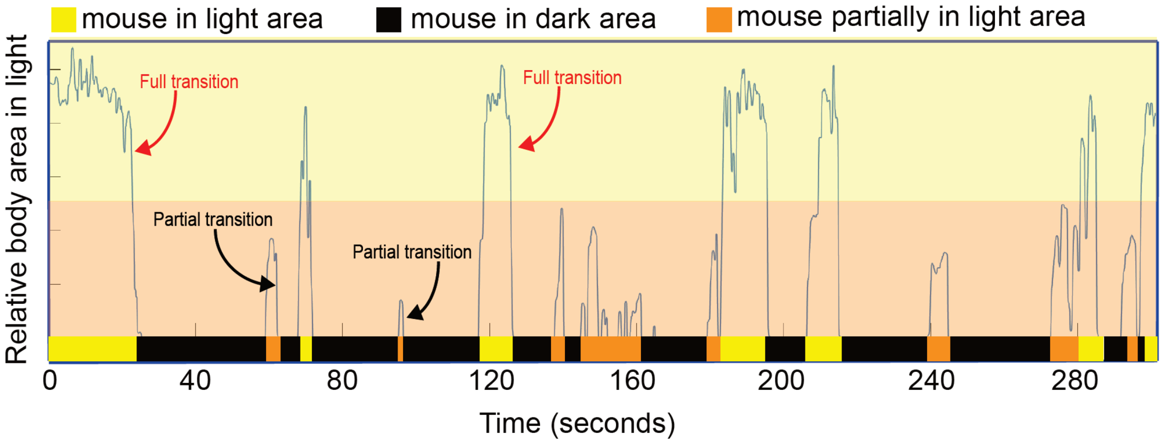
Results

The light/dark box test to measure anxiety

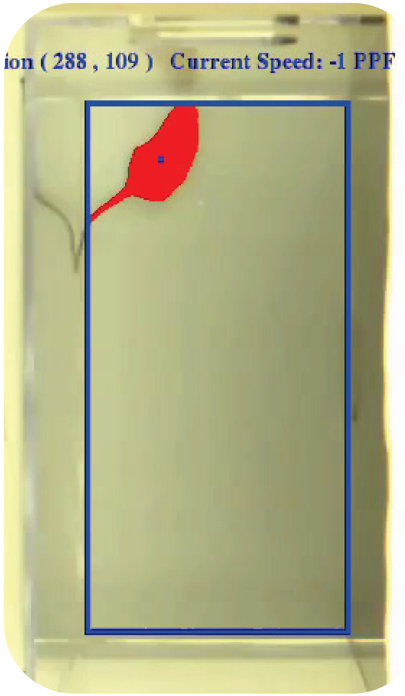


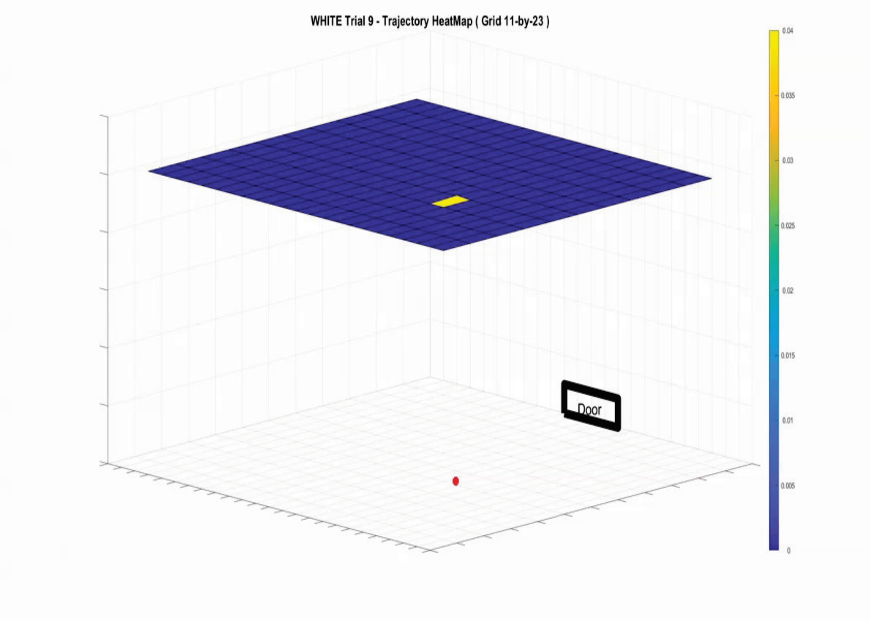




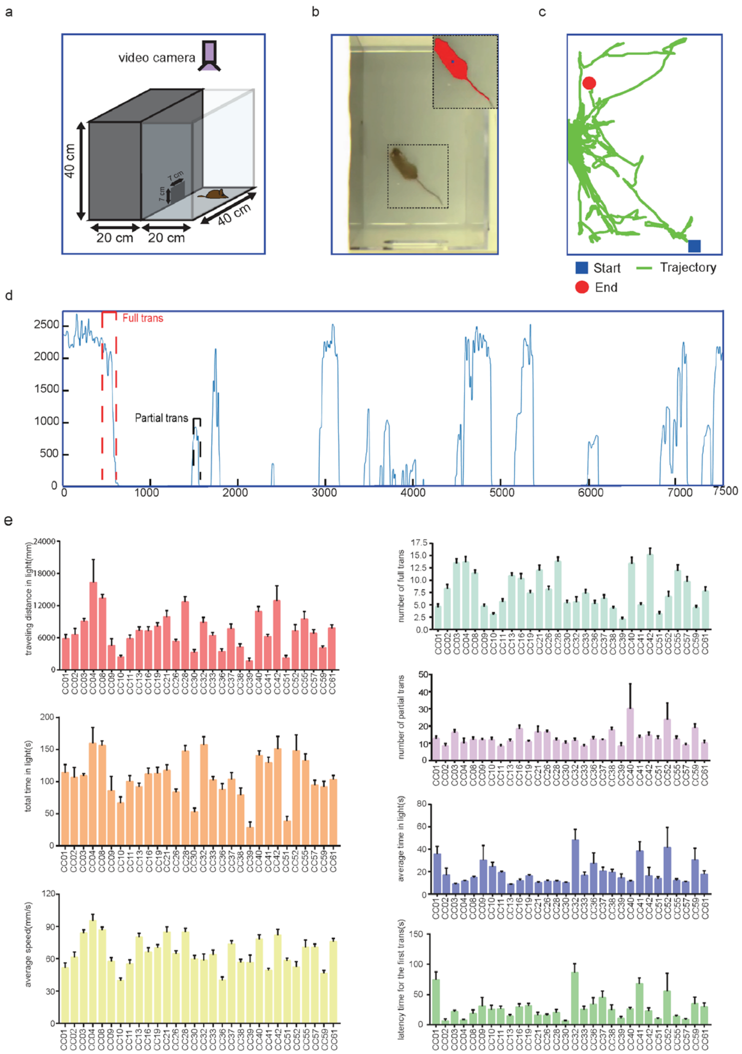


Anxiety Characterization – From Video to Anxiety-related Phenotypes



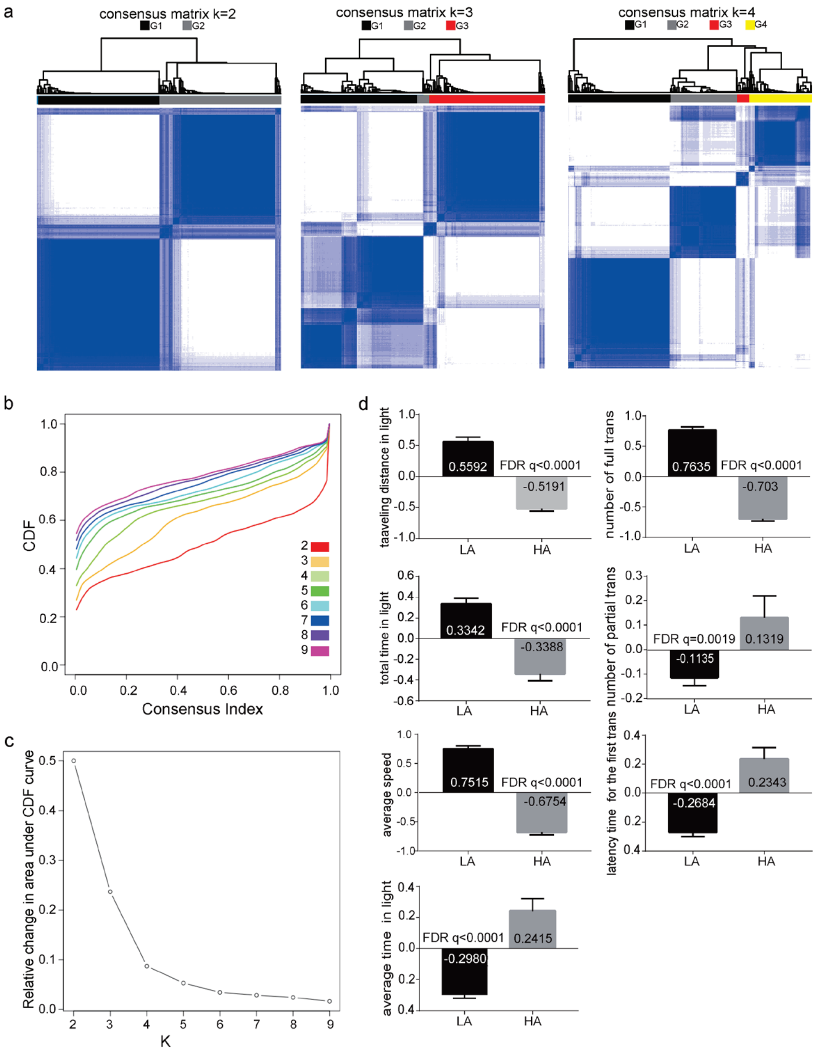


Anxiety Characterization – Anxiety related behavior phenotypes vary across CC strains



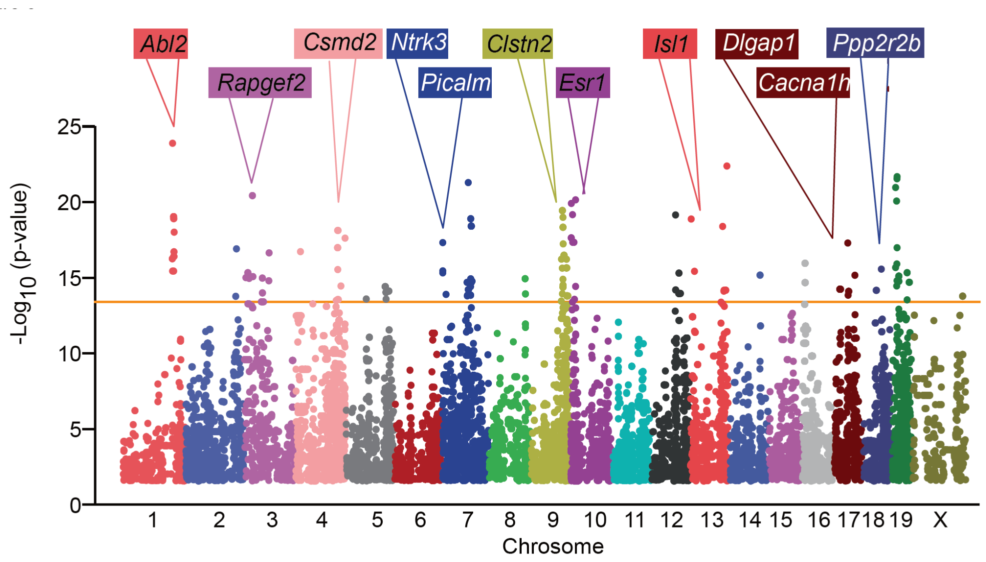
Phenotype Name	Phenotype Description
Traveling distance in light	Total distance travelled in light
Number of full translations	Number of whole-body transitions from light to dark
Total time in light	Total time spent in light
Number of partial translations	Number of partial-body transitions from dark to light and then back to dark
Average speed	Average speed in light
Average time in light	Average time spent in light
Latency time for the first time	Time spent in light before first transition from light to dark

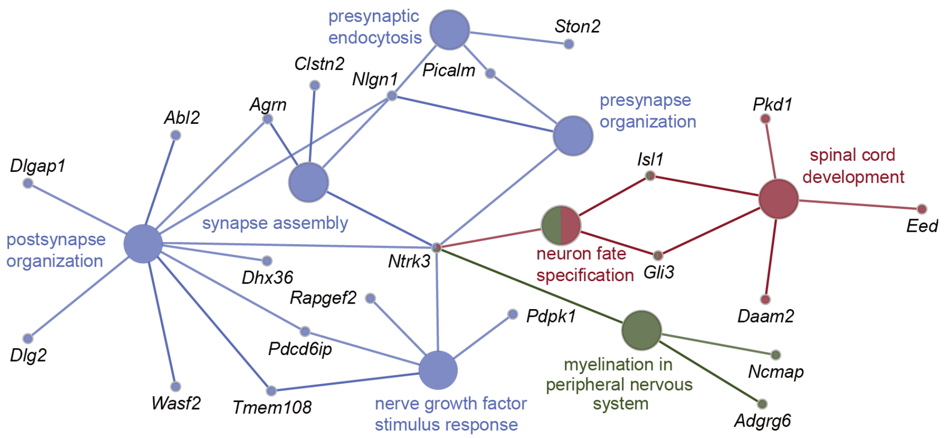
Anxiety level assessment based on anxiety-related phenotypes

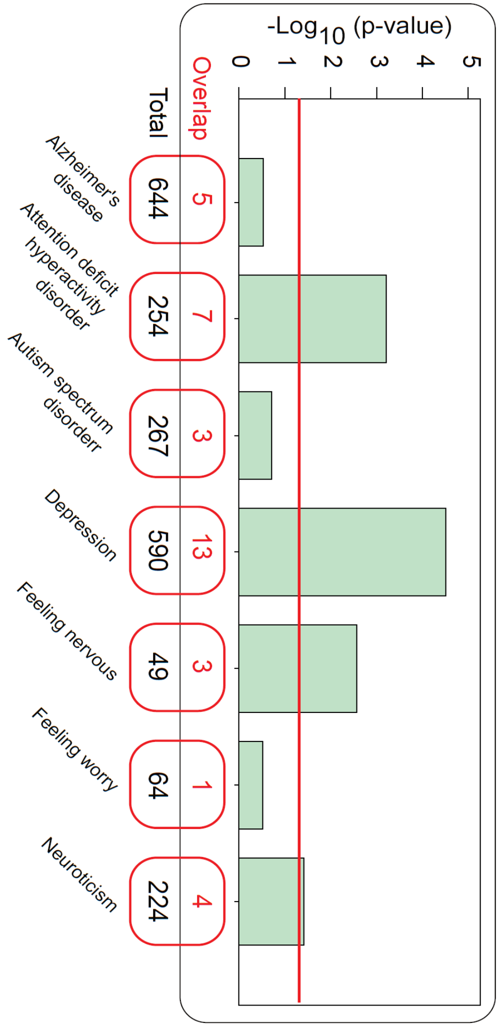


- Two Subgroups of mice identified with different level of anxiety: High Anxiety (HA, 198 mice) and Low Anxiety (LA, 247 mice)
- Phenotypes are significantly different across anxiety levels, which agrees with literature

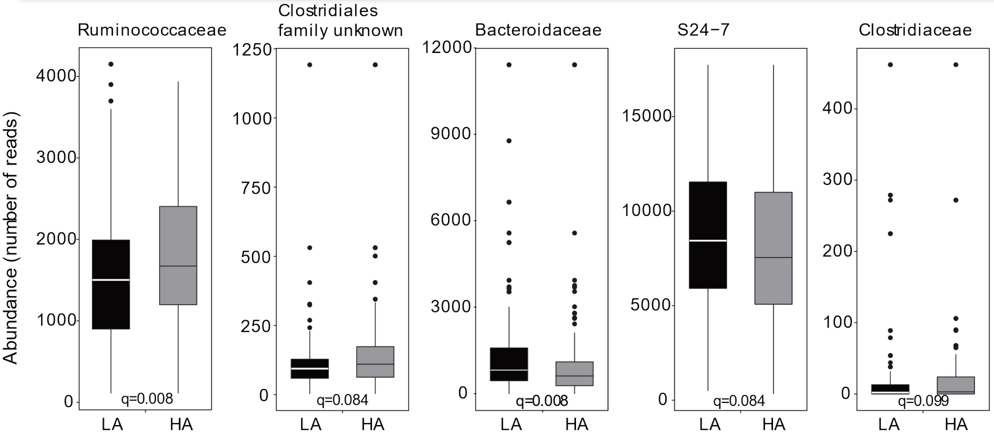
Genetic variations and candidate genes associated with anxiety in CC mice

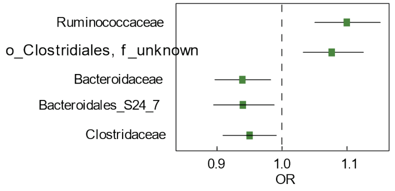


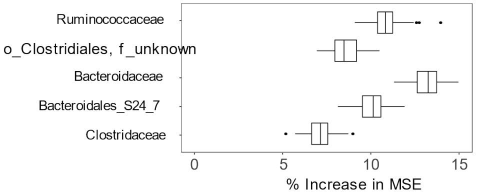


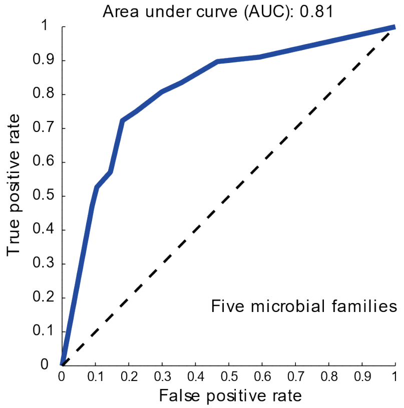


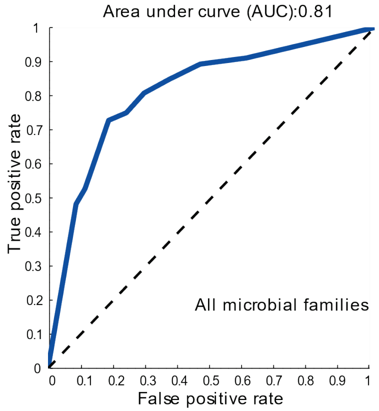
Association between microbiome and anxiety levels





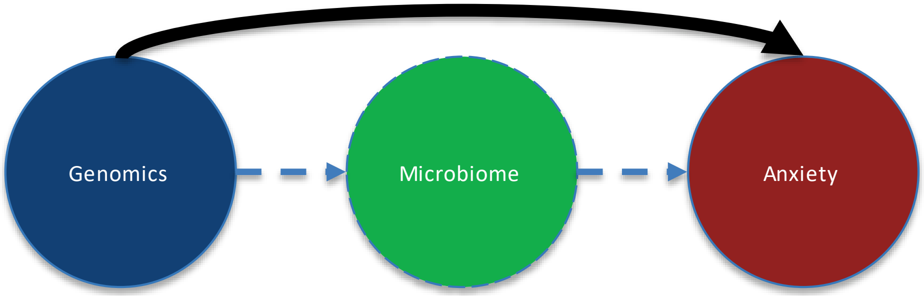


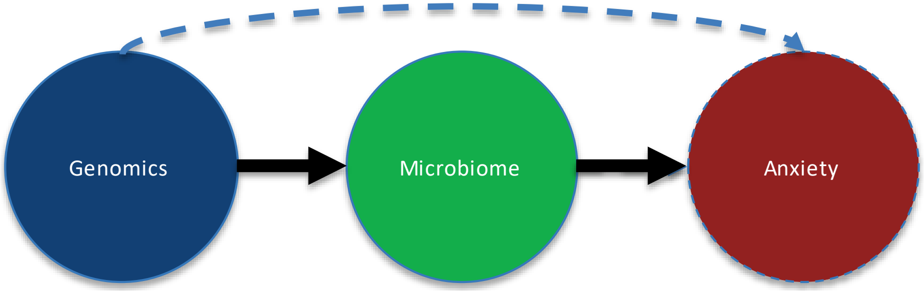


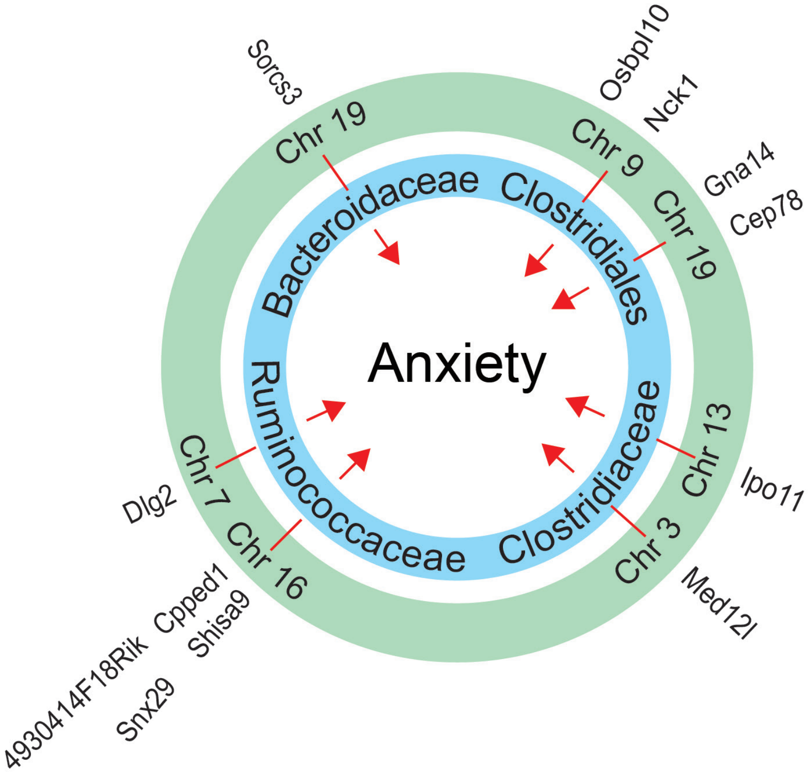


- We identified five families with significantly different abundance levels between high anxiety and low anxiety groups
- Logistic regression confirmed that these families were significantly correlated with anxiety
- Random forest classification based on the five families related to anxiety level led to the predictive accuracy around 79% with an AUC around 0.81

Mediation effect of microbiome between genetics and anxiety







Summary of key findings

- **Huge phenotypic diversity across CC strains**

- **Rapidly map genetic loci at high resolution and identify individual genes involved in disease.**

- **Wide range in the incidence and types of tumors across CC and identified a new mouse model for studying spontaneous human gastric tumourigenesis.**

- **Gut microbiome is associated with memory and anxiety.**

Collaborative Cross is an optimal model for advancing precision medicine

Acknowledgements

LBNL: Jian-Hua Mao, Hang Chang, Sue Celniker, Ben Brown, et al
PNNL: Janet Jansson, Colin Brislawn, Thomas Metz, Young-Mo Kim, et al
Texas A&M: David Threadgill
NMU: Pin Wang, Xiaoping Zou

NIH National Institute of Allergy and Infectious Diseases

NIH National Institute of Environmental Health Sciences

M2B microbes to biomes

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