The Influence of the Microbiome in Drug Development and Preclinical Studies

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The Microbiome – our "Other" Self

- Site-specific microbial communities residing in and on all free-living multi-cellular organisms

- Fecal microbiota harbors >10^{12} bacteria/gram, outnumbering somatic cells by up to 10:1

- Fecal microbiota harbors over 3 million unique gene sequences, outnumbering host genes by 100:1

- Highly dynamic populations comprising bacteria, archaea, viruses, fungi, protists, and multicellular parasites

The Microbiome – our "Other" Self
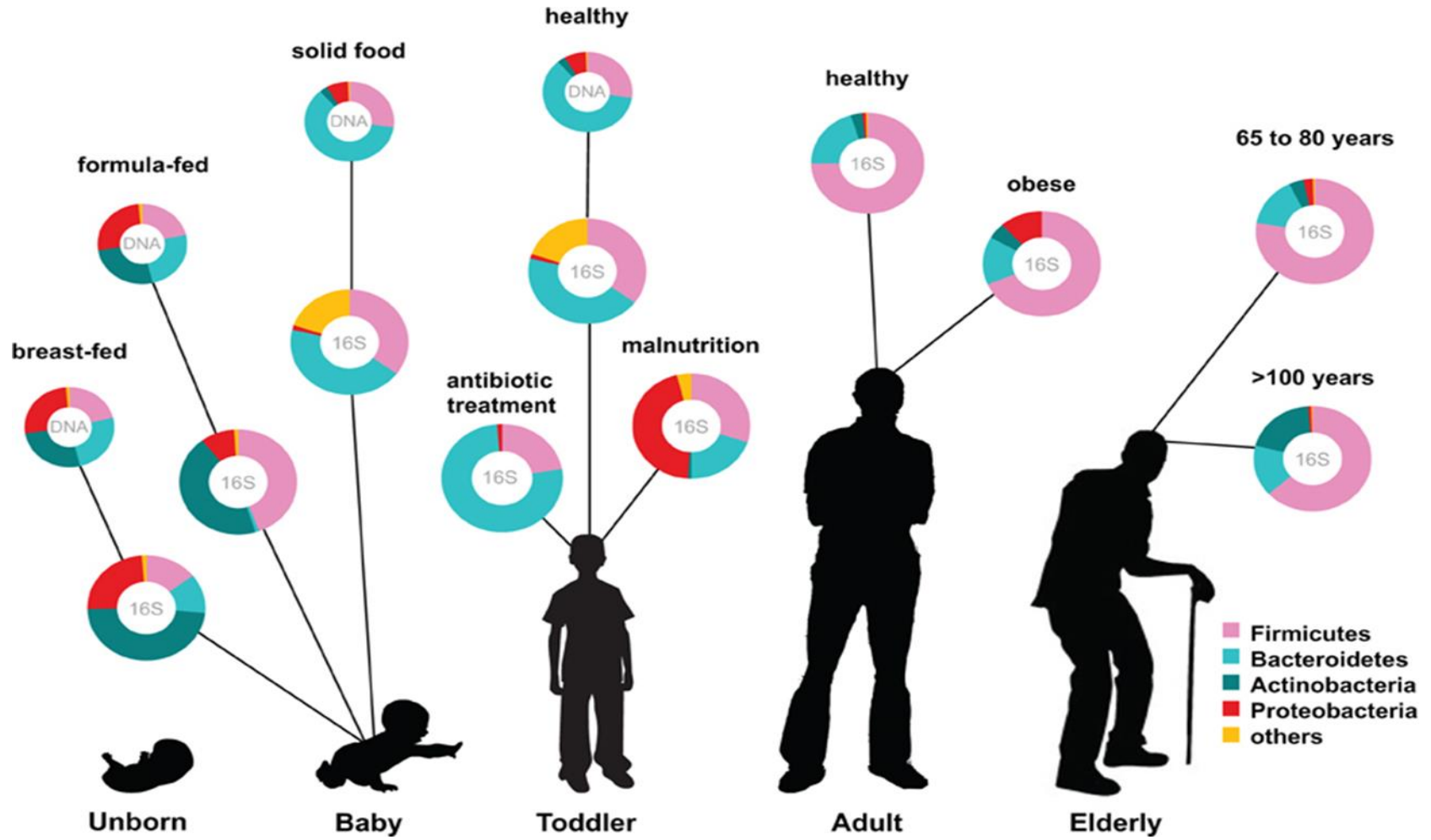
Ottman et al. 2012 *Front Cell Infect Microbiol* 2(104): 1-11

The Human Body is an Extended Community
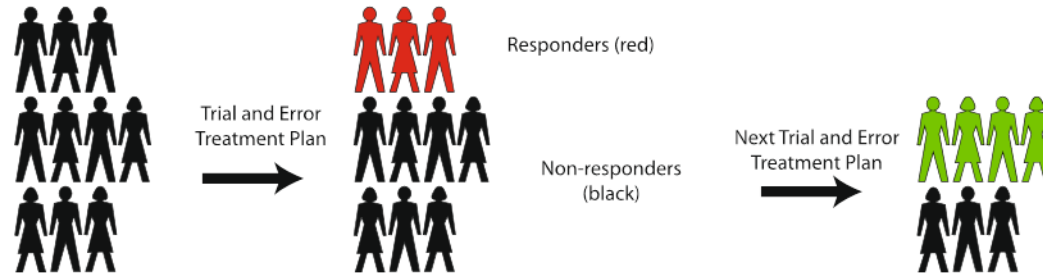
- We are a composite of:
  - Bacteria
  - Archaea
  - Eukaryotes
  - Viruses

- ... and human cells

- 100 trillion cells total:
  - 10% are human
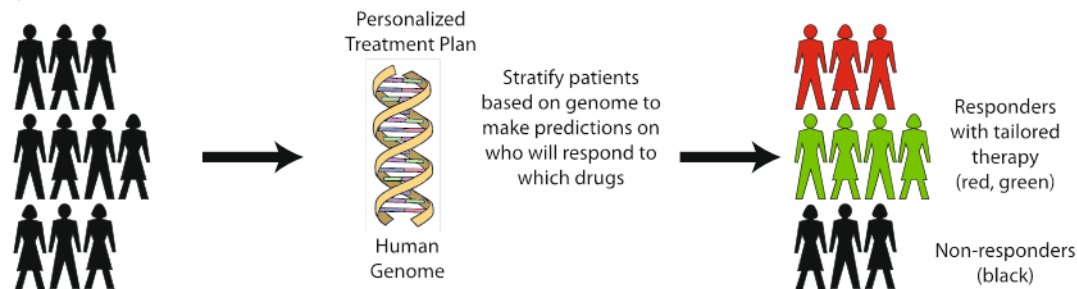 - Most are bacterial

**Human Microbiome
>3,000,000 genes**

**Human Genome
~20,000 genes**

Gut Microbiome as the "Second Genome" for Precision Medicine: the Pharmaco-metagenomic Approach
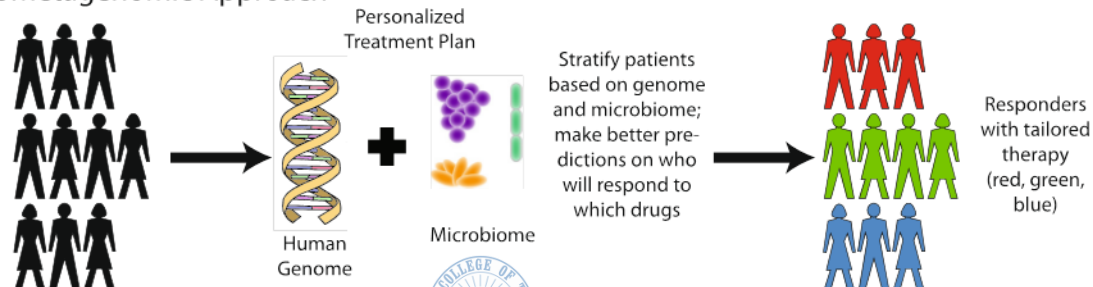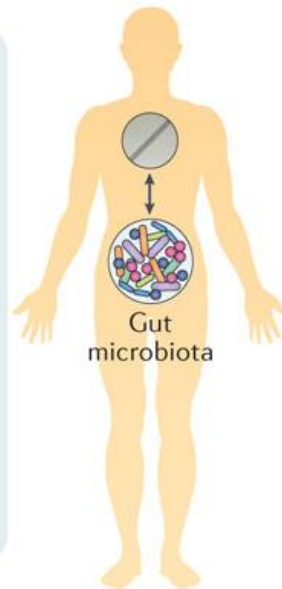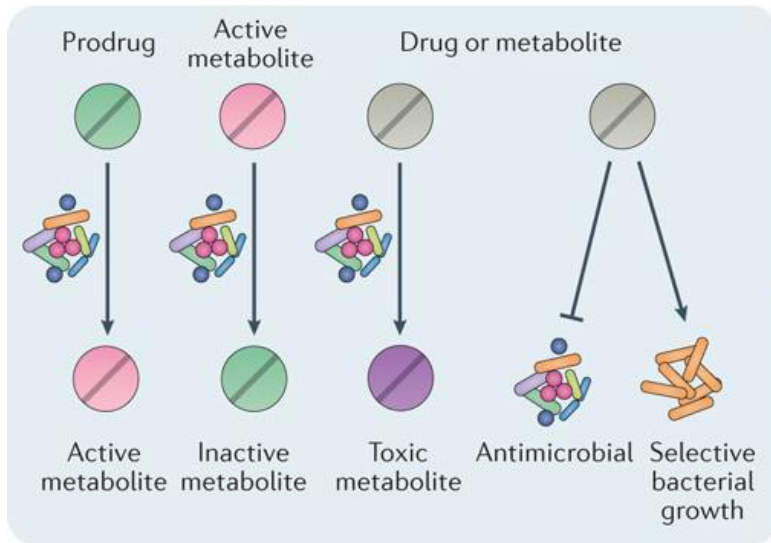

Nayak and Turnbaugh, 2016 BMC Medicine

A Metagenomic View of Xenobiotic Biotransformation

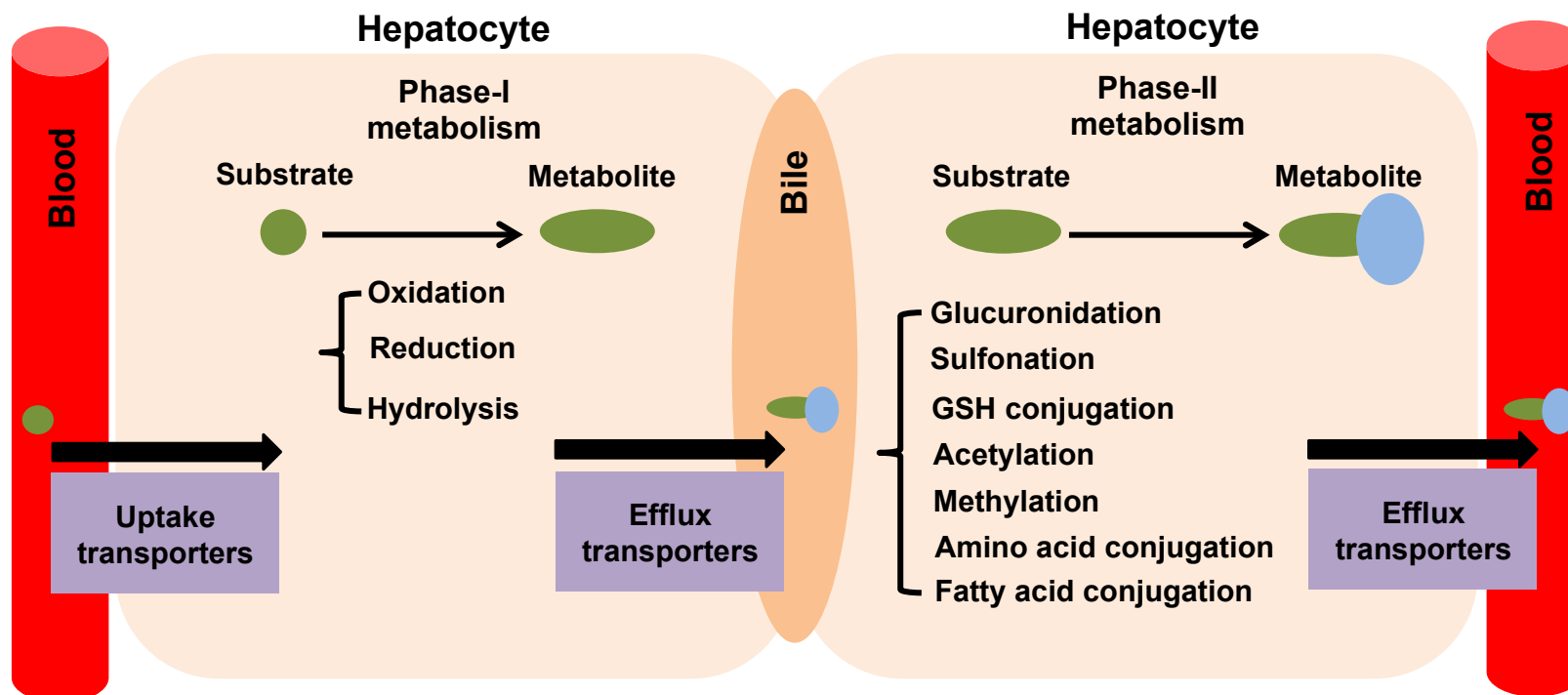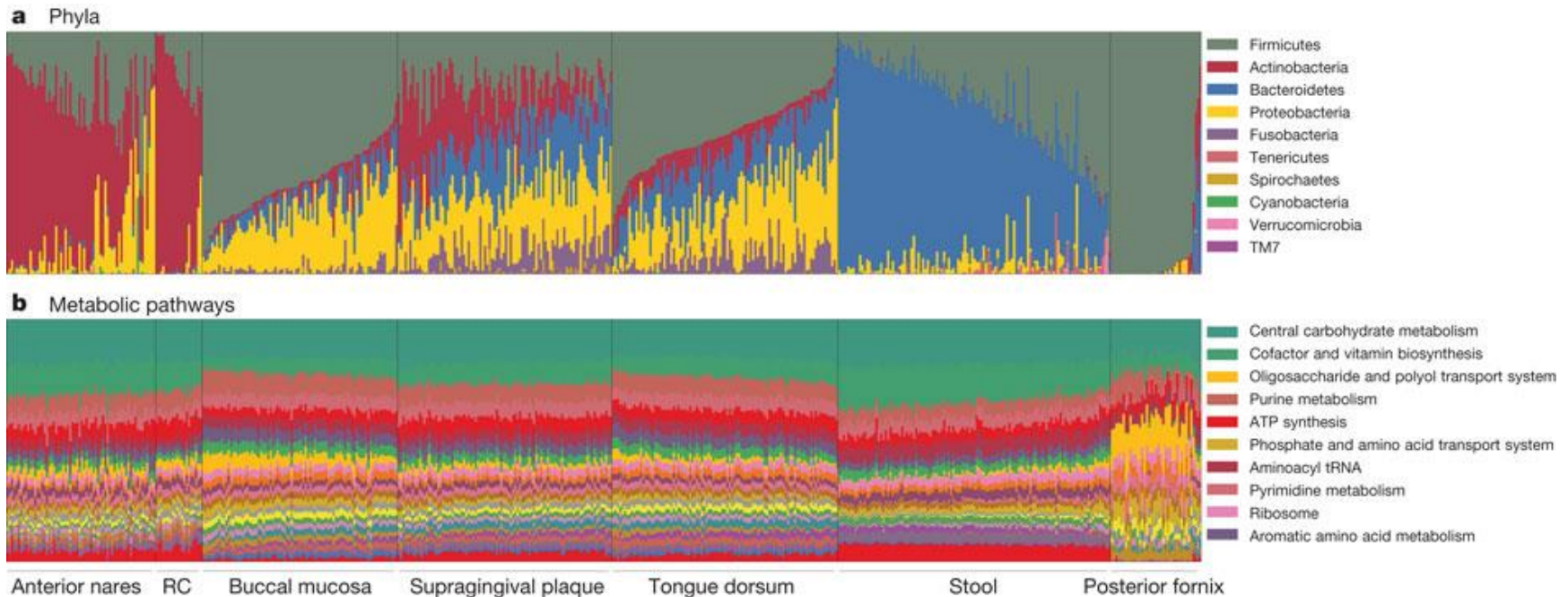Spanogiannopoulos et al., 2016 Nature Reviews
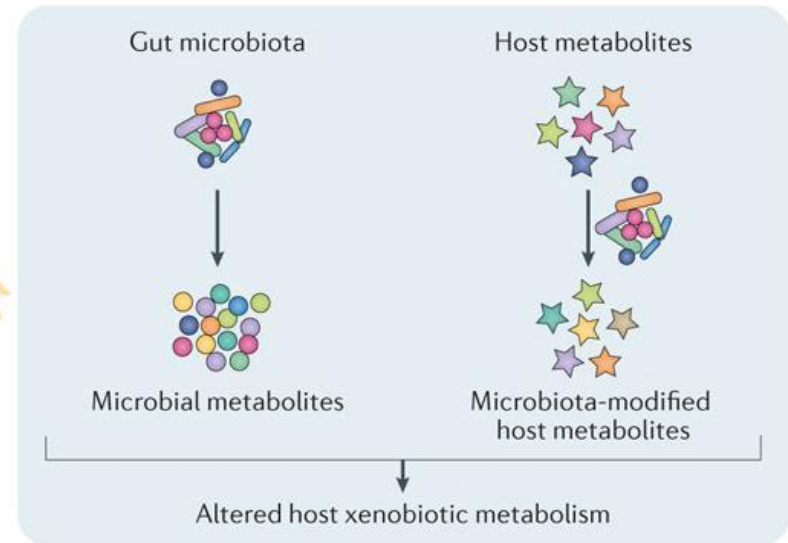
Introducing the "Xenobiotic-Processing Genome"

Julia Cui and Cindy Li, 2016 Comprehensive Toxicology 3rd Edition

Compositional differences belie common function

Huttenhower et al. 2012 *Nature* 486: 207-214

Associations with GI conditions

Table 1 **Intestinal microbiota-associated diseases, syndromes, or other aberrations, with summaries of multiple studies that support an association between the microbiota and the indicated aberration.**

Aberration	Most relevant observations and potential correlation	References
Crohn's disease	Diversity decrease – reduced *F. prausnitzii*	Kaser et al. 2010[51]; Sokol et al. 2009[52]; Willing et al. 2010[53]
Ulcerative colitis	Diversity decrease – reduced *A. muciniphila*	Png et al. 2010[54]; Kaser et al. 2010[51]; Lepage et al. 2011[55]
Irritable bowel syndrome	Global signatures – increased *Dorea* and *Ruminococcus*	Salonen et al. 2010[36]; Saulnier et al. 2011[56]; Rajilić-Stojanović et al. 2011[13]
Clostridium difficile infection	Strong diversity decrease – presence of *C. difficile*	Grehan et al. 2010[57]; Khoruts et al. 2010[58]
Colorectal cancer	Variation in *Bacteroides* spp. – increased fusobacteria	Sobhani et al. 2011[59]; Wang et al. 2012[60]; Marchesi et al. 2011[61]
Allergy/atopy	Altered diversity – specific signatures	Stsepetova et al. 2007[62]; Bisgaard et al. 2011[63]; Storrø et al. 2011[64]
Celiac disease	Altered composition, notably in small intestine	Nistal et al. 2012[65]; Di Cagno et al. 2011[66]; Kalliomäki et al. 2012[67]
Type 1 diabetes	Signature differences	Vaarela 2011[68]; Giongo et al. 2011[69]; Brown et al. 2011[70]
Type 2 diabetes	Signature differences	Larssen et al. 2010[71]; Wu et al. 2010[72]; Kootte et al. 2012[73]
Obesity	Specific bacterial ratios (*Bacteroidetes/Firmicutes*)	Ley et al. 2006[74]; Turnbaugh et al. 2009[10]; Musso et al. 2011[75]

de Vos and de Vos 2012 *Nutr Rev* 70(suppl 1): S45-S56

Associations with extra-GI conditions

Table 2 **Indications for associations between the microbiota and health aberrations, provided as an alphabetical listing of the aberrations suggested to be associated with the intestinal microbiota, along with support for such an association.**

Disease or aberration	Type of support	Reference*
Alzheimer's disease	Microbiota in a mouse model of Alzheimer's disease	Karri et al. 2010[103]
Atherosclerosis	Analysis of plaques in humans	Koren et al. 2011[104]
Autistic spectrum disorders	Analysis of mucosa in children with autism spectrum disorders	Williams et al. 2011[105]
Chronic fatigue syndrome	Cultured microbiota in patients with chronic fatigue syndrome	Sheedy et al. 2009[106]
Colic babies	Longitudinal analysis of colic babies cohort	de Weerth et al. 2012 unpublished data
Cardiovascular disease	Cardiovascular-diseased mice and microbial metabolism	Wang et al. 2011[48]
Depression and anxiety	Probiotic intervention in stressed mice	Bravo et al. 2011[34]
Frailty	Analysis of elderly and high frailty scores	van Tongeren et al. 2005[107]
Graft-vs-host disease	Review of human data on graft-vs-host disease	Murphy et al. 2011[108]
Multiple sclerosis	Involvement of microbiota in mice with multiple sclerosis	Berer et al. 2011[109]
Nonalcoholic fatty liver disease	Effect of choline depletion in humans	Spencer et al. 2011[101]
Parkinson's disease	Role of enteric nervous system and review of Parkinson's disease development	Braak et al. 2003[110]
Rheumatoid arthritis	Microbiota as predisposing factor in rheumatoid arthritis	Scher and Abramson 2011[111]
Retrovirus infection	Mouse retrovirus infection relies on microbiota	Kane et al. 2011[112]
Poliovirus infection	Mouse microbiota promotes poliovirus infection	Kuss et al. 2011[113]

* The most recent single reference is given.

de Vos and de Vos 2012 *Nutr Rev* 70(suppl 1): S45-S56

Multiple bi-directional pathways
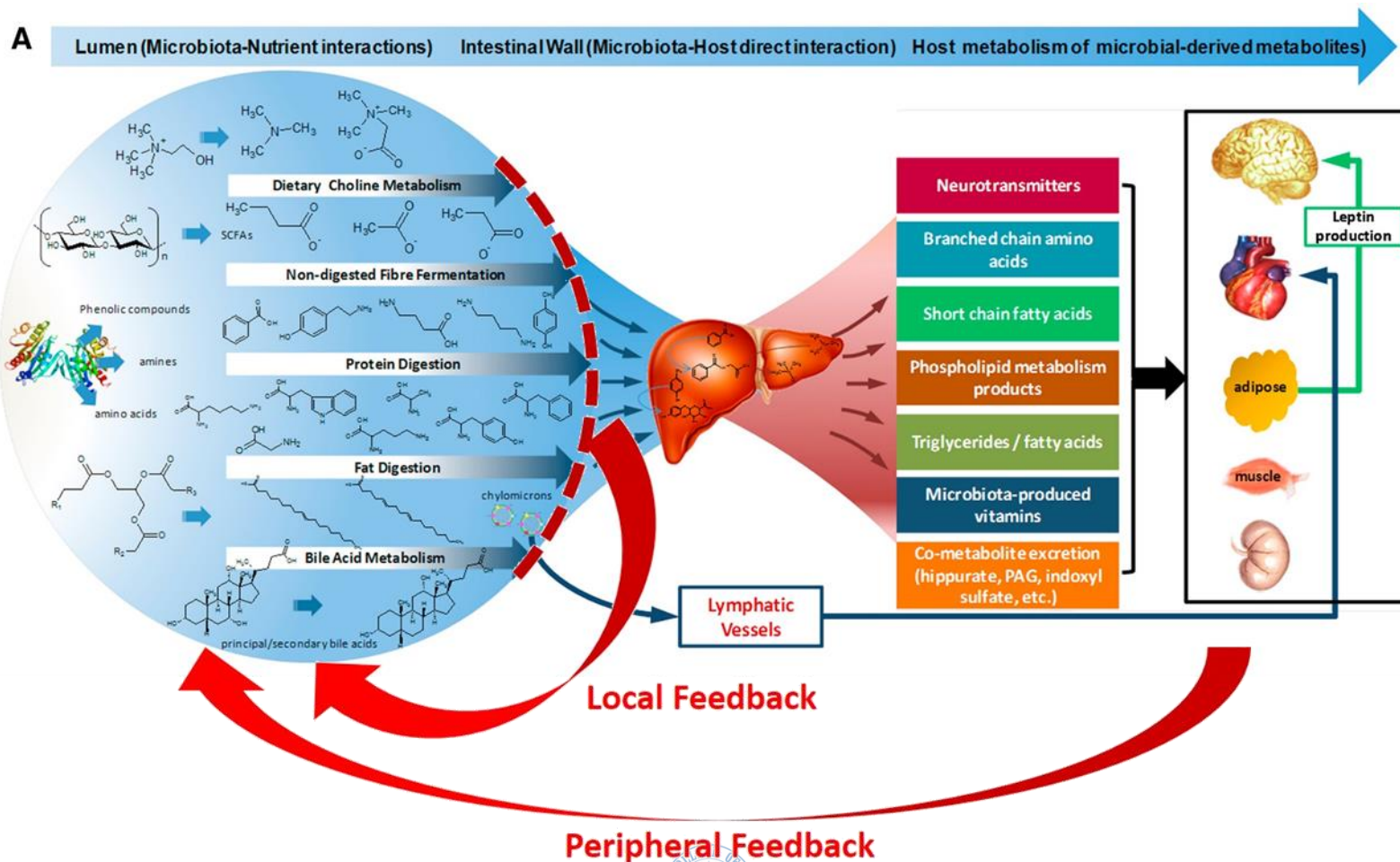
Holmes et al. 2012 *Cell Metab* 16(5): 559-564

Implications of microbiome in research

- Reproducibility
  - Which factors/procedures influence the composition or function of the Gut Microbiota of research animals?
    - Commercial source of mice
    - Husbandry
    - Rederivation
  - Which changes in Gut Microbiota influence model phenotypes?

- Translatability
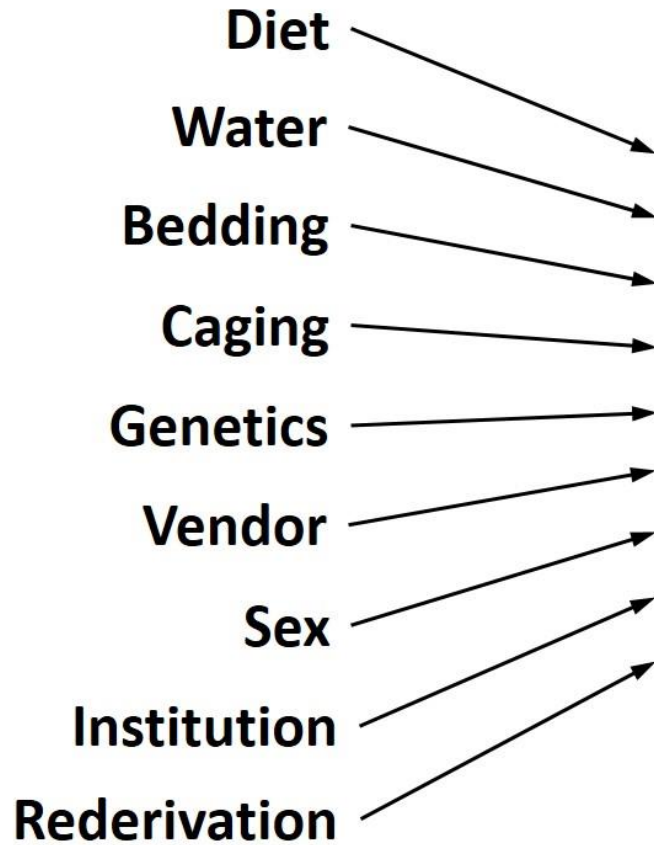 - How do we exploit the Gut Microbiota in research animals to generate the most translatable data?

Mice harbor same microbial families/genera
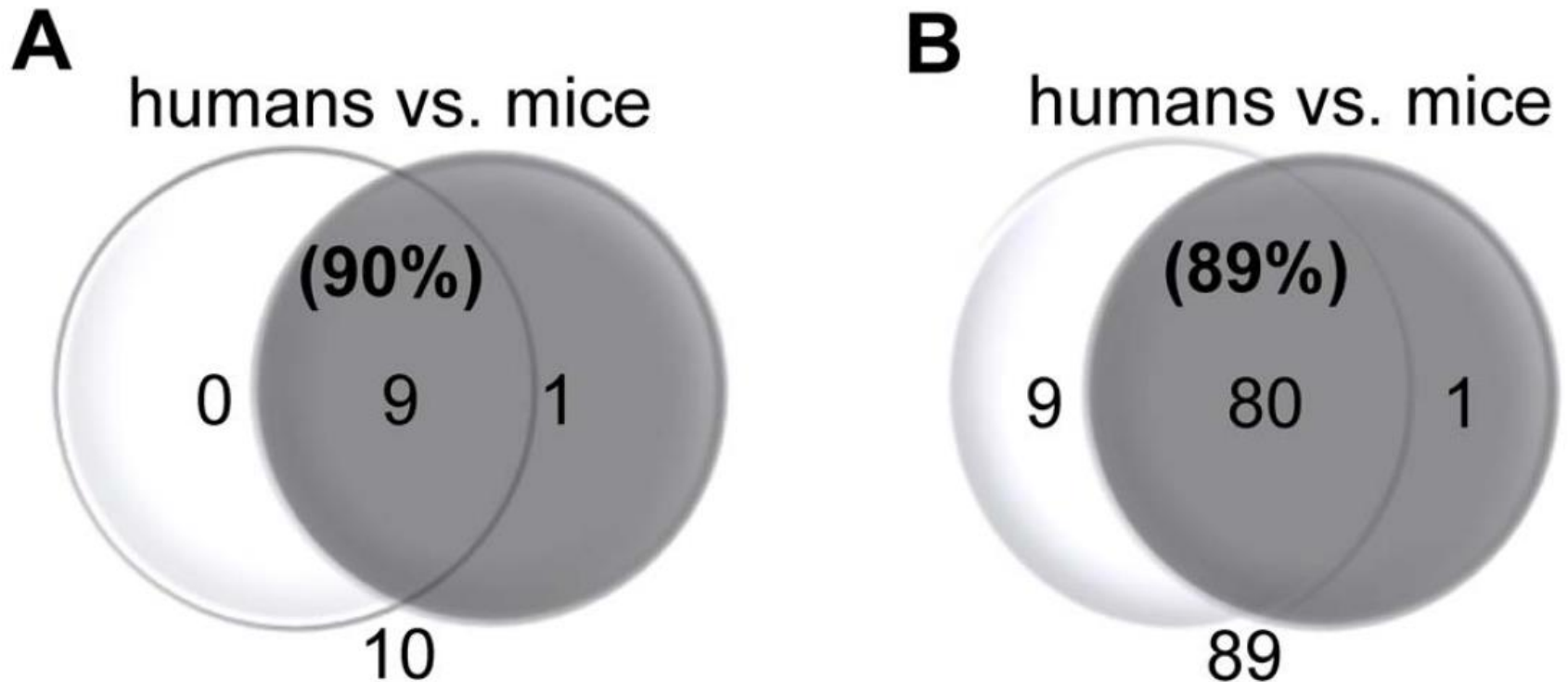
Number of Gut Microbiota taxa shared between human and mouse at level of **phylum** (**A**) and **genus** (**B**)

Krych et al. 2013 *PLoS One* 8(5): e62578

The Gut Microbiota as dependent variable

Diet
Water
Bedding
Caging
Genetics
Vendor
Sex
Institution
Rederivation

Mice seeded at vendor; Gut Microbiota shaped by genetics
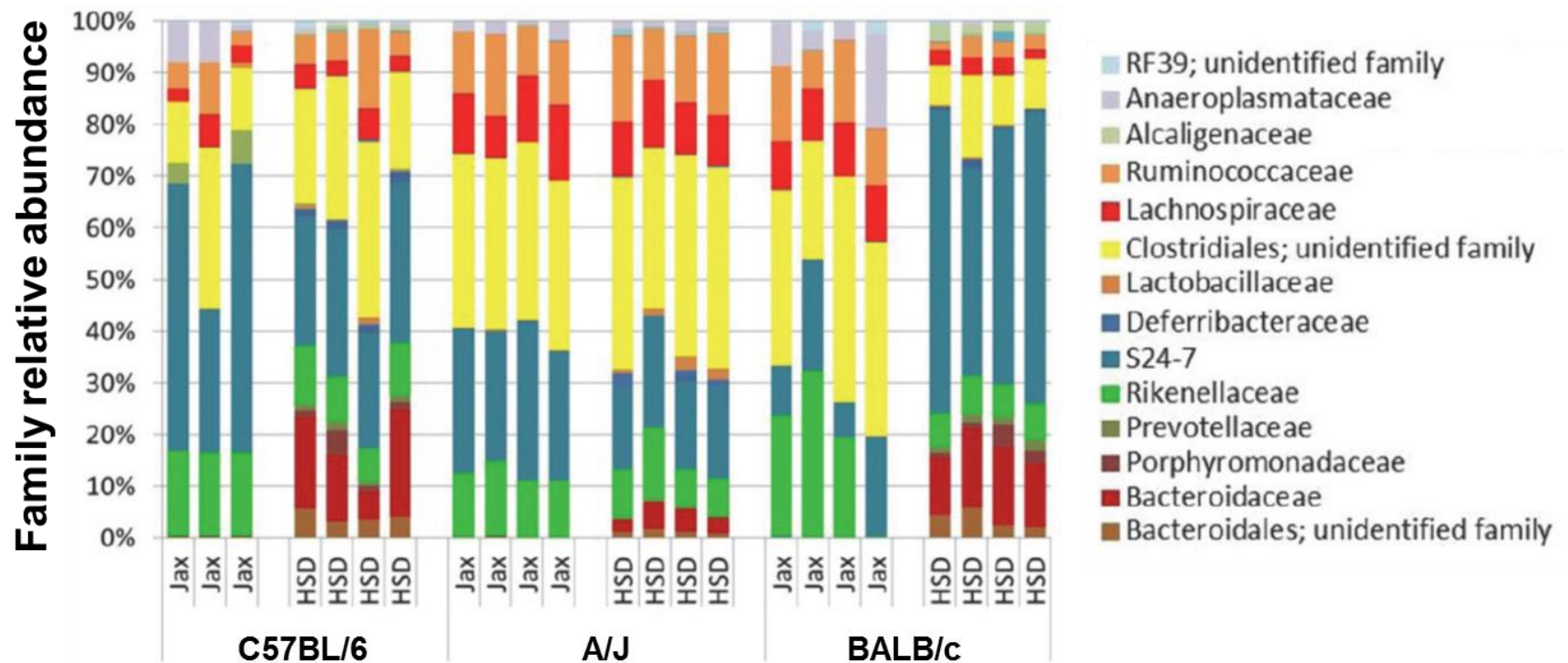
Rederivation leads to shift in colony Gut Microbiota
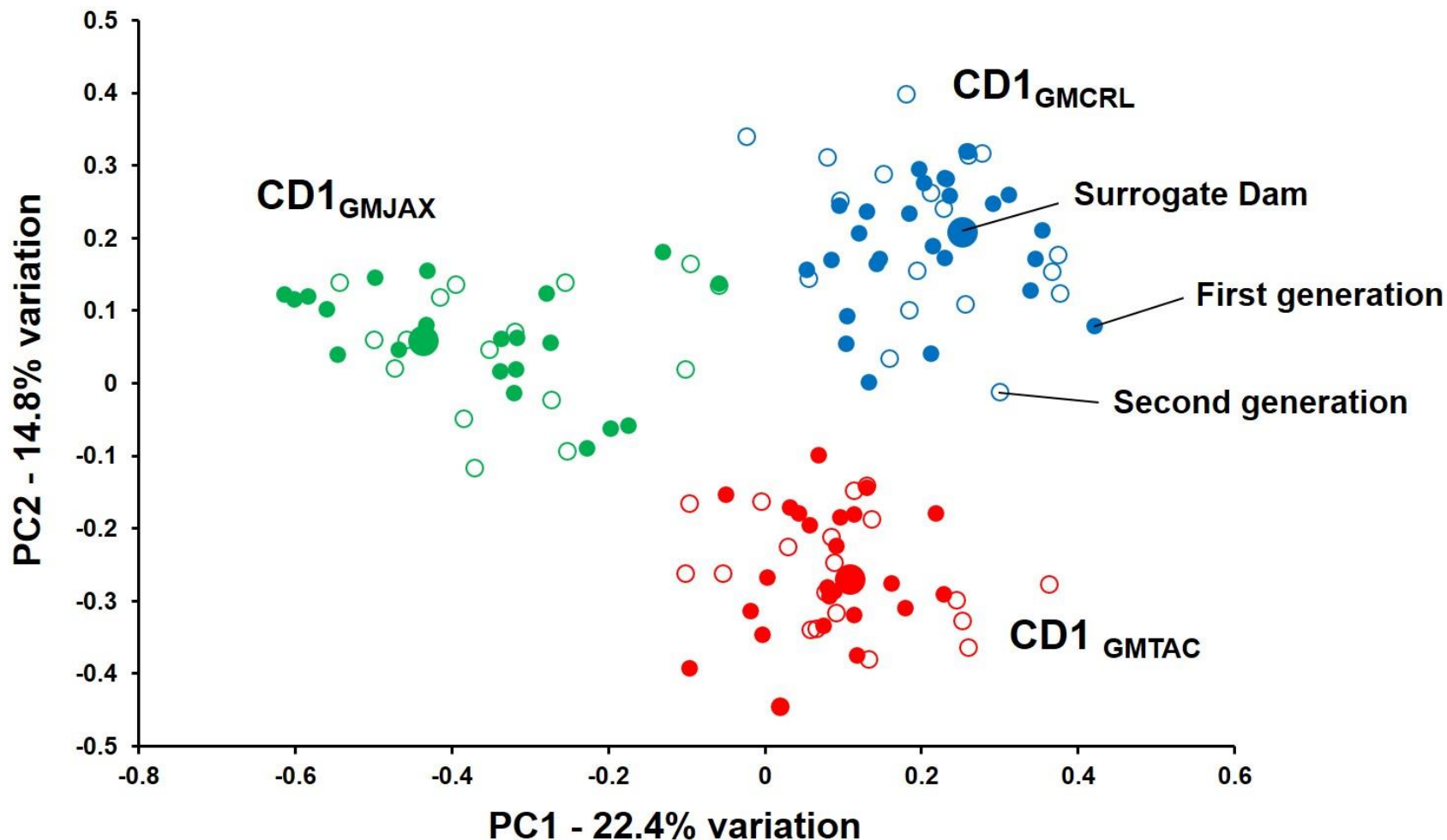
Hart et al. (manuscript in preparation)
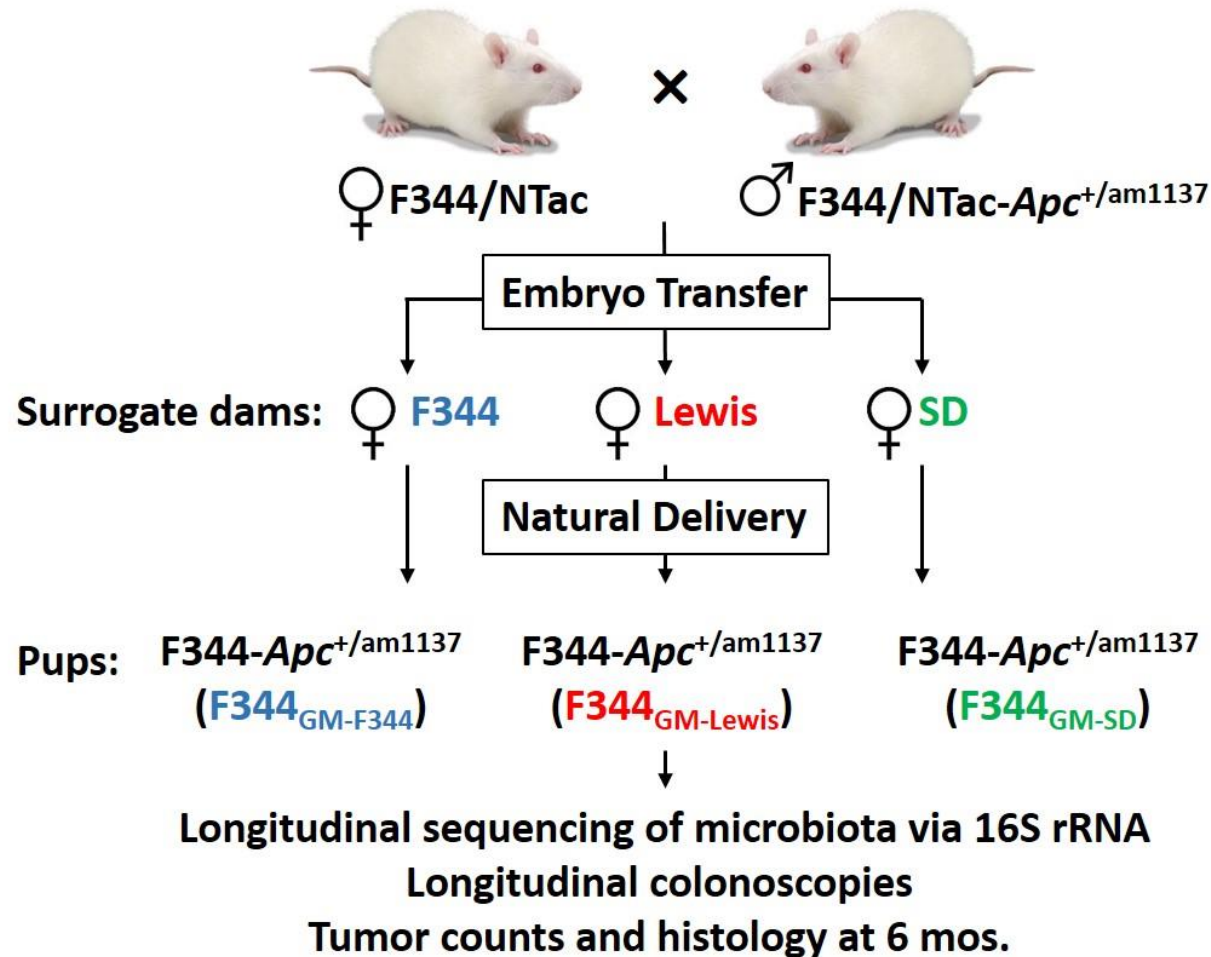
Genetic background shapes composition

Hart et al. (manuscript in preparation)
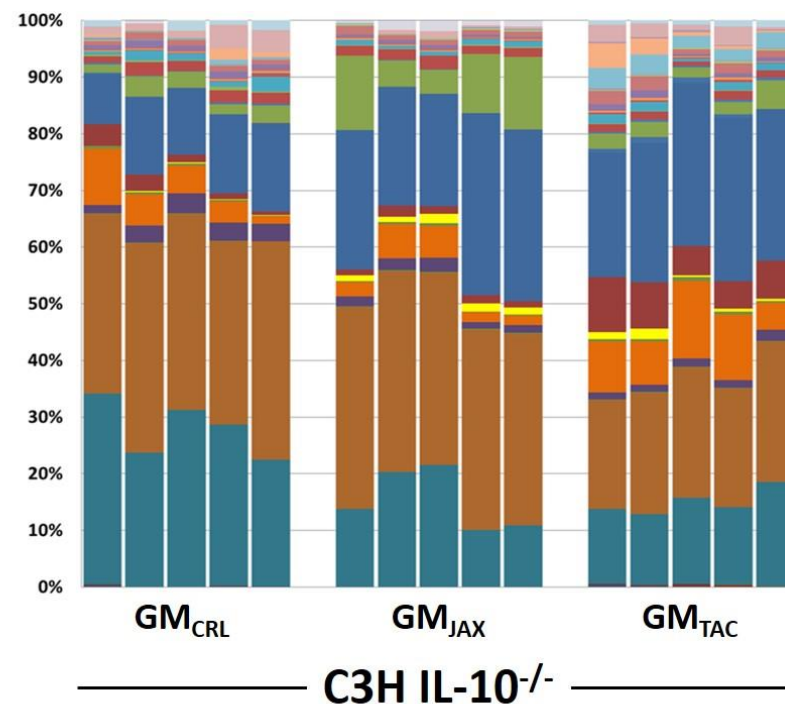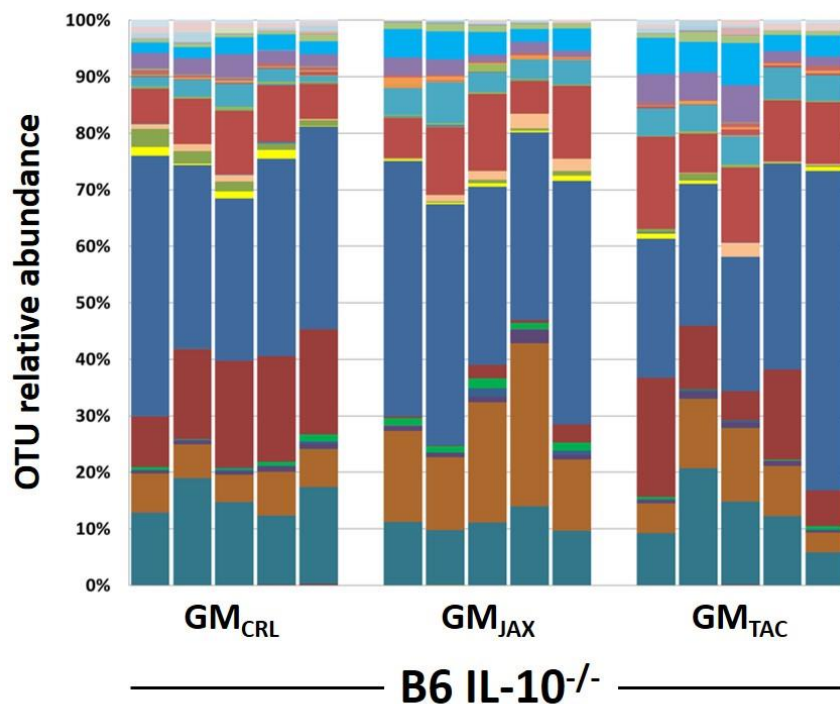
Fecal microbiota ≠ "gut" microbiota
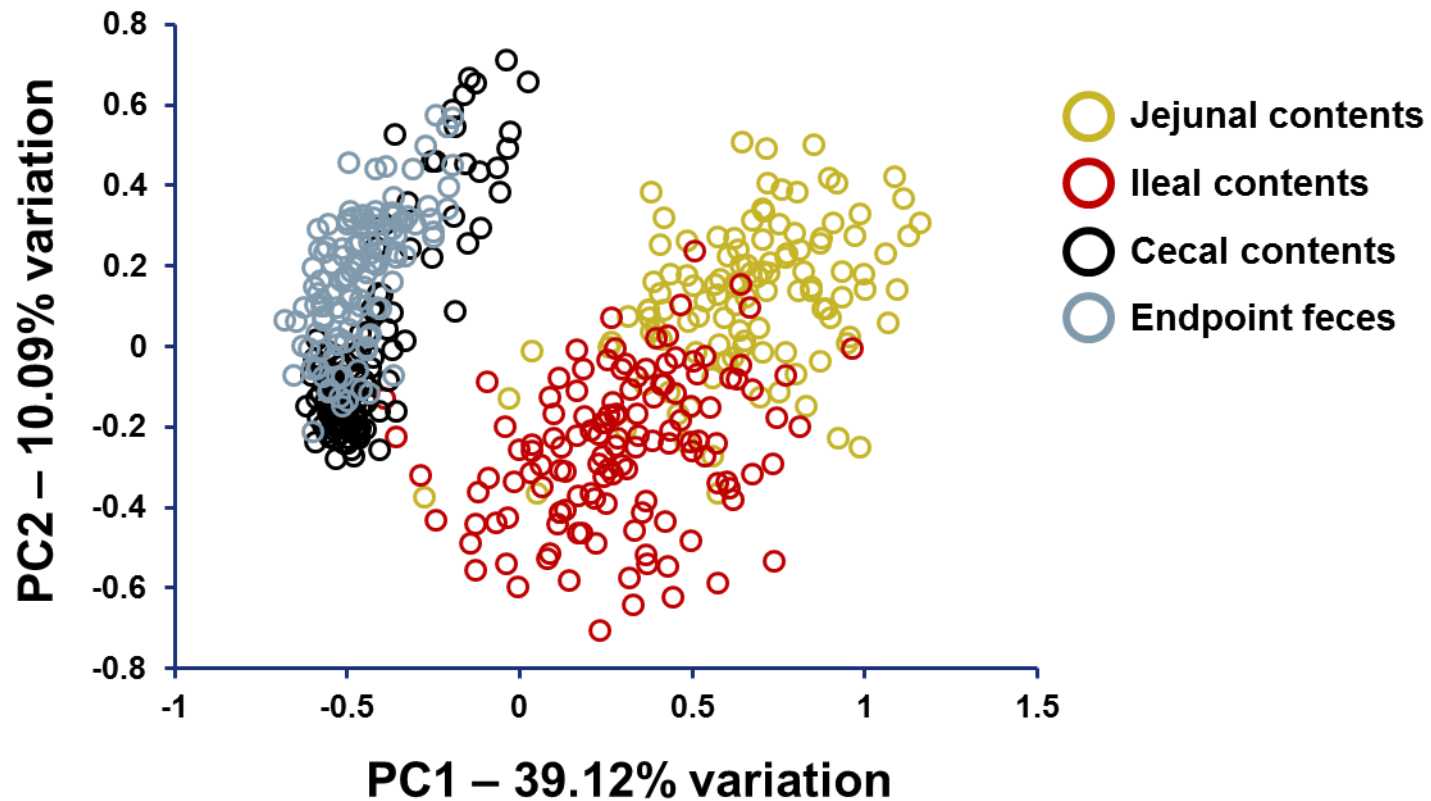
Influence of husbandry on cecal microbiota
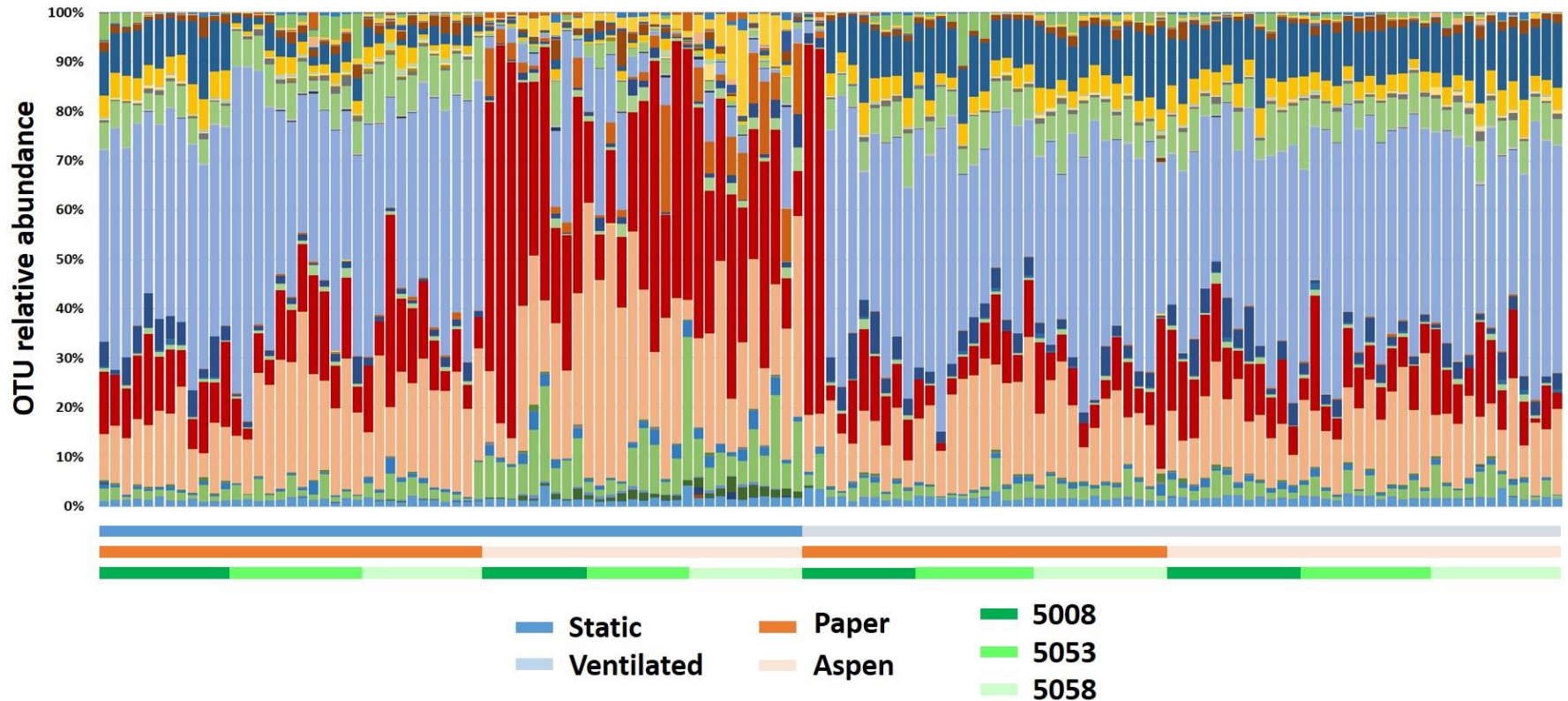
Ericsson et al. (manuscript in preparation)

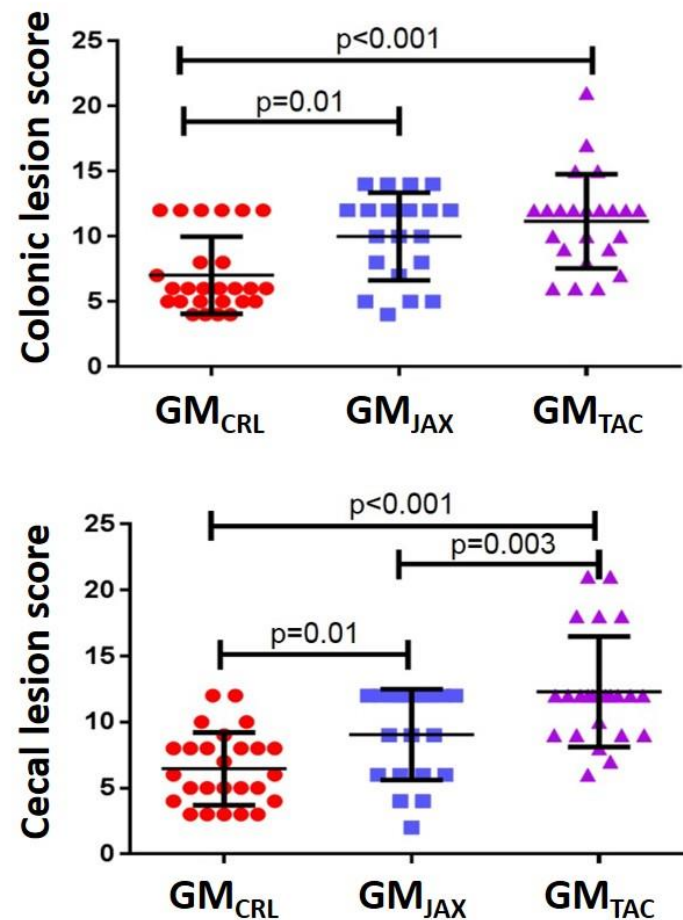# Complex Microbiota Targeted Rederivation
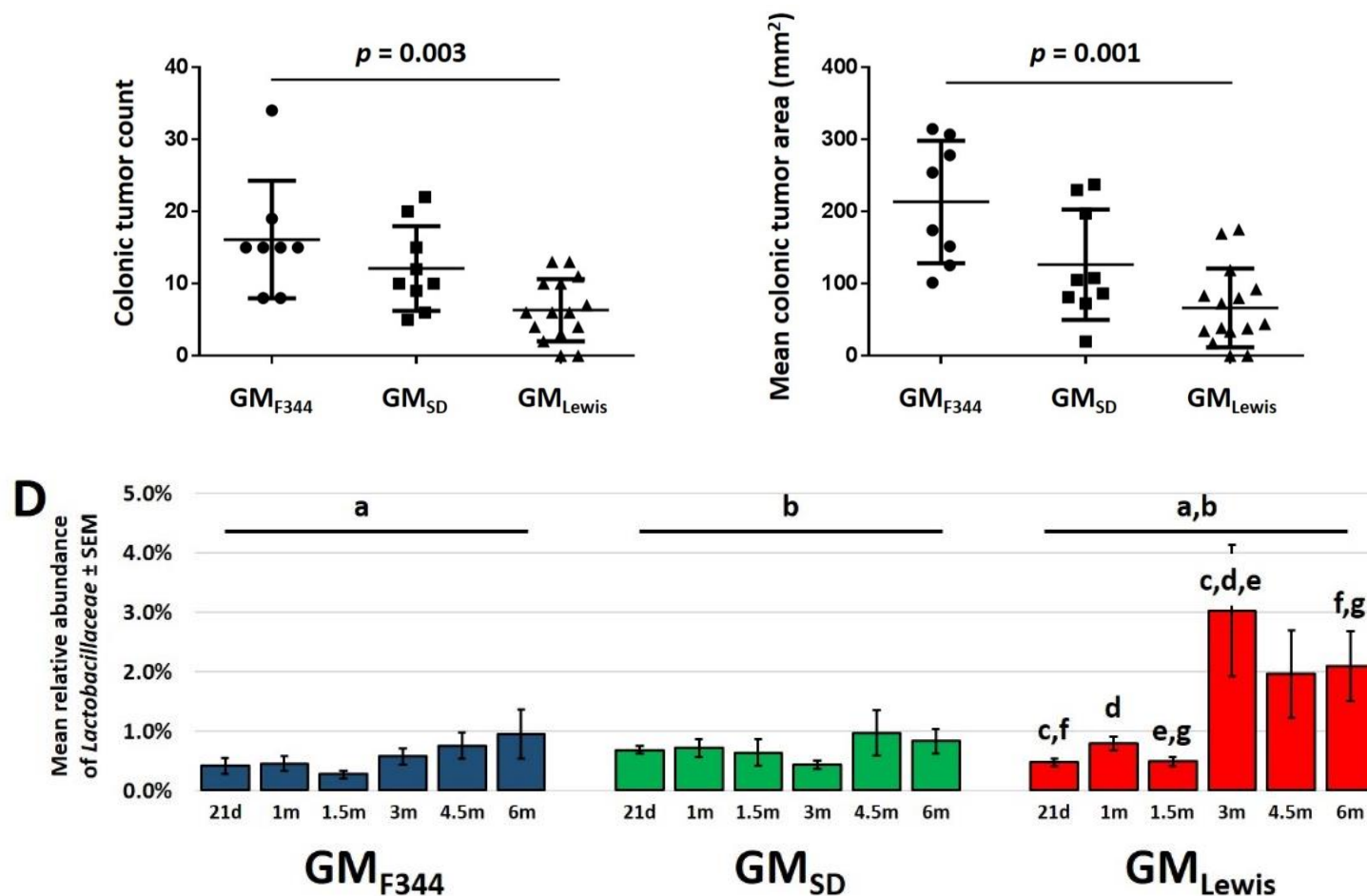
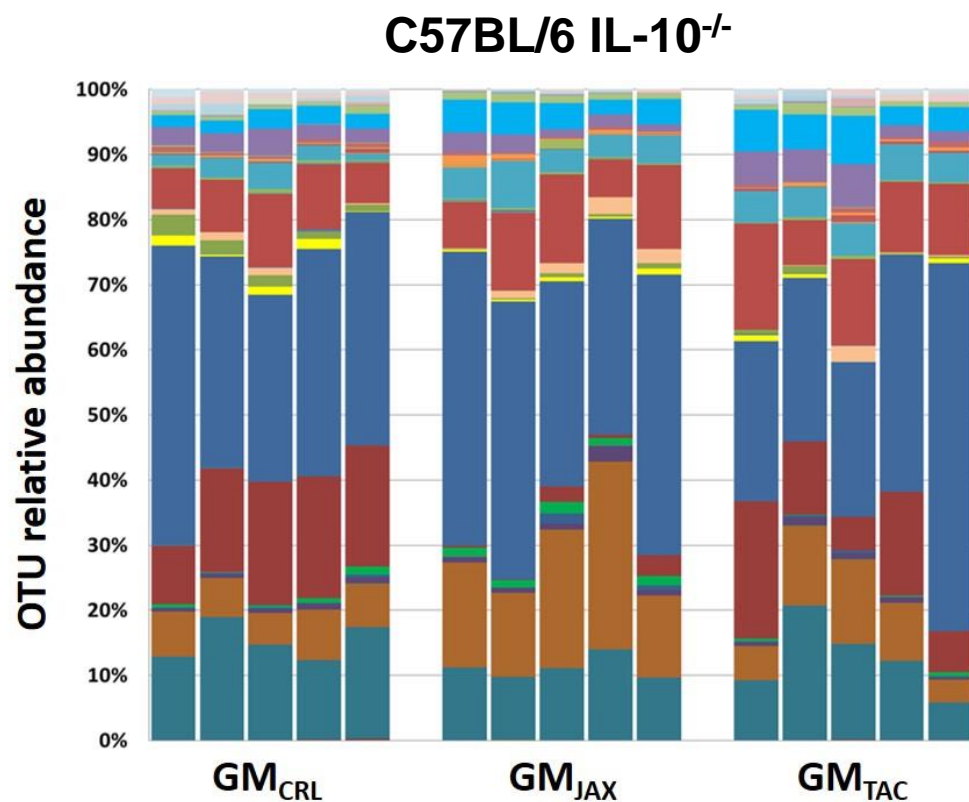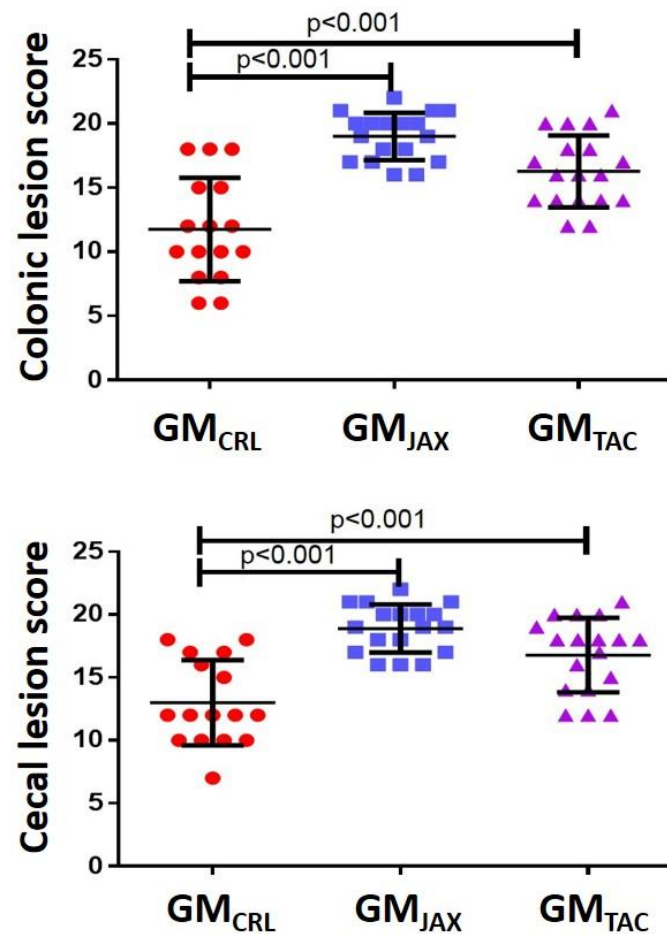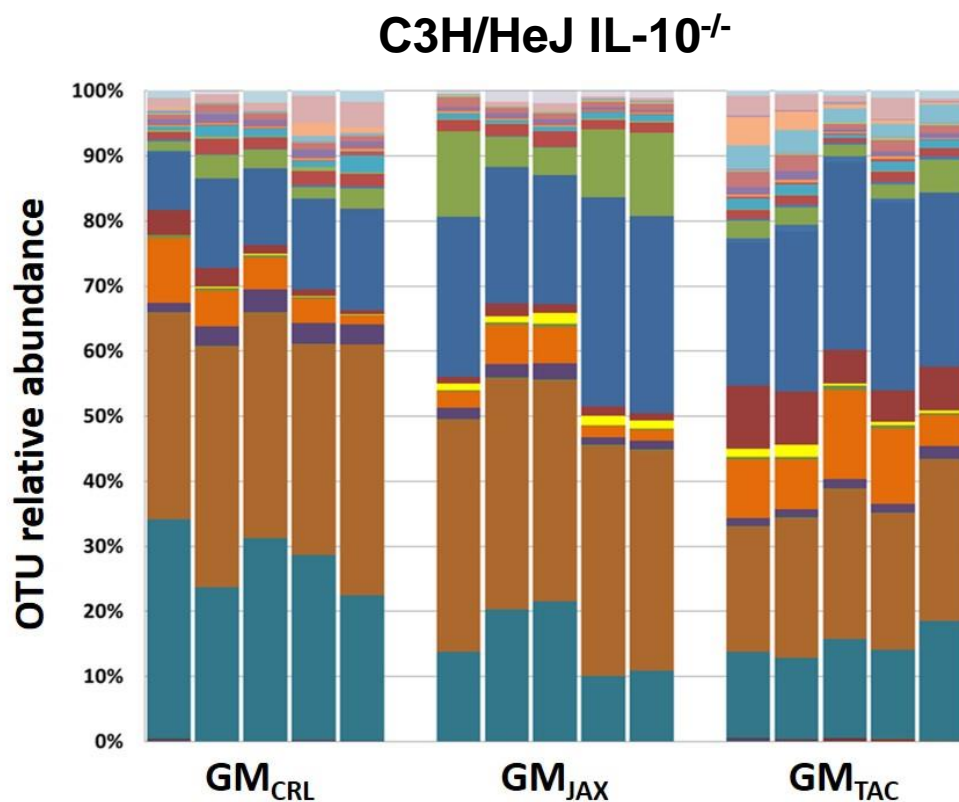Ericsson et al. 2015 *Oncotarget* 6(32): 33689-33704

Different Gut Microbiota → different disease

Ericsson et al. 2015 *Oncotarget* 6(32): 33689-33704
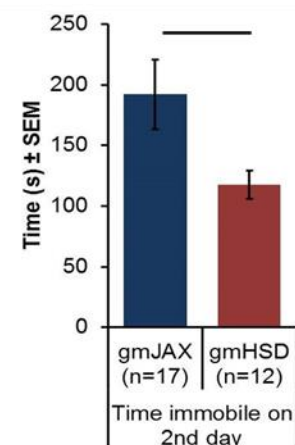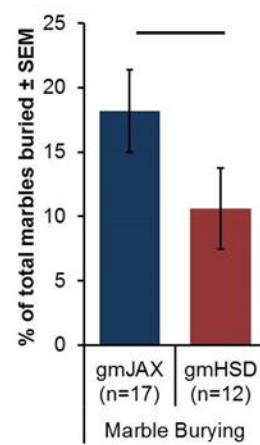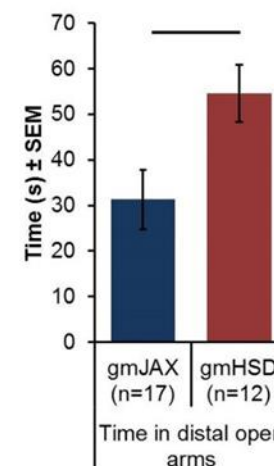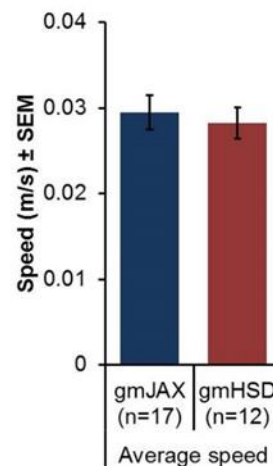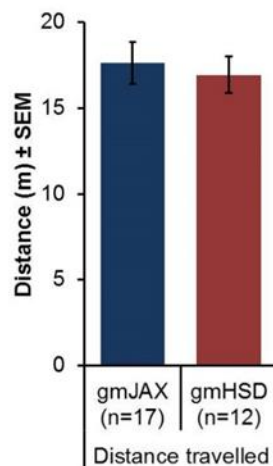
Different Gut Microbiota → different disease

Hart et al. (manuscript in preparation)

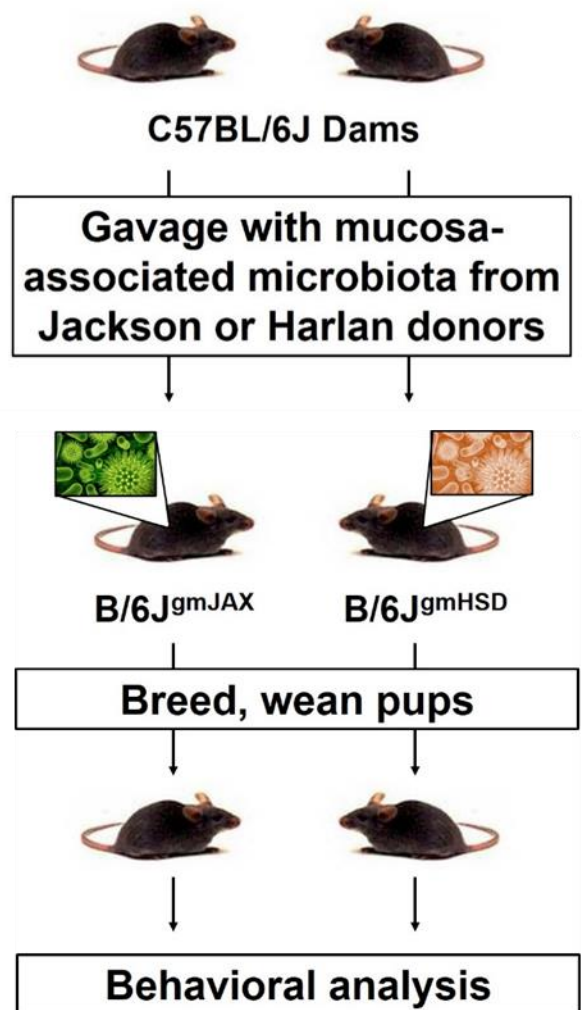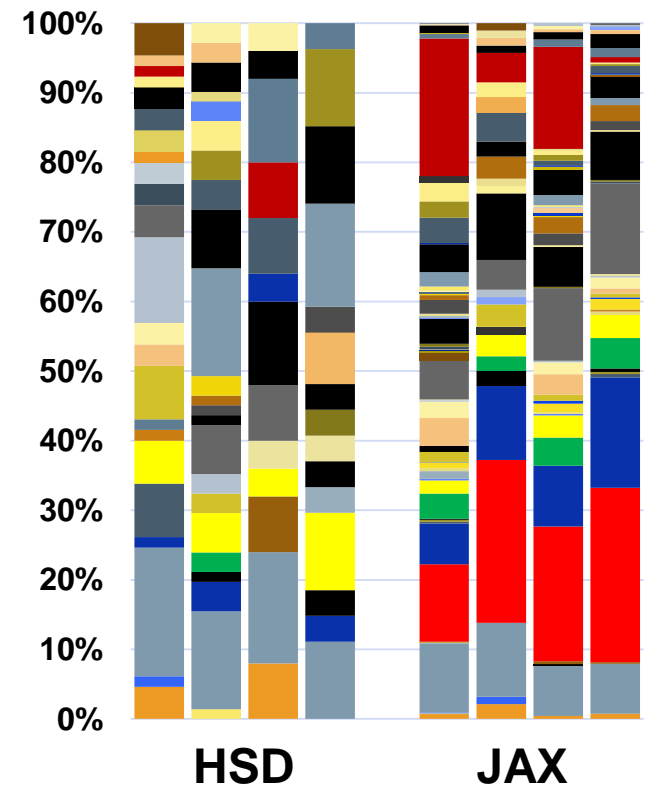# Different Gut Microbiota → different disease

C3H/HeJ IL-10⁻/⁻

Hart et al. (manuscript in preparation)

Different Gut Microbiota → different behavior

Davis et al. (manuscript in preparation)

Skin microbiota affects local immune response
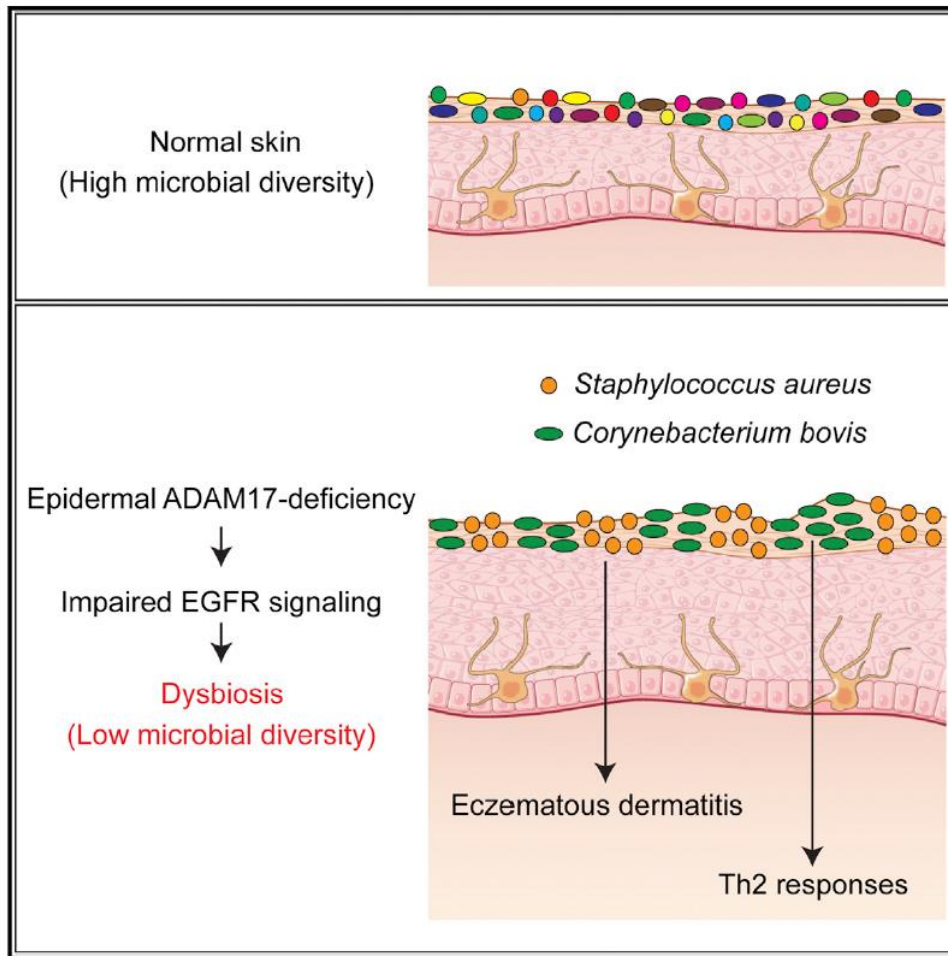
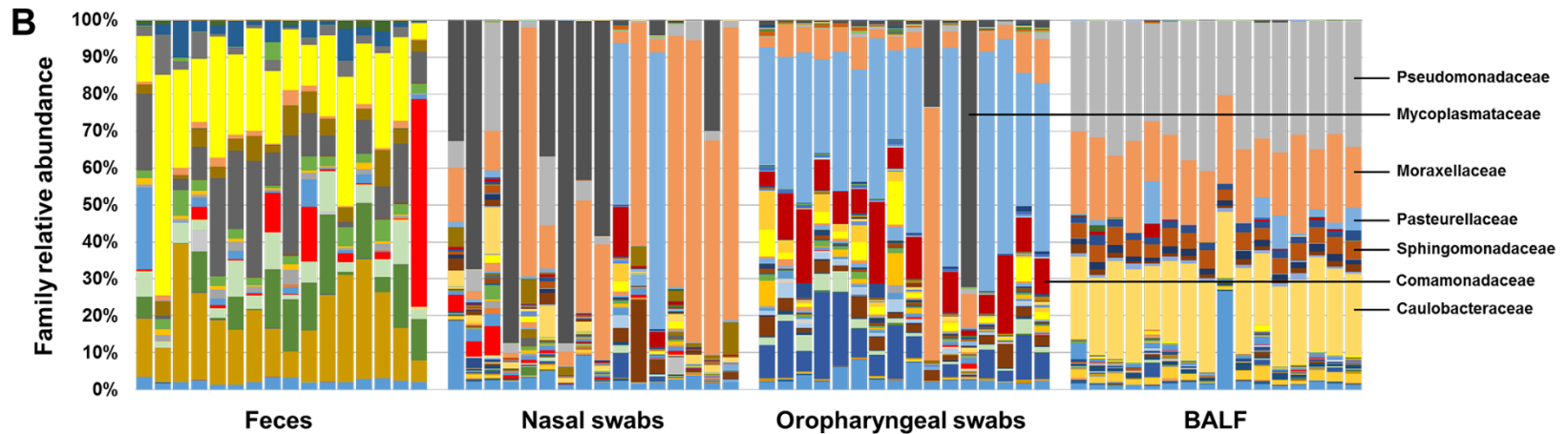

Kobayashi et al. 2015 *Immunity* 42: 756-766

Respiratory microbiota

- Airways harbor uniform microbiota
- Composition differs in inflamed airways (canine chronic bronchitis, feline asthma)
- Influenced by micro-aspirations
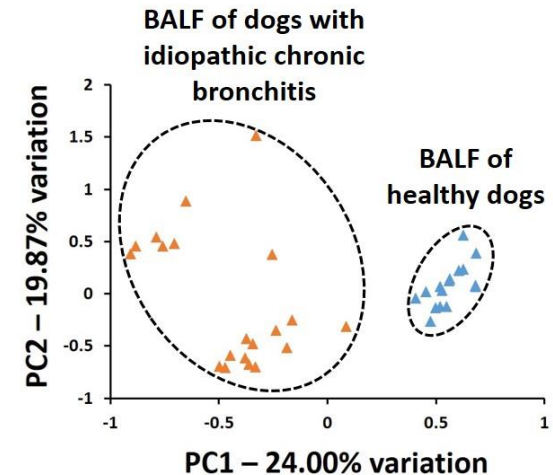
Ericsson et al. 2016 *PLoS One* 11(5): e0154646

Do "ultra-clean" mice limit translatability?

Gnotobiotic rederivation
Reduction in bacterial pathogens

Domestication
Pathogens common

Improved diagnostics
Reduction in viral pathogens

1900   1950   1980   2016

Overall "pathogen" exposure

Microbial diversity?
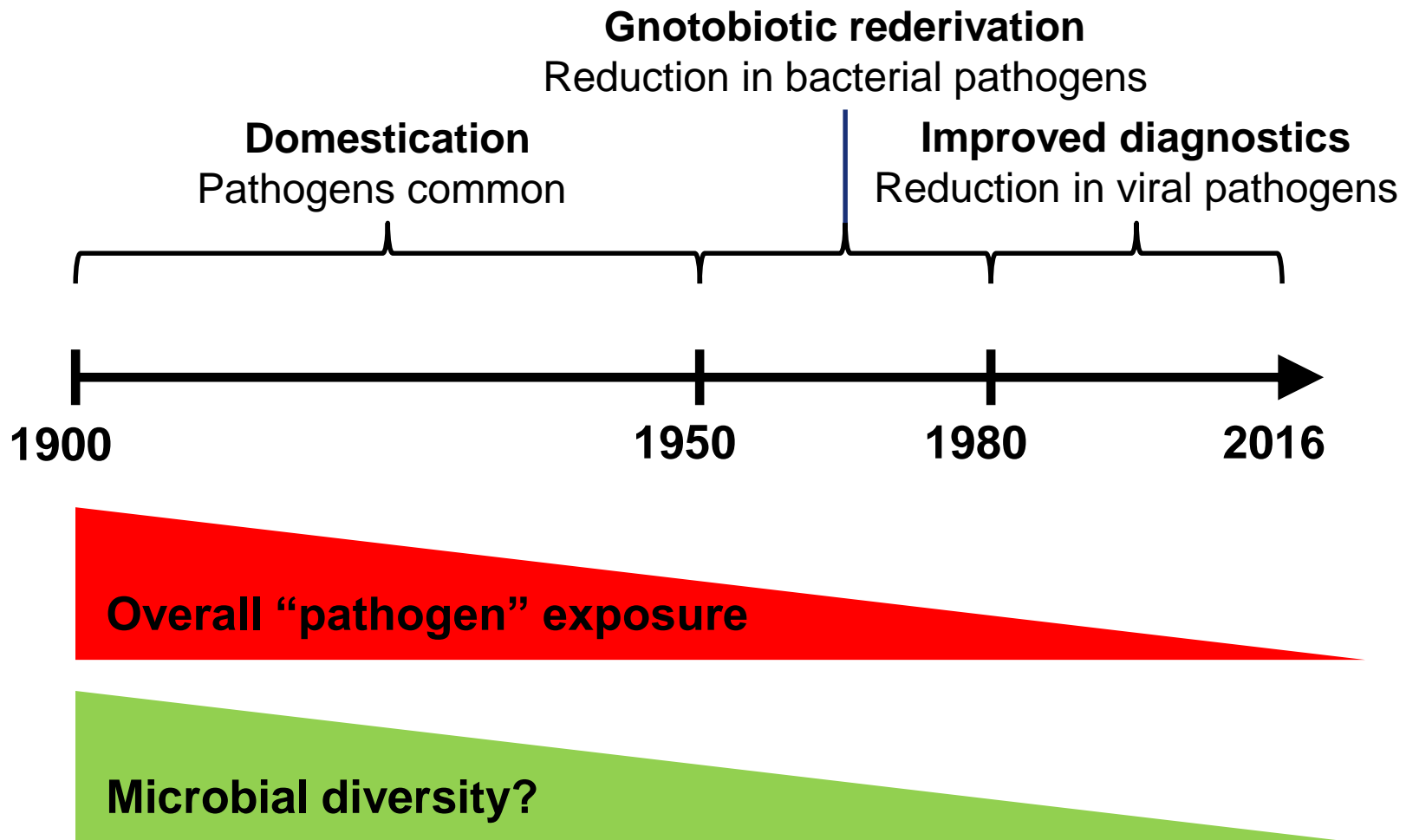
Weisbroth et al. 1996 *Lab Anim* 25: 25-33

How do we model microbial diversity?
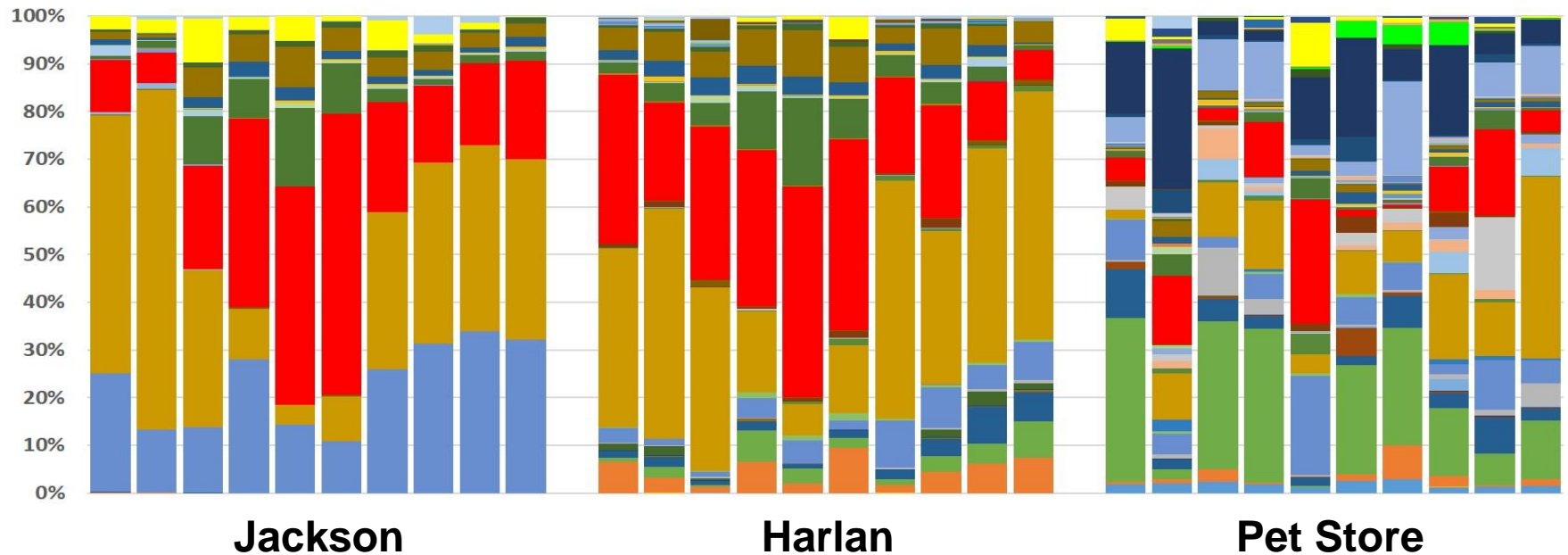
SPF mice model *neonatal* immunity

Specific-Pathogen Free

Immature, neonate-like

Barrier-raised laboratory mice

Gene expression

vaccination   Response A

A   B

Infect with specific viruses, bacteria and parasites

vaccination   Response B

Gene expression

Co-infected

Mature, adult-like
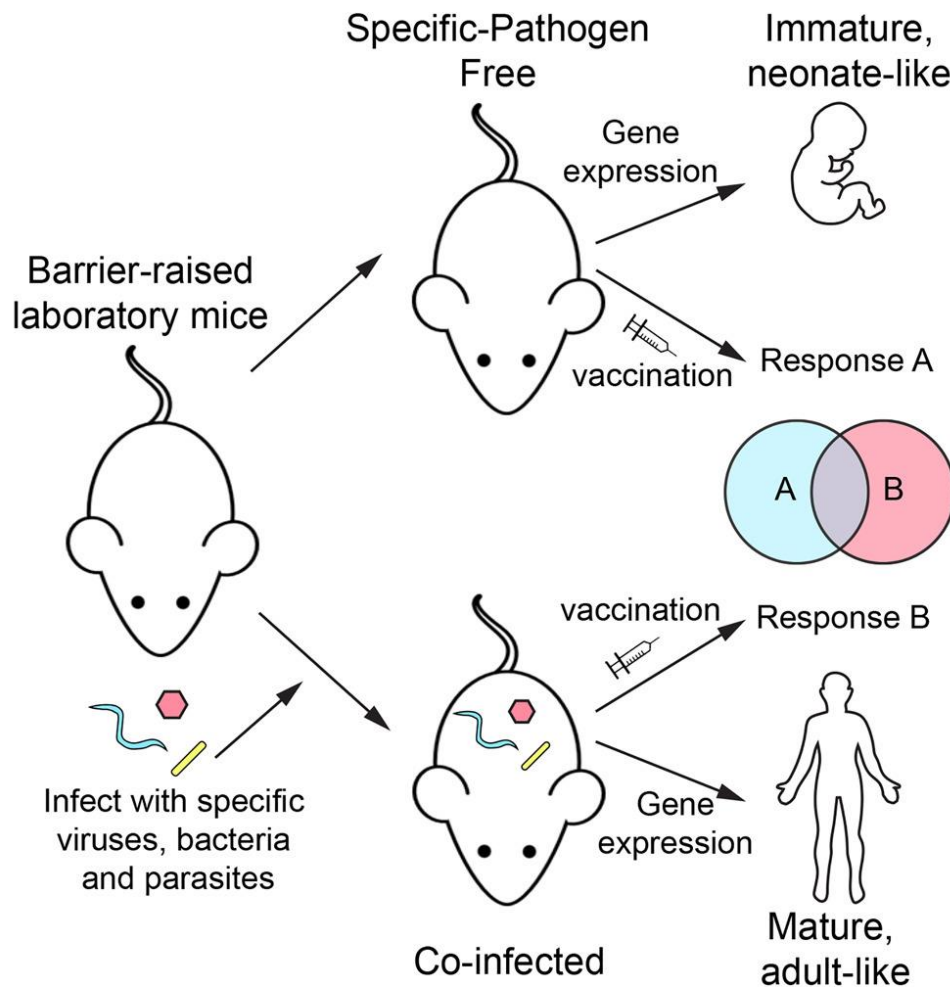
Reese et al. 2016 *Cell Host Microbe* 19(5): 713-719

Are feral/pet store mice more appropriate?

Beura et al. 2016 *Nature* 532: 512-518
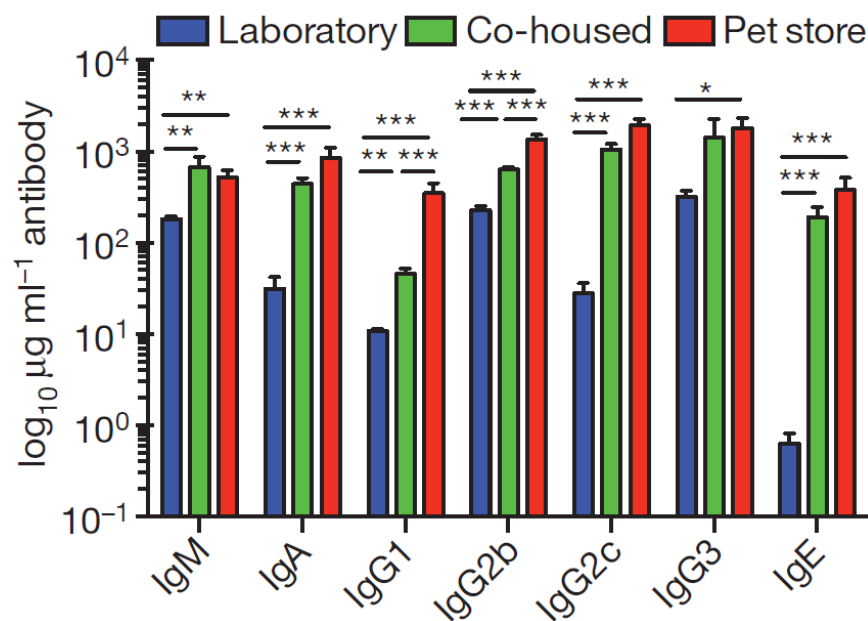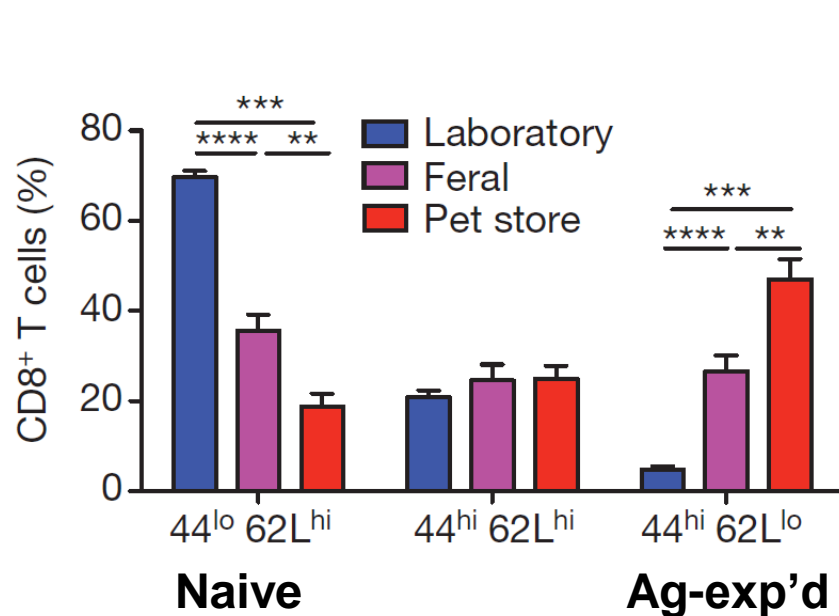
Summary

- Gut microbiota seeded early after birth but shaped by myriad factors specific to each institution

- Differences in gut microbiota associated with source or husbandry of animals can lead to differences in model phenotype

- Overly "clean" mice may not model humans accurately

Reproducibility   **Translatability**
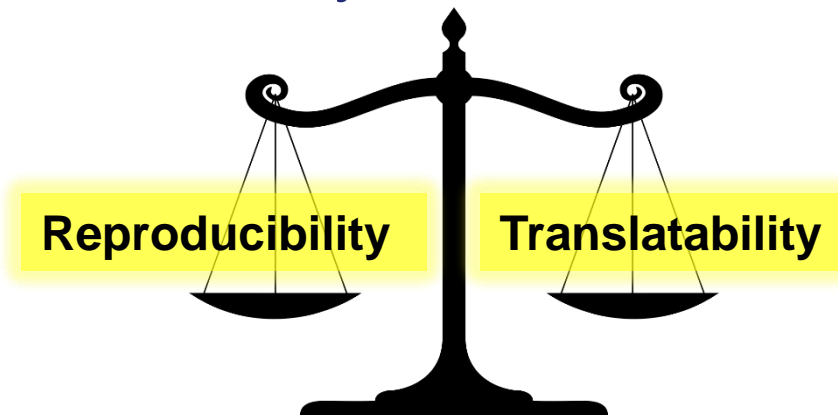
Acknowledgements

- **Craig Franklin and MMRRC**

- **Elizabeth Bryda and RRRC**

- **MU Comparative Metagenomics Laboratory**
  - **Marcia Hart, DVM, MS**
  - **Annie Wolfe**
  - **Dan Montonye, DVM**
  - **Matt Burch**
  - **Jasmine Hall**
  - **Giedre Turner**
  - **Becky Dorfmeyer**
  - **Willie Bidot, DVM**

- **Nathan Bivens and the MU DNA Core**

- **Bill Spollen and the MU Informatics Research Core Facility**

- **Yuksel Agca and MMRRC Cryobiology Lab**

- **Jim Amos-Landgraf and Lab**

- **Carol Reinero and Lab**

- **Leah Cohn and Lab**

- **Catherine Hagan (Gillespie) and Lab**

MMRRC
Mutant Mouse Resource & Research Centers
supported by NIH

NIH 2U42 OD010918-16

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NIH P40 OD011062-13

MUMC
MU Metagenomics Center

Mizzou Advantage – One Health