

Differences in gut microbiota significantly modulate colon cancer susceptibility in the rat genetic model of familial colon cancer

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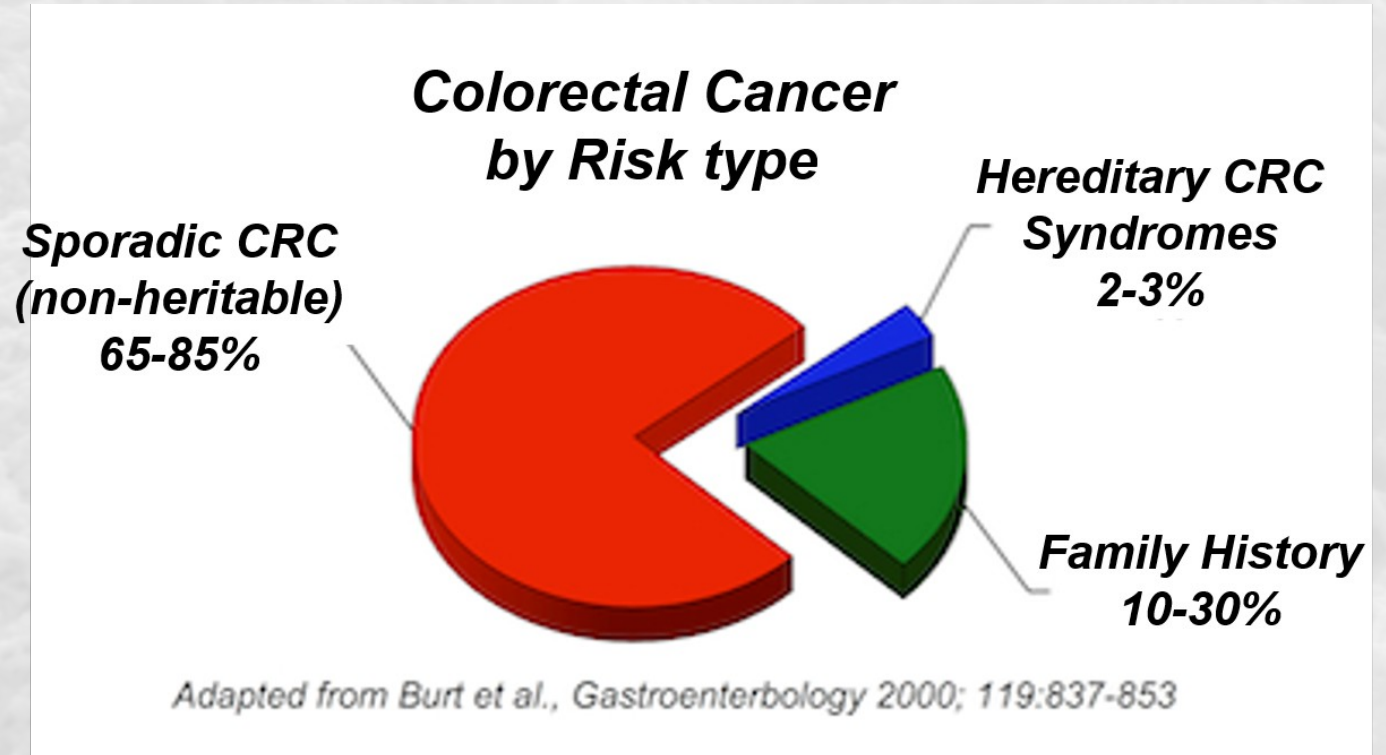
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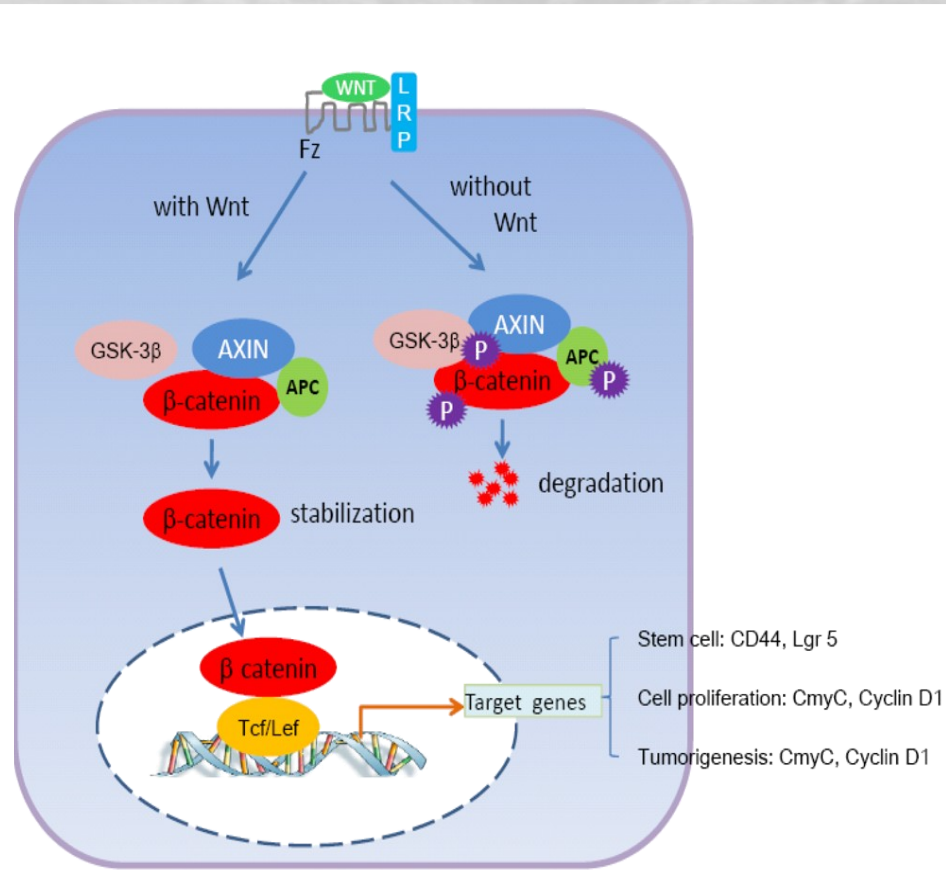


Colorectal cancer

- Multifactorial: Genetics, environment, lifestyle
- Familial Adenomatous Polyposis (FAP), Lynch syndrome
- ~80% of CRC is sporadic
- Smoking, dietary factors
- Pre-existing inflammatory conditions



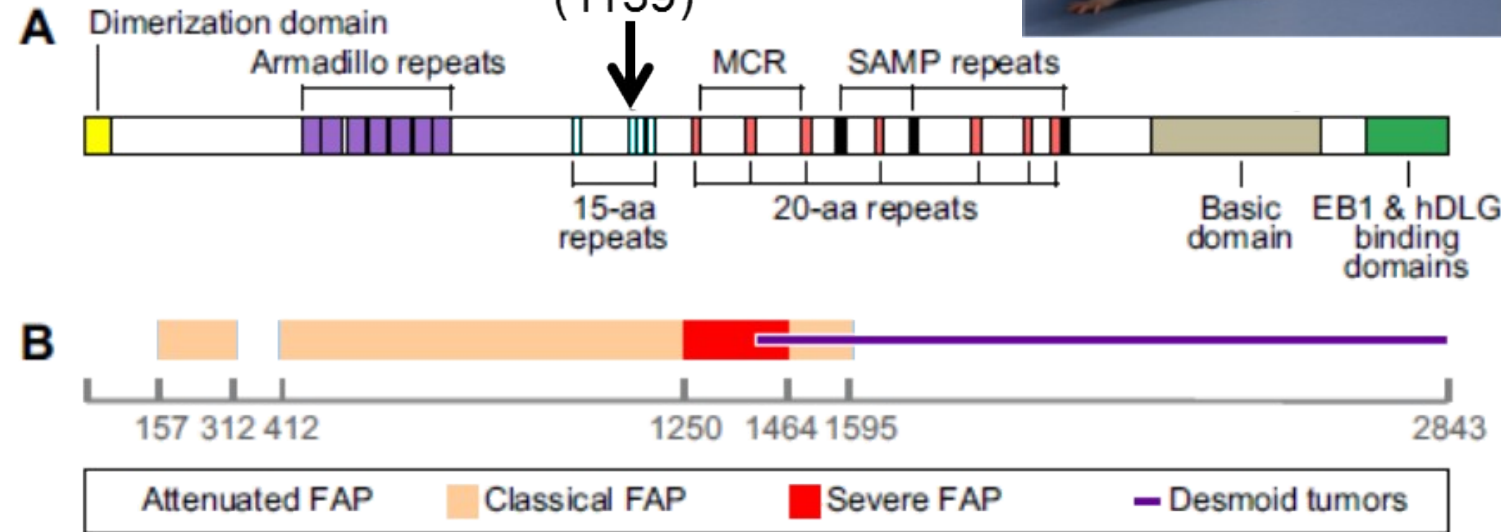
F344 Polyposis In Rat Colon (PIRC) model



Apc

Pirc

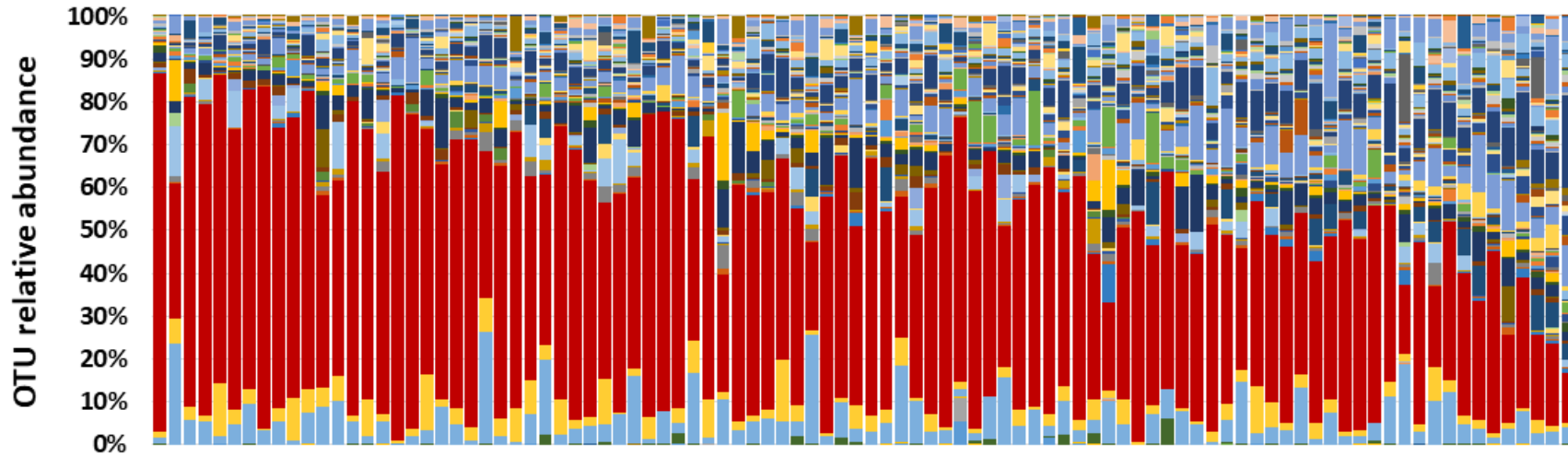
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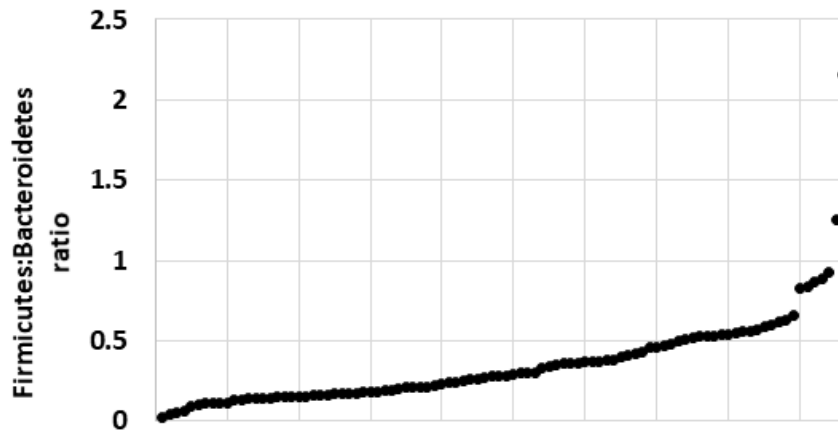
Genetic control of the F344-*Apc*^{Pirc} rat phenotype

		Tumors		
Strain	Sex	Minimum	Maximum	Average Total
F344/NTac	M	6	24	14
(ACI x F)F1	M	14	81	31.8
ACI (N10)	M	12	56	30.3
[(AxF)F1xACI]N2	M	23	93	52.7
[(AxF)F1 x F344NTac]N2	M	4	129	39.0
(BN x F)F1	M	3	48	17.1
BN (N8)	M	0	3	0.8
(WFxF344NTac)F1	M	1	6	3.5

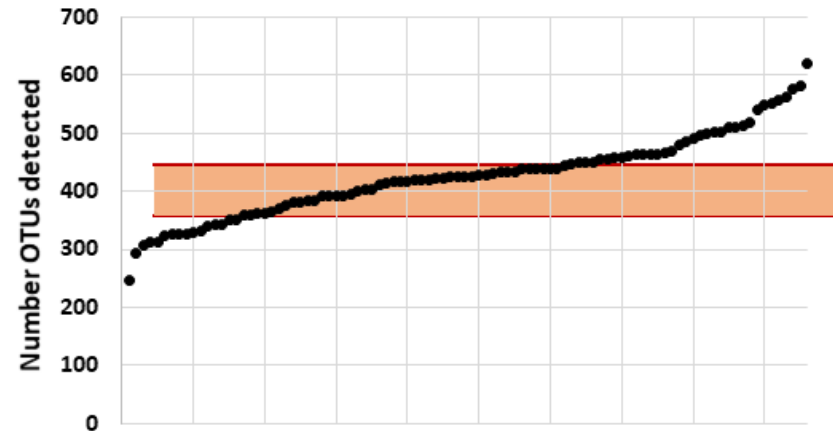
Variability gut of microbiome in “academic” colonies



Individual mice donated to the MMRRC for cryopreservation

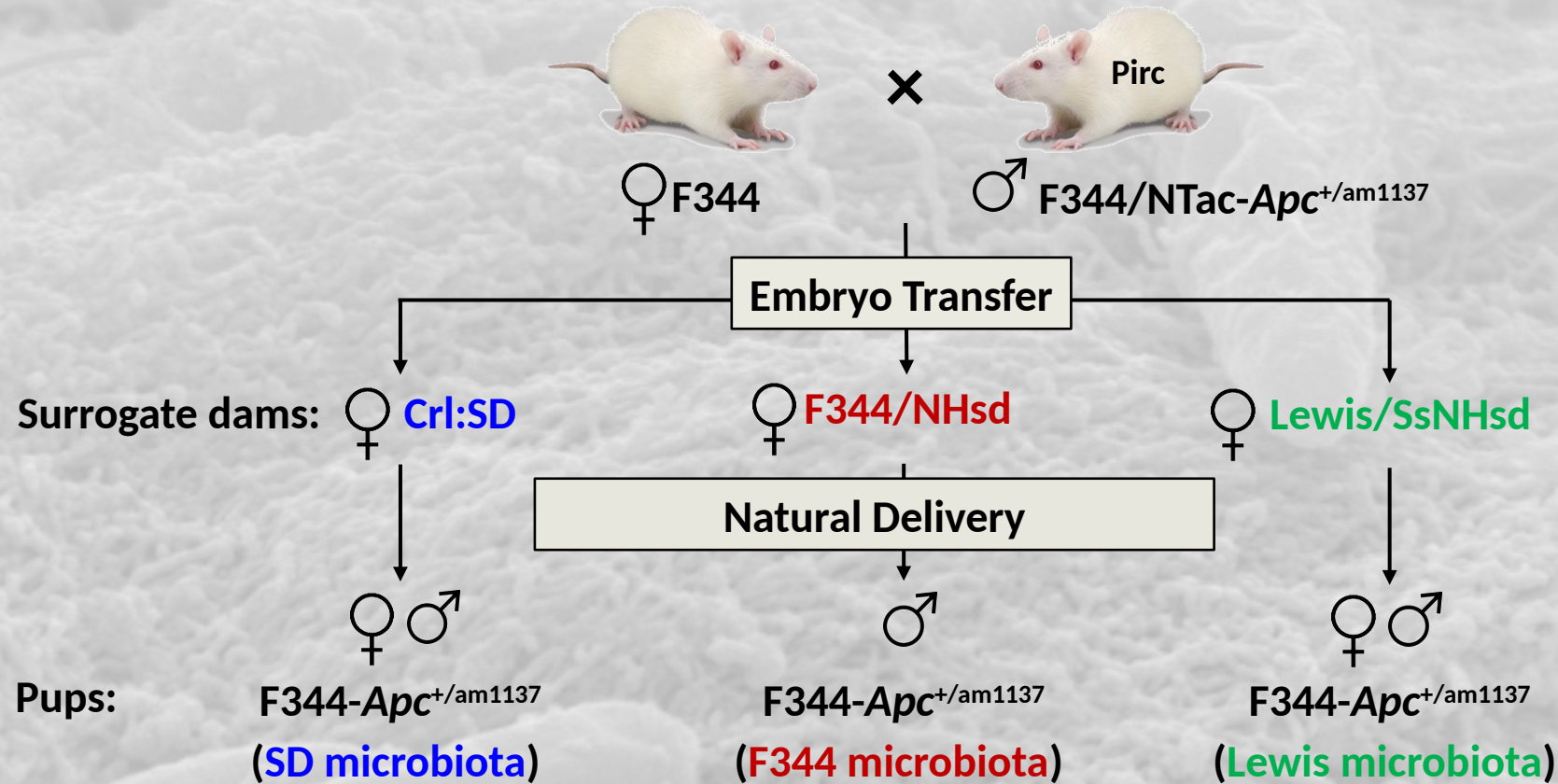


Individual mice donated to the MMRRC
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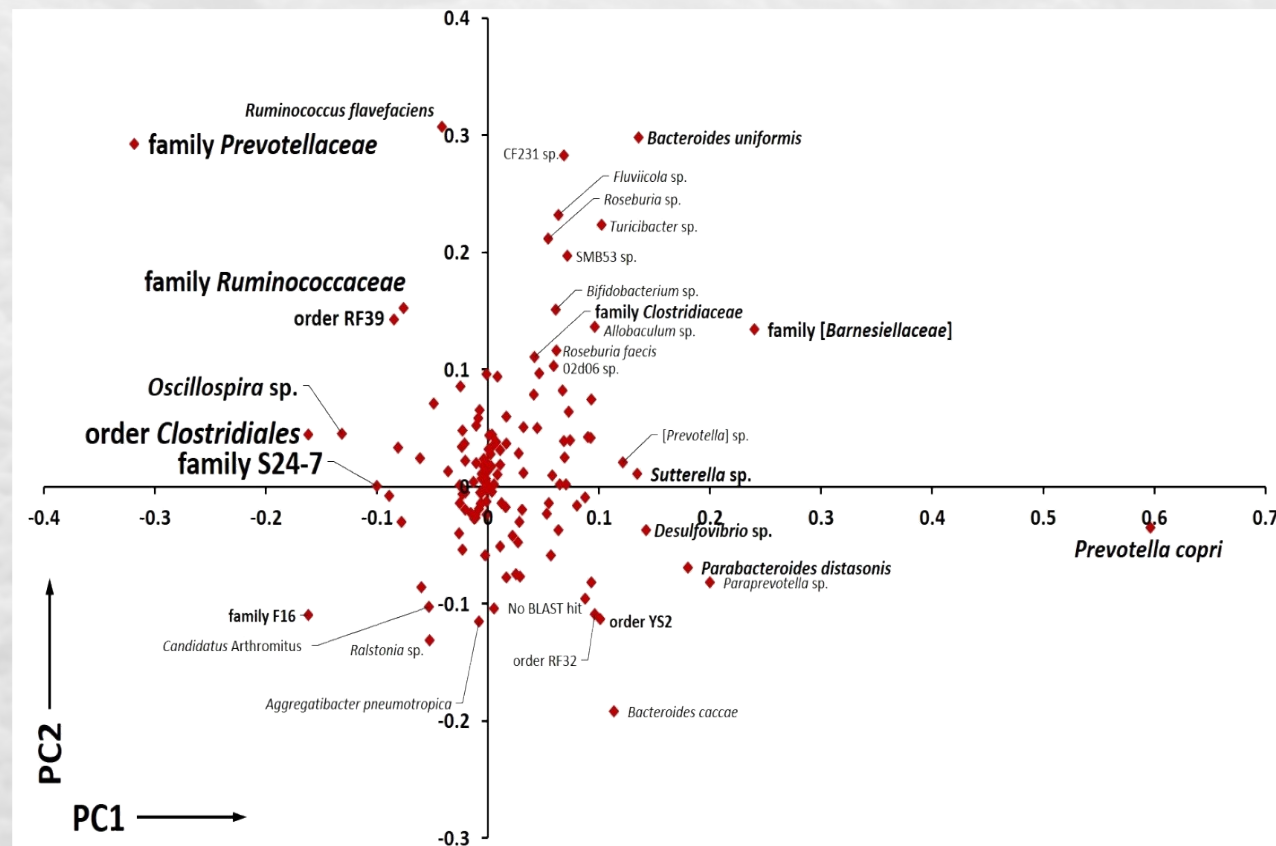
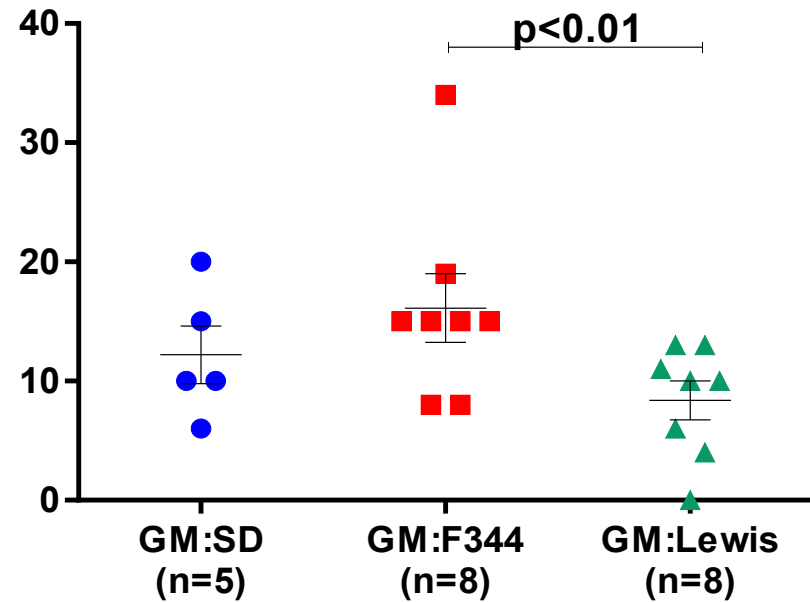
Complex Microbiota Targeted Rederivation (CMTR) Isogenic Pups: Different Complex Gut Microbiota



Longitudinal colonoscopies

Longitudinal sequencing of microbiota via 16S rRNA

Tumor Multiplicity + SEM



Differential susceptibility to colorectal cancer due to naturally occurring gut microbiota

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CRASF rats: Charles River Altered Schaedler Flora

- CRL refreshes rat genetics every 5 years
 - Importation of global CRL units
 - Rederivation
 - Surrogate dams w/ CR-ASF
- “It never hurts to ask, what’s the worst they can say.”
- ” You can always ask again”
- Received LEW/CrI- CRASF Dams
- Maintained on IVC rack

1. SCHAEGLER 19X BACTEROIDES - This species of bacteroides produces a small to medium-sized convex, gray colony consisting of pleomorphic rods which can appear as coccobacilli. It has been identified as B.distasonis.

2. SCHAEGLER L1 LACTOBACILLUS - This species of lactobacillus is referred to as "rhizoid" because of the typical colonial morphology with an irregular border. The cellular morphology consists of a large rod with blunt ends. It has been identified as L. acidophilus.

3. SCHAEGLER L3 LACTOBACILLUS - This species is referred to as a compact lactobacillus because of the non-rhizoid colonial morphology. Colonies are round, raised, and of a yeasty consistency. The cells are smaller rods than those of the L1 lactobacillus and they can form branching arrangements. It has been identified as L. salivarius.

4. SCHAEGLER FUSIFORM-SHAPED BACTERIUM (Stock #356)* - This species is a large, cigar-shaped bacterium that often appears in two's resembling an airplane propeller. Colonies are small to medium-sized and have a yeasty appearance with white streaks running throughout the colony giving it a flame-like appearance. This bacterium forms spores and measures 10-12 micrometers x 2-4 micrometers. *Also known as Fat Boy.

5. CRL FUSIFORM-SHAPED BACTERIUM (Stock #492) - This species closely resembles the Schaedler fusiform-shaped bacterium but differs in that the individual cells are not perfectly straight and the colonies can grow to be 5-7mm in diameter. Like the Schaedler fusiform-shaped bacterium, it is also motile and a spore former but does not have as rigid a cell wall as does #356.

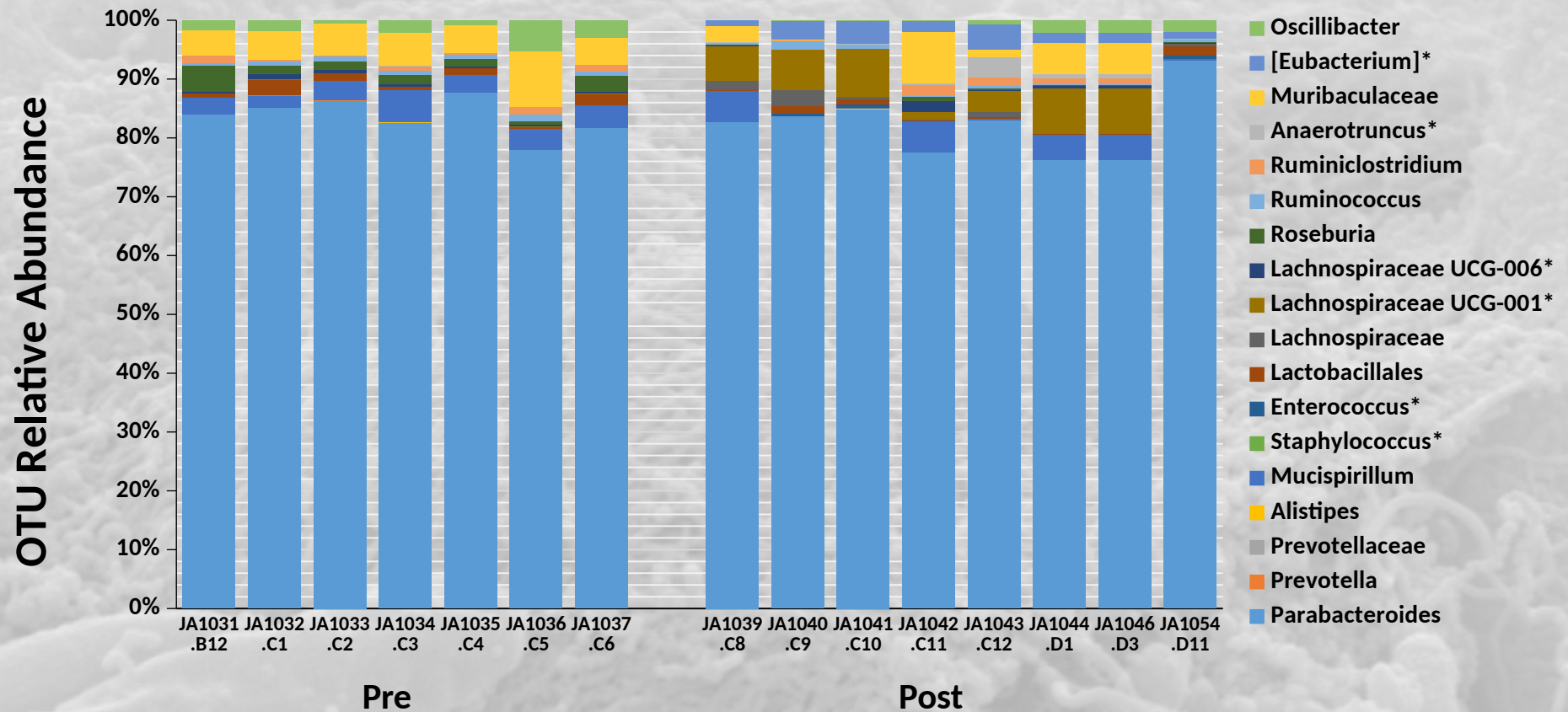
6. CRL FUSIFORM-SHAPED BACTERIUM (Stock #500) - This species is a very long, thin fusiform-shaped bacterium with a brilliant green speckled colony which can be small, medium or very large depending on the age of the colonies.

7. CRL FUSIFORM-SHAPED BACTERIUM (Stock #502) - This species is a small, short, cigar-shaped bacterium. The bacterium will demonstrate pleomorphism in older cultures, often containing long chains of short tapered rods. The colony is white, speckled and medium-sized.

8. CRL MOUSE SPIROCHETE (Stock #457) - The spirochete appears as a tiny greenish speckled colony on Schaedler agar supplemented with 5% sheep blood. It takes at least four days to grow and upon making a wet mount of a colony, one sees much debris along with the characteristic crooked spirochetes.

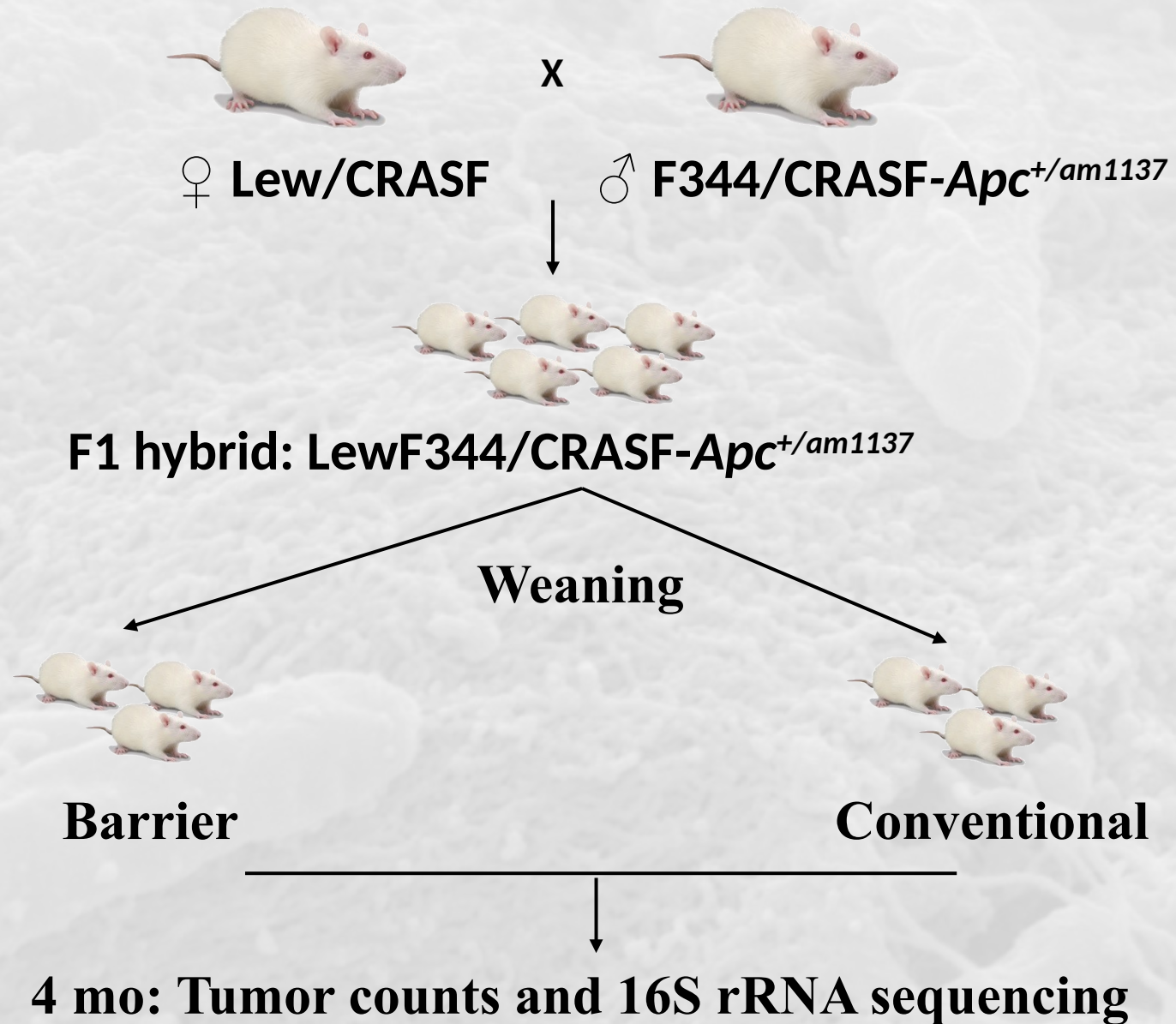
Lew/CRASFS – Pre and Post 16S data

Supp.Fig.1



*bacteria picked up after arrival

Experimental Design



OTU Relative Abundance

Weaning

Legend:

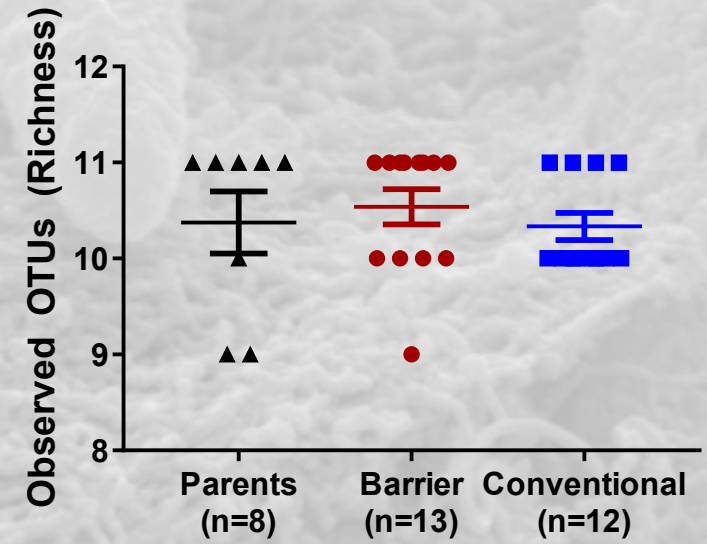
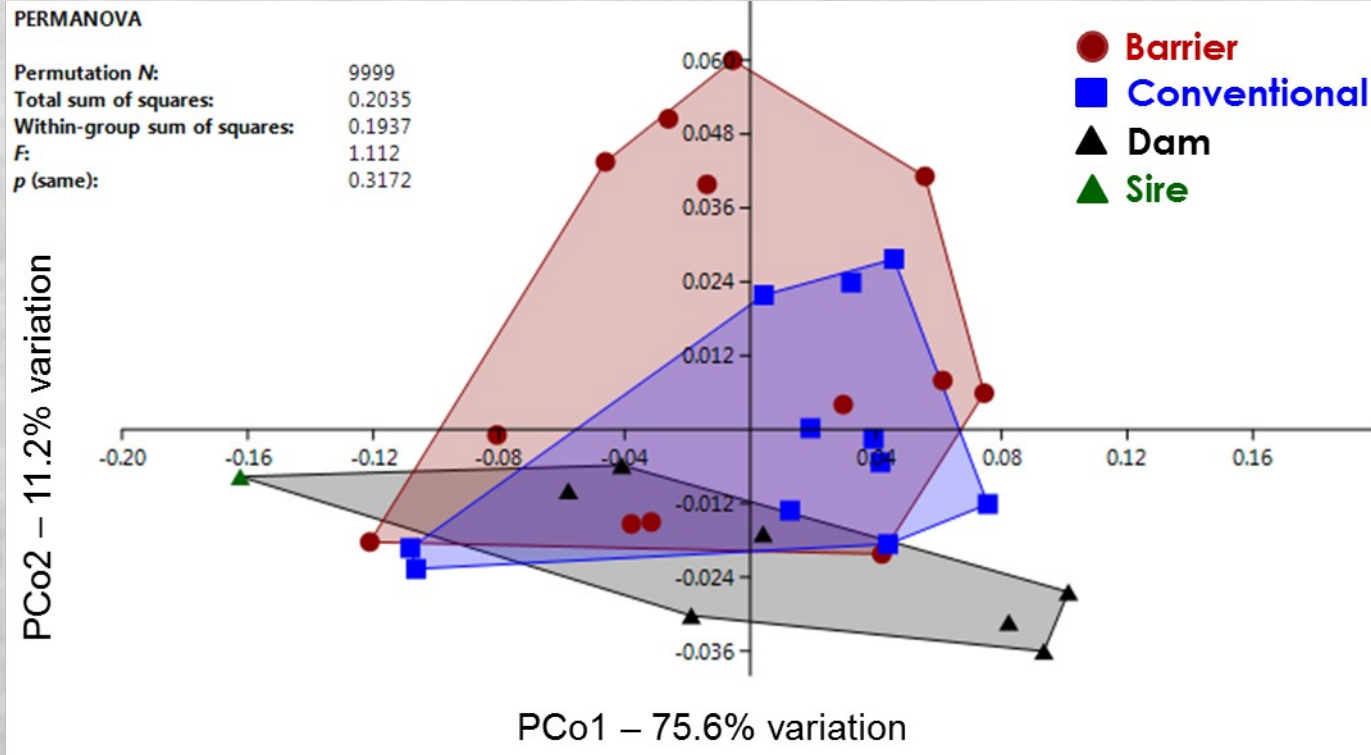
- Oscillibacter
- Eubacterium
- Peptostreptococcaceae
- Lachnospiraceae
- Ruminococcus
- Blautia
- ASF356
- Lactobacillus
- Enterococcus
- Mucisprillum
- Parabacteroides

The chart displays the relative abundance of 12 bacterial taxa across 28 weaning stages. The y-axis represents OTU Relative Abundance from 0% to 100%. The x-axis categories are Dam1, Dam2, Dam3, Dam4, Dam5, Dam6, Dam7, Sire, Barrier (10 samples), and Conventional (18 samples). Parabacteroides is the dominant taxon in all samples, typically accounting for 80-95% of the relative abundance. Other taxa like Lactobacillus, Enterococcus, and Mucisprillum show varying degrees of presence across the different weaning stages.

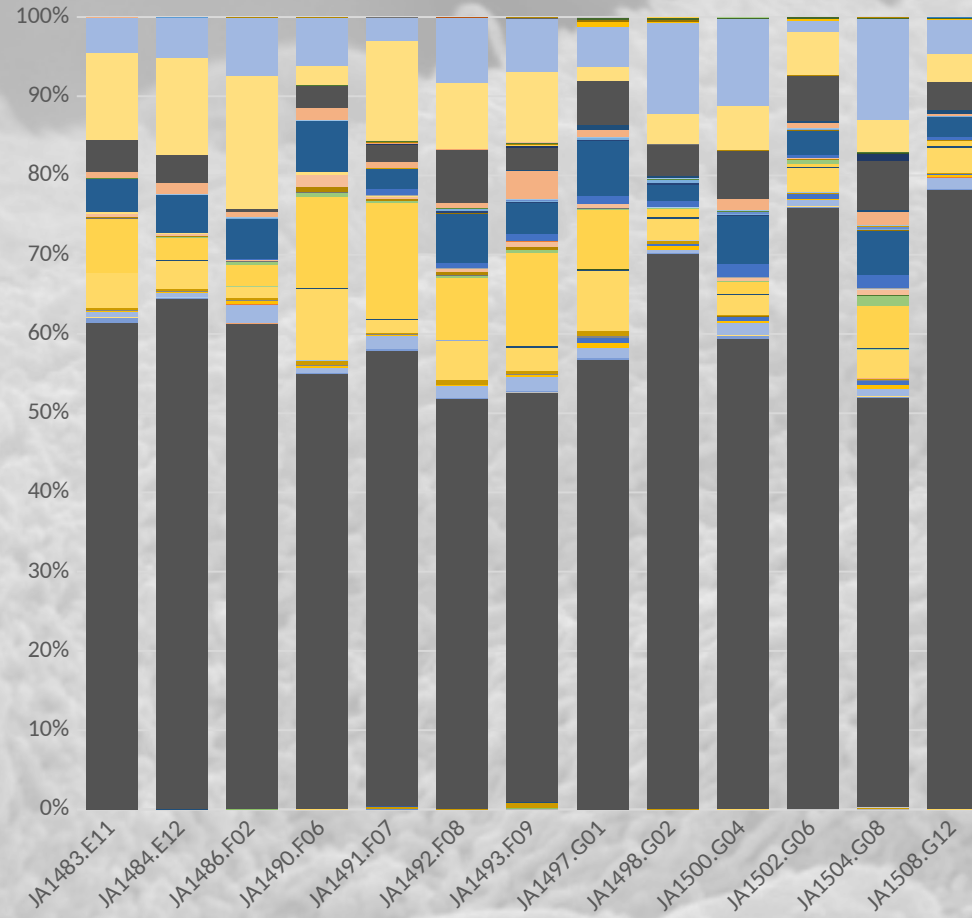
OTU Relative Abundance

Weaning

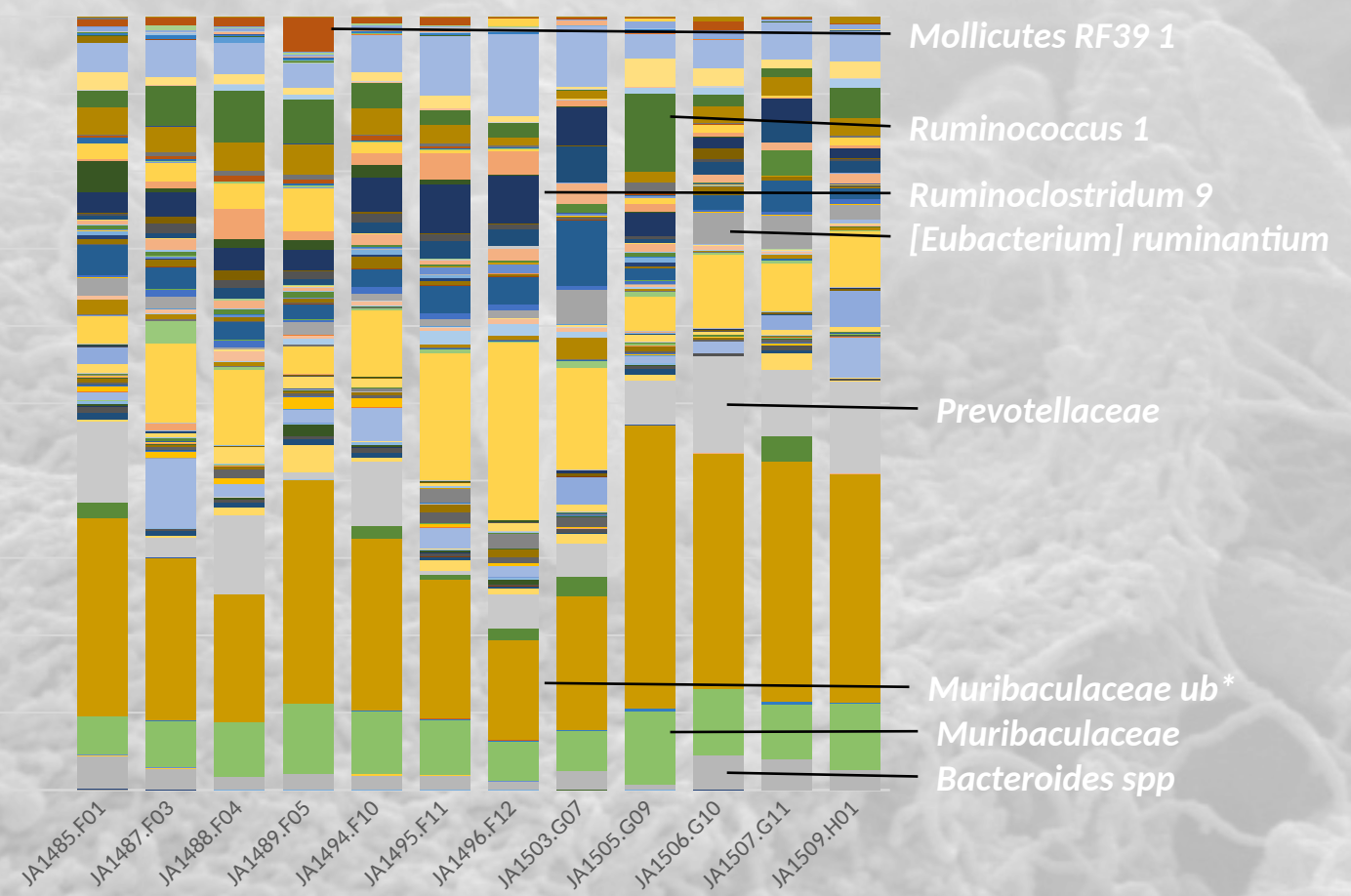
Weaning



4-month



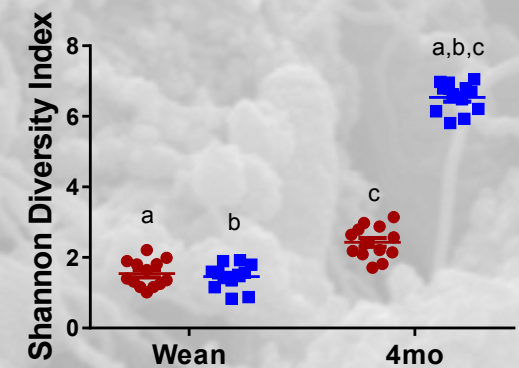
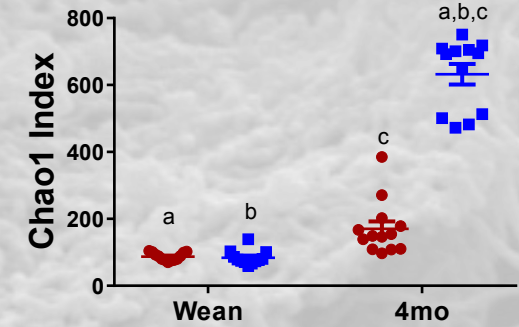
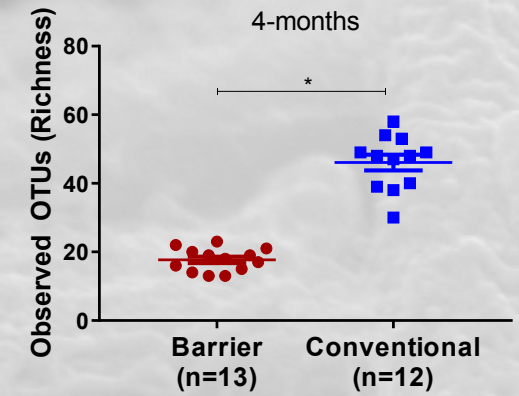
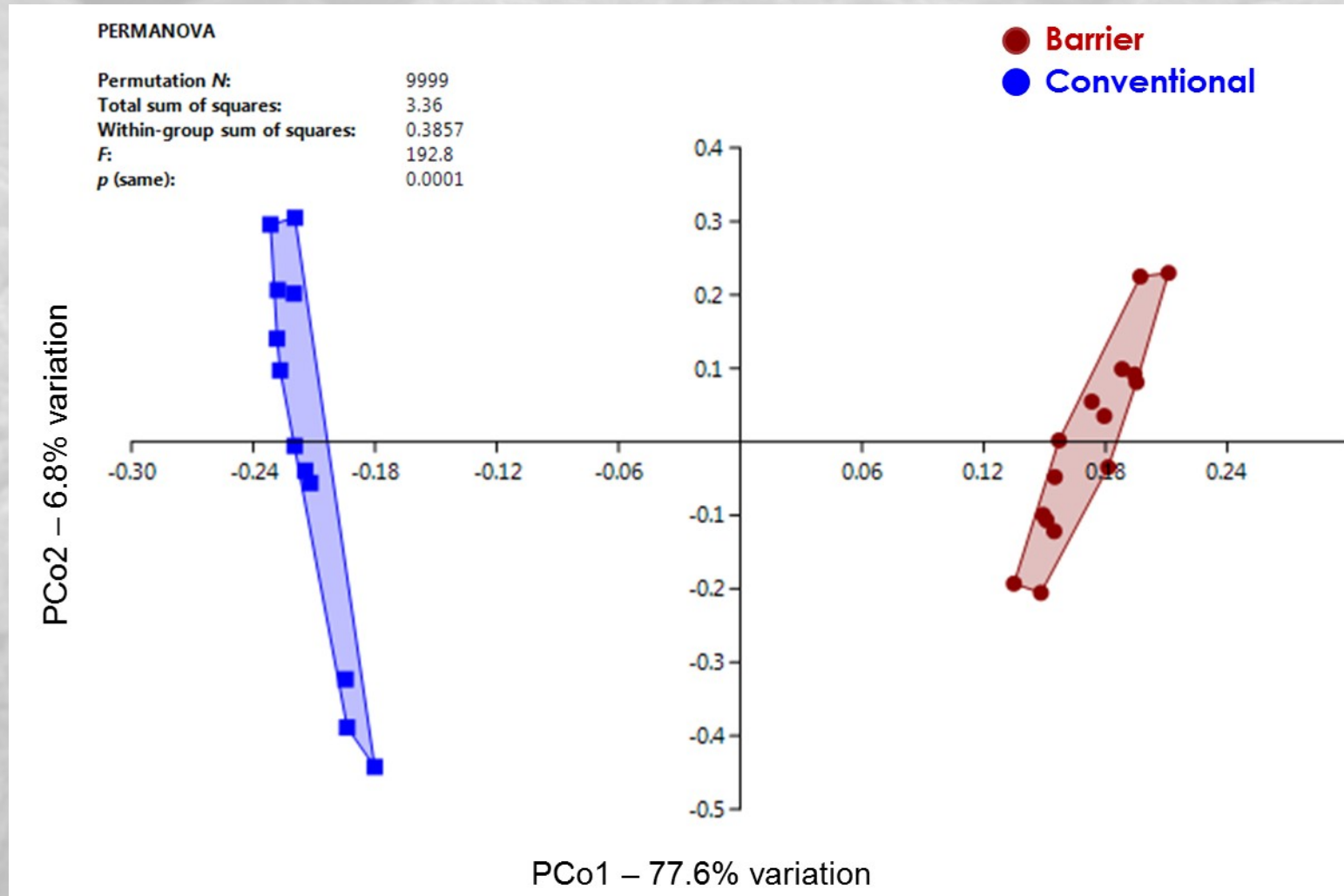
Barrier



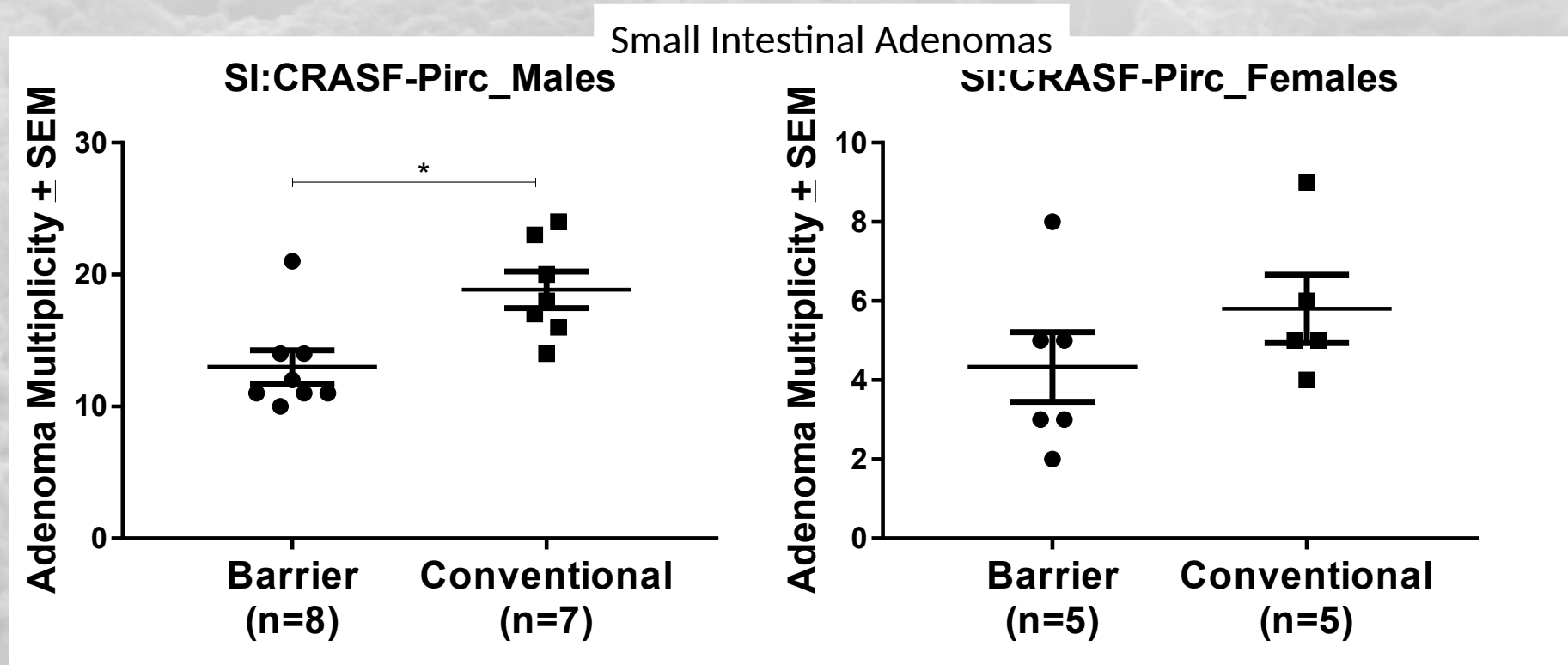
Conventional

- Mollicutes RF39 1
- Ruminococcus 1
- Ruminoclostridium 9
- [Eubacterium] ruminantium
- Prevotellaceae
- Muribaculaceae ub*
- Muribaculaceae
- Bacteroides spp

4-month



Barrier maintained vs. conventionalized Pirc rat tumor counts



What is driving differences tumor number

- Quantitative expression analysis of cancer related pathway genes
- Normal intestinal epithelium
- Genes
 - Mmp7 – extracellular matrix remodeling
 - Elevated in adenomas
 - Mgmt – DNA repair / response to alkylation
 - removal of methyl groups from O6-methylguanine
 - Cyp27a1 – Bile acid production
 - Production of CDCA 2^o bile acids

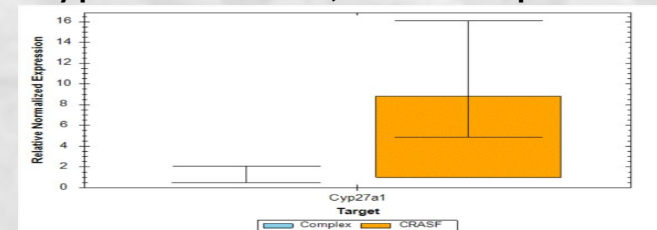
Mmp7 Gene (Extracellular Matrix Remodeling)



Mgmt Gene (DNA Repair)



Cyp27a1 Gene (Bile Acid production)

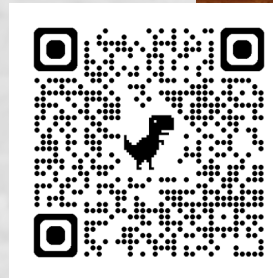


Conventional Barrier



What can you do?

- Ask – “Could it matter?”
 - Disease model
 - Data outcomes (Qualitative or Quantitative)
- Advise
 - Surrogate dam choice
 - Proper controls! (littermates, not purchased)
 - Metagenomic profile
- Be proactive
 - Document surrogate source
 - Fecal banking (pre rederivation)
- MU MUMC can help
 - Microbiota characterization
 - Microbiota manipulation

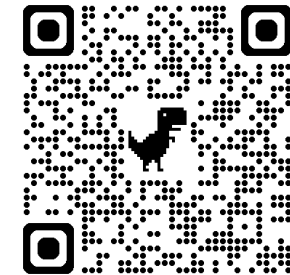




Acknowledgments



Share
your
rats



Share
your
mice



MMRRC

Craig Franklin

Aaron Ericsson

Elizabeth Bryda

MMRRC Consortium partners

JAX, UNC, UC Davis

Funding

MU CVM Faculty Research Award

CMP (NIH T32 OD011126-38)

MMRRC (NIH U42 OD010918 &S1)

Co-PIs: Craig Franklin, Jim Amos-Landgraf

RRRC (NIH P40 OD011062) Elizabeth Bryda, PI

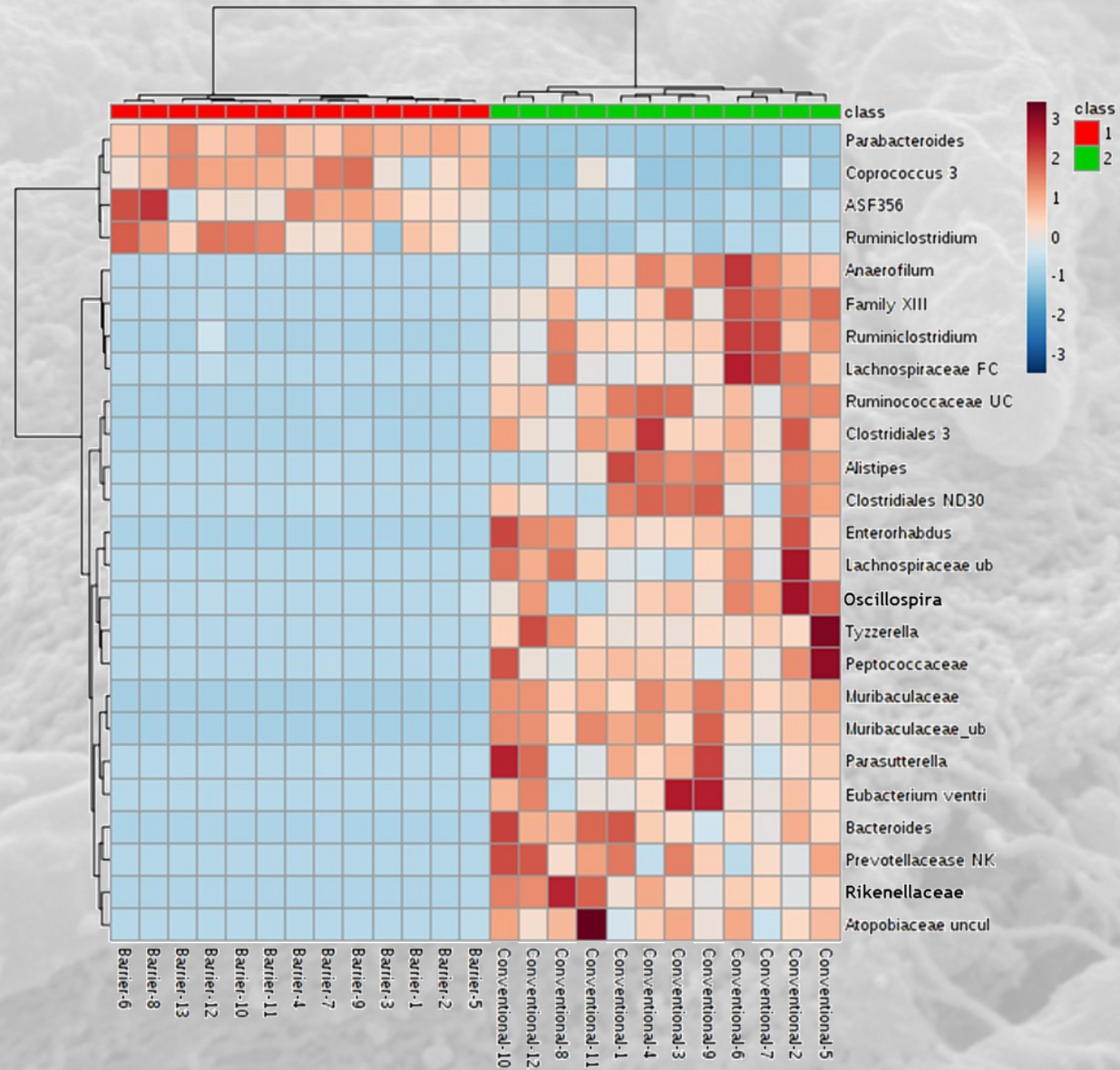
K01 (NIH OD018824) Aaron Ericsson, PI



MU METAGENOMICS CENTER (MUMC)



4-month



Proximal Tumor phenotype

Fig.2C. Weaning cecum

Barrier

Conventional



Fig.2D

Barrier

Conventional

