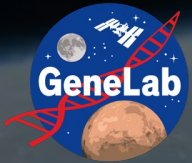


Murine host-gut microbiota interactions are modulated during spaceflight



Open Science for Life in Space

Université 
de Montréal



GeneLab Analysis Working Group – Microbes

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Rodent Research 6 – Research Question

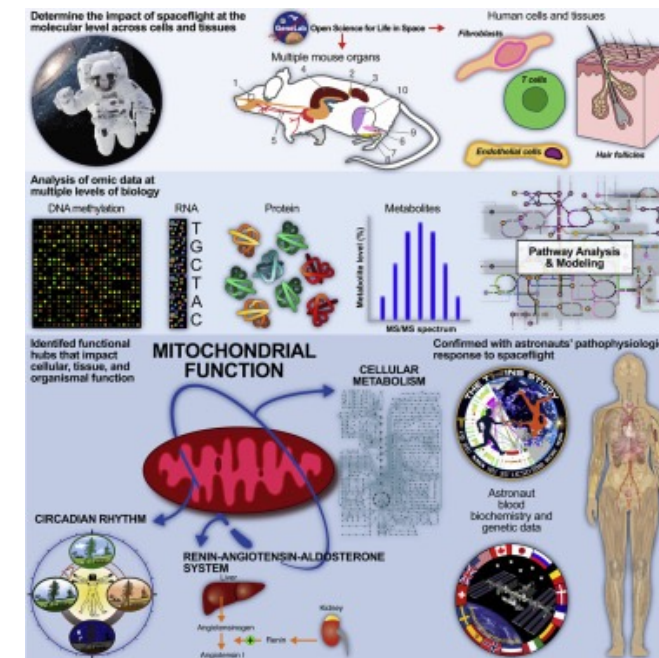
Why explore microbiota influence on spaceflight pathology?

GeneLab AWG – Multi-omics research on spaceflight:

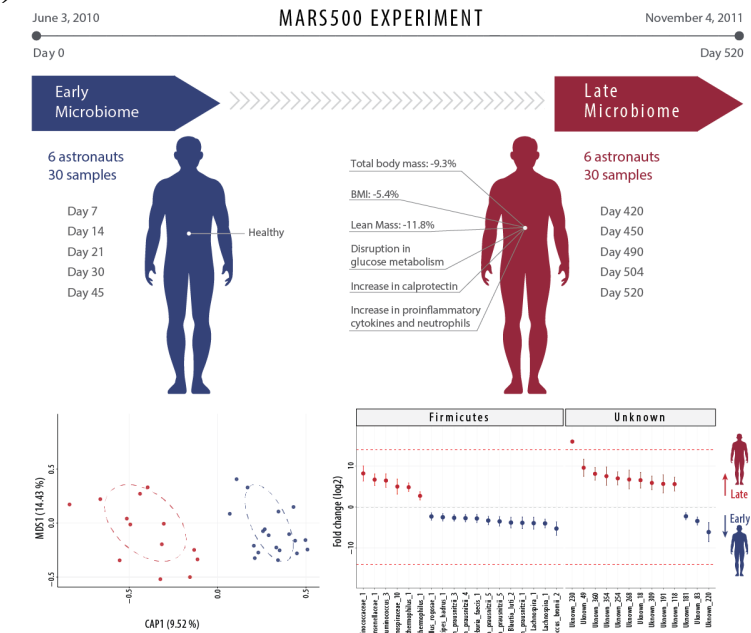
- Common impacts to innate immunity, inflammation, circadian rhythm, glucose, cholesterol and lipid metabolism
- Mitochondrial dysfunction unifies spaceflight pathology - why?

Mars500 experiment:

- 6 crew members isolated for 520 days in Martian analog habitat (longest controlled isolation experiment)
- Similar muscle mass loss, glucose homeostasis disruption phenotype
- Significant changes in gut microbiota associated with starch degradation, inflammation, nutrient absorption and glucose homeostasis



Silveira et al (2020)



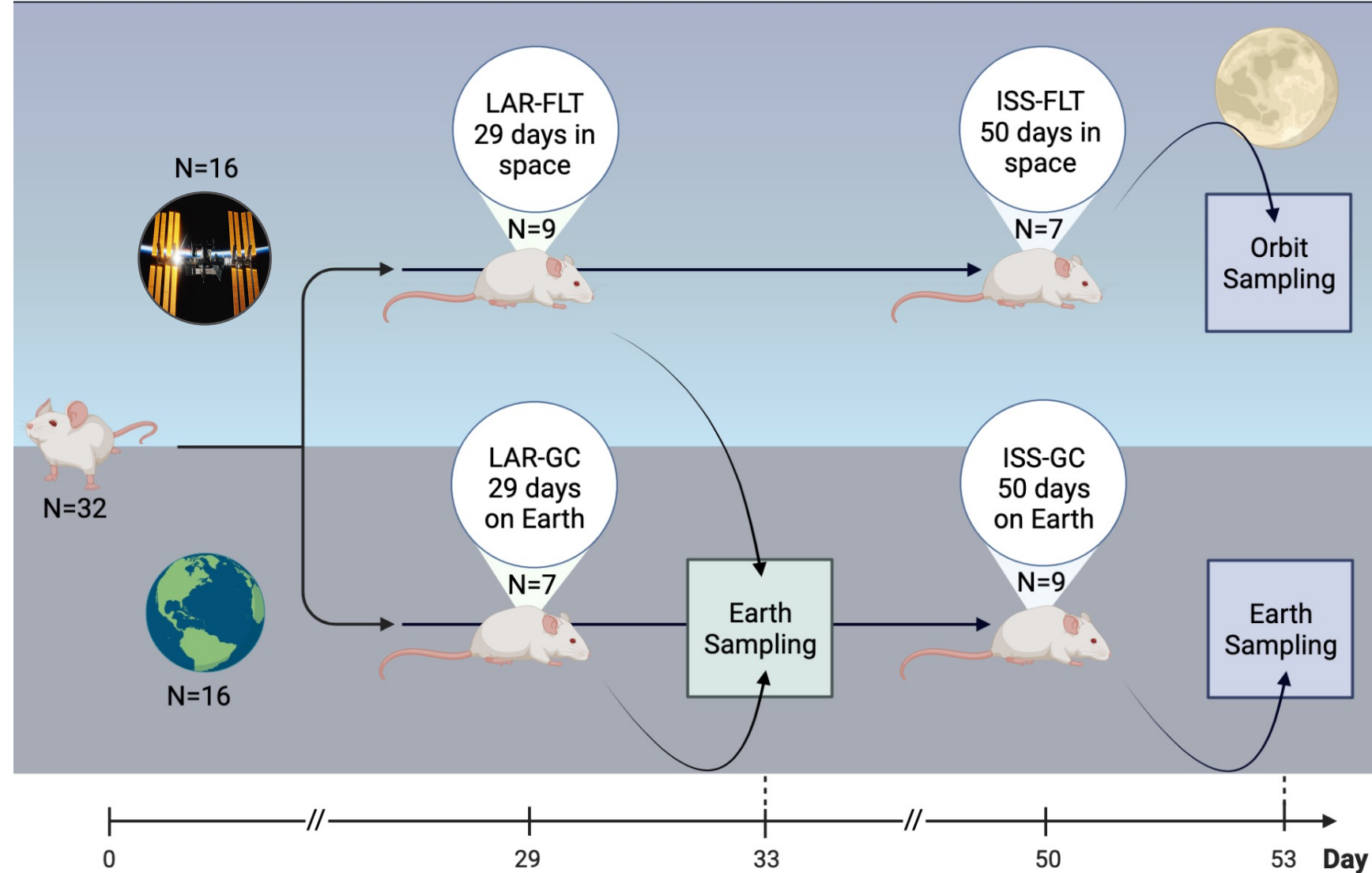
Brereton et al (2021)

Rodent Research 6 - design

Does FLT and ISS habitation alter the murine gut microbiome?

Microbiome interactions related to:

- Short duration space travel **high gravity** FLT
- Long duration space travel **microgravity, radiation, elevated CO₂** and the **extreme built environment (BE)**



ISS: International Space Station

FLT: Spaceflight

LAR: Live Animal Return

GC: Ground Controls

Analytical Comparisons

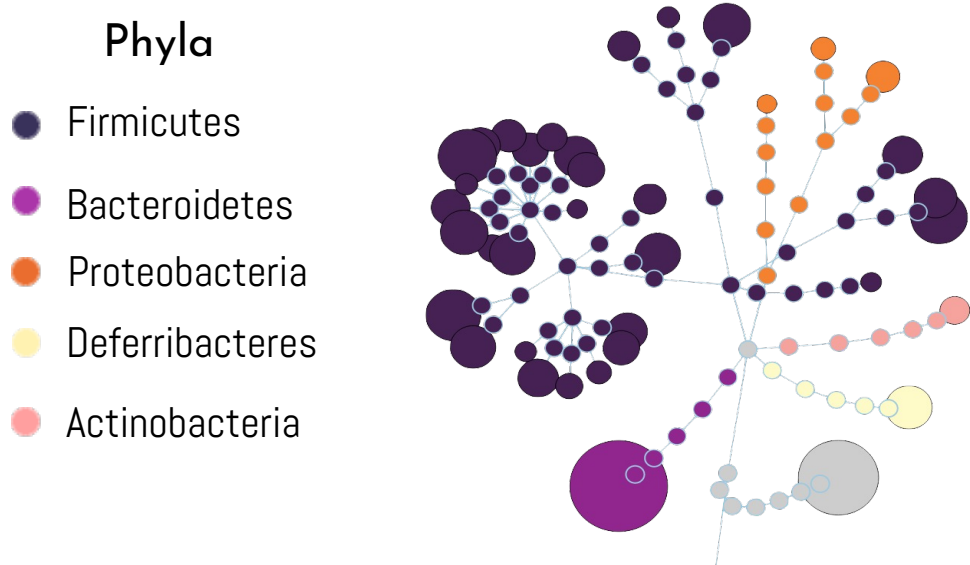
ISS-FLT v. ISS-GC (50 days FLT)

LAR-FLT v. LAR-GC (29 days FLT)

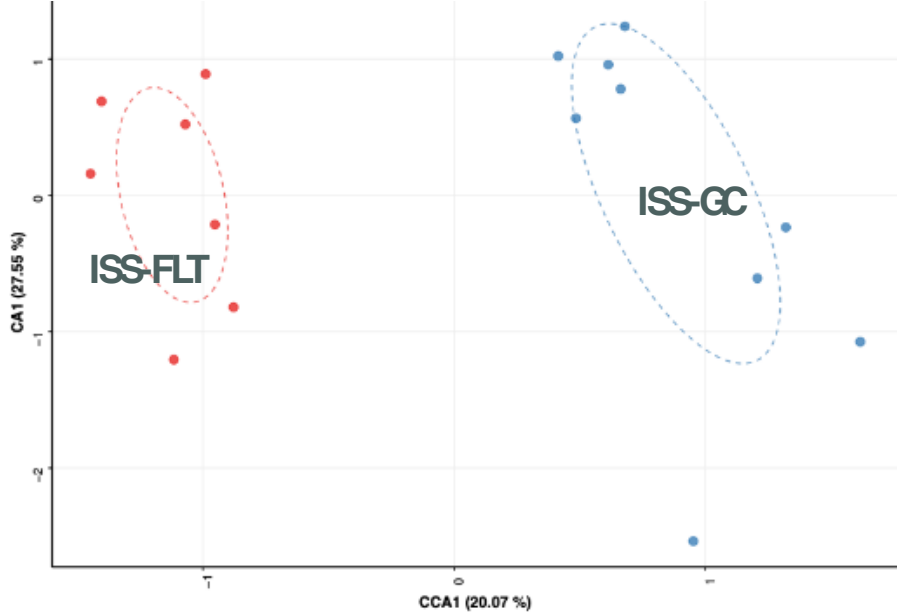
RR 6 faecal 16S rRNA gene barcoding overview

Anchor Exact Sequence Variants (ESVs) Inference and Abundance

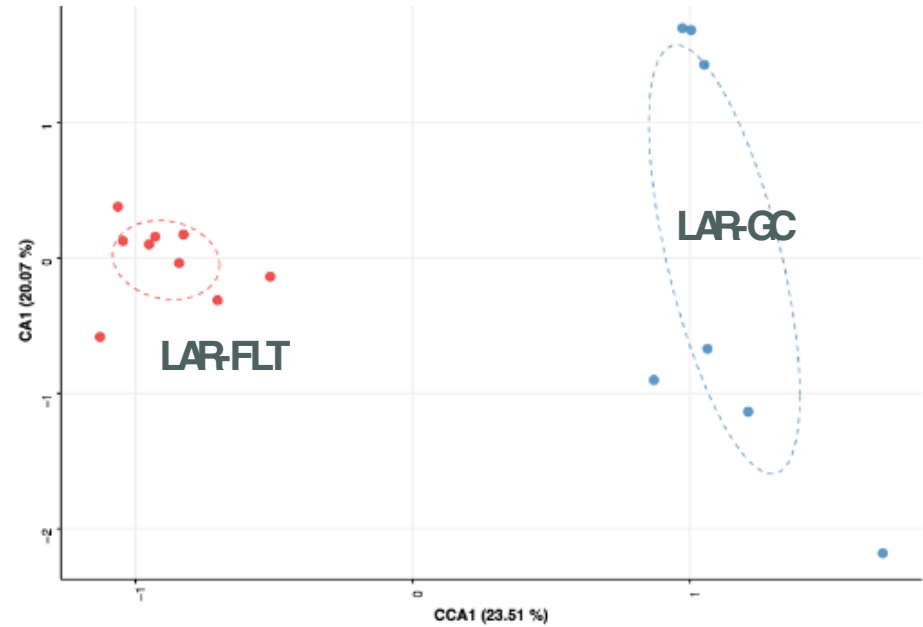
Tax Level	ESVs	ambiguity	identity	coverage	Counts	Counts (%)
Species	31	10	100	100	1429,884	73.05
Genus	9	0	100	100	60,088	3.07
Family	9	0	99.8	99.9	188,990	9.66
Unknown	63	0	0	0	278,351	14.22
Total	112	10	-	-	1,957,313	100.00



ISS-FLT VS ISS-GC



LAR-FLT VS LAR-GC



RR6 faecal microbiota differential abundance (16S)

Do taxa significantly differ due to spaceflight (DESeq2 +FDR)?

1. ISS-FLT v. ISS-GC

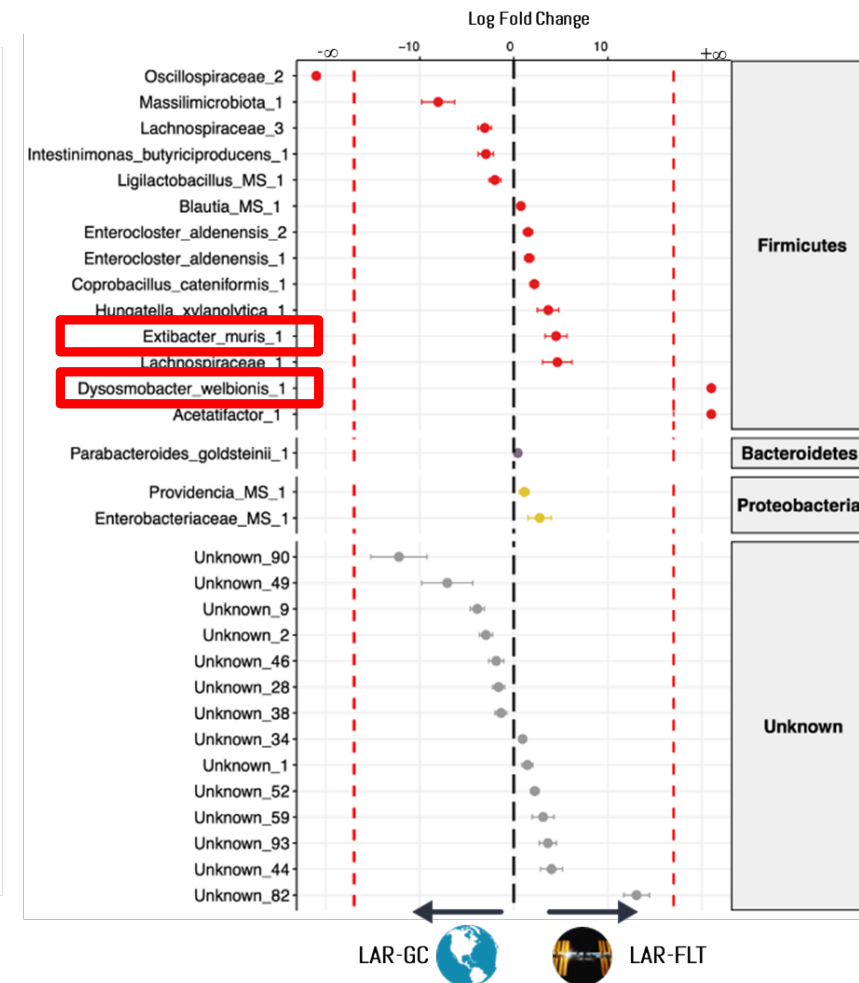
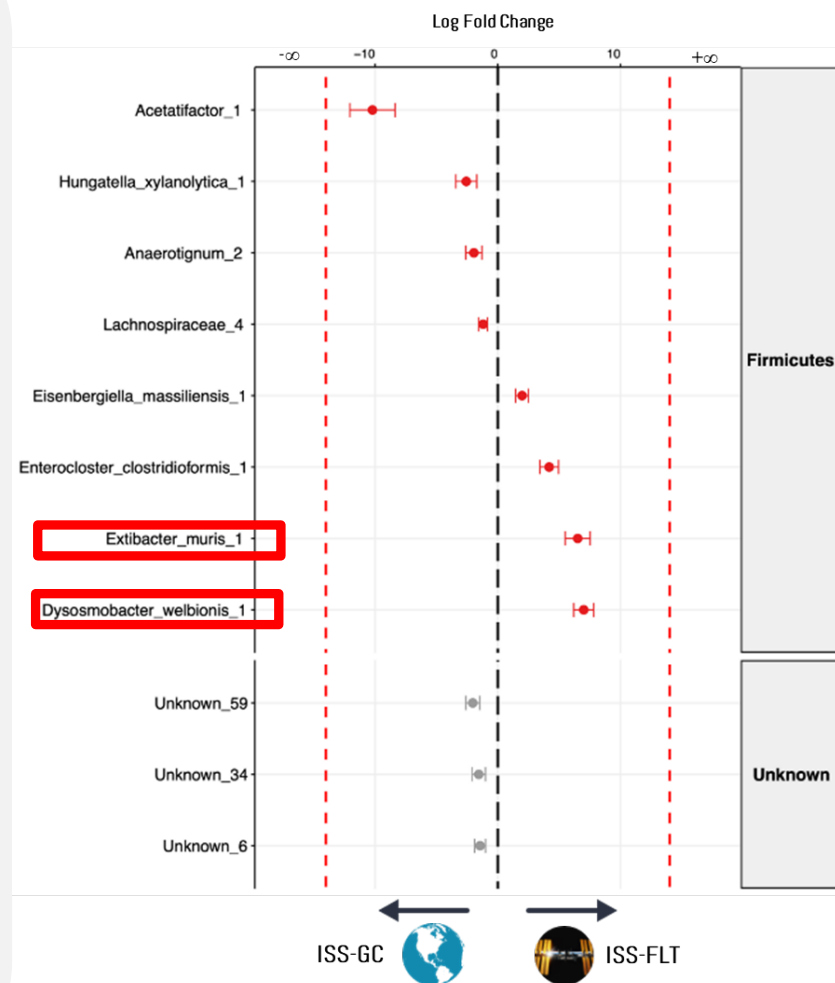
11 differentially abundant ESVs

2. LAR-FLT v. LAR-GC

31 differentially abundant ESVs

Are there common changes associated to spaceflight?

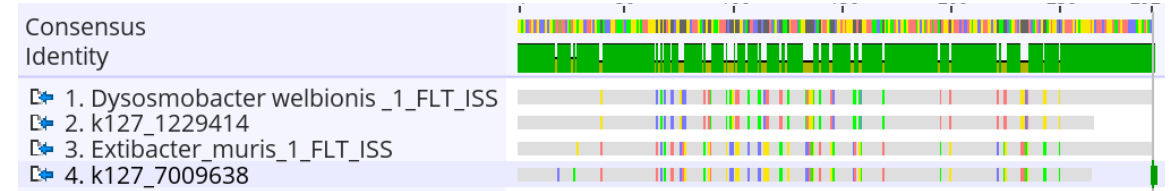
- *Dysosmobacter welbionis*
- *Extibacter muris*



What has 16S rRNA gene amplification missed?

WGS assembly:

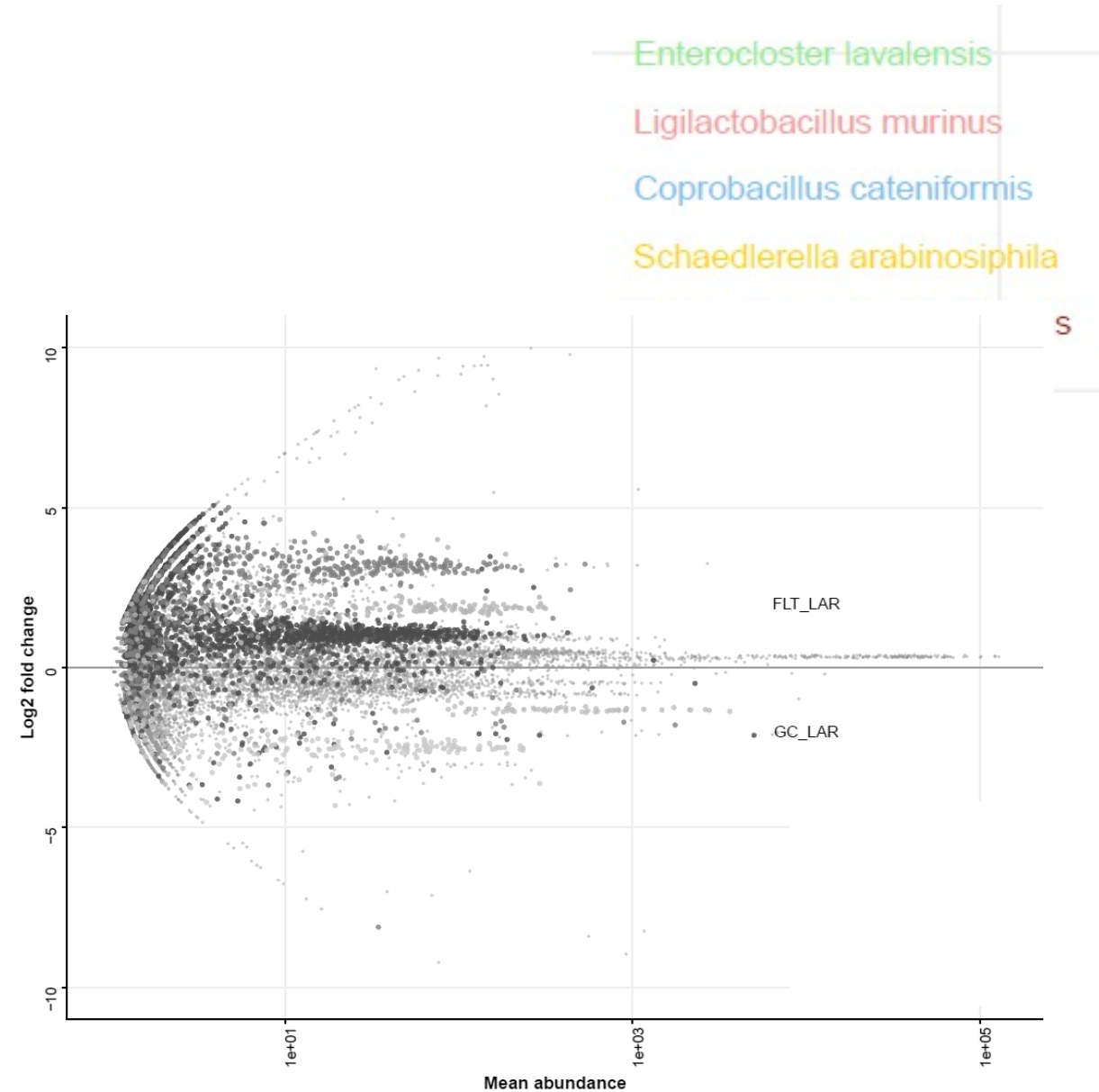
- *De novo* co-assembly across 32 samples – 3.5 million contigs
- 220,000 contigs capturing 65% reads after count and mouse filters
- ~30,000 contigs after sparsity control (inc >99.7 % counts)
- around 220 putative species and WGS – 16S agree (>99% sequence identity of bacteria species)
- Highly abundant *Parabacteroides goldsteinii*
- Ubiquitous nematodes (*Trichinella nativa* and *Trichinella pseudospiralis*)



RR6 faecal microbiota differential abundance (WGS)

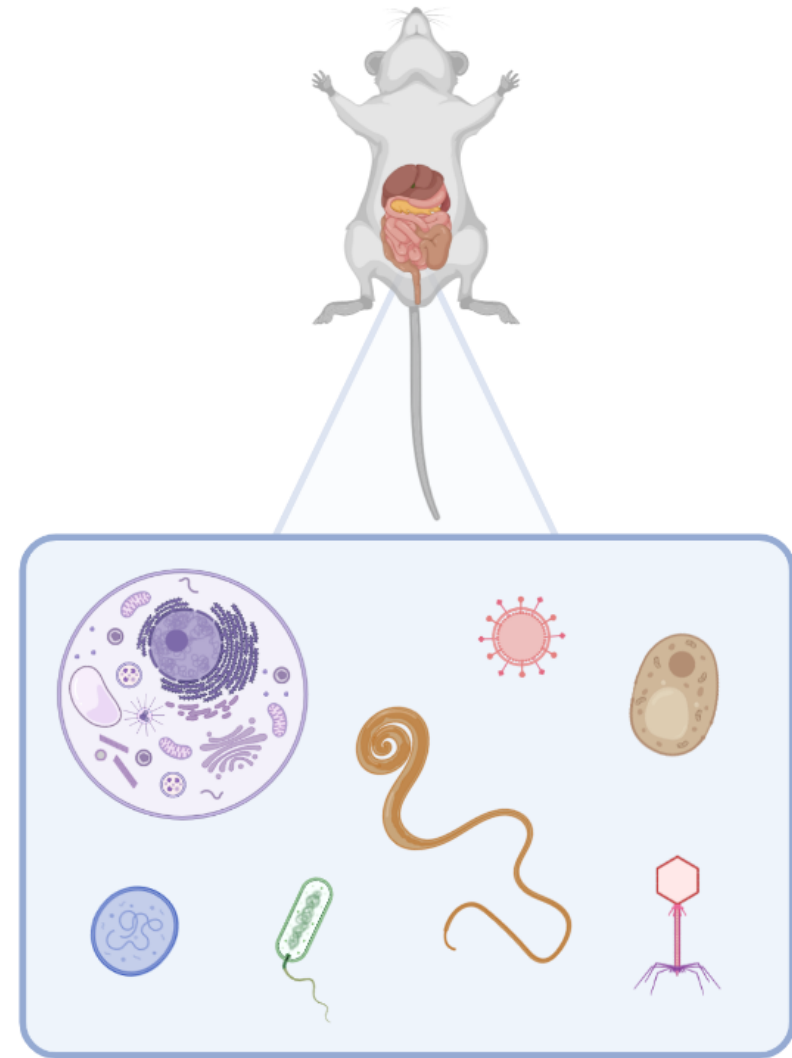
Do taxa significantly differ due to spaceflight (DESeq2 +FDR)?

- ~1000 DA contigs ISS vs GC
- ~2000 DA contigs for LAR vs GC
- ~ 80 Metagenome assembled genomes (MAGs)
- 16S - WGS DA largely aligns
- Up *Eisenbergiella massiliensis*, *Enterocloster clostridioformis*, *Dysosmobacter welbionis*, *Extibacter muris*,
- Down *Intestinimonas butyriciproducens*, *Ligilactobacillus murinus*
- No change in *Trichinella nativa*
- Functional prediction *ongoing*



Does spaceflight alter gut microbiota?

- Significant microbiome change associated to spaceflight
- *Dysosmobacter welbionis*: Butyrate producer which influences lipid metabolism
- *Extibacter muris*: 1° → 2° bile acid conversion (7α-dehydroxylating)
- BAs mediate host-microbiome metabolism: BA receptor interactions, BA pool and liver metabolism (enterohepatic recirculation >95% BAs)
- To test this we need metabolomics – research challenges in the space sciences....



Spaceflight Gut
Microbiome

Host-gut microbiome interface RNASeq

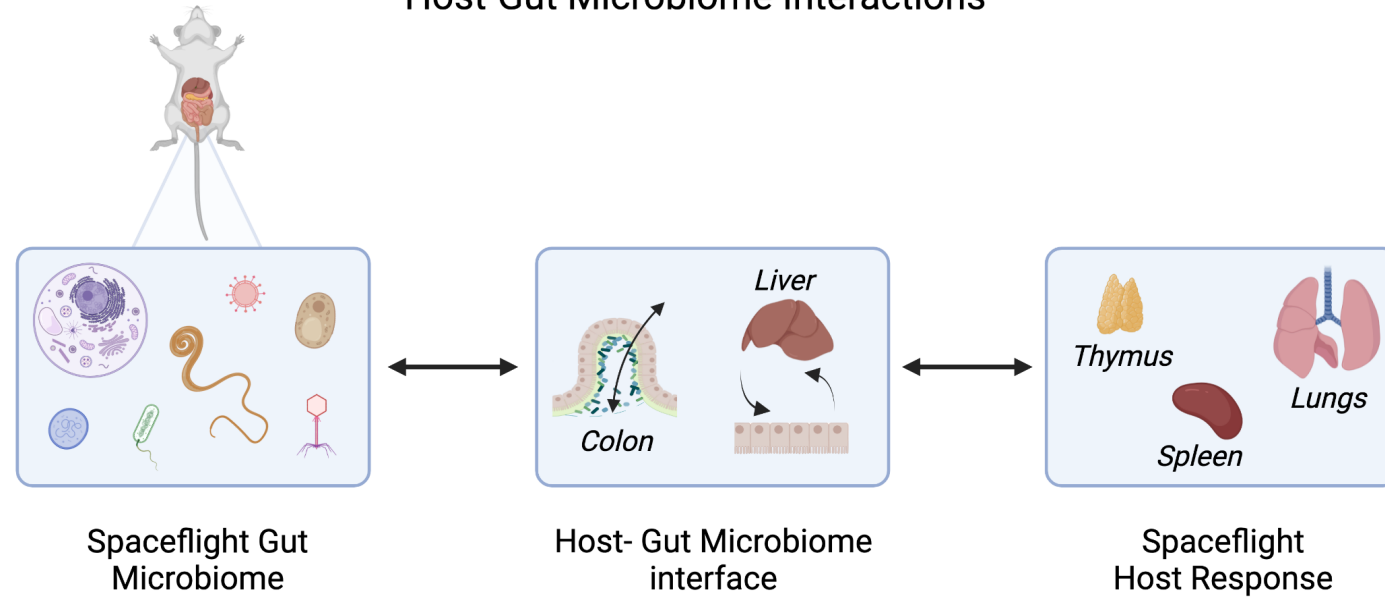
GeneLab data

- Host RNAseq colon, liver, thymus, spleen and lungs datasets available
- Host responses to spaceflight

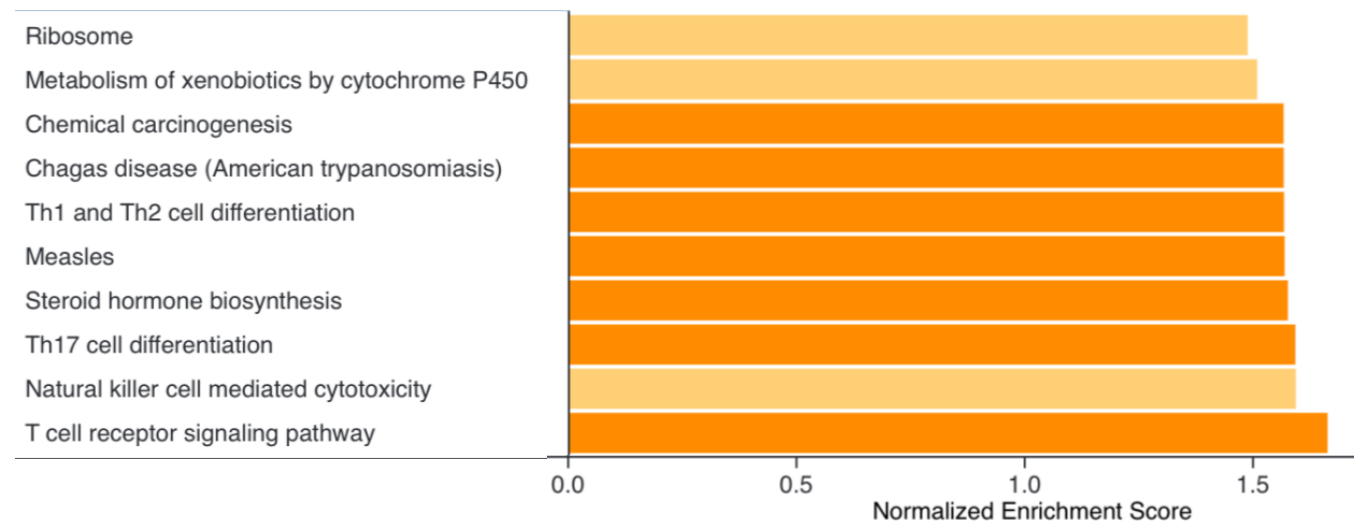
Expected changes at the host-microbiome interface?

- Expected changes to immune response, bile acid, cholesterol/steroidal and lipid metabolism
- Colon and liver differential expression *ongoing*

Spaceflight Modulation of Host-Gut Microbiome Interactions



Colon RNASeq pathway enrichment



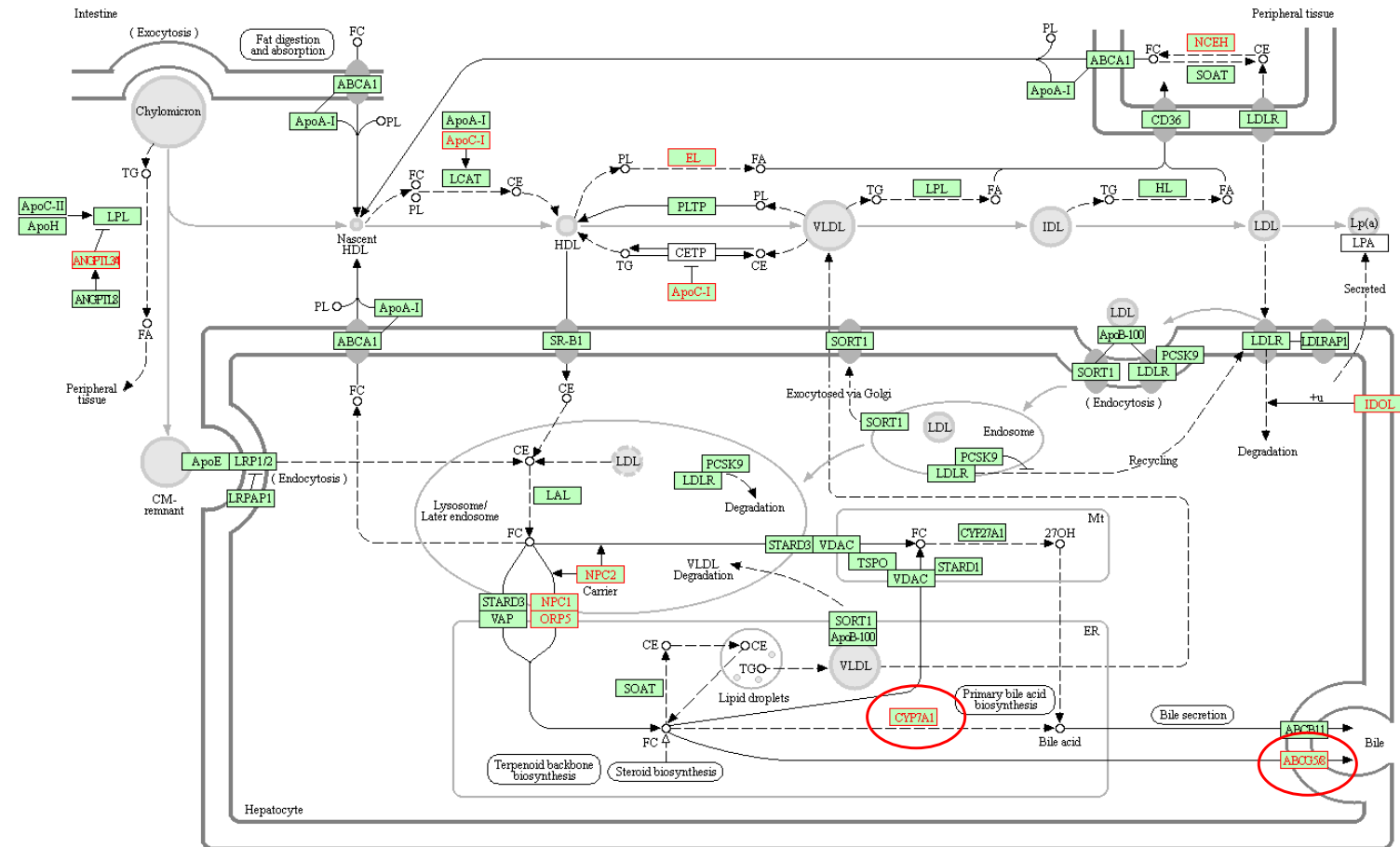
RR6 Liver gene expression

An example of liver changes in gene expression

CYP7A1 - Cholesterol 7- α -monooxygenase

- Critical point of regulation in bile acid synthesis from cholesterol
- Tightly regulated by bile acids entering the liver via the enterohepatic circulation
- Primary mechanism for cholesterol removal
- *ABCG5/G8* – Sterol exporter
- *ApoC* – FXR gene target (bile acid receptor)
- *NPC1+2* – Intracellular cholesterol transporter

CHOLESTEROL METABOLISM



Lipoprotein	HDL	LDL	Lp(a)	IDL	VLDL	CM-remnant	Chylomicron
Components (apoproteins & lipids)	ApoA-I, ApoA-II, ApoC, ApoE	ApoB-100, OCE	Apo(a), ApoB-100, OCE	ApoB-100, ApoE, OTG	ApoB-100, ApoC, ApoE, OTG	ApoB-48, ApoE, OCE, OTG	ApoA-I, ApoA-II, ApoA-IV, ApoC, ApoE, OTG

RR6 Summary

- Novel spaceflight induced gut microbiome change identified
- Microbiome functional prediction and host gene expression *ongoing*
- Change is coherent with spaceflight pathology; potential host-gut microbiome genetic interactions observed
- Challenge of spaceflight 'omics to highly trackable and replicated systems – *establishing causality*
- GeneLab AWGs can overcome these with diversity of data and people integrating multi-omics; here 16S, WGS and RNASeq
- Asking questions of public data – wealth of discovery left to be made

Thanks to

GeneLab Microbes Analysis Working Group members

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