UC Davis Microbiome Project

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UCDAVIS MMPC Mouse Metabolic Phenotyping Center

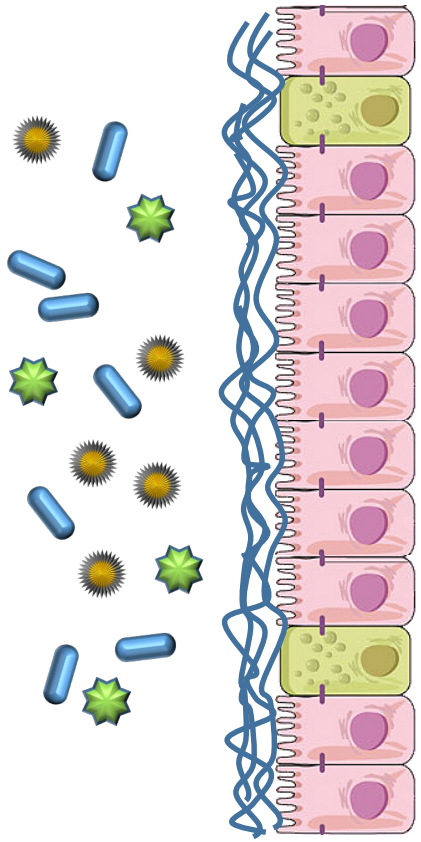
MMPC National Mouse Metabolic Phenotyping Center

Image from http://sustainablebalance.ca/the-human-microbiome-an-introduction/

Microbiome & Host Response

Integrated Physiology

Energy Balance
Food Intake
Behavior
Pathway Analysis



Microbiome & Metabolomics

- Microbiota community profiling
  - 16S rDNA sequencing
- Multi-omics pipeline development
  - Metabolomics
    - Microbiota-derived metabolites
  - Metagenomics
  - Metatranscriptomics
- Microbiome Project

Host Response

- Focus on gut & GI function
  - Regional effects
- Gut permeability:
  - *Ex vivo* Ussing chambers
  - Tight junction proteins
  - Leak/Metabolic endotoxemia
- Transit time
- Gastric emptying
- Gut-brain axis
- Inflammatory profiling
- Transcriptomics

Microbiome Research Project:

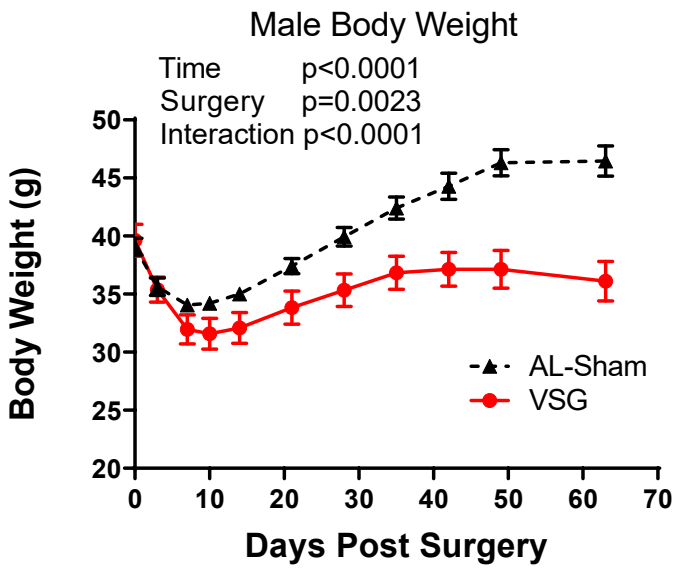
Targeted manipulation of microbiota vs host

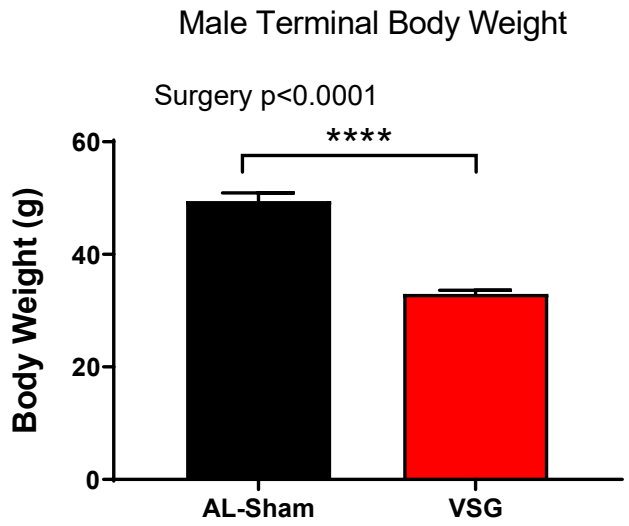
- Microbiota- e.g. antibiotics, prebiotics, probiotics

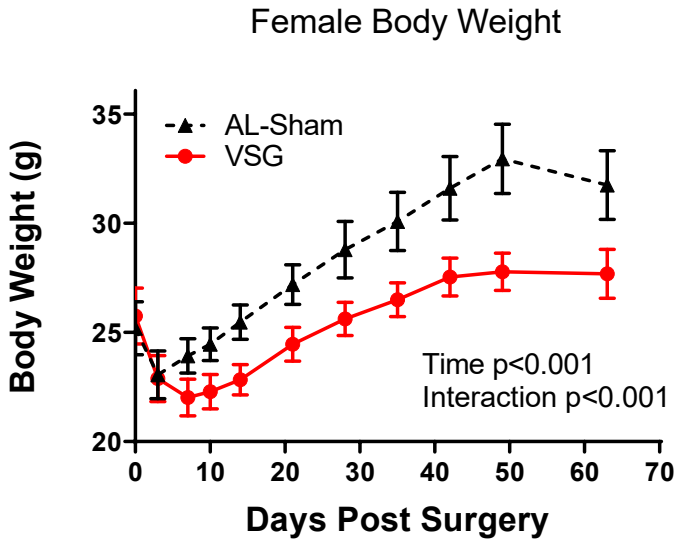
- Host- state of altered metabolism- examine effect on microbiota
  - Energy Balance
    - Positive Energy Balance- OBESITY/HFD
    - Negative Energy Balance- weight loss- calorie restriction, bariatric surgery

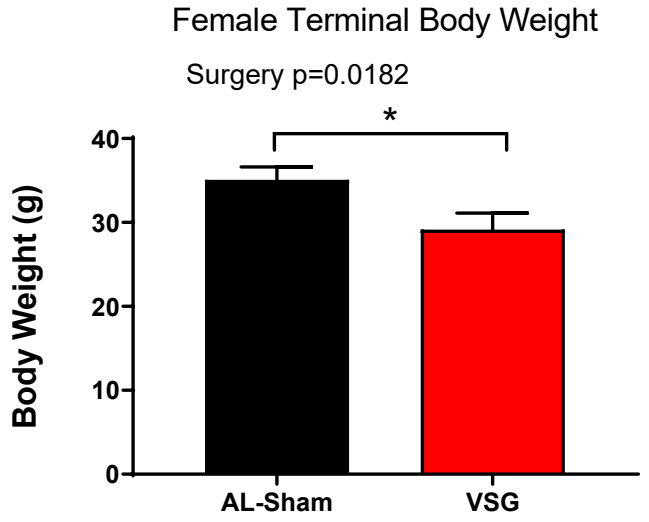
- Bariatric surgery model- VSG
  - Cecal tissue and contents
  - Sham vs VSG

VSG surgery results in sustained weight reduction in males and females compared to sham controls.

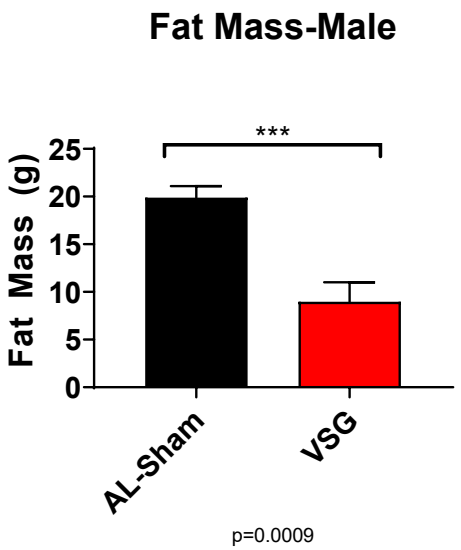


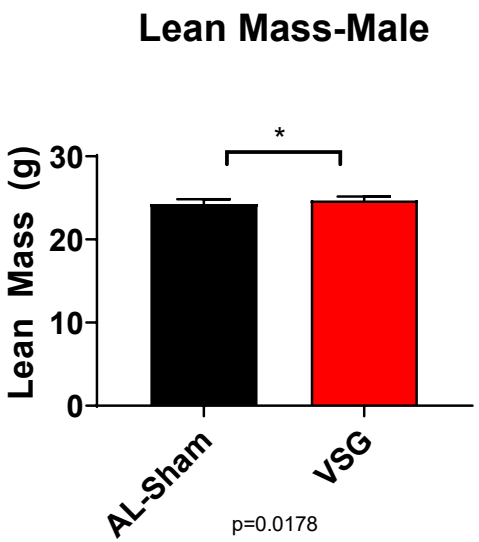


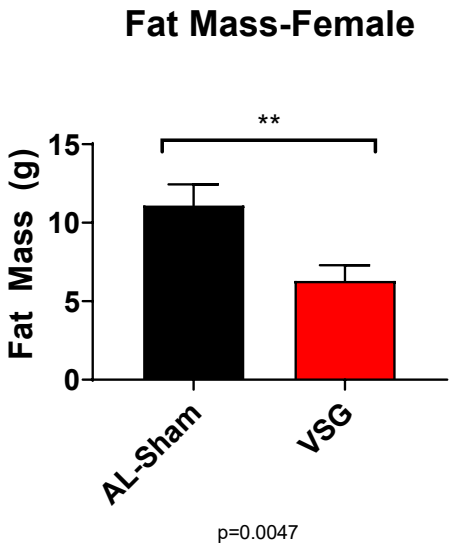


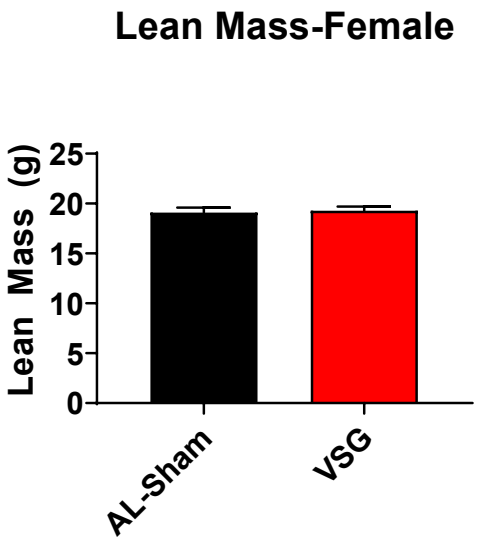


VSG surgery promotes reduction in fat depots in male and female mice compared to sham controls.

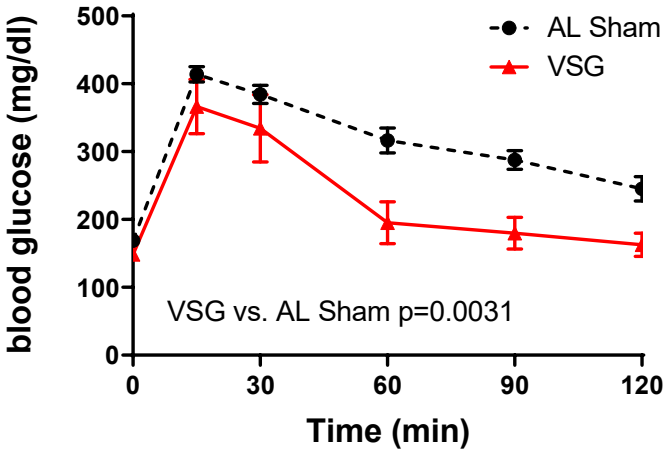


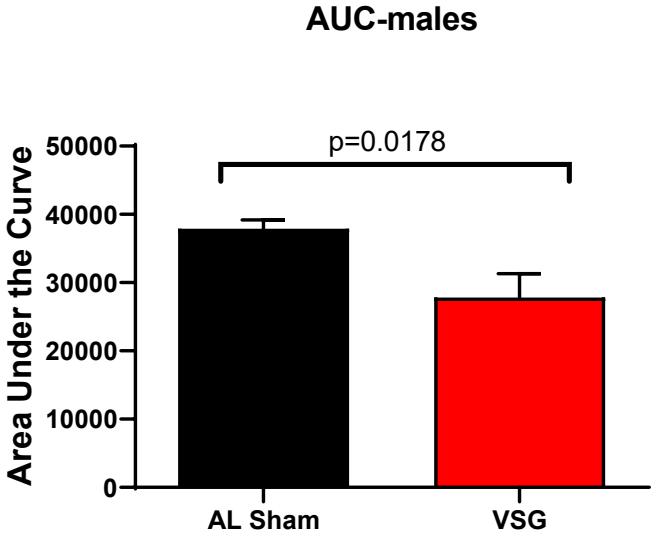


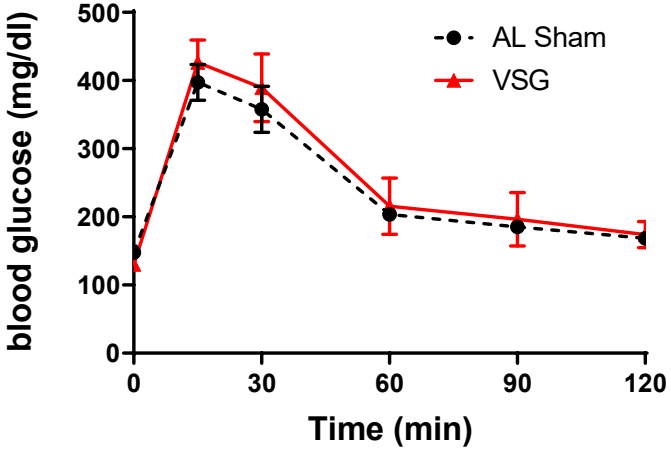


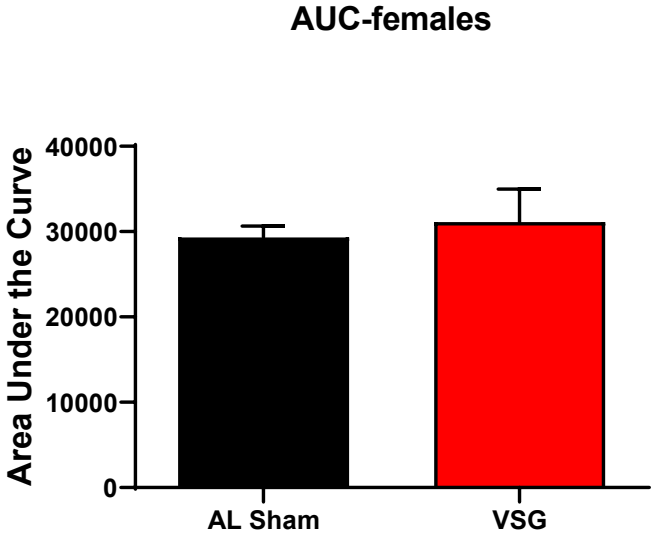


VSG surgery improved glucose tolerance in males. Glucose intolerance was not observed in females.









Study Goals

1. To understand how the changes in the intestinal milieu after intervention alters the microbial community structure, microbial and host transcription, and host-microbe interactions.
    1. **Cecal Tissue Transcriptomics**
    2. **Cecal Content Metatranscriptomics**
    3. **Cecal Content 16S Microbial Community Analysis**
    4. Cecal Content Metagenomics
    5. Targeted metabolomics for bile acids

2. To establish a pipeline to measure, analyze and integrate data from transcriptomics with the microbial community taxonomic profiles using bioinformatics tools and multivariate modeling

Hypotheses

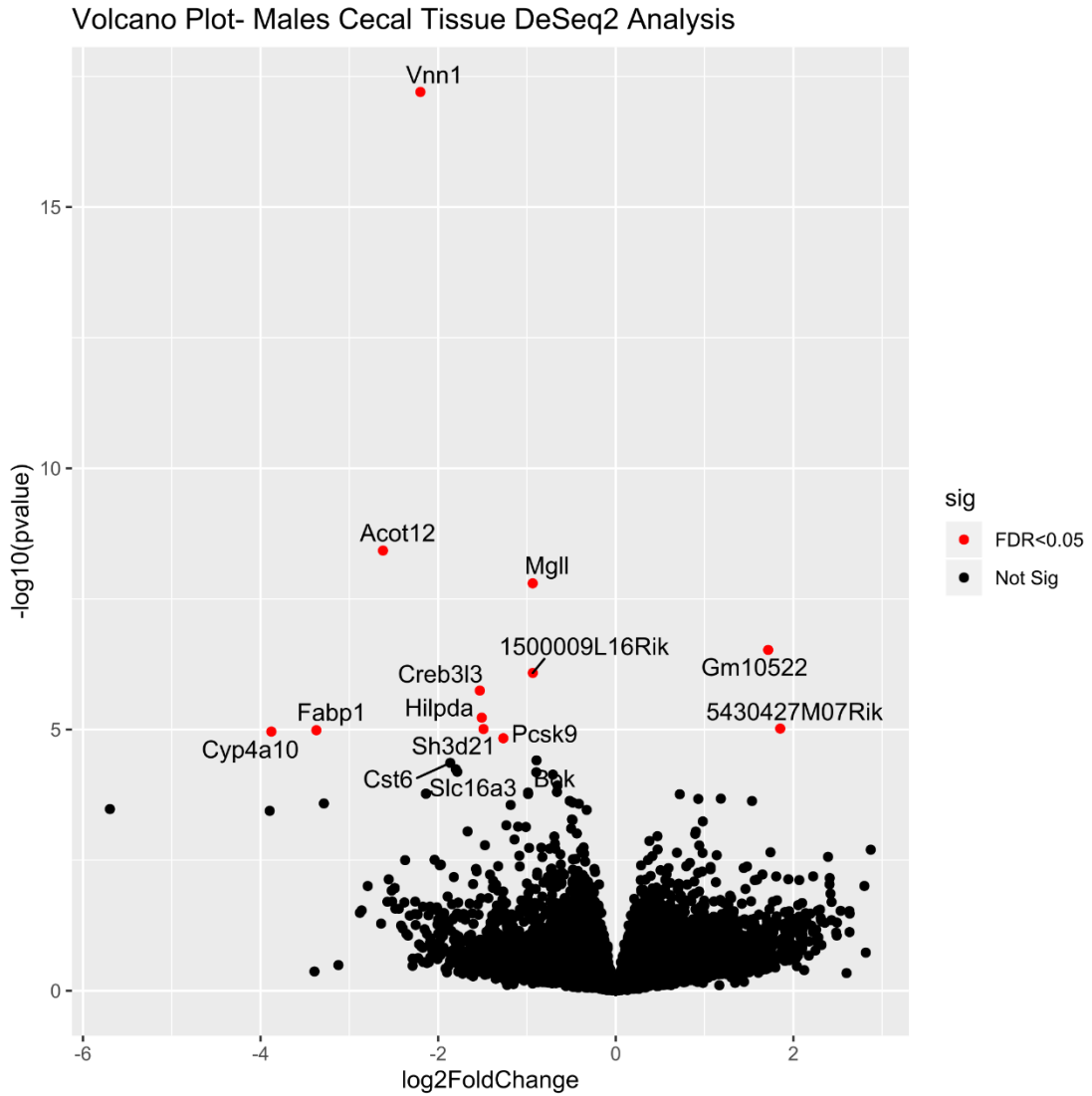
- The functional activity (gene expression and metabolism) of the microbiota induce changes in energy balance in the host.

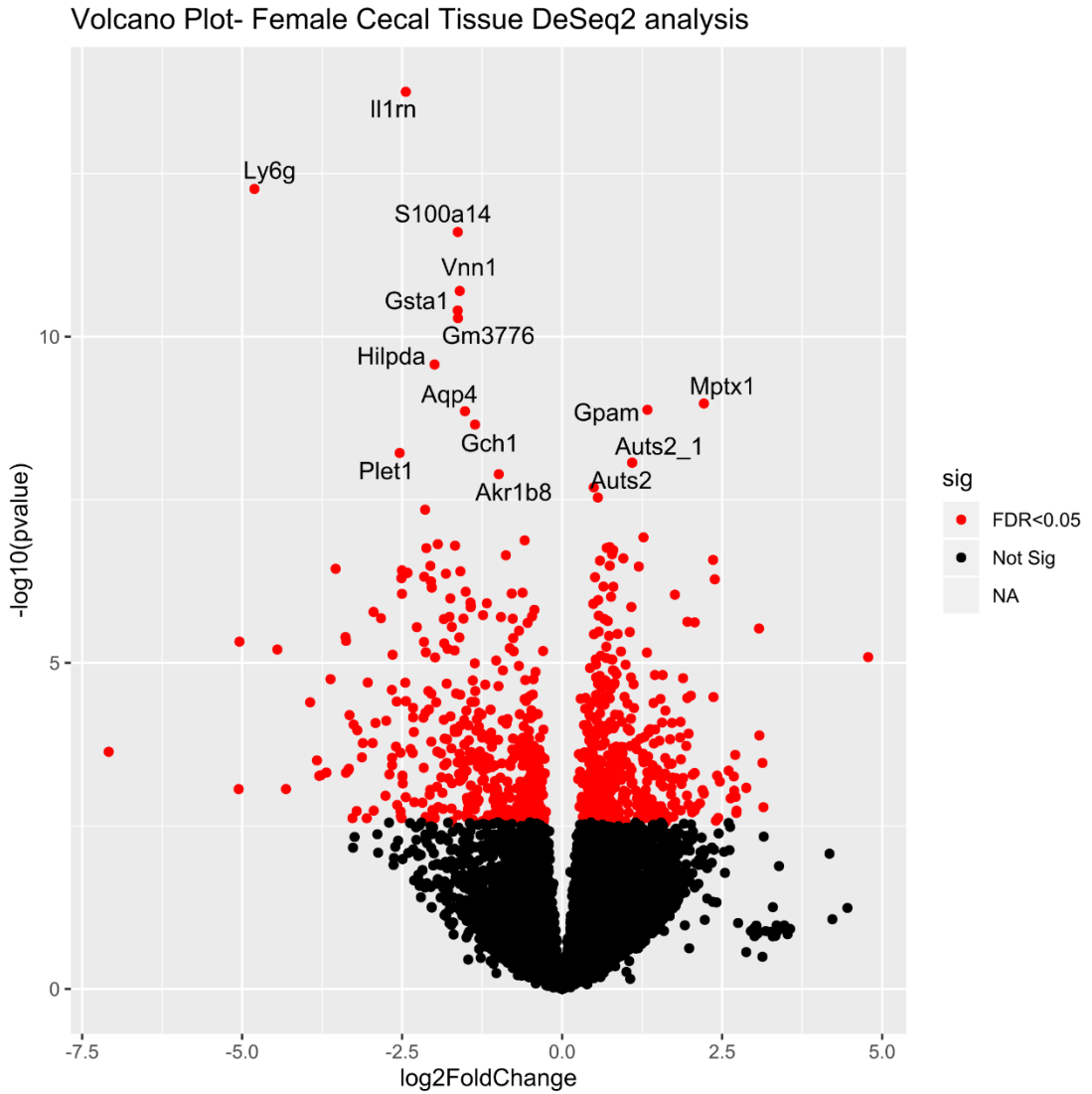
- Explore the sex differences in microbe-host responses in response to surgery

Cecal Tissue Transcriptomics- Tag-Seq

- Tag-seq transcriptome analysis of ribodepleted polyA RNA
  - Gene mapping/Annotation using STAR RNA-seq aligner (https://github.com/alexdobin/STAR/releases)
  - Differential gene expression (DGE) analysis using DeSeq2
- Post-analysis
  - Gene Set Enrichment Analysis (GSEA)- Broad Institute(MIT/Harvard)
  - Pathway analysis using ClusterProfileR using GO (Gene Ontology) and KEGG pathways

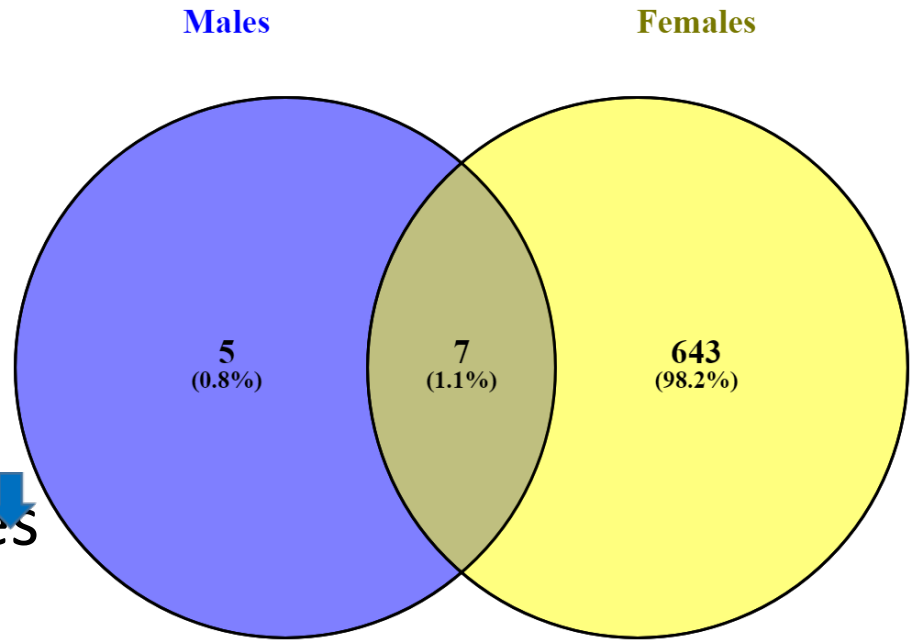
Cecal Tissue Transcriptomics- Tag-Seq (Host)





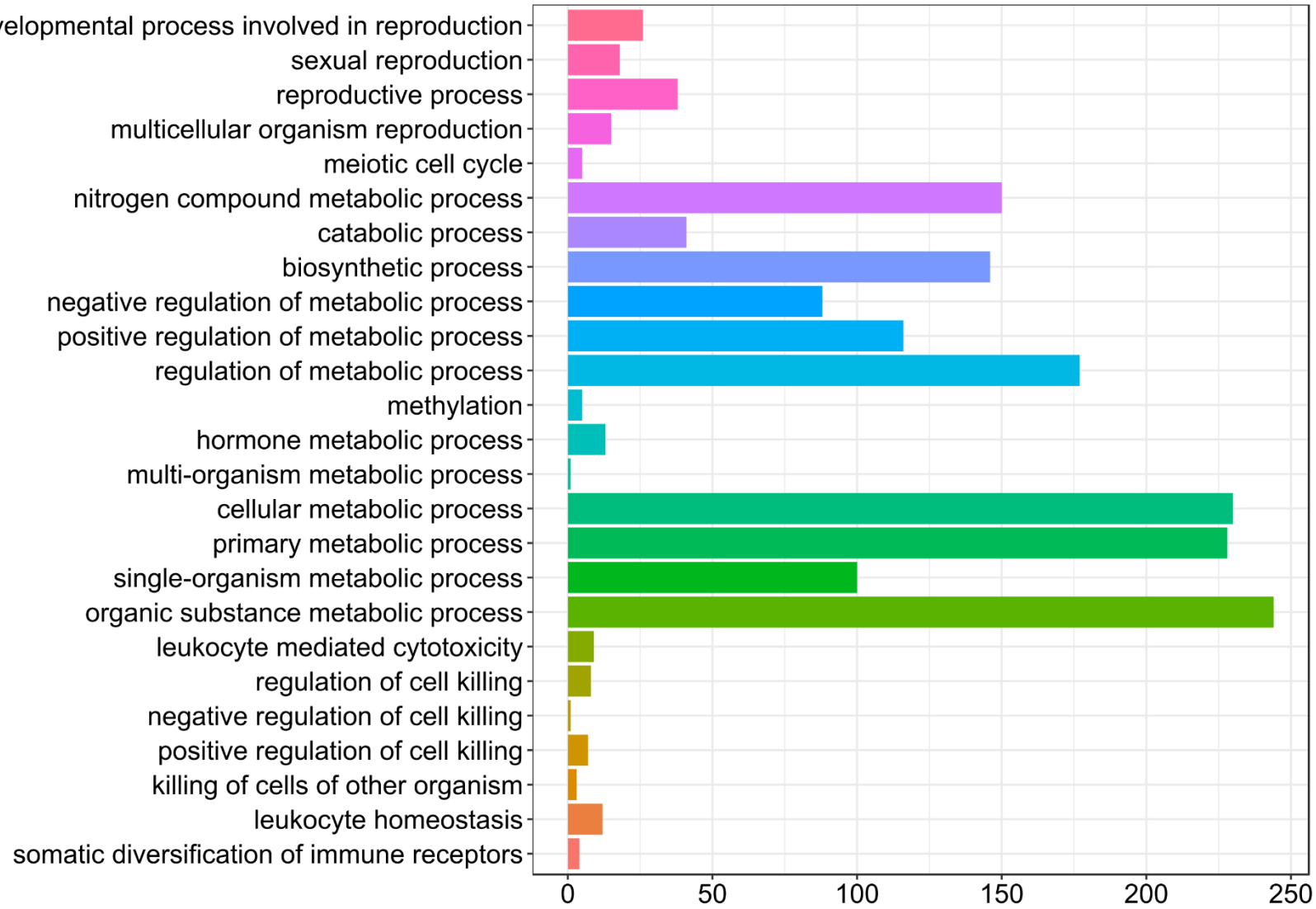
Cecal Tissue Transcriptomics- Tag-Seq (Host)

- Males- 12 genes as differentially expressed
- Females- 650 differentially expressed
- 7 common differentially expressed genes in males and females
  - Vnn1 (Vanin-1)
  - Acot12 (Acyl-CoA Thioesterase 12)
  - 1500009L16Rik
  - Hilpda (hypoxia inducible lipid droplet associated)
  - Cyp4a10 (Cytochrome P450 4A10) 5430427M07Rik
  - 5430427M07Rik (LncRNA)
  - Pcsk9 (Proprotein convertase subtilisin/kexin type 9)
- Significant number of predicted PPAR target genes



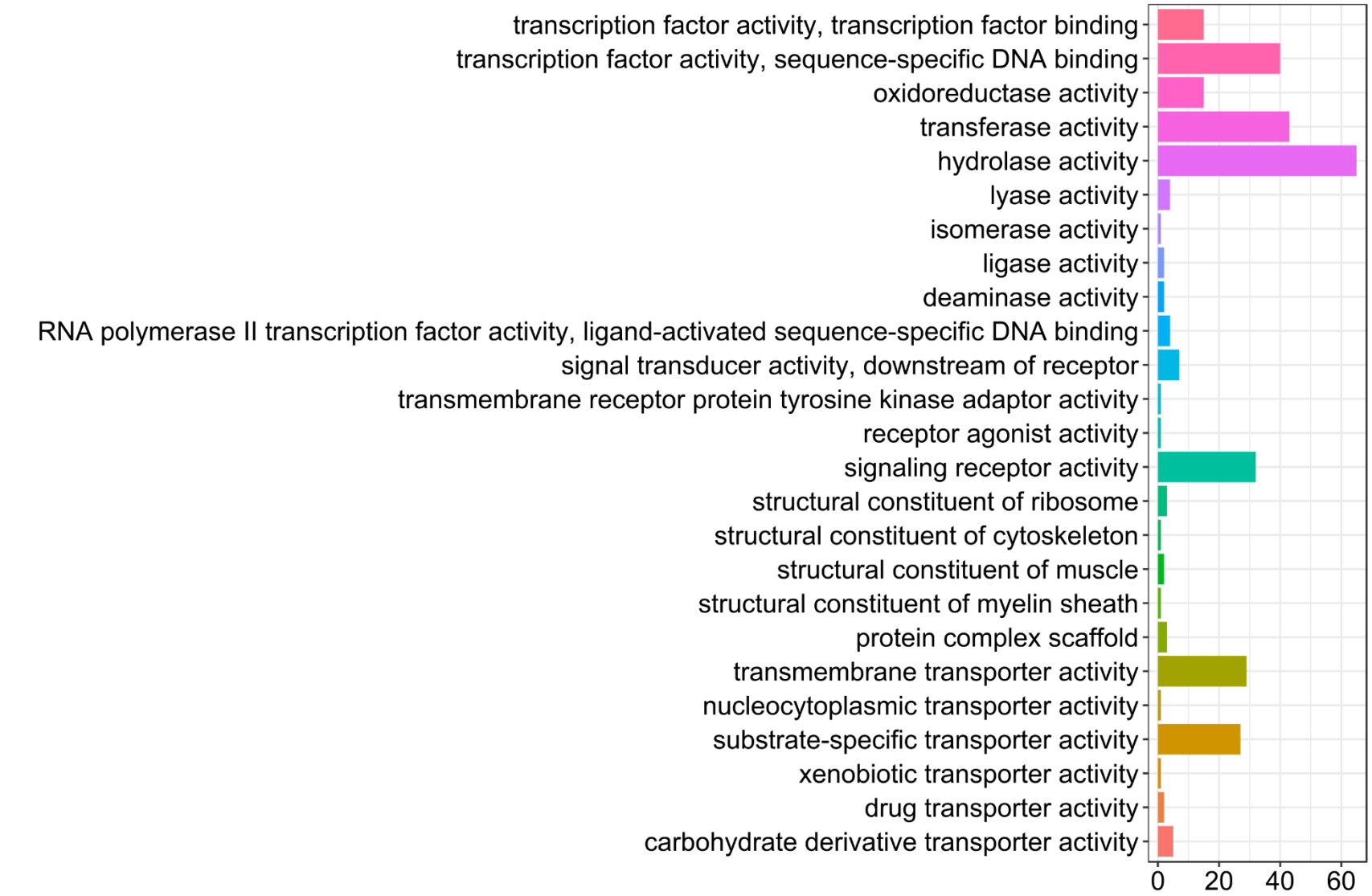
Cecal Tissue Transcriptomics- Tag-Seq (Host)

Gene Ontology (GO):
Biological Processes (BP)



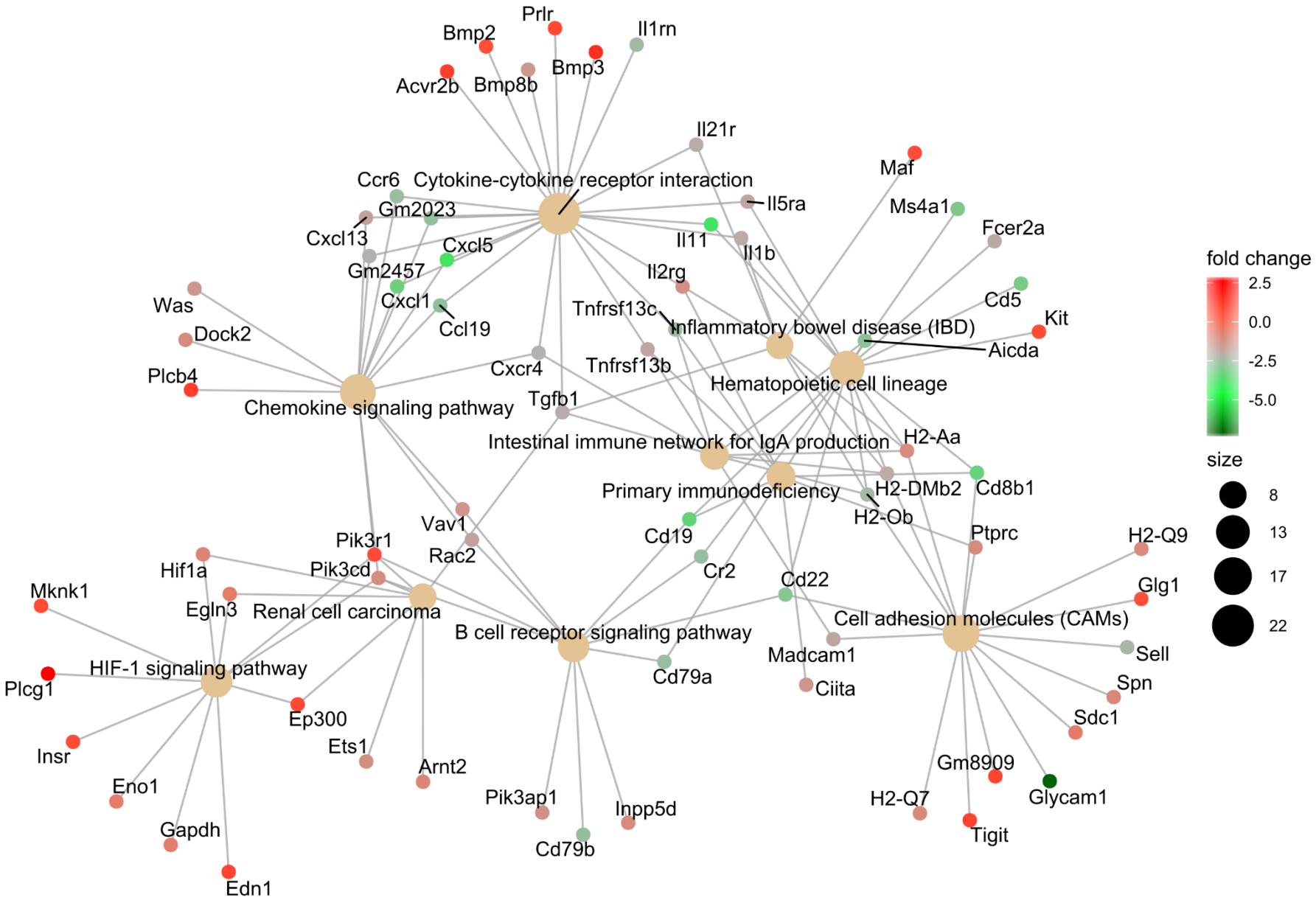
Cecal Tissue Transcriptomics- Tag-Seq (Host)

Gene Ontology (GO):
Molecular Function (MF)



Cecal Tissue Transcriptomics- Tag-Seq (Host)- Initial Pathway Analysis

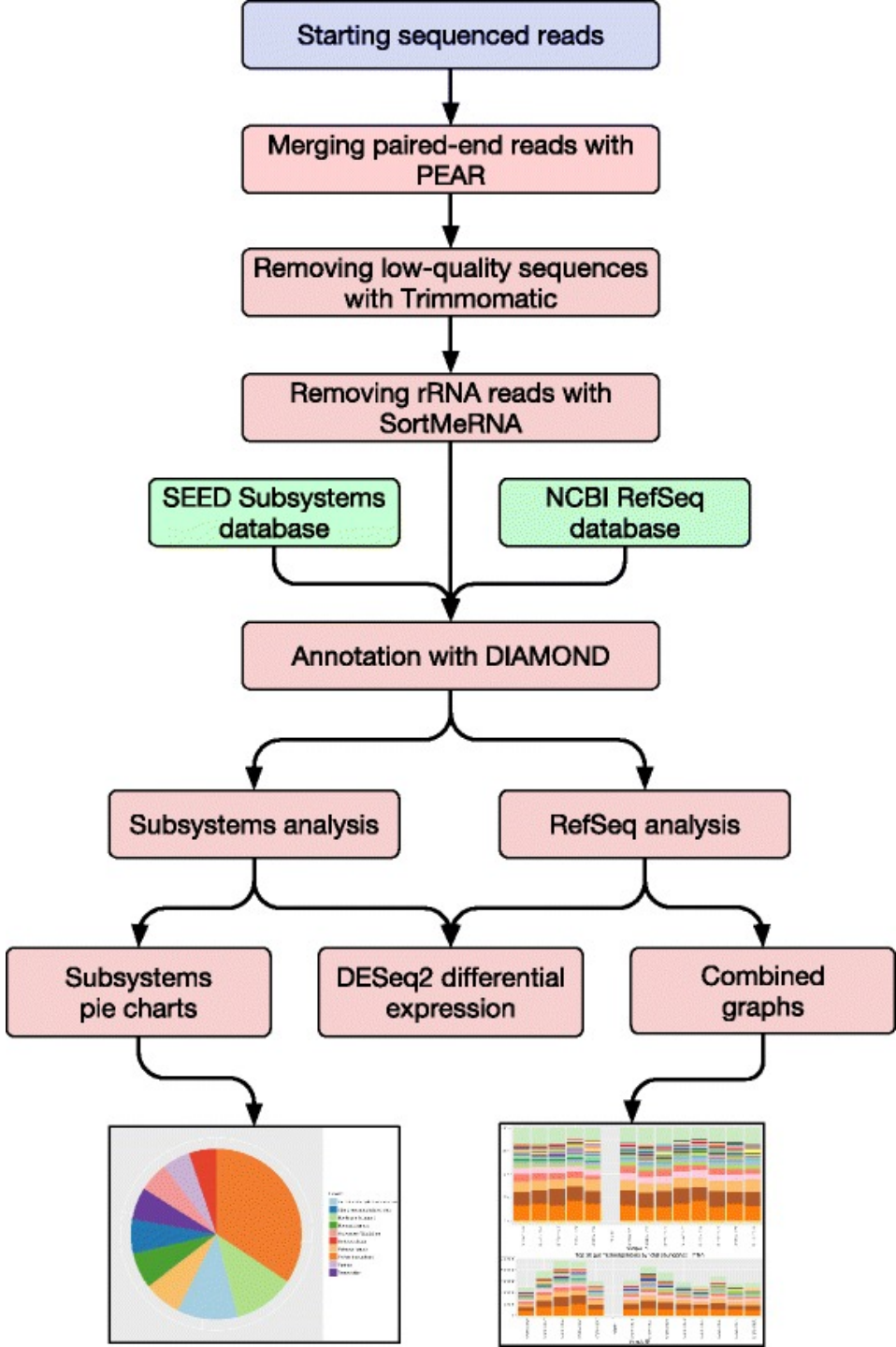
Gene function enrichment analysis using KEGG pathways



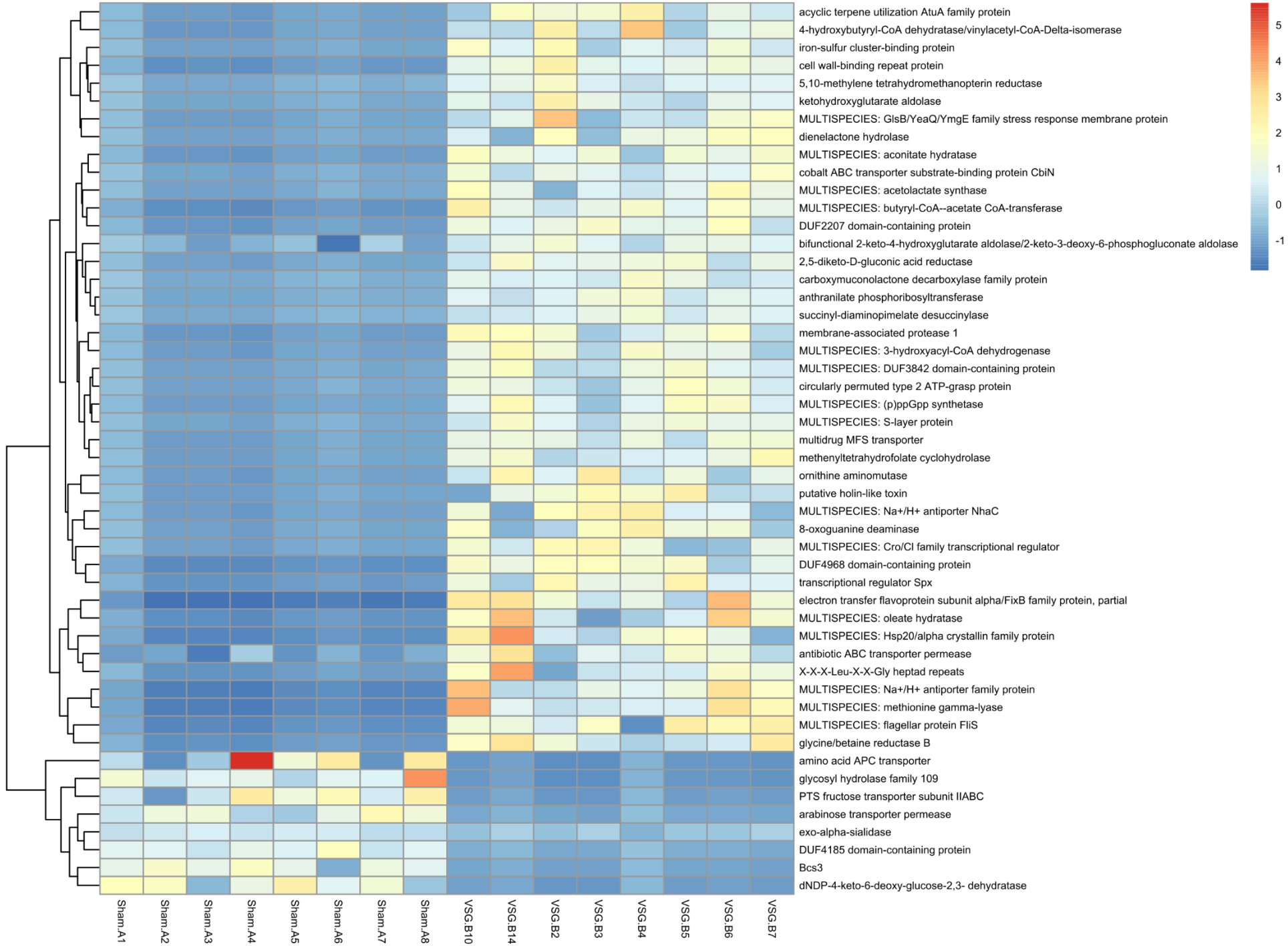
Cecal Content Metatranscriptomics

- Ribodepleted total RNA
- SAMSA2 (Simple Annotation of Metatranscriptomes by Sequence Analysis) pipeline for analysis (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5963165/)
- https://github.com/transcript/samsa2

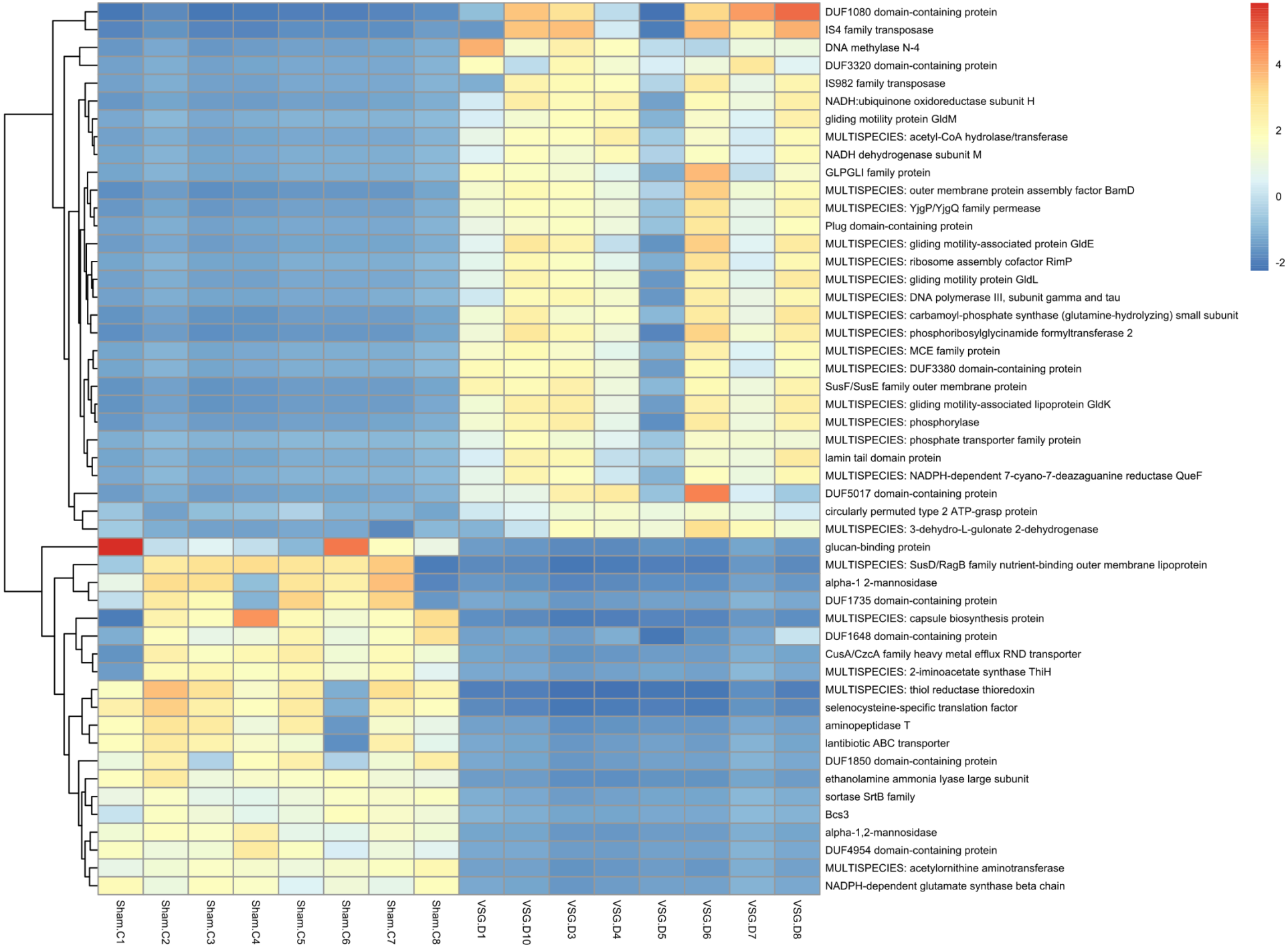
- Additional analysis in R



Males


acyclic terpene utilization AtuA family protein
4-hydroxybutyryl-CoA dehydratase/vinylacetyl-CoA-Delta-isomerase
iron-sulfur cluster-binding protein
cell wall-binding repeat protein
5,10-methylene tetrahydromethanopterin reductase
ketohydroxyglutarate aldolase
MULTISPECIES: GlsB/YeaQ/YmgE family stress response membrane protein
dienelactone hydrolase
MULTISPECIES: aconitate hydratase
cobalt ABC transporter substrate-binding protein CbiN
MULTISPECIES: acetolactate synthase
MULTISPECIES: butyryl-CoA--acetate CoA-transferase
DUF2207 domain-containing protein
bifunctional 2-keto-4-hydroxyglutarate aldolase/2-keto-3-deoxy-6-phosphogluconate aldolase
2,5-diketo-D-gluconic acid reductase
carboxymuconolactone decarboxylase family protein
anthranilate phosphoribosyltransferase
succinyl-diaminopimelate desuccinylase
membrane-associated protease 1
MULTISPECIES: 3-hydroxyacyl-CoA dehydrogenase
MULTISPECIES: DUF3842 domain-containing protein
circularly permuted type 2 ATP-grasp protein
MULTISPECIES: (p)ppGpp synthetase
MULTISPECIES: S-layer protein
multidrug MFS transporter
methenyltetrahydrofolate cyclohydrolase
ornithine aminomutase
putative holin-like toxin
MULTISPECIES: Na+/H+ antiporter NhaC
8-oxoguanine deaminase
MULTISPECIES: Cro/CI family transcriptional regulator
DUF4968 domain-containing protein
transcriptional regulator Spx
electron transfer flavoprotein subunit alpha/FixB family protein, partial
MULTISPECIES: oleate hydratase
MULTISPECIES: Hsp20/alpha crystallin family protein
antibiotic ABC transporter permease
X-X-X-Leu-X-X-Gly heptad repeats
MULTISPECIES: Na+/H+ antiporter family protein
MULTISPECIES: methionine gamma-lyase
MULTISPECIES: flagellar protein FliS
glycine/betaine reductase B
amino acid APC transporter
glycosyl hydrolase family 109
PTS fructose transporter subunit IIABC
arabinose transporter permease
exo-alpha-sialidase
DUF4185 domain-containing protein
Bcs3
dNDP-4-keto-6-deoxy-glucose-2,3- dehydratase
5
4
3
2
1
0
-1
Sham.A1
Sham.A2
Sham.A3
Sham.A4
Sham.A5
Sham.A6
Sham.A7
Sham.A8
VSG.B10
VSG.B14
VSG.B2
VSG.B3
VSG.B4
VSG.B5
VSG.B6
VSG.B7

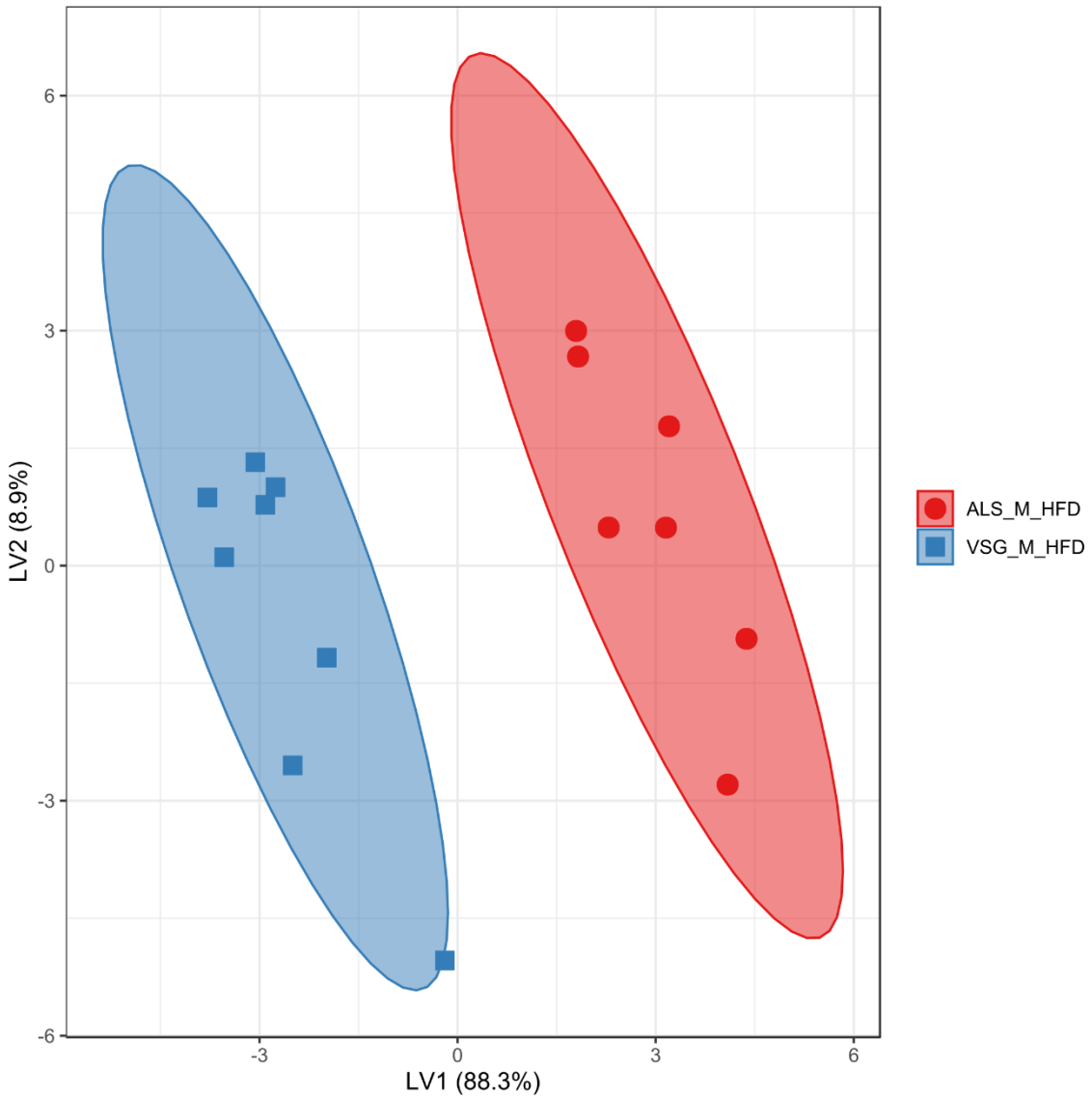
Females

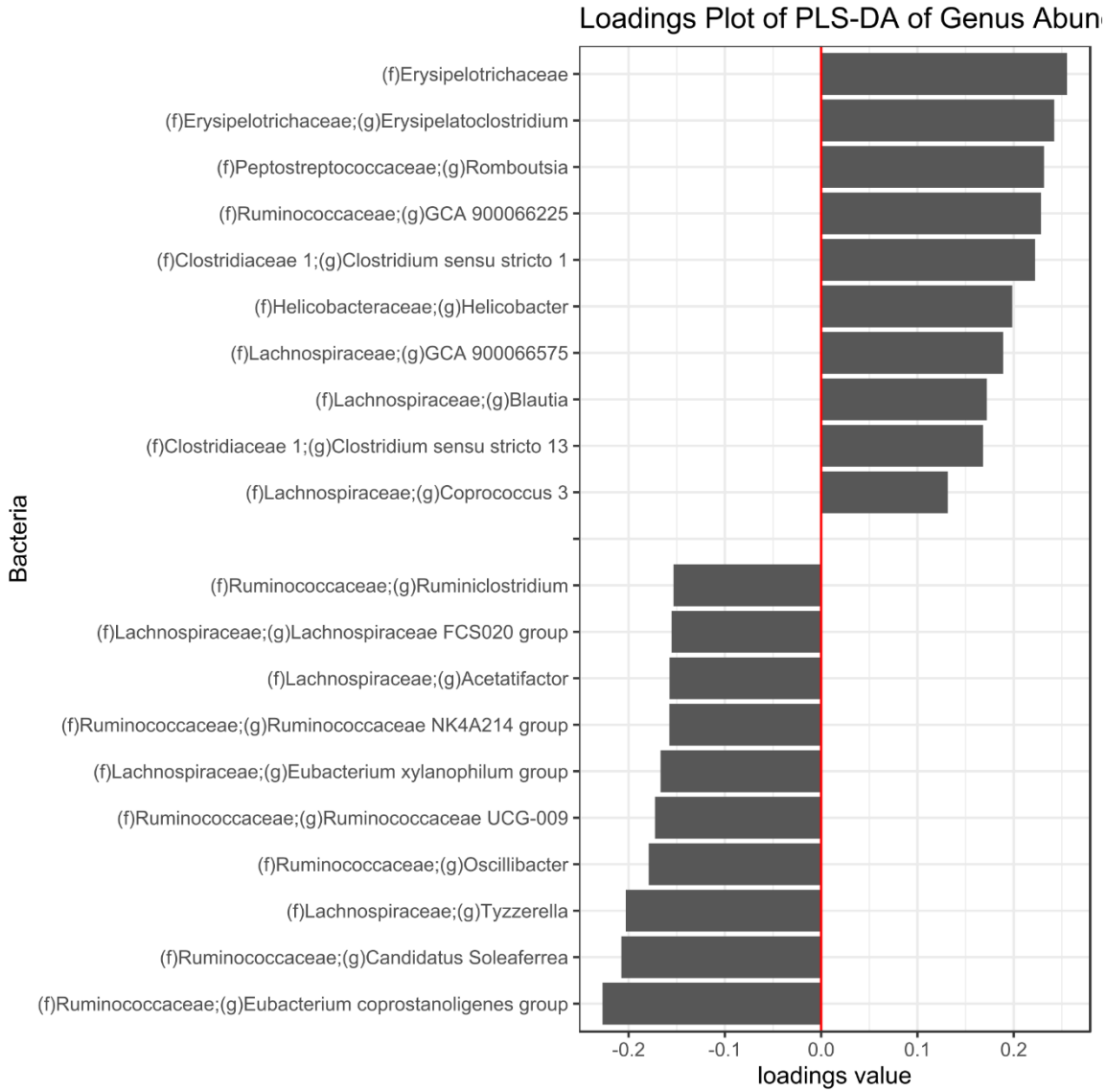

DUF1080 domain-containing protein
IS4 family transposase
DNA methylase N-4
DUF3320 domain-containing protein
IS982 family transposase
NADH:ubiquinone oxidoreductase subunit H
gliding motility protein GldM
MULTISPECIES: acetyl-CoA hydrolase/transferase
NADH dehydrogenase subunit M
GLPGLI family protein
MULTISPECIES: outer membrane protein assembly factor BamD
MULTISPECIES: YjgP/YjgQ family permease
Plug domain-containing protein
MULTISPECIES: gliding motility-associated protein GldE
MULTISPECIES: ribosome assembly cofactor RimP
MULTISPECIES: gliding motility protein GldL
MULTISPECIES: DNA polymerase III, subunit gamma and tau
MULTISPECIES: carbamoyl-phosphate synthase (glutamine-hydrolyzing) small subunit
MULTISPECIES: phosphoribosylglycinamide formyltransferase 2
MULTISPECIES: MCE family protein
MULTISPECIES: DUF3380 domain-containing protein
SusF/SusE family outer membrane protein
MULTISPECIES: gliding motility-associated lipoprotein GldK
MULTISPECIES: phosphorylase
MULTISPECIES: phosphate transporter family protein
lamin tail domain protein
MULTISPECIES: NADPH-dependent 7-cyano-7-deazaguanine reductase QueF
DUF5017 domain-containing protein
circularly permuted type 2 ATP-grasp protein
MULTISPECIES: 3-dehydro-L-gulonate 2-dehydrogenase
glucan-binding protein
MULTISPECIES: SusD/RagB family nutrient-binding outer membrane lipoprotein
alpha-1 2-mannosidase
DUF1735 domain-containing protein
MULTISPECIES: capsule biosynthesis protein
DUF1648 domain-containing protein
CusA/CzcA family heavy metal efflux RND transporter
MULTISPECIES: 2-iminoacetate synthase ThiH
MULTISPECIES: thiol reductase thioredoxin
selenocysteine-specific translation factor
aminopeptidase T
lantibiotic ABC transporter
DUF1850 domain-containing protein
ethanolamine ammonia lyase large subunit
sortase SrtB family
Bcs3
alpha-1,2-mannosidase
DUF4954 domain-containing protein
MULTISPECIES: acetylornithine aminotransferase
NADPH-dependent glutamate synthase beta chain
4
2
0
-2
Sham.C1
Sham.C2
Sham.C3
Sham.C4
Sham.C5
Sham.C6
Sham.C7
Sham.C8
VSG.D1
VSG.D10
VSG.D3
VSG.D4
VSG.D5
VSG.D6
VSG.D7
VSG.D8

Bacterial community profiling- 16S rDNA sequencing

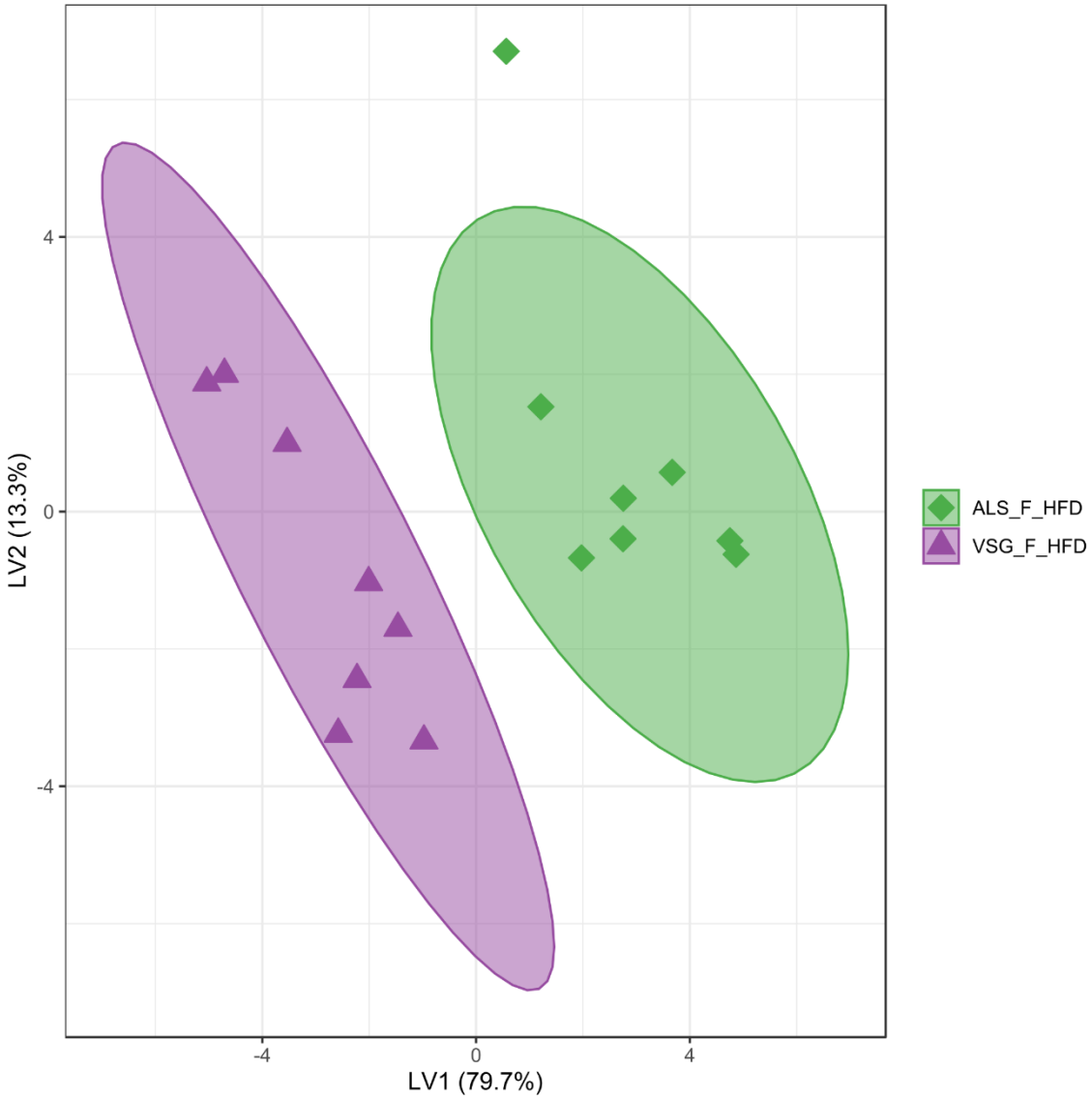
- Microbiota community composition & diversity
  - Illumina paired end 300bp reads (PE300)
  - QIIME2 pipeline using Silva alignment databases
  - Taxonomical classification- % Abundance
  - Diversity- phylogenetic and non-phylogenetic measures
  - Statistical analysis
- Multivariate Analysis
  - Partial least squares discriminant analysis (PLS-DA) using R software
- Metadata correlations
  - Microbiota composition with measured physiological parameters
  - Spearman correlation with heatmap visualization (R)

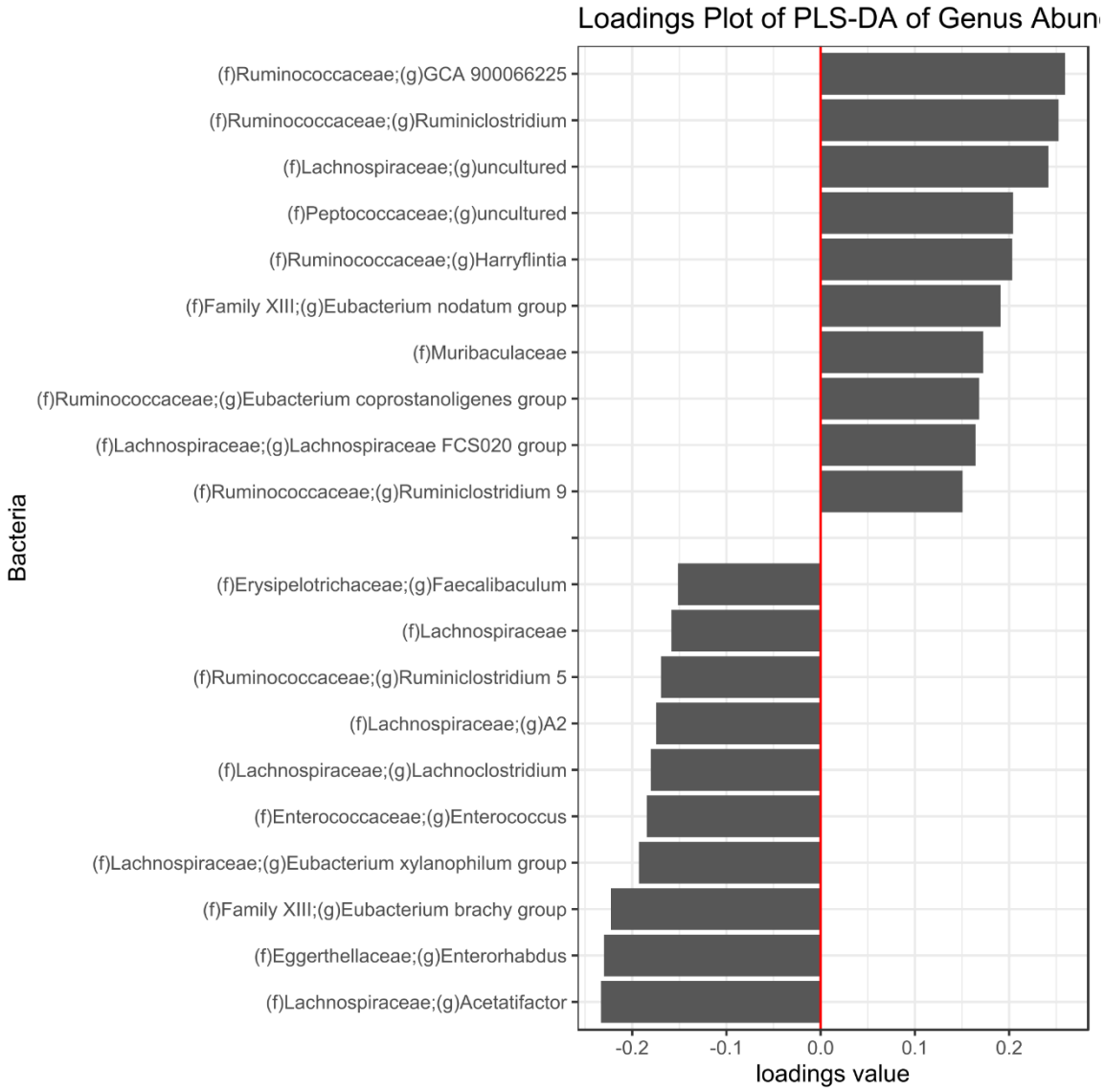
Males


6
3
0
-3
-6
LV2 (8.9%)
-3
0
3
6
LV1 (88.3%)
ALS_M_HFD
VSG_M_HFD


Loadings Plot of PLS-DA of Genus Abun
(f)Erysipelotrichaceae
(f)Erysipelotrichaceae;(g)Erysipelatoclostridium
(f)Peptostreptococcaceae;(g)Romboutsia
(f)Ruminococcaceae;(g)GCA 900066225
(f)Clostridiaceae 1;(g)Clostridium sensu stricto 1
(f)Helicobacteraceae;(g)Helicobacter
(f)Lachnospiraceae;(g)GCA 900066575
(f)Lachnospiraceae;(g)Blautia
(f)Clostridiaceae 1;(g)Clostridium sensu stricto 13
(f)Lachnospiraceae;(g)Coprococcus 3
(f)Ruminococcaceae;(g)Ruminiclostridium
(f)Lachnospiraceae;(g)Lachnospiraceae FCS020 group
(f)Lachnospiraceae;(g)Acetatifactor
(f)Ruminococcaceae;(g)Ruminococcaceae NK4A214 group
(f)Lachnospiraceae;(g)Eubacterium xylanophilum group
(f)Ruminococcaceae;(g)Ruminococcaceae UCG-009
(f)Ruminococcaceae;(g)Oscillibacter
(f)Lachnospiraceae;(g)Tyzzerella
(f)Ruminococcaceae;(g)Candidatus Soleaferrea
(f)Ruminococcaceae;(g)Eubacterium coprostanoligenes group
Bacteria
-0.2
-0.1
0.0
0.1
0.2
loadings value

Females


4
0
-4
LV2 (13.3%)
-4
0
4
LV1 (79.7%)
ALS_F_HFD
VSG_F_HFD


Loadings Plot of PLS-DA of Genus Abun
(f)Ruminococcaceae;(g)GCA 900066225
(f)Ruminococcaceae;(g)Ruminiclostridium
(f)Lachnospiraceae;(g)uncultured
(f)Peptococcaceae;(g)uncultured
(f)Ruminococcaceae;(g)Harryflintia
(f)Family XIII;(g)Eubacterium nodatum group
(f)Muribaculaceae
(f)Ruminococcaceae;(g)Eubacterium coprostanoligenes group
(f)Lachnospiraceae;(g)Lachnospiraceae FCS020 group
(f)Ruminococcaceae;(g)Ruminiclostridium 9
(f)Erysipelotrichaceae;(g)Faecalibaculum
(f)Lachnospiraceae
(f)Ruminococcaceae;(g)Ruminiclostridium 5
(f)Lachnospiraceae;(g)A2
(f)Lachnospiraceae;(g)Lachnoclostridium
(f)Enterococcaceae;(g)Enterococcus
(f)Lachnospiraceae;(g)Eubacterium xylanophilum group
(f)Family XIII;(g)Eubacterium brachy group
(f)Eggerthellaceae;(g)Enterorhabdus
(f)Lachnospiraceae;(g)Acetatifactor
Bacteria
-0.2
-0.1
0.0
0.1
0.2
loadings value

Moving forward

- Continue pathway analysis
- Correlations to the physiological metadata
  - BW, body comp, OGTT
  - Plasma analytes
    - TG
    - FFAs
    - Fasting glucose
  - Bile acids
  - Energy expenditure
- Combine with microbial community data
- Streamline analysis process for pipeline development

Acknowledgments

- MMPC Bariatric Working Group
  - Kristin Evans/David Wasserman
- MMPC Core B
  - Kristin Grimsrud
  - Leslie Stewart (surgeon/mouse whisperer)
  - MBP Pheno core- Lynette Bower, Todd Tolentino, Heather Tolentino
- Michael Goodson and Helen Raybould