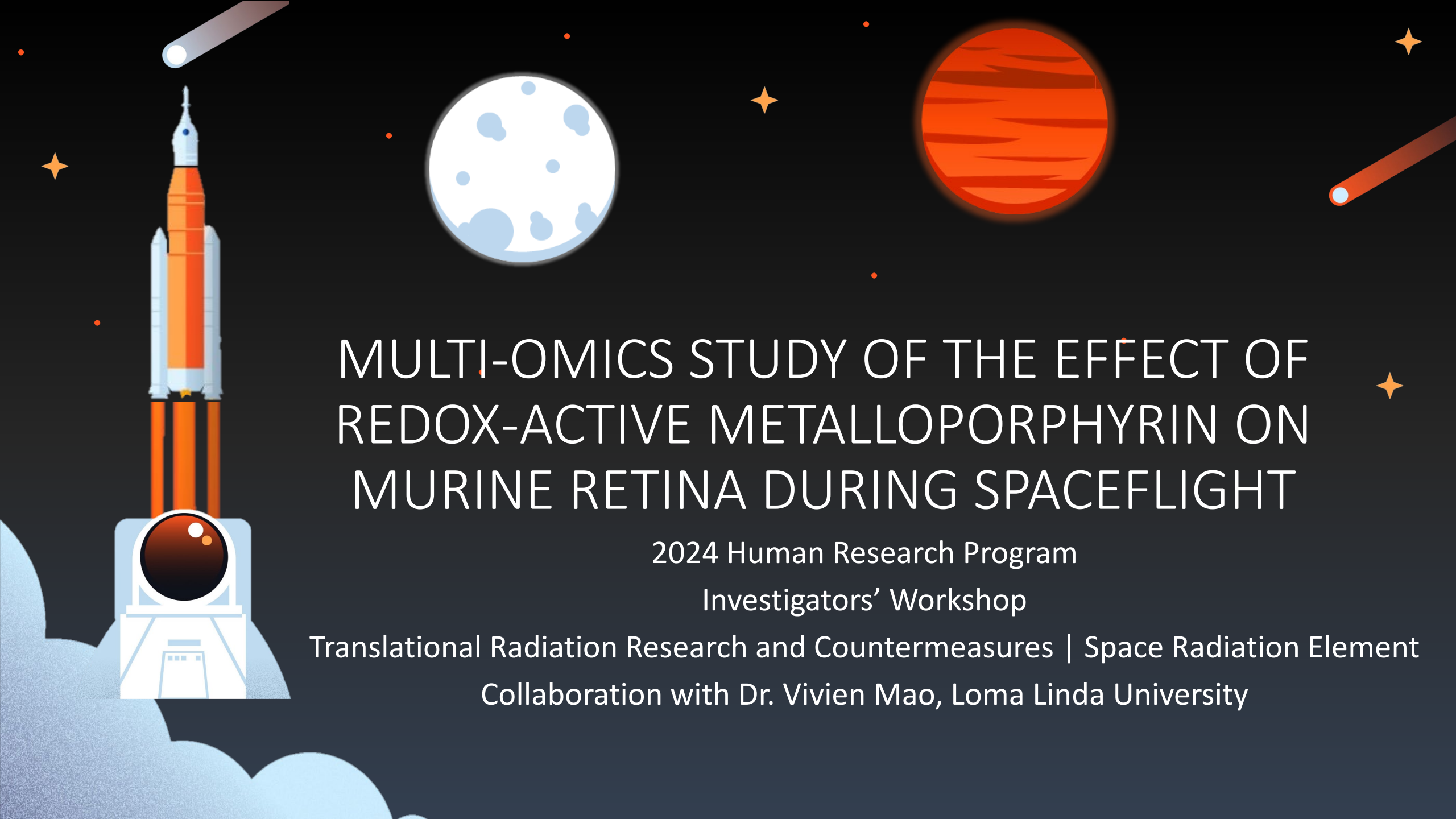


MULTI-OMICS STUDY OF THE EFFECT OF REDOX-ACTIVE METALLOPORPHYRIN ON MURINE RETINA DURING SPACEFLIGHT

2024 Human Research Program

Investigators' Workshop

Translational Radiation Research and Countermeasures | Space Radiation Element

Collaboration with Dr. Vivien Mao, Loma Linda University

Translational Radiation Research and Countermeasures (TRRaC)

- Mission to translate radiation research results based on animal studies to humans using bioinformatics and computational modeling
- Previously collaborated with Dr Vivien Mao on multi-omics longitudinal study of murine retina from HLU and GCRsim study
- Previous collaborative work:
  - Mao, Xiaowen, Seta Stanbouly, Jacob Holley, Michael Pecaut, and James Crapo. 2023. "Evidence of Spaceflight-Induced Adverse Effects on Photoreceptors and Retinal Function in the Mouse Eye" International Journal of Molecular Sciences 24, no. 8: 7362. https://doi.org/10.3390/ijms24087362
  - Kothiyal P, Eley G, Ilangovan H, et al. A multi-omics longitudinal study of the murine retinal response to chronic low-dose irradiation and simulated microgravity. Sci Rep. 2022;12(1):16825. Published 2022 Oct 7. doi:10.1038/s41598-022-19360-9

TRRaC Accomplishments

Deliverables

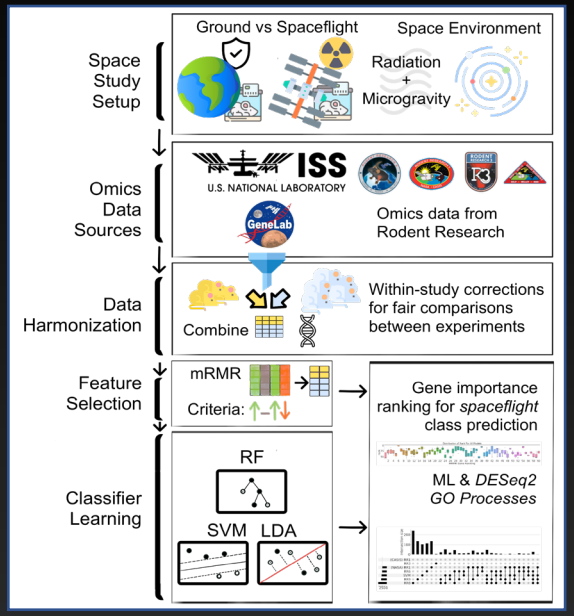
- NASA/JAXA Nature Package publication pending
  - Hari Ilangovan, Prachi Kothiyal, Katherine Hoadley et al. Spaced Out Data No More: Harmonizing Heterogeneous Transcriptomics Datasets for Machine Learning based Analysis to Identify Spaceflown Murine Liver-specific changes, 11 July 2023, PREPRINT (Version 1) available at Research Square [https://doi.org/10.21203/rs.3.rs-2827816/v1]
- Collaboration with Dr. Vivien Mao on RR-18 Study
  - *Multi-omics study of the effect of redox-active metalloporphyrin on murine retina during spaceflight (Oral presentation at IWS 24)*

Presentations

- ASGSR 2023, ICRR 2023, IWS 2023 on harmonized murine spaceflight studies and multi-omics murine retina analyses

In the Pipeline

- Sequencing minipig tissues from SRE x USAF radiation study
- AML (Spleen) hybridized murine study (RNA-seq & RRBS)

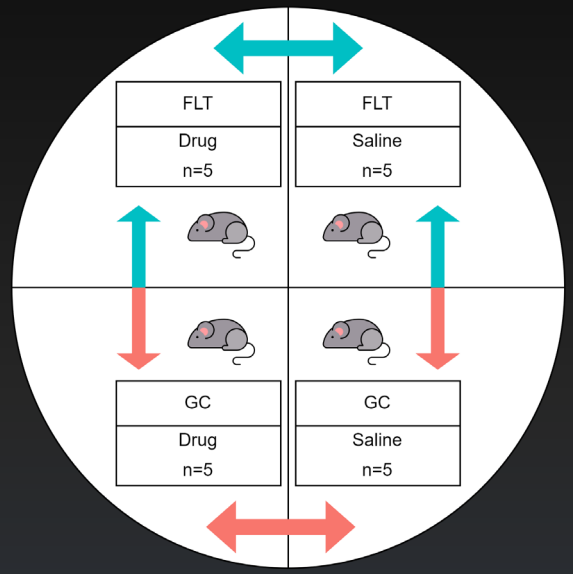


Team Capabilities

- Bioinformatics Analysis & Cloud Infrastructure
- Advanced Analytics (ML, GenAI) & Pipeline Development
- Sequencing Capability for RNA & DNA
- Space Radiation Focused Genomic Signatures

Murine Retinas from RR-18

C57BL/6J
Mouse Strain



35 days LEO

- Astronauts can experience eye problems after returning from space, along with SANS
- Spaceflight experiment with live animal return that investigated effect of antioxidant metalloporphyrin (BuOE)
  - BuOE protects cells against oxidative damage by controlling generation of mitochondrial ROS
- Multi omics data available including transcriptomics (RNA-Seq) and DNA methylation (RRBS)

Experiment Analysis Roadmap

Weekly treatment injection

Housed in Rodent Habitat **(35 days)**

C57BL/6J

Treatment

BuOE *Saline*

Murine samples spaceflown to ISS

Retina Extraction

Live Animal Return

Next Gen Sequencing

Transcriptomics Analysis

DNA Methylation Analysis

Differential Gene Expression

Significance

Fold Change

Pathways (GSEA)

GENEONTOLOGY Unifying Biology

BPs compared via Enriched Genes

RNA-Seq ↔ RRBS

Mapping DMRs to Genes

Expression versus Methylation

Weekly treatment injection

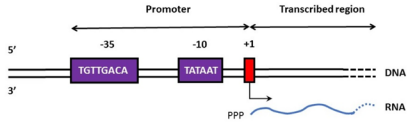
Housed in CASIS Rodent Habitat **(35 days)**

Differentially Methylated Regions (DMRs)

Num. DMRs

Chromosome

Annotation of DMRs

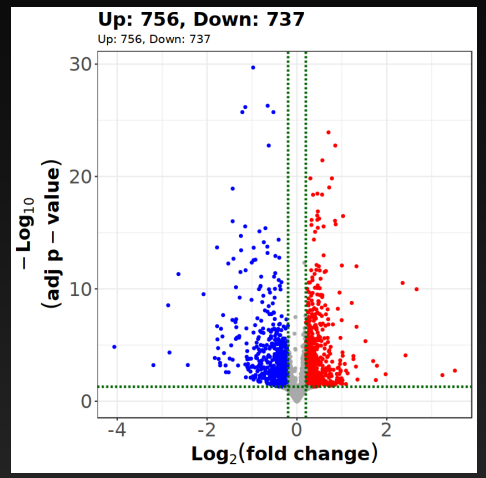


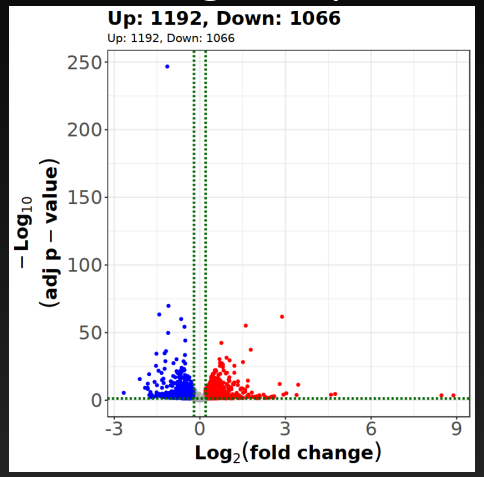
RNA-Seq Expression by Contrast

Saline Group

Drug Group

FLT vs GC

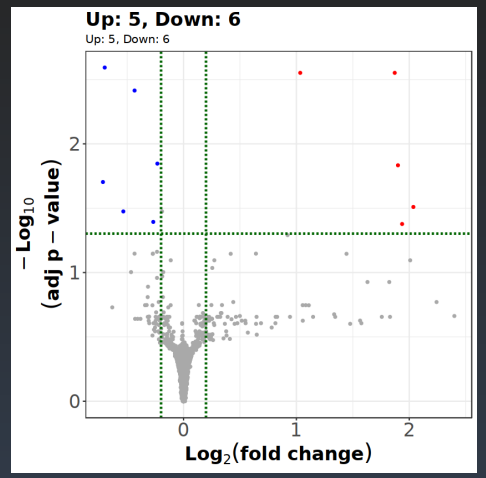


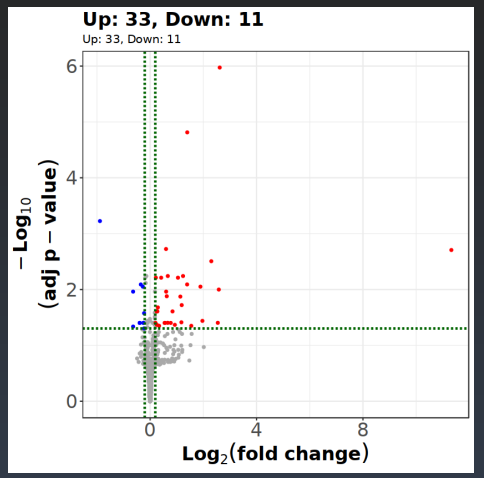


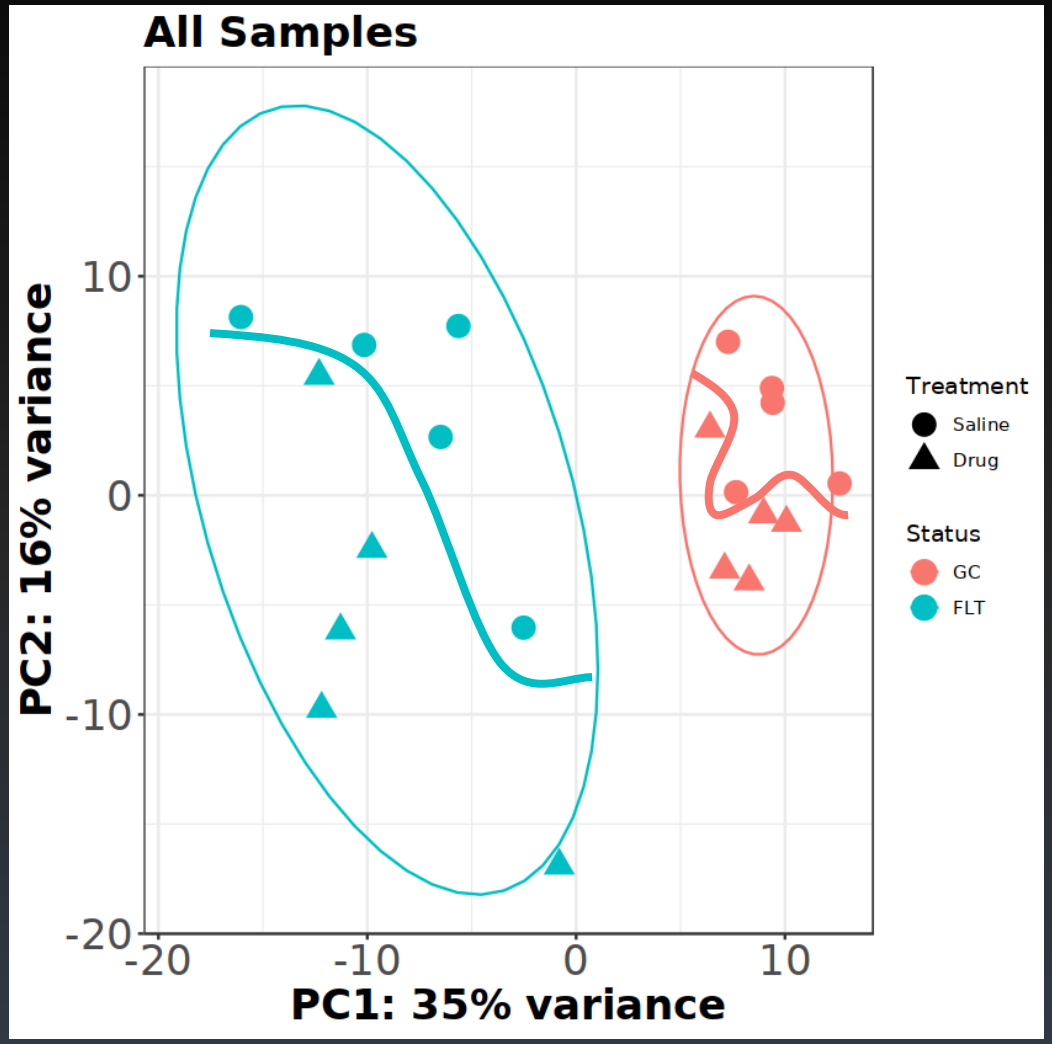
Spaceflown Group

Ground Group

Drug vs Saline







Status is larger driver of variance than BuOE treatment

Separability between Saline and Drug treatment

Differential Expression Results

DESeq2 - RNA-Seq | *MethylKit* & *Genomation* - RRBS

n = 5 per group (10 per contrast)	RNA-Seq	DEGs	RRBS	DMLs
Spaceflown (BuOE vs Saline)	11	(5↑ 6↓)	3890	(1654↑ 2236↓)
Ground (BuOE vs Saline)	44	(33↑ 11↓)	347	(251↑ 96↓)
Drug (FLT vs GC)	2258	(1192↑ 1066↓)	224	(140↑ 84↓)
Saline (FLT vs GC)	1493	(756↑ 737↓)	3590	(1786↑ 1804↓)

DEG ($|\log_2(\text{fold-change})| \geq 0.2$ and adjusted p-value ≤ 0.05)

DML (CpG loci or regions with |percent methylation difference| ≥ 10 and q-value ≤ 0.05) are listed for spaceflown and BuOE drug treated groups versus their matched ground controls

DEG counts are separated by up (↑) or down (↓) regulation; DML counts are separated by hyper (↑) and hypo (↓) methylation.

RNA-Seq

- FLT vs GC increased expression
- BuOE vs Saline minimal expression

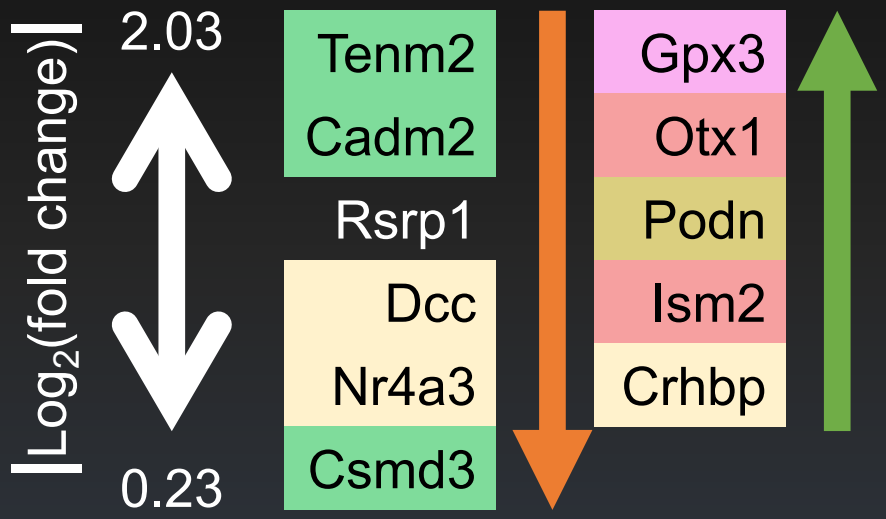
RRBS

- BuOE decreased DMLs relative to Saline in FLT vs GC
- FLT increased DMLs relative to GC

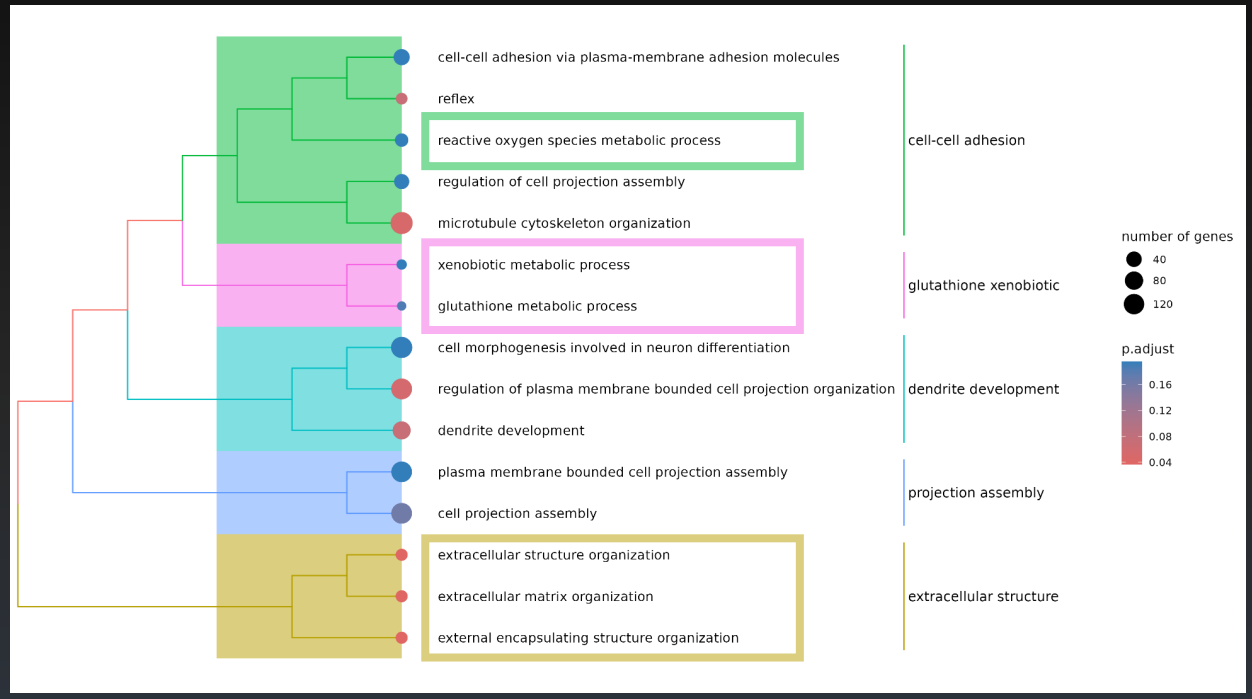
RNA-Seq Expression & Pathways

Spaceflown Group (Saline is reference)

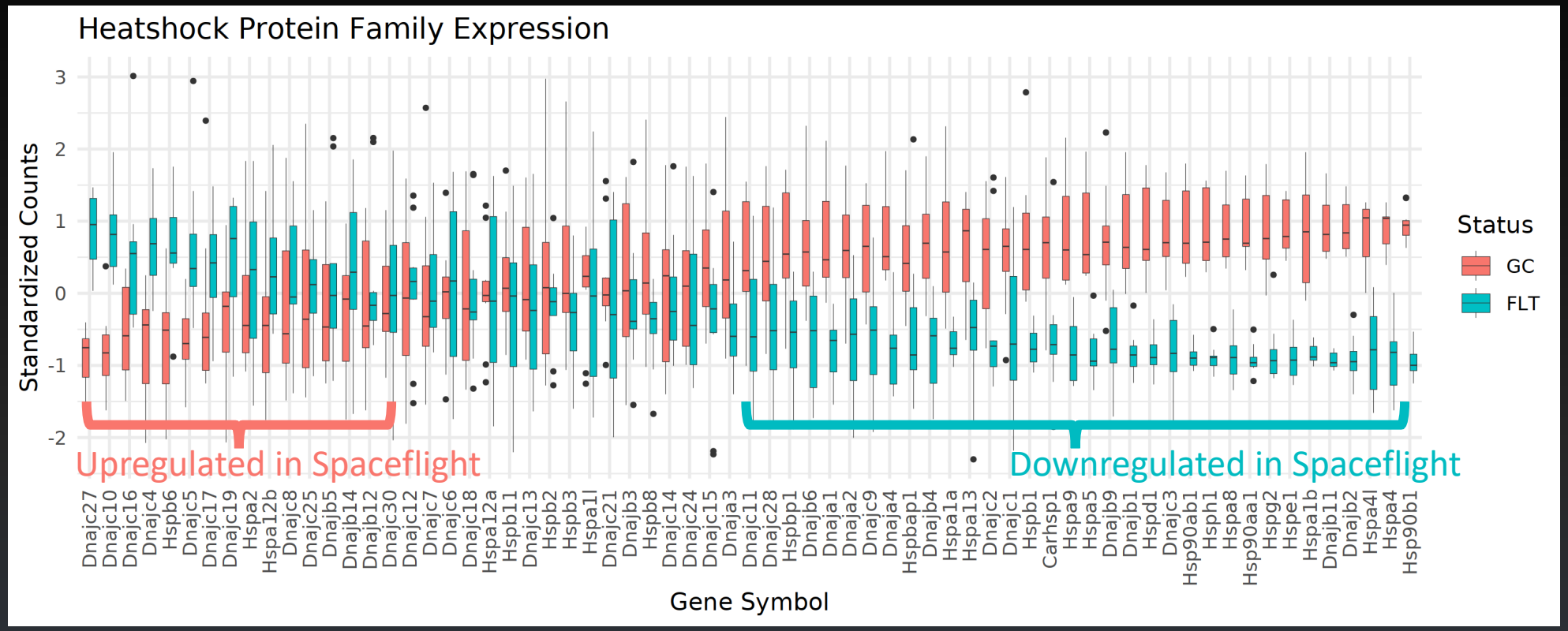
BuOE Treated vs Saline Control
Differential Expression (p-adjusted <0.05)



Gene Set Enrichment Analysis (GSEA)
Gene Ontology (p-adjusted < 0.05)



RNA-Seq Takeaways



- DEGs in center are highly variable and their significance driven by individual samples (dots)
- Heat shock proteins are triggered based on stress signals such as Oxidative Stress (ROS, ER Stress)

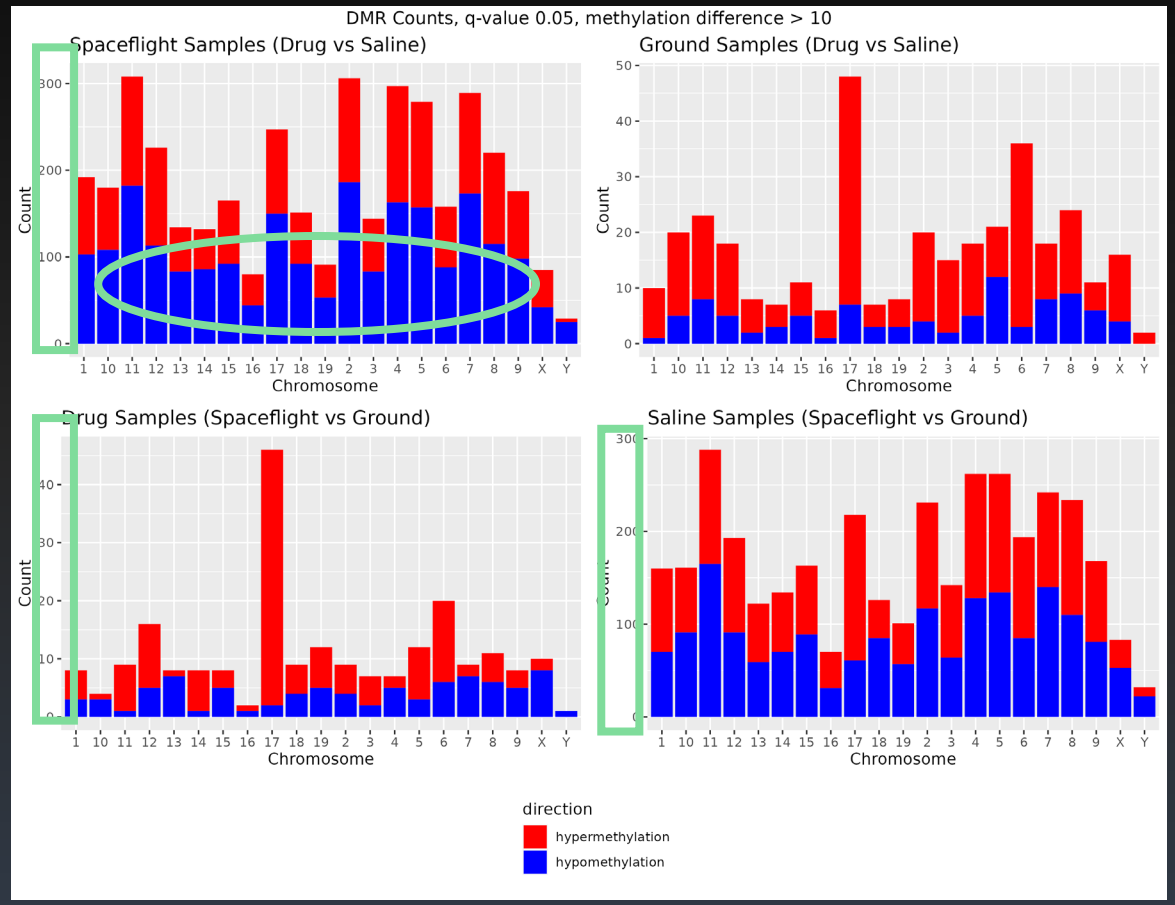
Heatshock protein list determined from pooled analysis DEGs

n = 10 per group (20 total)	**RNA-Seq**	**DEGs**
Spaceflown vs Ground Control	2604	(1296↑ 1308↓)

- Research also show that small heat shock proteins (Hspb1) are associated with retinal diseases. (Shepard et. al) (Rajeswaren e. al)

Chromosomal Region Breakdown

Differential Methylation Annotated



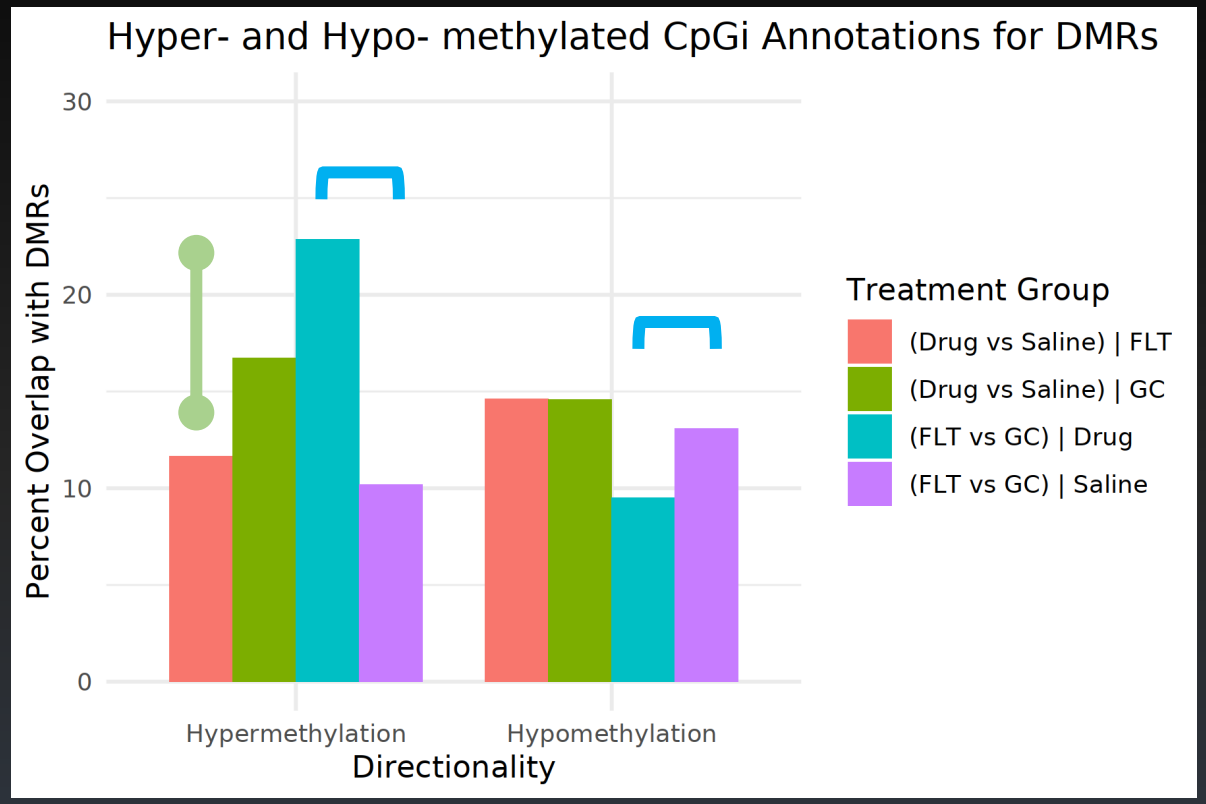
BuOE vs Saline: Spaceflight group more changes than Ground group, slight skew towards hypomethylation in Spaceflight

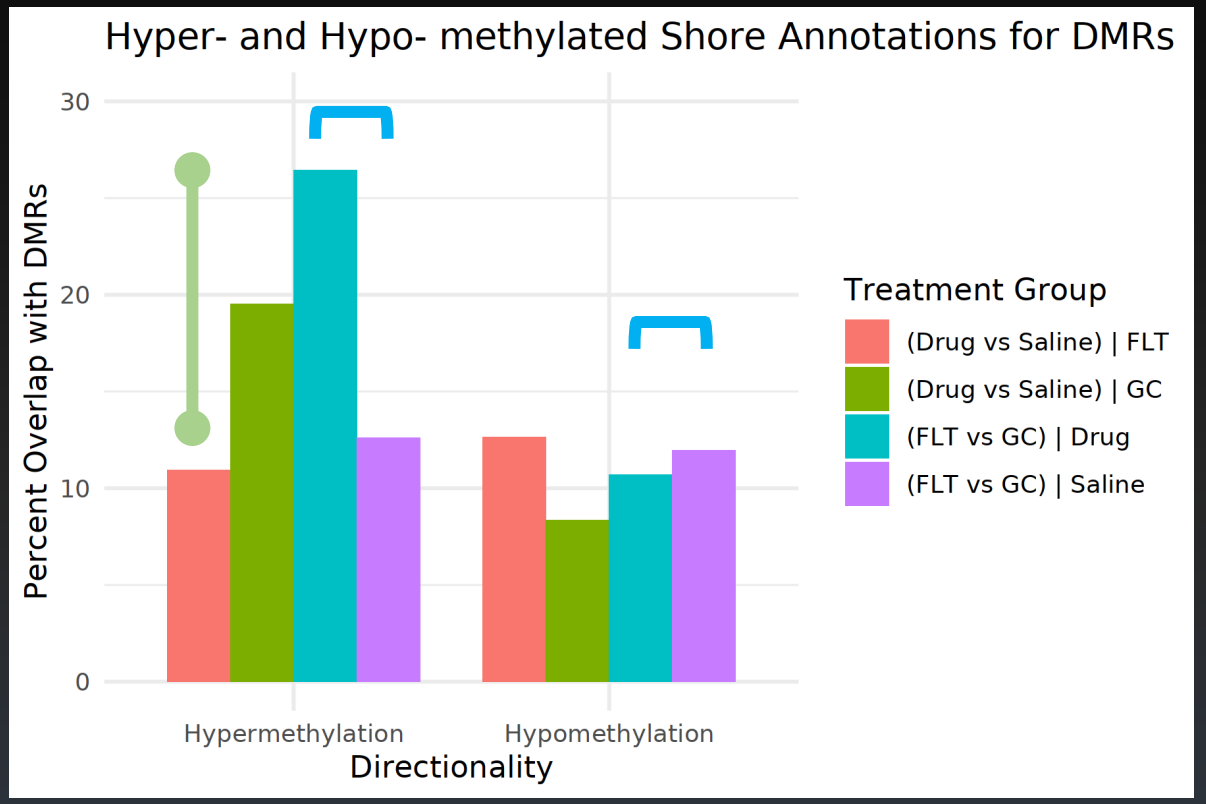
FLT vs GC: Saline group has more overall changes that Drug group

Mapping DMRs to Protein Coding Transcripts found spaceflight ubiquitin stress response related genes (*Trim47, Trim10, Tnfaip1, Acsbg3*)

[Ubiquitin Stress Response]
[Macrophage (Ubiquitin)]

CpGi and Shore Annotation

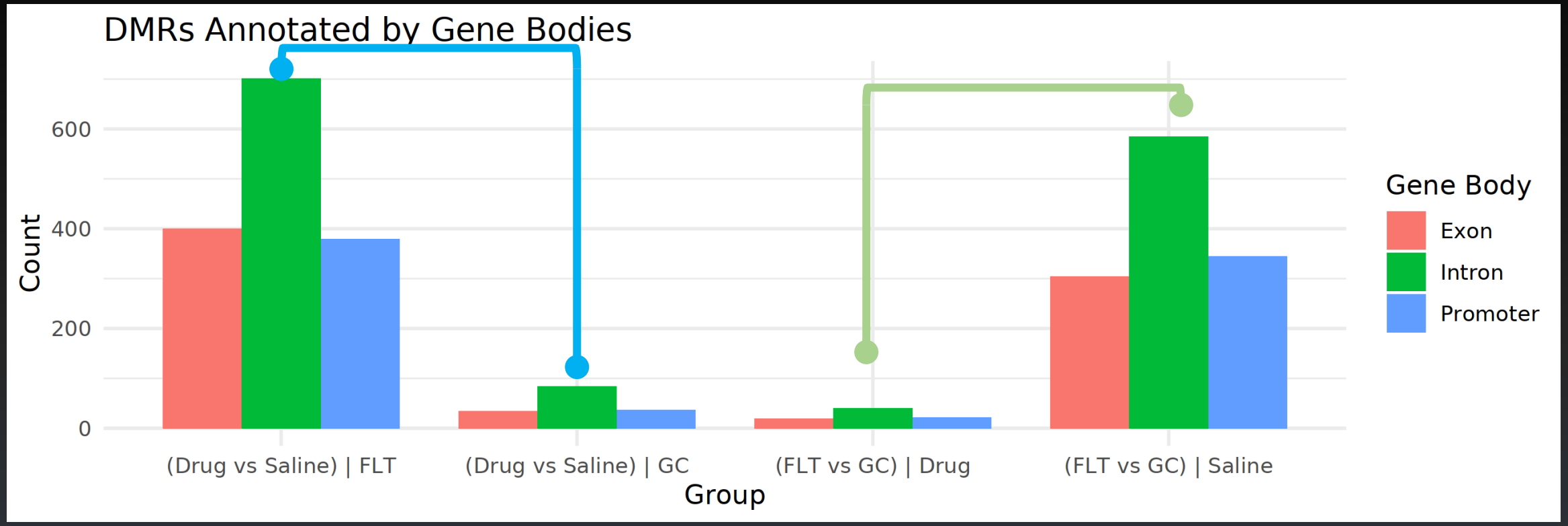




↑ variability in shores than CpGis

↑ modulation in FLT vs GC

Differential Methylation Gene Parts

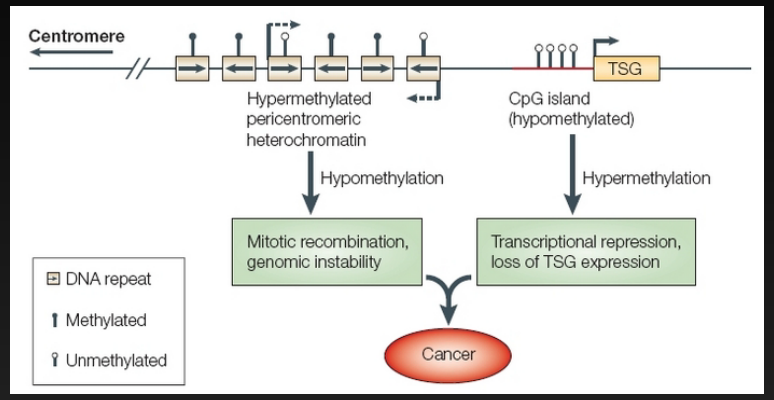


Spaceflight exposure increases overall methylation in gene bodies

Antioxidant BuOE decreases overall methylation in gene bodies

Methylation matched to RNA-Seq

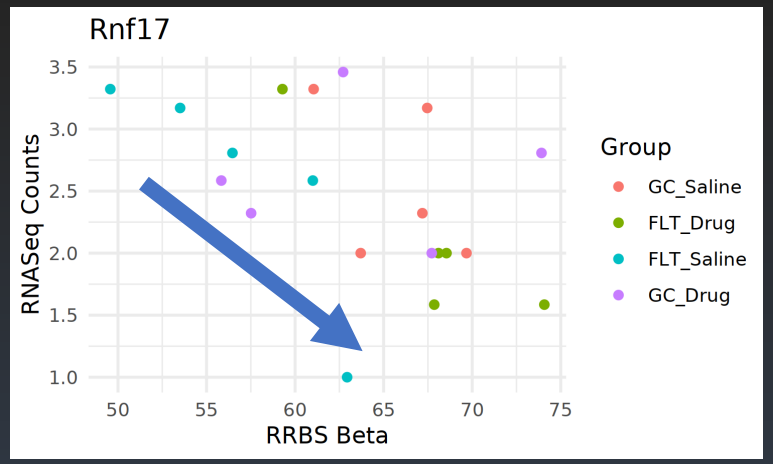
Classical Methylation and Expression Relationship



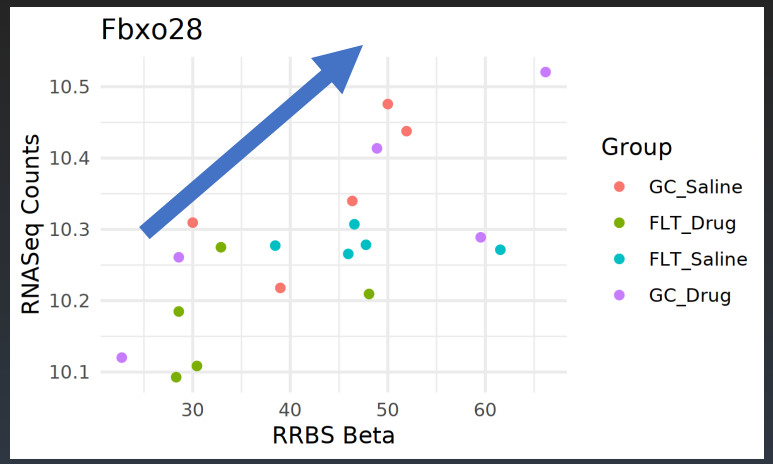
Phillips, T. (2008) The role of methylation in gene expression. Nature Education 1(1):116

We estimated the nearest transcription start site (TSS) to a DMR, and then map the TSS to a protein coding gene to compare methylation to expression directly

Top Correlations between RRBS and RNA-Seq highlight expected behavior



Promoter & CpGi tagged region



Intron & Shore tagged region

We also see more complex relationships among other gene body results

Future Work

- Manuscript pending for MULTI-OMICS STUDY OF THE EFFECT OF REDOX-ACTIVE METALLOPORPHYRIN ON MURINE RETINA DURING SPACEFLIGHT
  - TRRaC Team, Dr. Vivien Mao, Loma Linda University
- Harmonizing Heterogeneous Transcriptomics Datasets for Machine Learning based Analysis to Identify Spaceflown Murine Liver-specific changes
  - Manuscript under consideration NASA/JAXA Nature Package
- TRRaC Team Repository - github.com/nasa/trrac