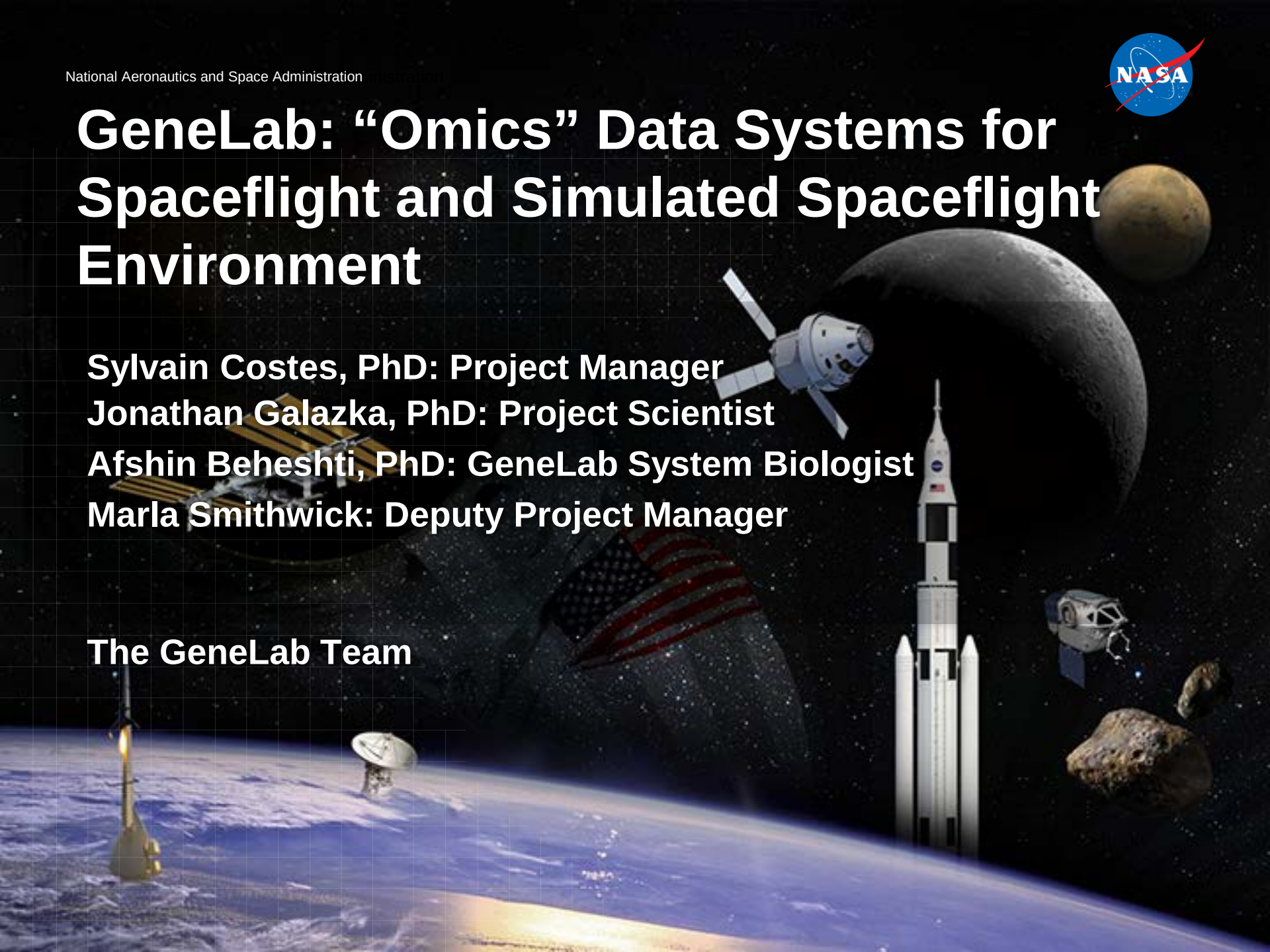
National Aeronautics and Space Administration



GeneLab: “Omics” Data Systems for Spaceflight and Simulated Spaceflight Environment

Sylvain Costes, PhD: Project Manager
Jonathan Galazka, PhD: Project Scientist
Afshin Beheshti, PhD: GeneLab System Biologist
Marla Smithwick: Deputy Project Manager

The GeneLab Team

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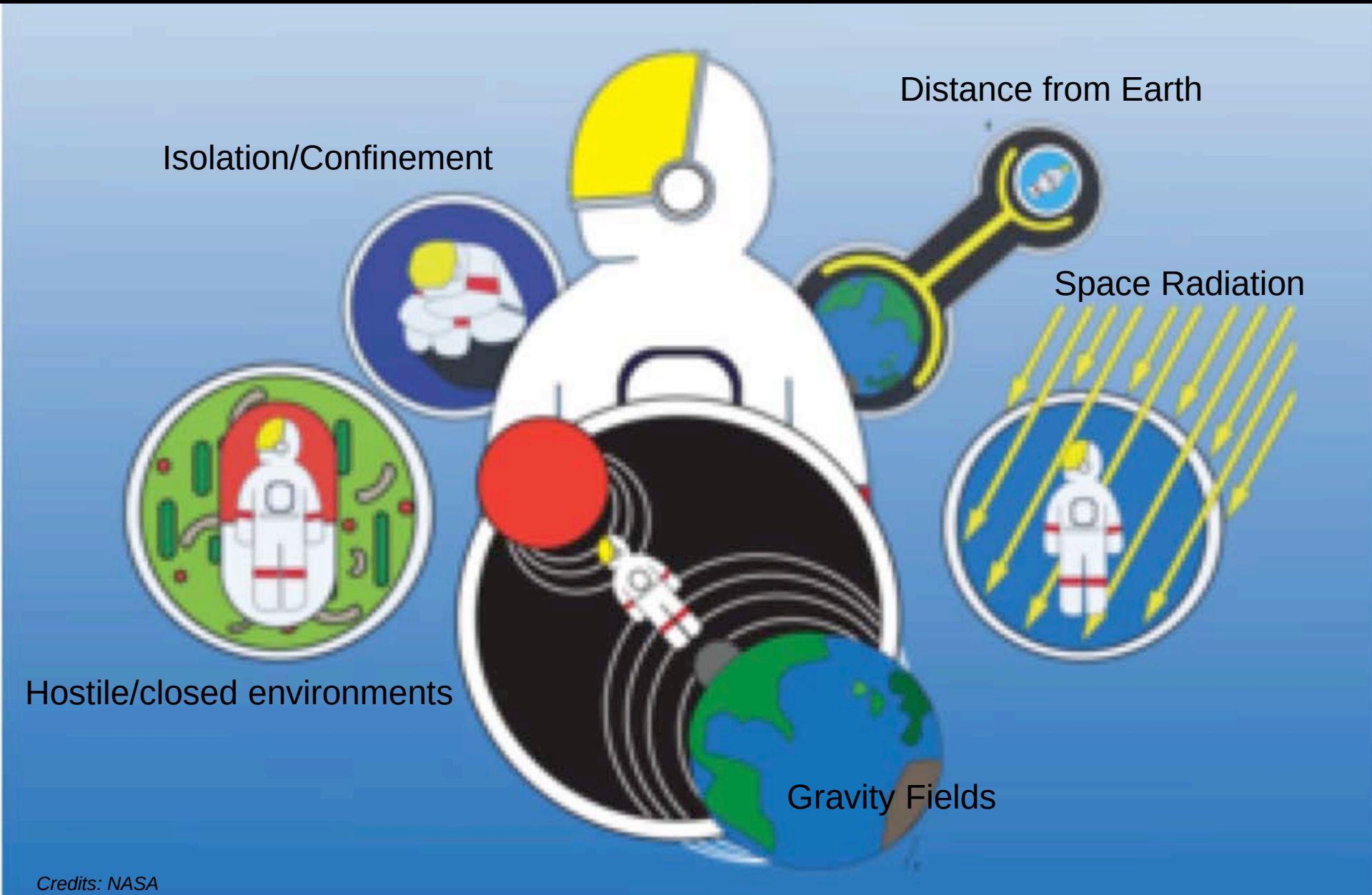
Challenges of Spaceflight



Credits: NASA

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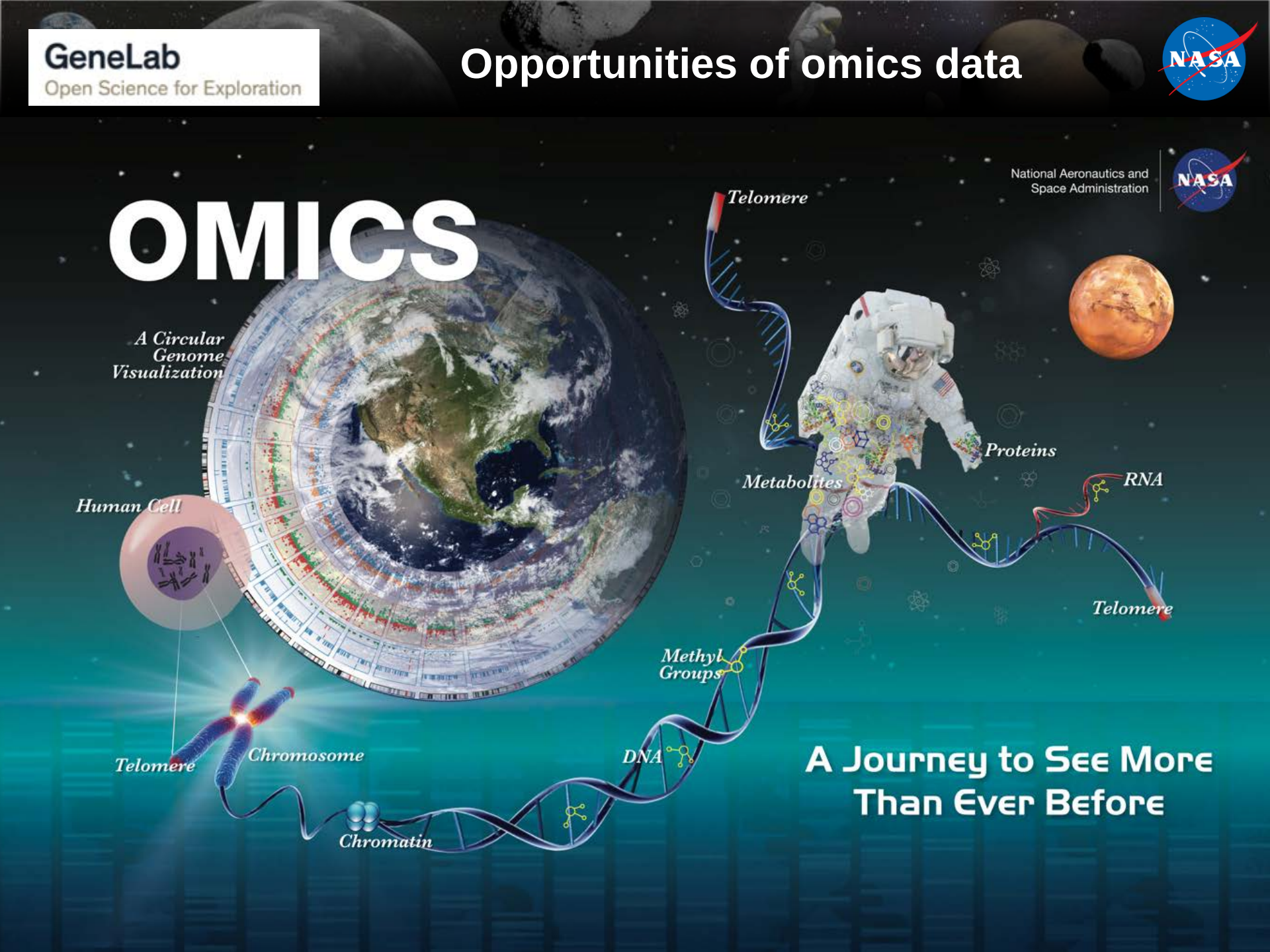
Opportunities of omics data



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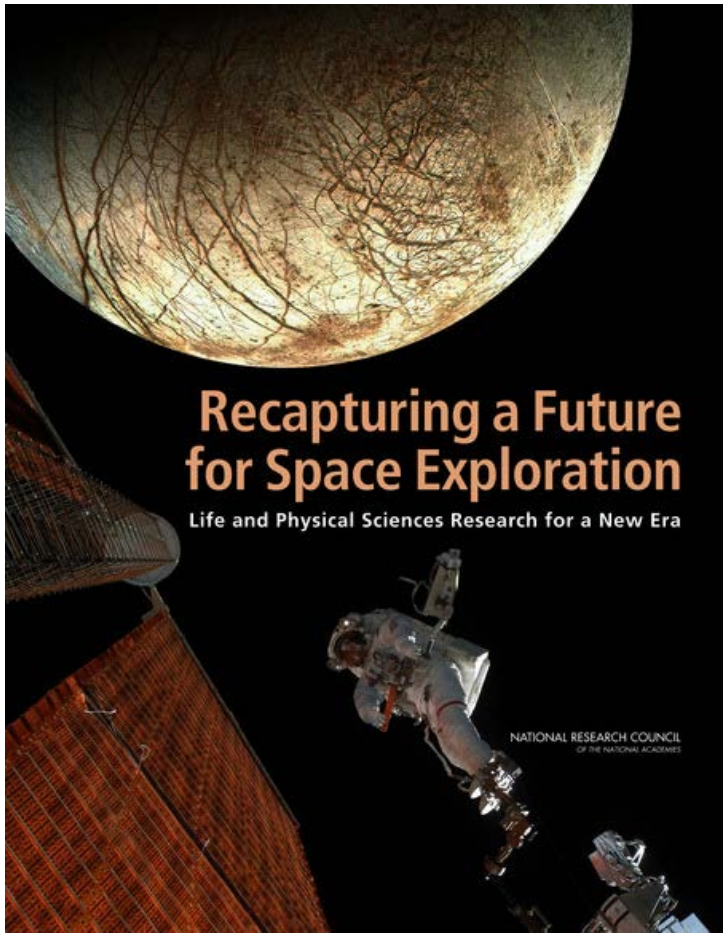
2011 NRC Decadal Survey



"*…**genomics, transcriptomics, proteomics, and metabolomics** offer an immense opportunity to understand the effects of spaceflight on biological systems…*"

"*…Such techniques generate considerable amounts of **data that can be mined and analyzed** for information by multiple researchers…*"

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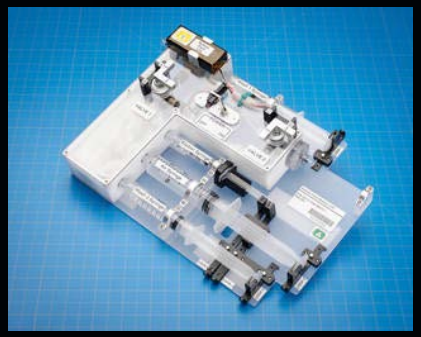
cquisition in Space is Now a Reality

This is truly an exciting time for cellular and molecular biology, omics and biomedicine research on ISS with these amazing additions to the suite of ISS Laboratory capabilities.

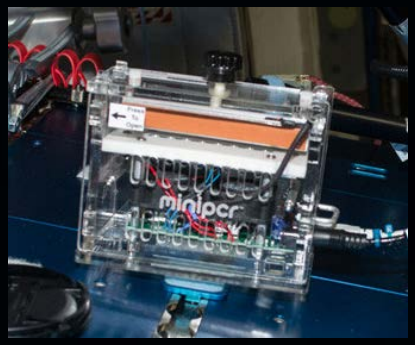


Sample Preparation Module

Oxford Nanopore MinION Gene Sequencer

Cepheid Smart Cycler qRT-PCR

Reaction tube containing lyophilized chemical assay bead (proprietary)



Mini-PCR

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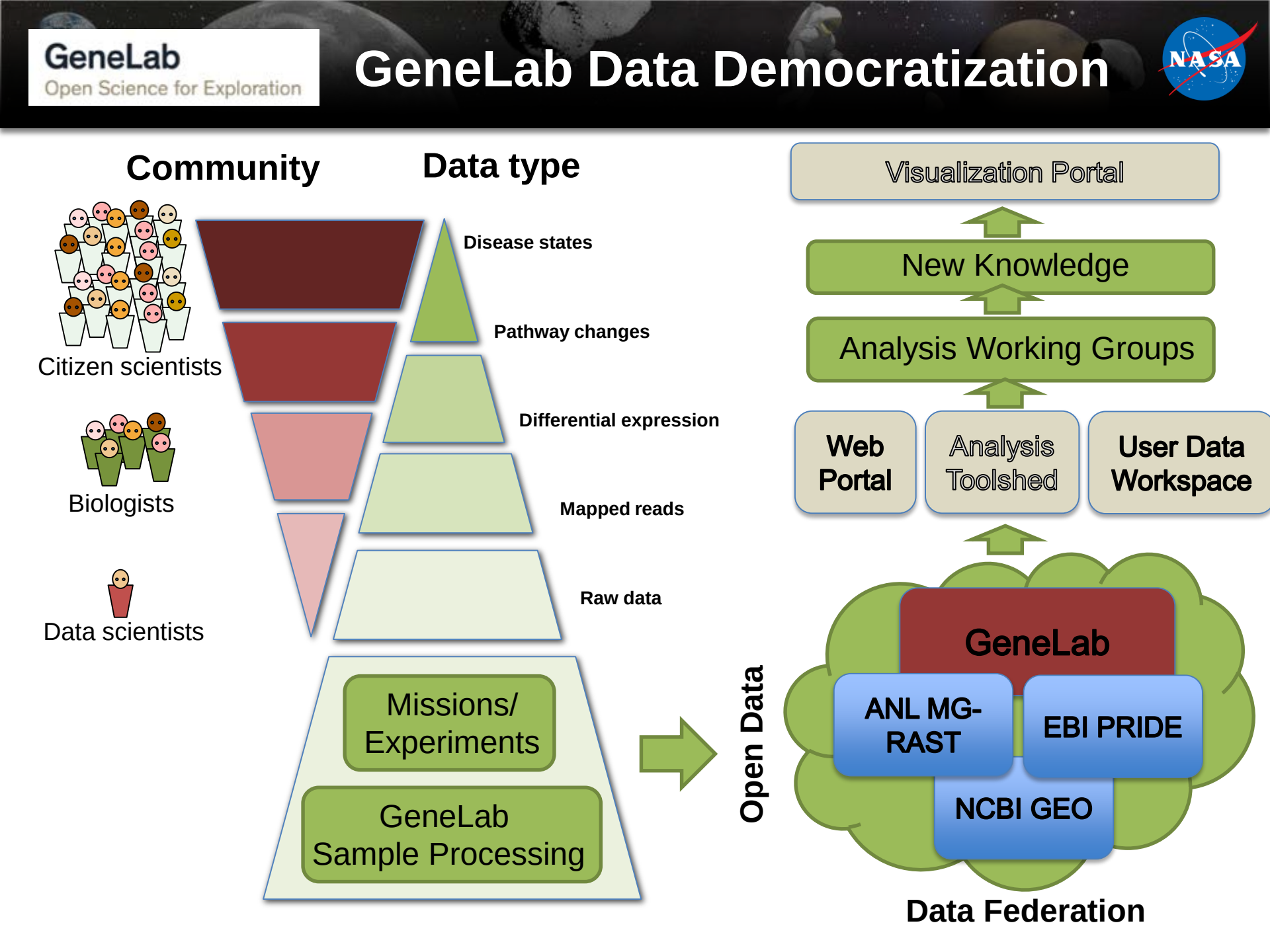
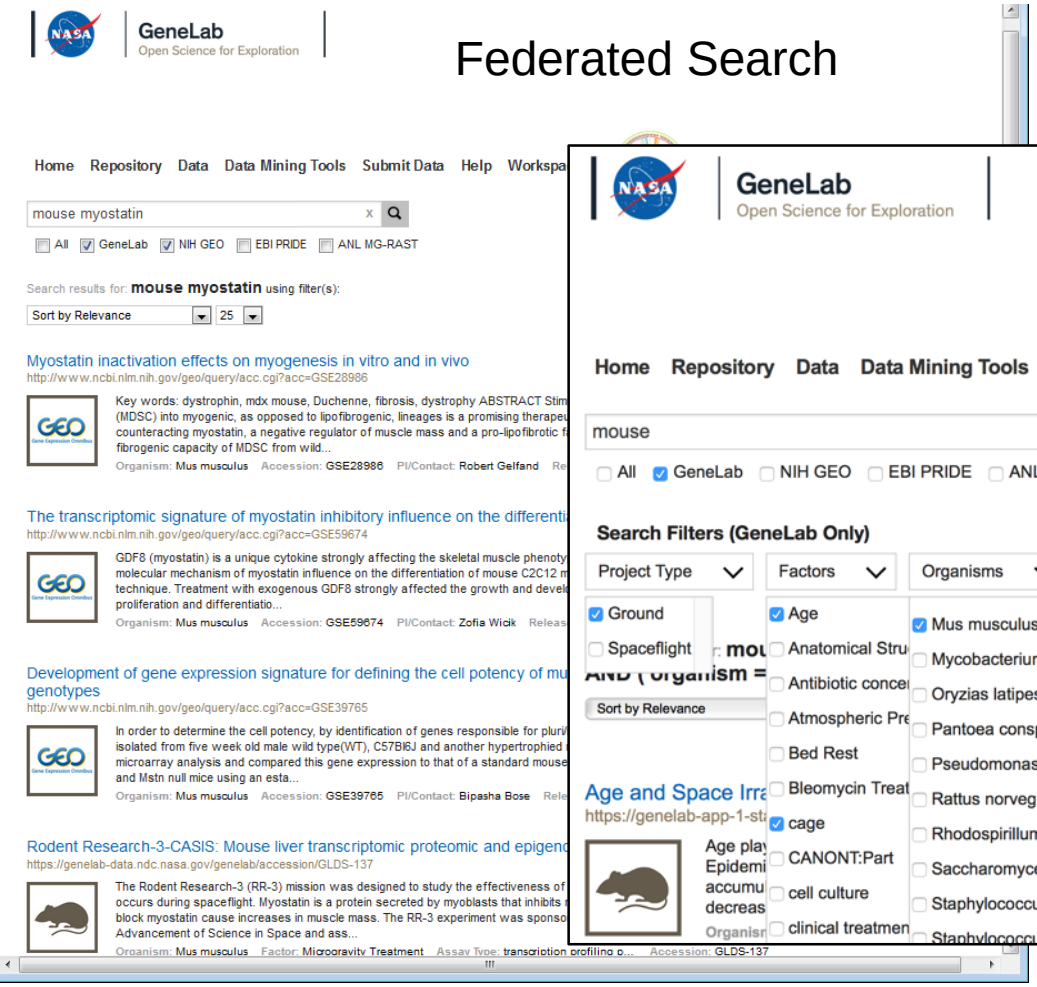
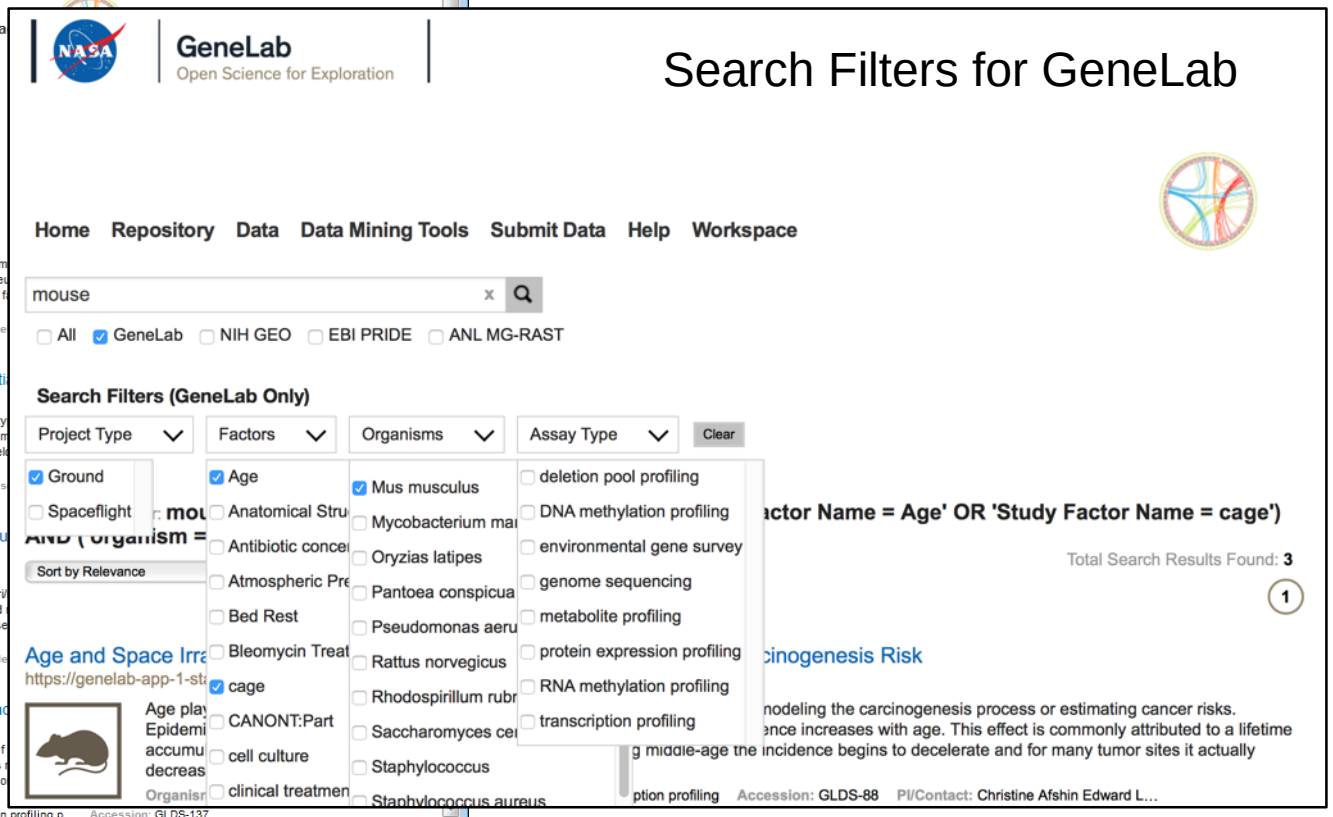
GeneLab Data Democratization



GLDS Phase 2 (Release 2.0)
Google-like Search, Federated Search

Data federation/integration with heterogeneous bioinformatics external databases (GEO, PRIDE, MG-RAST)





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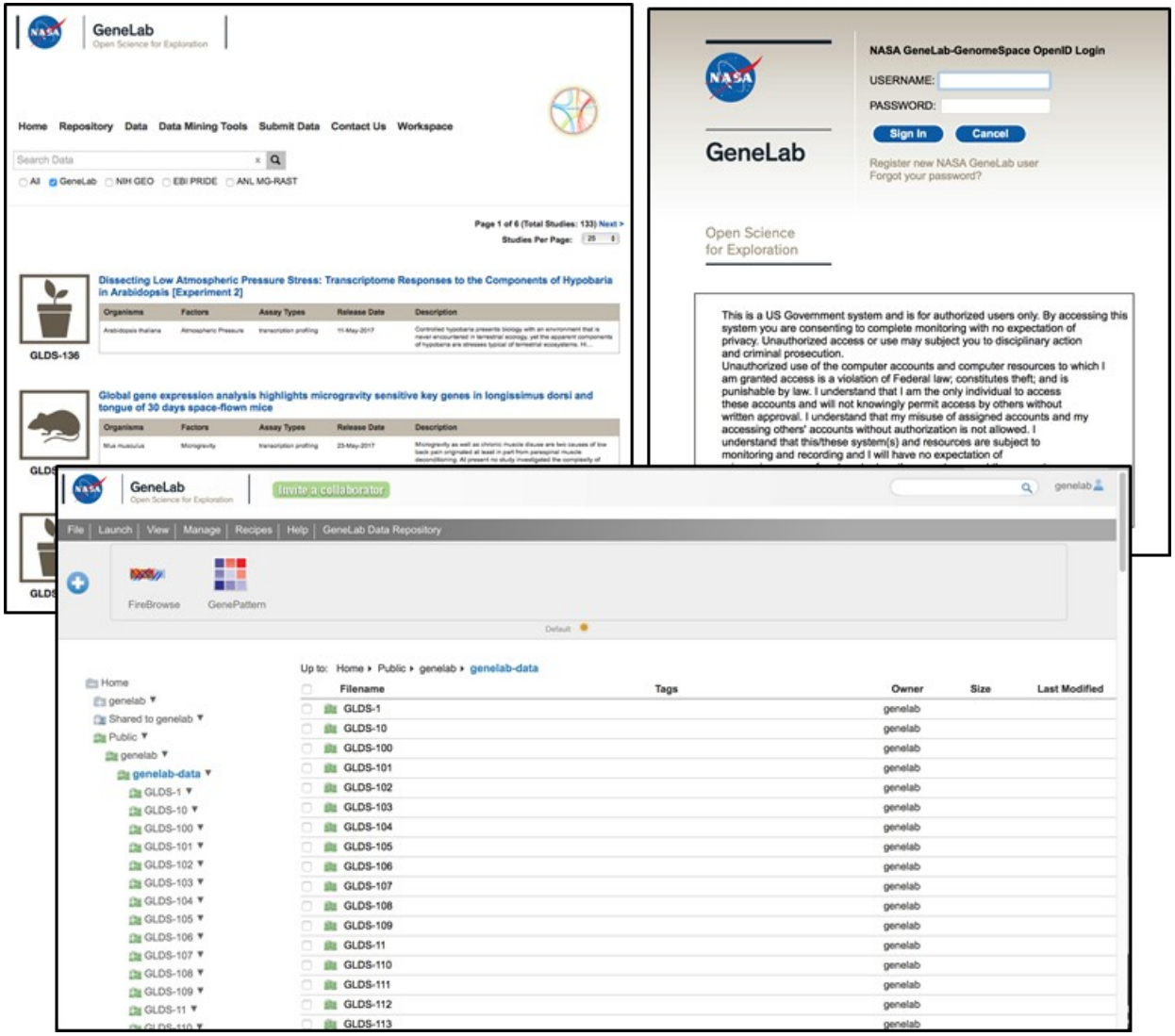
GLDS Phase 2 (Release 2.0)
Customized NASA Collaborative Workspace

User Account Mgmt., Access Controls (e.g., Private, Shared, Public Folders)



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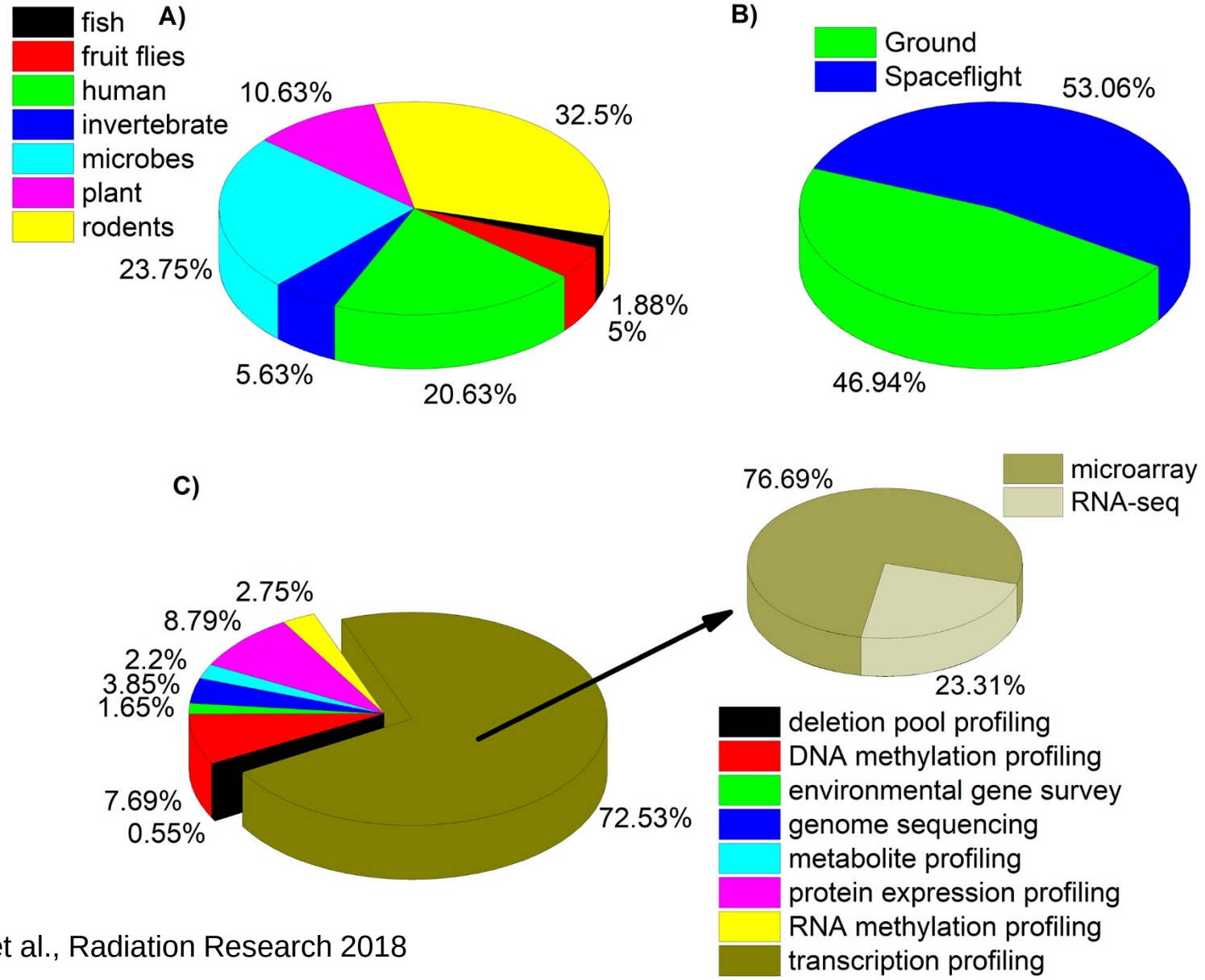
GeneLab Database: >190 data sets



Beheshti et al., Radiation Research 2018

Earth's magnetic field protects us from cosmic

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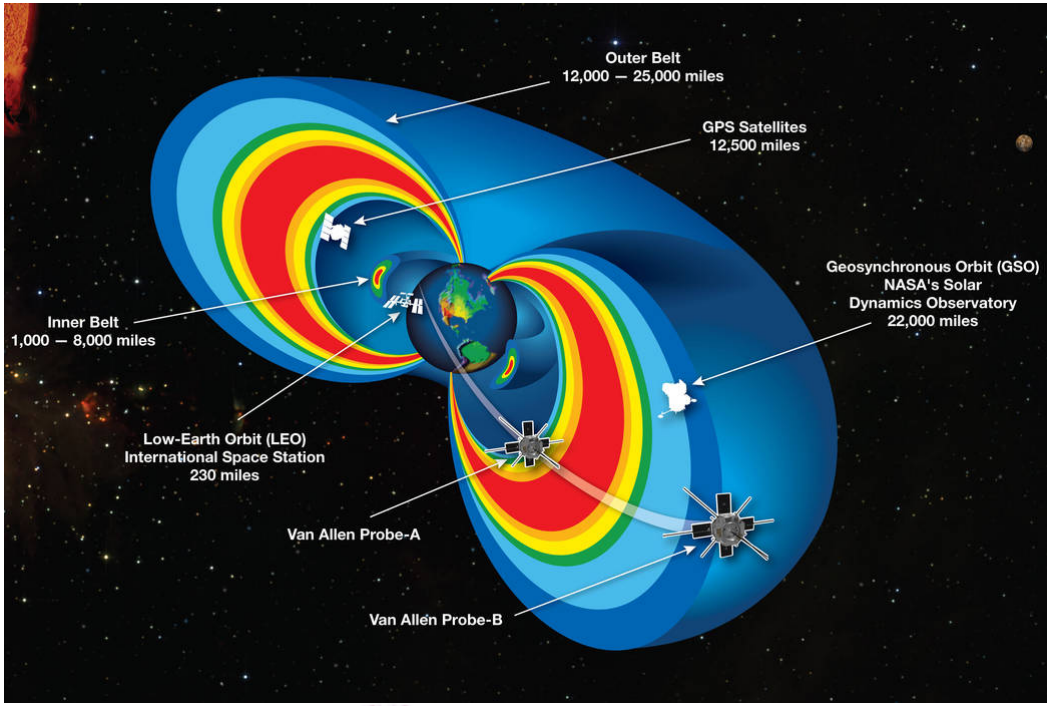
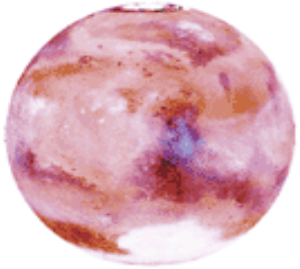


2½ Years, 2,600 X-Rays

Americans on average absorb the radiation equivalent of at least 7 chest X-rays each year.

Space missions, outside of Earth's protective atmosphere and magnetic field, expose astronauts to many times more.



NASA

Source: Brookhaven National Laboratory, U.S. Department of Energy


CAUTION
LASER RADIATION

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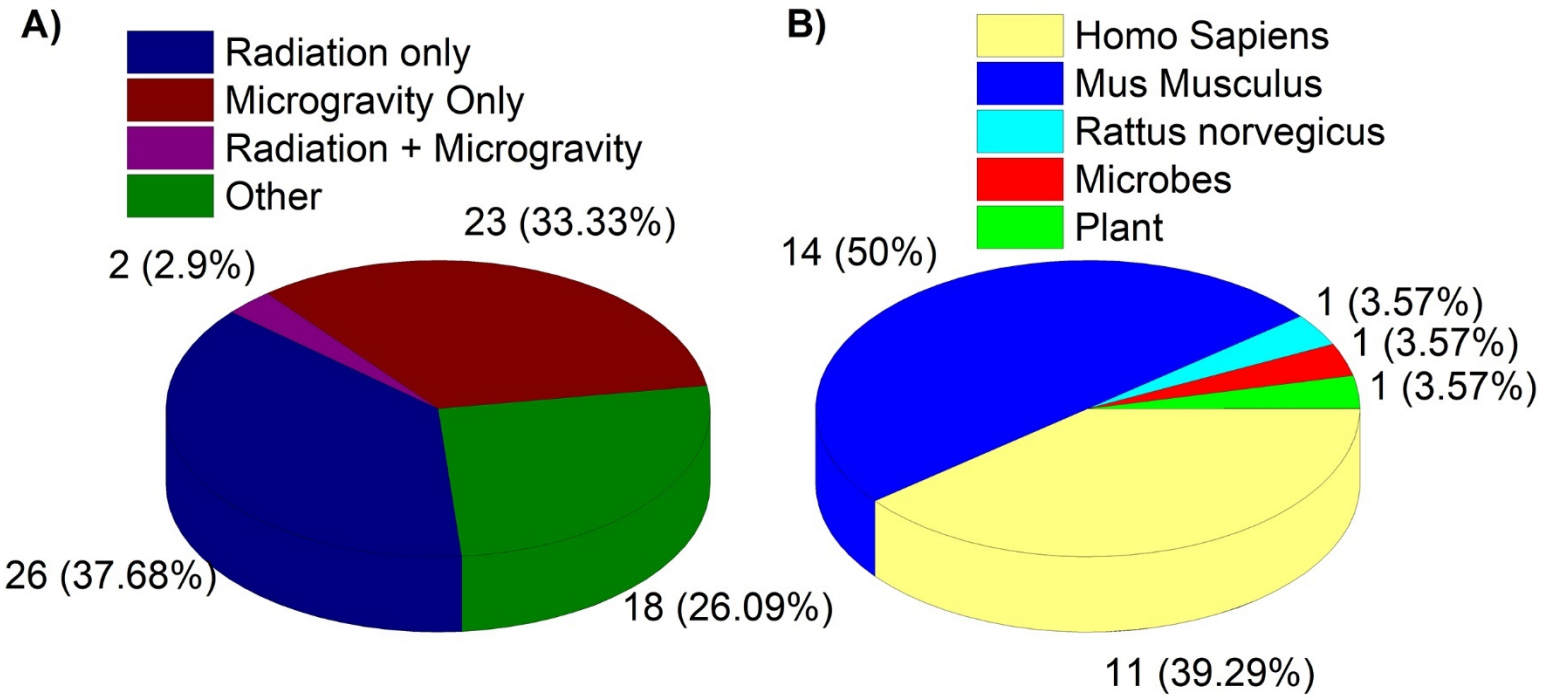
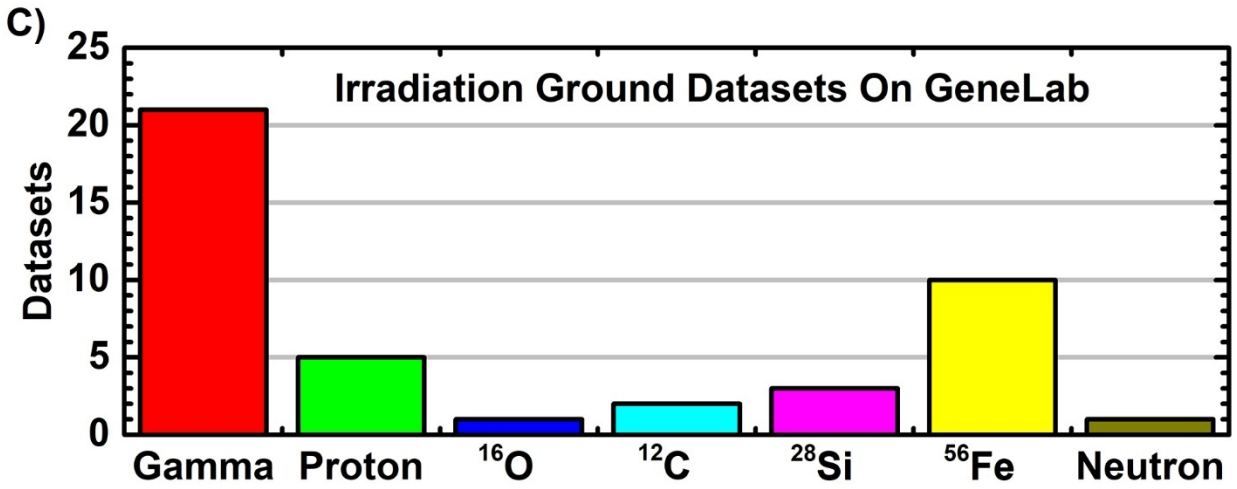
69 Ground Data Sets: Radiation and simulated microgravity





Beheshti et al., Radiation Research 2018

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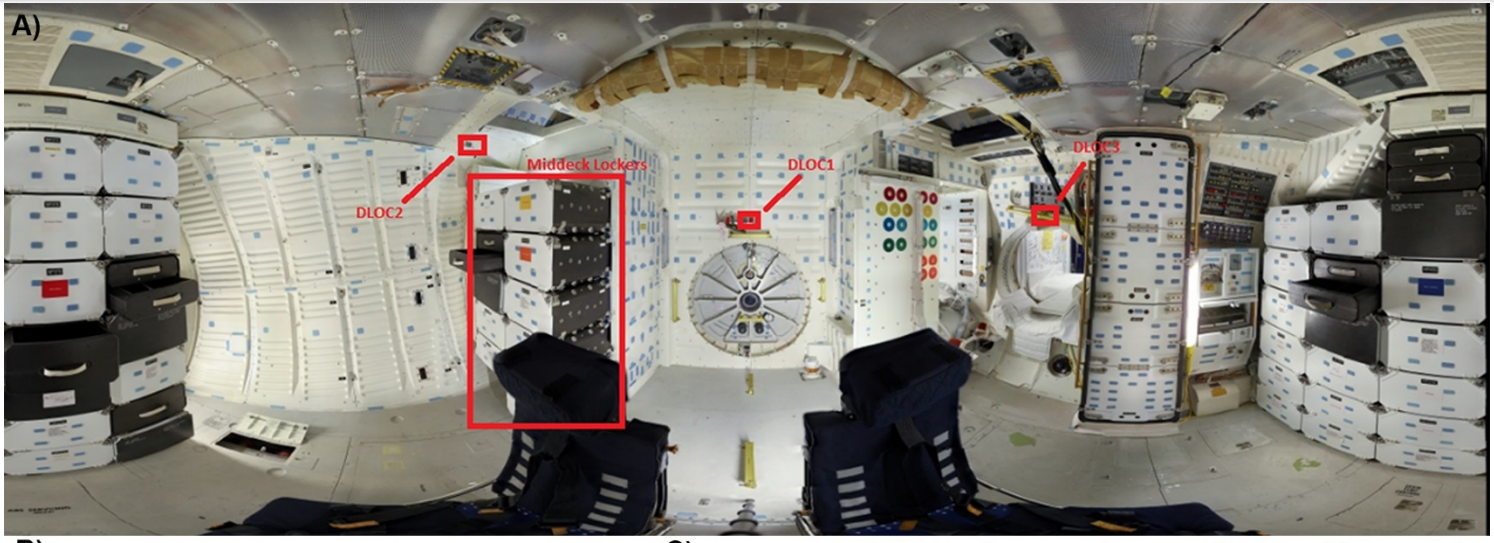
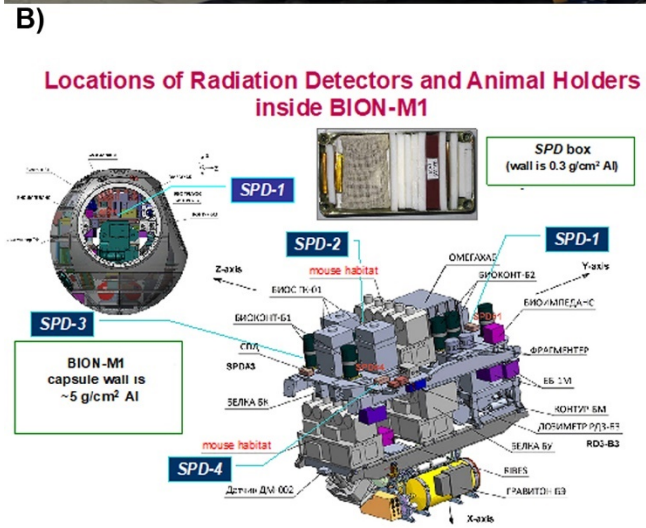
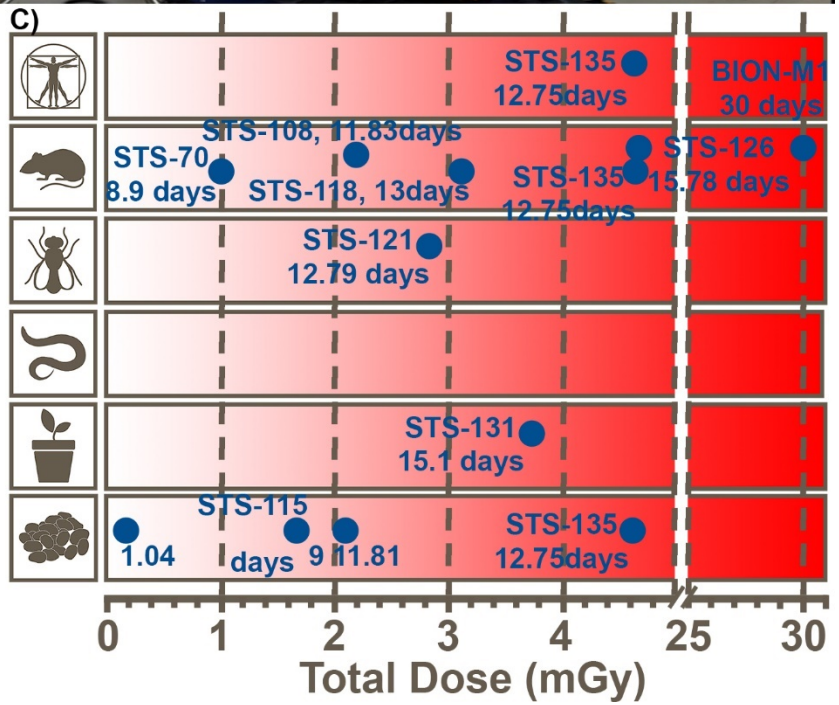
Radiation Dosimetry for STS samples (ISS to follow)







Beheshti et al., Radiation Research 2018

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GLD3.0 – Omics Analysis Toolshed

Barriers to reproducible analysis of omics data:

1. Large files are difficult to move around and process
2. Workflows vary from user to user and details are sometimes poorly documented

Galaxy platform:

1. Open source, extensible platform for cloud based analysis of omics data
2. Allows any command line tool or script to be run and chained together into workflows
3. Workflows can published, shared and downloaded

Galaxy PROJECT
galaxyproject.org

Afgan et al. The Galaxy platform for accessible, reproducible and collaborative biomedical analyses: 2016 update. Nucleic Acids Research (2016)

GeneLab Analysis Working Groups

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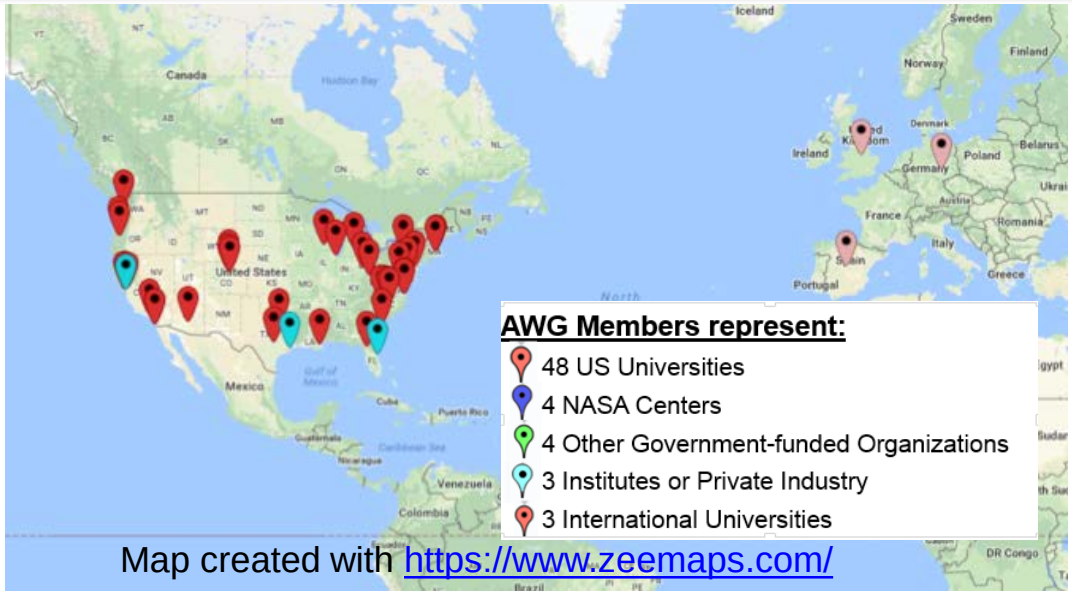
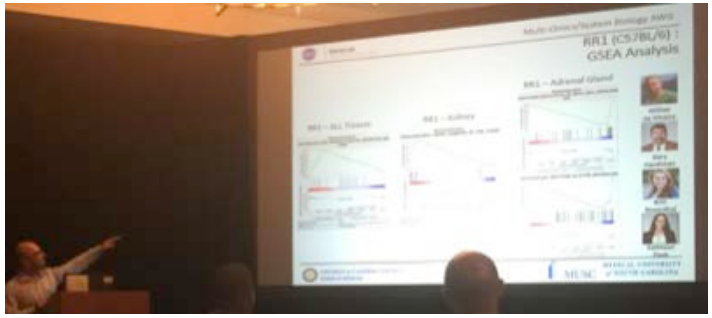


Total AWG Members:	**114**
AWG Members Per Group:	
Animal	47
Multi-Omics/System Biology	33
Plants	24
Microbes	21

**Some members are in multiple groups*



- **Monthly meetings + "Homework"**
- **One Annual Workshop (April)**
- **Summer internship (8 – 10 students for 10 weeks)**
- **Deliverables:**
  - Consensus pipelines for primary analysis of data (Microarray, RNASeq, Bisulfite sequencing, Proteomics, 16S metagenomics, Whole genome metagenomics)
  - Recommendations for visualization of data

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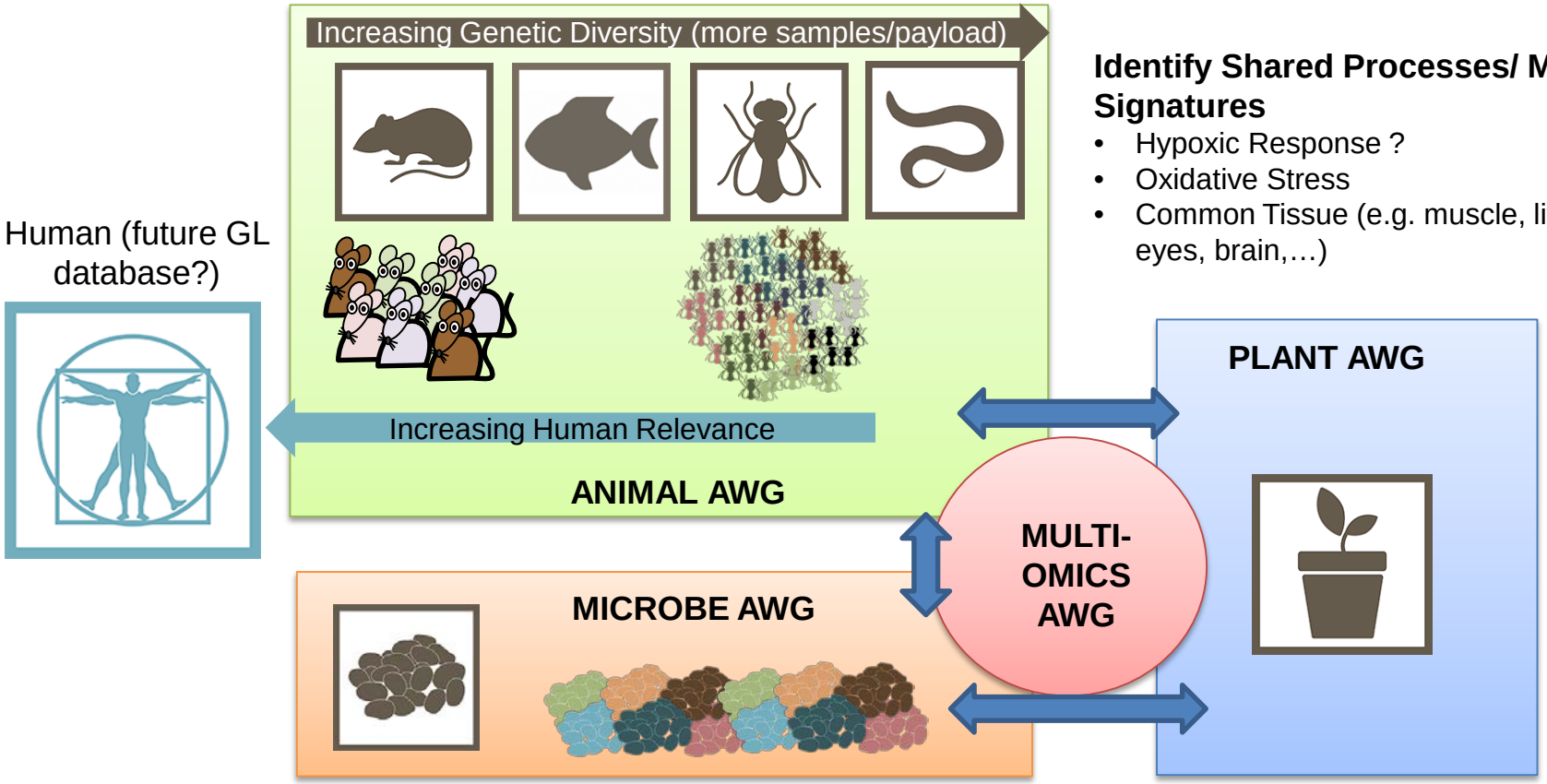
Guiding principles to look at GeneLab data



Identify Shared Processes/ Molecular Signatures

- Hypoxic Response ?
- Oxidative Stress
- Common Tissue (e.g. muscle, liver, heart, eyes, brain,…)

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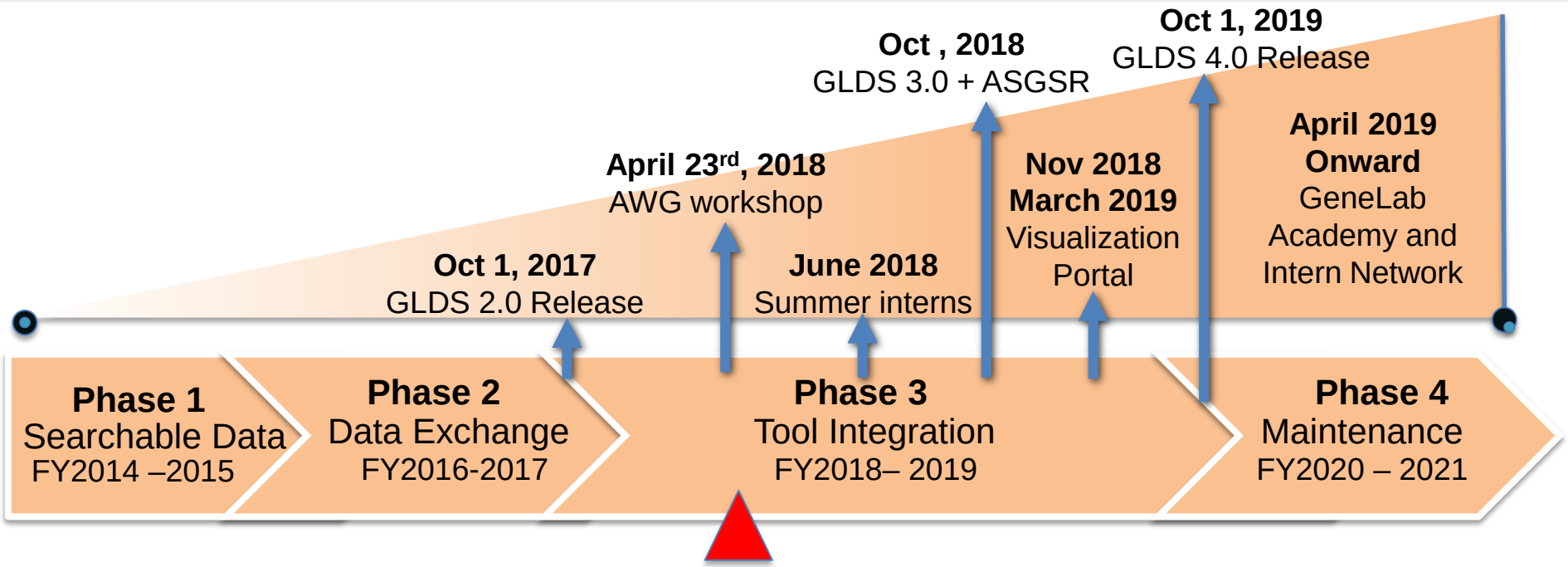
Phased Implementation



Data System
- ✓ Public Website
- ✓ Searchable Data Repository
- ✓ Top Level Requirements
- ✓ New Data and Legacy Data

Data System
- ✓ Link to Public Databases via Data Federation
- ✓ Integrated Search (e.g., data mashup)

Data System
- Integrated Platform across model organisms
- Build Community via AWG
- Provide access to biocomputational tools for omics analysis
- Provide collaboration framework and tools

Open Source Maintenance
- User community becomes primary provider of new tools/knowledge
- Maintain integrity of data, and data system

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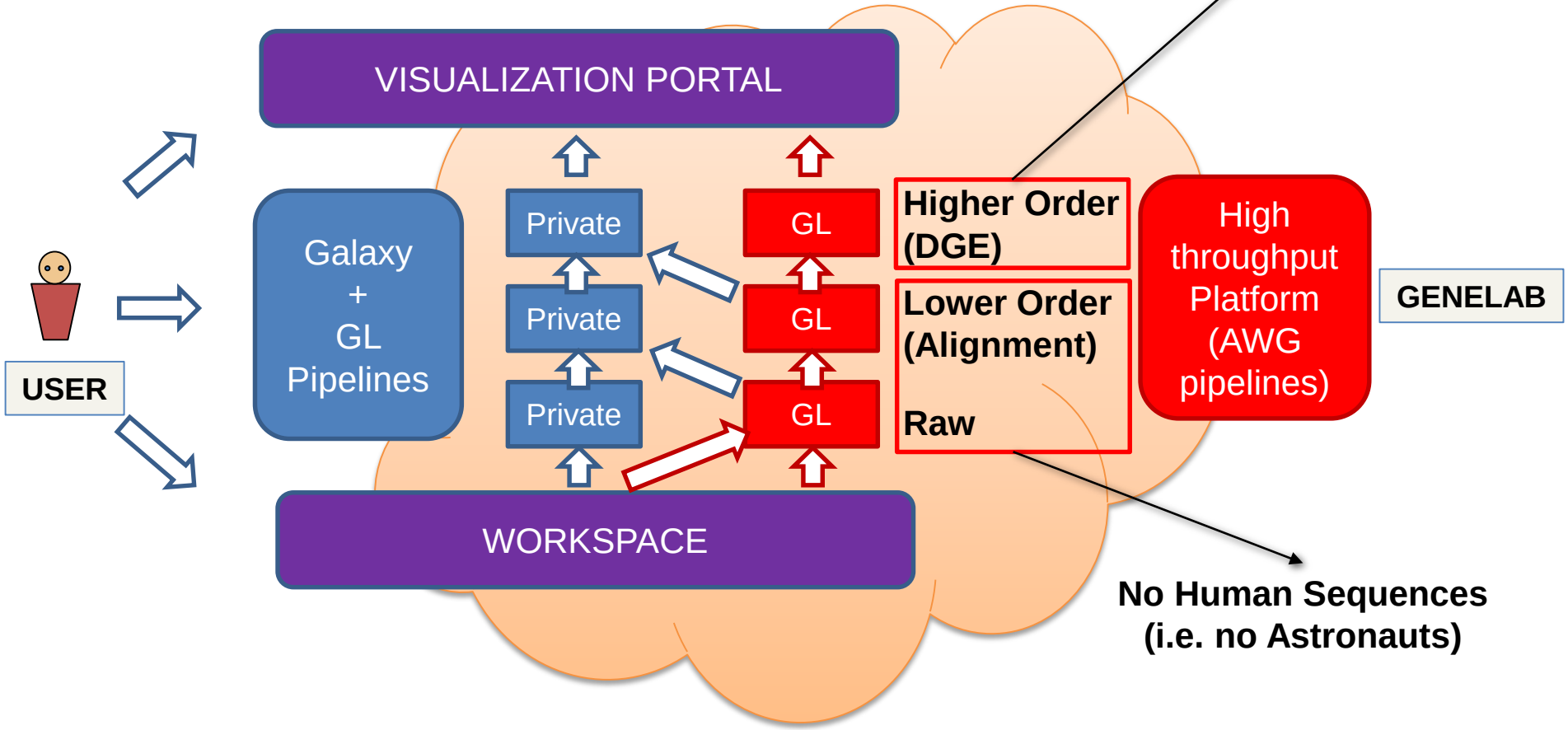
GLDS 4.0

Astronaut Data should be possible for Higher Order Data
Providing minimum metadata



No Human Sequences (i.e. no Astronauts)

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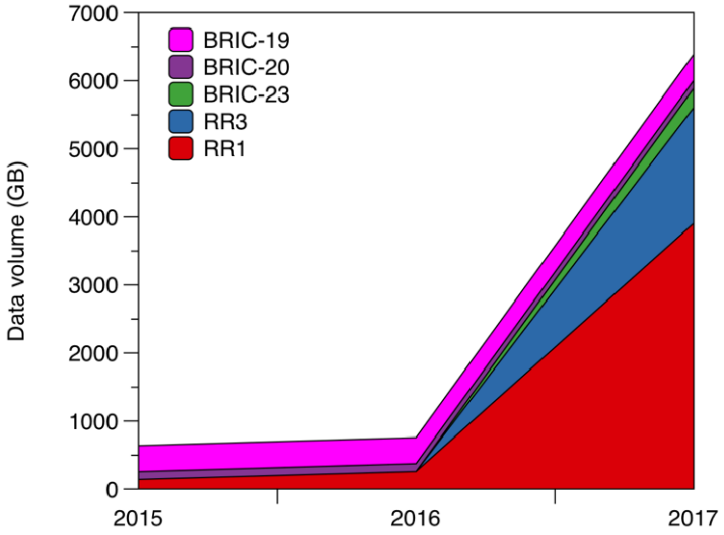
GeneLab Sample Processing Laboratory (SPL)

- Expertise:
  - DNA/RNA/protein extraction
  - Animal work
  - In-house Sequencing (including Library Prep)

- Develop standards for sample processing (species dependent)
- Responsible for ~50% of GeneLab data by volume – NCI TCGA model: keeping data as consistent as possible.



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Hypothesis and Science from GeneLab

- Cage Effects with rodent experiments: Carbon Dioxide as an Environmental Stressor in Spaceflight
- Systems Biology analysis reveals biological spaceflight master regulators
- AWG related work determines novel systemic biological factors causing damage due to spaceflight

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Cage Effects with rodent experiments: Carbon Dioxide as an Environmental Stressor in Spaceflight

Beheshti A, Cekanaviciute E, Smith DJ, Costes SV. Global transcriptomic analysis suggests carbon dioxide as an environmental stressor in spaceflight: A systems biology GeneLab case study. Sci Rep. 2018;8(1):4191. doi: 10.1038/s41598-018-22613-1. PubMed PMID: 29520055; PMCID: PMC5843582.

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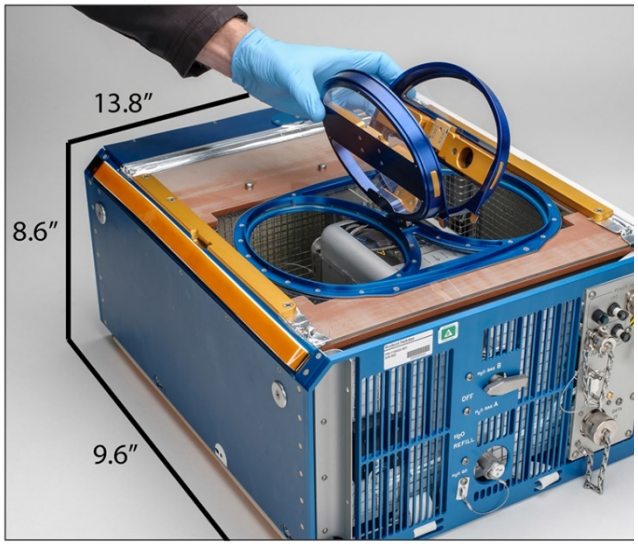
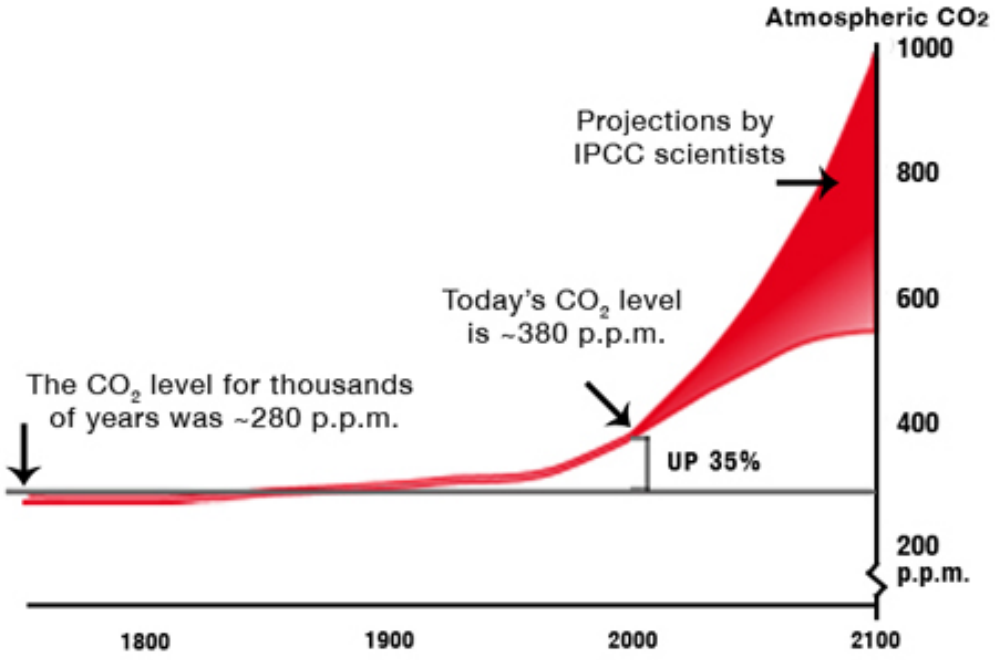
Carbon Dioxide as an Environmental Stressor in Spaceflight

A) Cage Types



Animal Enclosure Module (AEM)

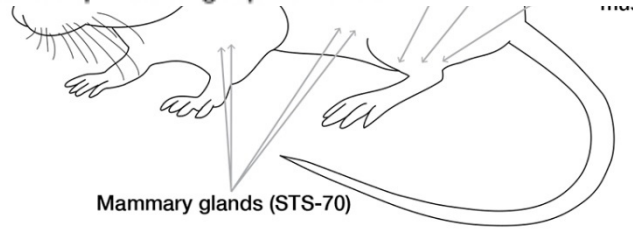
Historic and Projected CO2 Atmospheric Concentrations



Source: IPCC
http://www.ipcc.ch/present/graphics.htm

B)

GeneLab Study	Mission	Species	CO_2 (ppm)	Duration (days)	
GLDS-21	STS-108	mouse	~3000	11.8	
GLDS-111	BF	mouse	~600	30	
GLDS-111	BF	mouse	~600	30	extensor digitorum
GLDS-25	STS-135	mouse	~3000	13	liver
GLDS-63	STS-70	rat	~3000 (est)	9	mammary gland



Beheshti, et al., Scientific Reports, 2018

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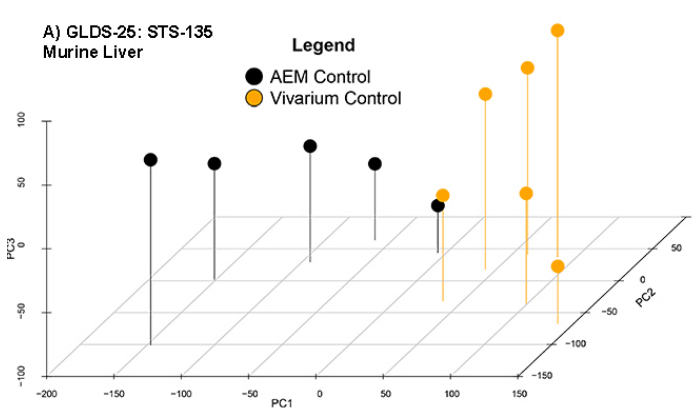
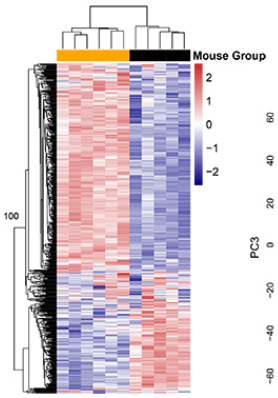
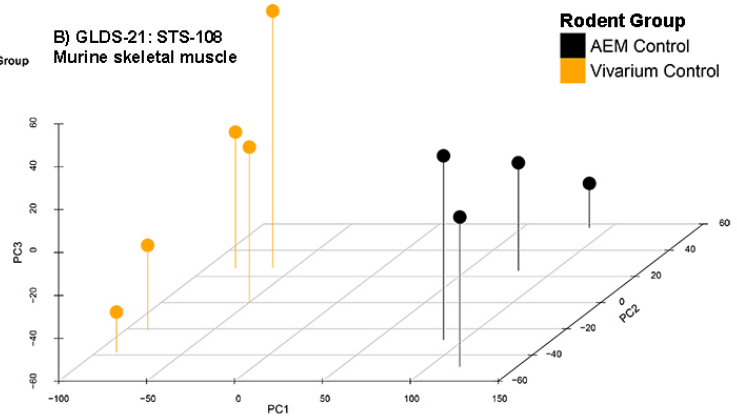
A Plots Suggest Strong Cage Effect

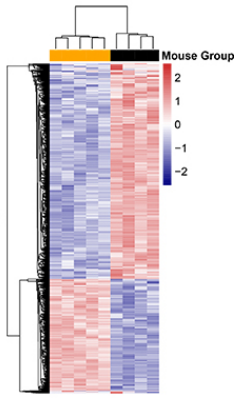
3000 ppm

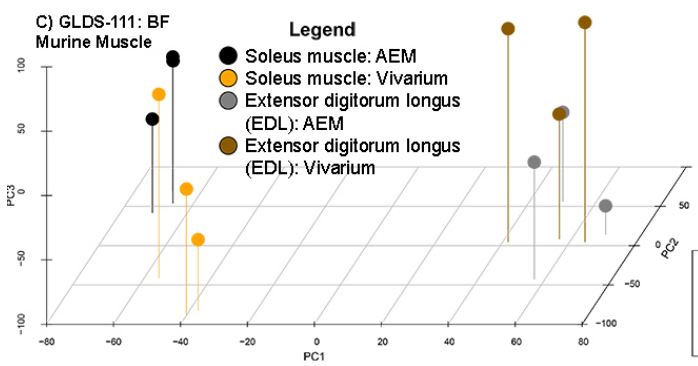


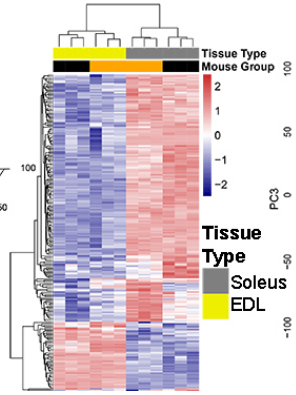


3000 ppm

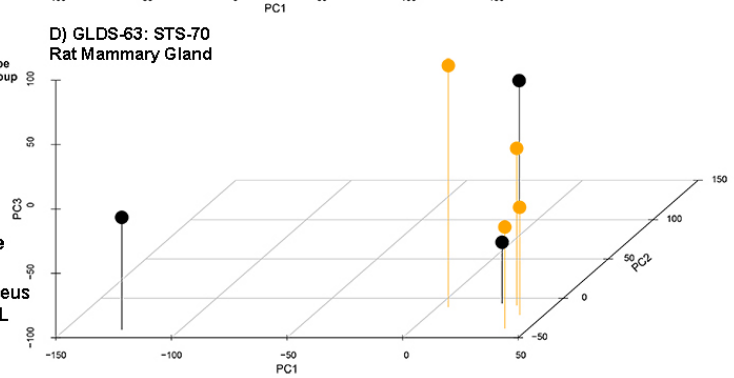


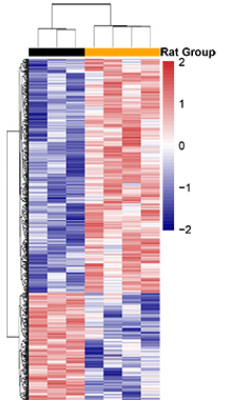






600 ppm



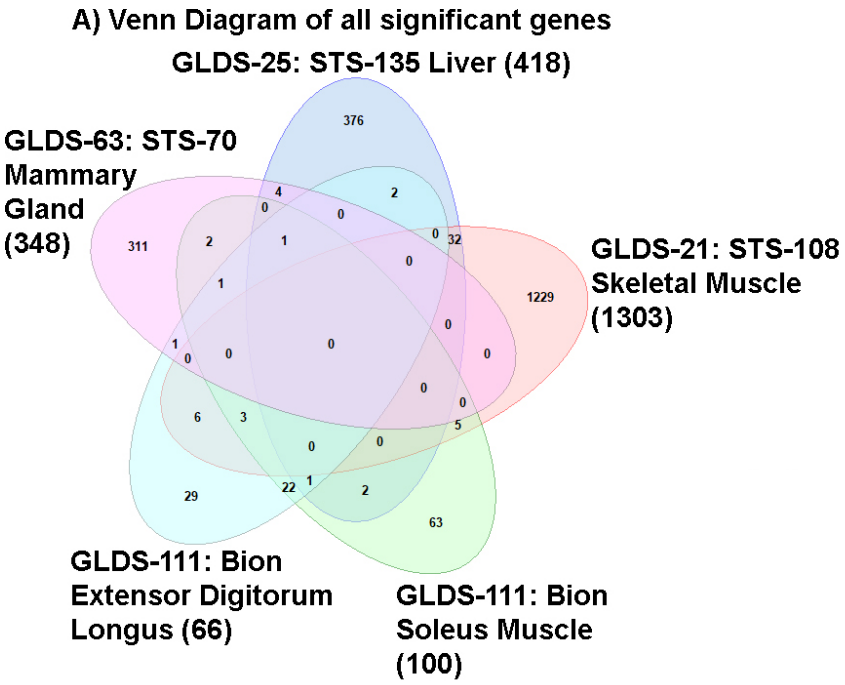


3000 ppm

AEM = Animal Enclosure Modules (now referred to as Rodent Habitats)
Vivarium = normal ground based rodent cages

Beheshti, et al., Scientific Reports, 2018

Differential Gene Expression: Cage or CO2 Effect?



An increase in aldosterone is associated with metabolic syndrome, which is characterized by chronic inflammation; aldosterone secretion can be triggered by hypoxia.

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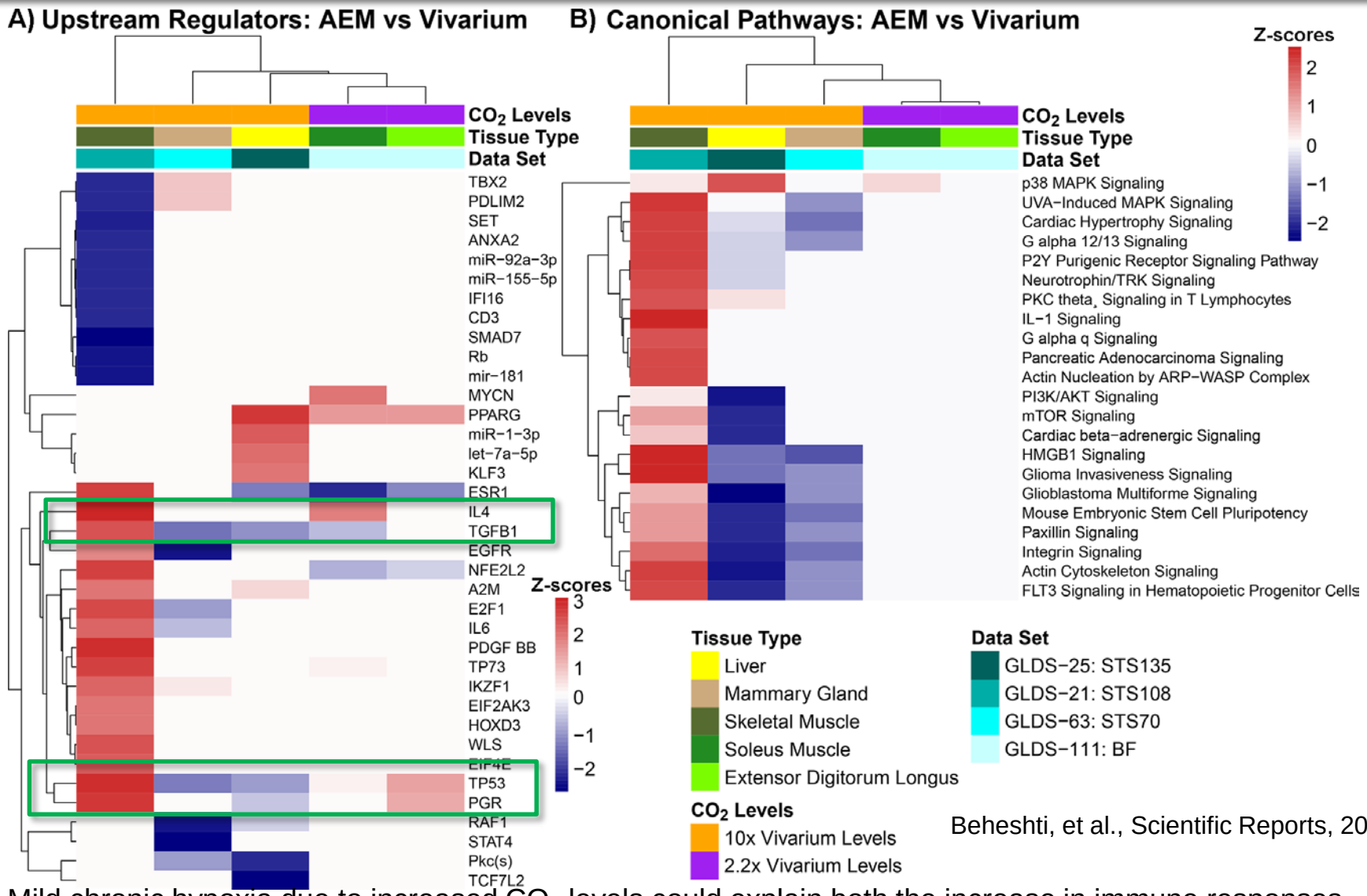
Upstream regulators and canonical pathways show response is tissue specific and highest for high CO_2



Beheshti, et al., Scientific Reports, 2018

Mild chronic hypoxia due to increased CO_2 levels could explain both the increase in immune responses and a reduction in metabolism – **Need to confirm with AEM experiments at ambient CO_2 levels.**

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Conclusions for this Study

- Through a systems biology approach we observed global transcriptomic changes in rodents induced by spaceflight-matched environment in AEM cages.
- Identify spaceflight CO_2 levels as a potential environmental stressor that merits experimental investigation
- Systematically changing one environmental aspect at a time (gas concentration, radiation, microgravity, etc.) and analyzing and comparing transcriptional responses could be used to create a network that could predict the most relevant causes and countermeasures for spaceflight-associated conditions, as well as confounding factors for spaceflight experiments.

Systems Biology analysis reveals biological spaceflight master regulators

Beheshti, et al., PLOS One, 2018

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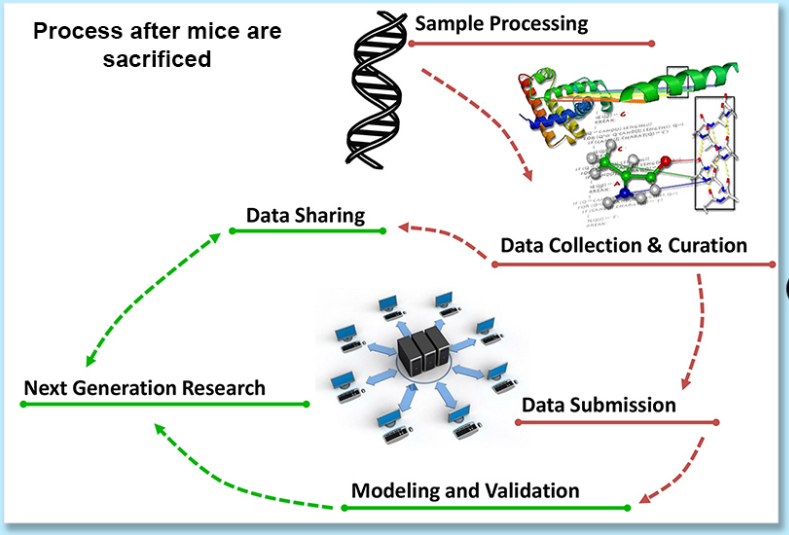
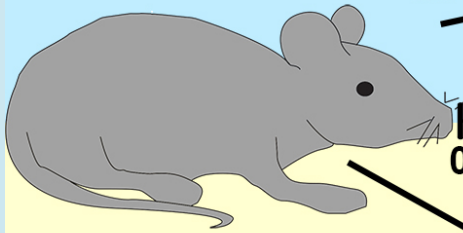
GeneLab Data Used to Generate Results



Mice Sacrificed on ISS



Extensor Digitorum Longus Muscle
Soleus Muscle
Gastrocnemius Muscle
Quadriceps
Tibialis Anterior Muscle
Adrenal Glands
Kidney
Liver
Skin

0 10 20 30 40 50 60 70 80 90 100

Time in Space for Mice (days)

Mice flown on STS and Sacrificed after Re-entry

Liver
Skeletal Muscle
Mammary Gland
Thymus
Soleus Muscle
Extensor Digitorum Longus Muscle

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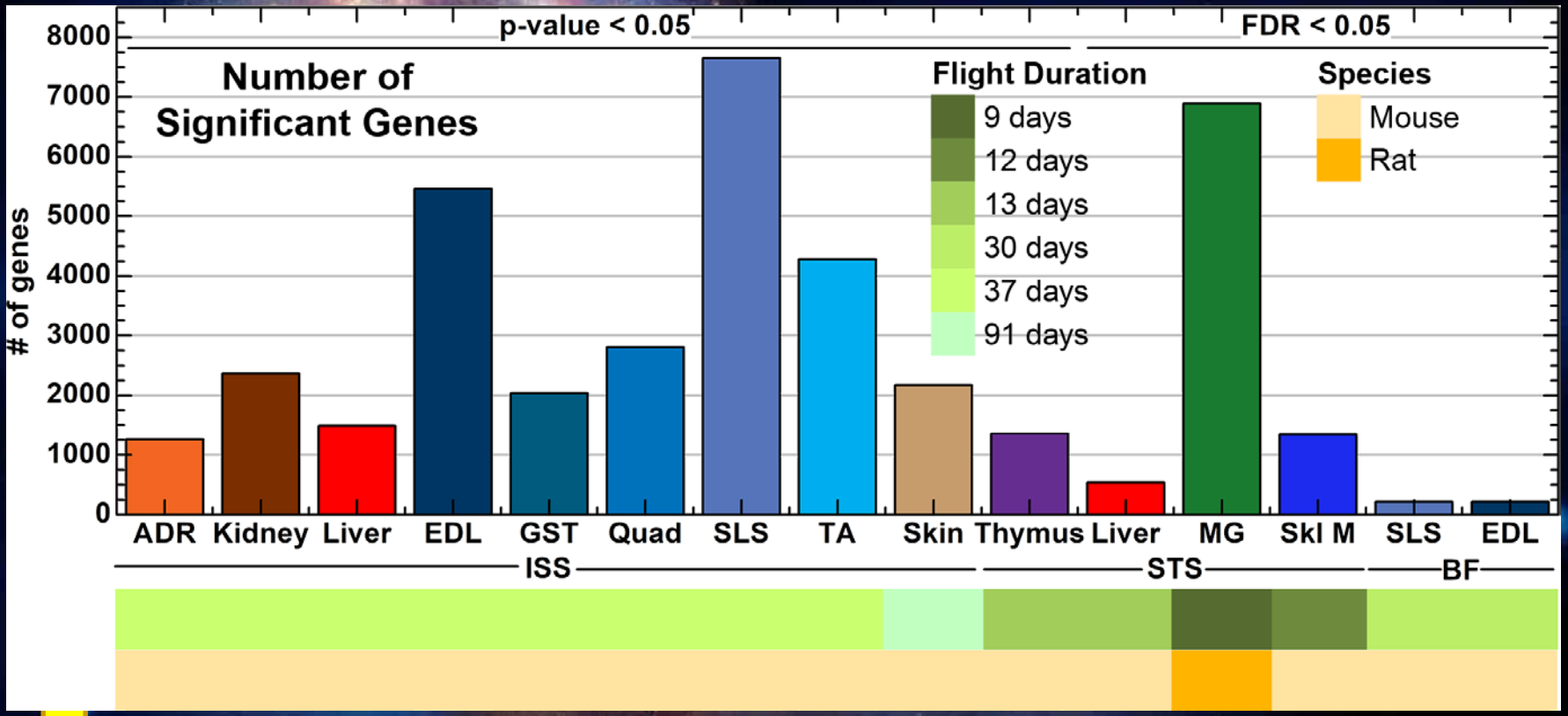
Number of Significant Genes from Each Dataset



Fold-Change ≥ |1.2|

Pathway/Functional Predictions:
Ingenuity Pathway Analysis (IPA)
Gene Set Enrichment Analysis (GSEA)

Beheshti, et al., PLOS One, 2018

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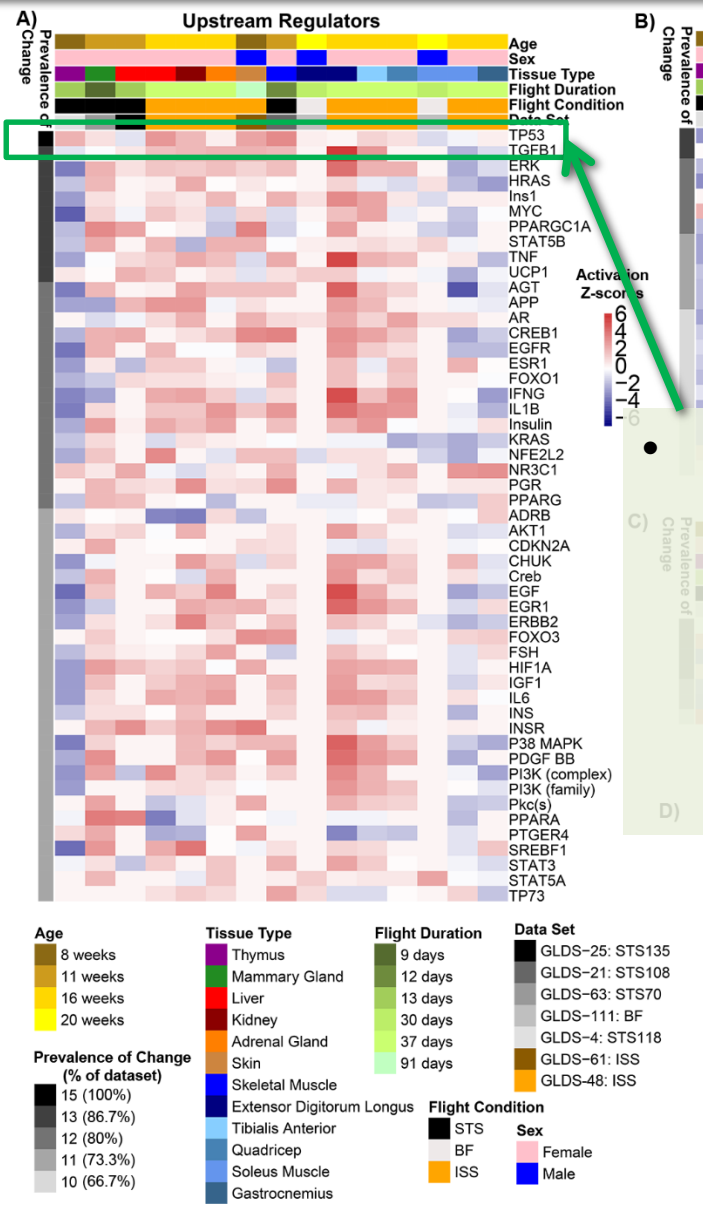
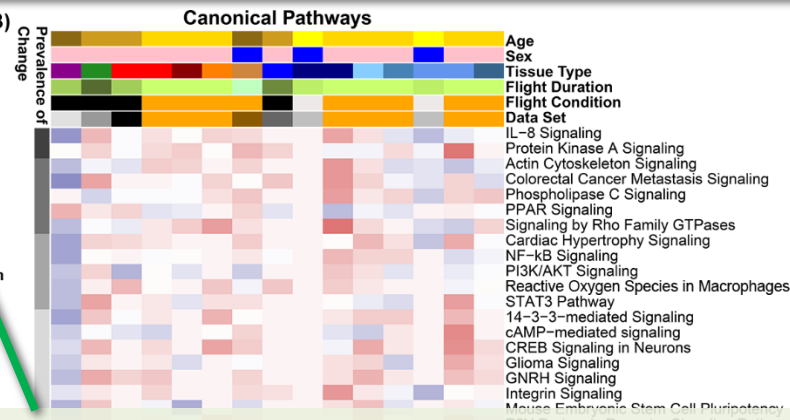
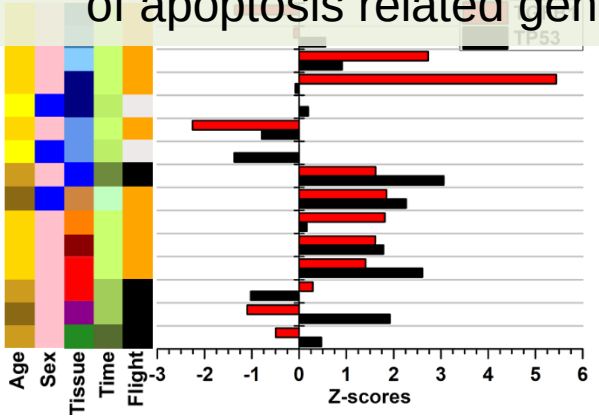
Predicted Master Regulators





- **p53 found in all tissues**
  - p53 is a transcription factor and in response to genotoxic stress, DNA damage, oncogene activation, and hypoxia, it is recruited to sites in chromatin, thus promoting transcription of apoptosis related genes



Beheshti, et al., PLOS One, 2018

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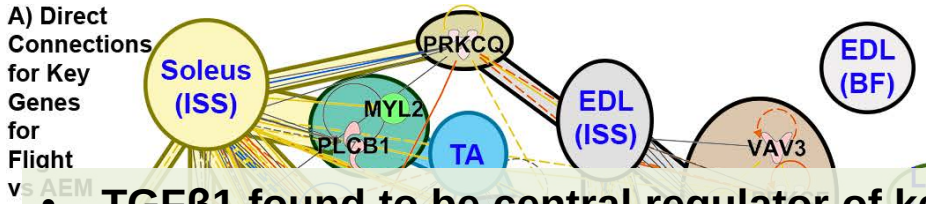
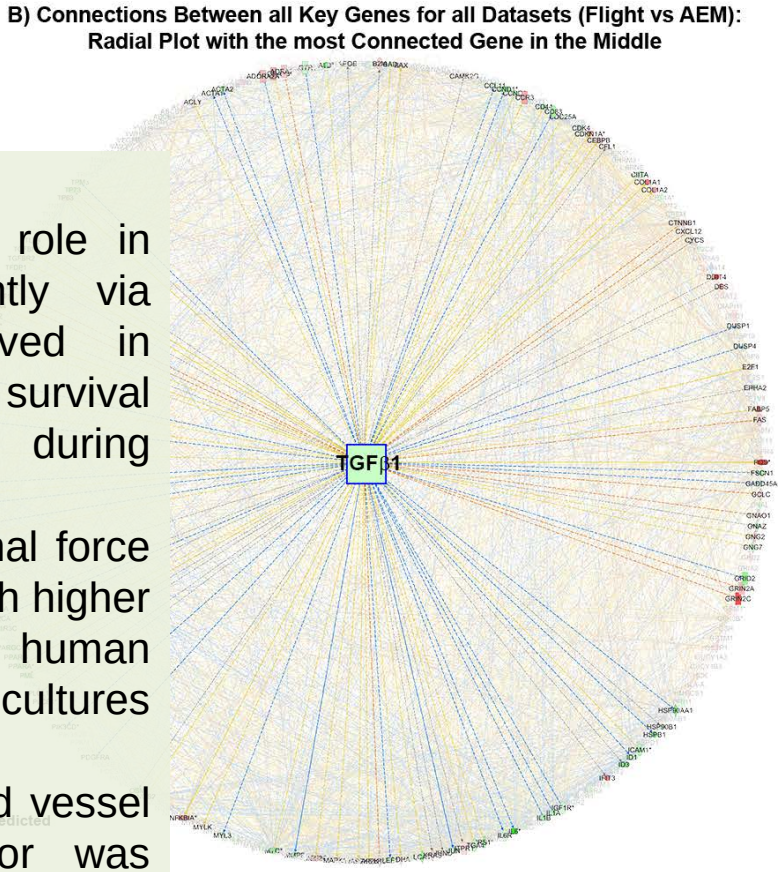
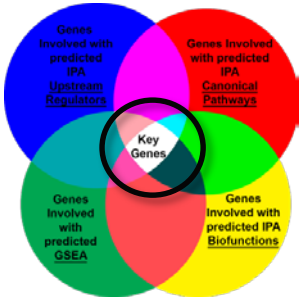
Key Genes and the Connections





- **TGFβ1 found to be central regulator of key genes**
  - TGFβ is known to play a context specific role in sustaining tissue homeostasis predominantly via transcriptional regulation of genes involved in differentiation, cell motility, proliferation, cell survival along with regulating immune responses during homeostasis and infection.
  - Previous Studies found reduction in gravitational force to diminish TGF-β expression and apoptosis with higher carcinoembryonic antigen expression in 3D human colorectal carcinoma cells, as compared to 3D cultures in unit gravity.
  - In another study, differential regulation of blood vessel growth using basic fibroblast growth factor was identified in modeled microgravity with induction early and late apoptosis, extracellular matrix proteins, endothelin-1 and TGFb1 expression



Beheshti, et al., PLOS One, 2018

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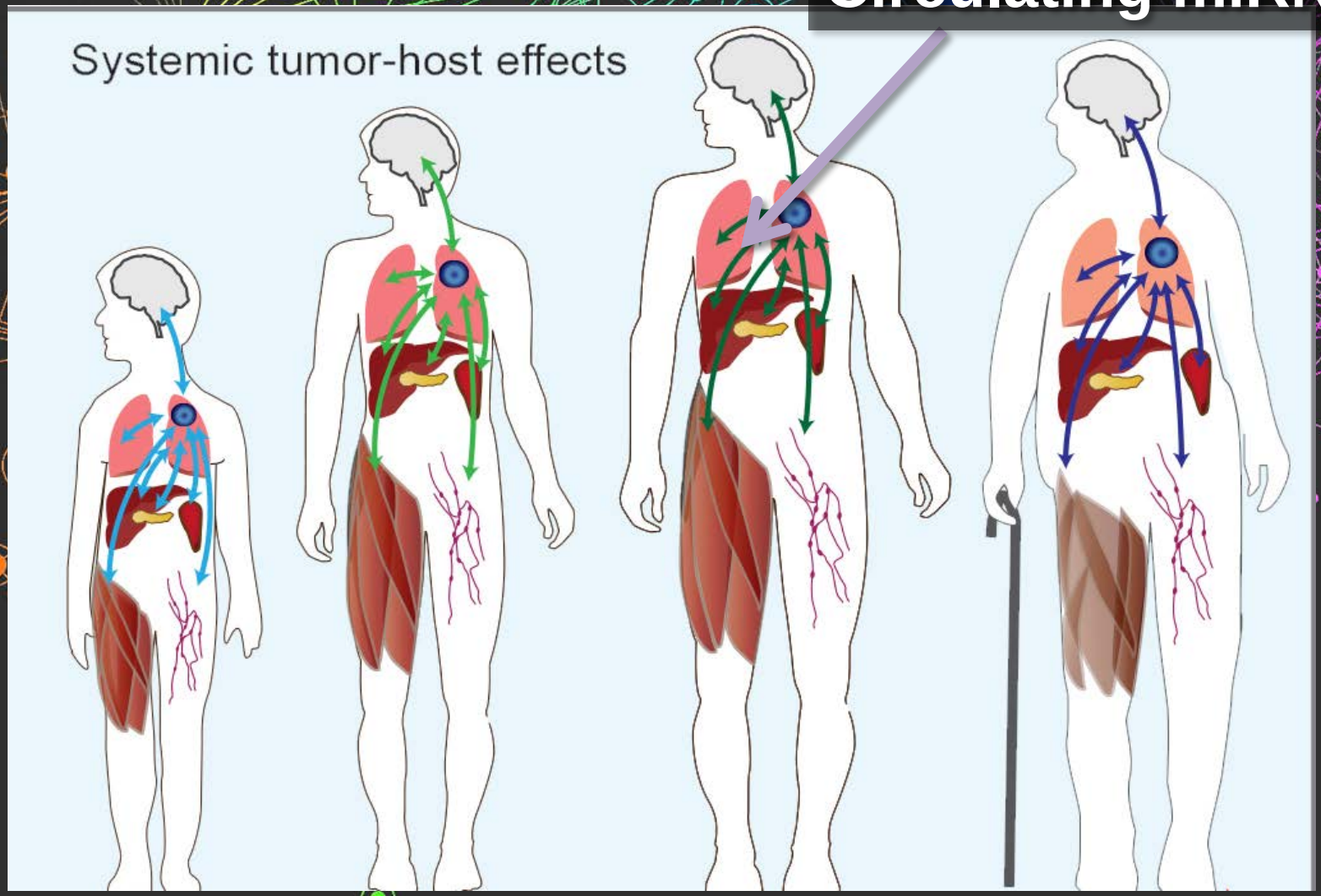
General Approach to Studying a Systematic Response in the Host

Circulating miRNAs



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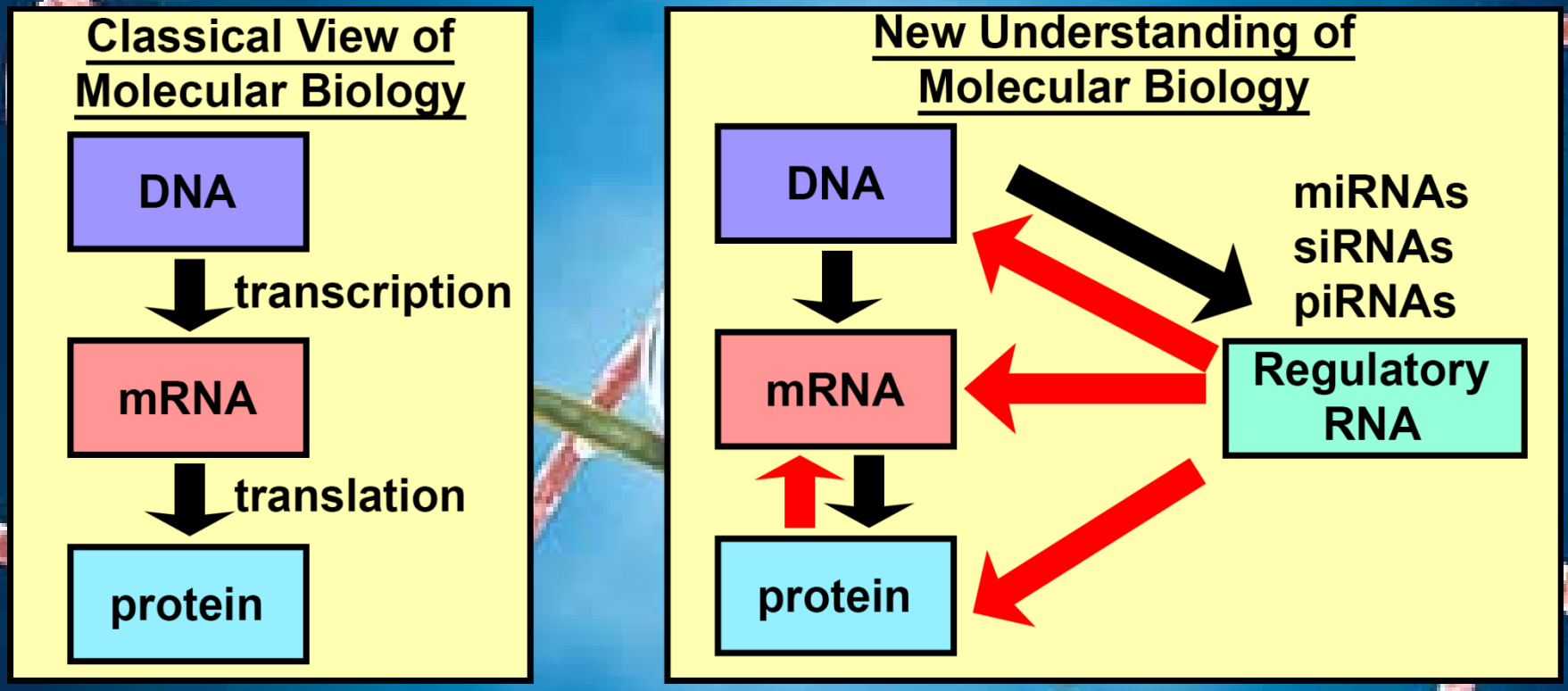
Revised View of Molecular Biology



- A single miRNA has been estimated to regulate up to 500 mRNAs
- miRNAs are single-stranded RNA sequences, of about 22 nucleotides in length, processed from longer transcripts.
- miRNAs are important regulators that repress the translation of mRNA transcripts

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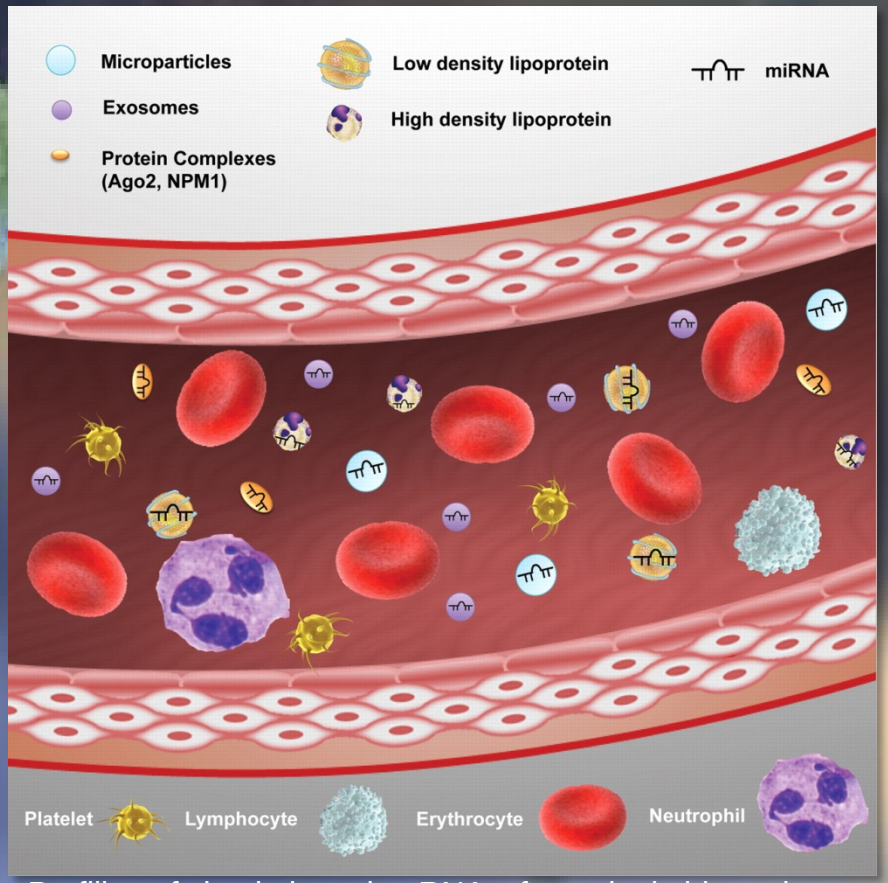
Impact of Circulating microRNAs

- Circulating miRNAs can carry signals from organs to other various parts of the body through the blood stream.
- The miRNAs can be transported in Exosomes, microparticles, lipoproteins, and outside any type of packaging.
- Our preliminary data shows that a miRNA signature is carried over from the spleen to the tumor with age.
  – Beheshti, et al. *PLoS ONE 2017*



Profiling of circulating microRNAs: from single biomarkers to re-wired networks Anna Zampetaki, Peter Willeit, Ignat Drozdov, Stefan Kiechl, Manuel Mayr. Cardiovascular Research , 2011.

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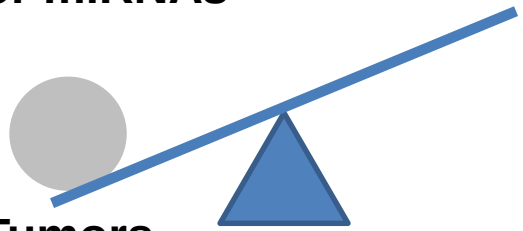
Systems Biology View of miRNAs

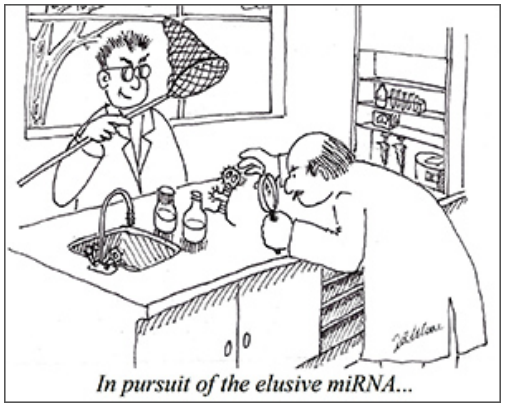
Tumor Suppressor miRNAs

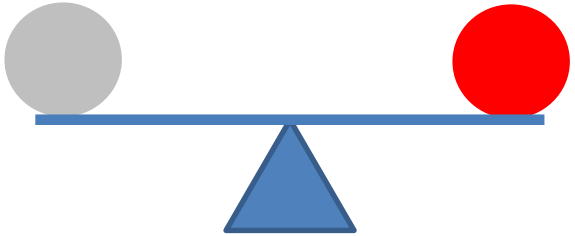
OncomiRNAs



Tumors Inhibited

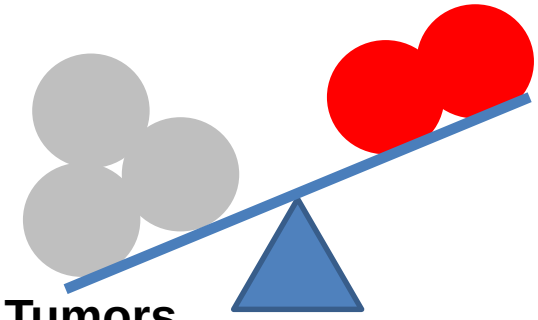
Only looking at a single miRNA



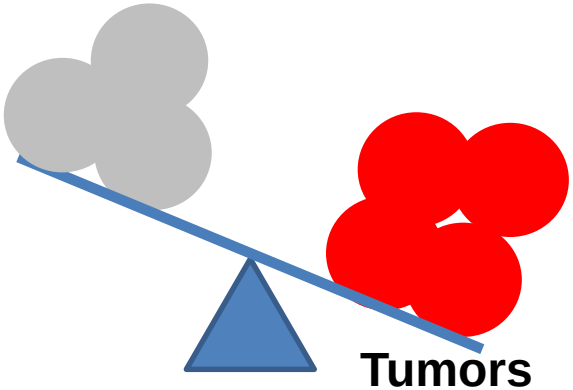


No Change in Tumors

looking at a pair of miRNAs



Tumors Inhibited



Tumors Promoted

<u>Systems Biology Approach:</u> Looking at how the entire system impacts the most Important miRNAs

Predicted miRNAs Involved with Microgravity Effects

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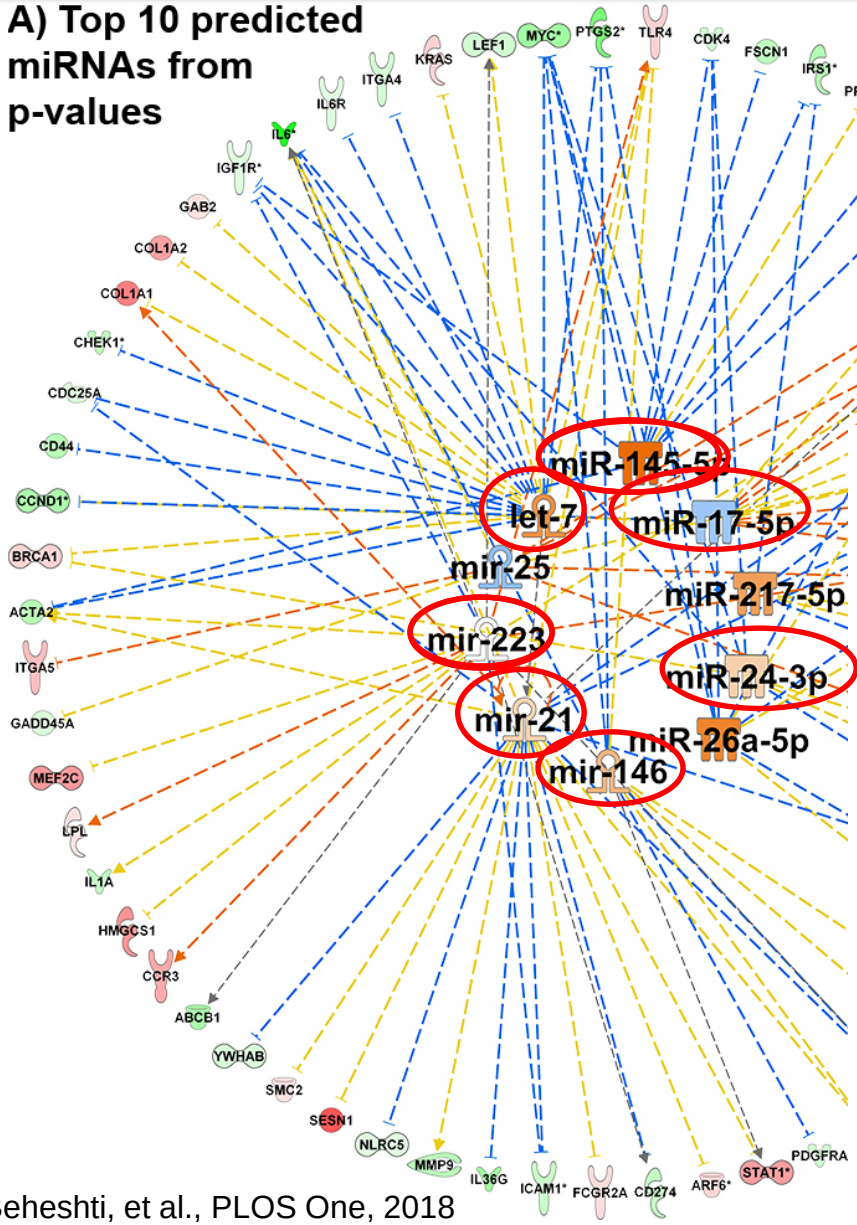
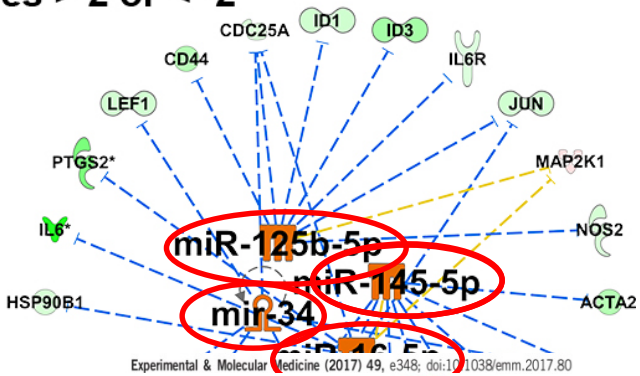
A) Top 10 predicted miRNAs from p-values



B) All miRNAs with Z-scores > 2 or < -2



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PLOS one

Research Article

Integration Analysis of MicroRNA and mRNA Expression Profiles in Human Peripheral Blood Lymphocytes Cultured in Modeled Microgravity

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Academic Editor: Mariano Bizzarri

We analyzed miRNA and mRNA expression profiles in human peripheral blood lymphocytes (PBLs) incubated in microgravity condition, simulated by a ground-based rotating wall vessel (RWV) bioreactor. Our results show that 42 miRNAs were differentially expressed in MMG-incubated PBLs compared with 1 g incubated ones. Among these, miR-9-5p, miR-9-3p, miR-155-5p, miR-150-3p, and miR-378-3p were the most dysregulated. To improve the detection of functional miRNA-mRNA pairs, we performed gene expression profiles on the same samples assayed for miRNA profiling and we integrated miRNA and mRNA expression data. The functional classification of miRNA-correlated genes evidenced significant enrichment in the biological processes of immune/inflammatory response, signal transduction, regulation of response to stress, regulation of programmed cell death, and regulation of cell proliferation. We identified the correlation of miR-9-3p, miR-155-5p, miR-150-3p, and miR-378-3p expression with that of genes involved in immune/inflammatory response (e.g., IFNG and IL17F), apoptosis (e.g., PDCD4 and PTEN), and cell proliferation (e.g., NKX3-1 and GADD45A). Experimental assays of cell viability and apoptosis induction validated the results obtained by bioinformatics analyses demonstrating that in human PBLs the exposure to reduced gravitational force increases the frequency of apoptosis and decreases cell proliferation.

Beheshti, et al., PLOS One, 2018

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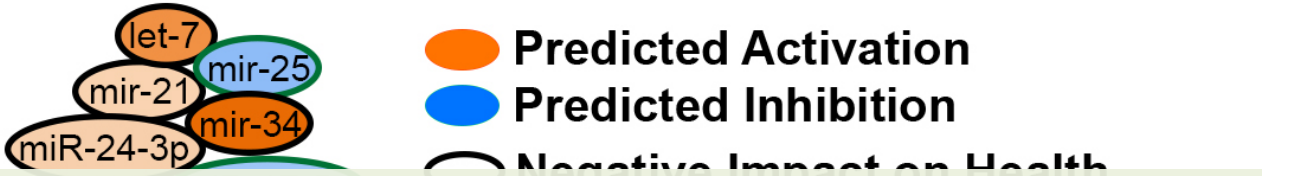
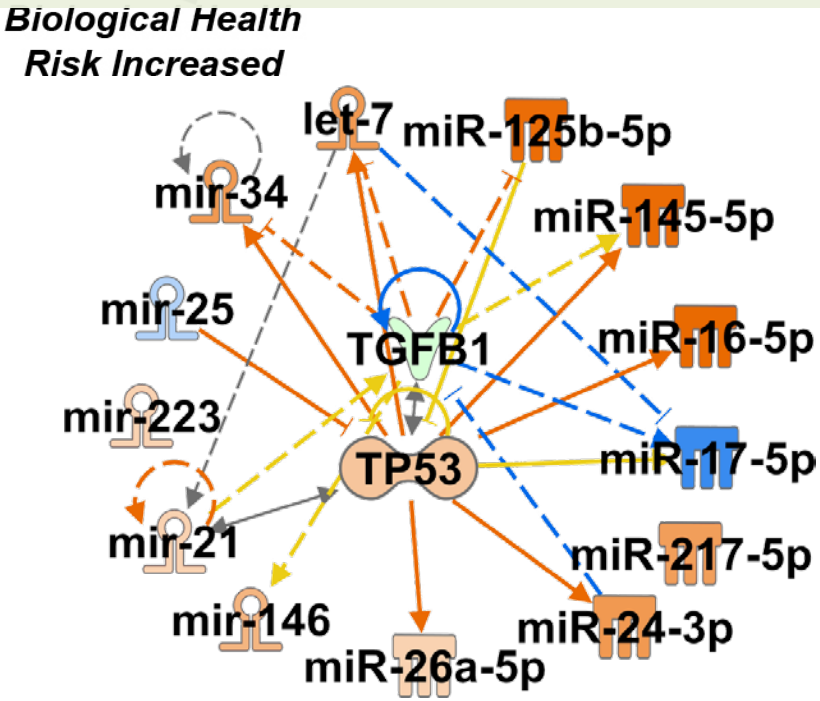
Predicted miRNAs Involved with Microgravity Effects

Health Risk Due to miRNAs



- A recent report showed that inactivation of p53 altered TGF-β signaling, which ironically displayed both tumor-suppressive and pro-oncogenic functions. p53 functions to integrate crosstalk between Ras/MAPK and TGF-β signaling via binding to Smad3, dislocating the Smad3/Smad4 complex formation and differentially regulating subsets of TGF-β target genes



Beheshti, et al., PLOS One, 2018

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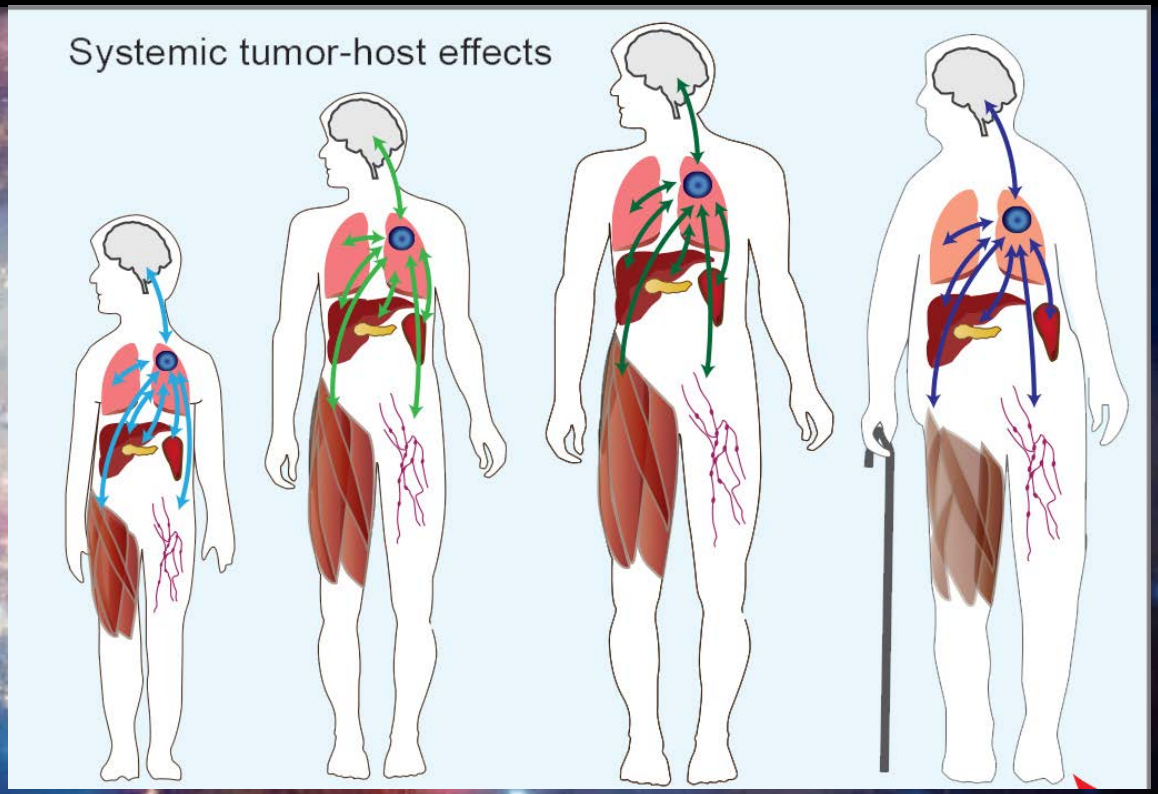
Conclusions for this Study



- Systems biology approach allows for systemic understanding of the impact of Microgravity.
- Circulating miRNAs can influence overall progression of health risk to the host.
- miRNAs can potentially be used for novel minimally invasive therapeutics and countermeasures
- GeneLab (genelab.nasa.gov) is a powerful tool to generate hypotheses and direct future space research

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Analysis Working Group (AWG) Member related work determines novel systemic biological factors causing damage due to spaceflight

Work in progress

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MEDICAL UNIVERSITY of SOUTH CAROLINA

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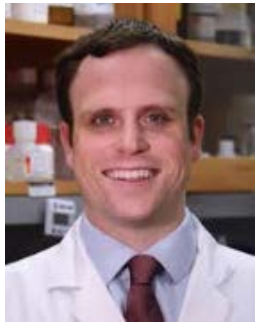
AWG Members Involved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Susana Zanello


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Space Center Houston
Manned Space Flight Education Foundation


Afshin Beheshti


Sylvain Costes

Ames Research Center

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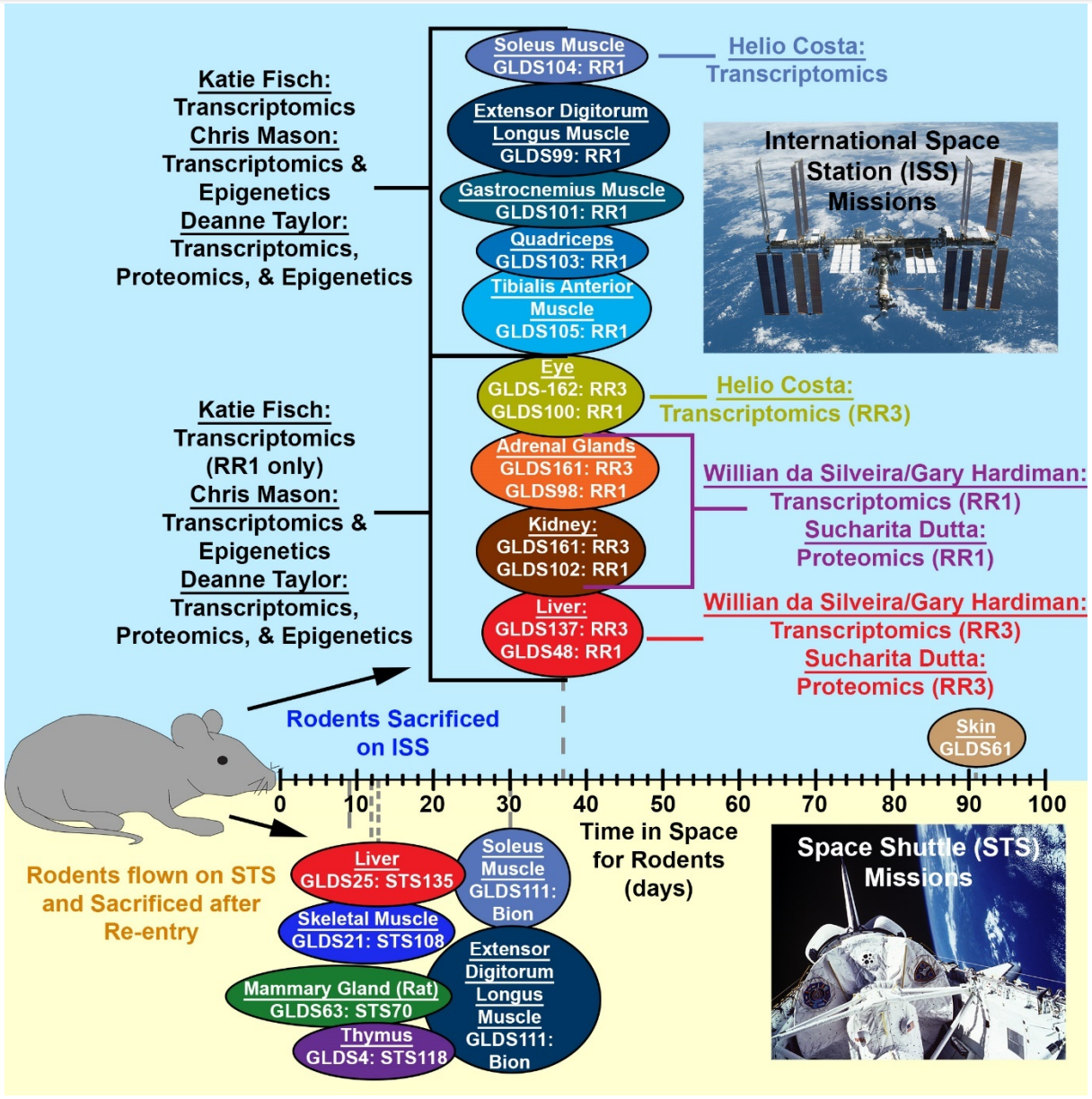
Specific Datasets and Tissues AWG Members Analyzed



- Additional Datasets that are being analyzed:
  - Human datasets
    - GLDS-54, GLDS-174, GLDS-86, GLDS-118, GLDS-53, GLDS-54, GLDS-13. GLDS-52, or GLDS-114 (Tyson McDonald and Yared Kidane)

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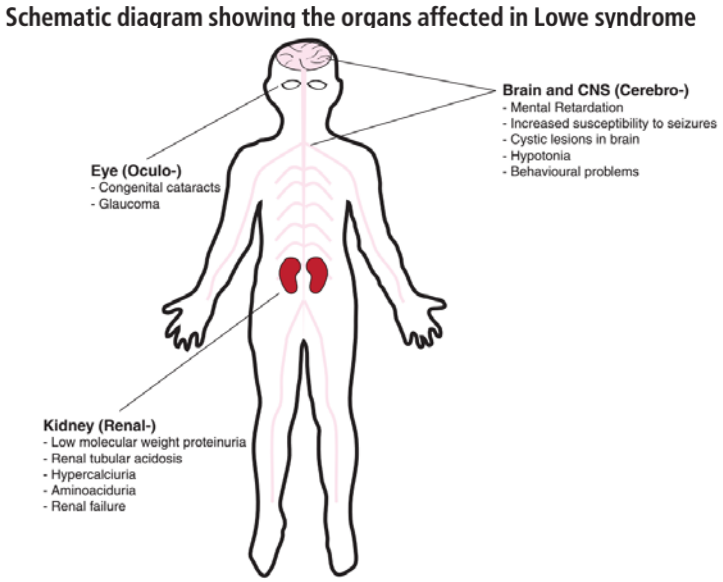
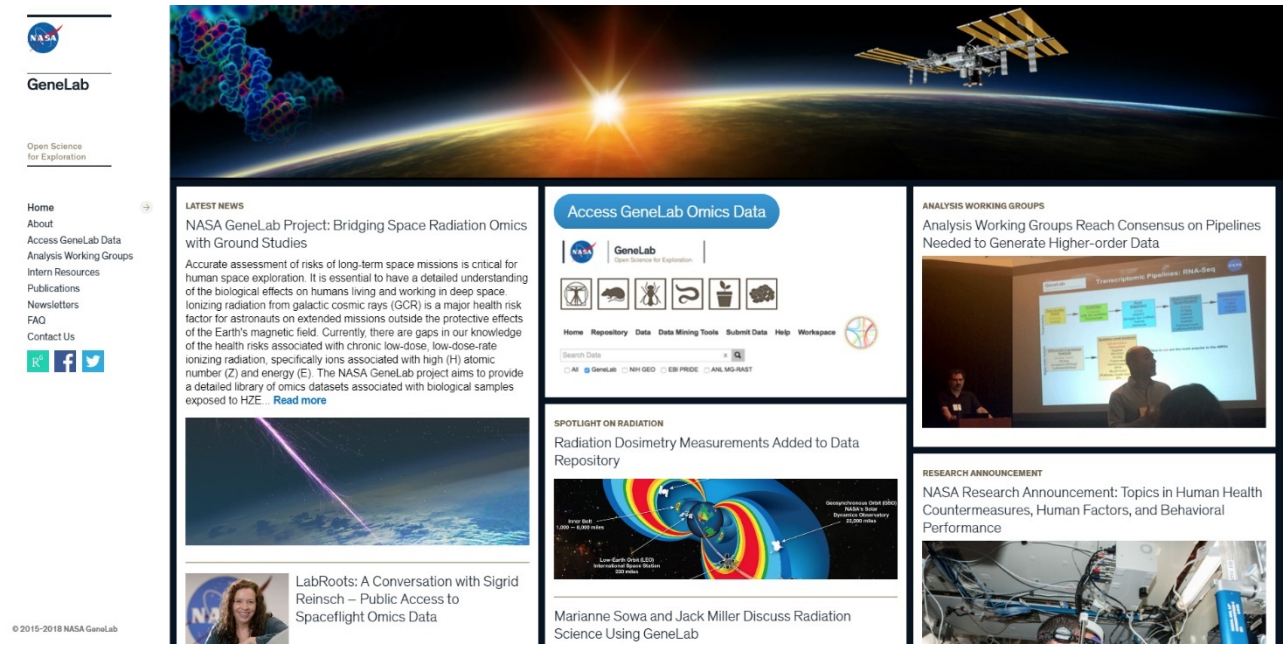
Hypothesis Developed and Being Worked On

- Spaceflight changes the physical properties of the cell components impacting from the molecular to the whole body level.
- The Mitochondria are the principal cellular component affect.
- The Liver is the principal organ affected in issues related to the metabolism.
- Possible disease that can be associated with liver damage and pathways is: Oculocerebrorenal Syndrome of Lowe
  - "Extensive research has demonstrated that OCRL-1 is involved in multiple intracellular processes involving endocytic trafficking and actin skeleton dynamics. This explains the multi-organ manifestations of the disease."
  - "The classic form of the oculocerebrorenal syndrome of Lowe (OMIM #309000), first described by Lowe et al. in 1952 [1], is characterized by the triad of congenital cataracts, severe intellectual impairment, and renal tubular dysfunction with slowly progressive renal failure"
  - Patients with this disease manifest Cataract, Glaucoma and Muscle hypotonia.



Mehta, Zenobia B et al. "The Cellular and Physiological Functions of the Lowe Syndrome Protein OCRL1." *Traffic* (2014).

Engaging with GeneLab



https://genelab.nasa.gov

Participate in GeneLab Analysis Working Groups

- Social media :
  - @NASAAmes **Facebook**
  - **Twitter** #GeneLab
  - **ResearchGate**: https://www.researchgate.net/project/Omics-for-Space-Biology-The-GeneLab-project

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Samrawit Gebre
Dennis Heher
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Tristan Le
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