National Aeronautics and Space Administration

GeneLab: “Omics” Data System for Space Biology Research

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Jonathan Galazka, PhD: Project Scientist

Marla Smithwick: Deputy Project Manager

The GeneLab Team

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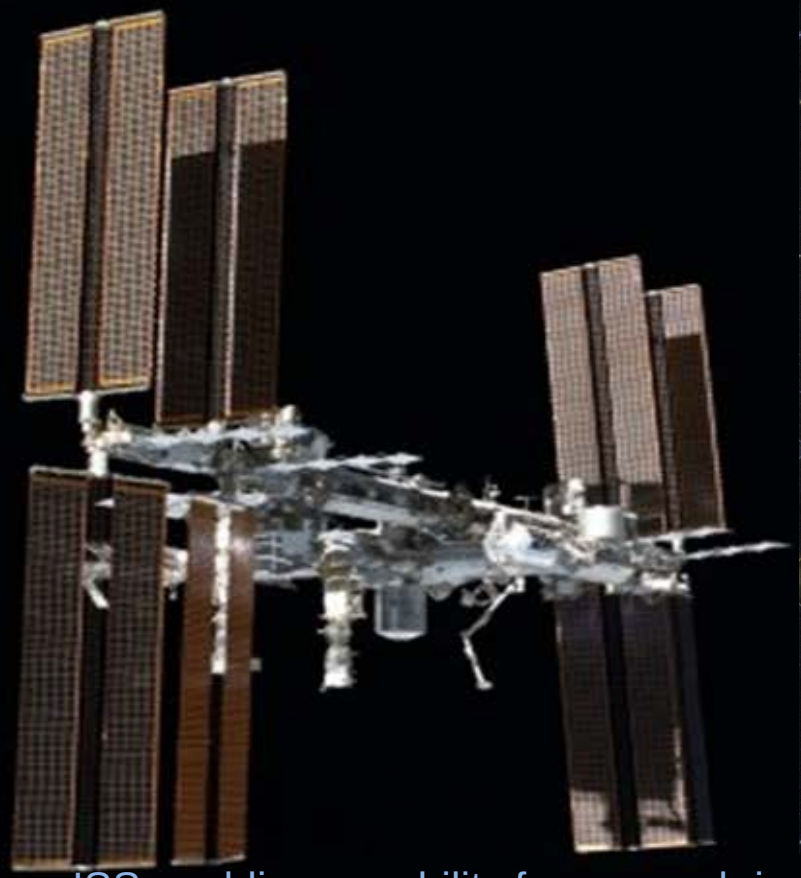
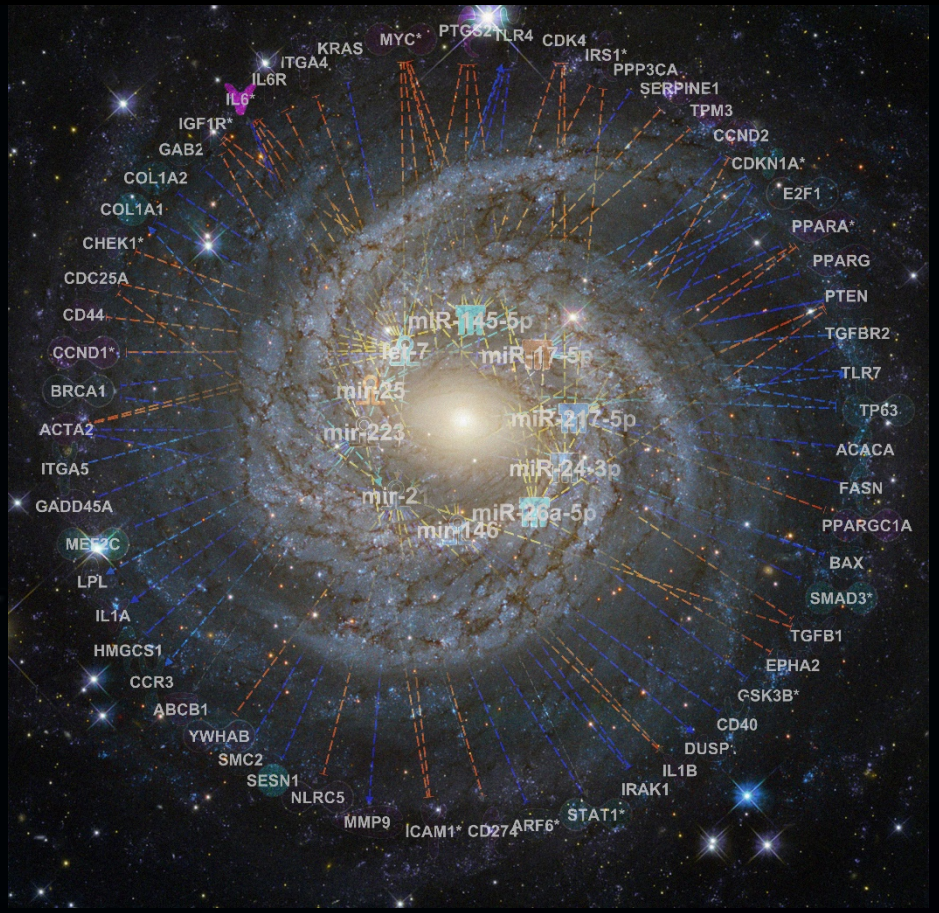
ISS: Our Orbiting Laboratory





ISS enabling capability for research in cellular and molecular biology includes equipment for *in situ*, on-orbit analysis of biomolecules

Applications of this growing capability range from biomedicine and biotechnology to the growing field of Omics

ASGSR 2016 - John Love, Vic Cooley, Yuri Guinart-Ramirez - NASA, JSC; Aaron Burton, Sarah Wallace, David S. Copeland, Cherie Oubre – NASA, JSC; Macarena Parra, David Smith, Julie Schonfeld - NASA, AMES; Christina Khodadad – NASA, KSC
Ronald Sicker, William Meyer, William Fletcher – NASA, GRC ; Luchino Cohen – CSA; Aldo Roda, Mara Mirasol – Univ. of Bologna

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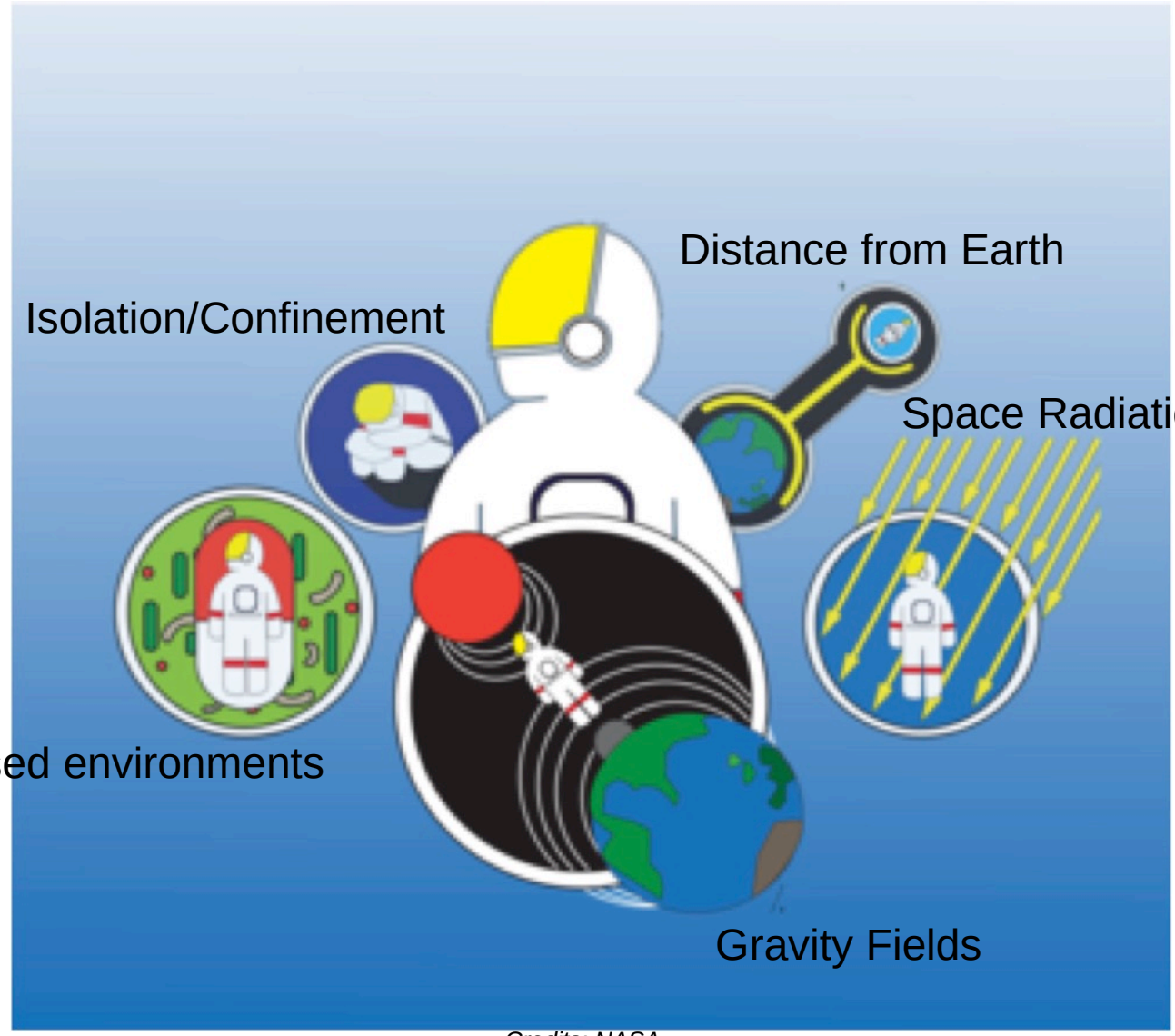
Challenges of Spaceflight



Credits: NASA

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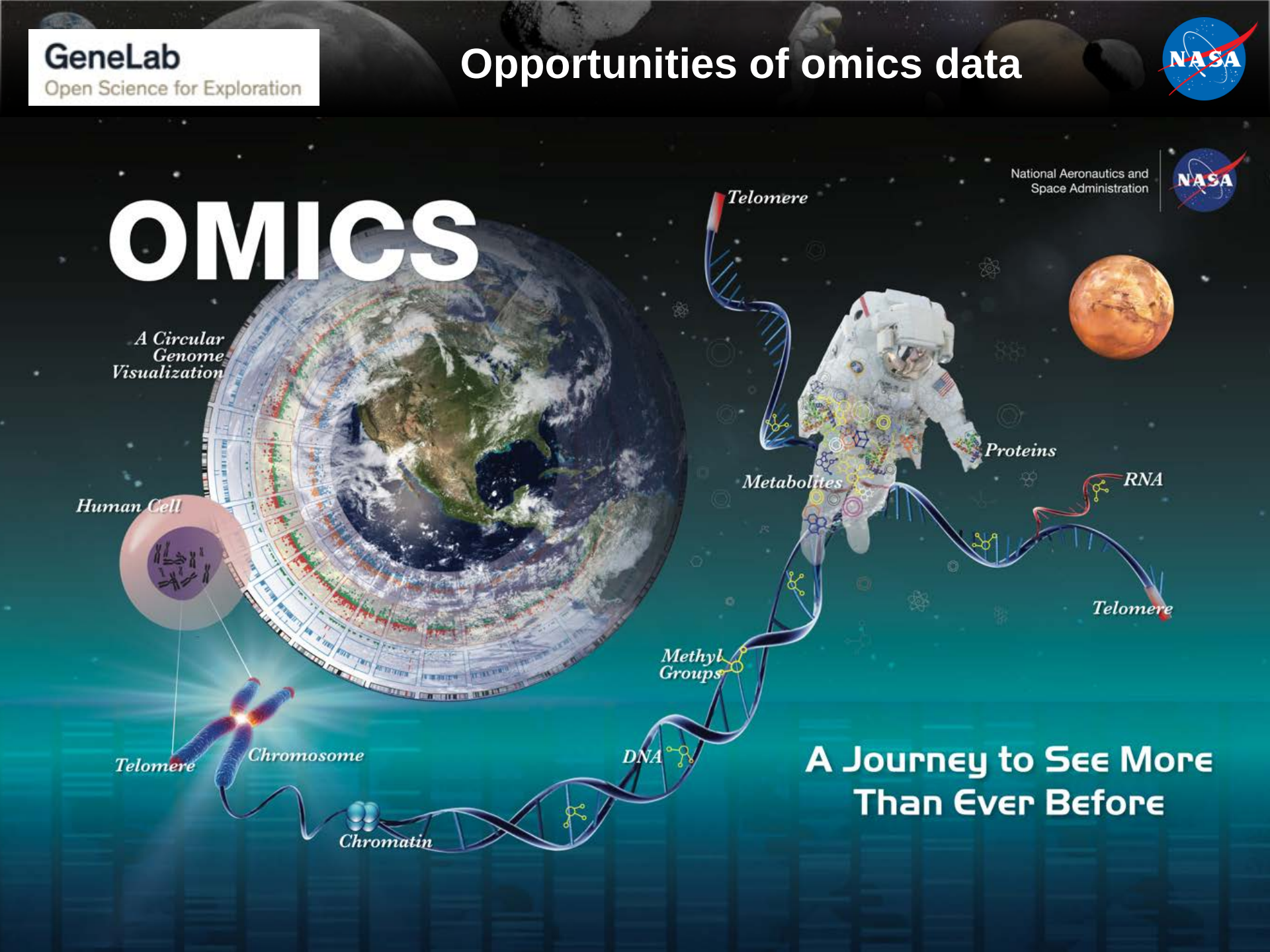
Opportunities of omics data



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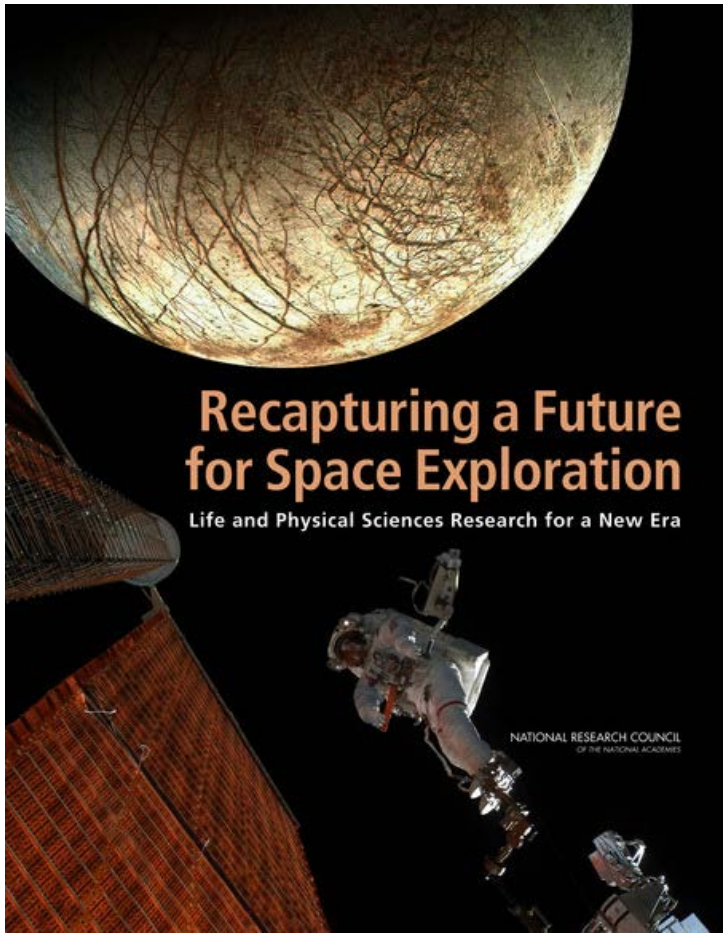
2011 NRC Decadal Survey



"*…**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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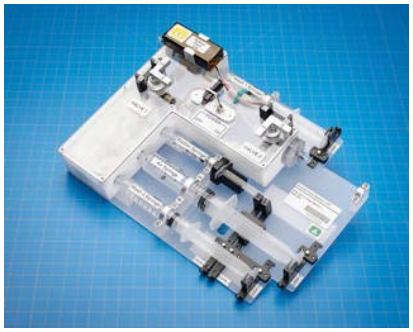
Omics Acquisition in Space is Now a Reality

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.

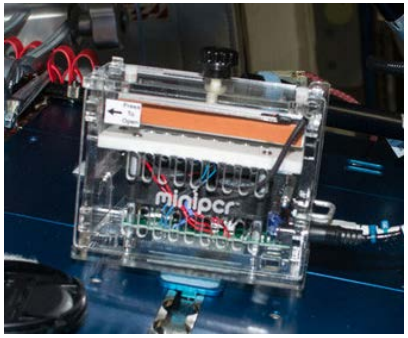


Sample Preparation Module

Oxford Nanopore MinION Gene Sequencer

Reaction tube containing lyophilized chemical assay bead (proprietary)

Cepheid Smart Cycler qRT-PCR



Mini-PCR

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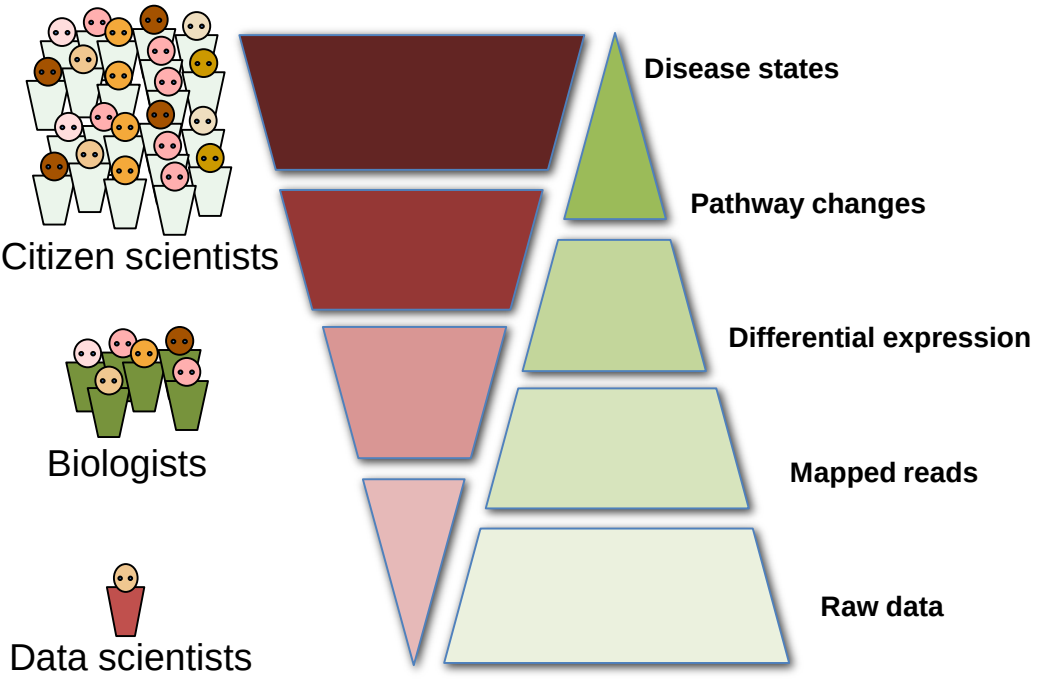

New Knowledge

Analysis Working Groups

Galaxy | GenomeSpace

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EBI PRIDE

ANL MG-RAST | NCBI GEO

Open Data

Missions/Experiments | GeneLab Sample Processing

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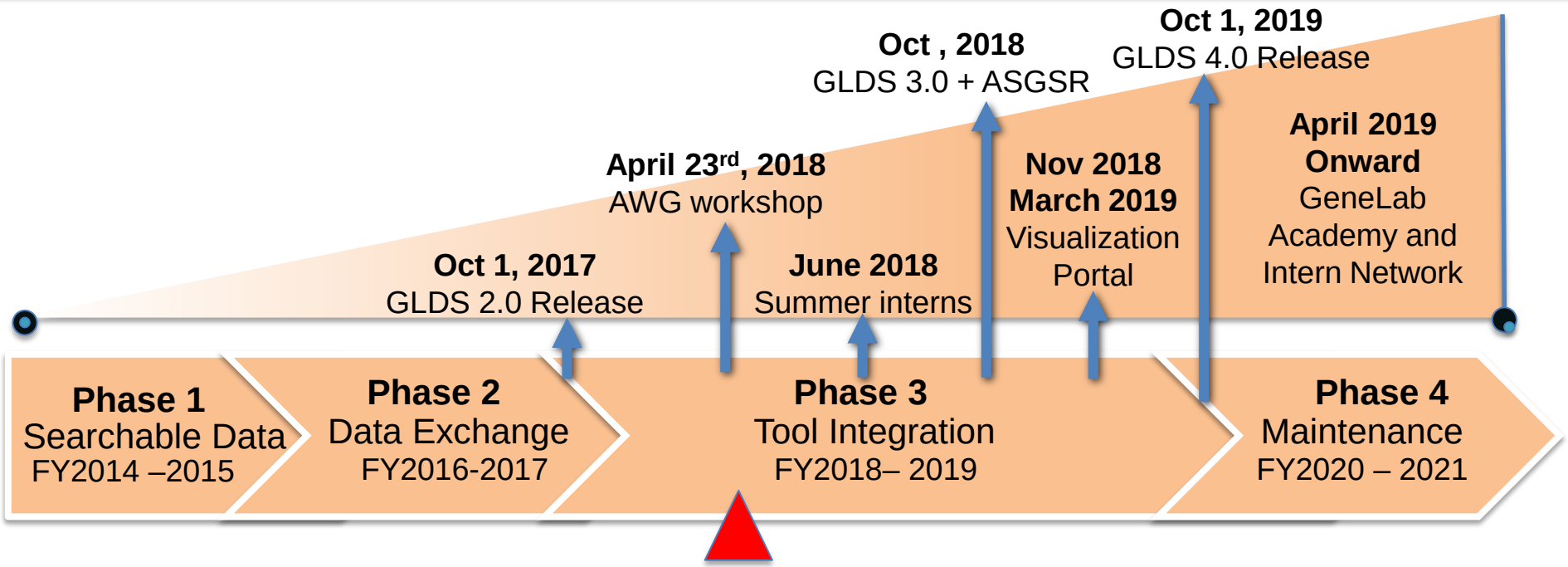
Phased Implementation



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

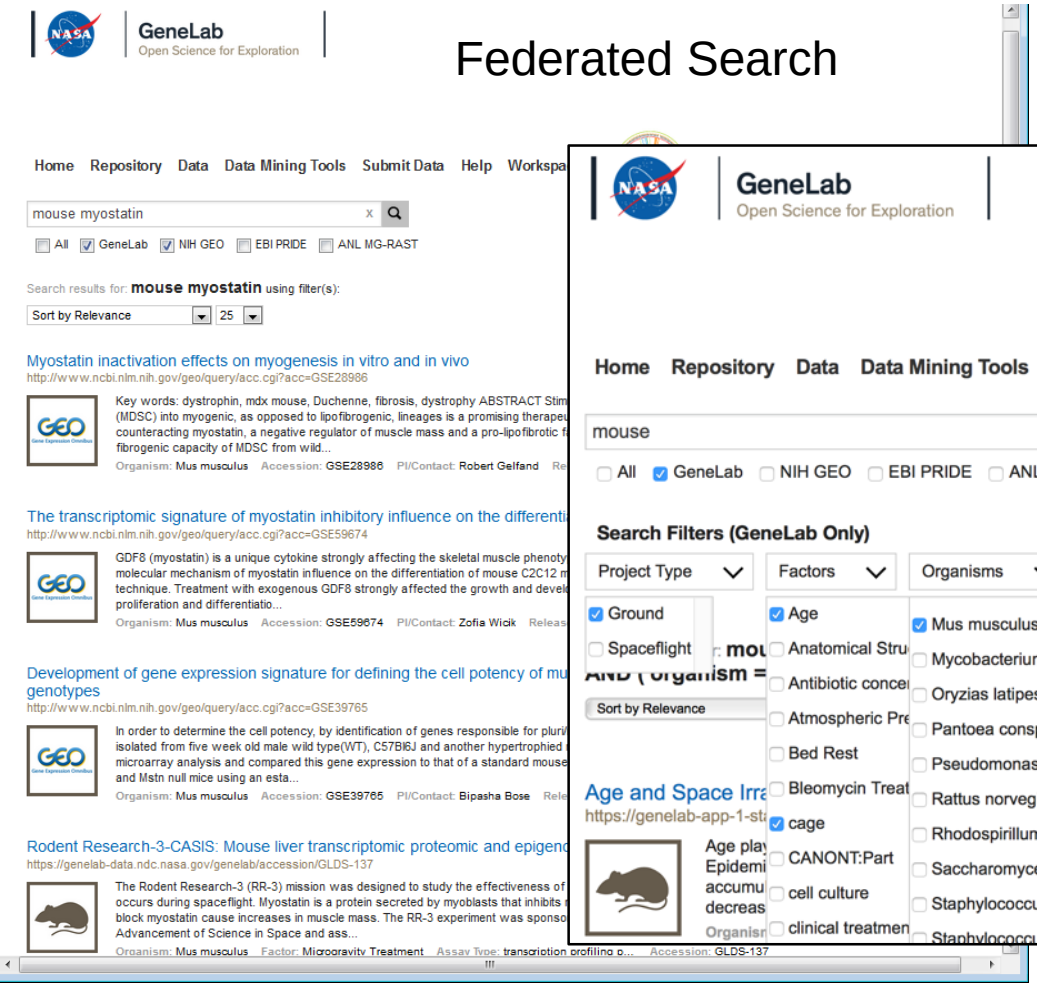
Data System
- ✓ Link to Public Databases via Data Federation
- ✓ Integrated Search (e.g., data mashup)

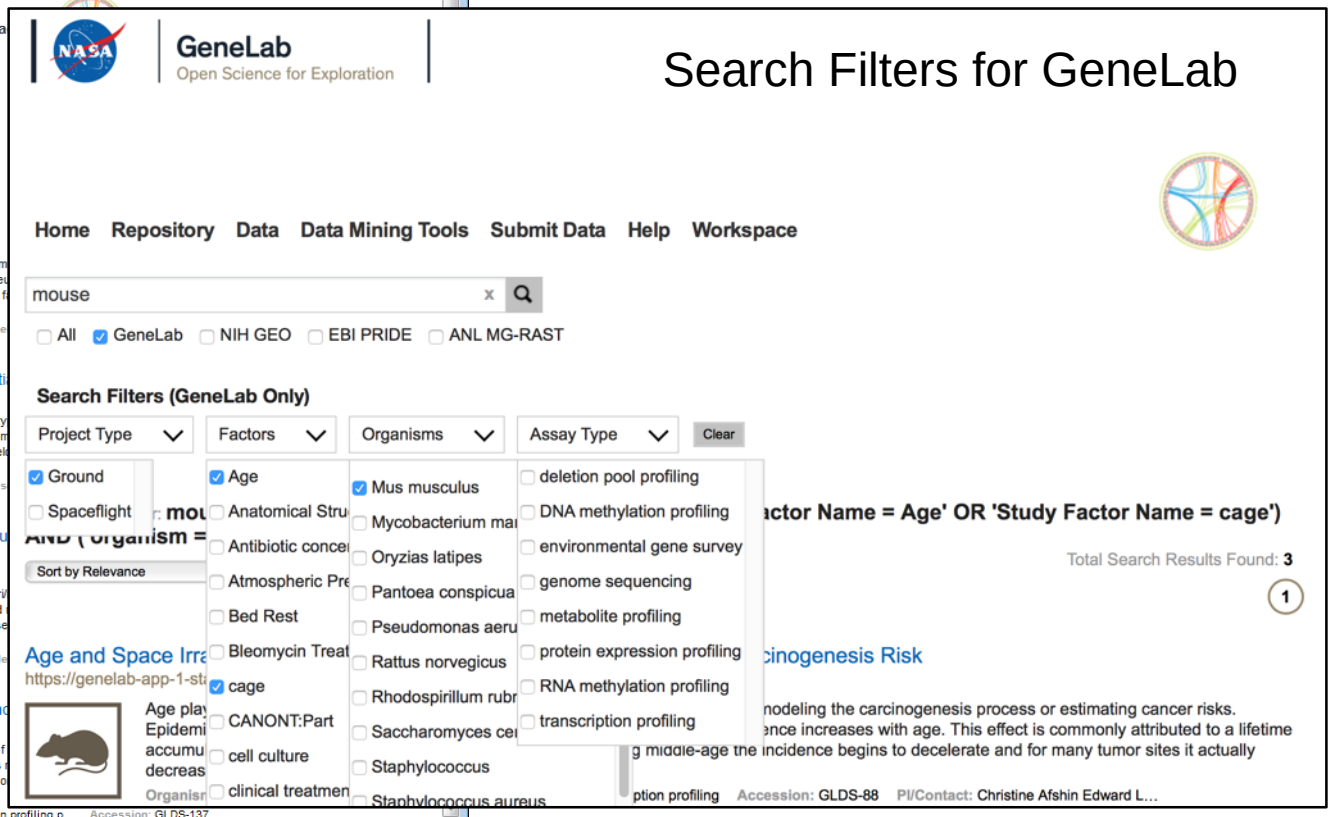
Data System
- Integrated Platform across model organisms
- Build Community via AWG
- Provide access to biocomputational tools for omics analysis
- Provide collaboration framework and tools

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

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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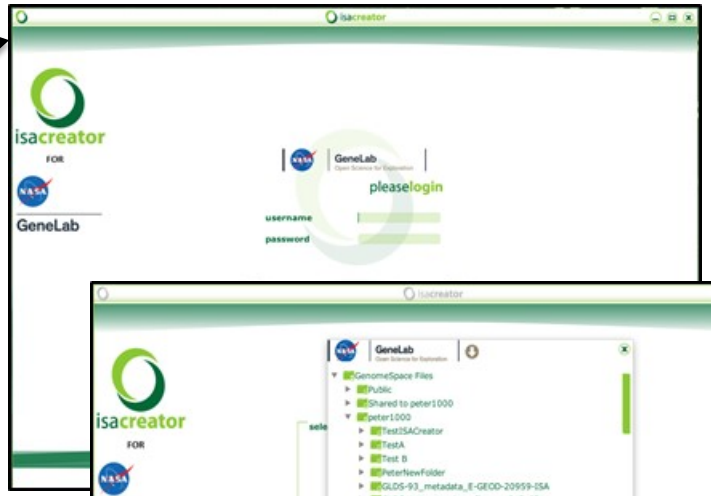
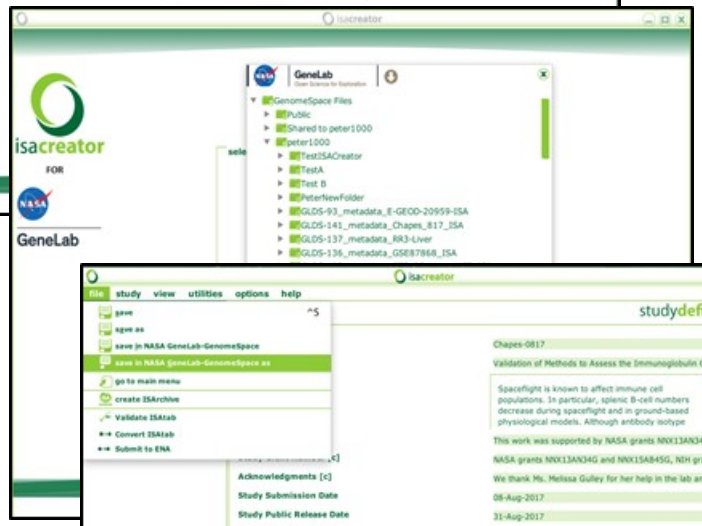
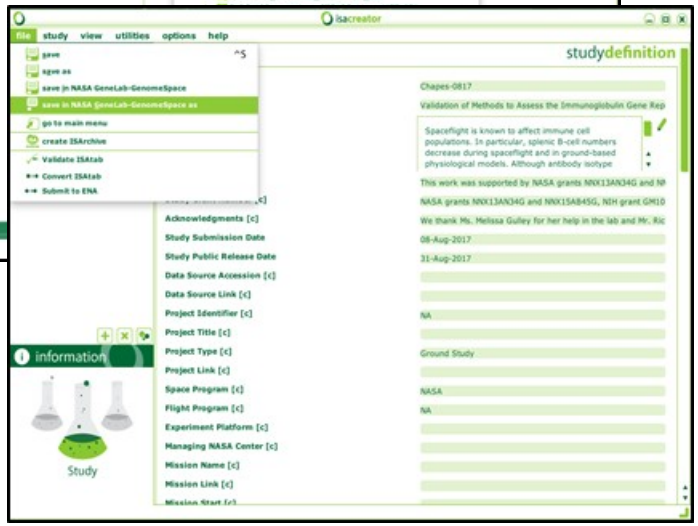
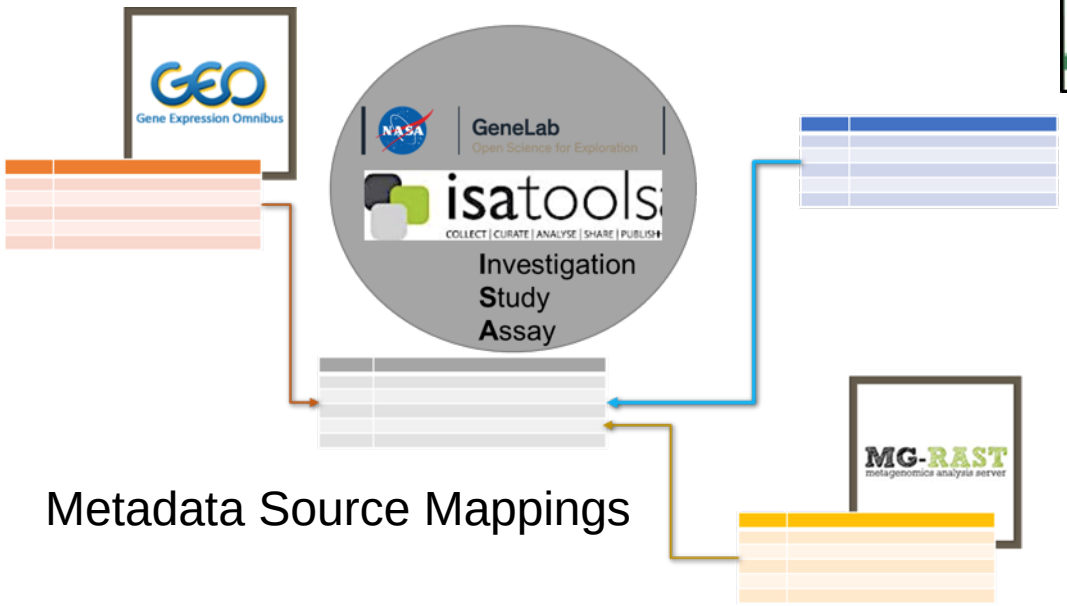
GLDS Phase 2 (Release 2.0) Metadata Curation via ISACreator Tool

GeneLab-GenomeSpace Integration with ISACreator for Streamlining Data Processing Operations









Metadata Source Mappings

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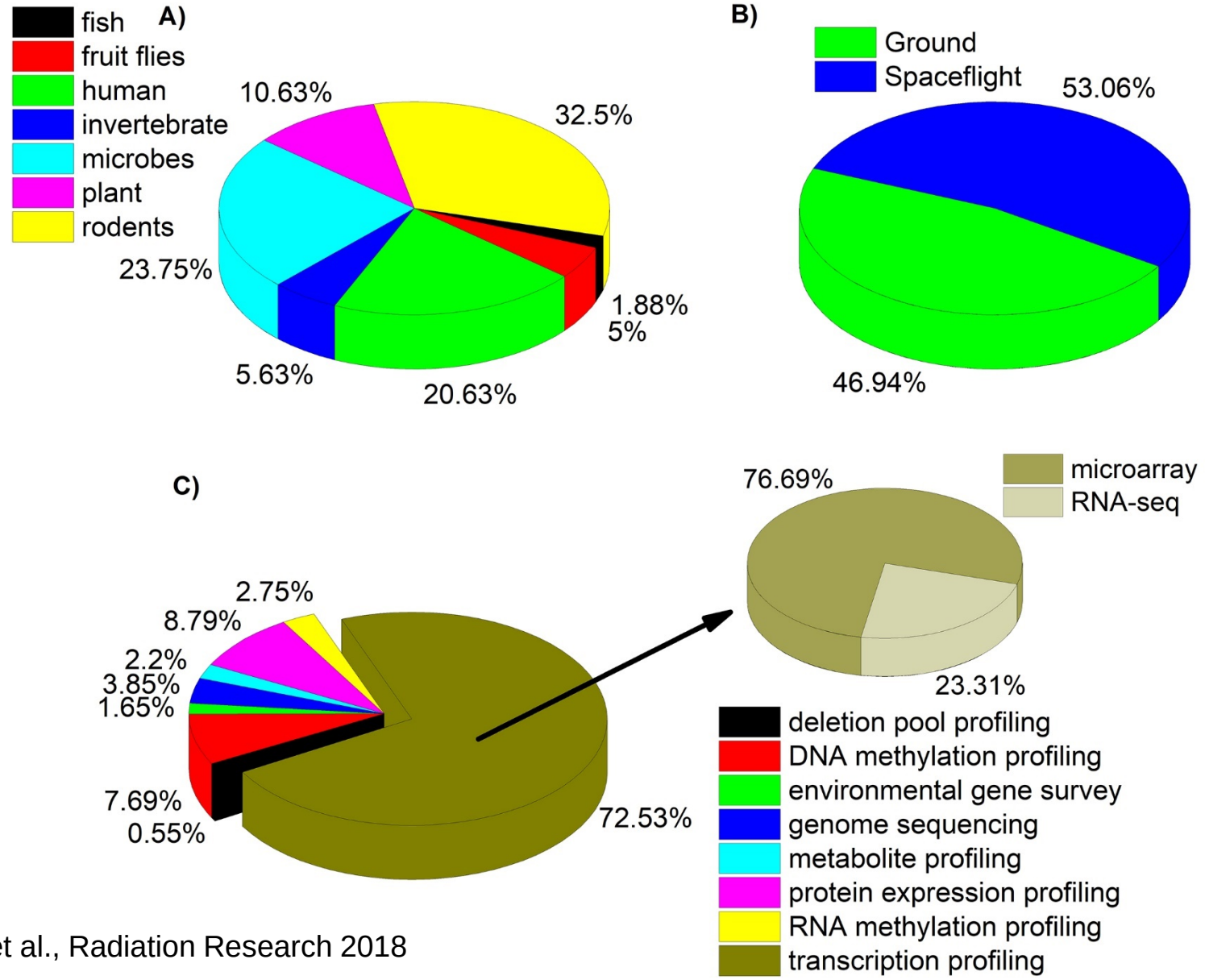
GeneLab Database: >190 data sets



Beheshti et al., Radiation Research 2018

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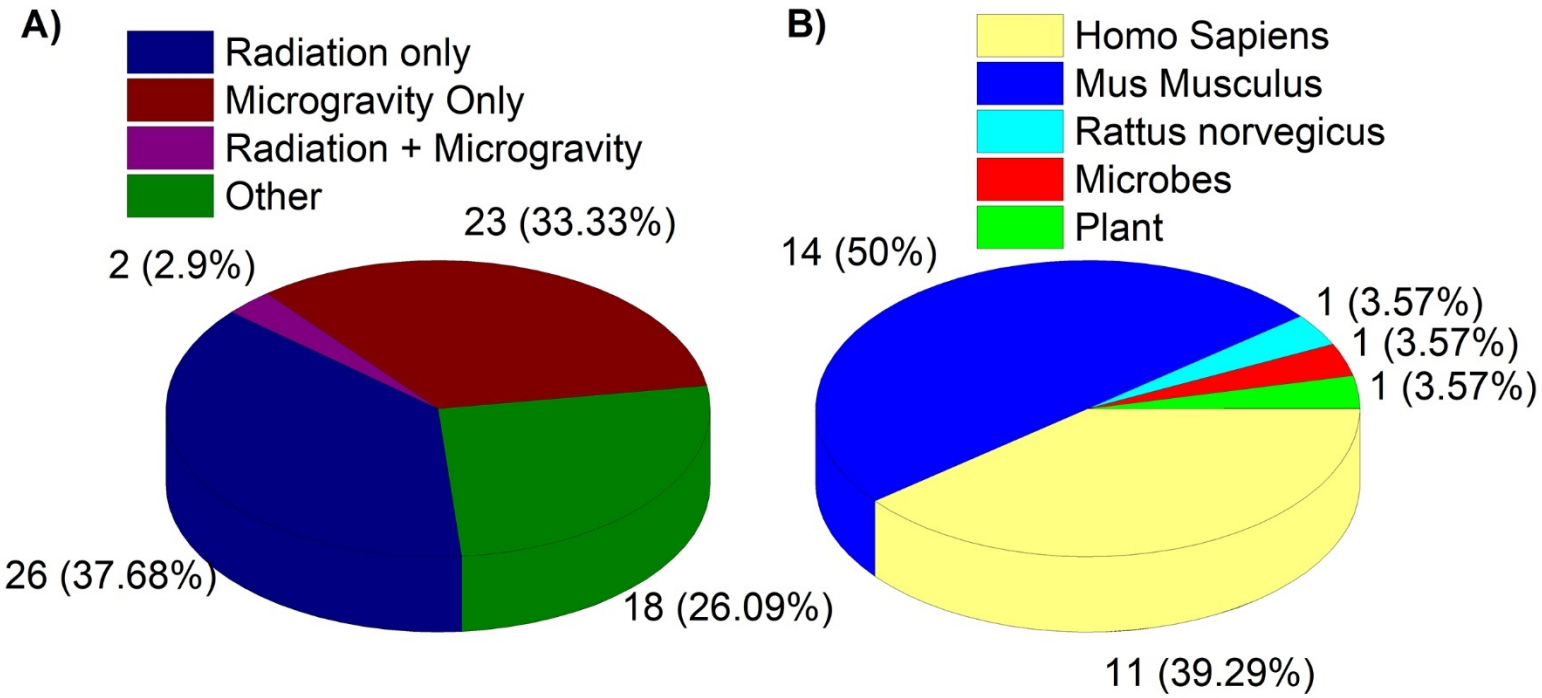
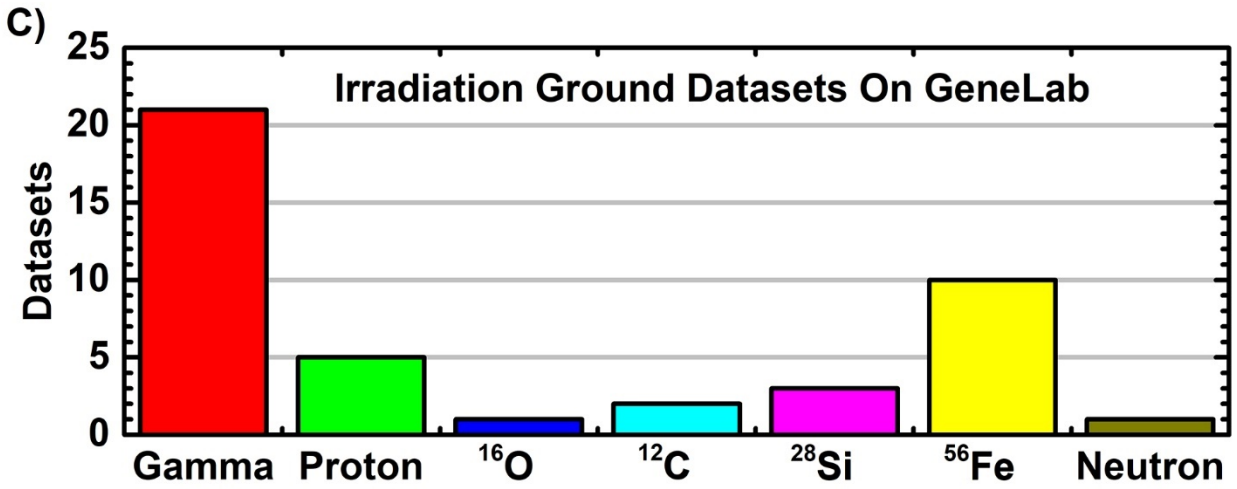
69 Ground Data Sets: Radiation and simulated microgravity





Beheshti et al., Radiation Research 2018

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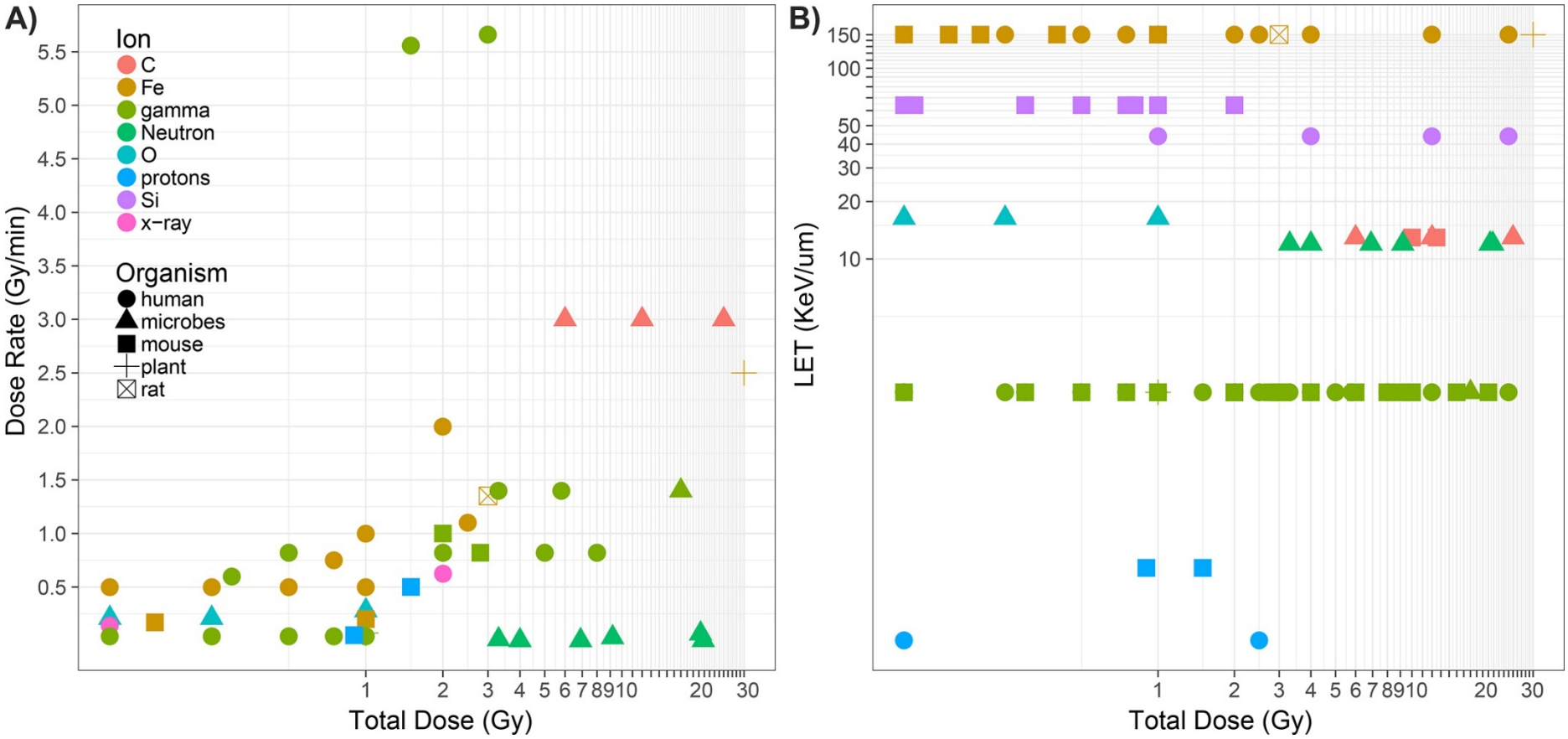
Radiation Ground Studies



Beheshti et al., Radiation Research 2018

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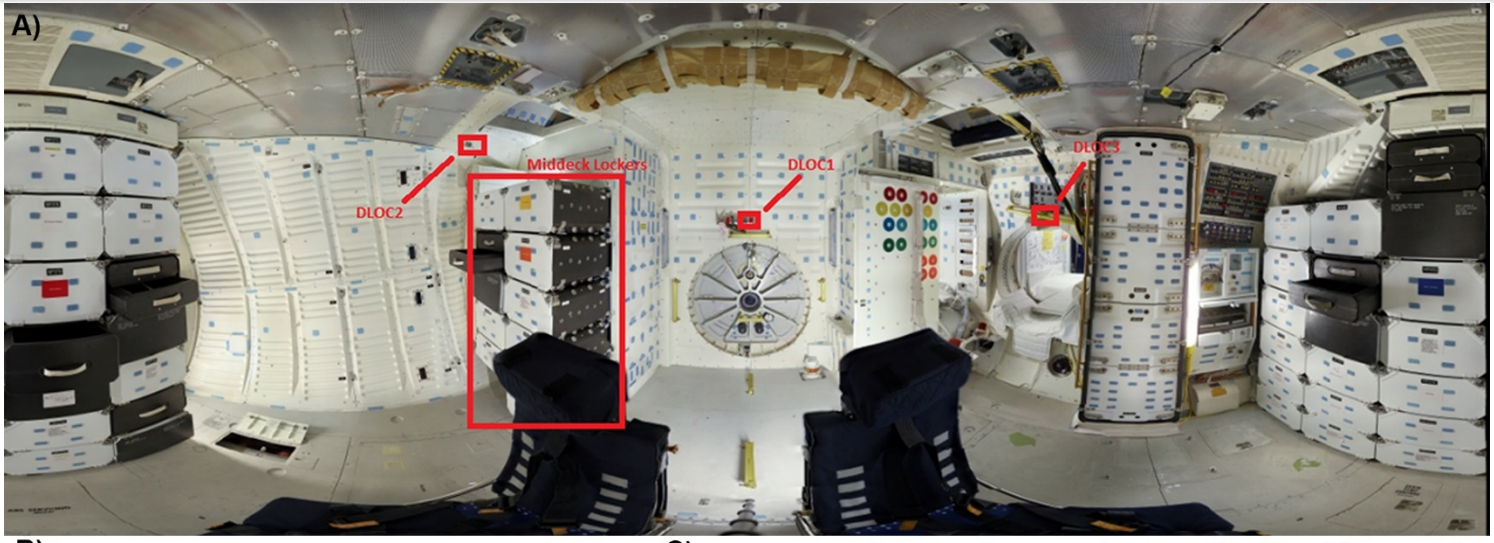
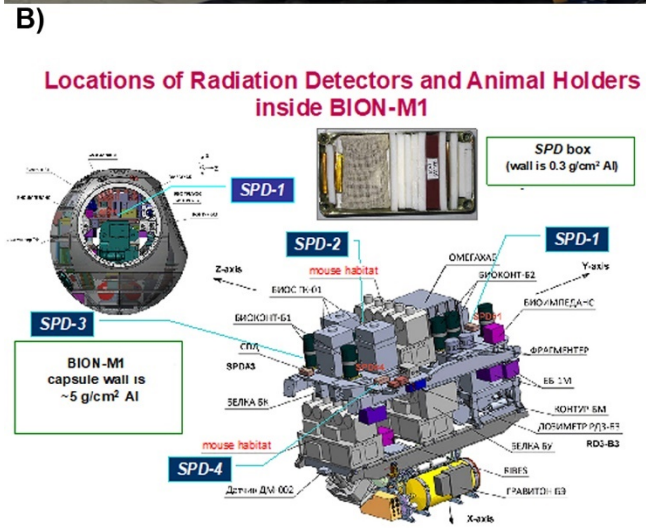
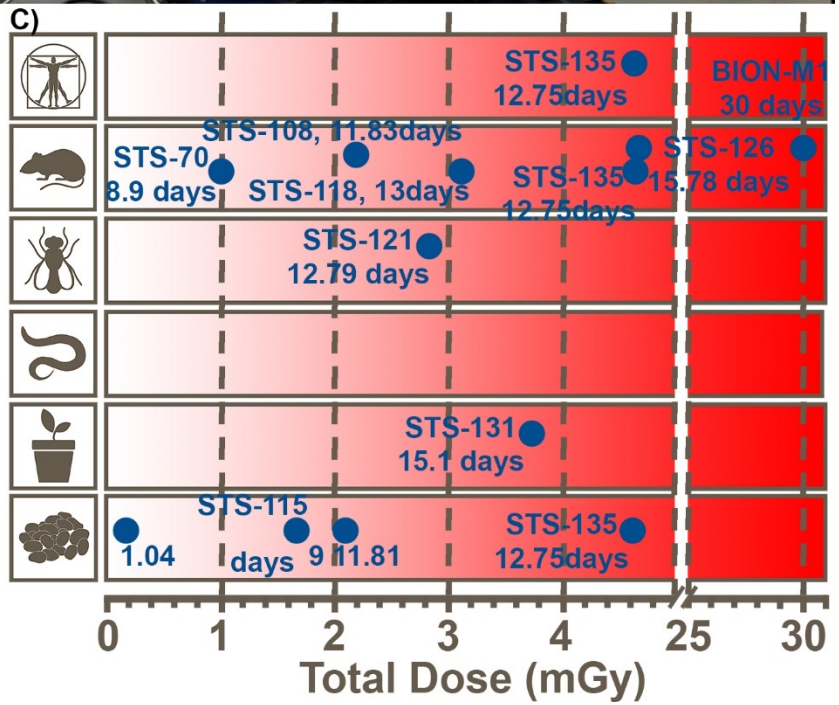
Radiation Dosimetry for STS samples (ISS to follow)







Beheshti et al., Radiation Research 2018

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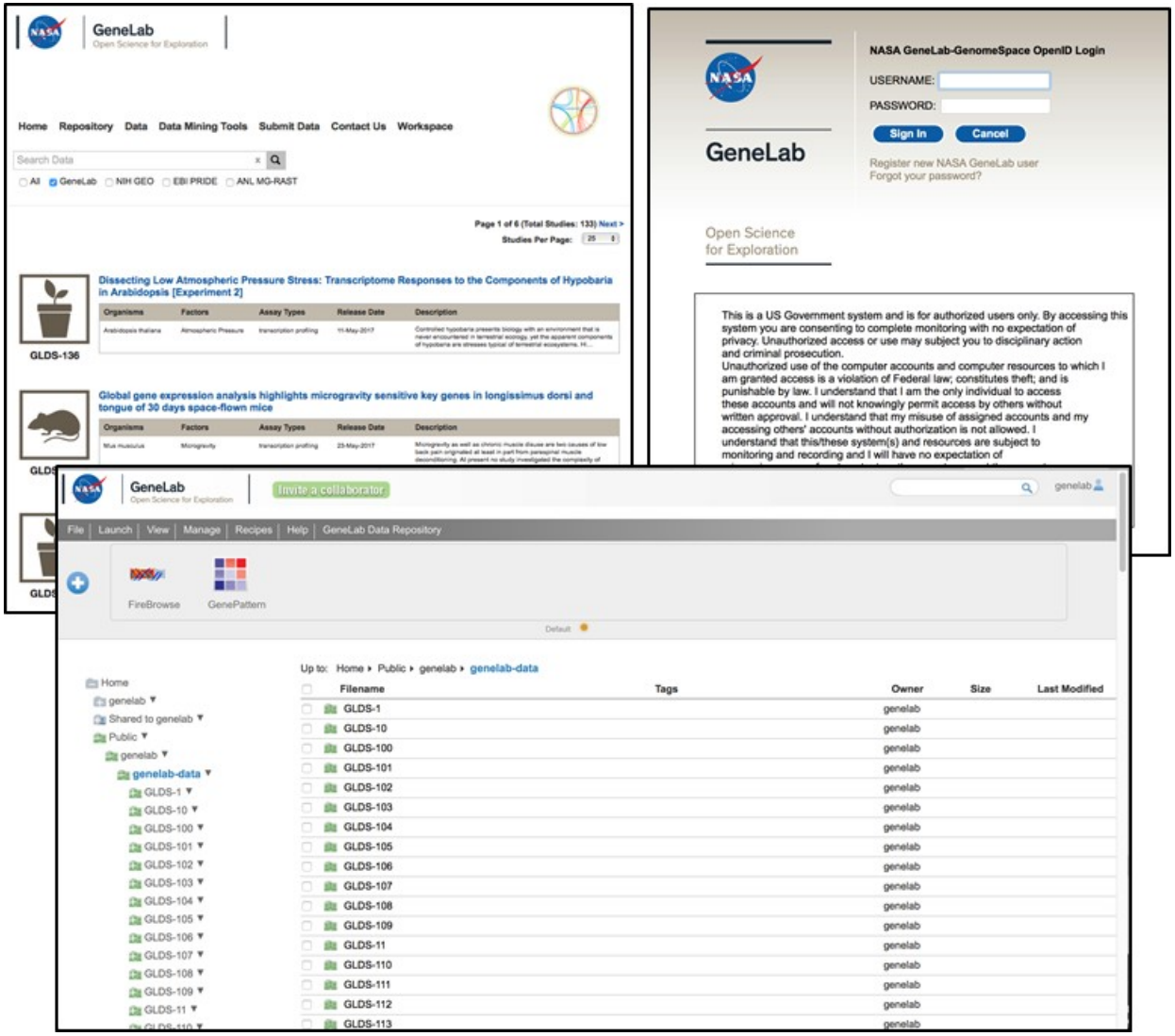
GLDS Phase 2 (Release 2.0)
Customized NASA Collaborative Workspace

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



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Galaxy Platform

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)

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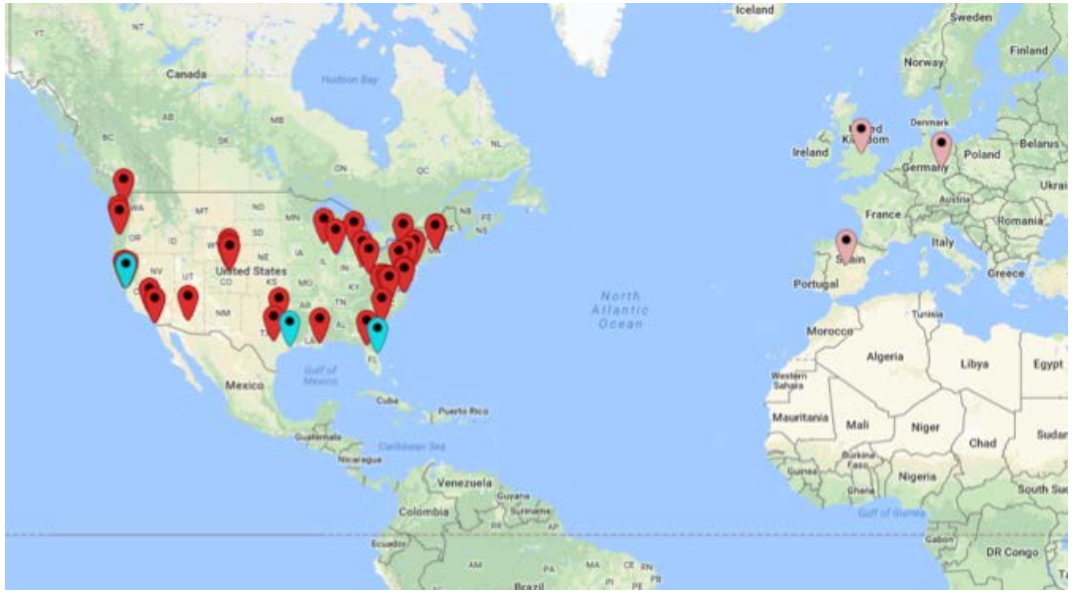
GeneLab Analysis Working Groups

- **~60 individuals**
- **4 Groups: Plants, Microbes, Animals, Multi-omics**
- **Monthly meetings**
- **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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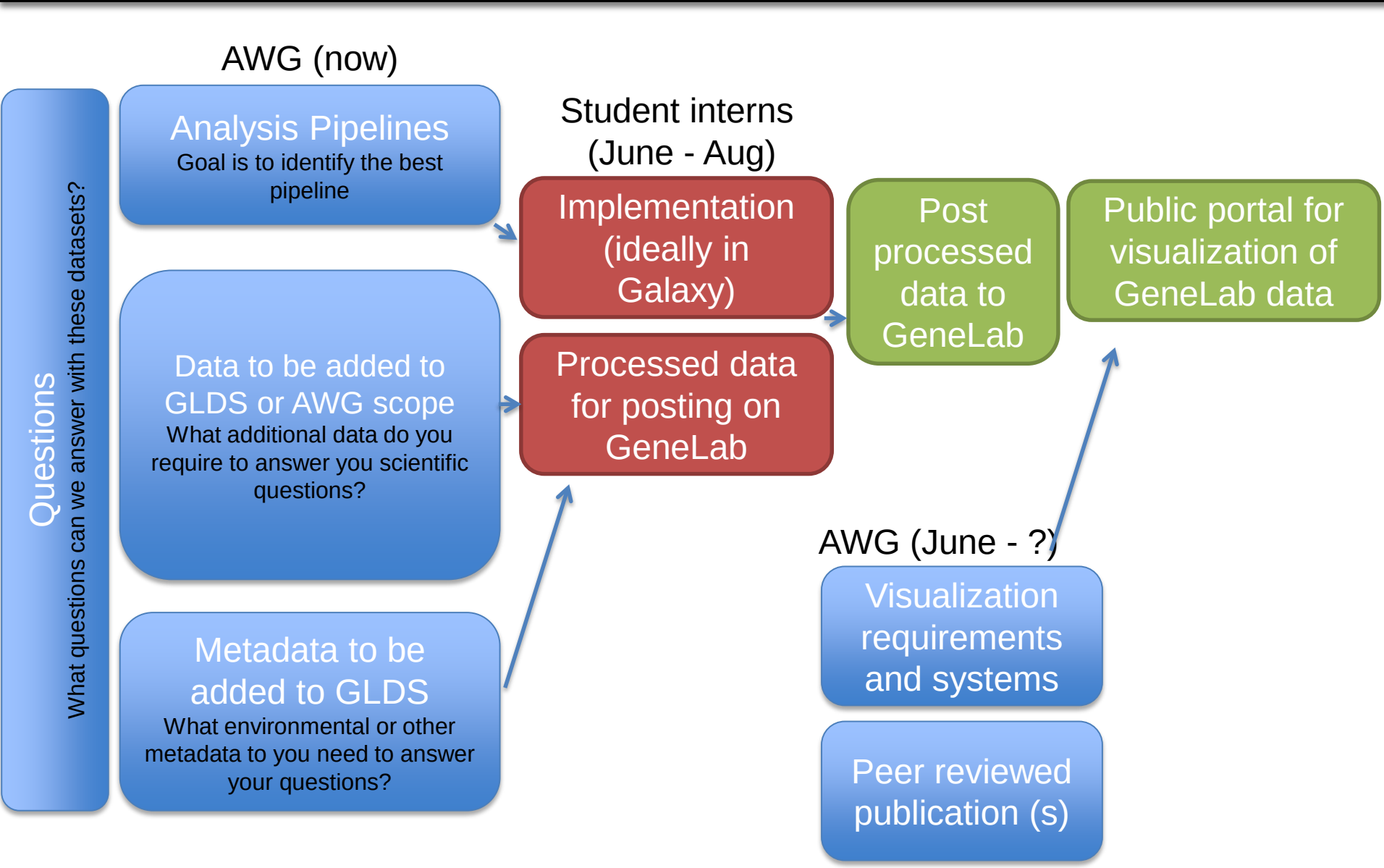
GeneLab Analysis Working Groups



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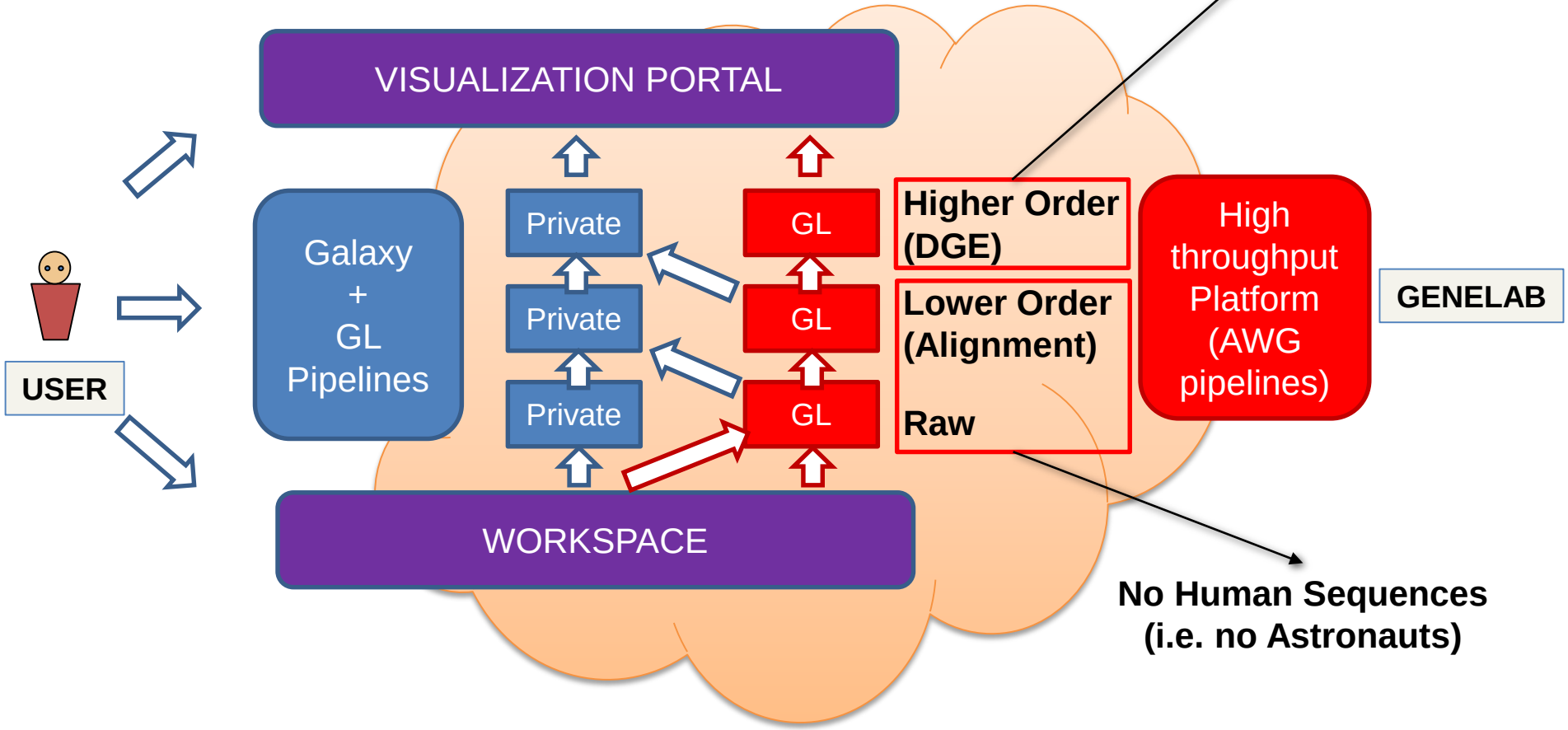
GLDS 4.0

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



No Human Sequences (i.e. no Astronauts)

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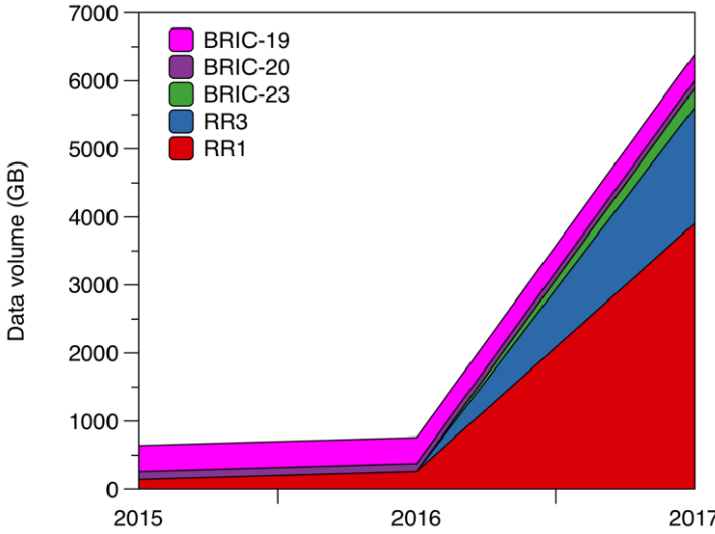
GeneLab Sample Processing Laboratory (SPL)

- Expertise:
  - DNA/RNA/protein extraction
  - Animal work

- Develop standards for sample processing (species dependent)
- Responsible for ~50% of GeneLab data by volume



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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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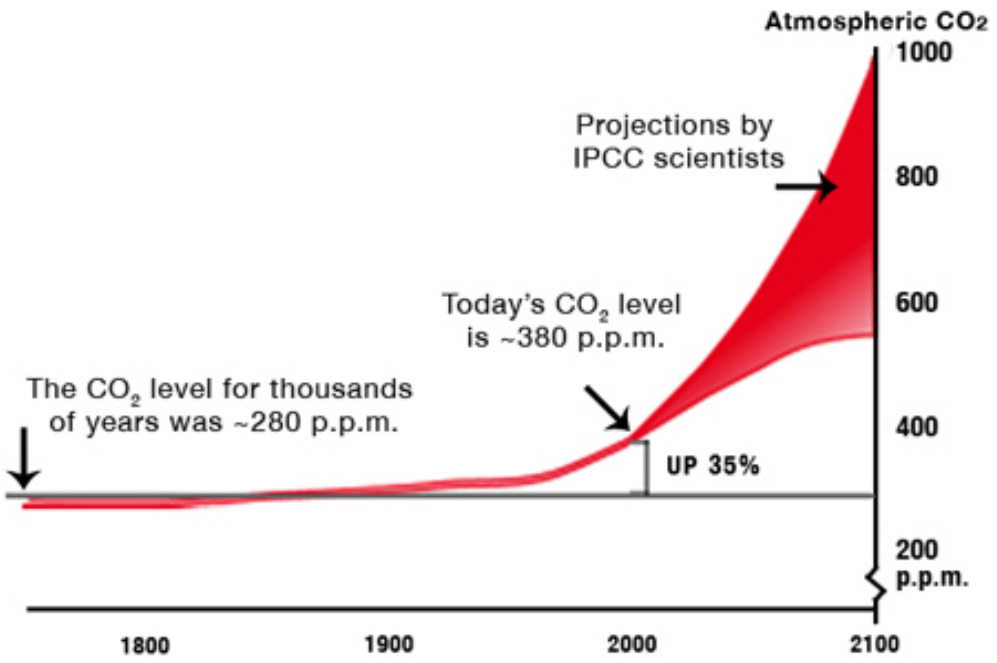
Carbon Dioxide as an Environmental Stressor in Spaceflight

A) Cage Types

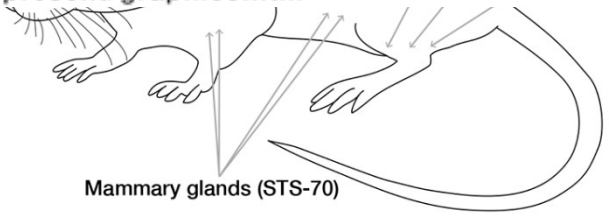
Animal Enclosure Module (AEM)



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

B)

GeneLab Study	Mission	Species	CO_2 (ppm)	Du (d	
GLDS-21	STS-108	mouse	~3000	1	
GLDS-111	BF	mouse	~600		
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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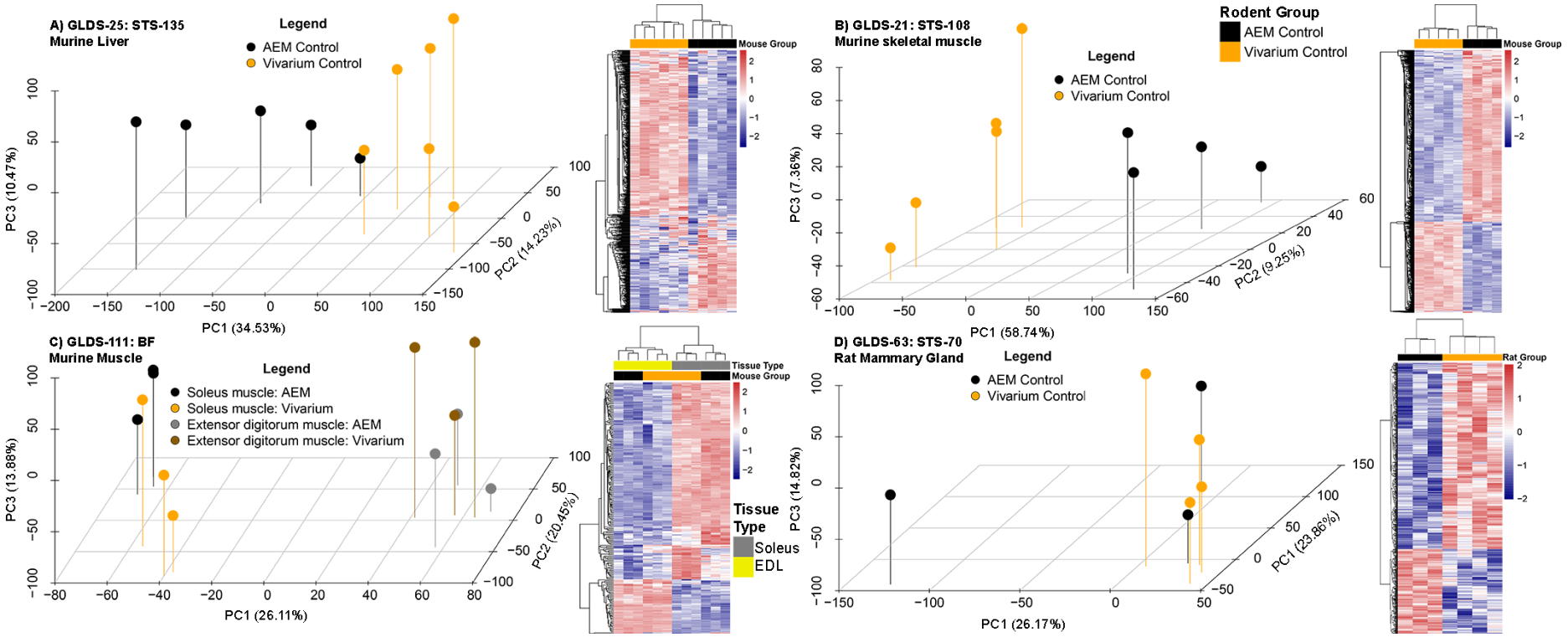
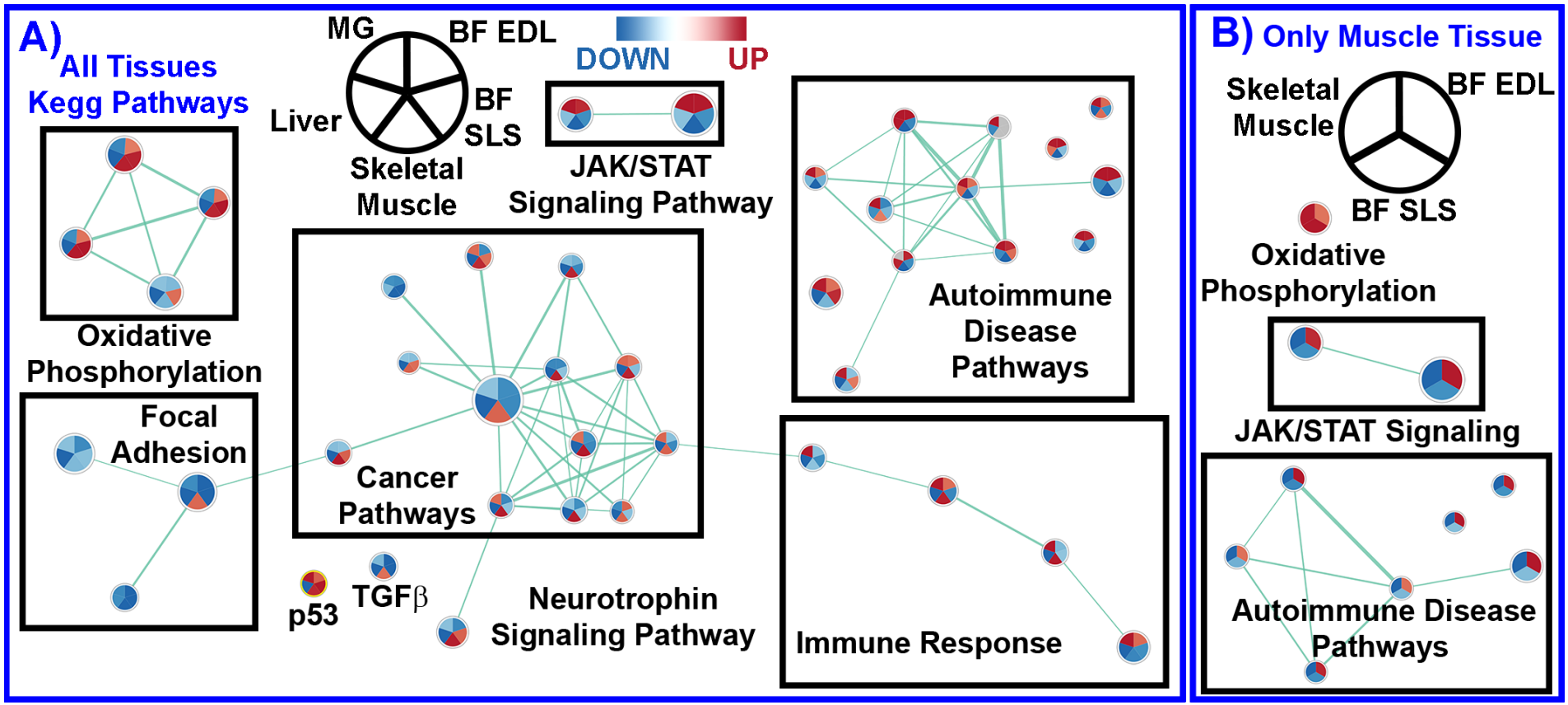
Global Cage Differences



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

Beheshti, et al., Scientific Reports, 2018

Major Pathways Regulated between Cages



GSEA: Kegg Pathways (network displayed using EnrichmentMap plugin for Cytoscape)

Beheshti, et al., Scientific Reports, 2018

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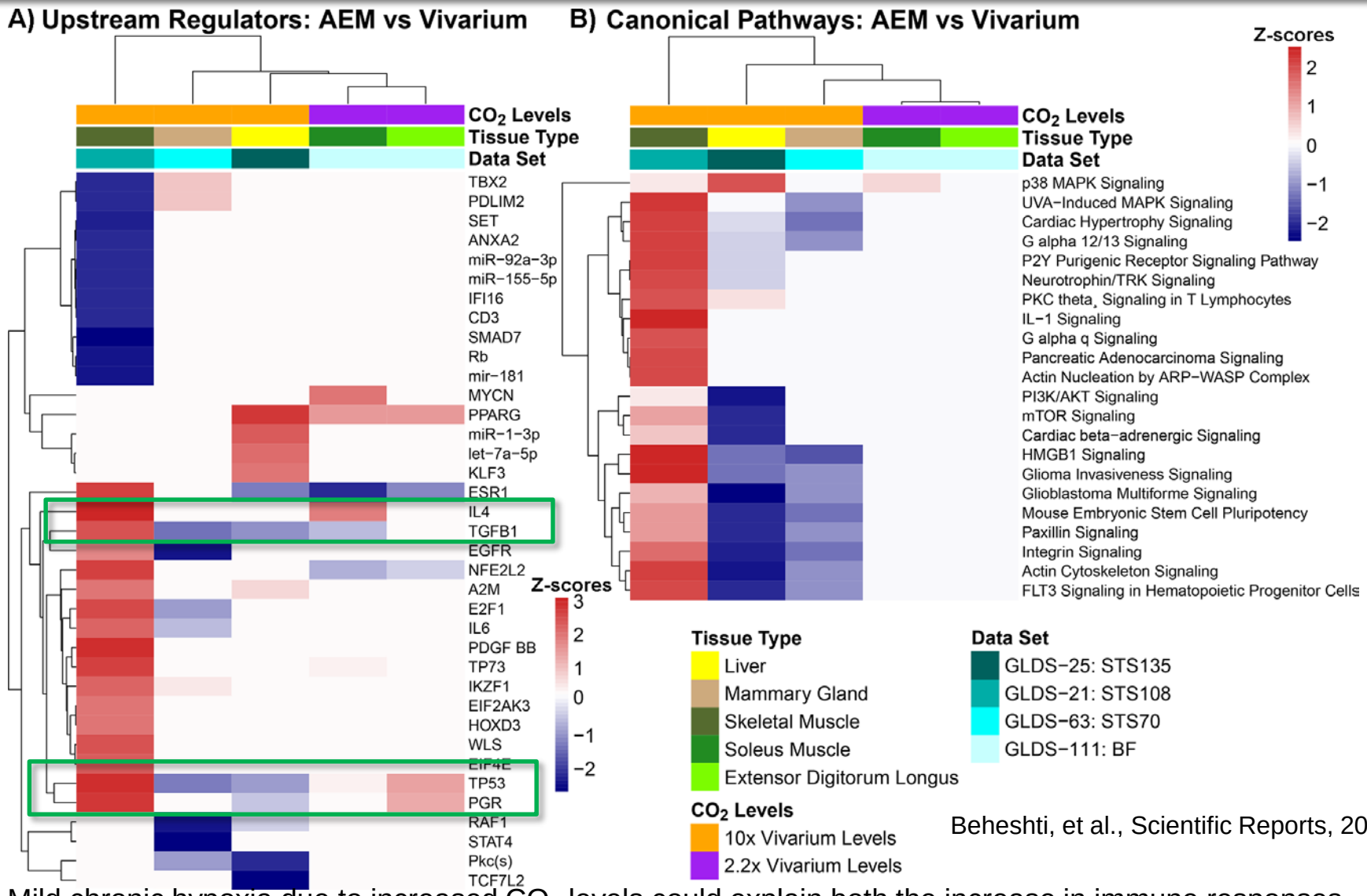
Upstream regulators and canonical pathways show response is tissue specific and highest for high CO_2



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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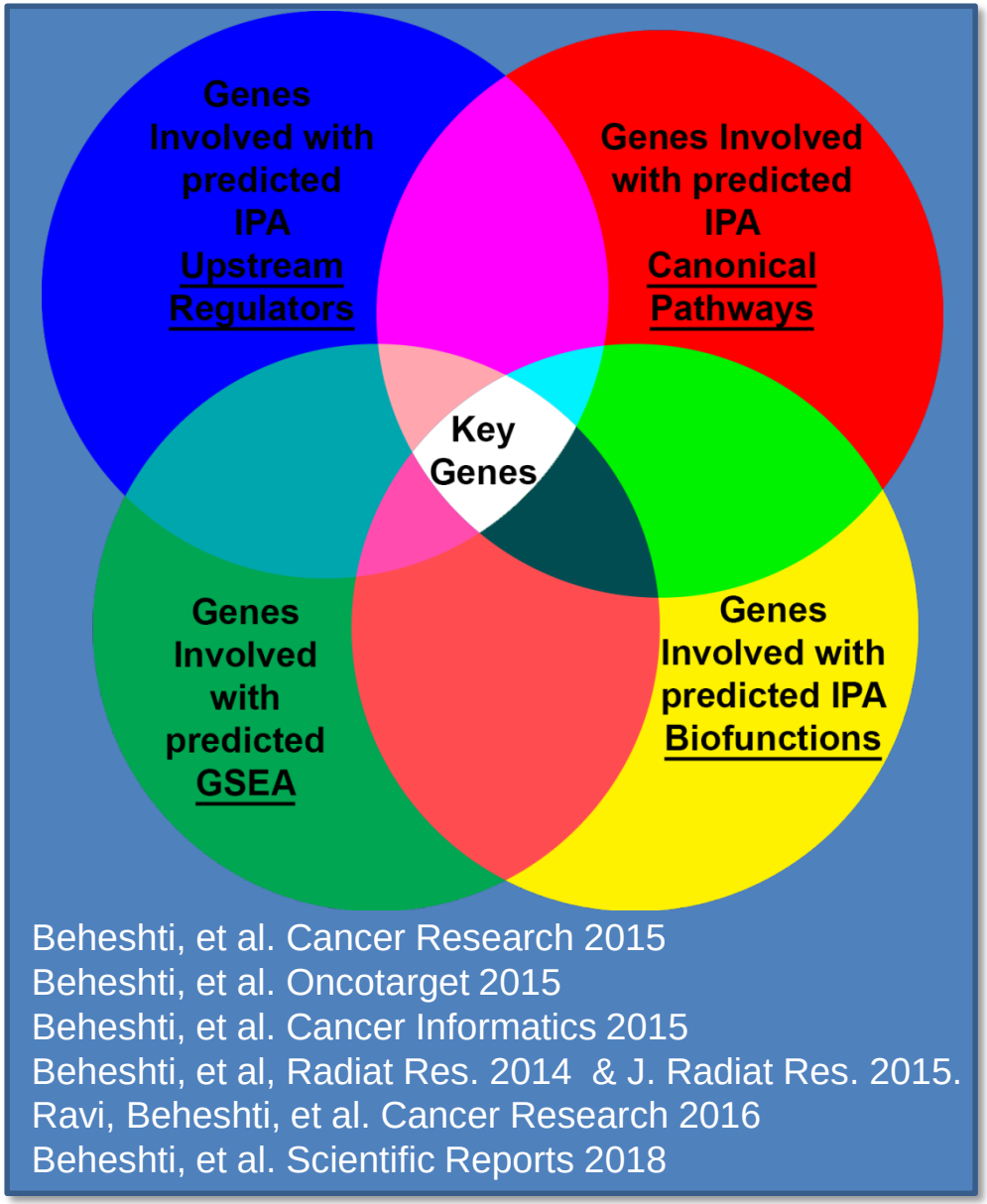
Determination of Key Driving Genes



Beheshti, et al. Cancer Research 2015
Beheshti, et al. Oncotarget 2015
Beheshti, et al. Cancer Informatics 2015
Beheshti, et al, Radiat Res. 2014 & J. Radiat Res. 2015.
Ravi, Beheshti, et al. Cancer Research 2016
Beheshti, et al. Scientific Reports 2018

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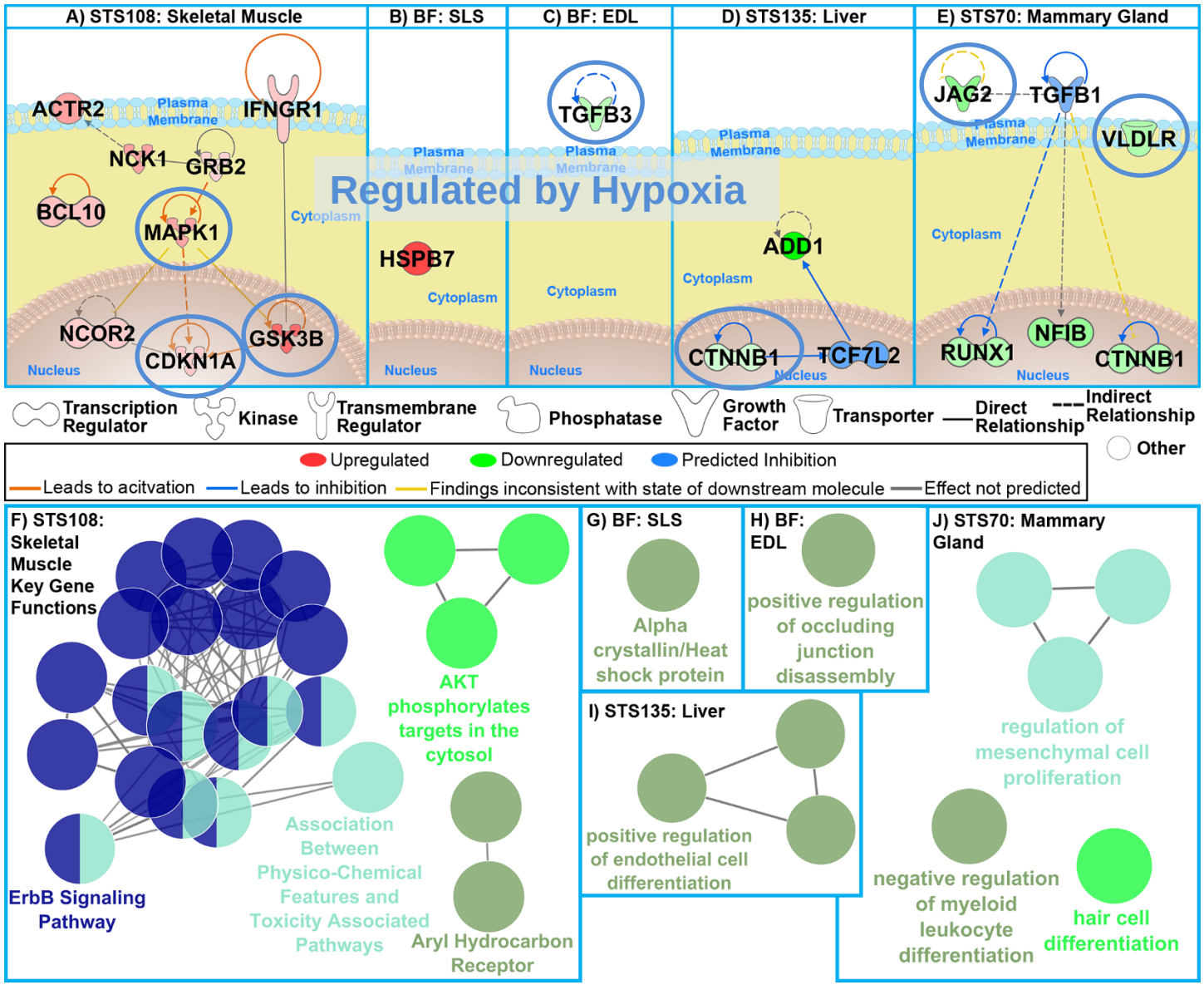
Identifying Key Cage-Dependent Drivers



Beheshti, et al., Scientific Reports, 2018

Systems Biology analysis reveals biological spaceflight master regulators

Beheshti, et al., PLOS One, in press

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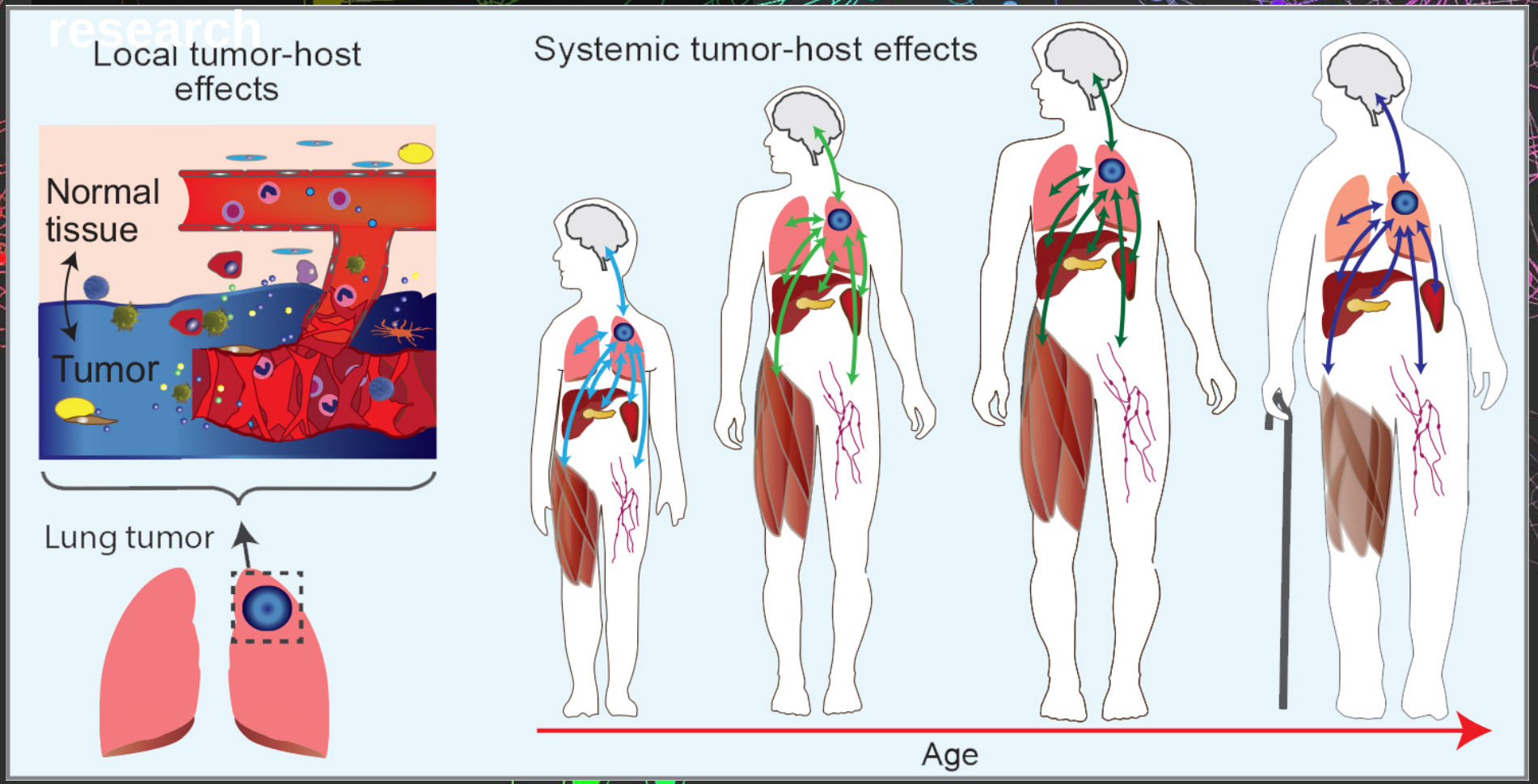
Systems Biology analysis reveals biological spaceflight master regulators

General Approach to Studying a Systematic Response in the Host

An example for cancer research



Beheshti, et al. Oncotarget, 2015.

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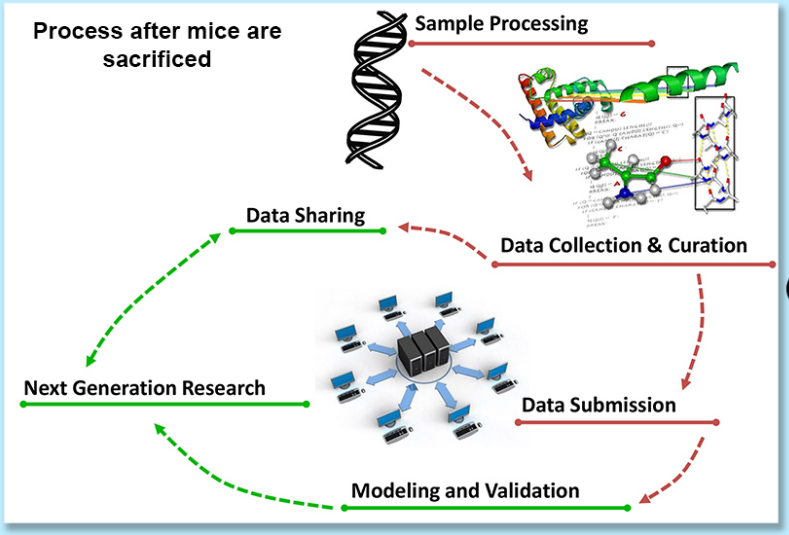
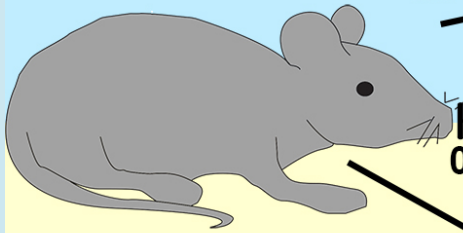
GeneLab Data Used to Generate Results



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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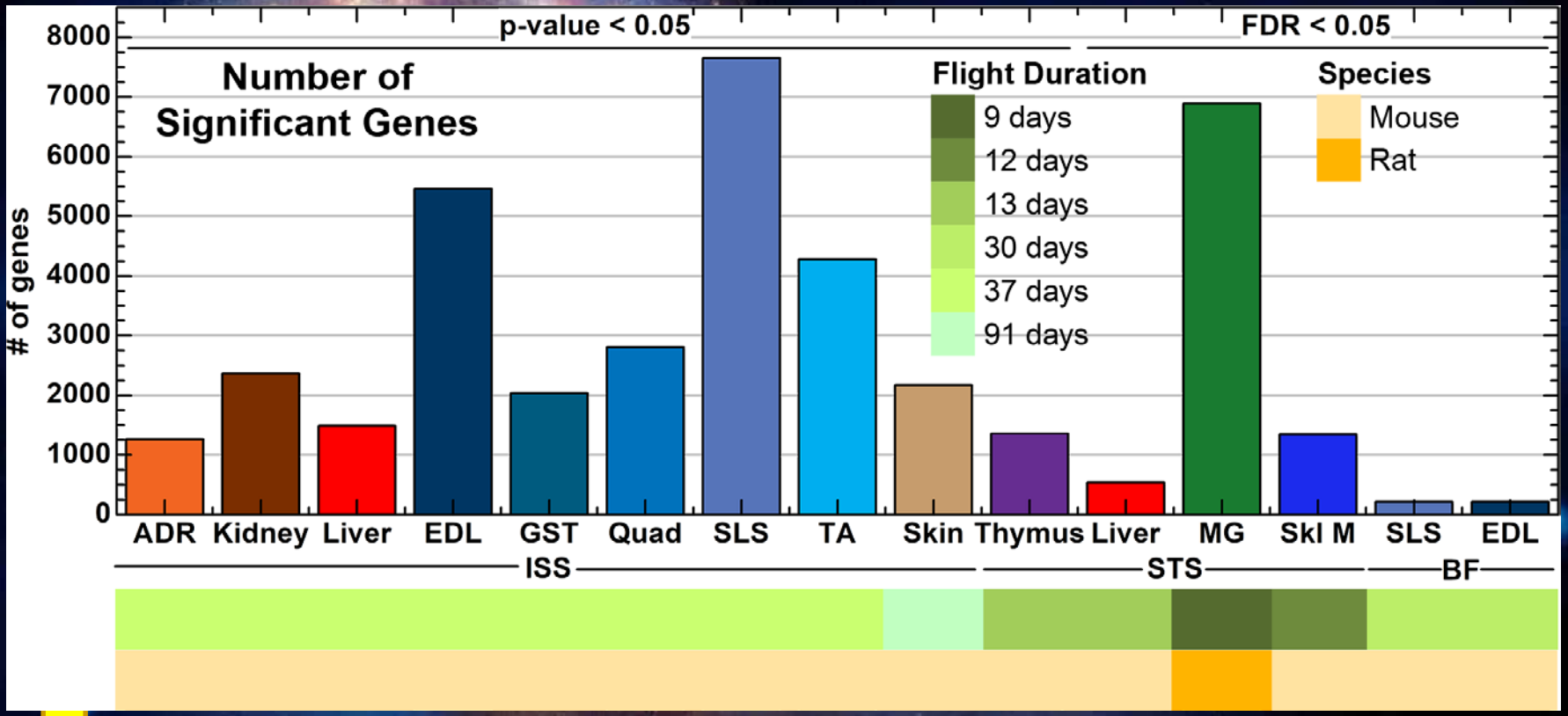
Number of Significant Genes from Each Dataset



Fold-Change ≥ |1.2|

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

Beheshti, et al., PLOS One, in press

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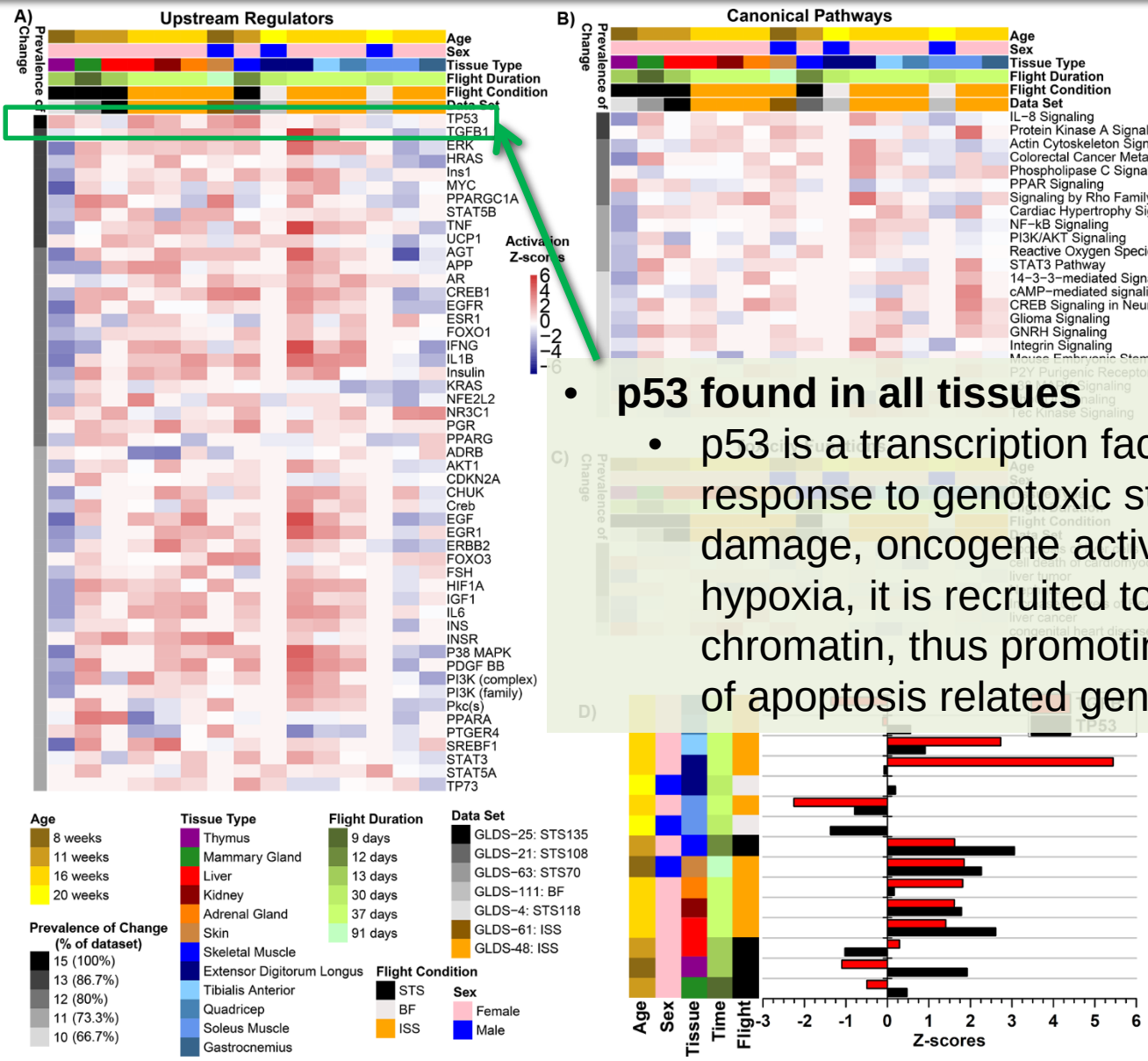
Predicted Master Regulators



- **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, in press

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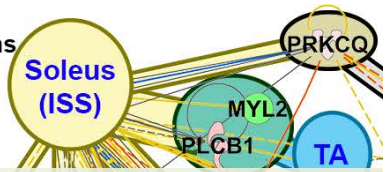
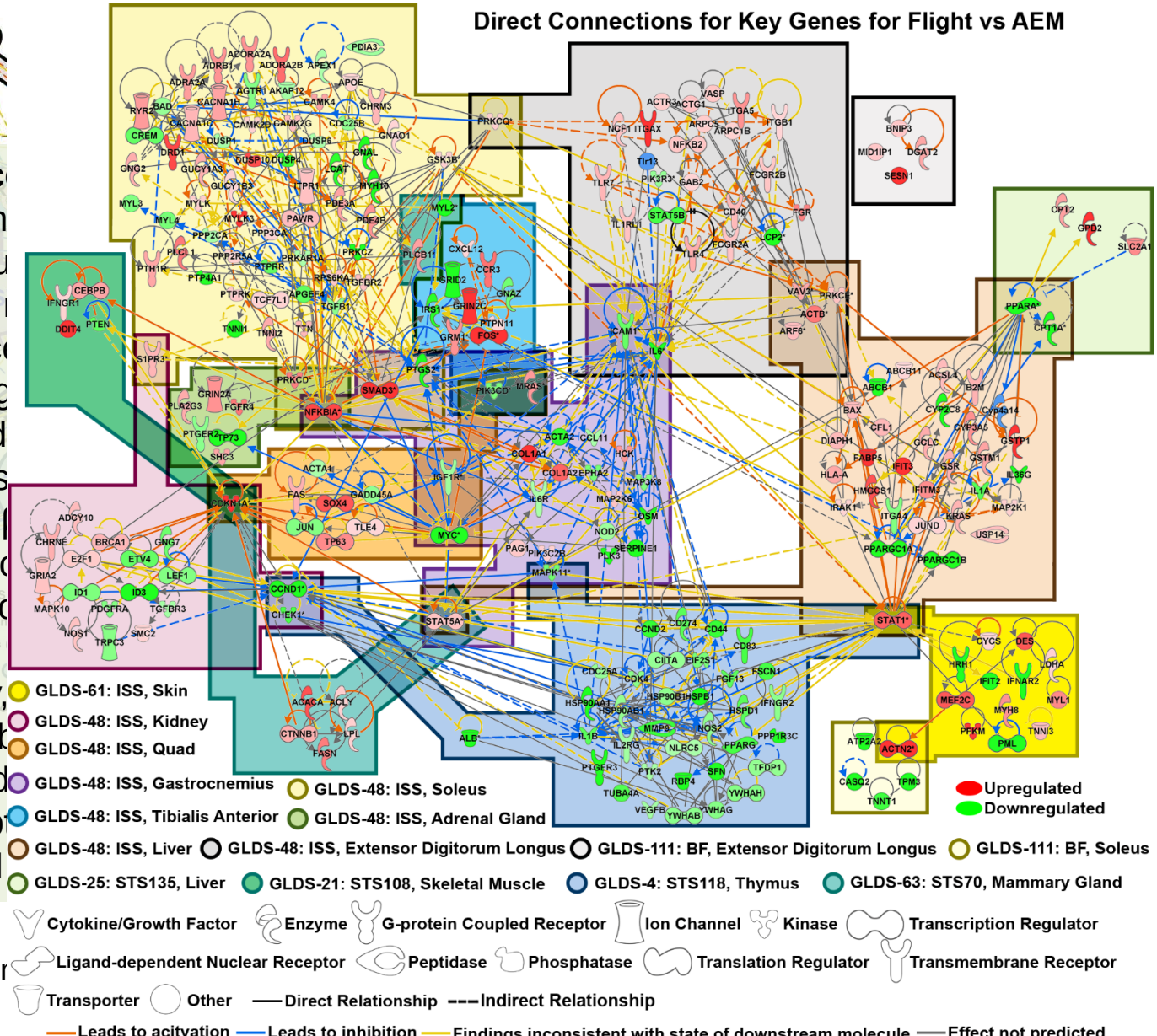
Key Genes and the Connections

A) Direct Connections for Key Genes for Flight vs AEM



- **TGFβ1 found to be c**
  - TGFβ is known sustaining tissu transcriptional differentiation, c along with reg homeostasis and
  - Previous Studies to diminish TGF-β carcinoembryonic colorectal carcino in unit gravity.
  - In another study, growth using b identified in mod and late apopt endothelin-1 and

Beheshti, et al., PLOS On



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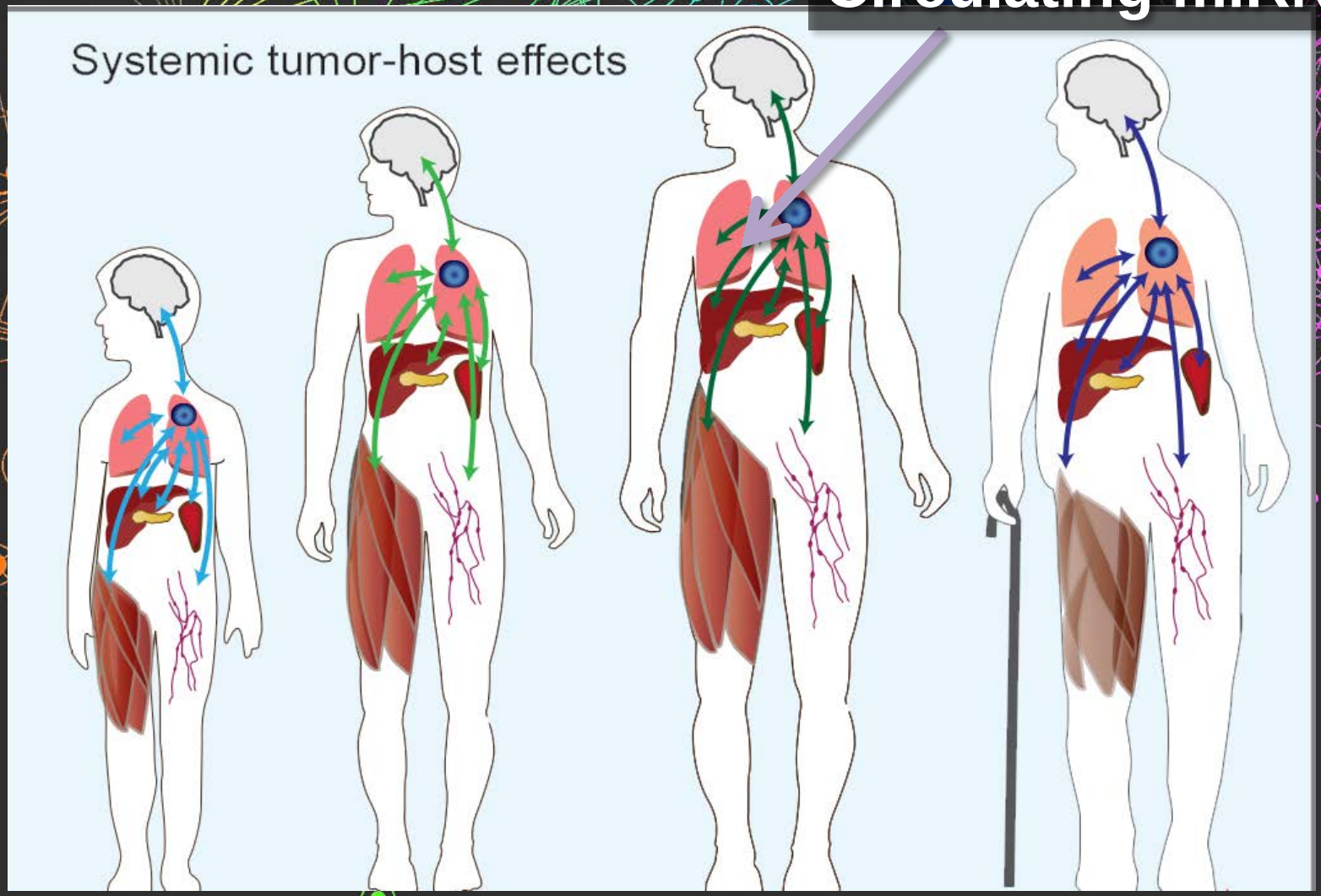
General Approach to Studying a Systematic Response in the Host

Circulating miRNAs



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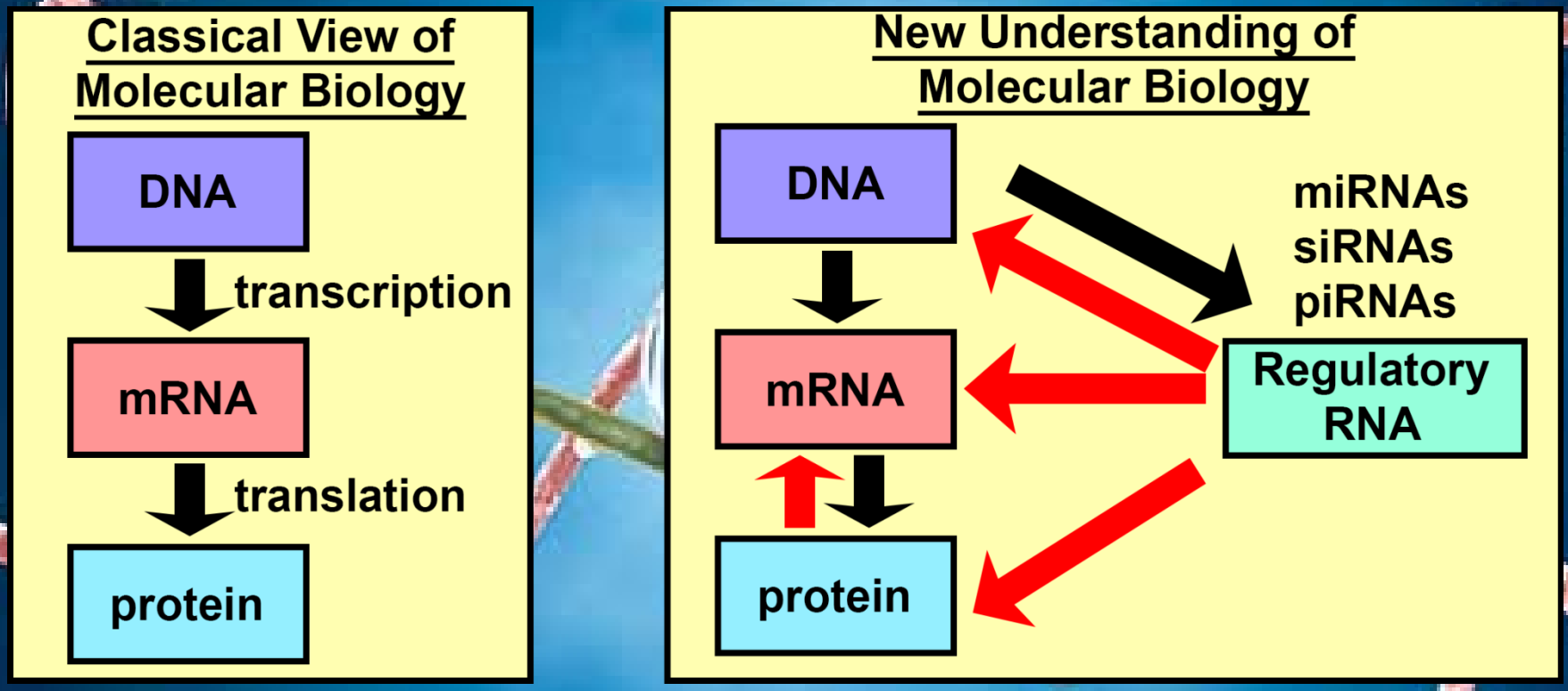
Revised View of Molecular Biology



- 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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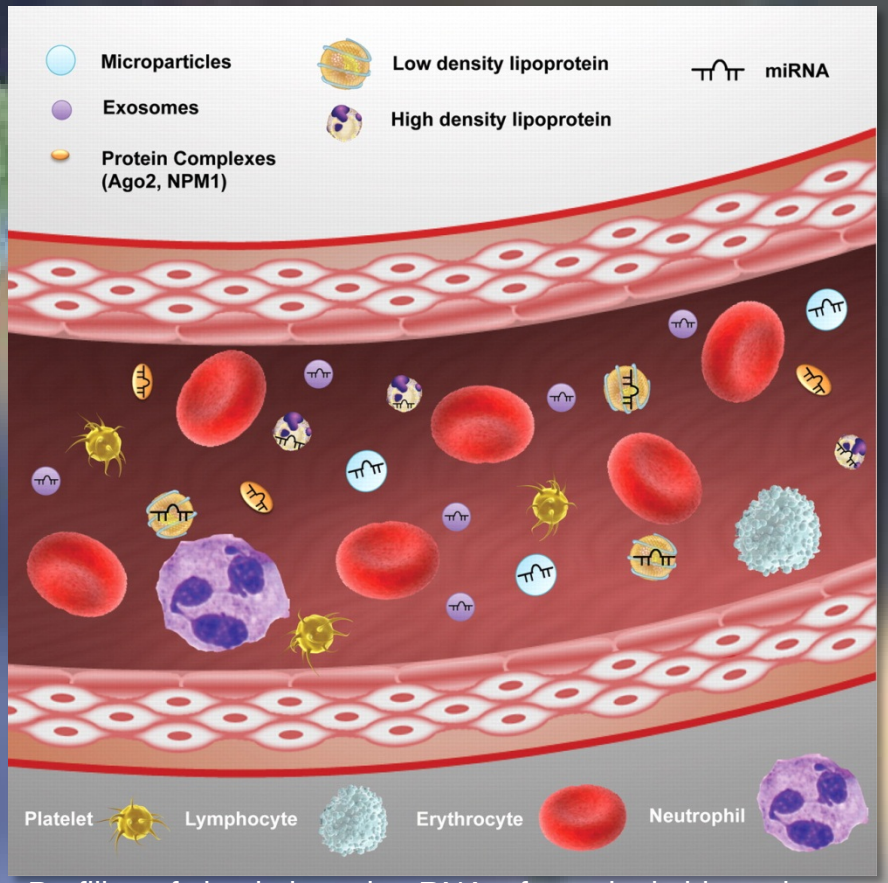
Impact of Circulating microRNAs

- 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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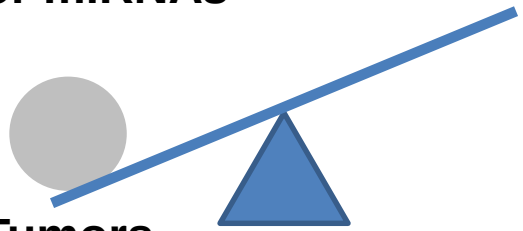
Systems Biology View of miRNAs

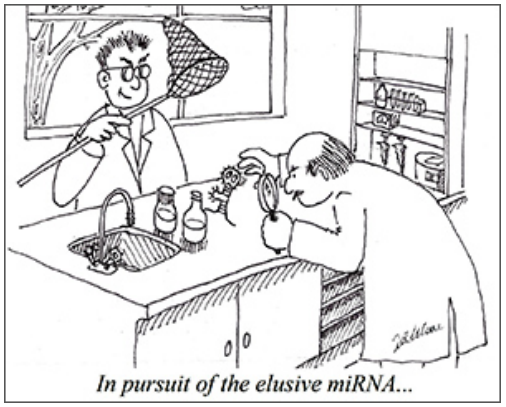
Tumor Suppressor miRNAs

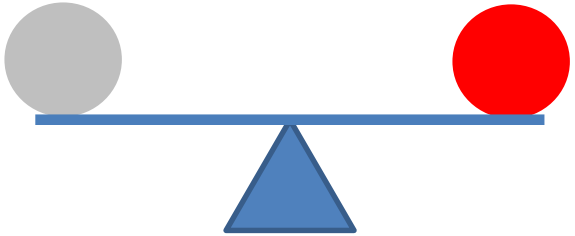
OncomiRNAs



Tumors Inhibited

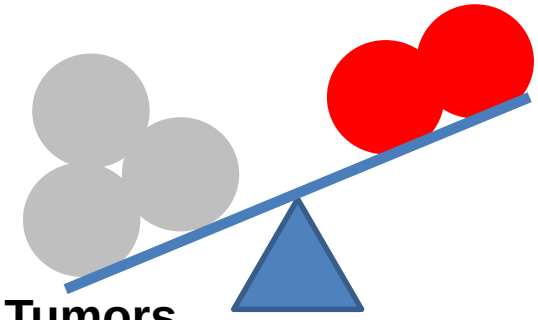
Only looking at a single miRNA



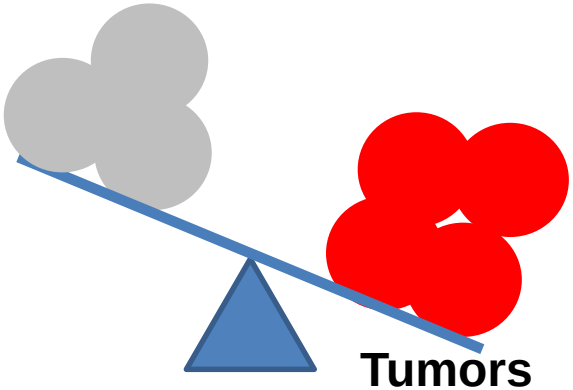


No Change in Tumors

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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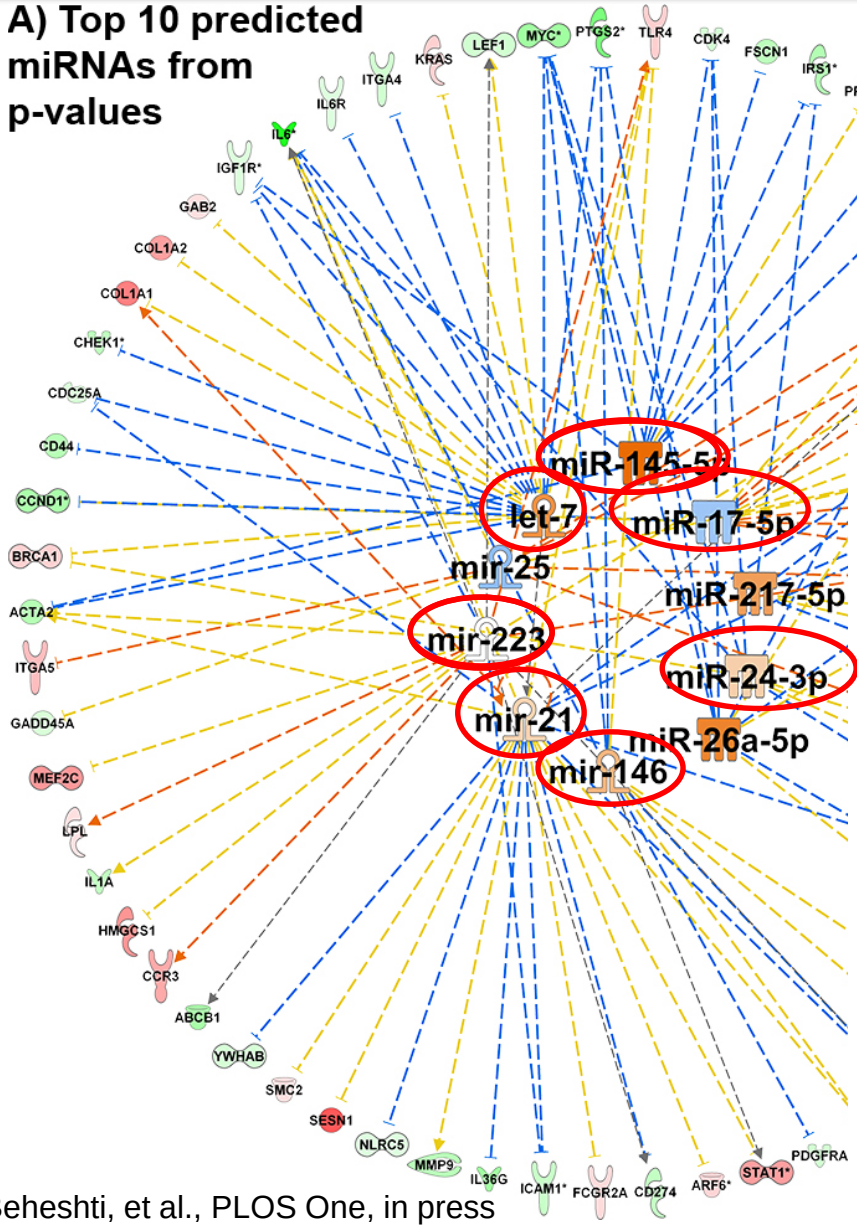
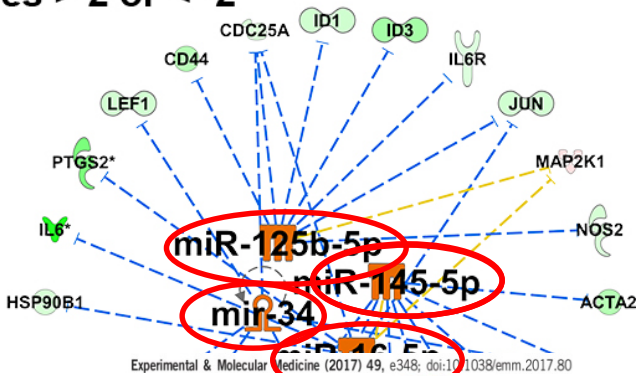
NASA

A) Top 10 predicted miRNAs from p-values



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



Experimental & Molecular Medicine (2017) 49, e348; doi:10.1038/emm.2017.80

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Volume 2014, Article ID 296747, 16 pages
http://dx.doi.org/10.1155/2014/296747

Hindawi

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, in press

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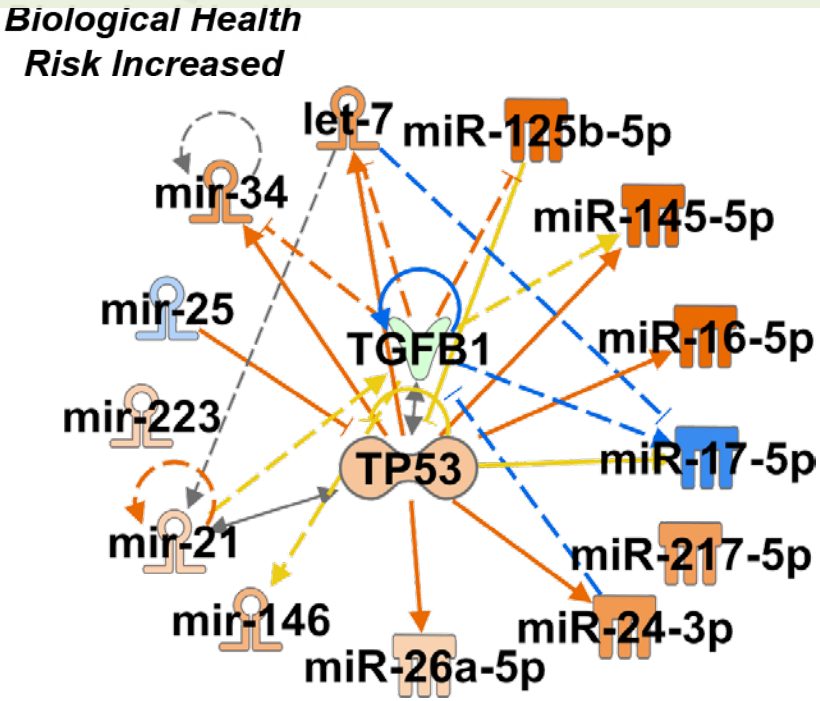
Predicted miRNAs Involved with Microgravity Effects

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, in press

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

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AWG Members Involved

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Perelman School of Medicine
University of Pennsylvania

Helio Costa

Kathryn Grabek

STANFORD UNIVERSITY

J. Tyson McDonald

HAMPTON UNIVERSITY
THE STANDARD OF EXCELLENCE

Gary Hardiman

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MUSC Health
MEDICAL UNIVERSITY of SOUTH CAROLINA

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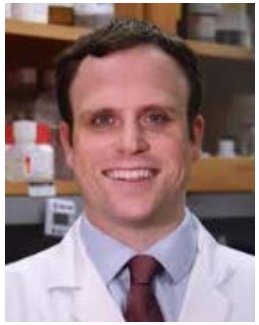
AWG Members Involved


Chris Mason


Cem Meydan


Jonathan Foox

Flavia Rius

Cornell University


Yared Kidane


Susana Zanello


Scott Smith


Sara Zwart

SPACE CENTER HOUSTON
Manned Space Flight Education Foundation


Afshin Beheshti


Sylvain Costes

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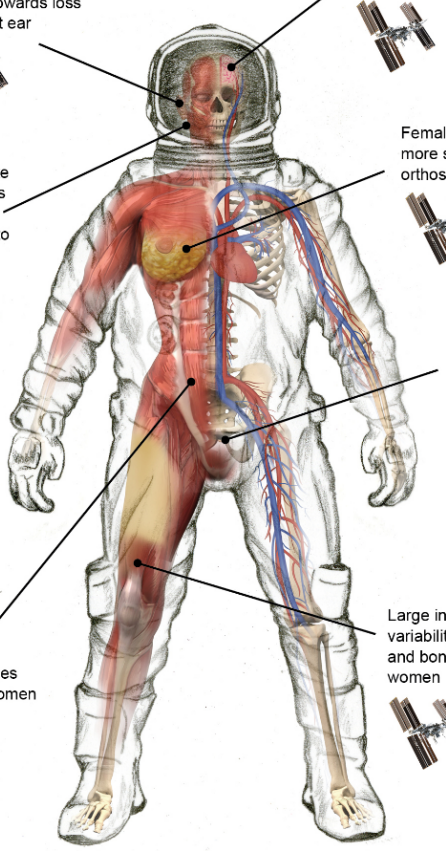
Astronauts health problems

FEMALE ASTRONAUT



Women suffer less from hearing loss with advancing age, and do not display a bias towards loss of hearing in the left ear

Female astronauts, (to date) do not exhibit clinically significant visual impairment

Women demonstrate a slight bias towards accuracy versus speed in response to an alertness test

Female astronauts are more susceptible to orthostatic intolerance

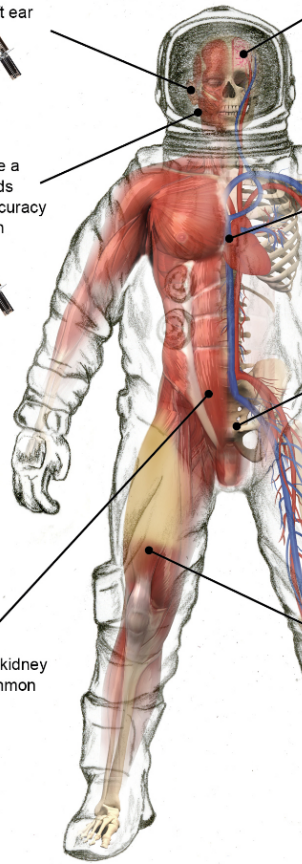
Urinary tract infections are more common in female astronauts

Women mount more potent immune responses

Large individual variability to muscle and bone loss in women

Struvite kidney stones more common in women

MALE ASTRONAUT



Men suffer more from hearing loss with advancing age, and display a bias towards loss of hearing in the left ear

Some male astronauts exhibit clinically significant visual impairment

Men demonstrate a slight bias towards speed versus accuracy in response to an alertness test

Male astronauts less susceptible to orthostatic intolerance

Urinary tract infections less common in male astronauts

Men mount less potent immune responses

Large individual variability to muscle and bone loss in men

Calcium oxalate kidney stones more common in men

Health effect observed on Earth

Health effect observed in space

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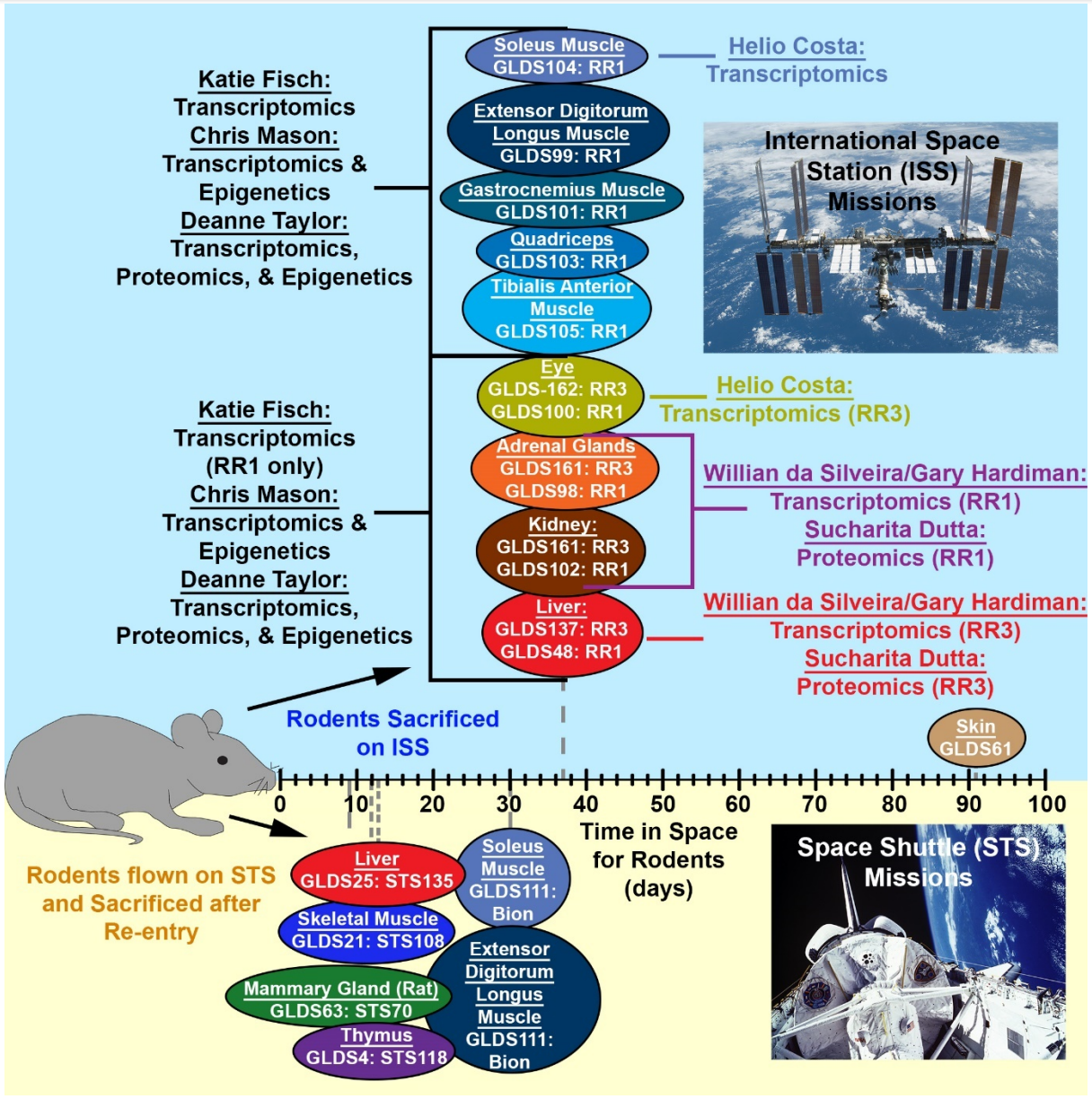
Specific Datasets and Tissues AWG Members Analyzed



- 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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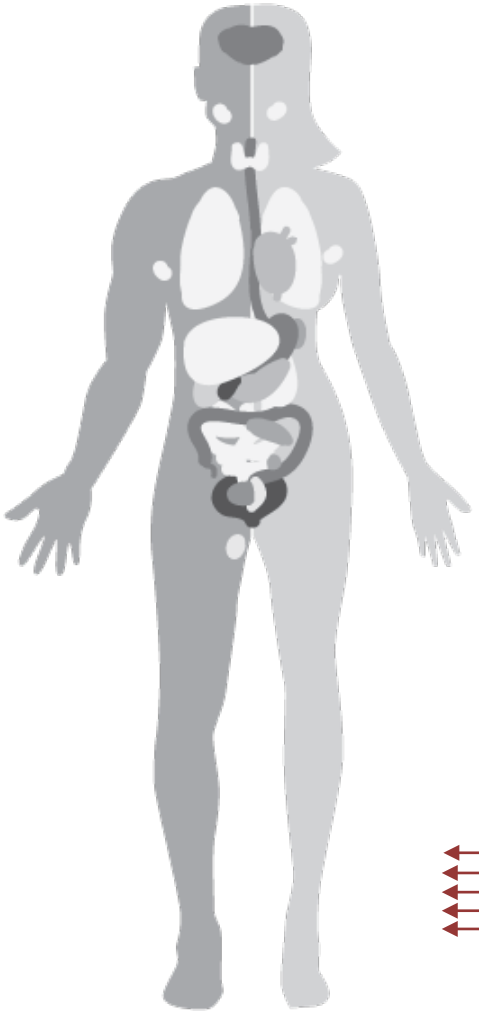
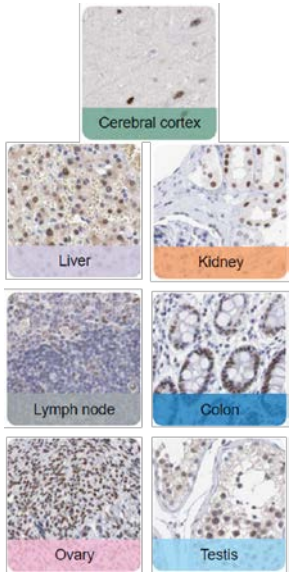
Structural Hierarchy of the Human Body

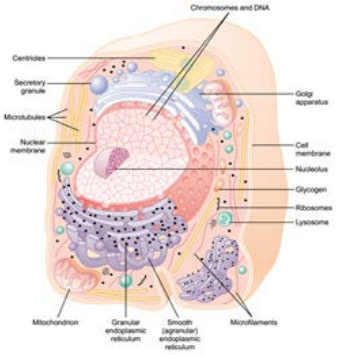
Level 7: The Body
Level 6: Organs



Level 5: Tissues



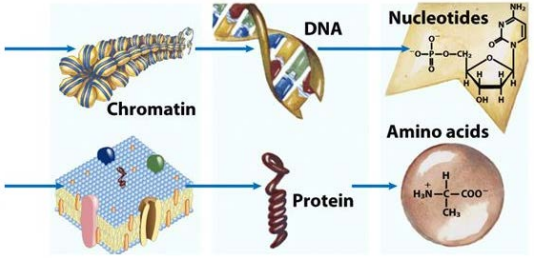
Level 4: The Cell and its Organelles



Level 3: Supra molecular Complexes

Level 2: Macro molecules

Level 1: Monomeric Units



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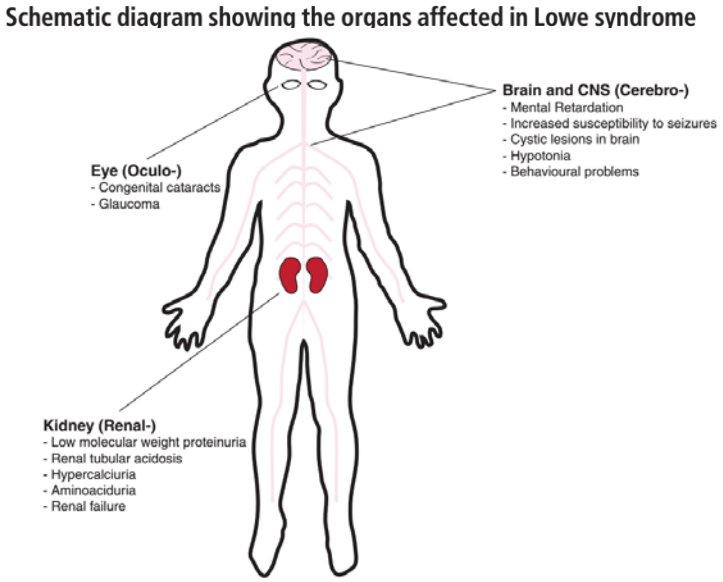
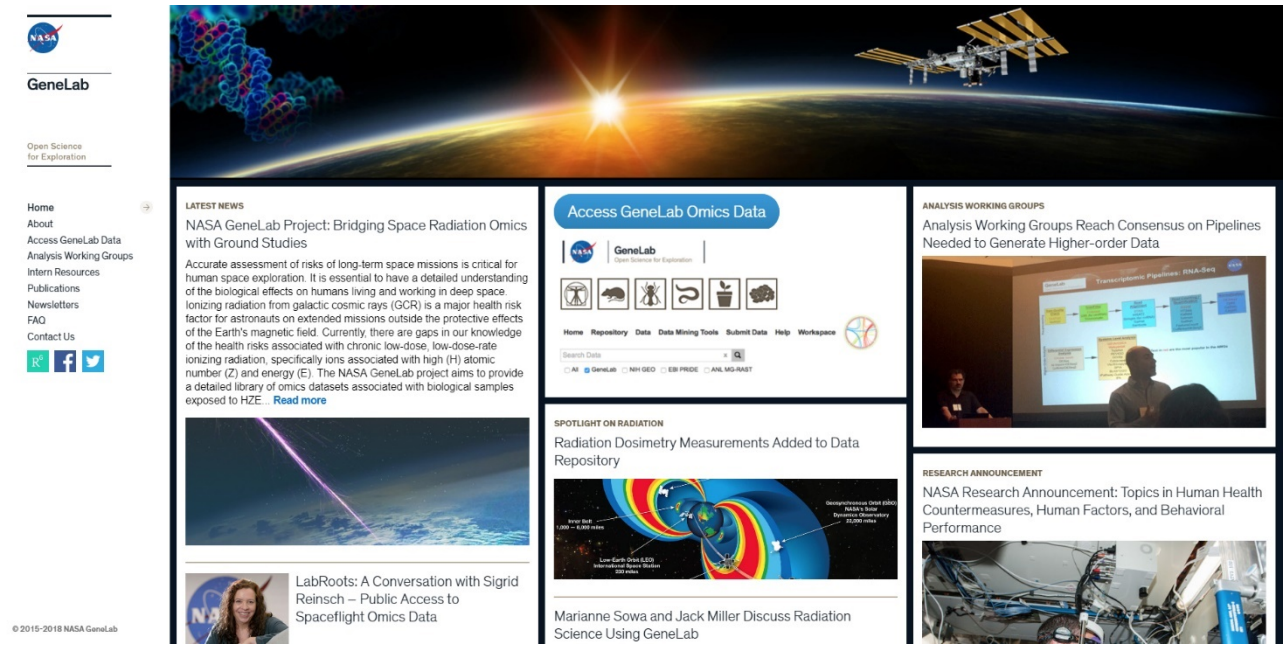
Hypothesis Developed and Being Worked On

- 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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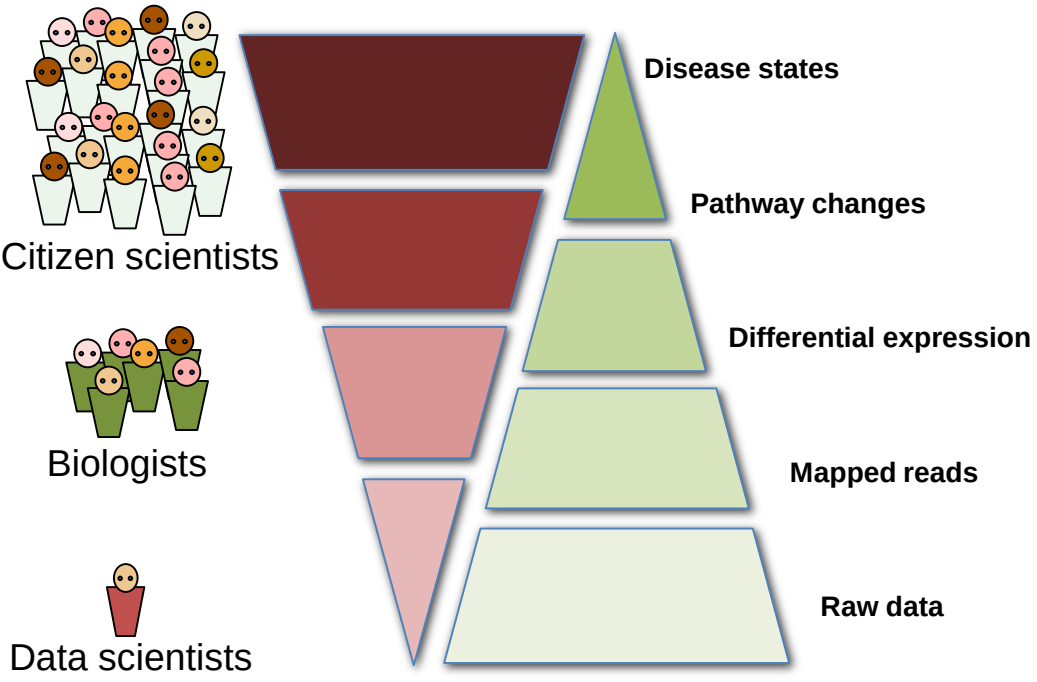

New Knowledge

Analysis Working Groups

Galaxy | GenomeSpace

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Open Data

EBI PRIDE

ANL MG-RAST | NCBI GEO

Missions/Experiments | GeneLab Sample Processing

GeneLab Acknowledgements

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Lynn Hutchison
Yared Kidane
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Tristan Le
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