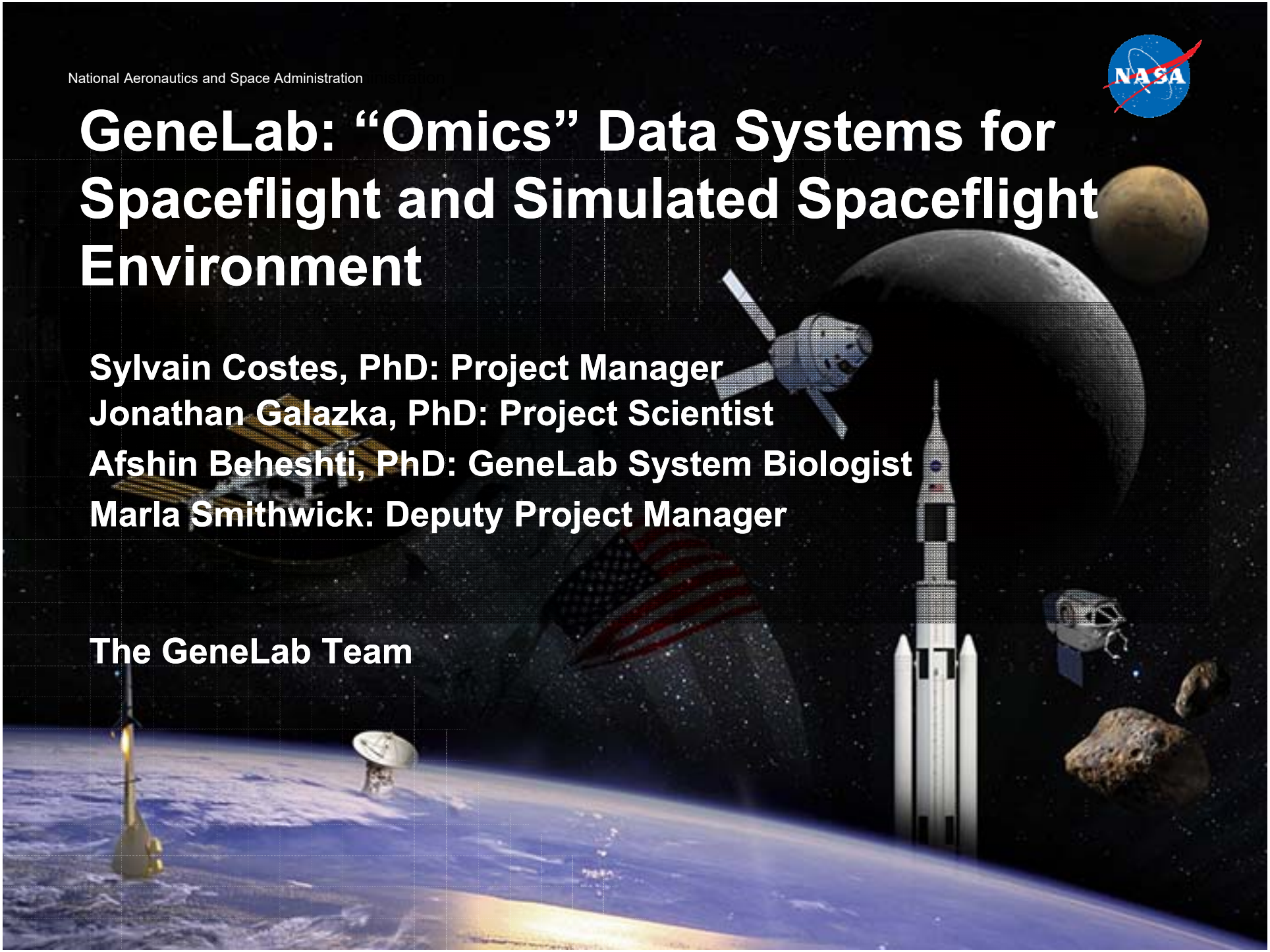
National Aeronautics and Space Administration



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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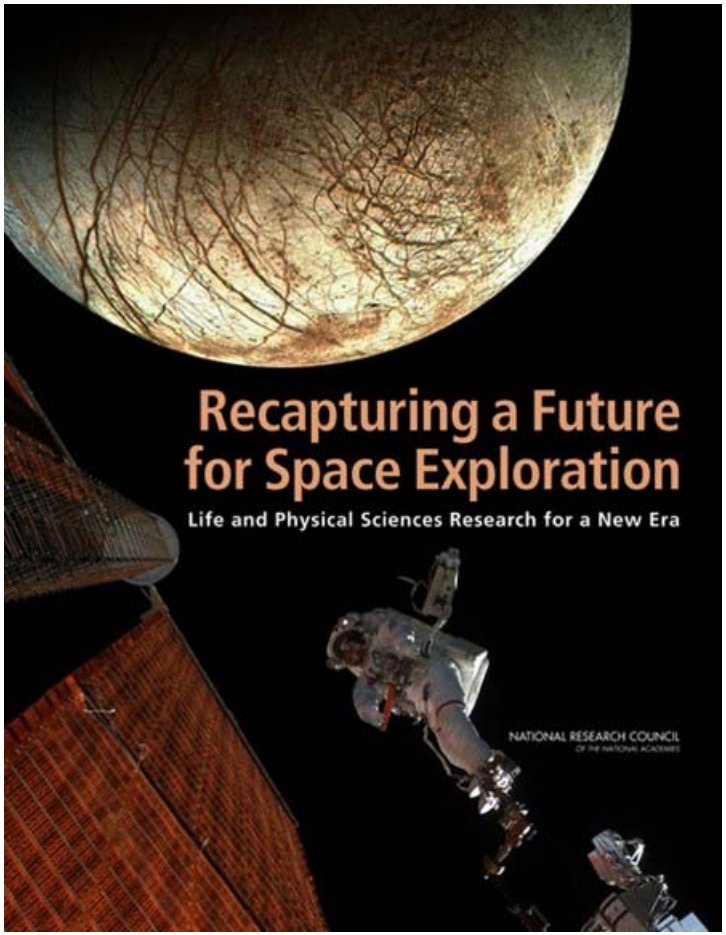
2011 NRC Decadal Survey



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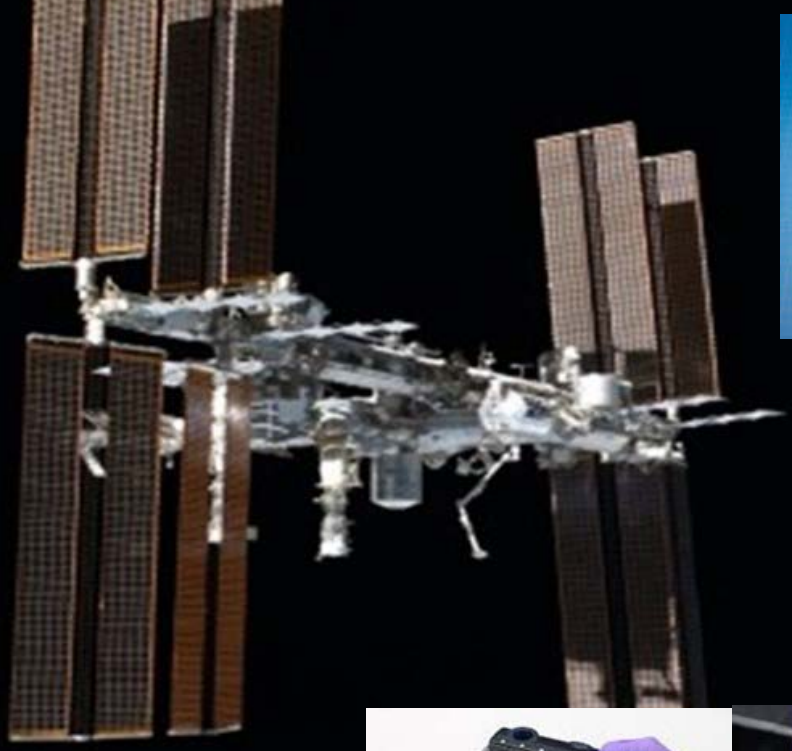
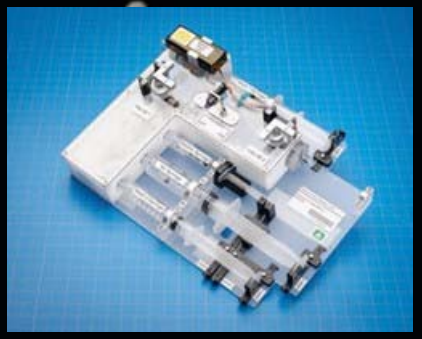
Omics Acquisition in Space is Now a Reality

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

Cepheid Smart Cycler qRT-PCR

Reaction tube
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yophilized
hemical assay
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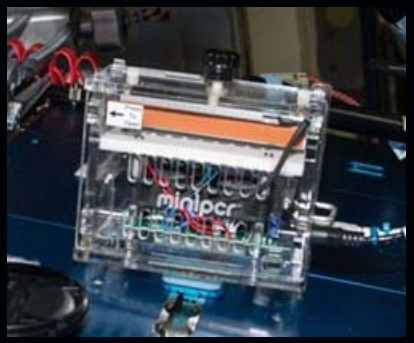
proprietary)



Mini-PCR

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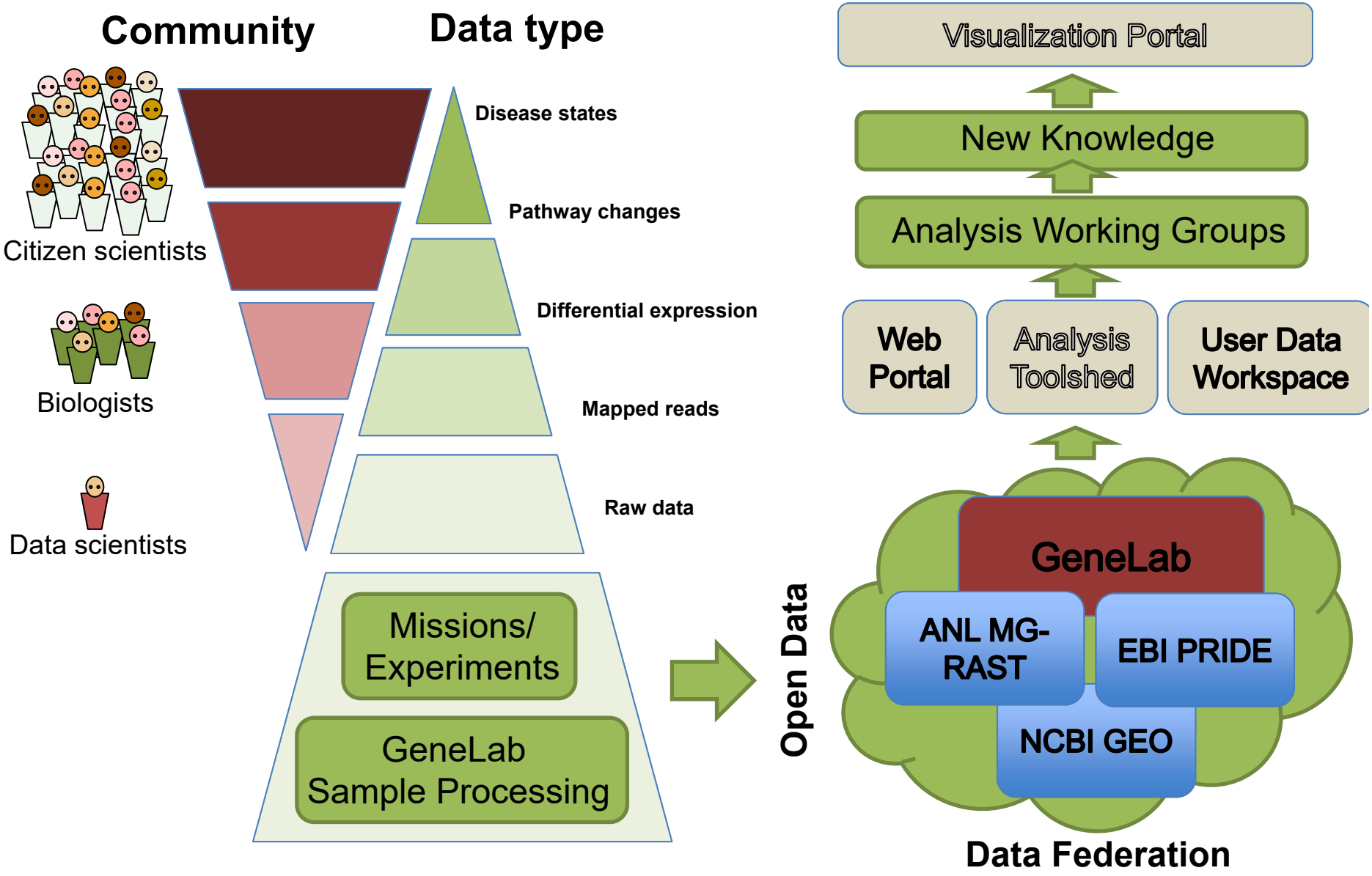
GeneLab Data Democratization



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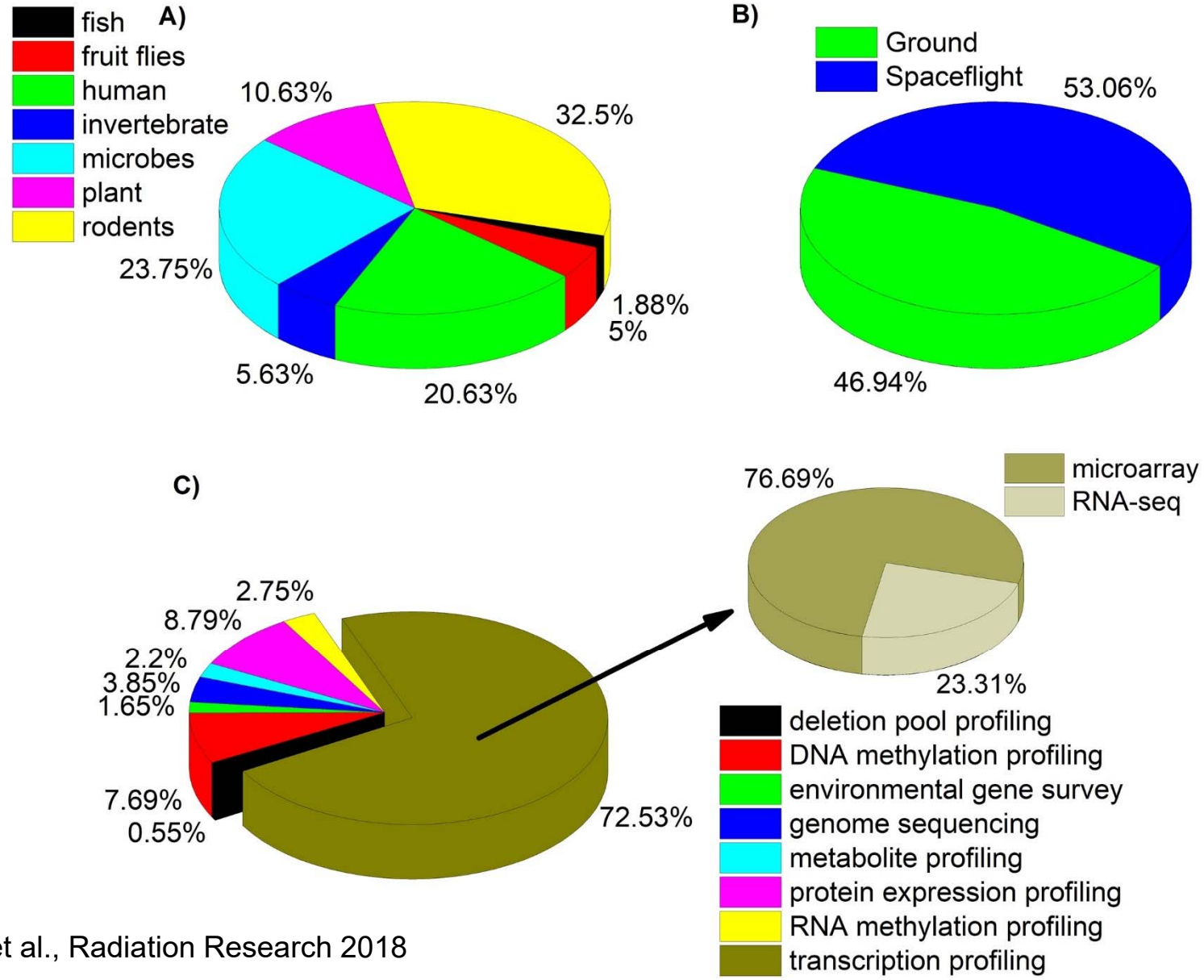
GeneLab Database: >190 data sets



Beheshti et al., Radiation Research 2018

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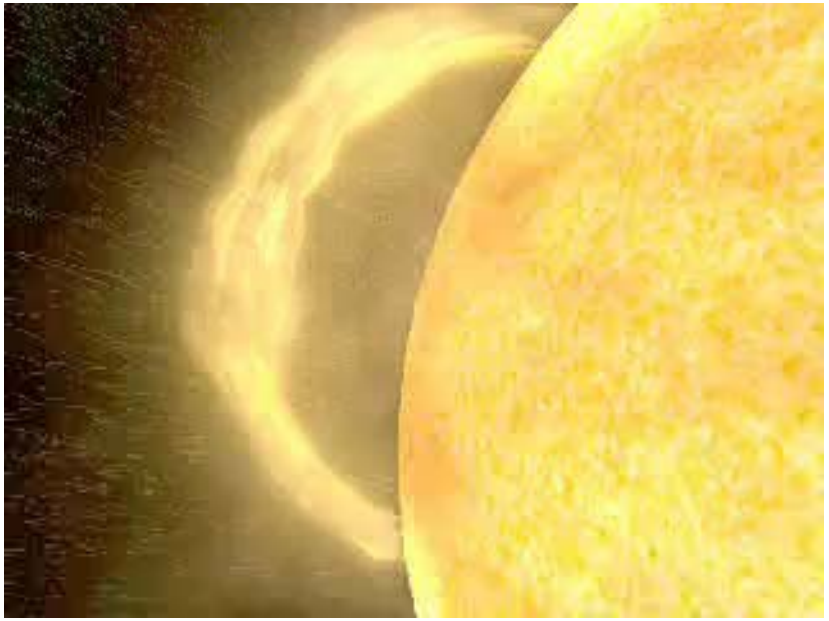
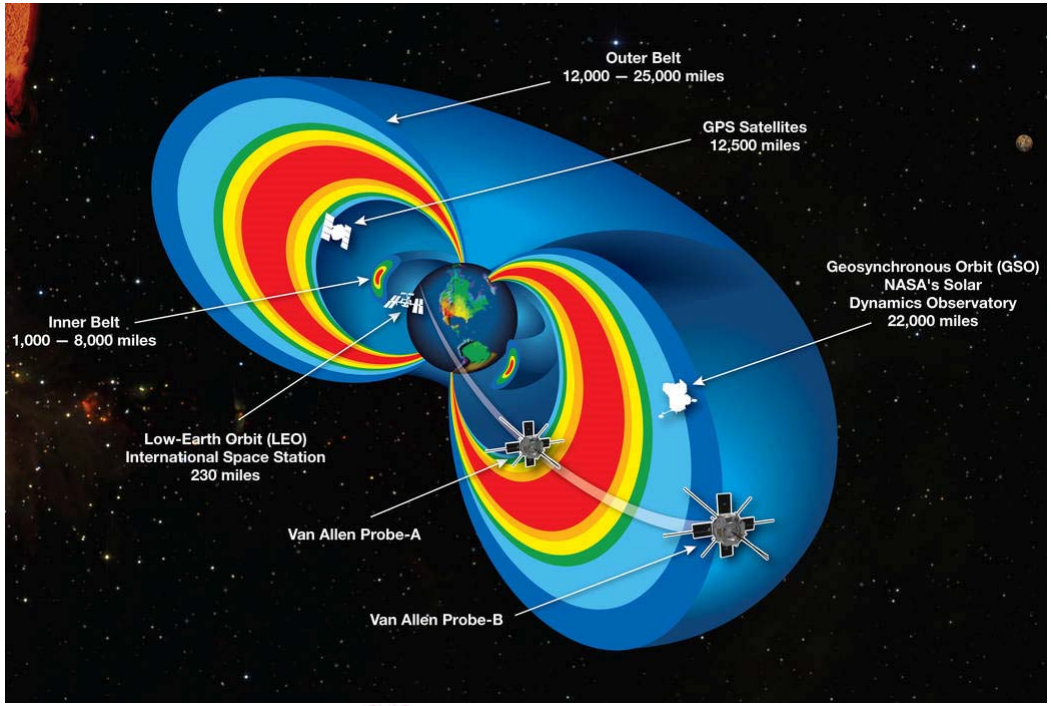
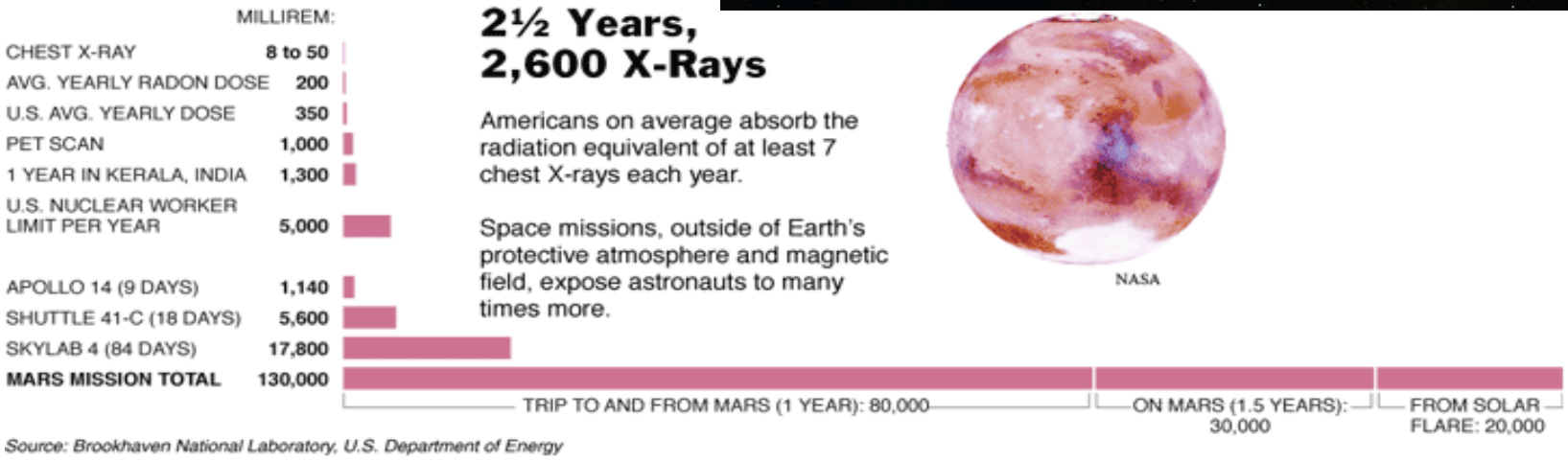
Earth's magnetic field protects us from cosmic radiation







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

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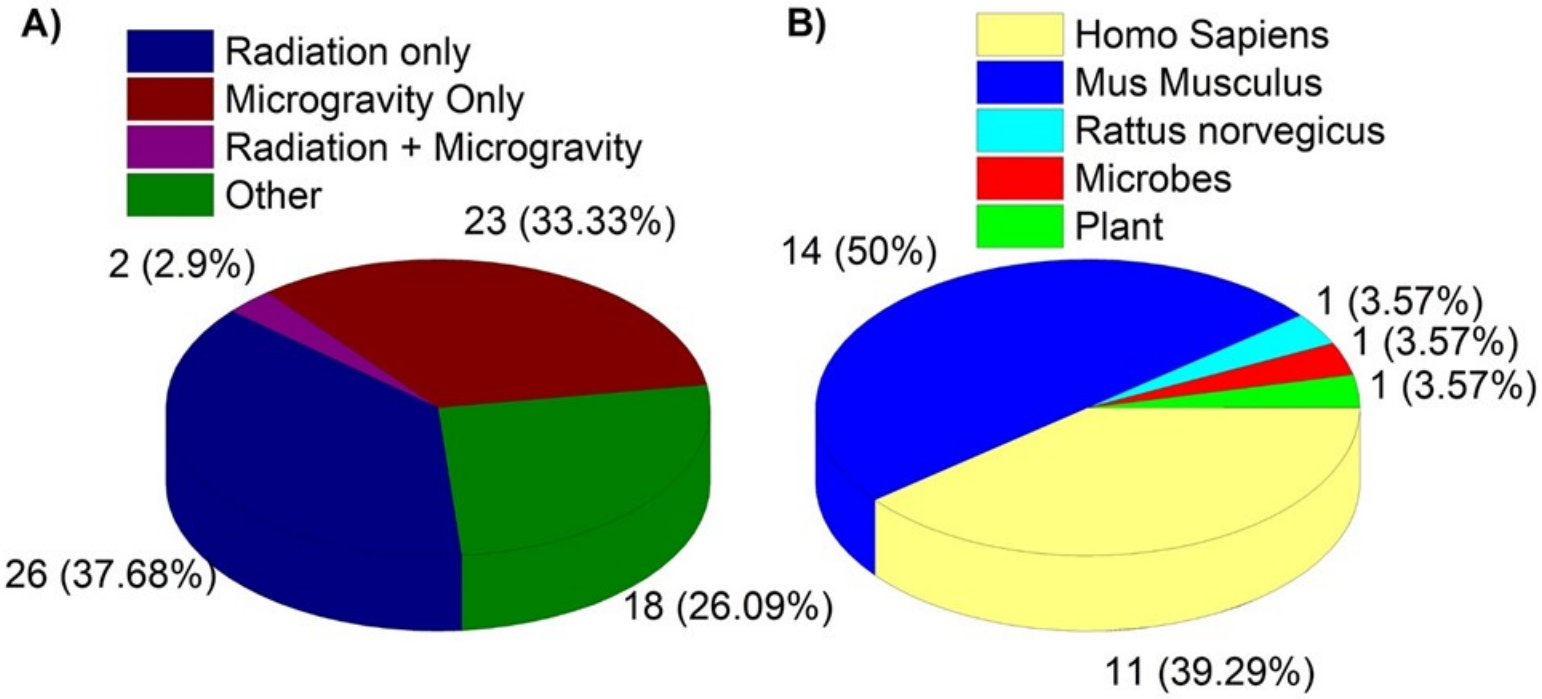
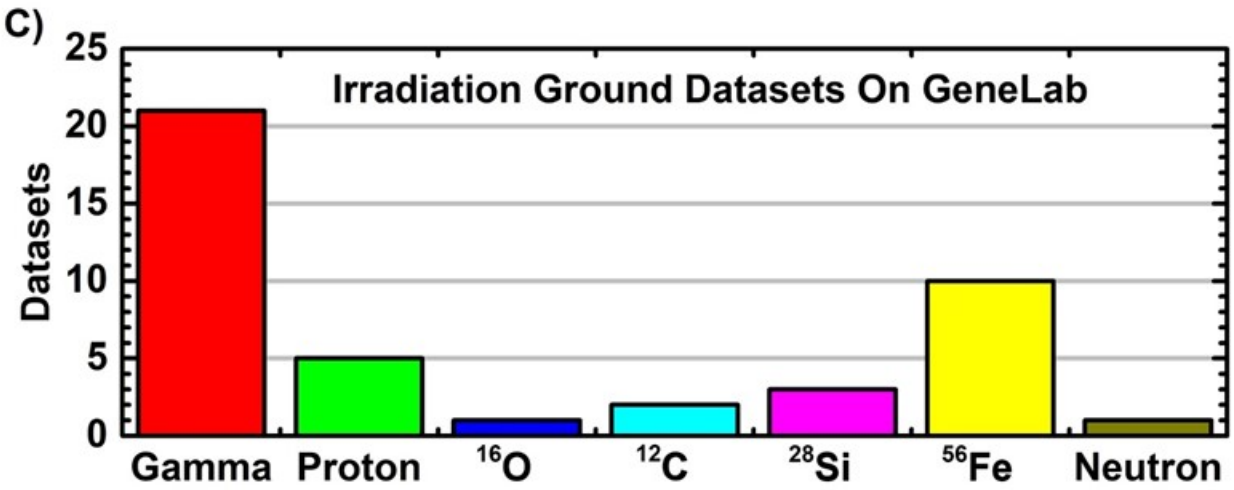
69 Ground Data Sets: Radiation and simulated microgravity





Beheshti et al.,
Radiation Research
2018

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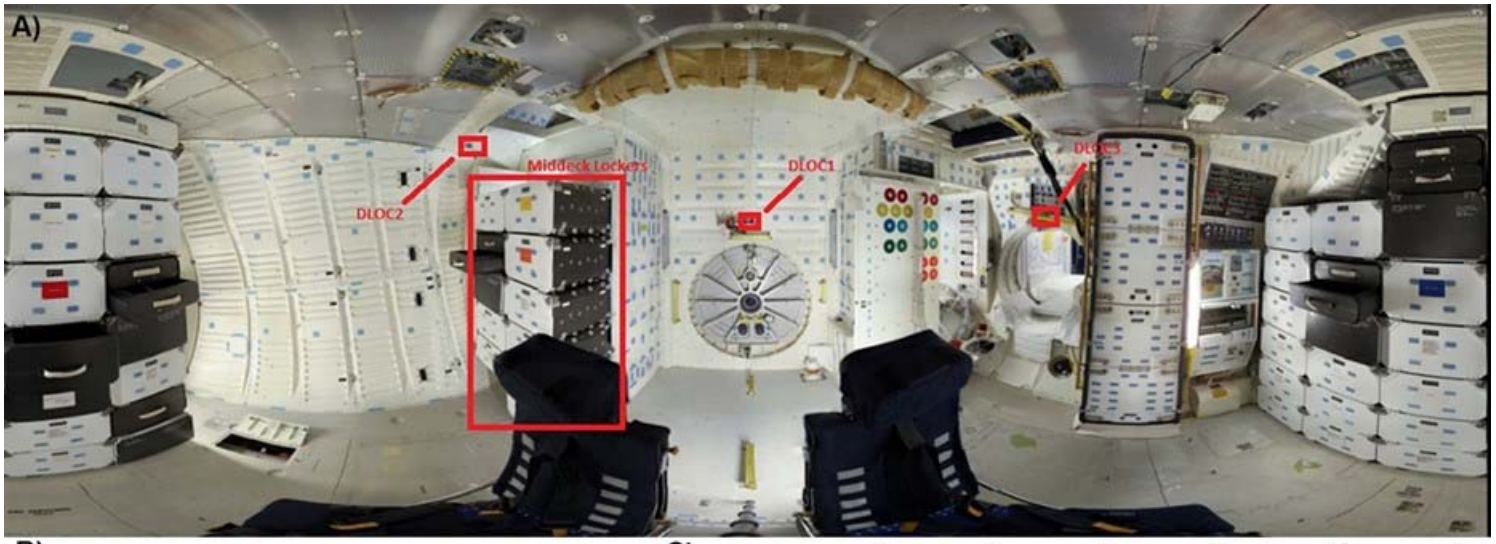
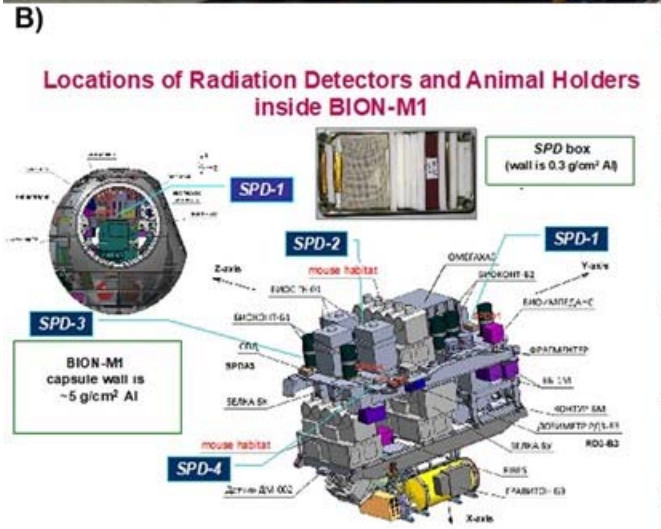
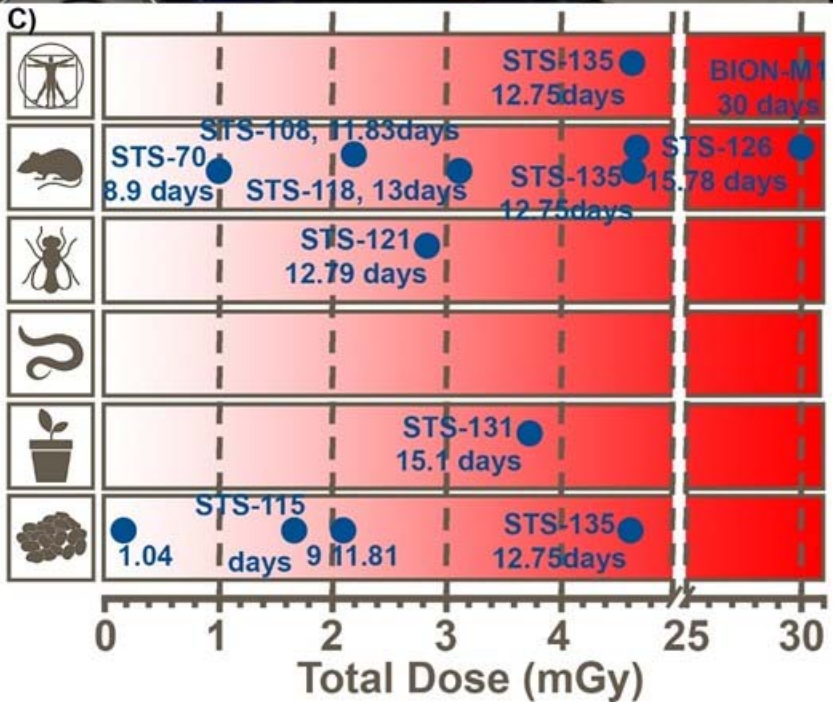
Radiation Dosimetry for STS samples (ISS to follow)







Beheshti et al., Radiation Research 2018

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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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Federated Search

Home Repository Data Data Mining Tools Submit Data Help Workspa

mouse myostatin

All GeneLab NIH GEO EBI PRIDE ANL MG-RAST

Search results for: **mouse myostatin** using filter(s):

Sort by Relevance 25

Myostatin inactivation effects on myogenesis in vitro and in vivo
http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE28986

Key words: dystrophin, mdx mouse, Duchenne, fibrosis, dystrophy ABSTRACT Stim (MDSC) into myogenic, as opposed to lipofibrogenic, lineages is a promising therapeu counteracting myostatin, a negative regulator of muscle mass and a pro-lipofibrotic f fibrogenic capacity of MDSC from wild...
Organism: Mus musculus Accession: GSE28986 PI/Contact: Robert Gelfand Re

The transcriptomic signature of myostatin inhibitory influence on the differenti
http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE59674

GDF8 (myostatin) is a unique cytokine strongly affecting the skeletal muscle phenoty molecular mechanism of myostatin influence on the differentiation of mouse C2C12 m technique. Treatment with exogenous GDF8 strongly affected the growth and devel proliferation and differentiatio...
Organism: Mus musculus Accession: GSE59674 PI/Contact: Zofia Wicik Releas

Development of gene expression signature for defining the cell potency of mu genotypes
http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE39765

In order to determine the cell potency, by identification of genes responsible for pluri isolated from five week old male wild type(WT), C57Bl6J and another hypertrophied microarray analysis and compared this gene expression to that of a standard mouse and Mstn null mice using an esta...
Organism: Mus musculus Accession: GSE39765 PI/Contact: Bipasha Bose Rele

Rodent Research-3-CASIS: Mouse liver transcriptomic proteomic and epigen
https://genelab-data.ndc.nasa.gov/genelab/accession/GLDS-137

The Rodent Research-3 (RR-3) mission was designed to study the effectiveness of occurs during spaceflight. Myostatin is a protein secreted by myoblasts that inhibits block myostatin cause increases in muscle mass. The RR-3 experiment was sponso Advancement of Science in Space and ass...
Organism: Mus musculus Factor: Microgravity Treatment Assay Type: transcription

GLDS-88: Age and Space Irradiation Modulate Tumor Progression: Implications for Carcinogenesis Risk

Source Accession Number E-GEOD-45606
Total Data Volume: 31.6 MB

Submitted Date: 28-Mar-2013
Release Date: 13-Jun-2013

DESCRIPTION | PROTOCOLS | SAMPLES | ASSAYS | PUBLICATIONS | STUDY FILES

DESCRIPTION

Study Description

Age plays a major role in tumor incidence and is an important consideration when modeling the carcinogenesis process or estimating cancer risks. Epidemiological data show that from adolescence through middle age, cancer incidence increases with age. This effect is commonly attributed to a lifetime accumulation of cellular, particularly DNA, damage. However, during middle-age, the incidence begins to decelerate and, for many tumor sites, it actually decreases at sufficiently advanced ages. We investigated if the observed deceleration and potential decrease in incidence could be attributed to a decreased capacity of older hosts to support tumor progression, and whether HZE (high atomic number (Z), high energy (E)) radiation differentially modulates tumor progression in young versus middle-age hosts, issues relevant to estimating carcinogenesis risk for astronauts. Lewis lung carcinoma (LLC) cells were injected into syngeneic mice (143 and 551 days old), which were then subject to whole-body 56Fe irradiation (1GeV/amu). Three findings emerged: 1) among unirradiated animals, substantial inhibition of tumor progression and significantly decreased tumor growth rates were seen for middle-aged mice compared to young mice; 2) whole-body 56Fe irradiation (1GeV/amu) inhibited tumor progression in both young and in middle-aged mice (with greater suppression seen in case of young animals), with little effect on tumor growth rates; and 3) 56Fe irradiation (1GeV/amu) suppressed tumor progression in young mice, to a degree not significantly different than transiting from young to middle-aged. Thus, 56Fe irradiation (1GeV/amu) acted similar to aging with respect to tumor progression. We further investigated the molecular underpinnings driving the radiation modulation of tumor dynamics in young and middle-aged mice. Through global gene expression analysis, the key players, FASN, AKT1, and the CXCL12/CXCR4 complex, were determined to be contributory. In sum, these findings demonstrate a reduced capacity of middle-aged hosts to support the progression phase of carcinogenesis and identify molecular factors contributory to HZE radiation modulation of tumor progression as a function of age. For genome-wide expression profiling of tumor tissue, Mouse WG-6 BeadArray chips (Illumina, San Diego, CA) were used. Total RNA was amplified with the Ambion Illumina TotalPrep Amplification Kit (Ambion, Austin, TX) and labeled from all replicate biological samples for each condition. The number of tumor sample replicates used from each condition is as follows: 10 samples from young unirradiated mice, 8 samples from young irradiated mice, 7 samples from middle-aged unirradiated mice, 5 samples from middle-aged irradiated mice. Total RNA was isolated and purified using Trizol (Invitrogen) or RNeasy (Qiagen), quantified and qualified using Agilent Bioanalyzer (Agilent) and samples were deemed suitable for amplification and hybridization if they had O.D. 260/280 = 1.7 - 2.1, 28s/18s = 2:1, RIN (RNA integrity number) >7. Total RNA of 500ng per sample was amplified using Ambion TotalPrep (Ambion), and 1.5ug of the product was loaded onto the chips. Following hybridization at 55C, the chips were washed and then scanned using the Illumina iScan (Illumina), and the data were analyzed using GenomeStudio (Illumina). Data were first analyzed for gene expression and then culled for present genes (genes that meet the criteria of detection p-value < 0.05). Expression above background was included in an expressed genes working data set for further analyses. Rank variant normalization was applied to the data before extensive analysis. Differential gene expression analysis was used to compare to the reference group, young unirradiated mice, and genes were then evaluated and validated.

Contacts

NAME	ROLE	ORGANIZATION	E-MAIL
Christine E Briggs	submitter	Tufts University School of Medicine	cebriggs7135@gmail.com
Afshin Beheshti			
Edward Rietman			
Lynn Hlatky	Principal Investigator	Tufts University School of Medicine	
Michael Peluso			
Philip Hahnfeldt			
Rainer K Sachs			

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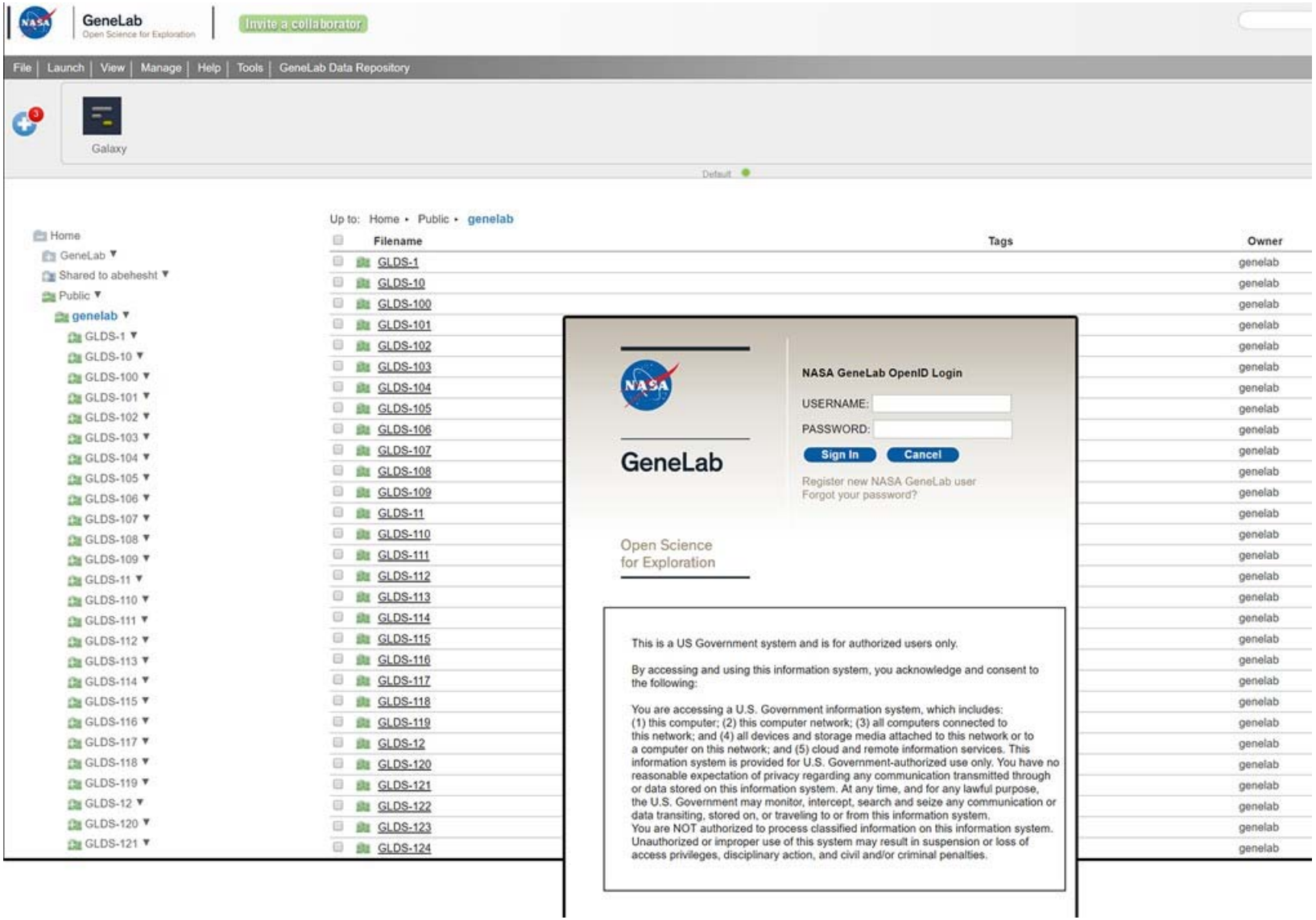
GLDS Phase 2 (Release 2.0)
Customized NASA Collaborative Workspace

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



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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)

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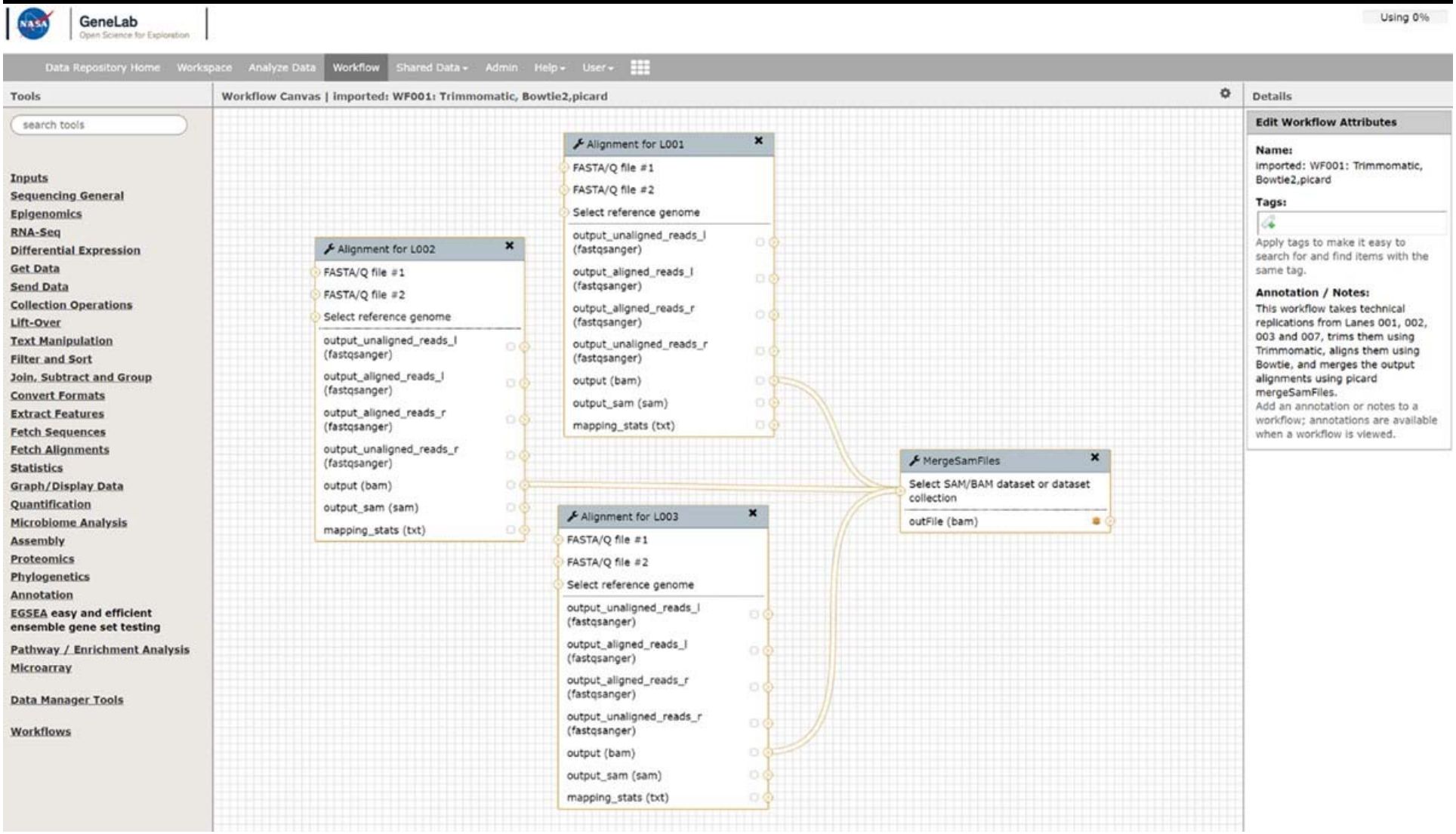
Preview of GLDS 3.0



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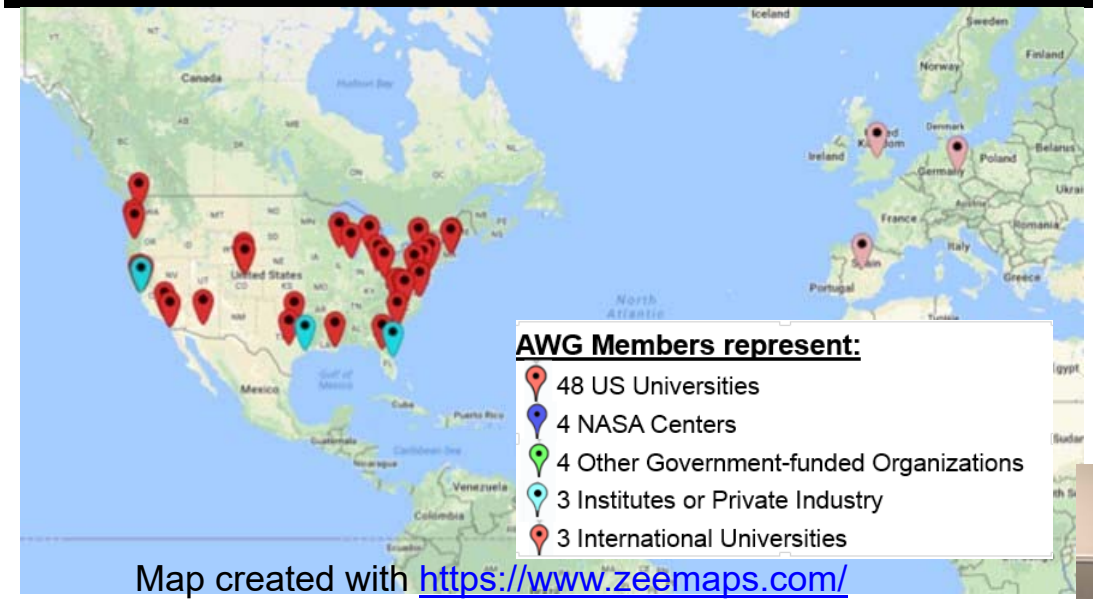
GeneLab Analysis Working Groups:
Letting the scientific community take the lead



Map created with https://www.zeemaps.com/

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*

Annual Workshop (April 2018)

2018 Summer Internship: Generate all higher order data

- **Monthly meetings + "Homework"**
- **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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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

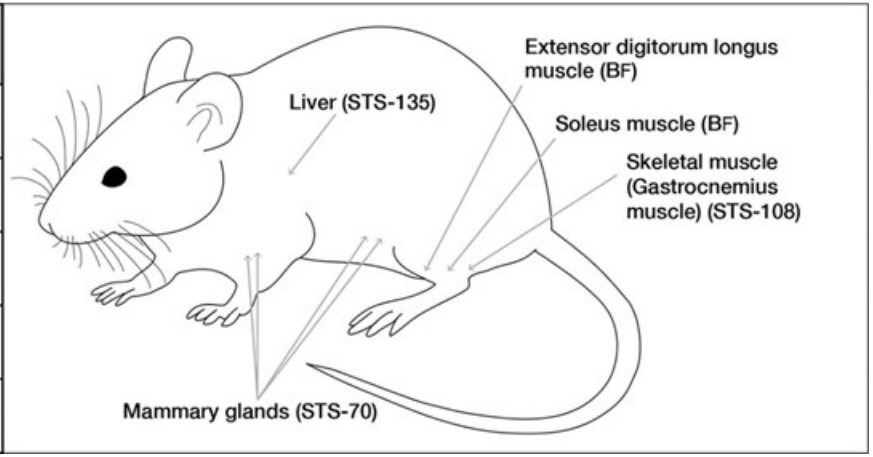
A) Cage Types

Animal Enclosure Module (AEM)

Sample vivarium cage

B)

GeneLab Study	Mission	Species	CO_2 (ppm)	Duration (days)	Tissue Type
GLDS-21	STS-108	mouse	~3000	11.8	skeletal muscle (gastrocnemius)
GLDS-111	BF	mouse	~600	30	soleus muscle
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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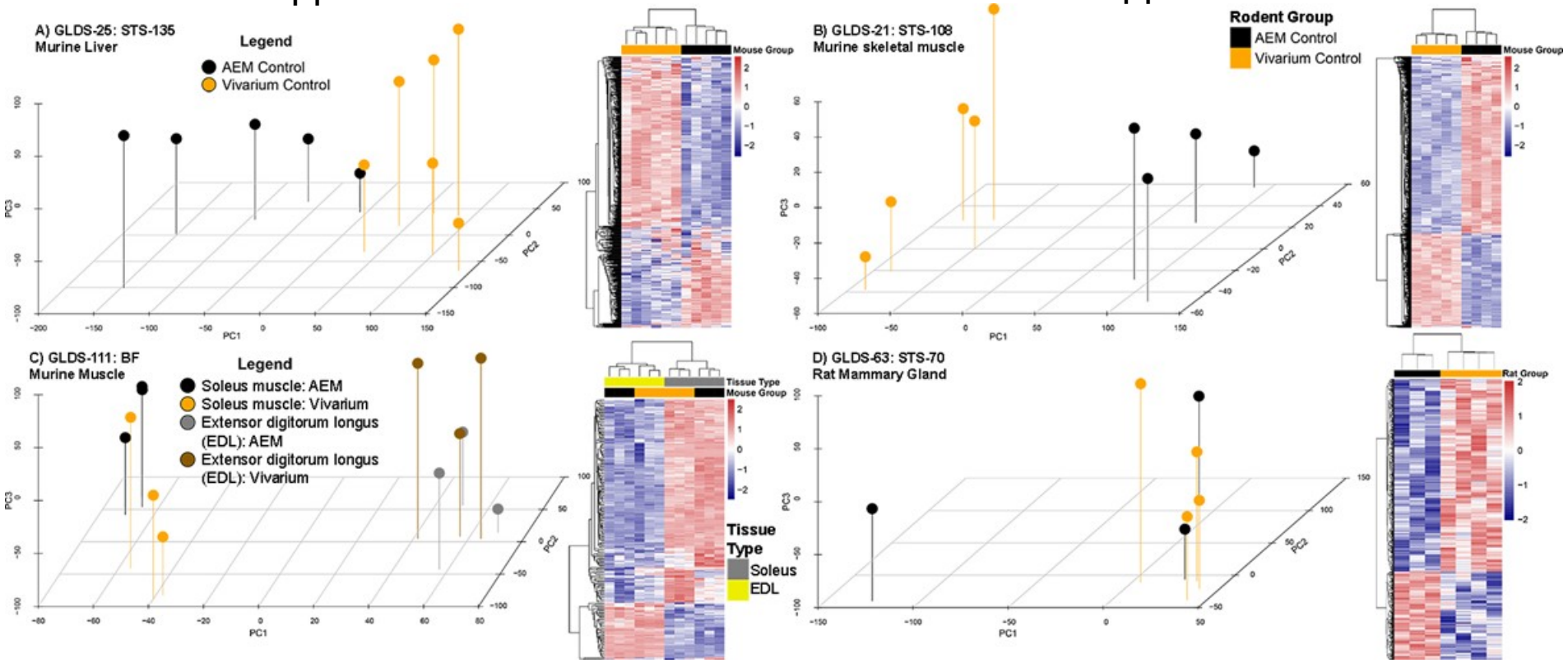
A Plots Suggest Strong Cage Effect

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

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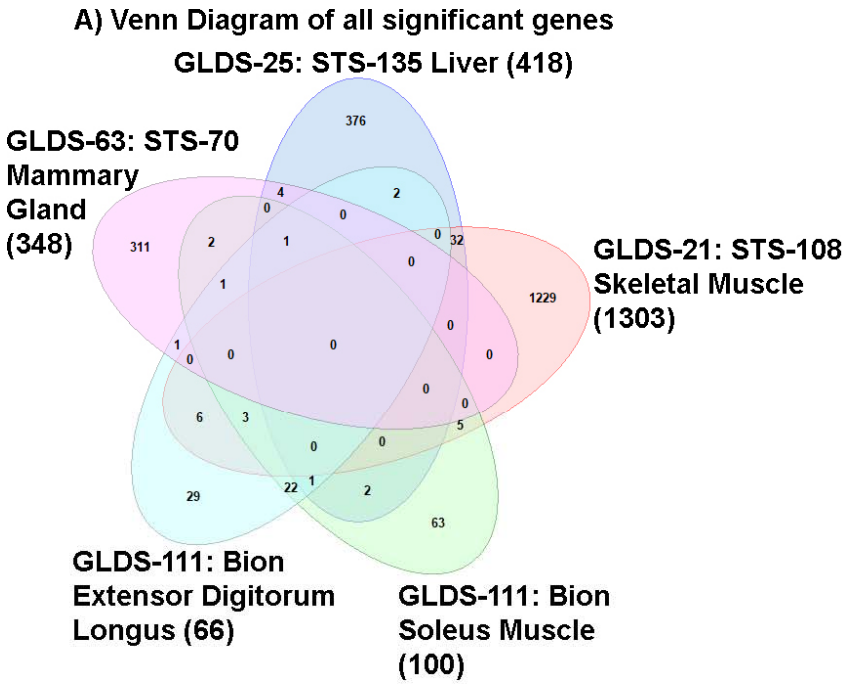
Differential Gene Expression: Cage or CO2 Effect?



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

Beheshti, et al., Scientific Reports, 2018

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Systems Biology analysis reveals biological spaceflight master regulators

Beheshti, et al., PLOS One, 2018

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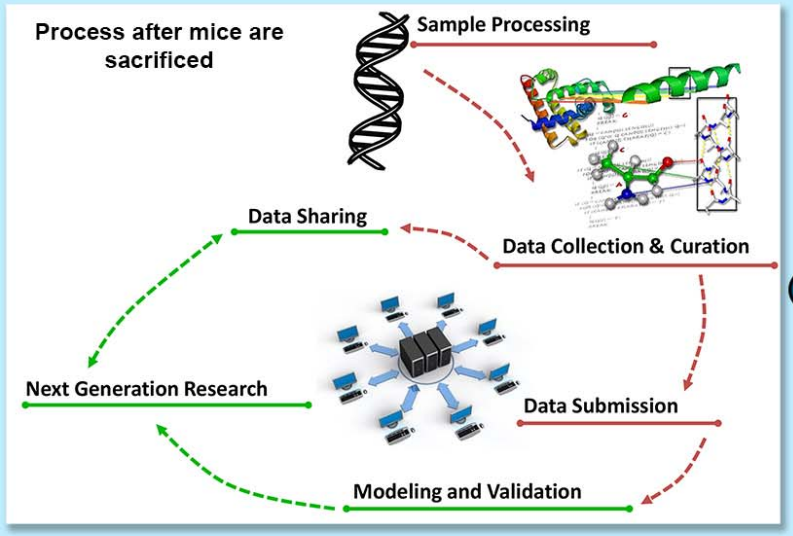
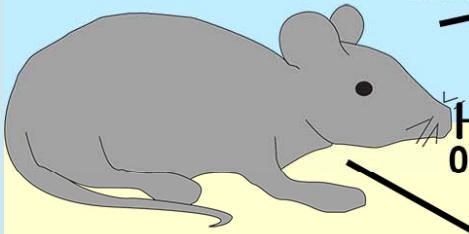
GeneLab Data Used to Generate Results



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

Mice Sacrificed on ISS



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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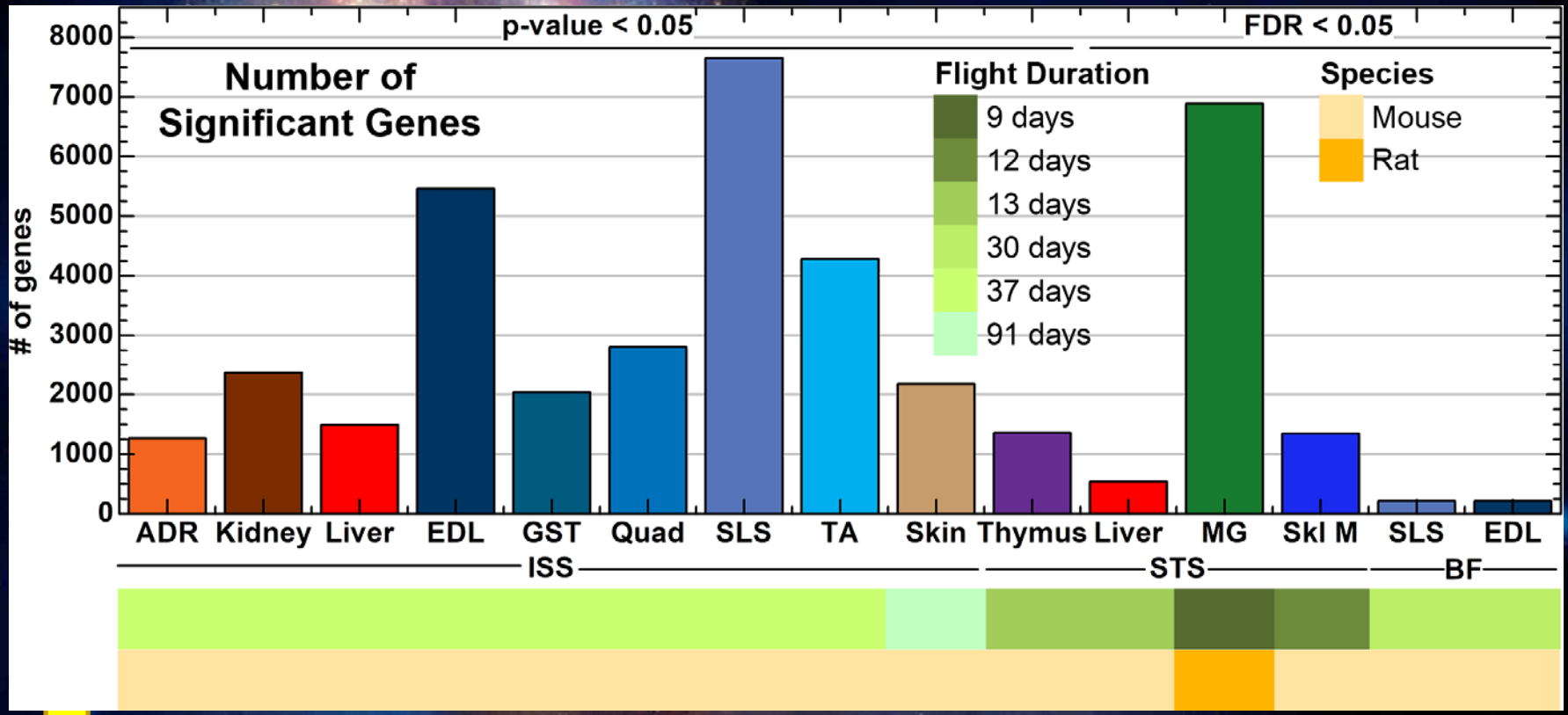
Number of Significant Genes from Each Dataset



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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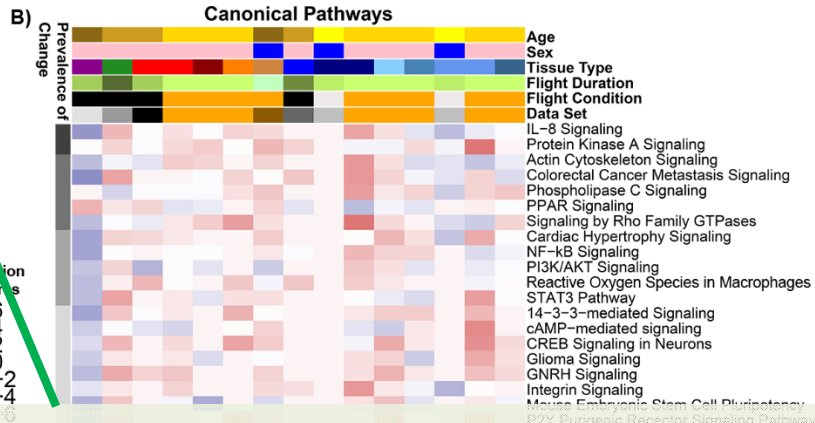
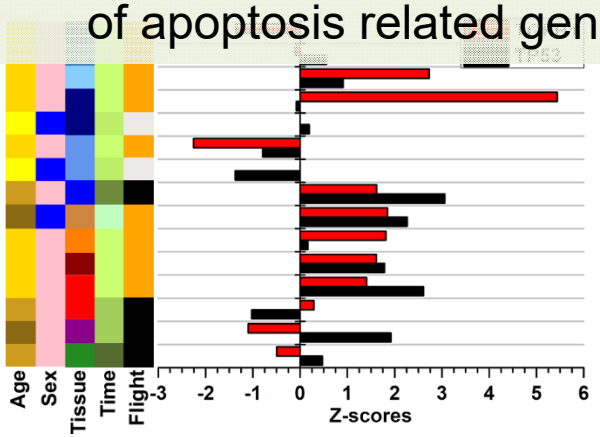
Predicted Master Regulators



- **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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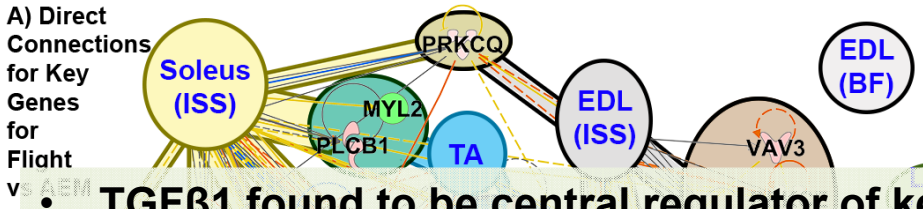
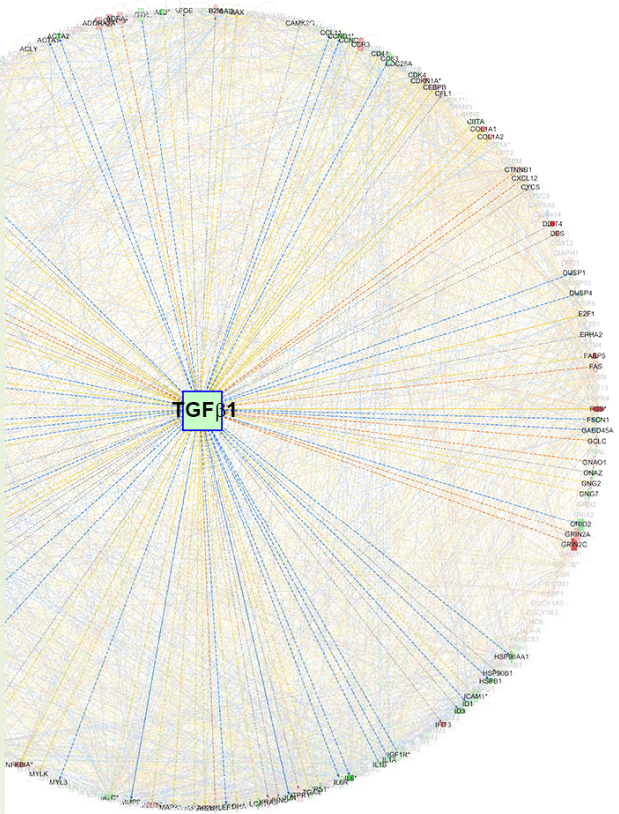
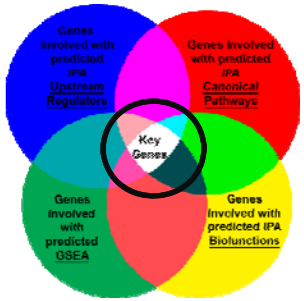
Key Genes and the Connections



B) Connections Between all Key Genes for all Datasets (Flight vs AEM): Radial Plot with the most Connected Gene in the Middle



- **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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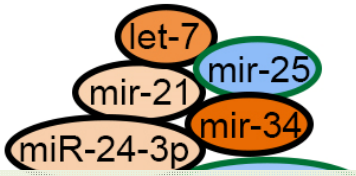
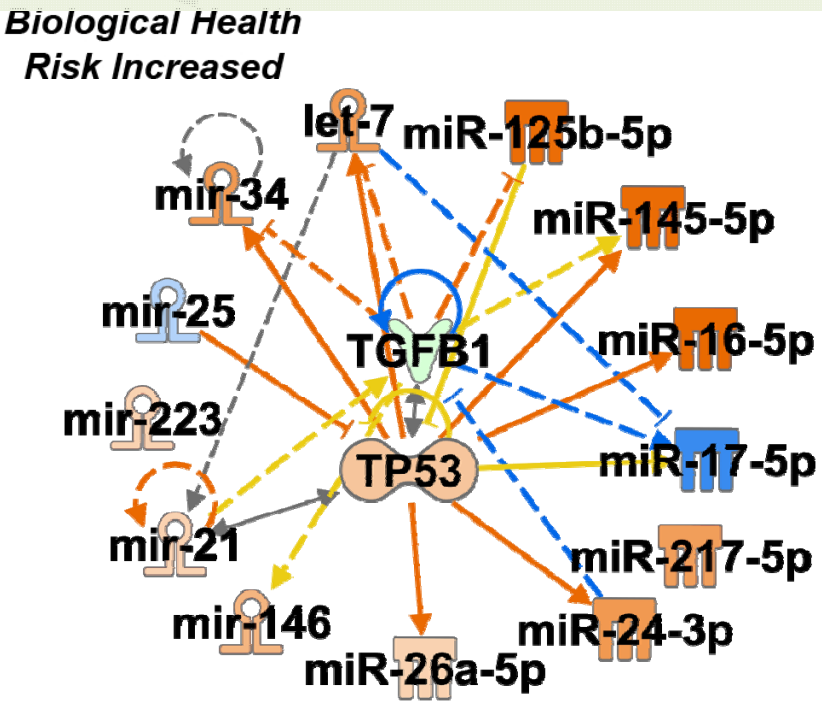
Predicted miRNAs Involved with Microgravity Effects

Health Risk Due to miRNAs



Predicted Activation
Predicted Inhibition

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

Work in progress

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AWG Members Involved

Kathleen Fisch

Brin Rosenthal

UNIVERSITY of CALIFORNIA, SAN DIEGO
SCHOOL OF MEDICINE

Deanne Taylor

Hossein Fazelinia

Komal Rathi

Children's Hospital of Philadelphia

Perelman School of Medicine
University of Pennsylvania

Helio Costa

Kathryn Grabek

STANFORD UNIVERSITY

J. Tyson McDonald

HAMPTON UNIVERSITY
THE STANDARD OF EXCELLENCE

Gary Hardiman

Willian da Silveira

MUSC Health
MEDICAL UNIVERSITY of SOUTH CAROLINA

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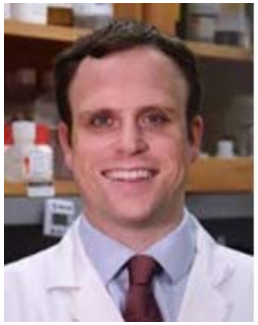
AWG Members Involved



Chris Mason

Cem Meydan

Jonathan Foox

Flavia Rius

Cornell University

Yared Kidane

NASA

Texas Scottish Rite Hospital for Children

Susana Zanello

Scott Smith

Sara Zwart

NASA

Space Center Houston
Manned Space Flight Education Foundation

Afshin Beheshti

Sylvain Costes

NASA

Ames Research Center

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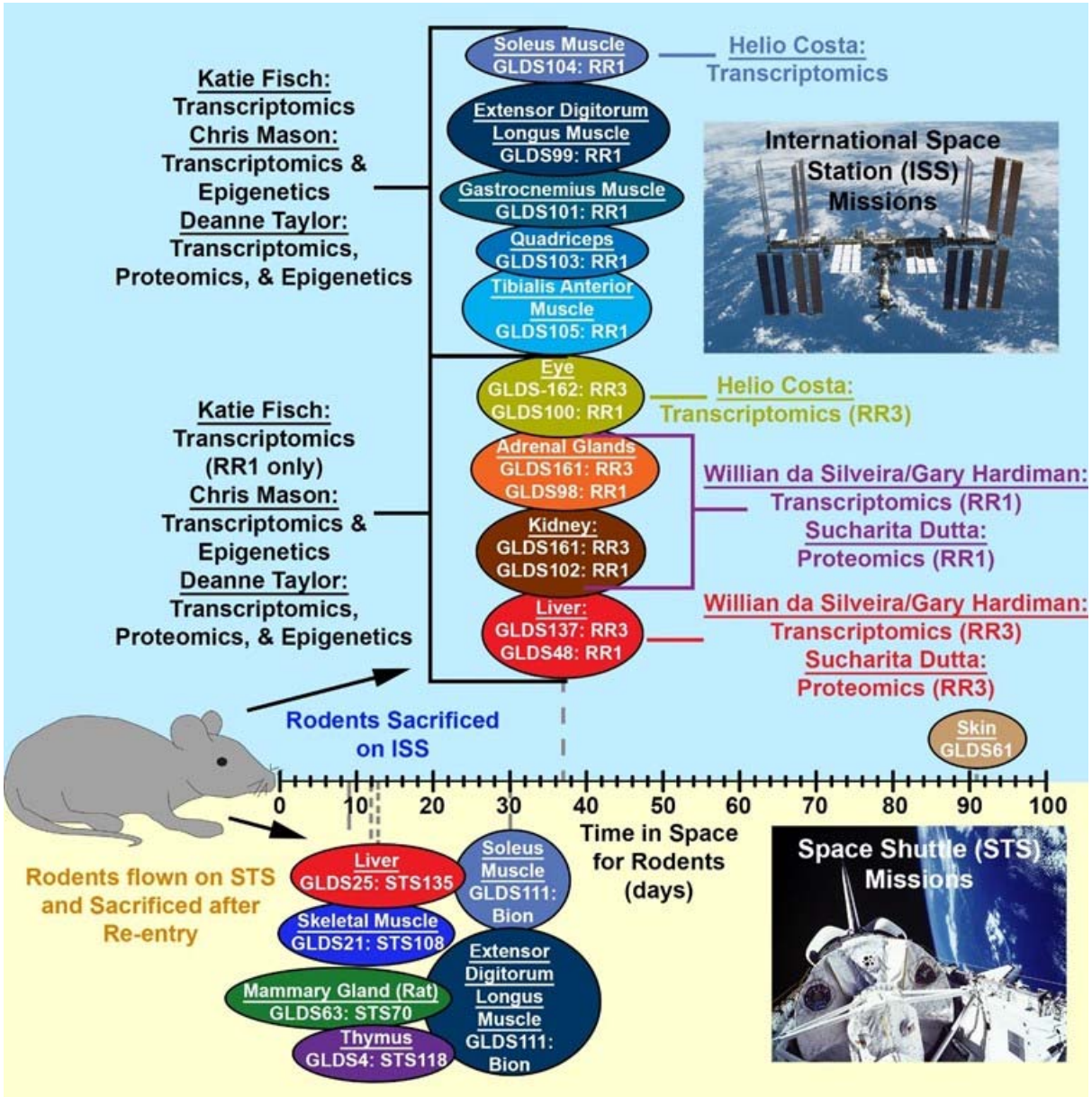
Specific Datasets and Tissues AWG Members Analyzed



In addition, human datasets are also included:

- 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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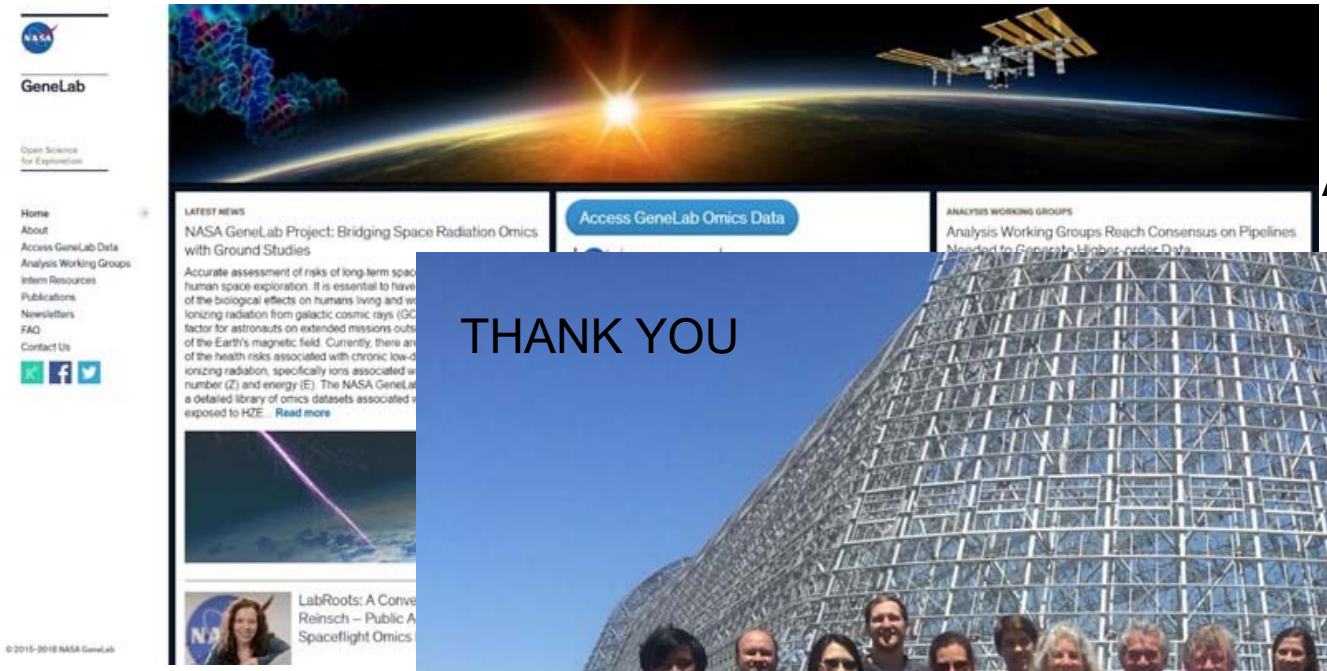
Engaging with GeneLab



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