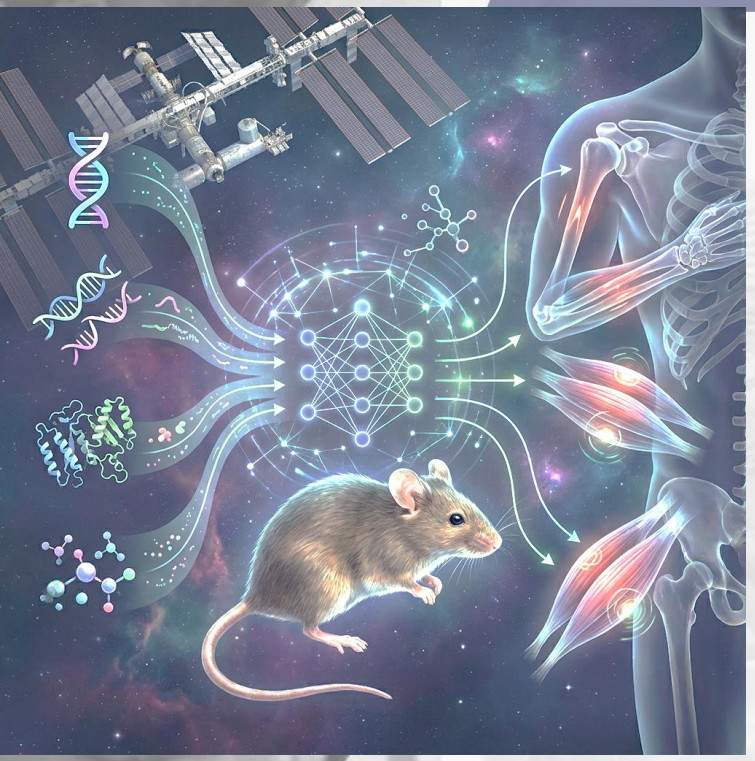

Bioenergy-Immune Response Axis is an Early Target of Spaceflight Induced Stress:

An Evidence Based Review of Five Space Projects

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Disclaimers

The views expressed in this presentation reflect the results of research conducted by the authors and do not necessarily reflect the official policy or position of the Defense Health Agency, Department of War, nor the U.S. Government.

The experiments reported herein were conducted in compliance with the Animal Welfare Act and per the principles set forth in the "Guide for Care and Use of Laboratory Animals," Institute of Laboratory Animals Resources, National Research Council, National Academy Press, 2011.

No endorsement of non-federal entity, product, service, or activity is approved or implied by this presentation.

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MedStar Washington Hospital Center (RR22)
Jeffry Shupp, MD
Lauren Moffatt, PhD

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Integrative Systems Biology

Core Capabilities & Strategic Ecosystem

Deep Discovery

- Next-Gen Sequencing
- Single Cell Omics
- Spectral Assays (LC/MS, OLINK)
- Automated Liquid Handling

Advanced Models

- 3D Cell Culture
- Gnotobiotic Vivarium
- Space Biology/ISS

Data Maneuvering

- DoD HPC Access
- Custom Scripts/Libraries
- Big Data Management

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ISB-Space Project Timeline

2011 STS-135: *in vitro* project

2017 Rodent Research 2 (RR2). Collaborating with Novartis, Inc.

2017 Rodent Research 4 (RR4): Collaborating with Indiana University

2019 Cell Science (CS-02): Collaborating with Indiana University

2021 Rodent Research Demo (RRD1): Collaborating with MedStar WHC
2022 Rodent Research 22 (RR22): Collaborating with MedStar WHC

STS-135

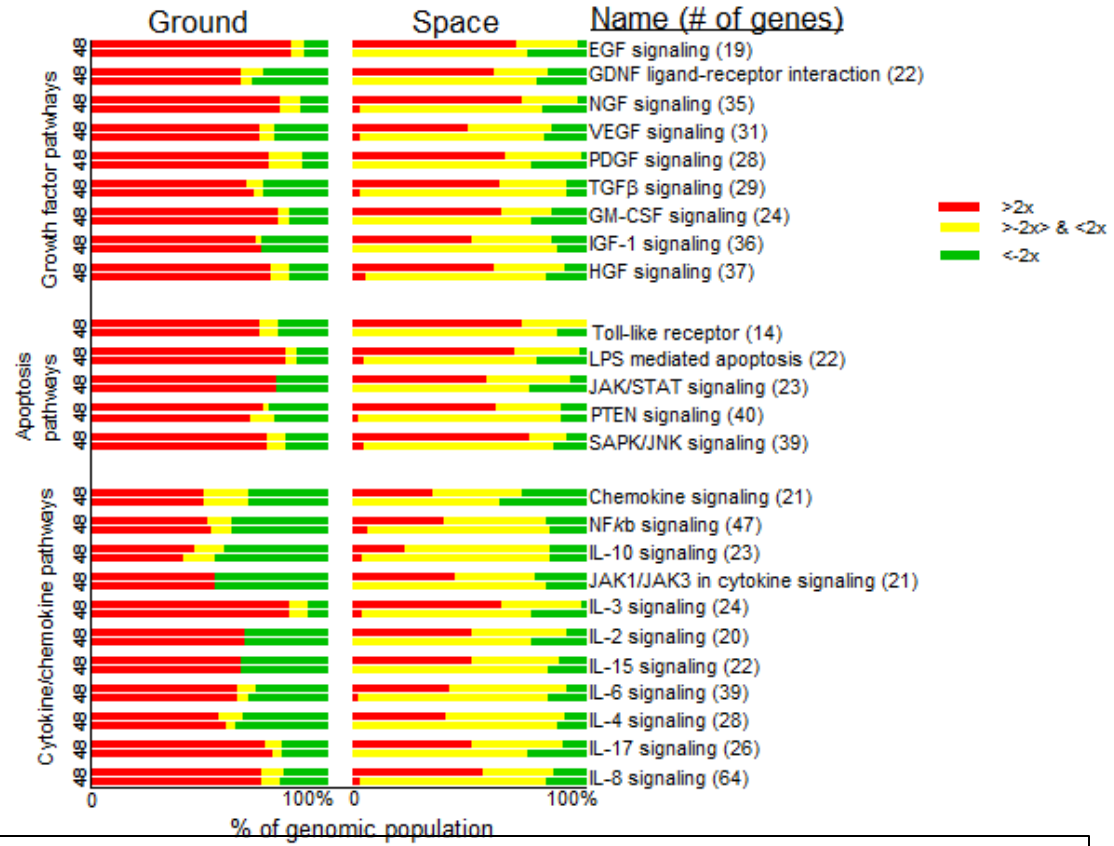
Response to Pathogens, Launched in 2011

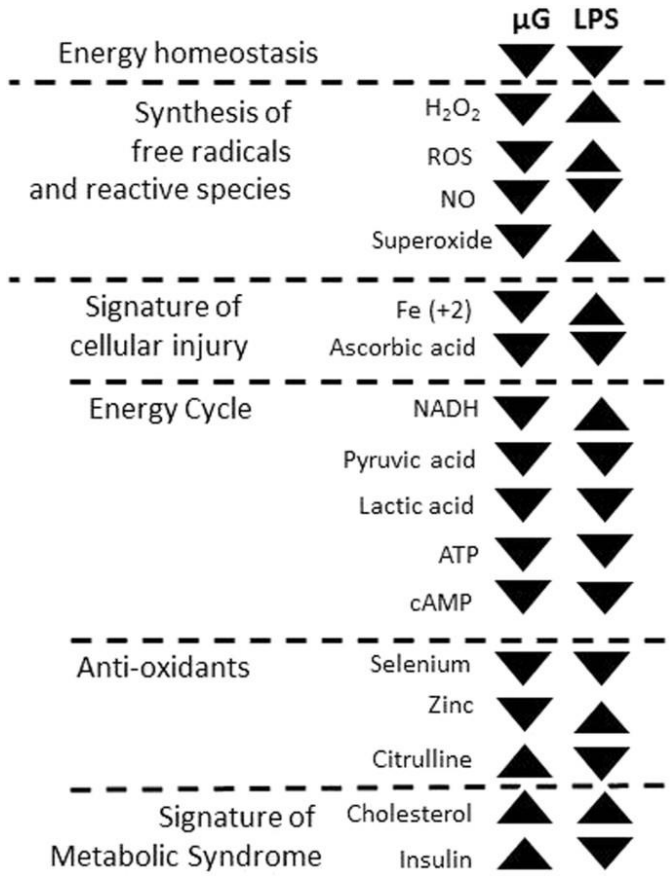
- 3-dimensional semi-automated cell culture system
- Ten bioreactors inoculated cells for 10 days followed by time- dependent LPS assaults
- Awarded by ISSRDC as “Most Compelling Research in Space in 2011”

Major findings

- Temporary *early* immune blunting in host cells in Space
- Early energy depletion attributed to immunosuppression

STS-135: Inhibited response to LPS coincides with energy suppression





Gene transcriptomic data showed comprehensive inhibited response to LPS

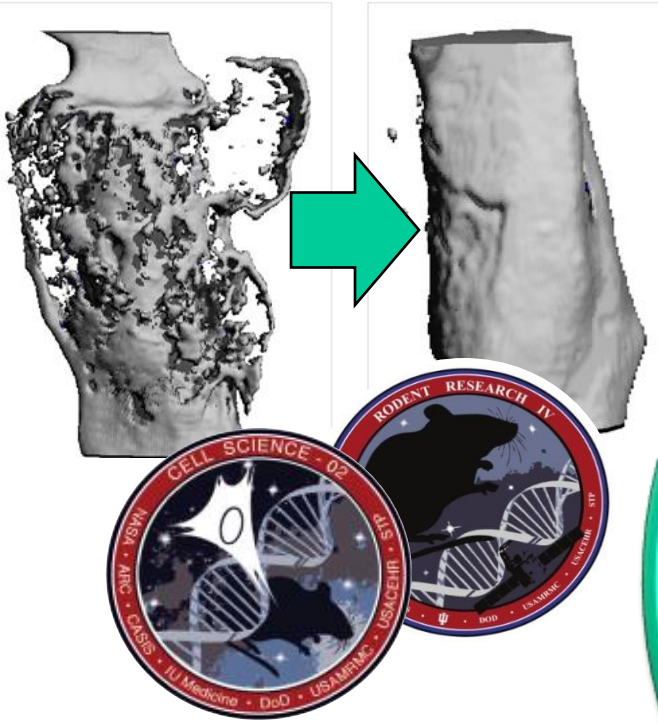
Gene metabolite integrative analysis showed parallel energy depletion

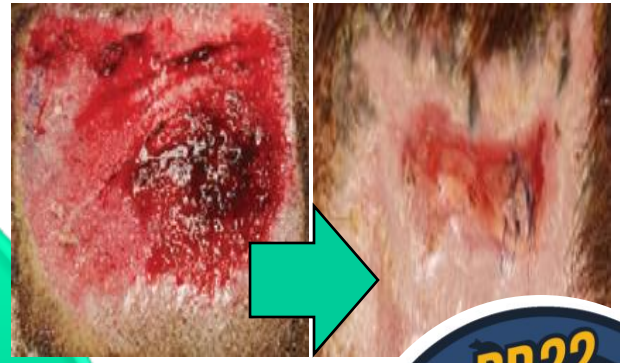
How STS-135 results shape the overarching goals of our subsequent studies

- STS-135 data suggested an adverse impact of Spaceflight-induced stress on immune response and energy synthesis- two key regulators of wound healing

- Musculoskeletal atrophy is a major challenge in Space mission.
  - Prolonged disuse and other spaceflight induced stress cause bone loss, but a significant knowledge gap about its biomechanism remains
  - **Hypothesis 1.** Microgravity could delay the fracture healing; and Spaceflight might trigger a different biomolecular path to heal
  - **Hypothesis 2.** Spaceflight would modulate cutaneous healing via alterations of normal healing phases and associated biomechanical mechanisms.

Overarching Goal





Wound in hard tissue/ bone (RR4, CS-02)

Wound in soft tissue/ skin (RRD1, RR22)

Therapeutic Approach?

Is Functional Immunity Effective in Space?

Is the Immunity-Bioenergy Axis vulnerable to Spaceflight-induced stress

Rodent Research 4 (RR4)

Animal study- Launched in 2017

- Forty (40) mice with segmental bone defects were launched in space
- Mice spent ~3 weeks at ISS
- It was the first animal study with male mice
- It was the first study that launched mice with surgery

Major findings

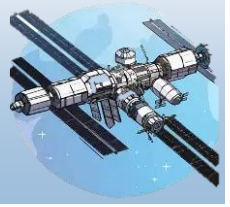
- Muscle and bone healing trajectories were delayed in Space
- Mitochondrial dysregulation and inhibited immune response co-occurred with delayed healing of musculoskeletal system in Space

RR4: Study design: 28d space mission

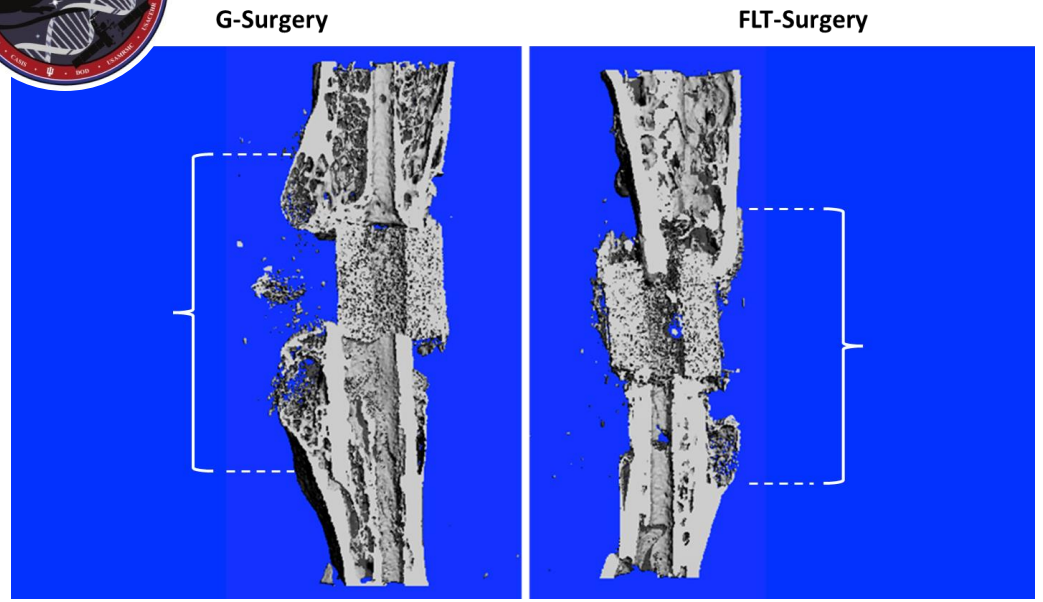
7 week old C57BL/6j

28-day space mission

- Spaceflight (FLT)
  - Sham (FLT-Sham)
    - Multi-omics (*N*= 3-4)
    - µCT (*N*= 5)
  - Surgery (FLT-Surgery)
    - Multi-omics (*N*= 3-4)
    - µCT (*N*= 5)
- Ground (G)
  - Sham (G-Sham)
    - Multi-omics (*N*= 3-4)
    - µCT (*N*= 5)
  - Surgery (G-Surgery)
    - Multi-omics (*N*= 3-4)
    - µCT (*N*= 5)

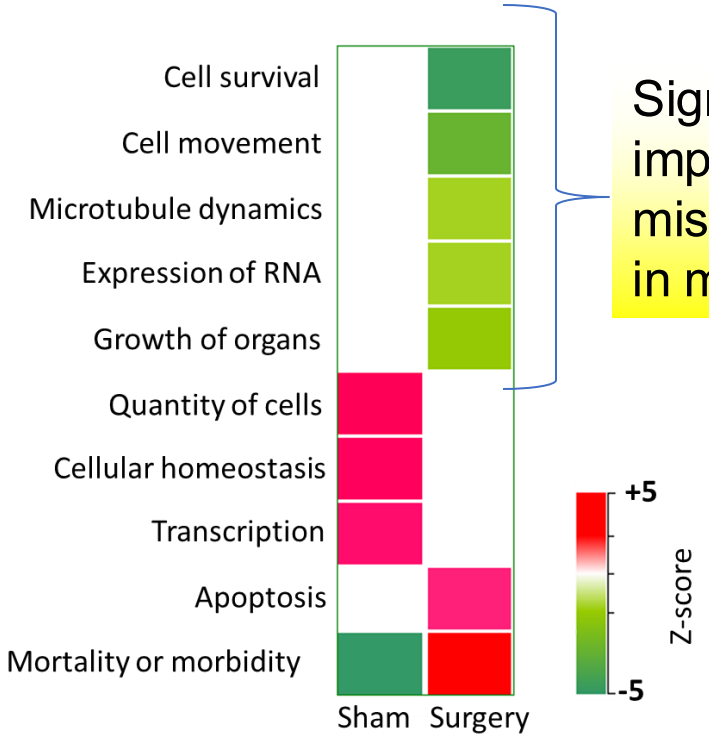


RR4: Fracture healing delayed in Space



	G-Surgery	FLT-Surgery	p-value
connectivity density (1/mm³)	185 ± 112	85 ± 34	p = 0.08 #
trabecular separation (mm),	0.22 ± 0.05	0.34 ± 0.08	p = 0.07 #

Bionetworks

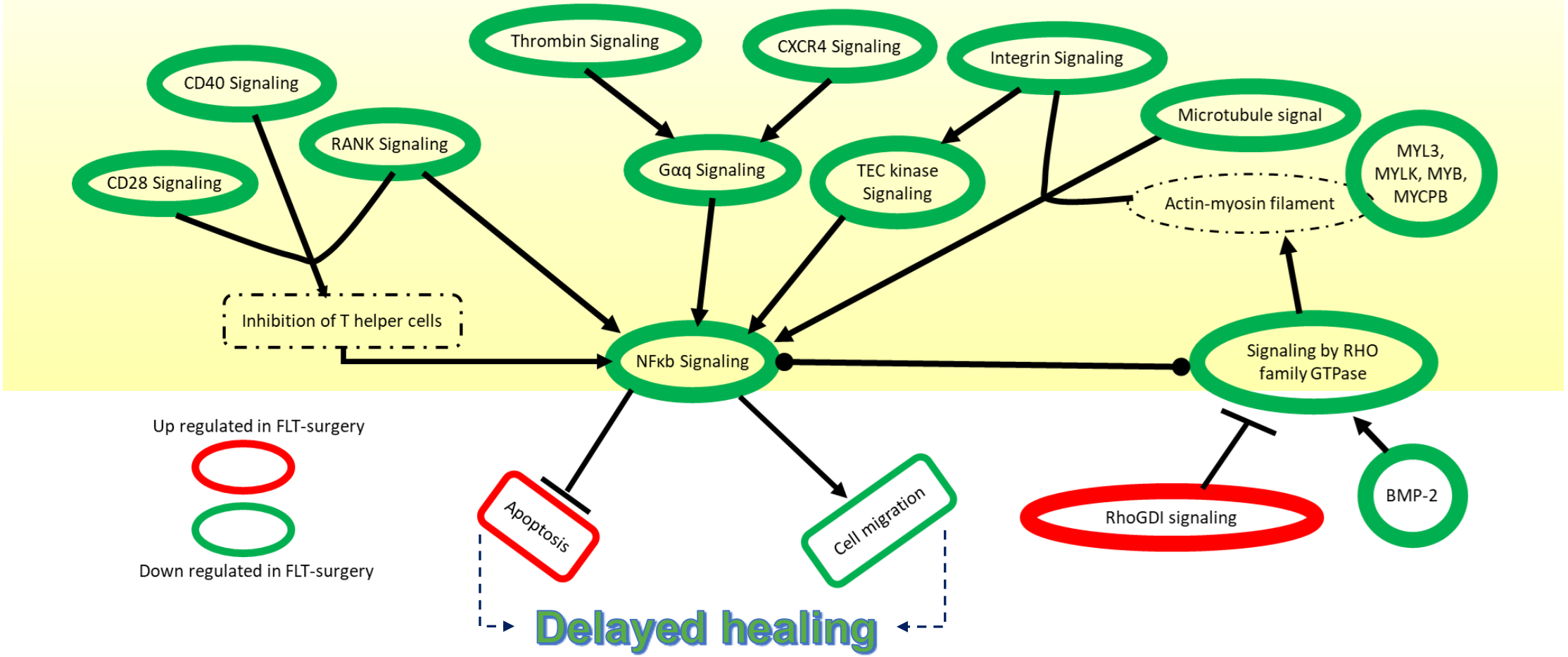


Sign of adverse impacts of 28d space mission in molecular landscape

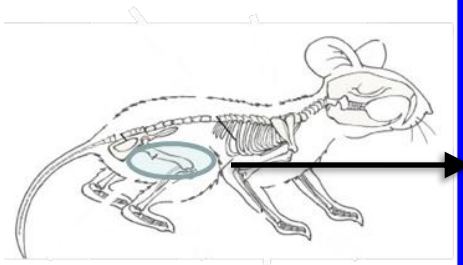
Sign of adverse effects of Spaceflight-induced stress

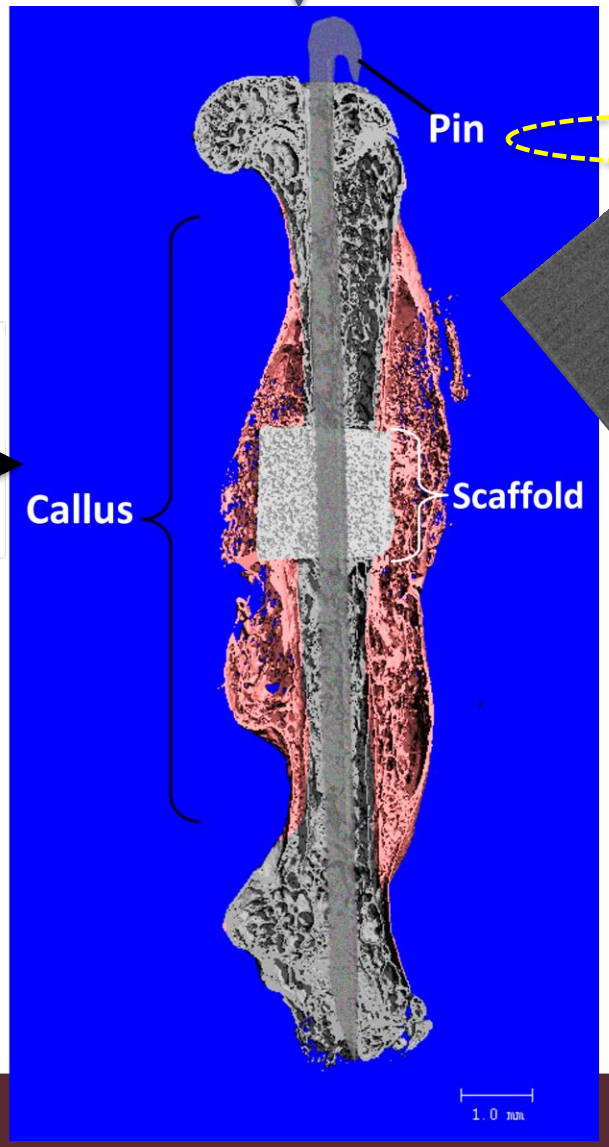
RR4: Delayed fracture healing and inhibited immune response

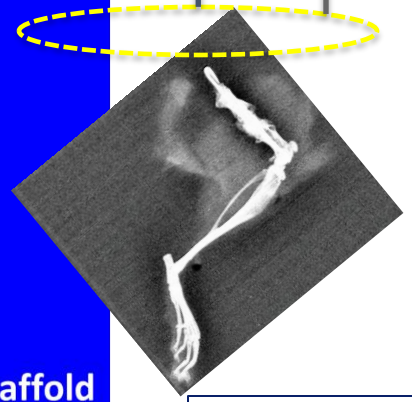
Inhibited immune response



RR4: Muscle weight loss in Spaceflight







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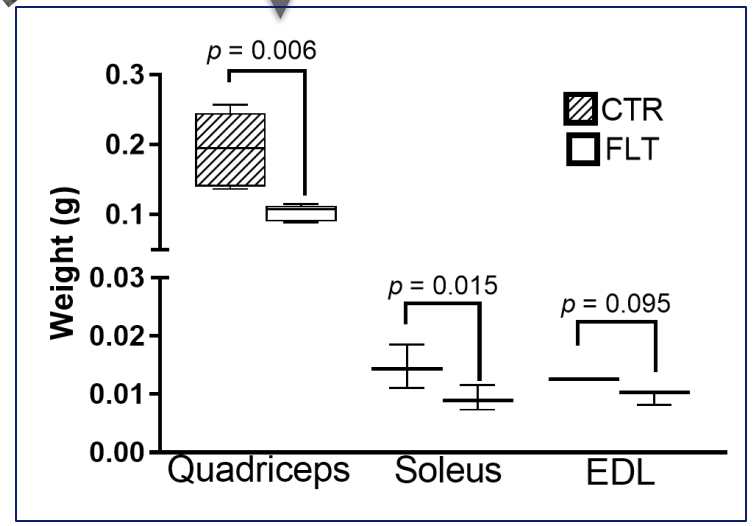
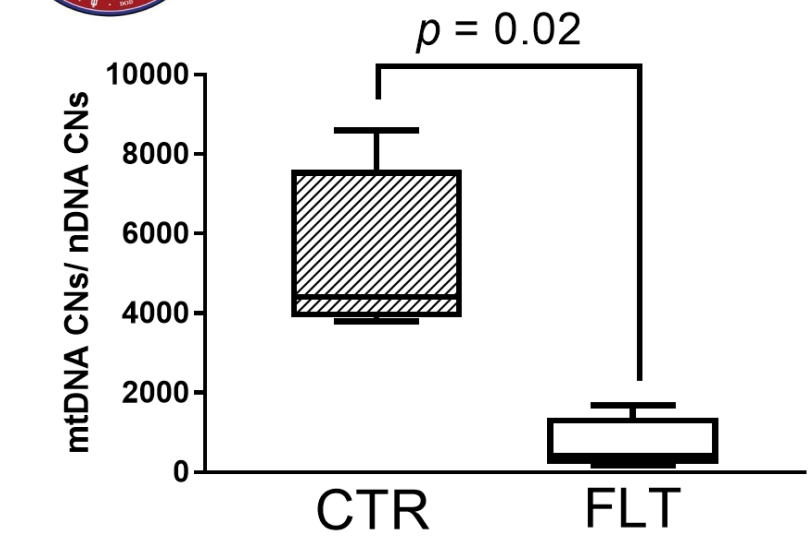
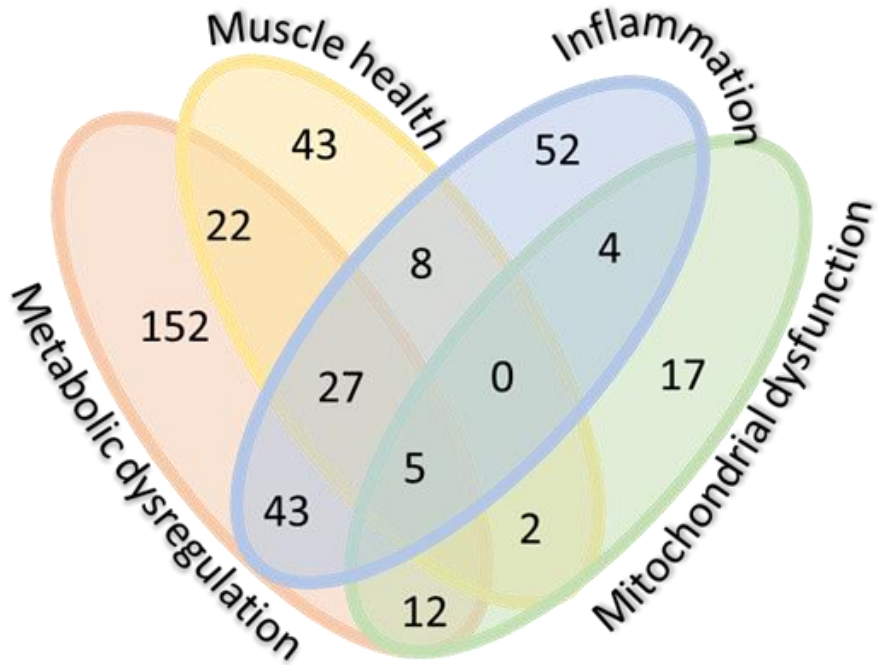


RR4: Muscle weight loss accompanied energy deprivation

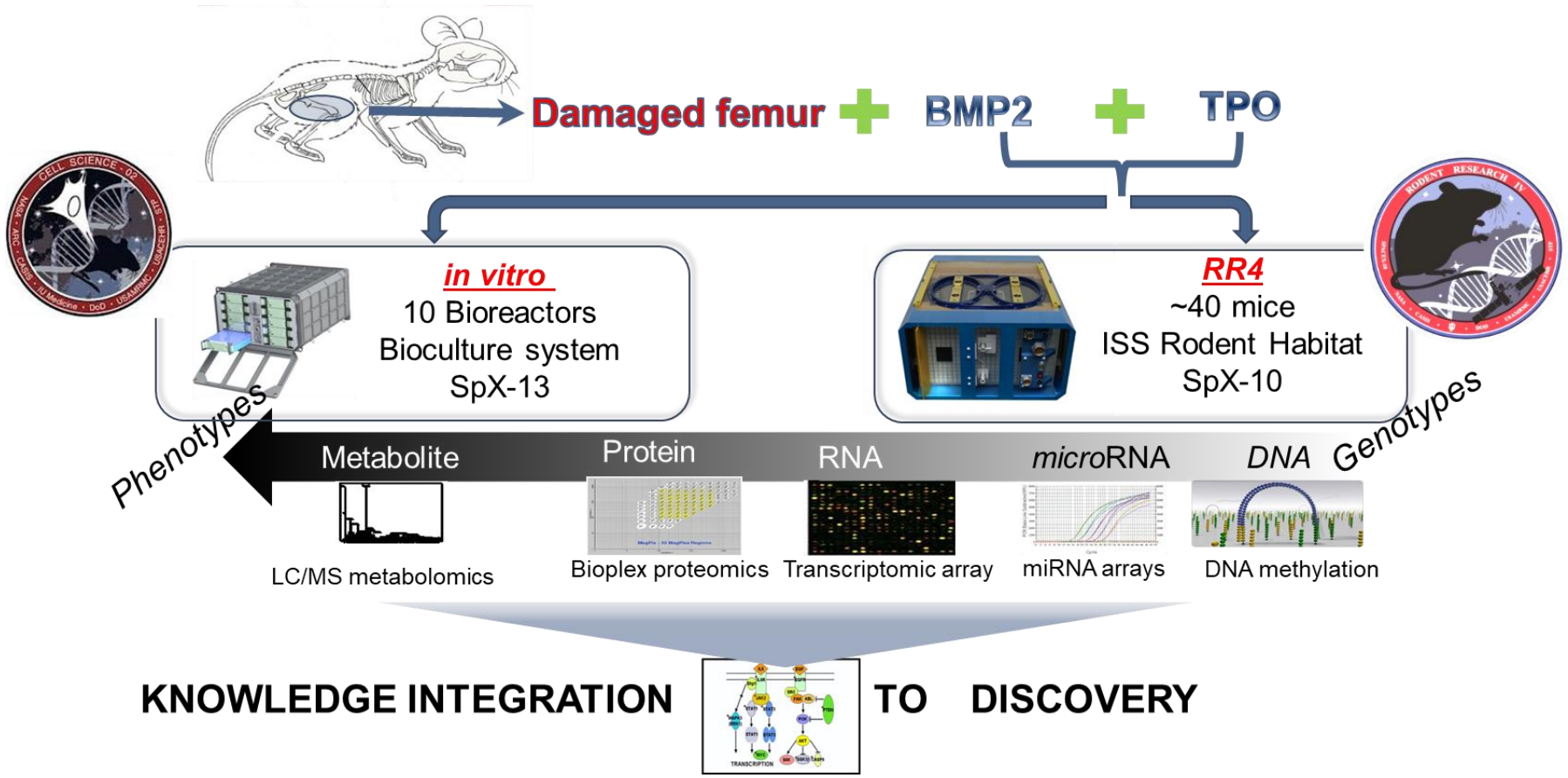


mtDNA copy number: a marker of bioenergy was reduced in Space



Functional analysis found muscle health in Space was associated with mitochondrial dysfunction and inhibited immune response

Moving forward:
Bone cell specific analysis to find Space-specific therapeutics



FDA-approved only oseoinductive agent, BMP-2 could be carcinogenic (dose-dependent).
We investigated TPO- promotes wound healing via enhancing megakaryocyte cell proliferation.

Cell Science-02 (CS-02)

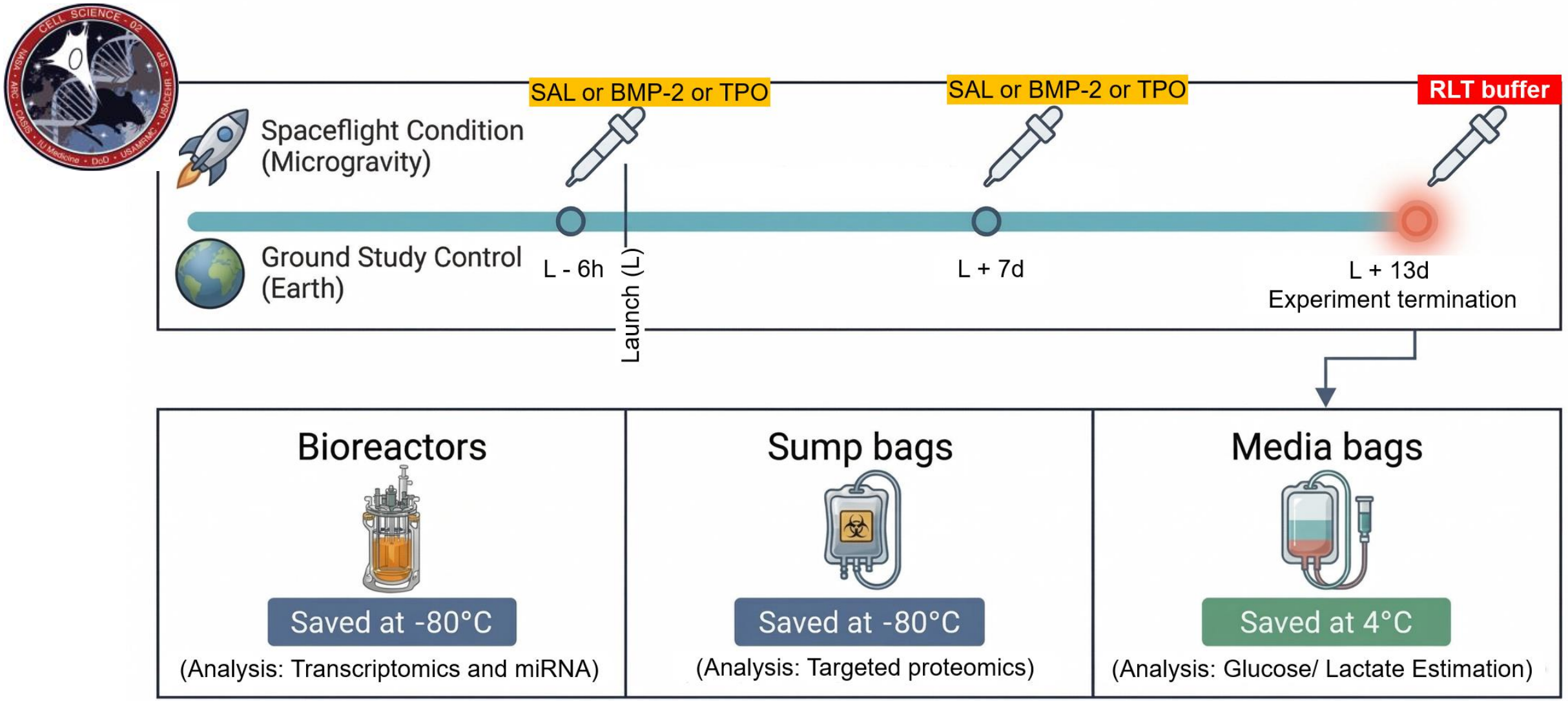
In vitro Project- Launched in 2019

- 3-dimensional semi-automated cell culture system
- Ten bioreactors harvested osteoblast cells in space for 21 days, and treated with BMP-2 and TPO
- Awarded by ISSRDC as “Most Compelling Research in Space in 2020”

Major findings

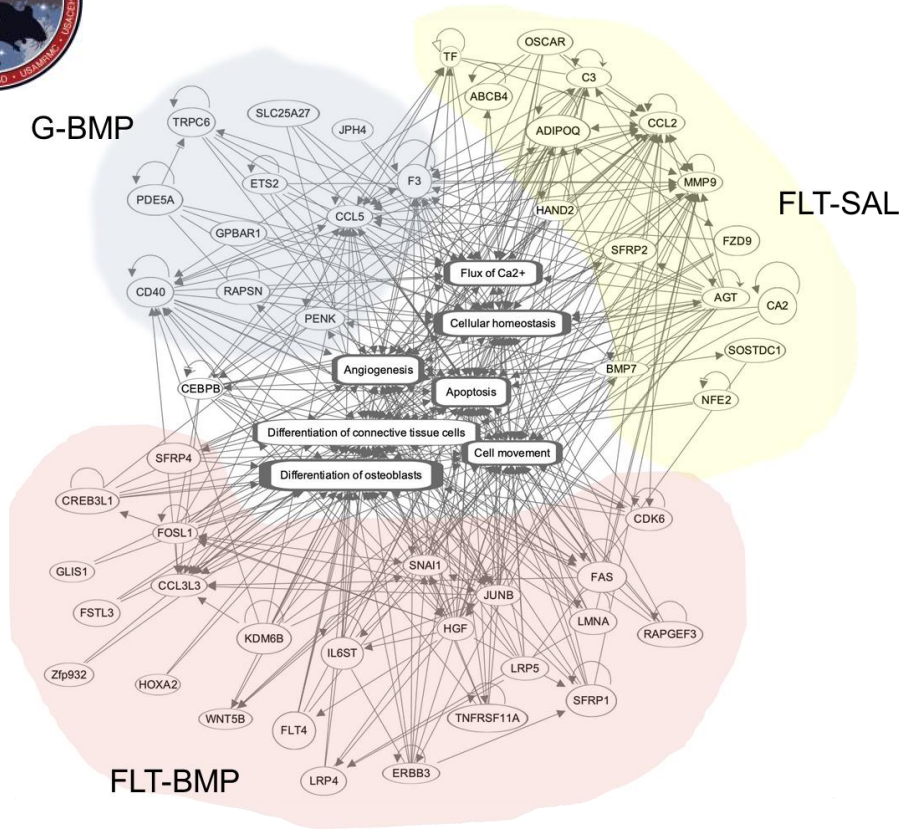
- Osteoblast continues play major roles in bone health in Space
- In Space the impact of BMP-2 came much faster than on Ground and TPO treatment

CS-02: Osteoblast cell and Osteoinductive agents in Space

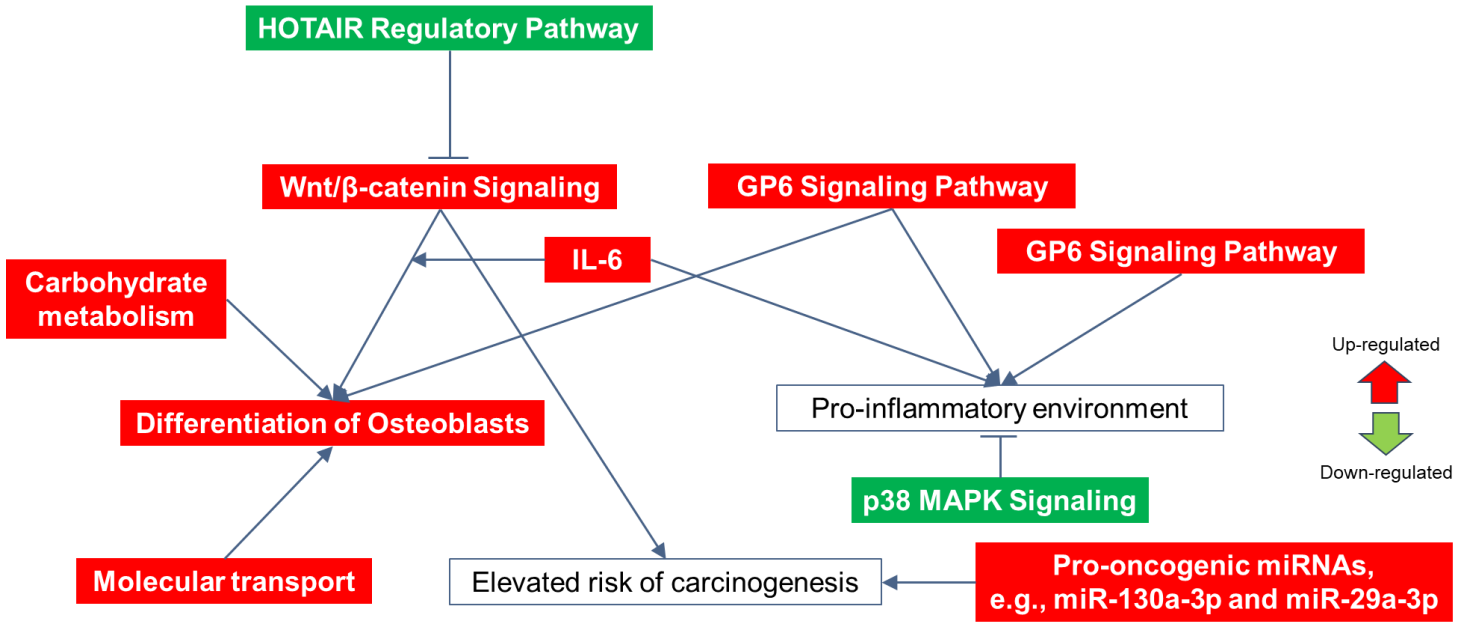


BMP-2 in Space operated via an independent pathway

Gene cluster linked to bone health



Network cluster regulated by BMP-2 in Space



BMP-2 in Spaceflight and on Ground operated via different path to regulate bone health

BMP-2 activated osteoblast cell differentiation network via creating a pro-inflammation environment and elevating risk of carcinogenesis

Rodent Research Demo- 1 and 22 (RRD1 and RR22)

Healing of cutaneous wound in Space

- Forty (40) mice with and without cutaneous wounds were probed in space
- Survival surgery on animal was conducted in Space **for the first time;** Astronauts and space crew were trained by our team to conduct this particular study
- In RR-22, mice were euthanized in 10 timepoints across 45-day mission
- A subgroup of 4 mice was live-returned

Major findings

- Wound histopathology data showed slow-paced reduction of wound gap in Space
- Omics study ongoing

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9. Spaceflight-induced stress caused atypical gut microbial composition (Submitted)
10. Spaceflight and Osteoinductive Agents: A Multi-Omics Study of Osteoblast Cellular Responses (Submitted)

Conclusions

- Spaceflight adversely impacts immune function and bioenergy production (STS-135)

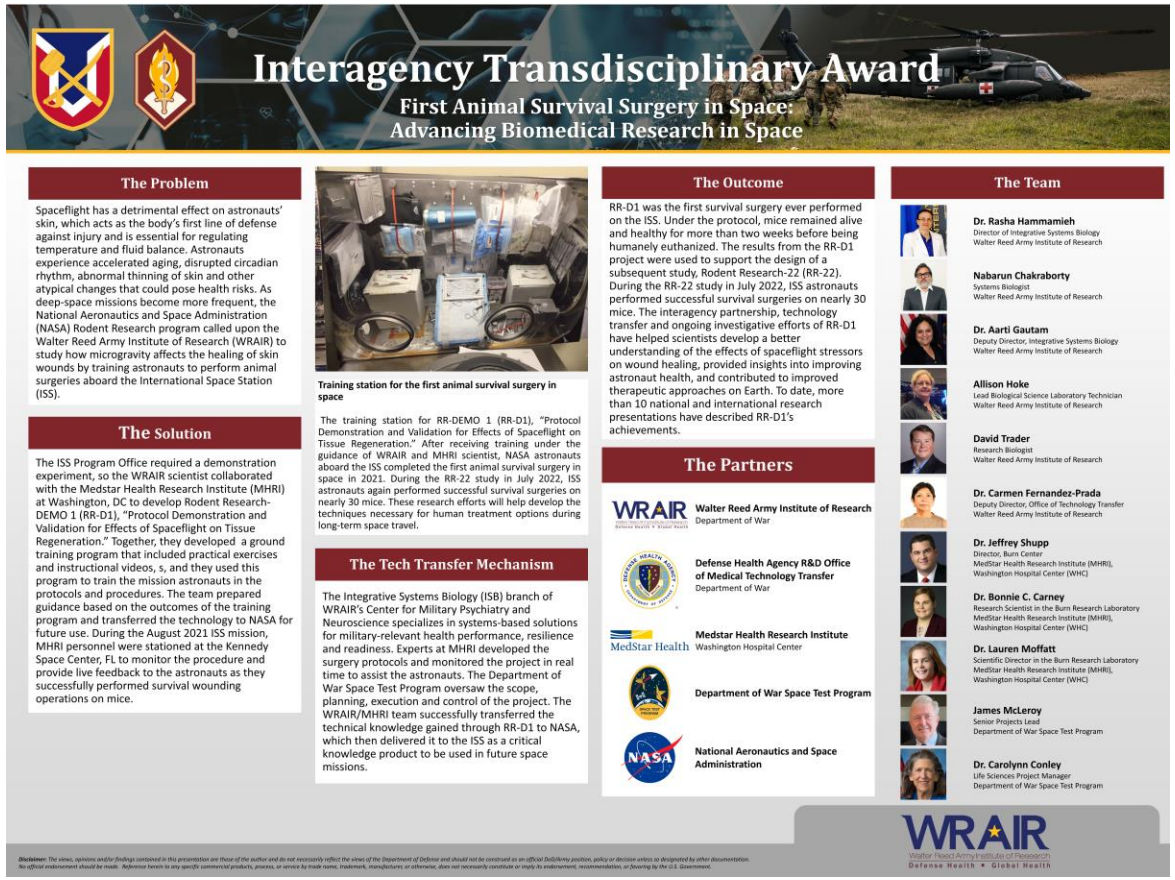
- Bone fracture healing in Space occurs under immune-deficient conditions with a concurrently activated apoptotic network (RR4)
  - FLT-Saline callus samples exhibited reduced immune responses despite expected strong foreign body reactions, suggesting spaceflight-induced immune suppression
- Bone healing phenotype displayed signs of non-union, with gene-metabolite network analyses revealing potential molecular underpinnings of impaired bone repair (RR4)
- Muscle networks showed significantly reduced adaptive trends compared to sham controls, with inhibited bioenergy production potentially compromising cell survival (RR4)

- Osteoblast-specific analyses revealed that BMP-2 signaling in Space operates independently from ground-based pathways, while activating a pro-inflammatory condition (CS-02)

- Collectively, these findings underscore a strong association between immune health and bioenergy synthesis in Space, both of which may critically influence wound healing dynamics during Spaceflight

Challenges and Rewards

WRAIR received FLC award 2026 for NASA/ RRD1 project



Interagency Transdisciplinary Award

First Animal Survival Surgery in Space: Advancing Biomedical Research in Space

The Problem

Spaceflight has a detrimental effect on astronauts' skin, which acts as the body's first line of defense against injury and is essential for regulating temperature and fluid balance. Astronauts experience accelerated aging, disrupted circadian rhythm, abnormal thinning of skin and other atypical changes that could pose health risks. As deep-space missions become more frequent, the National Aeronautics and Space Administration (NASA) Rodent Research program called upon the Walter Reed Army Institute of Research (WRAIR) to study how microgravity affects the healing of skin wounds by training astronauts to perform animal surgeries aboard the International Space Station (ISS).

The Solution

The ISS Program Office required a demonstration experiment, so the WRAIR scientist collaborated with the Medstar Health Research Institute (MHRI) at Washington, DC to develop Rodent Research-DEMO 1 (RR-D1), "Protocol Demonstration and Validation for Effects of Spaceflight on Tissue Regeneration." Together, they developed a ground training program that included practical exercises and instructional videos, s, and they used this program to train the mission astronauts in the protocols and procedures. The team prepared guidance based on the outcomes of the training program and transferred the technology to NASA for future use. During the August 2021 ISS mission, MHRI personnel were stationed at the Kennedy Space Center, FL to monitor the procedure and provide live feedback to the astronauts as they successfully performed survival wounding operations on mice.

Training station for the first animal survival surgery in space

The training station for RR-DEMO 1 (RR-D1), "Protocol Demonstration and Validation for Effects of Spaceflight on Tissue Regeneration." After receiving training under the guidance of WRAIR and MHRI scientist, NASA astronauts aboard the ISS completed the first animal survival surgery in space in 2021. During the RR-22 study in July 2022, ISS astronauts again performed successful survival surgeries on nearly 30 mice. These research efforts will help develop the techniques necessary for human treatment options during long-term space travel.

The Tech Transfer Mechanism

The Integrative Systems Biology (ISB) branch of WRAIR's Center for Military Psychiatry and Neuroscience specializes in systems-based solutions for military-relevant health performance, resilience and readiness. Experts at MHRI developed the surgery protocols and monitored the project in real time to assist the astronauts. The Department of War Space Test Program oversaw the scope, planning, execution and control of the project. The WRAIR/MHRI team successfully transferred the technical knowledge gained through RR-D1 to NASA, which then delivered it to the ISS as a critical knowledge product to be used in future space missions.

The Outcome

RR-D1 was the first survival surgery ever performed on the ISS. Under the protocol, mice remained alive and healthy for more than two weeks before being humanely euthanized. The results from the RR-D1 project were used to support the design of a subsequent study, Rodent Research-22 (RR-22). During the RR-22 study in July 2022, ISS astronauts performed successful survival surgeries on nearly 30 mice. The interagency partnership, technology transfer and ongoing investigative efforts of RR-D1 have helped scientists develop a better understanding of the effects of spaceflight stressors on wound healing, provided insights into improving astronaut health, and contributed to improved therapeutic approaches on Earth. To date, more than 10 national and international research presentations have described RR-D1's achievements.

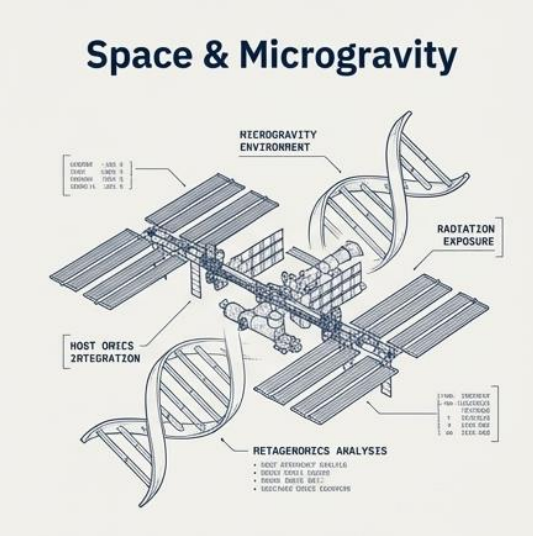
The Partners

- Walter Reed Army Institute of Research, Department of War
- Defense Health Agency R&D Office of Medical Technology Transfer, Department of War
- Medstar Health Research Institute, Washington Hospital Center
- Department of War Space Test Program
- National Aeronautics and Space Administration

The Team

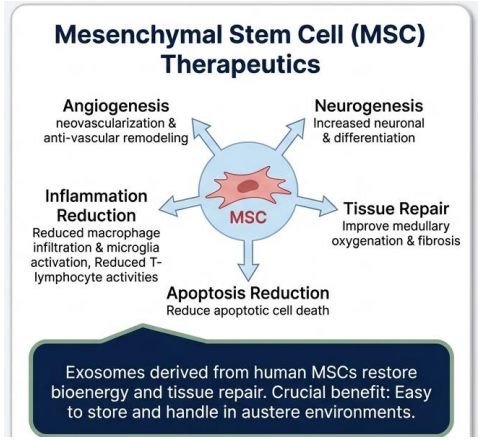
- Dr. Rasha Hammamieh, Director of Integrative Systems Biology, Walter Reed Army Institute of Research
- Nabarun Chakraborty, Systems Biologist, Walter Reed Army Institute of Research
- Dr. Aarti Gautam, Deputy Director, Integrative Systems Biology, Walter Reed Army Institute of Research
- Allison Hoke, Lead Biological Science Laboratory Technician, Walter Reed Army Institute of Research
- David Trader, Research Biologist, Walter Reed Army Institute of Research
- Dr. Carmen Fernandez-Prada, Deputy Director, Office of Technology Transfer, Walter Reed Army Institute of Research
- Dr. Jeffrey Shupp, Director, Burn Center, MedStar Health Research Institute (MHRI), Washington Hospital Center (WHC)
- Dr. Bonnie C. Carney, Research Scientist in the Burn Research Laboratory, MedStar Health Research Institute (MHRI), Washington Hospital Center (WHC)
- Dr. Lauren Moffatt, Scientific Director in the Burn Research Laboratory, MedStar Health Research Institute (MHRI), Washington Hospital Center (WHC)
- James McLeroy, Senior Projects Lead, Department of War Space Test Program
- Dr. Carolynn Conley, Life Sciences Project Manager, Department of War Space Test Program

Thank You



Review of our five Space studies

Session: Current Challenges in DoW Aerospace Medicine
Time: Monday, 1300-1500

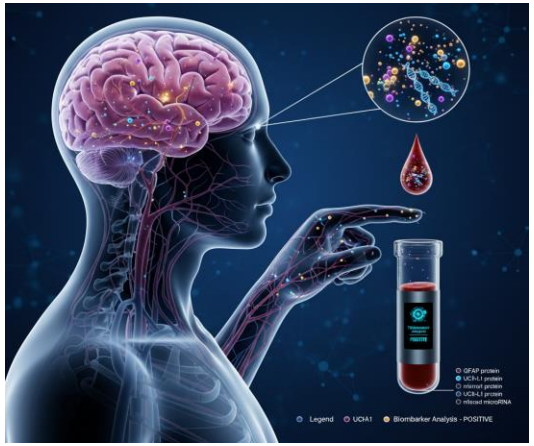


hMSC-ex in Cold stress

Session: Human Performance Optimization in Cold-Weather & High-Altitude Environments
Time: Monday, 1530-1730

hMSC-ex in ASD

Session: Cellular Therapeutics for Trauma
Time: Thursday, 1115-1315



TBI Biomarker

Session: Managing the Golden Hour & Beyond: Innovative TBI Care in Contested Combat Environments
Time: Wednesday, 0830-1000

Radiation Therapeutics

Session: Ops in a Nuclear Environment: Ensuring Medical Readiness & Preparing for Complex Injuries
Time: Wednesday, 1530:1730

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