miRNA-mRNA Interactions in Muscle and Bone Linked to Segmental Bone Defect Using a Mouse Model in Spaceflight

Rasha Hammamieh, PhD

Medical Readiness Systems Biology

Center For Military Psychiatry and Neuroscience

Walter Reed Army Institute of Research

March 24, 2023

WRAIR
Walter Reed Army Institute of Research

Disclaimer

The views, opinions, and findings contained in this report are those of the authors and should not be construed as official Department of the Army position, policy, or decision, unless so designated by other official documentation.

Citations of commercial organizations or trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

Research was conducted under an IACUC-approved animal use protocol in an AAALAC International - accredited facility with a Public Health Services Animal Welfare Assurance and in compliance with the Animal Welfare Act and other federal statutes and regulations relating to laboratory animals

Acknowledgements

WRAIR

Rasha Hammamieh

Nabarun Chakraborty

George Dimitrov

Stacy-Ann Miller

Allison Hoke

Stephen Butler

Bintu Sowe

Routing Yang

CPT Kevin Swift

CPL Phillip Urbanczyk

Pamela Spellman

Dana Youssef

Candace Moyler

Raina Kumar

Janice Gary

William Levangie

Jesse Lynch

DoD STP

Carolynn Conley
James McLeroy
Perry Ballard
John Scott

Indiana University

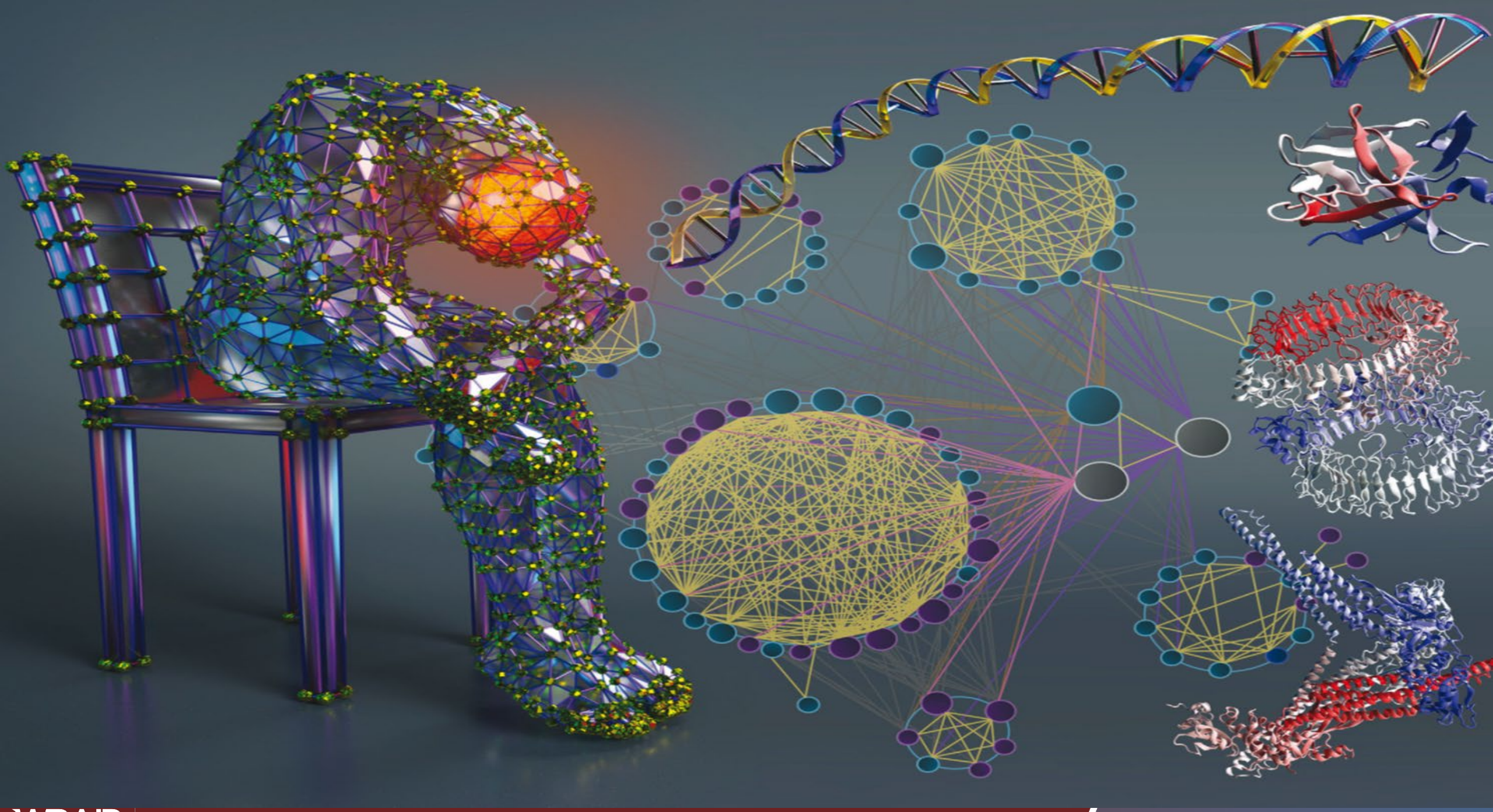
Melissa Kacena & team
Paul Childress

ISS National Lab Team

NASA ARC Rodent Research Team

USAMRICD

LTC Cary Honnold
MAJ Connie Schmitt



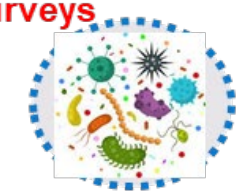
Integrative Biology Approaches to Diseases of Military Relevance

Exposures

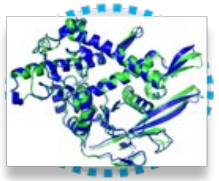
Psychological/clinical Surveys

Clinical

Genomics



Microbiome



Proteomics

Health Wearables/biosensors

Lab Procedures

ROC

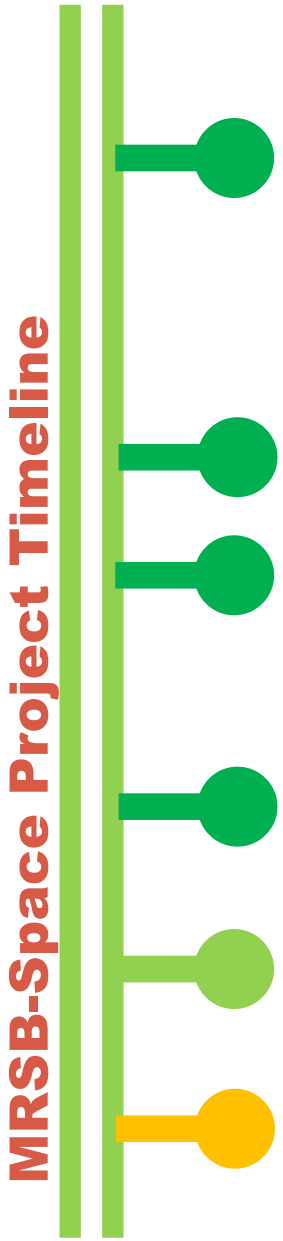
Force Health Protection

Disease Risk, Prognosis, Treatment

Human Performance and Resilience

Precision Operational Medicine

Our Efforts Onboard the International Space Station (ISS)



2011
STS-135: *in vitro* project: assess the effect of microgravity on host responses

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

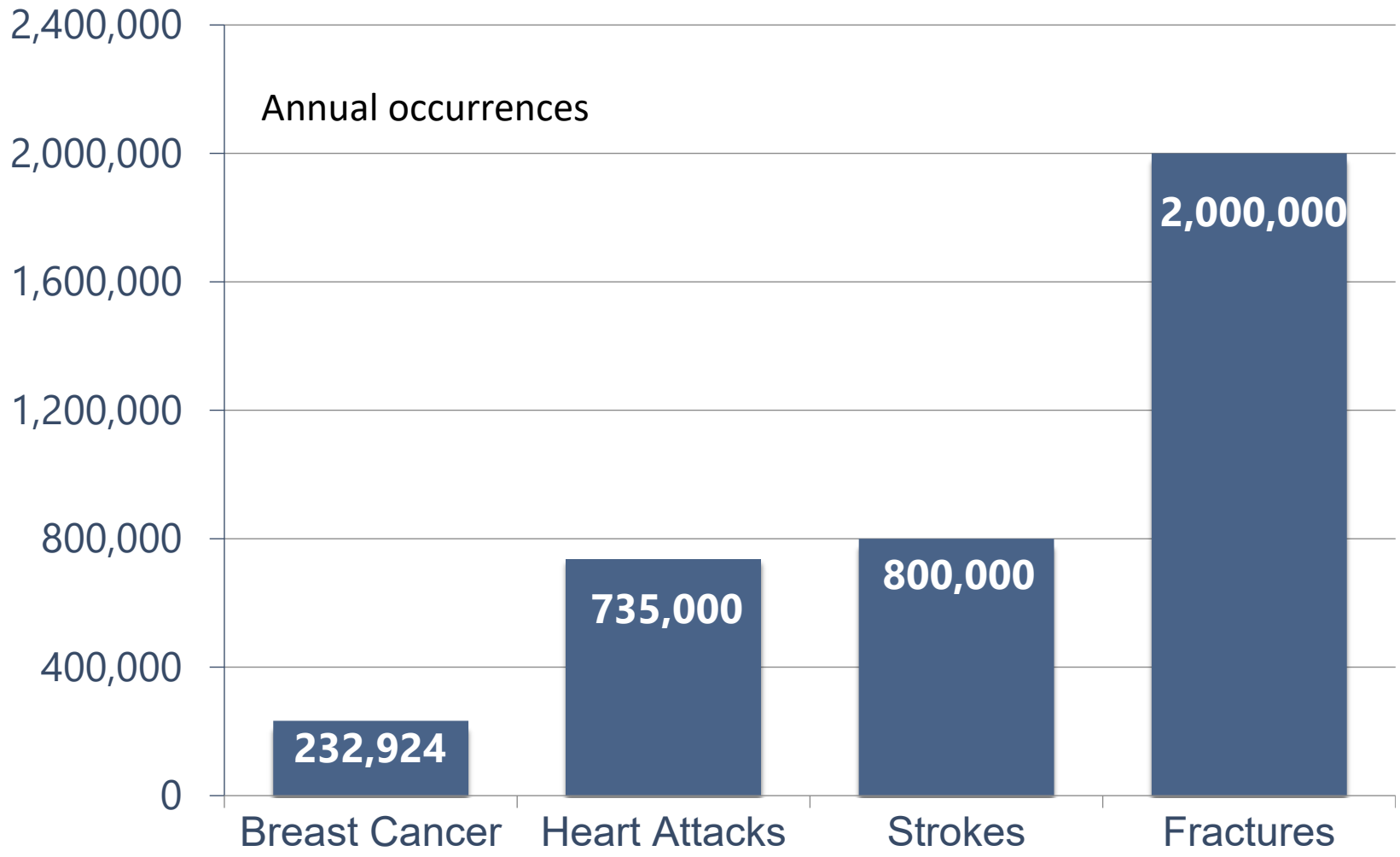
2017
Rodent Research 4 (RR4): (bone regeneration) Collaborating with Indiana University

2019
Cell Science-2 (CS-02): (bone regeneration) *in vitro* project,
Collaborating with Indiana University

2022
Rodent Research 22 (RR22): (wound healing) Collaborating with Washington Hospital Center

TBD: Vaccine Efficacy

Prevalence of Musculoskeletal injuries compared to other issues

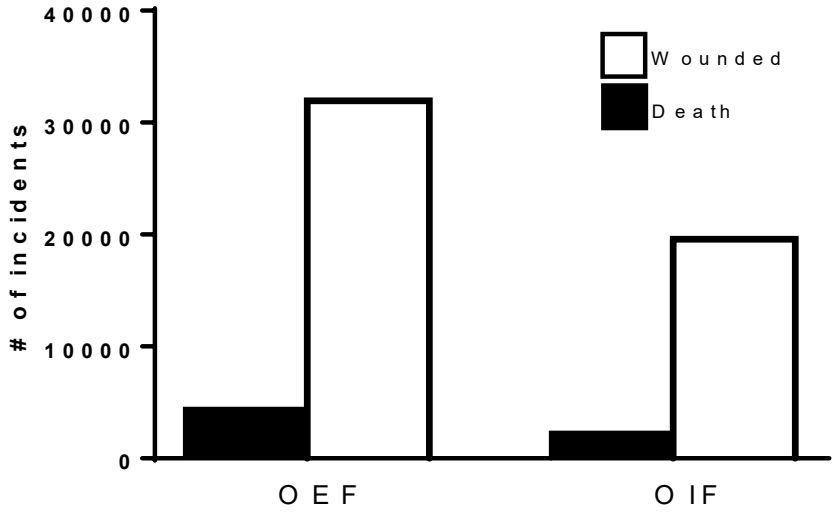


Centers for Disease Control & Prevention 2016
Centers for Disease Control & Prevention, 2015
National Osteoporosis Foundation, 2015

Slide adapted from American Bone health

Bone Defect: Cross-community Health Issue

- Modern warfare has reduced mortality, but comes with heavy cost
  - Healthcare burden increased 5x from Vietnam war to OIF/OEF war
- Orthopaedic injuries and bone fractures resulting from battlefield explosions and accidents are a focus in orthopaedic research
- Animal models don't fully mimic the weightlessness experienced by clinical patients



- Etiology of bone healing is still elusive
  - Bone defect is major career limiting factor of civilian community
  - Unhealed fractures lead to pain, loss of function, and potential amputation

Bone Healing in Microgravity

- Research has shown that bone healing in microgravity is slower and less effective than on Earth, with a higher risk of complications such as bone loss, delayed union, and non-union. This is due to several factors, including the reduced mechanical stress on the bone, altered bone remodeling, and changes in the balance of bone-forming and bone-resorbing cells.
- Various measures have been taken to promote bone healing in microgravity, such as exercise regimens, nutritional supplements, and the use of specialized medical devices.
- Medical devices such as low-intensity pulsed ultrasound (LIPUS) and bone morphogenetic proteins (BMPs) have also been investigated as potential treatments for bone healing in microgravity. LIPUS has been shown to accelerate bone healing and improve bone density in animal studies, while BMPs have been used successfully to promote bone growth in humans with bone defects.
- Research is needed to further understand the underlying mechanisms and to develop more effective treatments for bone healing in microgravity.

Objective and Approaches

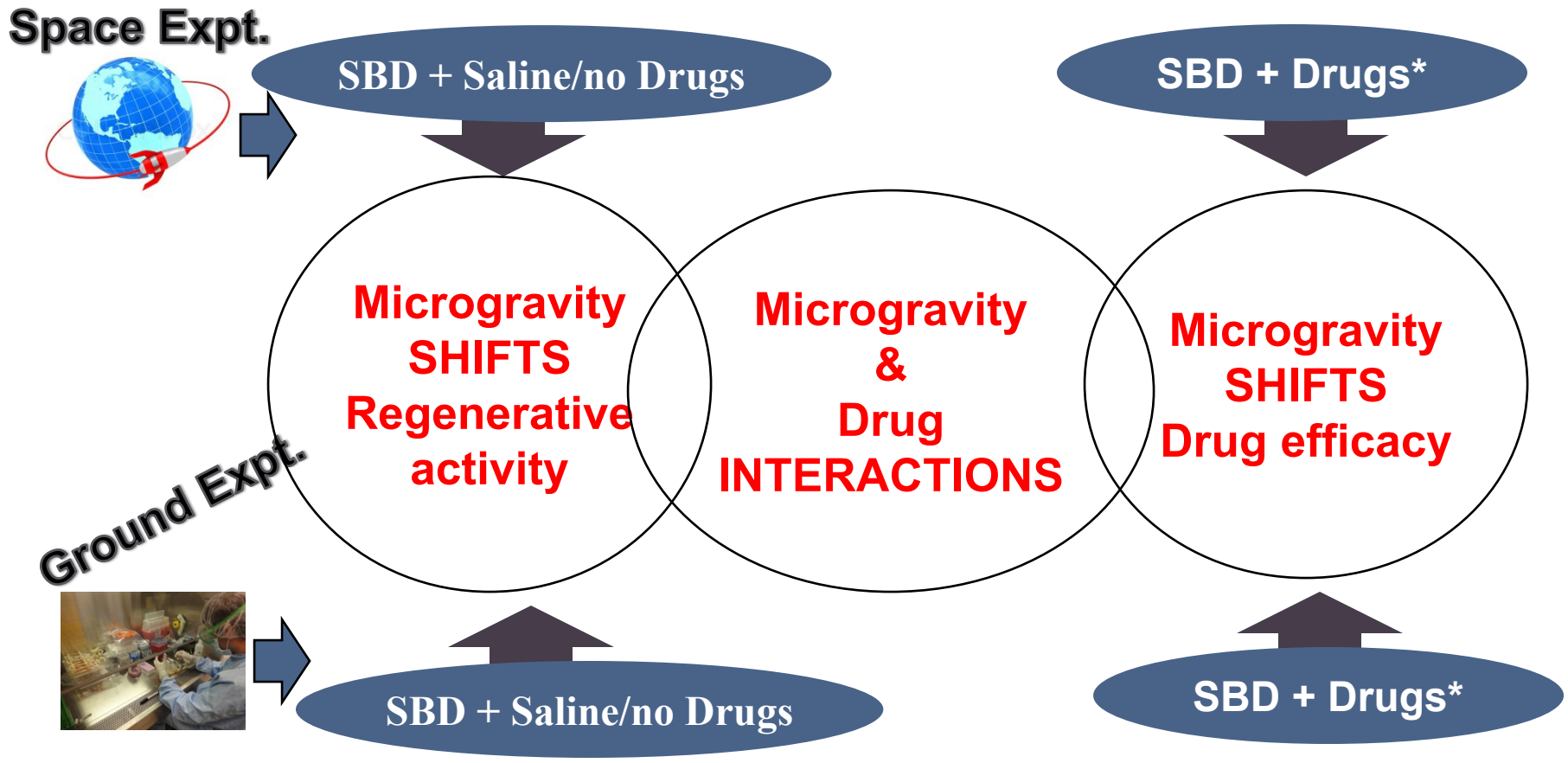
Objective

To gain a comprehensive understanding of the effects of µG and other comorbidities of spaceflight on mammalian tissue regeneration using a *in vivo* (mouse) segmental bone defect model. Further to check the efficacy of bone regeneration FDA approved drugs.

Approach

We will undertake a Systems Biology effort to integrate multi-omics reads: mRNA sequencing, miRNA profile, global changes in DNA methylation patterns, bioenergetics and changes in expression of targeted sets of proteins, and changes in the microbiome during the skin wound healing process for mice in on the ground, in spaceflight, and after return from spaceflight (post- flight).

Study Design: At a Glance

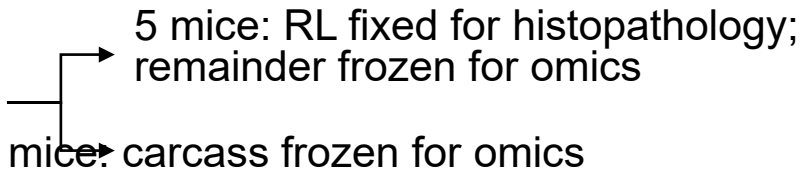


*Drugs:

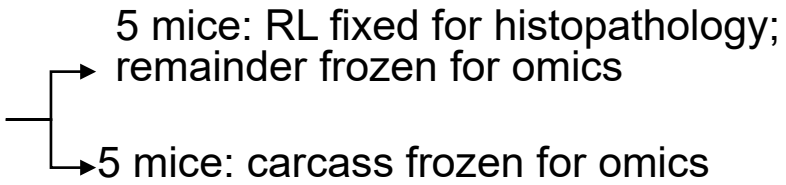
1. Bone Morphogenetic protein-2 (BMP-2): FDA-approved bone healing agent
2. Thrombopoietin (*TPO*): A recently patented *bone* healing agent

Experiment Design: Flight and Ground

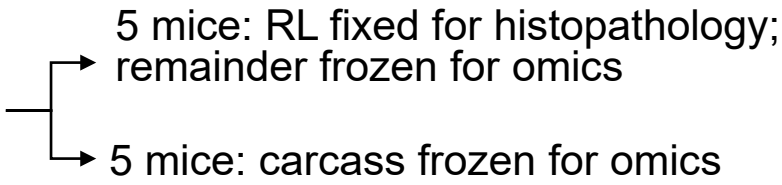
1) 10 mice with SBD + BMP2 treatment



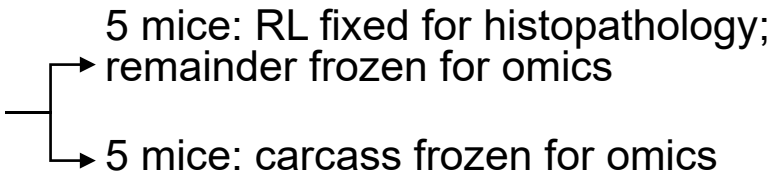
2) 10 mice with SBD + alternative treatment



3) 10 mice with SBD + Saline treatment



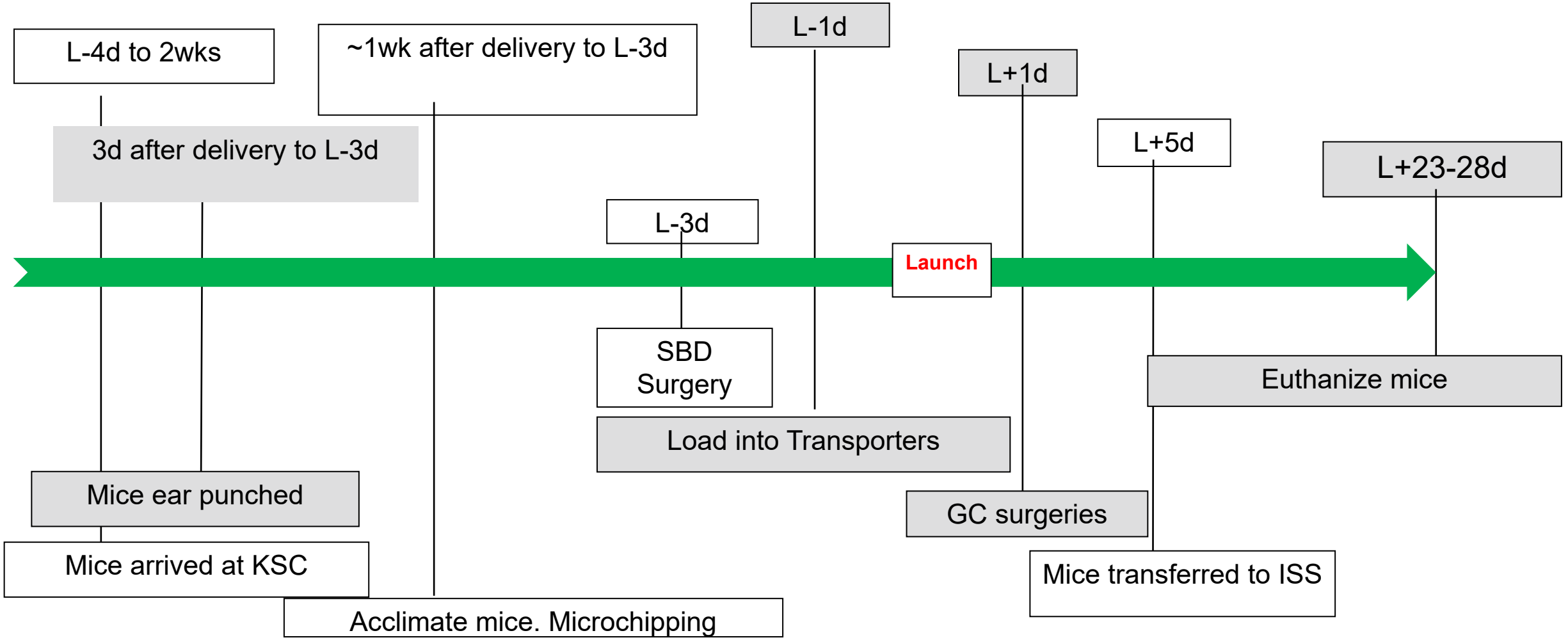
4) 10 mice no SBD (healthy/sham)



SBD- Segmental Bone Defect
RL- Right Limb

Rodent Research 4 (RR4)

Animal study- Launched in 2017

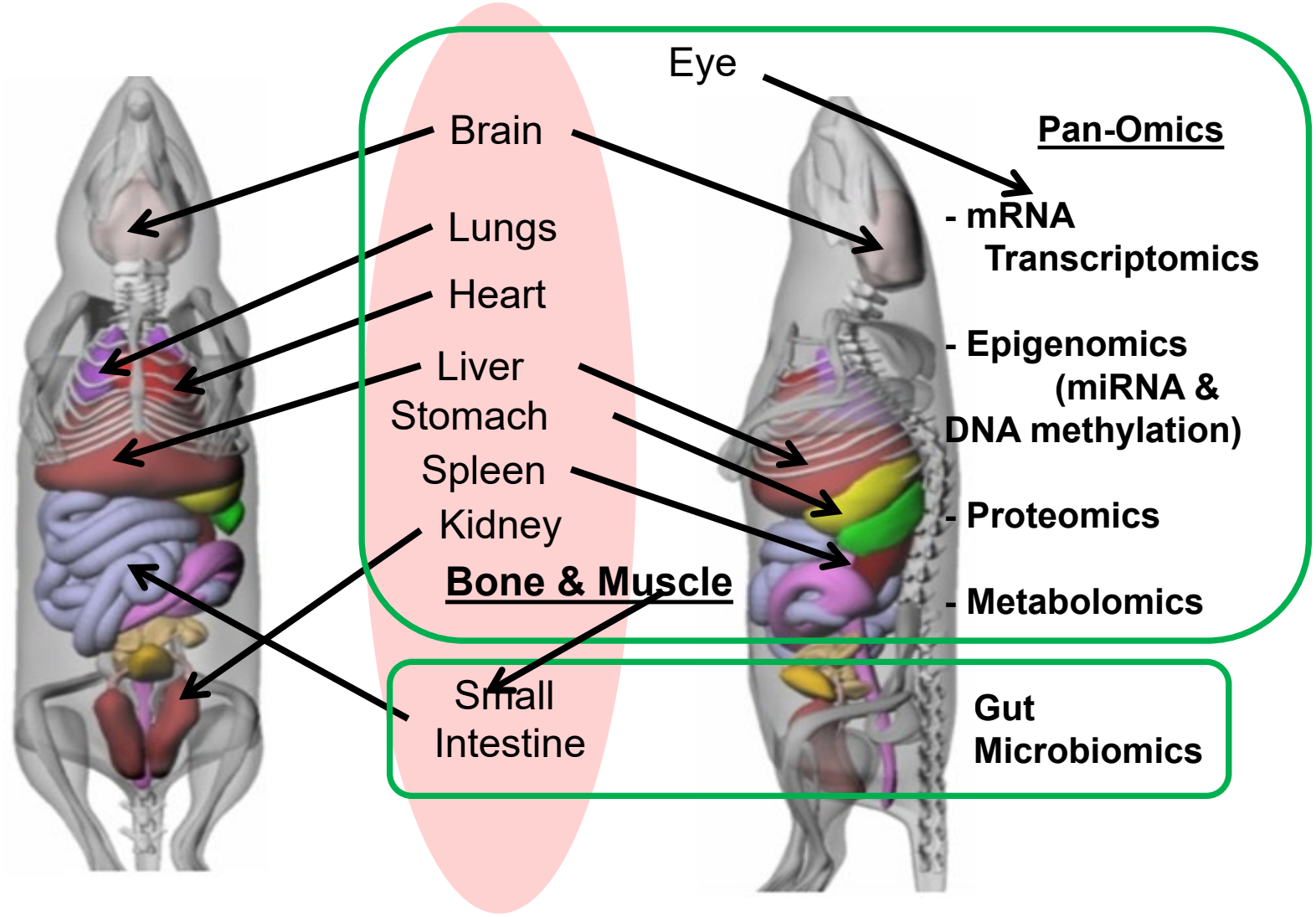


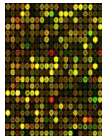
GC: Ground control; L: Launch; KSC: Kennedy Space Center; ISS: International Space Station, SBD: Segmental Bone Defect

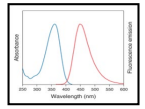
Animal cage used in Space

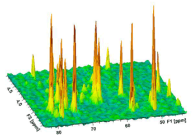
https://www.nasa.gov/mission_pages/station/research/news/rodent_research

RR4: Tissues/ Samples to Investigate









RR4: Molecular assay flow

Cryo-mill

Lyophilized Samples

RNA extraction

mRNA seq: Illumina HiSeq
miRNA Seq: Illumina small RNA seq

Q-TOF Premier mass spec (Waters, Inc.)

Metabolomics assays

Peaks annotation

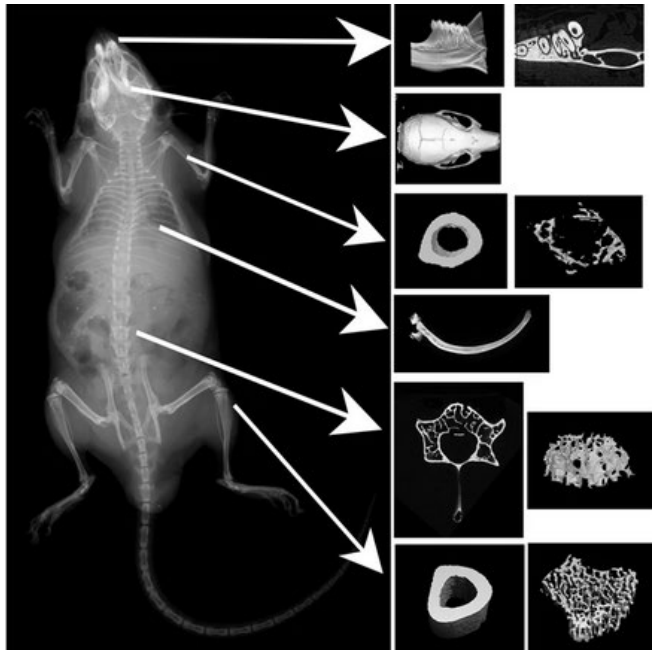
Protein preparation

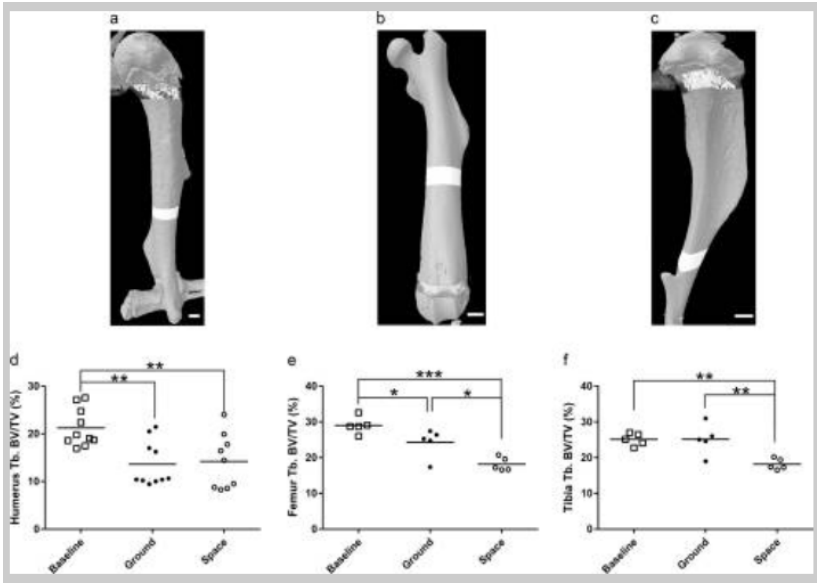
Olink Mouse panel

HD Biomark

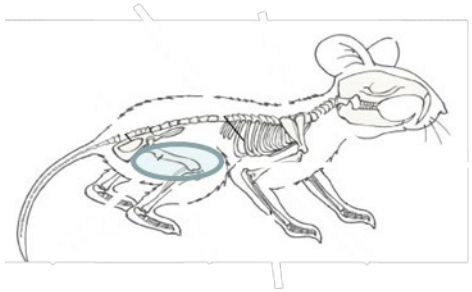
RR4: Major Findings

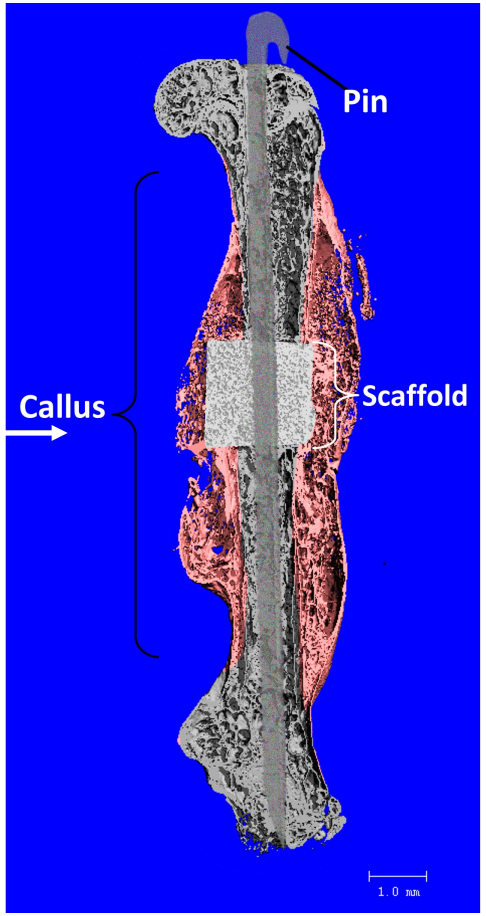
- Negative effects of spaceflight on each skeletal compartment was due to an enhanced loss of bone and sometimes due to a failure to accumulate bone mass





RR4: Spaceflight delayed bone healing





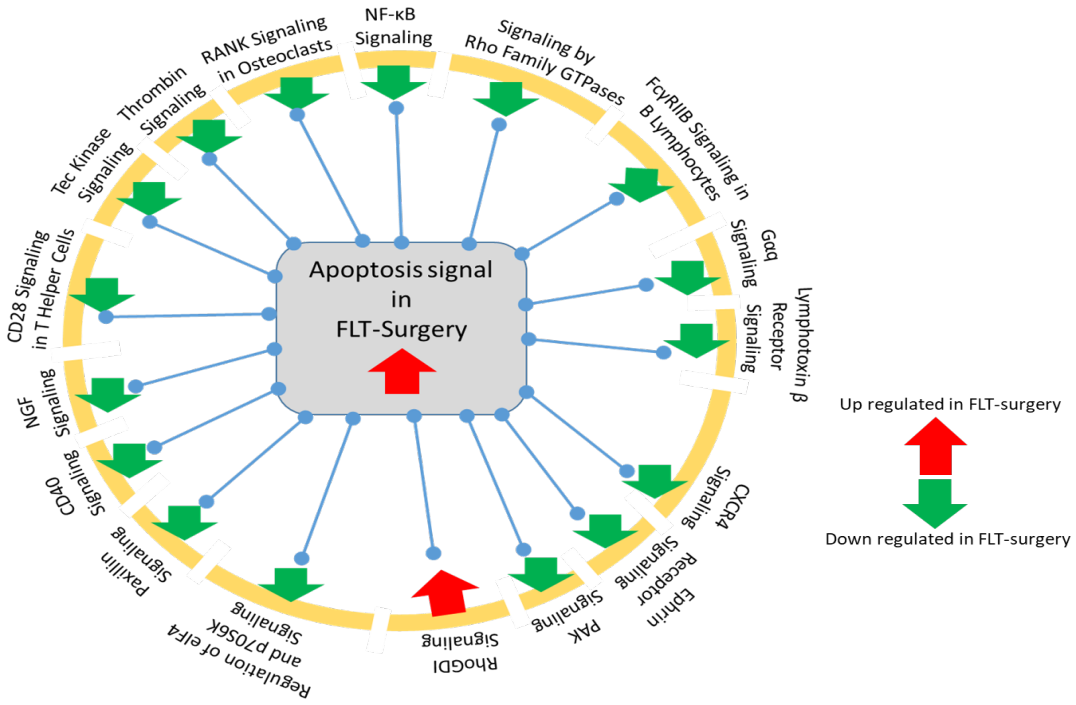
Saline

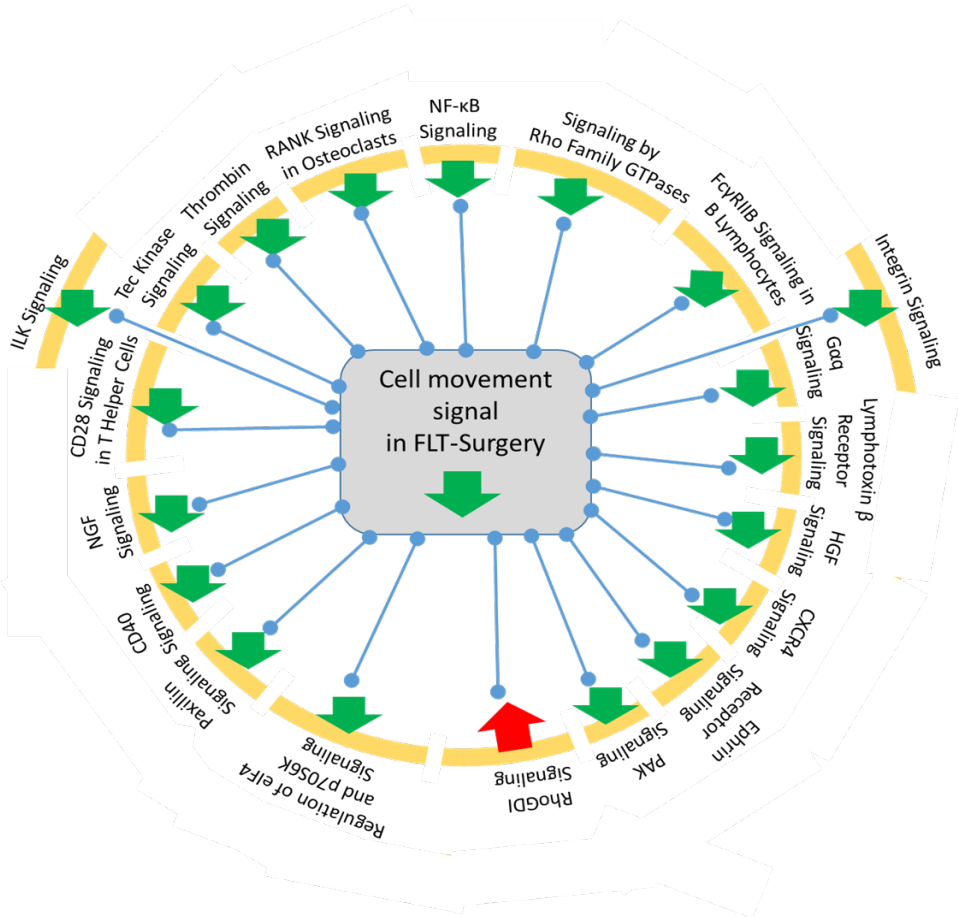
Ground

Flight

- Bone healing in spaceflight was delayed in comparison to on Ground
- Complete bone bridging was not observed in mice with bone defects
- Enhanced loss of bone due to a failure to accumulate bone mass

RR4 Bone: Cluster of Gene Expression Molecular Network

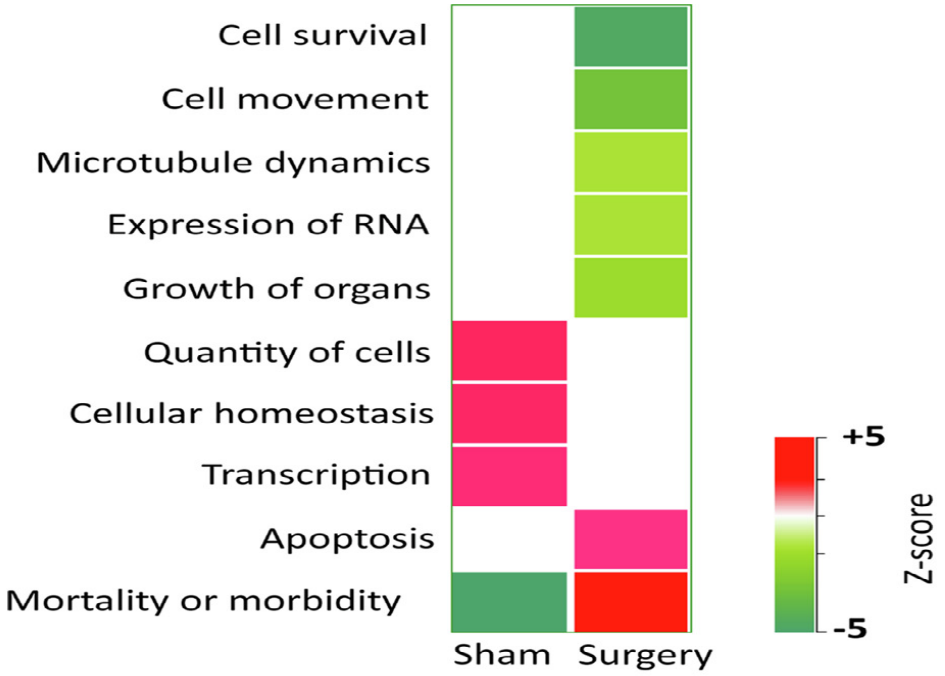




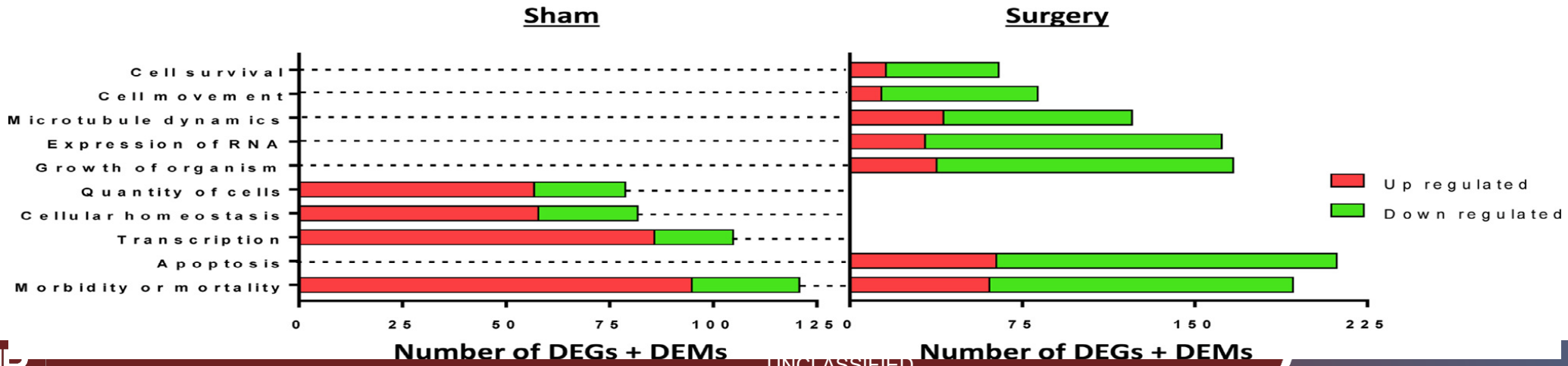
- Molecular analysis found a network cluster that activated apoptosis (cell death) but inhibited cell movement
- These data underlines the risk of delayed bone healing in space/ during chronic unloading

Biological functions in bone- Genes and Metabolites
Effect of microgravity on Sham and Surgery

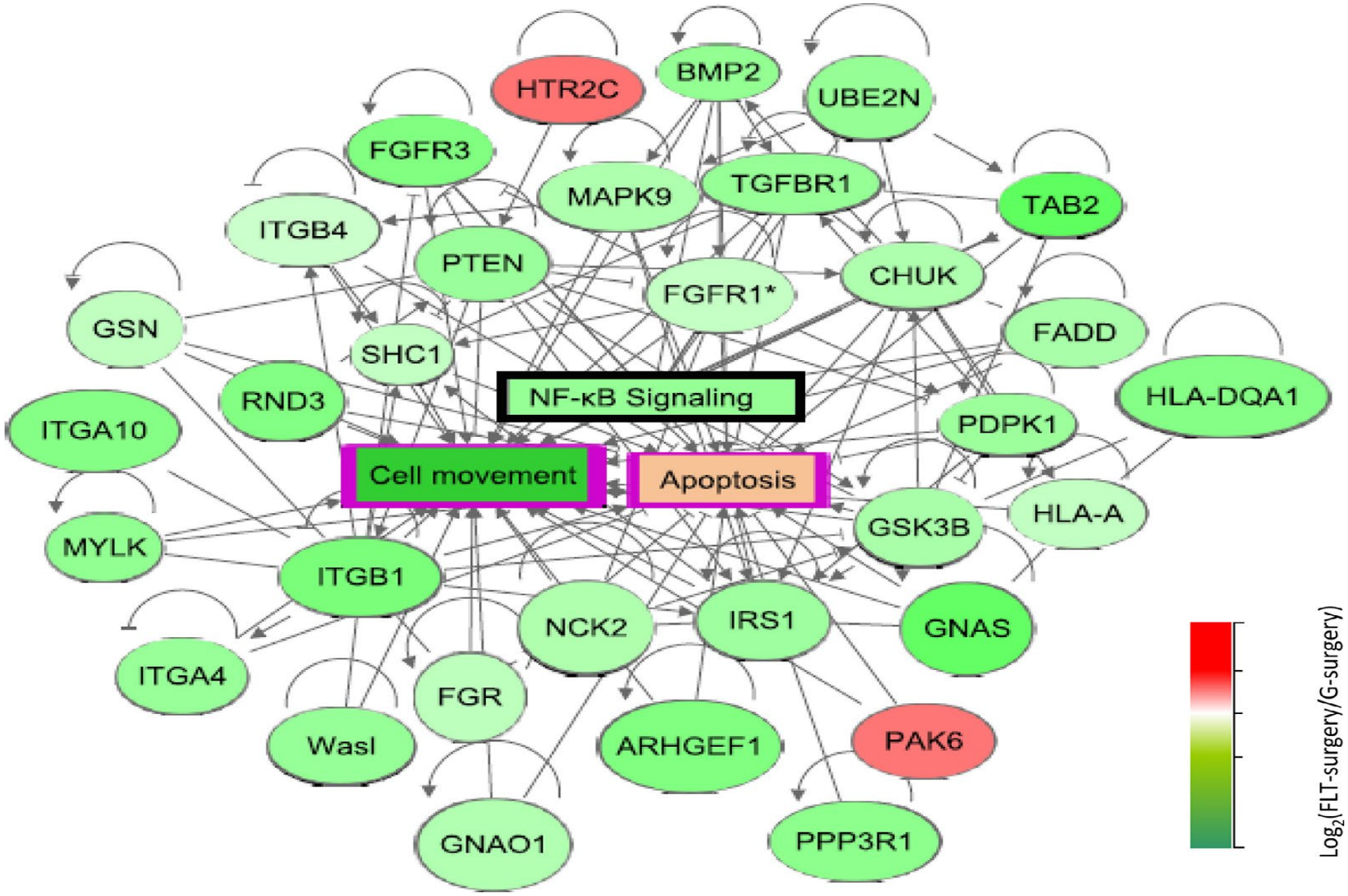
(A)



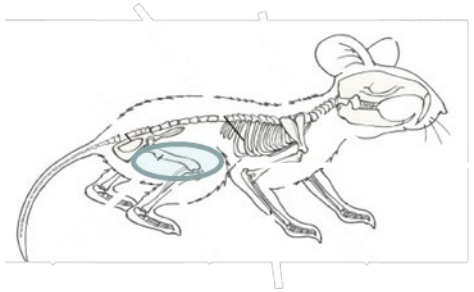
(B)

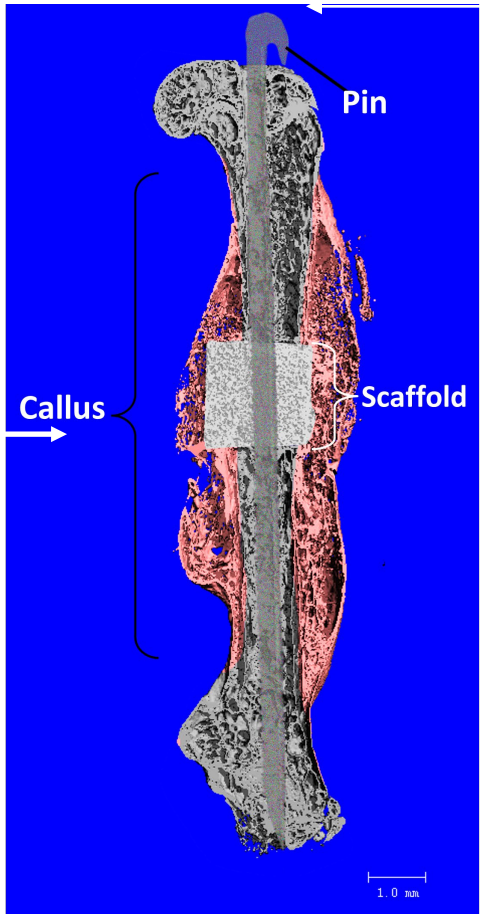


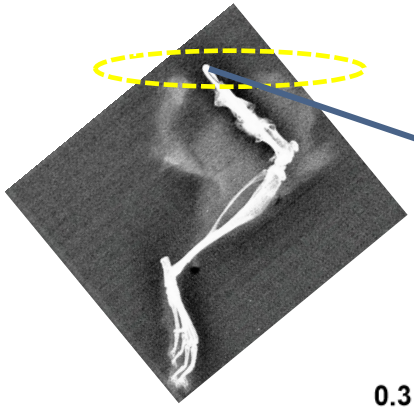
Bone: NFKB signaling in surgery mice



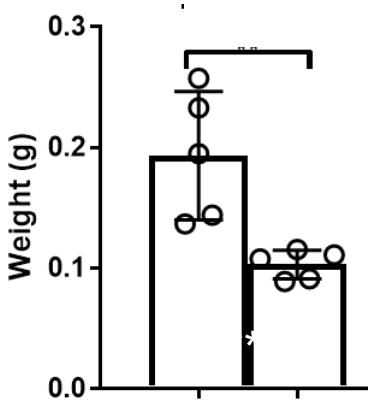
Spaceflight reduced quadriceps mass in healthy/sham mice





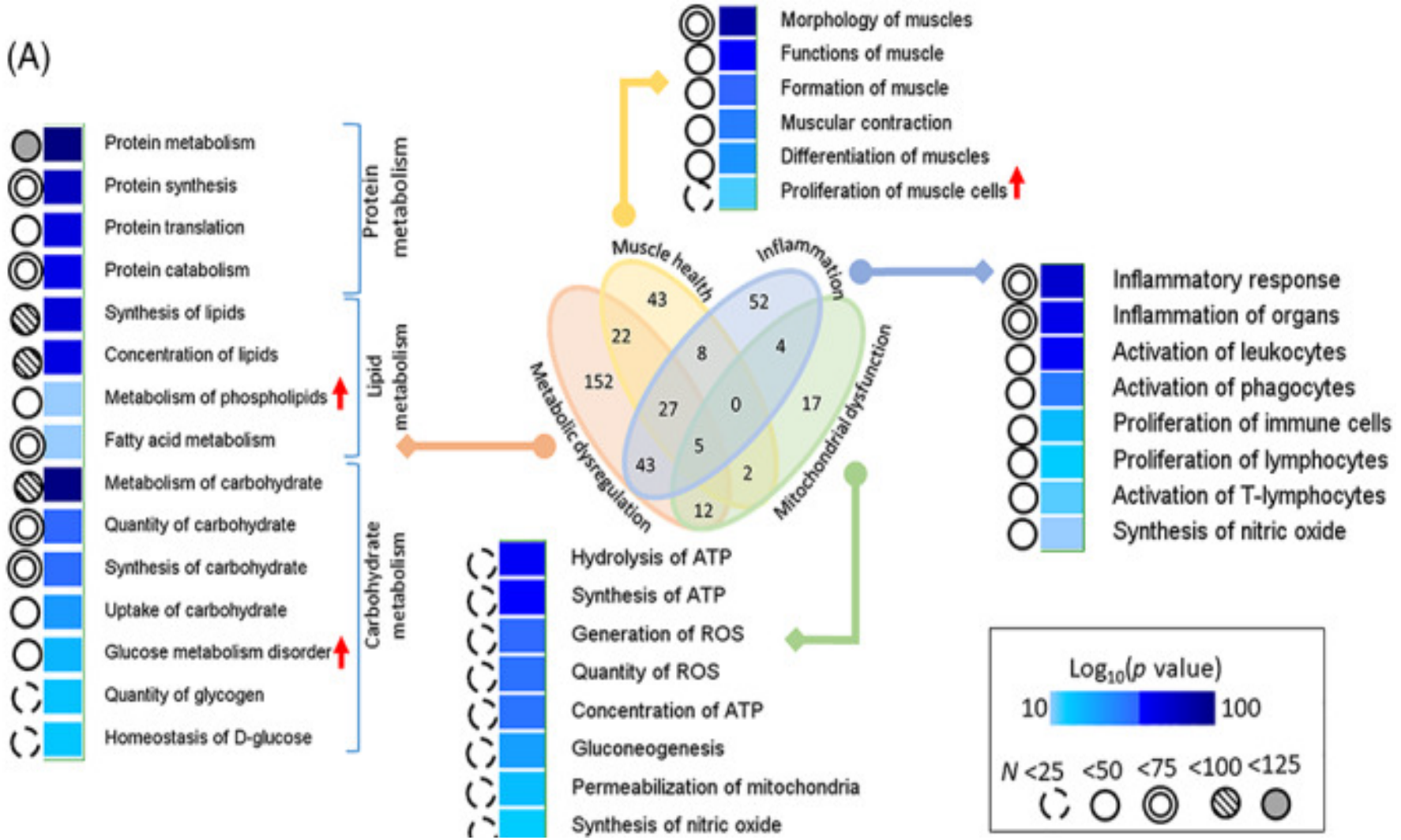


Quadriceps collected at this site



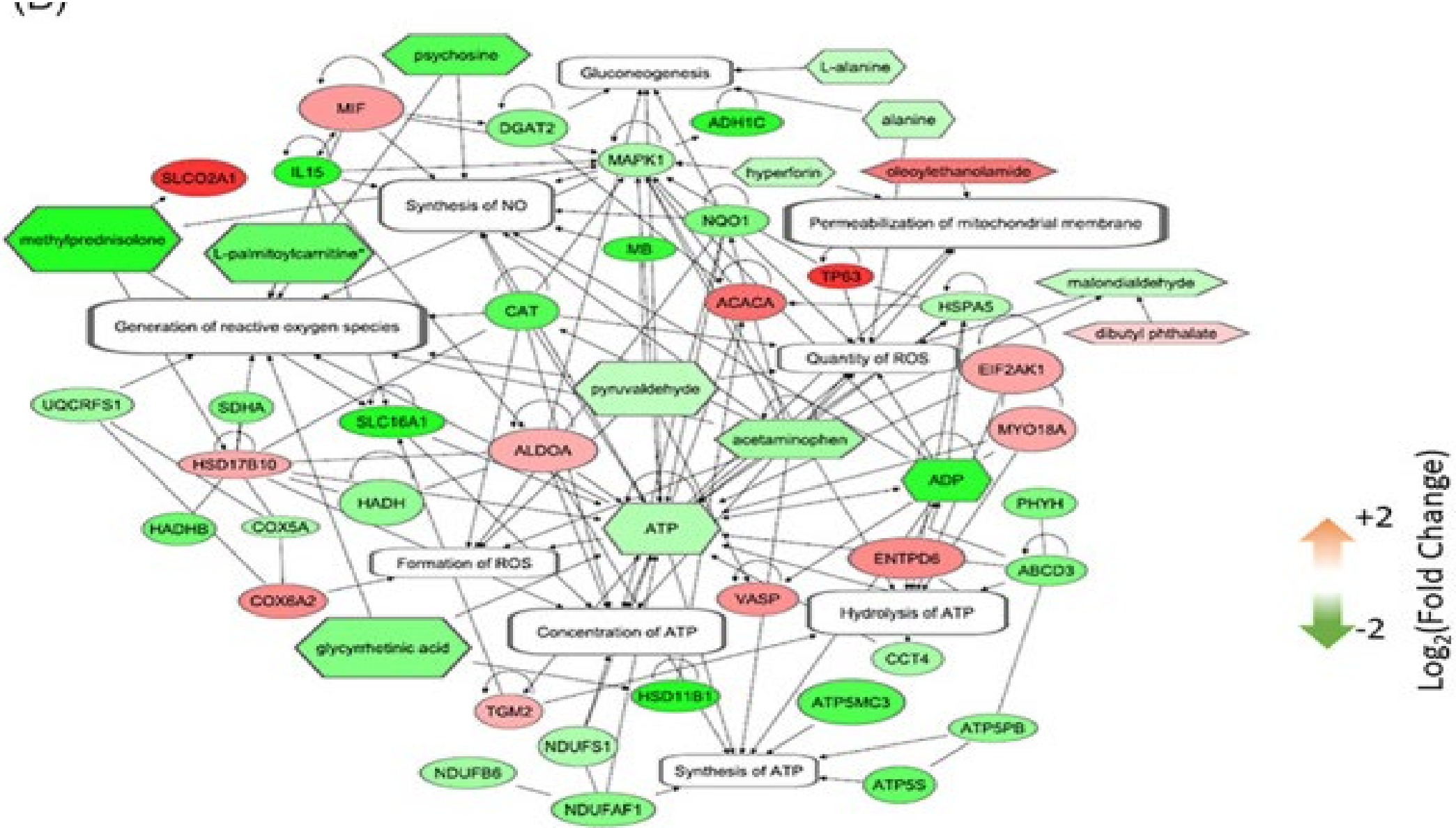
Quad: Non-Canonical networks

- Muscle tissues near the bone defect site were analyzed for gene expression regulation



- An intricate combinations of comorbidities towards muscular dystrophy in spaceflight

Quad: Bioenergetics networks



Gene-metabolite networks associated with impediment of bone fracture repair in spaceflight

Nabarun Chakraborty [a,1], Ariane Zamarioli [b,c,1], Aarti Gautam [a], Ross Campbell [a,d], Stephen K Mendenhall [f], Paul J. Childress [b], George Dimitrov [a,d], Bintu Sowe [b,e], Aamir Tucker [b], Liming Zhao [b], Rasha Hammamieh [a,*,2], Melissa A. Kacena [b,g,2]

Gene-Metabolite Network Linked to Inhibited Bioenergetics in Association With Spaceflight-Induced Loss of Male Mouse Quadriceps Muscle

Nabarun Chakraborty,[1,2] David L Waning,[3] Aarti Gautam,[2] Allison Hoke,[2,4] Bintu Sowe,[2,4] Dana Youssef,[2,4] Stephan Butler,[1,2] Michael Savaglio,[5] Paul J Childress,[5] Raina Kumar,[2] Candace Moyler,[2,4] George Dimitrov,[1,2] Melissa A Kacena,[5,6] and Rasha Hammamieh[2]

Follow-up study

How the underlying molecular events as well as phenotypic observations correlate to miRNA analysis?

1) 10 mice with SBD + Saline treatment

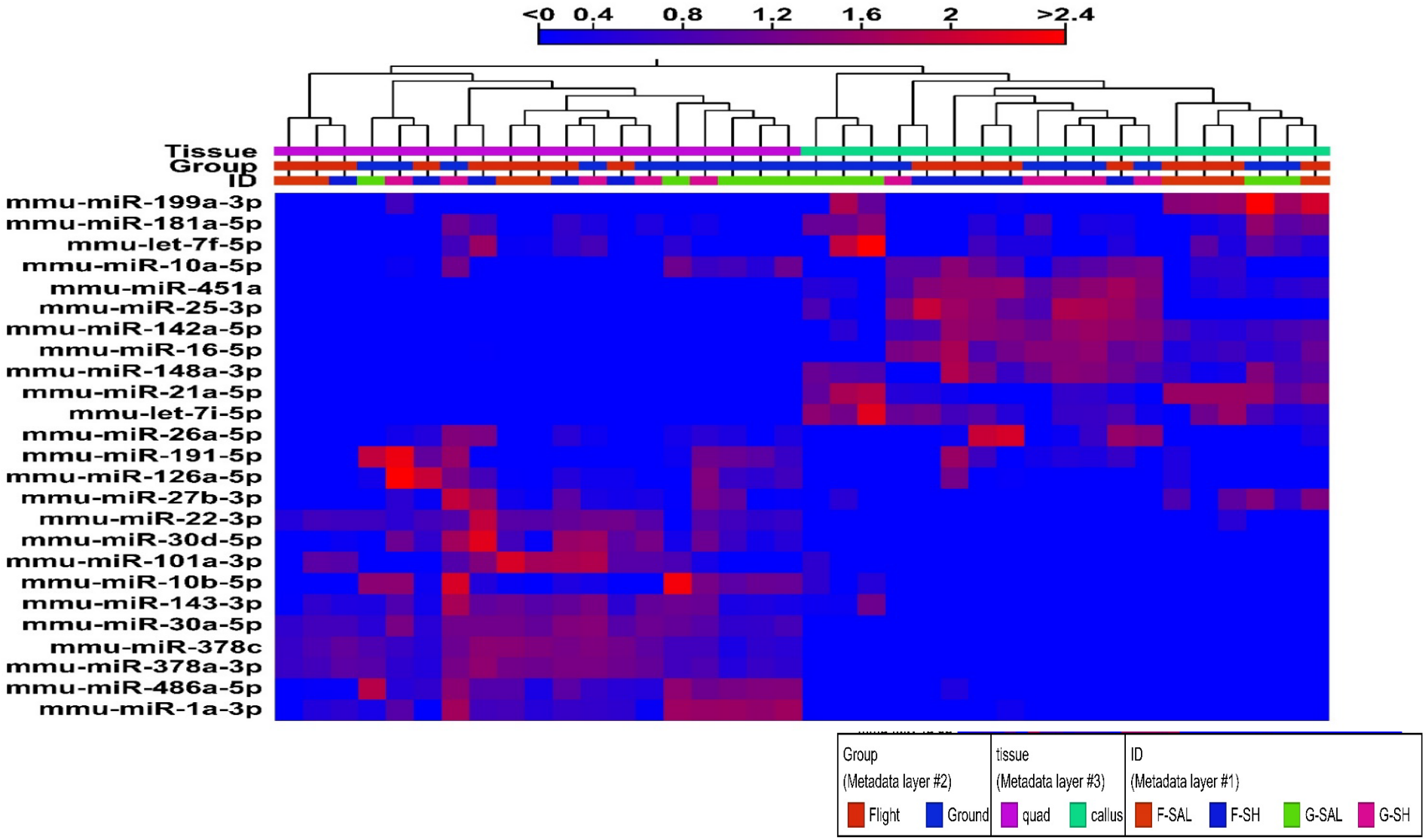
- **Flight**
- **Ground**

2) 10 mice no SBD (healthy/sham)

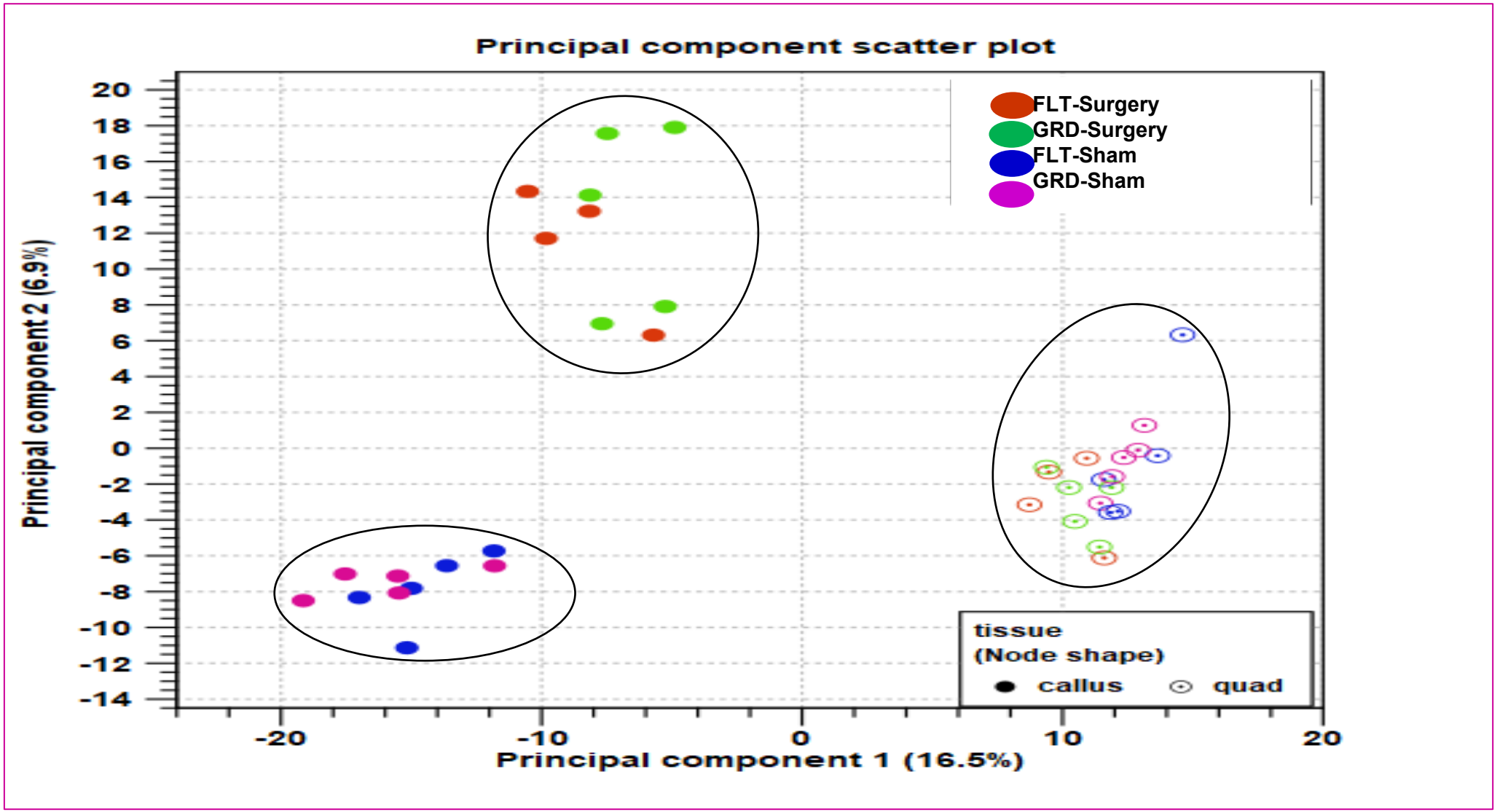
- **Flight**
- **Ground**

SBD- Segmental Bone Defect

Heatmap: miRNA in Callus and Quad



Quad-Callus:Principal Component Analysis-miRNA

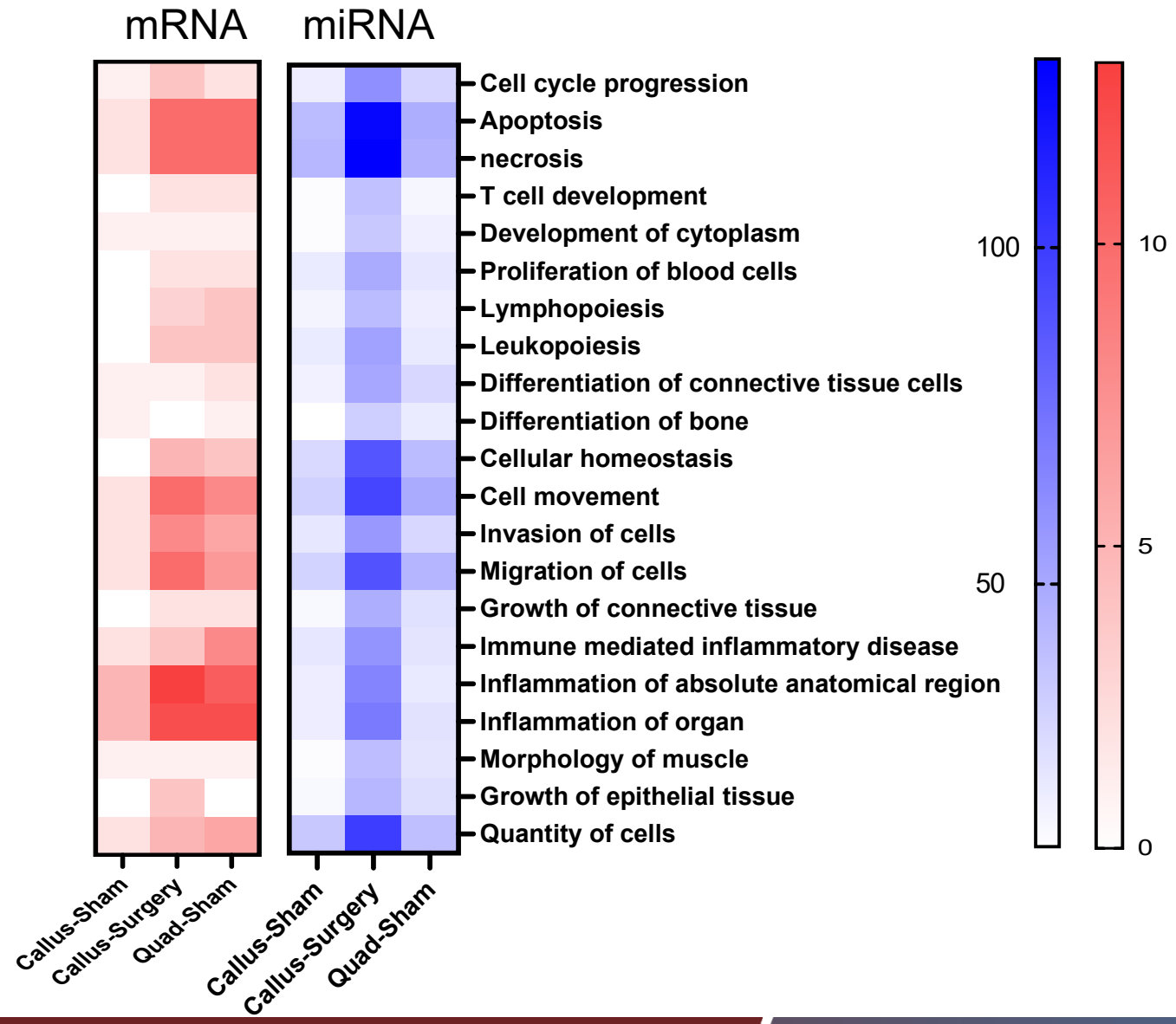


Diseases and Biofunctions: miRNA Analysis

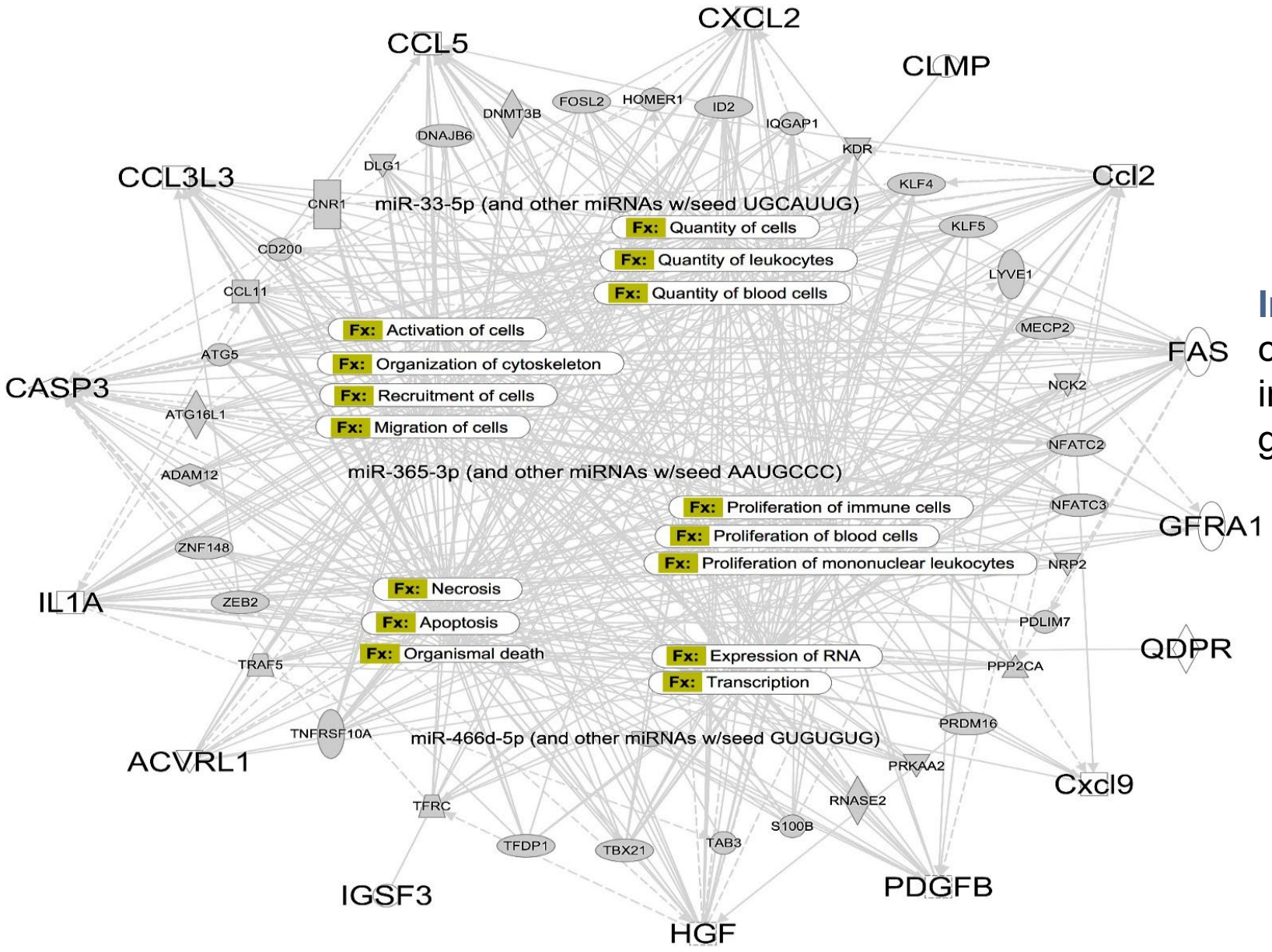
	Surgery +Flight	Surgery	Flight	Surgery +Flight	Surgery	Flight
Diseases and Bio Functions	**Callus**			**Quad**		
Leukopoiesis	-2.621	-2.621		-0.44		
Granulopoiesis	-2.425	-2.425				
Myelopoiesis of leukocytes	-2.425	-2.425				
Differentiation of myeloid leukocytes	-2.425	-2.425				
Differentiation of progenitor cells	-2.425					
Differentiation of hematopoietic progenitor	-2.213			-1.154		
Metastasis of cells	-1.082	-1.082				
Neoplasia of cells	-0.537	1.02				
Apoptosis	-0.364	-1.099		-0.392	-1.817	
Necrosis	-0.274	-0.967		-0.458	-1.506	
Migration of cells	0.642	1.12		1.027		
Cell viability	1.068	0.14		0.628		
Fibrosis		1.982				
Tubulation of cells		1.067				

miRNA and mRNA integrative analysis

Callus tissue of Surgery mice had maximum number of mRNA & miRNA in different networks followed by quad of sham mice and callus of sham mice

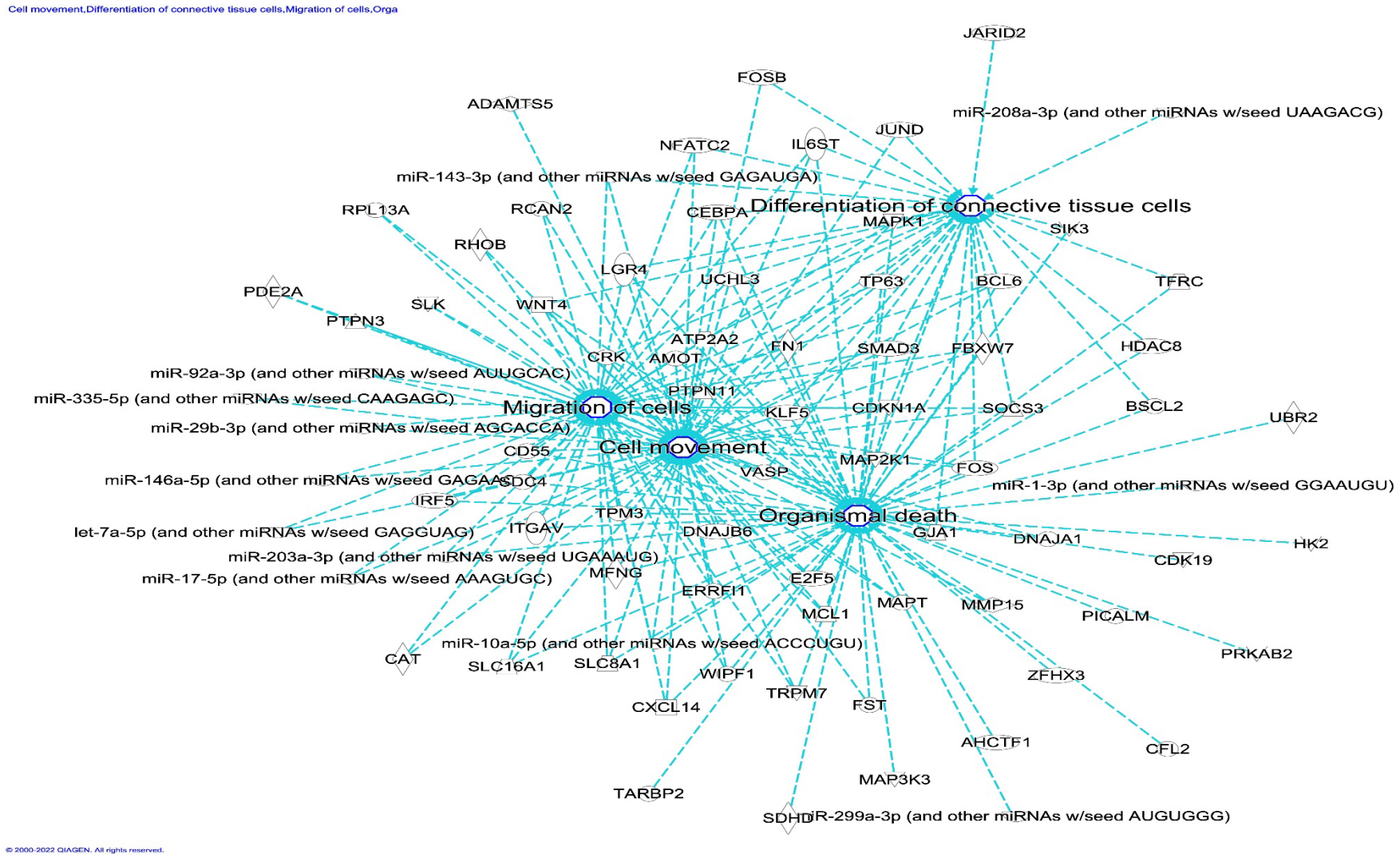


miRNA/mRNA/protein integrative analysis
Microgravity effects on callus



Imbalance of homeostatic functions
cell death, cell number, cell migration, immune response, and gene expression changes.

miRNA/mRNA/protein integrative analysis
Microgravity effects on Quad



Summary

- Adverse effects of spaceflight on musculoskeletal health
  - Spaceflight induced stress negatively impacted bone healing
  - Muscle mass was reduced in healthy/ sham mice in spaceflight

- Muscle (mRNA) : Inhibited protein synthesis and inflammation and elevated energy deficiency and metabolism dysfunction

- Bone (mRNA) Bone defect in space showed significantly higher adverse impact and met enhanced apoptosis and inhibited cellular migration/ immune response

- miRNA data: Callus and quad tissue have distinct miRNA profiles. Surgery had a greater impact on callus tissue as compared to quad tissue.

- miRNA data: Surgery as well as Flight plus surgery altered cell synthesis pathways, apoptosis, cell migration pathways in the tissues.

Summary

- miRNA:mRNA data :However, flight effect on surgery were much greater as observed by number of miRNA and mRNA in each of the networks.
- miRNA:mRNA data: Strongly validated the results from mRNA data where Apoptosis, quantity of cells, necrosis pathways are shown to be impacted.
- miRNA:mRNA data: Integrating the data revealed the networks altered due to flight as miRNA data by itself did not show major impact on changes in networks
- miRNA:mRNA: protein data: Microgravity perturbed cell homeostasis functions bone tissues

Thank-you

1: Chakraborty N, Gautam A, Muhie S, Miller SA, Jett M, Hammamieh R. An integrated omics analysis: impact of microgravity on host response to lipopolysaccharide in vitro. BMC Genomics. 2014 Aug 7;15(1)
2: Chakraborty N, Cheema A, Gautam A, Donohue D, Hoke A, Conley C, Jett M, Hammamieh R. Gene-metabolite profile integration to understand the cause of spaceflight induced immunodeficiency. NPJ Microgravity. 2018 Jan 29;4:4.
3: Childress P, Brinker A, Gong CS, Harris J, Olivos DJ 3rd, Rytlewski JD, Scofield DC, Choi SY, Shirazi-Fard Y, McKinley TO, Chu TG, Conley CL, Chakraborty N, Hammamieh R, Kacena MA. Forces associated with launch into space do not impact bone fracture healing. Life Sci Space Res (Amst). 2018 Feb;16:52-62. doi: 10.1016/j.lssr.2017.11.002
4: Dadwal UC, Maupin KA, Zamarioli A, Tucker A, Harris JS, Fischer JP, Rytlewski JD, Scofield DC, Wininger AE, Bhatti FUR, Alvarez M, Childress PJ, ChakrabortyN, Gautam A, Hammamieh R, Kacena MA. The effects of spaceflight and fracture healing on distant skeletal sites. Sci Rep. 2019 Aug 6;9(1):11419.
5: Maupin KA, Childress P, Brinker A, Khan F, Abeysekera I, Aguilar IN, Olivos DJ 3rd, Adam G, Savaglio MK, Ganesh V, Gorden R, Mannfeld R, Beckner E, Horan DJ, Robling AG, Chakraborty N, Gautam A, Hammamieh R, Kacena MA. Skeletal adaptations in young male mice after 4 weeks aboard the International Space Station. NPJ Microgravity. 2019 Sep 24;5:21.
6: Chakraborty N, Schmitt CW, Honnold CL, Moyler C, Butler S, Nachabe H, Gautam A, Hammamieh R. Protocol Improvement for RNA Extraction From Compromised Frozen Specimens Generated in Austere Conditions: A Path Forward to Transcriptomics-Pathology Systems Integration. Front Mol Biosci. 2020 Jul 22;7:142.
7: Chakraborty N, Waning DL, Gautam A, Hoke A, Sowe B, Youssef D, Butler S, Savaglio M, Childress PJ, Kumar R, Moyler C, Dimitrov G, Kacena MA, Hammamieh R. Gene-Metabolite Network Linked to Inhibited Bioenergetics in Association With Spaceflight-Induced Loss of Male Mouse Quadriceps Muscle. J Bone Miner Res. 2020 Oct;35(10):2049-2057.
8: Zamarioli A, Campbell ZR, Maupin KA, Childress PJ, Ximenez JPB, Adam G, Chakraborty N, Gautam A, Hammamieh R, Kacena MA. Analysis of the effects of spaceflight and local administration of thrombopoietin to a femoral defect injury on distal skeletal sites. NPJ Microgravity. 2021 Mar 26;7(1):12.
9: Chakraborty N, Zamarioli A, Gautam A, Campbell R, Mendenhall SK, Childress PJ, Dimitrov G, Sowe B, Tucker A, Zhao L, Hammamieh R, Kacena MA. Gene- metabolite networks associated with impediment of bone fracture repair in spaceflight. Comput Struct Biotechnol J. 2021 Jun 8;19:3507-3520