Circulating miRNA Signature Predicts Health Risks Associated with Cancer and Spaceflight

National Aeronautics and Space Administration

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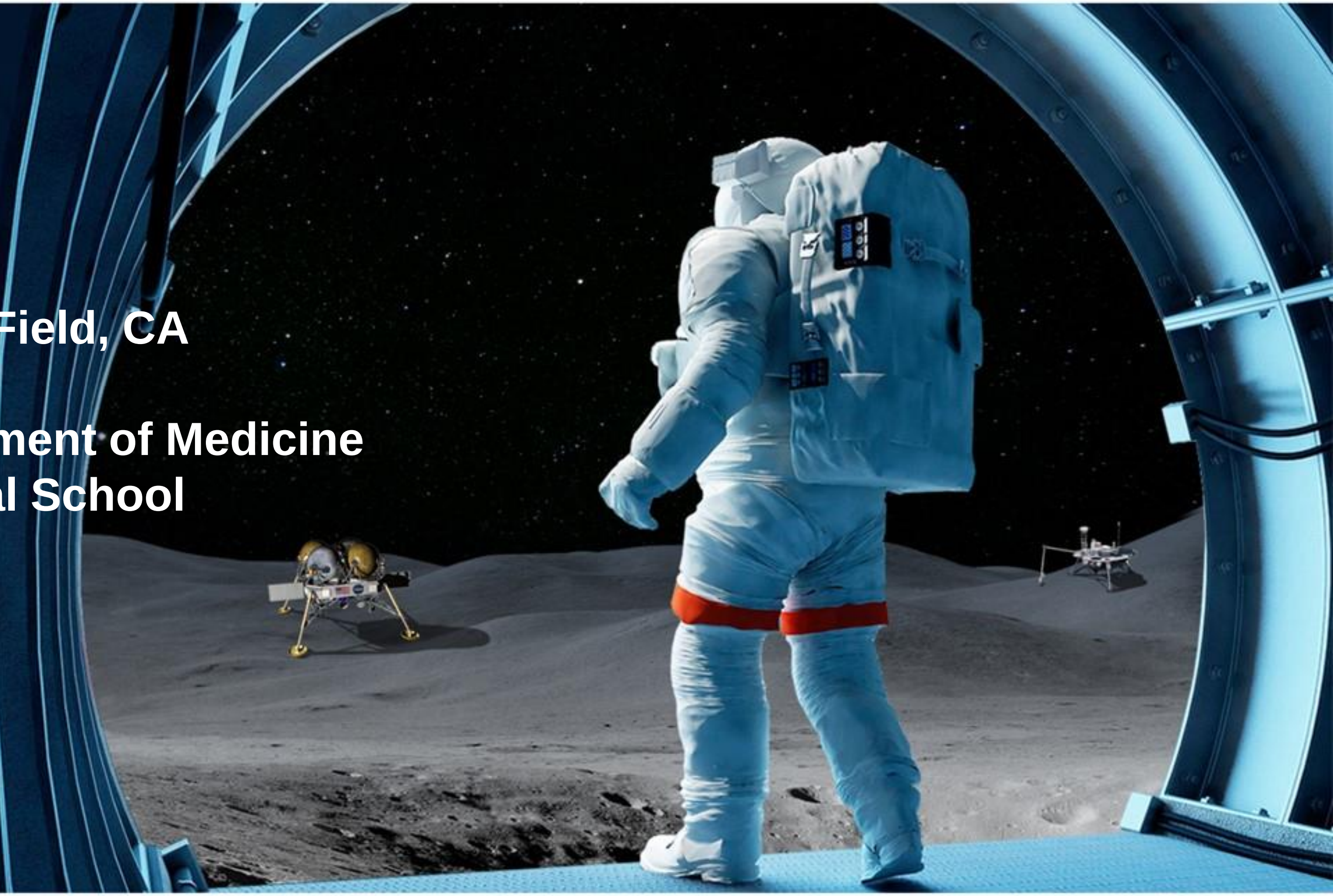
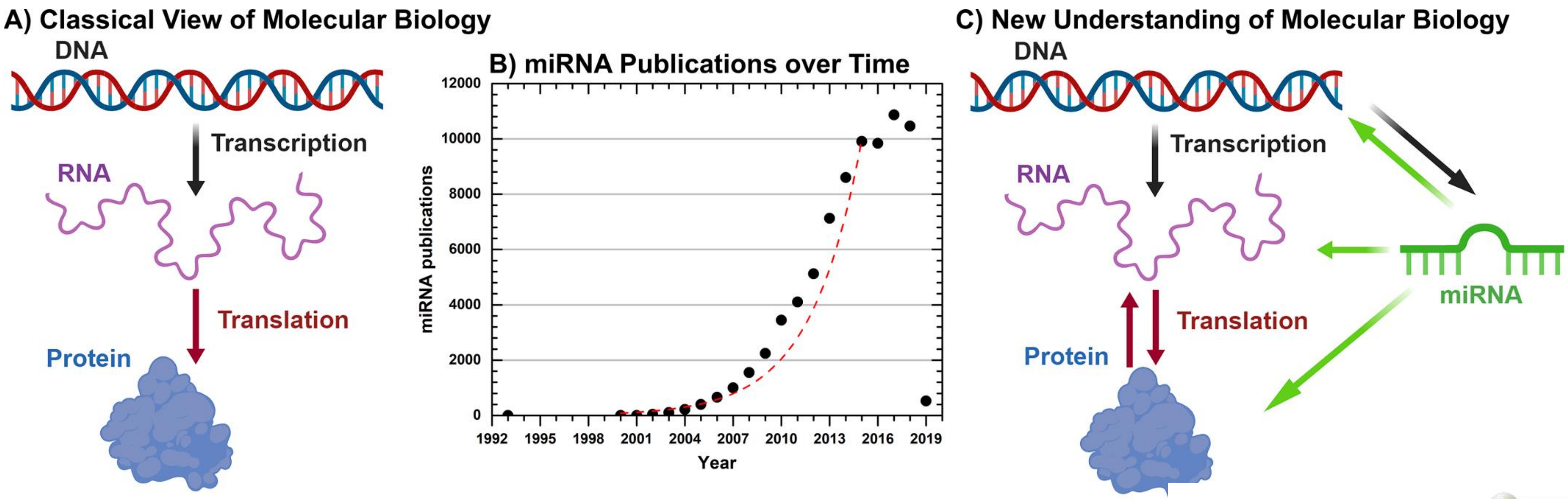
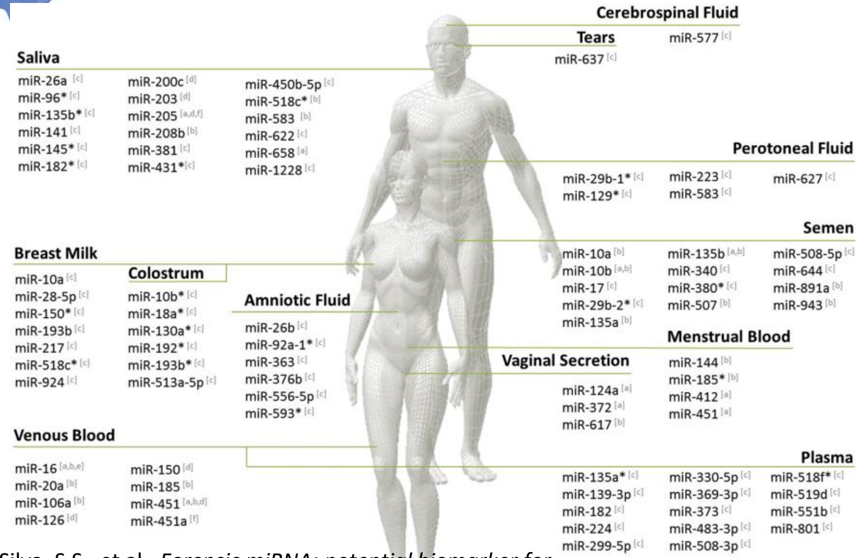


What are miRNAs and why study miRNAs



- **A single miRNA has been estimated to regulate up to 500 mRNAs.**
- **miRNAs are ~22nt**
- **Due to the size and stability of the miRNAs, it can float freely in the blood.**
- **miRNAs are now known to be involved in all aspects of diseases.**
- **miRNA are not only found in mammals, but everything else living: plants, microbes, fish, C. Elegans, fruit flies, insects, etc…**
- **miRNAs play a big role in radiation response (which also relates to space radiation).**



Silva, S.S., et al., *Forensic miRNA: potential biomarker for body fluids?* Forensic Sci Int Genet, 2015. **14**: p. 1-10.

Systems Biology View of miRNAs

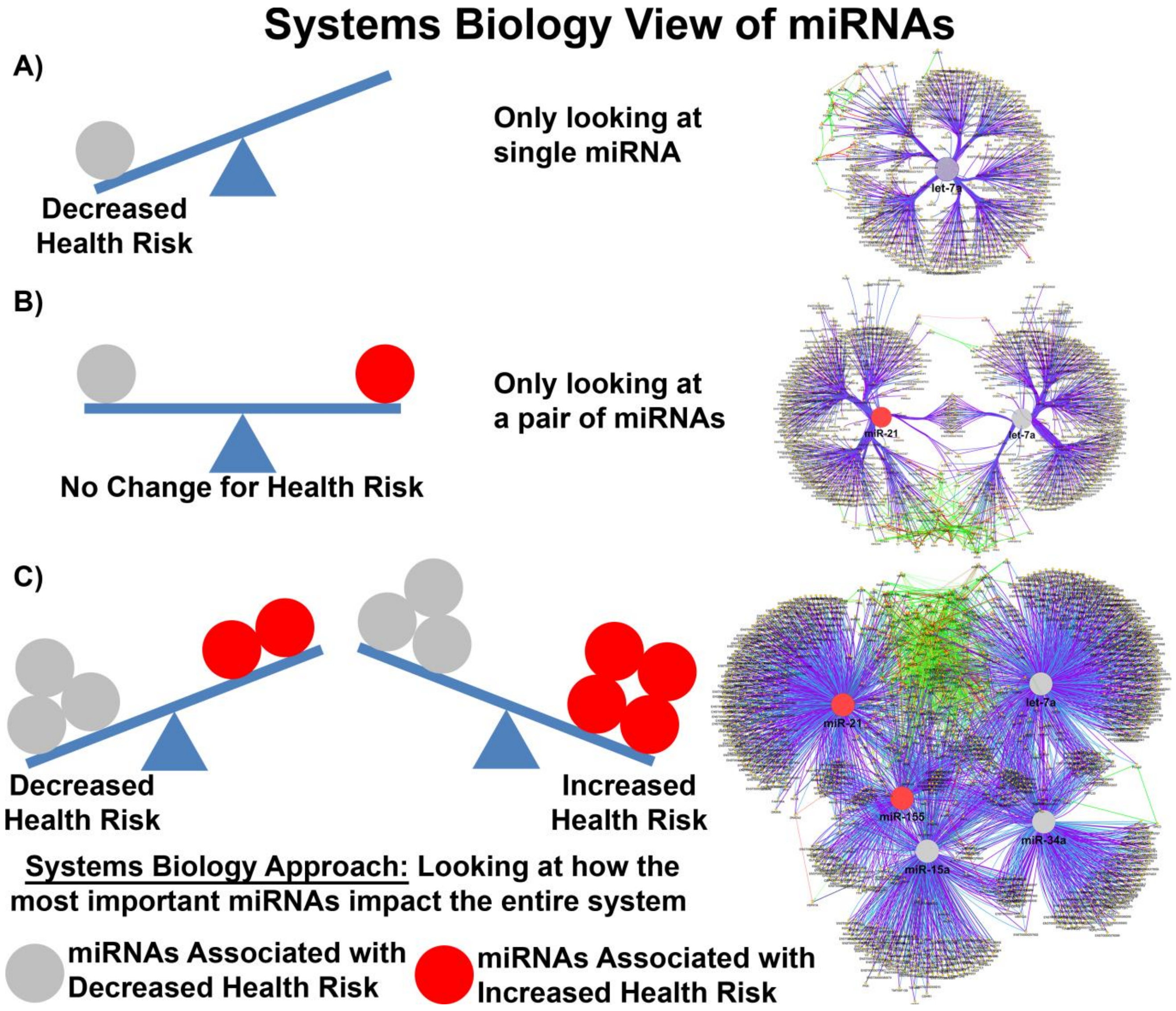
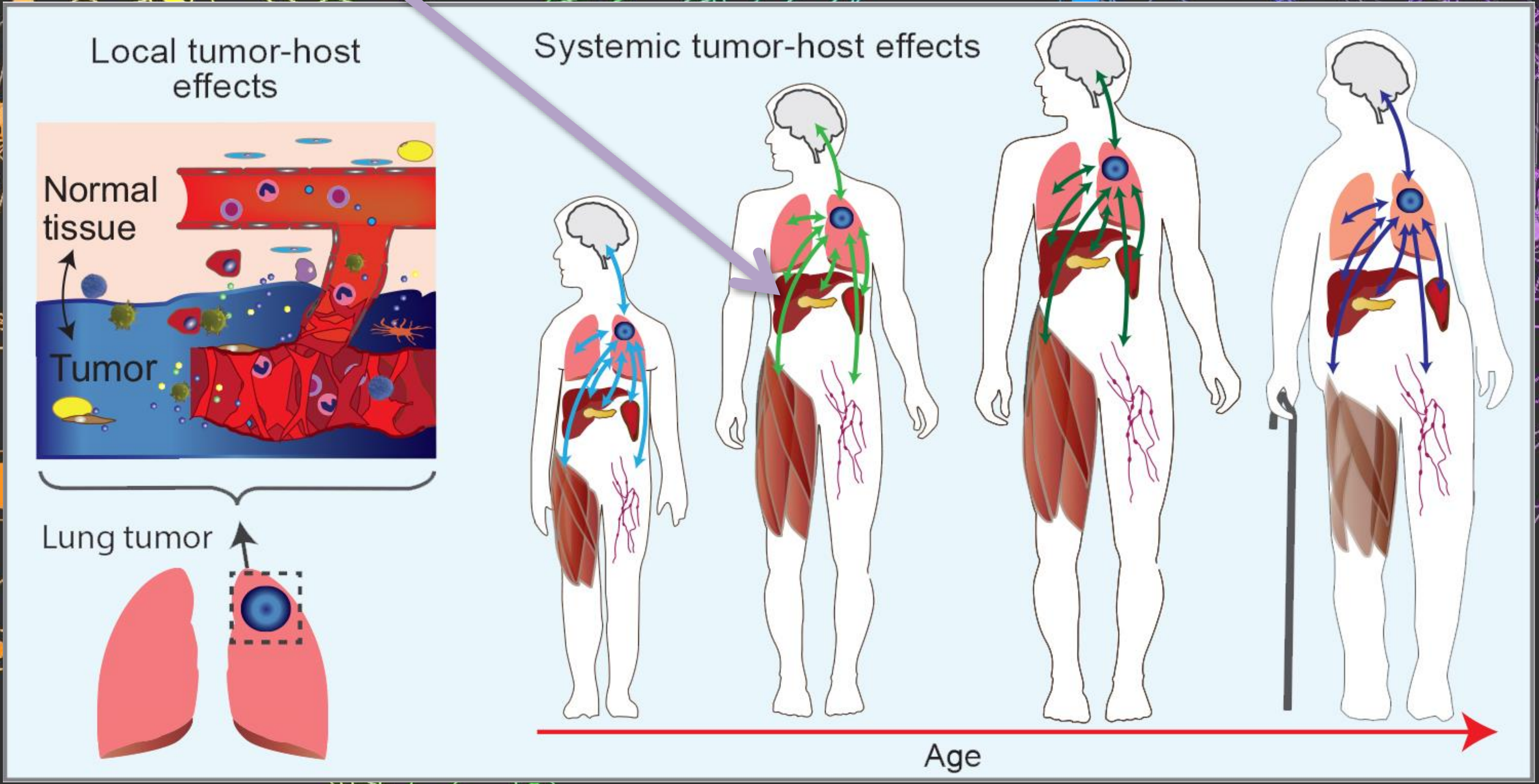


Figure from Vanderburg and Beheshti, MicroRNAs (miRNAs), the Final Frontier: The Hidden Master Regulators Impacting Biological Response in All Organisms Due to Spaceflight, THREE, 2020. In press.

Systems Biology Approach to Study the Impact of miRNAs

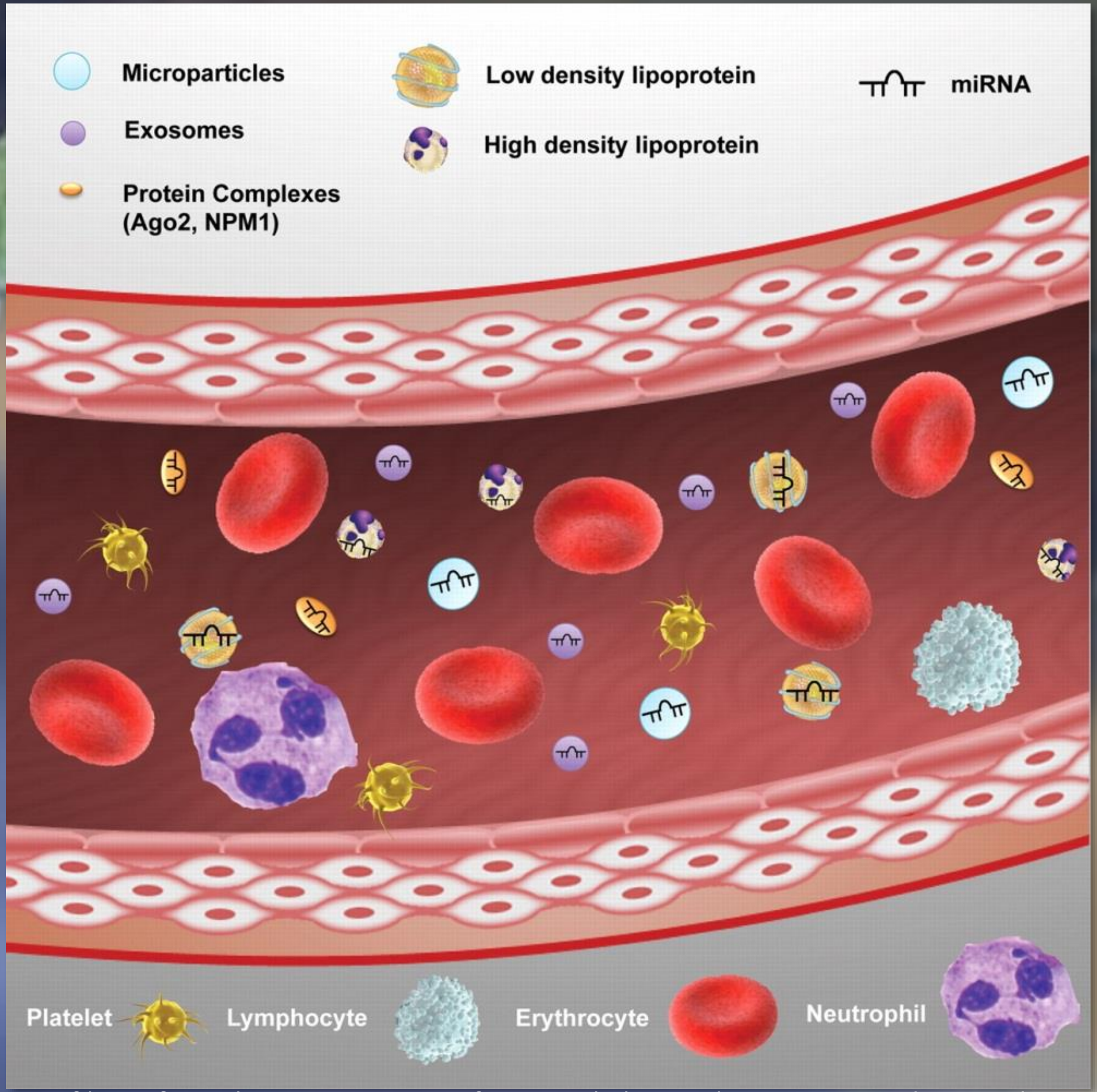
Circulating miRNAs



Beheshti, et al. Oncotarget 2015

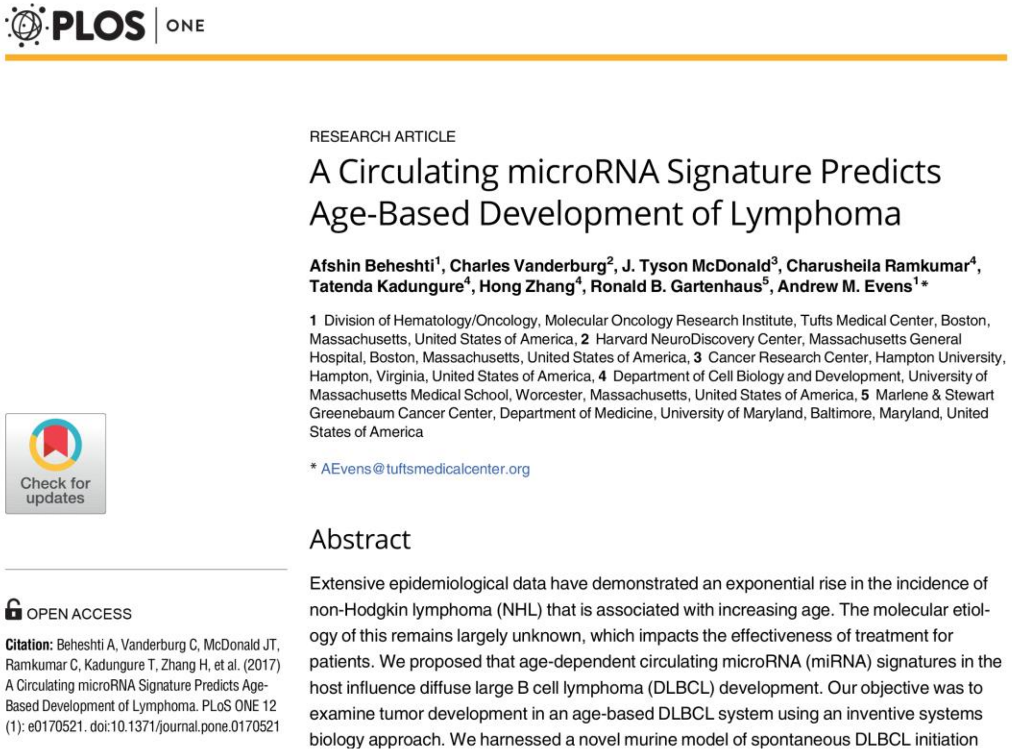
Impact of Circulating microRNAs

- 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.
- miRNAs can be conserved across multiple organs and in the blood



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

Determining miRNA signature associated with diseases: Lymphoma



PLOS | ONE

RESEARCH ARTICLE

A Circulating microRNA Signature Predicts Age-Based Development of Lymphoma

Afshin Beheshti[1], Charles Vanderburg[2], J. Tyson McDonald[3], Charusheila Ramkumar[4], Tatenda Kadungure[4], Hong Zhang[4], Ronald B. Gartenhaus[5], Andrew M. Evens[1]*

1 Division of Hematology/Oncology, Molecular Oncology Research Institute, Tufts Medical Center, Boston, Massachusetts, United States of America, 2 Harvard NeuroDiscovery Center, Massachusetts General Hospital, Boston, Massachusetts, United States of America, 3 Cancer Research Center, Hampton University, Hampton, Virginia, United States of America, 4 Department of Cell Biology and Development, University of Massachusetts Medical School, Worcester, Massachusetts, United States of America, 5 Marlene & Stewart Greenebaum Cancer Center, Department of Medicine, University of Maryland, Baltimore, Maryland, United States of America

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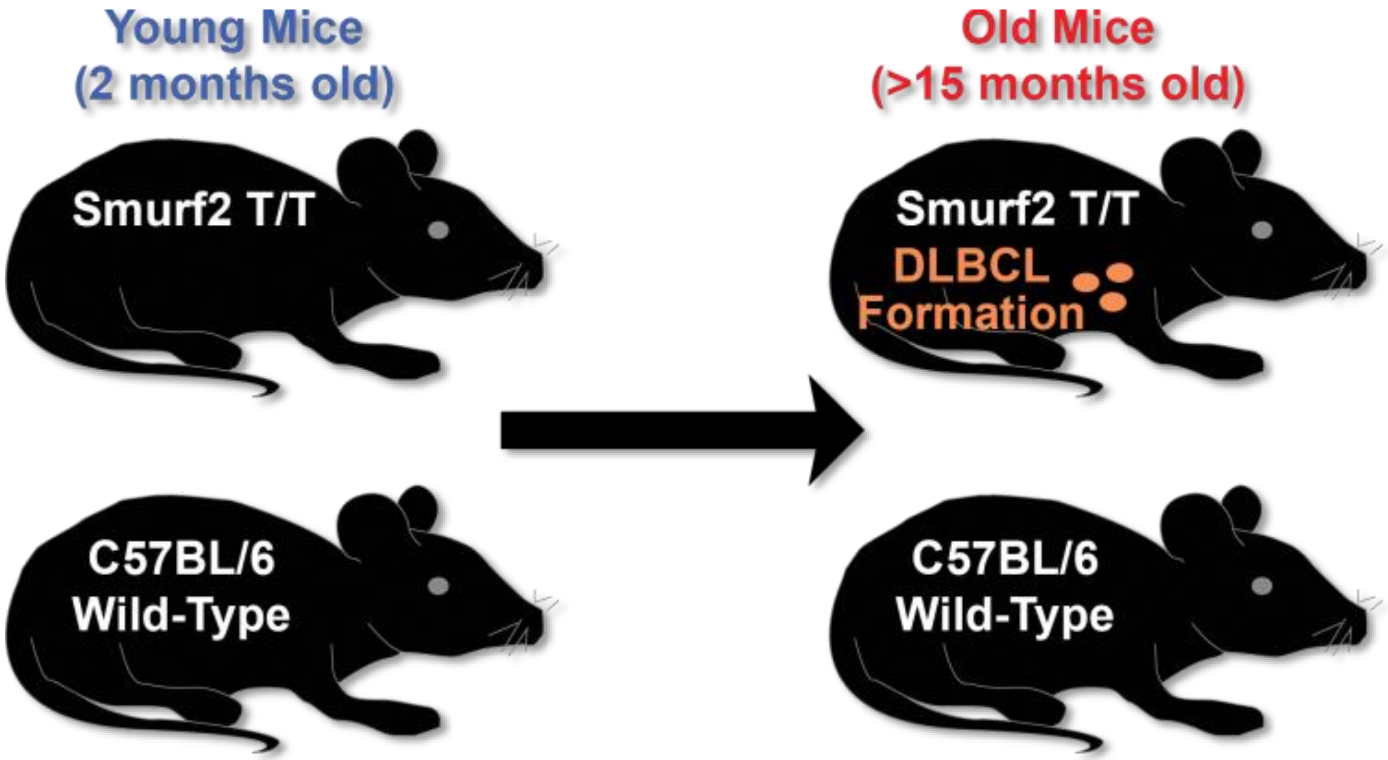
Citation: Beheshti A, Vanderburg C, McDonald JT, Ramkumar C, Kadungure T, Zhang H, et al. (2017) A Circulating microRNA Signature Predicts Age-Based Development of Lymphoma. PLoS ONE 12 (1): e0170521. doi:10.1371/journal.pone.0170521

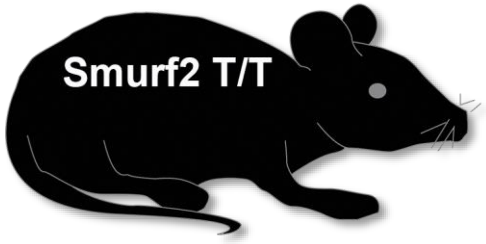
Abstract

Extensive epidemiological data have demonstrated an exponential rise in the incidence of non-Hodgkin lymphoma (NHL) that is associated with increasing age. The molecular etiology of this remains largely unknown, which impacts the effectiveness of treatment for patients. We proposed that age-dependent circulating microRNA (miRNA) signatures in the host influence diffuse large B cell lymphoma (DLBCL) development. Our objective was to examine tumor development in an age-based DLBCL system using an inventive systems biology approach. We harnessed a novel murine model of spontaneous DLBCL initiation

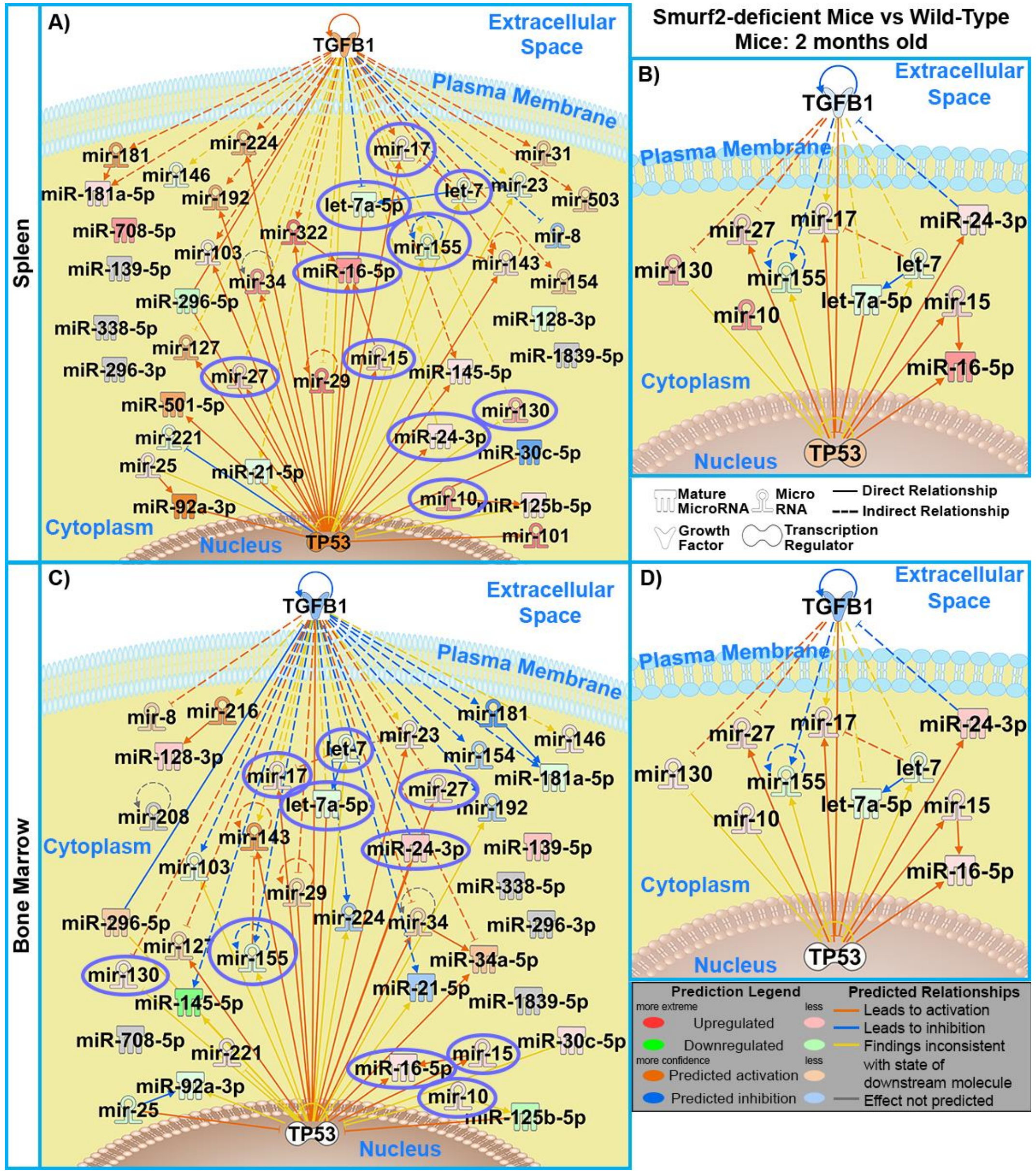


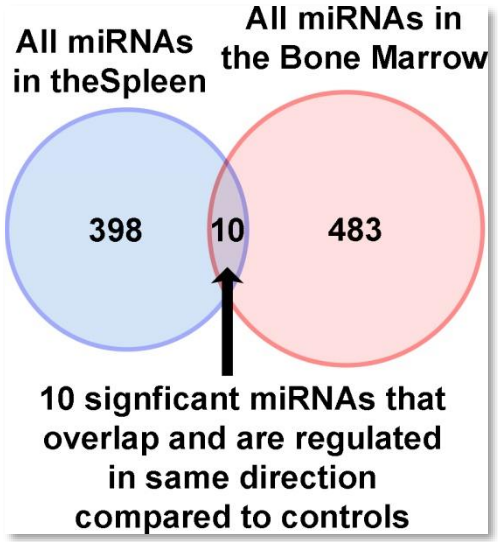
Determining miRNA signature associated with diseases: Lymphoma

Young Mice
(2 months old)

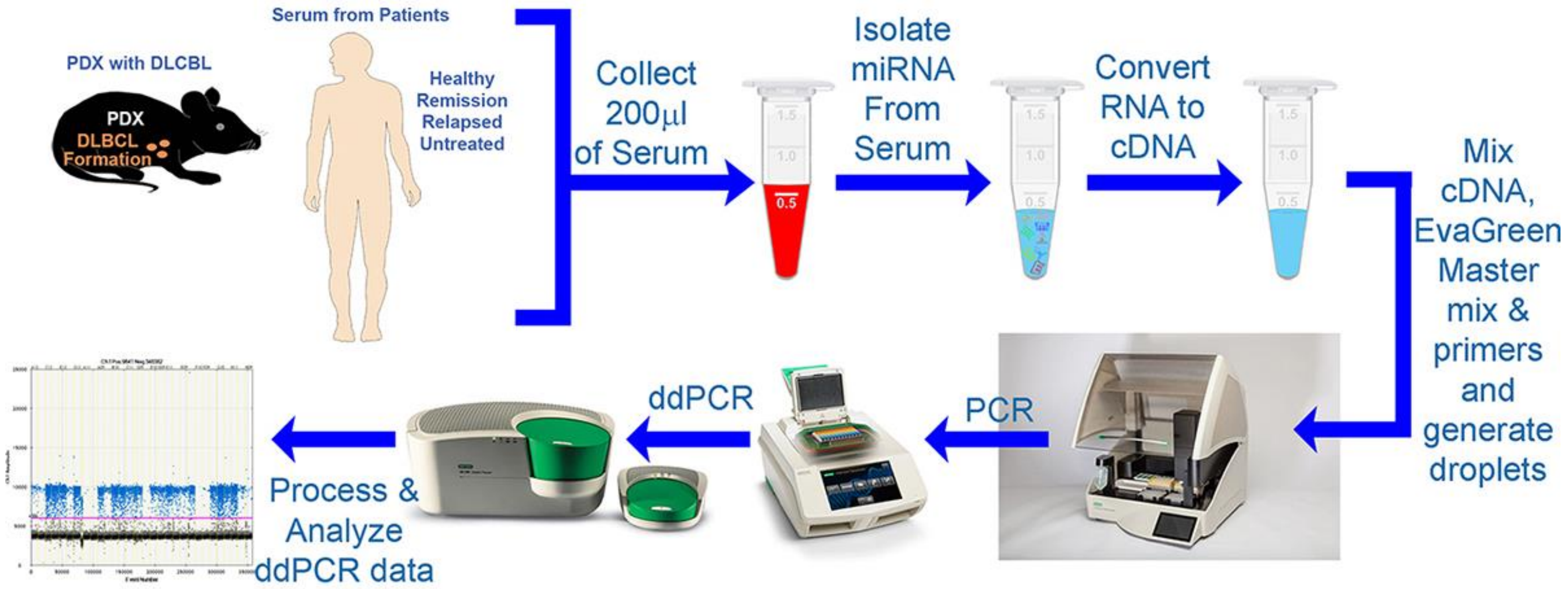


Smurf2-deficient Mice vs Wild-Type Mice: 2 months old

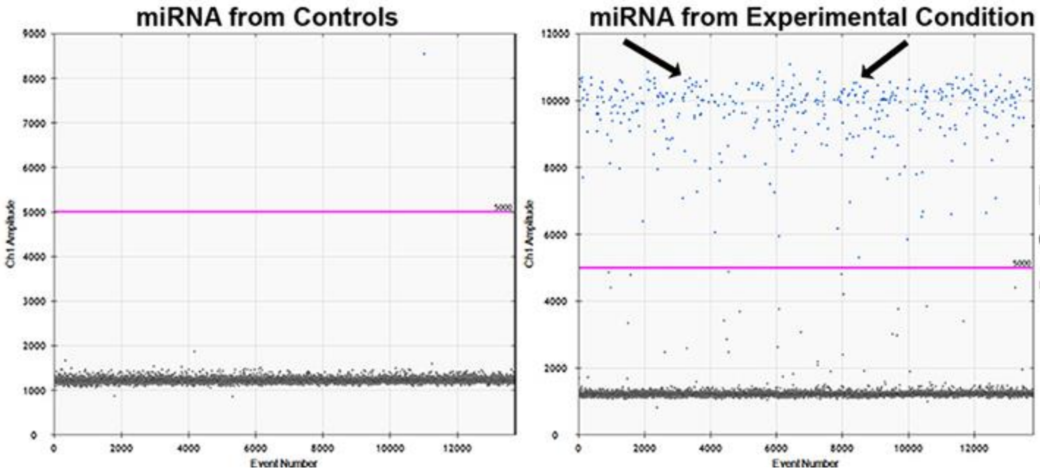




Quantifying miRNAs

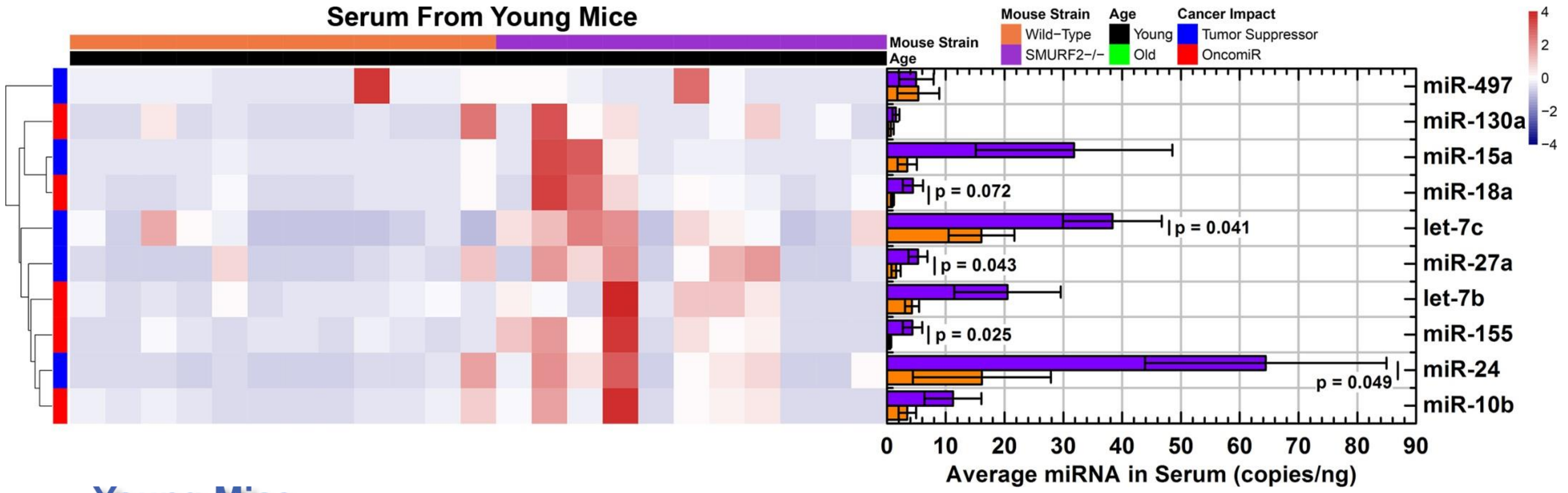


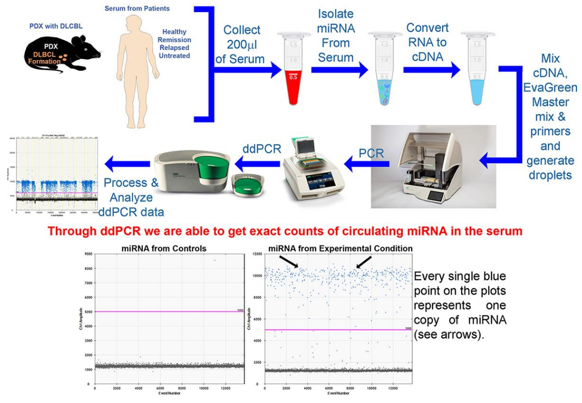
Through ddPCR we are able to get exact counts of circulating miRNA in the serum



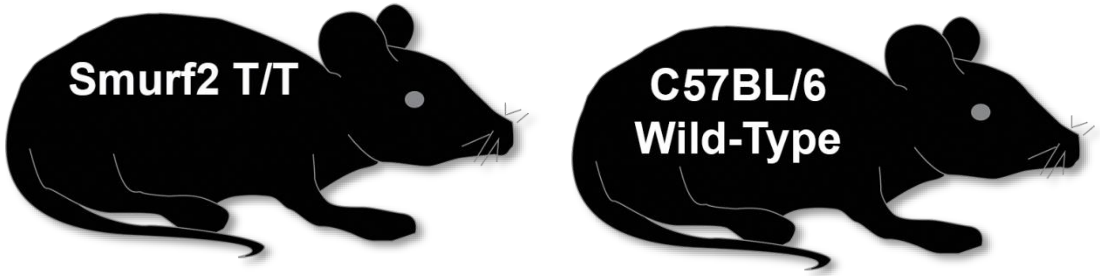
Every single blue point on the plots represents one copy of miRNA (see arrows).

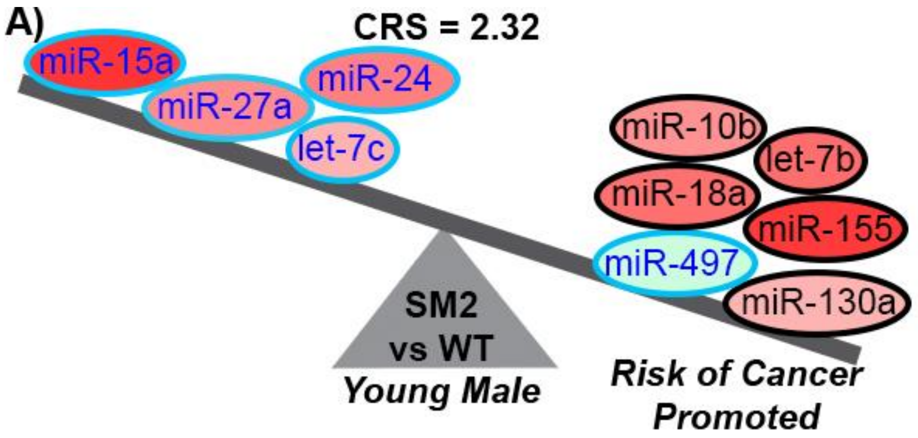
miRNAs Associated with DLBCL Development





Young Mice
(2 months old)



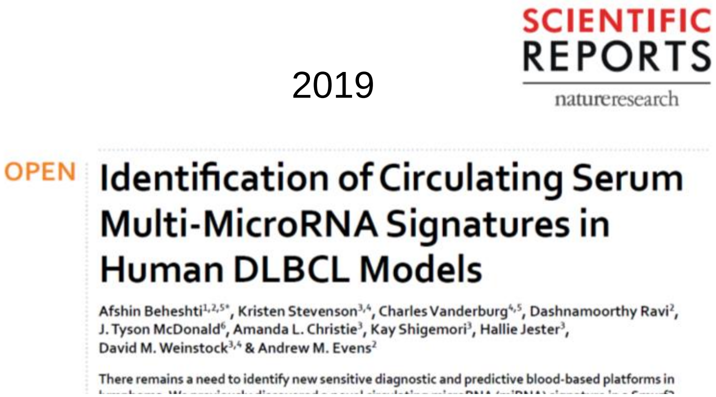


CRS = Cancer Risk Score

Beheshti et al. Plos One, 2017.

miRNAs Associated with DLBCL Development: in Humans

2019


SCIENTIFIC REPORTS

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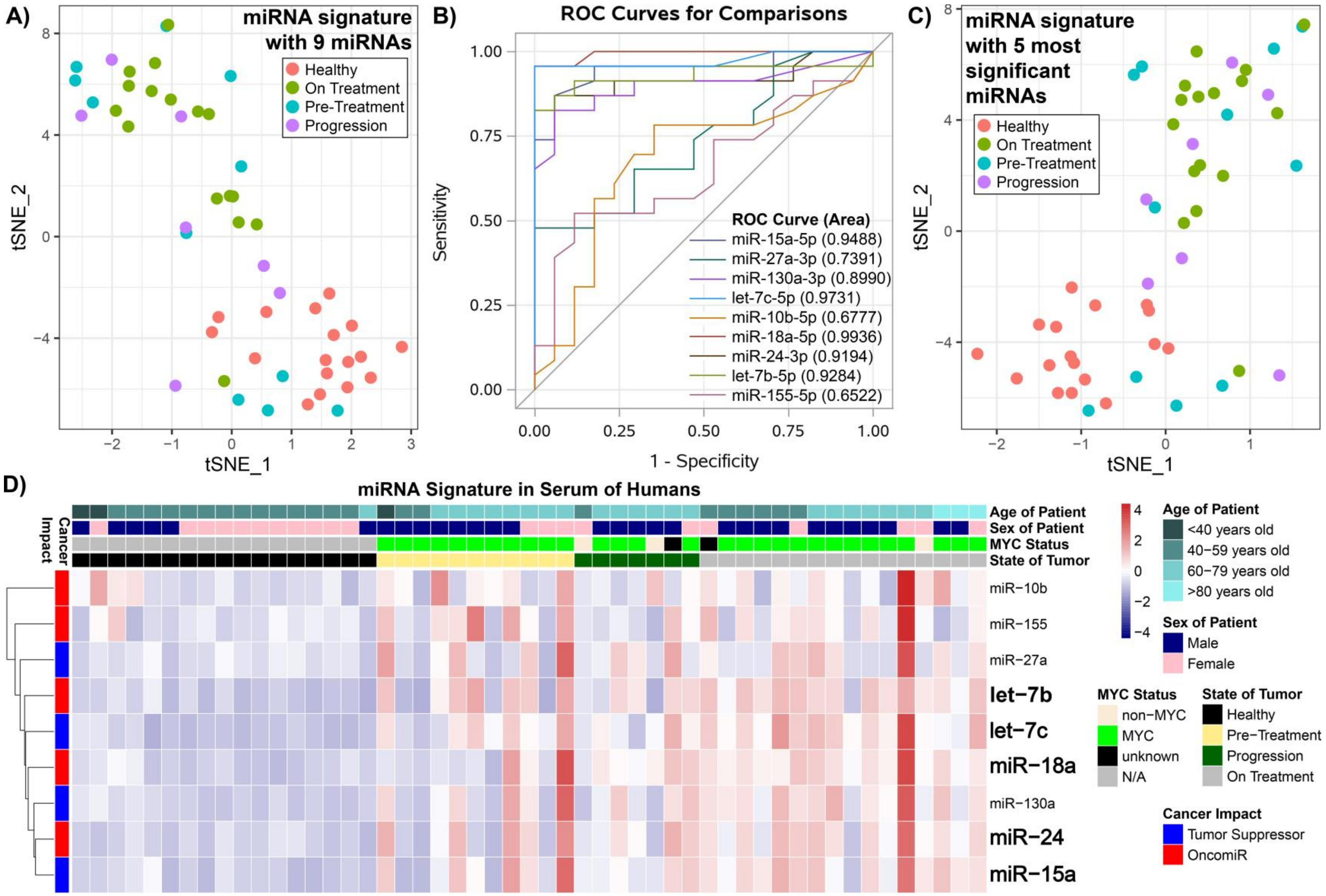
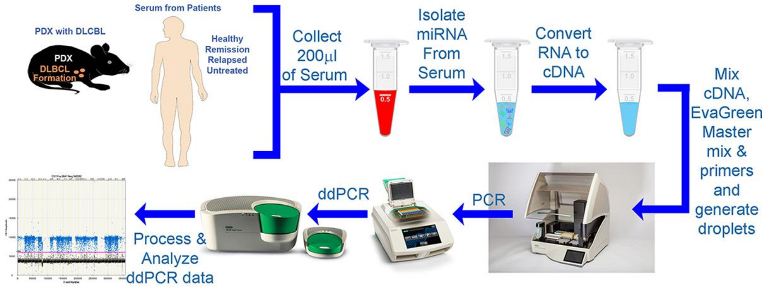
OPEN

Identification of Circulating Serum Multi-MicroRNA Signatures in Human DLBCL Models

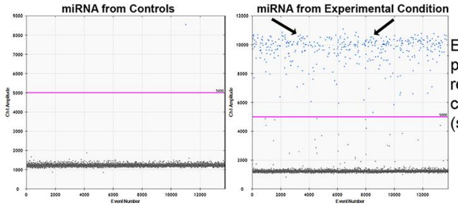
Afshin Beheshti[1,2,5*], Kristen Stevenson[3,4], Charles Vanderburg[4,5], Dashnamoorthy Ravi[2], J. Tyson McDonald[6], Amanda L. Christie[3], Kay Shigemori[3], Hallie Jester[3], David M. Weinstock[3,4] & Andrew M. Evens[2]

There remains a need to identify new sensitive diagnostic and predictive blood-based platforms in



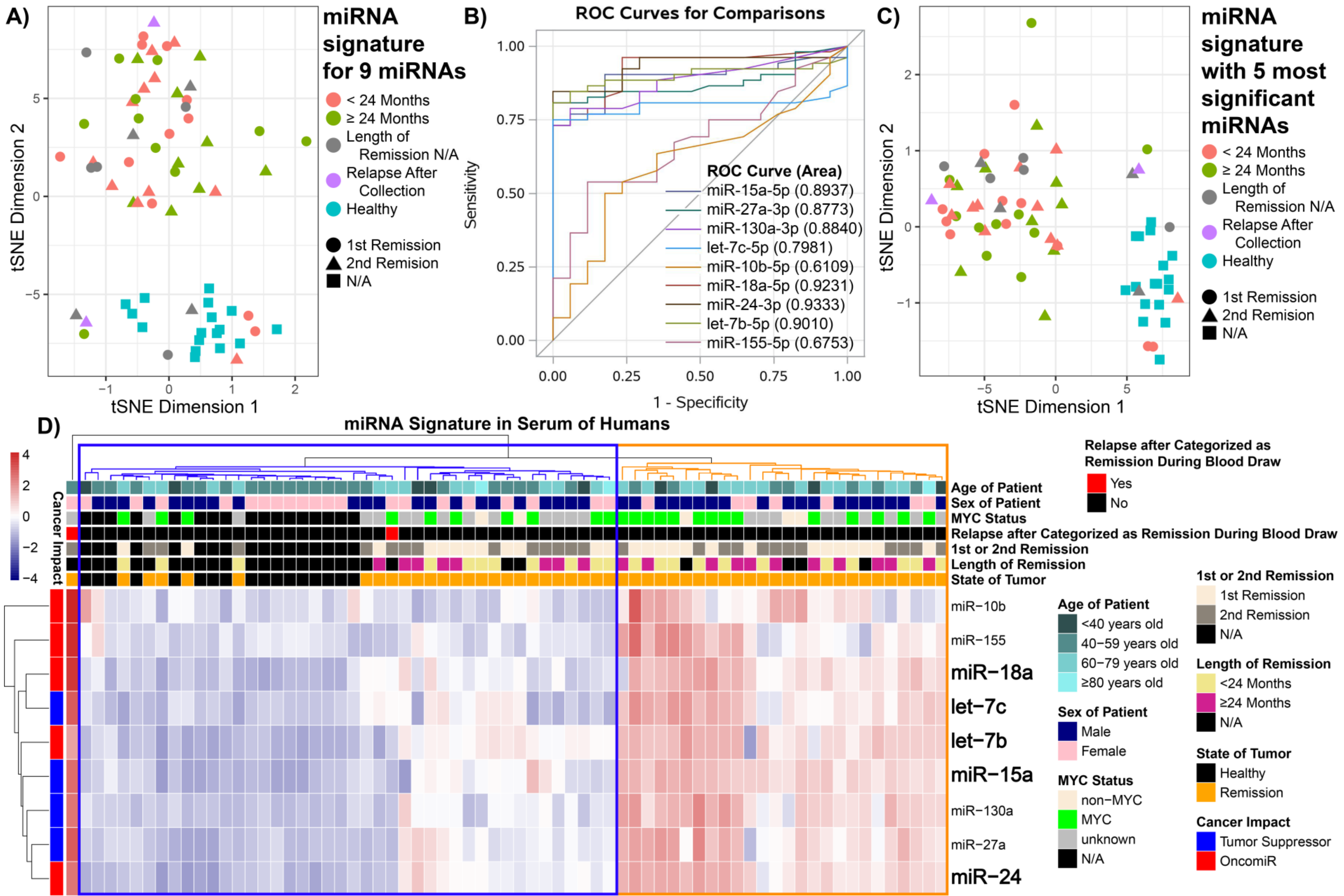


Through ddPCR we are able to get exact counts of circulating miRNA in the serum

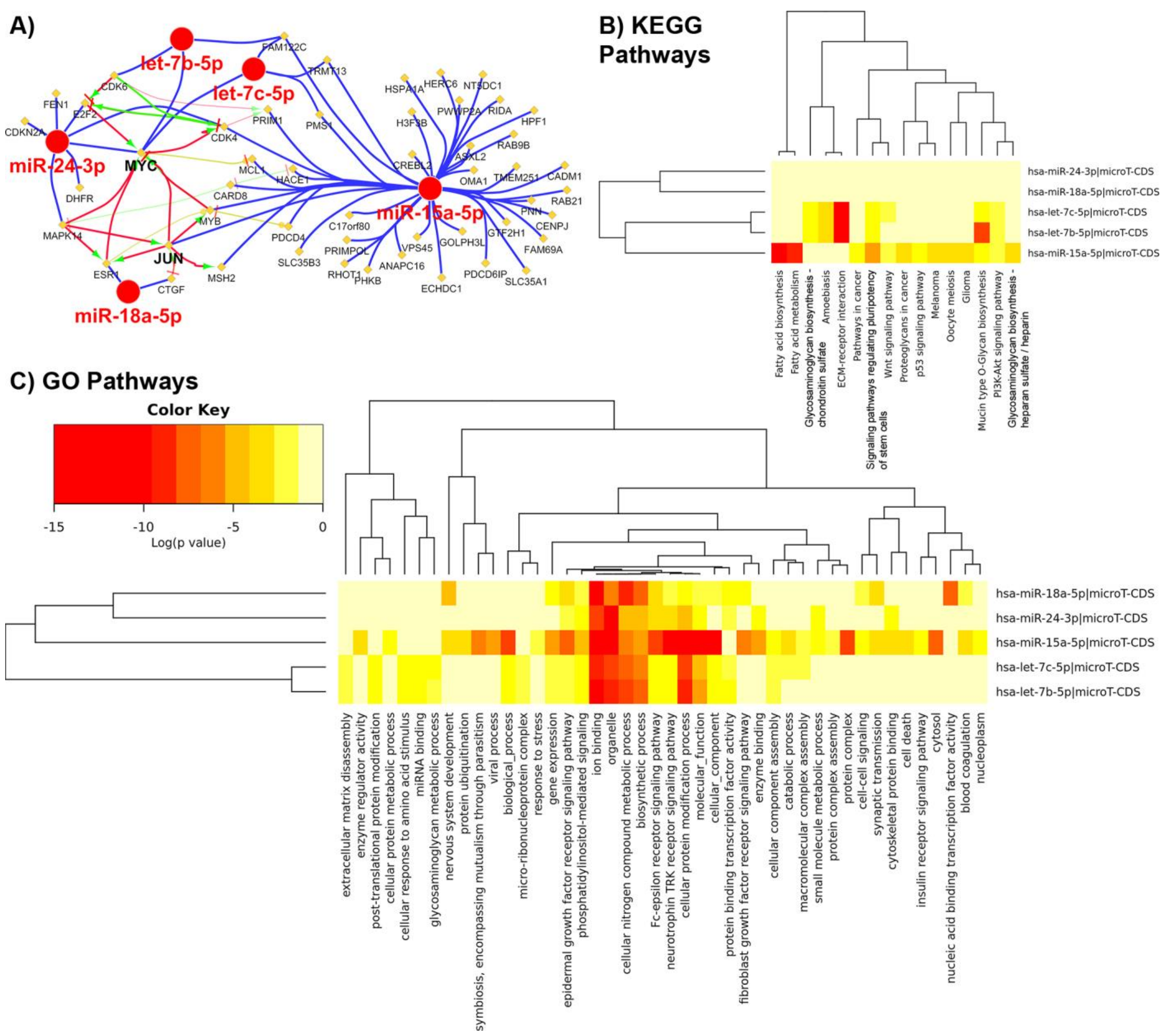


Every single blue point on the plots represents one copy of miRNA (see arrows).

miRNAs in DLBCL Patients After Remission



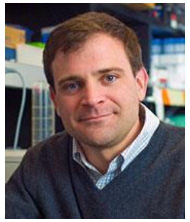
Targets for the DLBCL miRNA Signature



Conclusion Part 1: DLBCL miRNA Signature

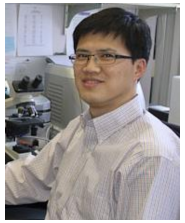
- This DLBCL miRNA Signature can potentially be utilized as a novel liquid biomarker to detect onset of DLBCL before any existing technology
- This DLBCL miRNA signature can be used to monitor patients after treatment to test true remission rate of cancer
- Apply same techniques for other cancers to determine specific miRNA signature for each cancer type.
- Possible miRNA-based therapeutic

Andrew Evens
RUTGERS Robert Wood Johnson Medical School



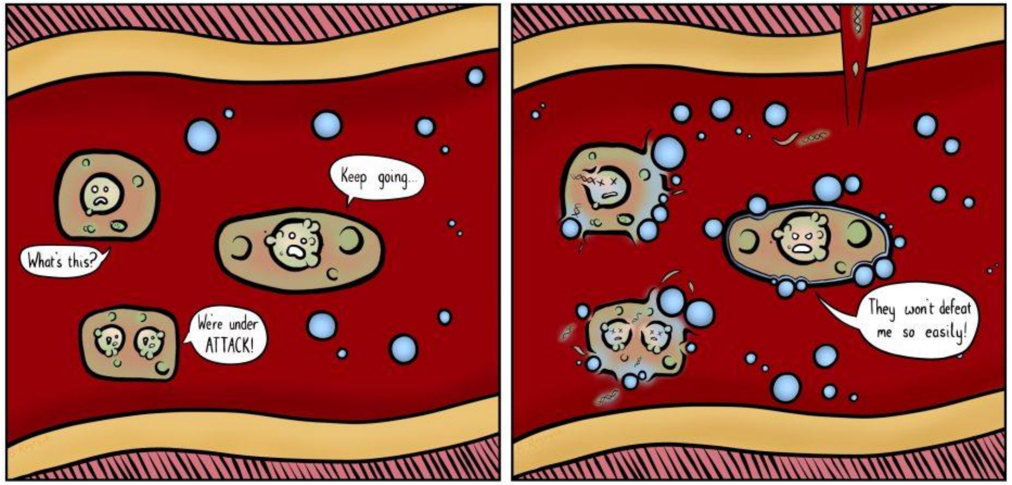
David Weinstock
DANA-FARBER CANCER INSTITUTE

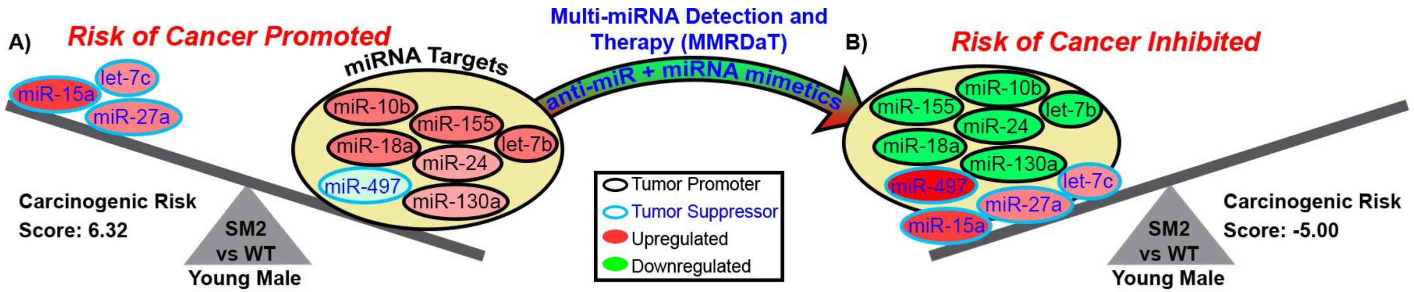
Charles Vanderburg
BROAD INSTITUTE



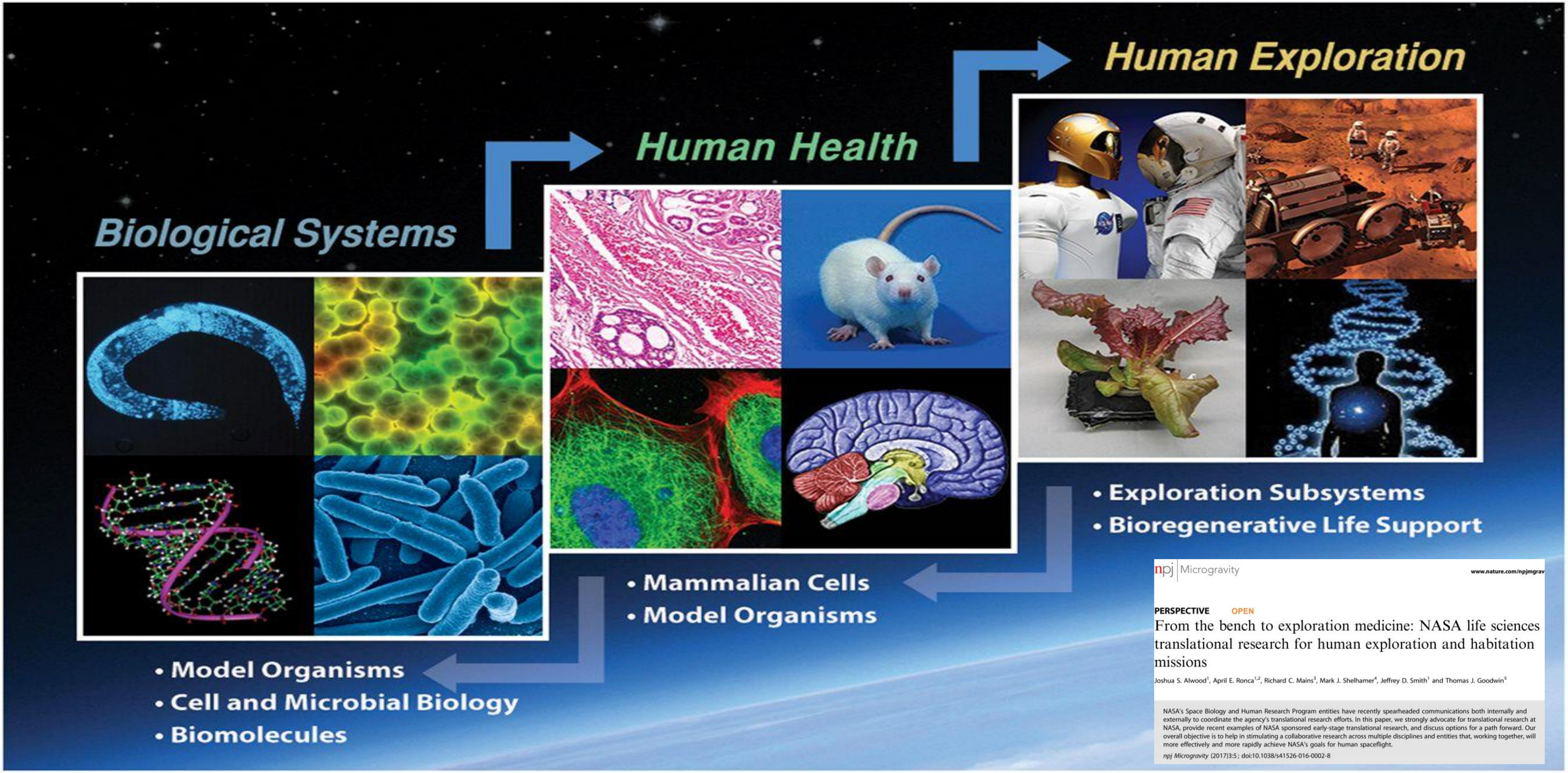
Hong Zhang
UMASS University of Massachusetts Medical School

J. Tyson McDonald
GEORGETOWN UNIVERSITY Georgetown University Medical Center





miRNAs related to Space Biology



npj | Microgravity www.nature.com/npjmgrav

PERSPECTIVE OPEN

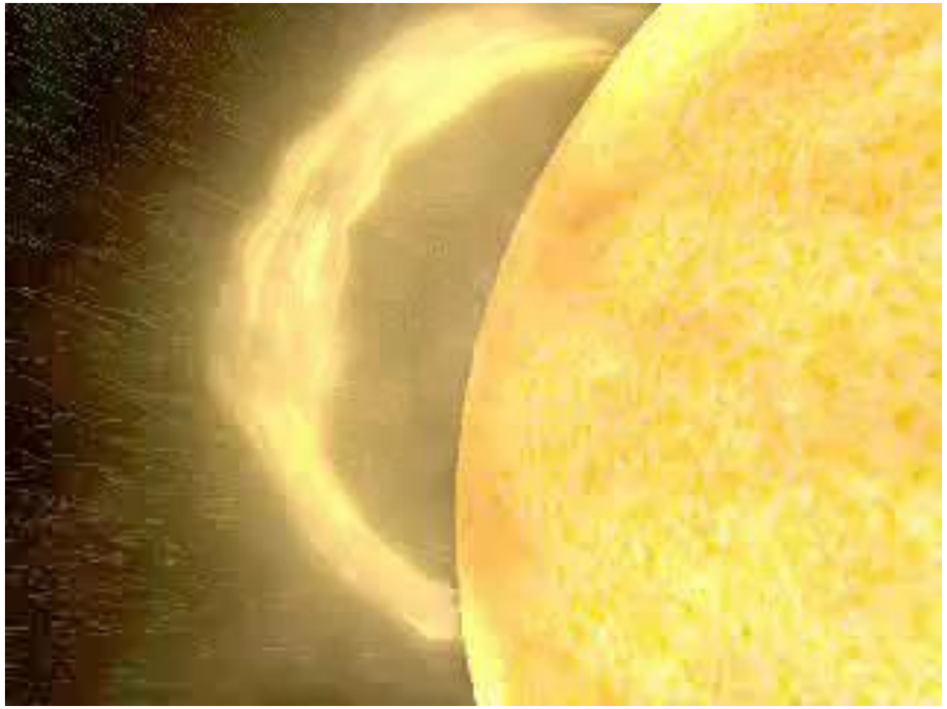
From the bench to exploration medicine: NASA life sciences translational research for human exploration and habitation missions

Joshua S. Alwood[1], April E. Ronca[1,2], Richard C. Mains[3], Mark J. Shelhamer[4], Jeffrey D. Smith[1] and Thomas J. Goodwin[5]

NASA's Space Biology and Human Research Program entities have recently spearheaded communications both internally and externally to coordinate the agency's translational research efforts. In this paper, we strongly advocate for translational research at NASA, provide recent examples of NASA sponsored early-stage translational research, and discuss options for a path forward. Our overall objective is to help in stimulating a collaborative research across multiple disciplines and entities that, working together, will more effectively and more rapidly achieve NASA's goals for human spaceflight.

npj Microgravity (2017)3:5 ; doi:10.1038/s41526-016-0002-8

Space Environment



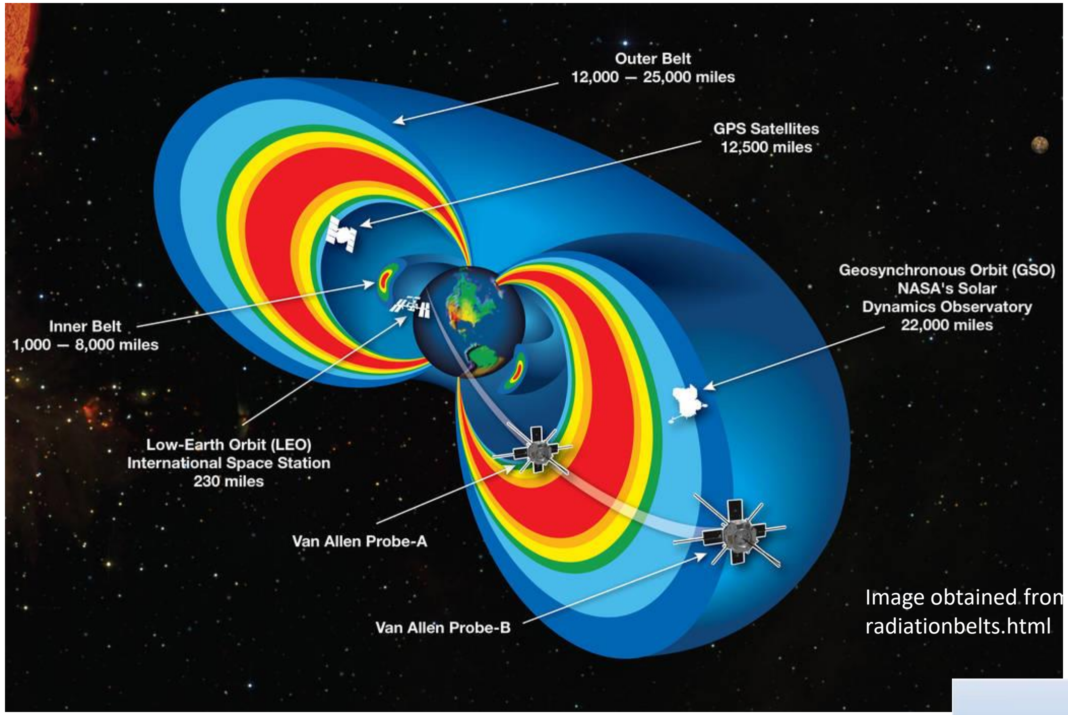
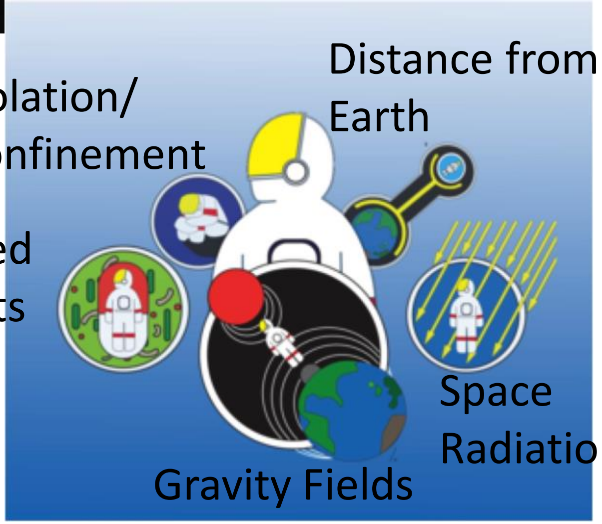
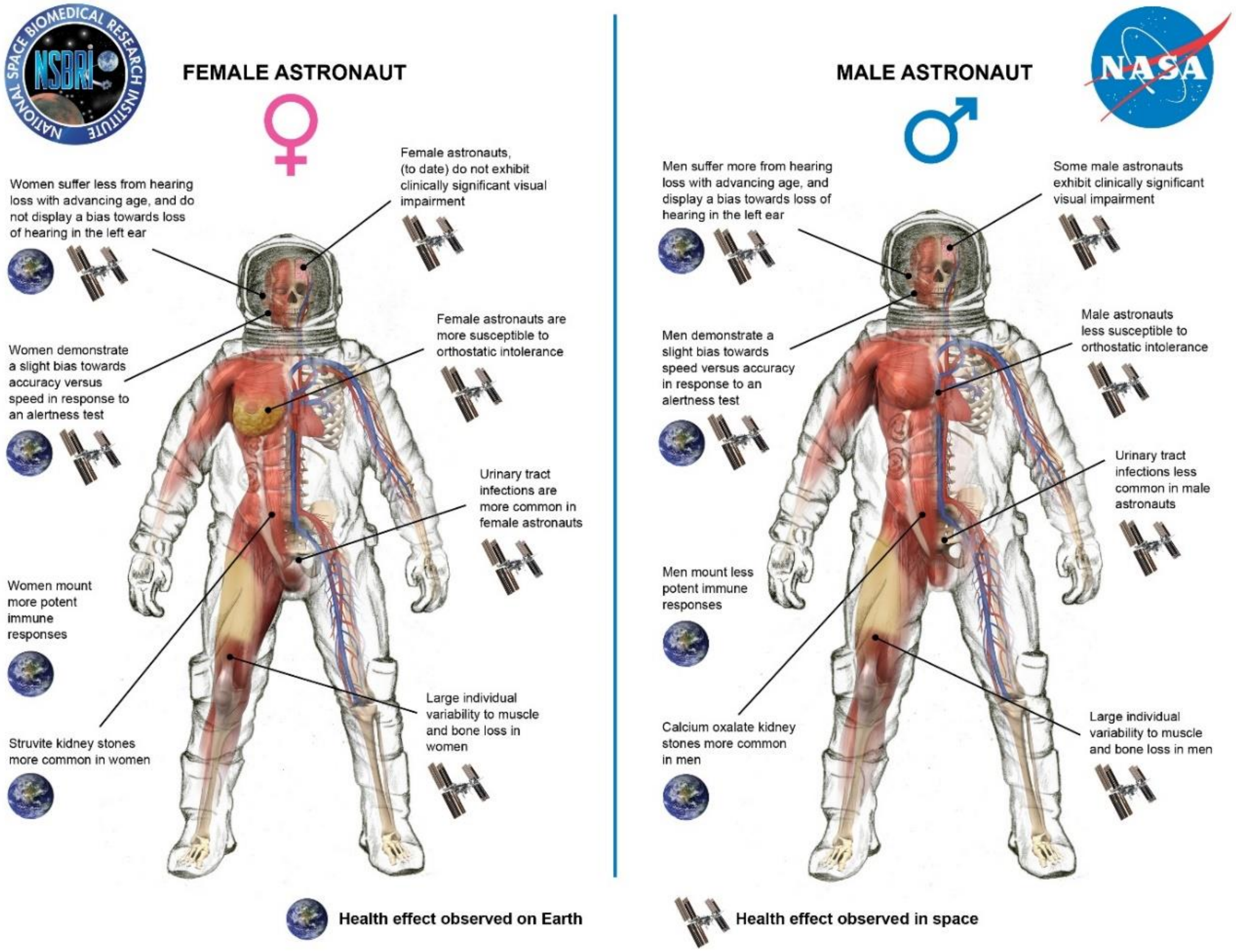


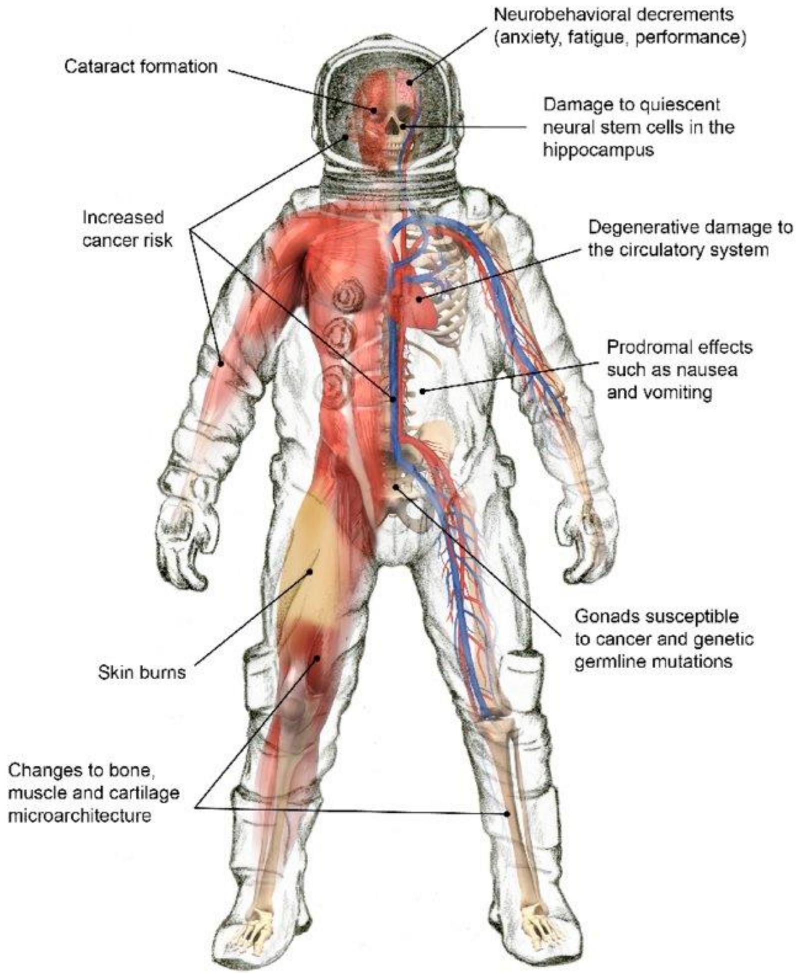
Image obtained from radiationbelts.html



Credits: NASA

Space Environment Health Risks On Astronauts



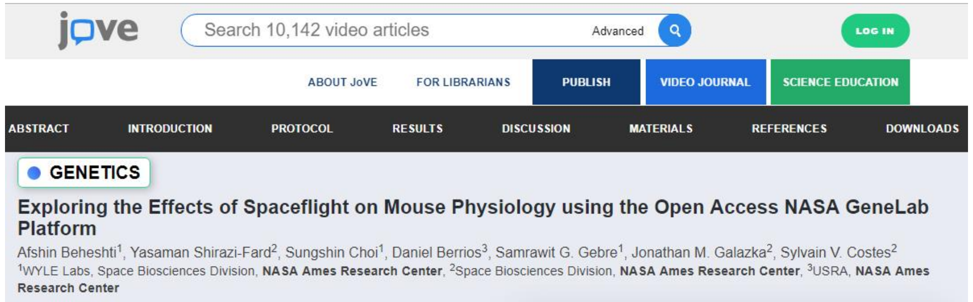


Select health effects due to space radiation exposures.

From: J. Chancellor et al., Space Radiation: The Number One Risk to Astronaut Health beyond Low Earth Orbit. *Life,* 4(3), 491-510;

Type of Experiments Related to Space Biology

Experiments Done in Space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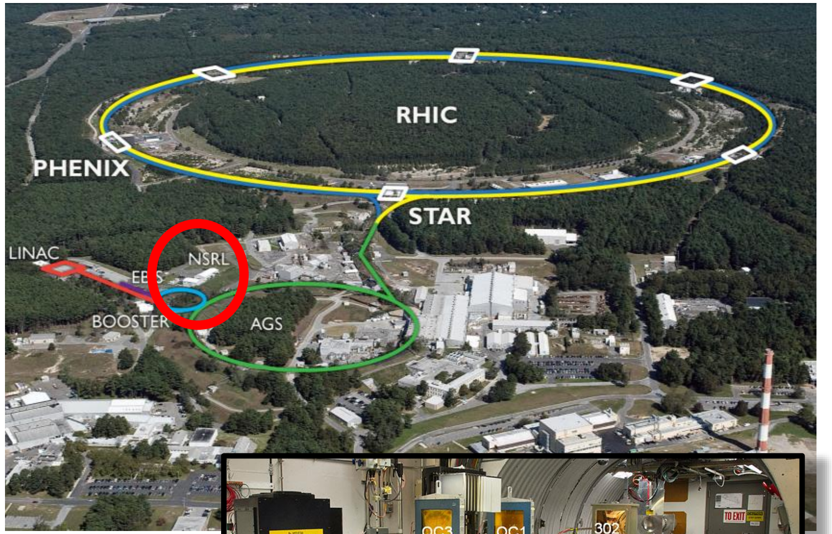
GENETICS

Exploring the Effects of Spaceflight on Mouse Physiology using the Open Access NASA GeneLab Platform

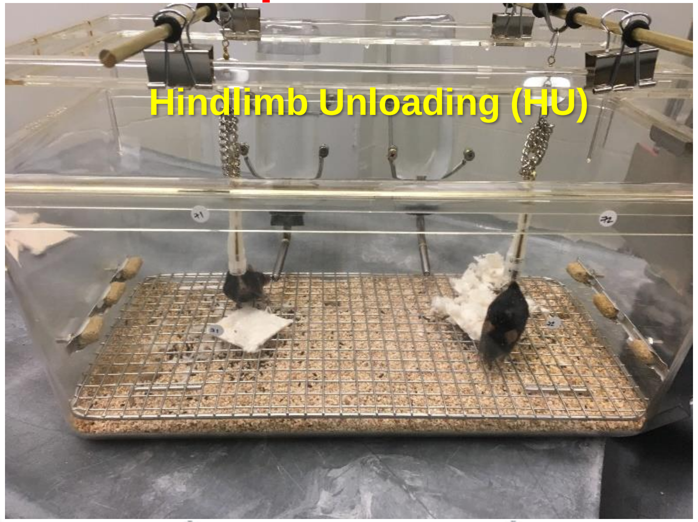
Afshin Beheshti[1], Yasaman Shirazi-Fard[2], Sungshin Choi[1], Daniel Berrios[3], Samrawit G. Gebre[1], Jonathan M. Galazka[2], Sylvain V. Costes[2]
[1]WYLE Labs, Space Biosciences Division, **NASA Ames Research Center**, [2]Space Biosciences Division, **NASA Ames Research Center**, [3]USRA, **NASA Ames Research Center**

Space Radiation Simulated Experiments

Brookhaven National Laboratory



Microgravity Simulated Experiments



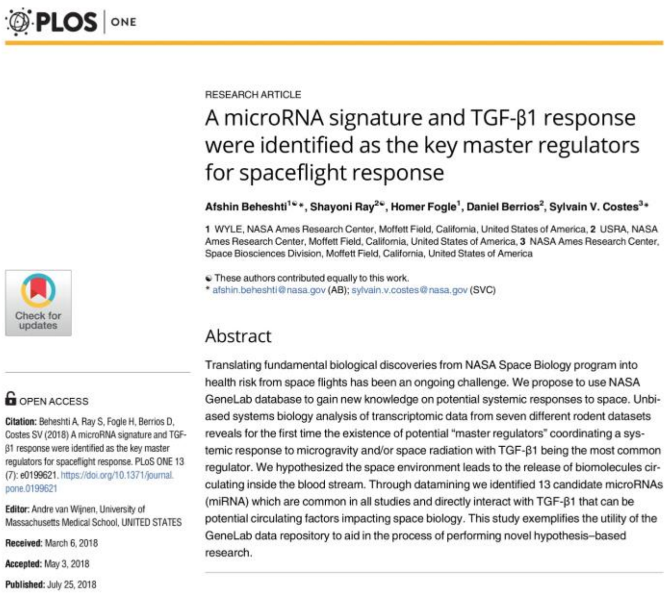
Partial Weight Bearing Rat Model

Seward Rutkove Marie Mortreux

Beth Israel Deaconess Medical Center
A teaching hospital of Harvard Medical School

https://www.rutkovelab.org/nasa/

miRNA Signature Prediction Associated with Space Flight


PLOS | ONE

RESEARCH ARTICLE

A microRNA signature and TGF-β1 response were identified as the key master regulators for spaceflight response

Afshin Beheshti[1☯]*, Shayoni Ray[2☯], Homer Fogle[1], Daniel Berrios[2], Sylvain V. Costes[3]*

1 WYLE, NASA Ames Research Center, Moffett Field, California, United States of America, 2 USRA, NASA Ames Research Center, Moffett Field, California, United States of America, 3 NASA Ames Research Center, Space Biosciences Division, Moffett Field, California, United States of America

☯ These authors contributed equally to this work.
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Citation: Beheshti A, Ray S, Fogle H, Berrios D, Costes SV (2018) A microRNA signature and TGF-β1 response were identified as the key master regulators for spaceflight response. PLoS ONE 13 (7): e0199621. https://doi.org/10.1371/journal.pone.0199621

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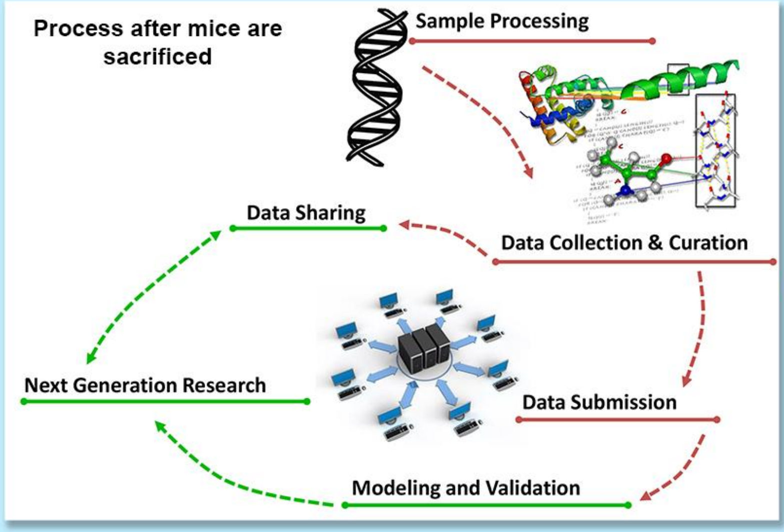
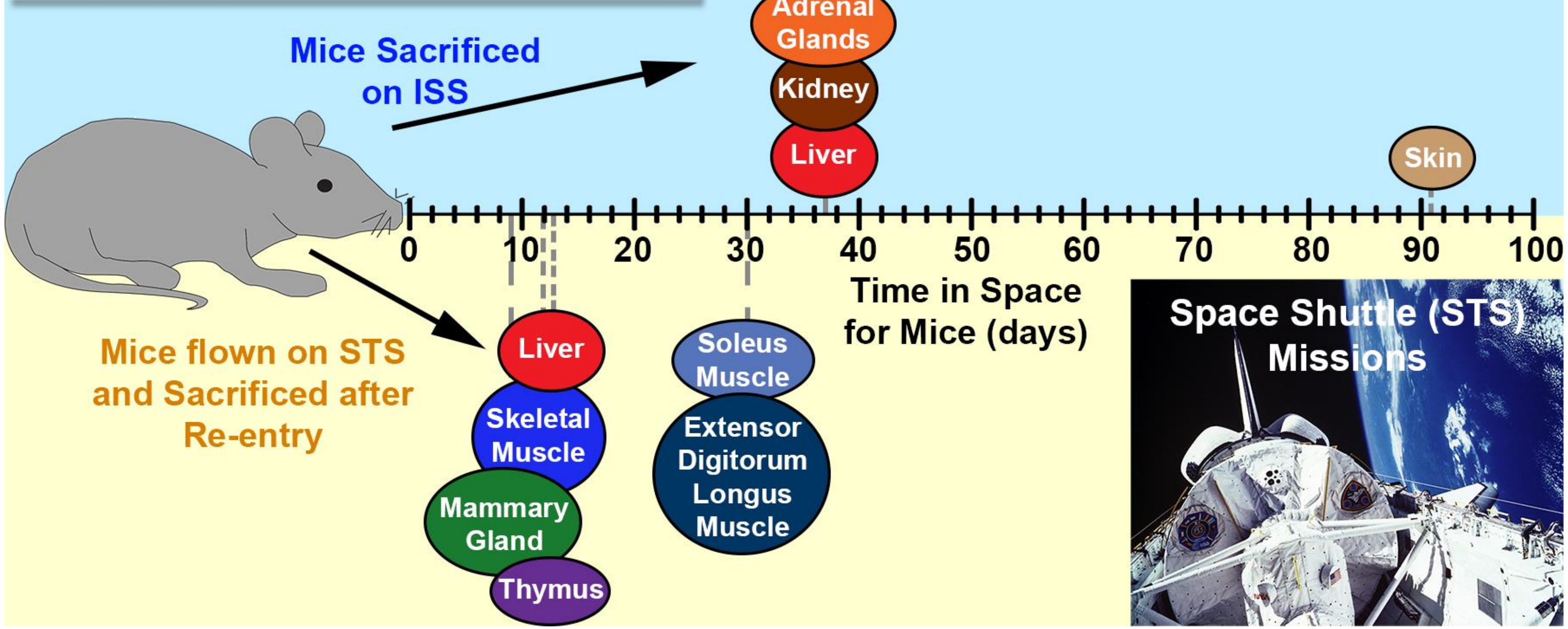
Abstract

Translating fundamental biological discoveries from NASA Space Biology program into health risk from space flights has been an ongoing challenge. We propose to use NASA GeneLab database to gain new knowledge on potential systemic responses to space. Unbiased systems biology analysis of transcriptomic data from seven different rodent datasets reveals for the first time the existence of potential "master regulators" coordinating a systemic response to microgravity and/or space radiation with TGF-β1 being the most common regulator. We hypothesized the space environment leads to the release of biomolecules circulating inside the blood stream. Through datamining we identified 13 candidate microRNAs (miRNA) which are common in all studies and directly interact with TGF-β1 that can be potential circulating factors impacting space biology. This study exemplifies the utility of the GeneLab data repository to aid in the process of performing novel hypothesis–based research.

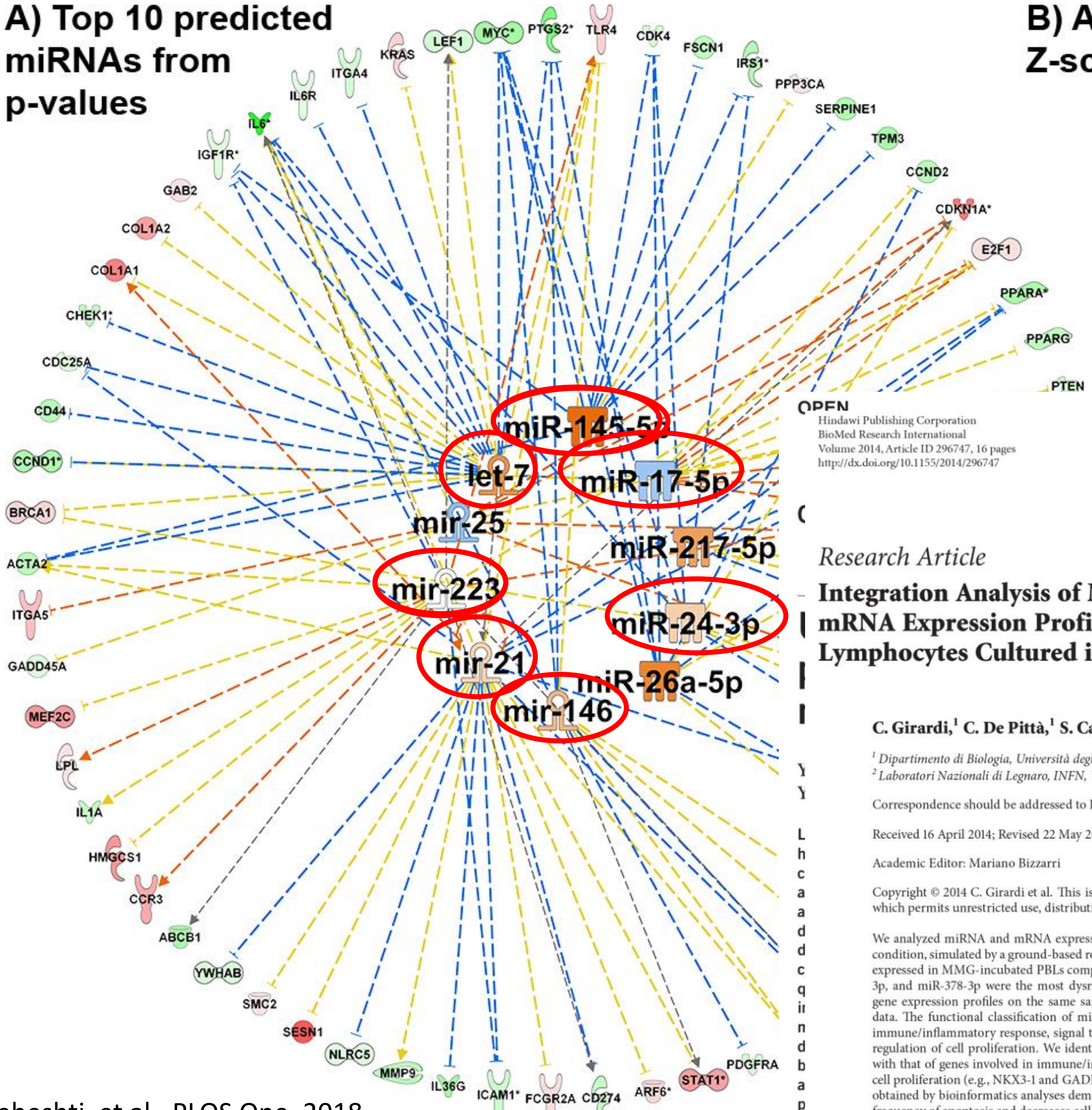
https://genelab.nasa.gov/



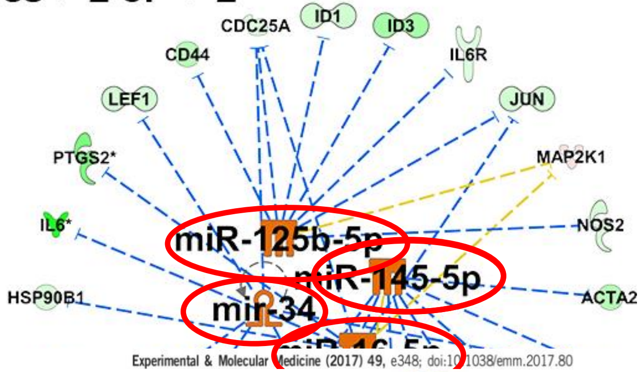


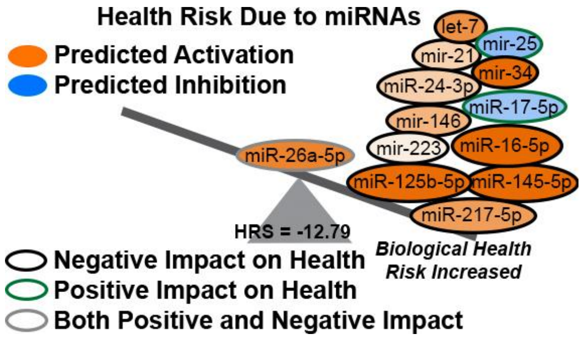
Predicted miRNAs Involved with Spaceflight

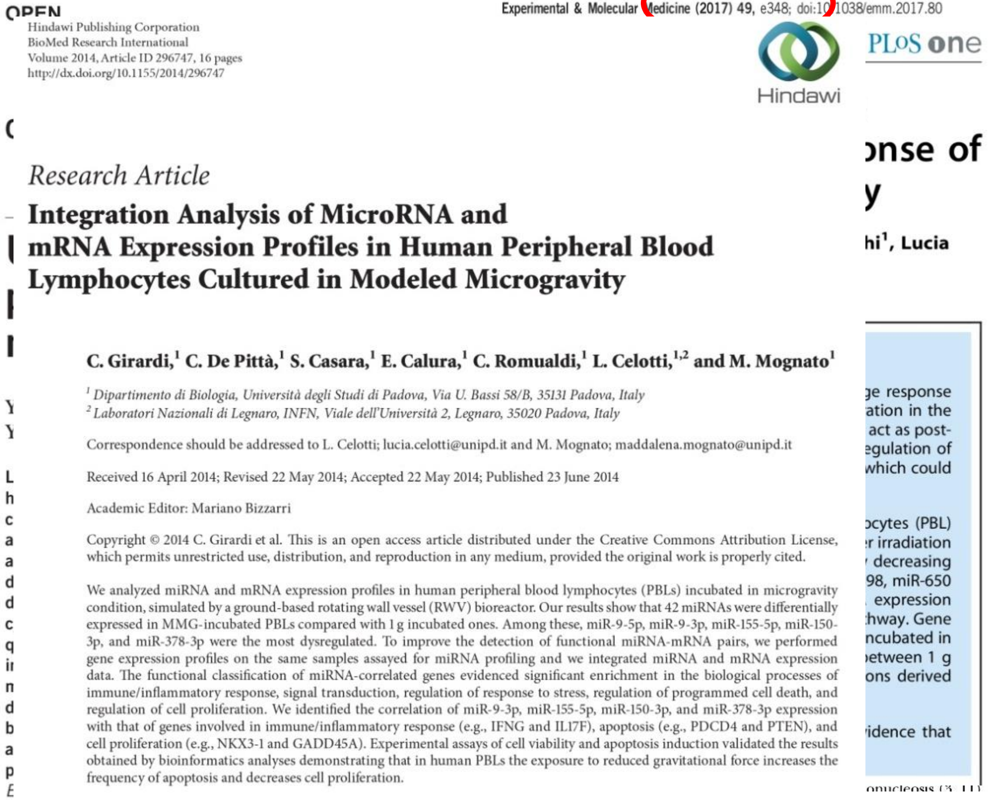
A) Top 10 predicted miRNAs from p-values



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






PLOS one
Hindawi

Hindawi Publishing Corporation
BioMed Research International
Volume 2014, Article ID 296747, 16 pages
http://dx.doi.org/10.1155/2014/296747

Research Article

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

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[1] Dipartimento di Biologia, Università degli Studi di Padova, Via U. Bassi 58/B, 35131 Padova, Italy
[2] Laboratori Nazionali di Legnaro, INFN, Viale dell'Università 2, Legnaro, 35020 Padova, Italy

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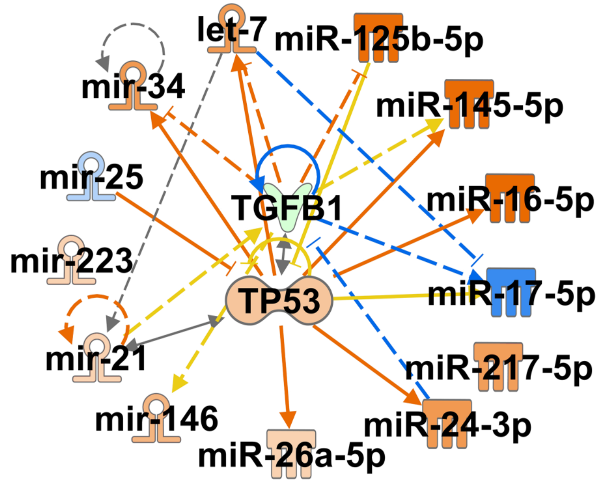
We analyzed miRNA and mRNA expression profiles in human peripheral blood lymphocytes (PBLs) incubated in microgravity condition, simulated by a ground-based rotating wall vessel (RWV) bioreactor. Our results show that 42 miRNAs were differentially expressed in MMG-incubated PBLs compared with 1 g incubated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



Beheshti, et al., PLOS One, 2018

Predict miRNAs with Space Radiation Cardiovascular Risk

International Journal of *Molecular Sciences*

MDPI

Article

GeneLab Database Analyses Suggest Long-Term Impact of Space Radiation on the Cardiovascular System by the Activation of *FYN* Through Reactive Oxygen Species

Afshin Beheshti [1,*], J. Tyson McDonald [2], Jack Miller [3], Peter Grabham [4] and Sylvain V. Costes [5,*]

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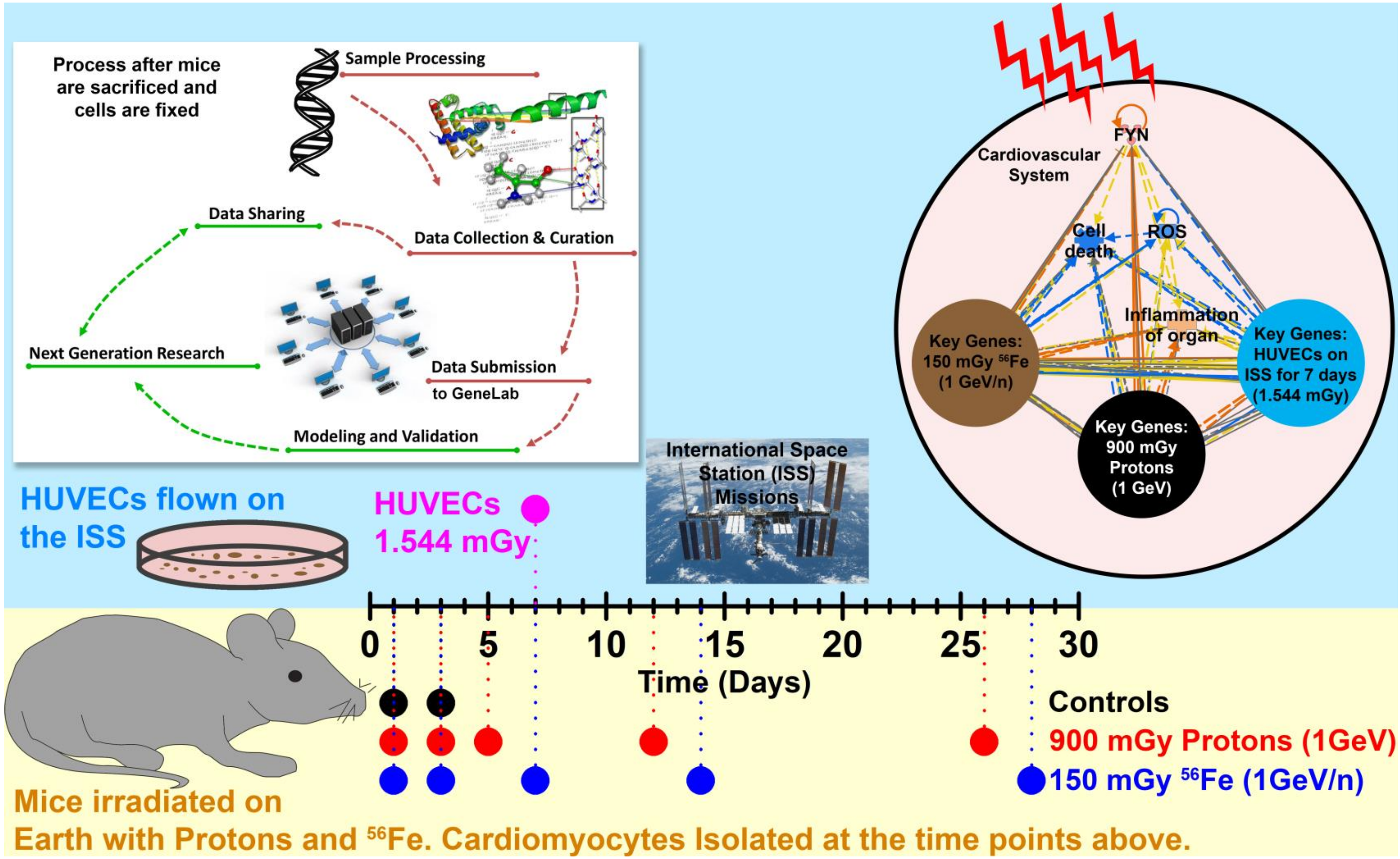
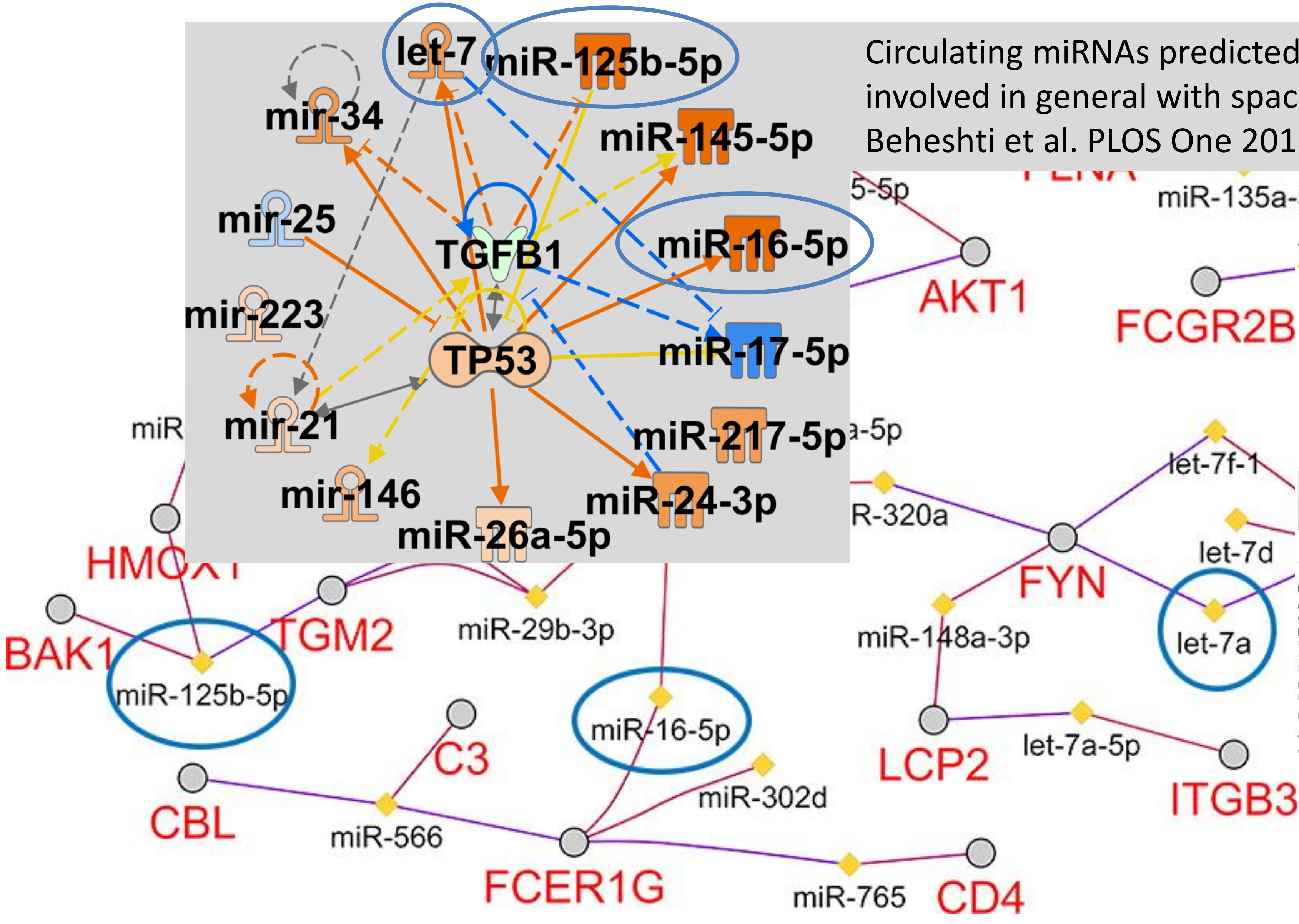
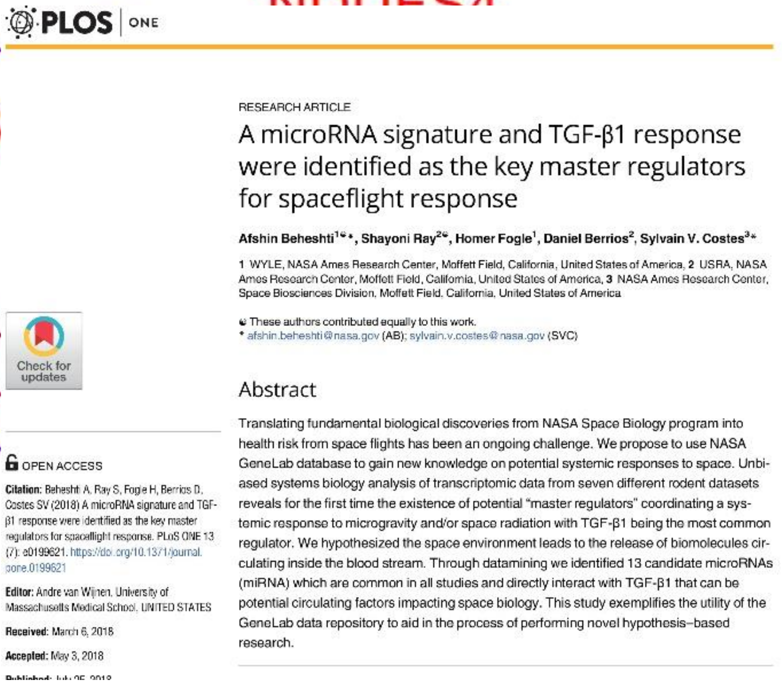
* Correspondence: afshin.beheshti@nasa.gov (A.B.); sylvain.v.costes@nasa.gov (S.V.C.); Tel.: +1-650-604-5343 (S.V.C.)



Predict miRNAs with Space Radiation Cardiovascular Risk



Circulating miRNAs predicted to be involved in general with spaceflight from Beheshti et al. PLOS One 2018



PLOS | ONE

RESEARCH ARTICLE

A microRNA signature and TGF-β1 response were identified as the key master regulators for spaceflight response

Afshin Beheshti[1☯*], Shayoni Ray[2☯], Homer Fogle[1], Daniel Berrios[2], Sylvain V. Costes[3*]

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Check for updates

OPEN ACCESS

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Received: March 6, 2018

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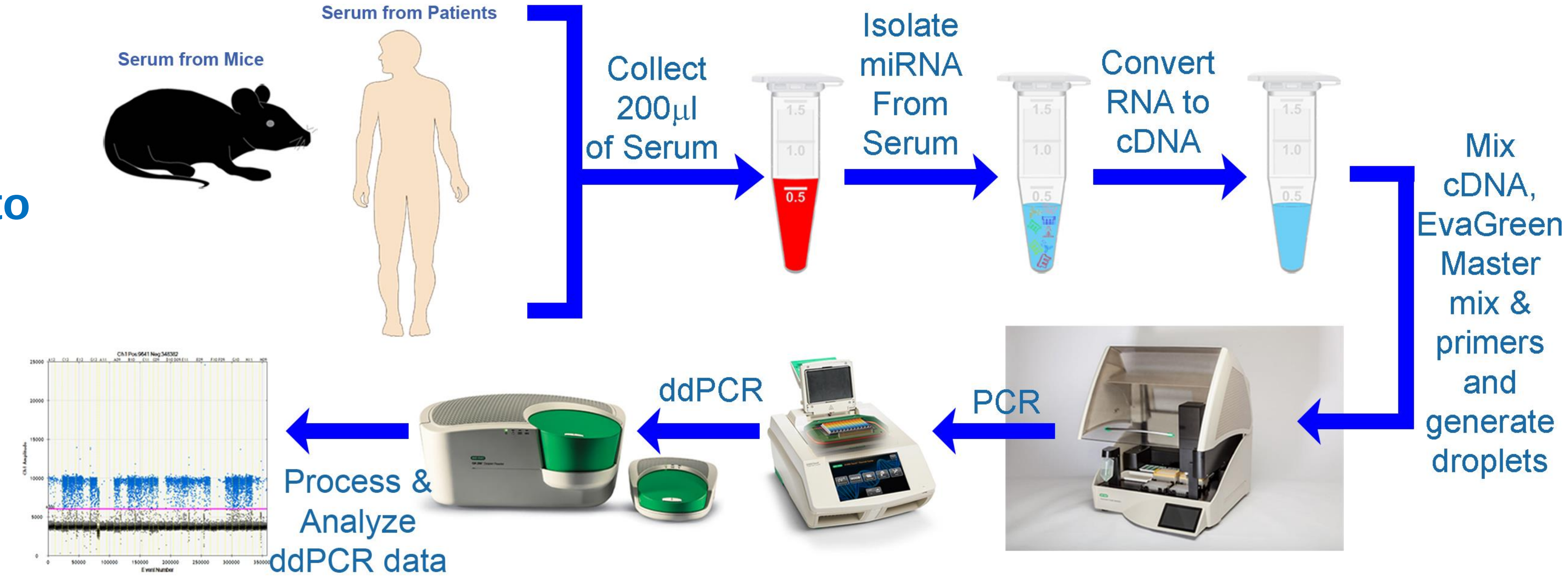
Abstract

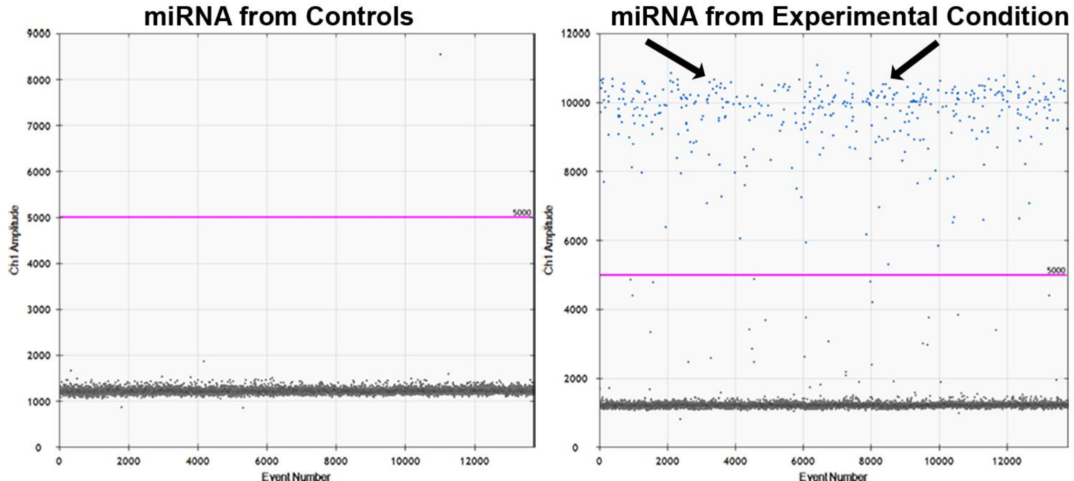
Translating fundamental biological discoveries from NASA Space Biology program into health risk from space flights has been an ongoing challenge. We propose to use NASA GeneLab database to gain new knowledge on potential systemic responses to space. Unbiased systems biology analysis of transcriptomic data from seven different rodent datasets reveals for the first time the existence of potential "master regulators" coordinating a systemic response to microgravity and/or space radiation with TGF-β1 being the most common regulator. We hypothesized the space environment leads to the release of biomolecules circulating inside the blood stream. Through datamining we identified 13 candidate microRNAs (miRNA) which are common in all studies and directly interact with TGF-β1 that can be potential circulating factors impacting space biology. This study exemplifies the utility of the GeneLab data repository to aid in the process of performing novel hypothesis–based research.

Technique to Quantify miRNAs

Gathered archived serum, plasma, and serum from various collaborators related to spaceflight experiments



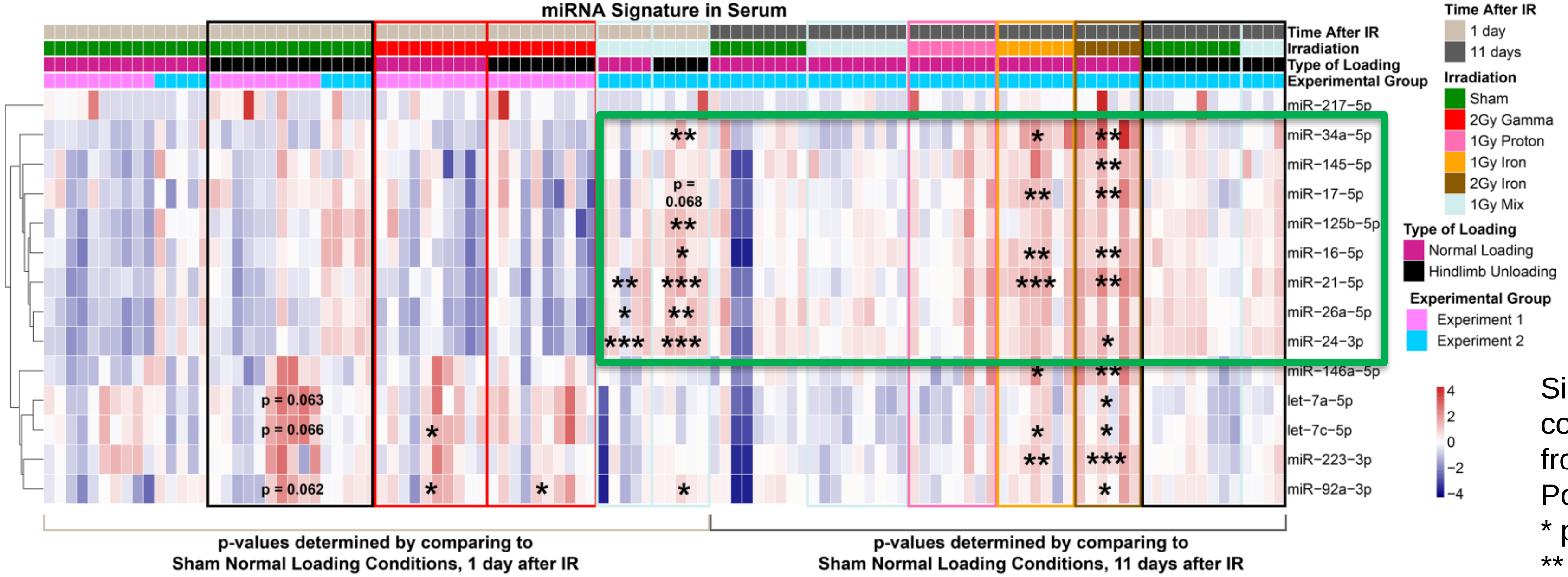
Through ddPCR we are able to get exact counts of circulating miRNA in the serum



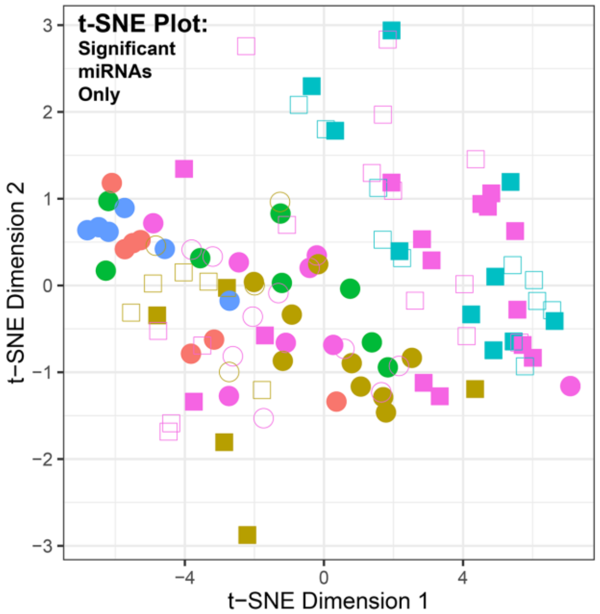
Every single blue point on the plots represents one copy of miRNA (see arrows).

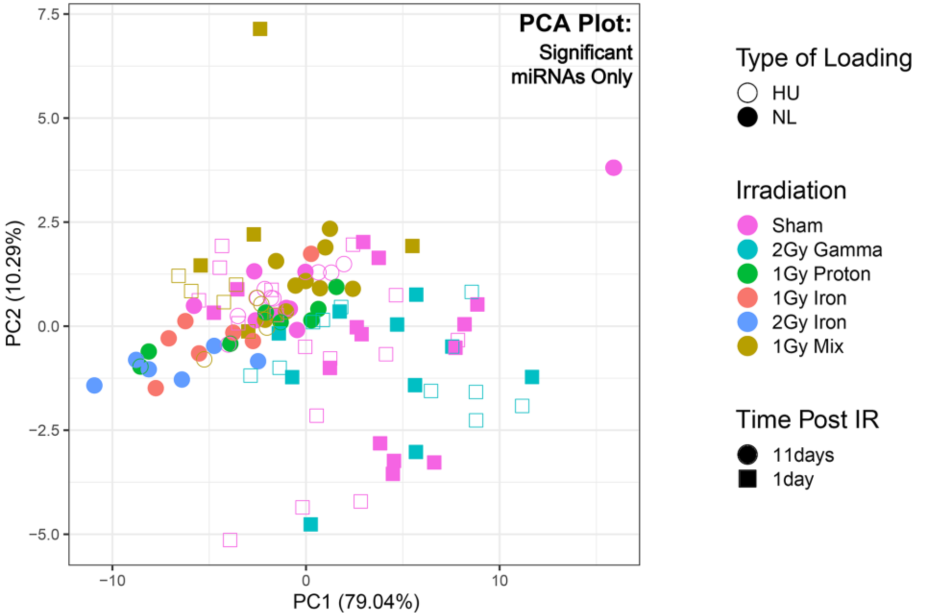
Presence of miRNA signature in Serum of Mice in Simulated Space Environment

- Female C57BL/6 mice
- HU for an initial three days followed by IR and continuation of HU for another 1 or 11 days
- Radiation exposure: Total body irradiation on conscious mice
  - 2Gy Gamma
  - 600 MeV/n ^{56}Fe (1 Gy and 2 Gy)
  - 150 MeV Proton (1Gy)
  - '1Gy Mix' (0.5Gy ^{56}Fe and 0.5Gy Proton)

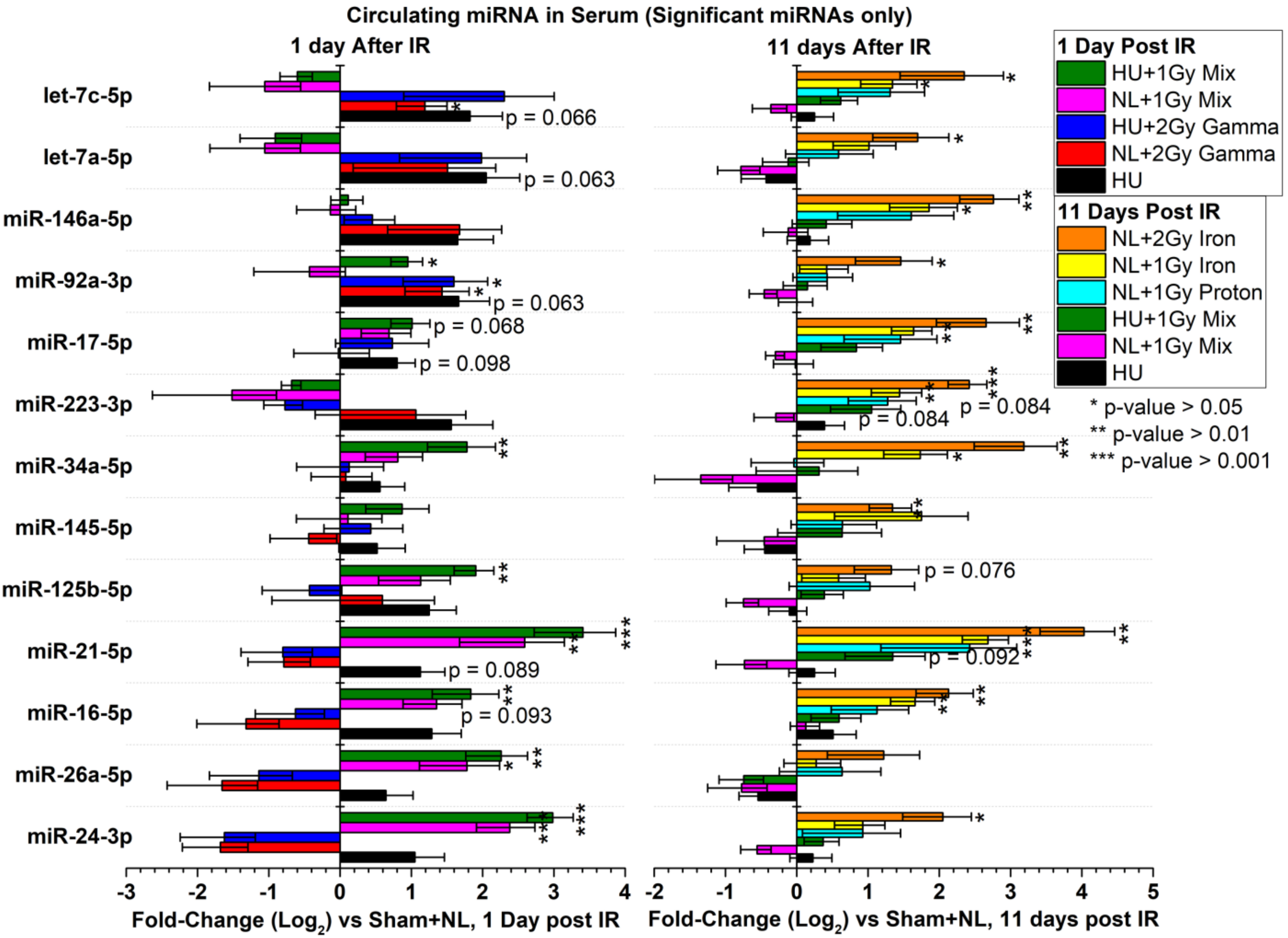


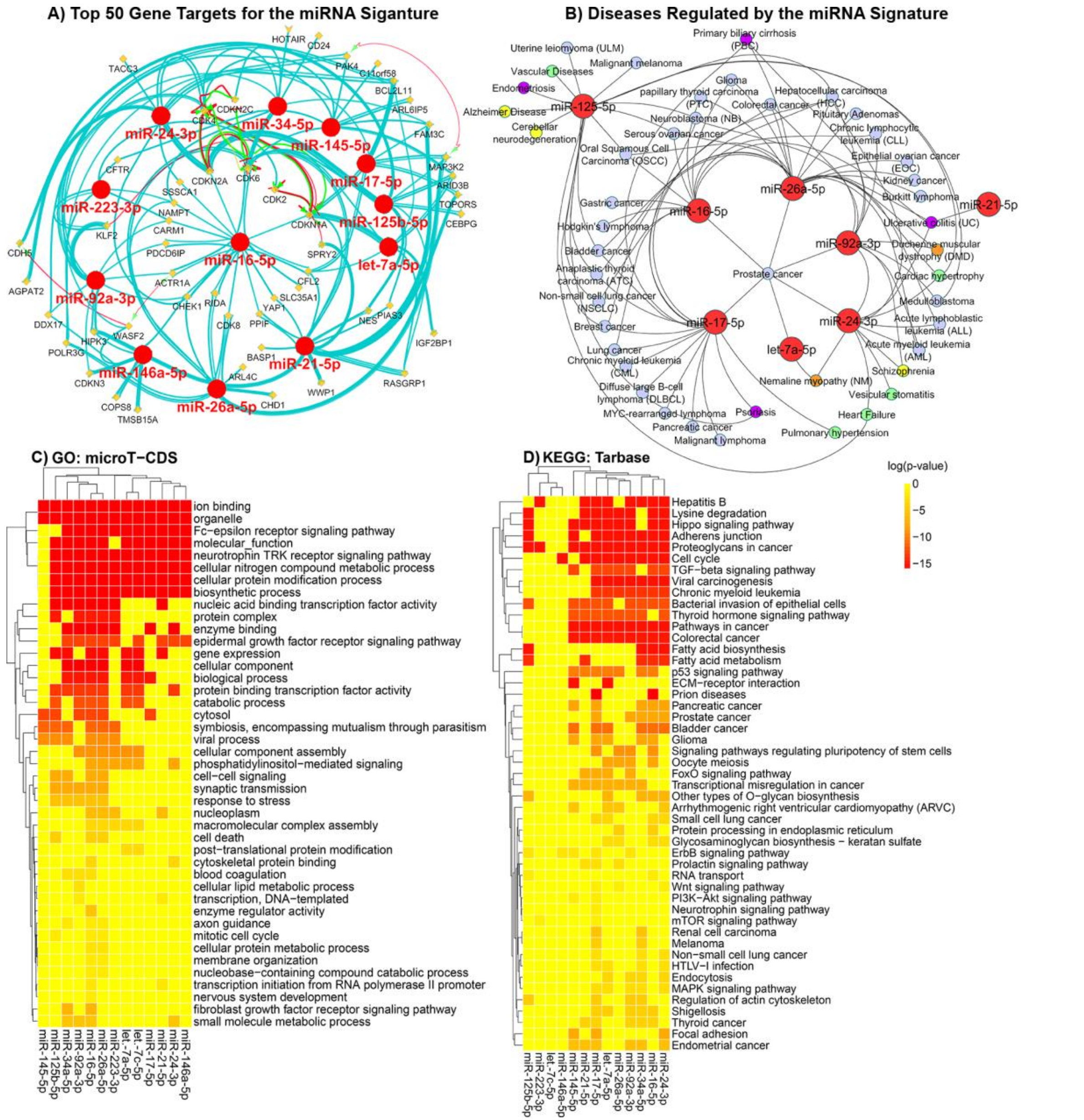
Significance compared to serum from Sham NL (Time Post IR)
* p-value < 0.05
** p-value < 0.01
*** p-value < 0.001





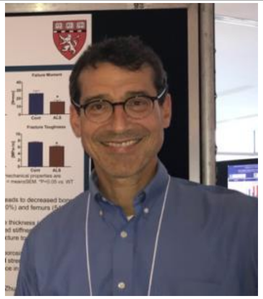
Predicted Targets for the Space Environment miRNA Signature





miRNAs Decrease with Increasing Gravity Conditions!

Partial Weight Bearing Rat Model



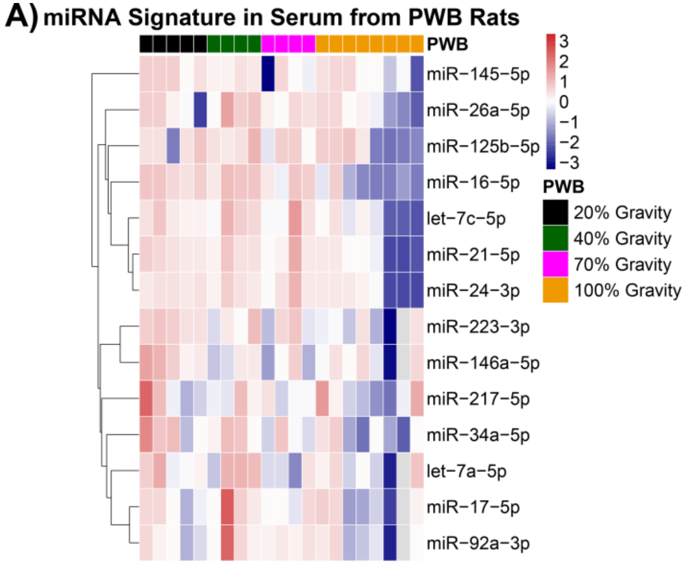
Seward Rutkove

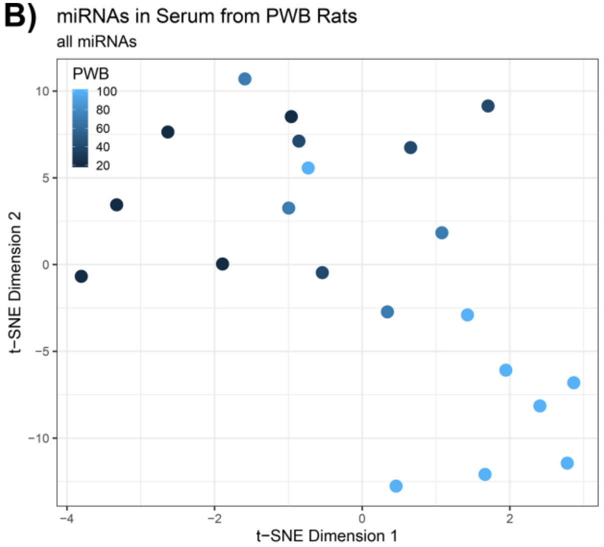
Marie Mortreux

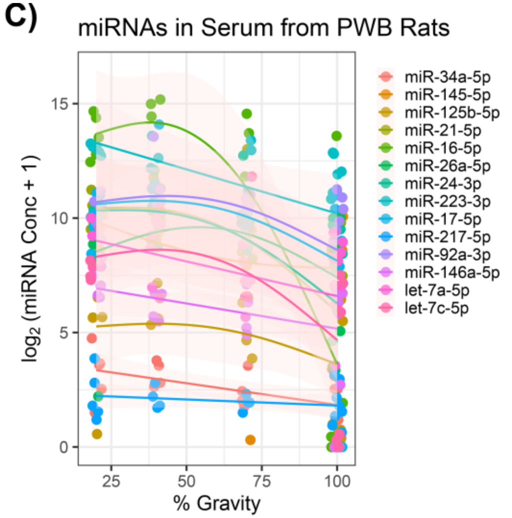
Beth Israel Deaconess Medical Center

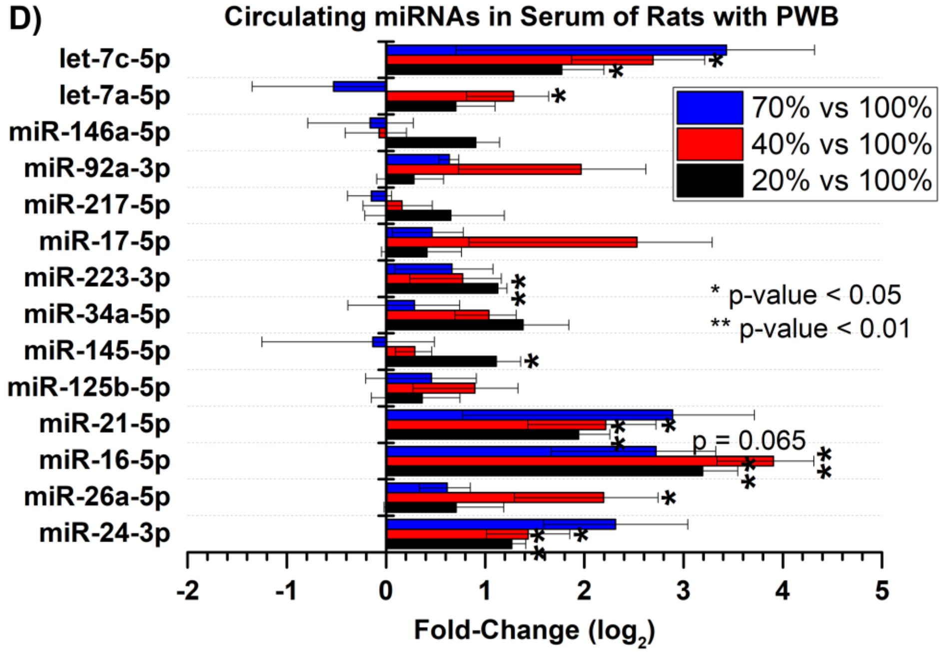
A teaching hospital of Harvard Medical School

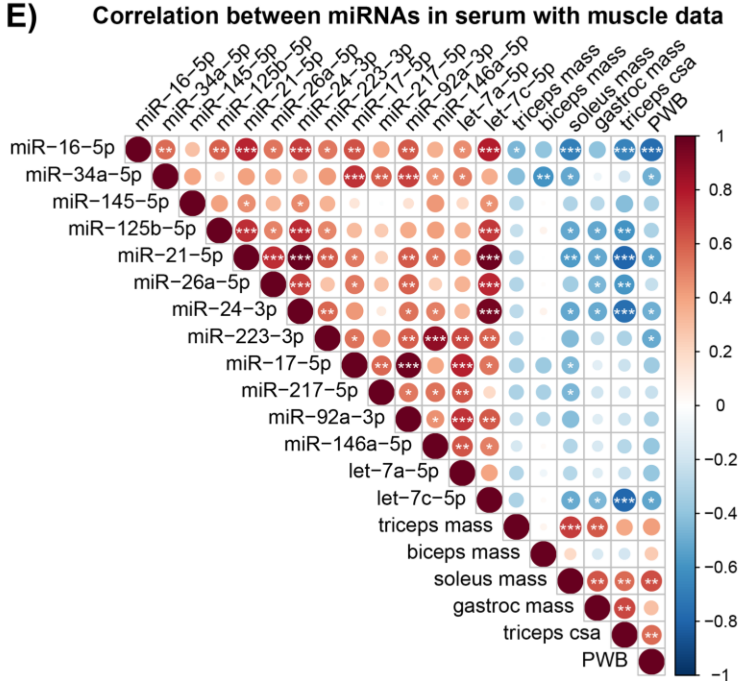
https://www.rutkovelab.org/nasa/



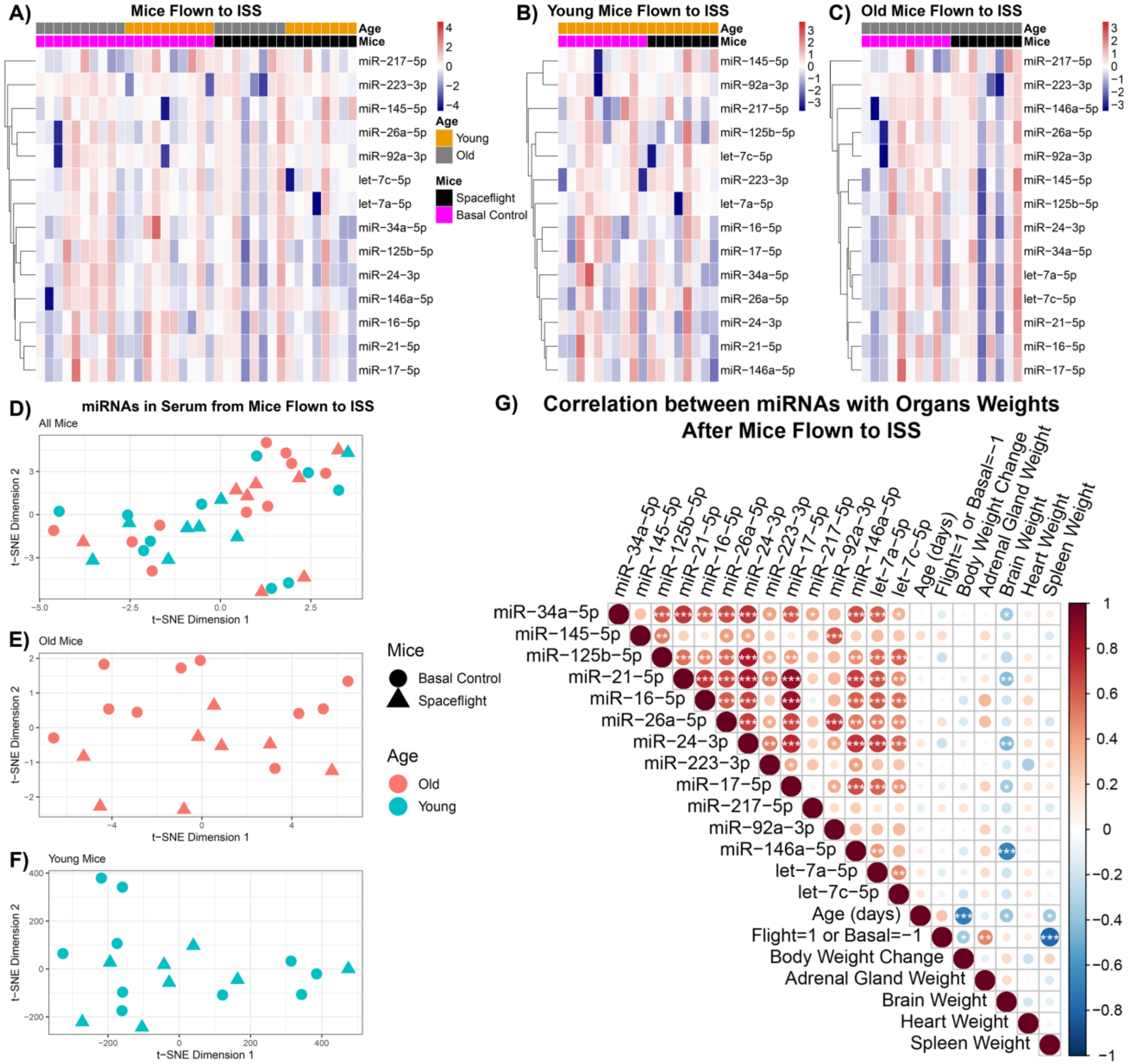


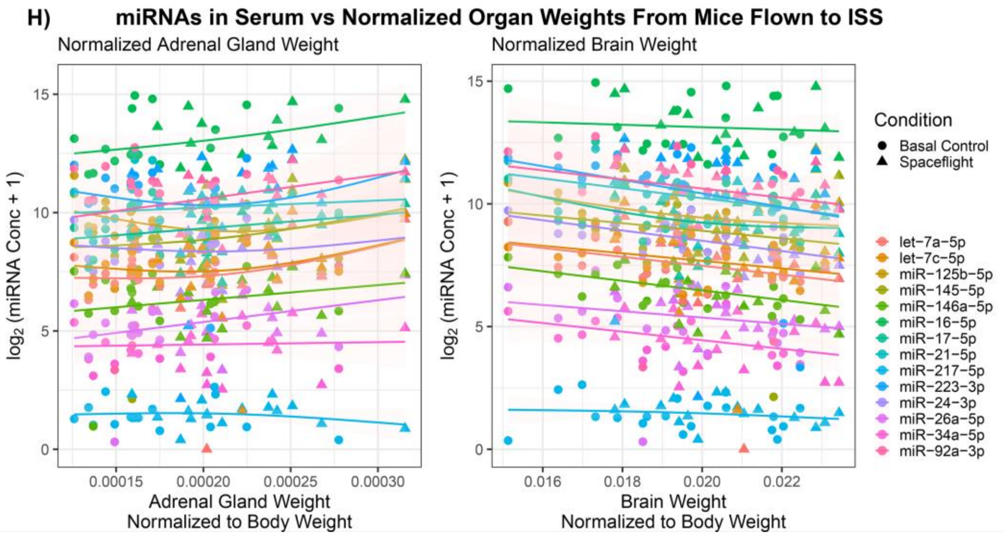






Direct Impact of the miRNAs After Mice Return from Space





- **Female BALB/c Mice on the ISS for 35 days**
- **Returned to Earth for 4 days before being sacrificed**
- **Approximate accumulated radiation dose = 7-9mGy**

Center for the Advancement of Science in Space

Spaceflight miRNAs Relevance to Humans

- Human peripheral blood mononuclear cells (PBMCs) were irradiated at BNL with 0.3Gy and 0.82Gy ^{56}Fe.
- PBMCs from different individuals.
- Cells were fixed 4 hrs after irradiation.

Sylvain Costes

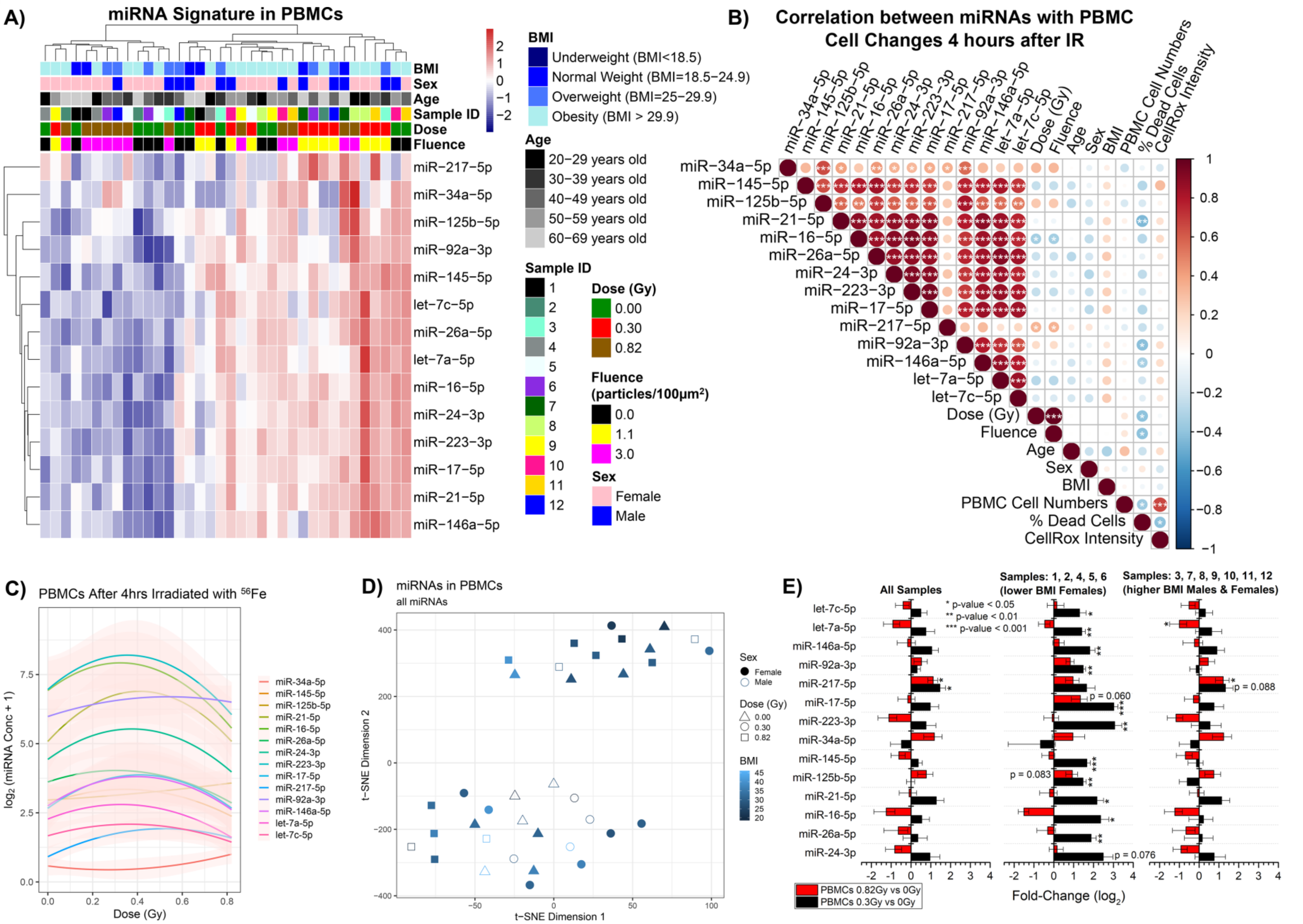
Egle Cekanaviciute

Sherina Malkani

Ames Research Center

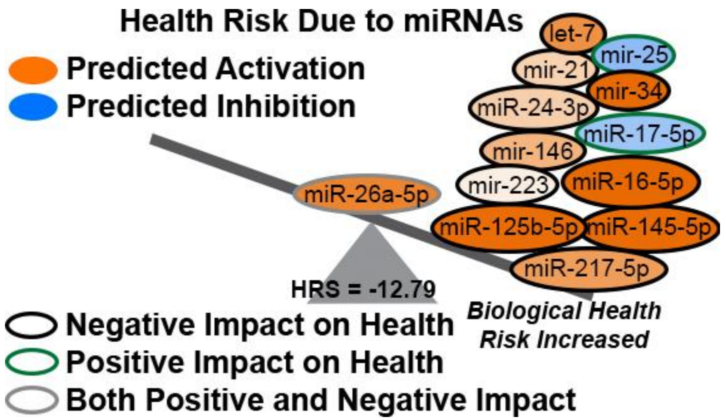
Space Biosciences
NASA AMES RESEARCH CENTER

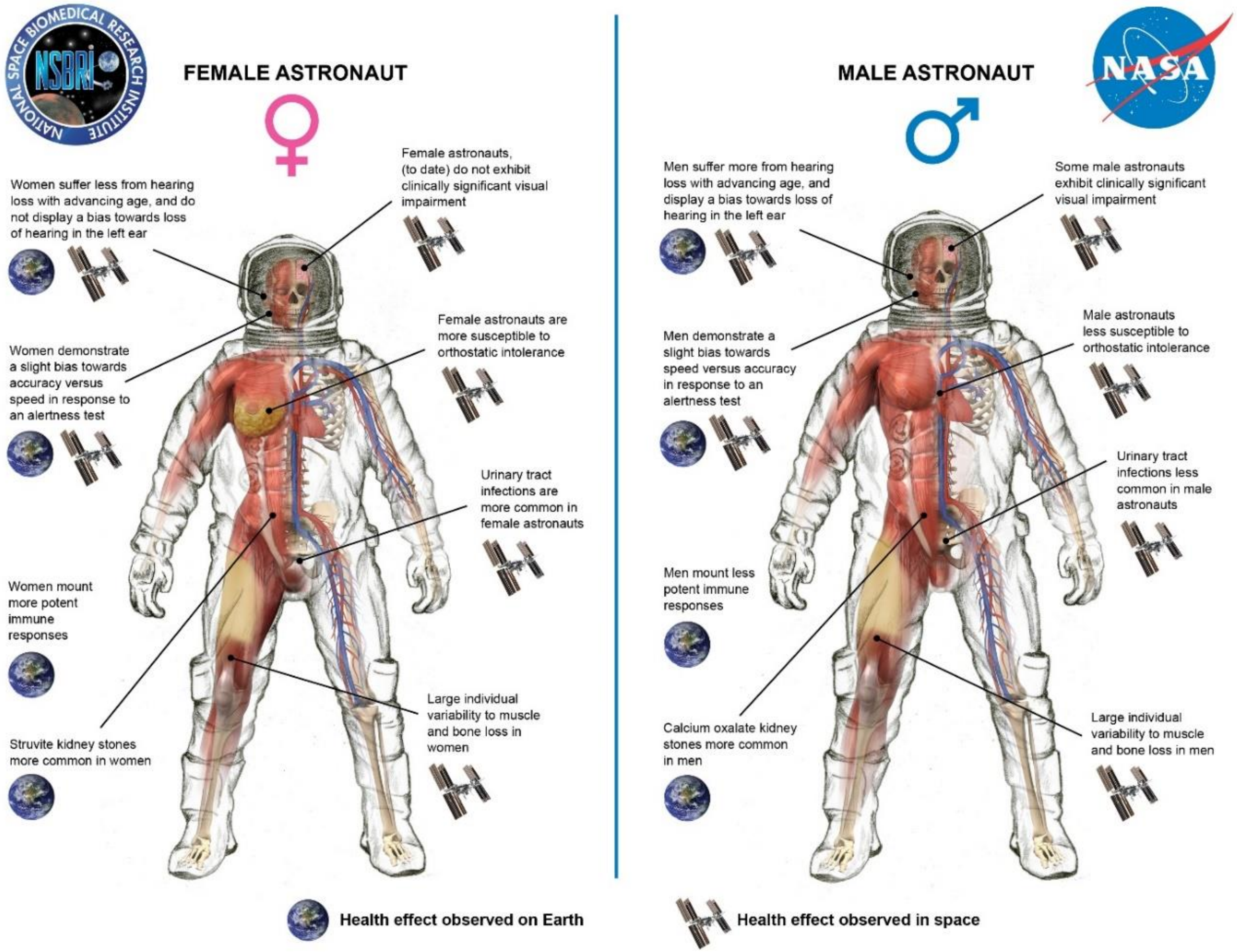
miRNA data from the NASA Twin Study also confirms that this miRNA signature does exist in astronauts flown in space!! (Unfortunately can't show results until getting final approvals from NASA)



Conclusion part 2: General Spaceflight miRNA Signature Can Be Utilized for Monitoring Spaceflight Health Risks

This spaceflight associated miRNA signature can be a novel minimally invasive biomarker to monitor increased health risks for long-term space missions





Acknowledgments for miRNA Space Biology Studies

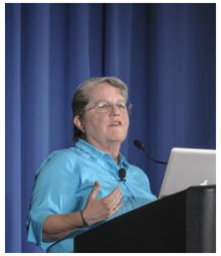

Sylvain Costes
PBMC samples


Egle Cekanaviciute
PBMC samples & Quantifying miRNAs


Sherina Malkani
Quantifying miRNAs


Ann-Sofie Schreurs
Provided Archived Tissues


Yasaman Shirazi
Provided Archived Tissues

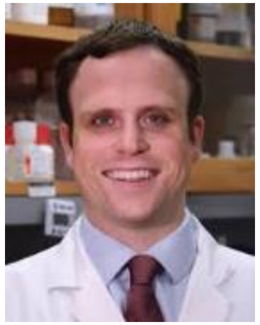

Ruth Globus
Provided Archived Tissues

NASA
Ames Research Center
Space Biosciences
NASA AMES RESEARCH CENTER

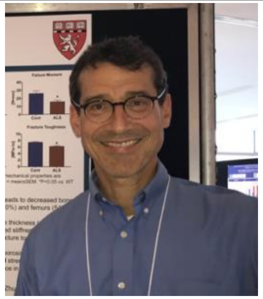

Elizabeth Blaber
Future Mice experiments


Margareth Cheng-Campbell
Quantifying miRNAs

NASA
Ames Research Center
Rensselaer


Chris Mason
Providing miRNA Twin Study Data

Cornell University


Seward Rutkove


Marie Mortreux

PWB samples

Beth Israel Deaconess Medical Center
A teaching hospital of Harvard Medical School


Peter Grabham
Columbia University Irving Medical Center


Charles Vanderburg
BROAD INSTITUTE


J. Tyson McDonald
Georgetown University
Georgetown University Medical Center

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NASA

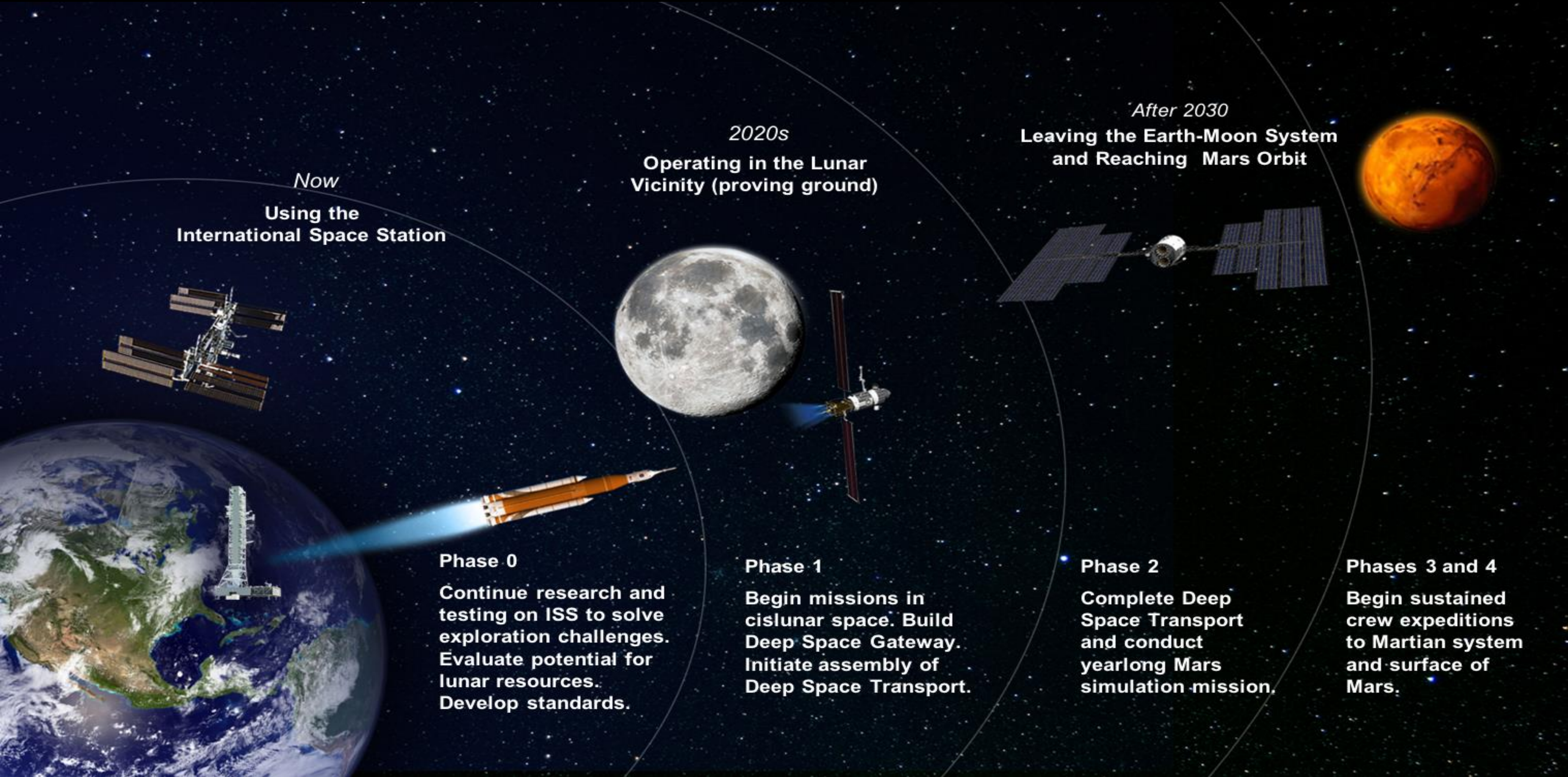
16-ROSBFP GL-0005: NNH16ZTT001N-FG Appendix G: Solicitation of Proposals for Flight and Ground Space Biology Research

CASIS

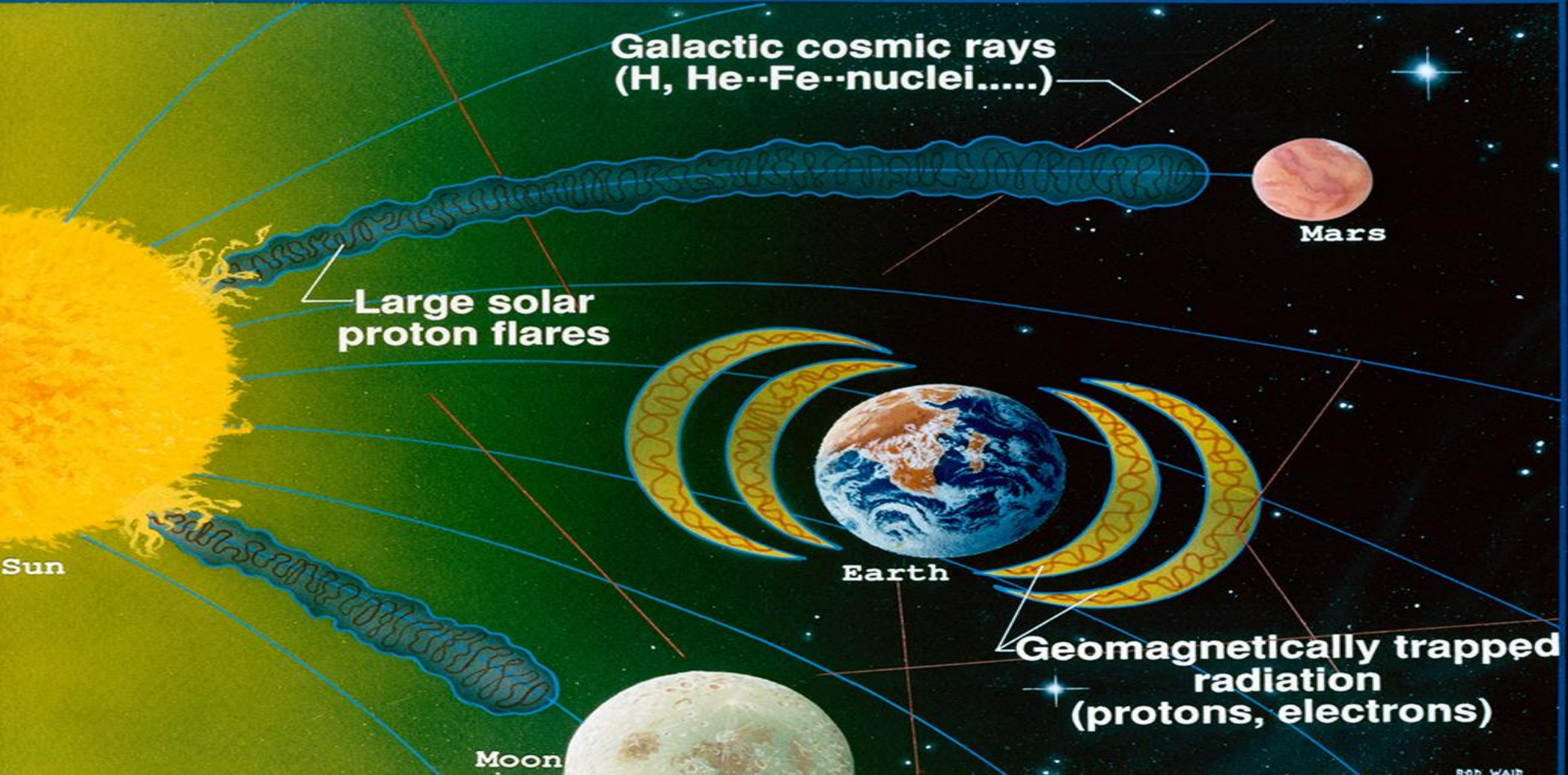
The Translational Research Institute through NASA Cooperative Agreement NNX16AO69A (T-0404)

TRANSLATIONAL RESEARCH INSTITUTE FOR SPACE HEALTH

Determining Deep Space miRNA signature Associated with Cardiovascular Health Risks



Determining Deep Space miRNA signature Associated with Cardiovascular Health Risks



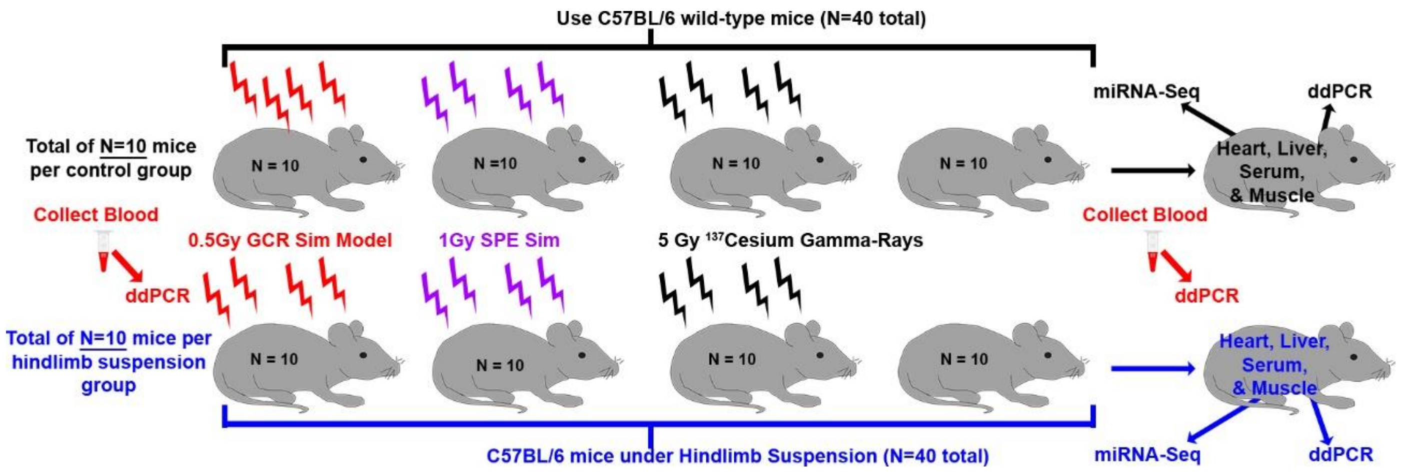
Determining Deep Space miRNA signature Associated with Cardiovascular Health Risks

Project Aims

SPECIFIC AIM 1: To determine the impact and mechanisms of circulating miRNA signatures that drive microvascular disease and muscle degeneration associated with and without space irradiation and simulated microgravity

SPECIFIC AIM 2: Establish functional significance and develop countermeasure strategies for circulating miRNAs and signaling pathways associated with microvascular disease and muscle degeneration with space irradiation and simulated microgravity (SMG).

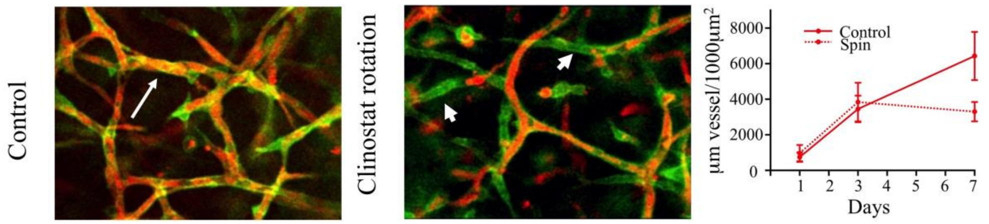
Specific Aim 1: Identify Key miRNA Signature

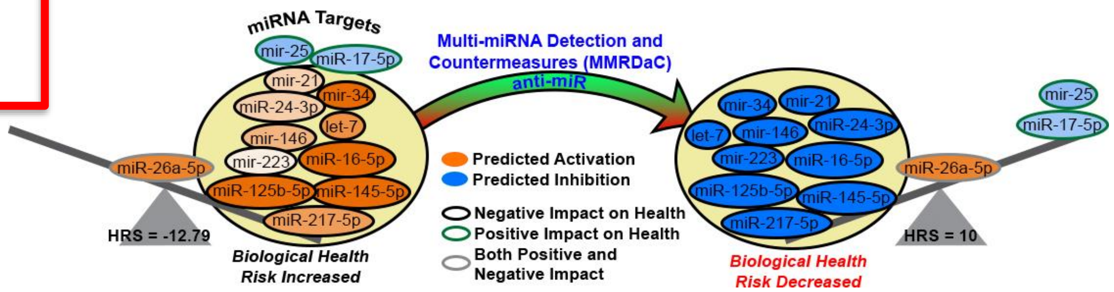


Specific Aim 2: Utilize 3D microvascular tissue models to determine functional impact of miRNAs and start development of miRNA based countermeasures with both *in vitro* and *in vivo* models.

Peter Grabham

Columbia University Irving Medical Center



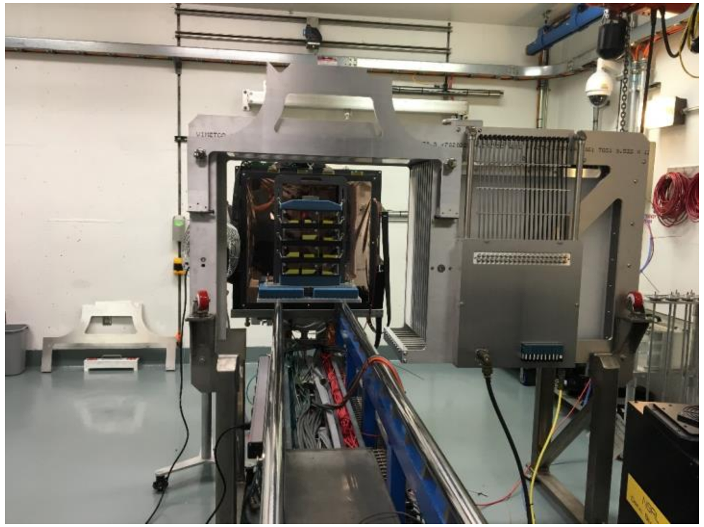


Mice Irradiated at NSRL at BNL

Hindlimb Unloaded (HU) Mice

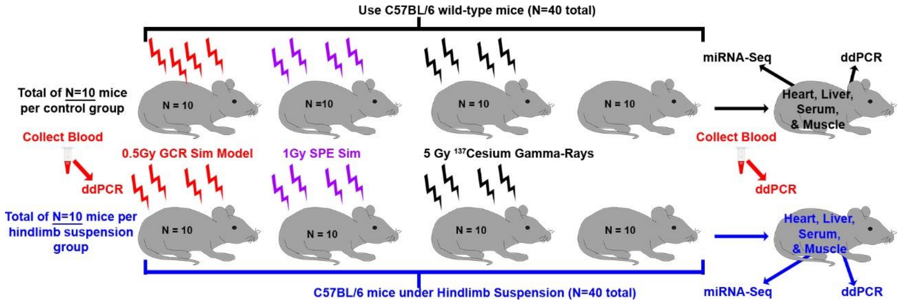
Experiment Group	Radiation Exposure	Dose
Sham	None	0.0 Gy
Sham + HU	None	0.0 Gy
Gamma	Gamma	5.0 Gy
Gamma + HU	Gamma	5.0 Gy
		1.0 Gy
		1.0 Gy
		0.5 Gy
GCR Sim + HU	GCR Sim	0.5 Gy

They're still smiling after our BNL run and finishing all the work!!



Simplified GCR Sim

Ion species	Energy (MeV/n)	LET (keV/µm)	Dose (mGy)	Dose fraction (mGy)
Proton	1000	0.2	175	0.35
^{28}Si	600	50.4	5	0.01
^{4}He	250	1.6	90	0.18
^{16}O	350	20.9	30	0.06
^{56}Fe	600	173.8	5	0.01
Proton	250	0.4	195	0.39

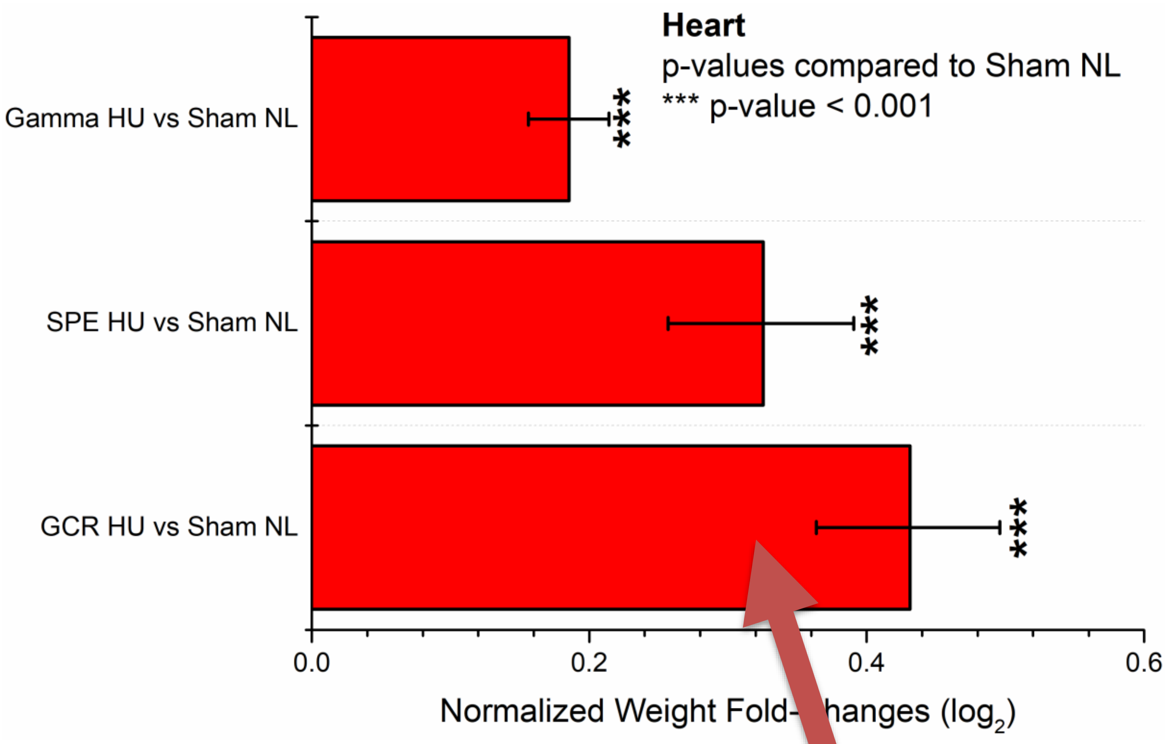


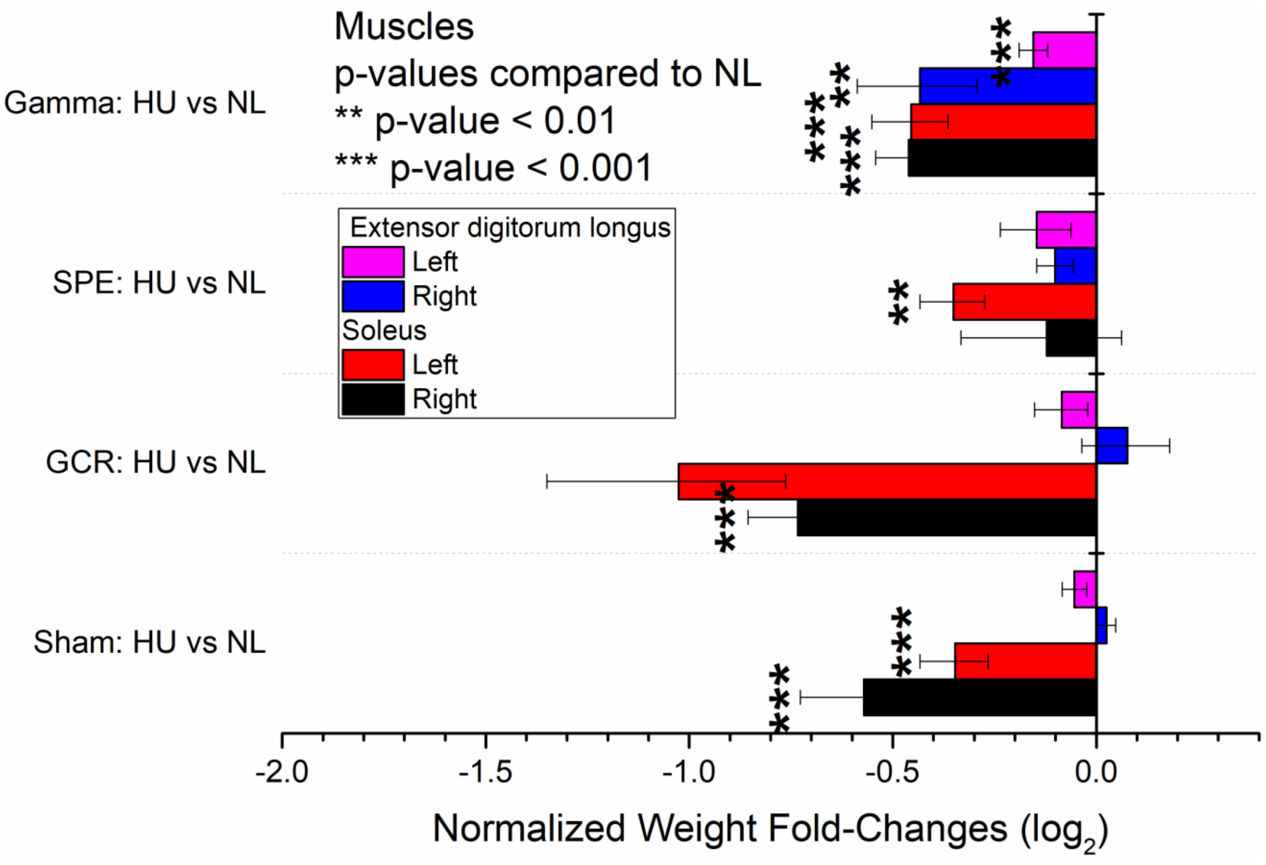
SPE Sim

Ion species	Energy (MeV/n)	Dose (cGy)
Proton	50	91.7
Proton	60	2.9
Proton	70	2.0
Proton	80	1.5
Proton	90	1.1
Proton	100	0.8
Proton	110	0.6
Proton	120	0.4
Proton	130	0.3
Proton	140	0.2
Proton	150	0.1

Thanks to Adam Rusek, Peter Guida, Mike Sivertz, BLAF, and NSRL!!!

Weights of Organs 24 hours after Irradiation





All weights normalized to total body weight

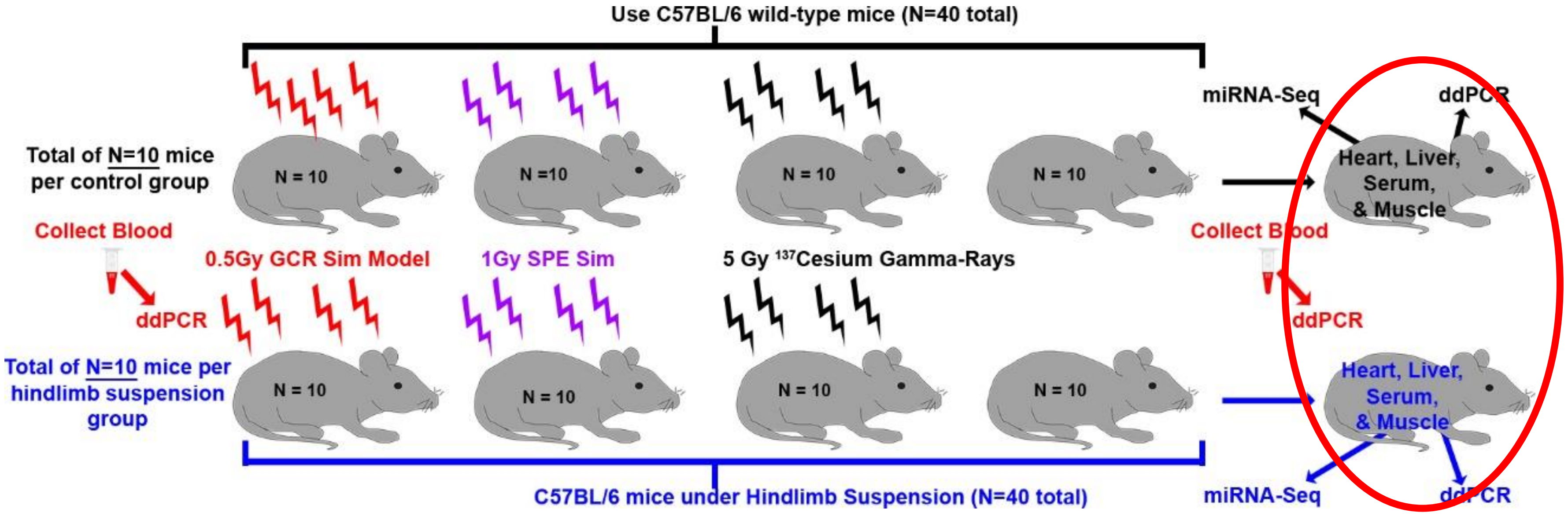
~35% increase in weight!

Samples that were miRNA-sequenced

Samples Sequenced

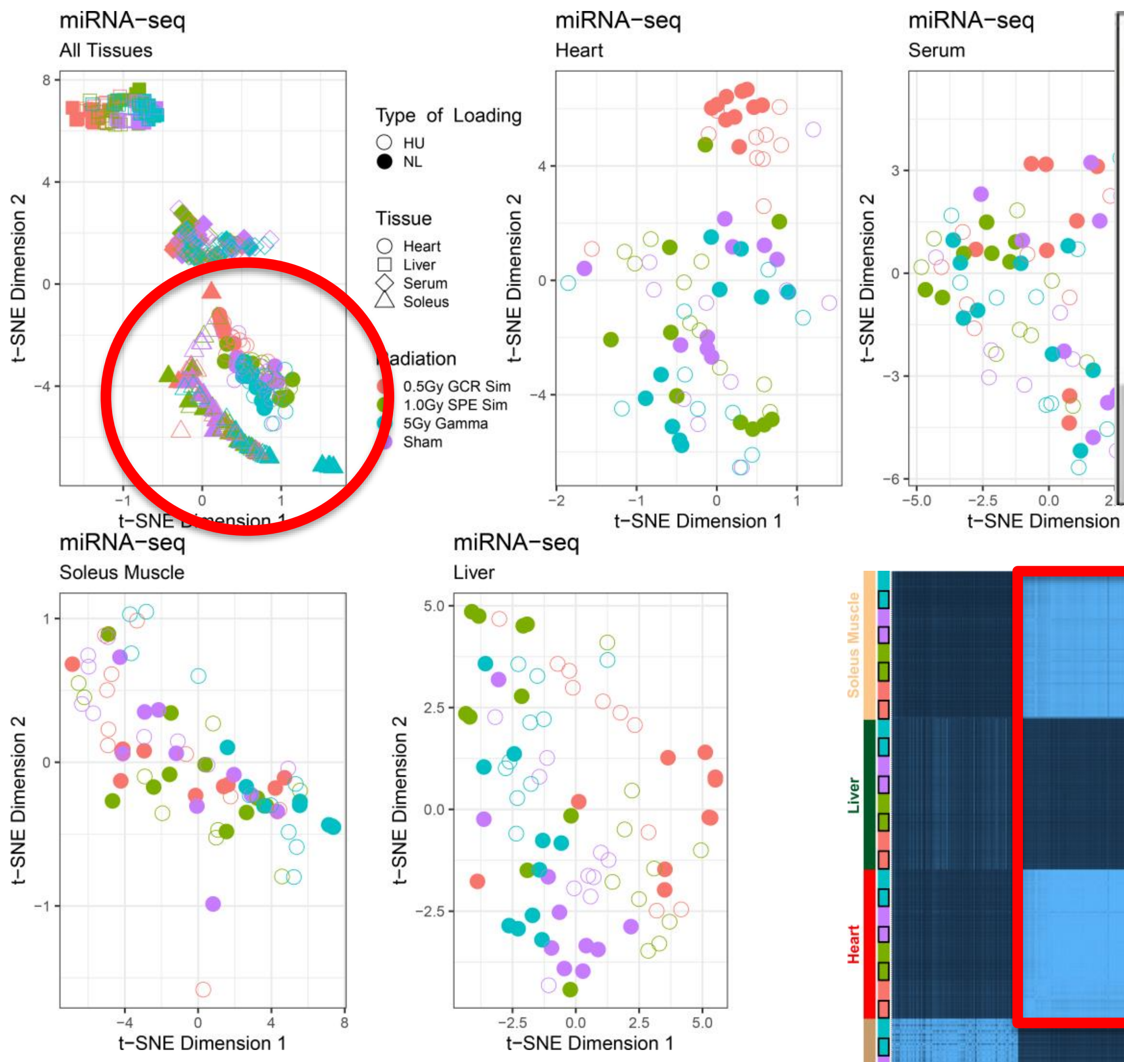
Sample	Total Number
Heart	80
Serum	80
Liver	80
Soleus Muscle	80

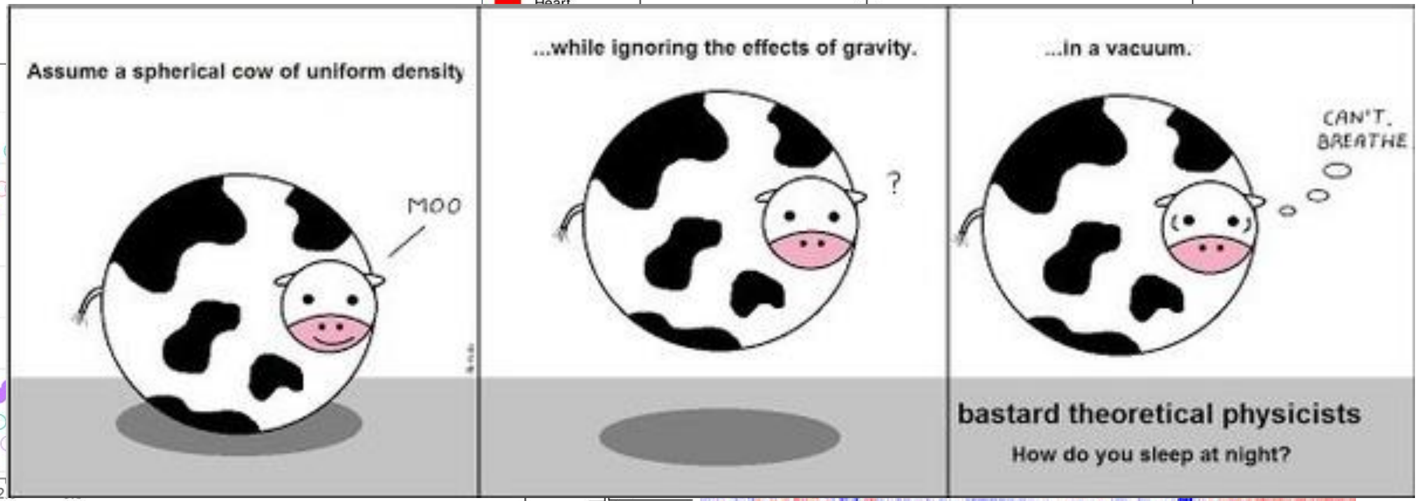
- **Total of 320 samples for miRNA-sequencing**

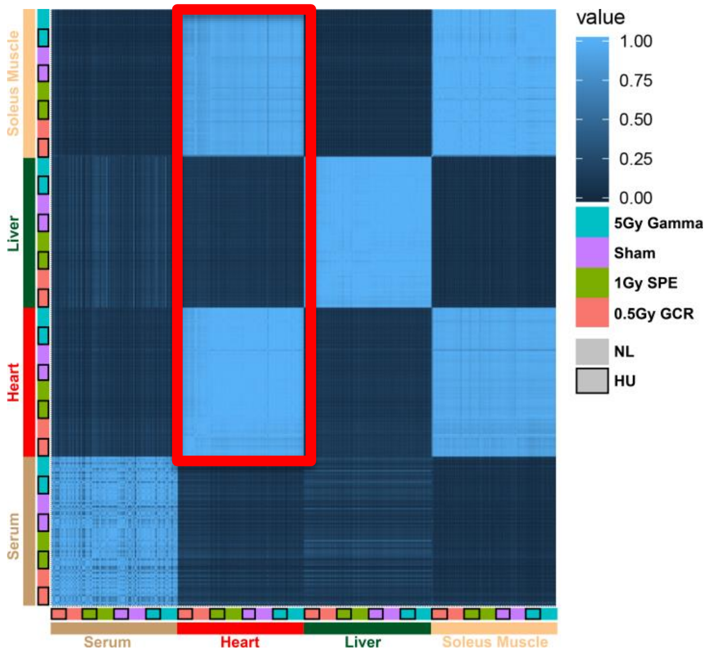


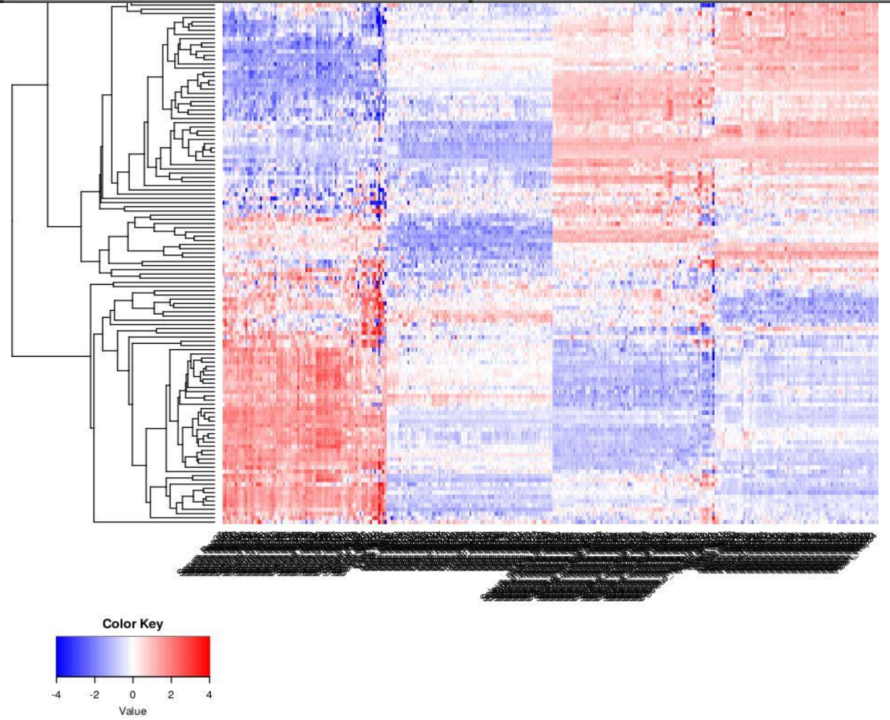
All miRNA-seq data will be deposited on GeneLab after first publication!!

Global View of miRNAs

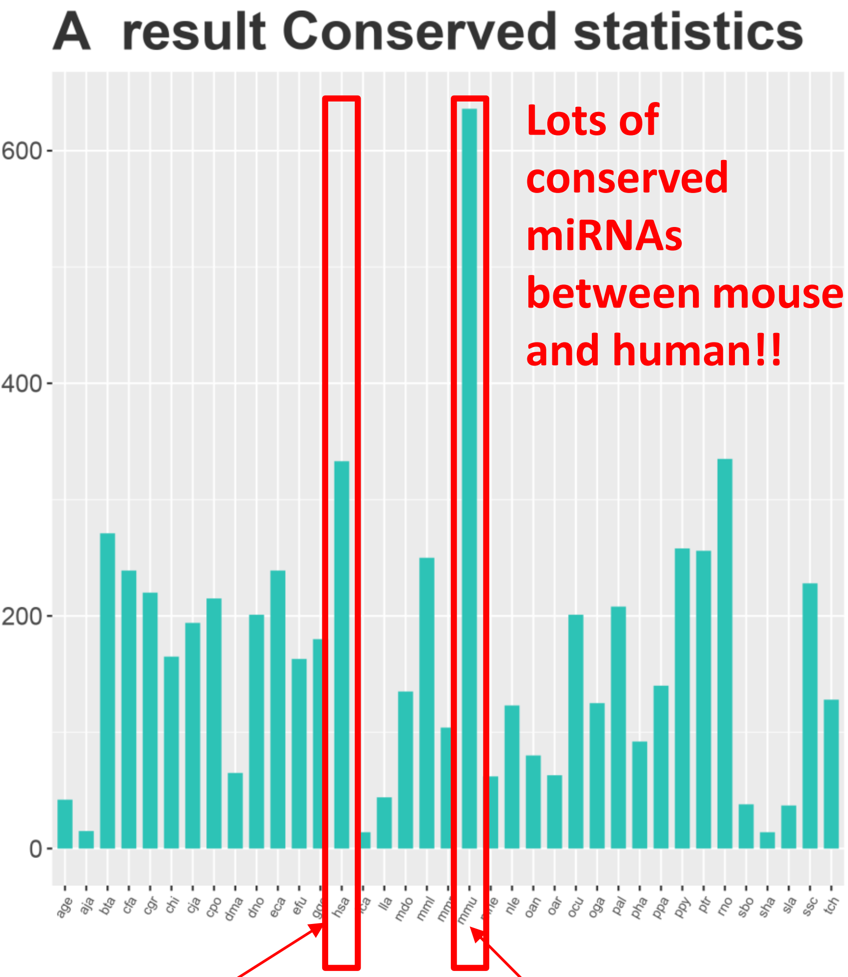








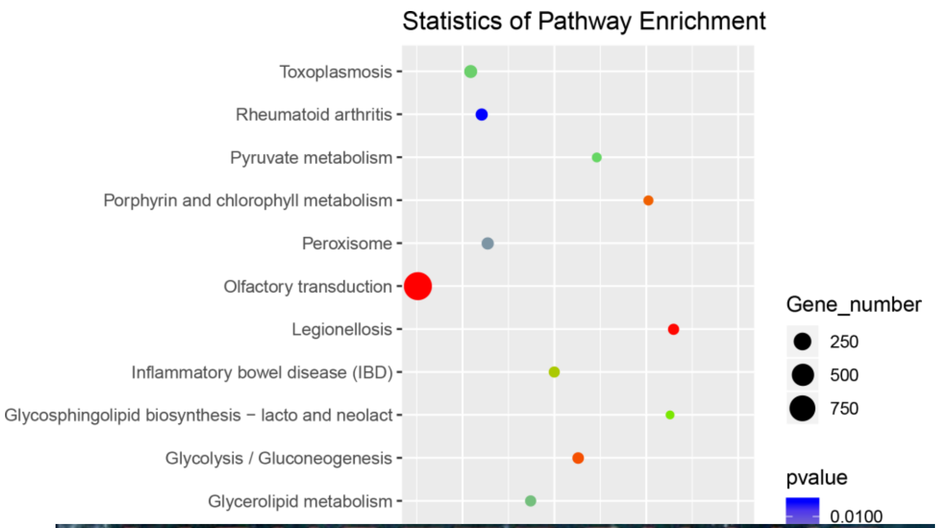
Global View of miRNAs

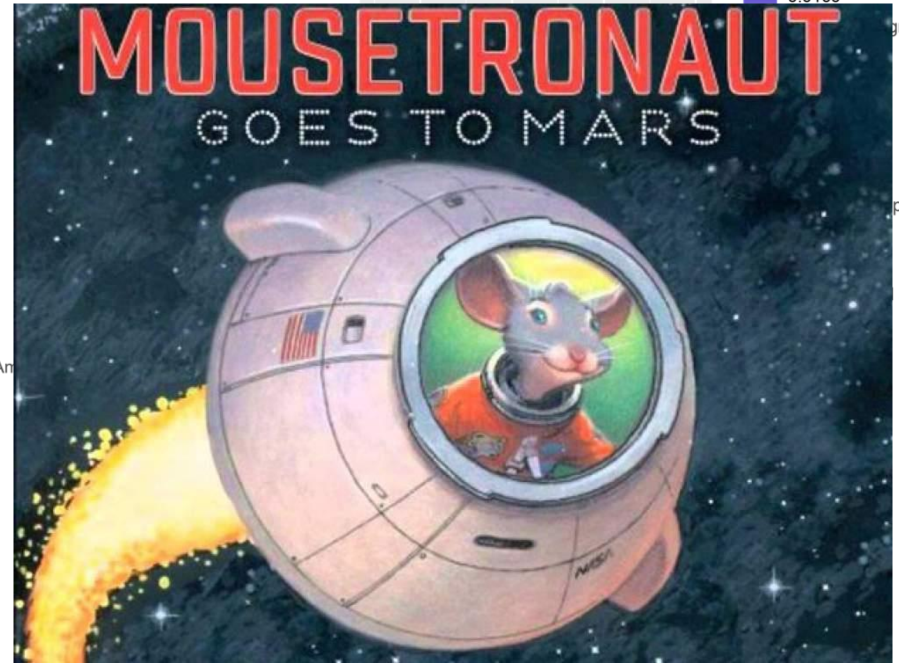


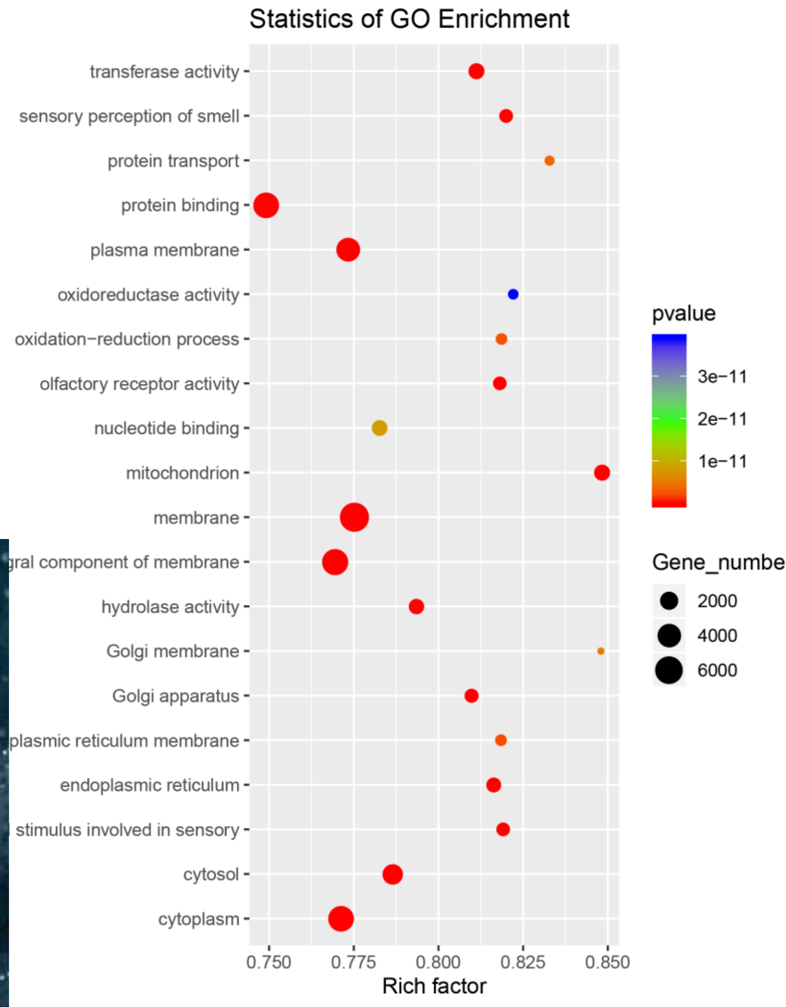
Lots of conserved miRNAs between mouse and human!!

Human miRNAs

Mouse miRNAs



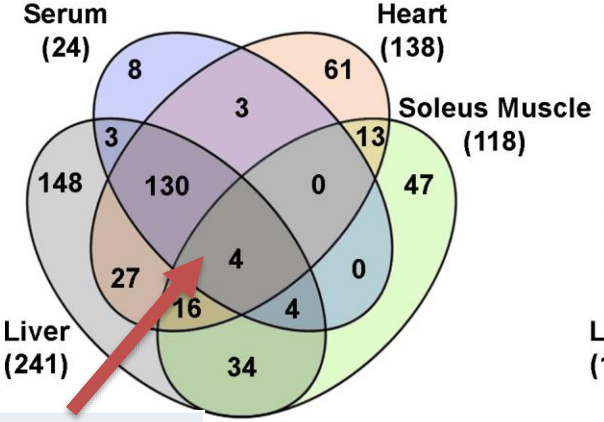




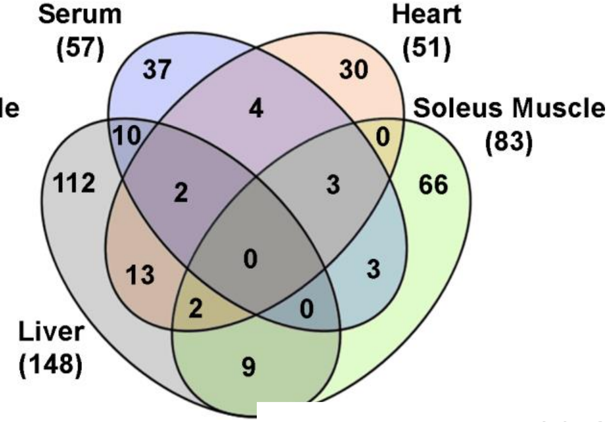
In the miRNA world this depiction is great!!

Potential Conserved Circulating miRNAs

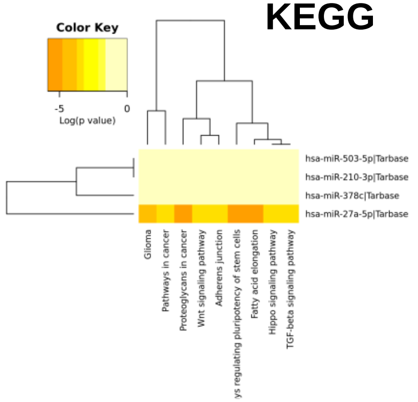
GCR HU vs Sham NL



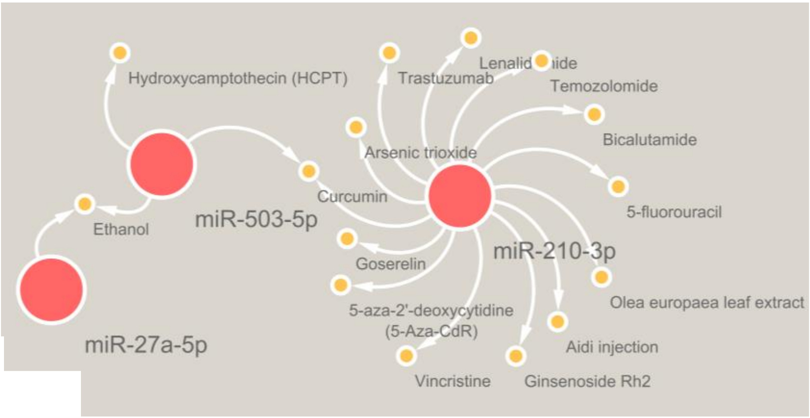
SPE HU vs Sham NL



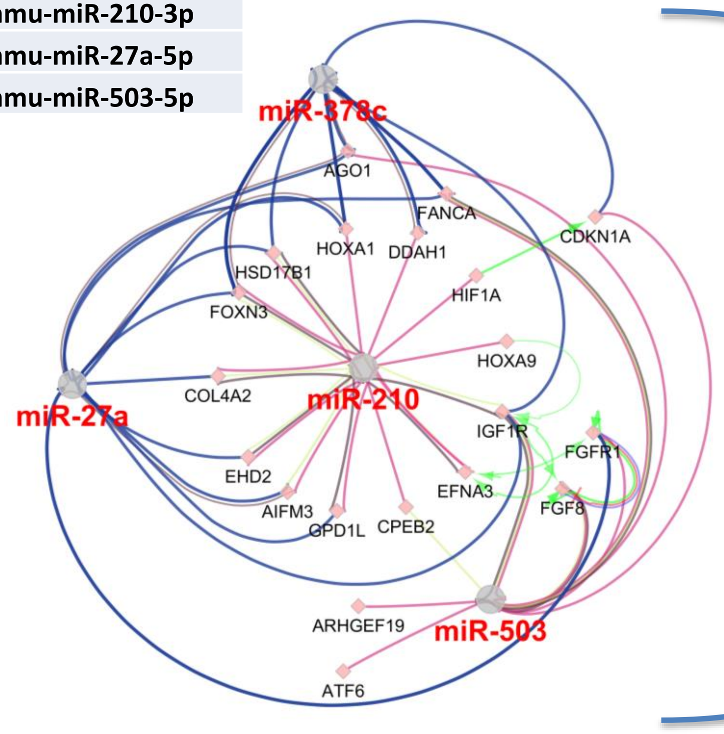
KEGG



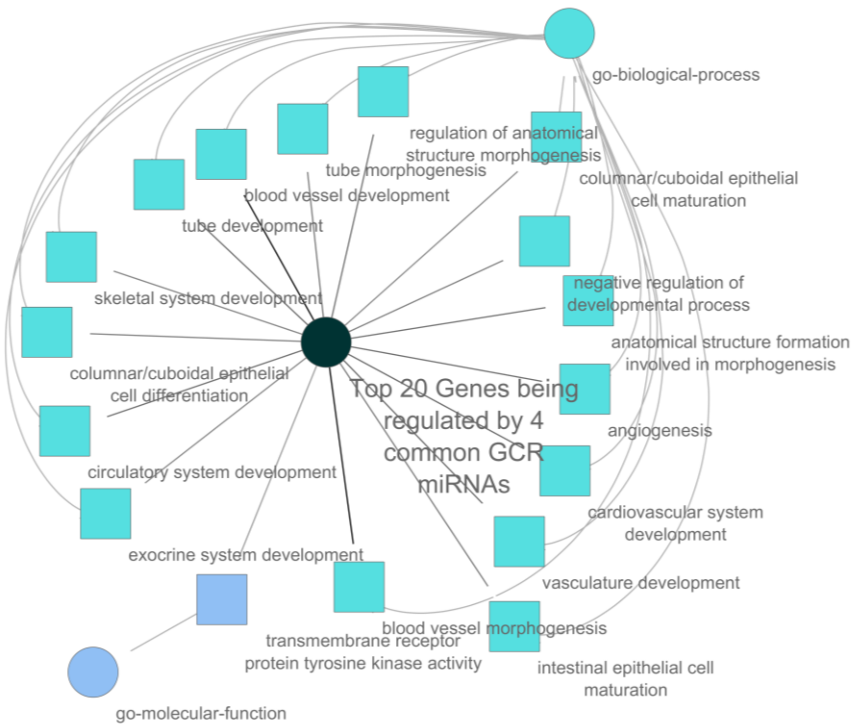
Impact on Small Molecules



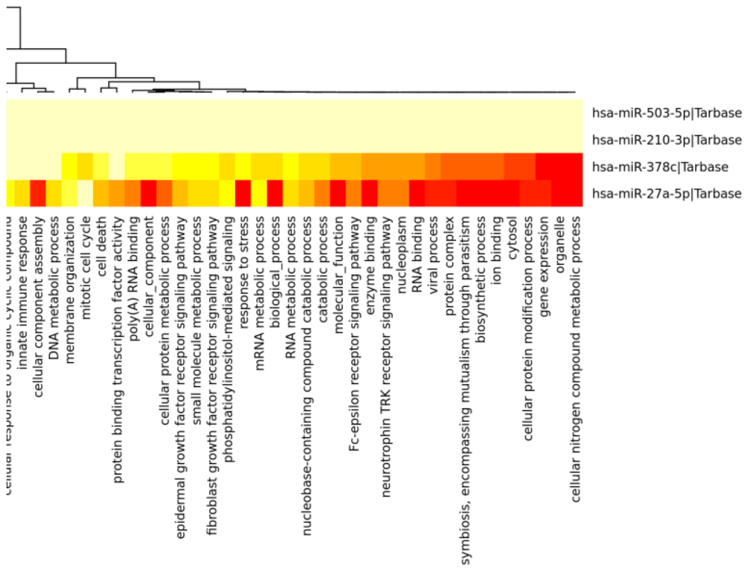
hsa-miR-378c_R-4
mmu-miR-210-3p
mmu-miR-27a-5p
mmu-miR-503-5p



Top 20 Genes Functions



Gene Ontology



Additional data that is being generated by Collaborators from other tissues: Initial data on the Whole blood related to in

Paladini et al. Journal of Experimental & Clinical Cancer Research (2016) 35:103
DOI 10.1186/s13046-016-0375-2
Journal of Experimental & Clinical Cancer Research
REVIEW Open Access

Targeting microRNAs as key modulators of tumor immune response

Laura Paladini[1], Linda Fabris[2], Giulia Bottai[1], Carlotta Raschioni[1], George A. Calin[2*] and Libero

hsa-miR-378c_R-4
mmu-miR-210-3p
mmu-miR-27a-5p
mmu-miR-503-5p

- Diff

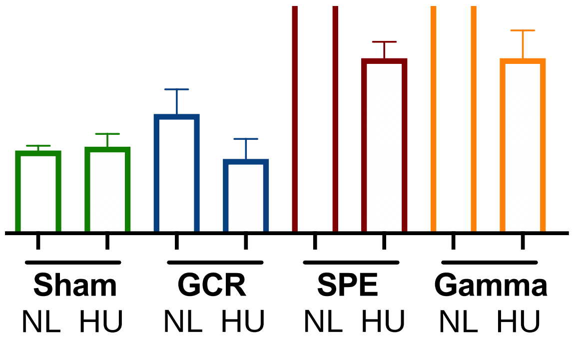
Table 1 MicroRNAs involved in Innate and Adaptive Immune System Functions

	Cell lineage	Cellular process	MicroRNAs
Immune cell progenitors			
	Hematopoietic stem cells	Cell maintenance	let-7e[a], miR-29a, miR-99b[a], **miR-125a**, **miR-126**, miR-212/132 cluster
	Multipotent progenitors	Cell development	**miR-10 family**, **miR-126**, **miR-196b**, miR-221/222
	Common myeloid progenitors	Cell development	**miR-17**, **miR-24**, miR-126, miR-128, **miR-155**, miR-181a
	Common lymphoid progenitors	Cell development	miR-126, miR-128, **miR-146**, miR-181a
	Granulocyte–macrophage progenitors	Cell development	miR-16, miR-103, miR-107
	Macrophage progenitors	Cell development	**miR-17-5p**, **miR-20a**, **miR-106a**
	Granulocyte progenitors	Cell development	**miR-223**
	Erythroid precursors	Cell development	miR-155, **miR-221/222**
	Megakaryocyte precursors	Cell development	**miR-10a**/b, miR-17, miR-20, miR-126
Innate immunity			
	Monocytes	Cell differentiation	**miR-17-5p**, **miR-20a**, miR-21, **miR-106a**, miR-155, miR-196b, miR-223, mi
		Cell activation	**miR-155**, **miR-424**
	Dendritic cells	Cell differentiation	**miR-21**, **miR-34a**
		Cell function	miR-10a, miR-148/152, **miR-155**, **miR-223**
	Macrophages	Cell differentiation	miR-15a, miR-16, miR-19a-3p, **miR-21**, miR-107, **miR-146a**, miR-424
		Cell function	**Let-7**, miR-9, **miR-21**, miR-101, **miR-125b**, **miR-146a**, miR-147, **miR-155**, miR-378, miR-487b, miR-1224
		Cell polarization	let-7c, let-7f, miR-9, miR-21, miR-33, miR-101, miR-124, miR-125, miR-146, miR-223, miR-342, miR-378, miR-511
	Granulocytes	Cell differentiation	miR-15a, miR-21, **miR-27**, miR-196b, **miR-223**
		Cell function	**miR-223**
	Neutrophils	Cell function	**miR-223**
	MDSCs	Cell function	miR-494, miR-17-5p/20a
	Megakaryocytes	Cell differentiation	miR-10a, miR-130a, miR-146a, **miR-150**, **miR-155**, **miR-223**
	Erythrocytes	Cell differentiation	miR-15a, miR-16, miR-24, miR-144, **miR-150**, **miR-155**, miR-221/222 cluster, **miR-223**, **miR-451**
	Natural killer cells	Cell differentiation	**miR-150**, **miR-181a/b**
		Cell function	miR-15/16, miR-27a, miR-29, miR-30c-1, miR-30e, **miR-155**, **miR-223**, miR-378
Adaptive immunity			
	B cells	Cell differentiation	**miR-17/92 cluster**, miR-23a, miR-34a, miR-142, **miR-150**, miR-155, miR-181 family, miR-212/132 cluster
		Cell activation	miR-9, **miR-17/92 cluster**, miR-30, miR-125b, **miR-155**, **miR-181b**, miR-223
	Plasma cells	Cell differentiation	miR-148a
	T cells	Cell differentiation	**miR-17/92 cluster**, miR-21, miR-142-3p, miR-150, **miR-181a**, miR-223

nvolved in Innate and Adaptive Immune System Functions *(Continued)*

Cell lineage	Cellular process	MicroRNAs
	Cell activation	**miR-155**, miR-181a, miR-182, miR-214
T helper cells	Cell differentiation	miR-125b, **miR-150**
	Cell function	miR-182, miR-214, miR-297, miR-669c
T helper 1 cells	Cell differentiation	miR-17/92 cluster, miR-29, miR-146a, miR-148a, **miR-155**, **miR-210**, **miR-326**
T helper 2 cells	Cell differentiation	miR-21, miR-27, miR-28
	Cell function	**miR-155**
T cytotoxic cells	Cell differentiation	Let-7f, miR-15b, miR-16, miR-17/92 cluster, miR-21, miR-139, miR-142, miR-150, miR-155, mi
	Cell function	**miR-17/92 cluster**, **miR-21**, miR-29, miR-23a, miR-24, miR-27a, **miR-30b**, **miR-130/301**, m miR-150, **miR-155**, miR-214
T regulatory cells	Cell differentiation	miR-17/92 cluster, miR-10, miR-99a/miR-150, **miR-155**
	Cell function	**miR-142-3p**, **miR-146a**, **miR-155**
T helper 17 cells	Cell differentiation	miR-10a, miR-19b, miR-17, **miR-155**, **miR-210**, **miR-212/132 cluster**, miR-301, **miR-326**
T follicular helper cells	Cell differentiation	**miR-10a**, **miR-17/92 cluster**

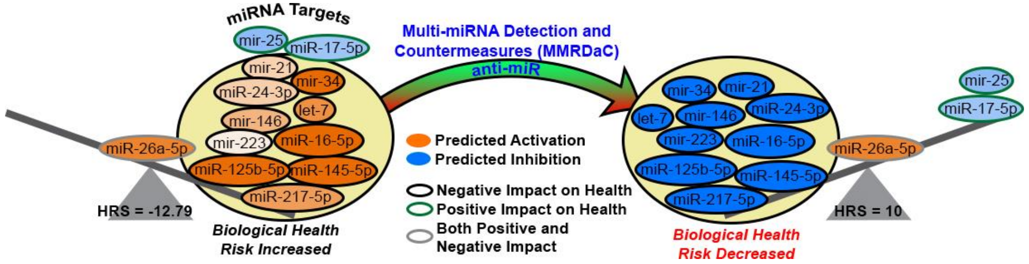
s are in bold. MDSCs, Myeloid-Derived Suppressor Cells
e required



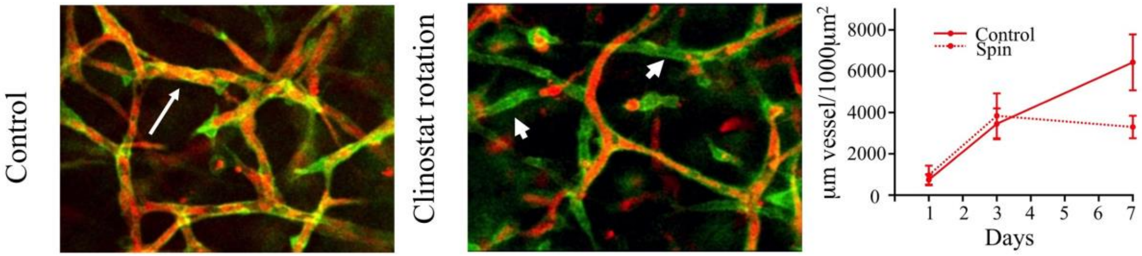
affect innate system (non-proliferating/ terminally differentiated cells) as much as **adaptive immune system** (proliferating cells)

Current On-Going Work and What to Expect to See Soon!

- **More analysis on the miRNA-seq data**
  - More Analysis on the GCR specific data
  - SPE specific analysis
  - Gamma specific analysis
  - Hindlimb unloading specific analysis
- **Countermeasure experiments**
  - Use antagomirs to potentially mitigate radiation effects



- **Complementary results on Deep RNA-sequencing on the whole blood**
  - Rob Meller is providing and analyzing complementary results on deep sequencing on the whole blood from these mice



Utilize 3D microvascular tissue models to determine functional impact of miRNAs and start development of miRNA based countermeasures.

- Irradiated 3D tissue model with 1Gy SPE sim
- Irradiated 3D tissue model with 0.5Gy GCR sim model with and without 3 antagomir countermeasure
- Promising results so far and more experiments and results to come soon!

Robert Meller

MOREHOUSE SCHOOL OF MEDICINE

Peter Grabham

COLUMBIA UNIVERSITY IRVING MEDICAL CENTER

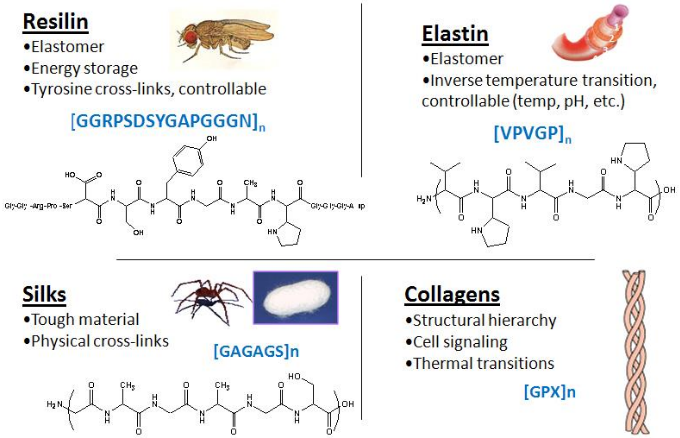
Current On-Going Work and What to Expect to See Soon!

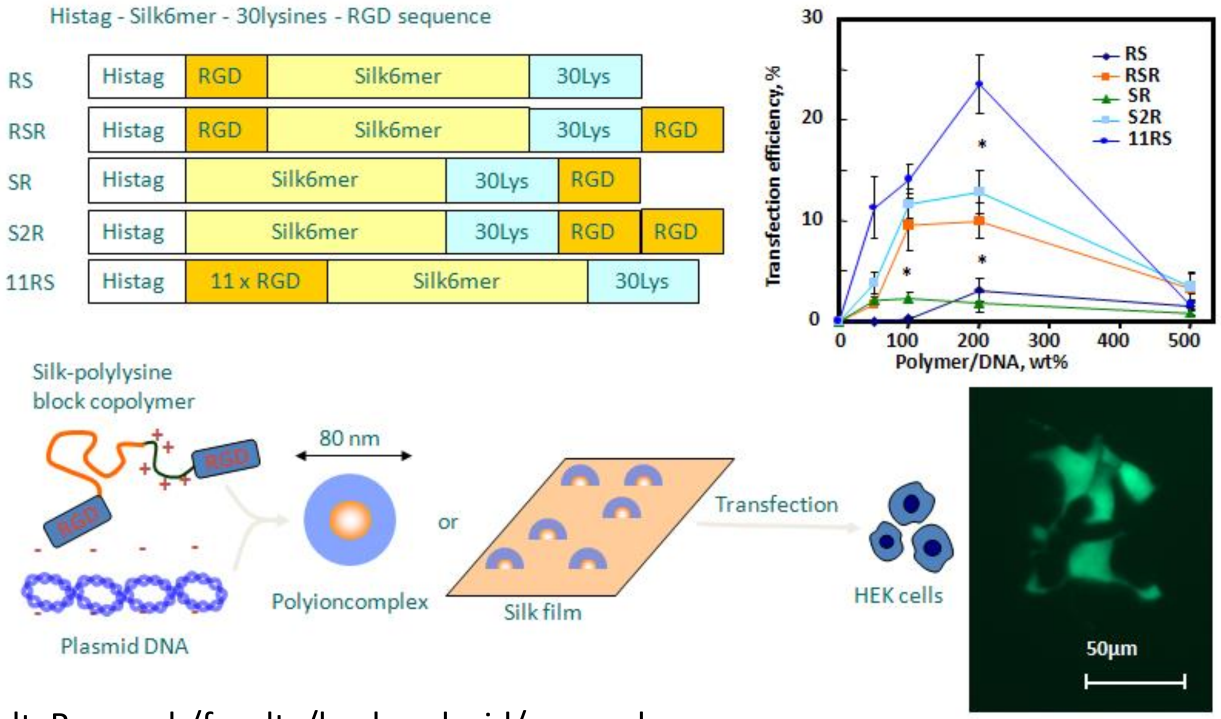
David Kaplan

Laura Chambre

Nafis Hasan

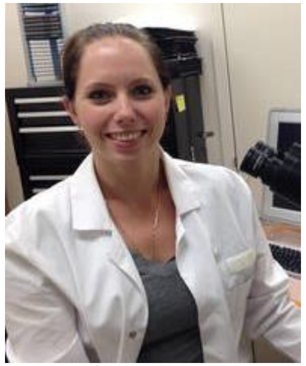
- Their team is assisting with designing a silk based drug delivery system to apply miRNA antagonists for countermeasures
- We are planning on testing the silk based antagomir capsules in the BNL spring run both in mice and the 3D tissue model





Images obtained from https://sackler.tufts.edu/facultyResearch/faculty/kaplan-david/research

Acknowledgments for miRNA work with Cardiovascular Risk and Deep Space Radiation



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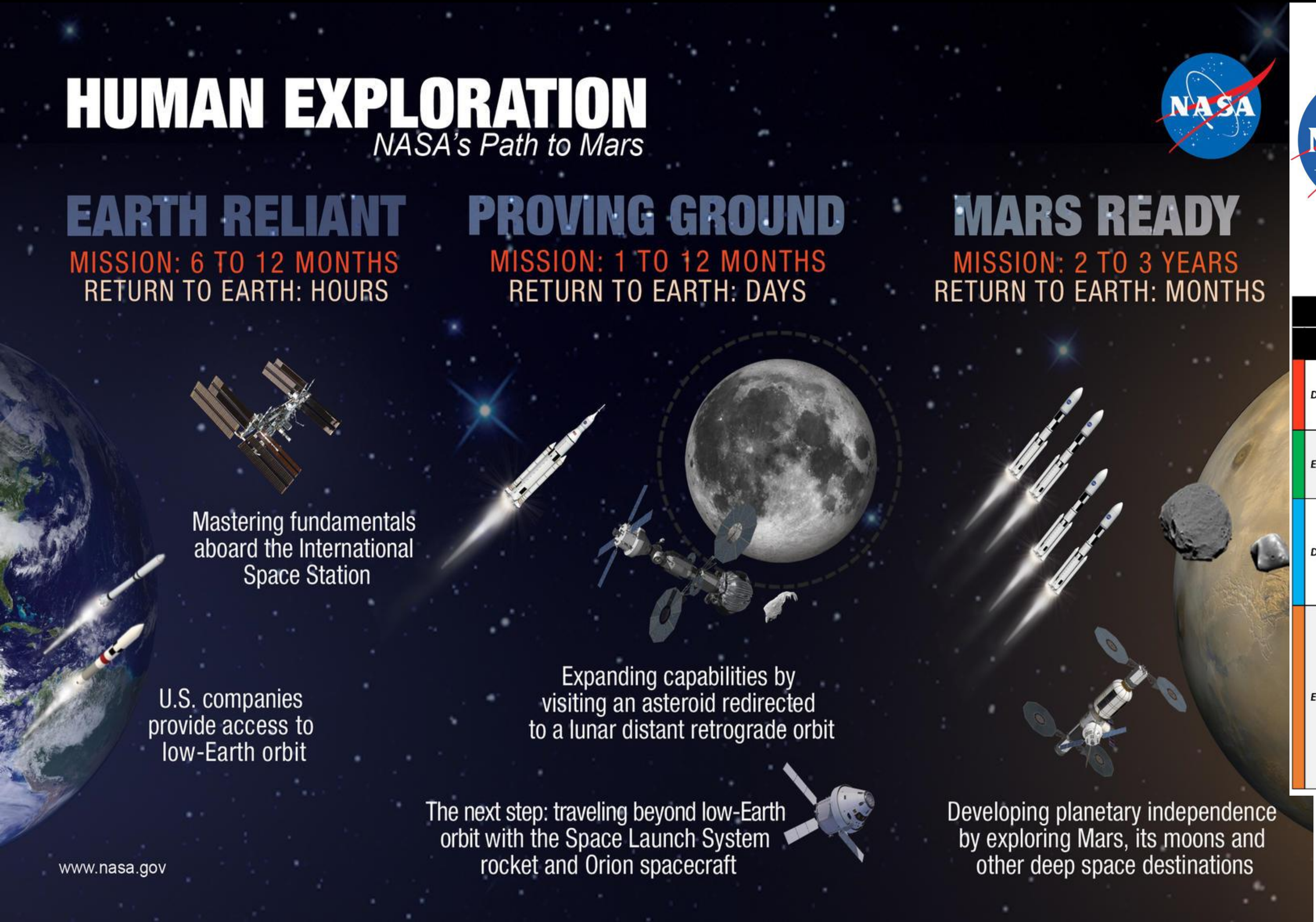
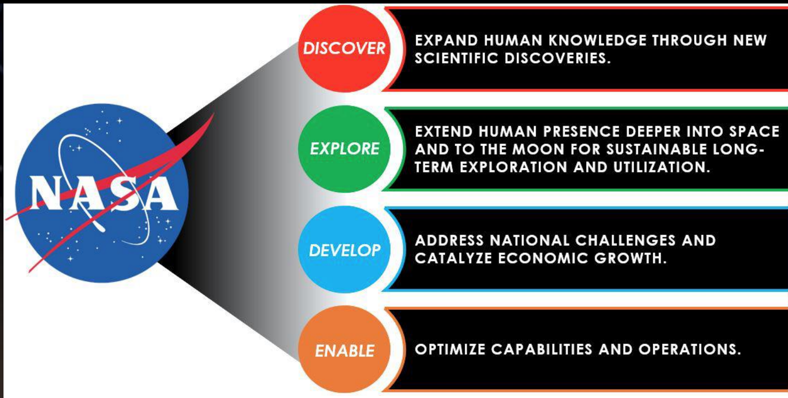
16-ROSBFP GL-0005: NNH16ZTT001N-FG Appendix G: Solicitation of Proposals for Flight and Ground Space Biology Research

miRNA Research will Further Assist with NASA's Future Missions





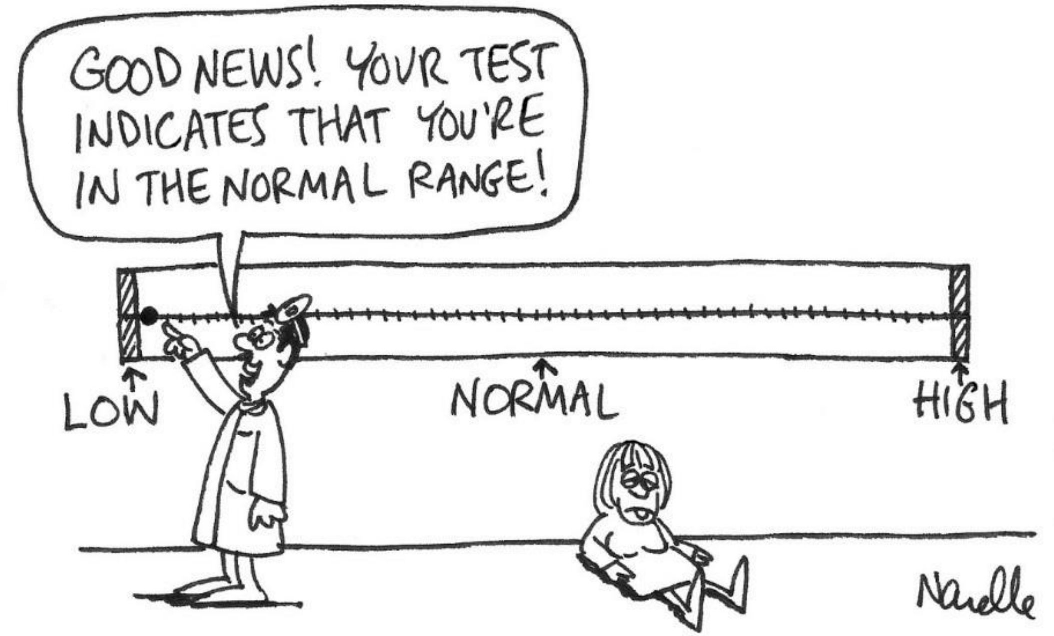
NASA 2018 Strategic Plan Framework

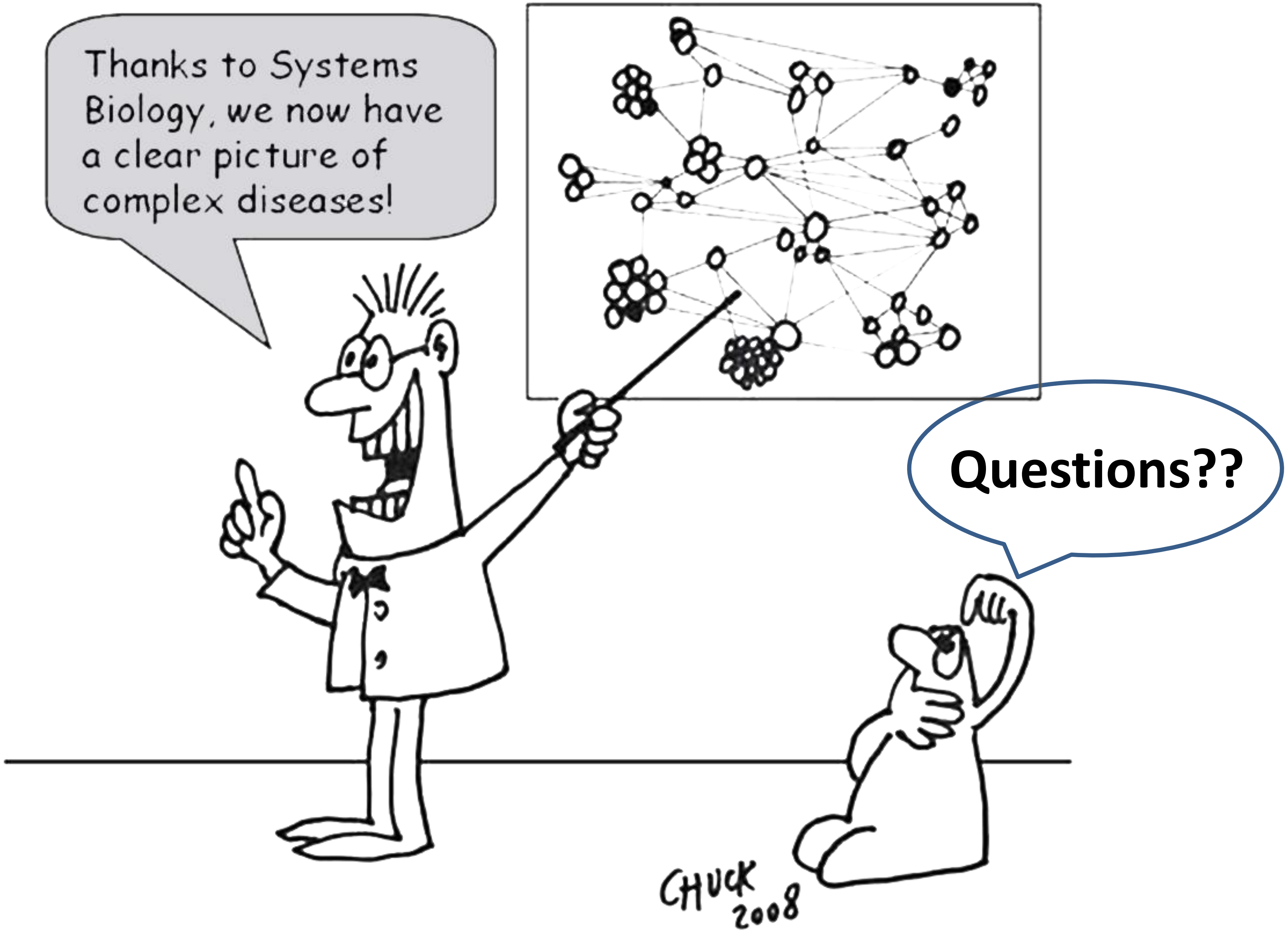
Theme	Strategic Goal	Strategic Objective
DISCOVER	EXPAND HUMAN KNOWLEDGE THROUGH NEW SCIENTIFIC DISCOVERIES.	1.1: Understand the Sun, Earth, Solar System, and Universe. 1.2: Understand Responses of Physical and Biological Systems to Spaceflight.
EXPLORE	EXTEND HUMAN PRESENCE DEEPER INTO SPACE AND TO THE MOON FOR SUSTAINABLE LONG-TERM EXPLORATION AND UTILIZATION.	2.1: Lay the Foundation for America to Maintain a Constant Human Presence in Low Earth Orbit Enabled by a Commercial Market. 2.2: Conduct Exploration in Deep Space, Including to the Surface of the Moon.
DEVELOP	ADDRESS NATIONAL CHALLENGES AND CATALYZE ECONOMIC GROWTH.	3.1: Develop and Transfer Revolutionary Technologies to Enable Exploration Capabilities for NASA and the Nation. 3.2: Transform Aviation Through Revolutionary Technology Research, Development, and Transfer. 3.3: Inspire and Engage the Public in Aeronautics, Space, and Science.
ENABLE	OPTIMIZE CAPABILITIES AND OPERATIONS.	4.1: Engage in Partnership Strategies. 4.2: Enable Space Access and Services. 4.3: Assure Safety and Mission Success. 4.4: Manage Human Capital. 4.5: Ensure Enterprise Protection. 4.6: Sustain Infrastructure Capabilities and Operations.

Overall Conclusion with miRNA studies

- **Can apply similar techniques for majority of diseases to determine circulating miRNA signature associated with each disease for liquid biomarker!**
- **Also can use similar techniques to inhibit the circulating miRNA signature**

- **OPEN FOR COLLABORATIONS!!**




Thanks to Systems Biology, we now have a clear picture of complex diseases!
Questions??
CHUCK 2008