Exploring the Genomic Effects of Ionizing Radiation on Cellular Aging

Chris Avery

Mentor: Dr. Ryan Norman

Durability Damage Tolerance and Reliability Branch

Space Radiation Group

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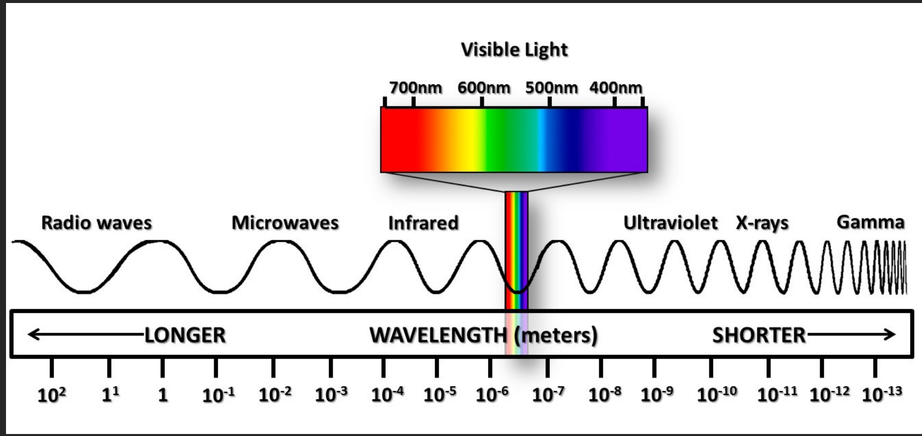
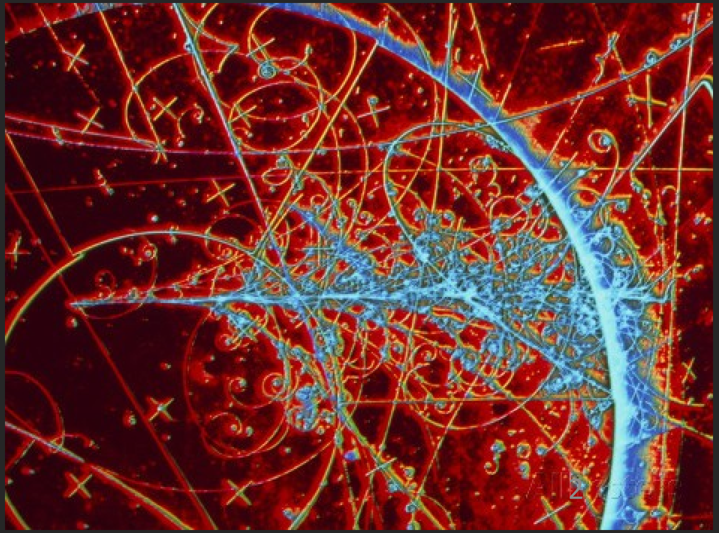
The Challenges of Radiation in Deep Space Travel

Radiation poses one of the greatest health related threats to astronauts in space!

The Earth is protected from dangerous space radiation by it's magnetosphere!

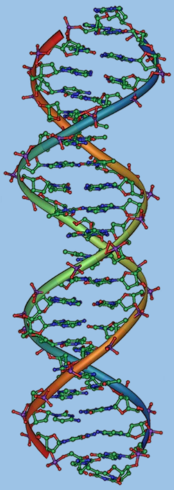
Radiation can take the form of electromagnetic photons or charged particles such as protons and electrons

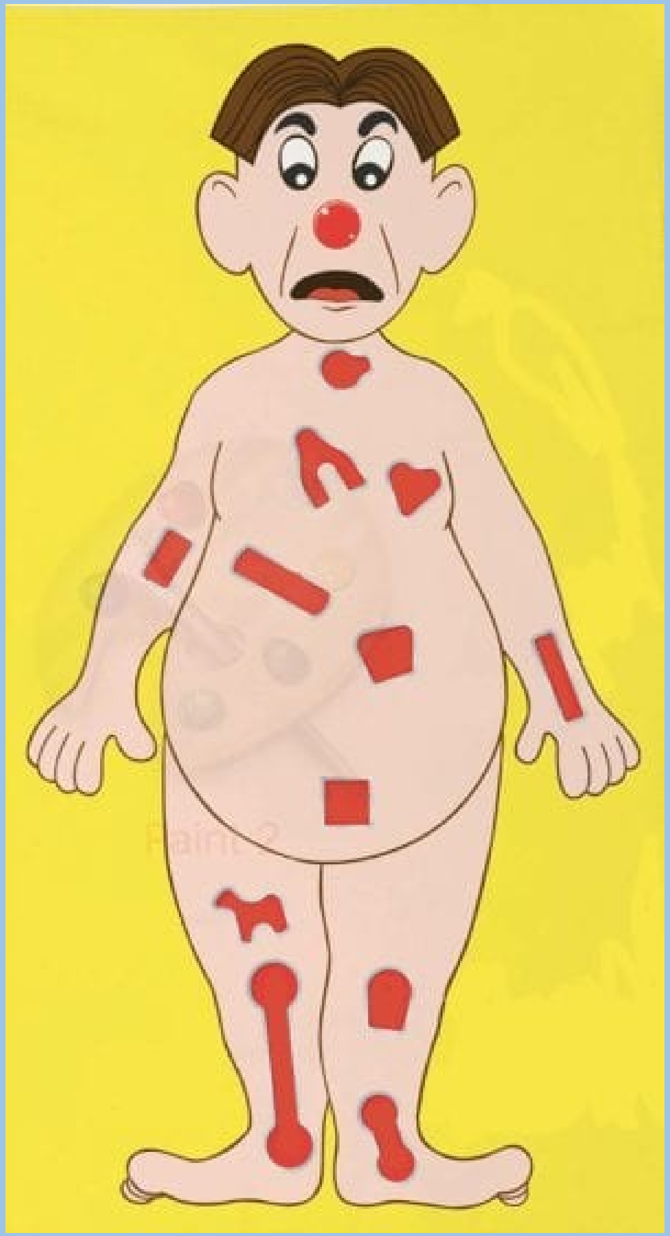




DNA Damage

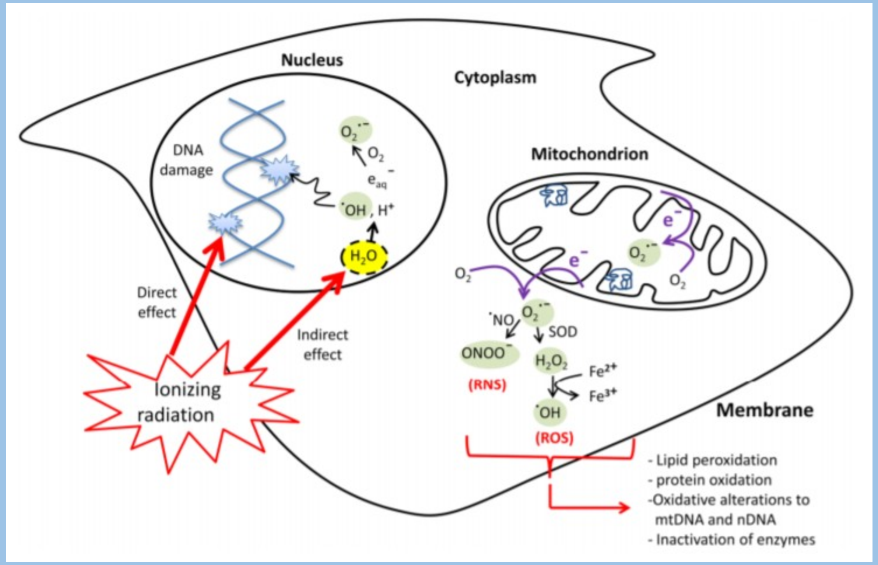
Ionized Radiation can directly hit DNA, which can cause double stranded breaks that lead to mutations and whole chromosome restructuring!





Reactive Oxygen Species (ROS)

ROS are generated when radiation interacts with water in the cell. These species can then interact and interfere with other molecules critical for biological processes



Cell Death

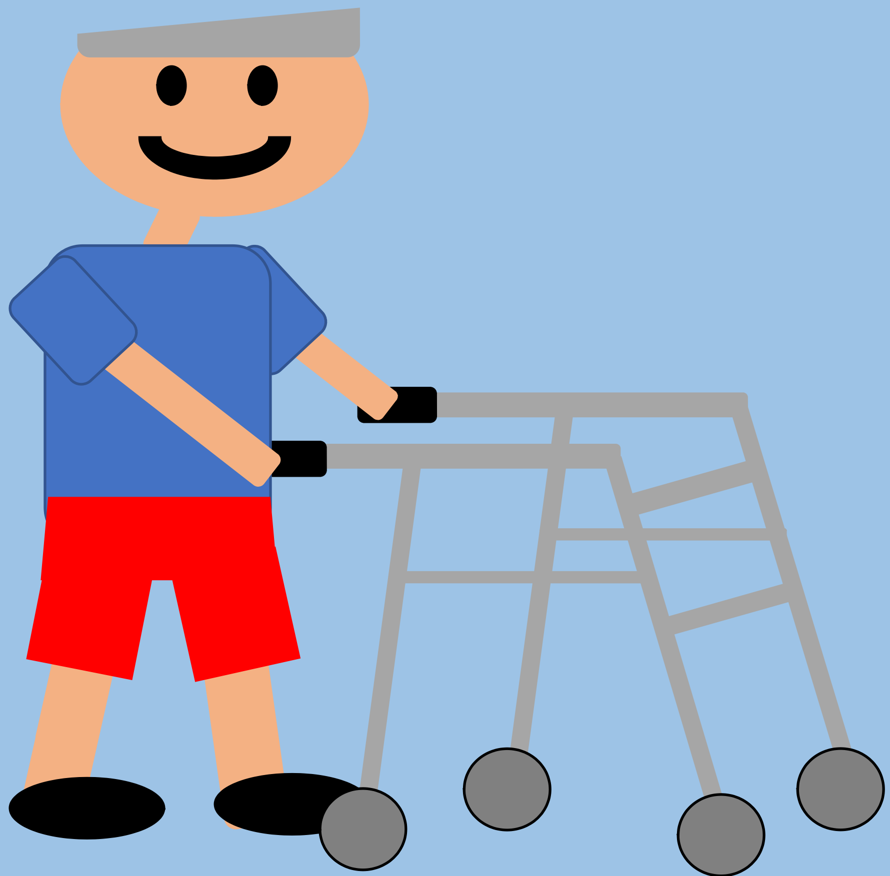
Radiation can trigger cells to die via regulated means.

Apoptosis is regulated cell death, while senescence is a state where cells cannot replicate but are metabolically active

These effects can lead to long lasting biological issues such as cancer development and cardiovascular disease

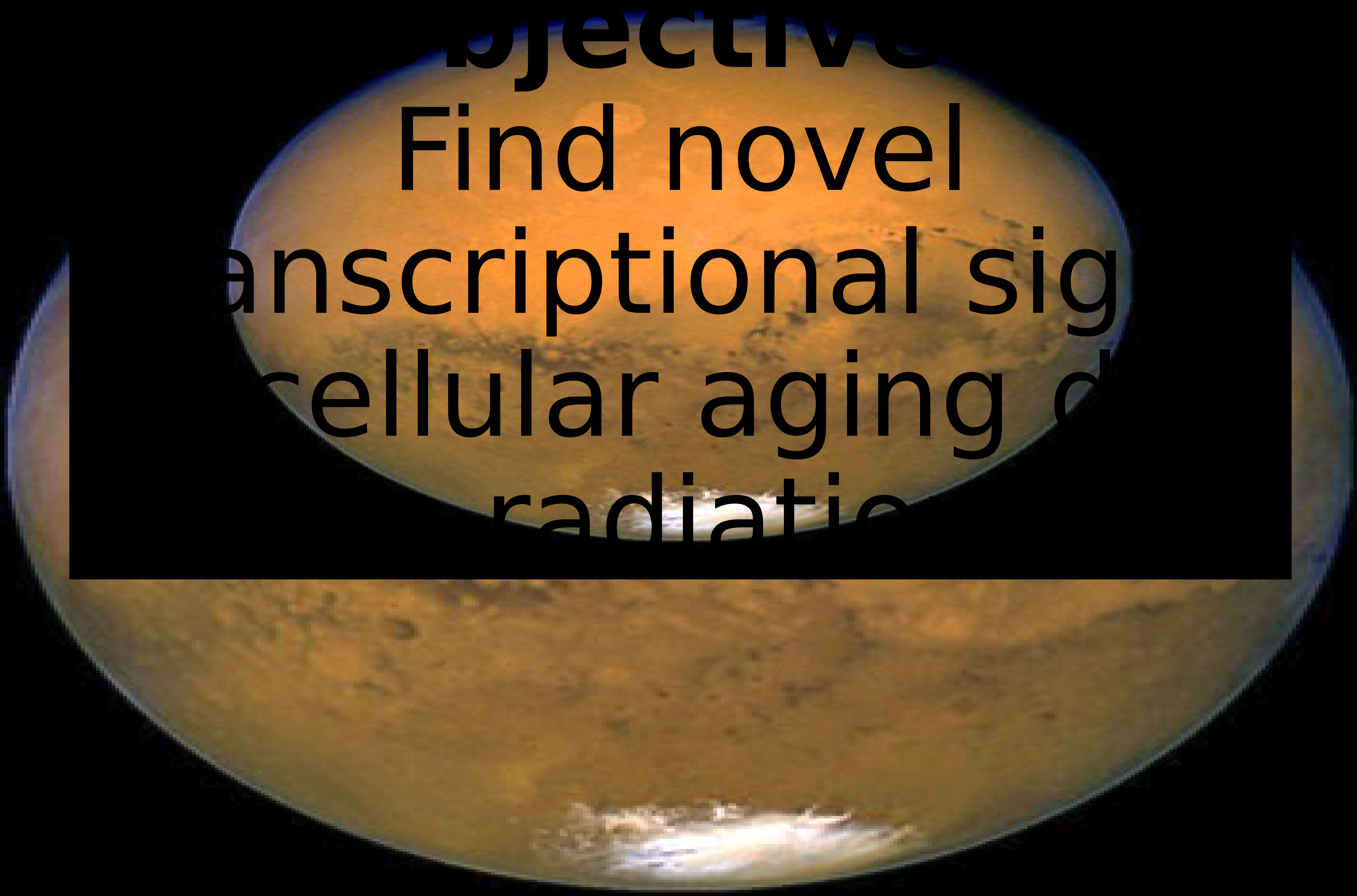
Azzam, Edouard I., Jean-Paul Jay-Gerin, and Debkumar Pain. "Ionizing radiation-induced metabolic oxidative stress and prolonged cell injury." *Cancer letters* 327.1-2 (2012): 48-60.

Transcriptional Hallmarks of Aging



1. **Downregulation of genes encoding mitochondrial proteins** – Mitochondrial activity has been noted to decrease with age, thus downregulation of associated processes like ATP synthesis is expected
2. **Downregulation of the protein synthesis machinery** – This includes ribosomal proteins and proteins involved in ribosome biogenesis
3. **Dysregulation of immune system genes** – The reduced or inappropriate regulation or expression or immune system related genes
4. **Reduction in growth factor signaling** – Growth factor are pivotal for signaling and proliferation activities. Genes in this hallmark are downregulated with age
5. **Constitutive responses to stress and DNA damage** – A general hallmark that could be activated by a number of environmental stress factors. (including radiation damage!)
6. **Dysregulation of gene expression and mRNA processing** – Regulation of transcription factors, chromatin level gene silencing, epigenetic and posttranscriptional modifications

Frenk, Stephen, and Jonathan Houseley. "Gene expression hallmarks of cellular ageing." *Biogerontology* 19.6 (2018): 547-566.



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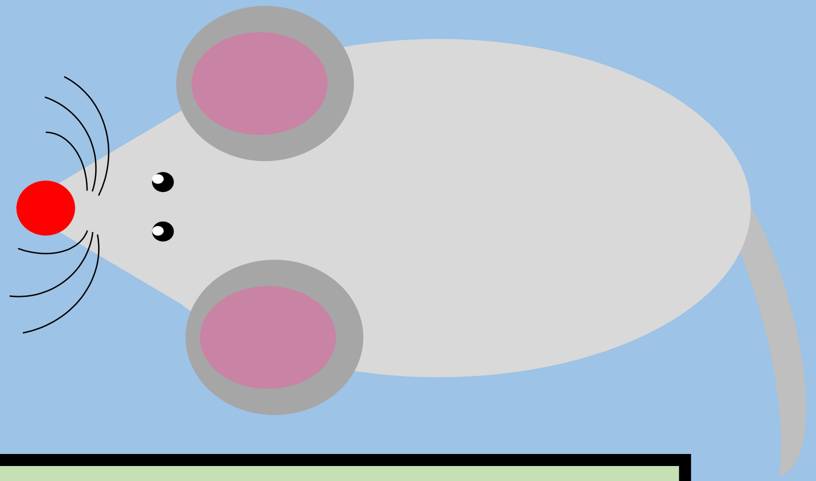
Data



Data Type: Microarray
Species: Mus Musculus
Tissue: Cardiomyocyte
Chip Type: [MoGene-1_0-st] Affymetrix Mouse Gene 1.0 ST Array

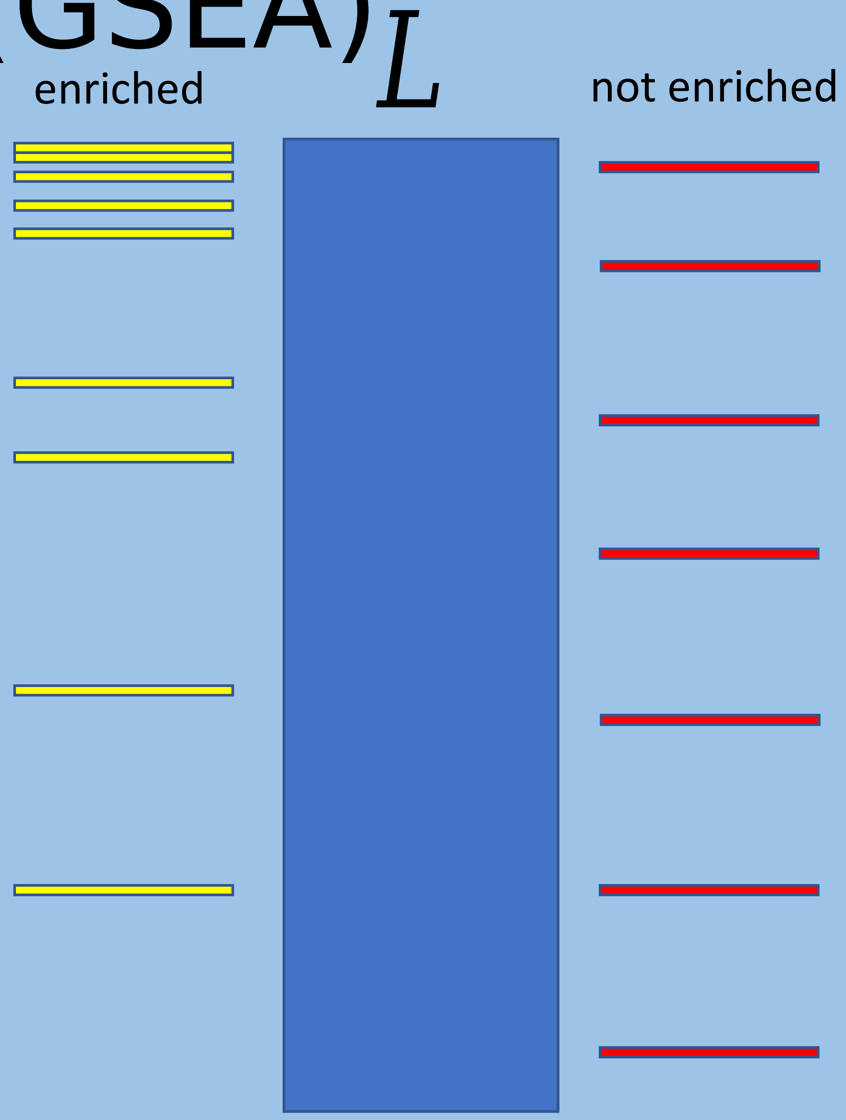
Fe Irradiation [15 cGy, 1 GeV/nucleon (n)]	Proton Irradiation [90 cGy, 1 GeV]
1 Day – 3 Replicates	1 Day – 2 Replicates
3 Days – 3 Replicates	3 Days – 3 Replicates
7 Days – 2 Replicates	5 Days – 3 Replicates
14 Days – 2 Replicates	12 Days – 2 Replicates
28 Days – 2 Replicates	26 Days – 2 Replicates
Control – 2 Replicates at Day 1 and 1 at Day 3	Control – 2 Replicates at Day 1 and 1 at Day 3

* Day 7 Irregularity – 2 or 3 replicates? This is addressed by (Beheshti et al.)

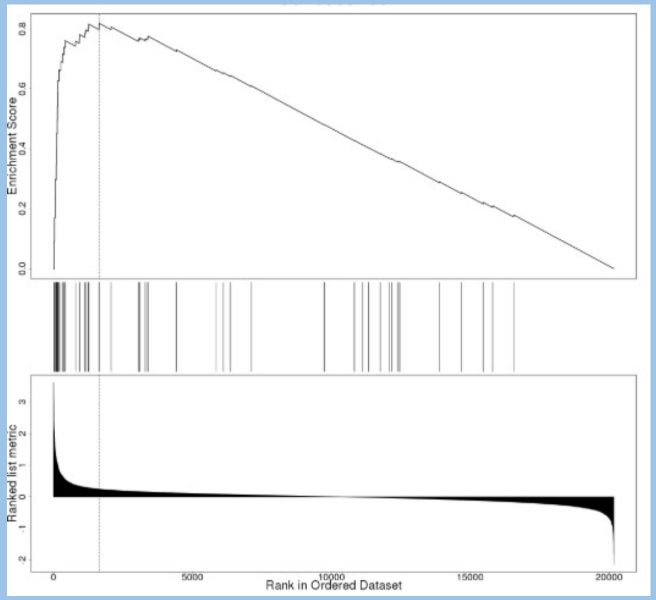
Data can be found on GEO or in GeneLab's database

- https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE68876
- https://genelab-data.ndc.nasa.gov/genelab/accession/GLDS-109/ (Fe)
- https://genelab-data.ndc.nasa.gov/genelab/accession/GLDS-117/ (H)
- Coleman, Matthew A., et al. "Low-dose radiation affects cardiac physiology: gene networks and molecular signaling in cardiomyocytes." *American Journal of Physiology-Heart and Circulatory Physiology* 309.11 (2015): H1947-H1963.
- Beheshti, Afshin, et al. "GeneLab database analyses suggest long-term impact of space radiation on the cardiovascular system by the activation of FYN through reactive oxygen species." *International journal of molecular sciences* 20.3 (2019): 661.

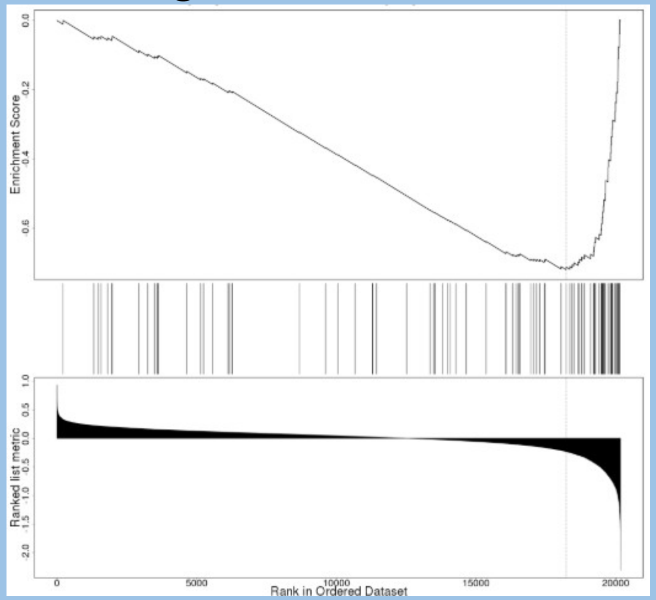
Gene Set Enrichment Analysis (GSEA)



Positive Enrichment



Negative Enrichment

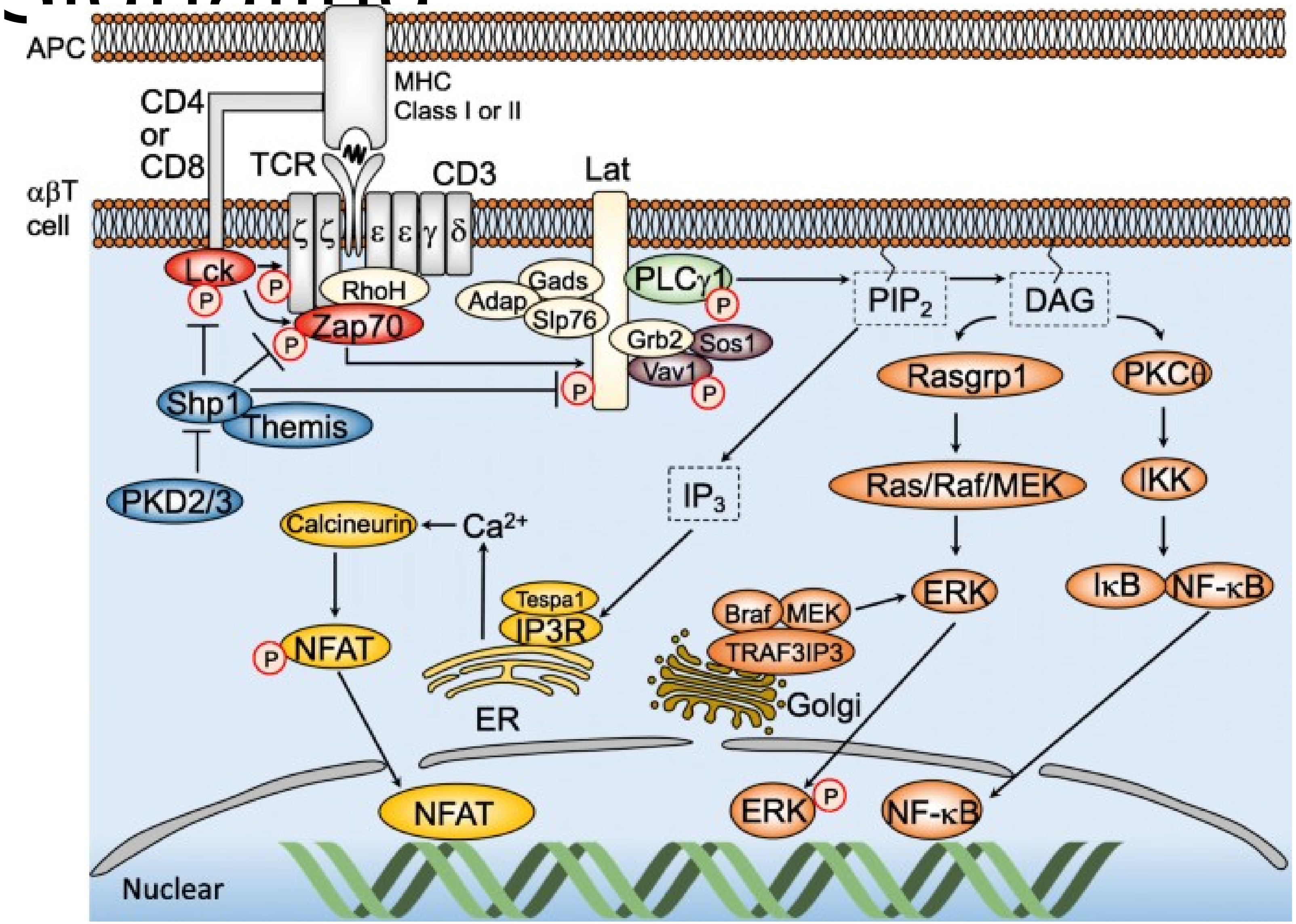


GSEA takes a ranked list of genes and searches a set of genes which are related in some way

The null hypothesis is that the genes of set S are uniformly distributed in the ranked list while the alternate hypothesis is that the genes are found to be over expressed at the extremes

Subramanian, Aravind, et al. "Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles." *Proceedings of the National Academy of Sciences* 102.43 (2005): 15545-15550.

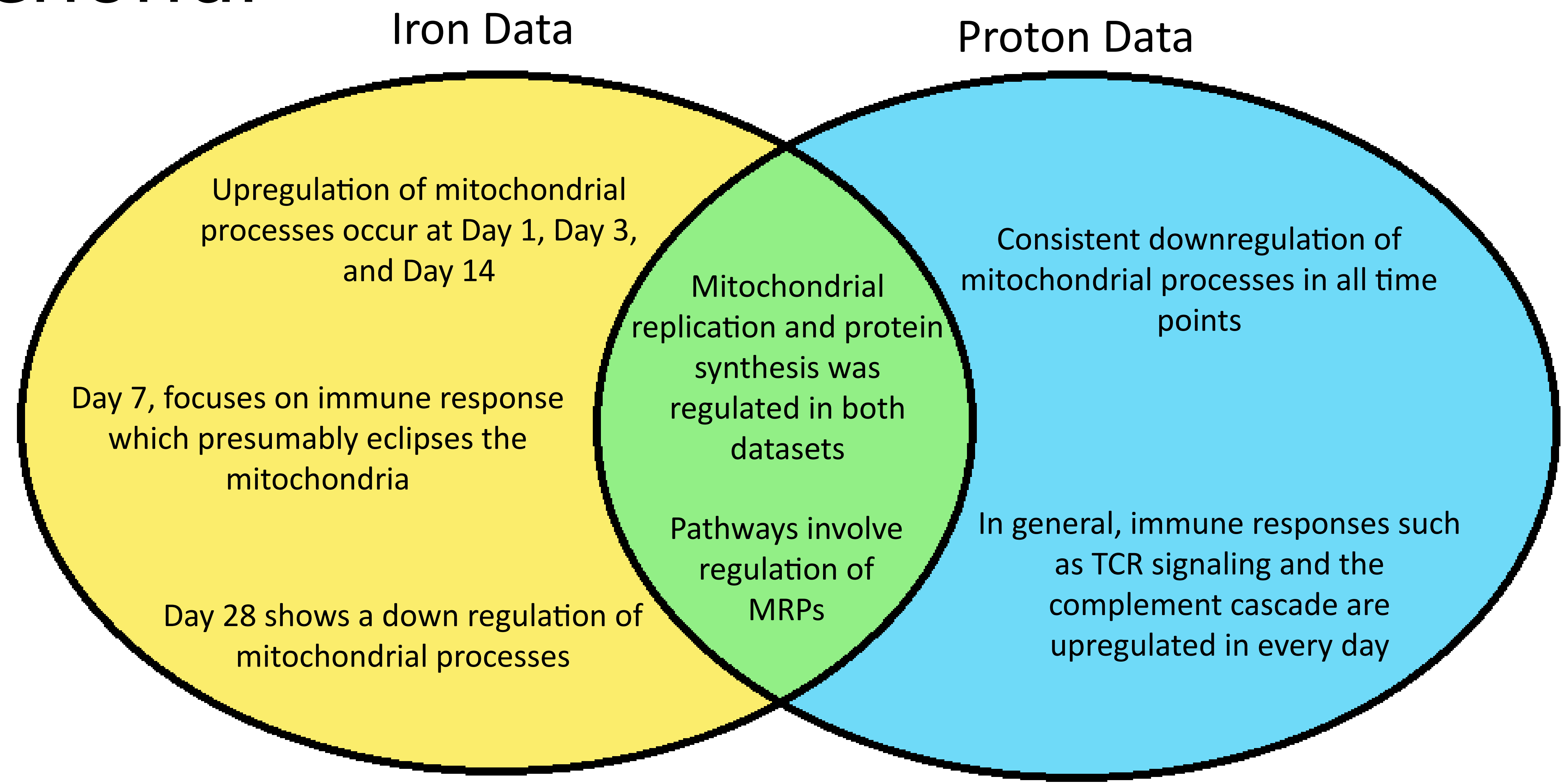
Immune System Responses: TCR Signaling



- TCR (T-Cell Receptor) Signaling pathway activated via ZAP-70 binding
- In Fe data, pathways to NF-kB, ERK, and NFAT show upstream activation via large fold changes
- The H data show similar gene expression qualitatively but smaller fold change
- NFAT and NF-kB are transcription factors that **regulate immune response** factors and ultimately can **lead to cell death**

Figure: Muro, Ryunosuke, Hiroshi Takayanagi, and Takeshi Nitta. "T cell receptor signaling for γδT cell development." *Inflammation and regeneration* 39.1 (2019): 1-11.

Mitochondria



Note that the cells react very differently to Iron and Proton irradiation. Protons cause a constant and immediate immune response compared to the delayed reaction to Iron particles

The mitochondrial reaction tends to be regulated opposite to the immune system!

https://www.rcsb.org/structure/3BQO

DNA/Telomere Damage

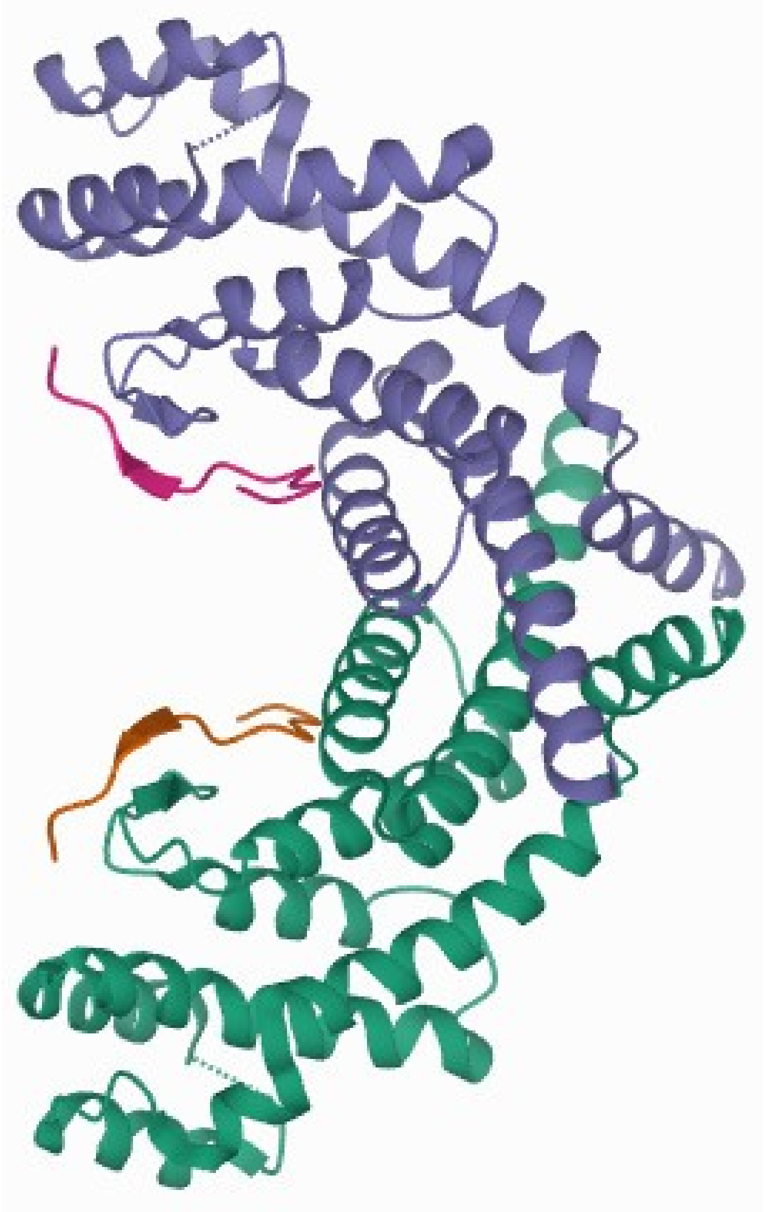
Genes involved in telomere maintenance are upregulated on day 28 in Fe data. Telomere damage/shortening is a sign factor in aging.

Shelterin is a complex of many proteins which protect against telomere shortening.

Genes involved are downregulated on days 1-14 then upregulated on day 28 indicating that damage was accrued over the month after radiation

TERF1, POT1b, TRAF, are part of the shelterin complex and differentially expressed in Fe data

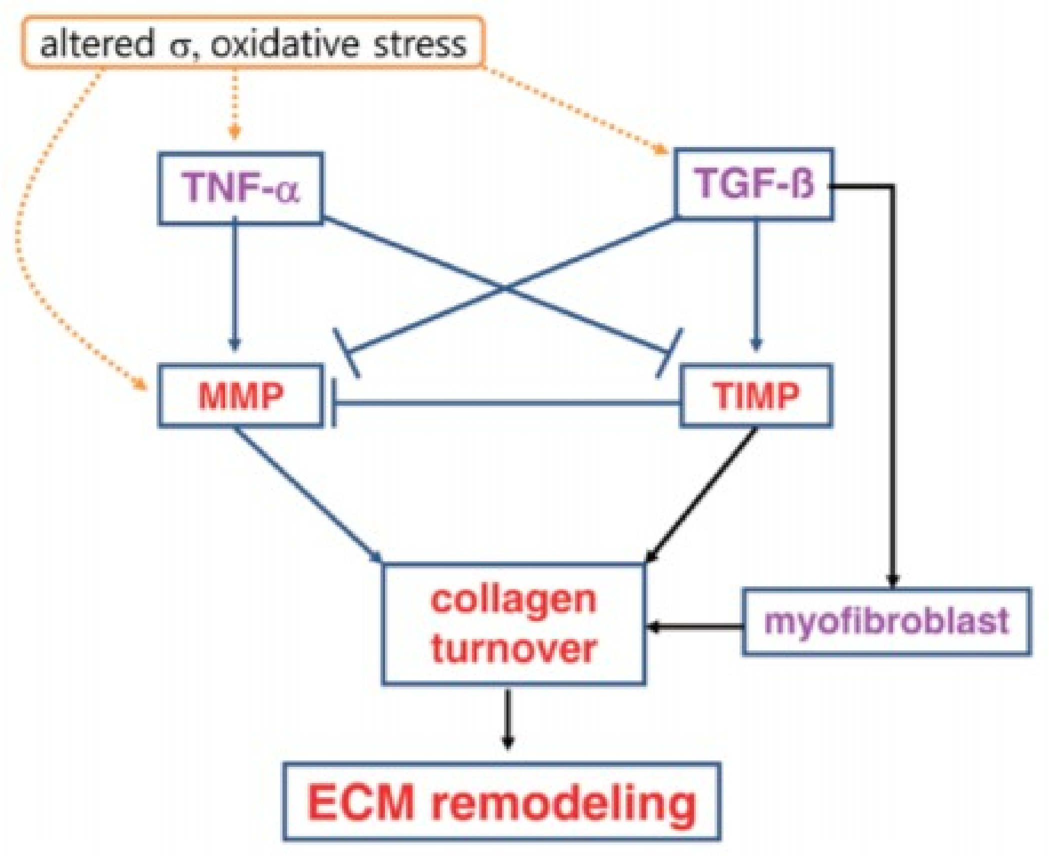
Only POT1b shows upregulation in the H data.



Crystal Structure of TRF1 TRFH domain and TIN2 peptide complex

Extracellular Matrix Remodeling

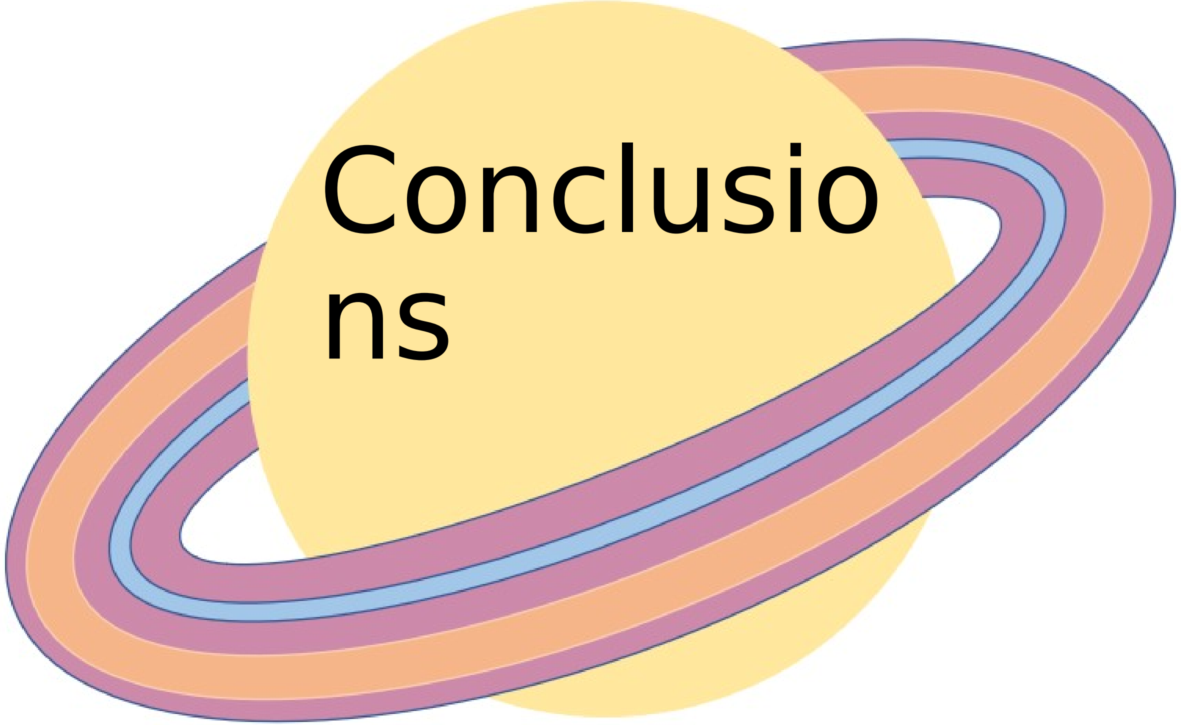
- ECM is the space between cells. Aging has been associated with increased collagen levels, and the development of fibrosis.
- Furthermore the ECM controls immune signaling system and tumor progression



- In Fe data we see the downregulation of the ECM components on days 1, 3, and 14
- For H data there is an upregulation of ECM regulation and structure
- TNF- and TGF- are only mildly differentially expressed indicating direct regulation of MMPs

Figure: Kwak, Hyo-Bum. "Aging, exercise, and extracellular matrix in the heart." *Journal of exercise rehabilitation* 9.3 (2013): 338.

Conclusions



- Differing radiation types affect cells very differently. Proton radiation triggered a immediate immune response that persisted over the 28 days while Iron ions had an delayed and sudden reaction starting 7 days after irradiation

- Radiation, specifically Iron radiation, induced a transcriptional response related to DNA damage repair

- Mitochondrial activity appeared to be downregulated by the upregulation of immune response pathways

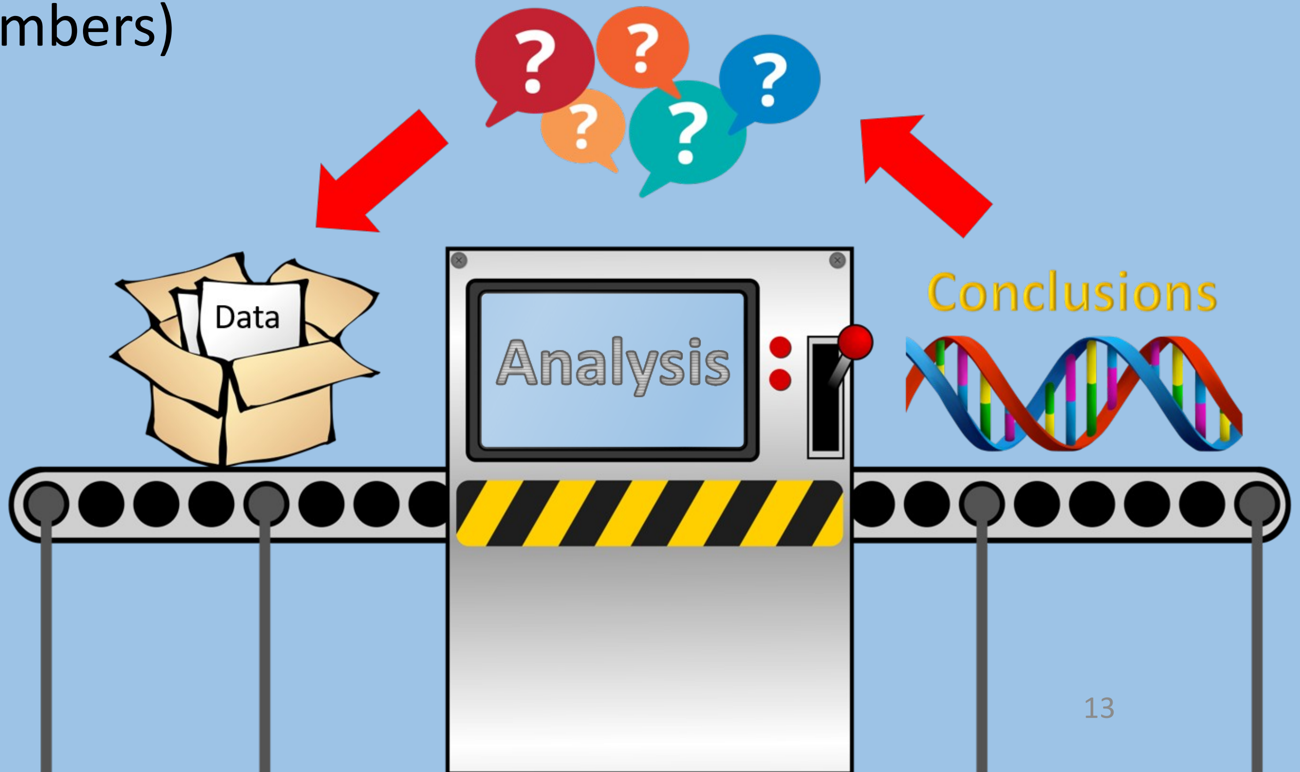
- The ECM structure is changed in order to facilitate stress and possibly signaling pathways such as the binding of TCRs to the cell membrane

Future Directions

- Further reading and follow-up studies showed that radiation effects may occur at timescales greater than the 28 day scope of what was presented here

- Check repeatability of this experiment. A major limitation was lack of controls at all time points and poor statistics (low replicate numbers)

- Compare expression results of mice with human transcripts for relevance



THANK YOU!

- Mentor: Dr Ryan Norman
- Dr Huff for your valuable insights!
- Human Research Project for the funding to do this research
- Space Radiation Group and Langley Research Center for the opportunity to work with amazing NASA researchers!
- The Internship Coordinators and team for putting the virtual internship experience together

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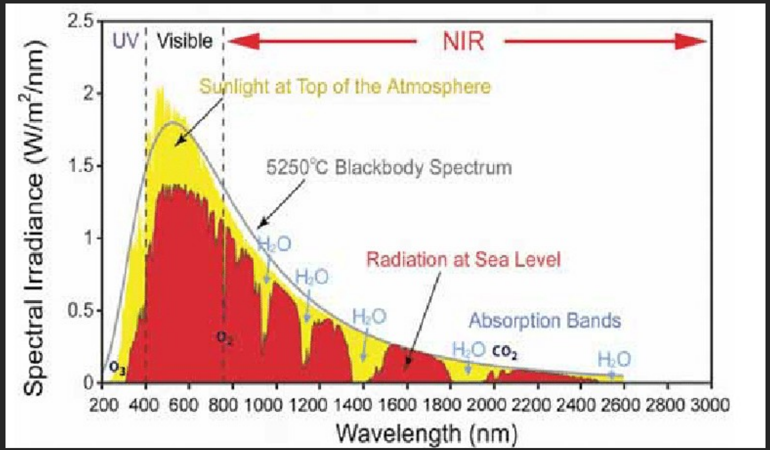
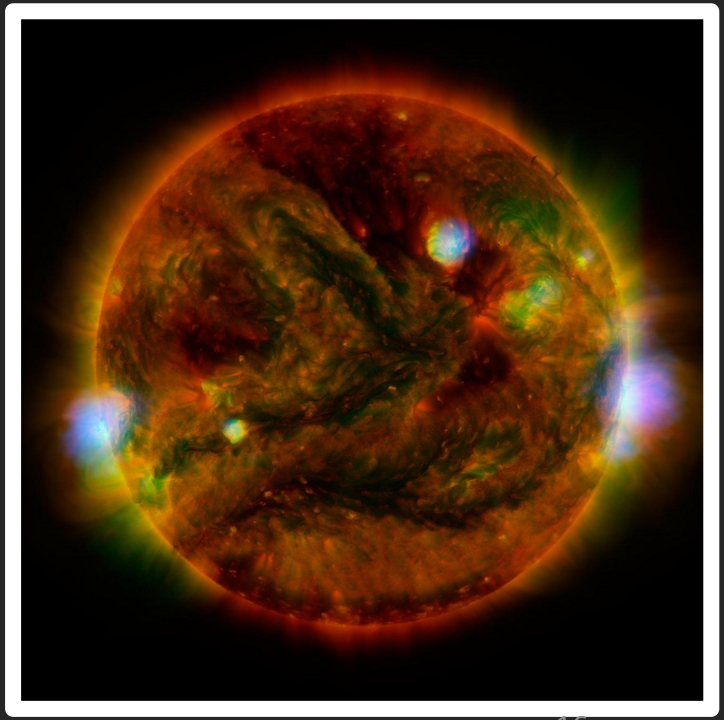
The Challenges of Radiation in Deep Space Travel

In deep space, one source of harmful radiation is **Solar Particle Events (SPE)**, or bursts of radiation released from the sun which releases high energy electromagnetic radiation as well as protons and electrons!





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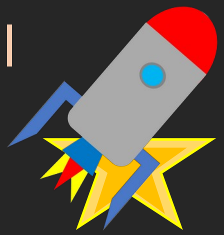
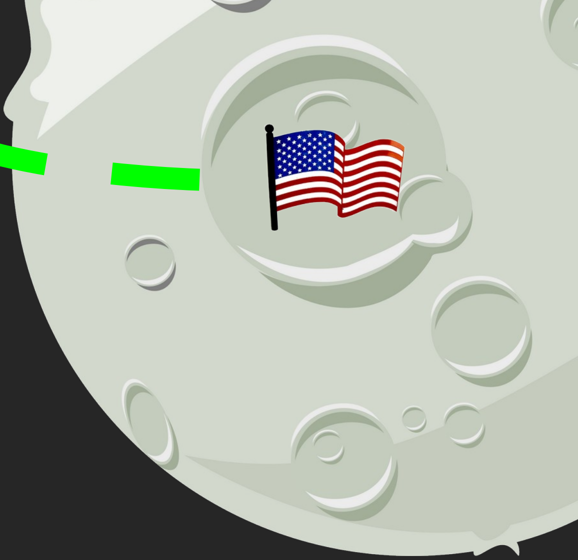
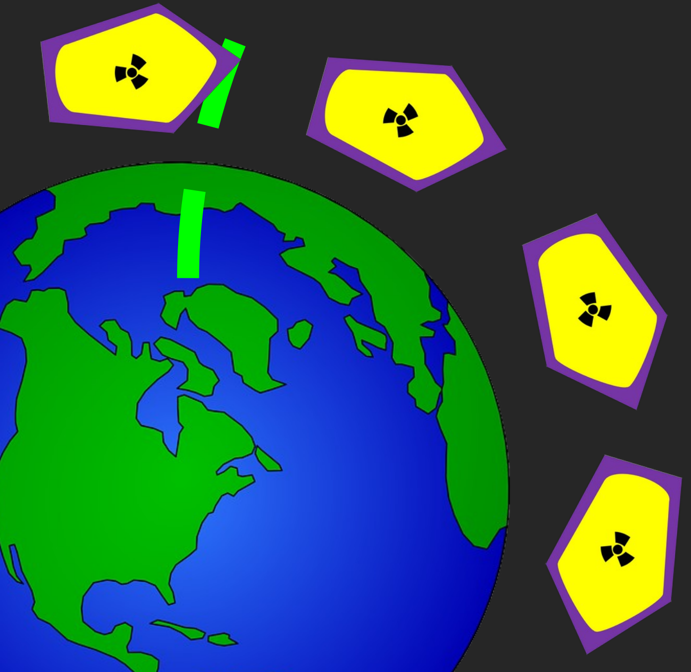
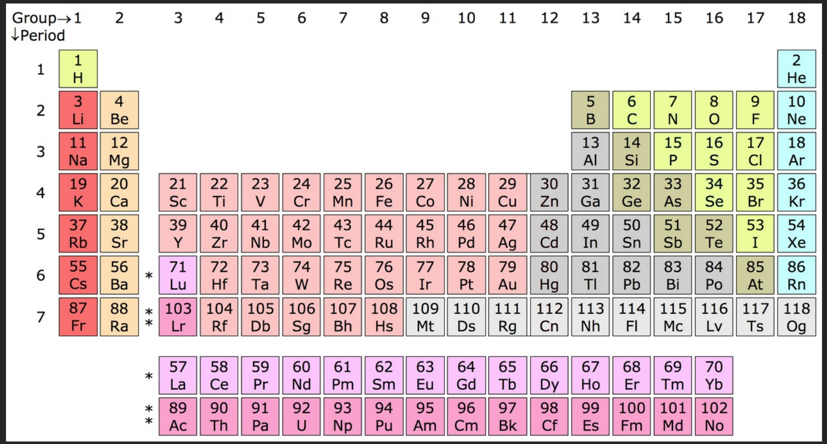




Galactic Cosmic Rays (GCR) provides a source of heavy ionized nuclei which present a real danger to astronauts outside of the magnetosphere





↓Period	Group→1	2		3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	1 H																		2 He
2	3 Li	4 Be												5 B	6 C	7 N	8 O	9 F	10 Ne
3	11 Na	12 Mg												13 Al	14 Si	15 P	16 S	17 Cl	18 Ar
4	19 K	20 Ca		21 Sc	22 Ti	23 V	24 Cr	25 Mn	26 Fe	27 Co	28 Ni	29 Cu	30 Zn	31 Ga	32 Ge	33 As	34 Se	35 Br	36 Kr
5	37 Rb	38 Sr		39 Y	40 Zr	41 Nb	42 Mo	43 Tc	44 Ru	45 Rh	46 Pd	47 Ag	48 Cd	49 In	50 Sn	51 Sb	52 Te	53 I	54 Xe
6	55 Cs	56 Ba	*	71 Lu	72 Hf	73 Ta	74 W	75 Re	76 Os	77 Ir	78 Pt	79 Au	80 Hg	81 Tl	82 Pb	83 Bi	84 Po	85 At	86 Rn
7	87 Fr	88 Ra	**	103 Lr	104 Rf	105 Db	106 Sg	107 Bh	108 Hs	109 Mt	110 Ds	111 Rg	112 Cn	113 Nh	114 Fl	115 Mc	116 Lv	117 Ts	118 Og
			*	57 La	58 Ce	59 Pr	60 Nd	61 Pm	62 Sm	63 Eu	64 Gd	65 Tb	66 Dy	67 Ho	68 Er	69 Tm	70 Yb		
			**	89 Ac	90 Th	91 Pa	92 U	93 Np	94 Pu	95 Am	96 Cm	97 Bk	98 Cf	99 Es	100 Fm	101 Md	102 No		

These nuclei travel incredibly fast and can be extremely massive!

Immune System Responses: TCR Signaling

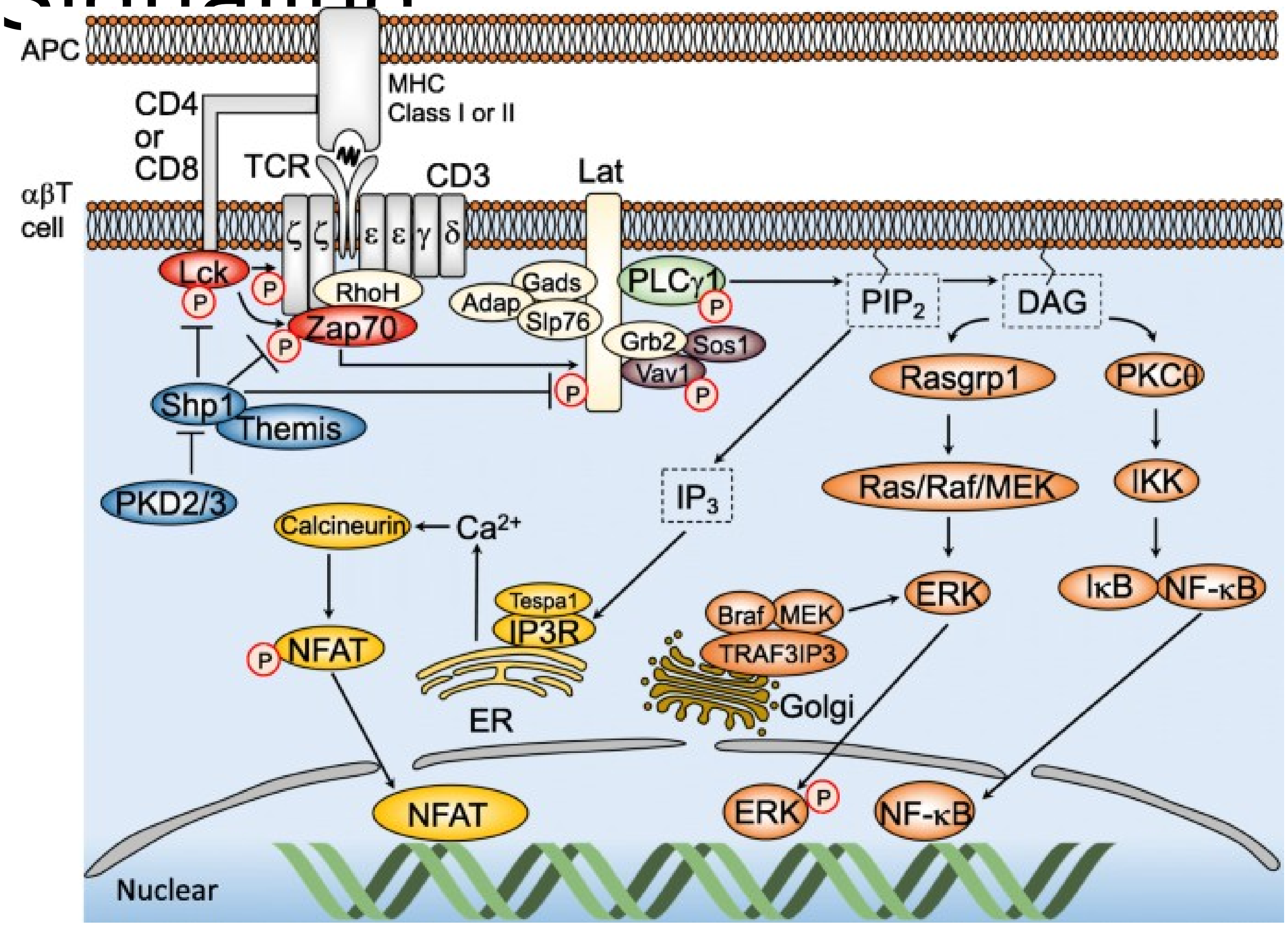


Figure: Muro, Ryunosuke, Hiroshi Takayanagi, and Takeshi Nitta. "T cell receptor signaling for γδT cell development." *Inflammation and regeneration* 39.1 (2019): 1-11.

Both Fe and H datasets showed significant upregulation of T-Cell Receptor (TCR) signaling pathways via ZAP-70

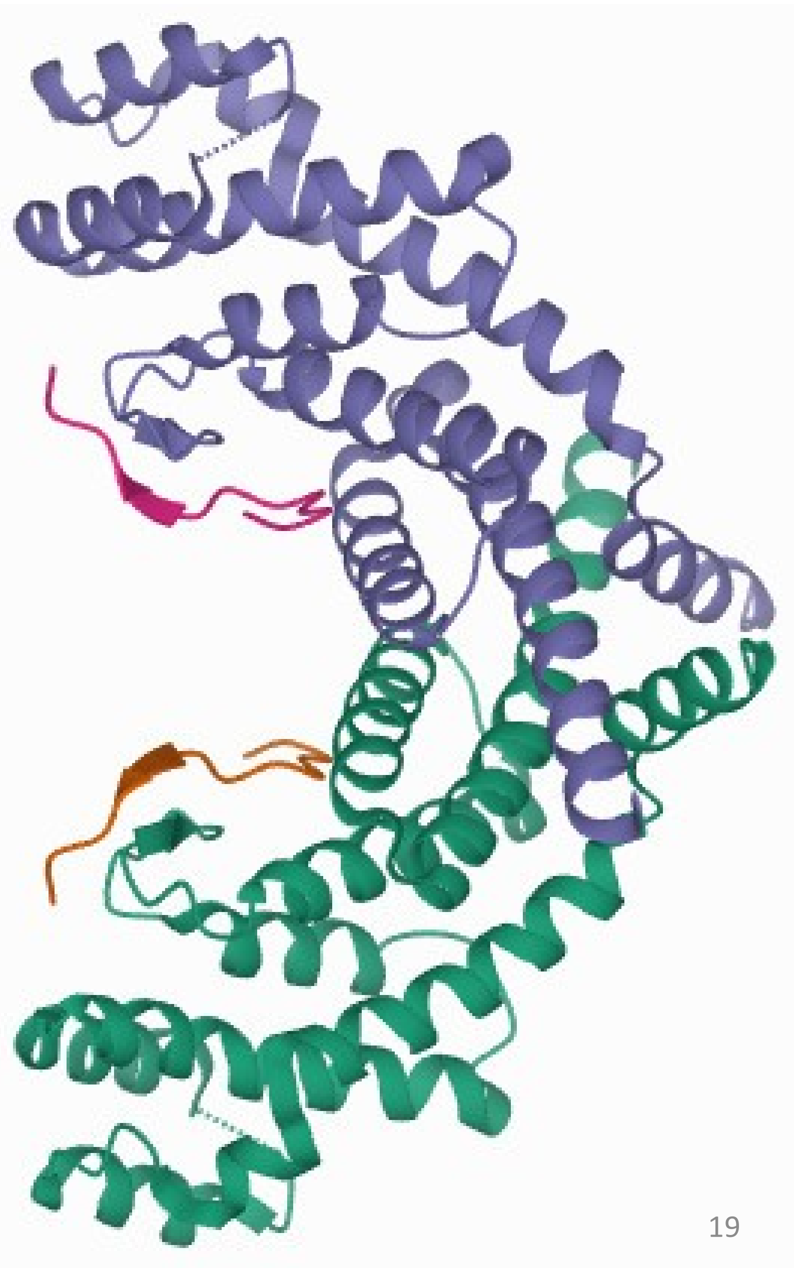
For the Fe data, some genes along this path were actually suppressed for the first few days, then later showed large fold changes including ZAP-70, LCK, LAT, RASGRP1, PKC, and NFAT. This indicates that the Fe radiation triggered multiple TCR signaling pathways related to immune response.

The H data show many of the similar genes to have qualitatively similar fold change trends, but less pronounced

NFAT and NF-kB are transcription factors that regulate the expression of important immune response factors such and ultimately can lead to cell death

Crystal Structure of TRF1 TRFH domain and TIN2 peptide complex https://www.rcsb.org/structure/3BQO

DNA/Telomere Damage



Genes involved in telomere maintenance are upregulated on day 28 in Fe data. Telomere damage/shortening is a sign factor in aging.

Shelterin is a complex of many proteins which protect against telomere shortening. Some of the genes involved are downregulated on days 1-14 then upregulated on day 28 indicating that damage was accrued over the month after radiation

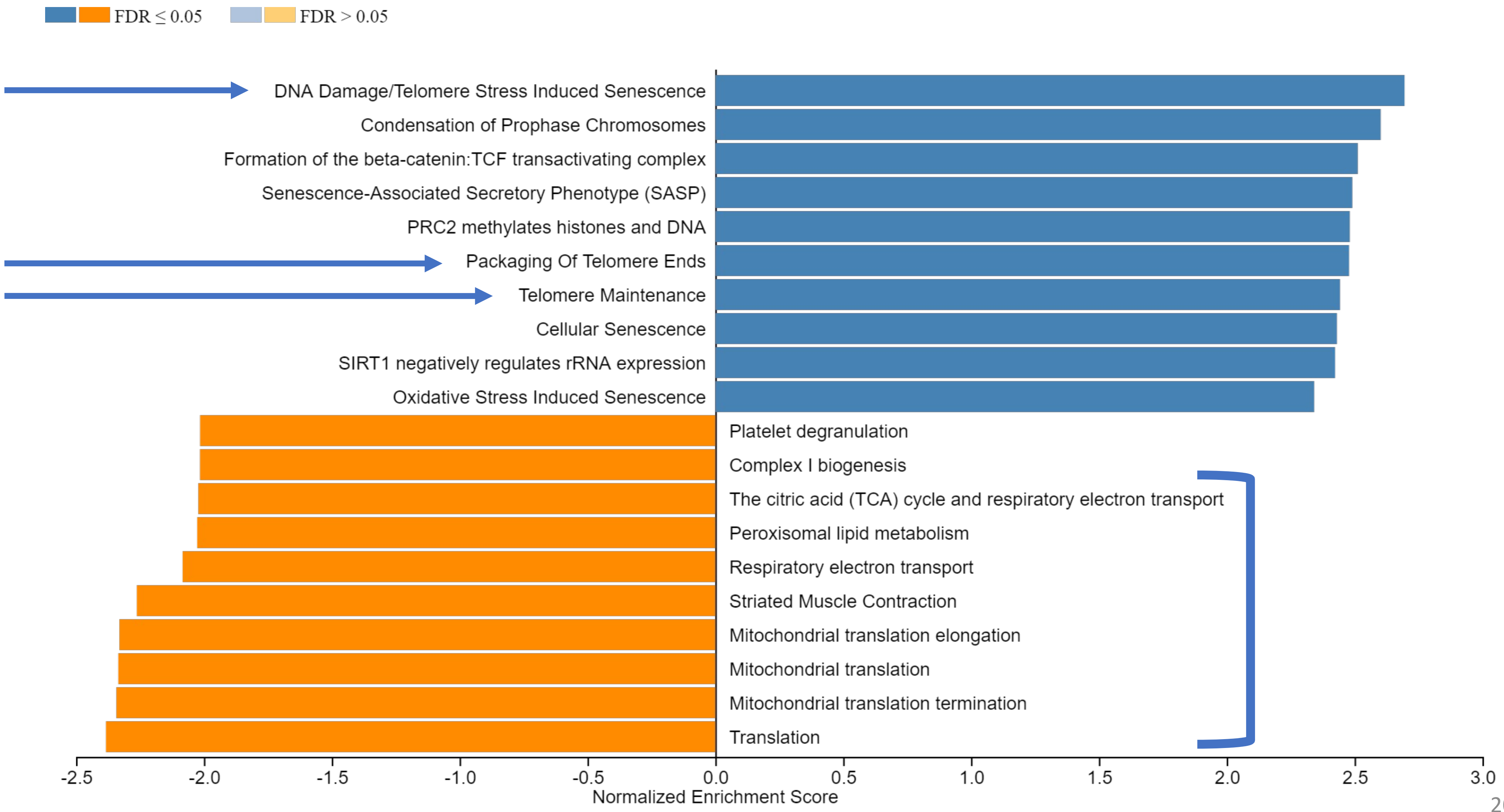
TERF1, POT1b, TRAF, are differentially expressed and part of the shelterin complex in Fe data, but only POT1b shows upregulation in the H data.

It seems that the Fe radiation has suppressed telomere maintenance which allows the telomeres to degrade until significant damage is sustained. At which point the cell attempts repair the damage.

DNA/Telomere Damage

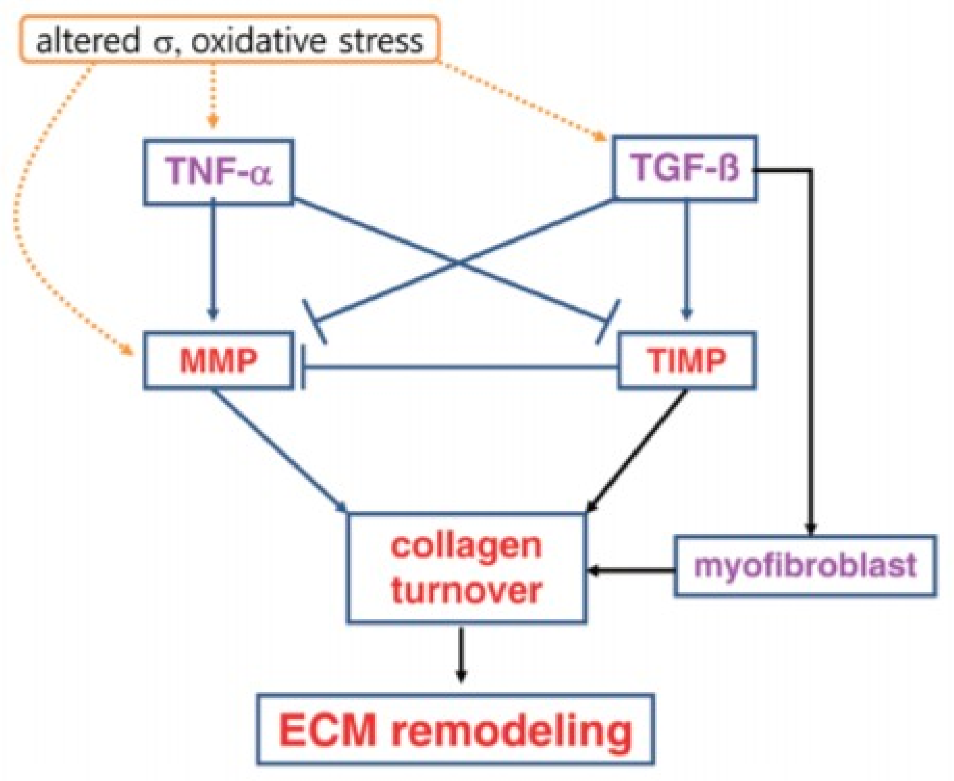
Fe Data: Day 28 GSEA Results

log of the fold change (logFC) is used as the ranking metric



Extracellular Matrix Remodeling

- ECM is the space between cells. Aging has been associated with increased collagen levels, and the development of fibrosis.
- Furthermore the ECM controls immune signaling system and tumor progression

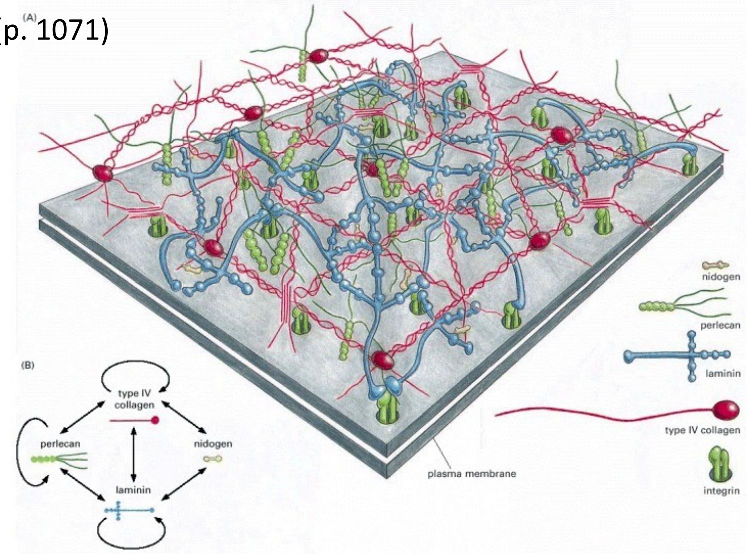


- In Fe data we see the downregulation of the ECM components on days 1, 3, and 14
  - Key genes such as Fibronectin, Fibrillin, Lumican, Versican, and MMPs are downregulated implicating dysregulation of collagen production.
  - Downregulation of Type III and VIII Collagen
- For H data there is an upregulation of ECM regulation and structure
  - This is constant for the whole experiment, and include the upregulation of various collagens
- A possible reason for ECM remodeling could be related to the TCR signal pathway seen in both datasets

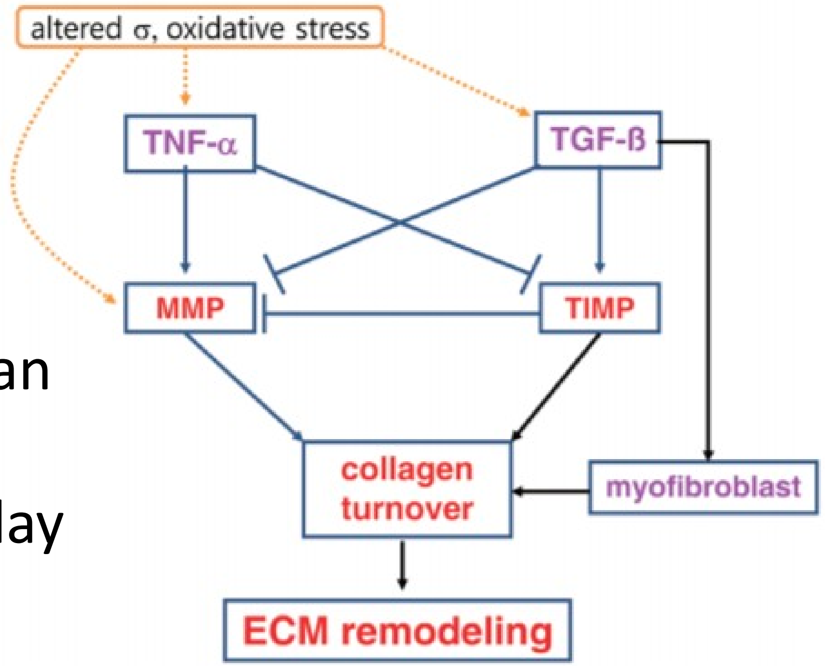
Figure: Kwak, Hyo-Bum. "Aging, exercise, and extracellular matrix in the heart." *Journal of exercise rehabilitation* 9.3 (2013): 338.

Figure Credit: Molecular Biology of The Cell (6th Ed.) 19-53 (p. 1071)

ECM Remodeling more detailed



- In both datasets MMPs are selectively regulated implicating a increase in collagen as they are responsible for collagen degradation
  - Fe: many MMPs (for example MMP2) are downregulated. Downregulation implies less collagen is being degraded. TIMPs inhibit MMPs and they are downregulated
  - The balance of MMP/TIMP is out of whack
  - In H: some MMP show slight if any upregulation. TIMPs are not significantly regulated
- Both data sets show mixed results for the activation of TNF as an upstream signal
- Fe data seems to show an under expression of TGF except on day 28 while some TGF products are over expressed in H data



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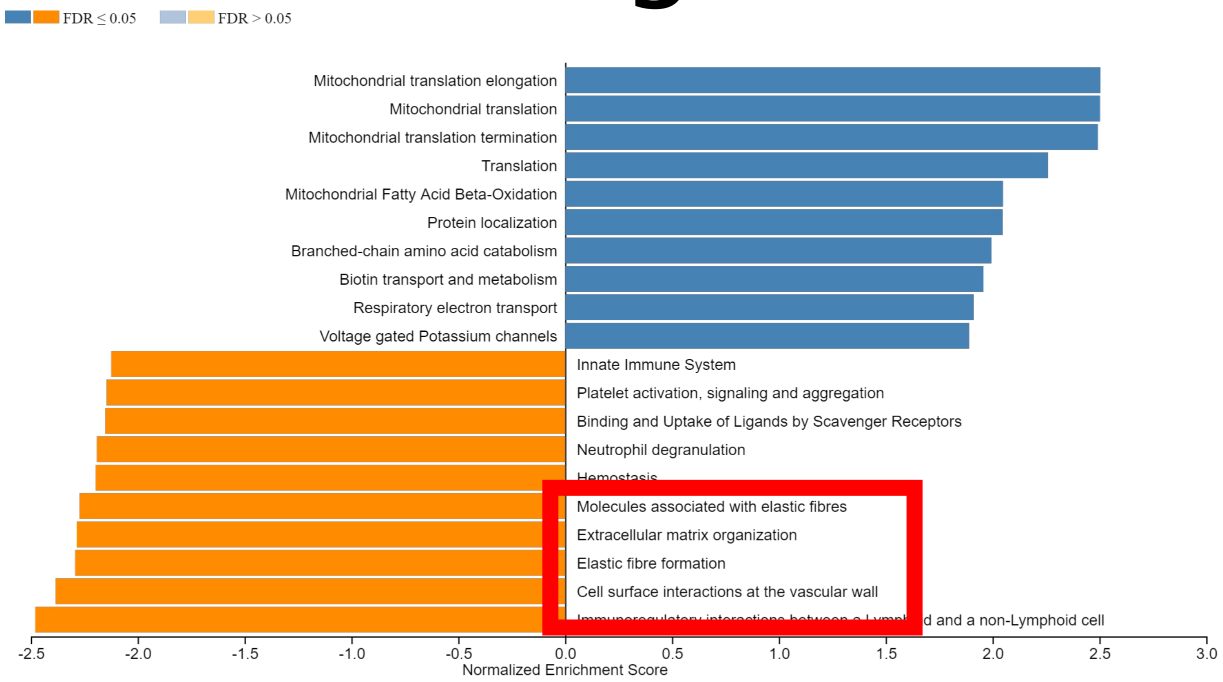
Extracellular Matrix Remodeling



Fe Data: Day 1 GSEA Results
logFC used as ranking metric

H Data: Day 1 GSEA Results
logFC used as ranking metric