

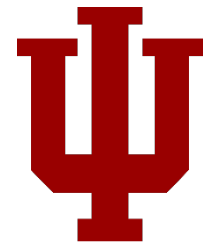
An Epigenetic Trigger for Induction of Genomic Plasticity Following Exposure to Space Environment

ISS RODENT MISSION 8

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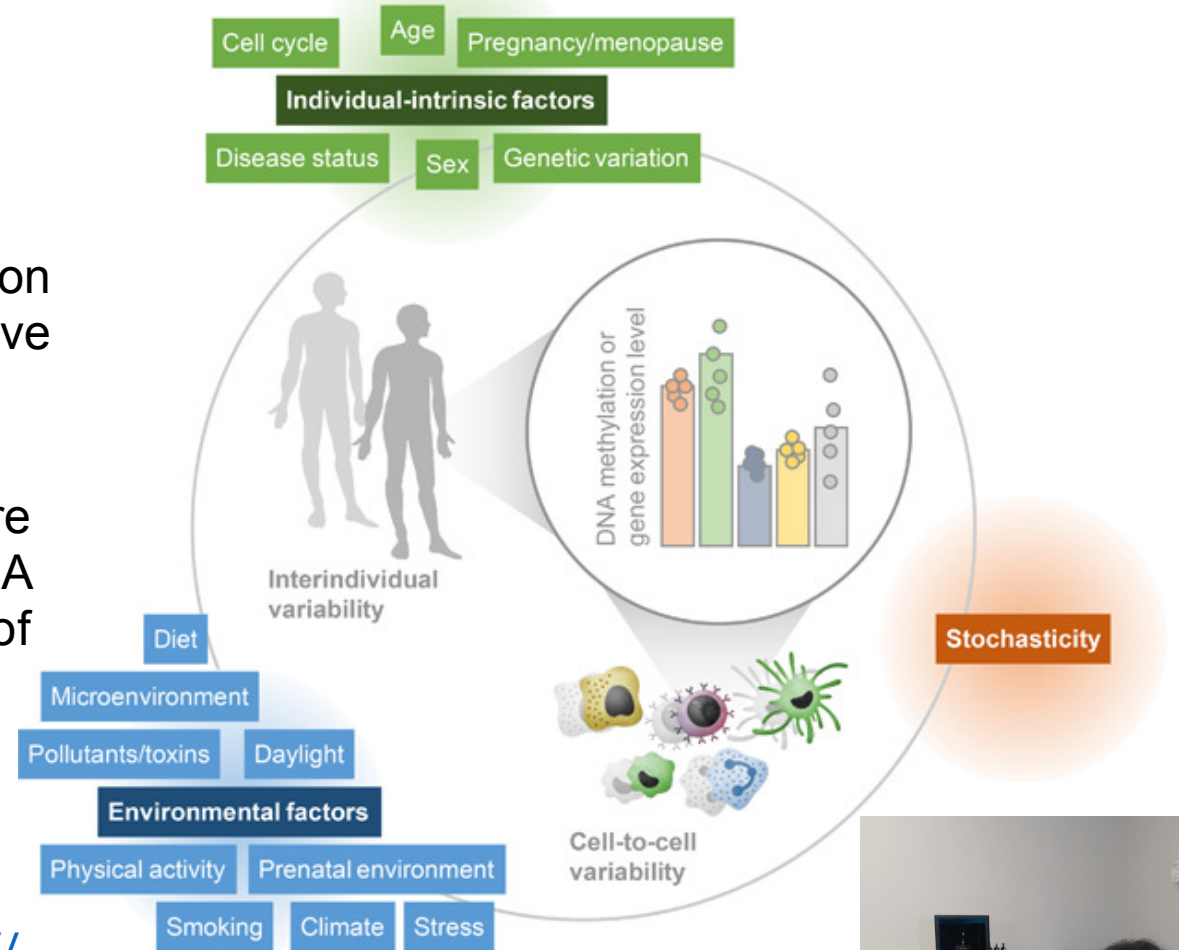


Genomic Plasticity: Foundation for Mammalian Life to Adapt to Extreme Natural Environment

Epigenetic alterations in the absence of genetic change can affect gene expression

If epigenetic signals producing phenotypic variation are inherited, they can form the basis of adaptive evolutionary change

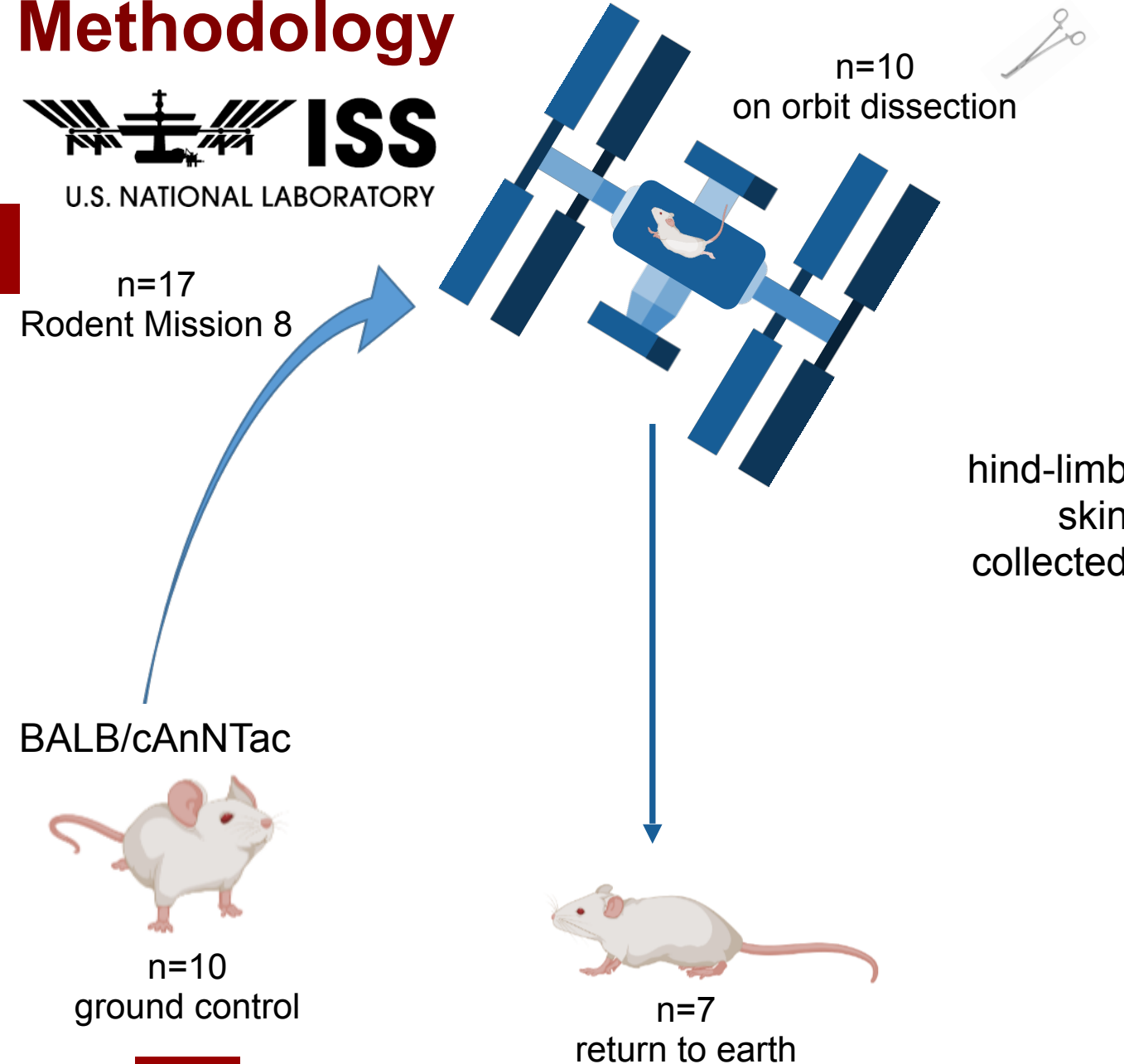
Rapid adaptive responses based on epigenetics are possible because the rate of epimutations from DNA methylation has been shown to be orders of magnitude higher than the rate of genetic mutations



The immune response, BigPicture (<http://bigpictureeducation.com>)



Methodology



Global 5mC levels analysis

MethylFlash™ Global DNA Methylation (5-mC) ELISA Easy Kit (Epigentek)

- ✓ comparative analysis of skin global 5mC levels between groups

Immunohistochemical analyses

- ✓ distribution of 5mC in different compartment of the skin

whole-genome Reduced Representation Bisulfite Sequencing (RRBS) methylome analysis

- ✓ unbiased whole genome methylation identification on individual genes

mRNAseq analysis

- ✓ observe the effect of DNA methylation over gene expression



ESE Lowered 5-mC Levels in Epithelial Compartment in Responder

- ground control
- non-responders
- responders

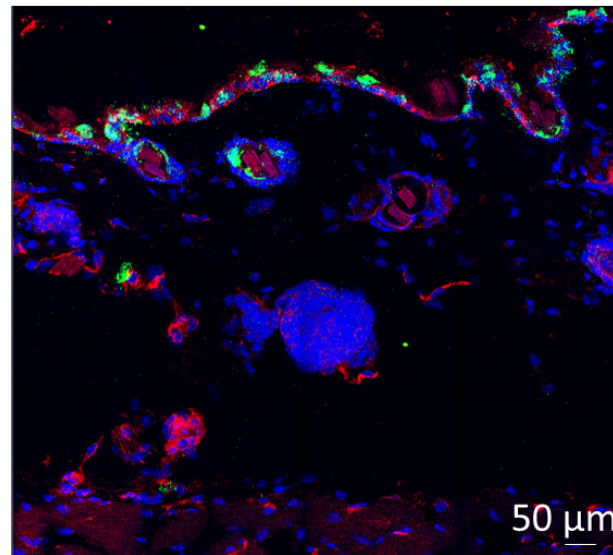
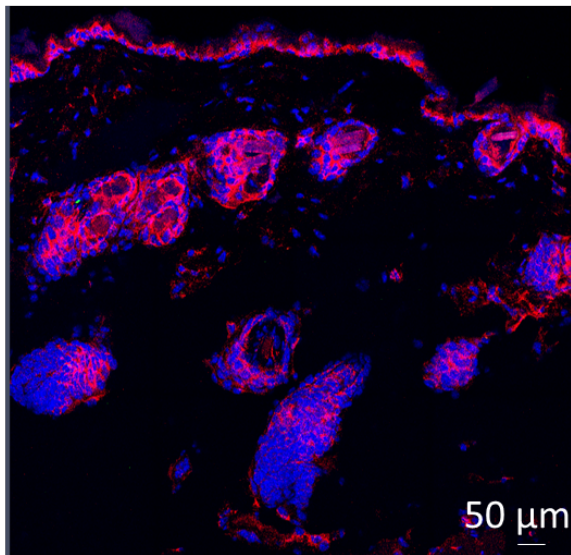
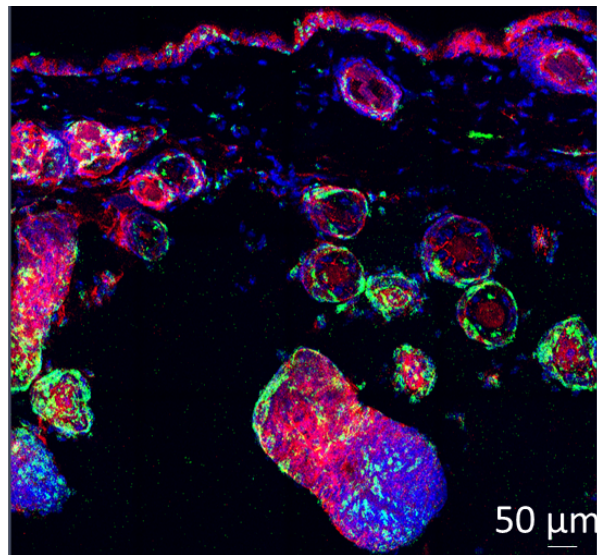
ground control

responder

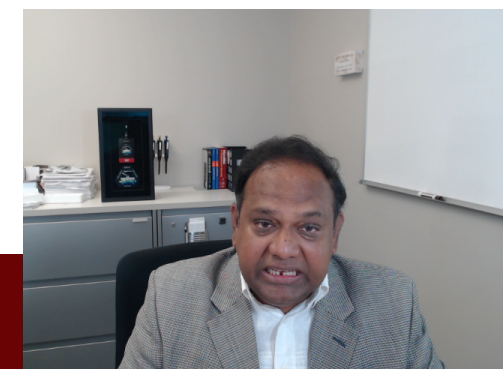
non-responder

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5mC/E-Cadherin/DAPI



ground control non responder responder
control responder ESE



ESE Lowered 5-mC Levels in Myeloid Compartment in Responder

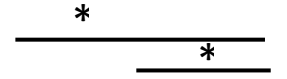
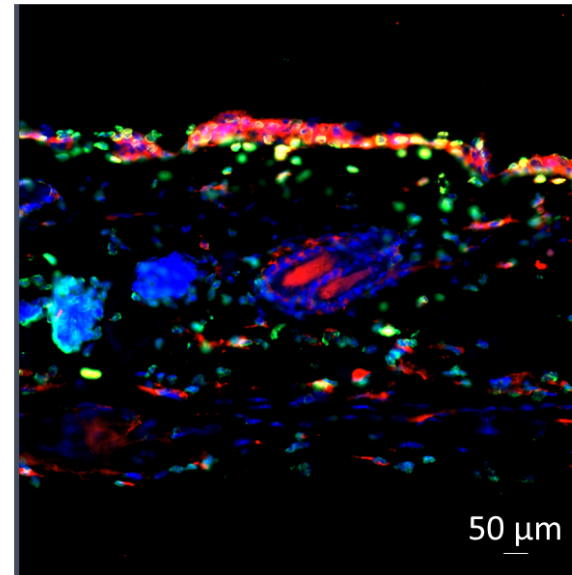
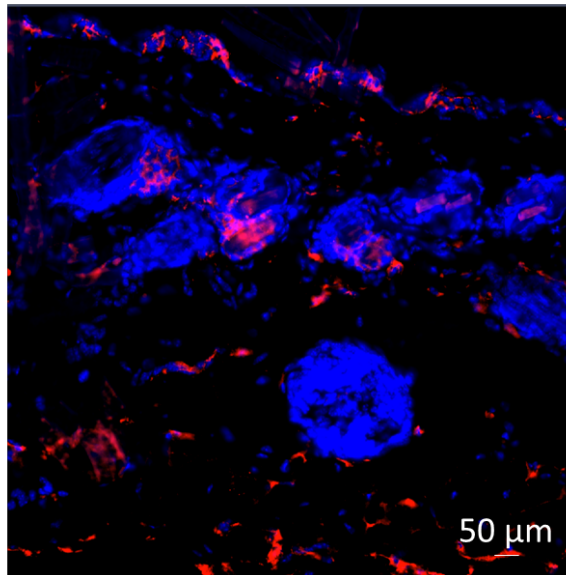
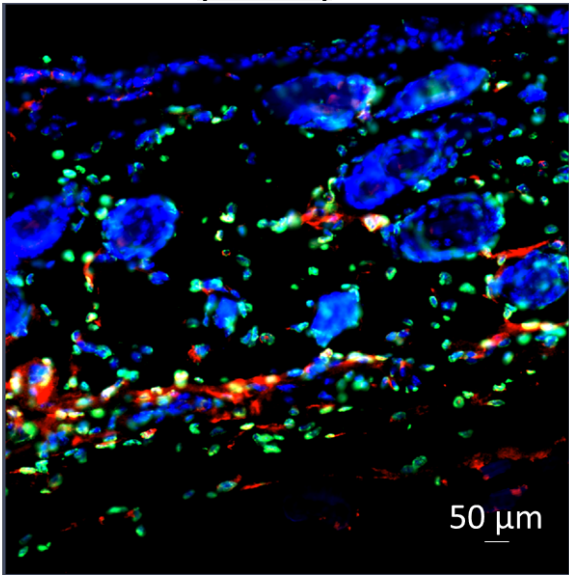
- ground control
- non-responders
- responders

ground control

responder

non-responder

5mC/F4/80/DAPI

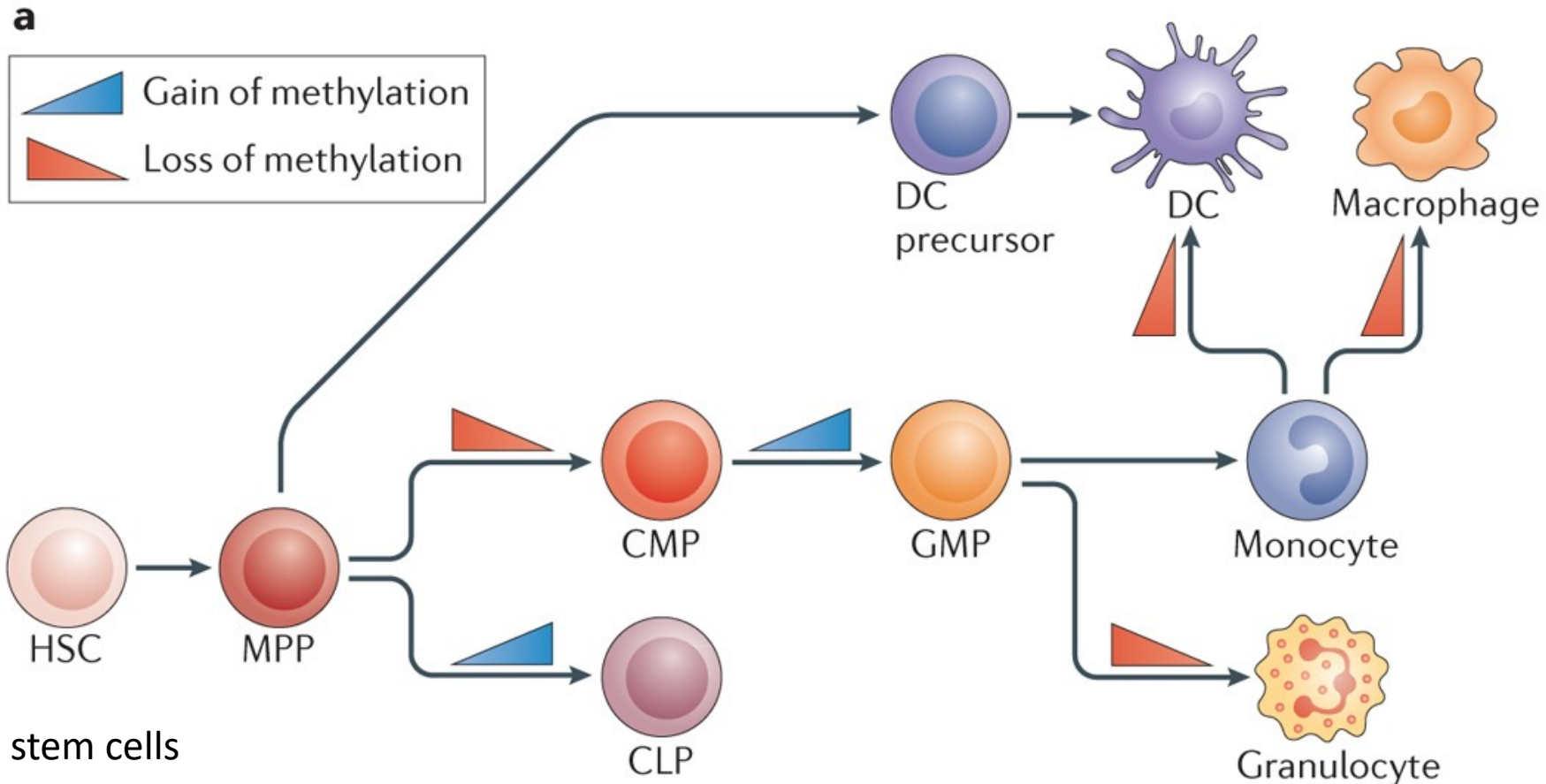


Note: No change in myeloid cell population

ground control non responder responder
control responder ESE



DNA Methylation Control of Myeloid Cell Development, Plasticity, Identity and Function



HSC - hematopoietic stem cells

MPP- multipotent progenitors

CMP - common myeloid progenitor

CLP - common lymphoid progenitor

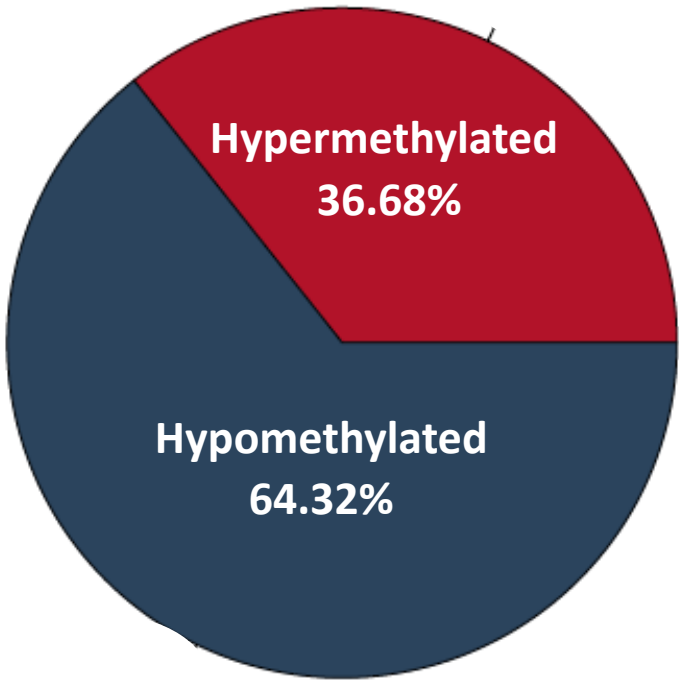
GMP - granulocyte-macrophage progenitor

Álvarez-Errico D et al. Nat Rev Immunol. 2015

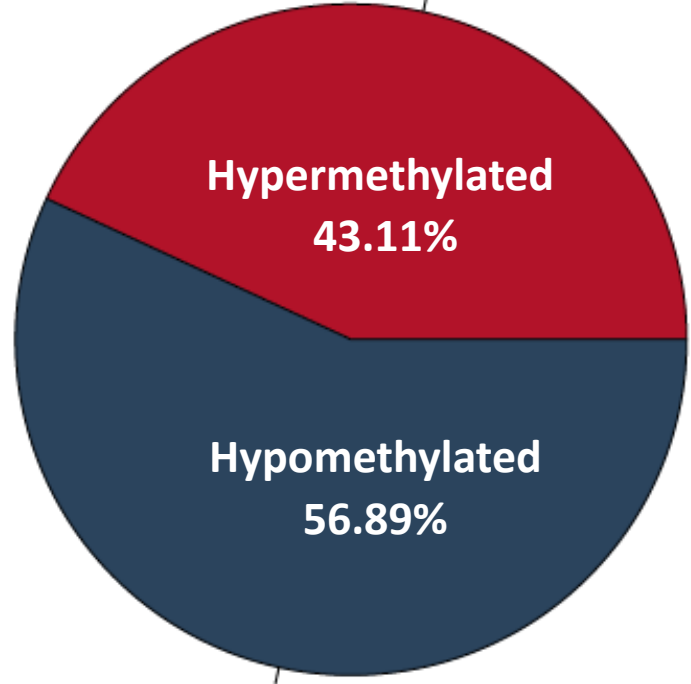


RRBS Analysis Showed a Difference of ~ 7.43 % of Differential Methylated Regions (DMRs) Towards Hypomethylation in Responders Compared to Non-responders

responders vs ground controls



non-responders vs ground controls



RRBS: Reduced Representation Bisulfite Sequencing



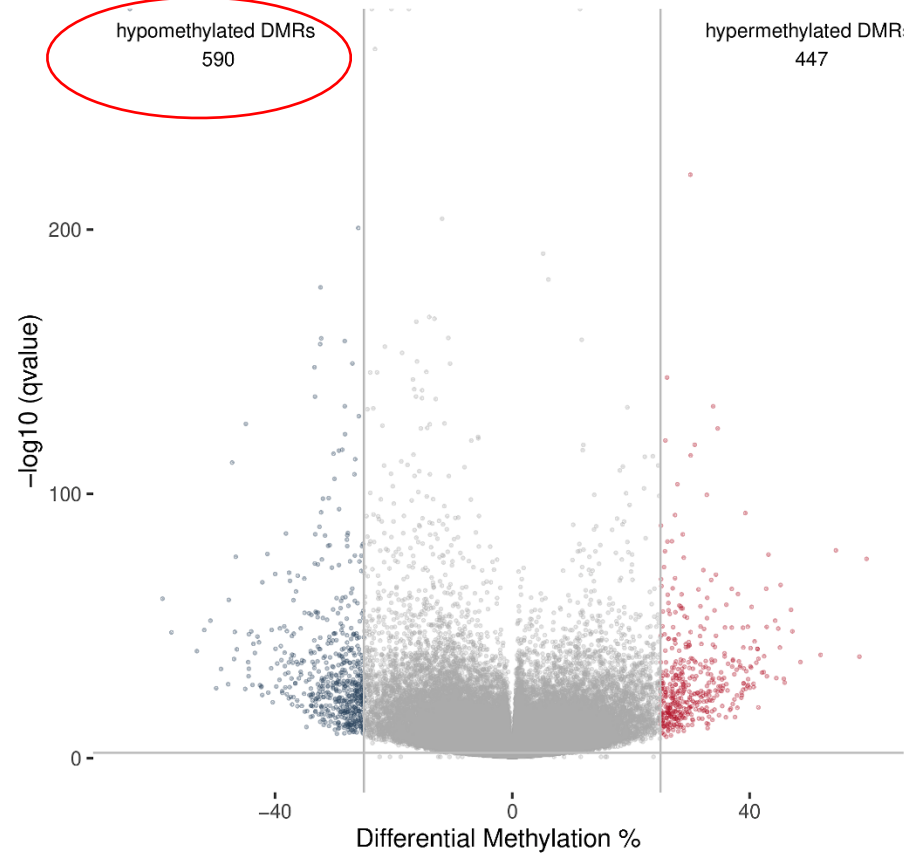
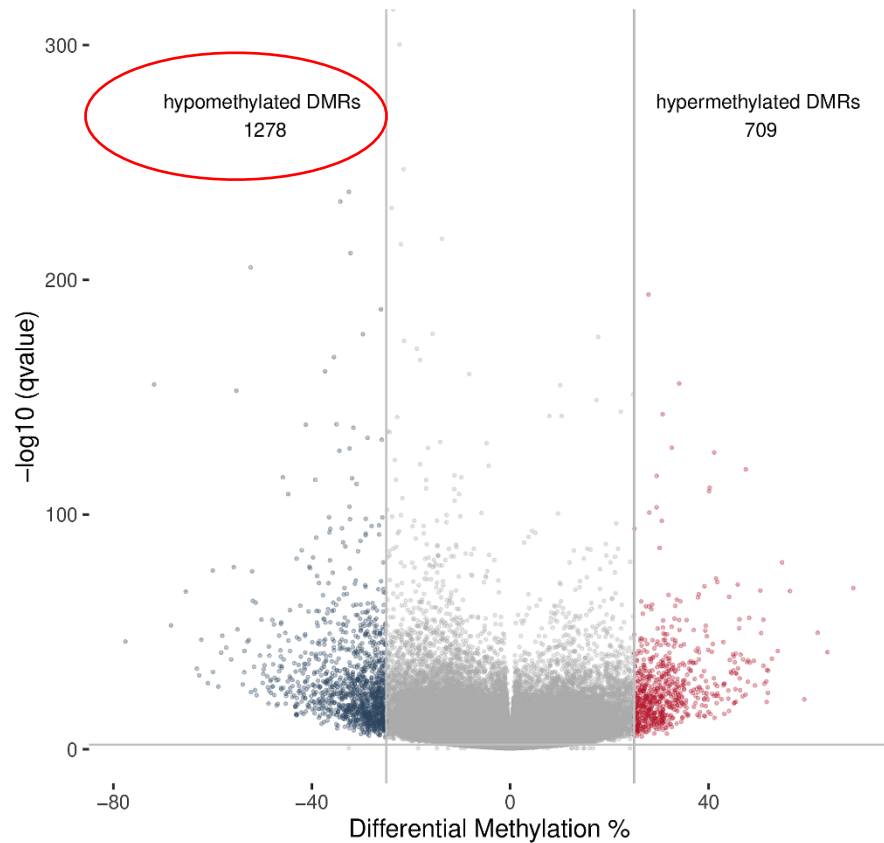
Hypomethylated Differentially Methylated Regions (DMRs) in Responders was Higher than 2-fold Compared to Non-responders

responders vs ground controls

non-responders vs ground controls

Volcano Plot of Differentially Methylated Regions

Volcano Plot of Differentially Methylated Regions



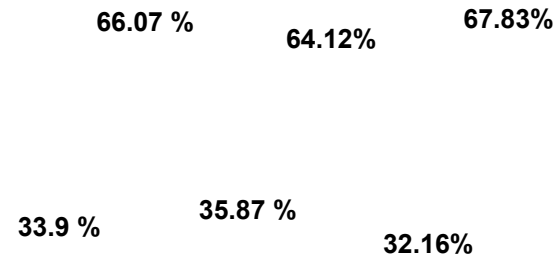
709 region gained methylation (Hyper DMR)
and 1278 region lost methylation (Hypo DMR)

447 region gained methylation (Hyper DMR)
and 590 region lost methylation (Hypo DMR)



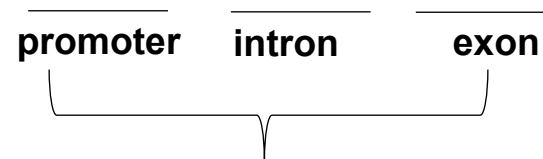
Hypomethylated DMRs were Distributed Throughout the Genome

responders vs ground controls



Hypermethylated

Hypomethylated



IPA analysis

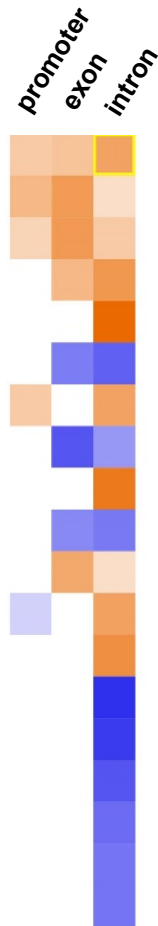
To identify the genetic pathways altered due to this hypomethylation, Ingenuity pathway analysis (IPA) was performed using regional annotations (promoter, exon and intron)



Ingenuity Pathway Analysis (IPA) Identified Pathways Enriched by Hypomethylated DMRs

canonical pathways enriched in methylation dataset

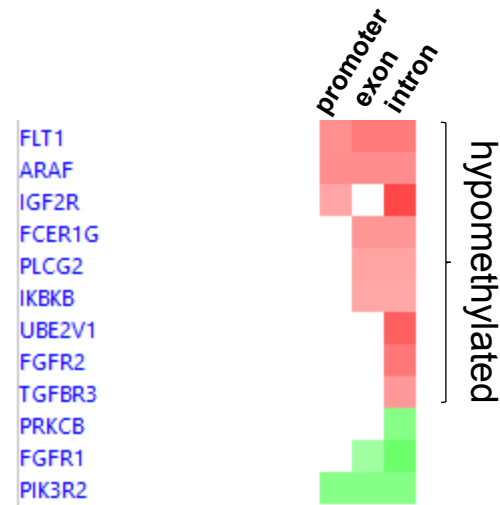
- NF-κB Signaling
- HIF1α Signaling
- Senescence Pathway
- Osteoarthritis Pathway
- Basal Cell Carcinoma Signaling
- Opioid Signaling Pathway
- Adrenomedullin signaling pathway
- MSP-ROn Signaling In Macrophages Pathway
- BEX2 Signaling Pathway
- GP6 Signaling Pathway
- PKCθ Signaling in T Lymphocytes
- CREB Signaling in Neurons
- Factors Promoting Cardiogenesis in Vertebrates
- CCR3 Signaling in Eosinophils
- CXCR4 Signaling
- Nur77 Signaling in T Lymphocytes
- Fcy Receptor-mediated Phagocytosis in Macrophag...
- ErbB4 Signaling
- Calcium-induced T Lymphocyte Apoptosis



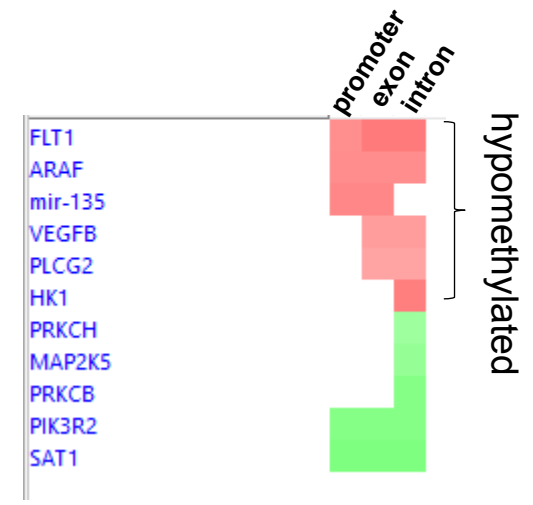
activation Z score infers likely activation state

top two pathways hypomethylated in responders

NF-κB



HIF-1α

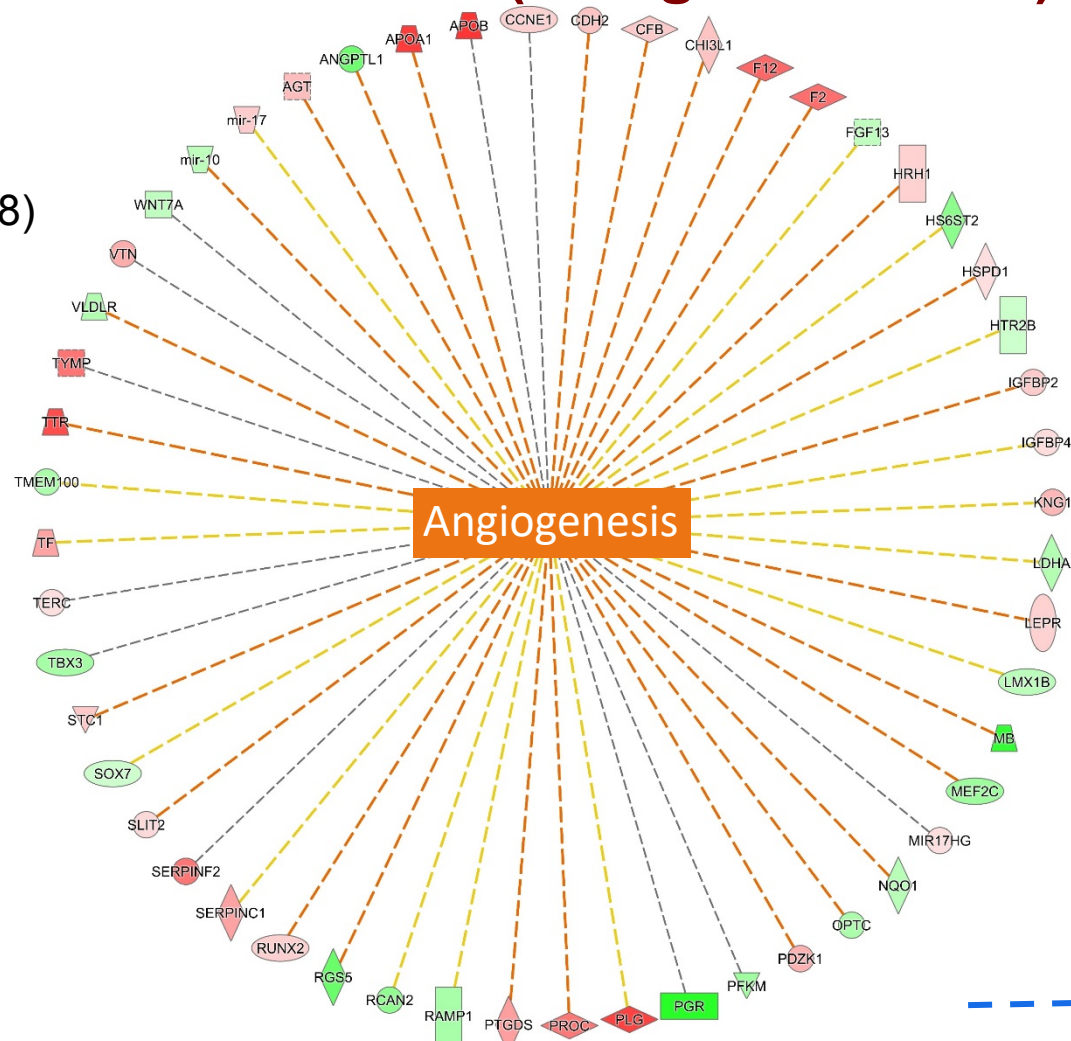


responders vs ground controls

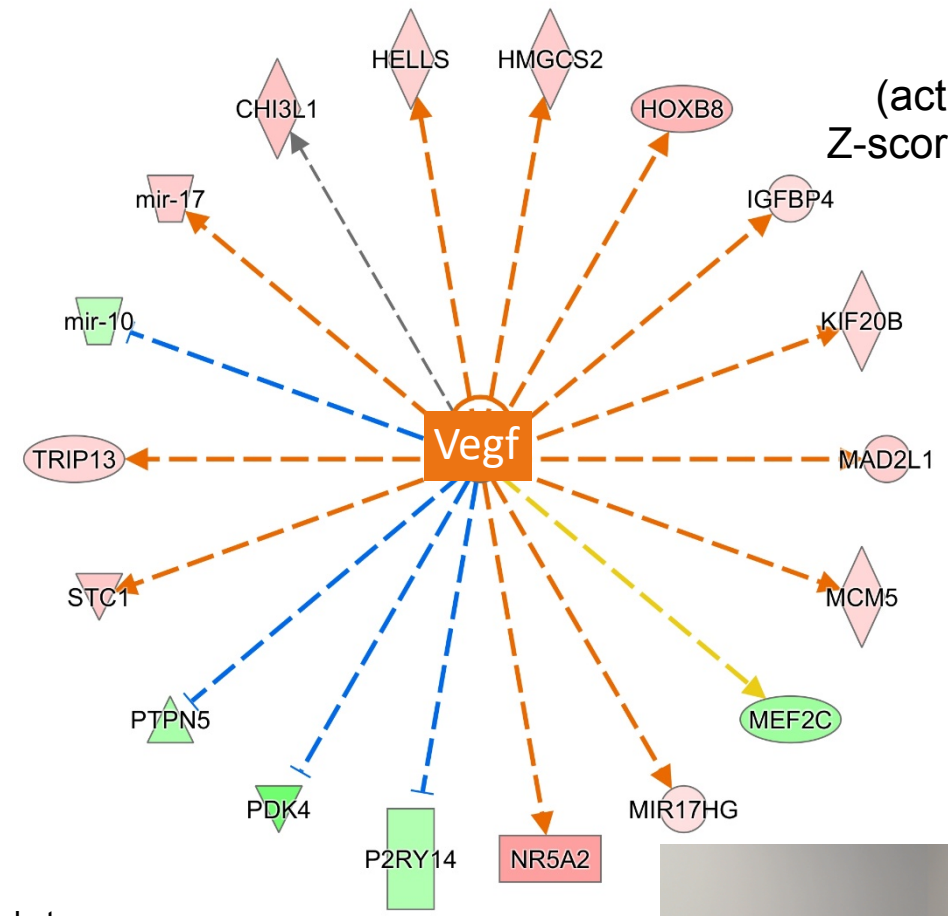


mRNA sequencing based differential expression analysis indicated angiogenesis biofunction (along with VEGF) was enriched in responders

(activation Z-score = +1.8)



(activation Z-score = +3.6)



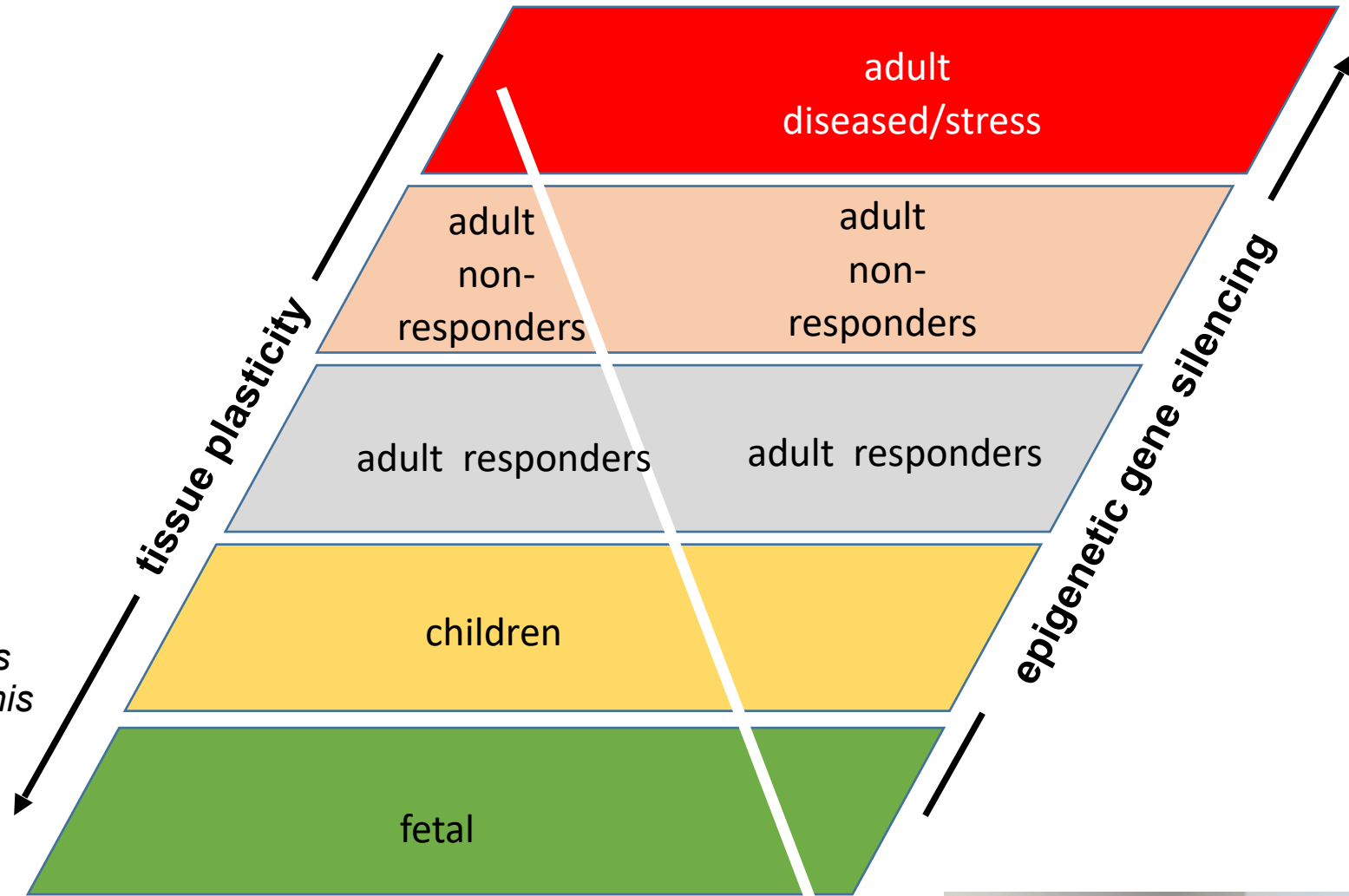
- predicted activation
- upregulated
- downregulated
- leads to inhibition
- finding inconsistent
- leads to activation
- effect not reported



Novel Paradigm

Exposure to space environment (ESE) profoundly impacts the epigenome as marked by DNA methylation to induce rodent genomic plasticity.

ESE involves numerous variables in play ranging from known factors such as microgravity to factors that remain to be identified. For the purposes of this work it is not of interest to isolate the variables in play and therefore we consider ESE as one variable.



Decrease in tissue plasticity due to increase in epigenetic gene silencing during development and disease

