

# HUMAN SPACEFLIGHT AND VISION IMPAIRMENT

## EFFECTS OF MICROGRAVITY ON GENE EXPRESSION IN RETINAL GLIAL CELLS

### INTRODUCTION

Human spaceflight has been shown time and again to impose a variety of negative effects on the body. Looking specifically at eye function, about 30% of astronauts on short-term flights and 60% on long-duration missions to the ISS have reported impairment to their vision. As future missions for astronauts are planned and space tourism nears the realm of possibility, it is essential to develop a deeper understanding of the possible repercussions to human safety.

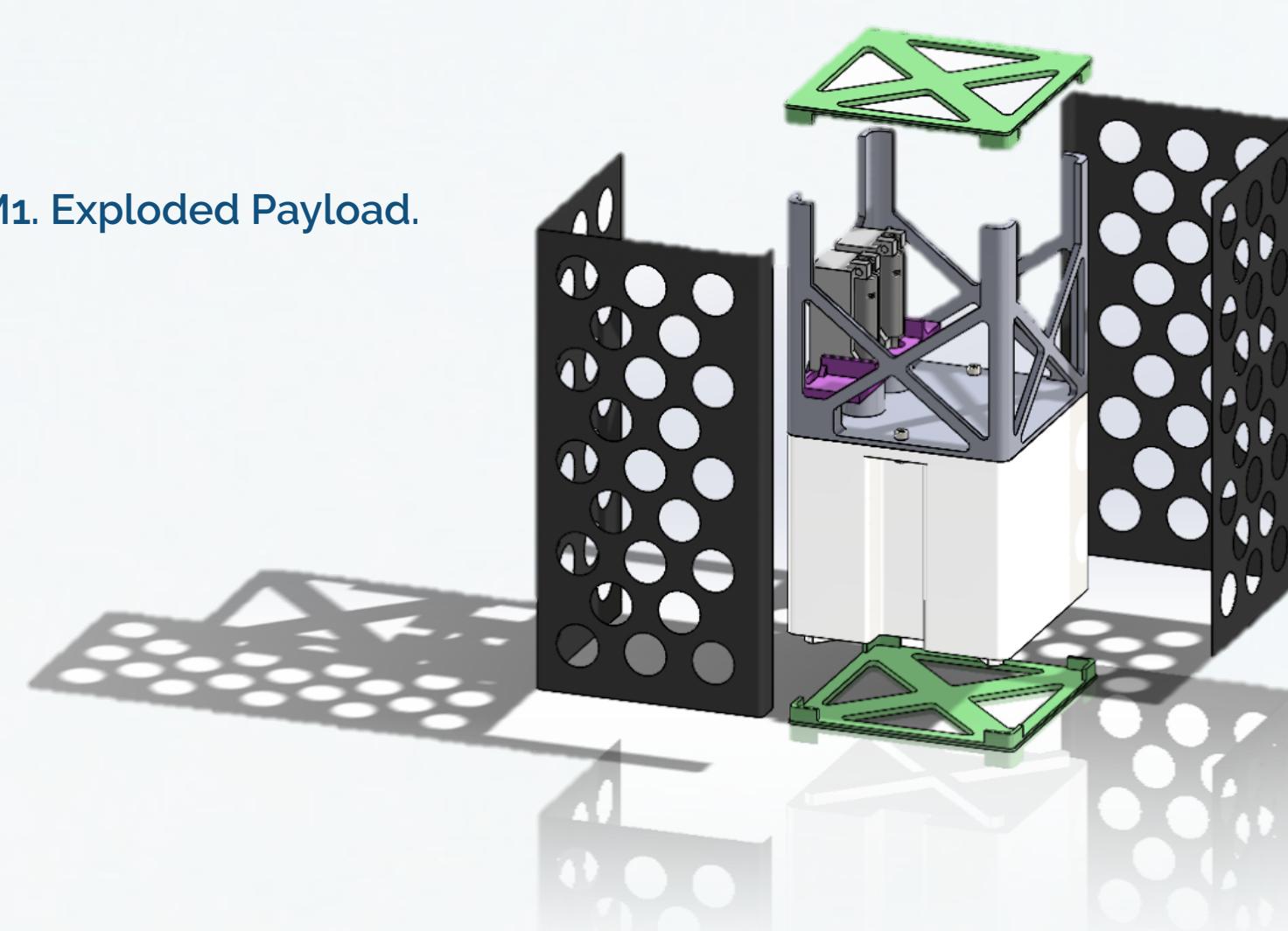
The extreme barriers to entry imposed on space travel, however, limit research regarding short term exposure to microgravity. Building off previous studies identifying cellular disorganization (Uva, et. al., 2002) and deterioration (Gringoryan, et. al., 2012) after simulated microgravity, this project aimed to further explore the effects of true short-term microgravity exposure on eye tissue at a gene expression level with particular interest in processes that affect the structural integrity of retinal glial cells. The cells were exposed to microgravity for several minutes during a Blue Origin New Shepard flight.

### MATERIALS & METHODS

#### PAYOUT DESIGN

- An automatic payload was designed to house cultured cells and inject a solution of RNA stabilizing solution at two time-points immediately before and after microgravity exposure
- All parts were 3D printed in PLA except the outside shell which was aluminum sheet metal with CNC machined holes. Material was removed based upon topology simulations to produce highest strength to weight ratio.
- Two linear actuators were used to push fluid into six cell cultures. These cell cultures were housed in a 3D printed container filled with aerogel to minimize heat loss from the system.

Figure M1. Exploded Payload.



#### CELL EXPERIMENT PREPARATION

- To account for limited weight, the rat retinal glial cells (Kerafast) were cultured in PermaLife cell culture bags with cytodex beads for the cells to adhere to. Cell culture media was supplemented with HEPES buffer to account for the lack of CO<sub>2</sub> in the payload.
- At L-1, three syringes were prepared with 9 mL of RNAlater Stabilization Solution (Thermo Fisher) and connected via tubing and customized tubing connectors to 3 cell culture bags each.
- Six samples and corresponding syringes were moved to the payload enclosure and approximately 37 degrees celsius was maintained until and during launch.
- Three control samples and an additional syringe of RNAlater were stored in a similar container that was to be kept on the Earth during launch.

#### DATA ANALYSIS

- Post launch, samples were then prepared for analysis by removing the cells from the cytodex beads. Cells were lysed in TRIzol Reagent, and lysates were sent to a 3rd party vendor for RNA isolation and mRNA sequencing.

Genes	Process_name	Total genes	Percent significant genes	P value	Padj value
Nelfe;	GO:000122-negative regulation of transcription from RNA polymerase II promoter	793	0.13	0.198	0.204
Gapdh;	GO:0000226-microtubule cytoskeleton organization	79	1.27	0.022	0.03
Gapdh;	GO:0005975-carbohydrate metabolic process	111	0.9	0.03	0.041
Gapdh;	GO:0006094-gluconeogenesis	27	3.7	0.008	0.02
Gapdh;	GO:0006996-glycolytic process	34	2.94	0.01	0.022
Gapdh;	GO:0006417-regulation of translation	63	1.59	0.017	0.028
Gapdh;	GO:0007275-multicellular organism development	364	0.27	0.095	0.104
Mt-atp6;	GO:0009409-apoptotic process	249	0.4	0.066	0.077
Mt-co2;	GO:0007595-lactation	321	0.31	0.085	0.096
Mt-co2;	GO:0009409-response to cold	59	1.69	0.016	0.028
Mt-atp6;	GO:0009409-response to cold	58	1.72	0.016	0.028
Mt-atp6;	GO:0015988-ATP synthesis coupled proton transport	22	4.55	0.006	0.02
Gapdh;	GO:0017148-negative regulation of translation	72	1.39	0.02	0.029
Mt-co2;	GO:0042773-ATP synthesis coupled electron transport	4	25	0.001	0.011
Gapdh;	GO:0050821-protein stabilization	160	0.63	0.043	0.054
Gapdh;	GO:0051402-neuron apoptotic process	45	2.22	0.012	0.026
Mt-atp6;	GO:0055093-response to hypoxia	43	2.33	0.012	0.026
Gapdh;	GO:0071346-cellular response to interferon-gamma	66	1.52	0.018	0.028

Table 1 (above). Selected portion of Gene Ontology Analysis for Group B vs Group A reporting processes specific to each significantly altered gene, total gene group counts, and percent significant genes.

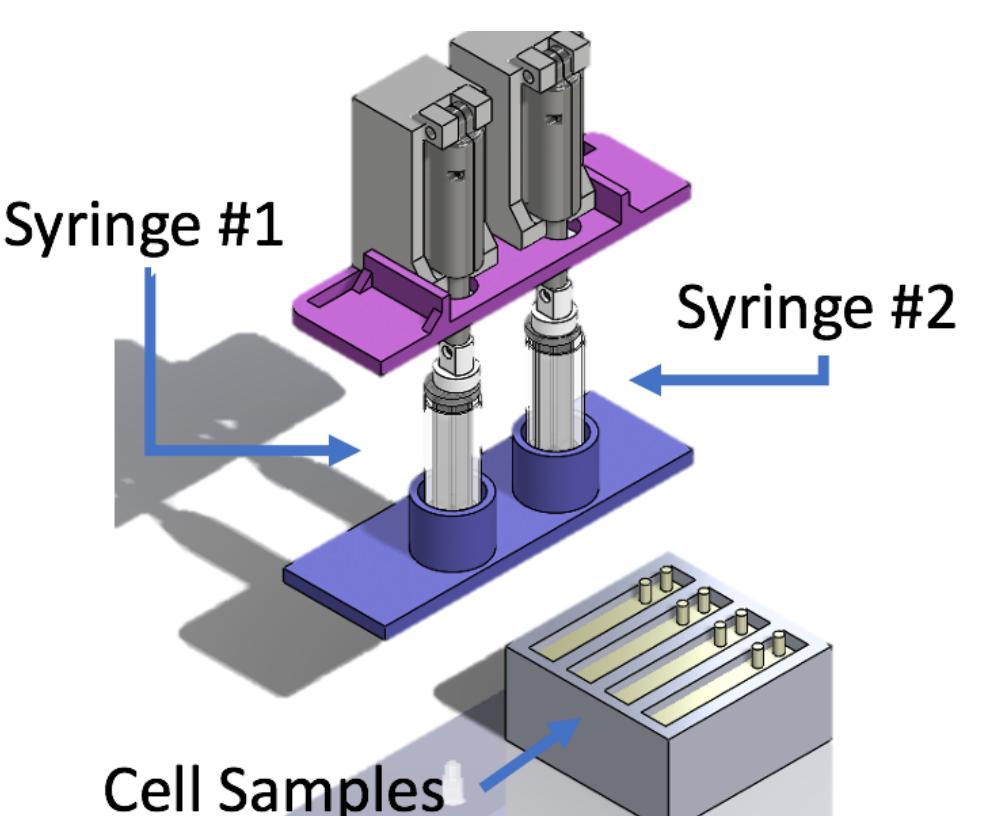
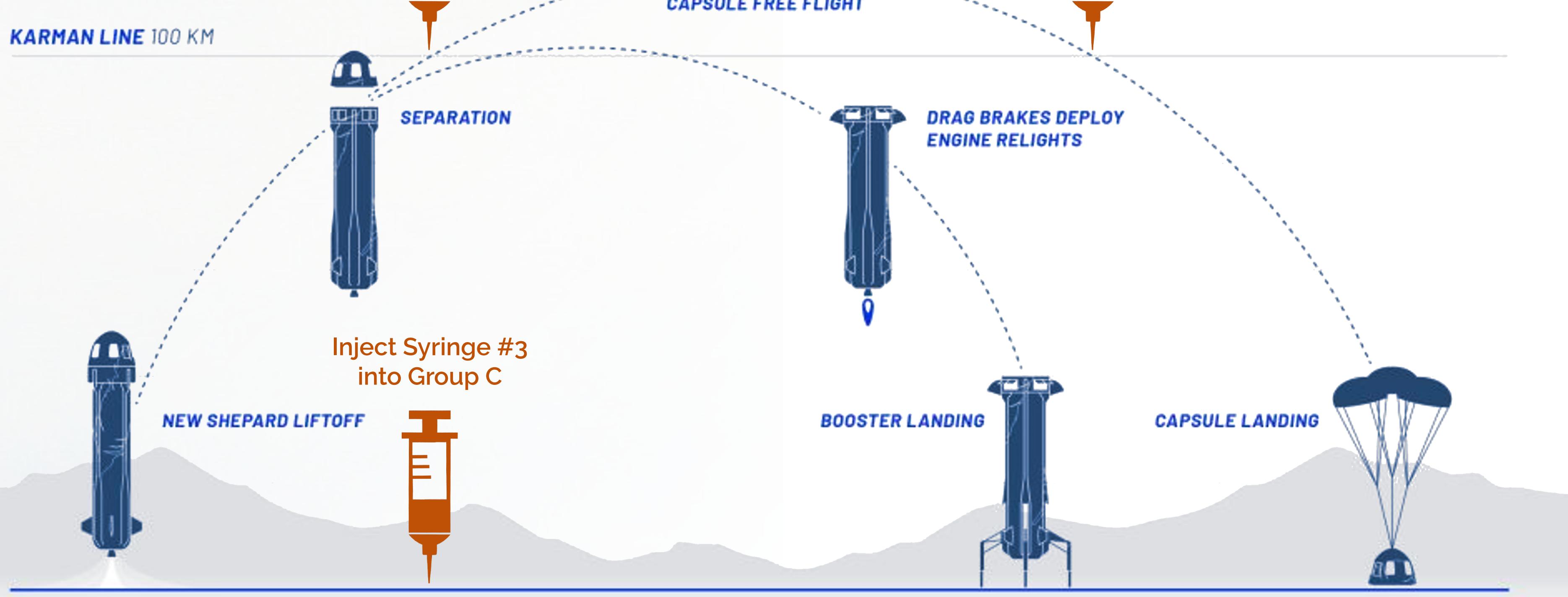


Figure M2 (right). Syringe & linear actuator system.

Figure M3 (below). Launch trajectory and sample injection timepoints.



WEST TEXAS LAUNCH SITE 3,700 FT ABOVE MSL

Credit: Blue Origin.

#### FINDING 1

In order to isolate the effects of true microgravity, two sets of cell samples were sent in a payload on a suborbital rocket and samples were injected with RNA stabilizing solution directly before and after microgravity exposure. The payload control (B) proved to be most similar to the experimental group (A) after experiencing the same launch forces as determined from looking at the sample distances between expression values from each sample (Figure R1). For this reason, Group B was identified as the best control to account for effects of launch conditions and all subsequent analyses focus on comparisons between groups B and A, reflecting gene expression before (B) and after (A) microgravity.

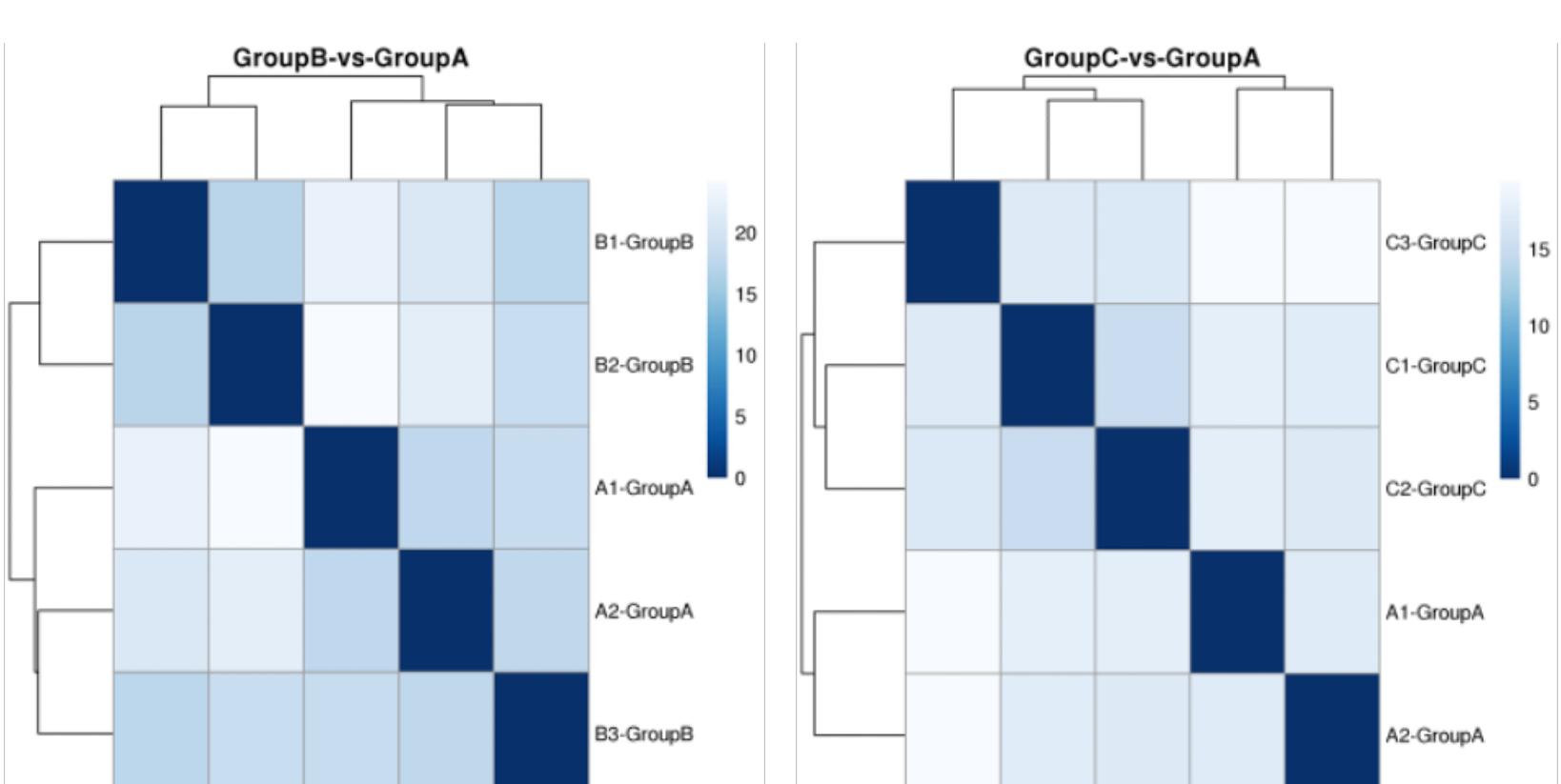


Figure R1. Sample distances between expression values from each sample. Darker blue corresponds to shorter distances and closer relationships.

#### FINDING 2

A visual of the gene expression profile in Figure R2 identifies co-regulated genes between Groups B and A, highlighting 6 genes significantly affected by microgravity exposure.

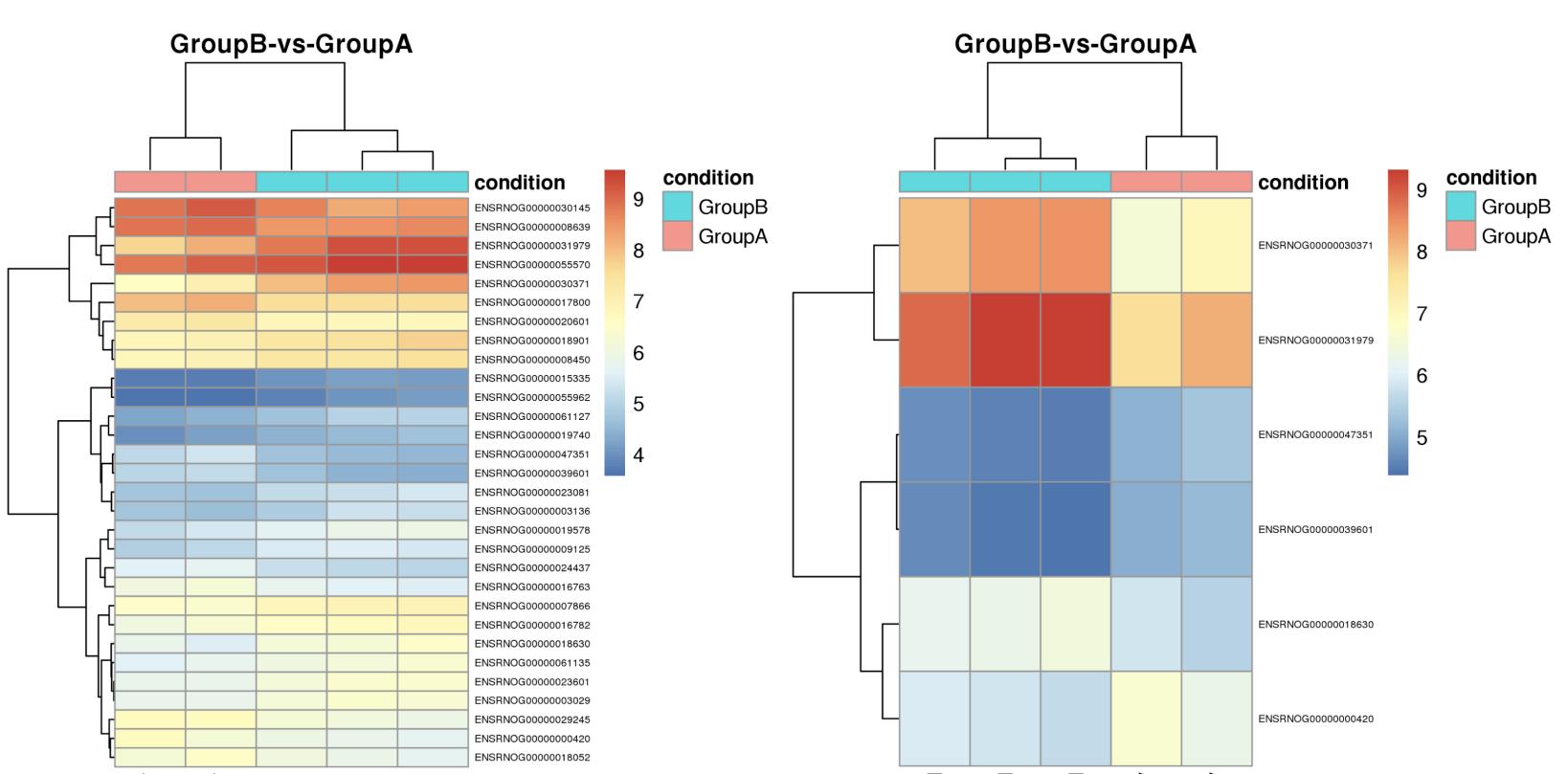


Figure R2A-B. Differentially expressed genes bi-clustering heat map containing the top 30 genes in A and only the top statistically significant differentially expressed genes in B.

As a result of microgravity, genes Nelfe, LOC683469, and AABR0715081.1 were downregulated and genes Gapdh, Mt-co2, Mt-atp6 were upregulated in Group A ( $p < 0.05$ ). These six genes are highlighted in both the volcano plot (Figure R3) and the bioclustering heat map of differential gene expression (Figure R2-B). A gene ontology analysis was performed on the statistically significant set of genes and the GO list was used to cluster the set of genes based on their biological processes and determine their statistical significance (Table 1).

GroupB-vs-GroupA

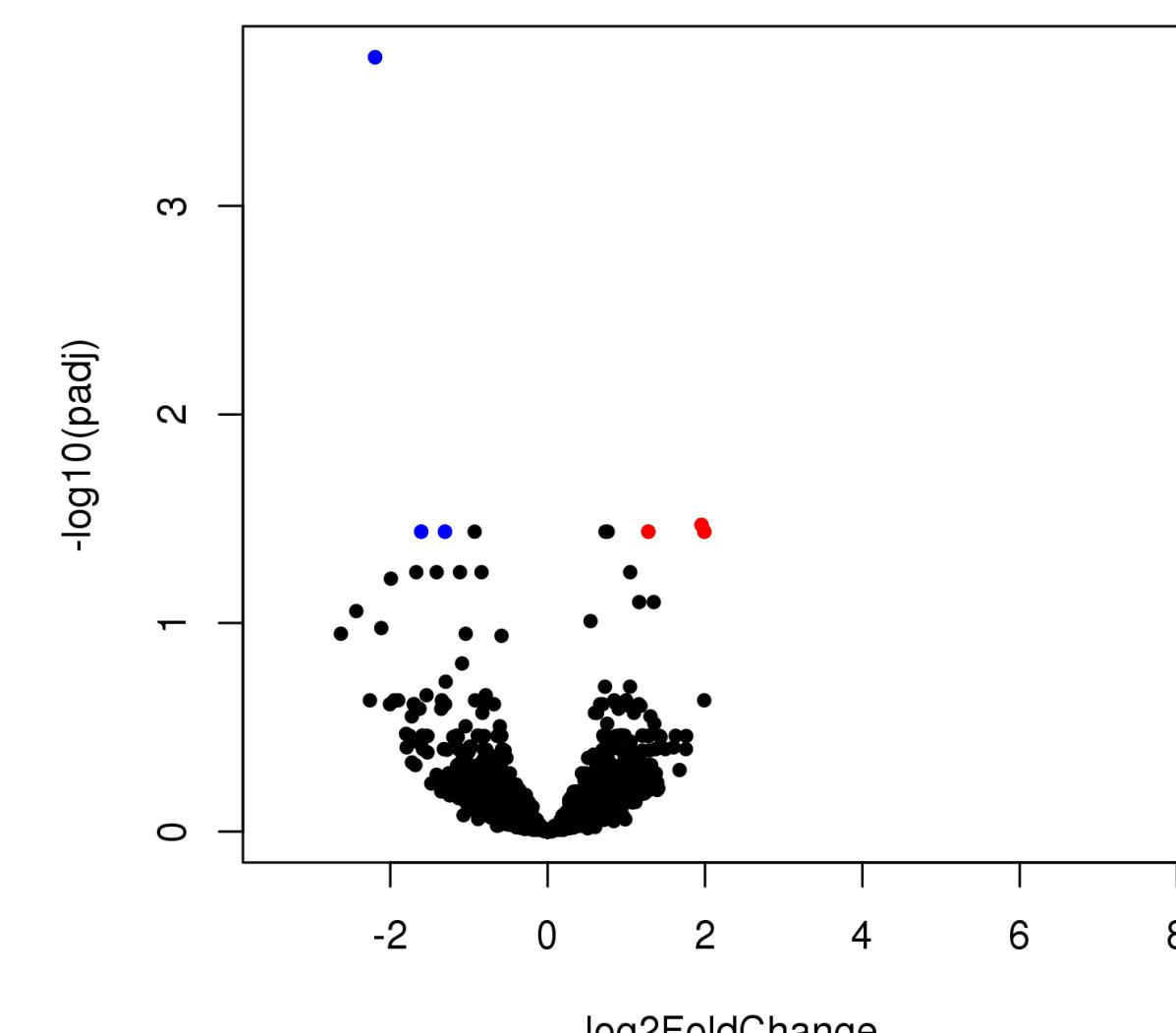


Figure R3. Volcano plot of global transcriptional change in all genes across groups. Genes with an adjusted p-value less than 0.05 and a log<sub>2</sub> fold change greater than 1 are indicated by red dots representing downregulation of genes in Group A. Genes with an adjusted p-value less than 0.05 and a log<sub>2</sub> fold change less than -1 are indicated by blue dots representing upregulation in Group A.

### CONCLUSION

Notably, increased activity of the gene Gadph and the process of microtubule cytoskeleton organization was observed.

- Microtubules are essential to the architecture of an animal cell, playing an integral role in load bearing and allowing the cell to perceive mechanical signals and maintain cell shape. (Hernandez, et al., 2014)

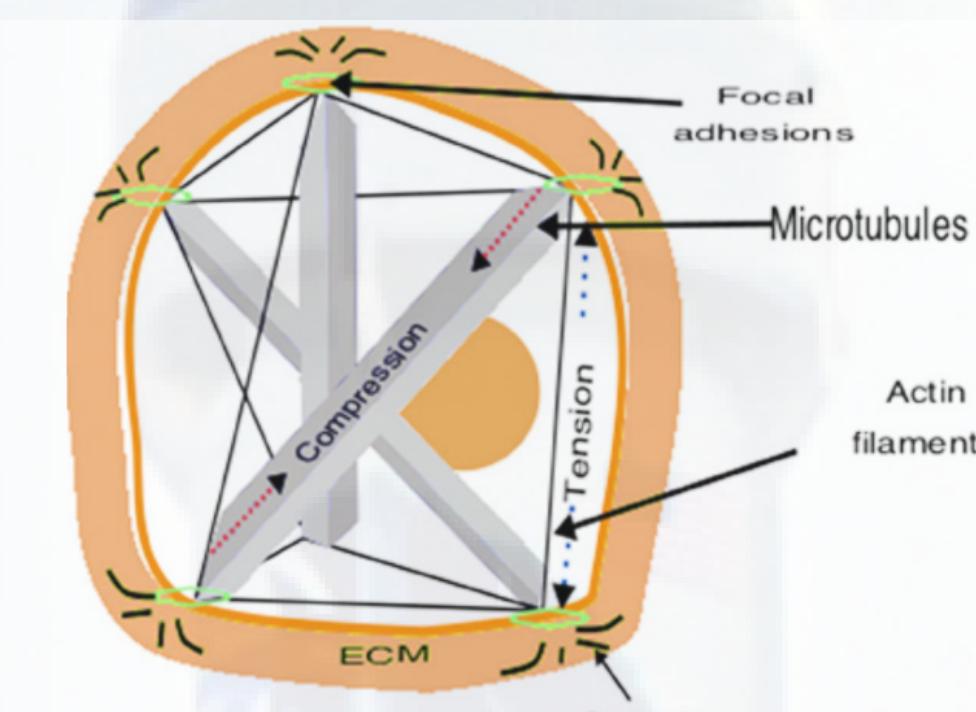


Figure C1. Schematic highlighting the role of microtubules in maintaining the structure of a cell (Hernandez et al., 2014)

- Elevated Gadph expression may indicate efforts by the cell to compensate for the changes experienced by the cell in microgravity.
- The increased effort by the cell to support microtubule cytoskeleton organization is especially interesting when considering results of earlier studies demonstrating highly disorganized cytoskeletal structure and alterations in glial cells in response to short-term simulated microgravity (Uva, et. al.).

Results of this study support the hypothesis that eye health is influenced not only at a systems level, but at a cellular level with attention paid specifically to the cytoskeletal structure of retinal glial cells.

Further studies are necessary to further understand the downstream effects of observed changes and to determine whether changes are sustained post launch. In the future, this newfound knowledge of the effect of microgravity on the structure of eye cells may contribute to inform clinical prevention of vision problems in astronauts.

Furthermore, the automated payload system that was designed to accomplish this research can be used as a basis for future biological payload experiments in a variety of different contexts.

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