To boldly go where no microRNAs have gone before: Spaceflight impact on risk for small-for-gestational-age infants

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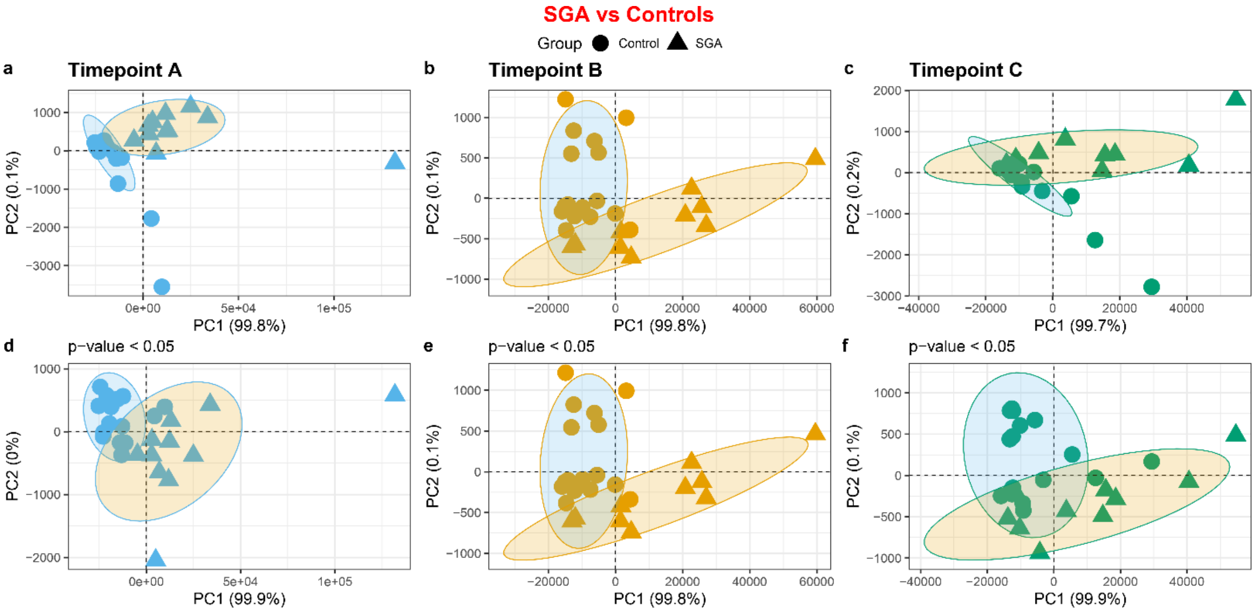

Figure S1. Global miRNA expression response in SGA patients vs. controls across gestation timepoints. Principal Component Analysis (PCA) plots illustrating the overall miRNA expression patterns in Small-for-Gestational-Age (SGA) patients (triangles) compared to control (healthy) patients (circles) at three gestation timepoints: A (blue), B (yellow), and C (green). Panels **a)** to **c)** display PCA plots for all miRNAs, while panels **d)** to **f)** focus on miRNAs with statistical significance ($p < 0.05$). The distinct clustering indicates differential miRNA expression profiles between SGA and control groups at each timepoint.

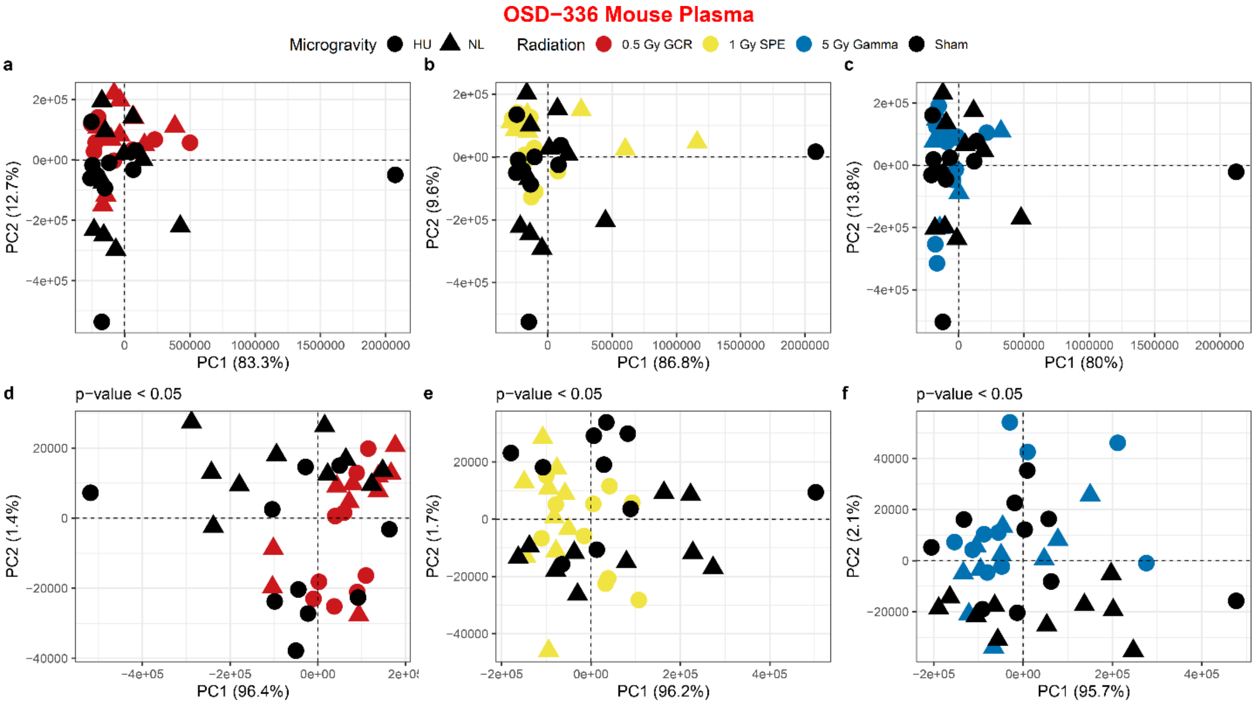

Figure S2. Global miRNA expression in irradiated mice vs. sham controls. Principal Component Analysis (PCA) plots depict overall miRNA expression patterns in mice exposed to different radiation doses: 0.5 Gy GCR (red), 1 Gy SPE (yellow), and 5 Gy gamma (blue), compared to 0 Gy sham controls (black). Panels (**a-c**) present PCA plots for all miRNAs, while panels (**d-f**) focus on miRNAs with statistical significance ($p < 0.05$). Distinct clustering signifies differential miRNA expression profiles between irradiated and 0 Gy sham groups

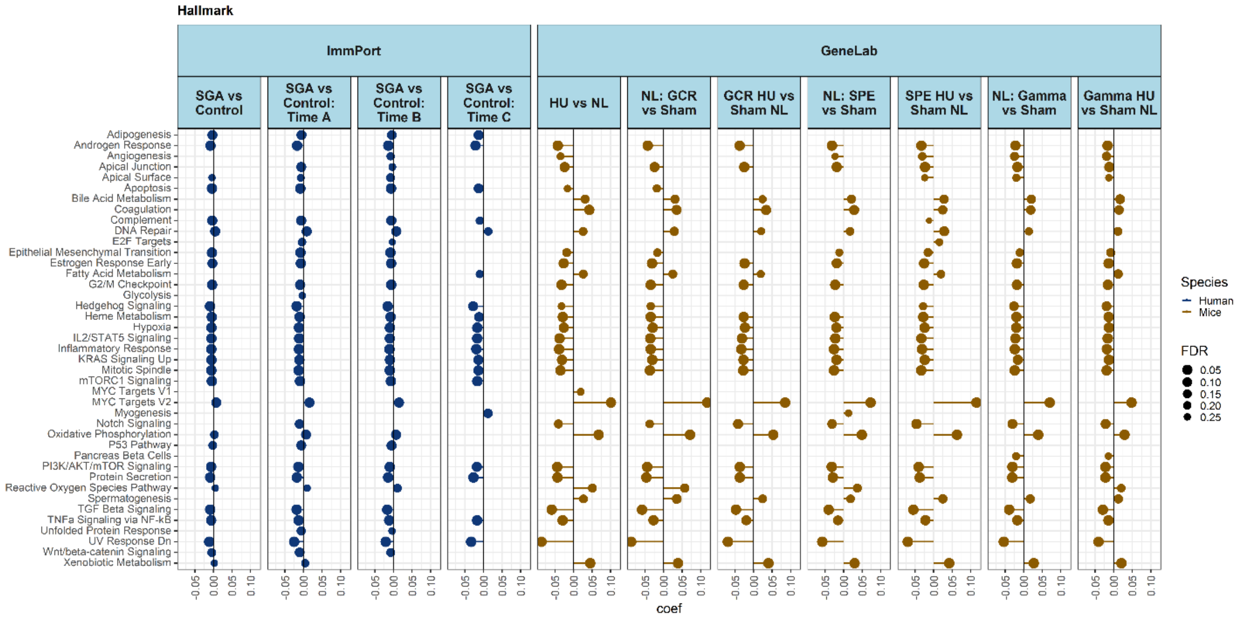

Figure S3. Global miRNA pathway analysis on Hallmark pathways. Gene set analysis of miRNAs on Hallmark pathways in Small for Gestational Age (SGA) and simulated spaceflight pathways compared to control. SGA human miRNA regulation is compared to control both time-independently and at different timepoints (left). Space radiation miRNAs with and without simulated microgravity are compared to Sham (right). The x-axis represents a coefficient term indicating pathway inhibition (negative value) or activation (positive value). The point size indicates the degree of significance, denoted by False Discovery Rate (FDR). Only significant values (FDR < 0.25) are displayed.

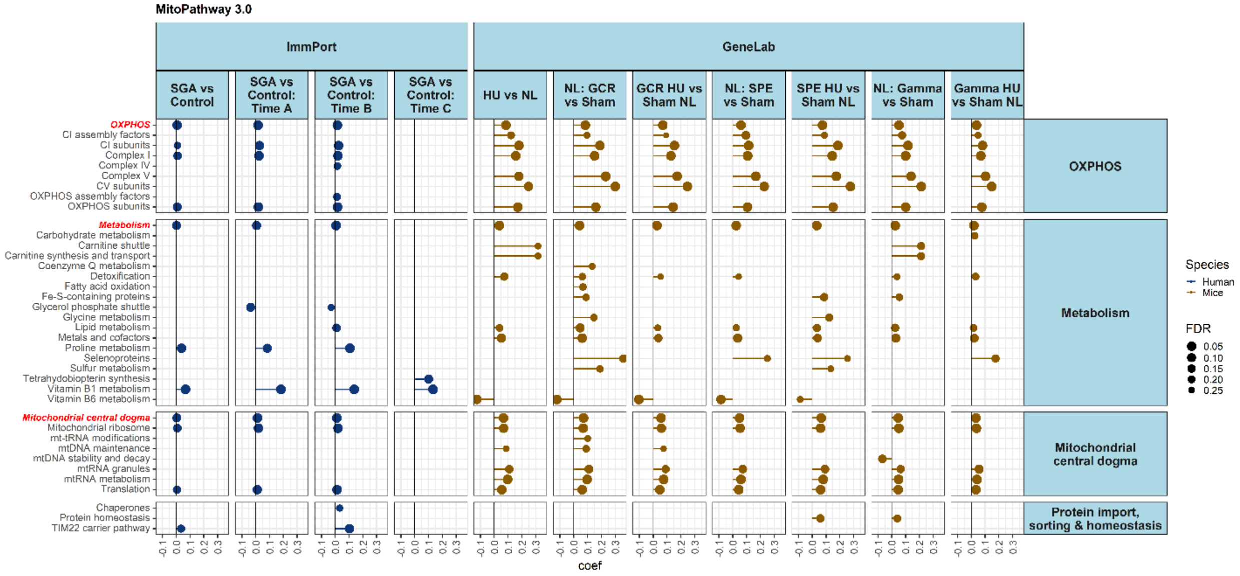

Figure S4. Global miRNA pathway analysis on MitoPathways. Gene set analysis of miRNAs on MitoPathways in Small for Gestational Age (SGA) and simulated spaceflight pathways compared to control. SGA human miRNA regulation is compared to control both time-independently and at different timepoints (left). Space radiation miRNAs with and without simulated microgravity are compared to Sham (right). The x-axis represents a coefficient term indicating pathway inhibition (negative value) or activation (positive value). The point size indicates the degree of significance, denoted by False Discovery Rate (FDR). Only significant values (FDR < 0.25) are displayed.

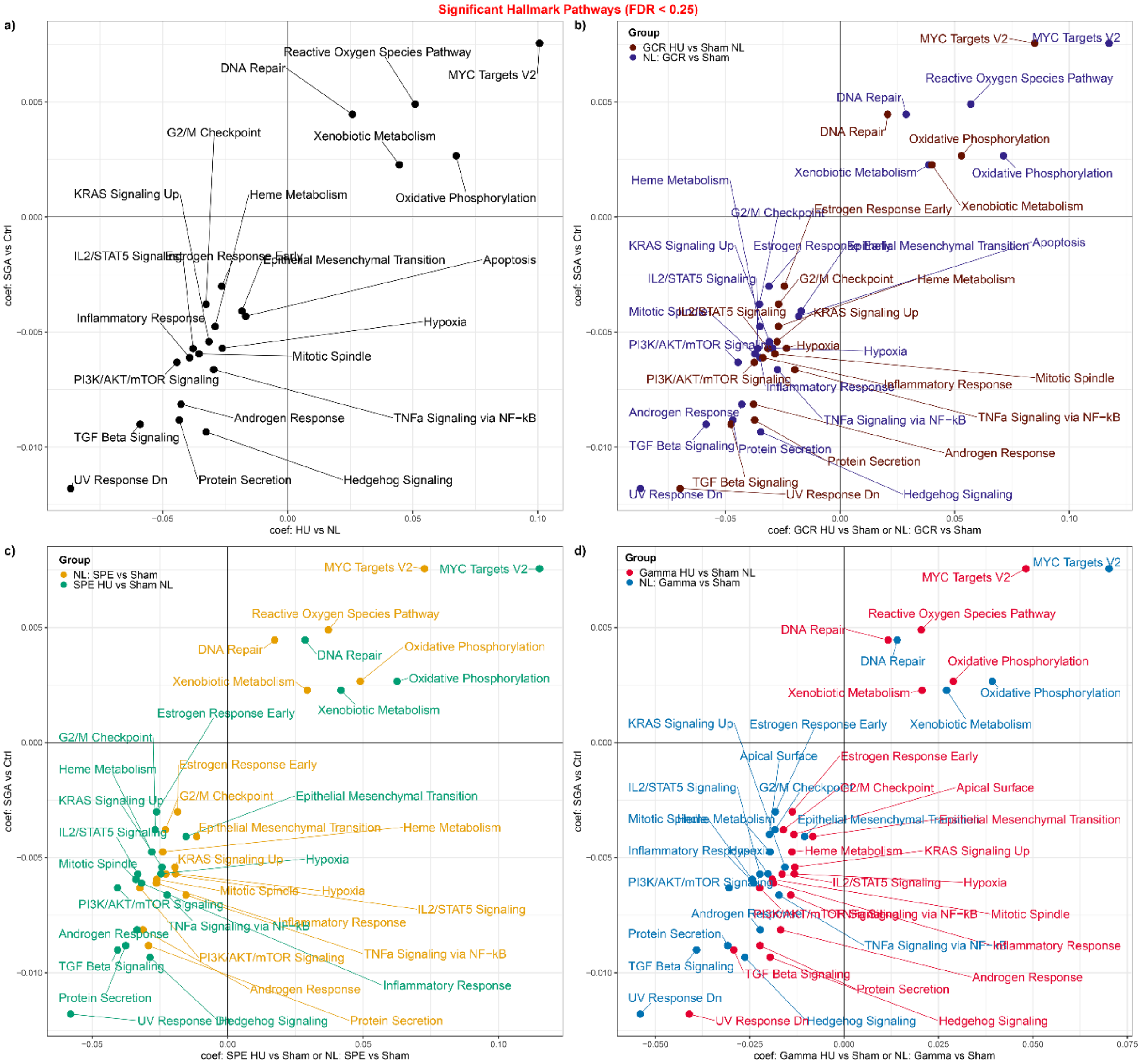

Figure S5. Common Global miRNA pathway analysis on Hallmark pathways. Scatter plot of the common significant gene sets (FDR < 0.25) analysis of miRNAs on Hallmark pathways in Small for Gestational Age (SGA) vs. Controls (y-axis) compared to **a)** Hindlimb Unloading (HU) vs. Normal Loaded (NL), **b)** all GCR conditions vs. Sham NL, **c)** all SPE conditions vs. Sham NL, and **d)** all gamma conditions vs Sham NL.

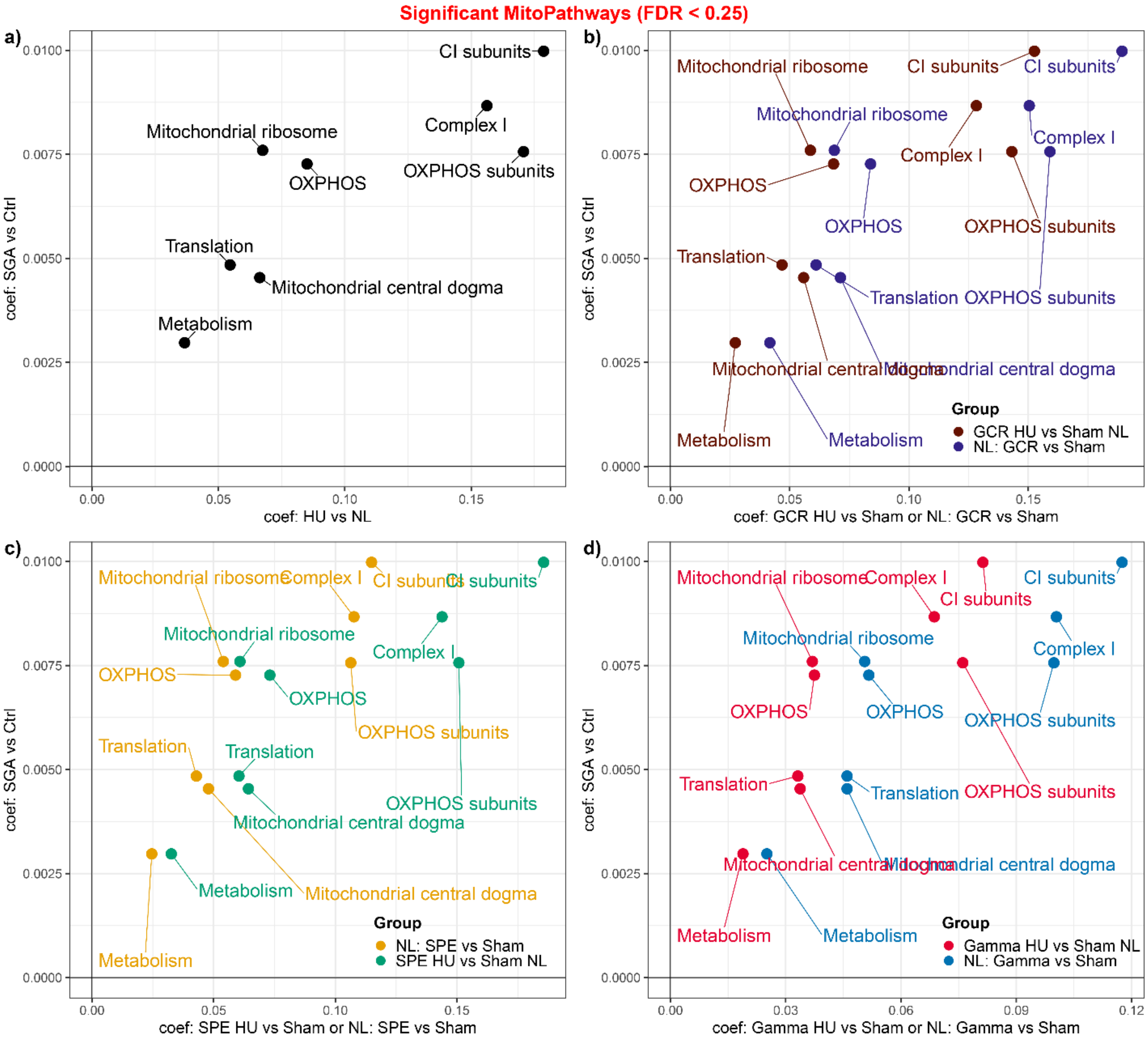

Figure S6. Common Global miRNA pathway analysis on MitoPathways. Scatter plot of the common significant gene sets (FDR < 0.25) analysis of miRNAs on MitoPathways in Small for Gestational Age (SGA) vs. Controls (y-axis) compared to **a)** Hindlimb Unloading (HU) vs. Normal Loaded (NL), **b)** all GCR conditions vs. Sham NL, **c)** all SPE conditions vs. Sham NL, and **d)** all gamma conditions vs Sham NL.

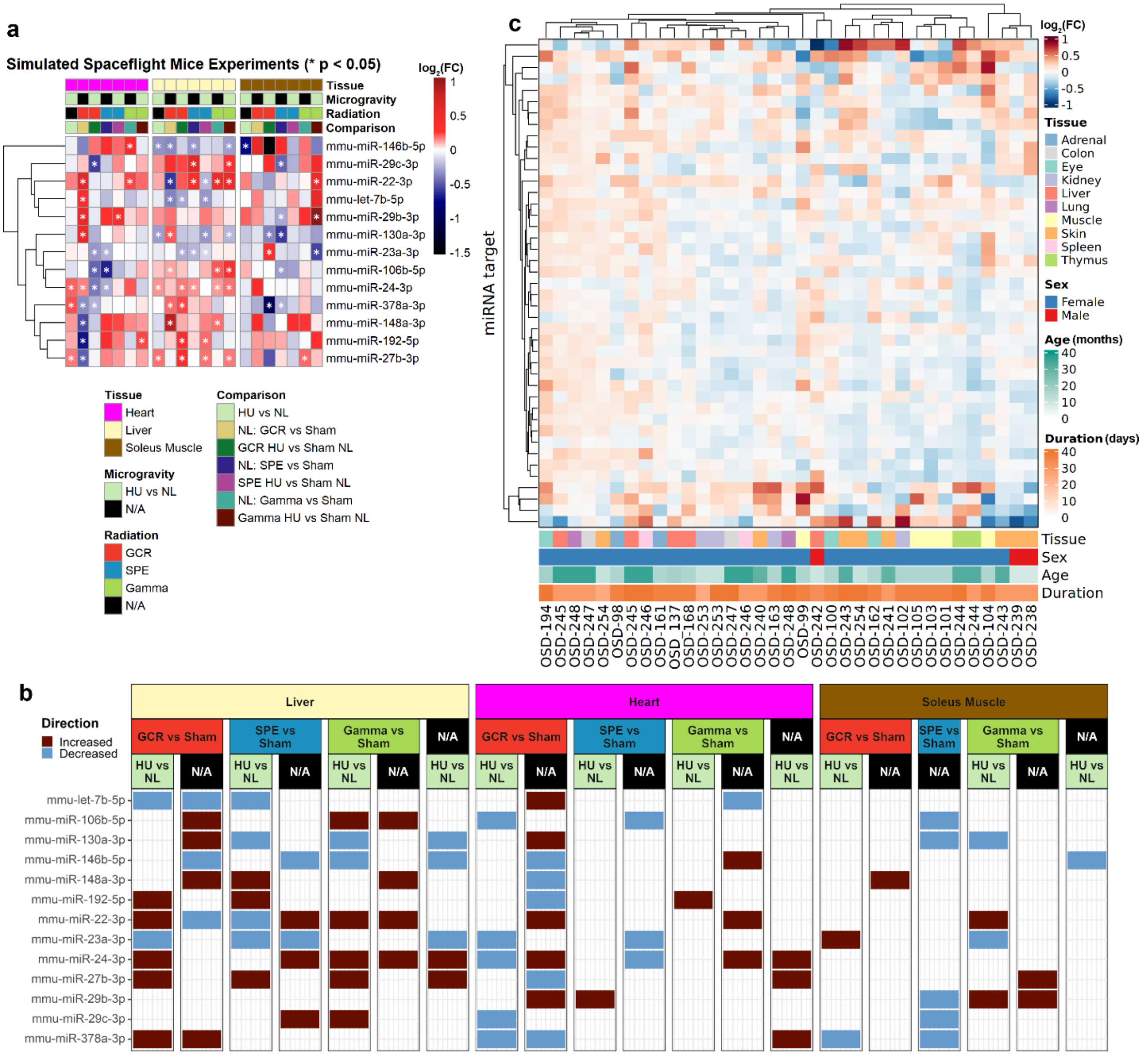

Figure S7. The impact of the 13 miRNAs and top 45 gene targets on other tissues exposed to the space environment. a) Heatmap displaying the $\log_2$(fold change) values for the 13 key miRNAs on heart, liver, and soleus muscle from mice exposed to simulated spaceflight experiments 24 hours after irradiation. For the heatmap, $\log_2$(Fold-Change) is color-coded, with red shades indicating upregulated genes and blue shades indicating downregulated genes. Significance is denoted by * p-value < 0.05. **b)** Bar plot displaying the 13 miRNAs significantly either increased (brick red) or decreased (blue) in expression in the liver, heart, and soleus muscle from mice exposed to the space environment 24 hours after irradiation. **c)** Top 45 gene targets for the 13 miRNAs across various tissues from mice exposed to the microgravity environment of the International Space Station (ISS). Analysis was conducted on the top 45 genes using 35 distinct datasets from NASA's Open Science Data Repository (OSDR), encompassing mice flown to the ISS at varying ages, durations in space, and sexes. The heatmap visually represents the $\log_2$(fold-change) values for the genes, with upregulated genes depicted in shades of red and downregulated genes in shades of blue. Of note, the majority of the mice included in the analysis were female.

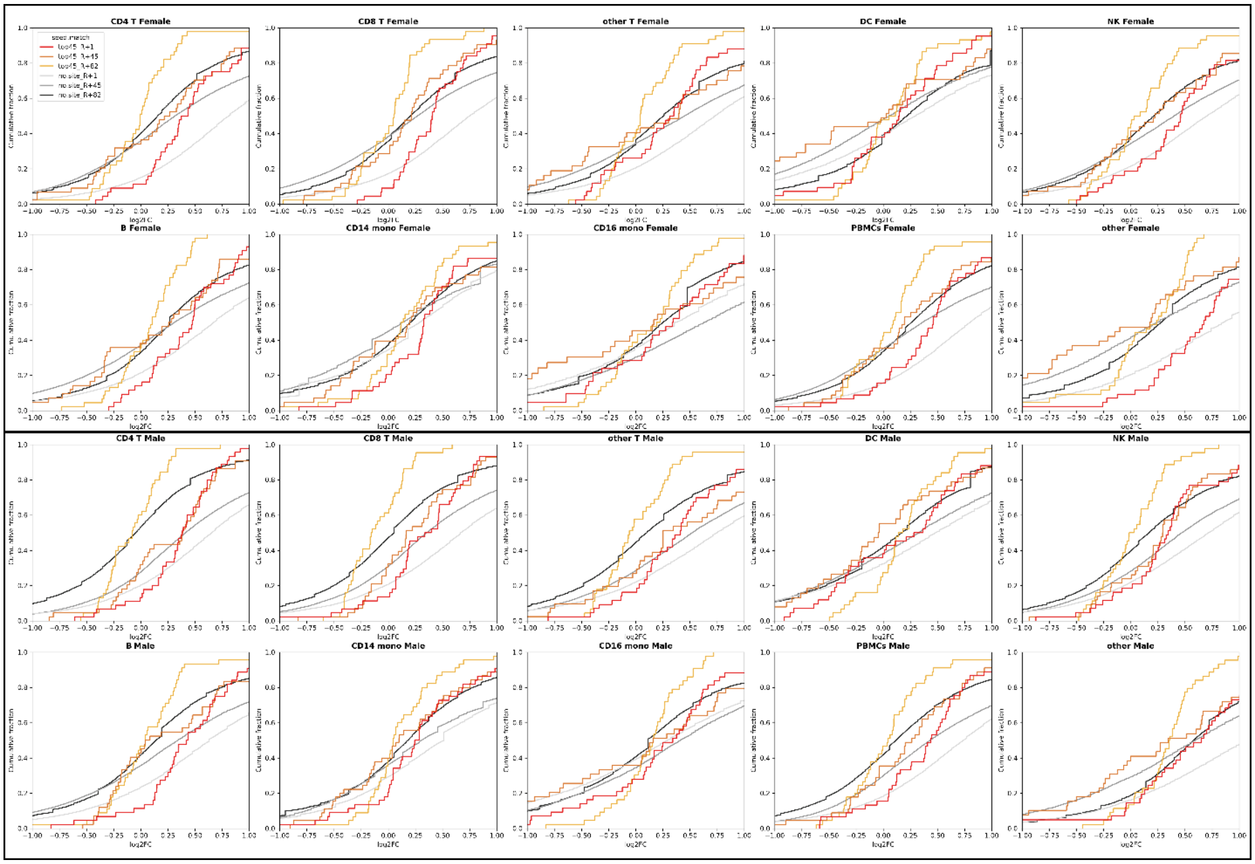

Figure S8. Sex-specific cumulative plots illustrate the impact on the top 45 gene targets of 13 specific miRNAs in Inspiration 4 (I4) astronaut data, derived from scRNA-sequence analysis of whole blood. Cumulative plots for the different cell types from the the I4 astronaut scRNA-seq data, comparing 1 day after return to Earth (R1) (red line), 45 days after return to Earth (R45) (orange line), and 82 days after return to Earth (R82) (gold line) to pre-flight levels. The x-axis represents $\log_2$(fold-change) values for the comparisons, while the "no-site" line serves as a baseline for genes without targets to the 13 miRNAs. Various shades of grey in the no-site lines correspond to specific comparisons, as indicated in the figure legend. The top cumulative plots are specifically for the female astronauts, while the bottom cumulative plots are specifically for the male astronauts

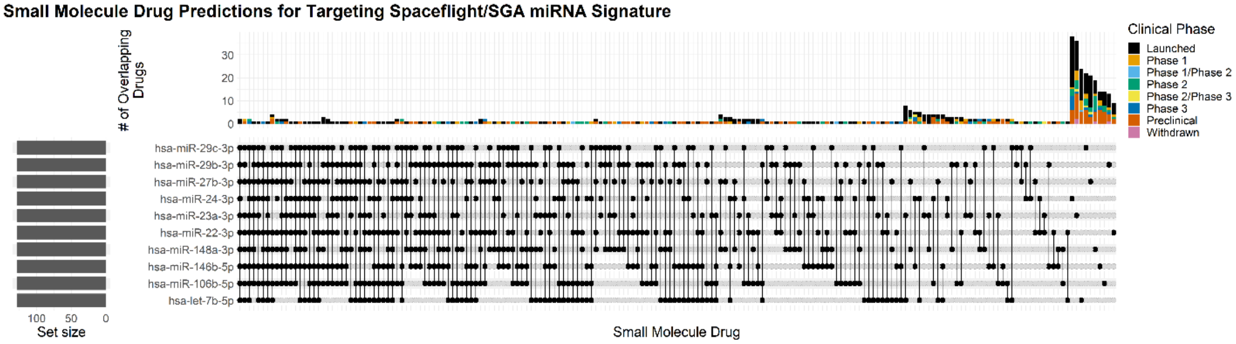

Figure S9. Predicted small molecule drugs for SGA-associated spaceflight miRNA signature. Upset plot revealing specific predicted small molecule drugs that target the miRNA signature associated with Small-for-Gestational-Age (SGA) in spaceflight. The drug names and details can be found in **Table S2**.