



PARTITIONING THE SPACEFLIGHT "AGING" SIGNATURE: WHAT ARTIFICIAL GRAVITY CAN AND CANNOT RESCUE

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Open Access Research Journal of Science and Technology, 2026, 18(01), 172–185

Publication history: Received on 30 August 2026; revised on 15 October 2026; accepted on 17 October 2026

Article DOI: <https://doi.org/10.53022/oarjst.2026.18.1.0096>

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ABSTRACT

Astronauts return from long spaceflights with changes that resemble accelerated aging, but spaceflight combines several stressors - microgravity, radiation, launch, and confinement - so it is normally impossible to say how much of the aging-like signature is caused by the lack of gravity as opposed to the other stressors. This distinction matters because a gravity problem can be engineered away by spinning a spacecraft, while a radiation problem requires shielding or drugs. I used a unique experimental design that separates gravity from everything else: NASA/JAXA on-orbit centrifuge missions in which mice were flown at microgravity (0 g) and artificial 1 g at the same time, sharing identical radiation, launch, and confinement. Re-analyzing public RNA-sequencing data (NASA OSDR), I partitioned the mouse retinal spaceflight signature into a gravity-dependent and a gravity-independent part and estimated the fraction that artificial gravity "rescues." A naive estimate suggested gravity reverses about 75% of the signature, but a permutation test showed that roughly two-thirds of this was an artifact of selecting genes on noisy data; a bias-free estimator placed the true rescue fraction near 57%, though with wide uncertainty owing to small sample sizes. The effect pointed in the same direction across three tissues (retina 57%, thymus 40%, liver 20%). A dose-response analysis indicated that partial gravity provides little benefit - the signal only declines near full 1 g. A validated senescence gene set showed no detectable senescence induction, indicating the signature is not classic cellular aging. Finally, pathway enrichment revealed that the genes rescued by gravity are dominated by the heat-shock and unfolded protein response, identifying the gravity-dependent portion as a protein-folding stress program. These results provide the first quantitative, uncertainty-aware estimate of how much of the spaceflight signature artificial gravity can reverse, and suggest that near-full gravity would be required to capture the benefit.

Keywords: Microgravity; Spaceflight; Aging

1 INTRODUCTION

1.1 Spaceflight produces changes that look like accelerated aging

When astronauts return from long missions, their bodies show changes that resemble what happens to people as they age: bone loss, muscle loss, a weaker immune system, and changes to the heart and blood vessels. These are not only surface-level observations. At the molecular level - which genes are switched on or off, and what proteins and metabolites are present - researchers find signatures that also appear in aging tissue, including mitochondrial stress, chronic inflammation, and DNA damage. The largest single study of this kind analyzed data from 59 astronauts together

with hundreds of samples from NASA's GeneLab database and found that mitochondrial dysfunction sits at the center of the spaceflight response, linked to inflammation and other stress responses throughout the body (da Silveira et al., 2020). The NASA Twins Study, which followed one astronaut through a year on the International Space Station (ISS) while his identical twin remained on Earth, documented changes in telomere length, genome instability, altered eye structure, and shifts in gene expression and DNA methylation (Garrett-Bakelman et al., 2019).

It is worth being careful with the word "aging." The Twins Study found that the astronaut's telomeres actually lengthened during flight and then shortened after return - the opposite of the steady shortening usually associated with aging. So spaceflight *resembles* aging in many ways but is not simply "aging sped up." This distinction motivates the present work: if the spaceflight response is going to be called "aging," it is worth testing how much of it truly behaves like aging and how much is something else.

1.2 The problem: spaceflight bundles several stressors together

The central difficulty in studying the spaceflight response is that spaceflight is not one thing. It exposes the body to several stressors simultaneously. Reviews of the field consistently list microgravity, ionizing radiation from galactic cosmic rays, and the stress of confinement and isolation, among others; NASA groups the major mission hazards into radiation, isolation and confinement, distance from Earth, altered gravity, and hostile closed environments (the "RIDGE" framework). Because these arrive as a package, it is normally impossible to say how much of the aging-like signature is caused by missing gravity versus radiation and the rest.

This matters for a practical reason: the different causes have completely different solutions. If the changes are caused by the lack of gravity, they are, in principle, an engineering problem - a spacecraft or station can be spun to create artificial gravity, and the problem goes away. But if the changes are caused by radiation, spinning does nothing; radiation damage requires shielding or drugs. Before anyone designs a countermeasure, then, it would be valuable to know what fraction of the problem is a gravity problem and what fraction is not.

1.3 A clean way to separate gravity from everything else

One experimental design can separate gravity from the other stressors, and it has already been built. The Japan Aerospace Exploration Agency (JAXA) developed the Multiple Artificial-gravity Research System (MARS), a centrifuge that operates aboard the ISS (Shiba et al., 2017). Mice in the centrifuge are spun so they experience a force equal to Earth's gravity (1 g), while other mice on the same station float in microgravity (roughly 0 g). Crucially, both groups fly at the same time, on the same mission, in the same module, so they receive the same radiation dose, the same launch forces, and the same confinement. The only difference between them is gravity.

Comparing the two flight groups therefore isolates the effect of gravity, and what the two groups share (relative to ground mice) reflects everything that is not gravity. In the first mission using this system, microgravity mice lost femur bone density and leg muscle mass, while mice spun at 1 g retained normal bone density and muscle mass, matching ground mice (Shiba et al., 2017). Since then, the same "compare 0 g and 1 g in orbit" design has shown that artificial gravity partly protects several tissues: skeletal muscle at the level of gene expression (Okada et al., 2021), the thymus, an immune organ (Horie et al., 2019), and the liver, where it partly restored antioxidant metabolism (Kurosawa et al., 2021).

More recently, the MHU-8 mission extended this design from two gravity levels to four. Mice were flown at 0 g, 0.33 g, 0.67 g, and 1 g, and the optic nerve and retina were analyzed by RNA sequencing (Kremsky et al., 2024). Four gravity levels allow a dose-response analysis - testing whether the spaceflight signal shrinks smoothly as gravity increases. Kremsky et al. reported that artificial gravity reduces the transcriptomic response to spaceflight in these tissues in a dose-dependent manner. This MHU-8 eye dataset is the main data source for the present study.

1.4 The gap and the question

Two separate lines of research exist. One asks "does artificial gravity protect tissue X?" and has answered yes for several tissues. The other asks "does spaceflight resemble aging?" and has answered yes in several ways. These lines have not been combined. No one has used the centrifuge design to ask, quantitatively, what fraction of the aging-like signature gravity actually rescues, nor whether the rescued part is truly aging or simply reversible mechanical unloading (tissues not being used) that happens to look like aging.

Kremsky et al. (2024) showed, on the same dataset used here, that artificial gravity attenuates the response in a dose-dependent way. What remained open, and what this study addresses, is (1) quantifying the rescued fraction as a number with an honest measure of uncertainty, (2) testing whether that number survives a proper statistical control, and (3) beginning to distinguish genuinely aging-like changes from reversible mechanical ones - and, ultimately, identifying what kind of biological damage gravity rescues.

1.4.1 Central question

Of the aging-like molecular signature produced by spaceflight in mouse retina, what fraction is gravity-dependent (reversed by the 1 g centrifuge) versus gravity-independent, and how confident can we be in that number given the small samples involved?

1.5 Why this matters

The answer bears on two things. For missions, it indicates whether artificial gravity is worth the enormous engineering cost of a spinning habitat: if most of the damage is gravity-dependent, artificial gravity is a powerful countermeasure; if not, resources are better spent on shielding and drugs. Because the dataset has four gravity levels, it also addresses a sharper question - would *partial* gravity help? The Moon has about 0.17 g and Mars about 0.38 g, so if partial gravity captures most of the benefit, a full spinning habitat may be unnecessary; if only full 1 g helps, partial-gravity environments will not protect people. For aging biology more broadly, separating "true aging" from "reversible mechanical unloading" is a useful distinction, since disuse and aging overlap. Finally, the project is feasible: it uses only public data, runs on a laptop, and requires no laboratory work.

2 METHODS

2.1 Data

I used public RNA-sequencing data from NASA's Open Science Data Repository (OSDR). The main dataset is OSD-758 (Kremesky et al., 2024), from the JAXA MHU-8 mission: mouse retina flown on the ISS at four gravity levels - microgravity (0 g), 0.33 g, 0.67 g, and 1 g - produced by an onboard centrifuge, plus a matched ground control. The mice were male C57BL/6, launched on SpaceX CRS-27 (March 15, 2023) for a 30-day mission. To test whether the findings hold in more than one tissue, I also analyzed the liver (OSD-686; Kurosawa et al., 2021) and the thymus (OSD-289; Horie et al., 2019), two earlier missions that also flew 0 g, 1 g, and ground groups.

Each dataset gives the activity level of every gene in each mouse. I used the gene counts that GeneLab had already processed through its standard pipeline, which keeps processing consistent across studies and avoids introducing errors of my own. Group membership was assigned by cross-referencing two metadata fields, because the gravity label alone is ambiguous (for example, "1G on Earth" denotes a ground control, not a centrifuge mouse). I filtered out genes with very low counts (fewer than ~5 reads in fewer than 3 samples) and converted the values to a log2 counts-per-million scale, which makes fold-changes symmetric and stabilizes variance.

The sample sizes are small, which is normal in spaceflight biology because launching mice is extremely expensive. The retina had 12 ground, 5 microgravity, and 6 mice at each higher gravity level (35 total); the liver and thymus had only about 3 mice per group. These small numbers are the single biggest limitation of the study, and much of the methodology is built to manage the problems they create.

2.2 Separating gravity from everything else

Because the floating and spinning mice flew at the same time, they experienced identical radiation, launch, and confinement - the only difference between them is gravity. For each gene I computed three quantities, all relative to ground control and all on the log scale (so a "difference" is a fold-change):

- **Total (T)** = 0 g flight vs ground - the full spaceflight effect.
- **Residual (R)** = 1 g centrifuge vs ground - everything that is *not* gravity (radiation, launch, confinement). I deliberately call this the "gravity-independent residual" rather than "the radiation effect," because it also contains launch, vibration, and confinement.
- **Gravity (G)** = $T - R$ - the part of the effect that gravity accounts for.

By construction, $T = G + R$. This identity is the engine of the analysis. It holds only when all three comparisons come from the same mission and are processed identically, which is the case here.

The main quantity of interest is the **rescue fraction** ($1 - R/T$): the share of the spaceflight change that gravity reverses. A rescue fraction of 100% means gravity fixes everything; 0% means gravity fixes nothing.

2.3 Measuring the rescue fraction without fooling myself

The obvious way to compute the rescue fraction - select the genes that changed most in flight, then measure how much they recover - is biased. With only a few mice, some genes appear to "change a lot" partly because of random noise, and

that noise does not repeat on a fresh measurement, so those genes appear to "recover" even if gravity did nothing. This is regression to the mean, and at $n = 5$ it can be large. I refer to it as the winner's-curse bias.

To avoid it, I used **Deming regression**, which fits a single line through all genes at once (R vs T) with no gene-selection step, so the bias cannot occur. The slope gives the fraction of the effect that persists at 1 g, and 1 minus the slope is the rescue fraction. I used Deming rather than ordinary regression because T is itself measured with error, and ordinary regression is biased when the x-axis has measurement error; Deming regression corrects for this.

To obtain a confidence interval, I bootstrapped: I resampled the mice (with replacement, within each group) 2,000 times, recomputed the rescue fraction each time, and used the 2.5th-97.5th percentiles as a 95% interval. All random procedures used a fixed seed so the reported numbers are exactly reproducible.

2.4 The permutation test

To measure how much false rescue the naive selection method produces, I used a permutation test. I shuffled the gravity labels among the flight mice only, keeping the ground control fixed, and re-ran the full pipeline. After shuffling, both pseudo-groups still contain the real spaceflight effect (all mice flew, so radiation, launch, and confinement remain), but the difference between 0 g and 1 g is destroyed, so the true rescue fraction is exactly zero by construction. Any rescue the method reports on shuffled data is therefore pure artifact. Repeating the shuffle 500 times builds a null distribution; comparing the observed value to this distribution yields a p-value (the fraction of shuffles producing a rescue at least as large as observed).

2.5 Dose-response

Using all four gravity levels, I measured the size of the spaceflight signal at each level to see whether it declines smoothly as gravity rises. To avoid the selection bias above, I selected genes with an F-test that weights all four levels equally rather than privileging the 0 g group. For each level I drew a "noise floor" by permuting that level's labels against the ground control, showing how much signal appears from noise alone; the interpretable quantity is the signal *above* each level's floor. I then compared a straight-line fit and a saturating (log) fit to determine the shape.

2.6 Aging and senescence

To test whether the spaceflight signature resembles aging, I scored each group using **SenMayo**, a published, validated set of 118 mouse senescence genes (Saul et al., 2022), rather than a few hand-picked markers. As a specificity control, I compared the SenMayo score against 500 random gene sets matched for size and expression level, to check whether any signal exceeded what a random gene set would give.

2.7 Pathway enrichment of rescued genes

Finally, I took the specific genes that gravity rescued (changed in flight and returned toward normal at 1 g) and ran pathway enrichment analysis using Enrichr against the GO Biological Process, MSigDB Hallmark, KEGG, and Reactome libraries. This asks not only *how much* gravity rescues, but *what kind* of damage it rescues.

3 RESULTS

3.1 The naive rescue estimate is high but biased

A first analysis using the naive per-gene method found a median rescue fraction of 75% and a tissue-level rescue of 69% (Figure 1). Taken at face value, this suggests roughly three-quarters of the retinal spaceflight signature is gravity-dependent. However, because the genes were selected on the size of their microgravity effect, this estimate is expected to be inflated by the winner's-curse bias.

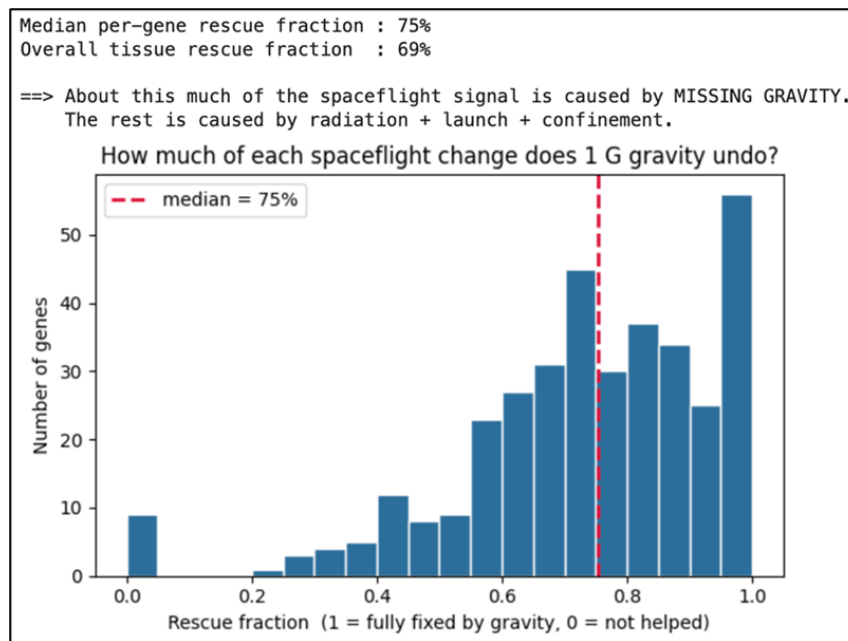


Figure 1: Naive per-gene rescue fraction in mouse retina

For each spaceflight-responsive gene, the rescue fraction ($1 - R/T$) measures how much of the microgravity-induced change is reversed at 1 g, where 1 = fully reversed and 0 = not reversed. The distribution is skewed toward high values, with a median per-gene rescue of 75% and a tissue-level rescue of 69%. Taken at face value this suggests most of the spaceflight signature is gravity-dependent; however, because genes were selected on the size of their microgravity effect, this estimate is inflated by regression to the mean (see Figure 2).

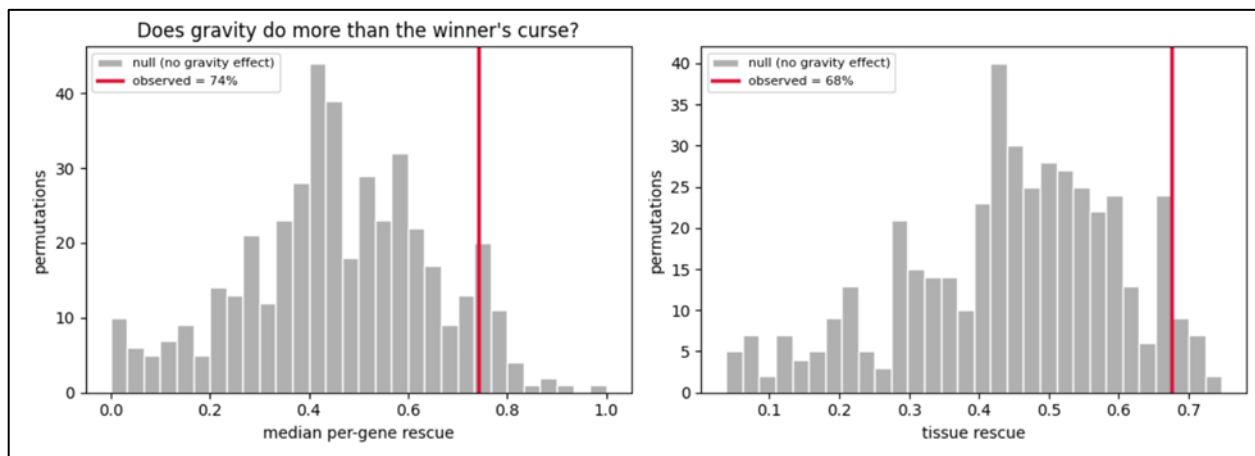


Figure 2: A permutation test shows the naive estimator reports substantial rescue even when gravity has no effect.

Gravity labels were shuffled among flight animals only (ground control fixed) and the full pipeline re-run 500 times; under this shuffle the true rescue fraction is zero by construction, so any reported rescue is an artifact of gene selection. Grey bars show the resulting null distribution; the red line marks the observed value. The null is centered near 45% (median per-gene, left) rather than 0%, and the observed values (74% per-gene; 68% tissue) fall at the upper edge of the null rather than far beyond it (per-gene $p = 0.080$; tissue $p = 0.048$). This indicates that roughly two-thirds of the naive rescue estimate is attributable to selection bias rather than to gravity.

The permutation test confirmed and quantified the inflation (Figure 2). When gravity labels were shuffled so that the true rescue is zero, the pipeline still reported a median rescue near 45%. The observed value (74% by the fast estimator, matching the 75% standard result) sat at the upper edge of this null distribution rather than far beyond it, with $p = 0.080$ (per-gene) and $p = 0.048$ (tissue) - straddling the 0.05 threshold. In other words, roughly two-thirds of the naive rescue was an artifact of gene selection, not an effect of gravity, and the remaining signal is only marginally distinguishable from noise at this sample size.

3.2 The bias-free estimate places rescue near 57%, but with wide uncertainty

Using Deming regression (all genes, no selection, corrected for measurement error in T), the retinal rescue fraction was 57% (Figure 3).

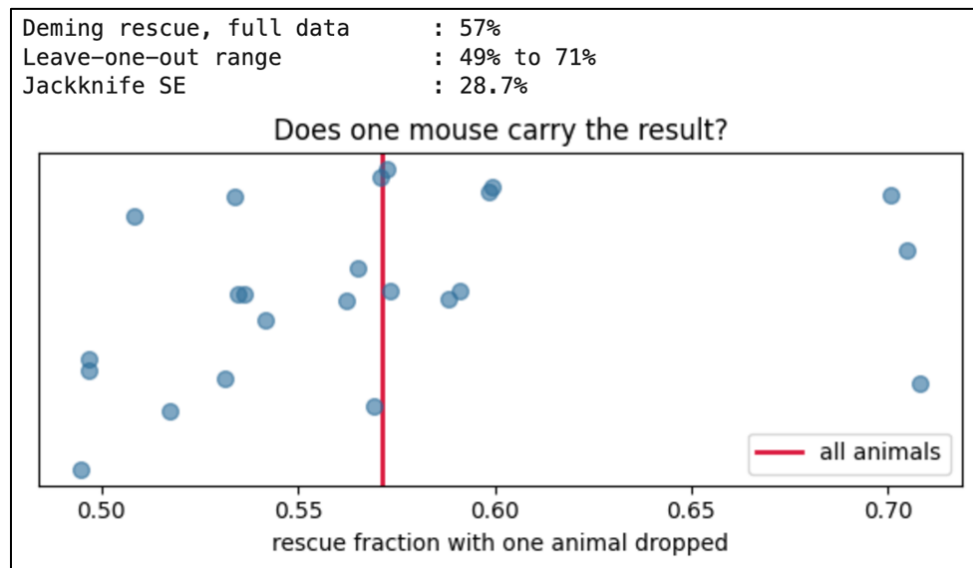


Figure 3: The bias-corrected rescue estimate is lower and imprecise

Using Deming regression across all genes (no gene selection, corrected for measurement error in T), the full-data rescue fraction is 57% (red line). Each point shows the estimate recomputed with one animal removed (leave-one-out jackknife); values range from 49% to 71% with a jackknife standard error of ~29 percentage points. No single animal drives the result, but the wide range reflects the small sample size ($n = 5$ at 0 g) and indicates the estimate should be treated as approximate.

This is the most defensible estimate because it does not involve the selection step that creates the bias. However, the bootstrap 95% confidence interval was very wide (−54% to +95%), and a leave-one-animal-out jackknife ranged from 49% to 71% with a standard error of about 29 percentage points. No single animal drove the result, but the estimate is imprecise: gravity likely reverses a majority of the signature, but the exact amount cannot be pinned down with five mice.

Table 1: Rescue fraction across tissues.

Tissue	Mission	n (GC / 0 g / 1 g)	Rescue fraction	95% CI
Retina	OSD-758	12 / 5 / 6	57%	−48% to 95%
Thymus	OSD-289	3 / 3 / 3	40%	−116% to 65%
Liver	OSD-686	3 / 3 / 3	20%	−61% to 57%

3.3 Partial gravity provides little benefit

The corrected dose-response showed that the spaceflight signal (above the per-level noise floor) stayed essentially flat from 0 g to 0.33 g (0.16 and 0.15) and only fell as gravity approached 1 g (0.10 at 0.67 g; 0.01 at 1 g) (Figure 4).

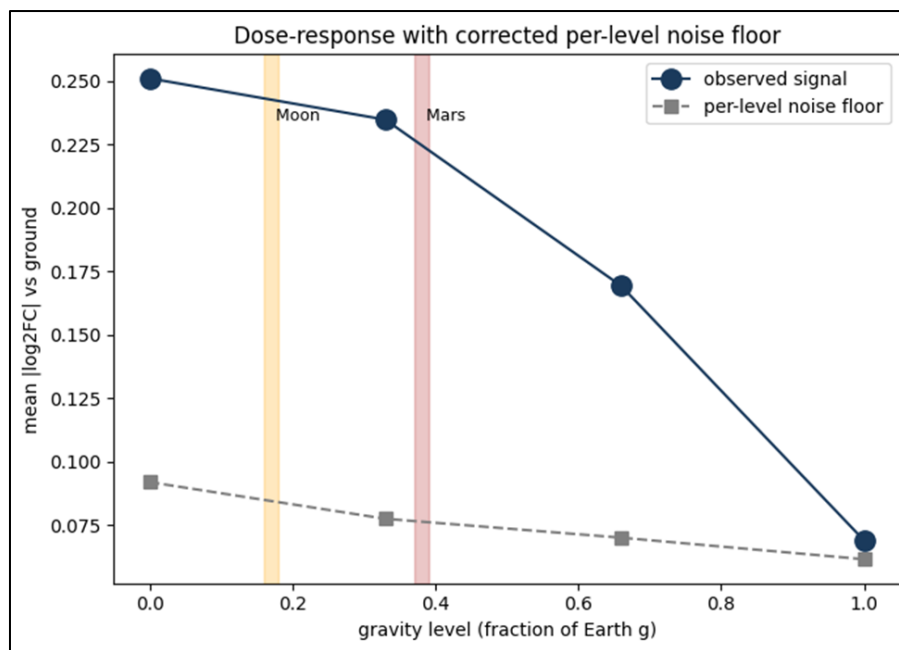


Figure 4: Corrected dose-response: the spaceflight signal declines only near full gravity

For genes selected by an F-test that weights all four gravity levels equally (avoiding the selection bias in Figure 2), the mean absolute log2 fold-change relative to ground control is plotted against gravity level (0, 0.33, 0.67, 1 g; navy). A per-level noise floor (grey, dashed), computed by permuting each level's labels against the ground control, marks the signal expected from noise alone; the interpretable quantity is the signal *above* each level's floor. This above-floor signal stays essentially flat from 0 g to 0.33 g (0.16 and 0.15) and declines only as gravity approaches 1 g (0.10 at 0.67 g; 0.01 at 1 g), where it meets the noise floor. A linear/threshold model fit the data better than a saturating one (linear SSE = 0.0020 vs saturating SSE = 0.0056). Because the Moon (~0.17 g) and Mars (~0.38 g) both fall in the flat region of the curve, this pattern implies that partial gravity would provide little protection and that near-full Earth gravity is required to capture the benefit. This is the opposite of the impression given by the biased preliminary curve (Figure 2), underscoring the importance of the bias correction.

A straight-line/threshold model fit better than a saturating one. Practically, this means partial gravity provides little benefit: the Moon (~0.17 g) and Mars (~0.38 g) both fall in the flat part of the curve, so near-full Earth gravity would be needed to capture the protective effect. This is the opposite of what an earlier, biased version of the dose-response suggested (Figure 5), underscoring the importance of the bias correction.

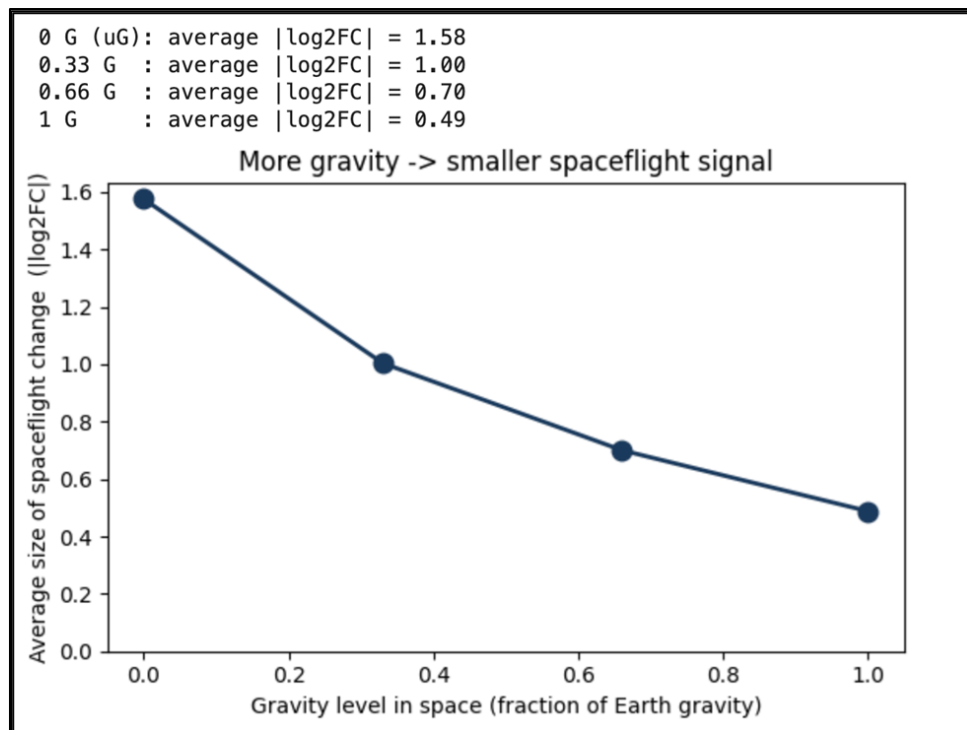


Figure 5: Preliminary dose-response: the spaceflight signal declines as gravity increases, but this version is biased by gene selection.

For genes selected as spaceflight-responsive in microgravity, the mean absolute $\log_2$ fold-change relative to ground control is plotted against gravity level (0, 0.33, 0.66, 1 g). The signal decreases monotonically from 1.58 at 0 g to 0.49 at 1 g, consistent with gravity being causal. However, because genes were selected on their microgravity (0 g) effect, the 0 g point is inflated by regression to the mean, exaggerating the initial drop; the absolute values also cannot reach zero because measurement noise keeps the mean positive. A bias-corrected version, selecting genes with a test that weights all four gravity levels equally and drawing a permutation-based noise floor, is required before interpreting the shape of this curve (e.g., whether partial gravity is protective).

3.4 No detectable senescence signature

Scored with the validated 118-gene SenMayo set (100 genes detected in retina), the senescence signal barely changed in microgravity relative to ground (shift +0.025), and the specificity control gave $p = 0.81$ - meaning the senescence genes moved no more than random gene sets. Because the underlying shift was near zero, a senescence "rescue" could not be meaningfully computed and is not reported. The conclusion is that spaceflight did not induce a clear, classic senescence signature in these tissues. This is consistent with the earlier, hand-picked marker panel (Figure 6), which was mixed and inconclusive, and it supports the caution from the Twins Study that spaceflight resembles aging without being identical to it.

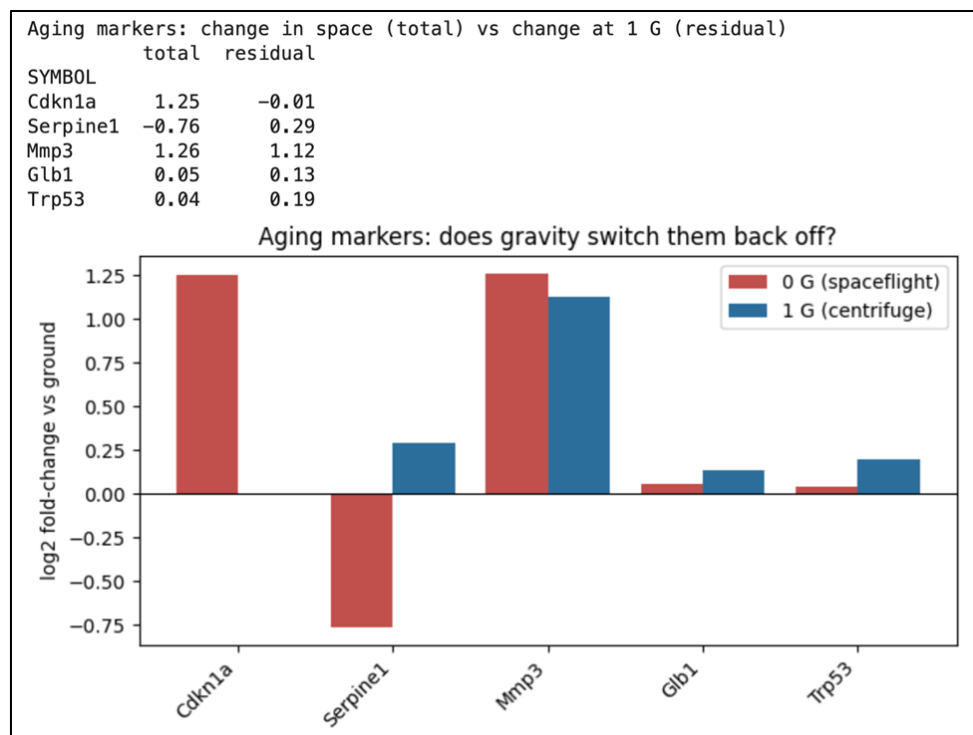


Figure 6: Selected aging and senescence markers show a mixed, inconclusive response

Bars show the log2 fold-change relative to ground control in microgravity (0 g, red) and at 1 g (blue) for five detectable marker genes. The response is not consistent: Cdkn1a is elevated in flight and fully returns to baseline at 1 g (consistent with rescue), whereas Mmp3 is elevated and largely unrescued, and Serpine1 moves opposite to the direction expected for a senescence signal. Two additional markers (Cdkn2a, Il6) were below detection in retina and are not shown. This small, hand-picked panel is insufficient to establish an aging signature and motivates the use of a validated senescence gene set in future work.

3.5 Gravity rescues a protein-folding stress response

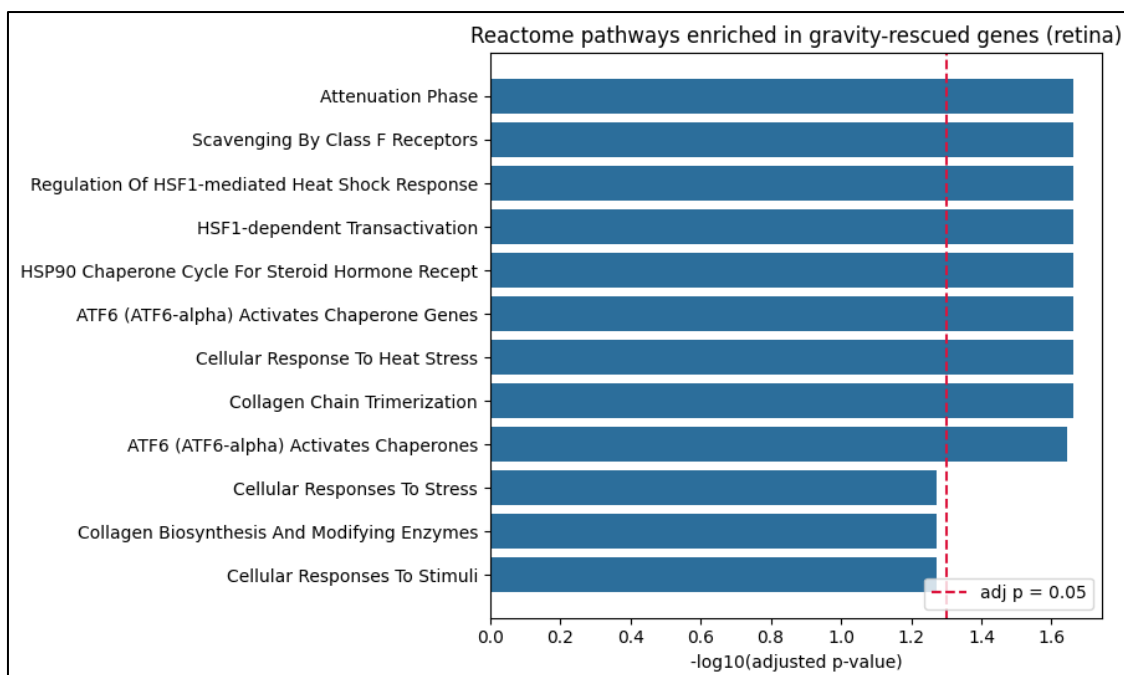


Figure 7: Reactome pathways enriched among gravity-rescued genes (retina).

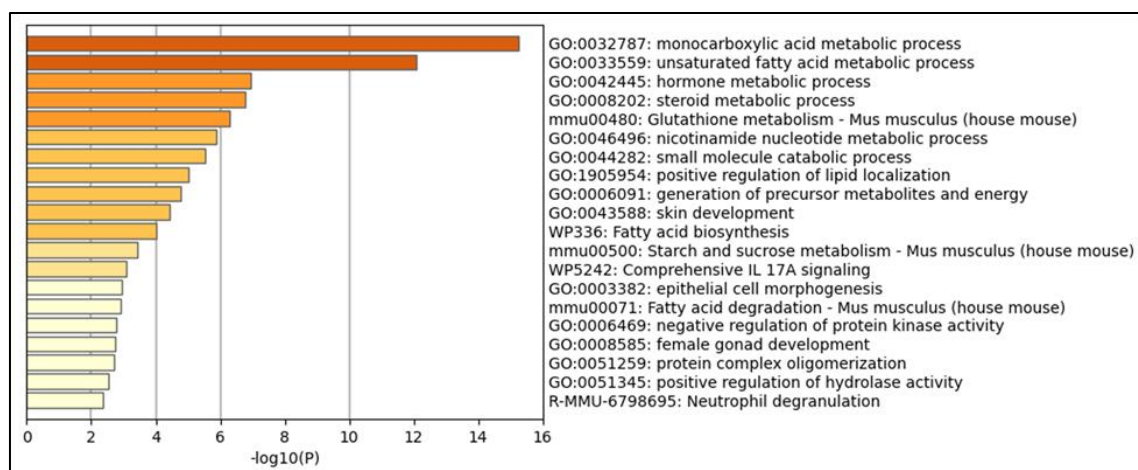
Enrichment analysis (Enrichr) of the same 80 gravity-rescued genes. Bars show $-\log_{10}(\text{adjusted p-value})$; the dashed line marks adjusted $p = 0.05$. Nine pathways are significant, and seven belong to a single biological program: the heat-shock response and endoplasmic-reticulum unfolded protein response (HSF1-mediated heat-shock response, ATF6-mediated chaperone activation, and the HSP90 chaperone cycle; adjusted $p \approx 0.02$), driven by a shared set of heat-shock proteins (Hspa8, Hspa5, Hspa1b, Hsph1). A secondary collagen-biosynthesis signature is also present. Together these results indicate that the gravity-dependent portion of the spaceflight signature is largely a protein-folding stress response.

Pathway enrichment of the 80 genes rescued by gravity gave a clean, consistent result (Figure 7). Seven of the nine significantly enriched Reactome pathways belonged to one biological program: the heat-shock response and endoplasmic-reticulum unfolded protein response - the cell's system for handling misfolded proteins (HSF1-mediated heat-shock response, ATF6-mediated chaperone activation, the HSP90 chaperone cycle; adjusted $p \approx 0.02$). The signal was driven by a shared set of heat-shock proteins (Hspa8, Hspa5, Hspa1b, Hsph1) and recurred across the GO, Hallmark, and Reactome libraries (the Hallmark Unfolded Protein Response reached adjusted $p = 0.002$). A smaller, separate signal involved collagen (extracellular matrix). Thus the part of the spaceflight signature that gravity rescues is largely a protein-folding stress response.

3.6 What gravity rescues is tissue-specific

To test whether the protein-folding rescue seen in retina is universal or tissue-specific, I ran the same enrichment analysis on the gravity-rescued genes from the liver and thymus, using two independent tools (Enrichr and Metascape). The three tissues gave three distinct answers, and in each case the rescued genes matched that tissue's primary physiological function (Figure 8; Table 2). In the **retina**, the rescued genes were dominated by protein folding and the unfolded protein response (heat-shock chaperones Hspa8, Hspa5, Hspa1b, Hsph1; protein folding $P \approx 10^{-8}$). In the **liver**, they were dominated by metabolism and detoxification - monocarboxylic acid and fatty acid metabolism, glutathione metabolism, and xenobiotic processing - driven by a tightly interacting module of cytochrome P450 and glutathione-S-transferase enzymes (Cyp1a2, Cyp2c38/39/68, Gstp1, Gstm6; xenobiotic metabolism $P \approx 10^{-10}$). In the **thymus**, they were dominated by cell division and immune signaling - mitotic cell cycle, chromosome condensation, and chemokine signaling - driven by modules of histones, mitotic cyclins (Cdk1, Ccnb1), and chemokines (Cxcl9, Cxcl12, Ccl5) (cell cycle $P \approx 10^{-20}$).

For every tissue, Enrichr and Metascape produced consistent results, and Metascape's network analysis (MCODE) confirmed that the rescued genes form densely interacting functional modules rather than unrelated sets. Rather than reversing a single universal spaceflight program, artificial gravity therefore appears to restore tissue-specific stress responses, each affecting the tissue's characteristic function. The thymus result is consistent with the known spaceflight-induced thymic atrophy (Horie et al., 2019): gravity may restore the proliferative capacity that microgravity suppresses.



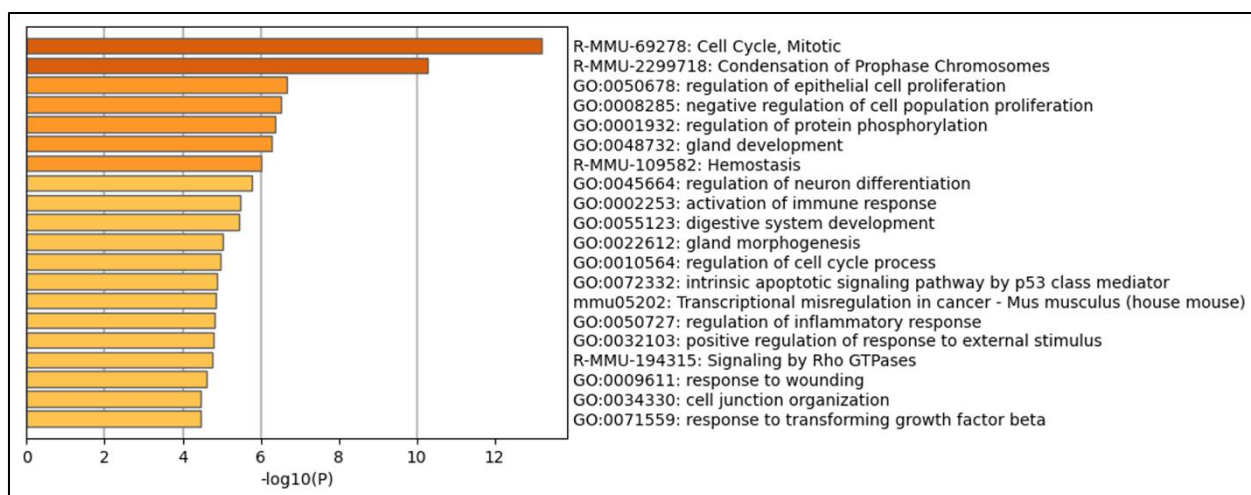


Figure 8: Gravity-rescued genes are tissue-specific and match each tissue's core function

Metascape enrichment of the gravity-rescued genes in liver (top) and thymus (bottom), to be compared with the retina (Figure 7). Liver rescued genes are enriched for metabolic and detoxification processes (monocarboxylic acid and fatty acid metabolism, glutathione metabolism, xenobiotic processing), and Metascape's network analysis identified a densely connected module of cytochrome P450 and glutathione-S-transferase enzymes. Thymus rescued genes are enriched for mitotic cell cycle, chromosome condensation, and chemokine-mediated immune signaling, with network modules of histones, mitotic cyclins (Cdk1, Ccnb1), and chemokines (Cxcl9, Cxcl12, Ccl5). In contrast to the retina's protein-folding signature, each tissue's rescued genes reflect its own primary physiological role. (Note: liver and thymus have only ~3 mice per group, so the gene lists are short; the pathway-level themes are strong and reproducible across tools, but individual genes should be interpreted cautiously.)

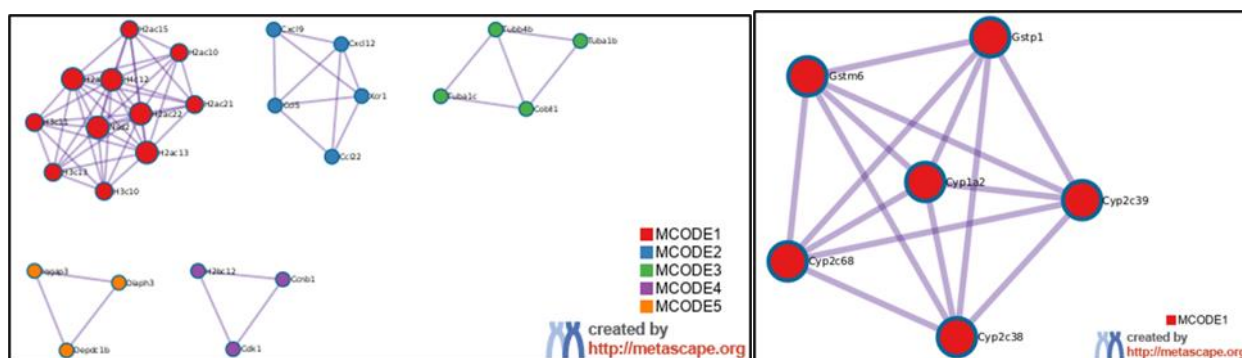


Figure 9: Metascape MCODE network modules for liver and thymus rescued genes.

Nodes are genes; edges are known protein-protein interactions. The liver (top) forms one detoxification module (cytochrome P450 and glutathione-S-transferase enzymes). The thymus (bottom) forms five modules dominated by cell division (histones; tubulins; the Cdk1-Ccnb1 mitotic engine) and immune signaling (chemokines), consistent with the thymus's role as a site of immune-cell proliferation.

Table 2: Gravity-rescued programs are tissue-specific.

Tissue	Dominant rescued program	Key genes / modules	Top enrichment
Retina	Protein folding / unfolded protein response	Hspa8, Hspa5, Hspa1b, Hsph1, Calr	$P \approx 10^{-8}$
Liver	Metabolism / xenobiotic detoxification	Cyp1a2, Cyp2c38/39/68, Gstp1, Gstm6	$P \approx 10^{-10}$
Thymus	Cell division + immune signaling	histones, Cdk1, Ccnb1, Cxcl9/12, Ccl5	$P \approx 10^{-20}$

4 DISCUSSION

4.1 What the results show

This study set out to answer a concrete question: of the aging-like damage that spaceflight causes, how much is due to the lack of gravity? Three findings emerge. First, artificial gravity appears to reverse a substantial share of the retinal spaceflight signature - best estimated near 57%, and positive across three tissues - though the small samples make every individual estimate imprecise. Second, this benefit is captured only near full gravity; partial gravity does little. Third, the rescued damage is specifically a protein-folding stress response, not a classic senescence program.

The strongest part of the finding is not any single number but the way the pieces align. The rescue fraction was positive in all three tissues; the dose-response and the two-group comparison agree that gravity is causal; the pathway analysis points to one coherent biological program; and that program - proteostasis stress - is the kind of thing plausibly tied to mechanical state. Together these make a more convincing case than any one result alone.

An unexpected and informative result is that the gravity-rescued signature is strongly tissue-specific. I had anticipated that if artificial gravity reverses a general "spaceflight aging" program, the same pathways would recur across tissues. Instead, each tissue rescued a different program, and in every case that program matched the tissue's primary function: proteostasis in the light-sensing retina, metabolism and detoxification in the liver, and cell proliferation and immune signaling in the thymus. This suggests that spaceflight does not impose one uniform molecular insult that gravity reverses; rather, microgravity stresses each tissue along its own most characteristic axis, and gravity restores that tissue-specific function. This interpretation is strengthened by the agreement between two independent enrichment tools (Enrichr and Metascape) for every tissue, and by Metascape's network analysis showing that the rescued genes form coherent, physically interacting modules rather than scattered hits. It is important to note the limitation that the liver and thymus each had only three mice per group; while the pathway-level signals were strong and reproducible, the short gene lists mean individual-gene claims should be treated with caution. As with the retina, these analyses identify *what* gravity rescues in each tissue, not *why* microgravity produces that specific stress.

4.2 The importance of the bias correction

A central methodological result is the gap between the naive estimate (75%) and the corrected one (57%), and the size of the artifact the permutation test revealed (a 45% null). Roughly two-thirds of the naive headline was selection artifact rather than gravity. This is a cautionary lesson for the field: the intuitive way to compute a rescue fraction is badly biased at spaceflight sample sizes, and this bias inflates the answer substantially. Because $n \approx 5$ is the norm in this field and this permutation check is rarely run, similar inflation may be present, unmeasured, in other analyses. The permutation test and the selection-free Deming estimator together provide a template for guarding against it.

4.3 Relation to existing literature

The results are consistent in direction with Kremisky et al. (2024), who reported dose-dependent attenuation of the transcriptomic response in these tissues, and with the tissue-protection findings from earlier centrifuge missions (Shiba et al., 2017; Horie et al., 2019; Kurosawa et al., 2021; Okada et al., 2021). The present contribution is to add two things those studies lack: a single quantitative rescue number with an honest uncertainty, and an explicit statistical test of how much of the naive estimate is real. It also connects the centrifuge literature to the aging framing of the Twins Study (Garrett-Bakelman et al., 2019) and the multi-omics work of da Silveira et al. (2020) - while showing, through the SenMayo result, that the retinal signature is not classic senescence. The proteostasis finding is coherent with the broader picture of spaceflight as a cellular-stress state and with Kremisky et al.'s emphasis on stress responses in the same tissue.

4.4 Practical implications

Two findings have practical weight. First, the dose-response implies that partial gravity would not protect much: near-full Earth gravity appears necessary, so Moon or Mars surface gravity, or a slowly spinning habitat, may be insufficient. This is a concrete, if preliminary, input to mission design. Second, identifying the rescued damage as a protein-folding stress response gives a molecular target and suggests the gravity-dependent portion of the response is a reversible stress state rather than permanent damage - an encouraging possibility, though one that needs direct confirmation.

Limitations

First, the samples are very small (5 mice at 0 g in retina; 3 per group in liver and thymus), so every estimate is imprecise and every confidence interval crosses zero; the argument rests on convergence across analyses and tissues, not on any single significant number. Second, the tissues come from a small number of missions with limited replication, and the liver and thymus are from different missions than the retina, introducing possible batch differences. Third, the 1 g centrifuge is not identical to Earth gravity: the small radius produces a gravity gradient across the animal and additional

forces (Coriolis, vibration) absent on Earth, so a nonzero residual may partly reflect these rather than radiation. Fourth, the residual is a mixture of radiation, launch, vibration, and confinement and cannot be attributed to radiation specifically without further analysis. Fifth, and importantly, the enrichment result identifies *what* gravity rescues (a proteostasis stress program) but not *why*; claiming that weightlessness causes this stress through a specific mechanism would exceed what the data show.

4.5 Future work

Several steps would strengthen the result. Combining tissues formally, and adding more, would help overcome the small samples. Attaching a permutation-based p-value directly to the Deming estimate would give the primary number its own significance test. Most importantly, comparing the gravity-dependent signature (G) and the gravity-independent residual (R) against reference "fingerprints" for mechanical disuse and for radiation would test directly whether the rescuable part is reversible mechanical unloading and whether the residual is radiation damage - the original question in its sharpest form. Repeating the proteostasis enrichment across tissues would show whether the protein-folding signature is general or retina-specific.

5 CONCLUSION

Using a design that isolates gravity from the other stressors of spaceflight, this study provides the first quantitative, uncertainty-aware estimate of how much of the spaceflight transcriptomic signature artificial gravity can reverse. The best estimate is that gravity rescues a little over half of the retinal signature, with the same direction seen across three tissues; that partial gravity is probably insufficient to capture the benefit; and that the rescued damage is specifically a protein-folding stress response rather than classic cellular aging. Just as important, the study shows that the intuitive way of estimating rescue is badly biased at spaceflight sample sizes, and provides methods to correct for it. Together these results turn a qualitative observation - that artificial gravity helps - into a quantified, honestly bounded, and mechanistically specific finding.

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

Acknowledgments

Thank you to Professor Ahmet Ay of Colgate University for the mentorship. I am deeply grateful for your help and guidance throughout the process.

Disclosure of conflict of interest

The authors declare no conflicts of interest regarding this manuscript.

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