

Gravitational and mechanical forces shape mitochondrial translation

Corresponding Author: Dr Shintaro Iwasaki

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

Eukaryotic cells contain two translation machineries, one in the cytosol and one in the mitochondrial matrix. In the current study, the authors investigated the effect of microgravity on cellular function and found that these conditions particularly affect mitochondrial activities. Following this up, they present evidence that microgravity reduces protein synthesis in mitochondria of HEK293 and C2C12 cells as well as in nematodes. Moreover, they showed a correlation with high levels of malonylated mitochondrial ribosome subunits under these conditions and the reduced translation rates. Finally, they present evidence that signaling by the RAC1 pathway influences mitochondrial translation via an effect on BCL2 family proteins and BAD. Even though the causal interactions are not always clear here, this is an interesting and somewhat surprising study. While cells normally will hardly encounter microgravity conditions in space, these observations suggest that the tension of the plasma membrane influences mitochondrial activity within cells. This might stimulate mitochondrial activity in selective mitochondria in cells, for example in growth zones. This points towards a novel concept of mitochondrial regulation. The study is generally of high quality and the experiments show the relevant controls – even if the evidence for some claims is rather thin. Still, this is an interesting study which fits well into the scope of Nature Comm.

Some minor aspects should be considered:

1. Mitochondrial translation can be analyzed *in vivo* (upon inhibition of cytosolic translation) or *in organello* (i.e. in isolated mitochondria) upon addition of 35S methionine. Both techniques are straight forward and require very little cellular material (particular the *in vivo* labeling). For this study, it would be important to show both: protein synthesis in cells and in isolated mitochondria. In organello, the mitochondria are incubated in high concentrations of ATP, amino acids and other reagents so that external metabolic conditions can be excluded as confounding factors for mitochondrial translation.
2. The translation products should be run out on SDS gels and presented. This would allow to see the synthesis of the 13 different mitochondrially encoded proteins. From the presented bar graph, it is not clear whether there is a general defect (e.g. via effects on the mitochondrial ribosome) or a specific one on a subset of transcripts. There are some protein patterns shown in the supplement, but no bands are labeled and controls are missing. This certainly needs to be improved.
3. On page 13 bottom, organello is miss-spelled
4. Loading controls should be added to Fig. 4C. Also a non-mitochondrial loading control should be added to Fig. 4G.
5. The experiment with the reconstituted system of mitochondrial translation (Fig. 5K) lacks controls. The data fit the proposed model, however, this single experiment is not particular strong. It also is unclear of whether the malonylation under these conditions is physiological. Presumably it is not. This part of the study is the weakest.
6. The mitochondrial transcripts shown in Fig. 6B are strongly affected. However, some go up and others down. Why is this? Does this correlate with translation? Again, the authors should show radiolabeled translation products in these cells.

Reviewer #2

(Remarks to the Author)

Wakigawa et al claim that microgravity, both in space and under simulated conditions on Earth, reduces mitochondrial translation. They propose a mechanism involving the FAK–RAC1–PAK1–BAD–Bcl-2 signaling axis, suggesting that cell adhesion, responding to mechanical and gravitational forces, activates mitochondrial fatty acid synthesis (mtFAS). This

mtFAS activation is proposed to lower mitochondrial malonyl-CoA levels, reduce demalonylation of translation-related proteins, and thereby enhance mitochondrial translation at both the initiation and elongation stages.

Major Comments:

- 1) The authors claim that alterations in mitochondrial translation occur at the L1 larvae samples. However, the ribo-seq, RNA-seq and translational efficiency data in Fig. S1L do not show significant changes in mitochondrial mRNAs. The lack of pronounced whole-body level effects reduces the significance of this study.
- 2) The manuscript proposes a signaling pathway—from FAK, RAC1, and PAK1 to BAD and Bcl-2, and subsequently to mtFAS—that influences mitochondrial translation. This pathway appears to be assembled from known signaling pathway modules. There is insufficient evidence that these components specifically transmit gravitational or mechanical forces to the mitochondrial translation machinery. In particular, the involvement of the Bcl-2 family, long associated with apoptosis, in translation regulation is not convincingly supported. The authors need to provide stronger evidence that these key nodes selectively regulate translation rather than representing a nonspecific stress or survival response.
- 3) The manuscript notes a substantial number of downregulated/upregulated cytoplasmic mRNAs, but provides insufficient analysis or discussion regarding their functional implications and potential relevance to the observed mitochondrial translation changes. Further examination of these cytoplasmic transcript alterations would help clarify their relationship to the proposed signaling pathway and overall translational dynamics under microgravity conditions.
- (4) The title of the manuscript is not supported by the data.

Minor Comments:

- 1) At the cellular level, mitochondrial translation inhibition is more pronounced after 24 hours in microgravity, while adaptation occurs by 48 hours. The manuscript should more explicitly differentiate between these two time points. For instance, in the final paragraph on page 6, when emphasizing translation reduction, it should be stated clearly that this is observed at 24 hours. Also, Figure S1D should not be cited in this context since it shows recovery at 48 hours.
- 2) In the last paragraph on page 6, the authors state: "The reduction in mitoribosome footprints could not be explained by a reduction in the corresponding mRNAs... Thus, the translation efficiency... still showed a decrease." To improve logical flow, replacing "Thus" with "Because" would make the reasoning clearer.
- 3) In Figures S1C and S1K, the authors should clarify how the Pearson's correlation coefficients were calculated. The footprints in the two replicates in Fig. S1I differ in distribution, yet they yield a correlation coefficient of 0.996. Additionally, in Fig. S1K (right panel), the microgravity condition replicates show a correlation of only 0.641, indicating weaker reproducibility. The authors should provide further explanation of these results.
- 4) The raw data underlying numerous plots presented in the manuscript are not provided in the supplementary files, limiting the transparency and reproducibility of the results. The authors should include these primary datasets to allow for independent verification and more in-depth analysis.

Reviewer #3

(Remarks to the Author)

Summary:

Overall, this study is methodologically sound and provides a novel and important alternative perspective on how mitochondrial function is regulated in space. While previous work, such as the multi-omics study published in *Cell*, highlighted mitochondrial stress and DNA damage as central features of spaceflight conditions, the current findings suggest that changes in mitochondrial translation are not merely a byproduct of damage. Instead, they point toward a regulated, integrin- and ECM-dependent signaling process that influences mtFAS and mitochondrial translation. This is a significant step beyond the damage-centric narrative, offering a mechanistic framework for how cells adapt their energy production under altered gravitational forces. I recommend accepting the paper on the condition that all data are deposited in a DOI-issuing, FAIR-compliant repository (e.g., NASA OSDR) and properly cited in the manuscript. Ensuring full data availability will facilitate replication and further exploration of these findings. Improving data visualization, adding temporal and contextual details to the figures, and refining certain mechanistic interpretations would further strengthen the study. Overall, this work is timely, relevant, and substantially contributes to our understanding of cellular adaptation in spaceflight conditions, complementing and extending the insights provided by previous large-scale omics studies.

Overall comments regarding data requirements and citations:

Reviewer could not review the study in detail as the data were not available. They were deposited in GEO, GSE222998 and GSE237154, but the data were kept private. GEO is not a fully FAIR-compliant repository and primarily serves as an archival database with limited metadata and no specialized ontology control for space-related samples. Given that these data pertain to Space Omics, they should be deposited in a specialized database, such as NASA GeneLab (genelab.nasa.gov), which is designed to handle rich metadata and controlled vocabularies, thereby reducing ambiguity in interpretation. Providing DOIs for these datasets is essential for proper FAIR compliance and long-term reusability. Currently, GEO typically doesn't assign DOIs, making reproducibility and proper citation more challenging. The authors should consider depositing the data in a repository that issues DOIs and then cite these datasets directly in the manuscript, not just the published articles. When authors use already published data—such as that from samples published in Higashitani et al. (2021)—they need to provide a DOI for the dataset, not just for the manuscript. For example, the mentioned dataset can be found in OSDR with DOI: [Higashibata A. "The effect of microgravity on the epigenetic modification in *C. elegans* - JAXA EPIGENETICS spaceflight experiment", NASA Open Science Data Repository, Version 6, <http://doi.org/10.26030/1rkt-t974>]. Referring to a DOI ensures that readers can access the exact dataset used for the study, adhering to open science principles and fostering reproducibility.

More details:

Figures 1C and 1E:

There is an inconsistency in data representation between human cell data (Figure 1C) and *C. elegans* data (Figure 1E). In Figure 1C, box plots are used, while Figure 1E employs cumulative distributions. The rationale for changing the metric isn't clear. The authors should maintain a consistent method for data presentation across species unless there is a strong justification. A direct comparison would be easier if the same visualization method were applied. Additionally, including a theoretical baseline curve for translation efficiency in the cumulative distribution plot would help interpret shifts in translation relative to a "no change" scenario. It is also important to clarify whether nuclear-encoded mRNAs shift similarly in these distributions, as it would put mitochondrial translation changes into better context. The subtle difference in response observed in *C. elegans* might be due to the protective effects of the native extracellular matrix (ECM) environment in the organism, as opposed to isolated cell cultures.

Figure 2:

When referring to Figure 2D in the text, please mention the specific time points (24 hours and the subsequent 24-hour recovery) explicitly in the main body, not just in the figure legend. Consider adding the 48- and 72-hour time points to Figure 2C (currently shown in Figure S2Q-R) to provide a clearer timeline of adaptation. This would help confirm whether the log2 fold changes in ribosome profiling converge to a steady state around -1, indicating a new equilibrium rather than a transient response. The idea of adaptation raised in the text implies that after an initial overshoot or strong response, cells reach a stable adjusted condition. Showing these additional time points can reinforce the notion that the mitochondrial translation changes persist over time and are not merely transient.

The data from 10G experiments (Figures 2F) also raise interesting points. While one might expect cells adapted to 1G to respond similarly to deviations in either direction, the finding that higher g-forces further enhance translation suggests a graded relationship between gravity and mitochondrial translation. Re-plotting Figures 2E-F-G with gravity as a continuous variable on the X-axis and mitochondrial translation levels on the Y-axis might provide a visual representation of this relationship. This would offer new insights into the gravity-translation curve, contributing to our understanding of gravitational influence on cellular metabolism.

Figure 3:

The findings in Section 3 are convincing. However, in Figure 3, Figure 3G is shown before Figure 3F, which can be confusing.

Cristae Formation Timing (related to Figure 5 and OPA1):

The discussion around OPA1 and cristae formation is interesting. The authors conclude that the increase in cristae is a result of enhanced mitochondrial translation rather than the cause. One would expect a time lag between laminin addition (which stimulates translation) and the subsequent formation of cristae structures. It would be helpful to examine time-course data to support this causal relationship. Even a simple time-series experiment showing cristae morphology evolving hours after translation changes would strengthen the argument that cristae formation is indeed downstream of increased mitochondrial translation activity.

Overall Study and Model Systems:

It is unfortunate the authors did not use 3D organoids for cell culture, as these can be grown in suspension and would serve as a better model for simulated microgravity in a clinostat. Clinostat creates a lot of sheer stress on adherent cells and could explain why adaptation was not observed. The response in mice was minimal and only apparent after 14 days, and the *C. elegans* response was also modest, which suggests the presence of the tissue matrix confer some protection.

In the discussion, the reference to mitochondrial damage in space needs clarification. There appears to be a citation error, as citation #3 is repeated. The authors state: "Our results closely aligned with the multiomics approach utilizing data from NASA's GeneLab platform³," but it should be referencing ⁷ and ⁸. In reference #3 (da Silveira et al., 2020), the authors reported disruption and lower metabolic activity of mitochondria, which aligns with the results shown here. The authors should be more specific when referring to this work and provide a deeper interpretation. They present an alternate hypothesis to the simple ROS-driven disruption previously reported, which is an important and novel insight from the submitted work here.

Another element that could strengthen the discussion is the fact that cosmic radiation has been shown to alter the ECM in breast and skin tissues (Costes et al., Radiation research 154 (4), 389-397). Considering that space conditions involve both cosmic radiation (affecting the basement membrane) and microgravity (reducing ECM interaction), the combined effects could be substantial. It would be interesting for the authors to consider a follow-up study using breast based organoids exposed to simulated cosmic radiation and/or simulated microgravity to investigate these combined stressors.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

The authors satisfactorily addressed all points raised on the previous version. I fully support publication of this study in its present form.

(Remarks on code availability)

Reviewer #2

(Remarks to the Author)

The authors have addressed my concerns. I support the publication of the manuscript.

(Remarks on code availability)

Reviewer #3

(Remarks to the Author)

I have carefully reviewed the resubmission, and the authors have addressed all of my comments. Thank you for a substantially improved manuscript.

(Remarks on code availability)

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Reviewer #1

Eukaryotic cells contain two translation machineries, one in the cytosol and one in the mitochondrial matrix. In the current study, the authors investigated the effect of microgravity on cellular function and found that these conditions particularly affect mitochondrial activities. Following this up, they present evidence that microgravity reduces protein synthesis in mitochondria of HEK293 and C2C12 cells as well as in nematodes. Moreover, they showed a correlation with high levels of malonylated mitochondrial ribosome subunits under these conditions and the reduced translation rates. Finally, they present evidence that signaling by the RAC1 pathway influences mitochondrial translation via an effect on BCL2 family proteins and BAD. Even though the causal interactions are not always clear here, this is an interesting and somewhat surprising study. While cells normally will hardly encounter microgravity conditions in space, these observations suggest that the tension of the plasma membrane influences mitochondrial activity within cells. This might stimulate mitochondrial activity in selective mitochondria in cells, for example in growth zones. This points towards a novel concept of mitochondrial regulation.

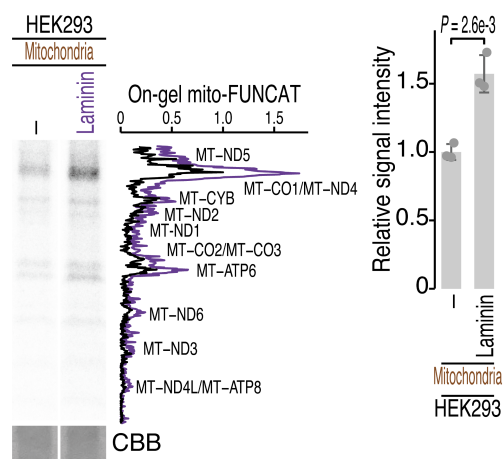
The study is generally of high quality and the experiments show the relevant controls – even if the evidence for some claims is rather thin. Still, this is an interesting study which fits well into the scope of Nature Comm.

We thank Reviewer #1 for their careful reading and positive comments.

Some minor aspects should be considered:

1. Mitochondrial translation can be analyzed in vivo (upon inhibition of cytosolic translation) or in organello (i.e. in isolated mitochondria) upon addition of ³⁵S-methionine. Both techniques are straight forward and require very little cellular material (particular the in vivo labeling). For this study, it would be important to show both: protein synthesis in cells and in isolated mitochondria. In organello, the mitochondria are incubated in high concentrations of ATP, amino acids and other reagents so that external metabolic conditions can be excluded as confounding factors for mitochondrial translation.

According to Reviewer #1's suggestion, we performed ³⁵S-methionine-mediated metabolic labeling to assess proteins synthesized in mitochondria, particularly under laminin treatment (Figure S3J–K; see also the figure below). Consistent with our HPG-based on-gel mito-FUNCAT results, these experiments demonstrated activation of mitochondrial translation.

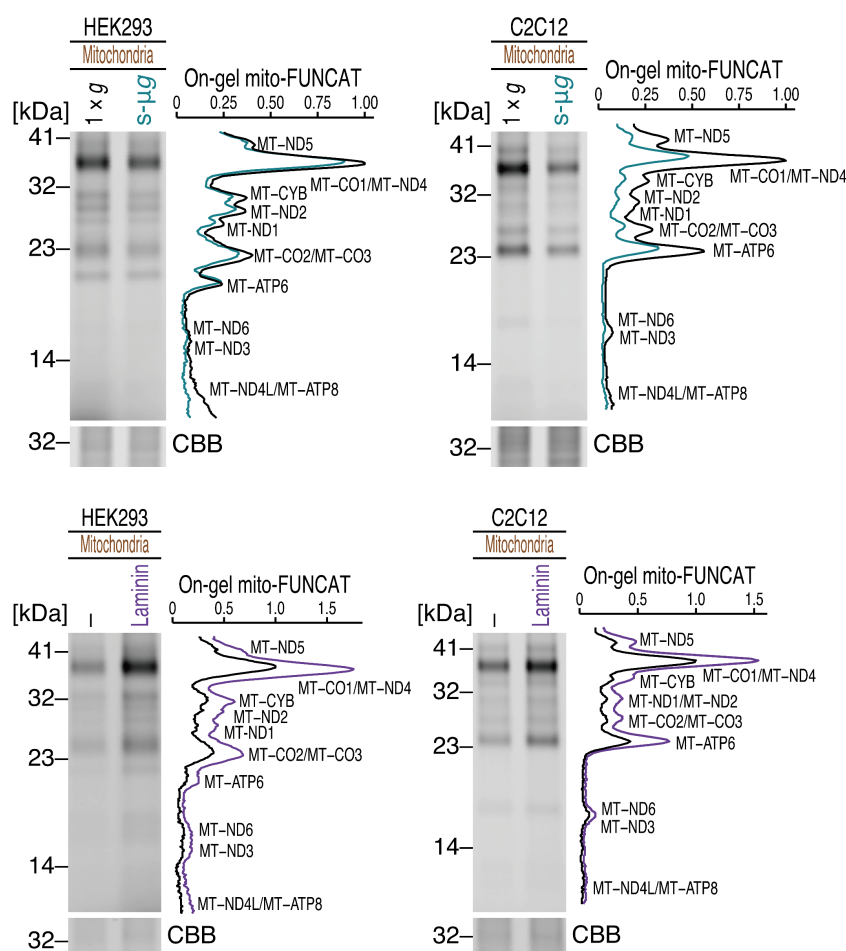


We agree that performing analogous experiments under simulated microgravity conditions would be ideal. However, due to strict regulations on radioisotope use in Japan, it is technically difficult to bring a 3D clinostat into the restricted facilities authorized for radioisotope experiments. These constraints make it technically infeasible to perform ^{35}S Met labeling under simulated microgravity conditions.

Experiments using purified organelles require milligram-scale quantities of mitochondria per reaction (Cruz-Zaragoza *et al.*, *Cell* 2021 DOI: 10.1016/j.cell.2021.09.033), corresponding to approximately 130×10^6 cells. In contrast, the disposable cultivation chamber (DCC, developed by JAXA and Chiyoda Corporation; Tanigawa *et al.*, *Biological Sciences in Space* 2014 DOI: 10.2187/bss.28.1), which is used for simulated microgravity experiments, accommodates only $2\text{--}2.5 \times 10^6$ cells (15.5 cm^2). Achieving this number of cells from DCC-based cultures under simulated microgravity is currently not technically feasible. We therefore appreciate Reviewer #1's understanding of these practical limitations. However, our ribosome profiling approach provided a complementary or even more quantitative/comprehensive assessment of mitochondrial protein synthesis, dissecting the net effect on translation over RNA abundance changes.

2. The translation products should be run out on SDS gels and presented. This would allow to see the synthesis of the 13 different mitochondrially encoded proteins. From the presented bar graph, it is not clear whether there is a general defect (e.g. via effects on the mitochondrial ribosome) or a specific one on a subset of transcripts. There are some protein patterns shown in the supplement, but no bands are labeled and controls are missing. This certainly needs to be improved.

According to Reviewer #1's suggestion, we assigned the 13 mitochondrial proteins on the gel and measured the signal intensities along the lanes (Figures S2H and S3I; see also the figure below). Generally, the synthesis of all the proteins was decreased.



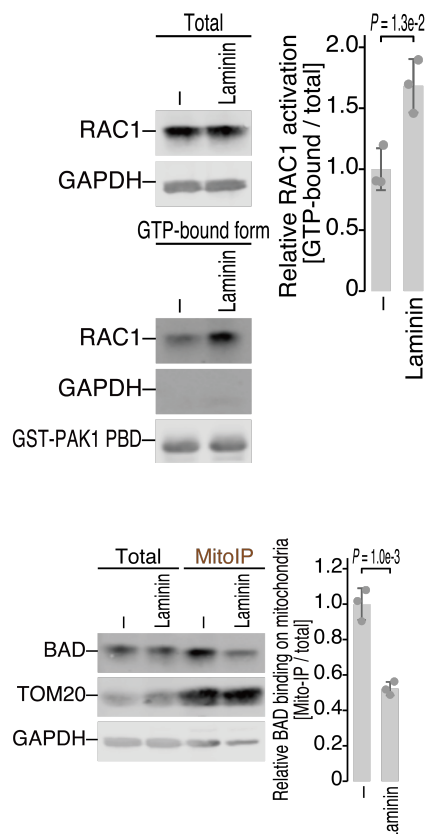
Congruent with this, our ribosome profiling-based methods showed the limited mRNA specificity in the mitochondrial translation control in (simulated) microgravity and in the laminin treatment (Figures 1B, 1C, 2B, 2C, 3E, S1D, and S2B).

3. On page 13 bottom, organello is miss-spelled

We thank Reviewer #1 for pointing this out. We amended this part accordingly.

4. Loading controls should be added to Fig. 4C. Also a non-mitochondrial loading control should be added to Fig. 4G.

According to Reviewer #1's suggestion, we added Western blots for the additional controls for Figure 4C and 4G. We note that the isolation of the GTP-bound form of RAC1 in Figure 4C depends on the co-pulldown assay with GST-fused PAK1 PBD (p21-binding domain), which only binds to the GTP-bound RAC1. Thus, the GST-PAK1 PBD was detected as a loading control.



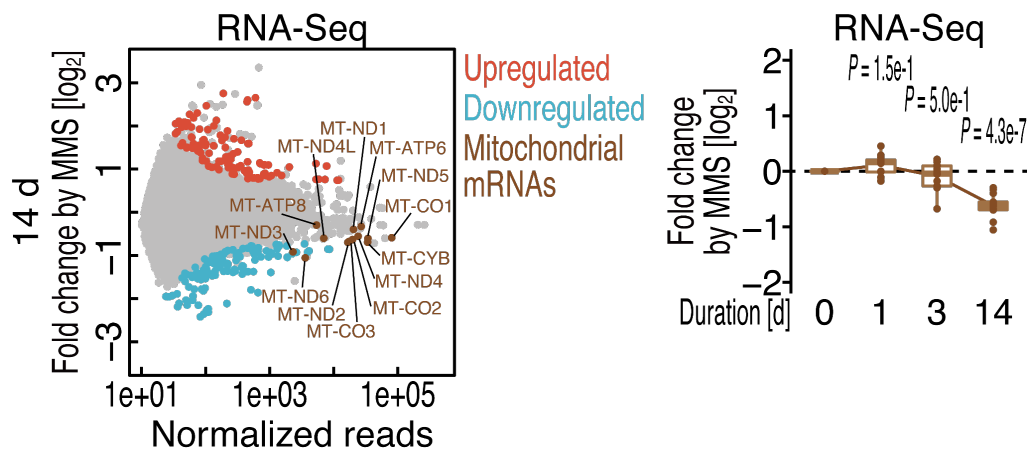
5. The experiment with the reconstituted system of mitochondrial translation (Fig. 5K) lacks controls. The data fit the proposed model, however, this single experiment is not particularly strong. It also is unclear of whether the malonylation under these conditions is physiological. Presumably it is not. This part of the study is the weakest.

We thank Reviewer #1 for pointing this out. As a control, we used acetyl-CoA, which may also lead to non-enzymatic acetylation of lysine. Nonetheless, we agree that, generally, biochemical assays should be interpreted carefully. Although millimolar order acetyl-CoA/malonyl-CoA, as used in this experiment, has been suggested in mitochondria, we added the caution of this data interpretation in the “Limitations of the study” section.

6. The mitochondrial transcripts shown in Fig. 6B are strongly affected. However, some go up and others down. Why is this? Does this correlate with translation? Again, the authors should show radiolabeled translation products in these cells.

We appreciate Reviewer #1’s constructive suggestion. However, we consider that these points are not pertinent to the corresponding data.

In Figure 6B and 6C (see also the figure below, brown points), mitochondrial transcripts were reduced. However, this trend was general across mitochondrial transcripts; we could not find any mitochondrial transcripts with upregulation at 14 d of MMS.



Also, these experiments were conducted in the mouse tissues. Technically, it is impossible to perform ^{35}S -Met (or HPG)-mediated metabolic labeling of newly synthesized proteins, which requires the depletion of Met from the condition, in mouse tissues.

Reviewer #2

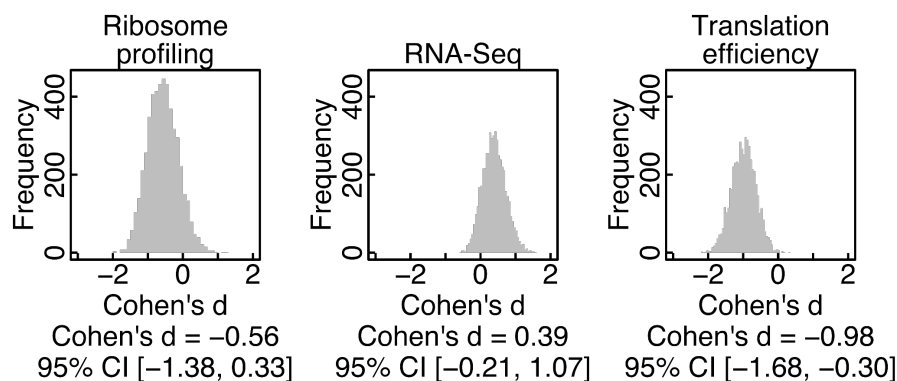
Wakigawa et al claim that microgravity, both in space and under simulated conditions on Earth, reduces mitochondrial translation. They propose a mechanism involving the FAK–RAC1–PAK1–BAD–Bcl-2 signaling axis, suggesting that cell adhesion, responding to mechanical and gravitational forces, activates mitochondrial fatty acid synthesis (mtFAS). This mtFAS activation is proposed to lower mitochondrial malonyl-CoA levels, reduce demalonylation of translation-related proteins, and thereby enhance mitochondrial translation at both the initiation and elongation stages.

We thank Reviewer #2 for their careful reading of our manuscript.

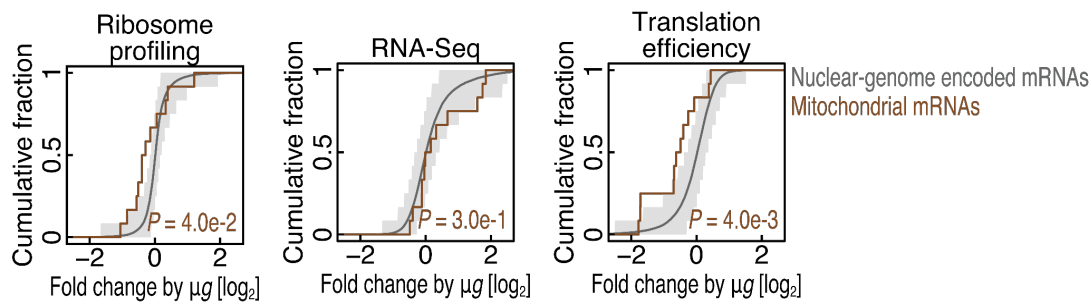
Major Comments:

1) The authors claim that alterations in mitochondrial translation occur at the L1 larvae samples. However, the ribo-seq, RNA-seq and translational efficiency data in Fig. S1L do not show significant changes in mitochondrial mRNAs. The lack of pronounced whole-body level effects reduces the significance of this study.

We thank Reviewer #2 for pointing this out. Although the translation efficiency changes of individual mitochondrial mRNAs did not reach the statistical threshold, mitochondrial mRNAs as a group exhibited significant changes. This group-level effect is supported by the effect size, quantified using Cohen's d , comparing cytoplasmic (nuclear genome–encoded) mRNAs and mitochondrial (mitochondrial genome–encoded) mRNAs. Statistical robustness was assessed by bootstrap analysis with 5,000 resampling iterations (see figure below).



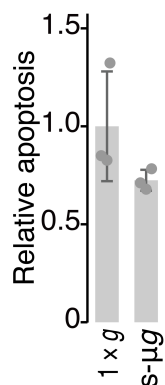
Similarly, according to the suggestion of Reviewer #3, we conducted a random sampling of 12 genes, which correspond to mitochondrial encoded ORFs in *C. elegans*, from the dataset, repeated 1000 times, and drew the range from 2.5 percentile to 97.5 percentile (shaded area in the figure below and Figure 1E). Mitochondrial mRNAs showed lower translation efficiency than this theoretical range, indicating further statistical evidence of reduced mitochondrial translation.



Both analyses supported the statistically significant translation changes of mitochondrial mRNAs at the group level. Nonetheless, we clarified the lack of statistical significance of individual mRNAs in the manuscript and toned down our claim.

2) The manuscript proposes a signaling pathway—from FAK, RAC1, and PAK1 to BAD and Bcl-2, and subsequently to mtFAS—that influences mitochondrial translation. This pathway appears to be assembled from known signaling pathway modules. There is insufficient evidence that these components specifically transmit gravitational or mechanical forces to the mitochondrial translation machinery. In particular, the involvement of the Bcl-2 family, long associated with apoptosis, in translation regulation is not convincingly supported. The authors need to provide stronger evidence that these key nodes selectively regulate translation rather than representing a nonspecific stress or survival response.

To address the point raised by Reviewer #2, we measured the apoptosis level (probing Annexin V binding to apoptotic cells) under simulated gravity, which reduced mitochondrial translation through the inhibition of the pathway of FAK-RAC1-PAK1-BAD-Bcl-2 family proteins. Indeed, the simulated gravity did not induce apoptosis (Figure S7N; see also the figure below). Thus, mitochondrial translation responses observed in this study were not associated with a general cell survival response. We added the discussion on this point in the manuscript.



Notably, the experiments using BAX activators/inhibitors (Figure S7J-M) also supported that the mitochondrial translation regulation is not dependent on the canonical apoptosis

pathway (at least, from the downstream of Bcl-2 family proteins).

3) The manuscript notes a substantial number of downregulated/upregulated cytoplasmic mRNAs, but provides insufficient analysis or discussion regarding their functional implications and potential relevance to the observed mitochondrial translation changes. Further examination of these cytoplasmic transcript alterations would help clarify their relationship to the proposed signaling pathway and overall translational dynamics under microgravity conditions.

According to Reviewer #2's suggestion, we added paragraphs to discuss alterations in the cytoplasmic mRNA as follows.

“Ultimately, the unbalanced subunit supply from the mitochondria matrix and cytoplasm for the OXPHOS complex (Figure S1F and S1M) may reduce ATP production of mitochondria and ultimately provide stress to the organelle. Although the cytosolic flux of OXPHOS subunits was not largely affected under microgravity in human cells (Figure S1F, left) and *C. elegans* (Figure S1M, left), whole body analysis of *C. elegans* showed a reduction at the RNA level (Figure S1M, middle), indicating compensation by translation efficiency (Figure S1M, right). Because this phenomenon was not observed in cultured cells (Figure S1F, middle and right), the complex response to microgravity may exist at the tissue level or may be specific to certain species.

Although this study mainly focused on mitochondrial mRNAs, we also observed alterations in cytoplasmic mRNAs under microgravity in ISS. While attenuated mRNA expression of actin filament-related genes was often found in the space experiments (Hwang et al. 2023; Wubshet et al. 2024; Di Filippo et al. 2024; Moreno-Villanueva et al. 2024), our analysis instead showed the reduction of mRNA related to intermediate filaments, another cytoskeleton (Figure S1E). This decreased RNA expression may explain, at least partially, the looser structure of intermediate filaments observed during microgravity (Wuest 2025).”

We avoided discussing the data in simulated microgravity since the low correspondence to microgravity in ribosome profiling/RNA-Seq/translation efficiency changes (Figure S2T).

(4) The title of the manuscript is not supported by the data.

According to Reviewer #2's suggestion, we modified the manuscript title to tone down.

Minor Comments:

1) At the cellular level, mitochondrial translation inhibition is more pronounced after 24 hours in microgravity, while adaptation occurs by 48 hours. The manuscript should more explicitly differentiate between these two time points. For instance, in the final paragraph on page 6, when emphasizing translation reduction, it should be stated clearly that this is observed at 24 hours. Also, Figure S1D should not be cited in this context since it shows recovery at 48 hours.

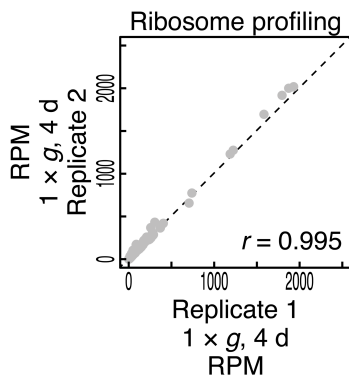
We thank Reviewer #2 for the constructive suggestion. We modified our text accordingly.

2) In the last paragraph on page 6, the authors state: “The reduction in mitoribosome footprints could not be explained by a reduction in the corresponding mRNAs... Thus, the translation efficiency... still showed a decrease.” To improve logical flow, replacing “Thus” with “Because” would make the reasoning clearer.

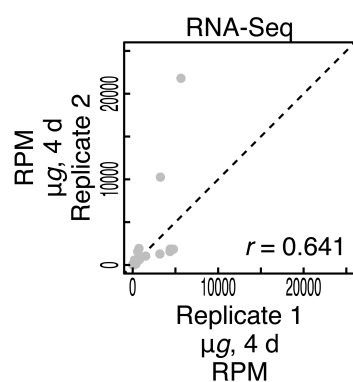
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We thank Reviewer #2 for pointing this out. Despite the difference in 1-nt trimming of the footprints (Figure S1I), read counting in each ORF was not perturbed, thus leading to the high reproducibility (see the figure below).



Regarding the RNA-Seq (Figure S1K), the moderate correlation may stem from the variability of mRNAs with exceptionally high expression (see the figure below). Generally, nearly all the mRNAs showed good reproducibility. We note that our data analysis by DESeq2 considered this variability in replicates and calculated the significance of the changes.



4) The raw data underlying numerous plots presented in the manuscript are not provided in the supplementary files, limiting the transparency and reproducibility of the results. The authors should include these primary datasets to allow for independent verification and more in-depth analysis.

According to Reviewer #2's suggestion, we also provided the Source Data files, following the journal's regulations.

Reviewer #3

Summary:

Overall, this study is methodologically sound and provides a novel and important alternative perspective on how mitochondrial function is regulated in space. While previous work, such as the multi-omics study published in *Cell*, highlighted mitochondrial stress and DNA damage as central features of spaceflight conditions, the current findings suggest that changes in mitochondrial translation are not merely a byproduct of damage. Instead, they point toward a regulated, integrin- and ECM-dependent signaling process that influences mtFAS and mitochondrial translation. This is a significant step beyond the damage-centric narrative, offering a mechanistic framework for how cells adapt their energy production under altered gravitational forces. I recommend accepting the paper on the condition that all data are deposited in a DOI-issuing, FAIR-compliant repository (e.g., NASA OSDR) and properly cited in the manuscript. Ensuring full data availability will facilitate replication and further exploration of these findings. Improving data visualization, adding temporal and contextual details to the figures, and refining certain mechanistic interpretations would further strengthen the study. Overall, this work is timely, relevant, and substantially contributes to our understanding of cellular adaptation in spaceflight conditions, complementing and extending the insights provided by previous large-scale omics studies.

We thank Reviewer #3 for their careful reading and positive comments.

Overall comments regarding data requirements and citations:

Reviewer could not review the study in detail as the data were not available. They were deposited in GEO, GSE222998 and GSE237154, but the data were kept private. GEO is not a fully FAIR-compliant repository and primarily serves as an archival database with limited metadata and no specialized ontology control for space-related samples. Given that these data pertain to Space Omics, they should be deposited in a specialized database, such as NASA GeneLab (genelab.nasa.gov), which is designed to handle rich metadata and controlled vocabularies, thereby reducing ambiguity in interpretation. Providing DOIs for these datasets is essential for proper FAIR compliance and long-term reusability. Currently, GEO typically doesn't assign DOIs, making reproducibility and proper citation more challenging. The authors should consider depositing the data in a repository that issues DOIs and then cite these datasets directly in the manuscript, not just the published articles.

We thank Reviewer #3 for constructive suggestions. We changed the status of the GEO data to be publicly available. Moreover, we deposited those deep sequencing data to the NASA Open Science Data Repository (OSDR) (OSD-936, DOI: 10.26030/gm17-vt61) (under the link for reviewers: <https://osdr.nasa.gov/bio/repo/data/studies/OSD-936/preview/SMIgfE0e0yJ81XCJRbNi5bsnWdPVaWMV>) for better reuse in future studies and cited it in the manuscript.

When authors use already published data—such as that from samples published in Higashitani et al. (2021)—they need to provide a DOI for the dataset, not just for the

manuscript. For example, the mentioned dataset can be found in OS DR with DOI: [Higashibata A. "The effect of microgravity on the epigenetic modification in *C. elegans* - JAXA EPIGENETICS spaceflight experiment", NASA Open Science Data Repository, Version 6, <http://doi.org/10.26030/1rkt-t974>]. Referring to a DOI ensures that readers can access the exact dataset used for the study, adhering to open science principles and fostering reproducibility.

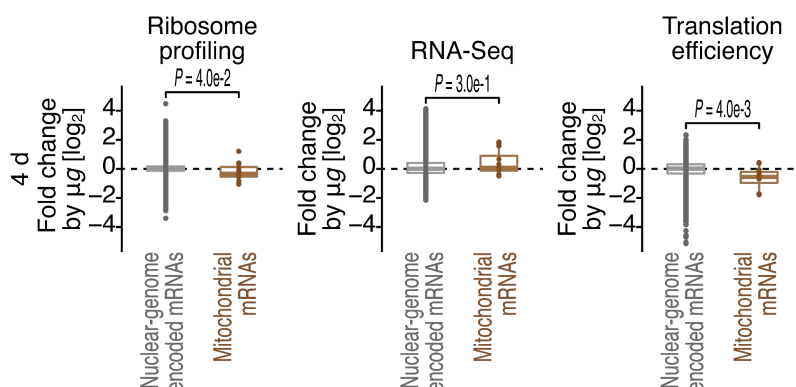
According to Reviewer #3's suggestion, we referred to the corresponding DOI of OS DR in the manuscript.

More details:

Figures 1C and 1E:

There is an inconsistency in data representation between human cell data (Figure 1C) and *C. elegans* data (Figure 1E). In Figure 1C, box plots are used, while Figure 1E employs cumulative distributions. The rationale for changing the metric isn't clear. The authors should maintain a consistent method for data presentation across species unless there is a strong justification. A direct comparison would be easier if the same visualization method were applied.

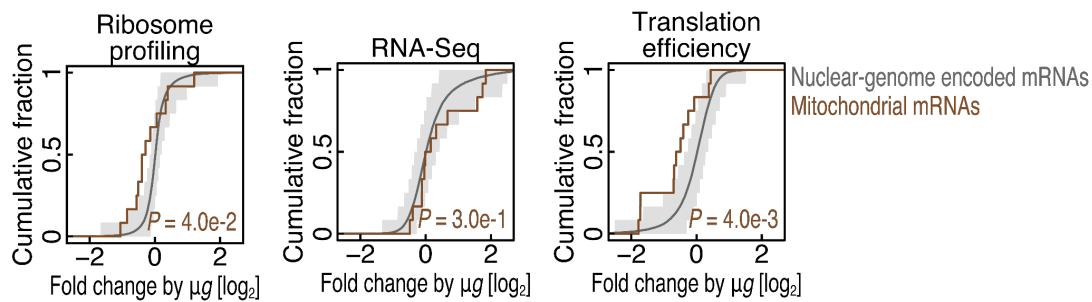
The reason for using a different visualization in Figure 1E stemmed from the only one time point to compare. According to Reviewer #3's suggestion, we added Figure S1N (see also the figure below) to show the box plots for consistent data presentation. We note that we kept the cumulative fraction plot in Figure 1C to draw the "baseline" (or random sampling), which is related to the points raised below.



Additionally, including a theoretical baseline curve for translation efficiency in the cumulative distribution plot would help interpret shifts in translation relative to a "no change" scenario.

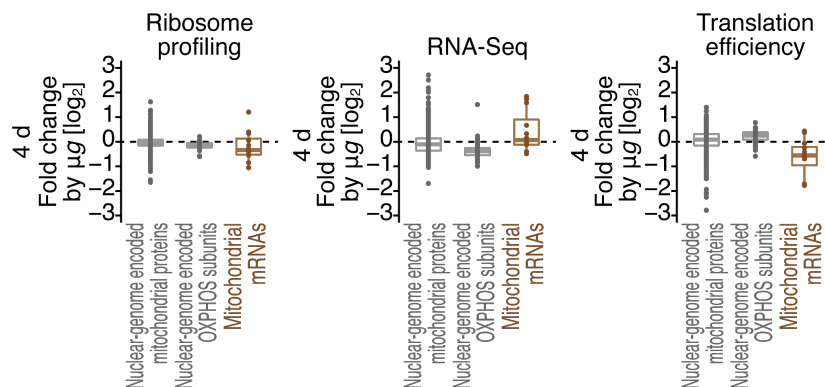
To answer Reviewer #3's point, we conducted a random sampling of 12 genes, which correspond to mitochondrial encoded ORFs in *C. elegans*, from the dataset, repeated 1000 times, and drew the range from 2.5 percentile to 97.5 percentile (shaded area in the figure below and Figure 1C). Mitochondrial mRNAs showed lower translation efficiency than this theoretical range, indicating further statistical evidence of reduced mitochondrial

translation.



It is also important to clarify whether nuclear-encoded mRNAs shift similarly in these distributions, as it would put mitochondrial translation changes into better context.

We added the corresponding figure panel in Figure S1O.



The subtle difference in response observed in *C. elegans* might be due to the protective effects of the native extracellular matrix (ECM) environment in the organism, as opposed to isolated cell cultures.

We thank Reviewer #3 for the interesting interpretation. We added this point in the "Limitations of the study" section.

Figure 2:

When referring to Figure 2D in the text, please mention the specific time points (24 hours and the subsequent 24-hour recovery) explicitly in the main body, not just in the figure legend.

We modified the text to state the incubation time (24 h) for Figure 2D and the recovery (further 24 h) for Figure 2F.

Consider adding the 48- and 72-hour time points to Figure 2C (currently shown in Figure

S2Q-R) to provide a clearer timeline of adaptation. This would help confirm whether the log2 fold changes in ribosome profiling converge to a steady state around -1, indicating a new equilibrium rather than a transient response. The idea of adaptation raised in the text implies that after an initial overshoot or strong response, cells reach a stable adjusted condition. Showing these additional time points can reinforce the notion that the mitochondrial translation changes persist over time and are not merely transient.

We thank Reviewer #3 for the constructive suggestion. We moved the previous Figure S2R (the time points with 48 h and 72 h) to the main figure (current Figure 2E) and highlighted that the steady state adaptation occurred over time in the main text.

The data from 10G experiments (Figures 2F) also raise interesting points. While one might expect cells adapted to 1G to respond similarly to deviations in either direction, the finding that higher g-forces further enhance translation suggests a graded relationship between gravity and mitochondrial translation. Re-plotting Figures 2E-F-G with gravity as a continuous variable on the X-axis and mitochondrial translation levels on the Y-axis might provide a visual representation of this relationship. This would offer new insights into the gravity-translation curve, contributing to our understanding of gravitational influence on cellular metabolism.

According to Reviewer #3's suggestion, we added a new panel (Figure S2L; see also the figure below) to depict the relationship between mitochondrial translation and the gravity force, for better visual representation.

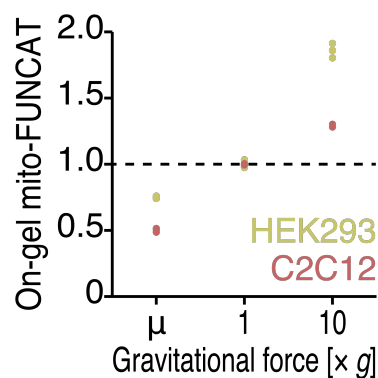


Figure 3:

The findings in Section 3 are convincing. However, in Figure 3, Figure 3G is shown before Figure 3F, which can be confusing.

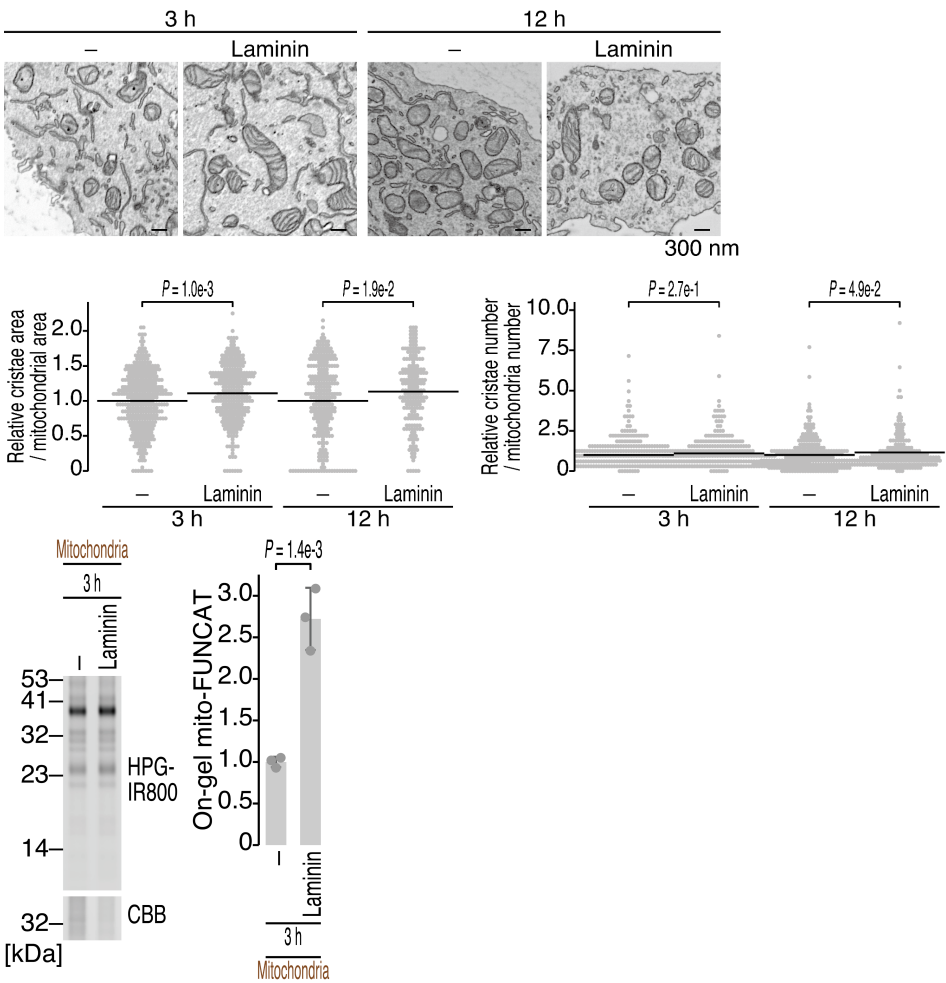
According to Reviewer #3's suggestion, we reordered the figure panels and the introduction of these data in the text.

Cristae Formation Timing (related to Figure 5 and OPA1):

The discussion around OPA1 and cristae formation is interesting. The authors conclude

that the increase in cristae is a result of enhanced mitochondrial translation rather than the cause. One would expect a time lag between laminin addition (which stimulates translation) and the subsequent formation of cristae structures. It would be helpful to examine time-course data to support this causal relationship. Even a simple time-series experiment showing cristae morphology evolving hours after translation changes would strengthen the argument that cristae formation is indeed downstream of increased mitochondrial translation activity.

We thank Reviewer #3 for the constructive suggestion. Here, we additionally conducted shorter laminin treatment (3 h) and performed 1) electron microscopy to measure cristae formation and 2) on-gel mito-FUNCAT (Figure S5G-K; see also the figure below). This 3-h laminin incubation is the shortest time point in practice, since mito-FUNCAT requires 3-h HPG incubation. Even in this early time point, both cristae formation and mitochondrial translation were enhanced (Figure S5G-K; see also the figure below). Thus, here, we could not conclude causality between cristae formation and mitochondrial translation. Although we are still actively working to understand the relationship between mitochondrial morphology and translation inside, this should be out of the scope of this manuscript. We modified the text to point out this limitation.



Overall Study and Model Systems:

It is unfortunate the authors did not use 3D organoids for cell culture, as these can be grown in suspension and would serve as a better model for simulated microgravity in a clinostat. Clinostat creates a lot of sheer stress on adherent cells and could explain why adaptation was not observed. The response in mice was minimal and only apparent after 14 days, and the *C. elegans* response was also modest, which suggests the presence of the tissue matrix confer some protection.

We thank Reviewer #3 for pointing this out. We agree that a 3D organoid may provide a better model for the gravitational response. However, this is out of scope for this work. Instead, we added the discussion in the “Limitations of the study” section to refer to this point of future study.

In the discussion, the reference to mitochondrial damage in space needs clarification. There appears to be a citation error, as citation #3 is repeated.

We thank Reviewer #3 for pointing this out. We deleted the repeated citation.

The authors state: “Our results closely aligned with the multiomics approach utilizing data from NASA’s GeneLab platform³,” but it should be referencing ⁷ and ⁸.

According to Reviewer #3’s suggestion, we added references 7 and 8 in the corresponding sentence.

In reference #3 (da Silveira et al., 2020), the authors reported disruption and lower metabolic activity of mitochondria, which aligns with the results shown here. The authors should be more specific when referring to this work and provide a deeper interpretation. They present an alternate hypothesis to the simple ROS-driven disruption previously reported, which is an important and novel insight from the submitted work here.

We thank Reviewer #3 for the constructive suggestion. We added more explanation in the Discussion to connect our results and the published study.

Another element that could strengthen the discussion is the fact that cosmic radiation has been shown to alter the ECM in breast and skin tissues (Costes et al., Radiation research 154 (4), 389-397). Considering that space conditions involve both cosmic radiation (affecting the basement membrane) and microgravity (reducing ECM interaction), the combined effects could be substantial.

Again, we thank Reviewer #3 for the constructive suggestion. We added the discussion regarding the synergistic effects of cosmic radiation on ECM.

It would be interesting for the authors to consider a follow-up study using breast organoids exposed to simulated cosmic radiation and/or simulated microgravity to investigate these combined stressors.

We thank Reviewer #3 for their encouraging words. We truly hope that we have another chance to follow up on our study using an organoid setup. We commented on the potential use of 3D organoids for future studies in the “Limitations of the study” section.