Author's Response to Peer Reviews

Authors' Response to Peer Reviews of "Machine Learning Ensemble Investigates Age in the Transcriptomic Response to Spaceflight in Murine Mammary Tissue: Observational Study"

James A Casaletto[1], BS, MS, PhD; Tyler Zhao[2]; Jay Yeung[2]; Abigail Lee[2]; Amaan Ansari[2,3], BSc; Amber Fry[2]; Arnav Mishra[2]; Ayush Raj[2]; Kathryn Sun[2]; Sofia Lendahl[2], BA; Willy Guan[2]; Melissa S Cline[4], PhD; Sylvain V Costes[5]

[1]Blue Marble Space Institute of Science, Seattle, WA, United States
[2]Student Association for Applied Statistics (SAAS), University of California, Berkeley, Berkeley, CA, United States
[3]University of Mannheim, Mannheim, Germany
[4]Genomics Institute, University of California, Santa Cruz, Santa Cruz, CA, United States
[5]NASA Ames, Mountain View, CA, United States

Corresponding Author:

James A Casaletto, BS, MS, PhD
Blue Marble Space Institute of Science
600 1st Ave, First Floor
Seattle, WA 98104
United States
Phone: 1 206 775 8787
Email: james.casaletto@gmail.com

Keywords: machine learning; spaceflight; mammary tissue; gene expression; mice; breast cancer; feature importance

This is the authors' response to peer review reports related to "Machine Learning Ensemble Investigates Age in the Transcriptomic Response to Spaceflight in Murine Mammary Tissue: Observational Study."

Live Review Round [1]

List of Major Comments

The title of this paper [2] should be more specific with respect to the source of mammary tissue: identify "mouse mammary gland tissue" in the title or, perhaps, simply "murine mammary tissue."

Response: We changed the title as suggested to "Machine learning ensemble investigates age in the transcriptomic response to spaceflight in murine mammary tissue: observational study."

While the methodology is interesting and the findings certainly warrant further study, this should be clearly identified as formative research: there was no preregistration of hypotheses and methods, and the findings (list of key genes and of pathways differing according to age) are just suggestive and not at all robust or convincing. Accordingly, some detail about the experiences of the mice and physiological values is beside the point, so we suggest it is moved to a "Supplements" section along with more specifics about machine learning parameters, etc, that could help researchers attempting similar approaches.

Response: We describe in the newly-added Strengths and Limitations section of the manuscript that our in silico findings need to be validated in vitro. We also make the software (as a Jupyter notebook) available so that our approach may be repurposed or reproduced.

With respect to the OSD-511 dataset, the details of Rodent Research Reference Mission 1 need revision, as it was mentioned that there are 40 female BALB/ cAnNTac mice, while the total number of animals used was 43: 21 younger mice and 22 older mice. Moreover, the 8 younger mice that were kept in standard cages were exposed to different conditions from the 7 older mice that were housed in flight hardware.

Response: We rectified the counts and created Table 1 for clarity.

In addition, it was mentioned that each group of space-flown mice had corresponding control groups (ground control), but it is not clear which basal controls (10 mice euthanized 1 day post launch) are used to compare which group. This is important to explain the single group called "non-flight" that is mentioned later in the paragraph, and indicate if these latter details from the original experiment are not available to the authors.

Response: We added explanations to specify which mice were used in which grouping.

In the Discussion section, or as a separate Limitations sections, consider explicitly pointing out that data of experimental mice that were collected just once after 40 days in space and 2 days post return recovery provides only cross-sectional data and does not capture changes in the mice that could be evident while in space or longer after return from space. Also, the description for Figure 1 mentions Figure 1E and F, which are not available in the figure.

Response: We added this and several others to a dedicated section called "Strengths and Limitations" in the Discussion.

The small sample size should be acknowledged, which means the outcome models may not be able to generalize well on unseen data in downstream tasks.

Response: We describe how we augmented the data in the Methods section. We also call out the paucity of data in the Strengths and Limitations part of the Discussion.

Minor Comments

The title could be enhanced to make it clear that this was an experiment based on a model organism (mouse) and not human.

Response: We changed the title as suggested to "Machine learning ensemble investigates age in the transcriptomic response to spaceflight in murine mammary tissue: observational study."

The reviewers acknowledge the availability of details that enable the reproducibility of the study, such as publicly accessible data sources and detailed description of data handling and analysis procedures. However, the reviewers wondered whether the source code used could be availed for enhancing the reproducibility.

Response: Per this suggestion, we made the code available to the reader.

The total number of mice stated that were used in the study does not correspond with the total number used, based on the breakdown of individual group numbers. Authors need to cross-check the numbers to ensure that they tally with the numbers used.

Response: We rectified the counts and created Table 1 for clarity.

Clarify the composition of the control cohort, refer to those mice in a consistent way, and discuss differences that were found to exist between the subsets of controls.

Response: We rectified the counts and created Table 1 for clarity.

In page 4, under the Data Transformation section, it is stated that "four filtering methods were performed," but Figure 2B only represents three filters. Kindly clarify if the fourth filtering method was used but not included in the figure or whether there was a mistake in either the figure or the text for the sake of consistency.

Response: We updated Figure 2B to include four filter icons.

On page 6, the last paragraph, a linear regression model was used to predict the weight of mice at euthanasia, but the significance of this prediction was not discussed. The significance should be discussed for a better understanding of its applicability. Add a brief discussion of the significance of the model, which may include a statistical test validation such as P values and/or CIs.

Response: We removed the linear regression model from the ensemble.

On page 15, under the Conclusion section, it is also mentioned that "The dysregulation of ECM [extracellular matrix] remodeling, cytoskeletal function, and stress response pathways was observed in radiation-exposed mice," but radiation exposure was not the intervention applied. Revise this statement to accurately reflect the intervention applied in this study (spaceflight) and ensure the conclusion is per the experimental conditions.

Response: We updated the model and the subsequent pathway results do not include extracellular matrix remodeling.

In the Discussion section, some results are repeated instead of being analyzed in depth. Focus more on interpreting the results, compare them with similar studies, and discuss their significance.

Response: We added a lot of content interpreting the results in the Discussion section, along with comparing to similar studies and discussing their relevance.

Only accuracy is reported for model performance metrics. Add other metrics, including area under the receiver operating characteristic curve, sensitivity, specificity, and F_1-score, to enhance the assessment of the model's predictive ability.

Response: We changed our model performance metric to use the F_1-score.

Under the algorithms discussion, remove possessive apostrophe from the "1950's."

Response: We removed the possessive apostrophe.

It may help to add a statement to make it explicit whether ethics approval was necessary for the study. In addition, it would add value in discussing ethical implications of collecting the dataset used in the manuscript with reference to any discussion in previous publications or from the authors who collected the original data.

Response: We added an entire section dedicated to ethics approval.

Concerns with Figures and Tables

Most figures have poor resolution, which makes them difficult to understand or interpret. It would be helpful to regenerate the figures with better resolution.

Response: We increased the resolution of all our images.

It would be helpful to add details to the captions to include what's represented in each panel and any elements of statistics.

Response: We added additional explanations to the captions of all figures and tables.

Creating a table to present the various groups and their characteristics, including ground control, would help improve readability.

Response: We created Table 1 for this purpose.

Figure 1 lacks an adequate explanation of each panel, which will clarify what they represent.

Response: We added additional explanations to the caption of Figure 1.

Table 1 is not clear, making it difficult to read.

Response: We made Table 1 more clear and legible.

The top and left parts of Figure 7 are cropped, and it is possible important information is omitted.

Response: We omitted Figure 7.

The legend refers to plots by layout (left/right), duplicating the role of (a)-(d) labels. Also, plot titles are not the most prominent text and are not referenced in the text.

Response: We removed the "left/right" language from the caption and removed the plot titles from the figure.

In Figure 4, the term "accuracy" is used without clarification.

Response: We replaced Figure 4 with Table 2. Also, we replaced "accuracy" with "F_1-score" as the performance metric.

Abbreviations used in Figures 2 and 3 are not explained.

Response: We added explanations for all abbreviations and created an abbreviation table at the end of the manuscript.

The Figure 3 legend does not clearly describe the difference between the left and right diagrams.

Response: We removed "left/right" language from the figure caption and replaced it with letters and colors to be more clear.

The manuscript refers to Table 1 subsections "e" and "f," which are not present. Some figures are also unclear and not explanatory enough.

Response: We added Figure 1E and F to Figure 1. We also added more explanations to all of the figure and table captions.

Figure 5: Fonts are too small to read, and part of the legend is cropped.

Response: Figure 5 is now Figure 4 and has been updated with larger fonts, and we removed the legend.

In Figure 1, the caption states that the left plots represent ground mice and the right plots represent space mice, which is not reflected in the figure.

Response: We removed "left" and "right" language from the figure caption.

On page 4, the principal components analysis statement interpreting Figure 1A and D is misleading. The statement suggests that both Figure 1A and D show principal components analysis for spaceflight, whereas Figure 1A only represents ground mice.

Response: We updated the figure caption and interpretation to properly reflect the principal components analysis plots.

The text for Figure 1 describes Figure 1E and F, but these panels are not present.

Response: We added Figure 1E and F to Figure 1.

Additional Comments

Consider revising the title and abstract to identify that the study was conducted with data collected in a model organism or murine model.

Response: We changed the title as suggested to "Machine learning ensemble investigates age in the transcriptomic response to spaceflight in murine mammary tissue: observational study."

The second page, second sentence of the first paragraph: "Female astronauts in particular have an increased risk of breast cancer due to exposure to galactic cosmic radiation (7)." Please revise the reference, as Kumar et al [3] did not investigate or conclude the mentioned data.

Response: We modified the text further to be more inclusive in terms of breast cancer risk from ionizing radiation, including cosmic radiation.

On the second page, in the last sentence of the first paragraph, "Female astronauts...this increased vulnerability." Please provide a reference for the mentioned data.

Response: It is a summary statement of the previous statements encompassing 20 references.

The second page, second paragraph: "Machine learning (ML) has been leveraged but to a much lesser extent (15)." Please revise the reference as Larrañaga et al [4], as ML's role in bioinformatics has been widely expanded since 2006.

Response: We updated the sentence and changed the reference to a more recent one.

Page 6, second paragraph: It was mentioned that "The support vector machine was created by Hava Siegelmann and Vladimir Vapnik," and there is a reference to Cortes and Vapnik [5], while this work [6] was published in 2001.

Response: We are not using support vector clustering in our method.

Page 11, pathway enrichment analysis: Please identify the abbreviation "KEGG" as "Kyoto Encyclopedia of Genes and Genomes."

Response: We expanded the acronym at its first use. We also created a table of acronyms at the end of the manuscript.

Page 11, pathway enrichment analysis: Please identify the abbreviation "FDR" as "False Discovery Rate."

Response: We expanded the acronym on first use. We also created a table of acronyms at the end of the manuscript.

Concluding Remarks

In the Data Transformation section, groups were introduced for the first time in the manuscript (FLT vs GC and YNG vs OLD); these categories are defined later, but it would be good to spell out the names the first time they are mentioned. That's true for any other acronym used.

Response: We added an explanation for those and all other acronyms on first mention. We also created a table at the end of the manuscript that defines each acronym.

The article did not introduce a Limitation section. It is helpful to the reader to emphasize the limitations of the methods.

Response: We added a Strengths and Limitations section to the Discussion.

References

1. Sakilay S, Collier M, Rahgozar A, et al. Peer review of "Machine Learning Ensemble Identifies Distinct Age-Related Response to Spaceflight in Mammary Tissue". JMIRx Bio. 2025;3:e75688. [doi: 10.2196/75688]

2. Casaletto JA, Zhao T, Yeung J, et al. Machine learning ensemble identifies distinct age-related response to spaceflight in mammary tissue. BioRxiv. Preprint posted online on Oct 6, 2025. [doi: 10.1101/2025.02.17.638732]

3. Kumar K, Angdisen J, Ma J, Datta K, Fornace AJ, Suman S. Simulated galactic cosmic radiation exposure-induced mammary tumorigenesis in $Apc^{Min/+}$ mice coincides with activation of ERα-ERRα-SPP1 signaling axis. Cancers (Basel). Nov 26, 2024;16(23):3954. [doi: 10.3390/cancers16233954] [Medline: 39682141]

4. Larrañaga P, Calvo B, Santana R, et al. Machine learning in bioinformatics. Brief Bioinform. Mar 2006;7(1):86-112. [doi: 10.1093/bib/bbk007] [Medline: 16761367]

5. Cortes C, Vapnik V. Support-vector networks. Mach Learn. Sep 1995;20(3):273-297. [doi: 10.1007/BF00994018]

6. Ben-Hur A, Horn D, Siegelmann HT, Vapnik V. Support vector clustering. J Mach Learn. Dec 2001:125-137. URL: https://www.jmlr.org/papers/volume2/horn01a/horn01a.pdf [Accessed 2025-12-03]