Reviewer #2:

The manuscript titled “Cognitive effects of simulated galactic cosmic radiation are mediated by ApoE status, sex, and environment in APP knock-in mice” by Wieg, L.; et al. is a scientific work where the authors study the effect of gamma rays and simulated galactic cosmic radiation in APP knock-in model mice at different irradiation doses. The most relevant outcomes found revealed the negative effect of this class of irradiation and the onset of neurodegenerative diseases and alterations in the microbiome in the examined specimens. This topic is interesting and the manuscript is generally well-written. However, it exists some points that need to be addressed (please, see them below detailed point-by-point) to improve the scientific quality of the submitted manuscript paper before this article will be consider for its publication in the *International Journal of Molecular Sciences*.

We thank the reviewer for taking time to review our manuscript and for their positive evaluation and suggestions to improve the quality of our paper.

1) Keyword. The authors should consider to add the term “APP mouse models” in the keyword list.

We thank the reviewer for the suggestion and have added this keyword to the list.

2) “There is an increasing demand for deep space exploration (…) astronauts will be exposed (…) Galactic Cosmic Radiation (…) (HZE) particles” (lines 50-56). Here, even if I agree with this statement provided by the authors it should not be neglected the potential impact of galactic cosmic radiation on the crew of commercial flights [1]. Some quantitative insights should be furnished in this regard.

[1] Toprani, S.M.; et al. Cosmic Ionizing Radiation: A DNA Damaging Agent That May Underly Excess Cancer in Flight Crews. *Int. J. Mol. Sci*. **2024**, *25*, 7670. https://doi.org/10.3390/ijms25147670.

We thank the reviewer for raising this important point. We have added a statement that we believe concisely and poignantly encapsulates the situation for commercial airline flight crews (Line 66) and added the reference above.

3) Results. Why did the authors only test the effect of 0.75 Gy and 2.00 Gy radiation (in addition to no radiation experiments) and not a broad range of values? (e.g. lower galactic cosmic radiation values around 0.2-0.3 Gy are received those astronauts who work at the International Space Station, among other situations). Some discussion should be provided in this regard.

We thank the reviewer for this insightful question. We agree that testing a broader range of radiation exposures could provide valuable insights, especially at lower doses expected for astronauts on long-duration deep-space missions. However, the exposure levels of 0.75 Gy and 2.00 Gy were selected based on specific guidelines provided by NASA for this study. These levels were chosen to align with anticipated radiation exposures relevant to expected exposures to astronauts traveling into deep space over long periods (i.e. a year or more).

Additionally, the number of mice available for this experiment was limited, as they were excess animals from a separate NASA study. This study was an add-on, taking advantage of the available animals to conduct additional research. This limitation constrained our ability to test additional exposure levels while maintaining sufficient statistical power. We acknowledge that testing a broader range of doses would be beneficial.

4) Then, this research examined the effect of gamma radiation but not X-ray radiation on the wild-type and APP knock-in mouse models. Could this aspect negatively interfere in the data interpretation?

We thank the reviewer for raising this important point. The decision to use gamma rays rather than X-ray radiation was based on several considerations, including the fact that gamma rays tend to be a higher-energy, deeper-penetrating form of radiation that more closely simulates the type of exposure expected in deep-space environments compared to X-rays. In addition, gamma ray exposure is available at Brookhaven

National Lab and its use is encouraged by NASA for NASA-funded research, such as this study. This choice is more relevant to the context of space missions and is used more frequently in similar research at BNL. We acknowledge that excluding X-ray radiation could potentially limit the generalizability of our findings to other types of radiation exposure, particularly those more common on Earth. We agree that further studies comparing the effects of gamma radiation with X-ray radiation could provide a more comprehensive understanding of radiation-induced effects in these mouse models. This could be an avenue for future research to explore potential differences in the biological responses elicited by different types of radiation.

5) "For the HZE particle they show higher oxidative stress in cortical cells (…) deoxyribonucleic acid (DNA) damage and cell death (…) reactive gliosis" (lines 585-588). Here, even if I also agree with this information stated by the authors it should be detailed the DNA molecular machinary which leads to DNA degradation inner the cells [2].

[2] Novo, N.; et al. Beyond a platform protein for the degradosome assembly: The Apoptosis-Inducing Factor as an efficient nuclease involved in chromatinolysis. *PNAS Nexus* **2022**, 2, pgac312. https://doi.org/10.1093/pnasnexus/pgac312

We thank the reviewer for providing us with this reference. We have added it to explain the process of DNA degradation in more detail (Line 597).

6) "In conclusion, this study is to our knowledge the first (…) ApoE and radiation" (lines 684-694). Here, the authors should consider to insert and additional "Conclusions" section and move all these information details.

We appreciate this suggestion and have added a "Conclusions" section.