

Response to Reviewer X Comments

27-Hydroxymangiferolic acid extends lifespan and improves neurodegeneration in *Caenorhabditis elegans* through activating nuclear receptors

Summary

Thank you very much for taking the time to review this manuscript. Please find the detailed responses below, and the revisions are marked in track changes in the re-submitted files.

Point-by-point response to Comments and Suggestions for Authors

Comments 1: Figure 1 (line 61), It would be nice to include one molecule of the dafachronic acids in that figure so readers can see it compared to 27-HMA, as both are putatively part of the dafachronic acid pathway. For Panel B and the legend, note the quantification was done with minigene reporters and not with the endogenous system. Did the authors measure endogenous expression of FXR by RT-qPCR?

Response 1: We thank the reviewer for the helpful comments and suggestions. We are sorry that we did not test the activity of dafachronic acid, so we cannot include dafachronic acid as a control compound. However, we have included the results of the FXR agonists screening for reference. Please refer to Figure S1 and Table S1.

Additionally, We have updated the legend for Figure 1b to specify that the quantification was done using dual-luciferase reporter assays. Regarding to the endogenous expression of FXR, we measured the mRNA expression of *nhr-8* and *daf-12* in CL4176 nematodes in section 2.7. Please refer to Page 3, Line 147, and Figure 6k.

Comments 2: Figure 2 (line 88), It is really busy in Panel A; I would have to remove some of the treatments so that it is easier to interpret.

Response 2: We have removed some of the concentrations of 27-HMA in Figure 2a and retain the optimal effective concentration to simplify the presentation. The survival curves for all concentrations are now included in the Supplementary Materials. Please refer to Figure 2a and Figure S2a.

Comments 3: Line 99, Perhaps the text that denotes that dietary restriction extends lifespan should also be included as a positive control in Figure 2A so readers may compare its influence with that of 27-HMA.

Response 3: We thank the reviewer for the helpful suggestion. We have included the *eat-2* strain, which mimics dietary restriction, as a positive control in Figure 2a to compare the effects of dietary restriction with those of 27-HMA treatment. Please refer to Figure 2a.

Comments 4: Line 95, The culling of Supplementary Data may move this panel to Figure 2, thereby tidying the primary text.

Response 4: We have moved the original Figure 2c (Chemotaxis of nematodes to *E. coli* OP50) and Figure 2f (The number of total progeny) to the Supplementary Materials to tidy up the main text. Please refer to Figure S2b, c.

Comments 5: Lines 102-103 (Figure 2G), The colors in the figure are ambiguous. The legend refers to A, B, and C, but there is no explanation for these in either the figure or its legend. Clarification is needed.

Response 5: We thank the reviewer for pointing out the mistake. The motion was classified into three groups: Motion A (moved freely), Motion B (moved slowly after stimulation), and Motion C (only moved head and tail after prodding). We have improved these clarifications in the corresponding legend. Please refer to Page 4, Line 199-200, and Figure 2e.

Comments 6: Line 105: Shall include similarity with DAF as mentioned earlier; otherwise, it is not bad to include this aspect as it would show how deep and supportive the results are.

Response 6: We have now included a comparison of the potential similarity between the endogenous ligand of DAF-12, dafachronic acid (DA), which influences lifespan through activation of DAF-12, and the 27-HMA in our manuscript. Please refer to Page 4, Line 185-188.

Comments 7: Figure 5 (line 213): In Figures where no significant change was observed, the displays should be confined to Supplementary Data. Other than that, different concentrations of the same treatment make too much information in one figure. Usually, four concentrations for each treatment would be better to present another graph.

Response 7: We thank the reviewer for the helpful suggestion. We have revised Figure 5 by presenting the detoxification-related results for treatment with a single concentration, while the data for treatment with multiple concentrations have been moved to the Supplementary Materials. The single-concentration results for the *nhr-8* and *daf-12* mutants have been retained, as the lack of significant differences in these mutants suggests that 27-HMA may activate detoxification functions through nuclear hormone receptors. Please refer to Figure 5a, b, c and Figure S4a, b, c.