

Answering Reviewers

Dear editor:

At first, we deeply appreciate all the approbations and the comments made by the three reviewers and we will present a point-by-point response to each comment in the next part.

Reviewer's code: 00053441

Reviewer's report 1: Discussion should be reformulated. It contains introductory material (paragraphs 1 and 2) and lacks a real discussion of data.

Response: We have reformulated the Discussion part. We moved the introductory material to the Introduction part partly and supplemented some materials discussing our results.

Reviewer's report 2: The authors should state that an *in vitro* and *in vivo* approach was used in the Abstract.

Response: We have made a statement that an *in vitro* and *in vivo* approach was used in our study.

Reviewer's report 3: Why was not included a control group having JZG. This would be interesting, because the dosage applied is much higher (994mg/kg).

Response: We are very grateful for this constructive suggestion. As the safety of JZG has been validated in the previous studies, and we focused on the efficacy in this research. Thus, we did not include a control group having JZG. However, as the review said, the dosage applied is high, we think it should be continually conducted to confirm the findings in the future research.

Reviewer number: 00646291

Reviewer's report 1: More details for the LC3, p62, p-mTORC1, mTORC1, p-PI3K, PI3K and Actin antibodies used in the study should be provided including their product numbers.

Response: We have supplemented the details for antibodies used in the study

including their product numbers in the Materials and methods part.

Reviewer's report 2: Reference should be provided for the statement “PA is a type of free fatty acid that is elevated in obese subjects and can induce NAFLD”.

Response: We have provided the reference for the statement in the References part as β -Caryophyllene attenuates palmitate-induced lipid accumulation through AMPK signaling by activating CB2 receptor in human HepG2 hepatocytes.

Reviewer's report 3: In the labeling of the figure 1B it is not clear what ALA indicates. Should it be JZG?

Response: We felt regret for the mistake that we signed an erroneous labeling and we have made a correction for it.

Reviewer's report 4: In the labeling of the figure 1D it should be clearly indicated that the cells were treated with combination of PA with either rapamycin or JZG.

Response: We have amended all the figures to indicate that the cells were treated with combination of PA with either rapamycin or JZG.

Reviewer's report 5: In the western blots shown in figure 5A it is not clear where the samples originate from.

Response: We have made a supplementary statement in the labeling to declare that the samples were come from liver tissue.

Reviewer's report 6: In the sentence “We found that JZG activated the autophagy process by either induction or co-localization and degradation to protect against metabolic stress-induced hepatocyte injury in NAFLD” induction, co-localization, and degradation should be specified.

Response: We have made a revision as we found that JZG could activate the autophagy process by either induction of autophagosomes or co-localization of autophagosomes and lysosomes as well as degradation of autolysosomes to protect against metabolic stress-induced hepatocyte injury in NAFLD.

Reviewer's report 7: In the labeling of the figure 4A ZJG should be replaced with JZG.

Response: We felt deeply regret for the mistake that we signed the erroneous labeling and we have made a correction for it.

Reviewer's report 8: Instead of Reactive Oxidative Species insert Reactive Oxygen Species.

Response: Thanks for the reminding and we have made a revision for it.

Reviewer number: 00004603

Reviewer's report 1: The results are not clear presented and will be very difficult to follow for those who do not specialized in autophagy.

Response: We have supplemented some more materials in details to discuss our results.

Reviewer's report 2: According to Autophagy guidelines, instead of LCII/LCI ratio, the upstream events should be presented as LCII/GAPDH or actin.

Response: We have made revisions in the Results and Discussion part. The LC3-II/actin expression was used to present the activation of autophagy, and the expressions of LC3-II/LC3-I and p62 were used to indicate the autophagic flux.

Reviewer's report 3: Yellow punctate does not always support the fusion between autophagosome and lysosome. It may happen if lysosomal enzymes are not active and GFP is not degraded.

Response: Just as the reviewer said, we revised the expression as we found that yellow fluorescence, which means GFP was not degraded by lysosomal enzymes, was largely seen in HepG2 cells treated with PA for 48 h.

Reviewer's report 4: More tests to characterize lysosome function (such as cathepsin activities) are necessary since p62 also is might be degraded by proteasome, and could

be stabilized if proteasome activity is impaired.

Response: As the reviewer said, more tests to examine the proteasome activity might be stabilized for this research. However, we thought that's not the highlight in our study and we have used the expressions of LC3-II/LC3-I and p62 to detect the autophagic flux as well as the stable fluorescent-expressing cell lines to monitor the cellular expression levels in real time. Nevertheless, we still made a supplementary statement in the Limitation part to declare that.

Reviewer's report 5: Discussion should be more detailed.

Response: We have reformulated the Discussion part. We moved the introductory material to the Introduction part partly and supplemented some materials discussing our results.

Reviewer's report 6: There are grammar mistakes, and the paper should undergo editing by English speaking person.

Response: This paper had been edited by **MedE Editing Group** at the first time, and we have made a re-editing in the revision.

To the editor

1. This paper had been edited by **MedE Editing Group** at the first time, and we have made a re-editing in the revision.
2. This paper has been revised according to peer-reviewers' comments.
3. ORCID numbers have been supplied.
4. The approved grant application form and institutional review board statement have been provided.
5. The Audio core tip has been supplemented.
6. The article highlights have been finished following the reminders.
7. The references have been re-edited, however, we are not very sure that the format is correct. If there are any problems, please point out and we will make the revision.
8. All the figures have been re-edited. The labeling has been amended and we

provided the decomposable figures as PPT format. However, as the figures were processed by different procedures and we conserved the original pictures as layers in the PS. We have to pick out each layer as an original part and put it in the PPT and we are not very sure that this is appropriate. If there are any problems, please point out and let us know how to amend it, and we will make the revision.

Thanks very much for the attention and the comments from the editor and the reviewers. And if there are any problems or questions regarding the manuscript, please point out and we will make the revision. We look forward to the reply.

Sincerely yours,

Guang Ji