

Dear Editor,

Thank you for your letter and for the reviewers' comments concerning our manuscript entitled "The role of Sirt1 in Attenuation of Acute Liver Failure by Reducing ROS via HIF-1 α ". We are truly grateful for the reviewers' critical comments and thoughtful suggestions. We feel fortunate that our manuscript went to these reviewers as their invaluable comments not only helped with the improvement of our manuscript, but also suggested some neat ideas for future studies. Please do forward our heartfelt thanks to these experts.

Based on these comments and suggestions, we have made carefully-considered modifications to the original manuscript. All changes made to the text are in color.

We hope the new manuscript will meet your Journal's standard. Below you will find our point-by-point responses to the editors' and reviewers' comments and questions.

With our best regards,

Zuo-jiong Gong

Pan Cao

Qian Chen

Chun-xia Shi

Lu-wen Wang

Comments from the Reviewers:

Reviewer: 1

Major concerns:

1. For induction of hypoxia animal model., mice should be present in a special environmental chamber (Oxycycler, BioSpherix, Redfield, NY). The ambient O₂ concentration within the chambers is continuously measured by an O₂ analyzer and adjusted according to computerized profiles set for the experiments with the use of a computerized servo-controlled system and not plastic chamber. So the lack of description of the method used for induction of hypoxia makes a doubt about the

whole work.

Response: We are truly grateful for the reviewer's valuable comments. Based on these comments and suggestions, we have made carefully-considered additions and modifications to the original manuscript. All changes made to the text are in color. In our experiment, we used COY Vinyl Anaerobic Chambers for the establishment of hypoxia model. The Coy hypoxic chamber is a device that is able to control aspired oxygen levels from 0 to 100%, allowing researchers to study hypoxic and or hyperoxic effects on animal organs. This chamber is also suitable for researching both acute and chronic effects. The Coy hypoxia chamber has an automatic waste filtration system that maintains a continuous clean air supply, making it safe for longterm usage in research as described ^[1].

1 Nakada Y, Sadek HA. Experimental Hypoxia as a Model for Cardiac Regeneration in Mice. *Methods Mol Biol* 2021 2021-01-20; 2158: 337-344.

2. If the procedure for induction of hypoxia was accurate, did authors measured blood gases?

Response: We are truly grateful for the reviewer's valuable comments. Due to the limitations of the experimental conditions, we did not continuously measure the blood gases of the animals. Only on the last day, we randomly selected one mouse from each hypoxia-treated group. The chest was opened indoors and the arterial blood was collected from the left ventricle through direct cardiac puncture. After blood sampling, in order to prevent gas leakage, the needle was sealed with a rubber cap and placed on ice, and then the blood gases was measured. The partial pressure of oxygen in the hypoxic model group were approximately between 60mmHg and 65mmHg, and we preliminarily judged the successful establishment of the hypoxia model.

3. How could animals survive for 2 weeks without developing pulmonary

edema, in my opinion this prolonged duration of hypoxia might induce severe complications that could influence survival of the animals.

Response: Thank you very much for bringing this issue to our attention. We have made carefully-considered additions and modifications to the original manuscript. All changes made to the text are in color. To avoid pulmonary and cerebral edema caused by a rapid drop in oxygenation, gradually decreased the fraction of inspired oxygen (FiO₂) (1%/day) from 21% normoxia (room-air oxygen) to 8% oxygen (severe hypoxia) over the course of 2 weeks, followed by continual exposure to 8% oxygen for an additional 2 weeks. We have added this description in the revised manuscript. Thanks again for your comments.

4. Authors are encouraged to present their time line study for each group?

Response: Thank you very much for bringing this issue to our attention. At the beginning, Hypoxia group and Hypoxia + LPS group first performed hypoxia pretreatment for one month, and then used LPS combined with D-Gal to intervene in LPS group and Hypoxia + LPS group, and finally saline control group, Hypoxia group, LPS group Euthanize with Hypoxia + LPS group and collect samples. After the in vitro molecular experiment was completed, we supplemented the Resveratrol group and LPS + Hypoxia + Resveratrol group, the latter also underwent hypoxic treatment for one month, and the subsequent experimental procedures were as described above.

5. What was the solvent substance used for dissolving resveratrol?

Response: Thank you very much for bringing this issue to our attention. In our experiment, we dissolved resveratrol in hot normal saline. Although there were some suspensions, we thought that this would not affect the absorption of the drug by the mice as we gave it by gavage.

6. Why did author choose 24 h time point after LPS administration for sacrifice?

Response: Thank you very much for bringing this issue to our attention. According to the previous research results of our research group ^[2, 3], the ALF model established by LPS combined with D-Gal has the most obvious sacrifice effect at the 24-hour time point.

2 Wang Y, Zhang H, Chen Q, Jiao F, Shi C, Pei M, Lv J, Zhang H, Wang L, Gong Z. TNF-alpha/HMGB1 inflammation signalling pathway regulates pyroptosis during liver failure and acute kidney injury. Cell Prolif 2020 2020-06-01; 53(6): e12829.

3 Chen Q, Wang Y, Jiao F, Shi C, Pei M, Wang L, Gong Z. Betaine inhibits Toll-like receptor 4 responses and restores intestinal microbiota in acute liver failure mice. Sci Rep 2020 2020-12-14; 10(1): 21850.

Minor corrections:

7. The manuscript still needs revision as it contains multiple typing and punctuation errors such as: Page 5; line 4: remove “which” Page 5; line 18: change “Approve number” to approval number Page 15; line 12 change “we examed” to we examined.

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Reviewer: 2

Method section: The sentence mentioned, “exposed or not to hypoxic conditions as the animal model.” This sentence is not clear and needs to say concretely which method followed the study. Should not keep “or” The reference should be revised in a

proper sequence. For example, this paper reference citation started with 20. Therefore, please need to edit it. Needs to write the separate section of conclusion. The whole manuscript needs reference formatting.

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Comments from the Editors:

Science editor:

1. The lack of enough description of the method used for induction of hypoxia.

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2. It's difficult to define that "Hypoxia reduced the expression of Sirt1 causing the activation and acetylation of HIF-1 α " according to Figures 2 and 3 because it's just a correlation between Sirt1 and Hif-1 α .

Response: We are truly grateful for the editor's valuable comments, and we have added relevant experimental data in Figure 2. The interaction between Sirt1 and HIF-1 α was determined by immunoprecipitation. After endogenous HIF-1 α was

induced by hypoxia, Sirt1-HIF-1 α binding was observed. We next examined whether Sirt1 deacetylates HIF-1 α . Lysyl acetylation of HIF-1 α was detected by immunoblotting with anti-acetyl-lysine in HIF-1 α immunoprecipitates. As shown in Figure 2, Sirt1 overexpression significantly decreased HIF-1 α acetylation suggesting that Sirt1 regulates lysyl acetylation of HIF-1 α .

3. Did they be encouraged to present their timeline study for each group?

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6. The reference should be revised in a proper sequence.

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