Dear Reviewers:

Thank you for your comments concerning our manuscript. Those comments are all valuable and very helpful for revising and improving our paper. We have studied comments carefully and have made correction which we hope meet with approval. Revised portion are marked in red in the paper. The corrections in the paper and the responds to your comments are as following:

Reviewer's code: 02822910

1. Thirty rats were randomly allocated to three groups (n=6 in each group): Sham, orthotopic liver transplantation (OLT) and remote ischemic perconditioning (RIC). What happened to 12 rats?

   Response: First of all, I apologize for the ignoring of details and thanks for your careful reading and proofreading. In rat RIC models, 18 of 30 rats were randomly allocated to three groups (n=6 in each group): Sham, orthotopic liver transplantation (OLT) and remote ischemic perconditioning (RIC), the rest of 12 rats were randomly allocated to OLT and RIC groups as liver donors (n=6 in each group).

2. More technical details needed such as how did you study in lab? A short explanation is needed for OLT and ischemia technique. Was the ischemia time between groups the same in terms of time? More technical details will help reader to make them believe that this study was really done in lab.

   Response: Thank you for your advice. In our operation, the warm ischemia times were about 2 minutes and the cold ischemia times were about 45 minutes. We make sure that both OLT group and RIC group has the same ischemia times (contains total ischemia time, cold and warm ischemia time). In addition, as negative control group, we also set up sham group in order to fit the mean time of the total ischemia in OLT/RIC group to

achieve homogenization. Because we have a mature team and our team has published several studies on liver transplantation in rats. In the early stage of manuscript we considering the number of words are limited, so we didn't describe too much technical details. But your proposal is very pertinent, we have added relevant technical details in the revised manuscript edition to help reader to make a clearer understanding of this research.

Reviewer's code: 01221925

1. This is an interesting paper looking at the role of mitofusin-2 in liver I/R injury.

   Response: First of all, thank you for your encouragement.

2. Could the authors please respond to the following questions/comments?

   (1) The paper could benefit from editorial language assistance.

   Response: We have revised the whole paper to correct the grammatical and spelling mistakes carefully and obtained help from professional English language editing company (meditorexpert) which recommended by the journal to check it. The editorial certificate number is Ref. MS2017072110 and we will upload the copy of this editorial certificate to journal manuscript system.

3. (2) Why were there only male rats used?

   Response: This is a very interesting and meaningful question although it looks very simple. There is little information to answer this question in published article and I believe that many authors are difficult to answer this question although most articles recommend the use of male rats. We have a mature technicians team in rats liver transplantation, combined with our extensive experience, we recommended that male rats are

selected for the following reasons: ①. To achieve homogenization, rats were choosing with the same sex. ②. Male rats are more tolerant of surgical injuries than female rats. ③. To achieve the weight for operation (250-300g), male rats grew faster than female rats. ④. Under the same anaesthesia conditions, the anesthesia recovery time of the male rats were about 30 minutes, but the female rats were extended for about 20 minutes. ⑤. In order to alleviate the acid-base imbalance of anhepatic phase, 1ml physiological saline injection is needed. For male rats, physiological saline can be injected via the dorsal penile vein, but for female rats, we need an extra surgical procedure to injected physiological saline via jugular vein.

4. (3) Could the authors describe in some more detail their technique of remote ischemic preconditioning in this model?

   Response: Thank you for your advice. In the early stage of manuscript we considering the number of words are limited, so we didn't describe too much technical details. But your proposal is very pertinent, we have added relevant technical details in the revised manuscript edition to help reader to have clearer cognition.

5. (4) Could the authors define AML12 cells?

   Response: The AML12 (alpha mouse liver 12) cell line was established from hepatocytes from male mouse (CD1 strain, line MT42) without tumorigenic and culture properties is adherent.

6. (5) What were the cold ischemia and the warm ischemia times in this model? Both cold ischemia time (especially) and warm ischemia time are important for approximating the I/R injury of liver transplantation.

   Response: indeed, both cold ischemia time and warm ischemia time are important for approximating the I/R injury of liver transplantation. Warm ischemia times means the time from the occlusion of the portal vein to the

graft was perfused through the portal vein with cold saline containing 25U/mL heparin. Cold ischemia times means the time from the graft placed into cold saline (4°C) to the graft being transplanted into the recipient when the portal vein was opened. In our operation, the warm ischemia times were about 2 minutes and the cold ischemia times were about 45 minutes. We make sure that both OLT group and RIC group has the same ischemia times (contains total ischemia time, cold and warm ischemia time). In addition, as negative control group, we also set up sham group in order to fit the mean time of the total ischemia in OLT/RIC group to achieve homogenization.

7. (6) Why were there different patterns in the comparison between the three groups regarding the mfn2 and the MICU?

   Response: We have used three different patterns in the comparison among these three groups regarding the mfn2 and the MICUs, the purpose is to discover phenomena → prepare conditions → test hypotheses. The first is in the surgical model, we use NC (sham)/OLT/RIC to explore the changes in the MFN2-MICUS axis. The second is in the cell line to detect the knock down efficiency of siRNA (only detect the expression of mfn2). The last is in the cell line that using NC/Hypoxia/Hypoxia+si under the related anoxic cultures to explore the changes in the MFN2-MICUS axis. At last, three different patterns confirm our hypothesis.

8. (7) Here the authors present two different sets of experiments with the surgical model on one hand and the experiments using the cell line on the other. Extrapolating between the two can create questions regarding any conclusions as the two have significant differences

   Response: This is a very pertinent question. For this experiment, the best choice is to use mfn2 genetically engineered rat, but the cost is high and need long time to wait. The second choice is the primary cell which obtain

form surgical model, but the focus of this experiment is to find the role of MFN2-MICUS axis, it is hard to perform gene knockout operations using primary cells. In addition, many literature uses in vitro experiments to simulate in vivo tests is also recognized. In summary, we designed use of vitro experiments to simulate in vivo tests and to revalidate it to prove our hypothesis.

9. (8) What happened to the rest of the 30 rats if only 6 were used per group (3 groups)?

   Response: I apologize for the ignoring of details and thanks for your careful reading and proofreading. In rat RIC models, 18 of 30 rats were randomly allocated to three groups (n=6 in each group): Sham, orthotopic liver transplantation (OLT) and remote ischemic perconditioning (RIC), the rest of 12 rats were randomly allocated to OLT and RIC groups as liver donors (n=6 in each group).

Reviewer's code: 02855928

1. This experimental model involves some invasive procedures. Ethical approval number from your IRB should be clearly mentioned.

   Response: First of all, thank you for your review and proofreading. We will add the ethical approval number from our IRB in the revised manuscript edition.

2. For journal readers, protocol of analgesic agent should be mentioned.

   Response: Thank you for your comments, more details will help reader to make a clearer understanding of this research. We will add the protocol of analgesic agent in the revised manuscript edition.