Dear editors and reviewers,

Thank you very much for your transfer offer and all the valuable and insightful comments on our manuscript. We benefit a lot from these constructive suggestions. We have studied comments carefully and revised paper thoroughly. In the new version of our manuscript, some changes have been made according to the advice from the editors and reviewers.

We appreciate editors and reviewers for such warm work earnestly and wish that the quality of the revised manuscript will meet the requirements of the journal.

The main corrections in the paper and responses to comments from science editor and reviewers are as following:

Responds to the science editor's comments:

The conclusion should not be more than 30 words in the abstract section.

Figure 1a should be revised to provide more precise and less blurred microscopic images.

Response:

We greatly appreciate your advice on our paper. We have simplified the conclusion more concisely in the abstract section to less than 30 words. Figure 1a has been updated to more precise images with higher resolution.

Responds to the reviewer's comments:

Reviewer #1:

1. In abstract, authors conclude that "Regulation of PPAR-γ alleviated NAFLD by modulating the crosstalk between hepatocytes and macrophages via the ROS-NLRP3-IL-1β signaling pathway". It would allow readers to understand if the term of downregulation and types of modulation are clearly written here.

Response:

Thank you very much for your suggestion. With the words “Up-regulation of PPAR-γ activity in hepatocytes” we hope to clearly clarify the meaning of this sentence for a better understanding. We have rewritten the sentence as “Up-regulation of PPAR-γ activity in hepatocytes alleviated NAFLD by modulating the cross-talk between hepatocytes and macrophages via the ROS-NLRP3-IL-1β pathway”.

2. In the introduction, research question or gap of knowledge is partially presented. It would be helpful to state what is unknown about PPAR-g regarding interaction between hepatocyte and macrophage in NAFLD.

Response:

We have added the limitations of current study about PPAR-γ in cross-talk between parenchymal (hepatocytes) and non-parenchymal cells (mainly macrophages, HSCs, etc.) in NAFLD in the introduction.

3. The term of "NAFLD hepatocytes" need to be verified by examining lipotoxicity, including ER stress or pyroptosis or ROS as explained in the introduction. There are assays for assessing pyroptosis and ER stress.

Response:

Thank you for your insightful comments. The establishment of the NAFLD hepatocytes model in this paper is mainly based on obvious lipid deposition (Figure 1) and the concentration of FFA as an intervention has been proven in previous study （PMID: 17188672）to be the classical, least cytotoxic but optimal concentration for successful NAFLD hepatocytes model in vitro.

4. For Fig. 1, it needs to explain what good condition in the cultures of primary hepatocytes was. Please provide higher magnified image if intend to show morphology of hepatocytes. Also insert scale bars.

Response:

Thank you for your suggestion. We have added a specific description about the good status of primary hepatocytes in the “Results” section (figure 1). Based on the editor's suggestion, we have updated figure 1A with higher resolution images. The maximum magnification obtainable is 400x, which allows not only to observe the approximate good morphology of the primary hepatocytes but also to present the density and purity of the hepatocytes.

5. Given key indicator of macrophage polarization, defining M1 and M2 must be accurate. In this study, M1 and M2 cells are characterized mainly based on mRNA level. Protein expression and functional assay would emphasize the existence of M1. This can be done using an immunocytochemistry or flow cytometry of iNOS, TNFa and IL-6 for M1 and Arg1, Mrc2 and IL-10 for M2. Moreover, release of pro-inflammatory cytokines directly indicates functioning M1, which can be examined using ELISA or WB analysis.

Response:

We greatly appreciate your constructive advice on improving our researches. As you mentioned, protein level and functional assays, flow cytometry, etc. are important to demonstrate the polarization shift in macrophages. Actually, we reported macrophages polarization during the NAFLD development in our previous study (2017 scientific report), so we didn’t repeat the work in this paper.

6. For data in Fig. 1, author wrote "Lipid-laden hepatocytes promoted M1 macrophage polarization and inflammation; however, the possible pathways of signal exchange between the two cell lines were unclear". Please ensure that primary hepatocyte and macrophage cells were used.

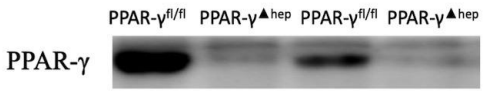
Response:

We apologize for the mistake we made here. It has been corrected in “Results” section now. We have corrected “the two cell lines” in this sentence into “the primary hepatocytes and macrophages”.

7. For Fig. 4, upregulation (? downregulation) of PPAR-γ activity in hepatocyte need to be verified. The mRNA expression of PPAR-γ in primary hepatocytes from hepatocyte-specific PPAR-γ knockout mice was fully knocked out. However, protein level of PPAR-γ need to be examined to verify the down-regulation of PPAR-γ in this study.

Response:

We have also assayed the protein level of PPAR-γ at the same time. We find one PPAR-γ western blotting strip currently on hand. It can be clearly shown that the protein expression of PPAR-γ was also completely reduced to the extent of being undetectable. It is reasonable and sufficient to conclude that PPAR-γ of hepatocytes was successfully knocked out.



8. Fig. 6, gene expression of NLRP3, Caspase-1 and IL-1β was from hepatocyte or bulk of cells in the liver genes of high-fat diet-induced NAFLD mice (Fig. 6A)? Clearly explain in the result.

Response:

The gene expression of NLRP3, Caspase-1 and IL-1β was from liver tissue (bulk of cells in the liver) of high-fat diet-induced NAFLD mice (Fig. 6A). We have added a clear explanation in the “Results” section.

9. Fig. 6, given many cell types in a liver, protein expression of NLRP3, Caspase-1 or IL-1β in the albumin-expressing hepatocytes. Also, is there any difference in the M1 polarization in the liver of high-fat diet-induced NAFLD mice and wild type mouse in this study?

Response:

Thank you for your constructive suggestion. In figure 6, we aimed to explore the possible effects of rosiglitazone on serum oxidative stress and hepatic NLRP3

inflammasome in NAFLD mice. Therefore, we did not consider changes in cell-matched markers, but focused on overall liver tissue alterations. Subsequent experiments can be considered to create a model of NAFLD mice again to extract the primary hepatocytes to detect these genes. We hope to carry out some experiments in the near future.

Actually, we reported macrophages polarization during the NAFLD development in our previous study (2017 scientific report), so we didn't repeat the work in this paper. We have addressed it in the discussion of our manuscript (Reference 17).

Reviewer #2:

1. M1 and M2 require definition, despite to be used in the past as indicators of pro-inflammatory and alternative activation of macrophages. Also, the wording in many parts of the text needs to be revised; for example, in the abstract, the authors use the construction 'manipulation of PPAR-γ'. This is not correct.

Response:

We greatly appreciate your advice. We have added the definition for macrophage M1 and M2 polarization in the "Introduction" section of our manuscript.

In addition, some inappropriate expressions in the text, such as "manipulation of PPAR-γ", have been corrected one by one.

2. Consider the use of an antagonist of PPAR-γ, in addition to the genetic models. It is known that targeting PPAR-γ alters the expression of other PPAR isoforms, in particular the delta. This needs to be assessed. One possibility is the use of antagonists.

Response:

Thank you for your suggestion. As you mentioned, an antagonist as opposed to an agonist would be a good choice in this study. Given that we have a mouse model of hepatocyte-specific PPAR-γ knockout, potential effects on other isoforms of PPAR-γ were also not considered. However, the significant PPAR-γ knockout in the present

study demonstrated significant deletion of PPAR-γ in hepatocytes, leading us to make the reliable and obvious conclusion. In future experiments, we will give more consideration to the rigorous questions you have raised.

3. Regarding the genes, authors should consider the use of standard annotations: in italics and the first letter in capital. iNOS2 is not a gene! Use Nos2 in italics. For other genes, please refer to the gene annotation, not for the protein encoded. This is general in almost all figures of the manuscript.

Response:

We are sorry for the incorrect gene annotations in original manuscript. We have corrected the gene annotations including the text and the figures according to your comments.

4. Why two activators of PPAR-γ are used? Which is the rationale? What about the viability of the cells after this strong load of FFAA? Lipotoxicity use to promote necrotic/apoptotic death in many cell types.

Response:

Thank you for your insightful comments. In vitro, we used a synthetic, non-TZD, novel PPAR-γ agonist GWA929 to upregulate PPAR-γ activity in hepatocytes. In vivo, in order to imitate the drug use condition in clinical practice, we treated high-fat diet-induced NAFLD mice with rosiglitazone to observe the effect of systemic PPAR-γ agonism. Rosiglitazone is more relevant to clinical practice, but the use of GW1929 in vitro is more reasonable for exploring the mechanism in depth.

The question about the concentration of FFA was as same as mentioned above.

5. The link between PPAR-γ and the inflammasome NLRP3 activation needs to be reinforced by pharmacological assays since it is the core of the work； Use correct works (words?) regarding PPAR-γ activity: activation, deletion, inhibition (not considered, etc.).

Response:

In the present experiments, we have shown that regulation of PPAR-γ activity in hepatocytes affects NLRP3 inflammasome expression by measuring the mRNA and protein levels of NLRP3 inflammasome in hepatocytes. Pharmacological assays between PPAR-γ and NLRP3 inflammasome are necessary to explore the in-depth relationship between them. We hope to carry out some experiments in the near future. We have carefully examined and corrected the words regarding PPAR-γ activity in the full text.

Special thanks for all constructive comments.

On behalf of all the authors of this article, I would like to express my gratitude to editors and reviewers for your profound opinions and comments again.

We look forward to hearing from you regarding our submission. We would be glad to respond to any further questions and comments that you may have.

Sincerely

The Corresponding Author

Jing Hua, MD, PhD,

Division of Gastroenterology and Hepatology, Key Laboratory of Gastroenterology and Hepatology, Ministry of Health, Ren ji Hospital, School of Medicine, Shanghai Jiao Tong University, No. 160 Pu Jian Road, Shanghai 200127, People's Republic of China