Dear Editor and Reviewers,

Thank you for your letter and for the reviewers' comments concerning our manuscript entitled "MANF ameliorates steatosis in HepG2 cells by regulating hepatic lipid metabolism" (manuscript No: 52534). Those comments are all valuable and very helpful for revising and improving our paper; they also provided future guidance to our researchers. We have studied the comments very carefully and have made the necessary corrections, which we hope that our paper more acceptable. The revised portions are marked in highlight yellow in the paper. 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. The main corrections in the paper and the responses to the reviewer's comments are as follows:

Reviewer #1:

1. *Discussion: Does MANF regulate lipid metabolism through SREBP -1 c? If so, please consider the relationship between MANF and SREBP.*

   Thank you for your kind comment. The SREBP protein is synthesized as a precursor that is attached to the nuclear membrane and endoplasmic reticulum. SREBP-1c is a major isoform related to the fatty acid metabolism in the liver and SREBP-1c overexpression raised the hepatic TG accumulation. We have found that over-expression of MANF could down regulated this gene while MANF knock-down could increase SREBP-1c expression, which implied that MANF could affect SREBP-1c expression and thus have a potential role in regulation of lipogenesis. And according your advice, we added some discussion to the Discussion part in the revised manuscript.

2. *Figure 3: In Lv-MANF+FFAs group, the content of cholesterol was decreased compared with Lv-GFP+FFAs group. However, no consideration has been given to lowering cholesterol. Please discuss the relationship between MANF and cholesterol levels.*

   We thank you for your constructive suggestion about this. As well, we add some information in the Discussion part. Since there is no related report and to our limit knowledge, our article maybe the first one found this fantastic results and more detail research need to do, we could not discuss it in deep, we hope our future research could provide more information about that.

3, 4. *Animal experiment: Please write how many weeks you kept mice. Oil red O staining: When I check the figure, I think other stains applied. Please describe the method exactly*

   We appreciate your kind reminder and have put that information in detail in the Methods part. The stain is Oil red O staining and sorry for the background contamination, we have replaced it with another high quality Fig.

Reviewer #2:

1. The author's purpose of the investigation is very interesting, also for scientists from related research fields. 1) The title should be short and concise. According to recent studies that would favor future citations to the paper. What is really new in the paper

Thank you very much for your suggestion. It is a novel role of MANF in regulating hepatic lipid metabolism and steatosis in an in vitro model of NAFLD. This study is the first to indicate that hepatic MANF expression was induced upon FFAs overload and gradually decreased thereafter. So we could not find a shorter title any more, if you have more suggestion about the title, we are glad to accept.

2. *Abstract should be also quantitative as possible for rapid comparison with similar studies. It should be a mirror of the paper. Avoid imprecise terms such as decrease or increase (but how much?). The results are not properly described. The authors should first describe in a quantitative manner the data before jump to conclusions. Avoid imprecise terms such as lower, higher, decrease, (but how much?)….. The results should be as quantitative described as possible according to data figures inserted. Avoid jumping immediately to conclusions, rather describing the data first.*

Thank you very much for your constructive suggestion. We have already revised the abstract as well as the result part to make the manuscript more informative.

3. Discussion should be more assertive and concise and eventually be divided in sections with titles highlighting the major results.

Thank you for your suggestion. We agree that highlighting titles would make the discussion more concise and easy to follow. However, we see no articles published in the journal did this (and many journals do the same way) and in order to make accordance with other articles, we just leave the divided sections and hope you could understand this. Of course, if you still think use short title in the discussion, we could add that. Thank you again.

Reviewer #3:

or a statistical analysis of the obtained data, the authors of the article used the t-test and ANOVA followed by the Tukey test to compare variables between groups. These criteria can only be used with normal distribution. Did the authors check the type of distribution? In addition, a number of features can only be compared by nonparametric criteria. Such signs include relative indicators, indices. To such data, the authors include relative gene expression, Western blot data.

Thank you very much for your suggestion. Sorry for the not so cleared statement maybe confused you. We agree with you that t-test and ANOVA can only be used with normal distribution. We definitely do the normal distribution test before t-test or ANOVA. And we did that again when revising the manuscript. And we also reedited the sentences to hope it more easily to understand. For the relative indicators, because they still normally

distributed, so we still used the parametric test and many other published articles using the parametric test to test relative indicators and we didn't see such limit (we provide some articles use the same methods as we did, please kindly find in the following references). In addition, we still compare them use the nonparametric test, and the results are the same with the parametric test, so if the editor and the reviewer all considered that these relative indicators should use nonparametrics, we will change it in the next revising edition.

Example: *relative indicators tested by parametric test like t-test and ANOVA*

1). **Goedeke L**, Bates J, Vatner DF, Perry RJ, Wang T, Ramirez R, Li L, Ellis MW, Zhang D, Wong KE, Beysen C, Cline GW, Ray AS, Shulman GI. Acetyl-CoA Carboxylase Inhibition Reverses NAFLD and Hepatic Insulin Resistance but Promotes Hypertriglyceridemia in Rodents. *Hepatology* 2018; **68**: 2197-2211

2). **Koonen DP**, Jacobs RL, Febbraio M, Young ME, Soltys CL, Ong H, Vance DE, Dyck JR. Increased hepatic CD36 expression contributes to dyslipidemia associated with diet-induced obesity. *Diabetes* 2007; **56**: 2863-2871

3). **Danilova T**, Belevich I, Li H, Palm E, Jokitalo E, Otonkoski T, Lindahl M. MANF is required for the postnatal expansion and maintenance of the pancreatic β-cell mass in mice. *Diabetes* 2019; **68**: 66-80

4). **Yang S,** Yang H, Chang R, Yin P, Yang Y, Yang W, Huang S, Gaertig MA, Li S, Li XJ. MANF regulates hypothalamic control of food intake and body weight. Nat Commun 2017; 8: 579

5). **Yan FJ**, Zhang XJ, Wang WX, Ji YX, Wang PX, Yang Y, Gong J, Shen LJ, Zhu XY, Huang Z, Li HL. The E3 ligase TRIM8 targets TAK1 to promote insulin resistance and steatohepatitis. 2017; **65**: 1492-1511

Sincerely

Lili zhang