Dear reviewers,

Thank you very much for your letter on October 11, 2017, with regard to our manuscript (Manuscript NO:35987). We greatly appreciate the comments and really have learned a lot from the reviewers' comment. Appropriate changes are made and highlighted in the revised manuscript. We made the following point-by-point responses to address the editors' and the reviewers' comments.

Reviewer 1：

Q1: Introduction: - Please, change epigenetic alterations for epigenetic mechanisms - Authors must review abbreviations list, i.e. SD in the abstract

A1: Thank you very much for the reminder. We have already changed the epigenetic alterations for epigenetic mechanisms, and the SD in the abstract has been changed to Sprague Dawley.

Q2: Material and methods:

-Please, clarify how authors isolated microRNA profile and the specific quantification method employed. Due to the small size of these microRNAs, targeted methods should be required. - Please, justify the employ of the microRNA housekeeping genes. - Please, detail the experiments performed to isolate and purify microRNAs from Huh 7 cells

A2: The qPCR methods in our manuscript are poorly described, so we have detailed the specific method for isolation and quantification of miRNA from liver tissue and Huh 7 cells. We isolated total RNA from liver tissue and Huh 7 cells using TRIzol (Invitrogen, Carlsbad, CA), and the total RNA is enriched with miRNA profile. The difference of quantification method between mRNA and miRNA is in the reverse transcription process. In the reverse-transcription reactions of miRNA, poly A modification and first-strand cDNA synthesis were performed with 500 ng of total RNA each reaction using the Mir-X miRNA First-Strand Synthesis Kit (Cat. No: 638313; Takara, Shiga, Japan). The Kit is used for converting miRNAs into cDNA to enable specific RNAs to be quantified by real-time PCR, and it contains the components needed to quantify miRNAs isolated from any source. In a simple, single-tube reaction, miRNA molecules are polyadenylated and reverse transcribed using poly(A) polymerase and SMART® MMLV Reverse Transcriptase. The SYBR

Advantage® qPCR Premix and mRQ 3' Primer are then used in real-time qPCR, along with the miRNA-specific 5' primer, to quantify specific miRNA sequences in the cDNA. The qPCR was performed in a total reaction volume of 20 µl using 2 µl of diluted (1:10) cDNA using SYBR Premix Ex Taq II (Cat. No: RR820A; Takara, Shiga, Japan). Sequences of primers used were: miR-192-5p: 5'-CTGACCTATGAATTGACAGCC-3'; U6: 5'-AGAGAAGATTAGCATGGCCCCTG-3'.

The microRNA housekeeping gene is U6 in our experiment. U6 small nuclear RNA is one of a family of U-rich snRNAs that are components of ribonucleoprotein structures known as spliceosomes involved in transcript processing, and it has been identified as housekeeping genes (*Gilson P R, Mcfadden G I. The miniaturized nuclear genome of eukaryotic endosymbiont contains genes that overlap, genes that are cotranscribed, and the smallest known spliceosomal introns.[J]. Proceedings of the National Academy of Sciences of the United States of America, 1996, 93(15):7737-42.* http://www.pnas.org/content/93/15/7737.long). Owing to its expression stability, U6 is the most widely used housekeeping gene in miRNA quantification studies in liver disease. (*Yang X, Zalzala M, Xu J, et al. A Metabolic Stress-inducible miR-34a-HNF4α Pathway Regulates Lipid and Lipoprotein Metabolism[J]. Nature Communications, 2015, 6:7466-7466.* https://www.nature.com/articles/ncomms8466 *; Ng R, Wu H, Xiao H, et al. Inhibition of microRNA-24 expression in liver prevents hepatic lipid accumulation and hyperlipidemia.[J]. Hepatology, 2014, 60(2):554.* http://onlinelibrary.wiley.com/doi/10.1002/hep.27153/abstract;jsessionid=321F4BD38AE624D0F4E106C63206182F.f02t02 *; Wang B, Hsu S H, Frankel W, et al. Stat3-mediated activation of microRNA-23a suppresses gluconeogenesis in hepatocellular carcinoma by down-regulating glucose-6-phosphatase and peroxisome proliferator-activated receptor gamma, coactivator 1 alpha[J]. Hepatology, 2012, 56(1):186–197.* http://onlinelibrary.wiley.com/doi/10.1002/hep.25632/abstract).

Q3: Results:

- Please, when referring to NAFLD in animal models, clarify if its regarding to simple steatosis or NASH, due to this disease comprised two distinct entities.
- Please, justify why after liraglutide injection, body weight decreased below control animals.
- Please, in figure 1B clarify whether hepatic TG in liraglutide vs control model was found to be statistically lower.
- Please, in figure 1D comparisons should be also performed among liraglutide and

control animals, as well as in figure 1E, protein levels.

- Please, change lipid drops by lipid droplets.
- Please, add the reference in figure 2A, y-axis
- Please, characterize the animal model, at least insert a table including biochemical and lipid panels, as well as liver evaluation.

A3: Thanks for the helpful advice. We have made the point-by-point change.

-The animal models in our experiment were all NASH models, and they have been diagnosed according to the SAF scoring system. We have changed the NAFLD in animal models into NASH.

- The liraglutide injection could suppress the appetite of rat models, so the body weight of HFD rats decreased after liraglutide injection. Compared with the control group, the body weight in liraglutide group also showed a little lower. In our study, we found the daily food-intake of rats in liraglutide group dropped dramatically, so the slight decrease of body weight in liraglutide group maybe caused by appetite repression or persistent injection stimulation.
- In figure 1C, the hepatic TG in liraglutide group showed statistically lower than HFD group, but it remained statistically higher than the control group. We have added the comparison line between the liraglutide and control groups.
- In figure 1D and 1E, the comparisons have been performed between liraglutide and control animals. Because there was no statistically difference between these two groups in figure 1D and 1E, so we didn't show the comparison line in the figure. We have supplemented the results in the manuscript in order to make it clear.
- We have changed lipid drops by lipid droplets.
- We have added the reference in figure 2A, y-axis (cell viability).
- We have characterized the animal model in the first paragraph of Results, and we have inserted Table 1 describing the biochemical parameters of animal models including liver evaluation and lipid panels.

Reviewer 2：

Q1: In the introduction, the association of NAFLD with mitochondrial dysfunction has been amply documented, and it was even related to epigenetic changes (Pirola et al., 2013)(Silvia Sookoian et al., 2016)(S. Sookoian et al., 2016)(S. Sookoian et al., 2010).

A1: Thanks for the recommendation of these references. We have learnt a lot from these studies and got a better understanding about the epigenetic modification of liver mitochondrial DNA in NAFLD. We have modified the expression in Introduction part and cited these references in our manuscript.

Q2: the role of miRs in NAFLD was found by many groups no cited in the introduction. In particular, the increase of miR-122, 194 and 34 in patients with NAFLD was previously described (cite 19), even before the citation 9 of the own authors. The power of discriminating between control, NAFL and NASH was also described in that work (cite 19). Please rephrase and cite the previous work properly in the introduction but also in the discussion, where proper credit should be given to the previous work.

A2: Thanks for the reminder of proper citation in our manuscript, and the proper credit should be given to the previous work. We have rephrased and cited the previous work properly in the introduction and discussion.

Q3: Please provide the rationale to use liraglutide as a treatment group. In addition, control group treated with liraglutide might be recommended, please explain. As the effect of liraglutide may be associated with the impairment of body weight gain, it would be important to adjust the differences in miR-192 expression and SCD-1 levels for and equivalent BMI in the rat, such as body weight divided by the distance between nose and tail. As the experiment is already performed, the body weight, although imperfect may be used.

A3: It is truly a good point that needs to be elaborate on. Liraglutide is a well-known therapeutic option for weight loss and NAFLD, so we choose liraglutide as a therapy in order to ensure weight loss and disease remission. The aim of our animal experiment is to identify the change of miR-192-5p and SCD-1 levels when animals develop NAFLD and when they are cured. The results showed that the hepatic miR-192-5p level decreased in NAFLD status and it reversed after disease remission and weight loss, which might indicate that hepatic miR-192-5p level had a negative correlation with disease status and lipid accumulation. Therefore, the use of liraglutide treatment group in our study is to identify the change of miR-192-5p levels when NAFLD develops and at remission, and supplement the evidence that hepatic

miR-192-5p levels might had a negative correlation with disease status and lipid accumulation. In order to make the rationale clear, we have rephrased the discussion and conclusion in our manuscript.

The control group should also be treated with liraglutide if the study aims to explore the therapeutic effect and functional mechanism of liraglutide in NAFLD. While our aim in this study is to identify the change of hepatic miR-192-5p levels when NAFLD develops and at remission, so we didn't treat the control rats with liraglutide. We believe that it is surely a good advice when we perform a study concerning the specific mechanism of liraglutide in NAFLD.

-The use of liraglutide in our study is to ensure weight loss and NAFLD remission, and consequently we could identify the change of miR-192-5p when animals develop NAFLD and when NAFLD is at remission. Since we were not meant to identify the relationship between liraglutide and miR-192-5p in the present study, we didn't adjust the differences in miR-192 expression for equivalent BMI or body weight. -In addition, the statistical comparison among control, HFD, and liraglutide group was made using ANOVA in our study, so the way to adjust for equivalent BMI or body weight is the stratification analysis. Concerning the study object of our study is model animals, the individual body weight differences within group were too small to make stratification analysis, so the stratification of BMI or body weight might compromise the statistical efficiency. We hope that the above two points might explain why we didn't adjust the differences in miR-192 expression and SCD-1 levels for equivalent BMI or body weight. We think that our experiment may not be optimal, but should be sufficient to identify the change of miR-192-5p when animals develop NAFLD and when NAFLD is at remission.

Q4: The mimics and antimiR used are poorly described. Please provide more details. If the sequence is known add it to the MS. In the case, it is commercially protected, provide more details as to how to obtain them. The qPCR are also poorly described, and more details about the specific assay should be provided.

A4: The sequences of the miR-192-5p mimics, inhibitors, SCD-1 siRNAs and their respective negative controls we used are commercially protected, so we have added the Cat. No of them, and how we obtain them. Meanwhile, we have provided detailed information about the method, specific assay No., and primer sequence used in qPCR

analysis.

Q5: There are some typos, i.e. Kuels is Keuls. please revise.

A5: Thanks for the reminder. We have reviewed the manuscript and changed these typos.

Q6: References suggested: Pirola, C. J., Fernandez Gianotti, T., Burgueño, A. L., Rey-Funes, M., Loidl, C. F., Mallardi, P., … Sookoian, S. (2013). Epigenetic modification of liver mitochondrial DNA is associated with histological severity of nonalcoholic fatty liver disease. Gut, 62(9). https://doi.org/10.1136/gutjnl-2012-302962 Sookoian, S., Castaño, G. O., Scian, R., Gianotti, T. F., Dopazo, H., Rohr, C., … Pirola, C. J. (2016). Serum aminotransferases in nonalcoholic fatty liver disease are a signature of liver metabolic perturbations at the amino acid and Krebs cycle level. American Journal of Clinical Nutrition, 103(2). https://doi.org/10.3945/ajcn.115.118695 Sookoian, S., Flichman, D., Scian, R., Rohr, C., Dopazo, H., Fernández Gianotti, T., … Pirola, C. J. (2016). Mitochondrial genome architecture in non-alcoholic fatty liver disease. The Journal of Pathology. https://doi.org/10.1002/path.4803 Sookoian, S., Rosselli, M. S., Gemma, C., Burgue?o, A. L., Fern?ndez Gianotti, T., Casta?o, G. O., & Pirola, C. J. (2010). Epigenetic regulation of insulin resistance in nonalcoholic fatty liver disease: Impact of liver methylation of the peroxisome proliferator-activated receptor ? coactivator 1? promoter. Hepatology, 52(6). https://doi.org/10.1002/hep.23927

A6: Thanks for the suggestion of these references. They are helpful for us to get a better understanding about the epigenetic modification of liver mitochondrial DNA in NAFLD. We have modified the expression in our manuscript and cited these references properly.

We believe that the comments by the respected reviewers and editor will surely improve our revised presentation, and we feel that these changes are more clear and persuasive. We hope the reviewers agree with our answers and the new version of this manuscript meets the standard of the prestigious journal of World Journal of Gastroenterology. Thank you very much for your kindly consideration.

With best regards.

Yours Sincerely,

Jian-Gao Fan