Response to Reviewer Comments

Dear Editor

We thank the reviewers for their generous comments regarding the manuscript. We have tried to answer the comments of the two reviewers and have edited the manuscript to address their concerns.

We believe that the manuscript is now suitable for publication in World Journal of Hepatology.

Reviewer #3472:

Kristiansen MNB et al, have investigated histological and biochemical analysis in two NASH models in mice. These data have been published in other journals. In addition, results presented here did not correspond to the aim of this study.

1) *Although the authors performed extensive analysis using two animal models, the merit to perform liver biopsy is not well documented in the present study. The authors only described histological and biochemical data in both models.*
   We acknowledge this comment and have now specified in the manuscript - the rationale for performing a baseline liver biopsy for individual disease staging and precise stratification and randomization into putative treatment groups.

2) *Study design is complicated. In addition, the purposes of carboxymethyl cellulose and subcutaneous injection of BSA were not mentioned.*
   We acknowledge this comment, and agree that the study design is complicated. We have outlined and explained the study design in more details and highlighted that the purpose of performing the dosing regimen was to test "vehicle" dosing including daily animal handling (stress) in these models. These models are designed for testing of novel NASH therapeutics, albeit the present study did not investigate pharmacological intervention.

3) *The author used 111 mice in DIO-NASH (Fig 1). Why the authors used too many mice?*
   We acknowledge this comment. In our preliminary model testing we have observed a heterogeneous NASH development and metabolic variation in DIO-NASH mice. The animals enrolled into the study period are representative for the DIO-NASH and ob/ob-NASH model. To avoid any confusion, we have edited the baseline steatosis score and fibrosis stage in Figure 1, to only conclude the specific animals used for the studies described in this paper. For the specific studies *n*=8-12 animals were used as indicated under results.

4) *The authors harvested 50 to 100mg liver tissue under general anesthesia. This size of tissue means partial hepatectomy rather than biopsy in mice. Thus, the authors take account for the effect of liver regeneration at the second evaluation of tissue.*
   This is a valid point. The edge of the liver contain more collagenous tissue, thus a cone shaped wedge was harvested. Previous studies harvested ~50 mg of liver tissue, from the left lateral lobe of mice. The size of the liver biopsy harvested was correlated with the size of the liver of the given mice, e.g. liver biopsy of lean chow was smaller than liver biopsy of ob/ob-NASH. The size of the biopsy was ~50-100 mg in order to avoid biased results as one could extract a bile duct or vessel in a needle biopsy and thereby the quantitative analysis would be biased.
   Clapper et al 2013 (AJP) performed studies on regeneration of the liver and found that the biopsy site appeared to have been replaced by collagenous scar tissue, however the remaining liver tissue did not seem to be affected[1].

5) *The author harvested edges of the left lobe whereas the right lobe is generally selected for liver biopsy in human. The author should add experiments whether left and right lobes have any difference in disease progression.*
   During optimization of the NASH model liver homogeneity was investigated in five NASH-mice. The inter lobe variation (left lateral lobe, median lobe and right lobe) and the intra lobe variation

(different levels of the lobe) was investigated using the following set-up: From each of the five livers, the left lateral lobe, the median lobe and the right lobe was isolated. The lobes were sampled using systematic uniform random sampling. Sections were stained with Sirius Red, for collagen quantification, and with H&E, for steatosis quantification. Collagen and H&E was quantified using image analysis. Results from the different lobes were compared and results from the different levels of the lobe were compared. These data are not shown in the manuscript.
In this study we found no difference in fibrosis amount or steatosis amount between the left lateral lobe, the median lobe or the right lobe neither did we see any differences between the levels of the lobes.

6) *Liver biopsy also helps the assessment of disease progression in individual. However, this information is lacking in the present study.*
We acknowledge this comment. The aim of this study was not to assess disease progression based on liver biopsy i.e. baseline vs. post-study. In contrast, the purpose of this study was to precisely enroll animals based on individual disease staging and to randomize the animals in intervention groups based on steatosis score and fibrosis stage.
It will be of interest in future studies to explore whether a more clinical approach can be applied to the present NASH mouse models – i.e. performing NAFLD Activity Score including fibrosis stage concomitantly in the baseline and post-study biopsies for evaluation of NASH "resolution" and fibrotic "worsening" as derived from the clinical endpoints.

Reviewer #1566894:

This is interesting but very descriptive. Two major concerns are noticed.

1. *Authors should provide all RNAseq data in the supplemental results.*
We acknowledge this comment and have provided additional RNAseq data in the supplemental results.

2. *Professional editing is strongly encouraged.*
The paper has been edited by a native English speaker.