Responses to the reviewer's comments

We wish to thank the reviewer for the valuable comments. Changes made in accordance with the comments are underlined in the revised manuscript. Please find below our point-by-point responses.

Reviewer# 2462668

This manuscript investigates the relationship between autophagy and lipotoxicity in both in vivo and in vitro models of NASH. This paper contains fascinating and novel data for understanding of pathophysiology of NASH; however there are some important issues that need attention.

Major comments:

1. Authors have to state why they chose AML 12 cell line.

Response: We confirmed autophagic status in the NASH mouse model in the study. To clarify the mechanism of the impaired autophagic process in cells in detail, we employed hepatocytes from same mouse species used in the *in vivo* experiment. Therefore, we used AML 12 cells. According to the reviewer's suggestion, we have mentioned the reason for employing AML 12 cells in the revised manuscript (line 13, page 7).

2. N values have to be mentioned for all experiments.

Response: We have presented the N values for all animal experiments. Additionally, we have added the number of repeats for each experiment in the *in vitro* study.

3. All results should be described in the Results and in the legends to figures (e. g. in the legend to figure 1D, p-JNK expression is missing, in the legend to figure 3 B, CHOP expression is missing; in the legend to figure 4A, p-c-jun expression is missing, description expression of Rubicon and JNK in the Results…)

Response: We have added accurate descriptions of all protein expression data in the figure legends and in the manuscript.

4. Sentence on page 12, line 16-18 should be revised. You have to state time of incubation and concentration of PA used in the experiment. Moreover, your statement, that 800 uM PA decreased Rubicon expression, is not true in general. It is true only in

some time intervals.

Response: For other investigators to recapitulate our experiments, we should indeed provide detailed descriptions of the conditions used in our study. Thus, we have mentioned such details in the revised manuscript per the reviewer's suggestion (line 6, page 13).

5. Why did authors choose 10 hour incubation interval (with 800 uM PA) for evaluation of the effect siRubicon on Rubicon expression? Expression of Rubicon is already lowered in this time interval.

Response: Thanks to the reviewer's comment, we noticed we had made a typographical error: the siRubicon experiment presented in Figure 2F was conducted using 4-h incubation. We have corrected the figure legend accordingly.

Minor comments:

1. You state that the age of mice at sacrifice was 20 weeks. Wasn't it 19 weeks? (6 weeks + 1 week of habitation + 12 weeks of experiments)

Response: We misrepresented the age of the mice. They were 5 week old at the start of the experiment. We thank the reviewer for pointing this out and have corrected this in the revised manuscript.

2. qRT-PCR is not quantitative reverse transcription but "quantitative real time"

Response: The widely accepted Minimum Information for Publication of Quantitative Real-Time PCR Experiments (MIQE) guidelines (http://www.clinchem.org/content/55/4/611.long) propose that the abbreviation "**qPCR**" be used for quantitative real-time PCR and that "**RT-qPCR**" be used for reverse transcription–qPCR. Therefore, we have chosen not to use the definition suggested by the reviewer, but we have changed the abbreviation (RT-qPCR) in accordance with that proposed in the MIQE guidelines.

3. Authors have to correct sentence on page 11, line 19-20 (Expression of the autophagy inhibitor Rubicon was significantly lower in the HFD mice than in the CT mice). It is vice versa.

Response: We thank the reviewer for pointing this out and have corrected this.

4. Authors have to correct sentence in figure 2 legend (page 25, line 15 – "and induces but impairs").

Response: PA induces initiation of autophagy, but inhibits progression of autophagy via Rubicon expression. Thus, we have changed the sentence as follows:

"Treatment with PA induces apoptosis in a dose- and a time-dependent manner, and initiates but impairs the autophagic process in AML12 cells."

5. Unify abbreviations (JNK vs. c-jun).
Response: JNK indicates c-jun N-terminal kinase, while c-jun is a transcription factor of JNK. In the original manuscript, we used "JNK phosphorylation" for both phosphorylated JNK and JNK signaling. To clearly distinguish these two different meanings, we have changed "JNK phosphorylation" to "JNK signaling" where appropriate.

6. Authors have to correct SP60012 on page 13, line 20.
Response: We have corrected this.