

Reviewer 1#:

Q&A:

Q:1. While the authors suggest that “Hepatic TM6SF2 levels are elevated in both NAFLD patients and mouse NAFLD models” in the first line of Results, they claim “In vivo and in vitro experiments confirmed that TM6SF2 knockdown increases intracellular lipid deposition”. There seems to be confusion with these opposing statements and needs clarification.

A: 1. In the course of our experiment, we found that wild type TM6SF2 protein improves NAFLD phenotype than the E167K TM6SF2 does in hepatic models of overexpression, suggesting the elevation of TM6SF2 plays an “anti-NAFLD” in the condition of overnutrition. These results were designed to demonstrate in our next article. Considering that the reviewers have questions about this, we decided to add parts of results to this article in order to reveal the role of reactive TM6SF2 overexpression.

Q:2. The primer sequence listed for Human TM6SF2 in the Supplementary table are F: 5'-GCATTGATGAGCGCCCTAATC-3' and R: 5'-AGTGGGTCATAGGAGACCTCG-3'. Both these primers are designed in Exon 2 of the gene. Usually, it is a norm to design the primers for qRT-PCR in the intron-exon boundaries or two different exons with an intervening large intron to avoid amplification from residual DNA in the converted cDNA. How would the authors justify the expression?

A:2. We extracted the RNA of cells by the standard Trizol method, during the process, all DNAs of cells will fall into the white layer between the RNA layer and the pink liquid layer. We are very careful when removing the supernatant to another 1.5 mL tube to ensure that the 260 / 280 value lies between 1.80-2.00. Therefore, there is no need to consider the problem of background DNAs when designing primers.

Q3: In Figure 1A, the authors depict the hepatic mRNA levels of TM6SF2 in liver specimens of Healthy subjects and subjects with simple steatosis or nonalcoholic steatohepatitis. They suggest that the expression of TM6SF2 was normalized to ACTB mRNA levels. It is a norm to represent the relative gene expression as fold change. It is confusing that they have represented as relative mRNA levels. How did they quantify the mRNA? They have to either change the representation in the figure or write the method clearly in the Figure legend.

A3: This is a mathematical problem. We choose the TM6SF2 level of one patient as the reference, and the TM6SF2 levels of all other patients are presented in several times of this reference.

Q4: Minor Comments In Abstract – Background third line add “in”, the sentence should read “resulting in no therapeutic strategy ...”

A4: Revisions have been made in the manuscript as requested.

Q5: In Abstract – Method – The method of evaluation of TM6SF2 expression in liver samples collected from both NAFLD mouse models and human subjects must be mentioned.

A5: Revisions have been made in the manuscript as requested.

Q6: In Abstract – Results – The number of liver samples collected from NAFLD patients and mouse models should be mentioned.

A6: Revisions have been made in the manuscript as requested.

Q7: The authors mention that the hepatic expression of TM6SF2 are elevated in both mouse models and human tissues. It is suggested to give the basis for this interpretation (Fold change, IHC result).

A7: Revisions have been made in the manuscript as requested.

Q8: In introduction, the authors have identified HSD17B13 gene as conferring susceptibility to NAFLD, while it is reported to protect against the phenotype.

A8: Thank for your preciseness, we have substitute HSD17B13 with PPARGC1A.

Q9: Main Text Materials and Methods The method of samples collection (RNA Later, TRIZOL, Snap frozen, FFPE etc.) of Liver specimens must be mentioned.

A9: Revisions have been made in the manuscript as requested.

Q10: Was the diagnosis of NAFLD in these specimens made by a single or multiple pathologists? This must be mentioned. Were the samples blinded for each of the pathologists if multiple pathologists have screened the sections.

A10: Revisions have been made in the manuscript as requested.

Q11: Bioinformatic analyses The software used to analyze the data sets is not mentioned. In the microarray analysis the authors set the cut off to identify

DEGs at a fold change of >1.2. Usually it is set at 2. How do the authors justify a more relaxed cut off?

A11: “edgeR” package in R platform was used to acquire the DEGs based on the R studio platform (R version 3.6.1). We once set this value as 2, but there were not enough genes for KEGG pathway analysis, so we set a more relaxed cut off for pathway analysis.

Review 2#:

Q:My most relevant comment is that Authors found that in liver tissue of NAFLD patients, TM6SF2 is overexpressed, while downregulation of the same gene leads to steatosis in mice and cell lines. These two results seem contradictory, and Authors did not comment on, nor tried to explain the result.

A: 1. In the course of our experiment, we found that wild type TM6SF2 protein improves NAFLD phenotype than the E167K TM6SF2 does in hepatic models of overexpression. These results were designed to demonstrate in our next article. Considering that the reviewers have questions about this, we decided to add some results to this article in order to answer the role of reactive TM6SF2 overexpression.

Minor comments:

Q1: Abstract: please explain the meaning of ACC.

A1: Revisions have been made in the manuscript as requested. Acetyl CoA carboxylase (ACC) is the rate-limiting enzyme of SPREBP1c/ACC pathway as demonstrated in Figure 7.

Q2: Some linguistic corrections are necessary (see for example page 4: “the enhanced the processes”).

A2: Revisions have been made in the manuscript as requested.

Q3: Page 4: the statements in the last 7 lines of the Introduction should be supported by references.

A3: Revisions have been made in the manuscript as requested.

Q4: Figure 1B: please explain the meaning of GSE abbreviations.

A4: Revisions have been made in the supplementary part as requested.