Responses and revisions

We would like to thank the reviewers for their encouraging comments and time to review our manuscript. We have carefully revised the manuscript accordingly, made point-by-point responses to each of reviewer's comment as detailed below, and included new data and references per reviewer's suggestions. Revised portions are marked in red in the revised manuscript. We believe that our work is a part of important journey to reveal the pathogenesis and our findings provide new and important information for this field. We appreciate the reviewers' comments, which helped us improving the significance of the manuscript.

Reviewer #1

Scientific Quality: Grade B (Very good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Minor revision

Comment-1: *Authors of this work have done a good job to relate TGFβ1 pathway and GLUT1 expression in liver fibrosis. This paper reveals new mechanisms involved in liver fibrosis which are mediated through nonparenchymal liver cells. Cannonical TGFβ1 pathway includes the targeting of SMAD4 by SMAD2 and SMAD3 proteins to form the the SMAD complex. This is not mentioned in the manuscript, please correct.*

Response-1: We thank the reviewer so much for the valuable comments

and the appreciation of our work. This has been corrected.

Yes, SMAD4 is very important in cannonical TGFβ1 pathway. We revised the sentence (in original Introduction) per suggestion. The new sentences are: “In the canonical TGFβ1/mothers-against -decapentaplegic-homolog 2/3 (Smad2/3) pathway, the ligands induce the assembly of the TGF β 1 receptor I (T β RI)/TGF β 1 receptor II (T β RII) heterocomplex, targeting of SMAD4 by SMAD2 and SMAD3 proteins to form the SMAD complex, leading to the phosphorylation and nuclear translocation of Smad2/3; and this R-Smad/Co-Smad4 complex translocates to the nucleus, where it binds to DNA either directly or in association with other DNA-binding proteins”. Please see page 5-6, line130-137.

Comment-2: *The sentence “However, the expression pattern and underlying mechanism of GLUT1 remain unclear “ and “This study first demonstrated that the GLUT1 expression was significantly increased in human and mouse liver fibrosis “ are partly true. The role of GLUT1 transportation through exosomes in HSCs has been studied. Wan et al. study (2019) entitled “Exosomes from activated hepatic stellate cells contain GLUT1 and PKM2: a role for exosomes in metabolic switch of liver nonparenchymal cells“ has revealed that activated HSCs by Hif-1 release exosomes which contain GLUT1. This mechanism is responsible for the metabolic switch of HSCs and other liver nonparenchymal cells.*

Please discuss.

Response-2: Thanks for the input and suggestion. We agree that Studies by Wan et al should be cited and are important to support our current work. To address this, First, we have deleted the "first" in the original sentence, and cited the work by Wan et al work to support our study. The revised sentence in the Discussion section is as the following: "This study has showed a significant increase in GLUT1 expression in human and mouse fibrotic liver tissues, which is consistent with the research results of Wan et al[24]." Please see revised "Discussion , page 18, line 520-522. Second, we revised the Introduction section and included the research results by Wan et al, as the following "Exosomes secreted by activated HSCs affect the metabolic switch of liver nonparenchymal cells via delivery of glycolysis-related proteins GLUT1 and PKM; GLUT1 is involved in metabolic reprogramming of hepatic stellate cells[24]". Please see revised "Introduction, page 7, line 173-176".

Comment-3: *Indicate which version of SPSS has been used during the statistical analysis.*

Response-3: We have used SPSS 17.0 during the statistical analysis. We have clarified the version of SPSS in the revised Statistical analysis section (please see page 12, line 328).

Comment-4: *In statistical analysis section, mean ±square error was used to express mean tendencies between groups, but in figures one sees that*

mean±SD was used. Please clarify!

Response-4: Thanks so much for the detailed checking. We apologize for the typo error, and it was corrected in all figures (to mean±SE).

Reviewer #2

Scientific Quality: Grade B (Very good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Minor revision

Comment-1: *This reviewer missed a toxicity analysis to support that RNA interference does not produce any effects on cell viability and proliferation rates, as well as the expression of relevant targets (TGF-beta receptors).*

Response-1: Thank you very much. The comments are all valuable and very helpful for revising and improving our paper. There was no noticeable effect of control siRNA on proliferation between TGF-β1 treated cells and TGF-β1/siRNA-control treated cells, and between control/saline treated cells and saline/siRNA-control treated cells (Fig. 5E). In addition, there is no noticeable effect of siRNA interference on cell viability (Supplementary figure S1) and expression of TGF-β receptors (Supplementary figure S2). We believe that RNA interference itself (control siRNA) does not produce any effects on cell viability, proliferation, and TGF-beta receptors.Please see page 9-10, line 249-263

Comment-2: *This reviewer missed a more detailed explanation about the*

procedure used to verify the purity of isolated HSCs.

Response-2: A great question, thanks. We clarified the procedure used to verify the purify of isolated HSCs in revised "Cells and cell culture" section under the "Materials and methods". We used Desmin expression (positive selection), and (without) E-cadherin expression (negative selection), to verify the purity. Please see page 9-10, line 249-263).

Comment-3: *The rationale for the selection of the TGF-β1 dose (3 ng/ml) is missed.*

Response-3: Thank you for pointing out this. This dose was selected based on the observation that doses from 3 to 5 ng/ml had similar effects on GLUT1 protein levels (Supplementary Figure S3). Hence, a concentration of 3 ng/ml TGF-β1 was used for subsequent experiments. We have clarified this and included the results for the selection as Supplementary Figure S3. Please see page13, lines 364-368.

Comment-4: *Please checked out all the Supplier' names, to get a uniform form throughout the text.*

Response-4: Thanks to the reviewer's question. We have unified the name of the reagent suppliers (please see page 8, lines 197-207). Thank you.

Comment-5: *Despite the relevance of the topic, the number of references corresponding to the last three years did not exceed 25%*

Response-5: Thanks for the suggestion. We have update and included the

literature of more recent studies to described published knowledge related to the topic in revision. A total of 5 new reports in the last 3 years have been included in updated references. Out of a total of 43 literatures, 14 are in the last 3 years now. Please see updated references in revision.

Comment-6: *Please check out the text for grammar.*

Response-6: Thanks. We have revised the manuscript thoroughly and tried to catch any grammar or syntax errors at our best efforts. as well as colloquial terms and have also involved native English speakers for language correction.We really hope that the language and grammar level have been substantially improved.All the grammatical and language changes have been highlighted in blue in the text.

Reviewer #3

Scientific Quality: Grade B (Very good)

Language Quality: Grade A (Priority publishing)

Conclusion: Minor revision

Comment-1*: This work presented by Ming-yu Zhou, et al. is clear and has a well-funded reasoning. The manuscript is well written. I have only some minor comments. 1. Figure 1E. quantifying the changes with software.*

Response-1: Thank you so much! Revision quantified the results in

Figure 1E showing the changes in revised Figure 1 F. Thanks again!

Comment-2*: Figure 7. add Figure legends; change "Figur7" to "Figure 7".*

Response-2: Thanks! Revision corrected this and added a brief legend for Figure 7.

Comment-3*: Materials and methods: provide sequences of siRNAs and primers used in this study.*

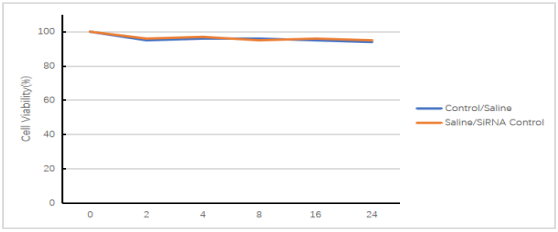
Response-3: Thank you! We have provided the sequences of siRNAs and primers in new Table 1 and new Table 2 in revision.

Science editor

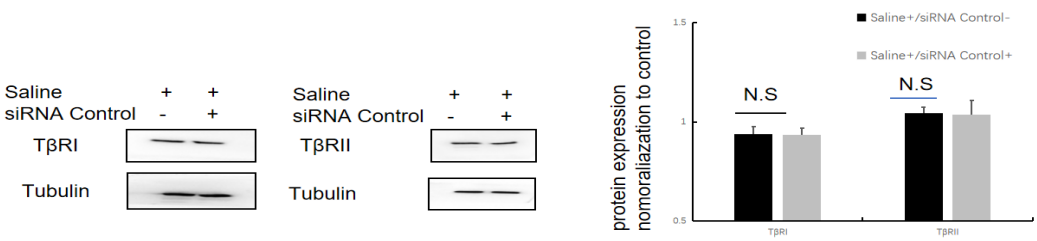
Comments: *(1)The title is too long, and it should be no more than 18 words; (2) The authors did not provide the approved grant application form(s). Please upload the approved grant application form(s) or funding agency copy of any approval document(s); (3) The authors did not provide original pictures.*

Responses: Thanks to the science editor's comments. We have made point-by-point responses to the three reviewer's comment. The number of literatures closely related to this topic has been updated to 43, and 13 in the last 3 years. We have shortened the title of the manuscript. "Transforming growth factor beta-1 upregulates GLUT1 and glycolysis through canonical and noncanonical pathways in hepatic stellate cells".

We have upload the approved grant application form(s) and funding agency copy of approval document(s). We have provided original pictures that can be reprocessed. The file name was “67468-Figures.pptx”



Supplementary Figure S1: Cell survival was measured with cell survival assay kit and Trypan blue stain



Supplementary Figure S2: Effect of siRNA interference on TGF-β receptors. Data reported as the mean ± se of 3 independent experiments (N.S: not significant; Student’s t test)
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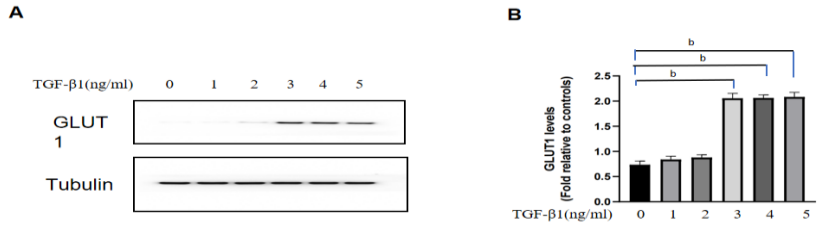

Supplementary Figure S3:A. Quantitative analysis ofGLUT1 protein levels and representative western blot showing the expression of GLUT1 . **B.**The values are expressed as the fold induction compared to untreated cells. The data represent the mean ± se for each condition ($^{b}P < 0.01$ determined by Student's t test).

Supplementary Table 1 Primer sequences for Real-time PCR

Gene	Forward sequence	Reverse sequence
HK2	5'-GGGTAGCCACGGAGTACAAA-3'	5'-TGGATTGAAAGCCAACTTCC-3'
GLUT1	5'-GGCTTCTCCAACTGGACCTC-3'	5'-AAGAAGAGCACGAGGAGCAC-3'
PKM2	5'-TGGGATGGAAACTGTGAAGAG-3'	5'-CGGAGTTCCTCGAATAGCTG-3'
α-SMA	5'-AAGAGCATCCGACACTGCTGAC-3'	5'-AGCACAGCCTGAATAGCCACATAC-3'

Supplementary Table 2 Sequences for transfection

siGlut1-1 Forward:
5′-CACCGGGAGTGACAAAGACTTTGTTCAAGCA-3′
Reverse:
5′-GATCCAAAAAAGGGAGTGACAAAGACTTCTC-3′
Negative control
Forward:
5′-ATCCGACTTCATAAGGCGCATGCT-3′
Reverse:
5′-AGTATTCCGCGTACGAAGTTCTGC-3′

siRNA targeting four different sequences of Smad4 :
(GCAAUUGAAAGUUUGGUAA,
CCCACAACCUUUAGACUGA,
GAAUCCAUAUCACUACGAA, and
GUACAGAGUUACUACUUAG)
purchased from Santa Cruz Biotechnology

siRNA Smad2/3
sc-37239 : Smad2/3 siRNA (m) is a pool of 4 different siRNA duplexes:
sc-37239A:

- Sense: CUUGCUGGAUUGAACUUCAtt Antisense: UGAAGUUCAAUCCAGCAAGtt

sc-37239B:

- Sense: CCGUCGUAGUAUUCAUGUAtt Antisense: UACAUGAAUACUACGACGGtt

sc-37239C:

- Sense: CUGACUCCUUGUUUAAUGAtt Antisense: UCAUUAAACAAGGAGUCAGtt

sc-37239D:

- Sense: GGAAGCUGAGAGUUAUAGAtt Antisense: UCUAUAACUCUCAGCUUCCtt

purchased from Santa Cruz Biotechnology