Dear Science Editor Jia-Ping Yan:

Thank you for your letter and for the reviewers' comments regarding our manuscript entitled "MiR-194 inactivates hepatic stellate cells and alleviates liver fibrosis by inhibiting AKT2" (Ref. No.: 47875). The comments are all valuable and were very helpful for improving our paper. We have made corrections as suggested by the reviewers and here by submit a revised manuscript. Corrections in the paper and responses to the reviewers' comments are listed below. We hope that the manuscript will now be accepted for publication in World Journal of Gastroenterology.

Corresponding author: Mingyi Xu, E-mail: xumingyi2014@163.com.

Once again, thank you very much for your comments and suggestions.

With all my best regards!

Sincerely yours,

Mingyi Xu

Revision Notes

The following is a point-by-point response to the reviewers' comments.

Reviewer 1：

In this manuscript, the authors at first reported that miR-194 down-regulated AKT2/cyclin D1 expression, which in turn suppressed G1/S transition in hepatic stellate cells leading to the reduction of α-smooth muscle actin and type I collagen expressions. After that, it was revealed that the introduction of miR-194 agomir through the tail vein diminished liver fibrosis in CCl4-treated mice in association with the reduction of AKT2/cyclin D1 expression. The strategy is straightforward, and the results are clear. However, most of the results are simply followed the observations that has been reported and expectable. The authors should focus the presentation on their original observations.

R1-1 The authors should summarize the story described above based on the literature in detail in the introduction section and focused in the results and

discussion sections on in vivo study using miR-194 agomir.

Answer: Thank you for kindly reminding us this point. We summarized our research in the introduction section as required. In page 5, line18-28: "Based on these previous findings, we examined the expression of miR-194 in experimental liver fibrosis models in vivo and in vitro, which showed that miR-194 was significantly downregulated in activated HSCs. Additionally, we investigated the role of miR-194 in HSCs. Our results showed that miR-194 attenuated the activation and proliferation of HSCs by suppressing AKT2. To explore the clinical implications, we reintroduced miR-194 in a mouse model of CCl_4-induced liver fibrosis by tail vein injection of a miR-194 agomir. In vivo application reduced the expression of AKT2 and ECM markers, and led to the recovery of fibrosis. Taken together, our results suggest that miR-194 can inhibit the activation and proliferation of HSCs by suppressing AKT2. Reintroduction of miR-194 might be a potential novel therapy for the treatment of liver fibrosis."

R1-2 Please describe the sequences used in mrR-194 and AKT targeting studies to specify OV-miR-NC, si-NC, respective controls, and so on.

Answer: All synthesized oligonucleotides in this study were listed in Suppl Table 3 in Supplementary materials.

Suppl Table 3 Synthesized oligonucleotides in this study

miR-194 mimics	F：5′ -UGUAACAGCAACUCCAUGUGGA-3′
	R：5′ -CACAUGGAGUUGCUGUUACAUU-3′
NC mimics	F：5′ -UUCUCCGAACGUGUCACGUTT-3′
	R：5′ -ACGUGACACGUUCGGAGAATT-3′
miR-194 inhibitors	F：5′ -UCCACAUGGAGUUGCUGUUACA-3′
NC inhibitors	R：5′ -CAGUACUUUUGUGUAGUACAA-3′
si-AKT2	F：5′ -CGUGGUGAAUACAUCAAGATT -3′
	R：5′ -UCUUGAUGUAUUCACCACGTT-3′
si-NC	F：5′ -UUCUCCGAACGUGUCACGUTT-3′
	R：5′ -ACGUGACACGUUCGGAGAATT-3′
NC agomir	F：5′ -UUCUCCGAACGUGUCACGUTT-3′
	R：5′ -ACGUGACACGUUCGGAGAATT-3′
miR-194 agomir	F：5′ -UGUAACAGCAACUCCAUGUGGA-3′
	R：5′ -CACAUGGAGUUGCUGUUACAUU-3′

R1-3 English should be revised.

Answer: The paper was revised with the help of the language editing company (AJE). We hope the reviewers will satisfy with the changes made in the revised manuscript.

R1-4 Liver cirrhosis is not an irreversible situation.
Answer: The sentence "However once cirrhosis formed there was no effective treatment to reverse the process of liver fibrosis." used is not appropriate, it should be "However, once liver cirrhosis develops, the process is difficult to reverse". We changed the sentence in the revised paper in page 12 line 13.

R1-5 Do not use an abbreviation from the first description.
Answer: Thank you for carefully review. We changed them in the revised paper.

Reviewer 2：

In this paper, the authors demonstrated the role and, at least partially, the mechanism of action of miR-194 as an inhibitor/control of liver fibrosis. The designs of all experiments were very elegant. The data demonstrated in this paper could be a promising new therapeutic approach of hepatic fibrosis.

R2-1 At Abstract (Results line 3): "suppressed cell viability" or "suppressed cell proliferation"?
Answer: Thank you for kindly reminding us this point. We changed it in the revised paper in page 3 line 21-22: "suppressed cell proliferation in HSCs by causing cell cycle arrest in G0/G1 phase".

R2-2 The text was written very objectively and in some parts it could a little hard to understand.
Answer: Thanks for the comment on the language of our paper. Spelling and grammar were modified throughout the manuscript.

Reviewer 3：

This work provides data about the protective role of miR-194 in liver fibrosis. The authors demonstrate its involvement in the development of liver fibrosis and a potential therapeutic role for the treatment of this pathology.

R3-1 Overall, the results support the conclusions, but sometimes it is not clear the understanding of the phrases. English revision is mandatory.
Answer: Thanks for the reviewer's valuable suggestion. Our manuscript was polished by a professional English-language editing company.

R3-2 The final phrase "In conclusion, miR-194 was essential in the development of liver fibrosis" is wrong. The correct version could be "In conclusion, miR-194 deregulation was essential in the development of liver fibrosis".

Answer: Thank you for pointing out the error. We changed it in the revised paper. In page 13 line 24-25: "In conclusion, miR-194 deregulation was essential in the development of liver fibrosis."

R3-3 Figure 1E, the q-pHSC image is shown at a magnification that is different from the others

Answer: Thanks for the careful review opinion. The q-pHSC used in our experiment were stained at 8 hours after isolation when cells had attached. The a-pHSC were stained at 7 days after isolation. The q-pHSC may not be fully extended and the nuclei became large during culture-activated, which might cause this confusion. Our IF images were similar as the previous literature [Thoen L F R et al, Journal of Hepatology, 2011, 55(6):0-1360; Ma MZ et al, Galler K et al, Integr. Biol. 2014, 6(10):946-956].

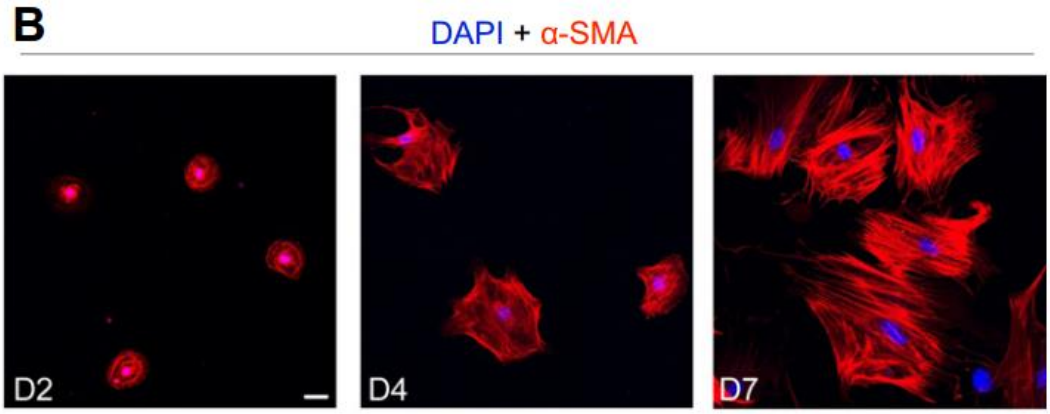

Figure A Fluorescence images of mouse pHSC [Thoen L F R et al]

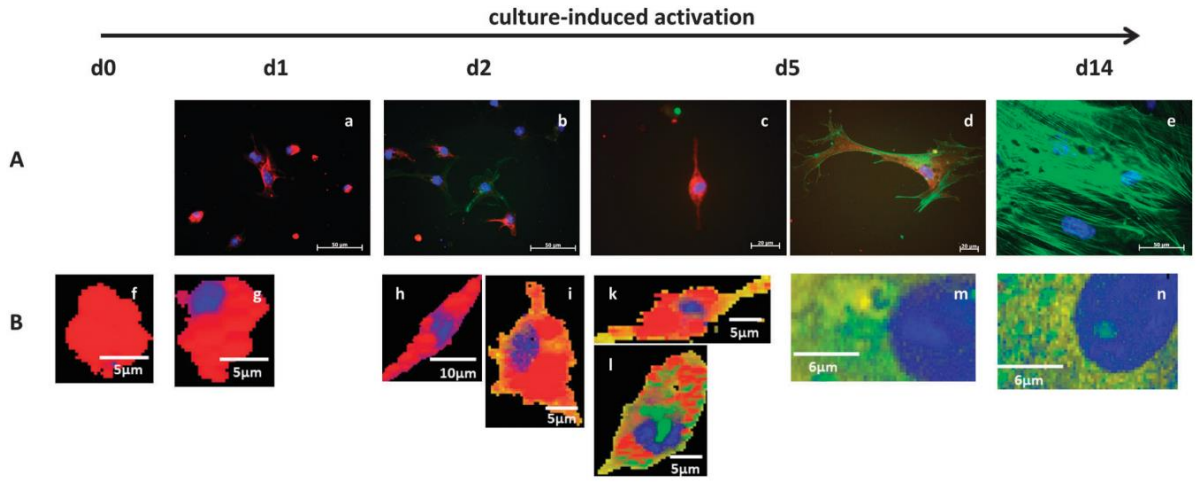

Figure B Fluorescence images of mouse pHSC [Ma MZ et al]

R3-4 Figure 3B, cell cycle graph, the legend of colours is lacking (e.g. what does red refer about?)

Answer: Thank you for kindly raising this important issue. The legend of colours in cell cycle graph was added in Figure 3B and Figure 4E in revised manuscript.

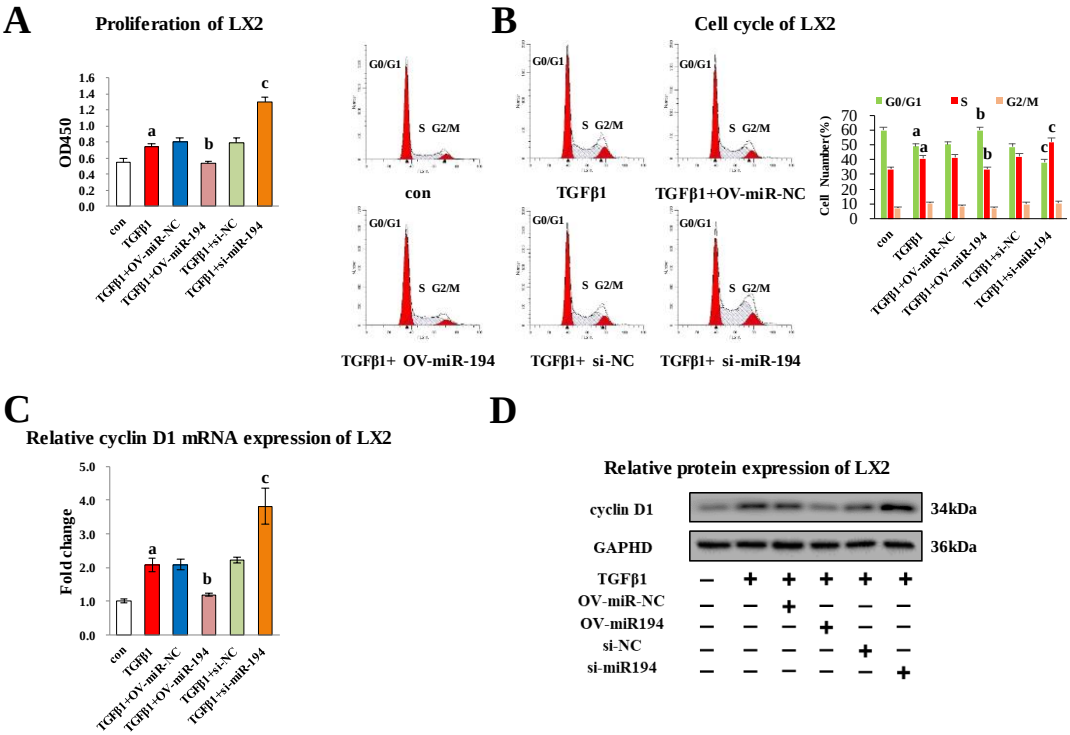

Figure 3 MiR-194 inhibited the proliferation of hepatic stellate cells

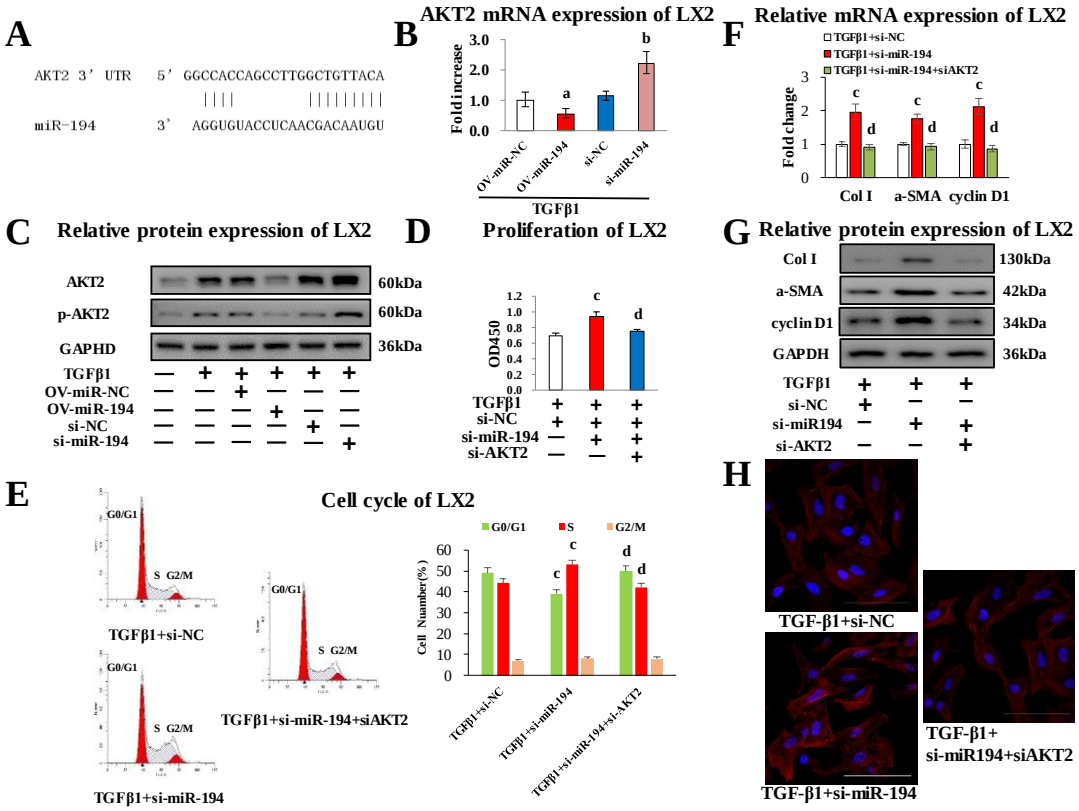

Figure 4 MiR-194 performed multiple functions by inhibiting AKT2 in hepatic stellate cells