

We thank the reviewers for their insightful and constructive comments. We have made revisions based on the suggestions by the reviewers, and as a result the manuscript is improved. The reviewer concerns and our responses are detailed below.

Reviewer 2 (#3674557):

1. Reviewer: *I would suggest including in the manuscript the PGC1- a protein levels quantification (through densitometric scans for the protein bands) normalized by GAPDH.*

Response: This has been done, and the data presented in Figure 4.

Reviewer 3 (#2763827):

1. Reviewer: *"The authors must indicate the real meanings in the symbols of figures. Otherwise, the audience cannot understand the significance. For example, in the figure 1B the authors mention different letters indicate significant differences. The "a" may be easy to understand that the meaning of significance is the comparison with "the control", but what are "b", "c", and "d"? The "b" can be the comparison with the "CCl4" or "Rosi". The similar situations also showed in the figure 2B, 2C, and 3B."*

We apologize for the confusion, and have now revised all relevant figures and legends to clearly state the specific statistical comparisons.

2. Reviewer: *"Is the Y axis of figure 1B wrong? Can the "Sirius red staining" indicate the "SMA-positive staining"?"*

The reviewer correctly pointed out that the graph accompanying the Sirius red stained liver micrographs was actually showing quantitation of SMA-positive staining. The correct graph has been added to Figure 1.

3. Reviewer: *"The authors did not explain why the relaxin has the better effects in the ALT and AST levels, and the combination of relaxin with rosiglitazone did not."*

Response: We apologize for the confusing way in which the data was presented and discussed. The important finding in this data was that none of the treatments resulted in a statistically significant change in ALT or AST compared to CCl₄ alone. Rosi and Rln alone each caused opposite, but statistically insignificant effects on ALT and AST. This resulted in a significant statistical difference between Rosi vs Rln. As per the guidelines of the journal, we have revised the text to discuss only the statistically significant differences. The new text reads: "None of the treatments resulted in a significant change in ALT or AST levels compared with CCl₄ treatment alone. A significant difference was detected between Rosi and Rln treatments alone, due to opposite but statistically insignificant differences caused by each treatment individually."

4. Reviewer: *"The authors' statement about that "Taken together, these data suggest that the combination of serelaxin and rosiglitazone may be a more rapid and effective treatment for hepatic fibrosis than either agent alone." is over-description. No evidence about the "rapid" effect was shown in this study."*

Response: Our intent when describing the "more rapid" effect was based on the findings of significant decreases in collagen content with combination treatment in 2 weeks. Our previous studies showed that Rln had no significant effect on fibrosis after 2 weeks treatment, and required longer (4 weeks) exposure for detection of antifibrotic effects. However, the reviewer is correct that the current study did not directly assess the rapidity of the effect. Therefore, we have revised the text as follows: In the Core Tip, the text now reads "These results suggest that relaxin and PPAR γ co-therapy could be a more **effective** treatment for hepatic fibrosis." In the final paragraph of the main text, the sentence now reads "Taken together, these data suggest that the combination of serelaxin and rosiglitazone may be a more effective treatment for hepatic fibrosis than either agent alone."

5. Reviewer: *"Furthermore, the single treatment of relaxin is better than the combination in the ALT and AST levels. Even in the collagen, SMA, or PGC1alpha levels, the data showed the single treatment of relaxin is effective enough."*

Response: As discussed above, none of the effects of Rln alone on ALT or AST (Table 1) were statistically significant compared to CCl₄ treatment. Furthermore, given the trend toward increased ALT and AST caused by rosiglitazone, the combination treatment appears to show that inclusion of relaxin reduces this effect, but did not reach statistical significance. Similarly, single treatment with relaxin did not significantly reduce Sirius red staining (corrected Figure 1), collagen I staining (Figure 2A & 2B) or gene expression (Figure 2C), and therefore did not reduce collagen content in the liver (fibrosis). On the other hand, there was a significant effect of Rln on SMA staining. We discussed these findings in the original manuscript, as follows: "While serelaxin alone had no effect on total collagen or type I collagen, it did significantly reduce α SMA content and therefore, HSC activation. This suggests that the effects of serelaxin on HSC activation precede the degradation of excess collagen." In regard to the PGC1 α data, we discussed this finding as follows: "Treatment with serelaxin, or the combination of serelaxin and rosiglitazone, restored PGC1 α levels. This finding supports the previous findings suggesting that relaxin acts to enhance PPAR γ activity through increased expression of PGC1 α . However, since relaxin treatment alone for 2 weeks failed to reduce collagen levels, induction of PGC1 α alone is not sufficient for resolution of hepatic fibrosis, and the presence of

PPAR γ agonists is necessary for maximum effectiveness." It is our hope that this clarifies our findings.