Peer Review File

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**Reviewer A**

1. In terms of basic reporting for this paper, in this study, the authors show that high levels of CXCR7 correlates with the remodeled pulmonary arterioles among hypoxic rats. This study also indicates that the inhibition of CXCR7 reduces PASMCs by downregulating MMP2, through p38 MAPK pathway.

2. As for the experimental design, the study is well planned; however, the data could be effectively presented with the sample size being inconsistent throughout different experiments and methodologies.

3. And speaking in terms of the validity of the findings, there are some concerns that need to be addressed as given below:
    a. The chemokine system among the rodents, is it the same as in humans? Adding the details about any rodents' study reported or studied so far in the introduction section would be informative.

    Reply3a: Thank you for your suggestion. The chemokine system among the rodents, is not it the same as in humans.

    My apologies for not understanding what you meant, research has been conducted on some rodents, could it be about CXCR7**?**

    Changes in the text:NONE

    b. Likewise, since it is a well-known fact that both SMCs and ECs contribute to vascular remodeling in PAH, I am wondering why the study just limited to PASMCs. Adding to this, there could have been more possibility to do the functional experiments like the wound healing/cell migration assay, the proliferation assay in response to the p38 inhibitor, SB203580, which is included in the study with the ECs from the rats.

    Reply b: Thank you for your suggestion. In the pre experiment, we found the target genes are mainly located in smooth muscle cells more than ECs.

    Changes in the text:None.

    c. Figure 2A, the band for CXCR7 in the control group does seem legit; I wonder what is going on with the band here? Would it be possible to provide a higher magnification image for this data?

    Reply c: Thank you for your suggestion. I have provided a higher magnification image for this data.

d. Similarly, the internal standard (α-tubulin) is missing in the western blotting data depicted in figure 3A.

Reply d: Thanks for your suggestion. We are so sorry to make the mistakes. We have revised the manuscript(Figure 3A).

Changes in the text:(Figure 3A).

e. I am wondering what is the solvent that is used to prepare the p38 inhibitor, SB203580, and how and why was the concentration for SB203580 finalized as 202 μM?

Reply e: Thank you for your suggestion. DMSO 43mg/ml warming(113.92mM), water insoluble and ethanol insoluble（Line 198-200）. Thanks for you correct the mistake (line 165).

Changes in the text:（Line 198-200）,(line 165).

f. Similarly, the vehicle control (DMSO) is missing in most of the data wherever SB203580 is used.

Reply f: Thanks for your suggestion. We are so sorry to make the mistakes. We have revised the manuscript (See FIG 3). All experiments were conducted with DMSO control added (See FIG 4).

g. Most importantly, addition or inclusion of immunofluorescence images for the validation for the PASMCs markers can be more validative.

Reply g: Thanks for your suggestion. We will pay attention to this issue of subsequent experiments.

h. Figure 5, adding or labeling the details about the pulmonary arterial histology depicting the plexiform lesions, medial hypertrophy, intimal hyperplasia or adventitial fibrosis can definitely add more value to this paper.

Reply h: Thank you for your reminder. We have redrawn Figure 5 and replaced it in the manuscript.

i. Check the spelling for “α-tubulin” throughout the draft (including the figures), spelt as “α- tublin”.

Reply i: Thank you for your reminder. We are so sorry to make the mistakes. We have revised the manuscript (see in Fig 1, Fig 2, Fig 3).

**Reviewer B**

The paper titled “CXCR7 promotes pulmonary vascular remodeling via targeting p38/MMP2 pathway in pulmonary arterial hypertension” is interesting. These findings suggest that CXCL12/CXCR7 play a critical role in PAH, the therapy of which can be developed further by targeting its potential targets. However, there are several minor issues that if addressed would significantly improve the manuscript.

1) There have been many studies on pulmonary arterial hypertension. What is the difference between this study and previous studies? What is the innovation? These need to be described in the introduction.

Reply 1): Thank you for your suggestion. This article mainly focuses on the role of chemokine systems in PAH. We demonstrated, for the first time, that the p38 MAPK/MMP-2 pathway is responsible for CXCL12/CXCR7-mediated proliferation in PASMCs. The findings of this study will assist further research into CXCR7 antagonists in PAH and offer an alternative pharmacological treatments for vascular diseases (See in Conclusions).

Changes in the text: (In Introduction line 78-81).

2) The description of some methods in this study is too simplistic, please describe in detail.

Reply 2): Thank you for your suggestion. We have improved the method description of the WB experiment and added a quantitative method. The revised content has been highlighted in line 183-185and line 195.

3) This study only focuses on animal models, and the study of CXCR7 in lung samples from PAH patients should be added.

Reply 3): Thank you for your reminder. We will carefully consider your suggestion.

4) It is proposed to add the functional study of CXCR7, which may make the whole study more complete.

Reply 4): The functional study of CXCR7, which need for follow-up experiments to confirm.

5) What new basis can the results of this study provide for the early diagnosis, disease evaluation and prognosis judgment of pulmonary hypertension? It is recommended to add relevant content to the discussion.

Reply 5): The findings of this study will assist further research into CXCR7 antagonists in PAH and offer an alternative pharmacological treatments for vascular diseases. CXCR7 expression may be related to prognosis and further clinical trials are needed**.**

6) The introduction part of this paper is not comprehensive enough, and the similar papers have not been cited, such as “Genetics and genomics of pulmonary arterial hypertension, Eur Respir J, PMID: 30545973”. It is recommended to quote this article.

Reply 6): Thank you for your reminder. We are so sorry to make the mistakes. The introduction is relatively concise. Reference has been added.

Changes in the text: (See in introduction, line 64-66 and reference 3)

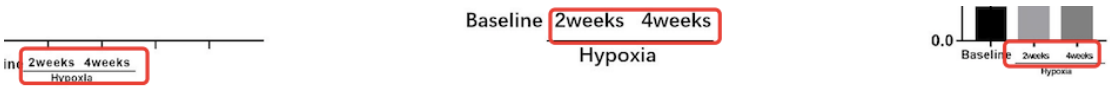
7) The description of the pathogenesis and clinical management of pulmonary arterial hypertension should be added.

Reply 7): Thank you for your reminder. As this article mainly belongs to basic research, we lack of emphasis on clinical management research. We will pay attention to correction in the next article.

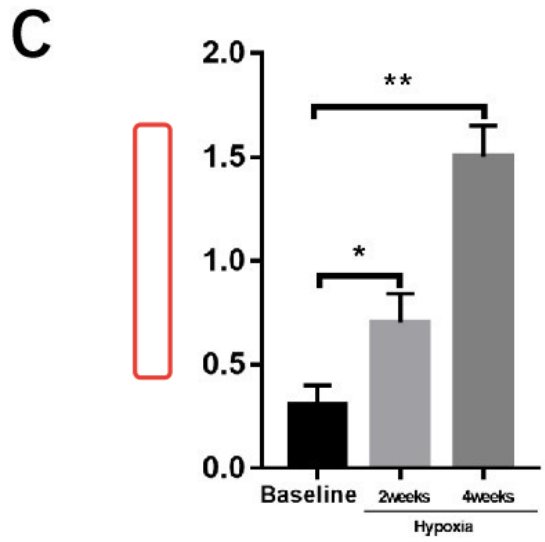
**Reviewer C**

1. **Figure 1**

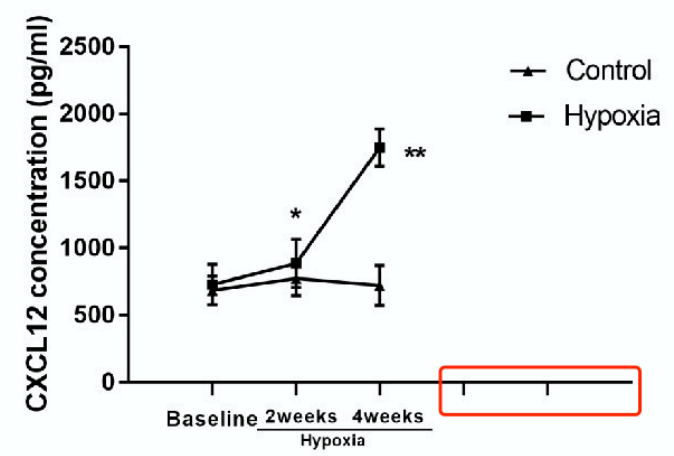
a. Please revise ‘2weeks, 4weeks’ to ‘2 weeks, 4 weeks’.



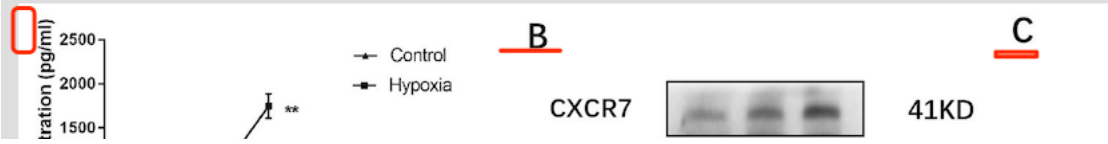

b. Please add the caption (with unit, if applicable) of the Y-axis.




c. Please check if contents are missing. If not, for the extra/unnecessary bars, please delete.




d. Please mark capital letter A.




Reply:Thanks for your suggestion. We have revised the manuscript.

**2. Figure 2B**

a. Please revise all “hypo” to “Hypoxia”.


b. No symbol *** in figure 2B but it was explained in figure legend (B); and no symbol * in figure 2D but it was explained in figure legend (D). Please check and revise.

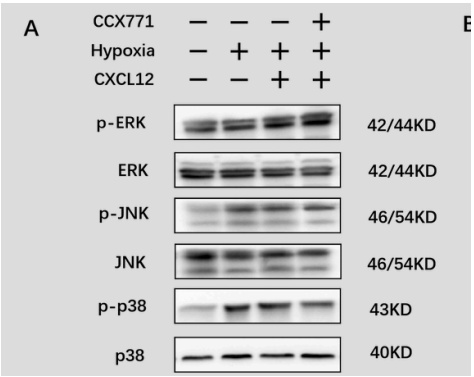
(B) The relative expression level of CXCR7 and MMP-2 was quantified by densitometry and normalized to α-Tubulin. n = 7; *p < 0.05, **p < 0.01, and ***p < 0.001; (C) Western blot analysis of CXCR7, MMP-2 in PASMCs treated with CXCL12; (D) The relative expression level of CXCR7 and MMP-2 was quantified by densitometry and normalized to α-Tubulin , n = 5; *p < 0.05, **p < 0.01, and ***p < 0.001. All values are represented as the mean ± SEM. Comparisons of parameters

Reply:Thanks for your suggestion. We have revised the manuscript.

**3. Figure 3**

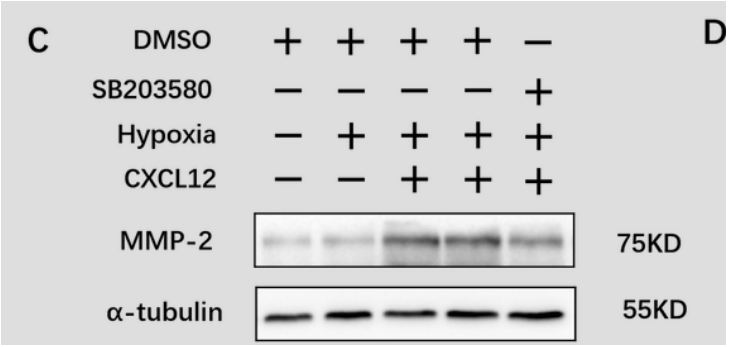
a. No symbols **, *** in figure 3A, but they were explained in figure legend (A), Please check and revise.

(A)PASMCs were preconditioned with CCX771, and then exposed to CXCL12(20ng/ml) for 6 h. The phosphorylation of MAPK protein in PASMCs was confirmed by western blotting. n = 3; **p < 0.01, and ***p < 0.001;(B)




b. No symbols **, *** in figure 3C, but they were explained in figure legend (C), Please check and revise.

***p < 0.001;(C) Following pretreatment with, or without, the p38 MAPK inhibitor SB203580 for 2 h reduced the expression levels of MMP-2 in PASMCs stimulated with CXCL12. n = 3; **p < 0.01, and ***p < 0.001. (D) The relative expression level

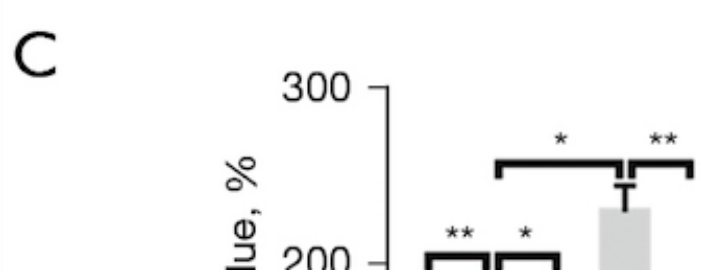



Reply:Thanks for your suggestion. We have revised the manuscript.

**4. Figure 4**

1) No symbol *** in figure 4C but it was explained in figure legend (C).

protein. n = 3; *p < 0.05, and ***p < 0.001; (C) CXCR4 inhibiton with CCX771 could block cell proliferation stimulated with hypoxia or CCX771, n = 3; *p < 0.05, **p < 0.01, and ***p < 0.001. (D) p38 MAPK signaling inhibitor SB203580




Reply:Thanks for your suggestion. We have revised the manuscript.

**2) Figure 4C**

Please revise this typo. Should it be "inhibition"?

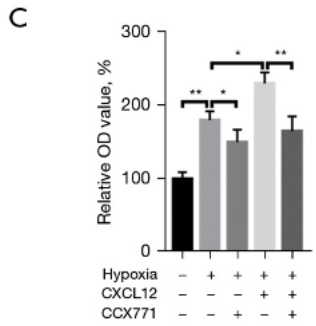
> protein. n = 3; *p < 0.05, and ***p < 0.001; (C) CXCR7 inhibiton with CCX771 could block cell proliferation stimulated with hypoxia or CCX771, n = 3; *p < 0.05, **p < 0.01; (D) p38 MAPK signaling inhibitor SB203580 abolished CXCL12

Thanks for your suggestion. We have revised the manuscript.

3) Figure 4C

Please check and confirm whether "CXCR7" is correct, since we did not find this word from Figure 4C

> protein. n = 3; *p < 0.05, and ***p < 0.001; (C) CXCR7 inhibiton with CCX771 could block cell proliferation stimulated with hypoxia or CCX771, n = 3; *p < 0.05, **p < 0.01; (D) p38 MAPK signaling inhibitor SB203580 abolished CXCL12

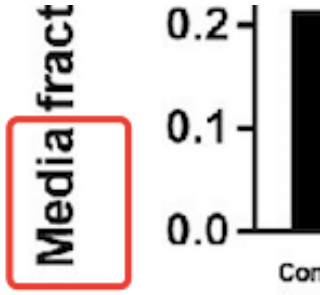



Thanks for your suggestion. We have revised the manuscript.

**5. Figure 5C**

a. Please indicate the scale bar in the figure legend (C).

b. Please check if "Media" should be "Medial".




*p < 0.05, **p < 0.01; (C) Pulmonary artery remodeling was also assessed by hematoxylin–eosin staining. Percentage of muscularization of hypoxia rats. Assessment of medial thickness in rats, n = 7, *p < 0.05, **p < 0.01; (D) Ratio of the

Reply:Thanks for your suggestion. We have revised the manuscript.

**6. Figure 5B-G**

Please revise all "hypo" to "Hypoxia".


Reply:Thanks for your suggestion. We have revised the manuscript.

**7. References**

a. References 14 and 33 are duplicated. Please delete one of them and update the citations in

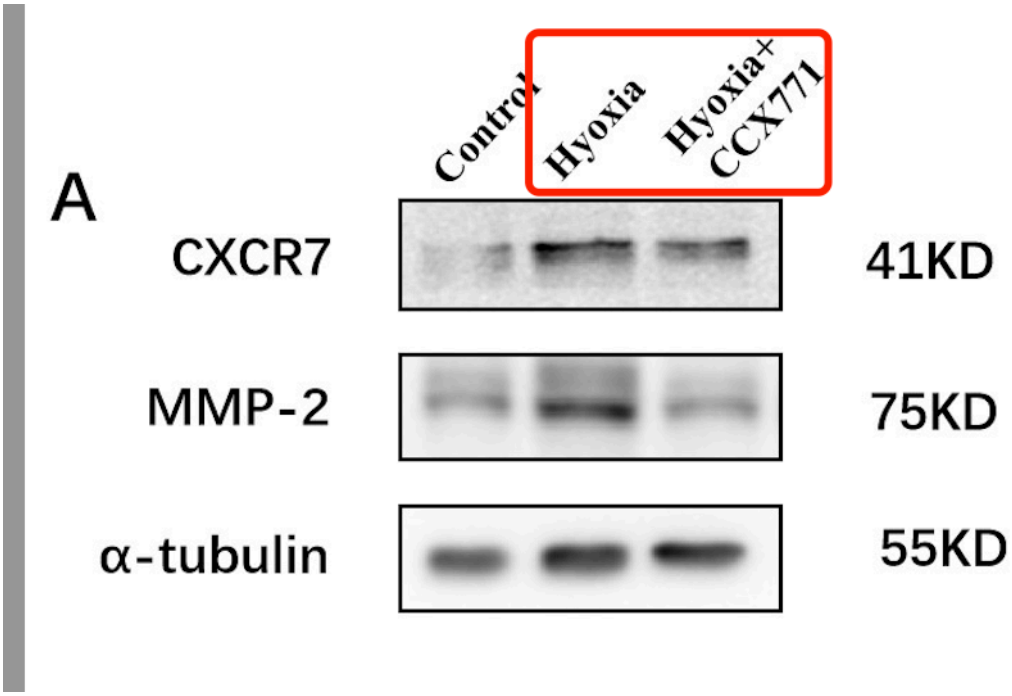
the paper. Please note that references should be cited consecutively and consistently according to the order in which they first appear in the text.

*14. Bai P, Lyu L, Yu T, et al. Macrophage-Derived Legumain Promotes Pulmonary Hypertension by Activating the MMP (Matrix Metalloproteinase)-2/TGF (Transforming Growth Factor)-β1 Signaling. Arterioscler Thromb Vasc Biol 2019;39:e130-45.*
*33. Bai P, Lyu L, Yu T, et al. Macrophage-Derived Legumain Promotes Pulmonary Hypertension by Activating the MMP (Matrix Metalloproteinase)-2/TGF (Transforming Growth Factor)-β1 Signaling. Arterioscler Thromb Vasc Biol 2019;39:e130-45.*

b. The authors mentioned "studies...", while only one reference was cited. Change "Studies" to "A study" or add more citations. Please revise. Please number references consecutively in the order in which they are first mentioned in the text.

*Related studies have shown that CXCL12 attracts bone marrow–derived progenitor cells or mesenchymal stem cells that express CXCR4 and CXCR7 to the subintimal layer (27).*
Reply:Thanks for your suggestion. We have revised the manuscript.

**8. Figure 2B**
Please revise all "Hyoxia" to "Hypoxia".




Thanks for your suggestion. We have revised the manuscript.