

Dear Editors and Reviewers,

I am very glad to receive the comments and suggestions on this manuscript from outside reviewers and the editorial board. We are resubmitting the revision of our manuscript by Chang Xu et al., "Conventional knockout, intestinal conditional knockout and inducible conditional knockout of Claudin-7 causes the destruction of intestinal structure and the death of mice" (Manuscript NO.: 43106) to the *World Journal of Gastroenterology*. We have revised the manuscript according to the reviewers' comments as described in our point-by-point response. All the amendments are highlighted in yellow in the revised manuscript.

Before replying to the reviewers and editors, I have to tell the editor that the name of the corresponding author was Lei Ding, not Ding Lei. I am sorry for our previous negligence, please help us to change it. Thank you!

Response to Reviewer #1:

1) How did they determine the mental status of the mice as authors stated in the abstract and elsewhere in the manuscript.

Response: Thank you very much for your suggestion. We have added the mental state description of CKO mice, cKO mice and ICKO mice. "The CKO mice were thin, lacked energy, showed signs of lethargy, exhibited decreased body temperature, activities were reduced, and the mice thus showing a state of dying." "Cldn7 cKO mice appeared to be languid, with reduced or even inactive activities, leaving only a slight breath. The body temperature of the mouse was reduced and it was in a state of dying." "The ICKO mice were lethargic, lack of activity, appeared thin, and exhibited a dying state." These sections are presented on page 10, 11 and 12.

2) Discussion is confusing should be shortened with clarity.

Response: Thanks for your suggestion, we have revised the discussion and removed the unnecessary sections.

Response to Reviewer #2:

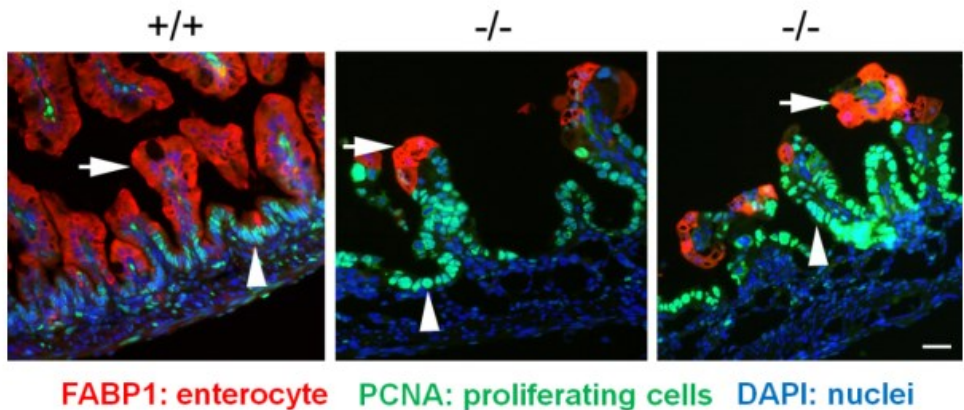
Major:

1) My major concern with this study is that while the findings are interesting, there is a lack of depth of investigation, particularly involving the inducible claudin-7 KO mice. The authors should expand their studies to better classify cell proliferation with determinations of which cells are proliferating and a more detailed analysis of the stem cell niche. In addition, cytokines and/or determination of the types of immune cells infiltrating into the intestine should be determined. Finally, gut function should be assessed to determine if uptake of nutrients is dysregulated, if endotoxins are entering portal tract, or other measures of gut function. This could help identify why the mice with claudin-7 KO are dying and better classify these mice would be very useful to the field.

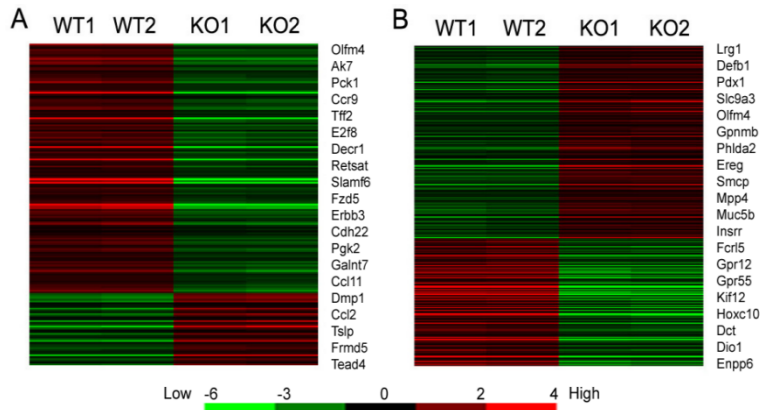
Response: At present, our team is conducting an in-depth study of Cldn7 on the basis of Cldn7 inducible mice. This manuscript mainly describes the construction methods and phenotypic analysis of three knockout mice, and provides animal models for the study of Cldn7 and other proteins. Researches related to the mechanism of Cldn7 on

intestinal inflammation, intestinal proliferation, and maintenance of intestinal stem cells is ongoing. If you are interested in this area, please follow our other articles and send email to me to communicate. Here, I will use our team's other research results to answer your questions.

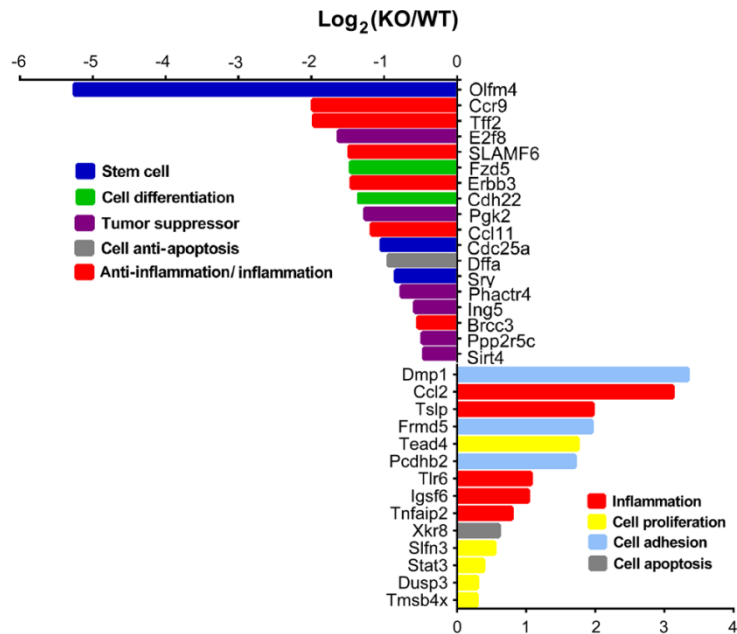
Firstly, we identified which cells in the intestine of mice are proliferating by immunofluorescence experiments. The picture shows immunofluorescence staining microscopy images of the intestine of *Cldn7* knockout mice (-/-) and control mice (+/+) (picture unpublished). Among the cells growing along the intestinal villi, intestinal cells are the predominant type (+/+, indicated by the arrow), while the continuously proliferating stem cells are located in the intestinal crypt area (+/+, indicated by the arrowhead). However, in the *Cldn7*^{-/-} intestinal tract, the number of intestinal cells is significantly reduced (-/-, indicated by the arrow), and the proliferating cells are distributed along the villi (-/-, indicated by the arrowhead). It is important that there is no overlap between proliferating cells and intestinal cells in the intestinal of *Cldn7* knockout mice, indicating that the proliferating cells are undifferentiated cells.



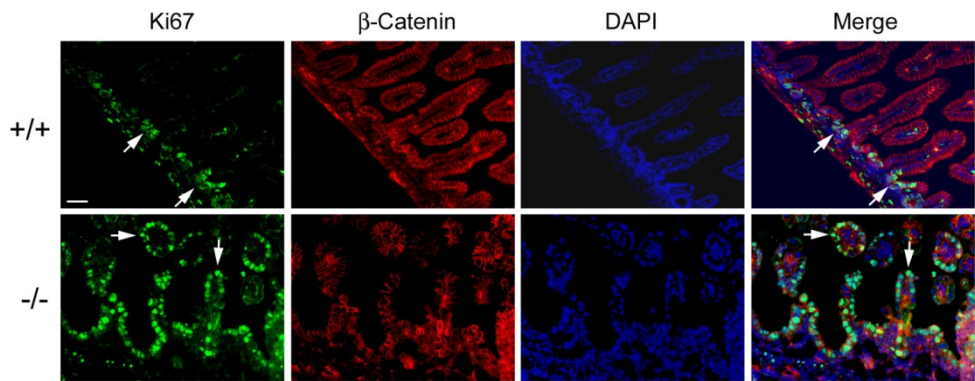
Based on knockout mice, we also conducted research on stem cells and proliferation. Gene changes in the gut of *Cldn7* knockout mice (-/-) and control mice (+/+) were examined by gene chip method. Compared with *Cldn7* +/+ small intestine, 213 gene expressions in *Cldn7*^{-/-} small intestine showed significant changes; 438 genes in the large intestine showed significant changes (picture unpublished).



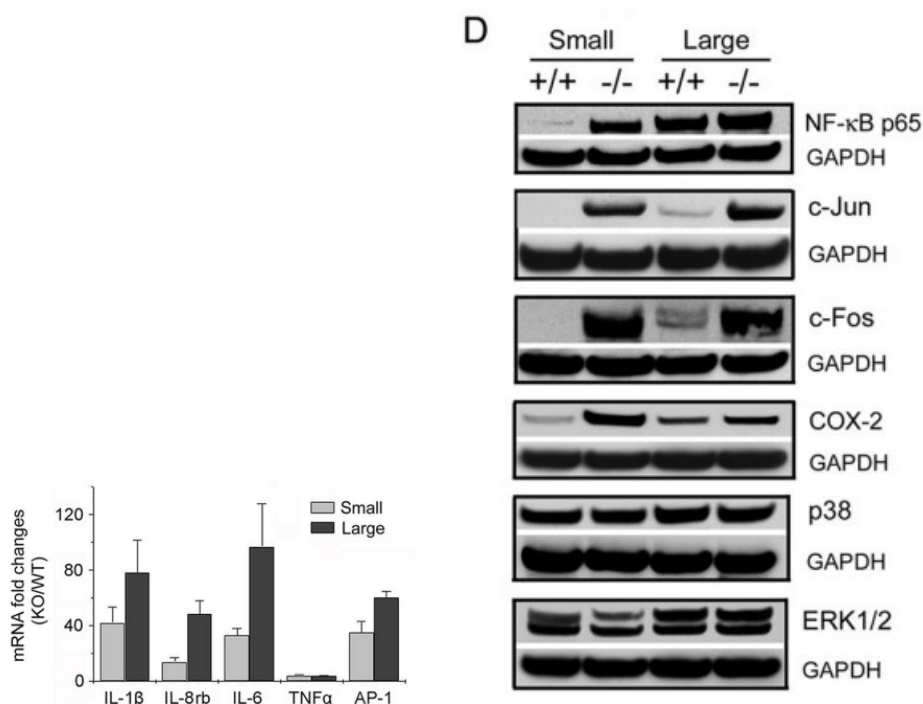
We found that stem cell marker genes, such as *Olfm4* and *Sry*, are significantly down-regulated in *Cldn7*^{-/-} intestine, and some genes related to inflammation and cell proliferation (such as *Ccl2*) are highly expressed in *Cldn7*^{-/-} intestine (picture unpublished).



On this basis, our team is investigating the effects of *Cldn7* expression on the proliferation of intestinal crypt stem cells. We found that in the *Cldn7*^{+/+} intestinal, most of the proliferating cells were located in the crypt area (^{+/+}, Ki67), while the proliferating cells in the *Cldn7*^{-/-} intestinal tract were no longer confined to the crypt area (^{-/-}, Ki67). In contrast, Ki67 proliferating cells can be observed throughout the intestinal villi. This suggests that the balance between cell proliferation and cell differentiation in the *Cldn7*^{-/-} intestinal tract has been disrupted. β -catenin is located in the junction of *Cldn7*^{+/+} intestinal cells and cells. However, in the *Cldn7*^{-/-} intestinal tract, β -catenin appears in the cytoplasmic, and the expression of β -catenin at the cell junction is also weakened. β -catenin is an important component of the Wnt signaling pathway. The migration of β -catenin from the cell junction to the cytosol causes abnormalities in transcriptional enhancement and cell proliferation.



Secondly, in our previous studies, we found that the expression of inflammatory factors in the intestinal of CKO Cldn7^{-/-} mice was higher than that of Cldn7^{+/+} mice by western blot and qPCR experiments (picture has been published). In addition, other members of our team are using immunofluorescence experiments to identify immune cell types in the gut of Cldn7 knockout mice, such as neutrophils, macrophages, T cells, and B cells.



Thirdly, other researchers in our team are conducting studies on intestinal function. We are performing FITC gavage on Cldn7 knockout mice and control mice, and measuring the concentration of FITC in serum to determine intestinal permeability. This simulates the absorption of nutrients by the intestines. In addition, the blood of the mice is taken for blood bacterial culture, and the amount of endotoxin in the plasma is also measured to determine whether the mouse has bacteremia and whether endotoxin enters the blood.

2) In the CKO mice the authors state that the mice had slow growth and died on the third day. Similar findings were reported in the cKO mice. Are these observations with growth due to changes in food intake, lack of absorption of macronutrients or some other effect?

Response: During the process of feeding the mice, there was no abnormality in the diet and excretion of the mice before they died. And the ongoing FITC gavage experiment in mice did not show that the absorption function of intestinal nutrients was destroyed. These results will be published later, and we look forward to your further attention.

3) The appropriate controls for the villin-CreERT2 mice would be to do tamoxifen injections in one group and sunflower oil injections into a second group of mice.

Response: Thank you for your rigorous advice. In a previous experimental study, we designed multiple control groups for Villin-CreERT2 mice injected with tamoxifen: Villin-CreERT2 mice injected with sun flower oil; Villin-CreW mice injected with tamoxifen; Villin-CreW mice injected with sun flower oil; C57BL/6N mice injected with tamoxifen, and C57BL/6N mice injected with sun flower oil (data not shown). Compared with the experimental group, Cldn7 intestinal knockout and intestinal inflammation did not occur in all the control groups. Therefore, the effect of sunflower oil on mice can be ruled out. This article focuses on the phenotypic differences between mice that can be induced to knock out Cldn7 and those that cannot be knocked out of Cldn7, rather than the effects of solvents. And the number of mouse models is limited, therefore, this article does not have the phenotypic analysis of the solvent group mice. We feel very sorry for our negligence, we will add solvent control in the following articles, thank you again for your suggestions!

Minor:

1) The authors use the words “poor spirited” or similar wording when the mice are sick. It would be better to describe this as lethargy or lack of activity or something along those lines to better describe the mice.

Response: We have added the mental state description of CKO mice, cKO mice and ICKO mice. “The CKO mice were thin, lacked energy, showed signs of lethargy, exhibited decreased body temperature, activities were reduced, and the mice thus showing a state of dying.” “Cldn7 cKO mice appeared to be languid, with reduced or even inactive activities, leaving only a slight breath. The body temperature of the mouse was reduced and it was in a state of dying.” “The ICKO mice were lethargic, lack of activity, appeared thin, and exhibited a dying state.” These sections are presented on page 10, 11 and 12.

2) On figure 2 the methodology used for marking the blots is inconsistent and fonts are different sizes. Please make figure larger and use larger fonts for 2C and 2D.

Response: We have modified the fonts in 2C and 2D, which will help reviewers and readers to read and understand the content of the picture more clearly. Thank you again for your suggestion.

3) For H&E images and IHC the authors should label in a manner that is consistent and easy to read. In particular, figure 4 it is difficult to see control listed in the bottom right panel.

Response: We have modified the marking method in Figure 4 and marked the control group. Thank you for your meticulous advice, which is very effective in improving our articles.

4) The nomenclature used to identify conventional vs conditional knockouts (CKO vs. cKO) could be improved.

Response: I am sorry that our terminology has caused your confusion, but CKO and cKO are commonly used expressions. In addition, in the abbreviations section, we

also explained the difference between the two. We will try to improve this statement in the later article. Thanks for your suggestion.

The above content is the point-by-point responses to reviewers based on the criticisms they raised. On behalf of my co-authors, we thank you very much for giving us an opportunity to revise our manuscript. We hope that the revision is acceptable and look forward to hearing from you soon. If you have any queries, please do not hesitate to contact me.

Thank you and best regards.

Yours sincerely, Lei Ding
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