Response letter

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Dear Editors,
We would firstly like to thank the reviewers and editors for donating time and efforts to our manuscript entitled “Fecal microbiota transplantation ameliorates experimental colitis via regulation of gut microbiota and T lymphocyte activation”.
Your comments and those from the reviewers were highly insightful and strengthened the logicality and rationality of our study. Our manuscript has been revised as recommended. The point-by-point responses to each of the comments of the reviewers are presented as follows.
We shall look forward to hearing from you at your earliest convenience.

Best Regards.
Yours sincerely,

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Responses to the comments of Reviewer #1:
This manuscript describes an interesting new assessment of the mechanisms involved in changes that occur after fecal microbiota transplant in mice that have DSS-induced colitis. The level of detail provided for the methods is very helpful for understanding the rigor of the study as well as the analysis goals of the authors.
1.However, in the Materials and Methods section, I was not sure I understood what "with specific pathogen free" referred to in the first paragraph. Could the authors please clarify in the text whether this is referring to the environment in which the mice were reared, the mice themselves, or another meaning?
Response: Thanks for your careful review. All mice were “specific-pathogen free” (SFP). SPF mice are mice that are demonstrated to be free of a specificlist of pathogens by routine testing. The list of organisms assessed typically includes disease-causing pathogens that can affect mouse health and research outcomes, as well as opportunistic and commensal organisms that typically do not cause illness in normal, healthy mice. We have modified the description and stated this point more clearly in the manuscript.

2.In the Results section, does n=7 refer to the DSS and DSS+FMT groups

combined? If n=7 is just for the DSS+FMT group, it would be helpful to have the n= for the DSS group mentioned as well. The level of detail provided in the figures is helpful.
Response: Sorry for our unclear description. Both DSS and DSS+FMT groups had 7 mice. We have stated this point more clearly in the manuscript.

3.However, Figure 4b would be more helpful if either the titles of the individual graphs or the figure legend was more clear about which groups were assessed in each graph shown; I am not sure I understand what the titles for the graphs are indicating about the groups.
Response: Thanks for your suggestion. We have modified the figure legend of Figure 4b.

4.I really like Figure 6; it is visually appealing and provides a helpful illustration of the concepts. I am not very familiar with the GO terms mentioned in this manuscript; it would be very helpful to have the importance of their use and their meaning for this study explained in the Discussion section.
Response: Really appreciate for your suggestion. The most enriched GO terms were immune system process and metabolic process based on the differentially expressed genes (DEGs) in the colon. We have explained the meaning in Discussion section.

5.Language in the Discussion section needs to be checked; for example, it is encouraging if the authors are planning to carry out future clinical studies on these concepts, but this does not sound like a limitation in itself for this study. It would be helpful to know what the authors mean by limitations of animal models for understanding the mechanism of FMT; I understand there may be limitations in understanding how humans are affected, as opposed to mice, but it would be helpful to have more detail on what makes the authors concerned about this limitation.
Response: Many thanks for your intelligent suggestions. As you said, we focused on the effect and mechanism of FMT on experimental colitis mice, which may be different from patients with ulcerative colitis. This is a limitation of animal models for understanding the mechanism of FMT. We have modified the language in Discussion section.

6.Overall, this is an interesting study that I believe has useful information to inform methods for future studies. For the most part, this manuscript appears to meet the criteria for review. Some revisions are needed for the language quality of this manuscript, but generally, it seems well-written. I would recommend the authors address the comments I have mentioned, but I believe that once revisions are complete, this manuscript should be accepted for publication.

Response: Greatly appreciate for your positive comments of our work and detailed suggestions to improve the quality of our manuscript. The manuscript has been carefully smoothed by language experts and we have already addressed the comments mentioned above. Many thanks.

Responses to the comments of Reviewer #2:

The submitted paper analyzed the effect of fecal transplantation in a mouse model of ulcerative colitis, i.e. the one using DSS for 7 days. The authors fistly observed the macroscopical aspect of the colon and the histogy, observing significant differences between mice treated with DSS and those that, after the DSS treatment, received fecal transplant. They also compared the microbiota of these animals, detecting differences at the genus level, with a transplant effect that changed the microbiota composition making it more similar to the sham animal. The analysis of the transcriptome revealed differentially expressed genes that the authors correlated with the presence of the bacteria identified as highly present in the DSS-only treated mice, namely Clostridium_sensu_stricto_1, Turicibacter and Ruminococcus. Although the presented data are interesting there are some points that need to be addressed by the authors:

1. Methods section: it is not clear whether they used the supernatant or the "sediment" for gavage; histological score should be defined; RNA used for sequencing was extracted from "inflammatory colonic tissue". If this is correct which percentage of the colon was regarded as having inflammation in the two groups? Was sequencing performed on all animals?

Response: Thank you for the comment and for interesting suggestions on research questions. In the sediment, the microbiota in the feces was collected and the sediment was used for gavage. Histological score has been defined in the Methods section. Colonic tissues for RNA sequencing were obtained from a position about 2 cm from the anus, regardless of whether there was visible inflammation. Four mice in each group were randomly selected for transcriptome sequencing.

2. Results: in Figure 3 results should be compared by ANOVA. Is there a significant difference in lactobacillus between control and transplanted mice? The same kind of comparison should be applied to Fig 5.

Response: Thanks for your suggestion. ANOVA have been used for statistical comparison. According to the analysis results, there is no significant difference in *Lactobacillus* between control and transplanted mice.

3. Although the GO and Metascape analysis identify the genes differentially expressed in FMT mice as involved in the regulation of T cells, the data provided are not enough to demonstrate this hypothesis "Furthermore, after

FMT treatment, the activation of T lymphocytes was significantly inhibited (Fig. 6)". To make this statement the authors should isolate T lymphocytes and test their activation status.

Response: Thanks for your careful review. We isolated the lamina propria mononuclear cells (LPMC) for immunephenotyping by flow cytometry (n=4 each group). As expected, T cell populations isolated from colons of FMT-treated mice showed a reduced activity as compared to those isolated from colitic mice that received DSS ($p < 0.01$). Phenotypically, mice receiving DSS displayed a higher proportion of $CD4^+$ and $CD8^+$ T cells. However, there was a significant decrease in $CD4^+$ and $CD8^+$ T cells in mice following FMT therapy, additionally supporting the observation that colonic T cells in FMT-treated mice manifest an activation decrease. We have improved the content of results and modified illustrations. Many thanks for your suggestions.

4. The entire discussion needs to be revised.

Response: Thanks for your suggestion. The Discussion has been revised and become more logical.

Responses to the comments of Reviewer #3:

Undoubtedly, the topic of the study presented by the authors is relevant due to the increasing prevalence of IBD and the potential effectiveness of FMT. The reviewer has no questions regarding the study's methodology, results, figures, and references.

1.However, it is desirable that the authors expand the discussion. The findings identified by the authors are very interesting, but the interpretation of changes in the microbiota after FMT is ambiguous. For example, a significant part of other studies shows not a decrease, but an increase in the relative abundance of *Lactobacillus* in IBD, which requires further study of their role in the pathogenesis of intestinal inflammation. *Turicibacter* has recently been discovered and cannot be considered unambiguously as a pathogen/pathobiont associated with IBD and other intestinal disorders. Recent research indicates an important role for this bacterium in host serotonin metabolism. *Turicibacter* was significantly less abundant in IBS-D patients. Similarly, the role of *Clostridium_sensu_stricto_1* also cannot be interpreted unambiguously.

Response: Truly thanks for your constructive comments. In this study, FMT changed the gut microbiota of colitis mice, including *Lactobacillus*, *Turicibacter*, *Clostridium_sensu_stricto_1* and other genus. Of these, *Lactobacillus* has been studied more frequently in colitis. In fact, *Lactobacillus* is considered to be a beneficial bacteria that can regulate intestinal immune homeostasis and has been used in the treatment of colitis. *Turicibacter* and

Clostridium_sensu_stricto_1 are currently unclear as to their role in balancing intestinal homeostasis. If the bacteria can be isolated, cultured and further used to treat colitis in mice, it may be possible to reveal its interaction with colitis. In this study, we propose the potential mechanism of FMT from the perspective of gut microbiota and intestinal mucosal immunity. It is true that the causal relationship between gut microbiota and immunity has yet to be confirmed by further studies. Many thanks for your suggestions.

2.In the title, I would recommend replacing "T lymphocyte activation" with "T-cell modulation".
Response: Thanks for your suggestion. We modified the title.

3.The manuscript may be published after a minor revision expanding and deepening the discussion regarding changes in the microbiota after FMT and its relationship with immune effects that could potentially improve colitis.
Response: Thank you for the positive comments. We modified the discussion carefully and stated the opinions clearly. Really appreciate for your suggestion.

Responses to the comments of Reviewer #4:
This manuscript demonstrates that fecal microbiota transplantation ameliorates experimental colitis through the mechanism of regulating gut microbiota and activating T lymphocytes. This study combined the animal study together with differentially expressed genes analysis, which is scientifically adequate enough for its research purpose. It provides scientific value to the treatment of colitis by FMT.
1.However, the language still needs some minor polishing before being accepted.
Response: Thanks for your suggestion. The quality of the English language has been improved by language experts. Many thanks.