Dear Editors and Reviewers:

Thank you for your letter and for the reviewers' comments concerning our manuscript (**Manuscript NO.:** 28397) entitled "MicroRNA-155 promotes the pathogenesis of experimental colitis by repressing SHIP-1 expression". Those comments are all valuable and very helpful for revising and improving our paper, as well as the important guiding significance to our researches. We have studied the comments carefully and have made correction which we hope meet with approval. Revised portion are marked in red in the paper. The main corrections in the paper and the responds to the reviewer's comments are as follows:

Point-to point response:

Reviewer #1:

In their manuscript, Dr. Lu and colleagues reported their studies on the potential mechanisms of miR-155 in the immunopathogenesis of inflammatory bowel diseases (IBD) using the mouse model of DSS-induced colitis. Previous studies by others have demonstrated the multiple physiological and immunological functions of miR-155 and have also implicated the involvement of this miRNA in the development of IBD both in patients and in mice. Therefore, further studies on the potential role and particularly the mechanisms of miR-155 in IBD are important for the clinical management of the disease and the development of novel therapeutic modalities.Since SHIP-1 is a well-established target of miR-155 and the suppression of SHIP-1 expression and function by miR-155 has been documented in several in vitro and in vivo studies (such as refs. 16-19), the real value of the experiments presented in Figs. 1 and 2 is arguable. Nevertheless, the reported study has convincingly and systematically demonstrated the beneficial effect of miR-155 inhibition on the development of DSS-induced colitis in this model. Similar results have also been reported or implicated in other mouse models (such as T cell mediated or TNBS) of IBD. Moreover, the current study demonstrated the involvement of Akt asa downstream signaling pathway in the SHIP-1-mediated inflammatory responses.The experiments were overall well designed with the appropriated controls included and statistical methods employed for data analysis. However, the significance of the study is somewhat diminished because the treatment of antagomiR-155 was initiated during the colitis-inducing phase (day 2 and 5 of DSS treatment), not after the completion of DSS treatment. To promote such approach as a potential novel therapeutic alternative, additional studies with the antogomiR-155 starting at day 7 or thereafter will be needed. At the minimal, the authors should discuss the limitation of their study in the

manuscript. In addition, the manuscript will be benefited by some language editing.

Response:

1. We really appreciate the review's valuable comment. We performed the experiments that are shown in Figure 1 and Figure 2 because previous studies are focusing on the relevance between miR-155 and SHIP-1 in cells like 293T and B cells (ref. 16, 17), but not the macrophages which are main functional cells in colitis; and neither did Wu's *in vivo* study (ref. 18) include related investigation. Therefore, this is the first report about the regulation of SHIP-1 by miR-155 in macrophages. So, from our point of view, these data are important for the investigations that followed.

2. Thanks for your suggestions. The reason why we conducted the antagomiR-155 treatment on day 2 and day 5 is that the classical colitis mouse model is established by a 7-day DSS induction, and after that, the repair and renew of colonic mucosa will start. If we perform the antagomiR-155 treatment after the completion of DSS induction, there would be a possible confusion about the cause of the decreased intestinal inflammation.

IBD is characterized by a chronic pathological course in colonic mucosa, and thus, a chronic colitis model established by 9 or more weeks' DSS induction would be better, we didn't carry out this in this study for the limitations around. We had added the related discussion in the end of paragraph 3 in the part of Discussion. Thanks again.

3. Thank you. We have polished the language in the revised manuscript.

Reviewer #2:

This work put focus on the role of SHIP-1 on the way how miR-155 associates with IBD.

Following the recent findings (one of miR-155's targets is SHIP-1, and SHIP-1 is associated with IBD), in this study, using DSS-induced IBD biological models, this work added evidences to clarify that the drop of SHIP-1, which is resulted from the increase in miR-155, is the reason why IBD patients have high level of miR-155.I would like to say this work offers new insight into the understanding of the inflammatory mechanisms in IBD. However, some conclusions in this manuscript are not fully supported and discussed, also, there are some typos and grammar errors in writing. After these issues get solved, I suggest this work would be considered to publish in World Journal of Gastroenterology.1. In figure 2A, why PTEN did not balance out the effect of the suppressed SHIP-1 on Raw264.7 cell proliferation?In PI3K/Akt pathway, PTEN acts as a gatekeeper, converting PIP3 to PIP2, in this regard, in your experiment, should the PTEN have consumed the extra PIP3 resulted from the less SHIP-1? And as a result, could the cell proliferation result from the other causes? I suggest an experiment on the expression of p-Akt should be added, so that we can have a closer lookat the effect of miR-155 suppressed SHIP-1 on cell proliferation.Also, it was reported that macrophages produce IL-4 (http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0046989, http://europepmc.org/abstract/med/15969673), and IL-4 contributes to macrophage proliferation (http://science.sciencemag.org/content/332/6035/1284.long), in this regard, is there any possibility that miR-155 would affect Raw264.7 proliferation through IL-4?Besides, what does it look like in BMDM in terms of cell proliferation? As primary macrophage cell, the result could be interesting.2. In figure 2B, have you normalized the concentration data based on the same cell number?From Fig 1A, the cell number was different in different groups in the end, as a result, the concentration of cytokines would vary with the cell number other than different treatments.And, what would be the possible explanations on the result that no significant difference was observed between group 'miR-155' and group 'miR-155+SHIP-1' in IL-6?3. In figure 5D, the expression of IL-10 should be included to fully support the effect of anti-miR-155, this is because, unlike pro-inflammatory cytokines, IL-10 is the anti-inflammatory cytokine that has been identified as being involved in IBD.4. Other than the above, the writing should be checked carefully, especially in the discussion section. The following are some examples:On p13, in line 20, "restoration of SHIP-1 could effectively inhibited or reversed them" should be "restoration of SHIP-1 could effectively inhibit or reverse them".On p24, in the description of Fig. 5, "lower than that of in mice from other colitis groups" should be "lower than that in mice from other colitis groups".

Response:

1. We really appreciate the reviewer's valuable comment. The pro-proliferation effect of miR-155 on raw264.7 cells has been documented previously (Tu J et al., Int Immunopharmacol, 2012), and our report had included the investigations on the relationship between miR-155, SHIP-1 and Akt/p-Akt (Fig.5B), which indicated that the level of miR-155 is

positively correlated with p-Akt expression. In this regard, the contribution of miR-155 on cell proliferation is possibly through p-Akt activation.

It has been reported that miR-155 has no significant relevance with IL-4 expression in mouse macrophages (Jablonski et al. Plos One, 2016), so we didn't conduct IL-4-related experiments. As to BMDM, it was hard to manage the experiments on cell proliferation since it was primarily isolated, so we didn't investigate on it. Thanks again.

2. Thanks for your suggestions. We performed the ELISA analyses in line with previous reports (Madera et al., Plos One, 2015; Lee et al., BIOL PHARM BULL, 2016). We seeded the cells onto the culture plates with a confluence of over 80%, thus the cell number would not vary between the groups. The reason why there was no significant difference between group miR-155 and group miR-155+SHIP-1 is hard to explain; however, SHIP-1 did decrease the IL-6 secretion, though no statistical signification was observed. Thank you again.

3. Thanks very much for your directions. We have added related experiments on the expression of IL-10 in the DSS mice model in the revised paper. As expected, the mRNA level of IL-10 was in contrast with the pro-inflammatory factors, which confirms its anti-inflammation role in colitis.

4. Thanks. We had done the corresponding corrections in the revised manuscript.

Reviewer #3:

major concerns:1.The logic of result section seems to be confused. Author should rearrange the presentation. eg, Fig3 and Fig 5 could be combined;2.Gene name should be italics. eg, SHIP-1 mRNA should be SHIP-1 mRNA3.Authors should add a luciferase reporters to confirm that SHIP-1 is one of targets of miR-155;4.The results seem not to be obvious in BMDM groups in Fig1b;5.It lacks one group that SHIP-1 inhibited the

proliferation of raw264.7 cells in Fig 2. Authors should also use SHIP-1 inhibitors to assess the secretion capacity of IL-6, TNF-α, and IL-1β;6.Author claimed that antagomiR-155 reduced disease active index (DAI) of experimental mice, whether this effect of antagomiR-155 was dose-dependant?7.Phosphorylated Akt was detected in Fig 5b, and which phosphorylated site(s) of Akt were analyzed?8.Authors claimed that Akt was obviously activated in Fig5b due to SHIP-1 up-regulation, however, the results of Akt were inconsistent with SHIP-1; Inhibition of Akt activation has better treatment effect compared to antagomiR-155?9.Authors found that SHIP-1 was slightly reduced after overexpression of miR-155 in Fig 1, and miR-155 was up-regulated about 2 folds in DSS or DSS+NC in Fig 3a, however, SHIP-1 showed more obvious change in Fig5a. Authors should try to explain this inconsistent;10.The quality of Fig5c was so poor. Negative control is needed;11.Just as Fig 5d, IFN-gamma and IL-17 should also be determined in Fig 2; The protein level of cytokines should also determined in Fig 5d.

Response:

1. Thanks for your suggestions. Figure3, 4 and 5 are the results in sequence from our *in vivo* studies. Figure 3 and 4 shows the phenotype changes between the differently treated groups while Figure 5 demonstrates the mechanism by which antagomiR-155 alleviates the DSS-induced colitis. So, from our point of view, fig. 3 and fig. 5 may not be combined. Still we appreciate your comments.

2. Thank you very much. We have checked the paper carefully and performed the corresponding corrections in the revised version.

3. Thanks for your constructive directions. We have added a luciferase reporter gene assay in Fig.1.

4. Thanks. The inhibition effect of miR-155 on SHIP-1 in BMDMs is lower than that in raw264.7 cells, but it's statistically significant.

5. Thank you. We have constructed SHIP-1 overexpression and knockdown vectors and assessed the effects of SHIP-1 upregulation/downregulation on cell proliferation and secretion in the revised paper.

6. Thanks for your valuable suggestions. We didn't perform such experiments for the limitations in our lab. We will further investigate on this issue in future.

7. The phosphorylated site of Akt we examined was T308.

8. Thanks for your comments. It has been well established that SHIP-1 negatively regulates the Akt signaling and our fig. 5B confirms that SHIP-1 upregulation leads to an inhibition of Akt activation.

9. Thanks. SHIP-1 is an endogenous phosphatase which is inactivated in normal colon tissues, and would be upregulated once the tissues or cells are stimulated by inflammation, injury or other factors. In our fig. 5a, the expression of SHIP-1 in the DSS+antagomiR-155 group increases about 150% compared with the SHIP-1 expression in the DSS or the DSS+NC group. Therefore, our results in fig. 5 were in line with the data in fig. 1 and fig. 3.

10. Thanks very much for your directions. We have replaced them with pictures with high resolutions.

11. Thank you. We have added the IFN-γ-related experiments in fig. 2 in the revised version., but we didn't obtained the IL-17 ELISA kit since the order was delayed for some reasons, we are very sorry for that. Anyway, the promotion of miR-155 on the pro-inflammatory secretions of mouse macrophages has been confirmed by our results. We didn't measure the protein levels of the cytokines because we didn't obtain all these antibodies, we will re-confirm these results by western blot analysis once we get the antibodies. We really appreciate your good comments.

Finally, we appreciate for the Editors/Reviewers' warm work earnestly, and hope that the

corrections will meet with approval.