Dear Editor,

Thanks a lot for your dealing with our manuscript entitled "***Sirtuin 1 alleviates the endoplasmic reticulum stress-mediated apoptosis of intestinal epithelial cells in ulcerative colitis***"(ID: 50161) and prompt response. The comments from you and the reviewers are really helpful for the improvement of our manuscript. We have prepared a revision strictly according to the comments and the alterations have been highlighted in the revised manuscript (red color). Please feel free to contact us if you have any further questions.

We look forward to your final decision.

Sincerely yours,
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Responses to reviewer :

Reviewer 1:

This is a basic study demonstrating that Sirtuin 1 (SIRT1) activation reduces apoptosis of intestinal epithelial cells via suppression of ER stress-mediated apoptosis-associated molecules CHOP and caspase-12.

Question 1: The methods and results in the abstract would be more concise.

Reply: Thank you very much for your advice. The methods and results in the abstract have been revised accordingly. (P4 line13-line23, P4 line26-P5 line2)

Question 2: The SIRT1 activation induced by the ulcerative colitis may be shown in figure 8.

Reply: Thanks a lot for the constructive advice. According to your suggestion, we

performed Western Blot to detect the protein levels of SIRT1 in colon tissues of DSS-treated mice. The results of experiments we have gained in the study have been added in the Result 8 and Figure 8. (P13 line24, P32)

Reviewer 2:

The study contribution is interesting . The authors show that Sirtuin 1 activation reduces apoptosis of IECs through the suppression of the endoplasmic reticulum (ER) stress-mediated apoptosis-associated molecules CHOP and caspase-12. SIRT1 activation could constitue a potential therapeutic strategy for ulcerative colitis . The Introduction Section is well documented .

Question 1: The authors must add one sentence about the role of cytokines and Nitric oxide in inflammatory process during ulcerative colitis (Soufil et al, 2016).

Reply: Thank you very much for your kind reminding. We are sorry that we did not find the literature you mentioned, yet we investigated relevant literatures and added a sentence about the role of cytokines and oxidative stress in inflammatory process during ulcerative colitis in the Introduction section in the revised manuscript. (P6 line6-line9)

Question 2: In Materiel and Methos Section, the choice of THP1 cell line(myeolo-monocytic lin in coculture must be argued in the text.

Reply: We really appreciate preciseness of the reviewer. We have consulted a large number of literatures in the early stage of our study. The coculture model of activated THP-1 cells and Caco-2 cells is a universally accepted model to investigate intestinal barrier and ulcerative colitis. Therefore, we chose THP-1 cell line based on previous studies which was mentioned in Materials and Methods section. (P7 line22-line23)

Question 3: The results are intersting. In Discussion, section the clinical relevance of the study in UC must be discused in one or more sentences.

Reply: It is a good suggestion. We have added sentences about the clinical application of the study in the Discussion section in the revised manuscript. (P14 line5-line8)

Reviewer 3:

After reading the entire submitted manuscript, it is a well-conducted article with potential clinical application that SIRT1 may serve as a novel therapeutic strategy for ulcerative colitis in future, to my opinion.

Reply: Thank you very much for your appreciation.

Reviewer 4:

Question 1: Authors applied the SIRT1 activator SRT1720 and inhibitor NAM to investigate the potential effects of SIRT1 on the intestinal barrier in a UC coculture model. Authors conclude that SIRT1 activation significantly increased the expression of occludin and ZO-1 in Caco-2 monolayers, suggesting that SIRT1 may exert protective effects on colitis by promoting intestinal barrier integrity. Please provide the SirT1 protein levels in the treatment of STR1720 and NAM. Most importantly, authors should provide the SirT1 activity to prove the activity of SirT1 was stimulated by STR1720 and inhibited by NAM well and truly, respectively.

Reply: We really appreciate the valuable advice from the reviewer. In fact, it is well-known that SRT1720 and NAM are specific SIRT1 activator and inhibitor, respectively, which are widely used in scientific researches. We investigated many previous literatures during our research and found that SIRT1 protein levels or SIRT1 activity was not detected when SIRT1 activator or inhibitor was applied in many studies. According to the reviewer's advice, we performed Western Blot to measure SIRT1 protein levels in Caco-2 cells and colon tissues of treated mice with SRT1720 and NAM treatment. The results have been added in the Result 4, Figure 4 Result 8 and Figure 8. (P11 line29-line30, P13 line24-line26, P28, P32)

Question 2: To prove the protective role of SirT1 on ER stress-mediated colitis, I suggest authors to design experiments to knock-down or express SirT1 in Caco-2 cells and then assay the inflammatory cytolines, the expression of occludin and ZO-1 and ER stress-related proteins.

Reply: Many thanks for the good suggestion. We agree with the reviewer that knock-down or overexpression could intervene SIRT1 expression in a more direct way. The suggestion will play a guiding role in our future study. In future studies, we will carry out experiments to knock down or overexpress SIRT1 in Caco-2 cells and then detect the inflammatory cytokines, the expression of occludin and ZO-1 as well as ER stress-related proteins.