Dear Editor and Reviewer,

Thank you for your highly instructive and valuable questions for revision. The manuscript "Schistosoma japonicum attenuates DSS-induced colitis in mice via reduction of endoplasmic reticulum stress" (ESPS Manuscript NO: 33558) has been revised again according to your suggestions, and the changes were listed as follow:

Comment 1: Does S. japonicum infect mice? Or are mice natural host of S. japonicum? This point may affect the results and their interpretation.

Response 1: The terminal host of Schistosoma japonicum includes a variety of mammals such as humans, cattle, dogs, mice, etc. In other words, mice are normal hosts of S. japonicum and they can be infected by S. japonicum. Your suggestion about whether mice are the natural host of S. japonicum is very rational. Thus, we revised and highlighted it in the updated version of the manuscript as "Here we infected mice with S. japonicum cercaria by contact of abdomen skin which is a classical and natural infection modeling method to infect host, such as mice".

Comment 2: Is intraperitoneal administration of S. japonicum cercariae a natural route of infection in human?

Response 2: In the natural state, terminal hosts (such as human) of S. japonicum get infected by skin contact with cercaria of S. japonicum. Infecting mice with S. japonicum by contact of abdomen skin is a classic infection modeling method that is widely used (Wu J et al., 2010). In addition to cercariae, other stages of S. japonicum can't infect the terminal host. Intraperitoneal administration of S. japonicum eggs is taken as an immune agent to stimulate the host immune response resulted in the release of relevant cytokines. Thus, we thanks for your suggestion and we also highlighted in the updated version of the manuscript as "Here we infected mice with S. japonicum cercaria by contact of abdomen skin which is a classical and natural infection modeling method to infect host, such as mice".

Comment 3: About the experimental model.

Response 3: After continuously drinking of dextran sulfate sodium (DSS) water, mice were presented as diarrhea, mucosal ulceration, rectal bleeding and bodyweight loss

which were characteristic performance of IBD. DSS-induced colitis model is a widely used model with good repetitiveness. In our previous research named "CARD3 deficiency protects against colitis through reduced epithelial cell apoptosis", we also used this DSS-induced colitis model and we are very familiar with this modeling method. Therefore, thanks for your suggestion about this DSS-induced colitis model and we revised it as "Then we investigated the effects of S. japonicum infection on the relatively simple and replicable DSS-induced colitis model due to the limitations of human clinical trials. DSS-induced colitis has been widely used as an experimental animal model of IBD, especially UC, owing to its characteristic IBD performance, such as diarrhea, mucosal ulceration, rectal bleeding and bodyweight loss" in the updated manuscript.

Comment 4: Please tell me the relationship between NF-κB and apoptosis in DSS?

Response 4: Thank you so much for the question. NF-κB does play an anti-apoptotosis role through transcriptional regulation of anti-apoptotic targets, such as IAPs, FLIP and Bcl-xL (Chen et al., 2001). However, many antiapoptotic targets of NF-kB show partial protective activity under conditions where NF-kB is inactivated: for example, it has been shown that co-expression of c-IAP1, c-IAP2, TRAF1 and TRAF2 is required to effectively suppress TNF-induced apoptosis in $RelA^{-/-}$ mouse embryo fibroblasts (MEFs) (Wang et al., 1998). The finding that hepatocyte apoptosis induced by TGF-β is accompanied by repression of NF-kB-dependent antiapoptotic targets Bcl-xL, X-chromosome-linked IAP (XIAP) and a-fetoprotein also supports this hypothesis (Cavin et al., 2004). In addition, NF-κB can also promote programmed cell death in response to certain death-inducing signals and in certain cell types (Kucharczak et al., 2003) which suggests a complicated relationship between NF-κB and apoptosis.

Even through NF-kB act as an anti-inflammatory role under some conditions, we also find the pro-inflammatory effect of NF-kB in IBD. Inhibition of RICK/NF-kB and p38 signaling attenuates the inflammatory response in a murine model of Crohn disease (Hollenbach et al., 2015). Shang et al reported that disruption of tumor necrosis factor receptor-associated factor 5 (TRAF5) could exacerbate DSS-induced mice and enhance the expression of phosphorylated-p65 (P-P65) (Shang et al., 2016). In our previous research, we also observed a down-regulation of inflammatory response in DSS-induced mice deficiency in CARD3 accompanying by decreased expression of P-P65 and

reduced epithelial cell apoptosis (Yu et al., 2015). Thus, we prefer to consider that NF-κB may play a pro-inflammatory and pro-apoptotosis role in DSS-induced colitis. And we revised is as "The high apoptosis level in DSS treated intestinal cells might be affected by the strong response of NF-κB which is consistent with our previous research that diminished NF-κB activation with decreased apoptosis in colon tissues, especially enterocytes in CARD3$^{-/-}$ mice induced by DSS" in the updated manuscript.

Comment 5: Please tell me the pathway and diagram from ER to Apoptosis in DSS via IRE1α、Chop and CRP78.

Response 5: Thanks for your question about the relationship between ER and Apoptosis.

ER stress can induce apoptosis in three major ways:

①CHOP pathway: The expression of CHOP, which mainly exists in the cytoplasm, is very low in general. However, increasing transcription of CHOP can be induced by IRE1 and accumulates in the nucleus under ER stress (Ron et al,. 1992). Excessive expression of CHOP promotes apoptosis (Mccullough et al,. 2001) through activating apoptosis response protein, such as GADD34, ERO1 and death receptor DR5 which encodes the death receptor anchored in the cell surface capable of activating the caspases cascade response (Yamaguchi et al,. 2004). Besides, the dimer which consists of CHOP and cAMP response element binding protein (CREB) can enhance mitochondria sensitivity to pro-apoptotic factors via inhibiting expression of Bcl-2. Enhanced expression of CHOP led to apoptosis by reducing expression of Bcl-2, decreasing endocelluar glutathione and increasing generation of reative oxygen intermediates (ROIs) (McCullough et al., 2001).

②Caspase pathway: Caspase-12 is a member of the Caspase subfamily and located in the outer membrane of endoplasmic reticulum which specially participates in the apoptosis mediated by ER (Gotoh et al., 2002). Under ER stress, chaperone protein GRP78 induced by ER stress, Caspase-7 and Caspase-12 can form a complex on the membrane to prevent Caspase-12 activation. However, the GRP78-Caspase-7-Caspase-12 complex can be dissociated by dATP. Then Caspase-12 can transfer to to cytoplasm and get activated to promote apoptosis (Peel et al. 2002). Besides, Caspase-12 may get activated by TRAF2 seperated from TRAF2-procaspase-12 complex and mediate phosphorylation of JNK by recruitment

JIK-IRE1 complex to TRAF2 (Rao et al. 2001).

③JNK pathway：C -Jun amino-terminal kinase belongs to the mitogen-activated protein kinases (MAPKs) family or stress-activated protein kinases (SAPKs) family. Overexpression of IRE-1 could promote apoptosis of human embryonic kidney cells HEK193 (Wang et al. 1998). When IRE1 is activated, its connection joint molecular TRAF2 (TNF-receptor-associated factor2) on the cytoplasmic enzyme structure domain and ASK1 (apoptosis signal-regulating kinase 1) together form IRE1-TRAF2-ASK1 complex to activate JNK. Then activated JNK transfers to the nucleus, and promotes apoptosis by phosphorylating transcription factors (c-jun, c-Fos, EIK-1) and regulating the expression of downstream apoptosis-related target genes. For example, activated JNK may start the death receptor pathway of apoptosis through increasing the expression of FasL, TNF and other ligand protein. Also pronounced expression of Bim, Bid can be induced by activated JNK and proapoptotic proteins such as Bax is activated to motivate apoptosis via mitochondrial pathway. In addition, activated JNK remaining in the cytoplasm can mediate the occurrence of apoptosis by phosphorylating the members of Bcl-2 family directly.

In this paper, we prefer to speculate that ER stress induce apoptosis through CHOP or JNK pathway. The expressions of Bcl-2 and ER stress-related proteins, especially CHOP support this hypothesis. Also, there is a chance that ER stress induces apoptosis through Caspase-12 pathway, which need much further research. And here is the revision about this issue in the updated manuscript “Wang et al [45] found that overexpression of IRE-1 could promote apoptosis of human embryonic kidney cells HEK193. Under ER stress, IRE1α could not only prevent cell apoptosis caused by sustained ER stress through activating Xbp1[36], but also promote mitochondrion-dependent cell death by binding to Bax on the outer membrane of mitochondrial[46,47]. If IRE-1 join together with TRAF2 and Aask1 and compose an IRE1-TRAF2-ASK1 complex, it can play a pro-apoptosis role by motiving JNK pathway. Besides, enhanced expression of CHOP might lead to apoptosis by reducing expression of Bcl-2, decreasing endocelluar glutathione and increasing generation of reative oxygen intermediates (ROIs) [48]. And by forming a dimer with cAMP response element binding protein (CREB), CHOP could also promote apoptosis through inhibiting the expression of Bcl-2 and enhancing the mitochondria sensitivity to pro-apoptotic factors[49]. Thus, we propose a hypothesis that less severe apoptosis in

CER+DSS mice was associated with lower level of ER stress in contrast to DSS mice."

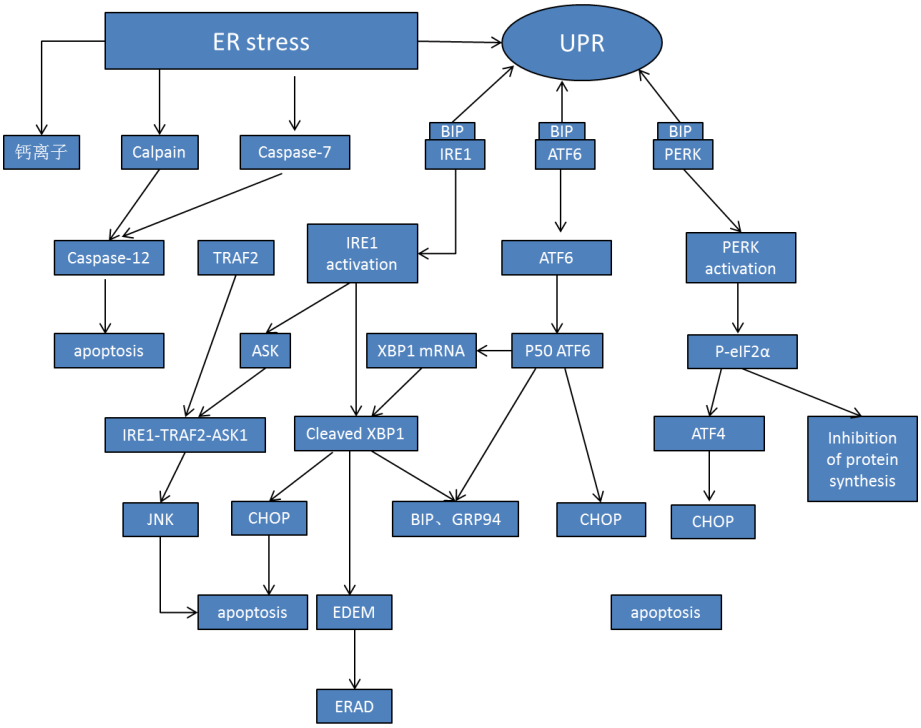

Diagram: ER stress and apoptosis

Comment 6: Please tell me the pathophysiology of DSS+CER can prevent enterocyte apoptosis of DSS induced colitis.

Response 6: Thank you so much for the question. Firstly, we're very sorry that when we used the word "apoptosis", we mean the apoptosis of enterocytes, immune cells and other cells in colon tissues. Thus we might have made some mistake by only used the words "enterocytes apoptosis". In this paper, we described remissive apoptosis in colon tissues of DSS induced colitis due to S japonicum infection including enterocytes and other cells. On one hand, we observed lots of Intestinal epithelial cell loss through H&E staining. On the other hand, we found lots of cleaved-caspase3 as well as TUNEL positive cells in colon tissues by immunofluorescence and TUNEL tests. Secondly, ER stress is strongly related with apoptosis. Therefore, in this paper, we speculate that S. japonicum reduced cell apoptosis in colon tissues via reduction of inflammatory response accompanying with low expression of inflammatory cytokines such as TNF-α. Not only NF-κB, but also ER stress probably takes part in the apoptosis including entercytes. Here, we prefer ER stress to NF-κB as a pro-apoptosis signal because of the expressions of relative proteins and the clearly correalated relationship between ER

stress and apoptosis. But NF-κB might also play a pro-apoptosis role in this research which needs further investigations. Thanks again for your instructive question and our partner will focus on this in the following research.

Thank you again for your careful works.

Sincerely yours,

Ya Liu

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