Dear Editor:

Enclosed please find our revised manuscript entitled "3,6-dichlorobenzo[b]thiophene-2-carboxylic acid alleviates ulcerative colitis by suppressing mammalian target of rapamycin complex 1 activation and regulating intestinal microbiota" (Manuscript NO: 78854)

We would like to thank you and the reviewers for your insightful and constructive critiques and comments. We have carefully revised the manuscript and reorganizing the figures.

Detailed, point-by-point responses to Editor and each individual reviewer are presented below.

Your consideration is greatly appreciated.

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Point-by-point responses:

Response to Reviewer #1:

Question 1: "Part 2.3. Induction of UC and experimental design： the other groups were given 3.5% DSS in drinking water …… Mice in the control group and DSS group were given 0.5% carboxymethyl cellulose sodium." It seems that 3.5% DSS

concentration a bit high, and there is no mouse death? And why mice were given 0.5% carboxymethylcellulose sodium?

Respond to comment: Thank you for your insightful comments. We set up several different DSS concentrations in the pre-experiment, and 3.5% DSS had the best modeling effect, with obvious inflammatory phenotype, and no mice died during the whole experiment. This concentration has also been reported in previous studies.

Citation: Li T, Wang C, Liu Y, et al. Neutrophil Extracellular Traps Induce Intestinal Damage and Thrombotic Tendency in Inflammatory Bowel Disease. *J Crohns Colitis*. 2020;14(2):240-253. doi:10.1093/ecco-jcc/jjz132.

Wu C, Yang H, Han C, et al. Quyu Shengxin Decoction Alleviates DSS-Induced Ulcerative Colitis in Mice by Suppressing RIP1/RIP3/NLRP3 Signalling. Evid Based Complement Alternat Med. 2021;2021:6682233. Published 2021 Aug 20. doi:10.1155/2021/6682233

Carboxymethyl cellulose sodium is a common drug co-solvent. In our experiment, 0.5% carboxymethyl cellulose sodium solution was used as the solvent for all drugs, so mice in the blank group and the model group were gavaged with equal volume of 0.5% carboxymethyl cellulose sodium solution.

Citation: Zhou J, Zhou Z, Ji P, Ma M, Guo J, Jiang S. Effect of fecal microbiota transplantation on experimental colitis in mice. Exp Ther Med. 2019;17(4):2581-2586. doi:10.3892/etm.2019.7263

Question 2: Fig4. BT2 suppressed the activity of mTORC1 signaling pathway. The experiment can only prove that he can inhibit the mTORC1 signaling pathway, not enough to prove that bt2 is inhibiting the mTORC1 signaling pathway by inhibiting bcaa metabolic disorder, suggesting that relevant experiments could be added if it is possible.

Respond to comment: Thanks for your constructive comments. A large number of previous studies have shown that BCAA plays an important role in activating the mTORC1 pathway. In our study, Immunoblotting and immunohistochemistry analysis were performed to detect the expression of kinases including BCKDK, P-BCKDHA,

BCKDHA and BCAT2, proving the ameliorative effect of BT2 on BCAA catabolism (Fig3 A-E). Moreover, elevated concentrations of leucine, isoleucine and valine were observed in the serum of DSS-treated mice, while BT2 treatment resulted in the downregulation of these amino acids (Fig3 F). In summary, BT2 could inhibit the mTORC1 signaling pathway by inhibiting BCAA metabolic disorder.

Citation: Zhang BK, Moran AM, Bailey CG, Rasko JEJ, Holst J, Wang Q. EGF-activated PI3K/Akt signalling coordinates leucine uptake by regulating LAT3 expression in prostate cancer. *Cell Commun Signal*. 2019;17(1):83. Published 2019 Jul 25. doi:10.1186/s12964-019-0400-0.

Goberdhan DC, Wilson C, Harris AL. Amino Acid Sensing by mTORC1: Intracellular Transporters Mark the Spot. Cell Metab. 2016;23(4):580-589. doi:10.1016/j.cmet.2016.03.013.

Lynch CJ, Adams SH. Branched-chain amino acids in metabolic signalling and insulin resistance. Nat Rev Endocrinol. 2014;10(12):723-736. doi:10.1038/nrendo.2014.171.

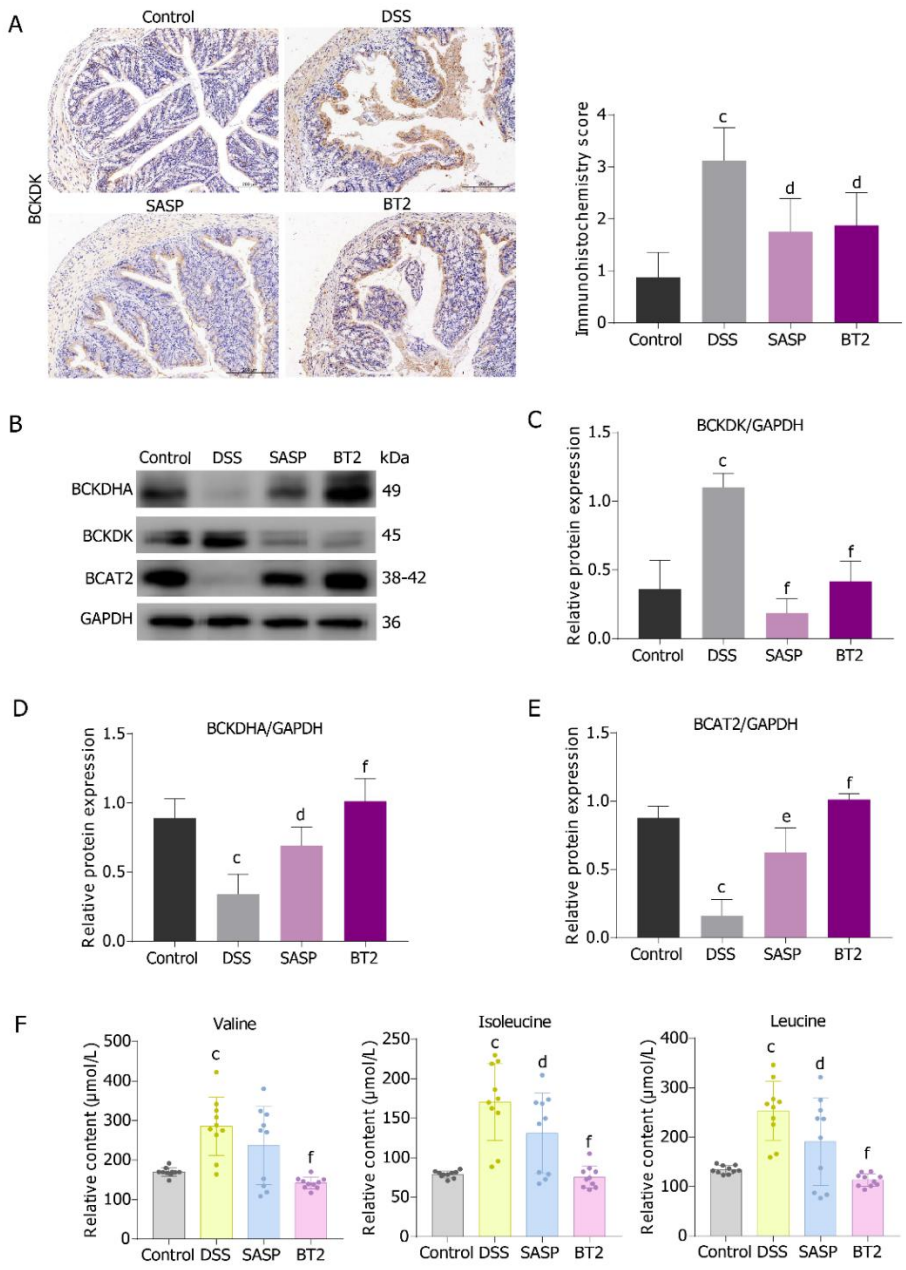

Figure 3 3,6-dichlorobenzo[b]thiophene-2-carboxylic acid improved branched-chain amino acid catabolism in dextran sodium sulfate-induced colitis mice. A: The expression of BCKDK in colon sections was detected by immunohistochemical assays (n=4, 200× magnification); B-E: Western blot images and quantitative data of branched-chain amino acid (BCAA) catabolic enzymes in colon tissues (n=3~4); F: The contents of BCAAs, including valine, leucine and isoleucine, in mouse serum were determined by liquid chromatography-tandem mass spectrometry (n=10). $^{d}P<0.05$, $^{e}P<0.01$, and $^{f}P<0.001$ *vs* the dextran sodium sulfate (DSS) group; $^{c}P<0.001$ *vs* the control group. The data are shown as the mean ± SD.

BCKDK: branched-chain α-keto acid dehydrogenase kinase.

Response to Reviewer #2:

Question 1: The state of the art should be extended.

Respond to comment: Thanks for your constructive comments. We have corrected the materials and methods section in detail to make sure the content is more accurate and the language in revised manuscript has been polished through professional company.

Question 1: A section of conclusions and future work should be included in which the scientific contribution of the work is indicated and a set of lines of future work are established.

Respond to comment: Thanks for your expert comments, we have supplemented the conclusion section and added the subsequent exploration in the discussion. In further investigations, we will combine the mTORC1 inhibitor rapamycin and gene knockout mouse models to validate the mechanism of BT2-regulated BCAA metabolism in anti-UC effect. Additionally, fecal microbiota transplantation will be employed to explore the specific flora concerning the pathogenesis of UC.