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Thank you very much for reviewing and positively considering our manuscript (Manuscript NO.: 39689) for its publication in World Journal of Gastroenterology

We are grateful to the reviewers for their useful and constructive comments and suggestions.

We have carefully revised our manuscript in term of all comments from the reviewers. Other minor corrections, including spelling and grammar checking, have been made and shown in the text. All the revisions are marked in Yellow fonts. Our point-by-point responses to each reviewer are shown below.

I am now submitted our revised manuscript for further consideration and publication in World Journal of Gastroenterology.

Sincerely □ yours

Lin Hai Kurahara, corresponding author

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Response to Reviewers:

First of all, we thank the reviewers for their constructive comments and suggestions. We have studied the comments carefully and made several amendments to the manuscript. Please see our point-by-point responses below.

Reviewer #1: This reviewer is very impressed with the study design, performance of the experiments and the way the data were collected, analyzed and presented. The effects of the well known Chinese medicine DKT on the expression and activation of the TRPA1 channel is well investigated in this study. The data clearly demonstrates DKT suppresses intestinal fibrosis which should be a step forward toward reducing intestinal inflammation. The technical design for running PCR, and collecting histopathology data were adequate. The manuscript is written concisely and nicely. The discussion is a bit long but it seems it was necessary to address all the data. Fig 9 (the Diagram) illustrates the proposed mechanism and it is well done. I have no reservations about this manuscript. I did not invest time on typos and minor issues.

Response: We are very grateful for your comments.

Reviewer #2: Hiraishi et al present a series of experiments in which they demonstrate that topical DKT treatment induced calcium influx in intestinal myofibroblasts, which appeared to be related to TRPA1 channel activation and upregulation. It was also shown that TRPA1 is increased in segments of stenosed bowel in specimens from patients with Crohn's disease and in the presence of collagen, suggesting a potential role in negative feedback for collagen production. The paper is well written with appropriate conclusions, and it is certainly an area of interest given the lack of anti-fibrotic therapies. I have a few suggestions: 1. You have not discussed any potential limitations of the techniques used in your discussion. These should be identified and discussed. Minor comments: - Figure 9 is not referenced in the text – it could be referenced in the introduction to improve understanding of the pathways that are being evaluated - For the statistics section “p” value should be in lower case. - For the results section “Chronic TNBS fibrosis model of TRPA1-KO mice”, the first paragraph can be moved to the TNBS model section in methods. - consider changing the wording of cold sensation to cold sensitivity in the discussion - Do you believe the fact that all patients with resection specimens were on anti-TNF therapy impacted your findings?

Response: Thank you very much for your critical comments.

- Potential limitations of the techniques used in this study are now described in the discussion.
- Figure 9 is referenced in the discussion on page 17.
- In the methods/statistics, “p” is now shown in lower case.
- Since all patients had long history of CD with anti-TNF therapies applied at various stages, with limited number of resected specimen, it is difficult to summarize the impact of anti-TNF  $\alpha$  treatment in this study. Our in-vitro data can only indicate that TNF $\alpha$  can up-regulate TRPA1 expression at InMyoFibs (Fig.6C).

Reviewer #3: The manuscript titled Daikenchuto (Da-Jian-Zhong-Tang) ameliorates intestinal fibrosis by activating myofibroblast TRPA1 channel described the properties of DKK in colonic fibrosis through TRPA1. The title reflect the main subject of the manuscript. The abstract summarize the work described but the conclusion is generic and has to be more concise. The key words reflect the focus of the manuscript. The background describe in a simple way the postoperative use of DKK. The Methods are adequate. However the authors have to explain how they measure the optical density of the blue-stained (with image J?). That item is not explained in the reference 18. The Results and experiments are well designed and improve knowledge. WB from Fig 2A WT can be improved. The authors interpret the findings adequately and the findings have relevance and the manuscript discuss the applicability. However, failed to explain what the pattern of treatment with DKK is. The illustrations and biostatistics are well, I haven't seen any errors. However, the genes of animals are named in italics and lowercase.

Response: Thank you very much for your nice comments.

- The discussion was partially modulated.
- The methods of evaluation blue stained section was explained in the [Histologic Evaluation] part in the method both of this paper and reference 18. All MT staining sections analyzed by a pathologist [K.K.] by blinded manner.
- WB from Fig 2A WT was improved.
- The pattern of treatment with DKT is descried at method part '*Trinitrobenzenesulfonic acid chronic colitis model*'. →DKT was administered daily (5 mg/kg/day; anesthetized by isoflurane) by enema for one week after the last TNBS treatment.
- The genes of animals are changed in italics and lowercase.