November 23rd, 2020

Reference: JoVE62093

Dear Dr. Bajaj

We thank you for your recent email inviting to resubmit a revised version of our manuscript entitled "Functional Assessment of Intestinal Permeability and Neutrophil Transepithelial Migration in Mice Using a Standardized Intestinal Loop Model".

Below is a point-by-point response to the editorial's comments and attached as a separate document a point-by-point response to the reviewers' comments.

All additional information and discussion points are included in the revised manuscript and are highlighted in red color.

Response to editorial comments:

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. Please define all abbreviations at first use.

Response: We have proofread and corrected the manuscript. Abbreviations are defined at first use.

2. Please shorten your title to "Functional Assessment of Intestinal Permeability and Neutrophil Transepithelial Migration in Mice Using a Standardized Intestinal Loop Model".

Response: As requested, we have shortened our title.

3. JoVE cannot publish manuscripts containing commercial language. This includes trademark symbols (TM), registered symbols (®), and company names before an instrument or reagent. Please remove all commercial language from your manuscript and use generic terms instead. All commercial products should be sufficiently referenced in the Table of Materials and Reagents.

For example: EZ-7000 anesthesia vaporizer; Kimwipe etc

Response: We have edited our manuscript to comply with JoVE's publication requirements.

4. Please note that your protocol will be used to generate the script for the video and must contain everything that you would like shown in the video. Please add more details to your protocol steps. Please ensure you answer the "how" question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action. Please add more specific details (e.g. button clicks for software actions, numerical values for settings, etc) to your protocol steps. There should be enough detail in each step to supplement the actions seen in the video so that viewers can easily replicate the protocol.

<u>Response</u>: We have included more details (including references) to our protocol in order to facilitate reproducibility of the methods. In addition, the specific points raised by reviewer #1 in the protocol section have also been addressed (see response to reviewers).

5. Please specify the number, strain, sex, and age of the mice. Please mention (even as notes) in the beginning if you are using Villin-cre; Jam-afl/fl and Jam-a-/- mice.

<u>Response</u>: We have provided more detailed information about the number, strain, sex, and age of the mice at the beginning of the protocol section (on line 125).

6. Please specify the method of euthanasia without highlighting it.

<u>Response</u>: We have clarified that animals are euthanized when being under anesthesia by using a physical method such as decapitation or cervical dislocation, and in accordance with the approved animal protocol (see note after step 4.5 and step 5.6.2).

7. Note after 1.2.5: What should the vitals and pedal reflex be (to help readers monitor)?

<u>Response</u>: We have now detailed what is expected for vitals and the pedal reflex when mice are under deep anesthesia and can undergo surgery (see steps 1.2.4 and 1.2.5).

8. Please use comma and not period as decimal (line 4.6).

Response: Done

9. Please include a one line space between each protocol step and then highlight up to 3 pages of protocol text for inclusion in the protocol section of the video.

Response: Done

10. Please do not abbreviate journal names in the reference list.

Response: Done

Respectfully,

Charles A. Parkos, MD, PhD