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Dr. Phillip Steindel Review Editor Journal of Visualized Experiments

July 6, 2018

Dear Dr. Steindel,

Re: JoVE58450 "In Vivo Imaging of Reactive Oxygen Species in a Murine Wound Model"

We would like to thank you for the thorough reading of our manuscript and valuable feedback. Please see below, our itemized point by point response to the editorial comments in bold. We hope that our revisions have satisfactorily addressed your concerns and questions, and we look forward to hearing from you.

Editorial comments:

- **1.** For both steps 1 and 4, we require more specific detail regarding the treatment of animals. Please address the following points, where applicable:
- **a.** Please specify the use of vet ointment on eyes to prevent dryness while under anesthesia.

Thank you to the editor for bringing this to our attention. We have added the detail to the manuscript.

b. Please discuss post-anesthesia treatment of animal, including recovery conditions and treatment for post-surgical pain.

Thank you for thorough evaluation of our manuscript. We have now added the pertaining details of post-op and post-anesthesia treatment of the animals to ensure their safety and recovery.

c. Please discuss maintenance of sterile conditions.

Thank you for bringing this detail to our attention. In this revised manuscript, we have added details including the personal protective equipment worn by all personnel and sterile surgical tools.

d. Please specify that the animal is not left unattended until it has regained sufficient consciousness to maintain sternal recumbency.

Thank you for pointing this out. We have revised the manuscript to include steps to monitor anesthetized animals post-op until conscious and mobile, including post-op housing conditions.



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e. Please specify that the animal that has undergone surgery is not returned to the company of other animals until fully recovered.

Thank you for attention to this important point. We do not return animals to the company of other animals after surgery to prevent adverse interactions and changes to wound healing trajectories. The revised manuscript includes these details.

2. Results/Figure 4D: What statistical tests were used?

Thank you to the editor for catching this critical point. We have added that we used Student's t- tests for Figure 4D.

3. Results: It is still somewhat unclear that Table 1 and Figure 4D show different results.

Thank you to the editor for bringing this to our attention. We have now revised Figure 4D to reflect the values in Table 1 to simplify the data presented.

4. Figure 3: You mention in your questionnaire this is reproduced; please indicate as such in the Figure legend, i.e. "This figure has been modified from [citation]." Also, please upload explicit permission (of a letter from the editor or a link to the editorial policy that allows reprints) to your Editorial Manager account.

Due to the high cost of reproducing our previously published figure, we have created a new Figure 3 with unpublished data.

5. Figure 4D: What is n here?

Thank you to the editor for bringing this to our attention. We have included n=4 in the figure legend.

6. Figure 5: Please explain more how panel A shows 'reduced evidence of inflammatory morphology' and how panel B shows 'decreased macrophages'. Also, please include scale bars in panel B.

Thank you to the reviewer for careful reading of our manuscript. We have re-worded and added more descriptive detail of the figures in Figure 5, both in the text and the figure legend.

Best Regards,

Piul Rabbani, PhD.