Dear Reviewer #1 (03478635),

We are pleased to receive your comments on our manuscript, proposed title “Do Paneth cells press a ‘button’ in controlling the pathogenesis of radiation enteropathy”. According to your suggestions, we believe that the scientific quality of this manuscript will be improved. The following information is our response to your comments.

As you mentioned, you believed the communications between intestinal stem cells and Paneth cells are mediated via molecules such as EGF, WNT3a and DLL are very interesting. So, to enable the readers to get direct information from this manuscript, we have revised the sentence into “epithermal growth factor (EGF), Wnt3 and Dll1/4 (Notch ligands) to neighboring ISCs” in the section of “PANETH CELL AND EPITHELIAL HOMEOSTASIS”, which has been labeled in yellow. We believe this revision will match with your intention.

Dear Reviewer #2 (02440884),

We are pleased to receive your comments on our manuscript, proposed titil “Do Paneth cells press a ‘button’ in controlling the pathogenesis of radiation enteropathy”. According to your suggestions, we believe that the scientific quality of this manuscript will be improved. The following

information is our response to your comments.

Thank you very much for your providing the reference *Annu. Rev. Physiol.* 2013. 75：289-311. We believe we get more useful information in this review article, presenting that ionizing irradiation can induce autophagy in Paneth cells, thus affecting their production of antimicrobial peptides. As you may concern, you suggest us to add information about the putative role of Wnt3 in irradiation induced Paneth cell damage. As we review the published data, we find that activation of Wnt signaling pathway can induce Paneth cell maturation, presenting by MMP7/defensin programme transcription (*Nat Cell Biol* 2005; **7**:381-6). Moreover, Wnt3-mutant miniguts will halt their growth in vitro (*Annu. Rev. Physiol*. 2013. 75： 289-311). So, we believe that loss of Paneth cells postirradiation will enable Wnt3 production to be decreased, somewhat affecting the epithelial regeneration process. Besides, although Math1-deletion induces elimination of Paneth cells in vivo, the Math1-mutant miniguts still halt their growth in vitro, demonstrating the specific role of Paneth cells in promoting ISC expansion. Meanwhile, due to the information you suggest us to add, we have revised some sentences in ABSTRACT and the section “THE MISSION OF PANETH CELL IN RADIATION ENTEROPATHY” to improve the logic flow of this manuscript. All the revised information is labeled in blue.

Dear Reviewer #3 (02567328),

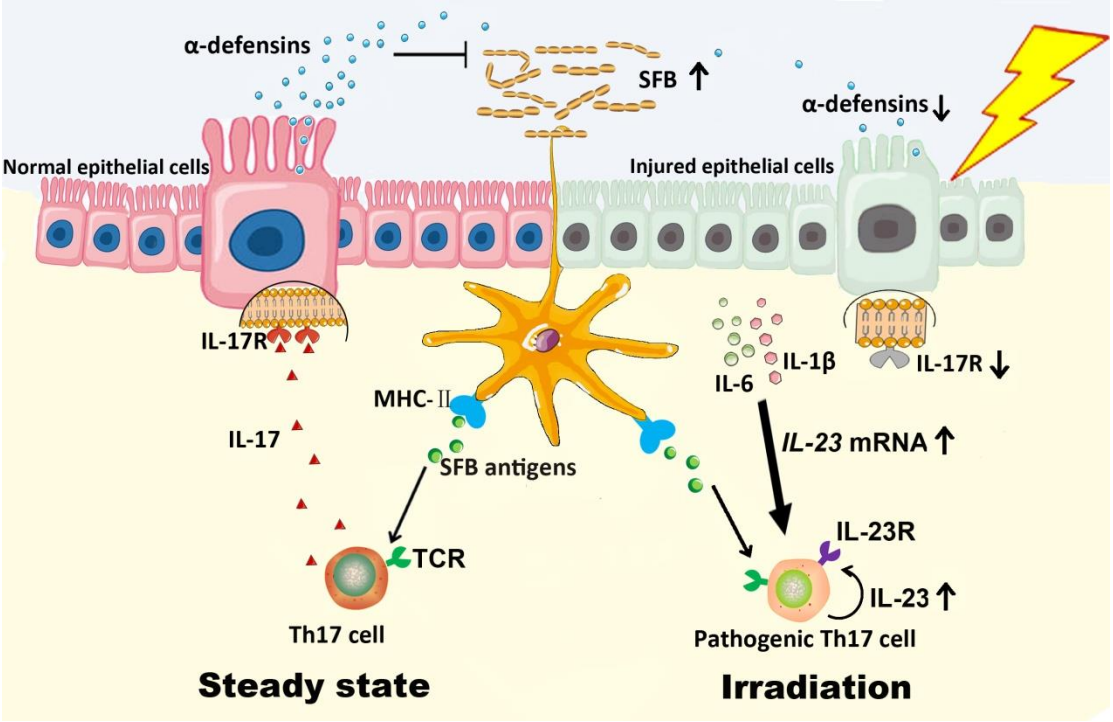
We are pleased to receive your comments on our manuscript, proposed title “Do Paneth cells press a ‘button’ in controlling the pathogenesis of radiation enteropathy”. According to your suggestions, we believe that the scientific quality of this manuscript will be improved. The following information are our responses to your comments point-by-point.

1. You believed the proposed title did not match with the contents of this manuscript. According to this suggestion, we have revised the title into “Gut commensal bacteria, Paneth cells and their relations to radiation enteropathy”. We believe this title will be more suitable for this manuscript, because we select gut commensal bacteria and Paneth cells as targets, and discuss the role of Paneth cells in controlling gut bacterial homeostasis along with their relations to radiation enteropathy. On this basis, to improve the logic flow of this manuscript, we have revised some sub-headlines of this manuscript. You can find them in the text, which have been labeled in pink color.
2. The second issue you may concern is that you believed that the term “intestinal epithelium” was incorrectly. As you depicted, Paneth cells are located in crypts of lamina propria. Of course, we believe this suggestion is useful. Moreover, you also believe that Paneth cells are

specialized epithelial cells of the small intestine, suggesting the Paneth cells are surely epithelial cells. But we can understand that you believe the intestinal epithelium is incorrect in depicting the intestinal barrier function. So, you can see in the review article (*Nat Rev Gastroenterol Hepatol* 2017; 14: 9-21), the term “intestinal epithelium” is used here. Moreover, to reflect epithelium is a part of mucosa, we revised the information in the section “PANETH CELL AND EPITHELIAL HOMEOSTASIS”, presenting that “Moreover, Paneth cells are derived from ISCs, and they are distributed in the basement of the crypts of Lieberkühn, tiny invaginations that line the mucosal surface all along the small intestine.” This sentence is cited from the Abstract of the reference *Annu. Rev. Physiol.* 2013. 75：289-311.

3. You believed the Fig.1 is not clear in showing scientific information. We have revised it to improve its quality. Besides, the figure legend has been revised as well. To elucidate how does radiation induce pathogenic Th17 cells in gut. We add information concerning Th17 polarization in gut in steady state. Functionally, dendritic cells in gut lamina propria are sufficient in Th17 cell polarization, because they can present *SFB* antigens to Th17 cells, while MHC-II molecule can provide all essential signals to Th17 cells for their polarization (*Immunity* 2014, 40:594-607). By using IL17/IL-17R interaction, Th17 cells will control the frequency of *SFB* in gut lumen by

stimulating epithelial generation of α-defensins, which protect against *SFB* overgrowth (*Immunity* 2016; 44:659-671). But under irradiated condition, ionizing irradiation will damage epithelial cells, thus causing local increasement of IL-1β and IL-6 in injured sites (*Gut* 2018; 67: 97-107). In this context, these cytokines will upregulate expression of gene encoding IL-23 (*Immunity* 2009; 30:576-87; *Nat Immunol* 2007, 8:967-74). IL-23 is able to promote Th17 cell expansion. By using IL-23/IL-17 loop, the inflammation in irradiated gut will persist (*World J Gastroenterol* 2015, 21:5823-30). Moreover, radiation-induced epithelial loss enables IL-17R to be decreased. In this context, epithelial generation of α-defensins will be decreased as well. On this basis, *SFB* overgrowth is facilitated, thus potentially promoting Th17 induction. All the newly-cited references have been added into the manuscript. The following is the revised Fig.1.



4. The full name of “CRC” has been added in INTRODUCTION by using “colorectal cancer”.
5. The section “COMMENSAL BACTERIA, ADAPTIVE IMMUNITY AND INTESTINAL RADIATION SENSITIVITY” has been divided. Moreover, because we only depict the information of Treg and Th17 polarization in gut in this manuscript, we revised the subheadline into “COMMENSAL BACTERIA AND GUT Th17/Treg BALANCE POSTIRRADIATION”. The revised information has been labeled in pink color.

Best regards,

Dr. Chang Peng-Yu on behalf of all contributors