Author's response to reviews

Title: Activation of transcription factor AP-1 in response to thermal injury in rat small intestine and IEC-6 cells

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Author's response to reviews: see over
Dear Editor and reviewers,

Thank you very much for your constructive suggestions on how to improve this manuscript. We have proofread the manuscript carefully and revised it accordingly. As corresponding author, I apologize for the delay of this revised manuscript. We sincerely wish the manuscript to be reconsidered for publication. The revisions are highlighted in the manuscript by colored text and our response to reviewers’ comments typed in bold-face below.

Response to reviewer Joerg Hoffmann

The results section in the Abstract is too short and should be expanded.

This section was expanded.

Response to reviewer Michael Schumann

Major Compulsory Revisions: Differences to previously published work on AP-1 and NF-κB should be delineated more specifically.

Done, see lines 197-204

Major points:

1. Figure 4: The reader can appreciate a higher proportion of shedded, presumably dead cells on a IEC6-layer. The %-survival needs to be clarified. A staining of the epithelial layer (e.g. IF for cadherin) in combination with an apoptosis assay would demonstrate the heat stress better. Determining a transepithelial electrical resistance (TER) for filter grown IEC-6 might be good global read-out to quantify the epithelial defect induced.

We do have pictures with higher proportion of damaged cells on a IEC6-layer (e.g. IEC-6 exposed to 42°C for 8h). It is not necessary to show the results of all time-points. The purpose of Figure 4 was just to tell readers that the morphology and viability of IEC-6 cell would change under heat stress. Actually, in our previously published papers, apoptosis assays (TUNEL staining and flow cytometry analysis) have demonstrated that heat stress could induce significant damage in intestinal epithelial cell. (Z Gao, et al, Inhibition of heat-induced apoptosis in rat small intestine and IEC-6 cells through the AKT signaling pathway, *BMC Veterinary Research* 2013, 9:241; J Yu, et al, Involvement of oxidative stress and mitogen-activated protein kinase signaling pathways in heat stress-induced injury in the rat small intestine, *Stress* 2013, 16:1) Hence, I think there is no need to post apoptosis assay result in this paper again. A new picture of AV/PI staining towards IEC-6 (42°C, 4h) was attached below. Limited to testing equipment, we are not able to do a transepithelial electrical resistance(TER).

Minor points:
1 Figure 3 should be incorporated in Figure 2

Done

2 Legend Figure 4 should include the information, how %-survival was actually analyzed.
Legend Figure 4 was expanded, see Legend Figure 3.
3 Figure 6 legend, 2nd sentence needs to be rewritten.

Done

4 Legend Figure 7B
Revised according to reviewer’s advice.

5 Consider to add Fig. S1A to Figure 5
Not necessary. Fig. S1A and Fig. S1B demonstrated NF-κB was not activated in vivo and in vitro respectively. Fig. 5 was the result of AP-1.