ANSWERING REVIEWERS

November 24, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: manuscript 14622 R1.doc).

Title: Intestinal genetic inactivation of caspase-8 diminishes migration of enterocytes

Authors: Elke Kaemmerer, Paula Kuhn, Ursula Schneider, Min Kyung Jeon, Christina Klaus, Miriam Schiffer, Danika Weisner, Christian Liedtke, Jörg Jäkel, Lieven Nils Kennes, Ralf-Dieter Hilgers, Norbert Wagner, and Nikolaus Gassler

Name of Journal: *World Journal of Gastroenterology*

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The manuscript has been improved according to the suggestions of reviewers:
1 Format has been updated

2 Revision has been made according to the suggestions of the reviewer

(1) Reviewer: How about cell migration using more differentiated Caco2 cells deleted for casp8?

Answer: We absolutely agree with you that cellular differentiation is a variable in cell migration. Differentiation of Caco2 cells is improvable by butyrate or other substitutes. However, we have never performed such differentiating experiments followed by casp8 deletion. In the present study, cell culture experiments were used to demonstrate a crucial link between casp8 and enterocyte migration. The intention was to demonstrate in vitro that cellular differentiation modifies casp8-dependent migration. In our opinion the differentiation experiments proposed should be included in a separate paper.

(2) Reviewer: What is the degree of caspase-8 expression in small intestine and large intestine? Perhaps, if differences exist, this could explain differences in migration seen between small intestine and colon.

Answer: Your interesting question addresses a very important point. Normalized to enterocyte housekeepers we were not able to demonstrate significant differences of casp8 expression between small and large intestinal mucosa. Consequently, we do not believe that inhomogeneous casp8 expression is crucial for differences in enterocyte migration. As discussed in the manuscript, migration distance could be the most important variable to explain the differences.

(3) Reviewer: Did the authors have an explanation about the loss of Paneth cells?

Answer: We have performed additional experiments to elucidate the molecular mechanisms behind. Some evidence is given now that the Notch pathway is activated in casp8 animals. The pathway mediates anti-secretory activities which could be delicious for Paneth cells. Further experiments are necessary to validate the working hypothesis.

(4) Reviewer: Did the authors check the evaluation, in capase-8 deficient mice, the presence of inflammatory cytokines such TNFalpha and interferon gamma that may explain the loss of Paneth cells?

Answer: In the casp8 knockout animals strong expression of inflammatory cytokines (TNF alpha and IL-1beta) was found in the large and small intestine. In the animal model we have never addressed the putative link between pro-inflammatory cytokines and cytotoxicity of Paneth cells. Following your recommendation we will address this interesting point in further experiments.

3 References and typesetting were corrected

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,

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