Author’s response to reviews

Title: MORG1+/- mice are protected from histological renal damage and inflammation in a murine model of endotoxemia

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Answers to the reviewers/ editorial points point-by-point in detail:

1. “In the Methods please clearly state the source of all animals used in your study including the mice with C57BL/6J genetic background. If the mice were obtained commercially please state the company name.”
   
   • We clearly state in the Methods section the source of our animals. The amendments in the text can be found in: Methods section, lines 119-122, page 6.

2. “In the ‘Ethics approval and consent to participate’ section of the Declarations we note that you state that all the animal experiments were performed according to the guidelines set by the local Animal Committee of the State of Thuringia. Please can you clarify whether your study protocol was submitted to and approved by this ethics committee or whether you followed local guidelines.”
   
   • We clarify in the ‘Ethics approval and consent to participate’ section of the Declarations that the protocols were submitted and approved from the local Animal Committee of the State of Thuringia and we presented as well the full name of the ethics committee. This can be found in the “Ethics approval and consent to participate” section of the Declarations, lines 622-625, page 27.

3. “In the Methods we note that you state that endotoxemia was induced by application of 5 mg/kg BW lipopolysaccharides from E. coli O111:B4 (LPS). Please can you clarify whether this is standard procedure and please provide a reference to this procedure in the
Methods. Please can you also clarify whether this was approved by an ethics committee and if so please clearly state this including the full name of the ethics committee.”

- We clarified the LPS application in the Methods section, lines 139-143, page 7. The citations are also shown and included to the “References” section, under numbers 15-17, lines 690-699, page 30.

- We also clarified that the LPS treatment was approved by an ethics committee and clearly stated this including the full name of the ethics committee. The amendments in the text can be found in Methods section, lines 136-139, page 7.

4. “In the Methods please can you clearly state how animals were sacrificed and provide a reference. Please clarify whether the method used to sacrifice the animals is standard and whether this was approved by an ethics committee including the full name of the ethics committee.”

- We clearly stated in the Methods section how the animals were sacrificed and refer to the European Community Council Directive for the Care and Use of Laboratory Animals (Directive 2010/63/EU; ANNEX IV, Methods of killing animals, point 3. Table, available online: http://ec.europa.eu/environment/chemicals/lab_animals/legislation_en.htm); The amendments to the text can be found in the Methods section, line 147-154, page 7.

- We as well clearly state that this was approved by an ethics committee including the full name of the ethics committee. The changes can be found in the Methods section, lines: 148-151, page 7.

5. “We also note that you performed a survival analysis for 72 hours. Please can you clarify if mice that survived the 72 hour LPS exposure were sacrificed and if so please clearly state the method used. Please can you also clarify whether all procedures followed in your survival analysis are considered standard and provide references justifying them. Please can you also clarify whether this was approved by an ethics committee and if so please clearly state this including the full name of the ethics committee.”

- We clarified in the “Methods” section Paragraph 2 “Survival analyses” how the mice that survived the 72 hours LPS exposure were sacrificed and also clearly state the method used. The amendments that have been made to the manuscript’s text can be found in Methods section, lines 175-181, page 8.

- We also clarify that all procedures followed in our survival analysis is a standard procedure and provided a reference for it, present in the Reference section under number 15, lines 690-692, page 30.

- We also clarified that the survival analyses were approved from the local ethics committee of the State of Thuringia and introduced the full name of ethics
committee as well as the file number 02-023/11 which was approved by the ethics committee. The provided information can be found in the text of the revised manuscript in Methods section, lines 161-166, page 8.

6. “Please ensure that your manuscript adheres to ARRIVE guidelines for reporting animal research. We suggest including an ARRIVE checklist with your revised submission. The ARRIVE checklist can be found here: https://www.nc3rs.org.uk/sites/default/files/documents/Guidelines/NC3Rs%20ARRIVE%20Guidelines%20Checklist%20%28fillable%29.pdf”

- The revised version BNEP-D-17-00200R2 submitted to the journal did not answer completely to some of the ARRIVE guidelines, therefore we completed and included in this revision the ARRIVE checklist, according to your suggestion.

- The completing of the ARRIVE checklist led to amendments which are listed in detail below:

- To answer more detailed to the point 14 from the ARRIVE guidelines: we added to the Methods section the information about the evaluation of the animal’s health. The well-being of the animals used in the LPS i.p. application as well as during the survival analyses was evaluated by the application of the Clinical Severity Score (CSS) system. The amendments in the text can be found in the Methods section lines 143-147, page 7 and lines 169-173 respectively. The records of the CSS evaluation are also presented in the Results section Paragraph 9 “Survival is improved in septic MORG1+/- mice. The changes can be found in the Results section lines 468-479, pp 20-21. And are presented in 3 Additional files as 2 Tables and a Suppl. Fig.2 in the new Section of the manuscript which is placed immediately after the References: Supplementary files: lines 815-843, pp 35-36.

7. “Unfortunately, there are some minor language issues throughout the manuscript. Please ensure that you thoroughly check your manuscript for any language errors and correct them. We recommend that you ask a native English speaking colleague to help you copyedit the paper. If this is not possible, you may need to use a professional language editing service. Use of an editing service is neither a requirement nor a guarantee of acceptance for publication.”

We thoroughly checked our manuscript for language issues and also found some errors and corrected them. The amendments are listed below:

Abstract section, line 31, page 2; deleted “an”;

Background section, line 82, page 4 localized was replaced by localised,

As well also some spelling mistakes and the name of the city Dusseldorf are corrected for English spelling.
“Please ensure that if you make any changes to the abstract, that the abstract in the system is also updated.”

- A minor correction was made to the Abstract section and it is also uploaded in the system.

8. ” Please add a “Supplementary files” section where you list the following information about your supplementary material:
   - File name
   - Title of data
   - Description of data

Please ensure also that all additional files have been cited in the main manuscript.”

- We added a “Supplementary files” section to the revised version of the manuscript. The supplementary files are cited in the manuscript.

The amendments are shown in the new Section of the revised manuscript place immediately after the Reference list, lines 798-843, pp 34-36.

9. “Please include the email addresses for all authors on the title page. The corresponding author should still be indicated.”

- The email addresses of all authors are included on the title page of the manuscript. The corresponding author is still indicated.

10. “Please submit your revised manuscript as a clean copy without any tracked changes, coloured or highlighted text, as these are no longer required at this stage of the editorial process.”

- The revised manuscript is submitted as a clean copy without any tracked changes, coloured or highlighted text and all amendments made on the manuscript are listed in details in this point-by-point answer to the editor.

Additional changes:

- Due to the introduction of several new references the Reference list was changed. The new references are with Numbers 15-18, and can be found in the Reference section lines690-703, page 30.

- The exact number of the animals per group included in the experimental sets is also added to the Figure legends section.
• In the Background section of the manuscript is not present a Paragraph "Objectives" as it was not involved in the BMC Nephrology Instructions for authors, but the relevant information is presented at the end of Background section of the manuscript, lines 102-105, page 5.

• We also added in the Methods section the complete name of the local ethic committee of Thuringia, and clearly state that the animal experiments were carried out in accordance with the NIH Guidelines for Care and Use of Laboratory animals and to the Directive 2010/63/EU from 22 September 2010 of the European Community Council Directive for the Care and use of Laboratory Animals. The statement can be found in lines 111-119.

We thanks to the editorials for the revision of our manuscript and believed that the made amendments to the text of the manuscript will improved the quality of our manuscript and hope that you will consider the manuscript for acceptance in your journal.

We would like to point also out that we are disappointed by the very long review process of this journal.

Prof Dr. Gunter Wolf, MHBA on behalf of all authors