

We are pleased to hear that our manuscript “**The polyglutamylase activity of TTLL4 is negatively regulated by the NIMA family kinase NEK5**”, by: Talita Diniz Melo-Hanchuk and Jörg Kobarg, is preliminary acceptable for publication for publication in the World Journal of Biological Chemistry and acceptable after thorough revision. We carefully considered each of the suggestions and revised our manuscript accordingly.

We believe that the revised version of our manuscript was improved and a detailed response to each of the reviewer’s concerns is listed below. We hope that the manuscript is now acceptable for publication and we would like to thank you for your time spent in considering our manuscript.

Sincerely,

Talita Hanchuk and Jorg Kobarg

Reviewer #1:

1. Abbreviation of NEK5 should be standardized though whole manuscript.

We appreciated the comment and all the abbreviations were standardized.

2. The phosphorylation sites of TTLL4 were identified by Mass spectrometry and point mutations were introduced (by what).

Point mutations were introduced by QuikChange II Site-Directed Mutagenesis Kit (Stratagene, LaJolla, CA) to generate the NEK5-KD (K33A - inactive mutant of NEK5), TTLL4-T813E, TTLL4-T815A, TTLL4-Y815F, TTLL4-Y815A TTLL4-S912E, TTLL4-S960E, TTLL4-S960A, TTLL4-T968E, TTLL4-S1136A, TTLL4-S1139E and TTLL4-S1136A. We improve the text to make it clear.

3. At the end of the introduction section, instead of mentioning the hypothesis of the study, the authors stating the conclusion, why?

The end of introduction was revised to catch the reader’s attention and only small part of results was presented.

4. Arrive guidelines is an animal research: reporting of in-vivo experiments.

All the experiments of this work were performed in cell lines or in vitro by using protein extract.

5. Minor English editing is recommended.

We have checked carefully the English language. We trust the text improved substantially.

(1) Science editor:

1 Scientific quality: The manuscript describes a basic study of the polyglutamylase activity of TTLL4 being negatively regulated by the NIMA family kinase NEK5. The topic is within the scope of the WJBC. (1) Classification: Grade B;

We appreciate your comment and we hope the data can be release soon to the scientific community.

(2) Summary of the Peer-Review Report: It is an interesting study. However, the abbreviation of NEK5 should be standardized though the whole manuscript.

We appreciated the comment and all the abbreviations were standardized.

The authors should provide more information about the factor(s) that were associated with the point mutations being introduced.

Point mutations were introduced by QuikChange II Site-Directed Mutagenesis Kit (Stratagene, LaJolla, CA) to generate the NEK5-KD (K33A - inactive mutant of NEK5), TTLL4-T813E, TTLL4-T815A, TTLL4-Y815F, TTLL4-Y815A TTLL4-S912E, TTLL4-S960E, TTLL4-S960A, TTLL4-T968E, TTLL4-S1136A, TTLL4-S1139E and TTLL4-S1136A. We improve the text to make it clear.

The Introduction section needs to be revised. The questions raised by the reviewers should be answered;

The conclusion of introduction was improved as suggested by the reviewer and the English was checked. We hope now the text in catching the reader's attention.

3 Academic norms and rules: The Institutional Review Board Approval Form, the Institutional Animal Care and Use Committee statement form, the Biostatistics Review Certificate, and the ARRIVE guidelines were waived. The authors need to provide the **signed Conflict-of-Interest Disclosure Form and Copyright License Agreement**. No academic misconduct was found in the Bing search.

Was provided as attachment

5 Issues raised:

(1) I found the authors did not **provide the approved grant application form(s)**. Please upload the approved grant application form(s) or funding agency copy of any approval document(s);

(2) Was now provided as attachment

(3) I found the authors did not **provide the original figures**. Please provide the original figure documents. Please prepare and arrange the figures using PowerPoint to ensure that all graphs or arrows or text portions can be reprocessed by the editor;

(4) Were provided as attachment

(3) I found the authors did not write the "article highlight" section. Please write the "article highlights" section at the end of the main text;

A new "article highlight" section was written.

(4) the in-text citation numbers need to be superscripted in square brackets at the end of the sentence with the citation content or after the cited author's name, with no spaces;

The citation numbers were formatted following the suggestion.

(5) please write the "Core Tip" section and provide an audio core tip file where the core tip content is recorded; and

The Core Tip was included in the main text and de audio was produced as well.

(6) please provide the signed Conflict-of-Interest Disclosure Form and Copyright License Agreement.

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