PIMIT
Processus Infectieux en Milieu Insulaire Tropical

December 29th, 2022
To Current Issues in Molecular Biology ***(CIMB)***
Section Biochemistry, Molecular and Cellular Biology

REF: Revised version of MS#cimb-2107494-R1 (Communication)

Dear Editors,
Dear Reviewers,

Thank you for your high consideration of our submitted manuscript.

Please in the revised document our point-to-point responses to Reviewers #1, #2, and #3.

Two major changes made to the article can be found below.

#1. In the revised version of the manuscript, we provided new Figures 1 and 6. A tridimensional structure prediction was performed on DENV-2 NS1 protein (Figure 1). A synthetic scheme on the biological behavior of DENV-2 NS1 mutant in Huh7 cells has been proposed as Figure 6.

#1. In the revised version of the manuscript, we provided new Figure S1. Immunofluorescence assay was performed on Huh7 cells expressing a recombinant DENV-2 NS1 protein.

We greatly appreciate the efforts of the three reviewers and the editorial team of *CIMB* to help us make this work the strongest possible, and we look forward to your reply.

Thank you very much for your assistance and we look forward to your earliest reply.

Sincerely yours,

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We greatly thank Reviewer #3 for his/her thoughtful comments and the opportunity to revise our manuscript.

Responses to Reviewer #3:

Q1. For the broader readership of the journal CIMB, the authors should describe a little more (at least 2-4 sentences) about the effect of single point mutations on different proteins of pathogenic origins other than dengue and some relevant journals.

We appreciate this point raised by the reviewer. According to the reviewer's comment, the text has been modified on Introduction section, page 2, line 47-49 and new references have been added in the revised manuscript.

Q2. The authors are recommended to provide the NS1 protein structure and the site of mutation in Figure 1.

We appreciate this point raised by the reviewer. A 3D structure prediction of DES-14 NS1 protein and mutants was performed using PHYRE² protein fold recognition server)L The predicted structures were analyzed with PyMOL 2.5, a program for interactive visualization of tridimensional proteins. Structural analysis of NS1 showed that mutations NS1-R272K, NS1-R324K, or NS1-(R272K, R324K) have no obvious effect on DENV-2 NS1 conformation. According to the reviewer's comment, a new Figure 1 has been added and the text has been modified in the revised manuscript (Results section, pages 3-4, lines 132-45).

Q3. A schematic explaining the findings drawn by the authors should be provided.

We appreciate this point raised by the reviewer. According to the reviewer's comment, a new Figure 6 has been added and the text has been modified in the revised manuscript (Concluding remarks section, page 9, lines 296-99).

Q4. What can be the physicochemical reason behind the alteration in function while arginine is replaces by lysine ?

Although arginine and lysine are two basic residues, only the latter is considered as substrate for protein ubiquitination, SUMOylation, or acetylation. According to the reviewer's comment, the text has been modified in the revised manuscript (Concluding remarks section, page 9, lines 301-02).