nature portfolio

Peer Review File

Zika virus modulates human fibroblasts to enhance transmission success in a controlled lab-setting

Corresponding Author: Professor S. Noushin Emami

This manuscript has been previously reviewed at another journal. This document only contains information relating to versions considered at Communications Biology.

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
This paper entitled "Zika virus modulates human skin cells to enhance transmission success" describes measurements of the VOCs in ZIKV-infected dermal fibroblasts in tissue culture. The authors found that upon ZIKV infection, at two timepoints (10h and 24h post-infection), various VOCs were differentially produced relative to uninfected cells. When using synthetic versions of these compounds, it was found that they altered mosquito behaviour (attraction and subsequent feeding). The authors propose that the virus is doing this to improve transmission success.

The scientific methods of the paper appear sound. The conclusions are mostly justified however I believe some additional commentary about the limitations of the study are necessary, as listed below.

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2. In the same vein, there has been work demonstrating the impact of the microbiota of the skin being involved with altering mosquito attraction (e.g. https://doi.org/10.1016/j.cell.2022.05.016). Perhaps some discussion on this, placing the results here in the context of the microbiota on the skin, would be beneficial to the readers.

Reviewer #2

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This manuscript suggests that ZIKV infection is modulated by VOCs in infected dermal fibroblasts and alters the emission of VOCs at the invasion and transmission stage. The authors used a human dermal fibroblast cell line to examine the VOC profile during ZIKV infection and analyzed related gene expression using RNA sequencing and proteomics. While the overall topic is interesting, weaknesses in experimental design and interpretation limit the impact of the results. Furthermore, the manuscript needs improvement in the writing of the introduction and results sections to enhance readability, although the Materials and Methods section is well-written.
Major point:
1. The authors should rewrite the introduction section. They need to include information about ZIKV, skin cells, VOCs, and the relationship between VOCs and mosquito attraction. At the end of the introduction section, the authors should clearly state the aim of the study and briefly describe how they intend to prove their hypothesis. Additionally, they should add appropriate references to support these topics, including relevant papers such as doi.org/10.1016/j.cell.2022.05.016.
2. In the results section, the authors need to include subtitles. They should also explain the rationale and purpose of each experiment in the first line of each paragraph or subtitle. Brief descriptions of the experimental methods are also necessary. For example, in the first paragraph (lines 71-82), they should provide the temporal definitions of invasion stage and the transmission stage, accompanied by appropriate references or brief descriptions of experiments, which are crucial for advancing their narrative. Additionally, the authors should verify the figure and panel numbers in this manuscript, ensuring

that the exact numbers are correctly referenced.
3. The authors performed most experiments in this study using immortalized human dermal fibroblast cells. They should not generalize their observations based on only this cell line. To confirm their observations, it is necessary to use primary human fibroblasts or several different cell lines.
4. In Figures 4 and 5, the authors analyzed gene expression and proteomes in ZIKV-infected fibroblasts. While these data present intriguing findings, they lack a clear connection between the VOCs and the identified genes or proteins. Moreover, the proteomic analysis was performed using data from ZIKV-infected human cells sourced from PubMed, rather than fibroblasts. This means that transcriptomic and proteomic analyses were not conducted on the same cells, highlighting the need for confirmation through direct experimental testing. Furthermore, a functional study, such as a loss-of-function or gain-of-function experiment, is necessary to support the conclusions presented in lines 179-180. The authors should clarify the significance of these gene expressions in lines 153-155.

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1. The exact figure and panel numbers should be specified.
2. The manuscript should provide information on the Anopheles gambiae sensu lato mosquitoes used as controls.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
The authors have addressed all of my comments to my satisfaction. I have no further comments to add.

Reviewer #2

(Remarks to the Author)
I am satisfied with the reply of the authors. In principle, all the necessary changes and experiments have been made or performed, respectively.

Point by point response to reviewers:

We sincerely thank all the reviewers for their valuable comments and suggestions. We've carefully revised the manuscript to address their feedback and made significant efforts to respond to the queries raised. We are pleased that the reviewer found our work scientifically sound and that the conclusions are largely justified. We are happy to address the suggested improvements (all additional and revised texts are highlighted in the main manuscript and supplementary information).

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