Zika virus depletes neural stem cells and evades selective autophagy by suppressing the Fanconi anemia protein FANCC

Shashi Tiwari, Jason Dang, Nianwei Lin, Yue Qin, Shaobo Wang and Tariq Rana
DOI: 10.15252/embr.201949183

Corresponding author(s): Tariq Rana (trana@ucsd.edu)

Review Timeline:

| Event                  | Date       |
|------------------------|------------|
| Submission Date        | 28th Aug 19|
| Editorial Decision     | 21st Oct 19|
| Revision Received      | 22nd Apr 20|
| Editorial Decision     | 16th Jun 20|
| Revision Received      | 7th Sep 20 |
| Accepted               | 17th Sep 20|

Editor: Esther Schnapp

Transaction Report:

No Peer Review Process File is available with this article, as the authors have chosen not to make the review process public.
### B-Statistics and general methods

| 1a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size? | Sample size for each experiment was clearly mentioned in manuscript. We did not use any statistical method to pre-specify the sample size. We determined the sample sizes similar to previous studies (PMID: 30126924, PMID: 31448519). |
| --- | --- |
| 1b. For animal studies, include a statement about sample size estimate even if no statistical methods were used. | We included at least three animals per group of treatment/conditions for each experimental set. |
| 2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established? | For all experiments including mice were excluded when we found health concerns including bleeding or significant changes in body weight. |
| 3. Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g., randomization procedure)? If yes, please describe. | No. |
| For animal studies, include a statement about randomization even if no randomization was used. | For all experiments, both male and female mice were assigned randomly without pre-determined criteria into each group. |
| 4a. Were any steps taken to minimize the effects of subjective bias during group allocation or/and when assessing results (e.g., blinding of the investigator)? If yes please describe. | Investigators were not blinded to experiments but data analysis was done by different investigators to assess the outcome. |
| 4b. For animal studies, include a statement about blinding even if no blinding was done | We were blinded to the group of animal during data collection and analysis for drug treatment and ZIKA virus infection. |
| 5. For every figure, are statistical tests justified as appropriate? | The reasoning behind the choice of tests is described in the Methods section and figure legends. |
| Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it. | Differences between group means were analyzed by Student’s t test. Differentially expressed genes in RNA-seq data were analyzed using ANOVA. |
| In there an estimate of variation within each group of data? | Either individual data points or error bars are shown in each figure to show the variation. |

---

### A- Figures

#### 1. Data

The data shown in figures should satisfy the following conditions:

- the data were obtained and processed according to the field’s best practice and are presented to reflect the results of the experiments in an accurate and unbiased manner.
- figure panels include only data points, measurements or observations that can be compared to each other in a scientifically meaningful way.
- graphs include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical replicates.
- if n > 5, the individual data points from each experiment should be plotted and any statistical test employed should be justified.
- source data should be included to report the data underlying graphs. Please follow the guidelines set out in the author ship guidelines on data presentation.

#### 2. Captions

Each figure caption should contain the following information, for each panel where they are relevant:

- a specific description of the experimental system investigated (e.g., cell line, species name).
- the assay(ies) and method(ies) used to carry out the reported observations and measurements.
- an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.
- the exact sample size (n) for each experimental group/condition, given as a number, not a range.
- an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.
- a statement of how many times the experiment shown was independently replicated in the laboratory.
- definitions of statistical methods and measures:
  - common terms, such as t-test (please specify whether paired or unpaired), simple (2 tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section).
  - are tests one-sided or two-sided?
  - are there adjustments for multiple comparisons?
  - exact statistical test results, e.g., P values < 0.05.
  - definition of “center value” as median or average.
  - definition of error bars as s.d. or s.e.m.

Any descriptions too long for the figure legend should be included in the methods section and/or with the source data.
### C- Reagents

5. To show that antibodies were profiled for use in the system under study (assay and species), provide a citation, catalog number and/or clone number, supplementary information or reference to an antibody validation profile. e.g., Antibodypedia (see link list at top-right), IDgenewbio (see link list at top-right). All the antibodies are well-characterized commercial antibodies. The specificity of these antibodies has been tested by the manufacturer and verified independently by previous published studies: Primary antibody GAPDH (Cell Signaling Technology, 5174S, PMID: 15769508), LC3 (Novus, NB100-2205, PMID: 22077625), Fancc (Novus, NBP1-16771), ZEVV envelope (WPS3, PMID: 24032216), ZEVV (Biofortin, BF-1225-06), ILAG (Sigma, M2, F1804), TMDM20 (Santa Cruz, SC-14153), PS2 (Abcam, ab61062) and hepsin (WPS3, RAB33A). Alexa Fluor 488, 594–conjugated secondary antibodies were purchased from Molecular Probes (Invitrogen, USA). (Page number: 23)

7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination. RH-KECs derived Human Keratinocytes (HKECs) were purchased from Gibco (Cat. No. C1760320). They tested the authentication and mycoplasma contamination. In addition we also checked for expression of their specific markers and healthy growth conditions.

### D- Animal Models

8. To report species, strain, gender, age of animals and genetic modification status where applicable. Please detail housing and husbandry conditions and the source of animals. We used both male and female Idr/rl-/- mice (4–5-week-old, MRRRC Jackson Laboratories, Fancc KO) 2–6-Week, from late Dr. Gregory Nalpas) and C57BL/6 mice (4–5-week-old, MRRRC Jackson Laboratories). Animal were housed under a light−dark cycle (lights on 06:00–18:00h) at a constant temperature (22 ± 1°C). Male and female were housed separately after weaning with all females feeding of food and water.

9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments. All animal work was performed in accordance with the guidelines of the Institutional Animal Care and Use Committee (IACUC) of the University of California, San Diego.

10. We recommend consulting the ARRIVE guidelines (see link list at top-right) (PloS Biol. 8(6), e1000412, 2010) to ensure that other relevant aspects of animal studies are adequately reported. See author guidelines, under ‘Reporting Guidelines’. Please confirm you have submitted this list.

### E- Human Subjects

12. Include a statement confirming that informed consent was obtained from all subjects and that the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the Department of Health and Human Services Belmont Report. N/A

13. For publication of patient photos, include a statement confirming that consent to publish was obtained. N/A

14. Report any restrictions on the availability (and/or on the use of) human data or samples. N/A

15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable. N/A

16. For phase II and III randomized controlled trials, please refer to the CONSORT flow diagram (see link list at top-right) and submit the CONSORT checklist (see link list at top-right) with your submission. See author guidelines, under ‘Reporting Guidelines’. Please confirm you have submitted this list.

17. For tumor marker prognostic studies, we recommend that you follow the REMARK reporting guidelines (see link list at top-right). See also: NCI (see link list at top-right) and MRC (see link list at top-right) recommendations. Please confirm compliance.

### F- Data Accessibility

18. Please provide a “Data Availability” section at the end of the Materials & Methods, listing the accession codes for data generated in this study and deposited in a public database (e.g. RNA-Seq data: Gene Expression Omnibus GSE13562, Proteomics data: PRIDE PX003328 etc.) Please refer to our author guidelines for ‘Data Deposition’. N/A

19. Data deposition in a public repository is mandatory for:
   a. Protein, DNA and RNA sequences
   b. Macromolecular structures
   c. Crystallographic data for small molecules
   d. Functional genomics data
   e. Proteomics and molecular interactions

   Data are available in expanded new dataset.

20. Access to human clinical and genomic datasets should be provided with as few restrictions as possible while respecting ethical obligations to the patients and relevant medical and legal issues. If practically possible and compatible with the individual consent agreement used in the study, such data should be deposited in one of the major public access-controlled repositories such as Dryad (see link list at top-right) or figshare (see link list at top-right). N/A

21. Computational models that are central and integral to a study should be shared without restrictions and provided in a machine-readable form. The relevant accession numbers or links should be provided. When possible, standardised formats (e.g., CellML) should be used instead of scripts (e.g., MATLAB). Authors are strongly encouraged to follow the MIMAM guidelines (see link list at top-right) and deposit their model in a public database such as BioModels (see link list at top-right) or JAX, Online (see link list at top-right). If computer source code is provided with the paper, it should be deposited in a public repository or included in supplementary information. N/A

### G- Dual use research of concern

22. Could your study fall under dual use research restrictions? Please check Biosecurity documents (see link list at top-right) and list select agents and toxins (APHS/CDC) (see link list at top-right). According to our biosecurity guidelines, provide a statement only if it could N/A