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☐ Initial submission ☐ Revised version ☒ Final submission

Life Sciences Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form is intended for publication with all accepted life science papers and provides structure for consistency and transparency in reporting. Every life science submission will use this form; some list items might not apply to an individual manuscript, but all fields must be completed for clarity.

For further information on the points included in this form, see Reporting Life Sciences Research. For further information on Nature Research policies, including our data availability policy, see Authors & Referees and the Editorial Policy Checklist.

Experimental design

1. Sample size

   Describe how sample size was determined.

   All statistical analyses were performed using GraphPad Prism 5.0 software. For analyses between 2 groups, Mann-Whitney U test were used. For comparisons among more than 2 groups, either one-way or two-way ANOVA, Bonferroni post-test was used. Sample size for each experiment was clearly indicated in the respective figure legends.

2. Data exclusions

   Describe any data exclusions.

   No data were excluded.

3. Replication

   Describe whether the experimental findings were reliably reproduced.

   Each experiment was repeated at least twice to ensure reproducibility of findings.

4. Randomization

   Describe how samples/organisms/participants were allocated into experimental groups.

   Blood specimens used in Fig. 1-3 were randomly obtained from equal number of males and females, aged between 20-40 years old, whom did not exhibit any signs of illness prior to blood collection. Blood specimens used in Fig. 4-6 were obtained from confirmed pregnant women of all 3 trimesters whom were aged between 20-40 years old, whom did not exhibit any signs of illness prior to blood collection. Sera specimens from ZIKV patients were collected from RT-PCR-confirmed infected pregnant patients of all 3 trimesters, whom exhibited rash prior to blood collection.

5. Blinding

   Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

   Blinding of group allocation were not performed as specimens identity (pregnant or non-pregnant; healthy or ZIKV patients) were essential for the allocation of correct group. However, identity of individuals and their specimens remained anonymous throughout the study.

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
☐	☒ A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ A statement indicating how many times each experiment was replicated
☐	☒ The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
☐	☒ A description of any assumptions or corrections, such as an adjustment for multiple comparisons
☐	☒ The test results (e.g. *P* values) given as exact values whenever possible and with confidence intervals noted
☐	☒ A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
☐	☒ Clearly defined error bars

See the web collection on statistics for biologists for further resources and guidance.

Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.	GraphPad Prism was used throughout the study for data analysis.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.	No restrictions on materials availability.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).	Antibody, Supplier, Cat. No., Clone, Lot no.: 1) CD45-BV421, Biolegend, 563879, HI30, 6018948 2) CD14-AF488, Biolegend, 557700, M5E2, 6034830 3) CD56-PerCP-Cy5.5, Biolegend, 560842, 5.1H11, 560842 4) CD3-AF647, Biolegend, 300322, HIT3a, B208649 5) CD19-PerCP, Biolegend, 302228, HIB19, B178735 6) Pan-flavivirus, EMD Millipore, MAB10216, D1-4G2-4-15, 2854249 7) CD16-AF647, Biolegend, 302020, 3G8, B201807 8) PE goat anti-mouse IgG, BD Pharmingen, 550589, 17891 9) AF488 goat anti-mouse IgG, Life Technologies, A11001, 1752514 10) Human BD Fc Block, BD Pharmingen, 564220, 5331582 All antibodies used have been validated and stated on suppliers' websites.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

Vero cell line was obtained from ATCC.

b. Describe the method of cell line authentication used.

Cell line authentication was performed by morphology check and growth curve analysis.

c. Report whether the cell lines were tested for mycoplasma contamination.

Cell lines were negative for mycoplasma contamination, validated by Hoechst staining.

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.

N/A.

Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.

N/A.

Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

Peripheral blood specimens were obtained from healthy donors aged 18 to 39 years old and stored temporarily at 37 °C in lithium heparin-containing tube (BD) prior to infection. Blood specimens used in Figure 1-4 were obtained from male and female subjects of equal distribution. Pregnant subjects were recruited at Los Angeles County (LAC) + University of Southern California (USC) Medical Center, with the following exclusion criteria: (i) vaccination within last 14 days, (ii) suspected/recent illness within last 14 days, (iii) steroid administration during pregnancy, (iv) immunodeficiency/immunocompromised conditions, (v) autoimmune disorders, and (vi) suspected fetal anomalies. Pregnancy was categorized into three trimesters based on the following: first trimester <12 weeks, second trimester 12-28 weeks and third trimester >28 weeks. Blood serum specimens from 30 symptomatic ZIKV+ pregnant women, 10 from each trimester, were collected from pregnant women who were admitted to the acute febrile illness clinic at the Oswaldo Cruz Foundation exhibiting rash that had developed within the previous 5 days. All patients were confirmed to be ZIKV-positive following the performance of reverse transcription-PCR (qRT-PCR) assay in blood or urine which utilized specific probe and primers for ZIKV detection59. Serum aliquots from these patients were stored at -80 oC until the performance of the present experiments. Written and oral informed consents were obtained prior to the collection of whole blood, sera and urine specimens from healthy individuals and ZIKV+ pregnant patients, and study protocols were approved by institutional review boards of the USC Institutional Review Board (IRB), Fundação Oswaldo Cruz (Fiocruz) and the University of California, Los Angeles (UCLA).