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Corresponding author(s): Shirit Einav

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Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☒	☐ A description of all covariates tested
☐	☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒ For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☐	☒ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐	☒ Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	- Single-cell RNA sequencing libraries were constructed at Stanford University via viscRNA-Seq 2 protocol (see manuscript) and sequenced on a Novaseq 6000 (Illumina) - Dengue virus (DENV) RNA copies number during the co-culture experiments were determined via RT-qPCR. - The spectral flow cytometry was conducted on an Aurara spectral analyzer (Cytek). - Serum cytokine concentration data was collected using a Luminex Assay Human 80 (R&D System) following manufacturer's instructions.
Data analysis	- Cell Ranger (v3.1.0) was used for demultiplexing, barcode processing, read alignment, gene counting, and generation of gene-barcode expression matrix. - Python 3 Jupyter (https://jupyter.org/) notebooks that use numpy (V1.21.2 https://numpy.org/doc/), pandas (V1.4.1 https://pandas.pydata.org/docs/), matplotlib (V3.6.2 https://matplotlib.org/stable/index.html#), seaborn (V0.11.1 https://seaborn.pydata.org/), anndataks (V0.1.3 https://github.com/iosonofabio/anndata_kolmogorov_smirnov), pysam (V0.18.0 https://pysam.readthedocs.io/en/latest/api.html) and scanpy (V1.9.1 https://scanpy.readthedocs.io/en/stable/) were used for the scRNA-Seq analysis. The code is available at https://github.com/echosun77/dengue. - Metascape (V3.5 https://metascape.org) and GSEAPY (V0.10.8 https://gseapy.readthedocs.io/en/latest/introduction.html) were used for pathway analysis. - FlowJo V10.9 (Tree Star) was used for cytometry analysis. - OmniPath (https://omnipathdb.org) was used as a baseline for cell interaction analysis and augmented by interactions - Prism 9 (GraphPad) was used for statistical analysis. - SoftMax pro 7.0.3 was used for ELISA analysis.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

The viscRNA-Seq 2 data reported in this paper have been deposited in the Gene Expression Omnibus database, https://www.ncbi.nlm.nih.gov/geo (accession no. GSE220969, already public). Fcs file used for CyTOF data reanalysis are available at FlowRepositiry: https://flowrepository.org/id/FR-FCM-Z5MQ, IDFR-FCM-Z5MQ. All other data needed to evaluate the conclusions of this study are present in the paper and/or the supplementary materials.

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	Samples were categorized solely based on the diagnosis (uncomplicated vs. severe dengue progressor), however, we do report the sex and age of all the participants whose samples were analyzed.
Reporting on race, ethnicity, or other socially relevant groupings	Samples were obtained from patients enrolled in the Colombia dengue cohort. Samples from the healthy controls used for validation assays were obtained from the Stanford Blood Bank. Ethnicity information is not available for the donors in this study.
Population characteristics	Samples were from pediatric patients enrolled to the Colombia dengue cohort. The Colombia cohort consists of individuals presenting to the emergency room or clinics of FVL or CDI between March 2016 and Jan 2020. Enrollment criteria consisted of: i) age equal to or greater than 2 years; ii) presentation with an acute febrile illness of less than 7 day duration associated with one or more of the following symptoms or signs: headache, rash, arthralgia, myalgia, retroorbital pain, abdominal pain, positive tourniquet test, petechiae, and bleeding; and iiia) a positive dengue IgM antibody and/or NS1 antigen by the SD BIOLINE Dengue Duo combo device (Standard Diagnostic Inc., Korea) test or iiib) clinical presentation highly consistent with dengue and subsequent confirmation of diagnosis via serological testing and rRT-qPCR at Stanford. PBMCs from 19 children infected with DENV (9 males and 10 females ranging, age: 4-17 years) and 4 healthy controls (2 males and 2 females, age: 5-12 years) were used for viscRNA-seq and cytokine analysis. Samples from 11 individuals were used for validation assays.
Recruitment	Participants meeting inclusion criteria were approached by our study coordinator after approval has been obtained by their primary physicians. Participants were only contacted after their physician either the ER or clinic doctor, or the infectious disease provider had obtained a verbal approval from them to allow our study staff to contact them. Participants were NOT cold called. Participants were then offered enrollment and IRB consent was obtained by members of the research team. The requirement for written informed consent might have introduced a selection bias. Patient recruitment was influenced by practical considerations, such as the time of day the patient presented to the ER or clinic and the seasonality of dengue outbreaks.
Ethics oversight	All work with human subjects was approved by the Stanford University Administrative Panel on Human Subjects in Medical Research (protocols #35460 and #50513) and the ethics committees in biomedical research of the Fundación Valle del Lili (FVL, Cali, Colombia) and Centro de Atención y Diagnóstico de Enfermedades Infecciosas (CDI, Bucaramanga, Colombia). The parents or legal guardians of subjects provided written informed consent, and subjects between 2 to 17 years of age provided assent. Subjects were not involved in previous procedures and were all test-naïve. The demographics and clinical parameters of the subjects are summarized in Table 1.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	ViscRNA-seq 2: 23 biologically independent samples (4 healthy, 8 dengue, 4 DWS, 7SD); Functional assays: 6 biologically independent samples (B cells); 11 biologically independent samples (flow cytometry) Total: 39 PBMC samples. Statistical methods were not used to predetermine sample size, but our sample size are similar to those reported in previous publications. ViscRNA-seq 2 and functional experiments included ≥ 4 donors per group. Orthogonal methods of analysis revealed concordant findings.

Data exclusions	No samples were excluded from this study.
Replication	scRNAseq was performed once with 23 donors grouped in 4 categories: 4 healthy, 8 dengue, 4 DWS, and 7 SD. Flow cyotmetry experiments and functional assays were performed at least twice, and all the biological replicates are included in the manuscript. All attempts at replication were successful.
Randomization	scRNA-seq experiments were each performed in 4 batches. Donors were randomly distributed across batches. Flow cytometry and functional assays were performed in two or more batches. Patient and healthy controls donors were randomly distributed across batches. Cytokine analysis was performed as a single batch. 19 samples including from patients and healthy controls were processed simultaneously.
Blinding	Measurements of viral load, serological parameters and cytokine levels were blinded for the diagnosis of the patients. The remaining experiments and analyses were not performed blinded, as the same investigators performed sample processing, data generation and data analysis.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used

Fluorophore; specificity; clone; manufacturer; catalog

BUV496 anti-CD19; SJ25C1;BD Biosciences; 612938
BUV563 anti-CD56; MY31;BD Biosciences; 612928
BUV615 anti-HLA-DR; G46-6; BD Biosciences; 751142
BUV661 anti-CD20; 2H7;BD Biosciences; 759952
BUV737 anti-CD16; 3G8;BD Biosciences; 612786
BUV805 anti-CD3; SK7;BD Biosciences; 612893
BV421 anti-CD1c; L161; Biolegend; 331526
BV605 anti CD11c; 3.9; Biolegend; 301636
BV711 anti CD163; GHI/61; Biolegend; 333630
PeCy5 anti-CD123; 6H6; Biolegend; 306008
PeCy7 anti-CD141; M80'; Biolegend; 344110
APC anti CD14; M5E2; Biolegend; 325608
AF700 anti CD303; 201A; Biolegend; 354228
APC-Cy7 anti CD15; W6D3;Biolegend; 323048
PeCy5.5 anti-Flavivirus; 4G2;Novus Biologicals; NBP2-52701PECY55
FITC anti-CD45; HI30;Biolegend; 304005
BV605 anti-CD3; OKT3;Biolegend; 317321
PE-Cy5 anti-CD19; HIB19;BD Biosciences; 302209
PE anti-CD20; 2H7;Biolegend; 302305
APC-Cy7 anti-HLA-DR; L243;Biolegend; 307617

Validation

All antibodies are commercially available and validation statements can be found on the manufacturers' websites using the catalogue number. Isotype and single staining controls were used to identify separation between negative and positive populations and specificity of labeling.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

Human hepatoma (Huh7) cells were obtained from Apath LLC (Brooklyn, NY).

Authentication	Huh7 cells identity was confirmed via phenotypic studies showing low grade infection with hepatitis C virus vs. high grade infection with dengue virus.
Mycoplasma contamination	Cells were tested negative for mycoplasma by the MycoAlert mycoplasma detection kit (Lonza, Morristown, NJ).
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used.

Flow Cytometry

Plots

Confirm that:

☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☒ All plots are contour plots with outliers or pseudocolor plots.

☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	Cryopreserved PBMCs were thawed at 37 °C, washed with cell culture media (RPMI, Gibco) supplemented with 10% FBS + 20 U/ml sodium heparin (Sigma) and 0.025 U/ml Benzonase (Sigma), and incubated with the Zombie Aqua™ live/dead fixable dye (Biolegend) at 1:1000 and with 5 µl of FcR blocking reagent (Miltenyi) for 15 min at 25 °C. Cells were labeled for 20 min at 4 °C with the specific antibodies, washed and fixed with 1% PFA for 24 hrs.
Instrument	Aurora spectral analyzer (Cytek).
Software	FlowJo V10.9 (Tree Star).
Cell population abundance	A minimum of 100,000 cells per sample was analyzed.
Gating strategy	The relevant gating strategies are shown in Extended Figure 6 and Extended Figure 7. Functional Flow cytometry: FSC-A SSC-A (cells), FSC-H FSC-A (singlets), Zombie Aqua (live), CD45+ (hematopoietic), CD19+ (B cells), CD3+ (T cells). CD19-CD3 were furthered discriminated into: CD16+CD14+ (intermediate monocytes), CD16-CD14+ (classical monocytes). CD16+CD16-CD14- was further discriminated into: CD16+CD56- (non-classical monocytes), CD16+CD56+ and CD56+CD16- (NK cells). CD56-CD16- was further discriminated into: CD123+CD303+ (pDC). CD123-CD303-was discriminated into: CD141-CD1c+ (cDC2), CD141+CD1c-(cDC1). For each of the identified cell populations, the following functional markers were analyzed: HLA-DR, CD64, CD163, CD32. The quantification of DENV infected cells was determined by positive anti-Flavivirus antibody staining. Flow Sorting of B cells and HLA-DR+ cells: FSC-A SSC-A (cells), FSC-H FSC-A (singlets), Dapi- (live), CD45+ (hematopoietic), CD20+CD19+ (sorted B cells). CD20-CD19-, CD3- HLA-DR+ (sorted HLA-DR+ cells).

☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.