

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 (<i>n</i>) 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 <i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>
☒	☐ 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. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
☒	☐ 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 <i>d</i> , Pearson's <i>r</i>), 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	Hematology parameters were measured by the clinical laboratory at the BPRC with a Sysmex XT-2000iV Automated Hematology Analyzer (Sysmex Nederland B.V., Etten-Leur, The Netherlands). The percentage of mCherry-expressing cells was quantified on a Cell Insight CX5/7 High Content Screening platform (Thermo Fischer Scientific) to determine neutralization half-maximal inhibitory concentrations (SNT50), using virus (100%) and cell controls (0%) for normalization, and curve fitting in GraphPad Prism. Flow cytometry was performed on the BD LSR Fortessa X20, and data were analyzed with FlowJo (LLC, V10). qPCR was carried out on a Bio-Rad CFX Connect real-time system. Digital transcriptomics (nCounter platform, Nanostring Ltd.) results were sequentially corrected for background (negative control probes), technical variation (positive control probes) and RNA content (housekeeping genes), followed by differential expression, cell type deconvolution, pathway enrichment and network analysis using nSolver 4.0 (NanoString), MSigDb(53), and STRING (https://string-db.org), in conjunction with publicly available bulk and single-cell transcriptomic datasets GEO (https://www.ncbi.nlm.nih.gov/geo/) and CZ CELLxGENE Discover (https://cellxgene.cziscience.com/).
Data analysis	GraphPad Prism Version 9 (GraphPad Software, Inc.) was used for all statistical analyses. Uniform Manifold Approximation and Projection (UMAP) and dot plot representations were performed in RStudio (version 2024.04.2+764), using the Seurat package (v5) and the Tabula Sapiens-Blood database. Digital transcriptomics data mining of demographic, serological and transcriptomic data with machine learning algorithms for prediction (attribute selection, decision trees) was performed using Weka (version 3.8.4).

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](#)

All data associated with this study are provided in the paper, the Supplementary materials or the Source Data file. Unique materials used in this study are available from the corresponding author upon reasonable request.

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

Reporting on race, ethnicity, or other socially relevant groupings

Population characteristics

Recruitment

Ethics oversight

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](https://www.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	Sample size was estimated on the basis of similar research reported in literature (PMID: 27492477; PMID: 29237759; PMID: 27708058; PMID: 32522807; PMID: 27355570). All sample sizes are stated in the manuscript and figure legends and the Source Data file.
Data exclusions	No data were excluded.
Replication	For NHP study, a single experiment was conducted due to high costs and ethical concerns. All other experiments were conducted with independent replicates.
Randomization	Randomization was performed while assigning the animals into experimental groups.
Blinding	The investigators were not blinded to allocation during the process of data collection and 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	CD45 BV786 (Clone D058-1283, BD, 563861) CD3 BUV 395 (Clone SP34-2, BD, 564117) CD4 BB515 (Clone L200, BD, 560837) CD8 PE (Clone SK1, BioLegend, 564116) IFN- γ BV711 (Clone 4S.B3, BD, 564793) IL-2 BV421 (Clone MQ1-17H12, BD, 564164) IL-4 APC (Clone 8D4-8, BD, 561233) Goat anti-monkey IgG, IgA, IgM (Fc Specific) (Nordic-MUBio cat. no. GAMon/Ig(Fc)/PO, 1:10000)
Validation	Validation data about the antibodies obtained from commercial sources are available on the manufacturers' website and/or data sheets.

Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

Cell line source(s)	Vero E6 (African green monkey kidney) and BHK-21J (baby hamster kidney) cells were provided by P. Bredenbeek at the Leiden University Medical Center (LUMC), the Netherlands).
Authentication	Cell lines were not authenticated.
Mycoplasma contamination	All cell lines tested negative for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	None of the commonly misidentified cell lines were used.

Animals and other research organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research, and [Sex and Gender in Research](#)

Laboratory animals	Rhesus macaques (Macaca mulatta, 7 males and 3 females, approximately 4 years old with a body weight of at least 6 kg) were used for immunization and challenge. 129/SvEv Ifnar1tm1Agt Ifngr1tm1Agt (AG129) mice deficient in interferon- α/β and γ receptors (PMID: 8009221; sourced from B&K Universal, Marshall Bio resources, UK) were used for the serum transfer experiment. Mice were housed in groups of up to n=5 in individually ventilated cages (IsoCage N Biocontainment System, Tecniplast) at a temperature of 21°C, humidity of 55%, and 12:12 dark/light cycles, with ad libitum access to food and water and cage enrichment (cardboard houses and wood blocks).
Wild animals	None.
Reporting on sex	Rhesus macaques (7 males; 3 females), were used for immunization and challenge. There were no sex-related significant differences in our correlation analysis, therefore, we did not do further research. For AG129 mice, sex was not considered in the study design since gender does not affect the outcome. The sex of animals used in the experiments depended on the availability of the animals.
Field-collected samples	Not applicable
Ethics oversight	All housing and animal procedures for NHPs took place at the BPRC, upon positive advice by the independent ethics committee (DEC-BPRC), under project license AVD502002016496 issued by the Dutch Central Committee for Animal Experiments and following approval of the detailed study protocol by the institutional animal welfare body. All experiments using mice strictly followed the Belgian guidelines for animal experimentation and the guidelines of the Federation of European Laboratory Animal Science Associations. The Ethical Committee of the Animal Research Center of the University of Leuven

pre-approved all experiments (project number P100/2019).

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

Plants

Seed stocks	N/A.
Novel plant genotypes	N/A.
Authentication	N/A.

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 peripheral blood mononuclear cells (PBMCs) were thawed at 37°C and 1×10 ⁶ cells were cultured in RPMI 1640 with L-glutamine (Lonza), supplemented with 10% fetal calf serum (FCS), penicillin (100 units/ml; Continental Pharma) and streptomycin (100 µg/ml; Continental Pharma). Cells were stimulated with interleukin-2 (IL-2) (50 ng/ml; PeproTech), anti-human CD28 (Clone CD28.2, 1 µg/ml; BD), and anti-human CD49d (Clone R1-2, 1 µg/ml; BD) for 72h at 37°C/5% CO ₂ . To measure vaccine-specific T cell responses, cells were either or not stimulated with PepMix ZIKV E protein peptide (PM-ZIKV-E, 5 µM; JPT Peptide Technologies). As a positive control, cells were stimulated with PMA (50 ng/ml; Sigma-Aldrich) and ionomycin (500 ng/ml; Sigma-Aldrich). Four hours prior to staining, cells were treated with Brefeldin A (GolgiPlug, 1/1000; BD) and Monensin (GolgiStop, 1/1500; BD). Then cells were incubated with FcR-blocking reagent (0.5 µl/well; Miltenyi Biotec) and extracellularly stained with CD45 BV786 (Clone D058-1283, BD), CD3 BUV 395 (Clone SP34-2, BD), CD4 BB515 (Clone L200, BD) and CD8 PE (Clone SK1, BioLegend) antibody in FACS buffer [PBS+2% FCS+2mM ethylenediaminetetraacetic acid (EDTA)]. Dead cells were excluded using Zombie Aqua 516 (BioLegend). Intracellular staining with IFNγ BV711 (Clone 4S.B3, BD), IL-2 BV421 (Clone MQ1-17H12, BD) and IL-4 APC (Clone 8D4-8, BD) was performed with the Fixation and Permeabilization kit (BD) according to the manufacturer's protocol. Flow cytometry was performed on the BD LSR Fortessa X20, and data were analyzed with FlowJo (LLC, V10).
Instrument	BD LSR Fortessa X20
Software	FlowJo (LLC, V10)
Cell population abundance	Cells were not sorted.
Gating strategy	First, dead cells were gated out as Zombie Aqua (ZA)-positive. Then, doublets were eliminated in an FSC-H versus FSC-A plot. T cells (CD45+CD3+) were stratified into CD4+ T cells and CD8+ T cells. Boundaries defining positive and negative populations for intracellular markers were set based on non-stimulated control samples.

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