nature portfolio

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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	REDCap (v13.1.30)
Data analysis	FlowJo (v10.10), nSolver analysis software (v4.0), Partek Genomics Suite (v7.21), GSEApy (https://github.com/zqfang/GSEApy), Graphpad Prism (v 9.0), R package (v4.3.2) pheatmap (v1.0.12)

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 data that support this study is available in the source data files are published alongside the manuscript.

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	Study population demographics are shown in extended data Table 1. Both males and females were recruited for the study and the sex of participants were balanced between the groups. Sex was determined based on information recorded in national identity documents. No information on gender was collected. No sex or gender specific analysis was conducted due to the small sample size.
Reporting on race, ethnicity, or other socially relevant groupings	Study population demographics are shown in extended data Table 1. Race was determined based on information recorded on national identity documents. No adjustment for potential confounders were made as this was a randomized trial.
Population characteristics	Study population demographics are shown in extended data Table 1. Essentially, study participants were all healthy adult volunteers aged between 21 and 45 years.
Recruitment	Study participants were recruited from within Singapore, through a combination of print and email advertisement. Potential sources of self-selection bias include the need to be available for the frequent study visits and the need to be dengue seronegative at the time of study enrolment.
Ethics oversight	The study was approved by the SingHealth Centralised Institutional Review Board (CIRB) (Ref: 2021/2738).

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	A sample size of 25 per arm was initially estimated to provide 80% power at a 5% type 1 error rate, in order to detect an effect size of 0.8 standard deviation in mean RNA levels on a log10 scale, between primary vaccination and challenge infection. However, a decision was made to terminate enrolment after the first 34 participants, as five participants did not seroconvert after JE/YF17D or YF17D challenge infection, indicating that a proportion of study participants would not be fully protected from a future JE or YF infection, despite being vaccinated.
Data exclusions	No data was excluded from this analysis.
Replication	Samples were run in technical duplicates for the viral RT-qPCR and the anti-NS1 and anti-E antibody ELISAs, and in triplicates for the whole-blood cytokine release assay. All attempts at replication were successful. Apart from these 3 assays, samples were only re-run if the assay controls indicated an experimental failure, due to the limited and unique nature of samples collected from study participants.
Randomization	Participants were randomized in a 1:1 ratio using opaque sealed envelopes, each containing a study arm, that were randomly picked by a non-study team member.
Blinding	Study investigators were blinded to group allocation during 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

From BD Biosciences
Anti-human CD3-PerCPCy5.5 (clone SK7, 340949, 2:50 dilution)
Anti-human CD4-PECy7 (clone SK3, 557852, 3:50 dilution)
Anti-human CD8-APCCy7 (clone SK1, 557834, 3:50 dilution)
Anti-human CD38-APC(clone HIT2, 555462, 10:50 dilution)

From Biolegend
Anti-human HLA-DR-BV605 (clone L243; 307640, 1:20 dilution)

Validation

Antibody validation is available at each manufacturer's website below:
Anti-human CD3-PerCPCy5.5 - https://www.bdbiosciences.com/en-sg/products/reagents/flow-cytometry-reagents/clinical-discovery-research/single-color-antibodies-ruo-gmp/percp-cy-5-5-mouse-anti-human-cd3.340949
Anti-human CD4-PECy7 - https://www.fishersci.com/shop/products/anti-cd4-pe-cy-7-clone-sk3-bd/BDB557852
Anti-human CD8-APCCy7 - https://www.bdbiosciences.com/en-sg/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/apc-cy-7-mouse-anti-human-cd8.557834
Anti-human CD38-APC - https://www.bdbiosciences.com/en-sg/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/apc-mouse-anti-human-cd38.555462
Anti-human HLA-DR-BV605 - https://www.biolegend.com/fr-lu/products/brilliant-violet-605-anti-human-hla-dr-antibody-7875

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	Baby Hamster Kidney (BHK) cell lines were used for the PRNT assay, obtained from ATCC.
Authentication	None
Mycoplasma contamination	Cell lines tested negative for mycoplasma contamination
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cells lines were used.

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration	NCT05568953
Study protocol	The study protocol has been previously published (https://doi.org/10.3389/fimmu.2023.1135979).
Data collection	Study enrolment, follow-up and data collection were conducted between 30 MAR 2023 and 31 OCT 2023 at the SingHealth Investigational Medicine Unit, Singapore
Outcomes	The primary outcome of the study was viral RNA levels after challenge infection. Secondary objectives included an assessment of surrogate measures of viral control, including post-challenge antibody titers and symptomatic outcome rates.

Plants

Seed stocks	NA
Novel plant genotypes	NA
Authentication	NA

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	PBMCs were isolated by density-gradient centrifugation using Ficoll–Paque. Fresh PBMCS were used for the AIM assay.Cells were suspended Yellow live/dead (invitrogen) was used to exclude dead cells in all experiments.
Instrument	CytoFLEX S (Beckman Coulter)
Software	FlowJo software (BD) v10.10
Cell population abundance	Sorting and collection of cells was not performed.
Gating strategy	Gating strategy is shown in Extended data Figure 2c. To exclude dead cells, Fixable Live/Dead Cell reactive dye+ cells were gated out. Single cells were gated on a forward scatter height vs. forward scatter area plot. Lymphocytes were gated on forward vs. side scatter area plot based on their size and granularity. CD3+ T cells were gated by selecting cells positive for the anti-CD3 antibody. CD4+ and CD8+ T cells were identified by gating on CD3+ cells that were positive for either CD4 or CD8 expression, respectively. A Boolean gating strategy was applied to ensure mutually exclusive subsets. Activation status was assessed within the CD4+ and CD8+ T cell subsets by gating on cells co-expressing HLA-DR and CD38.

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