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	Flow cytometry data acquisition was performed with FACSymphony A5 (BD Biosciences) driven by FACS DiVa software or a Cytek Aurora (Cytek) run by SpectroFlo. Sequencing was performed with Illumina NovaSeq 6000.
Data analysis	Flow cytometry data were analyzed using FlowJo version 10 (BD) and OMIQ (Dotmatics) software. Statistical and data visualization were performed using Grahpad Prism version 10. For Bulk RNA seq analysis: Alignment was performed using STAR version 2.7.9a and transcripts were annotated using a composite genomic assembly and annotation of the Mmul10 Indian rhesus macaque and SIVsm804ECL757 (Accession MF370842). Transcript abundance estimates were calculated internal to the STAR aligner using the algorithm of htseq-count. DESeq2 was used for normalization and differential expression analysis using the Wald test. Functional enrichment was performed using the Gene Set Enrichment Analysis (GSEA) method implemented in the fgsea R package against the Hallmark and Canonical Pathways collections from the Molecular Signatures Database (MSigDB). Virological modeling and decay analysis was performed with Monolix 2024R1 (Lixoft)

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

Bulk RNA-seq data have been deposited in the NCBI GEO database under accession number GSE301433. All other data are present in the article as individual data points, supplementary information, supplementary data sets, or 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	Not applicable
Reporting on race, ethnicity, or other socially relevant groupings	Not applicable
Population characteristics	Not applicable
Recruitment	Not applicable
Ethics oversight	Not applicable

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	Sample size was determined using power analysis based on previous in vivo Rhesus Macaque studies to ensure statistically significant results
Data exclusions	No data were exlcuded from analysis unless a specific data point was unavailable for that analysis due to samples availability
Replication	No attempts were made to replicate this data given the scale and resource investment required for these studies.
Randomization	Animals were stratified into experimental groups based on their age and viral load before starting ART.
Blinding	Blinding of primary investigators was not possible given the need to properly handle and identify samples. Secondary collaborators were blinded to treatment groups prior to analysis of shipped materials.

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

Material and methods contains all the following information: anti-CCR7-BB700 (clone 3D12, catalog no. 566437), anti-CXCR3-BV421 (clone 1C6/CXCR3, catalog no. 562558), anti-CCR4-PE-CF594 (clone 1G1, catalog no. 565391), anti-CD3-BUV395 (clone SP34-2, catalog no. 564117), anti-CD8-BUV496 (clone RPA-T8, catalog no. 612942), anti-NHP CD45-BUV563 (clone D058-1283, catalog no. 741414), anti-CD28-BUV737 (clone CD28.2, catalog no. 612815), anti-CD69-BUV805 (clone FN50, catalog no. 748763), anti-BCL-2-AF647 (clone Bcl-2/100, catalog no. 563600), anti-active caspase-3 (clone C92-605.rMAb, catalog no. 570185)(all from BD Bioscience); anti-CD95-BV605 (clone DX2, catalog no. 305628), anti-HLA-DR-BV650 (clone L243, catalog no. 307650), anti-CD25-BV711 (clone BC96, catalog no. 302636), anti-PD-1-BV785 (clone EH12.2H7, catalog no. 329930), anti-CCR6-PE (clone G034E3, catalog no. 353410), anti-CD4-APC-Cy7 (clone OKT4, catalog no. 317418)(all from Biolegend); anti-CD127-PE-Cy5 (clone eBioRDR5, catalog no. 15-1278-42), anti-CD185-PE-Cy7 (clone MU5UBEE, catalog no. 25-9185-42))(all from Thermo Fisher Scientific). Intracellular staining to evaluate BCL-2 expression and cleaved caspase-3 was performed using the BD Cytofix/Cytoperm™ Fixation/ Permeabilization Kit (catalog no. 554714, BD Bioscience).

Validation

All antibodies used for flow cytometry analysis of rhesus macaque samples were validated to be cross-reactive with rhesus macaque tissues through extensive in-house testing, including staining with FMO and isotype controls. Some antibodies are validated as rhesus-reactive through databases maintained by the NHP Reagent Resources.

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

All animals in this study were Indian rhesus macaques (Macaca mulatta) between 43 and 68 months of age at the time of infection.

Wild animals

This study did not include wild animals.

Reporting on sex

The study involved 24 male rhesus macaques. We only included male rhesus macaques because of the availability of animals at the start of the study.

Field-collected samples

This study did not include samples collected from the field.

Ethics oversight

This study was approved by the Emory University Institutional Animal Care and Use Committee (IACUC) via protocol 202100086. Experiments were conducted following guidelines set forth by the NIH and Animal Welfare Act in regard to the housing and welfare of laboratory Rhesus macaques (RMs). All procedures were performed under anesthesia, with appropriate pain management as necessary. Proper measures were taken to minimize animal suffering.

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

Plants

Seed stocks

Not applicable

Novel plant genotypes

Not applicable

Authentication

Not applicable

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 from EDTA-anticoagulated blood using density gradient centrifugation (Ficoll-Paque Premium, GE Healthcare). LNs were mechanically dissociated in culture medium and filtered through a 100-µm strainer to isolate mononuclear cells. Freshly isolated cells were used for flow cytometry analyses presented in Figures 1 and 4, Extended Data Figure 1, and Supplementary Figures 3 and 4. Single-cell suspensions were cryopreserved in 10% DMSO in FBS, then thawed and cultured prior to staining for the experiments shown in Figure 6.
Instrument	FACSymphony A5 for and Cytek Aurora
Software	Flow cytometry data acquisition was performed using a FACSymphony A5 (BD Biosciences) operated with FACS DiVa software or a Cytek Aurora (Cytek) run by SpectroFlo. Flow cytometry data were analyzed with FlowJo version 10 (BD) and OMIQ (Dotmatics) software.
Cell population abundance	Sorted cell purity following CD4+ magnetic enrichment ranged between 85% and 97%, as determined by flow cytometry analysis post-sort.
Gating strategy	Singlets were defined using the diagonal of FSC-H versus FSC-A, and lymphocytes were gated from SSC-A versus FSC-A. A live/dead gate based on viability dye was then applied. T cells were defined as $CD3^+$ within live cells. CD4 and CD8 T cells were gated from $CD3^+$ cells. Memory subsets were identified as $CD95^+$ (CD28 versus CD95). Subsequent gates for the different markers were applied to memory $CD4^+$ T cells. Gate boundaries were determined based on the expression of phenotypic markers within the naïve ($CD95^-$ $CD28^+$) subset.

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