

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 BD FACSChorus was used to collect flow cytometry data.

Data analysis For 10X genomics sequencing, cells were assigned to individual macaques using CITE-seq-Count in combination with the MULTISEQDemux function implemented in Seurat (v5.4.0) R package. V(D)J gene usage was annotated using IMG/HighV-QUEST in conjunction with the VDJbase. Cryo-EM data processing was performed in cryoSPARC v4.5 and EPU v2.9. Atomic models were built through iterative manual rebuilding in Coot (v1.1) and ISOLDE (v1.10), followed by real-space refinement in Phenix (v2.0). Initial Fab variable region (Fv) models were generated using the AlphaFold 3 server. Apo Env trimer and Fv models were fit into cryo-EM density maps using UCSF ChimeraX (v1.11). Model quality was iteratively assessed using MolProbity v4.0 and EMRinger v1.0 until satisfactory validation metrics were achieved. Protein interfaces were analyzed using PDBePISA v1.52. R packages LASSIE and Logomaker were used to visualize sequence evolution and identify sites under selection, respectively. Visualizations of Env evolution were generated using Matplotlib (v3.8.4), and statistical analyses were performed using the SciPy stats module (v1.13.1). EMPER image processing was performed using RELION (v3.0). Biolayer interferometry data were analyzed by quantifying the binding response at the end of the 200 s association phase using ForteBio Data Analysis HT (v12.0). Sequence assembly, alignment, and analysis were performed using Geneious (v2025.2). Statistical analyses were performed using GraphPad Prism (v11.0). Flow cytometry results were analyzed with FlowJo (v10.10.10).

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](#)

CryoEM model coordinates are available under Protein Data Bank (PDB) IDs 9ZRX, 9ZRY, and 9ZRZ. The associated cryo-EM reconstruction maps are deposited in the Electron Microscopy Data Bank (EMDB) under the accession codes EMDB-74654, EMDB-74655, and EMDB-74656. Env sequences generated in this study are available under Genbank accession numbers PX727509-PX729439, PX747763-PX747970, PX751872 - PX751937 and PX752574 - PX752824. SHIV sequences and plasmid DNA clones are available under Genbank accession number PZ137521, PZ137522, PX677578 and PX677579. Plasmids encoding prefusion-stabilized CAP256.OPT4-S Env (GenBank accession #PZ137520). B cell 10X genomics sequencing data are available under BioProject SRA PRJNA1450649, Biosample SAMN57163943 - SAMN57164009.

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	N/A
Reporting on race, ethnicity, or other socially relevant groupings	N/A
Population characteristics	N/A
Recruitment	N/A
Ethics oversight	N/A

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	No statistical methods were used to determine sample size. The number of macaques in each SHIV or immunization group was determined based on common standards in the field, and ranged from 4–8 animals per group (PMID 41686912; 42096521; 29170366). For experiments other than those involving macaques, no independent sample size calculation was performed because these analyses were conducted on samples obtained from the macaque cohorts. Replicate measurements were performed to ensure reproducibility and accurate quantification of virological and immunological parameters and were consistent with standard practice in the field.
Data exclusions	Macaques that developed rapid-progressor phenotypes (high persisting vRNA loads, early AIDS-related morbidity) were excluded from bNAb analyses. Rapid progressor animals were euthanized according to predefined IACUC-approved clinical endpoints.
Replication	Each SHIV and immunization group consisted of multiple macaques (range 4–8). Endpoint results were replicated and validated within and between groups. All neutralization assays were performed with two technical replicates and successfully repeated. B cell sorting was performed once per sampling timepoint for each rhesus macaque, with each animal representing an independent biological replicate. Sample sizes were determined by the number of animals included in each infection or immunization cohort: SHIV.CAP256.OPT4 (n = 8), SHIV.CAP256.OPT4-S (n = 6), SHIV.Q23 (n = 4), SHIV.CH505 (n = 4), SHIV.CAP256SU (n = 4), CAP256.OPT4-immunized (n = 4), and CAP256.OPT4-S-immunized (n = 4).
Randomization	Macaques were randomly assigned to different SHIV or immunization groups and were unselected with respect to age or sex. No other experiments were randomized.
Blinding	Investigators were not blinded to group allocation. The analysis performed included SGA which do not allows for blinded study.

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	CD3 (clone SP34-2, BD 557757, dilution 1:100), CD8a (clone SK1, Invitrogen 47-0087-41, dilution 1:200), CD14 (clone 61D3, Invitrogen 47-0149-42), dilution 1:200, CD16 (clone eBioCB16, Invitrogen 47-0168-41, dilution 1:200), CD20 (clone L27, BD 335793, dilution 1:100), IgG (clone G18-145, BD 550931, dilution 1:20) and hashing antibodies TotalSeq – C0251 (clone 2M2, Biolegend 394661; 394663; 394665; 394667; 394669; 394671; 394673; 394675; 394677; 394679; 394683; 394685; 394687; 394689; 394691; 394693; 394695; 394697; 394699, dilution 1:100). All other antibodies described in the text were synthesized in-house
Validation	Antibodies generated in-house were biophysically validated using protein gels and functionally validated through the use of controls in neutralization assays to ensure similar neutralization potency across batches and experiments. Commercial antibodies have been pre-titrated and tested by flow cytometric analysis of normal human peripheral blood cells by the manufacturer. All in house mAb were synthesized and cloned (Genscript) into rhesus IgG1, IgK, or IgL expression vectors previously characterized (PMID 41686912; 42096521; 33214287).

Eukaryotic cell lines

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

Cell line source(s)	HEK 293T/17 cells were obtained from the American Type Culture Collection (ATCC, CRL-11268). TZM-bl cells were obtained from BEI Resources (ARP-8129). Expi293F were obtained from ThermoFisher (A14527).
Authentication	All cell lines were authenticated by the original source. Cells were regularly monitored for unusual morphology or growth kinetics.
Mycoplasma contamination	All cell lines were certified mycoplasma free by the original source. Cell lines were not tested after receipt but were used at low passage numbers.
Commonly misidentified lines (See ICLAC register)	No 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	Macaca mulatta. Macaques used in this study were between 6 and 11 years old, as described in the Materials and Methods section. CH01 RUA3 (CH01 RUA3 VLJL+/+, VHDJH+/+) site-directed knock-in (KI) mice were used.
Wild animals	The study did not involve wild animals.
Reporting on sex	Macaques were randomly assigned to different SHIV or immunization groups and were unselected with respect to age or sex. The sexes of macaques used in this study are outlined in the Materials and Methods section.
Field-collected samples	The study did not involve samples collected from the field.
Ethics oversight	Macaques and mice were housed in AAALAC-accredited facilities, and all veterinary and study procedures were performed in accordance with the University of Pennsylvania, Duke University, University of Louisiana (NIRC) and Bioqual Institutional Animal Care and Use Committees.

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

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	Rhesus plasma was processed and stored as described in the Materials and Methods section. 5-10 million PBMCs were thawed, stained for 30 min with Zombie-NIR viability dye (Biolegend 77184), washed, and stained for 1 hour with a cocktail of antibodies. Cells were then washed, stained for 1 hour with BV421, AF647, and/or PE-conjugated soluble Env trimer, filtered through a sterile 100 µm strainer, and sorted on a BD FACSMelody Cell Sorter. B cells were sorted in bulk into FBS, counted, and immediately carried forward for single-cell analysis using the 10X Genomics platform.
Instrument	BD FACSMelody
Software	Flow cytometry data were analyzed in FlowJo (v10.10.1)
Cell population abundance	From lymphocytes gate, population is ranging from 26% to 83%. CD20+/Dump- ranged from 2.5%- 45.3%. IgG+ cells ranged from 20.1 - 46.8%
Gating strategy	All positive and negative populations were determined by compensation with single color controls. For sorting and analysis, all lymphocytes were first gated based on SSC-A vs FSC-A, followed by a singlet gate (FSC-H vs FSC-A). Antigen-specific (Env+ +) or epitope-specific (Env++V2KO-) CD3-CD8a-CD14-CD16-CD20+IgG+ were gated as outlined in Fig 2a.

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