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Reporting Summary

Nature Research 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 Research 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 FACSDiva Software version 9.0 for FACS Enspire Manager version for 413.3005.1482 for Elisa and Neutralization EPU version 2.2.0.65REL for NSEM data
Data analysis	FlowJo 10.7.1 for FACS analysis; Microsoft Excel 16.36; GraphPad Prism 8.4.2; Microsoft Excel 16.49; SPICE 6 version6.1(61001) MacVector version 17.0.5 (43) For statistical analysis, R software, version 4.0.3. Relion version 1.4 for NSEM data

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 Research 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 list of figures that have associated raw data
- A description of any restrictions on data availability

All mRNA sequence data are publicly available (Accession codes: MZ362872, MZ362873, MZ362874, MZ362875, MZ362876).

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	For the mouse study a Wilcoxon sum rank test with two-tailed significance level of 0.05 would have over 80% power to detect an effective size of 1.6 between the Env and Env-Gag vaccine arms, each with the sample size of 8, and an effect size of 2 between a vaccine arm and the control arm with the sample size of 8 and 4, respectively. For the macaque study, with seven rhesus macaques per arm, the study had over 80% power to detect the protective efficacy of the vaccine against HIV-1 infection if the protective efficacy, defined as one minus the hazard ratio, was no less than 0.90 from a Wilcoxon sum rank test on the time to infection with two-tailed significance level of 0.05.
Data exclusions	No data were excluded from this report
Replication	All critical assays were reliably replicated as indicated in figures or tables. Animal experiments were carried out in experimental groups. All immunized animals developed both trimer-binding and neutralizing antibodies.
Randomization	Mice were inbred from the same colony and the same age range. They were randomly assigned to the study groups. Macaques were randomly assigned to the study groups, which were balanced for age, weight, CBC (complete blood count) and chemistry profiles.
Blinding	Mice and macaques were identified by ear tags or skin tatoos, respectively, and therefore could not be handled blindly. However, operators were blinded to the substances administered to each group.

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
☒	☐ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

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

Antibodies

Antibodies used	Previously published human monoclonal antibodies were obtained from the AIDS Research Program, including CD4-Ig VRC01 VRC03 VRC07 VRC-PG04 VRC-CH31 N6 N49P7 3BNC117 NIH45-46 HJ16 F105 b6 b12 b13 10-1074 PGT121 PGT123 PGT125 PGT126 PGT128 PGT130 PGT135 PGT136 2G12 PG9 PG16 PGT145 CH01 CH02 CH03 VRC26.08 VRC38 447-52D 19B 39F b4e8 2219 3074 412D 48d 17b 35O22 8ANC195 PGT151 VRC34 2F5 4E10 10E8. VRC01-UCA CH31-UCA CH103-UCA CH01-UCA PG9-UCA RM20J RM20F RM20E1 RM20G RM20C RM20A3 RM54B1 RM15E were produced in our laboratories based on published sequences. The antigenic profile of these antibodies, assessed by ELISA and flow cytometry, agrees with published descriptions as referenced throughout the

manuscript. Directly conjugated antibodies purchased from BD Biosciences include CD107a BV510 (Clone H4A3; cat. 563078), CXCR3 PE-Cy5 (Clone 1C6/CXCR3; cat. 551128), CD14 BUV805 (Clone M5E2; cat. 612902), CD20 BUV805 (Clone 2H7; cat. 612905), CD4 BUV395 (Clone L200; cat 564107), CD28 BB515 (Clone CD28.2; cat. 564492), CD8 BUV496 (Clone RPA-T8; cat. 612942), CD3 APC-Cy7 (Clone SP34-2; cat. 557757), IFN-gamma V450 (Clone B27; cat. 560371), IL-2 BUV737 (Clone MQ1-17H12; cat. 612836) and IL-21 Alexa Fluor 647 (Clone 3A3N2.1; cat. 560493). Antibodies purchased from Biolegend include PD-1 BV785 (Clone EH12.2H7; cat. 329930), CD95 BV605 (Clone DX2; cat. 305628), CCR7 BV650 (Clone G043H7; cat. 353234); CD154 BV711 (Clone 24-31; cat. 310838), TNF-alpha Alexa Fluor 700 (Clone Mab11; cat. 502928) and CD69 PE (Clone FN50; cat. 310906). CXCR5 PE-eFluor 610 (Clone 5UMUBEE; cat. 61-9185-42), IL-4 PE-Cy7 (Clone 8D4-8; cat. 25-7049-82) and Granzyme B PE-Cy5.5 (Clone GB11; cat. GRB18) were purchased from Invitrogen along with LIVE/DEAD Fixable Blue Dead Cell Stain (cat. L34962).

Validation

The primary antibodies program were extensively validated on appropriate positive and negative controls in our experiments. The information about antibodies from HIV-1 reagent could be found at https://www.hivreagentprogram.org/Home.aspx. The catalog information is also listed here: ARP-857 Antibody (F105), ARP-1475 Antibody (2F5), ARP-1476 Antibody (2G12), ARP-1756 Antibody (48d),ARP-4030 Antibody (447-52D),ARP-4091 Antibody 17b, ARP-10091 Antibody (4E10),ARP-11437 Antibody (39F), ARP-11438 Antibody (A32),ARP-11682 gp120 Antibody (2191), ARP-12032 Antibody (VRC03),ARP-12033 Antibody (VRC01), ARP-12040 Antibody (3074), ARP-12138 Antibody (HJ16), ARP-12149 Antibody (PG9), ARP-12150 Antibody (PG16),ARP-12294 Antibody (10E8),ARP-12344 Antibody (PGT126),ARP-12474 Antibody (3BNC117),ARP-12477 Antibody (10-1074),ARP-12561 Antibody (CH01),ARP-12562 Antibody (CH02),ARP-12566 Antibody (CH106), ARP-12586 Antibody (35022), ARP-12703 Antibody (PGT145), ARP-12968 Antibody (N6). In-house produced monoclonal antibodies were validated by sequencing the heavy and light chain and Elisa assay. The conjugated FACS antibodies were validated either by BD (https://www.bdbiosciences.com/en-us#) or Biolegend(https://www.biolegend.com).
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Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

293T (ATCC CRL-11268)
293Freestyle (Thermo Fisher Scientific, R79007)
Expi293 (Thermo Fisher Scientific, A14528)
Homo sapiens TZM-bl Cells (ARP-8129)

Authentication

Commercial cell lines were not authenticated

Mycoplasma contamination

Mycoplasma contamination was not tested

Commonly misidentified lines
(See ICLAC register)

No commonly misidentified cell lines were used

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals

Fourteen 6- to 10-year old outbred naive female rhesus macaques (Macaca mulatta) of Indian origin were genotyped and selected as negative for the protective major histocompatibility complex (MHC) class I alleles Mamu-A*01, Mamu-B*08, and Mamu-B*17. The animals were randomly assigned to the study groups, which were balanced for age, weight, CBC (complete blood count) and chemistry profiles. Mice were 6- to 8-week-old female BALB/c obtained from Charles River.

Wild animals

No wild animals were used in the study.

Field-collected samples

No field collected samples were used in the study.

Ethics oversight

The macaque study protocol was approved by both the NIH and the Bioqual Institutional Animal Care and Use Committees (ACUCs). Mice experiments was approved by the Animal Care and Use Committee (ACUC) of Moderna Inc.
All animals studies were carried out in compliance with all pertinent US National Institutes of Health regulations.

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

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

Macaque PBMCs ficoll-purified from the whole blood. The rhesus Macaque PBMCs were thawed, rested and counted before

Sample preparation	stimulation with viral peptide pools. For stimulation, 1-1.5 million cells were plated into 96-well V-bottom plates in 200 µL R10 and stimulated with SIV Gag or HIV-1 clade B Env peptides (2 µg/mL; NIH AIDS Reagent Program) in the presence of Brefeldin A (10 µg/mL; Sigma-Aldrich) and monensin (GolgiStop; BD Biosciences). Titrated amounts of anti-CD107a and anti-CXCR3 were included in the culture during cell stimulation. A DMSO-only condition was included to determine background for unstimulated cells. Following stimulation for 6 hours, samples were stained with LIVE/DEAD Fixable Blue Dead Cell Stain for 10 minutes at room temperature and surface stained with titrated amounts of anti-CD14, anti-CD20, anti-CD4, anti-CXCR5, anti-CD28, anti-PD-1, anti-CD8, anti-CD95 and anti-CCR7 for 20 minutes at room temperature. Cells were washed in FACS Buffer (PBS + 2% FBS), and fixed and permeabilized (Cytofix/Cytoperm, BD Biosciences) for 20 minutes at room temperature. Cells were then washed with Perm/Wash buffer (BD Biosciences) and stained intracellularly with anti-CD3, anti-CD154, anti-IFN-g, anti-IL-2, anti-IL-4, anti-IL-21 anti-TNF-a, anti-Granzyme B and anti-CD69 for 20 minutes at room temperature. Following intracellular staining, cells were washed with Perm/Wash buffer, fixed with 1% paraformaldehyde and data were acquired on a modified BD FACSymphony. Transfected adherent cells were harvested by mechanical shaking and pipetting. All the cells were washed with PBS and incubated with antibodies (1 µg/mL) in flow cytometry buffer (1× PBS with 2% FBS, at RT for 30 min. After washing with PBS twice, the cells were incubated with PE-conjugated goat anti-human IgG (Southern Biotech) at RT for 15 min. Adherent cells were washed once with PBS, fixed in 2% paraformaldehyde (PFA) and analyzed on a BD LSRFortessa (BD Biosciences). Data analysis and geometric mean fluorescence intensity (MFI) measurement were performed using the FlowJo software.
Instrument	BD LSRFortessa and BD FACSymphony (BD Biosciences).
Software	FlowJo, version 10.7.1
Cell population abundance	All cell population tested were abundant (>50,000 events collected).
Gating strategy	Appropriate gating was applied in the FSC/SSC window to exclude nonviable cell fragments and apoptotic cells. Additional gating was applied for the analysis of specific cell subpopulations.

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

Magnetic resonance imaging

Experimental design

Design type	*Indicate task or resting state; event-related or block design.*
Design specifications	*Specify the number of blocks, trials or experimental units per session and/or subject, and specify the length of each trial or block (if trials are blocked) and interval between trials.*
Behavioral performance measures	*State number and/or type of variables recorded (e.g. correct button press, response time) and what statistics were used to establish that the subjects were performing the task as expected (e.g. mean, range, and/or standard deviation across subjects).*

Acquisition

Imaging type(s)	*Specify: functional, structural, diffusion, perfusion.*
Field strength	*Specify in Tesla*
Sequence & imaging parameters	*Specify the pulse sequence type (gradient echo, spin echo, etc.), imaging type (EPI, spiral, etc.), field of view, matrix size, slice thickness, orientation and TE/TR/flip angle.*
Area of acquisition	*State whether a whole brain scan was used OR define the area of acquisition, describing how the region was determined.*

Diffusion MRI ☐ Used ☐ Not used

Preprocessing

Preprocessing software	*Provide detail on software version and revision number and on specific parameters (model/functions, brain extraction, segmentation, smoothing kernel size, etc.).*
Normalization	*If data were normalized/standardized, describe the approach(es): specify linear or non-linear and define image types used for transformation OR indicate that data were not normalized and explain rationale for lack of normalization.*
Normalization template	*Describe the template used for normalization/transformation, specifying subject space or group standardized space (e.g. original Talairach, MNI305, ICBM152) OR indicate that the data were not normalized.*
Noise and artifact removal	*Describe your procedure(s) for artifact and structured noise removal, specifying motion parameters, tissue signals and physiological signals (heart rate, respiration).*
Volume censoring	*Define your software and/or method and criteria for volume censoring, and state the extent of such censoring.*

Statistical modeling & inference

Model type and settings	*Specify type (mass univariate, multivariate, RSA, predictive, etc.) and describe essential details of the model at the first and second levels (e.g. fixed, random or mixed effects; drift or auto-correlation).*
Effect(s) tested	*Define precise effect in terms of the task or stimulus conditions instead of psychological concepts and indicate whether ANOVA or factorial designs were used.*

Specify type of analysis: ☐ Whole brain ☐ ROI-based ☐ Both

Statistic type for inference (See Eklund et al. 2016)	*Specify voxel-wise or cluster-wise and report all relevant parameters for cluster-wise methods.*
Correction	*Describe the type of correction and how it is obtained for multiple comparisons (e.g. FWE, FDR, permutation or Monte Carlo).*

Models & analysis

n/a	Involved in the study
☐	☐ Functional and/or effective connectivity
☐	☐ Graph analysis
☐	☐ Multivariate modeling or predictive analysis

Functional and/or effective connectivity	*Report the measures of dependence used and the model details (e.g. Pearson correlation, partial correlation, mutual information).*
Graph analysis	*Report the dependent variable and connectivity measure, specifying weighted graph or binarized graph, subject- or group-level, and the global and/or node summaries used (e.g. clustering coefficient, efficiency, etc.).*
Multivariate modeling and predictive analysis	*Specify independent variables, features extraction and dimension reduction, model, training and evaluation metrics.*