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Reporting Summary

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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 were collected using FACS DiVa software (BD Biosciences) on an LSRFortessa. DNA/RNAscope data were scanned at 40x magnification on an Aperio AT2 (Leica Biosystems). To quantify the number of SIV vDNA+ lymphocytes or myeloid cells on stained tissues, scans were performed at 20X using the Zeiss Axioscan Z.1 slide scanner and representative images were taken using a Keyence BZ-X710 fluorescence microscope with the 20X objective. Single-genome sequencing of gp160 env sequences were performed on the MiSeq platform (Illumina).
Data analysis	Flow Cytometry data were processed using FlowJo version 9.9.6 (TreeStar). vDNA and vRNA scope images were analyzed with the CytoNuclear module (v1.6) within Halo software (v2.3.2089.27; Indica Labs). Multi-spectral images were analyzed using the HALO 2.3 platform (Indica Labs) and quantified with FISH v1.1. gp160 env sequencing reads were aligned using Geneious version R11; phylogenetic trees were generated in PhyML version 3.0 (ATGC Bioinformatics); and visualized in FigTree. Statistics were performed using GraphPad Prism version 8.1.2.

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/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

Data Availability
The data that support the findings of this study are available from the corresponding author on reasonable request.

Note: Sequencing reads of gp160 env are currently being submitted to Genbank (related to Fig. 6j and Supplementary Fig. 15). Accession numbers will be added to the data availability statement as they become available.

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	n=34 total; Group sizes (n=6 to 8 per arm) were selected to provide approximately 80% power to detect mean differences of 1.5 standard deviations or greater. We compared the parameters of interest longitudinally in the same animals before and during the specific interventions as well as between different groups. Repeated-measures analyses were performed using mixed-effect linear models or ANOVA with multiple comparison corrections. T-tests were used to compare the differences between the model-based treatment means at each time point within the framework of the mixed-effects linear model. Statistical tests will be 2-sided, and a P value ≤0.05 will be considered significant.
Data exclusions	For cell-associated SIV-DNA and -RNA measurements, data were excluded for samples if <10,000 cells were sorted and values fell outside the assay limit of detection. Select flow cytometry data points were excluded based on indications of aberrant staining. IPDA data with an associated DNA shearing index of greater than 25 were excluded.
Replication	No attempts were made to replicate data, as this is a long term (~2yr) in vivo study with substantial costs pertaining to animal acquisition and care. Sorted LN cells were subsequently purity sort (>96%) to confirm phenotypic identity.
Randomization	Animals were stratified into treatment groups based on acute ("peak") and set point ("chronic" at ART initiation) viral load; duration of virological suppression; Mamu-A*01 status (six out of 33 were Mamu-A*01+; all Mamu-B*08- and B*17-); and the lack of naturally arising, pre-existing cross-reactive anti-drug antibodies (ADAs) against the tested antibodies (i.e., anti-PD-1 and anti-CTLA-4) in plasma. The initiation of ICB was dependent on the duration of virological suppression in plasma during ART.
Blinding	Blinding of primary investigators was not possible as we were responsible of the distribution of compounds for administration and stratification of the animals into treatment groups. 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
☐	☒ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data

Methods

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

Antibodies

Antibodies used	Immune checkpoint receptor (ICR) blocking monoclonal antibodies utilized in this study were developed, formulated, and produced by GlaxoSmithKline and provided via Qura Therapeutics. The described therapeutics are intellectual property of GlaxoSmithKline and are not commercially available. VHDUM, the control, is a fully-human IgG1 mAbdAb with the Fc region disabled by the LAGA mutations: L235A and G237A. The mAb portion has the sequence of palivizumab (anti-respiratory syncytial virus, MedImmune) and is linked by a fibronectin linker to an irrelevant domain antibody that has no known specificity. The anti-PD-1 mAb (αPD-1) is a biosimilar formulation of nivolumab (Bristol-Myers Squibb) with a human variable region and a rhesus constant region. The anti-CTLA-4 mAb (αCTLA-4) is a fully-human, biosimilar formulation of ipilimumab (Bristol-Myers Squibb) that retains Fcγ binding activity. The αCTLA-4/PD-1 mAbdAb (BsAb) has a human variable and rhesus constant region with a LAGA mutation-disabled Fc receptor. The BsAb CTLA-4 is biosimilar to ipilimumab and the PD-1 is a proprietary formulation of GlaxoSmithKline (Supplementary Table 3).

The following antibodies were used at predetermined optimal concentrations for the longitudinal staining panel: anti-T-bet-PE/Dazzle-594 (clone 4B10), anti-FoxP3-APC (clone 150D), anti-CD4-APC-Cy7 (clone OKT4), anti-CD95-BV605 (clone DX2), anti-HLA-DR-BV650 (clone L243), anti-CD25-BV711 (clone BC96), anti-CD20-PerCp/Cy5.5 (clone 2H7), and anti-PD-1-BV785 (clone EH12.2H7) all from Biolegend; anti-CXCR5-PE (clone MU5UBEE) and anti-CD127-PE-Cy5 (clone eBioRDR5) both from eBioscience; anti-CCR7-PE-Cy7 (clone 3D12), anti-Ki-67-Alexa700 (clone B56), anti-CTLA-4-BV421 (clone BNI3), anti-CD3-BUV395 (clone SP34-2), anti-CD8-BUV496 (clone RPA-T8), and anti-CD28-BUV737 (clone CD28.2) all from BD Biosciences; anti-LAG-3-FITC (clone 17B4) from Novus Biologicals; and Aqua Live/Dead amine dye-AmCyan from Invitrogen.

The following antibodies were used at predetermined optimal concentrations for phenotyping cryo-preserved LN: anti-CD200-PE (clone OX104), anti-HLA-DR-PE-Cy5 (clone L243), anti-CD4-APC-Cy7 (clone OKT4), anti-CD25-BV711 (clone BC96), anti-CD101-PerCP/Cy5.5 (clone BB27), anti-CD95-BV605 (clone DX2), and anti-CD69-BV785 (clone FN50) all from Biolegend; anti-CD38-FITC (clone AT-1) from STEMCELL Technologies; anti-CCR7-PE-Cy7 (clone 3D12), anti-Ki-67-Alexa700 (clone B56), anti-CTLA-4-BV421 (clone BNI3), anti-CXCR3-BV650 (clone 1C6/CXCR3), anti-CD3-BUV395 (clone SP34-2), anti-CD8-BUV496 (clone RPA-T8), and anti-CD28-BUV737 (clone CD28.2) all from BD Biosciences; anti-PD-1-Alexa647 (clone 913429) from Novus Biologicals; and anti-Granzyme B-PE-Texas Red (clone GB11) and Aqua Live/Dead amine dye-AmCyan from Invitrogen.

The following panel of antibodies was utilized for measuring cytolytic and SIV-specific responses in cryo-preserved cells: anti-CD3-BUV395 (clone SP34-2), anti-CD8-BUV496 (clone RPA-T8), anti-Ki-67-AL700 (clone B56), anti-CD95-PE-Cy5 (clone DX2), anti-CCR7-BV480 (clone 3D12), anti-GranzymeB-BV421(clone GB11), and anti-CD28-BUV737 (clone CD28.2) all from BD Biosciences; anti-HLA-DR-BV750 (clone L243), anti-CD4-BV570 (clone OKT4), anti-PD-1-BV786 (clone EH12.2H7), and anti-CTLA-4-PE (clone BNI3) all from Biolegend; anti-Gag-APC (clone CM9) from the NIH Tetramer Facility at Emory University; anti-CD38-APC (clone AT-1) from STEMCELL Technologies; and anti-EOMES-PE-eFluor610 (clone WD1928) and LIVE/DEAD Fixable Near-IR Dead Cell Stain from Thermo Fisher Scientific.

Cryo-preserved, unstimulated mononuclear cells derived from LN biopsies were stained with the following combination of monoclonal antibodies: anti-CD200-PE (clone OX104), anti-CD25-Alexa700 (clone BC96), and anti-CD4-BV650 (clone OKT4) all from Biolegend; anti-CD28-PE-CF594 (clone CD28.2), anti-CCR7-PE-Cy7 (clone 3D12), anti-CD3-APC-Cy7 (clone SP34-2), and anti-CD95-BV421 (clone DX2) all from BD Biosciences; anti-CD8-FITC (clone 3B5) and Aqua Live/Dead amine dye-AmCyan from Invitrogen; anti-CD127-PE-Cy5 (clone eBioRDR5) from eBioscience; and anti-PD-1-Alexa647 (clone 913429) from Novus Biologicals. Purified anti-PD-1 was labeled using an Alexa Fluor 647 Antibody Labeling Kit (Invitrogen) using the manufacture's guidelines.

Upon SIV peptide stimulation in the presence of anti-CD107a-APC (clone H4A3; Biolegend), cells were washed and stained for cell surface antigens with the following combination of monoclonal antibodies: anti-CD3 Alexa-700 (clone SP34-2), anti-CD95 BB515 (clone DX2), and anti-CD4 BV711 (clone L200) all from BD Bioscience; anti-CD8 PerCP-Cy5.5 (clone RPA-T8/SK1) and anti-PD-1 BV421 (clone EH12) both from Biolegend; and the Live/Dead Amine Aqua Dye from Life Technologies. To detect intracellular expression of cytokines, mononuclear cells were fixed and permeabilized with a Cytofix/Cytoperm kit (BD Biosciences) and stained as follows: anti-TNFa BV505 (clone Mab11) and anti-IL-2 PE (clone MQ1-17H12) both from Biolegend, and anti-IFNg PE-CF594 (clone B27) from BD Biosciences.

For CellTrace dilution, cells with labeled with 5uM CellTrace Violet (Thermo Fisher Scientific) and following TCR stimulation were washed and stained with the following combination of monoclonal antibodies: anti-CD3-BUV395 (clone SP34-2), anti-CD8-BUV496 (clone RPA-T8), anti-Ki-67-AL700 (clone B56), anti-CD95-PE-Cy5 (clone DX2), anti-CCR7-PE-Cy7 (clone 3D12), and anti-CD28-BUV737 (clone CD28.2) all from BD Biosciences; anti-CD4-PerCP-Cy5.5 (clone OKT4), anti-PD-1-BV786 (clone EH12.2H7), and anti-CTLA-4-PE (clone BNI3) all from Biolegend; and LIVE/DEAD Fixable Near-IR Dead Cell Stain from Thermo Fisher Scientific.

Validation

Antibodies are validated as human reactive by the manufacturers with some being verified as macaque reactive via the NHP Reagent Resource. We have also previously demonstrated these clones to be reactive in macaques:

McGary, C.S., Deleage, C., Harper, J., Micci, L., Ribeiro, S.P., et al. CTLA-4(+)PD-1(-) Memory CD4(+) T Cells Critically Contribute to Viral Persistence in Antiretroviral Therapy-Suppressed, SIV-Infected Rhesus Macaques. Immunity 47, 776-788 e775 (2017).

Micci, L., Ryan, E.S., Fromentin, R., Bosinger, S.E., Harper, J.L., He, T., Paganini, S., Easley, K.A., Chahroudi, A., Benne, C., et al. (2015). Interleukin-21 combined with ART reduces inflammation and viral reservoir in SIV-infected macaques. J Clin Invest 125, 4497-4513.

Animals and other organisms

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

Laboratory animals

34 male Indian rhesus macaques (RMs; Macaca mulatta), all housed at the Yerkes National Primate Research Center (YNPRC) at Atlanta, Ga, were included in this study. Animals were between 25 and 44 months old at time of infection. See also data provided in Supplementary Table 1.

Wild animals

Study did not involve wild animals.

Field-collected samples

Study did not involve field-collected samples.

Ethics oversight

All animal experimentation was conducted following guidelines set forth by the Animal Welfare Act and by the NIH's Guide for the Care and Use of Laboratory Animals, 8th edition. All studies were conducted in accordance with institutional regulations including GSK's Policy on the Care, Welfare, and Treatment of Laboratory Animals, and were reviewed and approved by Emory's Institutional Animal Care and Use Committee (IACUC; permit numbers 3000065, 2003297, 2003470, and PROTO201700665).

Animal care facilities at YNPRC are accredited by the U.S. Department of Agriculture (USDA) and the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International. Proper steps were taken to minimize animal suffering and all procedures were conducted under anesthesia with follow up pain management as needed.

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	Phenotypic analyses were performed on cellular suspensions derived from fresh tissue; whereas, sorting was performed on cryo-preserved cells from lymph node biopsies. PB was used for complete blood counts and metabolic chemistry panels, and plasma was separated by centrifugation within 1 hr of phlebotomy. During ICB infusions, blood was drawn prior to and 5 mins afterwards for pharmacokinetic assays. Peripheral blood mononuclear cells (PBMCs) were isolated from PB by density gradient centrifugation (Ficoll-Paque Premium, GE Healthcare). For LN biopsies, the skin over the axillary or inguinal region was clipped and surgically prepped. An incision was then made in the skin and the LN was exposed by blunt dissection and excised over clamps. LNs were segmented using a sterile scalpel; macerated over RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 U/mL penicillin, and 100 µg/mL streptomycin; and filtered through a 100 µm strainer to isolate mononuclear cells.
Instrument	LSR Fortessa (BD Biosciences) or FACSymphony (BD Biosciences) for phenotyping, and FACS AriaII (BD Biosciences) for cell sorting.
Software	Data collected on LSR Fortessa driven by FACS DiVa software (BD Biosciences). FlowJo version 9.9.6 (TreeStar).
Cell population abundance	Post-sort fractions (>10,000 cells) were purify sorted (>96% purity).
Gating strategy	Mononuclear cells were defined by laying on the diagonal of FSC-H versus FSC-A, and lymphocytes were gated from SSC-A versus FSC-A. CD4+ and CD8+ T-cells were pre-gated as live CD3+, and memory subsets were gated as CD95+ (CD28 versus CD95). Gate boundaries were determined via expression of phenotypic markers within the Naive (CD95-CD28+) subset. For phenotypic discrimination of sorted populations, TFH were defined as PD-1hiCD200hi; whereas, TREG (CD25+CD127-) were pre-gated as PD-1loCD200lo to eliminate CD25+ TFH T-cells. Within the CD25-PD-1loCD200lo population the TEM and TCM were sorted based on their CCR7 expression. Gating strategies are supplied as Fig 5a and Supplementary Fig. 4.

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