nature portfolio

https://doi.org/10.1038/s41591-021-01574-5

Supplementary information

A multiclade *env–gag* VLP mRNA vaccine elicits tier-2 HIV-1-neutralizing antibodies and reduces the risk of heterologous SHIV infection in macaques

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Supplementary Materials for

A multiclade *env-gag* VLP mRNA vaccine elicits broad neutralization and reduces the risk of heterologous tier-2 SHIV infection in macaques

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Methods

Animals. BALB/c mice (6-8 weeks old) were obtained from Charles River. The experiments in mice were carried out in compliance with all pertinent US NIH regulations and were approved by the Animal Care and Use Committee (ACUC) of Moderna Inc. Fourteen outbred naive female rhesus macaques of Indian origin, 6-10 years of age, 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. All the animals were housed at Bioqual Inc., Rockville, MD, according to the guidelines of the Association for Assessment and Accreditation of Laboratory Animal Care standards, with the exception of three vaccine-naïve controls which were housed at the NIH Animal Facility in Poolesville, MD. During the challenge phase, the animals received the same dose of the same viral stock (SHIV AD8_EO) in both facilities, and the veterinary staff followed the same operating procedures. The study protocol was approved by both the NIH and the Bioqual ACUCs.

mRNA immunogens. Sequence-optimized mRNAs encoding the immunogens were synthesized *in vitro* using an optimized T7 RNA polymerase-mediated transcription reaction with N1-methylpseudouridine-5'-triphosphate utilized in place of uridine-5'-triphosphate[79]. The *in vitro* transcription DNA template contained the target open reading frame flanked by 5' and 3' untranslated regions and an encoded polyA tail. The GenBank accession numbers for the mRNA immunogens are: WITO4160.27.ENV.gp145.N276D.(1-745aa), MZ362872; WITO4160.27.ENV.gp145.G153E.(1-745aa), MZ362873; DU422.1.ENV.K295N.D386N.375Y.(1-745aa), MZ362874; BG505.W6M.ENV.C2.T332N.S375Y.gp160, MZ362875; SIVmac239.gag.P55, MZ362876. After transcription, a cap 1 structure was added enzymatically using vaccine capping enzyme and 2'O-methyltransferase (New England Biolabs, Ipswitch, MA). Purified mRNA was sterilized by filtration and co-formulated into the same lipid nanoparticle (LNP) using a method described previously[80]. Briefly, mRNA was diluted in acetate, pH 5.0, and mixed with lipids (SM-102 : DSPC : Cholesterol : PEG2000-DMG) dissolved in neat ethanol at a ratio of 3:1 (mRNA:lipids). The product was then dialyzed against PBS, pH 7.4, tested for encapsulation, particle size/polydispersity, mRNA purity and endotoxin, and was deemed acceptable for *in vivo* use. The Env:Gag ratio ranged between 1.7:1 and 2.2:1 in the various immunogen formulations (see Suppl, Table 2).

***In vitro* transfection and VLP capture and quantification.** mRNAs encoding different HIV-1 Env constructs (1 µg) and SIVmac239 Gag (2 µg) were co-transfected into HEK293T cells. The DNA mixtures were added to 6-well plates (10^6 cells per well) with 6 µL of transfection reagent and transfection booster from TransIT-mRNA Transfection Kit (Mirus). Supernatants were harvested 48 hours after transfection, centrifuged at 1,000 rpm and filtered through a 0.45 µM PES filter (Millipore). For VLP capture from culture supernatants, the anti-Env bNAb PG16 and PGT128 were incubated with Protein G-conjugated immunomagnetic beads (Dynabeads; Life Technologies) at 20 µg/ml. Antibody-armed beads were incubated with VLP-containing

supernatants at room temperature (RT) for 30 minutes and then washed 3 times with PBS containing 0.025% (vol/vol) casein (R&D Systems) to remove the unbound material. Captured VLPs were lysed with RIPA buffer on the beads, and the released p27 Gag protein concentration was measured using an SIV p27 antigen Elisa. Serial dilutions of reference p27 protein and lysed VLP p27 were coated onto 96-well Elisa plates (Corning) at 4ºC overnight. Then, the plates were blocked with 1x casein at RT for 1 hour, followed by the addition of mouse anti-p27 monoclonal antibody (AIDS Reagent Program cat. #3537) at 1 ug/ml. After washing, horseradish peroxidase (HRP)-conjugated goat anti-mouse IgG (Invitrogen Cat#A16072) was added for 1 hour at RT. The optical density (OD) at 450 nm was measured. The standard curves and VLP p27 concentration were calculated by dose-response curve fit with a 5-parameter nonlinear function using GraphPad Prism 8. For concentrating VLPs, 25 mL of VLP-containing supernatants were layered onto a 10~20% linear gradient sucrose PBS buffer and centrifuged at 29,300 rpm (Beckman Rotor Type 50.2 Ti, ~78,000 g) for 2 hours at 4ºC. After centrifugation, the supernatants were discarded and the VLP pellets were resuspended in PBS and stored at 4ºC for NSEM or gp120/p27 Elisa.

Mouse immunization protocol. BALB/c mice were injected with mRNA formulations in a volume of 0.05 mL using a sterile syringe by intramuscular injection in the hind leg. The animals were immunized at day 0 and 28 with 2.5 µg of HIV-1 Env 426c.D276.D460.D463(1-745) mRNA alone (group 1; n=8) or co-formulated with 2.5 µg of SIVmac239 Gag mRNA (group 2; n=8). Control mice (n=5) received PBS because previous studies have proven that the mRNA formulations being tested do not create substantial levels of nonspecific immunity[81]. Blood was collected on day -1, 15, 28, 43, 57. Blood collections did not to exceed 1% of the total body weight, or 10 mL/kg over a two-week period per ACUC protocol. Regarding the sample size, 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.

Macaque immunization protocol and live virus challenges. Formulated mRNA was diluted in sterile PBS immediately before the immunizations, as required to reach the desired volume. The protein immunization doses were prepared by mixing 100 µg of SOSIP trimer with 100 µL Adjuvant (Adjuplex™, Sigma) in PBS per animal. The final inoculation volume was 500 µL per animal. Animals were vaccinated intramuscularly at week 0, 11, 19, 27, 35, 43, 47, 51, 56 and 59 with either mRNA or protein into the right hind leg. At week 27, 35, 43 and 47, the animals received two separate inoculations (DU422 375Y.1-745 and BG505 375Y gp160 mRNA or protein), at least 1 inch apart, in the same quadriceps muscle. The mRNA and protein doses inoculated at each time point are listed in Suppl. Table 2. For live virus challenge, molecularly cloned SHIV AD8 previously titrated *in vivo* was inoculated intrarectally. All animals received 13 sequential low-dose intrarectal inoculations containing 10 $TCID_{50}$ of SHIV AD8_EO diluted in sterile PBS. For reference, a previous study conducted with 10 $TCID_{50}$ of the same viral stock via intrarectal inoculation in 12 naïve macaques yielded infection after a mean of 3.8±1.1 inoculations (range 2-6; median 3 inoculations)[61]. All blood samples (serum, plasma or PBMC)

were collected either on the day of or two weeks after vaccine inoculations. PBMC were isolated by Ficoll and frozen in 10% DMSO in CoolCell LX cell freezing containers (Corning), left at −80°C overnight and then transferred to vapor-phase liquid N2 freezer (Thermo Scientific) for long-term storage. Regarding the sample size, with 7 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.

Mutagenesis, protein expression and purification. Mutations were introduced by site-directed mutagenesis using the QuikChange Lightning Multi Site-Directed Mutagenesis Kit (Agilent Technologies, Part number 210514). The mutagenized trimers 426C.DS.SOSIP, WITO 501GCG SOSIP.664 (containing a modified furin cleavage site), BG505 375Y SOSIP.664 and JR-FL 375Y SOSIP.664 (containing a tyrosine substitution at position 375) were expressed by co-transfecting the relevant plasmids (from John P. Moore and the VRC) with a plasmid expressing the cellular protease furin into Free-style 293-F cells (ThermoFisher Scientific, Inc). For AD8, a non-SOS soluble trimer was used, devoid of the stabilizing 501-605 disulfide bond but containing a neo-disulfide bond between aa. 113 and 432[17]. This trimer design will be described elsewhere (Zhang et al., in preparation). Cell-free supernatants were harvested after 6-7 days by centrifugation and passed through a 0.22 μm filter. A *Galanthus nivalis* (GNA) lectin affinity column (Vector laboratories) and size-exclusion column were utilizd to purify the trimer proteins. The peak corresponding to the Env trimer protein was collected and concentrated, followed by a mAb 447-52D affinity column for negative selection of open trimer forms displaying the V3-loop tip. Purified trimers were concentrated to 0.5-2 mg/mL in PBS and stored at -80°C.

***In vitro* mRNA transfection and flow cytometry assays.** HEK 293T cells (female human embryonic kidney cells, obtained from ATCC) were transiently transfected with mRNA encoding different HIV-1 Envs using the TransIT-mRNA Transfection Kit (Mirus). After 48 hours, the cells were harvested by mechanical shaking and pipetting, washed with PBS and incubated with CD4-Ig or anti-gp120 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. The cells were then 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 (version 10.7.1).

Antibody-binding assays. HIV-1 Env binding by antibodies present in macaque plasma or serum was tested by ELISA as previously described[17]. For assessing endpoint binding titers, 96-well ELISA plates (Corning) were either coated with 4 μg/mL of lectin Galanthus nivalis (Sigma) for testing Env trimer binding or directly coated with smaller antigens (e.g. SIV p27 Gag, V3 peptides, gp70-V1V2 scaffold, or eOD-GT8 proteins) at 1 μg/ml in PBS at 4°C overnight. Then, the plates were blocked by 1x Casein (R&D Systems) at room temperature (RT) for 1 hour. All the antibodies and HIV-1 Env proteins used in ELISA experiments were diluted

in 0.1x casein solution in PBS (washing buffer). All samples were washed 3 times with washing buffer after each step. To evaluate macaque sera binding to soluble Env trimers, purified SOSIP trimers (1 μg/mL) were added to lectin-coated plates for 1 hour at RT. After washing, serially diluted rhesus macaque sera were added and incubated for 1 hour at RT. After washing, horseradish peroxidase (HRP)-conjugated goat anti human IgG (Jackson ImmunoResearch) was added for 1 hour at RT. The reaction was revealed by incubation with 50 μl of substrate (R&D Systems) for 15 min before addition 25 μl of stop solution. Light absorption was measured at a wavelength of 450 nm. All samples were tested in duplicate wells.

bNAb competition ELISA. BG505 SOSIP.664 trimers (1 μg/ml) were added to lectin-coated ELISA plates for 1 hour at RT. Macaque sera (at 1:100 dilution) and biotinylated bNAbs or sCD4-4d at pre-determined concentrations to yield 50-60% of maximum binding signal were added to the plates simultaneously. Bound bNAbs were detected using Streptavidin-HRP (R&D Systems). The OD values at 450 nm were measured, and binding data were analyzed with the Prism 8 software (GraphPad Prism Software, Inc.). The degree of competition was calculated as the percentage of signal reduction in the presence or absence of the immune macaque sera.

HIV-1 pseudovirus production and neutralization assays. Plasmids expressing various HIV-1 Envs were co-transfected with an HIV-1 backbone plasmid (pSG3Δenv, Env-deficient) into HEK293T cells. One μg of each Env-expressing plasmid and 1 μg of the backbone plasmid were mixed in Opti-MEM medium (Gibco), and 4 μL of LiFect293 Transfection Reagent were added at 1:2 DNA to reagent ratio. The DNA-LiFect293 Transfection Reagent complex was incubated for 15 minutes at RT and added to 10^6 HEK293T cells in 6-well plates with 2 mL of fresh 10% FBS-DMEM medium. After incubation for 2 days at 37˚C, the supernatants containing pseudoviruses were centrifuged, filtered by a 0.45 μm filter, aliquoted and titrated for further use. For single-round HIV-1 Env-pseudovirus infection of TZM-bl cells, heat-inactivated rhesus macaque sera were serially diluted 3-fold and incubated with the pseudoviruses for 30 min at RT; then, 10^4 TZM-bl cells were added to 96-well flat-bottom plates in 10% FBS-DMEM medium, in a total volume of 200 μL per well. All the samples were tested in duplicate or triplicate wells. After 72 hours, the medium was completely removed, and the reporter gene (luciferase) expression was detected by adding 50 μL per well of Luciferase Assay Reagent (Promega). The cell lysates were transferred to luminescence plates and measured with a luminometer (PerkinElmer). The viral stocks were used at a concentration yielding approximately 100,000-500,000 Relative Light Units (RLU). Half-maximal inhibitory concentrations (IC_{50}) were calculated by a dose-response curve fit with a 5-parameter nonlinear function using GraphPad Prism 8.

Macaque CD4 functional assay. Rhesus macaque CD4 (rhCD4) and HIV-1 coreceptor-expressing 293T were generated by transient transfection of rhCD4, rhCXCR4 and rhCCR5 expression vectors (HIV Reagent Program: Cat #3600, #3602 and #3601) on the day before the pseudovirus titration experiment. Titrated HIV-1 WT or 375Y pseudoviruses were incubated with transfected HEK293T cells. Seventy-two hours later, the cells were lysed and luciferase activity (RLU) was measured.

Cell-surface staining and ADCC assay. Briefly, SHIV AD8-infected primary human $CD4^+$ T cells were stained with AquaVivid viability dye and cell proliferation dye (eFluor670; eBioscience) and used as target cells and treated with and without 50 μM DIP for 1 h at 37°C. Autologous PBMC effector cells, stained with proliferation dye eFluor450 (eBioscience), were added at an effector/target ratio of 10:1 in 96-well V-bottom plates (Corning). Then, heat-inactivated macaque sera were added to appropriate wells, and the cells were incubated for 15 min at RT. Subsequently the cells were centrifuged for 1 min at 1,000 rpm and incubated at 37°C and 5% CO_2 for 5 hrs before being fixed in a 2% PFA solution in PBS. Samples were acquired on an LSRII cytometer (BD Biosciences), and data analysis was performed using FlowJo vX.0.7 (Tree Star). The percentage of cytotoxicity was calculated using the following formula: (% of GFP+ cells in targets plus effectors) − (% of GFP+ cells in targets plus effectors plus MAbs)/(% of GFP+ cells in targets) by gating on infected live target cells.

T-cell immunity assays. Cryopreserved PBMC were thawed into warm R10 media (RPMI 1640, 10% FBS, 2 mM L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin), washed and rested for 1 hour before stimulation. 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 washed with Perm/Wash buffer (BD Biosciences) and stained intracellularly with anti-CD3, anti-CD154, anti-IFN-γ, anti-IL-2, anti-IL-4, anti-IL-21 anti-TNF-α, anti-Granzyme B and anti-CD69 for 20 minutes at room temperature. The antibodies used for staining were the following: 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-γ 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-α 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). Following intracellular staining, the cells were

washed with Perm/Wash buffer, fixed with 1% PFA, and data were acquired on a modified BD FACSymphony. Data were analyzed using FlowJo software (version 10.7.1) and cytokine frequencies were background subtracted. Examples of the gating strategy are shown in Supplementary Figure 14. Polyfunctional T-cell responses were analyzed using SPICE, as reported[82].

Plasma viral RNA quantification assay. Plasma SHIV RNA levels were measured using a *gag*-targeted quantitative real-time RT-PCR assay. Plasma RNA was extracted by using a QIAamp Viral RNA Kit (Qiagen, USA) and reverse-transcribed with AgPath-ID One-Step RT-PCR Kit (Applied Biosystems, USA). The primers, probe and amplification conditions were as previously reported[83]. The samples were measured in triplicate reactions, and the copy numbers were calculated by interpolation of a standard curve generated with a digested SIV *gag* DNA fragment that contains the RT-PCR amplicon (serial 10-fold dilutions from 1 copy/reaction to 10^6 copies/reaction).

Polyclonal epitope mapping by NSEM. Fabs were generated from macaque sera using the Pierce™ Fab Preparation Kit following the manufacturer's protocol. Briefly, macaque IgG were purified by affinity columns of Protein A-Sepharose resin (GE). After washing with PBS (10 times the column volume), IgG was eluted by IgG-elution buffer (Thermo Fisher) and immediately neutralized with pH9.0 Tris-HCl buffer. Resin-immobilized papain (250 μL settled resin for 4 mg IgG, Thermo Fisher Scientific) were prepared and equilibrated in the digestion buffer with 20 mM cysteine, pH 7.4 for 3 h at 37°C with the end-over-end mixer. Fc and undigested IgGs were removed by passing protein A Sepharose. The Fab-containing supernatant was buffer exchanged into PBS by centrifugation with Amico MWCO 10,000 Da centrifuge filter unit (EMD Millipore). Polyclonal Fabs were diluted to the concentration that is 10 times higher than that of BG505 SOSIP.664 trimer, and the mixtures of Fab and BG505 were incubated at 4°C overnight in PBS. The complexes were diluted to a concentration of about 0.02 mg/mL, adsorbed to freshly glow-discharged carbon-coated grids for 15 seconds, washed with a buffer containing 10 mM HEPES, pH 7 and 150 mM NaCl, and stained with 0.7% uranyl formate. Images were collected on a Thermo Scientific Talos F200C electron microscope operating at 200 kV and equipped with a 4k×4k Ceta CCD camera at a nominal magnification of 57,000, corresponding to a pixel size of 0.253 nm. Particles were picked from micrographs automatically using in-house written software (YT, unpublished data). Reference-free two-dimensional classification was performed in Relion[84].

Statistical analysis. The protective efficacy of the vaccine was evaluated based on the time to infection over the course of 13 sequential weekly low-dose inoculations. The Kaplan-Meier curves of vaccinated and control macaques were provided and compared by Wilcoxon exact test. Vaccine efficacy was assessed by one minus the hazard ratio, estimated from a Cox proportional hazards regression via the exact partial likelihood with group (vaccinated versus control) as the regressor. Th association of various immunological parameters with vaccine protection was assessed via Spearman's rank correlation with the number of infection-free days post-challenge.

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Supplementary Table 1. Antigenic profile of the 5 HIV-1 Envs used for mRNA immunization in mice and macaques. The geometric mean fluorescence intensity (MFI) for each antibody is shown, as assessed by flow cytometry on mRNA-transfected 293T cells.

	WITO.27 Δ276	WITO.27 G153E	BG505 S375Y	DU422.1 S375Y	426c.3dg
CD4-BS					
CD4-Ig	8,363	4,232	1,533	86	729
VRC01	2,867	992	787	55	1,540
VRC03	5,698	1,835	1,208	54	2,229
VRC07	2,231	733	1,222	63	2,437
VRC-PG04	9	13	554	48	1,133
VRC-CH31	107	12	633	50	348
N6	6,291	3,666	768	233	1,892
N49P7	3,274	1,970	607	102	822
3BNC117	5,658	2,378	926	54	940
NIH45-46	7,579	3,515	1,338	57	-3
HJ16	594	35	913	67	-4
F105	4,317	2,562	-57	56	208
b6	-13	9	-16	54	-10
b12	7,330	3,992	80	134	433
b13	4,799	3,768	-37	49	-2
V3 glycan					
10-1074	12,766	7,962	1,164	143	437
PGT121	14,753	7,717	2,203	498	494
PGT123	1,254	923	391	184	9
PGT125	16,194	10,987	6,073	719	56
PGT126	11,674	6,587	3,944	338	14
PGT128	7,869	4,231	7,233	878	439
PGT130	7,610	9,702	3,060	318	122
PGT135	9,921	5,819	812	77	990
PGT136	13,974	11,162	1,932	280	1,434
2G12	27,483	17,974	3,557	89	61
V2-glycan					
PG9	11,129	6,015	2,171	129	74
PG16	11,046	6,211	2,918	221	386
PGT145	10,931	6,356	2,844	102	240
CH01	4,308	2,293	219	72	6
CH02	4,088	2,585	274	67	-10
CH03	4,171	2,401	317	77	-2
VRC26.08	31	29	3,179	643	55
VRC38	2,224	1,259	292	60	-7
V3 tip					
447-52D	16,823	10,228	93	48	345
19B	12,249	7,412	402	82	462
39F	6,780	3,777	315	46	-4
b4e8	13,691	8,271	384	58	257
2219	13,152	7,721	512	138	363
3074	11,547	6,432	776	545	1,070
Bridging sheet					
412D	6,553	5,355	90	48	528
48d	5,958	4,168	6	57	-10
17b	7,405	4,945	91	45	283
gp120/gp41 interface					
35O22	5,326	3,931	1,277	134	818
8ANC195	2,039	9	476	53	3
PGT151	12,571	9,184	3,201	965	2,079
VRC34	3,018	2,221	1,019	439	868
MPER					
2F5	207	1,644	104	50	-9
4E10	556	262	325	83	73
10E8	742	614	295	89	236
UCA					
VRC01-UCA	-3	45	29	42	215
CH31-UCA	0	13	11	40	-6
CH103-UCA	38	23	12	44	-6
CH01-UCA	1,575	603	25	48	-11
PG9-UCA	30	41	34	44	-10
IgG control	0	0	0	33	0

50-100	100-250	250-500	500-2000	>2000

Supplementary Table 2. Trimer-binding and neutralizing antibodies in serum of immunized mice.

Trimer-binding antibodies

Time (week)	Env alone								Env+Gag								Controls			
2	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50
4	695	556	341	759	607	634	463	722	758	931	3374	1076	563	836	966	511	<50	<50	<50	<50
6	933	1206	820	1240	960	1827	1220	978	1667	1310	4238	2618	798	1005	4325	609	<50	<50	<50	<50
8	84326	2E+05	58957	62135	42375	97913	24856	72529	78862	85593	50803	50798	47671	93749	2E+05	52727	<50	<50	<50	<50
10	1E+05	1E+05	85880	71692	67804	2E+05	82033	96305	1E+05	1E+05	76095	64819	88178	1E+05	1E+05	73416	<50	<50	<50	<50

Autologous neutralizing antibodies

Time (week)	Env alone								Env+Gag								Controls			
2	<40	<40	<40	<40	<40	<40	<40	<40	<40	<40	<40	<40	<40	<40	<40	<40	<40	<40	<40	<40
4	<40	<40	<40	<40	<40	<40	<40	<40	<40	<40	274	<40	<40	<40	<40	<40	<40	<40	<40	<40
6	<40	<40	<40	<40	1980	5029	4268	90	78	<40	20658	26417	10012	11047	<40	840	<40	<40	<40	<40
8	94	<40	<40	<40	4575	5269	4859	474	192	740	21591	26599	22101	12800	1360	2255	<40	<40	<40	<40

Supplementary Table 3. Immunogens used in the macaque study.

		Subgroup 1			Subgroup 2		
		HIV-1 Env	Dose*	Immunogen	HIV-1 Env	Dose*	Immunogen
Autologous phase	Week 0	WITO.27.276D gp150 SIV Gag	250μg 150μg	mRNA	WITO.27.276D gp150 SIV Gag	250μg 150μg	mRNA
	Week 11	WITO.27.153E gp150 SIV Gag	150μg 90μg	mRNA	WITO.27.153E gp150 SIV Gag	150μg 90μg	mRNA
	Week 19	WITO.27.153E SOSIP.664	100μg + Adjuplex 100μl	protein	WITO.27.153E gp150 SIV Gag	150μg 75μg	mRNA
Heterologous phase	Week 27	BG505.T332N.375Y gp160 SIV Gag	155μg 70μg	mRNA	BG505.T332N.375Y gp160 SIV Gag	155μg 70μg	mRNA
		DU422.1.386N.295N 375Y gp150 SIV Gag	155μg 70μg	mRNA	DU422.1.386N.295N 375Y gp150 SIV Gag	155μg 70μg	mRNA
	Week 35	BG505.T332N.375Y gp160 SIV Gag	155μg 70μg	mRNA	BG505.T332N.375Y gp160 SIV Gag	155μg 70μg	mRNA
		DU422.1.386N.295N 375Y gp150 SIV Gag	155μg 70μg	mRNA	DU422.1.386N.295N 375Y gp150 SIV Gag	155μg 70μg	mRNA
	Week 43	BG505.T332N.375Y gp160 SIV Gag	155μg 70μg	mRNA	BG505.T332N.375Y gp160 SIV Gag	155μg 70μg	mRNA
		DU422.1.386N.295N 375Y gp150 SIV Gag	155μg 70μg	mRNA	DU422.1.386N.295N 375Y gp150 SIV Gag	155μg 70μg	mRNA
	Week 47	BG505.332N.241N.289N 375Y SOSIP.664	100μg + Adjuplex 100μl	protein	BG505.T332N.375Y gp160 SIV Gag	155μg 70μg	mRNA
		DU422.1.386N.295N 375Y SOSIP.664	100μg + Adjuplex 100μl	protein	DU422.1.386N.295N 375Y gp150 SIV Gag	155μg 70μg	mRNA
Additional boosts	Week 51	JRFL.375Y SOSIP.664	100μg + Adjuplex 100μl	protein	JRFL.375Y SOSIP.664	100μg + Adjuplex 100μl	protein
	Week 56	WITO.27.153E SOSIP.664	50μg + Adjuplex 100μl	protein	WITO.27.153E gp150 SIV Gag	50μg 25μg	mRNA
	Week 59	WITO.27.153E SOSIP.664 JRFL.375Y SOSIP.664 BG505.332N.241N.289N 375Y SOSIP.664 DU422.1.386N.295N 375Y SOSIP.664	50μg each + Adjuplex 100μl	protein	WITO.27.153E SOSIP.664 JRFL.375Y SOSIP.664 BG505.332N.241N.289N 375Y SOSIP.664 DU422.1.386N.295N 375Y SOSIP.664	50μg each + Adjuplex 100μl	protein

*Variations in the doses used were dependent on the mRNA yield after synthesis, which was different for each form.

Supplementary Table 4. Sensitivity of HIV-1 WITO4160.clone 27 and variants thereof to neutralization by sCD4 and neutralizing antibodies. The presence of a glycine (G) at position 153 is typical of clone 27, while most published WITO4160 clones bear a glutamic acid (E) at this position. The N188 glycan, which enhances binding to the CH01 UCA antibody, was present in all of the Env immunogens used in this study.

		WITO4160.27	WITO4160.27.276D	WITO4160.27.153E	WITO4160.27.190G
Mutations	153G/E	G	G	E	G
	N188 glycan	+	+	+	-
Antibodies	sCD4	0.019	0.22	6.6	0.1
	VRC01	0.097	0.308	0.027	0.061
	N6	0.059	0.04	0.049	0.11
	b12	0.31	0.12	0.46	0.24
	F105	>20	>20	>20	>20
	PGT121	0.057	0.008	0.016	0.011
	PGT135	0.096	0.84	5.5	>20
	2G12	0.98	0.37	0.25	0.29
	PG9	0.005	0.006	0.007	0.005
	PG16	<0.001	0.002	0.002	0.002
	PGT145	<0.001	0.001	0.001	0.001
	CH01	0.019	0.029	0.097	0.01
	447-52D	0.005	0.012	>20	0.01
	19B	0.024	0.12	>20	0.019
	17b	1.03	1.69	>20	0.44
	CH01-UCA	1.4	2.6	5.8	24.5
	VRC01-UCA	>20	>20	>20	>20

Supplementary Table 5. Reactogenicity of mRNA immunizations.

Event	Dose 1	Dose 2	Dose 3*	Dose 4	Dose 5	Dose 6	Dose 7*	Dose 8**	Dose 9	Dose 10**
Injection-site redness/swelling	0/7	0/7	0/4	0/7	0/7	0/7	0/4	n.a.	0/4	n.a.
Itching	0/7	0/7	0/4	0/7	0/7	0/7	0/4	n.a.	0/4	n.a.
Pain	0/7	0/7	0/4	0/7	0/7	0/7	0/4	n.a.	0/4	n.a.
Diarrhea	0/7	0/7	0/4	0/7	0/7	1/7	0/4	n.a.	0/4	n.a.
Loss of appetite	1/7	3/7	4/4	1/7	2/7	6/7	3/4	n.a.	2/4	n.a.
Lethargy	0/7	0/7	0/4	0/7	0/7	0/7	0/4	n.a.	0/4	n.a.
Behavioral changes	0/7	0/7	0/4	0/7	0/7	0/7	0/4	n.a.	0/4	n.a.
Total number of events:	**1**	**3**	**4**	**1**	**2**	**7**	**3**	**n.a.**	**2**	**n.a.**

*** Only 4 animals received mRNA at these time points.**
**** All animals received protein-only boosts at these time points.**

Supplementary Table 6. Viral antigen-binding antibodies in immunized macaques and correlation with protection.

Animal	Vaccine subgroup	Week of infection
V1	1	>13
V2	1	5
V3	1	9
V4	2	6
V5	2	4
V6	2	5
V7	2	>13

Autologous trimer (WITO SOSIP.664)

Animal	Week 6	11	13	15	19	21	25	37	45	53	58
V1	3912	7515	427684	379370	25597	203473	156764	63731	130120	144157	24023
V2	14238	10894	397698	472564	41760	335301	221929	63556	91973	124895	209671
V3	14196	23632	371958	331749	76620	883543	192037	275431	133813	95371	155228
V4	12215	4576	326988	479555	35617	406411	177414	182931	132589	99705	168107
V5	7385	11192	149564	278870	39655	150889	75527	124469	69655	137958	68065
V6	14131	16120	847834	872047	162125	647545	152753	180436	149114	30838	306198
V7	10325	17002	1522350	1456691	101435	941065	150719	242265	156440	376967	52747
Mean:	**10915**	**12990**	**577725**	**610121**	**68973**	**509746**	**161020**	**161831**	**123386**	**144270**	**140577**
Correlation with week of infection:	-0.2910	0.1091	0.5455	0.3273	-0.1091	0.4910	0.1091	0.3819	0.5455	0.4364	-0.6183
p value:	0.5270	0.8238	0.2095	0.4762	0.8238	0.2698	0.8238	0.3952	0.2095	0.3238	0.1508

Heterologous trimer (AD8 IP)

Animal	Week 6	13	21	29	37	45	49	53	58
V1	424	3720	2153	6647	15902	29673	68270	64970	58243
V2	137	4999	2738	8250	9192	18833	139886	55928	84536
V3	169	4689	3921	15879	20328	7698	137106	68280	217558
V4	218	3522	3256	6434	10094	38485	18744	16249	18582
V5	133	2017	633	4373	9277	12386	13322	36940	31653
V6	115	7597	3518	11129	13754	13469	39580	59894	44865
V7	132	9588	2826	8430	18329	15638	33302	32318	20464
Mean:	**190**	**5162**	**2721**	**8735**	**13839**	**19455**	**64316**	**47797**	**67986**
Correlation with week of infection:	0.4001	0.3455	0.2000	0.3273	0.7638	0.2910	0.2364	0.1637	-0.0364
p value:	0.3683	0.4444	0.6730	0.4762	0.0619	0.5270	0.6063	0.7238	0.9508

Autologous V3-loop peptide (WITO V3)

Animal	Week 6	13	23	29	33	37	45	53	58
V1	451	7260	17660	12462	5322	4405	12452	11325	30573
V2	272	5812	11227	15300	8202	16270	8909	17930	61058
V3	229	6535	44382	36937	14054	15033	4121	5572	10592
V4	106	3477	5776	7549	3307	35763	12507	12798	52092
V5	244	2623	3847	3610	2257	4825	5893	6054	16409
V6	1046	9760	97646	21632	10076	11197	6963	6147	12185
V7	408	3585	8204	3216	2177	15997	7780	11324	10228
Mean:	**394**	**5579**	**26963**	**14387**	**6485**	**14784**	**8375**	**10164**	**27591**
Correlation with week of infection:	0.1455	0.2910	0.2364	-0.1091	-0.1091	-0.0182	0.2910	0.0909	-0.3637
p value:	0.7492	0.5270	0.6063	0.8238	0.8238	0.9794	0.5270	0.8508	0.4222

SIV p27 Gag

Animal	Week 58
V1	37893
V2	42224
V3	9378
V4	25731
V5	16554
V6	24218
V7	45419
Mean:	**28774**
Correlation with week of infection:	0.4183
p value:	0.3460

Heterologous V3 peptide (JR-FL)

Animal	Week 58
V1	30572.9
V2	61058.3
V3	10591.5
V4	52091.5
V5	16408.9
V6	12184.8
V7	10227.5
Mean:	**27591**
Correlation with week of infection:	-0.6728
p value:	0.1079

V1V2 scaffold (gp70)

Animal	Week 58
V1	49542
V2	6306
V3	44652
V4	39077
V5	37968
V6	7151
V7	30041
Mean:	**30677**
Correlation with week of infection:	0.5274
p value:	0.2254

Binding to SHIV-AD8-infected CD4+ T cells (MFI)

Animal	Week 58
V1	1844.8
V2	1381.7
V3	1760.7
V4	1550.0
V5	1363.3
V6	1238.5
V7	658.0
Mean:	**1399.6**
Correlation with week of infection:	0.2910
p value:	0.5270

ADCC against SHIV-AD8-infected CD4+ T cells

Animal	Week 60
V1	3.020
V2	3.301
V3	6.937
V4	6.194
V5	4.848
V6	5.275
V7	7.178
Mean:	**5.250**
Correlation with week of infection:	0.2910
p value:	0.5270

Supplementary Table 7. Neutralizing antibodies in immunized macaques and correlation with protection.

Animal	Vaccine subgroup	Week of infection
V1	1	>13
V2	1	5
V3	1	9
V4	2	6
V5	2	4
V6	2	5
V7	2	>13

Autologous neutralization (WITO.27)

Animal	Week 6	11	13	15	19	21	25	27	29	33	35	37	43	45	47	49	51	53	56	58
V1	<8	<8	105	47	<8	80	100	137	361	147	<8	585	112	1148	371	837	730	623	214	489
V2	<8	<8	151	101	<8	120	162	154	558	160	<8	447	403	245	409	438	369	433	176	798
V3	<8	<8	245	134	<8	282	179	107	1695	234	130	664	584	347	803	516	515	319	153	593
V4	<8	<8	105	78	<8	179	146	50	1227	169	74	1188	828	598	960	440	443	882	707	1216
V5	<8	<8	214	91	<8	215	145	103	249	113	82	186	338	247	373	164	290	432	291	603
V6	<8	<8	134	65	<8	56	23	<8	753	140	<8	1390	774	504	1504	202	555	645	173	530
V7	<8	<8	104	79	<8	98	160	44	706	142	76	388	439	521	1327	303	593	793	254	836
Mean:	**<8**	**<8**	**151**	**85**	**<8**	**147**	**131**	**99**	**793**	**158**	**91**	**693**	**497**	**516**	**821**	**414**	**499**	**590**	**281**	**724**
Correlation with week of infection:	n.a.	n.a.	-0.578	-0.273	n.a.	-0.218	0.182	0.036	0.255	0.400	0.019	0.073	-0.073	0.709	0.018	0.691	**0.837**	0.291	-0.073	-0.073
p value:	n.a.	n.a.	0.179	0.552	n.a.	0.648	0.698	0.951	0.579	0.368	0.991	0.897	0.897	0.086	0.979	0.095	**0.027**	0.527	0.897	0.897

Heterologous neutralization (SF162)

Animal	Week 11	21	29	33	37	43	45	47	53	56	58
V1	<8	<8	35	5	64	5	761	383	414	194	279
V2	<8	<8	39	5	38	5	223	88	219	80	449
V3	<8	<8	221	31	336	75	521	349	241	105	343
V4	<8	<8	159	25	414	35	597	366	509	286	1514
V5	<8	<8	37	5	69	139	226	77	404	273	646
V6	<8	<8	151	5	71	39	1607	2246	422	204	493
V7	<8	<8	85	5	94	5	477	381	517	189	449
Mean:	**<8**	**<8**	**104**	**12**	**155**	**43**	**630**	**556**	**389**	**190**	**596**
Correlation with week of infection:	n.a.	n.a.	0.055	0.181	0.255	-0.585	0.309	0.509	0.418	-0.273	-0.642
p value:	n.a.	n.a.	0.922	0.738	0.579	0.174	0.498	0.254	0.346	0.552	0.123

Heterologous neutralization (BaL)

Animal	Week 13	21	29	33	37	45	49	53	56	58
V1	<8	<8	33	<8	<8	106	170	43	35	52
V2	<8	<8	52	<8	<8	43	168	47	35	72
V3	<8	<8	123	41	22	76	144	33	58	49
V4	<8	<8	63	<8	118	117	136	92	148	105
V5	<8	<8	40	<8	<8	5	57	49	66	81
V6	<8	<8	56	50	<8	133	106	48	41	72
V7	<8	<8	34	34	<8	60	95	74	45	63
Mean:	**<8**	**<8**	**57**	**42**	**70**	**77**	**125**	**55**	**61**	**71**
Correlation with week of infection:	n.a.	n.a.	-0.291	0.120	0.136	0.273	0.400	-0.164	-0.220	-0.661
p value:	n.a.	n.a.	0.527	0.800	0.810	0.552	0.368	0.724	0.625	0.113

Heterologous neutralization (JR-FL)

Animal	Week 11	13	19	27	37	43	45	47	49	56	58
V1	<8	<8	<8	<8	<8	<8	<8	<8	55	26	44
V2	<8	<8	<8	<8	<8	<8	26	23	42	38	40
V3	<8	<8	<8	<8	<8	<8	50	44	29	80	52
V4	<8	<8	<8	<8	<8	<8	33	43	66	80	48
V5	<8	<8	<8	<8	<8	<8	30	34	14	36	48
V6	<8	<8	<8	<8	<8	<8	<8	24	70	35	58
V7	<8	<8	<8	<8	<8	<8	12	33	46	40	32
Mean:	**<8**	**<8**	**<8**	**<8**	**<8**	**<8**	**30**	**34**	**46**	**48**	**46**
Correlation with week of infection:	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	-0.184	-0.109	0.236	0.128	-0.404
p value:	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	0.683	0.824	0.606	0.779	0.364

Heterologous neutralization (AD8)

Animal	Week 11	13	19	27	37	43	47	49	51	53	58
V1	<8	<8	<8	<8	<8	<8	15	53	15	<8	8
V2	<8	<8	<8	<8	<8	<8	16	38	20	8	33
V3	<8	<8	<8	<8	<8	<8	23	33	44	36	44
V4	<8	<8	<8	<8	<8	<8	25	43	20	30	84
V5	<8	<8	<8	<8	<8	<8	24	13	27	22	46
V6	<8	<8	<8	<8	<8	<8	14	78	38	15	47
V7	<8	<8	<8	<8	<8	<8	23	58	40	18	32
Mean:	**<8**	**<8**	**<8**	**<8**	**<8**	**<8**	**20**	**45**	**29**	**22**	**42**
Correlation with week of infection:	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	-0.119	0.418	0.046	-0.109	-0.618
p value:	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	0.798	0.346	0.926	0.824	0.151

Tier-2 heterologous neutralization (week 58)

Animal	AD8 Clade B	JR-FL B	398F1 A	246F3 AC	CNE8 CRF01_AE	CNE55 CRF01_AE	TRO11 B	X2278 B	BJOXO CRF07_BC	CH119 CRF07_BC	25710 C	CE1176 C	CE0217 C	X1632 G	SIVmac251 n.a.
V1	8	44	89	44	35	<8	36	42	10	33	35	31	15	16	<8
V2	33	40	63	20	24	<8	46	38	20	20	31	22	12	9	<8
V3	44	52	114	62	51	<8	75	140	37	37	35	27	20	40	16
V4	84	48	126	56	44	<8	80	44	40	43	61	29	20	12	<8
V5	46	48	44	38	36	<8	32	10	37	30	32	21	18	14	<8
V6	47	58	77	37	44	13	48	80	76	56	46	33	75	42	10
V7	32	32	79	39	41	<8	47	40	18	53	60	28	20	9	<8
Mean:	**42**	**46**	**85**	**42**	**39**	**<8**	**52**	**56**	**34**	**39**	**43**	**27**	**26**	**20**	**<8**
Correlation with week of infection:	-0.618	-0.404	0.655	0.582	0.119	n.a.	0.218	0.346	-0.606	0.309	0.450	0.418	0.057	-0.092	n.a.
p value:	0.151	0.364	0.129	0.181	0.802	n.a.	0.648	0.444	0.161	0.498	0.315	0.346	0.919	0.845	n.a.

Supplementary Table 8a. Virus-specific CD4+ and CD8+ T-cell responses calculated as fraction of total CD4+ and CD8+ T cells.

Animal	Vaccine subgroup	Week of infection
V1	1	>13
V2	1	5
V3	1	9
V4	2	6
V5	2	4
V6	2	5
V7	2	>13

HIV-1 Env responses (% of total CD4 and CD8 T cells)

Week 27	CD4+ T cells						CD8+ T cells				
Animal	CD154+CD69+	CD154+IFN-γ+	CD154+IL-2+	CD154+IL-4+	CD154+IL-21+	CD154+TNF-α+	CD69+IFN-γ+	CD69+IL-2+	CD69+TNF-α+	CD69+CD107A+	IFN-γ, IL-2 or TNF-α
V1	0.103000	0.055430	0.051290	0.021580	0.036000	0.028430	0.019000	0.001270	0.003570	0.001350	0.018000
V2	0.245000	0.132350	0.058000	0.019000	0.099150	0.012000	0.010000	0.000000	0.002160	0.070000	0.008000
V3	0.194000	0.110000	0.085930	0.029380	0.072491	0.034870	0.038000	0.000000	0.003700	0.090000	0.036000
V4	0.099000	0.060000	0.023000	0.016420	0.030320	0.021000	0.021000	0.004437	0.021000	0.020000	0.029000
V5	0.258000	0.177360	0.040760	0.022400	0.067340	0.028760	0.009050	0.000000	0.005000	0.017000	0.008000
V6	0.074470	0.041000	0.013000	0.009254	0.025000	0.015000	0.020000	0.002760	0.007150	0.024100	0.023000
V7	0.258490	0.177730	0.075243	0.072000	0.081000	0.048243	0.023940	0.006990	0.016940	0.012000	0.025890
Mean:	**0.175994**	**0.107696**	**0.049603**	**0.027148**	**0.058757**	**0.026900**	**0.020141**	**0.002208**	**0.008503**	**0.033493**	**0.021127**
Correlation with week of infection:	0.073	-0.018	0.509	0.455	0.127	0.509	0.637	0.491	0.073	-0.400	0.523
p value:	0.897	0.979	0.254	0.303	0.786	0.254	0.140	0.250	0.897	0.368	0.229

Week 60	CD4+ T cells						CD8+ T cells				
Animal	CD154+CD69+	CD154+IFN-γ+	CD154+IL-2+	CD154+IL-4+	CD154+IL-21+	CD154+TNF-α+	CD69+IFN-γ+	CD69+IL-2+	CD69+TNF-α+	CD69+CD107A+	IFN-γ, IL-2 or TNF-α
V1	0.154000	0.067032	0.065000	0.149032	0.082032	0.045032	0.055000	0.014000	0.026000	0.021000	0.057000
V2	0.465000	0.304030	0.234030	0.284690	0.366020	0.100050	0.080000	0.006077	0.017000	0.070000	0.082000
V3	0.281000	0.160000	0.087340	0.125280	0.199056	0.058280	0.013000	0.009420	0.002000	0.019000	0.013000
V4	0.587000	0.493400	0.130000	0.149000	0.416230	0.127000	0.003000	0.000021	0.003000	0.025000	0.005000
V5	0.868000	0.747690	0.298460	0.358460	0.679230	0.337690	0.051000	0.003600	0.044000	0.049000	0.064000
V6	0.308630	0.168630	0.066317	0.094000	0.229317	0.062590	0.003000	0.000970	0.000000	0.037000	0.007000
V7	0.449219	0.380000	0.159219	0.170000	0.370000	0.189219	0.040000	0.003580	0.015000	0.013000	0.040000
Mean:	**0.444693**	**0.331540**	**0.148624**	**0.190066**	**0.334555**	**0.131409**	**0.035000**	**0.005381**	**0.015286**	**0.033429**	**0.038286**
Correlation with week of infection:	-0.673	-0.527	-0.546	-0.255	-0.527	-0.436	0.009	0.309	-0.109	**-0.873**	-0.218
p value:	0.108	0.225	0.210	0.579	0.225	0.324	0.989	0.498	0.824	**0.019**	0.648

SIV Gag responses (% of total CD4 and CD8 T cells)

Week 27	CD4+ T cells						CD8+ T cells				
Animal	CD154+CD69+	CD154+IFN-γ+	CD154+IL-2+	CD154+IL-4+	CD154+IL-21+	CD154+TNF-α+	CD69+IFN-γ+	CD69+IL-2+	CD69+TNF-α+	CD69+CD107A+	IFN-γ, IL-2 or TNF-α
V1	0.001000	0.001600	0.001070	0.000000	0.001390	0.002980	0.015000	0.000000	0.004570	0.000000	0.017000
V2	0.009000	0.000740	0.000000	0.000000	0.000000	0.000000	0.003000	0.001930	0.008160	0.010000	0.003000
V3	0.008000	0.002480	0.003880	0.000340	0.000000	0.002810	0.000000	0.000000	0.000000	0.000000	0.000000
V4	0.002000	0.001000	0.000000	0.000000	0.000000	0.000000	0.004000	0.000467	0.012000	0.003000	0.014000
V5	0.011000	0.003810	0.001760	0.000000	0.001280	0.000000	0.006050	0.000860	0.004000	0.003000	0.007000
V6	0.008470	0.003280	0.001970	0.000000	0.003930	0.000655	0.003000	0.000684	0.001350	0.002100	0.003000
V7	0.025490	0.014730	0.004763	0.003070	0.004910	0.008443	0.002870	0.001640	0.004520	0.011000	0.005760
Mean:	**0.009280**	**0.003949**	**0.001920**	**0.000487**	**0.001644**	**0.002127**	**0.004846**	**0.000797**	**0.004943**	**0.004157**	**0.007109**
Correlation with week of infection:	-0.309	0.073	0.340	0.590	0.302	**0.849**	-0.184	-0.413	0.091	-0.157	0.220
p value:	0.498	0.897	0.445	0.191	0.495	**0.031**	0.682	0.344	0.851	0.739	0.633

Week 60	CD4+ T cells						CD8+ T cells				
Animal	CD154+CD69+	CD154+IFN-γ+	CD154+IL-2+	CD154+IL-4+	CD154+IL-21+	CD154+TNF-α+	CD69+IFN-γ+	CD69+IL-2+	CD69+TNF-α+	CD69+CD107A+	IFN-γ, IL-2 or TNF-α
V1	0.000000	0.002752	0.003720	0.004612	0.000000	0.003682	0.002000	0.002140	0.005000	0.000000	0.008000
V2	0.004000	0.000000	0.002420	0.000980	0.000740	0.002050	0.000000	0.000000	0.000000	0.000000	0.000000
V3	0.000000	0.000000	0.000000	0.000000	0.000000	0.000240	0.000000	0.000000	0.000000	0.000000	0.000000
V4	0.000000	0.000000	0.000000	0.000000	0.000630	0.000000	0.003000	0.000000	0.000000	0.008000	0.000000
V5	0.000000	0.000000	0.001990	0.002700	0.001350	0.000510	0.000000	0.000680	0.015000	0.012000	0.011000
V6	0.001660	0.000450	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.004000	0.014000	0.000000
V7	0.002589	0.002020	0.000569	0.001350	0.001350	0.001239	0.000000	0.000000	0.000000	0.000000	0.000000
Mean:	**0.001178**	**0.000746**	**0.001243**	**0.001377**	**0.000581**	**0.001103**	**0.000714**	**0.000403**	**0.003429**	**0.004857**	**0.002714**
Correlation with week of infection:	-0.070	0.652	0.094	0.189	-0.257	0.358	0.340	0.045	-0.341	-0.692	-0.204
p value:	0.876	0.133	0.857	0.686	0.548	0.417	0.452	0.952	0.457	0.105	0.714

Supplementary Table 8b. Virus-specific CD4+ and CD8+ T-cell responses calculated as fraction of memory CD4+ and CD8+ T cells.

Animal	Vaccine subgroup	Week of infection
V1	1	>13
V2	1	5
V3	1	9
V4	2	6
V5	2	4
V6	2	5
V7	2	>13

HIV-1 Env responses (% of memory CD4 and CD8 T cells)

Week 27	CD4+ T cells						CD8+ T cells				
Animal	CD154+CD69+	CD154+IFN-γ+	CD154+IL-2+	CD154+IL-4+	CD154+IL-21+	CD154+TNF-α+	CD69+IFN-γ+	CD69+IL-2+	CD69+TNF-α+	CD69+CD107A+	IFN-γ, IL-2 or TNF-α
V1	0.175000	0.095610	0.089070	0.038000	0.061000	0.048610	0.024000	0.001620	0.004460	0.001000	0.023000
V2	0.508000	0.274000	0.126000	0.041000	0.208250	0.024000	0.015000	0.000000	0.004000	0.110000	0.010000
V3	0.435000	0.260000	0.190870	0.064000	0.158860	0.078000	0.057000	0.000000	0.006000	0.140000	0.061000
V4	0.220000	0.131000	0.050000	0.035000	0.067330	0.047000	0.030000	0.006327	0.030000	0.028000	0.042000
V5	0.488000	0.335010	0.076000	0.043000	0.128750	0.055000	0.011220	0.000000	0.008000	0.021000	0.011000
V6	0.114400	0.065000	0.021000	0.014880	0.039000	0.023000	0.023000	0.003040	0.008450	0.026000	0.026000
V7	0.407650	0.276470	0.118820	0.110000	0.120000	0.073820	0.032230	0.009320	0.023230	0.017000	0.034470
Mean:	**0.335436**	**0.205299**	**0.095966**	**0.049411**	**0.111884**	**0.049919**	**0.027493**	**0.002901**	**0.012020**	**0.049000**	**0.029639**
Correlation with week of infection:	-0.364	-0.218	0.364	0.309	-0.218	0.455	0.782	0.491	0.073	-0.346	0.491
p value:	0.422	0.648	0.422	0.498	0.648	0.303	0.048	0.250	0.897	0.444	0.270

Week 60	CD4+ T cells						CD8+ T cells				
Animal	CD154+CD69+	CD154+IFN-γ+	CD154+IL-2+	CD154+IL-4+	CD154+IL-21+	CD154+TNF-α+	CD69+IFN-γ+	CD69+IL-2+	CD69+TNF-α+	CD69+CD107A+	IFN-γ, IL-2 or TNF-α
V1	0.232000	0.098580	0.095000	0.218580	0.118580	0.065580	0.063000	0.016000	0.030000	0.024000	0.071000
V2	1.132000	0.755000	0.575000	0.707000	0.900140	0.245000	0.140000	0.010480	0.028000	0.120000	0.136000
V3	0.590000	0.340000	0.188000	0.260030	0.418010	0.130030	0.018000	0.013000	0.003000	0.026000	0.018000
V4	1.174000	0.987000	0.269000	0.307000	0.822440	0.254000	0.005000	0.000024	0.005000	0.034000	0.006000
V5	1.628000	1.395660	0.557110	0.667110	1.278550	0.625660	0.062000	0.004360	0.054000	0.060000	0.082000
V6	0.497760	0.277760	0.108880	0.150000	0.378880	0.104390	0.003000	0.001140	0.000000	0.042000	0.006000
V7	0.898530	0.760000	0.328530	0.340000	0.740000	0.388530	0.061000	0.005440	0.023000	0.018000	0.061000
Mean:	**0.878899**	**0.659143**	**0.303074**	**0.378531**	**0.665229**	**0.259027**	**0.050286**	**0.007206**	**0.020429**	**0.046286**	**0.054286**
Correlation with week of infection:	-0.582	-0.436	-0.491	-0.346	-0.637	-0.346	0.036	0.509	-0.109	**-0.927**	-0.193
p value:	0.181	0.324	0.270	0.444	0.140	0.444	0.951	0.254	0.824	**0.008**	0.665

SIV Gag responses (% of memory CD4 and CD8 T cells)

Week 27	CD4+ T cells						CD8+ T cells				
Animal	CD154+CD69+	CD154+IFN-γ+	CD154+IL-2+	CD154+IL-4+	CD154+IL-21+	CD154+TNF-α+	CD69+IFN-γ+	CD69+IL-2+	CD69+TNF-α+	CD69+CD107A+	IFN-γ, IL-2 or TNF-α
V1	0.004000	0.002800	0.001860	0.000000	0.002400	0.005190	0.019000	0.000000	0.006460	0.000000	0.021000
V2	0.020000	0.002000	0.001000	0.000000	0.000000	0.000000	0.004000	0.002850	0.012000	0.020000	0.003000
V3	0.006000	0.003000	0.000000	0.000000	0.000000	0.000000	0.007000	0.000687	0.017000	0.005000	0.021000
V4	0.019000	0.005600	0.008870	0.001000	0.000000	0.006000	0.000000	0.000000	0.000000	0.000000	0.000000
V5	0.020000	0.007010	0.004000	0.000000	0.002410	0.000000	0.007220	0.001070	0.006000	0.004000	0.009000
V6	0.014400	0.005140	0.003080	0.000000	0.006160	0.001030	0.004000	0.000752	0.001470	0.002000	0.003000
V7	0.039650	0.023470	0.007460	0.004800	0.007680	0.012820	0.003920	0.002230	0.006160	0.016000	0.007470
Mean:	**0.017579**	**0.007003**	**0.003753**	**0.000829**	**0.002664**	**0.003577**	**0.006449**	**0.001084**	**0.007013**	**0.006714**	**0.009210**
Correlation with week of infection:	-0.266	0.000	-0.018	0.454	0.094	0.642	-0.037	-0.358	0.309	-0.119	0.380
p value:	0.562	>0.999	0.979	0.357	0.857	0.133	0.948	0.417	0.498	0.801	0.379

Week 60	CD4+ T cells						CD8+ T cells				
Animal	CD154+CD69+	CD154+IFN-γ+	CD154+IL-2+	CD154+IL-4+	CD154+IL-21+	CD154+TNF-α+	CD69+IFN-γ+	CD69+IL-2+	CD69+TNF-α+	CD69+CD107A+	IFN-γ, IL-2 or TNF-α
V1	0.000000	0.004020	0.005440	0.006740	0.000000	0.005380	0.002000	0.002530	0.005000	0.000000	0.009000
V2	0.009000	0.000000	0.006000	0.003000	0.002140	0.004000	0.000000	0.000000	0.000000	0.000000	0.000000
V3	0.000000	0.000000	0.000000	0.000000	0.001410	0.000000	0.004000	0.000000	0.000000	0.012000	0.000000
V4	0.001000	0.000000	0.000000	0.000000	0.000000	0.001030	0.000000	0.000000	0.000000	0.000000	0.000000
V5	0.000000	0.000000	0.003780	0.005110	0.002550	0.001000	0.000000	0.000830	0.019000	0.014000	0.013000
V6	0.002700	0.000720	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.004000	0.015000	0.000000
V7	0.005070	0.003920	0.001140	0.002610	0.002610	0.002450	0.000000	0.000000	0.000000	0.000000	0.000000
Mean:	**0.002539**	**0.001237**	**0.002337**	**0.002494**	**0.001244**	**0.001980**	**0.000857**	**0.000480**	**0.004000**	**0.005857**	**0.003143**
Correlation with week of infection:	-0.075	0.652	-0.057	0.038	-0.075	0.413	0.522	0.045	-0.341	-0.592	-0.204
p value:	0.900	0.133	0.917	0.933	0.900	0.344	0.262	0.952	0.457	0.162	0.714

Supplementary Table 8c. Polyfunctionality of anti-Env CD4+ T-cell responses calculated as fraction of memory CD4+ T cells.

Animal	Vaccine subgroup	Week of infection
V1	1	>13
V2	1	5
V3	1	9
V4	2	6
V5	2	4
V6	2	5
V7	2	>13

Polyfunctional responses at week 27

Animal	G+2+4+ 21+T+	G+2+4+ 21+T-	G+2+4+ 21-T+	G+2+4+ 21-T-	G+2+4- 21+T+	G+2+4- 21+T-	G+2+4- 21-T+	G+2+4- 21-T-	G+2-4+ 21+T+	G+2-4+ 21+T-	G+2-4+ 21-T+	G+2-4+ 21-T-	G+2-4- 21+T+	G+2-4- 21+T-	G+2-4- 21-T+	G+2-4- 21-T-	G-2+4+ 21+T+	G-2+4+ 21+T-	G-2+4+ 21-T+	G-2+4+ 21-T-	G-2+4- 21+T+	G-2+4- 21+T-	G-2+4- 21-T+	G-2+4- 21-T-	G-2- 4+21+T+	G-2-4+ 21+T-	G-2-4+ 21-T+	G-2-4+ 21-T-	G-2-4- 21+T+	G-2-4- 21+T-	G-2-4- 21-T+
V1	0.00105	0.00421	0.00316	0.00211	0.00948	0.015	0.00316	0.016	0	0.00316	0.00211	0.00632	0.00316	0.00105	0.00843	0.01761	0.00105	0.00527	0	0.00527	0.00316	0.00316	0.00737	0.012	0.00105	0.00211	0	0.003	0	0.00843	0.00697
V2	0.00334	0.00334	0	0.00502	0.00669	0.043	0	0.017	0	0.01	0	0.00334	0.00334	0.06525	0	0.11476	0.00167	0.00334	0.003	0.00494	0	0.023	0.00145	0.01	0	0.00502	0	0.00302	0.00167	0.04	0.01151
V3	0.00331	0.00551	0	0.00992	0.02	0.05	0.00551	0.021	0	0.00441	0.0022	0.00331	0.0022	0.039	0.00882	0.082	0.0011	0.00661	0.01472	0.00544	0.00441	0.011	0.00944	0.023	0	0.0011	0.00209	0.00515	0	0.015	0.00536
V4	0	0.00534	0.00528	0.00172	0.00712	0.012	0	0.01616	0	0.00178	0.00178	0.00356	0.00534	0.021	0.01181	0.04049	0	0	0	0.0035	0	0.0035	0.00365	0	0.00178	0.00356	0.0035	0.00524	0.00178	0.0035	0.01081
V5	0	0.00498	0.00249	0.00125	0.00623	0.027	0.00623	0.019	0.00249	0.011	0.00373	0.00498	0.00623	0.047	0.015	0.17875	0	0	0	0.00498	0	0.00125	0	0.00373	0	0.00249	0	0.00747	0	0.01775	0.01575
V6	0	0.00315	0	0	0.00105	0.00734	0	0.0021	0	0.0021	0	0.0021	0.00105	0.00629	0.00524	0.035	0	0	0	0.00105	0	0.0021	0.00105	0.00315	0	0.00419	0	0.00203	0.00105	0.01	0.014
V7	0.003	0.006	0.00899	0.00899	0.007	0.022	0.00582	0.02	0.002	0.018	0.007	0.017	0.006	0.029	0.012	0.09765	0	0.002	0.002	0.00999	0	0.004	0.004	0.012	0	0.00899	0	0.017	0	0.017	0.016
Mean:	**0.001529**	**0.004647**	**0.002846**	**0.004144**	**0.008224**	**0.025191**	**0.002960**	**0.015894**	**0.000641**	**0.007207**	**0.002403**	**0.005801**	**0.003903**	**0.029799**	**0.008757**	**0.080894**	**0.000546**	**0.002460**	**0.002817**	**0.005024**	**0.001081**	**0.006859**	**0.003851**	**0.009126**	**0.000404**	**0.003923**	**0.000799**	**0.006130**	**0.000643**	**0.015954**	**0.011486**
Correlation with week of infection:	0.340	0.527	0.528	0.618	0.727	-0.036	0.113	0.164	-0.204	0.018	0.367	0.491	-0.146	-0.509	0.036	-0.509	0.120	0.585	0.221	0.655	0.522	0.346	0.873	0.606	0.340	-0.073	0.136	0.091	-0.361	-0.418	-0.291
p value:	0.448	0.225	0.224	0.151	0.071	0.951	0.817	0.724	0.714	0.979	0.404	0.270	0.749	0.254	0.951	0.254	0.800	0.174	0.638	0.129	0.262	0.444	0.019	0.159	0.452	0.897	0.810	0.851	0.419	0.346	0.527

Polyfunctional responses at week 60

Animal	G+2+4+ 21+T+	G+2+4+ 21+T-	G+2+4+ 21-T+	G+2+4+ 21-T-	G+2+4- 21+T+	G+2+4- 21+T-	G+2+4- 21-T+	G+2+4- 21-T-	G+2-4+ 21+T+	G+2-4+ 21+T-	G+2-4+ 21-T+	G+2-4+ 21-T-	G+2-4- 21+T+	G+2-4- 21+T-	G+2-4- 21-T+	G+2-4- 21-T-	G-2+4+ 21+T+	G-2+4+ 21+T-	G-2+4+ 21-T+	G-2+4+ 21-T-	G-2+4- 21+T+	G-2+4- 21+T-	G-2+4- 21-T+	G-2+4- 21-T-	G-2- 4+21+T+	G-2-4+ 21+T-	G-2-4+ 21-T+	G-2-4+ 21-T-	G-2-4- 21+T+	G-2-4- 21+T-	G-2-4- 21-T+
V1	0.00935	0.00935	0.00312	0.00623	0.00312	0.00312	0.00468	0.00156	0.00623	0.014	0.00312	0.011	0	0.00623	0.00312	0.01458	0.00779	0.019	0.00468	0.02	0	0	0.00312	0	0.00312	0.028	0.012	0.06258	0.00156	0.00958	0.00014
V2	0.049	0.13	0.00565	0.021	0.042	0.072	0.00424	0.013	0.02	0.065	0	0.052	0.04536	0.12671	0.00936	0.09342	0.018	0.12	0.00942	0.042	0.0099	0.024	0	0.00771	0.00707	0.081	0.00543	0.07771	0.00543	0.07571	0.01607
V3	0.014	0.03	0.00141	0.00563	0.017	0.039	0.00563	0.00704	0.00985	0.035	0.00704	0.00985	0.018	0.069	0.018	0.048	0.00422	0.03	0.00305	0.00505	0.00422	0.015	0.00023	0.00505	0.00282	0.058	0.00282	0.04701	0.00422	0.06601	0.01201
V4	0.012	0.025	0.00398	0.00388	0.056	0.09311	0.00199	0.016	0.025	0.1	0.012	0.047	0.066	0.29	0.02922	0.19811	0.00388	0.014	0	0.00194	0.0097	0.012	0.00204	0.01711	0.00194	0.016	0.00393	0.03355	0.014	0.08333	0.01722
V5	0.092	0.075	0.015	0.00801	0.099	0.1	0.016	0.031	0.092	0.12	0.031	0.047	0.13	0.28855	0.053	0.19711	0.00935	0.036	0.01055	0.00534	0.00935	0.028	0.00523	0.013	0.015	0.057	0.00935	0.04755	0.02	0.1	0.01755
V6	0.00302	0.019	0.00101	0.00101	0.01	0.023	0	0.0009	0.00504	0.033	0.00101	0.00907	0.028	0.097	0.00807	0.04188	0	0.016	0	0.00101	0.00706	0.016	0.00202	0.00706	0.00605	0.03	0	0.028	0.01388	0.068	0.01651
V7	0.033	0.033	0.00736	0.00491	0.068	0.063	0.014	0.014	0.043	0.054	0.012	0.023	0.11	0.17	0.022	0.096	0.00982	0.02	0.00368	0.011	0.017	0.015	0.00368	0.01353	0.00859	0.028	0.00491	0.047	0.014	0.058	0.02053
Mean:	**0.030339**	**0.045907**	**0.005361**	**0.007239**	**0.042160**	**0.056176**	**0.006649**	**0.011929**	**0.028731**	**0.060143**	**0.009453**	**0.028417**	**0.056766**	**0.149641**	**0.020396**	**0.098443**	**0.007580**	**0.036429**	**0.004483**	**0.012334**	**0.008176**	**0.015714**	**0.002331**	**0.009066**	**0.006370**	**0.042571**	**0.005491**	**0.049057**	**0.010441**	**0.065804**	**0.014290**
Correlation with week of infection:	-0.364	-0.455	-0.200	-0.218	-0.346	-0.582	0.109	-0.291	-0.200	-0.582	-0.009	-0.330	-0.436	-0.418	-0.346	-0.327	0.036	-0.327	-0.248	0.236	-0.127	-0.835	0.091	-0.218	-0.364	-0.477	0.091	-0.018	-0.486	-0.873	-0.236
p value:	0.422	0.303	0.673	0.648	0.444	0.181	0.824	0.527	0.673	0.181	0.985	0.462	0.324	0.346	0.444	0.476	0.951	0.476	0.595	0.606	0.786	**0.029**	0.851	0.648	0.422	0.275	0.851	0.979	0.271	**0.019**	0.606

Legend: G: IFN-γ; 2: IL-2; 4: IL-4; 21: IL-21; T: TNF-α

Supplementary Table 8d. Bulk T-follicular helper (TFH) CD4+ T cells in the circulation of vaccinated macaques.

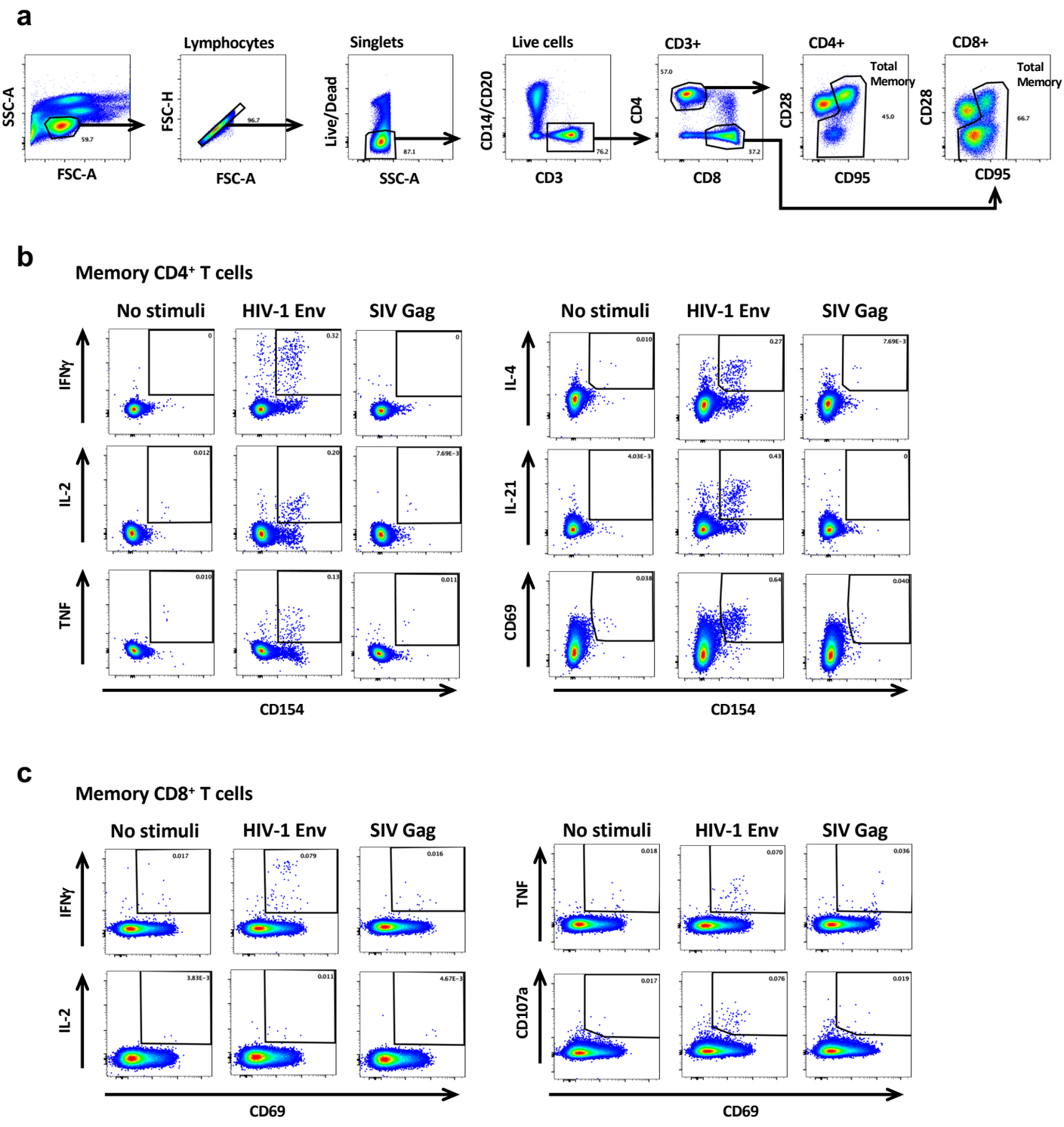
Week 27	% of CD4+						% of memory						% of CXCR5+					
Animal	CXCR5+	CXCR5+ PD1+	CXCR5+ CXCR3+ PD1+	CXCR5+ CXCR3- PD1+	CXCR5 +CXCR3 +PD1-	CXCR5 +CXCR3- PD1-	CXCR5+	CXCR5+ PD1+	CXCR5+ CXCR3+ PD1+	CXCR5+ CXCR3- PD1+	CXCR5 +CXCR3 +PD1-	CXCR5 +CXCR3- PD1-	PD1+	CXCR3+	CXCR3+ PD1+	CXCR3- PD1+	CXCR3 +PD1-	CXCR3- PD1-
V1	17.0	4.3	2.3	2.0	3.8	8.4	29.0	7.4	4.0	3.5	6.5	14.3	25.6	35.9	13.6	12.0	22.3	49.2
V2	16.0	4.8	3.1	1.8	4.7	5.9	33.5	10.1	6.5	3.8	9.9	12.4	30.6	48.8	19.3	11.3	29.5	37.1
V3	12.1	3.3	2.0	1.4	2.7	5.6	28.5	7.8	4.7	3.4	6.3	13.2	28.3	38.5	16.4	11.9	22.1	46.4
V4	11.0	2.5	1.4	1.3	2.6	5.5	23.8	5.5	3.1	2.7	5.5	11.9	24.2	36.1	12.8	11.4	23.3	49.8
V5	18.2	5.1	2.8	2.3	4.7	7.7	36.1	10.2	5.6	4.6	9.4	15.3	28.4	41.7	15.6	12.8	26.1	42.3
V6	23.1	4.4	2.6	2.0	6.0	12.0	36.6	7.0	4.1	3.1	9.5	19.0	19.7	37.1	11.1	8.6	26.0	52.0
V7	17.3	3.2	2.0	1.3	4.0	9.6	26.8	5.0	3.1	2.0	6.2	14.8	19.1	34.8	11.6	7.5	23.2	55.4
Mean:	**16.4**	**4.0**	**2.3**	**1.7**	**4.1**	**7.8**	**30.6**	**7.6**	**4.4**	**3.3**	**7.6**	**14.4**	**25.1**	**39.0**	**14.3**	**10.8**	**24.6**	**47.5**
Correlation with week of infection:	0.0104	0.1235	0.1444	0.1314	0.1190	0.0273	0.3072	0.2971	0.2905	0.2873	0.4292	0.0090	0.1323	0.3364	0.1182	0.0953	0.4600	0.3247
p value:	0.8277	0.4396	0.4004	0.4242	0.4486	0.7234	0.1967	0.2058	0.2119	0.2149	0.1102	0.8395	0.4225	0.1723	0.4502	0.5005	0.0940	0.1817

Week 60	% of CD4+						% of memory						% of CXCR5+					
Animal	CXCR5+	CXCR5+ PD1+	CXCR5+ CXCR3+ PD1+	CXCR5+ CXCR3- PD1+	CXCR5 +CXCR3 +PD1-	CXCR5 +CXCR3- PD1-	CXCR5+	CXCR5+ PD1+	CXCR5+ CXCR3+ PD1+	CXCR5+ CXCR3- PD1+	CXCR5 +CXCR3 +PD1-	CXCR5 +CXCR3- PD1-	PD1+	CXCR3+	CXCR3+ PD1+	CXCR3- PD1+	CXCR3 +PD1-	CXCR3- PD1-
V1	14.2	4.3	1.6	2.3	2.9	7.0	21.1	6.3	2.3	3.4	4.4	10.4	27.1	31.8	11.1	16.0	20.7	49.2
V2	13.4	3.8	2.3	1.2	4.2	5.4	32.8	9.4	5.6	3.0	10.2	13.1	26.1	48.2	17.1	9.0	31.1	40.0
V3	12.6	3.3	1.5	1.5	2.7	6.5	26.4	7.0	3.2	3.1	5.7	13.6	24.0	33.8	12.1	11.9	21.7	51.5
V4	10.6	2.7	1.3	1.2	2.5	5.4	21.4	5.4	2.6	2.3	5.0	10.9	23.1	35.7	12.2	10.9	23.5	50.9
V5	17.6	4.2	2.1	1.6	4.8	8.6	32.8	7.7	3.9	3.0	8.9	16.1	21.2	38.9	11.9	9.3	27.0	49.0
V6	25.7	4.8	2.5	1.8	6.3	14.5	33.5	6.3	3.3	2.4	8.2	19.0	16.9	34.2	9.8	7.1	24.4	56.6
V7	16.0	2.6	1.4	1.0	3.8	9.5	29.8	4.9	2.6	1.8	7.0	17.7	14.7	32.3	8.7	5.9	23.6	59.6
Mean:	**15.7**	**3.7**	**1.8**	**1.5**	**3.9**	**8.1**	**28.3**	**6.7**	**3.4**	**2.7**	**7.1**	**14.4**	**21.9**	**36.4**	**11.9**	**10.0**	**24.6**	**51.0**
Correlation with week of infection:	0.0584	0.1045	0.3922	0.0304	0.2269	0.0083	0.2511	0.2979	0.3947	0.0070	0.3709	0.0298	0.0035	0.3923	0.2406	0.0907	0.4189	0.1667
p value:	0.6017	0.4794	0.1324	0.7083	0.2799	0.8459	0.2520	0.2050	0.1308	0.8580	0.1467	0.7115	0.8993	0.1324	0.2637	0.5115	0.1161	0.3632

Supplementary Table 8e. Env-specific T-follicular helper (TFH) CD4+ T cells in vaccinated macaques.

Week 27	% of Env specific							% of Env-specific CXCR5+					
Animal	CXCR5+	CXCR5+ PD1+	CXCR5+ CXCR3+	CXCR5+ CXCR3+ PD1+	CXCR5+ CXCR3- PD1+	CXCR5+ CXCR3+ PD1-	CXCR5+ CXCR3- PD1-	PD1+	CXCR3+	CXCR3+ PD1+	CXCR3- PD1+	CXCR3+ PD1-	CXCR3- PD1-
V1	21.6	17.4	18.8	15.0	2.4	3.8	0.5	80.5	87.0	69.6	10.9	17.4	2.2
V2	31.1	21.3	26.9	22.6	0.9	4.3	2.7	75.4	86.2	72.5	2.9	13.7	8.8
V3	16.9	9.4	12.3	7.5	1.9	4.8	1.9	55.7	72.9	44.3	11.4	28.6	11.4
V4	13.9	10.4	12.1	10.4	0.6	1.7	1.2	79.2	87.5	75.0	4.2	12.5	8.3
V5	26.4	15.2	20.0	14.5	2.2	5.5	3.7	63.2	75.5	54.7	8.5	20.8	14.2
V6	16.2	10.3	8.5	7.7	3.4	0.9	3.4	68.5	52.7	47.4	21.1	5.3	21.1
V7	6.4	2.4	4.6	2.4	0.0	2.2	0.5	38.5	73.1	38.5	0.0	34.6	7.7
Mean:	**18.9**	**12.4**	**14.8**	**11.4**	**1.6**	**3.3**	**2.0**	**65.9**	**76.4**	**57.4**	**8.4**	**19.0**	**10.5**
Correlation with week of infection:	0.2663	0.1441	0.1365	0.1671	0.0729	0.0062	0.7528	0.1293	0.0395	0.0590	0.0648	0.3682	0.4514
p value:	0.2358	0.4010	0.4146	0.3626	0.5581	0.8664	**0.0114**	0.4283	0.6692	0.5996	0.5818	0.1485	0.0983

Week 60	% of Env specific							% of Env-specific CXCR5+					
Animal	CXCR5+	CXCR5+ PD1+	CXCR5+ CXCR3+	CXCR5+ CXCR3+ PD1+	CXCR5+ CXCR3- PD1+	CXCR5+ CXCR3+ PD1-	CXCR5+ CXCR3- PD1-	PD1+	CXCR3+	CXCR3+ PD1+	CXCR3- PD1+	CXCR3+ PD1-	CXCR3- PD1-
V1	6.4	4.7	4.1	3.5	1.2	0.6	1.2	72.7	63.6	54.5	18.2	9.1	18.2
V2	11.8	9.0	10.2	8.2	0.5	2.0	0.6	73.5	86.7	69.4	4.1	17.3	5.1
V3	10.9	7.1	6.4	4.7	2.2	1.8	2.0	63.3	59.2	42.9	20.4	16.3	18.4
V4	5.8	4.7	4.4	3.6	1.1	0.8	0.3	81.1	75.7	62.2	18.9	13.5	5.4
V5	7.7	6.4	6.4	5.5	0.7	0.9	0.4	81.1	83.2	71.6	9.5	11.6	5.3
V6	14.8	13.1	11.1	9.8	3.1	1.4	0.2	86.9	75.0	65.8	21.1	9.2	1.3
V7	9.1	8.0	6.9	6.2	1.6	0.7	0.4	86.6	76.2	68.7	17.9	7.5	4.5
Mean:	**9.5**	**7.6**	**7.1**	**5.9**	**1.5**	**1.2**	**0.7**	**77.9**	**74.2**	**62.2**	**15.7**	**12.1**	**8.3**
Correlation with week of infection:	0.1124	0.1159	0.2285	0.1867	0.0054	0.2314	0.1400	0.0146	0.3285	0.1338	0.1793	0.2542	0.2802
p value:	0.4623	0.4549	0.2779	0.3329	0.8761	0.2744	0.4083	0.7964	0.1786	0.4197	0.3438	0.2486	0.2218



Supplementary Fig. 1. Gating strategy used for flow cytometry analysis of antiviral T-cell responses. **a.** Representative gating strategy to identify memory CD4$^+$ and CD8$^+$ T cells. Memory cells were defined based on CD28 and CD95 expression. **b.** Representative flow plots showing cytokine responses for memory CD4$^+$ T cells following stimulation with HIV-1 Env or SIV Gag peptide pools. **c.** Representative flow plots showing cytokine responses and CD107a expression for memory CD8$^+$ T cells following stimulation with HIV-1 Env or SIV Gag peptide pools.