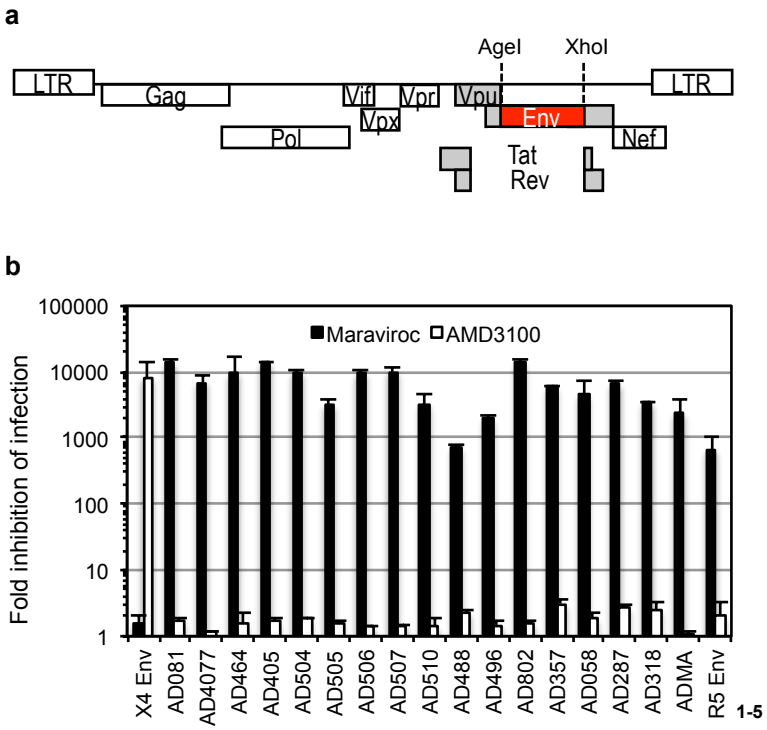

Figure S1. **Viral plasmids and *in vitro* tropism of R5-SHIVs.** (Related to Figure 1)

a. Schematic representation of the full length SHIV plasmid. White boxes represent SIV_{MAC239}-derived sequences, grey $SHIV_{KB9}$-derived sequences and red Group 1 or Group 2 Env-derived sequences. **b.** Fold inhibition of infectivity of SHIVs expressing the Env protein indicated on TZM-bl cells in the presence of Maraviroc (8μM) or AMD3100 (1μM) relative to no inhibitor. Control envelope proteins were from HIV-1_{NL4-3} Env (X4 Env) and HIV-1_{AD8} (R5 Env). The mean fold-inhibition and S.D. of two independent experiments is plotted.

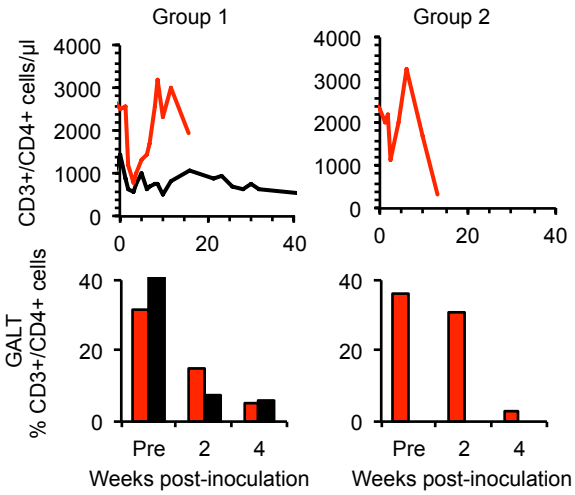

Figure S2. CD4+ T-cell depletion in animals inoculated with R5-SHIV cocktails. (Related to Figure 2)

Absolute numbers of CD4+, CD3+ cells in blood and % of CD3+ cells that were CD4+ in the GALT in animals inoculated with cocktails of Group 1 or Group 2 viruses were determined by flow cytometry. Corresponding viral loads are shown in Fig. 2a,b.

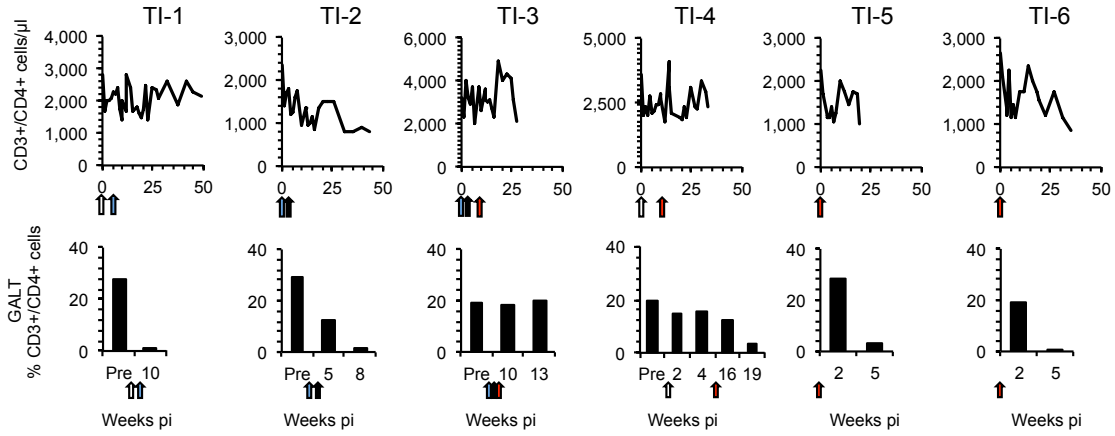

Figure S3. Mucosal challenges with increasing doses of SHIV$_{1054}$. (Related to Figure 3c)

Six animals were inoculated intrarectally with increasing SHIV$_{1054}$ doses. Absolute numbers of CD3/CD4+ cells in blood and percentage of CD3+ cells that were CD4+ in the GALT for each animal are shown. 'Pre' indicates samples obtained three weeks prior to the first virus challenge in each animal. Arrows indicate time and dose of viral challenge: white arrow is $3x10^3$ i.u., blue arrow is $3x10^4$ i.u., black arrow is $7.4x10^4$ i.u. and red arrow is $2.1x10^5$ i.u.. Corresponding viral loads are shown in Fig. 3c.

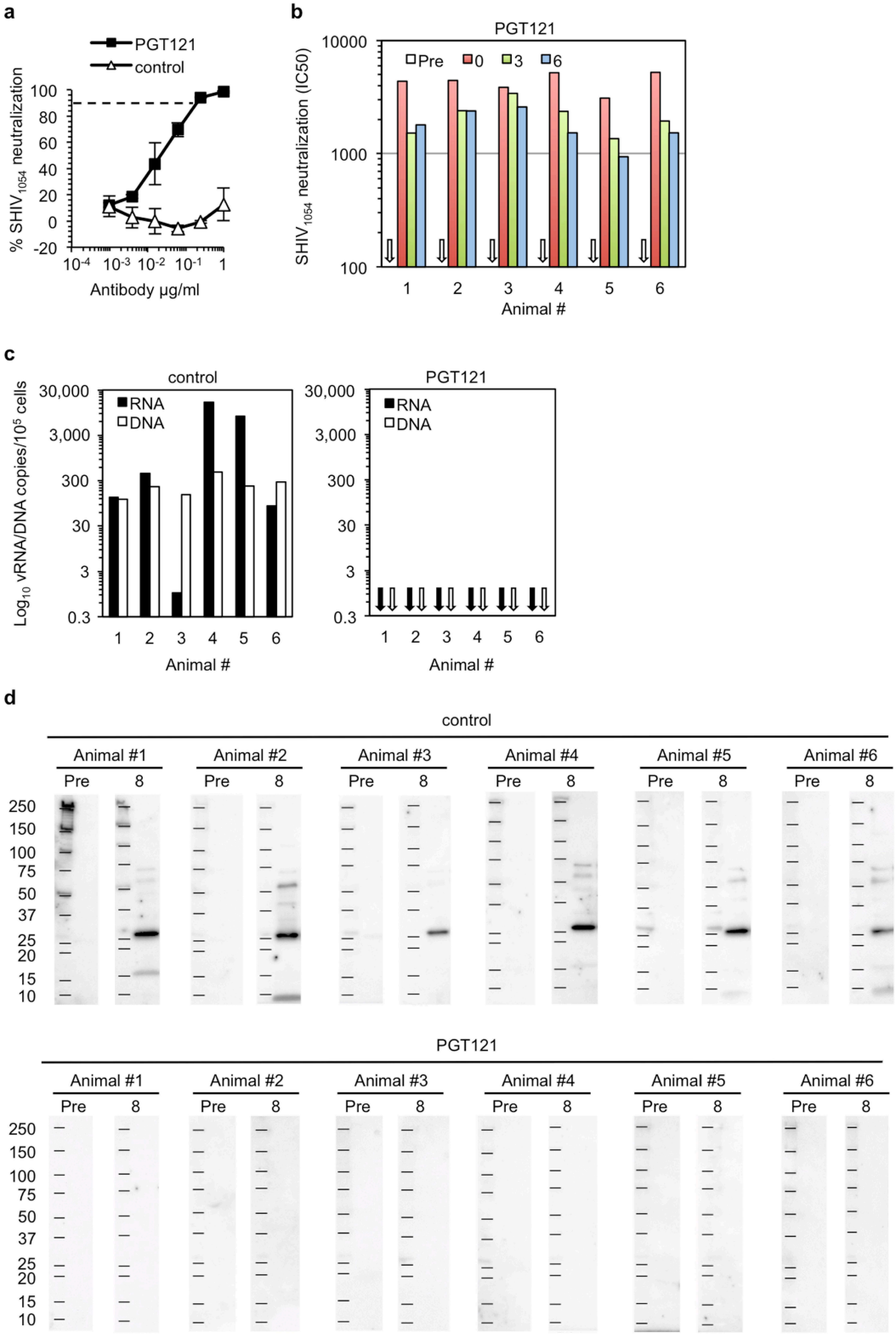
a
PGT121
control
% SHIV1054 neutralization
100
80
60
40
20
0
-20
10-4
10-3
10-2
10-1
1
Antibody µg/ml
b
PGT121
Pre
0
3
6
SHIV1054 neutralization (IC50)
10000
1000
100
1
2
3
4
5
6
Animal #
c
control
PGT121
RNA
DNA
Log10 vRNA/DNA copies/10^5 cells
30,000
3,000
300
30
3
0.3
1
2
3
4
5
6
Animal #
d
control
PGT121
Animal #1
Animal #2
Animal #3
Animal #4
Animal #5
Animal #6
Pre
8
250
150
100
75
50
37
25
20
15
10

Figure S4. HIV-1 monoclonal antibody protects against T/F Env virus challenge. (Related to Figure 4)

a. Neutralization of $SHIV_{1064}$ by anti-HIV-1 Env monoclonal antibody PGT121 and control (DEN3) antibody assayed in TZM-bl cells. Dotted line marks IC90. **b.** Neutralization titers of plasma samples obtained at the indicated days after antibody administration and given as reciprocal dilution at which 50% reduction of virus infectivity was achieved. Day 0 is the day of virus inoculation. Average of two independent experiments. **c**. PBMC cell-associated viral RNA and DNA copies sampled at the time of the plasma viral 'blip', week 3 p.i. for animals #1, 2 and 3 and week 4 p.i. for animals #4, 5 and 6 in both control and PGT-121 groups. **d.** Immunoblots performed using purified $SHIV_{1054}$ virions as the antigen, probed with plasma samples recovered from the indicated animals at 8 weeks prior to and 8 weeks after intrarectal challenge with $SHIV_{1054}$.

Table S1. Group 1 Env sequence origin and Genebank accession numbers. (Related to Figure 1)

Env name	original clone name	Accession #
1054	1054.TC4.1499	EU289185
AD17	AD17_3C_A9	GU331244
SC31	SC31 4E11A	EU576909
BORI2F	BORId9_2F8	EU576282
TT29	TT29P 3A1	EU577190
BORI4D	BORId9_4D7	EU576296
TT31	TT31P_2F10	EU577213
9015	9015.07_A1	EU575795
62615	62615.03_p4	EU575611
1051C	1051.12_C22	EU575148
1051TD	1051.12_TD12	EU575170
1006	1006_11.C3.1601	EU289183
62357	62357_14.D3.4589	EU289189
6244	6244_13.B5.4576	EU289191
63358	63358.p3.4013	EU289192
CH040	700010040.C9.4520	EU289193
9014	9014_01.TB1.4769	EU289195
SC45	SC45_45B	EU289201
PRB926	PRB926_04.A9.4237	EU289197
SC05	SC05.8C11.2344	EU289201
ADMA	ADMAG9b	KJ372191

Table S2. Neutralization of R5-SHIVs by reference plasmas, sCD4 and broadly neutralizing antibodies (TZM-bl system).

(Related to Experimental Procedures)

Neutralization sensitivity of R5-SHIVs given as reciprocal dilution for reference plasmas and sCD4 or antibody concentration (μg/ml) at which 50% reduction of virus infectivity was achieved.

Table S3. Plasma and serum viral loads in antibody-infused animals.

(Related to Figure 4)

	control		PGT121	
animal #	Plasma VL	Serum VL	Plasma VL	Serum VL
1	28,000	59,000	30	<30
2	260,000	200,000	190	<30
3	2,800	24,000	50	<30
4	1,900,000	1,800,000	40	<30
5	810,000	1,000,000	60	<30
6	3,000,000	2,400,000	<30	<30

Viral RNA copies/ml in plasma and serum obtained from animals infused with control (for comparison) or PGT121 antibodies. Samples were obtained from both groups of animals at the time of the 'blips' in PGT121 infused animals, 4 weeks post-inoculation for animals 1,2 and 3 and 3 weeks post-inoculation for animals 4, 5 and 6. 30 RNA copies/ml is the limit of detection for this assay.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Cloning strategy

HIV-1 Env-coding sequences were amplified using degenerate PCR primers that introduced restriction enzyme sites (forward: 5'-TTATGGGGTACCGGTRTGGAARGAAGCAACCACCAC-3' and reverse: 5'-GGACCCCTCGAGGYTGGGARGYGGGTCTGNCCYGAYAATGG-3') directly from

plasmids encoding the 3' half of Group 1 T/F viruses. For Group 2 viruses nested PCR was done using DNA extracted from patient PBMC samples (first round primers: forward: 5'-TAGAGCCCTGGAAGCATCCAGGAAG-3' and reverse: 5'-TTGCTACTTGTGATTGCTCCATGT-3'). The unique 5' restriction site, AgeI, was positioned immediately 3' to the sequence encoding the HIV-1 Env leader peptide and the 3' restriction site, XhoI, was positioned within the coding sequence of the HIV-1 gp41 cytoplasmic tail, proximal to the 5' end of sequences encoding the second exon of Tat and Rev (Figure S1a). Following introduction of the HIV-1 Env sequences into the 3' half SHIV plasmid, a semi-automated procedure was employed to introduce a large number of 3' half SHIV sequences into the plasmid expressing the 5' half SHIV sequences and then screened for infectious clones (outlined in Figure 1a). Specifically, bacterial colonies resulting from the ligation of the HIV-1 Env amplicons into the 3' half SHIV plasmid were pooled, plasmid DNA was extracted, digested and introduced into the plasmid containing the 5' half SHIV genome. Individual colonies resulting from this second ligation/transformation, containing the full-length SHIV genome, were picked and DNA extracted using an epMotion 5057 robot (Eppendorf) and Qiaprep 96 Turbo kit (Qiagen). Subsequently full-length SHIV clones were screened by transfection using PEI (Polyscience, Inc.) in 293T cells seeded in 96-well plates. Infectivity of all full-length viral clones was assayed on indicator TZM-bl cells, obtained from the NIH AIDS Research and Reference Reagent Program, as contributed by John Kappes and Xiaoyun Wu. Following selection of SHIV clones for further study, larger viral stocks for animal inoculations were produced by transfection of full-length SHIV clones in 293T cells.

Animal experiments

At the start of the study, all animals were free of cercopithicine herpesvirus 1, simian immunodeficiency virus (SIV), simian type-D retrovirus, and simian T-lymphotropic virus

type 1. Animals with Mamu alleles B08 and B17 were excluded from these studies. Plasma for viral RNA (vRNA) quantification, sequencing analysis, and immunoblots, and peripheral blood mononuclear cells (PBMCs) for flow cytometry assays were isolated from whole blood collected in EDTA-anticoagulated Vacutainer tubes (BD) at the time points indicated. Plasma was separated from the blood by centrifugation and was frozen at -80 °C in aliquots before analysis for the presence of vRNA or antibodies. PBMCs were isolated by Ficoll-Paque Plus (GE Healthcare) gradient centrifugation; serum was prepared from serum collection tubes.

Single-genome amplification/sequencing of SHIV *env*

A 3.5kb fragment that includes the entire *env* gene was amplified from the inoculum stock and each macaque at peak viremia. A limiting dilution, single-genome amplification PCR approach was used so that only one amplifiable molecule was present in each reaction. Reverse transcription of RNA to single-stranded cDNA was performed using SuperScript III reverse transcriptase according to manufacturer's recommendations (Invitrogen) and a gene specific primer: SIVEnvR1 5'-TGT AAT AAA TCC CTT CCA GTC CCC CC-3'. The *env* gene was then amplified using a 1× PCR buffer consisting of 2 mM $MgCl_2$, 0.2 mM of each deoxynucleoside triphosphate, 0.2 μM of each primer, and 0.025 U/μl Platinum *Taq* polymerase (Invitrogen) in a 20-μl reaction. First round PCR was performed with sense primer SIVEnvF1 5'-CCT CCC CCT CCA GGA CTA GC-3' and antisense primer SIVEnvR1 under the following conditions: 1 cycle of 94°C for 2 min, 35 cycles at 94°C for 15 sec, 55°C for 30 sec, and 72°C for 4 min, followed by a final extension of 72°C for 10 min. Nested PCR was performed with primers SIVEnvF2 5'-TAT AAT AGA CAT GGA GAC ACC CTT GAG GGA GC-3' and SIVEnvR2 5'-ATG AGA CAT RTC TAT TGC CAA TTT GTA-3' under the same conditions used for first-round PCR, but for a total of 45 cycles. Correctly sized amplicons were sequenced

directly using inner PCR primers and 6 additional HIV-1 specific primers using Big Dye Terminator technology (Applied Biosystems). To confirm PCR amplification from a single template, chromatograms were manually examined for multiple peaks, indicative of the presence of amplicons resulting from PCR-generated recombination events, *Taq* polymerase errors or multiple variant templates in a single reaction.

To determine the statistical significance of the outcome from animals infected with group 1 viruses the χ^2 test was used.

The 'winner' Env sequences were derived from samples obtained from (i) subject 1054, a male subject from South Carolina with unknown risk factors, plasma VL 320,000 RNA copies/ml at estimated 2-3 weeks post-infection, and (ii) subject AD081, a male subject from New York, with a history of intramuscular steroid use with shared needles, plasma VL 136,000 RNA copies/ml at estimated 6 weeks post-infection.

Flow cytometry

Absolute cell counts were performed on EDTA-anti-coagulated whole blood described (Del Prete et al., 2012; Hatziioannou et al., 2009; Tabb et al., 2013) using the following surface antigen staining panel: CD45 FITC (DO58-1283), CD3 PE (SP34-2), CD4 APC (L200), CD14 APC-Cy7 (M5E2; BioLegend), CD8α PE-Cy7 (SK1), and CD20 Pacific Blue (2H7; BioLegend). Lymphocyte immunophenotyping was performed on freshly isolated mononuclear cells using the following antibodies: CD4 Pacific Blue (OKT4; BioLegend), CCR5 PE (3A9), CD28 ECD (CD28.2; Beckman Coulter), CD95 PE-Cy5 (DX2), CD8 PE-Cy7 (SK1), CD38 APC (OK10; NIH Nonhuman Primate Resource), CD3 APC-Cy7 (SP34-2), and Ki67 FITC (B56).