

Supplementary Material for:
Therapeutic efficacy of combined active and passive immunization in ART-suppressed,
SHIV-infected rhesus macaques

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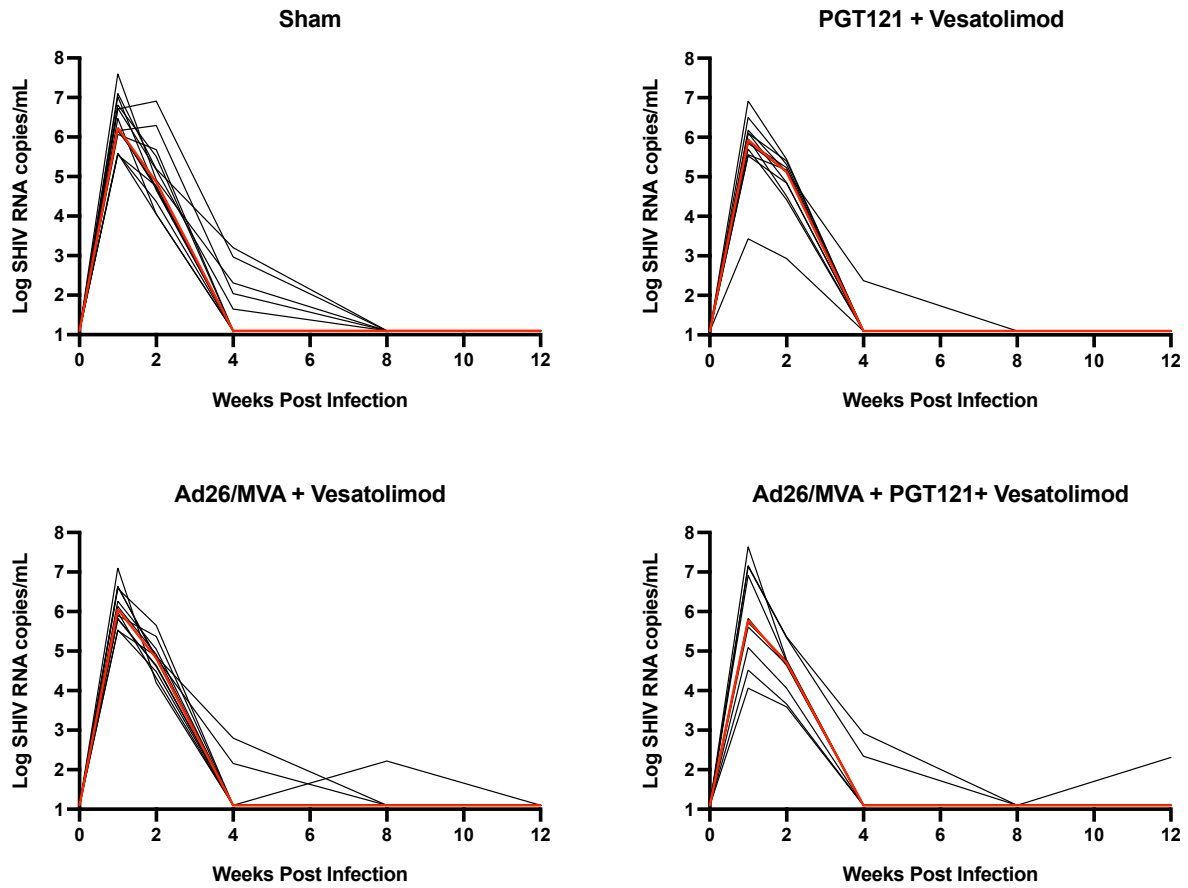
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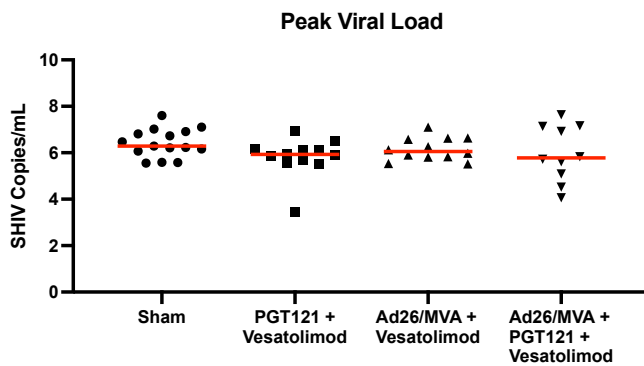
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a



b

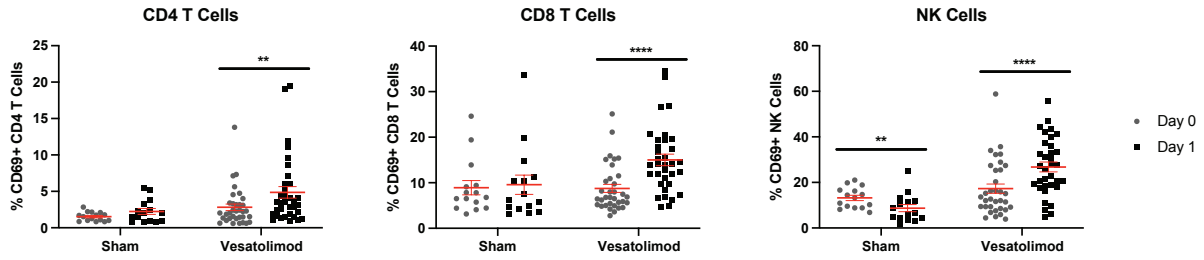


Supplementary Fig. 1. SHIV plasma viremia early in infection. a, SHIV viral loads from infection to week 10 of infection with the median viral load of each group indicated by a red line.

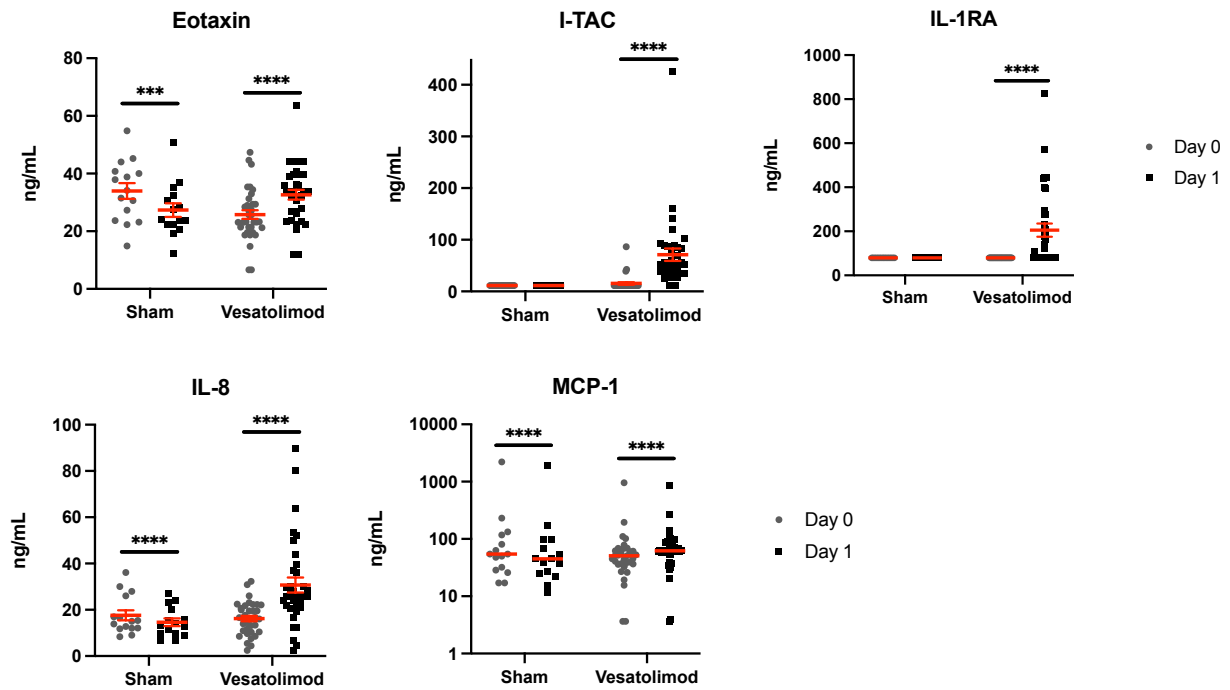
b, Peak viral loads with the median viral load indicated in red. N = 51 macaques; n_{Sham} = 15

macaques, $n_{\text{PGT121+Vesatolimod}} = 12$ macaques, $n_{\text{Ad26/MVA+Vesatolimod}} = 12$ macaques, and

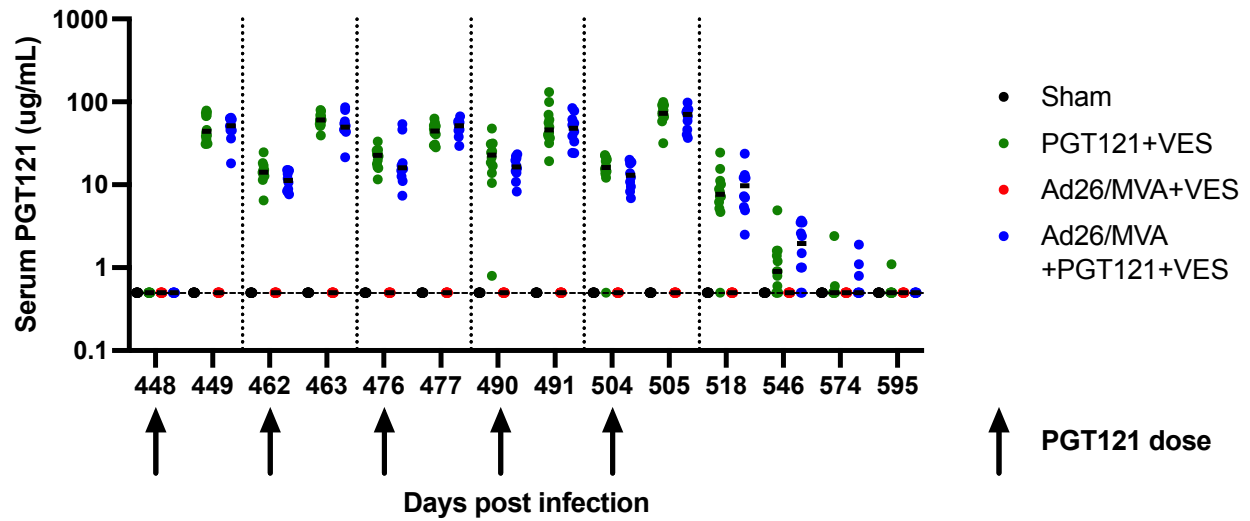
$n_{\text{Ad26/MVA+PGT121+Vesatolimod}} = 10$ macaques.



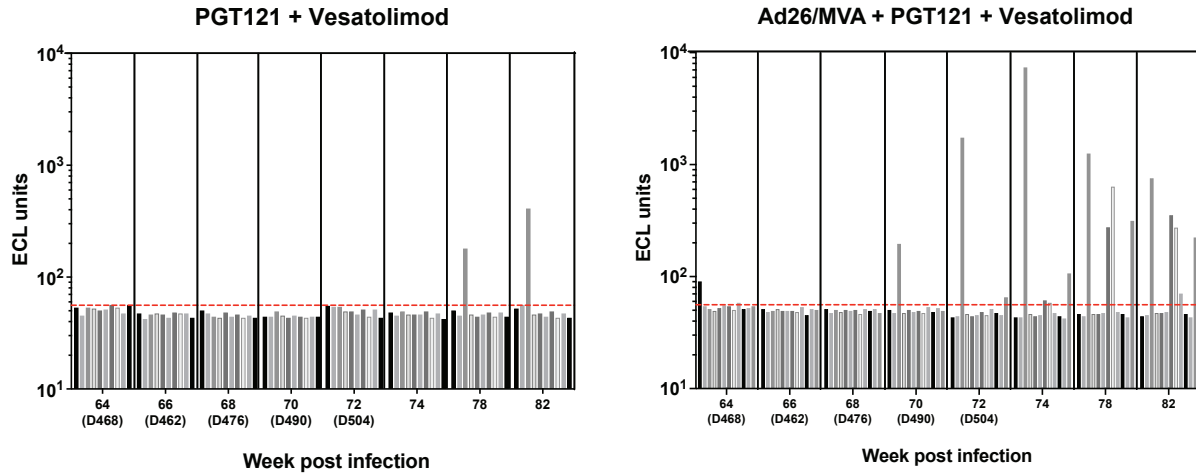
Supplementary Fig. 2. Vesatolimod induces cellular activation in vivo. Bulk PBMCs were analyzed via flow cytometry to determine the effect of the TLR7 agonist on activation in T and NK cells. CD69 expression was found to significantly increase in CD4 and CD8 T cells and NK cells as a result of TLR7 engagement. Representative data shown from week 64 on the day of but prior to injection with vesatolimod and the following day. Average \pm standard error of the mean is shown. Two-sided Wilcoxon signed rank tests used to determine significance. N = 51 macaques; $n_{\text{Sham}} = 15$ macaques, $n_{\text{Vesatolimod}} = 39$ macaques. ** $p < 0.01$ ($p = 0.0023$ (CD4 T cells, Vesatolimod) and 0.0067 (NK cells, Sham)), **** $p < 0.0001$



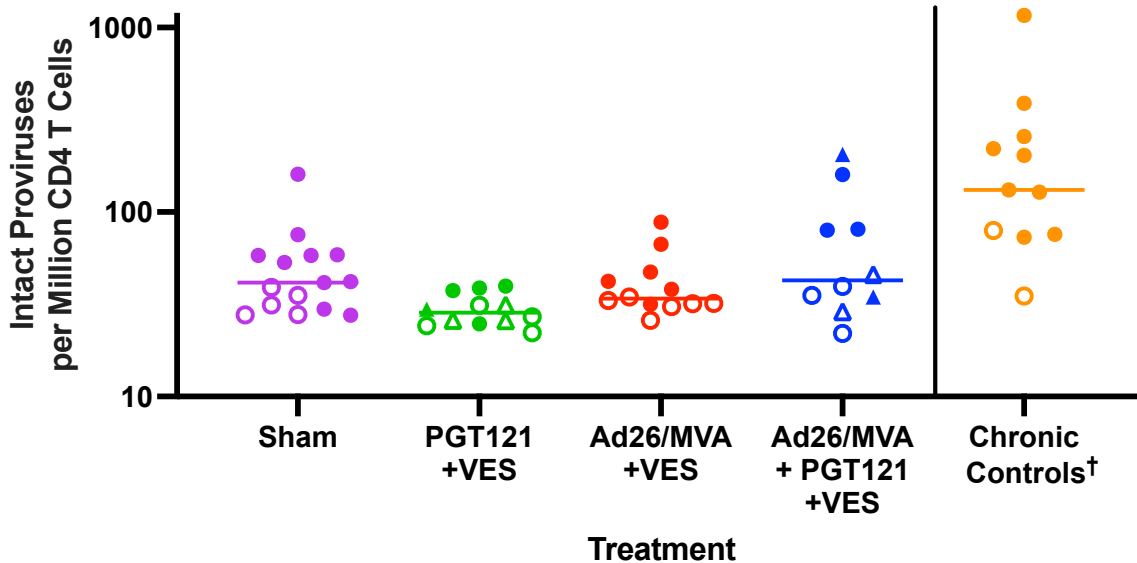
Supplementary Fig. 3. Vesatolimod induces cytokine production in vivo. Serum cytokines were measured before and after vesatolimod administration. A total of nine pairs of measurements were taken for each animal with the median values displayed for each animal. Average \pm standard error of the mean shown save for with MCP-1, where the median is shown. Two-sided Wilcoxon signed rank tests used to determine significance. $N = 51$ macaques; $n_{\text{Sham}} = 15$ macaques, $n_{\text{Vesatolimod}} = 39$ macaques. *** $p < 0.001$ ($p = 0.0002$ in Eotaxin), **** $p < 0.0001$



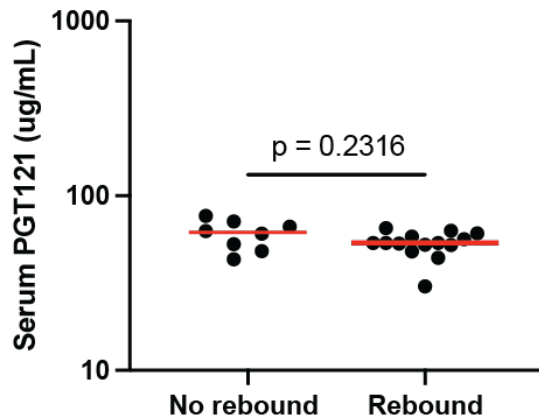
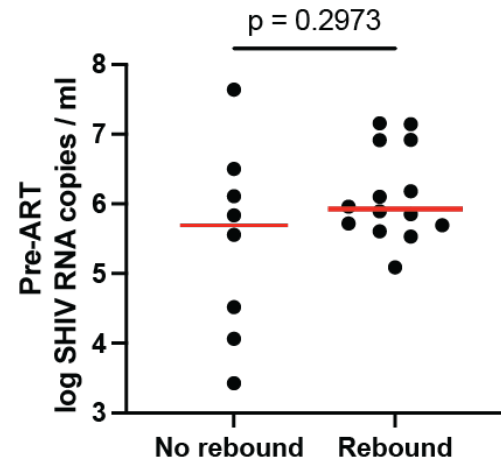
Supplementary Fig. 4. PGT121 serum pharmacokinetics. Serum concentration of PGT121 was measured by ELISA in all the animals from the first day of PGT121 treatment to one week prior to ART cessation. Arrows indicate the timing of PGT121 infusion. Sham = sham treatment; PGT121 + VES = PGT121 + vesatolimod treatment; Ad26/MVA + VES = Ad26/MVA + vesatolimod treatment; and Ad26/MVA + PGT121 + VES = Ad26/MVA, PGT121, + vesatolimod treatment. N = 51 macaques; $n_{\text{Sham}} = 15$ macaques, $n_{\text{PGT121+Vesatolimod}} = 12$ macaques, $n_{\text{Ad26/MVA+Vesatolimod}} = 12$ macaques, and $n_{\text{Ad26/MVA+PGT121+Vesatolimod}} = 10$ macaques.



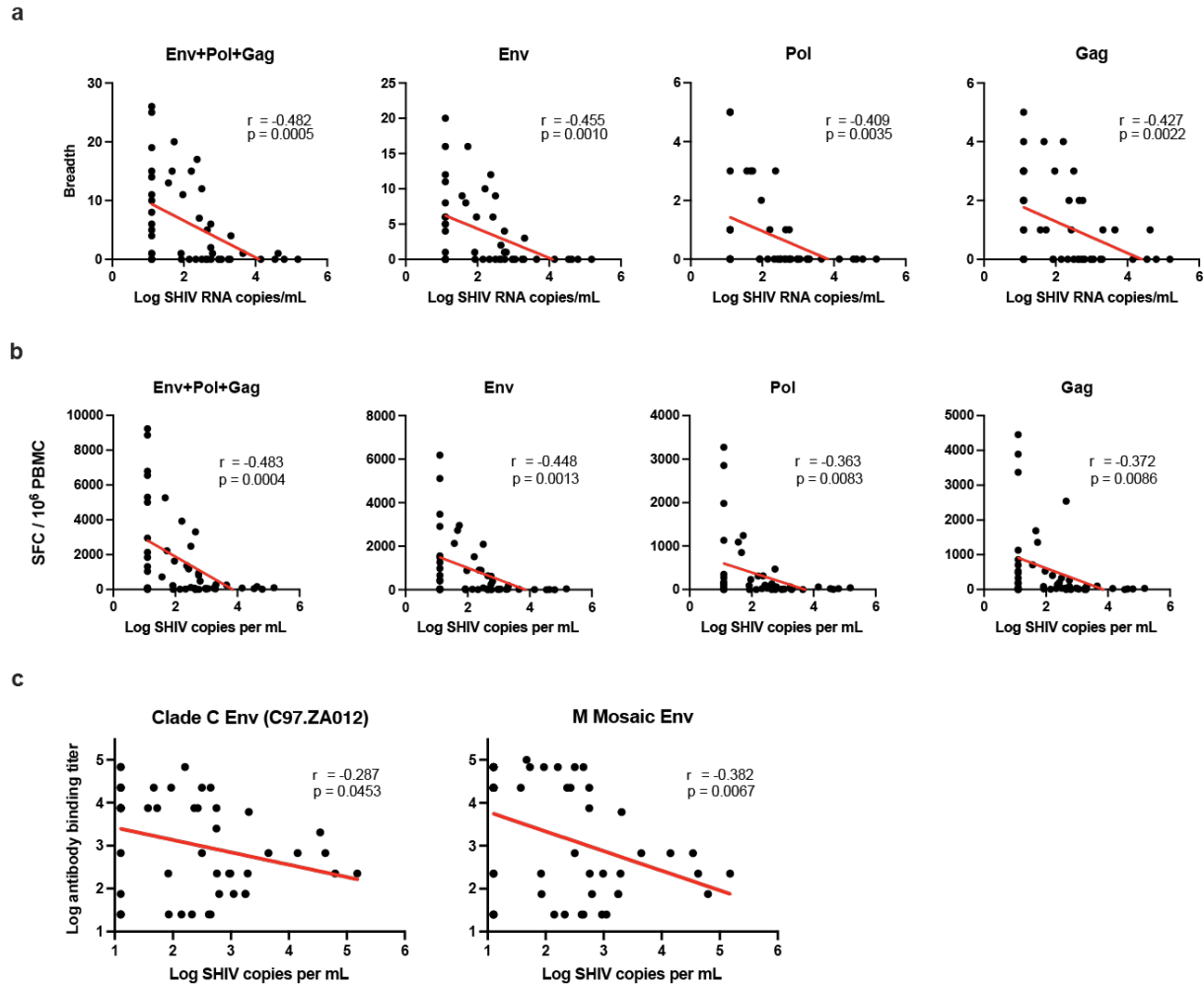
Supplementary Fig. 5. PGT121 anti-drug antibody reactivity. Serum reactivity of the treated animals to PGT121 was measured from the first administration of PGT121 to one month prior to the cessation of ART. ECL = electrochemiluminescence.



Supplementary Fig. 6. Intact proviral frequency of SHIV in circulating CD4 T cells. DNA extracted from peripheral blood CD4 T cells from week 82-85 post-ART initiation was analyzed for the frequency of intact provirus. All values were assayed with three replicate wells. Unfilled symbols represent the level of detection for animals in which the amount of virus was undetectable. Circles represent animals that rebounded after cessation of ART, and triangles represent animals that did not rebound for at least 168 days post cessation of ART. Lines indicate medians. [†]Reference values are from animals treated 58 weeks with ART following 89 weeks of chronic SHIV infection with the median value indicated. Sham = sham treatment; PGT121 + VES = PGT121 + vesatolimod treatment; Ad26/MVA + VES = Ad26/MVA + vesatolimod treatment; and Ad26/MVA + PGT121 + VES = Ad26/MVA, PGT121, + vesatolimod treatment. N = 51 macaques; n_{Sham} = 15 macaques, n_{PGT121+Vesatolimod} = 12 macaques, n_{Ad26/MVA+Vesatolimod} = 12 macaques, and n_{Ad26/MVA+PGT121+Vesatolimod} = 10 macaques.

a**b**

Supplementary Fig. 7. No difference in median serum PGT121 concentration and pre-ART viral loads in animals that did and did not rebound. a, Equivalent PGT121 concentrations in macaques that did and did not rebound. **b,** Equivalent pre-ART peak viral loads in macaques that did and did not rebound. Two-sided Mann-Whitney tests used to determine significance for both analyses. Median indicated by red bar. N = 22 macaques treated with PGT121 (PGT121 + vesatolimod and Ad26/MVA + PGT121 + vesatolimod groups); $n_{\text{Rebound}} = 8$ macaques, $n_{\text{No Rebound}} = 14$ macaques.



Supplementary Fig. 8. Strength and breadth of adaptive immune responses correlate with improved SHIV outcomes. a, Breadth of SHIV-specific cellular immune IFN- γ responses significantly correlate with set-point viral load post-ART cessation. **b,** Size of SHIV-specific cellular immune IFN- γ responses significantly correlate with set-point viral load post-ART cessation. **c,** Serum antibody binding to Env significantly correlates with set-point viral load post-ART cessation. Two-sided Spearman rank-correlation tests were used to determine significance. N = 51 macaques. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$

	10	20	30	40	50	60
HXB2	MRVK---EKY	QHLWRWGWRW	GTMLLGMLMI	CSATEKLWVT	VYYGVPVWKE	ATTTFLFCASD
SHIV-SF162GIRKN.----G	..L.....	...V.....
Mosaic 1	...TGIRKN.----.I...	...AG.....
Mosaic 2	...RGIQRNW	PQW.I----	.ILGFW.II.	.RVMGN....K.....
	70	80	90	100	110	120
HXB2	AKAYDTEVHN	VWATHACVPT	DPNPQEVVLV	NVTENFNMWK	NDMVEQMHEH	IISLWDQSLK
SHIV-SF162I..EN.....
Mosaic 1EN.....
Mosaic 2EK....M..ED....	..R.....
	130	140	150	160	170	180
HXB2	PCVKLTPLCV	SLKC----TD	LKNDTNTNSS	SGRMIMEKGE	IKNCSFNIST	SIRGKVQKEY
SHIV-SF162	T.H.----.N	.E.A...TG.	NWKE-.NR..VT.	..GN.M....
Mosaic 1	T.N.TDDVRN	VT.NATNTN.	.WGEP.....T.	...N...Q.
Mosaic 2	T.E.----RN	VR.VSSNGTY	NIIHNETYK.	M.....AT.	VVEDRK..VH
	190	200	210	220	230	240
HXB2	AFFYKLDIIP	ID-----	ND--TTSYKL	TSCNTSVITQ	ACPKVSFEPI	PIHYCAPAGF
SHIV-SF162	.L....VV.--N.X.N.	IN.....
Mosaic 1	.L....VV.SNN.N.R.	I.....
Mosaic 2	.L..R...V.	L.ENNSSEKS	SENSSEY.R.	IN...A...D..Y
	250	260	270	280	290	300
HXB2	AILKCNKNTF	NGTGPCTNVS	TVQCTHGIRP	VVSTQLLLNG	SLAEEVVIR	SVNFTDNAKT
SHIV-SF162D.K.	..S...I...G....	.E....V..
Mosaic 1D.K.E...N...
Mosaic 2N...K.II..	.E.L.N...
	310	320	330	340	350	360
HXB2	IIVQLNTSVE	INCTRPNNNT	RKRIRIQRGP	GRAFVTIGK-	IGNMRQAHCN	ISRAKWNNTL
SHIV-SF162KE...S.P.--..	.K..YAT.DI	..DI.....	..GE.....
Mosaic 1	..M...V...S.H.--..	...Y.A.DI	..DI.....	...N.....
Mosaic 2	...H..ET.N	.T.....	..S.....	.QT.YAT.DI	..DI.....	L..DG..K..
	370	380	390	400	410	420
HXB2	KQIASKLREQ	FGNNKTIIFK	QSSGGDPEIV	THSFNCGGEF	FYCNSTQLFN	STWF--NSTW
SHIV-SF162	..VT..QA.	.-E...V..	M.....	-----
Mosaic 1	R..VE..GK.V.N	H.....	M.....K...	...TWN...
Mosaic 2	QGVKK..A.H	.-P...N.T	S....L..TR...	...TSG..-	-----G.Y
	430	440	450	460	470	480
HXB2	STEGSNNTTEG	SDTITLPCRI	KQIINMWQKV	GKAMYAPPIS	GQIRCSSNIT	GLLLTRDGG-
SHIV-SF162	NNTIGP.-NT	NG.....R..E.
Mosaic 1	NNTKRS.-DT	EEH.....E.R
Mosaic 2	MPN.T.S-NS	.SN.....E.	.R.....A	.N.T.R....S
	490	500	510	520	530	540
HXB2	--NSNNESEI	FRPGGDMRD	NWRSELYKYK	VVKIEPLGVA	PTKAKRRVVQ	REKRAVGIGA
SHIV-SF162	--REVS.TT..TL..
Mosaic 1	--DTSGT..	S..S.....
Mosaic 2	NNGVP.DT.TNEVK.....	..E.....E	S..S.....
	550	560	570	580	590	600
HXB2	LFLGFLGAAG	STMGAASMTL	TVQARQLLSG	IVQQNNLLR	AIEAQHLLQ	LTVWGIKQLQ
SHIV-SF162	V.....L.
Mosaic 1	V.....L.
Mosaic 2	V...I.....I..S....M..
	610	620	630	640	650	660
HXB2	ARILAVERYL	KDQQLLGIWG	CSGKLICTTA	VPWNASWSNK	SLEQIWNHTT	WMEWDREINN
SHIV-SF162	..V.....D...NM.	...E...G.
Mosaic 1	..V.....TDK...NM.	...E.....
Mosaic 2	T.V..I....	Q.....L..T....	.QTD..DNM.	..Q..K..G.
	670	680	690	700	710	720
HXB2	YTSLIHLIE	ESQNQQEKNE	QELLELDKWA	SLWNWFNITN	WLWYIKLFIM	IVGGLVGLRI
SHIV-SF162	..N..YT...D.SKI...
Mosaic 1YT...D.S.
Mosaic 2	..GE.YR.L.	KD..A..S.K	N.....D...

	730	740	750	760	770	780
HXB2	VFAVLSIVNR	VRQGYSPFSF	QTHLPTPRGP	DRPEGIEEEG	GERDRDRSIR	LVNGSLALIW
SHIV-SF162	..T.....RF.A...LRP	..H.L.....
Mosaic 1	-----	-----	-----	-----	-----	-----
Mosaic 2	-----	-----	-----	-----	-----	-----
	790	800	810	820	830	840
HXB2	DDLRSCLCFS	YHRLRDLLLI	VTRIVELLGR	RGWEALKYWW	NLLQYWSQEL	KNSAVSLLNA
SHIV-SF162I..	AA.....GI...FG.
Mosaic 1	-----	-----	-----	-----	-----	-----
Mosaic 2	-----	-----	-----	-----	-----	-----
	850	860	870			
HXB2	TAIAVAEGTD	RVIEVVQGAC	RAIRHIPRRI	RQGLERILL		
SHIV-SF162	I.....	.I...A.RIG	..FL.....T..		
Mosaic 1	-----	-----	-----	-----		
Mosaic 2	-----	-----	-----	-----		

Supplementary Fig. 9. HIV Env protein alignment. The HIV mosaic-1 and mosaic-2 and SHIV-SF162 Env proteins are shown aligned to the reference HXB2 sequence.