Supplementary Material for:

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

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

 $n_{Ad26/MVA+PGT121+Vesatolimod} = 10$ 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_{Sham} = 15 macaques, n_{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_{Sham} = 15 macaques, n_{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; $n_{Sham} = 15$ macaques, $n_{PGT121+Vesatolimod} = 12$ macaques, and $n_{Ad26/MVA+PGT121+Vesatolimod} = 10$ 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. N = 51 macaques; $n_{Sham} = 15$ macaques, $n_{PGT121+Vesatolimod} = 12$ macaques, $n_{Ad26/MVA+PGT121+Vesatolimod} = 10$ macaques.



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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); $n_{Rebound} = 8$ macaques, $n_{No Rebound}$ = 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 < 0.001

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	73	30 74	40 75	50 7	60 7	70 780
HXB2	VFAVLSIVNR	VRQGYSPLSF	QTHLPTPRGP	DRPEGIEEEG	GERDRDRSIR	LVNGSLALIW
SHIV-SF162	T		RF.AL		RP	H.L
Mosaic 1						
Mosaic 2						
	79	90 80	00 83	10 8	20 83	30 840
HXB2	DDLRSLCLFS	YHRLRDLLLI	VTRIVELLGR	RGWEALKYWW	NLLQYWSQEL	KNSAVSLLNA
SHIV-SF162		I	AA	•••••G	I	FG.
Mosaic 1						
Mosaic 2						
	85	50 80	60 8'	70		
HXB2	TAIAVAEGTD	RVIEVVQGAC	RAIRHIPRRI	RQGLERILL		
SHIV-SF162	I	.IA.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.