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Supplemental information

**HIV-1 neutralizing antibodies provide
sterilizing immunity by blocking
infection of the first cells**

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Supplemental figures and tables

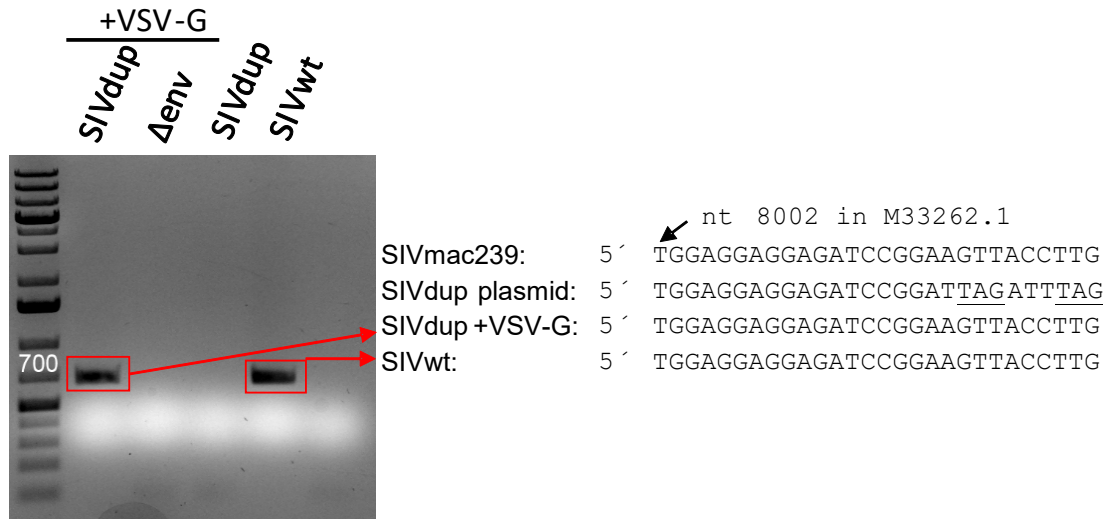


Fig. S1. Repair of SIVdup. Related to Fig. 2. RNA was extracted on day 7 from CEMxSEAP cells infected as described in Figure legend 2B. PCR amplicons with primers spanning the duplication site in *env* were separated by gel electrophoresis and bands marked in red were sequenced. The stop codons introduced into the *env* open reading frame of SIVdup are underlined.

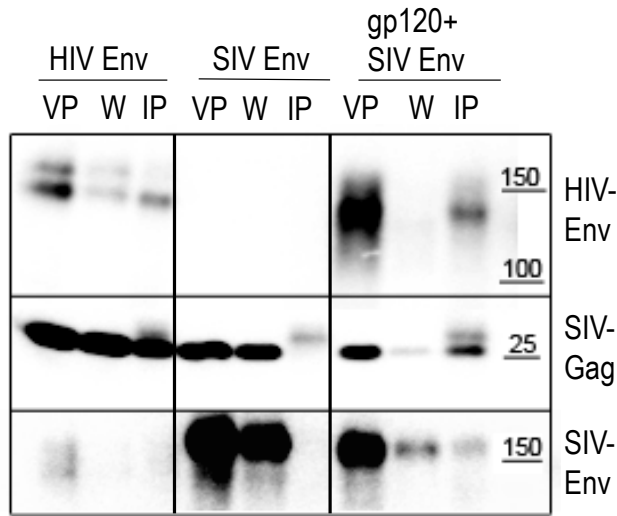


Fig. S2. Incorporation of Env proteins into SIVdup virions. Related to Figure 6.

Virion incorporation of HIV Env, SIV Env and gp120 was analysed by pelleting the whole cell culture supernatants of 293T cells cotransfected with SIVdup and the Env expression plasmids indicated through a 35% sucrose cushion. The pelleted virion preparations (VP) were analysed by Western blot with antibodies detecting the viral proteins indicated at the right of the figure. This revealed the presence of gp120, SIV-Gag and SIV-Env in pellets of gp120+SIV-Env pseudotyped SIVdup (Lane VP of gp120+SIV-Env panel). To test, whether PGT121 would bind to VPs of gp120+SIV-Env pseudotyped SIVdup, an aliquot of the same VP preparation analysed by Western blot was also immunoprecipitated by PGT121. The immunoprecipitates (IP) and the washing fraction (W) were then also analysed. The IP, but not the W fraction contained gp120. As expected from immunoprecipitation of entire particles by PGT121, the IP fraction also contained a clear SIV Gag band and a weak SIV Env band. Since immunoprecipitation of SIVdup particles pseudotyped with SIV Env only, did not reveal SIV Gag or SIV Env bands in the IP fraction (Lane IP of SIV Env panel), the SIV Gag and SIV Env bands detected in SIVdup pseudotyped with gp120+SIV Env cannot be due to cross-reaction of PGT121 with these antigens. The bands are rather explained by the coprecipitation of entire virions by PGT121.

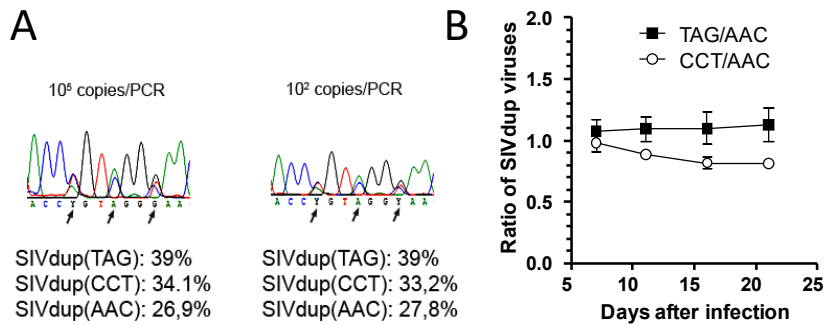


Figure S3. Tagging of SIVdup. Related to Fig. 6.

A) Sequence analysis of amplicons of an equimolar mix of SIVdup DNAs tagged at three consecutive wobble positions of *gag* (arrows) at the indicated template copy number. Percentage of reads of each tagged SIVdup as determined by NGS is shown. B) Replication efficiency of differentially tagged SIVdups. The three differentially tagged SIVdups were pseudotyped with HIV Env and titered on TZMbl cells. CEMxSEAP cells were co-infected in triplicates with 10⁴ IUs of each of the three differentially tagged, HIV-Env pseudotyped SIVdups. Cultures were split and fed with fresh CEMxSEAP cells 4 hours after infection and on day 7, 11, and 16. Viral RNA was extracted from the supernatant of infected cultures on day 7, 11, 16 and 21. Amplicons spanning the tag were analysed by NGS and the number of reads of each tag in each sample was determined. The mean and standard deviations of the ratio of reads for the CCT tag and the TAG tag to the wild type AAC tag at the indicated time points are shown.

Table S1. PGT121 serum concentrations. Related to Fig. 4 and 6

Days after injection	Low dose challenge (µg/ml)						Simultaneous challenge (µg/ml)			
	16115	2701	2782	2882	2744	2816	2783	2733	2752	2730
1	4.8	4.5	3.5	3.3	3.9	n.a.	n.a.	n.a.	n.a.	n.a.
8	2.0	1.7	0.9	1.6	1.2	n.a.	2.0	1.1	1.5	1.2
70	n.a.	0 ¹	0	n.a.	n.a.	0	n.a.	n.a.	n.a.	n.a.

n.a.: sera for analysis not available; ¹ detection limit: 0.09 µg/ml. Representative results of one out of three antibody measurements are shown.

Supplemental Table 2. Number of reads. Related to Fig. 6.

Animals	Tag (Env)	Cut-off	Plasma	PBMCs	Lymph nodes				
					axillary 1	axillary 2	inguinal	submandibular	mesenteric
Control									
2774	AAC (HIV Env)	238	37680	43886	34228	41404	20266	33070	31705
	CCT (gp120+SIV Env)	60	1250	2434	2001	2251	1112	2002	1770
	TAG (SIV Env)	926	872*	7837	6120	6898	3355	6443	5313
2712	AAC (HIV Env)	212	34864	39094	40836	33332	51021	48140	46460
	CCT (gp120+SIV Env)	36	1064	1573	1847	3275	2863	1750	1619
	TAG (SIV Env)	412	4331	4736	4865	8171	8136	5403	5896
2709	AAC (HIV Env)	212	14606	35470	4387	17994	25013	29277	30048
	CCT (gp120+SIV Env)	36	26	425	437	428	1530	761	157
	TAG (SIV Env)	412	220	1105	2434	956	2823	1343	438
16906	AAC (HIV Env)	212	13686	20192	18555	14736	26676	31945	24125
	CCT (gp120+SIV Env)	36	3444	6816	6636	4809	8751	16926	10909
	TAG (SIV Env)	412	937	1897	2624	2471	3712	1859	1237
PGT121									
2783	AAC (HIV Env)	238	5556	4852	3974	4270	3237	2838	3627
	CCT (gp120+SIV Env)	60	6432	5553	3767	4525	3523	3336	3625
	TAG (SIV Env)	926	53234	40340	26147	34601	24013	20202	27604
2733	AAC (HIV Env)	212	5013	2653	6238	316	4477	7737	9275
	CCT (gp120+SIV Env)	36	277	252	558	10	546	685	954
	TAG (SIV Env)	412	16424	8557	25673	1026	15977	28426	25335
2752	AAC (HIV Env)	212	2939	2994	4499	3464	2934	5152	7545
	CCT (gp120+SIV Env)	36	2566	2864	5179	3854	2919	7572	6990
	TAG (SIV Env)	412	16037	15839	28138	23760	18478	34079	32163
2730	AAC (HIV Env)	212	188	97	5593	88	215	186	205
	CCT (gp120+SIV Env)	36	412	513	992	84	290	433	291
	TAG (SIV Env)	412	38023	41490	29738	18129	43646	42723	33305

* shaded numbers are below cut-off

Supplemental Table 3. Ratio of reads. Related to Fig. 6.

Animals	Ratio of viruses	Plasma	PBMCs	Lymph nodes					Median
				axillary 1	axillary 2	inguinal	submandibular	mesenteric	
Control									
2774	HIV Env/SIV Env	n.a.	5,60	5,59	6,00	6,04	5,13	5,97	5,78
	gp120+SIV Env/SIV Env	n.a.	0,31	0,33	0,33	0,33	0,31	0,33	0,33
2712	HIV Env/SIV Env	8,05	8,25	8,39	4,08	6,27	8,91	7,88	8,05
	gp120+SIV Env/SIV Env	0,25	0,33	0,38	0,40	0,35	0,32	0,27	0,33
2709	HIV Env/SIV Env	n.a.	32,10	1,80	18,82	8,86	21,80	68,60	20,31
	gp120+SIV Env/SIV Env	n.a.	0,38	0,18	0,45	0,54	0,57	0,36	0,42
16906	HIV Env/SIV Env	14,61	10,64	7,07	5,96	7,19	17,18	19,50	10,64
	gp120+SIV Env/SIV Env	3,68	3,59	2,53	1,95	2,36	9,10	8,82	3,59
PGT121									
2783	HIV Env/SIV Env	0,10	0,12	0,15	0,12	0,13	0,14	0,13	0,13
	gp120+SIV Env/SIV Env	0,12	0,14	0,14	0,13	0,15	0,17	0,13	0,14
2733	HIV Env/SIV Env	0,31	0,31	0,24	0,31	0,28	0,27	0,37	0,31
	gp120+SIV Env/SIV Env	0,02	0,03	0,02	n.a.	0,03	0,02	0,04	0,03
2752	HIV Env/SIV Env	0,18	0,19	0,16	0,15	0,16	0,15	0,23	0,16
	gp120+SIV Env/SIV Env	0,16	0,18	0,18	0,16	0,16	0,22	0,22	0,18
2730	HIV Env/SIV Env	n.a.	n.a.	0,19	n.a.	0,005	n.a.	n.a.	0,10
	gp120+SIV Env/SIV Env	0,011	0,012	0,033	0,005	0,007	0,010	0,009	0,01

n.a.: not applicable since one value below threshold