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Supplemental information

Highly mutated antibodies capable of neutralizing

N276 glycan-deficient HIV after a single

immunization with an Env trimer

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 ${\tt GEFFYCNTSGLFNSTWISNTSVQGSNSTGSNDSITLPCRIKQIINMWQRIGQAMYAPPIQGVIRCVSNITGLILTRDGGSTNSTTET$ ${\tt GEFFYCNTSGLFNSTWISNTSVQGSNSTGSNDSITLPCRIKQIINMWQRIGQAMYAPPIQGVIRCVSNITGLILVRDGGYTNSNTET$ GEFFYCNTSGLFNSTWISNTSVOGSNSTGSNDSITLPCRIKOIINMWORIGOAMYAPPIOGVIRCVSNITGLILVRDGGYTNSNTET GEFFYCDTSGLFNSTWISNTSVQGSNSTGSNDSITLPCRIKQIINMWQRIGQAMYAPPIQGVIRCVSNITGLILTRDGGSTDSTTET

VQINCTRPNNNTRKSIRIGPGQAFYATGDIIGDIRQAHCNVSKATWNETLGKVVKQLRKHFGNNTIIRFANSSGGDLEVTHSFNCG VOINCTRPNNNTRKSIRIGPGOAFYATGDIIGDIROAHCNVSKATWNETLGKVVKOLRKHYGNDTTIRFAPSSGGDLEVMNHSFNCG VQINCTRPNNNTVKSIRIGPGQAFYYTGDIIGDIRQAHCNVSKATWNETLGKVVKQLRKHYGNDTTIRFAPSSGGDLEVMNHSFNCG VQINCTRPNNNTRKSIRIGPGQWFYATGDIIGDIRQAHCNVSKATWNETLGKVVKQLRKHFGNNTIIRFANSSGGDLEVTTHSFNCG

 $\texttt{C} \underline{\texttt{PKVSFEPIPIHYCAP} \texttt{AGFAILKCKDKKFNGTGPCPSVSTVQCTHGIKPVVSTQLLLNGSLAEEEVMIRSENITNNAKNILVQFNTP}$ CPKVSFEPIPIHYCAPAGFAILKCKDKKFNGTGPCPSVSTVQCTHGIKPVVSTQLLLNGSLAEEEVMIRSEDFRNNAKNILVQFNTP CPKVSFEPIPIHYCAPAGFAILKCKDKKFNGTGPCPSVSTVQCTHGIKPVVSTQLLLNGSLAEEEVIIRSEDFRNNAKNILVQLNTP CPKVSFEPIPIHYCAPAGFAILKCKDKKFNGTGPCPSVSTVOCTHGIKPVVSTOLLLNGSLAEEEVMIRSEDIRNNAKNILVOFNTP

 ${\tt PCVKLTPLCVTLQCTNVTNNITDDMRGELKNCSFNMTTELRDKKQKVYSLFYRLDVVQINENQGNRSNNSNKEYRLINCNTSAITQA}$ PCVKLTPLCVTLQCTNVTNNITDDMRGELKNCSFNMTTELRDKKQKVYSLFYRLDVVQINENQGNRSNNSNKEYRLINCNTSAITQA ${\tt PCVKLTPLCVTLQCTNVTNNITDDMRGELKNCSFNMTTELRDKKQKVYSLFYRLDVVQINENQGNRSNNSNKEYRLINCNTSAITQA}$ PCVKLTPLCVTLQCTNVTNNITDDMRGELKNCSFNMTTELRDKRQKVHALFYKLDIVPINENQNT-----SYRLINCNTAAITQA

AENLWVTVYYGVPVWKDAETTLFCASDAKAYETEKHNVWATHACVPTDPNPOEIHLENVTEEFNMWKNNMVEOMHTDIISLWDOSLK AENLWVTVYYGVPVWKDAETTLFCASDAKAYETEKHNVWATHACVPTDPNPQEIHLENVTEEFNMWKNNMVEQMHTDIISLWDQSLK AENLWVTVYYGVPVWKDAETTLFCASDAKAYETEKHNVWATHACVPTDPNPQEIHLENVTEEFNMWKNNMVEQMHEDIISLWDQSLK AENLWVTVYYGVPVWKDAETTLFCASDAKAYETKKHNVWATHACVPTDPNPQEIHLENVTEEFNMWKNNMVEQMHTDIISLWDQSLK

1 and 2.

A BG505 SOSIP

BG505 SOSIP-GT3

BG505 SOSIP-GT3 BG505 MD39-GT3.1

BG505 MD39-GT3.1

BG505 SOSTP-GT3

BG505 SOSIP-GT3

BG505 MD39-GT3.1

BG505 SOSIPv4.1-GT1

BG505 MD39-GT3.1

BG505 SOSIPv4.1-GT1

BG505 SOSTPv4.1-GT1

BG505 SOSIP

BG505 SOSIP BG505 SOSIP-GT3

BG505 SOSIP

BG505 SOSIP

BG505 MD39-GT3.1

BG505 SOSTPv4.1-GT1

BG505 SOSIPv4.1-GT1

(A) The sequence of BG505 SOSIP trimer and BG505 SOSIP-based CD4bs GT trimer constructs. Modifications from the parent BG505 SOSIP sequence is shown in red. The furin cleavage site between gp120 and gp41 is shown in bold font. The 15mer epitope of HYCAP Tg CD4 T cells is underlined.

(B) Size exclusion chromatography-multiangle light scattering (SEC-MALS) traces for BG505 MD39 and BG505 MD39-GT3.1. The left y-axis is normalized A280 and right y-axis is the molecular weight of protein and glycan. The MW assessed by protein-conjugate analysis is shown as the red line inside the peak. Theoretical protein MW is 214 kDa in both cases. (C) Thermal stability of BG505 MD39 and BG505 MD39-GT3.1 measured by differential scanning calorimetry (DSC). The raw data are shown as blue solid line, and the fit is shown as red line. Melting temperature (Tm) values from the fit are shown.

(D) Antigenic profiles measured by ELISA for BG505 MD39 and BG505 MD39-GT3.1, with trimers either captured by the base binding mAb RM19R (Cottrell et al., 2020) in order to preserve trimer quaternary structure or coated directly onto the ELISA plate so as to disrupt trimer quaternary structure.

(E) SPR kinetic analysis of glVRC01 Fab binding to MD39-GT3.1 trimer (left) and BG505 SOSIPv4.1-GT1 (right) captured on sensor by PGT121. The glVRC01 Fab analyte was tested at a top concentration of 30.347 μ M and four successive 4-fold dilutions. The dissociation constant from this kinetic fit was 86 nM and 1.4 μ M, respectively.



Figure S2. Serum IgG responses from MD39-GT3.1 immunized mice, related to Figure 2.

(A) MD39-GT3.1 trimer-binding serum IgG measured by ELISA and endpoint titers (ET). Biotinylated MD39-GT3.1 trimers were captured on to SA coated plates.

(B) As in (A), but serum IgG binding to the MD39-GT3.1 KO trimer.

(C) Comparison of SA-biotinylated trimer capture and GNL-trimer capture. SA-based trimer capture results in C-terminus oriented binding of the trimer on to ELISA plates, sterically occluding the trimer base epitope. GNL binds mannose residues and captures Env trimers in random orientations.

(D) BG505 SOSIP trimer-binding serum IgG measured by ELISA and ET. Plates were initially coated with GNL, and trimer was captured onto the GNL coated plates. P-values calculated by two-tailed Student's t-tests.

(E) IgG serum ELISA to detect monomeric BG505 gp120. Recombinant BG505 gp120 was directly coated onto ELISA plates.

(F) As in (D) for d22 and d36 (black lines), but with the respective trimer base blocking ELISA performed in parallel (red lines). To block the base epitope, RM19R Fab was added to the trimers prior to the addition of mouse serum.



Figure S3. Analysis of endogenous B_{GC} BCR sequences in MD39-GT3.1 trimer immunized mice, related to Figure 2.

(A) Endogenous B_{GC} cell and VRC01^{gHL} B_{GC} cell sort strategy for sequencing. Dump: CD4, CD8a, NK1.1, Gr-1.
(B) Diversity of endogenous B_{GC} cells. Each circle denotes a unique clonotype, defined by paired HC VDJ-LC VJ genes. The size of each circle is directly proportional to the number of clones observed for that clonotype. Circles are colored by IGHV-genes although the colors are not mutually exclusive due to the large number of IGHV genes observed. Distinct

clones using the same IGHV-genes are proximally clustered. N = total number of paired sequences obtained from two independent experiments; N1 = sequences from first experiment, pooled from 4 mice each for both control and HYCAP3 groups; N2 = sequences from a second experiment, pooled from 5 mice in the control group, pooled from 10 mice in the HYCAP group (5 mice from HYCAP1 and HYCAP3 groups each). B cells from d14 were only sequenced in the second experiment.

(C) The top 5 most used IGHV (VH, upper) and IGKV (VK, lower) genes among HC-LC paired endogenous B_{GC} cells after 10, 14 or 21 days post immunization in the control group. The reference V-gene usage frequencies were derived from V-gene usage observed unimmunized C57BL/6J splenic B cells (Rettig et al., 2018). Dotted line indicates 1%.

(D) As in (C) but analysis of sequences from mice that received HYCAP1 and HYCAP3 CD4 T cells by adoptive transfer.





(A) Number of B_{GC} VRC01^{gHL} HC and LC sequences analyzed. N1 and N2 indicate two independent experiments. D10, 14, 21 B cells were sequenced using the 10X Genomics platform and pooled from 4 mice per group for each experiment. D36 sequences were obtained by Sanger sequencing from 4 mice within each transfer group.

(B) Violin plots of % nucleotide (NT) mutations for the data shown in Figure 2A. The denominator for d10, 14, 21 sequences is 363 NT residues for HC and 312 residues for LC. The denominator for d36 sequences is 345 NT residues for HC and 290 residues for LC.

(C) Per residue HC AA mutations in the three experimental groups.(D) Per residue LC AA mutations in the three experimental groups.

			M1		M2		M3		M4		M5		M6		M7		M8		M9			
Virus Strain	Tier	Clade	т	S	т	s	т	s	т	S	т	S	т	S	т	s	т	S	т	S		
191084 B7-19_N276A	2	Α	0.02631	0.00295	0.02851	0.00276	1.07981	0.05221	0.02356	0.00146	26.43695	0.11549	9.25587	0.06637	1.24439	0.00542	0.07767	0.00225	0.23643	0.00289		
BG505_N276A	2	A	3.87232	NT	21.55737	NT	17.87130	NT	8.80939	NT	NN	NT	66.33857	NT	76.17481	NT	0.53851	NT	79.94976	NT		
BG505_N276D	2	A	3.06971	NT	40.76442	NT	3.46174	NT	0.49062	NT	95.17247	NT	17.85259	NT	3.84156	NT	0.05780	NT	1.14787	NT		
REJO4541.67_N276A	2	в	3.79945	0.01394	44.31766	0.10066	11.37000	0.39231	8.65472	0.01772	NN	NN	NN	22.88683	NN	NN	NN	38.42655	NN	93.06217		
1012_11_TC21_3257_N276A	1B	в	0.12607	0.02426	0.44165	0.01760	6.86379	0.22708	7.33115	0.09931	NN	95.86216	NN	37.24879	15.54891	0.45328	5.41503	0.03266	21.41162	0.11809		
HIV-001428-2.42_N276A	2	c	0.01680	0.00082	7.89591	0.01182	2.64889	0.03082	0.60076	0.00297	NN	15.01911	21.68769	0.15419	19.81655	0.14983	0.04261	0.00057	1.20000	0.00210		
3301.v1.c24_N276A		AC	NN	0.06797	NN	0.09463	NN	0.46500	NN	0.09445	NN	1.07127	NN	6.92608	NN	2.79699	NN	0.03651	NN	0.02381		
6041.v3.c23_N276A		AC	NN	0.62301	NN	NN	NN	NN	NN	5.48987	NN	NN	NN	NN	NN	NN	29.68087	0.11642	NN	5.32711		
92TH021_N276A	2	AE	NN	0.13069	NN	NN	NN	NN	NN	NN	NN	NN	NN	NN	NN	NN	NN	0.49901	NN	NN		
191084 B7-19_N276D	2	A	0.07461	0.00304	0.04816	0.00324	0.25399	0.00722	0.02596	0.00212	0.95622	0.01078	0.11903	0.00379	0.04188	0.00283	0.02837	0.00215	0.04985	0.00337		
SC422661.8_N276D	2	в	1.72409	NT	3.75924	NT	8.09772	NT	0.32041	NT	NN	NT	NN	NT	24.88707	NT	16.12163	NT	78.30517	NT		
HIV-001428-2.42_N276D	2	с	0.02598	0.00085	15.10990	0.01596	1.14572	0.00733	0.16154	0.00250	NN	2.77310	34.96976	0.14733	37.50414	0.03775	0.01913	0.00037	0.23609	0.00125		
HIV-0815.v3.c3_N276D	2	ACD	4.12250	NT	NN	NT	NN	NT	2.30058	NT	50.56341	NT	NN	NT	NN	NT	0.73764	NT	NN	NT		
MLV			NN	NN	NN	NN	NN	NN	NN	NN	NN	NN	NN	NN	NN	NN	NN	NN	NN	NN		
			м	10	м	11	м	12	N	113	м	14	м	15	E	7	E	11	glV	RC01	VR	C01
Virus Strain	Tier	Clade	т	10 s	<u>м</u>	11 S	т	12 S	<u>м</u>	113 S	м т	14 S	м т	15 S	E T	7 S	E	11 s	giV т	RC01 S	VR(T	C01 S
Virus Strain 191084 B7-19_N276A	Tier 2	Clade A	M T 5.50763	10 S 0.02893	M T 9.50712	11 S 0.03078	M T 0.05328	12 S 0.00272	T 0.00746	113 S 0.00049	M T NN	14 S 79.73813	T NN	15 S 28.77998	T 2.77038	7 S 0.02073	E T 0.02364	11 S 0.00149	gIV T NN	RC01 S NN	VR0 T 0.03100	C01 S 0.00092
Virus Strain 191084 B7-19_N276A BG505_N276A	Tier 2 2	Clade A A	T 5.50763 24.89845	10 S 0.02893 NT	M T 9.50712 NN	11 S 0.03078 NT	M T 0.05328 NN	12 S 0.00272 NT	T 0.00746 1.00591	113 S 0.00049 NT	T NN NN	14 S 79.73813 ND	T NN NN	S 28.77998 ND	T 2.77038 NN	5 0.02073 NT	E T 0.02364 NN	11 S 0.00149 NT	gIV T NN NN	RC01 S NN ND	VR0 T 0.03100 0.01300	C01 S 0.00092 NT
Virus Strain 191084 B7-19_N276A BG505_N276A BG505_N276D	Tier 2 2 2	Clade A A A	M T 5.50763 24.89845 0.10831	10 S 0.02893 NT NT	M T 9.50712 NN 0.64819	11 s 0.03078 NT NT	M T 0.05328 NN 3.72484	12 S 0.00272 NT NT	T 0.00746 1.00591 0.00850	13 S 0.00049 NT NT	M T NN NN NN	14 5 79.73813 ND ND	T NN NN NN	15 S 28.77998 ND ND	E T 2.77038 NN 30.75698	5 0.02073 NT NT	E T 0.02364 NN 15.40579	11 S 0.00149 NT NT	gIV T NN NN NN	RC01 S NN ND ND	VR0 T 0.03100 0.01300 0.01100	C01 S 0.00092 NT NT
Virus Strain 191084 B7-19_N276A BG505_N276A BG505_N276D REJO4541.67_N276A	Tier 2 2 2 2	Clade A A B	M T 5.50763 24.89845 0.10831 NN	10 S 0.02893 NT NT 63.44921	M T 9.50712 NN 0.64819 NN	11 S 0.03078 NT NT NN	M T 0.05328 NN 3.72484 NN	12 S 0.00272 NT NT 0.39616	T 0.00746 1.00591 0.00850 0.00805	13 S 0.00049 NT NT 0.00001	M T NN NN NN	14 \$ 79.73813 ND ND NN	T NN NN NN NN	15 S 28.77998 ND ND NN	T 2.77038 NN 30.75698 27.23893	5 5 0.02073 NT NT 0.50054	E T 0.02364 NN 15.40579 16.35902	11 S 0.00149 NT NT 0.57058	gIV T NN NN NN NN	RC01 S NN ND ND NN	VR0 T 0.03100 0.01300 0.01100 0.02100	C01 S 0.00092 NT NT 0.00350
Virus Strain 191084 B7-19_N276A BG505_N276A BG505_N276D REJO4541.67_N276A 1012_11_TC21_3257_N276A	Tier 2 2 2 2 1B	Clade A A B B B	M T 5.50763 24.89845 0.10831 NN NN	10 S 0.02893 NT NT 63.44921 NN	M T 9.50712 NN 0.64819 NN NN	11 S 0.03078 NT NN 21.65742	M T 0.05328 NN 3.72484 NN 0.28868	12 S 0.00272 NT NT 0.39616 0.03467	T 0.00746 1.00591 0.00850 0.00805 0.03405	S 0.00049 NT NT 0.00001 0.00393	T NN NN NN NN NN	14 S 79.73813 ND ND NN NN	T NN NN NN NN NN	15 S 28.77998 ND ND NN NN	T 2.77038 NN 30.75698 27.23893 89.60507	S 0.02073 NT NT 0.50054 6.88754	E 0.02364 NN 15.40579 16.35902 0.80414	11 S 0.00149 NT NT 0.57058 0.01075	gIV T NN NN NN NN NN	RC01 S NN ND ND NN NN	VR0 T 0.03100 0.01300 0.01100 0.02100 0.05400	C01 S 0.00092 NT NT 0.00350 0.81000
Virus Strain 191084 B7-19_N276A BG505_N276A BG506_N276D REJ04541.67_N276A 1012_11_TC21_3257_N276A HIV-001428-2.42_N276A	Tier 2 2 2 1B 2	Clade A A B B C	M T 5.50763 24.89845 0.10831 NN NN NN	10 S 0.02893 NT NT 63.44921 NN 0.53909	M 7 9.50712 NN 0.64819 NN NN 12.40000	11 S 0.03078 NT NN 21.65742 0.01435	M T 0.05328 NN 3.72484 NN 0.28868 0.53000	12 S 0.00272 NT 0.39616 0.03467 0.00155	T 0.00746 1.00591 0.00850 0.00805 0.03405 0.01100	S 0.00049 NT NT 0.00001 0.00393 0.00002	M T NN NN NN NN NN	14 5 79.73813 ND ND NN NN NN NN	M T NN NN NN NN NN	S 28.77998 ND ND ND NN NN 2.46747	E 7 2.77038 NN 30.75698 27.23893 89.60507 NN	5 0.02073 NT 0.50054 6.88754 2.00477	E 0.02364 NN 15.40579 16.35902 0.80414 ND	11 S 0.00149 NT 0.57058 0.01075 0.94445	gIV T NN NN NN NN NN NN	RC01 S NN ND ND NN NN NN	VR0 0.03100 0.01300 0.01100 0.02100 0.05400 0.00550	C01 S 0.00092 NT NT 0.00350 0.81000 0.00240
Virus Strain 191084 87-19_N276A BG505_N276A BG505_N276D REJO4541.67_N276A 1012_11_TC21_3257_N276A 101428-2.42_N276A 3301.v1.c24_N276A	Tier 2 2 2 1B 2	Clade A A B C AC	M 5.50763 24.89845 0.10831 NN NN NN NN NN	10 S 0.02893 NT NT 63.44921 NN 0.53909 39.31180	M 9.50712 NN 0.64819 NN NN 12.40000 NN	11 S 0.03078 NT NN 21.65742 0.01435 0.76253	M T 0.05328 NN 3.72484 NN 0.28868 0.53000 NN	12 S 0.00272 NT 0.39616 0.03467 0.00155 0.03244	T 0.00746 1.00591 0.00850 0.00805 0.03405 0.01100 0.20735	13 S 0.00049 NT 0.00001 0.00393 0.00002 0.00001	M T NN NN NN NN NN NN	14 5 79.73813 ND ND NN NN NN NN	M T NN NN NN NN NN NN	15 S 28.77998 ND ND NN NN 2.46747 12.53858	E 2.77038 NN 30.75698 27.23893 89.60507 NN NN	5 0.02073 NT NT 0.50054 6.88754 2.00477 1.56525	E 7 0.02364 NN 15.40579 16.35902 0.80414 ND NN	11 S 0.00149 NT 0.57058 0.01075 0.94445 0.13680	gIV T NN NN NN NN NN NN	RC01 S ND ND NN NN NN NN	T 0.03100 0.01300 0.01100 0.02100 0.05400 0.00550 0.09000	C01 S 0.00092 NT NT 0.00350 0.81000 0.00240 0.00240
Virus Strain 191084 B7-19_N276A BG505_N276A BG505_N276D REJ04541.57_N276A 1012_11_7221_3257_N276A HIV-001428-242_N276A 3301.v1.c24_N276A 6041.v3.c23_N276A	Tier 2 2 2 1B 2	Clade A A B C AC AC	M 5.50763 24.89845 0.10831 NN NN NN NN NN NN NN	10 S 0.02893 NT NT 63.44921 NN 0.53909 39.31180 7.14739	M 9.50712 NN 0.64819 NN NN 12.40000 NN NN	11 S 0.03078 NT NN 21.65742 0.01435 0.76253 NN	M T 0.05328 NN 3.72484 NN 0.28868 0.53000 NN NN	12 S 0.00272 NT 0.39616 0.03467 0.00155 0.03244 NN	T 0.00746 1.00591 0.00850 0.00805 0.03405 0.01100 0.20735 NN	13 S 0.00049 NT 0.00001 0.00393 0.00002 0.00001 2.56698	M T NN NN NN NN NN NN NN	14 5 79.73813 ND ND NN NN NN NN NN NN	M T NN NN NN NN NN NN NN	S 28.77998 ND ND NN NN 2.46747 12.53858 NN	E 2.77038 NN 30.75698 27.23893 89.60507 NN NN NN	S 0.02073 NT NT 0.50054 6.88754 2.00477 1.56525 NN	E 0.02364 NN 15.40579 16.35902 0.80414 ND NN NN	11 S 0.00149 NT 0.57058 0.01075 0.94445 0.13680 NN	gIV T NN NN NN NN NN NN NN	RC01 NN ND ND NN NN NN NN NN	T 0.03100 0.01300 0.01100 0.02100 0.05400 0.00550 0.09000 0.00900	C01 S 0.00092 NT NT 0.00350 0.81000 0.00240 0.00240 0.00340 0.00290
Virus Strain 191064 B7-19_N276A BG505_N276A BG505_N276D REJ04541.67_N276A 1012_11_TC21_3257_N276A HW-001428-2.42_N276A 3301.v1.c24_N276A 6041.v3.c23_N276A 9271H021_N276A	Tier 2 2 2 1B 2 2	Clade A A B C AC AC AE	M 5.50763 24.89845 0.10831 NN NN NN NN NN NN NN NN NN	10 S 0.02893 NT NT 63.44921 NN 0.53909 39.31180 7.14739 NN	M T 9.50712 NN 0.64819 NN NN 12.40000 NN NN NN NN	11 S 0.03078 NT NN 21.65742 0.01435 0.76253 NN NN	M T 0.05328 NN 3.72484 NN 0.28868 0.5300 NN NN NN	12 S 0.00272 NT 0.39616 0.03467 0.00155 0.03244 NN NN	T 0.00746 1.00591 0.00850 0.03405 0.03405 0.01100 0.20735 NN NN	S 0.00049 NT NT 0.00001 0.00001 0.00002 0.00001 2.56698 2.80218	M T NN NN NN NN NN NN NN	14 5 79.73813 ND ND NN NN NN NN NN NN NN NN	M T NN NN NN NN NN NN NN	12 28.77998 ND ND NN NN 2.46747 12.53858 NN NN	E 7 2.77038 NN 30.75698 27.23893 89.60507 NN NN NN NN NN	5 0.02073 NT 0.50054 6.88754 2.00477 1.56525 NN NN	E 0.02364 NN 15.40579 16.35902 0.80414 ND NN NN NN	11 S 0.00149 NT 0.57058 0.01075 0.94445 0.13680 NN NN	gIV T NN NN NN NN NN NN NN	RC01 S NN ND NN NN NN NN NN NN	VR0 T 0.03100 0.01300 0.02100 0.02100 0.05400 0.00550 0.09000 0.00900 0.00900	C01 S 0.00092 NT NT 0.00350 0.81000 0.00240 0.00240 0.00290 0.01000
Virus Strain 191084 B7-19, N276A BG305, N276A BG305, N276D REJ04541.67, N276A 1012, 11, TC21, 3257, N276A HIV-001428-242, N276A 501, v1-24, N276A 6041, v3-23, N276A 92TH021, N276A 910404 B7-19, N276D	Tier 2 2 2 1B 2 2 1B 2 2 2 2 2	Clade A A B C AC AC AE A	M 5.50763 24.89845 0.10831 NN NN NN NN NN NN NN NN NN N	10 S 0.02893 NT NT 63.44921 NN 0.53909 39.31180 7.14739 NN 0.00255	M 9.50712 NN 0.64819 NN NN 12.40000 NN NN NN NN 0.14182	11 S 0.03078 NT NN 21.65742 0.01435 0.76253 NN NN 0.00439	M T 0.05328 NN 3.72484 NN 0.28868 0.5300 NN NN NN NN 0.01806	12 S 0.00272 NU NT 0.39616 0.03467 0.00155 0.03244 NN NN NN 0.00262	T 0.00746 1.00591 0.00850 0.03405 0.01100 0.20735 NN NN NN	S 0.00049 NT NT 0.00001 0.00001 0.00002 0.00001 2.56698 2.80218 0.00024	M T NN NN NN NN NN NN NN NN	14 5 79.73813 ND ND NN NN NN NN NN NN NN 1.01321	M T NN NN NN NN NN NN NN NN	12 28.77998 ND ND NN NN 2.46747 12.53858 NN NN 0.29781	E 2.77038 NN 30.75698 27.23893 89.60507 NN NN NN NN NN NN NN NN	5 0.02073 NT 0.50054 6.88754 2.00477 1.56525 NN NN 0.00074	E 0.02364 NN 15.40579 16.35902 0.80414 ND NN NN NN NN 0.01150	11 S 0.00149 NT 0.57058 0.57058 0.94445 0.13680 NN NN NN 0.00127	gIVI T NN NN NN NN NN NN NN NN	8 NN ND NN NN NN NN NN NN 36.73057	VR0 T 0.03100 0.01300 0.02100 0.05400 0.00550 0.09000 0.00500 0.00000 0.06000 0.02700	C01 S 0.00092 NT 0.00350 0.81000 0.00240 0.00240 0.00290 0.01000 0.00520
Virus Strain 191054 87-19_N276A BG505_N276A REJ04541.67_N276A 1012_11_TC21_3257_N276A 1012_11_TC21_3257_N276A 13010_t1_c24_N276A 6041_v3.c23_N276A 9271H021_N276A 1910084 87-19_N276D SC422661.8_N276D	Tier 2 2 2 1B 2 2 2 2 2 2 2 2 2 2	Clade A A B C AC AC AE A B	M T 5.50763 24.89845 0.10831 NN NN NN NN NN NN NN NN NN N	10 S 0.02893 NT 63.44921 NN 0.53909 39.31180 7.14739 NN 0.00255 NT	M T 9.50712 NN 0.64819 NN NN 12.40000 NN NN NN NN 0.14182 NN	11 S 0.03078 NT NN 21.65742 0.01435 0.76253 NN NN 0.00439 NT	M T 0.05328 NN 3.72484 NN 0.28868 0.53000 NN NN NN NN 0.01806 0.25366	12 S 0.00272 NT 0.39616 0.03467 0.00155 0.03244 NN NN 0.00262 NT	N - T 0.00746 1.00591 0.00850 0.0805 0.03405 0.01100 0.20735 NN NN 0.00795 0.00585	S 0.00049 NT 0.00001 0.00093 0.00002 0.00001 2.56698 2.80218 0.00024 NT	M T NN NN NN NN NN NN NN NN NN	14 5 79.73813 ND ND NN NN NN NN NN NN NN NN	M T NN NN NN NN NN NN NN NN NN NN	15 S 28.77998 ND ND NN 2.46747 12.53858 NN NN 0.29781 ND	T 2.77038 NN 30.75698 27.23893 89.60507 NN NN NN NN NN NN NN NN NN NN NN	S 0.02073 NT 0.50054 6.88754 2.00477 1.56525 NN NN 0.00074 NT	E 7 0.02364 NN 15.40579 16.35902 0.80414 ND NN NN NN NN NN 0.01150 4.75373	11 S 0.00149 NT 0.57058 0.01075 0.94445 0.13680 NN NN 0.00127 NT	gIVI T NN NN NN NN NN NN NN NN NN	RC01 S NN ND ND NN NN NN NN NN NN NN	VR0 T 0.03100 0.01100 0.02100 0.05400 0.0550 0.09000 0.09000 0.06000 0.02700 0.01000	C01 S 0.00092 NT 0.00350 0.81000 0.00240 0.00240 0.00240 0.00290 0.01000 0.00520 NT
Virus Strain 191084 B7-19_N276A BG505_N276D REJO4541.67_N276A 1012_11_TC21_3257_N276A 1012_11_TC21_3257_N276A 101428-242_N276A 101428-242_N276A 101084 B7-19_N276D SC422661.8_N276D SC422661.8_N276D	Tier 2 2 2 1B 2 2 1B 2 2 2 2 2 2 2 2	Clade A A B C AC AC AE A B C	M 5.50763 24.89845 0.10831 NN NN NN NN NN NN NN NN NN N	10 S 0.02893 NT 63.44921 NN 0.53909 39.31180 7.14739 NN 0.00255 NT 0.00369	M 7 9.50712 NN 0.64819 NN 12.40000 NN NN NN 0.14182 NN 0.45619	11 S 0.03078 NT NN 21.65742 0.01435 0.02135 NN NN 0.0437 0.00137	M T 0.05328 NN 3.72464 NN 0.28868 0.53000 NN NN NN NN NN NN NN NN NN NN NN NN	12 S 0.00272 NT 0.39616 0.03647 0.00155 0.00244 NN NN 0.00262 NT 0.00123	N - - - - - - - - - - - - -	13 S 0.00049 NT 0.00001 0.00393 0.00002 0.00001 2.56698 2.800218 0.0024 NT 0.00001	M T NN NN NN NN NN NN NN NN NN	14 5 79.73813 ND NN NN NN NN NN 1.01321 ND 99.53962	M T NN NN NN NN NN NN NN NN	15 S 26.77998 ND NN NN NN 2.46747 12.53858 NN NN 0.29781 ND 1.30816	T 2.77038 NN 30.75698 38.60507 NN NN NN NN NN NN NN NN NN NN NN NN NN	7 S 0.02073 NT 0.50054 6.88754 2.00477 1.56525 NN NN NN 0.0074 NT 0.00392	E 7 0.02864 NN 15.40579 16.35992 0.80414 ND NN NN NN NN NN NN NN NN S NN S 18986	11 S 0.00149 NT 0.57058 0.01075 0.94445 0.13680 NN NN NN NN NN NN NN NN NN N	gIV T NN NN NN NN NN NN NN NN NN	8C01 S NN ND NN NN NN NN NN NN NN 36.73057 ND NN	VR0 T 0.03100 0.01100 0.02100 0.05400 0.05500 0.09000 0.09000 0.09000 0.09000 0.09000 0.09000 0.09000 0.02000 0.02000 0.01000 0.01000	C01 S 0.00092 NT 0.00350 0.81000 0.00240 0.00290 0.01000 0.00020 NT 0.00230
Virus Strain 191084 87-19_N276A 80505_N276A 80505_N276D 78L04541.67_N276A 1012_11_TC21_3257_N276A 1010_11_TC21_3257_N276A 3301.v1.c24_N276A 6041.v3.c23_N276A 92110021_N276A 191084 87-19_N276D 80542861.6_N276D HIV-001428-2.42_N276D HIV-001428-3.3_N276D	Tier 2 2 2 1B 2 1B 2 2 2 2 2 2 2 2 2 2 2	Clade A A B B C AC AC AC AC AC AC AC AC AC AC	M T 5.50763 24.89845 0.10831 NN NN NN NN NN NN NN NN NN 1.79904 1.79904 4.16580	10 S 0.02893 NT NT 63.44921 NN 0.53909 39.31180 7.14739 NN 0.00359 NT	M 9.50712 NN 0.64819 NN 12.40000 NN NN NN 0.14182 NN 0.45619 9.12986	11 S 0.03078 NT NN 21.65742 0.01435 0.74253 NN NN 0.00439 NT NT	M 	12 S 0.00272 NT NT 0.39616 0.03467 0.00155 0.03244 NN NN 0.00262 NT NT	T 0.00746 1.00591 0.00850 0.00805 0.03405 0.0100 0.20735 NN NN 0.00795 0.00585 0.00114 0.004374	13 S 0.00049 NT NT 0.00001 0.00001 0.00002 0.00002 0.00001 2.56698 2.80218 0.00024 NT NT	M T NN NN NN NN NN NN NN NN NN	14 5 79.73813 ND NN NN NN NN NN NN NN NN NN	M T NN NN NN NN NN NN NN NN NN	15 8 28.77998 ND NN NN 2.46747 12.53858 NN NN 0.29781 1.30816 ND	T 2.77038 NN 30.75698 27.23893 89.60507 NN NN NN NN NN 0.02558 7.62172 2.16156 NN	7 S 0.02073 NT 0.50054 6.88754 2.00477 1.56525 NN NN NN 0.00074 NT NT	E 0.02364 NN 15.40579 16.35902 0.80414 ND NN NN NN 0.01150 4.75373 5.18986 NN	11 S 0.00149 NT 0.57058 0.01075 0.94445 0.13680 NN NN 0.0127 NT NT	gIV T NN NN NN NN NN NN NN NN NN NN	RC01 S NN ND NN NN NN NN NN NN NN NN	T 0.03100 0.01300 0.05200 0.06500 0.00550 0.00900 0.00900 0.00900 0.02700 0.02700 0.01000 0.01000 0.01000	C01 S 0.00092 NT 0.00350 0.81000 0.00240 0.00240 0.00240 0.00290 0.00290 0.01000 0.00520 NT 0.00250 NT

Figure S5. Pseudovirus neutralization by post-immunization VRC01^{gHL} mAbs, related to Figure 6.

Individual pseudovirus neutralization assay IC₅₀ (μ g/mL) values graphed in Figure 6. The indicated Δ N276 viruses were produced in HEK293T (T) or HEK293S (S) cells. Murine leukemia virus (MLV) was used as a negative control. ND = Not determined; NN = IC₅₀ > 100 μ g/mL, no neutralization; NT = No virus titer. Data shown is an average of two experiments.