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Supplemental Information

Structural Survey of Broadly Neutralizing Antibodies Targeting the HIV-1 Env Trimer Delineates Epitope Categories and Characteristics of Recognition

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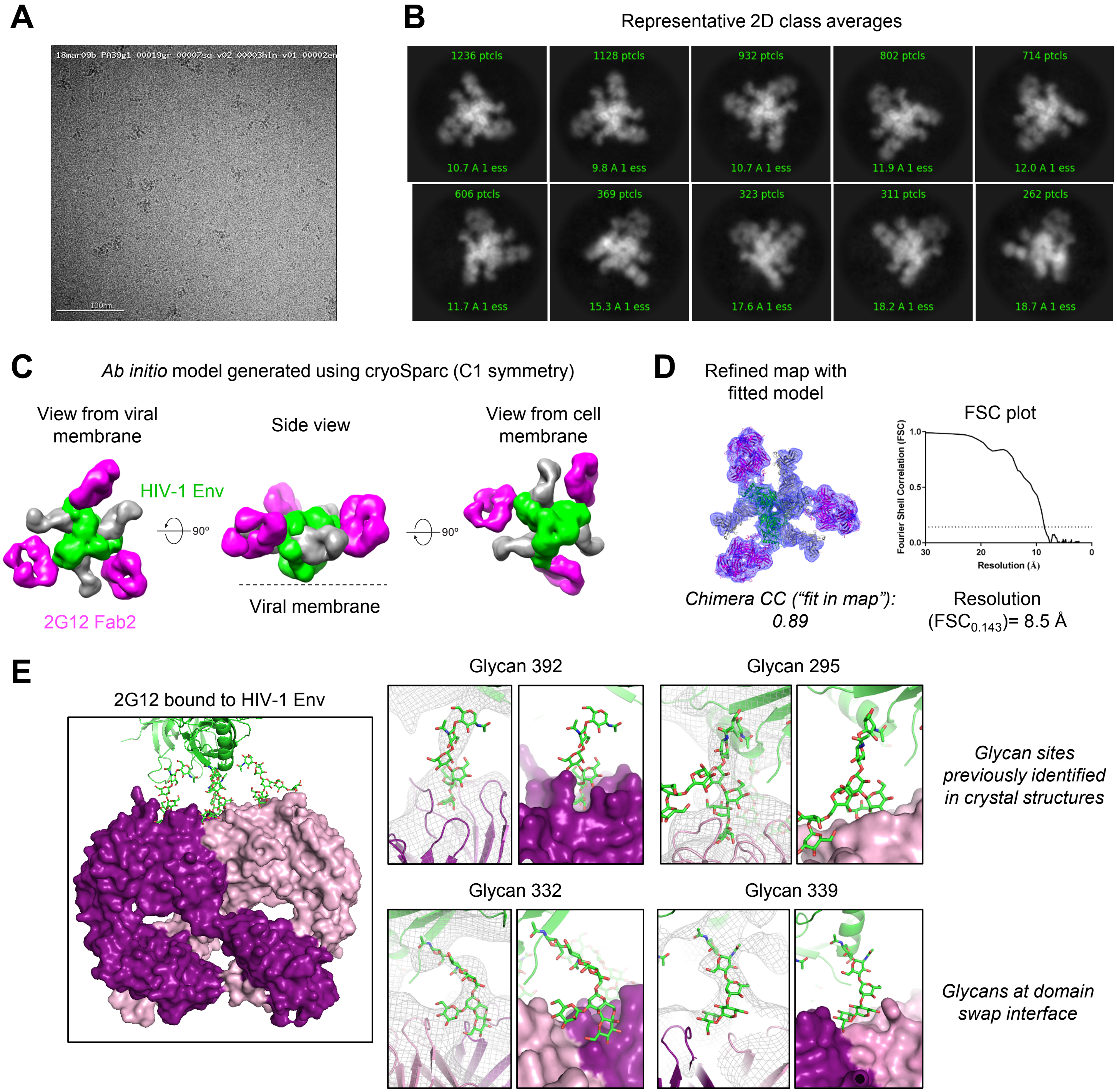

Figure S1. Details of cryo-EM structure of HIV-1 BG505 DS-SOSIP in complex with antibodies VRC03 and 2G12. Related to Figures 1-3. (**A**) Representative micrograph, (**B**) Representative 2D class averages (**C**) Ab initio model generated using cryoSparc showing HIV-1 Env in green VRC03 in grey and 2G12 in magenta. (**D**) Refined map fitted with model of BG505 DS-SOSIP (green) VRC03 (grey) and 2G12 (magenta) (left) Fourier shell correlation plotted as a function of resolution with resolution reported according to the gold standard FSC0.143 criterion (FSC0.143 shown as dotted line) (right). (**E**) Details of HIV-1 Env glycan interactions with 2G12. Left. Domain swapped 2G12 shown in surface representation (magenta and pink) bound to HIV-1 Env (green ribbon with glycans shown as sticks). Right. Details of the interactions of 2G12 with the four glycans in its binding site. For each pair of figures for the 4 glycans, the left image shows the Env and 2G12 as ribbons, glycans as sticks, and electron density as grey mesh. The right images show 2G12 as surface, Env as ribbon, and glycans as sticks.

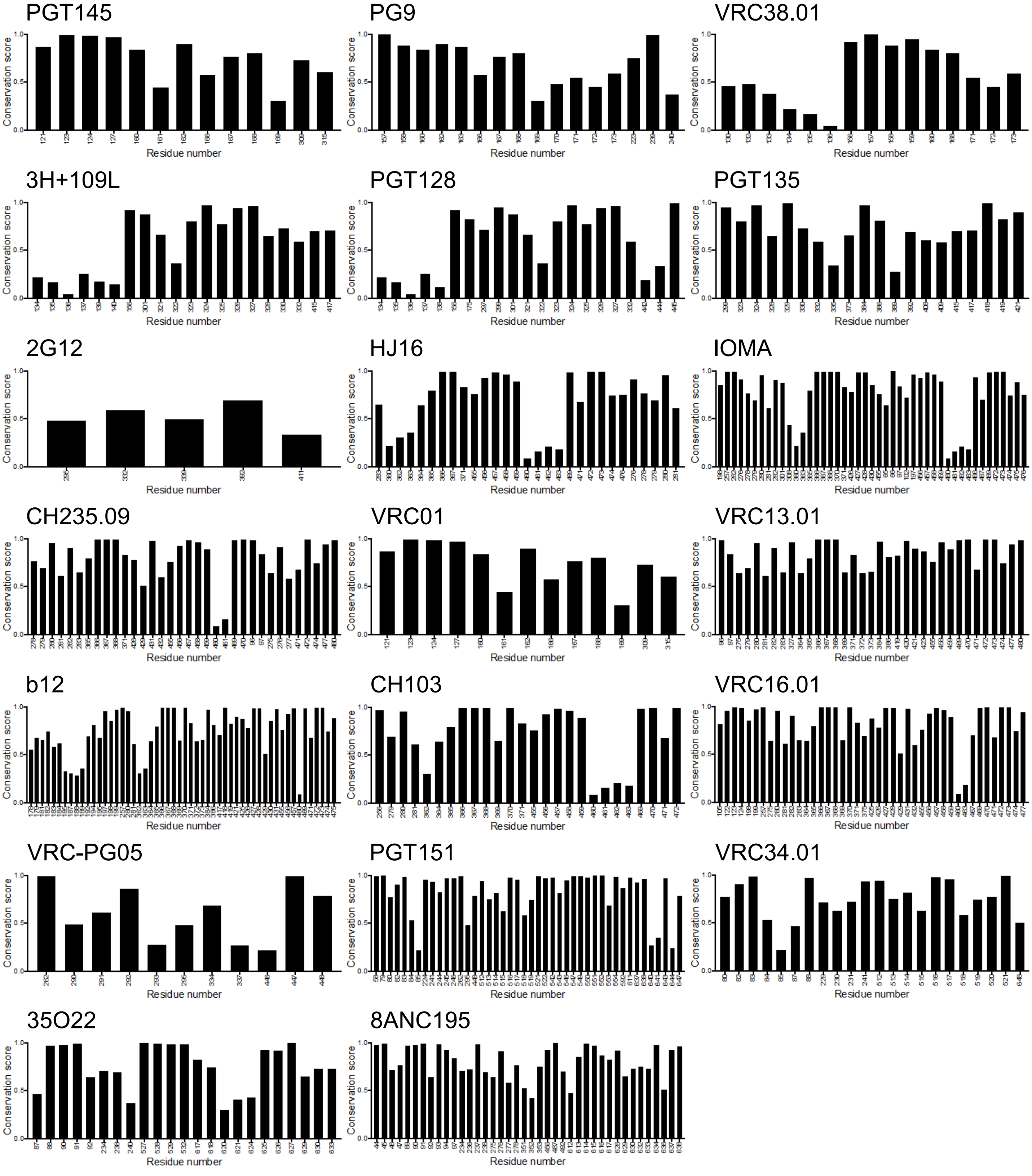

Figure S2. Conservation score for individual epitope residues. Related to Figure 3. Conservation score 0 indicates the most variable, whereas score 1 indicates the most conserved.

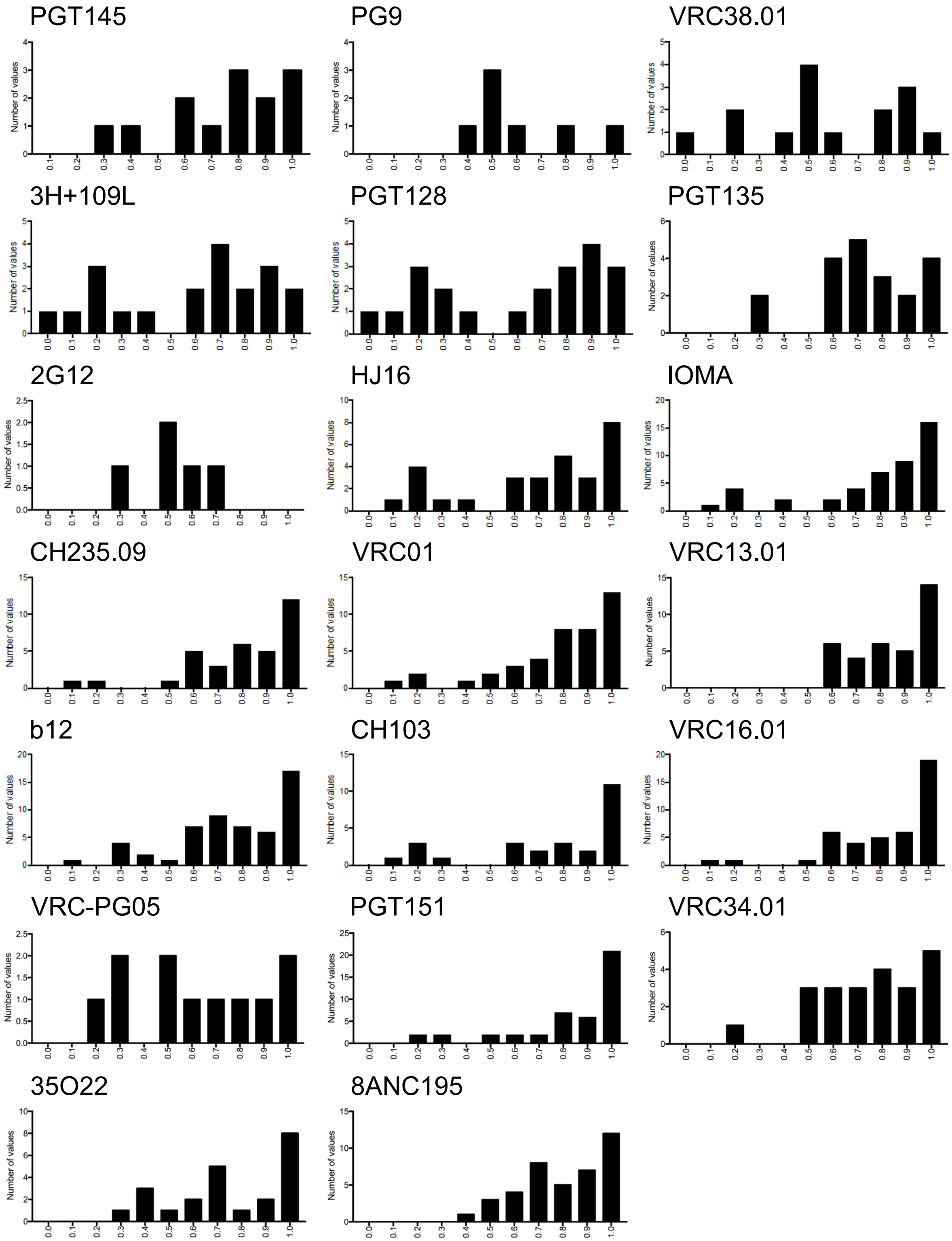

Figure S3. Histogram distribution of conservation score for individual epitope residues. Related to Figure 3.

Figure S4. WebLogo Plots for individual epitope residues. Related to Figure 3. X denotes putative N-linked glycosylation sequon. The plots were generated using https://www.hiv.lanl.gov/content/sequence/ANALYZEALIGN/analyze_align.html.

Class	Structure	PDB
PGT145	PGT145	5V8L
PG9	PG9	3U2S
VRC38.01	VRC38.01	5VGJ
PGT121	3H+109L	5CEZ
PGT128	PGT128	5ACO
PGT135	PGT135	4JM2
2G12	2G12	6E5P
HJ16	HJ16	4YE4
IOMA	IOMA	5T3Z
8ANC131	CH235.09	5F9O
VRC01	VRC01	5FYJ
VRC13.01	VRC13.01	4YDJ
b12	b12	5VN8
CH103	CH103	4JAN
VRC16.01	VRC16.01	4YDK
VRC-PG05	VRC-PG05	6BF4
PGT151	PGT151	5FUU
VRC34.01	VRC34.01	5I8H
35O22	35O22	5CEZ
8ANC195	8ANC195	5CJX
2F5	2F5	1TJI
10E8	10E8	4G6F
4E10	4E10	4XCF
DH511	DH511	5U3O
Z13e1	Z13e1	3FN0

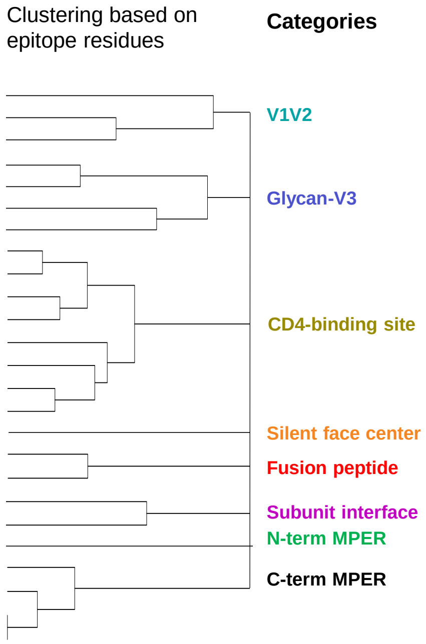

Figure S5. MPER antibodies segregate into two additional categories, N-term MPER and C-term MPER. Related to Figure 1. Representative antibody-Env structures and PDBs for MPER-directed HIV-1 neutralizing antibodies (10E8, 2F5, 4E10, DH511 and Z13e1) were analyzed with representative antibodies recognizing the prefusion-closed Env trimer by neighbor-joining analysis based on epitope-residue overlap (dendrogram). The MPER antibodies segregated into two additional categories, N-term MPER (containing the single antibody 2F5, which recognizes primarily residues 661-670) and C-term MPER (containing antibodies 10E8, 4DE10, DH5511 and Z13e1,which recognize primarily residues 671-683).

Table S1. Matrix of epitope distances among 20 unique antibodies. Epitope distance between each of the antibody is defined as $1 - \frac{n_o}{N_s}$, where n_o is the number of overlapping epitope residues, and N_s is the number of epitope residues for the antibody with the least number of epitope residues of the two. Related to Figure 1.

	PGT145	**PG9**	**VRC38.01**	**3H+109L**	**PGT128**	**PGT135**	**2G12**	**HJ16**	**IOMA**	**CH235.09**	**VRC01**	**VRC13.01**	**b12**	**CH103**	**VRC16.01**	**VRC-PG05**	**PGT151**	**VRC34.01**	**35O22**	**8ANC195**
PGT145	0	0.54	0.85	1	1	1	1	1	1	1	1	1	1	1	0.85	1	1	1	1	1
PG9	0.54	0	0.46	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
VRC38.01	0.85	0.46	0	0.73	0.73	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
3H+109L	1	1	0.73	0	0.3	0.65	0.8	1	0.95	1	0.95	0.95	0.95	1	1	1	1	1	1	1
PGT128	1	1	0.73	0.3	0	0.8	0.8	1	0.95	1	0.95	0.95	1	1	1	1	1	1	1	1
PGT135	1	1	1	0.65	0.8	0	0.6	1	1	1	0.95	0.75	0.65	1	1	1	1	1	1	1
2G12	1	1	1	0.8	0.8	0.6	0	1	1	1	1	1	1	1	1	0.8	0.8	1	1	1
HJ16	1	1	1	1	1	1	1	0	0.14	0.28	0.17	0.41	0.38	0.19	0.31	1	1	1	1	0.9
IOMA	1	1	1	0.95	0.95	1	1	0.14	0	0.32	0.26	0.51	0.44	0.23	0.4	0.91	0.98	1	1	0.9
CH235.09	1	1	1	1	1	1	1	0.28	0.32	0	0.21	0.32	0.47	0.27	0.24	1	1	1	1	0.82
VRC01	1	1	1	0.95	0.95	0.95	1	0.17	0.26	0.21	0	0.37	0.48	0.31	0.38	1	1	1	1	0.88
VRC13.01	1	1	1	0.95	0.95	0.75	1	0.41	0.51	0.32	0.37	0	0.4	0.35	0.37	1	1	1	1	0.94
b12	1	1	1	0.95	1	0.65	1	0.38	0.44	0.47	0.48	0.4	0	0.31	0.35	1	1	1	1	0.97
CH103	1	1	1	1	1	1	1	0.19	0.23	0.27	0.31	0.35	0.31	0	0.19	1	1	1	1	0.96
VRC16.01	0.85	1	1	1	1	1	1	0.31	0.4	0.24	0.38	0.37	0.35	0.19	0	1	1	1	1	0.95
VRC-PG05	1	1	1	1	1	1	0.8	1	0.91	1	1	1	1	1	1	0	0.73	1	1	1
PGT151	1	1	1	1	1	1	0.8	1	0.98	1	1	1	1	1	1	0.73	0	0.32	1	0.95
VRC34.01	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0.32	0	0.91	1
35O22	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0.91	0	0.57
8ANC195	1	1	1	1	1	1	1	0.9	0.9	0.82	0.88	0.94	0.97	0.96	0.95	1	0.95	1	0.57	0

Table S2. NGS reads statistics. Related to Figures 2 and 5.

Table S2A. Heavy chain Read statistics for normal donor data sets associated with Healthy Donor NGS.

	LP32647	LP23810	LP08248
RAW Reads	232,094	261,224	300,213
≥ 350 nucleotide	216,971	248,046	286,701
VH assignment	203,869	203,872	223,172
Average CDR H3 length	13.42	14.46	13.82
Average SHM	9.59	8.21	8.39

Table S2B. Lambda chain read statistics for normal donor data sets associated with Healthy Donor NGS.

	LP32647	LP23810	LP08248
RAW Reads	208,141	159,365	284,437
≥ 300 nucleotide	186,441	145,904	274,687
VL assignment	185,972	144,632	272,667
Average SHM	5.4	4.66	5.64

Table S2C. Kappa chain read statistics for normal donor data sets associated with Healthy Donor NGS.

	LP32647	LP23810	LP08248
RAW Reads	295,412	316,019	296,609
≥ 300 nucleotide	285,178	304,104	283,451
VK assignment	284,590	299,672	269,184
Average SHM	4.78	3.32	4.80

Table S2D. Vgene reads of 20 representative antibody for normal donor data sets associated with Healthy Donor NGS.

Antibody	Vgene	LP32647	LP23810	LP08248
PGT145	IGHV1-8	2090	734	2640
PG9	IGHV3-33	14	4533	1347
VRC38.01	IGHV3-13	89	443	9
PGT121	IGHV4-59	9984	735	7879
PGT128	IGHV4-39	57613	0	47240
PGT135	IGHV4-39	57613	0	47240
2G12	IGHV3-21	1742	24324	2376
HJ16	IGHV3-30	3784	6013	3911
IOMA	IGHV1-2	7151	18158	9054
8ANC131	IGHV1-46	2245	1334	2632
VRC01	IGHV1-2	7151	18158	9054
VRC13.01	IGHV1-69	11115	10453	6862
b12	IGHV1-3	1694	0	0
CH103	IGHV4-59	9984	735	7879
VRC16.01	IGHV3-23	2658	19767	8705
VRC-PG05	IGHV3-7	2634	16492	14298
PGT151	IGHV3-30	3784	6013	3911
VRC34	IGHV1-2	7151	18158	9054
35O22	IGHV1-18	3004	1514	2500
8ANC195	IGHV1-69	11115	10453	6862

Table S3. Structural details for MPER antibodies in complex with HIV-1 Env. Related to Figure 1.

Category	Class	Antibody with most informative structure	Most informative PDB	Experiment method	Resolution (Å)	Env component	Epitope Residues
N-term MPER	2F5	2F5	1TJI	X-ray	2.20	MPER peptide	E657, Q658, E659, L661, E662, L663, D664, K665, W666, A667, L669, W670
C-term MPER	10E8	10E8	4G6F	X-ray	2.10	MPER peptide	S668, L669, W670, N671, W672, F673, T676, N677, L679, W680, I682, R683
	4E10	4E10	4XCF	X-ray	1.43	MPER peptide	N671, W672, W673, D674, I675, T676, N677, W678, L679, W680, K683
	DH511	DH511	5U3O	X-ray	1.76	MPER peptide	K669, W670, N671, W672, F673, D674, I675, T676, N677, L679, W680, I682, R683
	Z13e1	Z13e1	3FN0	X-ray	1.80	MPER peptide	W670, N671, W672, F673, D674, I675, T676, N677