## **Supplementary Information**

Supplementary Note 1. Sequence alignment of gp120s from structures of the indicated complexes demonstrates similarities and differences in interactions.

E51-sCD4-BG505 CCR5-sCD4-gp120 17b-sCD4-8ANC195-BG505 21c-sCD4-8ANC195-B41 E51-sCD4-BG505 CCR5-sCD4-gp120 17b-sCD4-8ANC195-BG505 21c-sCD4-8ANC195-B41	$\begin{array}{c} \alpha 0 & \beta 0 \\ \\ \label{eq:relation} \\ \begin{tabular}{lllllllllllllllllllllllllllllllllll$
E51-sCD4-BG505 CCR5-sCD4-gp120 17b-sCD4-8ANC195-BG505 21c-sCD4-8ANC195-B41	V1V2 DDMRGELKNCSFNMTTEIRDKKQKVYSLFYRLDVVQINENQGNRSNNSNKEYRLINCNTS GIDKGEIKNCSFNTTTSVKDKEKKEYALFYNLDVVQIGNDNTSYRLTSCNTS DDMRGELKNCSFNMTTEIRDKKQKVYSLFYRLDVVQINENQGNRSNNSNKEYRLINCNTS KMETGEMKNCSFNVTTSIRDKIKKEYALFYKLDVVPLENKN-NINNTNITNYRLINCNTS I 164
E51-sCD4-BG505 CCR5-sCD4-gp120 17b-sCD4-8ANC195-BG505 21c-sCD4-8ANC195-B41	$\frac{\beta 3}{\textbf{AIT}OACPKVSFEPIPIHYCAPAGFAILKCKDKKFNGTGPCPSVSTVQCTHGIKPVVSTQL} VITQACPKVTFEPIPIHYCTPAGYAILKCNGKKFNGTGPCTNVSTVQCTHGIKPVVSTQL AITQACPKVSFEPIPIHYCAPAGFAILKCKDKKFNGTGPCPSVSTVQCTHGIKPVVSTQL VITQACPKVSFEPIPIHYCAPAGFAILKCNSKTFNGSGPCTNVSTVQCTHGIRPVVSTQL I 202$
E51-sCD4-BG505 CCR5-sCD4-gp120 17b-sCD4-8ANC195-BG505 21c-sCD4-8ANC195-B41	V3 LLNGSLAEEEVMIRSENITNNAKNILVQFNTPVQINCTRPNNNTRKSIRIGPGQAFYATG LLNGSLAEEDIVIRSENITNNAKTIIVQLKDPVDINCTRPNNTRKSIHIGPGRAFYATG LLNGSLAEEEVMIRSENITNNAKNILVQFNTPVQINCTRPNNNTRKSIRIGPGQAFYATG LLNGSLAEEEIVIRSENITDNAKTIIVQLNEAVEINCTRPNNNTRKSIHIGPGRAFYATG 1 261 300
E51-sCD4-BG505 CCR5-sCD4-gp120 17b-sCD4-8ANC195-BG505 21c-sCD4-8ANC195-B41	V3 CD4bs loop DIIGDIEQAHCNVSKATWNETLGKVVKQLRKHFGNNTIIRFANSSGGDLEVTTHSENCGG DIIGDIEQAHCNLSRAQWNDTLSKIVTKLREQFENKT-IKFQPPSGGDPEIVFHSENCGG DIIGDIEQAHCNVSKATWNETLGKVVKQLRKHFGNNTIIRFANSSGGDLEVTTHSENCGG DIIGNIEQAHCNISKARWNETLGQIVAKLEEQFPNKT-IIFNHSSGGDPEIVTHSENCGG 1 327 369
E51-sCD4-BG505 CCR5-sCD4-gp120 17b-sCD4-8ANC195-BG505 21c-sCD4-8ANC195-B41	V4 <u>B20</u> <u>B21</u> EFFYCNTSGLFNSTWISNTSVQGSNSTGSNDSITLPCRIKQIINMWQRIGQAMYAPPIQG EFFYCNTTQLFNSTWINNTEGTSNTTGNDTITLPCRIKQIVNMWQEVGKAMYAPPIQG EFFYCNTSGLFNSTWISNTSVQGSNSTGSNDSITLPCRIKQIINMWQRVGKAMYAPPIQG EFFYCNTTPLFNSTWNNTRTDDYPTGGEQNITLQCRIKQIINMWQGVGKAMYAPPIRG 1 381 421 432
E51-sCD4-BG505 CCR5-sCD4-gp120 17b-sCD4-8ANC195-BG505 21c-sCD4-8ANC195-B41	VIRCVSNITGLILTRDGGSTN-STTETFRPGGGDMRDNWRSELYKYKVVKIEPLGVAPTR KIKCSSNITGLLLTRDGGNNEMNTTEIFRPGGGDMRDNWRSELYKYKVVKIEPLGVAPTR VIRCVSNITGLILTRDGGSTN-STTETFRPGGGDMRDNWRSELYKYKVVKIEPLGVAPTR QIRCSSNITGLLLTRDGGRDQ-NGTETFRPGGGNMRDNWRSELYKYKVVKIEPLGVAPTR 442
E51-sCD4-BG505 CCR5-sCD4-gp120 17b-sCD4-8ANC195-BG505 21c-sCD4-8ANC195-B41	CKRRVVG X = residues contacted by Fabs or CCR5   CKRRVVG = residues contacted by sulfated tyrosines   CKRRV = disordered regions

## **Supplementary Tables**

Trimer state	Trimer type	Ligand(s)	Method	PDB	Resolution (Å)	Distance(s) between V3 (His330) (Å)	Distance(s) between V1V2 (Pro124) (Å)	Distance(s) between CD4bs (Asp368) (Å)
Closed	BG505 SOSIP.664	8ANC195	X-ray	5CJX	3.6	68	14	54
Closed	BG505 SOSIP.664	PGT122, 35O22	X-ray	4TVP	3.5	69	15	55
Closed	BG505 SOSIP.664	PGT122	X-ray	4NCO	4.7	70	14	56
Closed	BG505 SOSIP.664	3H+109L 35O22	X-ray	5CEZ	3	69	14	56
Closed	BG505 SOSIP.664	IOMA, 35022	X-ray	5T3Z	3.5	69	14	54
Closed	JR-FL Env∆CT	PGT151	cryo- EM	5FUU	4.2	69	16	56
Partially open	BG505 SOSIP.664	sCD4, 17b 8ANC195	cryo- EM	6CM3	3.5	76	67	79
Partially open	B41 SOSIP.664	sCD4, 21c 8ANC195	cryo- EM	6EDU	4.1	73	69	79
Open	B41 SOSIP.664	sCD4, 17b	cryo- EM	5VN8	3.6	73	79	84
Open (Class I)	BG505 SOSIP.664	sCD4, E51	cryo- EM	6U0L	3.3	75, 80, 70	67, 75, 70	79, 85, 78
Open (Class II)	BG505 SOSIP.664	sCD4, E51	cryo- EM	6U0N	3.5	81, 73, 70	76, 77, 70	85, 83, 79

**Supplementary Table 1. Distance comparisons in Env trimer structures.** Structures are grouped into four conformational states: closed (unliganded and bound to Fabs), partially open (bound to 8ANC195, sCD4, and either 17b or 21c), and open (bound to sCD4 and 17b), and the open class I and class II E51-sCD4-BG505 complexes (this study). The PDB identifier is given for each structure. PDB coordinates for gp120 subunits within a trimer were used to measure distances on adjacent protomers between V3 base residue His330<sub>gp120</sub>, V1V2 base residue Pro124<sub>gp120</sub>, and the CD4 binding site residue Asp368<sub>gp120</sub>.

Supplementary Table 2. PDB entries for structures presented in Extended Data Figure 5 and their corresponding references.

PDB	Reference
6CM3	Wang, H., Barnes, C.O., Yang, Z., Nussenzweig, M.C. & Bjorkman, P.J. Partially Open HIV-1
	Envelope Structures Exhibit Conformational Changes Relevant for Coreceptor Binding and
	Fusion. Cell Host Microbe 24, 579-592 e4 (2018)
5VN3	Ozorowski, G. et al. Open and closed structures reveal allostery and pliability in the HIV-1
	envelope spike. <i>Nature</i> <b>547</b> , 360-363 (2017)
4TVP	Pancera, M. et al. Structure and immune recognition of trimeric pre-fusion HIV-1 Env. Nature
	<b>514</b> , 455-61 (2014)
4ZMJ	Kwon, Y.D. et al. Crystal structure, conformational fixation and entry-related interactions of
	mature ligand-free HIV-1 Env. Nat Struct Mol Biol 22, 522-31 (2015)
5FYJ	Stewart-Jones, G.B.E. et al. Trimeric HIV-1-Env Structures Define Glycan Shields from Clades
	A, B, and G. <i>Cell</i> <b>165</b> , 813-26 (2016)
5FYK	Stewart-Jones, G.B.E. et al. Trimeric HIV-1-Env Structures Define Glycan Shields from Clades
	A, B, and G. <i>Cell</i> <b>165</b> , 813-26 (2016)
5 EVI	Stewart-Jones, G.B.E. et al. Trimeric HIV-1-Env Structures Define Glycan Shields from Clades
SFTL	A, B, and G. <i>Cell</i> <b>165</b> , 813-26 (2016)
БІОЦ	Kong, R. et al. Fusion peptide of HIV-1 as a site of vulnerability to neutralizing antibody.
51011	Science <b>352</b> , 828-33 (2016)
5 190	Kong, L. et al. Uncleaved prefusion-optimized gp140 trimers derived from analysis of HIV-1
0009	envelope metastability. Nat Commun 7, 12040 (2016)
5 ISA	Kong, L. et al. Uncleaved prefusion-optimized gp140 trimers derived from analysis of HIV-1
535A	envelope metastability. Nat Commun 7, 12040 (2016)
	Lee, J.H., de Val, N., Lyumkis, D. & Ward, A.B. Model Building and Refinement of a Natively
5ACO	Glycosylated HIV-1 Env Protein by High-Resolution Cryoelectron Microscopy. Structure 23,
	1943-51 (2015)
	Kong, L. et al. Complete epitopes for vaccine design derived from a crystal structure of the
5C7K	broadly neutralizing antibodies PGT128 and 8ANC195 in complex with an HIV-1 Env trimer.
	Acta Crystallogr D Biol Crystallogr <b>71</b> , 2099-108 (2015)
5T27	Gristick, H.B. et al. Natively glycosylated HIV-1 Env structure reveals new mode for antibody
513Z	recognition of the CD4-binding site. Nat Struct Mol Biol 23, 906-915 (2016)
5CEZ	Garces, F. et al. Affinity Maturation of a Potent Family of HIV Antibodies Is Primarily Focused
	on Accommodating or Avoiding Glycans. Immunity 43, 1053-63 (2015)
5CJX	Scharf, L. et al. Broadly Neutralizing Antibody 8ANC195 Recognizes Closed and Open States
	of HIV-1 Env. <i>Cell</i> <b>162</b> , 1379-90 (2015)
5D9Q	Jardine, J.G. et al. Minimally Mutated HIV-1 Broadly Neutralizing Antibodies to Guide

	Reductionist Vaccine Design. PLoS Pathog 12, e1005815 (2016)
5FUU	Lee, J.H., Ozorowski, G. & Ward, A.B. Cryo-EM structure of a native, fully glycosylated,
6MDT	Kumar, S. et al. Capturing the inherent structural dynamics of the HIV-1 envelope glycoprotein
	fusion peptide. Nat Commun 10, 763 (2019).
6NQD	Ananthaswamy, N. et al. A sequestered fusion peptide in the structure of an HIV-1 transmitted
	founder envelope trimer. Nat Commun 10, 873 (2019)
60KP	Schoofs, T. et al. Broad and Potent Neutralizing Antibodies Recognize the Silent Face of the
	HIV Envelope. <i>Immunity</i> <b>50</b> , 1513-1529 e9 (2019)
60RO	Barnes, C.O. et al. Structural characterization of a highly-potent V3-glycan broadly neutralizing
	antibody bound to natively-glycosylated HIV-1 envelope. Nat Commun 9, 1251 (2018)
6CH7	Escolano, A. et al. Immunization expands B cells specific to HIV-1 V3 glycan in mice and
	macaques. <i>Nature</i> 570, 468-473 (2019)

## References

- 1. Zheng, S.Q. et al. MotionCor2: anisotropic correction of beam-induced motion for improved cryo-electron microscopy. *Nat Methods* **14**, 331-332 (2017).
- 2. Zhang, K. Gctf: Real-time CTF determination and correction. *J Struct Biol* **193**, 1-12 (2016).
- 3. Zivanov, J. et al. New tools for automated high-resolution cryo-EM structure determination in RELION-3. *Elife* **7** (2018).
- 4. Scheres, S.H. RELION: implementation of a Bayesian approach to cryo-EM structure determination. *J Struct Biol* **180**, 519-30 (2012).