Figure S1

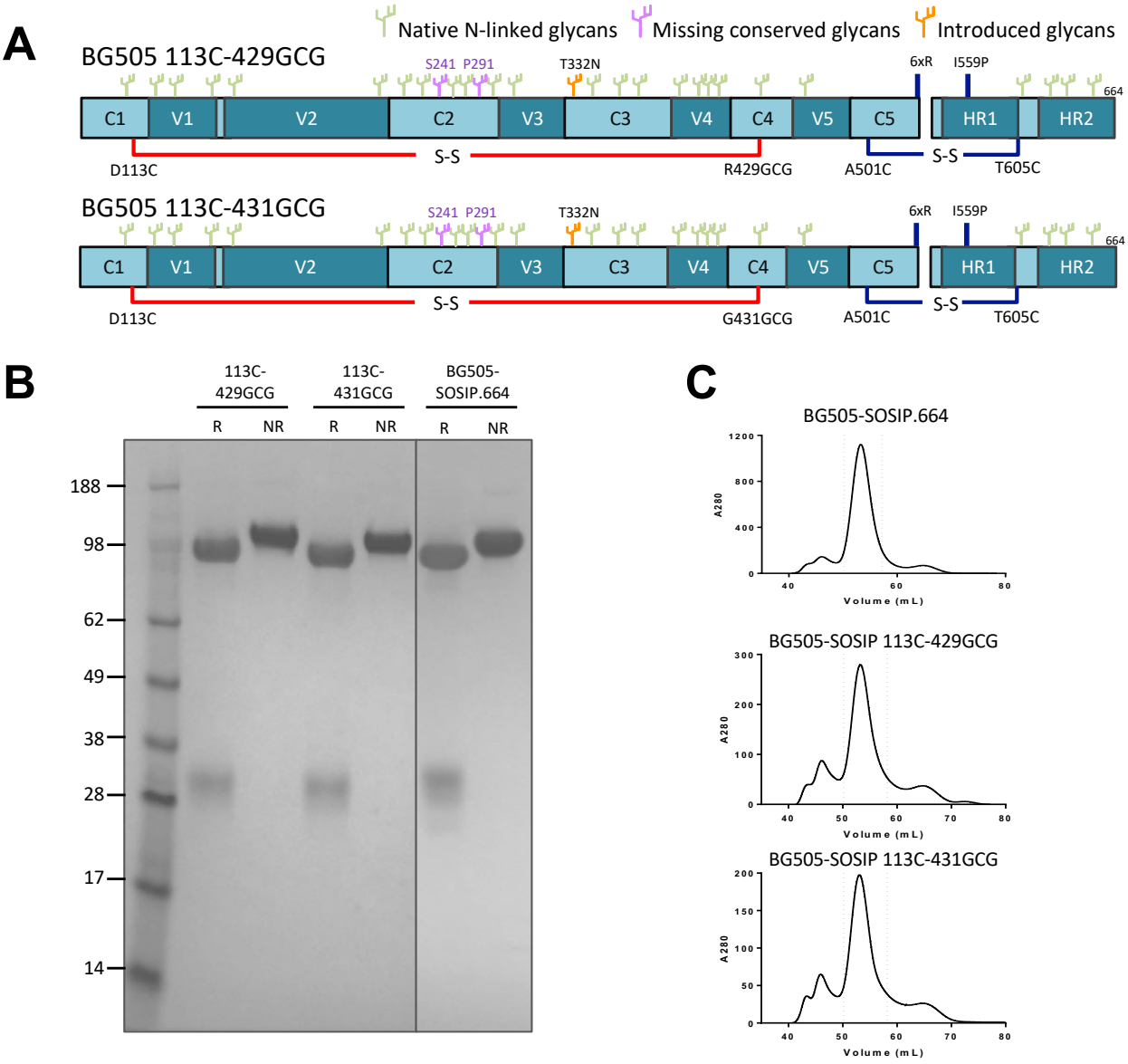


Figure S1. Characteristics of interdomain-locked BG505-SOSIP.664 trimers, Related to Figure 1
(A) Linear schematic of two locked BG505 SOSIP.664 trimers: 113C-429GCG and 113C-431GCG. The neo-disulfide bonds are indicated in red. Missing glycans that are conserved in more than 60% of HIV-1 isolates are indicated in purple. (B) SDS-PAGE analysis of unmutated and interdomain-locked BG505 SOSIP.664 trimers expressed in 293-FreeStyle cells using a 4-12% Bis-Tris Nu-PAGE gel under reducing and non-reducing conditions, followed by Coomassie blue staining. (C) SEC profile of unmutated and interdomain-locked BG505 SOSIP.664 trimers expressed in 293-FreeStyle cells.

Figure S2

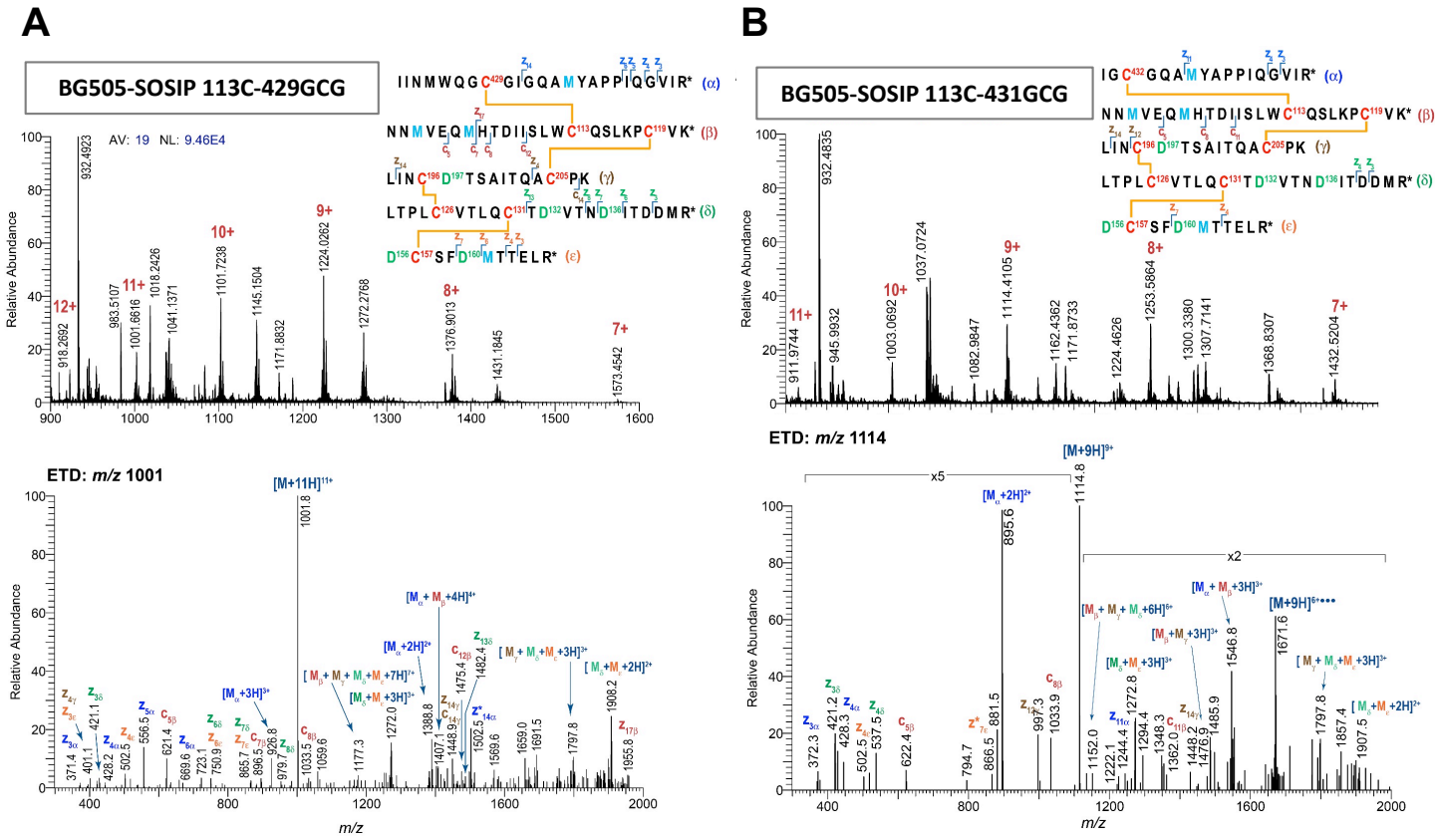


Figure S2. LC-MS analysis of disulfide bonds in two interdomain-locked BG505 SOSIP.664 trimers, Related to Figure 1

(A) Data for BG505-SOSIP 113C-429GCG. Top panel: Raw LC-MS data showing the elution of the relevant disulfide-linked peptide, abundantly ionizing in multiple charge states, as labeled on the spectrum. Bottom panel: ETD data for the ion in the 11+ charge state, m/z 1001. This spectrum clearly confirms the composition of the ion, as depicted on the top panel. C and Z ions are abundant in the spectrum, confirming the peptide sequence. Additionally, ions corresponding to cleavage across one or more disulfide bonds confirm the disulfide connectivity.

(B) Data for BG505-SOSIP 113C-431GCG. Top panel: Raw LC-MS data showing the elution of the relevant disulfide-linked peptide, abundantly ionizing in multiple charge states, as labeled on the spectrum. Bottom panel: ETD data for the ion in the 9+ charge state, m/z 1114. This spectrum clearly confirms the composition of the ion, as shown on the top panel. C and Z ions, along with ions resulting from disulfide cleavage, are labeled.

Figure S3

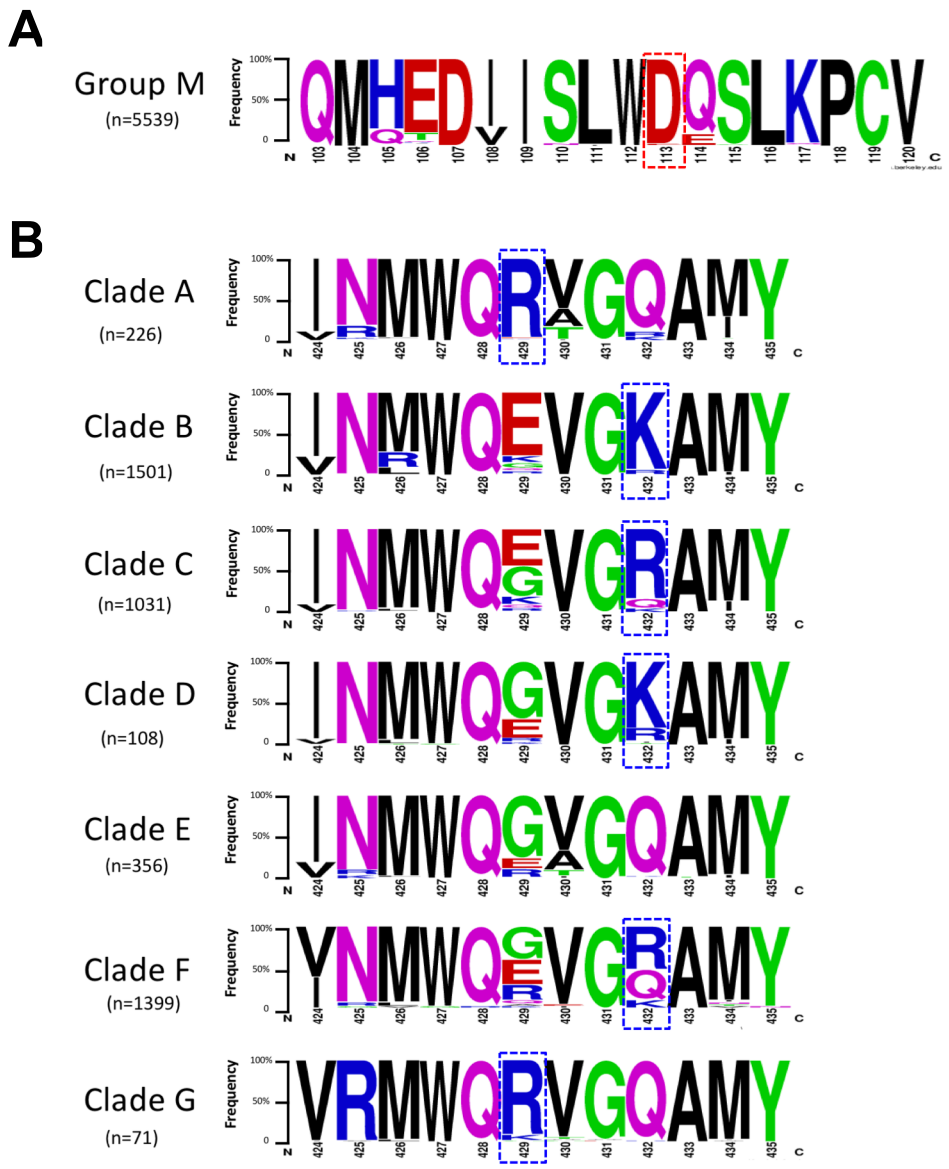
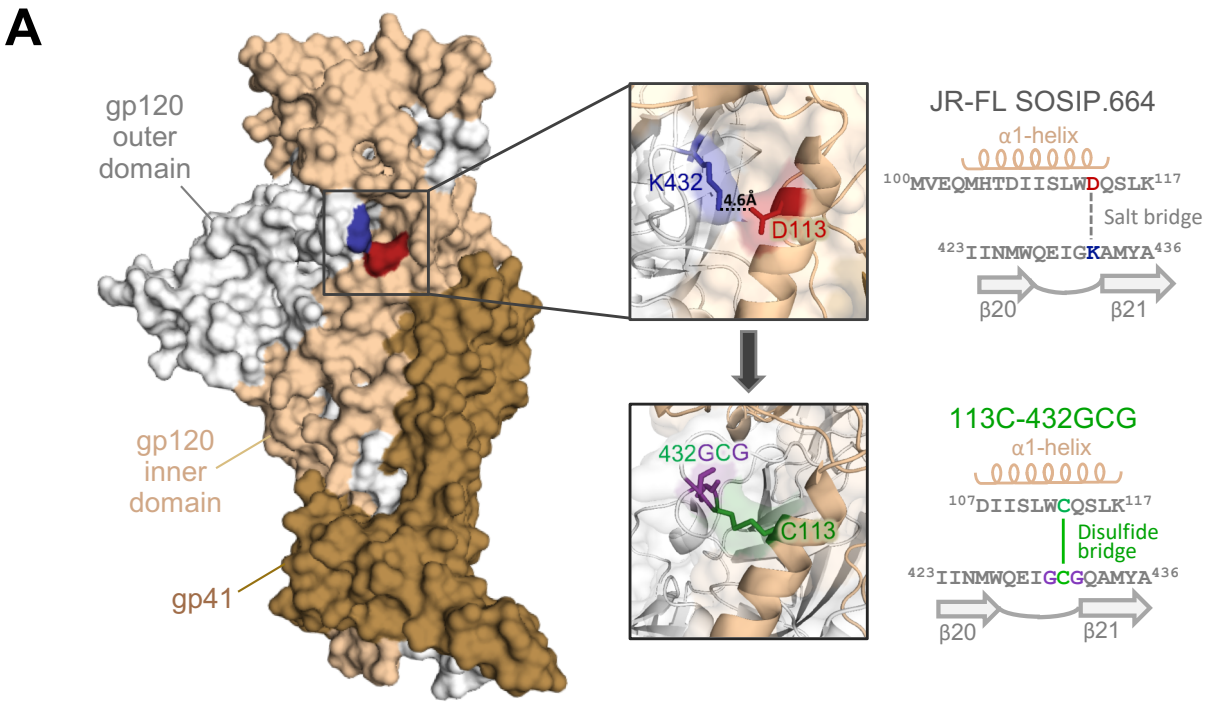


Figure S3. Amino acid conservation within the inner-domain α1-helix region and the outer-domain β20-β21 loop region of HIV-1 gp120, Related to Figure 1

(A) Residue D113 in the α1-helix is nearly universally conserved across all group-M HIV-1 isolates. (B) A conserved positively-charged amino acid (K or R) within the β20-β21 loop is frequently present at position 429 or 432 depending on the HIV-1 subtype. The height of each stack indicates the degree of conservation of each residue; the height of each letter represents the relative frequency of the indicated amino acid at that site.

Figure S4



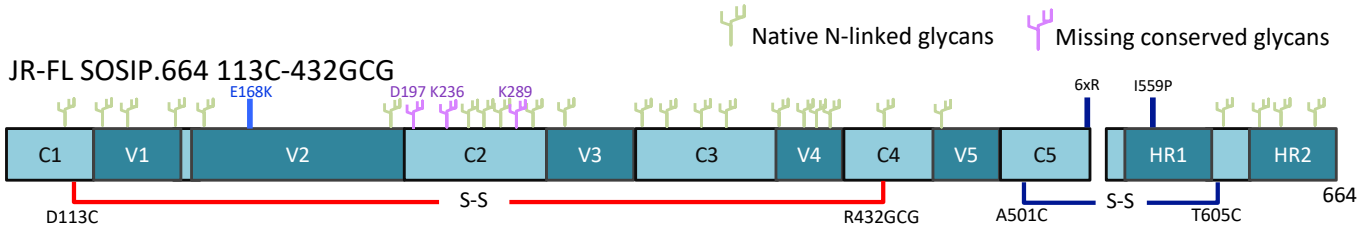
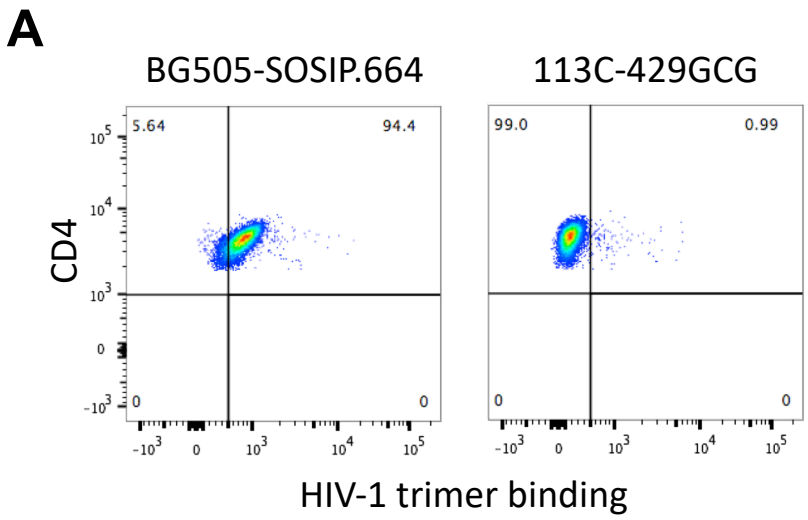


Figure S4. Structure-based design of an interdomain-locked HIV-1 Env trimer derived from a clade-B isolate (JR-FL), Related to Figure 1

(A) Intramolecular interaction between the gp120 inner-domain α1-helix and the outer-domain β20-β21 loop in a clade-B soluble trimer (JR-FL SOSIP.664) and structure-based design of an interdomain-locked HIV-1 Env trimer. A single protomer from a crystal structure of the JR-FL SOSIP.664 trimer (PDB ID: 5FYK; Stewart-Jones et al., 2016) is illustrated in surface representation, highlighting the intramolecular contact between R432 in the outer-domain β20-21 loop (blue) and D113 in the inner-domain a1-helix (red) of gp120. The upper inset shows a semi-transparent magnification of the interactive region with the side chains of K432 and D113 highlighted in stick representation, with the neighboring sequences reported in the scheme on the right. The design of an interdomain-locked mutant (D113C-R432GCG) bearing a neo-disulfide bridge (green) is illustrated in the lower inset, with the sequences reported in the scheme on the right. (B) Linear schematic of the interdomain-locked JR-FL mutant D113C-R432GCG. The neo-disulfide bond is indicated in red. Missing glycans that are conserved in more than 60% of HIV-1 isolates are indicated in purple.

Figure S5



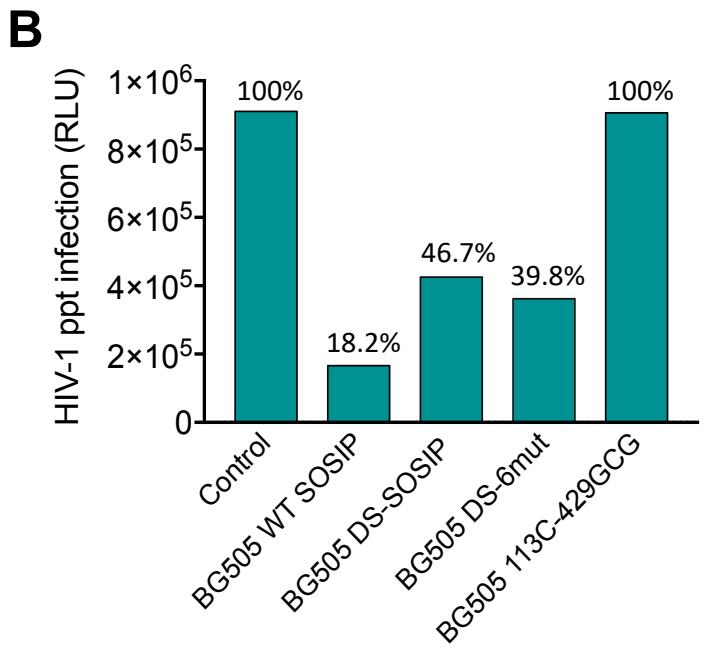


Figure S5. Lack of biologically-relevant interaction of stabilized trimers with the CD4 receptor, Related to Figure 2

(A) Binding of unmutated and interdomain-stabilized BG505 SOSIP.664 trimers to primary human CD4⁺ T cells as assessed by flow cytometry. (B) Inhibition of HIV-1 BG505 pseudovirus infectivity by soluble unmutated and interdomain-stabilized BG505 SOSIP.664 trimers in human CD4⁺ cells (TZM-bl). Two soluble trimers with reduced CD4-binding activity, i.e., DS-SOSIP (I201C-A433C) and DS-SOSIP.6mut (I201C-A433C + L154M, Y177W, N300M, N302M, T320L, I420M), were tested in parallel as controls.

Figure S6

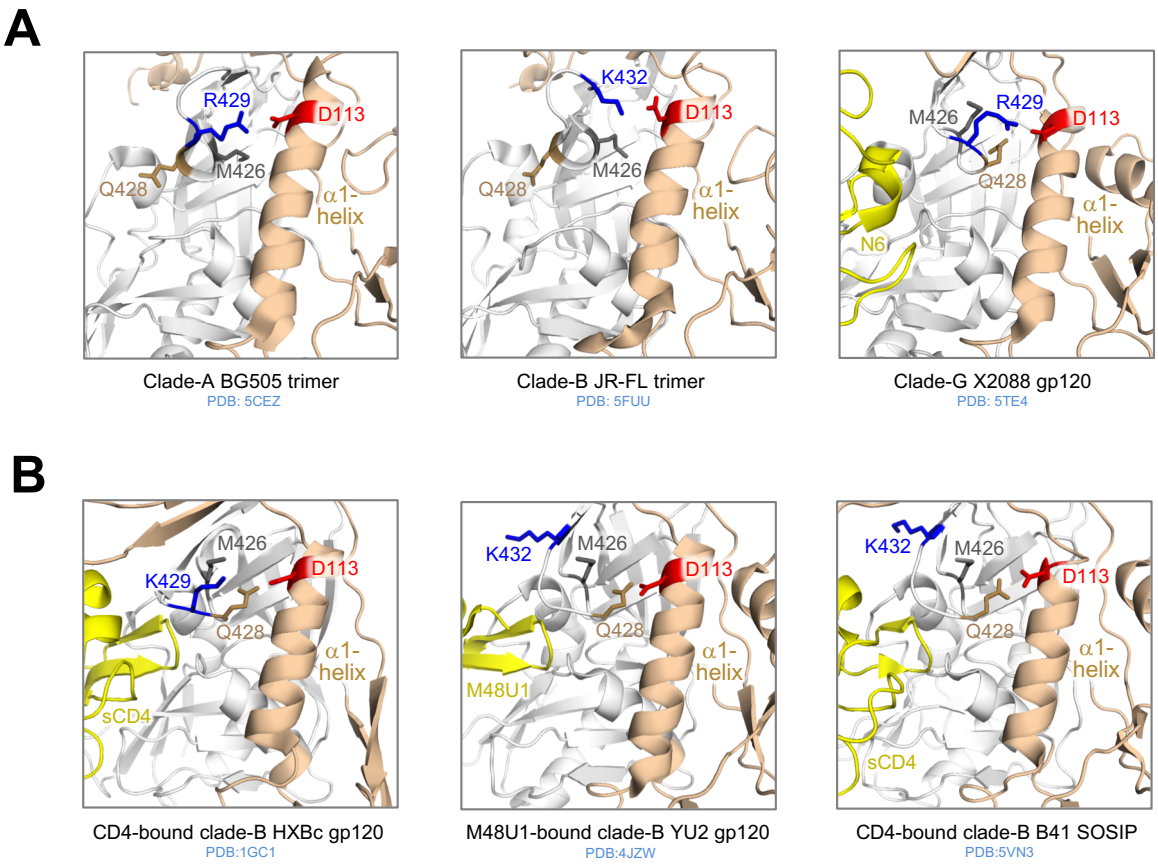


Figure S6. Interaction between the α1-helix and the β20-21 loop in different trimeric and monomeric HIV-1 gp120 structures, Related to Figure 3

(A) In the pre-fusion conformation from different structures, R429/K432 in the β20-21 loop points toward D113 in the α1-helix, in a favorable position for salt-bridge formation, while Q428 points in a different direction. Although the clade-G X2088 structure is based on monomeric gp120 and is not unliganded, it maintains the D113-R429 salt bridge because binding to the broadly neutralizing antibody N6 stabilizes the native gp120 conformation. (B) In the bound state from different structures (complexed with sCD4 or the CD4 miniprotein M48U1), K429/432 rotates outward, while Q428 moves close to D113 and M426 flips upwards. The outer domain of gp120 is shown in grey; inner domain in beige; gp120 ligands (N6, CD4 and M48U1) in yellow. Key residues are shown in stick representation.

Figure S7

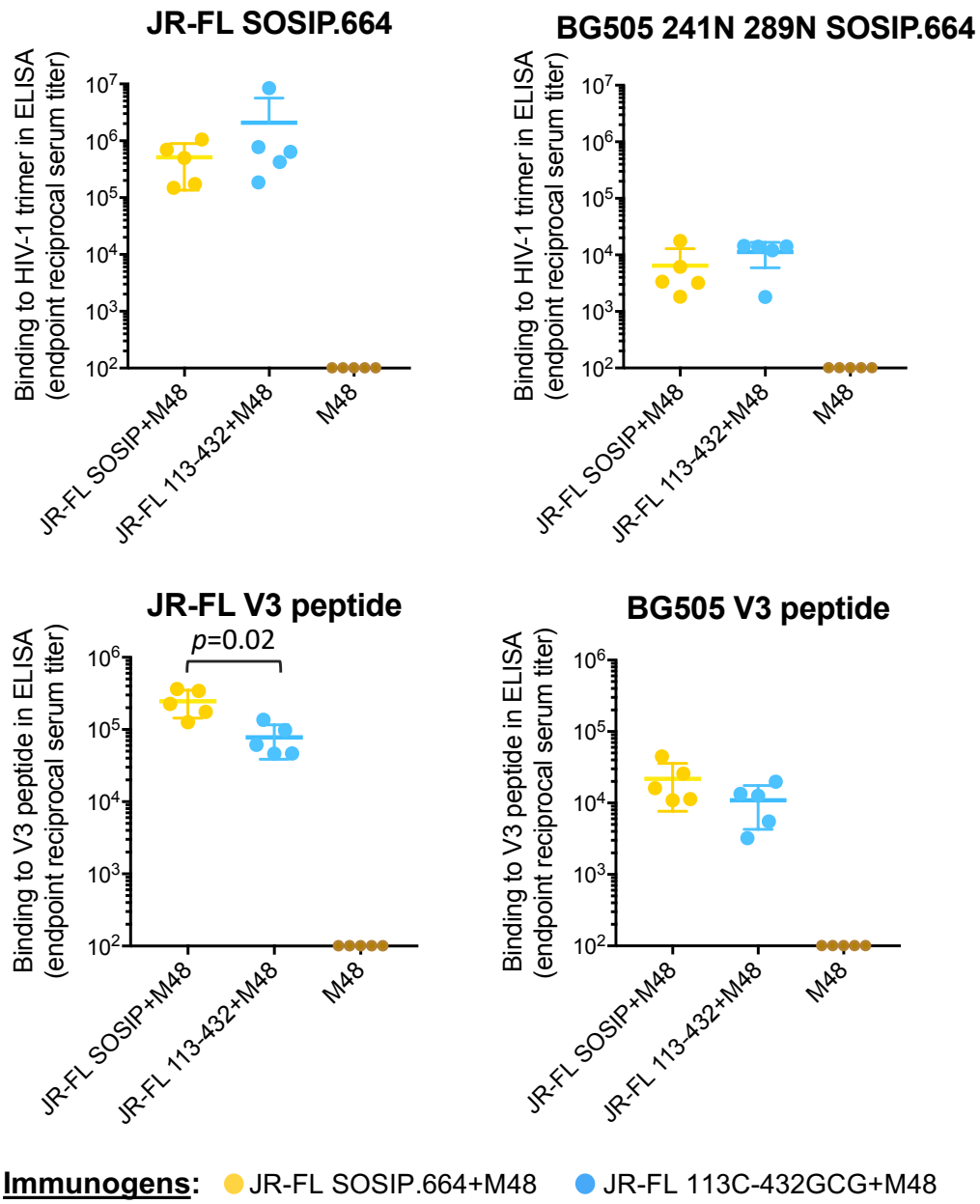


Figure S7. Reactivity of immune rabbit sera with immobilized HIV-1 Env trimers or V3 peptides, as tested by ELISA, Related to Figure 7.

Endpoint trimer- or peptide-binding titers are shown for week-16 sera obtained from rabbits immunized with unmutated or interdomain-locked HIV-1 JR-FL SOSIP.664 trimers in the presence of the CD4 mimic, M48U1. Serially-diluted rabbit sera were tested on JR-FL SOSIP.664 or glycan-repaired BG505 (241N 289N) trimers, as well as on both autologous (JR-FL) and heterologous (BG505) linear V3 peptides. The data show endpoint titers for individual rabbits with geometric mean and standard deviation. No statistically significant differences were found between groups as calculated by a two-tailed Mann-Whitney test using GraphPad Prism 7.

Table S1. Reactivity of WT and mutated BG505-SOSIP.664 trimers with a panel of anti-HIV-1 Env antibodies in ELISA, Related to Figure 1.

429 mutants		sCD4	2G12	VRC01	PG16	PGT121	PGT145	35O22
WT	WT	1.04*	1.00	1.00	0.61	0.85	0.80	0.11
D113C	WT	0.08	1.00	0.80	0.14	0.50	0.05	0.02
WT	R429C	0.10	1.00	0.78	0.22	0.55	0.21	0.06
D113C	R429C	0.11	1.00	0.23	0.03	0.27	0.04	0.02
D113C	R429GCG	0.03	1.00	0.97	0.66	0.75	0.72	0.11
D113C	R429GGCG	0.03	1.00	0.80	0.41	0.55	0.41	0.05
D113C	R429GCGG	0.28	1.00	0.90	0.44	0.74	0.48	0.06
D113C	R429GGCGG	0.09	1.00	0.99	0.53	0.68	0.39	0.04
D113C	R429GSCSG	0.34	1.00	0.99	0.37	0.68	0.29	0.05
D113C	R429GCGSG	0.49	1.00	0.92	0.45	0.74	0.48	0.06
D113C	R429GSGCGSG	0.79	1.00	0.94	0.60	0.79	0.63	0.07
D113C	R429GCIGG	0.24	1.00	0.92	0.31	0.62	0.23	0.05

432 mutants		sCD4	2G12	VRC01	PG16	PGT121	PGT145	35O22
WT	WT	1.04	1.00	1.00	0.61	0.85	0.80	0.11
D113C	G431GC	0.31	1.00	0.78	0.31	0.63	0.35	0.05
D113C	G431GCG	0.03	1.00	0.98	0.64	0.66	0.69	0.10
D113C	G431GCGG	0.66	1.00	0.98	0.18	0.58	0.11	0.05
D113C	G431GSGCGSG	0.23	1.00	0.90	0.44	0.63	0.38	0.05
D113C	Q432C	0.21	1.00	0.98	0.50	0.68	0.69	0.05
D113C	Q432CG	0.09	1.00	0.94	0.65	0.72	0.64	0.07
D113C	Q432CGG	0.15	1.00	0.90	0.52	0.66	0.43	0.05
D113C	Q432CI	0.41	1.00	0.85	0.06	0.27	0.11	0.02
D113C	Q432CGI	0.10	1.00	0.82	0.39	0.61	0.38	0.05
D113C	G431C Q432G	0.27	1.00	0.90	0.50	0.67	0.62	0.06
D113C	G431C Q432GG	0.47	1.00	0.97	0.32	0.53	0.21	0.03

*The results shown are optical density (OD) values at 450 nm, normalized relative to the value of the reference mAb 2G12 for each mutant. The unmutated BG505-SOSIP.664 trimer was expressed and tested in parallel as a control. The different shades of blue denote the intensity of binding relative to the wild-type (WT) trimer: dark blue, >90% of WT; medium dark blue, 70-90%; medium-light blue, 50-70%; light blue, 20-50%; white, <20%.

Table S2. X-ray structure statistics, Related to Figure 3.

	BG505-SOSIP 113C-429GCG
PDB ID	xxxx
Data collection	
Space group	$P6_3$
Cell dimensions	
a, *b*, *c* (Å)	128.65 128.65 312.92
a, b, g (°)	90.0, 90.0, 120.0
Resolution (Å)	50.0-4.30 (4.40-4.30)*; 50.0-4.10 (4.40-4.30, 4.30-4.20, 4.20-4.10)
$R_{merge\ (\%)}$	20.8 (48.8); 18.2 (43.9, 52.8, 72.6)
I / sI	7.4 (2.0); 8.7 (2.2, 1.8, 1.1)
Completeness (%)	81.0 (50.0); 75.6 (49.9, 45.7, 44.0)
Redundancy	7.3 (4.0); 6.6 (3.7, 3.6, 3.4)
Refinement	
Resolution (Å)	42.11-4.12 (4.27-4.12)
No. reflections	14133(503)
R_{work} / R_{free}	25.8/28.4
No. atoms (total)	11747
Protein	10964
Carbohydrate	783
B-factors (Å)^2	
Protein	74.4
Carbohydrate	79.3
R.m.s. deviations	
Bond lengths (Å)	0.006
Bond angles (°)	0.92

*Values in parentheses are for highest-resolution shell. Three crystals were used to determine the structure. Data processing statistics based on the overall resolution cutoff determined as: completeness greater than 50% and I/σI greater than 2, as mentioned in the Methods.

Table S3. Reactivity of unmutated and interdomain-locked SOSIP.664 trimers based on Env from different HIV-1 clades with anti-Env mAbs and sCD4 in ELISA, Related to Figure 2.

Env	Trimer	VRC01	2G12	PG9	PG16	PGT145	35O22	PGT122	sCD4
JR-FL	SOSIP.664	1.69*	1.52	0.38	0.41	1.20	0.63	1.13	1.56
	113C432GCG	1.79	1.59	0.45	0.48	1.33	0.75	1.55	0.07
B41	SOSIP.664	0.77	1.09	0.09	0.03	0.68	0.28	0.55	1.07
	113C432GCG	0.77	1.03	0.13	0.06	0.70	0.27	0.56	0.09
DU422.1	SOSIP.664	0.23	1.30	1.30	1.03	0.40	1.30	1.10	2.00
	113C432GCG	0.70	1.23	1.27	1.03	0.45	1.42	1.10	0.30

*Mean optical density (OD) values at 450 nm for each mAb and sCD4 are shown from a representative experiment performed in duplicate wells. The color codes denote increased (blue) or decreased (red) binding to each stabilized trimer compared to the unmutated form.

Table S4. Neutralization of BG505.T332N pseudoviruses by purified IgG from immunized rabbits, Related to Figure 6.

Viral pseudoparticles expressing WT BG505 (clade A, tier 2, with a large Env glycan hole) or a glycan-repaired form of BG505.T332N (N241 N289) were neutralized using purified serum IgG obtained from rabbits immunized with unmutated or interdomain-stabilized HIV-1 SOSIP.664 trimers in the presence or absence of the CD4 mimic M48U1 at the indicated weeks after the first immunization.

Immunogen	Rabbit ID	Week 0		Week 4		Week 8		Week 12		Week 16	
		BG505		BG505		BG505		BG505		BG505	
		WT	N241/ N289	WT	N241/ N289	WT	N241/ N289	WT	N241/ N289	WT	N241/ N289
BG505	1A	>400*	>400	41.0	79.7	3.5	9.5	3.1	6.5	1.7	4.7
	1B	>400	>400	>400	>400	9.0	90.1	1.4	24.9	1.5	20.2
	1C	>400	>400	>400	>400	41.1	>400	32.1	>400	10.5	>400
	1D	>400	>400	>400	>400	>400	>400	203	>400	288	>400
BG505 + M48U1	2A	>400	>400	>400	>400	7.2	6.8	2.7	6.3	3.4	5.8
	2B	>400	>400	>400	>400	23.4	>400	3.7	>400	2.1	>400
	2C	>400	>400	249	>400	4.9	7.5	0.8	2.9	1.3	3.6
	2D	>400	>400	>400	>400	73.0	>400	16.7	>400	10.5	>400
	2E	>400	>400	>400	>400	38.8	>400	6.7	>400	5.0	>400
BG505 113-429	3A	>400	>400	122	>400	24.8	>400	3.4	>400	5.3	>400
	3B	>400	>400	30.0	62.0	6.3	2.5	2.7	2.9	4.8	3.3
	3C	>400	>400	>400	>400	>400	83.5	0.8	53.5	88.2	54.5
	3D	>400	>400	>400	>400	48.1	20.4	11.7	36.8	1.6	6.5
	3E	>400	>400	>400	>400	19.6	36.4	3.1	2.8	1.4	1.1
BG505 113-429 + M48U1	4A	>400	>400	>400	>400	>400	>400	>400	>400	318	>400
	4B	>400	>400	>400	>400	4.9	5.4	5.6	5.8	2.8	1.9
	4C	>400	>400	>400	>400	20.1	24.3	22.2	10.6	4.7	2.9
	4D	>400	>400	>400	>400	5.1	12.5	18.7	4.9	4.4	7.7
	4E	>400	>400	>400	>400	90.0	301	2.9	33.2	2.8	5.4
M48U1	7A	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400
	7B	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400
	7C	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400
	7D	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400
	7E	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400

IC50 (IgG, mg/ml):	>100	20-100	4-20	0.8-4	<0.8

*Half-maximal inhibitory IgG concentrations (IC_{50}, μg/ml) are shown from a representative neutralization experiment performed in duplicate wells in TZMbl cells. For the immunization schedule, see Figure 6A.

Table S5. Neutralization of JR-FL pseudoviruses by purified IgG from immunized rabbits, Related to Figure 7.

Viral pseudoparticles expressing WT JR-FL pseudovirus (clade B, tier 2) or a glycan-repaired form of JR-FL (N197) were neutralized using purified serum IgG obtained from rabbits immunized with unmutated or interdomain-stabilized HIV-1 SOSIP.664 trimers in the presence or absence of the CD4 mimic M48U1 at the indicated weeks after the first immunization.

Immunogen	Rabbit ID	Week 0		Week 4		Week 8		Week 12		Week 16	
		JR-FL		JR-FL		JR-FL		JR-FL		JR-FL	
		WT *	N197	WT	N197	WT	N197	WT	N197	WT	N197
JR-FL + M48U1	5A	>400	nt	>400	nt	>400	>400	179	320	129	276
	5B	>400	nt	>400	nt	>400	>400	>400	>400	>400	>400
	5C	>400	nt	>400	nt	285	>400	187	178	68.2	284
	5D	>400	nt	>400	nt	188	24.9	166	75.2	143	81.4
	5E	>400	nt	>400	nt	>400	>400	>400	>400	220	>400
JR-FL 113-432 + M48U1	6A	>400	nt	>400	nt	26.5	121	23.3	98.8	48.3	250
	6B	>400	nt	>400	nt	63.7	128	20.0	26.6	30.1	14.6
	6C	>400	nt	>400	nt	28.3	260	22.1	94.1	7.1	11.9
	6D	>400	nt	>400	nt	266	29.1	154	66.3	54.6	11.6
	6E	>400	nt	>400	nt	120	>400	174	144	65.3	97.6
M48U1	7A	>400	nt	>400	nt	>400	nt	>400	nt	>400	nt
	7B	>400	nt	>400	nt	>400	nt	>400	nt	>400	nt
	7C	>400	nt	>400	nt	>400	nt	>400	nt	>400	nt
	7D	>400	nt	>400	nt	>400	nt	>400	nt	>400	nt
	7E	>400	nt	>400	nt	>400	nt	>400	nt	>400	nt

IC50 (IgG, mg/ml): >100 | 20-100 | 2-20 | 0.2-2 | 0.02-0.2

*Half-maximal inhibitory IgG concentrations (IC_{50}, μg/ml) are shown from a representative neutralization experiment performed in duplicate wells in TZMbl cells. nt = not tested. For the immunization schedule, see Figure 6A.

Table S6. Neutralization of heterologous pseudoviruses by purified IgG from immunized rabbit, Related to Figures 6 and 7.

Viral pseudoparticles expressing different HIV-1 Env were neutralized using purified serum IgG obtained from rabbits immunized with unmutated or interdomain-stabilized HIV-1 SOSIP.664 trimers in the presence or absence of the CD4 mimic M48U1 at week 16 after the first immunization.

Immunogen	Tier	1a	1b	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
	Clade	B	B	A	A	A	B	B	B	B	AC rec	CRF07_BC	CRF07_BC	C	C	C	C	C	C	AE	CRF01_AE	CRF01_AE	G
	Strain	SF162	Bal.01	BG505	RW020	Q769	JR-FL	Q168	TRO11	X2278	246F3	BJOX 002000	CH119	DU422.1	CE1176	WITO.33	TV1.29	CE1176	CE0217	CNE56	CNE55	CNE8	X1632
BG505	1A	43.5 *	82.6	1.7	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400
	1B	104	187	1.5	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400
	1C	26.3	51.6	10.5	>400	>400	>400	>400	>400	116.1	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400
	1D	251	>400	288	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400
BG505 + M48U1	2A	330	>400	3.4	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400
	2B	201	393	2.1	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400
	2C	94.7	56.7	1.3	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400
	2D	85.5	98.7	10.5	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400
	2E	60.9	110.8	5	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400
BG505 113-429	3A	>400	>400	5.3	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400
	3B	123	128	4.8	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400
	3C	57.3	69.2	88.2	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400
	3D	>400	207	1.6	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400
	3E	173	>400	1.4	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400
BG505 113-429 + M48U1	4A	72.1	123	318	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400
	4B	79.8	104	2.8	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400
	4C	67.0	56.9	4.7	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400
	4D	315.7	375.2	4.4	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400
	4E	39.5	88.9	2.8	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400
JR-FL + M48U1	5A	56.7	127	>400	>400	>400	129	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400
	5B	171	112	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400
	5C	23.9	21.7	>400	>400	>400	68.2	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400
	5D	159	135	>400	>400	>400	143	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400
	5E	56.4	161	>400	>400	>400	220	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400
JR-FL 113-432 + M48U1	6A	174	>400	>400	>400	>400	48.3	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400
	6B	105	250	237	>400	>400	30.1	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400
	6C	36.0	106	>400	>400	>400	7.1	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400
	6D	>400	>400	>400	>400	>400	54.6	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400
	6E	>400	>400	>400	>400	>400	65.3	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400
M48U1	7A	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400
	7B	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400
	7C	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400
	7D	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400
	7E	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400

IC50 (IgG, µg/ml): >100 | 20-100 | 4-20 | 0.8-4 | <0.8

*Half-maximal inhibitory IgG concentrations (IC_{50}, µg/ml) are shown from a representative neutralization experiment performed in duplicate wells in TZMbl cells. For the immunization schedule, see Figure 6A.