Supplementary information for

Co-crystal structures of antibody N60-i3 and antibody JR4 in complex with gp120 define more Cluster A epitopes involved in effective antibody-dependent effector function against HIV-1

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Table S1. Details of the N60-i3- and JR4-gp120$_{93TH057}$ core$_e$ interfaces as calculated by the EBI PISA server (http://www.ebi.ac.uk/msd-srv/prot_int/cgi-bin/piserver)

		N60-i3 Fab-gp120$_{93TH057}$ core$_e$-M48U1			JR4 Fab- gp120$_{93TH057}$ core$_e$-M48		
Buried Surface Area (BSA), Å^2		Heavy chain		Light chain	Heavy chain		Light chain
	gp120$_{93TH057}$ core$_e$ total	695			1145 (1148)*		
	7-stranded β-sandwich	2		0	121 (131)		0 (0)
	Layer 1	439		107	656 (655)		185 (198)
	Layer 2	144		3	183 (164)		0 (0)
		Layer 1	**Layer 2**	**7-stranded β-sandwich**	**Layer 1**	**Layer 2**	**7-stranded β-sandwich**
	Heavy chain total	657			988 (989)		
	FWR	4	12	1	192 (185)	14 (8)	0 (0)
	CDR H1	52	57	0	142 (144)	79 (65)	0 (1)
	CDR H2	154	0	0	63 (57)	48 (60)	21 (39)
	CDR H3	235	142	0	267 (279)	70 (61)	92 (90)
	Light chain total	114			208 (213)		
	FWR	0	0	0	31 (35)	0 (0)	0 (0)
	CDR L1	71	4	0	59 (49)	0 (0)	0 (0)
	CDR L2	0	0	0	118 (129)	0 (0)	0 (0)
	CDR L3	39	0	0	0 (0)	0 (0)	0 (0)
	Heavy and light chain total	771			1196 (1202)		
	Fab-gp120$_{93TH057}$ core$_e$ total	1466			2341 (2350)		

*** number in the brackets show BSA as calculated for second copy of JR4 Fab-gp120$_{93TH057}$ core$_e$-M48 complex in the asymmetric unit**

Table S2. Interactions at the N60-i3-gp120$_{93TH057}$ and JR4-gp120 core$_e$ interfaces. Accessible Surface Area, Å² (**ASA**) and Buried Surface Area, Å² (**BSA**) were calculated using the EBI PISA server (http://www.ebi.ac.uk/msd-srv/prot_int/cgi-bin/piserver) Vertical bars 'I' represent the buried area percentage, one bar per 10%.

N60-i3 Fab-gp120 93TH057 core$_e$-M48U1

	Heavy chain	ASA	BSA
	H: ILE 29	72.85	0.34 |
CDRH1	H:SER 30	66.54	16.09 |||
	H:SER 31	57.48	40.16 ||||||
	H:GLY 32	21.24	18.92 ||||||||
	H:GLY 33	24.18	24.18 ||||||||||
	H:TYR 34	36.53	10.69 |||
	H:PHE 35	20.18	15.32 |||||||
CDRH2	H:TYR 52	43.42	40.61 |||||||||
	H:TYR 53	68.62	36.74 ||||||
	H:ILE 54	98.99	52.24 ||||||
	H:ASN 56	64.79	17.84 |||
	H:TYR 58	88.33	6.31 |
CDRH3	H:ARG 97	113.20	76.08 |||||||
	H:LEU 98	120.56	84.50 |||||||
	H:ARG 99	239.92	194.91||||||||
	H:GLY 100	37.93	15.68 |||||
	H:ASN 100B	79.51	5.97 |

	gp120	ASA	BSA
Layer1	G:THR 51	112.44	47.77 |||||
	G:LEU 52	16.68	16.01 |||||||||
	G:PHE 53	80.16	70.31 ||||||||
	G:CYS 54	7.28	7.28 ||||||||||
	G:TRP 69	17.88	1.88 ||
	G:THR 71	77.17	9.56 ||
	G:HIS 72	101.61	21.96 |||
	G:ALA 73	60.28	50.80 ||||||||
	G:CYS 74	12.93	10.32 ||||||||
	G:VAL 75	65.92	61.92 |||||||||
	G:PRO 76	118.17	84.65 ||||||||
	G:THR 77	41.33	7.37 ||
	G:ASP 78	72.18	20.65 |||
	G:PRO 79	127.06	28.45 |||
Layer2	G:GLN 103	27.08	17.37 |||||||
	G:GLU 106	116.19	12.91 ||
	G:ASP 107	32.53	25.32 |||||||
	G:TYR 217	8.67	4.62 ||||||
	G:THR 219	10.55	5.89 ||||||
	G:PRO 220	45.37	34.28 |||||||
	G:ALA 221	92.55	43.36 |||||
7-strand β sand	G:GLN 246	74.22	1.65 |

	Light chain	ASA	BSA
CDRL1	L:TYR 30	67.79	28.38 |||||
	L:TYR 32	109.06	45.08 |||||
CDRL2	L:TYR 91	48.76	31.87 ||||||
	L:SER 94	101.18	0.17 |
	L:SER 95	73.32	7.03 |

	gp120	ASA	BSA
Layer1	G:ALA 60	86.29	0.25 |
	G:VAL 68	73.11	3.00 |
	G:THR 71	77.00	16.99 |||
	G:HIS 72	101.12	76.33 |||||||
	G:ALA 73	60.86	9.53 ||
Layer2	G:GLN 114	88.17	1.94 |

JR4 Fab- gp12093TH057 core$_e$-M48

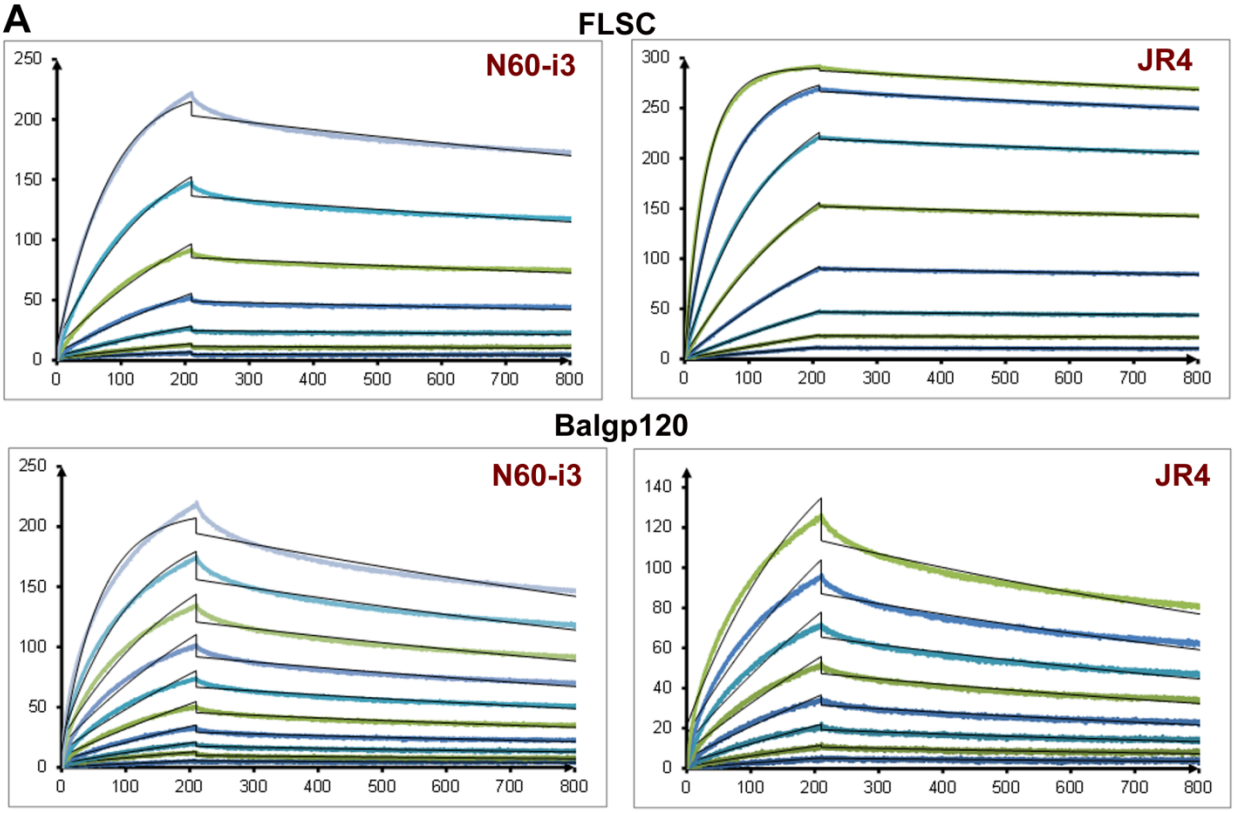
	Heavy chain	ASA	BSA
CDRH1	H:ARG 31	185.40	152.7 ||||||||
	H:ASN 32	26.39	24.86 ||||||||
	H:TYR 33	46.11	42.88 ||||||||
CDRH2	H:TYR 52	68.42	68.27 ||||||||
	H:SER 53	57.35	19.07 ||||
	H:GLY 54	54.64	13.87 |||
	H:SER 56	56.20	25.88 |||||
	H:ASN 58	69.48	4.74 |
CDRH3	H:THR 95	5.11	3.47 ||||||
	H:VAL 96	26.82	26.15 ||||||||
	H:TRP 97	66.80	21.06 ||||
	H:TYR 98	139.03	138.71||||||||
	H:TYR 99	179.31	71.83 ||||
	H:THR 100	127.12	60.00 ||||
	H:SER 100A	91.14	49.26 |||||
	H:THR 100C	55.48	19.08 ||||
	H:TYR 100E	108.14	28.72 |||
	H:ASP 101	56.06	9.56 ||
	H:HIS 102	42.22	0.87 |

	gp120	ASA	BSA
Layer1	G:THR 50	28.65	8.94 ||||
	G:THR 51	114.20	99.83 ||||||||
	G:LEU 52	15.37	15.04 ||||||||
	G:PHE 53	85.93	82.21 |||||||||
	G:CYS 54	10.95	10.65 |||||||||
	G:ALA 55	3.18	2.68 ||||||||
	G:ALA 60	94.50	10.09 ||
	G:VAL 68	51.91	0.83 |
	G:TRP 69	19.39	5.79 |||
	G:THR 71	41.66	21.07 |||||
	G:HIS 72	162.55	93.56 |||||
	G:ALA 73	57.95	49.24 ||||||||
	G:CYS 74	13.33	8.87 |||||
	G:VAL 75	70.22	68.83 ||||||||
	G:PRO 76	113.36	55.19 |||||
	G:THR 77	37.86	5.36 ||
	G:ASP 78	48.65	30.09 ||||||
	G:PRO 79	132.67	32.47 |||
	G:ASN 80	95.00	14.77 ||
	G:GLN 82	112.08	41.23 ||||
Layer2	G:GLN 103	28.14	1.75 |
	G:GLU 106	120.01	12.69 ||
	G:ASP 107	33.35	21.36 ||||||
	G:TYR 217	5.93	2.76 |||||
	G:PRO 220	54.67	48.86 ||||||||
	G:ALA 221	107.76	86.90 ||||||||
	G:GLY 222	17.61	8.15 |||||
7-stranded β sandwich	G:I84	108.38	4.84 |
	G:TYR 223	50.09	15.62 ||||
	G:VAL 224	32.56	5.27 ||
	G:GLN 246	101.64	80.71 ||||||||
	G:CYS 247	18.84	5.27 ||||
	G:GLU 492	153.88	9.18 |

	Light chain	ASA	BSA
CDRL1	L:TYR 32	137.99	58.83 |||||
CDRL2	L:ASP 50	57.83	28.01 |||||
	L:LYS 53	91.68	13.72 ||
	L:SER 56	119.44	76.04 |||||||

	gp120	ASA	BSA
Layer1	G:LYS 59	136.24	14.11 |
	G:ALA 60	94.50	19.35 |||
	G:HIS 61	121.99	12.17 ||
	G:PRO 76	113.36	33.65 |||
	G:THR 77	37.86	5.51 ||
	G:PRO 79	132.67	73.46 |||||
	G:ASN 80	95.00	26.56 |||

A



B

	FLSC	gp120	Fold change
mAb N60-i3			
K_D (M) x 10^{-9}	0.3 ± 0.01	2.0 ± 0.07	**6.7**
k_a(1/Ms) x 10^5	12.4 ± 0.01	2.5 ± 0.07	**5.0**
k_d(1/s) x10^{-5}	0.31 ± 0.01	0.5 ± 0.05	**1.6**
mAb JR4			
K_D (M) x 10^{-9}	0.2 ± 0.003	2.3 ± 0.20	**11.5**
k_a(1/Ms) x 10^5	6.4 ± 0.07	2.6 ± 0.09	**2.5**
k_d(1/s) x10^{-5}	0.12 ± 0.003	0.61 ± 0.02	**5.1**

Figure S1. mAb N60-i3 and mAb JR4 binding to monomeric gp120 and a single-chain $gp120_{BaL}$-CD4 complex (FLSC, (1)) as measured by Surface Plasmon Resonance (SPR). **(A)** Binding curves of different concentrations of $gp120_{BaL}$ and FLSC (0–50 nM, two-fold dilutions) injected over mAb N60-i3 or mAb JR4 captured on Protein A-coated sensor chips. The data were double referenced by subtraction of the blank surface (no antibody) and a blank injection (no analyte). Binding curves were globally fit to a 1:1 binding model. The experiments were repeated with similar results. **(B)** Binding kinetics of mAb N60-i3 and JR4 to monomeric $gp120_{BaL}$ and FLSC. Antibody association rates (k_{on}), dissociation rates (k_{off}), and affinity constants (K_D) were calculated with the BIAevaluation software.

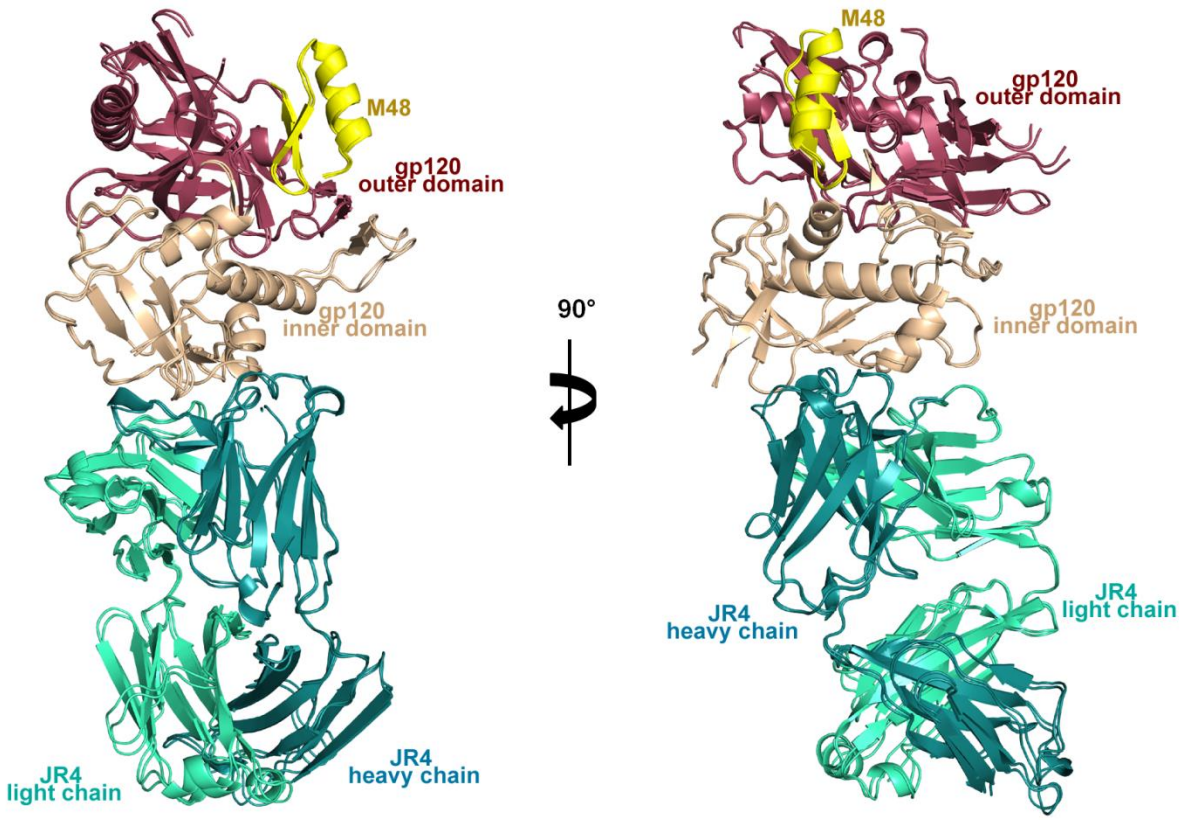


Figure S2. Ribbon diagram of superposition of the two copies of JR4 Fab-gp120$_{93TH057}$ core$_e$-M48 complex from the asymmetric unit of the crystal. The light and heavy chains of JR4 Fab are colored light cyan and dark cyan, respectively. The gp120 inner domain is shown in wheat, the outer domain in raspberry. The mimetic peptides M48U1 and M48 are colored yellow. The root mean square deviation (RMSD) between complexes for main chain atoms is 0.8 Å.

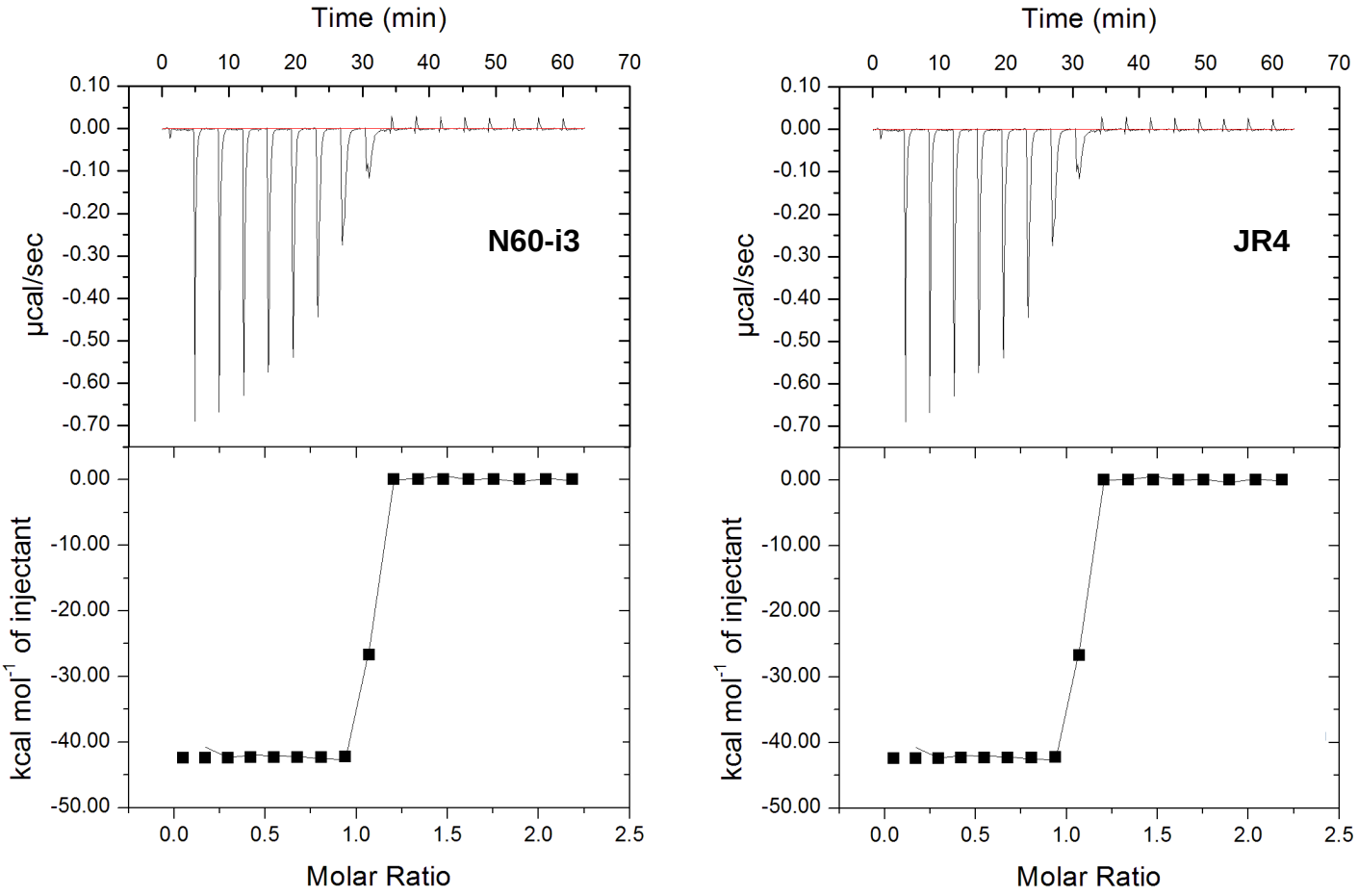


Figure S3. mAb N60-i3 and mAb JR4 binding to a single-chain gp120$_{BaL}$-CD4 complex (FLSC, (1)) as measured by ITC. Isothermal titration calorimetry (ITC) was used to characterize the interaction of FLSC with N60-i3 and JR4 IgGs. All proteins were dialyzed against 1X PBS pH 7.4 and experiments performed using an iTC200 instrument (GE Healthcare). A typical experiment had JR4 or N60-i3 in the syringe (111 µM) and FLSC in the cell (11 µM). Titrations were performed at 25 °C with 17 injections of 2.42 µl aliquots, with 210-240 second intervals between injections. Heats of dilutions were measured and subtracted from each data set. All data were analyzed using Origin 7.0 software. The results of the titration experiments, in which a fixed amount of FLSC was titrated against increasing concentration of N60-i3 or JR4 IgG, are shown. The ITC binding isotherm for both N60-i3 and JR4 against FLSC shows a "step" indicative of tight (<nM) binding, precluding an accurate determination of K_D, but allowing the determination of Δ*H* of −4.253(±0.23) and −2.19(±0.25) kcal/mol for the N60-i3 and JR4-bound complexes, respectively.

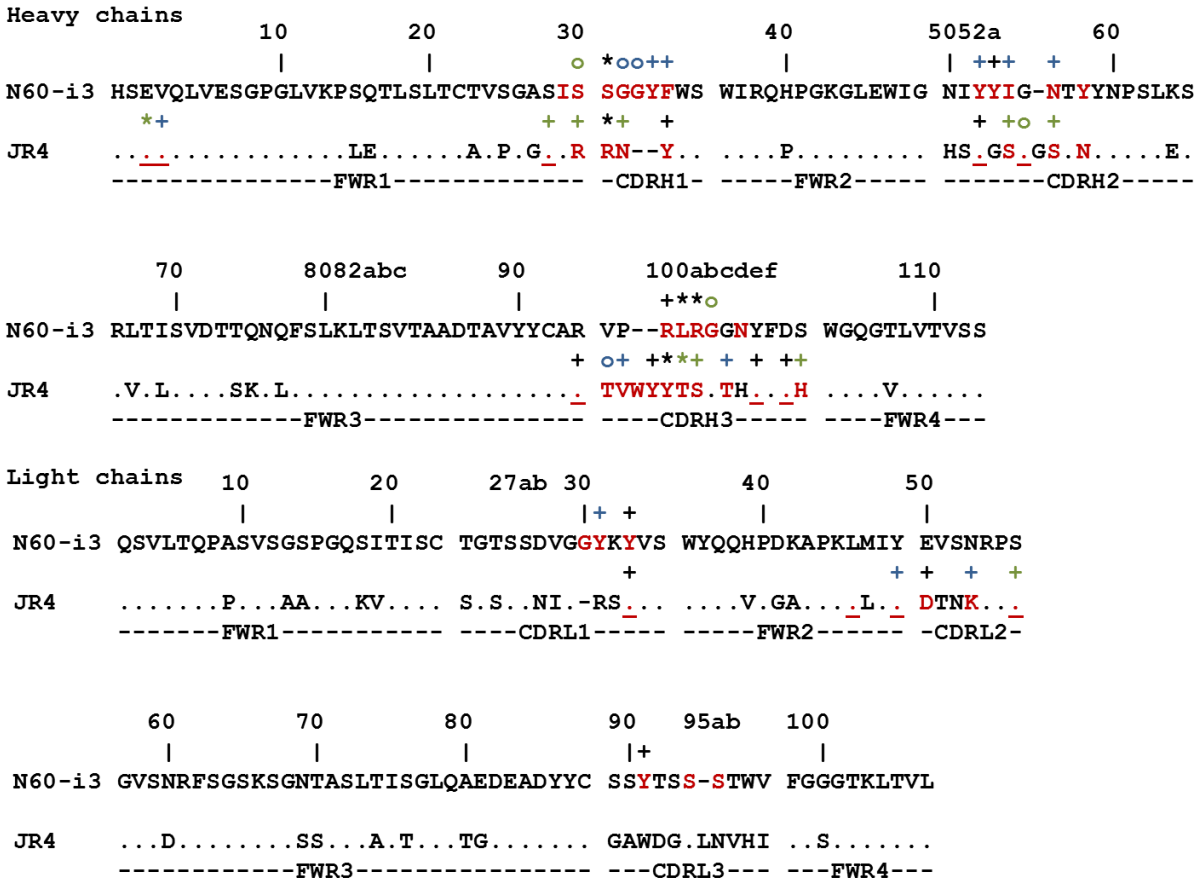


Figure S4. Residues in N60-i3 and JR4 involved the gp120 binding interface. Identical JR4 residues are shown with a dot in the alignment and gaps with a dash. Buried residues are highlighted red (and underlined in JR4 when a dot is used). Main chain (o), side chain (+), and both main and side chain (*) interactions are shown immediately above the residue as defined by a 5 Å distance criteria cutoff and colored based on contact type, hydrophobic (blue), hydrophilic (green), or both (black). Framework and complementary-determining regions are indicated below the alignment. Only the variable regions of the heavy and light chains for both antibodies are shown. The light chain contacts of N60-i3 Fab are largely confined to tyrosine residues; Tyr^{30} (framework region L1 [FWR L1]), Tyr^{32} (CDR L1), and Tyr^{91} (CDR L3) contacting α0-helix (Layer 1) in the Thr^{71}HisAlaCysValPro motif of gp120. JR4 involves Tyr^{32} (CDR L1), Tyr^{49} (FWR L1) and Asp^{50}, Leu^{53} and Ser^{56} (CDR L2) to contact the β$\bar{1}$-strand and β$\bar{1}$-β0-connecting coil (Layer 1) in the Thr^{71}HisAlaCysValPro and Asn^{80}ProGln motifs of gp120 (Figure 5). In both complexes, the rest of the binding contacts to Layer 1 and all contacts with Layer 2 and the 7-stranded β-sheet are provided by the heavy chain of the Fab with CDR H3 contributing the most to the contact interface. In N60-i3, Arg^{97} and Arg^{99} (CDR H3) represent 41% of the heavy chain binding surface and make up the bulk of N60-i3's electrostatic contribution to the binding interface including $Glu^{106}Asp^{107}$ in the α1-helix (Layer 2) of gp120. Hydrophobic contributions to the interface come from Phe^{35} (CDR H1), Tyr^{52}, Tyr^{53}, Ile^{54} (CDR H2), Leu^{98} (CDR H3), Tyr^{30}, Tyr^{32} (CDR L1), and Tyr^{91} (CDR L3). Main chain residues from Gly^{32}, Ser^{31}, Gly^{33} (CDR H1), Leu^{98}, Arg^{99}, and Gly^{100} (CDR H3) further stabilize the interface. Ile^{29} and Ser^{30} from FWR H1 contribute slightly to the interface, but the binding interface occurs primarily within the complementary-determining regions.

In JR4, Arg^{30} (FWR H1), Arg^{31} (CDR H1), and Arg^{94} (FWR H3) provide the bulk of the electrostatic contribution to the interface including $Glu^{106}Asp^{107}$ in the α1-helix (Layer 2) of gp120, but only makes up approximately 20% of the interface (25% of the total heavy chain contribution). JR4's contact with gp120 is more extensive than N60-i3 but with fewer main chain contributions to the interface, Glu^{1} (FWR H1), Arg^{31} (CDR H1), and Thr^{100} (CDR H3) being the exceptions. Approximately 18% of the buried surface area of JR4 comes from outside of the traditional complementary-determining regions with contributions from largely adjacent residues from FWR H1, FWR H3, and FWR L2. The hydrophobic contribution of Tyr^{33} (CDR H1), Tyr^{52} (CDR H2), Tyr^{98}, Tyr^{99}, Tyr^{100E} (CDR H3) Tyr^{32} (CDR L1), and Tyr^{49} (CDR L2) is coupled with residues Val^{2} (FWR H1), Val^{96}, Trp^{97} (CDR H3) and Leu^{46} (CDR L2) and represents a greater proportion of the total buried surface area implying that charge is not the major stabilizing factor in the JR4 binding interface. A full list of contacts can be found in supplemental Table S2.

1. **Fouts TR, Tuskan R, Godfrey K, Reitz M, Hone D, Lewis GK, DeVico AL.** 2000. Expression and characterization of a single-chain polypeptide analogue of the human immunodeficiency virus type 1 gp120-CD4 receptor complex. J Virol **74:**11427-11436.