Supporting Information

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SI Materials and Methods

Production of HIV-1 Envelope Proteins, CD4, and Antibodies. The expression constructs of 92UG037.8 and CZA97012 gp140 trimers were described previously (1, 2). The codon-optimized synthetic genes of the full-length gp120s with a C-terminal His-tag were produced by GeneArt. The 293T cell lines stably transfected with these envelope constructs were generated by Codex Biosolutions. The stable cell lines were grown in DMEM with 10% FBS to confluence and then were changed to Freestyle 293 expression medium (Invitrogen). The cell supernatants were harvested at 96–108 h after medium change. The His-tagged gp120 and gp140 proteins were purified by Ni-NTA (Qiagen) followed by gel-filtration chromatography as described (1). Briefly, after a clarifying spin and the addition of imidazole to the final concentration of 15 mM, the cell supernatant was loaded onto a nickel column at a flow rate of 0.8 mL/min and was washed with 20 mM imidazole in PBS followed by further washing with 40 mM imidazole in PBS. The protein then was eluted with 300 mM imidazole in PBS. The fractions containing the purified protein were pooled, concentrated, and further purified by gel-filtration chromatography on Superose 6 (GE Healthcare) for gp140 trimers and Superdex 200 (GE Healthcare) for gp120s in a column running buffer containing 25 mM Tris (pH 7.5) and 150 mM NaCl. The purified proteins were concentrated, frozen in liquid nitrogen, and stored at –80 °C. Soluble two-domain and four- domain CD4 were produced as described (3). The VRC01 expression construct was kindly provided by John Mascola (Vaccine Research Center, National Institutes of Health, Bethesda, MD); the CHO stable line expressing antibody b6 was provided by Dennis Burton (Scripps); the 17b hybridoma was provided by James Robinson (Tulane University, New Orleans, LA); and A244 gp120 gD (+) was provided by Larry Liao and Barton Haynes (Duke University, Winston Salem, NC). Antibodies PG9, PG16, and 2G12 used in the pilot experiments were kindly provided by Dennis Burton and Ian Wilson (The Scripps Research Institute). Subsequently, we generated expression constructs of these antibodies, as well as PGT123, CH31, 2F5, and 4E10, using synthetic genes made by GeneArt. The intact antibodies or Fab fragments then were expressed in 293T cells either by transient transfection or using selected stably transfected clones and were purified by affinity chromatography using Gammabind-Plus resin (GE Healthcare) followed by gel-filtration chromatography. Fab preparations by papain digestion were carried out as described (3).

Surface Plasmon Resonance Binding Assays. All experiments were performed in duplicate with a Biacore 3000 instrument (Biacore, Inc.) at 20 °C in HBS-EP running buffer [10 mM Hepes (pH 7.4), 150 mM NaCl, 3 mM EDTA, 0.005% P20], with immobilization levels of 250–600 response units (RU) to avoid rebinding events. Immobilization of CD4, protein A (Sigma), or human Fab binder (Biacore) to CM5 chips was performed following the standard amine coupling procedure as recommended by the manufacturer. For kinetic measurements, sensorgrams were recorded by passing various concentrations of an analyte over the immobilized ligand surface at a flow rate of 50 μL/min with a 2-min association phase followed by a 10-min dissociation phase. The surface was regenerated between each experiment with two consecutive injections (1 min) of 10 mM glycine·HCl (pH 2.1) at 50 μL/min followed by a 5-min equilibration phase in the HBS-EP buffer before the subsequent experiment. Identical injections over blank surfaces were subtracted from the data for kinetic analysis. Binding kinetics were analyzed by BiaEvaluation software (Biacore) using a 1:1 Langmuir binding model. All injections were carried out in duplicate and gave essentially identical results.

Multiangle Light Scattering. Purified envelope proteins or their complexes with four-domain CD4, or with VRC01 Fab, or with CD4 and 17b Fab were resolved on a Superdex 200 size-exclusion column (GE Healthcare) in a running buffer containing 25 mM Tris (pH 7.5) and 150 mM NaCl. The gel-filtration chromatography was coupled to a Wyatt miniDAWN TREOS three-angle light-scattering detector and an Optilab-rEX refractive index detector (Wyatt Technology). The recorded scattering data were evaluated using ASTRA 5.3.4 software.

Analytical Ultracentrifugation. Analytical ultracentrifugation was performed on a Beckman-Coulter ProteomeLab XL-A analytical ultracentrifuge as described previously (4). Briefly, experiments were carried out at 4 °C in a buffer containing 25 mM Tris (pH 7.5) and 150 mM NaCl with three protein concentrations of 0.25, 0.50, and 0.75 absorbance units at 280 nm, respectively. Three rotor speeds (5,000, 7,000, and 9,000 rpm) were used for the gp140s and their complexes, and three higher speeds (7,000, 10,000, and 13,000 rpm) were used for the gp120s with Beckman An-60 Ti rotor. Equilibrium was reached in 30 h. Data were fitted to a single-species model using the XL-A software package. The carbohydrate contents of the gp120s and gp140s were estimated to be ~43% and ~55%, respectively, based on their amino acid sequences and SDS/PAGE analysis. The average partial specific volumes of a *N*-linked oligosaccharide and the polypeptide chains of the envelope proteins were calculated to be 0.64 and 0.73 mL/g, respectively, according to the methods reported previously (5, 6). Thus, the calculated partial specific volume is 0.6769 mL/g for the gp120s and 0.6874 mL/g for the gp140s. For the complexes of gp140 and CD4 binding site ligands, the partial specific volumes varied only slightly when all possible stoichiometries for a given complex were considered and had very little impact on the determined molecular masses. For example, the partial specific volume of the 92UG037.8 gp140-CD4 complex is 0.6918 mL/g for the assumed stoichiometry of 1:1, 0.6955 mL/g for the stoichiometry of 1:2, and 0.6976 mL/g for the stoichiometry of 1:3; and the corresponding molecular mass is 456 ± 5 kDa, 458 ± 4 kDa, and 461 ± 3 kDa, respectively, all values that are consistent with a stoichiometry of 1:1. The partial specific-volume values used in the Table S1 are 0.6918 mL/g for the gp140-CD4 complex; 0.6981 mL/g for the gp140-VRC01 Fab complex; and 0.6955 mL/g for the gp140-CD4-17b Fab complex.

Animals and Immunizations. Outbred female Hartley guinea pigs (Elm Hill Labs) were housed at the Animal Research Facility of Beth Israel Deaconess Medical Center under protocols approved by the Institutional Animal Care and Use Committee. Guinea pigs were immunized by bilateral i.m. injections in the upper quadriceps of gp140s or gp120s (100 μg/animal) at 4-wk intervals (weeks 0, 4, and 8) using 500 μL of a dual adjuvant combination comprising 15% (vol/vol) oil-in-water Emulsigen (MVP Laboratories)/PBS and 50 μg of immunostimulatory di-nucleotide CpG DNA (5′-TCGTCGTTGTCGTTTTGTCGTT-3′) (Midland Reagent Company). Serum samples were obtained from the vena cava of anesthetized animals after each immunization.

ELISA. Serum-binding antibody titers against gp140 trimers and gp120 monomers were determined by endpoint ELISAs as previously described (2). Ninety-six–well Maxisorp ELISA plates

(Thermo Fisher Scientific) coated overnight with 100 μL per well of 92UG037.8 gp140, C97ZA012 gp140, 92UG037.8 gp120, or C97ZA012 gp120 at a concentration of 1 μg/mL in PBS were blocked for 3 h with PBS containing 2% BSA (Sigma) and 0.05% Tween 20 (Sigma). Then guinea pig sera were added in serial dilutions and incubated for 1 h at room temperature. The plates were washed three times with PBS containing 0.05% Tween 20 and were incubated for 1 h with a 1/2,000 dilution of an HRP-conjugated goat anti-guinea pig secondary antibody (Jackson ImmunoResearch Laboratories). The plates were washed three times and developed with SureBlue tetramethylbenzidine microwell peroxidase (KPL Research Products), stopped by the addition of stop solution (KPL Research Products), and analyzed at 450 nm/550 nm on a Spectramax Plus ELISA plate reader (Molecular Devices) using Softmax Pro-4.7.1 software. ELISA endpoint titers were defined as the highest reciprocal serum dilution that yielded absorbance greater than twofold background.

Neutralizing Antibody Assay in TZM.bl Cells. Neutralizing antibody responses against HIV-1 Env pseudoviruses were measured using luciferase-based virus neutralization assays in TZM.bl cells as previously described (2, 7, 8). These assays measure the reduction in luciferase reporter gene expression in TZM-bl cells following a single round of virus infection. The ID_{50} was calculated as the serum dilution that resulted in a 50% reduction in relative luminescence units compared with the virus control wells after the subtraction of cell control relative luminescence units. Briefly, threefold serial dilutions of serum samples were performed in duplicate (96-well flat-bottomed plate) in 10% DMEM growth medium (100 μL per well). Virus was added to each well in a volume of 50 μL, and the plates were incubated for 1 h at 37 °C. Then TZM.bl cells were added (1×10^4 per well in 100 μL volume) in 10% DMEM growth medium containing diethylaminoethyl-dextran (Sigma) at a final concentration of 11 μg/mL. Murine leukemia virus (MuLV) negative controls were included in all assays. HIV-1 Env pseudoviruses, including clade A (DJ263.8), clade B (SF162.LS and BaL.26), and clade C (MW965.26, TV1.21, ZM109F.PB4, ZM197M.PB7, ZM233M.PB6, and Du422.1) isolates, were prepared as previously described (7).

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4. Chen B, et al. (2000) Expression, purification, and characterization of gp160e, the soluble, trimeric ectodomain of the simian immunodeficiency virus envelope glycoprotein, gp160. *J Biol Chem* 275:34946–34953.
5. Lewis MS, Junghans RP (2000) Ultracentrifugal analysis of molecular mass of glycoproteins of unknown or ill-defined carbohydrate composition. *Methods Enzymol* 321:136–149.
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7. Li M, et al. (2005) Human immunodeficiency virus type 1 env clones from acute and early subtype B infections for standardized assessments of vaccine-elicited neutralizing antibodies. *J Virol* 79:10108–10125.
8. Mascola JR, et al. (2005) Recommendations for the design and use of standard virus panels to assess neutralizing antibody responses elicited by candidate human immunodeficiency virus type 1 vaccines. *J Virol* 79:10103–10107.

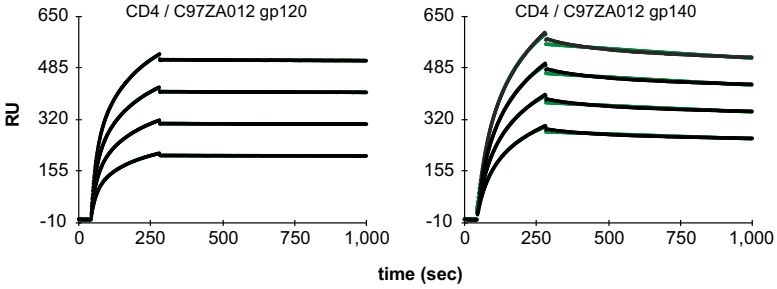

Fig. S1. Binding of soluble CD4 to C97ZA012 gp120 and gp140. Soluble four-domain CD4 was immobilized on a CM-5 chip, and various concentrations (50–1,000 nM) of C97ZA012 gp120 or C97ZA012 gp140 were passed over the chip surface. Binding kinetics was evaluated using a 1:1 Langmuir binding model; binding constants are summarized in Table S2. The sensorgrams are shown in black and the fits in green. All injections were carried out in duplicate and gave essentially identical results. Only one of the duplicates is shown.

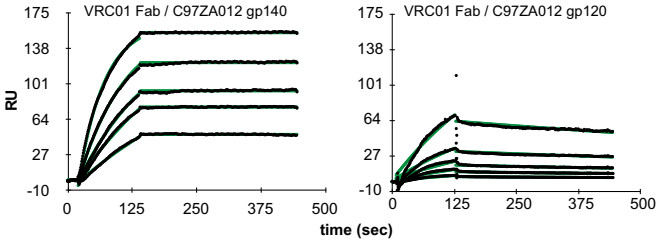

Fig. S2. Interaction of CD4 binding site antibody VRC01 with C97ZA012 gp140 and gp120. Fab of the CD4 binding site antibody VRC01 was captured on a surface immobilized with human Fab binder, and various concentrations (50–1,000 nM) of C97ZA012 gp120 or C97ZA012 gp140 were flowed over the chip surface.

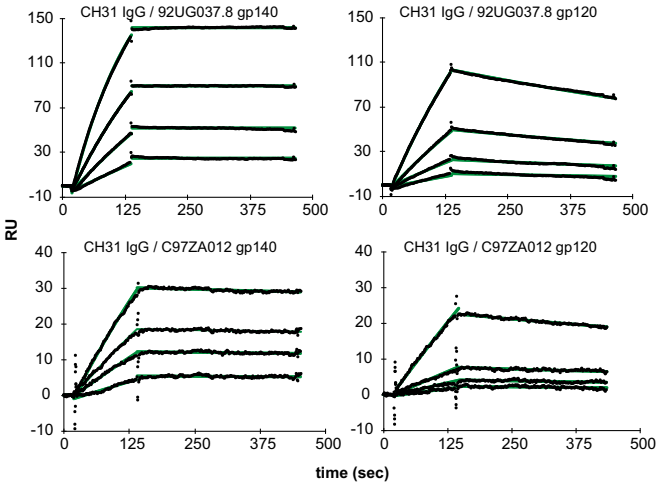

Fig. S3. Binding of CD4 binding site antibody CH31 with gp140 and gp120. CD4 binding site antibody CH31 was captured onto a surface immobilized with protein A, and various concentrations (50–1,000 nM) of gp120 or gp140 were flowed over the chip surface.

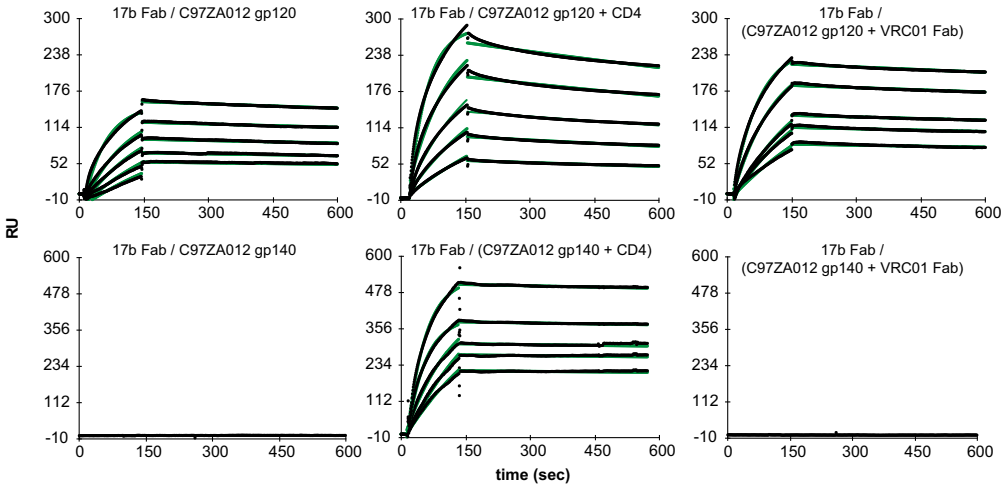

Fig. S4. Interactions of C97ZA012 gp140 and gp120 with CD4-induced antibody 17b. Fab of the CD4-induced antibody 17b was immobilized to a sensor chip surface using human Fab binder. Various concentrations (50–1,000 nM) of C97ZA012 gp120 and C97ZA012 gp140, purified C97ZA012 gp120-CD4 and C97ZA012 gp140-CD4 complexes, or purified C97ZA012 gp120-VRC01 Fab and C97ZA012 gp140-VRC01 Fab complexes were passed over the 17b surface.

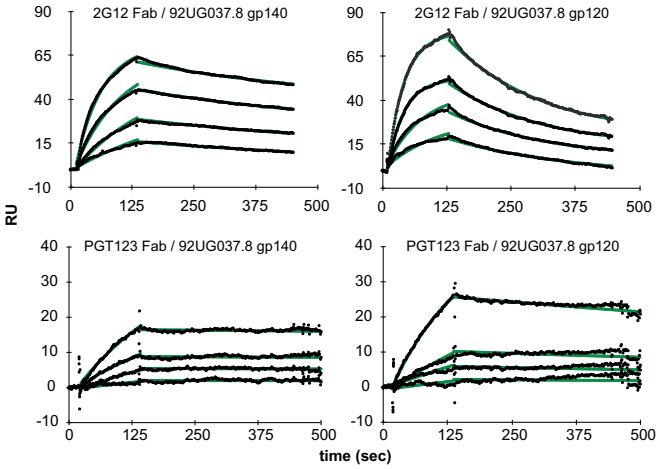

Fig. S5. Interactions of gp140 and gp120 with broadly neutralizing antibodies targeting the glycan-dependent epitopes. Fab of broadly neutralizing antibody 2G12 or PGT 123 was immobilized on a chip surface using human Fab binder. Then various concentrations (50–1,000 nM) of 92UG037.8 gp120 or 92UG037.8 gp140 were passed over the antibody surface. Binding kinetics was evaluated using a 1:1 Langmuir binding model; binding constants are summarized in Table S2. The sensorgrams are in black and the fits in green. All injections were carried out in duplicate and gave essentially identical results. Only one of the duplicates is shown.

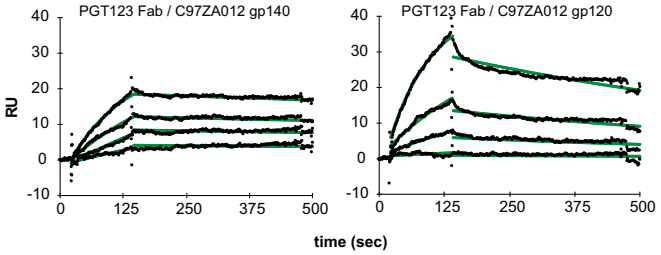

Fig. S6. Binding of PGT123 with C97ZA012 gp140 and gp120. Fab of broadly neutralizing antibody PGT 123 was immobilized on a chip surface using human Fab binder. Then various concentrations (50–1,000 nM) of C97ZA012 gp120 or C97ZA012 gp140 were passed over the antibody surface.

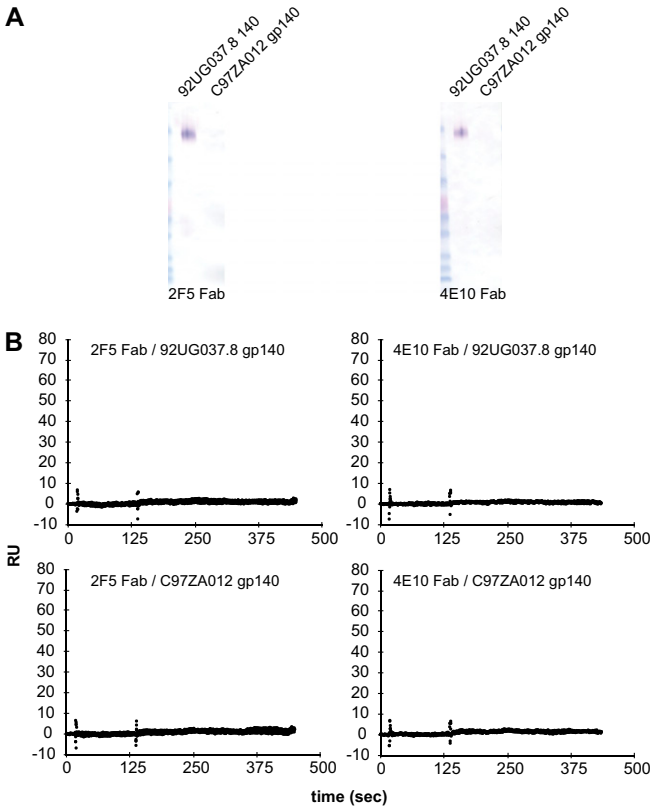

Fig. S7. There is no binding between native gp140s and anti-MPER antibodies. (*A*) 2F5 and 4E10 Fabs react on an immunoblot with 92UG037.8 gp140 but not with C97ZA012 gp140, because the C97ZA012 isolate is very resistant to both 2F5 and 4E10 (Table S3). (*B*) Fab of 2F5 or 4E10 was immobilized on the chip surface using human Fab binder. Then various concentrations (50–1,000 nM) of 92UG037.8 gp140 or C97ZA012 gp140 were passed over the antibody surface.

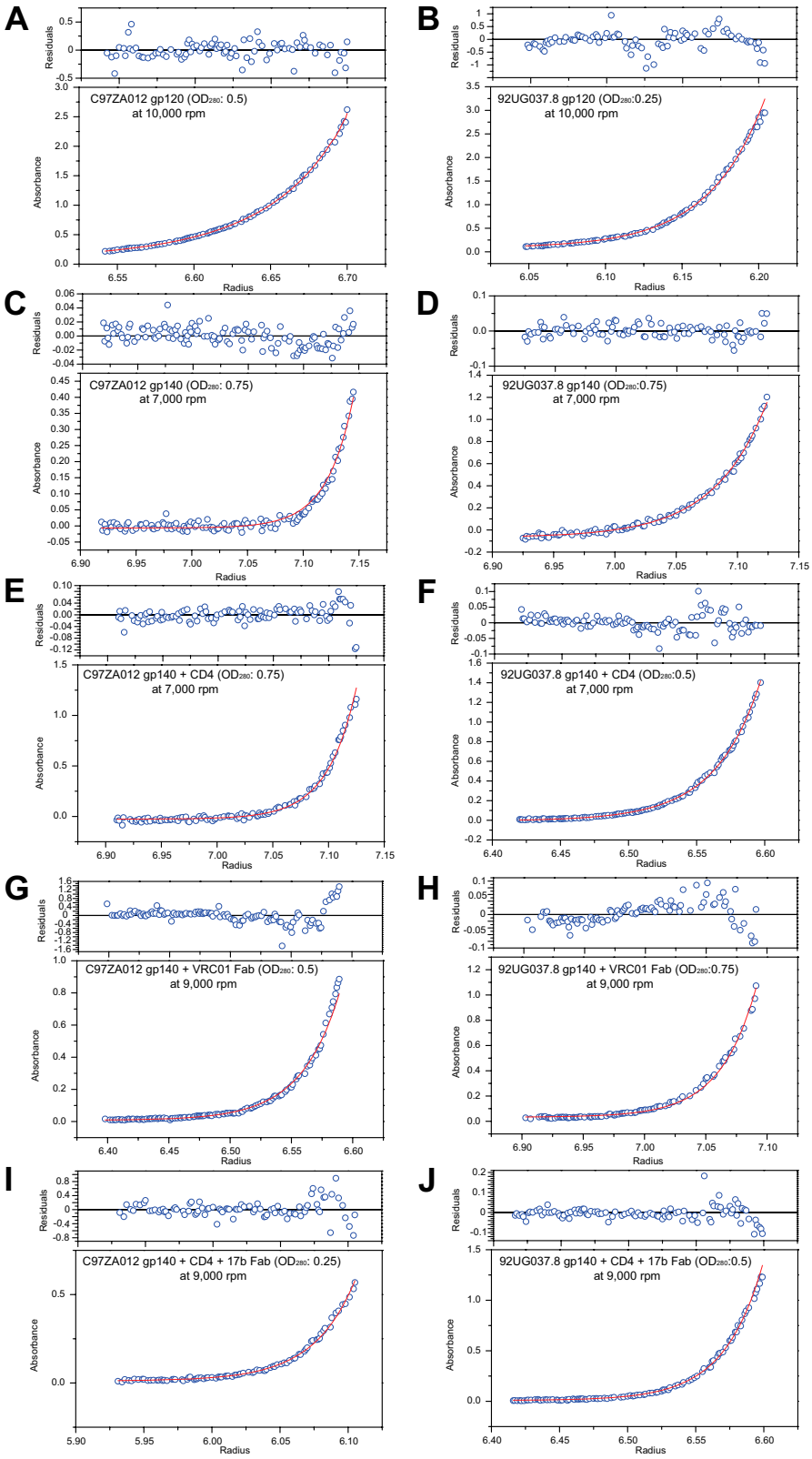

Fig. S8. Analytical ultracentrifugation analysis of envelope proteins and their complexes with CD4 binding site ligands. Analytical ultracentrifugation was performed with three protein concentrations and three rotor speeds for each protein or protein complex as described in *SI Materials and Methods*. The data shown for each sample were collected at the protein concentration and rotor speed indicated. All data sets were fitted to a single-species model. The partial specific volumes of the envelope glycoproteins and their complexes were calculated as described in *SI Materials and Methods*. The determined molecular masses are summarized in Table S1.

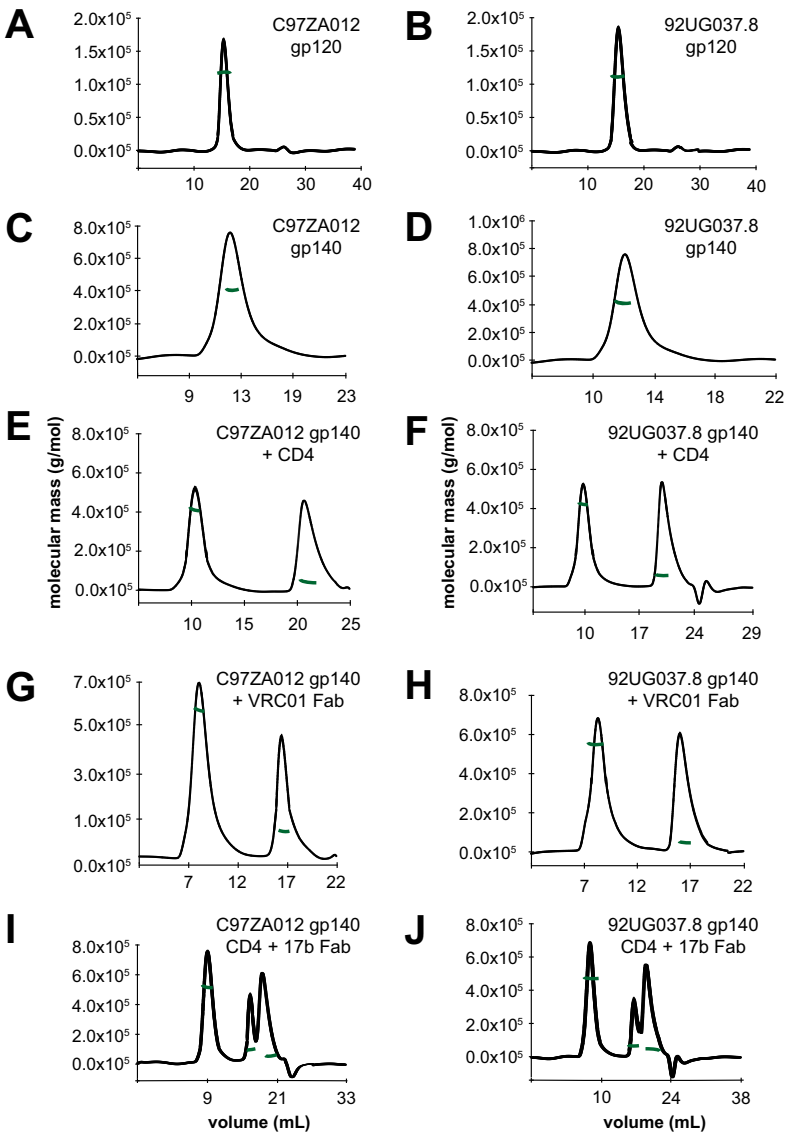

Fig. S9. Multiangle light-scattering analysis of envelope proteins and their complexes with CD4 binding site ligands. Purified 92UG037.8 and C97ZA012 envelope proteins and their complexes with CD4, VRC01 Fab, or CD4 and 17b were resolved on a Superdex 200 column, coupled to a Wyatt miniDAWN TREOS three-angle light-scattering detector and Optilab-rEX refractive index detector. The scattering data were evaluated using ASTRA 5.3.4 software. The determined molecular masses are summarized in Table S1.

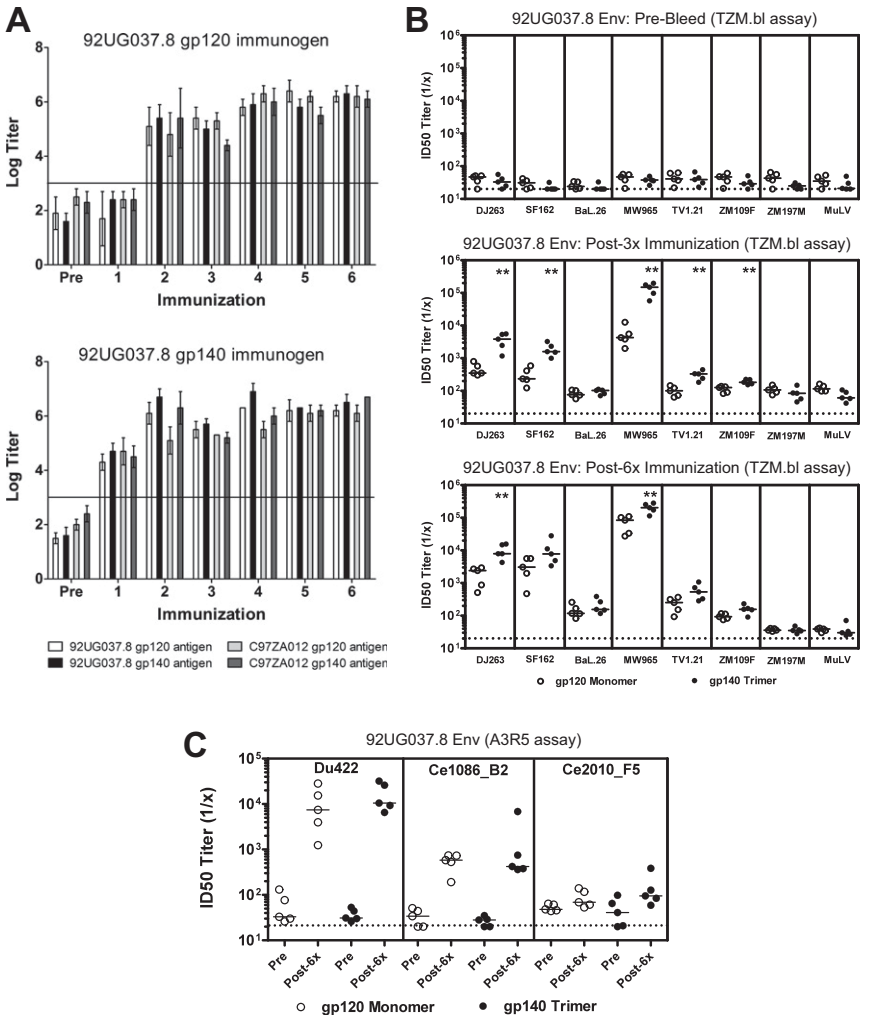

Fig. S10. Immunogenicity of the 92UG037.8 trimeric gp140 and monomeric gp120 in guinea pigs. (*A*) Sera of guinea pigs vaccinated with either 92UG037.8 gp120 or gp140 obtained prevaccination (Pre) and 4 wk after each immunization were tested in endpoint ELISAs against all 92UG037.8 and C97ZA012 antigens as indicated. Binding antibody responses against 92UG037.8 gp120 monomer (white columns), 92UG037.8 gp140 trimer (black columns), C97ZA012 gp120 monomer (light gray columns), and C97ZA012 gp140 trimer (dark gray columns) are presented as geometric mean titers at each time point ± SD. Horizontal lines indicate background threshold. (*B*) Guinea pig sera obtained prevaccination (Pre-Bleed), 4 wk after the third vaccination (Post-3×) and 4 wk after the sixth vaccination (Post-6×) were tested against a multiclade panel of tier 1 neutralization-sensitive isolates including clade A (DJ263.8), clade B (SF162.LS and Bal.26), and clade C (MW965.26, TV1.21, ZM109F, and ZM197M) HIV-1 Env pseudoviruses and MuLV (negative control) in TZM.bl neutralization assays. Neutralizing antibody titers induced by 92UG037.8 gp120 and gp140 are shown by graphical representation. $*P < 0.05$; $**P < 0.01$; unpaired two-tailed *t* test. Horizontal bars indicate median titers. (*C*) Sera obtained prevaccination (Pre) and 4 wk after the sixth vaccination (Post-6×) were tested against three tier 2 clade C isolates —Du422, Ce1086_B2, and Ce2010_F5 IMC viruses—in A3R5 neutralization assays. Neutralizing antibody titers induced by 92UG037.8 gp120 and gp140 are shown by graphical representation.

92UG037.8 gp120 & gp140 TZM.bl Titers

Timepoint	Tier 1A DJ263.8		Tier 1B SF162.LS		Tier 1B BaL.26		Tier 1C MW965.26		Tier 1C TV1.21		Tier 1C ZM109F.PB4		Tier 1C ZM197M.PB7		MuLV	
	gp120	gp140	gp120	gp140	gp120	gp140	gp120	gp140	gp120	gp140	gp120	gp140	gp120	gp140	gp120	gp140
Pre	51	33	31	<20	24	<20	47	38	41	31	48	27	43	25	35	20
Post-3rd	573	2,464	233	1,524	106	71	12,639	149,926	123	240	138	165	151	57	161	58
Post-6th	873	7,893	3,079	4,885	118	117	33,508	172,988	153	720	83	152	40	34	35	33
Pre	51	25	42	<20	34	<20	55	36	61	39	47	29	65	31	47	21
Post-3rd	795	5,364	222	3,248	102	112	5,523	175,523	145	445	133	221	119	149	139	107
Post-6th	2,941	14,854	5,661	28,075	259	388	83,821	280,286	366	1,079	113	231	33	37	42	30
Pre	47	<20	36	<20	33	<20	57	26	63	23	61	<20	56	<20	53	<20
Post-3rd	350	1,170	411	998	74	103	3,841	57,247	64	183	90	154	75	84	98	90
Post-6th	2,698	4,289	1,981	3,368	115	149	109,024	113,956	316	286	116	91	36	28	39	27
Pre	35	56	22	32	21	33	38	49	37	67	35	51	38	26	29	49
Post-3rd	303	5,557	123	1,593	57	82	4,295	196,648	73	336	83	183	107	46	99	42
Post-6th	2,416	15,628	472	11,140	82	156	101,670	254,084	252	534	93	157	36	35	30	25
Pre	<20	41	<20	<20	<20	<20	21	38	22	44	21	33	<20	<20	<20	30
Post-3rd	352	3,855	587	2,321	76	104	1,986	97,502	100	329	126	224	91	86	115	61
Post-6th	510	7,891	5,638	7,772	167	267	27,493	203,356	93	329	75	166	41	48	39	71

Timepoint	Tier 2C ZM233M.PB6		Tier 2C Du422.1		MuLV	
	gp120	gp140	gp120	gp140	gp120	gp140
Pre	45	21	37	25	35	20
Post-3rd	163	59	95	45	161	58
Post-6th	47	56	22	28	35	33
Pre	52	23	45	29	47	21
Post-3rd	129	117	90	77	139	107
Post-6th	42	74	27	28	42	30
Pre	58	<20	59	<20	53	<20
Post-3rd	79	84	55	80	98	90
Post-6th	44	43	28	22	39	27
Pre	34	50	42	58	29	49
Post-3rd	70	42	79	38	99	42
Post-6th	49	53	28	27	30	25
Pre	<20	26	22	31	<20	30
Post-3rd	107	79	103	111	115	61
Post-6th	58	76	32	33	39	71

C97ZA012 gp120 & gp140 TZM.bl Titers

Timepoint	Tier 1A DJ263.8		Tier 1B SF162.LS		Tier 1B BaL.26		Tier 1C MW965.26		Tier 1C TV1.21		Tier 1C ZM109F.PB4		Tier 1C ZM197M.PB7		MuLV	
	gp120	gp140	gp120	gp140	gp120	gp140	gp120	gp140	gp120	gp140	gp120	gp140	gp120	gp140	gp120	gp140
Pre	32	83	<20	<20	<20	<20	34	35	27	20	22	25	20	27	20	24
Post-3rd	514	3,743	102	2,874	25	141	14,727	77,195	24	369	32	147	<20	23	27	22
Post-6th	NT	11,256	NT	11,040	NT	302	NT	193,394	NT	907	NT	141	NT	35	NT	22
Pre	22	37	<20	<20	<20	<20	23	29	20	25	26	25	<20	<20	<20	25
Post-3rd	2,181	4,562	56	1,873	54	86	125,447	178,965	119	426	121	158	56	29	71	42
Post-6th	1,390	12,118	268	10,223	145	280		389,423	202	878	71	244	25	34	21	27
Pre	25	41	<20	<20	<20	<20	27	33	<20	25	<20	24	<20	23	<20	21
Post-3rd	293	3,431	118	5,243	51	225	3,478	147,353	61	293	70	165	56	67	78	66
Post-6th	756	7,387	102	12,133	57	433		275,335	119	548	50	115	22	28	25	21
Pre	28	35	<20	<20	<20	<20	33	31	27	29	29	29	25	<20	21	33
Post-3rd	1,089	2,922	341	2,427	50	162	23,474	136,667	79	378	56	285	63	133	67	115
Post-6th	2,456	20,358	5006	8,942	96	263		848,672	327	1,243	74	258	23	33	25	27
Pre	23	54	<20	36	<20	21	32	38	<20	61	21	65	<20	54	25	59
Post-3rd	611	4,846	301	1,521	41	147	36,318	124,229	95	681	62	308	52	156	52	134
Post-6th	496	12,037	583	24,998	112	632	27,309	349,392	102	987	67	182	36	40	33	36

Timepoint	Tier 2C ZM233M.PB6		Tier 2C Du422.1		MuLV	
	gp120	gp140	gp120	gp140	gp120	gp140
Pre	<20	<20	<20	<20	20	24
Post-3rd	<20	70	<20	<20	27	22
Post-6th	NT	60	NT	<20	NT	22
Pre	<20	<20	<20	<20	<20	25
Post-3rd	71	40	50	30	71	42
Post-6th	40	44	<20	<20	21	27
Pre	<20	<20	<20	<20	<20	21
Post-3rd	73	53	55	49	78	66
Post-6th	28	48	<20	<20	25	21
Pre	<20	<20	<20	<20	21	33
Post-3rd	68	130	46	95	67	115
Post-6th	50	91	22	23	25	27
Pre	<20	44	<20	54	25	59
Post-3rd	65	222	41	166	52	134
Post-6th	58	113	30	28	33	36

Color Code	Titer Range
	0-99
	100-999
	1000-9999
	>10,000

Fig. S11. Comparison of gp140 trimer versus gp120 monomer neutralizing antibody titers by the TZM.bl assay. Guinea pig sera obtained prevaccination (Pre), 4 wk after the third vaccination (Post-3rd), and 4 wk after the sixth vaccination (Post-6th) were tested against a multiclade panel of tier 1 neutralization-sensitive isolates including clade A (DJ263.8), clade B (SF162.LS and Bal.26), and clade C (MW965.26, TV1.21, ZM109F, and ZM197M), and two clade C tier 2 neutralization-resistant pseudoviruses (ZM233M.PB6 and ZM197M.PB7) in TZM.bl neutralization assays. Values shown are the serum dilutions representing the ID_{50} titers for each animal with titer ranges color-coded as indicated.

92UG037.8 gp120 & gp140 A3R5 Titers

Timepoint	Tier 2 (C) Du422.1		Tier 2 (C) Ce1086_B2		Tier 2 (C) Ce2010_F5	
	gp120	gp140	gp120	gp140	gp120	gp140
Pre	33	53	34	35	64	65
Post-6th	1,244	10,517	580	6,860	140	384
Pre	77	31	51	<20	44	<20
Post-6th	28,227	31,975	737	748	53	95
Pre	131	30	44	<20	61	21
Post-6th	15,386	25,953	192	421	117	127
Pre	30	44	<20	29	46	98
Post-6th	7,436	9,269	735	363	61	59
Pre	26	26	<20	28	48	41
Post-6th	3,969	6,526	530	380	69	84

C97ZA012 gp120 & gp140 A3R5 Titers

Timepoint	Tier 2 (C) Du422.1		Tier 2 (C) Ce1086_B2		Tier 2 (C) Ce2010_F5	
	gp120	gp140	gp120	gp140	gp120	gp140
Pre	NT	36	NT	<20	NT	39
Post-6th	NT	1,782	NT	11,675	NT	256
Pre	24	32	<20	<20	<20	26
Post-6th	1,770	6,200	1,185	4,152	95	210
Pre	<20	120	<20	<20	<20	115
Post-6th	933	1,907	105	729	58	83
Pre	69	37	<20	29	57	45
Post-6th	437	1,575	347	979	65	177
Pre	40	34	28	<20	29	50
Post-6th	433	3,850	109	1,924	62	572

Color Code	Titer Range
	0-99
	100-999
	1000-9999
	>10,000

Fig. S12. Comparison of gp140 trimer versus gp120 monomer tier 2 neutralizing antibody titers by the A3R5 assay. Guinea pig sera obtained prevaccination (Pre) and 4 wk after the sixth vaccination (Post-6th) were tested against three tier 2 clade C isolates–Du422, Ce1086_B2, and Ce2010_F5 IMC viruses—in A3R5 neutralization assays. Values shown are the serum dilutions representing the ID_{50} titers for each animal with titer ranges color-coded as indicated.

Table S1. Molecular mass determined by analytical ultracentrifugation and multiangle light scattering

Protein	Molecular mass (kDa) by analytical ultracentrifugation	Molecular mass (kDa) by MALS
92UG037.8 gp120	130 ± 7	126 ± 4
C97ZA012 gp120	128 ± 6	125 ± 6
92UG037.8 gp140	413 ± 9	419 ± 5
C97ZA012 gp140	408 ± 6	410 ± 4
92UG037.8 gp140 + 4D CD4	458 ± 4	460 ± 5
C97ZA012 gp140 + 4D CD4	454 ± 3	454 ± 4
92UG037.8 gp140 + VRC01 Fab	567 ± 5	570 ± 15
C97ZA012 gp140 + VRC01 Fab	563 ± 4	570 ± 6
92UG037.8 gp140 + 4D CD4 + 17b Fab	511 ± 2	510 ± 10
C97ZA012 gp140 + 4D CD4 + 17b Fab	506 ± 3	507 ± 6
4D CD4	n.d.	43 ± 5
VRC01 Fab	n.d.	49 ± 7
17b Fab	n.d.	50 ± 6

Detailed data analyses are shown in Figs. S7 and S8. MALS, multiangle light scattering; n.d., not determined.

Table S2. Binding rate constants derived from surface plasmon resonance analysis

Immobilized ligand	Flowing analyte	K_a (1/Ms)	K_d (1/s)	K_d (M)
VRC01	92UG037.8 gp120	8.3×10^3	7.9×10^{-4}	9.6×10^{-8}
	92UG037.8 gp140	1.6×10^4	6.4×10^{-5}	3.9×10^{-9}
	C97ZA012 gp120	3.2×10^3	6.6×10^{-4}	9.6×10^{-8}
	C97ZA012 gp140	1.2×10^3	7.6×10^{-5}	2.1×10^{-9}
B6	92UG037.8 gp120	1.2×10^5	6.1×10^{-3}	5.1×10^{-8}
	92UG037.8 gp140	nb	nb	nb
	C97ZA012 gp120	nb	nb	nb
	C97ZA012 gp140	nb	nb	nb
PG9	92UG037.8 gp120	nb	nb	nb
	92UG037.8 gp140	1.9×10^4	1.0×10^{-3}	9.3×10^{-8}
	C97ZA012 gp120	nb	nb	nb
	C97ZA012 gp140	1.4×10^4	2.5×10^{-3}	3.1×10^{-7}
PG16	92UG037.8 gp120	nb	nb	nb
	92UG037.8 gp140	2.4×10^4	1.8×10^{-3}	4.3×10^{-8}
	C97ZA012 gp120	nb	nb	nb
	C97ZA012 gp140	1.6×10^4	4.2×10^{-3}	1.1×10^{-7}
PGT123	92UG037.8 gp120	3.6×10^3	4.9×10^{-4}	1.4×10^{-7}
	92UG037.8 gp140	2.9×10^3	7.9×10^{-5}	2.7×10^{-8}
	C97ZA012 gp120	4.3×10^3	1.1×10^{-3}	2.6×10^{-7}
	C97ZA012 gp140	6.1×10^3	2.7×10^{-4}	4.3×10^{-8}
2G12	92UG037.8 gp120	3.6×10^4	3.6×10^{-3}	9.9×10^{-8}
	92UG037.8 gp140	8.4×10^3	6.0×10^{-3}	7.1×10^{-7}
	C97ZA012 gp120	nb	nb	nb
	C97ZA012 gp140	nb	nb	nb
4E10	92UG037.8 gp120	nb	nb	nb
	92UG037.8 gp140	nb	nb	nb
	C97ZA012 gp120	nb	nb	nb
	C97ZA012 gp140	nb	nb	nb
2F5	92UG037.8 gp120	nb	nb	nb
	92UG037.8 gp140	nb	nb	nb
	C97ZA012 gp120	nb	nb	nb
	C97ZA012 gp140	nb	nb	nb
CD4	92UG037.8 gp120	1.2×10^4	1.1×10^{-4}	9.5×10^{-9}
	92UG037.8 gp140	2.1×10^4	4.4×10^{-4}	2.1×10^{-8}
	C97ZA012 gp120	1.4×10^4	1.0×10^{-4}	7.5×10^{-9}
	C97ZA012 gp140	2.5×10^4	4.8×10^{-4}	1.1×10^{-8}
17b	92UG037.8 gp120	6.5×10^3	1.9×10^{-4}	3.9×10^{-8}
	92UG037.8 gp140	nb	nb	nb
	C97ZA012 gp120	8.5×10^3	2.2×10^{-4}	2.6×10^{-8}
	C97ZA012 gp140	nb	nb	nb
17b	92UG037.8 gp120 + CD4	2.1×10^4	6.7×10^{-5}	3.2×10^{-9}
	92UG037.8 gp140 + CD4	1.0×10^4	3.1×10^{-5}	$6.0 \times 1\ 0^{-9}$
	C97ZA012 gp120 + CD4	9.2×10^3	$5.8 \times 1\ 0^{-5}$	4.3×10^{-9}
	C97ZA012 gp140 + CD4	1.0×10^4	3.9×10^{-5}	$5.8 \times 1\ 0^{-9}$
17b	92UG037.8 gp120 + VRC01	8.6×10^3	7.8×10^{-5}	$9.1 \times 1\ 0^{-9}$
	92UG037.8 gp140 + VRC01	nb	nb	nb
	C97ZA012 gp120 + VRC01	8.4×10^3	7.9×10^{-5}	9.3×10^{-9}
	C97ZA012 gp140 + VRC01	nb	nb	nb
CH31	92UG037.8 gp120	2.8×10^3	8.7×10^{-4}	$3.2 \times 1\ 0^{-7}$
	92UG037.8 gp140	8.9×10^3	4.0×10^{-5}	4.5×10^{-9}
	C97ZA012 gp120	1.3×10^2	5.9×10^{-4}	4.5×10^{-6}
	C97ZA012 gp140	$9.7 \times 1\ 0^3$	1.3×10^{-4}	1.3×10^{-8}

nb, no binding.

Table S3. Neutralization of HIV-1 isolates C97ZA012 and 92UG037 by broadly neutralizing antibodies in TZM.bl cells

	Antibodies									
	PG9		PG16		CH31		2F5		4E10	
Isolate	IC_{50} (μg/mL)	IC_{80} (μg/mL)	IC_{50} (μg/mL)	IC_{80} (μg/mL)	IC_{50} (μg/mL)	IC_{80} (μg/mL)	IC_{50} (μg/mL)	IC_{80} (μg/mL)	IC_{50} (μg/mL)	IC_{80} (μg/mL)
C97ZA012	8.20	>25	2.90	>25	0.18	0.47	>50	>50	20.8	>50
92UG037.8	0.04	0.17	<0.01	0.03	0.04	0.08	0.97	4.40	2.11	7.70