www.sciencemag.org/cgi/content/full/1178746/DC1

Supporting Online Material for

Broad and Potent Neutralizing Antibodies from an African Donor Reveal a New HIV-1 Vaccine Target

Laura M. Walker, Sanjay K. Phogat,* Po-Ying Chan-Hui, Denise Wagner, Pham Phung, Julie L. Goss, Terri Wrin, Melissa D. Simek, Steven Fling, Jennifer L. Mitcham, Jennifer K. Lehrman, Frances H. Priddy, Ole A. Olsen, Steven M. Frey, Phillip W. Hammond, Protocol G Principal Investigators, Stephen Kaminsky, Timothy Zamb, Matthew Moyle, Wayne C. Koff, Pascal Poignard, Dennis R. Burton*

*To whom correspondence should be addressed. E-mail: burton@scripps.edu (D.R.B.); SPhogat@iavi.org (S.K.P.)

Published 3 September 2009 on *Science* Express
DOI: 10.1126/science.1178746

This PDF file includes

MATERIAL AND METHODS

Antibodies and Antigens. Recombinant antibodies IgG1 b12 and b6 were expressed in Chinese hamster ovary (CHO-K1) cells, purified using a Protein A affinity matrix, and monitored for endotoxin contamination. Fab b3 was expressed in *E.coli* and purified using an anti-human Fab specific affinity column. The following antibodies and reagents were procured by the IAVI Neutralizing Antibody Consortium: antibodies 2F5, 4E10, and 2G12 (Polymune Scientific, Vienna, Austria), antibodies A32, 23b, and 17b (Strategic BioSolutions), antibody F425/b4E8 (provided by Lisa Cavacini, Beth Israel Deaconess Medical Center), soluble CD4 and JR-FL gp120 (Progenics, Tarrytown, NY), and JR-CSF gp120 and antibody C11 (Advanced Product Enterprises). Purified ADA, HXB2, DU422, and YU2 gp120s were produced in the laboratory of Robert Doms, University of Pennsylvania.

Patients. The patient identified for this study was selected from the IAVI sponsored study, Protocol G *(S1)*. Eligibility for enrollment into Protocol G was defined as: male or female at least 18 years of age with documented HIV infection for a least three years, clinically asymptomatic at the time of enrollment, and not currently receiving antiretroviral therapy. Selection of individuals for monoclonal antibody generation was based on a rank-order high throughput analytical screening algorithm(*S1*). This volunteer was identified as a broad neutralizer based on broad and potent neutralizing activity against a cross-clade pseudovirus panel.

Isolation of mAbs. The human monoclonal antibody discovery platform utilized a short-term B cell culture system to interrogate the memory B cell repertoire. Surface IgG-expressing memory B cells were enriched from peripheral blood mononuclear cells of the HIV-1 infected donor by negative depletion with antibodies to CD3, CD14, CD16, IgM, IgA, IgD on magnetic beads (Miltenyi, Auburn, CA). To promote B cell activation, proliferation, terminal differentiation and antibody secretion, 30,300 CD19+ sIgG+ memory B cells were seeded in 384-well microtiter plates at an average density of 1.3 cells/well in the presence of feeder cells and conditioned media generated from mitogen-stimulated human T cells from healthy donors. This seeding cell density was important to facilitate the rescue of antigen-specific antibody variable domain sequences from the antibody pool derived from multiple B cells in each well. The culture supernatants were collected 8 days later and screened in a high throughput format for binding reactivity to recombinant gp120 and gp41 indirectly and directly immobilized on ELISA plates, respectively. In parallel, the culture supernatants were also screened for neutralization activity in a high throughput micro-neutralization assay. Heavy and light variable regions were isolated from lysate of selected neutralizing hits by RT-PCR amplification using family-specific primer sets. From positive family-specific PCR reactions, pools of the V_H or V_L-region clones were cloned into an expression vector upstream to human IgG1 constant domain sequence. Minipreps (Qiagen) of these DNA pools, derived from suspension bacterial cultures, were combined in all possible heavy and light chain family-specific pairs and used to transiently transfect 293 cells. All transfectant supernatants containing secreted recombinant antibodies were screened in ELISA and neutralization assays. For B-cell wells that contained more than one B cell clone per culture well, multiple V_H and V_L domain sequences were isolated. ELISA (for B-cell wells positive for

ELISA) and neutralization screens identified the heavy and light chain combination pools that reconstituted the binding and neutralizing activity as observed for the B-cell well. In most cases, IgG were rescued that showed similar neutralization profile as noted in the B cell wells. DNA sequences of the heavy and light chain variable domains for all neutralizing mAbs were confirmed by multiple sequencing reactions using purified DNA from maxipreps (Qiagen).

Neutralization assays. Neutralization by monoclonal antibodies and patient sera was performed by Monogram Biosciences using a single round of replication pseudovirus assay as previously described (*S2*). Briefly, pseudoviruses capable of a single round of infection were produced by co-transfection of HEK293cells with a subgenomic plasmid, pHIV-1lucΔu3, that incorporates a firefly luciferase indicator gene and a second plasmid, pCXAS that expressed HIV-1 Env libraries or clones. Following transfection, pseudoviruses were harvested and used to infect U87 cell lines expressing either the co-receptor CCR5 or CXCR4. Generation of pseudoviruses incorporating HIV-$1_{JR\text{-}CSF}$ alanine mutants is fully described elsewhere (*S3*). Neutralization activity of PG9 and PG16 against HIV-$1_{JR\text{-}CSF}$ alanine mutants was measured using a TZM-BL assay, as described (*S4*). Memory B cell supernatants were screened in a micro-neutralization assay against HIV-1_{SF162}, HIV-$1_{JR\text{-}CSF}$, and SIV_{mac239} (negative control). This assay was based on the 96-well pseudotyped HIV-1 neutralization assay (Monogram Biosciences) and was modified for screening 15 µl of B cell culture supernatants in a 384-well format. The neutralization index was expressed as the ratio of normalized relative luminescence units (RLU) of SIV_{mac239} to that of test viral strain derived from the same test B cell culture supernatant. The cut-off values used to distinguish neutralizing hits were determined by the neutralization index of a large number of negative control wells containing B cell culture supernatants derived from healthy donors. The false positive rate based on the cut-off value of 1.5 was very low (1-3%), and it was reduced to zero if the cut-off value of 2.0 was used.

Cell surface binding assays. Titrating amounts of PG9 and PG16 were added to HIV-1 Env transfected 293T cells, incubated for 1 h at 4°C, washed with FACS buffer, and stained with a 1:200 dilution of goat anti-human IgG $F(ab')_2$ conjugated to phycoerythrin (Jackson). Binding was analyzed using flow cytometry, and binding curves were generated by plotting the mean fluorescence intensity of antigen binding as a function of antibody concentration. For competition assays, competitor antibodies were added to the cells 15 min prior to adding 0.1 µg/ml biotinylated PG9 or PG16. For sCD4 inhibition assays, 40 µg/ml sCD4 was added to the cells and incubated for 1 h at 4°C prior to adding titrating amounts of antibodies. A FACSArray plate reader (BD biosciences) was used for flow cytometric analysis and FlowJo software was used for data interpretation.

ELISA assays. For B cell culture supernatant screening, ELISA hits were identified in a singlet screen based on optical density (OD) values above 3 times the assay background. For antigen binding ELISAs, ninety-six-well ELISA plates were coated overnight at 4ºC with 50 µl PBS containing 100 ng gp120 or gp140 per well. This coating density will provide the similar gp120 concentrations per well if it is assumed that the antibodies bind with a stoichiometry of three to each gp140 trimer. The wells were washed four times with PBS containing 0.025% Tween 20 and blocked with 3% BSA at room temperature for 1 h. Serial dilutions of PG9 were added to antigen-coated wells, incubated for 1 h at room temperature, and washed 4x with PBS supplemented with 0.025% Tween 20. Binding was probed with alkaline phosphatase-conjugated goat anti-human immunoglobulin G (IgG) $F(ab')_2$ Ab

(Pierce) diluted 1:1000 in PBS containing 1% BSA and 0.025% Tween 20. The plate was incubated at room temperature for 1 h, washed four times, and the plate was developed by adding 50 uL of alkaline phosphatase substrate (Sigma) to 5 mL alkaline phosphatase staining buffer (pH 9.8), according to the manufacturer's instructions. The optical density at 405 nm was read on a microplate reader (Molecular Devices). For competition ELISAs, competitor mAbs were added to $gp120_{HxB2}$ or $gp140_{YU2}$ coated ELISA wells and incubated for 15 min prior to adding 15 μg/ml biotinylated PG9 to each well. Biotinylated PG9 was detected using alkaline phosphatase-conjugated streptavidin (Pierce) and visualized using *p*-nitrophenol phosphate substrate (Sigma). For polyreactivity assays, double-stranded human placental DNA (dsDNA; Sigma) and ganglioside GD2 (Sigma) in 96% ethanol were coated at 37°C overnight onto ELISA wells. Bovine serum albumin (BSA; Sigma), Ovalbumin (OVA; sigma), and apo transferrin (Sigma) were resuspended in PBS and coated overnight at 4°C. All antigens were coated at 50 ng per well. The wells were then washed three times with PBS containing 0.025% Tween 20 and blocked for 1 h at room temperature with 3% BSA. Primary antibody, diluted to 5 μg/ml in 1% BSA/PBS, was then added to the antigen-coated ELISA wells for 1 h at room temperature, followed by washing 3 times with PBS/0.05% Tween 20. Bound antibody was detected by using an alkaline phosphatase-conjugated goat anti-human IgG F(ab′)2 antibody (Pierce) diluted 1:1000 in 1% BSA/PBS.

Gp120 deglycosylation. $Gp120_{Du422}$ and $gp120_{ADA}$ were treated with 40 mU/μg Endoglycosidase H (Endo H; New England Biolabs) in sodium acetate buffer for 24 hr at 37 ºC. Mock treated gp120s were treated under same conditions, but the enzyme was omitted from the reaction.

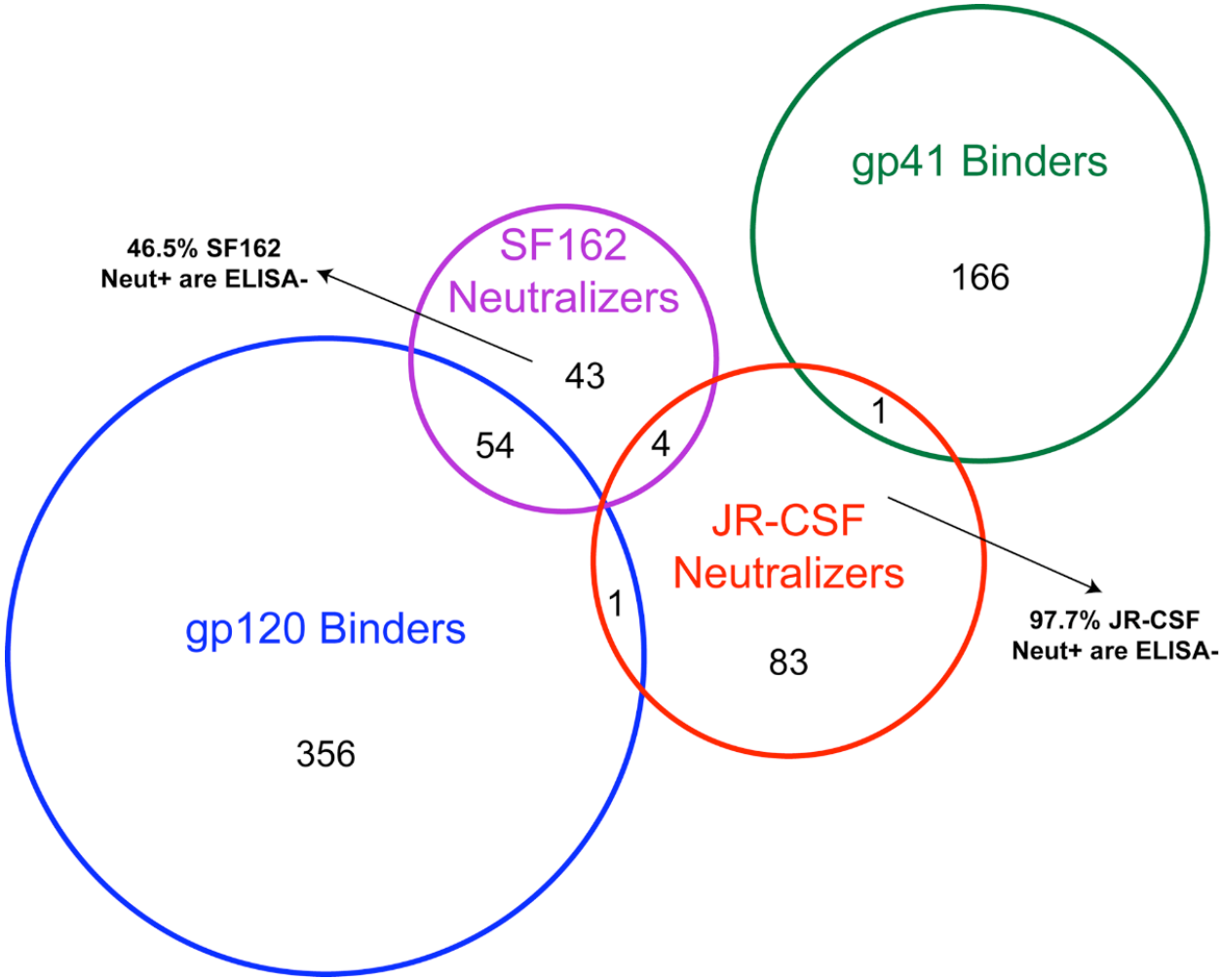


Figure S1. Summary of binding and neutralization activities of supernatants from B cell wells. Values inside of the circles represent the number of B cell supernatants with the indicated binding and/or neutralization profiles. 46.5% of B cell supernatants that neutralized HIV-1_{SF162} and 97.7% that neutralized HIV-1_{JR-CSF} did not bind to monomeric $gp120_{JR-CSF}$ or $gp41_{HxB2}$, as determined by ELISA.

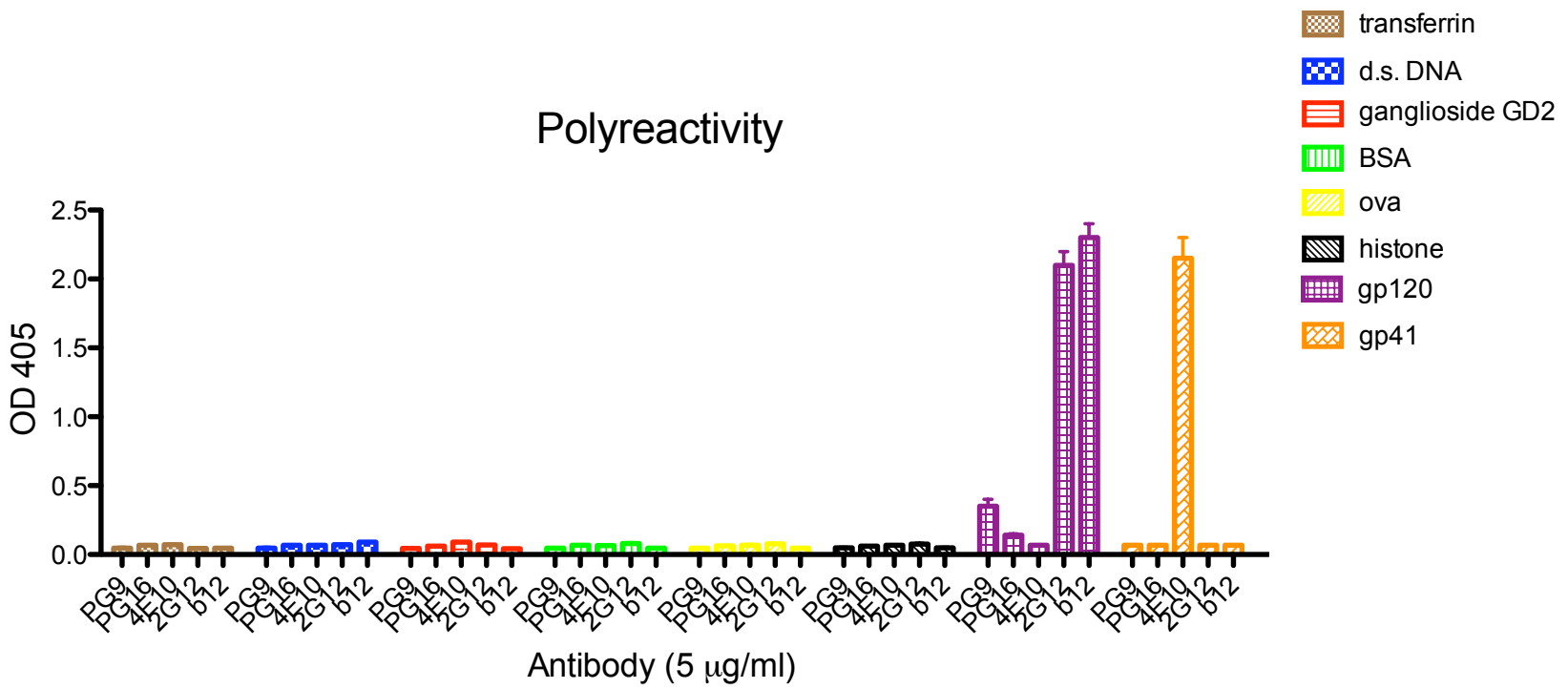


Figure S2. Polyreactivity ELISA assay. PG9 and PG16 were tested for ELISA reactivity against a panel of antigens. The bNAbs b12, 2G12, and 4E10 were also included for comparison. d.s, double-stranded.

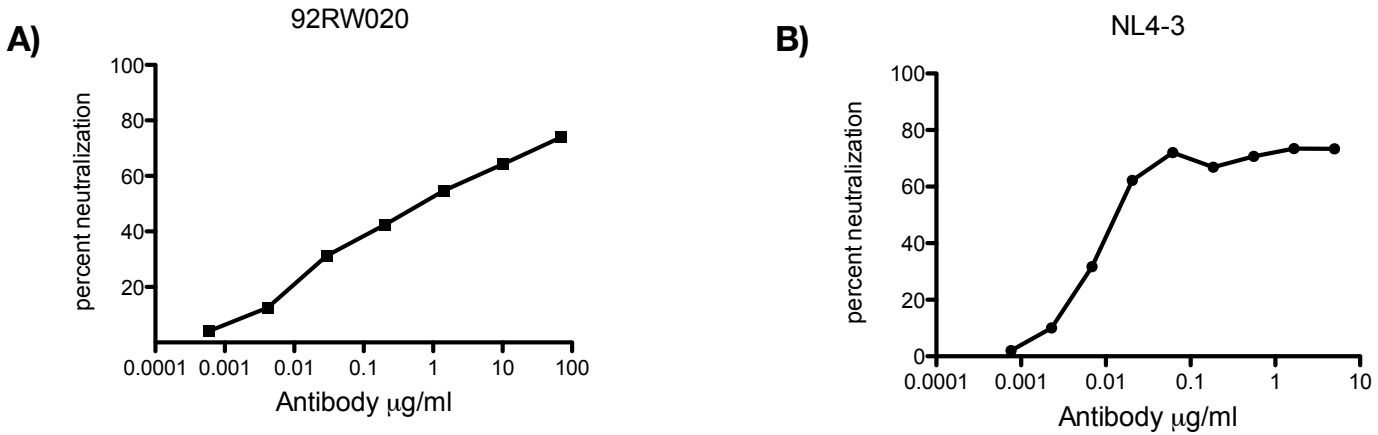


Figure S3. PG16 neutralization curves. For certain isolates, the PG16 neutralization curves exhibited gradual, shallow slopes (A) or plateaud at less than 100% neutralization (B). Neutralization activity was determined using a single-round replication luciferase reporter assay of pseudotyped virus.

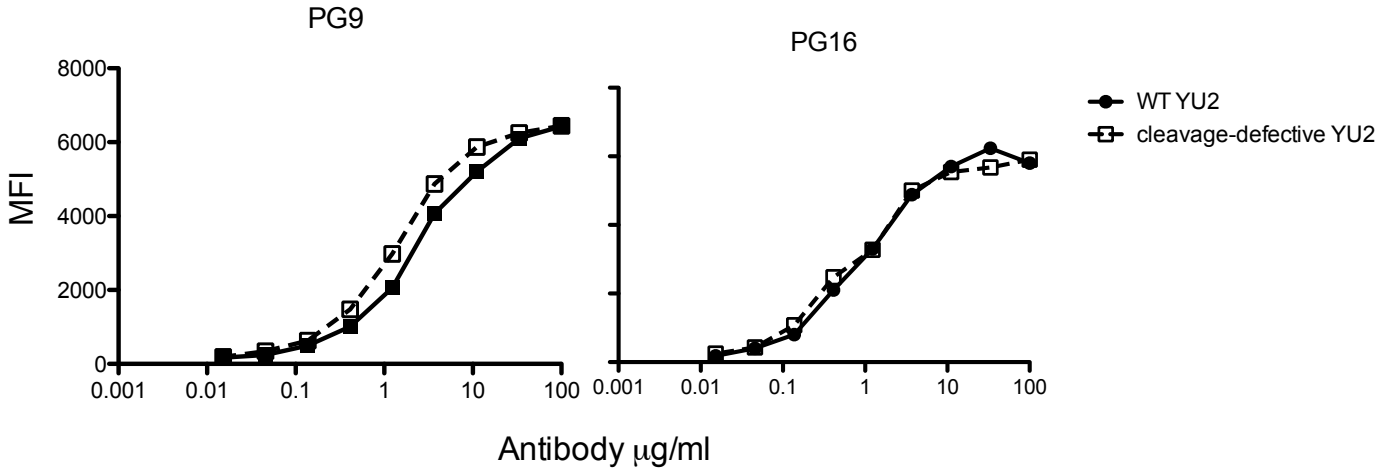


Figure S4. Binding of PG9 and PG16 to cleavage-defective HIV-1$_{YU2}$ trimers. PG9 and PG16 bind with high affinity to cleavage-defective HIV-1$_{YU2}$ trimers as determined by flow cytometry. The cleavage-defective HIV-1$_{YU2}$ construct used in this experiment has been described previously (*S5)*. Binding curves were generated by plotting the mean fluorescence intensity (MFI) of antigen binding as a function of antibody concentration.

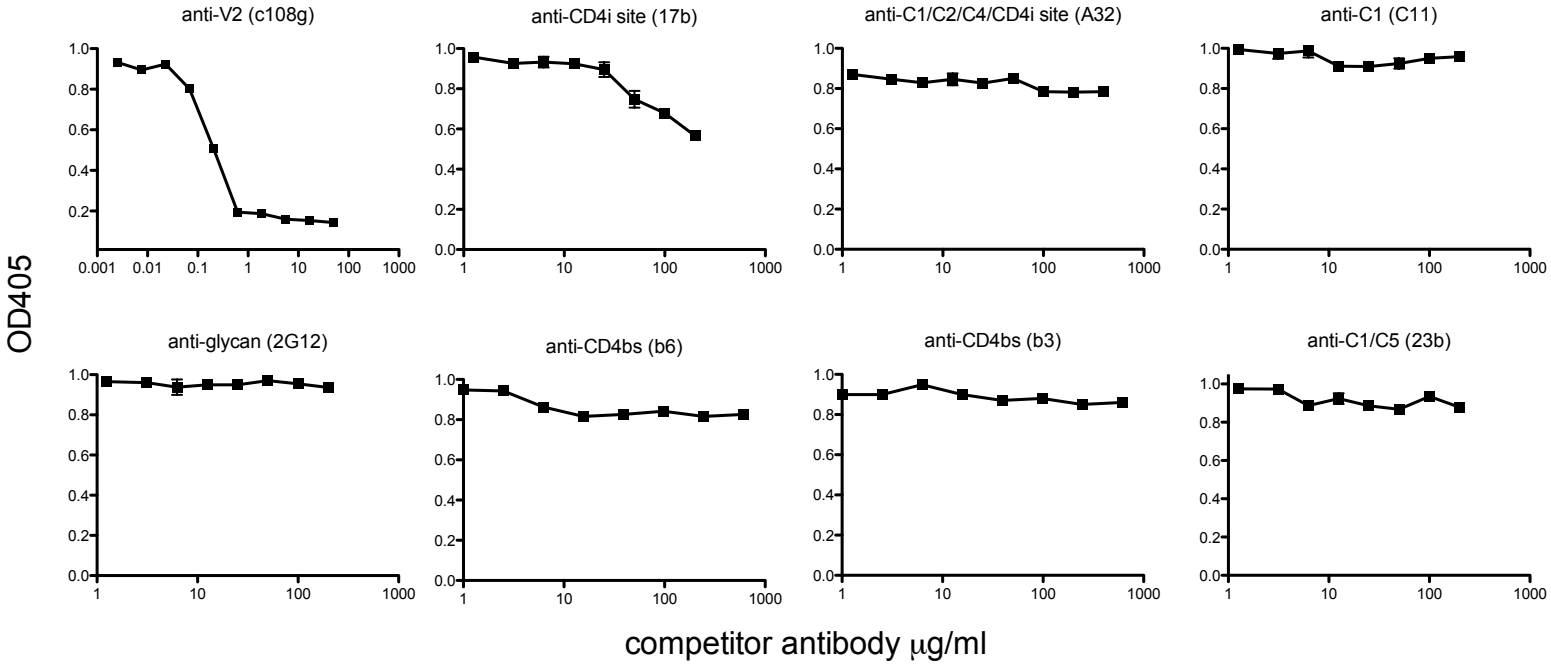


Figure S5. Competition ELISA assays using PG9. Competition of PG9 with antibodies against the V2 loop, CD4 induced epitopes (CD4i), regions of the first, second, and fourth constant domains and the CD4i site (C1/C2/C4/CD4i), the first constant domain (C1), the glycan shield, the CD4 binding site (CD4bs), and the first and fifth constant domains (C1/C5), for $gp120_{HxB2}$ binding.

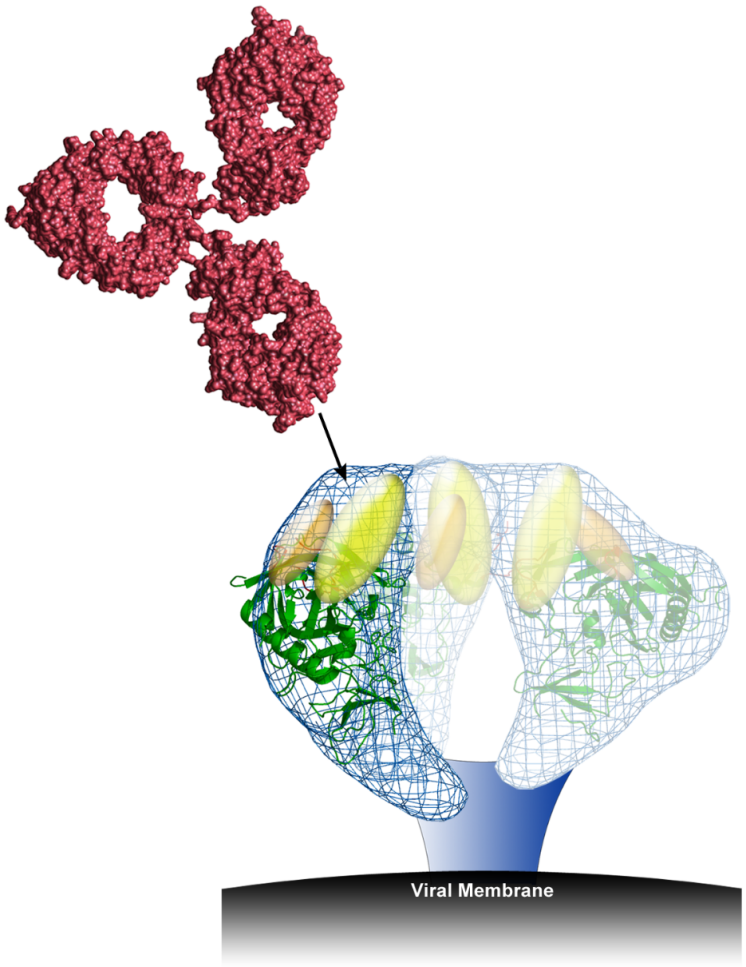


Fig S6. Modeling the PG9 and PG16 epitopes onto the HIV-1 trimer. The above model is adapted from a recent cryo-electron tomographic structure of the HIV-1 trimer *(S6)*. The crystal structure of the b12-bound monomeric gp120 core has been fitted into the density map *(S7)*. The V1/V2 and V3 loops, which are not resolved in the crystal structure, are represented as yellow and orange ovals, respectively. The approximate locations of gp41 and the viral membrane (not resolved in the structure) are shown in solid blue and black, respectively. The red structure located above the trimer is a human IgG molecule representative of PG9 and PG16, and the black arrow indicates schematically that the PG9 and PG16 epitopes are believed to involve residues in the V1/V2 and V3 loops of gp120.

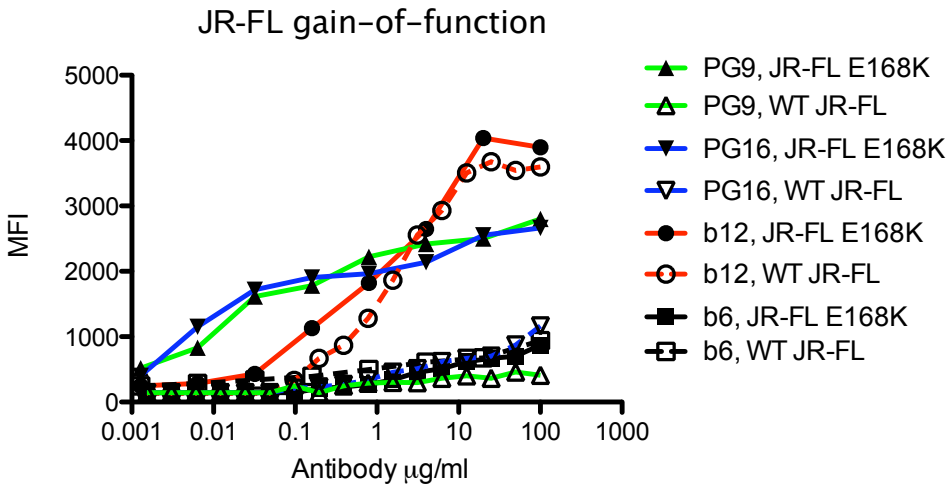


Figure S7. Binding of PG9 and PG16 to HIV-1$_{JR\text{-}FL}$ E168K. Antibody binding to HIV-1$_{JR\text{-}FL\Delta CT}$ E168K (mutation located in the V2 loop of gp120) and WT HIV-1$_{JR\text{-}FL\Delta CT}$ Env expressed on the surface of 293T cells as determined by flow cytometry. Cytoplasmic tail deleted constructs were used to increase cell surface expression. The bNAb b12, which binds to both cleaved and uncleaved forms of Env, and the non-neutralizing antibody b6, which only binds to uncleaved Env, are included in the cell surface binding assays to show the expected percentages of cleaved and uncleaved Env expressed on the cell surface *(S5)*. HIV-1$_{JR\text{-}FL}$ E168K was generated by site-directed mutagenesis. Binding curves were generated by plotting the MFI of antigen binding as a function of antibody concentration.

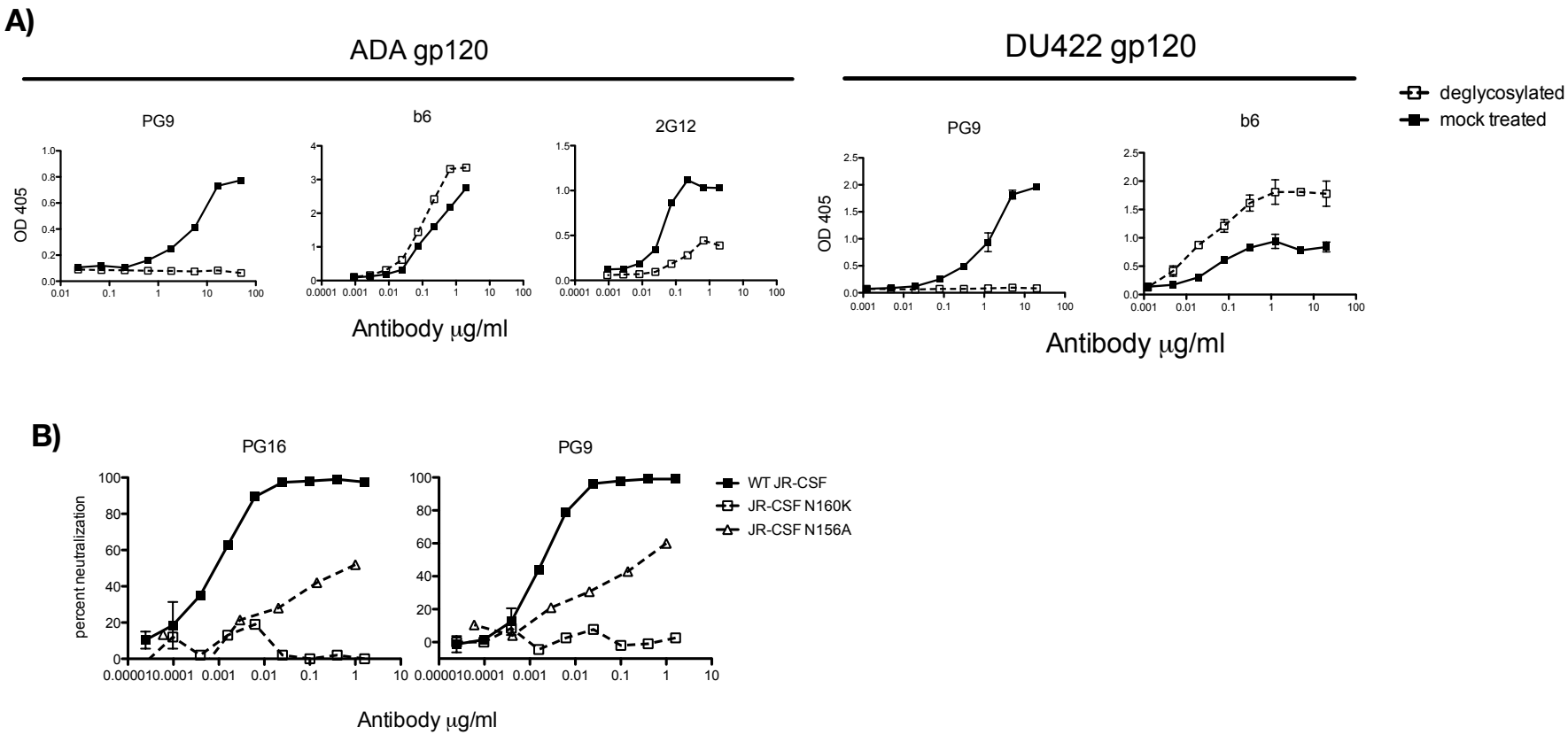


Figure S8. PG9 and PG16 bind to glycan-dependent epitopes. A) Binding of PG9, b6 (anti-CD4bs), and 2G12 (anti-glycan) to Endo H treated and mock treated gp120s as determined by ELISA. Binding of 2G12 to $gp120_{Du422}$ could not be determined because 2G12 does not bind to this isolate. B) The N-linked glycans at position 156 and 160 in the V2 loop of gp120 are required for potent neutralization by PG9 and PG16. Neutralization activity was determined using a single-round replication luciferase reporter assay of pseudotyped virus. $HIV\text{-}1_{JR\text{-}CSF}$ N160K was generated by site-directed mutagenesis.

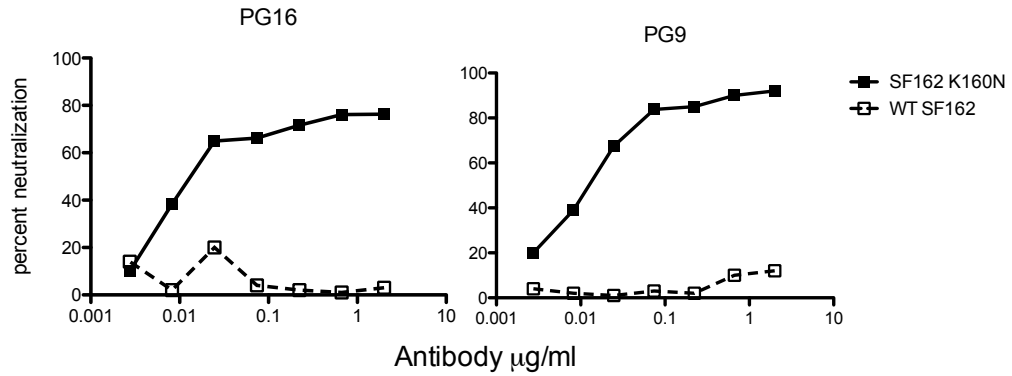
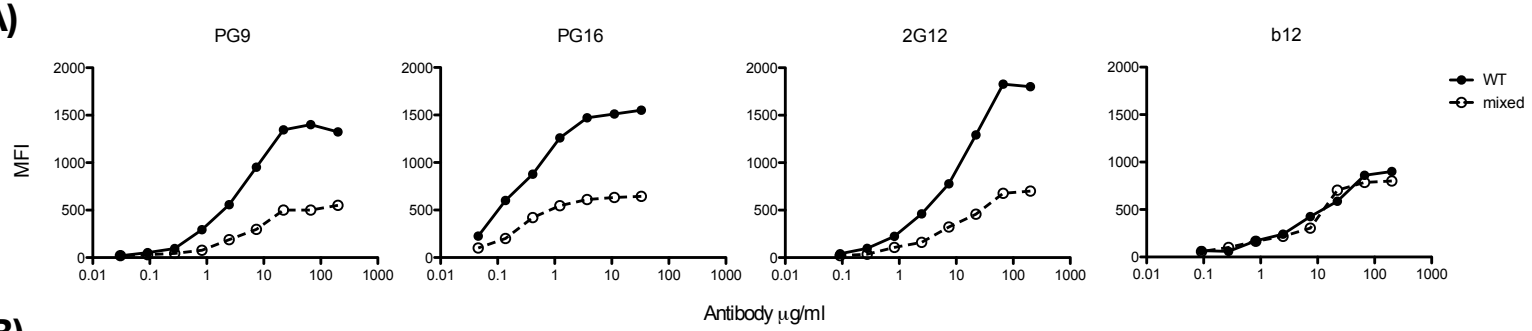


Figure S9. Neutralization activity against HIV-1$_{SF162}$ K160N. Neutralization activity of PG9 and PG16 against HIV-1$_{SF162}$ and HIV-1$_{SF162}$ K160N was determined using a single-round replication luciferase reporter assay of pseudotyped virus. HIV-1$_{SF162}$ K160N was generated by site-directed mutagenesis.

A)



B)

Number of each type of trimer (total = 100)			
WT homotrimer	Mutant homotrimer	2 mutant/1WT	1 mutant/2WT
4	29	44	23

C)

Number of Ab molecules bound (for a total of 300 gp120 subunits)					
	WT homotrimer	**Mutant homotrimer**	**2 mutant/1WT**	**1 mutant/2WT**	**Total Ab molecules bound**
Cross-linking	**4x3=12**	**0**	**0**	**23x1=23**	**35/300 = 12%**
No cross-linking	**4x3=12**	**0**	**44x1=44**	**23x2=46**	**102/300 = 34%**

Figure S10. Binding of PG9 and PG16 to mixed trimers. A) Alanine substitutions at positions 160 and 299 were introduced into HIV-1_{YU2} Env to abolish binding of PG9 and PG16. An alanine substitution at position 295 was also introduced into the same construct to abrogate binding of 2G12. Binding of Abs to Env trimers was determined by flow cytometry. b12 was included to control for Env cell surface expression. B) Co-transfection of 293T cells with WT and mutant plasmids in a 1:2 ratio results in the expression of approximately 4% wild-type homotrimers, 29% mutant homotrimers, 44% heterotrimers with two mutant subunits, and 23% heterotrimers with one mutant subunit. These proportions were calculated using the formula described previously *(S8)* and assumes that mutant and wild-type gp120s mix randomly to form trimers. C) Assuming that antibody (Ab) molecules bind to each available WT gp120, for a population composed of 100% WT homotrimers, the proportion of bound gp120 is 100% (eg. 300 Ab molecules bind to a total of 100 WT homotrimers). For mixed trimer expressing cells, for the same level of Env expression (b12 control), the binding signal measured is predicted to be approximately 12% of the signal measured for WT homotrimer expressing cells in the case of cross-linking (Ab binding to heterotrimers with one mutant gp120 only) and approximately 34% in absence of cross-linking (Ab binding to heterotrimers with one mutant gp120 and to heterotrimers with two mutant gp120s). Blue spheres represent WT gp120 and yellow spheres represent mutant gp120.

Table S1. Memory B cell screening

Total number of wells screened	23,328
Number of IgG^+ memory B cells screened	30,300
gp120 ELISA hits	411 (1.36%)
gp41 ELISA hits	167 (0.55%)
SF162 neutralization hits	101 (0.33%)
JR-CSF neutralization hits	89 (0.29%)

Table S2. Variable gene usage of rescued mAbs

Clone	Germline IGVL[a]	Germline IGVH[a]	CDRL3[b]	CDRH3[b]
PG16	VL2-14*01	VH3-33*05	SSLTDRSHRIF	EAGGPIWHDDVKYYDFNDGYYNYHYMDV
PG9	VL2-14*01	VH3-33*05	KSLTSTRRRVF	EAGGPDYRNGYNYYDFYDGYYNYHYMDV
PGG14	VK1-39*01	VH1-69*12	SYSTPRTF	DRRVVPMATDNWLDP
PG20	VK1-39*01	VH1-69*12	SFSTPRTF	DRRAVPIATDNWLDP
PGC14	VL3-1*01	VH1-24*01	AWETTTTTFVFF	GAVGADSGSWFDP

[a] Germ line gene sequences were determined using the IMGT database (http://imgt.cines.fr). “L” and “K” refer to lambda and kappa chains, respectively.

[b] Amino acids colored red denote differences between somatic variants.

Table S3. Neutralization activity of rescued mAbs on a multi-clade 16-pseudovirus panel

		IC50 (μg/ml)[a]				
	Isolate	PGC14	PG9	PG16	PGG14	PG20
Clade A	94UG103	>50	0.17	0.008	>50	>50
	92RW020	28.60	0.06	0.004[b]	>50	>50
	93UG077	>50	>50	>50	>50	>50
Clade B	92BR020	0.64	>50	>50	>50	>50
	APV-13	>50	>50	>50	>50	>50
	JRCSF	>50	<0.0025	<0.0025	>50	>50
	APV-17	>50	26.45	>50	>50	>50
	APV-6	7.41	0.09	0.08[b]	>50	25.770
Clade C	93IN905	>50	N/A	0.10[b]	>50	>50
	IAVI-C18	>50	0.05	0.007	>50	>50
	IAVI-C22	>50	N/A	0.069[b]	>50	>50
	IAVI-C3	9.50	12.91	14.80	>50	>50
Clade D	92UG024	>50	10.96	>50	>50	>50
	92UG005	>50	>50	>50	>50	>50
CRF01_AE	92TH021	>50	0.11	0.13[b]	>50	>50
	CMU02	>50	>50	>50	>50	>50
negative control	aMLV	>50	>50	>50	>50	>50

[a] White squares indicate an IC_{50} of >50 μg/ml, green squares indicate 50 μg/ml > IC_{50} >10 μg/ml, yellow squares indicate 10 μg/ml > IC_{50} >1 μg/ml, orange squares indicate 1 μg/ml > IC_{50} >0.1 μg/ml, and red squares indicate IC_{50} <0.1 μg/ml. N/A, not available.

[b] Plateau observed in curve.

Table S4. Neutralization activity of mAbs against a cross-clade 162-pseudovirus panel

Clade	Virus	IC50 (µg/ml)[a]							IC50 (1/Dil'n)[b]
		b12	2G12	2F5	4E10	PG9	PG16	PGC14	Donor Serum
A	MGRM-A-001	>50	>50	>50	15.08	>50	>50	>50	<100
	MGRM-A-002	>50	>50	>50	6.45	0.02	0.004	>50	804
	MGRM-A-003	>50	>50	7.37	5.94	0.65	2.65	>50	<100
	MGRM-A-004	>50	>50	7.49	3.14	0.02	0.04	>50	523
	MGRM-A-005	3.64	>50	5.70	4.09	0.28	0.09	>50	175
	MGRM-A-006	13.62	13.75	15.73	9.87	>50	>50	>50	131
	MGRM-A-007	>50	>50	16.33	1.82	0.37	5.91	>50	142
	MGRM-A-008	>50	>50	>50	7.59	>50	>50	>50	142
	MGRM-A-009	4.34	7.47	9.40	12.01	0.03	0.01	>50	941
	MGRM-A-010	>50	17.01	20.75	14.44	0.02	0.004	>50	1430
	MGRM-A-011	4.01	>50	>50	2.88	0.02	0.24	>50	404
	MGRM-A-012	>50	>50	2.36	4.27	11.18	20.72	>50	<100
	MGRM-A-013	7.04	>50	0.66	1.46	0.16	0.09	>50	350
	MGRM-A-014	>50	>50	1.43	1.74	0.62	20.33	>50	158
	94UG103	6.92	48.12	1.92	4.97	0.24	0.04	>50	350
	92RW020	>50	0.48	3.36	4.54	0.08	0.28	46	282
	93UG077	46.95	>50	3.30	10.60	>50	>50	>50	206
	94KE105	>50	7.22	>50	7.63	29.56	6.13	>50	<100
	93RW029	>50	>50	>50	15.52	1.19	3.83	42	256
	92RW009	>50	26.14	39.08	>50	0.03	0.11	>50	254
	92UG031	>50	>50	3.81	4.94	3.08	0.43	>50	259
	92RW026	>50	17.20	8.63	12.88	0.27	0.03	>50	361
	92UG037	>50	45.24	3.24	8.84	0.02	0.01	>50	1252
	92RW008	9.46	22.47	10.41	14.53	0.01	0.002	37	4067
	92RW021*	>50	>50	4.16	4.87	0.05	0.11	>50	316
	VLGCA1	>50	>50	3.90	4.58	0.07	0.18	>50	197
	92RW024	>50	>50	8.22	8.88	0.18	0.08	>50	241

Clade	Virus	IC50 (µg/ml)[a]							IC50 (1/Dil'n)[b]
		b12	2G12	2F5	4E10	PG9	PG16	PGC14	Donor Serum
B	6535.3 (Acute)	1.93	3.85	2.76	1.23	0.22	36.88	35	387
	QH0692.42 (Acute)	0.73	4.39	5.42	12.67	>50	>50	>50	<100
	SC422661.8 (Acute)	6.11	0.84	>50	6.35	0.79	1.13	>50	182
	PVO.4 (Acute)	>50	0.80	>50	18.32	4.01	5.43	>50	171
	TRO.11 (Acute)	>50	0.29	>50	1.39	5.43	0.22	>50	222
	CAAN.A2 (Acute)	>50	>50	23.05	17.89	5.67	8.83	>50	<100
	TRJ0.58 (Acute)	>50	>50	>50	11.94	0.43	1.16	>50	171
	THR0.18 (Acute)	3.62	>50	>50	4.68	12.39	1.34	>50	<100
	92BR020	>50	4.84	>50	>50	>50	>50	4	<100
	APV_13	>50	9.24	3.81	7.33	>50	>50	>50	<100
	APV_17	>50	>50	4.61	10.53	14.59	24.78	>50	<100
	APV_6	>50	1.90	0.25	1.10	0.12	0.29	23	394
	93TH305	4.17	0.55	7.61	12.33	2.08	19.34	6	133
	VLGCB3	0.15	7.90	>50	5.76	0.02	0.40	21	244
	JRCSF	0.21	0.37	1.85	3.30	0.003	0.001	15	8425
	NL43	0.17	0.49	2.02	4.67	0.32	0.02	40	1488
	JRFL	0.02	1.45	3.54	18.91	>50	>50	>50	<100
	SF162	0.02	1.67	2.52	4.28	>50	>50	<0.0025	9777
	MGRM-Chronic-B-001	0.75	0.08	0.55	1.46	>50	>50	>50	<100
	MGRM-Chronic-B-002	0.86	>50	1.25	2.19	1.41	3.06	>50	220
	MGRM-Chronic-B-003	>50	0.06	1.00	3.50	50.00	0.19	>50	280
	MGRM-Chronic-B-004	0.26	8.65	2.41	3.70	0.11	0.01	>50	1316
	MGRM-Chronic-B-008	2.82	0.55	>50	16.70	6.66	0.73	>50	140
	MGRM-Chronic-B-010	>50	1.50	0.96	1.69	0.004	0.01	27	1640
	MGRM-Chronic-B-011	2.11	>50	0.81	1.07	>50	>50	>50	249
	MGRM-Chronic-B-012	>50	0.22	17.65	48.05	0.91	3.74	>50	304
	MGRM-Chronic-B-017	2.59	>50	>50	2.77	0.32	0.02	>50	644
	MGRM-Chronic-B-018	0.66	>50	10.80	23.19	0.16	0.70	>50	180
	MGRM-Chronic-B-020	6.16	0.20	0.78	2.45	>50	>50	>50	<100
	MGRM-Chronic-B-023	>50	0.16	0.10	27.92	0.04	0.13	>50	286
	MGRM-Chronic-B-024	>50	>50	>50	9.19	0.18	0.01	>50	884

Clade	Virus	IC50 (µg/ml)[a]							IC50 (1/Dil'n)[b]
		b12	2G12	2F5	4E10	PG9	PG16	PGC14	Donor Serum
C	MGRM-C-001	>50	2.93	>50	5.66	>50	16.79	>50	175
	MGRM-C-002	>50	>50	44.68	18.19	>50	28.30	>50	<100
	MGRM-C-004	5.46	>50	>50	24.24	1.18	5.09	>50	183
	MGRM-C-005	2.66	>50	>50	16.41	2.98	2.55	>50	306
	MGRM-C-006	>50	>50	>50	4.94	0.23	2.62	>50	224
	MGRM-C-007	>50	>50	>50	5.84	0.09	0.05	>50	598
	MGRM-C-008	1.51	>50	>50	2.97	>50	>50	>50	160
	MGRM-C-009	>50	>50	>50	0.56	>50	>50	>50	<100
	MGRM-C-010	>50	>50	>50	10.96	12.45	>50	>50	<100
	MGRM-C-012	>50	>50	>50	0.44	0.24	0.48	>50	432
	MGRM-C-013	>50	>50	18.35	2.10	>50	>50	>50	105
	MGRM-C-014	>50	>50	>50	2.48	0.64	>50	>50	124
	MGRM-C-015	13.30	1.75	>50	2.52	0.50	0.26	>50	365
	MGRM-C-017	>50	>50	>50	1.47	1.52	1.80	>50	190
	MGRM-C-019	>50	>50	>50	3.49	0.01	0.002	12	6894
	MGRM-C-020	>50	18.58	>50	2.80	>50	>50	>50	<100
	MGRM-C-022	>50	>50	>50	5.71	0.19	0.25	>50	126
	MGRM-C-023	13.88	>50	>50	1.95	0.51	0.09	>50	220
	MGRM-C-024	>50	>50	>50	22.61	0.22	0.04	>50	494
	MGRM-C-025	>50	>50	>50	5.58	0.17	0.04	>50	434
	93IN905	21.38	>50	>50	1.26	0.03	0.25	19	647
	IAVIC_18	>50	>50	>50	>50	0.10	0.02	>50	577
	IAVI_C22	7.64	>50	>50	2.02	0.14	0.02	25	1002
	IAVI_C3	0.94	>50	>50	2.85	1.45	9.55	12	443
	98IN022	0.42	>50	>50	0.53	0.006	0.003	9	2708
	93MW959	>50	>50	>50	4.55	0.04	0.007	>50	976
	97ZA012	>50	>50	>50	4.70	1.27	2.55	>50	188
CRF08_BC	98CN006	>50	>50	>50	1.91	>50	>50	>50	397
CRF07_BC	98CN009	1.52	>50	>50	2.46	1.07	5.76	43	289

Clade	Virus	IC50 (µg/ml)[a]							IC50 (1/Dil'n)[b]
		b12	2G12	2F5	4E10	PG9	PG16	PGC14	Donor Serum
D	MGRM-D-001	>50	>50	0.63	1.84	>50	>50	>50	<100
	MGRM-D-002	>50	>50	24.64	9.44	0.027	0.01	29	515
	MGRM-D-003	>50	>50	>50	2.49	0.02	0.01	>50	363
	MGRM-D-004	>50	>50	2.30	1.58	0.03	0.01	>50	616
	MGRM-D-005	>50	25.66	>50	35.16	0.59	19.66	>50	<100
	MGRM-D-008	>50	>50	>50	42.90	6.86	>50	>50	<100
	MGRM-D-011	7.75	1.50	>50	0.91	0.06	0.01	>50	298
	MGRM-D-012	0.13	>50	1.70	1.13	9.31	0.35	>50	<100
	MGRM-D-013	>50	>50	2.12	5.38	0.06	0.11	>50	<100
	MGRM-D-014	>50	>50	2.22	3.24	0.02	0.003	48	5127
	MGRM-D-016	1.12	>50	9.85	15.45	0.10	0.02	>50	364
	MGRM-D-018	1.39	0.12	4.05	3.90	0.02	0.004	>50	883
	MGRM-D-019	>50	>50	0.14	0.04	0.03	0.01	>50	497
	MGRM-D-020	>50	>50	>50	>50	2.03	16.27	>50	<100
	MGRM-D-021	5.23	22.98	>50	13.26	>50	>50	>50	<100
	MGRM-D-022	17.63	>50	8.45	16.92	>50	>50	>50	<100
	MGRM-D-024	5.92	>50	>50	3.60	0.03	0.02	>50	239
	MGRM-D-026	1.55	>50	4.37	2.95	17.51	>50	>50	<100
	MGRM-D-028	0.78	>50	>50	1.28	4.39	>50	>50	<100
	MGRM-D-029	>50	>50	>50	5.30	>50	>50	>50	<100
	92UG024	45.64	0.42	0.95	2.17	1.91	23.98	>50	112
	92UG005	>50	>50	8.61	7.46	>50	>50	>50	<100
	92UG046	0.07	>50	>50	12.15	0.64	1.42	>50	114
	92UG001	1.01	>50	12.98	13.58	41.79	>50	>50	<100
	94UG114	>50	13.92	>50	9.72	>50	>50	>50	<100

Clade	Virus	IC50 (µg/ml)[a]							IC50 (1/Dil'n)[b]
		b12	2G12	2F5	4E10	PG9	PG16	PGC14	Donor Serum
CRF01_AE	MGRM-AE-001	25.95	>50	0.29	0.85	2.97	4.33	>50	<100
	MGRM-AE-002	17.10	>50	0.31	0.55	0.04	0.01	>50	653
	MGRM-AE-003	>50	>50	0.24	0.34	0.02	0.03	>50	211
	MGRM-AE-004	>50	>50	0.98	1.27	0.01	0.002	>50	1773
	MGRM-AE-005	0.63	>50	0.14	0.47	0.16	0.02	>50	233
	MGRM-AE-006	>50	>50	0.18	0.23	0.05	0.03	>50	151
	MGRM-AE-007	>50	>50	0.07	0.45	0.11	0.04	>50	176
	MGRM-AE-008	>50	>50	>50	0.94	10.58	3.25	>50	141
	92TH021	N/A	>50	N/A	1.17	0.09	0.10	>50	192
	CMU02	29.32	>50	0.60	0.72	7.69	43.63	>50	142
CRF_AG	MGRM-AG-001	11.87	0.69	0.75	1.12	8.83	0.03	>50	388
	MGRM-AG-002	0.89	0.54	0.54	0.80	0.04	0.03	>50	147
	MGRM-AG-003	>50	>50	0.14	0.64	9.71	>50	>50	<100
	MGRM-AG-005	>50	>50	>50	2.13	29.67	>50	>50	150
	MGRM-AG-006	>50	3.92	0.85	1.76	>50	>50	>50	<100
	MGRM-AG-008	>50	>50	0.54	1.48	0.02	0.002	45	1518
	MGRM-AG-009	>50	>50	24.80	31.39	>50	>50	>50	<100
	MGRM-AG-011	>50	>50	>50	1.36	0.01	0.002	>50	1427
	MGRM-AG-012	10.40	1.94	0.33	0.86	1.37	25.13	>50	<100
	MGRM-AG-013	>50	0.95	1.79	2.61	0.23	0.31	>50	<100
G	MGRM-G-001	>50	>50	4.10	2.04	0.16	0.15	>50	<100
	MGRM-G-004	>50	>50	>50	1.47	>50	>50	>50	<100
	MGRM-G-006	>50	>50	1.33	1.23	0.51	2.42	>50	116
	MGRM-G-009	>50	>50	7.21	1.34	4.90	>50	>50	<100
	MGRM-G-011	>50	>50	1.16	1.44	0.19	0.04	>50	150
	MGRM-G-013	>50	>50	0.59	1.15	>50	>50	>50	<100
	MGRM-G-014	>50	>50	9.65	13.67	6.32	6.98	>50	<100
	MGRM-G-015	>50	>50	0.43	1.07	1.51	5.33	>50	<100
	MGRM-G-016	>50	>50	16.82	1.02	0.40	11.35	>50	<100
	MGRM-G-017	>50	>50	0.60	1.14	0.03	0.02	>50	453
	MGRM-G-019	3.77	31.03	>50	6.53	0.67	1.21	>50	<100
	MGRM-G-024	2.38	>50	1.07	1.57	0.07	0.01	>50	236
	MGRM-G-025	>50	31.94	>50	1.70	>50	>50	>50	<100
	MGRM-G-027	>50	>50	0.28	1.19	0.01	0.01	>50	351
	MGRM-G-028	>50	28.25	2.24	6.32	0.13	3.09	>50	<100

Clade	Virus	IC50 (μg/ml)[a]							IC50 (1/Dil'n)[b]
		b12	2G12	2F5	4E10	PG9	PG16	PGC14	Donor Serum
F	MGRM-F1-004	>50	>50	4.31	2.74	0.11	0.43	>50	104
	MGRM-F1-006	>50	>50	1.10	1.01	1.45	0.27	>50	<100
	MGRM-F1-008	>50	>50	1.61	2.75	>50	>50	>50	<100
	MGRM-F1-010	>50	N/A	14.56	3.69	0.03	0.01	>50	634
	MGRM-F1-012	>50	1.81	>50	0.37	0.01	0.003	>50	866
	MGRM-F1-013	>50	>50	4.57	N/A	0.56	N/A	6	142
	MGRM-F1-014	>50	>50	15.13	7.36	0.01	0.01	>50	437
	MGRM-F1-015	>50	>50	0.10	0.53	>50	>50	>50	<100
	MGRM-F1-016	>50	>50	21.47	7.61	0.58	1.12	>50	<100
	MGRM-F1-017	>50	>50	>50	4.92	>50	>50	>50	<100
	MGRM-F1-018	>50	>50	3.91	3.60	0.03	0.01	>50	432
	MGRM-F1-020	>50	>50	0.59	0.66	4.55	4.35	>50	<100
	MGRM-F1-021	>50	14.09	1.37	1.87	>50	>50	46	<100
	MGRM-F1-022	>50	>50	1.26	1.01	0.06	0.08	>50	246
	MGRM-F1-023	>50	9.23	1.78	0.44	>50	>50	>50	101
neg.control	aMLV	>50	>50	>50	>50	>50	>50	>50	<100

[a] White squares indicate an IC_{50} of >50 μg/mL, green squares indicate 50 μg/mL > IC_{50} >10 μg/mL, yellow squares indicate 10 μg/mL > IC_{50} >1 μg/mL, orange squares indicate 1 μg/mL > IC_{50} >0.1 μg/mL, and red squares indicate IC_{50} <0.1 μg/mL. N/A, not available.

[b] White squares indicate an IC_{50} of <1:100 dilution, green squares indicate 1:50 > IC_{50} > 1:150, yellow squares indicate 1:150 > IC_{50} > 1:500, orange squares indicate 1:500 > IC_{50} > 1:1000, and red squares indicate IC_{50} >1:1000 dilution.

Table S5. Neutralization activity of mAbs

a) Neutralization potency.

		Median IC_{90} (µg/mL) against viruses neutralized with an IC_{90} <50 µg/ml						
Clade [a]	# viruses	b12	2G12	2F5	4E10	PG9	PG16	PGC14
A	27	48.45	17.77	28.82	40.62	0.99	0.81	>50
B	31	2.30	4.65	25.85	32.38	0.11	0.01	9.45
C	27	28.41	28.67	>50	23.37	2.94	5.10	>50
D	25	12.68	8.76	9.02	23.45	0.34	0.44	>50
CRF01_AE	10	12.68	>50	8.14	12.95	0.36	1.51	>50
CRF_AG	10	16.97	7.04	13.49	15.78	0.28	1.86	>50
G	15	23.62	>50	17.54	16.67	1.91	1.96	>50
F	15	>50	21.49	17.77	7.64	0.25	0.55	>50
total	162	20.30	13.27	17.54	23.37	0.36	1.16	9.45

White boxes indicate a median potency >50 µg/ml, green boxes between 20 and 50 µg/ml, yellow boxes between 2 and 20 µg/ml, orange boxes between 0.2 and 2 µg/ml, and red boxes <0.2 µg/ml.

[a]CRF_07BC and CRF_08BC viruses are not included in the clade analysis, but are counted towards the total # of neutralized viruses, because there was only one virus tested from each of these clades.

b) Neutralization breadth.

		% viruses neutralized with an IC_{90} <50 µg/ml						
Clade [a]	# viruses	b12	2G12	2F5	4E10	PG9	PG16	PGC14
A	27	4	4	33	22	74	41	0
B	31	45	52	45	23	42	26	6
C	27	15	4	0	41	52	41	0
D	25	28	12	12	20	44	36	0
CRF01_AE	10	11	0	67	70	60	60	0
CRF_AG	10	10	30	70	60	40	40	0
G	15	13	0	53	53	47	27	0
F	15	0	7	47	43	47	29	0
total	162	19	15	33	36	51	35	4
		% viruses neutralized with an IC_{90} <1.0 µg/ml						
Clade [a]	# viruses	b12	2G12	2F5	4E10	PG9	PG16	PGC14
A	27	0	0	0	0	36	27	0
B	31	10	6	0	0	13	19	3
C	27	0	0	0	0	15	15	0
D	25	0	4	0	0	32	20	0
CRF01_AE	10	0	0	0	0	40	30	0
CRF_AG	10	0	0	0	0	30	10	0
G	15	0	0	0	0	13	7	0
F	15	0	0	0	0	33	21	0
total	162	2	2	0	0	25	18	<1

White boxes indicate that no viruses were neutralized, green boxes indicate 1 to 30% of viruses were neutralized, yellow boxes indicate 30 to 60% of viruses were neutralized, orange boxes indicate 60 to 90% of viruses were neutralized, and red boxes indicate 90 to 100% of viruses were neutralized.

[a]CRF_07BC and CRF_08BC viruses are not included in the clade analysis, but are counted towards the total # of neutralized viruses, because there was only one virus tested from each of these clades.

Table S6. Neutralization activity of PG9 and PG16 against HIV-1_{JR-CSF} pseudoviruses containing alanine point mutations

Mutation[a,b]	gp120 domain[c]	Fold IC_{50} increase relative to wild-type[d]	
		PG9	PG16
W112A	C1	1	1
V120A	C1	2	1
K121A	C1 (V1/V2 stem)	1	1
L122A	C1 (V1/V2 stem)	2	1
L125A	C1 (V1/V2 stem)	1	1
V127A	C1 (V1/V2 stem)	30	57
N134A	V1	5	23
N156A	C1 (V1/V2 stem)	280	1500
S158A	C1 (V1/V2 stem)	>2000	>2000
F159A	C1 (V1/V2 stem)	>2000	>2500
N160K	V2	>2000	>2500
T162A	V2	>2000	>2500
I165A	V2	1	1
R166A	V2	2	1
D167A	V2	5	30
K168A	V2	1	3
K171A	V2	1	1
E172A	V2	1	1
Y173A	V2	1400	1000
F176A	V2	>5000	>7000
Y177A	V2	1	5
L179A	V2	1	3
D180A	V2	1	4
V181A	V2	200	250
V182A	V2	1	3
I184A	V2	1	1
D185A	V2	1	1
N188A	V2	3	3
T190A	V2	2	4
N197K	C2 (V1/V2 stem)	1	1
T198A	C2 (V1/V2 stem)	2	1
S199A	C2 (V1/V2 stem)	2	1
T202A	C2 (V1/V2 stem)	1	1
F210A	C2	3	1
I213A	C2	1	1
N241A	C2	4	3
N262A	C2	1	1
N276A	C2	1	1
N295A	C2	2	1
T297A	V3 (base)	1	1
P299A	V3 (base)	200	1400
N301A	V3 (base)	9	3
N302A	V3 (stem)	1	1
R304A	V3 (stem)	2	3
K305A	V3 (stem)	50	2800
S306A	V3 (tip)	1	1
I307A	V3 (tip)	10	3000
H308A	V3 (tip)	3	1
I309A	V3 (tip)	9	150
P313A	V3 (tip)	1	1
R315A	V3 (tip)	1	1
F317A	V3 (tip)	3	1400
Y318A	V3 (tip)	2	1000
T319A	V3 (tip)	1	1
T320A	V3 (tip)	2	1
E322A	V3 (stem)	2	3
D325A	V3 (stem)	1	1
H330A	V3 (base)	1	1
N332A	V3 (base)	1	1
Q337A	C3	1	1
N339A	C3	1	1
K343A	C3	1	1
R350A	C3	1	1
N355A	C3	9	3
S365A	C3	2	3
N386A	C3	1	1
T388A	C3	1	1
N392A	V4	7	23
W395A	V4	1	1
R419A	C4	3	3
I420A	C4	9	11
K421A	C4	1	1
Q422A	C4	9	5
I423A	C4	40	14
I424A	C4	10	9
I439A	C4	2	3
T450A	C4	1	1
L452A	C4	1	1
P470A	V5	1	1

[a] Amino acid numbering is based on the sequence of HIV-1_{HxB2}.

[b] White boxes indicate that the amino acid is identical among 0 to 49% of all HIV-1 isolates, light blue boxes indicate the amino acid is identical among 50 to 90% of all HIV-1 isolates, and dark blue boxes indicate the amino acid is identical among 90 to 100% of all HIV-1 isolates. Amino acid identity was determined based on a sequence alignment of HIV-1 isolates listed in the HIV sequence database at http://hiv-web.lanl.gov/content/hiv-db/mainpage.html.

[c] C refers to constant domains and V refers to variable loops.

[d] Neutralization activity is reported as fold increase in IC_{50} value relative to WT JR-CSF and was calculated using the equation (IC_{50} mutant / IC_{50} WT). Green: substitutions which had a negligible effect on neutralization activity, yellow: 4-9 fold IC_{50} increase, orange: 10-100 fold IC_{50} increase, red: >100 fold IC_{50} increase. Experiments were performed in triplicate and values represent an average of at least three independent experiments.

SUPPORTING REFERENCES

S1. M. D. Simek et al., J Virol, (2009).
S2. D. D. Richman, T. Wrin, S. J. Little, C. J. Petropoulos, Proc Natl Acad Sci U S A **100**, 4144 (2003).
S3. R. Pantophlet et al., J Virol **77**, 642 (2003).
S4. M. Li et al., J Virol **79**, 10108 (2005).
S5. M. Pancera, R. Wyatt, Virology **332**, 145 (2005).
S6. J. Liu, A. Bartesaghi, M. J. Borgnia, G. Sapiro, S. Subramaniam, Nature **455**, 109 (2008).
S7. T. Zhou et al., Nature **445**, 732 (2007).
S8. X. Yang, S. Kurteva, S. Lee, J. Sodroski, J Virol **79**, 3500 (2005).

SUPPORTING NOTES

Monogram Clinical reference Laboratory staff and research staff at Protocol G Clinical Centers : S. Sriplienchan, A. Franca, J. H Kim, M.S. de Souza, E. Hunter, P. Kaleebu, E. Chomba, O. Anzala, C. Vwalika, F. Henderson, S. Holman, A. Kelley, A. Kelleher, L. Gelgor, J. Bizimana, B. Farah, G. Omosa, K. Kayitenkore, K. Middelkoop, M. Kabran, J. Dhaitawat, Minga, S. Roux, and B.O Auma.

Protocol G project team members: J. Cox, T. Tarragona-Fiol, L. Seamons, S. Loewenbein, L. Nielsen, H. Thomson, R. Tladi, A. Balyegisawa, J. Sherwood, M. Onyango and T. Mebrahtu for all of the support that they have provided for this study.

Protocol G Principal Investigators and institutions: G. Miiro and J. Serwanga (MRC/UVRI Uganda Research Unit on AIDS, Uganda Virus Research Institute, Entebbe, Uganda), A. Pozniak (St Stephens AIDS Trust, Chelsea and Westminster NHS Foundation Trust, London UK), D. McPhee (NRL, St Vincent's Institute, Melbourne, Victoria, Australia), O. Manigart and L. Mwananyanda (Zambia Emory HIV Research Project, Lusaka, Zambia and the Rwanda-Zambia HIV Research Group, Emory University, Atlanta, GA, USA), E. Karita (Projet San Francisco, Kigali, Rwanda and the Rwanda-Zambia HIV Research Group, Emory University, Atlanta, GA, USA), A. Inwoley (CeDReS/CHU Treichville, Abidjan, Côte d'Ivoire), W. Jaoko (Kenya AIDS Vaccine Initiative, College of Health Sciences, University of Nairobi, Nairobi, Kenya), J. DeHovitz (SUNY Downstate Medical Center, Brooklyn, NY, USA), L.G. Bekker (Desmond Tutu HIV Centre, University of Cape Town, Cape Town, South Africa), P. Pitisuttithum (Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand), R. Paris (Dept. of Retrovirology, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand), and S. Allen (Rwanda-Zambia HIV Research Group, Emory University, Atlanta, GA, USA).