www.sciencemag.org/cgi/content/full/science.1187659/DC1

Supporting Online Material for

Rational Design of Envelope Identifies Broadly Neutralizing Human Monoclonal Antibodies to HIV-1

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Published 8 July 2010 on *Science* Express
DOI: 10.1126/science.1187659

This PDF file includes:

Supplementary Materials and Methods

Human specimens. The sera and peripheral blood mononuclear cells (PBMC) described in this study were from HIV-1 infected individuals enrolled in investigational review board approved clinical protocols at the National Institute of Allergy and Infectious Diseases. Donor 45, from whom mAbs VRC01, VRC02 and VRC03 were isolated, has been HIV-1 infected with a clade B virus for more than 15 years. He is a slow progressor with CD4 T-cell counts over 500 cells/μl, plasma HIV-1 RNA values less than 15,000 copies/ml. He has not initiated antiretroviral treatment.

Computational design of the antigenically resurfaced core (RC) and resurfaced stabilized core (RSC) proteins. The atomic level structures of HIV-1 gp120 in complex with CD4 (Protein Data Bank (PDB) ID: 2NXY) (*S1*), b12 (PDB ID: 2NY7) (*S2*), and F105 (PDB ID: 3HI1) (*S3*) defined the CD4-binding footprint, and neutralizing (b12) as well as non-neutralizing (F105) antibody epitopes on gp120. These structures were used to guide the computational design of new gp120 proteins that maintain the b12 neutralizing epitope but modify the antigenic surface outside the b12 epitope. Modifications outside the b12 epitope included, but were not limited to, mutations to eliminate CD4 and F105 binding and trimming the V1/V2 to eliminate co-receptor epitopes. The structure of gp120 in complex with another non-neutralizing antibody, b13, has been published recently (*S3*) but was not available to guide resurfacing at the time the work was carried out. Designs of most of the resurfaced proteins were based on the wild-type HXB2 core in PDB ID: 2NXY to optimize expression and folding. However, since the stabilized core version of gp120 HXB2 Ds12F123 (*S2, 4*) eliminates binding to most non-neutralizing antibodies and keeps b12 binding intact, some designs (including RSC3) were based on the stabilized core version of gp120 HXB2 Ds12F123.

The general algorithm of the resurfacing design is illustrated in Fig. S1A. First, candidate resurfacing positions on gp120 were identified as surface exposed positions that do not contact the antibody (b12) and are not within or near an N-glycosylation site. Next, the set of amino acids allowed at each resurfacing position was assigned semi-automatically, employing a combination of different types of information (evolutionary information (*S5-6*), structural and

solubility considerations, and similarity/differences with wild-type or pre-existing designs). Finally, RosettaDesign (*S7-8*) was used to select low energy sequences. Different final designs were generated largely by devising different sets of allowed amino acids at each design position, but also by modifying the design positions themselves. The genes of the resurfaced proteins were synthesized for cloning, and the RC and RSC proteins were expressed and characterized for antigenic properties. What follows are details on the process and the individual designs.

Identification of resurfacing positions. CD4- and b12-contacting residues as well as the surface accessibility of each residue on gp120 were determined based on the gp120-CD4 and gp120-b12 structures (PDB ID 2NXY and 2NY7). Surface exposed residues were defined using the program NACCESS (http://www.bioinf.manchester.ac.uk/naccess/) as residues with > 40% side-chain surface area exposed, relative to the same side-chain in an isolated tripeptide. Antibody contact residues were defined as any gp120 residue with at least one heavy atom within 8.0 angstroms of a heavy atom on the antibody. Residues near N-glycosylation sites were defined as any residue with at least one side-chain heavy atom within 6.0 angstroms of any heavy atom on either the N-acetyl-glucosamine (NAG) group or the asparagine of a N-glycosylation site (NXS/T, where X is any residues except proline). Initially, 49 candidate positions were identified on b12-bound gp120 (PDB ID: 2NY7), but the above criteria were relaxed in some cases to allow additional design positions, and in other cases design positions were restricted to generate pairs of molecules with resurfacing mutations at complementary sets of positions.

Semi-automatic assignment of amino acid libraries. Different strategies were used to assign libraries of allowed amino acids at each resurfacing position, in order to obtain different final sequences from RosettaDesign. For the design of RSC2, amino acids from a multiple sequence alignment of HIV-1 HXB2 with SIV (http://www.hiv.lanl.gov) were allowed (evolutionary information), but most hydrophobic residues were disallowed unless packed on the surface of a beta sheet, and all polar and the native HIV-1 residues were allowed (structural and solubility considerations). RC1 was derived from RSC2 by threading the final RSC2 sequence onto the CD4-bound structure (2NXY) and reverting mutations that would destabilize the CD4-bound conformation. The design of RSC3 was carried out following experimental feedback that RSC2 successfully maintained nM b12 affinity. For RSC3, most mutations from RSC2 were enforced,

a wider range of amino acids were allowed at some positions that had not been mutated in RSC2, and additional resurfacing positions were selected based on both exposure and distance from mutations in RSC2. The goal was to ensure that as many potential antibody footprints of area ~20 $Å^2$ outside the b12-binding site as possible contained at least one mutation. The criteria for resurfacing positions were relaxed for RSC3 – eight of the new positions were near a NAG, and six were slightly less than 40% exposed. Finally, the native amino acid was not allowed at the new RSC3 design positions, guaranteeing increased resurfacing surface coverage. Resurfacing positions and allowed amino acids for RC4-8 were designed to increase the resurfaced area and antigenic diversity of RC1, following experimental feedback that RC1 maintained high b12 affinity. RC8 was generated using an expanded set of design positions and nearly only polars were allowed at all design positions. RC7 was generated using the same design positions as RC8, but the amino acids chosen for RC8 were disallowed at most design positions, and native amino acids were disfavored directly by assigning them a small energetic penalty. RC4 and RC5 utilized different resurfacing positions compared to RC1, RC7, and RC8 wherever possible, were restricted to polar mutations, and RC5 was designed to be antigenically different than RC4 by disallowing amino acids chosen for RC4. RC6 used the same design positions as RC1, but expanded beyond those positions, and disallowed the amino acids used in RC1.

RosettaDesign parameters. In all cases non-exposed amino acids were held fixed at the native rotamer. In most cases surface exposed amino acids that were kept as native were also held fixed at the native rotamer. For design of RSC3, amino acids designed into the parent RSC2 were allowed to repack during design of RSC3. The lowest energy design for a particular combination of resurfacing positions and allowed amino acids was selected for experimental testing.

RSC2 and RSC3 sequences. The final RSC2 and RSC3 designs contained 34 and 61 mutations relative to the stabilized core (HXB2 Ds12F123) in PDB ID: 2NY7, respectively (not including the V1/V2 trim discussed below). The stabilized core has a total of 330 amino acids, so the resurfacing mutations in RSC2 and RSC3 amounted to modifications of 10% and 18% of the protein, respectively. For RSC3, 82% (50/61) of the mutations were contained in the SIV multiple sequence alignment and 18% (11/61) were not, illustrating that structural and solubility considerations contributed to the design. Only 11 (18%) of the final RSC3 mutations were

contained within the sequence of SIVmac32H, illustrating the importance of using a multiple sequence alignment rather than a single sequence. Mutations for all the resurfaced proteins are highlighted in the sequence alignment in fig. S1B.

Eliminating CD4 and F105 binding. RSC2 and RSC3 were designed on the stabilized core that already eliminates F105 binding. To eliminate CD4 binding, the resurfacing described above was expected to suffice due to mutations of two CD4 contact residues in the β20/21 (mutations were N425G and W427V) and due to the favored b12-bound conformation of the β20/21 over the bridging sheet conformation necessary for CD4 binding (by mutations in β20/21 and in α1 which is linked to β2021 by a disulfide in RSC3). To eliminate F105-binding for RC1 and RC4-8 mutations at key F105 contact locations were added to the resurfacing design. To eliminate CD4 binding for RC1 and RC4-8, the β20/21 was truncated to GG between I423 and Y435.

V1/V2 stem trimming. The V1/V2 was trimmed differently in different constructs, attempting to find minimal truncation while maintaining high protein expression. RSC2 utilized the same V1/V2 trim as the stabilized core in the b12-crystal structure (PDB ID: 2NY7), but RSC3 had a more aggressively truncated V1/V2 (see alignment in fig. S1B). RC1 and RC4-8 used a V1/V2 trim sequence of VKLTPLAGATSVITQA between C119 and C205, as previously described (*S4*).

Protein expression and purification. Genes for HXB2 core, the stabilized core (HXB2 Ds12F123) and the designed RC and RSCs were each synthesized with a C-terminal His tag by GeneArt (Regensburg, Germany), and cloned into a mamalian CMV/R expression vector (*S9*). Proteins were produced by transient tranfection using 293fectin (Invitrogen, Carlsbad, CA) in 293F cells, a human embryonic kidney cell line (Invitrogen) maintained in serum-free free-style medium (Invitrogen). Culture supernatants were harvested 4-5 days after transfection, filtered through a 0.45 μm filter, and concentrated with buffer-exchange into 500 mM NaCl, 50 mM Tris (pH 8.0). Proteins were first purified by Co-NTA (cobalt-nitrilotriacetic acid) chromatography method using a HiTrap IMAC HP column (GE Healthcare, Piscataway, NJ). The peak fractions were collected, and further purified by gel-filtration using a HiLoad 16/60 Superdex 200 pg column (GE Healthcare). The fractions containing monomers of each protein were combined, concentrated and flash frozen at -80°C. To generate the surface plasmon resonance (SPR) data

shown in Fig. 2C and fig. S4, both RSC3 and the stabilized core (Ds12F123) were further purified using b12 affinity column. Monomeric gp120s were expressed by transient transfection of 293F cells as previously described (*S10-11*). The CD4bs knockout mutant gp120 D368R was also previously described (*S10-11*).

Antibodies, plasmids, antibody expression and purification. Anti-gp120 mAb 2G12 was purchased from Polymun Scientific Inc. (Vienna, Austria). Anti-CD4bs mAbs were provided as follows: F91 and 1.5E were provided by James Robinson (Tulane University, New Orleans, LA); mAb F105 was provided by Marshall Posner (Dana Farber Cancer Institute, Boston, MA); mAb b6 was provided by Dennis Burton (Scripps Research Institute, La Jolla, CA); mAb m18 IgG and Fab were provided by Dimiter Dimitrov (National Cancer Institute, Frederick, MD). For mAbs directed to the co-receptor region of gp120, 17b, 48D and E51, were provided by James Robinson (Tulane University). The anti-V3 mAb 447-52D was provided by Susan Zolla-Pazner (New York University, New York, NY), and 39F, also reactive to V3, was provided by James Robinson (Tulane University). For mAbs that bind to the constant regions of gp120, 2.2C and 211C were provided by James Robinson (Tulane University). The anti-gp41 mAb 2F5 was provided by Hermann Katinger (Institute of Applied Microbiology, University of Agriculture, Vienna, Austria). HIV immune globulin (HIVIG) was obtained from the NIH AIDS Research and Reference Reagent Program (ARRRP). Two domain soluble CD4 (sCD4) was obtained from the ARRRP or purchased from Pharmacia. The heavy and light chain genes of mAbs b12 and b13 were synthesized and cloned into the CMV/R expression vector containing the constant regions of IgG1. Full-length IgGs were expressed from transient transfection of 293F cells, and purified by affinity chromatography using HiTrap Protein A HP Columns (GE Healthcare). The CD4-Ig plasmid construct was provided by Joseph Sodroski (Dana Farber Cancer Institute) and the fusion protein was expressed by transient transfection as described above. The Cf2Th/syn CCR5 cell line, a canine thymocyte line stably transfected to express human CCR5, was obtained from the NIH ARRRP, as contributed by Tajib Mizabekov and Joseph Sodroski (Dana Farber Cancer Institute).

Surface plasmon resonance (SPR). The binding kinetics of gp120 variants with different ligands were assessed by SPR analysis on Biacore 3000 or Biacore T-100 (GE Healthcare). HIV-

1 gp120-reactive ligands (CD4-Ig and specific mAbs) were either immobilized directly onto a CM5 sensor chip with standard amine coupling or captured with a mouse anti-human IgG Fc antibody supplied in the "human antibody capture kit" (GE Healthcare) to a surface density about 300 response units (RU). Variant gp120s at 5 - 200 nM were first passed over the modified sensor chips at 30 µl/min for 3 - 4 min, followed by a 5 min dissociation phase to identify rough binding affinities. Then a 2-fold increasing series of gp120 concentrations were passed over the chip, with the concentration of the series adjusted so that at least three runs resulted in maximum 10 - 150 RU. Accompanying each gp120 series, blank reference using buffer to mock gp120 was included. The buffer in all experiments was 10 mM HEPES, pH 7.4, 150 mM NaCl, 3 mM EDTA, and 0.01% surfactant P-20. Sensorgrams were corrected with blank reference and fit globally with Biacore Evaluation software using a 1:1 Langmuir model of binding. Although CD4-Ig and other ligands might formally be analyzed with a two-state binding model, such treatment should not affect the primary on-rates nor overall K_Ds reported here.

ELISA analyses. Each antigen in PBS (pH 7.4) at 2 µg/ml was used to coat plates overnight at 4°C. Coated plates were blocked with B3T buffer (150 mM NaCl, 50 mM Tris-HCl, 1 mM EDTA, 3.3% fetal bovine serum, 2% bovine albumin, 0.07% Tween 20) for 1 hour at 37°C, followed by incubation with sera or antibody serially diluted in B3T buffer for 1 hour at 37°C. Horseradish peroxidase (HRP)-conjugated goat anti-human IgG Fc antibody (Jackson ImmunoResearch Laboratories Inc., West Grove, PA) at 1:10,000 was added for 1 hour at 37°C. All volumes were 100 µl/well except that 200 µl/well was used for blocking. Plates were washed between each step with 0.1% Tween 20 in PBS. Plates were developed using either 3,3´,5,5´-tetramethylbenzidine (TMB) (Kirkegaard & Perry Laboratories) and read at 450 nm, or o-phenylenediamine dihydrochloride (Sigma) and read at 490 nm. For competitive ELISA analyses, plates were coated with 1 µg/ml of a sheep anti-gp120 C5 antibody, D7324 (Cliniqa Corp., Fallbrook, CA) or 10 µg/ml of *Galanthus nivalis* lectin (Sigma) to capture 2 µg/ml of purified YU2 gp120 or RSC3 respectively. After blocking, serial dilutions of the competitor antibodies or CD4-Ig were added to the captured gp120 or RSC3 in 50 µl of B3T buffer, followed by adding 50 µl of biotin-labeled antibody at fixed concentrations: 100 ng/ml for VRC01 and VRC02 for both proteins, 4 µg/ml for VRC03 for YU2 gp120 or 100 ng/ml for RSC3, and 20 ng/ml for 17b for YU2 gp120. The plates were incubated at 37^{o}C for 1 hour, followed by incubation with 250

ng/ml of streptavidin-HRP (Sigma) at ambient temperature for 30 min, and developed with TMB as described above.

Isothermal Titration Calorimetry (ITC). Isothermal titration calorimetry (ITC) was carried out using ITC200 microcalorimeter system from MicroCal, Inc. All proteins were thoroughly dialyzed against PBS before use. The dialysis buffer was filtered through a 0.2 μm membrane and used to dilute the protein samples. The concentration of gp120 in the sample cell was approximately 5 μM and that of CD4-Ig or mAbs in the syringe was approximately 25 μM. The molar concentrations of the proteins were calculated using the following molar extinction coefficients: gp120, 1.52; CD4-Ig, 1.2; b12, 1.46; VRC01, 1.53; VRC03, 1.57. Gp120 in the sample cell were titrated to saturation by the stepwise addition of 2 μl of ligand from the syringe at 120-sec intervals at 37°C. The heat evolved upon each injection was obtained from the integral of the calorimetric signal. The values for enthalpy (ΔH) and entropy (ΔS) were obtained by fitting the data to a nonlinear least-squares analysis with Origin software.

Viral entry, neutralization and protein competition assays. Neutralization was measured using single round infection by HIV-1 Env-pseudoviruses and TZM-bl target cells as described previously (*S10, 12-13*). Some assays were performed at the Vaccine Research Center laboratory and others were performed at the Beth Israel Deaconess Medical Center laboratory. Neutralization curves were fit by nonlinear regression using a 5-parameter hill slope equation as previously described (*S13*). The 50% and 80% inhibitory concentrations (IC_{50} and IC_{80}) were reported as the antibody concentrations required to inhibit infection by 50% and 80% respectively. Competition of serum or mAb neutralization was assessed by adding a fixed concentration (25 μg/ml) of the RSC3 or ΔRSC3 glycoprotein to serial dilutions of antibody for 15 min prior to the addition of virus. The resulting IC_{50} values were compared to the control with mock protein added. The neutralization blocking effect of the proteins was calculated as the percent reduction in the IC_{50} value of the antibody in the presence of protein compared to PBS. Synergistic or additive neutralization was assessed by mixing a fixed concentration (10 μg/ml) of the test antibody with serial dilutions of sCD4, CD4-Ig or VRC01 for 15 min prior to the addition of virus. The baseline of viral entry at each concentration of sCD4, CD4-Ig or VRC01 was used to calculate the adjusted percent neutralization (*S14*). Neutralization was also assessed

using Env-pseudoviruses generated by 293T transfection using the pNL4-3 ΔEnv HIV-1 backbone containing a luciferase reporter gene to infect activated PBMC. Neutralizations using uncloned PBMC-derived HIV-1 primary isolates were performed by single-round infection of either TZM-bl cells using luciferase as readout, or activated PBMC using flow cytometry staining for HIV-1 p24 antigen as previously described (*S15*). CD4-facilitated virus entry was performed in the $CCR5^{+}/CD4^{-}$ cell line Cf2Th/syn CCR5 (*S16-17*) with Env-pseudoviruses containing the luciferase pNL4-3 ΔEnv HIV-1 backbone. A mixture of 40 µl of viral stock and 10 µl of serial dilutions of sCD4, CD4-Ig or VRC01 was incubated at 37°C for 30 min before adding 1 x 10^4 Cf2Th/syn CCR5 cells. Virus entry was measured 2 days later by luciferase activity in cell lysates.

Construction of the HIV-1 envelope sequence dendrogram. HIV-1 gp160 protein sequences (excluding the signal peptide) of HXB2 and the 190 isolates used in the neutralization assays were aligned using MUSCLE, for multiple sequence comparison by log-expectation. The protein distance matrix was calculated by "protdist" and the dendrogram was constructed using the neighbor-joining method by "Neighbor". All analysis and the programs used were performed at the NIAID Biocluster (https://niaid-biocluster.niaid.nih.gov/). The tree was displayed with Dendroscope (*S18*).

Isolation of antigen-specific memory B cells by fluorescence activated cell sorting (FACS). The plasmid constructs for RSC3 and ΔRSC3 were modified by the addition of the sequence encoding the Avi-tag signal for biotinylation (LNDIFEAQKIEWHE) at the 3' end of the gene, and the modified genes were subcloned into the CMV/R expression vector. After expression and purification, the proteins were biotinylated at 40 µM utilizing biotin ligase Bir A (Avidity, Denver, CO) at 30˚C for 30 min, followed by removal of excess free biotin and buffer exchange with PBS (pH 7.4) using a 30-kDa Centricon plus filter (Millipore). Biotinylation of the RSC proteins was confirmed by ELISA. To conjugate proteins with the streptavidin-fluorochrome reagents, in a stepwise process, 1/5 of the molar equivalent of the streptavidin-fluorochrome reagent was added to the biotinylated RSC3 or ΔRSC3 at 20-min intervals until the molar ratio of streptavidin-fluorochrome reagent: biotinylated protein reached 1:1. The incubation was carried out at 4°C with gentle rocking. Streptavidin-allophycocyanin (SA-APC) (Invitrogen) was

mixed with biotinylated RSC3 and streptavidin-phycoerythrin (SA-PE) (Sigma) was mixed with biotinylated ΔRSC3. Thus, each protein carried a different fluorochrome: RSC3-SA-APC and ΔRSC3-SA-PE.

Antigen specific B cells were identified with a panel of ligands including fluorescently labeled antibodies for CD3, CD8, CD19, CD20, CD27, CD14, IgG and IgM. PBMC were stained with an antibody cocktail consisting of anti-CD3-APC-Cy7 (BD Pharmingen), CD8-Qdot705 (VRC), CD19-Qdot585 (VRC), CD20-Pacific Blue (VRC), CD27-APC-AlexaFluor700 (Beckman Coulter), CD14-Qdot800 (VRC), IgG-FITC (BD Pharmingen), and IgM-PE-Cy5 (BD Pharmingen). In addition, aqua blue (Invitrogen) was used to exclude dead cells. A total of 25 million cryopreserved PBMC were thawed and resuspended in 10 ml RPMI 1640 medium (Invitrogen) with 10% fetal bovine serum pre-warmed to 37˚C and treated with 20 μg/ml DNase I (New England Biolabs, Ipswich, MA), followed by centrifugation at 860 xG for 5 min. Medium was removed and the cells were resuspended in 10 ml chilled PBS followed by centrifugation at 860 xG for 5 min. The cell pellet was resuspended in 50 μl of chilled PBS with the aqua blue dye and stained at 4^{o}C in dark for 20 min. The antibody cocktail and the RSC3 and ΔRSC3 multimers, in a total volume of 50 μl, was added to the cells and incubated at 4˚C in dark for 1 hour. The cells were washed with 10 ml cold PBS, resuspended in 2 ml cold PBS and passed through a 70-μm cell mesh (BD Biosciences). The stained PBMC were analyzed and sorted using a modified 3-laser FACSAria cell sorter using the FACSDiva software (BD Biosciences). Fluorescence compensation was performed using anti-mouse Ig Kappa compensation beads (BD Biosciences) stained with each antibody in a separate tube. For the CD3-APC-Cy7 antibody, anti-mouse IgH&L COMPtrol beads (Spherotech, Lake Forrest, IL) were used and the aqua blue fluorescence was compensated using pre-labeled amine-beads. Single cells with the phenotype of CD3-, CD8-, aqua blue-, CD14-, CD19+, CD20+, IgG+, IgM-, RSC3+ and ΔRSC3- were defined as CD4bs directed antigen specific B cells, and single cells were sorted into 96-well PCR plates containing 20 μl of lysis buffer per well. The lysis buffer contained 0.5 μl of RNase Out (Invitrogen), 5 μl of 5x first strand buffer (Invitrogen), 1.25 μl of 0.1M DTT (Invitrogen) and 0.0625 μl of Igepal (Sigma). The PCR plates with sorted cells were quickly frozen on dry-ice and stored at -80˚C. The total content of the patient PBMC sample

passing through the sorter was saved in FCS files for further analysis with FlowJo software (TreeStar, Cupertino, CA).

Single B-cell RT-PCR and subsequent sequencing and cloning. For each sorted cell, the IgG heavy and the corresponding Ig light chain gene transcripts were amplified by RT-PCR and cloned into eukaryotic expression vectors to produce full IgG1 antibodies, using previously described methods with minor modifications (*S19-22*). The frozen plates with single B-cell RNA were thawed at room temperature, and the RT reaction was carried out by adding 3 μl of random hexamers at 150 ng/μl, 2 μl of dNTP mix, each at 10 mM, and 1 μl of SuperScript III (Invitrogen) into each well. The thermocycle program for RT was 42°C for 10 min, 25°C for 10 min, 50°C for 60 min and 94°C for 5 min. The cDNA plates were stored at -20°C, and the IgH, Igκ and Igλ variable region genes were amplified independently by nested PCR starting from 5 μl of cDNA as template. All PCRs were performed in 96-well PCR plates in a total volume of 50 μl containing water, 5 μl of 10x buffer, 1 μl of dNTP mix, each at 10 mM, 1 μl of $MgCl_2$ at 25 mM (Qiagen) for 1st round PCR or 10 μl 5x Q-Solution (Qiagen) for 2nd round PCR, 1 μl of primer or primer mix (*S20*) for each direction at 25 μM, and 0.4 μl of HotStar Taq DNA polymerase (Qiagen). Each round of PCR was initiated at 94°C for 5 min, followed by 50 cycles of 94°C for 30 sec, 58°C for IgH and Igκ or 60°C for Igλ for 30 sec, and 72°C for 1 min, followed by 72°C for 10 min. The positive 2nd round PCR products were cherry-picked for direct sequencing with both forward and reverse PCR primers. PCR products that gave a productive IgH, Igκ or Igλ rearranged sequence were re-amplified from the 1st round PCR using custom primers containing unique restriction digest sites and subsequently cloned into the corresponding Igγ1, Igκ and Igλ expression vectors as previously described (*S20*). The full-length IgG1 was expressed by co-transfection of 293F cells with equal amounts of the paired heavy and light chain plasmids, and purified using a recombinant protein-A column (GE Healthcare).

IgG gene family analysis. The IgG heavy and light chain nucleotide sequences of the variable region were analyzed with JoinSolver® (http://Joinsolver.niaid.nih.gov) (*S23*) and using the IMGT database (http://imgt.cines.fr). Normal donor peripheral blood data originated from 120 IgD+CD27+ and 97 IgD-CD27+ sequences pooled for heavy chain analysis (*S24*) and 167 mutated IgM+ sequences for kappa chain analysis (*S25*). The VRC mAb VK gene use was

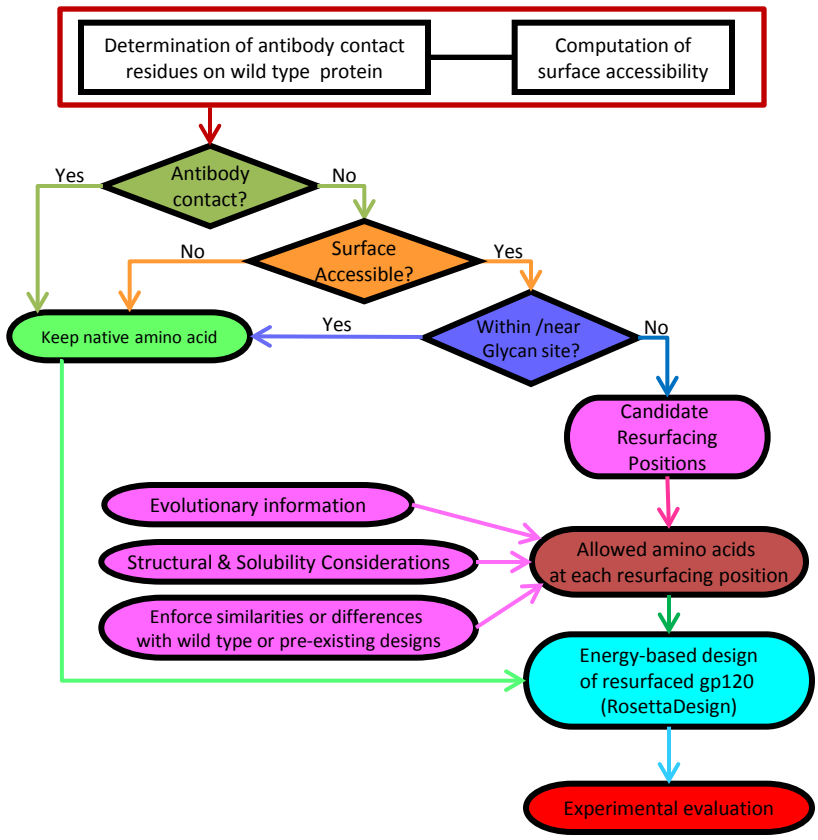
determined by homology to germline genes in the major 2p11.2 IGK locus (*S26*). VRC mAb D gene use was determined by homology to genes in the major 14q32.33 IGH locus.

Env and CCR5 cell surface staining. 293T cells were transfected with plasmid DNA encoding JRFL Env to express the envelope glycoprotein on the cell surface. Cells were stained with anti-Env mAbs as previously described (*S3, 27*). The FACS signal was generated by adding a secondary antibody, goat anti-human IgG $F(ab')_2$ conjugated with phycoerythrin (SouthernBiotech), at 1:125. Data were collected using flow cytometry with the BD LSR Flow Cytometer, and binding curves were generated by plotting the mean fluorescence intensity (MFI) as a function of antibody concentration.

To assess gp120 binding to CCR5 on the surface of cells, we used biotinylated gp120 at 5 μg/ml to stain the CCR5 expressing canine thymus cell line, Cf2Th/syn CCR5. Prior to the staining of Cf2Th/syn CCR5 cells, biotinylated gp120 was incubated with ligands including CD4-Ig, VRC01, VRC02, VRC03 and b12 at serial concentrations ranging from 0.04 - 25 μg/ml. A streptavidin-APC conjugate (Invitrogen) was used at 1 μg/ml to stain Cf2Th/syn CCR5 cells to generate FACS signal, and binding data were collected using flow cytometry with the BD LSR Flow Cytometer. All the staining and incubations were carried out at room temperature for 1 hour.

Statistical analysis. Statistical analyses were performed using GraphPad Prism version 5.0 (GraphPad Software Inc.). A two-sided Fisher's Exact Test at alpha=0.05 was used for assessing the relationship between the viral sensitivity to serum 45 IgG and to VRC01. Among the viruses that were sensitive to both, Deming Regression was used to model the relationship on the $\log_{10}$ scale, allowing for measurement error in the IC_{50}s for both the serum IgG and the mAb. These models were run under the assumption of equal error variance. As a sensitivity analysis, the regression models were rebuilt with an estimated variance ratio; although the slope estimate changed slightly, the conclusions were consistent.

A



B

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                    90        100       110       120       194   200       210       220       230       240       250
                    |         |         |         |         |     |         |         |         |         |         |
HXB2 Ds12F123       EVVLVNVTEN FNWCKNDMVE QMHEDICSLW DQSLKPCVKL TPLCVGAGSC NTSVITQACP KVSFEPIPIH YCAPAGFAIL KCNNKTFNGT GPCTNVSTVQ CTHGIRPVVS
RSC2                TTVLVNVTVT FDWCKNDMVA QMNTAICTLW KTSNKPCVKL TPLCVGAGSC NTSVITQACP TVSFEPIPIR YCAPPGYAIL KCNNKTFNGT GPCTNVSVVT CTDGIRPVVS
RSC3                TTVTVNVTVT FDWCADDMVA TMNTAICTLW KTSNDPCT-- ---------- -------KCP TVRFKPVPIR YCAPPGYAIL KCNNRDFNGT GPCTNVSVVT CTDGIHPVVS

HXB2 core           EVVLVNVTEN FNMWKNDMVE QMHEDIISLW DQSLKPCVKL TPLCVGAGSC NTSVITQACP KVSFEPIPIH YCAPAGFAIL KCNNKTFNGT GPCTNVSTVQ CTHGIRPVVS
RC1                 TTVLVNVTVT FDMWKNDMVE QMDEAIKTLL DTSLKPCVKL TPLAG----- ATSVITQACP TVSWEPIPIR YCAPPGYAIL KCNNKTFNGT GPCTNVSVVT CTHGIRPVVS
RC4                 QKVLVNVTEE FNMWNNMMVE LMHQKIASLI KQSLQPCVKL TPLAG----- ATSVITQACP KVDWEPQPIE YCAPDGFAIL KCNNSTFNGT GPCTNVSTVR CTHGIRPVVS
RC5                 REVLVNVTEQ FNMWRNQMVE AMHREIERLE RAKLNPCVKL TPLAG----- ATSVITQACP KVQFEPTPIT YCAPEGFAIL KCNNDTFNGT GPCTNVSTVD CTHGIRPVIS
RC6                 KQVLVNTTIH FNMWENSMVQ QMHEQIAKLK DQQLEPCVKL TPLAG----- ATSVITQACP VVSWSPEPIK YCAPQGYAIL KCNNNTFNGT GPCTNVSEVE CTHGIKPVVS
RC7                 KQPLQNVTVD FKMWDNDMVD DMHDQIAKEM DEKLSPCVKL TPLAG----- ATSVITQACP KTNWNPVPIK YCAPKGFAIL KCNNATFNGT GPCTNVSTVE CTHGIRPVVS
RC8                 KTPLPNVTQH FDMWNNNMVE EMHQTIQELL KQQLTPCVKL TPLAG----- ATSVITQACP KRKWDPLPIR YCAPPGFAIL KCNNKTFNGT GPCTNVSTVE CTHGIRPVVS

                      260       270       280       290       330       340       350       360       370       380       390
                      |         |         |         |         |         |         |         |         |         |         |
HXB2 Ds12F123       SQLLLNGSLA EEEVVIRSCN FTDNAKTIIV QLNTSVEINC TGAGHCNIAR AKWNNTLKQI ASKLREQFGN NKTIIFKQSS GGDPEIVTHW FNCGGEFFYC NSTQLFNSTW
RSC2                SQLLLNGTLA DEEVVIRSCN FTDNAKTIIV QLNTSVEINC TGAGHCNITR AKWNNTLKQI AEKLREQFGN NKTIIFKQSS GGDPEIVTHW FNCGGEFFYC NSTQLFNSTW
RSC3                SQLLLNGTLA DEKVVIRSCN FSDNAKTIIV QLNTSVEINC TGQGHCNITR AKWNQTLKQI AEKLREQFGN NKTIIFRPSS GGDPEIVTHW FNCGGKFFYC NSTQLFNSTW

HXB2 core           TQLLLNGSLA EEEVVIRSVN FTDNAKTIIV QLNTSVEINC TGAGHCNIAR AKWNNTLKQI ASKLREQFGN NKTIIFKQSS GGDPEIVTHS FNCGGEFFYC NSTQLFNSTW
RC1                 SQLLLNGSLA DEEVVIRSVN FTDNAKTIIV QLNTSVEINC TGAGHCNITR AKWNNTLKQI AEKLREQFGN NKTIIFKQSS GGDPEIVTHW FNCGGEFFYC NSTQLFNSTW
RC4                 SQLLLNGSLA SSEVVIRSVN FTDNAKTIIV QLNTSVEINC TGDGRCNIAR DKWNATLQQI ASKLRQQFGS NKTIIFEQSS GGDPEIVTHW FNCGGEFFYC NSTQLFNSTW
RC5                 SQLLLNGSLA KGEVVIRSVN FTDNAKTIIV QLNTSVEINC TGRGYCNIAR KKWNETLEQI ASKLRDQFGK NKTIIFSQSS GGDPEIVTHW FNCGGEFFYC NSTQLFNSTW
RC6                 SQLLLNGSLA NEEVVIRSVN FTDNAKTIIV QLNSSVEINC TGNGHCNITR AKWNQTLKQI AQKLREQFGE NKTIIFAQSS GGDPEIVTHW FNCGGEFFYC NSTQLFNSTW
RC7                 SQLLLNGSLA ETEVVIRSVN FTDNAKTIIV QLNTSVEINC TGDGSCNIAR QKWNQTLQQI AEKLRRQFGD NKTIIFRSSS GGDPEIVTHW FNCGGEFFYC NSTRLFNSTW
RC8                 SQLLLNGSLA NTEVVIRSVN FTDNAKTIIV QLNTSVEINC TGNGHCNIAR EKWNKTLKQI AKKLREQFGS NKTIIFKSSS GGDPEIVTHW FNCGGEFFYC NSTKLFNSTW

                      400       410       420       430       440       450       460       470       480       490
                      |         |         |         |         |         |         |         |         |         |
HXB2 Ds12F123       FNSTWSTEGS NNTEGSDTIT LPCRIKQIIN MWCKVGKMMY APPISGQIRC SSNITGLLLT RDGGNSNNES EIFRPGGGDM RDNWRSELYK YKVVKIE
RSC2                FNSTWSTKGS NNTEGSDTIT LPCRIKQITG MWCTVGKMMY APPVSGVITC SSNITGLLLT RDGGNDNNES EIFRPGGGDM RDNWRSELYK YKVVKLT
RSC3                FNSTWSTKGS NNTEGSDTIT LPCRIRSITG MVCTVGKMIY APPVEGVITC SSNITGLLLT RDGGNDNNES EIFRPGGGDM RDNWRSELYK YRVVRLT

HXB2 core           FNSTWSTEGS NNTEGSDTIT LPCRIKQIIN MWQKVGKAMY APPISGQIRC SSNITGLLLT RDGGNSNNES EIFRPGGGDM RDNWRSELYK YKVVKIE
RC1                 FNSTWSTKGS NNTEGSDTIT LPCRIKQIGG ---------Y APPVSGVITC SSNITGLLLT RDGGNDNNES EIFRPGGGDM RDNWRSELYK YKVVKLE
RC4                 FNSTWSTEGS NNTEGSDTIS LPCRIKQIGG ---------Y APPTRGQIRC SSNITGLLLT RDGGDSSNES EIFRPGGGDM RDNWRSELYK YKVTPIE
RC5                 FNSTWSTEGS NNTEGSDTIT LPCRIKQIGG ---------Y APPQNGQIRC SSNITGLLLT RDGGPSQNES EIFRPGGGDM RDNWRSELYK YKVKAIE
RC6                 FNSTWSTEGS NNTEGSDTIR LPCRIKQIGG ---------Y APPTSGNISC SSNITGLLLT RDGGNRNNNS EIFRPGGGDM RDNWRSELYK YKVVSR-
RC7                 FNSTWSTEGS NNTEGSQTIQ LPCRIKQIGG ---------Y APPTQNHIHC SSNITGLLLT RDGGNRNNDS EIFRPGGGDM RDNWRSELYK YKVVKEE
RC8                 FNSTWSTEGS NNTEGSNTIE LPCRIKQIGG ---------Y APPTEDNISC SSNITGLLLT RDGGNRDNNS EIFRPGGGDM RDNWRSELYK YKVVQRE
```

Figure S1. (A) Algorithm of structure-based design of the resurfaced core proteins. The design intent was to resurface non-CD4bs regions of the core protein, and to maintain the b12 contact surface while abrogating CD4 binding. **(B)** Protein sequence alignment of the resurfaced proteins and the HIV-1 HXB2 core or stabilized core (Ds12F123), which provided the framework for the resurfaced protein designs. Residue positions are marked according to the HXB2 sequence. Highlighted in red are amino acid substitutions made in the resurfaced proteins in comparison to the original HXB2 core or stabilized core sequences. Gaps are indicated as "-".

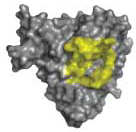
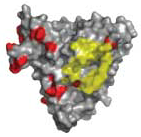
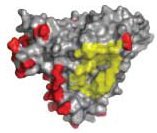
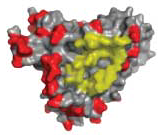
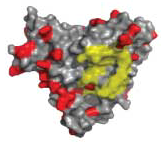
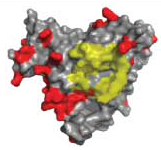
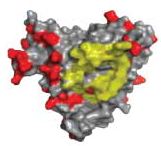
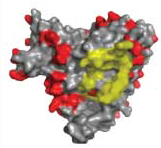
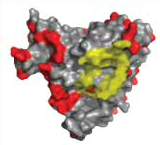
Name	Model	Percent Resurfaced	Antigenicity		
			CD4-Ig	b12	2G12
RC0		0	+++	+++	+++
ΔRC0			—	—	+++
RC1		12.7	+	+++	+++
ΔRC1			—	—	+++
RSC2		17.2	—	+++	+++
ΔRSC2				—	+++
RSC3		31.3	—	+++	+++
ΔRSC3			—	—	+++
RC4		24.6		—	—
ΔRC4				—	+
RC5		25.4	—	++	+
ΔRC5			—	—	—
RC6		26.0	—	++	+
ΔRC6			—	—	—
RC7		31.5		—	—
ΔRC7				—	—
RC8		29.3	—	++	—
ΔRC8			—	—	—

Figure S2. Design and expression of resurfaced core (RC) and resurfaced stabilized core (RSC) glycoproteins, and summary of antigenic reactivity. A panel of 8 resurfaced proteins with different degrees of resurfacing were designed and tested. The surface structural model of each resurfaced protein is shown. Yellow region indicates the outer domain contact site for CD4. The regions colored in red were antigenically substituted. Each protein was analyzed for binding to CD4-Ig, b12 and 2G12. 2G12 binding was used as a marker of conformational integrity of the purified protein. ELISA binding activity was categorized as strong (+++), moderate (++), weak (+) or negative (-). RSC3, used subsequently to isolate mAbs, was 31.3% antigenically resurfaced and maintained strong binding to b12.

Sample ID	Neutralization (ID_{50})						ELISA Binding	
	JRFL (B)	PVO.4 (B)	YU2 (B)	RW020.2 (A)	ZA012.29 (C)	MuLV	RSC3	ΔRSC3
45	4654	172	767	207	301	26	+++	+
N6	2097	245	210	865	205	31	+++	-
N27	558	126	93	2122	53	39	+++	-
N44	408	49	24	17	268	37	+++	-
N32	1250	1640	55	2088	150	44	+++	+
200-384	2468	949	341	582	120	< 5	+++	+
44	6121	182	217	237	89	18	+++	++
N17	810	617	134	566	290	20	+++	+++
1	650	60	139	280	78	27	++	+
N22	2702	87	211	374	160	13	++	++
20	231	113	42	34	47	19	+	-
N95	593	32	409	165	19	34	+++	-
N55	8602	624	96	3072	105	16	+++	++
N26	3666	1875	309	173	100	< 5	++	++
N53	2225	1712	1152	1259	125	34	+	+

Figure S3. Binding to RSC3 protein by a panel of neutralizing sera. Fifteen clade B sera with moderate to broad neutralizing activity were evaluated by ELISA for binding to RSC3 and ΔRSC3. Neutralization of five viral isolates is shown; the clade of each virus is indicated in parenthesis. Neutralization ID_{50} values greater than 1000 are highlighted in red; values between 100 - 1000 are highlighted in yellow. ELISA binding was categorized as strong (+++), moderate (++), weak (+) or negative (-). Preferential binding to RSC3, compared to ΔRSC3, is evidence of CD4bs directed antibodies in the sera. Donor 45 was chosen for additional serum analysis, and eventually for isolation of mAbs.

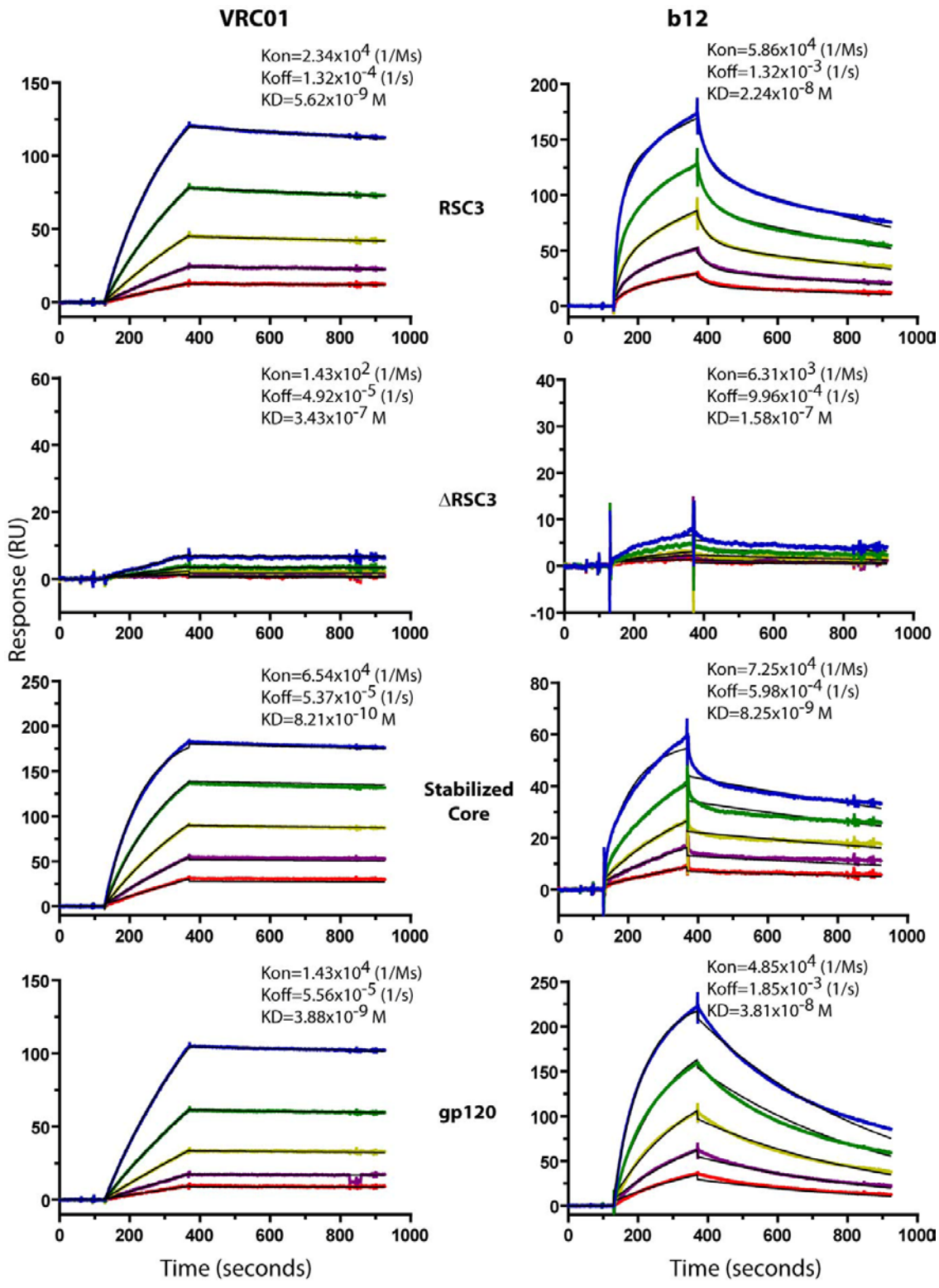


Figure S4A. Comparison of VRC01 and b12 binding kinetics by SPR. The mAbs were captured with a mouse anti-human IgG Fc antibody that was immobilized onto the chip matrix. The binding kinetics of ligands RSC3, ΔRSC3, stabilized core and full-length YU2 gp120 were analyzed.

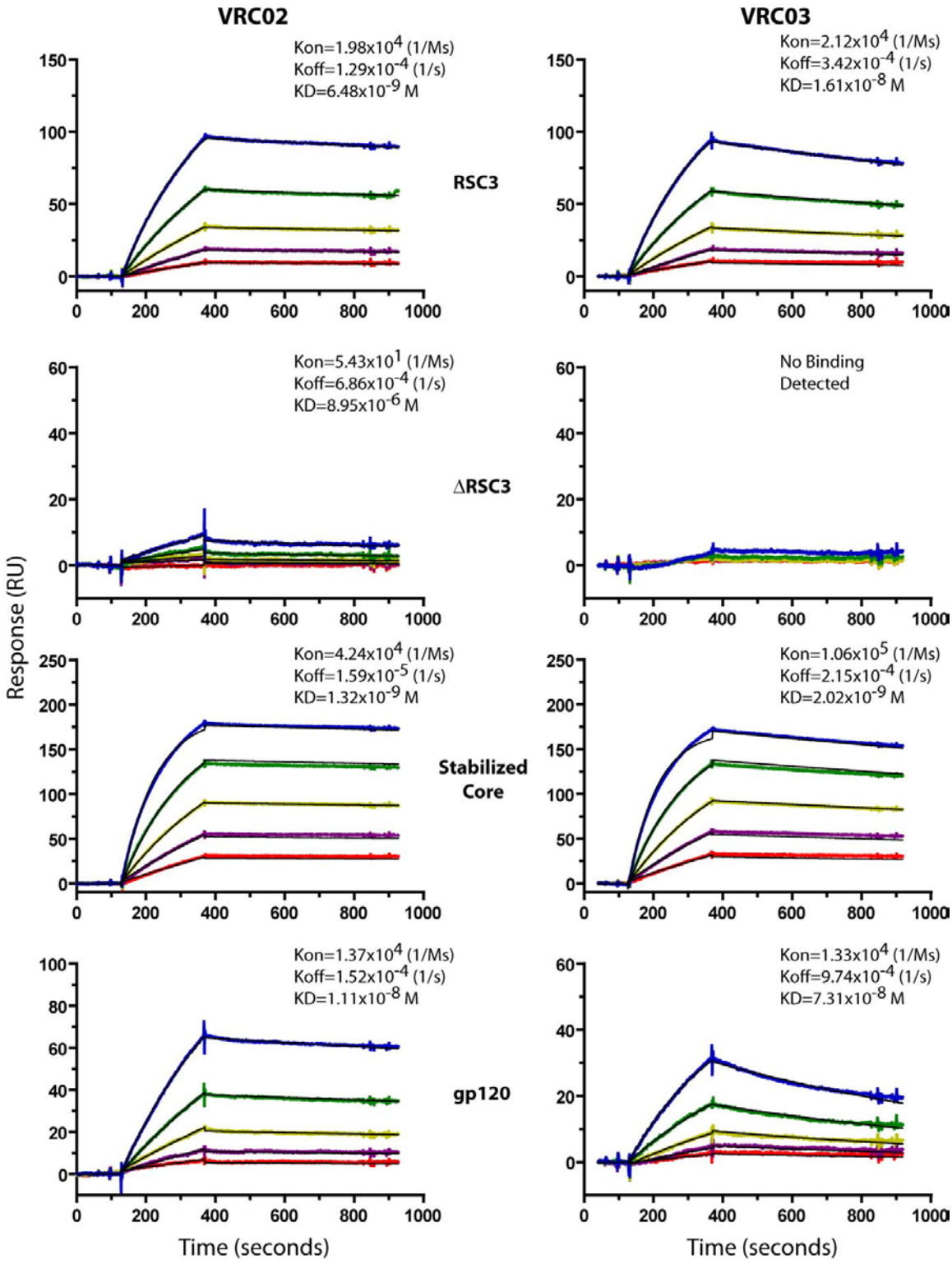


Figure S4B. VRC02 and VRC03 binding kinetics by SPR. The mAbs were captured with a mouse anti-human IgG Fc antibody that was immobilized onto the chip matrix. The binding kinetics of ligands RSC3, ΔRSC3, stabilized core and full-length YU2 gp120 were analyzed.

A

Heavy chain	IGHV	IGHD	IGHJ	CDR3 length (amino acid)	VH mutation frequency
VRC01	1-02*02	3-16*01 (or *02)	1*01[b]	14	91/288 (32%)
VRC02	1-02*02	3-16*01 (or *02)	1*01[b]	14	92/288 (32%)
VRC03	1-02*02	IGHD3 family[a]	1*01	16	86/288 (30%)
b12	1-03*01	3-10*02	6*03	20	39/288 (13%)
Normal donors				15	5.9%

Light chain	IGKV	IGKJ	CDR3 length (amino acid)	VK mutation frequency
VRC01	3-11*01[c]	2*01	5	45/264 (17%)
VRC02	3-11*01[c]	2*01	5	49/264 (19%)
VRC03	3-20*01[d]	2*01	5	53/267 (20%)
b12	3-20*01	2*01	9	35/267 (13%)
Normal donors			9	2.0%

B

Heavy chain

```
                                                                                                IGHD3-16*02          JH1*01
          ---------FR1-------------__CDR1__-------FR2-------__CDR2__--------------------FR3---------------_______CDR3___________---FR4-----
IGHV1-02*02 QVQLVQSGAEVKKPGASVKVSCKASGYTFTGYYMHWVRQAPGQGLEWMGWINPNSGGTNYAQKFQGRVTMTRDTSISTAYMELSRLRSDDTAVYYCAR    DYVW     AEYFQHWGQGTLVTVSS
VRC01       QVQLVQSGGQMKKPGESMRISCRASGYEFIDCTLNWIRLAPGKRPEWMGWLKPRGGAVNYARPLQGRVTMTRDVYSDTAFLELRSLTVDDTAVYFCTRGKNCDYNW       DFEHWGRGTPVIVSS
VRC02       QVQLVQSGGQMKKPGESMRISCQASGYEFIDCTLNWVRLAPGRRPEWMGWLKPRGGAVNYARPLQGRVTMTRDVYSDTAFLELRSLTADDTAVYYCTRGKNCDYNW       DFEHWGRGTPVTVSS
VRC03       QVQLVQSGAVIKTPGSSVKISCRASGYNFRDYSIHWVRLIPDKGFEWIGWIKPLWGAVSYARQLQGRVSMTRDPDWGVAYMEFSGLTPADTAEYFCVRRGSCDYCGDFPWQ     YWGQGTVVVVSS
                                                                                                ↑[QLSQDPD]
```

Light chain

```
          --------FR1---------------_CDR1__--------FR2------CDR2-----------------FR3---------------___CDR3___-----FR4--
IGKV3-11*01 EIVLTQSPATLSLSPGERATLSCRASQSVS SYLAWYQQKPGQAPRLLIYDASNRATGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQRSNWP YTFGQGTKLEIK  JK2*01
VRC01       EIVLTQSPGTLSLSPGETAIISCRTSQYGS ..LAWYQQRPGQAPRLVIYSGSTRAAGIPDRFSGSRWGPDYNLTISNLESGDFGVYYCQQ     YEFFGQGTKVDIK
                                           ^2 aa deletion                                                                ↑[VQ]
VRC02       EIVLTQSPGTLSLSPGETAIISCRTSQYGS ..LAWYQQRPGQAPRLVIYSGSTRAAGIPDRFSGSRWGPDYNLTIRNLESGDFGLYYCQQ     YEFFGQGTKVDIK
                                           ^2 aa deletion                                                                ↑[VQ]

IGKV3-20*01 EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSP
VRC03       EIVLTQSPGILSLSPGETATLFCKASQGGNA..MTWYQKRRGQVPRLLIYDTSRRASGVPDRFVGSGSGTDFFLTINKLDREDFAVYYCQQ     FEFFGLGSELEVH
                                           ^2 aa deletion
```

Figure S5. (A) Gene family analysis of VRC01, VRC02, VRC03 and b12. The VH and VK mutation frequency was calculated from the mutated nucleotides. Mean values from three normal donors consisted of 120 IgD^+CD27^+ and 97 IgD^-CD27^+ sequences for heavy chain analysis and 167 mutated IgM^+ sequences for kappa analysis are shown. Superscript notes: [a]A specific D gene could not be determined since the germline genes with the greatest homology (IGHD3/OR15-3, IGHD3-22 or IGHD3-16) each contained a mutation within a matching length of less than 11 nucleotides and the orphan IGHD3/OR15-3 gene on chromosome 15 cannot contribute to Ig chain synthesis; [b]IGHJ2*01 is an alternative possibility based on the third complementarity determining region 3 (CDR3) sequence analysis; [c]IGKV3-NL1*01 (NL = Not Located) showed greater homology than IGKV3-11*01 by one nucleotide ; [d]IGKV3-NL5*01 showed greater homology than IGKV3-20*01 by one nucleotide. **(B)** The deduced amino acid sequences of the variable regions of VRC01, VRC02 and VRC03. Framework (FR) and CDRs are indicated above the sequence alignment. The top sequence in each group represents the deduced germline sequence with identity to the expressed VH1, D3, JH1, VK3 and JK2 genes. VRC01, VRC02 and VRC03 were derived from the same VH germline gene (IGHV1-02*02), hence all 3 mAbs are aligned to this sequence. VRC01 and VRC02 are somatic variants of each other (they have the same V-D-J recombination). The arrow marks the position of a 7 amino acids insertion (QLSQDPD) in the VRC03 heavy chain FR3 region. A common motif is underlined in the heavy chain CDR1 (GYXFXD), CDR2 (KPXXGAV) and CDR3 (CDYXXDF). VRC01 and VRC02 have the same VK gene (IGKV3-11*01). While the closest inferred germline sequence match for VRC03 was IGKV3-20*01, IGKV3-11*01 was also a close match. The dot symbol marks an amino acid deletion in the VK CDR1. The arrows mark the position of a 2 amino acids insertion (VQ) in VRC01 and VRC02 FR4 (JK gene). Residues in red indicate replacement substitutions compared to germline sequence.

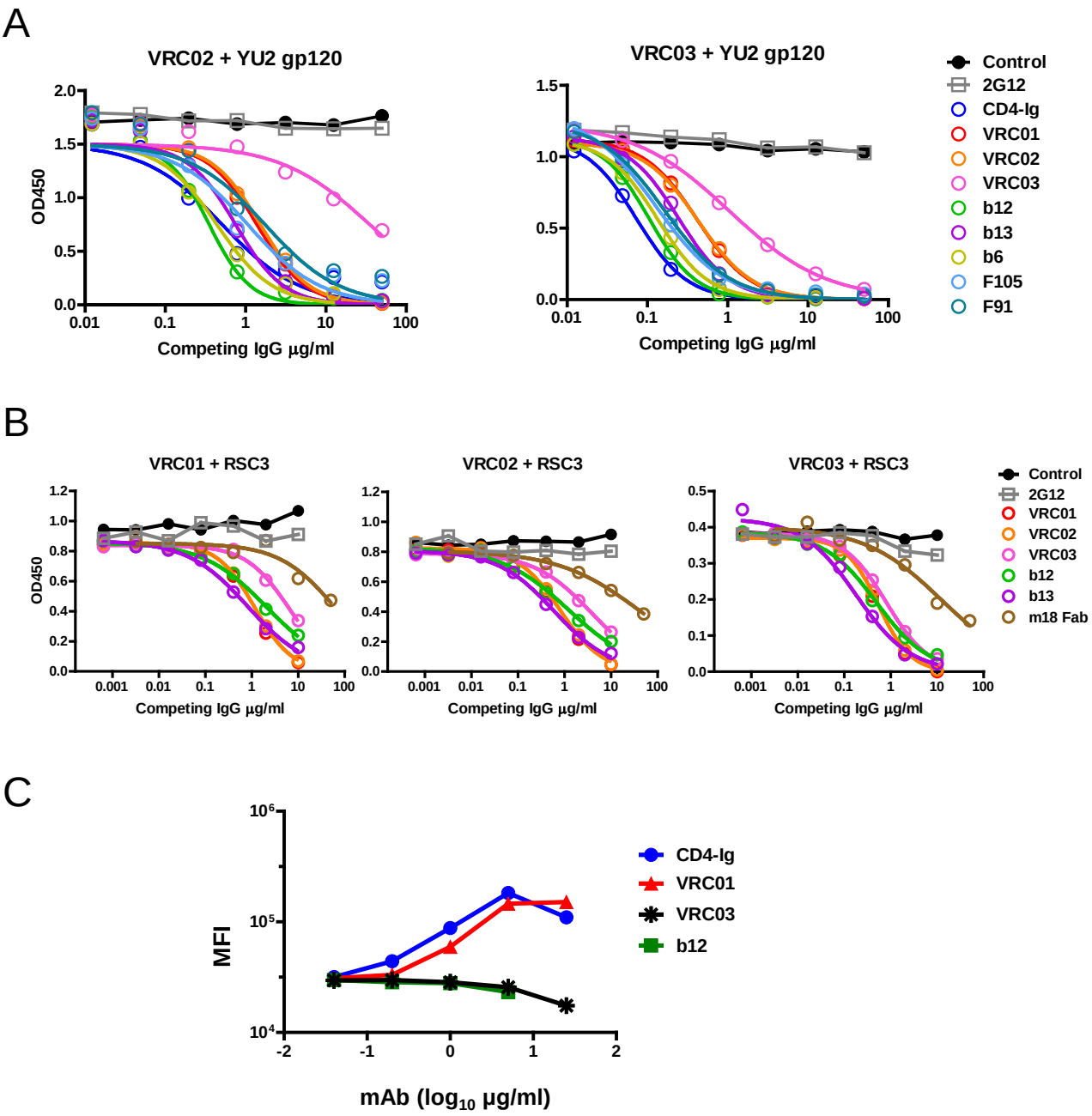


Figure S6. (A) Competition ELISA performed with a single concentration (50 ng/ml) of biotinylated VRC02 or VRC03 binding to YU2 gp120. The unlabeled competing mAbs were titrated into the ELISA at increasing concentrations to evaluate the effect on VRC02 and VRC03 binding respectively. **(B)** Competition ELISA performed with a single concentration (50 ng/ml) of biotinylated VRC01, VRC02 or VRC03 binding to RSC3. The unlabeled competing mAbs were limited to those that showed binding to RSC3. **(C)** Analysis of gp120 binding to cell surface expressed CCR5 by flow cytometry. Biotinylated gp120 at 5 µg/ml was used to stain the human CCR5 expressing canine thymus cell line, Cf2Th/syn CCR5. Prior to cell staining, biotinylated gp120 was incubated with CD4-Ig or mAbs VRC01, VRC03 or b12 with serial concentrations ranging from 0.04 - 25 µg/ml. Binding of gp120 was detected by streptavidin-APC and FACS analysis. Note that CD4-Ig and VRC01 enhanced gp120 binding to CCR5, while mAbs b12 and VRC03 did not.

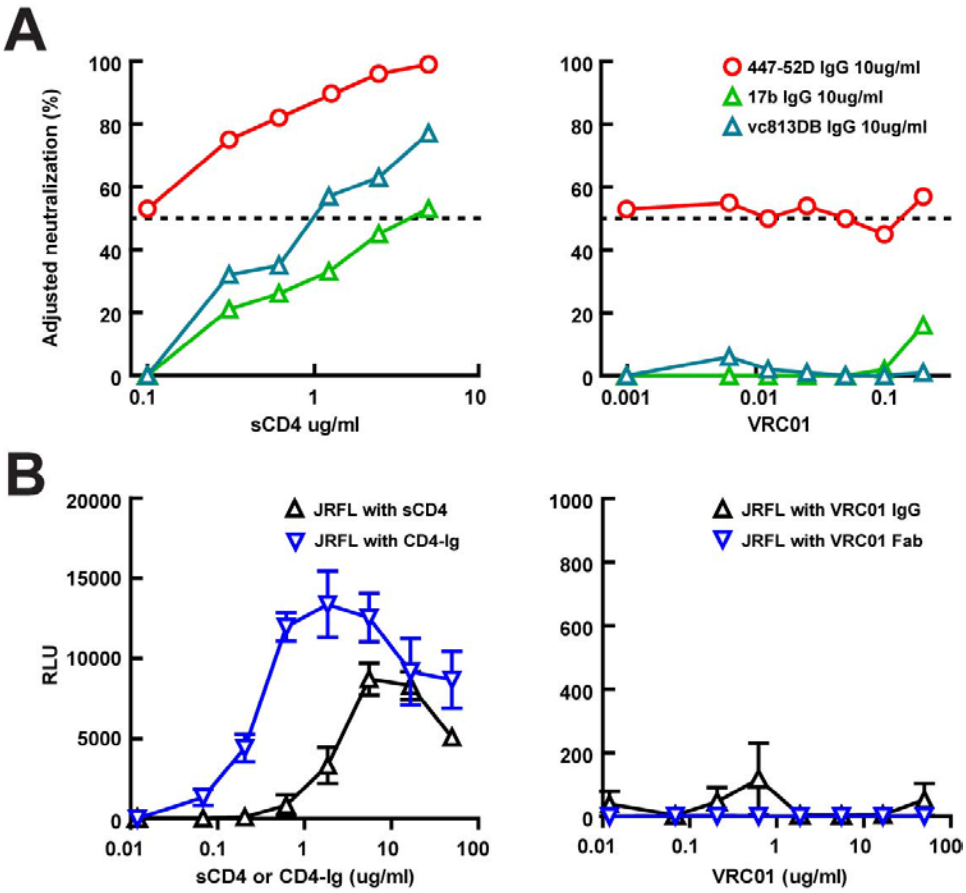


Figure S7. Analysis of the effect of VRC01 on the functional viral spike. (A) Neutralization of JRFL was performed with the mAbs shown in the legend. Antibody 447-52D is directed to the V3 region of gp120, and mAbs 17b and vc813DB are directed to the co-receptor binding region of gp120. The later mAb was isolated from donor 45 and has not previously been published. Graphs show the effect on neutralization as sCD4 (left panel) or VRC01 (right panel) were added to the assay. The adjusted neutralization was calculated using the baseline of viral entry at each concentration of sCD4 or VRC01 (*S14*). In contrast to sCD4, VRC01 did not enhance the neutralization by mAbs 447-52D, 17b and vc813DB. **(B)** JRFL entry into the CCR5+/CD4- cell line, Cf2Th/syn CCR5. CD4-Ig and sCD4 (left panel) promote entry of JRFL into CD4 negative cells. VRC01 (right panel) did not promote viral entry. Each infection was performed in triplicate, and the mean and standard error are shown.

A

	VRC01 sensitive	VRC01 resistant	Total
Serum 45 IgG sensitive	122	8	130
Serum 45 IgG resistant	6	4	10
Total	128	12	140

P=0.005 by Fisher's exact test

B

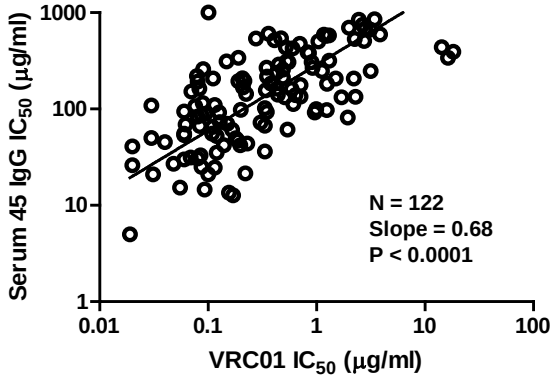


Figure S8. Correlation analysis of neutralization by serum 45 IgG and mAb VRC01. (A) Contingency table showing neutralization by serum 45 IgG and mAb VRC01. Fisher's exact test demonstrated a strong association between the number of viruses neutralized by serum 45 IgG and mAb VRC01. Serum 45 IgG sensitive was defined as an IC_{50} < 1000 µg/ml. VRC01 sensitivity was defined as an IC_{50} < 50 µg/ml. **(B)** Deming regression analysis of log transformed IC_{50} values of viruses neutralized by both VRC01 and serum 45 IgG. This showed a strong association between the potency of serum 45 IgG and mAb VRC01. The slope of the regression line is 0.68 (95% CI 0.07). Thus, while VRC01 accounts for a substantial portion of total serum 45 IgG neutralization, the slope of less than 1.0 suggests that VRC01 does not account for all serum 45 IgG neutralization activity.

Table S1. ELISA binding profiles of VRC01, VRC02 and VRC03 compared to a panel of known mAbs

		YU2 gp120 based mutants*						HXB2 core proteins		Antigenically resurfaced proteins		
		gp120 WT	gp120 D368R	gp120 I420R	gp120 K121D	gp120 D368R/ I420R	gp120 M475S/ R476A	gp120 core	stabilized core	RSC3	ΔRSC3 (Δ371I)	ΔRSC3** (P363N Δ371I)
CD4bs	CD4-Ig	++++[#]	-	++++	++++	-	-	+	++++	-	-	-
	VRC01	++++	+++	++++	++++	+++	+++	++++	++++	++++	++	+
	VRC02	++++	+++	++++	++++	+++	+++	++++	++++	++++	++	+
	VRC03	+++	-	++	+++	-	-	+++	++++	++++	-	-
	b12	++++	-	++++	++++	-	++	++++	++++	++++	-	-
	b13	++++	-	++++	++++	+	++++	++++	++++	++++	-	-
	m18	++++	+	++++	++++	+	++	++++	++++	++++	-	-
	b6	++++	++++	++++	++++	++++	-	++++	+	-	-	-
	1.5E	++++	++++	++++	++++	++++	+	++++	-	-	-	-
	F91	++++	-	++++	++++	-	-	++++	-	-	-	-
	F105	++++	-	++++	++++	-	-	++++	-	-	-	-
Co-receptor	17b	++++	++++	-	+	+	-	-	-	-	-	-
	48D	+++	++++	-	-	-	-	-	-	-	-	-
	E51	++++	++++	-	-	+	-	-	-	-	-	-
V3	447-52D	++++	++++	++++	++++	++++	++++	-	-	-	-	-
	39F	++++	++++	++++	++++	++++	++++	-	-	-	-	-
Constant regions	2.2C	++++	++++	++	+++	++	+	-	-	-	-	-
	211C	+++	+++	+	+	+	-	-	-	-	-	-
Other	2G12	++	+	+	+	+	+	+++	++++	+++	+++	++
	HIVIG	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	+
	2F5	-	-	-	-	-	-	-	-	-	-	-

*Mutant residue numbers are based on the HXB2 sequence.

** This is a double mutant of the Δ371I deletion together with the P363N mutation, which adds an N-linked glycan on the β15 strand near the CD4 binding loop.

[#]Binding was categorized based on the OD450 values at the highest concentration of antibody tested (5 μg/ml for mAbs and CD4-Ig, 50 μg/ml for HIVIG) and the 50% effective concentration (EC_{50}) values as shown below:

++++ $OD_{450} \geq 3.0$ and $EC_{50} \leq 0.1$

+++ $OD_{450} \geq 3.0$ and $EC_{50} > 0.1$

++ $1.0 \leq OD_{450} < 3.0$

\+ $0.2 \leq OD_{450} < 1.0$

\- $OD_{450} < 0.2$

Table S2a: Summary of the breadth and potency of antibody neutralization against 190 HIV-1 Env-pseudoviruses

Clade		Measured by IC_{50} (µg/ml)					Measured by IC_{80} (µg/ml)				
		VRC01	VRC02	VRC03	b12	CD4-Ig	VRC01	VRC02	VRC03	b12	CD4-Ig
Total	Titer < 50	91%	91%	57%	41%	94%	86%	88%	48%	27%	66%
(N = 190)	Titer < 1	72%	75%	39%	17%	30%	42%	44%	26%	5%	8%
	Median*	0.37	0.38	9.76	>50	3.35	1.44	1.37	>50	>50	20.50
	Geometric mean*	0.34	0.32	0.45	1.79	2.39	1.03	1.04	0.94	3.83	6.44
A	Titer < 50	100%	100%	64%	45%	91%	95%	95%	55%	27%	55%
(N = 22)	Titer < 1	95%	95%	45%	23%	32%	77%	82%	23%	5%	14%
	Median*	0.11	0.10	2.47	>50	8.30	0.50	0.39	19.5	>50	43.50
	Geometric mean*	0.15	0.13	0.40	1.29	2.66	0.45	0.40	0.97	2.67	5.46
B	Titer < 50	96%	94%	80%	63%	96%	94%	94%	78%	55%	63%
(N = 49)	Titer < 1	80%	82%	65%	39%	35%	39%	41%	49%	10%	12%
	Median*	0.39	0.44	0.29	1.80	2.49	1.67	1.46	1.11	19.10	15.88
	Geometric mean*	0.39	0.36	0.25	0.75	1.99	1.28	1.25	0.81	3.48	4.43
C	Titer < 50	87%	87%	58%	47%	95%	82%	79%	42%	26%	68%
(N = 38)	Titer < 1	66%	71%	29%	13%	39%	37%	39%	16%	0%	8%
	Median*	0.39	0.40	26.70	>50	1.42	1.50	1.39	>50	>50	18.85
	Geometric mean*	0.34	0.35	1.07	4.46	1.40	1.14	0.93	1.60	7.25	6.17
D	Titer < 50	88%	88%	25%	63%	100%	75%	88%	13%	50%	88%
(N = 8)	Titer < 1	50%	63%	13%	25%	38%	25%	25%	13%	13%	25%
	Median*	1.37	0.72	>50	10.25	1.82	3.91	5.11	>50	>50	6.93
	Geometric mean*	0.73	0.68	1.40	0.91	1.10	1.83	2.78	0.19	1.93	2.76
CRF01_AE	Titer < 50	89%	89%	28%	6%	78%	83%	89%	22%	0%	56%
(N = 18)	Titer < 1	61%	61%	11%	0%	22%	17%	22%	6%	0%	0%
	Median*	0.44	0.50	>50	>50	10.80	1.72	2.08	>50	>50	35.29
	Geometric mean*	0.61	0.56	2.01	41.2	4.89	1.77	2.15	5.67	>50	14.45
CRF02_AG	Titer < 50	81%	81%	19%	19%	100%	75%	81%	19%	6%	75%
(N = 16)	Titer < 1	56%	56%	19%	0%	25%	38%	38%	13%	0%	6%
	Median*	0.59	0.59	>50	>50	2.82	2.20	1.92	>50	>50	25.09
	Geometric mean*	0.35	0.35	0.06	8.04	2.28	0.95	1.18	0.47	42.73	7.96
G	Titer < 50	90%	90%	60%	0%	100%	90%	90%	40%	0%	60%
(N = 10)	Titer < 1	90%	90%	30%	0%	20%	50%	50%	20%	0%	0%
	Median*	0.34	0.42	24.91	>50	7.44	1.00	1.12	>50	>50	24.61
	Geometric mean*	0.25	0.29	1.14	>50	5.14	0.86	0.89	0.91	>50	7.10
CRF07_BC	Titer < 50	100%	100%	73%	27%	100%	91%	91%	45%	18%	91%
(N = 11)	Titer < 1	45%	55%	45%	9%	36%	18%	27%	18%	9%	0%
	Median*	1.25	0.95	1.47	>50	1.20	3.53	3.54	>50	>50	7.52
	Geometric mean*	1.23	0.96	0.74	2.57	1.95	3.05	2.39	0.83	2.32	7.03
Other recombinants	Titer < 50	83%	83%	56%	33%	94%	78%	83%	50%	11%	67%
(N = 18)	Titer < 1	78%	83%	44%	6%	6%	61%	61%	33%	6%	0%
	Median*	0.13	0.12	8.33	>50	7.87	0.41	0.46	>50	>50	25.67
	Geometric mean*	0.12	0.13	0.20	6.99	6.56	0.33	0.44	0.43	3.25	13.12

*Medians were calculated using 100 for any IC_{50} (or IC_{80}) values > 50 µg/ml; Geometric means were calculated for viruses neutralized with an IC_{50} (or IC_{80}) value < 50 µg/ml.

Table S2b: Antibody neutralization data against 22 HIV-1 clade A Env-pseudoviruses

			IC_{50} (µg/ml)*						IC_{80} (µg/ml)*					
Virus ID	**Clade**	**Origin**	**Serum45 IgG**	**VRC01**	**VRC02**	**VRC03**	**b12**	**CD4-Ig**	**Serum45 IgG**	**VRC01**	**VRC02**	**VRC03**	**b12**	**CD4-Ig**
BS208.B1	A	Kenya	5	0.019	0.014	0.297	0.042	0.246	46	0.078	0.050	2.6	0.224	20.8
Q842.d12	A	Kenya	50	0.030	0.025	>50	>50	>50	177	0.096	0.074	>50	>50	>50
DJ263.8	A	Kenya	31	0.080	0.055	>50	0.812	0.088	181	0.553	0.424	>50	>50	0.557
Q769.h5	A	Kenya	67	0.084	0.047	0.034	>50	1.3	679	0.289	0.204	0.140	>50	5.7
Q23.17	A	Kenya	33	0.085	0.071	0.065	>50	12.7	150	0.261	0.220	0.202	>50	>50
KNH1209.18	A	Kenya	87	0.087	0.095	45.0	0.227	6.0	274	0.296	0.260	>50	1.8	>50
MS208.A1	A	Kenya	1000	0.101	0.074	>50	0.201	7.9	>1000	0.462	0.353	>50	1.1	40.9
Q168.a2	A	Kenya	110	0.115	0.092	3.4	>50	11.6	559	0.362	0.310	27.8	>50	>50
Q259.w6	A	Kenya	13	0.170	0.147	0.055	>50	0.708	41	0.543	0.434	0.178	>50	2.8
KER2008.12	A	Kenya	184	0.379	0.265	0.403	>50	0.649	>1000	1.7	0.994	1.7	>50	4.0
Q461.e2	A	Kenya	>1000	0.492	0.463	>50	>50	25.4	>1000	1.6	1.4	>50	>50	>50
KER2018.11	A	Kenya	>1000	0.652	0.516	0.389	>50	3.3	>1000	2.3	1.9	1.3	>50	15.9
RW020.2	A	Rwanda	143	0.224	0.123	>50	10.1	11.7	887	0.883	0.492	>50	33.5	46.1
3415.v1.c1	A	Tanzania	54	0.060	0.060	0.020	2.5	12.6	200	0.150	0.200	0.060	14.1	>50
3365.v2.c20	A	Tanzania	94	0.060	0.070	0.980	10.7	0.050	266	0.170	0.190	9.7	>50	0.230
0330.v4.c3	A	Tanzania	152	0.070	0.100	>50	>50	0.970	628	0.210	0.290	>50	>50	4.7
783.v0.c51	A	Tanzania		0.240	0.290	1.6	23.3	8.7		0.680	0.840	11.2	>50	39.8
398-F1_F6_20	A	Tanzania		0.270	0.240	0.490	0.070	11.7		0.740	0.680	2.0	1.8	>50
216-F2_E3_5	A	Tanzania		0.280	0.350	>50	>50	30.1		0.760	0.900	>50	>50	>50
0260.v5.c36	A	Tanzania		0.760	1.1	0.020	>50	>50		2.0	3.1	0.070	>50	>50
3718.v3.c11	A	Tanzania	574	1.2	0.700	>50	18.9	14.6	>1000	>50	>50	>50	>50	>50
UG037.8	A	Uganda	220	0.079	0.082	12.1	>50	0.134	731	0.313	0.263	>50	>50	0.721
Breadth	**N=22**	**Titer < 50**		**100%**	**100%**	**64%**	**45%**	**91%**		**95%**	**95%**	**55%**	**27%**	**55%**
		Titer < 1		**95%**	**95%**	**45%**	**23%**	**32%**		**77%**	**82%**	**23%**	**5%**	**14%**
Median[#]				**0.11**	**0.10**	**2.47**	**>50**	**8.30**		**0.50**	**0.39**	**19.50**	**>50**	**43.50**
Geometric mean[#]				**0.15**	**0.13**	**0.40**	**1.29**	**2.66**		**0.45**	**0.40**	**0.97**	**2.67**	**5.46**

*Values < 1 µg/ml are highlighted in red, and values 1 – 50 µg/ml are in green. Blanks indicate not tested.
[#]Medians were calculated based on all viruses tested, including those with values > 50 µg/ml, which were assigned a value of 100; Geometric means were calculated for neutralization sensitive viruses with an IC_{50} (or IC_{80}) value < 50 µg/ml.

Table S2c: Antibody neutralization data against 49 HIV-1 clade B Env-pseudoviruses

			IC_{50} (µg/ml)*						IC_{80} (µg/ml)*					
Virus ID	Clade	Origin	Serum45 IgG	VRC01	VRC02	VRC03	b12	CD4-Ig	Serum45 IgG	VRC01	VRC02	VRC03	b12	CD4-Ig
HT593.1	B	Haiti	36	0.334	0.542	0.235	0.117	0.323	706	1.8	3.9	0.741	1.7	4.5
TRO.11	B	Italy	208	0.207	0.208	0.055	>50	>50	1000	0.832	0.876	0.342	>50	>50
PVO.4	B	Italy	195	0.216	0.168	0.328	>50	20.1	>1000	1.2	1.0	1.7	>50	>50
H077.31	B	Peru	50	0.180	0.170	0.050	>50	3.4	473	0.460	0.430	0.130	>50	>50
H078.14	B	Peru	217	0.350	0.260	5.7	>50	14.9	>1000	1.2	0.850	35.6	>50	>50
H029.12	B	Peru	307	0.550	0.410	0.270	>50	44.5	913	1.9	1.4	2.1	>50	>50
H061.14	B	Peru	160	0.590	0.570	0.190	1.1	4.1	827	1.7	1.6	0.480	3.2	15.9
H022.7	B	Peru	427	0.600	0.640	0.260	26.2	8.1	>1000	1.6	1.7	0.810	>50	25.4
H080.23	B	Peru	178	0.710	0.920	0.150	>50	4.8	599	2.4	3.2	0.680	>50	47.1
H079.2	B	Peru	382	0.850	0.830	>50	13.9	2.6	997	2.7	2.3	>50	36.8	8.4
H031.7	B	Peru	306	0.900	0.680	0.290	1.5	39.4	804	2.5	1.8	1.3	5.1	>50
H030.7	B	Peru	581	1.3	1.0	>50	>50	22.0	>1000	3.6	2.9	>50	>50	>50
H035.18	B	Peru	>1000	9.1	14.5	>50	>50	6.9	>1000	29.9	41.9	>50	>50	27.1
H086.8	B	Peru	37	>50	>50	>50	>50	9.8	272	>50	>50	>50	>50	>50
SC422661.8	B	Trinidad	107	0.076	0.084	0.036	0.440	5.2	1000	0.265	0.267	0.105	1.7	>50
QH0515.01	B	Trinidad	>1000	0.386	0.470	0.187	0.300	1.8	>1000	2.9	2.5	0.668	7.2	>50
QH0692.42	B	Trinidad	207	1.5	1.3	0.954	0.970	0.603	>1000	4.8	4.2	2.1	2.7	2.6
JRFL	B	USA	21	0.031	0.024	0.009	0.022	0.247	75	0.093	0.075	0.025	0.075	0.967
RHPA4259.7	B	USA	30	0.060	0.086	1.1	0.120	1.1	132	0.185	0.243	6.6	0.390	13.9
REJO4541.67	B	USA	69	0.062	0.056	0.059	5.9	1.2	228	0.251	0.240	0.196	>50	11.5
TRJO4551.58	B	USA	163	0.083	0.115	0.043	>50	22.1	594	0.207	0.284	0.098	>50	>50
JRCSF	B	USA	15	0.093	0.099	0.093	0.096	0.186	190	0.544	0.475	0.517	0.874	1.7
6101.10	B	USA	207	0.111	0.135	0.094	>50	2.7	749	0.315	0.384	0.184	>50	5.3
YU2	B	USA	92	0.126	0.115	0.037	2.2	0.102	222	0.372	0.359	0.115	7.8	0.314
WITO4160.33	B	USA	312	0.148	0.115	>50	8.5	2.2	>1000	0.412	0.350	>50	41.4	13.2
5768.04	B	USA	61	0.166	0.275	0.382	0.249	0.756	459	0.829	0.854	0.995	14.5	>50
R2	B	USA	42	0.198	0.242	0.035	1.2	0.016	149	0.931	1.2	0.126	9.3	0.063
3988.25	B	USA	21	0.220	0.243	2.5	0.378	49.4	269	1.2	0.881	12.0	4.1	>50
BG1168.01	B	USA	539	0.276	0.458	>50	>50	13.4	>1000	1.5	2.0	>50	>50	>50
89.6	B	USA	132	0.511	0.444	0.187	0.140	0.242	>500	2.3	1.5	0.589	0.560	0.752
6535.3	B	USA	61	0.539	0.733	0.438	0.429	2.5	284	2.7	3.8	2.4	19.1	16.3
CAAN5342.A2	B	USA	388	0.824	0.899	8.3	>50	>50	>1000	2.8	3.1	47.6	>50	>50
BR07	B	USA	97	1.2	0.948	3.4	0.096	0.046	889	5.2	4.2	12.8	0.898	0.211
AC10.0.29	B	USA	207	2.2	2.5	>50	1.8	10.7	>1000	6.5	7.0	>50	14.2	>50
THRO4156.18	B	USA	531	2.3	3.4	>50	1.2	0.509	>1000	23.0	21.7	>50	4.6	2.5
7165.18	B	USA	340	29.3	>50	>50	>50	2.9	>1000	>50	>50	>50	>50	33.0
BL01	B	USA	147	>50	>50	>50	1.7	0.100	>1000	>50	>50	>50	>50	0.625
SC05.8C11.2344	B-trans†	Trinidad	139	0.640	0.660	0.200	0.830	1.1	430	1.9	2.0	0.630	6.2	8.5
PRB926-04.A9.4237	B-trans	USA	21	0.100	0.100	0.040	0.360	0.640	58	0.340	0.340	0.160	1.6	4.6
WEAU-d15.410.787	B-trans	USA	52	0.120	0.130	0.120	0.960	0.720	182	0.260	0.350	0.470	6.7	5.5
1012-11.TC21.3257	B-trans	USA	35	0.120	0.100	0.050	>50	1.7	134	0.320	0.290	0.150	>50	14.8
1006-11.C3.1601	B-trans	USA	70	0.150	0.160	0.080	0.380	2.3	192	0.390	0.530	0.310	1.4	8.7
6244.13.B5.4567	B-trans	USA	168	0.210	0.310	22.8	>50	3.1	558	0.530	0.760	>50	>50	26.8
700010040.C9.4520	B-trans	USA	44	0.230	0.210	0.270	0.300	0.570	217	0.710	0.650	0.960	1.3	2.6
6240.08.TA5.4622	B-trans	USA	112	0.610	1.2	1.1	>50	20.5	443	1.8	3.3	4.2	>50	>50
1054-07.TC4.1499	B-trans	USA	134	0.710	0.950	0.920	0.900	0.990	826	2.9	4.5	3.4	16.7	7.9
1056-10.TA11.1826	B-trans	USA	266	0.920	0.720	0.350	0.510	15.7	952	3.3	3.2	1.5	2.2	>50
62357.14.D3.4589	B-trans	USA	92	0.960	0.360	0.240	17.7	0.220	364	4.7	1.2	0.900	>50	1.0
9021.14.B2.4571	B-trans	USA	132	1.7	0.650	0.310	>50	0.840	550	10.6	2.8	1.1	>50	2.9
Breadth	**N=49**	**Titer < 50**		**96%**	**94%**	**80%**	**63%**	**96%**		**94%**	**94%**	**78%**	**55%**	**63%**
		Titer < 1		**80%**	**82%**	**65%**	**39%**	**35%**		**39%**	**41%**	**49%**	**10%**	**12%**
Median#				**0.39**	**0.44**	**0.29**	**1.80**	**2.49**		**1.67**	**1.46**	**1.11**	**19.10**	**15.88**
Geometric mean#				**0.39**	**0.36**	**0.25**	**0.75**	**1.99**		**1.28**	**1.25**	**0.81**	**3.48**	**4.43**

*Values < 1 µg/ml are highlighted in red, and values 1 – 50 µg/ml are in green. Blanks indicate not tested.
†B-trans indicate viruses representing the transmitted strains during virus transmission (*S28*).
#Medians were calculated based on all viruses tested, including those with values > 50 µg/ml, which were assigned a value of 100; Geometric means were calculated for neutralization sensitive viruses with an IC_{50} (or IC_{80}) value < 50 µg/ml.

Table S2d: Antibody neutralization data against 38 HIV-1 clade C Env-pseudoviruses

			IC_{50} (µg/ml)*						IC_{80} (µg/ml)*					
Virus ID	**Clade**	**Origin**	**Serum45 IgG**	**VRC01**	**VRC02**	**VRC03**	**b12**	**CD4-Ig**	**Serum45 IgG**	**VRC01**	**VRC02**	**VRC03**	**b12**	**CD4-Ig**
BR025.9	C	Brazil	25	0.115	0.208	>50	>50	0.064	242	0.555	1.1	>50	>50	3.6
286.36	C	Ethiopia	47	0.188	0.193	1.8	0.701	7.3	141	0.839	0.868	18.4	2.7	39.1
288.38	C	Ethiopia	100	0.992	0.749	0.342	>50	0.459	484	4.0	2.6	1.2	>50	2.1
001428-2.42	C	India	26	0.020	0.030	0.020	39.2	0.490	58	0.060	0.070	0.020	>50	2.1
16055-2.3	C	India	83	0.080	0.090	0.110	>50	1.3	309	0.260	0.280	1.9	>50	11.6
0013095-2.11	C	India	65	0.110	0.160	0.800	>50	0.300	183	0.330	0.380	2.9	>50	1.3
16936-2.21	C	India	194	0.190	0.180	0.080	41.8	0.810	596	0.630	0.520	0.390	>50	5.3
26191-2.48	C	India	340	0.190	0.260	>50	1.5	1.3	927	0.670	0.710	>50	7.4	10.3
25710-2.43	C	India	162	0.430	0.430	0.170	>50	0.320	633	1.4	1.4	0.610	>50	2.1
25925-2.22	C	India	312	0.530	0.410	0.210	>50	2.6	679	1.6	1.2	1.0	>50	20.2
25711-2.4	C	India	504	1.0	0.730	0.990	8.7	13.8	>1000	2.4	1.7	3.7	49.8	>50
16845-2.22	C	India	509	2.8	4.0	48.5	>50	0.210	>1000	12.7	18.9	>50	>50	0.930
S018.18	C	Malawi	31	0.069	0.071	0.083	13.9	9.9	118	0.178	0.190	0.324	>50	>50
Du156.12	C	South Africa	115	0.089	0.091	>50	0.656	14.5	475	0.193	0.204	>50	2.8	>50
CAP45.2.00.G3	C	South Africa	134	2.3	5.7	>50	0.370	2.1	>500	>50	>50	>50	4.1	>50
ZA012.29	C	South Africa	72	0.305	0.176	9.2	>50	5.4	381	1.0	0.654	>50	>50	45.7
CAP244.2.00.D3	C	South Africa	238	0.428	0.688	47.1	>50	2.6	1000	2.7	2.1	>50	>50	17.5
Du151.2	C	South Africa	248	3.2	4.8	34.6	3.8	1.4	>1000	46.5	>50	>50	>50	6.0
Du123.6	C	South Africa	393	18.2	16.1	>50	1.8	0.142	>500	>50	>50	>50	9.2	0.938
Du172.17	C	South Africa	349	>50	>50	>50	0.300	0.260	>1000	>50	>50	>50	2.6	1.8
Du422.1	C	South Africa	>1000	>50	>50	>50	0.464	11.5	>1000	>50	>50	>50	1.8	>50
CAP210.2.00.E8	C	South Africa	423	>50	>50	>50	27.0	1.480	>1000	>50	>50	>50	>50	8.3
TV1.29	C	South Africa	>1000	>50	>50	>50	>50	0.405	>1000	>50	>50	>50	>50	1.0
TZBD.02	C	Tanzania	56	0.109	0.074	1.3	>50	0.895	220	0.328	0.225	22.1	>50	5.7
TZA125.17	C	Tanzania	87	>50	>50	>50	>50	0.125	>1000	>50	>50	>50	>50	39.5
ZM249M.PL1	C	Zambia	27	0.048	0.062	8.6	3.810	11.1	262	0.232	0.297	>50	20.3	>50
ZM176.66	C	Zambia	15	0.055	0.036	0.033	>50	0.212	151	0.258	0.154	0.15	>50	35.4
ZM215.8	C	Zambia	89	0.095	0.149	>50	>50	1.2	>1000	0.527	0.724	>50	>50	>50
ZM109F.PB4	C	Zambia	73	0.128	0.127	>50	>50	0.028	915	0.754	0.619	>50	>50	0.281
ZM146.7	C	Zambia	67	0.333	0.396	1.0	18.0	4.2	706	1.3	1.4	4.5	>50	>50
ZM55.28a	C	Zambia	156	0.340	0.326	>50	>50	>50	438	1.2	1.0	>50	>50	>50
ZM135M.PL10a	C	Zambia	268	0.346	0.140	>50	>50	0.296	>1000	2.7	1.6	>50	>50	9.8
ZM197M.PB7	C	Zambia	605	0.360	0.408	2.13	11.9	28.3	>1000	1.6	2.0	9.2	>50	>50
ZM214M.PL15	C	Zambia	141	0.440	0.750	18.8	13.6	26.6	>1000	2.6	3.2	>50	40.4	>50
ZM106.9	C	Zambia	212	0.489	0.378	0.150	>50	>50	623	1.3	0.927	0.428	>50	>50
ZM181.6	C	Zambia	247	1.1	0.574	>50	>50	4.9	>1000	6.5	3.8	>50	>50	31.6
ZM53M.PB12	C	Zambia	317	1.3	1.4	10.3	32.6	8.6	>1000	4.0	4.9	45.6	>50	32.2
ZM233M.PB6	C	Zambia	698	2.0	1.0	>50	>50	3.4	>1000	9.3	4.7	>50	>50	12.4
Breadth	**N=38**	**Titer < 50**		**87%**	**87%**	**58%**	**47%**	**95%**		**82%**	**79%**	**42%**	**26%**	**68%**
		Titer < 1		**66%**	**71%**	**29%**	**13%**	**39%**		**37%**	**39%**	**16%**	**0%**	**8%**
Median[#]				**0.39**	**0.40**	**26.7**	**>50**	**1.42**		**1.50**	**1.39**	**>50**	**>50**	**18.85**
Geometric mean[#]				**0.34**	**0.35**	**1.07**	**4.46**	**1.40**		**1.14**	**0.93**	**1.60**	**7.25**	**6.17**

*Values < 1 µg/ml are highlighted in red, and values 1 – 50 µg/ml are in green. Blanks indicate not tested.
[#]Medians were calculated based on all viruses tested, including those with values > 50 µg/ml, which were assigned a value of 100; Geometric means were calculated for neutralization sensitive viruses with an IC_{50} (or IC_{80}) value < 50 µg/ml.

Table S2e: Antibody neutralization data against 8 HIV-1 clade D Env-pseudoviruses

			IC_{50} (µg/ml)*						IC_{80} (µg/ml)*					
Virus ID	**Clade**	**Origin**	**Serum45 IgG**	**VRC01**	**VRC02**	**VRC03**	**b12**	**CD4-Ig**	**Serum45 IgG**	**VRC01**	**VRC02**	**VRC03**	**b12**	**CD4-Ig**
247-23	D	Cameroon		2.8	0.76	>50	>50	1.1		>50	10.7	>50	>50	6.7
NKU3006.ec1	D	Kenya		0.57	0.68	0.06	17.7	6.5		1.7	1.9	0.19	>50	21.4
3016.v5.c45	D	Tanzania		0.16	0.25	>50	1.1	0.72		0.42	0.54	>50	4.0	3.2
6405.v4.c34	D	Tanzania		2.2	3.0	>50	>50	41.8		6.1	8.3	>50	>50	>50
UG024.2	D	Uganda	14	0.16	0.10	>50	>50	0.01	49	0.67	0.62	>50	>50	0.03
231965.c1	D	Uganda		0.34	0.54	32.6	0.07	2.5		1.2	1.8	>50	0.16	7.1
A03349M1.vrc4a	D	Uganda		3.7	3.1	>50	2.8	4.0		11.0	12.4	>50	12.7	17.3
57128.02	D	Uganda	38	>50	>50	>50	0.17	0.11	308	>50	>50	>50	1.7	0.86
Breadth	**N=8**	**Titer < 50**		**88%**	**88%**	**25%**	**63%**	**100%**		**75%**	**88%**	**13%**	**50%**	**88%**
		Titer < 1		**50%**	**63%**	**13%**	**25%**	**38%**		**25%**	**25%**	**13%**	**13%**	**25%**
Median[#]				**1.37**	**0.72**	**>50**	**10.25**	**1.82**		**3.91**	**5.11**	**>50**	**>50**	**6.93**
Geometric mean[#]				**0.73**	**0.68**	**1.40**	**0.91**	**1.10**		**1.83**	**2.78**	**0.19**	**1.93**	**2.76**

*Values < 1 µg/ml are highlighted in red, and values 1 – 50 µg/ml are in green. Blanks indicate not tested.
[#]Medians were calculated based on all viruses tested, including those with values > 50 µg/ml, which were assigned a value of 100; Geometric means were calculated for neutralization sensitive viruses with an IC_{50} (or IC_{80}) value < 50 µg/ml.

Table S2f: Antibody neutralization data against 18 HIV-1 CRF01_AE Env-pseudoviruses

			IC_{50} (µg/ml)*						IC_{80} (µg/ml)*					
Virus ID	**Clade**	**Origin**	**Serum45 IgG**	**VRC01**	**VRC02**	**VRC03**	**b12**	**CD4-Ig**	**Serum45 IgG**	**VRC01**	**VRC02**	**VRC03**	**b12**	**CD4-Ig**
CNE5	CRF01_AE	China		0.37	0.54	6.03	41.2	12.3		1.1	2.0	29.8	>50	24.1
CNE55	CRF01_AE	China		0.39	0.35	0.99	>50	17.3		1.4	1.2	8.5	>50	>50
CNE56	CRF01_AE	China		0.43	0.38	>50	>50	14.1		1.6	1.3	>50	>50	>50
CNE59	CRF01_AE	China		0.54	0.62	>50	>50	0.56		1.8	2.2	>50	>50	4.8
CNE8	CRF01_AE	China		1.3	1.7	47.1	>50	>50		3.7	4.7	>50	>50	>50
CNE3	CRF01_AE	China		17.7	2.2	>50	>50	33.1		>50	15.9	>50	>50	>50
CNE28	CRF01_AE	China		>50	>50	>50	>50	8.9		>50	>50	>50	>50	29.8
R2184.c4	CRF01_AE	Thailand	192	0.08	0.10	0.05	>50	7.4	476	0.32	0.31	0.14	>50	25.3
TH976.17	CRF01_AE	Thailand	25	0.09	0.11	>50	>50	0.90	1000	0.49	0.59	>50	>50	40.8
C3347.c11	CRF01_AE	Thailand		0.17	0.15	2.4	>50	>50		0.58	0.55	29.1	>50	>50
TH966.8	CRF01_AE	Thailand	102	0.33	0.29	>50	>50	0.40	>1000	1.4	1.2	>50	>50	3.2
M02138	CRF01_AE	Thailand	93	0.35	0.45	>50	>50	0.30	392	1.6	2.0	>50	>50	2.8
C2101.c1	CRF01_AE	Thailand		0.36	0.30	>50	>50	9.3		1.2	0.97	>50	>50	26.2
R3265.c6	CRF01_AE	Thailand		0.45	0.38	>50	>50	8.1		1.9	2.2	>50	>50	26.0
R1166.c1	CRF01_AE	Thailand		1.7	1.8	>50	>50	36.4		4.6	4.9	>50	>50	>50
C1080.c3	CRF01_AE	Thailand	855	3.4	3.8	>50	>50	4.3	>1000	14.4	15.3	>50	>50	18.7
703357.2	CRF01_AE	Thailand	>1000	4.0	3.7	>50	>50	>50	>1000	11.1	10.6	>50	>50	>50
620345.c1	CRF01_AE	Thailand	>1000	>50	>50	>50	>50	>50	>1000	>50	>50	>50	>50	>50
Breadth	**N=18**	**Titer < 50**		**89%**	**89%**	**28%**	**6%**	**78%**		**83%**	**89%**	**22%**	**0%**	**56%**
		Titer < 1		**61%**	**61%**	**11%**	**0%**	**22%**		**17%**	**22%**	**6%**	**0%**	**0%**
Median[#]				**0.44**	**0.50**	**>50**	**>50**	**10.80**		**1.72**	**2.08**	**>50**	**>50**	**35.29**
Geometric mean[#]				**0.61**	**0.56**	**2.01**	**41.20**	**4.89**		**1.77**	**2.15**	**5.67**	**>50**	**14.45**

*Values < 1 µg/ml are highlighted in red, and values 1 – 50 µg/ml are in green. Blanks indicate not tested.
[#]Medians were calculated based on all viruses tested, including those with values > 50 µg/ml, which were assigned a value of 100; Geometric means were calculated for neutralization sensitive viruses with an IC_{50} (or IC_{80}) value < 50 µg/ml.

Table S2g: Antibody neutralization against 16 CRF02_AG Env-pseudoviruses

			IC_{50} (µg/ml)*						IC_{80} (µg/ml)*					
Virus ID	**Clade**	**Origin**	**Serum45 IgG**	**VRC01**	**VRC02**	**VRC03**	**b12**	**CD4-Ig**	**Serum45 IgG**	**VRC01**	**VRC02**	**VRC03**	**b12**	**CD4-Ig**
280-5	CRF02_AG	Cameroon	41	0.02	0.02	0.02	>50	4.2	129	0.15	0.10	0.16	>50	37.5
33-7	CRF02_AG	Cameroon	109	0.03	0.03	>50	>50	0.97	302	0.10	0.09	>50	>50	8.1
235-47	CRF02_AG	Cameroon	45	0.04	0.04	0.11	6.8	29.0	232	0.17	0.15	1.5	>50	>50
271-11	CRF02_AG	Cameroon	55	0.06	0.06	>50	>50	0.02	192	0.22	0.20	>50	>50	0.07
263-8	CRF02_AG	Cameroon	98	0.20	0.22	0.11	>50	1.0	465	0.55	0.59	0.41	>50	8.3
269-12	CRF02_AG	Cameroon		0.23	0.34	>50	>50	4.5		0.66	0.87	>50	>50	22.9
253-11	CRF02_AG	Cameroon	541	0.47	0.57	>50	>50	43.1	>1000	1.4	1.4	>50	>50	>50
255-34	CRF02_AG	Cameroon	476	0.70	0.61	>50	>50	0.19	>1000	2.7	1.9	>50	>50	1.1
266-60	CRF02_AG	Cameroon	592	1.2	1.4	>50	4.2	2.5	>1000	4.3	5.0	>50	42.7	>50
251-18	CRF02_AG	Cameroon	841	2.5	3.1	>50	>50	3.1	>1000	11.2	10.4	>50	>50	27.3
257-31	CRF02_AG	Cameroon	683	2.8	2.8	>50	>50	20.1	>1000	8.7	8.7	>50	>50	>50
211-9	CRF02_AG	Cameroon	438	14.3	5.5	>50	>50	5.6	>1000	>50	28.7	>50	>50	31.9
250-4	CRF02_AG	Cameroon	90	>50	>50	>50	>50	6.0	382	>50	>50	>50	>50	43.1
278-50	CRF02_AG	Cameroon	>1000	>50	>50	>50	18.4	1.6	>1000	>50	>50	>50	>50	8.0
242-14	CRF02_AG	Cameroon	322	>50	>50	>50	>50	1.9	>1000	>50	>50	>50	>50	15.5
928-28	CRF02_AG	Cote d'Ivoire	517	0.41	0.43	>50	>50	0.40	>1000	1.7	2.0	>50	>50	3.3
Breadth	**N=16**	**Titer < 50**		**81%**	**81%**	**19%**	**19%**	**100%**		**75%**	**81%**	**19%**	**6%**	**75%**
		Titer < 1		**56%**	**56%**	**19%**	**0%**	**25%**		**38%**	**38%**	**13%**	**0%**	**6%**
Median[#]				**0.59**	**0.59**	**>50**	**>50**	**2.82**		**2.20**	**1.92**	**>50**	**>50**	**25.09**
Geometric mean[#]				**0.35**	**0.35**	**0.06**	**8.04**	**2.28**		**0.95**	**1.18**	**0.47**	**42.7**	**7.96**

*Values < 1 µg/ml are highlighted in red, and values 1 – 50 µg/ml are in green. Blanks indicate not tested.
[#]Medians were calculated based on all viruses tested, including those with values > 50 µg/ml, which were assigned a value of 100; Geometric means were calculated for neutralization sensitive viruses with an IC_{50} (or IC_{80}) value < 50 µg/ml.

Table S2h: Antibody neutralization data against 10 HIV-1 clade G Env-pseudoviruses

			IC_{50} (µg/ml)*						IC_{80} (µg/ml)*					
Virus ID	**Clade**	**Origin**	**Serum45 IgG**	**VRC01**	**VRC02**	**VRC03**	**b12**	**CD4-Ig**	**Serum45 IgG**	**VRC01**	**VRC02**	**VRC03**	**b12**	**CD4-Ig**
X2088_c9	G	Ghana		>50	>50	>50	>50	2.4		>50	>50	>50	>50	8.8
P0402_c2_11	G	Portugal		0.21	0.29	>50	>50	11.4		0.59	0.77	>50	>50	>50
X1254_c3	G	Spain		0.07	0.09	>50	>50	9.2		0.19	0.25	>50	>50	29.7
X1193_c1	G	Spain		0.11	0.15	0.04	>50	21.3		0.32	0.44	0.11	>50	>50
X1632_S2_B10	G	Spain		0.12	0.11	0.08	>50	0.46		0.74	0.42	0.38	>50	1.6
X1854_c2_10	G	Spain		0.14	0.16	26.7	>50	5.7		0.45	0.58	>50	>50	19.5
P1981_C5_3	G	Spain		0.46	0.55	0.68	>50	41.8		1.3	1.5	1.9	>50	>50
X2131_C1_B5	G	Spain		0.51	0.57	1.7	>50	0.28		1.5	1.7	8.8	>50	1.3
X2160_c25	G	Spain		0.84	0.83	23.1	>50	3.9		4.6	3.1	>50	>50	12.1
252-7	G	West Africa		0.77	0.87	>50	>50	20.1		2.4	2.2	>50	>50	>50
Breadth	**N=10**	**Titer < 50**		**90%**	**90%**	**60%**	**0%**	**100%**		**90%**	**90%**	**40%**	**0%**	**60%**
		Titer < 1		**90%**	**90%**	**30%**	**0%**	**20%**		**50%**	**50%**	**20%**	**0%**	**0%**
Median[#]				**0.34**	**0.42**	**24.91**	**>50**	**7.44**		**1.00**	**1.12**	**>50**	**>50**	**24.61**
Geometric mean[#]				**0.25**	**0.29**	**1.14**	**>50**	**5.14**		**0.86**	**0.89**	**0.91**	**>50**	**7.10**

*Values < 1 µg/ml are highlighted in red, and values 1 – 50 µg/ml are in green. Blanks indicate not tested.
[#]Medians were calculated based on all viruses tested, including those with values > 50 µg/ml, which were assigned a value of 100; Geometric means were calculated for neutralization sensitive viruses with an IC_{50} (or IC_{80}) value < 50 µg/ml.

Table S2i: Antibody neutralization data against 11 HIV-1 CRF07_BC Env-pseudoviruses

			IC_{50} (µg/ml)*						IC_{80} (µg/ml)*					
Virus ID	Clade	Origin	Serum45 IgG	VRC01	VRC02	VRC03	b12	CD4-Ig	Serum45 IgG	VRC01	VRC02	VRC03	b12	CD4-Ig
CH117.4	CRF07_BC	China	42	0.14	0.12	0.14	>50	3.9	135	0.34	0.34	0.63	>50	16.9
CH114.8	CRF07_BC	China		0.21	0.20	0.04	>50	0.86		0.68	0.64	0.12	>50	3.9
CH064.2	CRF07_BC	China	289	0.45	0.29	0.29	35.2	1.4	629	1.4	0.89	1.9	>50	8.7
CH181.12	CRF07_BC	China	257	0.49	0.33	7.8	2.2	0.99	745	1.7	1.2	>50	8.3	3.5
CH038.12	CRF07_BC	China	436	0.52	0.45	>50	0.22	3.8	>1000	1.6	1.4	>50	0.65	17.1
CH119.1	CRF07_BC	China	182	1.3	1.3	18.9	>50	4.0	694	3.5	3.5	>50	>50	15.8
CH110.2	CRF07_BC	China	82	1.9	0.95	1.5	>50	1.1	235	18.7	4.9	>50	>50	5.7
CH115.12	CRF07_BC	China	746	2.7	3.2	0.35	>50	35.5	>1000	9.5	9.0	1.0	>50	>50
CH120.6	CRF07_BC	China	666	3.1	3.2	0.74	>50	1.2	>1000	11.4	11.4	2.6	>50	4.4
CH111.8	CRF07_BC	China	595	3.9	2.9	>50	>50	0.84	>1000	11.5	10.9	>50	>50	7.5
CH070.1	CRF07_BC	China	>1000	37.7	17.2	>50	>50	0.57	>1000	>50	>50	>50	>50	2.9
Breadth	**N=11**	**Titer < 50**		**100%**	**100%**	**73%**	**27%**	**100%**		**91%**	**91%**	**45%**	**18%**	**91%**
		Titer < 1		**45%**	**55%**	**45%**	**9%**	**36%**		**18%**	**27%**	**18%**	**9%**	**0%**
Median[#]				**1.25**	**0.95**	**1.47**	**>50**	**1.20**		**3.53**	**3.54**	**>50**	**>50**	**7.52**
Geometric mean[#]				**1.23**	**0.96**	**0.74**	**2.57**	**1.95**		**3.05**	**2.39**	**0.83**	**2.32**	**7.03**

*Values < 1 µg/ml are highlighted in red, and values 1 – 50 µg/ml are in green. Blanks indicate not tested.
[#]Medians were calculated based on all viruses tested, including those with values > 50 µg/ml, which were assigned a value of 100; Geometric means were calculated for neutralization sensitive viruses with an IC_{50} (or IC_{80}) value < 50 µg/ml.

Table S2j: Antibody neutralization against 18 HIV-1 recombinant Env-pseudoviruses

			IC_{50} (µg/ml)*						IC_{80} (µg/ml)*					
Virus ID	Clade	Origin	Serum45 IgG	VRC01	VRC02	VRC03	b12	CD4-Ig	Serum45 IgG	VRC01	VRC02	VRC03	b12	CD4-Ig
6041.v3.c23	AC	Tanzania		0.02	0.02	14.4	>50	10.0		0.08	0.08	>50	>50	31.7
3589.v1.c4	AC	Tanzania		0.12	0.12	>50	>50	13.0		0.47	0.45	>50	>50	47.1
3301.v1.c24	AC	Tanzania		0.14	0.19	0.09	26.6	2.9		0.32	0.46	0.23	>50	10.1
6545.v4.c1	AC	Tanzania		>50	>50	>50	31.5	3.0		>50	>50	>50	>50	11.4
6540.v4.c1	AC	Tanzania		>50	>50	>50	16.1	3.7		>50	>50	>50	>50	11.7
0815.v3.c3	ACD	Tanzania		0.06	0.09	0.02	11.2	3.0		0.13	0.19	0.07	>50	9.4
3103.v3.c10	ACD	Tanzania		0.93	0.83	0.59	>50	30.5		2.5	2.0	2.3	>50	>50
3468-v1.c12	AD	Tanzania		0.05	0.05	>50	>50	7.8		0.17	0.13	>50	>50	>50
0907.v4.c12	AD	Tanzania		0.20	0.18	0.08	>50	19.1		0.72	0.46	0.27	>50	>50
6480.v4.c25	CD	Tanzania		0.04	0.04	0.03	>50	6.4		0.09	0.13	0.09	>50	17.6
6952.v1.c20	CD	Tanzania		0.04	0.11	0.05	6.4	2.0		0.12	0.23	0.17	24.5	7.7
6650.v1.c8	CD	Tanzania		0.05	0.10	0.05	>50	29.1		0.13	0.27	0.12	>50	>50
6811.v5.c20	CD	Tanzania		0.09	0.12	2.2	>50	7.9		0.26	0.33	20.3	>50	19.6
3326.v4.c3	CD	Tanzania		0.10	0.10	>50	0.12	50.0		2.3	1.4	>50	0.43	>50
3337.v2.c6	CD	Tanzania		0.14	0.12	>50	>50	0.32		0.34	0.30	>50	>50	1.3
3817.v2.c59	CD	Tanzania		>50	>50	>50	>50	>50		>50	>50	>50	>50	>50
X2252_c7	CRF14_BG	Portugal		0.36	0.62	0.4	>50	2.9		1.1	1.6	1.3	>50	12.1
X1100_c7	CRF14_BG	Switzerland		1.3	0.31	>50	>50	10.0		>50	6.6	>50	>50	32.3
Breadth	**N=18**	**Titer < 50**		**83%**	**83%**	**56%**	**33%**	**94%**		**78%**	**83%**	**50%**	**11%**	**67%**
		Titer < 1		**78%**	**83%**	**44%**	**6%**	**6%**		**61%**	**61%**	**33%**	**6%**	**0%**
Median[#]				**0.13**	**0.12**	**8.33**	**>50**	**7.87**		**0.41**	**0.46**	**>50**	**>50**	**25.67**
Geometric mean[#]				**0.12**	**0.13**	**0.20**	**6.99**	**6.56**		**0.33**	**0.44**	**0.43**	**3.25**	**13.12**

*Values < 1 µg/ml are highlighted in red, and values 1 – 50 µg/ml are in green. Blanks indicate not tested.
[#]Medians were calculated based on all viruses tested, including those with values > 50 µg/ml, which were assigned a value of 100; Geometric means were calculated for neutralization sensitive viruses with an IC_{50} (or IC_{80}) value < 50 µg/ml.

Table S3: IC_{50} titers (µg/ml) of antibody neutralization against selected HIV-1 clade B and C viruses using Env-pseudoviruses to infect TZM-bl or activated PBMC, and using PBMC-derived uncloned primary isolates to infect TZM-bl or activated PBMC

Virus*	Virus type	Target cell	Serum45 IgG	VRC01	VRC02	VRC03	b12	CD4-Ig
Clade B n=9								
BaL	Primary	TZM-bl	31	0.215	0.182	3.7	0.138	0.032
BaL	Primary	PBMC		0.142		4.1	4.8	0.208
BaL.01.SG3	Pseudo	TZM-bl	31	0.055	0.053	20	0.093	0.030
BaL.01.LUC	Pseudo	PBMC	130	0.054	0.051	11	0.343	0.134
MN	Primary	TZM-bl	48	0.283	0.271	0.054	0.081	0.082
MN	Primary	PBMC		0.105		0.020	0.060	0.024
MN.3.SG3	Pseudo	TZM-bl	1.7	0.022	0.024	0.027	0.003	0.006
JRFL	Primary	TZM-bl	76	0.142	0.104	0.029	0.181	1.2
JRFL	Primary	PBMC		0.020		0.014	0.221	1.9
JRFL.SG3	Pseudo	TZM-bl	21	0.031	0.024	0.009	0.022	0.247
JRFL.LUC	Pseudo	PBMC	67	0.035	0.010	0.004	0.014	0.560
SF162	Primary	TZM-bl	5.0	0.289	0.289	0.079	0.021	0.036
SF162	Primary	PBMC		0.250		0.040	0.062	0.062
SF162.SG3	Pseudo	TZM-bl	11	0.139	0.112	0.033	0.070	0.153
89.6	Primary	TZM-bl	14	0.813	0.640	0.110	1.2	0.289
89.6	Primary	PBMC		0.046		0.014	<0.003	0.003
89.6.SG3	Pseudo	TZM-bl	132	0.511	0.444	0.187	0.140	0.242
BL01	Primary	TZM-bl	>1000	>50	>50	>50	>50	2.0
BL01	Primary	PBMC		>50		>50	5.3	0.184
BL01.SG3	Pseudo	TZM-bl	147	>50	>50	>50	1.7	0.100
BR07	Primary	TZM-bl	>1000	6.8	7.1	2.9	3.7	0.212
BR07	Primary	PBMC		0.446		0.866	0.012	0.007
BR07.SG3	Pseudo	TZM-bl	97	1.2	0.948	3.4	0.096	0.046
QH0692.42.SG3	Pseudo	TZM-bl	207	1.5	1.3	0.954	0.970	0.603
QH0692.42.LUC	Pseudo	PBMC	>1000	1.9	2.5	0.236	2.4	0.643
AC10.29.SG3	Pseudo	TZM-bl	207	2.2	2.5	>50	1.8	10.7
AC10.29.LUC	Pseudo	PBMC	>1000	2.3	2.9	>50	18	>50
Clade C n=3								
BR025	Primary	TZM-bl	111	1.6	1.1	0.169	0.384	0.653
BR025	Primary	PBMC		0.568		0.040	0.196	0.192
BR025.9.SG3	Pseudo	TZM-bl	25	0.115	0.208	>50	>50	0.064
ZA012	Primary	TZM-bl	736	1.3	0.951	21	>50	>50
ZA012	Primary	PBMC		2.1		29.2	>50	9.4
ZA012.29.SG3	Pseudo	TZM-bl	72	0.305	0.176	9.2	>50	5.4
Du156	Primary	TZM-bl	246	1.1	1.2	>50	>50	11.0
Du156	Primary	PBMC		0.356		>50	0.917	0.271
Du156.12.SG3	Pseudo	TZM-bl	115	0.089	0.091	>50	0.656	14.5

*Viruses with suffix of SG3 are Env-pseudoviruses made with the SG3 ΔEnv HIV-1 backbone. These pseudoviruses were used to infect the TZM-bl cell line containing a Tat sensitive luciferase reporter gene. Viruses with suffix of LUC are Env-pseudoviruses made in the pNL4-3 ΔEnv HIV-1 backbone that contained a luciferase reporter gene. This allowed assays with these Env-pseudoviruses on PBMC target cells. Viral isolates with no suffix are primary replication competent PBMC derived viruses. Blanks indicate not tested.

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