Supplemental Experimental Procedures

Donor and Sample Information.

The CH505 donor, from which the CH103 and the CH235 antibody lineages were isolated, is an African male enrolled in the CHAVI001 acute HIV-1 infection cohort (Tomaras et al., 2008) and followed for over 6 years. During this time viral load ranged from 14,460 to 847,279 copies/ml (median = 173,667 copies/ml), and CD4 counts ranged from 69 to 431 cells/mm^3 (median = 294 cells/ mm^3).

The time of infection was estimated by analyzing the sequence diversity in the first available sample using the Poisson Fitter tool (Giorgi et al., 2010) as described in (Liao et al., Nature 2013). Results were consistent with a single founder virus establishing the infection and with the earliest isolated virus sequences being taken 4 weeks post-transmission.

Flow Cytometry, Memory B Cell Cultures and mAb Isolation.

The HIV-1 CH505.TF gp120 envelope glycoprotein was produced and used in flow cytometry on PBMC collected from donor CH505 at week 264 and 323 post-transmission using a two-color technique as described (Gray et al., 2011).

CH505.TF gp120 Env-positive memory B cells were cultured as described (Bonsignori et al., 2011) with the following modifications: non-irradiated MS40L cells were used as feeder cells at a concentration of 3,000 cells/well and were added to wells in which memory B cells were sorted in bulk; 50 ng/ml of recombinant human (rHu) IL-21 (200-21; Peprotech, Rocky Hill, NJ) were added to the complete medium; memory B cells were distributed by limiting dilution at a calculated concentration of 2 cells/well; culture medium was refreshed every 5 days.

Cell culture supernatants were screened for neutralization of autologous CH505.TF virus using the tzm-bl neutralization assay (Bonsignori et al., 2011; Montefiori, 2005) and for binding to CH505.TF gp120 Env, CH505.TF Δ371I gp120 Env mutant, HIV-1 Env resurface core protein 3 (RSC3) and RSC3 Δ371I (Wu et al., 2010).

MAbs CH235.10 through CH235.13 were isolated from cultures that displayed differential binding of CH505.TF and CH505 TF Δ371I gp120 Env, did not bind to RSC3 (Gao et al., 2014) and neutralized 13 to 99% CH505.TF infectivity.

CH235 lineage antibody frequency over total memory B cells was calculated by dividing the number of CH235 lineage antibodies isolated at week 41 (n = 5; Gao et al., 2014) for the number of memory B cells analyzed (n = 27,950). CH235 lineage antibody frequency over CH505.TF gp120 Env-specific memory B cells was calculated by dividing the number of CH235 lineage antibodies isolated at weeks 264 and 323 (n = 4) for the number of CH505.Env gp120-specific memory B cells analyzed (n = 794).

454 Pyrosequencing of CH235 lineage heavy chains.

Antibody heavy chain gene rearrangements were PCR amplified from 6 independent 100ng genomic DNA aliquots to generate 6 barcoded libraries per sample. Multiplexed primers complementary to the IGHV FR1 or FR2 framework regions, and an IGHJ-primer were modified from the BIOMED-2 consortium primers (Boyd et al., 2009; van Dongen et al., 2003). 10-nucleotide 'barcode' sequences in the primer sets encoded sample identity and replicate library identity. AmpliTaq Gold (Roche) enzyme was used for PCR following the manufacturer's instructions, with a thermocycler program: 94°C 5 min; 35 cycles of (94°C 30 sec, 60°C 45 sec, 72°C 90 sec); and final extension at 72°C for 10 min. Following quantitation, PCR products from each replicate library were pooled in equimolar amounts, then the pooled library was run on a 1.5% agarose gel and gel extracted (Qiagen). High-throughput sequencing was performed on the 454 (Roche) platform using Titanium chemistry.

Antibody production.

Immunoglobulin genes of mAbs CH235.10 through CH235.13 were amplified from RNA from isolated cells, expression cassettes made, and mAbs expressed as described (Gao et al. 2014). The V_H genes of mAbs CH235.6 through CH235.9 were retrieved from sequences obtained through genomic DNA 454 sequencing, which were restored to full length and complemented with the V_L of the phylogenetically closest isolated antibody in the CH235 lineage (i.e. CH241 for CH235.6 and CH235.8, and CH236 for CH235.7 and CH235.9). We have previously described the isolation of mAbs CH235, CH236, CH239, CH240 and CH241and the inference of unmutated common ancestor (UCA) and intermediate antibodies IA1 through IA4 (Gao et al., 2014; Kepler, 2013).
Heavy chain plasmids were co-transfected with appropriate light chain plasmids at an equal ratio in Expi 293 cells using either 293Fectin or ExpiFectamine 293 transfection reagents (Thermo Fisher Scientific) according to the manufacturer's protocols. Cultures were supplemented with AbBooster antibody expression enhancer media (ABI

Scientific) at 10% of the final culture volume 24 h after transfection. . Cultures were then incubated at 33°C for 5 more days, and supernatants were harvested and passed over a protein A affinity column. Following PBS wash and low pH elution, the pH of eluate was neutralized with 1MTris pH 8.5 and samples were dialyzed against PBS. Antibodies were then aliquoted and stored at -80°C prior to use. Alternatively, for ExpiFectamine transfections we used the enhancer provided with the kit, transfected cultures were incubated at 37°C 8% CO_2 for 2-6 days, harvested, concentrated and incubated overnight with Protein A beads at 4°C on a rotating shaker before loading the bead mixture in columns for purification; following PBS/NaCl wash, eluate was neutralized with trizma hydrochloride and antibody concentration was determined by Nanodrop. Purified antibodies were tested in SDS-Page Coomassie and western blots, and stored at 4°C.

Direct-binding ELISA.

Direct-binding ELISAs were performed as described previously (Bonsignori et al., 2011) with the following modifications: plates were blocked for 1 h at room temperature (RT) or overnight at 4°C (both procedures were previously validated); primary purified antibodies were tested at a starting concentrations of 100µg/ml, serially three-fold diluted and incubated for 1 h at RT; HRP-conjugated human IgG antibody was added at optimized concentration of 1:30,000 in assay diluent for 1 hour and developed using TMB substrate; plates were read at 450 nm in a SpectraMax 384 PLUS reader (Molecular Devices, Sunnyvale, CA); results are reported as logarithm area under the curve (LogAUC) unless otherwise noted.

For cell culture supernatant screening of RSC3 and RSC3 Δ371I HIV-1 Env core proteins reactivity, plates were coated with streptavidin (2µg/ml); blocked plates were stored at -20°C until used; 10 µl/well of biotinylated avi-tagged RSC3 and RSC3 Δ371I were added at 2 µg/ml for 30 minutes at RT and culture supernatants were added at 1:3 dilution in assay diluent; plates were developed for 10 min using SureBlue Reserve TMB (53-00-03; KPL, Gaithersburg, MD) equilibrated at RT.

Competition ELISAs were performed using 10µl of primary purified monoclonal antibody, starting at 100µg/ml and diluted in a two-fold concentration, incubated for 1 h at RT; for CD4 binding site blocking assays, 10 µl of a saturating concentration soluble CD4 (Progenics Pharm Inc.) was added following antibody incubation step. Ten µl of biotinylated target Mab was added at the EC50 determined by a direct binding of biotinylated-Mab for one hour at RT. After background subtractions, percent inhibition was calculated as follows: 100-(sera triplicate mean/no inhibition control mean)*100.

Autoimmune purified antigens histones (whole), Jo-1, RNP/Sm, Scl-70, Sm, SSA (Ro), SSB (all from ImmunoVision) and centromere B (Prospec) were coated at optimal concentrations determined by lot-specific checkerboard with positive controls. All plasma antibody positive controls were purchased from ImmunoVision; lot-specific optimal ranges for standard curves were determined. All antibodies were tested using the same lots for each antigen and positive controls with the protocol described above. For DNA ELISA, plates were coated with 2µg/ml poly-lysine (Sigma-Aldrich) for 2 h at RT, washed 3X with PBS and blocked with PBS/2%BSA/0.05% Tween-20 for 2h at RT. After 3X wash, DNA (LS002195, Worthington) in saline sodium citrate buffer was added for 1 h, washed and antibodies were incubated for 1 h. Secondary antibody was diluted in PBS/0.05% Tween-20. Plates were developed for 30 min. Human recombinant monoclonal antibody Ab008391 (courteously provided by David Easterhoff, Duke Human Vaccine Institute) was used as positive control. For all autoantigen ELISAs, palivizumab was used as negative control. For each antibody, LogAUC was calculated and data are presented semi-quantitatively: no binding = $logAUC_{Ab} \leq 2X$ negative control $logAUC_{neg\ ctrl}$; to quantify antibody binding we divided ($logAUC_{pos\ ctrl}$ - 2X $logAUC_{neg\ ctrl}$) in tertiles and expressed test antibody binding as weak (+), intermediate (+ +) or strong (+ + +) if $logAUC_{Ab}$ was in the first, second or higher tertile, respectively.

Anti-cardiolipin ELISA was performed using the QUANTA Lite ACA IgG III kit (708625; INOVA Diagnostics) following manufacturer's protocol.

Assessment of virus neutralization using a large panel and calculation of neutralization dendrograms.

Neutralizing antibody assays in TZM-bl cells were performed as described previously (Montefiori, 2005). Neutralization breadth of CH235 UCA, CH235, CH235.9 and CH235.12 neutralization breadth was assessed using the 384-well plate declination of the assay using an updated panel of 199 geographically and genetically diverse Env-pseudoviruses representing the major circulating genetic subtypes and recombinant forms as described (Seaman et al., 2010; Wu et al., 2010). The data were calculated as a reduction in luminescence units compared with control wells, and reported as IC_{50} in µg/ml (Montefiori, 2005).

Dendrograms were calculated using the neighbor-joining method, showing the protein sequence distance from the HIV-1 Env gp160 sequences of 190 HIV-1 primary isolates. The clades of HIV-1, including circulating recombinant forms (CRFs) are indicated.

Antibody neutralization fingerprinting analysis.

Neutralization fingerprints were computed and compared for CH235, CH235.9 and CH235.12 from the CH235 lineage, other CD4-binding-site antibodies, and antibodies targeting other sites of vulnerability on HIV-1 Env. The fingerprints were computed over a common panel of 165 HIV-1 strains with neutralization data for all antibodies, and a hierarchical clustering procedure was applied for building the tree, as described in (Georgiev et al., 2013). Briefly, for each antibody, the neutralization data for the common set of 165 HIV-1 strains formed that antibody's neutralization fingerprint. The Spearman correlation coefficients for all pairs of antibody neutralization fingerprints were then computed, transforming the antibody-virus neutralization matrix into an antibody-antibody correlation matrix. This correlation matrix was then input into a hierarchical clustering procedure as a way to visualize the similarities between the neutralization fingerprints for the different antibodies. The distances in the resulting tree are thus a function of the differences between fingerprints.

VH1-46 and VH1-2 antibody dendrogram calculation.

Phylogenic trees for multiple antibodies derived from VH1-46 and VH1-2 heavy chain variable genes were calculated using the neighbor-joining method. The sequences are aligned by Clustal Omega, calculated using ClustalW2. Dendrograms were drawn in Figtree.

Production and purification of HIV-1 Env protein complexed to antigen-binding fragments.

HIV-1 gp120 protein from clade AE 93TH057 and antibodies of CH235, CH235.9 and CH235.12 were produced and purified as described previously (Zhou et al., 2010). Fab fragments of antibodies were prepared by digesting purified IgG with Lys-C at 37°C for 2-4 h. The digestion reaction was quenched by the addition of cOmplete protase inhibitors (Roche). The digested antibodies were passed over Protein A agarose to remove the Fc fragment. The Fab was further purified over a Superdex 200 gel filtration column and concentrated aliquots were stored at −80 °C.

X-ray crystallography.

The gp120-antibody complexes were formed by mixing deglycosylated gp120 with the antibody Fab in a 1:1.5 molar ratio. The complexes were purified by size exclusion chromatography (Hiload 26/60 Superdex S200 prep grade; GE Healthcare) with buffer containing 0.35 M NaCl, 2.5 mM Tris (pH 7.0), and 0.02% NaN_3. Fractions with gp120-antibody complexes were concentrated to ~10 mg/ml and used for crystallization experiments. All gp120-Fab complexes were screened against 576 crystallization conditions using a Cartesian Honeybee crystallization robot. Initial crystals were grown by the vapor diffusion method in sitting drops at 20 °C by mixing 0.2 µl of protein complex with 0.2 µl of reservoir solution. Crystals were manually reproduced in hanging drops by mixing 0.50 µl protein complex solution with 0.5 µl reservoir solution.

The 93TH057 $core_e$ gp120-CH235 complex was crystallized with a reservoir solution of 25% (w/v) of PEG2000, 0.2 M of Li_2SO_4, 0.1 M of Tris-HCl pH 8.5 and 5% (v/v) of isopropanol and was flash frozen in liquid nitrogen in mother liquor supplemented with 15% of 2R,3R-butanediol as a cryoprotectant. The 93TH057 $core_e$ gp120-CH235.9 complex was crystallized with a reservoir solution of 9% (w/v) of PEG8000, 19% (w/v) of PEG400, 0.1 M HEPES pH 7.5 and was flash frozen in mother liquor supplemented with an additional 15% PEG 400 as a cryoprotectant. The 93TH057 $core_e$ gp120-CH235.12 complex was crystallized with a reservoir solution of 10% PEG 8000, 20% PEG 400 and 100 mM HEPES, pH7.5 and was flash frozen in mother liquor supplemented with an additional 15 - 20% PEG 400 as a cryoprotectant.

Data for all crystals were collected at a wavelength of 1.00Å at SER-CAT beamlines ID-22 and BM-22 (Advanced Photon Source, Argonne National Laboratory). All diffraction data were processed with the HKL2000 suite, structures were solved by molecular replacement using PHASER, and iterative model building and refinement were performed in COOT and PHENIX, respectively. For 93TH057$core_e$ complexes with CH235.9 and CH235.12, molecular replacement solutions were obtained using EAF31403.1-CH235 complex as a search model.

Throughout the refinement processes, a cross validation (R_{free}) test set consisting of 5% of the data was used and hydrogen atoms were included in the refinement model. Structure validations were performed periodically during the model building/refinement process with MolProbity. The 93TH057 $core_e$-CH235 structure was refined to a final R_{free} value of 22.9% with 96% residues in the favored region of the Ramachandran plot, and 0.1% outliers. The 93TH057 $core_e$-CH235.9 structure was refined to a final R_{free} value of 22% with 97.1% residues in the favored region of the Ramachandran plot, and 0% outliers. The 93TH057 $core_e$-CH235.12 structure was refined to a final R_{free} value of 23% with 97.0% residues in the favored region of the Ramachandran plot, and 0.1% outliers. All figures containing representations of the protein crystal structures were made with PyMOL. Gp120 and antibody interactions were analyzed with the PISA server.

Surface Plasmon Resonance Affinity and Kinetics Measurements.

For kinetic measurement, each antibody was captured on an anti-human IgFc immobilized sensor surface (200-500RU) and gp120 proteins at varying concentrations were injected to monitor association and dissociation phases. Buffer reference and non-specific binding to a control antibody (palivizumab) captured surface were used to derive specific binding signals. Kinetic rate constants and dissociation constant (Kd) were derived from global curve fitting analysis using a Langmuir 1:1 interaction model using the BIAevaluation 4.1 software (GE Healthcare).

Electron microscopy data collection and processing.

BG505 SOSIP.664 and B41 SOSIP.664 gp140 trimers were expressed in HEK293F cells and purified by 2G12-affinity and gel filtration chromatography as described elsewhere (Pugach et al., 2015; Sanders et al., 2013).Trimers were incubated with a 10 molar excess of Fab (CH235, CH235.9, or CH235.12) overnight at room temperature and the complexes were diluted to ~0.03 mg/mL prior to application onto a carbon-coated 400 Cu mesh grid that had been glow discharged at 20 mA for 30 s. The grids were stained with 2% (w/v) uranyl formate for 60 seconds. Samples were imaged using a FEI Tecnai T12 electron microscope operating at 120 keV, with an electron dose of ~ 25 $e^-/Å^2$ and a magnification of 52,000x that resulted in a pixel size of 2.05 Å at the specimen plane. Images were acquired with Leginon (Suloway et al., 2005) using a Tietz TemCam-F416 CMOS camera with a nominal defocus range of 1000-1500 nm. Automated particle picking, stack creation, and initial 2D classification was performed in the Appion software suite (Lander et al., 2009). Noise and junk particles were discarded and the remaining stack was subjected to 3D classification using Relion (Scheres, 2012) with an EM volume created from the x-ray structure of ligand-free BG505 SOSIP.664 (PDB: 4zmj) low pass filtered to 60 Å as the reference model. While both CH235.9 and CH235.12 bound to either B41 or BG505 at predominantly full stoichiometry (3 Fabs per trimer), CH235 bound to either trimer at sub-stoichiometric ratios (1 Fab per BG505 trimer and 2 Fabs per B41 trimer). The 3D classes representing the predominant stoichiometry for each complex were used as the initial models (low pass filtered to 40 Å) for further refinement using Relion, with C3 symmetry imposed for complexes with CH235.9 or CH235.12. The total number of particles used in refinement and final resolution of the map using a Fourier shell correlation of 0.5 are as follows: BG505 in complex with CH235 – 3,467 particles (~25 Å); B41 in complex with CH235 – 4,248 particles (~24 Å); BG505 in complex with CH235.9 – 2,567 particles (25 Å); B41 in complex with CH235.9 – 8,061 particles (19 Å); BG505 in complex with CH235.12 – 15,565 particles (17 Å); B41 in complex with CH235.12 – 17,023 particles (16 Å).

To create figures of each Fab in complex with a representative trimer, the 3D reconstructions for each complex were fit into an EM volume created from the x-ray structure of unliganded BG505 SOSIP.664 (PDB: 4ZMJ) low pass filtered to 30 Å in UCSF Chimera (Pettersen et al., 2004) and using the "segment map" option to isolate the density of the Fab components alone. Two-dimensional back projections of the final 3D models were generated using EMAN (Tang et al., 2007).

Epitope visualization.

The HIV-1 gp120 epitopes targeted by donor CH235 antibodies were visualized using PyMOL (Schrodinger, 2010). In this graphic program, we used 5.5-Å distance for selection of epitope atom sets which were virtually identical to those defined by protein interface analysis program PISA.

Monoclonal antibody CH235.9 amino acid reversion.

Primers were designed with the online Agilent Quikchange primer designer tool (www.thermofisher.com) and were as follows:

$CH235.9_{N30T}$: CGTGGCGTCTGGATACAACTTCACCGACTACTATATAC;
$CH235.9_{D31T}$: CGTCTGGATACAACTTCAACACCTACTATATACACTGGGTGC;
$CH235.9_{G62Q}$: GGTCGCACAGATTACGCACAGGCGTTTGGGGA;
$CH235.9_{G65Q}$: GATTACGCAGGGGCGTTTCAGGACAGAGTGTCCA;
$CH235.9_{A103E}$: GTTAGAAATGTGGGAACGGAGGGCAGCTTGCTCCACTATG;
$CH235.9_{G62Q/G65Q}$: GGTCGCACAGATTACGCACAGGCGTTTCAGGACAGAGTGTCCA;
$CH235.9_{S54R}$: GGATCGACCCTAGGGGTGGTCGCACAG;
$CH235.9_{A61S}$: GTGGTCGCACAGATTACTCAGGGGCGTTTG.

Presence of mutations in plasmid products was confirmed by single-colony sequencing.

Structural bioinformatics.

Average buried surface area (BSA) on gp120 was calculated for residues with BSA > 1Å^2 for the gp120-antibody complexes, and the corresponding antibody neutralization potencies were averaged for each of those residues based on data from neutralization assays. Spearman correlation between BSA on gp120 and antibody potencies was calculated for BSA cutoffs = 0 to 85 Å^2 and potency $logIC_{50}$ cutoffs = 0.60 to 1.62 µg/ml.

Sample preparation for 5' RACE method and 454 pyrosequencing.

Human PBMCs (6×10^7) were obtained from three HIV-1 and hepatitis C negative individuals (LP32647, LP08248 and LP23810). A 5'RACE approach was developed to amplify immunoglobulin genes based on previously described methods (Venturi et al., 2011). Briefly, the PBMCs were pelleted at 1200 rpm for 8 min. mRNA was then extracted and eluted in 50 µl elution buffer using µMACS mRNA isolation kit (Miltenyi Biotec) according to manufacturer's instructions. To synthesize cDNA, 10 µl mRNA was mixed with 1 µl 5'CDS Oligo dT primers (12 µM) and incubated at 70 °C for 1 min and then -20 °C for 1 min. Then 1 µl SMARTER Oligo Primer (12 µM) (Clontech), 4 µl 5X RT buffer, 1 µl DTT 20 (20mM), 1 µl dNTP (10mM), 1 µl RNAse out and 1 µl SuperScript II reverse transcriptase (Invitrogen) were added to the reaction. After 2 hours incubation at 42 °C, the cDNA products were purified using Nucleospin II kit (Macherey-Nagel) and eluted in 50 µl water. 454 pyrosequencing was performed as described previously (Wu et al., 2011). The first PCR amplification was performed with a common 5' primer II A (Clontech) and an Ig gene specific 3' primer (5'GGGGAAGACCGATGGGCCCTTGGTGG3') using KAPA HIFI qPCR kit (Kapa Biosystems). The PCR products were purified with 2% Size Select Clonewell E-gel (Invitrogen) and Agencourt AMPure XP beads (Beckman Coulter). The second PCR amplification was performed with primers with 454 sequencing adapters (454-RACE-F: 5'CCATCTCATCCCTGCGTGTCTCCGACTCAGAAGCAGTGGTATCAACGCAGAGT3'; 454-IgG-R: 5'CCTATCCCCTGTGTGCCTTGGCAGTCTCAGGGGGAAGACCGATGGGCCCTTGGTGG3'). The PCR products were again purified with 2% Size Select Clonewell E-gel and Agencourt AMPure XP beads.

Germline V gene specific substitution profile.

The raw reads from three healthy donors shorter than 300 nucleotides or longer than 600 nucleotides in length were not analyzed. Germline V gene was then assigned to each read using an in-house bioinformatics pipeline (Wu et al., 2015). We removed reads containing stop codons. Functional reads were then clustered using Usearch at 97% sequence identity, and one unique sequence was selected from each cluster to derive a curated dataset. To further reduce reads containing sequencing errors in the curated dataset, unique sequences having only one read in the clustering step were excluded. Finally, the curated dataset of the three donors were pooled for substitution frequency analyses.

Reads from the curated dataset that were assigned to germline V genes of interest were extracted, and were aligned using MUSCLE (Edgar, 2004). The amino acid substitution frequency or mutability of a V gene position was calculated by counting how many reads contain amino acids that are different from the germline V gene, and normalized by the total number of reads. We further calculated the frequency of the 19 types of amino acid substitutions at a position, which was used to generate positional substitution logo. The similarity of positional substitution frequency profiles between V genes of interest was measured by Pearson correlation coefficient.

Conformity analysis.

The positional conformity of a conforming antibody sequence A to a reference sequence B is defined as the number of mutated positions shared by both sequences divided by the total number of mutations in the conforming sequence. Thus:

$$c_p(A;B) = \frac{|M_A \cap M_B|}{|M_A|}$$

where M_i represents the set of amino acid positions in sequence i which are mutated from the germline V residue. Insertions and missing data are ignored, but deletions relative to the germline V are counted as mutations. For 8ANC131 and CH235 (Figure S3A):

$M_{8ANC131}$ = {2, 9, 10, 11, 16, 19, 20, 23, 26, 30, 31, 32, 33, 34, 37, 45, 46, 48, 50, 52, 53, 55, 57, 58, 59, 60, 62, 63, 66, 68, 69, 70, 71, 74, 77, 80, 84, 85, 88, 89}

M_{CH235} = {19, 23, 31, 34, 46, 47, 50, 52, 55, 57, 59, 63, 68, 83, 84}

$M_{8ANC131} \cap M_{CH235}$ = {19, 23, 31, 34, 46, 50, 52, 55, 57, 59, 63, 68, 84}

c_p(8ANC131, CH235) = 13 / 15 = 86.7%

Identity conformity was defined the number of positionally conforming sites in conforming antibody A which were also mutated to the same residue as in the reference antibody B. Thus:

$$c_i(A;B) = \frac{\sum_{x \in \{M_A \cap M_B\}} \delta_{A_x B_x}}{|M_A|}$$

where δ is the Kronecker delta function and A_x is the identity of the residue at position x of sequence A. For 8ANC131 and CH235 (Figure S3B): c_i(8ANC131, CH235) = 4 / 15 = 26.7%

Targeting precision of CD4bs-directed antibodies.

The targeting precision of the CD4bs-directed antibodies was defined as the buried surface area inside of the CD4 binding site minus the buried surface area outside of the CD4 binding site. The buried surface area of each antigen residue was determined by NACCESS. The buried surface area from the following residue numbers were considered inside of the CD4 binding site: 257, 279, 280, 281, 282, 283, 365, 366, 367, 368, 370, 371, 455, 456, 457, 458, 459, 460, 469, 472, 473, 474, 475, 476, and 477 (Zhou et al., 2007). The buried surface areas from the rest of the residues were considered outside of the CD4 binding site. Somatic hypermutation was defined using nucleotide sequences and *P* values were calculated based on linear regression.

Antibody binding orientation calculation.

To calculate the relative rotation angles and translation to gp120-bound CD4 for gp120-bound CD4-binding site antibodies, all antibody-gp120 complexes to be analyzed were first superposed over the outer domain of gp120 (residue ranges: 252-392, 412-422, 437-476) with gp120 in its CD4 complex (PDB ID: 2NXY). The calculations of rotation angles and translation were then carried out with the gp120-aligned structures. For comparison of position of heavy chain variable domain relative to gp120-bound CD4, the frame work regions (residues 46-52, 56-59, 66-71 and 76-82) were superimposed to regions of CD4 domain 1 (residues 34-40, 43-46, 54-59, 65-71). The superposition procedures were performed with the Superpose Molecules module in CCP4 (Collaborative Computational Project, 1994). The Chi angle and distance between centroids in the Superpose output was taken as the rotation angle and translation distance between CD4 and a CD4-binding site antibody.

Table S1. Characteristics of the V(D)J rearrangements of key CH235 lineage antibodies. Related to Figure 1.

Antibody ID	VH	D	JH	Mutation frequency	CDRH3 length	VK	JK	Mutation frequency	CDRL3 length	Week of isolation
UCA	1-46*01	3-10*01	4*02	0.0%	15	3-15*01	1*01	0.0%	8	-
CH235	1-46*01	3-10*01	4*02	7.9%	15	3-15*01	1*01	3.8%	8	41
CH236	1-46*01	3-10*01	4*02	8.2%	15	3-15*01	1*01	2.8%	8	41
CH239	1-46*01	3-10*01	4*02	7.9%	15	3-15*01	1*01	4.7%	8	41
CH240	1-46*01	3-10*01	4*02	7.4%	15	3-15*01	1*01	3.1%	8	41
CH241	1-46*01	3-10*01	4*02	11.5%	15	3-15*01	1*01	3.5%	8	41
CH235.6	1-46*01	3-10*01	4*02	12.6%	15	3-15*01	1*01	3.5%	8	66[^]
CH235.7	1-46*01	3-10*01	4*02	14.8%	15	3-15*01	1*01	2.8%	8	100[#]
CH235.8	1-46*01	3-10*01	4*02	12.0%	15	3-15*01	1*01	3.5%	8	100[^]
CH235.9	1-46*01	3-10*01	4*02	19.6%	15	3-15*01	1*01	2.8%	8	152[#]
CH235.10	1-46*01	3-10*01	4*02	21.6%	15	3-15*01	1*01	16.7%	8	264
CH235.11	1-46*01	3-10*01	4*02	25.1%	15	3-15*01	1*01	17.6%	8	323
CH235.12	1-46*01	3-10*01	4*02	25.7%	15	3-15*01	1*01	12.9%	8	323
CH235.13	1-46*01	3-10*01	4*02	23.5%	15	3-15*01	1*01	11.6%	8	323

[^] Paired with CH241 V-light chain and complemented with CH241 V-heavy.

[#] Paired with CH236 V-light chain and complemented with CH236 V-heavy.

Mutation frequency is calculated on nucleotide sequences of the whole V(D)J rearrangement compared to UCA.

CDR H3 and CDR L3 lengths are expressed in amino acids.

Table S2. Summary of the Breadth and Potency of Antibody Neutralization Against 199 HIV-1 Env-Pseudoviruses. Related to Figure 1.

Virus ID	Clade	CH235	CH235.9	CH235.12	VRC01
0260.v5.c36	A	>50	10.5	1.02	0.468
0330.v4.c3	A	>50	1.88	0.313	0.047
0439.v5.c1	A	>50	3.49	0.374	0.129
3365.v2.c20	A	>50	1.29	0.068	0.030
3415.v1.c1	A	>50	3.20	0.450	0.084
3718.v3.c11	A	12.3	1.80	0.360	0.165
398-F1_F6_20	A	>50	5.48	1.76	0.181
BB201.B42	A	>50	7.20	0.573	0.316
BG505.W6M.C2	A	>50	0.823	0.111	0.053
BI369.9A	A	>50	1.95	0.290	0.224
BS208.B1	A	>50	1.77	0.263	0.022
KER2008.12	A	>50	>50	>50	0.591
KER2018.11	A	>50	9.89	2.52	0.555
KNH1209.18	A	>50	1.21	0.251	0.099
MB201.A1	A	>50	12.9	0.333	0.212
MB539.2B7	A	>50	11.7	1.71	0.500
MI369.A5	A	>50	2.64	0.416	0.269
MS208.A1	A	>50	2.77	0.463	0.178
Q23.17	A	1.35	0.405	0.132	0.052
Q259.17	A	>50	7.46	0.100	0.075
Q769.d22	A	>50	0.981	0.110	0.035
Q769.h5	A	>50	2.55	0.139	0.062
Q842.d12	A	8.15	0.378	0.091	0.038
QH209.14M.A2	A	>50	5.76	0.374	0.060
RW020.2	A	1.20	1.05	0.301	0.203
UG037.8	A	>50	1.10	0.188	0.089
246-F3.C10.2	AC		1.33		
3301.V1.C24	AC	20.9	1.91	0.473	0.097
3589.V1.C4	AC	>50	>50	0.309	0.047
6540.v4.c1	AC	>50	>50	>50	>50
6545.V4.C1	AC	>50	>50	>50	>50
0815.V3.C3	ACD	>50	0.549	0.056	0.015
6095.V1.C10	ACD	>50	3.29	1.33	0.506
3468.V1.C12	AD	2.47	0.659	0.070	0.050
Q168.a2	AD	>50	1.10	0.261	0.098
Q461.e2	AD	>50	6.95	0.818	0.497
620345.c1	AE	>50	8.61	1.94	>50
BJOX009000.02.4	AE	>50	>50	5.50	1.54
BJOX010000.06.2	AE	>50	>50	10.6	6.79
BJOX025000.01.1	AE	40.6	0.586	0.271	8.46
BJOX028000.10.3	AE	>50	0.886	0.168	0.256
C1080.c3	AE	>50	13.3	2.69	2.10
C2101.c1	AE	12.6	3.37	0.261	0.179
C3347.c11	AE	>50	0.482	0.117	0.095
C4118.09	AE	3.30	1.04	0.084	0.248
CM244.ec1	AE	1.19		0.160	0.089
CNE3	AE	>50	>50	2.45	1.63
CNE5	AE	17.6	2.94	1.03	0.323
CNE55	AE	>50	1.90	0.400	0.359
CNE56	AE	42.9	2.96	1.10	0.343
CNE59	AE	13.6	3.79	0.943	0.623
CNE8	AE	>50	3.22	1.10	0.510
R1166.c1	AE	>50	34.4	0.758	3.00
R2184.c4	AE	5.82	6.83	0.563	0.133
R3265.c6	AE	>50	35.0	0.172	0.710
TH966.8	AE	0.732	1.70	0.304	0.284
TH976.17	AE	0.975	0.935	0.286	0.332
235-47	AG	>50	2.25	0.293	0.061
242-14	AG	>50	>50	2.83	>50
263-8	AG	>50	2.93	0.447	0.168
269-12	AG	>50	>50	>50	0.293
271-11	AG	>50	0.652	0.090	0.054
928-28	AG	>50	3.55	0.542	0.476
DJ263.8	AG	>50	2.90	0.276	0.066
T250-4	AG	>50	>50	>50	>50
T251-18	AG	>50	>50	4.02	4.42
T253-11	AG	>50	>50	1.65	0.501
T255-34	AG	>50	7.83	0.608	0.725
T257-31	AG	>50	13.3	2.66	2.47
T266-60	AG	>50	>50	10.3	2.37
T278-50	AG	>50	>50	>50	>50
T280-5	AG	>50	0.308	0.109	0.059
T33-7	AG	>50	0.469	0.039	0.019
3988.25	B	>50	3.49	0.917	0.369
5768.04	B	>50	3.75	0.715	0.354
6101.10	B	>50	3.14	0.467	0.023
6535.3	B	>50	>50	4.85	2.10
7165.18	B	>50	>50	>50	45.0
45_01dG5	B	>50	0.507	0.058	0.011
89.6.DG	B	>50	27.7	2.23	1.30
AC10.29	B	>50	9.55	2.13	1.41
ADA.DG	B	>50	2.88	0.907	0.563
Bal.01	B	>50	0.326	0.237	0.124
BaL.26	B	>50	1.10	0.214	0.060
BG1168.01	B	>50	4.06	1.42	0.738
BL01.DG	B	>50	>50	>50	>50
BR07.DG	B	>50	4.66	1.51	1.81
BX08.16	B	>50	>50	2.35	0.389
CAAN.A2	B	>50	7.47	2.23	0.963
CNE10	B	>50	23.2	5.26	0.689
CNE12	B	>50	8.19	2.56	0.695
CNE14	B	>50	12.5	0.594	0.199
CNE4	B	>50	5.97	1.16	0.639
CNE57	B	>50	>50	1.25	0.496
HO86.8	B	>50	1.35	0.174	>50
HT593.1	B	>50	2.23	0.984	0.606
HXB2.DG	B	18.1	0.243	0.173	0.063
JRCSF.JB	B	>50	1.65	0.596	0.436
JRFL.JB	B	1.82	2.13	0.127	0.051
MN.3	B	>50	1.27	0.142	0.011
PVO.04	B	>50	3.53	1.47	0.552
QH0515.01	B	26.4	7.95	1.40	1.43
QH0692.42	B	>50	10.8	2.25	1.37
REJO.67	B	>50	>50	1.09	0.113
RHPA.7	B	16.6	0.300	0.091	0.051
SC422.8	B	>50	3.73	0.798	0.127
SF162.LS	B	>50	2.21	0.534	0.228
SS1196.01	B	>50	>50	0.827	0.246
THRO.18	B	>50	>50	>50	4.63
TRJO.58	B	>50	1.76	0.524	0.116
TRO.11	B	14.8	4.68	0.714	0.502
WITO.33	B	>50	3.65	0.418	0.140
X2278.C2.B6	B	>50	5.96	0.425	0.133
YU2.DG	B	>50	0.761	0.235	0.113
BJOX002000.03.2	BC	>50	2.74	0.739	>50
CH038.12	BC	>50	>50	17.3	0.519
CH070.1	BC	>50	>50	2.39	9.99
CH117.4	BC	>50	1.03	0.340	0.095
CH119.10	BC	>50	3.68	1.24	0.577
CH181.12	BC	>50	3.44	0.612	0.481

Virus ID	Clade	CH235	CH235.9	CH235.12	VRC01
CNE15	BC	15.2	1.16	0.249	0.100
CNE19	BC	27.5	0.488	0.134	0.169
CNE20	BC	>50	1.09	0.254	9.25
CNE21	BC	>50	1.81	0.527	0.357
CNE40	BC	>50	0.477	0.207	0.370
CNE7	BC	>50	>50	1.36	0.286
286.36	C	>50	3.00	0.699	0.322
288.38	C	>50	3.62	1.62	1.49
0013095-2.11	C	>50	>50	29.7	0.088
001428-2.42	C	>50	0.417	0.087	0.008
0077_V1.C16	C	>50	41.7	6.84	1.28
00836-2.5	C	>50	>50	1.09	0.119
0921.V2.C14	C	10.9	1.76	0.344	0.182
16055-2.3	C	>50	0.768	0.159	0.063
16845-2.22	C	>50	28.0	7.47	3.60
16936-2.21	C	>50	1.85	0.500	0.110
25710-2.43	C	>50	0.983	0.382	0.594
25711-2.4	C	>50	4.57	0.974	0.555
25925-2.22	C	>50	2.51	0.641	0.474
26191-2.48	C	>50	1.65	0.583	0.166
3168.V4.C10	C	>50	6.56	0.372	0.255
3637.V5.C3	C	>50	10.5	12.2	1.45
3873.V1.C24	C	>50	>50	>50	0.791
6322.V4.C1	C	>50	4.74	0.944	>50
6471.V1.C16	C	>50	>50	>50	>50
6631.V3.C10	C	>50	>50	5.83	>50
6644.V2.C33	C	>50	>50	>50	0.243
6785.V5.C14	C	>50	>50	>50	0.286
6838.V1.C35	C	>50	4.54	1.08	0.210
96ZM651.02	C	>50	4.37	1.18	0.570
BR025.9	C	>50	>50	>50	0.593
CAP210.E8	C	>50	>50	>50	>50
CAP244.D3	C	>50	13.2	1.52	1.33
CAP256.206.C9	C	14.6	3.73	1.32	0.971
CAP45.G3	C	>50	4.00	0.568	7.00
Ce1176.A3	C	>50	7.71	1.24	2.60
CE703010217.B6	C	>50	1.70	0.319	0.366
CNE30	C	>50	4.31	1.21	0.525
CNE31	C	>50	>50	2.78	0.786
CNE53	C	1.77	0.781	0.274	0.087
CNE58	C	>50	>50	1.95	0.225
DU123.06	C	>50	17.5	4.25	7.92
DU151.02	C	3.94	1.33	0.287	14.8
DU156.12	C	9.48	1.65	0.285	0.086
DU172.17	C	1.92	1.74	0.361	>50
DU422.01	C	>50	2.85	0.944	>50
MW965.26	C	6.10	3.03	0.573	0.029
SO18.18	C	>50	1.24	0.110	0.058
TV1.29	C	>50	11.0	4.63	>50
TZA125.17	C	>50	>50	>50	>50
TZBD.02	C	>50	38.9	0.219	0.078
ZA012.29	C	13.2	11.5	0.971	0.384
ZM106.9	C	>50	2.09	0.620	0.311
ZM109.4	C	>50	2.50	0.416	0.177
ZM135.10a	C	>50	>50	>50	2.25
ZM176.66	C	>50	1.21	0.183	0.083
ZM197.7	C	>50	10.5	1.40	0.428
ZM214.15	C	>50	10.1	2.22	0.893
ZM215.8	C	6.19	1.71	0.315	0.215
ZM233.6	C	5.71	5.02	1.25	1.02

Virus ID	Clade	CH235	CH235.9	CH235.12	VRC01
ZM249.1	C	9.99	0.598	0.273	0.057
ZM53.12	C	>50	4.44	0.558	0.625
ZM55.28a	C	>50	4.20	0.665	0.285
3326.V4.C3	CD	>50	1.54	0.114	0.068
3337.V2.C6	CD	>50	10.8	0.429	0.090
3817.v2.c59	CD	>50	14.6	3.63	>50
231965.c1	D	>50	>50	13.9	0.353
247-23	D	>50	3.32	0.691	1.84
3016.v5.c45	D	>50	>50	>50	0.155
57128.vrc15	D	>50	>50	6.59	>50
6405.v4.c34	D	>50	>50	>50	1.55
A03349M1.vrc4a	D	>50	7.54	4.08	4.10
A07412M1.vrc12	D	>50		0.351	0.082
NKU3006.ec1	D	4.61	1.29	0.466	0.596
P0402.c2.11	G		3.65		
P1981.C5.3	G	>50	>50	2.19	0.330
X1193.c1	G	>50	4.08	0.972	0.154
X1254.c3	G	>50	>50	1.98	0.059
X1632.S2.B10	G	>50	1.18	0.484	0.130
X2088.c9	G	>50	>50	>50	>50
X2131.C1.B5	G	>50	10.3	2.58	0.537
SIVmac251.30.SG3	NA	>50	>50	>50	>50
SVA.MLV	NA	>50	>50	>50	>50

IC50 (µg/ml)
- <0.100
- 0.100-1.00
- 1.00-10.0
- >10.0
- >50

	CH235	CH235.9	CH235.12	VRC01
# Viruses	202	202	202	202
Total VS Neutralized				
IC50 <50 µg/ml	35	153	179	179
IC50 <10 µg/ml	19	130	173	177
IC50 <1.0 µg/ml	2	25	115	146
IC50 <0.1 µg/ml	0	0	10	47
IC50 <0.01 µg/ml	0	0	0	1
% VS Neutralized				
IC50 <50 µg/ml	17	76	89	89

Table S3. Crystallographic Data Collection and Refinement Statistics. Related to Figure 2.

Complex (antibody-gp120)	CH235-93TH057	CH235.9-93TH057	CH235.12-93TH057
PDB ID	5F9W	5F9O	5F96
Data collection			
Space group	$P3_2$	$P\ 2_1\ 2_1\ 2_1$	$P\ 2_1$
Cell dimensions			
a, b, c (Å)	123.4, 123.4, 127.3	63.5, 67.8, 225.6	53.7, 69.9, 127.3
α, β, γ (°)	90.0, 90.0, 120.0	90.0, 90.0, 90.0	90.0, 94.6, 90.0
Resolution (Å)	40.94-2.89 (2.99-2.89)*	50.0-1.86 (2.00-1.93; 1.93-1.86)	2.25 (2.29-2.25)
R_{sym} or R_{merge}	0.22 (0.68)	14.1 (41.8; 48.4)	12.5 (41.4)
$I/\sigma I$	8.9 (1.9)	7.06 (1.79; 1.25)	15.5 (2.1)
Completeness (%)	100 (100)	89.7 (73.4; 46.8)	97.6 (86.7)
Redundancy	7.6 (6.7)	3.4 (1.6; 1.2)	3.0 (2.1)
Refinement			
Resolution (Å)	40.9 – 2.89	35.8-1.86	34.5-2.25
No. reflections	48360	73935	43920
R_{work}/R_{free}	17.5/22.9	20.4/22.0	18.3/23.0
No. atoms			
Protein	11932	6137	5976
Ligand/ion	393	53	213
Water	74	461	196
B-factors (Å^2)			
Protein	92.5	43.5	63.4
Ligand/ion	120.9	87.0	103.4
Water	77.5	47.2	59.3
R.m.s deviations			
Bond lengths (Å)	0.004	0.008	0.006
Bond angles (º)	0.78	1.27	0.92
Ramachandran statistics			
Favored (%)	96.0	97.1	97.0
Outliers (%)	0.1	0.0	0.1

*Values in parenthesis denote highest resolution shell.

Table S4. Sequence Similarity Between VH1-2 and VH1-46 Broadly Neutralizing Antibodies and Mutability of Germline Genes. Related to Figure 3.

A. The probability of a conforming VH1-46 antibody with *x* V_H mutations, having *c* common mutation positions with a reference antibody were estimated based on 100,000 simulated events, with the likelihood of each residue being mutated based on uniform distribution (position) ($P_{uniform}$), or the mutation frequency at each residue position derived from the VH1-46 antibodies (P_{VH1-46}).

				Probability of seeing *c* based on:		
Conforming antibody	**# mutations (*x*)**	**Reference antibody**	**# sharing mutation positions (*c*)**	**Uniform distribution ($P_{uniform}$)**	**VH1-46 mutation frequencies (P_{VH1-46})**	PVH1-46/$P_{uniform}$
CH235	15	1B2530	11	0.00432	0.17751	41.1
CH235	15	8ANC131	13	0.00010	0.01744	174.4
1B2530	39	8ANC131	26	0.00001	0.04274	4274.0

B. The probability of a conforming VH1-46 antibody with *x* V_H mutations, having *i* identical mutations with a reference antibody were estimated based on 100,000 simulated events, with the likelihood of each residue being mutated based on uniform distribution (position and mutation type) ($P_{uniform}$), or the mutation frequency at each residue position derived from the VH1-46 antibodies (P_{VH1-46}).

				Probability of seeing *i* based on:		
Conforming antibody	**# mutations (*x*)**	**Reference antibody**	**# identical mutations (*i*)**	**Uniform distribution ($P_{uniform}$)**	**VH1-46 mutation frequencies (P_{VH1-46})**	PVH1-46/$P_{uniform}$
CH235	15	1B2530	4	0.00022	0.10433	474.2
CH235	15	8ANC131	4	0.00019	0.11546	607.7
1B2530	39	8ANC131	7	0.00001	0.14622	14622.0

C. Pearson correlation coefficients of positional somatic mutation frequency between VH1-46, VH1-2 and three others.

	IGHV1-2	**IGHV1-46**	**IGHV1-69**	**IGHV3-23**	**IGHV3-30**
IGHV1-46	0.84				
IGHV1-69	0.74	0.74			
IGHV3-23	0.54	0.63	0.68		
IGHV3-30	0.53	0.57	0.62	0.83	
IGHV4-59	0.47	0.53	0.55	0.67	0.57

Table S5 - CH235 Lineage and CH106 Monoclonal Antibodies Cross-Blocking. Related to Figure 4.

A. CH235 lineage antibodies blocking of sCD4 and CH106 binding to CH505 TF gp120 and B.63521 gp120 Envs. Results expressed as IC50 ug/ml. nb= no blocking.

	UCA	IA4	IA3	IA2	IA1	CH240	CH236	CH235	CH239	CH241
sCD4	nb	nb	nb	nb	>100	nb	nb	26.3	92.6	2.6
CH106	nb	nb	nb	nb	82.4	68.9	82.2	16.5	45.4	1.5

B. Monoclonal antibody CH106 blocking of CH235 lineage antibodies to CH505 TF gp120. Results expressed as IC50 ug/ml. nb= no blocking.

	CH235	CH236	CH239	CH240	CH241	CH106
CH106	4.3	6.8	4.6	2.3	14.3	2.5

Table S6 - CH235 lineage autologous neutralization. Related to Figure 5.

		Neutralization IC50, ug/ml															
Virus ID	Week	UCA	IA4	IA3	CH235.11	IA2	IA1	CH240	CH236	CH235	CH239	CH241	CH235.7	CH235.13	CH235.10	CH235.9	CH235.12
T/F		>50	>50	5.22	2.19	0.97	0.91	0.94	0.61	0.63	0.48	0.14	0.39	0.91	0.22	0.51	0.22
CH505.w4.10	4	>50	20.51	1.02	0.12	0.24	0.20	0.15	0.10	0.10	<0.02	<0.02	0.07	0.17	0.10	0.08	<0.02
CH505.w4.26	4	>50	>50	3.67	1.59	1.36	0.32	0.67	0.78	0.46	0.30	0.05	0.09	0.16	0.04	0.16	0.03
CH505.w4.3	4	>50	>50	1.65	9.58	0.35	0.30	0.25	0.40	0.11	0.10	<0.02	0.06	0.15	0.11	0.11	<0.02
CH505.w8.e12	14	>50	>50	34.99	0.06	2.22	0.85	0.93	0.53	0.42	0.41	0.09	0.07	0.18	0.02	0.08	<0.02
CH505.w8.e21	14	>50	>50	>50	5.52	38.08	0.79	0.58	0.96	0.38	0.14	0.04	0.10	0.17	0.24	0.14	<0.02
CH505.w8.e29	14	>50	>50	5.34	6.19	1.02	0.86	0.88	1.05	0.75	0.61	0.17	0.17	0.35	0.15	0.21	0.06
CH505.w8.e34	14	>50	>50	4.84	7.07	0.94	0.84	0.89	1.09	0.52	0.78	0.12	0.14	0.31	0.12	0.16	0.05
CH505.w8.e6	14	>50	>50	5.73	17.42	1.64	0.92	0.73	0.63	0.31	0.43	0.17	0.21	0.34	0.21	0.30	0.09
CH505.w8.e3	14	>50	>50	6.28	27.80	1.40	0.79	1.05	0.75	0.49	0.40	0.09	0.19	0.27	0.24	0.25	0.11
CH505.w8.e4	14	>50	>50	3.60	35.26	1.00	0.72	0.60	0.88	0.27	0.40	0.08	0.20	0.34	0.13	0.27	0.06
CH505.w12.e4	20	>50	>50	>50	2.66	47.61	0.94	1.00	1.81	0.65	0.18	0.06	0.08	0.19	0.18	0.08	<0.02
CH505.w12.e19	20	>50	>50	8.99	>50	2.12	1.99	1.63	1.24	1.16	1.11	0.27	0.29	0.47	0.24	0.27	0.05
CH505.w12.e25	20	>50	>50	8.54	>50	1.69	1.85	1.41	0.85	1.08	0.75	0.18	0.29	0.37	0.23	0.38	0.07
CH505.w12.e27	20	>50	>50	10.28	>50	2.06	0.98	1.13	0.46	0.39	0.27	0.10	0.16	0.30	0.12	0.15	0.05
CH505.w24.e5	30	>50	>50	>50	2.08	7.72	1.83	2.08	0.58	0.80	0.67	1.17	0.35	0.38	0.44	0.26	0.07
CH505.w24.e34	30	>50	>50	>50	2.18	4.98	5.18	5.61	2.27	3.23	3.02	3.68	0.74	0.60	0.38	0.62	0.12
CH505.w24.e37	30	>50	>50	>50	3.56	6.45	6.92	7.57	2.60	4.25	2.79	3.99	0.94	1.19	0.43	0.70	0.19
CH505.w24.e24	30	>50	>50	29.77	>50	27.95	4.62	4.12	3.69	2.06	1.54	0.49	0.64	0.72	0.43	0.67	0.17
CH505.w24.e28	30	>50	>50	>50	>50	>50	5.28	5.28	9.14	2.16	2.07	0.40	0.11	0.53	45.05	0.37	0.17
CH505.w24.e13	30	>50	>50	>50	>50	>50	4.73	5.11	4.39	1.15	1.49	2.96	0.16	0.39	>50	0.46	0.10
CH505.w48.e6	53	>50	>50	>50	>50	>50	>50	>50	>50	>50	19.95	>50	1.76	2.66	3.69	1.25	0.46
CH505.w48.e22	53	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	12.55	5.43	3.06	1.30	0.58
CH505.w48.e13	53	>50	>50	>50	>50	>50	>50	>50	>50	>50	33.78	>50	27.04	5.03	2.42	1.39	0.48
CH505.w48.e28	53	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	25.29	5.08	2.58	1.23	0.66
CH505.w48.e11	53	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	34.23	3.26	3.10	2.48	0.75
CH505.w48.e31	53	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	25.92	1.53	1.44	0.11
CH505.w96.A5	100	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	3.68
CH505.w96.B8	100	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	3.02
CH505.w96.B6	100	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	8.35
CH505.w96.A10	100	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	19.82
CH505.w96.A3	100	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	45.37	>50	>50	>50	>50	>50
CH505.w96.B4	100	>50	>50	>50	>50	>50	>50	>50	>50	>50	48.62	41.17	>50	>50	>50	>50	>50
CH0505.C.w136.e.B23	136	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	29.73	11.41	10.23	1.75
CH0505.C.w136.e.B18	136	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	32.27	27.81	12.41	3.33
CH0505.C.w136.e.B24	136	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	43.03	13.58	4.09
CH0505.C.w136.e.B33	136	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	32.79	16.05	3.62
CH0505.C.w136.e.B38	136	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	32.68	16.17	3.88
CH0505.C.w136.e.B2	136	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	28.62	5.95
CH0505.C.w136.e.B3	136	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
CH0505.C.w136.e.B4	136	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
CH0505.C.w136.e.B5	136	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
CH0505.C.w136.e.B12	136	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
CH0505.C.w136.e.B27	136	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
CH0505.C.w160.C2	160	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
CH0505.C.w24.C9	160	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
CH0505.C.w24.C10	160	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
CH0505.C.w24.C11	160	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
CH0505.C.w24.C12	160	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
CH0505.C.w24.D2	160	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
CH0505.C.w24.T3	160	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
CH0505.w176.e11	176	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	15.43
CH0505.w176.e12	176	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	26.20
CH0505.w176.e13	176	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	30.25
CH0505.w176.e1	176	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	35.54
CH0505.w176.e2	176	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	26.11
CH0505.w176.e10	176	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
CH0505.w233.e1	233	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
CH0505.w233.e3	233	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
CH0505.w233.e4	233	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
CH0505.w233.e7	233	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
CH0505.w233.e17	233	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
CH0505.w233.e18	233	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
CH0505.w258.e4	258	>50	>50	34.10	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
CH0505.w258.e1	258	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
CH0505.w258.e7	258	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
CH0505.w258.e6	258	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
CH0505.w258.e5	258	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
CH0505.w323.e1	323	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
CH0505.w323.e11	323	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
CH0505.w323.e13	323	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
CH0505.w323.e14	323	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
CH0505.w323.e15	323	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
CH0505.w323.e16	323	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
CH0505.w323.e17	323	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
CH0505.w323.e18	323	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50

Table S7. CH235 lineage antibodies and CH235.9 mutants neutralization of CH505 TF loop D mutant viruses. Related to Figure 5.

		Neutralization IC50 ug/ml)												
Virus ID	Virus Mutations	CH236	CH235.7	CH235.10	CH235.11	CH235.13	CH235.12	CH235.9	CH235.9 N30T	CH235.9 D31T	CH235.9 G62Q	CH235.9 G65Q	CH235.9 G62Q+ G65Q	CH235.9 A103E
CH505.TF	-	**0.61**	**0.39**	**0.22**	**2.19**	**0.91**	**0.22**	**0.51**	**4.26**	**0.43**	**0.30**	**0.26**	**0.15**	**0.43**
CH505.TF.M5	N279K	**0.26**	**0.16**	**0.17**	**0.31**	**0.18**	**0.02**	**0.19**	**0.90**	**0.11**	**0.12**	**0.14**	**0.12**	**0.03**
CH505.TF.M6	V281A	**0.80**	**0.34**	**0.11**	**2.30**	**0.30**	**0.08**	**0.40**	**1.06**	**0.12**	**0.02**	**0.08**	**0.04**	**0.16**
CH505.TF.M10	V281G	**1.75**	**0.18**	**0.19**	**1.52**	**0.14**	**0.02**	**0.24**	**0.83**	**0.07**	**0.02**	**0.09**	**0.08**	**0.07**
CH505.TF.M19	V281D	**7.53**	**0.40**	>50	>50	**0.57**	**0.17**	**0.63**	**4.04**	**0.89**	**1.36**	**1.58**	**1.54**	**0.43**
CH505.TF.M11	N279D+V281A	>50	**0.50**	**0.21**	**15.35**	**0.25**	**0.08**	**0.39**	**1.25**	**0.12**	**0.02**	**0.14**	**0.13**	**0.24**
CH505.TF.M8	N280S+V281A	>50	>50	>50	**1.91**	**0.86**	**0.05**	**0.66**	**24.31**	**0.15**	**0.14**	**0.12**	**0.03**	**0.13**
CH505.TF.M21	N280T+V281A	>50	>50	>50	**10.16**	**16.72**	**0.13**	**0.74**	>50	**0.23**	**0.15**	**0.25**	**0.27**	**0.19**
CH505.TF.M20	N280S+V281G	>50	>50	>50	**7.86**	**11.58**	**0.07**	**0.94**	44.29	**0.08**	**0.11**	**0.26**	**0.26**	**0.10**
CH505.TF.M7	E275K+N279D+V281S	>50	>50	**0.61**	>50	**2.12**	**0.13**	**0.77**	44.21	**0.38**	**0.37**	**0.49**	**0.38**	**0.19**
CH505.TF.M9	E275K+N279D+V281G	>50	>50	**0.24**	**0.46**	**5.02**	**0.11**	**0.85**	42.75	**0.25**	**0.25**	**0.24**	**0.14**	**0.14**

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