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Revised 15 September 2011: Author name corrections: Olivera to Oliveira and initial added to Dennis R. Burton. Typographical errors were corrected in Table S3, A and B, and Tabl8 S8. In Table S7 "Geometric Mean" was changed to "Average."

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Materials and Methods

Samples. Human samples were collected after signed informed consent in accordance with Institutional Review Board (IRB)-reviewed protocols by all participating institutions. Patient 1 was selected from a cohort of long term non progressors followed at the Aaron Diamond Aids Research Center, New York. Patients 3 and 8 were selected from a group of elite controllers that were followed at the Ragon Institute in Boston. Patients 1, 3 and 8 were selected based on their broad neutralizing serum activity against a standard panel of HIV isolates (*20*). Patient 12 was selected from the Protocol G Cohort of the "International Aids Vaccine Initiative" based on broad serum neutralizing activity (*1*).

Staining, single-cell sorting and antibody cloning. Staining and single cell sorting of 2CC-core and gp140 specific IgG+ memory B cells was performed as previously described (*7, 9, 38*). Briefly, CD19+ B cells were enriched from peripheral blood mononuclear cells using anti human CD19 magnetic MACS beads (Miltenyi Biotec) and subsequently stained with anti human CD20 and anti human IgG antibodies (Becton Dickinson) as well as biotinylated 2CC-core (*11*) or YU2-gp140 trimer (*13*) followed by detection with streptavidin coupled phycoerythrin (PE, Beckton Dickinson). Single cells were sorted on a FACSAria III cell sorter (Becton Dickinson), excluding cell doublets, into 96-well PCR plates (Denville) containing 4 µl/well of ice-cold 0.5× phosphate-buffered saline (PBS) containing 10 mM DTT, 8 U RNAsin® (Promega), 0.4 U 5′-3′ Prime RNAse Inhibitor™ (Eppendorf). Plates were sealed with Microseal® ´F` Film (BioRad), immediately frozen on dry ice before storage at −80 °C.

cDNA synthesis and Ig amplification were performed as previously described (*9*) with following modifications:
Instead of using the original primer sets (*9*), first and second immunoglobulin specific PCRs were carried out using the primers described in table S1 in a semi-nested approach (fig. S1). In order to amplify immunoglobulin genes that are highly somatically mutated the 5’ primers (table S1) are set further upstream in the leader region to avoid the potentially mutated region. These 5' primers (table S1) are used in both PCR steps with different constant region primers in each step (fig. S1, table S1). The cloning of heavy and light chain PCR products into their respective expression vectors was performed as previously described (*9*) and 100% identity of cloned expression plasmids with the original PCR product confirmed by sequencing before expression of the antibodies in HEK 293 cells as previously described (*9*). Clonal relationships are identified by immunoglobulin sequence analysis. Antibodies with shared V and J genes for heavy and light chains as well as shared CDR3 region characteristics and somatic hypermutations are considered members of an expanded clone.

ELISAs. High-binding 96-well ELISA plates (Costar) were coated overnight with 100 ng/well of purified antigens (gp140, gp120, gp41, $gp120^{core}$ and 2CC-core) (*11, 13, 25*) and mutant proteins (gp120 D368R, gp120 I420R) (*14-17*) in PBS. After washing, plates were blocked 2 h with 2% BSA, 1µM EDTA, 0.05% Tween-PBS (blocking buffer) and

then incubated for 2 hours with IgG antibodies diluted at 4 μg/ml and several consecutive 1:4 dilutions in PBS. After washing, the plates were developed by incubation for 1 h with goat HRP-conjugated anti-human IgG (Jackson ImmunoReseach) (at 0.8 μg/ml in blocking buffer) and by adding 100 μl of HRP chromogenic substrate (ABTS solution, Invitrogen). Optical densities were measured at 405nm (OD_{405nm}) using an ELISA microplate reader (Molecular Devices). Background values given by incubation of PBS alone in coated wells were subtracted. IgG Antibodies were tested for polyreactivity as previously described (*8*) and considered polyreactive when they recognized at least two structurally different antigens out of the four tested; ssDNA, dsDNA, insulin, and LPS. Threshold values for reactivity were determined by using control antibodies mGO53 (negative), eiJB40 (low positive), and ED38 (high positive) (*8, 9*).

Neutralization assays. Neutralization screens were performed as described (*39*). In brief, neutralization was detected as reduction in luciferase reporter gene expression after single round infection in Tzm-bl cells. In order to rule out unspecific antiviral activity in antibody samples MuLV (murine leukemia virus) was used as a negative control.

Clone specific identification of bone marrow plasma cells. Bone marrow plasma cells were stained with anti human CD138 and anti CD19 antibodies (Becton Dickinson) after Ficoll purification of mononuclear cells from bone marrow aspirates using Ficoll-Paque (GE Healthcare). CD138+ CD19+ human plasma cells were bulk sorted on a FACSAriaIII cell sorter (Becton Dickinson) and RNA isolation performed on 100,000 cells using Trizol LS reagent (Invitrogen) according to the manufacturers instructions. RNA was reverse transcribed using Superscript III reverse transcriptase (Invitrogen) according to manufacturers instructions. cDNA was then subjected to Immunoglobulin specific PCR as previously described (*9*) with following modifications: 1μl of cDNA was amplified in 2 rounds of nested immunoglobulin heavy chain clone specific PCR using first round forward leader and constant region reverse primers (table S1) followed by clone specific forward and reverse primers, designed based on sequencing results from single cell analysis. PCR products were gel purified and cloned into TOPO TA vectors (Invitrogen) according to the manufacturers instructions. Colonies were screened by PCR with clone specific primers and sequenced.

Surface plasmon resonance. All experiments were performed with a Biacore T100 (Biacore, Inc) in HBS-EP+ running buffer (Biacore, Inc) at 25ºC as described previously (*8*). YU2-gp140 and 2CC-core proteins at 12.5 μg/mL were immobilized on CM5 chips (Biacore, Inc.) by amine coupling at pH 4.5 resulting in an immobilization level of 100 RUs. For kinetic measurements on the gp140- and 2CC-core-derivatized chips, IgGs were injected through flow cells at 700 nM and 4 successive 1:2-dilutions in HBS-EP+ running buffer (Biacore, Inc.) at flow rates of 40 μL/min with 3 min association and 5 min dissociation. The sensor surface was regenerated between each experiment with a 30 second injection of 10 mM glycine-HCl pH 2.5 at a flow rate of 50 μL/min. Off rate (k_d (s^{-1})), on rate (k_a (M^{-1} s^{-1}) and binding constants (K_D (M) or K_A (M^{-1}) were calculated after subtraction of backgrounds (binding to control flow cells and signal of the HBS-EP+ running buffer) using Biacore T100 Evaluation software using the kinetic analysis and the 1:1 binding model. The sensorgrams showed in Fig. 3, fig. S7

and S10 are derived from the Biacore data processing using Scrubber 2 software (Center for Biomolecular Interaction Analysis, University of Utah).

CD4i site induction. 293T cells were transfected with $gp160^{BAL}\Delta c$ or $gp160^{YU.2}\Delta c$ in a pMX-IRES-GFP construct (*24*) using Fugene™6 (Roche) at a 1:2 plasmid/Fugene ratio. After 48 hours 293T cells were washed with PBS, detached with trypsin-free cell dissociation buffer (Gibco) and resuspended at a concentration of 10^7 cells/ml in FACS buffer (PBS, 2 % fetal bovine serum (FBS), 2 mM EDTA). sCD4 (Progenics Pharmaceuticals, Inc.) and monoclonal antibodies were added to gp160-expressing 293T cells in a 1:4 dilution series starting with a concentration of 40 µg/ml. mGO53 (*8, 9*)is a non-polyreactive negative control antibody that does not bind to gp160Δc. After incubation for 15 min on ice cells were stained for 25 min on ice with an Alexa647-labeled CD4-induced site antibody (3-67; (*7*)) or an Alexa647-labeled control antibody (PG16 (*29*) or 2G12 (*30*)). Antibody labeling was performed by using Alexa Fluor® 647 Microscale Protein Labeling Kit (Invitrogen). Cells were analyzed on an LSRFortessa cell analyzer (BD Bioscience).

Crystallization. The 3BNC60 IgG was expressed by transient expression in HEK293-6E cells and the Fab fragment was prepared by papain cleavage as described (*40*). Crystallization screens were conducted at 20°C by vapor diffusion in nL sitting drops using a Mosquito™ (TTP LabTech) crystallization robot on MRC crystallization plates (Jena Bioscience). We combined 3BNC60 Fab at a concentration of 9.5 mg/ml with reservoir solution in a 1:1 ratio to create 400 nL drops. Initial crystallization hits were obtained using the PEGRx HT™ (Hampton Research) crystallization screen and further optimized manually. Crystals suitable for data collection grew after several weeks in 11.7% polyethylene glycol 20,000, 0.1 M sodium acetate pH 5.0, 100 mM potassium/sodium tartrate, 20 mM lithium sulfate, 10 mM N-Cyclohexyl-2-aminoethanesulfonic acid (CHES) pH 9.5 in the monoclinic space group $P2_1$ with two Fabs in the asymmetric unit. Crystals were soaked in reservoir solution supplemented with 15% glycerol for 2 hours before immersing in reservoir solution supplemented with 30% glycerol and flash cooling in liquid nitrogen. Diffraction data were collected at the Stanford Synchrotron Radiation Lightsource (SSRL) beam-line 12-2 at 100 K using a Pilatus 6M detector. Data were indexed, integrated, and scaled using XDS (*41*) (table S11). Molecular replacement was conducted using Phaser (*42*) with the V_H and C_H1 domains from the anti-tumor antibody CTM01 (PDB code 1AD9) and with the V_L and C_L domains of the gp120 b13 antibody (PDB code 3IDX) as search models. Model building and refinement to 2.65 Å resolution was done iteratively using Phenix (*43*)and Coot (*44*). The structure was refined using a maximum-likelihood target function and non-crystallographic symmetry restraints. The final model (R_{work} = 20.7%; R_{free} = 25.7%) includes 6478 protein atoms, 146 water molecules and 28 sugar atoms (table S11). 91.9%, 7.6% and 0.5% of the residues were in the favored, allowed, and disallowed regions, respectively, of the Ramachandran plot. Structural analyses and visualization were done using PyMol (The PyMOL Molecular Graphics System, Version 1.3, Schrödinger, LLC). The 3BNC60 structure consists of residues 3–205 for the light chain (including the first N-acetylglucosamine within an N-linked carbohydrate attached to

Asn72) and 2–217 for the heavy-chain. Residues at the termini residues and residues 133–140 within the C_H1 domain are disordered.

Mass Spectrometry. IgG was purified from serum using Protein G Sepharose (GE Healthcare) according to the manufacturers instructions. IgGs were then digested with immobilized papain (Pierce) and digested Fab-Fc fragment mixes incubated with saturating quantities of biotinylated 2CC-core protein (*11*). Streptavidin coupled Dynabeads (Invitrogen) were added after incubation for 15 minutes at room temperature and subjected to 10 rounds of washing with Phosphate Buffered Saline (Gibco). Bound Fab fragments were eluted with lithium dodecyl sulfate buffer (Invitrogen) at 95˚C and sample purity confirmed with SDS-polyacrylamide gel electrophoresis followed by silver stain or coomassie staining before analysis by mass spectrometry.

Isolated Fab fragments were reduced with dithiothreitol, alkylated using iodoacetamide, resolved by 1D gel electrophoresis on a 4-12% NuPAGE Novex Bis-Tris gel (Invitrogen), and stained with Coomassie Blue (Thermo Fisher). The Fab fragments were excised from the gel, and digested using 200ng of trypsin (Promega). The resulting peptides were isolated using reverse phase resin (PORS 20 R2, Applied Biosystem) (*45*) and eluted using an aliquot of 40% acetonitrile in 0.5% acetic acid and a second aliquot of 80% acetonitrile in 0.5% acetic acid. Acetonitrile was removed using a speedvac (Thermo Fisher Scientific) and aliquots of the remaining solution pressure loaded onto self-packed PicoFrit® column (New Objective, Woburn, MA) with integrated emitter tip (360 μm O.D., 50μm I.D., 10 μm tip), packed with 6 cm of reverse-phase C18 material (ReproSil-Pur C18-AQ, 3 μm beads from Dr. Maisch GmbH) and interfaced to a Agilent 1200 series HPLC system (Agilent) with either a LTQ Orbitrap™ XL mass spectrometer or a LTQ Orbitrap Velos™ mass spectrometer (Thermo Fisher Scientific) using a home-built micro electrospray source. The peptides were eluted into the mass spectrometer with the following gradient: 0 to 5% B in 5min, 40% B in 125 min, 60% B in 150 min, 100% B in 165 min (A = 0.1 M acetic acid, B = 70% acetonitrile in 0.1 M acetic acid, flow rate 90 nL/min). Both instruments were operated in the data dependent mode and for both mass spectrometers the target value was set to 5e5 ions and a resolution of 60,000 (at 400 m/z). For analysis on the LTQ Orbitrap™ XL a full scan was followed by 8 MS/MS scans on the 8 most abundant ions from that full scan. The peptides (only charge states >1) were isolated with a 2 Da window, target window of 1e4 ions, dissociated via CAD (normalized collision energy = 35, activation Q = 0.25, activation time 30 msec) and mass analyzed in the LTQ. For analysis on the LTQ Orbitrap™ Velos a full scan was followed by 10 MS/MS scans at 7,500 resolution on the 10 most abundant ions from the immediate preceding full scan. The peptides (only charge state >2) were isolated with a 3 Da window, target window of 2e5 ions, dissociated via HCD (normalized collision energy = 40, activation time 0.100 msec) and mass analyzed in the Orbitrap. For either instrument the ions selected for MS/MS were set on an exclusion list for 30 seconds. The resulting MS/MS spectra were searched against the Human IPI and in-house patient specific IgG database using Xtandem! (*46*). Peptides were automatically compared to tryptic peptides in the human IPI and our in-house patient specific database. Peptide hits corresponding to patient specific IgG were manually confirmed.

Multiple sequence alignments. All multiple sequence alignments were conducted using CLUSTALW2 with default parameters (weight matrix: GONNET for proteins and UIB for DNA, gap open= 10, gap extension 0.1). Alignment shadings were generated using TeXshade package.

Alignment consensus. The consensus sequences for multiple alignments were generated based on identity and similarity between residues (≥70%). The amino acids were grouped based on biochemical similarity as: FYW, ILVM, RK, DE, GA, ST and NQ.

Phylogenetic Germline Trees. The relationship between sequences was generated using the Neighbor-Joining method (*47*). The bootstrap consensus tree inferred from 1,000 replicates (*48*) was taken to represent the relationship. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated sequence clustered together in the bootstrap test (1,000 replicates) are shown next to the branches (*48*). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the number of differences method (*49*) and are in the units of the number of amino acid differences per sequence. All ambiguous positions were removed for each sequence pair. Evolutionary analyses were conducted in MEGA5 (*50*).

R/S Ratio Calculation. DNA sequences were superposed over the protein alignments for replacement/substitution calculation. All gap positions were removed from the analysis. The R/S ratio analysis was conducted using Perl scripts.

Statistical analysis. P value for mutation analysis was calculated using an unpaired two-tailed students t-test.

Figure S1

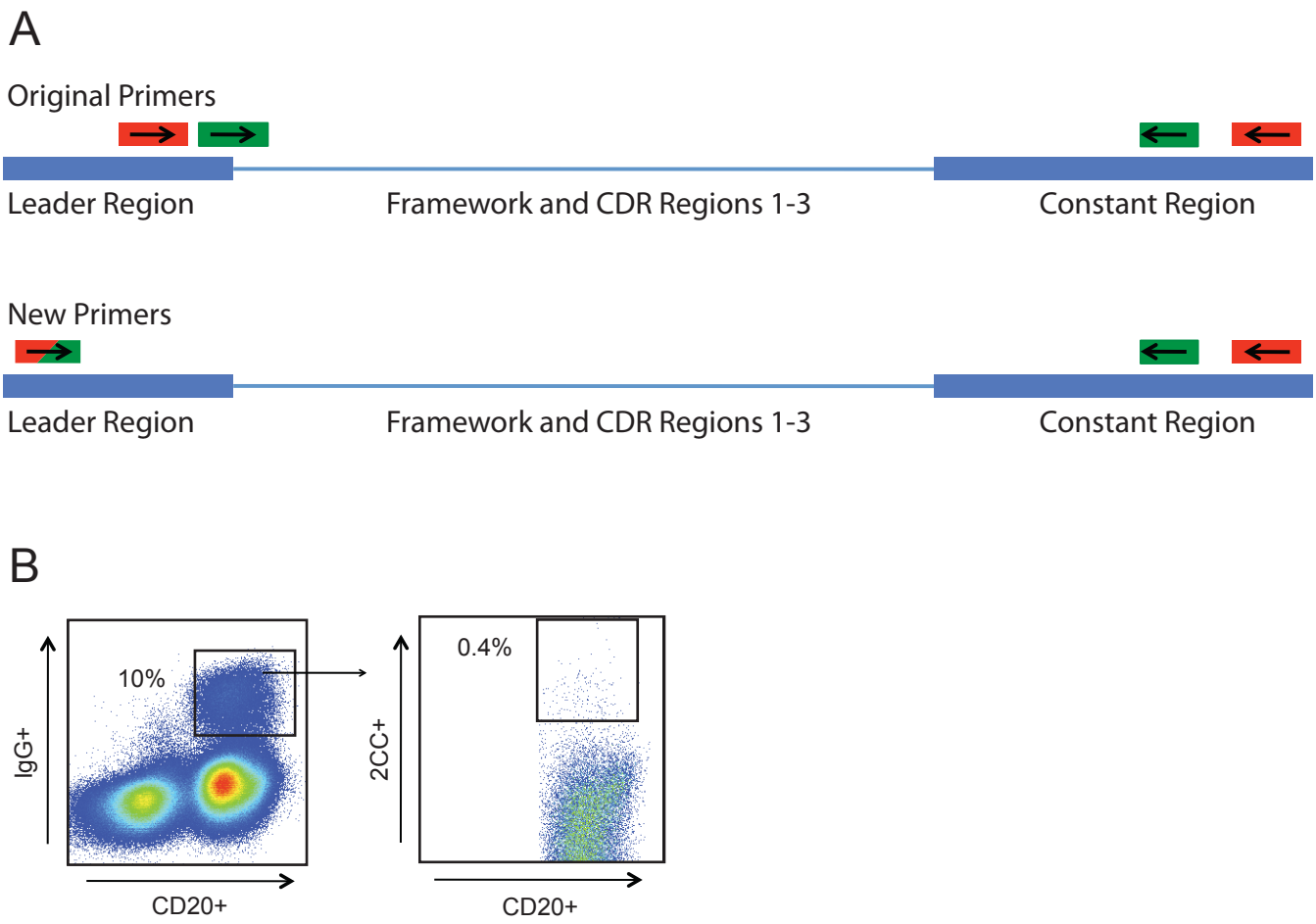


Fig. S1 Highly mutated antibodies captured with new primers. (**A**) Amplification strategy of the original (above(9)) and new (below(table S1)) primer sets. Red boxes indicate the location of the first primer pair on the heavy chain immunoglobulin gene, green boxes the location of the second primer pair in the nested PCR approach. Amplification with the new primer set follows a semi-nested approach with the same forward primers in the first and second PCR. (**B**) Representative flow cytometry plots show sorting strategy on peripheral blood mononuclear cells from Pt 8 stained with anti-CD20, anti-IgG and biotinylated 2CC-core.

Figure S2

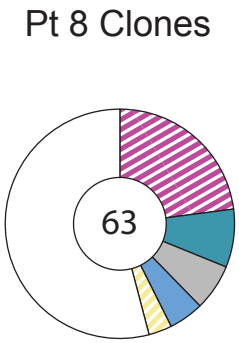
A

ORIGINAL PRIMERS

NEW PRIMERS

8A	HEAVY					
	VH	JH	CDR3 (aa)	NR OF NUCL MUTATIONS	NEW PRIMERS	OLD PRIMERS
8A1	1-46	6	D G L G E V A P D Y R Y G I D V	85	X	
8A2	4-61	4/5	Q S L S W Y R P S G Y F E S	57	X	
8A3	1-69	6	D R G D T R L L D Y G D Y E D E R Y Y Y G M D V	40	X	X
8A4	1-69	6	S I N A A V P G L E G V Y Y Y Y Y G M A V	27	X	X
8A5	1-69	6	D R G D T R L L D Y G D Y E D E R Y Y Y G M D V	37	X	
8A6	1-69	6	D R G D T R L L D Y G D Y E D E R Y Y Y G M D V	35	X	X
8A7	1-69	1/2	W D Y Y D S R G Y Y Y G E Y F D L	23	X	
8A8	3-21	6	D T K V G A P R Q D C Y A M D L	29	X	X
8A9	1-46	6	D G L G E V A P D Y R Y G I D V	75	X	
8A10	1-24	4	A D R F K V A Q D E G L F V I F D Y	11	X	X
8A11	1-69	3	D R S S A I G Y C S S I S C Y K G S F D I	12	X	X
8A12	3-48	6	L A E V P P A I R G S Y Y Y G M D V	18	X	X
8A13	3-11	6	A Y G T G N W R G L Y Y Y Y Y G M D V	23	X	X
8A14	3-30	4	S P S Y Y F D Y	9	X	X
8A15	1-46	6	D G L G E V A P A Y L Y G I D A	85	X	
8A16	1-24	6	G G L Y C S S I S C I M D V	12	X	X
8A17	1-24	6	G G L Y C S S I S C I M D V	6	X	X
8A18	1-46	6	D G L G E V A P A Y L Y G I D A	76	X	
8A19	1-24	4	A D R F K V A Q D E G L F V I F D Y	9	X	X
8A20	1-46		D G L G E V A P A Y L Y G I D A	81	X	
8A21	3-30	4/5	E G G L R F L E W L F	13	X	X
8A22	3-21	6	S R P P Q R L Y G M D V	19	X	X
8A23	1-24	4	A D P F K V A Q D E G L Y V I F D Y	10	X	
8A24	3-30	4	D S S G S N W F D Y	22	X	
8A25	1-46	6	D G L G E V A P A Y L Y G I D A	82	X	
8A26	3-43	5	N G F D V	70	X	
8A27	1-24	4	A D P F K V A Q D E G L Y V I F D Y	12	X	
8A28	1-46	6	D G L G E V A P A Y L Y G I D A	85	X	
8A29	1-46	6	D G L G E L A P A Y H Y G I D V	71	X	
8A30	1-69	3	A R A D S H T P I D A F D I	23	X	X
8A31	1-46	6	D G L G E L A P A Y H Y G I D V	71	X	
8A32	1-69	3	S S G N F E F A F E I	30	X	X
8A33	1-69	6	D R W L P Q Y Y Y Y G M D V	3	X	X
8A34	3-7	2	N P E S R C I V G R N R G W C R Y F D	11	X	X
8A35	1-46	6	D G L G E L A P A Y Q Y G I D V	71	X	
8A36	3-30	4	P K F L P G A D I V V V V A A T P F D	2	X	X
8A37	1-46	6	D G L G E L A P A Y H Y G I D V	71	X	
8A38	1-46	6	D G L G E V A P A Y L Y G I D A	83	X	
8A39	3-43	5	N G F D V	70	X	
8A40	1-24	4	A D P F K V A Q D E G L Y V I F D Y	18	X	
8A41	3-33	4/5	E M A V G G T K A L D H	10	X	X
8A42	1-46	4/5	G V S F	41	X	X
8A43	3-11	4/5	D L L H A H D F	13	X	
8A44	3-33	4	D S V A F V L E G P I D Y	23	X	X
8A45	1-2	6	Y S T R Q F F H Y Y Y V T D V	26	X	X
8A46	4-34	6	G K V W G I T A R P R D A G L D	38	X	X
8A47	3-7	4	V R D P N Y N L H F D S	11	X	X
8A48	3-53	4/5	G L R V Y F D L	17	X	X
8A49	1-69	3	D R S S A I G Y C S S I S C Y K G S F D I	8	X	X
8A50	4-39	4/5	Q K G S G T S L L Y	8	X	X
8A51	7-4-1	4/5	D L L E S R T Y Y N D I R D C	7	X	X
8A52	1-69	6	D R G D T R L L D Y G D Y E D E R Y Y Y G M D V	50	X	X
8A53	4-4	4	V R G S W N F D Y	15	X	X
8A54	1-24	5	T Y L A V V P D G F D G Y S S S W Y W F D P	19	X	X
8A55	1-69	3	D R S S A I G Y C S S I S C Y K G S F D I	8	X	X
8A56	4-31	4/5	C Q D G L A S R P I D F	44	X	X
8A57	3-30	4/5	D S V S K S Y S A P P E F	39	X	
8A58	1-46	6	D G L G E V A P D Y R Y G I D V	73	X	
8A59	4-39	5	H V R P Y D R S G Y P E R P N W F D	32	X	X
8A60	1-69	3	N A G A Y F Y P F D I	35	X	
8A61	1-46	6	E M G T F T L L G V V I D H Y D F Y P M D V	24	X	X
8A62	4-34	4	G R G K R C S G A Y C F A G Y F D S	37	X	
8A63	1-46	6	D G L G E V A P A Y L Y G I D A	83	X	

B



C

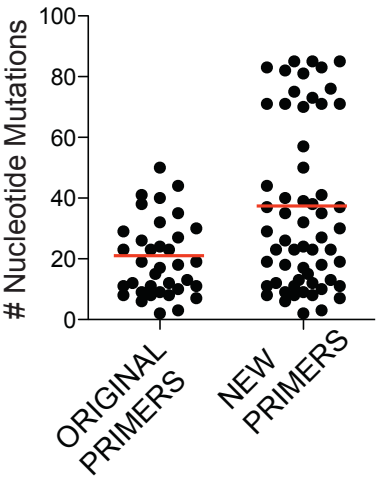


Fig. S2 Recovery of highly mutated immunoglobulin heavy chains with specific primers. (**A**) Side by side comparison of new and original(9) primer set on 2CC-core sorted single cells from Pt 8. Each row represents one amplified heavy chain sequence and rows with the same color identify clonally related B cells. Red boxes and (x) in the last two columns indicate successful amplification of IgVH genes either with the new primers (table S1), original primers(9), or both. (**B**) Sequences from (**A**) summarized as pie chart. Clonal families are shown by differently expanded pie slices. The two highly mutated clones that were not amplified with the original primer set are shown in striped pie slices. (**C**) IgVH mutations from the same experiment captured by original and new primer sets. Each dot represents one IgVH sequence.

Figure S3

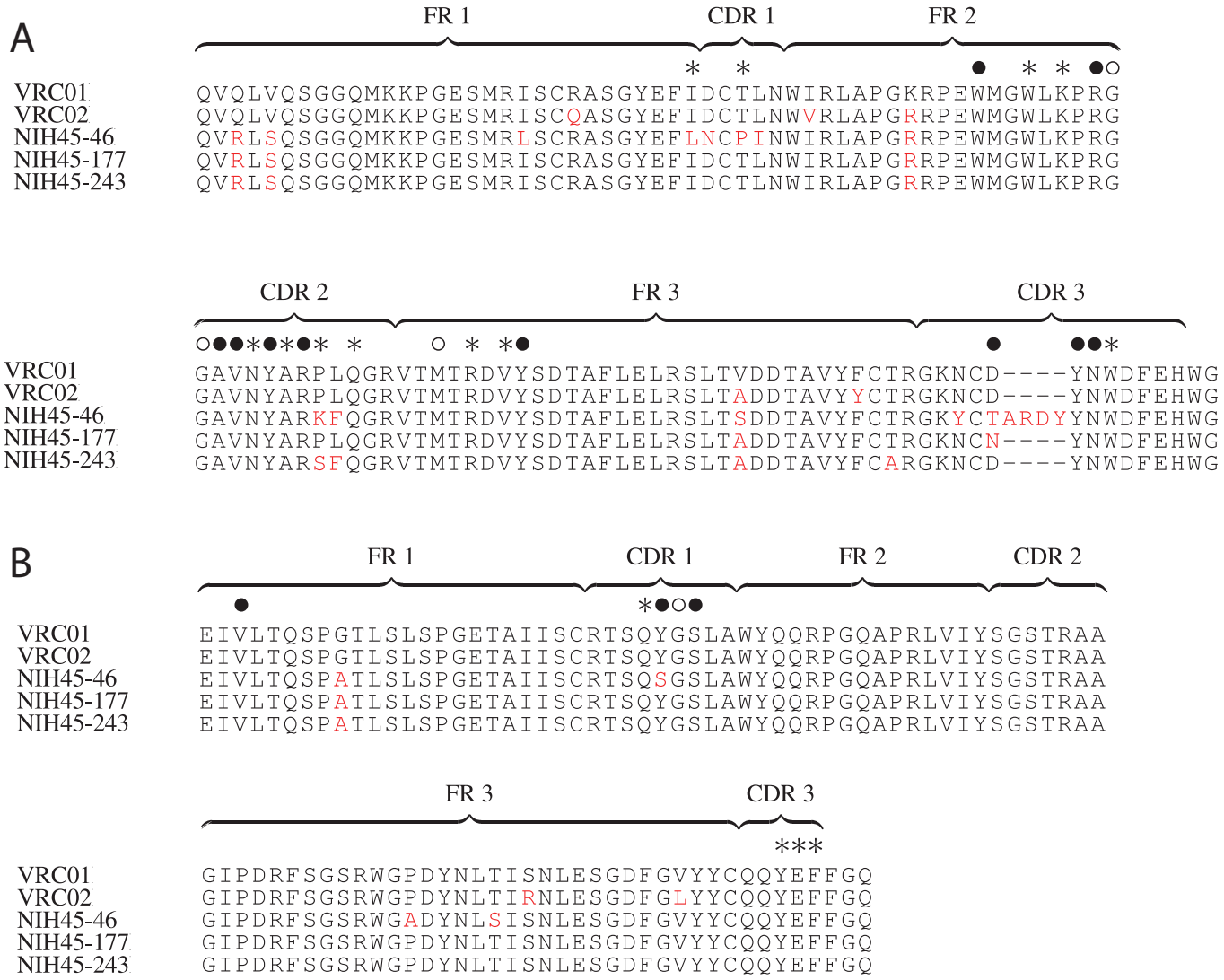


Fig. S3 Ig V heavy (**A**) and light chain (**B**) sequences of new VRC01 clonal members. Framework (FR) and CDR regions are indicated. Red shading shows amino acid differences from VRC01. Contact residues between VRC01 and gp120 are shown above as closed circles for main and side chain interactions, open circles main chain only, and stars side chains only(5).

Figure S4

A

	IC_{50}	Pt 1	Pt 3	Pt 8	NIH45	Pt 12
Clade B	6535.3	88	400.4	23.2	61	101.3
	RHPA4259.7	113	16.6	154.1	30	30.1
	SC422661.8	49	25.9	16.6	107	62.7
	PVO.4	89	78.1	74.1	195	116.3
	TRO.11	72	24.5	62.2	208	53.6
	YU2.DG	131	25.4	32.7	92	50.6
	H086.8	>132	>132	>132	37	
Clade C	Du172.17	228.42	418.62	86.463	349	
	ZM53M.PB12	60.70	383.37	>227	317	
	ZM109F.PB4	86.82	12.97	>227	73	
Clade A	Q842.d12	12.196	6.168	4.056	50	
	3415.v1.c1	48.26	38.88	16.63	54	
	3365.v2.c20	111.54	28.46	>227	94	
CRF02_AG	250-4	>132	560.58	55.09	90	
	251-18	>340	104.58	92.28	841	
	278-50	>132	>132	>132	>1000	
CRF01_AE	620345.c1	>132	>132	>132	>1000	
Clade D	3016.v5.c45	>340	185.62	>227	ND	
	231965.c1	304.48	86.54	171.56	ND	
Clade G	X1254_c3	222.01	81.48	>227	ND	
CRF01_AE	R1166.c1	>340	52.01	>227	ND	

B

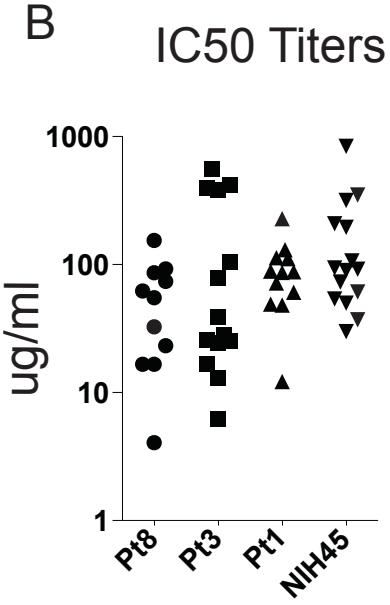


Fig. S4 Serum neutralizing activity from indicated patients. (**A**) Table summarizes purified serum IgG neutralizing activity against a panel of tier 2 viruses in a Tzm-bl assay(39). Dark red boxes indicate IC50 values below 10µg/ml, orange 10-100µg/ml and yellow above 100 µg/ml. (**B**) dot plot summarizes the IC50 values shown in (**A**) for 4 of the tested patients.

Figure S5

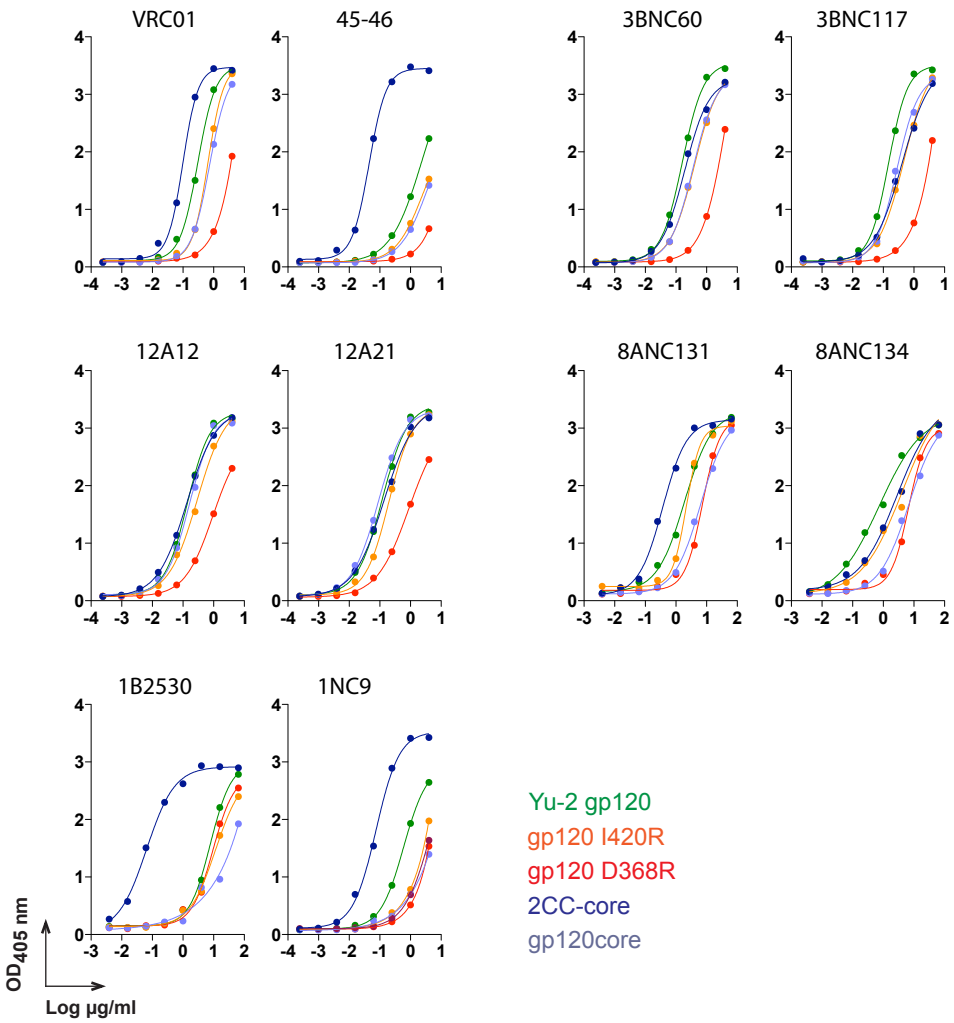


Fig. S5 Epitope mapping of 2CC-core specific antibodies. Graphs show antibody binding in enzyme-linked immunosorbent assays (ELISA) to YU2-gp120, gp120core, gp120(D368R) (14-16), gp120(I420R) (17) and 2CC-core(11).

Figure S6

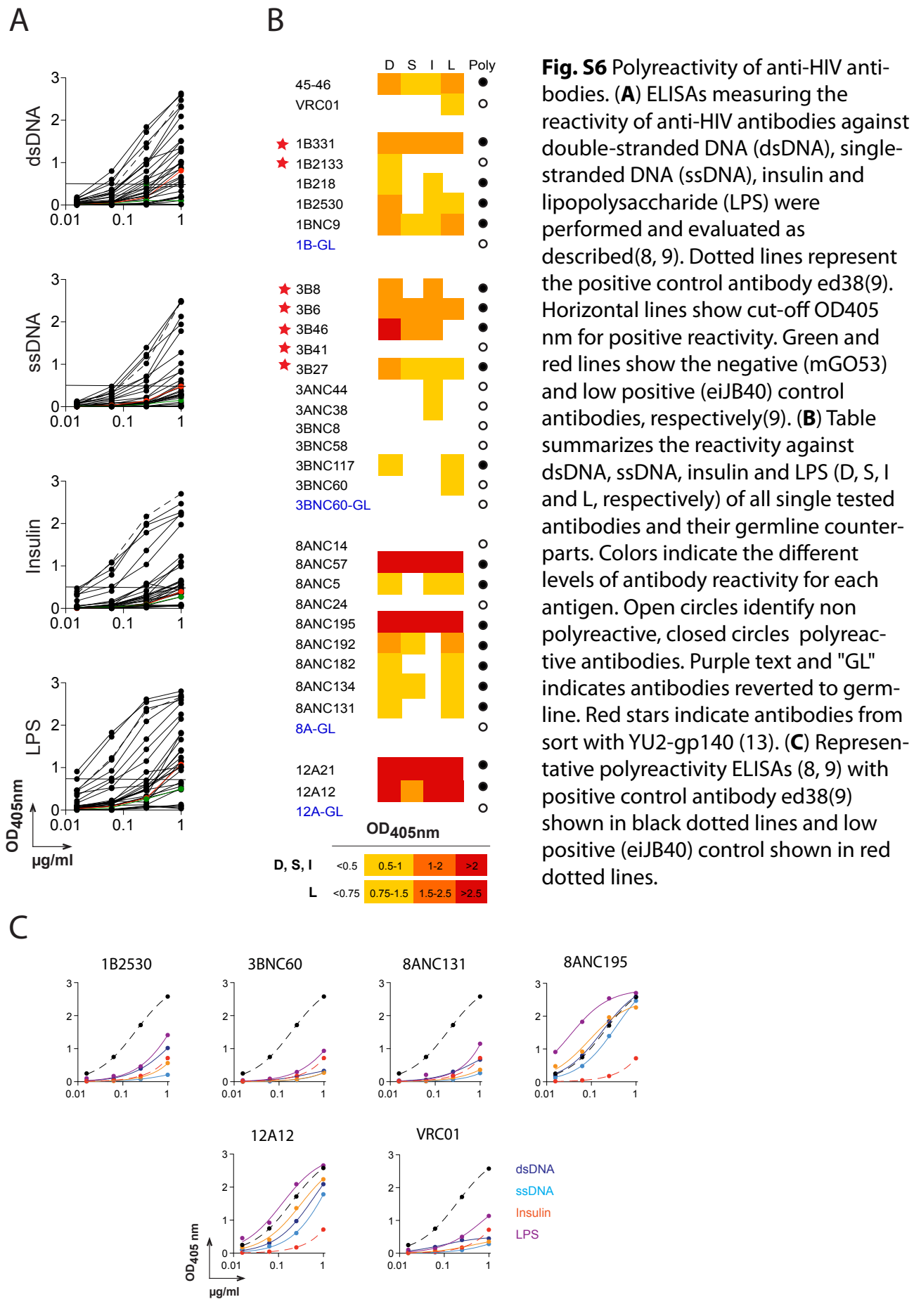
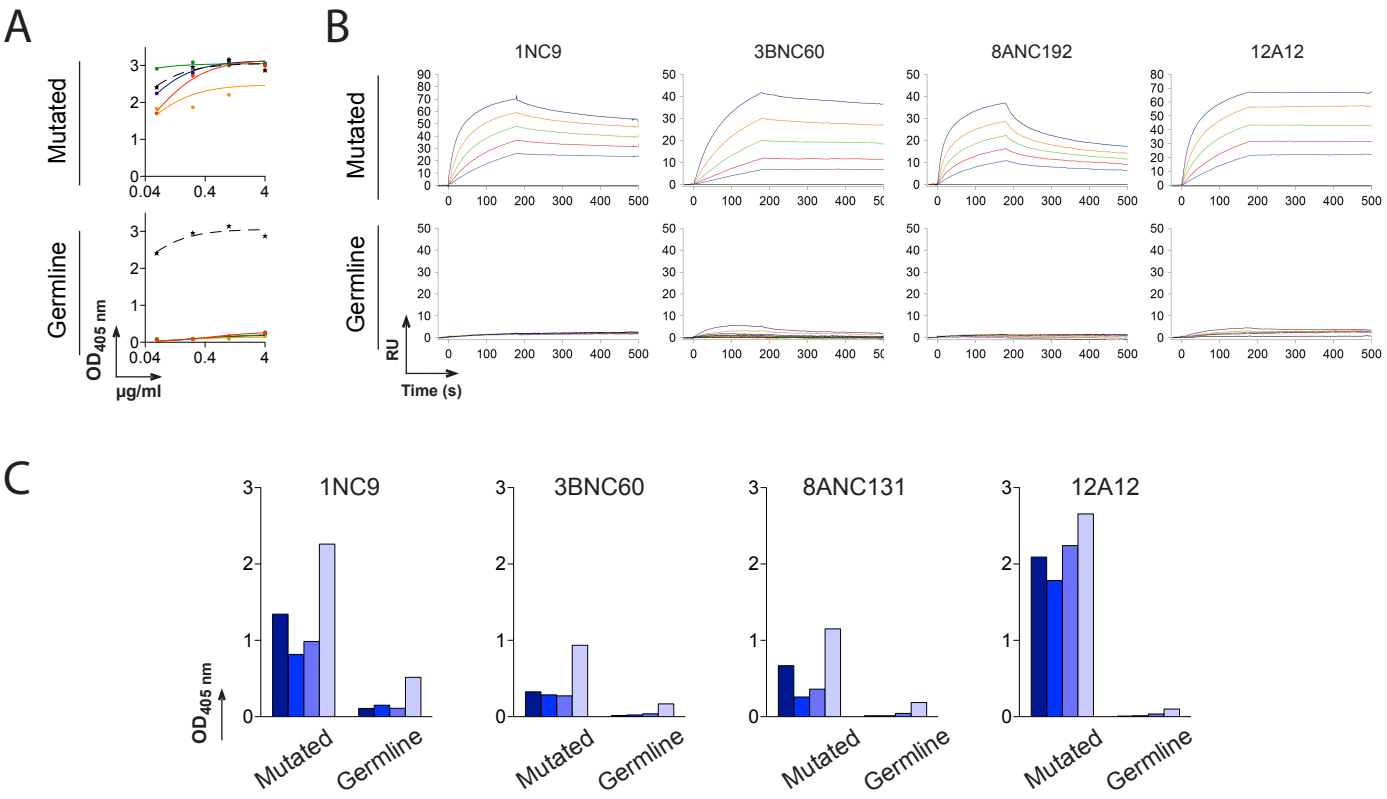


Fig. S6 Polyreactivity of anti-HIV antibodies. (**A**) ELISAs measuring the reactivity of anti-HIV antibodies against double-stranded DNA (dsDNA), single-stranded DNA (ssDNA), insulin and lipopolysaccharide (LPS) were performed and evaluated as described(8, 9). Dotted lines represent the positive control antibody ed38(9). Horizontal lines show cut-off OD405 nm for positive reactivity. Green and red lines show the negative (mGO53) and low positive (eiJB40) control antibodies, respectively(9). (**B**) Table summarizes the reactivity against dsDNA, ssDNA, insulin and LPS (D, S, I and L, respectively) of all single tested antibodies and their germline counterparts. Colors indicate the different levels of antibody reactivity for each antigen. Open circles identify non polyreactive, closed circles polyreactive antibodies. Purple text and "GL" indicates antibodies reverted to germline. Red stars indicate antibodies from sort with YU2-gp140 (13). (**C**) Representative polyreactivity ELISAs (8, 9) with positive control antibody ed38(9) shown in black dotted lines and low positive (eiJB40) control shown in red dotted lines.

Figure S7



D

IC50	1NC9 (RU08)	3BNC60 (RU01)	8ANC131 (RU10)	12A12 (RU16)	RU08 Germline	RU01 Germline	RU10 Germline	RU16 Germline
MW965.26	>50	<0.04	>50	0.042	>50	>50	>50	>50
BAL.26	1.37	<0.04	0.06	0.017	>50	>50	>50	>50
DJ263.8	>50	<0.04	0.004	0.002	>50	>50	>50	>50
6535.3	>50	0.54	0.20	21.97	>50	>50	>50	>50
RHPA4259	0.09	<0.05	>50	0.086	>50	>50	>50	>50
TRO.11	0.20	<0.05	>50	0.288	>50	>50	>50	>50
PVO.4	0.34	0.09	0.81	0.928	>50	>50	>50	>50
YU2	0.13	<0.05	0.18	0.084	>50	>50	>50	>50

Fig. S7 Binding and neutralization of mutated and germline antibodies. (**A**) Binding of selected antibodies (table S5) and their germline versions against YU2-gp140 as measured by ELISA. Dotted black line shows b12 (22), solid blue line 3BNC60, green 8ANC192, orange 12A12 and red 1NC9 (table S3 and S5). (**B**) Antibody binding to YU2-gp140 measured by surface plasmon resonance (SPR). The SPR sensorgrams for antibody binding of the selected antibodies and their germline counterparts are shown. The antibodies were tested at concentrations ranging from 44.8 nM (light blue curve) to 700 nM (dark blue curve). RU, response units. (**C**) Bar graphs show the binding of the selected antibodies (table S5) and their germline versions to ssDNA, dsDNA, Insulin and LPS (from dark to light blue) (OD405nm) at an antibody concentration of 1µg/ml. (**D**) IC50 neutralization titers of the selected antibodies and germline versions against a basic virus panel (table S4). Colors indicate concentration at IC50: red ≤ 0.1µg/ml; orange 0.1-1 µg/ml; yellow 1-10µg/ml; green ≥10µg/ml; white not neutralized at any concentration tested.

Figure S8

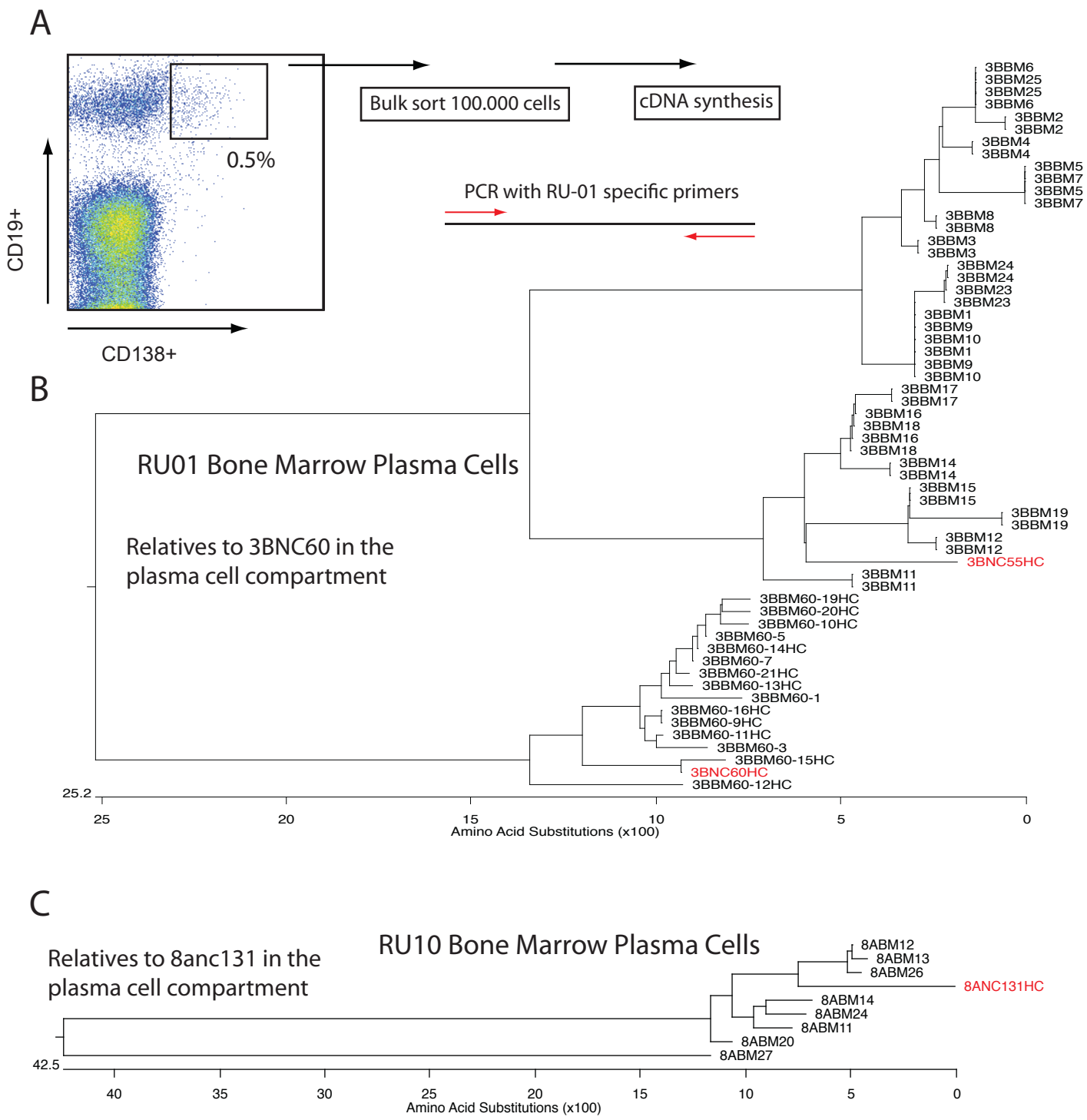


Fig. S8 Clone specific amplification of IgG genes from CD138+ bone marrow plasma cells. (**A**) FACS plot showing sorting strategy for bulk sort of CD19+, CD138+ bone marrow plasma cells. (**B** and **C**) Phylogenetic trees based on predicted amino acid sequences of members of the (**B**) RU01 clone (Pt 3) and (**C**) RU10 clone (Pt 8). Antibodies that were also isolated from the peripheral memory B cell compartment are shown in red. Clone specific primers were: for RU01 CTGCAACCGGTGTACATTCTCAAGTGCAACTGGTGC (FWRD), CTGCAACCGGTGTACATTCTCAGGTCCATTTGTCACAG (FWRD), TGCGAAGTCGACGCTGACGAGACAGTGACCTGC (REV), TGCGAAGTCGACGCTGAAGAGACAATAATTTG (REV), TGCGAAGTCGACGCTGACGAGACAATAACT (REV) and for RU10: CTGCAACCGGTGTACATTTTCAGGGGCACTTGGTG (FWRD), TGCGAAGTCGACGCTGAGGTGACGATGACCGTG (REV).

Figure S9

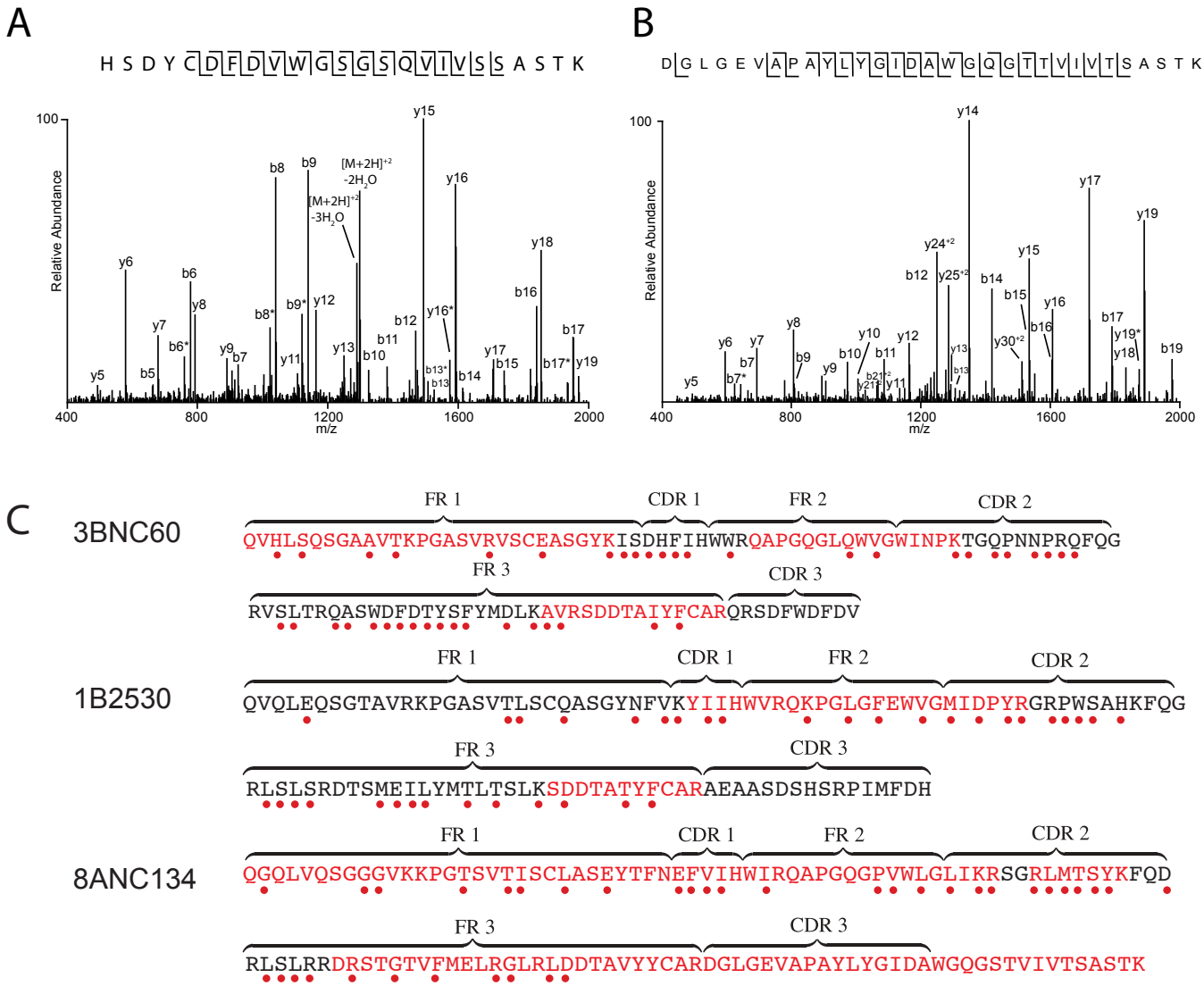


Fig. S9 Detection of antibodies by mass spectrometry. (**A** and **B**) Two sample spectra of collision activated dissociation MS/MS recorded on the doubly charged peptides HSDYCDFDVWGSGSQVIVSSASTK from 3BNC153HC (**A**) (table S3) and DGLGEVAPAYLYGIDAWGQGTTVIVTSASTK from 8ANC134HC (**B**) (table S3). Observed b-type fragment ions (containing the N-terminus) and y-type fragment ions (containing the C-terminus) are labeled in the spectrum. Loss of water from fragment ions is indicated by *. Ions corresponding to the loss of water from the parent ion are labeled in the spectrum. Observed backbone cleavages are indicated in the sequence with ⌝ for b-type ions and ⌞ for y type ions. The complete data set for the peptides detected is shown in table S8.
(**C**) Amino acid sequences of 3BNC60, 1B2530 and 8ANC134 (Fig. 2B, 4A, tables S4, 5 and 6) heavy chains with peptides found by Mass Spec in red. Red dots indicate differences from respective germline sequences.

Figure S10

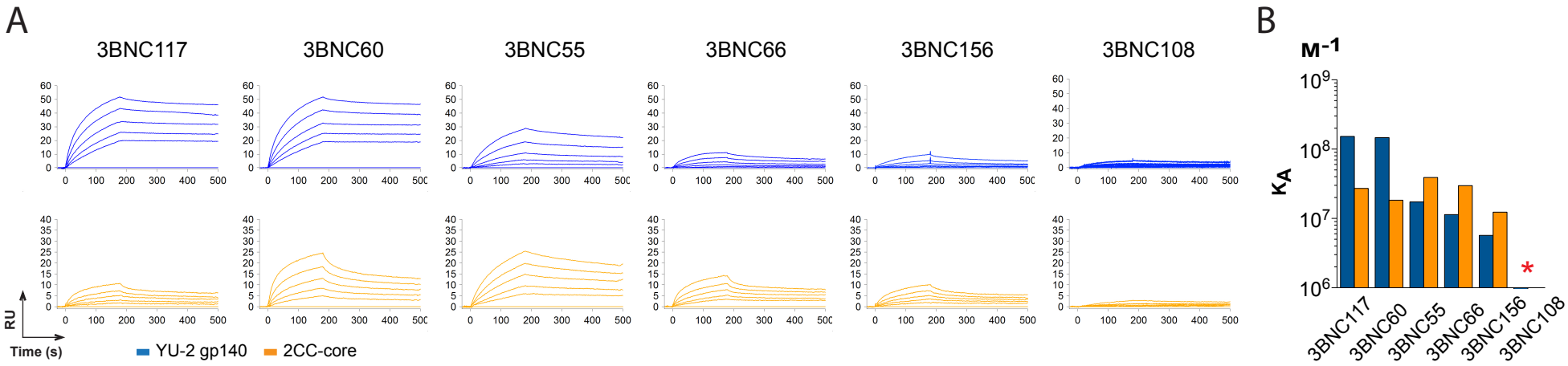


Fig. S10 Affinity of RU01 clone members (Pt 3). (**A**) Antibody binding to YU2-gp140(13) and 2CC-core(11) measured by surface plasmon resonance (SPR). The SPR sensograms for antibody binding of the selected 3BNC-antibodies (table S3) are shown over time. (**B**) Bar graphs show the binding affinity (KA) for gp140 and 2CC-core antigens for the selected IgG antibodies shown in (**A**). RU, response units.

Figure S11, A (BAL)

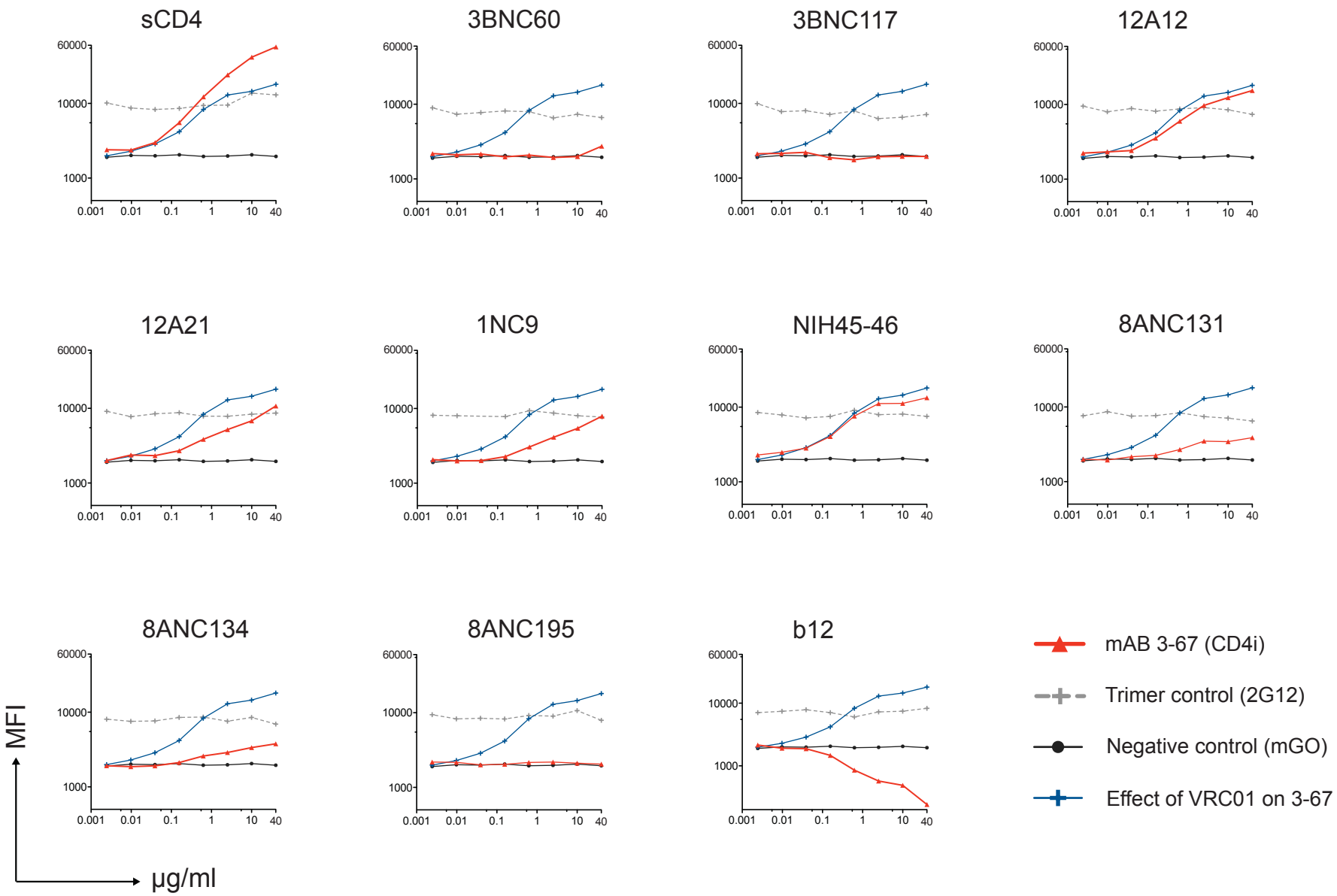


Figure S11, B (YU2)

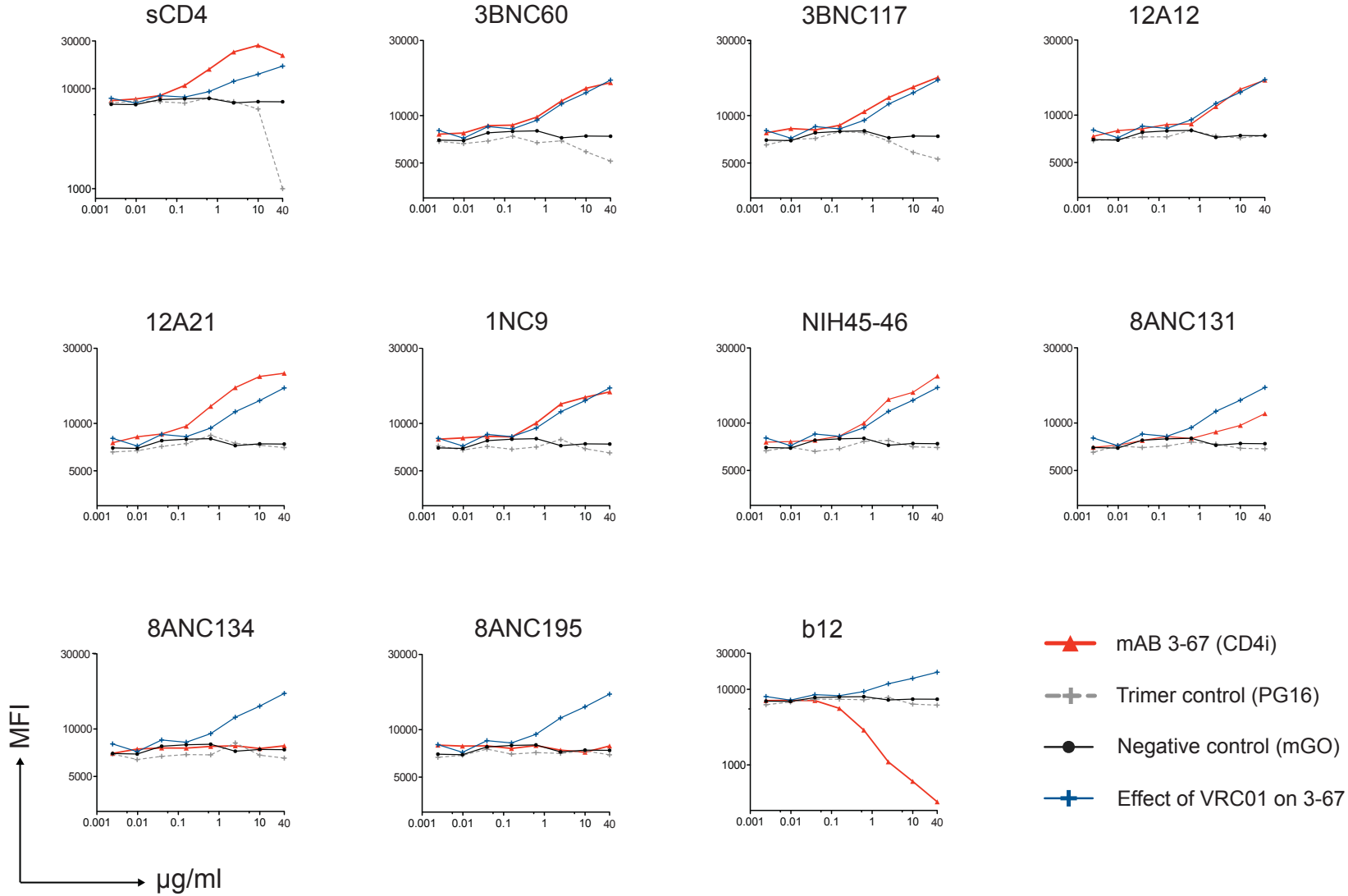
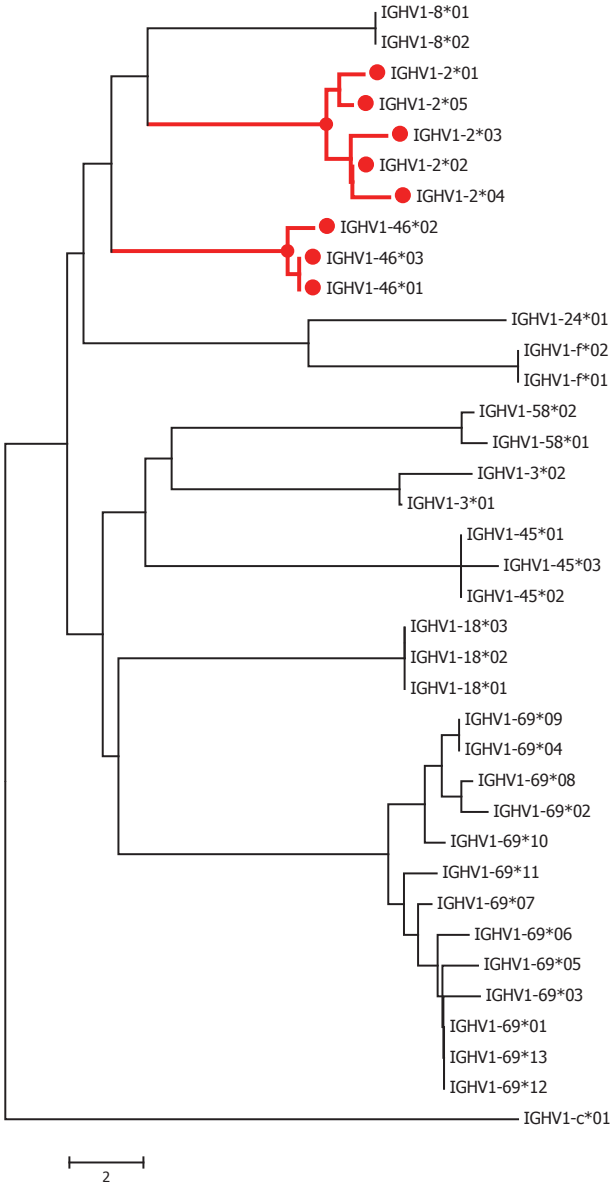


Fig. S11 Effect of sCD4 or monoclonal antibodies on binding of a CD4i antibody (3-67(7)) to 293T cells expressing two different HIV spikes. To characterize the newly identified antibodies we measured changes in binding of the CD4i antibody (3-67(7)) in the presence of soluble CD4 or the selected antibodies (table S5). sCD4 or antibodies of interest were added to (**A**) BAL gp160Δc or (**B**) YU2 gp160Δc expressing 293T cells at different concentrations (1:4 dilutions, starting with 40µg/ml), followed by incubation with an Alexa647-labeled CD4-induced-site antibody (3-67, red line). In addition, surface expression of gp160Δc was tested with Alexa-647-labeled 2G12(30) or PG16(29)(grey lines). As a reference the binding curves of 3-67(7) in the presence of the CD4bs antibody VRC01(6)(blue line) and the non-HIV reactive antibody mGO (black line(9)) is illustrated in all graphs.

Figure S12

A



B

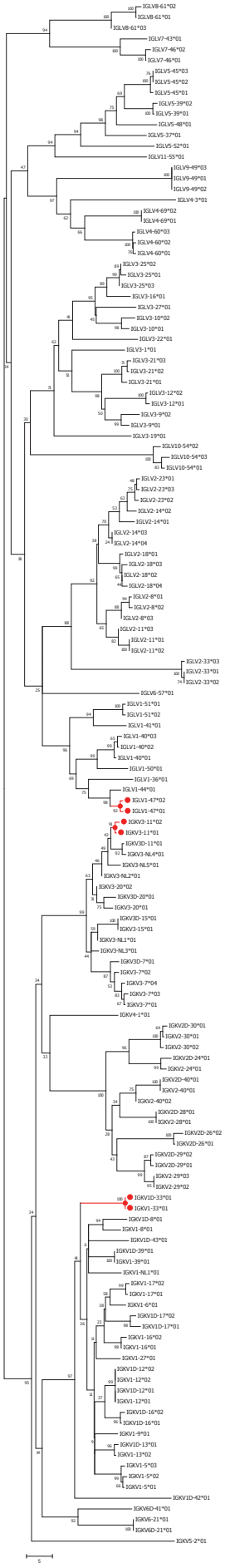


Fig. S12 Phylogenetic tree of (**A**) IgVH and (**B**) Ig light chain germline sequences. Red shows position of germline genes used by highly active agonistic anti-CD4bs antibodies.

Figure S13

A


FR 1
CDR 1
FR 2
CDR 2
FR 3
IgVH1-2
3BNC60
3BNC117
NIH45-46
VRC01
12A12
12A21
8ANC131
8ANC134
1B2530
1NC9

Figure S13

B

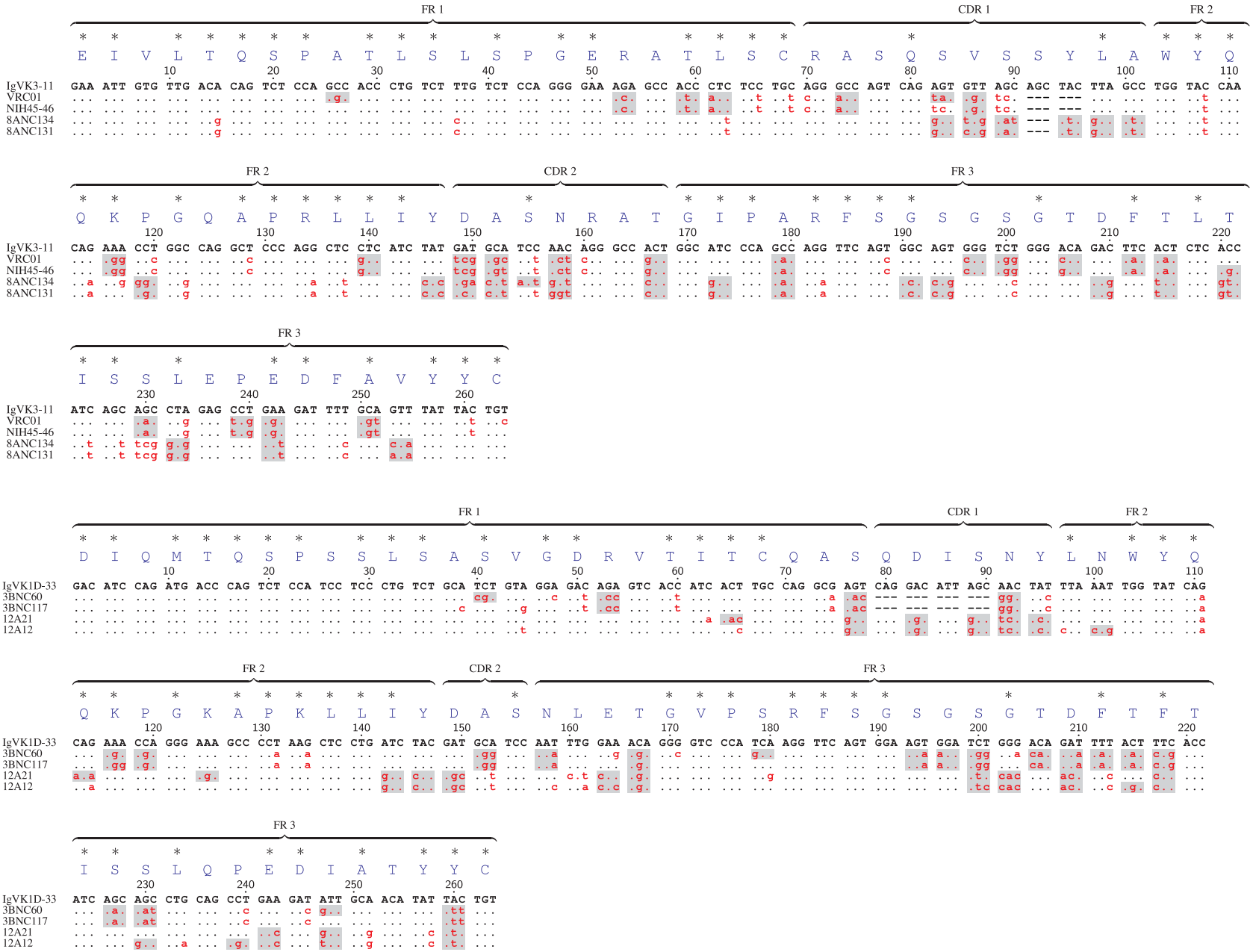

FR 1
CDR 1
FR 2
CDR 2
FR 3
IgVK3-11
VRC01
NIH45-46
8ANC134
8ANC131
IgVK1D-33
3BNC60
3BNC117
12A21
12A12

Figure S13

C

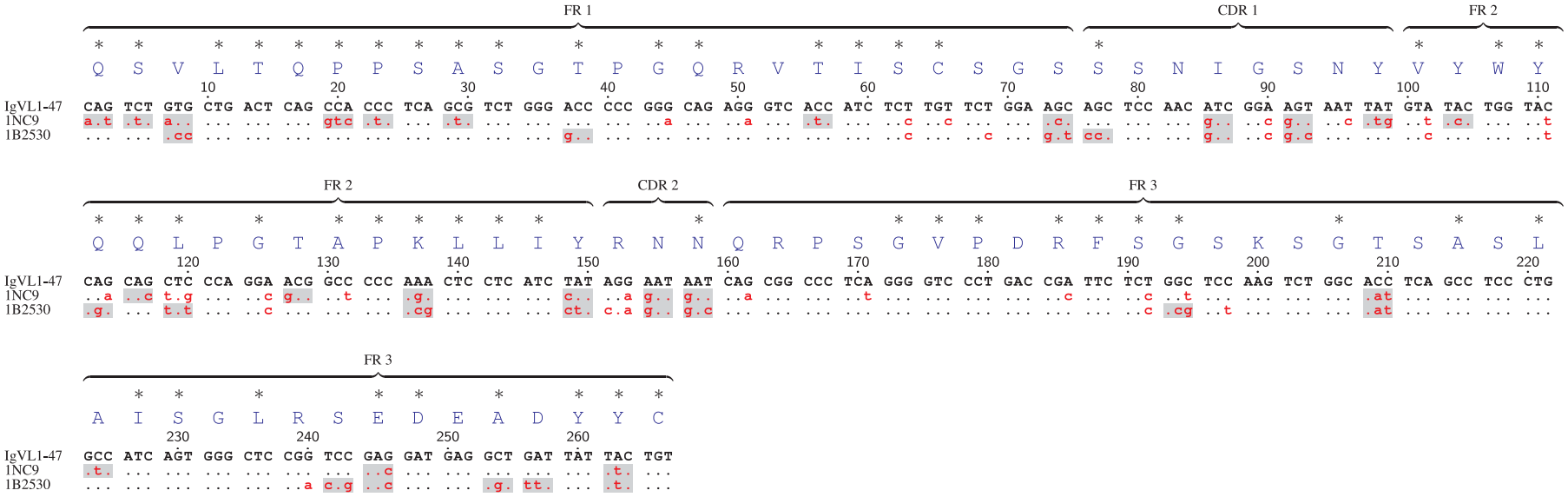


Fig. S13 Somatic hypermutation analysis of selected antibodies for (**A**) immunoglobulin heavy chain gene, (**B**) light chain kappa and (**C**) light chain lambda gene sequences. Sequences are aligned with their respective germline nucleotide sequences. Somatic mutations are shown in red letters, additionally gray boxes designate replacement mutations. Germline amino acid sequences with (∗) indicating consensus residues are shown above the nucleotide alignment.

Figure S14

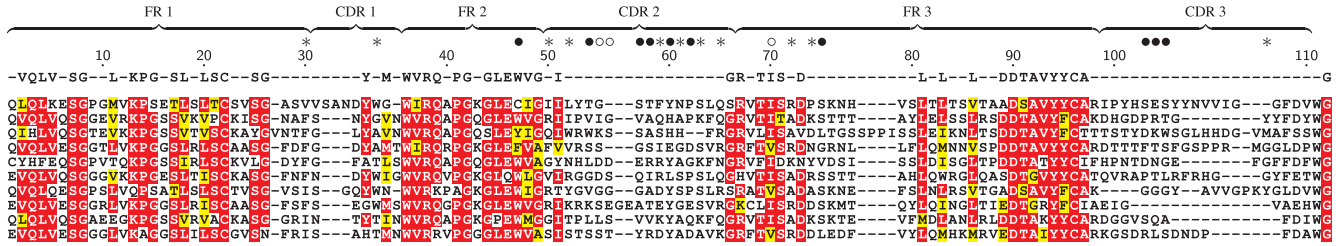


Fig. S14 Negative control alignment of HIV specific antibodies. Ig heavy chain sequences of highly mutated non-HAAD, HIV-specific antibodies (2g12(*30*), antibodies from earlier studies(7, 8), this study (tableS3) and unpublished HIV antibodies 9ANC112 and 10A205) were aligned as in Fig. 4A for HAAD antibodies.
Residues are numbered according to the 3BNC60 structure. Framework (FR) and CDR regions are indicated. Red shading shows amino acid identity, yellow shows biochemical similarity. 70% similarity between the 10 selected antibodies defined the consensus. The consensus sequence is shown above, dashes in this sequence indicate non-conserved residues. Contact residues between VRC01 and gp120 are shown above the consensus as closed circles for main and side chain interactions, open circles main chain only, and stars side chains only(5).

Table S1

Forward Leader Sequence Primers

VH1 LEADER-A	ATGGACTGGACCTGGAGGAT
VH1 LEADER-B	ATGGACTGGACCTGGAGCAT
VH1 LEADER-C	ATGGACTGGACCTGGAGAAT
VH1 LEADER-D	GGTTCCTCTTTGTGGTGGC
VH1 LEADER-E	ATGGACTGGACCTGGAGGGT
VH1-LEADER-F	ATGGACTGGATTTGGAGGAT
VH1-LEADER-G	AGGTTCCTCTTTGTGGTGGCAG
VH3 LEADER-A	TAAAAGGTGTCCAGTGT
VH3 LEADER-B	TAAGAGGTGTCCAGTGT
VH3 LEADER-C	TAGAAGGTGTCCAGTGT
VH3 LEADER-D	GCTATTTTTAAAGGTGTCCAGTGT
VH3 LEADER-E	TACAAGGTGTCCAGTGT
VH3 LEADER-F	TTAAAGCTGTCCAGTGT
VH4 LEADER-A	ATGAAACACCTGTGGTTCTTCC
VH4 LEADER-B	ATGAAACACCTGTGGTTCTT
VH4 LEADER-C	ATGAAGCACCTGTGGTTCTT
VH4 LEADER-D	ATGAAACATCTGTGGTTCTT
VH5 LEADER-A	TTCTCCAAGGAGTCTGT
VH5 LEADER-B	CCTCCACAGTGAGAGTCTG
VH6 LEADER-A	ATGTCTGTCTCCTTCCTCATC
VH7 LEADER-A	GGCAGCAGCAACAGGTGCCCA

Reverse Constant Region Primers

First PCR	Second PCR
3´ Cg CH1 (gamma)	3´ IgG (internal)
GGAAGGTGTGCACGCCGCTGGTC	GTTCGGGGAAGTAGTCCTTGAC

Primers for amplification of highly mutated Immunoglobulin V heavy chain genes;
Forward priming sites are located in the leader region of the respective VH genes and allow amplification of highly mutated VH genes in a semi-nested protocol with 2 reverse priming sites located in the IgG constant region.

Table S2

Clinical Information

	gender	ethnicity	HIV clade	year of birth	year of diagnosis	CD4+ T cells/ul	Virus copies/ml	clinical status
pt1	male	caucasian	B	1948	1985	354	4722	non progressor
pt3	male	hispanic	B	1965	2002	427	880	non progressor
pt8	male	caucasian	B	1962	1989	580	<50	elite controller
pt12	male	african	ND	ND	ND	ND	ND	ND

Pt1 and Pt3 correspond to Pt1 and Pt3 in (7), Pt12 corresponds to #57 in (1).

Table S3A. Repertoire and Reactivity of 2CC binding Antibodies, patient 3

Clone R U01

Ab name	VH	D	JH	(-)	CDR3 (aa)	(+)	Length	Mutations HC	Primer set	k/l	Vk/l	Jk/l	(-)	CDR3 (aa)	(+)	Length	Mutations LC	Binding	NEUT	# of Relatives	
3BNC4	1-2	7-27	2/6	3	R H S D Y C D F D V	2	10	72	new	k	1D-33	3	1	Q V Y E F	0	5	38		+	7	a
3BNC23	1-2	6-25/3-3	2/6	3	Q R S D F W D F D V	1	10	79	new	k	1D-33	3	1	Q V Y E F	0	5	50	CD4BS	+	5	b
3BNC42	1-2	7-27	2/6	3	R H S D Y C D F D V	2	10	69	new	k	1D-33	3	1	Q V Y E F	0	5	42		-	1	
3BNC53	1-2	3-3	2/6	3	R H S D Y C D F D V	2	10	74	new	k	1D-33	3	1	Q V Y E V	0	5	42		+	1	
3BNC55	1-2	3-3/6-19/5-12	2/6	3	R H S D Y C D F D I	2	10	64	new	k	1D-33	1/3	1	Q V Y E F	0	5	32		+	1	
3BNC62	1-2	6-25/6-13/6-6	2/6	3	Q R S D Y W D F D V	1	10	81	new	k	1D-33	3	1	Q V Y E F	0	5	43		+	3	c
3BNC65	1-2	6-25/6-6	2/6	3	Q R S D Y W D F D V	1	10	82	new	k	1D-33	3	1	Q V Y E F	0	5	44		ND	1	
3BNC66	1-2	7-27	2/6	3	R H T D Y C D F D V	2	10	69	new	k	1D-33	3	1	Q V Y E F	0	5	38		+	1	
3BNC72	1-2	7-27	2/6	3	R H S D Y C D F D V	2	10	69	new	k	1D-33	3	1	Q V Y E F	0	5	38		+	1	
3BNC79	1-2	6-25/6-6	2/6	3	Q R S D Y W D F D V	1	10	76	new	k	1D-33	3	1	Q V Y E F	0	5	44		ND	2	d
3BNC81	1-2	7-27	2/6	3	R H S D Y C D F D V	2	10	71	new	k	1D-33	3	1	Q V Y E F	0	5	38		ND	2	e
3BNC89	1-2	3-3/6-19/5-12	2/6	3	R H S D Y C D F D I	2	10	68	new	k	1D-33	3	1	Q V Y E F	0	5	35		+	1	
3BNC91	1-2	2-21/6-25	2/6	3	R R S D Y C D F D V	2	10	76	new	k	1D-33	3	1	Q V Y E F	0	5	42		+	1	
3BNC95	1-2	6-25/2-8	2/6	3	Q R S D Y W D F D V	1	10	74	new	k	1D-33	3	1	Q V Y E F	0	5	41		+	1	
3BNC105	1-2	6-6/6-25	2/6	3	Q R S D Y W D F D V	1	10	77	new	k	1D-33	3	1	Q V Y E F	0	5	43		ND	1	
3BNC107	1-2	7-27/3-3	2/6	3	R H S D Y C D F D V	2	10	69	new					ND					ND	1	
3BNC108	1-2	3-3/6-19/6-25	2/6	3	R H S D Y C D F D I	2	10	62	new	k	1D-33	3	1	Q V Y E F	0	5	38		+	2	f
3BNC117	1-2	6-25/2-8	2/6	3	Q R S D Y W D F D V	1	10	72	new	k	1D-33	3	1	Q V Y E F	0	5	41	CD4BS	+	9	g
3BNC134	1-2	7-27	2/6	3	R H S D Y C D F D V	2	10	72	new	k	1D-33	3	1	Q V Y E F	0	5	38		ND	1	
3BNC142	1-2	3-3	2/6	3	R H S D Y C D F D V	2	10	71	new	k	1D-33	3	1	Q V Y E V	0	5	42		+	1	
3BNC151	1-2	7-27/4-17/3-3	2/6	3	R H S D Y C D L D V	2	10	69	new	k	1D-33	3	1	Q V Y E F	0	5	40		ND	1	
3BNC156	1-2	3-3/7-27	2/6	3	R H S D Y C D F D V	2	10	72	new	k	1D-33	3	1	Q V Y E F	0	5	37		+	1	
3BNC159	1-2	7-27	2/6	3	R H S D Y C D F D V	2	10	71	new	k	1D-33	3	1	Q V Y E F	0	5	39		ND	1	
3BNC176	1-2	6-25/6-6	2/6	3	Q R S D Y W D F D V	1	10	72	new	k	1D-33	3	1	Q V Y E F	0	5	41		+	3	h
3BNC196	1-2	6-25/6-6/6-13	2/6	3	Q R S D Y W D F D V	1	10	78	new	k	1D-33	3	1	Q V Y E F	0	5	43		ND	1	
3BNC6	1-2	3-16/1-7	2	1	P L R G G D T W H Y H S	3	12	55	new	k	1D-33	1/3	1	Q H Y E F	1	5	44		+	19	i
3BNC101	1-2	1-7/3-16	2	1	P L R G G D T W H Y H S	3	12	54	new					ND					ND	1	
3BNC102	1-2	3-22/1-26/1-20	2	3	P H S P D D A W S L D V	1	12	63	new	k	1D-33	1/3	1	Q H N E F	1	5	34		-	1	
3BNC126	1-2	3-22/1-26/1-20	2	3	P H S P D D A W S L D V	1	12	65	new					ND					ND	1	
3BNC149	1-2	3-22/1-26/1-20	2	3	P H S P D D A W S L D V	1	12	68	new					ND					ND	1	
3ANC3	1-2	2-21/2-15	1/2	1	P R G G R D N W S F H V	3	12	59	new	k	1D-33	3	1	Q N Y E F	0	5	47		+	1	
3ANC42	1-2	ND	2	2	P K S G R D Y W S F D L	2	12	53	new	k	1D-33	3	1	Q Q Y E F	0	5	41		ND	4	j
3BNC3	1-69	5-5/5-18/5-24	3	2	A T G Y S Y G Y L D A F D I	0	14	22	new	l	1-44	1	2	A A W D D T L Y V	0	9	19	CD4i	+	6	k
3BNC8	1-24	5-24/4-17	4	3	E P R E M G T L T A G F E Y	1	14	21	old	k	3-11	2	0	Q H R S I W P L M C T	2	11	10	CD4i	+	3	l
3BNC48	1-69	3-3	4	5	G Q T D L N D D L W S D Y S T P G F D Y	0	20	18	new					ND					ND	2	m
3ANC38	1-69	3-3	4	5	G Q T D L N D D F W S E Y S T P G F D Y	0	20	12	new	l	1-47	1/6	2	G A W D D T L Y V	0	9	8	CD4i	-	2	n
3BNC49	1-69	3-22/6-19/5-12	6	5	G E F D S S G F D Y E S W Y P Y Y M D V	0	20	23	old	k	3-20	3		ND				CD4i	ND	1	q
3BNC58	1-24	3-16/3-10	4/5	2	A P R L E L G E L S S G F H Y	2	15	15	old	k	3-11	2	0	Q Q R T I W P P D C S	1	11	10	CD4i	ND	2	o
3BNC78	1-24		4/5	2	A P R L D L G E L S S G F H F	2	15	39	old					ND					ND	1	
3BNC82	1-24		4/5	2	A P R L D L G E L S S G F H F	2	15	39	old					ND					ND	2	p
3BNC71	1-24	1-24	4/5	3	D N P L L Q S G E F S S S L D N	0	16	22	old	k	3-11	5		ND				CD4i	ND	1	r
3BNC109	1-24	1-24	4/5	3	D N P L L Q S G E F S S S L E N	0	16	17	old	k	3-11	5						CD4i	ND	1	
3BNC144	1-69	3-9/5-5	4	3	A Q G D I L T E G Y F D Y	0	13	15	old	k/l	1-44/1-47	1	2	ND	1	9		CD4i	ND	1	s

t

a 3BNC15, 3BNC80, 3BNC158, 3BNC31, 3BNC123, 3BNC153

b 3BNC60, 3BNC128, 3BNC64, 3BNC34

c 3B21, 3B183

d 3B180

e 3BNC84

f 3BNC192

g 3B106, 3BNC87, 3BNC1, 3BNC115, 3BNC129, 3BNC75, 3BNC38, 3B16

h 3BNC195, 3B188

i 3BNC44, 3BNC54, 3BNC10, 3BNC18, 3BNC20, 3BNC29, 3BNC33, 3BNC45, 3BNC186, 3BNC59, 3BNC173, 3BNC175, 3BNC86, 3BNC104, 3BNC106, 3BNC114, 3BNC148, 3BNC181

j 3ANC41, 3ANC75, 3ANC87

k 3ANC44, 3BNC16, 3BNC63, 3BNC125, 3BNC185

l 3BNC17, 3BNC130

m 3BNC124

n 3ANC85

o 3BNC177

p 3B60

q 3A383 ★

r 3A67 ★

s 3A576 ★

t 11 additional unique B cells

Table S3B. Repertoire and Reactivity of 2CC binding Antibodies, patient 1

Clone RU08

Ab name	VH	D	JH	(-)	CDR3 (aa)	(+)	Length	Mutations HC	Primer set	k/l	Vk/l	Jk/l	(-)	CDR3 (aa)	(+)	Length	Mutations LC	Binding	NEUT	# of Relatives	
1NC2	1-46	3-22/5-5	4/5	4	N E A D Y H D G N G H S L R G M F D Y	3	19	74	new	l	1-47	3	1	A V Y D S S L S L G L	0	11	47		+	15	a
1NC3	1-46	6-19	4/5	3	A E A E S Q S H S R P I M F D F	2	16	86	new	l	1-47	6/7	1	A T Y D S Q R S I R L	2	11	55		+	1	
1NC7	1-46	6-19/1-14	4/5	3	A E A E S Q S H S R P I M F D S	2	16	77	new	l	1-47	6/7	1	A T Y D S Q G S T R L	1	11	51		+	1	
1NC9	1-46	5-12/2-8	4/5	4	Q D S D F H D G H G H T L R G M F D S	4	19	67	new	l	1-47	3	1	A A Y D S T F S L P V	0	11	53	?	+	2	b
1NC18	1-46	1-14/2-21	4/5	2	N E P Q Y H S L P G M F D Y	1	14	85	new					ND					ND	1	
1NC24	1-46	3-16	4/5	3	N E P Q Y H D G N G H S L P G M F D Y	2	19	79	new	l	1-47	3	1	A A Y D S S L S L R L	1	11	30		+	2	c
1NC29	1-46	3-16/6-19	4/5	3	N E P Q Y Y D G S G H S L P G M F D Y	1	19	87	new					ND					ND	1	
1NC33	1-46	5-12	4/5	5	L E A D G D D Y S P K M V D Y	1	15	84	new	l	1-47	3	2	A T Y D T D L S L R L	1	11	49		+	1	
1NC46	1-46	3-9/3-16	4/5	4	R E A D Y H D G N G H T L P G M F D F	3	19	85	new	l	1-47	3	1	A A Y D S A V S L P V	0	11	52		ND	1	
1NC48	1-46	3-9/6-19	4/5	3	N E P Q Y F D G S G H S L P G M F D Y	1	19	88	new	l	1-47	3	1	A A Y D S T L S L R L	1	11	37		ND	1	
1NC52	1-46	3-16/6-19	4/5	3	N E P Q Y Y D G S G H S L P G M F D Y	1	19	82	new	l	1-47	3	1	A A Y D S T F S L P V	0	11	54		ND	1	
1NC56	1-46	5-12/3-9	4/5	5	L E A D G D D Y S P K M F D H	2	15	91	new	l	1-47	3	1	A T Y D T G L S L R L	1	11	58		ND	1	
1NC60	1-46	3-22/1-26	1/5	4	L E A E S D S H S R P I M F D H	3	16	72	new	l	1-47	3	1	A T Y D S G W S I R L	1	11	46		+	3	d
1NC66	1-46	3-16	4/5	3	N E P Q Y H D G N G H S L P G M F D F	2	19	91	new	l	1-47	3	1	A A Y D S T L S L R L	1	11	33		ND	1	
1NC70	1-46	3-16/6-19	4/5	3	N E P Q Y Y D G S G H S L P G M F D Y	1	19	85	new	l	1-47	3	1	A A Y D S T L S L R L	1	11	40		ND	1	
1NC72	1-46	6-19/1-14	4/5	3	A E A E S Q S H S R P I M F D F	2	16	77	new	l	1-47	6/7	1	A T Y D S Q G S T R L	1	11	51		+	2	e
1NC94	1-46	6-13/6-19	4/5	3	A E A A S D S H S R P I M F D H	3	16	81	new	l	1-47	3	2	A T Y D S D G S I R L	1	11	41		-	5	f
1NC95	1-46	3-16/6-19	4/5	4	L E A D G S D Y S P K M F D F	1	15	93	new					ND					ND	1	
1NC107	1-46	3-3/5-12	4/5	5	L E A D G D D Y S P K M F D Y	1	15	90	new	l	1-47	3	1	A T Y D T G L S L R L	1	11	58		ND	1	
1NC108	1-46	3-9/3-16	4/5	4	R E A D Y H D G N G H T L P G M F D F	3	19	85	new	l	1-47	3	1	A A F D S A L S L P L	0	11	51		+	1	
1NC109	1-46	5-12/6-19	4/5	5	L E A D G D D Y S P K M F D Y	1	15	85	new					ND					ND	1	
1NC110	1-46	5-24/6-19	4/5	4	L E A D G D N Y S P K M V D Y	1	15	88	new					ND					ND	1	
1NC116	1-46	2-21	4	2	N E P Q Y H S L P G M F D Y	1	14	83	new					ND					ND	1	
1NC118	1-46	3-9/5-12	4	4	L E A D G G D Y S P K M F D Y	1	15	86	new	l	1-47	3	1	A T Y D T G L S L R L	1	11	54		ND	1	
1NC122	1-46	3-16/3-3	4	4	L E A D G A D Y S P K M F D F	1	15	94	new	l	1-47	3	1	G T Y D T S L S L R L	1	11	57		ND	1	
1NC123	1-46	6-19	4	3	A E A E S Q S H S R P I M F D Y	2	16	78	new	l	1-47	3	1	A T Y D S H G S I R L	2	11	48		-	1	
1NC127	1-46	6-13/6-19	4/5	3	A E A A S D S H S R P I M F D H	3	16	81	new	l	1-47	3	2	A T Y D S D G S I R L	1	11	41	?	+	5	g
1B344	1-46	3-22/1-26	1/5	4	L E A E S D S H S R P I M F D H	3	16	79	new	l	1-47	3	1	A T Y D S G W S I R L	1	11	46		+	1	
1B2416	1-46	1-14/3-16	4	4	N E P Q Y H D D N G H S L P G M I D Y	2	19	81	new					ND					ND	1	
1B2503	1-46	6-19	5	3	A E A E S Q S H S R P I M F D S	2	16	78	new	l	1-47	3	1	G T Y D S Q G S T R L	1	11	49		ND	1	
1B2573	1-46	3-22	4/5	3	N E P Q Y H D G N G H S L P G M F D S	2	19	81	new					ND					-	2	h
1NC5	1-69	3-3	3	1	G R Q T F R A I W S G P P V V F D I	2	18	47	new	k	3-11	2	0	Q H R S N W P W T	2	9		CD4BS	+	1	
1NC126	1-69	3-3	3	1	G R Q T F R A I W S G P P A V F D I	2	18	47	new					ND					ND	1	
1NC16	4-34	3-10	5	2	A V A G L W F E D A Y N W F G P	0	16	75	new	k	1D-39	2/3	0	Q Q S F A V P Y T	0	9	35	ND	ND	1	
1NC21	4-34	3-10	5	2	A V K G L W F D E T Y T W F G P	1	16	58	new					ND				ND	ND	1	
1NC54	4-34	3-10	5	2	A V K G F W F D E P S T W F G P	1	16	59	new					ND				ND	ND	1	
1NC57	4-34	3-10	5	2	A V K G F W F D D P Y T W F G P	1	16	61	new					ND				ND	ND	1	
1NC115	4-34	3-10	5	2	A V K G F W F D E V Y N W F G P	1	16	58	new					ND				ND	ND	1	

i

a 1B2436, 1B2361, 1B2483, 1NC11, 1B2538, 1B2578, 1B2609, 1B2630, 1B2665, 1B2490, 1B2640, 1NC8, 1NC34, 1NC82

b 1NC117

c 1B2367

d 1B2351, 1B2525

e 1NC2364

f 1B2586, 1B2612, 1B2683, 1B2339

g 1B2530, 1B2666, 1B2669, 1B2554

h 1B2644

i 13 additional unique B cells

Table S3C. Repertoire and Reactivity of 2CC binding antibodies, patient 8

Clone RU10

Ab name	VH	D	JH	(-)	CDR3 (aa)	(+)	Length	Mutations HC	Primer set	k/l	Vk/l	Jk/l	(-)	CDR3 (aa)	(+)	Length	Mutations LC	Binding	NEUT	# of Relatives	
8ANC13	1-46	3-16	6	4	D G L G E V A P D Y R Y G I D V	1	16	75	new	k	3-11	2/3	1	Q E Y S S T P Y N	0	9	50		+	1	a
8ANC22	1-46	3-16	6	3	D G L G E V A P A Y L Y G I D A	0	16	85	new					ND					ND	1	
8ANC26	1-46	3-16	6	3	D G L G E V A P A Y L Y G I D A	0	16	76	new	k	3-11	2/3	1	Q E Y S S T P Y N	0	9	55	CD4BS	+	2	
8ANC37	1-46	3-16	6	3	D G L G E V A P A Y L Y G I D A	0	16	82	new	k	3-11	2/3	1	Q E Y S S T P Y N	0	9	50	CD4BS	+	8	
8ANC41	1-46	3-16	6	3	D G L G E L A P A Y H Y G I D V	1	16	71	new	k	3-11	2/3	1	Q E Y S S T P Y N	0	9	42		+	2	
8ANC50	1-46	3-16	6	3	D G L G E L A P A Y Q Y G I D V	0	16	71	new	k	3-11	2/3	1	Q E Y S S T P Y N	0	9	46	CD4BS	+	2	
8ANC88	1-46	3-16	6	4	D G L G E V A P D Y R Y G I D V	1	16	73	new	k	3-11	2/3	1	Q E Y S S T P Y N	0	9	46		ND	1	
8ANC127	1-46	3-16	6	3	D G L G E V A P A Y L Y G I D A	0	16	86	new					ND					ND	1	
8ANC131	1-46	3-16	6	4	D G L G E V A P D Y R Y G I D V	1	16	75	new	k	3-11	2/3	1	Q E Y S S T P Y N	0	9	45	CD4BS	+	1	
8ANC142	1-69	3-3	ND	2	T S T Y D Q W S G L H H D G V M A F S S	2	20	72	new	k	1-5	1/5	1	Q Q Y D T Y P G T	0	9	43	?	+	2	
8ANC46	1-69	3-22/3-15	3	2	S S G N F E F A F E I	0	11	30	old	l	1-40	3	1	Q S Y D R S L R G S V	2	11	30	ND	ND	1	
8ANC191	1-69	3-22/3-15	3	2	S S G N Y D F A Y D I	0	11	28	old					ND					ND	1	
8ANC196	1-69	3-22/3-15	3	2	S S G N Y D F A F D I	0	11	25	old					ND					ND	1	
8ANC14	1-24	6-13/5-5	4	4	A D R F K V A Q D E G L F V I F D Y	2	18	11	old	k	3-11	4	0	Q Q R A N W R L L T	2	10	9	CD4i	+	2	f
8ANC34	1-24	6-13/5-5	4	4	A D P F K V A Q D E G L Y V I F D Y	1	18	10	new					ND					ND	5	g
8ANC58	1-24	6-13/5-5	4	4	A D P F K V A Q D E G L Y V I F D Y	1	18	18	new					ND					ND	3	
8ANC168	1-24	6-13/5-5	4	4	A D P F K V A Q D E G L F V I F D Y	1	18	11	new					ND					ND	1	
8ANC5	1-69	4-17/3-10	6	8	D R G D T R L L D Y G D Y E D E R Y Y Y G M D V	3	24	40	old	k	1D-33	2	0	Q Q Y S N L P Y T	0	9	17	CD4i	-	2	h
8ANC7	1-69	4-17/3-10	6	8	D R G D T R L L D Y G D Y E D E R Y Y Y G M D V	3	24	37	new					ND					ND	2	
8ANC9	1-69	4-17/3-10	6	8	D R G D T R L L D Y G D Y E D E R Y Y Y G M D V	3	24	35	old					ND					ND	1	i
8ANC77	1-69	4-17/3-10	6	8	D R G D T R L L D Y G D Y E D E R Y Y Y G M D V	3	24	50	old					ND					ND	3	j
8ANC107	1-69	4-17/3-10	6	8	D R G D T R L L D Y G D Y E D E R Y Y Y G M D V	3	24	38	old					ND					ND	2	k
8ANC108	1-69	4-17/3-10	6	8	D R G D T R L L D Y G D Y E D E R Y Y Y G M D V	3	24	37	old					ND					ND	4	l
8ANC137	1-69	4-17/3-10	6	8	D R G D T R L L D Y G D Y E D E R Y Y Y G M D V	3	24	37	new					ND					ND	1	m
8ANC16	1-69	2-2	3	2	D R S S A I G Y C S S I S C Y K G S F D I	2	21	12	old	k	3-15	2	0	Q Q Y Y Q W L S Y T	0	10	13	ND	ND	8	n
8ANC24	1-24	2-2	6	1	G G L Y C S S I S C I M D V	0	14	12	old	k	3-15	1	0	Q Q Y N H W P Q T	1	9	7	CD4i	+	1	
8ANC25	1-24	2-2	6	1	G G L Y C S S I S C I M D V	0	14	6	old					ND					ND	1	
8ANC38	3-43	3-16	5	1	N G F D V	0	5	70	new	l	2-11	3	0	C L K K T S S Y V	2	9	41	CORE	+	2	o

p

a 8ANC134

b 8ANC116, 8ANC105, 8ANC53, 8ANC40, 8A275, 8ANC171, 8ANC192

c 8ANC45

d 8ANC182

e 8ANC195

f 8ANC27

g 8ANC39, 8ANC146, 8ANC150, 8ANC184

h 8ANC133, 8ANC163

i 8ANC156

j 8ANC126

k 8ANC158, 8ANC166

l 8ANC153

m 8ANC115, 8ANC143, 8ANC154

n 8ANC74, 8ANC80, 8ANC114, 8ANC117, 8ANC157, 8ANC169, 8ANC193

o 8ANC57

p 61 additional unique B cells

Table S3D. Repertoire and Reactivity of 2CC binding Antibodies, patient 12

Clone RU16

Ab name	VH	D	JH	(-)	CDR3 (aa)	(+)	Length	# Mutations HC	Primer set	k/l	Vk/l	Jk/l	(-)	CDR3 (aa)	(+)	Length	Mutations LC	Binding	NEUT	# of Relatives	
12A1	1-2	5-12/3-10	4/5	4	D E S G D D L K W H L H P	3	13	60	new	k	1D-33	3	0	A A F Q W	0	5	39		ND	1	
12A2	1-2	4-17	4/5	3	D G S G D D T S W H L H P	2	13	67	new	k	1D-33	3	1	A V L E F	0	5	44		+	3	a
12A4	1-2	5-12/3-10	4/5	4	D E S G D D L K W H L H P	3	13	59	new	k	1D-33	3	0	A V F Q W	0	5	36	CD4BS	+	3	b
12A6	1-2	1-26/3-10	4/5	2	D G S G D A T S W H L H P	2	13	61	new	k	1D-33	3	1	A V L E F	0	5	39		+	1	
12A7	1-2	1-26	4/5	4	D G S G D A R D W H L D P	2	13	62	new	k	1D-33	3	1	A V L E F	0	5	41		ND	2	c
12A9	1-2	3-3	4/5	5	D R R D D D R A W L L D P	3	13	62	new	k	1D-33	3	1	Q L F E F	0	5	39		ND	1	
12A12	1-2	1-26/3-10	4/5	4	D G S G D D T S W H L D P	1	13	60	new	k	1D-33	3	1	A V L E F	0	5	41	CD4BS	+	1	
12A13	1-2	1-26	4/5	4	D G S G D D T S W Y L D P	0	13	61	new	k	1D-33	3	1	A V V E F	0	5	41		ND	1	
12A20	1-2	1-26	4/5	3	D G S G D A R D W H L H P	3	13	61	new	k	1D-33	3	1	A A L E F	0	5	40		+	1	
12A22	1-2	3-16	4/5	4	D G G G D D R T W L L D A	1	13	61	new	k	1D-33	3	1	S V Y E F	0	5	39		+	2	d
12A23	1-2	3-3	4/5	5	D R R D D G L D W L L D P	2	13	51	new	k	1D-33	3	1	Q L F E F	0	5	39		+	1	
12A27	1-2	1-26/3-10	4/5	3	D G S G D D T S W H L H P	2	13	68	new	k	1D-33	3	1	A V L E F	0	5	40		ND	1	
12A46	1-2	3-10	4/5	1	G G G D G R N W H L H P	3	12	62	new	k	1D-33	3	1	A S L E F	0	5	43		+	1	
12A55	1-2	1-26	4/5	4	D G S G D D R N W H L D P	2	13	63	new	k	1D-33	3	2	E V Y E F	0	5	37		+	1	
12A56	1-2	1-26	4/5	4	D E S G Y D L N W H L D S	1	13	66	new	k	1D-33	3	1	E S F Q W	0	5	37		ND	1	

e

a 12A30, 12A16

b 12A10, 12A21

c 12A17

d 12A37

e 12 additional unique B cells

Table S3E. Repertoire and Reactivity of GP140 binding Antibodies, patient 3

Ab name	VH	D	JH	(-)	CDR3 (aa)	(+)	Length	Mutations HC	Primer set	k/l	Vk/l	Jk/l	(-)	CDR3 (aa)	(+)	Length	Binding	NEUT	# of Relatives	
3B191	1-2	6-25/6-13/6-6	2/6	3	Q R S D Y W D F D V	1	10	81	new	k	1D-33	3	1	Q V Y E F	0	5	CD4BS	+	7	
3B6	4-39	3-9/3-10	3	2	I P Y H S E S Y Y K V V I G G F D V	2	18	50	new	k	1-9	1/3	0	Q Q L A T	0	5	GP41	+	11	
3B8	1-69	4-17/3-22	4	3	D H G D P R T G Y Y F D Y	2	13	50	new	k	3-20	1/5	2	Q Q Y D D A P I T	0	9	GP41	-	9	
3B27	3-64	3-9/1-26/4-17	5	1	G P L L R Y L D S	1	9	18	old	k	3-11	1/5	0	Q H R T N W P P S I T	2	11	CD4i	-	3	
3B41	1-24	3-16	6	4	K A K D Y Y Y E S S D Y S P Y Y Y Y Y M D V	2	22	17	old	k	3-20	2	0	Q Q Y G T S S C T	0	9	CD4i	-	2	
3B46	4-31	3-3/2-8	4/5	1	G S G R W T I G A R I Y F D N	2	15	22	old	k	3-20	1/4	0	Q Q Y G S S P P T	0	9	GP41	ND	2	
3B144	3-30	3-3/3-10/3-16	4/5	2	T P P H Y D V L T G Y P S S V L E F	1	18	23	old	k	3-15	1/5	0	Q Q Y N N W P P I T	0	10	ND	ND	4	
3B117	1-69	5-5/5-18/5-24	3	2	A T G Y S Y G Y L D A F D I	0	14	22	new	l	1-44	1	2	A A W D D T L Y V	0	9	ND	ND	1	
3A869	4-4/4-59	6-19/5-12/1-26	4	2	E K G Q W L T V P P Y Y F D S	1	15	33	old	k	1D-39	5	0	Q Q S H S P S	1	7	CD4BS	+	1	★
3A228	5-51	3-3/2-2	6	1	T R C F G A N C F N F M D V	1	14	34	old	k	4-1	3	0	Q Q Y Y I S P	0	7	VAR	+	4	★
3A461	1-46	2-2	4	1	P E P S S I V A P L Y Y	0	12	15	old	k	3-20	1	0	Q Q Y G T L H P R T	2	10	GP41	-	3	★
3A18	1-69	3-10/5-24	3	3	D P Q V E V R G N A F D I	1	13	40	old	k	1D-39	5	0	Q Q T Y T S P I T	0	9	GP41	-	2	★
3A125	1-46	1-20/1-7/3-10	3	2	P Q Y N L G R D P L D V	1	12	22	old	k	3-20	1	0	Q Q Y G L S P W T	0	9	GP41	-	4	★
3A255	4-59	3-3/3-9	4	3	A D Y D L L T S S Y H F D S	1	14	35	old	l	7-43	3	0	L L L P Y Y G G P W I	0	11	GP41	-	2	★
3A233	4-59/4-61	3-3/4-17	4/5	3	L D G E A F R Y Y L D L	1	12	32	old	l	2-14	2/3	0	S S F T P T N T L V	0	10	GP41	-	2	★

21 additional unique B cells

Table S3F. Repertoire and Reactivity of GP140 binding Antibodies, patient 1

Ab name	VH	D	JH	(-)	CDR3 (aa)	(+)	Length	Mutations HC	Primer set	k/l	Vk/l	Jk/l	(-)	CDR3 (aa)	(+)	Length	Binding	NEUT	# of Relatives	
1B2434	1-46	3-22/5-5	1	4	N E A D Y H D G N G H S L R G M F D Y	3	19	74	new	l	1-47	3	1	A V Y D S S L S L G L	0	11	?	+	7	★
1B218	1-69	3-3	3	1	G R Q T F R A I W S G P P V V F D I	2	18	47	new	k	3-11	2	0	Q H R S N W P W T	2	9	CD4BS	+	10	★
1B331	4-34	3-9/3-3	6	3	R Y F D W S P F R R D T Y G T D V	3	17	40	new	k	4-1	1/4	0	H Q Y F S T P R T	2	9	CORE	+	4	
1B2174	4-34	3-9/3-3	6	3	R Y L D W S P I G R D T Y G T D V	2	17	41	new	k	4-1	1/4	0	H Q Y F N T P R T	2	9		ND	1	
1B2155	1-69	2-21	2/5	1	G L C R G G N C R L G P S G W L D P	2	18	62	new	k	3-15	1	2	Q Q Y E D P P W T	0	9	ND	ND	3	
1B2133	1-3	4-17/2-21	4	1	V A Y V H V V T T R S L D N	1	14	22	new	k	1D-39	1	0	Q Q T Y S N P R M	1	9	CD4i	-	2	
1A64	4-59	5-5/5-18	6	2	H E A P R Y S Y A F R R Y Y H Y G L D V	5	20	20	old	l	1-44	3	2	A S W D D S L S G W V	0	11	CD4BS	+	24	★
1A621	4-59	3-3/3-9	6	1	V I S G R I T I F Y Y N Y I D V	1	16	30	old	l	1-47	3	1	A S W D N S L S G P V	0	11	CD4BS	+	3	★
1A577	3-48	3-10/3-16	1	2	G T L W F G E S G L R L D H	2	14	15	old	k	1-16	2	0	Q Q Y N S F P P T	0	9	CD4BS	+	8	★
1A732	3-72/3-73	3-22/3-10	6	2	N R R V A M P E A M I L S F Y M D V	2	18	9	old	k	3-20	3	0	Q Q Y G R S P	1	7	CD4BS	+	1	★
1A74	4-34	3-3/3-9	4	1	V V P M F S I F G V V K A N Y F D Y	1	18	23	old	l	1-51	3	1	G T W D S S L S A V L	0	11	CORE	+	2	★
1A695	4-59	3-3/3-9	3	2	A G L D Y N F W N G K G R K G A F D V	3	19	9	old	k	1-5	1	1	Q Q Y D S	0	5	CORE	+	2	★
1A479	1-69	3-22	4	1	G F R G S P F S S G S L Y F D S	1	16	25	old	k	3-20	1	0	H Q Y A Y S P R T	2	9	CORE	+	11	★
1A182	1-69	4-17/1-26	6	6	A V I T D L H T F G D Y E L E D P S Y Y Y M D V	1	24	28	old	k	1-5	1	0	Q Q Y K S Y S G T	1	9	CD4i	+	3	★
1A693	3-23	7-27/3-22	4	1	R G R R Q I G D Y	3	9	17	old	k	1D-39	2	0	Q H S F G S P P W T	1	10	CD4i	-	1	★
1A79	5-51	3-9/3-3	3	4	S Y Y D F S I G D G N D A F D V	0	16	30	old	l	1-47	1	3	A A W D D S F D Y V	0	10	V3	+	27	★
1A27	3-11	3-3/5-5	5	2	D T T T F T T F G G G P N M G G F D P	0	19	50	old	k	1-9	1	0	Q Q L R T	1	5	GP41	-	8	★

★ identical to 1NC2
★ identical to 1NC5

35 additional unique B cells

Table S3 IgH and IgL chain gene sequence, antibody reactivity, neutralization assay results for cloned antibodies.
ND indicates not determined. Letters on the right side of the table indicate close clonal relatives shown below. Previously published antibodies (7) are indicated with a red star. **A**-**D**, Antibodies from 2CC-core reactive IgG B cells from patients 1, 3, 8 and 12.
The number of clone members with 95% IgH and IgL chain gene sequence homology is indicated and clonal relatives with various degrees of somatic mutations are shown in the same color. **E**, **F**, Antibodies from gp140 reactive IgG B cells from patients 3 and 1.
Each clone is represented once with the number of relatives indicated.

Table S4, In vitro Tzm-bl neutralization assay, basic panel IC50 values

A

Patient 3, Clone RU01

	3BNC62	3BNC176	3BNC60	3BNC117	3BNC95	3BNC104	3BNC91	3BNC55	3BNC89	3ANC3	3BNC53	3BNC72	3BNC156	3BNC158	3BNC153	3BNC108	3BNC142	3BNC66	3BNC42	3BNC102
MW965.26	<0.09	<0.10	<0.04	<0.09	<0.07	>50	<0.08	0.04	<0.05	0.18	0.09	<0.06	0.08	0.11	0.15	ND	0.14	1.24	ND	>50
BaL.26	<0.09	<0.10	<0.04	<0.09	<0.07	0.025	>178	>30	>110	>50	>30	>139	>111	>109	>100	20.6	>172	>189	>26	>50
DJ263.8	<0.09	<0.10	<0.04	<0.09	<0.07	0.054	>178	>30	>110	>50	>30	>139	>111	>109	>100	>55	>172	>189	>26	>50
6535.3	0.68	0.46	0.54	0.55	1.0	>50	1.0	2.6	1.7	>50	13.6	8.49	11.1	9.9	28.9	>55	>172	>189	>26	>50
RHPA4259.7	<0.09	<0.10	<0.05	0.041	<0.07	0.022	<0.08	2.2	12.4	7.66	100.6	>139	>111	>109	>100	45.91	>172	>189	>26	>50
TRO.11	<0.09	<0.10	<0.05	0.077	<0.07	3.791	3.06	18.4	52.4	10.76	>155	>139	>111	>109	>100	>55	>172	>189	>26	>50
PVO.4	<0.09	<0.10	0.09	<0.09	<0.07	0.348	0.44	3.9	2.7	36.77	>155	>139	>111	>109	>100	>55	>172	>189	ND	>50
YU2.DG	<0.09	<0.10	<0.05	0.054	<0.07	0.034	<0.08	0.9	0.39	35.01	>155	>139	>111	>109	>100	25.5	>172	>189	>26	>50

Patient 3, Clones RU02-07

	3A67	3A383	3BNC8	3ANC44	3A576	3ANC38
MW965.26	0.1	0.5	0.74	25.49	>50	>50
BaL.26	19.2	5.3	>50	27.91	27	>50
DJ263.8	>50	>50	>50	>50	>50	>50
6535.3	>50	ND	>50	>50	>50	>50
RHPA4259.7	>50	ND	>50	>50	>50	>50
TRO.11	>50	ND	>50	>50	>50	>50
PVO.4	>50	ND	>50	>50	>50	>50
YU2.DG	>50	ND	>50	>50	>50	>50

B12 and NIH45 Clone

	B12	VRC01	NIH45-46
MW965.26	0.2	<0.08	0.04
BaL.26	0.2	0.1	<0.04
DJ263.8	>50	0.08	<0.04
6535.3	1.4	0.539	0.14
RHPA4259.7	0.1	0.06	0.034
TRO.11	>50	0.2	1.9
PVO.4	>50	0.2	0.17
YU2.DG	2.2	0.12	<0.05

Patient Serum IgG

	Pt 1	Pt 3	Pt 8	NIH45	Pt 12
6535.3	88	400.4	23.2	61	101.3
RHPA4259.7	113	16.6	154.1	30	30.1
TRO.11	72	24.5	62.2	208	53.6
PVO.4	89	78.1	74.1	195	116.3
YU2.DG	131	25.4	32.7	92	50.6

B

Patient 1, Clone RU08

	1B2640	1B2530	1B2364	1NC9	1B2490	1B2351	1B344	1NC24	1NC3	1NC7	1NC33	1NC108	1B2644	1B2339	1NC123
MW965.26	41.76	0.762	1.85	>50	>50	>50	>50	>50	>25	>50	>50	>50	>25	>25	>50
BaL.26	0.08	>50	>25	1.37	0.058	>50	>50	>50	>25	>50	>50	>50	>25	>25	>50
DJ263.8	>50	2.71	3.75	>50	>50	8.76	12.62	>50	>25	>50	>50	>50	>25	>25	>50
6535.3	>50	>50	>25	>50	>50	>50	>50	>50	>25	>50	22.04	>50	>25	>25	>50
RHPA4259.7	0.04	3.60	2.18	0.09	0.414	36.48	29.98	>50	>25	34.27	>50	>50	>25	>25	>50
TRO.11	0.23	0.516	0.27	0.20	1.06	0.331	0.27	0.20	3.37	16.57	>50	19.37	>25	>25	>50
PVO.4	1.05	0.275	0.161	0.34	2.97	0.250	0.27	0.19	6.68	1.39	1.84	3.13	>25	>25	>50
YU2.DG	0.20	0.209	2.46	0.13	0.125	0.058	0.25	0.16	18.26	>50	>50	>50	>25	>25	>50

Clone RU09

1B218
>119
1.1
>119
3.6
>100
>100
>100
>100

Table S4, In vitro Tzm-bl neutralization assay, basic panel IC50 values

C

Patient 8, Clone RU10

	8ANC192	8ANC134	8ANC13	8ANC131	8ANC182	8ANC45
MW965.26	>73	>50	>50	>50	>115	>50
BaL.26	0.08	0.02	0.04	0.06	0.08	0.296
DJ263.8	<0.03	0.003	0.008	0.004	<0.05	0.041
6535.3	0.34	0.06	0.27	0.20	0.89	0.813
RHPA4259.7	>50	>50	>50	>50	>100	>50
TRO.11	>100	>50	>50	>50	>100	>50
PVO.4	0.89	0.46	0.63	0.81	1.2	4.259
YU2.DG	0.09	0.15	0.21	0.18	0.22	0.499

Patient 8, Clones RU11-15

8ANC57	8ANC195	8ANC24	8ANC14	8ACN5
24.1	>50	0.29	2.01	>50
4.35	>50	47.53	>50	>50
30.19	>50	>50	>50	>50
>103	0.20	>50	>50	>50
1.65	0.34	>50	>50	>50
32.07	0.18	>50	>50	>50
101.15	0.52	>50	>50	>50
27.52	0.79	>50	>50	>50

D

Patient 12, Clone RU16

	12A12	12A21	12A22	12A16	12A20	12A6	12A23	12A46	12A55
MW965.26	0.042	0.075	0.06	0.167	0.192	0.112	5.10	>50	0.58
BaL.26	0.017	<0.001	0.04	0.042	0.035	0.072	0.570	0.013	2.87
DJ263.8	0.002	0.035	0.08	0.012	0.05	0.004	0.630	5.79	>50
6535.3	21.97	>50	>25	15.44	48.73	>24	14.73	48.85	>50
RHPA4259.7	0.086	0.038	0.04	0.207	0.109	0.227	0.496	>50	>50
TRO.11	0.288	0.164	0.56	0.751	0.689	1.52	2.88	>50	21.45
PVO.4	0.928	0.584	0.45	2.44	3.04	3.32	2.24	2.18	0.99
YU2.DG	0.084	0.015	0.11	0.234	0.142	0.222	0.053	0.049	0.10

B12 and NIH45 Clone

	B12	VRC01	NIH45-46
MW965.26	0.2	<0.08	0.04
BaL.26	0.2	0.1	<0.04
DJ263.8	>50	0.08	<0.04
6535.3	1.4	0.539	0.14
RHPA4259.7	0.1	0.06	0.034
TRO.11	>50	0.2	1.9
PVO.4	>50	0.2	0.17
YU2.DG	2.2	0.12	<0.05

Table S4, In vitro Tzm-bl neutralization assay, basic panel IC80 values

E

Patient 3, Clone RU01

	3BNC62	3BNC176	3BNC60	3BNC117	3BNC95	3BNC104	3BNC91	3BNC55	3BNC89	3ANC3	3BNC53	3BNC72	3BNC156	3BNC158	3BNC153	3BNC108	3BNC142	3BNC66	3BNC42	3BNC102
MW965.26	<0.09	<0.10	0.09	<0.09	<0.07	>50	<0.08	0.15	0.16	0.64	0.61	0.37	0.47	0.60	0.63	ND	0.80	28.98	ND	>50
BaL.26	<0.09	<0.10	<0.04	<0.09	<0.07	0.09	>178	>30	>110	>50	>30	>139	>111	>109	>100	>55	>172	>189	>26	>50
DJ263.8	0.10	<0.10	0.10	0.10	0.1	0.187	>178	>30	>110	>50	>30	>139	>111	>109	>100	>55	>172	>189	>26	>50
6535.3	2.24	1.70	1.77	2.44	4.5	>50	6.7	5.53	5.92	>50	73.38	133.65	69.66	97.75	>100	>55	>172	>189	>26	>50
RHPA4259.7	<0.09	<0.10	0.07	0.137	<0.07	0.060	0.52	8.03	>110	>50	>155	>139	>111	>109	>100	>55	>172	>189	>26	>50
TRO.11	<0.09	<0.10	0.12	0.077	<0.07	30.847	32.31	41.67	>110	>50	>155	>139	>111	>109	>100	>55	>172	>189	>26	>50
PVO.4	0.23	0.16	0.27	0.19	0.23	0.901	2.65	6.5	10.18	>50	>155	>139	>111	>109	>100	>55	>172	>189	ND	>50
YU2.DG	<0.09	<0.10	0.07	0.054	<0.07	0.097	<0.08	1.07	1.49	>50	>155	>139	>111	>109	>100	>55	>172	>189	>26	>50

Patient 3, Clones RU02-07

	3A67	3A383	3BNC8	3ANC44	3A576	3ANC38
MW965.26	16	>25	0.74	>50	>50	>50
BaL.26	>50	>25	>50	>50	>50	>50
DJ263.8	>50	>25	>50	>50	>50	>50
6535.3	>50	ND	>50	>50	>50	>50
RHPA4259.7	>50	ND	>50	>50	>50	>50
TRO.11	>50	ND	>50	>50	>50	>50
PVO.4	>50	ND	>50	>50	>50	>50
YU2.DG	>50	ND	>50	>50	>50	>50

B12 and NIH45 Clone

	B12	VRC01	45-46
MW965.26	ND	<0.08	0.21
BaL.26	ND	0.1	0.06
DJ263.8	ND	0.553	0.06
6535.3	ND	2.7	0.28
RHPA4259.7	0.39	0.185	0.146
TRO.11	>50	0.832	9.56
PVO.4	>50	1.2	0.47
YU2.DG	7.8	0.372	0.08

F

Patient 1, Clone RU08

	1B2640	1B2530	1B2364	1NC9	1B2490	1B2351	1B344	1NC24	1NC3	1NC7	1NC33	1NC108	1B2644	1B2339	1NC123
MW965.26	>50	>50	>25	>50	>50	>50	>50	>50	>25	>50	>50	>50	>25	>25	>50
BaL.26	0.32	>50	>25	19.92	0.30	>50	>50	>50	>25	>50	>50	>50	>25	>25	>50
DJ263.8	>50	>50	>25	>50	>50	>50	>50	>50	>25	>50	>50	>50	>25	>25	>50
6535.3	>50	>50	>25	>50	>50	>50	>50	>50	>25	>50	>50	>50	>25	>25	>50
RHPA4259.7	0.25	>50	>25	0.40	1.97	>50	>50	>50	>25	>50	>50	>50	>25	>25	>50
TRO.11	1.62	2.46	1.77	0.65	3.58	1.13	0.89	0.66	>25	>50	>50	>50	>25	>25	>50
PVO.4	2.97	1.25	0.65	1.32	10.57	0.880	0.94	0.60	>25	7.17	10.12	25.08	>25	>25	>50
YU2.DG	0.70	7.74	>25	0.56	0.59	0.48	1.29	0.55	>25	>50	>50	>50	>25	>25	>50

Clone RU09

1B218
>119
5.61
>119
35.12
>100
>100
>100
>100

Table S4, In vitro Tzm-bl neutralization assay, basic panel IC80 values

G Patient 8, Clone RU10

	8ANC192	8ANC134	8ANC13	8ANC131	8ANC182	8ANC45
MW965.26	>73	>50	>50	>50	>115	>50
BaL.26	0.43	0.11	0.18	0.31	0.73	7.45
DJ263.8	0.10	0.044	0.069	0.046	0.11	0.166
6535.3	1.43	2	2.3	1.9	3.93	10.473
RHPA4259.7	>100	>50	>50	>50	>100	>50
TRO.11	>100	>50	>50	>50	>100	>50
PVO.4	3.94	2.5	3.7	4.9	4.43	17.315
YU2.DG	0.51	0.616	1.07	0.92	1.46	2.942

Patient 8, Clones RU11-15

8ANC57	8ANC195	8ANC24	8ANC14	8ANC5
>103	>50	0.76	6.64	>50
24.76	>50	>50	>50	>50
>103	>50	>50	>50	>50
>103	0.91	>50	>50	>50
14.44	1.56	>50	>50	>50
>103	0.89	>50	>50	>50
>103	1.87	>50	>50	>50
91.49	2.77	>50	>50	>50

H Patient 12, Clone RU16

	12A12	12A21	12A22	12A16	12A20	12A6	12A23	12A46	12A55
MW965.26	0.20	0.85	0.21	0.58	2.20	0.52	>50	>50	4.49
BaL.26	0.08	0.004	0.14	0.25	0.23	0.47	3.470	0.08	>50
DJ263.8	0.31	0.42	1.86	0.12	ND	0.08	30.81	>50	>50
6535.3	>50	>50	>25	>42	ND	>24	>50	>50	>50
RHPA4259.7	0.40	0.13	0.13	0.93	0.49	1.02	1.69	>50	>50
TRO.11	0.98	0.57	1.94	2.57	2.41	5.15	10.11	>50	>50
PVO.4	3.15	2.09	1.49	8.72	11.20	17.34	7.81	7.97	4.30
YU2.DG	0.31	0.06	0.36	1.13	0.67	1.20	0.19	0.25	0.29

B12 and NIH45 Clone

	B12	VRCO1	45-46
MW965.26	ND	<0.08	0.21
BaL.26	ND	0.1	0.06
DJ263.8	ND	0.553	0.06
6535.3	ND	2.7	0.28
RHPA4259.7	0.39	0.185	0.146
TRO.11	>50	0.832	9.56
PVO.4	>50	1.2	0.47
YU2.DG	7.8	0.372	0.08

Table S4 Neutralizing activity of 2CC-core binding antibodies against a basic virus panel of 3 Tier 1 (Clades A,B, C) and 5 Tier 2 (Clade B) viruses.
Each column represents one antibody and antibody clones are grouped for each source patient (Table S3).
Tier 1 viruses are: MW965.26 (Clade C), BaL.26 (Clade B), DJ263.8 (Clade A); Tier 2 (Clade B) viruses are: 6535.3, RHPA4259.7, TRO.11, PVO.4, YU2.DG.
Colors indicate concentration at IC50 (a-d) and IC80 (e-h): for monoclonal antibodies: red ≤ 0.1µg/ml; orange 0.1-1 µg/ml; yellow 1-10µg/ml; green ≥10µg/ml; white not neutralized at any concentration tested. Previously published data from antibodies B12 (22,5,7) and VRC01 (5) is added as control; for serum IgG: orange 10-100µg/ml; yellow >100µg/ml; white not neutralized at any concentration tested.

Table S5

ANTIBODY NAME	CLONE	PATIENT	EXTENDED PANEL (15 isolates)	EXTENDED PANEL (118 isolates)	CD4 AGONIST	MEMBER OF CONSENSUS
VRC01		NIH45	X	X	+	X
NIH45-46		NIH45	X	X	+	X
3BNC55	RU01	Pt3	X	X	ND	
3BNC60	RU01	Pt3	X	ND	+	X
3BNC117	RU01	Pt3	X	X	+	X
1NC9	RU08	Pt1	X	ND	+	X
1B2530	RU08	Pt1	X	ND	+	X
8ANC131	RU10	Pt8	X	ND	+	X
8ANC134	RU10	Pt8	X	ND	+	X
8ANC195	RU11	Pt8	X	X	-	
12A12	RU16	Pt12	X	X	+	X
12A21	RU16	Pt12	X	ND	+	X

Summary table of antibody representatives selected for extended neutralization testing, CD4 agonistic function and sequence alignment. Membership of an antibody to a particular clone is identified by heavy and light chain sequence analysis (Table S3). VRC01 neutralization on the extended virus panels has been published previously(5).

Table S6, In vitro Tzm-bl neutralization assay, extended panel IC50 values

A

	B12	VRC01	NIH45-46	3BNC60	3BNC62	3BNC117	3BNC55	1NC9	1B2530	8ANC131	8ANC134	8ANC195	12A12	12A21
Q842.d12	>50	0.03	0.008	0.01	<0.01	<0.01	0.011	0.020	0.249	0.053	0.061	>30	0.014	0.015
3415.v1.c1	2.5	0.06	0.017	0.10	0.17	0.17	0.110	0.266	0.065	0.299	0.323	2.404	0.124	0.082
3365.v2.c20	>50	0.06	0.029	0.02	0.03	0.03	0.221	0.329	4.357	>30	>30	>30	0.068	0.045
H086.8*	>50	>50	>30	>15	>15	>15	>30	>30	>30	>50	>50	0.095	>30	>30
ZM53M.PB12	>50	1.3	0.187	0.22	0.30	0.21	12.549	0.705	0.912	>30	>30	9.626	0.593	0.420
Du172.17*	0.3	>50	>30	3.81	1.72	1.19	3.518	>30	>30	>30	>30	10.797	0.196	0.126
ZM109F.PB4	>50	0.128	0.059	0.22	0.14	0.14	0.083	0.023	>30	>30	>30	>30	0.148	2.104
3016.v5.c45	1.1	0.16	>30	1.40	0.42	1.38	>30	>30	>30	>30	>30	0.195	1.163	0.097
231965.c1	0.07	0.34	0.021	0.07	0.05	0.05	0.505	0.393	0.168	6.346	>30	0.514	2.217	>30
X1254_c3	>50	0.07	0.027	0.09	0.08	0.08	0.138	>30	>30	>30	>30	1.524	1.032	26.793
250-4*	>50	>50	>30	>15	>15	>15	0.236	>30	>30	>50	>50	>50	>30	>30
251-18	>50	2.5	1.445	0.35	0.32	0.26	>30	1.234	9.847	0.968	1.560	0.284	2.622	1.713
278-50*	>50	>50	>30	>15	>15	>15	>30	>30	>30	>50	>50	>50	>30	>30
620345.c1*	>50	>50	>30	>15	>15	>15	>30	>30	>30	>50	>50	>50	>30	>30
R1166.c1	>50	1.7	0.445	0.14	0.32	0.17	0.298	0.651	0.119	>30	>30	0.986	0.342	0.292

Patient Serum IgG

	pt1	pt3	pt8	NIH45
Q842.d12	12.196	6.168	4.056	50
3415.v1.c1	48.26	38.88	16.63	54
3365.v2.c20	111.54	28.46	>227	94
H086.8*	>132	>132	>132	37
ZM53M.PB12	60.70	383.37	>227	317
Du172.17*	228.42	418.62	86.463	349
ZM109F.PB4	86.82	12.97	>227	73
3016.v5.c45	>340	185.62	>227	ND
231965.c1	304.48	86.54	171.56	ND
X1254_c3	222.01	81.48	>227	ND
250-4*	>132	560.58	55.09	90
251-18	>340	104.58	92.28	841
278-50*	>132	>132	>132	>1000
620345.c1*	>132	>132	>132	>1000
R1166.c1	>340	52.01	>227	ND

Table S6, In vitro Tzm-bl neutralization assay, extended panel IC80 values

B

	B12	VRC01	45-46	3BNC60	3BNC62	3BNC117	3BNC55	1NC9	1B2530	8ANC131	8ANC134	8ANC195	12A12	12A21
Q842.d12	>50	0.096	0.026	0.03	0.03	0.01	0.062	0.133	2.191	0.179	0.205	>30	0.060	0.066
3415.v1.c1	14.1	0.15	0.069	0.37	0.40	0.47	0.388	1.002	0.35	1.555	2.643	17.743	0.418	0.296
3365.v2.c20	>50	0.17	0.114	0.08	0.09	0.10	2.341	2.163	>30	>30	>30	>30	0.192	0.166
H086.8*	>50	>50	>30	>15	>15	>15	>30	>30	>30	>50	>50	5.328	>30	>30
ZM53M.PB12	>50	4	0.652	0.76	1.10	0.85	>30	2.771	4.022	>30	>30	>30	2.069	1.458
Du172.17*	2.6	>50	>30	>15	12.18	8.90	>30	>30	>30	>30	>30	>30	0.992	0.637
ZM109F.PB4	>50	0.754	0.220	1.23	0.78	0.88	0.396	0.146	>30	>30	>30	>30	0.698	13.686
3016.v5.c45	4	0.42	>30	7.38	2.35	>15	>30	>30	>30	>30	>30	0.872	11.864	0.358
231965.c1	0.16	1.2	0.100	0.25	0.22	0.22	2.780	2.276	0.963	>30	>30	2.355	15.102	>30
X1254_c3	>50	0.19	0.078	0.29	0.27	0.27	0.571	>30	>30	>30	>30	6.949	5.777	>30
250-4*	>50	>50	>30	>15	>15	>15	1.922	>30	>30	>50	>50	>50	>30	>30
251-18	>50	11.2	5.255	0.96	1.00	0.82	>30	6.291	>30	5.550	6.281	1.511	9.39	6.063
278-50*	>50	>50	>30	>15	>15	>15	>30	>30	>30	>50	>50	>50	>30	>30
620345.c1*	>50	>50	>30	>15	>15	>15	>30	>30	>30	>50	>50	>50	>30	>30
R1166.c1	>50	4.6	1.679	0.51	0.89	0.64	2.351	2.669	0.684	>30	>30	4.830	1.850	2.137

Table S6 Neutralizing activity of selected antibodies and serum IgG against an extended Tier 2 virus panel.

* indicate VRC01 resistant viral strains. Colours indicate concentration at IC50 (**A**) and IC80 (**B**): for monoclonal antibodies: red ≤ 0.1µg/ml; orange 0.1-1 µg/ml; yellow 1-10µg/ml; green ≥10µg/ml; white not neutralized at any concentration tested. for serum IgG: red ≤10µg/ml; orange 10-100µg/ml; yellow >100µg/ml; white not neutralized at any concentration tested. Previously published data from antibodies B12 (22,5) and VRC01 (5) is added as control.

Table S7, IC50

A

Virus ID	Clade	3BNC117	3BNC55	45-46	12A12	8ANC195		VRC01
MS208.A1	A	0.01	0.52	0.09	0.12	>50		0.10
Q23.17	A	0.01	3.49	0.14	0.07	2.55		0.09
Q461.e2	A	0.03	36.51	0.06	0.12	0.63		0.49
Q769.d22	A	0.01	0.20	0.01	0.03	0.26		0.08
Q259.d2.17	A	0.01	0.16	0.01	0.01	>50		0.17
Q842.d12	A	<0.01	0.01	0.01	0.01	>30		0.03
3415.v1.c1	A	0.17	0.11	0.02	0.12	2.40		0.06
3365.v2.c2	A	0.03	0.22	0.03	0.07	>30		0.06
191955_A11	A (T/F)	>50	>50	0.11	>50	>50		NT
191084 B7-19	A (T/F)	0.07	0.18	0.02	0.07	>50		NT
9004SS_A3_4	A (T/F)	0.07	>50	0.18	0.09	0.17		NT
T257-31	CRF02_AG	0.06	>50	0.23	0.30	0.32		2.80
928-28	CRF02_AG	0.15	>50	0.10	0.21	>50		0.41
263-8	CRF02_AG	0.01	0.28	0.05	0.04	>50		0.20
T250-4	CRF02_AG	>15	0.24	>30	>30	>50		>50
T251-18	CRF02_AG	0.26	>30	1.45	2.62	0.28		2.50
T278-50	CRF02_AG	>15	>30	>30	>30	>50		>50
T255-34	CRF02_AG	0.02	0.24	0.23	0.03	0.54		0.70
211-9	CRF02_AG	0.35	>50	>50	7.41	1.34		14.30
235-47	CRF02_AG	0.03	1.24	0.30	0.03	>50		0.04
620345.c01	CRF01_AE	>15	>30	>30	>30	>50		>50
703357.c02	CRF01_AE	33.93	>50	13.91	1.90	6.55		4.00
C1080.c03	CRF01_AE	0.25	>50	1.79	0.94	0.17		3.40
R2184.c04	CRF01_AE	0.03	0.03	0.03	0.09	0.09		0.08
R1166.c01	CRF01_AE	0.17	0.30	0.45	0.34	0.99		1.70
R3265.c06	CRF01_AE	0.48	>50	0.04	0.16	50.00		0.45
C2101.c01	CRF01_AE	0.05	>50	12.78	0.05	0.48		0.36
C3347.c11	CRF01_AE	0.03	0.12	0.03	0.03	>50		0.17
C4118.c09	CRF01_AE	0.09	>50	0.14	0.11	0.20		NT
CNE5	CRF01_AE	0.28	0.48	0.09	0.81	2.12		0.37
BJOX009000.02.4	CRF01_AE	0.40	1.17	0.26	>50	0.64		NT
BJOX015000.11.5	CRF01_AE (T/F)	0.05	3.89	0.06	0.11	0.97		NT
BJOX010000.06.2	CRF01_AE (T/F)	1.58	1.75	0.87	21.25	0.85		NT
BJOX025000.01.1	CRF01_AE (T/F)	0.05	>50	0.18	0.10	4.96		NT
BJOX028000.10.3	CRF01_AE (T/F)	0.01	>50	0.07	0.01	0.45		NT
X1193_c1	G	0.06	0.09	0.04	0.14	0.27		0.11
P0402_c2_11	G	0.06	7.35	0.06	0.11	0.62		0.21
X1254_c3	G	0.08	0.14	0.03	1.03	1.52		0.07
X2088_c9	G	>50	>50	>50	>50	>50		>50
X2131_C1_B5	G	0.43	0.48	0.20	0.63	3.47		0.51
P1981_C5_3	G	0.74	0.72	0.05	0.25	0.14		0.46
X1632_S2_B10	G	15.69	>50	0.07	0.13	0.47		0.12
3016.v5.c45	D	1.38	>30	>30	1.16	0.20		0.16
A07412M1.vrc12	D	0.02	33.16	0.03	0.05	2.25		NT
231965.c01	D	0.05	0.51	0.02	2.22	0.51		0.34
231966.c02	D	0.29	5.05	1.11	0.10	0.54		NT
191821_E6_1	D (T/F)	0.09	0.57	0.09	1.20	0.36		NT
3817.v2.c59	CD	0.15	>50	>50	23.74	1.00		>50
6480.v4.c25	CD	0.01	0.05	0.02	0.05	0.16		0.04
6952.v1.c20	CD	0.15	0.13	0.02	4.82	1.38		0.04
6811.v7.c18	CD	0.03	2.36	0.03	0.22	5.34		0.09
89-F1_2_25	CD	>50	>50	>50	>50	>50		NT
3301.v1.c24	AC	0.01	0.08	0.01	0.05	>50		0.14
6041.v3.c23	AC	0.01	>50	0.01	0.01	>50		0.02
6540.v4.c1	AC	>50	>50	>50	0.11	>50		>50
6545.v4.c1	AC	>50	>50	>50	0.25	26.94		>50
0815.v3.c3	ACD	0.01	0.80	0.01	0.02	1.94		0.06
3103.v3.c10	ACD	0.22	0.26	1.66	0.70	48.07		0.93

Table S7, IC50

A

Virus ID	Clade	3BNC117	3BNC55	45-46	12A12	8ANC195		VRC01
6535.3	B	0.55	2.60	0.14	21.97	0.20		0.54
QH0692.42	B	0.13	2.45	0.55	1.92	2.71		1.50
SC422661.8	B	0.02	0.26	0.01	0.24	0.29		0.08
PVO.4	B	<0.09	3.90	0.17	0.93	0.52		0.22
TRO.11	B	<0.09	18.40	1.90	0.29	0.18		0.21
AC10.0.29	B	13.84	>50	0.42	1.15	0.88		2.20
RHPA4259.7	B	<0.09	2.20	<0.05	0.09	0.34		0.06
THRO4156.18	B	1.76	>50	1.59	3.05	>50		2.30
REJO4541.67	B	0.01	0.13	<0.05	0.19	0.08		0.06
TRJO4551.58	B	0.05	0.10	0.01	0.14	0.19		0.08
WITO4160.33	B	0.01	1.71	0.01	0.06	>50		0.15
CAAN5342.A2	B	0.42	4.10	0.11	1.32	>50		0.82
WEAU_d15_410_787	B (T/F)	0.05	0.10	0.01	0.05	>50		0.12
1006_11_C3_1601	B (T/F)	0.03	>50	0.05	0.22	0.43		0.15
1054_07_TC4_1499	B (T/F)	0.07	4.05	0.07	2.55	1.02		0.71
1056_10_TA11_1826	B (T/F)	0.30	1.25	0.12	0.58	>50		0.92
1012_11_TC21_3257	B (T/F)	0.02	0.22	0.01	0.27	>50		0.12
6240_08_TA5_4622	B (T/F)	0.33	2.51	0.44	1.21	>50		0.61
6244_13_B5_4576	B (T/F)	0.04	7.24	0.07	0.47	>50		0.21
62357_14_D3_4589	B (T/F)	0.06	>50	0.05	0.62	>50		0.96
SC05_8C11_2344	B (T/F)	0.15	1.93	0.14	2.03	0.47		0.64
Du156.12	C	0.02	0.07	0.01	0.04	0.22		0.09
Du172.17	C	1.19	3.52	>30	0.20	10.80		>50
Du422.1	C	>50	>50	>50	>50	>50		>50
ZM197M.PB7	C	0.22	0.53	0.14	1.24	23.45		0.36
ZM214M.PL15	C	0.06	3.10	0.05	0.37	0.91		0.44
ZM233M.PB6	C	0.13	0.64	1.86	39.19	7.39		2.00
ZM249M.PL1	C	0.03	0.06	0.02	0.06	>50		0.05
ZM53M.PB12	C	0.21	12.55	0.19	0.59	9.63		1.30
ZM109F.PB4	C	0.14	0.08	0.06	0.15	>30		0.13
ZM135M.PL10a	C	0.03	0.29	0.36	1.35	>50		0.35
CAP45.2.00.G3	C	3.88	>50	>50	1.83	28.30		2.30
CAP210.2.00.E8	C	17.22	>50	>50	>50	>50		>50
HIV-001428-2.42	C	<0.05	0.03	<0.001	0.02	>50		0.02
HIV-0013095-2.11	C	0.33	0.57	0.01	0.20	0.32		0.11
HIV-16055-2.3	C	5.60	>50	0.02	0.04	15.30		0.08
HIV-16845-2.22	C	27.46	>50	2.05	1.93	3.30		2.80
Ce1086_B2	C (T/F)	0.09	>50	0.04	0.12	4.42		NT
Ce0393_C3	C (T/F)	0.20	14.03	0.32	0.33	6.77		NT
Ce1176_A3	C (T/F)	0.22	>50	1.29	1.44	4.45		NT
Ce2010_F5	C (T/F)	0.05	1.32	0.13	0.33	>50		NT
Ce0682_E4	C (T/F)	0.03	1.71	0.07	0.11	0.20		NT
Ce1172_H1	C (T/F)	>50	>50	>50	>50	42.30		NT
Ce2060_G9	C (T/F)	0.24	37.85	0.14	0.48	6.20		NT
Ce703010054_2A2	C (T/F)	0.37	0.22	0.22	0.25	13.62		NT
BF1266.431a	C (T/F)	0.03	0.07	0.01	2.07	>50		NT
246F C1G	C (T/F)	19.32	0.28	>50	0.37	12.81		NT
249M B10	C (T/F)	0.10	0.12	0.04	0.11	>50		NT
ZM247v1(Rev-)	C (T/F)	>50	>50	1.64	0.27	3.41		NT
7030102001E5(Rev-)	C (T/F)	0.29	1.08	0.14	0.60	0.86		NT
1394C9G1(Rev-)	C (T/F)	>50	>50	0.09	0.32	>50		NT
Ce704809221_1B3	C (T/F)	0.08	>50	0.15	0.36	0.48		NT
CNE19	BC	0.02	10.38	0.07	0.13	0.29		NT
CNE20	BC	>50	>50	8.71	0.31	0.50		NT
CNE21	BC	45.38	>50	7.53	0.34	0.27		NT
CNE17	BC	5.76	2.39	0.15	1.74	2.59		NT
CNE30	BC	0.26	>50	0.34	0.68	7.52		NT
CNE52	BC	0.02	>50	0.03	0.08	5.59		NT
CNE53	BC	0.08	3.54	0.01	0.09	>50		NT
CNE58	BC	0.25	>50	0.25	0.07	>50		NT

Table S7, IC80

B

Virus ID	Clade*	3BNC117	3BNC55	45-46	12A12	8ANC195		VRC01
MS208.A1	A	0.09	2.41	0.60	0.44	>50		0.462
Q23.17	A	0.02	32.12	0.60	0.18	2.55		0.261
Q461.e2	A	0.09	>50	0.32	0.45	2.51		1.6
Q769.d22	A	0.04	2.55	0.04	0.11	1.20		0.289
Q259.d2.17	A	0.05	2.16	0.06	0.06	>50		0.543
Q842.d12	A	0.01	0.06	0.03	0.06	>30		0.096
3415.v1.c1	A	0.47	0.39	0.07	0.42	17.74		0.15
3365.v2.c2	A	0.10	2.34	0.11	0.19	>30		0.17
191955_A11	A (T/F)	>50	>50	0.43	>50	>50		NT
191084 B7-19	A (T/F)	0.23	0.55	0.07	0.22	>50		NT
9004SS_A3_4	A (T/F)	0.18	>50	0.65	0.30	0.81		NT
T257-31	CRF02_AG	0.37	>50	1.11	1.63	3.89		8.7
928-28	CRF02_AG	0.55	>50	0.39	0.79	>50		1.7
263-8	CRF02_AG	0.04	2.22	0.10	0.23	>50		0.55
T250-4	CRF02_AG	>15	1.92	>30	>30	>50		>50
T251-18	CRF02_AG	0.82	>30	5.26	9.39	1.51		11.2
T278-50	CRF02_AG	>15	>30	>30	>30	>50		>50
T255-34	CRF02_AG	0.14	1.58	2.38	0.26	8.09		2.7
211-9	CRF02_AG	1.28	>50	>50	47.27	8.43		>50
235-47	CRF02_AG	0.12	14.94	1.49	0.12	>50		0.17
620345.c01	CRF01_AE	>15	>30	>30	>30	>50		>50
703357.c02	CRF01_AE	>50	>50	>50	9.62	30.52		11.1
C1080.c03	CRF01_AE	1.73	>50	18.04	7.05	1.12		14.4
R2184.c04	CRF01_AE	0.10	0.16	0.12	0.37	12.98		0.32
R1166.c01	CRF01_AE	0.64	2.35	1.68	1.85	4.83		4.6
R3265.c06	CRF01_AE	13.04	>50	0.44	1.61	>50		1.9
C2101.c01	CRF01_AE	0.31	>50	>50	0.24	3.54		1.2
C3347.c11	CRF01_AE	0.13	1.16	0.18	0.19	>50		0.58
C4118.c09	CRF01_AE	0.52	>50	0.91	0.72	0.71		NT
CNE5	CRF01_AE	1.28	3.56	0.41	6.28	9.67		1.1
BJOX009000.02.4	CRF01_AE	1.79	5.58	1.16	>50	3.09		NT
BJOX015000.11.5	CRF01_AE (T/F)	0.32	>50	0.38	0.76	4.30		NT
BJOX010000.06.2	CRF01_AE (T/F)	10.45	10.75	6.37	>50	5.84		NT
BJOX025000.01.1	CRF01_AE (T/F)	0.20	>50	10.00	1.54	>50		NT
BJOX028000.10.3	CRF01_AE (T/F)	0.05	>50	>50	0.06	2.83		NT
X1193_c1	G	0.25	0.54	0.16	0.87	1.66		0.32
P0402_c2_11	G	0.38	>50	0.26	0.49	6.35		0.59
X1254_c3	G	0.27	0.57	0.08	5.78	6.95		0.19
X2088_c9	G	>50	>50	>50	>50	>50		>50
X2131_C1_B5	G	1.96	2.12	0.90	2.94	>50		1.5
P1981_C5_3	G	3.62	3.26	0.22	1.18	1.15		1.3
X1632_S2_B10	G	>50	>50	>50	0.87	4.11		0.74
3016.v5.c45	D	>30	>30	>30	11.86	0.87		0.42
A07412M1.vrc12	D	0.10	>50	0.13	0.27	7.49		NT
231965.c01	D	0.22	2.78	0.10	15.10	2.36		1.2
231966.c02	D	2.37	>50	11.36	0.51	2.98		NT
191821_E6_1	D (T/F)	0.51	3.17	0.46	6.66	1.61		NT
3817.v2.c59	CD	0.52	>50	>50	>50	5.30		>50
6480.v4.c25	CD	0.04	0.18	0.08	0.18	1.13		0.09
6952.v1.c20	CD	0.75	0.46	0.07	>50	7.22		0.12
6811.v7.c18	CD	0.17	15.22	0.13	0.83	27.63		0.26
89-F1_2_25	CD	>50	>50	>50	>50	>50		NT
3301.v1.c24	AC	0.05	0.37	0.03	0.18	>50		0.32
6041.v3.c23	AC	0.07	>50	0.04	0.04	>50		0.08
6540.v4.c1	AC	>50	>50	>50	>50	>50		>50
6545.v4.c1	AC	>50	>50	>50	7.42	>50		>50
0815.v3.c3	ACD	0.02	34.86	0.03	0.05	1.94		0.13
3103.v3.c10	ACD	0.85	0.78	6.57	2.49	>50		2.5

Table S7, IC80

B

Virus ID	Clade*	3BNC117	3BNC55	45-46	12A12	8ANC195		VRC01
6535.3	B	2.44	22.70	0.28	>50	0.91		2.7
QH0692.42	B	0.49	9.04	1.56	7.12	17.08		4.8
SC422661.8	B	0.08	1.24	0.07	1.51	4.67		0.265
PVO.4	B	0.19	14.70	0.47	3.15	1.87		1.2
TRO.11	B	<0.09	>50	9.56	0.98	0.89		0.832
AC10.0.29	B	>50	>50	1.49	5.08	7.00		6.5
RHPA4259.7	B	<0.09	21.20	<0.05	0.40	1.56		0.185
THRO4156.18	B	10.14	>50	6.02	12.79	>50		23
REJO4541.67	B	0.05	1.34	0.010	1.13	0.68		0.251
TRJO4551.58	B	0.19	0.41	0.05	0.44	1.17		0.207
WITO4160.33	B	0.04	26.55	0.08	0.33	>50		0.412
CAAN5342.A2	B	1.51	15.32	0.33	4.93	>50		2.8
WEAU_d15_410_787	B (T/F)	0.19	0.49	0.03	0.23	>50		0.26
1006_11_C3_1601	B (T/F)	0.10	>50	0.31	0.96	1.98		0.39
1054_07_TC4_1499	B (T/F)	0.49	23.30	0.61	9.64	6.75		2.9
1056_10_TA11_1826	B (T/F)	1.82	7.00	0.59	3.65	>50		3.3
1012_11_TC21_3257	B (T/F)	0.07	1.58	0.04	1.19	>50		0.32
6240_08_TA5_4622	B (T/F)	1.17	9.30	1.55	4.31	>50		1.8
6244_13_B5_4576	B (T/F)	0.15	43.45	0.26	1.64	>50		0.53
62357_14_D3_4589	B (T/F)	0.26	>50	0.22	4.15	>50		4.7
SC05_8C11_2344	B (T/F)	0.51	6.83	0.38	7.00	3.06		1.9
Du156.12	C	0.08	0.24	0.05	0.16	1.56		0.193
Du172.17	C	8.90	>30	>30	0.99	>30		>50
Du422.1	C	>50	>50	>50	>50	>50		>50
ZM197M.PB7	C	1.03	2.64	0.64	6.16	>50		1.6
ZM214M.PL15	C	0.52	>50	0.39	1.45	12.30		2.6
ZM233M.PB6	C	0.85	4.18	16.52	>50	43.45		9.3
ZM249M.PL1	C	0.11	0.27	0.06	0.22	>50		0.232
ZM53M.PB12	C	0.85	>30	0.65	2.07	>30		4
ZM109F.PB4	C	0.88	0.40	0.22	0.70	>30		0.754
ZM135M.PL10a	C	0.13	1.37	3.11	6.45	>50		2.7
CAP45.2.00.G3	C	>50	>50	>50	36.51	>50		>50
CAP210.2.00.E8	C	>50	>50	>50	>50	>50		>50
HIV-001428-2.42	C	0.02	0.10	0.01	0.07	>50		0.06
HIV-0013095-2.11	C	2.53	2.14	0.03	0.97	1.85		0.33
HIV-16055-2.3	C	>50	>50	0.06	0.16	>50		0.26
HIV-16845-2.22	C	>50	>50	12.10	10.02	20.06		12.7
Ce1086_B2	C (T/F)	0.31	>50	0.28	0.62	19.11		NT
Ce0393_C3	C (T/F)	0.67	>50	1.07	1.52	>50		NT
Ce1176_A3	C (T/F)	0.75	>50	5.55	4.69	35.17		NT
Ce2010_F5	C (T/F)	0.21	6.20	0.43	1.12	>50		NT
Ce0682_E4	C (T/F)	0.15	18.38	0.25	0.45	1.29		NT
Ce1172_H1	C (T/F)	>50	>50	>50	>50	>50		NT
Ce2060_G9	C (T/F)	0.81	>50	0.48	2.18	>50		NT
Ce703010054_2A2	C (T/F)	1.67	1.08	0.97	1.16	>50		NT
BF1266.431a	C (T/F)	0.09	0.22	0.03	26.46	>50		NT
246F C1G	C (T/F)	>50	1.00	>50	1.81	>50		NT
249M B10	C (T/F)	0.40	0.47	0.17	0.41	>50		NT
ZM247v1(Rev-)	C (T/F)	>50	>50	>50	1.73	25.12		NT
7030102001E5(Rev-)	C (T/F)	1.44	5.78	0.63	4.40	8.32		NT
1394C9G1(Rev-)	C (T/F)	>50	>50	0.38	2.51	>50		NT
Ce704809221_1B3	C (T/F)	0.31	<50	0.88	2.39	3.52		NT
CNE19	BC	0.08	>50	0.37	0.46	1.73		NT
CNE20	BC	>50	>50	47.01	1.94	3.19		NT
CNE21	BC	>50	>50	>50	1.72	1.52		NT
CNE17	BC	42.98	8.93	0.55	6.23	11.07		NT
CNE30	BC	0.85	>50	1.48	2.23	40.45		NT
CNE52	BC	0.06	>50	0.12	0.34	43.09		NT
CNE53	BC	0.99	>50	0.04	0.32	>50		NT
CNE58	BC	2.03	>50	1.49	0.26	>50		NT

Table S7

C

Average (μg/ml)

IC50

		3BNC117	3BNC55	45-46	12A12	8ANC195		VRC01
CLADE B								
Titer <50μg/ml (%)		100.0	81.0	100.0	100.0	57.1		100.0
Titer <1μg/ml (%)		90.5	23.8	90.5	61.9	47.6		85.7
Average (μg/ml)		0.9	3.1	0.3	1.9	0.6		0.6
CLADE C								
Titer <50μg/ml (%)		87.10	64.52	80.65	90.32	67.74		81.25
Titer <1μg/ml (%)		67.74	38.71	67.74	67.74	19.35		56.25
Average (μg/ml)		2.98	3.72	0.36	1.94	9.29		0.77
CLADE BC								
Titer <50μg/ml (%)		87.50	37.50	100.00	100.00	75.00		
Titer <1μg/ml (%)		62.50	0.00	75.00	87.50	37.50		
Average (μg/ml)		6.47	5.44	2.14	0.43	2.79		
CLADE A								
Titer <50μg/ml (%)		90.91	81.82	100.00	90.91	45.45		100.00
Titer <1μg/ml (%)		90.91	63.64	100.00	90.91	27.27		100.00
Average (μg/ml)		0.04	4.14	0.06	0.07	1.20		0.14
CRF02_AG								
Titer <50μg/ml (%)		77.78	44.44	66.67	77.78	44.44		77.78
Titer <1μg/ml (%)		77.78	33.33	55.56	55.56	33.33		44.44
Average (μg/ml)		0.13	0.50	0.39	1.52	0.50		2.99
CRF01_AE								
Titer <50μg/ml (%)		93.33	46.67	93.33	86.67	86.67		88.89
Titer <1μg/ml (%)		80.00	26.67	73.33	73.33	60.00		55.56
Average (μg/ml)		2.67	0.97	2.19	1.99	5.27		1.32
CLADE G								
Titer <50μg/ml (%)		85.71	71.43	85.71	85.71	85.71		85.71
Titer <1μg/ml (%)		71.43	57.14	85.71	71.43	57.14		85.71
Average (μg/ml)		2.84	1.76	0.07	0.38	1.08		0.25
CLADE D								
Titer <50μg/ml (%)		100.00	80.00	80.00	100.00	100.00		
Titer <1μg/ml (%)		80.00	40.00	60.00	40.00	80.00		
Average (μg/ml)		0.37	9.82	0.31	0.95	0.77		
CD/AC/ACD								
Titer <50μg/ml (%)		72.73	54.55	63.64	90.91	63.64		70.00
Titer <1μg/ml (%)		72.73	45.45	54.55	72.73	18.18		70.00
Average (μg/ml)		0.07	0.61	0.25	3.00	12.12		0.19

IC80

		3BNC117	3BNC55	45-46	12A12	8ANC195		VRC01
CLADE B								
Titer <50μg/ml (%)		95.2	76.2	100.0	95.2	57.1		100.0
Titer <1μg/ml (%)		71.4	9.5	76.2	28.6	14.3		47.6
Average (μg/ml)		1.0	12.8	1.1	3.5	4.0		2.8
CLADE C								
Titer <50μg/ml (%)		71.0	48.4	77.4	87.1	35.5		75.0
Titer <1μg/ml (%)		54.8	22.6	61.3	32.3	0.0		37.5
Average (μg/ml)		1.0	2.8	1.9	4.3	15.6		2.9
CLADE BC								
Titer <50μg/ml (%)		75.0	12.5	87.5	100.0	75.0		
Titer <1μg/ml (%)		50.0	0.0	50.0	50.0	0.0		
Average (μg/ml)		7.8	8.9	7.3	1.7	16.8		
CLADE A								
Titer <50μg/ml (%)		90.9	72.7	100.0	90.9	45.5		100.0
Titer <1μg/ml (%)		90.9	27.3	100.0	90.9	9.1		87.5
Average (μg/ml)		0.1	5.3	0.3	0.2	5.0		0.4
CRF02_AG								
Titer <50μg/ml (%)		77.8	44.4	66.7	77.8	44.4		66.7
Titer <1μg/ml (%)		66.7	0.0	22.2	44.4	0.0		22.2
Average (μg/ml)		0.5	5.2	1.8	8.5	4.4		4.2
CRF01_AE								
Titer <50μg/ml (%)		86.7	40.0	73.3	80.0	73.3		88.9
Titer <1μg/ml (%)		53.3	6.7	40.0	40.0	6.7		22.2
Average (μg/ml)		2.4	3.9	3.6	2.5	7.2		4.4
CLADE G								
Titer <50μg/ml (%)		71.4	57.1	71.4	85.7	71.4		85.7
Titer <1μg/ml (%)		42.9	28.6	71.4	42.9	0.0		57.1
Average (μg/ml)		1.3	1.6	0.3	2.0	4.0		0.8
CLADE D								
Titer <50μg/ml (%)		80.0	40.0	80.0	100.0	100.0		
Titer <1μg/ml (%)		60.0	0.0	60.0	40.0	20.0		
Average (μg/ml)		0.8	3.0	3.0	6.9	3.1		
CD/AC/ACD								
Titer <50μg/ml (%)		72.7	54.5	63.6	63.6	45.5		70.0
Titer <1μg/ml (%)		72.7	36.4	54.5	45.5	0.0		54.5
Average (μg/ml)		0.3	8.6	1.0	1.6	8.6		0.5

Table S7 Neutralizing activity of selected antibodies against an extended Tier 2 virus panel.

Colors indicate concentration at IC50 (**A**) and IC80 (**B**):
red ≤ 0.1μg/ml; orange 0.1-1 μg/ml; yellow 1-10μg/ml; green ≥10μg/ml; white not neutralized at any concentration tested. Previously published neutralization data from antibody VRC01 (*5*) is added as control. (**C**) Summary table of neutralizing activity shows the % of viruses from the indicated clades neutralized <50 and <1μg/ml respectively. The Average expresses the arithemtic mean concentrations needed to neutralize the viruses that are sensitive to the respective antibodies.

Table S8

A

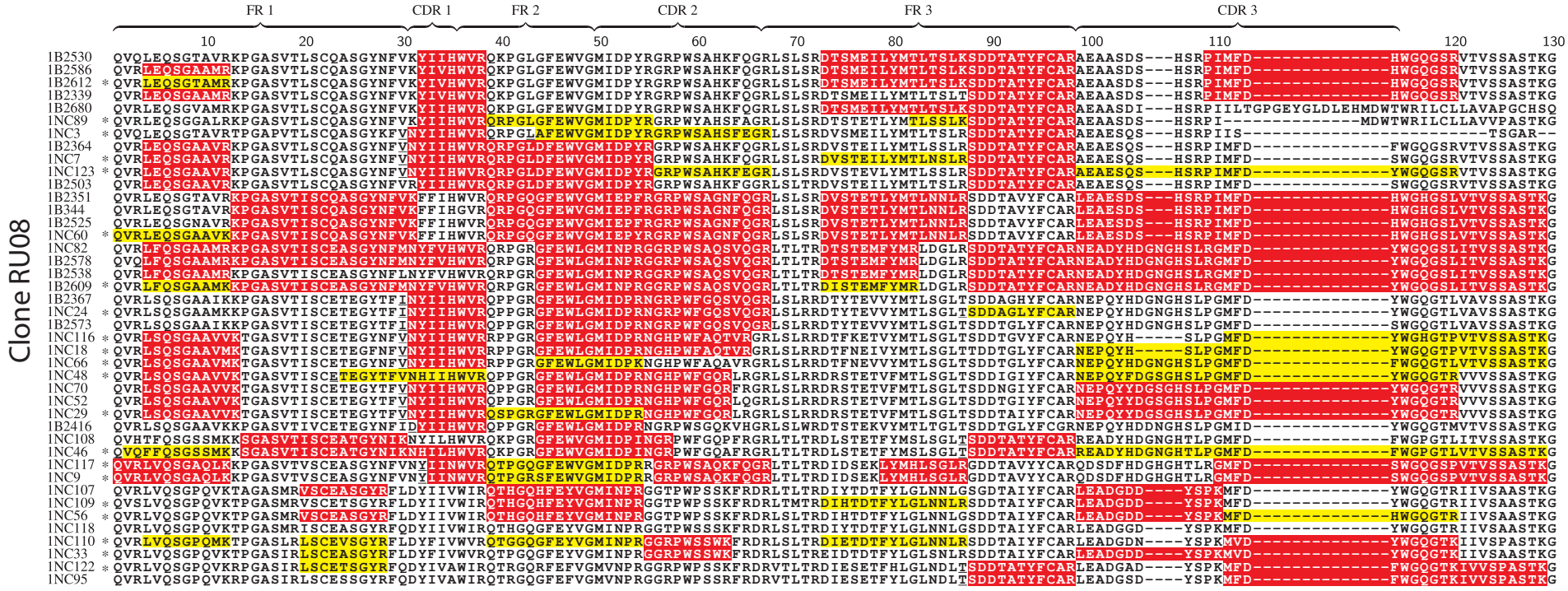

Clone RU08
FR 1
CDR 1
FR 2
CDR 2
FR 3
CDR 3
10
20
30
40
50
60
70
80
90
100
110
120
130
1B2530
1B2586
1B2612
1B2339
1B2680
1NC89
1NC3
1B2364
1NC7
1NC123
1B2503
1B2351
1B344
1B2525
1NC60
1NC82
1B2578
1B2538
1B2609
1B2367
1NC24
1B2573
1NC116
1NC18
1NC66
1NC48
1NC70
1NC52
1NC29
1B2416
1NC108
1NC46
1NC117
1NC9
1NC107
1NC109
1NC56
1NC118
1NC110
1NC33
1NC122
1NC95

Table S8
B

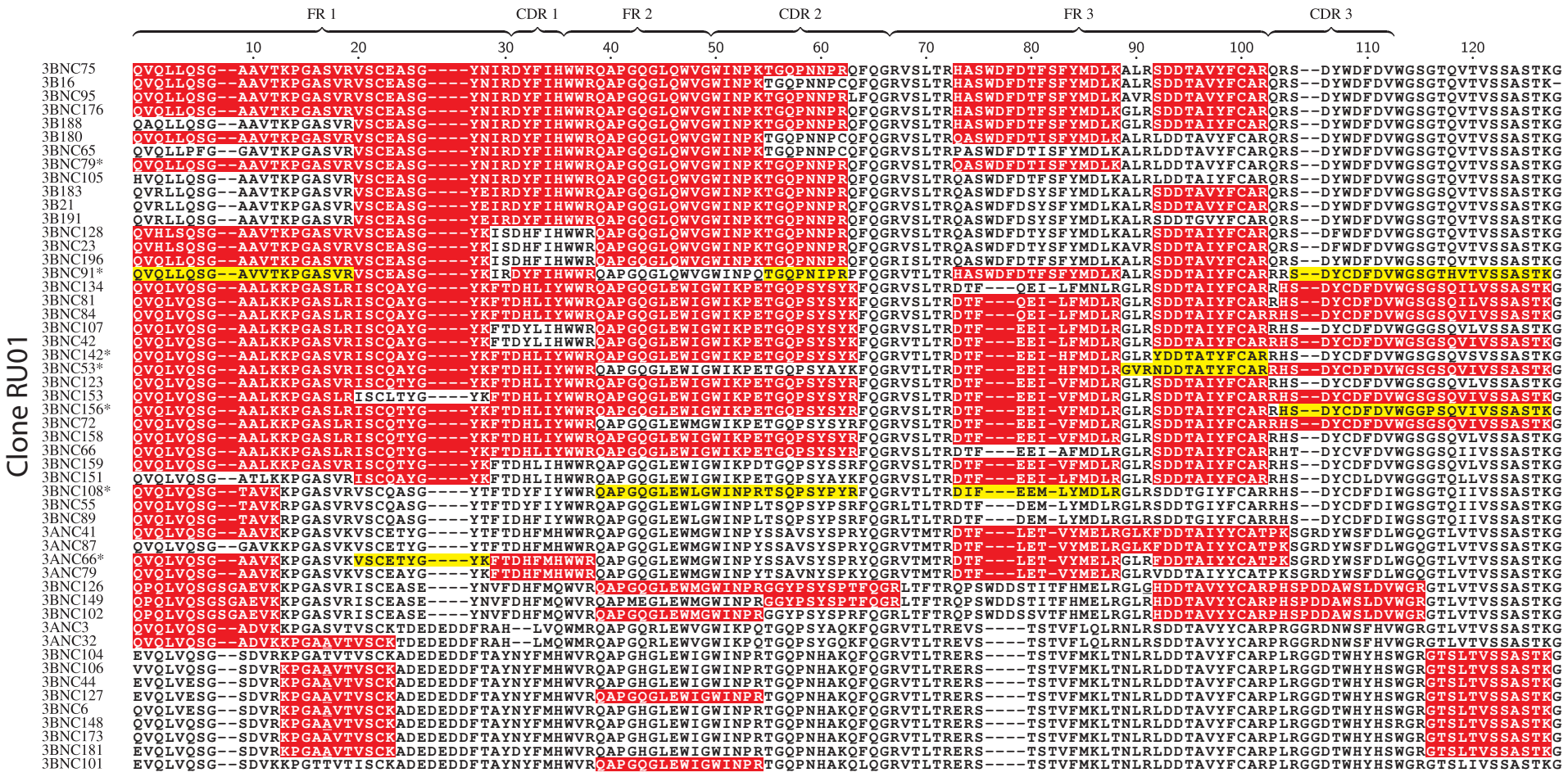
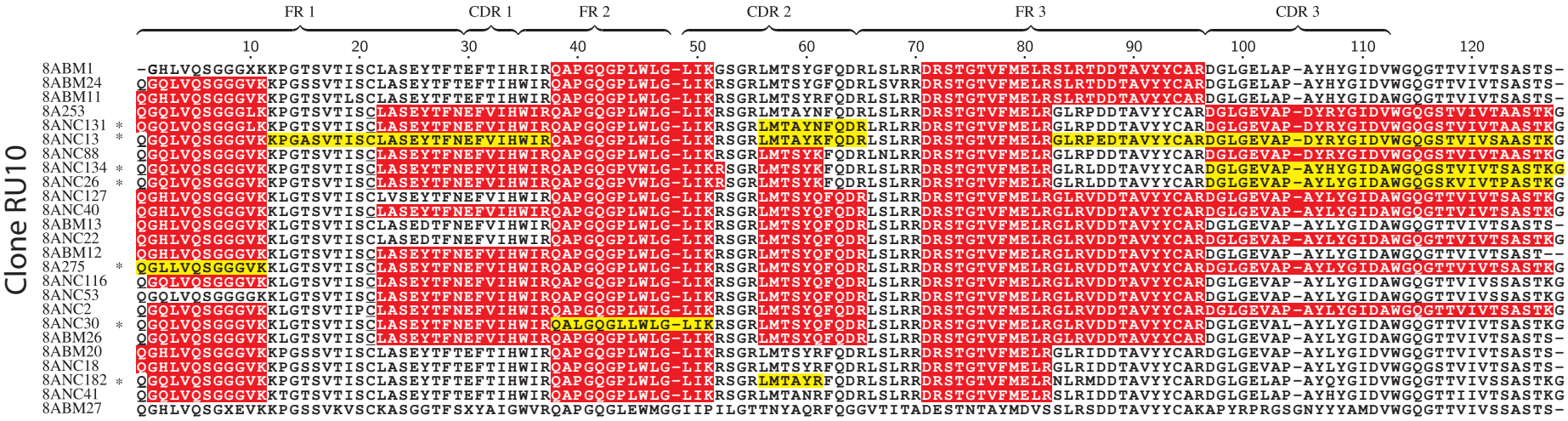

FR 1
CDR 1
FR 2
CDR 2
FR 3
CDR 3
10
20
30
40
50
60
70
80
90
100
110
120
Clone RU01
3BNC75 QVQLLQSG--AAVTKPGASVRVSCEASG----YNIRDYFIHWWRQAPGQGLQWVGWINPKTGQPNNPRQFQGRVSLTRHASWDFDTFSFYMDLKALRSDDTAVYFCARQRS--DYWDFDVWGSGTQVTVSSASTKG
3B16 QVQLLQSG--AAVTKPGASVRVSCEASG----YNIRDYFIHWWRQAPGQGLQWVGWINPKTGQPNNPCQFQGRVSLTRHASWDFDTFSFYMDLKALRSDDTAVYFCARQRS--DYWDFDVWGSGTQVTVSSASTK-
3BNC95 QVQLLQSG--AAVTKPGASVRVSCEASG----YNIRDYFIHWWRQAPGQGLQWVGWINPKTGQPNNPRLFQGRVSLTRHASWDFDTFSFYMDLKAVRSDDTAVYFCARQRS--DYWDFDVWGSGTQVTVSSASTKG
3BNC176 QVQLLQSG--AAVTKPGASVRVSCEASG----YNIRDYFIHWWRQAPGQGLQWVGWINPKTGQPNNPRQFQGRVSLTRHASWDFDTFSFYMDLKGLRSDDTAIYFCARQRS--DYWDFDVWGSGTQVTVSSASTKG
3B188 QAQLLQSG--AAVTKPGASVRVSCEASG----YNIRDYFIHWWRQAPGQGLQWVGWINPKTGQPNNPRQFQGRVSLTRHASWDFDTFSFYMDLKGLRSDDTAIYFCARQRS--DYWDFDVWGSGTQVTVSSASTKG
3B180 QVQLLQSG--AAVTKPGASVRVSCEASG----YNIRDYFIHWWRQAPGQGLQWVGWINPKTGQPNNPCQFQGRVSLTRQASWDFDTISFYMDLKALRLDDTAVYFCARQRS--DYWDFDVWGSGTQVTVSSASTKG
3BNC65 QVQLLPFG--GAVTKPGASVRVSCEASG----YNIRDYFIHWWRQAPGQGLQWVGWINPKTGQPNNPCQFQGRVSLTRPASWDFDTISFYMDLKALRLDDTAVYFCARQRS--DYWDFDVWGSGTQVTVSSASTKG
3BNC79* QVQLLQSG--AAVTKPGASVRVSCEASG----YNIRDYFIHWWRQAPGQGLQWVGWINPKTGQPNNPRQFQGRVSLTRQASWDFDTISFYMDLKALRLDDTAVYFCARQRS--DYWDFDVWGSGTQVTVSSASTKG
3BNC105 HVQLLQSG--AAVTKPGASVRVSCEASG----YNIRDYFIHWWRQAPGQGLQWVGWINPKTGQPNNPRQFQGRVSLTRQASWDFDTFSFYMDLKALRLDDTAIYFCARQRS--DYWDFDVWGSGTQVTVSSASTKG
3B183 QVRLLQSG--AAVTKPGASVRVSCEASG----YEIRDYFIHWWRQAPGQGLQWVGWINPKTGQPNNPRQFQGRVSLTRQASWDFDSYSFYMDLKALRSDDTAVYFCARQRS--DYWDFDVWGSGSQVTVSSASTKG
3B21 QVRLLQSG--AAVTKPGASVRVSCEASG----YEIRDYFIHWWRQAPGQGLQWVGWINPKTGQPNNPRQFQGRVSLTRQASWDFDSYSFYMDLKALRSDDTAVYFCARQRS--DYWDFDVWGSGTQVTVSSASTKG
3B191 QVRLLQSG--AAVTKPGASVRVSCEASG----YEIRDYFIHWWRQAPGQGLQWVGWINPKTGQPNNPRQFQGRVSLTRQASWDFDSYSFYMDLKALRSDDTGVYFCARQRS--DYWDFDVWGSGTQVTVSSASTKG
3BNC128 QVHLSQSG--AAVTKPGASVRVSCEASG----YKISDHFIHWWRQAPGQGLQWVGWINPKTGQPNNPRQFQGRVSLTRQASWDFDTYSFYMDLKALRSDDTAIYFCARQRS--DFWDFDVWGSGTQVTVSSASTKG
3BNC23 QVHLSQSG--AAVTKPGASVRVSCEASG----YKISDHFIHWWRQAPGQGLQWVGWINPKTGQPNNPRQFQGRVSLTRQASWDFDTYSFYMDLKAVRSDDTAIYFCARQRS--DFWDFDVWGSGTQVTVSSASTKG
3BNC196 QVQLLQSG--AAVTKPGASVRVSCEASG----YKISDHFIHWWRQAPGQGLQWVGWINPKTGQPNNPRQFQGRISLTRQASWDFDTFSFYMDLKALRSDDTAVYFCARQRS--DYWDFDVWGSGTQVTVSSASTKG
3BNC91* QVQLLQSG--AAVTKPGASVRVSCEASG----YKIRDYFIHWWRQAPGQGLQWVGWINPQTGQPNIPRPFQGRVTLTRHASWDFDTFSFYMDLKALRSDDTAIYFCARRRS--DYCDFDVWGSGTHVTVSSASTKG
3BNC134 QVQLVQSG--AALKKPGASLRISCQAYG----YKFTDHLIYWWRQAPGQGLEWIGWIKPETGQPSYSYKFQGRVSLTRDTF---QEI-LFMNLRGLRSDDTAIYFCARRHS--DYCDFDVWGSGSQILVSSASTKG
3BNC81 QVQLVQSG--AALKKPGASLRISCQAYG----YKFTDHLIYWWRQAPGQGLEWIGWIKPETGQPSYSYKFQGRVSLTRDTF---QEI-LFMDLRGLRSDDTAIYFCARRHS--DYCDFDVWGSGSQILVSSASTKG
3BNC84 QVQLVQSG--AALKKPGASLRISCQAYG----YKFTDHLIYWWRQAPGQGLEWIGWIKPETGQPSYSYKFQGRVSLTRDTF---QEI-LFMDLRGLRSDDTAIYFCARRHS--DYCDFDVWGSGSQVIVSSASTKG
3BNC107 QVQLVQSG--AALKKPGASLRISCQAYG----YKFTDYLIHWWRQAPGQGLEWIGWIKPETGQPSYSYKFQGRVSLTRDTF---EEI-LFMDLRGLRSDDTAIYFCARRHS--DYCDFDVWGGGSQVLVSSASTKG
3BNC42 QVQLVQSG--AALKKPGASVRISCQAYG----YKFTDYLIHWWRQAPGQGLEWIGWIKPETGQPSYSYKFQGRVTLTRDTF---EEI-LFMDLRGLRSDDTAIYFCARRHS--DYCDFDVWGSGSQVIVSSASTKG
3BNC142* QVQLVQSG--AALKKPGASVRISCQAYG----YKFTDHLIYWWRQAPGQGLEWIGWIKPETGQPSYSYKFQGRVTLTRDTF---EEI-HFMDLRGLRYDDTATYFCARRHS--DYCDFDVWGSGSQVSVSSASTKG
3BNC53* QVQLVQSG--AALKKPGASVRISCQAYG----YKFTDHLIYWWRQAPGQGLEWIGWIKPETGQPSYAYKFQGRVTLTRDTF---EEI-HFMDLRGVRNDDTATYFCARRHS--DYCDFDVWGSGSQVIVSSASTKG
3BNC123 QVQLVQSG--AALKKPGASVRISCQTYG----YKFTDHLIYWWRQAPGQGLEWIGWIKPETGQPSYSYRFQGRVSLTRDTF---EEI-VFMDLRGLRSDDTAIYFCARRHS--DYCDFDVWGSGSQVLVSSASTKG
3BNC153 QVQLVQSG--AALKKPGASLRISCLTYG----YKFTDHLIYWWRQAPGQGLEWIGWIKPETGQPSYSYRFQGRVSLTRDTF---EEI-VFMDLRGLRSDDTAIYFCARRHS--DYCDFDVWGSGSQVIVSSASTKG
3BNC156* QVQLVQSG--AALKKPGASLRISCQTYG----YKFTDHLIYWWRQAPGQGLEWIGWIKPETGQPSYSYRFQGRVSLTRDTF---EEI-VFMDLRGLRSDDTAIYFCARRHS--DYCDFDVWGGPSQVIVSSASTKG
3BNC72 QVQLVQSG--AALKKPGASLRISCQTYG----YKFTDHLIYWWRQAPGQGLEWMGWIKPETGQPSYSYRFQGRVSLTRDTF---EEI-VFMDLRGLRSDDTAIYFCARRHS--DYCDFDVWGSGSQVIVSSASTKG
3BNC158 QVQLVQSG--AALKKPGASLRISCQTYG----YKFTDHLIYWWRQAPGQGLEWIGWIKPETGQPSYSYRFQGRVSLTRDTF---EEI-VFMDLRGLRSDDTAIYFCARRHS--DYCDFDVWGSGSQVLVSSASTKG
3BNC66 QVQLVQSG--AALKKPGASLRISCQTYG----YKFTDHLIYWWRQAPGQGLEWIGWIKPETGQPSYSYRFQGRVSLTRDTF---EEI-AFMDLRGLRSDDTAIYFCARRHT--DYCVFDVWGSGSQIIVSSASTKG
3BNC159 QVQLVQSG--AALKKPGASVRISCQTYG----YKFTDHLIHWWRQAPGQGLEWIGWIKPDTGQPSYSSSRFQGRVSLTRDTF---EEI-VFMDLRGLRSDDTAIYFCARRHS--DYCDFDVWGSGSQVLVSSASTKG
3BNC151 QVQLVQSG--ATLKKPGASVRISCQAYG----YKFTDHLIHWWRQAPGQGLEWIGWIKPETGQPSYAYKFQGRVSLTRDTF---EEI-LFMDLRGLRSDDTAIYFCARRHS--DYCDLDVWGGGTQLLVSSASTKG
3BNC108* QVQLVQSG--TAVKKPGASVRVSCQASG----YTFTDYFIYWWRQAPGQGLEWLGWINPRTSQPSYPYRFQGRVTLTRDIF---EEM-LYMDLRGLRSDDTGIYFCARRHS--DYCDFDIWGSGTQIIVSSASTKG
3BNC55 QVQLVQSG--TAVKRPGASVRVSCQASG----YTFTDYFIYWWRQAPGQGLEWLGWINPLTSQPSYPSRFQGRLTLTRDTF---DEM-LYMDLRGLRSDDTGIYFCARRHS--DYCDFDIWGSGTQIIVSSASTKG
3BNC89 QVQLVQSG--TAVKRPGASVRVSCQASG----YTFIDHFIYWWRQAPGQGLEWLGWINPLTSQPSYPSRFQGRLTLTRDTF---DEM-LYMDLRGLRSDDTGIYFCARRHS--DYCDFDIWGSGTQIIVSSASTKG
3ANC41 QVQLVQSG--AAVKKPGASVKVSCETYG----YTFTDHFMHWWRQAPGQGLEWMGWINPYSSAVSYSPRYQGRVTMTRDTF---LET-VYMELRGLKFDDTAIYYCATPKSGRDYWSFDLWGQGTLVTVSSASTKG
3ANC87 QVQLVQSG--GAVKKPGASVKVSCETYG----YTFTDHFMHWWRQAPGQGLEWMGWINPYSSAVSYSPRYQGRVTMTRDTF---LET-VYMELRGLKFDDTAIYYCATPKSGRDYWSFDLWGQGTLVTVSSASTKG
3ANC66* QVQLVQSG--AAVKKPGASVKVSCETYG----YKFTDHFMHWWRQAPGQGLEWMGWINPYSSAVSYSPRYQGRVTMTRDTF---LET-VYMELRGLRFDDTAIYYCATPKSGRDYWSFDLWGQGTLVTVSSASTKG
3ANC79 QVQLVQSG--AAVKKPGASVKVSCEAYG----YKFTDHFMHWWRQAPGQGLEWMGWINPYTSAVNYSPKYQGRVTMTRDTF---LET-VYMELRGLRVDDTAIYYCATPKSGRDYWSFDLWGQGTLVTVSSASTKG
3BNC126 QPQLVQSGSGAEVKKPGASVRISCEASE----YNVFDHFMQWVRQAPGQGLEWMGWINPRGGYPSYSPTFQGRLTFTRQPSWDDSTITFHMELRGLGHDDTAVYYCARPHSPDDAWSLDVWGRGTLVTVSSASTKG
3BNC149 QPQLVQSGSGAEVKKPGASVRISCEASE----YNVFDHFMQWVRQAPMEGLEWMGWINPRGGYPSYSPTFQGRLTFTRQPSWDDSTITFHMELRGLRHDDTAVYYCARPHSPDDAWSLDVWGRGTLVTVSSASTKG
3BNC102 QPQLVQSGSGAEVKKPGASVRISCEASE----YNVFDHFMQWVRQAPGQGLEWMGWINPRGGYPSYSPRFQGRLTFTRQPSWDDSSVTFHMELRGLRHDDTAVYYCARPHSPDDAWSLDVWGRGTLVTVSSASTKG
3ANC3 QVQLVQSG--ADVKKPGASVTVSCKTDEDEDDFRAH--LVQWMRQAPGQRLEWVGWIKPQTGQPSYAQKFQGRVTLTREVS----TSTVFLQLRNLRSDDTAVYYCARPRGGRDNWSFHVWGRGTLVTVSSASTKG
3ANC32 QVQLVQSG--ADVKKPGAAVTVSCKTDEDEDDFRAH--LMQWMRQAPGQRLEWVGWIKPQTGQPSYGQKFQGRVTLTREVS----TSTVFLQLRNLRSDDTAVYYCARPRGGRDNWSFHVWGRGTLVTVSSASTKG
3BNC104 EVQLVQSG--SDVRKPGATVTVSCKADEDEDDFTAYNYFMHWVRQAPGHGLEWIGWINPRTGQPNHAKQFQGRVTLTRERS----TSTVFMKLTNLRLDDTAVYFCARPLRGGDTWHYHSWGRGTSLTVSSASTKG
3BNC106 VVQLVQSG--SDVRKPGAAVTVSCKADEDEDDFTAYNYFMHWVRQAPGHGLEWIGWINPRTGQPNHAKQFQGRVTLTRERS----TSTVFMKLTNLRLDDTAVYFCARPLRGGDTWHYHSWGRGTSLTVSSASTKG
3BNC44 EVQLVESG--SDVRKPGAAVTVSCKADEDEDDFTAYNYFMHWVRQAPGHGLEWIGWINPRTGQPNHAKQFQGRVTLTRERS----TSTVFMKLTNLRLDDTAVYFCARPLRGGDTWHYHSWGRGTSLTVSSASTKG
3BNC127 EVQLVESG--SDVRKPGAAVTVSCKADEDEDDFTAYNYFMHWVRQAPGQGLEWIGWINPRTGQPNHAKQFQGRVTLTRERS----TSTVFMKLTNLRLDDTAVYFCARPLRGGDTWHYHSWGRGTSLTVSSASTKG
3BNC6 QVQLVESG--SDVRKPGAAVTVSCKADEDEDDFTAYNYFMHWVRQAPGHGLEWIGWINPRTGQPNHAKQFQGRVTLTRERS----TSTVFMKLTNLRLDDTAVYFCARPLRGGDTWHYHSWGRGTSLTVSSASTKG
3BNC148 QVQLVQSG--SDVRKPGAAVTVSCKADEDEDDFTAYNYFMHWVRQAPGHGLEWIGWINPRTGQPNHAKQFQGRVTLTRERS----TSTVFMKLTNLRLDDTAVYFCARPLRGGDTWHYHSRGRGTSLTVSSASTKG
3BNC173 QVQLVQSG--SDVRKPGAAVTVSCKADEDEDDFTAYNYFMHWVRQAPGHGLEWIGWINPRTGQPNHAKQFQGRVTLTRERS----TSTVFMKLTNLRLDDTAVYFCARPLRGGDTWHYHSWGRGTSLTVSSASTKG
3BNC181 EVQLVQSG--SDVRKPGAAVTVSCKADEDEDDFTAYDYFMHWVRQAPGHGLEWIGWINPRTGQPNHAKQFQGRVTLTRERS----TSTVFMKLTNLRLDDTAVYFCARPLRGGDTWHYHSWGRGTSLTVSSASTKG
3BNC101 EVQLVQSG--SDVKKPGTTVTISCKADEDEDDFTAYNYFMHWVRQAPGQGLEWIGWINPRTGQPNHAKQLQGRVTLTRERS----TSTVFMKLTNLRLDDTAVYFCARPLRGGDTWHYHSWGRGTSLIVSSASTKG

Table S8
C



Antibody sequences from one expanded neutralizing clone in each (A) Pt1, (B) Pt3 and (C) Pt8. Clone labeling as in table S3. Peptides identified by mass spectrometry are indicated in color. The variants marked with an asterisk are uniquely defined by one or more mass spectrometrically observed peptides (shown in yellow). The remaining mass spectrometrically observed peptides map non-uniquely to multiple variants as shown in red. Underlined amino acids indicate non-tryptic cleavage sites in the variants shown. The cleavages are presumed to occur through chymotryptic cleavage or additional mutations (not observed among the cloned variants) that place a lysine or arginine residue at these sites.

Table S9

	gp140					2CC-core				
	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (M)	K_A (M^{-1})	χ^2	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (M)	K_A (M^{-1})	χ^2
12A12	4.59E+04	1.44E-05	**3.15E-10**	**3.18E+09**	0.77	6.33E+04	1.70E-06	**2.69E-11**	**3.71E+10**	0.68
12A21	9.18E+04	3.44E-07	**3.75E-12**	**2.67E+11**	4.99	1.82E+05	3.30E-04	**1.81E-09**	**5.53E+08**	3.06
12AGL	/	/	/	/		/	/	/	/	/
3BNC60	2.73E+04	1.86E-04	**6.81E-09**	**1.47E+08**	0.32	3.02E+04	1.64E-03	**5.45E-08**	**1.84E+07**	0.38
3BNC117	3.04E+04	1.99E-04	**6.54E-09**	**1.53E+08**	0.48	4.05E+04	1.49E-03	**3.68E-08**	**2.72E+07**	0.33
3BNC55	1.31E+04	7.55E-04	**5.78E-08**	**1.73E+07**	0.19	3.16E+04	8.15E-04	**2.57E-08**	**3.88E+07**	0.55
3BNC66	1.60E+04	1.41E-03	**8.81E-08**	**1.14E+07**	0.10	3.96E+04	1.33E-03	**3.36E-08**	**2.98E+07**	0.12
3BNC156	1.13E+04	1.98E-03	**1.75E-07**	**5.70E+06**	0.07	1.88E+04	1.53E-03	**8.12E-08**	**1.23E+07**	0.12
3BNC108	/	/	/	/	/	/	/	/	/	/
3BNC60Gl	/	/	/	/	/	/	/	/	/	/
8ANC131	6.59E+04	1.09E-03	**1.65E-08**	**6.05E+07**	2.64	4.88E+04	3.23E-03	**6.61E-08**	**1.51E+07**	0.31
8ANC134	1.55E+04	1.74E-03	**1.13E-07**	**8.86E+06**	0.07	2.08E+04	9.57E-04	**4.61E-08**	**2.17E+07**	0.09
8AGL	/	/	/	/		/	/	/	/	/
8ANC195	4.88E+04	1.67E-03	**3.43E-08**	**2.92E+07**	0.24	2.41E+04	1.32E-03	**5.47E-08**	**1.83E+07**	0.17
LSSNEC9	4.83E+04	5.81E-04	**1.20E-08**	**8.31E+07**	1.19	5.11E+04	2.36E-04	**4.61E-09**	**2.17E+08**	0.29
LSSB2530	4.74E+04	1.62E-03	**3.42E-08**	**2.93E+07**	0.61	6.83E+04	4.02E-04	**5.90E-09**	**1.70E+08**	0.36
LSSGL	/	/	/	/		/	/	/	/	/
45-46	4.26E+04	2.87E-04	**6.75E-09**	**1.48E+08**	0.88	1.12E+05	4.94E-04	**4.40E-09**	**2.27E+08**	0.94
VRC01	1.83E+04	8.08E-06	**4.41E-10**	**2.27E+09**	0.08	2.84E+04	3.25E-05	**1.15E-09**	**8.73E+08**	0.10

M, mol/l; s, seconds. /, no binding detected or too low to be determined with accuracy.

A Khi² value (χ^2) < 10 indicates that the fitting model used adequately describes the experimental data.

Affinity of IgG antibodies to YU2-gp140 and 2CC-core ligands measured by surface plasmon resonance.

Table S10

A

	All Nucleotides	Consensus Nucleotides	Non Consensus Nucleotides
3BNC117HC	1.8	1.0	3.5
3BNC60HC	2.0	1.1	4.4
12A12HC	2.8	1.7	6.3
12A21HC	2.6	1.5	4.8
NIH45-46HC	1.7	0.9	5.5
VRCO1HC	2.2	1.1	22.0
8ANC131HC	2.7	1.3	8.0
8ANC134HC	2.2	1.5	3.7
1B2530HC	2.0	0.9	11.0
1NC9HC	1.9	0.7	12.0

B

	All Nucleotides	Consensus Nucleotides	Non Consensus Nucleotides
3BNC117KC	1.7	0.8	2.8
3BNC60KC	1.7	0.7	4.0
12A12KC	1.7	0.6	4.0
12A21KC	2.5	1.4	4.3
NIH45-46KC	1.7	0.9	3.0
VRCO1KC	1.8	0.8	4.0
8ANC131KC	1.5	0.5	4.2
8ANC134KC	1.5	0.5	4.2
1B2530LC	1.9	2.0	1.8
1NC9LC	1.2	0.9	1.8

Replacement/Silent mutation ratios for heavy (A) and light chain (B) sequences of 10 selected antibodies.

Table S11. Crystallization data collection and refinement statistics

Crystal	3BN60 Fab
Data collection*	
Wavelength (Å)	0.9537
Space group	$P2_1$
Unit Cell dimensions	
a (Å)	63.6
b (Å)	155.7
c (Å)	74.8
α, β, γ (ϒ)	90.0, 110.4, 90.0
Resolution, (Å)	39.17-2.65
R_{mrgd}-F (%)§	8.3 (55.5)
R_{meas} (%)§	7.7 (53.4)
I / σI	15.7 (2.5)
Completeness (%)	96.0 (68.1)
Multiplicity	5.0 (3.6)
Reflections	192709
Unique reflections	38111
Refinement	
Resolution (Å)	39.17-2.65
No. reflections	37086
R_{work} / R_{free} (%)†	20.7 / 25.7
RMSD Bond lengths (Å)	0.01
RMSD Bond angles (ϒ)	1.3
Average B-factor Å^2	64.9
Ramachandran analysis	
Favored (%)	91.9
Allowed (%)	7.6
Outlier (%)	0.5

* Values in parentheses are for the highest resolution shell (2.72-2.65).
† 5% of unique reflections were removed as a test set for the Rfree calculation.
§ Rmrgd and Rmeas are described in (*51*)

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