Supporting Information

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SI Materials and Methods

Antibody Nomenclature. The previously isolated PGT antibodies from the Protocol G cohort were isolated through Theraclone screening, and thus were named "PGT." To distinguish between antibodies isolated from Theraclone (by B-cell culture) and antibodies isolated by investigators of this study (by antigen selection), we have named the newly isolated variants "PGDM." We start the series with "14" to indicate that they are related to the PGT140 series, but we have added an extra digit to the end of the name because we have >10 antibodies. The somatic variants therefore are named PGDM1400–1412.

Human Specimens. PBMCs were obtained from donor 84, an HIV-1–infected donor from the IAVI Protocol G cohort (1). All human samples were collected with written informed consent under clinical protocols approved by the Republic of Rwanda National Ethics Committee, the Emory University Institutional Review Board, the University of Zambia Research Ethics Committee, the Charing Cross Research Ethics Committee, the Uganda Virus Research Institute Science and Ethics Committee, the University of New South Wales Research Ethics Committee, St. Vincent's Hospital and Eastern Sydney Area Health Service, Kenyatta National Hospital Ethics and Research Committee, University of Cape Town Research Ethics Committee, the International Institutional Review Board, the Mahidol University Ethics Committee, the Walter Reed Army Institute of Research Institutional Review Board, and the Ivory Coast Comité National d'Éthique des Sciences de la Vie et de la Santé.

Data and Materials Availability. Gene sequences of the reported antibodies have been deposited in GenBank under the accession numbers KP006370–KP006382 for heavy-chain sequences and KP006383–KP006395 for kappa-chain sequences.

Construct Design. For this study, a variant of the recombinant envelope protein BG505 SOSIP.664 gp140 trimer (2) bearing an avi tag at the C terminus of the gp41 ectodomain (gp41ECTO) was made by adding the amino acid sequence GSGLNDIFEAQKIEWHE after residue 664 in gp41ECTO and preceding the stop codon. This protein is designated "SOSIP.664-Avi gp140." A monomeric BG505 gp120 with a sequence identical to the gp120 components of the gp140 trimer also was constructed with an avi tag, designated "BG505-avi" and includes the L111A substitution to decrease gp120 dimer formation (3, 4). BG505 SOSIP.664 containing a polyhistidine tag (BG505 SOSIP.664-His gp140) was used as described previously (2).

Recombinant Env Trimer Expression. The recombinant envelope proteins BG505 SOSIP.664-avi gp140, BG505 SOSIP.664-His gp140, and BG505-avi gp120 were expressed in HEK293F cells (Invitrogen) as described previously (2). Briefly, HEK293F cells were maintained in FreeStyle medium (Invitrogen). For gp140 trimer production, HEK293F cells were seeded at a density of 0.5×10^6/mL. After 24 h, cells were transfected with 1 mg of 293Fectin (Invitrogen) with 300 μg of Env plasmid and 75 μg of furin plasmid in Opti-MEM (Life Technologies) according to the manufacturer's protocol. Kifunensine-treated proteins were produced by adding kifunensine (Tocris) to HEK293F cells at a final concentration of 25 μM on the day of transfection. Culture supernatants were harvested 6–7 d after transfection. SDS/PAGE and Blue Native-PAGE were performed as described previously (2).

Recombinant Env Trimer Purification. BG505 SOSIP.664-avi gp140 and BG505 SOSIP.664-His gp140 were purified by affinity chromatography using a 2G12 column as described previously (2). BG505-avi gp120 proteins were purified using a *Galanthus nivalis* lectin (Vector Labs) column (2). Briefly, transfection supernatants were vacuum-filtered through 0.2-μm filters and then passed over the column at a 0.5–1 mL/min flow rate. The 2G12 column was made from cyanogen bromide-activated Sepharose 4B beads (GE Healthcare) coupled to the bnAb 2G12 (Polymun Sciences). Purification using this column was performed as follows: The beads were washed with two column volumes of buffer [0.5 M NaCl, 20 mM Tris (pH 8.0)] before eluting bound Env proteins using one column volume of 3 M $MgCl_2$ into one column volume of 75 mM NaCl, 10 mM Tris (pH 8.0). The eluted Env proteins were concentrated using Vivaspin columns with a 100-kDa cut off (GE Healthcare). The affinity-purified Env proteins were purified further to size homogeneity using size-exclusion chromatography on a Superose 6 10/300 GL column (GE Healthcare) in PBS. The trimer fractions were collected and pooled, and protein concentrations were determined using either a bicinchonic acid-based assay (Thermo Scientific) or UV280 absorbance using theoretical extinction coefficients (5). The Env proteins were biotinylated in vitro using the BirA enzyme (Avidity) according to the manufacturer's protocol.

ELISA for Monomeric and Trimeric BG505 Env Proteins. ELISAs were performed as described previously (2) with minor modifications. Microlon 96-well plates (Corning) were coated overnight with streptavidin (Thermo Scientific), anti-biotin antibody (Roche), or anti-His antibody (Thermo Scientific) at 2.5 μg/mL in PBS (50 μL per well). After washing and blocking with 3% BSA for 1 h at room temperature, biotinylated BG505 SOSIP.664-Avi gp140, BG505 SOSIP.664-His gp140, or BG505-avi gp120 proteins were added at 1 μg/mL in PBS/1% BSA for 2 h at 37 °C. Unbound Env proteins were washed away, and serially diluted mAbs in PBS/1% BSA then were added for 2 h at room temperature. Unbound mAbs were washed away, and alkaline phosphatase-labeled goat anti-human IgG (Jackson ImmunoResearch) was added for 1 h at a 1:1,000 dilution (final concentration 0.33 μg/mL) in PBS/1% BSA at room temperature. After washing, absorption was measured at 405 nm. For binding to gp120 extracted from lysed virions, plates were coated with 5 ng/uL of sheep D7324 anti-gp120 antibody (Aalto Bioreagents). Virus supernatants were lysed using a final concentration of 1% Nonidet P-40 and incubated on coated plates for 2 h at 37 °C. Detection was measured using goat anti-human IgG $F(ab')_2$ conjugated to alkaline phosphatase (Pierce). Antibody concentration was calculated by linear regression using a standard concentration curve of purified IgG protein.

Competition ELISA. For competition ELISA experiments, competing antibodies were biotinylated using an antibody biotinylation kit (Thermo Scientific). Plates were coated with an anti-His antibody (Roche) at 5 μg/mL overnight. After washing, plates were blocked with 3% BSA for 1 h at room temperature. BG505 SOSIP.664-His gp120 then was captured at 2.5 μg/mL in PBS (50 μL per well) for 2 h at 37 °C. After washing, serially diluted antibodies in PBS/1% BSA were added for 30 min. The biotinylated antibody was added at a constant EC_{70} concentration for 1 h. Plates were washed, and detection was measured using alkaline phosphatase-conjugated streptavidin (Pierce) at

1:1,000 for 1 h at room temperature. Absorption was measured at 405 nm.

Flow Cytometry Staining of WEHI B-Cell Lines. Previously described mouse B-cell WEHI cell lines (6), which express cell-surface bnAbs, were used in flow cytometric binding assays to evaluate the antigenicity of BG505 SOSIP-AviB trimers. The bnAb-expressing cells were induced by overnight incubation with doxycycline (1 μg/mL) as described previously (6). Cells (1×10^6) were stained simultaneously with 50 nM of BG505 SOSIP-AviB conjugated to streptavidin-PE (Invitrogen) and 50 nM of JR-CSF gp120-AviB conjugated to streptavidin-APC (Invitrogen). Staining was performed in a final volume of 100 μL at 4 °C in 1× PBS with 1 mM EDTA and 1% FBS, before washing and was analyzed on a BD LSR II FACS machine.

Single B-Cell RT-PCR, Gene Amplification, and Cloning. Reverse transcription and subsequent PCR amplification of heavy- and light-chain variable genes were performed according to previous protocols (7, 8). All PCR reactions were performed in a 25-μL volume with 2.5 μL of cDNA transcript using HotStar Taq DNA polymerase master mix (Qiagen). Previous primer mixes were supplemented with additional specific V_H1-8 primers and V_K2-28 primers: heavy-chain PCR 1 (ATGGACTGGATTTGGAG-GAT), heavy-chain PCR 2 (ATGGACTGGATTTGGAGGATC-CTCTTCTTGG), kappa-chain PCR 1 (ATGAGGCTCCCTGCT-GCCATCCTGGGGCTGCTAATGC), and kappa-chain PCR 2 (GCTCCTGGGGCTGCTAATGCTCTGGGTCTCTGG). Amplified IgG heavy- and light-chain variable regions were sequenced and analyzed using the international ImMunoGeneTics information system (IMGT) V-quest webserver (www.IMGT.org) (9). Wells for which heavy-chain (V_H1-8 gene) and light-chain (V_K2-28) sequences were deemed productive rearrangements by IMGT analysis were selected for cloning into corresponding Igγ1, Igκ, and Igλ expression vectors as previously described (7).

Antibody Production. Heavy- and light-chain plasmids were cotransfected (1:1 ratio) in either HEK 293T or 293 FreeStyle cells using Fugene 6 (Promega) or 293fectin (Invitrogen), respectively. Transfections were performed according to the manufacturer's protocol, and antibody supernatants were harvested 4 d after transfection. Antibodies produced in 293T cells were quantified by ELISA and used directly in neutralization assays. Antibodies produced in 293 freestyle cells were purified over a protein A column as described previously (10).

Cell Surface-Binding Assays. Titrating amounts of mAbs were added to HIV-1 Env-transfected 293T cells and were incubated for 1 h at 4 °C in 1× PBS. After washing, cells were fixed with 2% paraformaldehyde (PolySciences) for 20 min at room temperature. The cells then were washed and stained with a 1:200 dilution of PE-conjugated goat anti-human IgG $F(ab')_2$ (Jackson) for 1 h at room temperature. Binding was analyzed using flow cytometry. Binding competitions were performed by titrating amounts of competitor mAbs before adding biotinylated antibody at the concentration required to achieve IC_{70} and then measuring binding with PE-labeled streptavidin (Invitrogen). FlowJo software was used for data interpretation.

Autoreactivity Assays. Antibodies were assayed at 100 μg/mL for autoreactivity to HEp-2 cells (Aesku Diagnostics) by immunofluorescence according to the manufacturer's instructions. Briefly, 2.5 μg or 25 μL of 100 μg/mL mAb was incubated on HEP-2 slides in a moist chamber at room temperature for 30 min. The slides then were placed in staining dishes and washed with PBS. FITC-conjugated goat anti-human IgG (20 μL) was applied to each well, and slides were incubated for another 30 min. After washing, slides were photographed on an EVOS f1 fluorescence microscope at a 250-ms exposure with 70% intensity. Reactivity to HIV-1$^-$ human epithelial HEp-2 cells was interpreted by the staining patterns. Positive and negative control sera were provided by the vendor. ELISAs were performed as described previously (11). Briefly, human placental dsDNA (Sigma) and ganglioside GD1a (Sigma) in 96% ethanol were coated at 37 °C overnight onto ELISA wells. BSA (Sigma), ovalbumin (Sigma), apo transferrin, and histone (Sigma) were resuspended in PBS and coated overnight at 4 °C. All antigens were coated at 50 ng per well. Then the wells were washed and blocked for 1 h at room temperature with 3% BSA. Serial dilutions of antibodies were added for 1 h at room temperature. After washing, bound antibody was detected by using an alkaline phosphatase-conjugated goat anti-human IgG $F(ab')_2$ antibody (Pierce) diluted 1:1,000 in 1% BSA/PBS.

Octet Measurements. Binding curves were determined by bio-layer interferometry using an Octet RED instrument (ForteBio, Inc.) as previously described (12, 13). Briefly, IgG antibodies were immobilized onto anti-human IgG Fc biosensors. Varying concentrations of BG505-SOSIP.664-AviB were flowed as analyte in solution. Binding-affinity constants (K_D; on-rate, k_a; off-rate, k_d) were determined using Octet Analysis version 7 software (ForteBio; Pall Life Sciences).

Determination of PGDM1400 Fab Crystal Structure. Expression and purification of the PGDM1400 Fab was executed following a protocol similar to that previously described (14). Briefly, the Fab was produced by cotransfection of the heavy- and light-chain genes into HEK 293F cells. Six days after transfection, the supernatant was recovered and flowed over an anti-human kappa light-chain affinity matrix (CaptureSelect Fab κ; BAC). The eluted fraction containing the Fab was purified further by MonoS cation exchange chromatography (GE Healthcare). PGDM1400 Fab at a concentration of 6 mg/mL in 20 mM sodium acetate (pH 5.6) was crystallized from nonbuffered mother liquor containing 0.04 M potassium dihydrogen phosphate, 16% (wt/vol) polyethylene glycol 8000, and 20% (vol/vol) glycerol. Crystals were flash cooled in liquid nitrogen, and data were collected at the Stanford Synchrotron Radiation Lightsource (SSRL) beamline 11-1. Statistics for data collection and data processing in XDS (15) are summarized in Table S1. The monoclinic space group $P2_1$ and the structure was solved by molecular replacement using the program Phaser (16) with the PGT145 Fab structure (PDB ID code 3U1S) as a search model. Refinement of three Fab copies in the asymmetric unit was performed using a combination of Phenix (17) and Coot (18). Final refinement statistics are reported in Table S1.

Electron Microscopy. Sample grids were prepared as described previously (19). Data were collected on an FEI Tecnai T12 electron microscope operating at 120 keV coupled with a 4 × 4 k Tietz TemCam-F416 camera. Images were taken using the LEGINON interface (20). The imaging magnification was 52,000× with a pixel size of 2.05 Å at the specimen plane. The data were collected using an electron dose of ~30 $e^-/Å^2$. Particles were picked using DoG picker in the Appion interface (21, 22), and class averages were generated using the SPARX software package (23).

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10. Sok D, et al. (2013) The effects of somatic hypermutation on neutralization and binding in the PGT121 family of broadly neutralizing HIV antibodies. *PLoS Pathog* 9(11):e1003754.
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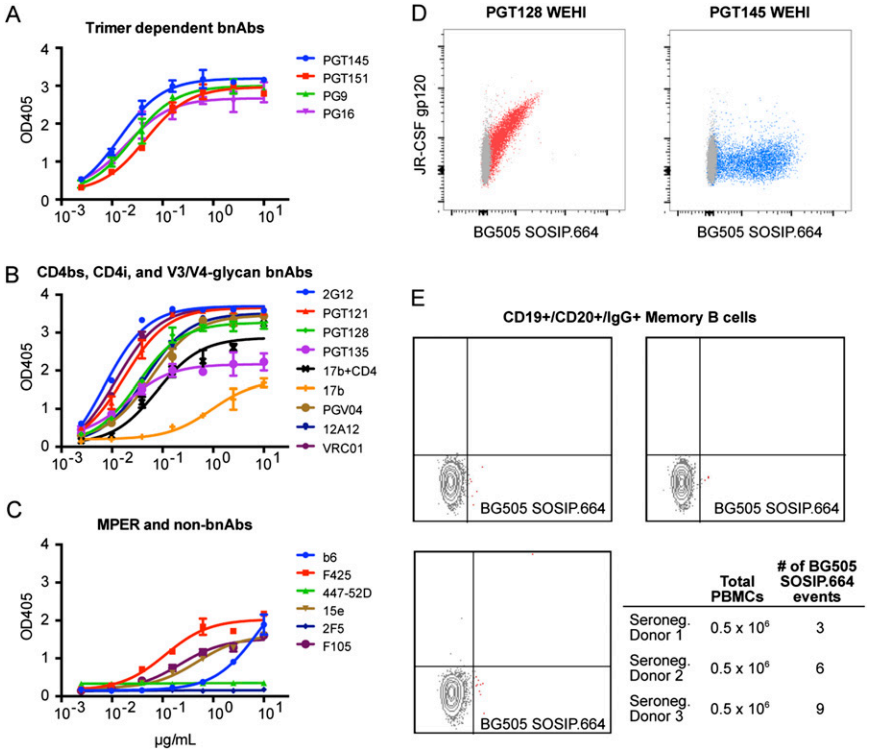

Fig. S1. BG505 SOSIP.664-AviB is an appropriate probe for bnAbs. (*A*–*C*) Biotinylated BG505 SOSIP.664-Avi (BG505 SOSIP.664-AviB) was tested in a streptavidin-capture ELISA against trimer-dependent bnAbs (*A*); CD4bs, CD4i, and V3/V4-glycan bnAbs (*B*); and MPER and non-bnAbs (*C*). (*D*) Binding of BG505 SOSIP.664-AviB and JR-CSF gp120-AviB to PGT128 and PGT145 WEHI mouse B-cell lines (1) was detected via flow cytometry using streptavidin-PE and streptavidin-Alexa 488, respectively. Values are presented in mean fluorescence intensity (MFI). (*E*) PBMCs from HIV-seronegative donors were isolated by Ficoll gradient, and IgG^+ memory B cells were stained with BG505 SOSIP.664-AviB and streptavidin-PE to evaluate the extent of nonspecific trimer binding.

1. Ota T, et al. (2012) Anti-HIV B Cell lines as candidate vaccine biosensors. *J Immunol* 189(10):4816–4824.

	mAb	V-GENE	J-GENE	CDRH3 Length (aa)	CDRH3 Sequence (aa)	V_HJ_H % mut (nt)	Insertion/Deletions
HEAVY CHAIN	PGDM1400	IGHV1-8	IGHJ6	34	CAKGSKHRLRDYALYDDDGALNWAVDVDYLSNLEFW	73%	
	PGDM1401			34	CARGSKHRLRDYVMYDDYGALQWAVYVDYLSNLDVW	73%	
	PGDM1402			34	CAKGSKHRLRDYALYDDIGALQWAVDVDYLSTLEFW	73%	
	PGDM1403			33	CVKGSKFRLREWADYNEWGLVSAQHGDYVTQLGIW	74%	
	PGDM1404			34	CVRGAKFRLRHDATYDYWNDLLWADDRDYVTQLDLW	77%	
	PGDM1405			34	CVRGAKFRLRHDATYDYYNDLLWADDRDYVTQLDLW	76%	
	PGDM1406			33	CVKGQKFRLTEWADYNEFGLVAAQKGDYVTQLDVW	76%	
	PGDM1407			33	CVKGQKFRLTEWADYNEFGLVAAEKGDYVTQLDVW	75%	
	PGDM1408			34	CARGSKHKLRDYVMYDDYGALQWAVYVDYLSNLDVW	73%	
	PGDM1409			34	CVRGAKFRLRHDATYDYWNDLLWADDRDYVTQLDLW	75%	
	PGDM1410			34	CVRGSKFRLRNDAIYDYWNDLLWADDGDYVTKLDLW	75%	
	PGDM1411			33	CARRTEKQLRAEYVLDQEDGFYREEAIYITVLDVW	78%	
	PGDM1412			33	CVKGLKFRLREWSDYNEFGLVAAQHGDYVTQMEVW	78%	
	PGT141			34	CTRGSKHRLRDYVLYDDYGLINYQEWNDYLEFLDVW	82%	
	PGT142			34	CTRGSKHRLRDYVLYDDYGLINYQEWNDYLEFLDVW	82%	
	PGT143			34	CTRGSKHRLRDYVLYDDYGLINYQEWNDYLEFLDVW	82%	
	PGT144			34	CTGGSKHRLRDYVLYDDYGLINQQEWNDYLEFLDVW	82%	
	PGT145			33	CLTGSKHRLRDYFLYNEYGPNYEEWGDYLATLDVW	77%	
LIGHT CHAIN	PGDM1400	IGKV2-28	IGKJ1	9	CMQGRESPWTF	89%	
	PGDM1401			9	CMQGRESPWTF	88%	
	PGDM1402			9	CMQGRESPWTF	89%	
	PGDM1403			9	CMQGLQSPTTF	88%	+7 (CDR1)
	PGDM1404			9	CMQGRHIPLTF	85%	+3 (CDR1)
	PGDM1405			9	CMQGRHIPLTF	84%	+3 (CDR1)
	PGDM1406			9	CMQGLRTPMTF	84%	+7 (CDR1)
	PGDM1407			9	CMQGLRTPMTF	82%	+7 (CDR1)
	PGDM1408			9	CMQGRESPWTF	88%	
	PGDM1409			9	CMQGRHIPLTF	85%	+3 (CDR1)
	PGDM1410			9	CFQGRHTPLTF	86%	+3 (CDR1)
	PGDM1411			9	CMDTLRPPYAF	78%	-1 (CDR1)
	PGDM1412			9	CMQGLQVPMTF	88%	+7 (CDR1)
	PGT141			9	CMQGLNRPWTF	86%	
	PGT142			9	CMQGLNRPWTF	86%	
	PGT143			9	CMQGLNRPWTF	86%	
	PGT144			9	CMQGLNRPWTF	87%	
	PGT145			9	CMQGLHSPWTF	84%	

Fig. S2. Summary of mutation frequency and gene family of newly isolated somatic variants. PGDM1400–1412 were analyzed for germline gene, CDRH3 length, CDRH3 sequence, percent identity (in nucleotides), and possible insertions or deletions. Genetic analyses were performed using the IMGT system (1).

1. Lefranc M-P, et al. (2009) IMGT, the international ImMunoGeneTics information system. *Nucleic Acids Res* 37(Database issue):D1006–D1012.

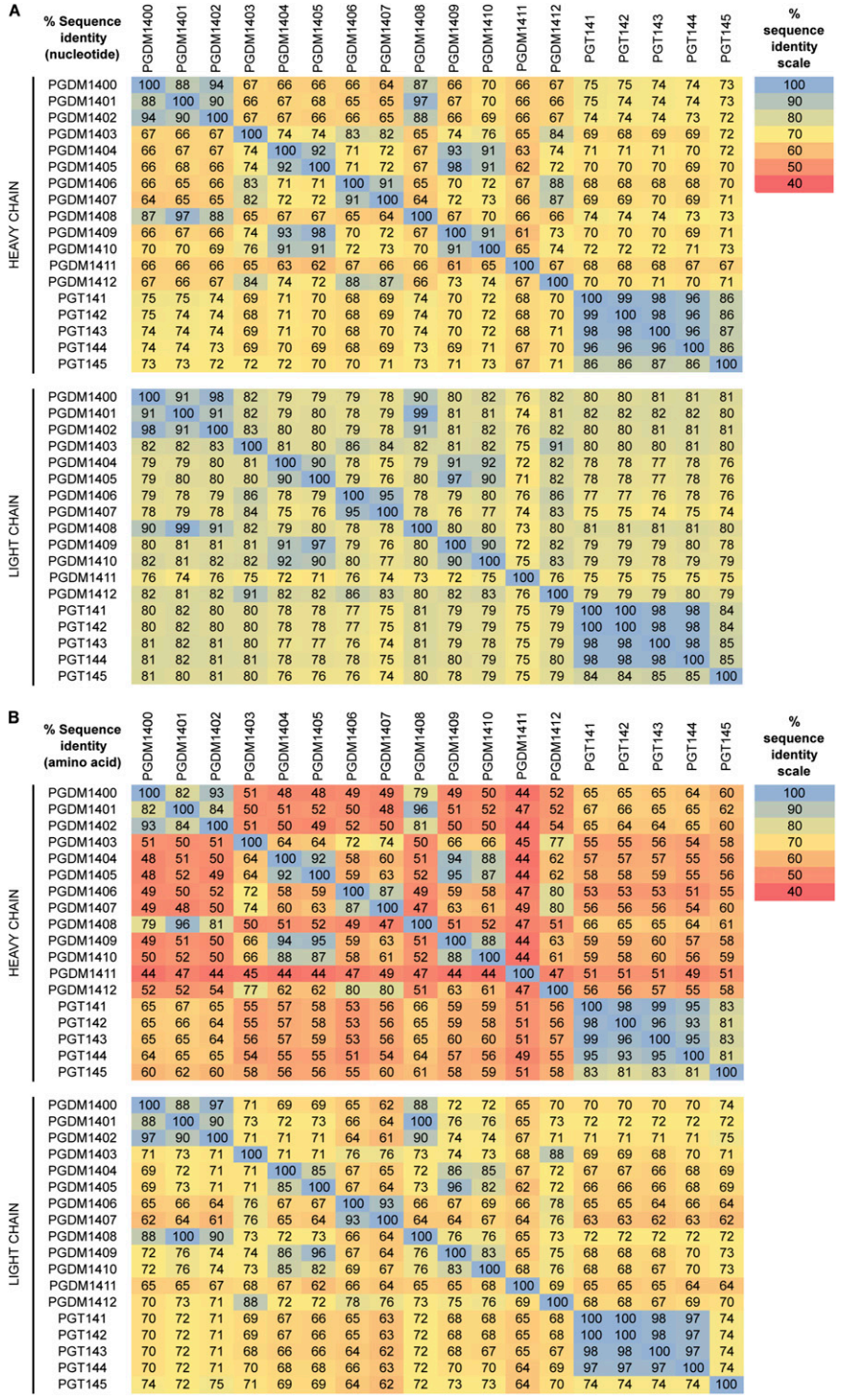

Fig. S3. Newly isolated somatic variants are highly divergent from the PGT141–145 sequences. A sequence identity matrix was created using the somatic variants PGDM1400–1412 and PGT141–145. Values listed are (*A*) nucleotide percent identity and (*B*) amino acid percent identity colored according to the key. Sequence identities were calculated using Clustal Omega (1).

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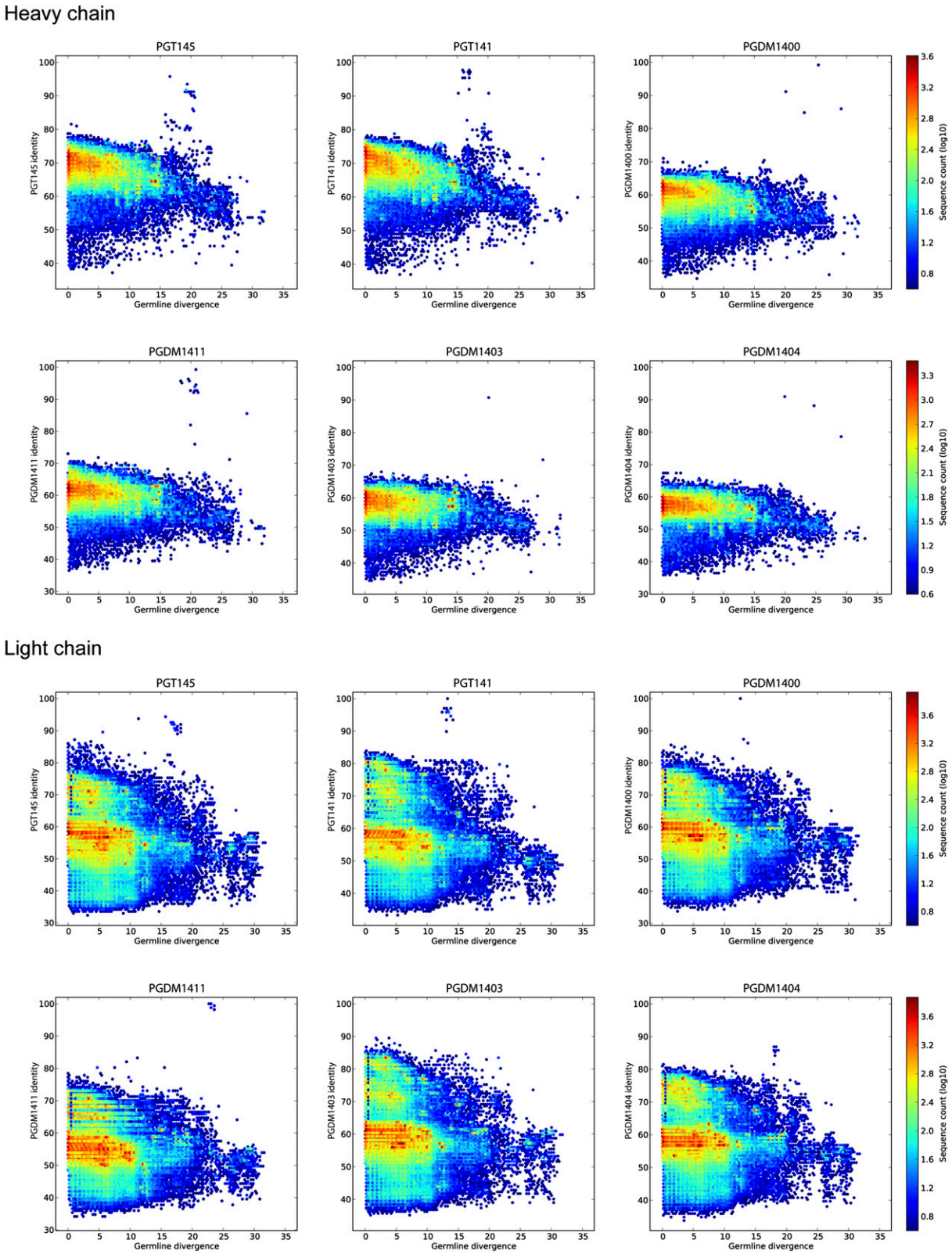
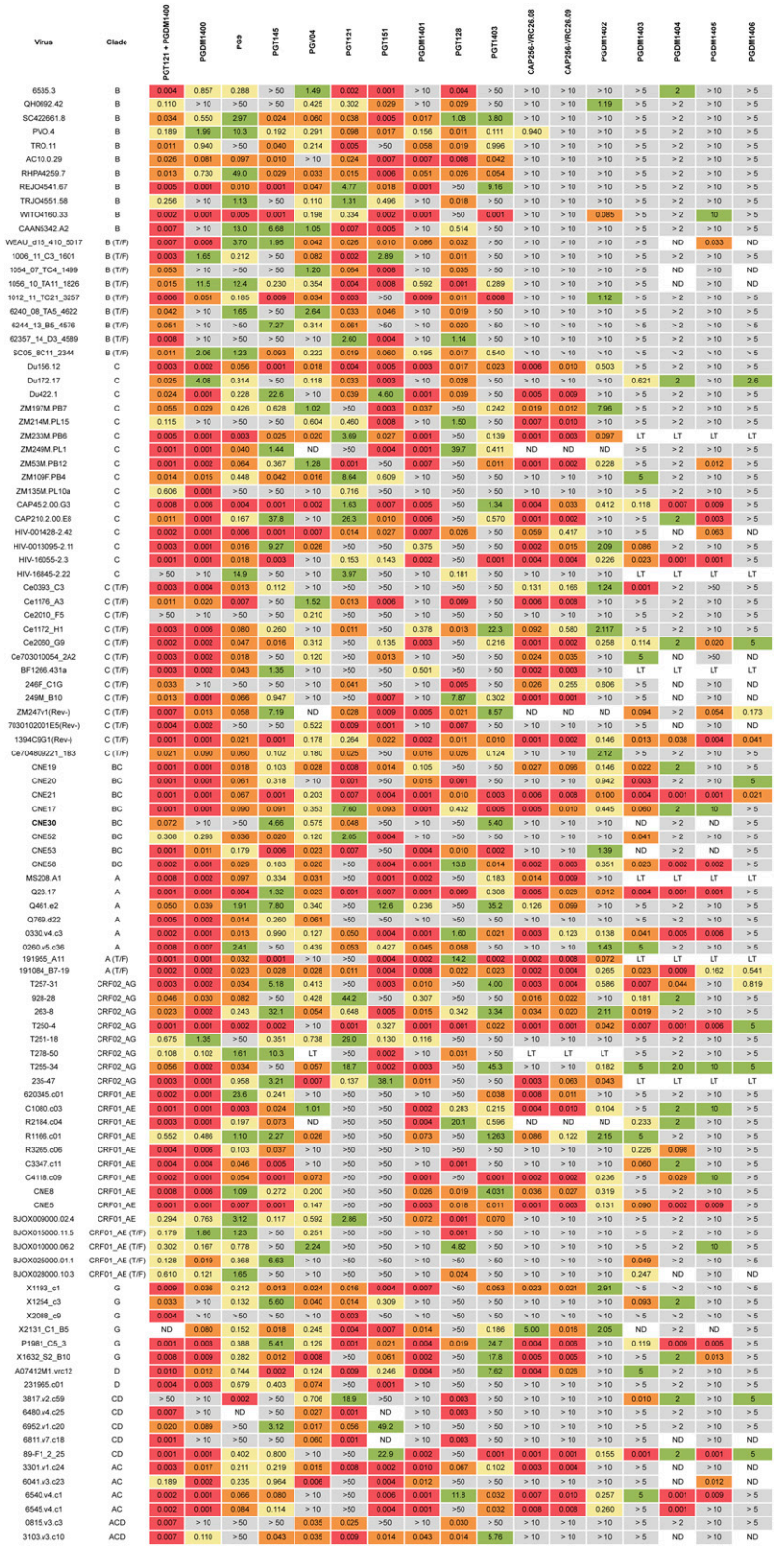


Fig. S4. Sequences of the newly isolated somatic variants can be found in a next-generation sequencing (NGS) dataset. Heavy-chain and light-chain sequences of the newly isolated somatic variants were queried in a previously published NGS dataset (1). The frequency of sequences is plotted in terms of percent identity to the listed antibody sequence and percent divergence from the germline gene. The number of sequences is colored according to the key.

1. Zhu J, et al.; NISC Comparative Sequencing Program (2013) Mining the antibodyome for HIV-1-neutralizing antibodies with next-generation sequencing and phylogenetic pairing of heavy/light chains. *Proc Natl Acad Sci USA* 110(16):6470–6475.



Virus	Clade	PGT121 + PGDM1400	PGDM1400	PG9	PGT145	PGV04	PGT121	PGT151	PGDM1401	PGT128	PGT1403	CAP256-VRC26.08	CAP256-VRC26.09	PGDM1402	PGDM1403	PGDM1404	PGDM1405	PGDM1406
6535.3	B	0.004	0.857	0.288	> 50	1.49	0.002	0.001	> 10	0.004	> 50	> 10	> 10	> 10	> 5	2	> 10	> 5
QH0692.42	B	0.110	> 10	> 50	> 50	0.425	0.302	0.029	> 10	0.029	> 50	> 10	> 10	1.19	> 5	> 2	> 10	> 5
SC422661.8	B	0.034	0.550	2.97	0.024	0.060	0.038	0.005	0.017	1.08	3.80	> 10	> 10	> 10	> 5	> 2	> 10	> 5
PVO.4	B	0.189	1.99	10.3	0.192	0.291	0.098	0.017	0.156	0.011	0.111	0.940	> 10	> 10	> 5	> 2	> 10	> 5
TRO.11	B	0.011	0.940	> 50	0.040	0.214	0.005	>50	0.058	0.019	0.996	> 10	> 10	> 10	> 5	> 2	> 10	> 5
AC10.0.29	B	0.026	0.081	0.097	0.010	> 10	0.024	0.007	0.007	0.008	0.042	> 10	> 10	> 10	> 5	> 2	> 10	> 5
RHPA4259.7	B	0.013	0.730	49.0	0.029	0.033	0.015	0.006	0.051	0.026	0.054	> 10	> 10	> 10	> 5	> 2	> 10	> 5
REJO4541.67	B	0.005	0.001	0.010	0.001	0.047	4.77	0.018	0.001	>50	9.16	> 10	> 10	> 10	> 5	> 2	> 10	> 5
TRJO4551.58	B	0.256	> 10	1.13	> 50	0.110	1.31	0.496	> 10	0.018	> 50	> 10	> 10	> 10	> 5	> 2	> 10	> 5
WITO4160.33	B	0.002	0.001	0.005	0.001	0.198	0.334	0.002	0.001	>50	0.001	> 10	> 10	0.085	> 5	> 2	10	> 5
CAAN5342.A2	B	0.007	> 10	13.0	6.68	1.05	0.007	0.005	> 10	0.514	> 50	> 10	> 10	> 10	> 5	> 2	> 10	> 5
WEAU_d15_410_5017	B (T/F)	0.007	0.008	3.70	1.95	0.042	0.026	0.010	0.086	0.032	> 50	> 10	> 10	> 10	> 5	ND	0.033	ND
1006_11_C3_1601	B (T/F)	0.003	1.65	0.212	> 50	0.082	0.002	2.89	> 10	0.011	> 50	> 10	> 10	> 10	> 5	> 2	> 10	> 5
1054_07_TC4_1499	B (T/F)	0.053	> 10	> 50	> 50	1.20	0.064	0.008	> 10	0.035	> 50	> 10	> 10	> 10	> 5	ND	> 10	ND
1056_10_TA11_1826	B (T/F)	0.015	11.5	12.4	0.230	0.354	0.004	0.008	0.592	0.001	0.289	> 10	> 10	> 10	> 5	ND	> 10	ND
1012_11_TC21_3257	B (T/F)	0.006	0.051	0.185	0.009	0.034	0.003	>50	0.009	0.011	0.008	> 10	> 10	1.12	> 5	> 2	> 10	> 5
6240_08_TA5_4622	B (T/F)	0.042	> 10	1.65	> 50	2.64	0.033	0.046	> 10	0.019	> 50	> 10	> 10	> 10	> 5	> 2	> 10	> 5
6244_13_B5_4576	B (T/F)	0.051	> 10	> 50	7.27	0.314	0.061	>50	> 10	0.020	> 50	> 10	> 10	> 10	> 5	> 2	> 10	> 5
62357_14_D3_4589	B (T/F)	0.008	> 10	> 50	> 50	> 10	2.60	0.004	> 10	1.14	> 50	> 10	> 10	> 10	> 5	> 2	> 10	> 5
SC05_8C11_2344	B (T/F)	0.011	2.06	1.23	0.093	0.222	0.019	0.060	0.195	0.017	0.540	> 10	> 10	> 10	> 5	> 2	> 10	> 5
Du156.12	C	0.003	0.002	0.056	0.001	0.018	0.004	0.005	0.003	0.017	0.023	0.006	0.010	0.503	> 5	> 2	> 10	> 5
Du172.17	C	0.025	4.08	0.314	> 50	0.118	0.033	0.003	> 10	0.028	> 50	> 10	> 10	> 10	0.621	2	> 10	2.6
Du422.1	C	0.024	0.001	0.228	22.6	> 10	0.039	4.60	0.001	0.039	> 50	0.005	0.009	> 10	> 5	> 2	> 10	> 5
ZM197M.PB7	C	0.055	0.029	0.426	0.628	1.02	>50	0.003	0.037	>50	0.242	0.019	0.012	7.96	> 5	> 2	> 10	> 5
ZM214M.PL15	C	0.115	> 10	> 50	> 50	0.604	0.460	0.009	> 10	1.50	> 50	0.007	0.010	> 10	> 5	> 2	> 10	> 5
ZM233M.PB6	C	0.005	0.001	0.003	0.025	0.020	3.69	0.027	0.001	>50	0.139	0.001	0.003	0.097	LT	LT	LT	LT
ZM249M.PL1	C	0.001	0.001	0.040	1.44	ND	>50	0.004	0.001	39.7	0.411	ND	ND	ND	> 5	> 2	> 10	> 5
ZM53M.PB12	C	0.001	0.002	0.064	0.367	1.28	0.001	>50	0.007	>50	0.011	0.001	0.002	0.228	> 5	> 2	0.012	> 5
ZM109F.PB4	C	0.014	0.015	0.448	0.042	0.016	8.84	0.609	> 10	>50	> 50	> 10	> 10	> 10	5	> 2	> 10	> 5
ZM135M.PL10a	C	0.606	0.001	> 50	> 50	> 10	0.716	>50	> 10	>50	> 50	> 10	> 10	> 10	> 5	> 2	> 10	> 5
CAP45.2.00.G3	C	0.008	0.006	0.004	0.001	0.002	1.63	0.007	0.005	>50	1.34	0.004	0.033	0.412	0.118	0.007	0.009	> 5
CAP210.2.00.E8	C	0.011	0.001	0.167	37.8	> 10	26.3	0.010	0.005	>50	0.570	0.001	0.002	> 10	> 5	2	0.003	> 5
HIV-001428-2.42	C	0.002	0.001	0.006	0.001	0.007	0.014	0.027	0.007	0.026	> 50	0.059	0.417	> 10	> 5	ND	0.063	ND
HIV-0013095-2.11	C	0.003	0.001	0.016	9.27	0.026	>50	>50	0.375	>50	> 50	0.002	0.015	2.09	0.086	> 2	> 10	> 5
HIV-16055-2.3	C	0.001	0.001	0.018	0.003	> 10	0.153	0.143	0.002	>50	0.001	0.004	0.004	0.226	0.023	0.001	0.001	> 5
HIV-16845-2.22	C	> 50	> 10	14.9	> 50	> 10	3.97	>50	> 10	0.181	> 50	> 10	> 10	> 10	LT	LT	LT	LT
Ce0393_C3	C (T/F)	0.003	0.004	0.013	0.112	> 10	>50	>50	> 10	>50	> 50	0.131	0.166	1.24	0.001	> 2	>50	> 5
Ce1176_A3	C (T/F)	0.011	0.020	0.007	> 50	1.52	0.013	0.006	> 10	0.009	> 50	0.006	0.008	> 10	> 5	> 2	> 10	> 5
Ce2010_F5	C (T/F)	> 50	> 10	> 50	> 50	0.210	>50	>50	> 10	>50	> 50	> 10	> 10	> 10	> 5	> 2	> 10	> 5
Ce1172_H1	C (T/F)	0.003	0.006	0.080	0.260	> 10	0.011	>50	0.378	0.013	22.3	0.092	0.580	2.117	> 5	> 2	> 10	> 5
Ce2060_G9	C (T/F)	0.002	0.002	0.047	0.016	0.312	>50	0.135	0.003	>50	0.216	0.001	0.002	0.258	0.114	2	0.020	5
Ce703010054_2A2	C (T/F)	0.003	0.002	0.018	> 50	0.120	>50	0.013	> 10	>50	> 50	0.024	0.035	> 10	5	ND	>50	ND
BF1266.431a	C (T/F)	0.003	0.002	0.043	1.35	> 10	>50	>50	0.501	>50	> 50	0.002	0.003	> 10	LT	LT	LT	LT
246F_C1G	C (T/F)	0.033	> 10	> 50	> 50	> 10	0.041	>50	> 10	0.005	> 50	0.026	0.255	0.606	> 5	ND	> 10	ND
249M_B10	C (T/F)	0.013	0.001	0.066	0.947	> 10	>50	0.007	> 10	7.87	0.302	0.001	0.001	> 10	> 5	ND	> 10	ND
ZM247v1(Rev-)	C (T/F)	0.007	0.013	0.058	7.19	ND	0.028	0.009	0.005	0.021	8.57	ND	ND	ND	0.094	> 2	0.054	0.173
7030102001E5(Rev-)	C (T/F)	0.004	0.002	> 50	> 50	0.522	0.009	0.001	> 10	0.007	> 50	> 10	> 10	> 10	> 5	ND	> 10	ND
1394C9G1(Rev-)	C (T/F)	0.001	0.001	0.021	0.001	0.178	0.264	0.022	0.002	0.011	0.010	0.001	0.002	0.146	0.013	0.038	0.004	0.041
Ce704809221_1B3	C (T/F)	0.021	0.090	0.060	0.102	0.180	0.025	>50	0.016	0.026	0.124	> 10	> 10	2.12	> 5	> 2	> 10	> 5
CNE19	BC	0.001	0.001	0.018	0.103	0.028	0.008	0.014	0.105	>50	> 50	0.027	0.096	0.146	0.022	2	> 10	> 5
CNE20	BC	0.001	0.001	0.061	0.318	> 10	0.001	>50	0.015	0.001	> 50	> 10	> 10	0.942	0.003	> 2	> 10	5
CNE21	BC	0.001	0.001	0.067	0.001	0.203	0.007	0.004	0.001	0.010	0.003	0.006	0.008	0.100	0.004	0.001	0.001	0.021
CNE17	BC	0.001	0.001	0.090	0.091	0.353	7.60	0.093	0.001	0.432	0.005	0.005	0.010	0.445	0.060	2	10	> 5
CNE30	BC	0.072	> 10	> 50	4.66	0.575	0.048	>50	> 10	>50	5.40	> 10	> 10	> 10	ND	> 2	ND	> 5
CNE52	BC	0.308	0.293	0.036	0.020	0.120	2.05	0.004	> 10	>50	> 50	> 10	> 10	> 10	0.041	> 2	> 10	> 5
CNE53	BC	0.001	0.011	0.179	0.006	0.023	0.007	>50	0.004	0.010	0.002	> 10	> 10	1.39	ND	> 2	ND	> 5
CNE58	BC	0.002	0.001	0.029	0.183	0.020	>50	0.004	0.001	13.8	0.014	0.002	0.003	0.351	0.023	0.002	0.002	> 5
MS208.A1	A	0.008	0.002	0.097	0.334	0.031	>50	0.001	0.002	>50	0.183	0.014	0.009	> 10	LT	LT	LT	LT
Q23.17	A	0.001	0.001	0.004	1.32	0.023	0.001	0.007	0.001	0.009	0.308	0.005	0.028	0.012	0.004	0.001	0.001	> 5
Q461.e2	A	0.050	0.039	1.91	7.80	0.340	>50	12.6	0.236	>50	35.2	0.126	0.099	> 10	> 5	> 2	> 10	> 5
Q769.d22	A	0.005	0.002	0.014	0.260	0.061	>50	>50	> 10	>50	> 50	> 10	> 10	> 10	> 5	> 2	> 10	> 5
0330.v4.c3	A	0.002	0.001	0.013	0.990	0.127	0.050	0.004	0.001	1.60	0.021	0.003	0.123	0.138	0.041	0.005	0.006	> 5
0260.v5.c36	A	0.008	0.007	2.41	> 50	0.439	0.053	0.427	0.045	0.058	> 50	> 10	> 10	1.43	5	> 2	> 10	> 5
191955_A11	A (T/F)	0.001	0.001	0.032	0.001	> 10	>50	0.004	0.002	14.2	0.002	0.002	0.008	0.072	LT	LT	LT	LT
191084_B7-19	A (T/F)	0.002	0.002	0.023	0.028	0.028	0.011	0.004	0.008	0.022	0.023	0.002	0.004	0.265	0.023	0.009	0.162	0.541
T257-31	CRF02_AG	0.003	0.002	0.034	5.18	0.413	>50	0.003	0.010	>50	4.00	0.003	0.004	0.586	0.007	0.044	> 10	0.819
928-28	CRF02_AG	0.046	0.030	0.082	> 50	0.428	44.2	>50	0.307	>50	> 50	0.016	0.022	> 10	0.181	2	> 10	> 5
263-8	CRF02_AG	0.023	0.002	0.243	32.1	0.054	0.648	0.005	0.015	0.342	3.34	0.034	0.020	2.11	0.019	> 2	> 10	> 5
T250-4	CRF02_AG	0.001	0.001	0.002	0.002	> 10	0.001	0.327	0.001	0.001	0.022	0.001	0.001	0.042	0.007	0.001	0.006	5
T251-18	CRF02_AG	0.675	1.35	> 50	0.351	0.738	29.0	0.130	0.116	>50	> 50	> 10	> 10	> 10	> 5	> 2	> 10	> 5
T278-50	CRF02_AG	0.108	0.102	1.61	10.3	LT	>50	0.002	> 10	0.031	> 50	LT	LT	LT	> 5	> 2	> 10	> 5
T255-34	CRF02_AG	0.056	0.002	0.034	> 50	0.057	18.7	0.002	0.003	>50	45.3	> 10	> 10	0.182	5	2.0	10	5
235-47	CRF02_AG	0.003	0.001	0.958	3.21	0.007	0.137	38.1	0.011	>50	> 50	0.003	0.063	0.043	LT	LT	LT	LT
620345.c01	CRF01_AE	0.002	0.001	23.6	0.241	> 10	>50	>50	> 10	>50	0.038	0.008	0.011	> 10	> 5	> 2	> 10	> 5
C1080.c03	CRF01_AE	0.001	0.001	0.003	0.024	1.01	>50	>50	0.002	0.283	0.215	0.004	0.010	0.104	> 5	2	10	> 5
R2184.c04	CRF01_AE	0.003	0.001	0.197	0.073	ND	>50	>50	0.004	20.1	0.596	ND	ND	ND	0.233	2	> 10	> 5
R1166.c01	CRF01_AE	0.552	0.486	1.10	2.27	0.026	>50	>50	0.073	>50	1.263	0.086	0.122	2.15	5	> 2	> 10	> 5
R3265.c06	CRF01_AE	0.004	0.006	0.103	0.037	> 10	>50	>50	> 10	>50	> 50	> 10	> 10	> 10	0.226	0.098	> 10	> 5
C3347.c11	CRF01_AE	0.004	0.004	0.046	0.005	> 10	>50	>50	> 10	0.001	> 50	> 10	> 10	> 10	0.060	2	> 10	> 5
C4118.c09	CRF01_AE	0.002	0.001	0.054	0.001	0.073	>50	>50	0.001	>50	0.001	0.002	0.002	0.236	> 5	0.029	10	> 5
CNE8	CRF01_AE	0.008	0.006	1.09	0.272	0.200	>50	>50	0.026	0.019	4.031	0.036	0.027	0.319	> 5	> 2	> 10	> 5
CNE5	CRF01_AE	0.001	0.001	0.007	0.001	0.147	>50	>50	0.003	0.018	0.011	0.001	0.003	0.131	0.090	0.002	0.009	> 5
BJOX009000.02.4	CRF01_AE	0.294	0.763	3.12	0.117	0.592	2.86	>50	0.072	0.001	0.070	> 10	> 10	> 10	> 5	> 2	> 10	> 5
BJOX015000.11.5	CRF01_AE (T/F)	0.179	1.86	1.23	> 50	0.251	>50	>50	> 10	0.001	> 50	> 10	> 10	> 10	> 5	> 2	> 10	> 5
BJOX010000.06.2	CRF01_AE (T/F)	0.302	0.167	0.778	> 50	2.24	>50	>50	> 10	4.82	> 50	> 10	> 10	> 10	> 5	> 2	10	> 5
BJOX025000.01.1	CRF01_AE (T/F)	0.128	0.019	0.368	6.63	> 10	>50	>50	> 10	>50	> 50	> 10	> 10	> 10	0.049	> 2	> 10	> 5
BJOX028000.10.3	CRF01_AE (T/F)	0.610	0.121	1.65	> 50	> 10	>50	>50	> 10	0.024	> 50	> 10	> 10	> 10	0.247	ND	> 10	ND
X1193_c1	G	0.009	0.036	0.212	0.013	0.024	0.016	0.004	0.007	>50	0.053	0.023	0.021	2.91	> 5	> 2	> 10	> 5
X1254_c3	G	0.033	> 10	0.132	5.60	0.040	0.014	0.309	> 10	>50	> 50	> 10	> 10	> 10	0.093	2	> 10	> 5
X2088_c9	G	0.004	> 10	> 50	> 50	> 10	0.003	>50	> 10	>50	> 50	> 10	> 10	> 10	> 5	> 2	> 10	> 5
X2131_C1_B5	G	ND	0.080	0.152	0.018	0.245	0.004	0.007	0.014	>50	0.186	5.00	0.016	2.05	ND	> 2	ND	> 5
P1981_C5_3	G	0.001	0.003	0.388	5.41	0.129	0.001	0.021	0.004	0.019	24.7	0.004	0.006	> 10	0.119	0.009	0.005	> 5
X1632_S2_B10	G	0.008	0.009	0.282	0.012	0.008	>50	0.061	0.002	>50	17.8	0.005	0.005	> 10	> 5	2	0.013	> 5
A07412M1.vrc12	D	0.010	0.012	0.744	0.002	0.124	0.009	0.246	0.004	>50	7.62	0.004	0.026	> 10	5	> 2	> 10	> 5
231965.c01	D	0.004	0.003	0.679	0.403	0.074	>50	0.001	> 10	>50	> 50	> 10	> 10	> 10	> 5	> 2	> 10	> 5
3817.v2.c59	CD	> 50	> 10	0.002	> 50	0.706	18.9	>50	> 10	0.003	> 50	> 10	> 10	> 10	0.010	2	> 10	5
6480.v4.c25	CD	0.007	> 10	ND	> 50	0.027	0.001	ND	> 10	0.003	> 50	> 10	> 10	> 10	> 5	> 2	> 10	> 5
6952.v1.c20	CD	0.020	0.089	> 50	3.12	0.017	0.056	49.2	> 10	>50	> 50	> 10	> 10	> 10	> 5	> 2	> 10	> 5
6811.v7.c18	CD	0.001	> 10	> 50	> 50	0.060	0.001	ND	> 10	0.003	> 50	> 10	> 10	> 10	> 5	ND	> 10	ND
89-F1_2_25	CD	0.001	0.001	0.402	0.800	> 10	>50	22.9	0.002	>50	0.001	0.001	0.001	0.155	0.001	2	0.001	5
3301.v1.c24	AC	0.003	0.017	0.211	0.219	0.015	0.008	0.002	0.010	0.067	0.102	0.003	0.004	> 10	> 5	ND	> 10	ND
6041.v3.c23	AC	0.189	0.002	0.235	0.964	0.006	>50	0.004	0.012	>50	> 50	> 10	> 10	> 10	> 5	ND	0.012	ND
6540.v4.c1	AC	0.002	0.001	0.066	0.080	> 10	>50	0.006	0.001	11.8	0.032	0.007	0.010	0.257	5	0.001	0.009	> 5
6545.v4.c1	AC	0.002	0.001	0.084	0.114	> 10	>50	0.004	0.001	>50	0.032	0.008	0.008	0.260	> 5	0.001	> 10	> 5
0815.v3.c3	ACD	0.007	> 10	> 50	> 50	0.035	0.025	>50	> 10	0.030	> 50	> 10	> 10	> 10	> 5	> 2	> 10	> 5
3103.v3.c10	ACD	0.007	0.110	> 50	0.043	0.035	0.009	0.014	0.043	0.014	5.76	> 10	> 10	> 10	> 5	ND	> 10	ND

Fig. S5. Neutralization breadth and potency of somatic variants compared with other HIV-1 broadly neutralizing antibodies. Somatic variants were tested on a cross-clade pseudovirus panel. Neutralization IC_{50} values are colored from highly potent (red) to less potent (green). LT, viruses for which there were low titers; ND, not determined.

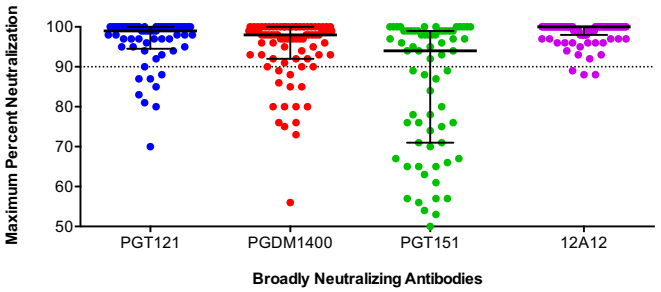
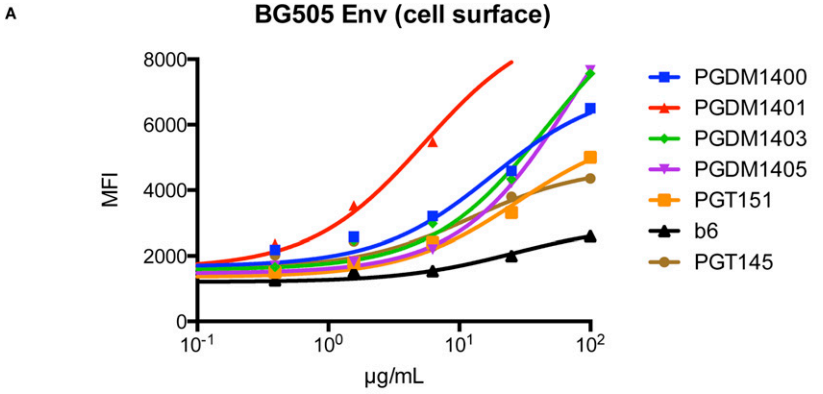


Fig. S6. PGDM1400 neutralizes viruses to completion as much as PGT121. MPNs were determined for PGDM1400 on a 106-virus panel (Fig. S5). For comparison, PGT151, which was reported to exhibit incomplete neutralization activity (1), and PGT121 and 12A12, which have been shown to achieve generally complete neutralization for the majority of viruses, were measured in parallel. Each data point represents a different virus on a cross-clade 106-virus panel (Fig. S5). The median MPN with interquartile range is shown for each antibody.

1. Falkowska E, et al. (2014) Broadly neutralizing HIV antibodies define a glycan-dependent epitope on the prefusion conformation of gp41 on cleaved envelope trimers. *Immunity* 40(5): 657–668.



B

IC_{50} (µg/mL)	PGDM1400	PGDM1401	PGDM1403	PGDM1404	PGDM1405	PGDM1406	PGDM1407	PGT145
BG505	0.002	0.004	> 10	> 50	> 50	> 50	> 50	0.027

Fig. S7. Somatic variants bind to cell-surface BG505 Env despite not neutralizing the BG505 pseudovirus. (*A*) Somatic variants were tested for binding to BG505 Env expressed on the surface of 293T cells. Antibody PGT151, which binds specifically to cleaved Env, and antibody b6, which binds to uncleaved Env, were included for comparison. (*B*) Antibodies also were tested for neutralization against the BG505 pseudovirus. Values shown are neutralization IC_{50} in micrograms per milliliter.

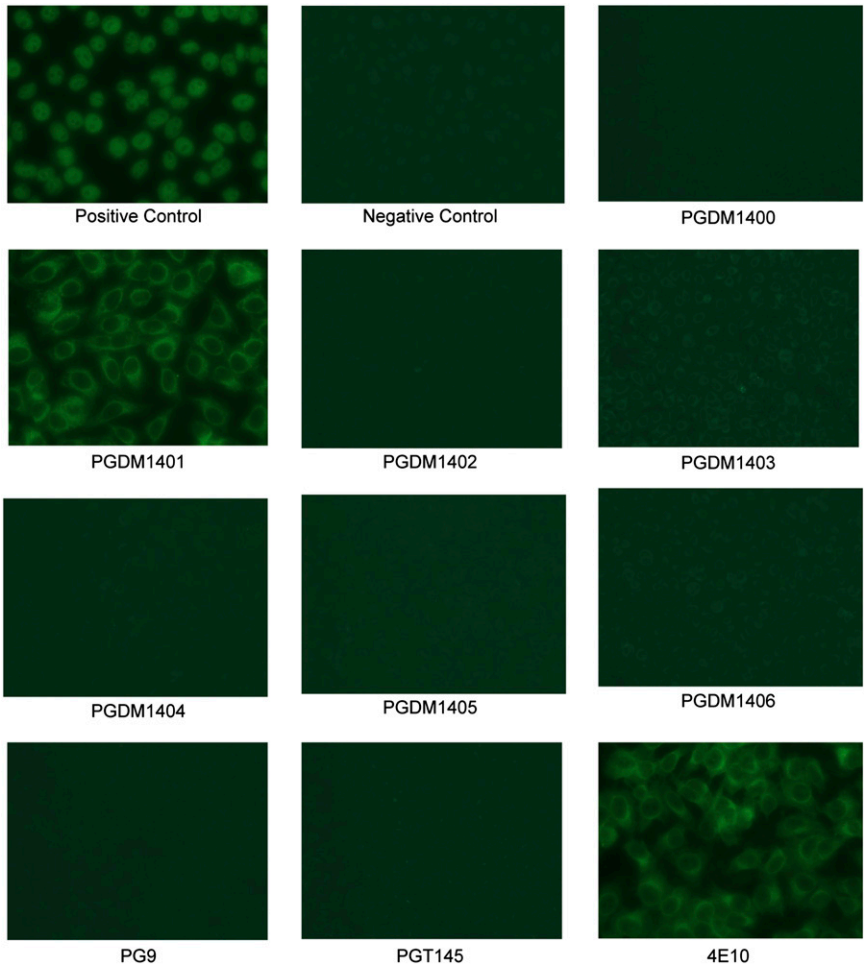


Fig. S8. Among the newly isolated somatic variants, only PGDM1401 is autoreactive by the HEp2 assay. Somatic variants were tested for autoreactivity using the HEp2 cell assay kit according to the manufacturer's protocol. 4E10 was included as a positive control.

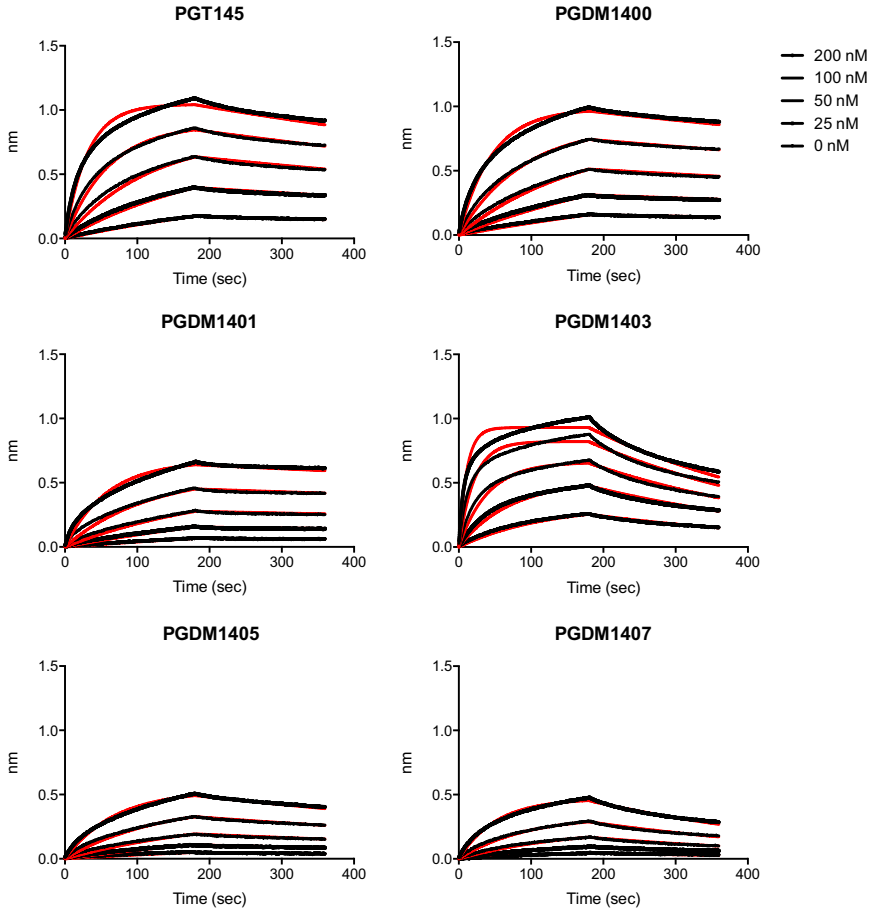


Fig. S9. Binding of mAbs to BG505 SOSIP.664-aviB by Octet. Antibodies PGT145, PGDM1400, PGDM1401, PGDM1403, PGDM1405, and PGDM1407 were analyzed for binding to BG505 SOSIP.664-AviB by Octet. Black curves represent measured data points; red curves represent best-fit lines following analysis.

Table S1. Primers used for amplification of variable heavy- and light-chain genes

Heavy chain (V_H1-8)	
Nested PCR reaction	5′ to 3′ sequence
PCR 1 Forward	ATGGACTGGATTTGGAGGAT
PCR 1 Reverse	GGAAGGTGTGCACGCCGCTGGTC
PCR 2 Forward	ATGGACTGGATTTGGAGGATCCTCTTCTTGG
PCR 2 Reverse	GTTCGGGGAAGTAGTCCTTGAC
Light chain ($V_κ$2-28)	
Nested PCR reaction	5′ to 3′ sequence
PCR 1 Forward	ATGAGGCTCCCTGCTCAGCTCCTGGGGCTGCTAATGC
PCR 1 Reverse	GTTTCTCGTAGTCTGCTTTGCTCA
PCR 2 Forward	GCTCCTGGGGCTGCTAATGCTCTGGGTCTCTGG
PCR 2 Reverse	GTGCTGTCCTTGCTGTCCTGCT

Table S2. Data collection and refinement statistics for SSRL 11-1

Data collection	
Wavelength, Å	0.97947
Space group	$P2_1$
Unit cell a, b, c, Å	55.7, 149.2, 109.9
α, β, γ, °	90, 100.9, 90
Resolution (Å)*	40–3.1 (3.2–3.1)
Completeness*	96.7 (98.6)
Redundancy*	3.4 (3.5)
No. total reflections	108,438
No. unique reflections	30,950
I/σ*	6.8 (1.7)
R_{merge}*,†	16.7 (58.4)
R_{pim}*,‡	10.4 (35.7)
$CC_{1/2}$*,§	97.4 (50.1)
Refinement statistics	
Resolution, Å	40–3.1
No. reflections total/R_{free}	30,926/1,544
R_{cryst}¶/R_{free}‖	22.1/25.3
Rmsd bond length, Å	0.003
Rmsd bond angles, °	0.8
Protein atoms	10,427
Wilson B-value, $Å^2$	57.7
B-value overall, $Å^2$	61.5
Ramachandran favored, %	95.4
Ramachandran allowed, %	99.9
Molprobity all-atom clashscore	7.8
PDB ID code	4RQQ

*Values in parentheses are for the highest resolution shell.

†R_{merge} = Σ|I-<I>|/Σ<I>, where *I* is the observed intensity, and <*I*> is the average intensity of multiple observations of related reflections.

‡R_{pim} = Σhkl (1/(n – 1))1/2 Σi | Ihkl,i - <Ihkl> |/Σhkl Σi Ihkl,I, where *Ihkl,i* is the scaled intensity of the i^{th} measurement of reflection *h*, *k*, *l*, <*Ihkl* > is the average intensity for that reflection, and *n* is the redundancy.

§$CC_{1/2}$ = correlation coefficient of half-datasets (1).

¶R_{cryst} = Σhkl||Fobs|-|Fcalc||/Σhkl|Fobs|.

‖R_{free} calculated as for R_{cryst} but for 5% of the reflections excluded from refinement.

1. Karplus PA, Diederichs K (2012) Linking crystallographic model and data quality. *Science* 336(6084):1030–1033.