Supplementary Materials for

CD4-binding site immunogens elicit heterologous anti-HIV-1 neutralizing antibodies in transgenic and wildtype animals

Harry B. Gristick and Harald Hartweger et al.

Corresponding authors:
Michel C. Nussenzweig nussen@rockefeller.edu
Pamela J. Bjorkman bjorkman@caltech.edu,

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Supplementary Materials and Methods

Generation of anti-idiotypic monoclonal antibodies

Mice were injected three times with purified IOMA iGL. 3 days after the final injection spleens were harvested and used to generate hybridomas at the Fred Hutchinson Antibody Technology Center. Hybridoma supernatants were initially screened against IOMA iGL to identify antigen-specific hybridomas. Supernatants from positive wells were then screened against a panel of monoclonal antibodies that included IOMA, IOMA iGL, and inferred germlines of other anti-HIV-1 antibodies that served as isotype controls using a high throughput bead array. We identified two hybridomas of interest; 3D3, which bound specifically to IOMA iGL, and 3D7, which bound to IOMA and IOMA iGL, which were subcloned from single cells. To produce recombinant anti-idiotypes, RNA was extracted from 1 × 10^6 cells using the RNeasy kit (Qiagen), and the heavy and light chain sequences of the murine hybridomas were by obtained using the mouse Ig-primer set (69831; EMD Millipore) as described (*98*). Sequences were codon optimized, cloned into pTT3-based IgG expression vectors with human constant regions (*99*) using In-Fusion cloning (Clontech), expressed in 293 cells, and purified using Protein A chromatography.

X-ray crystallography

Crystallization screens for IOMA iGL Fab were performed using the sitting drop vapor diffusion method at room temperature (RT) by mixing 0.2 µL Fabs with 0.2 µL of reservoir solution (Hampton Research) using a TTP Labtech Mosquito automatic microliter pipetting robot. IOMA iGL Fab crystals were obtained in 20% (v/v) PEG 2000, 0.1 M Sodium Acetate (pH 4.6). Crystals were looped and cryopreserved in reservoir solution supplemented with 20% glycerol and flash frozen in liquid nitrogen.

The crystal structure of IOMA iGL Fab was solved with data sets. A 1.9 Å-resolution structure of IOMA – 10-1074 – BG505 was solved with a single data set collected at 100 K and 1 Å resolution on Beamline 12-2 at the Stanford Synchrotron Radiation Lightsource (SSRL) with a Pilatus 6M pixel detector (Dectris) that was indexed and integrated with iMosflm v7.4, and then merged with AIMLESS in the CCP4 software package v7.1.018. The structure was determined by molecular replacement using Phaser with one copy of IOMA Fab (PDB 5T3Z). Coordinates were refined with PHENIX v1.19.2-4158 (*100*) with group B factor and TLS restraints. Manual rebuilding was performed iteratively with Coot v1.0.0 (*101*). Data refinement statistics are shown in Table S2, with > 98% of the residues in the favored region of the Ramachandran plot and < 1% in the disallowed regions.

Cloning yeast libraries

Crystal structures of IOMA in complex with BG505 SOSIP.664 (PDB ID 5T3X and 5T3Z) were analyzed to determine mutations on gp120 that potentially could be beneficial for IOMA iGL binding. In addition, we modeled the crystal structure of IOMA iGL (PDB ID 7TQG) onto 426c.TM4ΔV1-3 (426c.TM4) gp120 (PDB ID 5FEC) and selected positions within gp120 that we predicted to be favorable for IOMA iGL binding. We chose 426c.TM4ΔV1-3 (426c TM4), an engineered clade C Env previously shown to activate B cell precursors of HIV-1 bNAbs targeting the CD4bs (*25*) as the starting point for our library design.

Yeast libraries were generated as described (*102*). Specifically, to generate the libraries of 426c gp120 variants we used degenerate oligos in conjunction with an overlap assembly polymerase chain reaction (PCR) method. Overlapping primers for the PCR assembly reactions were designed using Primerize (*103*) and shown in Table S6. NNK codons (where N = A/C/G/T and K = G/T) were utilized that encode for all 20 amino acids but decrease the chances of introducing a premature stop codon. Two different DNA fragments (426c library fragment 1 and 2) were synthesized first and then linearized in a final PCR step to generate the full-length 426c gp120 library used in yeast transformation. To obtain the full-length 426c gp120, a final PCR reaction was performed in which the PCR products of the 426c Library Fragment 1 and 2 were used as a template. Primers were used with overhangs complementary to the yeast display vector pCTCON-2 necessary for the homologous recombination in yeast. Library 2 was cloned in a similar manner as Library 1, but using a different set of primers as shown in Table S6 based on results from Library 1.

Yeast transformation

The yeast display vector pCTCON-2 was used for cell surface display of the 426c gp120 proteins in *Saccharomyces cerevisiae* (*S. cerevisiae*) strain EBY100. A primary culture of 5 mL 2x YPD (40 g/L glucose, 20 g/L peptone, 20 g/L yeast extract) media was inoculated with a single *S. cerevisiae* EBY100 colony (freshly streaked on a YPD plate) and incubated overnight in a shaker at 30 °C and 250 rpm. 100 µL of the overnight yeast *S. cerevisiae* EBY100 cultures was transferred into 5 mL 2x YPD media and incubated overnight at 30 °C, 250 rpm. The following day, 300 mL 2x YPD media was inoculated with the overnight precultures to an OD_{600} ~0.3 and was grown until an OD_{600} ~1.6. 3 mL of sterile filtered Tris/DTT (0.462 g 1,4-dithiothreitol in 3 mL 1 M Tris, pH 8.0) and 15 mL sterile filtered 2 M LiAc/TE (1.98 g LiAc in 10 mL of TE (10 mM Tris, 1 mM EDTA) was added and the culture incubated for 15 min at 30 °C and 250 rpm. Yeast cells were then pelleted at 3,500 g for 3 min and washed with 50 mL ice-cold sterile filtered NewE buffer (0.6 g Tris base, 91.09 g Sorbitol (1 M), 73.50 mg $CaCl_2$ in

ddH_2O to a final volume of 500 mL, pH 7.5). After two additional wash steps, the pellet was re-suspended in 3 mL NewE buffer and 50 µg 426c library DNA insert and 10 µg pCTCON-2 vector (digested with NheI and BamHI) was added. 200 µL of this transformation mix was then aliquoted into pre-chilled 2 mm electroporation cuvettes (Bio-Rad) and electroporated at 1500 V with an average time constant of ~4.5 ms using a Gene Pulser Xcell Electroporation System (Bio-Rad), which was repeated for the entire transformation mix. After electroporation, yeast cells were directly recovered with 2 mL 2x YPD media and transferred into 50 mL cold 2x YPD media (final volume up to 200 mL 2x YPD media) and grown for 1 h at 30 °C and 250 rpm. Serial dilutions of the freshly transformed yeast culture were plated on SDCAA (20 g/L glucose, 6.7 g/L Difco yeast nitrogen base, 1.4 g/L Yeast Synthetic Drop-out Medium Supplements without histidine, leucine, tryptophan and uracil, 20 mg/L uracil, 50 mg/L histidine, 100 mg/L leucine) agarose plates to test the viability and size of the library. After 1 h, the culture was removed and the cells were pelleted and resuspended in 500 mL SDCAA media + carbenicillin (100 µg/mL final concentration) and grown for two days at 30 °C and 250 rpm. To confirm the genetic diversity of the library, a yeast colony PCR was performed on the liquid culture and the PCR product was sequenced. Sequencing reactions were performed at Laragen Inc (Culver City, CA). The sequence data was analyzed using SeqMan Pro (DNASTAR, v13.02). After two days, cells were pelleted and glycerol stocks were made by suspending ~10^9 yeast cells in 1 mL of freezing buffer (0.335 g Yeast Nitrogen Base, 1 mL glycerol in 50 mL H_2O, sterilized by filtration). Aliquots were flash frozen in liquid nitrogen and stored at -80 °C.

Magnetic-activated cell sorting

Magnetic-activated cell sorting (MACS) was used to remove transformants containing stop codons. After growing up the freshly transformed cells for two days in SDCAA, cells were pelleted and induced at an OD_{600} ~1.0 in 100 mL SGCAA-carb (SDCAA prepared with 20 g/L galactose instead of glucose and supplemented with 100 µg/mL carbenicillin final concentration) for 20 h at 20 °C and 250 rpm. Yeast cells were washed 5 times with PBSF (PBS + 0.1% bovine serum albumin (BSA)) and 10^8 cells were incubated with 400 µL PBSF and 100 µL µMACS™ anti-c-Myc MicroBeads (Miltenyi Biotec) for 45 min on a rotator at 4 °C. Cells were then pelleted and resuspended in 5 mL PBSF and sorted using a MidiMACS Separator magnet (Miltenyi Biotec) in combination with an LS column (Miltenyi Biotec) equilibrated in PBSF. Isolated cells were then grown for 2 days in 100 mL SDCAA-carb at 30 °C and 250 rpm and then induced again with SGCAA-carb for 20 h at 20 °C and 250 rpm.

Yeast flow cytometry and cell sorting

To prepare the yeast library for FACS analysis, cells were pelleted at 3000 rpm for 2 min and washed 5 times with PBSF. Cells were then stained at a density of 10^7 cells/mL with 1:500 anti-c-Myc antibody conjugated to AlexaFluor488 (Abcam, ab190026) and 1 μM IOMA iGL and incubated for 1 – 2 h on a rotator at 4 °C. Cells were then washed twice with PBSF and resuspended in 200 μL PBSF with 1:1000 goat anti-human antibody conjugated to AlexaFluor647 (Abcam, ab190560, RRID:AB_2876372) and incubated for 30 min at 4 °C. Cells were then analyzed on a MACSQuant Analyzer (Miltenyi Biotec) or sorted using an SY3200 cell sorter system (Sony). In either case, non-transformed yeast cells and single-stained transformed samples stained with either anti-c-Myc or IOMA iGL IgG were used to set the gates for analysis and collection. Cells that stained double-positive for both c-Myc and IOMA iGL were collected and grown in 5 mL SDCAA-carb for 1 - 2 days at 30 °C and 250 rpm and then transferred to 100 mL SDCAA-carb for an additional 1 - 2 days at 30 °C and 250 rpm. Cells were then pelleted and resuspended in H_2O and plated onto SDCAA-carb for 2 - 3 days at 30 °C. After multiple iterative rounds of sorting (three rounds for Library 1 and seven rounds for Library 2), sequences were recovered by colony PCR and sequence confirmed (Laragen). Primers were used with specific complementary regions to enable ligation of the linear product into the expression vector pTT5 using the Gibson assembly method for protein production. After construction, plasmids were isolated from *E.coli* using the QIAprep Miniprep kit (Qiagen) and confirmed by Sanger sequencing (Laragen).

Generation of IOMA-expressing RAMOS cells by CRISPR/Cas9 gene editing

A targeting vector was constructed using the NEB Hifi DNA assembly kit to clone a gBlock (IDT) into pUCmu (*104*). The gBlock (IDT) contained ~0.5 kb homology arms to the human IgH locus which flanked an expression cassette consisting of the C_μ splice acceptor, the entire IOMA LC gene, a furin-GSG-P2A sequence (*105*) followed by the IOMA HC Leader-VDJ and the J_H4 splice donor based on previously-described designs (*106*) (Figure S5C). Vectors were maxi-prepped (Machery-Nagle) for transfection.

RAMOS (RA 1) cells were purchased from ATCC (CRL-1596) and maintained in RPMI-1640 supplemented with 10 FCS, 1x antibiotic/antimycotic, 2 mM glutamine, 1 mM sodium pyruvate, 10 mM HEPES and 55 μM β-mercaptoethanol. Before transfection, cells were harvested, washed once in PBS and resuspended at $6x10^7$ cells/mL in Neon kit buffer T (ThermoFisher). Three ribonucleoprotein complexes (RNPs) were prepared using 3 different sgRNAs. AGGCATCGGAAAATCCACAG was used to target the IgH locus in the intron 3' of *IGHJ6* to integrate the sequence flanked by the appropriate homology arms from the targeting vector; CTGGGAGTTACCCGATTGGA was used to ablate the human *IGKC* exon and CACGCATGAAGGGAGCACCG was used to ablate all functional *IGLC* genes

(*IgLC1, IGL2, IGLC3* and *IGLC7*). Complexes were prepared by mixing 1.875 µL of 100 µM sgRNA with 1 µL of 61 µM Cas9 (all IDT) for a molar ratio of ~3:1 followed by incubation for 20 min at RT. *IGH:IGK:IGL* RNPs were then mixed at a 2:1:1 v/v/v ratio. 2.6 µg targeting vector (at 4 mg/mL) were mixed with 1.5 µL *IGH:IGK:IGL* RNP mix and 11 µL RAMOS cells in buffer T. 10 µL of the final mix were transfected in a 10 µL Neon tip in a Neon device at 1350 V 30 ms 1 pulse. Cells were immediately transferred into 50 µL RAMOS medium without 1x antibiotic/antimycotic in a 48-well plate and 2 h later 450 µL full RAMOS medium was added. Cells were then cultured as before. Edited IOMA-expressing cells were bulk sorted by flow cytometry as live, singlet, $CD19^{+}$, RC1 $antigen^{hi}$, IgL^{+}, IgK^{+} IgM^{+} (Table S7) and cultured as before. IOMA-expression was further verified by staining with 426c-, CNE8- and CNE20-derived SOSIPs and 426c-CD4bs-KO proteins to show specificity.

10x Genomics single cell processing and next generation V(D)J sequencing

Cells were counted in the final injection volume, and 18,000 cells loaded onto a Chromium Controller (10x Genomics). Single-cell RNA-seq libraries were prepared using the Chromium Single Cell 5 v2 Reagent Kit (PN-1000265) according to manufacturer's protocol. Chromium Single Cell Mouse BCR Amplification Kit (PN-1000255) was used for VDJ cDNA amplification. After QC, 5' expression and VDJ Libraries were pooled 1:1 and sequenced on an Illumina NOVAseq S1 flowcell at the Rockefeller University Genomics Core.

Computational Analyses of V(D)J sequences derived from IOMAgl mice by next generation sequencing

The single-cell V(D)J assembly was carried out by Cell Ranger 6.0.1. A customized reference was created by adding the knocked-in IOMA iGL V(D)J genes to the mouse GRCm38 V(D)J reference so Cell Ranger could recognize and assemble the human/mouse chimera transcripts. Contigs associated with a valid cell barcode according to Cell Ranger were selected for downstream processing using seqtk version 1.3-r106 (https://github.com/lh3/seqtk).

IgBlast standalone version 1.14 (*107*) was used to annotate the immunoglobulin sequences based on a custom database with mouse and human V(D)J genes. Productive IG sequences with more than 20 reads of coverage and with any identified isotype were selected for downstream processing. Unexpectedly, although the IgBlast algorithm identified the V and J genes for 8010 LC sequences, it failed to annotate the CDR3, and consequently, the information regarding their functionality was missing. We extracted and submitted 7782 (97.15%) sequences corresponding to the knock-in LC to

IMGT/V-QUEST (*108*), which successfully identified the CDR3 and provided the productivity information.

Cell barcodes associated with sequences coded by different V genes for either HC or LC were considered doublets and were subsequently removed from downstream analysis. HCs and LCs derived from the same cell were paired, and clones were assigned using our previously-described IgPipeline (*96, 97*) (https://github.com/stratust/igpipeline/tree/igpipeline2_timepoint_v2).

Single cell antibody cloning

The following modifications were applied to the described protocol from reference (*92*) Briefly, single cell RNA in 96-well plates was purified using magnetic beads (RNAClean XP, Beckman Coulter, Cat # A63987). RNA was eluted from the magnetic beads with 11 µL of a solution containing 14.5 ng/µL of random primers (Invitrogen, Cat # 48190011), 0.5% of Igepal Ca-630 (type NP-40, 10% in dH_2O, MP Biomedicals, Cat # 198596) and 0.6 U/µL of RNase inhibitor (Promega, Cat# N2615) in nuclease-free water (Qiagen, Cat # 129117), and incubated at 65 °C for 3 min. cDNA was synthesized by reverse transcription (SuperScript™ III Reverse Transcriptase 10,000 U, Invitrogen, Cat# 18080-044). cDNA was stored at −80 °C or used for antibody gene amplification by nested polymerase chain reaction (PCR) after addition of 10 µL of nuclease-free water.

Mouse antibody genes were amplified using HotstarTaq DNA polymerase (Qiagen Cat # 203209) with the primer sets specific for the $Igh^{IOMAiGL}$ and $Igk^{IOMAiGL}$ transgenes. Primer sequences and reaction mixes are provided in Table S8. Thermocycler conditions were as follows for annealing (°C)/elongation (s)/number of cycles: PCR1 (IgG, IgM and IgK): 51/55/50; PCR2 (IgG and IgM): 54/55/50; PCR2 (IgK): 50/55/50.

PCR products of antibody HC and LC genes were purified and Sanger-sequenced (Genewiz) and *ab1 files analyzed using our previously described IgPipeline (https://github.com/stratust/igpipeline/tree/igpipeline2_timepoint_v2) (*96, 97*). V(D)J sequences were ordered as eBlocks (IDT) with short homologies for Gibson assembly and cloned into human IgG1 or human IgL2 expression vectors using the NEB Hifi DNA Assembly mix (NEB, Cat#E2621L). Plasmid sequences were verified by Sanger sequencing (Genewiz).

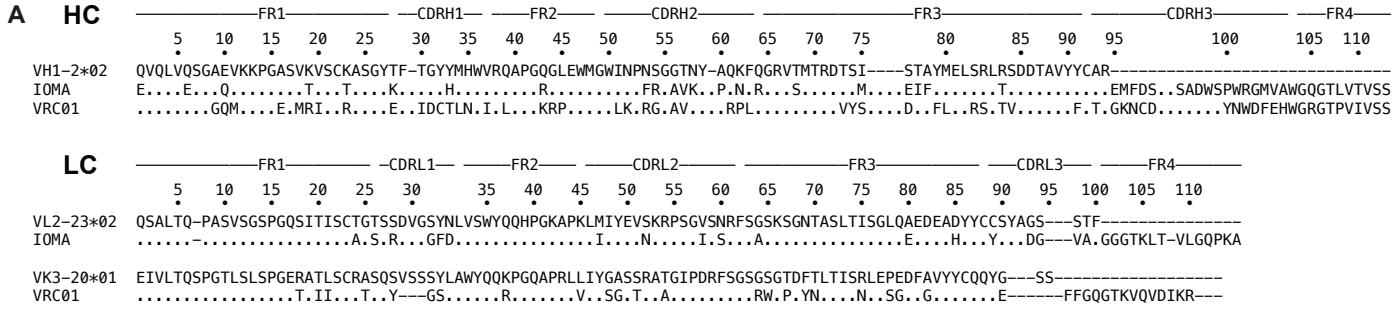
SPR binding studies

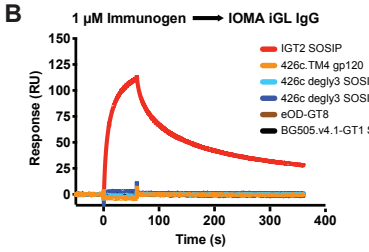
All SPR measurements were performed on a Biacore T200 (GE Healthcare) at 20 °C in HBS-EP+ (GE Healthcare) running buffer. IgGs were directly immobilized onto a CM5 chip (GE Healthcare) to ~3000 resonance units (RUs) using primary amine chemistry. A concentration series of monomeric gp120 core constructs (IGT2, IGT1, 426c TM4) were injected over the flow cells at increasing concentrations (top concentrations ranging from 600 μM to 10 μM) at a flow rate of 60 μL/min for 60 s and allowed to dissociate for 300 s. Regeneration of flow cells was achieved by injecting one pulse each of 10 mM glycine pH 2.0 at a flow rate of 90 μL/min. Kinetic analyses were used after subtraction of reference curves to derive on/off rates (k_a/k_d) and binding constants (K_Ds) using a 1:1 binding model with or without bulk refractive index change (RI) correction as appropriate (Biacore T200 Evaluation software v3.0). Reported affinities represent the average of two independent experiments. SPR experiments that were not used to derive binding affinities or kinetic constants were done using a single high concentration (1 μM) to qualitatively determine binding versus no binding.

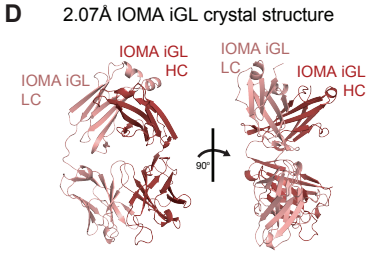
Analysis Software

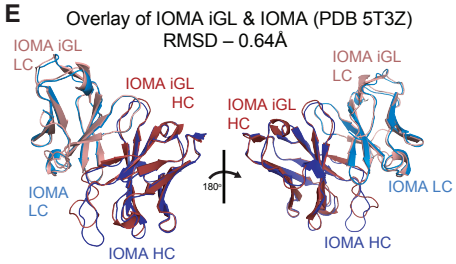
Unless stated otherwise, Geneious Prime 2021.2.2, MacVector 18.2.0 and DNAStar SeqMan Pro 17.1.1 were used for sequence analysis and graphs were created using R language. Flow cytometry data were processed using Mac versions of FlowJo 10.7.2. and GraphPad Prism 9.3 and Microsoft Excel for Mac 16.54 were used for data analysis. Structural figures were made using PyMOL (Schrödinger, LLC) or ChimeraX (*109*). V(D)J gene assignments of NHP and murine antibodies were done using IMGT/V-QUEST (*108*). Sequence alignments were done using Clustal Omega (*110*).

Figs. S1 to S8



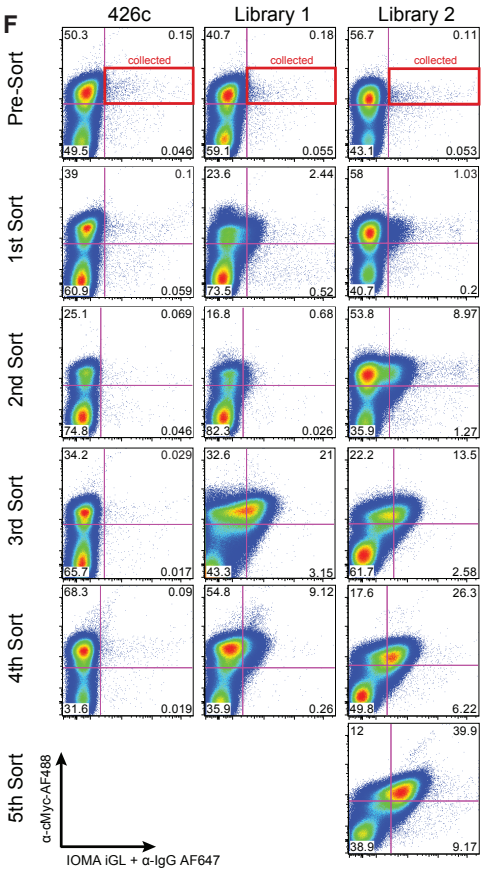


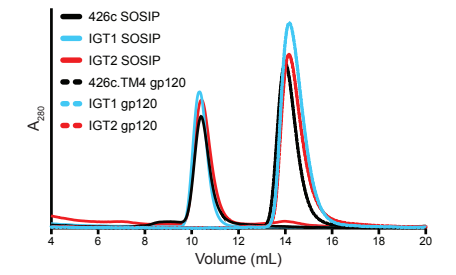


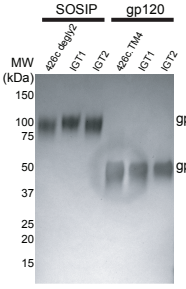


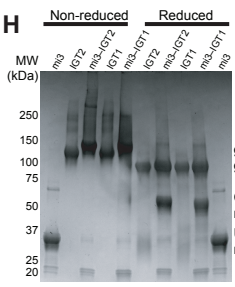
C

Virus	Clade	Tier	IC50 (μg/mL) IOMA	IC50 (μg/mL) IOMA iGL
QH0692.42	B	2	12.9	>100
SC422661.8	B	2	17.3	>100
PVO.4	B	3	0.5	>100
TRO.11	B	2	0.5	>100
RHPA4259.7	B	2	9.6	>100
THRO4156.18	B	2	29.7	>100
TRJO4551.58	B	3	11.7	>100
CAAN5342.A2	B	2	1.7	>100
WEAU_d15_410_787	B (T/F)	NC	3.0	>100
1006_11_C3_1601	B (T/F)	2	0.3	>100
1054_07_TC4_1499	B (T/F)	2	2.2	>100
1056_10_TA11_1826	B (T/F)	1	2.1	>100
1012_11_TC21_3257	B (T/F)	1	0.3	>100
6240_08_TA5_4622	B (T/F)	2	4.0	>100
6244_13_B5_4576	B (T/F)	2	0.6	>100
62357_14_D3_4589	B (T/F)	2	11.2	>100
SC05_8C11_2344	B (T/F)	2	1.5	>100
ZM214M.PL15	C	2	1.7	>100
Ce1086_B2	C (T/F)	2	0.6	>100
Ce1176_A3	C (T/F)	2	3.0	>100
Ce2010_F5	C (T/F)	3	20.4	>100
Ce0682_E4	C (T/F)	2	1.2	>100
7030102001ES(Rev-)	C (T/F)	2	13.3	>100
Ce704809221_1B3	C (T/F)	2	2.5	>100
CNE30	BC	NC	8.3	>100
CNE53	BC	NC	0.1	>100
0260.v5.c36	A	NC	1.6	>100
3415.v1.c1	A	2	0.7	>100
3365.v2.c2	A	2	5.0	>100
R1166.c01	CRF01_AE	NC	8.4	>100
C2101.c01	CRF01_AE	NC	1.5	>100
C3347.c11	CRF01_AE	NC	0.1	>100
BJOX025000.01.1	CRF01_AE (T/F)	NC	1.5	>100
BJOX028000.10.3	CRF01_AE (T/F)	NC	20.5	>100
P0402_c2_11	G	NC	16.0	>100
X1632_S2_B10	G	NC	0.6	>100
A07412M1.vrc12	D	NC	8.4	>100
3817.v2.c59	CD	NC	14.2	>100
3301.v1.c24	AC	NC	34.9	>100
MLV	–	–	>50	>50









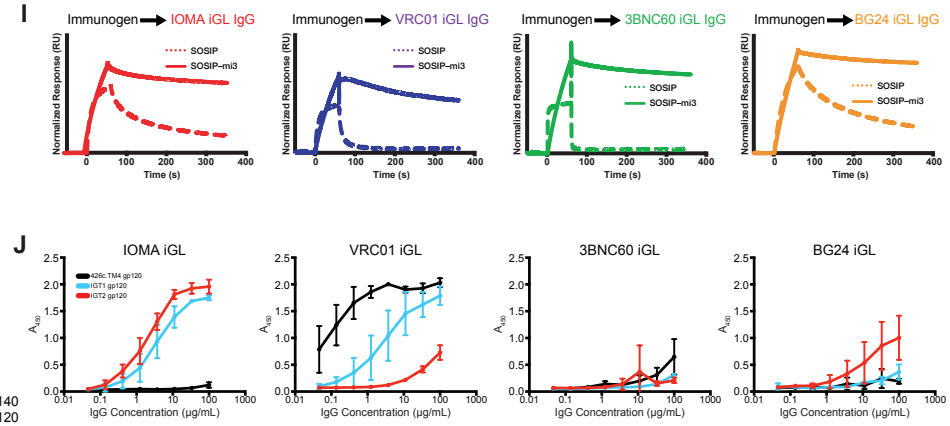
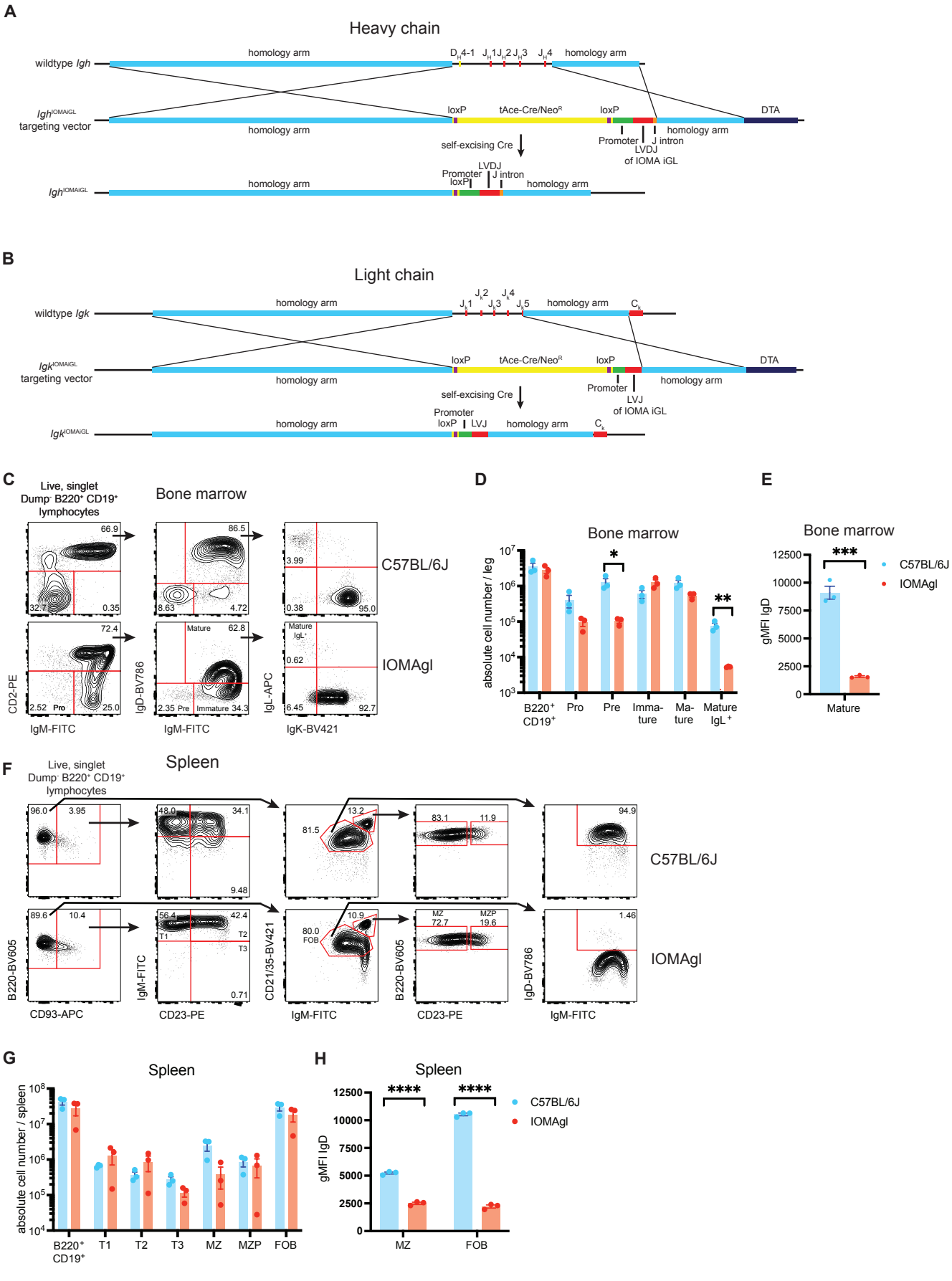


Figure S1. Development and characterization of IGT1 and IGT2 immunogens. (A) Amino acid alignment of IOMA and VRC01 to their respective germline V genes. **(B)** Representative SPR sensorgrams demonstrating no detectable binding of IOMA iGL to previously described immunogens (eOD-GT8, 426c.TM4, BG505.v4.1-GT1). This experiment was performed to qualitatively evaluate binding of IGT2 and previously described CD4bs immunogens to IOMA iGL rather than to derive affinity or kinetic constants. **(C)** Neutralization titers (IC_{50}s) of IOMA and IOMA iGL against a panel of 38 viruses and an MLV control. **(D)** 2.07 Å crystal structure of IOMA iGL Fab shown in two views. **(E)** Structural overlay of IOMA iGL Fab and IOMA Fab from BG505-bound structure (PDB 5T3Z).**(F)** Flow cytometric analysis of yeast cells expressing 426c.TM4 starting protein (left), Library 1 (middle), or Library 2 (right) stained with IOMA iGL IgG/anti-IgG AF647 (x-axis) and anti-cMyc AF488 (y-axis). **(G)** Representative size exclusion chromatography profiles and Coomassie-stained SDS-PAGE analysis for 426c.TM4 gp120, IGT1 gp120, and IGT2 gp120, 426c SOSIP, IGT1 SOSIP, and IGT2 SOSIP demonstrating that all of these proteins are monodispersed samples and that the selected mutations do not alter the stability or behavior of the immunogens compared to the starting proteins. **(H)** Coomassie-stained SDS–PAGE analysis for mi3, IGT2, IGT2-mi3, IGT1, and IGT1-mi3 under non-reducing and reducing conditions. **(I)** SPR sensorgrams demonstrating binding of IGT2 (dashed line) and IGT2-mi3 (solid line) to IOMA iGL IgG (red), VRC01 iGL IgG (purple), 3BNC60 iGL IgG (green), and BG24 iGL IgG (orange). IgG was immobilized to the CM5 chip and 1 μM SOSIP or 1 μM SOSIP-mi3 was flowed over the chip surface. **(J)** Representative ELISA binding curves measuring binding of 426c.TM4 gp120, IGT1 gp120, and IGT2 gp120 to the same iGL IgGs as in (I). Dots indicate mean and error bars indicate 95% confidence interval.

Figure S2


A
Heavy chain
wildtype Igh
homology arm
DH4-1 JH1 JH2 JH3 JH4
homology arm
IghIOMAiGL targeting vector
loxP
tAce-Cre/NeoR
loxP
DTA
homology arm
Promoter
J intron
homology arm
self-excising Cre
LVDJ of IOMA iGL
LVDJ
Promoter
J intron
loxP
IghIOMAiGL
homology arm
homology arm
B
Light chain
wildtype Igk
homology arm
Jk1 Jk2 Jk3 Jk4 Jk5
homology arm
Ck
IgkIOMAiGL targeting vector
loxP
tAce-Cre/NeoR
loxP
DTA
homology arm
homology arm
Promoter
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Promoter
loxP
LVJ
homology arm
Ck
IgkIOMAiGL
C
Live, singlet Dump- B220+ CD19+ lymphocytes
Bone marrow
C57BL/6J
IOMAgI
CD2-PE
IgM-FITC
IgD-BV786
IgM-FITC
IgL-APC
IgK-BV421
Pro
Pre
Immature
Mature
IgL+
D
Bone marrow
absolute cell number / leg
B220+ CD19+
Pro
Pre
Imma-ture
Ma-ture
Mature IgL+
E
Bone marrow
gMFI IgD
C57BL/6J
IOMAgI
Mature
F
Live, singlet Dump- B220+ CD19+ lymphocytes
Spleen
C57BL/6J
IOMAgI
B220-BV605
CD93-APC
IgM-FITC
CD23-PE
CD21/35-BV421
IgM-FITC
B220-BV605
CD23-PE
IgD-BV786
IgM-FITC
T1
T2
T3
MZ
MZP
FOB
G
Spleen
absolute cell number / spleen
B220+ CD19+
T1
T2
T3
MZ
MZP
FOB
H
Spleen
gMFI IgD
C57BL/6J
IOMAgI
MZ
FOB

Figure S2. Targeting strategy and characterization of IOMAgl mice. (A) In *Igh*^{IOMAiGL} mice *Ighd4-1* to *Ighj4* are replaced by a self-excising Neomycin cassette followed by the mouse *Ighv9-4* promoter, a leader sequence (L) followed by the iGL version of the IOMA HC VDJ sequence and a *Ighj1* splice donor sequence. **(B)** In *Igk*^{IOMAiGL} mice *Igkj1* to *Igkj5* are replaced by a self-excising Neomycin cassette followed by a mouse *Igkv3-12* promoter, a leader sequence followed by the iGL version of the IOMA lambda LC VDJ sequence and a *Igkj5* splice donor sequence. DTA, diphtheria toxin A **(C)** Flow cytometric analysis of B cell development in the bone marrow of control (C57BL/6J) or IOMAgl (*Igh*$^{IOMAiGL/IOMAiGL}$ *Igk*$^{IOMAiGL/IOMAiGL}$) mice. **(D)** Absolute cell number quantification from (C). **(E)** Geometric mean fluorescence intensity (gMFI) of IgD in mature recirculating B cells from the bone marrow. **(F)** Flow cytometric analysis of peripheral B cell development in the spleens of control (C57BL/6J) or IOMAgl mice. **(G)** Absolute cell number quantification from (F). **(H)** gMFI of IgD in marginal zone and follicular B cell. MZ, marginal zone B cells; MZP, marginal zone precursors; FOB, follicular B cells. Data from 1 of 2 independent experiments, each dot represents a data from 1 mouse. Bars represent mean ± SEM. Statistical analysis used unpaired t test.

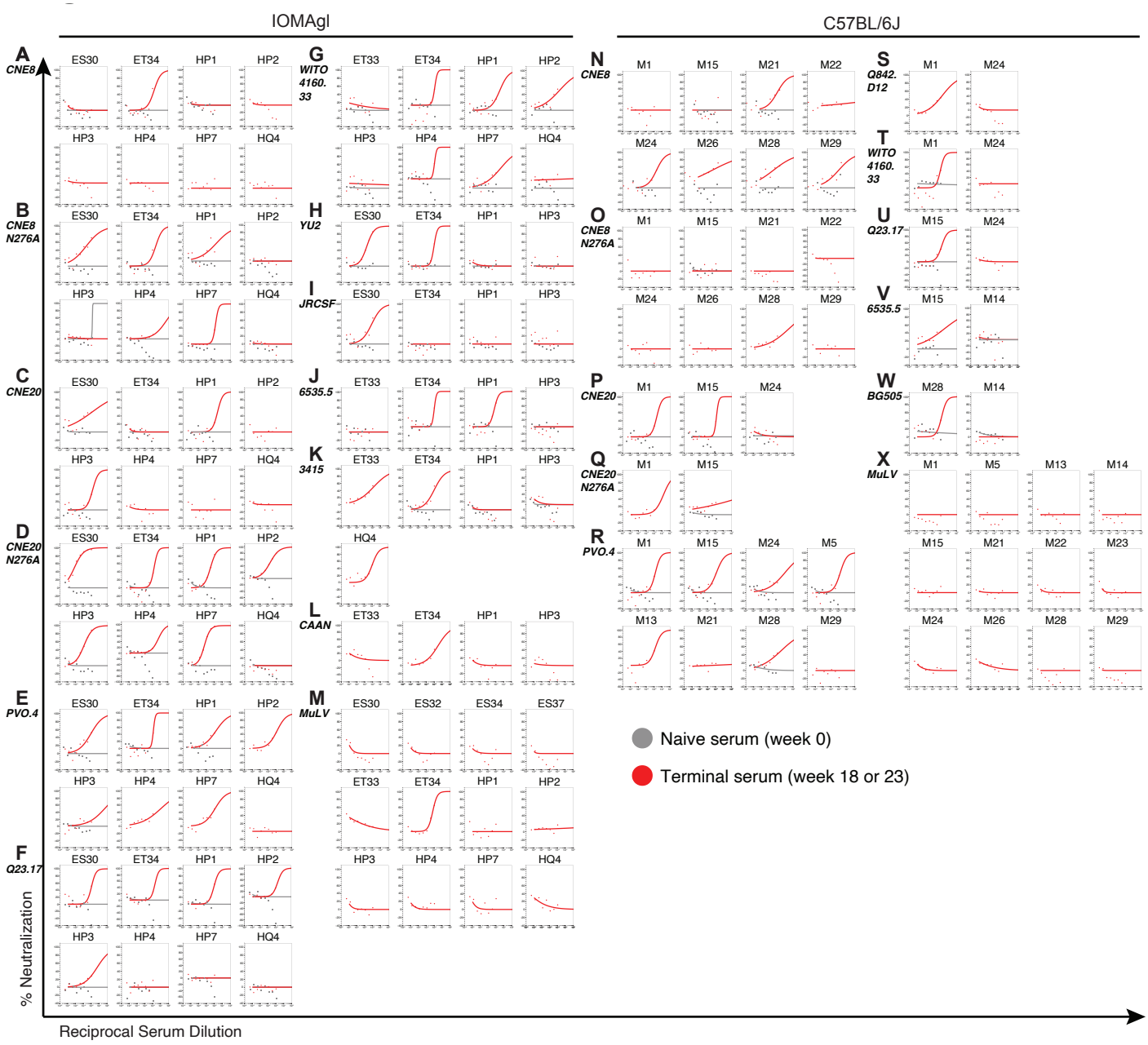


Figure S3. Serum neutralization from immunized mice. Neutralization curves of serum isolated from IOMA iGL transgenic mice (**A-M**) or C57BL/6J wildtype mice (**N-X**) against the following HIV strains or control MuLV: **(A,N)** CNE8, **(B,O)** CNE8 N276A, **(C,P)** CNE20, **(D,Q)** CNE20 N276A, **(E,R)** PVO.4, **(F,U)** Q23.17, **(G,T)** WITO4160.33, **(H)** YU2, **(I)** JRCSF, **(J, V)** 6535.5, **(K)** 3415_V1_C1, **(L)** CAAN5342.A2, **(M,X)** MuLV, (**S**) Q842.D12 and (**W**) BG505. Naïve serum was also tested against the same strains when available. Note that sera which showed neutralization activity of < 40% as listed in Table S3 are presented in Figure 2G as white rectangles; several of these sera neutralized strains above background including ET33 against PVO.4; ET34 against CNE20 N276A and Q23.17; HP1 against CNE8 N276A, CNE20, and WITO4160.33; HP2 against Q23.17; HP3 against Q23.17 and PVO.4; HP4 against CNE8 N276A, CNE20 N276A, and PVO.4.

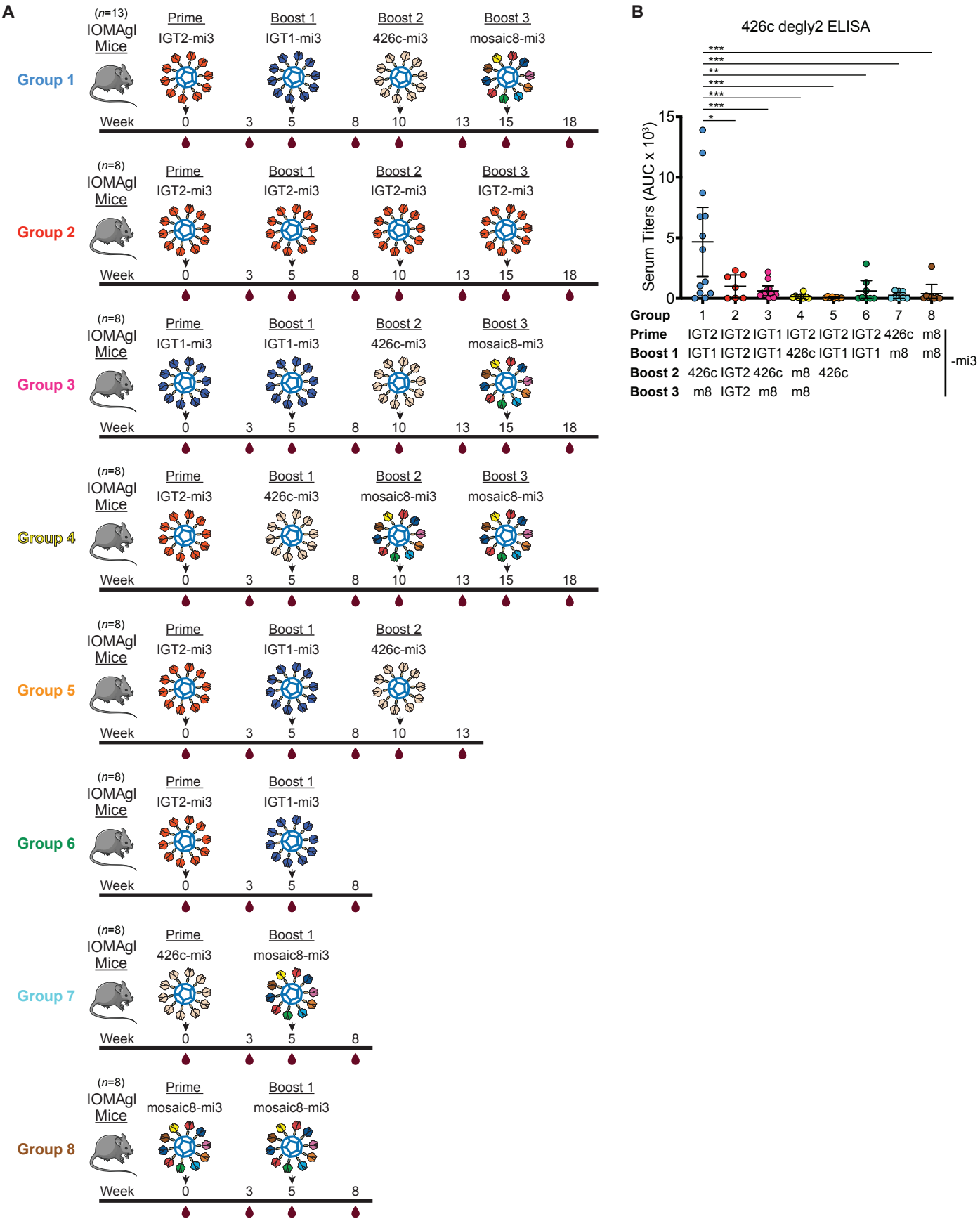

A
(n=13)
IOMAgl Mice
Group 1
Prime
IGT2-mi3
Boost 1
IGT1-mi3
Boost 2
426c-mi3
Boost 3
mosaic8-mi3
Week 0 3 5 8 10 13 15 18
(n=8)
IOMAgl Mice
Group 2
Prime
IGT2-mi3
Boost 1
IGT2-mi3
Boost 2
IGT2-mi3
Boost 3
IGT2-mi3
Week 0 3 5 8 10 13 15 18
(n=8)
IOMAgl Mice
Group 3
Prime
IGT1-mi3
Boost 1
IGT1-mi3
Boost 2
426c-mi3
Boost 3
mosaic8-mi3
Week 0 3 5 8 10 13 15 18
(n=8)
IOMAgl Mice
Group 4
Prime
IGT2-mi3
Boost 1
426c-mi3
Boost 2
mosaic8-mi3
Boost 3
mosaic8-mi3
Week 0 3 5 8 10 13 15 18
(n=8)
IOMAgl Mice
Group 5
Prime
IGT2-mi3
Boost 1
IGT1-mi3
Boost 2
426c-mi3
Week 0 3 5 8 10 13
(n=8)
IOMAgl Mice
Group 6
Prime
IGT2-mi3
Boost 1
IGT1-mi3
Week 0 3 5 8
(n=8)
IOMAgl Mice
Group 7
Prime
426c-mi3
Boost 1
mosaic8-mi3
Week 0 3 5 8
(n=8)
IOMAgl Mice
Group 8
Prime
mosaic8-mi3
Boost 1
mosaic8-mi3
Week 0 3 5 8
B
426c degly2 ELISA
Serum Titers (AUC x 10³)
0 5 10 15
***
***
**
***
***
***
*
Group 1 2 3 4 5 6 7 8
Prime IGT2 IGT2 IGT1 IGT2 IGT2 IGT2 426c m8
Boost 1 IGT1 IGT2 IGT1 426c IGT1 IGT1 m8 m8
Boost 2 426c IGT2 426c m8 426c
Boost 3 m8 IGT2 m8 m8
-mi3

Figure S4. Screening immunization regimens to determine the optimal boosting strategy. (A) Schematic and timeline of immunization strategies to determine the optimal regimen to elicit IOMA-like bNAbs. **(B)** Serum ELISA binding to 426c degly2 represented as AUC using serum samples isolated from mice at the end of the regimen. m8, mosaic8.

Figure S5

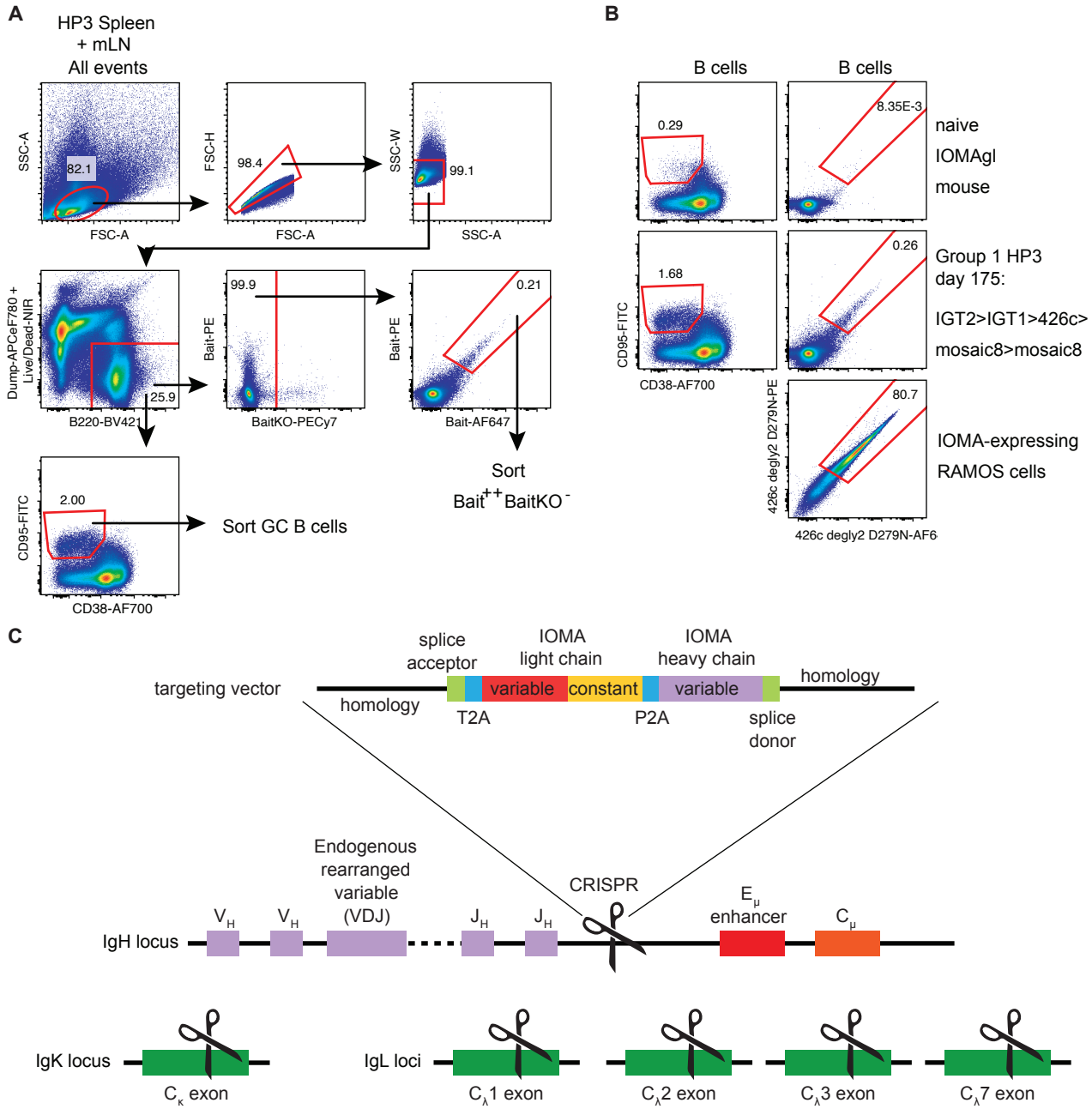


Figure S5. Cell sorting strategies and sorting controls. (A) Representative full gating of cell sorts for single cell Bait++ BaitKO^{-} B cell cloning and 10x Genomics next generation VDJ sequencing of bulk-sorted GC B cells from splenic and mesenteric lymph nodes. Baits used were 426c degly2 D279N or CNE8 N276A with 426c degly2 D279N-CD4bs KO, the former is shown. **(B)** Induction of germinal center response and wt SOSIP-binding cells by immunization regimen (group 1). Naïve IOMAgl mouse splenocytes and IOMA-expressing RAMOS cells served as negative and positive control, respectively. (**C**) Gene editing strategy to generate IOMA-expressing RAMOS cells.

Simultaneous targeting of IgH, IgK and IgL loci with CRISPR/Cas9 to delete endogenous LCs and edit a promoterless tricistronic expression cassette into the IgH locus to express IOMA on the surface of RAMOS cells. A polycistronic mRNA was created using T2A and P2A sequences to induce ribosomal skipping (*96*).


A
Color code
Identity
100 %
<100 - 30 %
<30 %
Kabat #
Mouse
Sorted Population
Identity [%]
Antibody ID
IOMA
IOMA IGL
Domains
CDRH1
CDRH2
CDRH3
Structurally important residues
B
CDRL1
CDRL2
CDRL3

Figure S6. Amino acid alignments of selected IOMAgl mouse-derived antibodies. (A) V_H alignment of cloned antibodies IO-001 to IO-067 that were expressed and tested for Env binding. IOMA iGL and IOMA sequence at the bottom as reference. Mouse ID and population sorted are indicated. Differences to IOMA iGL are highlighted using chemically similar color coding; dots indicate identical residues to IOMA iGL. Kabat numbering and percent identity of residues are indicated on top. Domains and residues of structural importance are annotated below. **(B)** as above but corresponding V_L alignment.

Figure S7

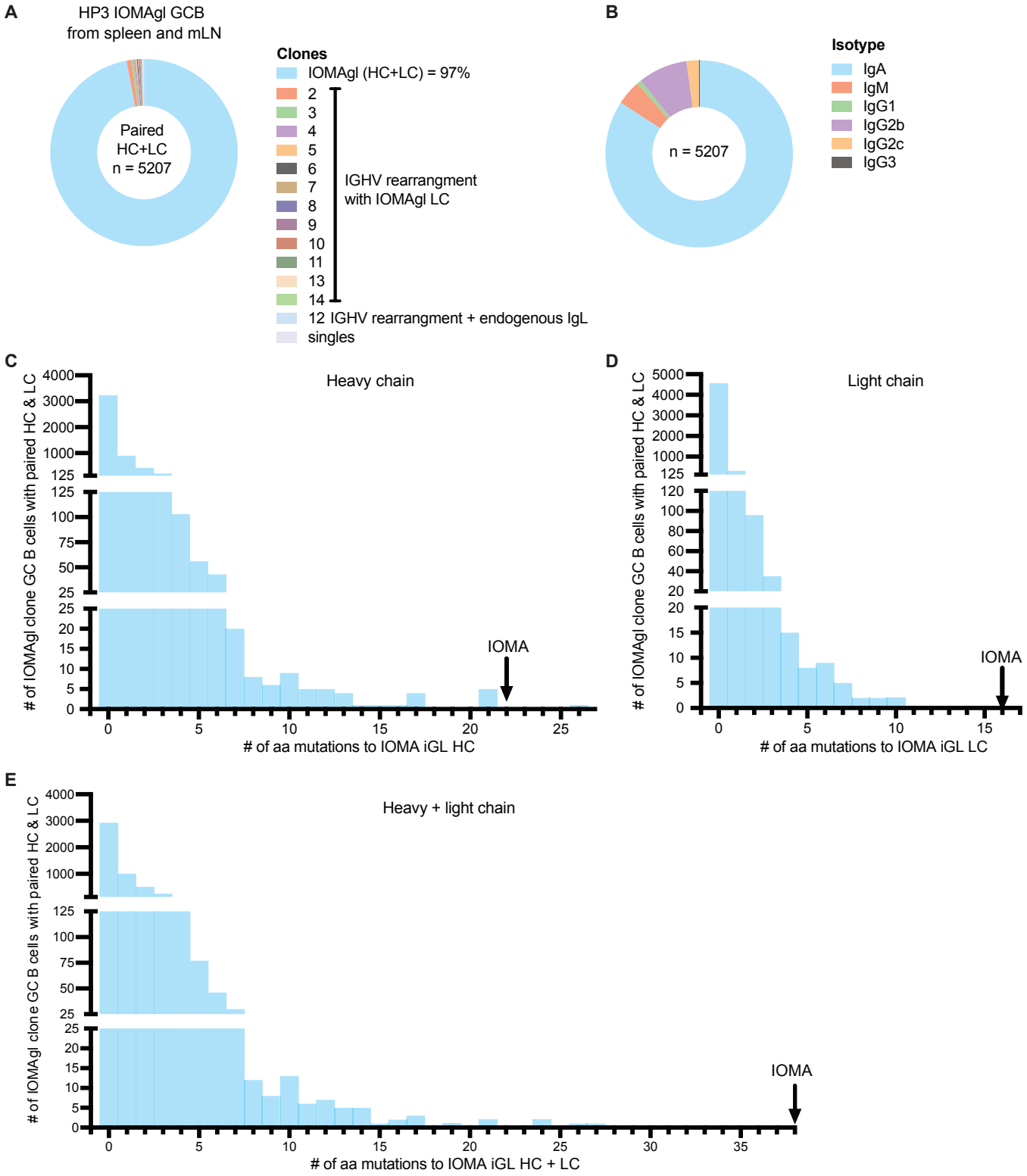
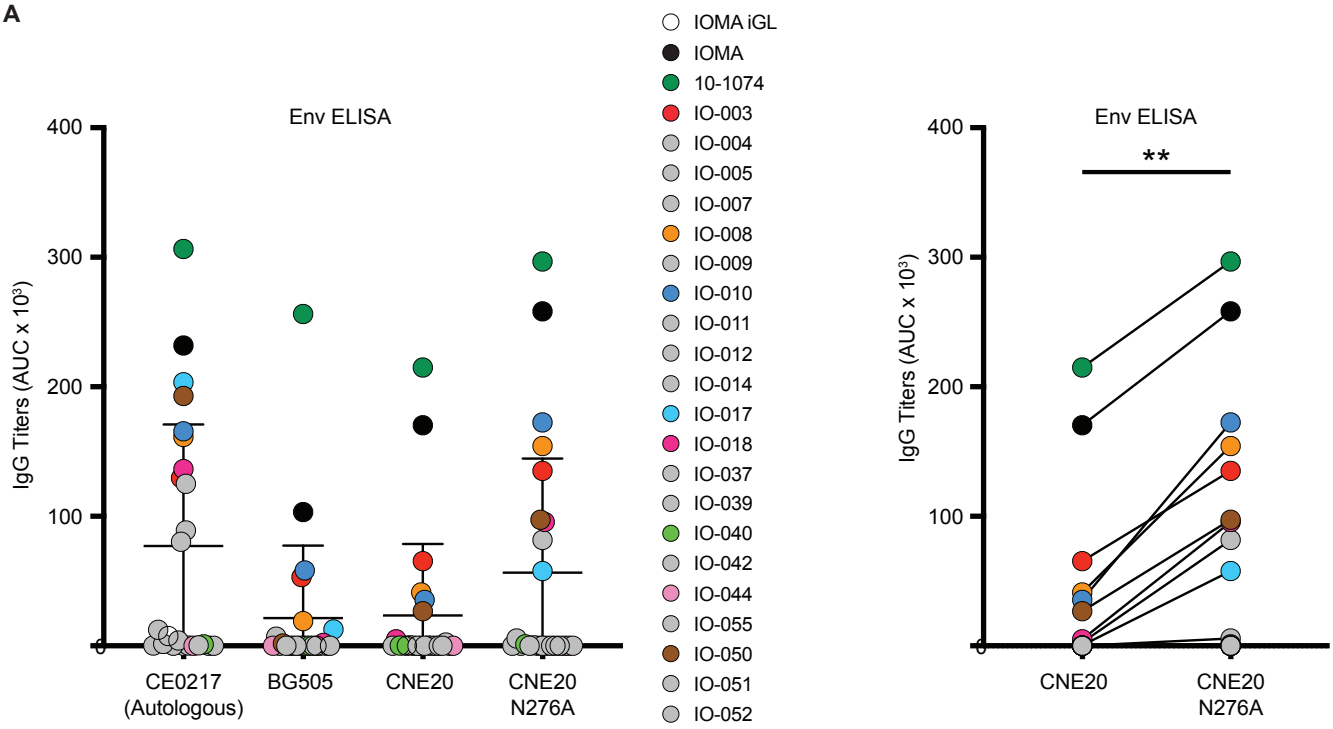


Figure S7. Next generation single cell VDJ analysis determines the extent of mutations in germinal centers of IOMAgl mice. (A) Clonal analysis of paired HC and LC sequences from splenic and mesenteric lymph node germinal center B cells of IOMAgl mouse HP3. **(B)** Isotype distribution among these cells. **(C)** Frequency distribution of the number of amino acid mutations to IOMA iGL in

the HC sequences of these cells. **(D)** Frequency distribution of the number of amino acid mutations to IOMA iGL in the LC sequences of these cells. **(E)** Frequency distribution of the number of amino acid mutations to IOMA iGL in the paired HC and LC sequences of these cells.

Figure S8

A



B

Reference		This Study	Chen et al, *Immunity* 2021			
Immunogen		IGT2-mi3	eOD-GT6 60mer		eOD-GT8 60mer	
Immunizations		4 or 5	6	9	4	9
Residue	SHM					
Heavy Chain						
K19	T/R	67	N/A	N/A	N/A	N/A
Y33	H/D/E/F (V/F/L/I)	58	7.5	56	25	85
V37	(M/L/I)	N/A	3.7	23	7.50	52
N52	(K/R)	N/A	0	9.3	4.3	23
N53	F/Y/K/R/T (Y/K/R/T/V/L)	73	31	56	20	65
S54	R/F/N/T (R/F/N/T/Y/W/H/G)	82	41	65	46	88
G56	A (A)	30	1.5	40	2.2	54
T57	V/I/P/R (V/I/P/R)	66	13	67	12	88
N58	K/E/D	58	N/A	N/A	N/A	N/A
Q61	(R/H/K/W)	N/A	13	40	37	58
V89	(L/I/M/T)	N/A	4.5	26	5.9	77
Light Chain						
CDR L1	(deletion)	N/A	2.6	21	0	15
CDR L1	(G/S)	N/A	11.8	47	7.2	69
Q27	(Y/F/H)	N/A	0	5.3	0	17
S27A	R/N (F/Y)	5.9	0	18	0	17
S31	G	6.0	N/A	N/A	N/A	N/A
Y32	F	10	N/A	N/A	N/A	N/A
N33	D	42	N/A	N/A	N/A	N/A
G93	D	33	N/A	N/A	N/A	N/A
Average # Key SHMs per HC		4.3	1.6	3.8	1.6	6.5

Figure S8. Monoclonal antibodies cloned from IOMA iGL transgenic mice bind to heterologous Envs. (A) AUC of ELISA binding curves of selected monoclonal antibodies isolated from IOMA iGL knock-in mice to BG505, CE0217, CNE20 and CNE20 N276A SOSIPs. **(B)** Comparison of the occurrence frequency of key mutations among IOMA-like antibody sequences selected for cloning and VRC01-class antibody sequences from reference 37 at different time points throughout the respective sequential immunization regimen. Mutations essential for IOMA-class antibody binding to gp120 are listed first, while mutations essential for VRC01-class antibody binding to gp120 are listed second in brackets. Values for each residue represent the percentage of antibodies containing one of the essential mutations at that position.

Tables S1 to S8

Table S1: Amino acid sequences for HIV Envs and antibodies used in this study.

Protein Name	Sequence
IGT2 gp120	VWKEAKTTLFCASDAKAYEKECHNVWATHACVPTDPNPQEVVLENVTENFNMWKNDMVDQMQEDVISIWDQ CLKPCVKLTNTSTLTQACPKVTFDPIPIHYCAPAGYAILKCNNKTFNGKGPCNNVSTVQCTHGIKPVVSTQ LLLNGSLAEEEIVIRSKNLRNNAKIIIVQLNKSVEIVCTRPNNGGSGSGGDIRQAYCNISGRNWSEAVNQV KKKLKEHFPHKNISFQSSSGGDLEITTHSFNCGGEFFYCNTSGLFNDTISNATIMLPCRIKQIINMWQEPG KAIYAPPIKGNITCKSDITGLLLLRDGGNALRPTEIFRPSGGDMRDNWRSELYKYKVVEIKPLHHHHHH
IGT1 gp120	VWKEAKTTLFCASDAKAYEKECHNVWATHACVPTDPNPQEVVLENVTENFNMWKNDMVDQMQEDVISIWDQ CLKPCVKLTNTSTLTQACPKVTFDPIPIHYCAPAGYAILKCNNKTFNGKGPCNNVSTVQCTHGIKPVVSTQ LLLNGSLAEEEIVIRSKNLRNNAKIIIVQLNKSVEIVCTRPNNGGSGSGGDIRQAYCNISGRNWSEAVNQV KKKLKEHFPHKNISFQSSSGGDLEITTHSFNCGGEFFYCNTSGLFNDTISNATIMLPCRIKQIINMWQEPG KAIYAPPIKGNITCKSDITGLLLLRDGGNSQRETEIFRPSGGDMRDNWRSELYKYKVVEIKPLHHHHHH
426c.TM4 gp120	VWKEAKTTLFCASDAKAYEKECHNVWATHACVPTDPNPQEVVLENVTENFNMWKNDMVDQMQEDVISIWDQ CLKPCVKLTNTSTLTQACPKVTFDPIPIHYCAPAGYAILKCNNKTFNGKGPCNNVSTVQCTHGIKPVVSTQ LLLNGSLAEEEIVIRSKNLRDNAKIIIVQLNKSVEIVCTRPNNGGSGSGGDIRQAYCNISGRNWSEAVNQV KKKLKEHFPHKNISFQSSSGGDLEITTHSFNCGGEFFYCNTSGLFNDTISNATIMLPCRIKQIINMWQEVG KAIYAPPIKGNITCKSDITGLLLLRDGGDTTDNTEIFRPSGGDMRDNWRSELYKYKVVEIKPLHHHHHH
IGT2 SOSIP	GSNLWVTVYYGVPVWKEAKTTLFCASDAKAYEKEVHNVWATHACVPTDPNPQEVVLENVTENFNMWKNDMV DQMQEDVISIWDQSLKPCVKLTPLCVTLNCTNVNVTSNSTNVNSSSTDNTTLGEIKNCSFDITTEIRDKTR KEYALFYRLDIVPLDNSSNPNSSNTYRLINCNTSTCTQACPKVTFDPIPIHYCAPAGYAILKCNNKTFNGK GPCNNVSTVQCTHGIKPVVSTQLLLNGSLAEEEIVIRSKNLRNNAKIIIVQLNKSVEIVCTRPNNNTRRSI RIGPGQTFYATDIIGDIRQAYCNISGRNWSEAVNQVKKKLKEHFPHKNISFQSSSGGDLEITTHSFNCGGE FFYCNTSGLFNDTISNATIMLPCRIKQIINMWQEVGKCIYAPPIKGNITCKSDITGLLLLRDGGNALRPTE IFRPSGGDMRDNWRSELYKYKVVKIEPLGVAPTRCKRRVVGRRRRRRAVGIGAVFLGFLGAAGSTMGAASM TLTVQARNLLSGIVQQQSNLLRAPEAQQHLLKLTVWGIKQLQARVLAVERYLRDQQLLGIWGCSGKLICCT NVPWNSSWSNRNLSEIWDNMTWLQWDKEISNYTQIIYGLLEESQNQQEKNEQDLLALD
IGT1 SOSIP	GSNLWVTVYYGVPVWKEAKTTLFCASDAKAYEKEVHNVWATHACVPTDPNPQEVVLENVTENFNMWKNDMV DQMQEDVISIWDQSLKPCVKLTPLCVTLNCTNVNVTSNSTNVNSSSTDNTTLGEIKNCSFDITTEIRDKTR KEYALFYRLDIVPLDNSSNPNSSNTYRLINCNTSTCTQACPKVTFDPIPIHYCAPAGYAILKCNNKTFNGK GPCNNVSTVQCTHGIKPVVSTQLLLNGSLAEEEIVIRSKNLRNNAKIIIVQLNKSVEIVCTRPNNNTRRSI RIGPGQTFYATDIIGDIRQAYCNISGRNWSEAVNQVKKKLKEHFPHKNISFQSSSGGDLEITTHSFNCGGE FFYCNTSGLFNDTISNATIMLPCRIKQIINMWQEVGKCIYAPPIKGNITCKSDITGLLLLRDGGNSQRETE IFRPSGGDMRDNWRSELYKYKVVKIEPLGVAPTRCKRRVVGRRRRRRAVGIGAVFLGFLGAAGSTMGAASM TLTVQARNLLSGIVQQQSNLLRAPEAQQHLLKLTVWGIKQLQARVLAVERYLRDQQLLGIWGCSGKLICCT NVPWNSSWSNRNLSEIWDNMTWLQWDKEISNYTQIIYGLLEESQNQQEKNEQDLLALD
426c SOSIP	AENLWVTVYYGVPVWKEAKTTLFCASDAKAYEKEVHNVWATHACVPTDPNPQEVVLENVTENFNMWKNDMV DQMQEDVISIWDQSLKPCVKLTPLCVTLNCTNVNVTSNSTNVNSSSTDNTTLGEIKNCSFDITTEIRDKTR KEYALFYRLDIVPLDNSSNPNSSNTYRLINCNTSTCTQACPKVTFDPIPIHYCAPAGYAILKCNNKTFNGK GPCNNVSTVQCTHGIKPVVSTQLLLNGSLAEEEIVIRSKNLSDNAKIIIVQLNKSVEIVCTRPNNNTRRSI RIGPGQTFYATDIIGDIRQAYCNISGRNWSEAVNQVKKKLKEHFPHKNISFQSSSGGDLEITTHSFNCGGE FFYCNTSGLFNDTISNATIMLPCRIKQIINMWQEVGKCIYAPPIKGNITCKSDITGLLLLRDGGNTTNNTE IFRPGGGDMRDNWRSELYKYKVVKIEPLGVAPTRCKRRVVGRRRRRRAVGIGAVFLGFLGAAGSTMGAASM TLTVQARNLLSGIVQQQSNLLRAPEAQQHLLKLTVWGIKQLQARVLAVERYLRDQQLLGIWGCSGKLICCT NVPWNSSWSNRNLSEIWDNMTWLQWDKEISNYTQIIYGLLEESQNQQEKNEQDLLALD
426c D279N SOSIP	AENLWVTVYYGVPVWKEAKTTLFCASDAKAYEKEVHNVWATHACVPTDPNPQEVVLENVTENFNMWKNDMV DQMQEDVISIWDQSLKPCVKLTPLCVTLNCTNVNVTSNSTNVNSSSTDNTTLGEIKNCSFDITTEIRDKTR KEYALFYRLDIVPLDNSSNPNSSNTYRLINCNTSTCTQACPKVTFDPIPIHYCAPAGYAILKCNNKTFNGK GPCNNVSTVQCTHGIKPVVSTQLLLNGSLAEEEIVIRSKNLSNNAKIIIVQLNKSVEIVCTRPNNNTRRSI RIGPGQTFYATDIIGDIRQAYCNISGRNWSEAVNQVKKKLKEHFPHKNISFQSSSGGDLEITTHSFNCGGE FFYCNTSGLFNDTISNATIMLPCRIKQIINMWQEVGKCIYAPPIKGNITCKSDITGLLLLRDGGNTTNNTE IFRPGGGDMRDNWRSELYKYKVVKIEPLGVAPTRCKRRVVGRRRRRRAVGIGAVFLGFLGAAGSTMGAASM TLTVQARNLLSGIVQQQSNLLRAPEAQQHLLKLTVWGIKQLQARVLAVERYLRDQQLLGIWGCSGKLICCT NVPWNSSWSNRNLSEIWDNMTWLQWDKEISNYTQIIYGLLEESQNQQEKNEQDLLALD

426c degly2 SOSIP	AENLWVTVYYGVPVWKEAKTTLFCASDAKAYEKEVHNVWATHACVPTDPNPQEVVLENVTENFNMWKNDMV DQMQEDVISIWDQSLKPCVKLTPLCVTLNCTNVNVTSNSTNVNSSSTDNTTLGEIKNCSFDITTEIRDKTR KEYALFYRLDIVPLDNSSNPNSSNTYRLINCNTSTCTQACPKVTFDPIPIHYCAPAGYAILKCNNKTFNGK GPCNNVSTVQCTHGIKPVVSTQLLLNGSLAEEEIVIRSKNLTDNAKIIIVQLNKSVEIVCTRPNNNTRRSI RIGPGQTFYATDIIGDIRQAYCNISGRNWSEAVNQVKKKLKEHFPHKNISFQSSSGGDLEITTHSFNCGGE FFYCNTSGLFNDTISNATIMLPCRIKQIINMWQEVGKCIYAPPIKGNITCKSDITGLLLLRDGGNTANNAE IFRPGGGDMRDNWRSELYKYKVVKIEPLGVAPTRCKRRVVGRRRRRRAVGIGAVFLGFLGAAGSTMGAASM TLTVQARNLLSGIVQQQSNLLRAPEAQQHLLKLTVWGIKQLQARVLAVERYLRDQQLLGIWGCSGKLICCT NVPWNSSWSNRNLSEIWDNMTWLQWDKEISNYTQIIYGLLEESQNQQEKNEQDLLALD
426c degly2 D279N SOSIP	AENLWVTVYYGVPVWKEAKTTLFCASDAKAYEKEVHNVWATHACVPTDPNPQEVVLENVTENFNMWKNDMV DQMQEDVISIWDQSLKPCVKLTPLCVTLNCTNVNVTSNSTNVNSSSTDNTTLGEIKNCSFDITTEIRDKTR KEYALFYRLDIVPLDNSSNPNSSNTYRLINCNTSTCTQACPKVTFDPIPIHYCAPAGYAILKCNNKTFNGK GPCNNVSTVQCTHGIKPVVSTQLLLNGSLAEEEIVIRSKNLTNNAKIIIVQLNKSVEIVCTRPNNNTRRSI RIGPGQTFYATDIIGDIRQAYCNISGRNWSEAVNQVKKKLKEHFPHKNISFQSSSGGDLEITTHSFNCGGE FFYCNTSGLFNDTISNATIMLPCRIKQIINMWQEVGKCIYAPPIKGNITCKSDITGLLLLRDGGNTANNAE IFRPGGGDMRDNWRSELYKYKVVKIEPLGVAPTRCKRRVVGRRRRRRAVGIGAVFLGFLGAAGSTMGAASM TLTVQARNLLSGIVQQQSNLLRAPEAQQHLLKLTVWGIKQLQARVLAVERYLRDQQLLGIWGCSGKLICCT NVPWNSSWSNRNLSEIWDNMTWLQWDKEISNYTQIIYGLLEESQNQQEKNEQDLLALD
426c degly3 SOSIP	AENLWVTVYYGVPVWKEAKTTLFCASDAKAYEKEVHNVWATHACVPTDPNPQEVVLENVTENFNMWKNDMV DQMQEDVISIWDQSLKPCVKLTPLCVTLNCTNVNVTSNSTNVNSSSTDNTTLGEIKNCSFDITTEIRDKTR KEYALFYRLDIVPLDNSSNPNSSNTYRLINCNTSTCTQACPKVTFDPIPIHYCAPAGYAILKCNNKTFNGK GPCNNVSTVQCTHGIKPVVSTQLLLNGSLAEEEIVIRSKALTDNAKIIIVQLNKSVEIVCTRPNNNTRRSI RIGPGQTFYATDIIGDIRQAYCNISGRNWSEAVNQVKKKLKEHFPHKNISFQSSSGGDLEITTHSFNCGGE FFYCNTSGLFNDTISNATIMLPCRIKQIINMWQEVGKCIYAPPIKGNITCKSDITGLLLLRDGGNTANNAE IFRPGGGDMRDNWRSELYKYKVVKIEPLGVAPTRCKRRVVGRRRRRRAVGIGAVFLGFLGAAGSTMGAASM TLTVQARNLLSGIVQQQSNLLRAPEAQQHLLKLTVWGIKQLQARVLAVERYLRDQQLLGIWGCSGKLICCT NVPWNSSWSNRNLSEIWDNMTWLQWDKEISNYTQIIYGLLEESQNQQEKNEQDLLALD
BG505.v4.1-GT1 SOSIP	AENLWVTVYYGVPVWKDAETTLFCASDAKAYETKKHNVWATHACVPTDPNPQEIHLENVTEEFNMWKNNMV EQMHTDIISLWDQSLKPCVKLTPLCVTLQCTNVTNAITDDMRGELKNCSFNMTTELRDKRQKVHALFYKLD IVPINENQNTSYRLINCNTAAITQACPKVSFEPIPIHYCAPAGFAILKCKDKKFNGTGPCPSVSTVQCTHG IKPVVSTQLLLNGSLAEEEVMIRSEDIRNNAKNILVQFNTPVQINCTRPNNNTRKSIRIGPGQWFYATGDI IGDIRQAHCNVSKATWNETLGKVVKQLRKHFGNNTIIRFANSSGGDLEVTTHSFNCGGEFFYCDTSGLFNS TWISNTSVQGSNSTGSNDSITLPCRIKQIINMWQRIGQAMYAPPIQGVIRCVSNITGLILTRDGGSTDSTT ETFRPSGGDMRDNWRSELYKYKVVKIEPLGVAPTRCKRRVVGRRRRRRAVGIGAVFLGFLGAAGSTMGAAS MTLTVQARNLLSGIVQQQSNLLRAPEAQQHLLKLTVWGIKQLQARVLAVERYLRDQQLLGIWGCSGKLICC TNVPWNSSWSNRNLSEIWDNMTWLQWDKEISNYTQIIYGLLEESQNQQEKNEQDLLALD
eOD-GT8	DTITLPCRPAPPPHCSSNITGLILTRQGGYSNANTVIFRPSGGDWRDIARCQIAGTVVSTQLFLNGSLAEE EVVIRSEDWRDNAKSICVQLATSVEIACTGAGHCAISRAKWANTLKQIASKLREQYGAKTIIFKPSSGGDP EFVNHSFNCGGEFFYCASTQLFASTWFASTGTGTK
IGT2 SOSIP SpyTag	GSNLWVTVYYGVPVWKEAKTTLFCASDAKAYEKEVHNVWATHACVPTDPNPQEVVLENVTENFNMWKNDMV DQMQEDVISIWDQSLKPCVKLTPLCVTLNCTNVNVTSNSTNVNSSSTDNTTLGEIKNCSFDITTEIRDKTR KEYALFYRLDIVPLDNSSNPNSSNTYRLINCNTSTCTQACPKVTFDPIPIHYCAPAGYAILKCNNKTFNGK GPCNNVSTVQCTHGIKPVVSTQLLLNGSLAEEEIVIRSKNLRNNAKIIIVQLNKSVEIVCTRPNNNTRRSI RIGPGQTFYATDIIGDIRQAYCNISGRNWSEAVNQVKKKLKEHFPHKNISFQSSSGGDLEITTHSFNCGGE FFYCNTSGLFNDTISNATIMLPCRIKQIINMWQEVGKCIYAPPIKGNITCKSDITGLLLLRDGGNALRPTE IFRPSGGDMRDNWRSELYKYKVVKIEPLGVAPTRCKRRVVGRRRRRRAVGIGAVFLGFLGAAGSTMGAASM TLTVQARNLLSGIVQQQSNLLRAPEAQQHLLKLTVWGIKQLQARVLAVERYLRDQQLLGIWGCSGKLICCT NVPWNSSWSNRNLSEIWDNMTWLQWDKEISNYTQIIYGLLEESQNQQEKNEQDLLALDGGGGSGGGSGGGS GSGAHIVMVDAYKPTK
IGT1 SOSIP SpyTag	GSNLWVTVYYGVPVWKEAKTTLFCASDAKAYEKEVHNVWATHACVPTDPNPQEVVLENVTENFNMWKNDMV DQMQEDVISIWDQSLKPCVKLTPLCVTLNCTNVNVTSNSTNVNSSSTDNTTLGEIKNCSFDITTEIRDKTR KEYALFYRLDIVPLDNSSNPNSSNTYRLINCNTSTCTQACPKVTFDPIPIHYCAPAGYAILKCNNKTFNGK GPCNNVSTVQCTHGIKPVVSTQLLLNGSLAEEEIVIRSKNLRNNAKIIIVQLNKSVEIVCTRPNNNTRRSI RIGPGQTFYATDIIGDIRQAYCNISGRNWSEAVNQVKKKLKEHFPHKNISFQSSSGGDLEITTHSFNCGGE FFYCNTSGLFNDTISNATIMLPCRIKQIINMWQEVGKCIYAPPIKGNITCKSDITGLLLLRDGGNSQRETE IFRPSGGDMRDNWRSELYKYKVVKIEPLGVAPTRCKRRVVGRRRRRRAVGIGAVFLGFLGAAGSTMGAASM TLTVQARNLLSGIVQQQSNLLRAPEAQQHLLKLTVWGIKQLQARVLAVERYLRDQQLLGIWGCSGKLICCT NVPWNSSWSNRNLSEIWDNMTWLQWDKEISNYTQIIYGLLEESQNQQEKNEQDLLALDGGGGSGGGSGGGS GSGAHIVMVDAYKPTK

426c degly2 D279N SOSIP SpyTag	AENLWVTVYYGVPVWKEAKTTLFCASDAKAYEKEVHNVWATHACVPTDPNPQEVVLENVTENFNMWKNDMV DQMQEDVISIWDQSLKPCVKLTPLCVTLNCTNVNVTSNSTNVNSSSTDNTTLGEIKNCSFDITTEIRDKTR KEYALFYRLDIVPLDNSSNPNSSNTYRLINCNTSTCTQACPKVTFDPIPIHYCAPAGYAILKCNNKTFNGK GPCNNVSTVQCTHGIKPVVSTQLLLNGSLAEEEIVIRSKNLTNNAKIIIVQLNKSVEIVCTRPNNNTRRSI RIGPGQTFYATDIIGDIRQAYCNISGRNWSEAVNQVKKKLKEHFPHKNISFQSSSGGDLEITTHSFNCGGE FFYCNTSGLFNDTISNATIMLPCRIKQIINMWQEVGKCIYAPPIKGNITCKSDITGLLLLRDGGNTANNAE IFRPGGGDMRDNWRSELYKYKVVKIEPLGVAPTRCKRRVVGRRRRRRAVGIGAVFLGFLGAAGSTMGAASM TLTVQARNLLSGIVQQQSNLLRAPEAQQHLLKLTVWGIKQLQARVLAVERYLRDQQLLGIWGCSGKLICCT NVPWNSSWSNRNLSEIWDNMTWLQWDKEISNYTQIIYGLLEESQNQQEKNEQDLLALDGGGGSGGGSGGGS GSGRGVPHIVMVDAYKRYK
398F1 SOSIP SpyTag	AENLWVTVYYGVPVWKDAETTLFCASDAKAYHTEVHNVWATHACVPTDPNPQEINLENVTEEFNMWKNKMV EQMHTDIISLWDQSLKPCVQLTPLCVTLDCQYNVTNINSTSDMAREINNCSYNITTELRDREQKVYSLFYR SDIVQMNSDNSSKYRLINCNTSACKQACPKVTFEPIPIHYCAPAGFAILKCKDKEFNGTGPCKNVSTVQCT HGIKPVVSTQLLLNGSLAEEKVMIRSENITDNAKNIIVQFKEPVKINCTRPNNNTRKSVRIGPGQTFYATG EIIGDIRQAHCNVSKAHWENTLQEVANQLKLMIHSNKTIIFANSSGGDLEITTHSFNCGGEFFYCYTSGLF NYTFNDTSTNSTESKSNDTITLQCRIKQIINMWQRAGQCVYAPPIPGIIRCESNITGLILTRDGGNNNSNT NETFRPGGGDMRDNWRSELYRYKVVKIEPLGVAPTRCKRRVVGRRRRRRAVGIGAVSLGFLGAAGSTMGAA SMTLTVQARNLLSGIVQQQSNLLRAPEPQQHLLKDTHWGIKQLQARVLAVEHYLRDQQLLGIWGCSGKLIC CTNVPWNSSWSNRNLSEIWDNMTWLQWDKEISNYTQIIYGLLEESQNQQEKNEQDLLALDGGGGSGGGSGG GSGSGAHIVMVDAYKPTK
BJOX2000 SOSIP SpyTag	AENLWVTVYYGVPVWKEATTTLFCASDAKAYDTEVHNVWATHACVPTDPDPQEMFLENVTENFNMWKNNMV DQMHEDVISLWDQSLKPCVKLTPLCVTLECKNVNSSSSDTKNGTDPEMKNCSFNATTELRDRKQKVYALFY KLDIVPLNEKNSSEYRLINCNTSTCTQACPKVTFDPIPIHYCTPAGYAILKCNDEKFNGTGPCSNVSTVQC THGIKPVVSTQLLLNGSLAEKGIVIRSENLTNNVKTIIVHLNQSVEILCIRPNNNTRKSIRIGPGQTFYAT GEIIGDIRQAHCNISGKVWNETLQRVGEKLAEYFPNKTIKFNSSSGGDLEITTHSFNCGGEFFYCNTSKLF NGTFNGTYMPNVTEGNSTISIPCRIKQIINMWQKVGRCMYAPPIEGNITCKSKITGLLLERDGGPENDTEI FRPGGGDMRNNWRSELYKYKVVEIKPLGVAPTRCKRRVVGRRRRRRAVGIGAVSLGFLGAAGSTMGAASMT LTVQARNLLSGIVQQQSNLLRAPEPQQHLLKDTHWGIKQLQARVLAVEHYLRDQQLLGIWGCSGKLICCTN VPWNSSWSNRNLSEIWDNMTWLQWDKEISNYTQIIYGLLEESQNQQEKNEQDLLALDGGGGSGGGSGGGSG SGAHIVMVDAYKPTK
CE1176 SOSIP SpyTag	AENLWVTVYYGVPVWKEAKTTLFCASDAKAYEKEVHNVWATHACVPTDPNPQEMVLENVTENFNMWKNDMV DQMHEDVISLWDQSLKPCVKLTPLCVTLTCTNTTVSNGSSNSNANFEEMKNCSFNATTEIKDKKKNEYALF YKLDIVPLNNSSGKYRLINCNTSACAQACPKVTFEPIPIHYCAPAGYAILKCNNKTFNGTGPCNNVSTVQC THGIKPVVSTQLLLNGSLAEKEIIIRSENLTNNAKTIIIHFNESVGIVCTRPSNNTRKSIRIGPGQTFYAT GDIIGDIRQAHCNVSKQNWNRTLQQVGRKLAEHFPNRNITFNHSSGGDLEITTHSFNCRGEFFYCNTSGLF NGTYHPNGTYNETAVNSSDTITLQCRIKQIINMWQEVGRCMYAPPIAGNITCNSTITGLLLTRDGGINQTG EEIFRPGGGDMRDNWRNELYKYKVVEIKPLGVAPTRCKRRVVGRRRRRRAVGIGAVSLGFLGAAGSTMGAA SMTLTVQARNLLSGIVQQQSNLLRAPEPQQHLLKDTHWGIKQLQARVLAVEHYLRDQQLLGIWGCSGKLIC CTNVPWNSSWSNRNLSEIWDNMTWLQWDKEISNYTQIIYGLLEESQNQQEKNEQDLLALDGGGGSGGGSGG GSGSGAHIVMVDAYKPTK
CE0217 SOSIP SpyTag	AENLWVTVYYGVPVWREAKTTLFCASDAKAYEREVHNVWATHACVPTDPNPQERVLENVTENFNMWKNNMV DQMHEDIISLWDESLKPCIKLTPLCVTLNCGNAIVNESTIEGMKNCSFNVTTELKDKKKKEYALFYKLDVV PLNGENNNSNSKNFSEYRLINCNTSTCTQACPKVSFDPIPIHYCAPAGFAILKCNNETFNGTGPCNNVSTV QCTHGIKPVVSTQLLLNGSLAEKEIIIRSENLTNNAKIIIVHLNNPVKIICTRPGNNTRKSMRIGPGQTFY ATGDIIGDIRRAYCNISEKTWYDTLKNVSDKFQEHFPNASIEFKPSAGGDLEITTHSFNCRGEFFYCDTSE LFNGTYNNSTYNSSNNITLQCKIKQIINMWQGVGRCMYAPPIAGNITCESNITGLLLTRDGGNNKSTPETF RPGGGDMRDNWRSELYKYKVVEIKPLGVAPTRCKRRVVGRRRRRRAVGIGAVSLGFLGAAGSTMGAASMTL TVQARNLLSGIVQQQSNLLRAPEPQQHLLKDTHWGIKQLQARVLAVEHYLRDQQLLGIWGCSGKLICCTNV PWNSSWSNRNLSEIWDNMTWLQWDKEISNYTQIIYGLLEESQNQQEKNEQDLLALDGGGGSGGGSGGGSGS GAHIVMVDAYKPTK
CNE55 SOSIP SpyTag	AENLWVTVYYGVPVWRDADTTLFCASDAKAHETEVHNVWATHACVPTDPNPQEIHLVNVTENFNMWKNKMV EQMQEDVISLWDESLKPCVKLTPLCVTLNCTTANTNETKNNTTDDNIKDEMKNCTFNMTTEIRDKKQRVSA LFYKLDIVPIDDSKNNSEYRLINCNTSVCKQACPKVSFDPIPIHYCTPAGYVILKCNDKNFNGTGPCKNVS SVQCTHGIKPVVSTQLLLNGSLAEEEIIIRSENLTDNAKNIIVHLNKSVEINCTRPSNNTRTSVRIGPGQV FYRTGDITGDIRKAYCNISGTEWNKTLTQVAEKLKEHFNKTIVYQPPSGGDLEITMHHFNCRGEFFYCNTT QLFNNSVGNSTIKLPCRIKQIINMWQGVGQCMYAPPISGAINCLSNITGILLTRDGGNNRSNETFRPGGG NIKDNWRSELYKYKVVEIEPLGVAPTRCKRRVVGRRRRRRAVGIGAVSLGFLGAAGSTMGAASMTLTVQAR NLLSGIVQQQSNLLRAPEPQQHLLKDTHWGIKQLQARVLAVEHYLRDQQLLGIWGCSGKLICCTNVPWNSS WSNRNLSEIWDNMTWLQWDKEISNYTQIIYGLLEESQNQQEKNEQDLLALDGGGGSGGGSGGGSGSGAHIV MVDAYKPTK

Tro11 SOSIP SpyTag	AENLWVTVYYGVPVWKDASTTLFCASDAKAYDTEVHNVWATHACVPTDPNPQEVVLGNVTENFNMWKNNMV DQMHEDIISLWDQSLKPCVKLTPLCVTLNCTDNITNTNTNSSKNSSTHSYNNSLEGEMKNCSFNITAGIRD KVKKEYALFYKLDVVPIEEDKDTNKTTYRLRSCNTSVCTQACPKVTFEPIPIHYCAPAGFAILKCNDKKFN GTGPCTNVSTVQCTHGIRPVVSTQLLLNGSLAEEEVVIRSENFTNNAKTIIVQLNESIAINCTRPNNNTRR SIHIGPGRAFYATGDIIGDIRQAHCNISRTEWNSTLRQIVTKLREQLGDPNKTIIFNQSSGGDTEITMHSF NCGGEFFYCNTTKLFNSTWNGNNTTESDSTGENITLPCRIKQIINLWQEVGKCMYAPPIKGQISCSSNITG LLLTRDGGNNNSSGPETFRPGGGNMKDNWRSELYKYKVIKIEPLGVAPTRCKRRVVGRRRRRRAVGIGAVS LGFLGAAGSTMGAASMTLTVQARNLLSGIVQQQSNLLRAPEPQQHLLKDTHWGIKQLQARVLAVEHYLRDQ QLLGIWGCSGKLICCTNVPWNSSWSNRNLSEIWDNMTWLQWDKEISNYTQIIYGLLEESQNQQEKNEQDLL ALDGGGGSGGGSGGGSGSGAHIVMVDAYKPTK
X1632 SOSIP SpyTag	AENLWVTVYYGVPVWEDADTTLFCASDAKAYSTESHNVWATHACVPTDPNPQEIYLENVTEDFNMWENNMV EQMQEDIISLWDESLKPCVKLTPLCVTLTCTNVTNVTDSVGTNSRLKGYKEELKNCSFNTTTEIRDKKKQE YALFYKLDIVPINDNSNNSNGYRLINCNVSTCKQACPKVSFDPIPIHYCAPAGFAILKCRDKEFNGTGTCR NVSTVQCTHGIKPVVSTQLLLNGSLAEGDIVIRSENITDNAKTIIVHLNKTVSITCTRPNNNTRKSIRIGP GQALYATGAIIGDTRQAHCNISGSEWYEMIQNVKNKLNETFKKNITFNPSSGGDLEITTHSFNCRGEFFYC NTSELFNSSHLFNGSTLSTNGTITLPCRIKQIVRMWQRVGQCMYAPPIAGNITCRSNITGLLLTRDGGTNK DTNEAETFRPGGGDMRDNWRSELYKYKVVKIKPLGVAPTRCKRRVVGRRRRRRAVGIGAVSLGFLGAAGST MGAASMTLTVQARNLLSGIVQQQSNLLRAPEPQQHLLKDTHWGIKQLQARVLAVEHYLRDQQLLGIWGCSG KLICCTNVPWNSSWSNRNLSEIWDNMTWLQWDKEISNYTQIIYGLLEESQNQQEKNEQDLLALDGGGGSGG GSGGGSGSGAHIVMVDAYKPTK
X2278 SOSIP SpyTag	AENLWVTVYYGVPVWKEATTTLFCASEAKAYDTEVHNIWATHACVPTDPNPQEMELKNVTENFNMWKNNMV EQMHQDIISLWDQSLKPCVKLTPLCVTLDCTNINSTNSTNNTSSNSKMEETIGVIKNCSFNVTTNIRDKVK KENALFYSLDLVSIGNSNTSYRLISCNTSICTQACPKVSFDPIPIHYCAPAGFAILKCRDKKFNGTGPCRN VSSVQCTHGIRPVVSTQLLLNGSLAEEEIVIRSANLTDNAKTIIIQLNETIQINCTRPNNNTRRSIPIGPG RTFYATGDIIGDIRKAYCNISATKWNNTLRQIAEKLREKFNKTIIFNQSSGGDPEVVRHTFNCGGEFFYCN SSQLFNSTWYSNGTSNGGLNNSANITLPCRIKQIINLWQEVGKCMYAPPIKGVINCLSNITGIILTRDGGE NNGTTETFRPGGGDMRDNWRSELYKYKVVKIEPLGVAPTRCKRRVVGRRRRRRAVGIGAVSLGFLGAAGST MGAASMTLTVQARNLLSGIVQQQSNLLRAPEPQQHLLKDTHWGIKQLQARVLAVEHYLRDQQLLGIWGCSG KLICCTNVPWNSSWSNRNLSEIWDNMTWLQWDKEISNYTQIIYGLLEESQNQQEKNEQDLLALDGGGGSGG GSGGGSGSGAHIVMVDAYKPTK
BG505 SOSIP	NLWVTVYYGVPVWKDAETTLFCASDAKAYETEKHNVWATHACVPTDPNPQEIHLENVTEEFNMWKNNMVEQ MHTDIISLWDQSLKPCVKLTPLCVTLQCTNVTNNITDDMRGELKNCSFNMTTELRDKKQKVYSLFYRLDVV QINENQGNRSNNSNKEYRLINCNTSAITQACPKVSFEPIPIHYCAPAGFAILKCKDKKFNGTGPCPSVSTV QCTHGIKPVVSTQLLLNGSLAEEEVMIRSENITNNAKNILVQFNTPVQINCTRPNNNTRKSIRIGPGQAFY ATGDIIGDIRQAHCNVSKATWNETLGKVVKQLRKHFGNNTIIRFANSSGGDLEVTTHSFNCGGEFFYCNTS GLFNSTWISNTSVQGSNSTGSNDSITLPCRIKQIINMWQRIGQAMYAPPIQGVIRCVSNITGLILTRDGGS TNSTTETFRPGGGDMRDNWRSELYKYKVVKIEPLGVAPTRCKRRVVGRRRRRRAVGIGAVSLGFLGAAGST MGAASMTLTVQARNLLSGIVQQQSNLLRAPEPQQHLLKDTHWGIKQLQARVLAVEHYLRDQQLLGIWGCSG KLICCTNVPWNSSWSNRNLSEIWDNMTWLQWDKEISNYTQIIYGLLEESQNQQEKNEQDLLALD
AMC011 SOSIP	AEQLWVTVYYGVPVWKEATTTLFCASDARAYDTEVRNVWATHCCVPTDPNPQEVVLENVTENFNMWKNNMV EQMHEDIISLWDQSLKPCVKLTPLCVTLNCTDLRNATNTNATNTTSSSRGTMEGGEIKNCSFNITTSMRDK VQKEYALFYKLDVVPIKNDNTSYRLISCNTSVITQACPKVSFEPIPIHYCAPAGFAILKCNNKTFNGTGPC TNVSTVQCTHGIRPVVSTQLLLNGSLAEEEVVIRSANFTDNAKIIIVQLNKSVEINCTRPNNNTRKSIHIG PGRWFYTTGEIIGDIRQAHCNISGTKWNDTLKQIVVKLKEQFGNKTIVFNHSSGGDPEIVMHSFNCGGEFF YCNSTQLFNSTWNDTTGSNYTGTIVLPCRIKQIVNMWQEVGKAMYAPPIKGQIRCSSNITGLILIRDGGKN RSENTEIFRPGGGDMRDNWRSELYKYKVVKIEPLGIAPTKCKRRVVQRRRRRRAVGIGAVFLGFLGAAGST MGAASMTLTVQARQLLSGIVQQQNNLLRAPECQQHLLKLTVWGIKQLQARVLAVERYLKDQQLLGIWGCSG KLICCTAVPWNTSWSNKSYNQIWNNMTWMEWEREIDNYTSLIYTLIEDSQNQQEKNEQELLELD
B41 SOSIP	AAKKWVTVYYGVPVWKEATTTLFCASDAKAYDTEVHNVWATHACVPTDPNPQEIVLGNVTENFNMWKNNMV EQMHEDIISLWDQSLKPCVKLTPLCVTLNCNNVNTNNTNNSTNATISDWEKMETGEMKNCSFNVTTSIRDK IKKEYALFYKLDVVPLENKNNINNTNITNYRLINCNTSVITQACPKVSFEPIPIHYCAPAGFAILKCNSKT FNGSGPCTNVSTVQCTHGIRPVVSTQLLLNGSLAEEEIVIRSENITDNAKTIIVQLNEAVEINCTRPNNNT RKSIHIGPGRWFYATGDIIGNIRQAHCNISKARWNETLGQIVAKLEEQFPNKTIIFNHSSGGDPEIVTHSF NCGGEFFYCNTTPLFNSTWNNTRTDDYPTGGEQNITLQCRIKQIINMWQGVGKAMYAPPIRGQIRCSSNIT GLLLTRDGGRDQNGTETFRPGGGNMRDNWRSELYKYKVVKIEPLGIAPTACKRRVVQRRRRRRAVGLGAFI LGFLGAAGSTMGAASMALTVQARLLLSGIVQQQNNLLRAPEAQQHMLQLTVWGIKQLQARVLAVERYLRDQ QLLGIWGCSGKIICCTNVPWNDSWSNKTINEIWDNMTWMQWEKEIDNYTQHIYTLLEVSQIQQEKNEQELL ELD

CH119 SOSIP	AENLWVTVYYGVPVWKEATTTLFCASDAKAYDTEVHNVWATHACVPTDPSPQELVLENVTENFNMWKNEMV NQMHEDVISLWDQSLKPCVKLTPLCVTLECSKVSNNETDKYNGTEEMKNCSFNATTVVRDRQQKVYALFYR LDIVPLTEKNSSENSSKYYRLINCNTSACTQACPKVSFEPIPIHYCTPAGYAILKCNDKTFNGTGPCHNVS TVQCTHGIKPVVSTQLLLNGSLAEGEIIIRSENLTNNVKTILVHLNQSVEIVCTRPNNNTRKSIRIGPGQT FYATGDIIGDIRQAHCNISKWHETLKRVSEKLAEHFPNKTINFTSSSGGDLEITTHSFTCRGEFFYCNTSG LFNSTYMPNGTYLHGDTNSNSSITIPCRIKQIINMWQEVGRCMYAPPIEGNITCKSNITGLLLVRDGGTES NNTETNNTEIFRPGGGDMRDNWRSELYKYKVVEIKPLGVAPTRCKRRVVGRRRRRRAVGIGAVSLGFLGAA GSTMGAASMTLTVQARNLLSGIVQQQSNLLRAPEPQQHLLKDTHWGIKQLQARVLAVEHYLRDQQLLGIWG CSGKLICCTNVPWNSSWSNRNLSEIWDNMTWLQWDKEISNYTQIIYGLLEESQNQQEKNEQDLLALD
CE0217 SOSIP	AENLWVTVYYGVPVWREAKTTLFCASDAKAYEREVHNVWATHACVPTDPNPQERVLENVTENFNMWKNNMV DQMHEDIISLWDESLKPCIKLTPLCVTLNCGNAIVNESTIEGMKNCSFNVTTELKDKKKKEYALFYKLDVV PLNGENNNSNSKNFSEYRLINCNTSTCTQACPKVSFDPIPIHYCAPAGFAILKCNNETFNGTGPCNNVSTV QCTHGIKPVVSTQLLLNGSLAEKEIIIRSENLTNNAKIIIVHLNNPVKIICTRPGNNTRKSMRIGPGQTFY ATGDIIGDIRRAYCNISEKTWYDTLKNVSDKFQEHFPNASIEFKPSAGGDLEITTHSFNCRGEFFYCDTSE LFNGTYNNSTYNSSNNITLQCKIKQIINMWQGVGRCMYAPPIAGNITCESNITGLLLTRDGGNNKSTPETF RPGGGDMRDNWRSELYKYKVVEIKPLGVAPTRCKRRVVGRRRRRRAVGIGAVSLGFLGAAGSTMGAASMTL TVQARNLLSGIVQQQSNLLRAPEPQQHLLKDTHWGIKQLQARVLAVEHYLRDQQLLGIWGCSGKLICCTNV PWNSSWSNRNLSEIWDNMTWLQWDKEISNYTQIIYGLLEESQNQQEKNEQDLLALD
CNE8 SOSIP	AENLWVTVYYGVPVWRDADTTLFCASDAKAYDTEVHNVWATHACVPTDPNPQEIHLENVTENFNMWKNKMA EQMQEDVISLWDESLKPCVQLTPLCVTLNCTNANLNATVNASTTIGNITDEVRNCSFNTTTELRDKKQNVY ALFYKLDIVPINNNSEYRLINCNTSVCKQACPKVSFDPIPIHYCAPAGYAILRCNDKNFNGTGPCKNVSSV QCTHGIKPVVSTQLLLNGSLAEDEIIIRSENLTDNVKTIIVHLNKSVEINCTRPSNNTRTSVRIGPGQVFY RTGDIIGDIRKAYCNISRTKWHETLKQVATKLREHFNKTIIFQPPSGGDIEITMHHFNCRGEFFYCNTTKL FNSTWGENTTMEGHNDTIVLPCRIKQIVNMWQGVGQCMYAPPIRGSINCVSNITGILLTRDGGTNMSNETF RPGGGNIKDNWRSELYKYKVVEIEPLGVAPTRCKRRVVGRRRRRRAVGIGAVSLGFLGAAGSTMGAASMTL TVQARNLLSGIVQQQSNLLRAPEPQQHLLKDTHWGIKQLQARVLAVEHYLRDQQLLGIWGCSGKLICCTNV PWNSSWSNRNLSEIWDNMTWLQWDKEISNYTQIIYGLLEESQNQQEKNEQDLLALD
CNE8 N276A SOSIP	AENLWVTVYYGVPVWRDADTTLFCASDAKAYDTEVHNVWATHACVPTDPNPQEIHLENVTENFNMWKNKMA EQMQEDVISLWDESLKPCVQLTPLCVTLNCTNANLNATVNASTTIGNITDEVRNCSFNTTTELRDKKQNVY ALFYKLDIVPINNNSEYRLINCNTSVCKQACPKVSFDPIPIHYCAPAGYAILRCNDKNFNGTGPCKNVSSV QCTHGIKPVVSTQLLLNGSLAEDEIIIRSEALTDNVKTIIVHLNKSVEINCTRPSNNTRTSVRIGPGQVFY RTGDIIGDIRKAYCNISRTKWHETLKQVATKLREHFNKTIIFQPPSGGDIEITMHHFNCRGEFFYCNTTKL FNSTWGENTTMEGHNDTIVLPCRIKQIVNMWQGVGQCMYAPPIRGSINCVSNITGILLTRDGGTNMSNETF RPGGGNIKDNWRSELYKYKVVEIEPLGVAPTRCKRRVVGRRRRRRAVGIGAVSLGFLGAAGSTMGAASMTL TVQARNLLSGIVQQQSNLLRAPEPQQHLLKDTHWGIKQLQARVLAVEHYLRDQQLLGIWGCSGKLICCTNV PWNSSWSNRNLSEIWDNMTWLQWDKEISNYTQIIYGLLEESQNQQEKNEQDLLALD
CNE20 SOSIP	NLWVTVYYGVPVWKEATTTLFCASDAKAYDTEVHNVWATHACVPTDPNPHELVLENVTENFNMWKNEMVNQ MHEDVISLWDQSLKPCVKLTPLCVTLECGNITTRKESMTEMKNCSFNATTVVKDRKQTVYALFYKLDIVPL SGKNSSGYYRLINCNTSACTQACPKVNFDPIPIHYCTPAGYAILKCNDKTFNGTGPCHNVSTVQCTHGIKP VISTQLLLNGSLAEGEIVIRSENLTNNAKIIIVHLNQTVEIVCTRPGNNTRKSIRIGPGQTFYATGEIIGN IRQAHCNISENQWHKTLQNVSKKLAEHFQNKTITFASSSGGDLEITTHSFNCRGEFFYCNTSGLFNGTYMS NNTEGNSSSIITIPCRIKQIINMWQEVGRCIYAPPIEGNITCKSNITGLLLERDGGTESNDTEIFRPGGGD MRNNWRSELYKYKVVEIKPLGVAPTRCKRRVVGRRRRRRAVGIGAVSLGFLGAAGSTMGAASMTLTVQARN LLSGIVQQQSNLLRAPEPQQHLLKDTHWGIKQLQARVLAVEHYLRDQQLLGIWGCSGKLICCTNVPWNSSW SNRNLSEIWDNMTWLQWDKEISNYTQIIYGLLEESQNQQEKNEQDLLALD
CNE20 N276A SOSIP	NLWVTVYYGVPVWKEATTTLFCASDAKAYDTEVHNVWATHACVPTDPNPHELVLENVTENFNMWKNEMVNQ MHEDVISLWDQSLKPCVKLTPLCVTLECGNITTRKESMTEMKNCSFNATTVVKDRKQTVYALFYKLDIVPL SGKNSSGYYRLINCNTSACTQACPKVNFDPIPIHYCTPAGYAILKCNDKTFNGTGPCHNVSTVQCTHGIKP VISTQLLLNGSLAEGEIVIRSEALTNNAKIIIVHLNQTVEIVCTRPGNNTRKSIRIGPGQTFYATGEIIGN IRQAHCNISENQWHKTLQNVSKKLAEHFQNKTITFASSSGGDLEITTHSFNCRGEFFYCNTSGLFNGTYMS NNTEGNSSSIITIPCRIKQIINMWQEVGRCIYAPPIEGNITCKSNITGLLLERDGGTESNDTEIFRPGGGD MRNNWRSELYKYKVVEIKPLGVAPTRCKRRVVGRRRRRRAVGIGAVSLGFLGAAGSTMGAASMTLTVQARN LLSGIVQQQSNLLRAPEPQQHLLKDTHWGIKQLQARVLAVEHYLRDQQLLGIWGCSGKLICCTNVPWNSSW SNRNLSEIWDNMTWLQWDKEISNYTQIIYGLLEESQNQQEKNEQDLLALD
IOMA HC Fab	EVQLVESGAQVKKPGASVTVSCTASGYKFTGYHMHWVRQAPGRGLEWMGWINPFRGAVKYPQNFRGRVSMT RDTSMEIFYMELSRLTSDDTAVYYCAREMFDSSADWSPWRGMVAWGQGTLVTVSSASTKGPSVFPLAPSSK STSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNH KPSNTKVDKRVEPKSCDKT

IOMA HC	EVQLVESGAQVKKPGASVTVSCTASGYKFTGYHMHWVRQAPGRGLEWMGWINPFRGAVKYPQNFRGRVSMT RDTSMEIFYMELSRLTSDDTAVYYCAREMFDSSADWSPWRGMVAWGQGTLVTVSSASTKGPSVFPLAPSSK STSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNH KPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP QVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPGK
IOMA LC	QSALTQPASVSGSPGQSITISCAGSSRDVGGFDLVSWYQQHPGKAPKLIIYEVNKRPSGISSRFSASKSGN TASLTISGLQEEDEAHYYCYSYADGVAFGGGTKLTVLGQPKAAPSVTLFPPSSEELQANKATLVCLISDFY PGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTEC S
IOMA iGL HC Fab	QVQLVQSGAEVKKPGASVKVSCKASGYTFTGYYMHWVRQAPGQGLEWMGWINPNSGGTNYAQKFQGRVTMT RDTSISTAYMELSRLRSDDTAVYYCARDFTSSYDSSGYYHEGYWGQGTLVTVSSASTKGPSVFPLAPSSKS TSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHK PSNTKVDKRVEPKSCDKT
IOMA iGL HC	QVQLVQSGAEVKKPGASVKVSCKASGYTFTGYYMHWVRQAPGQGLEWMGWINPNSGGTNYAQKFQGRVTMT RDTSISTAYMELSRLRSDDTAVYYCARDFTSSYDSSGYYHEGYWGQGTLVTVSSASTKGPSVFPLAPSSKS TSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHK PSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ VYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ QGNVFSCSVMHEALHNHYTQKSLSLSPGK
IOMA iGL LC	QSALTQPASVSGSPGQSITISCTGTSSDVGSYNLVSWYQQHPGKAPKLMIYEVSKRPSGVSNRFSGSKSGN TASLTISGLQAEDEADYYCCSYAGSVAFGGGTKLTVLGQPKAAPSVTLFPPSSEELQANKATLVCLISDFY PGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTEC S
VRC01 iGL HC	QVQLVQSGAEVKKPGASVKVSCKASGYTFTGYYMHWVRQAPGQGLEWMGWINPNSGGTNYAQKFQGRVTMT RDTSISTAYMELSRLRSDDTAVYYCARGKNSDYNWDFQHWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGG TAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNT KVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSVMHEALHNHYTQKSLSLSPGK
VRC01 iGL LC	EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASNRATGIPARFSGSGSGTDF TLTISSLEPEDFAVYYCQQYEFFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV QWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
3BNC60 iGL HC	QVQLVQSGAEVKKPGASVKVSCKASGYTFTGYYMHWVRQAPGQGLEWMGWINPNSGGTNYAQKFQGRVTMT RDTSISTAYMELSRLRSDDTAVYYCARERSDFWDFDLWGRGTLVTVSSASTKGPSVFPLAPSSKSTSGGTA ALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKV DKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGV EVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPP SREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFS CSVMHEALHNHYTQKSLSLSPGK
3BNC60 iGL LC	DIQMTQSPSSLSASVGDRVTITCQASQDISNYLNWYQQKPGKAPKLLIYDASNLETGVPSRFSGSGSGTDF TFTISSLQPEDIATYYCQQYEFIGPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV QWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
BG24 iGL HC	QVQLVQSGAEVKKPGASVKVSCKASGYTFTGYYMHWVRQAPGQGLEWMGWINPNSGGTNYAQKFQGRVTMT RDTSISTAYMELSRLRSDDTAVYYCATQLELDSSAGYAFDIWGQGTMVTVSSASTKGPSVFPLAPSSKSTS GGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPS NTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY TLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG NVFSCSVMHEALHNHYTQKSLSLSPGK

BG24 iGL LC	QSALTQPRSVSGSPGQSVTISCTGTSSDVGGYNYVSWYQQHPGKAPKLMIYDVSKRPSGVPDRFSGSKSGN TASLTISGLQAEDEADYYCSSYEYFGGGTKLTVLSQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGA VTVAWKADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS

Table S2: X-ray data collection for IOMA iGL Fab crystals

Space group	P 2_1 2_1 2_1
Cell dimensions	
a, *b*, *c* (Å)	57.7, 66.7, 166.3
α, *β*, *γ* (°)	90, 90, 90
Resolution (Å)	38.6–2.07 (2.15–2.07)[a]
R_{merge}	0.08 (0.58)
R_{pim}	0.05 (0.36)
$I/\sigma(I)$	9.7 (2.5)
$CC_{1/2}$	0.99 (0.92)
Completeness (%)	99 (99)
Redundancy	6.3 (6.6)
Refinement	
Resolution (Å)	38.6–2.07
No. reflections	39,372
R_{work} / R_{free}	0.224 / 0.257
No. atoms	
Protein	3,241
Ligand/ion	N/A
B factors (Å^2)	
Protein	46.7
Ligand/ion	N/A
R.m.s. deviations	
Bond lengths (Å)	0.008
Bond angles (°)	1.00

[a] Values in parentheses are for the highest-resolution shell.

Table S3: Serum neutralization data for IOMA iGL transgenic mice

			ES30		ES32		ES34		ES37		ET33		ET34	
			B3 (week 18)		B3 (week 18)		B3 (week 18)		B3 (week 18)		B3 (week 18)		B3 (week 18)	
Virus	Clade	Tier	ID_{50}	% 1:100	ID_{50}	% 1:100	ID_{50}	% 1:100	ID_{50}	% 1:100	ID_{50}	% 1:100	ID_{50}	% 1:100
426c	C	2	–	–	–	–	–	–	–	–	<100	0	<100	0
25710	B	2	–	–	–	–	–	–	–	–	<100	0	<100	0
CNE8	AE	1	<100	0	<100	0	<100	0	<100	0	<100	0	104	54
CNE8 N276A	AE	1	463	71	<100	0	<100	0	<100	0	<100	0	<100	40
CNE20	BC	2	<100	49	<100	0	<100	0	<100	0	<100	0	<100	0
CNE20 N276A	BC	2	14,922	95	<100	0	<100	0	<100	0	<100	0	<100	36
JRCSF	B	2	136	65	<100	0	<100	0	<100	0	<100	0	<100	0
Q23.17	A	1	100	51	<100	0	<100	0	<100	0	<100	0	<100	26
YU2	B	2	571	86	<100	0	<100	0	<100	0	<100	0	112	56
BG505 T332N	A	2	–	–	–	–	–	–	–	–	<100	0	<100	0
6535.5	B	1	–	–	–	–	–	–	–	–	<100	0	104	53
3415_V1_C1	A	2	–	–	–	–	–	–	–	–	154	53	102	59
CAAN5342.A2	B	2	–	–	–	–	–	–	–	–	<100	0	<100	41
PVO.4	B	3	113	52	<100	0	<100	0	<100	0	<100	22	<100	57
Q842.D12	A	2	–	–	–	–	–	–	–	–	<100	0	<100	0
RHPA4259.7	B	2	–	–	–	–	–	–	–	–	<100	0	<100	43
WITO4160.33	B	2	<100	0	<100	0	<100	0	<100	0	<100	0	<100	45
ZM214M.PL15	C	2	–	–	–	–	–	–	–	–	<100	0	<100	0
MuLV			<100	0	<100	0	<100	0	<100	0	<100	0	162	65

			HP1		HP2		HP3		HP4		HP6		HP7		HQ4	
			B4 (week 23)		B4 (week 23)		B4 (week 23)		B4 (week 23)		B4 (week 23)		B4 (week 23)		B4 (week 23)	
Virus	Clade	Tier	ID_{50}	% 1:100	ID_{50}	% 1:100	ID_{50}	% 1:100	ID_{50}	% 1:100	ID_{50}	% 1:100	ID_{50}	% 1:100	ID_{50}	% 1:100
426c	C	2	<100	0	<100	0	<100	0	<100	0	–	–	<100	0	<100	0
25710	B	2	<100	0	<100	0	<100	0	<100	0	–	–	<100	0	<100	0
CNE8	AE	1	<100	0	<100	0	<100	0	<100	0	–	–	<100	0	<100	0
CNE8 N276A	AE	1	<100	26	<100	0	<100	0	<100	12	–	–	<100	0	<100	0
CNE20	BC	2	<100	23	<100	0	<100	40	<100	0	–	–	<100	0	<100	0
CNE20 N276A	BC	2	338	83	610	79	903	88	<100	16	–	–	2,017	98	<100	0
JRCSF	B	2	<100	0	<100	0	<100	0	<100	0	–	–	<100	0	<100	0
Q23.17	A	1	120	57	<100	30	<100	28	<100	0	–	–	<100	0	<100	0
YU2	B	2	<100	0	<100	0	<100	0	<100	0	–	–	<100	0	<100	0
BG505 T332N	A	2	<100	0	<100	0	<100	0	<100	0	–	–	<100	0	<100	0
6535.5	B	1	165	67	<100	0	<100	0	<100	0	–	–	<100	0	<100	0
3415_V1_C1	A	2	<100	0	<100	40	<100	0	<100	0	–	–	<100	0	108	50
CAAN5342.A2	B	2	<100	0	<100	0	<100	0	<100	0	–	–	<100	0	<100	0
PVO.4	B	3	<100	43	<100	45	<100	12	<100	35	–	–	115	50	<100	0
Q842.D12	A	2	<100	0	<100	0	<100	0	<100	0	–	–	<100	0	<100	0
RHPA4259.7	B	2	<100	0	<100	0	<100	0	<100	0	–	–	<100	0	<100	0
WITO4160.33	B	2	<100	28	145	53	<100	0	<100	48	–	–	<100	40	<100	0
ZM214M.PL15	C	2	<100	0	<100	0	<100	0	<100	0	–	–	<100	0	<100	0
MuLV			<100	0	<100	0	<100	0	<100	0	–	–	<100	0	<100	0

Table S4: Mutational analysis of antibodies isolated from IOMA iGL transgenic mice.

VH1-2*02 amino acid	VH Position	amino acid substitution	Random Frequency	# of IGT2-induced mAbs with this SHM	IGT2 Frequency	IOMA Substitution	Critical Interaction	VRC01 Substitution
Q	1	 E	– 0.0	67 0	100.0 0.0	E		
V	2			67	100.0	V		
Q	3			67	100.0	Q		
L	4			67	100.0	L		
V	5			67	100.0	V		
Q	6	 E	 0.1	67 0	100.0 0.0	E		
S	7			67	100.0	S		
G	8			67	100.0	G		
A	9			67	100.0	A		G
E	10	 Q	 0.2	67 0	100.0 0.0	Q		Q
V	11	 M	 3.3	66 1	98.5 1.5	V		M
K	12	 R	 6.9	56 11	83.6 16.4	K		
K	13			67	100.0	K		
P	14			67	100.0	P		
G	15			67	100.0	G		E
A	16			67	100.0	A		
S	17			67	100.0	S		
V	18			67	100.0	V		M
K	19	 T R	 2.3 9.6	22 31 14	32.8 46.3 20.9	T	**YES**	R
V	20			67	100.0	V		I
S	21			67	100.0	S		
C	22			67	100.0	C		
K	23	 A T E R	 0.2 2.3 3.3 4.6	55 1 0 5 6	82.1 1.5 0.0 7.5 9.0	T		R
A	24	 T	 13.5	61 6	91.0 9.0	A		
S	25			67	100.0	S		
G	26			67	100.0	G		

Y	27			67	100.0	Y		
T	28			65	97.0	K		E
		K	0.6	0	0.0			
		N	2.0	2	3.0			
F	29			66	98.5	F		
		L	3.8	1	1.5			
T	30			58	86.6	T		I
		A	1.7	1	1.5			
		I	7.2	8	11.9			
G	31			32	47.8	G		D
		E	0.8	1	1.5			
		A	11.7	6	9.0			
		D	34.3	28	41.8			
Y	32			65	97.0	Y		C
		H	8.5	2	3.0			
Y	33			27	40.3	H	**YES**	T
		E	0.1	14	20.9			
		D	0.6	9	13.4			
		S	1.9	2	3.0			
		H	4.5	8	11.9			
		F	8.4	7	10.4			
M	34			33	49.3	M		L
		L	13.8	7	10.4			
		I	48.4	27	40.3			
H	35			61	91.0	H		N
		Q	2.2	1	1.5			
		Y	3.5	5	7.5			
W	36			67	100.0	W		
V	37			67	100.0	V		I
R	38			67	100.0	R		
Q	39			66	98.5	Q		L
		R	1.1	1	1.5			
A	40			65	97.0	A		
		V	2.0	2	3.0			
P	41			67	100.0	P		
G	42			67	100.0	G		
Q	43			66	98.5	R		K
		R	1.5	1	1.5			
G	44			67	100.0	G		R
L	45			64	95.5	L		P
		F	1.9	3	4.5			
E	46			66	98.5	E		

		D	0.5	1	1.5			
W	47			67	100.0	W		
M	48			62	92.5	M		
		L	4.9	4	6.0			
		V	6.4	1	1.5			
G	49			67	100.0	G		
W	50			52	77.6	W		
		R	0.0	14	20.9			
		L	0.8	1	1.5			
I	51			66	98.5	I		L
		S	0.5	1	1.5			
N	52			58	86.6	N		K
		H	2.2	3	4.5			
		S	4.4	6	9.0			
P	(52A)			67	100.0	P		
N	53			15	22.4	F	**YES**	R
		F	0.1	30	44.8			
		E	1.0	2	3.0			
		T	1.4	5	7.5			
		R	1.8	7	10.4			
		Y	3.5	5	7.5			
		D	8.0	1	1.5			
		K	13.5	2	3.0			
S	54			12	17.9	R	**YES**	G
		F	0.2	7	10.4			
		R	2.7	45	67.2			
		N	11.9	1	1.5			
		T	14.8	2	3.0			
G	55			67	100.0	G		
G	56			31	46.3	A	**YES**	A
		R	0.9	2	3.0			
		N	0.9	8	11.9			
		V	6.3	5	7.5			
		A	11.3	20	29.9			
		D	22.4	1	1.5			
T	57			21	31.3	V	**YES**	V
		V	0.4	21	31.3			
		R	0.9	3	4.5			
		P	1.3	2	3.0			
		I	1.4	18	26.9			
		S	1.9	2	3.0			
N	58			18	26.9	K	**YES**	

		G E D K	0.7 1.4 7.2 16.5	10 15 8 16	14.9 22.4 11.9 23.9			
Y	59	 C S	 0.7 5.6	52 2 13	77.6 3.0 19.4	Y		
A	60	 R E T P V S	 0.1 1.6 1.9 2.1 2.2 3.0	42 3 3 6 0 8 5	62.7 4.5 4.5 9.0 0.0 11.9 7.5	P		
Q	61	 R E	 2.8 4.3	56 3 8	83.6 4.5 11.9	Q		R
K	62	 R N	 7.5 9.4	64 1 2	95.5 1.5 3.0	N		P
F	63	 L	 2.4	66 1	98.5 1.5	F		L
Q	64	 R	 4.8	56 11	83.6 16.4	R		
G	65			67	100.0	G		
R	66			67	100.0	R		
V	67	 L	 2.3	66 1	98.5 1.5	V		
T	68	 I S	 2.4 4.4	64 2 1	95.5 3.0 1.5	S		
M	69	 L	 12.3	64 3	95.5 4.5	M		
T	70			67	100.0	T		
R	71			67	100.0	R	**YES**	
D	72			67	100.0	D		
T	73	 P	 0.8	66 1	98.5 1.5	T		V
S	74	 T	 0.7	66 1	98.5 1.5	S		Y
I	75	 M	 1.5	67 0	100.0 0.0	M		S

S	76			37	55.2	E		D
		E	0.1	0	0.0			
		I	0.3	1	1.5			
		K	0.6	1	1.5			
		R	3.1	1	1.5			
		T	13.3	20	29.9			
		N	18.0	7	10.4			
T	77			64	95.5	I		
		I	0.6	3	4.5			
A	78			60	89.6	F		
		F	0.5	0	0.0			
		T	2.1	1	1.5			
		V	15.3	6	9.0			
Y	79			67	100.0	Y		F
M	80			64	95.5	M		L
		L	9.1	3	4.5			
E	81			65	97.0	E		
		V	0.4	2	3.0			
L	82			66	98.5	L		
		M	2.3	1	1.5			
S	(82A)			45	67.2	S		R
		K	1.2	1	1.5			
		R	8.1	2	3.0			
		N	8.9	16	23.9			
		T	12.8	3	4.5			
R	(82B)			59	88.1	R		S
		G	13.4	8	11.9			
L	(82C)			66	98.5	L		
		V	0.9	1	1.5			
R	83			60	89.6	T		T
		N	0.7	1	1.5			
		I	1.7	1	1.5			
		K	7.9	2	3.0			
		T	29.6	3	4.5			
S	84			66	98.5	S		V
		Y	2.3	1	1.5			
D	85			66	98.5	D		
		N	1.0	1	1.5			
D	86			67	100.0	D		
T	87			67	100.0	T		
A	88			67	100.0	A		
V	89			60	89.6	V		

		R A M I	0.1 0.3 4.6 10.8	1 1 1 4	1.5 1.5 1.5 6.0			
Y	90			67	100.0	Y		
Y	91	 N F	 0.0 14.6	48 2 17	71.6 3.0 25.4	Y		F
C	92			67	100.0	C		
A	93	 T V	 3.5 5.1	65 1 1	97.0 1.5 1.5	A		T
R	94			67	100.0	R		

VL2-23*02 amino acid	VL Position	amino acid substitution	Random Frequency	# of IGT2 induced mAbs with this SHM	IGT2 Frequency	IOMA Substitution	Critical Interaction
Q	1			67	100.0	Q	
S	2			66	98.5	S	
		F	0.1	1	1.5		
A	3			67	100.0	A	
L	4			67	100.0	L	
T	5			67	100.0	T	
Q	6			67	100.0	Q	
P	7			67	100.0	P	
A	8			67	100.0	A	
S	9			67	100.0	S	
V	11			67	100.0	V	
S	12			66	98.5	S	
		F	0.1	1	1.5		
G	13			67	100.0	G	
S	14			67	100.0	S	
P	15			67	100.0	P	
G	16			62	92.5	G	
		E	0.1	5	7.5		
Q	17			67	100.0	Q	
S	18			67	100.0	S	
I	19			65	97.0	I	
		S	0.1	1	1.5		
		T	0.1	1	1.5		
T	20			67	100.0	T	
I	21			67	100.0	I	
S	22			67	100.0	S	
C	23			67	100.0	C	
T	24			67	100.0	A	
		A	2.3	0	0.0		
G	25			63	94.0	G	
		V	0.1	4	6.0		
T	26			65	97.0	S	
		P	0.6	1	1.5		
		A	2.6	1	1.5		
		S	7.2	1	1.5		
S	27			65	97.0	S	
		G	2.2	1	1.5		

		R	2.5	1	1.5		
S	(27A)			63	94.0	R	**YES**
		R	2.4	2	3.0		
		N	6.1	2	3.0		
D	(27B)			67	100.0	D	
V	(27C)			53	79.1	V	
		F	1.9	1	1.5		
		I	17.0	13	19.4		
G	28			67	100.0	G	
S	29			51	76.1	G	**YES**
		I	2.0	1	1.5		
		R	3.2	4	6.0		
		G	5.3	4	6.0		
		T	14.3	1	1.5		
		N	14.5	6	9.0		
Y	30			56	83.6	F	**YES**
		S	3.0	4	6.0		
		F	4.1	7	10.4		
N	31			35	52.2	D	**YES**
		Y	1.3	4	6.0		
		D	10.2	28	41.8		
L	32			65	97.0	L	
		F	8.2	2	3.0		
V	33			67	100.0	V	
S	34			66	98.5	S	
		P	0.0	1	1.5		
W	35			67	100.0	W	
Y	36			67	100.0	Y	
Q	37			67	100.0	Q	
Q	38			67	100.0	Q	
H	39			67	100.0	H	
P	40			67	100.0	P	
G	41			67	100.0	G	
K	42			66	98.5	K	
		N	0.7	1	1.5		
A	43			61	91.0	A	
		T	0.8	5	7.5		
		V	7.6	1	1.5		
P	44			67	100.0	P	

K	45			67	100.0	K	
L	46			66	98.5	L	
		F	2.4	1	1.5		
M	47			63	94.0	I	
		L	10.7	2	3.0		
		I	34.4	2	3.0		
I	48			66	98.5	I	
		L	2.6	1	1.5		
Y	49			66	98.5	Y	
		H	1.8	1	1.5		
E	50			51	76.1	E	
		K	0.3	4	6.0		
		D	6.5	12	17.9		
V	51			67	100.0	V	
S	52			46	68.7	N	
		I	3.2	1	1.5		
		N	19.8	14	20.9		
		T	29.2	6	9.0		
K	53			38	56.7	K	
		A	0.2	1	1.5		
		R	3.8	23	34.3		
		Q	4.9	4	6.0		
		E	7.1	1	1.5		
R	54			67	100.0	R	
P	55			67	100.0	P	
S	56			67	100.0	S	
G	57			67	100.0	G	
V	58			61	91.0	I	
		I	10.9	6	9.0		
S	59			66	98.5	S	
		Y	0.0	1	1.5		
N	60			65	97.0	S	
		S	7.3	1	1.5		
		D	17.3	1	1.5		
R	61			67	100.0	R	
F	62			67	100.0	F	
S	63			65	97.0	S	
		A	0.4	2	3.0		
G	64			65	97.0	A	

		D	0.1	1	1.5		
		A	5.3	1	1.5		
S	65			67	100.0	S	
K	66			67	100.0	K	
S	67			65	97.0	S	
		C	0.0	1	1.5		
		A	0.5	1	1.5		
G	68			65	97.0	G	
		D	3.3	2	3.0		
N	69			66	98.5	N	
		K	0.8	1	1.5		
T	70			64	95.5	T	
		M	0.8	2	3.0		
		S	0.8	1	1.5		
A	71			67	100.0	A	
S	72			67	100.0	S	
L	73			67	100.0	L	
T	74			56	83.6	T	
		P	0.0	2	3.0		
		I	0.5	9	13.4		
I	75			67	100.0	I	
S	76			67	100.0	S	
G	77			66	98.5	G	
		D	0.5	1	1.5		
L	78			32	47.8	L	
		F	0.0	35	52.2		
Q	79			65	97.0	Q	
		R	2.5	2	3.0		
A	80			59	88.1	E	
		E	0.1	0	0.0		
		D	0.3	1	1.5		
		T	4.9	6	9.0		
		P	5.1	1	1.5		
E	81			67	100.0	E	
D	82			67	100.0	D	
E	83			57	85.1	E	
		V	0.0	3	4.5		
		K	0.1	2	3.0		
		G	0.2	5	7.5		

A	84			66	98.5	A	
		G	4.2	1	1.5		
D	85			61	91.0	H	
		G	0.2	2	3.0		
		Y	0.9	1	1.5		
		N	2.1	1	1.5		
		H	2.6	0	0.0		
		E	4.1	2	3.0		
Y	86			65	97.0	Y	
		N		2	3.0		
Y	87			45	67.2	Y	
		E	0.0	1	1.5		
		D	0.0	5	7.5		
		H	4.3	12	17.9		
		F	6.2	4	6.0		
C	88			67	100.0	C	
C	89			57	85.1	Y	
		W	0.8	1	1.5		
		Y	1.8	4	6.0		
		S	10.3	5	7.5		
S	90			60	89.6	S	
		L	0.7	6	9.0		
		T	1.0	1	1.5		
Y	96			61	91.0	Y	
		C	0.7	1	1.5		
		S	2.1	3	4.5		
		F	7.4	2	3.0		
A	97			48	71.6	A	
		E	1.4	1	1.5		
		T	5.8	3	4.5		
		G	7.2	9	13.4		
		V	8.8	6	9.0		

Table S5: Serum neutralization in wildtype mice

			M1		M3		M4		M5		M13		M14		M15		M21	
			B3 (week 18)		B3 (week 18)		B3 (week 18)		B3 (week 18)		B3 (week 18)		B3 (week 18)		B3 (week 18)		B4 (week 23)	
Virus	**Clade**	**Tier**	**ID_{50}**	**% 1:100**	**ID_{50}**	**% 1:100**	**ID_{50}**	**% 1:100**	**ID_{50}**	**% 1:100**	**ID_{50}**	**% 1:100**	**ID_{50}**	**% 1:100**	**ID_{50}**	**% 1:100**	**ID_{50}**	**% 1:100**
426c	C	2	<100	0	<100	0	<100	0	<100	0	<100	0	<100	0	<100	0	<100	0
25710	B	2	<100	0	<100	0	<100	0	<100	0	<100	0	<100	0	<100	0	<100	0
CNE8	AE	1	<100	0	<100	0	<100	0	<100	0	<100	0	<100	0	<100	0	225	56
CNE8 N276A	AE	1	<100	0	<100	0	<100	0	<100	0	<100	0	<100	0	<100	0	<100	0
CNE20	BC	2	<100	0	<100	0	<100	0	<100	0	<100	0	<100	0	<100	40	<100	0
CNE20 N276A	BC	2	<100	0	<100	0	<100	0	<100	0	<100	0	<100	0	<100	0	<100	0
JRCSF	B	2	<100	0	<100	0	<100	0	<100	0	<100	0	<100	0	<100	0	<100	0
Q23.17	A	1	<100	0	<100	0	<100	0	<100	0	<100	0	<100	0	<100	40	<100	0
YU2	B	2	<100	0	<100	0	<100	0	<100	0	<100	0	<100	0	<100	0	<100	0
BG505 T332N	A	2	<100	0	<100	0	<100	0	<100	0	<100	0	<100	0	<100	0	<100	0
6535.5	B	1	<100	0	<100	0	<100	0	<100	0	<100	0	<100	0	<100	47	<100	0
3415_V1_C1	A	2	<100	0	<100	0	<100	0	<100	0	<100	0	<100	0	<100	0	<100	0
CAAN5342.A2	B	2	<100	0	–	–	<100	0	<100	0	<100	0	<100	0	<100	0	<100	0
PVO.4	B	3	113	57	–	–	<100	0	<100	0	<100	41	<100	0	145	58	<100	0
Q842.D12	A	2	<100	43	–	–	<100	0	<100	0	<100	0	<100	0	<100	0	<100	0
RHPA4259.7	B	2	<100	0	–	–	<100	0	<100	0	<100	0	<100	0	<100	0	<100	0
WITO4160.33	B	2	<100	47	–	–	<100	0	<100	0	<100	0	<100	0	<100	0	<100	0
ZM214M.PL15	C	2	<100	0	–	–	<100	0	<100	0	<100	0	<100	0	<100	0	<100	0
MuLV			<100	0	–	–	<100	0	<100	0	<100	0	<100	0	<100	0	<100	0
			M22		**M23**		**M24**		**M25**		**M26**		**M27**		**M28**		**M29**	
			B4 (week 23)		**B4 (week 23)**		**B4 (week 23)**		**B4 (week 23)**		**B4 (week 23)**		**B4 (week 23)**		**B4 (week 23)**		**B4 (week 23)**	
Virus	**Clade**	**Tier**	**ID_{50}**	**% 1:100**	**ID_{50}**	**% 1:100**	**ID_{50}**	**% 1:100**	**ID_{50}**	**% 1:100**	**ID_{50}**	**% 1:100**	**ID_{50}**	**% 1:100**	**ID_{50}**	**% 1:100**	**ID_{50}**	**% 1:100**
426c	C	2	<100	0	<100	0	<100	0	<100	0	<100	0	<100	0	<100	0	<100	0
25710	B	2	<100	0	<100	0	<100	0	<100	0	<100	0	<100	0	<100	0	<100	0
CNE8	AE	1	<100	0	<100	0	100	50	<100	0	729	70	<100	0	841	61	242	71
CNE8 N276A	AE	1	<100	0	<100	0	<100	0	<100	0	<100	0	<100	0	<100	0	<100	0
CNE20	BC	2	<100	0	<100	0	<100	0	<100	0	<100	0	<100	0	<100	0	<100	0
CNE20 N276A	BC	2	<100	0	<100	0	<100	0	<100	0	<100	0	<100	0	<100	0	<100	0

JRCSF	B	2	<100	0	<100	0	<100	0	<100	0	<100	0	<100	0	<100	0	<100	0
Q23.17	A	1	<100	0	<100	0	<100	0	<100	0	<100	0	<100	0	<100	0	<100	0
YU2	B	2	<100	0	<100	0	<100	0	<100	0	<100	0	<100	0	<100	0	<100	0
BG505 T332N	A	2	<100	0	<100	0	<100	0	<100	0	<100	0	<100	0	<100	41	<100	0
6535.5	B	1	<100	0	<100	0	<100	0	<100	0	<100	0	<100	0	<100	0	<100	0
3415_V1_C1	A	2	<100	0	<100	0	<100	0	<100	0	<100	0	<100	0	<100	0	<100	0
CAAN5342.A2	B	2	<100	0	<100	0	<100	0	<100	0	<100	0	<100	0	<100	0	<100	0
PVO.4	B	3	<100	0	<100	0	<100	0	<100	0	<100	0	<100	0	<100	43	<100	0
Q842.D12	A	2	<100	0	<100	0	<100	0	<100	0	<100	0	<100	0	<100	0	<100	0
RHPA4259.7	B	2	<100	0	<100	0	<100	0	<100	0	<100	0	<100	0	<100	0	<100	0
WITO4160.33	B	2	<100	0	<100	0	<100	0	<100	0	<100	0	<100	0	<100	0	<100	0
ZM214M.PL15	C	2	<100	0	<100	0	<100	0	<100	0	<100	0	<100	0	<100	0	<100	0
MuLV			<100	0	<100	0	<100	0	<100	0	<100	0	<100	0	<100	0	<100	0

Table S6: Oligonucleotides used to generate yeast display gp120 libraries.

Oligo Name	Fragment	Sequence
426c Library 1 For	1	GTCTGGAAAGAGGCTAAGACCACACTG
426c Library 1 Rev	1	CAGGTTTTTTGATCTGATCACAATCTCTTC
426c Library 1 - 1 For	2	GAAGAGATTGTGATCAGATCAAAAAACCTGNNKAACAATGCCAAGATCATTATCGTGC
426c Library 1 - 2 Rev	2	ATCTCCACACTCTTATTCAGCTGCACGATAATGATCTTGGCATT
426c Library 1 - 3 For	2	AGCTGAATAAGAGTGTGGAGATCGTCTGCACACGACCTAACA
426c Library 1 - 4 Rev	2	GCCTGCCGAATATCTCCCCCAGATCCGCTGCCGCCATTGTTAGGTCGTGTGCAGACG
426c Library 1 - 5 For	2	GGAGATATTCGGCAGGCTTATTGTAACATCAGTGGCAGAAATTGGTCAGAAGCCGTGAA
426c Library 1 - 6 Rev	2	TGGGGGAAGTGCTCTTTCAGCTTTTTCTTGACCTGGTTCACGGCTTCTGACCAATTT
426c Library 1 - 7 For	2	AAAGAGCACTTCCCCCATAAGAATATTAGCTTTCAGTCTAGTTCAGGCGGGGAC
426c Library 1 - 8 Rev	2	TCGCCTCCGCAGTTGAAGGAGTGTGTGGTGATTTCCAGGTCCCCGCCTGAACTA
426c Library 1 - 9 For	2	ACTGCGGAGGCGAGTTCTTTTACTGTAATACATCCGGCCTGTTTAACG
426c Library 1 - 10 Rev	2	CCGGCAAGGCAGCATGATTGTGGCATTAGAAATGGTATCGTTAAACAGGCCGGATGTA
426c Library 1 - 11 For	2	GCTGCCTTGCCGGATCAAGCAGATTATCAACATGTGGCAGGAA
426c Library 1 - 12 Rev	2	TGCCCTTGATGGGTGGTGCATAGATAGCCTTTCCMNNTTCCTGCCACATGTTGATAATC
426c Library 1 - 13 For	2	CCACCCATCAAGGGCAATATCACCTGTAAGAGTGACATTACAGGGCTGCTGCTGCTGAGA
426c Library 1 - 14 Rev	2	GCCGGAAAATCTCGGTmnnmnnmnnmnnmnnTCCCCCATCTCTCAGCAGCAGCAGCC
426c Library 1 - 15 For	2	ACCGAGATTTTCCGGCCTAGCGGAGGAGACATGCGAGATAATTGGCGGTCTGAACTG
426c Library 1 - 16 Rev	2	GGATCCCAGAGGCTTGATCTCGACCACCTTATATTTGTACAGTTCAGACCGCCAATTA
426c Library 1 For	3	CTGGTGGAGGCGGTAGCGGAGGCGGAGGGTCGGCTAGCGTCTGGAAAGAGGCTAAGACCA
426c Library 1 Rev	3	TTACAAGTCCTCTTCAGAAATAAGCTTTTGTTCGGATCCCAGAGGCTTGATCTCGACCAC
426c Library 2 For	1	GTCTGGAAAGAGGCTAAGACCACACTG
426c Library 2 Rev	1	CAGGTTTTTTGATCTGATCACAATCTCTTC
426c Library 2 - 1 For	2	GAAGAGATTGTGATCAGATCAAAAAACCTGNNKAACAATGCCAAGATCATTATCGTGC
426c Library 2 - 2 Rev	2	ATCTCCACACTCTTATTCAGCTGCACGATAATGATCTTGGCATT
426c Library 2 - 3 For	2	AGCTGAATAAGAGTGTGGAGATCGTCTGCACACGACCTAACA
426c Library 2 - 4 Rev	2	GCCTGCCGAATATCTCCCCCAGATCCGCTGCCGCCATTGTTAGGTCGTGTGCAGACG
426c Library 2 - 5 For	2	GGAGATATTCGGCAGGCTTATTGTAACATCAGTGGCAGAAATTGGTCAGAAGCCGTGAA
426c Library 2 - 6 Rev	2	TGGGGGAAGTGCTCTTTCAGCTTTTTCTTGACCTGGTTCACGGCTTCTGACCAATTT
426c Library 2 - 7 For	2	AAAGAGCACTTCCCCCATAAGAATATTAGCTTTCAGTCTAGTTCAGGCGGGGAC
426c Library 2 - 8 Rev	2	TCGCCTCCGCAGTTGAAGGAGTGTGTGGTGATTTCCAGGTCCCCGCCTGAACTA
426c Library 2 - 9 For	2	ACTGCGGAGGCGAGTTCTTTTACTGTAATACATCCGGCCTGTTTAACG
426c Library 2 - 10 Rev	2	CCGGCAAGGCAGCATGATTGTGGCATTAGAAATGGTATCGTTAAACAGGCCGGATGTA
426c Library 2 - 11 For	2	GCTGCCTTGCCGGATCAAGCAGATTATCAACATGTGGCAGGAA
426c Library 2 - 12 Rev	2	TGCCCTTGATGGGTGGTGCATAGATAGCCTTTCCMNNTTCCTGCCACATGTTGATAATC
426c Library 2 - 13 For	2	CCACCCATCAAGGGCAATATCACCTGTAAGAGTGACATTACAGGGCTGCTGCTGCTGAGA
426c Library 2 - 14 Rev	2	GCCGGAAAATCTCGGTmnnmnnmnnmnnmnnTCCCCCATCTCTCAGCAGCAGCAGCC
426c Library 2 - 15 For	2	ACCGAGATTTTCCGGCCTAGCGGAGGAGACATGCGAGATAATTGGCGGTCTGAACTG
426c Library 2 - 16 Rev	2	GGATCCCAGAGGCTTGATCTCGACCACCTTATATTTGTACAGTTCAGACCGCCAATTA
426c Library 2 For	3	CTGGTGGAGGCGGTAGCGGAGGCGGAGGGTCGGCTAGCGTCTGGAAAGAGGCTAAGACCA
426c Library 2 Rev	3	TTACAAGTCCTCTTCAGAAATAAGCTTTTGTTCGGATCCCAGAGGCTTGATCTCGACCAC

Table S7: Flow cytometric reagents

Reagent	Target species	Antibody clone	Company / Source	Cat.#	RRID
CD16/32	mouse	2.4G2	BD Biosciences	553142	AB_394657
CD4-APCeF780	mouse	RM4-5	Thermo Fisher	47-0042-82	AB_1272183
CD8a-APCeF780	mouse	53-6.7	Thermo Fisher	47-0081-82	AB_1272185
NK1.1-APCeF780	mouse	PK136	Thermo Fisher	47-5941-82	AB_2735070
F4/80-APCeF780	mouse	BM8	Thermo Fisher	47-4801-82	AB_2735036
Ly-6G/C (Gr1)-APCeF780	mouse	RB6-8C5	Thermo Fisher	47-5931-82	AB_1518804
CD11b-APCeF780	mouse	M1/70	Thermo Fisher	47-0112-82	AB_1603193
CD11c-APCeF780	mouse	N418	Thermo Fisher	47-0114-82	AB_1548652
CD93-APC	mouse	AA4.1	Thermo Fisher	17-5892-82	AB_469466
TER-119-APCCy0	mouse	TER-119	BD Pharmingen	560509	AB_1645230
CD95 (FAS)-FITC	mouse	SA367H8	BioLegend	152606	AB_2632901
CD38-AF700	mouse	90	Thermo Fisher	56-0381-82	AB_657740
CD45R/B220-BV421	mouse / human	RA3-6B2	BD Horizon	562922	AB_2737894
CD45R/B220-BV605	mouse / human	RA3-6B2	BioLegend	103244	AB_2563312
IgD-BV786	mouse	11-26c.2a	BD Horizon	563618	AB_2738322
CD19-PECy7	mouse	6D5	BioLegend	115520	AB_313655
CD2-PE	mouse	RM2-5	BioLegend	100108	AB_2073690
CD23-PE	mouse	B3B4	BioLegend	101607	AB_312832
Ig light chain lambda-APC	mouse	RML-42	BioLegend	407306	AB_961363
Ig light chain kappa-BV421	mouse	187.1	BD Horizon	562888	AB_2737867
CD21/CD35	mouse	7G6	BD Horizon	562756	AB_2737772
IgM Fab-FITC	mouse	polyclonal	Jackson Immunoresearch	115-097-020	AB_2338618
Zombie NIR	N/A*	N/A	BioLegend	423105	N/A
Streptavidin-PE	N/A	N/A	BD Pharmingen	554061	AB_10053328
Streptavidin-AF647	N/A	N/A	BioLegend	405237	N/A
Streptavidin-PECy7	N/A	N/A	BioLegend	405206	N/A

RC1-biotin	N/A	N/A	in house	N/A	N/A
CNE8 N276A-biotin	N/A	N/A	in house	N/A	N/A
426c degly2 D279N-biotin	N/A	N/A	in house	N/A	N/A
426c degly2 D279N CD4bs-KO -biotin	N/A	N/A	in house	N/A	N/A
Human Fc Block	human	N/A	BD Horizon	564220	AB_2869554
Ig light chain lambda-APC	human	MHL38	BioLegend	316610	AB_493629
CD19-PECy7	human	SJ25C1	BioLegend	363012	AB_2564203
IgM-FITC	human	MHM88	BioLegend	314506	AB_493009
Ig light chain kappa-BV421	human	MHK-49	BioLegend	316518	AB_2561581

*N/A not applicable

Table S8: Single cell antibody cloning reaction conditions.

PCR1 IgH	Primer sequence
HH_1FL (forward, leader)	CCATGGGATGGTCATGTATCA
HH_1RG (reverse, IgG)	GGACAGGGATCCAGAGTTCC
HH_1RM (reverse, IgM)	CCCATGGCCACCAGATTCTT
PCR1 IgK	**Primer sequence**
HH_1FL (forward, leader)	CCATGGGATGGTCATGTATCA
HH_1RK (reverse, IgK)	GACTGAGGCACCTCCAGATG

PCR1 mastermix		
Reagent	**Volume/plate (µL)**	**Concentration**
nuclease free water	3328	
10x buffer	384	1x
dNTP (25 mM)	48	0.3 mM
5' forward Primer (50 µM)	HC 15; LC 19	HC 0.25 µM; LC 0.25 µM
3' reverse Primer (50 µM)	HC 23 (IgG/IgM 1:1); LC 19	HC 0.30 µM; LC 0.25 µM
HotStar DNA Polymerase (5 U/µL)	42	0.055 U/µL
total	3840	

PCR2 IgH	Primer sequence
HH_2FL (forward, leader)	GTAGCAACTGCAACCGGTGTACATTCT
HH_2RG (reverse, IgG)	GCTCAGGGAARTAGCCCTTGAC
HH_2RM (reverse, IgM)	AGGGGGAAGACATTTGGGAAGGAC
PCR2 IgK	**Primer sequence**
HH_2FL (forward, leader)	GTAGCAACTGCAACCGGTGTACATTCT
HH_2RK (reverse, IgK)	AACTGCTCACTGGATGGTGG

PCR2 mastermix		
Reagent	**Volume/plate (µL)**	**Concentration**
nuclease free water	2536	
loading buffer*	800	
10x buffer	384	1x
dNTP (25 mM)	48	0.3 mM
5' forward Primer (50 µM)	HC 12; LC 15	HC 0.16 µM; LC 0.2 µM
3' reverse Primer (50 µM)	HC 18 (IgG/IgM 1:1); LC 15	HC 0.23 µM; LC 0.2 µM
HotStar DNA Polymerase (5 U/µL)	42	0.055 U/µL
total	3840	
*loading buffer: 40% (w/v) sucrose in nuclease free water with cresol red added to dark red color.		