Supporting Information

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SI Materials and Methods

Flow Cytometry. hCCR5 and hCD4 surface expression on 293T cells was confirmed by flow cytometry using an LSRFortessa cytometer (BD Biosciences). Cells were stained with fluorescein isothiocyanate (FITC) mouse anti-human CD195 (CCR5) (BD Pharmingen) and allophycocyanin (APC) mouse anti-human CD4 (BD Pharmingen) according to manufacturer's instructions. Data were analyzed using FlowJo software (Treestar).

Production of Recombinant Adenoviruses. Adenoviral stocks were generated as previously described (1). Briefly, AdV-hCCR5-2A-hCD4 (AdV serotype 5) was transfected into HEK293 cells (ATCC) using the calcium-phosphate method. At full cytophatic effects, transfected cultures were harvested and freeze–thawed. Supernatants were serially passaged two more times. For virus purification, cell pellets were resuspended in 0.01 M sodium phosphate buffer (pH 7.2) and lysed in 5% sodium deoxycholate, followed by DNase I digestion. Lysates were centrifuged and the supernatant was layered onto a 1.2- to 1.46-g/mL^{-1} CsCl gradient, then spun at 95,133 × *g* on a Beckman Optima 100K-Ultra centrifuge using an SW28 spinning-bucket rotor (Beckman-Coulter). Adenovirus bands were isolated and further purified on a second CsCl gradient using an SW41.Ti spinning-bucket rotor. Resulting purified adenoviral bands were isolated and twice dialyzed against 4% (wt/vol) sucrose. Adenovirus concentrations were determined at 10^{12} times the OD_{260} reading on a FLUOstar Omega plate reader (BMG Labtech). Adenovirus stocks were aliquoted and stored at −80 °C. For in vivo transduction, 10^{11} particles were injected through the lateral tail vein.

HIV Pseudovirus Production and in Vivo Infection. HIV_{YU-2} pseudovirus was generated by cotransfection of pTRIPcre [nls-cre cloned into pTRIP (2, 3) using XbaI/XhoI sites], HIV gag-pol (4), and $pSVIIIenv_{YU-2}$ (5) in HEK293T cells (ATCC) using X-tremeGENE 9 (Roche) according to the manufacturer's instructions. Plasmids were transfected at a 1.42:1:1.68 ratio (pTRIPcre:HIV gag-pol:$pSVIIIenv_{YU-2}$). Pooled supernatants were clarified by centrifugation at 300 × *g*, filtered through a 0.45-μm filter unit (Thermo Scientific) to remove cell debris and concentrated 20-fold using a stirred cell (Millipore) with a 300,000 nominal molecular weight limit (NMWL) cutoff membrane. Viral preparations were quantified using a p24 ELISA kit (PerkinElmer) according to manufacturer's instruction. For in vivo infection, 500 μL of concentrated pseudovirus (c_{p24} = 1.835 ± 0.3918 μg mL^{-1}, mean ± SEM) were injected through the lateral tail vein.

Histological Detection of hCCR5 and hCD4. Livers of mice injected with AdV-hCCR5-2A-hCD4 were harvested 24 h after infection and fixed in 4% (vol/vol) paraformaldehyde, 10% (wt/vol) sucrose for 1 h at 4 °C, followed by incubation in 30% (wt/vol) sucrose overnight at 4 °C. Tissue was frozen in optimal cutting temperature compound (Ted Pella, Inc.) at −80 °C. Tissue sections (8 μm) were cut on Superfrost Plus microscope slides (Fisher Scientific). Sections were immunostained with phycoerythrin (PE) mouse anti-human CD4 and FITC mouse anti-human CCR5 (both BD Pharmingen). Nuclei were detected using ProLong Gold + DAPI (Invitrogen). Images were captured on an Axioplan 2 imaging fluorescence microscope (Zeiss) using Metavue Software (Molecular Devices) and processed in ImageJ (National Institutes of Health).

Serum Antibody ELISA. ELISA plates (Corning) were coated with goat anti-human IgG (Jackson ImmunoResearch Laboratories) at 2.5 μg mL^{-1} overnight. Plates were blocked with 2 mM EDTA and 0.05% Tween-20 in PBS (blocking buffer) for 1 h at room temperature. Serial dilutions of mouse serum in PBS were incubated and detected with an HRP-conjugated goat anti-human IgG (Jackson ImmunoResearch Laboratories) at a 1:1,000 dilution in blocking buffer. Samples were subsequently developed with ABTS [2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)] (Invitrogen). Purified human IgG was included to generate the standard curve.

Bioluminescence Imaging. Mice were injected with 10^{11} adenoviral particles i.v. and mAbs s.c. 24 h before i.v. injection with HIV_{YU-2} pseudovirus. At day 4 after pseudovirus infection mice were anesthetized with 2% (vol/vol) isoflurane and injected i.p. with 4.5 mg D-Luciferin (Caliper Life Sciences). Bioluminescence was acquired using an IVIS Lumina II platform (Caliper Life Sciences).

Neutralization Assays. TZM-bl neutralization screens were performed as previously described (6).

Briefly, neutralization was detected as reduction in luciferase reporter gene expression after single round infection in Tzm.bl cells. Murine leukemia virus was used as a negative control.

Recombinant Protein Expression and Purification. Recombinant proteins were expressed in HEK293T cells (ATCC). Human monoclonal antibodies were purified using Protein G Sepharose 4 Fast Flow (GE Healthcare) as previously described (7). Antibodies were dialyzed against PBS and sterile filtered (0.22 μm). Endotoxin (LPS) contamination was quantified by the Limulus amebocyte lysate assay (Associates of Cape Cod) and levels were <0.005 EU mg^{-1}. His-tagged recombinant proteins were purified using His-Tag isolation and pull-down dynabeads (Invitrogen). Purity was assessed by SDS/PAGE and Coomassie staining and was estimated to be >90%.

Site-Directed Mutagenesis. G236R/L328R mutations of the hIgG1 constant region were introduced using the QuikChange site-directed mutagenesis Kit II (Agilent Technologies). For the introduction of the G236R mutation the following primer pairs were used: forward: 5′-GCACCTGAACTCCTGAGGGGACCGTCAGTCTTCCTC; reverse: 5′-GAGGAAGACTGACGGTCCCCTCAGGAGTTCAGGTGC. For the L328R mutation: forward: 5′-GGTCTCCAACAAAGCCCGCCCAGCCCCCATCGAG; reverse: 5′-CTCGATGGGGGCTGGGCGGGCTTTGTTGGAGACC. Mutated plasmid sequences were validated by direct sequencing (Genewiz).

gp140 ELISA. Recombinant gp140 (8) (50 ng per well) was immobilized onto high-binding 96-well microtiter plates (Nunc). After blocking with PBS + 2% (wt/vol) BSA + 0.05% Tween 20 for 2 h, plates were incubated for 1 h with serially diluted IgG antibodies in PBS, followed by HRP-conjugated goat anti-human IgG (1 h; 1:5,000; Sigma). Plates were developed using the 3,3′,5,5′-Tetramethylbenzidine (TMB) two-component peroxidase substrate kit (KPL).

Surface Plasmon Resonance. All experiments were performed with a Biacore T100 SPR system (Biacore, GE Healthcare) at 25 °C in HBS-EP^{+} buffer [10 mM Hepes (pH 7.4), 150 mM NaCl, 3.4 mM EDTA, and 0.005% (vol/vol) surfactant P20]. For the determination of the affinity of mouse FcγRs for human IgG1

and Fc domain variants, soluble ectodomains of mouse FcγRI, FcγRII, FcγRIII, and FcγRIV diluted at 20 μg mL^{-1} in 10 mM sodium acetate (pH 4.5, or pH 5 for FcγRI) were immobilized on Series S CM5 chips by amine coupling, resulting in a density of 2,000 response units (RU). Recombinant IgG samples were injected through flow cells at seven different concentrations (ranging from 2,000 to 31.25 nM; 1:2 successive dilutions) at a flow rate of 30 μL min^{-1} for 120 s, followed by a 300-s dissociation step. After each assay cycle, the sensor surface was regenerated with a 30-s injection of 25 mM NaOH at a flow rate of 30 μL min^{-1}. For the measurement of anti-gp140 IgG affinity for gp140 or 2-CC core (9), IgG antibodies (diluted at 20 μg mL^{-1} in 10 mM sodium acetate, pH 4.5) were immobilized on Series S CM5 chips by amine coupling at a density of 1,000 RU. Recombinant gp140 trimer or 2-CC core were injected through flow cells at a flow rate of 20 μL min^{-1}, with the concentration ranging from 31.25 to 1,000 nM (1:2 successive dilutions). Association time was 120 s, followed by 300 s dissociation. At the end of each cycle, sensor surface was regenerated with 50 mM NaOH (50 μL min^{-1}; 30 s). Background binding to blank immobilized flow cells was subtracted, and affinity constants were calculated using BIAcore T100 Evaluation software using the 1:1 Langmuir binding model.

C1q Binding and C3 Fixation Assays. Mouse C1q binding to mAbs and mAb-mediated fixation of mouse serum C3 were measured by ELISA. Antibodies were serially diluted (100–0.1 μg mL^{-1}) in PBS and coated overnight (4 °C) onto high-binding 96-well microtiter plates (50 μL per well). After washing with PBST [PBS + 0.05% (vol/vol) Tween-20], plates were blocked with protein-free blocking buffer (Pierce). Normal mouse serum [3% (vol/vol)] was added and incubated for 60 min with gentle shaking. For the detection of C1q binding, biotinylated mouse monoclonal anti-C1q antibody (JL-1, Abcam) was added at 0.5 μg mL^{-1}, whereas for the C3 fixation assay, biotinylated chicken polyclonal anti-C3 antibody (ab14232, Abcam) was used at a final concentration of 1 μg mL^{-1}. Streptavidin HRP (Invitrogen) was used at a dilution of 1:2,500, and plates were developed and analyzed as described above.

mAb Binding to Cell-Surface gp160. HEK293T cells were transfected with pMX-$gp160^{YU.2}$Δc-IRES-GFP (8). Mock-transfected cells (carrying pMX-IRES-GFP) were used as controls. Cells were maintained in DMEM supplemented with 10% (vol/vol) FBS and 10 μg mL^{-1} puromycin. Cells were detached (5 mM EDTA in PBS), resuspended at 10^7/mL in PBS + 5% (vol/vol) FBS + 2 mM EDTA, and incubated with the various anti-gp140 mAbs (mGO53 was used as a negative control) at a final concentration of 20 μg mL^{-1} for 30 min at 4 °C. Cells were then incubated for 20 min at 4 °C with Alexa Fluor 647-conjugated goat anti-human IgG (1 μg mL^{-1}; Invitrogen) and analyzed on a BD FACSCalibur cytometer (BD Biosciences). Data were analyzed using FlowJo (Treestar) software.

Immune Complex (IC) Binding Assay. CHO cells expressing mouse FcγR were previously described (10). Cells were maintained in DMEM supplemented with 10% (vol/vol) FBS and 0.1 mM MEM nonessential amino acids with the corresponding selection agent. ICs were generated by incubating mGO53 hIgG1 WT and GR/LR mAb (1 mg mL^{-1}) with Alexa Fluor 647-conjugated goat $F(ab')_2$ anti-human $F(ab')_2$ (200 μg mL^{-1}; Jackson Immunoresearch) for 20 min at 37 °C. ICs were centrifuged for 1 min at 14,000 × *g* to remove large aggregates, and supernatant was serially diluted (100-0.1 μg mL^{-1} based on hIgG concentration) in PBS +0.5% (wt/vol) IgG-free BSA (PBS-B) and immediately used for the IC binding assay. FcγR-CHO cells were detached using 0.01% trypsin + 5 mM EDTA and resuspended at 5×10^6 cells mL^{-1} in PBS-B. Cells were incubated for 30 min on ice, washed twice with PBS-B, and IC binding was quantified using a BD FACSCalibur cytometer (BD Biosciences). As control to determine the specificity of the FcγR-IC interaction, cells were preincubated with function blocking anti-FcγRII/III (2.4G2) or anti-FcγRIV (9E9) antibodies (final concentration 10 μg mL^{-1}) 10 min before the addition of the ICs. Data were analyzed using FlowJo (Treestar) software.

Statistical Analysis. Statistical analysis was performed using Prism software (GraphPad). Statistics were calculated using an unpaired one-tailed *t* test. Asterisks ($^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.0001$) indicate significant difference from the control group. Columns and error bars represent mean ± SEM.

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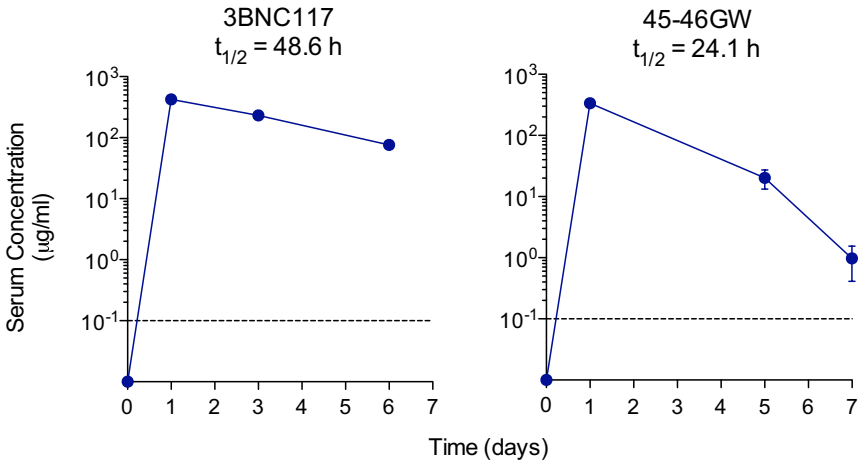

Fig. S1. Antibody half-life. Antibody serum concentration in NOD Rag1$^{-/-}$ IL2Rγ^{NULL} mice was determined by ELISA after i.v. injection of 0.5 mg antibody at day 0. Graphs show the actual serum concentration (mean ± SEM, three mice each) of 3BNC117 (*Left*) and 45-46^{G54W} (*Right*) plotted against the time after monoclonal antibody injection. Dotted line indicates limit of detection. The mean half-life ($t_{1/2}$) was calculated by using a one-phase exponential decay model between day 1 and day 5–7.

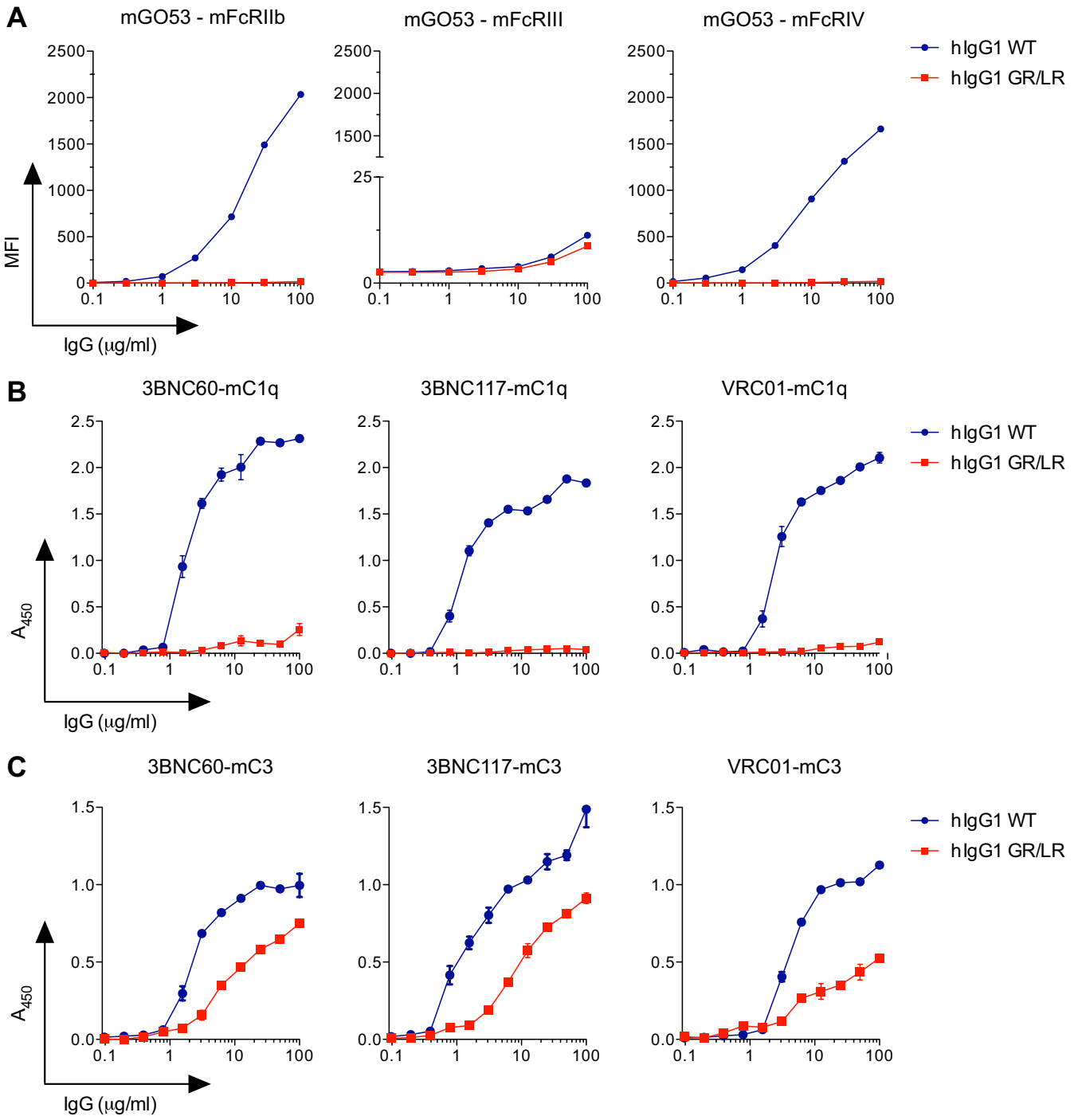

Fig. S2. Antibody Fc: hIgG1 WT vs. hIgG1 G236R/L328R. (*A*) Binding of hIgG1 (WT or G236R/L328R) after forming immune complexes with Alexa Fluor 647-conjugated goat F(ab')$_2$ anti-human F(ab')$_2$ to mouse FcγRIIb, mFcγRIII, and mFcγRIV stably expressed on CHO cells. Graph shows the mean fluorescent intensity (MFI) vs. the monoclonal antibody concentration. (*B* and *C*) Binding of 3BNC60, 3BNC117, and VRC01 hIgG1 WT and GR/LR mutant to mouse C1q (*B*) or mouse C3 (*C*) as determined by ELISA. Graph shows the absorption at 450 nm vs. the monoclonal antibody concentration.

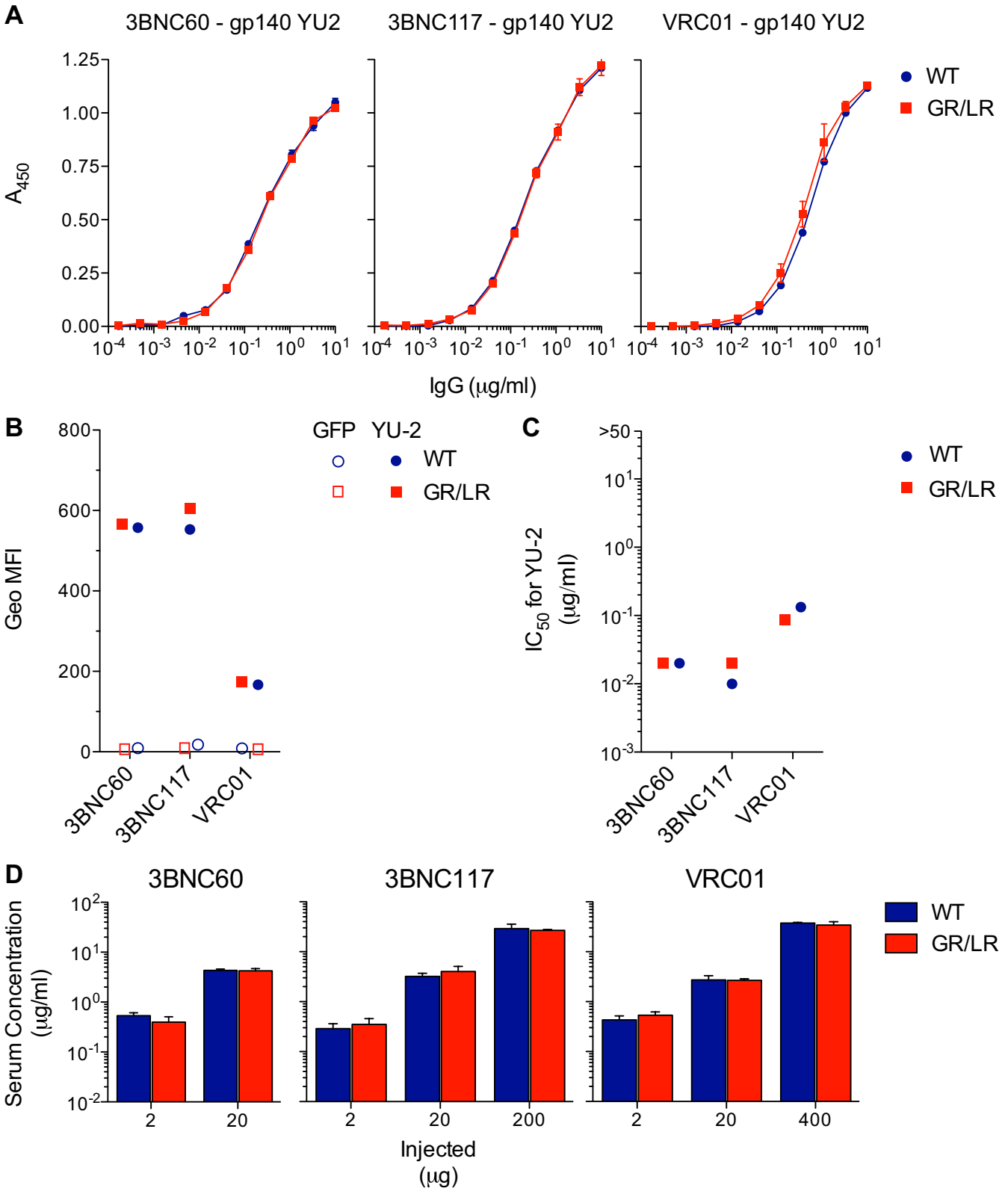

Fig. S3. Antibody functionality: hIgG1 WT vs. hIgG1 G236R/L328R. (*A*) ELISA of 3BNC60, 3BNC117, and VRC01 show comparable binding to gp140-YU2 for WT and GR/LR. Graph shows the absorption at 450 nm vs. the monoclonal antibody concentration. (*B*) 3BNC60, 3BNC117, and VRC01 were tested for binding to HIV_{YU-2} Env expressed on 293T cells. Binding was detected with anti-human IgG AF 647. 293T cells expressing GFP served as negative control. Representative graph shows the MFI for the individual antibodies. (*C*) In vitro neutralization of HIV_{YU-2} by WT and GR/LR mutants of 3BNC60, 3BNC117, and VRC01, as assessed by the TZM-bl neutralization assay. The IC_{50} in μg/mL against HIV_{YU-2} for the individual monoclonal antibodies is shown. (*D*) Serum antibody concentration after 1 d, the time point of pseudovirus injection, as determined by ELISA. Graph compares the actual monoclonal antibody concentration in serum (μg/mL) between WT and GR/LR mutant of 3BNC60, 3BNC117, and VRC01 (three to four mice per group, mean ± SEM).

Table S1. Comparison of the affinities of anti-gp140 antibodies in Fc domain variants

mAb	Fc variant	ka (1/Ms)	kd (1/s)	KD (M)
3BNC60	WT	1.4×10^{4}	1.73×10^{-4}	1.2×10^{-8}
	G236R/L328R	1.36×10^{4}	1.79×10^{-4}	1.31×10^{-8}
3BNC117	WT	1.5×10^{4}	1.29×10^{-4}	8.63×10^{-9}
	G236R/L328R	1.78×10^{4}	1.18×10^{-4}	7.9×10^{-9}
VRC01*	WT	2.5×10^{4}	6.25×10^{-5}	2.5×10^{-9}
	G236R/L328R	2.28×10^{4}	7.2×10^{-5}	3.17×10^{-9}

Affinities were determined using recombinant gp140 trimer or 2-CC core (*) and calculated by fitting the SPR sensograms to the 1:1 Langmuir binding model.

Table S2. Affinities of Fc domain variants for mouse FcγR

Variable	mFcγRI	mFcγRIIb	mFcγRIII	mFcγRIV
hIgG1 WT	4.2×10^{-7}	1.2×10^{-5}	$>10^{-4}$	9.4×10^{-7}
hIgG1 G236R/L328R	n.d.b.	n.d.b.	n.d.b.	n.d.b.

n.d.b., no detectable binding.