The scale on the y-axis spans from the smallest dissociation constant (16 pM) measureable by our SPR instrument (as stated by the manufacturer) to the highest dissociation constant (10 μM) measureable based on the analyte concentration used in the experiment. (B) Dissociation constants measured by SPR between selected eOD-GT8 60mer-elicited antibodies and candidate boost immunogens. Among the 115 Abs in (A), the 29 antibodies with highest affinity for eOD-GT8 ($K_D$ < 1 nM), along with 8 unmutated antibodies with lower affinity for eOD-GT8, were selected for binding to candidate boosting immunogens (HxB2 core-e 2CC N276D and core BG505 N276D) by SPR. High analyte concentration was used to determine $K_D$s up to 100 μM. HxB2 core-e 2CC N276D 60mer nanoparticles were also assayed, with values presented as apparent affinity, due to the avidity between particles and IgG. Mutated antibodies are shown as green open squares while germline antibodies are shown as black open diamonds.

**Supplementary Materials:**

Materials and Methods

Figures S1-S18

Table S1-S8

References (41-52)


# Supplementary Materials for

## Priming A Broadly Neutralizing Antibody Response To HIV-1 Using A Germline-Targeting Immunogen

Joseph G. Jardine, Takayuki Ota, Devin Sok, Matthias Pauthner, Daniel W. Kulp, Oleksandr Kalyuzhniy, Patrick D. Skog, Theresa C. Thinnes, Deepika Bhullar, Bryan Briney, Sergey Menis, Meaghan Jones, Mike Kubitz, Skye Spencer, Yumiko Adachi, Dennis R. Burton, William R. Schief, David Nemazee

correspondence to: David Nemazee (nemazee@scripps.edu), William R. Schief (schief@scripps.edu) and Dennis R. Burton (burton@scripps.edu)

**This PDF file includes:**

## Materials And Methods

### Study design

The overall objectives of this study were: (a) to develop a knock-in mouse model for the germline-reverted heavy chain of the HIV broadly neutralizing antibody VRC01 (VRC01 gH mouse model); and (b) to test germline-targeting and native-like immunogens in this mouse model. Specifically, we sought to test the immunogens for their ability to induce immune responses, activate VRC01-class precursors using the VRC01 gH-chain and a short (5aa) CDRL3, select favorable VRC01-like mutations, and produce memory antibodies with affinity for candidate boost immunogens. To make these assessments, we employed ELISA to evaluate polyclonal serum responses, antigen-specific B cell sorting and subsequent antibody cloning to evaluate sequences of induced antibodies, antibody expression and purification and SPR to evaluate antibody binding affinities to various immunogens, and neutralization assays to assess whether elicited antibodies had the capacity to neutralize HIV. We also employed next-generation sequencing and B cell sorting to assess the naive VRC01 gH repertoire. Randomization: VRC01 gH and wild-type littermates of similar ages were assigned to the same immunogens. Blinding: the studies were not blinded. Replication: the main experiment that focused on analysis by ELISA and B cell sorting included 14 groups of 5 mice each (Fig 1D), and this experiment was conducted once; this experiment did include internal controls by using wild-type littermate mice and it did also have some redundancy in that several immunogens were tested in more than one adjuvant; additional experiments for hybridoma generation were conducted in 13 additional animals for two of the experimental groups (Fig 1D).

### Knock-in mouse generation

Generation of VRC01 gH mice was carried out essentially as described (*41*), by substituting the VRC01 gH VDJ exon in the targeting construct by overlap PCR. Briefly, linearized targeting construct DNA was introduced into C57Bl/6-derived embryonic stem cells and selected in media supplemented with G418. Cells that insert the gene non specifically carry the DTA gene, which is toxic and cells lacking the neomycin resistance gene are counterselected. Positive clones were identified initially by a PCR strategy and confirmed by southern blot analysis as indicated in Fig S4.

Polymerase chain reaction conditions were as follows. Reactions were carried out with Q5 Hot start Master mix (NEB) according to the instructions of the manufacturer, using primers at a final concentration of 0.25 mM and ~200 ng genomic DNA template. Reaction conditions were 98°C 45 sec (1 cycle), followed by 98°C 15 sec, 67°C 30 sec, 72°C 1 min (35 cycles) then 72°C 10 min. Primers used were as follows:

gv3_13: GTG CAG TCT GGG GCT GAG GTG AAG
gv3_3123: AGC ACT CAG AGA AGC CCA CCC ATC T

The sequence used for VRC01 gH encodes the following predicted mature protein V region:

QVQLVQSGAEVKKPGASVKVSCKASGYTFTGYYMHWVRQAPGQGLEWMGWI
NPNSGGTNYAQKFQGRVTMTRDTSISTAYMELSRLRSDDTAVYYCARGKNCDY
NWDFQHWGQGTLVTVSS.

The upstream promoter, leader and intron elements were from VHJ558.85.191. Targeting, embryonic stem cell screening and mouse generation were as described (*41*).

Generation and screening of B cell hybridomas from VRC01 gH mice

Hybridomas were prepared by fusion of spleen or lymph node cells with sp2/0 cell line and selected in HAT medium (*42*). Fusions done on days 5 and 10 were carried out directly, whereas those done on day 31 were from mice boosted 3d previously with GT8-60mer in saline. Supernatants were screened for GT8 binding using ELISA assay with GT8-60mer applied to 96-well microtiter plates, followed by blocking with 1% BSA and application of undiluted culture supernatants. Antibody binding was developed with HRP coupled-antibodies (BD Biosciences) specific to the relevant mouse isotypes, followed by incubation with chromogenic substrate (Millipore). Analysis of hybridoma V gene sequences was carried out using a 5' RACE approach as described (*43, 44*).

Serum binding titers by ELISA

eOD ELISAs were performed as described previously (2) with minor modifications. Microlon 96-well plates (Corning) were coated overnight with antigen (eOD-GT8, eOD-GT8 KO, eOD-17, eOD-17 KO) at 2 µg/mL in PBS (25 µl/well). After washing and blocking with 1% FBS + 5% skim milk + 0.2% Tween20 for 1 h at RT, serially diluted serum in PBS + 1% FBS + 0.2% Tween20 were then added for 2 h at RT. Plates were then washed and alkaline phosphatase-labeled goat-anti-mouse immunoglobulin G (IgG) (Jackson Immunoresearch, Suffolk, England) was added for 1 h at a 1:2000 dilution in PBS + 1% FBS + 0.2% Tween20 at RT. After washing, absorption was measured at 450 nm.

BG505 SOSIP ELISA

Microlon 96-well plates (Corning) were coated overnight with anti-HIS epitope tag antibody (Thermo Scientific) at a concentration of 2µg/mL in PBS (25 µL per well). After washing (PBS + 0.2% Tween20) and blocking (5% skim milk in PBS with 1% FBS and 0.2% Tween20), 25 µL of C-terminally HIS-tagged SOSIP trimers were added to each well and incubated for 2 hours. Serially diluted serum in PBS/1% FBS + 0.2% Tween20 were then added for 2 h at RT. Plates were then washed and alkaline phosphatase-labeled goat-anti-mouse immunoglobulin G (IgG) (Jackson Immunoresearch, Suffolk, England) was added for 1 h at a 1:2000 dilution in PBS + 1% FBS + 0.2% Tween20 at RT. After washing, absorption was measured at 450 nm.

Single-cell sorting by flow cytometry

Mice spleen and lymph node samples were processed for single B cell sorting based on previously described methods (wu et al science 2013, tiller at al 2008, sok et al pnas 2014). In brief, mice spleen were stained with primary fluorophore-conjugated antibodies to murine CD4, CD8, F4/80, CD11c, Gr-1, CD19, B220, IgD, IgM, CD38, and GL7 markers. Memory B cells were selected for the phenotype CD19+, B220+, CD4-, CD8-, F4/80-, CD11c-, Gr-1-, IgM-, IgD-, while CD38 and GL7 markers were monitored to measure germinal center B cell frequencies (CD38-, GL7+). For antigen-specific staining, 50 nM of biotinylated AviTag eOD-GT8 monomer and its CD4bs KO varaint (eOD-GT8-KO) were coupled to Streptavidin-AF488 and Streptavidin-PE (Life Technologoies) in equimolar ratios, respectively. Similarly, eOD17 immunized mice were sorted using 50 nM of biotinylated AviTag eOD17 monomer and its CD4bs KO varaint (eOD17-KO), while BG505 SOSIP immunized mice were sorted with 50 nM of biotinylated AviTag BG505 SOSIP trimer (*45*). B cells of interest were single-cell sorted into 96 well plates containing lysis buffer on a BD FACSAria III sorter and immediately stored at -80°C (*9, 45, 46*).

Single B-cell RT-PCR, gene amplification, and cloning

Reverse transcription and subsequent PCR amplification of heavy and light chain variable genes were performed using SuperScript III (Life Technologies) according to published protocols (*9, 45, 46*). All PCR reactions were performed in 25 µl volume with 2.5 µl of cDNA transcript using HotStar Taq DNA polymerase master mix (Qiagen) and mixtures of previously described primers (*47*) that were supplemented with a human VH1-2 primer (CAGGTGCAGCTGGTGCAGTCTGG). Second round nested-PCR reactions were performed using Phusion proof reading polymerase (NEB). PCR products were then directly cloned into respective human Igγ1, Igκ and Igλ pFuse expression vectors via Gibson assembly (5). Multiple clones for each heavy and light chain pair were sequenced using Sanger sequencing and corrected for PCR errors before further analysis and expression.

Antibody production

Heavy and light chain plasmids were co-transfected (1:1 ratio) in 293 FreeStyle cells using 293fectin (Invitrogen) according to the manufacturer's protocol, and antibody supernatants were harvested four to five days following transfection. Supernatants were further purified using protein A Sepharose (GE Healthcare) and dialysed overnight into PBS (0.01 M sodium phosphate, pH 7.4, 0.137 M sodium chloride).

Protein production and purification

eOD monomers and 60mers were produced and purified as described previously (*17*). BG505 SOSIP D664 gp140 trimers (*33-36*) were produced in mammalian cells (HEK-293F) by co-transfection of the trimer gene and furin protease, at a trimer to furin ratio of 2:1. The pre-transfected cells were maintained in 293 Freestyle media (Life Technologies) in a humidified 37°C $C0_2$ incubator (8%), rotating at 135rpm at a density of ~2.4 x $10^6$ cells/ml. The genes were transfected using 293fectin (Invitrogen) and harvested 4-5 days later. The cells were centrifuged at 4000rpm for 15min, filtered using 0.2 µm filter (Millipore) and a protease inhibitor was added at ratio of 1ml per liter of supernant (Protease Arrest, GBiosciences). The supernants were purified by nickel affinity purification using His-Trap columns (GE), starting with a wash buffer (20mM

Imidizole, 500 mM NaCl, 20 mM Na2HPO4) and mixing with elution buffer (500 mM Imidizole, 500 mM NaCl, 20 mM Na2HPO4) using a linear gradient. The trimers were then purified by semi-analytical size exclusion chromatography on a S200Increase 10-300 column (GE) in HBS (10mM HEPES, 150mM NaCl). The trimer fractions were pooled, concentrated to 1mg/ml by using Ultracel 30K centrifugal spin concentrators (Millipore) and measuring concentration on a NanoDrop 2000c Spectrophotometer using the absorption signal at 280 nm, frozen in thin-walled PCR tubes using liquid nitrogen, and then stored at -80°C. BG505 SOSIP trimers produced by this in-house process have been thawed and analyzed by SECMALS, SPR, differential scanning calorimetry, and electron microscopy and have been found to possess the native-like antigenic profile, thermal stability and closed trimeric structure that have been reported by others for BG505 SOSIP purified by an antibody-affinity column followed by SEC (*33-36*) (data not shown).

Surface plasmon resonance (SPR)

We measured kinetics and affinities of antibody-antigen interactions on a ProteOn XPR36 (Bio-Rad) using GLC Sensor Chip (Bio-Rad) and 1x HBS-EP+ pH 7.4 running buffer (20x stock from Teknova, Cat. No H8022) supplemented with BSA at 1mg/ml. We followed the Human Antibody Capture Kit instructions (Cat. No BR-1008-39 from GE) to prepare chip surfaces for ligand capture. In a typical experiment, about 6000 RU of capture antibody was amine-coupled in all 6 flow cells of the GLC Chip. Regeneration was accomplished using 3M Magnesium Chloride with 180 seconds contact time and injected four times per each cycle. Raw sensograms were analyzed using ProteOn Manager software (Bio-Rad), including interspot and column double referencing, and either Equilibrium fits or Kinetic fits with Langmuir model, or both, were employed when applicable. Analyte concentrations were measured on a NanoDrop 2000c Spectrophotometer using Absorption signal at 280 nm.

Pseudovirus production and neutralization assays

Pseudoviruses were generated by transfection of 293T cells with an HIV-1 Env expressing plasmid and an Env-deficient genomic backbone plasmid (pSG3ΔEnv), as described previously (*48*). Pseudoviruses were harvested 72 hrs post-transfection for use in neutralization assays. Neutralizing activity was assessed using a single round of replication pseudovirus assay and TZM-Bl target cells, as described previously (*48*). Briefly, TZM-bl cells were seeded in a 96-well flat bottom plate. To this plate was added pseudovirus, which was preincubated with serial dilutions of antibody for 1 hr at 37°C. Luciferase reporter gene expression was quantified 72 hrs after infection upon lysis and addition of Bright-GloTM Luciferase substrate (Promega). To determine IC50 values, dose-response curves were fit by nonlinear regression.

Processing of DeKosky/Georgiou paired antibody sequences

Raw sequence data from DeKosky *et al*. (*19*) was downloaded from the Short Read Archive (SRA) and, because SRA files are generated by concatenating paired Illumina reads into a single file, each SRA file was split into two 'read' files corresponding to the paired sequencing reads. In each pair of read files, paired reads were assigned the same sequence ID to enable reconstitution of native heavy/light pairs.

Quality and length trimming was performed on each read file using Sickle (*49*) (with options -q 25 and -l 200) such that the 3' end of each read was trimmed until a 25-base sliding window contained an average sequence quality of 25 and trimmed reads of less than 200 bases were discarded. Germline V(D)J gene assignment and junction identification was performed with an in-house BLASTn-based pipeline and resulting assignments were stored in a MongoDB database. Heavy chain junctions were clustered at 96% sequence identity to collapse duplicate sequencing reads and centroid sequences were calculated for each cluster with at least 2 heavy chain junctions. For each centroid heavy chain sequence, the sequence ID was used to retrieve the appropriate paired light chain sequence. Length distributions for heavy, kappa light, and lambda light chains were measured using IMGT (*50*) conventions.

Healthy human subjects

Peripheral blood was obtained from healthy adult donors following informed consent, under a protocol (IRB# 12-5951) approved by the Scripps Institutional Review Board. Peripheral blood mononuclear cells (PBMCs) were isolated from the whole blood of four healthy donors by gradient centrifugation (Histopaque-1077; Sigma-Aldrich).

NGS sample preparation

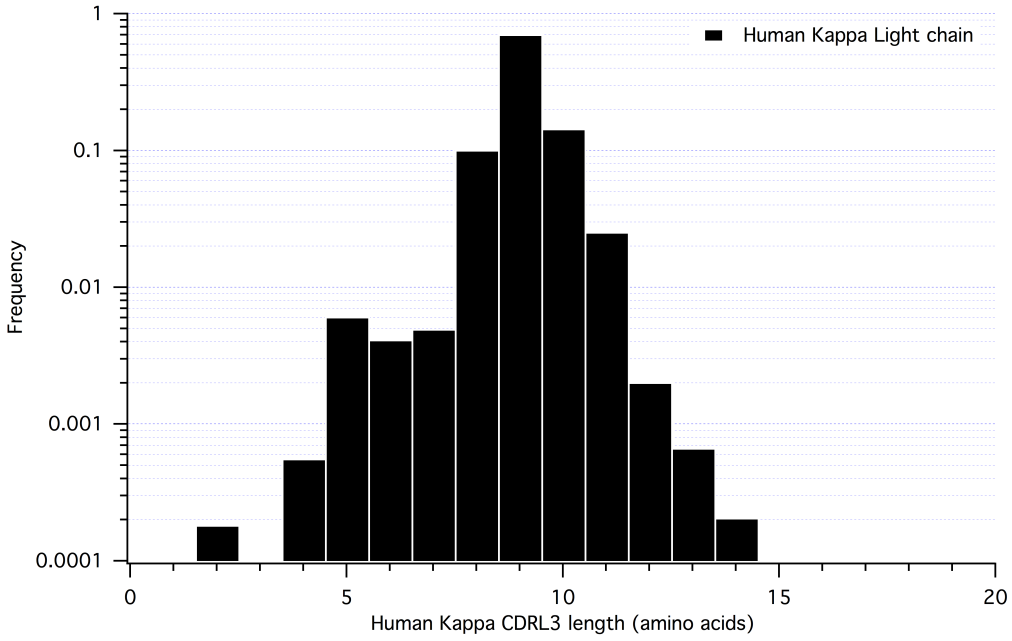
Total RNA was isolated from PBMCs (human subjects) or splenocytes (mouse subjects) (RNeasy; Qiagen). Approximately 5% of each total RNA sample was separately subjected to template-switching reverse transcription (SMARTer RACE kit; Clonetech) using the manufacturer's protocol, except that an alternate template-switching primer was used (TS-Illumina: AAGCAGTGGTATCAACGCAGAGTAGACGTGTGCTCTTCCGATCTrGrGrGrGrG, where rG = riboguanosine). 2.5uL of each RT product was used in a 50uL touch-down PCR reaction (Advantage2 Polymerase; Clonetech), with the following cycling conditions: 94C for 1 min; 5 cycles of 94C for 30 sec, 72C for 2 min; 5 cycles of 94C for 30 sec, 70C for 30 sec, 72C for 2 minutes; 25 cycles of 94C for 30 sec, 68C for 30 sec, 72C for 2 min; 72C for 5 min. PCR products were purified using 0.7 volumes of SPRI beads (SPRIselect; Beckman Coulter Genomics) using the manufacturer's standard protocol and eluted in 50uL of water. 2uL of each purified PCR product was used in a 50uL indexing PCR reaction (HotStarTaq Plus; Qiagen) with the following cycling conditions: 95C for 5 min; 15 cycles of 95C for 30 sec, 58C for 30 sec, 72C for 2 min; 72C for 10 min. Indexing PCR products were again purified using 0.7 volumes of SPRI beads and eluted in 50uL of water. Each purified product was quantified by fluorometry (Qubit; Life Technologies), products were pooled at approximately equimolar concentrations, and the pool was re-quantified (Qubit).

Next-generation antibody sequencing

Amplicons were loaded onto a MiSeq sequencer (MiSeq v3 Reagent Kit, 600-cycles; Illumina) with a target loading concentration of 40pM. To ensure that full-length 5'RACE products were sequenced (including the leader and 5' UTR region), we modified the run setup such that the first read was 350bp, followed by a second read of 300bp. Paired sequencing reads were merged with PANDAseq (*51*) and sequences were annotated with in-house antibody analysis software based on BLASTn, using human and

mouse germline V(D)J databases from IMGT (*52*). Following annotation, sequences were loaded into a MongoDB database for querying and additional analysis.

**A.**




B.

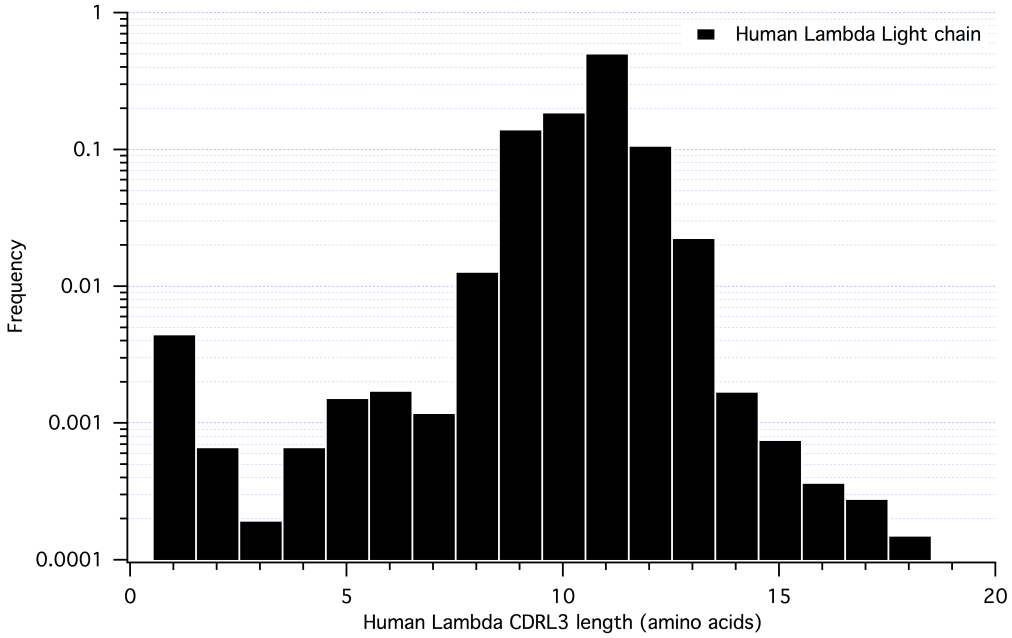
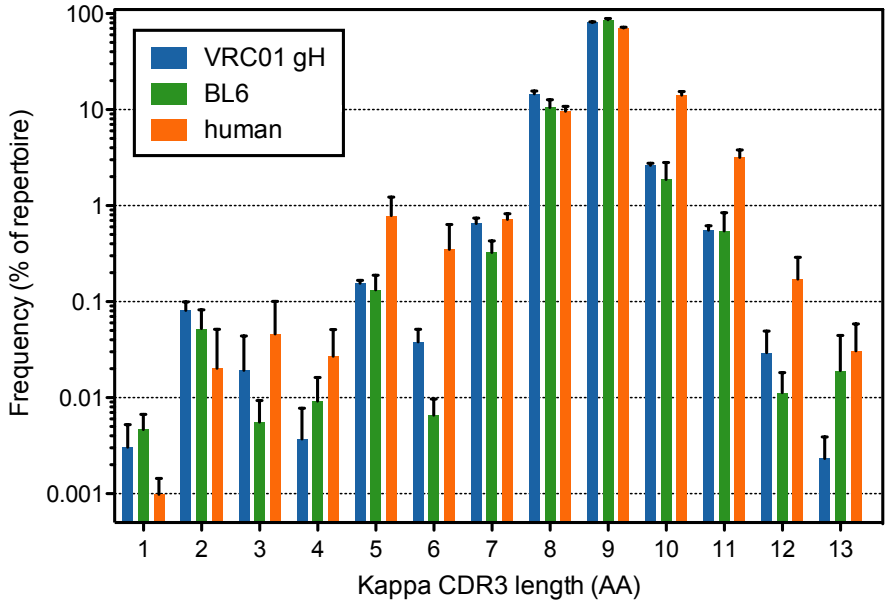



**Fig. S1.**

Human light chain CDRL3 length distributions for kappa (A.) and lambda (B.) chains. Histograms are based on 127,701 antibody sequences (81,910 of which had kappa light chains and 45,791 of which had lambda light chains) from three donors determined by DeKosky *et al.* (*19*).

**A.**




**B.**

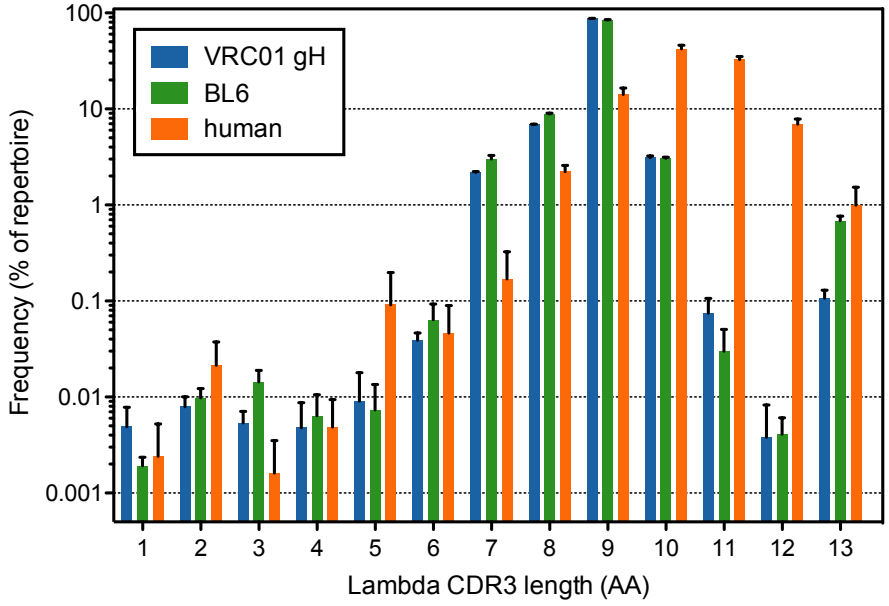



**Fig. S2.**

**Light chain CDRL3 length distributions in VRC01 gH mice, WT littermates, and humans, for kappa chains (A) and lambda chains (B). A.** Next-generation sequencing (NGS) of spleen from 4 VRC01 gH mice and 4 WT littermate mice show similar kappa chain CDRL3 length distributions compared to sequences from PBMCs from 4 humans, with a peak in the distribution at a length of 9. **B.** NGS of spleen from 4 VRC01 gH mice

and 4 C57B/6 WT littermate mice show similar lambda chain CDRL3 length distributions, indicating the repertoire is not biased by the knock-in VRC01 gH. The human lambda distribution is broader. The frequency of 5 aa CDRL3 is lower in mouse lambda chains compared to mouse kappa chains by a factor of ~8. CDRL3 lengths (amino acid) are shown based on percent frequency in the repertoire. Values plotted are mean and standard deviation for each sample (N=4 in all cases). The total number of sequences for each histogram was 1,169,886 (VRC01 gH kappa), 1,220,394 (WT kappa), and 799,458 (human kappa), 2,522,669 (VRC01 gH lambda), 1,800,093 (WT lambda), and 1,061,959 (human kappa)

**A.**

**VH Gene Alignment**

```
VRC01 AA         Q   V   Q   L   V   Q   S   G   G       Q   M   K   K   P   G   E   S
VRC01 NT       CAG GTG CAG CTG GTG CAG TCT GGG GGT --- CAG ATG AAG AAG CCT GGC GAG TCG
IGHV1-02*02    ... ... ... ... ... ... ... ... .C. --- G.. G.. ... ... ... ..G .CC ..A

VRC01 AA         M   R   I   S   C   R   A   S   G   Y   E   F   I   D   C   T
VRC01 NT       ATG AGA ATT TCT TGT CGG GCT TCT GGA TAT GAA TTT ATT GAT TGT ACG --- ---
IGHV1-02*02    G.. .AG G.C ..C ..C AA. ... ... ... ..C ACC ..C .CC .GC .AC TAT --- ---

VRC01 AA         L   N   W   I   R   L   A   P   G   K   R   P   E   W   M   G
VRC01 NT       --- --- CTA AAT TGG ATT CGT CTG GCC CCC GGA AAA AGG CCT GAG TGG ATG GGA
IGHV1-02*02    --- --- A.G C.C ... G.G ..A .A. ... ..T ... C.. G.. .T. ... ... ... ...

VRC01 AA         W   L   K   P   R   G   G   A   V           N   Y   A   R   P   L   Q
VRC01 NT       TGG CTG AAG CCT CGG GGG GGG GCC GTC --- --- AAC TAC GCA CGT CCA CTT CAG
IGHV1-02*02    ... A.C ..C ... AAC A.T ..T .G. ACA --- --- ... ..T ... .AG AAG T.. ...

VRC01 AA         G   R   V   T   M   T   R   D   V   Y   S   D   T   A   F   L   E
VRC01 NT       --- GGC AGA GTG ACC ATG ACT CGA GAC GTT TAT TCC GAC ACA GCC TTT TTG GAG
IGHV1-02*02    --- ... ..G ..C ... ... ..C A.G ... ACG .CC AT. AG. ... ... .AC A.. ...

VRC01 AA         L   R   S   L   T   V   D   D   T   A   V   Y   F   C   T   R   G
VRC01 NT       CTG CGC TCG TTG ACA GTA GAC GAC ACG GCC GTC TAC TTT TGT ACT AGG GG
IGHV1-02*02    ... A.. AG. C.. .G. TCT ... ... ... ... ..G ..T .AC ... G.G ..A .A
```

**DH Gene Alignment**

```
VRC01 AA         T   R   G   K   N   C   D   Y   N   W   D   F   E   H   W   G   R   G   T
VRC01 NT       ACT AGG GGA AAA AAC TGT GAT TAC AAT TGG GAC TTC GAA CAC TGG GGC CGG GGC ACC
IGHD3-16*02                G T.T .A. ... ... GT. ... .GG AGT T.T .GT .AT AC.
IGHD3-16*01                G T.T .A. ... ... GT. ... .GG AGT T.T GCT .AT AC.
IGHD2/OR15-2R  GG CAT A.. ..G T.G .AC T.. ... ... ATT CT
IGHD5-24*01                GT .GA GA. .GC ... ... .AC
IGHD1-01*01                           GG ... ..C ... A.. GA.
```

**JH Gene Alignment**

```
VRC01 AA     C   D   Y   N   W   D   F   E   H   W   G   R   G   T   P   V   I   V   S   S
VRC01 NT   TGT GAT TAC AAT TGG GAC TTC GAA CAC TGG GGC CGG GGC ACC CCG GTC ATC GTC TCA TCA
IGHJ1*01         GC. GAA T.. ... C.G ... ... ... .A. ... ... .T. ... .C. ... ..C ...
IGHJ2*01       . T.C ... T.. ... ..T .T. ... ... ..T ... ... .T. ... .CT ... ..C ...
IGHJ5*02         .C AAC TGG ... ..C .C. ... ... .A. ..A ... .T. ... .C. ... ..C ...
IGHJ4*02            AC T.. ..T ..C T.. ... ... .A. ..A ... .T. ... .C. ... ..C ...
IGHJ4*03            .C T.. ..T ..C T.. ... ... .AA ..G ... .T. ... .C. ... ..C ...
```

**B.**

```
VRC01      QVQLVQSGGQMKKPGESMRISCRASGYEFIDCTLNWIRLAPGKRPEWMGWLKPRGGAVNYARPLQGRV
IGHV1-2*02 ........AEV....A.VKV..K....T.TGYYMH.V.Q...QGL.....IN.NS.GT...QKF....
VRC01 gH   ........AEV....A.VKV..K....T.TGYYMH.V.Q...QGL.....IN.NS.GT...QKF....

VRC01      TMTRDVYSDTAFLELRSLTVDDTAVYFCTRGKNCDYNWDFEHWGRGTPVIVSS
IGHV1-2*02 .....TSIS..YM..SR.RS......Y.A.
IGHJ1*01                                  .Q...Q..L.T...
VRC01 gH   .....TSIS..YM..SR.RS......Y.A...S.....  Q...Q..L.T...
```

**Fig. S3.**

**Sequence of VRC01 gH. (A)** Alignments of the VRC01 H chain to the closest human V, D, and J gene segments are shown, as determined by JoinSolver (*31*). Multiple DH and JH genes are shown in the order ranked by JoinSolver to reflect the uncertainty in those assignments. Highlighted in red is the region of the CDRH3 encoded by the D gene and N/P addition where the determination of the true germline sequence is particularly uncertain. Based on this analysis, we selected IGHV1-02*02 and IGHJ1*01 as the V and J genes, respectively, for VRC01 gH. These choices are supported by recent analysis of the VRC01 lineage over 15 years of chronic infection in the VRC01 donor **(*30*)**. **(B)** Amino acid alignment of VRC01 and VRC01 gH. The CDRH3 for VRC01 gH used the

same CDRH3 as VRC01, except that we mutated an unpaired cysteine in the CDRH3 to serine to avoid potential solubility and B cell development problems (in VRC01 this cysteine in CDRH3 makes a disulfide bond with a cysteine that arose by affinity maturation in CDRH1, but germline reversion of the V gene, including the CDRH1, leaves the CDRH3 cysteine without a disulfide-bonding partner).

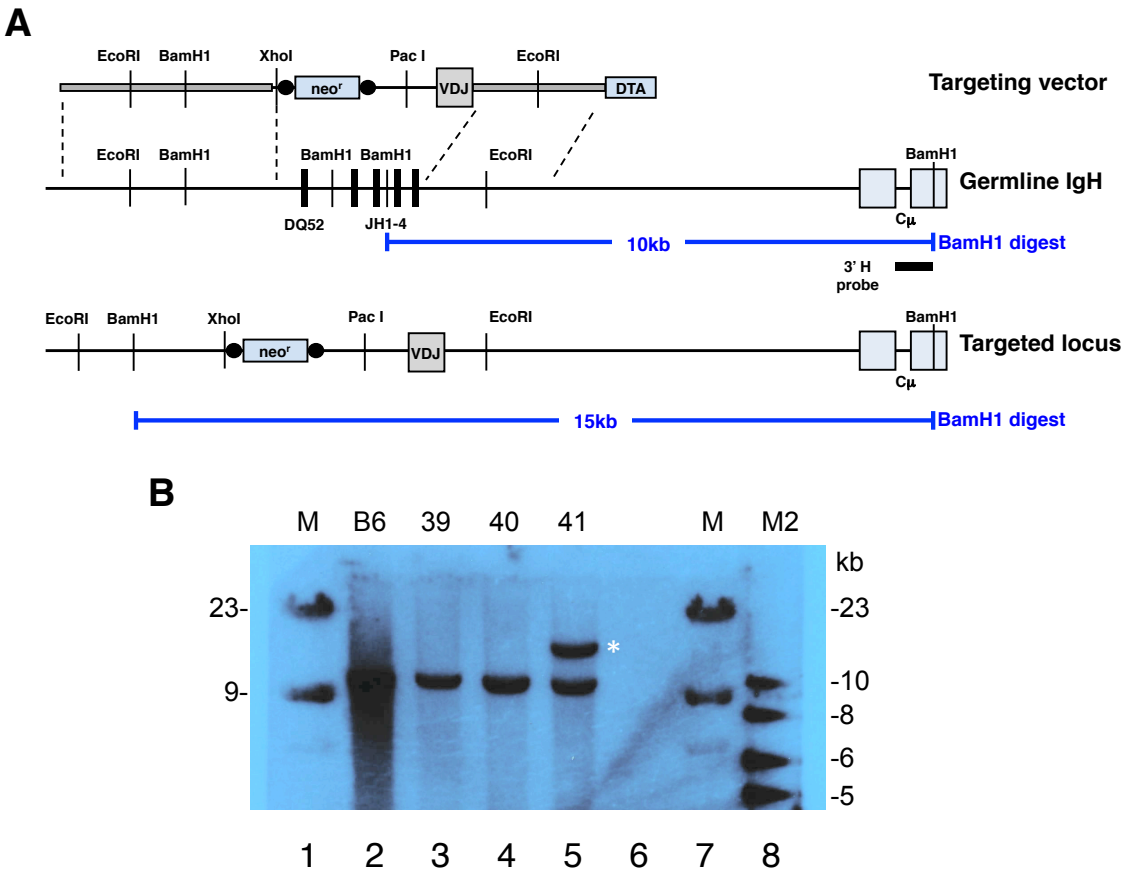



**Fig. S4.**

**Approach for knock-in of VRC01 germline heavy chain (gH) gene.** (**A**) Schematic of targeting strategy and analysis. Shown are partial restriction maps of the targeting vector, germline locus, and the predicted targeted allele before and after neomycin resistance gene (neo$^r$) removal by cre/lox recombination. Gray horizontal bars indicate arms of homology of the targeting vector. Dotted lines show areas of homology involved in recombination between the targeting vector and host locus to generate the knock-in allele. Note that targeting eliminates the host Jh locus and Dq52 gene and replaces it with the VRC01 gH variable gene, upstream elements and promoter, along with the neo gene which allows selection for the desired allele. Black circles represent loxP sites; white and lighter gray boxes indicate the elements of the inserted coding sequences including leader, intron and VDJ exon with upstream promoter elements not specifically shown. Blue boxes represent other coding sequences, including neo$^r$, diphtheria toxin A (DTA), and Cm exons. The germline locus shows JH and nearby $D_{Q52}$ elements in black boxes. Horizontal black bar below the lower part of the figure indicates location of probe used in southern blot analysis. Blue lines indicate predicted fragments generated with BamH1 restriction enzyme. (**B**) Shown is southern blotting analysis of genomic DNA of individual ES cell clones tail DNA from C57Bl/6 (B6) mice of the indicated genotypes. Asterisk shows positive clone used in blastocyst injections for mouse production. Breeding to EIIa-cre transgenic mice was used to remove neo$^r$.

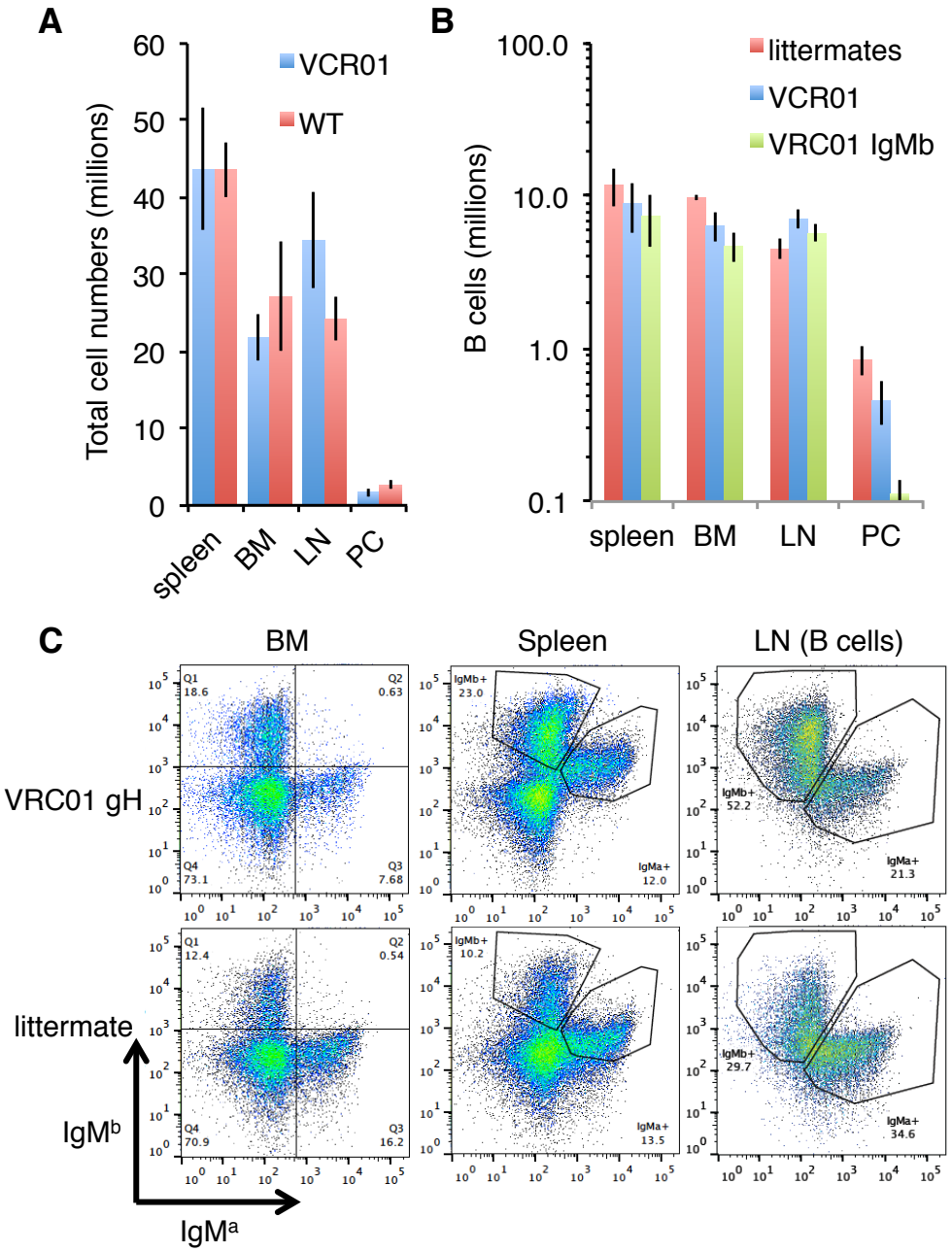



**Fig. S5.**

**VRC01 gH mice have significant numbers of B cells in secondary lymphoid tissues expressing the targeted allele.** To track B cells carrying the knock-in allele, VRC01 gH mice, carrying a single knock-in locus, were bred one generation to mice carrying the "a" allelic haplotype of the Igh locus. In these mice, $IgM^a$ is carried only by cells expressing the endogenous allele whereas $IgM^b$ cells almost invariably express the VRC01 gH transgene. Controls were non-transgenic littermates from the same cross where

expression of the two allele is expected to be ~50:50. Lymphoid tissue of the indicated mice were analyzed at 8 weeks of age for **(A)** total cell numbers, **(B)** B cells (CD19+B220+; marked blue), and cells carrying IgMb (green), which is the knock-in H-chain allele of surface IgM. Cells tested were from the following tissues: spleen, bone marrow (BM), lymph nodes (LN), peritoneal cavity (PC). As the knockin replaces the Jh cluster on the $Igh^b$ allele, in VRC01 gH mice virtually all B cells expressing $IgM^b$ use VRC01 gH. The allele usage frequency found here was consistent with the deep sequencing data shown in Fig 1. **(C)** Examples of flow cytometry plots enumerating B cells carrying IgMa and IgMb. Dh regions can rearrange into the knock-in VDJ, leading usually to loss of function. This likely explains the presence of $IgM^a$ cells in the VRC01 gH mice. LN plots were from gated B220+ cells.

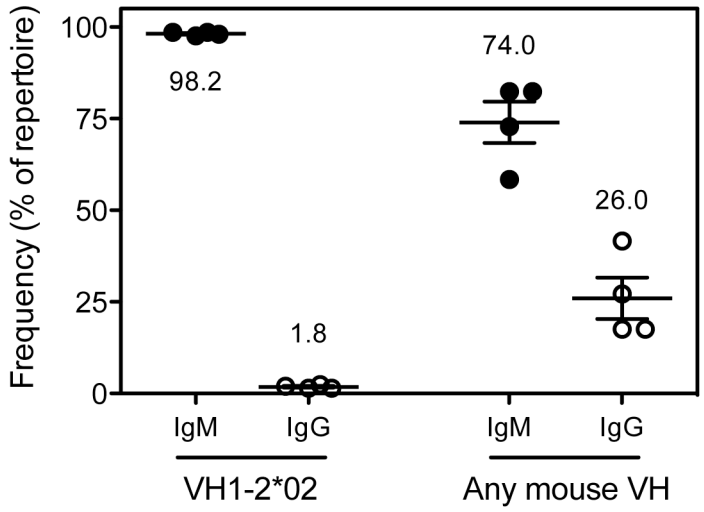



**Fig. S6.**

**The antibody repertoire of naïve VRV01 gH mice contains a high proportion of IgM.** Next-generation sequencing of spleen from VRC01 gH mice show that, for the VH1-2*02 gene, 98.2% of sequences are IgM while 1.8% of sequences are IgG. Different primers were used to amplify IgG vs IgM, introducing some uncertainty into the comparison of absolute frequencies.

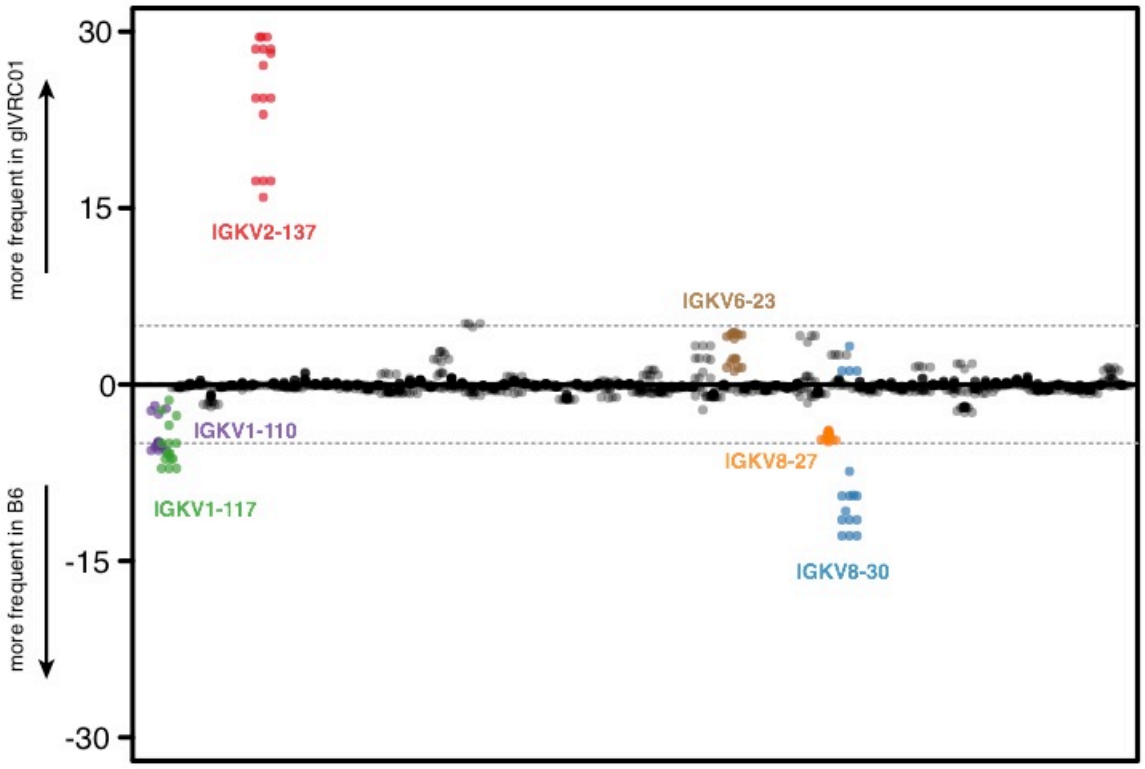



**Fig. S7.**

**The majority of Vκ genes are used similarly between VRC01 gH mice and littermate mice.** Next-generation sequencing of spleen from VRC01 gH mice and littermate mice show largely the same Vκ gene usage. Vκ genes that show different frequencies are highlighted with color, with Vκ genes used more frequency by VRC01 gH mice shown above the center axis and Vκ genes used more frequency by littermate mice shown below the center axis.

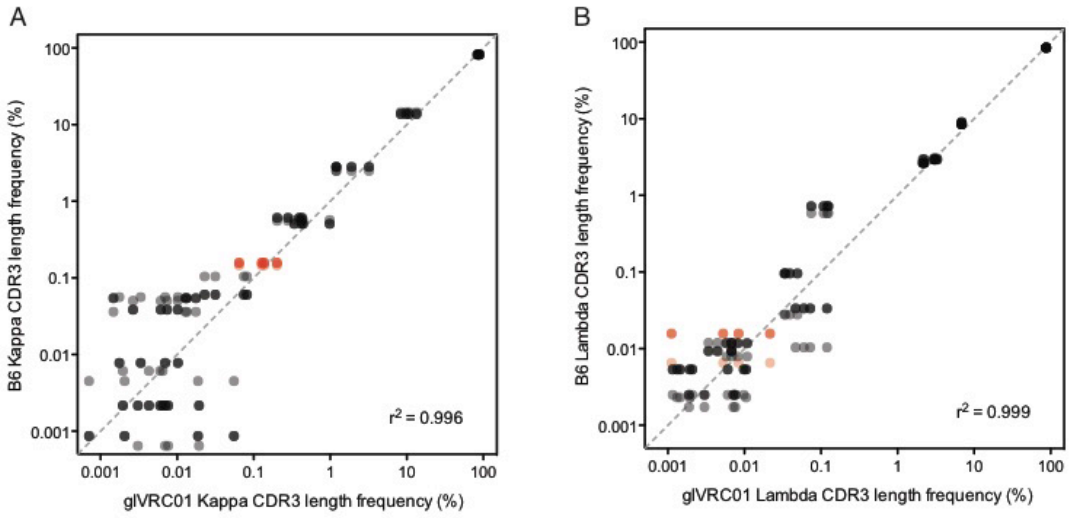



**Fig. S8.**

**VRC01 gH mice and littermates have similar CDRL3 length distributions for both kappa and lambda chains.** Next-generation sequencing of spleen from VRC01 gH mice and littermate mice show highly similar CDRL3 length distributions for both (A) kappa and (B) lambda chains.

eOD-GT8 60mer

**Fig. S9.**

Amino acid sequence of eOD-GT8 60mer nanoparticle used in this study. The particle base is shown in red, the G/S linker in black and the eOD-GT8 shown in blue. The engineering that led to the development of these particles will be described elsewhere.

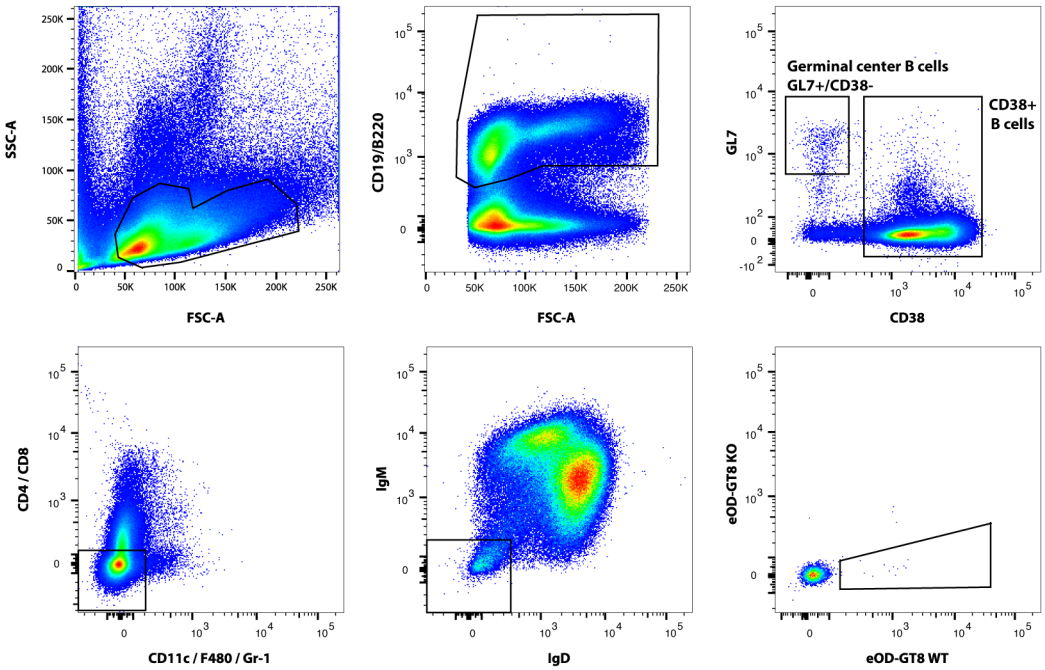



**Fig. S10.**

**Epitope-specific memory B cell sorting by flow cytometry.** Example gating strategy for sorting of epitope specific memory phenotype B cells. B cells were selected for a CD19+/B220+/CD38+/CD4-/CD8-/IgM-/IgD-/eOD-GT8+/eOD-GT8 KO- events. Frequency of germinal center phenotype B cell events could also be distinguished using GL7 and CD38 markers (GL7+/CD38-).

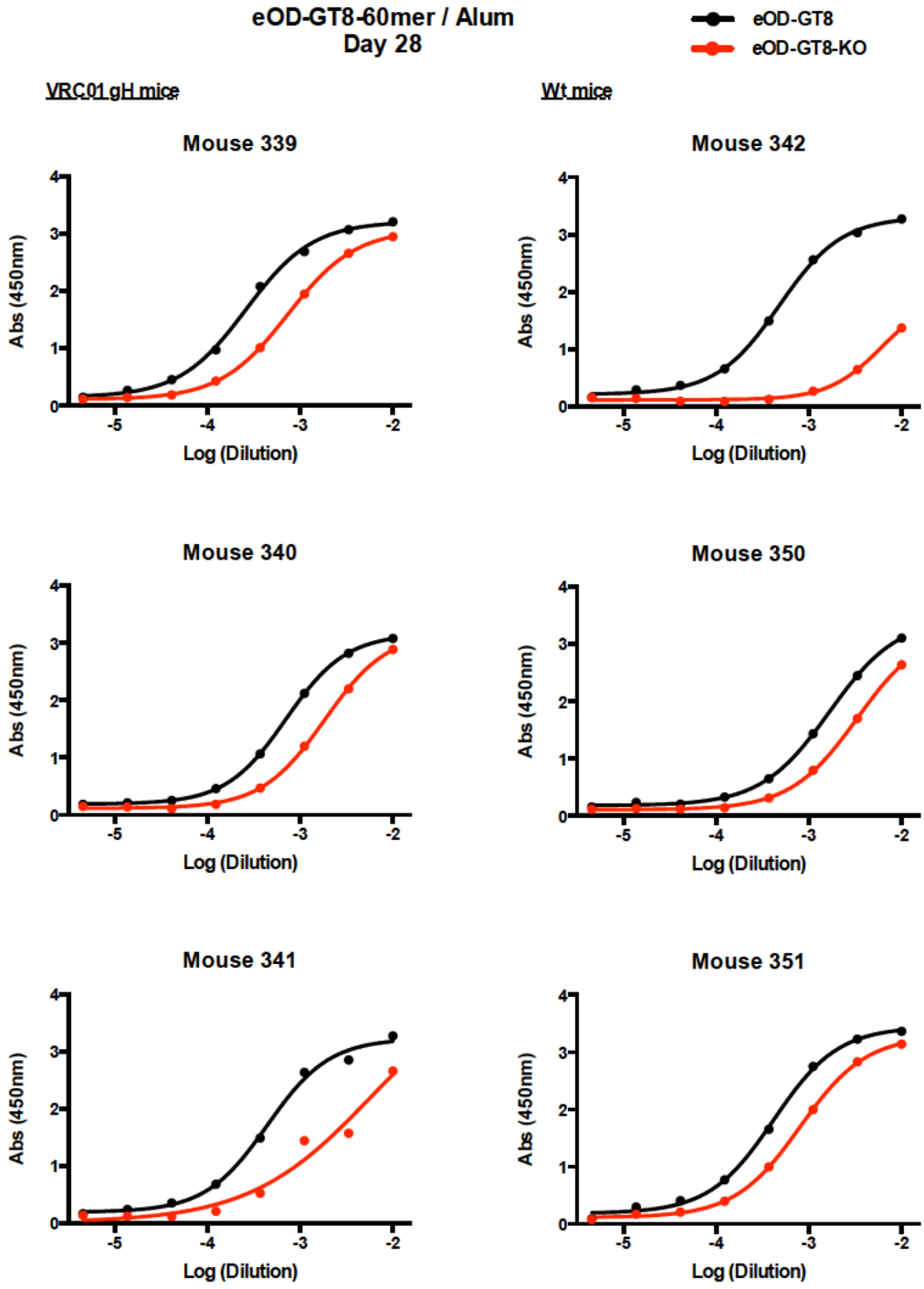



**Fig. S11.**

**ELISA titrations of serum from immunized mice against eOD-GT8 and eOD-GT8-KO, and against BG505 SOSIP.**

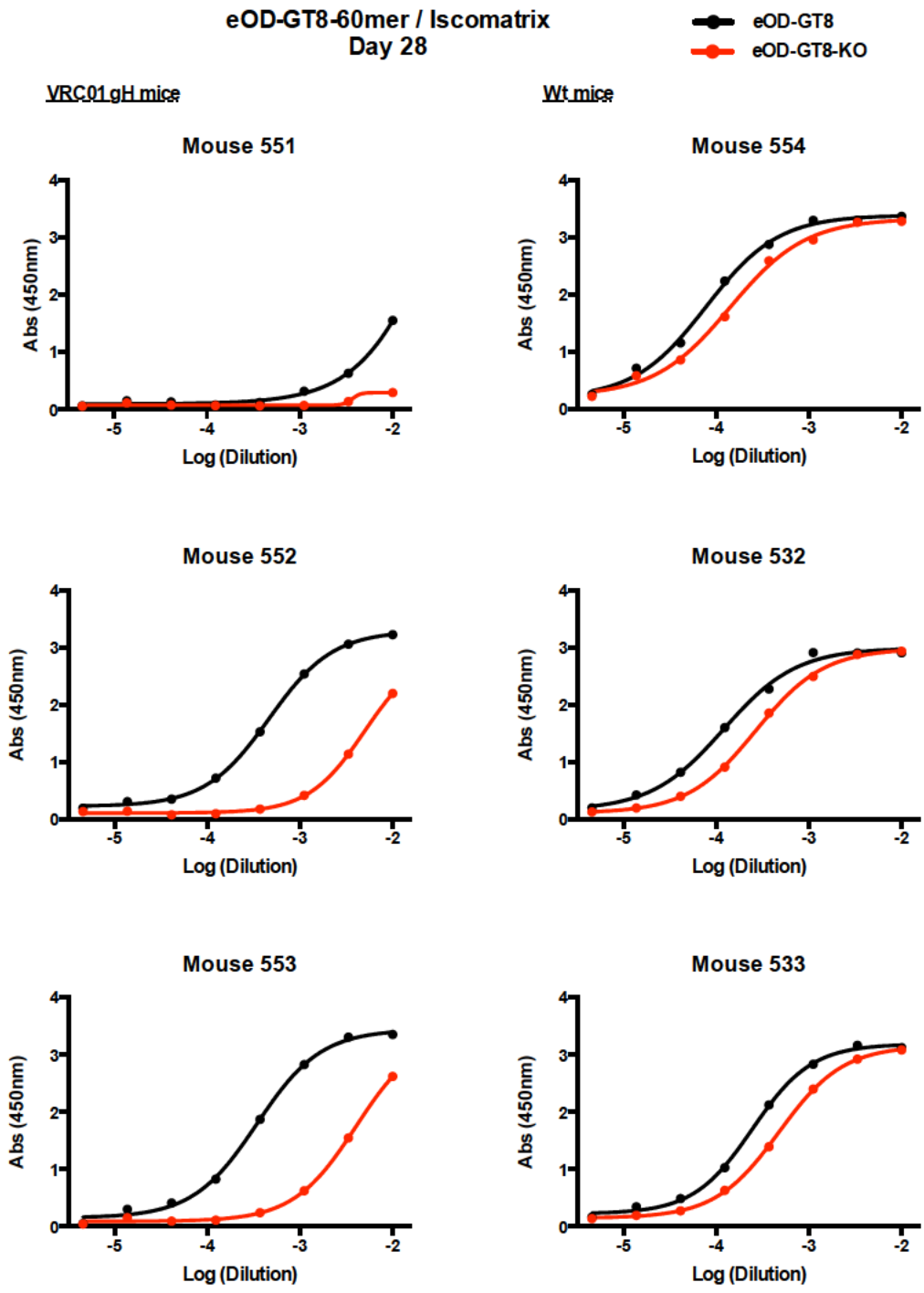



**Fig. S11. (continued)**

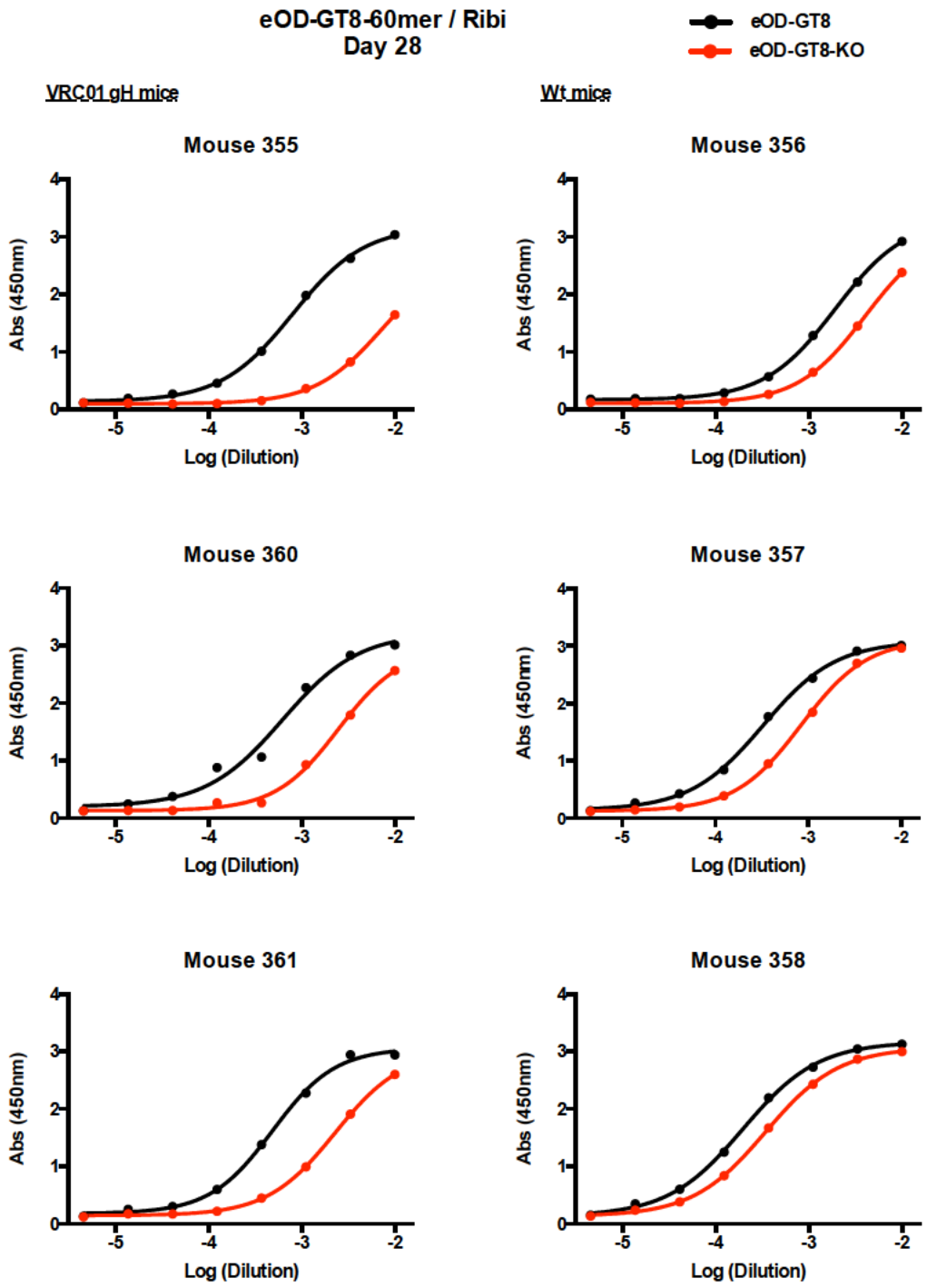



**Fig. S11. (continued)**

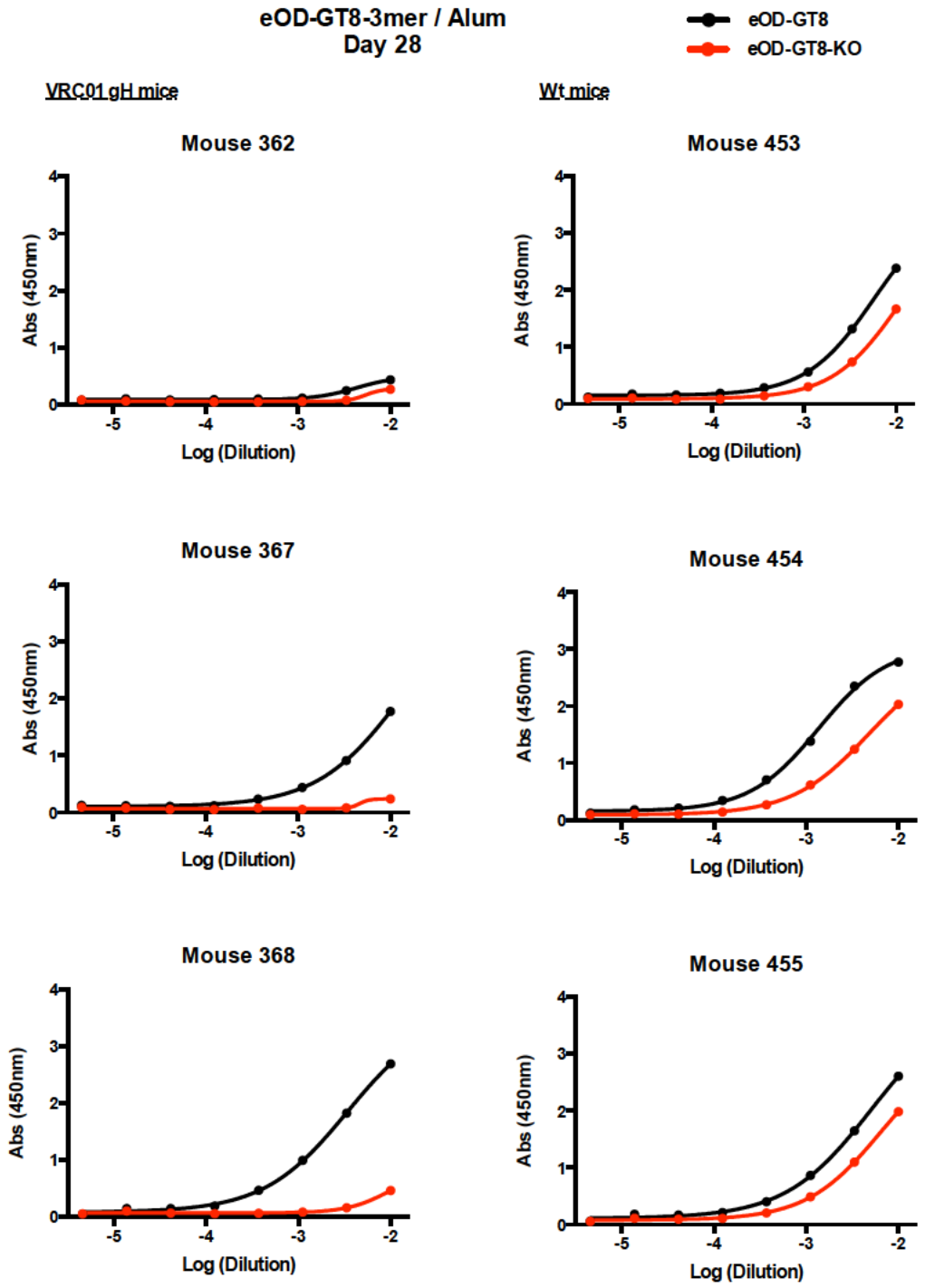



**Fig. S11. (continued)**

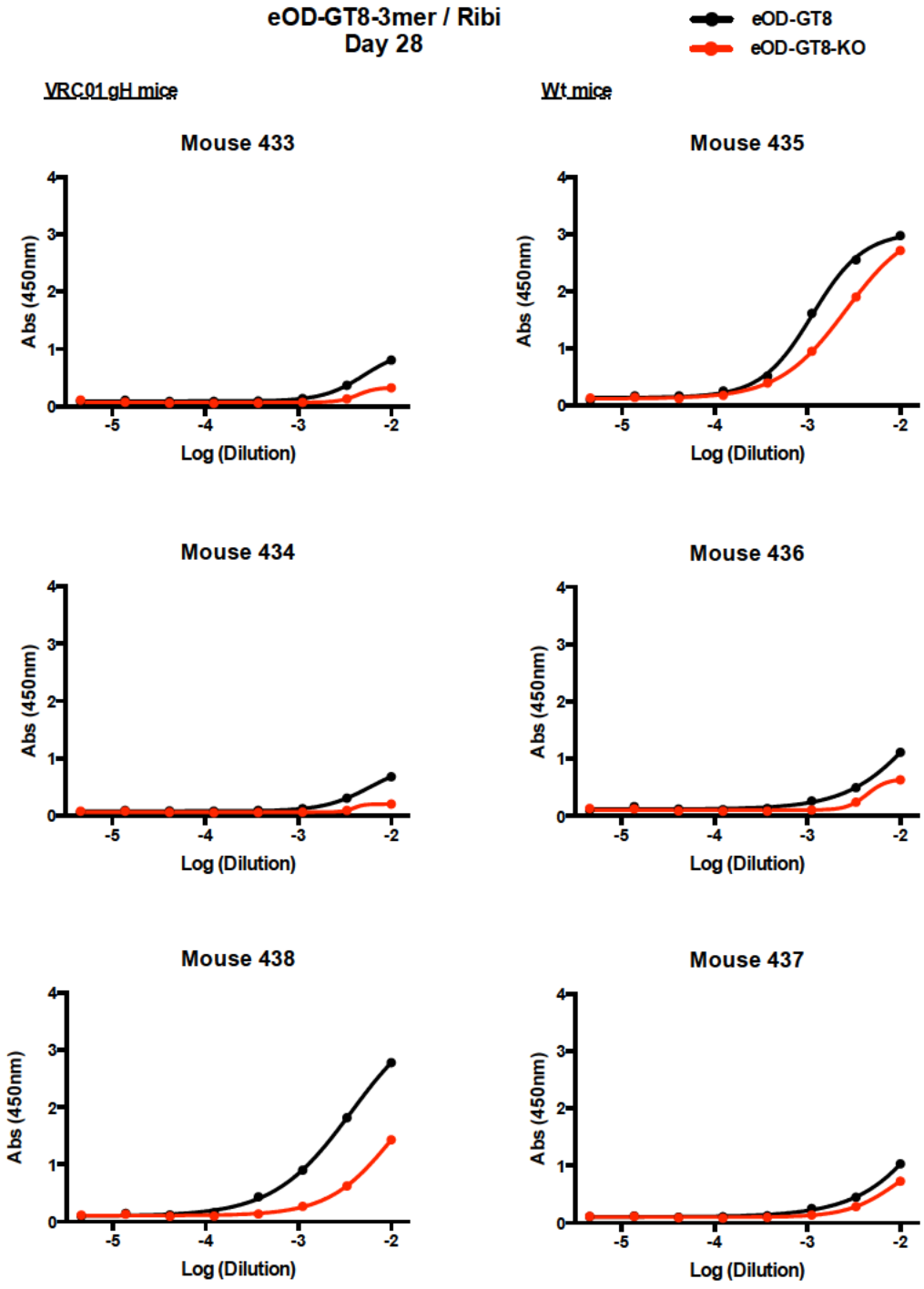



**Fig. S11. (continued)**

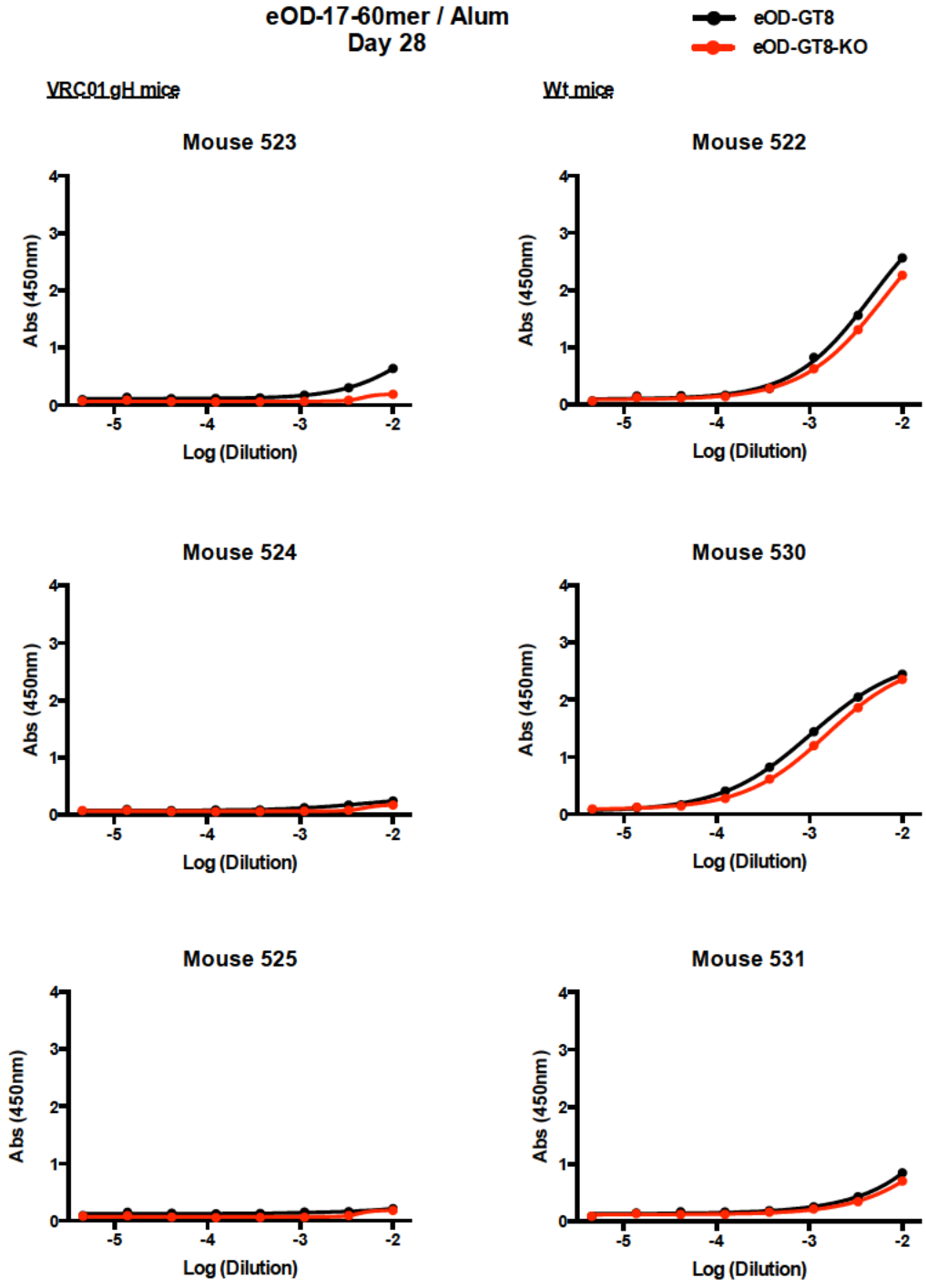



**Fig. S11. (continued)**

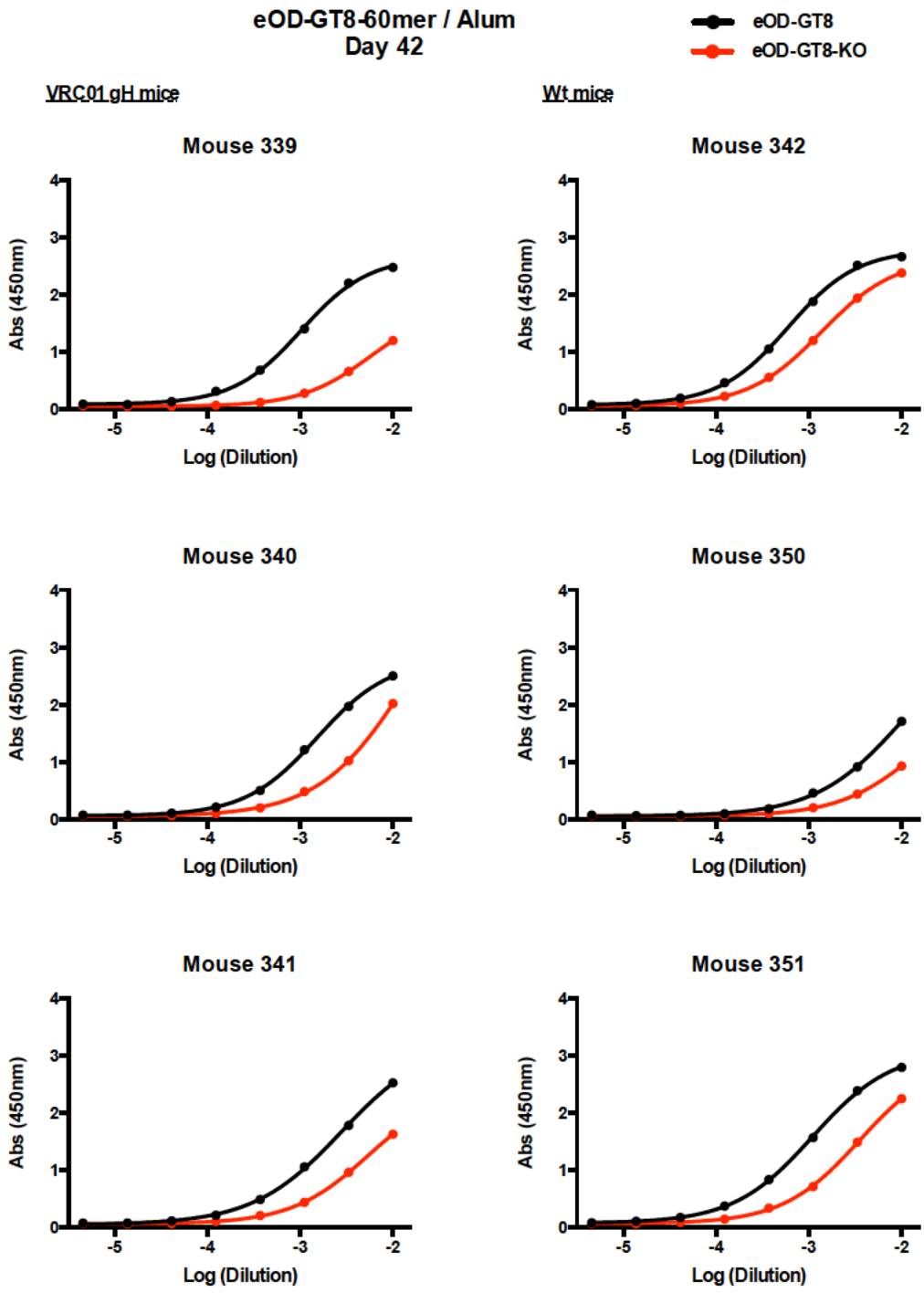



**Fig. S11. (continued)**

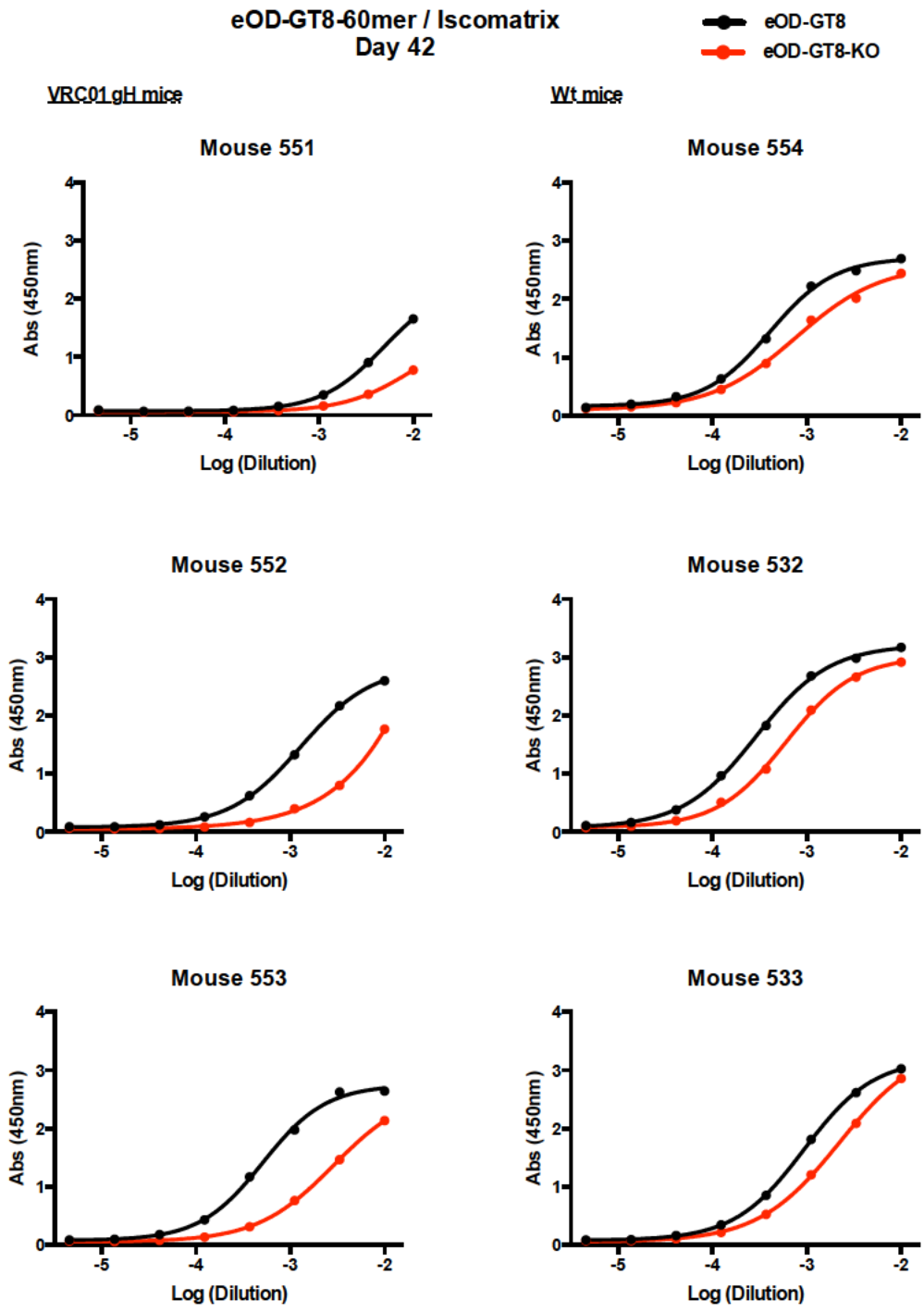



**Fig. S11. (continued)**

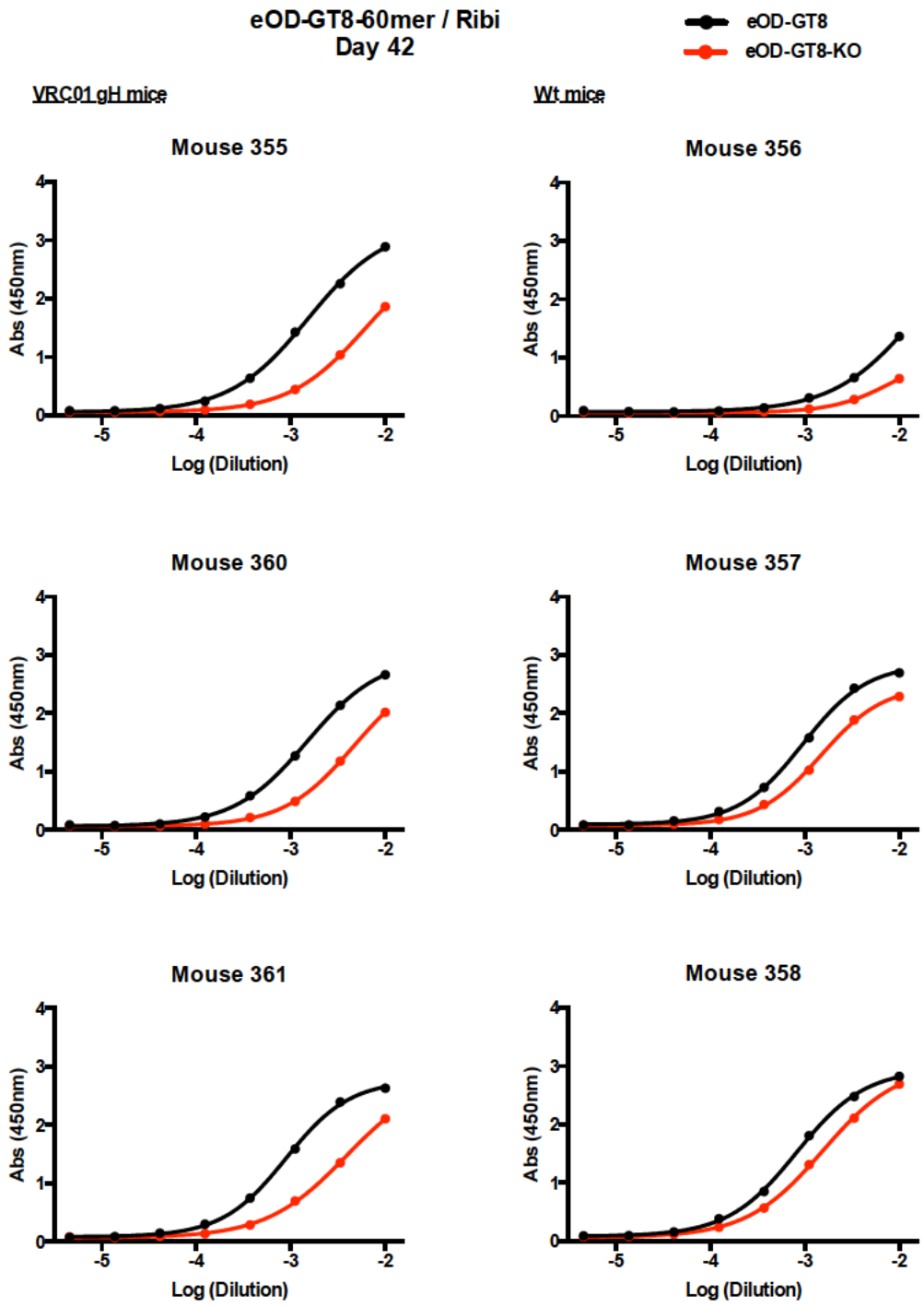



**Fig. S11. (continued)**

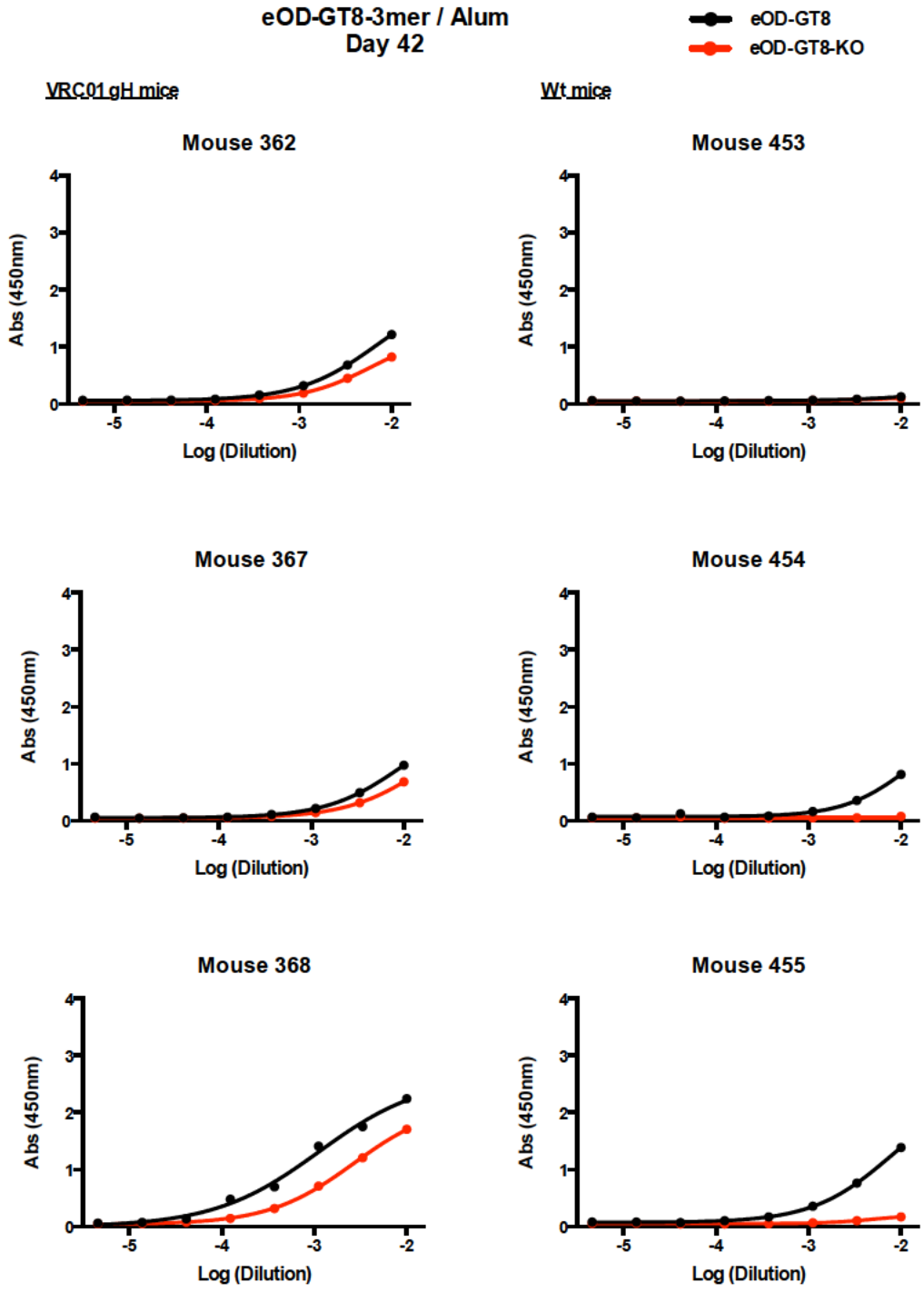



**Fig. S11. (continued)**

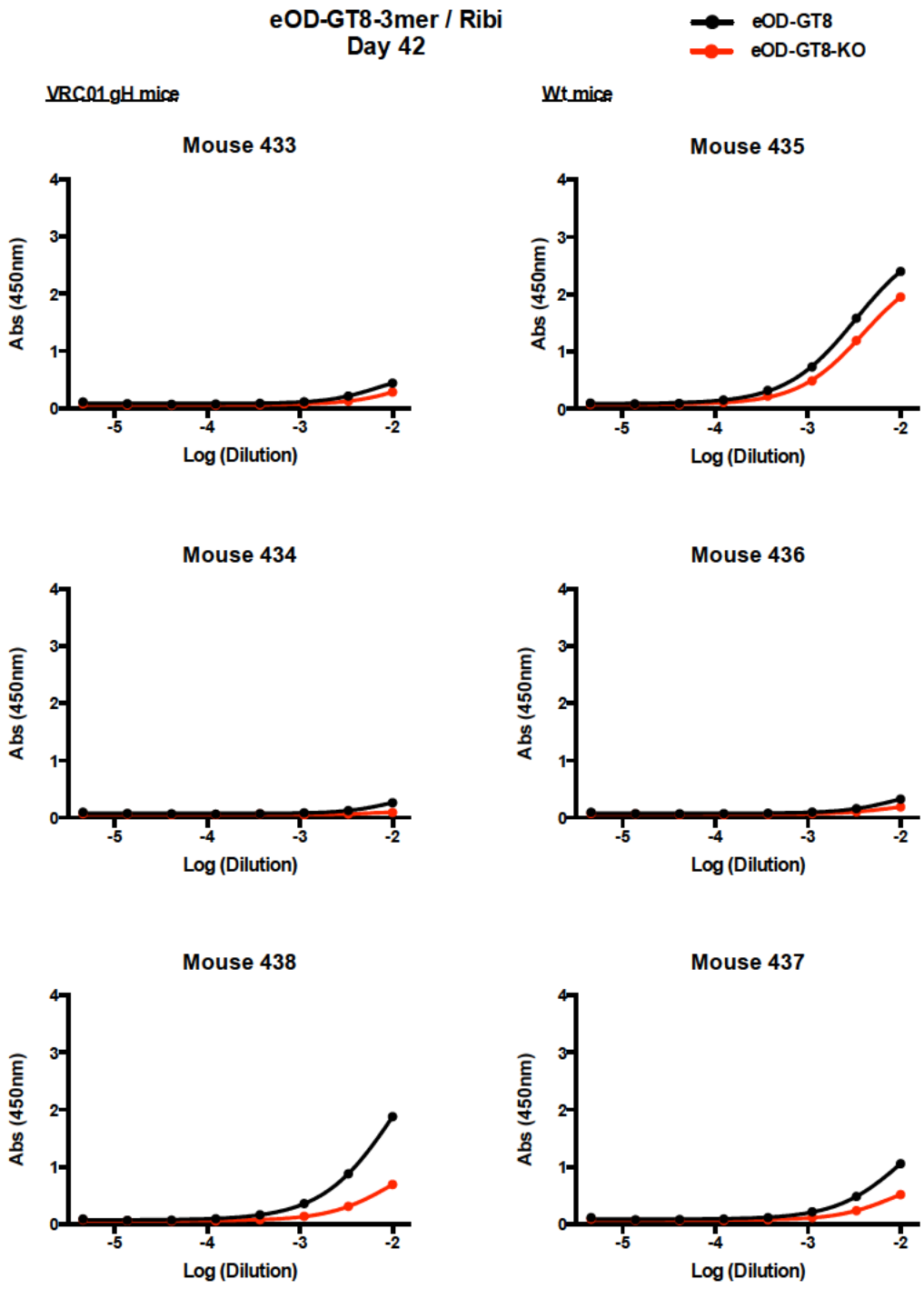



**Fig. S11. (continued)**

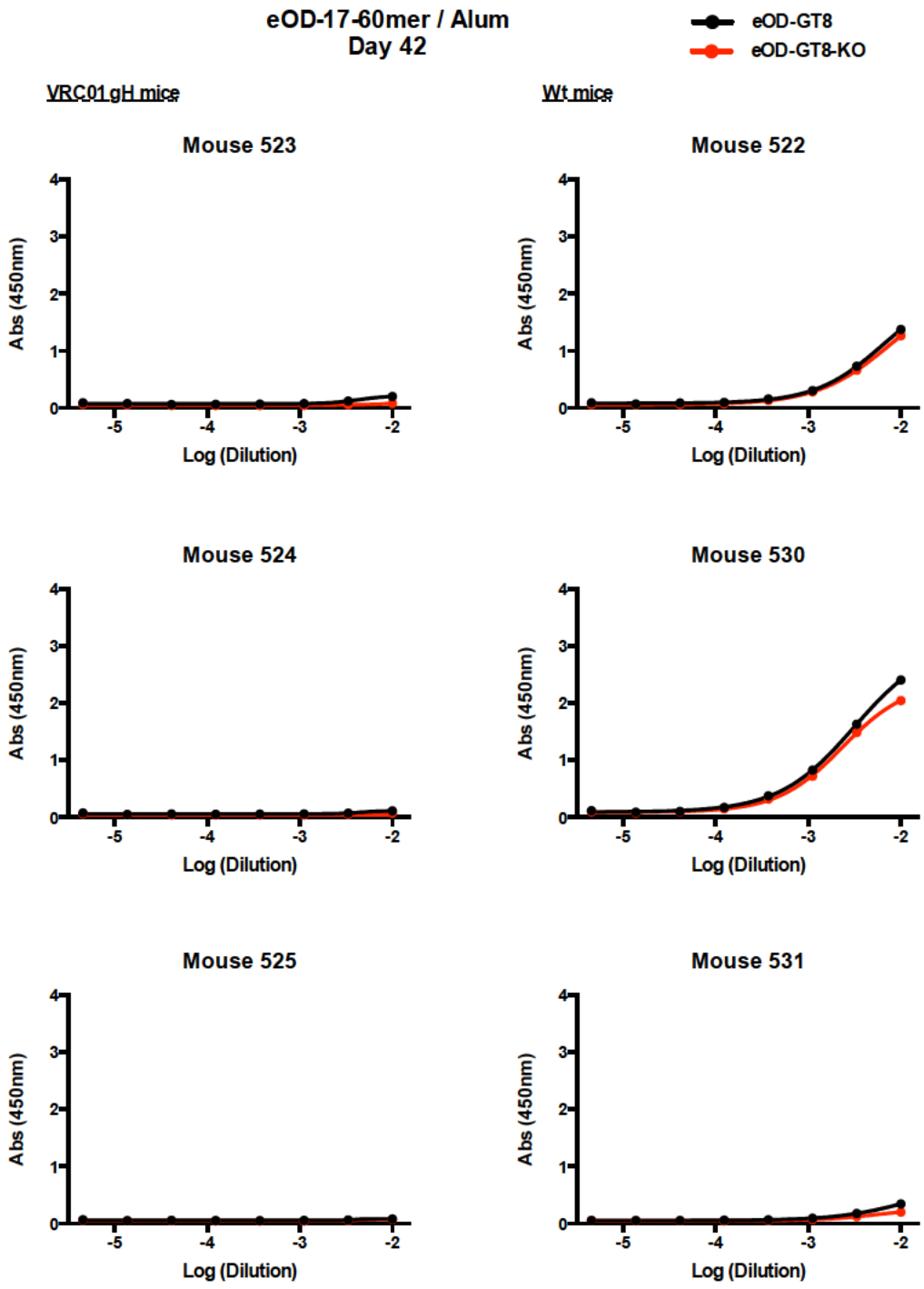



**Fig. S11. (continued)**

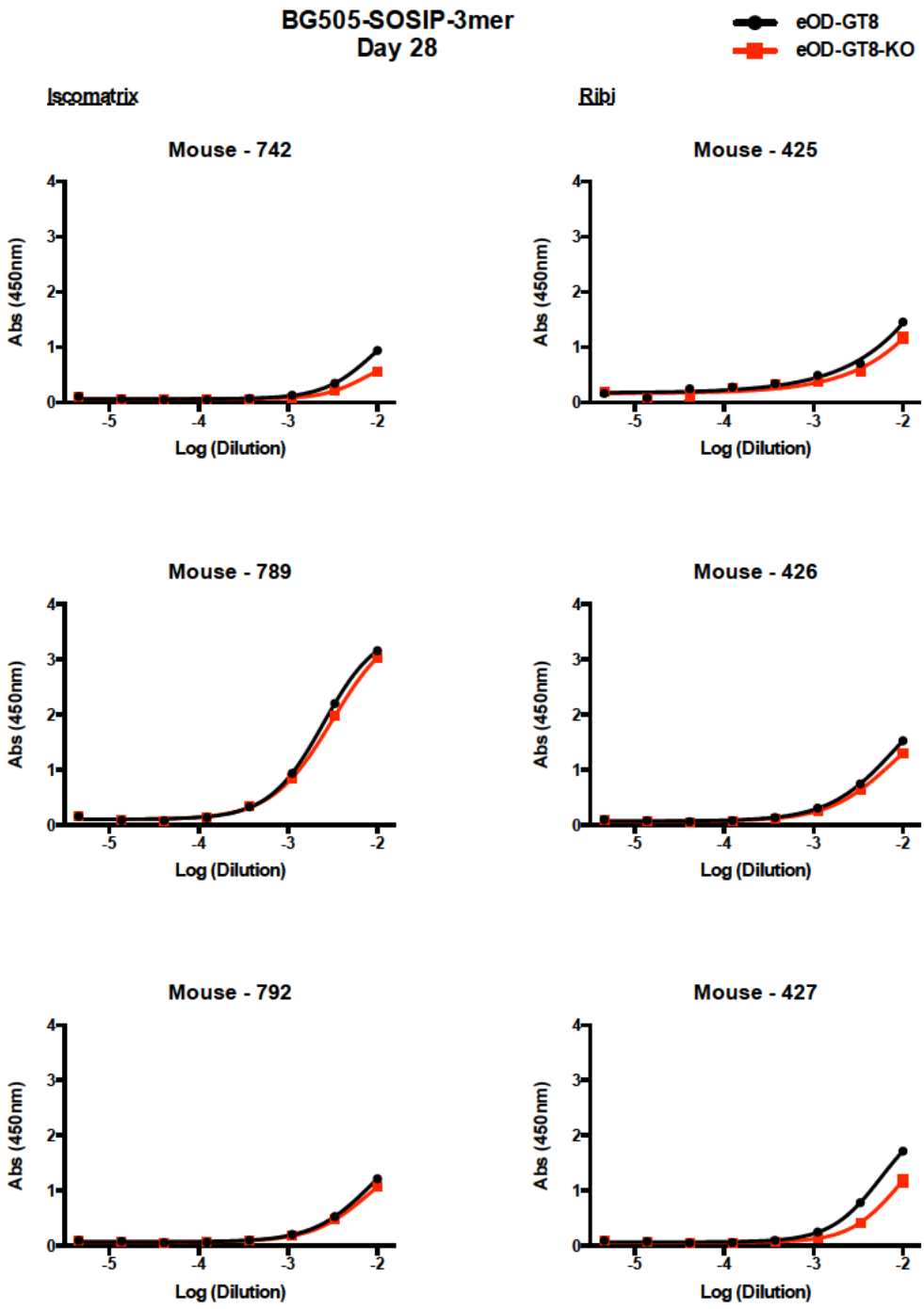



**Fig. S11. (continued)**

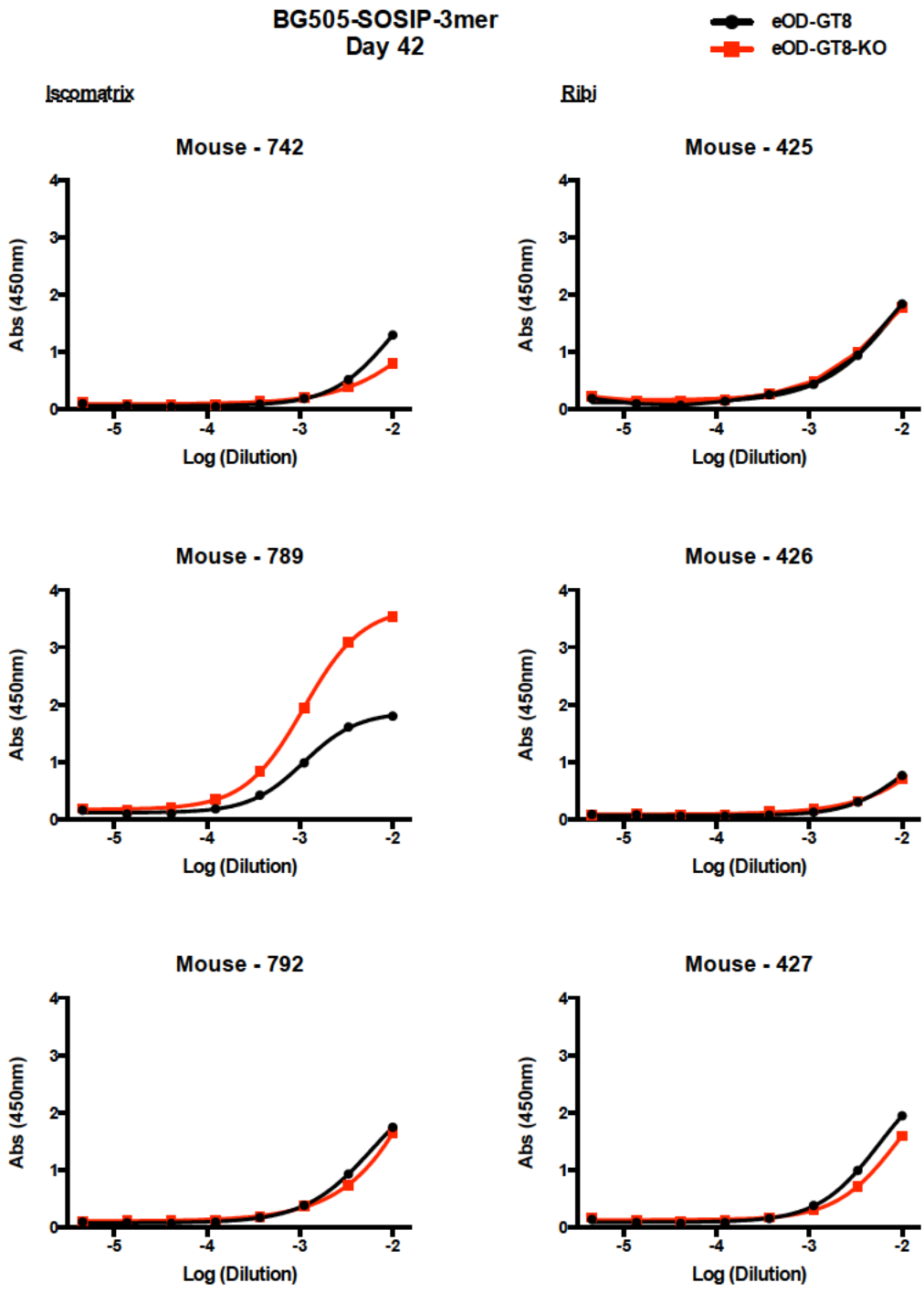



**Fig. S11. (continued)**

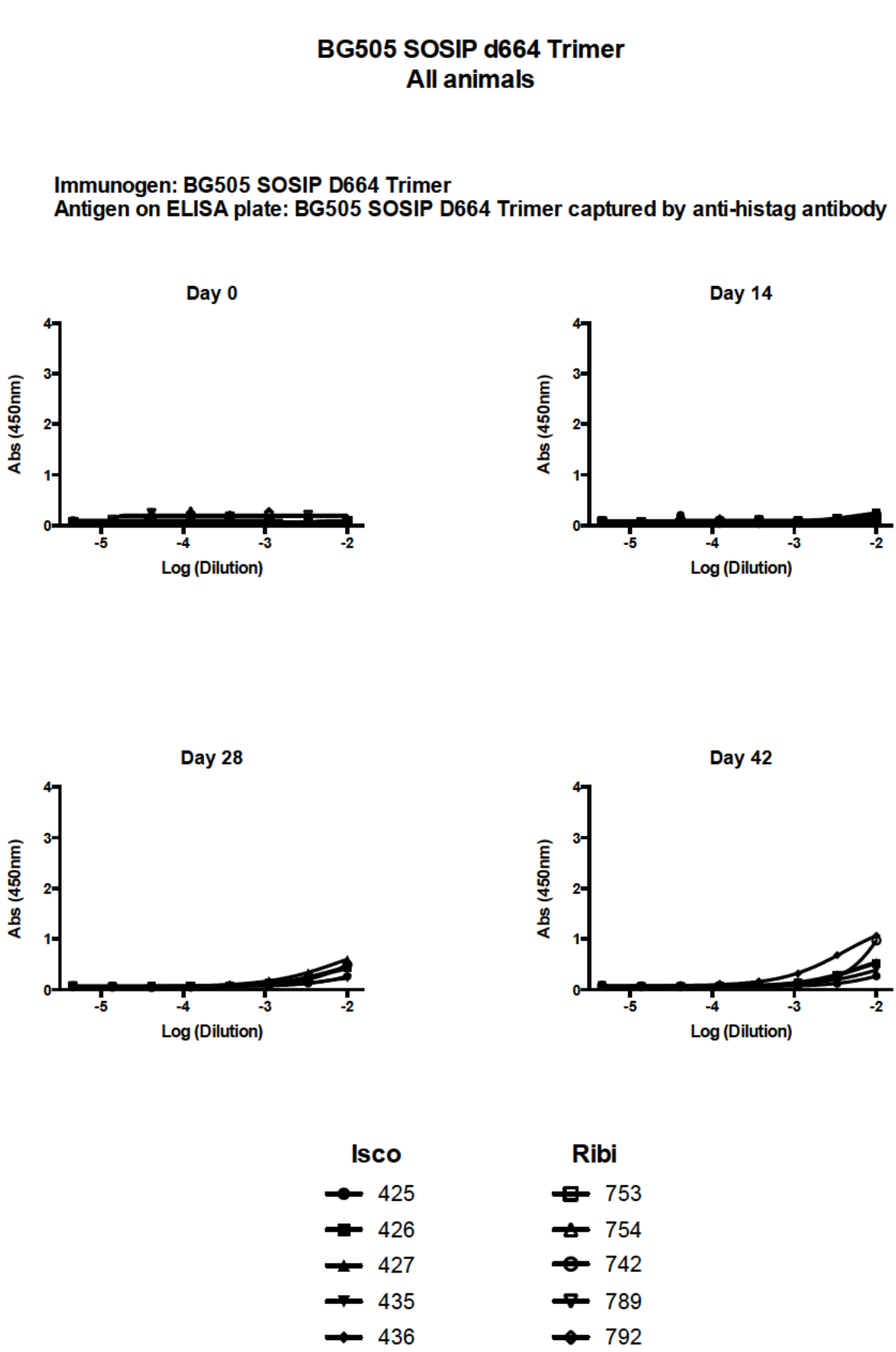



**Fig. S11. (continued)**

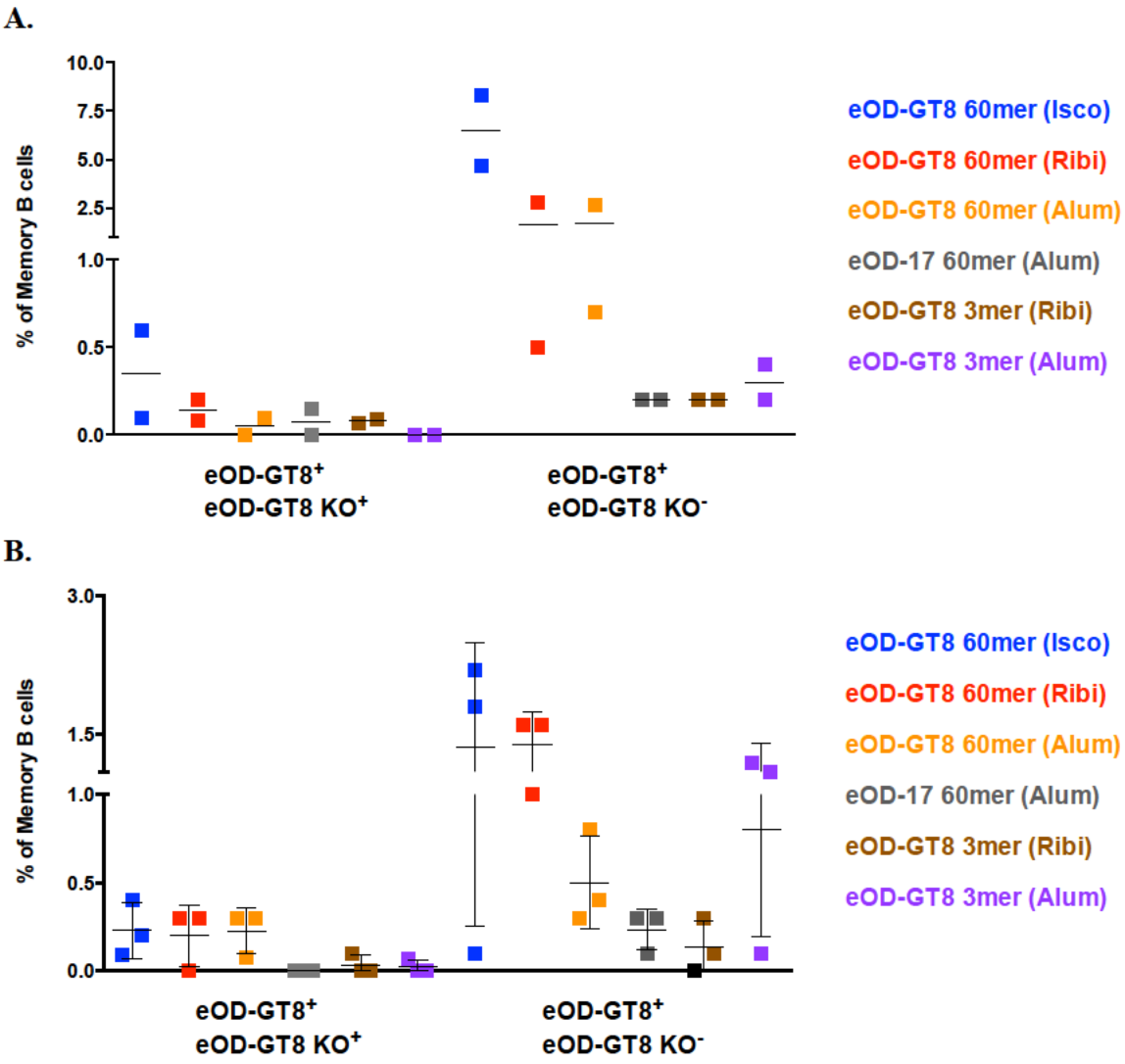



**Fig. S12.**
Frequencies of memory phenotype B cells at day 14 **(A)** and day 42 **(B)** that either bind eOD-GT8 but not eOD-GT8-KO (eOD-GT8$^{(+)}$/eOD-GT8-KO$^{(-)}$) and hence are epitope specific (right), or that bind both eOD-GT8 and eOD-GT8-KO (eOD-GT8$^{(+)}$/eOD-GT8-KO$^{(+)}$) and hence are not epitope specific (left). Data for different immunogen/adjuvant combination are shown. Bars represent mean (N=2) in (A); bars represent mean and standard deviation (N=3) in (B).

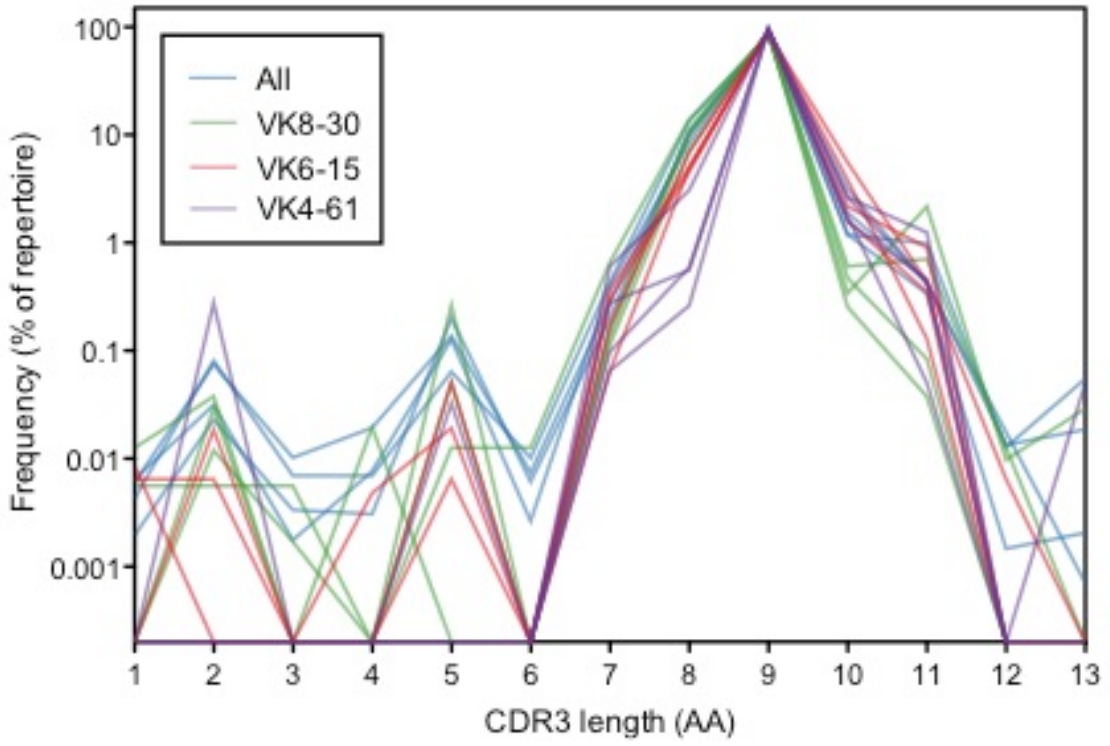



**Fig. S13.**

**CDRL3 length distribution for selected Vκ genes from non-immunized mice.**
CDRL3 length distribution from non-immunized mice, for antibodies derived from the three $V_k$ genes most common in eOD-GT8 60mer-induced Abs isolated by cell sorting. There is no apparent bias in 5 aa CDRL3s within these families.

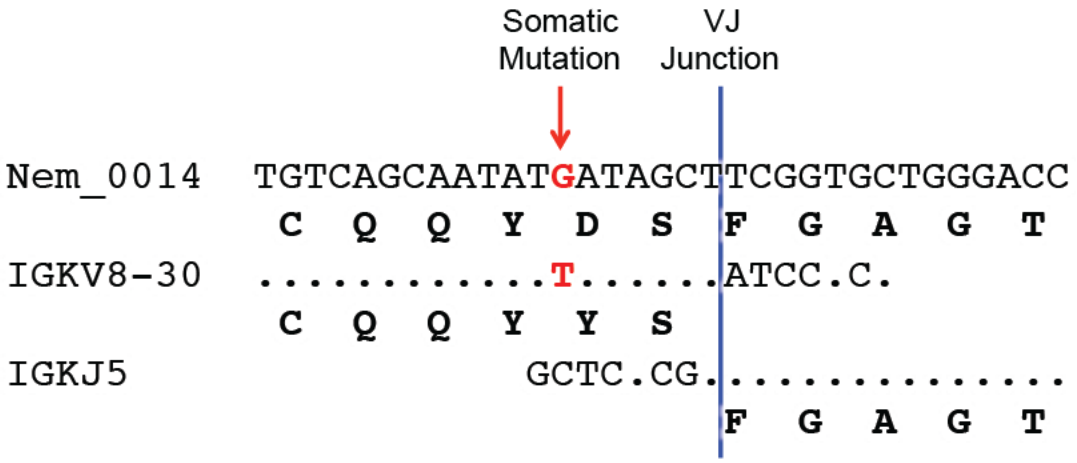



**Fig. S14.**

**Evidence for productive somatic mutation in the CDRL3 of an eOD-GT8 60mer induced Ab.** Example of the T:G mutation found in the CDRL3 codons. The probable VJ junction is marked with a blue line, therefore, the D likely arose through somatic mutation.

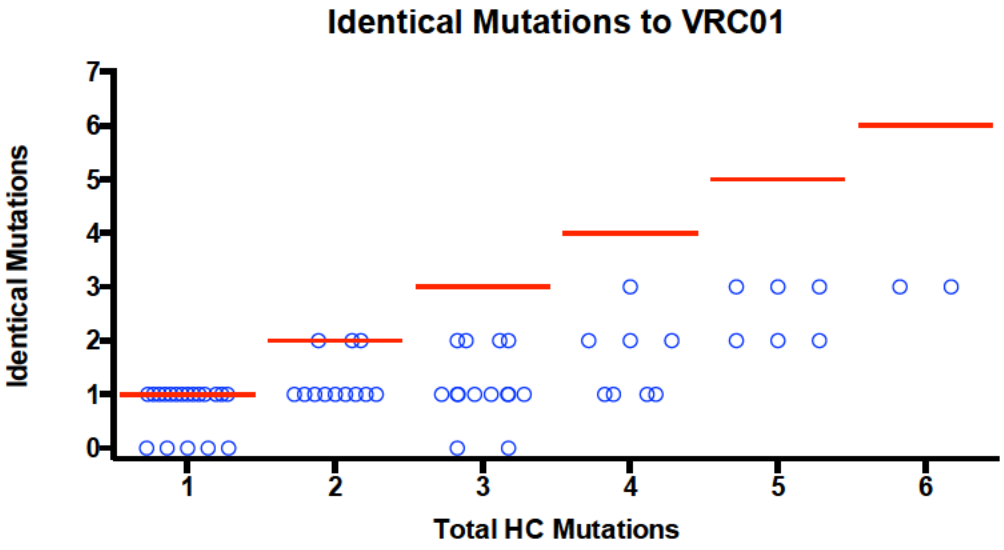
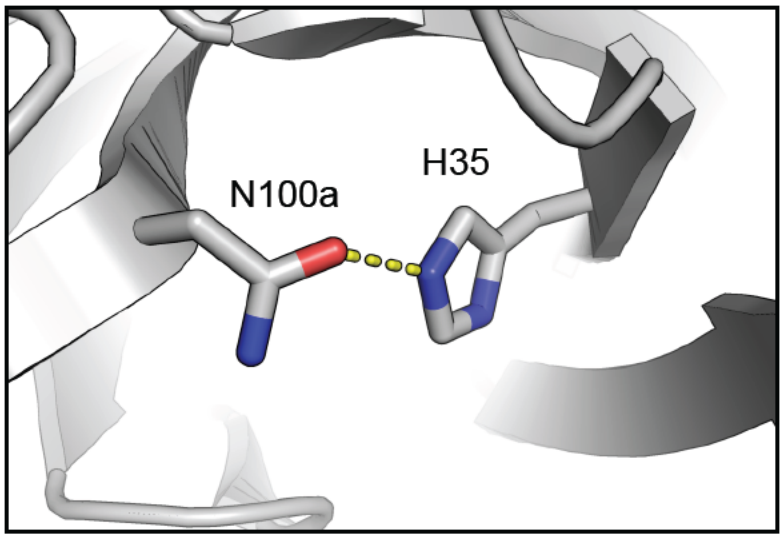
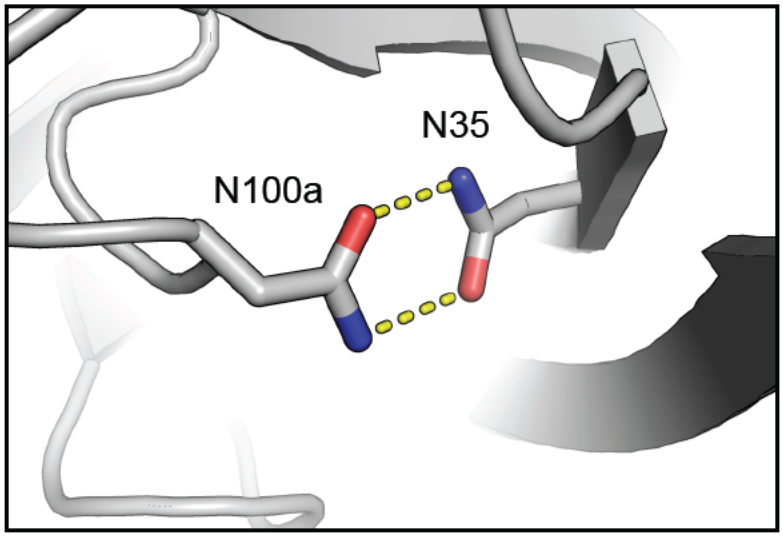



**Fig. S15.**

**Plot of the number of VRC01-identical heavy chain mutations in eOD-GT8 60mer-induced Abs versus the total number of mutations in those Abs.** A total of 61 mutated heavy chain sequences were evaluated for the number of amino acids that match the mutations found in only VRC01 compared to total heavy chain amino acid mutations from germline. Each circle represents a single heavy chain sequence that was isolated by antigen-specific sorting of memory phenotype B cells.




GL-VRC01 HC (PDBID:4jpk)




VRC01 HC (PDBID:3ngb)

**Fig. S16.**

**Visualization of the structural role of a His residue in germline VRC01, or an Asn residue in mature VRC01, at position 35 in the heavy chain.** The H35N mutation commonly found in mutated Abs from the crystal structure of the cGL VRC01 and VRC01.

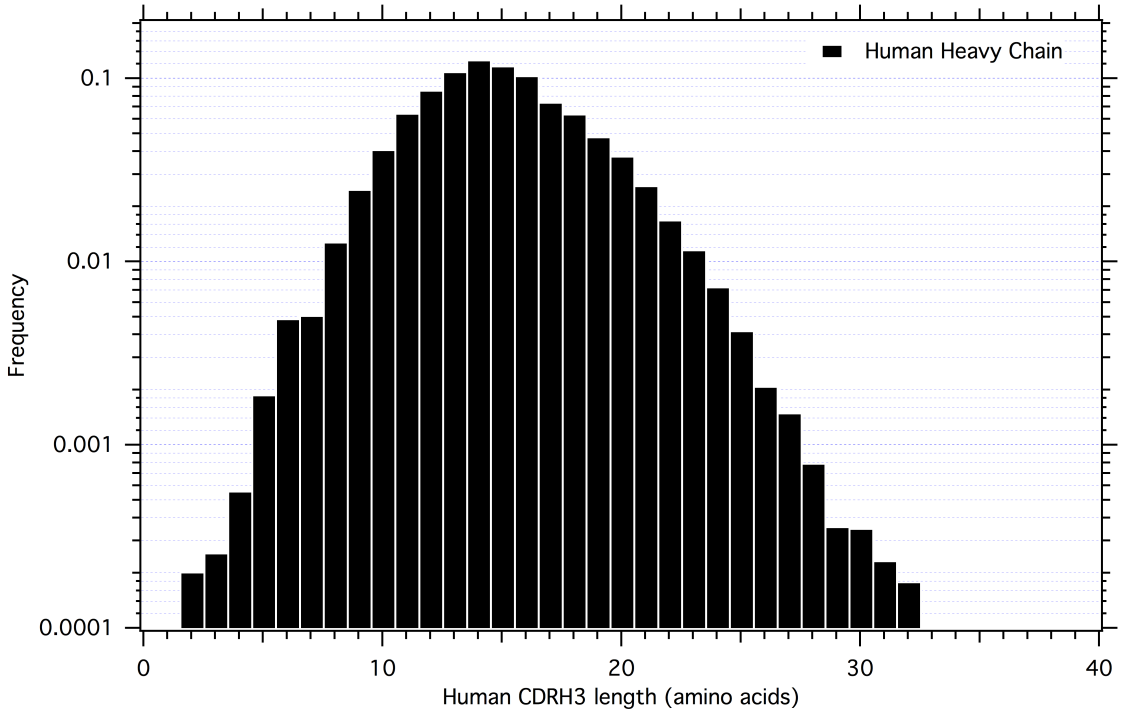



**Fig. S17.**

Human heavy chain CDRH3 length distribution, based on 127,701 antibody sequences from three donors determined by DeKosky *et al.* (*19*).

| Neutralization $IC_{50}$ (µg/mL) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Nem 0072 | Nem 0098 | Nem 0103 | Nem 0110 | Nem 0164 | Nem 0109 | Nem 0071 | Nem 0080 | VRC01 |
| BG505 | > 50 | > 50 | > 50 | > 50 | > 50 | > 50 | > 50 | > 50 | 0.039 |
| HXB2 | > 50 | > 50 | > 50 | > 50 | > 50 | > 50 | > 50 | > 50 | 0.100 |
| BG505 N276A | > 50 | > 50 | > 50 | > 50 | > 50 | > 50 | > 50 | > 50 | 0.007 |
| HXB2 N276A | > 50 | > 50 | > 50 | > 50 | > 50 | > 50 | > 50 | > 50 | 0.004 |

**Fig. S18.**

**Neutralization measurements of select eOD-GT8 60mer-induced antibodies.** Antibodies demonstrating modest affinity to core-e-2CC HxB2 N276D (Nem_0072, Nem_0098, Nem_0103, Nem_0110, Nem_0164) and antibodies with lower affinity to the same protein (Nem_0109, Nem_0071, Nem_080) were tested for neutralization activity against isolates BG505 and HXB2 as well as the corresponding viruses with the glycan site at N276 removed by alanine mutagenesis (N276A).

**Table S1.**

Summary of IgG B cells

| Mouse | IgG cells isolated | IgG cells isolated with 5aa CDRL3 | Different Vks with 5 aa CDRL3 | Adjuvant | Day post priming | Mutations per cell | | |
|---|---|---|---|---|---|---|---|---|
| ***Single cell cloning*** | | | | | | **H** | **L** | **Average** |
| 363 | 1 | 1 | 1 | Alum | 14 | 0 | 2 | 2 |
| 457 | 13 | 13 | 3 | | | 0 | 0 | 0 |
| 339 | 10 | 10 | 3 | | 42 | 20 | 10 | 3 |
| 340 | 3 | 3 | 2 | | | 7 | 15 | 5 |
| 341 | 1 | 1 | 1 | | | 2 | 1 | 3 |
| Bulk sorted | 6 | 4 | 2 | | | 0 | 2 | 0.5 |
| 521 | 19 | 17 | 3 | Isco | 14 | 9 | 6 | 0.88 |
| 526 | 13 | 13 | 5 | | | 3 | 2 | 0.385 |
| 552 | 5 | 5 | 1 | | 42 | 0 | 0 | 0 |
| 553 | 2 | 1 | 1 | | | 3 | 3 | 6 |
| Bulk sorted | 21 | 11 | 3 | | | 7 | 9 | 1.45 |
| 331 | 2 | 2 | 2 | Ribi | 14 | 1 | 1 | 1 |
| 353 | 10 | 10 | 6 | | | 2 | 2 | 0.4 |
| 355 | 23 | 21 | 3 | | 42 | 72 | 88 | 7.62 |
| 360 | 8 | 6 | 2 | | | 6 | 8 | 2.33 |
| 361 | 21 | 19 | 3 | | | 59 | 62 | 6.37 |
| Bulk sorted | 19 | 17 | 4 | | | 33 | 36 | 4.06 |
| ***Hybridomas*** | | | | | | | | |
| 483 | 1 | 1 | 1 | Alum | 5 | 0 | 0 | 0 |
| 512 | 1 | 0 | 0 | Alum | 10 | 1 | 0 | 1 |
| 443 | 1 | 1 | 1 | Ribi | 10 | 0 | 2 | 2 |
| 513 | 1 | 1 | 1 | Ribi | 10 | 2 | 7 | 9 |
| 401 | 1 | 1 | 1 | Ribi | 31 | 5 | 2 | 7 |
| 406 | 1 | 1 | 1 | Ribi | 31 | 3 | 2 | 5 |
| 407 | 1 | 1 | 1 | Ribi | 31 | 4 | 10 | 14 |
| **Total** | | | | | | | | |
| **21*** | **184** | **160** | **51** | | | **239** | **270** | |

* Mice marked "bulk" were pools of three d42 lymph nodes; other samples were from spleens of individual mice.

**Table S2.**

Antigen-sorted IgG Sequences

| Name | VDJ sequence | Vk | Jk | CDRL3 |
|---|---|---|---|---|
| VRC01 | --------GQM----E-MRI--R----E-IDCTLN-I-L---KRP-----LK-RG-AV---RPL---------VYSD--FL--RS-TV------F-T----C------E---R--P-I--- | Human | Human | QQYEF |
| **VRC01 gH** | **QVQLVQSGAEVKKPGASVKVSCKASGYTFTGYYMHWVRQAPGQGLEWMGWINPNSGGTNYAQKFQGRVTMTRDTSISTAYMELSRLRSDDTAVYYCARGKNSDYNWDFQHWGQGTLVTVSS** | Human | Human | **QQYEF** |
| **Mouse 363 Day 14 Alum** | ---------- | 8-30 | 1 | QQYYS |
| **Mouse 457 Day 14 Alum** | ---------- | 4-50 | 1 | QQFTT |
| | ---------- | 6-15 | 4 | QQYNS |
| | ---------- | 8-30 | 1 | QQYWT |
| | ---------- | 8-30 | 1 | QQYWT |
| | ---------- | 8-30 | 1 | QQYWT |
| | ---------- | 8-30 | 1 | QQYWT |
| | ---------- | 8-30 | 1 | QQYWT |
| | ---------- | 8-30 | 1 | QQYWT |
| | ---------- | 8-30 | 1 | QQYWT |
| | ---------- | 8-30 | 2 | QQYYT |
| | ---------- | 8-30 | 4 | QQYYT |
| | ---------- | 8-30 | 4 | QQYFT |
| | ---------- | 8-30 | 5 | QQYAT |
| **Mouse 339 Day 42 Alum** | ----------D---N----------L----------V---------- | 4-61 | 5 | QQYHT |
| | ----------N----------M---------- | 6-15 | 2 | QQYNT |
| | ---------- | 6-15 | 2 | QQYNT |
| | ----------N-------N---------- | 6-15 | 5 | QQYNS |
| | ---------- | 6-15 | 5 | QQYNS |
| | ---------- | 8-30 | 1 | QQYWT |
| | ----------N----------N----------F---------- | 8-30 | 2 | QQYDT |
| | ----------N--N----------N-L---------- | 8-30 | 4 | QQYDS |
| | ---------- | 8-30 | 4 | QQYST |
| | ---------- | 8-30 | 5 | QQYFT |

## Table S2. (continued)

| Name | VDJ sequence | Vk | Jk | CDRL3 |
|---|---|---|---|---|
| VRC01 | --------GQM----E-MRI--R----E-IDCTLN-I-L---KRP-----LK-RG-AV---RPL---------VYSD--FL--RS-TV------F-T----C------E---R--P-I--- | Human | Human | QQYEF |
| **VRC01 gH** | **QVQLVQSGAEVKKPGASVKVSCKASGYTFTGYYMHWVRQAPGQGLEWMGWINPNSGGTNYAQKFQGRVTMTRDTSISTAYMELSRLRSDDTAVYYCARGKNSDYNWDFQHWGQGTLVTVSS** | Human | Human | **QQYEF** |
| **Mouse 340 Day 42 Alum** | ----------N----------T---------- | 6-15 | 1 | QQYDS |
| | ----------LN----------D----------D----------Y---------- | 8-30 | 4 | QQYHS |
| | ----------N----------I---------- | 8-30 | 4 | QQYHS |
| **Mouse 341 Day 42 Alum** | ----------N----------T---------- | 6-15 | 5 | QQYET |
| **Bulk Alum Day 42** | ---------- | 6-15 | 5 | QQYNS |
| | ---------- | 8-30 | 2 | QQYYT |
| | ---------- | 8-30 | 2 | QQYYT |
| | ---------- | 8-30 | 2 | QQYYT |
| **Mouse 521 Day 14 Isco** | ---------- | 4-59 | 5 | QQYWT |
| | ---------- | 4-61 | 1 | QQYHS |
| | ---------- | 8-30 | 4 | QQYYT |
| | ----------V---------- | 8-30 | 2 | QQYYT |
| | ---------- | 8-30 | 4 | QQYYS |
| | ---------- | 8-30 | 2 | QQYYS |
| | ---------- | 8-30 | 1 | QQYWT |
| | ---------- | 8-30 | 1 | QQYWT |
| | ---------- | 8-30 | 1 | QQYWT |
| | ---------- | 8-30 | 2 | QQYYT |
| | ----------N---------- | 6-15 | 4 | QQYNT |
| | ---------- | 8-30 | 5 | QQYYS |
| | ---------- | 8-30 | 5 | QQYLT |
| | ----------V---------- | 8-30 | 4 | QQYFT |
| | ---------- | 4-61 | 2 | QQYHS |
| | ---------- | 8-30 | 1 | QQYWT |
| | ---------- | 8-30 | 1 | QQYYT |
| | ----------N---------- | 8-30 | 1 | QQYYS |
| | ---------- | 9-120 | 5 | QQYNS |

## Table S2. (continued)

| Name | VDJ sequence | Vk | Jk | CDRL3 |
|---|---|---|---|---|
| VRC01 | --------GQM----E-MRI--R----E-IDCTLN-I-L---KRP-----LK-RG-AV---RPL---------VYSD--FL--RS-TV------F-T----C------E---R--P-I--- | Human | Human | QQYEF |
| **VRC01 gH** | **QVQLVQSGAEVKKPGASVKVSCKASGYTFTGYYMHWVRQAPGQGLEWMGWINPNSGGTNYAQKFQGRVTMTRDTSISTAYMELSRLRSDDTAVYYCARGKNSDYNWDFQHWGQGTLVTVSS** | Human | Human | **QQYEF** |
| **Mouse 526 Day 14 Isco** | ---------- | 12-46 | 5 | QQYDS |
| | ---------- | 4-61 | 1 | QQYYS |
| | ----------N---------- | 6-15 | 1 | QQYYS |
| | ---------- | 6-15 | 2 | QQYYT |
| | ---------- | 6-23 | 5 | QQYYS |
| | ---------- | 8-30 | 2 | QQYWT |
| | ---------- | 8-30 | 1 | QQYNT |
| | ---------- | 8-30 | 2 | QQYYT |
| | ---------- | 8-30 | 2 | CQHFW |
| | ---------- | 8-30 | 4 | QQYYT |
| | ---------- | 8-30 | 4 | QQYNS |
| | ---------- | 8-30 | 4 | QQYNT |
| | ----------N---------- | 8-30 | 4 | QQYHT |
| **Mouse 552 Day 42 Isco** | ---------- | 8-30 | 1 | QQYWT |
| | ---------- | 8-30 | 1 | QQYWT |
| | ---------- | 8-30 | 5 | QQYYT |
| | ---------- | 8-30 | 5 | QQYYT |
| | ---------- | 8-30 | 5 | QQYYT |
| **Mouse 553 Day 42 Isco** | ---------- | 4-55 | 5 | QQWSS |
| | ----------N-------N----------G---------- | 8-30 | 1 | QQYET |

## Table S2. (continued)

| Name | VDJ sequence | Vk | Jk | CDRL3 |
|---|---|---|---|---|
| VRC01 | --------GQM----E-MRI--R----E-IDCTLN-I-L---KRP-----LK-RG-AV---RPL---------VYSD--FL--RS-TV------F-T----C------E---R--P-I--- | Human | Human | QQYEF |
| **VRC01 gH** | **QVQLVQSGAEVKKPGASVKVSCKASGYTFTGYYMHWVRQAPGQGLEWMGWINPNSGGTNYAQKFQGRVTMTRDTSISTAYMELSRLRSDDTAVYYCARGKNSDYNWDFQHWGQGTLVTVSS** | Human | Human | **QQYEF** |
| **Bulk Isco Day 42** | ----------H----V----------S----------D---------- | 3-4 | 4 | QQSSE |
| | ----------N----------V---------- | 3-4 | 2 | QQSNE |
| | ---------- | 4-61 | 1 | QQYHS |
| | ---------- | 8-24 | 1 | QQHYSTPPT |
| | ---------- | 8-24 | 1 | QQHYSTPPT |
| | ---------- | 8-24 | 5 | QQHYSTPPT |
| | ---------- | 8-30 | 1 | QQYWT |
| | ---------- | 8-30 | 1 | QQYWT |
| | ---------- | 8-30 | 1 | QQYCS |
| | ----------L---------- | 8-30 | 1 | QQYST |
| | ---------- | 8-30 | 2 | QQYYT |
| | ---------- | 8-30 | 2 | QQYYT |
| | ----------N----------L----------R---D----------S----------I---------- | 8-30 | 2 | QQYDS |
| | ---------- | 8-30 | 5 | QQYYS |
| | ---------- | 8-30 | 5 | QQYYT |
| | ---------- | 12-46 | 1 | QHFWT |
| **Mouse 331 Day 14 Ribi** | ---------- | 6-15 | 2 | QQYNT |
| | ----------P---------- | 8-30 | 4 | QQYFT |
| | | | | |
| **Mouse 353 Day 14 Ribi** | ---------- | 10-94 | 4 | QQYST |
| | ---------- | 4-61 | 1 | QQYHT |
| | ----------N---------- | 6-15 | 2 | QQYNN |
| | ---------- | 6-23 | 2 | QQYYS |
| | ---------- | 8-28 | 5 | QNDLT |
| | ---------- | 8-30 | 1 | QQYWT |
| | ---------- | 8-30 | 1 | QQYWT |
| | ---------- | 8-30 | 1 | QQYWT |
| | ---------- | 8-30 | 4 | QQYFT |
| | ---------- | 8-30 | 5 | QQYYS |

## Table S2. (continued)

| Name | VDJ sequence | Vk | Jk | CDRL3 |
|---|---|---|---|---|
| VRC01 | --------GQM----E-MRI--R----E-IDCTLN-I-L---KRP-----LK-RG-AV---RPL---------VYSD--FL--RS-TV------F-T----C------E---R--P-I--- | Human | Human | QQYEF |
| **VRC01 gH** | **QVQLVQSGAEVKKPGASVKVSCKASGYTFTGYYMHWVRQAPGQGLEWMGWINPNSGGTNYAQKFQGRVTMTRDTSISTAYMELSRLRSDDTAVYYCARGKNSDYNWDFQHWGQGTLVTVSS** | Human | Human | **QQYEF** |
| **Mouse 360** | ---------- | 3-4 | 2 | QQSNEDT |
| **Day 42** | ---------- | 6-15 | 2 | QQYNS |
| **Ribi** | ----------N----------L---------- | 8-30 | 1 | QQYST |
| | ---------- | 8-30 | 2 | QQYYT |
| | ---------- | 8-30 | 2 | QQYYT |
| | ----------N---------- | 8-30 | 4 | QQYYT |
| | ---------- | 8-30 | 5 | QQYYT |
| **Mouse 361** | ----------N----N----------L--------H---------- | 19-93 | 5 | LQYDNLLT |
| **Day 42** | ----------N----------V----------D-NS---------- | 4-73 | 5 | QQWSSKPLT |
| **Ribi** | ----------R----------D---N----------Q----------R--N---------- | 6-15 | 1 | QQYDT |
| | ----------D---N----------D---------- | 6-15 | 2 | QQYNT |
| | ----------L----------A----------M----------A---------- | 8-27 | 2 | HQYHT |
| | ----------N----N----------L--------H---------- | 8-30 | 1 | QQYDT |
| | ----------M----------VN----------V----------T---------- | 8-30 | 2 | QQYDN |
| | ----------N----------V---------- | 8-30 | 2 | QQYDS |
| | ----------N--N---------- | 8-30 | 2 | QQYDS |
| | ----------N---------- | 8-30 | 2 | QQYDT |
| | ----------N----------L---------- | 8-30 | 4 | QQYDS |
| | ----------D---N---------- | 8-30 | 4 | QQYDS |
| | ----------N----------M---------- | 8-30 | 4 | QEYDS |
| | ---------- | 8-30 | 5 | QQSYS |
| | ----------N---------- | 8-30 | 5 | QQYHT |
| | ----------N---------- | 8-30 | 5 | QQYNT |
| | ----------N----------SV----------T---------- | 8-30 | 5 | QQYDE |
| | ----------E----------LN----------T---------- | 8-30 | 5 | QQYDS |
| | ---------- | 8-30 | 5 | RQYYT |
| | ---------- | 8-30 | 5 | QQYST |
| | ----------N----------T----------H---------- | 8-30 | 5 | QQYSS |

**Table S2. (continued)**

| Name | VDJ sequence | Vk | Jk | CDRL3 |
|---|---|---|---|---|
| VRC01 | --------GQM----E-MRI--R----E-IDCTLN-I-L---KRP-----LK-RG-AV---RPL---------VYSD--FL--RS-TV------F-T----C------E---R--P-I--- | Human | Human | QQYEF |
| **VRC01 gH** | **QVQLVQSGAEVKKPGASVKVSCKASGYTFTGYYMHWVRQAPGQGLEWMGWINPNSGGTNYAQKFQGRVTMTRDTSISTAYMELSRLRSDDTAVYYCARGKNSDYNWDFQHWGQGTLVTVSS** | Human | Human | **QQYEF** |
| **Mouse 355** | ---------- | 4-55 | 5 | QQWSSYPT |
| **Day 42** | ----------N----------F----------I----------D---------- | 4-61 | 1 | QQYET |
| **Ribi** | ----------LN----------S----------D----------T---------- | 4-61 | 1 | QQYET |
| | ----------N---------- | 6-15 | 5 | QQYNN |
| | ---------- | 8-30 | 1 | QQYYR |
| | ----------V----------G--------F----------T---------- | 8-30 | 1 | QQYEA |
| | ----------H-------N----------D---------- | 8-30 | 1 | QQYWT |
| | ----------N----------D----------F---------- | 8-30 | 2 | QQYSS |
| | ----------N----------V---------- | 8-30 | 2 | QQYST |
| | ----------D---N----------V------D----------T---------- | 8-30 | 2 | QQYYS |
| | ----------N---------- | 8-30 | 4 | QQYFT |
| | ----------N---------- | 8-30 | 4 | QQYFT |
| | ---------- | 8-30 | 4 | QQYYS |
| | ----------N----------D----------W---------- | 8-30 | 4 | QQYFT |
| | ----------I---------- | 8-30 | 5 | QQYFS |
| | ----------N----------N----------R---------- | 8-30 | 5 | QQYDT |
| | ----------N----V----------D----------N--T---------- | 8-30 | 5 | QQYHS |
| | ----------N----------L----------N----W---------- | 8-30 | 5 | QQYWT |
| | ---------- | 8-30 | 5 | QQYLT |
| | ----------N----------D----------N--T---------- | 8-30 | 5 | QQYHS |
| | ----------N----------L----------F--------S-----S---------T-------R---------- | 8-30 | 5 | QQYDS |
| | ----------D----------A---------- | 8-30 | 5 | QQYSS |
| | ---------- | 9-120 | 1 | LQYASSPWT |

## Table S2. (continued)

| Name | VDJ sequence | Vk | Jk | CDRL3 |
|---|---|---|---|---|
| VRC01 | --------GQM----E-MRI--R----E-IDCTLN-I-L---KRP-----LK-RG-AV---RPL---------VYSD--FL--RS-TV------F-T----C------E---R--P-I--- | Human | Human | QQYEF |
| **VRC01 gH** | **QVQLVQSGAEVKKPGASVKVSCKASGYTFTGYYMHWVRQAPGQGLEWMGWINPNSGGTNYAQKFQGRVTMTRDTSISTAYMELSRLRSDDTAVYYCARGKNSDYNWDFQHWGQGTLVTVSS** | Human | Human | **QQYEF** |
| **Bulk Ribi Day 42 Ribi** | ----------N----G----------Q----------T----------D-------H---------- | 4-61 | 5 | QQYDM |
| | ----------R---E----------IN----------L----------Q---------- | 6-15 | 2 | QQYDT |
| | ---------- | 6-15 | 2 | QQYNT |
| | ---------- | 6-15 | 5 | QQYNT |
| | ---------- | 8-30 | 1 | QQYYS |
| | ---------- | 8-30 | 1 | QQLYT |
| | ----------F----------D----------F---------- | 8-30 | 1 | QQYWT |
| | ---------- | 8-30 | 2 | QQYWT |
| | ---------- | 8-30 | 2 | QQYWT |
| | --------T----------A--------N----------R---------- | 8-30 | 2 | QQYDS |
| | ---------- | 8-30 | 2 | QQYYT |
| | ----------N---------- | 8-30 | 5 | EQYDS |
| | ---------- | 8-30 | 5 | QQYST |
| | ---------- | 8-30 | 5 | QQYYK |
| | ---------- | 8-30 | 5 | QQYYS |
| | ----------LN----------R----------R---------- | 8-30 | 5 | QQYST |

**Table S3.**

Hybridoma IgG Sequences

| Name | VDJ sequence | Vk | Jk | CDRL3 |
|---|---|---|---|---|
| VRC01 | --------GQM----E-MRI--R----E-IDCTLN-I-L---KRP-----LK-RG-AV---RPL---------VYSD--FL--RS-TV------F-T----C------E---R--P-I--- | Human | Human | QQYEF |
| **VRC01 gH** | **QVQLVQSGAEVKKPGASVKVSCKASGYTFTGYYMHWVRQAPGQGLEWMGWINPNSGGTNYAQKFQGRVTMTRDTSISTAYMELSRLRSDDTAVYYCARGKNSDYNWDFQHWGQGTLVTVSS** | Human | Human | **QQYEF** |
| **IgG hybridomas** | ---------- | 8-30 | 1 | QQYWT |
| | ---------- | 6-32 | 2 | QQDYSSPYT |
| | ---------- | 8-30 | 1 | QQYDC |
| | ---V----------F---------- | 6-15 | 2 | QQYNT |
| | ----------D---N----------N----------T---------- | 8-30 | 2 | QQYDT |
| | ----------A----A-----N---------- | 8-30 | 5 | QQYDS |
| | ----------P----------N---------- | 6-15 | 4 | QQYDS |

**Table S4.**

Properties of VRC01 gH hybridomas raised by eOD-GT8 60mer.

| Mouse # | Adjuvant | Day of response | Hybrids tested | ELISA binding | Sequenced | Class | | | | | Cells with 5 aa CDRL3 | | | Mutations H chain | | | Mutations L chain | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | k | l | IgM | IgG | IgA | IgM | IgG | IgA | IgM | IgG | IgA | IgM | IgG | IgA |
| 482 | Alum | 5 | 400 | 33 | 32 | 23 | 10 | 33 | 0 | nd | | | | 0 | | | 2 | | |
| 483 | Alum | 5 | 158 | 12 | 11 | 10 | 1 | 11 | 1 | nd | 1 | 1 | | 0 | 0 | | 0 | 0 | |
| 511 | Alum | 10 | 12 | 3 | 2 | 1 | 1 | 2 | 0 | nd | | | | 0 | | | 0 | | |
| 512 | Alum | 10 | 44 | 9 | 9 | 9 | 0 | 8 | 1 | nd | | | | 0 | 1 | | 15 | 0 | |
| 514 | Alum | 10 | 31 | 3 | 2 | 2 | 0 | 2 | 0 | nd | | | | 0 | | | 0 | | |
| 443 | Ribi | 10 | 96 | 8 | 8 | 6 | 3 | 8 | 1 | nd | | 1 | | 0 | 0 | | 2 | 4 | |
| 513 | Ribi | 10 | 143 | 12 | 12 | 9 | 4 | 12 | 1 | nd | | 1 | | 0 | 2 | | 8 | 9 | |
| 480 | Alum | d46 (2nd boost) | 200 | 35 | 32 | 23 | 12 | 34 | 0 | 1 | | | 1 | 0 | | 1 | 0 | | 0 |
| Hybridomas from fusions only screened for IgG binding: | | | | | | | | | | | | | | | | | | | |
| 396 | Ribi | 31 | 192 | 0 | | | | | | | | | | | | | | | |
| 401 | Ribi | 31 | 165 | 1 | 1 | 1 | 0 | nd | 1 | nd | | 1 | | | 5 | | | 2 | |
| 402 | Ribi | 31 | 243 | 0 | | | | | | | | | | | | | | | |
| 406 | Ribi | 31 | 282 | 4 | 1 | 1 | 0 | nd | 1 | nd | | 1 | | | 3 | | | 2 | |
| 407 | Ribi | 31 | 242 | 1 | 1 | 1 | 0 | nd | 1 | nd | | 1 | | | 4 | | | 10 | |
| | **Totals:** | | | | | | | | | | **1/88** | **6/7** | | 0/88 | 15/7 | 1/1 | 27/88 | 27/7 | 0/1 |
| | | | | | | | | | | Frequency: | 0.01 | 0.86 | | 0 | 2.14 | | 0.31 | 3.86 | 0 |
| VRC01 gH hybridomas obtained in vitro after stimulation with LPS: | | | | | | | | | | | | | | | | | | | |
| 1 | LPS | 3 | ~500 | 2 | 2 | 2 | | 2 | | | | | | 0 | | | 0 | | |

**Table S5.**

Sequences and sequence analysis of VRC01 gH IgG hybridomas raised by eOD-GT8 60mer

| | | | | | L- chain | | | L-chain mutations | | | H-chain | H-chain mutations | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Name** | **day** | **adj** | **isotypes** | **Kd** | **Vk** | **Jk** | **CDRL3** | **total** | **R** | **S** | | **total** | **R** | **S** |
| **483-40** | d5 | alum | IgG3,k | 2.88E-10 | Vk8-30 | Jk1 | CQQYWT | 0 | | | VRC01 gH | 0 | | |
| **512-9** | d10 | alum | IgG2c,k | not detected | Vk6-32 | Jk2 | CQQDYSSPYT | 0 | | | VRC01 gH | 1 | | 1 |
| **443-19** | d10 | Ribi | IgG2b,k | 2.88E-10 | Vk8-30 | Jk1 | CQQYDC | 2 | 2 | 0 | VRC01 gH | 0 | | |
| **513-28** | d10 | Ribi | IgG3,k | 9.60E-07 | Vk6-15 | Jk2 | CQQYNT | 0 | | | VRC01 gH | 2 | 2 | 0 |
| **401-87** | d31 | Ribi | IgG2c,k | 1.30E-10 | Vk8-30 | Jk2 | CQQYDT | 2 | 2 | 0 | VRC01 gH | 5 | 4 | 1 |
| **406-143** | d31 | Ribi | IgG2b,k | 1.20E-09 | Vk8-30 | Jk5 | CQQYDS | 2 | 1 | 1 | VRC01 gH | 3 | 3 | 0 |
| **407-77** | d31 | Ribi | IgG2b,k | not done | Vk6-15 | Jk4 | CQQYDS | 10 | 8 | 2 | VRC01 gH | 4 | 2 | 2 |

| **Name** | **H-chain protein sequence** |
|---|---|
| VRC01 gH | QVQLVQSGAEVKKPGASVKVSCKASGYTFTGYYMHWVRQAPGQGLEWMGWINPNSGGTNYAQKFQGRVTMTRDTSISTAYMELSRLRSDDTAVYYCARGKNSDYNWDFQHWGQGTLVTVSS |
| VRC01 | --------GQM----E-MRI--R----E-IDCTLN-I-L---KRP-----LK-RF-AV---RPL---------VYSD--FL--RS-TV------F-T----C------E---R--P-I--- |
| **483-40** | ---------- |
| **512-9** | ---------- |
| **443-19** | ---------- |
| **513-28** | ---V----------F---------- |
| **401-87** | ----------D---N----------N---------- |
| **406-143** | ----------A----A-----N---------- |
| **407-77** | ----------P----------N---------- |

| **Name** | **Jk** | **L-chain protein sequence** |
|---|---|---|
| Vk8-30 | | DIVMSQSPSSLAVSVGEKVTMSCKSSQSLLYSSNQKNYLAWYQQKPGQSPKLLIYWASTRESGVPDRFTGSGSGTDFTLTISSVKAEDLAVYYCQQYYSYP |
| **483-40** | Jk1 | ----------CQQYWT/FGGGTKLEIK |
| **443-19** | Jk1 | ----------R----------T----------CQQYDC/FGGGTKLEIK |
| **406-143** | Jk5 | ----------K----------CQQYDS/FGAGTKLELK |
| **401-87** | Jk2 | ----------H-I----------CQQYDT/FGGGTKLEIK |
| Vk6-15 | | DIVMTQSQKFMSTSVGDRVSVTCKASQNVGTNVAWYQQKPGQSPKALIYSASYRYSGVPDRFTGSGSGTDFTLTISNVQSEDLAEYFCQQYNSYP |
| **513-28** | Jk5 | ----------CQQYNT/FGAGTKLELK |
| **407-77** | Jk4 | ----------I----------H----------T---F---------G----------T--------K--CQQYDT/FGSGTKLEIK |
| Vk6-32 | | SIVMTQTPKFLLVSAGDRVTITCKASQSVSNDVAWYQQKPGQSPKLLIYYASNRYTGVPDRFTGSGYGTDFTFTISTVQAEDLAVYFCQQDYSSP |
| **512-9** | Jk2 | ----------CQQDYSSPYT/FGGGTKLEIK |

**Table S6.**

L-chain sequence analysis of IgM hybridomas positive for binding to GT8-60mer. All hybridomas carried a VRC01 gH chain.

| Hybridoma number | VL/JL | CDRL3 | Length |
|---|---|---|---|
| **Lps hybridomas** | | | |
| LPS-1D9 | Vk12-46/Jk1 | CQHFWGTPWT | 9 |
| LPS-2D10 | Vk12-44/Jk5 | CHQHYGTPT | 8 |
| **Day 10 Alum eOD-GT8 60mers** | | | |
| 511-3 | Vk6-20/Jk5 | CGQSYSYPLT | 9 |
| 511-6 | Vl2/Jl2 | CALWYSTHFV | 9 |
| 512-5 | Vk4-91/Jk5 | CQQGSSIPT | 8 |
| 512-15 | Vk12-41/Jk1 | CQHFWSTPWT | 9 |
| 512-18 | Vk9-120/Jk1 | CLQYXSSPPT | 9 |
| 512-25 | Vk3-4/Jk2 | CQQSNEDPYT | 9 |
| 512-26 | Vk12-41/Jk1 | CQHFWSTPRA | 9 |
| 512-28 | Vk12-41/Jk1 | CQHFWSTPRA | 9 |
| 512-32 | Vk1-110/Jk2 | CSQSTHVPYT | 9 |
| 512-35 | Vk1-110/Jk2 | CSQSTHVPYT | 9 |
| 514-17 | Vk6-23/Jk4 | CHQYSYPLT | 9 |
| 514-L9 | Vk12-44/Jk2 | CQHHYGTPYT | 9 |
| **Day 5 Ribi eOD-GT8 60mers** | | | |
| 482-B | Vk12-46/Jk2 | CQHFWGTPYT | 9 |
| 482-C | Vk12-46/Jk2 | CQHFWGTPYT | 9 |
| 482-D | Vk8-27/Jk2 | CHQYLSYT | 7 |
| 482-E | Vk8-28/Jk5 | CQNDHSYPLT | 9 |
| 482-I | Vk8-28/Jk5 | CQNDHSYPLT | 9 |
| 482-L | Vk8-28/Jk5 | CQNDHSYPLT | 9 |
| 482-N | Vk8-28/Jk5 | CQNDHSYPLT | 9 |
| 482-AA | Vk8-28/Jk5 | CQNDHSYPLT | 9 |
| 482-BB | Vk8-28/Jk5 | CQNDHSYPLT | 9 |
| 482-DD | Vk8-28/Jk5 | CQNDHSYPLT | 9 |
| 482-X | Vk8-28/Jk5 | CQNDHSYPLT | 9 |
| 482-P | Vk8-28/Jk5 | CQNDHSYPLT | 9 |
| 482-U | Vk3-4/Jk2 | CQQSNEDPYT | 9;9 |
| | Vk8-28/Jk2 | CQKNHRYPFT | |
| 482-H | Vk8-30/Jk4 | CQQYYSYPFT | 8 |
| 482-M | Vk8-21/Jk2 | CKQSYNLYT | 8 |
| 482-S | Vk1-117/Jk1 | CLQGSHVPWT | 9 |
| 482-W | Vk1-117/Jk2 | CFQGSHVPYT | 9 |
| 482-A | Vl1/Jl1 | CALWYSNHWV | 9 |
| 482-O | Vl1/Jl1 | CALWYSNLWV | 9 |
| 482-T | Vl1/Jl1 | CALWYSNHLV | 9 |
| 482-CC | Vl1/Jl1 | CALWYSNHLV | 9 |
| 482-F | Vl2/Jl2 | CALWYSTHYV | 9 |
| 482-R | Vl2/Jl2 | CALWYSTHYV | 9 |
| 482-V | Vl2/Jl2 | CALWYSTHYV | 9 |
| 482-FF | Vl2/Jl2 | CALWYSTHYV | 9 |

| | | | |
|---|---|---|---|
| 482-J | Vl2/Jl2 | CALWYSTHYV | 9 |
| 482-II | Vl2/Jl2 | CALWYSTHYV | 9 |
| 483-23 | Vk8-21/Jk2 | CKQSYNLYT | 8 |
| 483-63 | Vk8-21/Jk2 | CKQSYNLYT | 8 |
| 483-69 | Vk6-15/Jk2 | CQQYNRFGSGT | 10 |
| 483-77 | Vk1-117/Jk4 | CFQGSHVPFT | 9 |
| 483-132 | Vk4-61/Jk1 | CQQYHT | 5 |
| 483-135 | Vk6-23/Jk5 | CQQYSSYPT | 8 |
| 483-149 | Vk12-38/Jk4 | CKQAYDVPPT | 9 |
| 483-154 | Vk3-5/Jk4 | CQQSNEDPFT | 9 |
| 483-6 | Vl1/Jl1 | CALWYSNHLV | 9 |
| **Day 10 Ribi eOD-GT8 60mers** | | | |
| 443-3 | Vk4-59/Jk5 | CQQWSSNPIT | 9 |
| 443-19M | Vl1/Jl1 | CALWYSNHTFWV | 10 |
| 443-21 | Vk6-15/Jk2 | CQQYNSYPYT | 9 |
| 443-36 | Vl/Jl2 | CALWYSTHYV | 9 |
| 443-40 | Vk3-10/Jk5 | CQQNNEDLT | 8 |
| 443-82 | Vl1/Jl1 | CALWYSNTHYV | 9 |
| 443-88 | Vk4-55/Jk2 | CQQWSSYPPT | 8 |
| 513-16 | Vl1/Jl1 | CALWYSNHWV | 9 |
| 513-28M | Vl1/Jl1 | CALWYSNHWV | 9 |
| 513-29 | Vk5-39/ | CQXGHXFXFT | 9 |
| 513-41 | Vk6-14/Jk1 | CLQHWNYPWT | 9 |
| 513-52 | Vk1-110/Jk2 | CSQSTHVPYT | 9 |
| 513-58 | Vk19-93/Jk2 | CLQYECVFT | 9 |
| 513-63 | Vk3-2/Jk2 | CQQSKEVPYT | 9 |
| 513-69 | Vl2/Jl2 | CALWYSTHYV | 9 |
| 513-79 | Vk4-91/Jk2 | CQQNSIIPRT | 9 |
| 513-91 | Vk8-28/Jk5 | CQNDHSYPLT | 9 |
| 513-93 | Vk6-15/Jk5 | CQQYNSYPLT | 9 |
| 513-124 | Vl1/Jl1 | CALWYSNHWV | 9 |
| **Day 49 Alum 3x eOD-GT8 60mer** | | | |
| 480-13 | Vk3-4/Jk5 | CQQSNEDPLT | 9 |
| 480-62 | Vk3-4/Jk5 | CQQSNEDPLT | 9 |
| 480-42 | Vk8-21/Jk4 | CKQSYNLPT | 8 |
| 480-50 | Vk8-28/Jk5 | CQNDHSYPLT | 9;9 |
| | Vk12-44/Jk1 | CQHHYGTPPT | |
| 480-76 | Vk8-24/Jk5 | CQQHYSTPLT | 9 |
| 480-98 | Vk8-24/Jk5 | CQQHYSTPLT | 9 |
| 480-87 | Vk6-23/Jk5 | CQQYSSYPT | 8 |
| 480-90 | Vk8-21/Jk1 | CKQSYNLWT | 8 |
| 480-94 | Vk4-72/Jk5 | CQQWSSNPPMGT | 11 |
| 480-118 | Vk4-57/Jk5 | CQQRSSYPLT | 9 |
| 480-146 | Vk19-93/Jk2 | CLQYDNLYT | 8 |
| 480-183 | Vk12-46/Jk2 | CQHFWGTPYT | 9 |
| 480-199 | Vk19-93/Jk5 | CLQYDNLLT | 8;9 |
| | Vk10-96/Jk2 | CQQGNTLPYT | |
| 480-160 | Vk3-4/Jk2 | CQQSNEDPYT | 9 |
| 480-25 | Vl1/Jl1 | CALWYSNHLV | 9 |
| 480-73 | Vl1/Jl1 | CALWYSNHLV | 9 |
| 480-80 | Vl1/Jl1 | CALWYSNHLV | 9 |

| | | | |
|---|---|---|---|
| 480-144 | Vl1/Jl1 | CALWYSNHLV | 9 |
| 480-44 | Vl1/Jl1 | CALWYSNHWV | 9 |
| 480-207 | Vl1/Jl1 | CALWYSNHWV | 9 |

**Table S7.**

Summary of the numbers of complete H/L paired antibody sequences recovered by B cell sorting from VRC01 gH mice immunized with eOD-GT8 60-mers.

**All antibodies**

| | VRC01 gH | | Mouse HCs | Total |
|---|---|---|---|---|
| | Paired with 5 aa CDR-L3 | Paired with non 5 aa CDR-L3 | | |
| Day 14 | 56 | 2 | 0 | 58 |
| Day 42 | 98 | 11 | 10 | 119 |
| Total | 154 | 13 | 10 | 177 |

**VRC01-class antibodies (using VRC01 gH and 5aa CDRL3)**

| | Unmutated VRC01 gH | Mutated VRC01 gH | Total |
|---|---|---|---|
| Day 14 | 48 | 8 | 56 |
| Day 42 | 45 | 53 | 98 |
| Total | 93 | 61 | 154 |

**VRC01-class antibodies (using VRC01 gH and 5aa CDRL3) that were successfully expressed and tested by SPR**

| | No mutations in VRC01 gH or mouse L-chain | At least one mutation in either VRC01 gH or mouse L-chain | Total |
|---|---|---|---|
| Day 14 | 38 | 4 | 42 |
| Day 42 | 34 | 39 | 73 |
| Total | 72 | 43 | 115 |

**Table S8.**

Summary of SPR results for eOD-GT8 analyte binding to eOD-GT8 60mer-induced Abs isolated by cell sorting.

| Ab Name | Rmax | Kon | Koff | Kd |
|---|---|---|---|---|
| Nem_0016 | Pass | 1.10E+05 | 1.90E-02 | 1.72E-07 |
| Nem_0018 | Pass | 2.20E+05 | 5.90E-03 | 2.66E-08 |
| Nem_0021 | Pass | 1.90E+05 | 5.80E-03 | 3.05E-08 |
| Nem_0024 | Pass | 2.00E+05 | 3.00E-04 | 1.49E-09 |
| Nem_0025 | Pass | 1.50E+05 | 5.80E-03 | 3.84E-08 |
| Nem_0026 | Pass | 1.00E+05 | 1.40E-01 | 1.33E-06 |
| Nem_0027 | Pass | 2.50E+05 | 6.60E-03 | 2.70E-08 |
| Nem_0028 | Pass | 1.60E+05 | 3.00E-03 | 1.86E-08 |
| Nem_0030 | Pass | 4.40E+05 | 1.80E-02 | 3.94E-08 |
| Nem_0031 | Pass | 2.40E+05 | 5.90E-03 | 2.45E-08 |
| Nem_0032 | Pass | 2.40E+05 | 6.80E-03 | 2.85E-08 |
| Nem_0033 | Pass | 2.20E+05 | 1.20E-02 | 5.74E-08 |
| Nem_0037 | Pass | 2.00E+05 | 6.70E-03 | 3.27E-08 |
| Nem_0038 | Pass | 2.00E+05 | 7.00E-03 | 3.53E-08 |
| Nem_0039 | Pass | 1.00E+05 | 8.80E-03 | 8.78E-08 |
| Nem_0040 | Pass | 2.00E+05 | 7.50E-03 | 3.73E-08 |
| Nem_0041 | Pass | 1.80E+05 | 7.20E-03 | 3.93E-08 |
| Nem_0042 | Pass | 2.10E+05 | 7.40E-03 | 3.58E-08 |
| Nem_0043 | Pass | 2.00E+05 | 7.40E-03 | 3.71E-08 |
| Nem_0044 | Pass | 2.00E+05 | 6.40E-03 | 3.25E-08 |
| Nem_0045 | Pass | 2.10E+05 | 6.90E-03 | 3.27E-08 |
| Nem_0046 | Pass | 2.40E+05 | 4.80E-04 | 2.00E-09 |
| Nem_0048 | Pass | 2.00E+05 | 7.40E-03 | 3.71E-08 |
| Nem_0049 | Pass | 7.30E+04 | 2.70E-02 | 3.73E-07 |
| Nem_0050 | Pass | 2.10E+05 | 7.70E-03 | 3.63E-08 |
| Nem_0052 | Pass | 2.00E+05 | 7.10E-03 | 3.51E-08 |
| Nem_0054 | Pass | 1.90E+05 | 5.60E-03 | 2.93E-08 |
| Nem_0055 | Pass | 6.40E+05 | <5.0E-05 | <1.6e-11 |
| Nem_0057 | Pass | 1.00E+05 | 1.10E-02 | 1.10E-07 |
| Nem_0058 | Pass | 1.90E+05 | 3.30E-03 | 1.74E-08 |
| Nem_0061 | Pass | 1.20E+06 | <5.0E-05 | <1.6e-11 |
| Nem_0062 | Pass | 1.10E+05 | 7.20E-03 | 6.39E-08 |
| Nem_0063 | Pass | 2.80E+05 | 5.40E-04 | 1.89E-09 |
| Nem_0066 | Pass | 8.30E+05 | 2.50E-02 | 3.01E-08 |

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| Nem_0067 | Pass | 1.40E+06 | <5.0E-05 | <1.6e-11 |
| Nem_0068 | Pass | 1.80E+05 | 4.60E-03 | 2.59E-08 |
| Nem_0069 | Pass | 6.60E+05 | 5.60E-05 | 8.49E-11 |
| Nem_0070 | Pass | 1.60E+05 | 1.70E-03 | 1.08E-08 |
| Nem_0071 | Pass | 9.90E+05 | <5.0E-05 | <1.6e-11 |
| Nem_0072 | Pass | 1.20E+06 | <5.0E-05 | <1.6e-11 |
| Nem_0074 | Pass | 6.90E+05 | 1.00E-04 | 1.51E-10 |
| Nem_0077 | Pass | 1.60E+05 | 1.80E-03 | 1.15E-08 |
| Nem_0078 | Pass | 5.20E+05 | 6.00E-05 | 1.16E-10 |
| Nem_0079 | Pass | 7.10E+05 | <5.0E-05 | <1.6e-11 |
| Nem_0080 | Pass | 1.20E+06 | <5.0E-05 | <1.6e-11 |
| Nem_0081 | Pass | 2.10E+05 | 3.30E-03 | 1.54E-08 |
| Nem_0083 | Pass | 1.00E+06 | <5.0E-05 | <1.6e-11 |
| Nem_0086 | Pass | 8.90E+05 | 9.60E-05 | 1.08E-10 |
| Nem_0087 | Pass | 1.10E+06 | 3.10E-04 | 2.94E-10 |
| Nem_0088 | Fail | 3.30E+04 | 1.10E-01 | >1.0E-05 |
| Nem_0089 | Pass | 2.10E+05 | 2.50E-01 | 1.16E-06 |
| Nem_0096 | Pass | 4.60E+05 | <5.0E-05 | <1.6e-11 |
| Nem_0097 | Pass | 6.40E+05 | <5.0E-05 | <1.6e-11 |
| Nem_0098 | Pass | 5.10E+05 | <5.0E-05 | <1.6e-11 |
| Nem_0101 | Pass | 3.80E+05 | <5.0E-05 | <1.6e-11 |
| Nem_0103 | Pass | 4.20E+05 | <5.0E-05 | <1.6e-11 |
| Nem_0104 | Pass | 7.90E+05 | <5.0E-05 | <1.6e-11 |
| Nem_0105 | Pass | 9.40E+04 | 3.80E-01 | 4.08E-06 |
| Nem_0106 | Pass | 1.60E+05 | 7.80E-04 | 4.80E-09 |
| Nem_0108 | Pass | 3.30E+05 | 9.20E-05 | 2.75E-10 |
| Nem_0109 | Pass | 4.80E+05 | <5.0E-05 | <1.6e-11 |
| Nem_0110 | Pass | 6.20E+05 | <5.0E-05 | <1.6e-11 |
| Nem_0112 | Pass | 1.10E+05 | 7.10E-03 | 6.46E-08 |
| Nem_0113 | Pass | 1.90E+05 | 5.30E-03 | 2.77E-08 |
| Nem_0114 | Pass | 1.90E+05 | 5.80E-03 | 3.03E-08 |
| Nem_0115 | Pass | 2.10E+05 | 7.20E-03 | 3.37E-08 |
| Nem_0116 | Pass | 8.60E+04 | 1.70E-01 | 1.98E-06 |
| Nem_0117 | Pass | 2.00E+05 | 5.90E-03 | 2.99E-08 |
| Nem_0118 | Pass | 5.60E+05 | 9.90E-05 | 1.76E-10 |
| Nem_0119 | Pass | 2.00E+05 | 3.60E-03 | 1.78E-08 |
| Nem_0120 | Pass | 2.10E+05 | 3.50E-03 | 1.64E-08 |
| Nem_0121 | Pass | 1.30E+05 | 2.50E-02 | 1.93E-07 |
| Nem_0122 | Pass | 1.50E+05 | 2.60E-01 | 1.72E-06 |
| Nem_0123 | Pass | 3.90E+05 | 1.60E-02 | 4.19E-08 |
| Nem_0124 | Pass | 1.40E+05 | 3.50E-03 | 2.56E-08 |
| Nem_0126 | Pass | 2.30E+05 | 5.30E-03 | 2.31E-08 |
| Nem_0128 | Pass | 2.10E+05 | 6.90E-03 | 3.37E-08 |

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| Nem_0129 | Pass | 5.90E+05 | 1.10E-04 | 1.85E-10 |
| Nem_0130 | Fail | 2.40E+04 | 1.50E-01 | >1.0E-05 |
| Nem_0132 | Fail | 1.20E+06 | >6.0E-01 | >1.0E-05 |
| Nem_0135 | Fail | 6.70E+05 | >6.0E-01 | >1.0E-05 |
| Nem_0138 | Pass | 5.20E+05 | 1.80E-01 | 3.50E-07 |
| Nem_0139 | Pass | 1.50E+05 | 6.30E-03 | 4.16E-08 |
| Nem_0141 | Pass | 3.70E+05 | <5.0E-05 | <1.6e-11 |
| Nem_0143 | Fail | 5.70E+05 | >6.0E-01 | >1.0E-05 |
| Nem_0144 | Pass | 2.00E+05 | 6.40E-03 | 3.10E-08 |
| Nem_0145 | Pass | 3.00E+05 | 1.30E-02 | 4.34E-08 |
| Nem_0146 | Pass | >3.0E06 | >6.0E-01 | >1.0E-05 |
| Nem_0147 | Fail | 1.50E+05 | 6.00E-03 | >1.0E-05 |
| Nem_0149 | Pass | 7.60E+04 | >6.0E-01 | >1.0E-05 |
| Nem_0150 | Pass | 1.30E+05 | 6.20E-03 | 4.92E-08 |
| Nem_0151 | Pass | 9.30E+04 | >6.0E-01 | >1.0E-05 |
| Nem_0152 | Pass | 1.20E+05 | 2.40E-03 | 1.96E-08 |
| Nem_0153 | Fail | 1.30E+05 | 3.80E-01 | >1.0E-05 |
| Nem_0155 | Pass | 1.70E+05 | 2.90E-04 | 1.69E-09 |
| Nem_0156 | Pass | 2.60E+06 | 2.80E-02 | 1.09E-08 |
| Nem_0159 | Pass | 1.80E+05 | 2.60E-03 | 1.45E-08 |
| Nem_0160 | Fail | 7.50E+04 | 1.90E-03 | >1.0E-05 |
| Nem_0161 | Pass | 1.40E+05 | 7.10E-03 | 5.09E-08 |
| Nem_0162 | Pass | 8.70E+04 | 9.80E-02 | 1.12E-06 |
| Nem_0164 | Pass | 6.10E+05 | <5.0E-05 | <1.6e-11 |
| Nem_0169 | Pass | 2.30E+05 | 3.40E-03 | 1.48E-08 |
| Nem_0175 | Pass | 1.20E+05 | 8.60E-02 | 7.02E-07 |
| Nem_0176 | Pass | 2.20E+05 | 2.90E-03 | 1.33E-08 |
| Nem_0177 | Pass | 2.00E+05 | 1.50E-01 | 7.54E-07 |
| Nem_0178 | Pass | 3.60E+05 | 8.30E-05 | 2.28E-10 |
| Nem_0179 | Pass | 2.20E+05 | 5.20E-03 | 2.44E-08 |
| Nem_0181 | Pass | 4.30E+05 | <5.0E-05 | <1.6e-11 |
| Nem_0182 | Pass | 2.10E+05 | 5.70E-03 | 2.67E-08 |
| Nem_0186 | Pass | 2.10E+05 | 7.80E-04 | 3.63E-09 |
| Nem_0187 | Pass | 1.80E+05 | 3.80E-03 | 2.04E-08 |
| Nem_0188 | Pass | 9.50E+05 | <5.0E-05 | <1.6e-11 |
| Nem_0190 | Pass | 2.20E+05 | 7.50E-03 | 3.37E-08 |
| Nem_0191 | Pass | 2.90E+05 | 1.50E-03 | 5.20E-09 |
| Nem_0195 | Pass | 1.10E+05 | 1.20E-02 | 1.09E-07 |
| Nem_0201 | Pass | 2.20E+05 | 3.60E-03 | 1.68E-08 |
| Nem_0203 | Fail | 1.50E+06 | 1.90E-04 | >1.0E-05 |
| Nem_0204 | Fail | 8.00E+05 | 2.00E-02 | >1.0E-05 |
| Nem_0205 | Pass | 4.10E+05 | 1.50E-02 | 3.57E-08 |
| Nem_0206 | Pass | 2.10E+05 | 5.30E-03 | 2.54E-08 |

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|---|---|---|---|---|
| Nem_0209 | Pass | 2.50E+05 | 2.60E-03 | 1.02E-08 |
| Nem_0211 | Fail | 5.20E+04 | 2.60E-02 | >1.0E-05 |
| Nem_0214 | Pass | 2.20E+05 | 6.30E-03 | 2.88E-08 |
| Nem_0217 | Pass | 1.40E+05 | 1.70E-03 | 1.19E-08 |
| Nem_0219 | Pass | 4.00E+05 | 1.80E-02 | 4.56E-08 |
| Nem_0220 | Pass | 5.70E+05 | 6.40E-04 | 1.13E-09 |
| Nem_0221 | Pass | 2.20E+05 | 5.80E-03 | 2.66E-08 |
| Nem_0223 | Pass | 2.40E+04 | 1.10E-02 | 4.48E-07 |
| Nem_0224 | Pass | 7.30E+05 | <5.0E-05 | <1.6e-11 |