Supplementary Materials for

B cells expressing authentic naive human VRC01-class BCRs can be recruited to germinal centers and affinity mature in multiple independent mouse models

Deli Huang[1,*], Robert K. Abbott[2,3,*], Colin Havenar-Daughton[2,3,4], Patrick D. Skog[1], Rita Al-Kolla[2], Bettina Groschel[1,3,5], Tanya R. Blane[1], Sergey Menis[1,3,5], Jenny Tuyet Tran[1], Theresa C. Thinnes[1], Sabrina A. Volpi[1], Alessia Liguori[1,3,5], Torben Schiffner[1,3,5], Sophia M. Villegas[1,3,5], Oleksandr Kalyuzhniy[1,3,5], Mark Pintea[1], James E Voss[1], Nicole Phelps[1,3,5], Ryan Tingle[1,3,5], Alberto R. Rodriguez[1], Greg Martin[1], Sergey Kupryianov[1], Allan deCamp[6], William R. Schief[1,3,5,7], David Nemazee[1,§], Shane Crotty[2,3,8§]

* These authors contributed equally.

§ Correspondence to nemazee@scripps.edu and shane@lji.org.

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Extended Methods

Generation of Mice

Paired HC and LC sequences were selected from a panel of cloned BCRs that were selected from individual healthy HIV$^-$ human donors. B cells were selected for VRC01-class criteria, namely, co-expressing the VH1-2*02 allele and a LC with a 5 amino acid-long CDR3. All experimental mice were heterozygous for indicated HCs and LCs. All HuGL mice were on CD45.2 background while recipient mice were CD45.1 background. CD45.1 mice (B6.SJL-*Ptprc*^{a}*Pepc*^{b}*/BoyJ, stock No:002014)* were originally purchased from Jackson Labs (During breeding transgenic mice were maintained as heavy or light only. In some experiments donor heterozygous mice were generated from outbreeding to B6 females. HuGL mice used in experiments were bred by mating homozygous HC knock-in mice with corresponding homozygous LC knock-ins.

HC knock-in mouse generation

Generation of HuGL 16, 17 and 18 HC mice was carried out essentially as described (1-3), by substituting the HuGL 16, 17 and 18 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. The upstream promoter, leader and intron elements were from

VHJ558.85.191. Targeting, embryonic stem cell screening, and mouse generation were as described (1-3).

Light chain knock-in mouse generation

gRNAs design and Donor DNA plasmid preparation. gRNAs targeting the *IgKJ* region of the reference C57BL/6J genome (GRCm38) were designed using the GPP webtool (https://portals.broadinstitute.org/gpp/public/analysis-tools/sgrna-design). Selected gRNAs shown in Figure 1 B and C were synthesized by IDT and cloned into eSpCas9(1.1) (a gift from Feng Zhang Addgene plasmid # 71814). The LC donor DNA of HuGL18 was designed as follows from 5′ to 3′: a 2 kb homology arm upstream of the mouse *IgK-J5;* the mouse *IGKV4-53* LC promoter starting 1553-basepair upstream of, and including, the leader exon and intron; HuGL18 VJ; and a 1934-base region of DNA downstream of Jκ5 including the splicing signal. As for HuGL16 and 17 donor plasmids, the only difference in the backbone was the 5′ arm homology, which included a stretch of 2105 bp upstream of the IgKJ1-gRNA#2 targeting site. The homology regions and promoter were amplified from C57BL/6J genomic DNA. All LC fragments were synthesized (IDT). All fragments were assembled using the ligase-independent cloning method into pBKS (Addgene, 212207) and transformed into NEB® Stable Competent E. coli (NEB, C3040H). All plasmids were sequenced using several primers to generate high quality coverage of the entire insertions. Donor DNA plasmids were purified using EndoFree plasmid maxi kit (Qiagen, #12362) for engineering test and zygote microinjection.

gRNAs optimization in pro-B cells. *Rag1*$^{-/-}$ pro-B cells were cultured in complete medium, RPMI-1640 (Invitrogen, 21870076) supplemented with non-essential amino acids (Invitrogen,11140050), Sodium pyruvate (Invitrogen, 11360070), 55 μM 2-mercaptoethanol (Invitrogen, 21985023), 10% FBS (Invitrogen, 26140-079) and 100 units of Penicillin and 0.1 mg/ml of Streptomycin (P/S) (Invitrogen, 15140122). The cultured cells were washed twice with DPBS (Invitrogen, 14190-144) and resuspended at 0.5 million cells/ 10 μl in Neon R buffer (Invitrogen, MPK10096). 1 μg of each gRNA, 1 μg Donor DNA and 1 μg EGFP were mixed and added to the resuspended cells. The cells were electroporated using a 10 μl tip (Invitrogen, MPK1096) with 1400 V, 25 ms, 1 pulse using a Neon electroporation machine (Invitrogen). Transfected cells were immediately cultured in complete medium without P/S for 1 h before P/S was added for further culture. 48 hours later, the engineered cells were fixed with 4% PFA in DPBS at RT for 15 minutes and washed twice with FACS buffer (2% FBS in DPBS). Anti-mouse kappa LC antibody clone 187.1 was used to detect the engineering efficiency. EGFP-positive cells were gated for further engineering efficiency analysis.

Zygote injections. The gRNAs giving the highest engineering efficiency were chosen to generate LC knock-in mice using a Cas9 ribonucleoprotein approach along with the same donor plasmids described above. Female C57BL/6J mice (24-28 days old) were super-ovulated by the i.p. injection of 5 IU of pregnant mare serum gonadotropin (PMSG), followed by the 5 IU i.p. injection of human chorionic gonadotropin (hCG) 47 h later, then the mice were mated to C57BL/6J stud males. Fertilized oocytes were collected from the

oviducts 21-22 h post hCG injection. Cas9 protein (50 ng/μl), sgRNA (5 ng/μl) and donor plasmid (10 ng/μl) were mixed in IDTE 1X TE nuclease-free buffer (pH 7.5) and injected into the pronuclei of fertilized eggs in a droplet of FHM medium containing 5 μg/ ml of cytochalasin B, using a FemtoJet microinjector (Eppendorf) with a constant flow setting. The injected zygotes were cultured in KSOM medium with amino acids at 37°C under 5% CO2 overnight to 2-cell stage and surgically transferred into oviducts of pseudopregnant CD-1 recipient females at 0.5 dpc.

Flow cytometry analysis

Spleen samples were prepared as previously described (4). In brief, spleen suspensions were generated by smashing the spleen between frosted glass slides. Red blood cells were lysed with ammonium chloride (0.83%) before filtering cells through a 40 μM cell strainer to generate single-cell suspensions. Fc Blocker (homemade mAb 2G4) was added to single-cell suspensions at 1 μg per 10^6 cells before antibody staining. For antigen-specific staining, tetramerized GT8 and GT8-KO11 were made by mixing biotinylated AviTagged GT8 and GT8-KO11 monomer with Streptavidin-AF647 and Streptavidin-AF488 respectively. Fluorophore-conjugated antibodies CD19 (Biolegend, 115540, 152408; BD Biosciences, 566107), CD45.1 (Biolegend, 110728, 110738), TCRb (Biolegend, 109228), GL7 (Biolegend, 144608, 144610), F4/80 (Biolegend, 123128), Ter119 (Biolegend, 116228), CD38 (Biolegend, 102718; BD Biosciences, 740245), CD45.2 (Biolegend, 109806, 109822), IgM (Biolegend, 314524), IgD (Biolegend, 405721), IgG1 (Biolegend, 406620, 406616), CD80 (Biolegend, 104712), CD73 (Biolegend, 127210), PD-L2 (Biolegend, 107216), CXCR4 (Biolegend, 146511), CD86

(Biolegend, 105008), PD1 (Biolegend, 135214), CD8 (Biolegend, 100722), CD4 (homemade AF-647), CXCR5 (Biolegend, 145512), BCL6 (Biolegend, 648304), B220 (Biolegend, 103236), CD21 (Biolegend, 123414) CD23 (BD Biosciences, 553139) were used to define different cellular populations. CD45.1 and CD45.2 mAbs were used to distinguish host and transferred HuGL^{HL} B cells respectively. IgG1 staining was used to identify class-switched B cells; Germinal center (GC) B cells were gated as CD19^{+}TCRb^{-} CD38^{-}GL7^{+} and HuGL^{HL} fraction further gated as CD45.1^{-} CD45.2^{+}; HuGL^{HL} Memory B cells (MCs) were gated as Live, CD45.1^{-} CD45.2^{+} CD19^{+} CD38^{+} TCRβ^{-} IgD^{-}.

Calcium flux analysis

Calcium flux was monitored as previously described with minor modification (5). Primary B cells from HuGL16^{HL}, HuGL17^{HL}, HuGL18^{HL}, and WT mice were isolated using negative isolation kit (Miltenyi, 130-090-862). B cells were suspended at 4 million cells/ml in Advanced DMEM, labeled with 1 μM Indo-1 (Invitrogen, I1223) for 1 hour at 37°C, washed twice with DPBS, and resuspended in Advanced DMEM supplemented with 10% FBS. Basal Indo-1 fluorescence was recorded for 60 seconds, then antigens were added at 10 μg/ml final concentration and Ca^{++} signals were recorded for 180 s before ionomycin was added. Calcium flux analysis was performed on an LSRII cytometer (BD, San Jose, CA). Kinetic analysis was performed using FlowJo (Tree Star, Ashland, OR).

Single-cell sorting and sequencing

Single cell sequencing was performed using one of two methods. Most sequencing was done using the RT-PCR based method. The results between the methods highly agreed and were considered equivalent.

Method 1: Single cell PCR for HuGL18 and HuGL17 was performed as in (6) which was adapted from Von Boehmer et al. HuGL18 HC sequencing primers were as previously reported (6). Briefly, cDNA was generated from RNA from single cell sorted $IgG1^+$ GC B cells and then nested PCRs were performed to amplify both heavy and light chains. HuGL18 HCs and HuGL17 HCs were sequenced using 2MRG and 2MFG from Abbott et al 2018. HuGL18 LC sequencing primers were optimized and as follows VK320FM1 CATATTGTCCAGTGGAGAA and VK320RM3 CCGTTTGATTTCCACCTT. HuGL17 LC sequencing primers were as follows: VK15FM1 GACATCCAGATGACCCAG, VK15RM2 TTGATTTCCACCTTGGTCC. Sequences were aligned and trimmed in Sequencher v5.1. Analysis of VRC01-class mutations was performed as in (6).

Method 2: Class-switched $IgG1^+HuGL^{HL}$ GCs from spleen were individually sorted into wells of 96-well plate containing 10 μl of DPBS. Cells were spun down, frozen on dry ice and stored at -80 °C. To amplify HuGL heavy and LCs from single frozen cells, gDNA was prepared by adding 11 μl 2 X lysis buffer (20 mM Tris-HCl (pH 7.6), 100mM NaCl, 12.5 mM $MgCl_2$, 0.09% Tween-20) containing 0.2 mg/ml protease K (NEB, P8107S) and incubating at 56°C for 1 hour. Protease was heat inactivated at 98°C for 15 min. The nested PCR is used for amplifying the HC and LC. For outer PCR, the 30 μl outer Phusion (Thermo, F530L) PCR master including both HC and LC outer sets of primers was directly added to the 96-well plate for amplifying HC and LC. 1 μl PCR products from outer PCR

amplification was used for inner PCR amplification. The PCR products were subjected to electrophoresis through 1.5% agarose.

B cell isolation and transfers

HuGL16, HuGL17, and HuGL18 B cells were all treated as in (6). Briefly spleens were collected, manually dissociated and filtered. Lymphocytes were separated by Ficoll gradient. Lymphocytes were then stained with anti-CD43 FITC (clone S7, BD Biosciences) for 30 minutes. Cells were washed, stained with anti-FITC microbeads (Miltenyi Biotech), washed, filtered and then passed through a LS Column on a Quadromacs magnet. EDTA was specifically avoided to maintain cell health. LS column was washed once with 2ml of buffer which was discarded prior to running cells. Collected cells were then checked by flow cytometry for B cell purity and antigen-positive cells using fluorescently labeled eOD-GT8. Cells were enumerated on a hemocytometer. Final tubes, syringes, and pipette tips were pre-coated for at least 1 hour with buffer. All steps were performed with 5% horse serum/DPBS (with Ca^{++} and Mg^{++}).

Immunogen Production

eOD-GT8 d41m3 was produced as previously described (7). Care was taken to evaluate immunogens for low endotoxin. The eOD-GT8-KO used was eOD-GT8-KO11 (8). Affinities for HuGL16, HuGL17, and HuGL18 for eOD-GT8 were previously published, and each were subsequently re-measured on multiple occasions, against different batches of eOD-GT8, and a representative K_d is shown.

ELISAs

ELISAs for eOD-GT8 and eOD-GT8-KO were performed as in (6) with the only modification being the use of 96-well polysorb NUNC plates (Thermo). 60mers were used for all ELISAs at a coating concentration of 2 μg/ml. For negative control ELISA in Figure 2A (bottom) mice received a low number of HuGL18 B cells (1 in 10^6 B cells).

FACS and cell sorting

All flow cytometry analysis was in general performed as in (6). Briefly, all single cell suspensions were FC blocked for 10 minutes (clone 2.4G2) prior to all stains. Primary stains were carried out for 30 minutes. If no secondary stains were in a given panel, cells were washed twice with 10x volume (1 ml FACS buffer for 100 μL staining buffer) before fixation or collection on cytometer. 3-laser 12-color FACSCelesta was used for most experiments. Some experiments were collected while sorting on Aria Fusion. Data was processed using Flowjo 10. All mAb clones used were as listed in (6).

For sorting, 4-way purity mode was used and single cells were sorted into 96 well plate with collection buffer. Collection buffer and plates were the same as reported in Abbott et al 2018. Single cell mode was avoided as it was too restrictive. Cells were sorted with the 70 μ nozzle with custom pressure (61.5PSI) and sort rate was kept at 2.4 or below to ensure targeting. ACDU was checked for targeting after each plate and adjusted if necessary. Cells were kept at 4C (not on ice) while awaiting sort to avoid cell death. No

in-line filter was used during these sorts.

Histology

Histology was done essentially as in (6). Briefly 5-8 μM frozen splenic sections were cut, fixed in 1:1 mixture of acetone/methanol at -20C for 10 minutes and dried. Upon rehydration, slides were blocked (FC block and 0.5% BSA in PBS) and then stained with indicated antibodies. Zeiss axioscan was used for acquisition. Zen software was used for evaluation images.

Antibody Production

Antibodies were produced using the ExpiCHO Expression System (Thermo Fisher) in baffled 96 deep-well blocks (Thomas Scientific) according to manufacturer's instructions, with the following adjustments: the cell density was increased to $10x10^6$ viable cells/mL, cell culture volumes were increased to 1.0 mL/well, ExpiFectamine Reagent (Thermo Fisher) volume was increased to 6 µL/well, and incubator shake speed was set to 1300 RPM. After 7 days incubation, supernatants were supplemented with 40 µL 1M Tris, pH 8.0, harvested by centrifugation and purified using Protein A HP MultiTrap plates (GE Healthcare) per manufacturer's instructions.

SPR

We measured kinetics and affinity of antibody-antigen interactions on a ProteOn XPR36 (Bio-Rad) instrument using the 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 Human Antibody Capture Kit instructions (Cat. No BR-1008-39 from GE Healthcare) to prepare the chip surface for ligand capture. In a typical experiment, about 5700 RU of capture antibody was amine coupled in all flow cells of the GLC Chip. 3M Magnesium Chloride was our regeneration solution with 180 seconds contact time and injected four times per each cycle. The solution concentration of ligands was around 1ug/ml and contact time was 60 seconds. Raw sensograms were analyzed using ProteOn Manager software (Bio-Rad), interspot and column double referencing, Equilibrium or Kinetic with Langmuir model or both where applicable. Analyte concentrations were measured on NanoDrop 2000c Spectrophotometer using Absorption signal at 280 nm. Six concentrations were tested for each analyte, representing a dilution series from the top concentration using a dilution factor of 4. Analyte top concentrations were 50-62 uM for eOD-GT8 and 50-77 uM for eOD-GT8-KO11, except in two cases where no binding was detected to eOD-GT8 and the experiment was carried out a second time with a top concentration of 112 uM eOD-GT8. Higher concentrations of eOD-GT8 were tested but caused increases in bulk refractive index that precluded SPR analysis.

Statistical Analysis

Unless otherwise noted (e.g. VRC01-class mutation analysis), unpaired nonparametric Mann-Whitney T tests were run to determine statistical significance (Prism 7 or later).

Statistical Analysis of VRC01-class mutations

Data was collected on the number of VRC01-class amino acid mutations versus the number of total amino acid mutations in VH1-2 heavy chain genes. The data were from different mouse experiments, and were compared to a computationally generated "random antigen line". Data were from eight different experimental conditions given by two different mouse models (knockin mouse = HuGL17 or HuGL18) with the VRC01-class counts computed two different ways (standard and expanded) measured 16 or 36 days after vaccination. Either 4 or 5 mice were available for each experimental condition. The random antigen line was computationally generated from two mock human donors using the same two VRC01-class count methods (standard and expanded), following previous work (9). The 'standard' method of counting VRC01-class mutations was carried out as previously described, in which a VRC01-class mutation was any mutation observed in a representative set of VRC01-class bnAbs (12a12, 3BNC60, PGV04, PGV20, VRC-CH31, and VRC01)(3, 9), and the 'expanded' method of counting was the same as the 'standard' method but also included mutations observed in the VRC01-class bnAb PCIN63-71I1a (10) that utilizes k1-5 on the light chain as does HuGL17. The significance of the number of VRC01-class mutations compared to the random antigen line was analyzed for each experimental condition as follows.

Analysis for each experimental condition ('pooled analysis')

Under each experimental condition the pooled analysis measures the average mutation rate across all B-cells. The average is computed separately for each mouse indexed by $j \in J$ and defined as the sum of the number of VRC01-class mutations divided by the sum of the total mutations. For the jth mouse, where $i \in I_j$ indexes B-cells, we define V_{ij} as the

number of VRC01-class mutations and T_{ij} as the total number of mutations. The average mutation rate for the *j*th mouse is defined as:

$$r_j^T = \frac{\sum_{i \in I_j} V_{ij}}{\sum_{i \in I_j} T}$$

and mu is defined as the mean of the average mutations rates

$$\mathrm{mu}^{\mathrm{pooled}} = \frac{1}{|J|} \sum_{j \in J} r_j^{\mathrm{pooled}}$$

where |*J*| is the number of mice analyzed under the experimental condition.

The random mutation rate for the pooled analysis is the average mutation rate across both human donors where $i \in I$ indexes the B-cells. Under a given experimental condition, the maximum number of total mutations observed among mice is *M*. We compute the random mutation rate restricting to B-cells with at most *M* total mutations. For the *i*th B-cell the total mutations is T_i and $T_i \leq M$. The random mutation rate is defined as

$$\mathrm{rand}^{\mathrm{pooled}} = \frac{\sum_{i \in I} Vi}{\sum_{i \in I} T_i}$$

where *Vi* is the number of VRC01-class mutations for the *i*th human B-cell.

Under each experimental condition, a t-test is used to test the hypothesis that the mutation rate among mice significantly differs from $\mathrm{rand}^{\mathrm{pooled}}$.

Analysis for each number of Total Mutations ('binned analysis')

Under each experimental condition for a given number of Total Mutations (*T*), the average mutation rate is computed for each mouse indexed by *j* as the sum of the number of VRC01-class mutations divided by the sum of the total mutations where summation is across the sequenced B-cells. Let V_{ij} be the number of VRC01-class mutations for the *i*th B-cell from mouse *j*. The average rate of mutations, among B-cells with *T* total mutations, is defined as

$$r_j^T = \frac{\sum_{i \in I_j} V_{ij}}{\sum_{i \in I_j} T}$$

and mu^T is defined as the mean of the average mutations rates

$$\text{mu}^T = \frac{1}{|J|} \sum_{j \in J} r_j^T$$

The value of random antigen line for *T* is the average mutation rate across both human donors where $i \in I$ indexes the B-cells and the random mutation rate is defined as

$$\text{rand}^T = \frac{\sum_{i \in I} Vi}{\sum_{i \in I} T}$$

where V_i is the number of VRC01-class mutations for the *i*th human B-cell.

Under each experimental condition and each value of *T*, a t-test is used to test the hypothesis that the mutation rate among mice significantly differs from rand^T .

1. C. Doyle-Cooper *et al.*, Immune tolerance negatively regulates B cells in knock-in mice expressing broadly neutralizing HIV antibody 4E10. *J. Immunol.* **191**, 3186-3191 (2013).
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4. A. L. Gavin *et al.*, PLD3 and PLD4 are single-stranded acid exonucleases that regulate endosomal nucleic-acid sensing. *Nat. Immunol.* **19**, 942-953 (2018).
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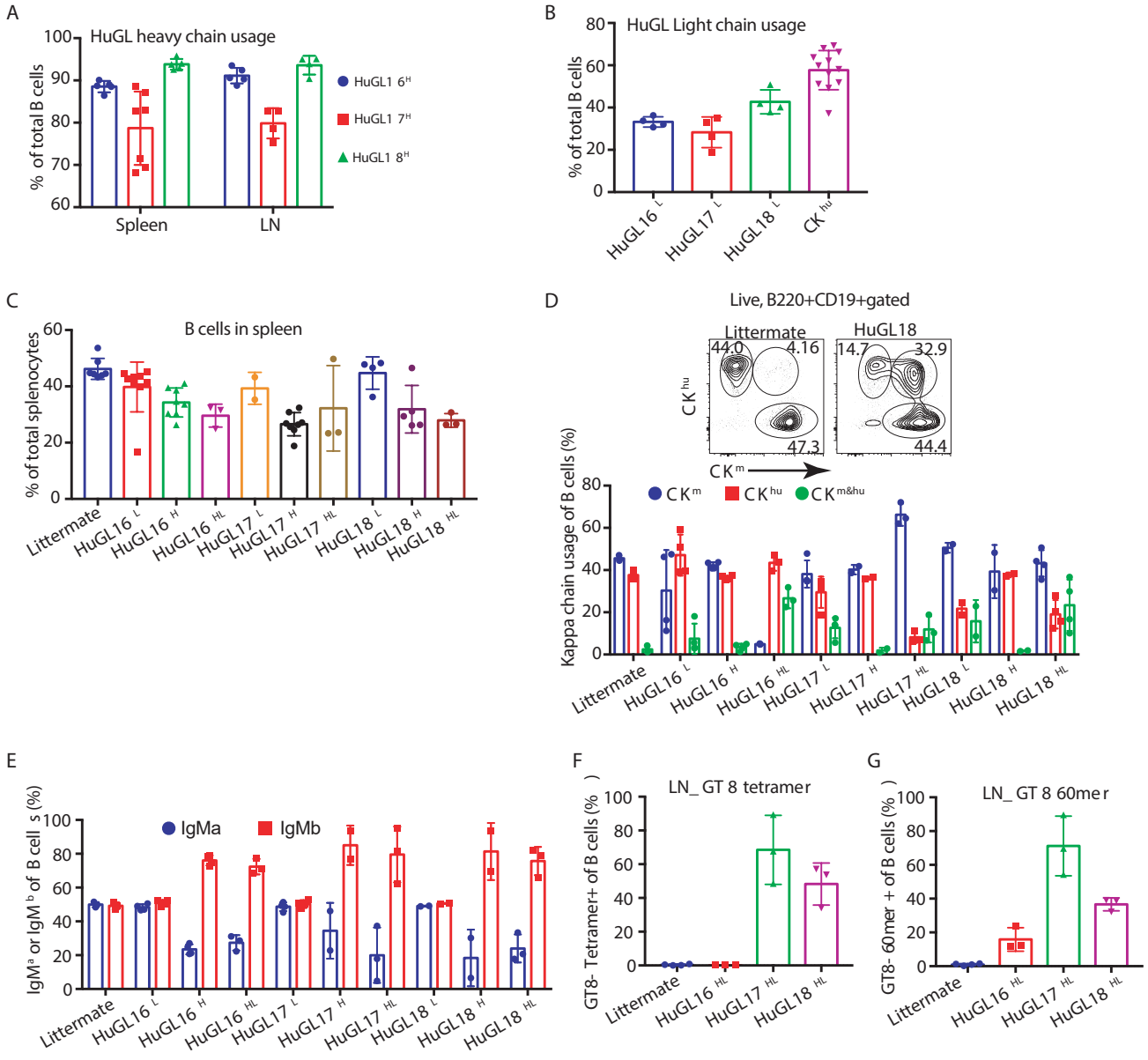

Figure S1. Analysis of pre-immune knock-in HuGL mice. The indicated BCR knock-in strains generated on the C57BL/6 background ($Igh^{b/b}C\kappa^{m/m}$) were bred to $IgH^{a/a}C\kappa^{hu/hu}$ mice and analyzed by flow cytometry for allelic exclusion and B cell numbers. (A) Analysis of HC allelic exclusion in the indicated HC-only mice. Spleen and lymph node B cells were assessed for expression of IgM^b by flow cytometry. **(B)** LC-only strain mice were similarly assessed for expression of the knock-in allele. **(C)** Enumeration of B cell numbers as a percentage of spleen cells. **(D)** LC allelic exclusion analysis of H, L and H/L strains. Flow plot shows gating enumerating cells expressing knock-in vs endogenous κ allele (horizontal vs vertical axis, respectively); double positive cells are in the upper right quadrant. **(E)** IgH allelic exclusion of H, L and H/L bone marrow B cells. **(F,G)** Enumeration of GT8-binding cells quantitated with streptavidin tetramers **(F)** or 60mer nanoparticles **(G).**

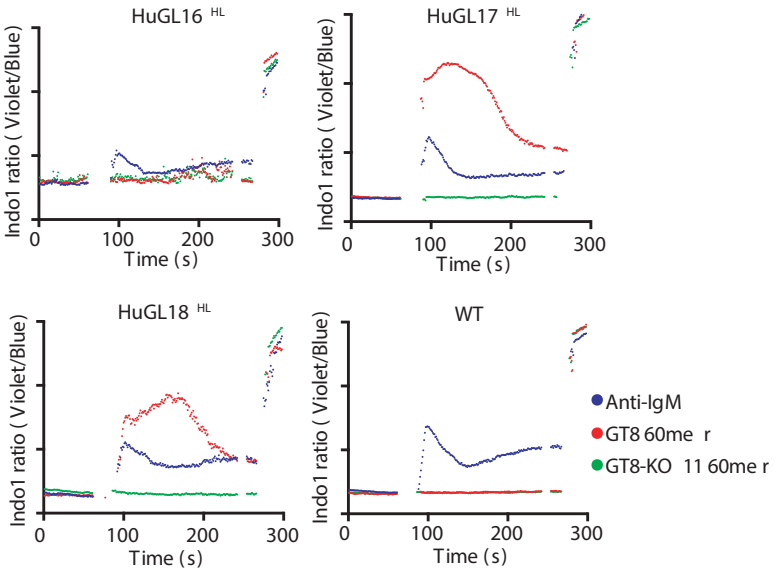

Figure S2. Calcium flux analysis of splenic B cells from HuGL16^{HL}, HuGL17^{HL} and HuGL18^{HL} mice treated with eOD-GT8 60mer nanoparticles, eOD-GT8-KO 60mer nanoparticles, or anti-IgM. Stimuli were applied at 10 μg/ml after 90 s equilibration followed by treatment with ionomycin to assess Indo1 loading.

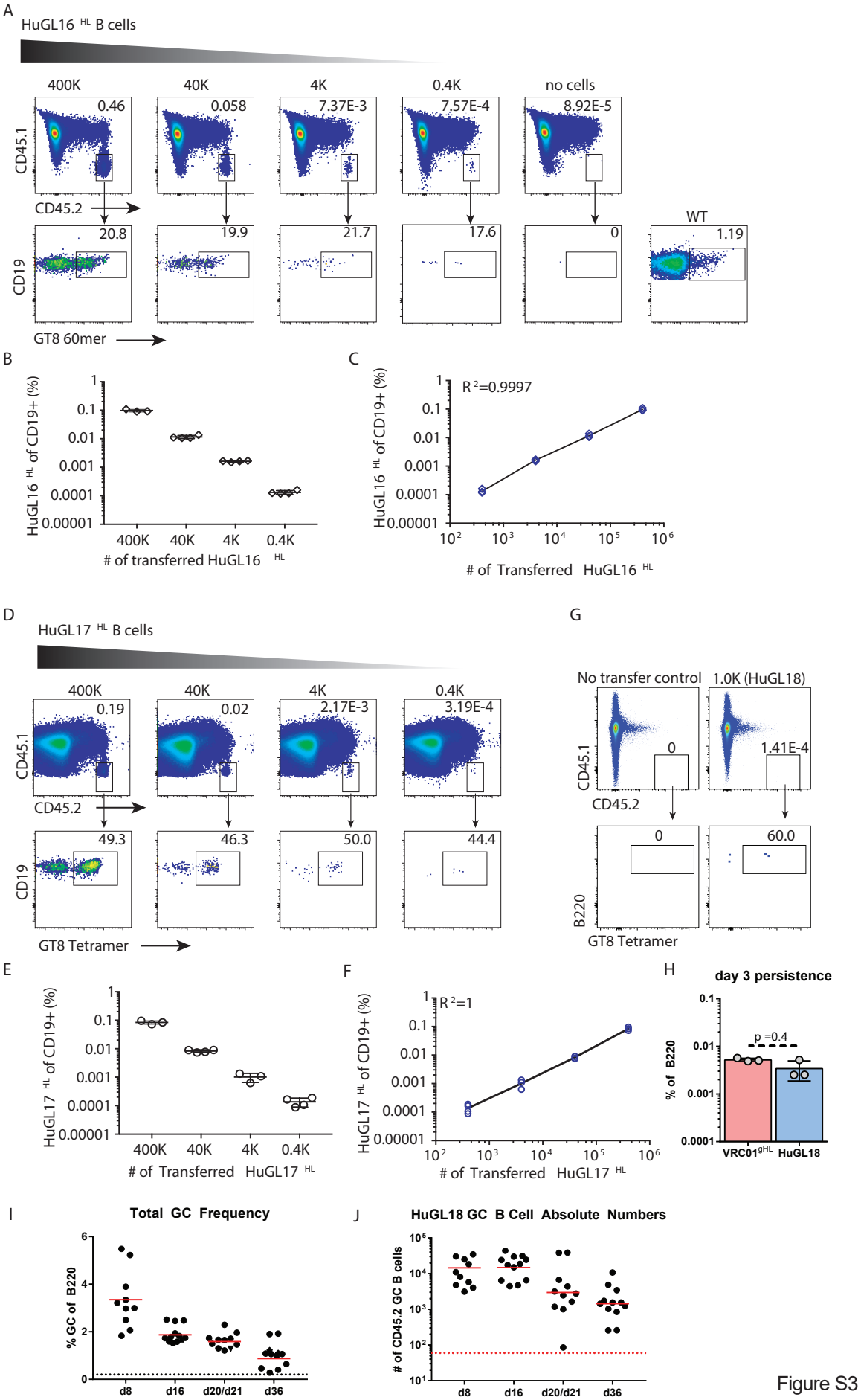
A
HuGL16 HL B cells
400K
40K
4K
0.4K
no cells
0.46
0.058
7.37E-3
7.57E-4
8.92E-5
CD45.1
CD45.2
20.8
19.9
21.7
17.6
0
WT
1.19
CD19
GT8 60mer
B
HuGL16 HL of CD19+ (%)
# of transferred HuGL16 HL
C
R 2=0.9997
HuGL16 HL of CD19+ (%)
# of Transferred HuGL16 HL
D
HuGL17 HL B cells
0.19
0.02
2.17E-3
3.19E-4
49.3
46.3
50.0
44.4
GT8 Tetramer
G
No transfer control
1.0K (HuGL18)
1.41E-4
60.0
B220
E
HuGL17 HL of CD19+ (%)
# of Transferred HuGL17 HL
F
R 2=1
H
day 3 persistence
p =0.4
% of B220
VRC01gHL
HuGL18
I
Total GC Frequency
% GC of B220
d8
d16
d20/d21
d36
J
HuGL18 GC B Cell Absolute Numbers
# of CD45.2 GC B cells

Figure S3

Figure S3. Analysis of seeding efficiency upon in vivo B cell transfer of **(A-C)** HuGL16 or **(D-F)** HuGL17 cells or (**G-H**) HuGL18 cells. For HuGL16 or HuGL17, Cohorts of 3-4 B6.CD45.1 mice/group were transferred with from 400 to 400,000 $GT8^+$ B cells, as indicated. Identification of GT8-binding cells in donor and recipient spleens was carried out using eOD-GT8 nanoparticles in (A-C) and with eOD-GT8 tetramers in (D-F). Seeding efficiency of HuGL18 B cells transferred to achieve 1 in 10^6 precursor frequency (**G**). All recipient mice were analyzed at 24 h post transfer. (**H**) Persistence of HuGL18 B cells in unimmunized mice at day 3 post transfer of high numbers of HuGL18 B cells showing these B cells are not rapidly cleared from the periphery post transfer. Total GC B cell frequencies are shown (gated as $SSL/B220^+/CD4^-/CD38^-/GL7^+$ for HuGL18 low cell (10^3) transfers over time (**I**). Red dotted line represents average background GC frequency. Absolute HuGL18 GC B cell frequencies over time when starting from rare precursors (**J**). Red dotted line indicates calculated numbers at seeding.

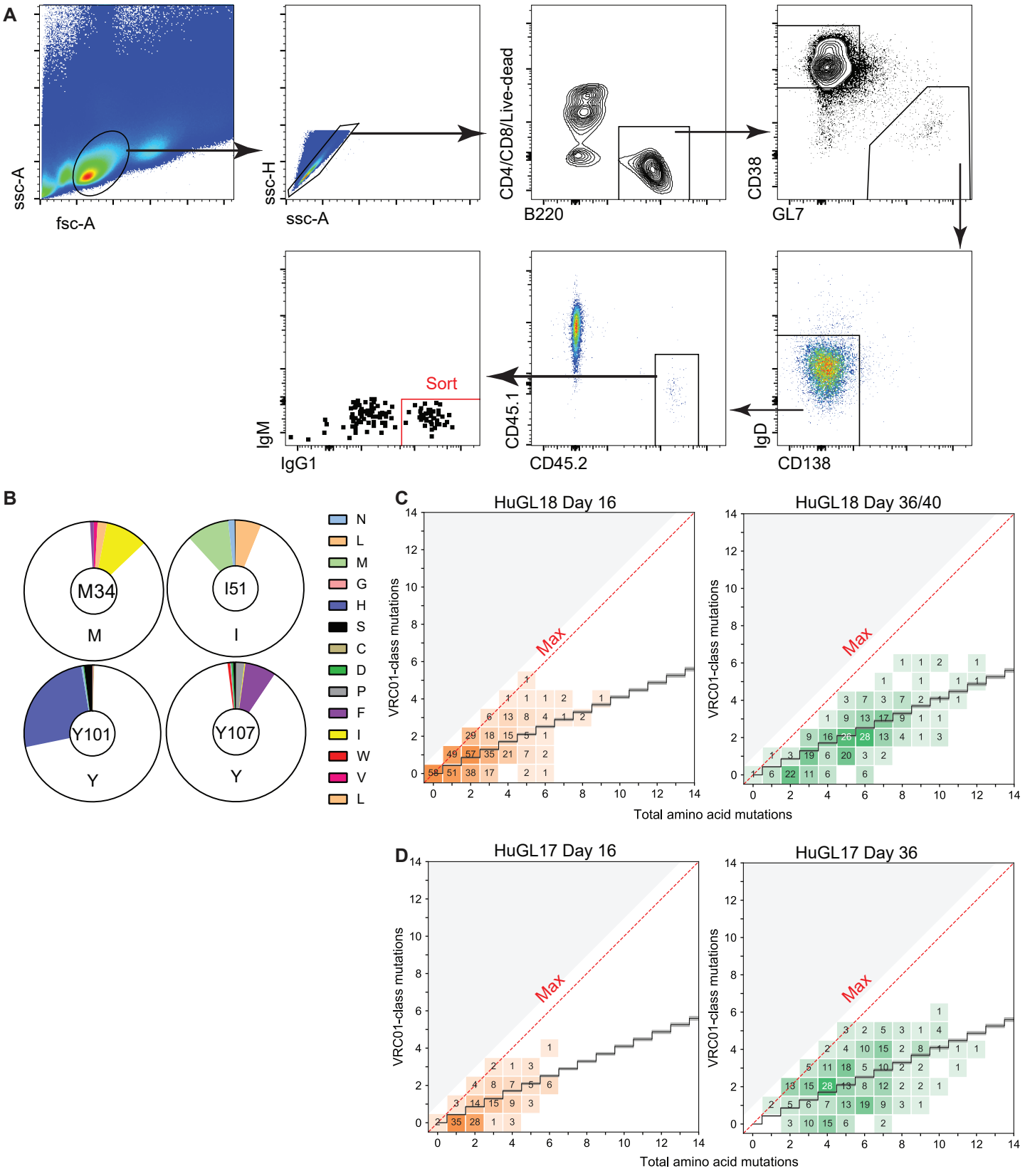

Figure S4. HuGL18 day 16 extended mutation data and sort layout. (A) Sort layout. (B) Amino acid replacements in VH1-2 heavy chain are shown for HuGL18 GC B cells sorted from day 16 mice, as per Figure 5. (C-D) VRC01-class analysis of HuGL18 and HuGL17 GC B cells with expanded VRC01-class antibody set.

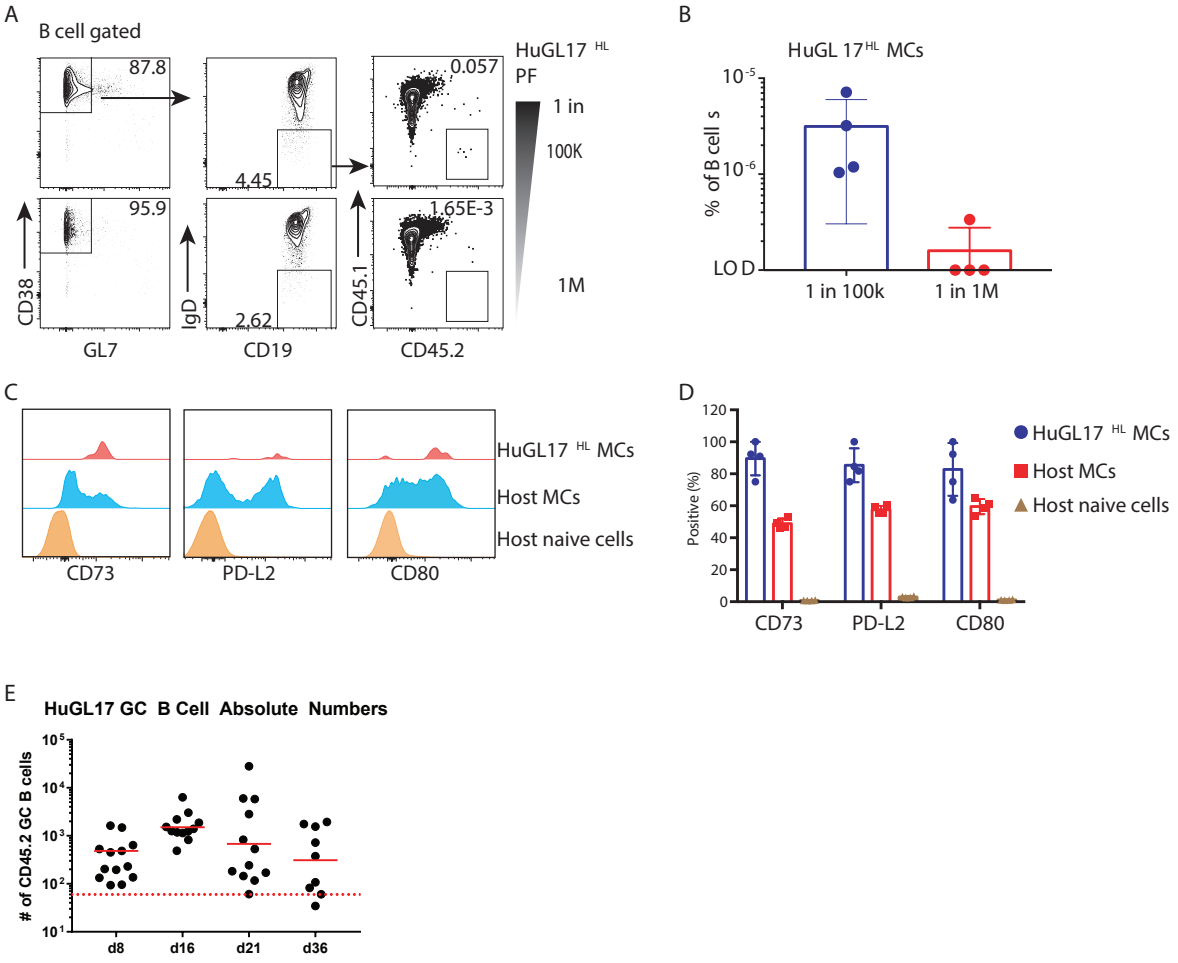

Fig S5. HuGL17 B cells were seeded at 1 in 1 million or 1 in 100,000, challenged with eOD-GT8, and HuGL17 CD45.2 memory B cells were analyzed at d49. **(A)** Gating strategy to enumerate $CD45.2^{+}CD19^{+}CD38^{+}IgD^{-}GL7^{-}$ ("memory B cells"). **(B)** Enumeration of memory B cells as a percentage of total B cells. Each datapoint represents the value obtain in one mouse. **(C,D)** Evaluation of memory B cell expression of CD73, PD-L2 and CD80 showing that HuGL17 cells were largely triple positive. (**E**) Absolute numbers of HuGL17 GC B cells over time when starting from rare precursors (1 in 10^6). Red dotted line represents seeding number.

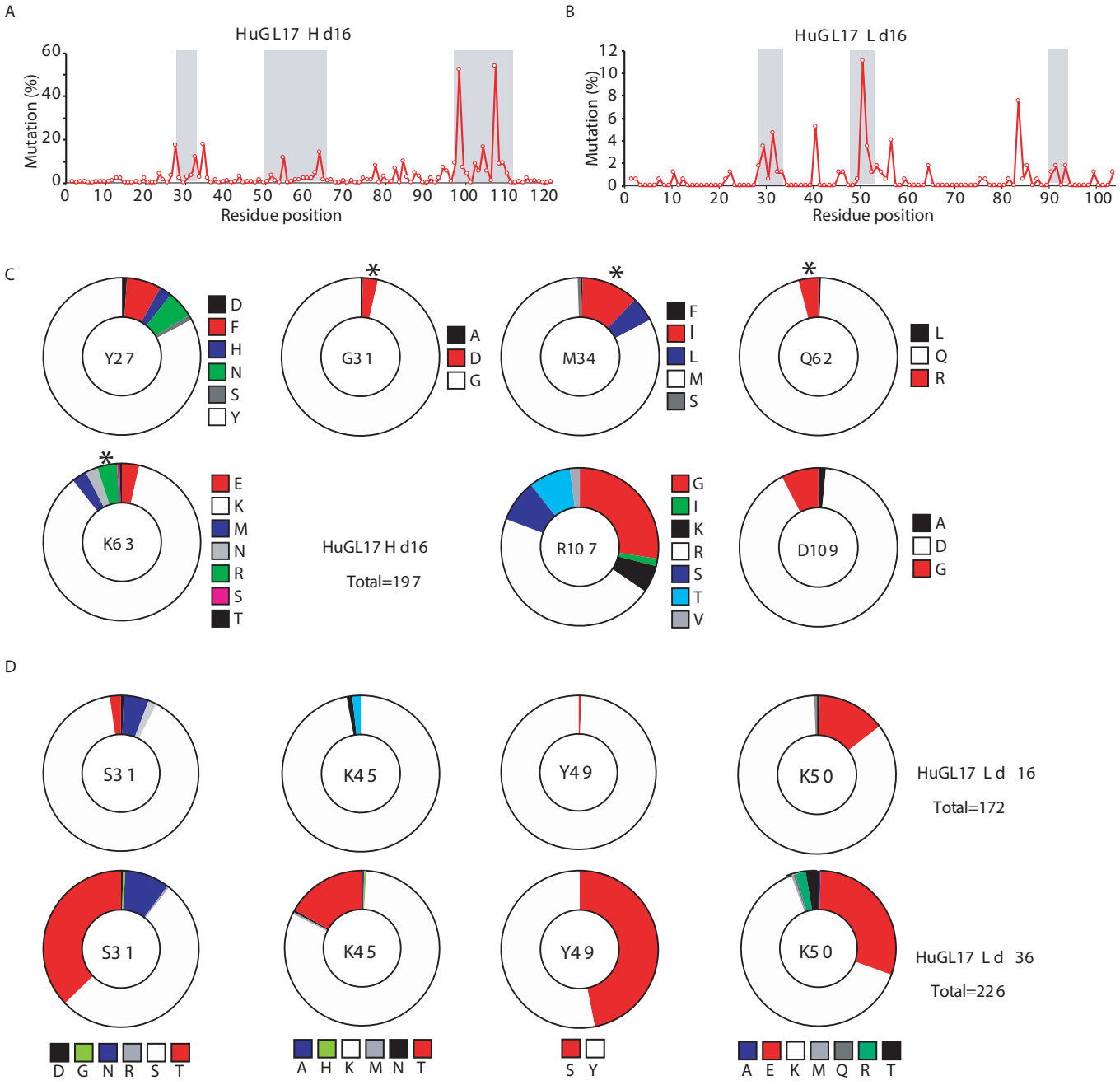

Fig S6. Further analysis of somatic mutations of HuGL17 cells. **(A,B)** Analysis of the extent of mutation on HC and LC on d16 as a function of amino acid position. **(C)** Prominent HC mutations at d16. Asterisks indicate VRC01-class mutations. The extent and variety of changes at R107 suggested selection against R at this position. **(D)** Comparison of prominent LC mutations at d16 and d36. Total sequences analyzed are indicated.

Table S1. Features of VRC01-class germline BCRs identified in human PBMC compared to a VRC01 iGL.

Name	H-chain V/D/J	CDRH3	L-chain V/J	CDRL3	GT8 K_D
HuGL16	VH1-2*02 D6-13*01 JH4*02	ARVRYGSWTGYYFDY	VK1-33*01 JK4*01	CQQYDLF	18.5 μM
HuGL17	VH1-2*02 D6-13*01 JH4*02	ARVIRSSSSWRYDY	VK1-05*03 JK1*01	CQQYETF	1.3 μM
HuGL18	VH1-2*02 D6-13*01 JH4*02	ARDHQGHSSSWSKRFDY	VK3-20*01 JK1*01	CQQYETF	125 nM
gl-VRC01	VH1-2*02 D2 JH1*01	ARGKNSDYNWDFQH	VK3-15*01 JK2*01	CQQYEFF	30 pM

Table S2: Example flow cytometry staining panel.

Marker	Fluorophore	Laser	Clone	Vendor	Cat No	Dilution
CD80	BV421	405	16-10A1	Biolegend	104726	1:50
IgD	BV510	405	11-26c.a2	Biolegend	405723	1:400
CD73	BV605	405	TY/11.8	Biolegend	127215	1:50
CD138	BV650	405	281-2	Beckton Dickenson	564068	1:400
CD45.1	BV711	405	A20	Biolegend	110739	1:200
B220	BV786	405	RA3-6B2	Biolegend	103245	1:200
CD45.2	FITC	488	104	Biolegend	109806	1:200
GL7	Percp-cy5.5	488	GL7	Biolegend	144610	1:200
PDL2	PE	561	TY25	Biolegend	107206	1:50
IgG1	PE-CF594	561	A85-1	Beckton Dickenson	562559	1:200
CD38	PE-Cy7	561	90	Biolegend	102718	1:400
IgM	APC	640	II/41	Beckton Dickenson	550676	1:200
CD4	APC-Fire 750	640	GK1.5	Biolegend	100460	1:400
CD8	APC-Fire 750	640	53-6.7	Biolegend	100766	1:400
Live/dead	eFluor 780	640	N/A	Thermo Fisher	65-0865-18	1:500