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Supplementary Materials for

mRNA-LNP HIV-1 trimer boosters elicit precursors to broad neutralizing antibodies

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The PDF file includes:

Figure S1

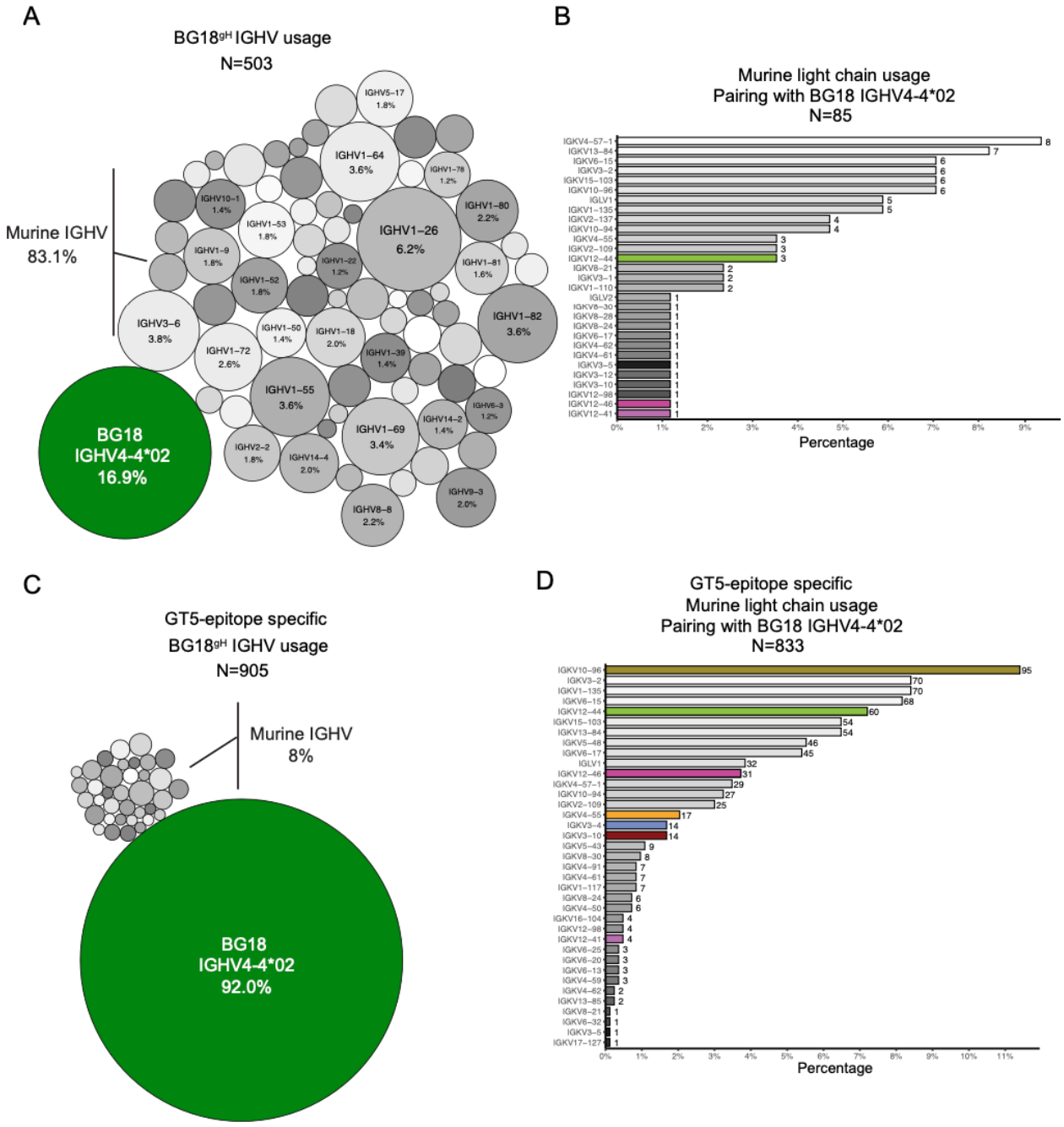

Figure S1. Characterization of naive BG18^{gH} mouse BCR repertoire, related to Figure 1. (**A, C**) 10x Genomics single-cell BCR sequences pooled from two naive BG18^{gH} mice. (**A**) total (B220^{+}) B cells and (**C**) (B220^{+}GT5^{+}KO^{-}) epitope-specific binders. Relative bubble size shows

the frequency of IGHV gene usage in splenic B cells. Human BG18 in green; murine IGHVs are presented with greyscale. (**B, D**) Bar graphs representing frequency and diversity of murine light chain V genes paired with BG18 IGH from (**A**) total sample and (**C**) GT5-epitope-specific binders. Murine light chains found in the GC after immunization are marked with bright colors; all others are in greyscale.

Figure S2

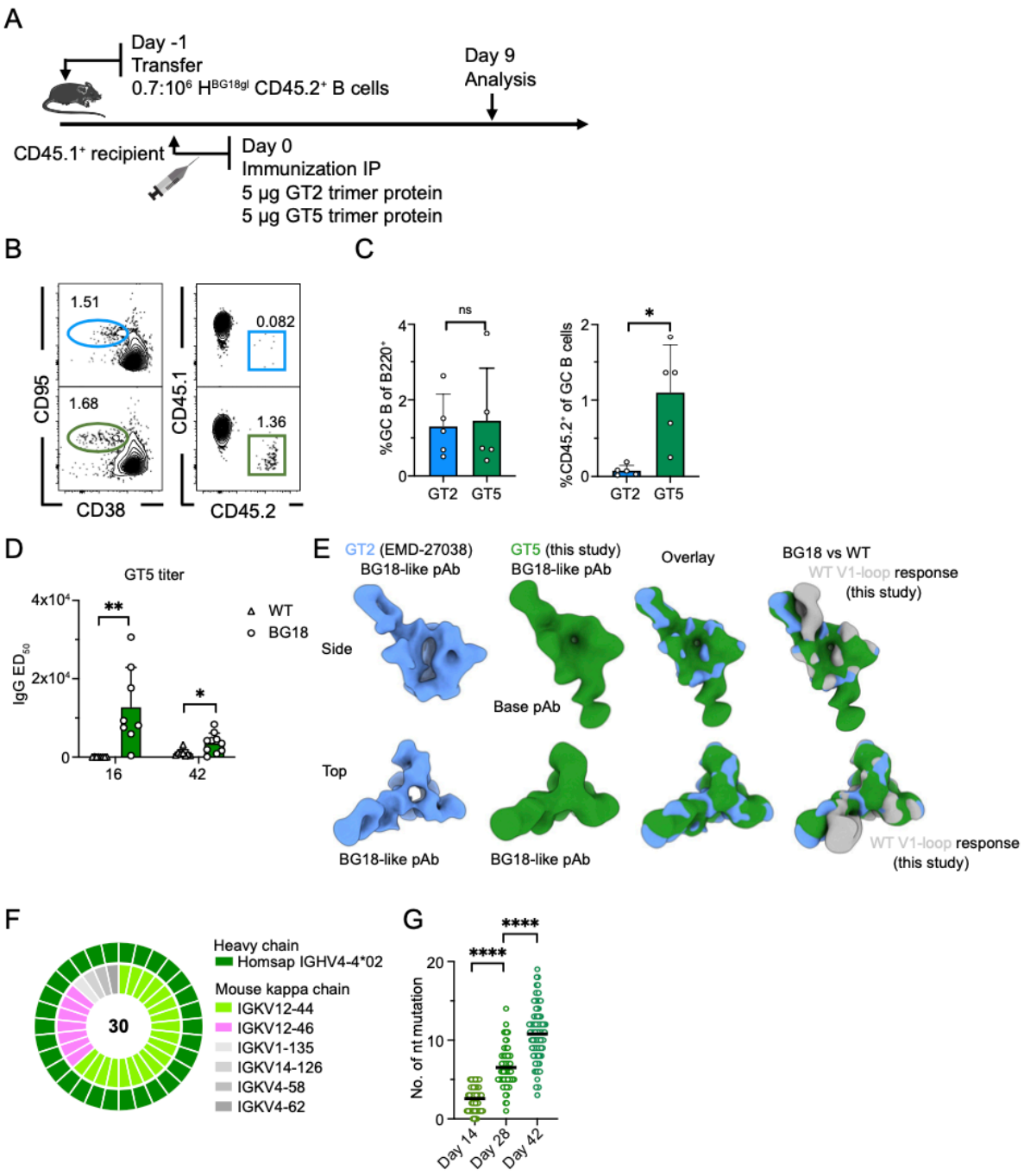

Figure S2. N332-GT5 trimer protein is sufficient to activate BG18^{gH} precursors, related to Figures 1 and 2. (**A**) Schematic showing BG18^{gH} B cells adoptive transfer recipients (0.7:10^6)

primed with GT2 or GT5 trimer protein. Spleen samples were collected at day 9 for analysis; results in B and C. Data collected from a single experimental run. (**B**) Representative flow cytometry plots of GC and CD45.2^{+} cells in the GC. Blue represents GT2 and green represents GT5 immunization. (**C**) GC as a percentage of total B cells and percentage of CD45.2^{+} B cells in GC. Bars indicate mean + SD. *$p < 0.05$. (**D–F**) Related to **Figure 2.** (**D**) Serum IgG ELISA 50% equilibrium dilution values for GT5 16 and 42 days after GT5 protein immunization. Each symbol represents a different mouse from experiments in each condition: WT (GT5), BG18^{gH} (GT5). Samples were pooled from 2–3 experiment with 8–10 mice per group. Statistical analysis was made using 2-way ANOVA with Bonferroni multiple comparison test. Bars indicate mean + SD. *$p < 0.05$, **$p < 0.01$. (**E**) Composite nsEMPEM maps of serum pAb responses in immunized animals after N332-GT2 or N332-GT5 trimer protein immunization. (**F**) Paired BCR V region sequence isolated from CD45.2 epitope specific binders (GT5^{+}KO^{-}) 14 days after GT5 protein immunization (see main **Figure 2F**). (**G**) Heavy chain nucleotide (nt) mutations of all sites of BCRs isolated from CD45.2^{+}GT5^{+}KO^{-}B cells 14, 28 and 42 dpi. Statistical analysis was made using Kruskal-Wallis test. Bars indicate mean. ****$p < 0.0001$.

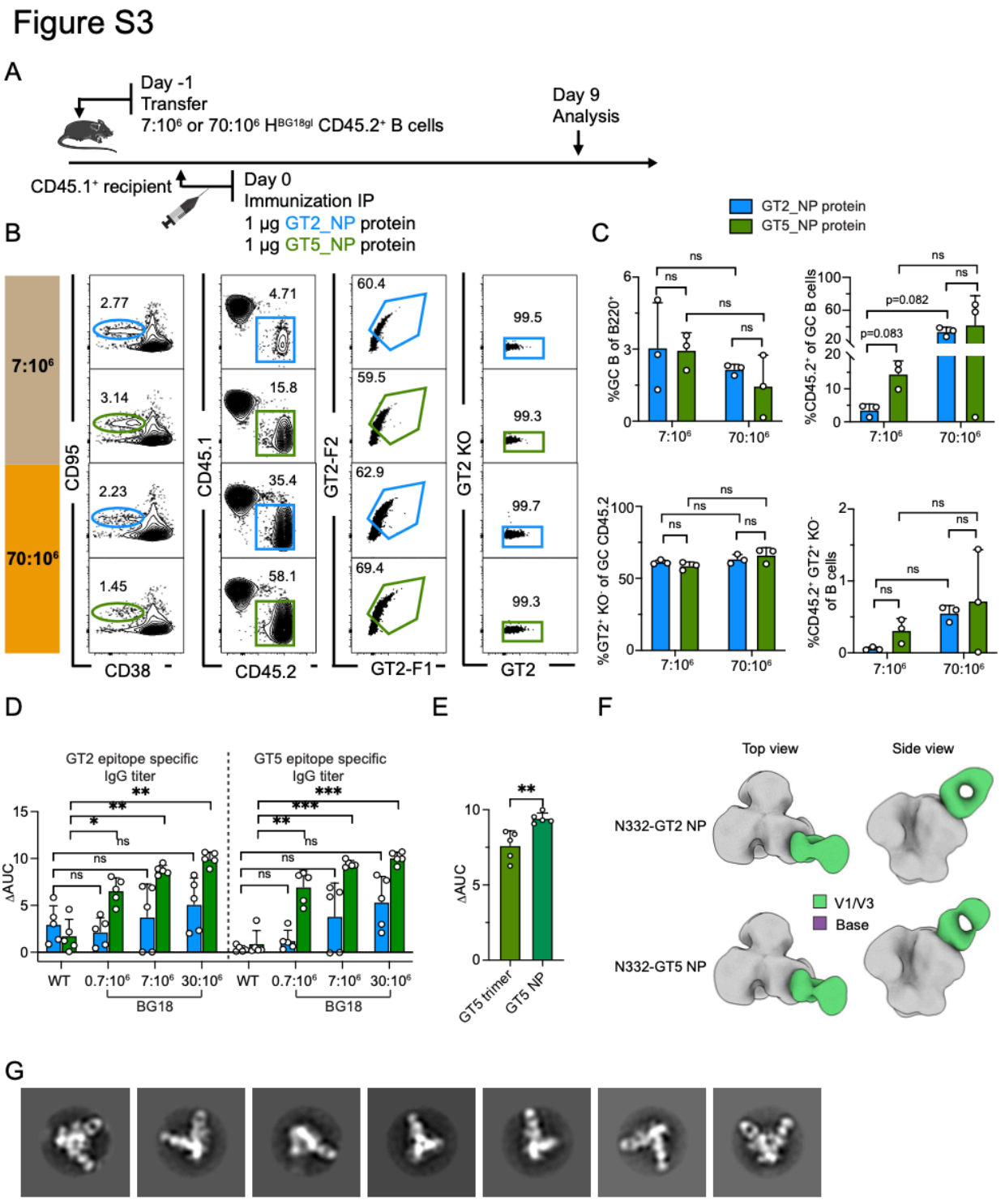

Figure S3. N332-GT2 and N332-GT5 nanoparticle proteins induce robust BG18^{gH} precursor activation, related to Figure 1. (**A**) Schematic. Mice were adoptively transferred with variable frequencies of BG18^{gH} B cells (day -1) and primed with either 1 µg of GT2 or GT5 nanoparticle (NP) proteins (day 0). Spleen samples were collected on day 9 for analysis. Data collected from a single experimental run. (**B**) Flow cytometry plots showing GC, CD45.2^{+} cell in GC, GT2 binders

of CD45.2 cells in GC and binding specificity. Upper panels $7{:}10^6$; lower panels $70{:}10^6$. (**C**) Frequencies of total GC B cells, CD45.2 cells in GC, GT2 specific binders of $CD45.2^+$ GC B cell and GT2 specific $CD45.2^+$ B cell binders in total B cells 9 dpi. Statistical analysis used 2-way ANOVA with Bonferroni multiple comparisons test. Bars indicate mean + SD. $^{ns}p > 0.05$; note scale change above axis break. (**D**) ELISA quantification of N332-GT2 or N332-GT5-epitope specific IgG in WT mice or $BG18^{gH}$ adoptively transferred mice 15 dpi by N332-GT2 or N332-GT5 NP protein. ΔAUC compared for either N332-GT2 and N332-GT2-KO (left) or N332-GT5 and N332-GT5-KO (right) broken by dashed line. Each symbol represents a different mouse. Blue bar represents GT2 NP and Green bar represents GT5 NP immunization. The $BG18^{gH}$ precursor frequency was applied as shown in x axis, n=5. Brown-Forsythe and Welch ANOVA with Dunnett's T3 multiple comparison test used; bars indicate mean + SD. $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. (**E**) ELISA quantification of N332-GT5-epitope specific IgG in $BG18^{gH}$ ($7{:}10^6$) adoptively transferred mice 15 dpi by either N332-GT5 trimer or N332-GT5 NP protein. ΔAUC compared for N332-GT5 and N332-GT5-KO. Each symbol represents a different mouse, n=5. Unpaired t test used; bars indicate mean + SD. $^{**}p < 0.01$. Note: values of N332-GT5 NP are as presented in (**D**). (**F**) Composite nsEMPEM maps of serum pAb responses in immunized animals, 14 or 15 days after N332-GT2_NP (top) or N332-GT5_NP (bottom) immunization, respectively, with no antibodies against the base visible in 3D classification. (**G**) Representative 2D class averages shown below. Sera were pooled for each group: N332-GT2_NP, n=8; N332-GT5_NP, n=5. Both groups have detectable antibodies against the V1/V3 epitope, with no antibodies against the base visible in 2D classification.

Figure S4

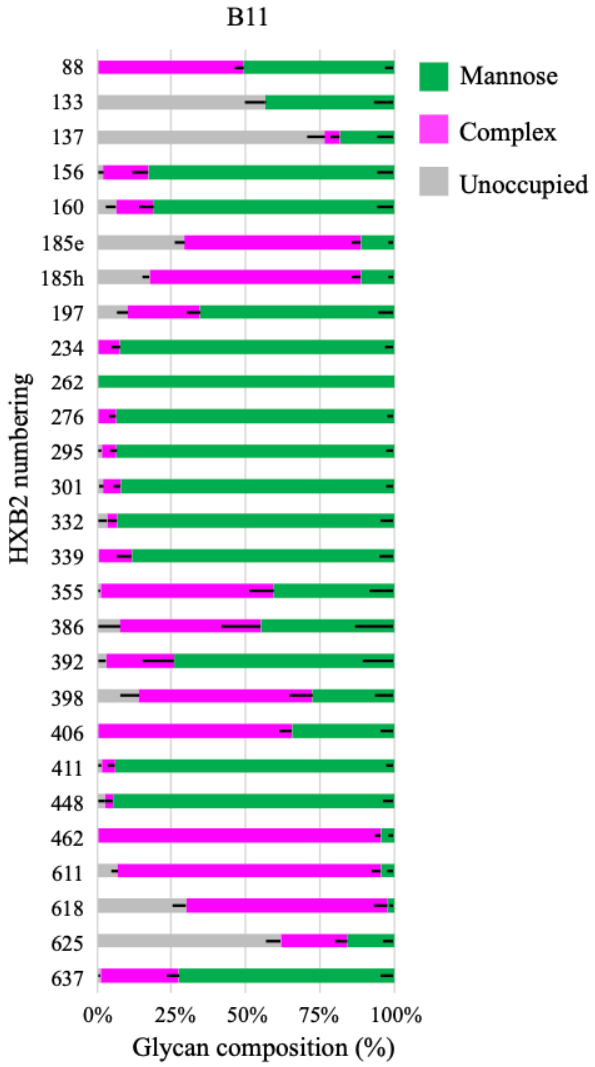

Figure S4. Glycan composition of the B11 trimer, related to Fig 3. Mass spectrometry analysis of glycan composition of the B11 trimer. Green (high mannose), pink (complex); gray (unoccupied).

Fig S5

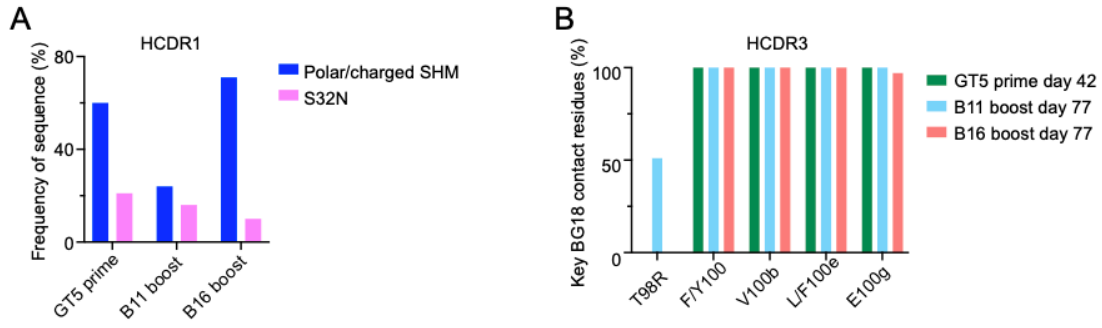

Figure S5. Mutation analysis of key residues after protein prime or boost, related to Figures 2 and 4. (**A**) Frequency of BCR sequences from BG18^{gH} derived HCs, derived as in **Fig. 4**, enriched with polar/charged AA mutations in the HCDR1, including S32N, after priming with GT5 protein or boosting with either B11 or B16 protein. (**B**) Frequency of BCR sequences from BG18^{gH} derived HCs as above containing key BG18 contact residues that were gained or maintained in HCDR3.

Fig S6

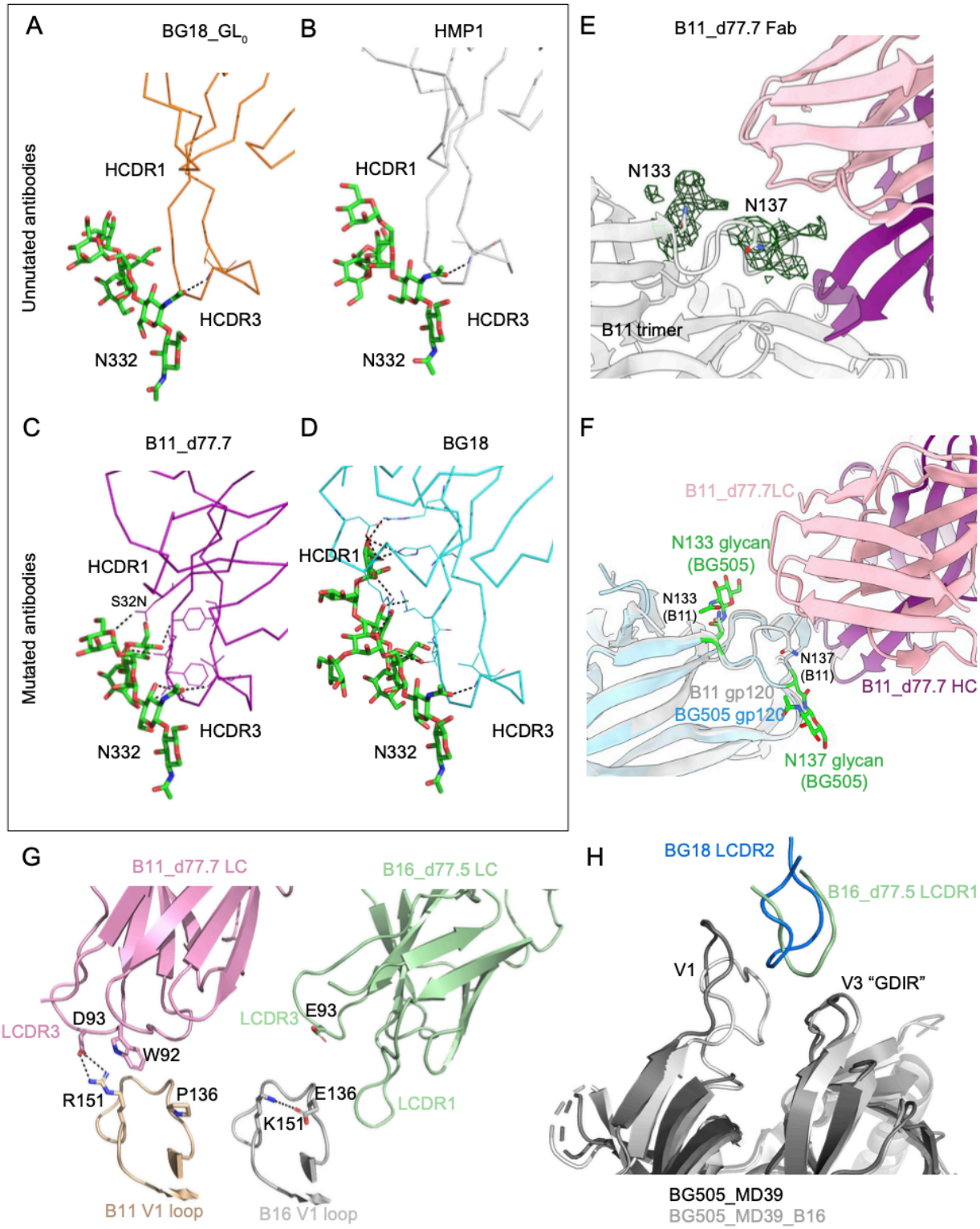
A
BG18_GL0
B
HMP1
Unmutated antibodies
HCDR1
HCDR3
N332
HCDR1
HCDR3
N332
C
B11_d77.7
D
BG18
Mutated antibodies
HCDR1
S32N
HCDR3
N332
HCDR1
HCDR3
N332
E
B11_d77.7 Fab
N133
N137
B11 trimer
F
B11_d77.7LC
N133 glycan
(BG505)
N133
(B11)
N137
(B11)
B11_d77.7 HC
B11 gp120
BG505 gp120
N137 glycan
(BG505)
G
B11_d77.7 LC
B16_d77.5 LC
LCDR3
D93
W92
R151
P136
B11 V1 loop
LCDR3
E93
E136
K151
LCDR1
B16 V1 loop
H
BG18 LCDR2
B16_d77.5 LCDR1
V1
V3 "GDIR"
BG505_MD39
BG505_MD39_B16

Figure S6. Increased polar interactions to the N332 glycan in mutated antibody B11_d77.7, related to Figure 4. Polar interactions to the N332 glycan are shown from the HCDR1 and HCDR3 of (**A**) BG18_GL0 (PDB ID: 6DFH); (**B**) HMP1 (PDB ID: 6NF5); (**C**) B11_d77.7 (this study); (**D**) BG18 (PDB ID: 6DFG); (**E**) The N133 and N137 side chains project away from the B11_d77.7 Fab. Partial cryo-EM density (green mesh) of core sugars of N133 and N137 shown (5σ). (**F**) Alignment of BG505 SOSIP gp120 (PDB ID: 6X9R) onto B11 SOSIP gp120 with key modeled V1 glycans displayed as sticks. (**G**) Comparison of the LC interactions of B11_d77.7_Fab and B16_d77.5_Fab with the B11 and B16 V1 loops, respectively. Dashed lines represent distances of < 4 Å. (**H**) A comparison of the LCDR1 of B16_d77.5_Fab (green) and the LCDR2 of mature BG18 (light blue). Both loops make interactions to the conserved base of V3.

Figure S7

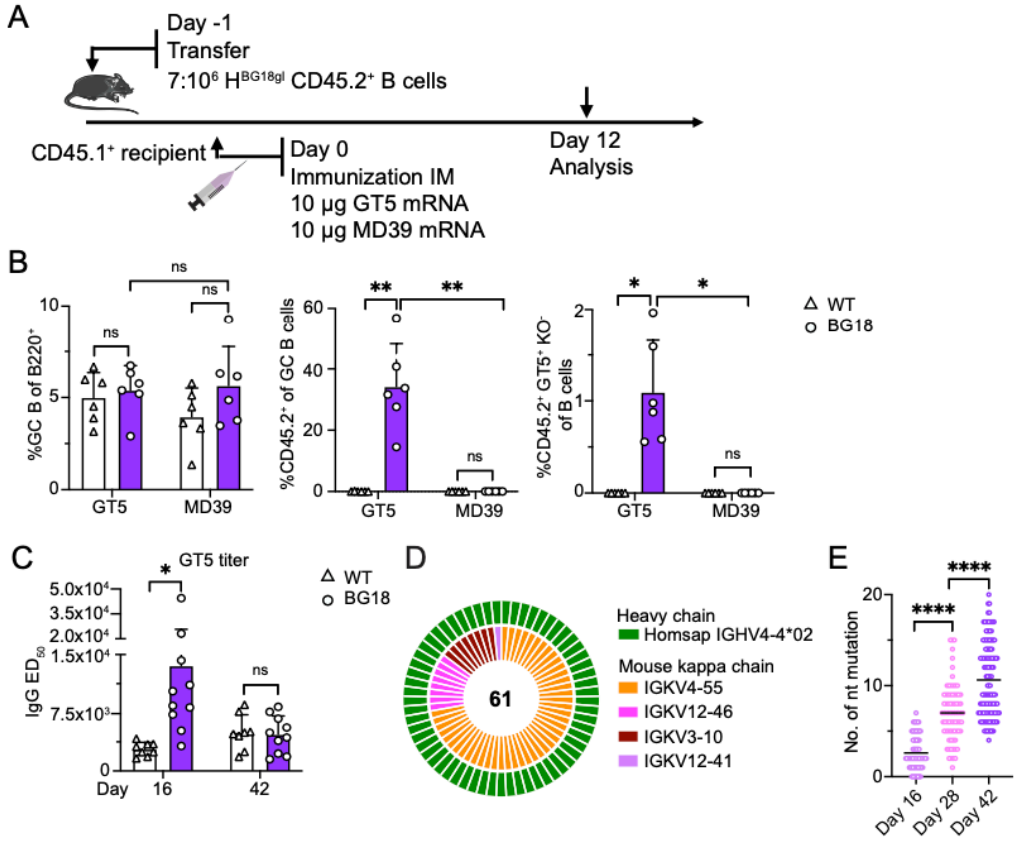

Figure. S7. Membrane-anchored N332-GT5 mRNA but not MD39 mRNA can trigger BG18^{gH} precursor activation and SHM, related to Figure 5. (**A**) Schematic showing BG18^{gH} and WT B cell adoptive transfer recipients immunized with membrane-anchored GT5 or MD39 mRNA. Lymph node samples were collected on day 12 for analysis. Data collected from a single experimental run. (**B**) Frequencies of GC B cells, CD45.2$^+$ B cell in GCs and epitope-specific (GT5$^+$KO$^-$) CD45.2$^+$ cells as a proportion of total B cells. WT (GT5 mRNA), BG18^{gH} (GT5 mRNA), WT (MD39 mRNA), BG18^{gH} (MD39 mRNA); n=6 in each group. Statistical analysis using 2-way ANOVA with Bonferroni multiple comparisons test. Bars indicate mean + SD, ^{ns}p >0.05, *p < 0.05, **p < 0.01. (**C**) Serum IgG ELISA 50% equilibrium dilution values for GT5 16 and 42 days after GT5 mRNA immunization (see main **Figure 5D**). Each symbol represents a different mouse from experiments in each condition: WT (GT5 mRNA), BG18^{gH} (GT5 mRNA). Samples were pooled from 2–3 experiments with 8–10 mice per group. Statistical analysis used 2-way ANOVA with Bonferroni multiple comparisons test. Bars indicate mean + SD. ^{ns}p > 0.05, *p < 0.05; note scale change above axis break. (**D**) Paired BCR V region sequence isolated from CD45.2 epitope specific binders (GT5$^+$KO$^-$) 16 days after GT5 mRNA immunization (see main **Figure 5F**). (**E**) Heavy chain nt mutations across all sites (see main **Figure 5G**). Statistical analysis used Kruskal-Wallis test. Bars indicate mean. ****p < 0.0001.

Figure S8

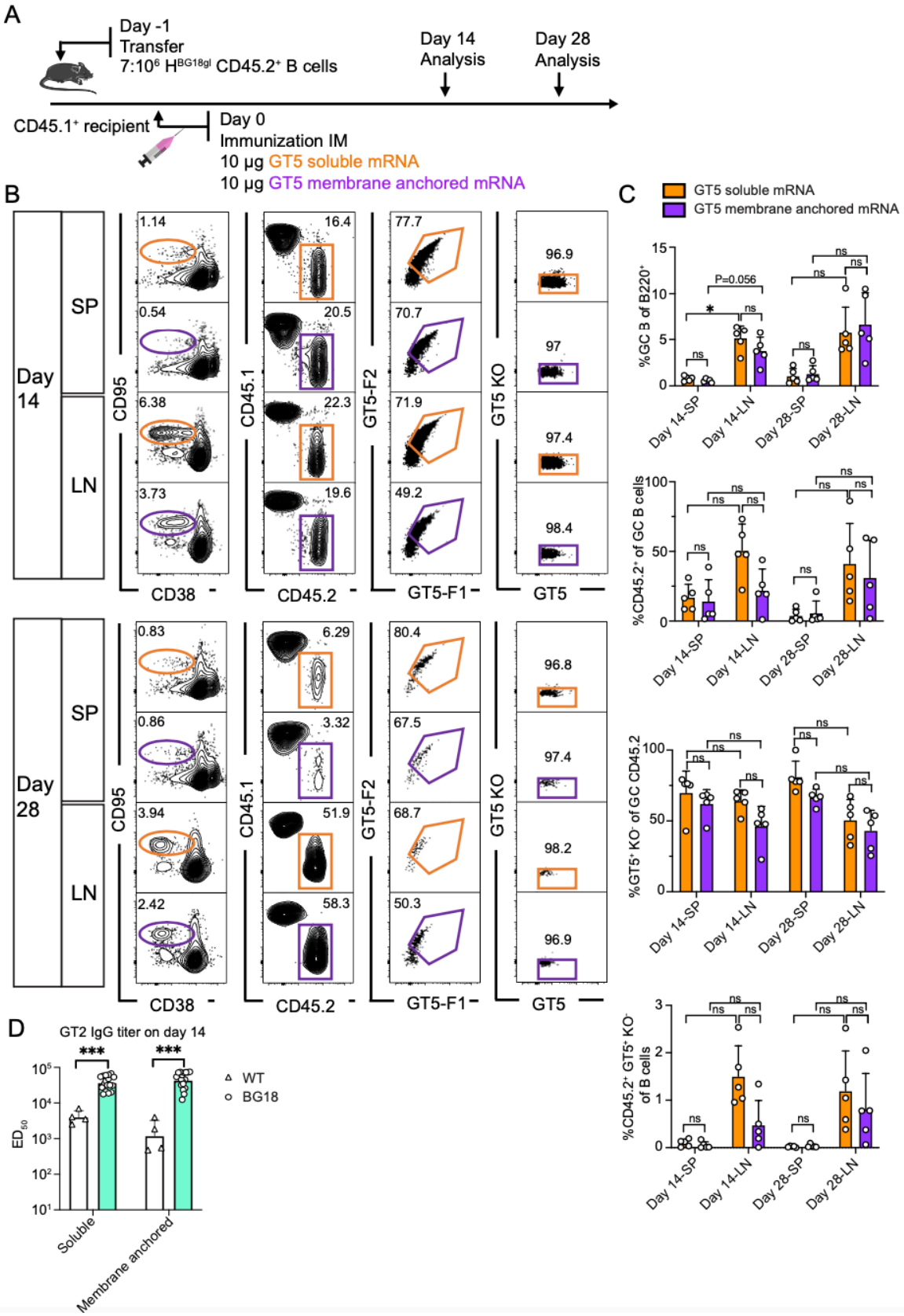
A
Day -1
Transfer
7:10⁶ H^BG18gl CD45.2⁺ B cells
CD45.1⁺ recipient
Day 14
Analysis
Day 28
Analysis
Day 0
Immunization IM
10 µg GT5 soluble mRNA
10 µg GT5 membrane anchored mRNA
B
Day 14
SP
LN
Day 28
SP
LN
CD95
CD38
CD45.1
CD45.2
GT5-F2
GT5-F1
GT5 KO
GT5
1.14
0.54
6.38
3.73
16.4
20.5
22.3
19.6
77.7
70.7
71.9
49.2
96.9
97
97.4
98.4
0.83
0.86
3.94
2.42
6.29
3.32
51.9
58.3
80.4
67.5
68.7
50.3
96.8
97.4
98.2
96.9
C
GT5 soluble mRNA
GT5 membrane anchored mRNA
%GC B of B220⁺
%CD45.2⁺ of GC B cells
%GT5⁺ KO⁻ of GC CD45.2
%CD45.2⁺ GT5⁺ KO⁻ of B cells
P=0.056
ns
Day 14-SP
Day 14-LN
Day 28-SP
Day 28-LN
D
GT2 IgG titer on day 14
***
ED50
WT
BG18
Soluble
Membrane anchored

Figure S8. Soluble and membrane-anchored N332-GT5 mRNA potentiate BG18^{gH} cells for activation efficiently and equivalently, related to Figure 5. (**A**) Schematic showing BG18^{gH} B cell adoptive transfer recipients primed with soluble or membrane-anchored GT5 mRNA via IM on day 0. Spleen (SP), lymph nodes (LN) and serum samples were collected on day 14 and 28 for analysis. Data collected from a single experimental run. (**B**) Representative flow cytometry plots for SP and LN samples showing GC, CD45.2 cells in GC, GT5 binders of CD45.2^{+} cells in GC and GT5 binding specificity 14 days (upper) and 28 days (lower) after soluble or membrane-anchored GT5 mRNA priming. (**C**) Frequencies of GC B cells, CD45.2^{+} cells in GCs, GT5 specific binders among CD45.2^{+} GC B cells, and CD45.2^{+}GT5^{+}KO^{-} B cell in total B cells. (**D**) Serum IgG ELISA 50% equilibrium dilution values for GT2 14 days after GT5 mRNA immunization. Each symbol represents a different mouse from experiments in each condition: WT (soluble GT5 mRNA), n=4; BG18^{gH} (soluble GT5 mRNA), n=17; WT (membrane-anchored GT5 mRNA), n=4; BG18^{gH} (membrane-anchored GT5 mRNA), n=18; Samples are pooled from 2–3 experiments. Statistical analysis performed using 2-way ANOVA with Bonferroni multiple comparisons test (C) or multiple t-tests (D). Bars indicate mean + SD (C) or geometrical mean + geometrical SD (D). $^{ns}p > 0.05$, *$p < 0.05$, ***$p<0.001$

Figure S9

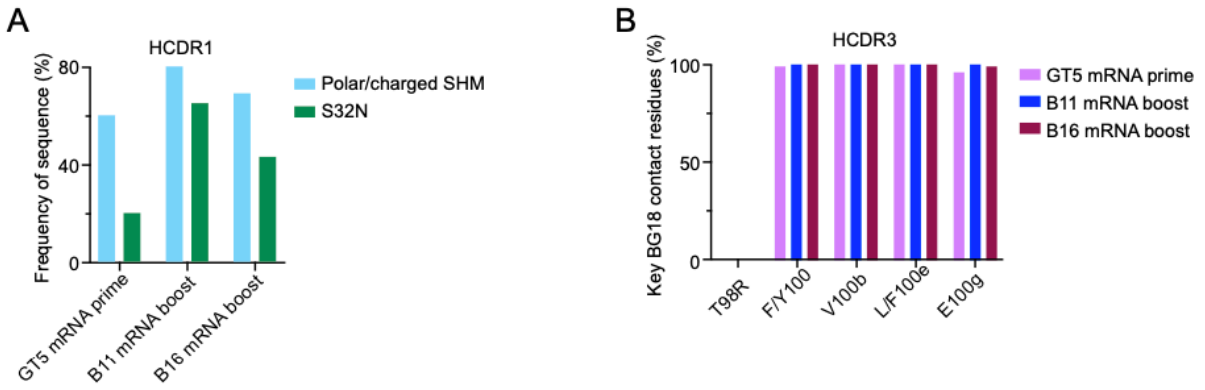

Figure S9. Mutation analysis after mRNA immunization of key contact residues, related to Fig 7. (**A**) Frequency of BCR sequences from BG18^{gH} derived HCs, obtained as described in **Fig. 7,** enriched for polar/charged AA mutations in the HCDR1, including S32N, after priming with GT5 mRNA or boosting with either B11 or B16 mRNA. (**B**) Frequency of BCR sequences from BG18^{gH}-derived HCs, as above, which have gained or maintained key BG18 contact residues in HCDR3.

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BG505_MD65_N332-GT5      AENLWVTVYYGVPVWKDAETTLFCASDAKAYETEKHNVWATHACVPTDPNPQEIHLENVT
BG505_MD39               AENLWVTVYYGVPVWKDAETTLFCASDAKAYETEKHNVWATHACVPTDPNPQEIHLENVT
BG505_MD39_B11           AENLWVTVYYGVPVWKDAETTLFCASDAKAYETEKHNVWATHACVPTDPNPQEIHLENVT
BG505_MD39_B16           AENLWVTVYYGVPVWKDAETTLFCASDAKAYETEKHNVWATHACVPTDPNPQEIHLENVT

BG505_MD65_N332-GT5      EEFNMWKNNMVEQMHEDIISLWDQSLKPCVKLTPLCVTLQCTNYAPKLRSMMRGEIKNCS
BG505_MD39               EEFNMWKNNMVEQMHEDIISLWDQSLKPCVKLTPLCVTLQCTNVTNNITDDMRGELKNCS
BG505_MD39_B11           EEFNMWKNNMVEQMHEDIISLWDQSLKPCVKLTPLCVTLQCTNYTPNLTSMMRGELKNCS
BG505_MD39_B16           EEFNMWKNNMVEQMHEDIISLWDQSLKPCVKLTPLCVTLQCTNYTEKLRSMMKGELKNCS

BG505_MD65_N332-GT5      FNMTTELRDKKQKVYSLFYRLDVVQINENQGNRSNNSNKEYRLINCNTSAITQACPKVSF
BG505_MD39               FNMTTELRDKKQKVYSLFYRLDVVQINENQGNRSNNSNKEYRLINCNTSAITQACPKVSF
BG505_MD39_B11           FNMTTELRDKKQKVYSLFYRLDVVQINENQGNRSNNSNKEYRLINCNTSAITQACPKVSF
BG505_MD39_B16           FNMTTELRDKKQKVYSLFYRLDVVQINENQGNRSNNSNKEYRLINCNTSAITQACPKVSF

BG505_MD65_N332-GT5      EPIPIHYCAPAGFAILKCKDKKFNGTGPCPSVSTVQCTHGIKPVVSTQLLLNGSLAEEEV
BG505_MD39               EPIPIHYCAPAGFAILKCKDKKFNGTGPCPSVSTVQCTHGIKPVVSTQLLLNGSLAEEEV
BG505_MD39_B11           EPIPIHYCAPAGFAILKCKDKKFNGTGPCPSVSTVQCTHGIKPVVSTQLLLNGSLAEEEV
BG505_MD39_B16           EPIPIHYCAPAGFAILKCKDKKFNGTGPCPSVSTVQCTHGIKPVVSTQLLLNGSLAEEEV

BG505_MD65_N332-GT5      IIRSENITNNAKNILVQLNTPVQINCTRPSNNTVKSIRIGPGQAFYYFGDVLGHVRMAHC
BG505_MD39               IIRSENITNNAKNILVQLNTPVQINCTRPNNNTVKSIRIGPGQAFYYTGDIIGDIRQAHC
BG505_MD39_B11           IIRSENITNNAKNILVQLNTPVQINCTRPNNNTVKSIRIGPGQAFYYTGDIIGDIRMAHC
BG505_MD39_B16           IIRSENITNNAKNILVQLNTPVQINCTRPNNNTVKSIRIGPGQAFYYTGDIIGHIRQAHC

BG505_MD65_N332-GT5      NISKATWNETLGKVVKQLRKHFGNNTIIRFAQSSGGDLEVTTHSFNCGGEFFYCNTSGLF
BG505_MD39               NVSKATWNETLGKVVKQLRKHFGNNTIIRFAQSSGGDLEVTTHSFNCGGEFFYCNTSGLF
BG505_MD39_B11           NVSKATWNETLGKVVKQLRKHFGNNTIIRFAQSSGGDLEVTTHSFNCGGEFFYCNTSGLF
BG505_MD39_B16           NVSKATWNETLGKVVKQLRKHFGNNTIIRFAQSSGGDLEVTTHSFNCGGEFFYCNTSGLF

BG505_MD65_N332-GT5      NSTWISNTSVQGSNSTGSNDSLILPCWIKQIINMWQRIGQAMYAPPIQGVIRCVSNITGL
BG505_MD39               NSTWISNTSVQGSNSTGSNDSITLPCRIKQIINMWQRIGQAMYAPPIQGVIRCVSNITGL
BG505_MD39_B11           NSTWISNTSVQGSNSTGSNDSITLPCRIKQIINMWQRIGQAMYAPPIQGVIRCVSNITGL
BG505_MD39_B16           NSTWISNTSVQGSNSTGSNDSITLPCRIKQIINMWQRIGQAMYAPPIQGVIRCVSNITGL

BG505_MD65_N332-GT5      ILTRDGGSTNSTTETFRPGGGDMRDNWRSELYKYKVVKIEPLGVAPTRCKRRTVGRRRRR
BG505_MD39               ILTRDGGSTNSTTETFRPGGGDMRDNWRSELYKYKVVKIEPLGVAPTRCKRRVVGRRRRR
BG505_MD39_B11           ILTRDGGSTNSTTETFRPGGGDMRDNWRSELYKYKVVKIEPLGVAPTRCKRRVVGRRRRR
BG505_MD39_B16           ILTRDGGSTNSTTETFRPGGGDMRDNWRSELYKYKVVKIEPLGVAPTRCKRRVVGRRRRR

BG505_MD65_N332-GT5      RAAGIGASSDGFLGAAGSTMGAASMTLTVQARNLLSGIVQQQSNLLRAPEPQQHLLKDTH
BG505_MD39               RAVGIGAVSLGFLGAAGSTMGAASMTLTVQARNLLSGIVQQQSNLLRAPEPQQHLLKDTH
BG505_MD39_B11           RAVGIGAVSLGFLGAAGSTMGAASMTLTVQARNLLSGIVQQQSNLLRAPEPQQHLLKDTH
BG505_MD39_B16           RAVGIGAVSLGFLGAAGSTMGAASMTLTVQARNLLSGIVQQQSNLLRAPEPQQHLLKDTH

BG505_MD65_N332-GT5      WGIKQLQARVLAVEHYLRDQQLLGIWGCSGKLICCTNVPWNSSWSNRNLSEIWDNMTWLQ
BG505_MD39               WGIKQLQARVLAVEHYLRDQQLLGIWGCSGKLICCTNVPWNSSWSNRNLSEIWDNMTWLQ
BG505_MD39_B11           WGIKQLQARVLAVEHYLRDQQLLGIWGCSGKLICCTNVPWNSSWSNRNLSEIWDNMTWLQ
BG505_MD39_B16           WGIKQLQARVLAVEHYLRDQQLLGIWGCSGKLICCTNVPWNSSWSNRNLSEIWDNMTWLQ

BG505_MD65_N332-GT5      WDKEISNYTQIIYGLLEESQNQQEKNEQDLLALD
BG505_MD39               WDKEISNYTQIIYGLLEESQNQQEKNEQDLLALD
BG505_MD39_B11           WDKEISNYTQIIYGLLEESQNQQEKNEQDLLALD
BG505_MD39_B16           WDKEISNYTQIIYGLLEESQNQQEKNEQDLLALD

>N332-GT5-NP
AENLWVTVYYGVPVWKDAETTLFCASDAKAYETEKHNVWATHACVPTDPNPQEIHLENVTEEFNMWKNNMVEQMHEDIISLWDQSLKPCVKLTPLCVTLQCTNYAPKLRSMMRGEIKNC
SFNMTTELRDKKQKVYSLFYRLDVVQINENQGNRSNNSNKEYRLINCNTSAITQACPKVSFEPIPIHYCAPAGFAILKCKDKKFNGTGPCPSVSTVQCTHGIKPVVSTQLLLNGSLAEE
EVIIRSENITNNAKNILVQLNTPVQINCTRPSNNTVKSIRIGPGQAFYYFGDVLGHVRMAHCNISKATWNETLGKVVKQLRKHFGNNTIIRFAQSSGGDLEVTTHSFNCGGEFFYCNTS
GLFNSTWISNTSVQGSNSTGSNDSLILPCWIKQIINMWQRIGQAMYAPPIQGVIRCVSNITGLILTRDGGSTNSTTETFRPGGGDMRDNWRSELYKYKVVKIEPLGVAPTRCKREVVGR
RRRRRAVGIGAVSLGFLGAAGSTMGAASMTLTVQARNLLSGIVQQQSNLLRAPEPQQHLLKDTHWGIKQLQARVLAVEHYLRDQQLLGIWGCSGKLICCTNVPWNSSWSNRNLSEIWDN
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Figure S10. Amino acid sequence of protein constructs used in this study, related to Methods. Green highlights indicate amino acid changes relative to BG505_MD39. MD65 mutations have been reported in (59).

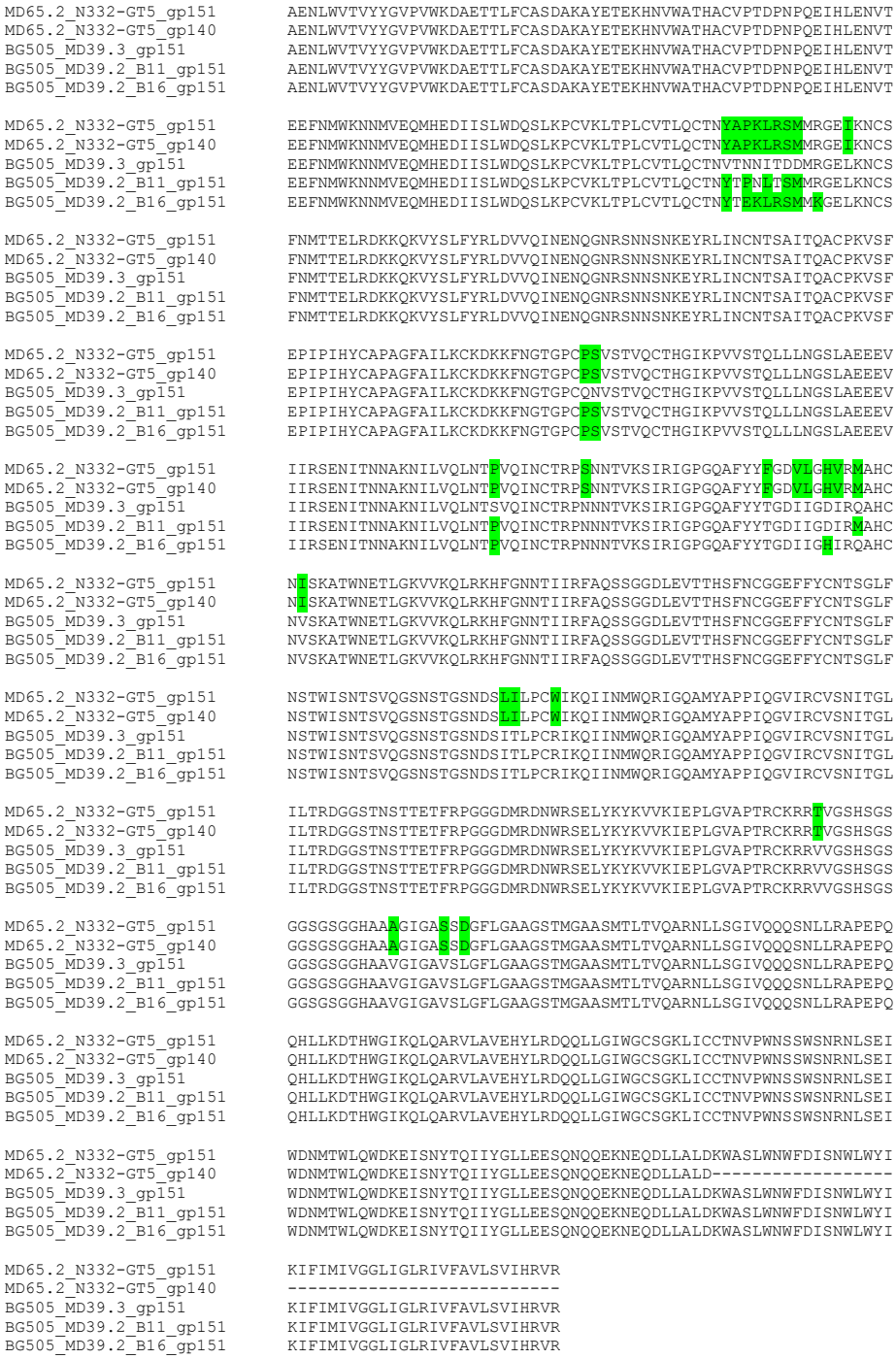

Figure S11. Amino acid sequence of mRNA constructs used in this study, related to Methods. gp151 indicates membrane bound trimer (*52*); gp140 indicates soluble trimer. Green highlights indicate amino acid changes relative to BG505_MD39.3_gp151. The addition of .2 to the name indicates the furin cleavage site has been replaced with a 14 aa linker (*19*). The addition of .3 to the name indicates the presence of the 14 aa linker at the furin cleavage site as well as the addition of glycosylation sites at positions 241 and 289 (*101*).

Table S1. CryoEM data collection, processing and model building statistics.

Map	**BG505_MD64_N332-GT5 + BG18HCgl mice polyclonal Fab**	**BG505_MD64_N332-GT5 + WT mice polyclonal Fab**	**BG505_MD39_B11 + B11_d77.7 Fab + RM20A3 Fab**	**BG505_MD39_B16 + B16_d77.5 Fab + RM20A3 Fab**
EMDB	EMD-28942	EMD-28945	EMD-28941	EMD-43190
Data collection				
Microscope	TFS Titan Krios	TFS Glacios	TFS Talos Arctica	TFS Glacios
Voltage (kV)	300	200	200	200
Detector	Gatan K3	TFS Falcon 4	Gatan K2 Summit	TFS Falcon 4
Recording mode	Super-resolution	Counting	Counting	Counting
Nominal magnification	29,000x	190,000x	36,000x	190,000x
Movie micrograph pixelsize (Å)	0.40075	0.725	1.15	0.725
Number of frames (Falcon 4 EER fractions)	60	40	49	40
Total dose ($e^-/Å^2$)	79	40	51	45
Defocus range (μm)	-0.8 to -2.5	-0.5 to -1.8	-0.7 to -1.5	-0.5 to -1.8
EM data processing				
Number of movie micrographs	9,459	9,547	2,072	3,510
Number of molecular projection images in map	23,292	11,058	129,821	39,822
Symmetry	C1	C1	C3	C1
Map pixel size	0.8015	1.0457	1.15	1.044
Map resolution (FSC 0.143; Å)	3.2	4.1	3.1	3.3
Map sharpening B-factor ($Å^2$)	-58.1	-22.3	-97.9	43
Structure building and validation				
Number of atoms in deposited model				
HIV Env trimer	13,080	13,136	13,191	13,221
Polyclonal Fv (as poly-alanine)	1,160	1,115	*n/a*	*n/a*
Monoclonal Fv	*n/a*	5,178	10,611	7,065
glycans	914	884	1,080	954
MolProbity score	0.74	1.18	0.97	1.14
Clashscore	0.76	2.24	0.84	2.86
Map correlation coefficient	0.74	0.75	0.83	0.84
EMRinger score	3.18	1.48	3.69	3.76
d FSC model (0.5; Å)	3.7	4.3	3.2	3.4
RMSD from ideal				
Bond length (Å)	0.006	0.005	0.006	0.007
Bond angles (°)	1.245	0.870	1.267	1.197
Ramachandran plot				
Favored (%)	98.26	96.97	96.70	97.69
Allowed (%)	1.74	3.03	3.30	2.31
Outliers (%)	0.00	0.00	0.00	0.00
Side chain rotamer outliers (%)	0.14	0.20	0.00	0.27
Cβ outliers (%)	0.00	0.00	0.00	0.04
PDB	8f9g	8f9m	8f92	8vfv

Table S2. Table of reagents

Antibodies	**Supplier**	**Cat_number**
BV786-B220	BD Bioscience	563894
Alexa Fluor 594-B220	BioLegend	103254
Alexa Fluor 700-Gr1	BioLegend	108422
Alexa Fluor 700-CD3	BioLegend	100216
Alexa Fluor 700-F4/80	BioLegend	123130
BV510-CD38	BD Bioscience	740129
PE-Cy7-CD95	BD Bioscience	557653
PerCP Cy5.5-CD45.1	BioLegend	110728
PE-CD45.2	BioLegend	109808
BV786-CD45.2	BD Bioscience	563686
BUV395-IgG1	BD Bioscience	742481
APC-Cy7-IgM	BioLegend	406516
BV650-IgD	BioLegend	405721
Commercial chemicals		
LIVE/DEAD™ Fixable Blue Dead Cell Stain Kit	Invitrogen	L23105
Alexa Fluor 647-Streptavidin	BioLegend	405237
BV421- Streptavidin	BD Bioscience	563259
Alexa Fluor 488-Streptavidin	BioLegend	405235
Alexa Fluor 594-Streptavidin	BioLegend	405240
Sigma Adjuvant	Sigma	S6322
ACK lysing buffer	Lonza	BP10-548E