Immunity, Volume *55*

Supplemental information

Antibodies from primary humoral responses modulate the recruitment of naive B cells during secondary responses

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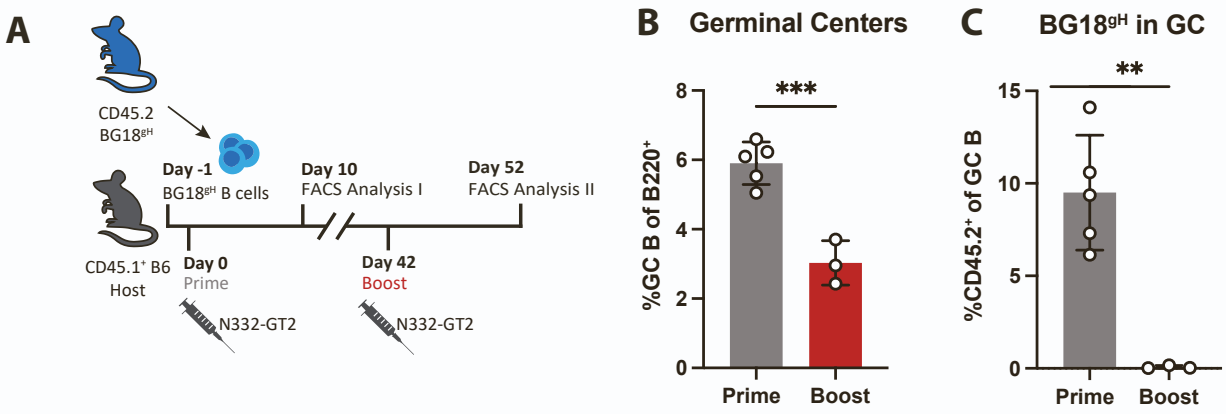

Figure S1. BG18^{gH} prime-boost response, related to Figure 1

A. Schematic representation of experimental design to compare the memory and naïve responses of BG18^{gH} B cells.

B. Quantification of GC cells as percentage of total B cells and **C.** Quantification of CD45.2^{+} BG18^{gH} cells as percentage of total GC B cells at experiment day 10 and 52 (10 days post primary and secondary immunization).

All p values were calculated by unpaired student's t test (** $p < 0.01$; *** $p < 0.001$).

Figures represent data from one of two experiments with 3-5 mice per condition. Data presented as mean ± SD

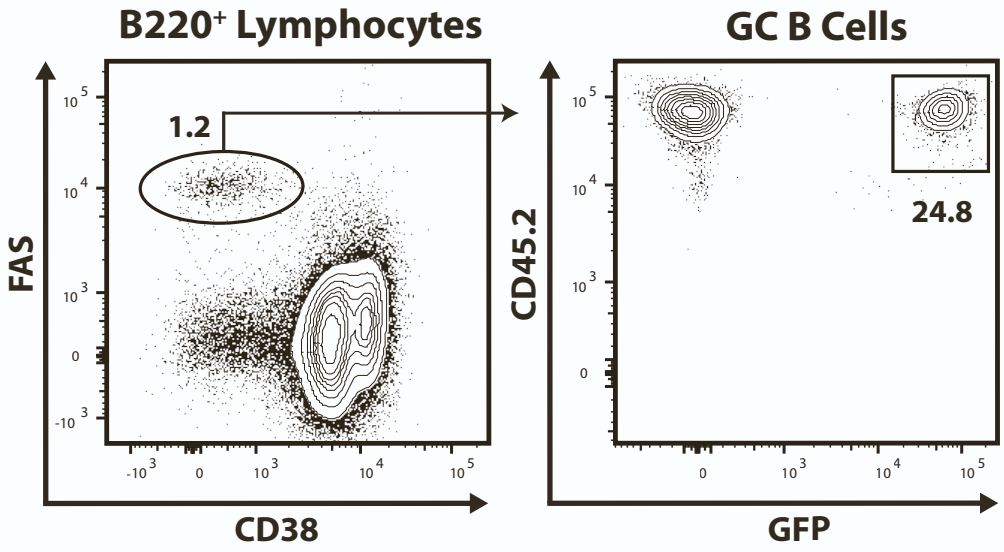

Figure S2. BG18gH-GFP gating strategy, related to Figure 1

Representative FACS plots at experiment day 52 (10 dpi) showing gating strategy to quantify $FAS^{+}CD38^{-}$ GC B cells and GFP^{+} $BG18^{gH}$ GC B cells.

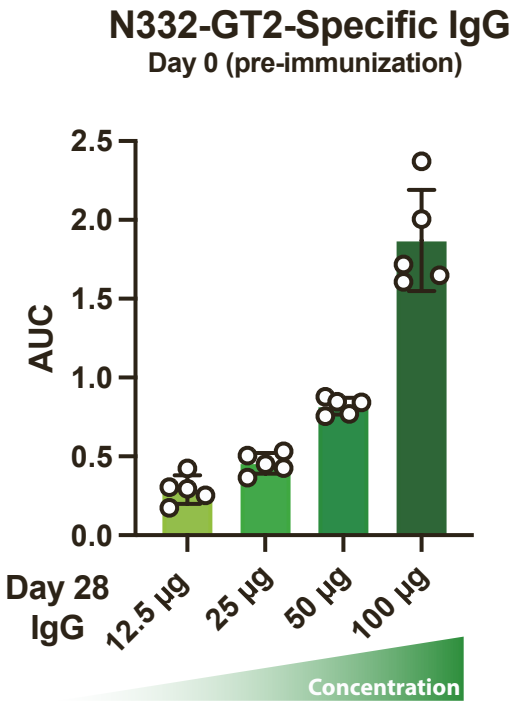

Figure S3. N332-GT2 specific IgG titers after transfer of isolated serum IgG, related to Figure 1

ELISA quantification of N332-GT2 binding serum IgG in naive recipients at day 0 after receiving intravenous serum IgG from previously immunized animal, prior to immunization. Data from one experiment with 5 mice per condition presented as mean ± SD

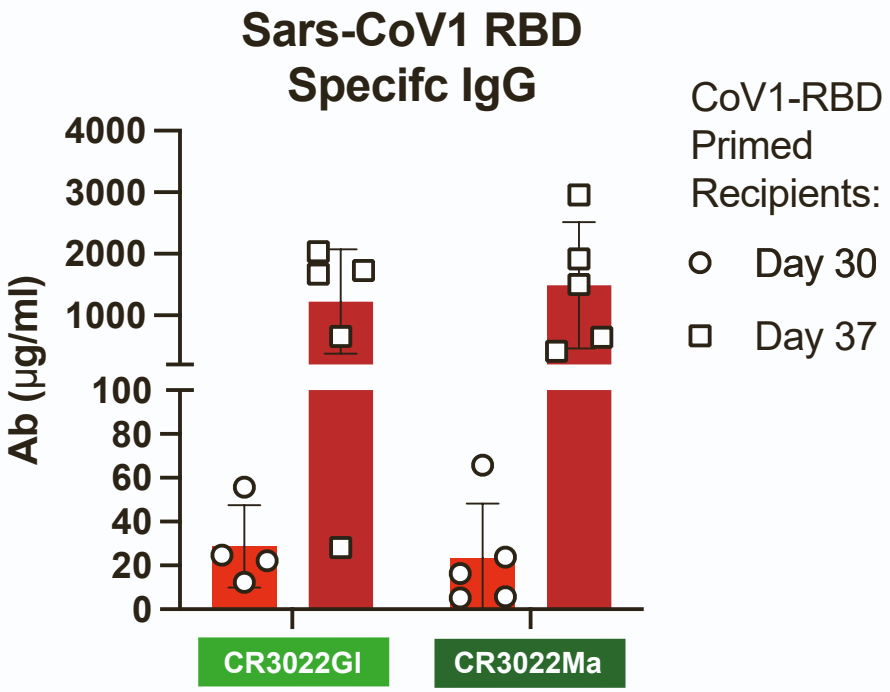

Figure S4. SARS-CoV-1 antibody titers in CR3022 recipients, related to Figure 2

SARS-CoV1-RBD-specific antibody titers determined by ELISA in primed CR3022Gl/Ma recipients pre- (day 30, light red) and post- (day 37, dark red) boost.

Data from one experiment with 4-5 mice per condition presented as mean ± SD

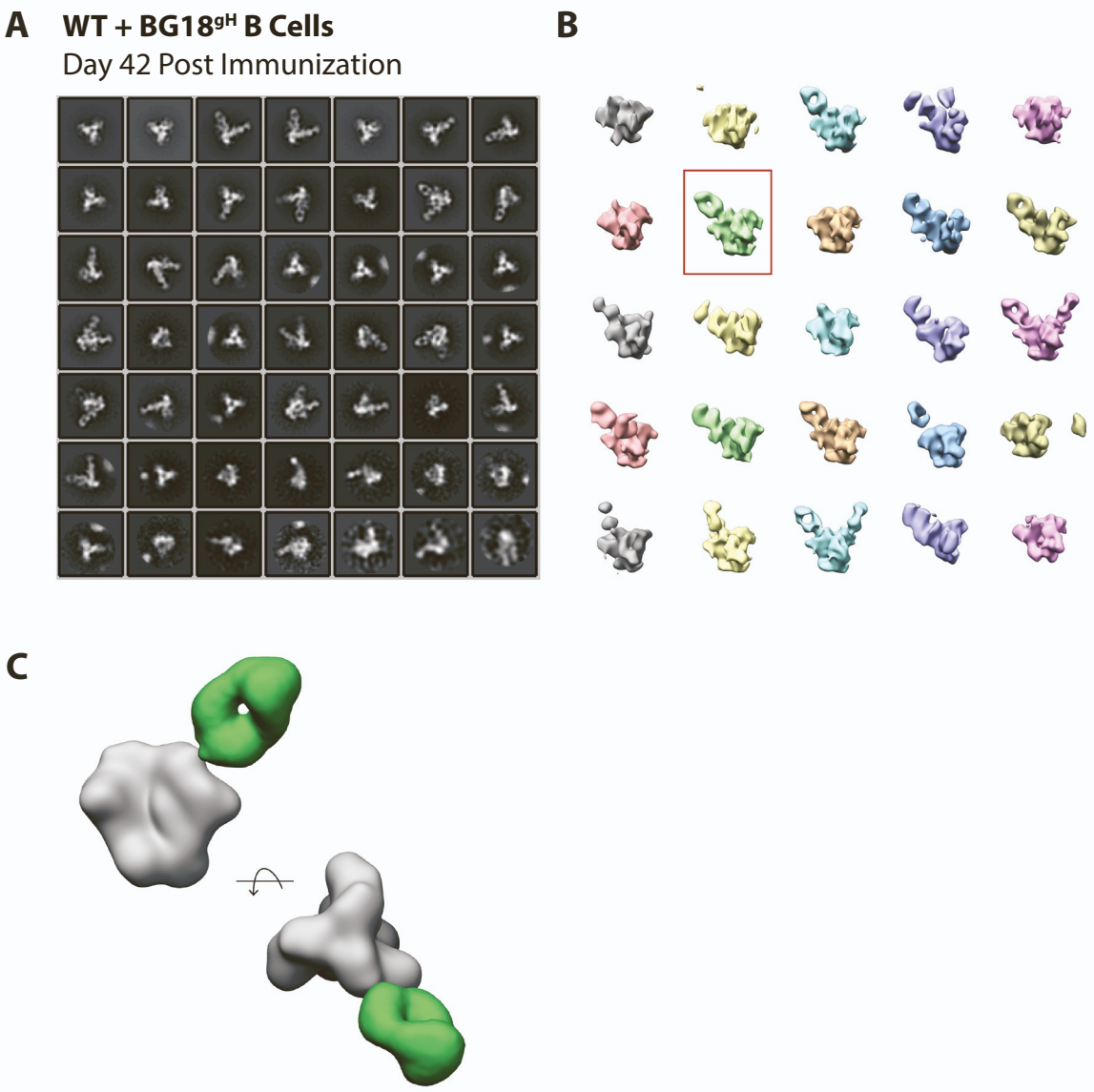

Figure S5. Data processing workflow for negative stain Electron Microscopy Based Polyclonal Epitope Mapping (nsEMPEM), related to Figure 3

A. Negative stain electron microscopy 2D class averages of polyclonal Fab from BG18 knock-in mice 42 days post immunization in complex with N332-GT2 trimer. **B.** Particles from the 2D class averages were sorted into 25 3D classes. Class 7 (boxed in red) was selected for refinement.

C. Composite model representing the refined Fab from Class 7 in complex with the N332-GT2 trimer.

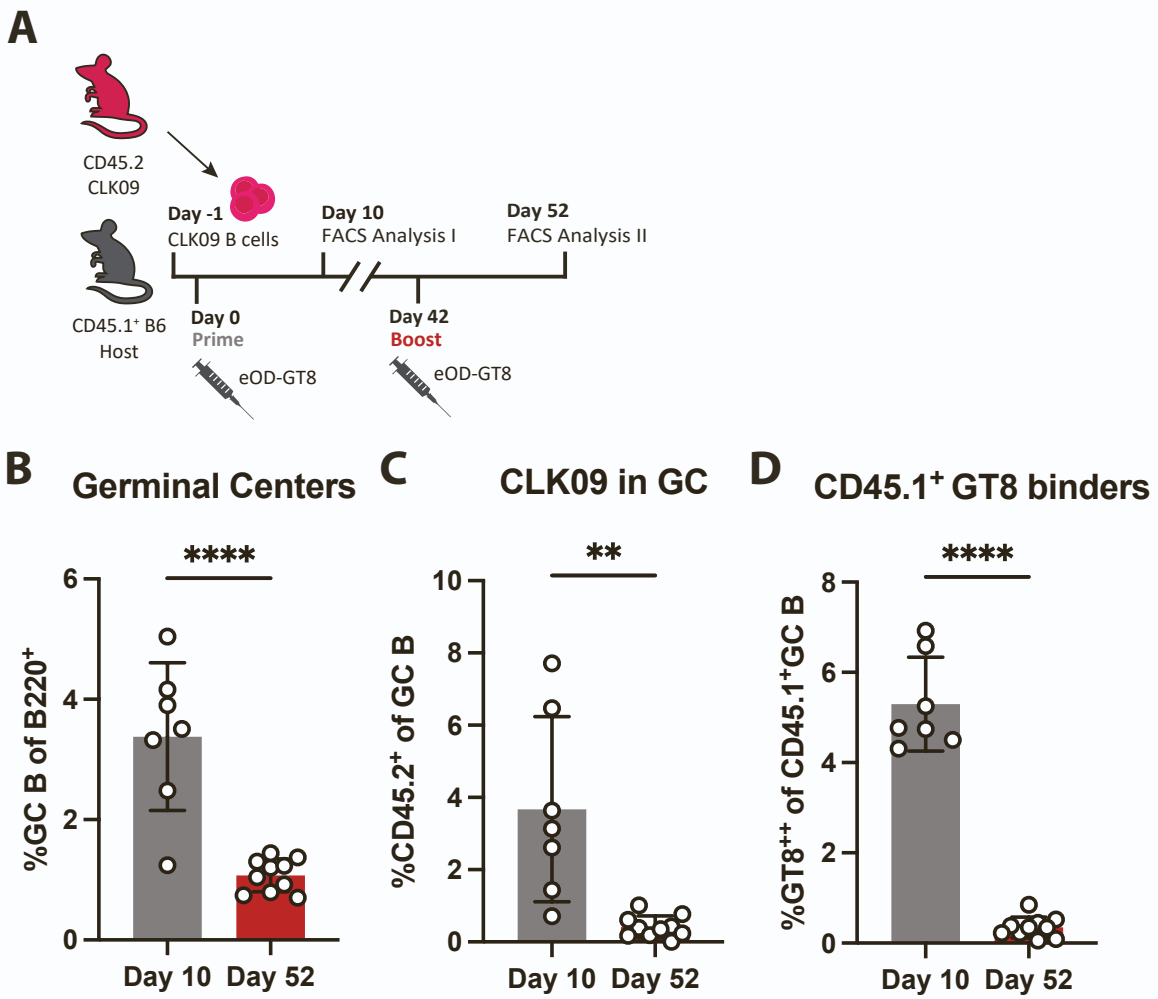

Figure S6. CLK09 prime-boost response, related to Figure 5

A. Schematic representation of experimental design to compare the memory and naïve responses of CLK09 B cells.

B. Quantification of GC cells as percentage of total B cells and **C.** Quantification of CD45.2+ CLK09 cells as percentage of total GC B cells at experiment day 10 and 52 (10 days post primary and secondary immunization).

D. Quantification of eOD-GT8 binding endogenous CD45.1+ GC B cells plots at experiment day 10 and day 52 (10 days post prime/boost).

All p values were calculated by unpaired student's t test (** $p < 0.01$; **** $p < 0.0001$).

Data from one experiment with 7-10 mice per condition presented as mean ± SD

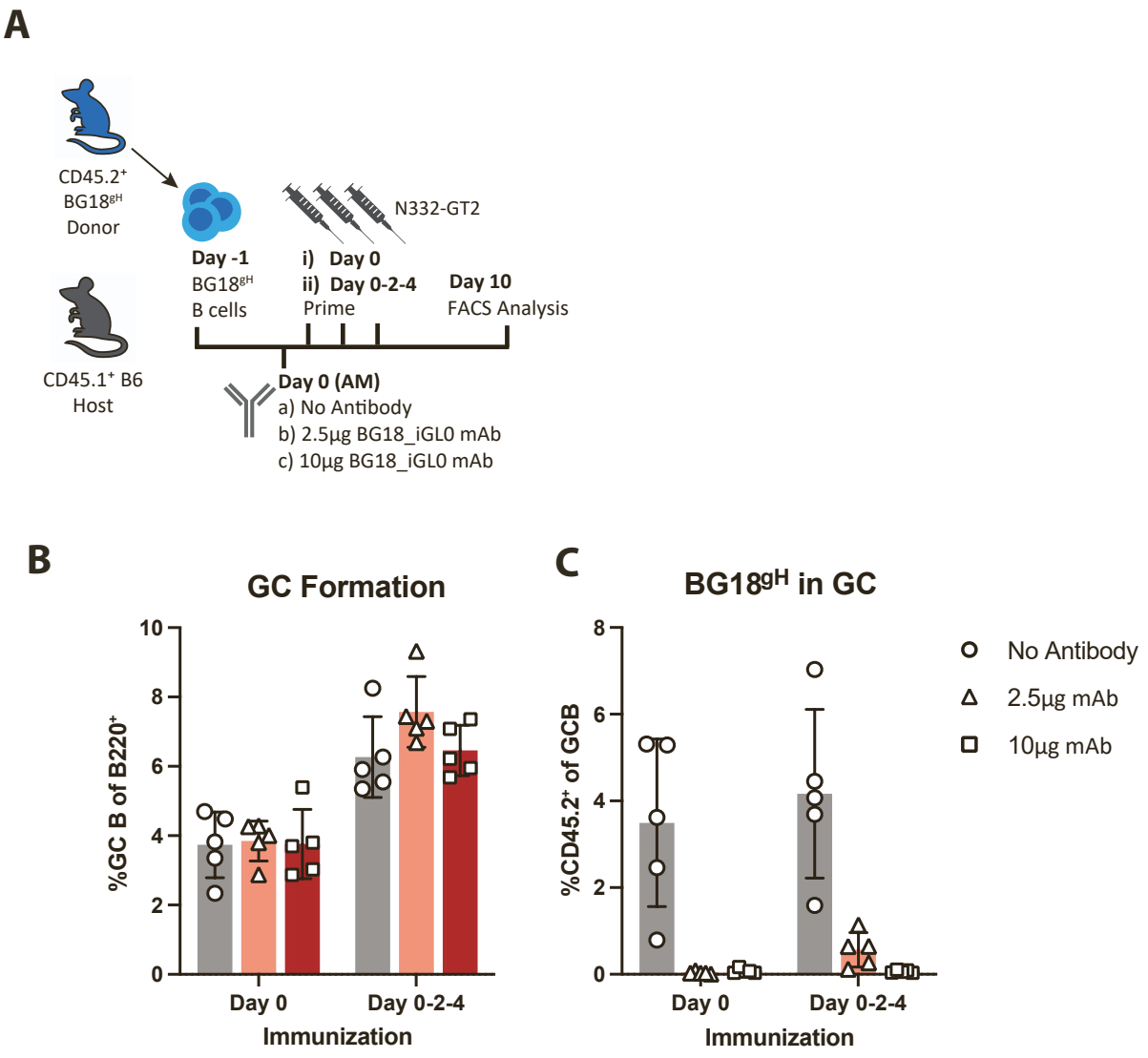

Figure S7. Response to sequential immunization with and without BG18_iGL0 mAb, related to Figure 7

A. Schematic representation of experimental design to test the effects of multiple doses of antigen on naïve BG18^{gH} B cell responses in BG18^{gH} recipients with or without mAb.

B Quantification of GC cells as percentage of total B cells and **C.** Quantification of CD45.2^{+} BG18^{gH} cells as percentage of total GC B cells at 10 dpi of BG18^{gH} recipients receiving a single or triple doses of N332-GT2 immunogen after receiving either 10µg BG18_iGL0 mAb (red), 2.5µg BG18_iGL0 mAb (orange) or no mAb (grey).

Data from one experiment with 5 mice per condition presented as mean ± SD