Cell Reports, Volume *42*

Supplemental information

Pre-existing immunity modulates responses to mRNA boosters

Tanushree Dangi, Sarah Sanchez, Min Han Lew, Bakare Awakoaiye, Lavanya Visvabharathy, Justin M. Richner, Igor J. Koralnik, and Pablo Penaloza-MacMaster

Supplemental Figures:

Figure S1

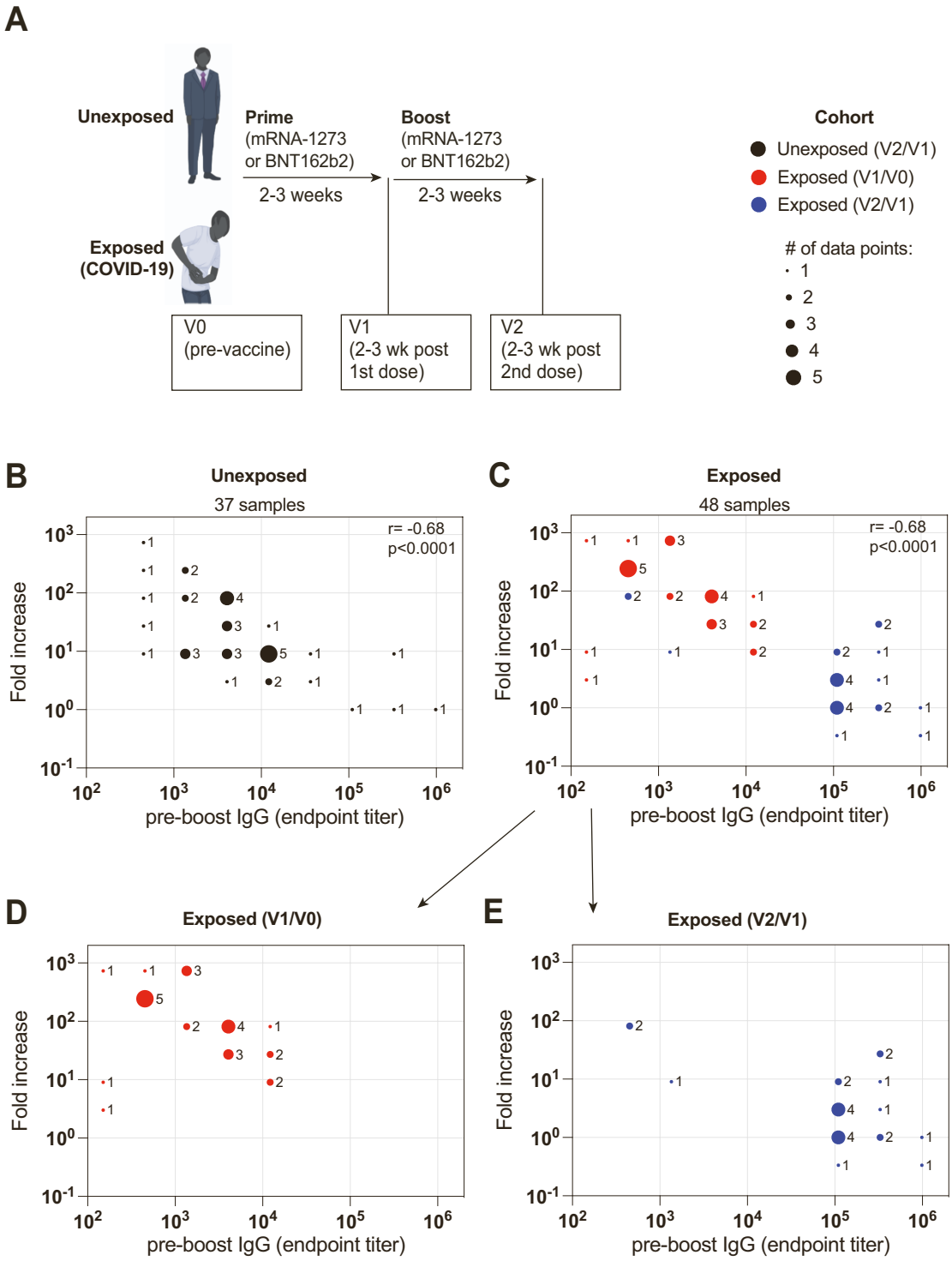

Figure S1. Pre-boost antibody levels are inversely correlated to fold-increase in antibody levels following mRNA vaccination in humans, Related to Figure 1. (A) Experimental layout for measuring antibody responses in 85 vaccine recipients. Participants were determined to be unexposed prior to vaccination (based on a negative serology test for SARS-CoV-2 spike and nucleocapsid). Participants were determined to be exposed to SARS-CoV-2 based on symptoms and confirmatory RT-PCR. (B) Summary of SARS-CoV-2 spike antibody responses in unexposed individuals. (C) Summary of SARS-CoV-2 spike antibody responses in all SARS-CoV-2 exposed individuals. (D) Summary of SARS-CoV-2 spike antibody responses in SARS-CoV-2 exposed individuals who received one vaccine shot. (E) Summary of SARS-CoV-2 spike antibody responses in SARS-CoV-2 exposed individuals who received two vaccine shots. Fold increase is represented in the y-axes, comparing the endpoint titers before and after boost (V2/V1) for both unexposed and exposed individuals; and before and after first vaccination (V1/V0) for exposed individuals only. As unexposed individuals had no detectable antibody prior to first vaccination, there is no fold increase pre- and post-first vaccination (V1/V0) to report for that cohort. Since endpoint titers fall on discrete values (multiples of 25), several values overlapped on the same data point, so bubble plots were utilized to depict overlapping data points. Fold increase was calculated by dividing the post-boost antibody titer by the pre-boost antibody titer (SARS-CoV-2-spike specific IgG). Data are from 3 visits or time points (V0, V1, V2). Non-parametric Spearman correlation was used to calculate correlation between pre-boost antibody titer and fold-increase in antibody titers (two-tailed test was used to calculate significance). P values are indicated. For source data, see Table 1.

Figure S2

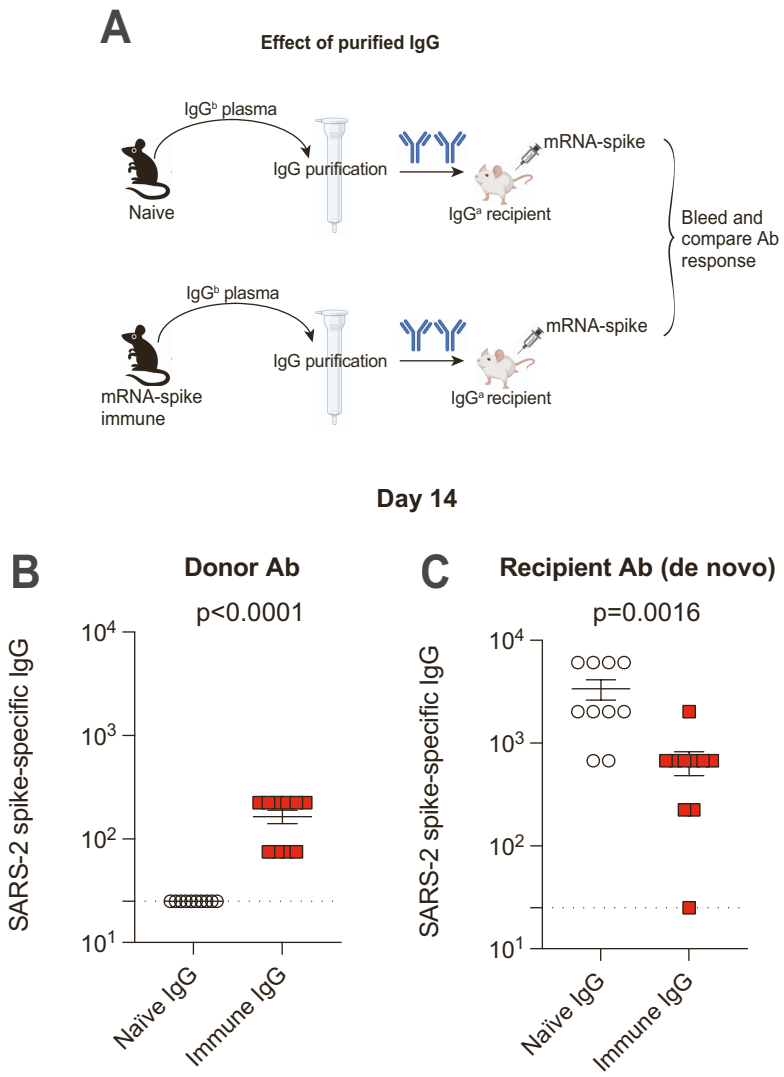

Figure S2. Purified immune IgG limits de novo IgG responses, Related to Figure 3. (A) Experimental layout. Plasmas were harvested from naïve or immune C57BL/6 mice, followed by IgG purification (see Methods). 1000 μg of purified IgG was adoptively transferred via the intraperitoneal route into BALB/c mice. On the following day, all mice were immunized intramuscularly with 3 μg of an mRNA expressing SARS-CoV-2 spike; and immune responses were quantified at week 2. (B) Donor-derived SARS-CoV-2 spike–specific antibody. (C) Recipient-derived SARS-CoV-2 spike–specific antibody. Two-tailed Mann Whitney test was used. Data are from two experiments with 5 mice per group; data from all experiments are shown. Dashed lines represent the limit of detection (LOD). P values are indicated. Data are represented as mean ± SEM.

Figure S3

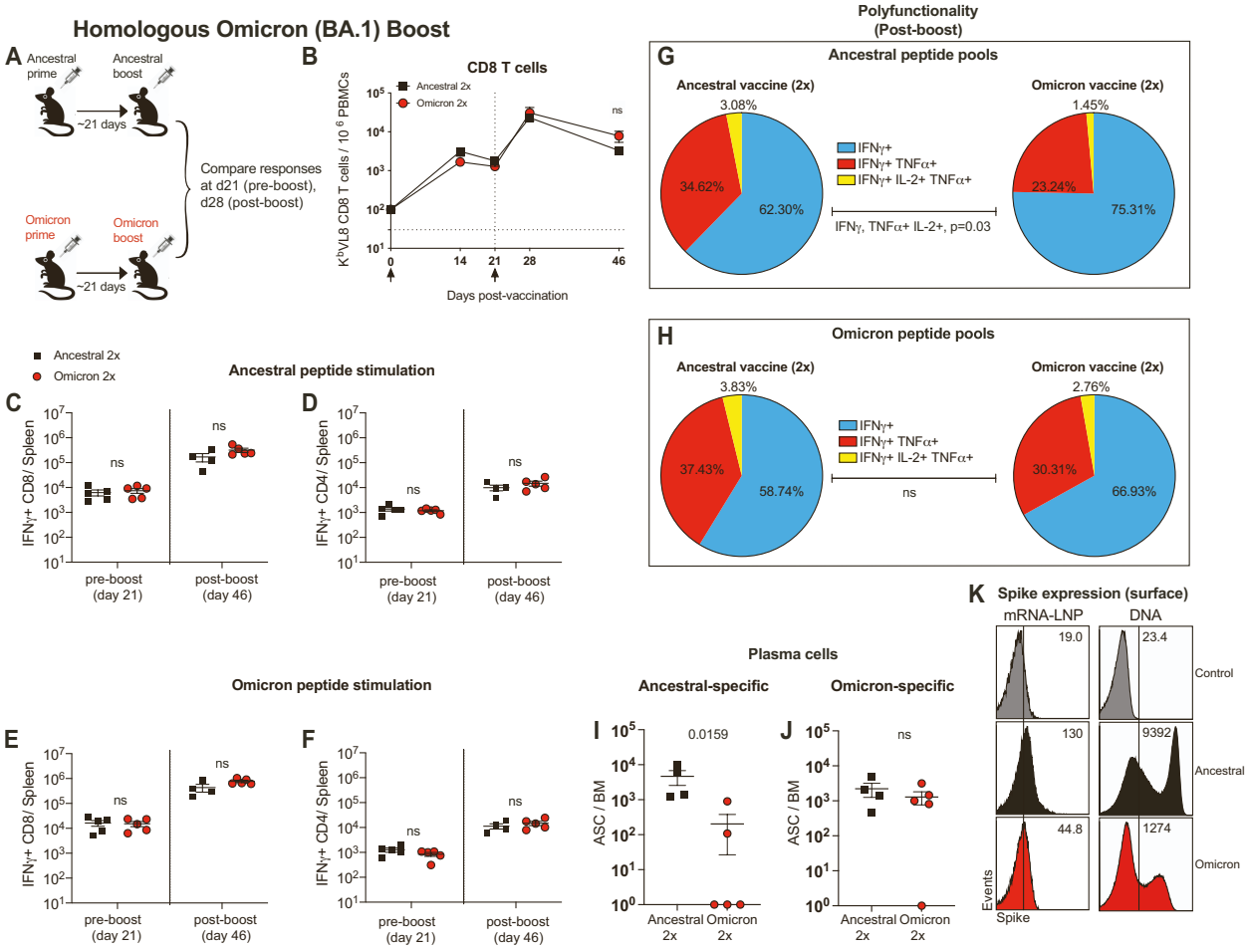

Figure S3. A homologous monovalent Omicron vaccine is not superior to a homologous monovalent ancestral vaccine, Related to Figure 4. (A) Experimental layout. C57BL/6 mice were immunized intramuscularly with 3 μg of an mRNA expressing ancestral spike. After 3 weeks, mice were boosted with a monovalent mRNA vaccine expressing ancestral spike or Omicron spike, and immune responses were quantified, as in Figure 4F. (B) Summary of SARS-CoV-2 spike–specific CD8 T cell responses by tetramer staining. (C-D) Summary of CD8 T cells and CD4 T cells after stimulation with ancestral spike peptide pools. (E-F) Summary of CD8 T cells and CD4 T cells after stimulation with Omicron spike peptide pools. (G) Polyfunctionality of CD8 T cells after stimulation with ancestral spike peptide pools. (H) Polyfunctionality of CD8 T

cells after stimulation with Omicron spike peptide pools. (I) Ancestral spike–specific plasma cells. (J) Omicron spike–specific plasma cells. (K) Representative FACS histograms comparing spike protein expression on 293T cells that were incubated with ancestral or Omicron mRNA-LNP vaccines (left), or 293T cells that were transfected with the respective DNA vectors used for *in vitro* transcription reaction (right). Two-tailed Mann Whitney test was used. Data are from an experiment with 4-5 mice that received an ancestral vaccine and 5 mice that received an Omicron vaccine; experiment was performed a total of 2 times, with similar results; dashed lines represent the LOD. P values are indicated. Data are represented as mean ± SEM.

Figure S4

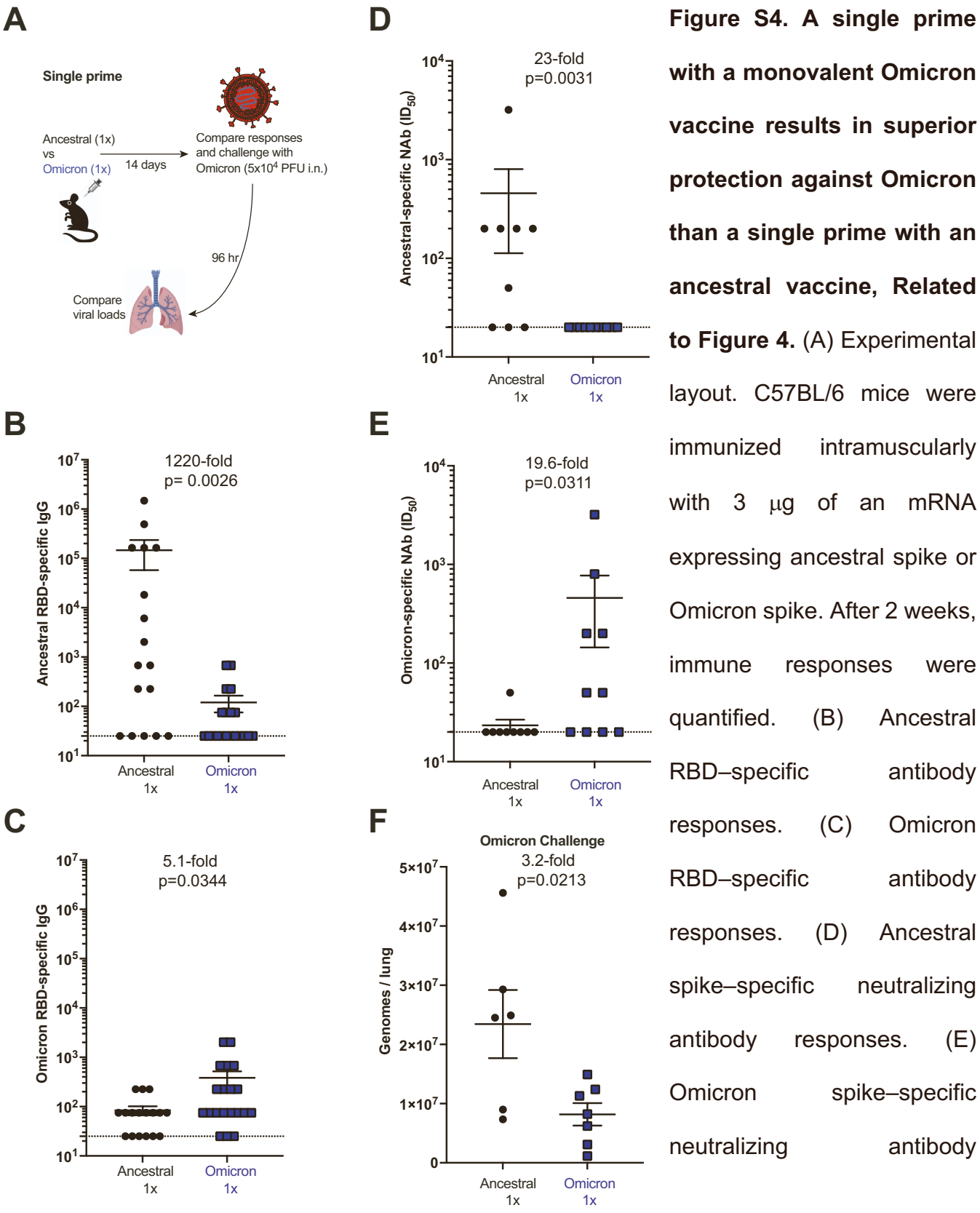

Figure S4. A single prime with a monovalent Omicron vaccine results in superior protection against Omicron than a single prime with an ancestral vaccine, Related to Figure 4. (A) Experimental layout. C57BL/6 mice were immunized intramuscularly with 3 μg of an mRNA expressing ancestral spike or Omicron spike. After 2 weeks, immune responses were quantified. (B) Ancestral RBD–specific antibody responses. (C) Omicron RBD–specific antibody responses. (D) Ancestral spike–specific neutralizing antibody responses. (E) Omicron spike–specific neutralizing antibody

responses. With only a single mRNA prime, there is substantial variability in antibody responses. (F) Viral loads 96 hr after Omicron challenge. Data from panels B-E used wild type C57BL/6 mice, whereas data from panel F used K18-hACE2 mice (on C57BL/6 background). Two-tailed Mann Whitney test was used. Data from panels B-C are from 3 experiments: the first experiment with 9-10 mice per group, the second experiment with 3-5 mice per group, and the third experiment with 5 mice per group. Data from panels D-E are from 1 experiment with 9-10 mice per group. Data from panel F are from 2 experiments with n=3-4 mice per group. All data are shown; dashed lines represent the LOD. P values are indicated. Data are represented as mean ± SEM.

Figure S5

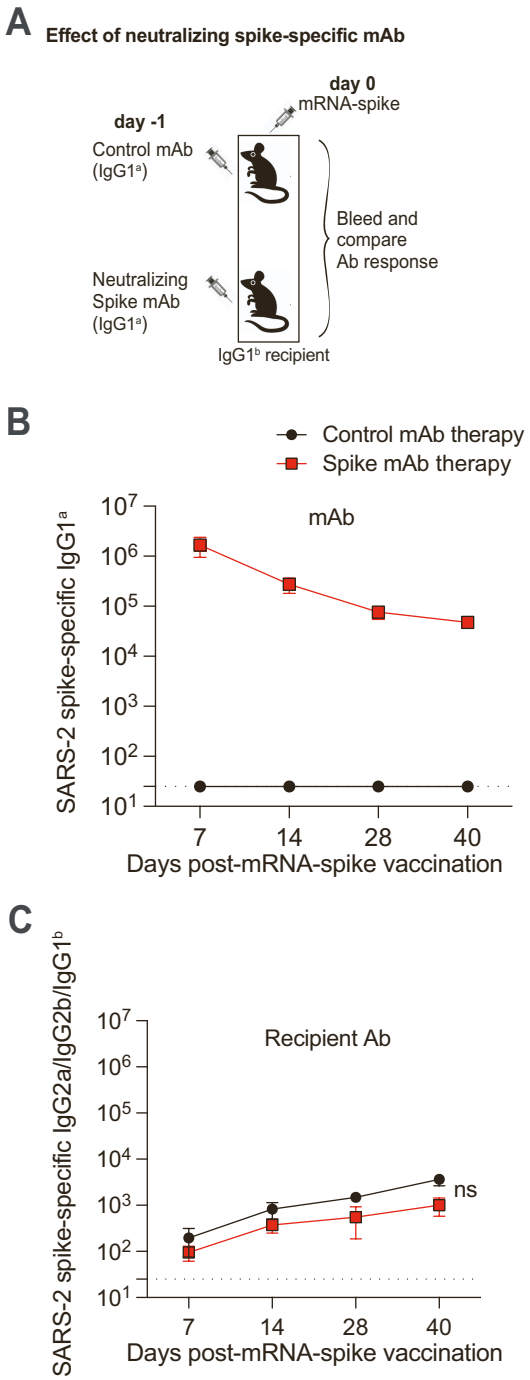

Figure S5. Neutralizing monoclonal antibodies do not significantly impair responses elicited by an mRNA vaccine, Related to Figure 5. (A) Experimental layout. C57BL/6 mice were treated with a cocktail of two neutralizing monoclonal antibodies targeting different epitopes on the spike protein (clones SARS2-34 and SARS2-01, 500 μg of each) and on the following day they were immunized intramuscularly with 3 μg of an mRNA expressing ancestral spike. Antibody responses were quantified longitudinally. (B) The monoclonal antibodies were distinguished from the host antibodies based on their allotype (neutralizing mAb are IgG1[a] allotype). (C) Recipient antibody responses (IgG1[b], IgG2a, IgG2b). Two-tailed Mann Whitney test was used. Data from an experiment with 5 mice per group. All data are shown; dashed lines represent the LOD. P values are indicated. Data are represented as mean ± SEM.

Figure S6

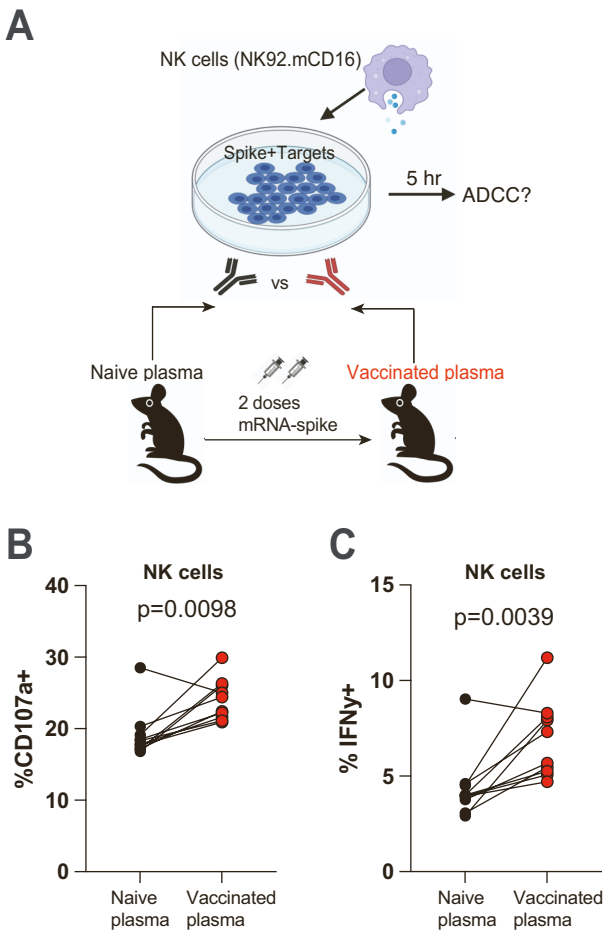

Figure S6. Vaccine-elicited plasma in mice mediate ADCC activity against target cells expressing vaccine antigen, Related to Figure 5. (A) Experimental layout for evaluating ADCC activity by vaccine-elicited antibodies using mouse plasma. The experimental layout was similar to that of Figure 5, but using effector NK92.mCD16 cells (see Methods for additional information). (B) CD107a expression by NK92 cells. (C) IFNγ expression by NK92 cells. Wilcoxon matched-pairs signed rank test was used. P values are indicated. Data are from two experiments, each with 5 mice per group (donor matched, pre- and post-vaccination).

Figure S7

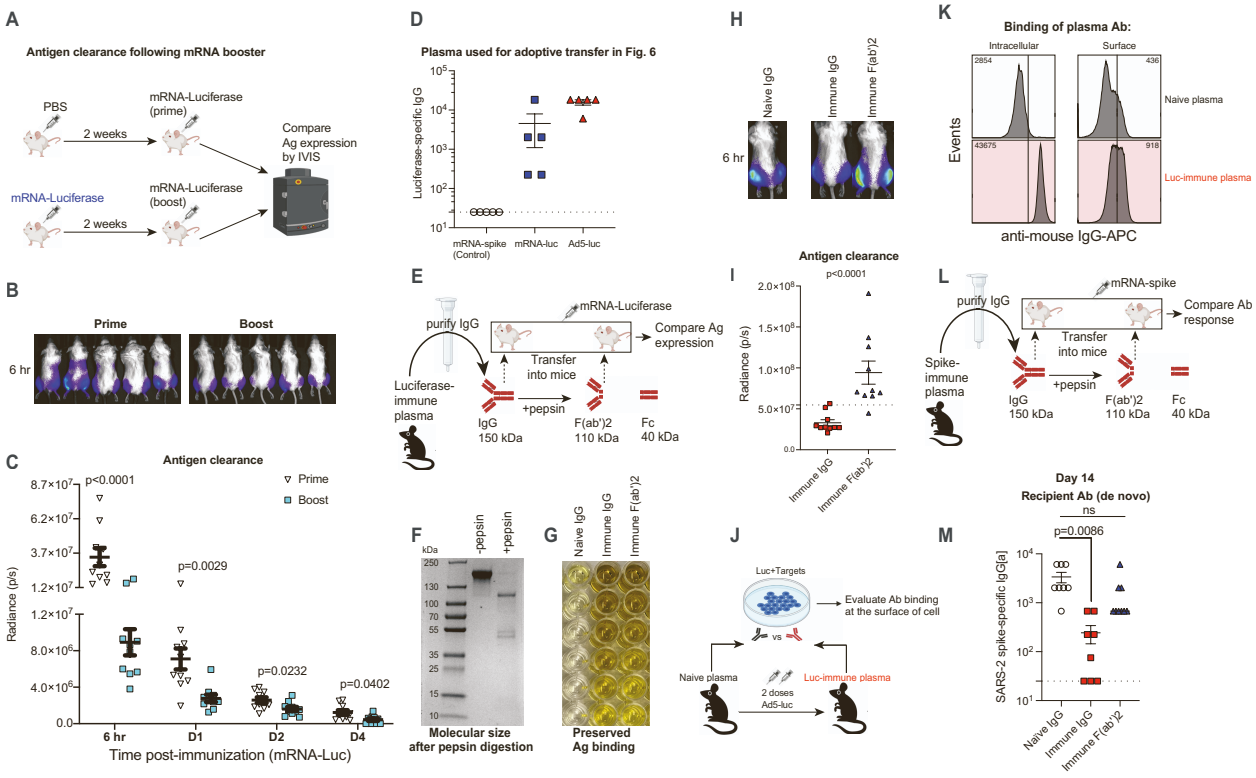

Figure S7. Kinetics of antigen clearance following an mRNA booster, Related to Figure 6.

(A) Experimental layout for quantifying antigen levels after a primary versus a secondary mRNA immunization. BALB/c mice were immunized intramuscularly with 3 μg of an mRNA expressing Luciferase (mRNA-Luc). After 2 weeks, mice were boosted homologously with the same mRNA, and luciferase expression was quantified by IVIS. (B) Bioluminescence images at 6 hr. (C) Summary of transgene expression by in vivo bioluminescence imaging. Data from A-C are from one experiment with 10 quadriceps per group (5 mice per group). Experiment was repeated for a total of 2 times, with similar results. (D) Luciferase-specific antibodies in donor mice from Figure 6. (E) Experimental layout for evaluating the role of antibody effector function in antigen clearance. mRNA-Luc immune mice were bled and IgG was purified from plasma. Purified IgG was digested with pepsin and transferred into naïve BALB/c mice (1000 μg per mouse). One

day later, recipient BALB/c mice were immunized intramuscularly with 3 μg of mRNA-Luc and luciferase expression was quantified by IVIS. (F) SDS-PAGE gel to confirm cleavage of IgG into smaller F(ab') and Fc fragments. (G) ELISA to confirm that the F(ab') fragment has retained antigen binding capacity. ELISA plates were coated with Luciferase protein, and the undigested IgG and F(ab')2 fragments were serially diluted to measure antigen binding, after adding a goat anti-mouse HRP polyclonal antibody. (H) Bioluminescence images at 6 hr. (I) Summary of transgene expression by in vivo bioluminescence imaging at 6 hr. Dashed lines indicate levels in mice that received naïve plasma. Data from H-I are from one experiment with 10 quadriceps per group (5 mice per group). Experiment was repeated for a total of 2 times, with similar results. (J) Experimental layout for evaluating surface binding of luciferase-specific IgG (see Methods for additional information). (K) Representative FACS histograms showing intracellular versus surface binding of luciferase-specific antibodies in cells expressing luciferase. (L) Experimental layout for evaluating the role of antibody effector function on *de novo* antibody responses. mRNA-spike immune mice were bled and IgG was purified from plasma. Purified IgG was then digested with pepsin and transferred into naïve BALB/c mice (1000 μg per mouse). One day later, recipient BALB/c mice were immunized intramuscularly with 3 μg of mRNA-spike and recipient-derived spike-specific antibodies were quantified at day 14. (M) Recipient-derived SARS-CoV-2 spike–specific antibody. Data from L-M are from two experiments with n=3-5 mice per group per experiment. Two-tailed Mann Whitney test was used in panel C and I; Two-way ANOVA test (Dunnett's multiple comparisons, with adjusted p-value) was used in panel K. P values are indicated. Data are represented as mean ± SEM.