Supplemental Figures:

Figure S1

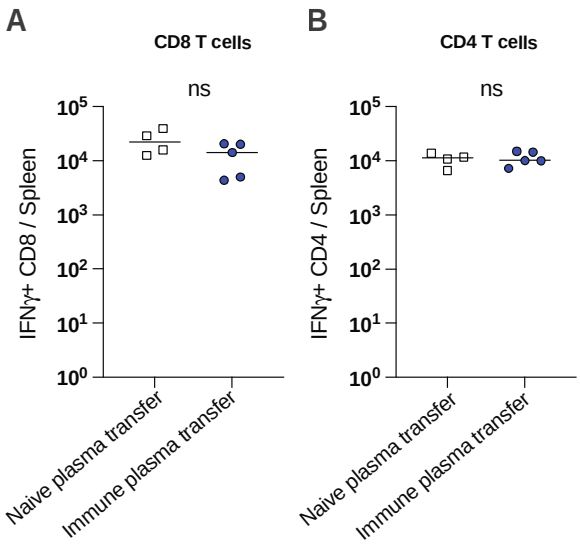

Figure S1. Plasma from vaccinated mice does not abrogate T cell responses. Plasmas were harvested from C57BL/6 mice that were vaccinated with mRNA-spike (two doses). 400 µL of plasma were adoptively transferred via the intraperitoneal route into BALB/c mice. On the following day, all mice were immunized intramuscularly with 3 µg of an mRNA expressing SARS-CoV-2 spike; and T cell responses were quantified by intracellular cytokine staining (ICS) at day 21 post-vaccination. Naïve plasmas were used as controls. Splenocytes were incubated with overlapping SARS-CoV-2 peptide pools for 5 hr at 37°C in the presence of GolgiStop and GolgiPlug to detect SARS-CoV-2–specific CD8+ T cell responses (A) and CD4+ T cell responses (B). Two-tailed Mann Whitney test was used. Data are from an experiment with 4 mice that received naïve plasma and 5 mice that received post-vaccine plasma (week 2 post-boost); experiment was repeated for a total of 2 times, with similar results; dashed lines represent the LOD. Error bars indicate the SEM.

Figure S2

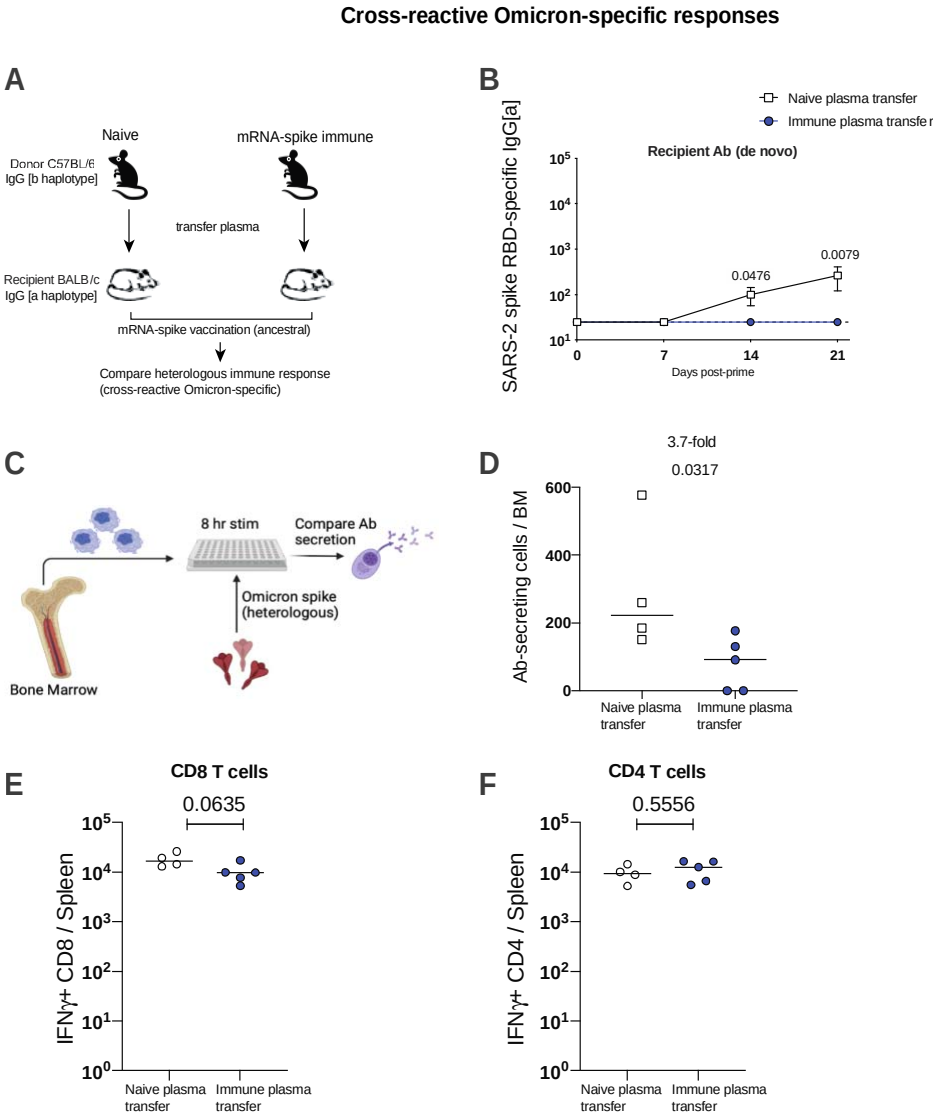

Figure S2. Plasma from vaccinated mice abrogates cross-reactive (Omicron-specific) antibody responses. Plasmas were harvested from C57BL/6 mice that were vaccinated with mRNA-spike (two doses). 400 µL of plasma was adoptively transferred via the intraperitoneal route into BALB/c mice. On the following day, all mice were immunized intramuscularly with 3 µg of an mRNA expressing SARS-CoV-2 spike (ancestral sequence); and cross-reactive Omicron-specific immune responses were quantified. Naïve plasmas were used as controls. (B) Recipient-derived Omicron-specific antibody. (C) Experimental layout for detection of antibody secreting cells specific for ancestral or Omicron spike. (D) Omicron-specific antibody secreting cells in bone marrow. (E-F) T cell responses were quantified by intracellular cytokine staining (ICS) at day 21 post-vaccination. Splenocytes were incubated with overlapping SARS-CoV-2 peptide pools (derived from the Omicron variant) for 5 hr at 37°C in the presence of

GolgiStop and GolgiPlug to detect SARS-CoV-2–specific CD8+ T cell responses (E) and CD4+ T cell responses (F). Two-tailed Mann Whitney test was used. Data are from an experiment with 4 mice that received naïve plasma and 5 mice that received post-vaccine plasma (week 2 post-boost); experiment was repeated for a total of 2 times, with similar results; dashed lines represent the LOD. Error bars indicate the SEM.

Figure S3

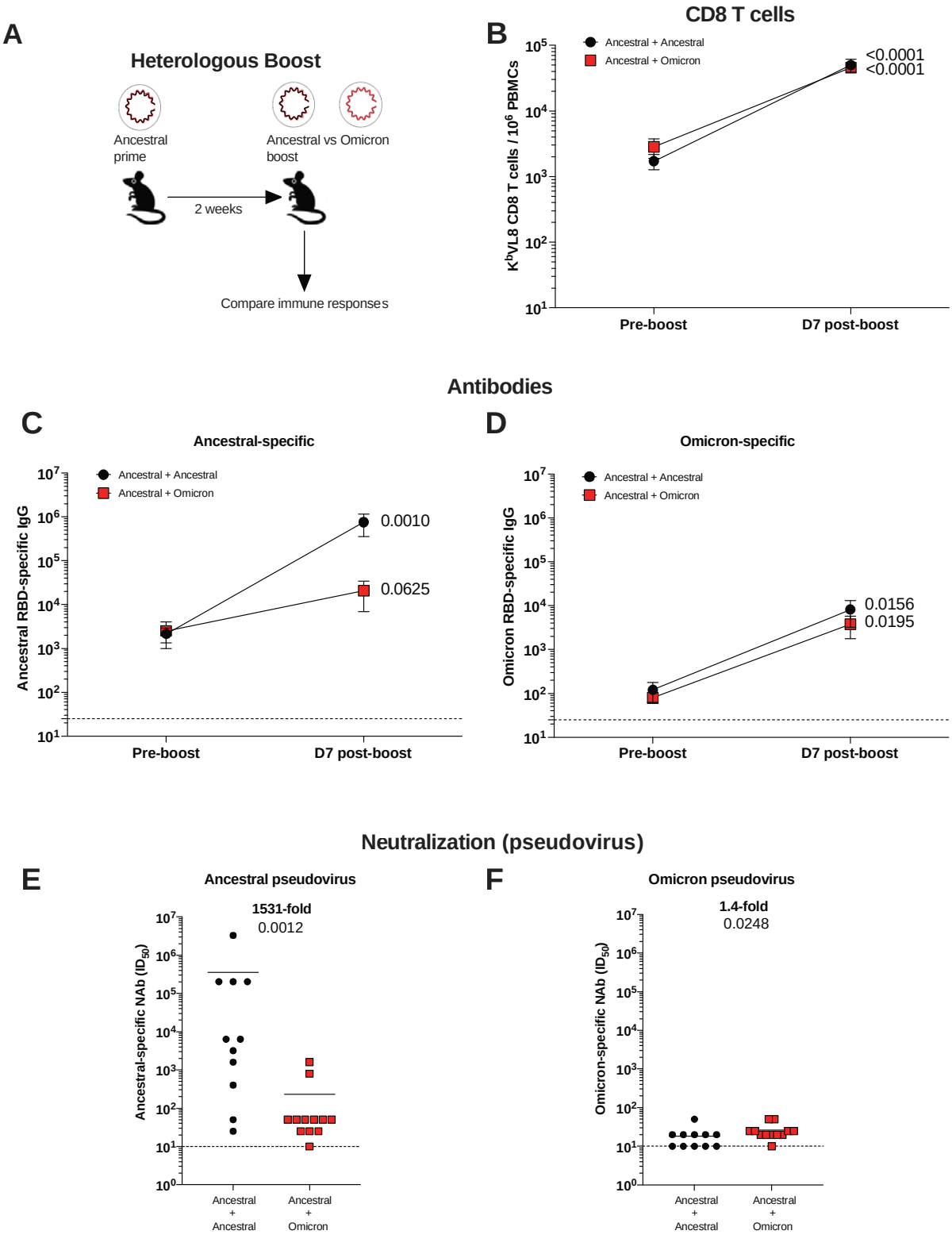

Figure S3. A heterologous Omicron boost is not superior to a homologous ancestral vaccine boost. (A) Experimental layout. C57BL/6 mice were immunized intramuscularly with 3 μg of an mRNA expressing ancestral spike. After 3 weeks, mice were boosted with either an mRNA expressing ancestral spike, or an mRNA expressing Omicron spike. Immune responses were quantified. (B) Summary of SARS-CoV-2 spike–specific CD8 T cells. These CD8 T cells were specific for a conserved CD8 T cell epitope present in both ancestral and Omicron viruses (K^bVL8). (C) Ancestral spike–specific antibody responses. (D) Omicron spike–specific antibody responses. (E) Ancestral spike–specific neutralizing antibody responses. (F) Omicron spike–specific neutralizing antibody responses. Wilcoxon matched-pairs signed rank test was used for panels B-D, comparing pre-boost and post-boost responses for each vaccine regimen. For all other plots, two-tailed Mann Whitney test was used. Data are combined from two experiments; one experiment with 6-7 mice per group and another experiment with 5 mice per group. Dashed lines represent the LOD (in one experiment the LOD was 25). Error bars indicate the SEM.

Figure S4

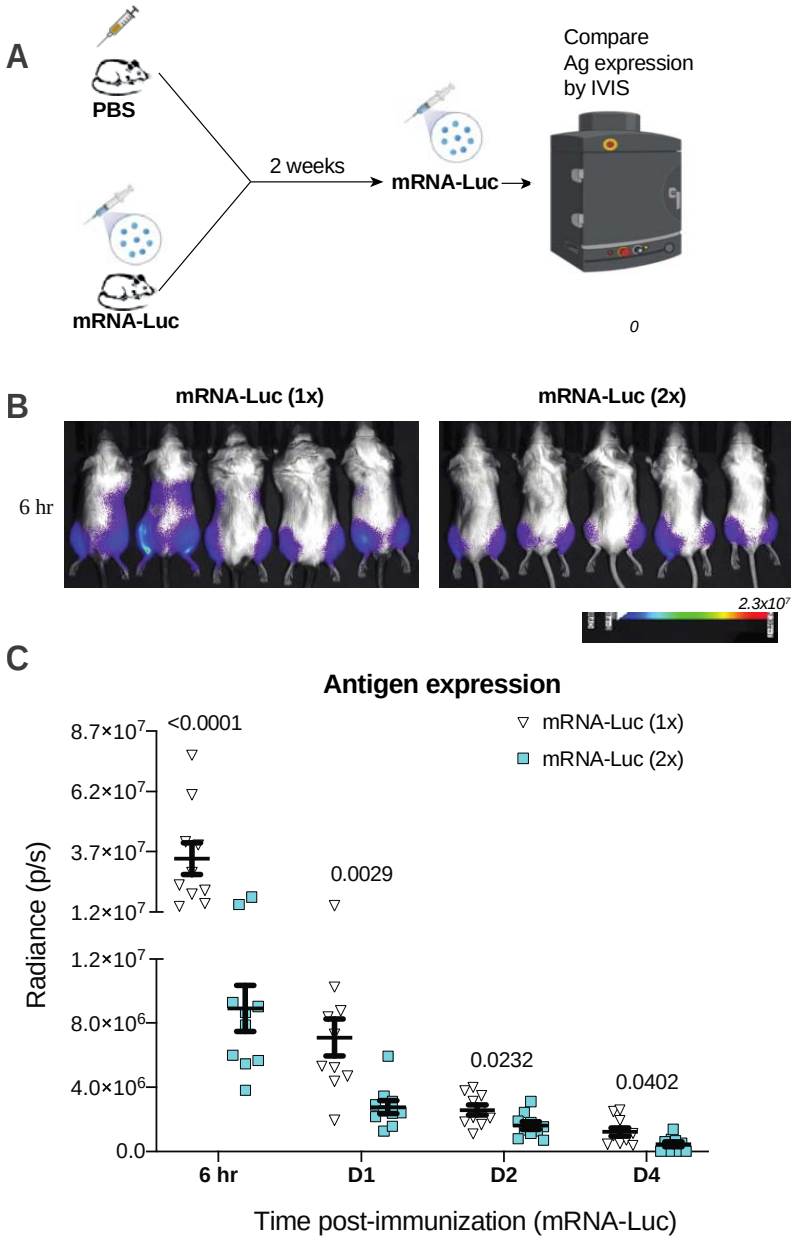

Figure S4. Boosting with mRNA results in lower antigen expression, relative to priming with mRNA. (A) Experimental layout. BALB/c mice were immunized intramuscularly with 3 µg of an mRNA expressing Luciferase (mRNA-Luc). After 2 weeks, mice were boosted with the same mRNA, and luciferase expression was quantified by IVIS. (B) Bioluminescence images at 6 hr. (C) Summary of transgene expression by in vivo bioluminescence imaging. BALB/c mice (with white coat) were used for improved visualization of luciferase. Two-tailed Mann Whitney test was used. Data 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. Error bars indicate the SEM.

Figure S5

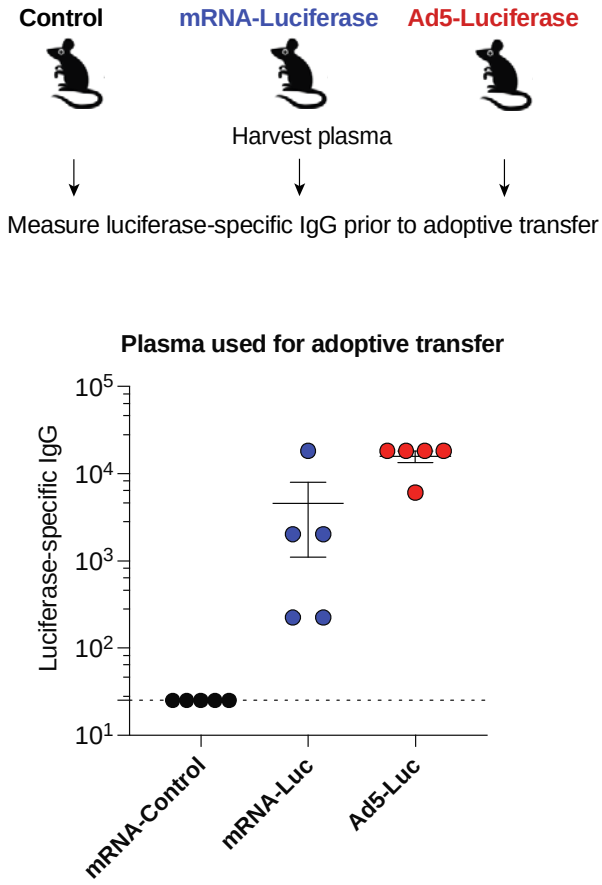

Figure S5. mRNA-Luciferase immunization induces luciferase-specific antibody responses. C57BL/6 mice were immunized intramuscularly with 3 μg of an mRNA expressing Luciferase, and after 3 weeks, mice were boosted. We immunized another group of mice with a different vaccine platform expressing the same transgene (Ad5-Luciferase) or with an irrelevant control mRNA vaccine expressing a different antigen (mRNA-spike). Two weeks after boost, mice were bled, and luciferase-specific antibodies were quantified in plasma. These plasma samples were used in adoptive transfers in Figure 6. Luciferase-specific antibody responses are shown. Data are from one experiment with 5 donor mice. Experiment was repeated for a total of 2 times, with similar results. Error bars indicate the SEM.