PNAS
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Supplementary Information for

A class II MHC-targeted vaccine elicits immunity against SARS-CoV-2 and its variants

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Figures S1 to S7

Figure S1.

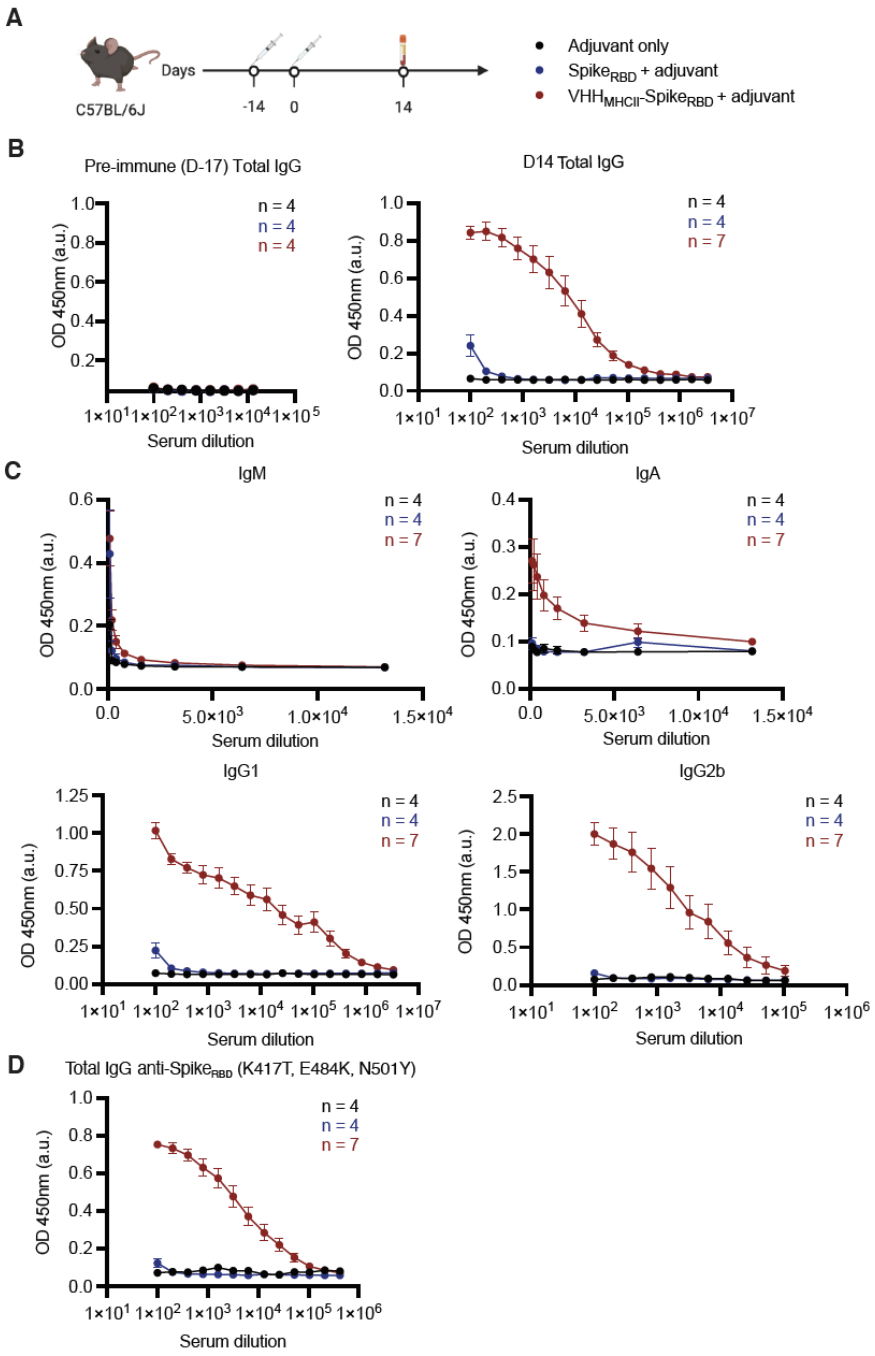

Fig. S1. Immunization with VHH$_{MHCII}$-Spike$_{RBD}$ induces high-titer anti-Spike$_{RBD}$ and neutralizing antibodies in C57BL/6J mice. (A). C57BL/6J mice were immunized intraperitoneally with adjuvant only, adjuvanted Spike$_{RBD}$, or adjuvanted VHH$_{MHCII}$-Spike$_{RBD}$ on the indicated days. Serum samples were collected as indicated. (B). Total IgG (days -13 and day 14) or (C). IgM, IgA, IgG1, and IgG2b (day 14) responses were evaluated from sera of immunized mice (n = 4-7/group) by ELISA against recombinant Spike$_{RBD}$. ELISA data were presented as means ± SEM. (D). Humoral responses in sera of immunized mice were evaluated (n = 4-7/group) by ELISA for anti-Spike$_{RBD}$ (K417T, E484K, N501Y mutations) IgG. ELISA data were presented as means ± SEM.

Figure S2.

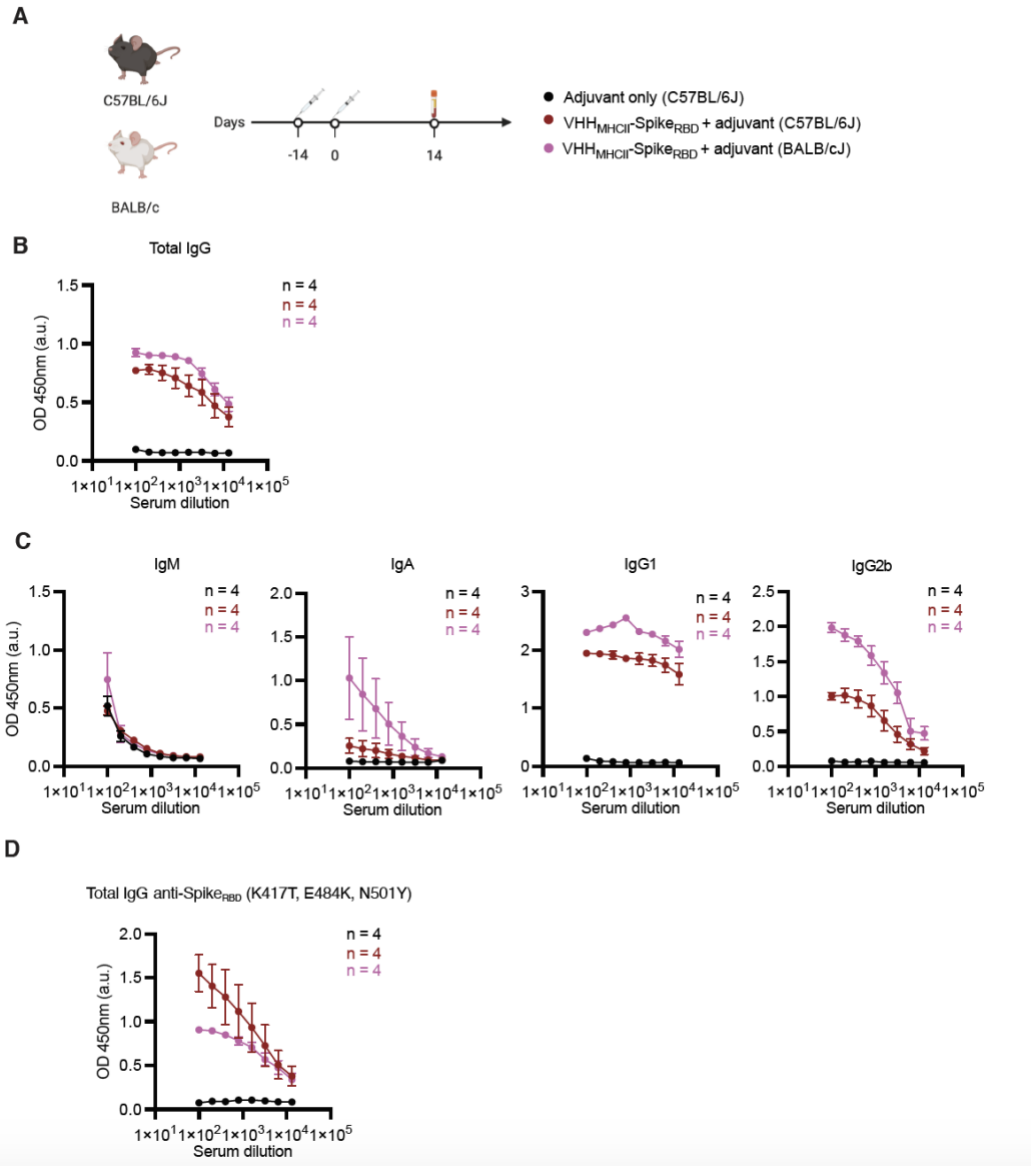

Fig. S2. Immunization with VHH$_{MHCII}$-Spike$_{RBD}$ induces high-titer anti-Spike$_{RBD}$ and neutralizing antibodies in BALB/c mice. (A). C57BL/6J and BALB/c mice were immunized intraperitoneally with adjuvant only or adjuvanted VHH$_{MHCII}$-Spike$_{RBD}$ on the indicated days. Serum samples were collected as indicated. (B). Total IgG or (C). IgM, IgA, IgG1, and IgG2b (day 14) responses were evaluated from sera of immunized mice (n = 4/group) by ELISA against recombinant Spike$_{RBD}$. ELISA data were presented as means ± SEM. (D). Humoral responses in sera of immunized mice were evaluated (n = 4/group) by ELISA for anti-Spike$_{RBD}$ (K417T, E484K, N501Y mutations) IgG. ELISA data were presented as means ± SEM.

Figure S3.

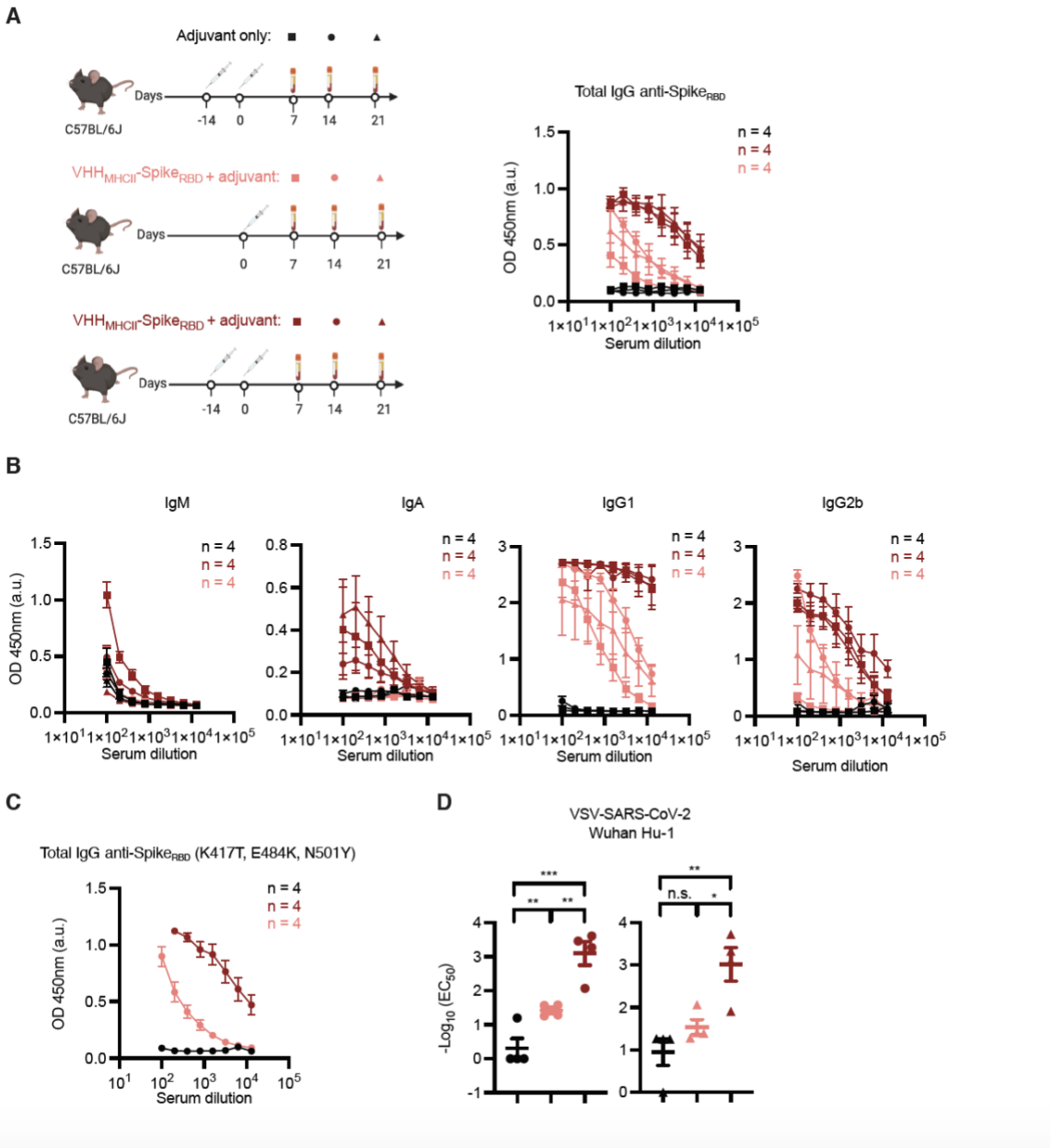

Fig. S3. Kinetics of humoral immune response upon immunization with 1 or 2 doses of adjuvanted VHH$_{MHCII}$-Spike$_{RBD}$. (A). C57BL/6J were immunized intraperitoneally with adjuvant only or adjuvanted VHH$_{MHCII}$-Spike$_{RBD}$ on the indicated days. Serum samples were collected as indicated. Total IgG responses were evaluated from sera of immunized mice (n = 4/group) by ELISA against recombinant Spike$_{RBD}$. ELISA data were presented as means ± SEM. (B). Similarly, IgM, IgA, IgG1, and IgG2b responses were evaluated from sera of immunized mice (n = 4/group) by ELISA against recombinant Spike$_{RBD}$. ELISA data were presented as means ± SEM. (C). Humoral responses in sera of immunized mice were evaluated (n = 4/group) by ELISA for anti-Spike$_{RBD}$ (K417T, E484K, N501Y mutations) IgG. ELISA data were presented as means ± SEM. (D). Neutralization data for VSV, pseudotyped with the SARS-CoV-2 Spike glycoprotein Wuhan Hu-1. All data presented as means ± SEM. n.s. not significant; *p<0.05, **p<0.01, ***p<0.001, unpaired t-test with Holm-Sidak adjustment.

Figure S4.

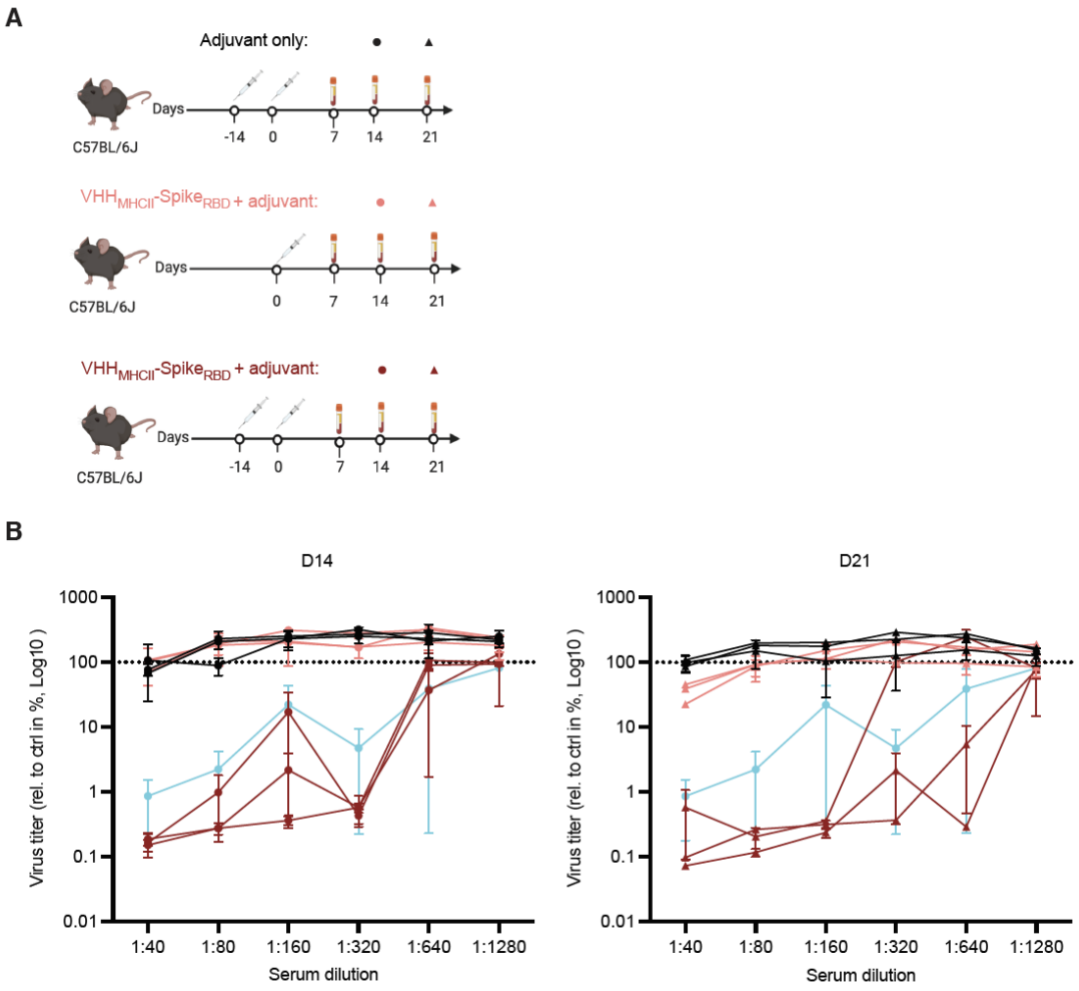

Fig. S4. **Neutralization of SARS-CoV-2 strain NL2020 following immunization with two doses of adjuvanted VHH$_{MHCII}$-Spike$_{RBD}$.** (A). Scheme of immunization and bleeding schedule of C57BL/6J mice. (B). qRT-PCR-based neutralization assay against SARS-CoV-2 strain /NL/2020 . Turquoise data points indicate neutralization by anti-Spike monoclonal antibody 47D11. Of note, mAB 47D11 (3.14mg/ml) was pre diluted at 1:100 (to 31.4ug/ml) and it was then used at indicated dilutions. All data presented as means of technical replicates ± SEM.

Figure S5.

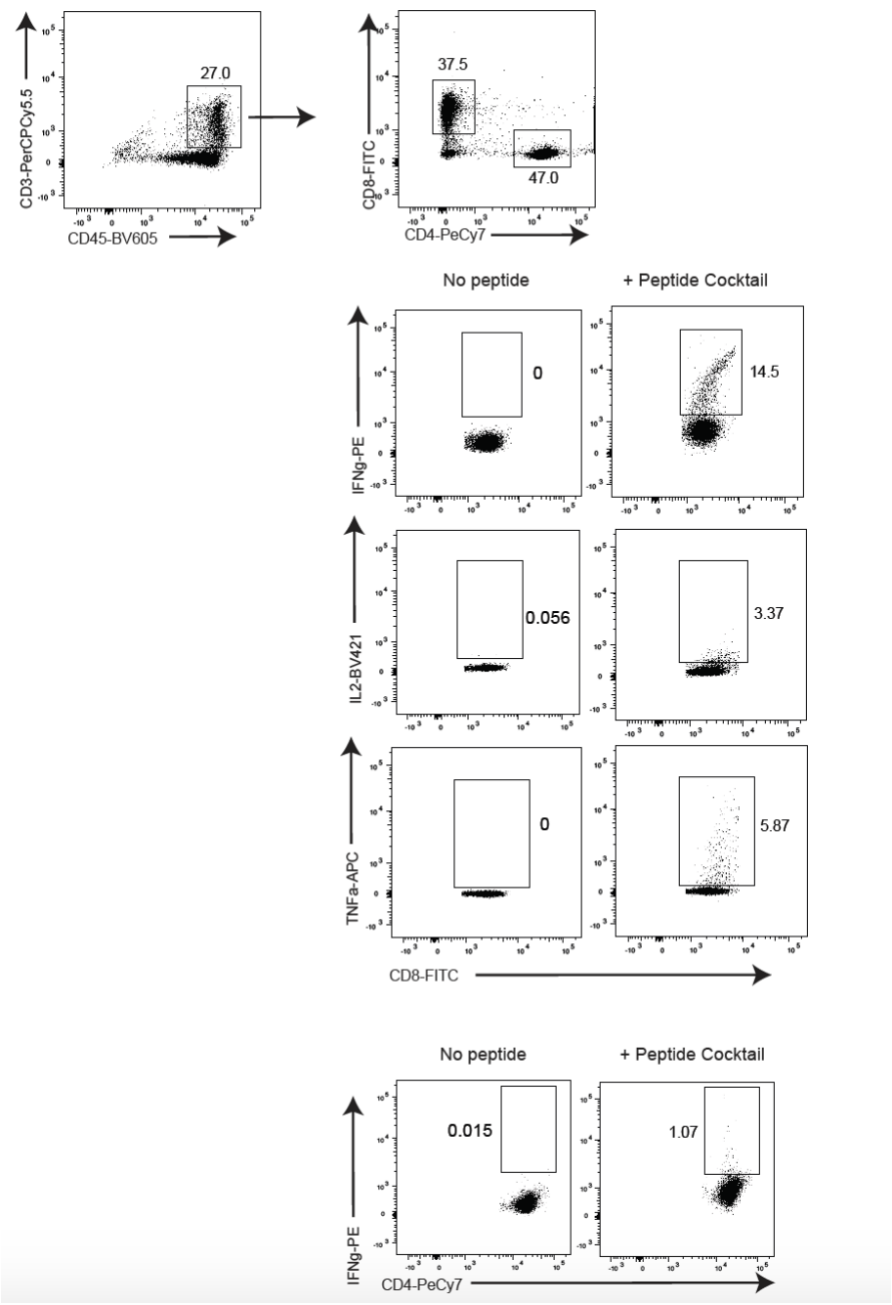

Fig. S5. Representative gating strategies of Spike$_{RBD}$-specific CD4 and CD8 T cell flow cytometry analyses. Flow cytometry analyses were conducted on the splenocytes from immunized mice in Figure 2, co-cultured for 6 h with or without cocktail of peptides (42 and 47-50) to identify subsets of CD4 and CD8 T cells that express pro-inflammatory cytokines.

Figure S6.

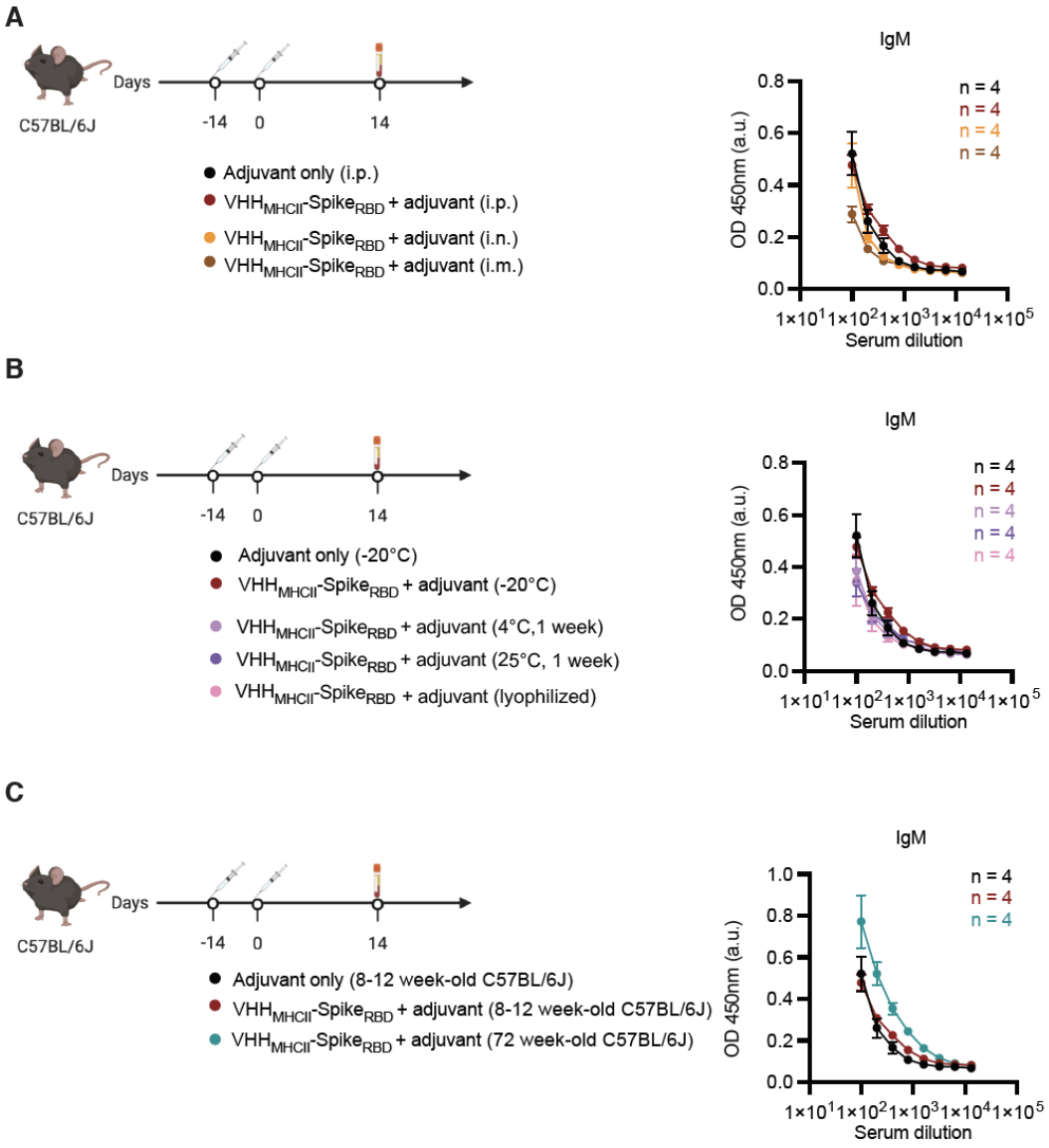

Fig. S6. VHH$_{MHCII}$-Spike$_{RBD}$ elicits a strong humoral response regardless of route of administration, storage temperature, lyophilization, or mouse age. (A). IgM levels were measured against recombinant Spike$_{RBD}$ following immunization with different routes of administration. (B). Anti-Spike$_{RBD}$ IgM levels upon immunization with vaccines maintained under different storage conditions. (C). Anti-Spike$_{RBD}$ IgM levels in immunized young and old mice. n = 4 for all conditions, and curves are plotted as means ± SEM of each condition.

Figure S7.

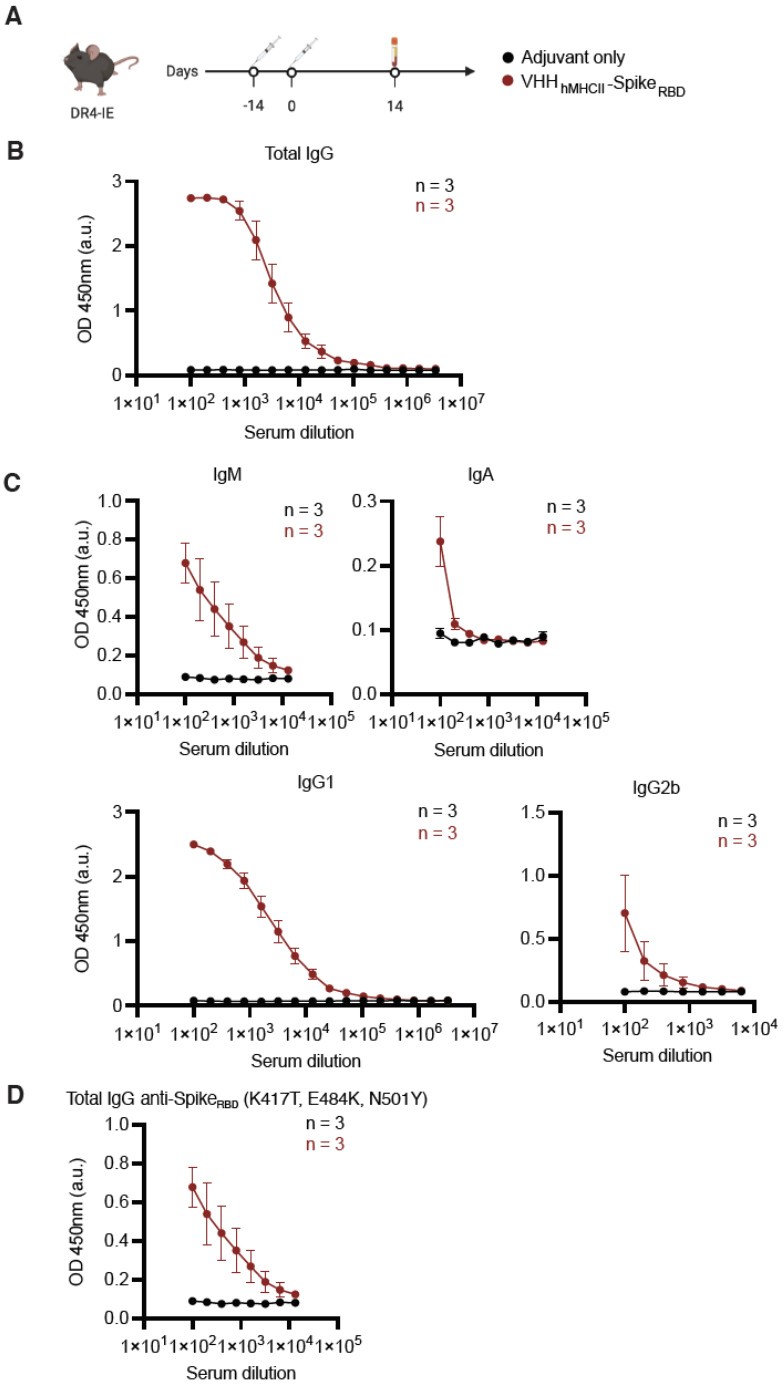

Fig. S7. Immunization with VHH$_{hMHCII}$-Spike$_{RBD}$ induces high-titer anti-Spike$_{RBD}$ in humanized DR4-IE mice. (A). DR4-IE mice were immunized intraperitoneally with adjuvant only or adjuvanted VHH$_{hMHCII}$-Spike$_{RBD}$ on the indicated days. Serum samples were collected as indicated. (B). Total IgG or (C). IgM, IgA, IgG1, and IgG2b (day 14) responses were evaluated from sera of immunized mice (n = 3/group) by ELISA against recombinant Spike$_{RBD}$. ELISA data were presented as means ± SEM. (D). Humoral responses in sera of immunized mice were evaluated (n = 3/group) by ELISA for anti-Spike$_{RBD}$ (K417T, E484K, N501Y mutations) IgG. ELISA data were presented as means ± SEM.