Cell Reports, Volume *38*

Supplemental information

A bivalent nanoparticle vaccine exhibits potent cross-protection against the variants of SARS-CoV-2

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SUPPLEMENTAL FIGURES

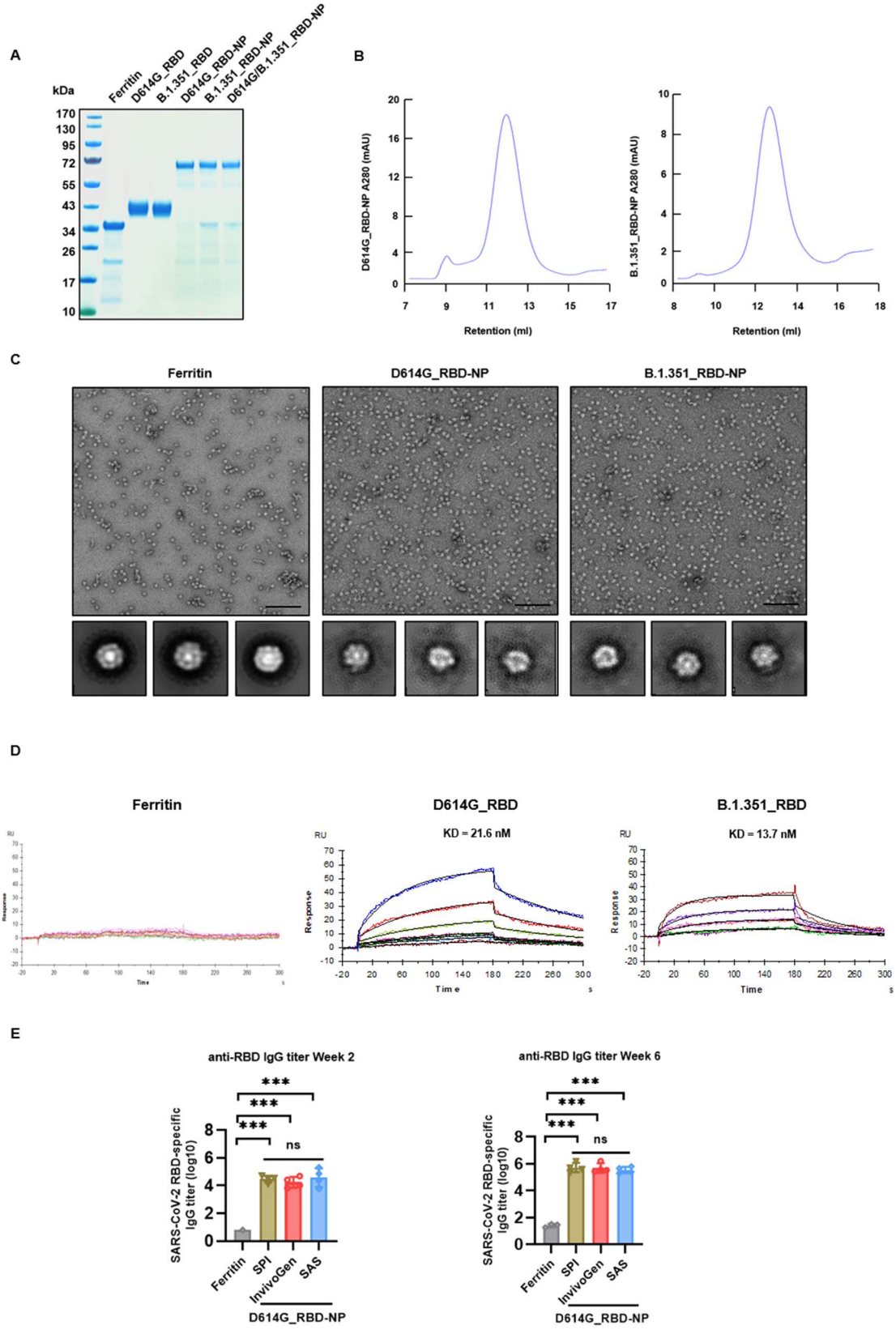

Fig. S1. Purification and characterization of bivalent D614G/B.1.351_RBD nanoparticles. Related to Figure 1.

(A) Coomassie blue staining of Ferritin, D614G_RBD, B.1.351_RBD, D614G_RBD-NP, B.1.351_RBD-NP and D614G/B.1.351_RBD-NP.

(B) SEC of D614G_RBD-NP and B.1.351_RBD-NP. The ultraviolet absorptions at 280 were shown. The retention volume represented peaks of each nanoparticle.

(C) TEM images and two-dimensional (2D) reconstruction of each nanoparticle.

(D) Representative BIAcore plots of Ferritin, D614G_RBD and B.1.351_RBD bound to hACE2.

(E) D614G_RBD- IgG titers of immunized BALB/c mice at week 2 and week 6 with different adjuvant were detected by ELISA. The data are represented as the reciprocal of the endpoint serum dilution. Scale bar in (C) represented 100 nm. Experiments were conducted independently in triplicates. Data represented as mean ± SD. Adjusted p values were calculated by one-way ANOVA with Tukey's multiple comparisons test. Significant differences between groups linked by horizontal lines are indicated by asterisks. ns, not significant, ***, $p < 0.001$.

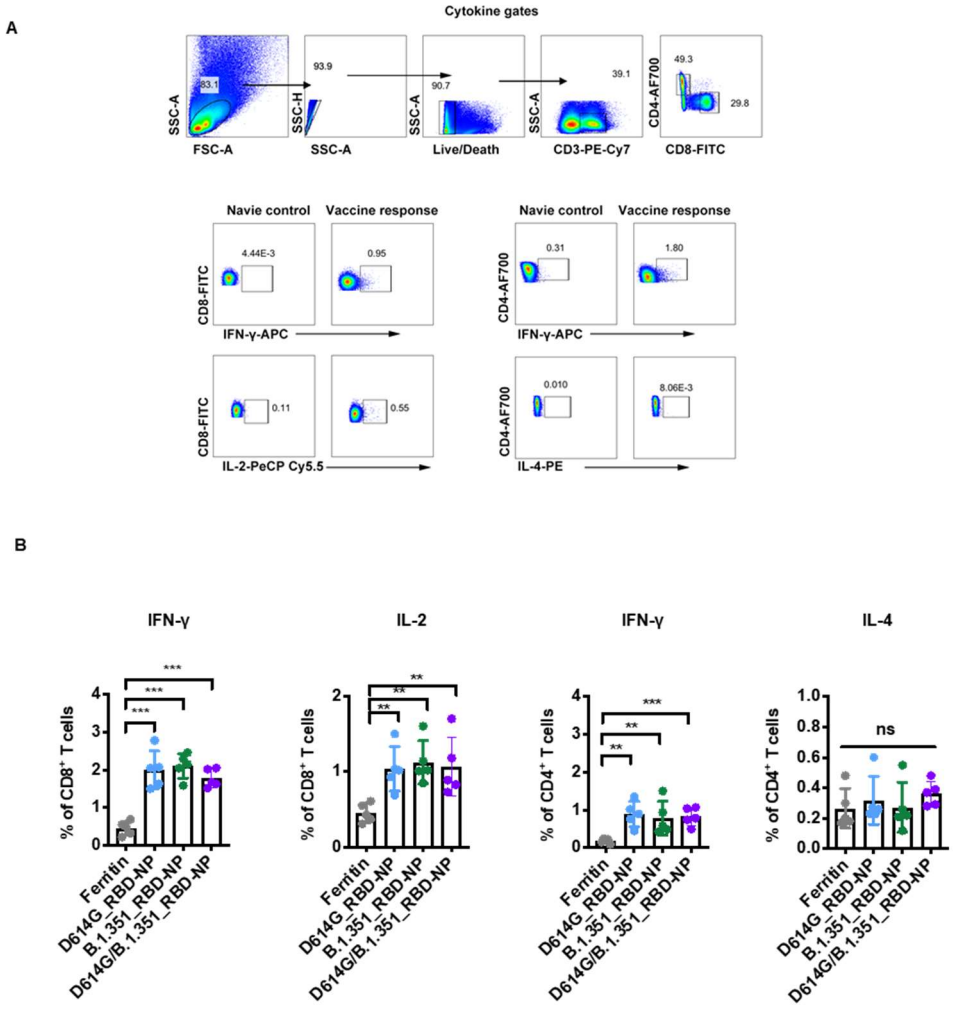

Fig. S2. T cell immune responses in two-dose D614G/B.1.351_RBD-NP vaccinated BALB/c Mice. Related to Figure 1.

(A)Gating strategy for intracellular cytokine staining in $CD8^+$ and $CD4^+$ T cells. Intracellular cytokine gating examples are spleen from a naïve mouse and a representative $CD8^+$ or $CD4^+$ T cell cytokine response to antigens.

(B) BALB/c mice were euthanized post two-dose vaccination at 42 days. Splenocytes were incubated with a peptides pool. The percentages of IFN-γ^+ and IL-2^+ $CD8^+$ T cells and the percentages of IFN-γ^+ and IL-4^+ $CD4^+$ T cells were determined by ICCS. Experiments were conducted independently in triplicates. Data represented as mean ± SD. Adjusted p values were calculated by one-way ANOVA with Tukey's multiple comparisons test. Significant differences between groups linked by horizontal lines are indicated by asterisks. ns, not significant, *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$.

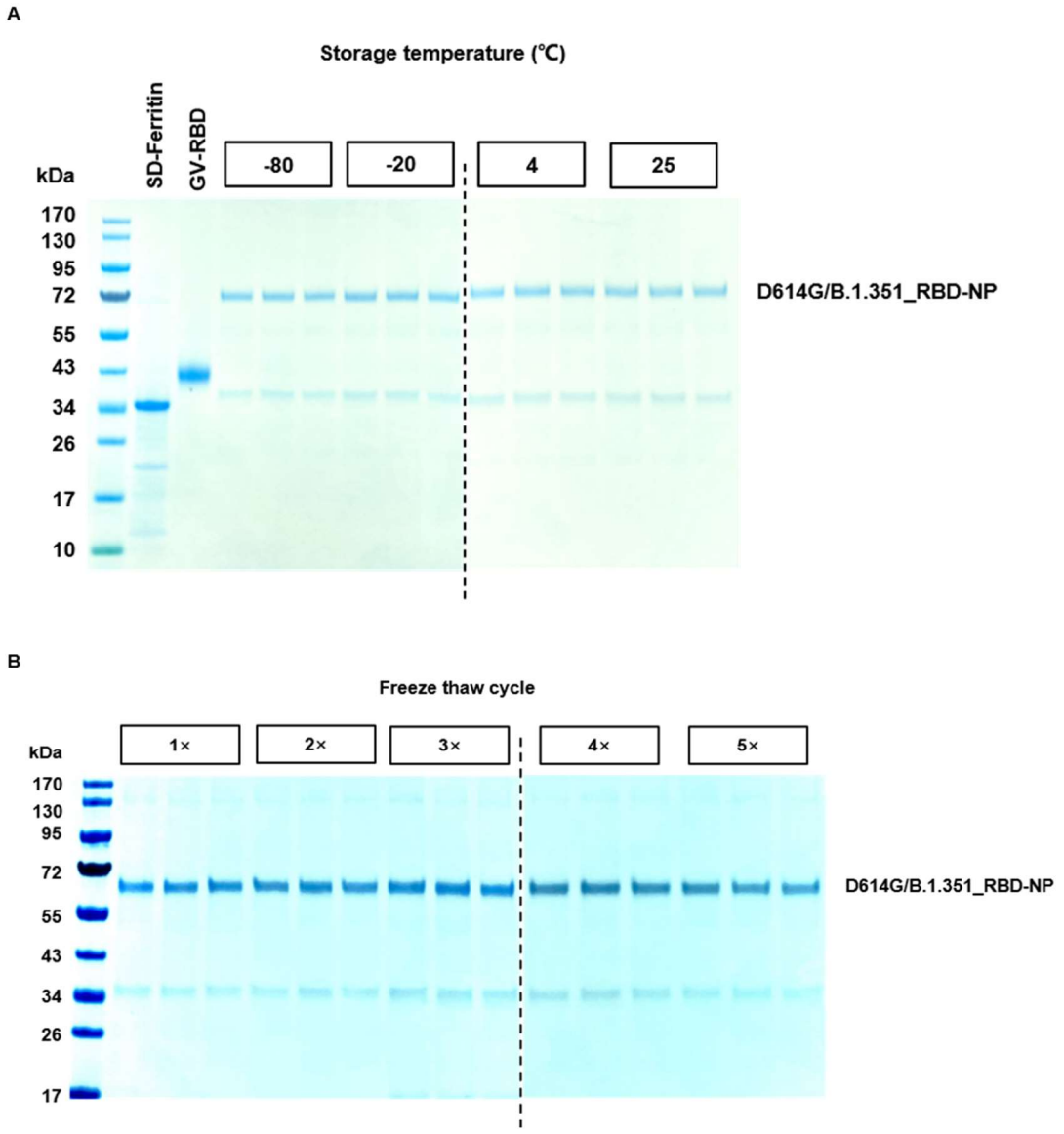

Fig. S3. The bivalent D614G/B.1.351_RBD-NP is thermostable and resilient. Related to Figure 1.

(A) Coomassie blue staining of D614G/B.1.351_RBD-NP after storage at various temperatures for 2 weeks.

(B) Coomassie blue staining of D614G/B.1.351_RBD-NP after one to five cycles of freeze-thawing.

Experiments were conducted independently in triplicates.

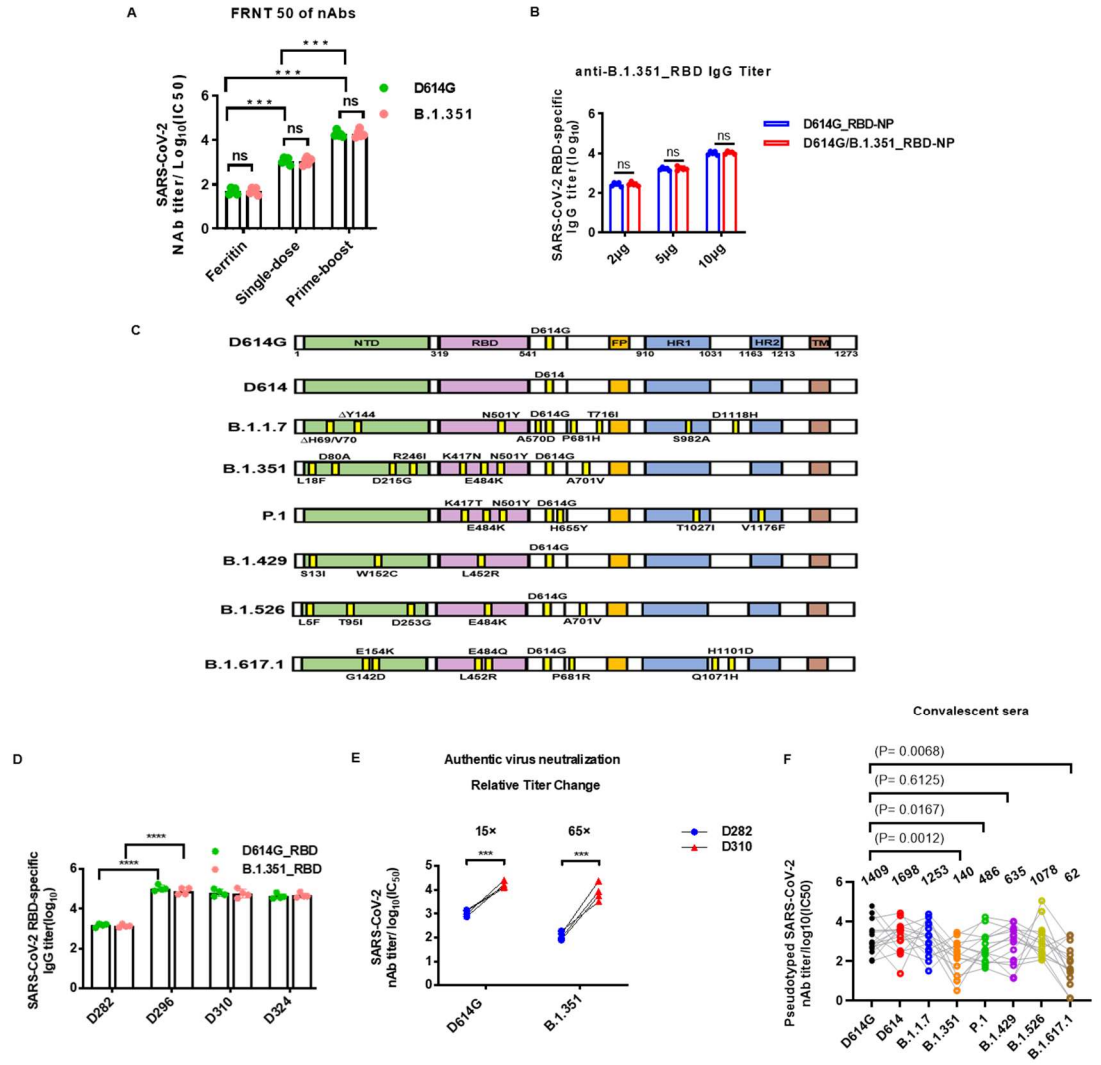

Fig. S4. The immune response to single-dose, prime-boost, and a third dose vaccine. Related to Figure 2, Figure 3 and Figure 4.

(A) The comparison of the neutralizing antibodies titers between single-dose and prime-boost utilizing FRNT assay.

(B) B.1.351_RBD-specific IgG antibodies titers of hACE2 mice immunized in a single-dose regimen with different dose were determined using ELISA by serial dilution.

(C) Schematic of mutations in the spike protein of SARS-CoV-2 among the recent mutated SARS-CoV-2 strains.

(D) D614G_RBD-specific and B.1.351_RBD-specific IgG antibodies titers of serum at indicated time were determined using ELISA by serial dilution.

(E) Fold changes in neutralization against both authentic SARS-CoV-2 viruses (D614G and B.1.351) from a third dose of bivalent D614G/B.1.351_RBD-NP vaccine.

(F) The nAbs titers of convalescent sera against SARS-CoV-2 pseudoviruses (D614G/D614/B.1.1.7/B.1.351/P.1/B.1.429/B.1.526/B.1.617.1) were determined and represented as half-maximal inhibitory concentrations (IC50).

The mean value of each group was annotated respectively. Experiments were conducted independently in triplicates. Data represented as mean ± SD. Adjusted p values were calculated by one-way ANOVA with Tukey's multiple comparisons test. ns, not significant, ***, $p < 0.001$, ****, $p < 0.0001$.