

## Supplementary information for

# **Durable cross-protective neutralizing antibody responses elicited by lipid nanoparticle-formulated SARS-CoV-2 mRNA vaccines**

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**Supplementary Table 1.**

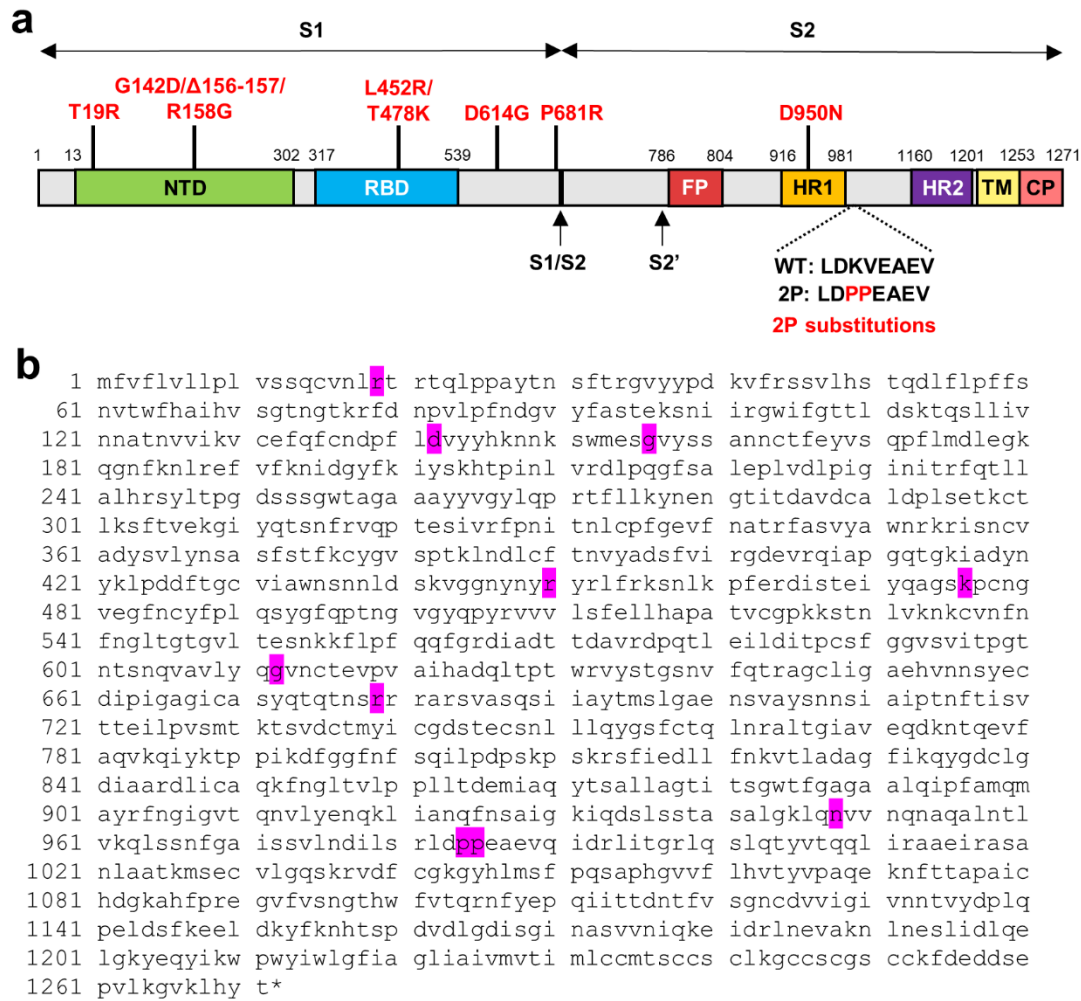
Polydispersity index and encapsulation efficiency of 4 different COVID-19 vaccine mRNA-LNPs.

Formulation	Polydispersity index (PDI)	Encapsulation efficiency (%)
PVX1010 mRNA-LNPs	$0.127 \pm 0.060$	94.3
PVX1020 mRNA-LNPs	$0.030 \pm 0.003$	93.5
PVX1040 mRNA-LNPs	$0.077 \pm 0.024$	90.9
PVX1050 mRNA-LNPs	$0.082 \pm 0.008$	93.7

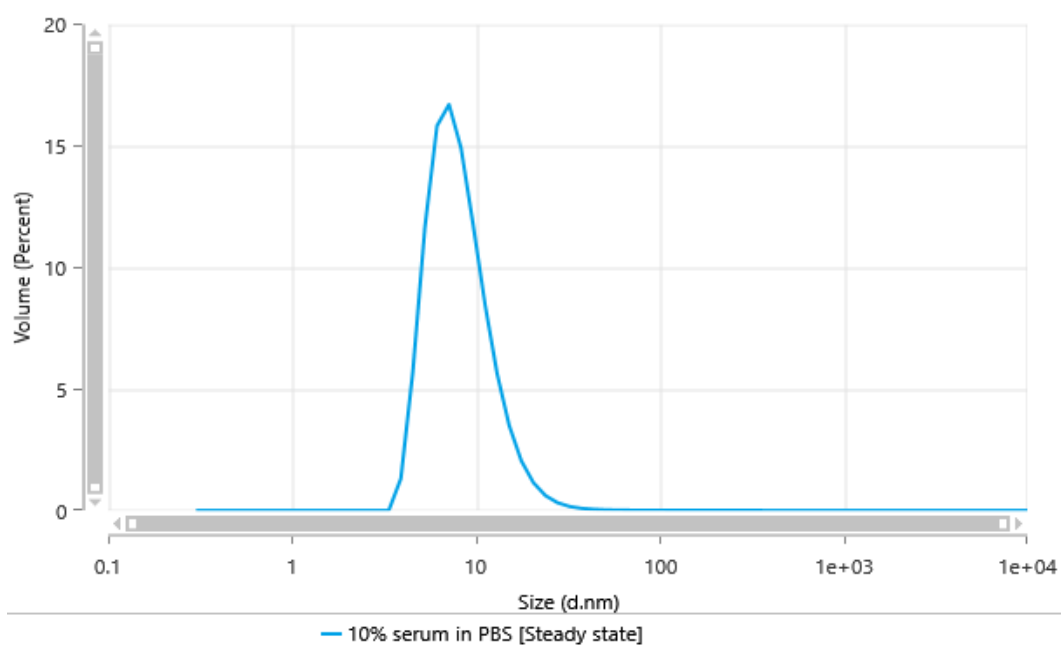
**Supplementary Table 2.**

Sequence of the primers used to generate constructs for RBD variants.

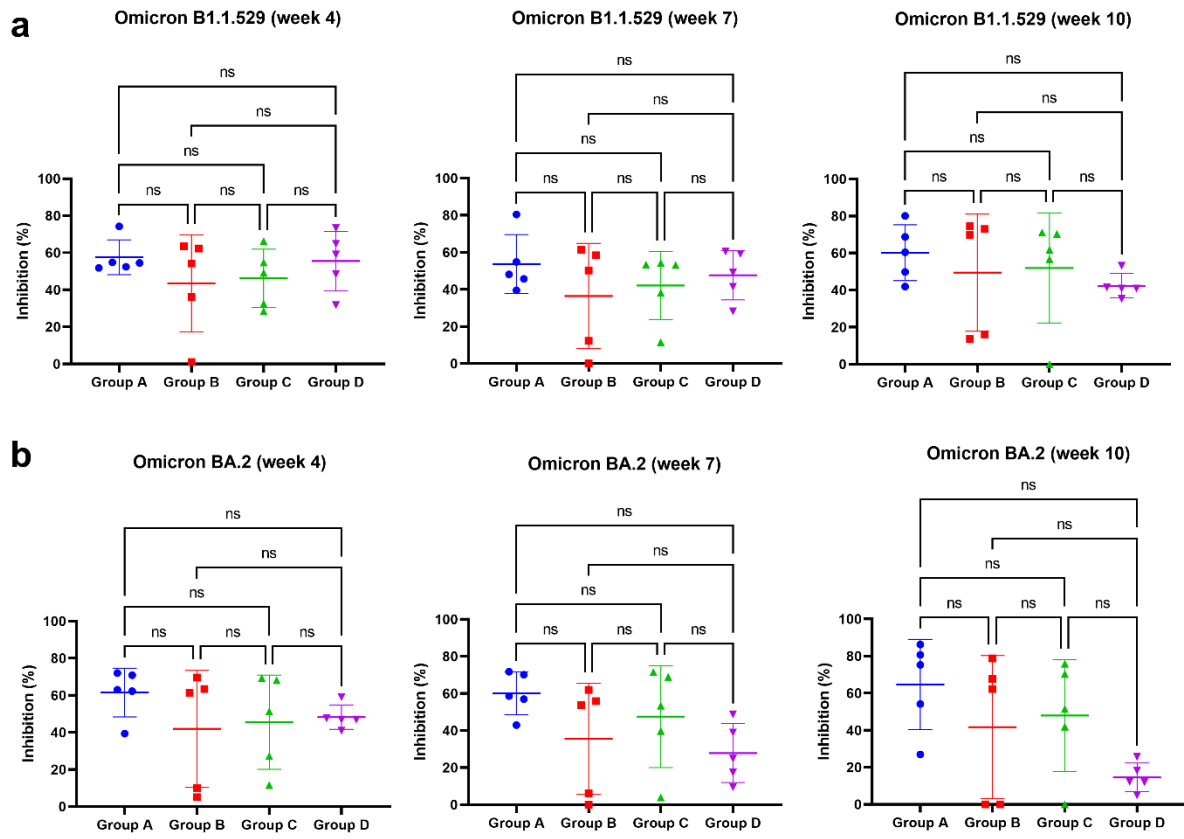
Primer	Sequence
D21 R	CAAAAGGGCACAAGTTTGTAATATTAGGAA
I14 F	ATTACAAACTTGTGCCCTTTTGATGAAG
G230 F	GCACAGGTGTTCTTACTGAGTCTAAC
E236 R	CTCAGTAAGAACACCTGTGCCTTTTAA
E236 T R	CTCAGTAAGAACACCTGTGCCTGTAA



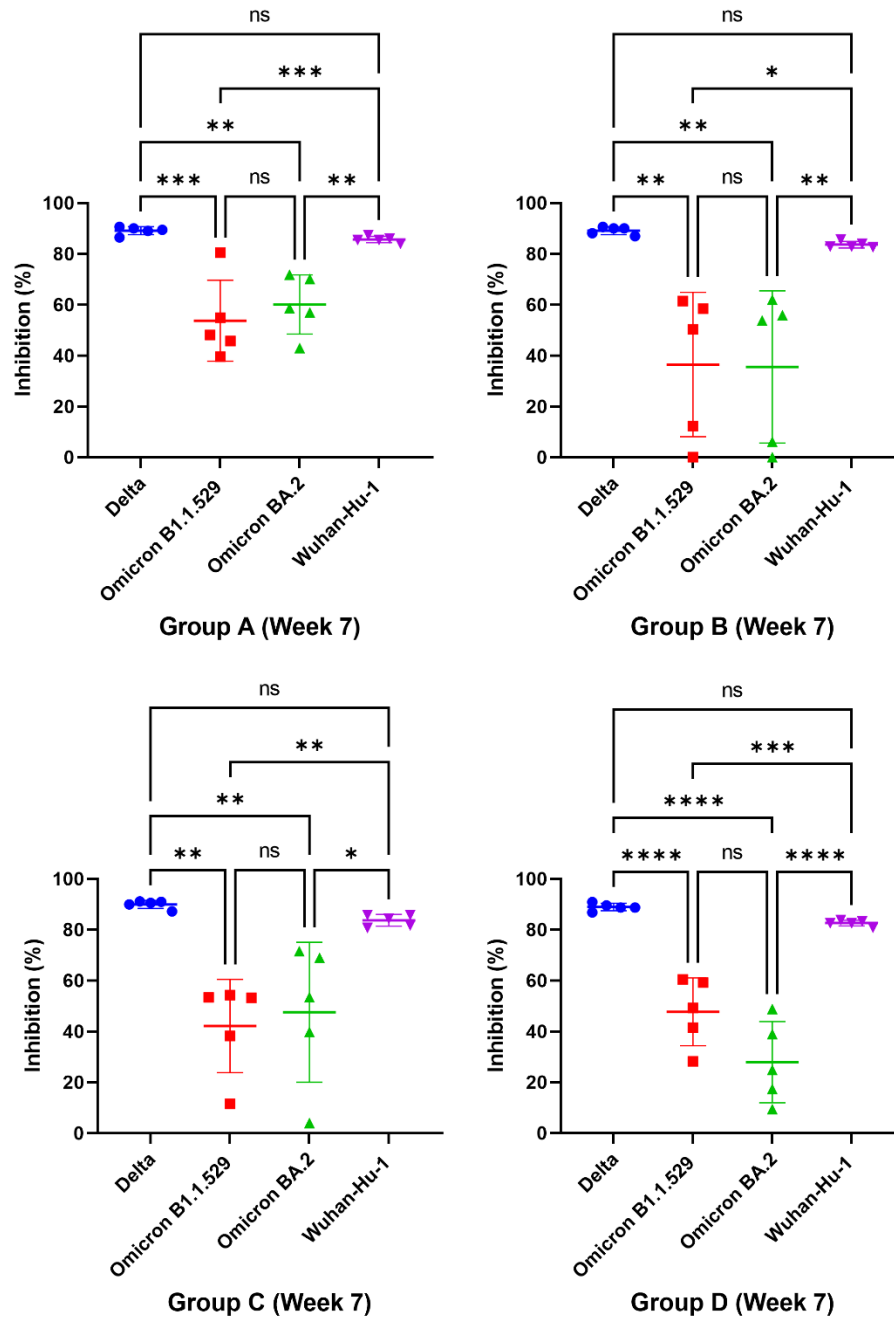
**Supplementary Figure 1.** Design of mRNA vaccine encoding the full-length spike glycoprotein of the Delta variant of SARS-CoV-2. **a** Linear diagram of the spike glycoprotein encoded by PVX1010 mRNA. N-terminal domain (NTD), receptor binding domain (RBD), S1/S2 cleavage site (S1/S2), S2 cleavage site (S2'), fusion peptide (FP), heptad repeat 1 (HR1), heptad repeat 2 (HR2), transmembrane domain (TM) and cytoplasmic tail domain (CP). Amino acid mutations, deletions and substitutions are shown in red text. **b** Protein sequence of the spike glycoprotein encoded by PVX1010 mRNA. Amino acid changes are highlighted in pink.



**Supplementary Figure 2.** Size distribution profile of 10% serum-supplemented PBS.



**Supplementary Figure 3.** ACE2-RBD binding inhibitory response observed for (a) Omicron B1.1.529 and (b) BA.2 variants at week 4, 7 and 10. The tested vaccination groups include Group A (a three-dose immunization of PVX1010 mRNA-LNPs), Group B (two doses of PVX1010 plus PVX1040 booster), Group C (two doses of PVX1010 plus PVX1050 booster) and Group D (two doses of PVX1010 plus PVX1020 booster). Mean  $\pm$  SD ( $n = 5$ ); ns: nonsignificant ( $P > 0.05$  from one-way ANOVA with Tukey's post hoc test).



**Supplementary Figure 4.** ACE2-RBD binding inhibitory responses observed at week 7. The tested vaccination groups include Group A (a three-dose immunization of PVX1010 mRNA-LNPs), Group B (two doses of PVX1010 plus PVX1040 booster), Group C (two doses of PVX1010 plus PVX1050 booster) and Group D (two doses of PVX1010 plus PVX1020 booster). Mean  $\pm$  SD ( $n = 5$ ); \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ ; \*\*\*\* $P < 0.0001$ ; ns: nonsignificant ( $P > 0.05$  from one-way ANOVA with Tukey's post hoc test).