Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Liu Y, Liu J, Xia H, et al. Neutralizing activity of BNT162b2-elicited serum. N Engl J Med. DOI: 10.1056/NEJMc2102017

(PDF updated on March 8, 2021)

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Materials and Methods

Construction of isogenic viruses. All recombinant SARS-CoV-2s with spike mutations (**Figure S1**) were prepared on the genetic background of an infectious cDNA clone derived from clinical strain USA-WA1/2020.[6] The mutations were introduced into the spike gene using a PCR-based mutagenesis protocol as reported previously.[7,8] The full-length infectious cDNAs were ligated and used as templates to *in vitro* transcribe full-length viral RNAs. The original viral stocks (P0) were recovered from Vero E6 cells on day 2 post electroporation of the *in vitro* transcribed RNAs. The P0 viruses were propagated on Vero E6 cells for another round to produce P1 viruses for the neutralization assays. The titers of P1 viruses were measured by plaque assay on Vero E6 cells (**Figure S2**). The complete spike sequences of the P1 viruses were confirmed by Sanger sequencing to have only the intended nucleotide changes from the USA-WA1/2020 sequence. A detailed protocol of the above experiments was recently reported.[9]

Serum specimens and neutralization assay. **Figure S3** illustrates the immunization and serum collection scheme. A conventional 50% plaque-reduction neutralization test ($PRNT_{50}$) was performed to quantify the serum-mediated virus suppression as previously reported.[10] Briefly, individual sera were 2-fold serially diluted in culture medium with a starting dilution of 1:40 (dilution range of 1:40 to 1:1280). The diluted sera were incubated with 100 PFU of USA-WA1/2020 or mutant SARS-CoV-2. After 1 h incubation at 37 °C, the serum-virus mixtures were inoculated onto a monolayer of Vero E6 cells pre-seeded on 6-well plates on the previous day. A minimal serum dilution that suppressed >50% of viral plaques is defined as $PRNT_{50}$. The neutralization titers are presented in **Table S1**.

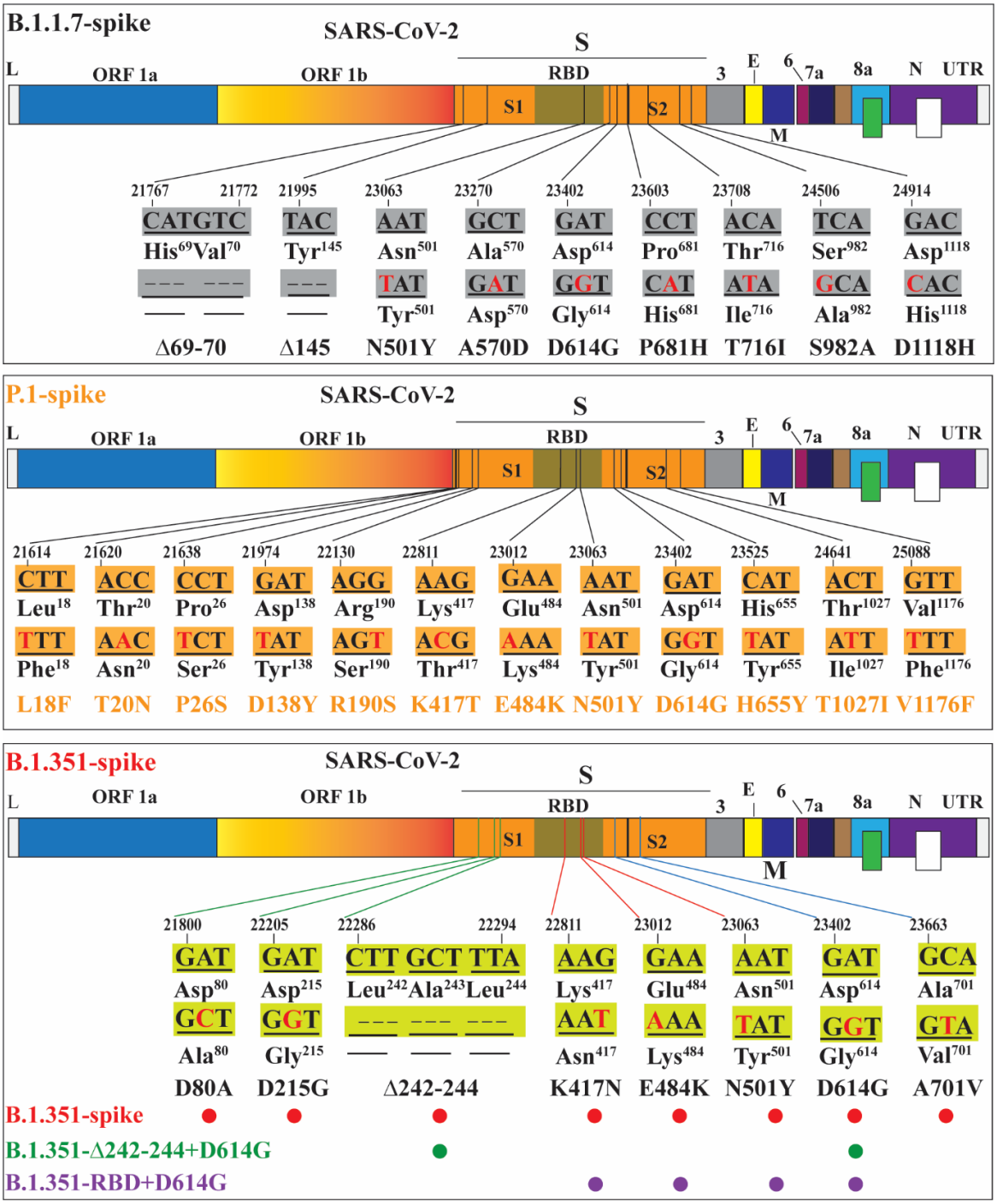

Figure S1. Diagram of engineered spike substitutions and deletions. The genome and sequence of clinical isolate USA-WA1/2020 are used as the wild-type virus in this study. Mutations from the United Kingdom B.1.1.7, Brazilian P.1, and South African B.1.351 lineages are presented. Deletions are indicated by dotted lines. Mutated nucleotides are in red. Nucleotide and amino acid positions are indicated. L – leader sequence; ORF – open reading frame; RBD – receptor binding domain; S – spike glycoprotein; S1 – N-terminal furin cleavage fragment of S; S2 – C-terminal furin cleavage fragment of S; E – envelope protein; M – membrane protein; N – nucleoprotein; UTR – untranslated region.

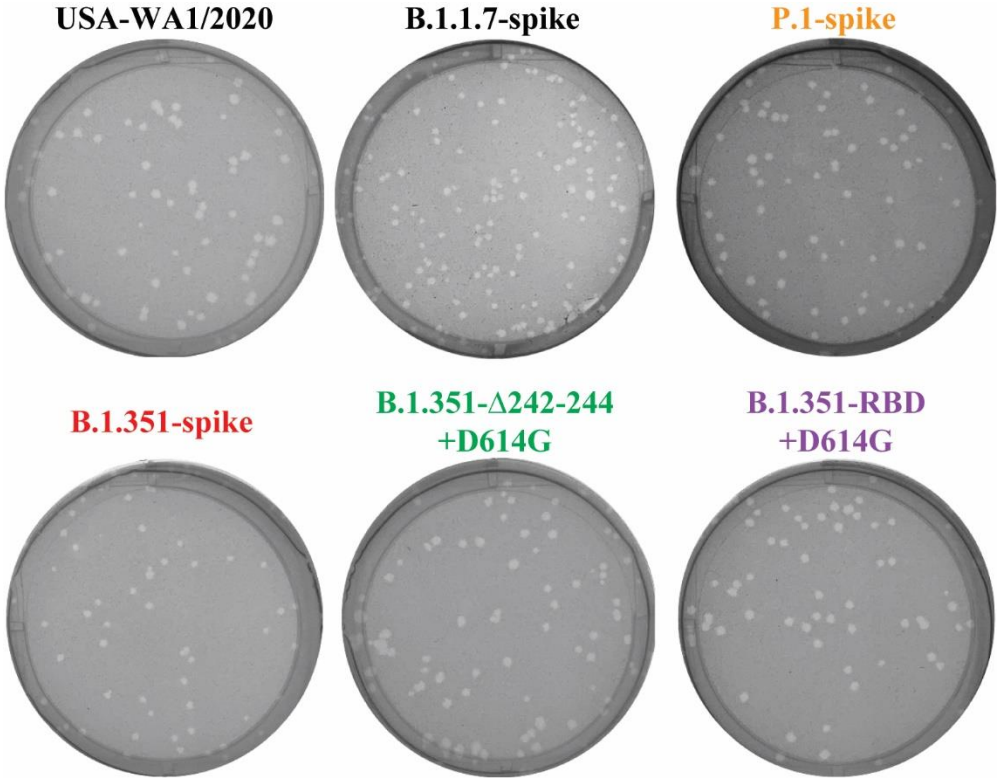

Figure S2. Plaque morphologies of USA-WA1/2020 and mutant SARS-CoV-2's. The plaque assays were performed on Vero E6 cells in 6-well plates.

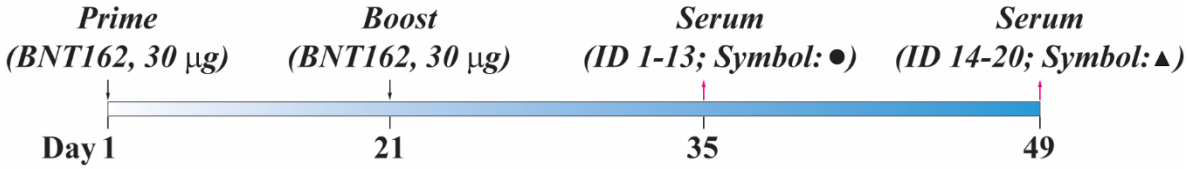

Figure S3. Scheme of BNT162 immunization and serum collection.

Table S1. $PRNT_{50}$'s of twenty BNT162b2 post-immunization sera against USA-WA1/2020 and mutant SARS-CoV-2.

Serum			$PRNT_{50}$*												
			USA-WA1/2020				B.1.1.7-spike	P.1-spike			B.1.351-spike			B.1.351-Δ242-244 +D614G	B.1.351-RBD +D614G
ID^	Age	Week	Exp1	Exp2	Exp3	GMT		Exp1	Exp2	GMT	Exp1	Exp2	GMT		
1	68	2	320	320	320	320	640	320	320	320	160	160	160	320	320
2	67	2	160	160	160	160	160	80	80	80	40	40	40	160	80
3	68	2	640	640	640	640	640	640	640	640	320	320	320	640	640
4	65	2	160	320	320	254	320	320	320	320	80	160	113	160	80
5	30	2	320	320	320	320	640	320	320	320	160	160	160	320	320
6	23	2	320	640	640	508	320	640	320	453	160	160	160	320	160
7	54	2	1280	640	640	806	1280	640	1280	905	320	320	320	1280	1280
8	69	2	320	320	320	320	320	160	160	160	80	80	80	160	160
9	65	2	1280	1280	1280	1280	1280	1280	640	905	640	640	640	1280	1280
10	38	2	640	640	1280	806	1280	640	320	453	640	320	453	1280	640
11	44	2	320	320	320	320	640	320	320	320	80	160	113	320	160
12	52	2	640	640	640	640	640	640	320	453	160	160	160	320	320
13	28	2	1280	1280	1280	1280	1280	640	640	640	160	320	226	1280	640
14	69	4	320	320	640	403	320	320	160	226	160	80	113	320	160
15	68	4	640	640	640	640	640	1280	640	905	320	320	320	640	320
16	26	4	320	320	640	403	1280	640	320	453	160	320	226	640	320
17	54	4	1280	1280	1280	1280	1280	1280	640	905	320	320	320	1280	640
18	35	4	640	640	640	640	640	640	320	453	160	320	226	320	320
19	44	4	640	640	640	640	640	1280	640	905	320	320	320	640	640
20	52	4	640	640	640	640	1280	640	320	453	160	160	160	640	320
	GMT†		502	520	577	532	663	520	368	437	184	204	194	485	331
	95% CI#		371-680	401-674	443-751	409-693	497-884	372-726	275-491	325-589	133-255	151-276	144-261	345-681	228-480

*The data for USA-WA1/2020 are from three experiments; the data for B.1.1.7-spike, B.1.351-Δ242-244+D614G, and B.1.351-RBD-D614G viruses are from one experiment each; and the data for P.1-spike and B.1.351-spike viruses are from two experiments. For each independent experiment, individual $PRNT_{50}$ value is the geometric mean of duplicate plaque assay results; no differences were observed between the duplicate assays.

^Pairs of serum samples were obtained from a single participant as follows: Serum ID 1 and 15, 7 and 17, 8 and 14, 11 and 19, and 12 and 20.

[†]Geometric mean neutralizing titers.

[#]95% confidence interval (95% CI) for the GMT.

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Supplemental References

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