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. BNT162b2-elicited neutralization against new SARS-CoV-2 spike variants. N Engl J Med. DOI: 10.1056/NEJMc2106083

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Materials and Methods

Construction and characterization of SARS-CoV-2s with variant spikes. Recombinant SARS-CoV-2s with variant spike mutations (**Figure S1**) were engineered into the genetic background of an infectious cDNA clone of clinical isolate USA-WA1/2020.[1] The spike mutations were introduced into the infectious cDNA clone using PCR-based mutagenesis as previously described.[2] The full-length cDNAs of viral genome containing the variant spike mutations were assembled via *in vitro* ligation; the resulting genome-length cDNAs were used as templates for *in vitro* transcription of full-length viral RNAs. Vero E6 cells were electroporated with the *in vitro* transcribed full-length viral RNAs. On day 2 post electroporation, the original viral stocks (P0) were harvested from the electroporated Vero E6 cells. The P0 viruses were amplified for another round on Vero E6 cells to produce the P1 stocks of viruses for neutralization experiments. The infectious titers of P1 viruses were quantified by plaque assay on Vero E6 cells (**Figure S2**). The complete sequences of spike genes from the P1 viruses were verified by Sanger sequencing to ensure no undesired changes. A detailed protocol for the mutagenesis of SARS-CoV-2 has been recently reported.[3] For determining the specific infectivity of each virus preparation, the P1 stocks were quantified for their genomic RNA content and plaque-forming units (PFU) by RT-qPCR and plaque assay on Vero E6 cells, respectively. The methods for RT-qPCR and plaque assay have been reported previously.[4] Genomic RNA to PFU ratios (genomes/PFU) were calculated to indicate the specific infectivity of each virus preparation.

Serum specimens and plaque-reduction neutralization assay. Serum specimens were collected from BTN162b2-immunized human participants as illustrated in **Figure S3**. The set of twenty sera, which has also been used in previous studies of SARS-CoV-2 neutralization by BNT162b2-elicited sera, was selected from available samples drawn 14 days or one month after two doses of 30 μg BNT162b2 were administered to participants in the phase 1 portion of the C4591001 clinical trial.[2,5,6] The sera were selected to represent a wide range of neutralization

titers and samples from younger adult (aged 18-55 years) and older adult (aged 65-85 years) participants in the study.

A conventional 50% plaque-reduction neutralization test ($PRNT_{50}$) was performed to measure the serum-mediated virus suppression as reported previously.[5,7] 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 (WT) or mutant SARS-CoV-2. After 1 h incubation at 37 °C, the serum-virus mixtures were inoculated onto 6-well plates with a monolayer of Vero E6 cells pre-seeded on the previous day. The minimal serum dilution that suppressed >50% of viral plaques is defined as $PRNT_{50}$. **Table S1** summarizes the $PRNT_{50}$ results.

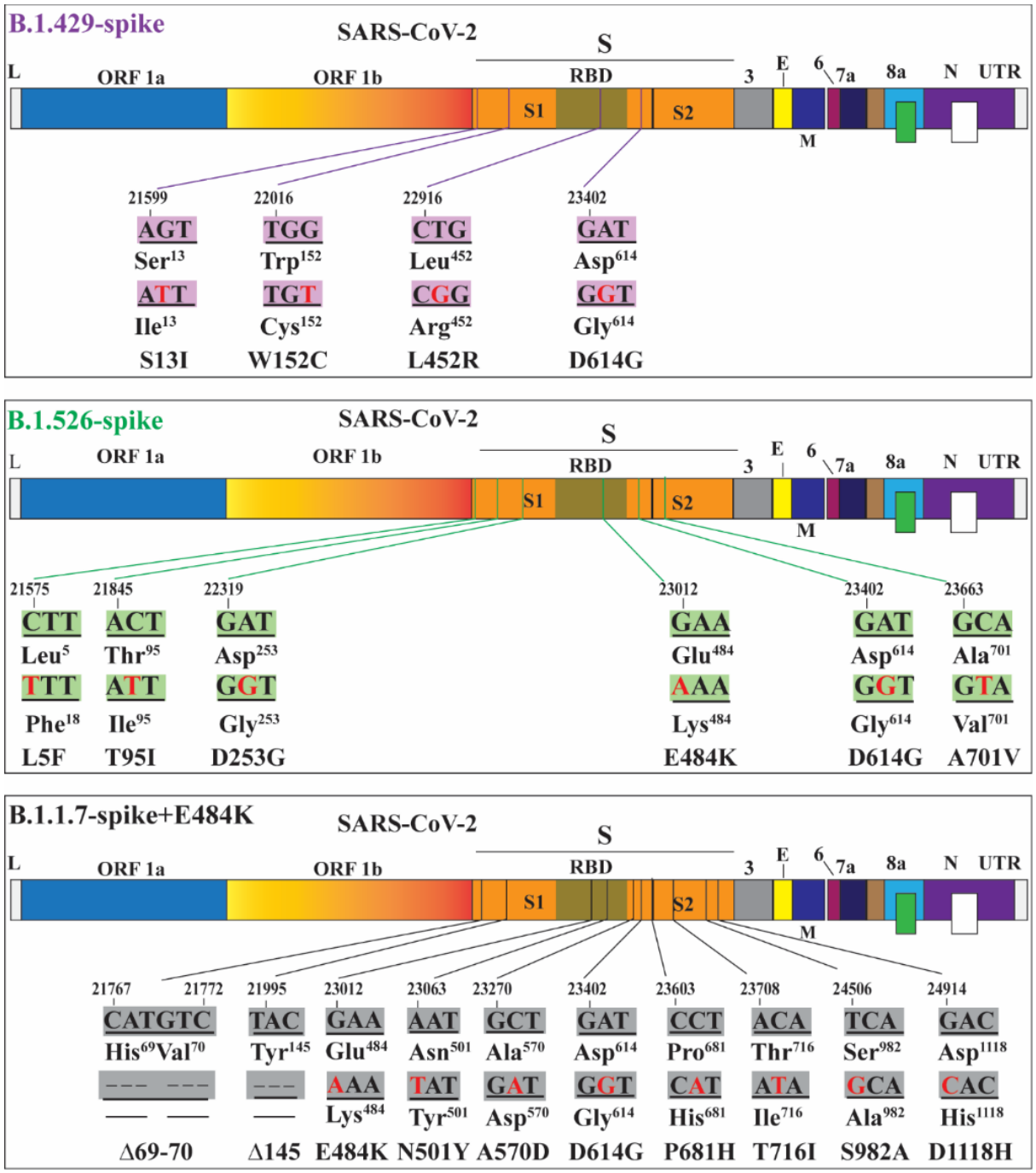

Figure S1. Diagram of engineered variant spike mutations. Clinical isolate USA-WA1/2020 was used as the wild-type virus to engineer variant spikes. Mutations and deletions are indicated in red and by dotted lines, respectively. Nucleotide and amino acid positions are indicated. L: leader sequence; ORF: 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.

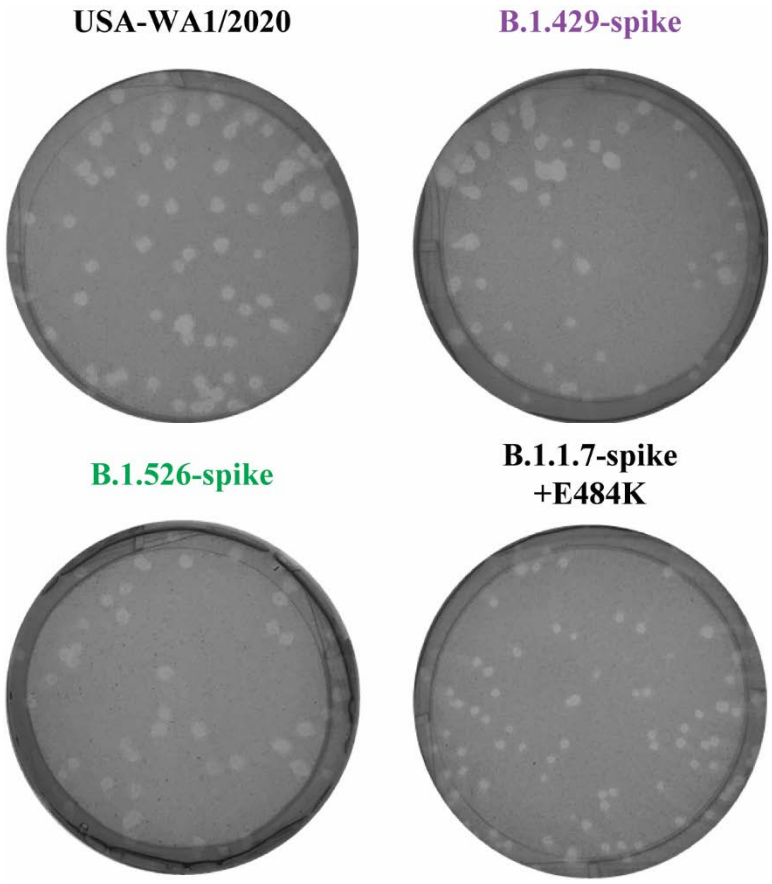

Figure S2. Plaque morphologies of USA-WA1/2020 and mutant SARS-CoV-2. The plaque assays were performed on Vero E6 cells in 6-well plates.

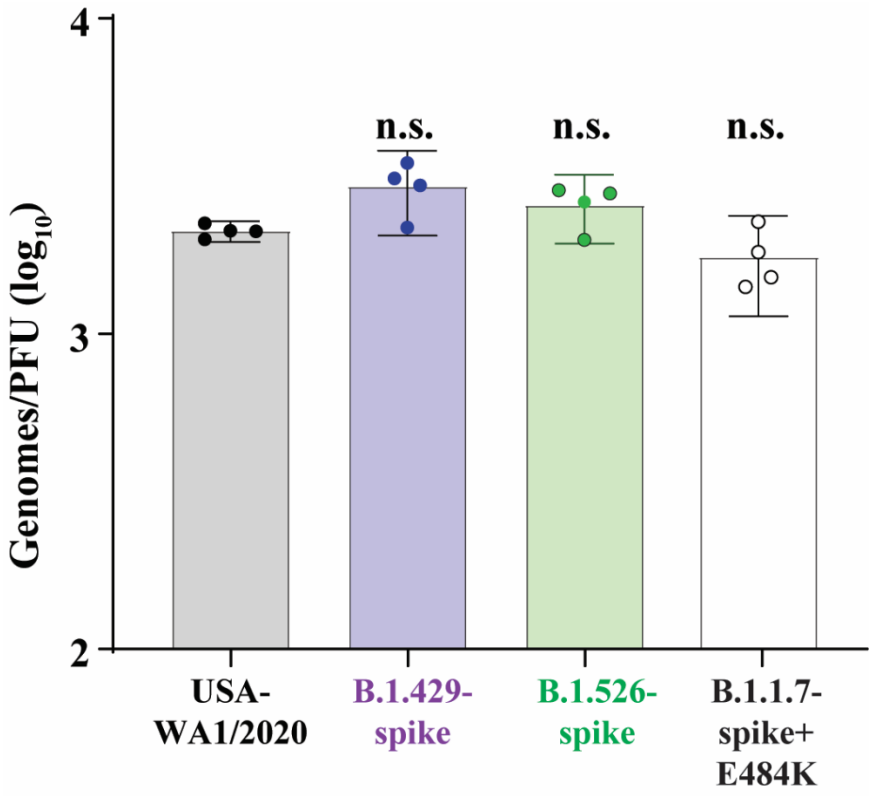

Figure S3. Comparison of viral genomic RNA versus plaque-forming unit ratios (genomes/PFU) of different recombinant SARS-CoV-2. Individual recombinant virus stocks were quantified for their genomic RNA and infectious plaque-forming units by RT-qPCR and plaque assay, respectively. The genomes/PFU ratio was calculated to determine specific infectivity. Dots represent individual biological replicates from 4 different aliquots of viruses. The values in the graph represent the means with 95% confidence intervals. A non-parametric Mann-Whitney test was used to determine significant differences between the variants and USA-WA1/2020. *P* values were adjusted using the Bonferroni correction to account for multiple comparisons. Differences were considered significant if $p < 0.05$; n.s., no statistical difference.

Figure S4. BNT162b2 immunization scheme and serum collection.

Table S1. $PRNT_{50}$ values of twenty BNT162b2-immunized sera against USA-WA1/2020 and variant SARS-CoV-2.

#Serum			*$PRNT_{50}$					
			USA-WA1/2020			B.1.429-spike	B.1.526-spike	B.1.1.7-spike+E484K
ID	Age	Week	Exp1	Exp2	GMT			
1	68	2	640	320	453	320	320	640
2	67	2	160	160	160	80	160	80
3	68	2	640	1280	905	640	1280	1280
4	65	2	640	640	640	320	640	640
5	30	2	320	320	320	320	320	320
6	23	2	320	640	453	320	640	320
7	54	2	640	640	640	640	320	640
8	69	2	320	320	320	320	320	320
9	65	2	1280	640	905	640	640	1280
10	38	2	640	640	640	640	640	640
11	44	2	640	640	640	640	640	640
12	52	2	640	640	640	320	640	1280
13	28	2	1280	1280	1280	640	640	1280
14	69	4	320	320	320	320	160	320
15	68	4	320	320	320	320	320	640
16	26	4	320	320	320	320	320	1280
17	54	4	640	640	640	640	640	640
18	35	4	640	640	640	640	640	640
19	44	4	640	640	640	320	640	640
20	52	4	640	640	640	320	640	640
†GMT			520	520	520	394	649	597
^95% CI			410-659	410-659	415-652	311-500	366-599	436-817

*The data for USA-WA1/2020 are from two independent experiments; the others are from one experiment each. For each independent experiment, the 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 at 2 and 4 weeks after the second dose 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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