

# BNT162b2-elicited neutralization of B.1.617 and other SARS-CoV-2 variants

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is continuing to evolve around the world, generating new variants that are of concern on the basis of their potential for altered transmissibility, pathogenicity, and coverage by vaccines and therapeutic agents<sup>1–5</sup>. Here we show that serum samples taken from twenty human volunteers, two or four weeks after their second dose of the BNT162b2 vaccine, neutralize engineered SARS-CoV-2 with a USA-WA1/2020 genetic background (a virus strain isolated in January 2020) and spike glycoproteins from the recently identified B.1.617.1, B.1.617.2, B.1.618 (all of which were first identified in India) or B.1.525 (first identified in Nigeria) lineages. Geometric mean plaque reduction neutralization titres against the variant viruses—particularly the B.1.617.1 variant—seemed to be lower than the titre against the USA-WA1/2020 virus, but all sera tested neutralized the variant viruses at titres of at least 1:40. The susceptibility of the variant strains to neutralization elicited by the BNT162b2 vaccine supports mass immunization as a central strategy to end the coronavirus disease 2019 (COVID-19) pandemic globally.

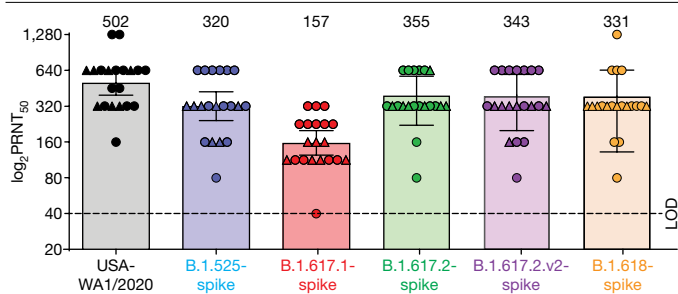
Since its emergence in late 2019, SARS-CoV-2 has caused more than 174 million infections and more than 3.7 million deaths resulting from COVID-19 worldwide (as of 9 June 2021; <https://coronavirus.jhu.edu/>). Although coronaviruses have a proofreading mechanism to maintain their long genomic RNAs<sup>6</sup>, mutations continuously emerge in circulating viruses. Because the viral spike protein (S) binds to angiotensin-converting enzyme 2 (ACE2), the cellular receptor for virus attachment, and mediates membrane fusion during viral entry, mutations in the spike protein can alter SARS-CoV-2 transmission, tissue tropism, and disease outcome<sup>7</sup>. Indeed, the first prevalent spike mutation, D614G, promotes spike binding to ACE2, leading to enhanced transmission of SARS-CoV-2<sup>3,8–11</sup>. Subsequently, another spike mutation, N501Y, emerged convergently in several variants that were first identified in different locations, including the UK (lineage B.1.1.7), Brazil (lineage P.1), and South Africa (lineage B.1.351)<sup>2</sup>. The N501Y mutation also increases the affinity of the spike for ACE2 and increases viral transmission<sup>12,13</sup>. Some mutations in the spike, such as E484K, contribute to evasion of antibody neutralization. The E484K mutation has emerged independently in many variants, such as P.1, B.1.351, B.1.526 (first identified in New York), B.1.525 (first identified in Nigeria), and P3 (first identified in the Philippines)<sup>1,2,14</sup>. Thus, as the COVID-19 pandemic continues, it is essential to closely monitor the effects of new mutations or combinations of mutations on viral transmission, pathogenesis, and vaccine and therapeutic efficacies.

BNT162b2, an mRNA vaccine that expresses the full prefusion spike glycoprotein of SARS-CoV-2, is 95% effective against COVID-19<sup>15</sup>. The US Food and Drug Administration has authorized BNT162b2 for

vaccination of individuals 12 years of age and older under emergency use provisions. Although the sequence of the mRNA in BNT162b2 is based on the original SARS-CoV-2 isolate<sup>16</sup>, it has previously been shown that sera from individuals immunized with BNT162b2 retained neutralizing activity against all tested variants, including the B.1.1.7, P.1, B.1.351, B.1.429, B.1.526, and B.1.1.7+E484K lineages<sup>1,2,4,5,17</sup>. Since then, a massive second wave of COVID-19 in India has been associated with the expansion of variant B.1.617.1 to 32 countries, B.1.617.2 to 49 countries, and B.1.618 to 6 countries (as of 31 May 2021; [https://cov-lineages.org/lineages/lineage\\_B.1.618.html](https://cov-lineages.org/lineages/lineage_B.1.618.html)). The B.1.617.2 variant has shown evidence of particularly high transmissibility in the UK<sup>18</sup>. In addition, variant B.1.525, which was initially detected in Nigeria, has spread to 49 countries. All of these variants are currently circulating in the USA. The World Health Organization has designated the B.1.617 lineage as a variant of concern and B.1.525 as a variant of interest<sup>18</sup>. This study analyses BNT162b2-elicited neutralization against these newly identified variants.

To examine the effects of the variants' mutations on neutralization, we used a reverse genetic system to swap the complete spike gene from different variants into an early SARS-CoV-2 isolate<sup>19</sup> (USA-WA1/2020, defined as wild-type) (Extended Data Fig. 1a). We prepared five chimeric viruses with different spike proteins, as follows: (1) B.1.525-spike (with Q52R, A67V, H69/Y70 deletion ( $\Delta$ 69/70), Y145 deletion ( $\Delta$ 145), E484K, D614G, Q677H, and F888L from the B.1.525 variant<sup>18</sup>); (2) B.1.617.1-spike (with G142D, E154K, L452R, E484Q, D614G, P618R, Q1071H, H1101D, and a synonymous mutation at D111 (nucleotide T21895C) from the B.1.617.1 variant); (3) B.1.617.2-spike (with T19R, G142D, L452R, T478K, D614G, P681R,

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**Fig. 1 | Neutralization of USA-WA1/2020 and variant SARS-CoV-2 viruses by BNT162b2-induced immune sera.** The PRNT<sub>50</sub> results for USA-WA1/2020 and variant viruses are plotted. Individual PRNT<sub>50</sub> values are presented in Extended Data Table 1. Each data point represents the geometric mean PRNT<sub>50</sub> against the indicated virus obtained with a serum sample collected two weeks (circles) or four weeks (triangles) after the second dose of vaccine. PRNT<sub>50</sub> values were determined in duplicate assays, and the geometric means were calculated ( $n=20$ , pooled from two independent experiments). The heights of bars and the numbers over the bars indicate geometric mean titres; error bars show 95% confidence intervals. LOD, limit of detection at 1:40. Statistical significance (two-tailed Wilcoxon matched-pairs signed-rank test) of the difference between geometric mean titres for USA-WA1/2020 and each variant:  $P=0.002$  for B.1.525-spike,  $P<0.0001$  for B.1.617.1-spike,  $P=0.001$  for B.1.617.2-spike,  $P=0.004$  for B.1.617.2-v2-spike,  $P=0.001$  for B.1.618-spike.

and D950N from an early B.1.617.2 variant (GISAID (<https://www.gisaid.org/>) accession number EPI\_ISL\_1663247); (4) B.1.617.2-v2-spike (with the mutations in B.1.617.2-spike plus an additional E156G substitution and F157–R158 deletion ( $\Delta 157-158$ ) found in currently circulating B.1.617.2 isolates<sup>18</sup>); and (5) B.1.618-spike (with H49Y, Y145–H146 deletion ( $\Delta 145-146$ ), E484K, and D614G from the B.1.618 variant<sup>20</sup>). All mutant viruses yielded infectious titres of more than  $10^7$  plaque-forming units (PFUs) per millilitre. The B.1.617.1-spike virus formed smaller plaques than other viruses on Vero E6 cells (Extended Data Fig. 1b). All viruses were quantified for their viral RNA genome-to-PFU ratios (a parameter that indicates virus infectivity). None of the variant spikes significantly altered the viral RNA-to-PFU ratio (Extended Data Fig. 1c), suggesting that the viruses had similar specific infectivities. The complete spikes of all viral stocks were sequenced to ensure that they contained no undesired mutations.

To compare the susceptibility of different variants to neutralization, we performed 50% plaque reduction neutralization (PRNT<sub>50</sub>) testing using a panel of 20 sera collected from volunteers who were immunized with BNT162b2 in a pivotal clinical trial<sup>15,21</sup>. The serum specimens were drawn two or four weeks after the second of two immunizations with 30  $\mu$ g of BNT162b2, which were spaced three weeks apart (Extended Data Fig. 2). Each serum was tested simultaneously for its PRNT<sub>50</sub> against the wild-type and mutant viruses (Extended Data Table 1). All the sera neutralized the wild-type and all mutant viruses with titres of 1:40 or higher (Fig. 1). The geometric mean neutralizing titres against the wild-type, B.1.525-spike, B.1.617.1-spike, B.1.617.2-spike, B.1.617.2-v2-spike, and B.1.618-spike viruses were 502, 320, 157, 355, 343, and 331, respectively (Fig. 1). The results indicate that neutralization of all variants, except the B.1.617.1 variant, was only modestly reduced relative to neutralization of the wild-type virus. Although neutralization of B.1.617.1 was reduced more strongly, BNT162b2 immune sera efficiently neutralized the B.1.617.1 virus and all of the other viruses.

In response to the global pandemic of COVID-19, the scientific community has increased surveillance to identify mutations in circulating SARS-CoV-2 strains that might increase infectivity, enhance pathogenicity, or alter coverage by therapeutic agents or vaccines. Such information is essential to guide public policy and the development of countermeasures. As part of ongoing diligence on coverage

of variants by the BNT162b2 vaccine, we have engineered variant spike genes into the backbone of the USA-WA1/2020 isolate, and, using the gold standard PRNT<sub>50</sub> assay, tested neutralization of the resulting viruses by a panel of BNT162b2-immunized human sera drawn two or four weeks after the second of two doses of BNT162b2 given three weeks apart<sup>4,5</sup>. Among all tested viruses, those with spike proteins from B.1.351<sup>4</sup> and B.1.617.1 (this study) exhibited the greatest reduction in neutralization by the sera, with PRNT<sub>50</sub> values 0.36 times and 0.31 times, respectively, that of USA-WA1/2020. Similarly, a recent study found that BNT162b2-induced immune sera neutralized a clinical B.1.617.1 isolate with 0.14 times the neutralization titre of the sera against the wild-type virus<sup>22</sup>. Other studies have found that BNT162b2-induced immune sera have 0.25 to 0.35 times the inhibitory titre against a pseudovirus with a B.1.617.1 spike compared to that against wild-type spike pseudovirus<sup>23</sup>, and that BNT162b2-induced immune sera inhibit a pseudovirus with a B.1.618 spike with 0.37 times the serum inhibition titre against a wild-type spike pseudovirus<sup>20</sup>. Our results showed that among the four tested variants that were first identified in India, B.1.617.1 was the least neutralized, probably owing to the presence of both L452R and E484Q substitutions at the receptor binding site (potentially under positive selection for resistance to neutralization by antibodies)<sup>14,24</sup>. Nevertheless, all variants were still neutralized by all tested sera at titres of at least 40. The reduction in neutralization could be a combined effect of mutation-mediated escape from antibody binding and mutation-altered spike function.

A recent real-world study in participants who had received two doses of BNT162b2 demonstrated an effectiveness of 75% against any documented infection and 100% against documented severe, critical, or fatal disease caused by the variant B.1.351<sup>25</sup>, which showed a similar reduction in neutralization titres to B.1.617.1. Consistent with the modest reduction in neutralization of the B.1.617.2 variants by BNT162b2-elicited sera reported here, a test-negative case–control study conducted in the UK found that the real-world effectiveness of two doses of BNT162b2 against B.1.617.2 virus was reduced only modestly to 87.9%, compared with 93.4% effectiveness against B.1.1.7 lineage virus<sup>26</sup>. Thus, reductions in neutralization such as those observed here have not been demonstrated to result in loss of vaccine efficacy against disease. BNT162b2 elicits not only neutralizing antibodies, but also spike-specific CD4<sup>+</sup> and CD8<sup>+</sup> T cells and non-neutralizing antibody-dependent cytotoxicity, which can also serve as immune effectors<sup>27,28</sup>. Because neutralization titres do not measure all potentially protective vaccine responses, they cannot substitute for studies of vaccine efficacy and the real-world effectiveness of COVID-19 vaccines against variants.

A limitation of the current study is the potential for mutations to alter neutralization by affecting spike function rather than antigenicity, even though the variant viruses exhibited similar infectious titres and specific infectivities to the original USA-WA1/2020 isolate. In addition, we examined the effect of mutations only in the spike glycoproteins. Mutations outside the spike gene could also affect viral replication and host immune response. We also did not examine the durability of neutralization titres against the variant viruses.

New variants will continue to emerge as the pandemic persists. To date, there is no evidence that virus variants have escaped BNT162b2-mediated protection from COVID-19. Therefore, increasing the proportion of the population immunized with current safe and effective authorized vaccines remains a key strategy to minimize the emergence of new variants and end the COVID-19 pandemic.

## Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at <https://doi.org/10.1038/s41586-021-03693-y>.

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## Methods

### Cells

African green monkey kidney epithelial Vero E6 cells (ATCC) were grown in Dulbecco's modified Eagle's medium (DMEM; Gibco/Thermo Fisher) with 10% fetal bovine serum (FBS; HyClone Laboratories) and 1% antibiotic/streptomycin (Gibco). The cell line was authenticated through STR profiling by ATCC and tested negative for mycoplasma.

### Construction of SARS-CoV-2s with variant spikes

All mutations from individual variant spike genes were engineered into an infectious cDNA clone of isolate USA-WA1/2020<sup>19</sup>. The spike mutations were introduced using a standard PCR-based mutagenesis method. A detailed protocol for construction of recombinant SARS-CoV-2 has previously been reported<sup>29</sup>. In brief, the full-length cDNAs of viral genome containing the variant spike mutations were assembled by T4 ligase-mediated in vitro ligation. The resulting genome-length cDNAs were used as templates to in vitro transcribe full-length viral RNAs. The in vitro transcribed full-length viral RNAs were electroporated into Vero E6 cells. When electroporated cells developed cytopathic effects (due to recombinant virus production and replication) on day 2 after electroporation, the original viral stocks (P0) were collected from the culture medium. The P0 viruses were amplified for another round on Vero E6 cells to produce the P1 stocks of viruses. The infectious titres of P1 viruses were measured by plaque assay on Vero E6 cells as previously described<sup>19</sup>. The complete sequences of spike genes from the P1 viruses were verified by Sanger sequencing to ensure that there were no undesired mutations. The P1 viruses were used for subsequent neutralization testing.

### Characterization of wild-type and mutant recombinant SARS-CoV-2s

To determine the specific infectivity of each virus, we quantified the P1 stocks for their genomic RNA content and PFUs by quantitative PCR with reverse transcription (RT-qPCR) and plaque assay on Vero E6 cells, respectively. The protocols for RT-qPCR and plaque assay have previously been reported<sup>3</sup>. Genomic viral RNA-to-PFU ratios (genomes/PFU) were calculated to indicate the specific infectivity of each virus preparation.

### BTN162b2 vaccine-immunized human sera

A panel of 20 serum specimens was collected from 15 BTN162b2-immunized participants in a clinical trial<sup>15,21</sup>. The sera were collected two or four weeks after the second of two doses of 30 µg BNT162b2 mRNA, spaced three weeks apart (Extended Data Fig. 2). Five of the 20 participants provided sera at both two and four weeks after the second dose of vaccine, as detailed in the footnote to Extended Data Table 1.

### Plaque-reduction neutralization assay

A PRNT<sub>50</sub> assay, which represents the gold standard neutralization assay, was performed to quantify serum-mediated virus suppression.

Individual sera were twofold serially diluted in culture medium with a starting dilution of 1:40. The diluted sera were mixed with 100 PFU of wild-type USA-WA1/2020 or variant mutant SARS-CoV-2. After 1 h incubation at 37 °C, the serum and virus mixtures were inoculated onto 6-well plates with a monolayer of Vero E6 cells pre-seeded the previous day. The minimal serum dilution that suppressed more than 50% of viral plaques is defined as PRNT<sub>50</sub>. A detailed PRNT<sub>50</sub> protocol has previously been reported<sup>21,30</sup>.

### Statistical analysis

Statistical analyses were performed by Graphpad Prism 9 for all experiments as detailed in the figure legends. No statistical methods were used to predetermine sample size. The experiments were not randomized and the investigators were not blinded to allocation during experiments and outcome assessment.

### Reporting summary

Further information on research design is available in the Nature Research Reporting Summary linked to this paper.

### Data availability

Source data for generating the main figures are available in the online version of the paper. Any other data are available upon request.

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**Author contributions** Conceptualization: K.U.J., U.S., X.X., K.A.S., A.M., P.R.D., P.-Y.S.; methodology: J.L., Y.L., H.X., J.Z., S.C.W., K.A.S., H.C., A.M., K.U.J., U.S., X.X., P.R.D., P.-Y.S.; investigation: J.L., Y.L., H.X., J.Z., S.C.W., K.A.S., H.C., M.C., D.C., K.U.J., U.S., X.X., P.R.D., P.-Y.S.; data curation: J.L., Y.L., M.C., D.C., X.X., P.-Y.S.; writing, original draft: J.L., Y.L., U.S., X.X., P.R.D., P.-Y.S.; writing, review and editing: S.C.W., K.A.S., A.M., K.U.J., U.S., X.X., P.R.D., P.-Y.S.; supervision: K.U.J., U.S., X.X., P.R.D., P.-Y.S.; funding acquisition: K.U.J., U.S., P.R.D., P.-Y.S.

**Competing interests** X.X. and P.-Y.S. have filed a patent on the reverse genetic system of SARS-CoV-2. K.A.S., H.C., M.C., D.C., K.U.J., and P.R.D. are employees of Pfizer and may hold stock options. A.M. and U.S. are employees of BioNTech and may hold stock options. Y.L., H.X., J.Z., X.X. and P.-Y.S. received compensation from Pfizer to perform the project.

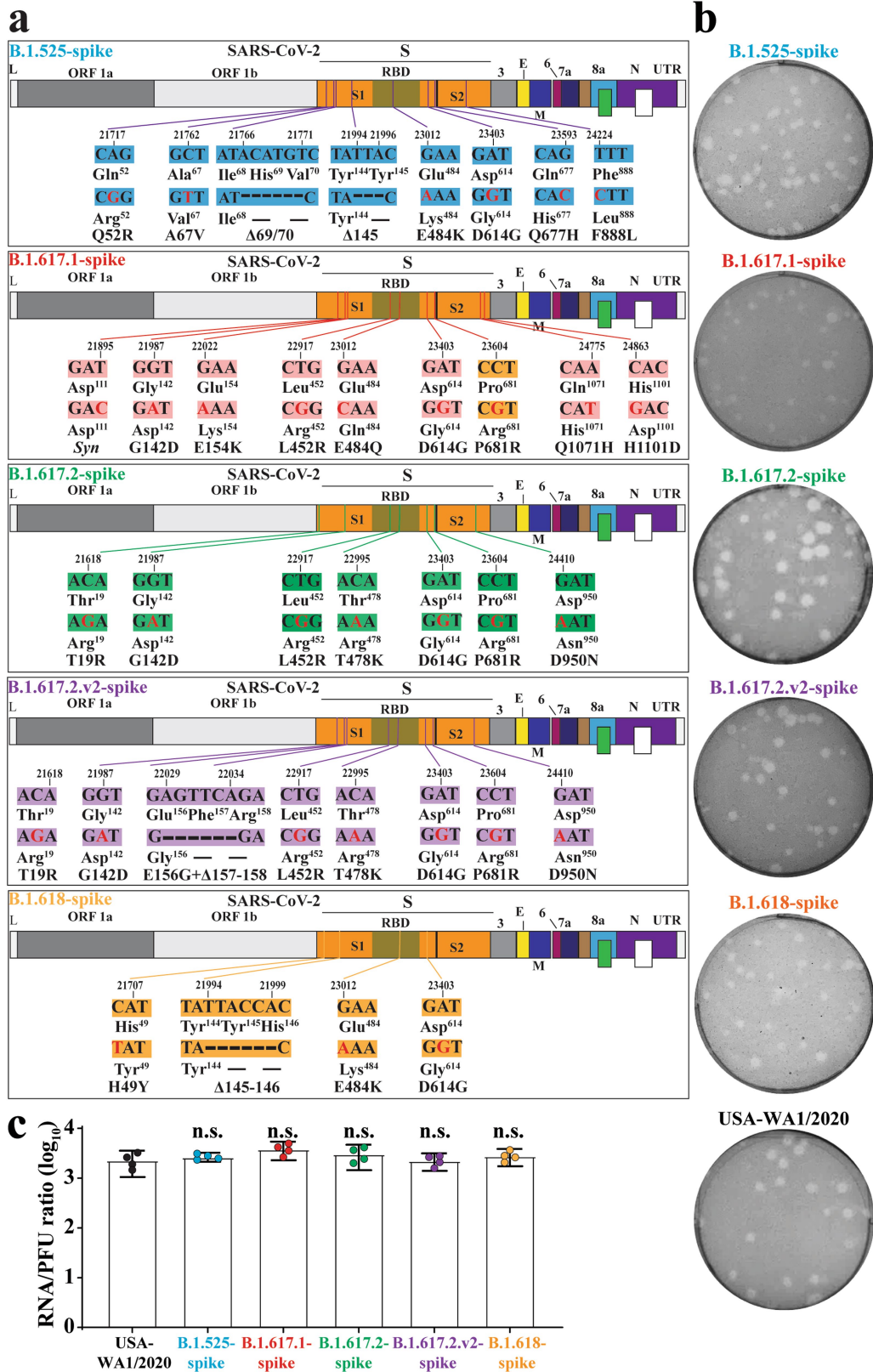
### Additional information

**Supplementary information** The online version contains supplementary material available at <https://doi.org/10.1038/s41586-021-03693-y>.

**Correspondence and requests for materials** should be addressed to U.S., X.X., P.R.D. or P.-Y.S.

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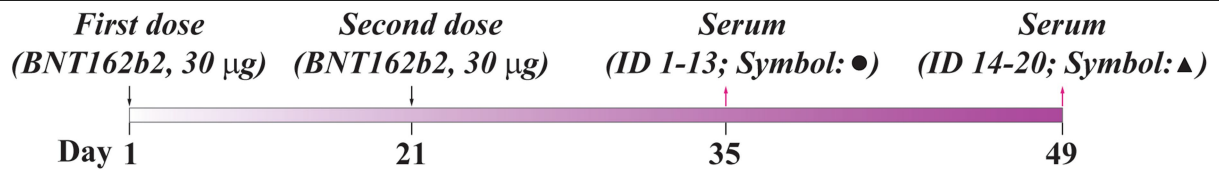
Extended Data Fig. 1 | See next page for caption.

# Article

## Extended Data Fig. 1 | Construction and characterization of SARS-CoV-2s with variant spikes. **a**, Diagram of engineered variant spike mutations.

Mutations from variant spikes were engineered into isolate USA-WA1/2020. Mutations and deletions are indicated in red and by dotted lines, respectively. Nucleotide and amino acid positions are also indicated. Different regions of SARS-CoV-2 genome 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), and UTR (untranslated region). **b**, Plaque morphologies of recombinant SARS-CoV-2s. Plaque assays were performed on Vero E6 cells in six-well plates. **c**, Comparison

of viral genomic RNA-to-PFU ratios ( $\log_{10}[\text{RNA}/\text{PFU}]$ ) of recombinant SARS-CoV-2s. The genomic RNA and PFU of individual virus stocks were measured by RT-qPCR and plaque assay, respectively. The RNA/PFU ratios were calculated to determine specific infectivities. Dots represent individual biological replicates from four aliquots of viruses ( $n = 4$ , one experiment). Bars and error bars show means with 95% confidence intervals. A non-parametric two-tailed Mann-Whitney test was used to determine the significance of differences between USA-WA1/2020 and variant viruses. *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.



**Extended Data Fig. 2 | BNT162b2 immunization scheme and serum collection.** Twenty human sera were obtained from 15 trial participants, 2 weeks (circles) or 4 weeks (triangles) after the second dose of BNT162b2

vaccine. Five of the 15 participants provided sera at both 2 and 4 weeks after the second dose of vaccine.

# Article

**Extended Data Table 1 | PRNT<sub>50</sub> values of sera from BNT162b2-immunized trial participants against USA-WA1/2020 and variant SARS-CoV-2 viruses**

| ID <sup>‡</sup> | Serum*               |     |      | PRNT <sub>50</sub> <sup>†</sup> |         |         |          |                 |         |         |            |            |          |         |
|-----------------|----------------------|-----|------|---------------------------------|---------|---------|----------|-----------------|---------|---------|------------|------------|----------|---------|
|                 | Age (years)          | Sex | Week | USA-WA1/2020                    |         |         | B.1.525- | B.1.617.1-spike |         |         | B.1.617.2- | B.1.617.2- | B.1.618- |         |
|                 |                      |     |      | Exp1                            | Exp2    | GMT     | spike    | Exp1            | Exp2    | GMT     | spike      | v2-spike   | spike    |         |
| 1               | 68                   | F   | 2    | 640                             | 640     | 640     | 640      | 320             | 320     | 320     | 320        | 320        | 320      | 320     |
| 2               | 67                   | M   | 2    | 160                             | 160     | 160     | 80       | 40              | 40      | 40      | 80         | 80         | 80       | 80      |
| 3               | 68                   | F   | 2    | 1280                            | 1280    | 1280    | 640      | 320             | 320     | 320     | 640        | 640        | 1280     | 1280    |
| 4               | 66                   | F   | 2    | 320                             | 320     | 320     | 320      | 80              | 160     | 113     | 320        | 160        | 160      | 160     |
| 5               | 30                   | M   | 2    | 320                             | 640     | 453     | 160      | 80              | 160     | 113     | 320        | 320        | 320      | 160     |
| 6               | 23                   | F   | 2    | 320                             | 320     | 320     | 320      | 80              | 160     | 113     | 160        | 160        | 320      | 320     |
| 7               | 54                   | M   | 2    | 640                             | 640     | 640     | 640      | 160             | 320     | 226     | 640        | 640        | 640      | 640     |
| 8               | 69                   | F   | 2    | 320                             | 320     | 320     | 160      | 80              | 160     | 113     | 320        | 320        | 320      | 320     |
| 9               | 65                   | M   | 2    | 640                             | 640     | 640     | 640      | 160             | 320     | 226     | 640        | 640        | 640      | 640     |
| 10              | 38                   | F   | 2    | 640                             | 640     | 640     | 640      | 320             | 320     | 320     | 640        | 640        | 640      | 640     |
| 11              | 44                   | F   | 2    | 320                             | 640     | 453     | 640      | 160             | 320     | 226     | 320        | 640        | 320      | 320     |
| 12              | 52                   | F   | 2    | 640                             | 640     | 640     | 320      | 160             | 320     | 226     | 320        | 640        | 320      | 320     |
| 13              | 28                   | M   | 2    | 1280                            | 1280    | 1280    | 320      | 160             | 320     | 226     | 640        | 640        | 320      | 320     |
| 14              | 69                   | F   | 4    | 320                             | 320     | 320     | 160      | 80              | 160     | 113     | 320        | 320        | 320      | 320     |
| 15              | 68                   | F   | 4    | 320                             | 320     | 320     | 160      | 80              | 160     | 113     | 320        | 320        | 320      | 320     |
| 16              | 26                   | F   | 4    | 320                             | 320     | 320     | 320      | 80              | 160     | 113     | 320        | 320        | 320      | 320     |
| 17              | 54                   | M   | 4    | 640                             | 640     | 640     | 320      | 160             | 160     | 160     | 640        | 320        | 320      | 320     |
| 18              | 35                   | F   | 4    | 640                             | 640     | 640     | 320      | 160             | 160     | 160     | 320        | 320        | 320      | 320     |
| 19              | 44                   | F   | 4    | 640                             | 640     | 640     | 320      | 80              | 160     | 113     | 320        | 320        | 320      | 320     |
| 20              | 52                   | F   | 4    | 640                             | 640     | 640     | 320      | 160             | 160     | 160     | 320        | 160        | 320      | 320     |
|                 | GMT <sup>§</sup>     |     |      | 485                             | 520     | 502     | 320      | 126             | 197     | 157     | 355        | 343        | 331      | 331     |
|                 | 95% CI <sup>  </sup> |     |      | 380-619                         | 410-659 | 397-636 | 242-423  | 96-163          | 155-245 | 124-199 | 278-452    | 260-452    | 253-433  | 253-433 |

\*Pairs of sera were obtained from five of the twenty participants at both 2 and 4 weeks after the second dose of vaccine. The paired sera have IDs 1 and 15, 7 and 17, 8 and 14, 11 and 19, and 12 and 20.

<sup>†</sup>The data for USA-WA1/2020 and B.1.617.1 are from two independent experiments. The results for other variants are from one experiment each. For each independent experiment, the individual PRNT<sub>50</sub> value is the geometric mean of duplicate plaque assay results; no differences were observed between the duplicate assays.

<sup>‡</sup>The serum donors were white, except for donor 10, who was Asian. All donors were of non-Latino/Latina ethnicity.

<sup>§</sup>Geometric mean neutralizing titres.

<sup>||</sup>95% confidence interval (95% CI) for the geometric mean neutralizing titres.



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- The exact sample size ( $n$ ) for each experimental group/condition, given as a discrete number and unit of measurement
- A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- The statistical test(s) used AND whether they are one- or two-sided  
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- For null hypothesis testing, the test statistic (e.g.  $F$ ,  $t$ ,  $r$ ) with confidence intervals, effect sizes, degrees of freedom and  $P$  value noted  
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## Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

|                 |  |
|-----------------|--|
| Sample size     | No sample size calculation was performed. Sample size was chosen based on previous experience and availability, 20 samples were collected from BNT162b2 vaccinees participating in the phase 1 portion of the ongoing phase 1/2/3 clinical trial (ClinicalTrials.gov identifier: NCT04368728). Those 20 samples had been tested as neutralizing positive against WT SARS-CoV-2 using the method according to the reference (Walsh EE, Frenck RW, Jr., Falsey AR, et al. Safety and Immunogenicity of Two RNA-Based Covid-19 Vaccine Candidates. N Engl J Med 2020.).                                 |
| Data exclusions | No data was excluded in the study.   |
| Replication     | The experiments were performed twice with 20 different samples. The averaged results from the duplication were reported in this study. All attempts at replication were successful.  |
| Randomization   | No randomization was performed. All samples were analyzed for the neutralizing activities against WT SARS-CoV-2 and variants in the same experimental settings.  |
| Blinding        | Patient information was blinded in the study. Those 20 samples had been tested as neutralizing positive against WT SARS-CoV-2 using the method according to the reference (Walsh EE, Frenck RW, Jr., Falsey AR, et al. Safety and Immunogenicity of Two RNA-Based Covid-19 Vaccine Candidates. N Engl J Med 2020.). The investigators were not blinded to the allocation during the experiments or to the outcome assessment. Blinding is not necessary because the results are quantitative and did not require subjective judgment or interpretation. Blinding is not typically used in the field. |

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### Materials & experimental systems

|                                     |   |
|-------------------------------------|---|
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| <input checked="" type="checkbox"/> | <input type="checkbox"/> Antibodies                       |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> Eukaryotic cell lines |
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### Methods

|                                     |   |
|-------------------------------------|---|
| n/a                                 | Involved in the study                           |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> ChIP-seq               |
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## Eukaryotic cell lines

Policy information about [cell lines](#)

|  |   |
|--|---|
| Cell line source(s)  | Vero E6 cells (ATCC® CRL-1586) were obtained from ATCC                                  |
| Authentication   | ATCC have comprehensively performed authentication on cell lines through STR profiling. |
| Mycoplasma contamination   | All cell lines were tested negative for mycoplasma.                                     |
| Commonly misidentified lines<br>(See <a href="#">ICLAC</a> register) | No commonly misidentified cell lines were used in the study.                            |