



## 28 **Main**

29 We previously reported that BNT162b2, a nucleoside modified RNA vaccine that  
30 encodes the SARS-CoV-2 full length, prefusion stabilized spike glycoprotein (S), elicited dose-  
31 dependent SARS-CoV-2-neutralizing geometric mean titers (GMTs) that were similar to or  
32 higher than the GMT of a panel of SARS-CoV-2 convalescent human serum samples.<sup>1</sup> We  
33 subsequently reported that, in a randomized, placebo-controlled trial in approximately 44,000  
34 participants 16 years of age or older, a two-dose regimen of BNT162b2 conferred 95%  
35 protection against COVID-19.<sup>2</sup>

36 Since the previously reported studies were conducted, rapidly spreading variants of  
37 SARS-CoV-2 have arisen in the United Kingdom (UK), South Africa (SA), and other regions.<sup>3,4</sup>  
38 These variants have multiple mutations in their spike glycoproteins, which are key targets of  
39 virus neutralizing antibodies. The emerged spike mutations have raised concerns of vaccine  
40 efficacy against these new strains. The goal of this study is to examine the effect of several key  
41 spike mutations from the UK and SA strains on BNT162b2 vaccine-elicited neutralization.

42 Using an infectious cDNA clone of SARS-CoV-2<sup>5</sup>, we engineered three spike mutant  
43 viruses on the genetic background of clinical strain USA-WA1/2020 (**Supplementary Fig. 1**). (i)  
44 Mutant N501Y virus contains the N501Y mutation that is shared by both the UK and SA variants.  
45 This mutation is located in the viral receptor binding domain (RBD) for cell entry, increases  
46 binding to the angiotensin converting enzyme 2 (ACE2) receptor, and enables the virus to  
47 expand its host range to infect mice.<sup>5,6</sup> (ii) Mutant  $\Delta 69/70$ +N501Y+D614G virus contains two  
48 additional changes present in the UK variants: amino acid 69 and 70 deletion ( $\Delta 69/70$ ) and  
49 D614G substitution. Amino acids 69 and 70 are located in the N-terminal domain of the spike S1  
50 fragment; deletion of these residues may allosterically change S1 conformation.<sup>6</sup> The D614G  
51 mutation is dominant in circulating strains around the world.<sup>7,8</sup> (iii) Mutant  
52 E484K+N501Y+D614G virus additionally contains the E484K substitution, which is also located

53 in the viral RBD. The E484K substitution alone confers resistance to several monoclonal  
54 antibodies.<sup>9,10</sup> Compared with the wild-type USA-WA1/2020 strain, the three mutant viruses  
55 showed similar plaque morphologies on Vero E6 cells (**Supplementary Fig. 2**).

56 We tested a panel of human sera from twenty participants in the previously reported  
57 clinical trial,<sup>1,2</sup> drawn 2 or 4 weeks after immunization with two 30- $\mu$ g doses of BNT162b2  
58 spaced three weeks apart (**Supplementary Fig. 3**). All neutralization assays were done with the  
59 same 20 sera samples, with the two experiments (as described in **Fig. 1** legend) done at  
60 different times. Each serum was tested for neutralization of wild-type USA-WA1/2020 strain and  
61 the three mutant viruses by a 50% plaque reduction neutralization assay (PRNT<sub>50</sub>;  
62 **Supplementary Tables 1 and 2**). All sera showed equivalent neutralization titers between the  
63 wild-type and mutant viruses, with differences of  $\leq 4$  fold (**Fig. 1**). Notably, ten out of the twenty  
64 sera had neutralization titers against mutant  $\Delta 69/70+N501Y+D614G$  virus that were twice their  
65 titers against the wild-type virus (**Fig. 1b**), whereas six out of the twenty sera had neutralization  
66 titers against mutant E484K+N501Y+D614G virus that were half their titers against the wild-type  
67 virus (**Fig. 1c**). The ratios of the neutralization GMTs of the sera against the N501Y,  
68  $\Delta 69/70+N501Y+D614G$ , and E484K+N501Y+D614G viruses to their GMTs against the USA-  
69 WA1/2020 virus were 1.46, 1.41, and 0.81, respectively (**Supplementary Fig. 4**).

70 Consistent with other recent reports of the neutralization of SARS-CoV-2 variants or  
71 corresponding pseudoviruses by convalescent or post-immunization sera,<sup>11,12</sup> the neutralization  
72 GMT of the serum panel against the virus with three mutations from the SA variant  
73 (E484K+N501Y+D614G) was slightly lower than the neutralization GMTs against the N501Y  
74 virus or the virus with three mutations from the UK variant ( $\Delta 69/70+N501Y+D614G$ ). However,  
75 the magnitude of the differences in neutralization GMTs against any of the mutant viruses in this  
76 study was small (0.81- to 1.41-fold), as compared to the 4-fold differences in hemagglutination-

77 inhibition titers that have been used to signal potential need for a strain change in influenza  
78 vaccines.<sup>13</sup>

79 A limitation of the current study is that the engineered viruses do not include the full set  
80 of spike mutations found in the UK or SA variants.<sup>3,4</sup> Nevertheless, preserved neutralization of  
81 N501Y,  $\Delta 69/70$ +N501Y+D614G, and E484K+N501Y+D614G viruses by BNT162b2 vaccine-  
82 elicited human sera is consistent with preserved neutralization of a panel of 15 pseudoviruses  
83 bearing spikes with other single mutations found in circulating SARS-CoV-2 strains.<sup>14</sup> The  
84 emergence of the common mutation N501Y from different geographical regions, as well as the  
85 previously emerged globally dominant D614G mutation, suggest that these mutations may  
86 improve viral fitness, as recently demonstrated for the increased viral transmission by the  
87 D614G mutation in animal models.<sup>7,15</sup> The biological functions of N501Y and the other mutations  
88 (such as  $\Delta 69/70$  and E484K) remain to be defined for viral replication, pathogenesis, and/or  
89 transmission in animal models. A second limitation of the study is that no serological correlate of  
90 protection against COVID-19 has been defined. Therefore, predictions about vaccine efficacy  
91 based on neutralization titers require assumptions about the levels of neutralization and roles of  
92 humoral and cell-mediated immunity in vaccine-mediated protection. Clinical data are needed  
93 for firm conclusions about vaccine effectiveness against variant viruses.

94 The ongoing evolution of SARS-CoV-2 necessitates continuous monitoring of the  
95 significance of changes for vaccine efficacy. This surveillance should be accompanied by  
96 preparations for the possibility that future mutations may necessitate changes to vaccine strains.  
97 The serological criteria for strain changes of influenza vaccine have been well-accepted.<sup>16</sup> For  
98 COVID-19, such vaccine updates would be facilitated by the flexibility of mRNA-based vaccine  
99 technology.

100

101 **Methods**

102           **Construction of isogenic viruses.** Three recombinant SARS-CoV-2 mutants (N501Y,  
103   Δ69/70-N501Y+D614G, E484K+N501Y+D614G in spike protein) were prepared on the genetic  
104   background of an infectious cDNA clone derived from clinical strain WA1 (2019-  
105   nCoV/USA\_WA1/2020)<sup>5</sup> by following the PCR-based mutagenesis protocol as reported  
106   previously<sup>7</sup>. The full-length infectious cDNAs were *in vitro* ligated and used as templates to  
107   transcribe full-length viral RNA. Mutant viruses (P0) were recovered on day 2 from Vero E6 cells  
108   after electroporation of the *in vitro* RNA transcripts. P1 viruses were harvested as stocks by  
109   passaging the P0 virus once on Vero E6 cells. The titers of P1 viruses were determined by  
110   plaque assay on Vero E6 cells. The genome sequences of the P1 viruses were validated by  
111   Sanger sequencing. The detailed protocol was recently reported<sup>17</sup>.

112           **Serum specimens and neutralization assay.** Serum samples were collected from  
113   BNT162b2 vaccinees participating in the phase 1 portion of the ongoing phase 1/2/3 clinical trial  
114   (ClinicalTrials.gov identifier: NCT04368728). The protocol and informed consent were approved  
115   by institutional review boards for each of the investigational centers participating in the study.  
116   The study was conducted in compliance with all International Council for Harmonisation (ICH)  
117   Good Clinical Practice (GCP) guidelines and the ethical principles of the Declaration of Helsinki.

118           The immunization and serum collection regimen are illustrated schematically in **Fig. S3**.  
119   A conventional (non-fluorescent) plaque reduction neutralization assay was performed to  
120   quantify the serum-mediated virus suppression as previously reported<sup>18</sup>. Briefly, each serum  
121   was 2-fold serially diluted in culture medium with the first dilution of 1:40 (dilution range of 1:40  
122   to 1:1280). The diluted sera were incubated with 100 plaque-forming units of wild-type or mutant  
123   viruses at 37°C for 1 h, after which the serum-virus mixtures were inoculated onto Vero E6 cell  
124   monolayer in 6-well plates. After 1 h of infection at 37°C, 2 ml of 2% Seaplaque agar (Lonza) in  
125   Dulbecco's modified Eagle medium (DMEM) containing 2% fetal bovine serum (FBS) and 1%  
126   penicillin/streptomycin (P/S) was added to the cells. After 2 days of incubation, 2 ml of 2%

127 Seaplaque agar (Lonza) in DMEM containing 2% FBS, 1% P/S and 0.01% neutral red (Sigma)  
128 were added on top of the first layer. After another 16 h of incubation at 37°C, plaque numbers  
129 were counted. The minimal serum dilution that inhibits 50% of plaque counts is defined as the  
130 50% plaque reduction neutralization titer (PRNT<sub>50</sub>). Each serum was tested in duplicates. The  
131 PRNT<sub>50</sub> assay was performed at the biosafety level-3 facility with the approval from the  
132 Institutional Biosafety Committee at the University of Texas Medical Branch.

133 **Statistics.** No statistics were performed in the study.

134

### 135 **Data availability**

136 The data that support the findings of this study are available from the corresponding  
137 authors upon request.

138

### 139 **References**

- 140 1. Walsh EE, Frenck RW, Jr., Falsey AR, et al. Safety and Immunogenicity of Two RNA-  
141 Based Covid-19 Vaccine Candidates. *N Engl J Med* 2020.
- 142 2. Polack FP, Thomas SJ, Kitchin N, et al. Safety and Efficacy of the BNT162b2 mRNA  
143 Covid-19 Vaccine. *N Engl J Med* 2020.
- 144 3. E. V, S. M, al. CMe. Report 42 - Transmission of SARS-CoV-2 Lineage B.1.1.7 in  
145 England: Insights from linking epidemiological and genetic data. [https://www.imperial.ac.uk/mrc-  
146 global-infectious-disease-analysis/covid-19/report-42-sars-cov-2-variant/](https://www.imperial.ac.uk/mrc-global-infectious-disease-analysis/covid-19/report-42-sars-cov-2-variant/) 2021.
- 147 4. Tegally H, Wilkinson E, Giovanetti M, al. e. Emergence and rapid spread of a new  
148 severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2) lineage with multiple  
149 spike mutations in South Africa. *medRxiv* 2020. :<https://doi.org/10.1101/2020.12.21.20248640>.
- 150 5. Xie X, Muruato A, Lokugamage KG, et al. An Infectious cDNA Clone of SARS-CoV-2.  
151 *Cell Host Microbe* 2020;27:841-8 e3.
- 152 6. Wrapp D, Wang N, Corbett KS, et al. Cryo-EM structure of the 2019-nCoV spike in the  
153 prefusion conformation. *Science* 2020;367:1260-3.
- 154 7. Plante JA, Liu Y, Liu J, et al. Spike mutation D614G alters SARS-CoV-2 fitness. *Nature*  
155 2020.
- 156 8. Korber B, Fischer WM, Gnanakaran S, et al. Tracking Changes in SARS-CoV-2 Spike:  
157 Evidence that D614G Increases Infectivity of the COVID-19 Virus. *Cell* 2020.
- 158 9. Ku Z, Xie X, Davidson E, et al. Molecular determinants and mechanism for antibody  
159 cocktail preventing SARS-CoV-2 escape. *Nat Commun* 2021;12:469.

- 160 10. Baum A, Fulton BO, Wloga E, et al. Antibody cocktail to SARS-CoV-2 spike protein  
161 prevents rapid mutational escape seen with individual antibodies. *Science* 2020;369:1014-8.
- 162 11. Wibmer CK, Ayres F, Hermanus T, et al. SARS-CoV-2 501Y.V2 escapes neutralization  
163 by South African COVID-19 donor plasma. *bioRxiv* 2021:doi:  
164 <https://doi.org/10.1101/2021.01.18.427166>.
- 165 12. Wang Z, Schmidt F, Weisblum Y, et al. mRNA vaccine-elicited antibodies to SARS-CoV-  
166 2 and circulating variants. *bioRxiv* 2021:doi: <https://doi.org/10.1101/2021.01.15.426911>.
- 167 13. Smith DJ, Lapedes AS, de Jong JC, et al. Mapping the antigenic and genetic evolution  
168 of influenza virus. *Science* 2004;305:371-6.
- 169 14. Sahin U, Alexander Muik, Vogler I, et al. BNT162b2 induces SARS-CoV-2-neutralising  
170 antibodies and T cells in humans. *medRxiv* 2020.
- 171 15. Hou YJ, Chiba S, Halfmann P, et al. SARS-CoV-2 D614G variant exhibits efficient  
172 replication ex vivo and transmission in vivo. *Science* 2020.
- 173 16. Trombetta CM, Perini D, Mather S, Temperton N, Montomoli E. Overview of Serological  
174 Techniques for Influenza Vaccine Evaluation: Past, Present and Future. *Vaccines (Basel)*  
175 2014;2:707-34.
- 176 17. Xie X, Lokugamage KG, Zhang X, et al. Engineering SARS-CoV-2 using a reverse  
177 genetic system. *Nature Protocols* 2021:<https://doi.org/10.1038/s41596-021-00491-8>.
- 178 18. Muruato AE, Fontes-Garfias CR, Ren P, et al. A high-throughput neutralizing antibody  
179 assay for COVID-19 diagnosis and vaccine evaluation. *Nat Commun* 2020;11:4059.

180

## 181 **Acknowledgments**

182 Supported by Pfizer and BioNTech. We thank colleagues at Pfizer, BioNTech, and  
183 UTMB for helpful discussions and support during the study. We thank the Pfizer-BioNTech  
184 clinical trial C4591001 participants, from whom the post-immunization human sera were  
185 obtained. We thank the many colleagues at Pfizer and BioNTech who developed and produced  
186 the BNT162b2 vaccine candidate. P.-Y.S. was supported by NIH grants AI142759, AI134907,  
187 AI145617, and UL1TR001439, and awards from the Sealy & Smith Foundation, Kleberg  
188 Foundation, the John S. Dunn Foundation, the Amon G. Carter Foundation, the Gilson  
189 Longenbaugh Foundation, and the Summerfield Robert Foundation.

190

## 191 **Author contributions**

192 Conceptualization, X.X., V.D.M., S.W., P.-Y.S.; Methodology, X.X., Y.L., J.L., J.Z.,  
193 C.R.F.G., H.X., P.-Y.S; Investigation, X.X., Y.L., J.L., J.Z., C.R.F.G., H.X., K.A.S., D.C., P.R.D.,

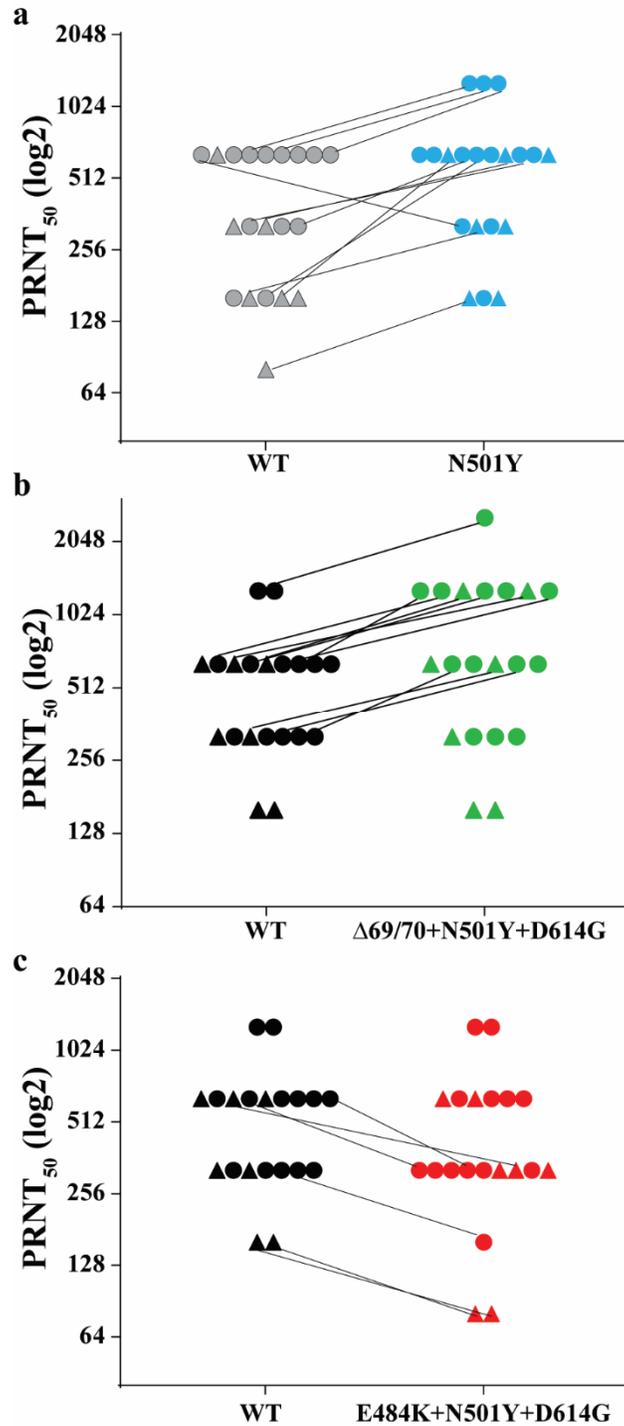
194 P.-Y.S; Resources, M.C., D.C., P.R.D., P.-Y.S; Data Curation, X.X., Y.L., J.L., J.Z., C.R.F.G.,  
195 P.-Y.S; Writing-Original Draft, X.X., P.-Y.S; Writing-Review & Editing, X.X., P.R.D., P.-Y.S.;  
196 Supervision, X.X., M.C., D.C., P.R.D., P.-Y.S.; Funding Acquisition P.-Y.S.

197

198 **Ethics declarations**

199 Competing interests

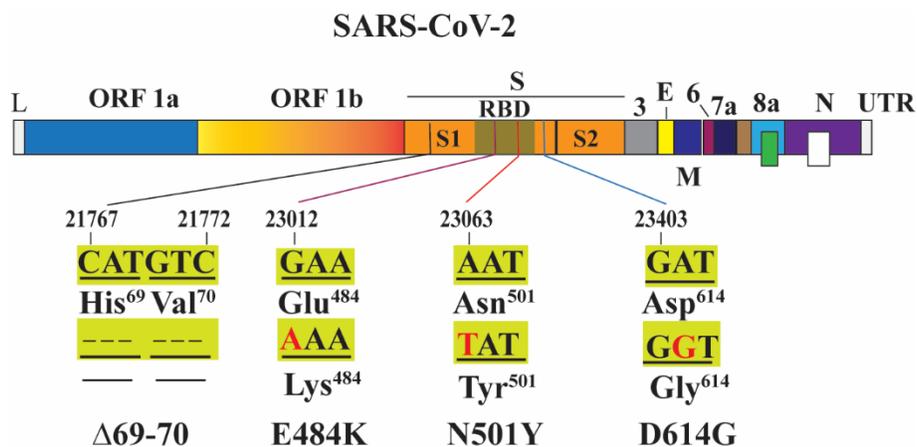
200 X.X., V.D.M., and P.-Y.S. have filed a patent on the reverse genetic system. K.A.S.,  
201 M.C., D.C., and P.R.D. are employees of Pfizer and may hold stock options. X.X., J.Z., C.R.F.G.,  
202 H.X., and P.-Y.S. received compensation from Pfizer to perform the neutralization assay. Other  
203 authors declare no competing interests.



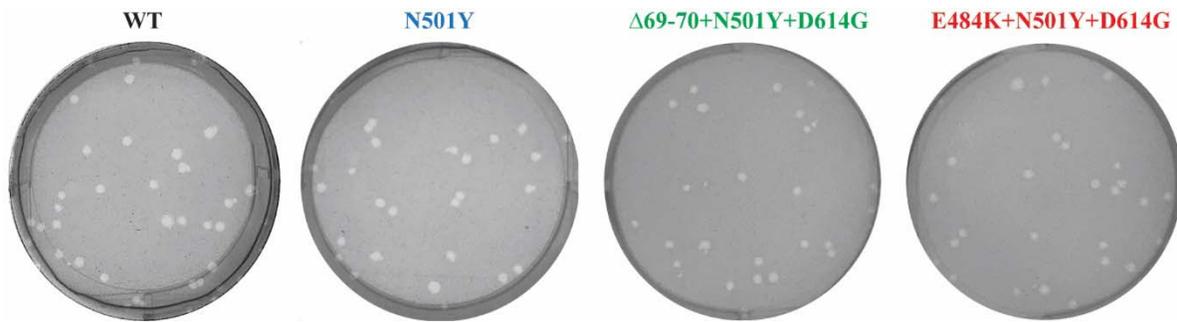
204

205 **Figure 1. PRNT<sub>50</sub>s of twenty BNT162b2-vaccinated human sera against wild-type (WT)**  
206 **and mutant SARS-CoV-2. (a) WT (USA-WA1/2020) and mutant N501Y. (b) WT and**  
207 **Δ69/70+N501Y+D614G. (c) WT and E484K+N501Y+D614G. Seven (triangles) and thirteen**  
208 **(circles) sera were drawn 2 and 4 weeks after the second dose of vaccination, respectively.**  
209 **Sera with different PRNT<sub>50</sub>s against WT and mutant viruses are connected by lines. Results in**  
210 **(a) were from one experiment; results in (b) and (c) were from another set of experiments. Each**  
211 **data point is the average of duplicate assay results.**

212 **Supplementary information**



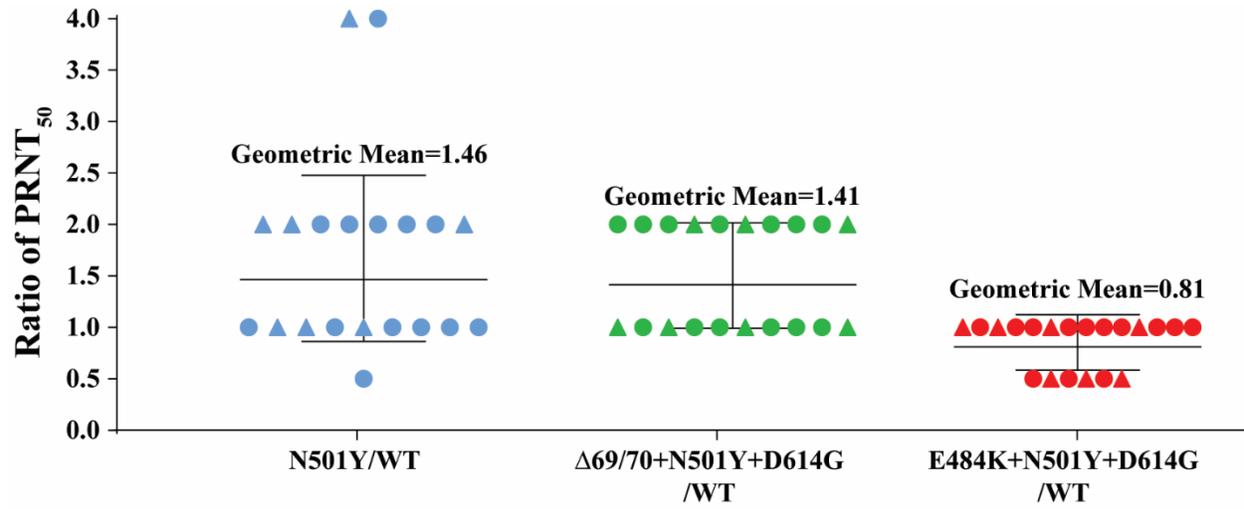
213  
 214 **Supplementary Figure 1. Engineered mutations.** Nucleotide and amino acid positions are  
 215 indicated. Deletions are depicted by dotted lines. Mutant nucleotides are in red. L, leader  
 216 sequence; ORF, open reading frame; RBD, receptor binding domain; S, spike glycoprotein; S1,  
 217 N-terminal furin cleavage fragment of S; S2, C-terminal furin cleavage fragment of S; E,  
 218 envelope protein; M, membrane protein; N, nucleoprotein; UTR, untranslated region.



219

220 **Supplementary Figure 2. Plaque morphologies of WT (USA-WA1/2020), mutant N501Y,**  
221 **Δ69/70+N501Y+D614G, and E484K+N501Y+D614G SARS-CoV-2s on Vero E6 cells.**





224

225

226

227

**Supplementary Figure 4. Ratios of neutralization GMTs against mutant viruses to GMTs against WT virus.** Triangles represent sera drawn two weeks after the second dose of vaccination; circles represent sera drawn four weeks after the second dose of vaccination.

228 **Supplementary Table 1. PRNT<sub>50</sub>s of twenty BNT162b2 post-immunization sera against**  
229 **wild-type (USA-WA1/2020) and mutant N501Y SARS-CoV-2s**

| Serum ID | PRNT <sub>50</sub> |       | PRNT <sub>50</sub> ratio<br>(N501Y/WT) |
|----------|--------------------|-------|--|
|          | WT                 | N501Y |  |
| 1        | 160                | 640   | 4                                      |
| 2        | 160                | 320   | 2                                      |
| 3        | 320                | 640   | 2                                      |
| 4        | 80                 | 160   | 2                                      |
| 5        | 160                | 160   | 1                                      |
| 6        | 320                | 320   | 1                                      |
| 7        | 640                | 640   | 1                                      |
| 8        | 160                | 160   | 1                                      |
| 9        | 640                | 640   | 1                                      |
| 10       | 640                | 1280  | 2                                      |
| 11       | 160                | 640   | 4                                      |
| 12       | 320                | 320   | 1                                      |
| 13       | 640                | 1280  | 2                                      |
| 14       | 640                | 320   | 0.5                                    |
| 15       | 320                | 640   | 2                                      |
| 16       | 320                | 640   | 2                                      |
| 17       | 640                | 640   | 1                                      |
| 18       | 640                | 1280  | 2                                      |
| 19       | 640                | 640   | 1                                      |
| 20       | 640                | 640   | 1                                      |

230

231

232 **Supplementary Table 2. PRNT<sub>50</sub>s of twenty BNT162b2 post-immunization sera against**  
 233 **wild-type (USA-WA1/2020), Δ69/70+N501Y+D614G, and E484K+N501Y+D614G SARS-CoV-**  
 234 **2s**

| Serum ID | PRNT <sub>50</sub> |                    |                   | PRNT <sub>50</sub> ratio |                      |
|----------|--------------------|--------------------|-------------------|--------------------------|----------------------|
|          | WT                 | Δ69/70+N501Y+D614G | E484K+N501Y+D614G | Δ69/70+N501Y+D614G/WT    | E484K+N501Y+D614G/WT |
| 1        | 320                | 640                | 320               | 2                        | 1                    |
| 2        | 160                | 160                | 80                | 1                        | 0.5                  |
| 3        | 640                | 1280               | 640               | 2                        | 1                    |
| 4        | 160                | 160                | 80                | 1                        | 0.5                  |
| 5        | 320                | 320                | 320               | 1                        | 1                    |
| 6        | 640                | 640                | 640               | 1                        | 1                    |
| 7        | 640                | 1280               | 320               | 2                        | 0.5                  |
| 8        | 320                | 320                | 160               | 1                        | 0.5                  |
| 9        | 1280               | 1280               | 1280              | 1                        | 1                    |
| 10       | 640                | 1280               | 640               | 2                        | 1                    |
| 11       | 320                | 320                | 320               | 1                        | 1                    |
| 12       | 640                | 1280               | 320               | 2                        | 0.5                  |
| 13       | 1280               | 2560               | 1280              | 2                        | 1                    |
| 14       | 320                | 320                | 320               | 1                        | 1                    |
| 15       | 320                | 640                | 320               | 2                        | 1                    |
| 16       | 640                | 640                | 640               | 1                        | 1                    |
| 17       | 640                | 1280               | 640               | 2                        | 1                    |
| 18       | 320                | 640                | 320               | 2                        | 1                    |
| 19       | 640                | 640                | 320               | 1                        | 0.5                  |
| 20       | 640                | 1280               | 640               | 2                        | 1                    |

235