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Antibody evasion properties of SARS-CoV-2 Omicron sublineages

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24
25

26 **The identification of the Omicron variant (B.1.1.529.1 or BA.1) of SARS-CoV-2 (severe**
27 **acute respiratory syndrome coronavirus 2) in Botswana in November 2021[1] immediately**
28 **raised alarms due to the sheer number of mutations in the spike glycoprotein that could**

lead to striking antibody evasion. We[2] and others[3-6] recently reported results in this Journal confirming such a concern. Continuing surveillance of Omicron evolution has since revealed the rise in prevalence of two sublineages, BA.1 with an R346K mutation (BA.1+R346K, also known as BA.1.1) and B.1.1.529.2 (BA.2), with the latter containing 8 unique spike mutations while lacking 13 spike mutations found in BA.1. We therefore extended our studies to include antigenic characterization of these new sublineages. Polyclonal sera from patients infected by wild-type SARS-CoV-2 or recipients of current mRNA vaccines showed a substantial loss in neutralizing activity against both BA.1+R346K and BA.2, with drops comparable to that already reported for BA.1[2,3,5,6]. These findings indicate that these three sublineages of Omicron are antigenically equidistant from the wild-type SARS-CoV-2 and thus similarly threaten the efficacies of current vaccines. BA.2 also exhibited marked resistance to 17 of 19 neutralizing monoclonal antibodies tested, including S309 (sotrovimab)[7], which had retained appreciable activity against BA.1 and BA.1+R346K[2-4,6]. This new finding shows that no authorized monoclonal antibody therapy could adequately cover all sublineages of the Omicron variant, except for the recently authorized LY-CoV1404 (bebtelovimab).

The meteoric rise of the B.1.1.529/Omicron to become the dominant SARS-CoV-2 variant globally has been truly remarkable[8]. Continuing surveillance of its evolution in the population in December 2021 and January 2022 has revealed that the proportion of the original form, BA.1, has been decreasing steadily while the proportions of two other sublineages have increased noticeably (**Fig. 1a**). In fact, the BA.1+R346K sublineage now accounts for ~40% of Omicron sequences globally, and ~35-60% in New Zealand, United Kingdom, and United States. On the other hand, the BA.2 sublineage accounts for only ~10% of Omicron sequences globally, but it is not only on the rise but also the dominant form in countries such as Denmark, India, and South Africa. These three sublineages of Omicron share 21 mutations in the spike protein, wherein BA.2 contains 8 unique mutations and BA.1 contains 13 unique mutations (**Fig. 1b**). Of course, BA.1+R346K has one mutation more than BA.1. Given these differences, their antigenic properties cannot be assumed to be the same or similar.

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60 **Serum neutralization of sublineages**

61

62 Therefore, we first investigated the neutralization sensitivity of the Omicron sublineages by
63 polyclonal sera from convalescent patients or individuals given mRNA vaccines, with or without
64 a booster shot. These serum samples, as well as the pseudovirus neutralization assay used, were
65 identical to ones previously reported[2]. The wild-type D614G pseudovirus was included as a
66 comparator. As was observed and reported for BA.1[2,3,5,6], a marked and significant loss of serum
67 neutralizing activity against BA.1+R346K and BA.2 relative to D614G was noted, with
68 neutralizing titers for numerous samples dropping below the limit of detection (**Fig. 1c**). The loss
69 of neutralizing activity against BA.1+R346K or BA.2 sublineages was less prominent for sera
70 obtained from individuals who received a booster vaccination (**Fig. 1c**, right panel), consistent
71 with reported findings for BA.1[2,3,6]. Among these samples, the mean serum neutralizing titers
72 against Omicron sublineages were significantly lower than the mean titer for D614G; although
73 the mean titer was slightly lower for BA.2, the difference from BA.1 sublineages did not reach
74 statistical significance ($P = 0.242$). Finally, we confirmed the pseudovirus neutralization data by
75 testing a separate set of sera from individuals given mRNA vaccines for neutralization of
76 authentic viruses (**Extended Data Fig. 1** and **Extended Data Table 1**). Similar to above,
77 neutralizing titers dropped significantly against authentic BA.2 virus relative to D614G.

78

79 **Antibody neutralization of sublineages**

80

81 To further examine antigenic differences in the spike protein of these Omicron sublineages, a
82 panel of 19 neutralizing monoclonal antibodies was used as probes. Seventeen were directed to
83 different epitope clusters (classes 1-4) within the receptor-binding domain (RBD), whereas two
84 were directed to the N-terminal domain (NTD). These antibodies included REGN10987
85 (imdevimab)[9], REGN10933 (casirivimab)[9], COV2-2196 (tixagevimab)[10], COV2-2130
86 (cilgavimab)[10], LY-CoV555 (bamlanivimab)[11], CB6 (etesevimab)[12], Brii-196 (amubarvimab)[13],
87 Brii-198 (romlusevimab)[13], S309 (sotrovimab)[7], LY-CoV1404 (bebtelovimab)[14], ADG-2[15],
88 DH1047[16], and S2X259[17], as well as 1-20, 2-15, 2-7, 4-18, 5-7[18] and 10-40[19] from our group.
89 Overall, 17 of 19 monoclonal antibodies were either totally inactive or severely impaired in
90 neutralizing BA.2 (**Fig. 2a**), somewhat like previous findings for BA.1 and BA.1+R346K[2] but

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91 with important differences (**Fig. 2b**). All class 4 antibodies tested lost greater neutralizing
92 potency against BA.2 versus BA.1 sublineages. Two class 3 antibodies, COV2-2130 and 2-7,
93 retained decent activity against BA.2 while having no activity against BA.1 viruses. S309 or
94 sotrovimab lost 27-fold neutralizing activity against BA.2; this is particularly important because
95 it is an authorized monoclonal antibody that was found to retain activity against the original form
96 of Omicron[2-4]. LY-CoV1404, the most recently authorized monoclonal antibody, remained
97 potent in neutralizing all Omicron sublineages, suggesting that there is still a patch within this
98 antibody-binding region that is unaffected by all spike mutations found in SARS-CoV-2 variants
99 to date. Although there was a lack of an observable difference among the Omicron sublineages in
100 neutralization by polyclonal sera (**Fig. 1c**), important antigenic differences do exist when probed
101 by monoclonal antibodies. Except for S309, BA.1 appears to be more resistant to class 3
102 antibodies than BA.2, while BA.2 is more resistant to all class 4 antibodies tested. Our recent
103 study[2] showed that previous SARS-CoV-2 variants, such as B.1.351/Beta and B.1.617.2/Delta,
104 evolved to resist class 1, class 2, and NTD antibodies first, and then the Omicron variant
105 seemingly has further evolved to resist class 3 and class 4 antibodies in addition. Our current
106 findings suggest that the Omicron sublineages may have diverged under slightly different
107 pressure from class 3 and class 4 antibodies to the RBD.
108
109 **Mutations conferring antibody resistance**
110
111 Finally, we constructed each of the eight BA.2-specific spike mutations alone as pseudoviruses
112 and tested them using the same panel of 19 monoclonal antibodies (**Fig. 2b**). S371F broadly
113 affected most of the RBD-directed antibodies, similar to what was observed for S371L in BA.1[2]
114 but with a greater negative impact, perhaps due to the bulkier side chain of phenylalanine.
115 Intriguingly but importantly, S371F appears to be majorly responsible for the loss in potency of
116 S309, although this mutation was not observed previously as a marker for clinical resistance to
117 sotrovimab[20]. CB6 was adversely affected by the D405N mutation, likely due to its position
118 within the epitope of this antibody[12]. It is not clear how T19I and L24S mutations in the NTD
119 subtly impaired the neutralizing activity of class 1 antibodies to RBD.
120
121 **Discussion**

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122

123 In summary, we have comprehensively evaluated the antigenic properties of two sublineages of
124 the Omicron variant, BA.1+R346K and BA.2, and we believe our results have important clinical
125 implications. First, polyclonal sera showed a substantial loss in neutralizing activity against both
126 sublineages, with drops comparable to that of BA.1 (**Fig. 1c**). These three sublineages of
127 Omicron, therefore, seem to be antigenically equidistant from the wild-type SARS-CoV-2, likely
128 threatening the efficacies of current COVID-19 (coronavirus disease 2019) vaccines to a similar
129 extent. The present study, however, does not address the antigenic distance between BA.1 and
130 BA.2, which will require cross-neutralization experiments using sublineage-specific sera to
131 determine. Second, monoclonal antibodies were affected in a disparate manner for the different
132 Omicron sublineages. For clinically approved or authorized antibodies, S309 (sotrovimab)
133 retained activity against both BA.1 and BA.1+R346K, but its activity against BA.2 has dropped
134 27-fold (**Fig. 2b**) to a 50% inhibitory concentration (IC_{50}) of ~1 μg/mL (**Fig. 2a**). COV2-2130
135 (cilgavimab) and its combination with COV2-2196 (tixagevimab) retained activity against BA.2,
136 but this antibody combination is only authorized for preventive use. Only the recently authorized
137 LY-CoV1404 (bebtelovimab) could adequately treat all sublineages of the Omicron variant. As
138 COVID-19 treatment options are narrowed by the emergence of more and more variants, it is
139 imperative that we continue to devise novel strategies to contain this ever-evolving pathogen.

140 **Online content** Any methods, additional references, Nature Research reporting summaries, source data,
141 extended data, supplementary information, acknowledgements, peer review information; details of
142 author contributions and competing interests; and statements of data and code availability are available
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149 1 Viana, R. *et al.* Rapid epidemic expansion of the SARS-CoV-2 Omicron variant in
150 southern Africa. *Nature*, doi:10.1038/s41586-022-04411-y (2022).

151 2 Liu, L. *et al.* Striking antibody evasion manifested by the Omicron variant of SARS-
152 CoV-2. *Nature* **602**, 676-681, doi:10.1038/s41586-021-04388-0 (2022).

ACCELERATED ARTICLE PREVIEW

153 3 Cameroni, E. *et al.* Broadly neutralizing antibodies overcome SARS-CoV-2 Omicron
154 antigenic shift. *Nature* **602**, 664-670, doi:10.1038/s41586-021-04386-2 (2022).
155 4 Cao, Y. *et al.* Omicron escapes the majority of existing SARS-CoV-2 neutralizing
156 antibodies. *Nature* **602**, 657-663, doi:10.1038/s41586-021-04385-3 (2022).
157 5 Cele, S. *et al.* Omicron extensively but incompletely escapes Pfizer BNT162b2
158 neutralization. *Nature* **602**, 654-656, doi:10.1038/s41586-021-04387-1 (2022).
159 6 Planas, D. *et al.* Considerable escape of SARS-CoV-2 Omicron to antibody neutralization.
160 *Nature* **602**, 671-675, doi:10.1038/s41586-021-04389-z (2022).
161 7 Pinto, D. *et al.* Cross-neutralization of SARS-CoV-2 by a human monoclonal SARS-CoV
162 antibody. *Nature* **583**, 290-295, doi:10.1038/s41586-020-2349-y (2020).
163 8 Shu, Y. & McCauley, J. GISAID: Global initiative on sharing all influenza data - from
164 vision to reality. *Euro Surveill* **22**, doi:10.2807/1560-7917.ES.2017.22.13.30494 (2017).
165 9 Hansen, J. *et al.* Studies in humanized mice and convalescent humans yield a SARS-
166 CoV-2 antibody cocktail. *Science* **369**, 1010-1014, doi:10.1126/science.abd0827 (2020).
167 10 Zost, S. J. *et al.* Potently neutralizing and protective human antibodies against SARS-
168 CoV-2. *Nature* **584**, 443-449, doi:10.1038/s41586-020-2548-6 (2020).
169 11 Jones, B. E. *et al.* The neutralizing antibody, LY-CoV555, protects against SARS-CoV-2
170 infection in nonhuman primates. *Sci Transl Med* **13**, doi:10.1126/scitranslmed.abf1906
171 (2021).
172 12 Shi, R. *et al.* A human neutralizing antibody targets the receptor-binding site of SARS-
173 CoV-2. *Nature* **584**, 120-124, doi:10.1038/s41586-020-2381-y (2020).
174 13 Ju, B. *et al.* Human neutralizing antibodies elicited by SARS-CoV-2 infection. *Nature*
175 **584**, 115-119, doi:10.1038/s41586-020-2380-z (2020).
ACCELERATED ARTICLE PREVIEW

176 14 Westendorf, K. *et al.* LY-CoV1404 (bebtelovimab) potently neutralizes SARS-CoV-2
177 variants. *bioRxiv*, doi:10.1101/2021.04.30.442182 (2022).
178 15 Rappazzo, C. G. *et al.* Broad and potent activity against SARS-like viruses by an
179 engineered human monoclonal antibody. *Science* **371**, 823-829,
180 doi:10.1126/science.abf4830 (2021).
181 16 Li, D. *et al.* In vitro and in vivo functions of SARS-CoV-2 infection-enhancing and
182 neutralizing antibodies. *Cell* **184**, 4203-4219 e4232, doi:10.1016/j.cell.2021.06.021
183 (2021).
184 17 Tortorici, M. A. *et al.* Broad sarbecovirus neutralization by a human monoclonal
185 antibody. *Nature* **597**, 103-108, doi:10.1038/s41586-021-03817-4 (2021).
186 18 Liu, L. *et al.* Potent neutralizing antibodies against multiple epitopes on SARS-CoV-2
187 spike. *Nature* **584**, 450-456, doi:10.1038/s41586-020-2571-7 (2020).
188 19 Liu, L. *et al.* Isolation and comparative analysis of antibodies that broadly neutralize
189 sarbecoviruses. *bioRxiv*, doi:https://doi.org/10.1101/2021.12.11.472236 (2021).
190 20 Rockett, R. J. *et al.* RESISTANCE CONFERRING MUTATIONS IN SARS-CoV-2
191 DELTA FOLLOWING SOTROVIMAB INFUSION. *medRxiv*,
192 doi:https://doi.org/10.1101/2021.12.18.21267628 (2021).
193

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199 **Methods**

200

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201 **Data reporting**

202 No statistical methods were used to predetermine sample size. The experiments were not
203 randomized and the investigators were not blinded to allocation during experiments and outcome
204 assessment.

205

206 **Serum samples**

207 For the pseudovirus neutralization experiments, identical samples from a previous study were
208 utilized[2]. For the authentic virus neutralization experiments, the samples are described in
209 **Extended Data Table 1**. All collections were conducted under protocols reviewed and approved
210 by the Institutional Review Board of Columbia University. All of the participants provided
211 written informed consent.

212

213 **Antibodies**

214 Antibodies were expressed as previously described[18]. Briefly, VH and VL genes for each
215 antibody were codon optimized and synthesized (GenScript), then inserted into mammalian
216 expression vectors. These plasmids were transiently transfected into Expi293 cells (Thermo
217 Fisher) using polyethylenimine and cultured for 5 days, and then the antibody was purified by
218 affinity chromatography using rProtein A Sepharose® (GE). REGN10933, REGN10987, COV2-
219 2130, and COV2-2196 were provided by Regeneron Pharmaceuticals, Brii-196 and Brii-198
220 were provided by Brii Biosciences, and CB6 was provided by B. Zhang and P. Kwong (NIAID).

221

222 **Cells**

223 Expi293 cells were obtained from Thermo Fisher (Catalog #A14527), Vero E6 cells were
224 obtained from ATCC (Catalog #CRL-1586), HEK293T cells were obtained from ATCC
225 (Catalog #CRL-3216), and Vero-E6-TMPRSS2 cells were obtained from JCRB (Catalog
226 #JCRB1819). All cells were purchased from authenticated vendors and morphology was visually
227 confirmed prior to use. All cell lines tested mycoplasma negative.

228

229 **Pseudovirus production**

230 Spike expression constructs for variant SARS-CoV-2 spikes were produced by an in-house gene
231 synthesis method as previously described[2]. Constructs were confirmed by sequencing, and then

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232 transfected into HEK293T cells using Lipofectamine 3000 (Thermo Fisher) according to the
233 manufacturer's instructions. Cells were washed 24 h post-transfection with complete media
234 (DMEM + 10% FBS + penicillin/streptomycin) and then infected with rVSV-G pseudotyped
235 ΔG-luciferase (G*ΔG-luciferase, Kerafast). Cells were thoroughly washed 2 h post-infection
236 with complete media, then incubated for an additional 24 h at 37 °C under 5% CO_2.
237 Pseudoviruses were then harvested and incubated with anti-VSV-G hybridoma supernatant for 1
238 h at 37 °C (I1-Hybridoma, ATCC) to neutralize residual rVSV-G. The titer of each pseudovirus
239 was determined by serially diluting the virus in complete media in 96 well-plates, and then
240 incubating with 40,000 Vero E6 cells for approximately 12 h at 37 °C under 5% CO_2. Following
241 infection, luminescence was quantified using the Luciferase Assay System (Promega) according
242 to the manufacturer's instructions and measured with a SpectraMax i3x Multi-Mode Microplate
243 Reader (Molecular Devices) using SoftMax Pro 7.0.2 (Molecular Devices), and then the titer was
244 determined by comparison to control wells with cells alone. Pseudoviruses were aliquoted and
245 stored at -80 °C until use.
246
247 **Pseudovirus neutralization assay**
248 Neutralization assays were conducted in 96 well-plates by serially diluting sera or antibodies in
249 complete media, starting at 1:100 dilution or 10 μg/mL respectively, and incubating with
250 pseudoviruses for 1 h at 37 °C. Following incubation, 40,000 Vero E6 cells were added to each
251 well, and further incubated for approximately 12 h at 37 °C under 5% CO_2. Luminescence was
252 quantified using the Luciferase Assay System according to the manufacturer's instructions and
253 measured with a SpectraMax i3x Multi-Mode Microplate Reader using SoftMax Pro 7.0.2.
254 Neutralization was determined by comparison to control wells with cells alone and with virus
255 alone. IC_{50} values were calculated by fitting a non-linear five-parameter dose-response curve in
256 GraphPad Prism version 9.2.
257
258 **Authentic virus isolation and propagation**
259 SARS-CoV-2 variants D614G (GISAID: EPI_ISL_497840) and BA.2 (GISAID:
260 EPI_ISL_9845731) were isolated from respiratory tract specimens of patients with COVID-19 in
261 Hong Kong by J.F.-W.C., K.-Y. Yuen, and colleagues at the Department of Microbiology, The

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262 University of Hong Kong. The viruses were propagated in Vero-E6-TMPRSS2 cells and the
263 sequence was confirmed by next-generation sequencing before use.
264
265 **Authentic virus neutralization assay**
266 Vero-E6-TMPRSS2 cells were seeded in 96 well-plates in complete media overnight at 37 °C
267 under 5% CO_2 to establish a monolayer. The following day, sera were serially diluted starting at
268 1:500 dilution in 96 well-plates in triplicate in DMEM + 2% FBS and then incubated with 0.01
269 MOI of either virus at 37 °C for 1 h. Afterwards, the mixture was overlaid onto cells and further
270 incubated at 37 °C under 5% CO_2 for approximately 72 h. Cytopathic effects were then visually
271 assessed in all wells and scored as either negative or positive for infection by comparison to
272 control uninfected or infected wells in a blinded manner. Neutralization curves and IC_{50} values
273 were derived by fitting a non-linear five-parameter dose-response curve to the data in GraphPad
274 Prism version 9.2.
275
276 **Reporting summary**
277 Further information on research design is available in the Nature Research Reporting Summary
278 linked to this paper.
279 **Data availability**
280 All experimental data are provided in the manuscript. Omicron prevalence analyses utilized
281 sequences submitted to and available from GISAID[8]. The sequences of the authentic viruses used
282 in this study have been deposited to GISAID under accession numbers EPI_ISL_497840
283 (D614G) and EPI_ISL_9845731 (BA.2). Materials use in this study will be made available under
284 an appropriate Materials Transfer Agreement.
285
286
287
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292 Viral Evolution (SAVE) Program.
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Author contributions D.D.H. conceived this project. S.I. and Lihong Liu conducted pseudovirus neutralization experiments. Y.G. and Z.Z. conducted bioinformatic analyses. Liyuan Liu and Yiming Huang constructed the spike expression plasmids. J.F.-W.C., H.C., K.K.-H.C., and T.T.-T.Y. conducted authentic virus neutralization experiments. M.W. aided sample collections. Y.L. managed the project. J.Y. expressed and purified antibodies. M.T.Y. and M.E.S. provided clinical samples. Yaoxing Huang contributed to discussions. K.-Y.Y., H.H.W., and D.D.H. directed and supervised the project. S.I, Lihong Liu, and D.D.H. analyzed the results and wrote the manuscript.

Competing interests S.I, Lihong Liu, J.Y., Yaoxing Huang, and D.D.H. are inventors on patent applications (WO2021236998) or provisional patent applications (63/271,627) filed by Columbia University for a number of SARS-CoV-2 neutralizing antibodies described in this manuscript. Both sets of applications are under review. D.D.H. is a co-founder of TaiMed Biologics and RenBio, consultant to WuXi Biologics and Brii Biosciences, and board director for Vicarious Surgical.

Additional information

Supplementary information is available for this paper at

Correspondence and requests for materials should be addressed to David D. Ho.

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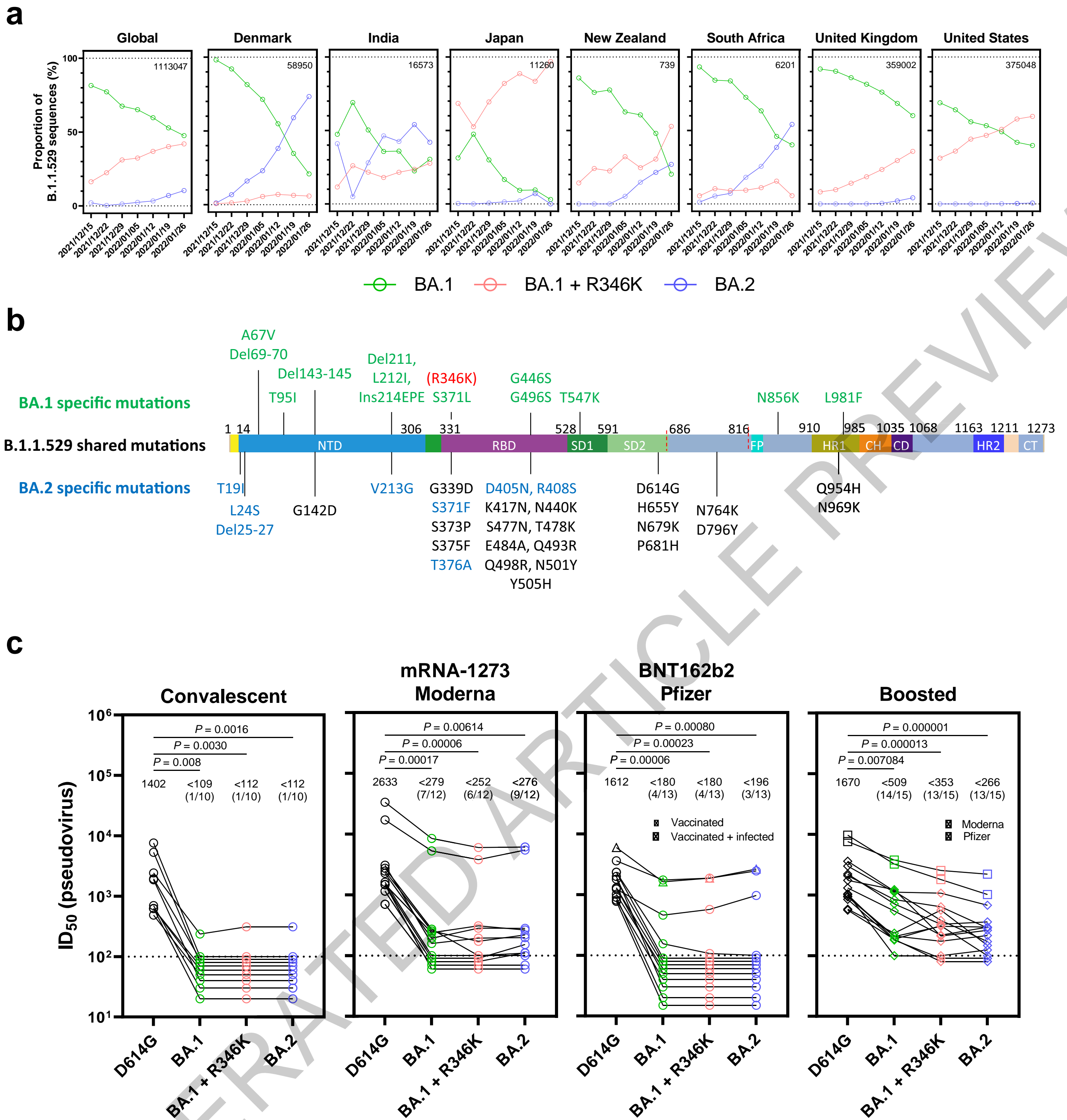

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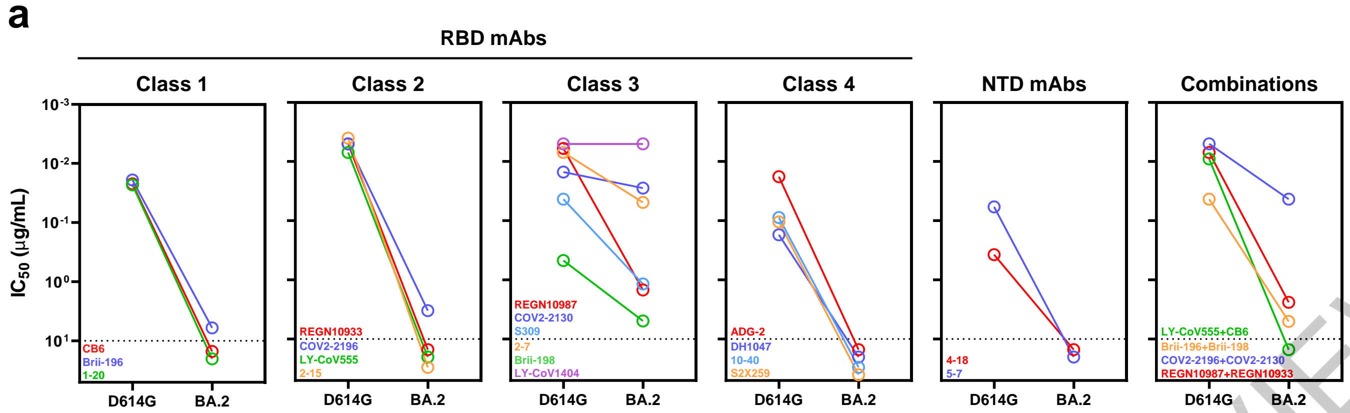
Fig. 1 | BA.2 exhibits a similar serum neutralization profile as BA.1 sublineages. a, Proportions of BA.1, BA.1+R346K, and BA.2 within B.1.1.529 sequences on GISAID over the latter half of December 2021 through January 2022. Values in the upper right corner of each box denote cumulative number of Omicron sequences. **b,** Mutations within the B.1.1.529 lineage. **c,** Pseudovirus neutralization by convalescent and vaccinee sera. n = 10, 12, 13, and 15 biologically independent serum samples, respectively, for convalescent, mRNA-1273, BNT162b2, and boosted groups. Values above points indicate the geometric mean. Numbers in parentheses denote the number of samples above the limit of detection (LOD) of 100. Values below the LOD are arbitrarily plotted to allow for visualization of each sample. P values were determined by two-sided Friedman test followed by Dunn's multiple comparisons test.

a



b

Fold change in IC_{50} relative to D614G	RBD mAbs																	NTD mAbs	
	Class 1			Class 2				Class 3						Class 4					
	CB6	Brii-196	1-20	REGN 10933	COV2-2196	LY-CoV 555	2-15	REGN 10987	COV2-2130	S309	2-7	Brii-198	LY-CoV 1404	ADG-2	DH1047	10-40	S2X259	4-18	5-7
BA.1	<-428	-298	<-429	<-2201	-306	<-1496	<-2716	<-1716	-83.5	-6.9	-195	2.3	1.4	-11.0	-14.2	-21.1	-13.7	<-26.7	-4.1
BA.1 + R346K	<-428	-135	<-429	-415	-187	<-1496	<-2716	<-1716	<-687	-4.5	-82.1	<-22	1.5	-15.7	-7.9	-20.5	-7.5	<-26.7	-5.5
BA.2	<-428	-322	<-429	<-2201	-680	<-1496	<-2716	-253	-1.9	-27.0	-7.3	-10.5	1.1	<-555	<-58.0	<-114	<-96	<-26.7	<-171
T19I	-3.1	-4.9	-5.3	-3.7	-1.9	-2.2	-2.0	-2.1	-1.5	-1.8	-5.1	-1.6	-1.7	-1.7	-1.5	-2.7	-2.9	-6.1	-3.3
L24S	-2.9	-4.0	-4.6	-3.2	-2.4	-2.4	-2.8	-4.2	-2.1	-1.5	-2.6	-2.2	-1.6	-1.3	-1.1	-2.4	-2.0	-3.1	-1.1
Del25-27	-1.2	-2.6	-2.0	-1.3	-1.0	-1.4	-1.2	-1.3	1.0	-1.3	-2.8	2.0	-1.2	1.1	1.6	-1.8	1.1	-23.1	-16.8
V213G	-2.5	-3.1	-3.0	-3.1	-1.5	-1.1	-1.6	-2.2	-2.0	-1.2	-3.2	-1.1	-1.5	1.1	1.0	-2.0	-1.7	1.9	-2.8
S371F	-143	-126	-95.1	-27.9	-26.1	-5.1	-6.3	-86.6	-1.3	-20.5	-30.6	<-22	-2.4	-43.0	-60.9	<-114	-77.5	7.8	2.3
T376A	-1.9	-3.1	-2.5	-2.1	-1.3	-1.7	-1.3	-1.9	-1.8	1.0	-2.7	2.0	-1.7	1.1	1.1	-1.5	-2.3	1.3	-1.3
D405N	-25.6	-2.3	-2.9	-2.8	-2.1	-1.9	-1.7	-1.6	1.0	1.5	-3.1	-1.6	1.3	3.3	-1.2	-3.9	-2.2	5.6	1.5
R408S	1.4	-1.1	-1.3	-1.1	1.5	-1.6	-1.3	1.2	1.0	1.0	1.2	1.4	-1.4	-1.6	-2.1	-1.2	-3.6	1.1	-1.3

>3	<-3	<-10	<-100

Fig. 2 | BA.2 differs in resistance profile to monoclonal antibodies. a, Pseudovirus neutralization by monoclonal antibodies. Values above the LOD of 10 μg/mL are arbitrarily plotted to allow for visualization of each sample**. b,** Fold change in IC_{50} values relative to D614G of neutralization of Omicron variants, as well as point mutants unique to BA.2.

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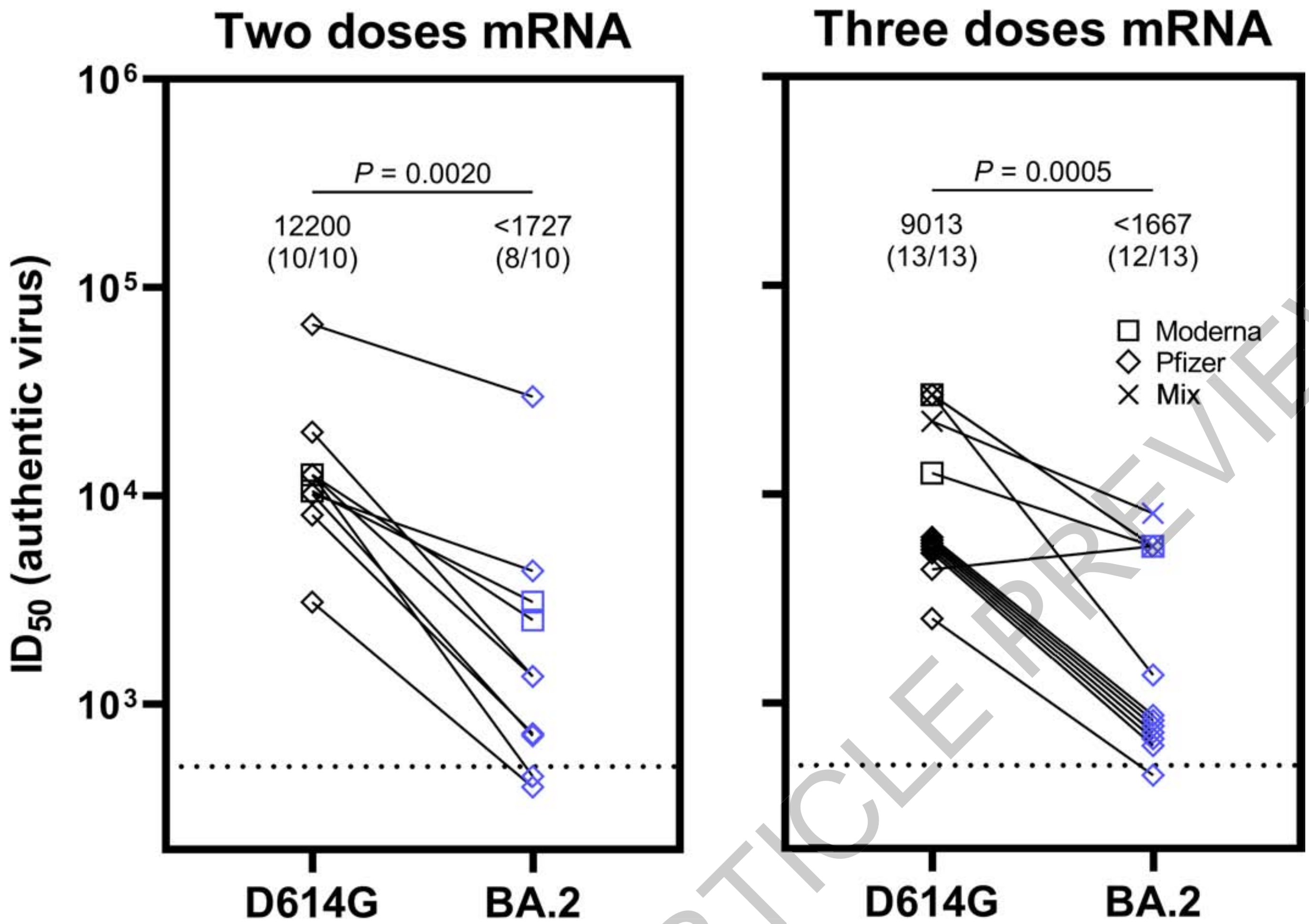

Extended Data Fig. 1 | Serum neutralization of authentic viruses. Authentic virus neutralization by vaccinee sera. n = 10 and 13 biologically independent serum samples, respectively, for two doses mRNA and three doses mRNA groups. Values above points indicate the geometric mean. Numbers in parentheses denote the number of samples above the limit of detection (LOD) of 500. Values below the LOD and those that overlap are plotted to allow for visualization of each sample. P values were determined by two-sided Wilcoxon matched-pairs signed rank test.

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Extended Data Table 1 | Demographics and vaccination information for serum samples from vaccinated individuals used in authentic virus neutralization experiments.

Vaccine Sample	Vaccine type	Days post-vaccination (after last dose)	Documented COVID Infection	Age	Gender
Two doses #1	mRNA-1273 + mRNA-1273	32	No	68	Male
Two doses #2	BNT162b2 + BNT162b2	22	No	57	Female
Two doses #3	BNT162b2 + BNT162b2	16	No	64	Female
Two doses #4	BNT162b2 + BNT162b2	21	No	45	Male
Two doses #5	mRNA-1273 + mRNA-1273	32	No	66	Female
Two doses #6	BNT162b2 + BNT162b2	213	Yes	66	Male
Two doses #7	BNT162b2 + BNT162b2	14	No	52	Female
Two doses #8	BNT162b2 + BNT162b2	8	No	67	Male
Two doses #9	BNT162b2 + BNT162b2	50	No	61	Female
Two doses #10	BNT162b2 + BNT162b2	102	No	39	Male
Three doses #1	mRNA-1273 + mRNA-1273 + mRNA-1273	29	No	66	Female
Three doses #2	BNT162b2 + BNT162b2 + BNT162b2	14	No	64	Female
Three doses #3	BNT162b2 + BNT162b2 + BNT162b2	34	No	45	Male
Three doses #4	BNT162b2 + BNT162b2 + BNT162b2	15	No	50	Female
Three doses #5	BNT162b2 + BNT162b2 + BNT162b2	15	No	48	Female
Three doses #6	BNT162b2 + BNT162b2 + BNT162b2	90	No	59	Male
Three doses #7	BNT162b2 + BNT162b2 + BNT162b2	87	No	66	Female
Three doses #8	mRNA-1273 + mRNA-1273 + mRNA-1273	23	Yes	28	Female
Three doses #9	BNT162b2 + BNT162b2 + BNT162b2	14	No	75	Female
Three doses #10	BNT162b2 + BNT162b2 + BNT162b2	14	No	78	Male
Three doses #11	mRNA-1273 + mRNA-1273 + BNT162b2	60	Yes	64	Male
Three doses #12	BNT162b2 + BNT162b2 + mRNA-1273	15	Yes	39	Male
Three doses #13	BNT162b2 + BNT162b2 + BNT162b2	53	No	30	Male

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nature portfolio

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For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed	
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☐	☒	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 *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☒	☐	A description of all covariates tested
☒	☐	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒	For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐	Estimates of effect sizes (e.g. Cohen's d, Pearson's r), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	SoftMax Pro 7.0.2 (Molecular Devices, LLC) was used to measure luminescence in the pseudovirus neutralization assays.
Data analysis	GraphPad Prism (version 9.2) was used for data visualization and for statistical tests.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

All experimental data are provided in the manuscript. Omicron prevalence analyses utilized sequences submitted to and available from GISAID. The sequences of the authentic viruses used in this study are available at GISAID under accession numbers EPI_ISL_497840 (D614G) and EPI_ISL_9845731 (BA.2).

Field-specific reporting

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For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

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

Sample size	We used analogous sample sizes as in previous work (e.g. Wang et al 2021, Nature, Liu et al 2021, Nature), which we had previously determined to be sufficient sample sizes for comparisons between groups for these experiments.
Data exclusions	No data were excluded.
Replication	The key results, the serum neutralization of D614G and BA.2 (both in pseudoviruses and authentic viruses), and the neutralization of all of the viruses in Fig. 2b by S309, were repeated twice independently in technical triplicate with similar results. The results that are shown are representative. Other experiments were conducted in technical triplicate and not repeated, as these results were consistent with bioinformatic and structural analyses.
Randomization	As this is an observational study, randomization is not relevant.
Blinding	As this is an observational study, investigators were not blinded.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study	
☐	☒	Antibodies
☐	☒	Eukaryotic cell lines
☒	☐	Palaeontology and archaeology
☒	☐	Animals and other organisms
☐	☒	Human research participants
☒	☐	Clinical data
☒	☐	Dual use research of concern

Methods

n/a	Involved in the study	
☒	☐	ChIP-seq
☒	☐	Flow cytometry
☒	☐	MRI-based neuroimaging

Antibodies

Antibodies used	All of the antibodies used in this study were produced in our laboratory or received from other laboratories. 1-20, LY-CoV555, 2-15, S309, 2-7, LY-CoV1404, ADG-2, DH1047, 10-40, S2X259, 4-18, and 5-7 were expressed and purified in-house as described previously in Liu et al 2020, Nature and in the Methods section of this manuscript. REGN10987, REGN10933, COV2-2196, and COV2-2130 were produced and provided by Regeneron Pharmaceuticals, Brii-196 and Brii-198 were produced and provided by Brii Biosciences, CB6 was produced and provided by Baoshan Zhang and Peter Kwong (NIAID).
Validation	All of the antibodies except LY-CoV1404 have been validated in previous studies both by binding to SARS-CoV-2 spike and neutralization of SARS-CoV-2 (both pseudovirus and authentic virus), and when applicable, have been confirmed to give similar results as that described in publications by other groups. Specifically, 1-20 and 4-18 were tested in Liu et al 2020, Nature, CB6, Brii-196, 910-30, REGN10933, COV2-2196, LY-CoV555, 2-15, REGN10987, COV2-2130, S309, 2-7, Brii-198, and 5-7 were tested in Wang et al 2021, Nature, and ADG-2, DH1047, 10-40, and S2X259 were tested in Liu et al 2021, bioRxiv. LY-CoV1404 was newly produced and tested prior to use in this study and confirmed to have similar results as that of the original publication from which it is derived (Westendorf et al 2022, bioRxiv).

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	Expi293 cells were obtained from Thermo Fisher (Catalog #A14527), Vero E6 cells were obtained from ATCC (Catalog

Cell line source(s)	#CRL-1586), HEK293T cells were obtained from ATCC (Catalog #CRL-3216), and Vero-E6-TMPRSS2 cells were obtained from JCRB (Catalog #JCRB1819).
Authentication	Cell lines were purchased from authenticated vendors, and morphology was also confirmed visually prior to use.
Mycoplasma contamination	Cell lines tested mycoplasma negative.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used in this study.

Human research participants

Policy information about studies involving human research participants

Population characteristics	Population characteristics for the sera utilized in the pseudovirus neutralization assays are described in Extended Data Table 1 of Liu et al 2021, Nature (ref 2). Convalescent samples had the following ranges: 9-120 days post-symptoms, 45-79 years old, 4/10 female, 6/10 male. We presume all of these individuals were infected with the wild-type strain of SARS-CoV-2 as these samples were collected in Spring of 2020. Vaccinee samples had the following ranges: 6-213 days post-vaccination, 26-78 years old, 12/40 two mRNA-1273 vaccinations, 13/40 two BNT162b2 vaccinations, 2/40 three mRNA-1273 vaccinations, 13/40 three BNT162b2 vaccinations, 1/40 previously infected, 39/40 uninfected, 20/40 female, 20/40 male. Population characteristics for the vaccinee sera utilized in the authentic virus neutralization assays are described in Extended Data Table 1 of this manuscript. These samples had the following ranges: 8-213 days post-vaccination, 28-78 years old, 2/23 two mRNA-1273 vaccinations, 8/23 two BNT162b2 vaccinations, 2/23 three mRNA-1273 vaccinations, 9/23 three BNT162b2 vaccinations, 1/23 two mRNA-1273 vaccinations followed by one BNT162b2 vaccination, 1/23 two BNT162b2 vaccination followed by one mRNA-1273 vaccination, 4/23 previously infected, 19/23 uninfected, 12/23 female, 11/23 male.
Recruitment	For convalescent sera, convalescing patients volunteered and were enrolled in an observational cohort study at Columbia University Irving Medical Center in Spring of 2020. For the vaccinee sera, individuals volunteered and were enrolled in an observational cohort study at Columbia University Irving Medical Center to study the immunological responses to SARS-CoV-2 in individuals who had received COVID-19 vaccines. Self-selection biases may have affected the demographics of the enrolled population, but are not expected to have impacted the results of this study. High titer samples were specifically chosen within each of the serum groups so that fold-change in titer could be better determined.
Ethics oversight	All collections were conducted under protocols reviewed and approved by the Institutional Review Board of Columbia University. All of the participants provided written informed consent.

Note that full information on the approval of the study protocol must also be provided in the manuscript.