

## Antibody Evasion Properties of SARS-CoV-2 Omicron Sublineages

1 Sho Iketani<sup>1,2\*</sup>, Lihong Liu<sup>1\*</sup>, Yicheng Guo<sup>1\*</sup>, Liyuan Liu<sup>3\*</sup>, Yiming Huang<sup>3</sup>, Maple Wang<sup>1</sup>,  
2 Yang Luo<sup>1</sup>, Jian Yu<sup>1</sup>, Michael T. Yin<sup>1,4</sup>, Magdalena E. Sobieszczyk<sup>1,4</sup>, Yaoxing Huang<sup>1</sup>,  
3 Harris H. Wang<sup>3,5</sup>, Zizhang Sheng<sup>1</sup>, David D. Ho<sup>1,2,4^</sup>  
4

5 <sup>1</sup>Aaron Diamond AIDS Research Center, Columbia University Vagelos College of Physicians  
6 and Surgeons, New York, NY 10032, USA

7 <sup>2</sup>Department of Microbiology and Immunology, Columbia University Vagelos College of  
8 Physicians and Surgeons, New York, NY 10032, USA

9 <sup>3</sup>Department of Systems Biology, Columbia University Vagelos College of Physicians and  
10 Surgeons, New York, NY 10032, USA

11 <sup>4</sup>Division of Infectious Diseases, Department of Medicine, Columbia University Vagelos College  
12 of Physicians and Surgeons, New York, NY 10032, USA

13 <sup>5</sup>Department of Pathology and Cell Biology, Columbia University Vagelos College of Physicians  
14 and Surgeons, New York, NY 10032, USA  
15

16 \* These authors contributed equally

17 ^ Address correspondence to David D. Ho ([dh2994@cumc.columbia.edu](mailto:dh2994@cumc.columbia.edu), 212-304-6101, 701 W  
18 168th St, 11th Floor, New York, NY 10032)  
19

20 Word count: 1267  
21

## 22 **Abstract**

23  
24 The identification of the Omicron variant (B.1.1.529.1 or BA.1) of SARS-CoV-2 (severe acute  
25 respiratory syndrome coronavirus 2) in Botswana in November 2021<sup>1</sup> immediately raised alarms  
26 due to the sheer number of mutations in the spike glycoprotein that could lead to striking  
27 antibody evasion. We<sup>2</sup> and others<sup>3-6</sup> recently reported results in this Journal confirming such a  
28 concern. Continuing surveillance of Omicron evolution has since revealed the rise in prevalence  
29 of two sublineages, BA.1 with an R346K mutation (BA.1+R346K) and B.1.1.529.2 (BA.2), with  
30 the latter containing 8 unique spike mutations while lacking 13 spike mutations found in BA.1.  
31 We therefore extended our studies to include antigenic characterization of these new sublineages.  
32 Polyclonal sera from patients infected by wild-type SARS-CoV-2 or recipients of current mRNA  
33 vaccines showed a substantial loss in neutralizing activity against both BA.1+R346K and BA.2,  
34 with drops comparable to that already reported for BA.1<sup>2,3,5,6</sup>. These findings indicate that these  
35 three sublineages of Omicron are antigenically equidistant from the wild-type SARS-CoV-2 and  
36 thus similarly threaten the efficacies of current vaccines. BA.2 also exhibited marked resistance  
37 to 17 of 19 neutralizing monoclonal antibodies tested, including S309 (sotrovimab)<sup>7</sup>, which had  
38 retained appreciable activity against BA.1 and BA.1+R346K<sup>2-4,6</sup>. This new finding shows that  
39 no presently approved or authorized monoclonal antibody therapy could adequately cover all  
40 sublineages of the Omicron variant.

41  
42 **Main Text**

43  
44 The meteoric rise of the B.1.1.529/Omicron to become the dominant SARS-CoV-2 variant  
45 globally has been truly remarkable<sup>8</sup>. Continuing surveillance of its evolution in the population  
46 over the past six weeks has revealed that the proportion of the original form, BA.1, has been  
47 decreasing steadily while the proportions of two other sublineages have increased noticeably  
48 (**Fig. 1a**). In fact, the BA.1+R346K sublineage now accounts for ~30% of Omicron sequences  
49 globally, and ~30-45% in South Africa, United Kingdom, and United States. On the other hand,  
50 the BA.2 sublineage accounts for only ~13% of Omicron sequences globally, but it is not only on  
51 the rise but also the dominant form in countries such as Denmark and India. These three  
52 sublineages of Omicron share 21 mutations in the spike protein, wherein BA.2 contains 8 unique

53 mutations and BA.1 contains 13 unique mutations (**Fig. 1b**). Of course, BA.1+R346K has one  
54 mutation more than BA.1. Given these differences, their antigenic properties cannot be assumed  
55 to be the same or similar.

56  
57 Therefore, we first investigated the neutralization sensitivity of the Omicron sublineages by  
58 polyclonal sera from convalescent patients or individuals given mRNA vaccines, with or without  
59 a booster shot. These serum samples, as well as the pseudovirus neutralization assay used, were  
60 identical to ones previously reported<sup>2</sup>. The wild-type D614G pseudovirus was included as a  
61 comparator. As was observed and reported for BA.1<sup>2,3,5,6</sup>, a marked and significant loss of serum  
62 neutralizing activity against BA.1+R346K and BA.2 relative to D614G was noted, with  
63 neutralizing titers for numerous samples dropping below the limit of detection (**Fig. 1c**). The loss  
64 of neutralizing activity against BA.1+R346K or BA.2 sublineages was less prominent for sera  
65 obtained from individuals who received a booster vaccination (**Fig. 1c**, right panel), consistent  
66 with reported findings for BA.1<sup>2,3,6</sup>. Among these samples, the mean serum neutralizing titers  
67 against Omicron sublineages were significantly lower than the mean titer for D614G; although  
68 the mean titer was slightly lower for BA.2, the difference from BA.1 sublineages did not reach  
69 statistical significance ( $P = 0.242$ ).

70  
71 To further examine antigenic differences in the spike protein of these Omicron sublineages, a  
72 panel of 19 neutralizing monoclonal antibodies was used as probes. Seventeen were directed to  
73 different epitope clusters (classes 1-4) within the receptor-binding domain (RBD), whereas two  
74 were directed to the N-terminal domain (NTD). These antibodies included REGN10987  
75 (imdevimab)<sup>9</sup>, REGN10933 (casirivimab)<sup>9</sup>, COV2-2196 (tixagevimab)<sup>10</sup>, COV2-2130  
76 (cilgavimab)<sup>10</sup>, LY-CoV555 (bamlanivimab)<sup>11</sup>, CB6 (etesevimab)<sup>12</sup>, Brie-196 (amubarvimab)<sup>13</sup>,  
77 Brie-198 (romlusevimab)<sup>13</sup>, S309 (sotrovimab)<sup>7</sup>, LY-CoV1404 (bebtelovimab)<sup>14</sup>, ADG-2<sup>15</sup>,  
78 DH1047<sup>16</sup>, and S2X259<sup>17</sup>, as well as 1-20, 2-15, 2-7, 4-18, 5-7<sup>18</sup> and 10-40<sup>19</sup> from our group.  
79 Overall, 17 of 19 monoclonal antibodies were either totally inactive or severely impaired in  
80 neutralizing BA.2 (**Fig. 2a**), somewhat like previous findings for BA.1 and BA.1+R346K<sup>2</sup> but  
81 with important differences (**Fig. 2b**). All class 4 antibodies tested lost greater neutralizing  
82 potency against BA.2 versus BA.1 sublineages. Two class 3 antibodies, COV2-2130 and 2-7,  
83 retained decent activity against BA.2 while having no activity against BA.1 viruses. S309 or

84 sotrovimab lost 27-fold neutralizing activity against BA.2; this is particularly important because  
85 it was found to be the only clinically approved or authorized monoclonal antibody to retain  
86 activity against the original form of Omicron<sup>2-4</sup>. LY-CoV1404, another class 3 antibody in  
87 development, remained potent in neutralizing all Omicron sublineages, suggesting that there is  
88 still a patch within this antibody-binding region that is unaffected by all spike mutations found in  
89 SARS-CoV-2 variants to date. Although there was a lack of an observable difference among the  
90 Omicron sublineages in neutralization by polyclonal sera (**Fig. 1c**), important antigenic  
91 differences do exist when probed by monoclonal antibodies. Except for S309, BA.1 appears to  
92 be more resistant to class 3 antibodies than BA.2, while BA.2 is more resistant to all class 4  
93 antibodies tested. Our recent study<sup>2</sup> showed that previous SARS-CoV-2 variants, such as  
94 B.1.351/Beta and B.1.617.2/Delta, evolved to resist class 1, class 2, and NTD antibodies first,  
95 and then the Omicron variant seemingly has further evolved to resist class 3 and class 4  
96 antibodies in addition. Our current findings suggest that the Omicron sublineages may have  
97 diverged under slightly different pressure from class 3 and class 4 antibodies to the RBD.

98  
99 Finally, we constructed each of the eight BA.2-specific spike mutations alone as pseudoviruses  
100 and tested them using the same panel of 19 monoclonal antibodies (**Fig. 2b**). S371F broadly  
101 affected most of the RBD-directed antibodies, similar to what was observed for S371L in BA.1<sup>2</sup>  
102 but with a greater negative impact, perhaps due to the bulkier side chain of phenylalanine.  
103 Intriguingly but importantly, S371F appears to be majorly responsible for the loss in potency of  
104 S309, although this mutation was not observed previously as a marker for clinical resistance to  
105 sotrovimab<sup>20</sup>. CB6 was adversely affected by the D405N mutation, likely due to its position  
106 within the epitope of this antibody<sup>12</sup>. It is not clear how T19I and L24S mutations in the NTD  
107 subtly impaired the neutralizing activity of class 1 antibodies to RBD.

108  
109 In summary, we have comprehensively evaluated the antigenic properties of two sublineages of  
110 the Omicron variant, BA.1+R346K and BA.2, and we believe our results have important clinical  
111 implications. First, polyclonal sera showed a substantial loss in neutralizing activity against both  
112 sublineages, with drops comparable to that of BA.1 (**Fig. 1c**). These three sublineages of  
113 Omicron, therefore, seem to be antigenically equidistant from the wild-type SARS-CoV-2, likely  
114 threatening the efficacies of current COVID-19 vaccines to a similar extent. The present study,

115 however, does not address the antigenic distance between BA.1 and BA.2, which will require  
116 cross-neutralization experiments using sublineage-specific sera to determine. Second,  
117 monoclonal antibodies were affected in a disparate manner for the different Omicron sublineages.  
118 For clinically approved or authorized antibodies, only S309 (sotrovimab) retained activity  
119 against both BA.1 and BA.1+R346K, but its activity against BA.2 has dropped 27-fold (**Fig. 2b**)  
120 to a 50% inhibitory concentration (IC<sub>50</sub>) of ~1 µg/mL (**Fig. 2a**). Only COV2-2130 (cilgavimab)  
121 and its combination with COV2-2196 (tixagevimab) retained activity against BA.2, but this  
122 antibody combination is only authorized for preventive use. Presently, no authorized therapeutic  
123 monoclonal antibody could adequately treat all sublineages of the Omicron variant. This finding  
124 poses a therapeutic dilemma in geographic regions where all three sublineages are present in  
125 sufficient numbers. As COVID-19 treatment options are narrowed by the emergence of more and  
126 more variants, it is imperative that we continue to devise novel strategies to contain this ever-  
127 evolving pathogen.

128 **Figure Legends**

129

130 **Fig. 1 | BA.2 exhibits a similar serum neutralization profile as BA.1 sublineages. a,**  
131 Proportions of BA.1, BA.1+R346K, and BA.2 within B.1.1.529 sequences on GISAID over the  
132 past six weeks. Values in the upper right corner of each box denote cumulative number of  
133 Omicron sequences. **b,** Mutations within the B.1.1.529 lineage. **c,** Pseudovirus neutralization by  
134 convalescent and vaccinee sera. Values above points indicate the geometric mean. Numbers in  
135 parentheses denote the number of samples above the limit of detection (LOD) of 100. Values  
136 below the LOD are arbitrarily plotted to allow for visualization of each sample. P values were  
137 determined by two-sided Friedman test followed by Dunn's multiple comparisons test.

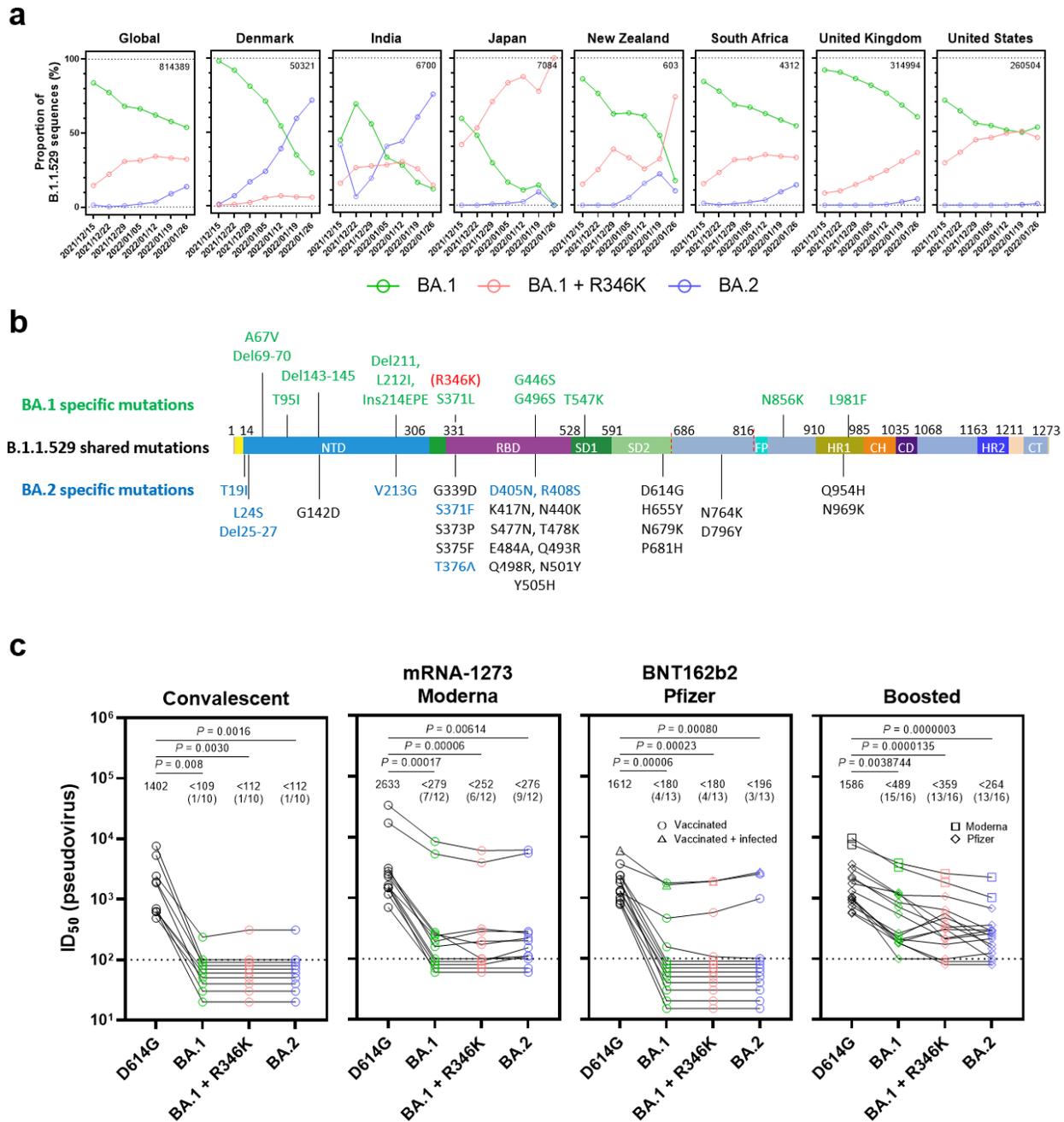
138

139 **Fig. 2 | BA.2 differs in resistance profile to monoclonal antibodies. a,** Pseudovirus  
140 neutralization by monoclonal antibodies. Values above the LOD of 10  $\mu\text{g/mL}$  are arbitrarily  
141 plotted to allow for visualization of each sample. **b,** Fold change in  $\text{IC}_{50}$  values relative to D614G  
142 of neutralization of Omicron variants, as well as point mutants unique to BA.2.

143

144 **Figure 1**

145

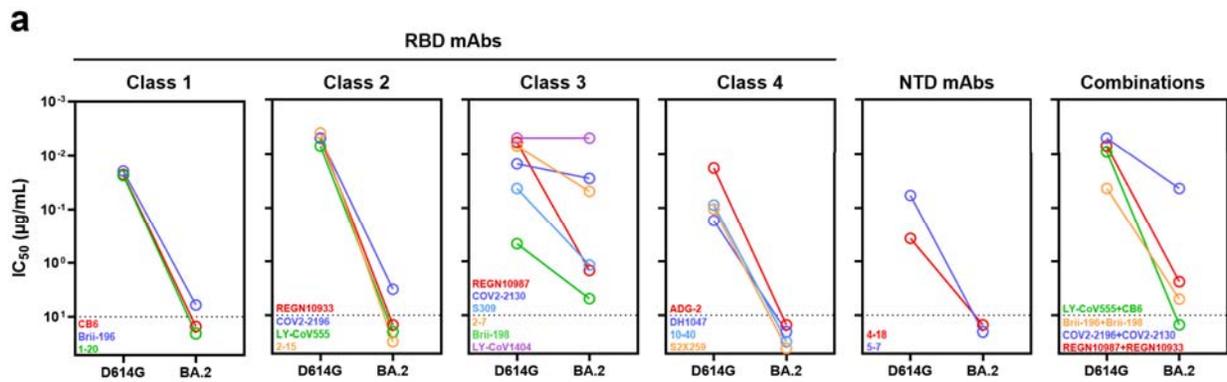


146

147

148 **Figure 2**

149



**b**

Fold change in IC <sub>50</sub> relative to D614G	RBD mAbs															NTD mAbs			
	Class 1			Class 2				Class 3					Class 4			4-18	5-7		
	CB6	Brij-196	1-20	REGN 10933	COV2-2196	LY-CoV 555	2-15	REGN 10987	COV2-2130	S309	2-7	Brij-196	LY-CoV 1404	ADG-2	DH1047			10-40	S2X259
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

150

>3   <-3   <-10   <-100

151 **References**

152

- 153 1 Viana, R. *et al.* Rapid epidemic expansion of the SARS-CoV-2 Omicron variant in  
154 southern Africa. *Nature*, doi:10.1038/s41586-022-04411-y (2022).
- 155 2 Liu, L. *et al.* Striking Antibody Evasion Manifested by the Omicron Variant of SARS-  
156 CoV-2. *Nature*, doi:10.1038/s41586-021-04388-0 (2021).
- 157 3 Cameroni, E. *et al.* Broadly neutralizing antibodies overcome SARS-CoV-2 Omicron  
158 antigenic shift. *Nature*, doi:10.1038/s41586-021-04386-2 (2021).
- 159 4 Cao, Y. *et al.* Omicron escapes the majority of existing SARS-CoV-2 neutralizing  
160 antibodies. *Nature*, doi:10.1038/s41586-021-04385-3 (2021).
- 161 5 Cele, S. *et al.* Omicron extensively but incompletely escapes Pfizer BNT162b2  
162 neutralization. *Nature*, doi:10.1038/s41586-021-04387-1 (2021).
- 163 6 Planas, D. *et al.* Considerable escape of SARS-CoV-2 Omicron to antibody neutralization.  
164 *Nature*, doi:10.1038/s41586-021-04389-z (2021).
- 165 7 Pinto, D. *et al.* Cross-neutralization of SARS-CoV-2 by a human monoclonal SARS-CoV  
166 antibody. *Nature* **583**, 290-295, doi:10.1038/s41586-020-2349-y (2020).
- 167 8 Shu, Y. & McCauley, J. GISAID: Global initiative on sharing all influenza data - from  
168 vision to reality. *Euro Surveill* **22**, doi:10.2807/1560-7917.ES.2017.22.13.30494 (2017).
- 169 9 Hansen, J. *et al.* Studies in humanized mice and convalescent humans yield a SARS-  
170 CoV-2 antibody cocktail. *Science* **369**, 1010-1014, doi:10.1126/science.abd0827 (2020).
- 171 10 Zost, S. J. *et al.* Potently neutralizing and protective human antibodies against SARS-  
172 CoV-2. *Nature* **584**, 443-449, doi:10.1038/s41586-020-2548-6 (2020).
- 173 11 Jones, B. E. *et al.* The neutralizing antibody, LY-CoV555, protects against SARS-CoV-2  
174 infection in nonhuman primates. *Sci Transl Med* **13**, doi:10.1126/scitranslmed.abf1906  
175 (2021).
- 176 12 Shi, R. *et al.* A human neutralizing antibody targets the receptor-binding site of SARS-  
177 CoV-2. *Nature* **584**, 120-124, doi:10.1038/s41586-020-2381-y (2020).
- 178 13 Ju, B. *et al.* Human neutralizing antibodies elicited by SARS-CoV-2 infection. *Nature*  
179 **584**, 115-119, doi:10.1038/s41586-020-2380-z (2020).
- 180 14 Westendorf, K. *et al.* LY-CoV1404 (bebtelovimab) potently neutralizes SARS-CoV-2  
181 variants. *bioRxiv*, doi:10.1101/2021.04.30.442182 (2022).

- 182 15 Rappazzo, C. G. *et al.* Broad and potent activity against SARS-like viruses by an  
183 engineered human monoclonal antibody. *Science* **371**, 823-829,  
184 doi:10.1126/science.abf4830 (2021).
- 185 16 Li, D. *et al.* In vitro and in vivo functions of SARS-CoV-2 infection-enhancing and  
186 neutralizing antibodies. *Cell* **184**, 4203-4219 e4232, doi:10.1016/j.cell.2021.06.021  
187 (2021).
- 188 17 Tortorici, M. A. *et al.* Broad sarbecovirus neutralization by a human monoclonal  
189 antibody. *Nature* **597**, 103-108, doi:10.1038/s41586-021-03817-4 (2021).
- 190 18 Liu, L. *et al.* Potent neutralizing antibodies against multiple epitopes on SARS-CoV-2  
191 spike. *Nature* **584**, 450-456, doi:10.1038/s41586-020-2571-7 (2020).
- 192 19 Liu, L. *et al.* Isolation and comparative analysis of antibodies that broadly neutralize  
193 sarbecoviruses. *bioRxiv*, doi:<https://doi.org/10.1101/2021.12.11.472236> (2021).
- 194 20 Rockett, R. J. *et al.* RESISTANCE CONFERRING MUTATIONS IN SARS-CoV-2  
195 DELTA FOLLOWING SOTROVIMAB INFUSION. *medRxiv*,  
196 doi:<https://doi.org/10.1101/2021.12.18.21267628> (2021).  
197

198 **Methods**

199

200 **Data reporting**

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

204

205 **Serum samples**

206 Identical samples from a previous study were utilized<sup>2</sup>. All collections were conducted under  
207 protocols reviewed and approved by the Institutional Review Board of Columbia University.

208

209 **Antibodies and pseudovirus neutralization**

210 The expression of antibodies, construction of variant SARS-CoV-2 spike plasmids, production  
211 and neutralization of pseudoviruses, were conducted as previously described<sup>2</sup>.

212

213 **Acknowledgements**

214 This study was supported by funding from the Gates Foundation, JPB Foundation, Andrew and  
215 Peggy Cherng, Samuel Yin, Carol Ludwig, David and Roger Wu, Regeneron Pharmaceuticals,  
216 the National Science Foundation (MCB-2032259), and the NIH SARS-CoV-2 Assessment of  
217 Viral Evolution (SAVE) Program.

218

219 **Author contributions**

220 D.D.H. conceived this project. S.I. and Lihong Liu conducted pseudovirus neutralization  
221 experiments. Y.G. and Z.Z. conducted bioinformatic analyses. Liyuan Liu and Yiming Huang  
222 constructed the spike expression plasmids. M.W. aided sample collections. Y.L. managed the  
223 project. J.Y. expressed and purified antibodies. M.T.Y. and M.E.S. provided clinical samples.  
224 Yaoxing Huang contributed to discussions. H.H.W. and D.D.H. directed and supervised the  
225 project. S.I, Lihong Liu, and D.D.H. analyzed the results and wrote the manuscript.

226

227 **Competing interests**

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

233

234 **Data and materials availability**

235 All data are provided in the manuscript.

236