bioRxiv preprint doi: https://doi.org/10.1101/2021.01.27.428353; this version posted January 30, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

1 # Circulating SARS-CoV-2 variants B.1.1.7, 501Y.V2, and P.1 have gained ability
2 to utilize rat and mouse Ace2 and altered in vitro sensitivity to neutralizing
3 antibodies and ACE2-Ig

4 Weitong Yao[1,2], Yifei Wang[1], Danting Ma[1], Xiaojuan Tang[1,2], Haimin Wang[1], Chao Li[1], Hua
5 Lin[3], Yujun Li[1]*, Guocai Zhong[1,2]*

6 **Affiliations:**
7 [1] Shenzhen Bay Laboratory, Shenzhen 518132, China
8 [2] School of Chemical Biology and Biotechnology, Peking University Shenzhen Graduate
9 School, Shenzhen 518055, China
10 [3] Biomedical Research Center of South China, Fujian Normal University, Fuzhou 350117,
11 China
12 * Corresponding author. Email: liyj@szbl.ac.cn; zhonggc@szbl.ac.cn

13 **Running title:** Receptor usage and neutralization sensitivity of SARS-CoV-2 variants

14 **Keywords:** SARS-CoV-2; Spike mutation; mouse Ace2; rat Ace2; ACE2-Ig; neutralizing
15 antibody

16

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.27.428353; this version posted January 30, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

17 # Abstract

18 Spontaneous and selection-pressure-driven evolution of SARS-CoV-2 has started to pose more
19 challenges to controlling the pandemic. Here, we first investigated cross-species receptor
20 usage of multiple SARS-CoV-2 variants that emerged during the pandemic. We found that, in
21 contrast to an early isolate WHU01, the circulating variants B.1.1.7/501Y.V1,
22 B.1.351/501Y.V2, and P.1/501Y.V3 were able to use rat and mouse Ace2 orthologs as entry
23 receptors, suggesting that rats and mice might be able to harbor and spread these variants. We
24 then evaluated in vitro sensitivity of these variants to three therapeutic antibodies in clinics
25 (etesevimab/LY-CoV016, casirivimab/REGN10933, and imdevimab/REGN10987) and an
26 ACE2-Ig variant we developed recently. We found that all the tested SARS-CoV-2 variants
27 showed reduced sensitivity to at least one of the tested antibodies but slightly increased
28 sensitivity to the ACE2-Ig protein. These data demonstrate that the ACE2-Ig is a good drug
29 candidate against SARS-CoV-2 variants that emerge over the course of the pandemic.

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.27.428353; this version posted January 30, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

30 # Introduction

31 The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the etiological agent of
32 the ongoing coronavirus disease 2019 (COVID-19) pandemic, has already caused about 100
33 million confirmed infections and over 2 million documented deaths across 224 countries,
34 according to World Health Organization's online updates
35 (https://www.who.int/emergencies/diseases/novel-coronavirus-2019). The pandemic has
36 triggered unprecedentedly extensive worldwide efforts to develop countermeasures against
37 COVID-19, and a number of encouraging progresses have been achieved in developing
38 prophylactic vaccines and antibody therapeutics[1-9]. So far, there are at least six prophylactic
39 COVID-19 vaccines that have been approved by different countries for clinical use. These
40 include two mRNA vaccines (Pfizer-BioNTech, US; Moderna, US)[1,2], two inactivated vaccines
41 (Sinopharm, China; Sinovac, China)[3-5], and two adenoviral vectored vaccines (Sputnik V,
42 Russia; AstraZeneca-Oxford, UK)[6,7]. There are also some convalescent patient-derived
43 antibodies that have been approved for clinical use, such as Regeneron's antibody cocktail
44 consisting of casirivimab (REGN10933) and imdevimab (REGN10987)[8].

45 SARS-CoV-2 is a betacoronavirus that has broad host ranges[10-13]. We recently found that
46 SARS-CoV-2 can use human ACE2 and a wide range of animal-Ace2 orthologs, but not rat or
47 mouse Ace2, for cell entry [11]. However, a single amino-acid change within the Spike receptor-
48 binding domain (RBD; Q498H, Q498Y, or N501Y) could be sufficient to confer SARS-CoV-
49 2 the ability to utilize mouse Ace2[ref.14-17]. SARS-CoV-2 is a single-stranded RNA virus with
50 moderate mutation and recombination frequencies[18,19]. Spontaneous and selection-pressure-
51 driven mutations, especially those within the Spike RBD region, might alter cross-species
52 receptor usage by SARS-CoV-2, or compromise the efficacy of neutralizing antibodies and
53 vaccines based on the Spike sequence of early SARS-CoV-2 strains, posing additional
54 challenges to controlling the pandemic. Therefore, in this study, we investigated cross-species

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.27.428353; this version posted January 30, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

55 receptor usage, as well as neutralization sensitivity, of multiple SARS-CoV-2 variants that
56 emerged during the pandemic.

57 ## Results

58 Spike RBD is the major determinant of viral receptor usage and neutralization sensitivity, we
59 thus primarily focused on RBD mutations found in different variants, especially the Variants
60 of Concern (VOC) that emerged from the UK (VOC202012/01, also known as B.1.1.7 or
61 501Y.V1), South Africa (VOC202012/02, also known as 501Y.V2 or B.1.351), and Brazil
62 (VOC202101/02, also known as P.1 or 501Y.V3), respectively. These include an N439K
63 mutation from the circulating variant B.1.141[ref.20], an N501Y mutation from B.1.1.7/501Y.V1
64 [21,22], E484K and N501Y mutations from B.1.351/501Y.V2[23] and P.1/501Y.V3 [24], and a Y453F
65 mutation from two mink-associated variants that emerged in Netherlands and Denmark mink
66 farms[25-27] (Figure 1). We further included a Spike Y453F-Q498H double mutant that has not
67 been detected in patient samples yet, but could evolve from the early isolate WHU01 through
68 adopting only two nucleotide mutations, or from the mink-associated Y453F variant through
69 adopting a single nucleotide mutation. To evaluate Spike protein-mediated viral entry of the
70 above-mentioned variants, we constructed Spike expression plasmids for these variants and
71 produced luciferase reporter retroviruses pseudotyped with one of these different Spike variants.
72 As rats and mice are rodent species that have huge population size and could have close
73 contacts with human and domestic animal populations, it's important to monitor SARS-CoV-
74 2's ability to utilize rat and mouse Ace2 as receptor. We therefore used the Spike variant-
75 pseudotyped reporter viruses to infect 293T cells transfected with an expression plasmid of
76 human, rat, or mouse ACE2 ortholog. Parallel infection experiments using 293T cells
77 transfected with an empty vector plasmid were included as controls. Consistent with our
78 previous report, the early strain WHU01 only infected 293T cells expressing human ACE2, but

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.27.428353; this version posted January 30, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

79 not rat or mouse Ace2 (Figure 2A). Though the variant carrying Δ(69-70)-N439K mutations
80 and the mink-associated variant carrying Δ(69-70)-Y453F mutations did so too (Figures 2B
81 and C), the mink-associated variant that carries Δ(69-70)-Y453F-F486L-N501T-M1229I
82 mutations also infected 293T cells expressing mouse Ace2, albeit with much lower efficiency
83 than infecting cells expressing human ACE2 (Figure 2D). It is of note that, in addition to the
84 Y453F-Q498H variant, all the variants that carry an N501Y mutation efficiently utilized all the
85 three Ace2 orthologs (Figures 2E-H). The variant that carries K417N-E484K-N501Y
86 mutations utilized rat Ace2 even more efficiently than using human ACE2 (Figure 2G). These
87 data suggest that the circulating SARS-CoV-2 variants B.1.1.7/501Y.V1, B.1.351/501Y.V2,
88 and P.1/501Y.V3 may be able to efficiently infect mice and rats, adding one more concerning
89 factor to these VOC strains.

90 We then performed neutralization assays to evaluate sensitivity of these SARS-CoV-2 variants
91 to three therapeutic antibodies in clinics (etesevimab/LY-CoV016, casirivimab/REGN10933,
92 and imdevimab/REGN10987)[8,9] and an ACE2-Ig variant we developed recently[11]. When
93 tested against the early strain WHU01, the Regeneron antibody casirivimab neutralized the
94 virus with the best potency, about 3-fold more potent than our ACE2-Ig, and about 20-fold
95 more potent than the monoclonal antibody etesevimab (Figure 3A and Table 1). When tested
96 against the variants, all the antibodies in general showed decreased sensitivity to at least one
97 variant (Figures 3B-H). Specifically, variants carrying an N501Y or N501T mutation were
98 found to be highly resistant to etesevimab (Figures 3D-G). Variants carrying a Y453F mutation
99 were found to be highly resistant to casirivimab (Figures 3C and D). It is of note that the Δ(69-
100 70)-Y453F-F486L-N501T-M1229I mutations carried by mink-associated variants and the
101 K417N-E484K-N501Y mutations carried by the South Africa VOC strain B.1.351/501Y.V2
102 made the virus highly resistant to both etesevimab and casirivimab, adding another concerning
103 factor to these two variants (Figures 3D and G). In addition, the Spike Y453F-Q498H double

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.27.428353; this version posted January 30, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

104 mutation made the virus partially resistant to etesevimab and highly resistant to both of the
105 Regeneron antibodies, casirivimab and imdevimab (Figure 3H). Consistent with these
106 neutralization data, structural data analysis revealed contact of etesevimab with RBD residues
107 K417 and N501, casirivimab with RBD residues Y453, E484, and F486, and imdevimab with
108 RBD residues N439 and Q498 (Figures 3I-K). On the other hand, when tested against the
109 variants, ACE2-Ig protein did not lose efficacy at all. Rather, it seems that ACE2-Ig gains
110 potency along with the course of the pandemic, consistent with the neutralizing mechanism of
111 this protein (Figures 3A-G and Table 1).

112 ## Discussion

113 The Spike protein is the major host-range determinant and neutralizing antigen of SARS-CoV-
114 2. It has been undergoing a number of mutations since the outbreak of the COVID-19 epidemic
115 in the end of 2019[ref.10,20-25,27-29]. With more and more SARS-CoV-2 variants being identified
116 to carry diverse Spike mutations within the RBD region, especially the Variants of Concern
117 initially identified in the UK (B.1.1.7 lineage)[21,22], South Africa (501Y.V2 lineage)[23], and
118 Brazil (P.1 lineage)[24], it is critical to understand whether the host range and neutralization
119 sensitivity of these variants have changed. In this study, we first investigated cross-species
120 receptor usage of an early SARS-CoV-2 isolate and multiple SARS-CoV-2 variants that
121 emerged during the pandemic (Figure 2). A mink-associated variant that carries Δ(69-70)-
122 Y453F-F486L-N501T-M1229I mutations was found also able to utilize mouse Ace2. In
123 addition, all the variants that carry an N501Y mutation, a shared feature of the UK, South
124 Africa, and Brazil VOC strains, were found able to efficiently use rat and mouse Ace2
125 orthologs for entry (Figures 2E-G), consistent with the identification of N501Y as the mouse-
126 susceptibility determinant of a mouse-adapted SARS-CoV-2 strain[16]. Moreover, the K417N-
127 E484K-N501Y mutations found in the South Africa variant 501Y.V2 even enable the virus to

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.27.428353; this version posted January 30, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

128 utilize rat Ace2 more efficiently than using human ACE2. These data suggest that rats and
129 mice may be able to harbor and spread these variants. Rats and mice are vaccine-inaccessible
130 rodent species that have large population size and could have close contacts with both human
131 and domestic animal populations. It is possible that the N501Y-associated circulating variants
132 might have emerged as an adaptation to rats before being passed back to humans. It is therefore
133 critical to closely monitor cross-species transmission and evolution of these SARS-CoV-2
134 variants.

135 We also evaluated sensitivity of these variants to three therapeutic antibodies in clinics
136 (etesevimab/LY-CoV016, casirivimab/REGN10933, and imdevimab/REGN10987) and an
137 ACE2-Ig variant we developed recently (Figure 3 and Table 1). We found that all the tested
138 Spike mutations were able to confer resistance to at least one of the three tested antibodies. A
139 mink-associated strain and the South Africa VOC strain 501Y.V2 are highly resistant to both
140 etesevimab and casirivimab. The South Africa VOC strain 501Y.V2 and the Brazil VOC strain
141 P.1/501Y.V3 are identical within the RBD region except that 501Y.V2 RBD adopted a K417N
142 mutation and P.1/501Y.V3 RBD adopted a K417T mutation, it's therefore likely that the Brazil
143 VOC strain P.1/501Y.V3 is also highly resistant to both etesevimab and casirivimab. More
144 importantly, two simple amino-acid mutations in the Spike, Y453F-Q498H, made the virus
145 partially resistant to etesevimab and highly resistant to both casirivimab and imdevimab, the
146 two monoclonal antibodies constituting Regeneron's FDA-approved antibody cocktail. The
147 Y453F-Q498H double mutant has not been detected in patient samples yet. However, it could
148 easily evolve from the early isolate WHU01 through adopting only two nucleotide mutations,
149 or evolve from mink-associated Y453F variant or mouse-adapted Q498H variant through
150 adopting a single nucleotide mutation. Potential cross-species transmission of SARS-CoV-2
151 variants, such as the South Africa VOC strain 501Y.V2, from humans to rats or mice, to
152 domestic animals, and then back to humans, might accelerate the emergence of new variants.

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.27.428353; this version posted January 30, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

153 Immune pressures, such as clinical applications of non-broadly neutralizing antibodies or
154 vaccines, or patients' immune reactions to SARS-CoV-2 infections, are also prone to drive
155 more and more immune-evasion mutations. These mutation-driving factors altogether will
156 likely accelerate the loss of efficacy of the therapeutic antibodies and possibly the vaccines in
157 clinics, posing additional challenges to the ongoing devastating pandemic. Indeed, during
158 preparation of this manuscript, multiple preprint reports have shown that the 501Y.V2 variant
159 significantly reduced the potency of vaccine-induced neutralization antibodies[30-32]. It is
160 therefore urgent to develop broadly anti-SARS-CoV-2 therapeutics or vaccines.

161 Soluble ACE2 protein, which neutralizes SARS-CoV-2 infection through competitive blocking
162 the interaction between viral Spike protein and cell-surface receptor ACE2, represents a good
163 candidate that will be hardly escaped by SARS-CoV-2 variants. It could also be a good
164 alternative anti-SARS-CoV-2 agent to the populations who are reluctant to take, not responsive
165 to, or don't have access to any prophylactic vaccines. Recently we have developed a panel of
166 ACE2-Ig variants that potently neutralize SARS-CoV-2 early isolate at picomolar range, and
167 have demonstrated that the protein is broadly effective against four distinct coronaviruses,
168 suggesting that the protein could be used to protect from SARS-CoV-2 and some other SARS-
169 CoV-2-like viruses that might spillover into humans in the future[11]. In this study, we
170 demonstrated that the ACE2-Ig protein is broadly effective against all the tested SARS-CoV-2
171 variants, including the ones simultaneously resistant to two or three neutralizing antibodies.
172 Perhaps more importantly, it seems that SARS-CoV-2 evolution tend to reduce sensitivity to
173 neutralizing antibodies while increasing sensitivity to ACE2-Ig (Figure 3 and Table 1). These
174 data suggest that ACE2 is still likely an essential receptor for SARS-CoV-2, and that ACE2-Ig
175 could be used to treat and prevent infection of any SARS-CoV-2 variant that emerges over the
176 course of the pandemic. Animal studies to test and optimize pharmacokinetics, as well as *in*
177 *vivo* anti-SARS-CoV-2 efficacy, of the ACE2-Ig protein are ongoing.

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.27.428353; this version posted January 30, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

178 ## Methods

179 Methods and associated references are provided below.

180 ## Acknowlegements

181 We thank Dr. Yu J. Cao (School of Chemical Biology and Biotechnology, Peking University
182 Shenzhen Graduate School, Shenzhen, China) for generously providing the 293F cells used in
183 this study for the production of ACE2-Ig protein and SARS-CoV-2 antibodies. We thank Dr.
184 Michael D. Alpert (Emmune, Inc., USA) for sharing useful comments on this manuscript.

185 This work was supported by Guangdong Provincial Department of Science & Technology
186 COVID-19 Contingency Funds (2020B1111340063, G.Z.), Shenzhen Bay Laboratory Startup
187 Funds (21230041, G.Z.), and Major Program of Shenzhen Bay Laboratory (S201101001-2,
188 G.Z.).

189 ## Contributions

190 G.Z. conceived and designed this study. W.Y., Y.W., D.M., X.T., H.W., and Y.L. generated
191 all experimental materials. W.Y. and Y.L. performed all experiments, acquired and analyzed
192 all data. C.L. and H.L. contributed key resources. G.Z. wrote the manuscript.

193 ## Competing interests

194 Shenzhen Bay Laboratory has filed a PCT patent application for ACE2-Ig variants.

195 ## References

196 1 Polack, F. P. *et al.* Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine. *N*
197 *Engl J Med* **383**, 2603-2615, (2020).

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.27.428353; this version posted January 30, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

198 2 Baden, L. R. *et al.* Efficacy and Safety of the mRNA-1273 SARS-CoV-2 Vaccine. *N*
199 *Engl J Med*, (2020).
200 3 Wang, H. *et al.* Development of an Inactivated Vaccine Candidate, BBIBP-CorV, with
201 Potent Protection against SARS-CoV-2. *Cell* **182**, 713-721 e719, (2020).
202 4 Xia, S. *et al.* Safety and immunogenicity of an inactivated SARS-CoV-2 vaccine,
203 BBIBP-CorV: a randomised, double-blind, placebo-controlled, phase 1/2 trial. *Lancet*
204 *Infect Dis* **21**, 39-51, (2021).
205 5 Gao, Q. *et al.* Development of an inactivated vaccine candidate for SARS-CoV-2.
206 *Science* **369**, 77-81, (2020).
207 6 Logunov, D. Y. *et al.* Safety and immunogenicity of an rAd26 and rAd5 vector-based
208 heterologous prime-boost COVID-19 vaccine in two formulations: two open, non-
209 randomised phase 1/2 studies from Russia. *Lancet* **396**, 887-897, (2020).
210 7 Voysey, M. *et al.* Safety and efficacy of the ChAdOx1 nCoV-19 vaccine (AZD1222)
211 against SARS-CoV-2: an interim analysis of four randomised controlled trials in Brazil,
212 South Africa, and the UK. *Lancet* **397**, 99-111, (2021).
213 8 Baum, A. *et al.* REGN-COV2 antibodies prevent and treat SARS-CoV-2 infection in
214 rhesus macaques and hamsters. *Science* **370**, 1110-1115, (2020).
215 9 Shi, R. *et al.* A human neutralizing antibody targets the receptor-binding site of SARS-
216 CoV-2. *Nature* **584**, 120-124, (2020).
217 10 Zhou, P. *et al.* A pneumonia outbreak associated with a new coronavirus of probable
218 bat origin. *Nature* **579**, 270-273, (2020).
219 11 Li, Y. *et al.* SARS-CoV-2 and Three Related Coronaviruses Utilize Multiple ACE2
220 Orthologs and Are Potently Blocked by an Improved ACE2-Ig. *J Virol* **94**, (2020).
221 12 Shi, J. *et al.* Susceptibility of ferrets, cats, dogs, and other domesticated animals to
222 SARS-coronavirus 2. *Science* **368**, 1016-1020, (2020).

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.27.428353; this version posted January 30, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

223 13 Cui, J., Li, F. & Shi, Z. L. Origin and evolution of pathogenic coronaviruses. *Nat Rev*
224 *Microbiol* **17**, 181-192, (2019).
225 14 Wang, J. *et al.* Mouse-adapted SARS-CoV-2 replicates efficiently in the upper and
226 lower respiratory tract of BALB/c and C57BL/6J mice. *Protein Cell* **11**, 776-782,
227 (2020).
228 15 Dinnon, K. H., 3rd *et al.* A mouse-adapted model of SARS-CoV-2 to test COVID-19
229 countermeasures. *Nature* **586**, 560-566, (2020).
230 16 Gu, H. *et al.* Adaptation of SARS-CoV-2 in BALB/c mice for testing vaccine efficacy.
231 *Science* **369**, 1603-1607, (2020).
232 17 Li, Y. *et al.* Single amino-acid change in mouse Ace2 or viral spike enables SARS-
233 CoV-2 to utilize mouse Ace2. (2021. manuscript submitted).
234 18 Kaushal, N. *et al.* Mutational Frequencies of SARS-CoV-2 Genome during the
235 Beginning Months of the Outbreak in USA. *Pathogens* **9**, (2020).
236 19 Gribble, J. *et al.* The coronavirus proofreading exoribonuclease mediates extensive
237 viral recombination. *PLoS Pathog* **17**, e1009226, (2021).
238 20 Thomson, E. C. *et al.* The circulating SARS-CoV-2 spike variant N439K maintains
239 fitness while evading antibody-mediated immunity. *bioRxiv*, (2020).
240 21 Davies, N. G. *et al.* Estimated transmissibility and severity of novel SARS-CoV-2
241 Variant of Concern 202012/01 in England. *medRxiv*, (2020).
242 22 Laydon, D. J. *et al.* Transmission of SARS-CoV-2 Lineage B.1.1.7 in England: Insights
243 from linking epidemiological and genetic data. *MedRxiv*, (2020).
244 23 Tegally, H. *et al.* Emergence and rapid spread of a new severe acute respiratory
245 syndrome-related coronavirus 2 (SARS-CoV-2) lineage with multiple spike mutations
246 in South Africa. *medRxiv*, (2020).

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.27.428353; this version posted January 30, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

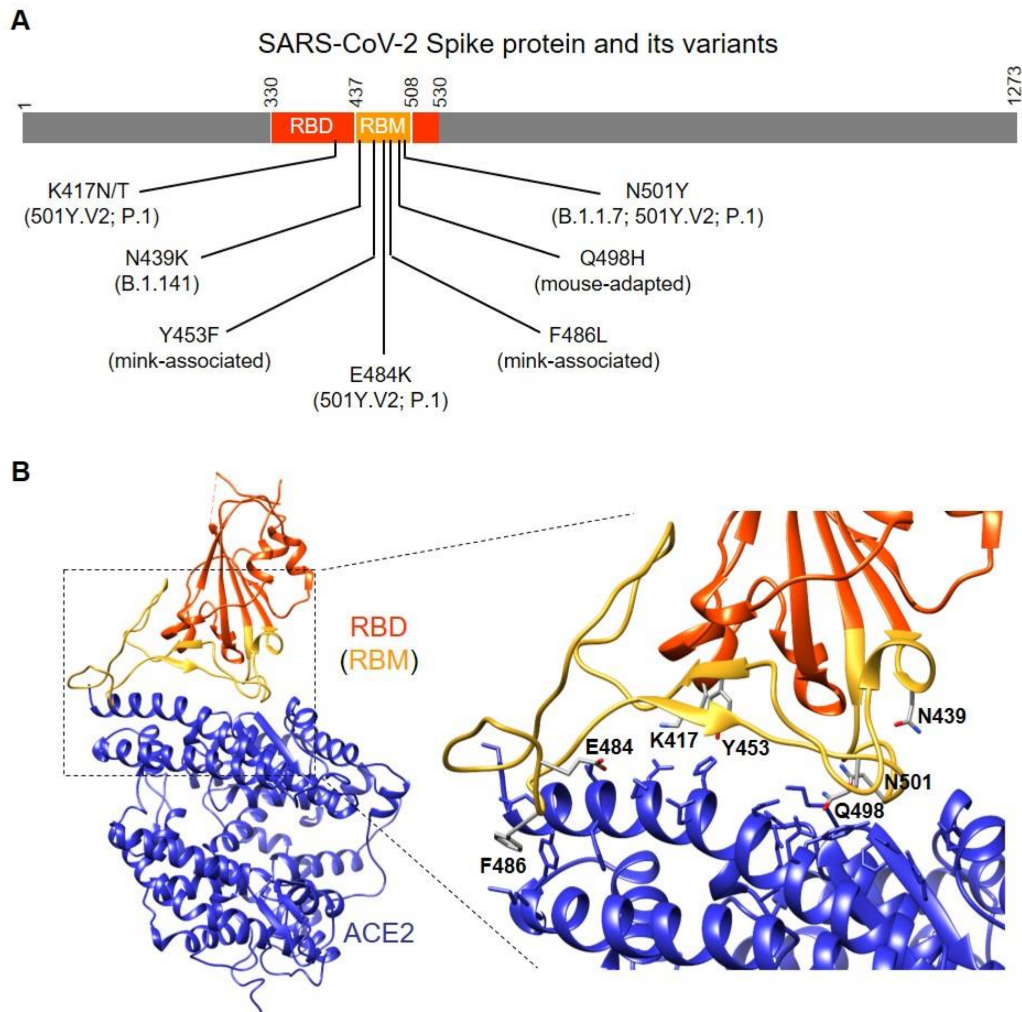
247 24 Diseases, J. N. I. o. I. Brief report: New Variant Strain of SARS-CoV-2 Identified in
248 Travelers from Brazil. doi:https://www.niid.go.jp/niid/images/epi/corona/covid19-33-
249 en-210112, (2021).
250 25 Oude Munnink, B. B. *et al.* Transmission of SARS-CoV-2 on mink farms between
251 humans and mink and back to humans. *Science* **371**, 172-177, (2021).
252 26 Welkers, M. R. A., Han, A. X., Reusken, C. B. E. M. & Eggink, D. Possible host-
253 adaptation of SARS-CoV-2 due to improved ACE2 receptor binding in mink. *Virus*
254 *evolution*, (2020).
255 27 Elaswad, A., Fawzy, M., Basiouni, S. & Shehata, A. A. Mutational spectra of SARS-
256 CoV-2 isolated from animals. *PeerJ* **8**, e10609, (2020).
257 28 Kemp, S. A. *et al.* Neutralising antibodies drive Spike mediated SARS-CoV-2 evasion.
258 *MedRxiv*, (2020).
259 29 Li, Q. *et al.* The Impact of Mutations in SARS-CoV-2 Spike on Viral Infectivity and
260 Antigenicity. *Cell* **182**, 1284-1294 e1289, (2020).
261 30 Wu, K. *et al.* mRNA-1273 vaccine induces neutralizing antibodies against spike
262 mutants from global SARS-CoV-2 variants. *bioRxiv*, (2021).
263 31 Wang, Z. *et al.* mRNA vaccine-elicited antibodies to SARS-CoV-2 and circulating
264 variants. *bioRxiv*, (2021).
265 32 Rees-Spear, C. *et al.* The impact of Spike mutations on SARS-1 CoV-2 neutralization.
266 *bioRxiv*, (2021).

267

268

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.27.428353; this version posted January 30, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

269 # Figures and legends

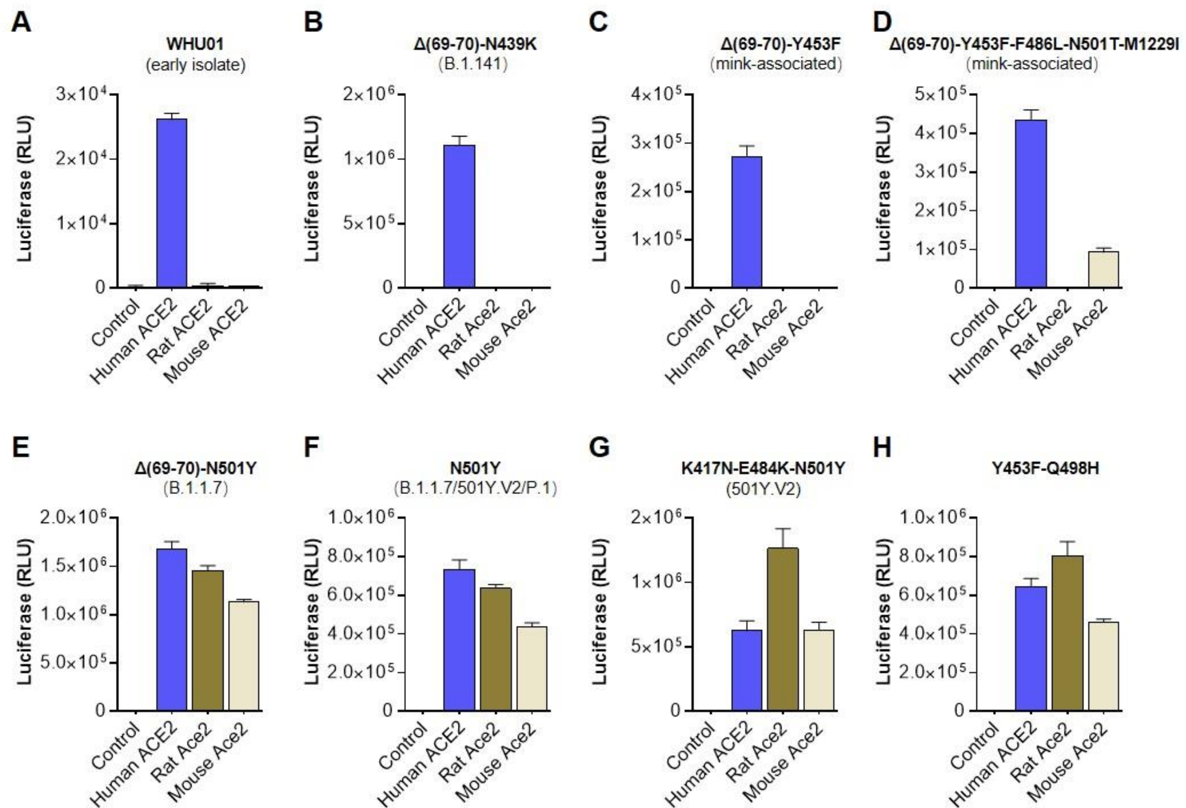


270

271 **Figure 1. SARS-CoV-2 Spike variants.** (**A**) SARS-CoV-2 Spike amino acid numbers are
272 indicated. The receptor binding domain (RBD) is in red and the receptor binding motif (RBM)
273 is in yellow. Spike mutations investigated in this study are indicated, with the associated
274 SARS-CoV-2 variant number shown in parentheses. (**B**) Interactions between SARS-CoV-2
275 RBD (red) and ACE2 (PDB code 6M0J). The ACE2 residues in less than 4 Å from RBD atoms
276 are shown. SARS-CoV-2 variant-associated RBD mutations that were investigated in
277 following study are shown and labelled, including residues K417N, N439K, Y453F, E484K,
278 F486L, Q498H, and N501Y.

279

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.27.428353; this version posted January 30, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

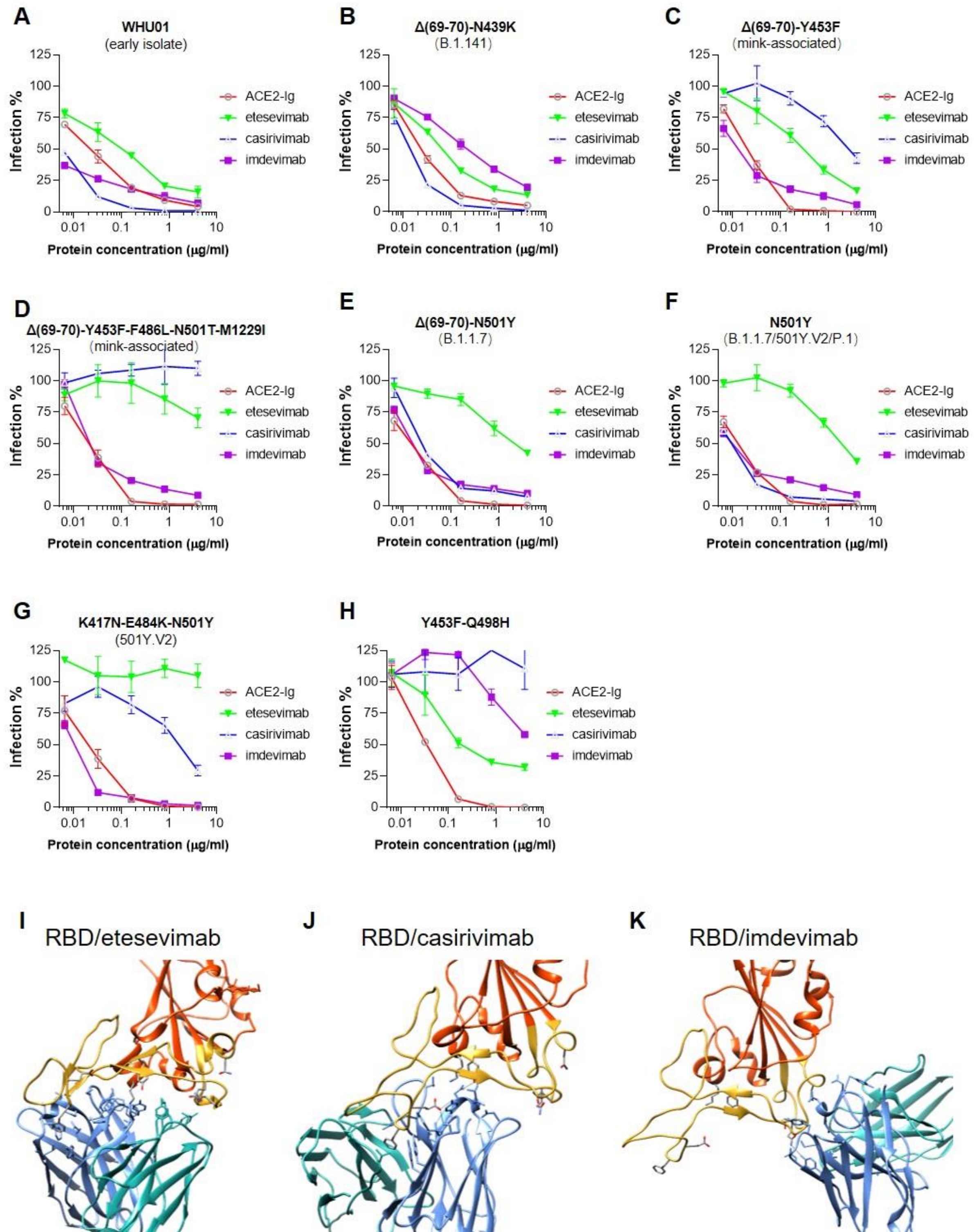


280

281 **Figure 2. Cross-species receptor usage of multiple SARS-CoV-2 variants that emerged**
282 **during the pandemic.** 293T cells in 96-well plates were transfected with 60 ng of a vector
283 control plasmid, or a plasmid expressing an ACE2 ortholog of human, rat, or mouse. Cells
284 were then infected with reporter retroviruses pseudotyped with the indicated SARS-CoV-2
285 Spike variants. ACE2 ortholog-mediated viral entry was measured by luciferase reporter
286 expression at 48 hours post infection. Data shown are representative of two or three
287 experiments independently performed by two different people with similar results, and data
288 points represent mean ± s.d. of three biological replicates.

289

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.27.428353; this version posted January 30, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.



290

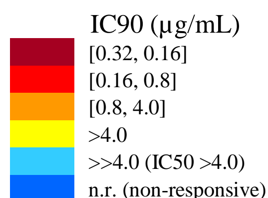
291 **Figure 3. Sensitivity of SARS-CoV-2 variants to neutralizing antibodies and ACE2-Ig.**
292 (**A-H**) HeLa-hACE2 cells in 96-well plates were infected with SARS-CoV-2 Spike variant-
293 pseudotyped retroviruses in the presence of an ACE2-Ig variant we developed recently or a
294 clinical-stage anti-SARS-CoV-2 antibody (etesevimab, casirivimab, or imdevimab) at the

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.27.428353; this version posted January 30, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

indicated concentrations. Etesevimab was originally named as CB6[ref.9], casirivimab as REGN10933[ref.8], and imdevimab as REGN10987[ref.8]. Viral entry was measured by luciferase reporter expression at 48 hours post infection. Luminescence values observed at each concentration were divided by the values observed at concentration zero to calculate percentage-of-infection (Infection%) values. Data shown are representative of two experiments independently performed by two different people with similar results, and data points represent mean ± s.d. of three biological replicates. Estimated IC50 and IC90 values for each protein are summarized in Table 1. (**I-K**) Interactions between SARS-CoV-2 RBD and monoclonal antibody etesevimab (**I**; PDB code 7C01), casirivimab (**J**; PDB code 6XDG), and imdevimab (**K**; PDB code 6XDG). The RBD is shown in red and yellow, and antibodies are shown in blue and green. The antibody residues in less than 4 Å from RBD atoms and SARS-CoV-2 variant-associated RBD mutations are shown. SARS-CoV-2 variant-associated RBD mutations that were investigated in this study are shown, including residues K417N, N439K, Y453F, E484K, F486L, Q498H, and N501Y, as shown in Figure 1B.

Table 1. IC50 (in parentheses) and IC90 values of the tested proteins (μg/mL)

	WHU01	Δ(69-70)-N439K	Δ(69-70)-Y453F	Δ(69-70)-Y453F-F486L-N501T-M1229I	Δ(69-70)-N501Y	N501Y	K417N-E484K-N501Y	Y453F-Q498H
etesevimab	>4.0 (0.1)	>4.0 (0.065)	>4.0 (0.3)	>>4.0 (>4.0)	>4.0 (2.2)	>4.0 (2.0)	n.r.	>4.0 (0.18)
casirivimab	0.045 (<0.0064)	0.1 (0.015)	>4.0 (1.6)	n.r.	1.5 (0.25)	0.11 (0.009)	>4.0 (1.7)	n.r.
imdevimab	1.5 (<0.0064)	>4.0 (0.22)	1.5 (0.015)	2.5 (0.02)	3.5 (0.16)	3 (0.1)	0.06 (0.01)	>>4.0 (>4.0)
ACE2-Ig	0.7 (0.022)	0.45 (0.025)	0.12 (0.02)	0.12 (0.02)	0.12 (0.015)	0.11 (0.013)	0.15 (0.02)	0.15 (0.035)



bioRxiv preprint doi: https://doi.org/10.1101/2021.01.27.428353; this version posted January 30, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

314 # Materials and methods

315 ## Cells

316 293T and HeLa cells were kindly provided by Stem Cell Bank, Chinese Academy of Sciences,
317 confirmed mycoplasma-free by the provider, and maintained in Dulbecco's Modified Eagle
318 Medium (DMEM, Life Technologies) at 37 °C in a 5% CO_2-humidified incubator. Growth
319 medium was supplemented with 2 mM Glutamax-I (Gibco, Cat. No. 35050061), 100 μM non-
320 essential amino acids (Gibco, Cat. No. 11140050), 100 U/mL penicillin and 100 μg/mL
321 streptomycin (Gibco, Cat. No. 15140122), and 10% FBS (Gibco, Cat. No. 10099141C). HeLa-
322 based stable cells expressing human ACE2 (HeLa-hACE2) were maintained under the same
323 culture condition as HeLa, except that 3 μg/mL of puromycin was added to the growth medium.
324 293F cells for the production of ACE2-Ig protein and SARS-CoV-2 antibodies were
325 generously provided by Dr. Yu J. Cao (School of Chemical Biology and Biotechnology, Peking
326 University Shenzhen Graduate School) and maintained in SMM 293-TII serum-free medium
327 (Sino Biological, Cat. No. M293TII) at 37 °C, 8% CO_2, in a shaker incubator at 125 rpm.

328 ## Plasmids

329 DNA fragments encoding Spike proteins of SARS-CoV-2 WHU01 (GenBank: MN988668.1)
330 was synthesized by the Beijing Genomic Institute (BGI, China) and then cloned into
331 pcDNA3.1(+) plasmid between EcoRI and XhoI restriction sites. Plasmids encoding SARS-
332 CoV-2 Spike variants were generated according the in-fusion cloning protocol. To facilitate
333 SARS-CoV-2 pseudovirus production, Spike sequences for WHU01 and all the variants
334 investigated in this study all contain a furin-cleavage site mutation (ΔPRRA). We had shown
335 in our previous study that the ΔPRRA mutation does not affect SARS-CoV-2 cross-species
336 receptor usage or neutralization sensitivity[11]. Plasmids encoding the ACE2-Ig variant were
337 generated in our previous study[11]. DNA fragments encoding heavy and light chains of SARS-

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.27.428353; this version posted January 30, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

338 CoV-2 antibodies (etesevimab, casirivimab, and imdevimab) were synthesized by Sangon
339 Biotech (Shanghai, China) and then cloned into a pCAGGS plasmid. The retroviral reporter
340 plasmids encoding a Gaussia luciferase reporter gene were constructed by cloning the reporter
341 genes into the pQCXIP plasmid (Clontech). DNA fragments encoding C-terminally S-tagged
342 ACE2 orthologs were synthesized in pUC57 backbone plasmid by Sangon Biotech (Shanghai,
343 China). These fragments were then cloned into pQCXIP plasmid (Clontech) between SbfI and
344 NotI restriction sites.

345 **Production and Purification of ACE2-Ig protein and SARS-CoV-2 antibodies**

346 293F cells at the density of 6×10^5 cells/mL were seeded into 100 mL SMM 293-TII serum-
347 free medium (Sino Biological, Cat. No. M293TII) one day before transfection. Cells were then
348 transfected with 100 μg plasmid in complex with 250 μg PEI MAX 4000 (Polysciences, Inc,
349 Cat. No. 24765-1). Cell culture supernatants were collected at 48 to 72 hours post transfection.
350 Human IgG1 Fc-containing proteins were purified using Protein A Sepharose CL-4B (GE
351 Healthcare, Cat. No. 17-0780-01), eluted with 0.1 M citric acid at pH 4.5 and neutralized with
352 1 M Tris-HCl at pH 9.0. Buffers were then exchanged to PBS and proteins were concentrated
353 by 30 kDa cut-off Amicon Ultra-15 Centrifugal Filter Units (Millipore, Cat. No. UFC903096).

354 **Production of reporter retroviruses pseudotyped with SARS-CoV-2 Spike variants**

355 MLV retroviral vector-based SARS-CoV-2 Spike pseudotypes were produced according to our
356 previous study[11], with minor changes. In brief, 293T cells were seeded at 30% density in 150
357 mm dish at 12-15 hours before transfection. Cells were then transfected with 67.5 μg of
358 polyethylenimine (PEI) Max 40,000 (Polysciences, Inc, Cat. No. 24765-1) in complex with
359 3.15 μg of plasmid encoding a Spike variant, 15.75 μg of plasmid encoding murine leukemia
360 virus (MLV) Gag and Pol proteins, and 15.75 μg of a pQCXIP-based luciferase reporter
361 plasmid. Eight hours after transfection, cell culture medium was refreshed and changed to

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.27.428353; this version posted January 30, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

362 growth medium containing 2% FBS (Gibco, Cat. No. 10099141C) and 25 mM HEPES (Gibco,
363 Cat. No. 15630080). Cell culture supernatants were collected at 36-48 hours post transfection,
364 spun down at 3000×g for 10 min, and filtered through 0.45 μm filter units to remove cell debris.
365 SARS-CoV-2 Spike-pseudotyped viruses were then concentrated 10 times at 2000×g using 100
366 kDa cut-off Amicon Ultra-15 Centrifugal Filter Units (Millipore. Cat. No. UFC910024).

367 **SARS-CoV-2 pseudovirus infection of 293T cells expressing ACE2 orthologs**

368 Pseudovirus infection assay was performed according to our previous study[11]. In brief, 293T
369 cells were seeded at 20% density in poly-lysine pre-coated 96-well plates 12-15 hours before
370 transfection. Cells in each well were then transfected with 0.2 μL of lipofectamine 2000 (Life
371 Technologies, Cat. No. 11668019) in complex with 60 ng of a vector control plasmid or a
372 plasmid encoding an ACE2 ortholog. Cell culture medium was refreshed at 12 hours post
373 transfection. Additional 18 hours later, cells in each well were infected with 10 μL of SARS-
374 CoV-2 pseudovirus (10×concentrated) diluted in 100 μL of culture medium containing 2%
375 FBS (Gibco, Cat. No. 10099141C). Culture medium was refreshed at 2 hours post pseudovirus
376 infection and the medium was refreshed every 12 hours. Cell culture supernatants were
377 collected and subjected to a Gaussia luciferase assay at 48 hours post infection.

378 **Gaussia luciferase luminescence flash assay**

379 To measure Gaussia luciferase expression, 20 μL of cell culture supernatant of each sample
380 and 100 μL of assay buffer containing 4 μM coelenterazine native (Biosynth Carbosynth, Cat.
381 No. C-7001) were added to one well of a 96-well black opaque assay plate (Corning, Cat. No.
382 3915), and measured with Centro LB 960 microplate luminometer (Berthold Technologies) for
383 0.1 second/well.

384 **SARS-CoV-2 pseudovirus neutralization assay**

bioRxiv preprint doi: https://doi.org/10.1101/2021.01.27.428353; this version posted January 30, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

385 Pseudovirus neutralization experiments were performed following our previous study[11]. In
386 brief, SARS-CoV-2 Spike variant-pseudotyped luciferase reporter viruses were pre-diluted in
387 DMEM (2% FBS, heat-inactivated) containing titrated amounts of the ACE2-Ig or one of the
388 three anti-SARS-CoV-2 antibodies. Virus-inhibitor mixtures were incubated at 37 °C for
389 30min, then added to HeLa-hACE2 cells in 96-well plates and incubated overnight at 37 °C.
390 Virus-inhibitor-containing supernatant was then removed and changed with 150 μL of fresh
391 DMEM (2% FBS) and incubated at 37 °C. Cell culture supernatants were collected for Gaussia
392 luciferase assay at 48 h post infection.

393 **Data collection and analysis**

394 All the experiments were repeated at least twice with two different people. GraphPad Prism
395 6.0 software was used for figure preparation and statistical analyses.

396 **Statistical analysis**

397 Data expressed as mean values ± s.d. Statistical analyses were performed using two-sided two-
398 sample Student's t-test using GraphPad Prism 6.0 software when applicable. Differences were
399 considered significant at $P < 0.01$.

400 **Data availability**

401 The study did not generate unique datasets or code. Our research resources, including methods,
402 plasmids, and protocols, are available upon reasonable request to qualified academic
403 investigators for noncommercial research purposes. All reagents developed in this study, such
404 as vector plasmids, as well as detailed methods, will be made available upon written request.