1 **Virological characteristics of the SARS-CoV-2 Omicron BA.2.75**

2

3 Akatsuki Saito[1-3,32], Tomokazu Tamura[4,32], Jiri Zahradnik[5,32], Sayaka Deguchi[6,32],

4 Koshiro Tabata[7,32], Izumi Kimura[8,32], Jumpei Ito[8,32], Hesham Nasser[9,10], Mako

5 Toyoda[11], Kayoko Nagata[12], Keiya Uriu[8,13], Yusuke Kosugi[8,13], Shigeru Fujita[8,13],

6 Daichi Yamasoba[8,14], Maya Shofa[1,2], MST Monira Begum[9], Yoshitaka Oda[15],

7 Rigel Suzuki[4], Hayato Ito[4], Naganori Nao[16], Lei Wang[15,17], Masumi Tsuda[15,17],

8 Kumiko Yoshimatsu[18], Yuki Yamamoto[19], Tetsuharu Nagamoto[19], Hiroyuki

9 Asakura[20], Mami Nagashima[20], Kenji Sadamasu[20], Kazuhisa Yoshimura[20],

10 Takamasa Ueno[11], Gideon Schreiber[5], Akifumi Takaori-Kondo[12], The Genotype

11 to Phenotype Japan (G2P-Japan) Consortium, Kotaro Shirakawa[12], Hirofumi

12 Sawa[7,16,21], Takashi Irie[22], Kazuo Takayama[6,23], Keita Matsuno[21,24,25], Shinya

13 Tanaka[15,17]*, Terumasa Ikeda[9]*, Takasuke Fukuhara[4,26]*, Kei Sato[8,13,27-31,33,34]*

14

15 [1] Department of Veterinary Science, Faculty of Agriculture, University of

16 Miyazaki, Miyazaki, Japan

17 [2] Graduate School of Medicine and Veterinary Medicine, University of Miyazaki,

18 Miyazaki, Japan

19 [3] Center for Animal Disease Control, University of Miyazaki, Miyazaki, Japan

20 [4] Department of Microbiology and Immunology, Faculty of Medicine, Hokkaido

21 University, Sapporo, Japan.

22 [5] Department of Biomolecular Sciences, Weizmann Institute of Science,

23 Rehovot, Israel

24 [6] Center for iPS Cell Research and Application (CiRA), Kyoto University, Kyoto,

25 Japan

26 [7] Division of Molecular Pathobiology, International Institute for Zoonosis Control,

27 Hokkaido University, Sapporo, Japan

28 [8] Division of Systems Virology, Department of Microbiology and Immunology,

29 The Institute of Medical Science, The University of Tokyo, Tokyo, Japan

30 [9] Division of Molecular Virology and Genetics, Joint Research Center for Human

31 Retrovirus infection, Kumamoto University, Kumamoto, Japan

32 [10] Department of Clinical Pathology, Faculty of Medicine, Suez Canal University,

33 Ismailia, Egypt

34 [11] Division of Infection and immunity, Joint Research Center for Human

35 Retrovirus infection, Kumamoto University, Japan

36 [12] Department of Hematology and Oncology, Graduate School of Medicine,

37 Kyoto University, Kyoto, Japan

38 [13] Graduate School of Medicine, The University of Tokyo, Tokyo, Japan

39 [14] Faculty of Medicine, Kobe University, Kobe, Japan

40 [15] Department of Cancer Pathology, Faculty of Medicine, Hokkaido University,

41 Sapporo, Japan

42   [16] Division of International Research Promotion, International Institute for

43   Zoonosis Control, Hokkaido University, Sapporo, Japan

44   [17] Institute for Chemical Reaction Design and Discovery (WPI-ICReDD),

45   Hokkaido University, Sapporo, Japan

46   [18] Institute for Genetic Medicine, Hokkaido University, Sapporo, Japan

47   [19] HiLung Inc., Kyoto, Japan

48   [20] Tokyo Metropolitan Institute of Public Health, Tokyo, Japan

49   [21] One Health Research Center, Hokkaido University, Sapporo, Japan.

50   [22] Institute of Biomedical and Health Sciences, Hiroshima University, Hiroshima,

51   Japan.

52   [23] AMED-CREST, Japan Agency for Medical Research and Development

53   (AMED), Tokyo, Japan

54   [24] International Collaboration Unit, International Institute for Zoonosis Control,

55   Hokkaido University, Sapporo, Japan

56   [25] Division of Risk Analysis and Management, International Institute for Zoonosis

57   Control, Hokkaido University, Sapporo, Japan

58   [26] Laboratory of Virus Control, Research Institute for Microbial Diseases, Osaka

59   University, Suita, Japan.

60

61   [27] International Research Center for Infectious Diseases, The Institute of Medical

62   Science, The University of Tokyo, Tokyo, Japan

63   [28] International Vaccine Design Center, The Institute of Medical Science, The

64   University of Tokyo, Tokyo, Japan

65   [29] Graduate School of Frontier Sciences, The University of Tokyo, Kashiwa,

66   Japan

67   [30] Collaboration Unit for Infection, Joint Research Center for Human Retrovirus

68   infection, Kumamoto University, Kumamoto, Japan

69   [31] CREST, Japan Science and Technology Agency, Kawaguchi, Japan

70   [32] These authors contributed equally

71   [33] Twitter: @SystemsVirology

72   [34] Lead Contact

73

74   *Corresponding authors:

75   tanaka@med.hokudai.ac.jp (Shinya Tanaka),

76   ikedat@kumamoto-u.ac.jp (Terumasa Ikeda),

77   fukut@pop.med.hokudai.ac.jp (Takasuke Fukuhara),

78   KeiSato@g.ecc.u-tokyo.ac.jp (Kei Sato)

79

2

83   cells", PCT/JP2016/059786, "Method of producing airway epithelial cells"). The
84   other authors declare that no competing interests exist.
85
86   **Short title**: Characteristics of SARS-CoV-2 BA.2.75 (37/50 characters)
87   **Keywords**: SARS-CoV-2; COVID-19; Omicron; BA.2.75; transmissibility;
88   immune resistance; antiviral drug resistance; pathogenicity

89   **Abstract** (150/150 words)

90   SARS-CoV-2 Omicron BA.2.75 emerged in May 2022. BA.2.75 is a BA.2
91   descendant but is phylogenetically different from BA.5, the currently
92   predominant BA.2 descendant. Here, we showed that the effective reproduction
93   number of BA.2.75 is greater than that of BA.5. While the sensitivity of BA.2.75
94   to vaccination- and BA.1/2 breakthrough infection-induced humoral immunity
95   was comparable to that of BA.2, the immunogenicity of BA.2.75 was different
96   from that of BA.2 and BA.5. Three clinically-available antiviral drugs were
97   effective against BA.2.75. BA.2.75 spike exhibited a profound higher affinity to
98   human ACE2 than BA.2 and BA.5 spikes. The fusogenicity, growth efficiency in
99   human alveolar epithelial cells, and intrinsic pathogenicity in hamsters of
100   BA.2.75 were comparable to those of BA.5 but were greater than those of BA.2.
101   Our multiscale investigations suggest that BA.2.75 acquired virological
102   properties independently of BA.5, and the potential risk of BA.2.75 to global
103   health is greater than that of BA.5.

Introduction

By the end of 2021, five SARS-CoV-2 variants-of-concern (VOCs) were classified by the WHO (WHO, 2022). These are the Alpha [also known as lineage B.1.1.7 based on the PANGO classification (https://cov-lineages.org); clade 20I based on the Nextstrain classification (https://nextstrain.org)], Beta (lineage B.1.351; clade 20H), Gamma (lineage P.1; clade 20J), Delta (lineages B.1.617.2 and AY; clades 21I and 21J), and Omicron (lineages B.1.1.529 and BA; clade 21K) variants. Since these five VOCs are phylogenetically unrelated to each other, SARS-CoV-2 evolution until the end of 2021 was posed by the antigenic shift. At the beginning of 2022, Omicron BA.1 variant (clade 21K) outcompeted the other variants and spread globally. Thereafter, BA.2 (clade 21L) and BA.4/5 (clades 22A and 22B) continuously emerged from South Africa, while BA.2.12.1 (clade 22C) emerged in the USA. As of the beginning of August 2022, Omicron BA.5 (clade 22B) is the most predominant SARS-CoV-2 variant in the world. In contrast to the five VOCs detected in 2021, the Omicron subvariants are phylogenetically related. Therefore, the evolution of SARS-CoV-2 Omicron subvariants since the end of 2021 is posed by the antigenic drift.

Newly emerging SARS-CoV-2 variants need to be carefully and rapidly assessed for a potential increase in their growth efficiency in the human population [i.e., relative effective reproduction number (R_e)], their evasion from antiviral immunity, and their pathogenicity. Resistance to antiviral humoral immunity can be mainly determined by substitutions in the spike (S) protein. For instance, Omicron BA.1 (Cao et al., 2021; Cele et al., 2021; Dejnirattisai et al., 2022; Garcia-Beltran et al., 2021; Liu et al., 2021; Meng et al., 2022; Planas et al., 2021; Takashita et al., 2022a; VanBlargan et al., 2022) , BA.2 (Bruel et al., 2022; Takashita et al., 2022b; Yamasoba et al., 2022c), and BA.5 (Arora et al., 2022; Cao et al., 2022; Gruell et al., 2022; Hachmann et al., 2022; Khan et al., 2022; Kimura et al., 2022c; Lyke et al., 2022; Qu et al., 2022; Tuekprakhon et al., 2022; Wang et al., 2022; Yamasoba *et al.*, 2022c) exhibit profound resistance to neutralizing antibodies induced by vaccination, natural SARS-CoV-2 infection, and therapeutic monoclonal antibodies. Particularly, newly spreading SARS-CoV-2 variants tend to be resistant to the humoral immunity induced by the infection with prior variant; for instance, BA.2 is resistant to BA.1 breakthrough infection sera (Qu *et al.*, 2022; Tuekprakhon *et al.*, 2022; Yamasoba et al., 2022b), and BA.5 is resistant to BA.2 breakthrough infection sera (Hachmann *et al.*, 2022; Kimura *et al.*, 2022c; Wang *et al.*, 2022). Therefore, acquiring immune resistance to previously dominant variant is a key factor in outcompeting previous variants, thereby obtaining relatively increased R_e compared to the previously dominant variant. Viral pathogenicity is also closely associated with the phenotype of viral S protein. Particularly, we have proposed

5

145 that the fusogenicity of viral S protein in *in vitro* cell cultures is associated with
146 viral pathogenicity *in vivo* (Kimura *et al.*, 2022c; Saito et al., 2022; Suzuki et al.,
147 2022; Yamasoba *et al.*, 2022b).

148   As mentioned above, major SARS-CoV-2 phenotypes can be defined
149 by the function of the viral S protein. SARS-CoV-2 S protein bears two major
150 domains, receptor binding domain (RBD) and N-terminal domain (NTD)
151 [reviewed in (Harvey et al., 2021; Mittal et al., 2022)]. RBD is crucial for the
152 binding to human angiotensin-converting enzyme 2 (ACE2) receptor for the cell
153 attachment and entry, and therefore, this domain has been considered a major
154 target for neutralizing antibodies to block viral infection [reviewed in (Barnes et
155 al., 2020; Harvey *et al.*, 2021; Jackson et al., 2022)]. On the other hand, NTD is
156 an immunodominant domain that can be recognized by antibodies, and some
157 antibodies targeting NTD potentially neutralize viral infection (Cerutti et al., 2021;
158 Chi et al., 2020; Liu et al., 2020; Lok, 2021; McCallum et al., 2021; Suryadevara
159 et al., 2021; Voss et al., 2021), despite our limited understanding of its virological
160 function.

161   The Omicron BA.2.75 variant, a new BA.2 subvariant, was first
162 detected in India in May 2022 (WHO, 2022). Because an early preliminary
163 investigation suggested the potential increase in the relative R_e value of BA.2.75
164 compared to BA.5 and the original BA.2 (GitHub, 2022), BA.2.75 has been
165 flagged as the most concerning variant that can potentially outcompete BA.5 and
166 be the next predominant variant in the future. In fact, on July 19, 2022, the WHO
167 classified this variant as a VOC lineage under monitoring (VOC-LUM) together
168 with the other BA.2 subvariants, including BA.5, which bear the substitution at
169 the L452 residue in their S proteins (WHO, 2022). On July 23, 2022, Nextstrain
170 (https://nextstrain.org) classified BA.2.75 as a new clade, 22D. Compared to the
171 BA.2 S, BA.4/5 bears four mutations in its S protein (Kimura *et al.*, 2022c;
172 Yamasoba *et al.*, 2022b). On the other hand, the majority of BA.2.75 S bears
173 nine mutations: K147E, W152R, F157L, I210V, and G257S substitutions are
174 located in the NTD, while D339H, G446S, N460K, and R493Q substitutions are
175 located in the RBD. The mutation number in the BA.2.75 S is larger than that in
176 the BA.4/5 S, and notably, some of the substitutions detected in the BA.2.75 S
177 show the signs of convergent evolution (Zahradnik et al., 2022). These notions
178 raise the possibility that the phenotype of BA.2.75 S is critically different from
179 previous BA.2 subvariants. In fact, we have recently revealed that the S protein
180 of BA.2.75 exhibits different sensitivity towards several therapeutic monoclonal
181 antibodies from those of BA.2 and BA.5 (Yamasoba et al., 2022a). However, the
182 virological phenotype of BA.2.75, including its R_e, potential evasion from antiviral
183 humoral immunity, sensitivity to currently recommended antiviral small
184 compounds, virological properties of its S protein, and intrinsic pathogenicity

185   remains unclear. Here, we elucidate the features of newly emerging

186   SARS-CoV-2 Omicron BA.2.75 subvariant.

187 **Results**

188 **Epidemics of BA.2.75 in India**

189 As of the beginning of August 2022, the Omicron BA.5 variant is predominant in

190 the world and is outcompeting the BA.2 variant. However, a novel BA.2

191 subvariant, BA.2.75, emerged and rapidly spread in India since May 2022.

192 Although BA.2.75 and BA.5 (and BA.4) belong to the BA.2 subvariant clade,

193 BA.2.75 is phylogenetically distinct from the BA.4/5 clade (**Figure 1A**).

194 Compared to BA.2, BA.2.75 harbors 14 amino acid substitutions, including nine

195 substitutions in the S protein (**Figures 1B and S1A**). Of these, only one

196 revertant mutation (S:R493Q) is shared with BA.5. In India, BA.5 and BA.2.75

197 spread in different regions each other: BA.5 spreads in the south part including

198 Tamil Nadu and Telangana states, while BA.2.75 spreads the other parts

199 including Himachal Pradesh, Odisha, Haryana, Rajasthan, and Maharashtra

200 states (**Figures 1C and 1D**). To compare the relative R_e between BA.5 and

201 BA.2.75 in India with adjusting the regional differences, we constructed a

202 Bayesian hierarchical model that can estimate both state-specific R_e values and

203 the value averaged in India (**Figures 1E and S1B and Table S1**). The R_e value

204 of BA.5 is 1.19-fold higher than that of BA.2 [95% credible interval (CI):

205 1.14–1.24] on average in India (**Figure 1E**). This value is comparative to the

206 relative R_e value of BA.5 in South Africa (1.21) estimated in our recent study

207 (Kimura *et al.*, 2022c). Of note, the R_e value of BA.2.75 is 1.34-fold higher than

208 that of BA.2 (95% CI: 1.29–1.38), and the R_e value of BA.2.75 is 1.13-fold higher

209 than that of BA.5 (95% CI: 1.06–1.20) (**Figures 1E and S1C**). Furthermore, in

210 the Indian states analyzed, where both BA.5 and BA.2.75 are dominant, such as

211 Telangana and Tamil Nadu (for BA. 5-dominant states) and Odisha, Haryana,

212 Rajasthan, and Maharashtra (for BA.2.75-dominant states), the R_e value of

213 BA.2.75 was greater than that of BA.5 (**Figures S1B and S1C**). Together, our

214 data suggest that BA.2.75 bears the potential to spread more rapidly than BA.5

215 and will be predominant in some regions including India in the near future.

216

217 **Sensitivity of BA.2.75 to antiviral humoral immunity and antiviral drugs**

218 Recent studies, including ours, showed that newly emerging Omicron

219 subvariants such as BA.5 exhibit higher resistance to the humoral immunity

220 induced by vaccination and natural infections with prior SARS-CoV-2 variants

221 including BA.1 and BA.2 (Hachmann *et al.*, 2022; Kimura *et al.*, 2022c; Wang *et*

222 *al.*, 2022). Additionally, we have recently demonstrated that BA.2.75 is more

223 resistant to a therapeutic monoclonal antibody, bebtelovimab, compared to BA.2

224 and BA.5 (Yamasoba *et al.*, 2022a). To investigate the sensitivity of BA.2.75 to

225 antiviral humoral immunity, we prepared pseudoviruses bearing the S proteins of

226 D614G-bearing ancestral B.1.1, BA.2, BA.5 and BA.2.75. Human sera were

227 collected from vaccinated and infected individuals (listed in **Table S2**). The

228   2-dose vaccine sera were ineffective against all Omicron subvariants tested,
229   including BA.2.75 (**Figure 2A**). Although BA.5 was significantly more resistant to
230   3-dose vaccine sera than BA.2, which is consistent with previous studies
231   (Hachmann *et al.*, 2022; Kimura *et al.*, 2022c; Wang *et al.*, 2022), the sensitivity
232   of BA.2.75 to these sera was comparable to that of BA.2 (**Figures 2B and 2C**).
233   We then assessed the sensitivity of BA.2.75 to the convalescent sera from
234   individuals who were infected with BA.1 and BA.2 after 2-dose or 3-dose
235   vaccination (i.e., breakthrough infection). Similar to the previous reports
236   including ours (Hachmann *et al.*, 2022; Kimura *et al.*, 2022c; Wang *et al.*, 2022),
237   BA.5 exhibited significant resistance to breakthrough infection sera compared to
238   BA.2, while the sensitivity of BA.2.75 to these sera was comparable to that of
239   BA.2 (**Figures 2D and 2E**). These results suggest that BA.2.75 is not resistant
240   to the humoral immunity induced by vaccination and the infection with prior
241   Omicron subvariants including BA.1 and BA.2. Since the Delta variant emerged
242   and caused a huge surge of infection in India in the middle of 2021 (Mlcochova
243   et al., 2021), it is hypothesized that BA.2.75 evades the immunity induced by
244   Delta. To address this possibility, we used Delta infection sera. However, the
245   sensitivity of all Omicron subvariants tested, including BA.2.75, to Delta infection
246   sera was similar (**Figure 2F**), implying that previous Delta infection is not
247   associated with the emergence of BA.2.75 in India.
248   To further address the difference in immunogenicity among Omicron
249   subvariants, we used the sera obtained from infected hamsters at 16 days
250   postinfection (d.p.i., i.e., after recovery) (Kimura *et al.*, 2022c; Suzuki *et al.*,
251   2022; Yamasoba *et al.*, 2022b). While BA.1 infection hamster sera were
252   ineffective against BA.2, BA.5 and BA.2.75 (**Figure 2G**), both BA.5 (17-fold,
253   *P*=0.031 by Wilcoxon signed-rank test) and BA.2.75 (23-fold, *P*=0.031 by
254   Wilcoxon signed-rank test) exhibited significant resistance to BA.2 infection
255   hamster sera than BA.2 (**Figure 2H**). These results suggest that the
256   immunogenicity of BA.5 and BA.2.75 is different from BA.2. Notably, BA.2
257   (5.1-fold, *P*=0.031 by Wilcoxon signed-rank test) and BA.2.75 (12-fold, *P*=0.031
258   by Wilcoxon signed-rank test) exhibited significant resistance to BA.5 infection
259   hamster sera (**Figure 2I**). These results suggest that the immunogenicity of BA.5
260   and BA.2.75 is also different. To identify the substitutions responsible for the
261   different immunogenicity of BA.2.75 S from BA.2 S and BA.5 S, we prepared the
262   BA.2 S-based derivatives that bear respective BA.2.75 substitutions. The
263   neutralization assay using BA.2-infected hamster sera showed that the G446S
264   and R493Q substitutions contribute to the resistance of BA.2.75 to BA.2-induced
265   immunity (**Figure 2H**). Because the R493Q substitution is shared with BA.5
266   (**Figures 1B and S1A**), it can be suggested that this substitution contributes to
267   the resistance of BA.5 to BA.2-induced immunity (**Figure 2H**). In the case of
268   BA.5-infected hamster sera, multiple substitutions, including the K147E, W152R,

9

269 F157L, I210V, G446S and N460K, associated with the resistance of BA.2.75 to
270 BA.5-induced immunity (**Figure 2I**).

271   To evaluate the sensitivity of BA.2.75 to three antiviral drugs,
272 Remdesivir, EIDD-1931 (an active metabolite of Molnupiravir) and Nirmatrelvir
273 (also known as PF-07321332), we used a clinical isolate of BA.2.75 (strain
274 TY41-716; GISAID ID: EPI_ISL_13969765). As controls, we also used clinical
275 isolates of B.1.1 (strain TKYE610670; GISAID ID: EPI_ISL_479681) (Suzuki *et*
276 *al.*, 2022), BA.2 (strain TY40-385; GISAID ID: EPI_ISL_9595859) (Kimura *et al.*,
277 2022c), BA.5 (strain TKYS14631; GISAID ID: EPI_ISL_12812500) (Tamura et
278 al., 2022). These viruses were inoculated into human airway organoids (AO), a
279 physiologically relevant model (Sano et al., 2022), and treated with three
280 antiviral drugs. As shown in **Table 1** and **Figure S2A**, Remdesivir had a stronger
281 antiviral effect (EC_{50}=0.63 μM) against B.2.75 than other variants, B.1.1, BA.2
282 and BA.5. EIDD-1931 inhibited BA.2 and BA.2.75 (EC_{50}=0.02 μM and 0.08 μM,
283 respectively) more potently than B.1.1 and BA.5 (EC_{50}=0.24 μM and 0.21 μM,
284 respectively). For Nirmatrelvir, no differences in antiviral efficacy were observed
285 between four variants (EC_{50}=0.84 μM, 0.85 μM, 0.63 μM and 0.81 μM for B.1.1,
286 BA.2, BA.5 and BA.2.75, respectively). Altogether, it is suggested that all three
287 drugs exhibit antiviral effects against BA.2.75, and particularly, EIDD-1931 is
288 effective against BA.2.75.

289

290 **Virological characteristics of BA.2.75 S *in vitro***
291 To investigate the virological properties of BA.2.75 S, we measured the
292 pseudovirus infectivity. As shown in **Figure 3A**, the pseudovirus infectivity of
293 BA.2.75 was significantly (12.5-fold) higher than that of BA.2. To assess the
294 association of TMPRSS2 usage with the increased pseudovirus infectivity of
295 BA.2.75, we used both HEK293-ACE2/TMPRSS2 cells and HEK293-ACE2 cells,
296 on which endogenous surface TMPRSS2 is undetectable (Yamasoba *et al.*,
297 2022b), as target cells. Consistent with our recent study (Kimura *et al.*, 2022c),
298 the fold increase in pseudovirus infectivity of BA.5 caused by TMPRSS2
299 expression on the target cells was not observed (**Figure S3A**). Similarly, the
300 infectivity of BA.2.75 pseudovirus was not increased by TMPRSS2 expression
301 (**Figure S3A**), suggesting that TMPRSS2 is not associated with an increase in
302 pseudovirus infectivity of BA.2.75. To determine the substitutions that are
303 responsible for the increased pseudovirus infectivity of BA.2.75, we used a
304 series of BA.2 derivatives that bears the BA.2.75-specific substitutions. Three
305 substitutions in the NTD, K147E, F157L, and I210V, and two substitutions in the
306 RBD, N460K and R493Q, significantly increased infectivity (**Figure 3A**). Notably,
307 the N460K substitution increased infectivity by 44-fold (**Figure 3A**). On the other
308 hand, a substitution in the NTD, W152R, significantly (8.9-fold) decreased
309 infectivity (**Figure 3A**). The BA.2 derivative bearing the three substitutions in the

10

310 NTD in close proximity to each other, K147E, W152R and F157L, exhibited
311 comparable infectivity to BA.2 (**Figure 3A**).

312 To decipher the binding properties of BA.2.75 S RBD to human ACE2
313 and the role of each substitution, we measured the ACE2 binding affinity of the S
314 RBDs of BA.2.75 as well as those of BA.2 derivatives bearing D339H, G446S,
315 N460K and R493Q substitutions by an enhanced surface display system
316 (Zahradnik et al., 2021a). Intriguingly, the BA.2.75 S RBD showed a strongly
317 tight binding with 146 ± 6 pM affinity (**Figure 3B**). Out of the four BA.2-based
318 derivatives, only the BA.2 N460K substitution exhibited a significantly increased
319 binding affinity than BA.2 (**Figure 3B**). Consistent with the results of pseudovirus
320 assay (**Figure 3A**), these observations suggest that the N460K substitution is
321 critical to characterize the virological phenotype of BA.2.75 S. To reveal the
322 structural effect of the N460K substitution, we generated a structural model of
323 BA.2.75 S RBD using AlphaFold2 (Mirdita et al., 2022). Calculating the
324 electrostatic potential of this model in comparison with the S RBDs of B.1.1 and
325 BA.2 showed that K460 of BA.2.75 S RBD is positively charged (**Figure 3C**),
326 and the K460 is complementary to the negative charged binding site on human
327 ACE2 (**Figure 3D**). These structural observations suggest that N460K
328 substitution contributes to increased electrostatic complementary binding
329 between the BA.2.75 S RBD and human ACE2.

330 Although the N460K substitution significantly increased binding affinity
331 (**Figure 3B**), the binding affinity of the BA.2 N460K was still 5-fold lower than
332 that of BA.2.75 (**Figure 3B**). Therefore, the extraordinary tight binding of
333 BA.2.75 cannot be explained by the N460K alone, and it is hypothesized that the
334 additional substitutions conferred negative effects in the BA.2 background. In
335 particular, the D339H substitution requires two nucleotide changes in the codon
336 to occur. Such changes are still relatively rare in the evolution of SARS-CoV-2,
337 reinforcing the importance and corresponding fitness advantage. To analyze the
338 potential impact of this substitution, we additionally prepared the BA.2.75 H339D
339 derivative and measured its affinity. The K_D value of this mutant was significantly
340 (3-fold) lower than that of the parental BA.2.75 (**Figure 3B**). The structural model
341 computed by AlphaFold2 (Mirdita *et al.*, 2022) suggested that the loss of
342 ion-dipole interaction between the D339 and the N343 allowed for the N343 side
343 chain repositioning (**Figure S3B**). These data suggest that the D339H
344 substitution potentially influences the position of the linoleic acid binding loop
345 between residues 367–378 (Toelzer et al., 2020) and thereby increases binding
346 affinity to ACE2.

347 To further reveal the virological property of BA.2.75 S, we performed a
348 cell-based fusion assay (Kimura et al., 2022b; Kimura *et al.*, 2022c; Motozono et
349 al., 2021; Saito *et al.*, 2022; Suzuki *et al.*, 2022; Yamasoba *et al.*, 2022b) using
350 Calu-3 cells as target cells. Flow cytometry analysis showed that the surface

351   expression level of BA.2.75 is comparable to that of BA.2 (**Figure 3E**).
352   Consistent with our recent study (Kimura *et al.*, 2022c), the fusogenicity of BA.5
353   was significantly higher than that of BA.2, and notably, the BA.2.75 S was also
354   significantly more fusogenic than the BA.2 S (**Figure 3F**). Altogether, these
355   results suggest that BA.2.75 S exhibits higher binding affinity to human ACE2
356   and higher fusogenicity.

357

358   **Virological characteristics of BA.2.75 clinical isolate *in vitro***
359   To evaluate the growth capacity of BA.2.75, a clinical isolate of BA.2.75 (strain
360   TY41-716; GISAID ID: EPI_ISL_13969765) was inoculated in a variety of *in vitro*
361   cell culture systems. As controls, we also used clinical isolates of B.1.1 (strain
362   TKYE610670; GISAID ID: EPI_ISL_479681) (Suzuki *et al.*, 2022), Delta
363   (B.1.617.2, strain TKYTK1734; GISAID ID: EPI_ISL_2378732) (Saito *et al.*,
364   2022), BA.2 (strain TY40-385; GISAID ID: EPI_ISL_9595859) (Kimura *et al.*,
365   2022c) and BA.5 (strain TKYS14631; GISAID ID: EPI_ISL_12812500) (Tamura
366   *et al.*, 2022). The growth efficacy of B.1.1 and Delta was significantly higher than
367   that of BA.2 in Vero cells (**Figure 4A**), VeroE6/TMPRSS2 cells (**Figure 4B**),
368   HEK293-ACE2/TMPRSS2 cells (**Figure 4C**), AO-derived air-liquid interface
369   (AO-ALI) model (**Figure 4D**), human iPS cell (iPSC)-derived airway epithelial
370   cells (**Figure 4E**) and lung epithelial cells (**Figure 4F**). BA.5 replicated more
371   efficiently than BA.2 with statistically significant differences in the five cell culture
372   systems except AO-ALI (**Figures 4A–4F**). The growth efficacy of BA.2.75 was
373   significantly higher than that of BA.2 in Vero cells (**Figure 4A**),
374   VeroE6/TMPRSS2 cells (**Figure 4B**), HEK293-ACE2/TMPRSS2 cells (**Figure
375   4C**), and iPSC-derived lung epithelial cells (**Figure 4F**), while the growth efficacy
376   of BA.2.75 and BA.2 were comparable in the two airway epithelial cell systems
377   (**Figures 4D and 4E**).
378   To evaluate the effect of BA.2.75 on the airway epithelial and
379   endothelial barriers, airway-on-a-chips (**Figure S3C**) were used. By measuring
380   the amount of virus that invades from the top channel (airway channel; **Figure
381   4G**) to the bottom channel (blood vessel channel; **Figure 4H**), the ability of
382   viruses to disrupt the airway epithelial and endothelial barriers can be evaluated.
383   Notably, the amount of virus that invades to the blood vessel channel of
384   BA.2.75-, BA.5- and B.1.1-infected airway-on-chips was significantly higher than
385   that of BA.2-infected one (**Figure 4I**). These results suggest that BA.2.75
386   exhibits more severe airway epithelial and endothelial barrier disruption than
387   BA.2.
388   To further address the fusogenic capacity of BA.2.75, we performed
389   plaque assay using VeroE6/TMPRSS2 cells. Consistent with our previous
390   studies using a Delta isolate (Saito *et al.*, 2022) as well as the recombinant
391   SARS-CoV-2 bearing the B.1.1 S (Yamasoba *et al.*, 2022a), BA.2 S (Yamasoba

12

392 *et al.*, 2022a), and BA.5 S (Kimura *et al.*, 2022c), the plaques formed by the
393 infections of clinical isolates of B.1.1, Delta and BA.5 were significantly bigger
394 than those formed by the infection of BA.2 (**Figure 4J**). Notably, BA.2.75
395 infection also showed significantly bigger plaques than BA.2 infection (**Figure**
396 **4J**). Together with the results of cell-based fusion assay (**Figure 3F**) and
397 airway-on-a-chip infection experiments (**Figures 4G–4I**), these observations
398 suggest that BA.2.75 is more fusogenic than BA.2, and the fusogenicity of
399 BA.2.75 is comparable to that of BA.5.
400

401 **Virological characteristics of BA.2.75 *in vivo***
402 As we proposed in our prior studies (Kimura *et al.*, 2022c; Saito *et al.*, 2022;
403 Suzuki *et al.*, 2022; Yamasoba *et al.*, 2022b), the fusogenicity of the S proteins
404 of SARS-CoV-2 variants is closely associated with the intrinsic pathogenicity in
405 an experimental hamster model. Here we revealed that both BA.5 and BA.2.75
406 are more fusogenic than BA.2 in the *in vitro* cell culture systems (**Figures 3 and**
407 **4**). Given that the recombinant SARS-CoV-2 bearing the BA.5 S (Kimura *et al.*,
408 2022c) as well as a clinical isolate of BA.5 (Tamura *et al.*, 2022) exhibited
409 relatively higher pathogenicity than BA.2 in hamsters, it is hypothesized that
410 BA.2.75 is also intrinsically more pathogenic than BA.2. To address this
411 possibility, we intranasally inoculated a BA.2.75 isolate into hamsters. As
412 controls, we also used clinical isolates of Delta, BA.2 and BA.5. While we
413 followed our established experimental protocol (Kimura *et al.*, 2022c; Saito *et al.*,
414 2022; Suzuki *et al.*, 2022; Yamasoba *et al.*, 2022b), the viral titers of clinical
415 isolates of Omicron subvariants were relatively low. Therefore, we set out to
416 conduct animal experiments in this study with relatively lower titer inoculum
417 (1,000 $TCID_{50}$ per hamster) than our previous studies (10,000 $TCID_{50}$ per
418 hamster) (Kimura *et al.*, 2022c; Saito *et al.*, 2022; Suzuki *et al.*, 2022; Yamasoba
419 *et al.*, 2022b). Nevertheless, consistent with our previous study (Saito *et al.*,
420 2022), the Delta infection exhibited the most severe weight changes among the
421 five groups (**Figure 5A**). While the body weight of BA.2-infected hamsters was
422 similar to that of uninfected hamsters, those of BA.5- and BA.2.75-infected
423 hamsters were significantly lower than that of uninfected hamsters (**Figure 5A**).
424 We then quantitatively analyzed the pulmonary function of infected
425 hamsters as reflected by three parameters, enhanced pause (Penh), the ratio of
426 time to peak expiratory follow relative to the total expiratory time (Rpef), and
427 breath per minute (BPM), which are surrogate markers for bronchoconstriction
428 or airway obstruction. Subcutaneous oxygen saturation (SpO_2) was also
429 routinely measured. Although the SpO_2 values were comparable among the five
430 groups, Delta infection resulted in significant differences in the other three
431 respiratory parameters compared to BA.2 (**Figure 5A**), suggesting that Delta is
432 more pathogenic than BA.2. There were no differences in the values of Penh,

13

433 Rpef and BPM between BA.5 and BA.2, and the values of Penh and Rpef of
434 BA.2.75-infected hamsters were comparable to those of BA.2 (**Figure 5A**).
435 However, the BPM value of BA.2.75 was significantly lower than that of BA.2
436 (**Figure 5A**), suggesting that BA.2.75 is slightly more pathogenic than BA.2.

437 To address the viral spread in infected hamsters, we routinely
438 measured the viral RNA load in the oral swab. Although the viral RNA loads of
439 the hamsters infected with Delta, BA.2 and BA.5 were comparable, the viral load
440 in the swabs of BA.2.75-infected hamsters was relatively highly maintained by 7
441 d.p.i. and was significantly higher than that of BA.2-infected hamsters (**Figure
442 5B**). To address the possibility that BA.2.75 more efficiently spread in the
443 respiratory tissues, we collected the lungs of infected hamsters at 2 and 5 d.p.i.,
444 and the collected tissues were separated into the hilum and periphery regions.
445 Although the viral RNA loads in both the hilum and periphery of four infection
446 groups were comparable at 2 d.p.i. (**Figure 5C, top**), those of the hamsters
447 infected with Delta, BA.5 and BA.2.75 were significantly higher than those
448 infected with BA.2 at 5 d.p.i. (**Figure 5C, bottom**).

449 To further address the virus spread in the respiratory tissues,
450 immunohistochemical (IHC) analysis targeting viral nucleocapsid (N) protein was
451 conducted. Similar to our previous studies (Kimura *et al.*, 2022c; Suzuki *et al.*,
452 2022; Yamasoba *et al.*, 2022b), epithelial cells in the upper tracheae of infected
453 hamsters were sporadically positive for viral N protein at 2 d.p.i., but there were
454 no significant differences among four viruses including BA.2.75 (**Figure S4A**). In
455 the alveolar space around the bronchi/bronchioles at 2 d.p.i., the N-positive cells
456 were detected in Delta-infected hamsters. On the other hand, the N proteins
457 strongly remained in the lobar bronchi in BA.5- and BA.2.75-infected hamsters
458 (**Figures 5D, top, and S4B**). While few N-positive cells were detected in the
459 alveolar space of BA.2- and BA.5-infected hamsters, it was notable that the N
460 positivity spread into the alveolar space in BA.2.75-infected hamsters (**Figures
461 5D, top, and S4B**). The quantification of the N-positive area in total of four lung
462 lobes at 2 d.p.i. (**Figure S4B**) showed that the N-positive areas of Delta- and
463 BA.2.75-infected hamsters were significantly greater than that of BA.2-infected
464 hamsters (**Figure 5E, top**). At 5 d.p.i., although the N-positive cells were hardly
465 detected in the lungs infected with BA.2, a few N-positive cells were detected in
466 the peripheral alveolar space in Delta, BA.5, BA.2.75 (**Figures 5D, bottom, and
467 S4C**). The quantification of the N-positive area in the four lung lobes at 5 d.p.i.
468 (**Figure S4C**) further showed that the N-positive areas of Delta- and BA.5- and
469 BA.2.75-infected hamsters were significantly greater than that of BA.2-infected
470 hamsters (**Figure 5E, bottom**). These data suggest that BA.2 targets only a
471 portion of bronchial/bronchiolar epithelium and was less efficiently transmitted to
472 the neighboring epithelial cells. On the other hand, BA.5 and BA.2.75 infections
473 seemed to persist in the bronchial/bronchiolar epithelium, and particularly,

14

474   BA.2.75 invaded the alveolar space more efficiently than BA.5 at the early stage
475   of infection. Altogether, the IHC data suggest that among Omicron subvariants,
476   BA.2.75 more efficiently spread into the alveolar space than BA.2 and BA.5, with
477   persistent infection in the bronchi/bronchioles.

478

479   **Pathogenicity of BA.2.75**

480   To investigate the intrinsic pathogenicity of BA.2.75, the formalin-fixed right
481   lungs of infected hamsters at 2 and 5 d.p.i. were analyzed by carefully identifying
482   the four lobules and main bronchus and lobar bronchi sectioning each lobe
483   along with the bronchial branches. Histopathological scoring was performed
484   according to the criteria described in our previous studies (Kimura *et al.*, 2022c;
485   Saito *et al.*, 2022; Suzuki *et al.*, 2022; Yamasoba *et al.*, 2022b): (i)
486   bronchitis/bronchiolitis (an inflammatory indicator at early stage of infection), (ii)
487   hemorrhage/congestion, (iii) alveolar damage with epithelial apoptosis and
488   macrophage infiltration, (iv) emergence of type II pneumocytes, and (v)
489   hyperplasia of type II pneumocytes were evaluated by certified pathologists and
490   the degree of these pathological findings were arbitrarily scored using four-tiered
491   system as 0 (negative), 1 (weak), 2 (moderate), and 3 (severe). Consistent with
492   our previous studies (Saito *et al.*, 2022; Suzuki *et al.*, 2022), all five parameters
493   as well as the total score of Delta-infected hamsters were significantly higher
494   than those of BA.2-infected hamsters (**Figures 5F and 5G**), suggesting that
495   Delta is more pathogenic than BA.2. When we compare the histopathological
496   scores of Omicron subvariants, the scores indicating hemorrhage or congestion
497   and total histology scores of BA.5 and BA.2.75 were significantly greater than
498   those of BA.2 (**Figures 5F and 5G**). Similar to our recent studies (Kimura *et al.*,
499   2022c; Tamura *et al.*, 2022), BA.5 is intrinsically more pathogenic than BA.2,
500   and notably, our results suggest that BA.2.75 exhibits more significant
501   inflammation than BA.2. To clarify the area of pneumonia, the inflammatory area,
502   which is mainly composed of the type II pneumocytes with some inflammatory
503   cell types, such as neutrophils, lymphocytes, and macrophages, is termed the
504   area of type II pneumocytes and was morphometrically analyzed (**Figure S4D**).
505   As summarized in **Figure 5H**, at 5 d.p.i., the percentages of the area of type II
506   pneumocytes of Delta, BA.5 and BA.2.75 were significantly higher than that of
507   BA.2. Altogether, these findings suggest that BA.2.75 infection intrinsically
508   induces greater inflammation and exhibits higher pathogenicity than BA.2.

Discussion

509

510 Here, we characterized the virological property of the Omicron BA.2.75 variant,

511 such as the growth rate in the human population, resistance to antiviral humoral

512 immunity and antiviral drugs, functions of S protein *in vitro*, and intrinsic

513 pathogenicity.

514 In terms of the emergence geography and phylogeny, BA.5 and

515 BA.2.75 emerged independently. Nevertheless, the results of cell-based fusion

516 assay, airway-on-a-chip assay and plaque assay suggested that both BA.5 and

517 BA.2.75 acquired higher fusogenicity after the divergence from BA.2. Our data

518 including a recent study (Kimura *et al.*, 2022c) suggest that the critical

519 substitution responsible for the higher fusogenicity of BA.5 and BA.2.75 S

520 proteins are different: the L452R substitution for BA.5 S, and the D339H/N460K

521 substitution for BA.2.75 S.

522 The higher fusogenicity attributed by the increased binding affinity of

523 the L452R-bearing S RBD to human ACE2 was reported in previous studies

524 focusing on the S proteins of previous SARS-CoV-2 variants including Epsilon

525 (Motozono *et al.*, 2021), Delta (Saito *et al.*, 2022) and Omicron BA.5 (Kimura *et*

526 *al.*, 2022c) variants. The prominently increased ACE2 binding affinity caused by

527 the N460K substitution was also reported in our previous study (Zahradnik et al.,

528 2021b). We also demonstrated that the D339H, which is unique in the BA.2.75 S,

529 contributes to increased ACE2 binding affinity. Our data suggest that the N460K

530 and D339H substitutions cooperatively determine the higher fusogenicity of

531 BA.2.75 S. In our previous studies focusing on Delta (Saito *et al.*, 2022),

532 Omicron BA.1 (Suzuki *et al.*, 2022), BA.2 (Yamasoba *et al.*, 2022b) and BA.5

533 (Kimura *et al.*, 2022c), we proposed a close association between the S-mediated

534 fusogenicity *in vitro* and the pathogenicity in a hamster model. Consistent with

535 our hypothesis, here we demonstrated that, compared to BA.2, BA.2.75 exhibits

536 higher fusogenicity *in vitro* and efficient viral spread in the lungs of infected

537 hamsters, which leads to enhanced inflammation in the lung and higher

538 pathogenicity *in vivo*. Moreover, *in vitro* experiments using a variety of cell

539 culture systems showed that BA.2.75 replicates more efficiently than BA.2 in

540 alveolar epithelial cells but not in airway epithelial cells. Altogether, our results

541 suggest that BA.2.75 exhibits higher fusogenicity and pathogenicity via evolution

542 of its S protein independently of BA.5.

543 Consistent with our previous study (Yamasoba *et al.*, 2022b),

544 neutralization experiments showed that BA.5 was significantly more resistant to

545 the humoral immunity induced by vaccination and breakthrough infections of

546 prior Omicron subvariants. On the other hand, the sensitivity of BA.2.75 to these

547 antisera was comparable to BA.2. More importantly, BA.2.75 was highly

548 resistant to the BA.5-induced immunity. These results suggest that, although

549 both BA.2.75 and BA.5 are descendants of BA.2, their immunogenicity is

16

550 different from each other. Furthermore, compared to BA.2, the sensitivity of
551 BA.2.75 and BA.5 to therapeutic monoclonal antibodies was also different
552 (Yamasoba *et al.*, 2022a). The G446S was also closely associated with the
553 resistance of BA.2.75 to the antiviral effects of BA.2- and BA.5-infected hamster
554 sera. Because the G446S significantly decreases ACE2 affinity of S RBD, this
555 substitution was acquired to evade antiviral immunity, and the other substitutions
556 in RBD, particularly N460K, contributed to compensate for the decreased ACE2
557 binding affinity by G446S.
558   Another remarkable substitution pattern in the BA.2.75 S is the multiple
559 substitutions in the S NTD. Particularly, three out of the five substitutions in the
560 NTD (K147E, W152R and F157L) are located in a well-studied region, the NTD
561 supersite. Previous studies showed that the mutations in the NTD supersite are
562 responsible for the resistance to antiviral monoclonal antibodies (Cerutti *et al.*,
563 2021; Chi *et al.*, 2020; Liu *et al.*, 2020; Lok, 2021; McCallum *et al.*, 2021;
564 Suryadevara *et al.*, 2021; Voss *et al.*, 2021). In fact, our results suggested that
565 these three substitutions in the NTD supersite are closely associated with the
566 evasion from BA.5-induced humoral immunity, in addition to the G446S in RBD.
567 In fact, the W152 has been shown as a mutational hot spot of SARS-CoV-2
568 (Kubik et al., 2021). Therefore, BA.2.75 might mutate this specific residue to
569 evade neutralization by sera of convalescent or vaccinated individuals.
570   According to the "COVID-19 Treatment Guidelines" issued by NIH (NIH,
571 2022), the use of Paxlovid (Ritonavir and Nirmatrelvir), Remdesivir and
572 Molnupiravir (a prodrug of EIDD-1931) is highly recommended as treatment of
573 patients who do not require hospitalization or oxygen supplement. Because the
574 evolution of SARS-CoV-2 is unpredictable, timely and accurate testing of the
575 efficacy of currently available antiviral drugs is indispensable to treat patients
576 infected with a new variant. Our results using physiologically relevant human AO
577 demonstrated that BA.2.75 retained the sensitivity to major small-molecule
578 anti-SARS-CoV-2 drugs including Remdesivir, EIDD-1931 and Nirmatrelvir.
579 Interestingly, BA.2.75 was more sensitive to Remdesivir than other stains, and a
580 similar tendency was observed with EIDD-1931. In terms of drug testing,
581 previous studies addressed the antiviral activity of these drugs against BA.2 and
582 BA.5 using immortalized cell lines such as VeroE6/TMPRSS2 cells (Takashita et
583 al., 2022c), Caco-2-F03 cells (Bojkova et al., 2022) and Calu-3 cells (Carlin et al.,
584 2022) but the effects of these antiviral drugs were different each other, and these
585 results were also different from ours (**Table 1**). In addition, a previous study
586 demonstrated that VeroE6 cells have a low capacity to metabolize Remdesivir,
587 leading to a weak antiviral activity (Pruijssers et al., 2020). These results suggest
588 that the experimental system significantly affects the outcome of antiviral drug
589 efficacy, raising the importance to evaluate the efficacy of antiviral drugs using

17

590 physiologically relevant systems, such as organoids and organ-on-a-chip
591 systems.
592   Our investigation using the viral genome surveillance data reported
593 from India suggested that BA.2.75 bears the potential to outcompete BA.2 as
594 well as BA.5, the most predominant variant in the world as of August 2022.
595 Following the worldwide spread of BA.5, it is probable that the number of
596 individuals infected with BA.5 will increase. Together with our findings showing
597 the higher resistance of BA.2.75 to the BA.5-induced immunity, there appears to
598 be sufficient plausibility that BA.2.75 evades the BA.5-induced immunity, and
599 this property will confer this variant to more efficient spread in the countries
600 where BA.5 has been widely spreading, such as Australia and Japan.
601 Additionally, here we showed that the intrinsic pathogenicity of BA.2.75 in
602 hamsters is comparable to BA.5 and higher than that of BA.2. Since a recent
603 study showed that the hospitalization risk of BA.5 was significantly higher than
604 that of BA.2 in the once-boosted vaccinated population (Kislaya et al., 2022), it is
605 not unreasonable to infer that the intrinsic pathogenicity in infected hamsters
606 reflects to the severity and outcome in infected humans to a meaningful extent.
607   In summary, our multiscale investigations revealed the growth rate in
608 the human population, fusogenicity and intrinsic pathogenicity of BA.2.75 are
609 greater than BA.2. These features of BA.2.75 suggests the potential risk of this
610 variant to global health. Since BA.2.75 shows significantly higher R_e than BA.2
611 and BA.5 in India, this variant will probably transmit to and initiate outcompeting
612 BA.2 and BA.5 in some countries other than India in the near future. To assess
613 the potential risk of BA.2.75 to global health, this variant should be under
614 monitoring carefully and continuously through worldwide cooperation of in-depth
615 viral genomic surveillance.

18

616 **STAR⬛METHODS**

617 ● KEY RESOURCES TABLE

618 ● RESOURCE AVAILABILITY

619   ○ Lead Contact

620   ○ Materials Availability

621   ○ Data and Code Availability

622 ● EXPERIMENTAL MODEL AND SUBJECT DETAILS

623   ○ Ethics Statement

624   ○ Human serum collection

625   ○ Cell culture

626 ● METHOD DETAILS

627   ○ Viral genome sequencing

628   ○ Phylogenetic analyses

629   ○ Modelling the epidemic dynamics of SARS-CoV-2 lineages

630   ○ Plasmid construction

631   ○ Neutralization assay

632   ○ Airway organoids

633   ○ SARS-CoV-2 preparation and titration

634   ○ Antiviral drug assay using SARS-CoV-2 clinical isolates and AO

635   ○ Cytotoxicity assay

636   ○ Pseudovirus infection

637   ○ Yeast surface display

638   ○ AlphaFold2

639   ○ SARS-CoV-2 S-based fusion assay

640   ○ AO-ALI model

641   ○ Preparation of human airway and alveolar epithelial cells from human

642 iPSC

643   ○ Airway-on-a-chips

644   ○ Microfluidic device

645   ○ SARS-CoV-2 infection

646   ○ RT–qPCR

647   ○ Plaque assay

648   ○ Animal experiments

649   ○ Lung function test

650   ○ Immunohistochemistry

651   ○ H&E staining

652   ○ Histopathological scoring

653 ● QUANTIFICATION AND STATISTICAL ANALYSIS

654 **Supplemental Information**

655 Additional Supplemental Items are available upon request.

656

657 **Author Contributions**

658 Akatsuki Saito, Sayaka Deguchi, Izumi Kimura, Hesham Nasser, Mako Toyoda,
659 Kayoko Nagata, Keiya Uriu, Yusuke Kosugi, Shigeru Fujita, Daichi Yamasoba,
660 Maya Shofa, MST Monira Begum, Takashi Irie, Takamasa Ueno, and Terumasa
661 Ikeda performed cell culture experiments.

662 Tomokazu Tamura, Koshiro Tabata, Rigel Suzuki, Hayato Ito, Naganori Nao,
663 Kumiko Yoshimatsu, Hirofumi Sawa, Keita Matsuno, and Takasuke Fukuhara
664 performed animal experiments.

665 Yoshitaka Oda, Lei Wang, Masumi Tsuda, and Shinya Tanaka performed
666 histopathological analysis.

667 Jiri Zahradnik and Gideon Schreiber performed yeast surface display assay.

668 Jiri Zahradnik and Yusuke Kosugi performed structural analysis.

669 Sayaka Deguchi and Kazuo Takayama prepared AO, AO-ALI and
670 airway-on-a-chip systems.

671 Yuki Yamamoto and Tetsuharu Nagamoto performed generation and provision
672 of human iPSC-derived airway and alveolar epithelial cells.

673 Hiroyuki Asakura, Mami Nagashima, Kenji Sadamasu, Kazuhisa Yoshimura
674 performed viral genome sequencing analysis.

675 Akifumi Takaori-Kondo and Kotaro Shirakawa contributed clinical sample
676 collection.

677 Jumpei Ito performed statistical, modelling, and bioinformatics analyses.

678 Jumpei Ito, Kazuo Takayama, Keita Matsuno, Shinya Tanaka, Terumasa Ikeda,
679 Takasuke Fukuhara, and Kei Sato designed the experiments and interpreted the
680 results.

681 Jumpei Ito, Terumasa Ikeda, Takasuke Fukuhara and Kei Sato wrote the original
682 manuscript.

683 All authors reviewed and proofread the manuscript.

684 The Genotype to Phenotype Japan (G2P-Japan) Consortium contributed to the
685 project administration.

686

687 **Conflict of interest**

688 The authors declare that no competing interests exist.

689

690 **Acknowledgments**

735

736   **Consortia**
737   Mai Kishimoto, Marie Kato, Zannatul Ferdous, Hiromi Mouri, Kenji Shishido,
738   Naoko Misawa, Mai Suganami, Mika Chiba, Ryo Yoshimura, So Nakagawa,

739   Jiaqi Wu, Yasuhiro Kazuma, Ryosuke Nomura, Yoshihito Horisawa, Yusuke

740   Tashiro, Yugo Kawai, Ryoko Kawabata, Ryo Shimizu, Otowa Takahashi, Kimiko

741   Ichihara, Chihiro Motozono, Yuri L. Tanaka, Erika P. Butlertanaka, Rina

742   Hashimoto, Takao Hashiguchi, Tateki Suzuki, Kanako Kimura, Jiei Sasaki,

743   Yukari Nakajima, Kaori Tabata

744 **References**

745 Arora, P., Kempf, A., Nehlmeier, I., Schulz, S.R., Cossmann, A., Stankov, M.V.,
746 Jack, H.M., Behrens, G.M.N., Pohlmann, S., and Hoffmann, M. (2022).
747 Augmented neutralisation resistance of emerging omicron subvariants
748 BA.2.12.1, BA.4, and BA.5. Lancet Infect Dis. 10.1016/S1473-3099(22)00422-4.
749 Barnes, C.O., Jette, C.A., Abernathy, M.E., Dam, K.A., Esswein, S.R., Gristick,
750 H.B., Malyutin, A.G., Sharaf, N.G., Huey-Tubman, K.E., Lee, Y.E., et al. (2020).
751 SARS-CoV-2 neutralizing antibody structures inform therapeutic strategies.
752 Nature *588*, 682-687. 10.1038/s41586-020-2852-1.
753 Bojkova, D., Stack, R., Rothenburger, T., Kandler, J.D., Ciesek, S., Wass, M.N.,
754 Michaelis, M., and Cinatl, J., Jr. (2022). Synergism of interferon-beta with
755 antiviral drugs against SARS-CoV-2 variants. J Infect.
756 10.1016/j.jinf.2022.07.023.
757 Bruel, T., Hadjadj, J., Maes, P., Planas, D., Seve, A., Staropoli, I.,
758 Guivel-Benhassine, F., Porrot, F., Bolland, W.H., Nguyen, Y., et al. (2022).
759 Serum neutralization of SARS-CoV-2 Omicron sublineages BA.1 and BA.2 in
760 patients receiving monoclonal antibodies. Nat Med.
761 10.1038/s41591-022-01792-5.
762 Cao, Y., Wang, J., Jian, F., Xiao, T., Song, W., Yisimayi, A., Huang, W., Li, Q.,
763 Wang, P., An, R., et al. (2021). Omicron escapes the majority of existing
764 SARS-CoV-2 neutralizing antibodies. Nature, doi:
765 https://doi.org/10.1038/d41586-41021-03796-41586.
766 Cao, Y., Yisimayi, A., Jian, F., Song, W., Xiao, T., Wang, L., Du, S., Wang, J., Li,
767 Q., Chen, X., et al. (2022). BA.2.12.1, BA.4 and BA.5 escape antibodies elicited
768 by Omicron infection. Nature. 10.1038/s41586-022-04980-y.
769 Capella-Gutierrez, S., Silla-Martinez, J.M., and Gabaldon, T. (2009). trimAl: a
770 tool for automated alignment trimming in large-scale phylogenetic analyses.
771 Bioinformatics *25*, 1972-1973. 10.1093/bioinformatics/btp348.
772 Carlin, A.F., Clark, A.E., Chaillon, A., Garretson, A.F., Bray, W., Porrachia, M.,
773 Santos, A.T., Rana, T.M., and Smith, D.M. (2022). Virologic and Immunologic
774 Characterization of COVID-19 Recrudescence after Nirmatrelvir/Ritonavir
775 Treatment. Clin Infect Dis. 10.1093/cid/ciac496.
776 Cele, S., Jackson, L., Khoury, D.S., Khan, K., Moyo-Gwete, T., Tegally, H., San,
777 J.E., Cromer, D., Scheepers, C., Amoako, D., et al. (2021). Omicron extensively
778 but incompletely escapes Pfizer BNT162b2 neutralization. Nature, doi:
779 https://doi.org/10.1038/d41586-41021-03824-41585.
780 Cerutti, G., Guo, Y., Zhou, T., Gorman, J., Lee, M., Rapp, M., Reddem, E.R., Yu,
781 J., Bahna, F., Bimela, J., et al. (2021). Potent SARS-CoV-2 neutralizing
782 antibodies directed against spike N-terminal domain target a single supersite.
783 Cell Host Microbe *29*, 819-833 e817. 10.1016/j.chom.2021.03.005.
784 Chen, S., Zhou, Y., Chen, Y., and Gu, J. (2018). fastp: an ultra-fast all-in-one

785   FASTQ   preprocessor.   Bioinformatics   *34*,   i884-i890.
786   10.1093/bioinformatics/bty560.

787   Chi, X., Yan, R., Zhang, J., Zhang, G., Zhang, Y., Hao, M., Zhang, Z., Fan, P.,
788   Dong, Y., Yang, Y., et al. (2020). A neutralizing human antibody binds to the
789   N-terminal domain of the Spike protein of SARS-CoV-2. Science *369*, 650-655.
790   10.1126/science.abc6952.

791   Cingolani, P., Platts, A., Wang le, L., Coon, M., Nguyen, T., Wang, L., Land, S.J.,
792   Lu, X., and Ruden, D.M. (2012). A program for annotating and predicting the
793   effects of single nucleotide polymorphisms, SnpEff: SNPs in the genome of
794   Drosophila melanogaster strain w1118; iso-2; iso-3. Fly (Austin) *6*, 80-92.
795   10.4161/fly.19695.

796   Deguchi, S., Tsuda, M., Kosugi, K., Sakamoto, A., Mimura, N., Negoro, R., Sano,
797   E., Nobe, T., Maeda, K., Kusuhara, H., et al. (2021). Usability of
798   Polydimethylsiloxane-Based Microfluidic Devices in Pharmaceutical Research
799   Using Human Hepatocytes. ACS Biomater Sci Eng *7*, 3648-3657.
800   10.1021/acsbiomaterials.1c00642.

801   Dejnirattisai, W., Huo, J., Zhou, D., Zahradnik, J., Supasa, P., Liu, C.,
802   Duyvesteyn, H.M.E., Ginn, H.M., Mentzer, A.J., Tuekprakhon, A., et al. (2022).
803   SARS-CoV-2 Omicron-B.1.1.529 leads to widespread escape from neutralizing
804   antibody responses. Cell *185*, 467-484 e415. 10.1016/j.cell.2021.12.046.

805   Dolinsky, T.J., Czodrowski, P., Li, H., Nielsen, J.E., Jensen, J.H., Klebe, G., and
806   Baker, N.A. (2007). PDB2PQR: expanding and upgrading automated
807   preparation of biomolecular structures for molecular simulations. Nucleic Acids
808   Res *35*, W522-525. 10.1093/nar/gkm276.

809   Ferreira, I., Kemp, S.A., Datir, R., Saito, A., Meng, B., Rakshit, P.,
810   Takaori-Kondo, A., Kosugi, Y., Uriu, K., Kimura, I., et al. (2021). SARS-CoV-2
811   B.1.617 mutations L452R and E484Q are not synergistic for antibody evasion. J
812   Infect Dis *224*, 989-994. 10.1093/infdis/jiab368.

813   Garcia-Beltran, W.F., Lam, E.C., St Denis, K., Nitido, A.D., Garcia, Z.H., Hauser,
814   B.M., Feldman, J., Pavlovic, M.N., Gregory, D.J., Poznansky, M.C., et al. (2021).
815   Multiple SARS-CoV-2 variants escape neutralization by vaccine-induced
816   humoral immunity. Cell *184*, 2372-2383 e2379. 10.1016/j.cell.2021.03.013.

817   GitHub (2022). "BA.2 sublineage with S:K147E, W152R, F157L, I210V, G257S,
818   D339H, G446S, N460K, R493Q (73 seq as of 2022-06-29, mainly India) (June
819   21, 2022)". https://github.com/cov-lineages/pango-designation/issues/773.

820   Gotoh, S., Ito, I., Nagasaki, T., Yamamoto, Y., Konishi, S., Korogi, Y.,
821   Matsumoto, H., Muro, S., Hirai, T., Funato, M., et al. (2014). Generation of
822   alveolar epithelial spheroids via isolated progenitor cells from human pluripotent
823   stem cells. Stem Cell Reports *3*, 394-403. 10.1016/j.stemcr.2014.07.005.

824   Gruell, H., Vanshylla, K., Korenkov, M., Tober-Lau, P., Zehner, M., Münn, F.,
825   Janicki, H., Augustin, M., Schommers, P., ErikSander, L., et al. (2022).

24

826   SARS-CoV-2 Omicron sublineages exhibit distinct antibody escape patterns.
827   Cell Host Microbe *in press*, https://doi.org/10.1016/j.chom.2022.1007.1002.
828   Hachmann, N.P., Miller, J., Collier, A.Y., Ventura, J.D., Yu, J., Rowe, M.,
829   Bondzie, E.A., Powers, O., Surve, N., Hall, K., and Barouch, D.H. (2022).
830   Neutralization Escape by SARS-CoV-2 Omicron Subvariants BA.2.12.1, BA.4,
831   and BA.5. N Engl J Med *387*, 86-88. 10.1056/NEJMc2206576.
832   Harvey, W.T., Carabelli, A.M., Jackson, B., Gupta, R.K., Thomson, E.C.,
833   Harrison, E.M., Ludden, C., Reeve, R., Rambaut, A., Consortium, C.-G.U., et al.
834   (2021). SARS-CoV-2 variants, spike mutations and immune escape. Nat Rev
835   Microbiol *19*, 409-424. 10.1038/s41579-021-00573-0.
836   Hashimoto, R., Takahashi, J., Shirakura, K., Funatsu, R., Kosugi, K., Deguchi,
837   S., Yamamoto, M., Muraoka, K., Morita, M., Tanaka, M., et al. (2022).
838   SARS-CoV-2 disrupts the respiratory vascular barrier by suppressing Claudin-5
839   expression. Sci Adv.
840   Jackson, C.B., Farzan, M., Chen, B., and Choe, H. (2022). Mechanisms of
841   SARS-CoV-2 entry into cells. Nat Rev Mol Cell Biol *23*, 3-20.
842   10.1038/s41580-021-00418-x.
843   Khan, K., Karim, F., Ganga, Y., Bernstein, M., Jule, Z., Reedoy, K., Cele, S.,
844   Lustig, G., Amoako, D., Wolter, N., et al. (2022). Omicron sub-lineages
845   BA.4/BA.5 escape BA.1 infection elicited neutralizing immunity. MedRxiv, doi:
846   https://doi.org/10.1101/2022.1104.1129.22274477.
847   Khare, S., Gurry, C., Freitas, L., Schultz, M.B., Bach, G., Diallo, A., Akite, N., Ho,
848   J., Lee, R.T., Yeo, W., et al. (2021). GISAID's Role in Pandemic Response.
849   China CDC Wkly *3*, 1049-1051. 10.46234/ccdcw2021.255.
850   Kimura, I., Kosugi, Y., Wu, J., Zahradnik, J., Yamasoba, D., Butlertanaka, E.P.,
851   Tanaka, Y.L., Uriu, K., Liu, Y., Morizako, N., et al. (2022a). The SARS-CoV-2
852   Lambda variant exhibits enhanced infectivity and immune resistance. Cell Rep
853   *38*, 110218. 10.1016/j.celrep.2021.110218.
854   Kimura, I., Yamasoba, D., Nasser, H., Zahradnik, J., Kosugi, Y., Wu, J., Nagata,
855   K., Uriu, K., Tanaka, Y.L., Ito, J., et al. (2022b). SARS-CoV-2 spike S375F
856   mutation characterizes the Omicron BA.1 variant. BioRxiv, doi:
857   https://doi.org/10.1101/2022.1104.1103.486864.
858   Kimura, I., Yamasoba, D., Tamura, T., Nao, N., Suzuki, T., Oda, Y., Mitoma, S.,
859   Ito, J., Nasser, H., Zahradnik, J., et al. (2022c). Virological characteristics of the
860   novel SARS-CoV-2 Omicron variants including BA.2.12.1, BA.4 and BA.5.
861   BioRxiv, doi: https://doi.org/10.1101/2022.1105.1126.493539.
862   Kislaya, I., Casaca, P., Borges, V., Sousa, C., Ferreira, B.I., Fernandes, E., Dias,
863   C.M., Duarte, S., Almeida, J.P., Grenho, I., et al. (2022). SARS-CoV-2 BA.5
864   vaccine breakthrough risk and severity compared with BA.2: a case-case and
865   cohort study using Electronic Health Records in Portugal. MedRxiv, doi:
866   https://doi.org/10.1101/2022.1107.1125.22277996.

867 Kondo, N., Miyauchi, K., and Matsuda, Z. (2011). Monitoring viral-mediated
868 membrane fusion using fluorescent reporter methods. Curr Protoc Cell Biol
869 *Chapter 26*, Unit 26 29. 10.1002/0471143030.cb2609s50.
870 Konishi, S., Gotoh, S., Tateishi, K., Yamamoto, Y., Korogi, Y., Nagasaki, T.,
871 Matsumoto, H., Muro, S., Hirai, T., Ito, I., et al. (2016). Directed Induction of
872 Functional Multi-ciliated Cells in Proximal Airway Epithelial Spheroids from
873 Human Pluripotent Stem Cells. Stem Cell Reports *6*, 18-25.
874 10.1016/j.stemcr.2015.11.010.
875 Kubik, S., Arrigo, N., Bonet, J., and Xu, Z. (2021). Mutational Hotspot in the
876 SARS-CoV-2 Spike Protein N-Terminal Domain Conferring Immune Escape
877 Potential. Viruses *13*. 10.3390/v13112114.
878 Li, H. (2018). Minimap2: pairwise alignment for nucleotide sequences.
879 Bioinformatics *34*, 3094-3100. 10.1093/bioinformatics/bty191.
880 Li, H., and Durbin, R. (2009). Fast and accurate short read alignment with
881 Burrows-Wheeler transform. Bioinformatics *25*, 1754-1760.
882 10.1093/bioinformatics/btp324.
883 Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G.,
884 Abecasis, G., Durbin, R., and Genome Project Data Processing Subgroup
885 (2009). The sequence alignment/map format and SAMtools. Bioinformatics *25*,
886 2078-2079. 10.1093/bioinformatics/btp352.
887 Liu, L., Iketani, S., Guo, Y., Chan, J.F.-W., Wang, M., Liu, L., Luo, Y., Chu, H.,
888 Huang, Y., Nair, M.S., et al. (2021). Striking antibody evasion manifested by the
889 Omicron variant of SARS-CoV-2. Nature, doi:
890 https://doi.org/10.1038/d41586-41021-03826-41583.
891 Liu, L., Wang, P., Nair, M.S., Yu, J., Rapp, M., Wang, Q., Luo, Y., Chan, J.F.,
892 Sahi, V., Figueroa, A., et al. (2020). Potent neutralizing antibodies against
893 multiple epitopes on SARS-CoV-2 spike. Nature *584*, 450-456.
894 10.1038/s41586-020-2571-7.
895 Lok, S.M. (2021). An NTD supersite of attack. Cell Host Microbe *29*, 744-746.
896 10.1016/j.chom.2021.04.010.
897 Lyke, K.E., Atmar, R.L., Islas, C.D., Posavad, C.M., Szydlo, D., Paul Chourdhury,
898 R., Deming, M.E., Eaton, A., Jackson, L.A., Branche, A.R., et al. (2022). Rapid
899 decline in vaccine-boosted neutralizing antibodies against SARS-CoV-2
900 Omicron variant. Cell Rep Med, 100679. 10.1016/j.xcrm.2022.100679.
901 Matsuyama, S., Nao, N., Shirato, K., Kawase, M., Saito, S., Takayama, I.,
902 Nagata, N., Sekizuka, T., Katoh, H., Kato, F., et al. (2020). Enhanced isolation of
903 SARS-CoV-2 by TMPRSS2-expressing cells. Proc Natl Acad Sci U S A *117*,
904 7001-7003. 10.1073/pnas.2002589117.
905 McCallum, M., De Marco, A., Lempp, F.A., Tortorici, M.A., Pinto, D., Walls, A.C.,
906 Beltramello, M., Chen, A., Liu, Z., Zatta, F., et al. (2021). N-terminal domain
907 antigenic mapping reveals a site of vulnerability for SARS-CoV-2. Cell *184*,

908   2332-2347 e2316. 10.1016/j.cell.2021.03.028.

909   Meng, B., Abdullahi, A., Ferreira, I.A.T.M., Goonawardane, N., Saito, A., Kimura,
910   I., Yamasoba, D., Gerber, P.P., Fatihi, S., Rathore, S., et al. (2022). Altered
911   TMPRSS2 usage by SARS-CoV-2 Omicron impacts tropism and fusogenicity.
912   Nature. 10.1038/s41586-022-04474-x.

913   Mirdita, M., Schutze, K., Moriwaki, Y., Heo, L., Ovchinnikov, S., and Steinegger,
914   M. (2022). ColabFold: making protein folding accessible to all. Nat Methods *19*,
915   679-682. 10.1038/s41592-022-01488-1.

916   Mittal, A., Khattri, A., and Verma, V. (2022). Structural and antigenic variations in
917   the spike protein of emerging SARS-CoV-2 variants. PLoS Pathog *18*, e1010260.
918   10.1371/journal.ppat.1010260.

919   Mlcochova, P., Kemp, S.A., Dhar, M.S., Papa, G., Meng, B., Ferreira, I., Datir, R.,
920   Collier, D.A., Albecka, A., Singh, S., et al. (2021). SARS-CoV-2 B.1.617.2 Delta
921   variant replication and immune evasion. Nature *599*, 114-119.
922   10.1038/s41586-021-03944-y.

923   Motozono, C., Toyoda, M., Zahradnik, J., Saito, A., Nasser, H., Tan, T.S., Ngare,
924   I., Kimura, I., Uriu, K., Kosugi, Y., et al. (2021). SARS-CoV-2 spike L452R
925   variant evades cellular immunity and increases infectivity. Cell Host Microbe *29*,
926   1124-1136. 10.1016/j.chom.2021.06.006.

927   NIH (2022). "Clinical Management Summary (April 8, 2022)"
928   https://www.covid19treatmentguidelines.nih.gov/management/clinical-managem
929   ent/clinical-management-summary/?utm_source=site&utm_medium=home&utm
930   _campaign=highlights.

931   Niwa, H., Yamamura, K., and Miyazaki, J. (1991). Efficient selection for
932   high-expression transfectants with a novel eukaryotic vector. Gene *108*, 193-199.
933   10.1016/0378-1119(91)90434-d.

934   Ozono, S., Zhang, Y., Ode, H., Sano, K., Tan, T.S., Imai, K., Miyoshi, K.,
935   Kishigami, S., Ueno, T., Iwatani, Y., et al. (2021). SARS-CoV-2 D614G spike
936   mutation increases entry efficiency with enhanced ACE2-binding affinity. Nat
937   Commun *12*, 848. 10.1038/s41467-021-21118-2.

938   Ozono, S., Zhang, Y., Tobiume, M., Kishigami, S., and Tokunaga, K. (2020).
939   Super-rapid quantitation of the production of HIV-1 harboring a luminescent
940   peptide tag. J Biol Chem *295*, 13023-13030. 10.1074/jbc.RA120.013887.

941   Planas, D., Saunders, N., Maes, P., Guivel-Benhassine, F., Planchais, C.,
942   Buchrieser, J., Bolland, W.-H., Porrot, F., Staropoli, I., Lemoine, F., et al. (2021).
943   Considerable escape of SARS-CoV-2 Omicron to antibody neutralization.
944   Nature, doi: https://doi.org/10.1038/d41586-41021-03827-41582.

945   Pruijssers, A.J., George, A.S., Schafer, A., Leist, S.R., Gralinksi, L.E., Dinnon,
946   K.H., 3rd, Yount, B.L., Agostini, M.L., Stevens, L.J., Chappell, J.D., et al. (2020).
947   Remdesivir Inhibits SARS-CoV-2 in Human Lung Cells and Chimeric SARS-CoV
948   Expressing the SARS-CoV-2 RNA Polymerase in Mice. Cell Rep *32*, 107940.

949   10.1016/j.celrep.2020.107940.

950   Qu, P., Faraone, J., Evans, J.P., Zou, X., Zheng, Y.M., Carlin, C., Bednash, J.S.,

951   Lozanski, G., Mallampalli, R.K., Saif, L.J., et al. (2022). Neutralization of the

952   SARS-CoV-2 Omicron BA.4/5 and BA.2.12.1 Subvariants. N Engl J Med *386*,

953   2526-2528. 10.1056/NEJMc2206725.

954   Reed, L.J., and Muench, H. (1938). A simple method of estimating fifty percent

955   endpoints. Am J Hygiene *27*, 493-497.

956   Saito, A., Irie, T., Suzuki, R., Maemura, T., Nasser, H., Uriu, K., Kosugi, Y.,

957   Shirakawa, K., Sadamasu, K., Kimura, I., et al. (2022). Enhanced fusogenicity

958   and pathogenicity of SARS-CoV-2 Delta P681R mutation. Nature *602*, 300-306.

959   10.1038/s41586-021-04266-9.

960   Sano, E., Suzuki, T., Hashimoto, R., Itoh, Y., Sakamoto, A., Sakai, Y., Saito, A.,

961   Okuzaki, D., Motooka, D., Muramoto, Y., et al. (2022). Cell response analysis in

962   SARS-CoV-2 infected bronchial organoids. Commun Biol *5*, 516.

963   10.1038/s42003-022-03499-2.

964   Stalls, V., Lindenberger, J., Gobeil, S.M., Henderson, R., Parks, R., Barr, M.,

965   Deyton, M., Martin, M., Janowska, K., Huang, X., et al. (2022). Cryo-EM

966   structures of SARS-CoV-2 Omicron BA.2 spike. Cell Rep *39*, 111009.

967   10.1016/j.celrep.2022.111009.

968   Stamatakis, A. (2014). RAxML version 8: a tool for phylogenetic analysis and

969   post-analysis of large phylogenies. Bioinformatics *30*, 1312-1313.

970   10.1093/bioinformatics/btu033.

971   Suryadevara, N., Shrihari, S., Gilchuk, P., VanBlargan, L.A., Binshtein, E., Zost,

972   S.J., Nargi, R.S., Sutton, R.E., Winkler, E.S., Chen, E.C., et al. (2021).

973   Neutralizing and protective human monoclonal antibodies recognizing the

974   N-terminal domain of the SARS-CoV-2 spike protein. Cell *184*, 2316-2331 e2315.

975   10.1016/j.cell.2021.03.029.

976   Suzuki, R., Yamasoba, D., Kimura, I., Wang, L., Kishimoto, M., Ito, J., Morioka,

977   Y., Nao, N., Nasser, H., Uriu, K., et al. (2022). Attenuated fusogenicity and

978   pathogenicity of SARS-CoV-2 Omicron variant. Nature.

979   10.1038/s41586-022-04462-1.

980   Takashita, E., Kinoshita, N., Yamayoshi, S., Sakai-Tagawa, Y., Fujisaki, S., Ito,

981   M., Iwatsuki-Horimoto, K., Chiba, S., Halfmann, P., Nagai, H., et al. (2022a).

982   Efficacy of antibodies and antiviral drugs against Covid-19 Omicron variant. N

983   Engl J Med. 10.1056/NEJMc2119407.

984   Takashita, E., Kinoshita, N., Yamayoshi, S., Sakai-Tagawa, Y., Fujisaki, S., Ito,

985   M., Iwatsuki-Horimoto, K., Halfmann, P., Watanabe, S., Maeda, K., et al. (2022b).

986   Efficacy of Antiviral Agents against the SARS-CoV-2 Omicron Subvariant BA.2.

987   N Engl J Med *386*, 1475-1477. 10.1056/NEJMc2201933.

988   Takashita, E., Yamayoshi, S., Simon, V., van Bakel, H., Sordillo, E.M., Pekosz,

989   A., Fukushi, S., Suzuki, T., Maeda, K., Halfmann, P., et al. (2022c). Efficacy of

990   Antibodies and Antiviral Drugs against Omicron BA.2.12.1, BA.4, and BA.5
991   Subvariants. N Engl J Med *387*, 468-470. 10.1056/NEJMc2207519.
992   Tamura, T., Yamasoba, D., Oda, Y., Ito, J., Kamasaki, T., Nao, N., Hashimoto,
993   R., Fujioka, Y., Suzuki, R., Wang, L., et al. (2022). Comparative pathogenicity of
994   SARS-CoV-2 Omicron subvariants including BA.1, BA.2, and BA.5. BioRxiv, doi:
995   https://doi.org/10.1101/2022.1108.1105.502758.
996   Toelzer, C., Gupta, K., Yadav, S.K.N., Borucu, U., Davidson, A.D., Kavanagh
997   Williamson, M., Shoemark, D.K., Garzoni, F., Staufer, O., Milligan, R., et al.
998   (2020). Free fatty acid binding pocket in the locked structure of SARS-CoV-2
999   spike protein. Science *370*, 725-730. 10.1126/science.abd3255.
1000 Tuekprakhon, A., Nutalai, R., Dijokaite-Guraliuc, A., Zhou, D., Ginn, H.M.,
1001 Selvaraj, M., Liu, C., Mentzer, A.J., Supasa, P., Duyvesteyn, H.M.E., et al.
1002 (2022). Antibody escape of SARS-CoV-2 Omicron BA.4 and BA.5 from vaccine
1003 and BA.1 serum. Cell *185*, 2422-2433 e2413. 10.1016/j.cell.2022.06.005.
1004 Uriu, K., Cardenas, P., Munoz, E., Barragan, V., Kosugi, Y., Shirakawa, K.,
1005 Takaori-Kondo, A., Sato, K., Ecuador-Covid19 Consortium, and The Genotype
1006 to Phenotype Japan (G2P-Japan) Consortium (2022). Characterization of the
1007 immune resistance of SARS-CoV-2 Mu variant and the robust immunity induced
1008 by Mu infection. J Infect Dis. 10.1093/infdis/jiac053.
1009 Uriu, K., Kimura, I., Shirakawa, K., Takaori-Kondo, A., Nakada, T.A., Kaneda, A.,
1010 Nakagawa, S., Sato, K., and The Genotype to Phenotype Japan (G2P-Japan)
1011 Consortium (2021). Neutralization of the SARS-CoV-2 Mu variant by
1012 convalescent and vaccine serum. N Engl J Med *385*, 2397-2399.
1013 10.1056/NEJMc2114706.
1014 VanBlargan, L.A., Errico, J.M., Halfmann, P.J., Zost, S.J., Crowe, J.E., Jr.,
1015 Purcell, L.A., Kawaoka, Y., Corti, D., Fremont, D.H., and Diamond, M.S. (2022).
1016 An infectious SARS-CoV-2 B.1.1.529 Omicron virus escapes neutralization by
1017 therapeutic monoclonal antibodies. Nat Med. 10.1038/s41591-021-01678-y.
1018 Voss, W.N., Hou, Y.J., Johnson, N.V., Delidakis, G., Kim, J.E., Javanmardi, K.,
1019 Horton, A.P., Bartzoka, F., Paresi, C.J., Tanno, Y., et al. (2021). Prevalent,
1020 protective, and convergent IgG recognition of SARS-CoV-2 non-RBD spike
1021 epitopes. Science *372*, 1108-1112. 10.1126/science.abg5268.
1022 Wang, Q., Guo, Y., Iketani, S., Nair, M.S., Li, Z., Mohri, H., Wang, M., Yu, J.,
1023 Bowen, A.D., Chang, J.Y., et al. (2022). Antibody evasion by SARS-CoV-2
1024 Omicron subvariants BA.2.12.1, BA.4, & BA.5. Nature.
1025 10.1038/s41586-022-05053-w.
1026 WHO (2022). "Tracking SARS-CoV-2 variants (July 19, 2022)"
1027 https://www.who.int/en/activities/tracking-SARS-CoV-2-variants.
1028 Yamamoto, M., Kiso, M., Sakai-Tagawa, Y., Iwatsuki-Horimoto, K., Imai, M.,
1029 Takeda, M., Kinoshita, N., Ohmagari, N., Gohda, J., Semba, K., et al. (2020).
1030 The Anticoagulant Nafamostat Potently Inhibits SARS-CoV-2 S

1031 Protein-Mediated Fusion in a Cell Fusion Assay System and Viral Infection In
1032 Vitro in a Cell-Type-Dependent Manner. Viruses *12*. 10.3390/v12060629.
1033 Yamamoto, Y., Gotoh, S., Korogi, Y., Seki, M., Konishi, S., Ikeo, S., Sone, N.,
1034 Nagasaki, T., Matsumoto, H., Muro, S., et al. (2017). Long-term expansion of
1035 alveolar stem cells derived from human iPS cells in organoids. Nat Methods *14*,
1036 1097-1106. 10.1038/nmeth.4448.
1037 Yamasoba, D., Kimura, I., Kosugi, Y., Fujita, S., Uriu, K., Ito, J., Sato, K., and
1038 The Genotype to Phenotype Japan (G2P-Japan) Consortium (2022a).
1039 Neutralization sensitivity of Omicron BA.2.75 to therapeutic monoclonal
1040 antibodies. BioRxiv, doi: https://doi.org/10.1101/2022.1107.1114.500041.
1041 Yamasoba, D., Kimura, I., Nasser, H., Morioka, Y., Nao, N., Ito, J., Uriu, K.,
1042 Tsuda, M., Zahradnik, J., Shirakawa, K., et al. (2022b). Virological
1043 characteristics of the SARS-CoV-2 Omicron BA.2 spike. Cell.
1044 10.1016/j.cell.2022.04.035.
1045 Yamasoba, D., Kosugi, Y., Kimura, I., Fujita, S., Uriu, K., Ito, J., Sato, K., and
1046 The Genotype to Phenotype Japan (G2P-Japan) Consortium (2022c).
1047 Neutralisation sensitivity of SARS-CoV-2 omicron subvariants to therapeutic
1048 monoclonal antibodies. Lancet Infect Dis *22*, 942-943.
1049 10.1016/S1473-3099(22)00365-6.
1050 Yan, R., Zhang, Y., Li, Y., Xia, L., Guo, Y., and Zhou, Q. (2020). Structural basis
1051 for the recognition of SARS-CoV-2 by full-length human ACE2. Science *367*,
1052 1444-1448. 10.1126/science.abb2762.
1053 Zahradnik, J., Dey, D., Marciano, S., Kolarova, L., Charendoff, C.I., Subtil, A.,
1054 and Schreiber, G. (2021a). A Protein-Engineered, Enhanced Yeast Display
1055 Platform for Rapid Evolution of Challenging Targets. ACS Synth Biol *10*,
1056 3445-3460. 10.1021/acssynbio.1c00395.
1057 Zahradnik, J., Marciano, S., Shemesh, M., Zoler, E., Harari, D., Chiaravalli, J.,
1058 Meyer, B., Rudich, Y., Li, C., Marton, I., et al. (2021b). SARS-CoV-2 variant
1059 prediction and antiviral drug design are enabled by RBD in vitro evolution. Nat
1060 Microbiol *6*, 1188-1198. 10.1038/s41564-021-00954-4.
1061 Zahradnik, J., Nunvar, J., and Schreiber, G. (2022). Perspectives: SARS-CoV-2
1062 Spike Convergent Evolution as a Guide to Explore Adaptive Advantage. Front
1063 Cell Infect Microbiol *12*, 748948. 10.3389/fcimb.2022.748948.
1064
1065

1066   **Table 1. Effects of three antiviral drugs against BA.2.75 in AO**

	EC_{50} (µM)				EC_{50} ratio				
	B.1.1	BA.2	BA.5	BA.2.75	BA.5/BA.2	BA.2.75/BA.2	BA.2/B.1.1	BA.5/B.1.1	BA.2.75/B.1.1
Remdesivir	1.08	1.89	1.31	0.63	0.70	0.34	1.75	1.21	0.59
EIDD-1931	0.24	0.02	0.21	0.08	8.82	3.53	0.10	0.89	0.36
Nirmatrelvir	0.84	0.85	0.63	0.81	0.74	0.95	1.02	0.75	0.97

1067

1068 **Figure legends**
1069
1070 **Figure 1. Epidemics of BA.2.75 in India**
1071 (**A**) A maximum likelihood tree of Omicron sublineages. Sequences of
1072 BA.1–BA.5 sampled from South Africa and BA.2.75 are included. The mutations
1073 acquired in the S protein of BA.2.75 are indicated in the panel. Note that R493Q
1074 is a reversion [i.e., back mutation from the BA.1–BA.3 lineages (R493) to the
1075 B.1.1 lineage (Q493)]. Bootstrap values, *, ≥ 0.8; **, ≥ 0.95.
1076 (**B**) Amino acid differences among BA.2, BA.2.75, and BA.5. Heatmap color
1077 indicates the frequency of amino acid substitutions.
1078 (**C**) Lineage frequencies of BA.5 (left) and BA.2.75 (right) in each Indian state.
1079 SARS-CoV-2 sequences collected from June 15, 2022, to July 15, 2022, were
1080 analyzed.
1081 (**D**) Epidemic dynamics of SARS-CoV-2 lineages in Indian states. Results for
1082 BA.2.75 and BA.5 are shown. The observed daily sequence frequency (dot) and
1083 the dynamics (posterior mean, line; 95% CI, ribbon) are shown. The dot size is
1084 proportional to the number of sequences.
1085 (**E**) Estimated relative R_e of each viral lineage, assuming a fixed generation time
1086 of 2.1 days. The R_e value of BA.2 is set at 1. The posterior (violin), posterior
1087 mean (dot), and 95% CI (line) are shown. The average values across India
1088 estimated by a Bayesian hierarchical model are shown, and the state-specific R_e
1089 values are shown in **Figure S1B**. The dynamics of the top seven predominant
1090 lineages in India were estimated. BA.5 sublineages are summarized as "BA.5",
1091 and non-predominant BA.2 sublineages are summarized as "other BA.2".
1092 See also **Figure S1 and Table S1**.
1093
1094 **Figure 2. Immune resistance of BA.2.75**
1095 Neutralization assays were performed with pseudoviruses harboring the S
1096 proteins of B.1.1 (the D614G-bearing ancestral virus), BA.1, BA.2, BA.2.75.
1097 Delta pseudovirus is included only in the experiment shown in **F**. The BA.2
1098 S-based derivatives are included in **H and I**. The following sera were used.
1099 (**A–C**) BNT162b2 vaccine sera (15 donors) collected at 1 month after 2nd-dose
1100 vaccination (**A**), 1 month after 3rd-dose vaccination (**B**), and 4 months after
1101 3rd-dose vaccination (**C**).
1102 (**D**) Convalescent sera from fully vaccinated individuals who had been infected
1103 with BA.1 after full vaccination (16 2-dose vaccinated donors).
1104 (**E**) Convalescent sera from fully vaccinated individuals who had been infected
1105 with BA.2 after full vaccination (9 2-dose vaccinated and 5 3-dose vaccinated. 14
1106 donors in total).
1107 (**F**) Convalescent sera from unvaccinated individuals who had been infected with
1108 Delta (18 donors).

32

1109   (**G–I**) Sera from hamsters infected with BA.1 (6 hamsters) (**G**), BA.2 (6

1110   hamsters) (**H**), and BA.5 (6 hamsters) (**I**).

1111   Assays for each serum sample were performed in triplicate to determine the

1112   50% neutralization titer (NT50). Each dot represents one NT50 value, and the

1113   geometric mean and 95% CI are shown. The numbers in the panels indicate the

1114   fold change resistance versus BA.2 (**B–E, G and H**) or BA.5 (**I**). The horizontal

1115   dashed line indicates the detection limit (120-fold in other than **F**, 40-fold in **F**).

1116   Statistically significant differences were determined by two-sided Wilcoxon

1117   signed-rank tests. The *P* values versus BA.2 (**B–E, G and H**) or BA.5 (**I**) are

1118   indicated in the panels. For the BA.2 derivatives and B.1.1 (**H and I**), statistically

1119   significant differences versus BA.2 (*P* < 0.05) are indicated with asterisks.

1120   Information on the vaccinated/convalescent donors is summarized in **Table S2**.

1121   See also **Table S2**.

1122

1123   **Figure 3. Virological features of BA.2.75 S *in vitro***

1124   (**A**) Pseudovirus assay. The percent infectivity compared to that of the virus

1125   pseudotyped with the BA.2 S protein are shown.

1126   (**B**) Binding affinity of the RBD of SARS-CoV-2 S protein to ACE2 by yeast

1127   surface display. The K_D value indicating the binding affinity of the RBD of the

1128   SARS-CoV-2 S protein to soluble ACE2 when expressed on yeast is shown.

1129   (**C**) Electrostatic potential of B.1.1 S RBD (PDB: 6M17) (Yan et al., 2020), BA.2

1130   S RBD (PDB: 7UB0) (Stalls et al., 2022) and BA.2.75 S RBD. The structure of

1131   BA.2.75 S RBD was prepared using AlphaFold2 (Mirdita *et al.*, 2022).

1132   Electrostatic potential surface depictions calculated by PDB2PQR tool (Dolinsky

1133   et al., 2007) with the positions of BA.2.75 characteristic mutations. The scale bar

1134   shows the electrostatic charge [kT/e].

1135   (**D**) The binding of BA.2.75 S RBD and human ACE2 (PDB: 6M17) (Yan *et al.*,

1136   2020). Left, the four substitutions in BA.2.75 S RBD compared to BA.2 S RBD

1137   are highlighted. Right, binding of BA.2.75 S RBD (top) and human ACE2

1138   (bottom). The electrostatic potential surface of human ACE2 is shown.

1139   (**E and F**) S-based fusion assay. (**E**) S protein expression on the cell surface.

1140   The summarized data are shown. (**F**) S-based fusion assay in Calu-3 cells. The

1141   recorded fusion activity (arbitrary units) is shown. The dashed green line

1142   indicates the results of BA.2.

1143   Assays were performed in quadruplicate (**A and F**) or triplicate (**B and E**), and

1144   the presented data are expressed as the average ± SD. In **A and B**, the dashed

1145   horizontal lines indicated the value of BA.2. In **A, B and E**, each dot indicates the

1146   result of an individual replicate. In **A, B and E**, statistically significant differences

1147   between BA.2 and other variants (*, *P* < 0.05) were determined by two-sided

1148   Student's *t* tests. In **F**, statistically significant differences between BA.2 and other

33

1149 variants across timepoints were determined by multiple regression. The FWERs

1150 calculated using the Holm method are indicated in the figures.

1151 See also **Figure S3**.

1152

1153 **Figure 4. Growth capacity of BA.2.75 *in vitro***

1154 (**A–I**) Growth kinetics of B.1.1, Delta, BA.2, BA.5 and BA.2.75. Clinical isolates of

1155 B.1.1 (strain TKYE610670; GISAID ID: EPI_ISL_479681), Delta (B.1.617.2,

1156 strain TKYTK1734; GISAID ID: EPI_ISL_2378732), BA.2 (strain TY40-385;

1157 GISAID ID: EPI_ISL_9595859), BA.5 (strain TKYS14631; GISAID ID:

1158 EPI_ISL_12812500), and BA.2.75 (strain TY41-716; GISAID ID:

1159 EPI_ISL_13969765) were inoculated into Vero cells (**A**), VeroE6/TMPRSS2

1160 cells (**B**), HEK293-ACE2/TMPRSS2 cells (**C**), AO-ALI (**D**), iPSC-derived airway

1161 epithelial cells (**E**), iPSC-derived lung epithelial cells (**F**), and an

1162 airway-on-a-chip system (**G and H**; the scheme of experimental system is

1163 illustrated in **Figure S3C**). The copy numbers of viral RNA in the culture

1164 supernatant (**A–C**), the apical sides of cultures (**D–F**), the top (**G**) and bottom (**H**)

1165 channels of an airway-on-a-chip were routinely quantified by RT–qPCR. The

1166 dashed green line in each panel indicates the results of BA.2. In **I**, the

1167 percentage of viral RNA load in the bottom channel per top channel at 6 d.p.i.

1168 (i.e., % invaded virus from the top channel to the bottom channel) is shown.

1169 (**J**) Plaque assay. VeroE6/TMPRSS2 cells were used for the target cells.

1170 Representative panels (left) and a summary of the recorded plaque diameters

1171 (20 plaques per virus) (right) are shown.

1172 Assays were performed in quadruplicate, and the presented data are expressed

1173 as the average ± SD. In **A–H**, statistically significant differences between BA.2

1174 and the other variants across timepoints were determined by multiple regression.

1175 The FWERs calculated using the Holm method are indicated in the figures. In **I**

1176 **and J (right)**, statistically significant differences versus BA.2 (*, $P < 0.05$) were

1177 determined by two-sided Mann–Whitney U tests. Each dot indicates the result of

1178 an individual replicate.

1179 See also **Figure S3**.

1180

1181 **Figure 5. Virological characteristics of BA.2.75 *in vivo***

1182 Syrian hamsters were intranasally inoculated with Delta, BA.2, BA.5 and

1183 BA.2.75. Six hamsters at the same age were intranasally inoculated with saline

1184 (uninfected). Six hamsters per each group were used to routinely measure

1185 respective parameters (**A and B**). Four hamsters per each group were

1186 euthanized at 2 and 5 d.p.i and used for virological and pathological analysis

1187 (**C–G**).

1188 (**A**) Body weight, Penh, Rpef, BPM, and SpO$_2$ values of infected hamsters (n = 6

1189 per infection group).

34

1190   (**B**) Viral RNA loads in the oral swab (n = 6 per infection group).

1191   (**C**) Viral RNA loads in the lung hilum (left) and lung periphery (right) of infected

1192   hamsters (n = 4 per infection group) at 2 d.p.i. (top) and 5 d.p.i. (bottom).

1193   (**D and E**) IHC of the viral N protein in the lungs at 2 d.p.i. (top) and 5 d.p.i.

1194   (bottom) of all infected hamsters. (**D**) Representative figures. (**E**) Percentage of

1195   N-positive cells in whole lung lobes (n = 4 per infection group). The raw data are

1196   shown in **Figure S4B and S4C**.

1197   (**F and G**) (**F**) H&E staining of the lungs of infected hamsters. Representative

1198   figures are shown. Uninfected lung alveolar space and bronchioles are also

1199   shown. (**G**) Histopathological scoring of lung lesions (n = 4 per infection group).

1200   Representative pathological features are reported in our previous studies

1201   (Kimura *et al.*, 2022c; Saito *et al.*, 2022; Suzuki *et al.*, 2022; Yamasoba *et al.*,

1202   2022b).

1203   (**H**) Type II pneumocytes in the lungs of infected hamsters. The percentage of

1204   the area of type II pneumocytes in the lung at 5 d.p.i. is summarized. The raw

1205   data are shown in **Figure S4D**.

1206   In **A–C, E, G and H**, data are presented as the average ± SEM. In **C, E** and **H**,

1207   each dot indicates the result of an individual hamster.

1208   In **A, B and G**, statistically significant differences between BA.2 and other

1209   variants across timepoints were determined by multiple regression. In **A**, the 0

1210   d.p.i. data were excluded from the analyses. The FWERs calculated using the

1211   Holm method are indicated in the figures.

1212   In **C, E and G**, the statistically significant differences between BA.2 and other

1213   variants were determined by a two-sided Mann–Whitney *U* test.

1214   In **D and F**, each panel shows a representative result from an individual infected

1215   hamster. Scale bars, 500 μm (**D**); 200 μm (**F**).

1216   See also **Figure S4**.

1217

1218   **Table S1.** Estimated relative R_e values of viral lineages in India, related to

1219   **Figure 1**

1220

1221   **Table S2.** Human sera used in this study, related to **Figure 2**

1222

1223   **Table S3.** Primers used for the construction of SARS-CoV-2 S expression

1224   plasmids, related to **Figures 2 and 3**

1225

1226   **Table S4.** Summary of unexpected amino acid mutations detected in the

1227   working virus stocks, related to **Figures 4 and 5 and Table 1**

1228

1229   **Figure S1. Epidemic dynamics of BA.2.75 in India, related to Figure 1**

35

1230   (**A**) Amino acid differences in B.1.1, Delta, BA.2, BA.5 and BA.2.75 compared to
1231   the SARS-CoV-2 A lineage. Heatmap color indicates the frequency of amino
1232   acid mutations.
1233   (**B**) Estimated relative R_e of each viral lineage, assuming a fixed generation time
1234   of 2.1 days. The R_e value of BA.2 is set at 1. The posterior (violin), posterior
1235   mean (dot), and 95% CI (line) are shown. The R_e values for respective Indian
1236   states are shown. The dynamics of the top seven predominant lineages in India
1237   were estimated. BA.5 sublineages are summarized as "BA.5", and
1238   non-predominant BA.2 sublineages are summarized as "other BA.2". Raw data
1239   are summarized in **Table S1**.
1240   (**C**) Fold change in R_e values between BA.2.75 and BA.5. Posterior mean (dot)
1241   and 95% CI (line) are shown. Red indicates that the 95% CI does not overlap
1242   with the value of 1.
1243
1244   **Figure S2. Effects of antiviral drugs in AO, related to Table 1**
1245   (**A**) Antiviral effects of the three drugs in AO culture. The assay of each antiviral
1246   drugs was performed in quadruplicate, and the 50% effective concentration
1247   (EC_{50}) was calculated. The data are summarized in **Table 1**.
1248   (**B**) Cytotoxic effects of the three drugs in AO culture. The assay of each antiviral
1249   drugs was performed in quadruplicate, and the 50% cytotoxic concentration
1250   (CC_{50}) was calculated. The CC_{50} values are indicated in the panels.
1251
1252   **Figure S3. Virological features of BA.2.75 *in vitro*, related to Figures 3 and**
1253   **4**
1254   (**A**) Fold increase in pseudovirus infectivity based on TMPRSS2 expression.
1255   (**B**) The structural effect of the D339H substitution in the BA.2.75 S RBD. The
1256   BA.2 S RBD (PDB: 7UB0) (Stalls *et al.*, 2022) and an AlphaFold2 structural
1257   model of BA.2.75 S RBD (bottom) are shown. The residues 339 and 343 are
1258   indicated in stick. The squared regions are enlarged in the right panel. A dashed
1259   line in the top panel indicates ion-dipole interaction between the D339 and the
1260   N343 residues.
1261   (**C**) A scheme of airway-on-a-chip system.
1262
1263   **Figure S4. Histological observations in infected hamsters, related to**
1264   **Figure 5**
1265   (**A**) IHC of the viral N protein in the middle portion of the tracheas of all infected
1266   hamsters at 2 d.p.i (4 hamsters per infection group). Each panel shows a
1267   representative result from an individual infected hamster.
1268   (**B and C**) IHC of the SARS-CoV-2 N protein in the lungs of infected hamsters at
1269   2 d.p.i. (**B**) and 5 d.p.i (**C**) (4 hamsters per infection group). In each panel, IHC
1270   staining (top) and the digitalized N-positive area (bottom, indicated in red) are

1271   shown. The red numbers in the bottom panels indicate the percentage of the

1272   N-positive area. Summarized data are shown in **Figure 5E**.

1273   (**D**) Type II pneumocytes in the lungs of infected hamsters (4 hamsters per

1274   infection group). H&E staining (top) and the digitalized inflammatory area with

1275   type II pneumocytes (bottom, indicated in red) are shown. The red numbers in

1276   the bottom panels indicate the percentage of inflammatory area with type II

1277   pneumocytes. Summarized data are shown in **Figure 5H**.

1278   Scale bars, 1 mm (**A**); 5 mm (**B–D**).

1279 **STAR⧫METHODS**

1280

1281 **KEY RESOURCES TABLE**

1282 **RESOURCE AVAILABILITY**

1283 **Lead Contact**

1284 Further information and requests for resources and reagents should be directed
1285 to and will be fulfilled by the Lead Contact, Kei Sato
1286 (KeiSato@g.ecc.u-tokyo.ac.jp).

1287

1288 **Materials Availability**

1289 All unique reagents generated in this study are listed in the Key Resources
1290 Table and available from the Lead Contact with a completed Materials Transfer
1291 Agreement.

1292

1293 **Data and Software Availability**

1294 All databases/datasets used in this study are available from GISAID database
1295 (https://www.gisaid.org) and GenBank database (https://www.gisaid.org;
1296 EPI_SET ID: EPI_SET_220804hy).

1297 The computational codes used in the present study, the raw data of
1298 virus sequences, and the GISAID supplemental table for EPI_SET ID:
1299 EPI_SET_220804hy are available in the GitHub repository
1300 (https://github.com/TheSatoLab/Omicron_BA.2.75).

1301

1302 **EXPERIMENTAL MODEL AND SUBJECT DETAILS**

1303 **Ethics statement**

1304 All experiments with hamsters were performed in accordance with the Science
1305 Council of Japan's Guidelines for the Proper Conduct of Animal Experiments.
1306 The protocols were approved by the Institutional Animal Care and Use
1307 Committee of National University Corporation Hokkaido University (approval ID:
1308 20-0123 and 20-0060). All experiments with mice were also performed in
1309 accordance with the Science Council of Japan's Guidelines for the Proper
1310 Conduct of Animal Experiments. All protocols involving specimens from human
1311 subjects recruited at Kyoto University were reviewed and approved by the
1312 Institutional Review Boards of Kyoto University (approval ID: G1309) and Chiba
1313 University (approval ID: HS202103-03). All human subjects provided written
1314 informed consent. All protocols for the use of human specimens were reviewed
1315 and approved by the Institutional Review Boards of The Institute of Medical
1316 Science, The University of Tokyo (approval IDs: 2021-1-0416 and
1317 2021-18-0617), Kyoto University (approval ID: G0697), Kumamoto University
1318 (approval IDs: 2066 and 2074), and University of Miyazaki (approval ID:
1319 O-1021).

1320
1321   **Human serum collection**
1322   Vaccine sera of fifteen individuals who had BNT162b2 vaccine
1323   (Pfizer/BioNTech) (average age: 38, range: 24–48; 53% male) (**Figures 2A–2C**)
1324   were obtained at one month after the second dose, one month after the third
1325   dose, and four months after the third dose. The details of the vaccine sera are
1326   summarized in **Table S2**.
1327   Convalescent sera were collected from the following donors: fully
1328   vaccinated individuals who had been infected with BA.1 (16 2-dose vaccinated.
1329   10–27 days after testing; average age: 48, range: 20–76, 44% male) (**Figure
1330   2D**), fully vaccinated individuals who had been infected with BA.2 (9 2-dose
1331   vaccinated and 5 3-dose vaccinated. 11–61 days after testing. n=14 in total;
1332   average age: 47, range: 24–84, 64% male) (**Figure 2E**), and unvaccinated
1333   individuals who had been infected with Delta (6–55 days after testing. n=18 in
1334   total; average age: 50, range: 22–67, 78% male) (**Figure 2F**). The SARS-CoV-2
1335   variants were identified as previously described (Kimura *et al.*, 2022c;
1336   Yamasoba *et al.*, 2022b). Sera were inactivated at 56°C for 30 minutes and
1337   stored at –80°C until use. The details of the convalescent sera are summarized
1338   in **Table S2**.
1339
1340   **Cell culture**
1341   HEK293T cells (a human embryonic kidney cell line; ATCC, CRL-3216),
1342   HEK293 cells (a human embryonic kidney cell line; ATCC, CRL-1573) and
1343   HOS-ACE2/TMPRSS2 cells (HOS cells stably expressing human ACE2 and
1344   TMPRSS2) (Ferreira et al., 2021; Ozono et al., 2021) were maintained in DMEM
1345   (high glucose) (Sigma-Aldrich, Cat# 6429-500ML) containing 10% fetal bovine
1346   serum (FBS, Sigma-Aldrich Cat# 172012-500ML) and 1%
1347   penicillin-streptomycin (PS) (Sigma-Aldrich, Cat# P4333-100ML).
1348   HEK293-ACE2 cells (HEK293 cells stably expressing human ACE2) (Motozono
1349   *et al.*, 2021) were maintained in DMEM (high glucose) containing 10% FBS, 1
1350   μg/ml puromycin (InvivoGen, Cat# ant-pr-1) and 1% PS.
1351   HEK293-ACE2/TMPRSS2 cells (HEK293 cells stably expressing human ACE2
1352   and TMPRSS2) (Motozono *et al.*, 2021) were maintained in DMEM (high
1353   glucose) containing 10% FBS, 1 μg/ml puromycin, 200 ng/ml hygromycin
1354   (Nacalai Tesque, Cat# 09287-84) and 1% PS.
1355   Vero cells [an African green monkey (*Chlorocebus sabaeus*) kidney cell line;
1356   JCRB Cell Bank, JCRB0111] were maintained in Eagle's minimum essential
1357   medium (EMEM) (Sigma-Aldrich, Cat# M4655-500ML) containing 10% FBS and
1358   1% PS.
1359   VeroE6/TMPRSS2 cells (VeroE6 cells stably expressing human TMPRSS2;
1360   JCRB Cell Bank, JCRB1819) (Matsuyama et al., 2020) were maintained in

39

1361 DMEM (low glucose) (Wako, Cat# 041-29775) containing 10% FBS, G418 (1

1362 mg/ml; Nacalai Tesque, Cat# G8168-10ML) and 1% PS.

1363 Calu-3/DSP$_{1-7}$ cells (Calu-3 cells stably expressing DSP$_{1-7}$) (Yamamoto et al.,

1364 2020) were maintained in EMEM (Wako, Cat# 056-08385) containing 20% FBS

1365 and 1% PS.

1366 Human airway and alveolar epithelial cells derived from human induced

1367 pluripotent stem cells (iPSCs) were manufactured according to established

1368 protocols as described below (see "Preparation of human airway and alveolar

1369 epithelial cells from human iPSCs" section) and provided by HiLung Inc.

1370 Airway organoids (AO) and AO-derived air-liquid interface model (AO-ALI) were

1371 generated according to established protocols as described below (see "Airway

1372 organoids" and "AO-ALI model" sections).

1373

1374 **METHOD DETAILS**

1375 **Viral genome sequencing**

1376 Viral genome sequencing was performed as previously described (Meng *et al.*,

1377 2022; Motozono *et al.*, 2021; Saito *et al.*, 2022; Suzuki *et al.*, 2022; Yamasoba *et*

1378 *al.*, 2022b). Briefly, the virus sequences were verified by viral RNA-sequencing

1379 analysis. Viral RNA was extracted using a QIAamp viral RNA mini kit (Qiagen,

1380 Cat# 52906). The sequencing library employed for total RNA sequencing was

1381 prepared using the NEB next ultra RNA library prep kit for Illumina (New England

1382 Biolabs, Cat# E7530). Paired-end 76-bp sequencing was performed using a

1383 MiSeq system (Illumina) with MiSeq reagent kit v3 (Illumina, Cat# MS-102-3001).

1384 Sequencing reads were trimmed using fastp v0.21.0 (Chen et al., 2018) and

1385 subsequently mapped to the viral genome sequences of a lineage B isolate

1386 (strain Wuhan-Hu-1; GenBank accession number: NC_045512.2) (Matsuyama

1387 *et al.*, 2020) using BWA-MEM v0.7.17 (Li and Durbin, 2009). Variant calling,

1388 filtering, and annotation were performed using SAMtools v1.9 (Li et al., 2009)

1389 and snpEff v5.0e (Cingolani et al., 2012).

1390

1391 **Phylogenetic analyses**

1392 To construct an ML tree of Omicron lineages (BA.1–BA.5) sampled from South

1393 Africa and BA.2.75 (shown in **Figure 1A**), the genome sequence data of

1394 SARS-CoV-2 and its metadata were downloaded from the GISAID database

1395 (https://www.gisaid.org/) (Khare et al., 2021) on July 23, 2022. We excluded the

1396 data of viral strains with the following features from the analysis: i) a lack

1397 collection date information; ii) sampling from animals other than humans, iii) >2%

1398 undetermined nucleotide characters, or iv) sampling by quarantine. From each

1399 viral lineage, 30 sequences were randomly sampled and used for tree

1400 construction, in addition to an outgroup sequence, EPI_ISL_466615,

1401 representing the oldest isolate of B.1.1 obtained in the UK. The viral genome

40

1402 sequences were mapped to the reference sequence of Wuhan-Hu-1 (GenBank
1403 accession number: NC_045512.2) using Minimap2 v2.17 (Li, 2018) and
1404 subsequently converted to a multiple sequence alignment according to the
1405 GISAID phylogenetic analysis pipeline
1406 (https://github.com/roblanf/sarscov2phylo). The alignment sites corresponding to
1407 the 1–265 and 29674–29903 positions in the reference genome were masked
1408 (i.e., converted to NNN). Alignment sites at which >50% of sequences contained
1409 a gap or undetermined/ambiguous nucleotide were trimmed using trimAl v1.2
1410 (Capella-Gutierrez et al., 2009). Phylogenetic tree construction was performed
1411 via a three-step protocol: i) the first tree was constructed; ii) tips with longer
1412 external branches (Z score > 4) were removed from the dataset; iii) and the final
1413 tree was constructed. Tree reconstruction was performed by RAxML v8.2.12
1414 (Stamatakis, 2014) under the GTRCAT substitution model. The node support
1415 value was calculated by 100 times bootstrap analysis.
1416

1417 **Modelling the epidemic dynamics of SARS-CoV-2 lineages**
1418 To quantify the spread rate of each SARS-CoV-2 lineage in the human
1419 population in India, we estimated the relative R_e of each viral lineage according
1420 to the epidemic dynamics, calculated on the basis of viral genomic surveillance
1421 data. The data were downloaded from the GISAID database
1422 (https://www.gisaid.org/) on August 1, 2022. We excluded the data of viral
1423 strains with the following features from the analysis: i) a lack of collection date
1424 information; ii) sampling in animals other than humans; or iii) sampling by
1425 quarantine. We analyzed the datasets of the ten states of India, where ≥20
1426 sequences of either BA.2.75 or BA.5 are reported (i.e., Himachal Pradesh,
1427 Odisha, Haryana, Rajasthan, and Maharashtra, Gujarat, West Bengal, Delhi,
1428 Tamil Nadu, and Telangana). BA.5 sublineages are summarized as "BA.5", and
1429 BA.2 sublineages with ≤400 sequences are summarized as "other BA.2".
1430 Subsequently, the dynamics of the top seven predominant lineages in India were
1431 estimated from April 24, 2022, to August 1, 2022, were analyzed. The number of
1432 viral sequences of each viral lineage collected on each day in each country was
1433 counted, and the count matrix was constructed as an input for the statistical
1434 model below.
1435 　　　　We constructed a Bayesian hierarchical model to represent relative
1436 lineage growth dynamics with multinomial logistic regression as described in our
1437 previous study (Yamasoba *et al.*, 2022b). In brief, we incorporated a hierarchical
1438 structure into the slope parameter over time, which enabled us to estimate the
1439 global average relative R_e of each viral lineage in India as well as the average
1440 value for each country. Arrays in the model index over one or more indices: L = 7
1441 viral lineages l; S = 10 states s; and T = 100 days t. The model is:

$$\beta_{ls} \sim \text{Student\_t}(6, \beta_l, \sigma_l)$$

41

$$\mu_{lst} = \alpha_{ls} + \beta_{ls}t$$
$$\theta_{.st} = \mathrm{softmax}(\mu_{.st})$$

$$y_{lst} \sim \mathrm{Multinomial}\left(\sum_l y_{lst}, \theta_{.st}\right)$$

1442 The explanatory variable was time, t, and the outcome variable was y_{lst}, which
1443 represented the count of viral lineage l in state s at time t. The slope
1444 parameter of lineage l in state s, β_{ls}, was generated from a Student's t
1445 distribution with hyperparameters of the mean, β_l, and the standard deviation,
1446 σ_l. As the distribution generating β_{ls}, we used a Student's t distribution with six
1447 degrees of freedom instead of a normal distribution to reduce the effects of
1448 outlier values of β_{ls}. In the model, the linear estimator μ_{st}, consisting of the
1449 intercept $\alpha_{.s}$ and the slope $\beta_{.s}$, was converted to the simplex $\theta_{.st}$, which
1450 represented the probability of occurrence of each viral lineage at time t in state
1451 s, based on the softmax link function defined as:

$$\mathrm{softmax}(x) = \frac{\exp(x)}{\sum_i \exp(x_i)}$$

1452 y_{lst} is generated from $\theta_{.st}$ and the total count of all lineages at time t in state s
1453 according to a multinomial distribution.
1454   The relative R_e of each viral lineage in each county (r_{ls}) was calculated
1455 according to the slope parameter β_{ls} as:

$$r_{ls} = \exp(\gamma\beta_{ls})$$

1456 where γ is the average viral generation time (2.1 days)
1457 (http://sonorouschocolate.com/covid19/index.php?title=Estimating_Generation_
1458 Time_Of_Omicron). Similarly, the global average relative R_e of each viral lineage
1459 was calculated according to the slope hyperparameter β_l as:

$$r_l = \exp(\gamma\beta_l)$$

1460 For parameter estimation, the intercept and slope parameters of the BA.2 variant
1461 were fixed at 0. Consequently, the relative R_e of BA.2 was fixed at 1, and those
1462 of the other lineages were estimated relative to that of BA.2.
1463   Parameter estimation was performed via the MCMC approach
1464 implemented in CmdStan v2.28.1 (https://mc-stan.org) with CmdStanr v0.4.0
1465 (https://mc-stan.org/cmdstanr/). Noninformative priors were set for all
1466 parameters. Four independent MCMC chains were run with 1,000 and 2,000
1467 steps in the warmup and sampling iterations, respectively. We confirmed that all
1468 estimated parameters showed <1.01 R-hat convergence diagnostic values and
1469 >200 effective sampling size values, indicating that the MCMC runs were
1470 successfully convergent. The above analyses were performed in R v4.1.3
1471 (https://www.r-project.org/). Information on the relative R_e estimated in the
1472 present study is summarized in **Table S1**.
1473

Plasmid construction

Plasmids expressing the codon-optimized SARS-CoV-2 S proteins of B.1.1 (the parental D614G-bearing variant), BA.2 and BA.5 were prepared in our previous studies (Kimura et al., 2022a; Ozono *et al.*, 2021; Saito *et al.*, 2022; Suzuki *et al.*, 2022; Yamasoba *et al.*, 2022b). Plasmids expressing the codon-optimized S proteins of BA.2.75 and the BA.2 S-based derivatives were generated by site-directed overlap extension PCR using the primers listed in **Table S3**. The resulting PCR fragment was digested with KpnI and NotI and inserted into the corresponding site of the pCAGGS vector (Niwa et al., 1991). Nucleotide sequences were determined by DNA sequencing services (Eurofins), and the sequence data were analyzed by Sequencher v5.1 software (Gene Codes Corporation).

Neutralization assay

Pseudoviruses were prepared as previously described (Kimura *et al.*, 2022a; Kimura *et al.*, 2022b; Kimura *et al.*, 2022c; Motozono *et al.*, 2021; Uriu et al., 2022; Uriu et al., 2021; Yamasoba *et al.*, 2022a; Yamasoba *et al.*, 2022b; Yamasoba *et al.*, 2022c). Briefly, lentivirus (HIV-1)-based, luciferase-expressing reporter viruses were pseudotyped with the SARS-CoV-2 S proteins. HEK293T cells (1,000,000 cells) were cotransfected with 1 µg psPAX2-IN/HiBiT (Ozono et al., 2020), 1 µg pWPI-Luc2 (Ozono *et al.*, 2020), and 500 ng plasmids expressing parental S or its derivatives using PEI Max (Polysciences, Cat# 24765-1) according to the manufacturer's protocol. Two days posttransfection, the culture supernatants were harvested and centrifuged. The pseudoviruses were stored at –80°C until use.

Neutralization assay (**Figure 2**) was prepared as previously described (Kimura *et al.*, 2022a; Kimura *et al.*, 2022b; Kimura *et al.*, 2022c; Saito *et al.*, 2022; Uriu *et al.*, 2022; Uriu *et al.*, 2021; Yamasoba *et al.*, 2022a; Yamasoba *et al.*, 2022b; Yamasoba *et al.*, 2022c). Briefly, the SARS-CoV-2 S pseudoviruses (counting ~20,000 relative light units) were incubated with serially diluted (120-fold to 87,480-fold dilution at the final concentration) heat-inactivated sera at 37°C for 1 hour. Pseudoviruses without sera were included as controls. Then, a 40 µl mixture of pseudovirus and serum/antibody was added to HOS-ACE2/TMPRSS2 cells (10,000 cells/50 µl) in a 96-well white plate. At 2 d.p.i., the infected cells were lysed with a One-Glo luciferase assay system (Promega, Cat# E6130) or a Bright-Glo luciferase assay system (Promega, Cat# E2650), and the luminescent signal was measured using a GloMax explorer multimode microplate reader 3500 (Promega) or CentroXS3 (Berthhold Technologies). The assay of each serum was performed in triplicate, and the 50% neutralization titer (NT_{50}) was calculated using Prism 9 software v9.1.1 (GraphPad Software).

43

1515

1516 **Airway organoids**

1517 Airway organoids (AO) model was generated according to our previous report
1518 (Sano *et al.*, 2022). Briefly, normal human bronchial epithelial cells (NHBE, Cat#
1519 CC-2540, Lonza) were used to generate AO. NHBE were suspended in 10
1520 mg/ml cold Matrigel growth factor reduced basement membrane matrix
1521 (Corning). 50 µl of cell suspension was solidified on pre-warmed cell-culture
1522 treated multi-dishes (24-well plates; Thermo Fisher Scientific) at 37 °C for 10
1523 min, and then 500 µl of expansion medium was added to each well. AO were
1524 cultured with AO expansion medium for 10 days. To mature the AO, expanded
1525 AO were cultured with AO differentiation medium for 5 days. In experiments
1526 evaluating the antiviral drugs (see "Antiviral drug assay using SARS-CoV-2
1527 clinical isolates and AO" section below), AO were dissociated into single cells,
1528 and then were seeded into 96-well plates.

1529

1530 **SARS-CoV-2 preparation and titration**

1531 The working virus stocks of SARS-CoV-2 were prepared and titrated as
1532 previously described (Kimura *et al.*, 2022b; Kimura *et al.*, 2022c; Meng *et al.*,
1533 2022; Motozono *et al.*, 2021; Saito *et al.*, 2022; Suzuki *et al.*, 2022; Yamasoba *et*
1534 *al.*, 2022b). In this study, clinical isolates of B.1.1 (strain TKYE610670; GISAID
1535 ID: EPI_ISL_479681) (Suzuki *et al.*, 2022), Delta (B.1.617.2, strain TKYTK1734;
1536 GISAID ID: EPI_ISL_2378732) (Saito *et al.*, 2022), BA.2 (strain TY40-385;
1537 GISAID ID: EPI_ISL_9595859) (Kimura *et al.*, 2022c) and BA.5 (strain
1538 TKYS14631; GISAID ID: EPI_ISL_12812500) (Tamura *et al.*, 2022), and
1539 BA.2.75 (strain TY41-716; GISAID ID: EPI_ISL_13969765) were used. In brief,
1540 20 µl of the seed virus was inoculated into VeroE6/TMPRSS2 cells (5,000,000
1541 cells in a T-75 flask). One h.p.i., the culture medium was replaced with DMEM
1542 (low glucose) (Wako, Cat# 041-29775) containing 2% FBS and 1% PS. At 3
1543 d.p.i., the culture medium was harvested and centrifuged, and the supernatants
1544 were collected as the working virus stock.

1545 The titer of the prepared working virus was measured as the 50%
1546 tissue culture infectious dose ($TCID_{50}$). Briefly, one day before infection,
1547 VeroE6/TMPRSS2 cells (10,000 cells) were seeded into a 96-well plate. Serially
1548 diluted virus stocks were inoculated into the cells and incubated at 37°C for 4
1549 days. The cells were observed under microscopy to judge the CPE appearance.
1550 The value of $TCID_{50}$/ml was calculated with the Reed–Muench method (Reed
1551 and Muench, 1938).

1552 To verify the sequences of SARS-CoV-2 working viruses, viral RNA
1553 was extracted from the working viruses using a QIAamp viral RNA mini kit
1554 (Qiagen, Cat# 52906) and viral genome sequences were analyzed as described
1555 above (see "Viral genome sequencing" section). Information on the unexpected

44

1556 substitutions detected is summarized in **Table S4**, and the raw data are

1557 deposited in the GitHub repository

1558 (https://github.com/TheSatoLab/Omicron_BA.2.75).

1559

1560 **Antiviral drug assay using SARS-CoV-2 clinical isolates and AO**

1561 Antiviral drug assay (**Table 1 and Figure S2A**) was performed as previously

1562 described (Meng *et al.*, 2022). Briefly, one day before infection, AO (10,000

1563 cells) was dissociated, and then seeded into a 96-well plate. The cells were

1564 infected with either B.1.1, BA.2, BA.5 or BA.2.75 isolate (100 $TCID_{50}$) at 37 °C

1565 for 2 hours. The cells were washed with DMEM and cultured in DMEM

1566 supplemented with 10% FCS, 1% PS and the serially diluted Remdesivir

1567 (Clinisciences, Cat# A17170), EIDD-1931 (an active metabolite of Molnupiravir;

1568 Cell Signalling Technology, Cat# 81178S), or Nirmatrelvir (PF-07321332;

1569 MedChemExpress, Cat# HY-138687). At 24 hours after infection, the culture

1570 supernatants were collected, and viral RNA was quantified using RT–qPCR (see

1571 "RT–qPCR" section below). The assay of each compound was performed in

1572 quadruplicate, and the 50% effective concentration (EC_{50}) was calculated using

1573 Prism 9 software v9.1.1 (GraphPad Software).

1574

1575 **Cytotoxicity assay**

1576 The cytotoxicity of Remdesivir, EIDD-1931 or Nirmatrelvir (**Figure S2B**) was

1577 performed as previously described (Meng *et al.*, 2022). Briefly, one day before

1578 the assay, AO (10,000 cells) was dissociated and then seeded into a 96-well

1579 plate. The cells were cultured with the serially diluted antiviral drugs for

1580 24 hours. The cell counting kit-8 (Dojindo, Cat# CK04-11) solution (10 µl) was

1581 added to each well, and the cells were incubated at 37 °C for 90 min.

1582 Absorbance was measured at 450 nm using the Multiskan FC (Thermo Fisher

1583 Scientific). The assay of each compound was performed in quadruplicate, and

1584 the 50% cytotoxic concentration (CC_{50}) was calculated using Prism 9 software

1585 v9.1.1 (GraphPad Software).

1586

1587 **Pseudovirus infection**

1588 Pseudovirus infection (**Figure 3A**) was performed as previously described

1589 (Ferreira *et al.*, 2021; Kimura *et al.*, 2022a; Kimura *et al.*, 2022b; Kimura *et al.*,

1590 2022c; Motozono *et al.*, 2021; Uriu *et al.*, 2022; Uriu *et al.*, 2021; Yamasoba *et*

1591 *al.*, 2022a; Yamasoba *et al.*, 2022b; Yamasoba *et al.*, 2022c). Briefly, the

1592 amount of pseudoviruses prepared was quantified by the HiBiT assay using

1593 Nano Glo HiBiT lytic detection system (Promega,Cat# N3040) as previously

1594 described (Ozono *et al.*, 2021; Ozono *et al.*, 2020), and the same amount of

1595 pseudoviruses (normalized to the HiBiT value, which indicates the amount of

1596 p24 HIV-1 antigen) was inoculated into HOS-ACE2/TMPRSS2 cells,

45

1597 HEK293-ACE2 cells or HEK293-ACE2/TMPRSS2 and viral infectivity was
1598 measured as described above (see "Neutralization assay" section). To analyze
1599 the effect of TMPRSS2 for pseudovirus infectivity (**Figure S3A**), the fold change
1600 of the values of HEK293-ACE2/TMPRSS2 to HEK293-ACE2 was calculated.
1601

1602 **Yeast surface display**
1603 Yeast surface display (**Figure 3B**) was performed as previously described
1604 (Dejnirattisai *et al.*, 2022; Kimura *et al.*, 2022a; Kimura *et al.*, 2022b; Kimura *et*
1605 *al.*, 2022c; Motozono *et al.*, 2021; Yamasoba *et al.*, 2022b; Zahradnik *et al.*,
1606 2021b). Briefly, the RBD genes ["construct 3" in (Zahradnik *et al.*, 2021b),
1607 covering residues 330–528] in pJYDC1 plasmid were cloned by restriction
1608 enzyme-free cloning and transformed into the EBY100 Saccharomyces
1609 cerevisiae. Primers are listed in **Table S3**. The expression media 1/9 (Zahradnik
1610 *et al.*, 2021a) was inoculated (OD 1) by overnight (220 rpm, 30°C, SD-CAA
1611 media) grown culture and cultivated for 24 hours at 20°C. The media was
1612 supplemented by 10 mM DMSO solubilized bilirubin (Sigma-Aldrich, Cat#
1613 14370-1G) for expression co-cultivation labeling (pJYDC1, eUnaG2 reporter
1614 holo-form formation, green/yellow fluorescence (Ex. 498 nm, Em. 527 nm). Cells
1615 (100 ul aliquots) were collected by centrifugation (3000 g, 3 minutes), washed in
1616 ice-cold PBSB buffer (PBS with 1 g/L BSA), and resuspended in an analysis
1617 solution with a series of CF®640R succinimidyl ester labeled (Biotium, USA,
1618 Cat# 92108) ACE2 peptidase domain (residues 18–740) concentrations. The
1619 reaction volume was adjusted (1–100 ml) to avoid the ligand depletion effect,
1620 and the suspension was incubated overnight in a rotator shaker (10 rpm, 4°C).
1621 Incubated samples were washed by PBSB buffer, transferred into 96 well plates
1622 (Thermo, USA, Nunc, Cat# 268200), and analyzed by the CytoFLEX S Flow
1623 Cytometer (Beckman Coulter, USA, Cat#. N0-V4-B2-Y4) with the gating strategy
1624 described previously (Zahradnik *et al.*, 2021b). The eUnaG2 signals were
1625 compensated by the instrument CytExpert software (Beckman Coulter). The
1626 mean binding signal (FL4-A) values of RBD expressing cells, subtracted by
1627 signals of non-expressing populations, were subjected for the determination of
1628 binding constant KD, YD by non-cooperative Hill equation fitted by nonlinear
1629 least-squares regression using Python v3.7 fitted together with two additional
1630 parameters describing titration curve (Zahradnik *et al.*, 2021b).
1631

1632 **AlphaFold2**
1633 To generate the structure model of BA.2.75 S RBD (**Figures 3C, 3D and S3B**),
1634 the AlphaFold2 structural prediction was performed using ColabFold (Mirdita *et*
1635 *al.*, 2022) using the BA.2 S RBD template (PDB: 7UB0) (Stalls *et al.*, 2022). The
1636 MMseqs2 and HHsearch parameters were set as default. The models were
1637 manually inspected, and those exhibiting poor parameters and models that did

1638   not adopt the classical RBD interface conformation were eliminated. The two
1639   highest score models were analyzed in detail. Three-dimensional visualization
1640   and analyses were performed using PyMOL v2.1.1 (Schrödinger,
1641   https://pymol.org/2/). In **Figure 3D**, the wild-type SARS-CoV-2 S RBD of the
1642   crystal structure of RBD-ACE2 complex (PDB:6M17) (Yan *et al.*, 2020) was
1643   replaced with the BA.2.75 S RBD structure generated by AlphaFold2.
1644

1645   **SARS-CoV-2 S-based fusion assay**
1646   SARS-CoV-2 S-based fusion assay (**Figures 3E and 3F**) was performed as
1647   previously described (Kimura *et al.*, 2022b; Kimura *et al.*, 2022c; Motozono *et al.*,
1648   2021; Saito *et al.*, 2022; Suzuki *et al.*, 2022; Yamasoba *et al.*, 2022b). Briefly, on
1649   day 1, effector cells (i.e., S-expressing cells) and target cells (Calu-3/DSP$_{1-7}$
1650   cells) were prepared at a density of 0.6–0.8 × 10^6 cells in a 6-well plate. On day
1651   2, to prepare effector cells, HEK293 cells were cotransfected with the S
1652   expression plasmids (400 ng) and pDSP$_{8-11}$ (Kondo et al., 2011) (400 ng) using
1653   TransIT-LT1 (Takara, Cat# MIR2300). On day 3 (24 hours posttransfection),
1654   16,000 effector cells were detached and reseeded into 96-well black plates
1655   (PerkinElmer, Cat# 6005225), and target cells were reseeded at a density of
1656   1,000,000 cells/2 ml/well in 6-well plates. On day 4 (48 hours posttransfection),
1657   target cells were incubated with EnduRen live cell substrate (Promega, Cat#
1658   E6481) for 3 hours and then detached, and 32,000 target cells were added to a
1659   96-well plate with effector cells. *Renilla* luciferase activity was measured at the
1660   indicated time points using Centro XS3 LB960 (Berthhold Technologies). To
1661   measure the surface expression level of S protein, effector cells were stained
1662   with rabbit anti-SARS-CoV-2 S S1/S2 polyclonal antibody (Thermo Fisher
1663   Scientific, Cat# PA5-112048, 1:100). Normal rabbit IgG (SouthernBiotech, Cat#
1664   0111-01, 1:100) was used as negative controls, and APC-conjugated goat
1665   anti-rabbit IgG polyclonal antibody (Jackson ImmunoResearch, Cat#
1666   111-136-144, 1:50) was used as a secondary antibody. Surface expression level
1667   of S proteins (**Figure 3E**) was measured using FACS Canto II (BD Biosciences)
1668   and the data were analyzed using FlowJo software v10.7.1 (BD Biosciences). To
1669   calculate fusion activity, *Renilla* luciferase activity was normalized to the MFI of
1670   surface S proteins. The normalized value (i.e., *Renilla* luciferase activity per the
1671   surface S MFI) is shown as fusion activity.
1672

1673   **AO-ALI model**
1674   AO-ALI model (**Figure 4D**) was generated according to our previous report
1675   (Sano *et al.*, 2022). To generate AO-ALI, expanding AO were dissociated into
1676   single cells, and then were seeded into Transwell inserts (Corning) in a 24-well
1677   plate. To promote their maturation, AO-ALI were cultured with AO differentiation
1678   medium for 5 days. AO-ALI were infected with SARS-CoV-2 from the apical side.

1679

1680 **Preparation of human airway and alveolar epithelial cells from human iPSC**

1681 The air-liquid interface culture of airway and alveolar epithelial cells (**Figures 4E**

1682 **and 4F**) were differentiated from human iPSC-derived lung progenitor cells as

1683 previously described (Gotoh et al., 2014; Kimura *et al.*, 2022c; Konishi et al.,

1684 2016; Tamura *et al.*, 2022; Yamamoto et al., 2017). Briefly, lung progenitor cells

1685 were stepwise induced from human iPSCs referring a 21-days and 4-steps

1686 protocol (Yamamoto *et al.*, 2017). At day 21, lung progenitor cells were isolated

1687 with specific surface antigen carboxypeptidase M and seeded onto upper

1688 chamber of 24-well Cell Culture Insert (Falcon, #353104), followed by 28-day

1689 and 7-day differentiation of airway and alveolar epithelial cells, respectively.

1690 Alveolar differentiation medium supplemented with dexamethasone

1691 (Sigma-Aldrich, Cat# D4902), KGF (PeproTech, Cat# 100-19), 8-Br-cAMP

1692 (Biolog, Cat# B007), 3-Isobutyl 1-methylxanthine (IBMX) (FUJIFILM Wako, Cat#

1693 095-03413), CHIR99021 (Axon Medchem, Cat# 1386), and SB431542

1694 (FUJIFILM Wako, Cat# 198-16543) was used for induction of alveolar epithelial

1695 cells. PneumaCult ALI (STEMCELL Technologies, Cat# ST-05001)

1696 supplemented with heparin (Nacalai Tesque, Cat# 17513-96) and Y-27632 (LC

1697 Laboratories, Cat# Y-5301) hydrocortisone (Sigma-Aldrich, Cat# H0135) was

1698 used for induction of airway epithelial cells.

1699

1700 **Airway-on-a-chips**

1701 Airway-on-a-chips (**Figure S3C**) were prepared as previously described

1702 (Hashimoto et al., 2022). Human lung microvascular endothelial cells

1703 (HMVEC-L) were obtained from Lonza (Cat# CC-2527) and cultured with

1704 EGM-2-MV medium (Lonza, Cat# CC-3202). To prepare the airway-on-a-chip,

1705 first, the bottom channel of a polydimethylsiloxane (PDMS) device was

1706 pre-coated with fibronectin (3 µg/ml, Sigma, Cat# F1141). The microfluidic

1707 device was generated according to our previous report (Deguchi et al., 2021).

1708 HMVEC-L were suspended at 5,000,000 cells/ml in EGM2-MV medium. Then,

1709 10 µl suspension medium was injected into the fibronectin-coated bottom

1710 channel of the PDMS device. Then, the PDMS device was turned upside down

1711 and incubated for 1 h. After 1 hour, the device was turned over, and the

1712 EGM2-MV medium was added into the bottom channel. After 4 days, AO were

1713 dissociated and seeded into the top channel. The AO was generated according

1714 to our previous report (Sano *et al.*, 2022). AO were dissociated into single cells

1715 and then suspended at 5,000,000 cells/ml in the AO differentiation medium. Ten

1716 microliter suspension medium was injected into the top channel. After 1 hour, the

1717 AO differentiation medium was added to the top channel. In the infection

1718 experiments (**Figures 4G–4I**), the AO differentiation medium containing either

1719 B.1.1, Delta, BA.2, BA.5 or BA.2.75 isolate (500 TCID$_{50}$) was inoculated from the

48

1720 top channel (**Figure S3C**). At 2 h.p.i., the top and bottom channels were washed
1721 and cultured with AO differentiation and EGM2-MV medium, respectively. The
1722 culture supernatants were collected, and viral RNA was quantified using
1723 RT–qPCR (see "RT–qPCR" section above).
1724

1725 **Microfluidic device**
1726 The microfluidic device was generated according to our previous report (Deguchi
1727 *et al.*, 2021). Briefly, the microfluidic device consisted of two layers of
1728 microchannels separated by a semipermeable membrane. The microchannel
1729 layers were fabricated from PDMS using a soft lithographic method. PDMS
1730 prepolymer (SYLGARD 184, Dow Corning) at a base to curing agent ratio of
1731 10:1 was cast against a mold composed of SU-8 2150 (MicroChem) patterns
1732 formed on a silicon wafer. The cross-sectional size of the microchannels was 1
1733 mm in width and 330 µm in height. To introduce solutions into the microchannels,
1734 access holes were punched through the PDMS using a 6-mm biopsy punch (Kai
1735 Corporation). Two PDMS layers were bonded to a PET membrane containing
1736 3.0 µm pores (Cat# 353091, Falcon) using a thin layer of liquid PDMS
1737 prepolymer as the mortar. PDMS prepolymer was spin-coated (4000 rpm for 60
1738 sec) onto a glass slide. Subsequently, both the top and bottom channel layers
1739 were placed on the glass slide to transfer the thin layer of PDMS prepolymer
1740 onto the embossed PDMS surfaces. The membrane was then placed onto the
1741 bottom layer and sandwiched with the top layer. The combined layers were left
1742 at room temperature for 1 day to remove air bubbles and then placed in an oven
1743 at 60°C overnight to cure the PDMS glue. The PDMS devices were sterilized by
1744 placing them under UV light for 1 hour before the cell culture.
1745

1746 **SARS-CoV-2 infection**
1747 One day before infection, Vero cells (10,000 cells), VeroE6/TMPRSS2 cells
1748 (10,000 cells), and HEK293-ACE2/TMPRSS2 cells were seeded into a 96-well
1749 plate. SARS-CoV-2 [1,000 $TCID_{50}$ for Vero cells (**Figure 4A**); 100 $TCID_{50}$ for
1750 VeroE6/TMPRSS2 cells (**Figure 4B**) and HEK293-ACE2/TMPRSS2 cells
1751 (**Figure 4C**)] was inoculated and incubated at 37°C for 1 hour. The infected cells
1752 were washed, and 180 µl culture medium was added. The culture supernatant
1753 (10 µl) was harvested at the indicated timepoints and used for RT–qPCR to
1754 quantify the viral RNA copy number (see "RT–qPCR" section below). In the
1755 infection experiments using human iPSC-derived airway and alveolar epithelial
1756 cells (**Figures 4E and 4F**), working viruses were diluted with Opti-MEM (Thermo
1757 Fisher Scientific, 11058021). The diluted viruses (1,000 $TCID_{50}$ in 100 µl) were
1758 inoculated onto the apical side of the culture and incubated at 37 °C for 1 hour.
1759 The inoculated viruses were removed and washed twice with Opti-MEM. To
1760 collect the viruses, 100 µl Opti-MEM was applied onto the apical side of the

49

1761 culture and incubated at 37□°C for 10□minutes. The Opti-MEM was collected
1762 and used for RT–qPCR to quantify the viral RNA copy number (see "RT–qPCR"
1763 section below). The infection experiments using an airway-on-a-chip system
1764 (**Figures 4G–4I**) was performed as described above (see "Airway-on-a-chips"
1765 section).
1766
1767 **RT–qPCR**
1768 RT–qPCR was performed as previously described (Kimura *et al.*, 2022b; Kimura
1769 *et al.*, 2022c; Meng *et al.*, 2022; Motozono *et al.*, 2021; Saito *et al.*, 2022; Suzuki
1770 *et al.*, 2022; Yamasoba *et al.*, 2022b). Briefly, 5 µl culture supernatant was mixed
1771 with 5 µl 2 × RNA lysis buffer [2% Triton X-100 (Nacalai Tesque, Cat# 35501-15),
1772 50 mM KCl, 100 mM Tris-HCl (pH 7.4), 40% glycerol, 0.8 U/µl recombinant
1773 RNase inhibitor (Takara, Cat# 2313B)] and incubated at room temperature for
1774 10 min. RNase-free water (90 µl) was added, and the diluted sample (2.5 µl) was
1775 used as the template for real-time RT-PCR performed according to the
1776 manufacturer's protocol using One Step TB Green PrimeScript PLUS RT-PCR
1777 kit (Takara, Cat# RR096A) and the following primers: Forward *N*, 5'-AGC CTC
1778 TTC TCG TTC CTC ATC AC-3'; and Reverse *N*, 5'-CCG CCA TTG CCA GCC
1779 ATT C-3'. The viral RNA copy number was standardized with a SARS-CoV-2
1780 direct detection RT-qPCR kit (Takara, Cat# RC300A). Fluorescent signals were
1781 acquired using QuantStudio 1 Real-Time PCR system (Thermo Fisher Scientific),
1782 QuantStudio 3 Real-Time PCR system (Thermo Fisher Scientific), QuantStudio
1783 5 Real-Time PCR system (Thermo Fisher Scientific), CFX Connect Real-Time
1784 PCR Detection system (Bio-Rad), Eco Real-Time PCR System (Illumina),
1785 qTOWER3 G Real-Time System (Analytik Jena) Thermal Cycler Dice Real Time
1786 System III (Takara) or 7500 Real-Time PCR System (Thermo Fisher Scientific).
1787
1788 **Plaque assay**
1789 Plaque assay (**Figure 4J**) was performed as previously described (Kimura *et al.*,
1790 2022b; Kimura *et al.*, 2022c; Suzuki *et al.*, 2022; Yamasoba *et al.*, 2022b).
1791 Briefly, one day before infection, VeroE6/TMPRSS2 cells (100,000 cells) were
1792 seeded into a 24-well plate and infected with SARS-CoV-2 (0.5, 5, 50 and 500
1793 $TCID_{50}$) at 37°C for 1 hour. Mounting solution containing 3% FBS and 1.5%
1794 carboxymethyl cellulose (Wako, Cat# 039-01335) was overlaid, followed by
1795 incubation at 37°C. At 3 d.p.i., the culture medium was removed, and the cells
1796 were washed with PBS three times and fixed with 4% paraformaldehyde
1797 phosphate (Nacalai Tesque, Cat# 09154-85). The fixed cells were washed with
1798 tap water, dried, and stained with staining solution [0.1% methylene blue
1799 (Nacalai Tesque, Cat# 22412-14) in water] for 30 minutes. The stained cells
1800 were washed with tap water and dried, and the size of plaques was measured
1801 using Fiji software v2.2.0 (ImageJ).

50

1802

1803 **Animal experiments**

1804 Animal experiments (**Figure 5**) were performed as previously described (Kimura

1805 *et al.*, 2022b; Kimura *et al.*, 2022c; Saito *et al.*, 2022; Suzuki *et al.*, 2022; Tamura

1806 *et al.*, 2022; Yamasoba *et al.*, 2022b). Syrian hamsters (male, 4 weeks old) were

1807 purchased from Japan SLC Inc. (Shizuoka, Japan). For the virus infection

1808 experiments, hamsters were euthanized by intramuscular injection of a mixture

1809 of 0.15 mg/kg medetomidine hydrochloride (Domitor$^®$, Nippon Zenyaku Kogyo),

1810 2.0 mg/kg midazolam (Dormicum$^®$, FUJIFILM Wako Chemicals) and 2.5 mg/kg

1811 butorphanol (Vetorphale$^®$, Meiji Seika Pharma) or 0.15 mg/kg medetomidine

1812 hydrochloride, 4.0 mg/kg alphaxaone (Alfaxan$^®$, Jurox) and 2.5 mg/kg

1813 butorphanol. The Delta, BA.2, BA.5 and BA.2.75 (1,000 $TCID_{50}$ in 100 µl), or

1814 saline (100 µl) were intranasally inoculated under anesthesia. Oral swabs were

1815 collected at indicated timepoints. Body weight was recorded daily by 7 d.p.i.

1816 Enhanced pause (Penh), the ratio of time to peak expiratory follow relative to the

1817 total expiratory time (Rpef), and BPM were measured every day until 7 d.p.i.

1818 (see below). Subcutaneous oxygen saturation (SpO_2, see below) was monitored

1819 at 0, 1, 3, 5, and 7 d.p.i. Lung tissues were anatomically collected at 2 and 5 d.p.i.

1820 Viral RNA load in the oral swabs and respiratory tissues were determined by

1821 RT–qPCR. These tissues were also used for IHC and histopathological analyses

1822 (see below).

1823

1824 **Lung function test**

1825 Lung function test (**Figure 5A**) was routinely performed as previously described

1826 (Kimura *et al.*, 2022b; Kimura *et al.*, 2022c; Saito *et al.*, 2022; Suzuki *et al.*,

1827 2022; Tamura *et al.*, 2022; Yamasoba *et al.*, 2022b). The three respiratory

1828 parameters (Penh, Rpef and BPM) were measured by using a whole-body

1829 plethysmography system (DSI) according to the manufacturer's instructions. In

1830 brief, a hamster was placed in an unrestrained plethysmography chamber and

1831 allowed to acclimatize for 30 seconds, then, data were acquired over a

1832 2.5-minute period by using FinePointe Station and Review softwares

1833 v2.9.2.12849 (STARR). The state of oxygenation was examined by measuring

1834 SpO_2 using pulse oximeter, MouseOx PLUS (STARR). SpO_2 was measured by

1835 attaching a measuring chip to the neck of hamsters sedated by 0.25 mg/kg

1836 medetomidine hydrochloride.

1837

1838 **Immunohistochemistry**

1839 Immunohistochemistry (IHC) (**Figures 5D, S4A–S4C**) was performed as

1840 previously described (Kimura *et al.*, 2022b; Kimura *et al.*, 2022c; Saito *et al.*,

1841 2022; Suzuki *et al.*, 2022; Tamura *et al.*, 2022; Yamasoba *et al.*, 2022b) using an

1842 Autostainer Link 48 (Dako). The deparaffinized sections were exposed to

51

1843 EnVision FLEX target retrieval solution high pH (Agilent, Cat# K8004) for 20
1844 minutes at 97°C to activate, and mouse anti-SARS-CoV-2 N monoclonal
1845 antibody (clone 1035111, R&D systems, Cat# MAB10474-SP, 1:400) was used
1846 as a primary antibody. The sections were sensitized using EnVision FLEX
1847 (Agilent) for 15 minutes and visualized by peroxidase-based enzymatic reaction
1848 with 3,3'-diaminobenzidine tetrahydrochloride (Dako, Cat# DM827) as substrate
1849 for 5 minutes. The N protein positivity (**Figures 5E, S4A and S4B**) was
1850 evaluated by certificated pathologists as previously described (Kimura *et al.*,
1851 2022b; Kimura *et al.*, 2022c; Saito *et al.*, 2022; Suzuki *et al.*, 2022; Tamura *et al.*,
1852 2022; Yamasoba *et al.*, 2022b). Images were incorporated as virtual slide by
1853 NDP.scan software v3.2.4 (Hamamatsu Photonics). The N-protein positivity was
1854 measured as the area using Fiji software v2.2.0 (ImageJ).
1855

1856 **H&E staining**
1857 H&E staining (**Figures 5F and S4D**) was performed as previously described
1858 (Kimura *et al.*, 2022b; Kimura *et al.*, 2022c; Saito *et al.*, 2022; Suzuki *et al.*,
1859 2022; Tamura *et al.*, 2022; Yamasoba *et al.*, 2022b). Briefly, excised animal
1860 tissues were fixed with 10% formalin neutral buffer solution, and processed for
1861 paraffin embedding. The paraffin blocks were sectioned with 3 µm-thickness and
1862 then mounted on MAS-GP-coated glass slides (Matsunami Glass, Cat# S9901).
1863 H&E staining was performed according to a standard protocol.
1864

1865 **Histopathological scoring**
1866 Histopathological scoring (**Figure 5G**) was performed as previously described
1867 (Kimura *et al.*, 2022b; Kimura *et al.*, 2022c; Saito *et al.*, 2022; Suzuki *et al.*,
1868 2022; Tamura *et al.*, 2022; Yamasoba *et al.*, 2022b). Pathological features
1869 including (i) bronchitis or bronchiolitis, (ii) hemorrhage with congestive edema,
1870 (iii) alveolar damage with epithelial apoptosis and macrophage infiltration, (iv)
1871 hyperplasia of type II pneumocytes, and (v) the area of the hyperplasia of large
1872 type II pneumocytes were evaluated by certified pathologists and the degree of
1873 these pathological findings were arbitrarily scored using four-tiered system as 0
1874 (negative), 1 (weak), 2 (moderate), and 3 (severe). The "large type II
1875 pneumocytes" are the hyperplasia of type II pneumocytes exhibiting more than
1876 10-µm-diameter nucleus. We described "large type II pneumocytes" as one of
1877 the remarkable histopathological features reacting SARS-CoV-2 infection in our
1878 previous studies (Kimura *et al.*, 2022b; Kimura *et al.*, 2022c; Saito *et al.*, 2022;
1879 Suzuki *et al.*, 2022; Tamura *et al.*, 2022; Yamasoba *et al.*, 2022b). Total
1880 histology score is the sum of these five indices.
1881 To measure the inflammation area in the infected lungs (**Figures 5H**
1882 **and S4D**), four hamsters infected with each virus were sacrificed at 5 d.p.i., and
1883 all four right lung lobes, including upper (anterior/cranial), middle, lower

1884 (posterior/caudal), and accessory lobes, were sectioned along with their bronchi.
1885 The tissue sections were stained by H&E, and the digital microscopic images
1886 were incorporated into virtual slides using NDP.scan software v3.2.4
1887 (Hamamatsu Photonics). The inflammatory area including type II pneumocyte
1888 hyperplasia in the infected whole lungs was morphometrically analyzed using Fiji
1889 software v2.2.0 (ImageJ).
1890
1891 **QUANTIFICATION AND STATISTICAL ANALYSIS**
1892 Statistical significance was tested using a two-sided Mann–Whitney *U*-test, a
1893 two-sided Student's *t*-test or a two-sided paired *t*-test unless otherwise noted.
1894 The tests above were performed using Prism 9 software v9.1.1 (GraphPad
1895 Software).
1896 In the time-course experiments (**Figures 3F, 4A–4H, 5A, 5B, and 5G**),
1897 a multiple regression analysis including experimental conditions (i.e., the types
1898 of infected viruses) as explanatory variables and timepoints as qualitative control
1899 variables was performed to evaluate the difference between experimental
1900 conditions thorough all timepoints. The initial time point was removed from the
1901 analysis. *P* value was calculated by a two-sided Wald test. Subsequently,
1902 familywise error rates (FWERs) were calculated by the Holm method. These
1903 analyses were performed in R v4.1.2 (https://www.r-project.org/).
1904 In **Figures 5D, 5F and S4**, photographs shown are the representative
1905 areas of at least two independent experiments by using four hamsters at each
1906 timepoint.

A

HV69-70del
L452R
F486V
R493Q

BA.5

BA.4

BA.2.75

BA.2 subvariants

K147E
W152R
F157L
I210V
G257S
D339H
G446S
N460K
R493Q

BA.2

BA.3

BA.1

B.1.1

0.001

B

	BA.2	BA.5	BA.2.75
E:T11A			■
M:D3N			■
NSP12:G671S			■
NSP3:S403L			■
NSP3:P822S			■
NSP4:L438F	■		■
NSP8:N118S			■
S:HV69-70del		■	
S:K147E			■
S:W152R			■
S:F157L			■
S:I210V			■
S:G257S			■
S:G339D/H	D	D	H
S:G446S			■
S:L452R		■	
S:N460K			■
S:F486V		■	
S:Q493R	■		

Frequency: 0 — 1

C

BA.5

BA.2.75

Telangana

Tamil Nadu

Delhi — Himachal Pradesh
Haryana
Rajasthan — West Bengal
Gujarat — Odisha
Maharashtra

Lineage frequency: 0 — 0.3

D

Himachal Pradesh | Odisha | Haryana | Rajasthan | Maharashtra

Gujarat | West Bengal | Delhi | Tamil Nadu | Telangana

Month

BA.5
BA.2.75

Sequence:
25
50
75
100
125

E

Average in India

Relative R_e (BA.2 = 1)

BA.2 BA.2.38 BA.2.74 BA.2.75 BA.2.76 BA.5 Other BA.2

A 2nd-dose vaccine sera (1 month) (n=15)

B 3rd-dose vaccine sera (1 month) (n=15)
- ×1.3 (*P*=0.090)
- ×2.1 (*P*=0.0046)

C 3rd-dose vaccine sera (4 months) (n=15)
- ×1.2 (*P*=0.75)
- ×1.9 (*P*=0.0020)

D BA.1 breakthrough infection sera n=16
- ×1.2 (*P*=0.27)
- ×1.5 (*P*=0.0010)

E BA.2 breakthrough infection sera (n=14)
- ×1.3 (*P*=0.55)
- ×2.5 (*P*=0.0002)

F Delta infection sera (n=18)

G BA.1 infection sera (hamster) (n=6)
- ×1.7 (*P*=0.25)
- ×1.7 (*P*=0.25)

H BA.2 infection sera (hamster) (n=6)
- ×17 (*P*=0.031)
- ×23 (*P*=0.031)

I BA.5 infection sera (hamster) (n=6)
- ×5.1 (*P*=0.031)
- ×12 (*P*=0.031)

A — % Infectivity (BA.2 = 100)

B.1.1, BA.2, BA.5, BA.2.75, K147E, W152R, F157L, K147E/W152R/F157L, I210V, G257S, D339H, G446S, N460K, R493Q (BA.2-based)

B — K_D (nM)

B.1.1, BA.2, BA.2 D339H, BA.2 G446S, BA.2 N460K, BA.2 R493Q, BA.5, BA.2.75, BA.2.75 H339D

C — Electrostatic potential (kT/e)

B.1.1 (N460), BA.2 (N460), BA.2.75 (K460)

D — BA.2.75 S RBD: D339H, G446S, N460K, R493Q; Human ACE2

E — Surface S (MFI × 1,000)

Mock, B.1.1, BA.2, BA.5, BA.2.75

F — Fusion activity (arbitrary unit)

B.1.1 (P=5.6E−6), BA.2, BA.5 (P=5.6E−6), BA.2.75 (P=1.6E−5)

Hours post coculture: 0, 6, 12, 18, 24

A Vero cells

B VeroE6/TMPRSS2 cells

C HEK293-ACE2/TMPRSS2 cells

D AO-ALI

E iPSC-derived airway epithelial cells

F iPSC-derived lung epithelial cells

G Airway-on-a-chip (top channel)

H Airway-on-a-chip (bottom channel)

I

J

A (left to right panels):
- % Body weight — 2.8E−8, 0.00056, 0.00017; d.p.i.; Uninfected, Delta, BA.2, BA.5, BA.2.75
- Penh — 0.049, 0.68, 0.26; d.p.i.
- Rpef — 0.0040, 0.14, 0.23; d.p.i.
- BPM — 0.00031, 0.36, 0.0058; d.p.i.
- SpO2 — 1.0, 1.0, 1.0; d.p.i.

B: Viral RNA in the oral swab (copies/μg RNA) (log) — 0.19, 0.051, 1.5E−5; d.p.i.; Uninfected, Delta, BA.2, BA.5, BA.2.75

C: Lung hilum / Lung periphery; Viral RNA at 2 d.p.i. (copies/μg RNA) (log) — NS, NS; Viral RNA at 5 d.p.i. (copies/μg RNA) (log); Delta, BA.2, BA.5, BA.2.75

D: Delta, BA.2, BA.5, BA.2.75; 2, 5 d.p.i.

E: % N-positive cells in the lung at 2 d.p.i.; % N-positive cells in the lung at 5 d.p.i.; Delta, BA.2, BA.5, BA.2.75

F: Delta, BA.2, BA.5, BA.2.75, Uninfected; 2, 5 d.p.i.

G:
- Bronchitis/bronchiolitis — 0.00041, 0.33, 0.20
- Hemorrhage/congestion — 1.8E−6, 0.0052, 0.0014
- Alveolar damage — 0.0021, 1.0, 1.0
- Type II pneumocytes — 0.0096, 0.34, 0.26
- Area of large type II pneumocytes — 0.031, 0.34, 0.26
- Total histology score — 6.3E−6, 0.029, 0.029
Delta, BA.2, BA.5, BA.2.75; d.p.i. 2, 5

H: % Area of type II pneumocytes in the lung at 5 d.p.i.; Delta, BA.2, BA.5, BA.2.75