1 **Virological characteristics of SARS-CoV-2 BA.2 variant**

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85   **Abstract**

86   Soon after the emergence and global spread of a new severe acute respiratory

87   syndrome coronavirus 2 (SARS-CoV-2) Omicron lineage, BA.1 (ref[1,2]), another

88   Omicron lineage, BA.2, has initiated outcompeting BA.1. Statistical analysis

89   shows that the effective reproduction number of BA.2 is 1.4-fold higher than that

90   of BA.1. Neutralisation experiments show that the vaccine-induced humoral

91   immunity fails to function against BA.2 like BA.1, and notably, the antigenicity of

92   BA.2 is different from BA.1. Cell culture experiments show that BA.2 is more

93   replicative in human nasal epithelial cells and more fusogenic than BA.1.

94   Furthermore, infection experiments using hamsters show that BA.2 is more

95   pathogenic than BA.1. Our multiscale investigations suggest that the risk of BA.2

96   for global health is potentially higher than that of BA.1.

Introduction

Virological characteristics of newly emerging SARS-CoV-2 variants, such as transmissibility, pathogenicity and resistance to the vaccine-induced immunity and antiviral drugs, is an urgent global health concern. In February 2022, the Omicron variant (B.1.1.529 and BA lineages) spreads worldwide and represents the most recently recognised variant of concern[2]. Omicron was first reported from South Africa at the end of November 2021[1]. Then, a variant of Omicron, the BA.1 lineage, has rapidly spread worldwide and outcompeted other variants such as Delta.

As of February 2022, another variant of Omicron, the BA.2 lineage, has detected in multiple countries such as Denmark and UK[3]. Notably, BA.2 has initiated outcompeting BA.1[3], suggesting that BA.2 is more transmissible than BA.1.

In a few months since the emergence of BA.1, we and others revealed the virological characteristics of BA.1[4-19]. For instance, BA.1 is highly resistant to the vaccine-induced humoral immunity and antiviral drugs[4-11,16-19]. Also, the spike (S) protein of BA.1 is less efficiently cleaved by furin and less fusogenic than those of Delta and an ancestral SARS-CoV-2 belonging to the B.1.1 lineage[11,12]. Further, the pathogenicity of BA.1 is attenuated when compared to Delta and an ancestral B.1.1 virus[12-14]. However, the virological characteristics of BA.2 remains unaddressed.

Phylogenetic and epidemic dynamics of BA.2

As of February 2022, Omicron is classified into three main lineages, BA.1, BA.2, BA.3, and a sublineage of BA.1, BA1.1, which harbours the R346K substitution in S (**Fig. 1a**). Although these lineages are monophyletic, their sequences have been greatly diversified. For example, BA.1 differs from BA.2 by 50 amino acids, which is approximately twice as much as the numbers of amino acid differences between four VOCs (Alpha, Beta, Gamma and Delta) and Wuhan-Hu-1, a prototypic SARS-CoV-2 isolate (**Fig. 1b**). Phylodynamics analysis suggests that BA.1 emerged first, followed by BA.2 and BA.3 (**Extended Data Fig. 1**). In addition to BA.1, the earlier strains of BA.2, BA.3, and BA.1.1 were isolated from Gauteng Province, South Africa, the place of the earliest Omicron (BA.1) epidemic (**Extended Data Fig. 1**)[20]. These results suggest that the remarkable diversification of Omicron occurred in Gauteng Province and all Omicron lineages emerged there.

Although BA.1 spread worldwide earlier than BA.2, the lineage frequency of BA.2 increased and exceeded that of BA.1 since January 2022 in multiple countries, such as Philippines, India, Denmark, Singapore, Austria, and South Africa (**Fig. 1c and Extended Data Fig. 2**). To quantify the growth advantage of BA.2 in the human population, we constructed a Bayesian model

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138 representing the epidemic dynamics of SARS-CoV-2 lineages. This hierarchical
139 model can estimate the global average of the relative effective reproduction
140 numbers of viral lineages (**Fig. 1d**) as well as those in each country (**Extended
141 Data Fig. 2**). The effective reproduction number of BA.2 is 1.40-fold higher than
142 that of BA.1 on average in the world [95% confidence interval (CI), 1.29–1.52;
143 **Fig. 1d**]. Furthermore, the effective reproduction number of BA.2 was even
144 higher than that of BA.1.1, which spread more rapidly than BA.1 in several
145 countries such as the UK and USA (**Fig. 1d and Extended Data Fig. 2d**). These
146 results suggest that the BA.2 epidemic will more expand around the world,
147 raising the importance of elucidating virological features of BA.2 in depth.
148
149 **Immune resistance of BA.2**
150 Since the sequence of BA.2, particularly in S protein, is substantially different
151 from that of BA.1 (**Fig. 1b** and **Fig. 2a**), it is reasonable to assume that the
152 virological properties of BA.2, such as immune resistance and pathogenicity, are
153 different from those of BA.1. To reveal the virological features of BA.2, we set
154 out to perform neutralisation assay using pseudoviruses and the neutralising
155 antibodies elicited by vaccination. Consistent with recent studies[4-11,16-19], BA.1 is
156 highly resistant to the antisera elicited by mRNA-1273 and ChAdOx1 vaccines
157 (**Fig. 2b,c**). Similar to BA.1, BA.2 was also highly resistant to the
158 vaccine-induced antisera (**Fig. 2b,c**). Also, BA.2 was almost completely
159 resistant to two therapeutic monoclonal antibodies, Casirivimab and Imdevimab,
160 and was 35-fold more resistant to another therapeutic antibody, Sotrovimab,
161 when compared to the ancestral D614G-bearing B.1.1 virus (**Fig. 2d**). Moreover,
162 both BA.1 and BA.2 were highly resistant to the convalescent sera who had
163 infected with early pandemic virus (before May 2020; **Fig. 2e**), Alpha (**Extended
164 data Fig. 3a**) and Delta (**Extended data Fig. 3b**). These data suggest that,
165 similar to BA.1, BA.2 is highly resistant to the antisera induced by vaccination
166 and infection with other SARS-CoV-2 variants as well as three antiviral
167 therapeutic antibodies.
168   We then tested the 17 sera infected with BA.1: 13 convalescents were
169 fully vaccinated (2 shots), 1 convalescent was 1-dose vaccinated, and 3
170 convalescents were not vaccinated. BA.1 convalescent sera exhibited the
171 strongest antiviral effect against BA.1 (**Fig. 2f**). Although BA.2 was 1.4-fold more
172 resistant to the BA.1-infected sera than BA.1, there was no statistical difference
173 (**Fig. 2f**; *P*=0.091 by Wilcoxon signed-rank test). Importantly, the BA.1
174 convalescent sera with full vaccination exhibited significantly stronger antiviral
175 effects against all variants tested than unvaccinated or 1-dose vaccinated
176 convalescents (**Extended Data Fig. 3c**).
177   To address the possibility that the BA.1-induced humoral immunity is
178 less effective against BA.2, we used the convalescent sera obtained from

179   infected hamsters at 16 days postinfection (d.p.i.). Similar to the results of
180   convalescent human sera (**Fig. 2e** and **Extended Data Fig. 2b**), both BA.1 and
181   BA.2 exhibited pronounced resistances against B.1.1- and Delta-infected
182   convalescent hamster sera (**Fig. 2g** and **Extended Data Fig. 3d**). Interestingly,
183   BA.2 was significantly (2.9-fold) more resistant to BA.1-infected convalescent
184   hamster sera than BA.1 (**Fig. 2g**). To further verify the resistance of BA.2
185   against BA.1-induced immunity, mice were immunised with the cells expressing
186   the S proteins of ancestral B.1.1 and BA.1 and obtained murine antisera. Again,
187   the neutralisation assay using murine sera showed that BA.2 is more
188   significantly (6.4-fold) resistant to the BA.1 S-immunised sera than BA.1 (**Fig.
189   2h**). These findings suggest that BA.1-induced humoral immunity is less
190   effective against BA.2.
191
192   **Virological features of BA.2 in vitro**
193   To investigate the virological characteristics of BA.2, we generated chimeric
194   recombinant SARS-CoV-2 that expresses GFP and harbours the *S* gene of
195   ancestral B.1.1, Delta, BA.1 and BA.2 by reverse genetics (**Extended Data Fig.
196   4**)[21]. Although the growth of BA.1 and BA.2 was comparable in
197   VeroE6/TMPRSS2 cells, BA.2 was more replicative than BA.1 in Calu-3 cells
198   and primary human nasal epithelial cells (**Fig. 3a**). Notably, the morphology of
199   infected cells was different; BA.2 formed significantly (1.52-fold) larger syncytia
200   than BA.1 (**Fig. 3b** and **Extended Data Fig. 5a**). Whereas the plaque size in
201   VeroE6/TMPRSS2 cells infected with BA.1 and BA.2 was significantly smaller
202   than those of cells infected with B.1.1, the plaques formed by BA.2 infection are
203   significantly (1.27-fold) larger than those by BA.1 infection (**Fig. 3c** and
204   **Extended Data Fig. 5b**). Moreover, the coculture of S-expressing cells with
205   HEK293-ACE2/TMPRSS2 cells showed that BA.2 S induces significantly
206   (2.9-fold) larger multinuclear syncytia formation when compared to BA.1 S
207   (**Extended Data Fig. 5c**). These data suggest that BA.2 is more fusogenic than
208   BA.1. To further address this possibility, we analysed the fusogenicity of the S
209   proteins of BA.2 S by a cell-based fusion assay[12,22,23]. The expression level of
210   BA.2 S on the cell surface was significantly lower than that of BA.1 S (**Extended
211   Data Fig. 6a**). Nevertheless, our fusion assay using VeroE6/TMPRSS2 cells and
212   Calu-3 cells showed that BA.2 S is significantly more fusogenic than BA.1 S (**Fig.
213   3d**). We then analysed the binding affinity of BA.2 S receptor binding domain
214   (RBD) to ACE2 by an yeast surface display assay[16,22,24]. Although the binding
215   affinity of BA.1 S RBD to ACE2 is controversial[11,15-17,25,26], our yeast surface
216   display showed that the binding affinity of the RBD of BA.1 and BA.2 is
217   comparable (**Extended Data Fig. 6b**).
218   Because we have proposed that the SARS-CoV-2 S-mediated
219   fusogenicity is closely associated with the efficacy of S1/S2 cleavage[12,23], we

220 hypothesized that BA.2 S is more efficiently cleaved than BA.1 S. However, an

221 western blotting analysis showed that BA.2 S is less cleaved than BA.1 S (**Fig.**

222 **3e**), suggesting that BA.2 S exhibits a higher fusogenicity independently of

223 S1/S2 cleavage.

224   We have recently revealed that BA.1 poorly utilizes TMPRSS2 for the

225 infection[11]. To analyse the TMPRSS2 usage by BA.2 S, we performed

226 cell-based fusion assay using 293-ACE2 cells with or without TMPRSS2

227 expression. We verified that 293-ACE2 cells do not express endogenous

228 TMPRSS2 on the cell surface (**Extended Data Fig. 6c**). As shown in **Fig. 3f**, the

229 fusogenicity of BA.2 S was significantly higher in both cell lines than that of BA.1.

230 However, although BA.2 S was less fusogenic than B.1.1 S in 293-ACE2 cells,

231 the fusogenicity of BA.2 S and B.1.1 S was comparable in 293-ACE2/TMPRSS2

232 cells (**Fig. 3f**). These results suggest that the relatively higher fusogenicity of

233 BA.2 is dependent on TMPRSS2 expression on the surface of target cells. To

234 further assess whether the TMPRSS2-dependent infection enhancement was

235 also observed in cell-free virus, we inoculated pseudoviruses into 293-ACE2

236 cells and 293-ACE2/TMPRSS2 cells. Although the infectivity of B.1.1 and Delta

237 was 15.3-fold and 24.6-fold increased by TMPRSS2 expression, respectively,

238 the TMPRSS2 expression on the target cells did not affect the infectivity of both

239 BA.1 and BA.2 (**Fig. 3g**). These results suggest that TMPRSS2 does not affect

240 the infectivity of cell-free BA.2 virus. However, although the growth of BA.2 and

241 BA.1 was comparable in 293-ACE2 cells, BA.2 was more replicative than BA.1

242 in 293-ACE2/TMPRSS2 cells (**Fig. 3h**). Overall, our data suggest that BA.2 is

243 more fusogenic and replicative than BA.1 in a TMPRSS2-dependent fashion.

244

245 **Virological features of BA.2 in vivo**

246 To investigate the dynamics of viral replication of BA.2 *in vivo*, we conducted

247 hamster infection experiments. Consistent with our recent study[12], B.1.1-infected

248 hamsters exhibited decreased body weight and respiratory disorders that are

249 reflected by two surrogate markers for bronchoconstriction or airway obstruction,

250 enhanced pause (Penh) and the ratio of time to peak expiratory follow relative to

251 the total expiratory time (Rpef), and subcutaneous oxygen saturation (SpO_2),

252 whereas BA.1-infected hamsters exhibited no or weak disorders (**Fig. 4a**).

253 Notably, all parameters routinely measured, including body weight, Penh, Rpef

254 and SpO_2, of BA.2-infected hamsters were significantly different from uninfected

255 and BA.1-infected hamsters, and these values were comparable to those of

256 B.1.1-infected hamsters (**Fig. 4a**). These data suggest that BA.2 is more

257 pathogenic than BA.1.

258   To analyse viral spread in the respiratory organs of infected hamsters,

259 viral RNA load and nucleocapsid (N) expression were assessed by RT-qPCR

260 of viral RNA and immunohistochemistry (IHC), respectively. As shown in **Fig. 4b**,

261 viral RNA loads in the two lung regions, hilum and periphery, of BA.2-infected
262 hamsters were significantly higher than those of BA.1-infected hamsters. In the
263 lung periphery, the viral RNA load of BA.2 was significantly higher than that of
264 B.1.1, and the viral RNA load of BA.2 at 1 d.p.i. was 11-fold and 9.3-fold higher
265 than those of B.1.1 and BA.1 at the same timepoint, respectively (**Fig. 4b**). To
266 address the possibility that BA.2 more efficiently spreads than BA.1, we
267 investigated the positivity for N protein in the trachea and lung area close to the
268 hilum. At 1 d.p.i., N protein was detectable in the lower tracheal epithelium in all
269 infected hamsters, and particularly, was clearly detectable in the middle part of
270 trachea in BA.2-infected hamsters (**Extended Data Fig. 7a**). The positivity of N
271 protein was observed in both bronchial and bronchiolar epithelium in all infected
272 lungs (**Extended Data Fig. 7b**). Notably, alveolar positivities were observed in
273 B.1.1- and BA.2-infected lungs but not in BA.1-infected lungs (**Extended Data
274 Fig. 7b**). Morphometry showed that the percentage of N-positive cells in
275 BA.2-infected lungs is significantly higher than that of BA.1-infected lungs and
276 peaked at 3 d.p.i. (**Fig. 4c** and **Extended Data Fig. 7c**). On the other hand, at 3
277 d.p.i., the percentage of N-positive cells in the bronchus/bronchioles of
278 BA.2-infected hamsters was 5.4-fold lower than that of BA.1-infected hamsters
279 (**Fig. 4d**). At 5 d.p.i., N protein was almost disappeared in BA.1-infected lungs,
280 whereas alveolar staining was still detectable in B.1.1- and BA.2-infected lungs
281 (**Fig. 4c** and **Extended Data Fig. 7c**). These data suggest that BA.2 is more
282 rapidly and efficiently spread in the lung tissues than BA.1.
283
284 **Pathogenicity of BA.2**
285 To investigate the pathogenicity of BA.2, the right lungs of infected hamsters
286 were collected at 1, 3, and 5 d.p.i. and used them for haematoxylin and eosin
287 (H&E) staining and histopathological analysis[12,23]. All histopathological
288 parameters including bronchitis/bronchiolitis, haemorrhage, alveolar damage,
289 and the levels of type II pneumocytes, of BA.2-infected hamsters were
290 significantly higher than those in BA.1 (**Fig 4e** and **Extended Data Fig. 8a**). The
291 score indicating haemorrhage including congestive edema of BA.2 was
292 significantly higher than that of B.1.1 (**Fig. 4e**). As shown in our previous
293 studies[12,23], hyperplastic large type II pneumocytes suggesting the severity of
294 inflammation were observed in all infected hamsters at 5 d.p.i., and particularly,
295 the area of large type II pneumocytes in BA.2-infected hamsters was
296 significantly larger than those in B.1.1- and BA.1-infected hamsters (**Fig. 4e**).
297 Total histology score of BA.2 was significantly higher than that of BA.1 (**Fig. 4e**).
298 Furthermore, in the BA.2- and B.1.1-infected lungs, the inflammation with type II
299 alveolar pneumocyte hyperplasia was found in each lobe especially
300 frontal/upper and accessary lobes (**Extended Data Fig. 8b**).

Discussion

301 Although BA.2 is considered as an Omicron variant, its genomic sequence is
302 heavily different from BA.1, which suggests that the virological characteristics of
303 BA.2 is different from that of BA.1. Here, we elucidated the virological
304 characteristics of BA.2, such as its higher effective reproduction number, higher
305 fusogenicity, higher pathogenicity when compared to BA.1. Moreover, we
306 demonstrated that BA.2 is resistant to the BA.1-induced humoral immunity. Our
307 data indicate that BA.2 is virologically different from BA.1 and raise a proposal
308 that BA.2 should be given a letter of the Greek alphabet and be distinguished
309 from BA.1, a commonly recognized Omicron variant.
310
311 We showed evidence suggesting that BA.2 is virologically different and
312 distinguishable from BA.1. First, by using the two different types of antisera that
313 were obtained from experimental animals, convalescent hamsters infected with
314 BA.1 and mice immunized with the BA.1 S protein, we showed that the
315 resistance of BA.2 to the BA.1-induced humoral immunity. Our results indicate
316 that the antigenicity of BA.2 is different from BA.1. Although similar tendency
317 was observed in human samples as well, a statistically significant difference was
318 not observed probably because a relatively lower number of vaccine-naïve
319 individuals who were infected with BA.1 (3 unvaccinated donors and a 1-dose
320 vaccinated donor) were tested in the present study. The effect of BA.1-induced
321 humoral immunity against BA.2 in humans should deeply be verified in the future
322 investigation.
323 Second, the higher fusogenicity of BA.2 S is a pronounced
324 characteristics in in vitro experiments. We have demonstrated that Delta S is
325 highly fusogenic than BA.1 S and B.1.1 S, and we considered that the higher
326 fusogenicity is attributed to the higher efficacy of S cleavage[11,12,23,27]. However,
327 BA.2 S exhibited higher fusogenicity than BA.1 S without the increase of S
328 cleavage efficacy. In recent studies[12,23], we have proposed that the fusogenicity
329 of SARS-CoV-2 variant is closely related to its pathogenicity. This hypothesis is
330 further supported by the observations in BA.2 in the present study. However,
331 unlike Delta, the higher fusogenicity of BA.2 appears to be not attributed to the
332 higher efficacy of S cleavage[11,12,23,27]. Moreover, although TMPRSS2 increased
333 the efficacies of both cell-cell fusion[23] and cell-free infection mediated by B.1.1 S
334 and Delta S, TMPRSS2 increased the efficacy of BA.2 S-mediated cell-cell
335 fusion but did not affect that of BA.2 S-mediated cell-free infection. These
336 observations suggest that TMPRSS2 contributes to the cell-cell fusion and
337 cell-free infection mediated by BA.2 S with different mechanisms of action.
338 Third, it would be most critical for global health that BA.2 exhibits higher
339 pathogenicity than BA.1. Although clinical researches on the BA.2 pathogenicity
340 are needed, our investigations using a hamster model showed that the
341 pathogenicity of BA.2 is similar to that of an ancestral B.1.1 and higher than that

10

342 of BA.1. More importantly, the viral RNA load in the lung periphery and
343 histopathological disorders of BA.2 were more severe than those of BA.1 and
344 even B.1.1. Together with a higher effective reproduction number and
345 pronounced immune resistance of BA.2, it is evident that the spread of BA.2 can
346 be a serious issue for global health in the near future.
347   In summary, our data suggest the possibility that BA.2 would be the
348 most concerned variant to global health. Currently, both BA.2 and BA.1 are
349 recognised together as Omicron and these are almost undistinguishable. Based
350 on our findings, we propose that BA.2 should be recognised as a unique variant
351 of concern, and this SARS-CoV-2 variant should be monitored in depth.

352 **Methods**

353

354 **Ethics statement**

355 All experiments with hamsters were performed in accordance with the Science
356 Council of Japan's Guidelines for the Proper Conduct of Animal Experiments.
357 The protocols were approved by the Institutional Animal Care and Use
358 Committee of National University Corporation Hokkaido University (approval ID:
359 20-0123 and 20-0060). All experiments with mice were also performed in
360 accordance with the Science Council of Japan's Guidelines for the Proper
361 Conduct of Animal Experiments. The protocols were approved by the
362 Institutional Animal Experiment Committee of The Institute of Medical Science,
363 The University of Tokyo (approval ID: PA21-39). All protocols involving
364 specimens from human subjects recruited at Kyoto University, Kuramochi Clinic
365 Interpark and Chiba University were reviewed and approved by the Institutional
366 Review Boards of Kyoto University (approval ID: G1309), Kuramochi Clinic
367 Interpark (approval ID: G2021-004) and Chiba University (approval ID:
368 HS202103-03). All human subjects provided written informed consent. All
369 protocols for the use of human specimens were reviewed and approved by the
370 Institutional Review Boards of The Institute of Medical Science, The University of
371 Tokyo (approval IDs: 2021-1-0416 and 2021-18-0617), Kyoto University
372 (approval ID: G0697), Kumamoto University (approval IDs: 2066 and 2074), and
373 University of Miyazaki (approval ID: O-1021).

374

375 **Human sera collection**

376 Vaccine sera were collected from sixteen vaccinees four weeks after the second
377 vaccination with mRNA-1273 (Moderna) (average age: 27, range: 20-47, 38%
378 male). The sera obtained from nine vaccinees 10-25 d after the second
379 vaccination with ChAdOx1 (Oxford-AstraZeneca) (average age: 45, range:
380 35-54, 67% male) were purchased from BioIVT. The detail of vaccine sera is
381 summarized in **Supplementary Table 1**.

382 Convalescent sera were collected from vaccine-naive individuals who
383 had infected with Alpha variant (n=8; average age: 41, range: 21-57, 63% male)
384 and Delta variant (n=15; average age: 51, range: 22-67, 80% male).
385 Convalescent sera of BA.1-infected individuals (n=17; average age: 39, range:
386 20-65, 47% male, 76% received the second vaccination) were also collected. To
387 determine the SARS-CoV-2 variants infected, salivas were collected from
388 COVID-19 patients during onset and RNA was extracted using a QIAamp viral
389 RNA mini kit (Qiagen, Cat# 52906) according to the manufacturer's protocol. To
390 determine Alpha and Delta variants, viral genome sequencing was performed as
391 previously described[11]. For the detail, see "Viral genome sequencing" section
392 below. To determine the BA.1 variant, mutation-targeting RT-qPCR was

12

393   performed. To detect the S E484A substitution (common in all Omicron variants

394   including BA.1 and BA.2), a set of primer/probe E484A (SARS-CoV-2) (Takara,

395   Cat# RC322A) was used. To detect the S R214EPE insertion (specific for

396   B.1.1.529 and BA.1, undetectable in BA.2), a hand-made protocol was used with

397   the following primers and probe: Omi_ins214s-F1, TTC TAA GCA CAC GCC

398   TAT TAT AGT GC; Omi_ins214s-R1, TAA AGC CGA AAA ACC CTG AGG; and

399   Omi_ins214s, FAM-TGA GCC AGA AGA TC-MGB. The twelve convalescent

400   sera during early pandemic (until May 2020) (average age: 71, range: 52-92, 8%

401   male) were purchased from RayBiotech. Sera were inactivated at 56°C for 30

402   min and stored at –80°C until use. The detail of convalescent sera is

403   summarized in **Supplementary Table 2**.

404

405   **Viral genome sequencing**

406   Viral genome sequencing was performed as previously described[11,12,22,23] with

407   some modifications. Briefly, the sequences of the working viruses were verified

408   by viral RNA-sequencing analysis. Viral RNA was extracted using QIAamp viral

409   RNA mini kit (Qiagen, Cat# 52906). The sequencing library for total

410   RNA-sequencing was prepared using NEB Next Ultra RNA Library Prep Kit for

411   Illumina (New England Biolabs, Cat# E7530). Paired-end, 76-bp sequencing

412   was performed using MiSeq (Illumina) with MiSeq reagent kit v3 (Illumina, Cat#

413   MS-102-3001). Sequencing reads were trimmed using fastp v0.21.0[28] and

414   subsequently mapped to the viral genome sequences of a lineage A isolate

415   (strain WK-521; GISAID ID: EPI_ISL_408667)[29] using BWA-MEM v0.7.17[30].

416   Variant calling, filtering, and annotation were performed using SAMtools v1.9[31]

417   and snpEff v5.0e[32].

418

419   **Phylogenetic and comparative genome analyses**

420   To construct a maximum likelihood tree of the Omicron lineages (BA.1, BA.1.1,

421   BA.2, and BA.3) sampled from South Africa, the genome sequence data of

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

423   (https://www.gisaid.org/) on January 26, 2022. We excluded the data of viral

424   strains with the following features from the analysis: i) lacking the collection date

425   information; ii) sampled from animals other than humans; iii) having the flag of

426   low coverage sequencing; or iv) having >2% of undetermined nucleotide

427   characters. All the BA.2 and BA.3 sequences and 200 randomly sampled BA.1

428   (including 20 BA.1.1) sequences were used for the tree construction in addition

429   to an outgroup sequence, EPI_ISL_466615, the oldest isolate of B.1.1 in the UK.

430   The viral genome sequences were mapped to the reference sequence of

431   Wuhan-Hu-1 (GenBank accession no.: NC_045512.2) using minimap2 v2.17[33]

432   and subsequently converted to the multiple sequence alignment according to the

433   GISAID phylogenetic analysis pipeline

13

434 (https://github.com/roblanf/sarscov2phylo). The alignment sites corresponding to
435 the 1–265 and 29674–29903 positions in the reference genome were masked
436 (i.e., converted to NNN). The alignment sites with >50% sequences having a
437 gap or undetermined/ambiguous nucleotide were trimmed using trimAl v1.2[34].
438 The phylogenetic tree construction was performed by a two-step protocol: i) The
439 first tree was constructed; ii) tips with a longer external branch (Z score > 4) were
440 removed from the dataset; iii) and the final tree was constructed. The tree
441 reconstruction was performed by RAxML v8.2.12[35] under the GTRCAT
442 substitution model. Node support value was calculated by 100-times of bootstrap
443 analysis.

444   We performed phylodynamics analysis of Omicron lineages (BA.1,
445 BA.1.1, BA.2 and BA.3) sampled from South Africa as below. The SARS-CoV-2
446 genome sequence dataset used above was split into each Omicron lineage. As
447 an outgroup sequence, the oldest BA.2 (GISAID ID: EPI_ISL_8128463) was
448 added to the BA.1 and BA.3 datasets, and the oldest BA.3 (GISAID ID:
449 EPI_ISL_8616600) was added to the BA.2 dataset. The multiple sequence
450 alignment was constructed as the procedures described above. A
451 time-calibrated tree of each lineage was constructed by BEAST2 v.2.6.6[36]. The
452 HKY model[37] with the four categories of discrete gamma rate variation was
453 selected as a nucleotide substitution model. A relaxed molecular clock modelled
454 by a log-normal distribution was selected. The exponential growth coalescent
455 model was used. For BA.1, BA.2, and BA.3 datasets, nineteen, four, and three
456 independent chains of Markov chain Monte Carlo (MCMC) were respectively run
457 with 2,000,000 warmup and 18,000,000 sampling iterations. We confirmed that
458 the effective sampling sizes for all parameters were greater than 200, indicating
459 the MCMC runs were successfully convergent. The maximum credible trees with
460 common ancestor heights are shown in **Extended Data Fig. 1**.

461   The number of amino acid differences (including nonsynonymous
462 substitutions, insertions, and deletions) between SARS-CoV-2 lineages was
463 defined as below. Information on amino acid differences of each viral strain
464 compared with the reference sequence of Wuhan-Hu-1 (GenBank accession
465 no.: NC_045512.2) was extracted from the GISAID metadata (downloaded on
466 January 26, 2022). In each viral lineage, the amino acid differences that were
467 present in >10% sequences were extracted and subsequently counted. For the
468 comparison of BA.1 and BA.2, the set of the symmetric difference of the amino
469 acid differences compared with the reference was determined and subsequently
470 counted.

471

472 **Modelling the epidemic dynamics of SARS-CoV-2 lineages**
473 To quantify the spread speed of each SARS-CoV-2 lineage in the human
474 population, we estimated the relative effective reproduction number of each viral

475   lineage according to the epidemic dynamics calculated on the basis of viral
476   genomic surveillance data. The data were downloaded from the GISAID
477   database (https://www.gisaid.org/) on February 1, 2022. We excluded the data
478   of viral strains with the following features from the analysis: i) lacking the
479   collection date information; ii) sampled from animals other than humans; or iii)
480   sampled by quarantine. We analysed the datasets of the eleven countries with
481   >100 BA.2 sequences (Austria, Denmark, Germany, India, Israel, Philippines,
482   Singapore, South Africa, Sweden, the UK, and the USA) (**Fig. 1 and Extended**
483   **Data Fig. 2**). The dynamics of BA.1, BA.1.1, BA.2, and Delta (B.1.617.2 and AY
484   lineages) in each country from October 1, 2021, to January 25, 2022, were
485   analysed. The number of viral sequences of each viral lineage collected on each
486   day in each country was counted. Finally, we constructed the L (lineage) × C
487   (country) × T (time) = 4 × 11 × 117 shaped array, which comprises the count of
488   each viral lineage in each country in each day. This array was used as input data
489   for the statistical model described below.
490   We constructed a Bayesian hierarchical model to represent the relative
491   lineage growth dynamics with a multinomial logistic regression. The
492   mathematical theory underlying the model is described in detail elsewhere[38,39],
493   and this model is basically similar to the one used in our previous study[12]. In the
494   present study, we incorporated a hierarchical structure into the slope parameter
495   over time, which enable us to estimate the global average of the relative
496   effective reproduction number of each viral lineage as well as that in each
497   country. Arrays in the model index over one or more indices: L = 4 viral lineages
498   l; C = 11 countries c; and T = 117 days t. The model is:

$$\beta_{lc} \sim \mathrm{Student\_t}(6, \beta_l, \sigma_l)$$
$$\mu_{lct} = \alpha_{lc} + \beta_{lc}\mathrm{t}$$
$$\theta_{.ct} = \mathrm{softmax}(\mu_{.ct})$$

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

499   The explanatory variable is time t, and the outcome variable is y_{lct}, which
500   represents the count of viral lineage l in country c at time t. The slope
501   parameter of lineage l in country c, β_{lc}, is generated from a student's t
502   distribution with the hyper parameters of the mean β_l and the standard
503   deviation σ_l. As the distribution generating β_{lc}, we used a student's t distribution
504   with six degrees of freedom instead of a normal distribution to reduce the effects
505   of outlier values of β_{lc}. In the model, the linear estimator $\mu_{.ct}$, consisting of the
506   intercept $\alpha_{.c}$ and the slope $\beta_{.c}$, is converted to the simplex $\theta_{.ct}$, which
507   represents the probability of occurrence of each viral lineage at time t in
508   country c, by the softmax link function defined as:

15

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

509   y_{lct} is generated from θ_{ct} and the total count of all lineages at t in country c

510   according to a multinomial distribution.

511   The relative effective reproduction number of each viral lineage in each

512   county (r_{lc}) was calculated according to the slope parameter β_{lc} as:

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

513   where γ is the average viral generation time (2.1 day)

514   (http://sonorouschocolate.com/covid19/index.php?title=Estimating_Generation_

515   Time_Of_Omicron). Similarly, the global average of the relative effective

516   reproduction number of each viral lineage was calculated according to the slope

517   hyper parameter β_l as:

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

518   For the parameter estimation, the intercept and slope parameters of the BA.1

519   variant were fixed at 0. Consequently, the relative effective reproduction number

520   of BA.1 was fixed at 1, and those of the respective lineages were estimated

521   relative to that of BA.1.

522   Parameter estimation was performed by MCMC implemented in

523   CmdStan v2.28.1 (https://mc-stan.org) with cmdstanr v0.4.0

524   (https://mc-stan.org/cmdstanr/). Noninformative priors were set for all

525   parameters. Four independent MCMC chains were run with 1,000 and 2,000

526   steps in the warmup and sampling iterations, respectively. We confirmed that all

527   estimated parameters had <1.01 R-hat convergence diagnostic and >200

528   effective sampling size values, indicating that the MCMC runs were successfully

529   convergent. The fitted model closely recapitulated the observed viral lineage

530   dynamics in each country (**Extended Data Fig. 2c**). The analyses above were

531   performed in R v4.1.2 (https://www.r-project.org/).

532

533   **Cell culture**

534   HEK293 cells (a human embryonic kidney cell line; ATCC CRL-1573) and

535   HEK293-ACE2 cells [HEK293 cells (ATCC CRL-1573) stably expressing human

536   ACE2][22] were maintained in DMEM (high glucose) (Sigma-Aldrich, Cat#

537   6429-500ML) containing 10% FBS, 1 µg/ml puromycin (InvivoGen, Cat#

538   ant-pr-1) and 1% PS. HEK293-ACE2/TMPRSS2 cells [HEK293 cells (ATCC

539   CRL-1573) stably expressing human ACE2 and TMPRSS2][22] were maintained

540   in Dulbecco's modified Eagle's medium (DMEM) (high glucose) (Wako, Cat#

541   044-29765) containing 10% foetal bovine serum (FBS) and 1%

542   penicillin-streptomycin (PS). HEK293-C34 cells, *IFNAR1* KO HEK293 cells

543   expressing human ACE2 and TMPRSS2 by doxycycline treatment[21], were

544   maintained in Dulbecco's modified Eagle's medium (high glucose)

545   (Sigma-Aldrich, Cat# R8758-500ML) containing 10% FBS, 10 µg/ml blasticidin

546 (InvivoGen, Cat# ant-bl-1) and 1% PS. Vero cells [an African green monkey
547 (*Chlorocebus sabaeus*) kidney cell line; JCRB0111] were maintained in Eagle's
548 minimum essential medium (EMEM) (Wako, Cat# 051-07615) containing 10%
549 FBS and 1% PS. VeroE6/TMPRSS2 cells (VeroE6 cells stably expressing
550 human TMPRSS2; JCRB1819)[29] were maintained in DMEM (low glucose)
551 (Wako, Cat# 041-29775) containing 10% FBS, G418 (1 mg/ml; Nacalai Tesque,
552 Cat# G8168-10ML) and 1% PS. Calu-3 cells (a human lung epithelial cell line;
553 ATCC HTB-55) were maintained in EMEM (Sigma-Aldrich, Cat# M4655-500ML)
554 containing 20% FBS and 1% PS. Calu-3/DSP$_{1-7}$ cells [Calu-3 cells (ATCC
555 HTB-55) stably expressing DSP$_{1-7}$][40] were maintained in EMEM (Wako, Cat#
556 056-08385) supplemented with 20% FBS and 1% PS. B16F10 cells (a mouse
557 melanoma cell line; RCB2630) were maintained in DMEM (high glucose)
558 (Sigma-Aldrich, Cat# D6429-500ML) containing 10% FBS and 1% PS. Expi293
559 cells (Thermo Fisher Scientific, Cat# A14527) were maintained in Expi293
560 expression medium (Thermo Fisher Scientific, Cat# A1435101). Primary human
561 nasal epithelial cells (Cat# EP02, Batch# MP0010) were purchased from
562 Epithelix and maintained according to the manufacturer's procedure.
563

Plasmid construction

565 Plasmids expressing the SARS-CoV-2 S proteins of B.1.1 (the parental
566 D614G-bearing variant), Alpha (B.1.1.7), Delta (B.1.617.2) and BA.1 variants
567 were prepared in our previous studies[11,12,22,23,41]. Plasmids expressing the
568 codon-optimised S proteins of BA.2 and a BA.2 derivative that loses its
569 cytoplasmic tail were generated by site-directed overlap extension PCR using
570 the primers listed in **Supplementary Table 3**. The resulting PCR fragment was
571 digested with KpnI and NotI and inserted into the corresponding site of the
572 pCAGGS vector. To construct the plasmids expressing anti-SARS-CoV-2
573 monoclonal antibodies (Casirivimab, Imdevimab or Sotrovimab), the sequences
574 of the variable regions of these antibodies were obtained from KEGG Drug
575 Database (https://www.genome.jp/kegg/drug/) and were artificially synthesized
576 (Fasmac). The obtained coding sequences of the variable regions of the heavy
577 and light chains were cloned into the pCAGGS vector containing the sequences
578 of the human immunoglobulin 1 and kappa constant region [kindly provided by
579 Dr. Hisashi Arase (Osaka University, Japan)]. Nucleotide sequences were
580 determined by DNA sequencing services (Eurofins), and the sequence data
581 were analyzed by Sequencher v5.1 software (Gene Codes Corporation).
582

Preparation of monoclonal antibodies

584 Casirivimab, Imdevimab and Sotrovimab were prepared as previously
585 described[11,42]. Briefly, the pCAGGS vectors containing the sequences encoding
586 the immunoglobulin heavy and light chains were cotransfected into HEK293T

17

587 cells using PEI Max (Polysciences, Cat# 24765-1). At 48 h posttransfection, the
588 cell culture supernatants were harvested, and the antibodies were purified using
589 NAb protein A plus spin kit (Thermo Fisher Scientific, Cat# 89948) according to
590 the manufacturer's protocol.
591
592 **Preparation of mouse sera**
593 The SARS-CoV-2 S-immunised mouse sera were prepared as previously
594 described[42]. To prepare the immunogen, B16F10 cells (2,500,000 cells) were
595 transfected with 5 µg S expression plasmid by PEI Max (Polysciences, Cat#
596 24765-1) according to the manufacturer's protocol. Two days posttransfection,
597 the transfected cells were washed twice with PBS, and then the cell pellets were
598 stored at –80°C (10,000,000 cells per stock). The expression of transfected S
599 protein was verified by flow cytometry and western blot. BALB/c mice (female, 7
600 weeks old) were purchased from Japan SLC Inc. (Shizuoka, Japan). The mice
601 were maintained under specific pathogen-free conditions. For the immunisation,
602 mice were subcutaneously immunized with the freeze-thawed S-expressing
603 B16F10 cells in complete Freund's adjuvant (50%) (Sigma-Aldrich, Cat# F5881).
604 Three weeks after immunisation, blood was collected in BD Microtainer blood
605 collection tubes (BD Biosciences, Cat# 365967) and sera were collected by
606 centrifugation.
607
608 **Neutralisation assay**
609 Pseudoviruses were prepared as previously described[11,23,24,41,43]. Briefly,
610 lentivirus (HIV-1)-based, luciferase-expressing reporter viruses were
611 pseudotyped with the SARS-CoV-2 spikes. HEK293T cells (1,000,000 cells)
612 were cotransfected with 1 µg psPAX2-IN/HiBiT[44], 1 µg pWPI-Luc2[44], and 500 ng
613 plasmids expressing parental S or its derivatives using PEI Max (Polysciences,
614 Cat# 24765-1) according to the manufacturer's protocol. Two days
615 posttransfection, the culture supernatants were harvested and centrifuged. The
616 pseudoviruses were stored at –80°C until use.
617   For the neutralisation assay, the SARS-CoV-2 S pseudoviruses
618 (counting ~20,000 relative light units) were incubated with serially diluted
619 (40-fold to 29,160-fold dilution at the final concentration) heat-inactivated sera or
620 monoclonal antibodies (Casirivimab, Imdevimab or Sotrovimab) at 37°C for 1 h.
621 Pseudoviruses without sera were included as controls. Then, an 80 µl mixture of
622 pseudovirus and serum/antibody was added to HOS-ACE2/TMPRSS2 cells
623 (10,000 cells/50 µl) in a 96-well white plate. At 2 d.p.i., the infected cells were
624 lysed with a One-Glo luciferase assay system (Promega, Cat# E6130) or a
625 Bright-Glo™ Luciferase Assay System (Promega, Cat# E2650), and the
626 luminescent signal was measured using a GloMax explorer multimode

18

627 microplate reader 3500 (Promega) or CentroXS3 (Berthhold Technologies). The
628 assay of each serum was performed in triplicate, and the 50% neutralisation titre
629 (NT50) was calculated using Prism 9 (GraphPad Software).
630

SARS-CoV-2 reverse genetics
632 Recombinant SARS-CoV-2 was generated by circular polymerase extension
633 reaction (CPER) as previously described[21-23]. In brief, 9 DNA fragments
634 encoding the partial genome of SARS-CoV-2 (strain WK-521, PANGO lineage
635 A; GISAID ID: EPI_ISL_408667)[29] were prepared by PCR using PrimeSTAR
636 GXL DNA polymerase (Takara, Cat# R050A). A linker fragment encoding
637 hepatitis delta virus ribozyme, bovine growth hormone poly A signal and
638 cytomegalovirus promoter was also prepared by PCR. The corresponding
639 SARS-CoV-2 genomic region and the PCR templates and primers used for this
640 procedure are summarised in **Supplementary Table 4**. The 10 obtained DNA
641 fragments were mixed and used for CPER[21]. To prepare GFP-expressing
642 replication-competent recombinant SARS-CoV-2, we used fragment 9, in which
643 the *GFP* gene was inserted in the *ORF7a* frame, instead of the authentic F9
644 fragment (see **Extended Data Fig. 4** and **Supplementary Table 4**)[21].

645   To produce recombinant SARS-CoV-2, the CPER products were
646 transfected into HEK293-C34 cells using TransIT-LT1 (Takara, Cat# MIR2300)
647 according to the manufacturer's protocol. At one day posttransfection, the
648 culture medium was replaced with Dulbecco's modified Eagle's medium (high
649 glucose) (Sigma-Aldrich, Cat# R8758-500ML) containing 2% FCS, 1% PS and
650 doxycycline (1 µg/ml; Takara, Cat# 1311N). At six days posttransfection, the
651 culture medium was harvested and centrifuged, and the supernatants were
652 collected as the seed virus. To remove the CPER products (i.e.,
653 SARS-CoV-2-related DNA), 1 ml of the seed virus was treated with 2 µl TURBO
654 DNase (Thermo Fisher Scientific, Cat# AM2238) and incubated at 37°C for 1 h.
655 Complete removal of the CPER products (i.e., SARS-CoV-2-related DNA) from
656 the seed virus was verified by PCR. The working virus stock was prepared from
657 the seed virus as described above.

658   To generate chimeric recombinant SARS-CoV-2 that encodes the S
659 proteins of B.1.1, BA.1 and BA.2 (**Extended Data Fig. 4**), mutations were
660 inserted in fragment 8 (**Supplementary Table 4**) using the GENEART
661 site-directed mutagenesis system (Thermo Fisher Scientific, Cat# A13312)
662 according to the manufacturer's protocol with the primers listed in
663 **Supplementary Table 5**. A recombinant SARS-CoV-2 that bears D614G S
664 (corresponds to B.1.1 S) was prepared in our previous study[23]. To prepare a
665 chimeric recombinant SARS-CoV-2 that bears Delta S (**Extended Data Fig. 4**),
666 the fragment of viral genome that corresponds to the region of fragment 8
667 (**Supplementary Table 4**) was subcloned from a Delta isolate (strain

19

668 TKYTK1734; GISAID ID: EPI_ISL_2378732[23]. Nucleotide sequences were
669 determined by a DNA sequencing service (Fasmac), and the sequence data
670 were analysed by Sequencher software v5.1 (Gene Codes Corporation).
671 To produce chimeric recombinant SARS-CoV-2, the CPER products
672 were transfected into HEK293-C34 cells using TransIT-LT1 (Takara, Cat#
673 MIR2300) according to the manufacturer's protocol. At 1 day posttransfection,
674 the culture medium was replaced with Dulbecco's modified Eagle's medium
675 (high glucose) (Sigma-Aldrich, Cat# R8758-500ML) containing 2% FCS, 1% PS
676 and doxycycline (1 µg/ml; Takara, Cat# 1311N). At 7 days posttransfection, the
677 culture medium was harvested and centrifuged, and the supernatants were
678 collected as the seed virus. To remove the CPER products (i.e.,
679 SARS-CoV-2-related DNA), 1 ml of the seed virus was treated with 2 µl TURBO
680 DNase (Thermo Fisher Scientific, Cat# AM2238) and incubated at 37°C for 1 h.
681 Complete removal of the CPER products (i.e., SARS-CoV-2-related DNA) from
682 the seed virus was verified by PCR. The working virus stock was prepared from
683 the seed virus as described below (see "SARS-CoV-2 preparation and titration"
684 section).
685

686 **SARS-CoV-2 preparation and titration**
687 The working virus stocks of chimeric recombinant SARS-CoV-2 that encodes the
688 S proteins of B.1.1, Delta, BA.1 and BA.2 were prepared and titrated as
689 previously described[21-23]. In brief, 20 µl of the seed virus was inoculated into
690 VeroE6/TMPRSS2 cells (5,000,000 cells in a T-75 flask). One hour after
691 infection (h.p.i.), the culture medium was replaced with DMEM (low glucose)
692 (Wako, Cat# 041-29775) containing 2% FBS and 1% PS. At 3 d.p.i., the culture
693 medium was harvested and centrifuged, and the supernatants were collected as
694 the working virus stock.
695 The titre of the prepared working virus was measured as the 50%
696 tissue culture infectious dose ($TCID_{50}$). Briefly, one day before infection,
697 VeroE6/TMPRSS2 cells (10,000 cells) were seeded into a 96-well plate. Serially
698 diluted virus stocks were inoculated into the cells and incubated at 37°C for 4 d.
699 The cells were observed under microscopy to judge the CPE appearance. The
700 value of $TCID_{50}$/ml was calculated with the Reed–Muench method[45].
701 To verify the sequence of chimeric recombinant SARS-CoV-2, viral
702 RNA was extracted from the working viruses using a QIAamp viral RNA mini kit
703 (Qiagen, Cat# 52906) and viral genome sequence was analysed as described
704 above (see "Viral genome sequencing" section). In brief, the viral sequences of
705 *GFP*-encoding recombinant SARS-CoV-2 (strain WK-521; GISIAD ID:
706 EPI_ISL_408667)[21,29] that harbour the *S* genes of respective variants (B.1.1,
707 BA.1 or BA.2) were used for the reference. Information on the unexpected

708   mutations detected is summarized in **Supplementary Table 6**, and the raw data
709   are deposited in Gene Expression Omnibus (accession number: GSE196649).
710

711   **SARS-CoV-2 infection**
712   One day before infection, Vero cells (10,000 cells), VeroE6/TMPRSS2 cells
713   (10,000 cells), Calu-3 cells (20,000 cells), HEK293-ACE2 cells (10,000 cells),
714   HEK293-ACE2/TMPRSS2 cells (10,000 cells), were seeded into a 96-well plate.
715   SARS-CoV-2 [100 $TCID_{50}$ for VeroE6/TMPRSS2 cells (**Fig. 3a**), 1,000 $TCID_{50}$
716   for Vero cells (**Fig. 3a**), HEK293-ACE2 cells (10,000 cells) (**Fig. 3j**), and
717   HEK293-ACE2/TMPRSS2 cells (10,000 cells) (**Fig. 3j**); and 2,000 $TCID_{50}$ for
718   Calu-3 cells (**Fig. 3a**)] was inoculated and incubated at 37°C for 1 h. The
719   infected cells were washed, and 180 µl of culture medium was added. The
720   culture supernatant (10 µl) was harvested at the indicated timepoints and used
721   for RT–qPCR to quantify the viral RNA copy number (see "RT–qPCR" section
722   below).
723   The infection experiment primary human nasal epithelial cells (**Fig. 3a**)
724   was performed as previously described[11,23]. Briefly, the working viruses were
725   diluted with Opti-MEM (Thermo Fisher Scientific, Cat# 11058021). The diluted
726   viruses (1,000 $TCID_{50}$ in 100 µl) were inoculated onto the apical side of the
727   culture and incubated at 37°C for 1 h. The inoculated viruses were removed and
728   washed twice with Opti-MEM. To harvest the viruses on the apical side of the
729   culture, 100 µl Opti-MEM was applied onto the apical side of the culture and
730   incubated at 37°C for 10 m. The Opti-MEM applied was harvested and used for
731   RT–qPCR to quantify the viral RNA copy number (see "RT–qPCR" section
732   below).
733

734   **RT–qPCR**
735   RT–qPCR was performed as previously described[11,12,22,23]. Briefly, 5 µl of culture
736   supernatant was mixed with 5 µl of 2 × RNA lysis buffer [2% Triton X-100, 50
737   mM KCl, 100 mM Tris-HCl (pH 7.4), 40% glycerol, 0.8 U/µl recombinant RNase
738   inhibitor (Takara, Cat# 2313B)] and incubated at room temperature for 10 min.
739   RNase-free water (90 µl) was added, and the diluted sample (2.5 µl) was used
740   as the template for real-time RT-PCR performed according to the manufacturer's
741   protocol using the One Step TB Green PrimeScript PLUS RT-PCR kit (Takara,
742   Cat# RR096A) and the following primers: Forward *N*, 5'-AGC CTC TTC TCG
743   TTC CTC ATC AC-3'; and Reverse *N*, 5'-CCG CCA TTG CCA GCC ATT C-3'.
744   The viral RNA copy number was standardized with a SARS-CoV-2 direct
745   detection RT-qPCR kit (Takara, Cat# RC300A). Fluorescent signals were
746   acquired using QuantStudio 3 Real-Time PCR system (Thermo Fisher Scientific),
747   CFX Connect Real-Time PCR Detection system (Bio-Rad), Eco Real-Time PCR

748 System (Illumina), qTOWER3 G Real-Time System (Analytik Jena) or 7500
749 Real-Time PCR System (Thermo Fisher Scientific).

750

751 **Fluorescence microscopy**
752 Fluorescence microscopy (**Fig. 3b** and **Extended Data Fig. 5a**) was performed
753 as previously described[23]. Briefly, one day before infection, VeroE6/TMPRSS2
754 cells (10,000 cells) were seeded into 96-well, glass bottom, black plates and
755 infected with SARS-CoV-2 (100 $TCID_{50}$). At 24, 48, and 72 h.p.i., GFP
756 fluorescence was observed under an All-in-One Fluorescence Microscope
757 BZ-X800 (Keyence) in living cells, and a 13-square-millimeter area of each
758 sample was scanned. Images were reconstructed using an BZ-X800 analyzer
759 software (Keyence), and the area of the GFP-positive cells was measured using
760 this software.

761

762 **Plaque assay**
763 Plaque assay (**Fig. 3c** and **Extended Data Fig. 5b**) was performed as
764 previously described[12,22,23]. Briefly, one day before infection, VeroE6/TMPRSS2
765 cells (100,000 cells) were seeded into a 24-well plate and infected with
766 SARS-CoV-2 (1, 10, 100 and 1,000 $TCID_{50}$) at 37°C for 2 h. Mounting solution
767 containing 3% FBS and 1.5% carboxymethyl cellulose (Wako, Cat# 039-01335)
768 was overlaid, followed by incubation at 37°C. At 3 d.p.i., the culture medium was
769 removed, and the cells were washed with PBS three times and fixed with 4%
770 paraformaldehyde phosphate (Nacalai Tesque, Cat# 09154-85). The fixed cells
771 were washed with tap water, dried, and stained with staining solution [0.1%
772 methylene blue (Nacalai Tesque, Cat# 22412-14) in water] for 30 m. The stained
773 cells were washed with tap water and dried, and the size of plaques was
774 measured using Fiji software v2.2.0 (ImageJ).

775

776 **Coculture experiment**
777 Coculture experiment (**Extended Data Fig. 5c**) was performed as previously
778 described[12]. Briefly, one day before transfection, effector cells (i.e., S-expressing
779 cells) were seeded on the poly-L-lysine (Sigma, Cat# P4832) coated coverslips
780 put in a 12-well plate, and target cells were prepared at a density of 100,000
781 cells in a 12-well plate. To prepare effector cells, HEK293 cells were
782 cotransfected with the S-expression plasmids (500 ng) and $pDSP_{8-11}$ (500 ng)
783 using PEI Max (Polysciences, Cat# 24765-1). To prepare target cells, HEK293
784 and HEK293-ACE2/TMPRSS2 cells were transfected with $pDSP_{1-7}$ (500 ng). At
785 24 h posttransfection, target cells were detached and cocultured with effector
786 cells in a 1:2 ratio. At 9 h post-coculture, cells were fixed with 4%
787 parafprmaldehyde in PBS (Nacalai Tesque, Cat# 09154-85) for 15 m at room
788 temperature. Nuclei were stained with Hoechst 33342 (Thermo Fisher Scientific,

789   Cat# H3570). The coverslips were mounted on glass slides using
790   Fluoromount-G (Southern Biotechnology, Cat# 0100-01) with Hoechst 33342
791   and observed using an A1Rsi Confocal Microscope (Nikon). The size of
792   syncytium (GFP-positive area) was measured using Fiji software v2.2.0
793   (ImageJ) as previously described[12].

794

795   **SARS-CoV-2 S-based fusion assay**
796   SARS-CoV-2 S-based fusion assay (**Fig. 3d and 3f**) was performed as
797   previously described[12,22,23]. This assay utilizes a dual split protein (DSP)
798   encoding *Renilla* luciferase and *GFP* genes; the respective split proteins,
799   DSP_{8-11} and DSP_{1-7}, are expressed in effector and target cells by transfection.
800   Briefly, on day 1, effector cells (i.e., S-expressing cells) and target cells (see
801   below) were prepared at a density of 0.6–0.8×10^6 cells in a 6-well plate. To
802   prepare effector cells, HEK293 cells were cotransfected with the S expression
803   plasmids (400 ng) and $pDSP_{8-11}$ (400 ng) using TransIT-LT1 (Takara, Cat#
804   MIR2300). To prepare target cells, HEK293 cells were cotransfected with
805   pC-ACE2 (200 ng) and $pDSP_{1-7}$ (400 ng). Target HEK293 cells in selected wells
806   were cotransfected with pC-TMPRSS2 (40 ng) in addition to the plasmids above.
807   VeroE6/TMPRSS2 cells, Calu-3 cells, HEK293-ACE2 cells and
808   HEK293-ACE2/TMPRSS2 cells were transfected with $pDSP_{1-7}$ (400ng). On day
809   3 (24 h posttransfection), 16,000 effector cells were detached and reseeded into
810   96-well black plates (PerkinElmer, Cat# 6005225), and target cells (HEK293,
811   VeroE6/TMPRSS2 or Calu-3/DSP_{1-7} cells) were reseeded at a density of
812   1,000,000 cells/2 ml/well in 6-well plates. On day 4 (48 h posttransfection), target
813   cells were incubated with EnduRen live cell substrate (Promega, Cat# E6481)
814   for 3 h and then detached, and 32,000 target cells were added to a 96-well plate
815   with effector cells. *Renilla* luciferase activity was measured at the indicated time
816   points using Centro XS3 LB960 (Berthhold Technologies). To measure the
817   surface expression level of S protein, effector cells were stained with rabbit
818   anti-SARS-CoV-2 S S1/S2 polyclonal antibody (Thermo Fisher Scientific, Cat#
819   PA5-112048, 1:100). Normal rabbit IgG (SouthernBiotech, Cat# 0111-01, 1:100)
820   was used as negative controls, and APC-conjugated goat anti-rabbit IgG
821   polyclonal antibody (Jackson ImmunoResearch, Cat# 111-136-144, 1:50) was
822   used as a secondary antibody. Surface expression level of S proteins
823   (**Extended Data Fig. 6a**) was measured using FACS Canto II (BD Biosciences)
824   and the data were analysed using FlowJo software v10.7.1 (BD Biosciences).
825   Gating strategy for flow cytometry is shown in **Supplementary Fig. 1**. To
826   calculate fusion activity, *Renilla* luciferase activity was normalized to the MFI of
827   surface S proteins. The normalized value (i.e., *Renilla* luciferase activity per the
828   surface S MFI) is shown as fusion activity.

829

Yeast surface display

Yeast surface display (**Extended Data Fig. 6b**) was performed as previously described as previously described[22,24,46]. Briefly, the peptidase domain of human ACE2 (residues 18-740) was expressed in Expi293 cells and purified by a 5-ml HisTrap Fast Flow column (Cytiva, Cat# 17-5255-01) and Superdex 200 16/600 (Cytiva, Cat# 28-9893-35) using an ÄKTA pure chromatography system (Cytiva), and the purified soluble ACE2 was labelled with CF640 (Biotium, Cat# 92108). Protein quality was verified using a Tycho NT.6 system (NanoTemper) and ACE2 activity assay kit (SensoLyte, Cat# AS-72086).

An enhanced yeast display platform for SARS-CoV-2 RBD [wild-type (B.1.1), residues 336-528] yeast surface expression was established using *Saccharomyces cerevisiae* EBY100 strain and pJYDC1 plasmid (Addgene, Cat# 162458) as previously described[22,24,47]. The yeast-optimized SARS-CoV-2_RBD-Omicron-BA.1 gene (**Supplementary Table 7**) was obtained from Twist Biosciences. The site-directed mutagenesis was performed using the KAPA HiFi HotStart ReadyMix kit (Roche, Cat# KK2601) by restriction enzyme-free cloning procedure[48]. Primers for mutagenesis are listed in **Supplementary Table 8**.

The binding affinity of SARS-CoV-2 S B.1.1, BA.1, and BA.2 RBDs to human ACE2 were titrated by flow cytometry. The CF640-labelled ACE2 at 12–14 different concentrations (200 nM to 13 pM in PBS supplemented with bovine serum albumin at 1 g/l) per measurement were incubated with expressed yeast aliquots and 1 nM bilirubin (Sigma-Aldrich, Cat# 14370-1G) and analysed by using FACS S3e Cell Sorter device (Bio-Rad) as previously described[22,24,47]. The background binding subtracted fluorescent signal was fitted to a standard noncooperative Hill equation by nonlinear least-squares regression using Python v3.7 (https://www.python.org) as previously described[47].

TMPRSS2 expression on the cell surface

To measure the surface expression level of TMPRSS2 (**Extended Data Fig. 6c**), HEK293-ACE2 cells and HEK293-ACE2/TMPRSS2 cells were stained with rabbit anti-TMPRSS2 polyclonal antibody (BIOSS, Cat# BS-6285R, 1:100). Normal rabbit IgG (SouthernBiotech, Cat# 0111-01, 1:100) was used as negative controls, and APC-conjugated goat anti-rabbit IgG polyclonal antibody (Jackson ImmunoResearch, Cat# 111-136-144, 1:50) was used as a secondary antibody. Surface expression level of TMPRSS2 was measured using FACS Canto II (BD Biosciences) and the data were analysed using FlowJo software v10.7.1 (BD Biosciences). Gating strategy for flow cytometry is shown in **Supplementary Fig. 1**.

Western blot

871 Western blot (**Fig. 3e**) was performed as previously described[12,22,23]. The
872 HEK293 cells cotransfected with the S expression plasmids and pDSP$_{8-11}$ (see
873 "SARS-CoV-2 S-based fusion assay" section above) were used. To quantify the
874 level of the cleaved S2 protein in the cells, the harvested cells were washed and
875 lysed in lysis buffer [25 mM HEPES (pH 7.2), 20% glycerol, 125 mM NaCl, 1%
876 Nonidet P40 substitute (Nacalai Tesque, Cat# 18558-54), protease inhibitor
877 cocktail (Nacalai Tesque, Cat# 03969-21)]. After quantification of total protein by
878 protein assay dye (Bio-Rad, Cat# 5000006), lysates were diluted with 2 ×
879 sample buffer [100 mM Tris-HCl (pH 6.8), 4% SDS, 12% β-mercaptoethanol,
880 20% glycerol, 0.05% bromophenol blue] and boiled for 10 m. Then, 10 μl
881 samples (50 μg of total protein) were subjected to Western blot. For protein
882 detection, the following antibodies were used: mouse anti-SARS-CoV-2 S
883 monoclonal antibody (clone 1A9, GeneTex, Cat# GTX632604, 1:10,000), rabbit
884 anti-beta actin (ACTB) monoclonal antibody (clone 13E5, Cell Signalling, Cat#
885 4970, 1:5,000), horseradish peroxidase (HRP)-conjugated donkey anti-rabbit
886 IgG polyclonal antibody (Jackson ImmunoResearch, Cat# 711-035-152,
887 1:10,000) and HRP-conjugated donkey anti-mouse IgG polyclonal antibody
888 (Jackson ImmunoResearch, Cat# 715-035-150, 1:10,000). Chemiluminescence
889 was detected using SuperSignal West Femto Maximum Sensitivity Substrate
890 (Thermo Fisher Scientific, Cat# 34095) according to the manufacturer's
891 instruction. Bands were visualized using an Amersham Imager 600 (GE
892 Healthcare), and the band intensity was quantified using Image Studio Lite v5.2
893 (LI-COR Biosciences) or Fiji software v2.2.0 (ImageJ). Uncropped blots are
894 shown in **Supplementary Fig. 2**.

895

896 **Pseudovirus infection**
897 Pseudovirus infection was (**Fig. 3g**) performed as previously
898 described[11,12,22-24,43]. Briefly, the same amount of pseudoviruses (normalized to
899 the HiBiT value, which indicates the amount of p24 HIV-1 antigen) was
900 inoculated into HEK293-ACE2 cells or HEK293-ACE2/TMPRSS2 and viral
901 infectivity was measured as described above (see "Neutralisation assay"
902 section). To analyse the effect of TMPRSS2 for pseudovirus infectivity, the fold
903 change of the values of HEK293-ACE2/TMPRSS2 to HEK293-ACE2 was
904 calculated.

905

906 **Animal experiments**
907 Animal experiments (**Fig. 4**) were performed as previously described[12,23]. Syrian
908 hamsters (male, 4 weeks old) were purchased from Japan SLC Inc. (Shizuoka,
909 Japan). Baseline body weights were measured before infection. For the virus
910 infection experiments, hamsters were euthanized by intramuscular injection of a
911 mixture of either 0.15 mg/kg medetomidine hydrochloride (Domitor®, Nippon

25

912 Zenyaku Kogyo), 2.0 mg/kg midazolam (Dormicum®, FUJIFILM Wako
913 Chemicals) and 2.5 mg/kg butorphanol (Vetorphale®, Meiji Seika Pharma), or
914 0.15 mg/kg medetomidine hydrochloride, 2.0 mg/kg alphaxaone (Alfaxan®,
915 Jurox) and 2.5 mg/kg butorphanol. The B.1.1 virus, Delta, Omicron (10,000
916 $TCID_{50}$ in 100 µl), or saline (100 µl) were intranasally inoculated under
917 anaesthesia. Oral swabs were daily collected under anaesthesia with isoflurane
918 (Sumitomo Dainippon Pharma). Body weight, enhanced pause (Penh), the ratio
919 of time to peak expiratory follow relative to the total expiratory time (Rpef) and
920 subcutaneous oxygen saturation (SpO_2) were routinely monitored at indicated
921 timepoints (see "Lung function test" section below). Respiratory organs were
922 anatomically collected at 1, 3 and 5 d.p.i (for lung) or 1 d.p.i. (for trachea). Viral
923 RNA load in the respiratory tissues were determined by RT–qPCR. The
924 respiratory tissues were also used for histopathological and IHC analyses (see
925 "H&E staining" and "IHC" sections below).
926

927 **Lung function test**
928 Lung function test (**Fig. 4a**) was performed as previously described[12].
929 Respiratory parameters (Penh and Rpef) were measured by using a whole-body
930 plethysmography system (DSI) according to the manufacturer's instructions. In
931 brief, a hamster was placed in an unrestrained plethysmography chamber and
932 allowed to acclimatize for 30 s, then, data were acquired over a 4-m period by
933 using FinePointe Station and Review softwares v2.9.2.12849 (STARR). The
934 state of oxygenation was examined by measuring SpO_2 using pulse oximeter,
935 MouseOx PLUS (STARR). SpO_2 was measured by attaching a measuring chip
936 to the neck of hamsters sedated by 0.25 mg/kg medetomidine hydrochloride.
937

938 **IHC**
939 IHC (**Extended Data Fig. 7**) was performed as previously described[12,23] using
940 an Autostainer Link 48 (Dako). The deparaffinized sections were exposed to
941 EnVision FLEX target retrieval solution high pH (Agilent, Cat# K8004) for 20 m at
942 97°C to activate, and mouse anti-SARS-CoV-2 N monoclonal antibody (R&D
943 systems, Clone 1035111, Cat# MAB10474-SP, 1:400) was used as a primary
944 antibody. The sections were sensitized using EnVision FLEX (Agilent) for 15 m
945 and visualised by peroxidase-based enzymatic reaction with
946 3,3'-diaminobenzidine tetrahydrochloride as substrate for 5 m. The N protein
947 positivity (**Fig. 4c and 4d**) was evaluated by certificated pathologists as
948 previously described[12]. Images were incorporated as virtual slide by
949 NDRscan3.2 software (Hamamatsu Photonics). The N-protein positivity was
950 measured as the area using Fiji software v2.2.0 (ImageJ).
951

952 **H&E staining**

953 H&E staining (**Extended Data Fig. 8**) was performed as previously
954 described[12,23]. Briefly, excised animal tissues were fixed with 10% formalin
955 neutral buffer solution, and processed for paraffin embedding. The paraffin
956 blocks were sectioned with 3 μm-thickness and then mounted on silane-coated
957 glass slides (MAS-GP, Matsunami). H&E staining was performed according to a
958 standard protocol.
959

960 **Histopathological scoring**
961 Histopathological scoring (**Fig. 4e** and **Extended Data Fig. 8a**) was performed
962 as previously described[12,23]. Pathological features including bronchitis or
963 bronchiolitis, haemorrhage with congestive edema, alveolar damage with
964 epithelial apoptosis and macrophage infiltration, hyperplasia of type II
965 pneumocytes, and the area of the hyperplasia of large type II pneumocytes were
966 evaluated by certified pathologists and the degree of these pathological findings
967 were arbitrarily scored using four-tiered system as 0 (negative), 1 (weak), 2
968 (moderate), and 3 (severe). The "large type II pneumocytes" are the hyperplasia
969 of type II pneumocytes exhibiting more than 10-μm-diameter nucleus. We
970 described "large type II pneumocytes" as one of the remarkable
971 histopathological features reacting SARS-CoV-2 infection in our previous
972 studies[12,23]. Total histology score is the sum of these five indices.
973 To measure the inflammation area in the infected lungs (**Extended
974 Data Fig. 8b**), four hamsters infected with each virus were sacrificed at the 1, 3
975 and 5 d.p.i., and all four right lung lobes, including upper (anterior/cranial),
976 middle, lower (posterior/caudal), and accessory lobes, were sectioned along
977 with their bronchi. The tissue sections were stained by H&E, and the digital
978 microscopic images were incorporated into virtual slides using NDRscan3.2
979 software (Hamamatsu Photonics). The inflammatory area including type II
980 pneumocyte hyperplasia in the infected whole lungs was morphometrically
981 analysed using Fiji software v2.2.0 (ImageJ).
982

983 **Statistics and reproducibility**
984 Statistical significance was tested using a two-sided Student's *t*-test or a
985 two-sided Mann–Whitney *U*-test unless otherwise noted. The tests above were
986 performed using Prism 9 software v9.1.1 (GraphPad Software).
987 In the time-course experiments (**Fig. 3a, 3d, 3f, 3h, 4a–4c,** and **4e**), a
988 multiple regression analysis including experimental conditions (i.e., the types of
989 infected viruses) as explanatory variables and timepoints as qualitative control
990 variables was performed to evaluate the difference between experimental
991 conditions thorough all timepoints. *P* value was calculated by a two-sided Wald
992 test. Subsequently, familywise error rates (FWERs) were calculated by the Holm
993 method. These analyses were performed in v4.1.2 (https://www.r-project.org/).

27

994   In **Extended Data Fig. 7 and 8**, photographs shown are the
995 representative areas of at least two independent experiments by using four
996 hamsters at each timepoint. In **Extended Data Fig. 5**, assays were performed in
997 triplicate. Photographs shown are the representatives of >20 fields of view taken
998 for each sample.

999

1000 **Data availability**
1001 The raw data of virus sequences analysed in this study are deposited in Gene
1002 Expression Omnibus (accession number: GSE196649). All databases/datasets
1003 used in this study are available from GISAID database (https://www.gisaid.org)
1004 and Genbank database (https://www.ncbi.nlm.nih.gov/genbank/). The accession
1005 numbers of viral sequences used in this study are listed in Method section.

1006

1007 **Code availability**
1008 The computational code to estimate the relative effective reproduction number of
1009 each viral lineage (**Fig. 1**) is available in the GitHub repository
1010 (https://github.com/TheSatoLab/Omicron_BA2/tree/main/lineage_growth_hierar
1011 chical_model).

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Author Contributions

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Jiri Zahradnik, Gideon Schreiber performed an yeast surface display assay.

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

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Jumpei Ito performed statistical, modelling, and bioinformatics analyses.

Jumpei Ito, Akatsuki Saito, Takashi Irie, Shinya Tanaka, Keita Matsuno, Takasuke Fukuhara, Terumasa Ikeda, and Kei Sato designed the experiments and interpreted the results.

Jumpei Ito and Kei Sato wrote the original manuscript.

All authors reviewed and proofread the manuscript.

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

Conflict of interest

The authors declare that no competing interests exist.

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Figure legends

Fig. 1 | Epidemic of BA.2.

a, Maximum likelihood tree of the Omicron lineages sampled from South Africa. Asterisk denote nodes with ≥0.95 bootstrap values. **b**, Number of amino acid differences between viral lineages detected in S (filled) or the other regions (opened). **c**, Relative frequency of BA.2 in the genome surveillance data during January 2022. The values for the countries with ≥20 SARS-CoV-2 sequences are shown. **d**, Estimated relative effective reproduction number of each viral lineage, assuming a fixed generation time of 2.1 days. The global average value estimated by a Bayesian hierarchical model is shown. The value in each country is shown in **Extended Data Fig. 2d**. The posterior distribution (violin), 95% CI (line), and posterior mean (dot) are shown.

Fig. 2 | Immune resistance of BA.2.

a, Amino acid substitutions in S. Left, primary structure and respective domains. Numbers indicate amino acid position. NTD, N-terminal domain; RBM, receptor-binding motif; HR, heptad repeat; TMD, transmembrane domain. Right, heatmap showing the frequency of amino acid substitutions. Substitutions detected in >10% sequences of any lineages are shown. **b–h**, Neutralisation assays. Neutralisation assays were performed with the pseudoviruses harbouring the S proteins of B.1.1 (the D614G-bearing ancestral virus), Delta, BA.1 and BA.2. Vaccinated sera [**b**, mRNA-1273 (16 donors); **c**, ChAdOx1 (9 donors)], monoclonal antibodies (**d**), convalescent sera of the individuals infected with early pandemic virus (until May 2020) (**e**, 12 donors), and BA.1 (**f**, 17 donors), convalescent sera of the hamsters infected B.1.1 (**g**, left; 6 hamsters) and BA.1 (**g**, right; 6 hamsters), and murine sera immunised with the cells expressing B.1.1 S (**h**, left; 10 mice) and BA.1 S (**h**, right; 10 mice) were used. In **b,c,e–h**, assay of each serum was performed in triplicate to determine 50% neutralisation titre (NT50). Each dot represents each NT50 value, and geometric mean and 95% CI are shown. Number indicates the fold change of resistance versus each antigenic variant. Statistically significant differences between BA.1 and BA.2 were determined by two-sided Wilcoxon signed-rank test. Information of vaccinated/convalescent donors are summarized in **Supplementary Tables 1 and 2**. In **f**, the dots of not-fully vaccinated 4 donors are filled. In **d**, assay of each concentration was performed in triplicate, and data are the average ± s.d. IC50, 50% inhibitory concentration; ND, not determined.

Fig. 3 | Virological features of BA.2 in vitro.

a,h, Growth kinetics of SARS-CoV-2 variants. **b**, Fluorescence microscopy. GFP area of infected VeroE6/TMPRSS2 cells at 48 h.p.i were measured. Numbers in

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1289 the panel indicate the number of GFP-positive cells counted. **c**, Plaque assay.
1290 Diameter of plaques (20 plaques per virus) are summarized. **d,f**, S-based fusion
1291 assay. The fusion activity (arbitrary units) is shown. **e**, Western blot. Left,
1292 representative blots of S-expressing cells. ACTB is an internal control. Right, the
1293 ratio of S2 to the full-length S plus S2 proteins. **g**, Fold increase of pseudovirus
1294 infectivity by TMPRSS2 expression. Assays were performed in quadruplicate
1295 (**a,g,h**), octuplicate (**a**, most left) or triplicate (**d–f**) and data are the average ± s.d.
1296 Each dot indicates the result from an individual plaque (**c**) and an individual
1297 replicate (**e,g**). In **b** and **c**, raw data are shown in **Extended Data Fig. 5 and 6**.
1298 Statistically significant differences between BA.2 and other variants through
1299 timepoints were determined by multiple regression (**a,d,f,h**). Familywise error
1300 rates (FWERs) calculated using the Holm method are indicated in the figures.
1301 Statistically significant differences between BA.1 and BA.2 were determined by
1302 two-sided Mann–Whitney *U*-tests (**b,d**) or two-sided paired Student's *t*-tests (**e**).
1303
1304 **Fig. 4 | Virological features of BA.2 in vivo.**
1305 Syrian hamsters were intranasally inoculated with B.1.1, BA.1 and BA.2. **a**, Body
1306 weight, Penh, Rpef, SpO_2 values routinely measured. Hamsters of the same age
1307 were intranasally inoculated with PBS (uninfected). **b**, Viral RNA load in the lung
1308 hilum (left) and periphery (right). **c,d**, Percentage of N-positive cells in the whole
1309 lobes of lung (**c**) and bronchiole in the frontal/upper lobe of lung (**d**) measured by
1310 IHC. **e**, Histopathological scoring of lung lesions. Representative pathological
1311 features are shown in our previous studies[12,23]. Data are the average (**a**, 6
1312 hamsters per each group; **b–e**, 4 hamsters per each group) ± s.e.m. In **a–c,e**,
1313 statistically significant differences between BA.2 and other variants or uninfected
1314 hamsters through timepoints were determined by multiple regression. The data
1315 at 0 d.p.i. was excluded from the analyses. FWERs calculated using the Holm
1316 method are indicated in the figures. In **d**, each dot indicates the result from an
1317 individual hamster. In **c–e**, raw data are shown in **Extended Data Fig. 7 and 8**.

Extended Data

Extended Data Fig. 1. Estimated emergence dates of the Omicron lineages.
a, Phylodynamics of BA.1 (top), BA.2 (middle), and BA.3 (bottom) sampled by January 26, 2022 in South Africa. All the BA.2 and BA.3 sequences and 200 randomly sampled BA.1 (including 20 BA.1.1) sequences were used. The time-resolved trees were constructed by BEAST2. Regarding a node with ≥0.95 posterior value (denoted by an asterisk), the 95% CI of the divergence time is shown. **b**, Estimated emergence dates of the Omicron lineages. The 95% CI (error bar) and posterior mean (dot) are shown.

Extended Data Fig. 2. Epidemic dynamics of SARS-CoV-2 lineages in countries with the BA.2 epidemic.
a, Daily sequence frequency of each viral lineage in eleven countries where ≥100 BA.2 sequences have been reported by January 25, 2022. This data was used as an input of a Bayesian hierarchical model to estimate the epidemic dynamics.
b,c, Epidemic dynamics of SARS-CoV-2 viral lineages. The observed daily frequency (dot) and the dynamics estimated by the Bayesian model (posterior mean; line) are shown. Additionally, 95% CI (**b**) and 90% prediction interval (**c**) are shown. **d,** Estimated relative effective reproduction number of each viral lineage in each country. The posterior distribution (violin), 95% CI (line), and posterior mean (dot) are shown.

Extended Data Fig. 3. Immune resistance of BA.2.
Neutralisation assays were performed with the pseudoviruses harbouring the S proteins of B.1.1 (the D614G-bearing ancestral virus), Alpha, Delta, BA.1 and BA.2. Assay of each serum sample was performed in triplicate to determine NT50, and each dot represents each NT50 value. Geometric mean and 95% CI are shown. The number indicates the fold change of resistance versus each antigenic variant. In **a** and **b**, statistically significant differences between BA.1 and BA.2 were determined by two-sided Wilcoxon signed-rank test. In **c**, statistically significant differences between fully-vaccinated (13 donors) and not-fully-vaccinated (4 donors) were determined by two-sided Mann-Whitney U-test. Information of vaccinated and convalescent donors are summarized in **Supplementary Tables 1 and 2**, respectively.

Extended Data Fig. 4. Scheme of chimeric recombinant SARS-CoV-2 used in this study.
The SARS-CoV-2 genome and respective genes are shown. The template is SARS-CoV-2 strain WK-521 (lineage A, GISAID ID: EPI_ISL_408667), and the

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1359   *S* genes were swapped with respective lineage/strain (GISAID IDs are indicated
1360   in the figure). Additionally, the *ORF7b* was swapped with *sfGFP* gene.
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1362   **Extended Data Fig. 5. Syncytia and plaque formations by BA.2.**
1363   **a,** Fluorescence microscopy. GFP area of infected VeroE6/TMPRSS2 cells
1364   (m.o.i. 0.01) at 24, 48, and 72 h.p.i were measured. Higher-magnification views
1365   of the regions indicated by squares are shown at bottom. **b**, Plaque assay. **c**,
1366   Coculture of S-expressing cells with HEK293-ACE2/TMPRSS2 cells. Left,
1367   representative images of S-expressing cells cocultured with HEK293 cells (top)
1368   or HEK293-ACE2/TMPRSS2 cells (bottom). Nuclei were stained with Hoechst
1369   33342 (blue). Right, the size distribution of syncytia (green). Numbers in the
1370   panel indicate the numbers of GFP-positive syncytia counted. Data are the
1371   average ± s.d. A statistically significant difference between BA.1 an BA.2 was
1372   determined by two-sided Mann–Whitney *U*-test. In **a** and **b**, summarized data
1373   are shown in **Fig. 3b** and **3c**. Scale bars, 500 μm (**a**) or 200 μm (**c**).
1374
1375   **Extended Data Fig. 6. Expression and binding properties of BA.2 S.**
1376   **a**, S expression on the cell surface. Representative histograms stained with
1377   anti-S1/S2 polyclonal antibody (left) and the summarised data (right) are
1378   respectively shown. The number in the histogram indicates MFI. Grey
1379   histograms indicate isotype controls. **b**, Binding affinity of SARS-CoV-2 S RBD
1380   to ACE2 by yeast surface display. Left, The percentage of the binding of the
1381   SARS-CoV-2 S RBD expressed on yeast to soluble ACE2 (left) and the
1382   summarised data (right) are respectively shown. **c**, TMPRSS2 expression on the
1383   cell surface. Left, representative histograms stained with anti-TMPRSS2
1384   polyclonal antibody are shown. The number in the histogram indicates MFI. Grey
1385   histograms indicate isotype controls. Right, summarized data. Assays were
1386   performed in triplicate, and data are the average ± s.d. Each dot indicates the
1387   result from an individual replicate. A statistically significant difference between
1388   BA.1 an BA.2 was determined by two-sided unpaired Student's *t*-test (**a**) or
1389   two-sided Mann–Whitney *U*-test (**b**).
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1391   **Extended Data Fig. 7. IHC of SARS-CoV-2 N protein.**
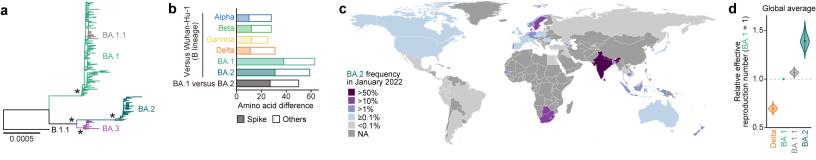1392   **a,b,** IHC of viral N protein in the middle part of trachea of all infected hamsters (n
1393   = 4 per each viral strain) at 1 d.p.i. (**a**) and the lung at 1, 3 and 5 d.p.i (**b**). Each
1394   panel indicates the representative result from an individual infected hamster. **c**,
1395   Lung lobes of the hamsters infected with B.1.1, BA.1 or BA.2 (n = 4 for each
1396   virus) at 1, 3 and 5 d.p.i. were immunohistochemically stained with
1397   anti-SARS-CoV-2 N monoclonal antibody. In each panel, IHC staining (top) and
1398   the digitalized N-positive area (bottom, indicated in red) are shown. The number
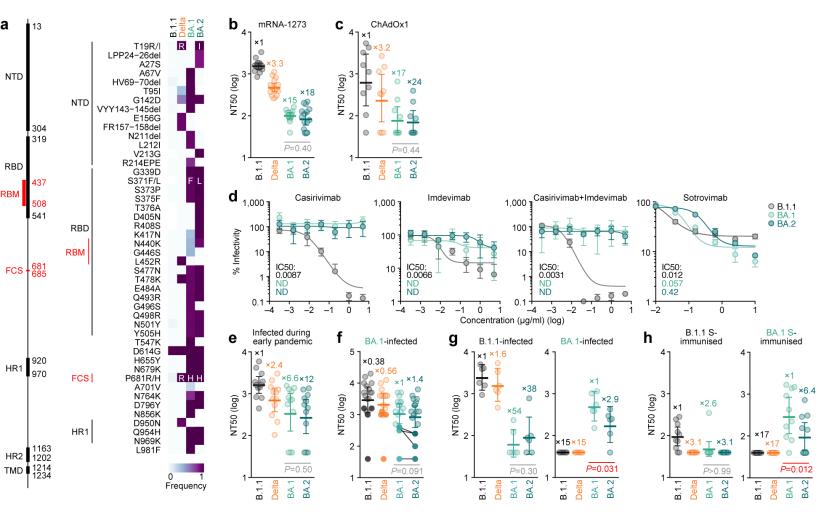
1399　in the bottom panel indicates the percentage of N-positive area. Summarized

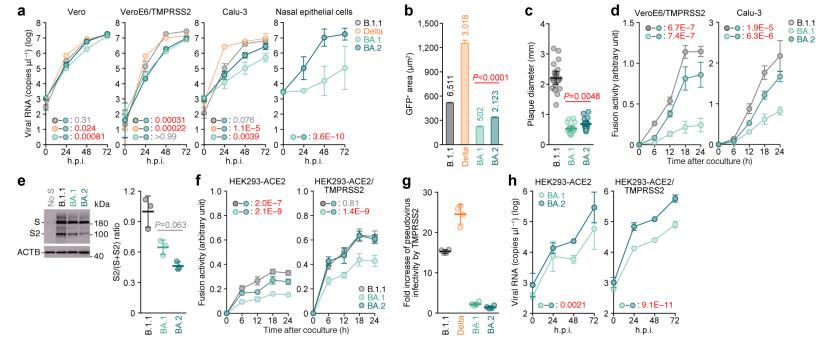1400　data is shown in **Fig. 4c**. Scale bars, 1 mm (**a**); 500 µm (**b**); or 5 mm (**c**).

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1402　**Extended Data Fig. 8. Pathological features of BA.2.**

1403　**a**, H&E staining of the lungs of infected hamsters. Uninfected lung alveolar

1404　space and bronchioles are also shown. **b**, Type II pneumocytes in the lungs of

1405　infected hamsters. Left, lung lobes of the hamsters infected with B.1.1 (n = 4),

1406　BA.1 (n = 4), and BA.2 (n = 4) at 5 d.p.i. In each panel, H&E staining (top) and

1407　the digitalized inflammation area (bottom, indicated in red) are shown. The

1408　number in the bottom panel indicates the percentage of the section represented

1409　by the indicated area (i.e., the area indicated with red colour per the total area of

1410　the lung lobes). Right, summarized data. Each dot indicates the result from an

1411　individual hamster.

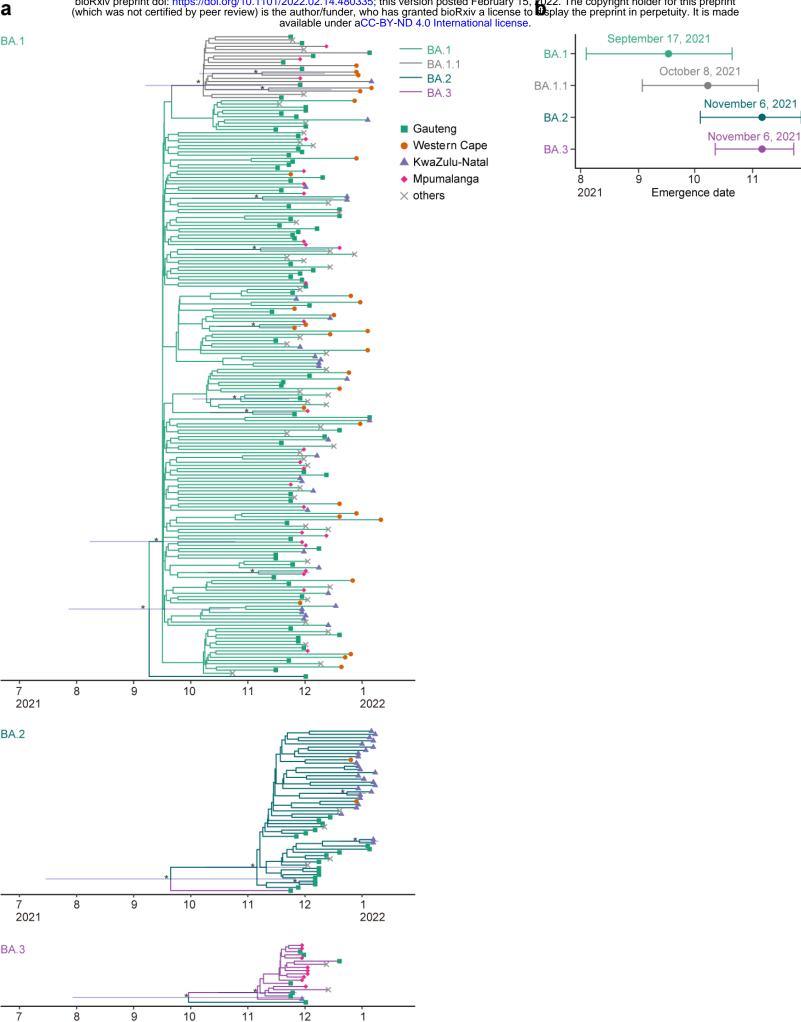1412　Scale bars, 250 µm (**a**) or 5 mm (**b**).