

1 **Characterization of SARS-CoV-2 Omicron BA.2.75 clinical isolates**

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37 **Abstract (150 words)**

38 The prevalence of the Omicron subvariant BA.2.75 is rapidly increasing in India and Nepal. In  
39 addition, BA.2.75 has been detected in at least 34 other countries and is spreading globally.  
40 However, the virological features of BA.2.75 are largely unknown. Here, we evaluated the  
41 replicative ability and pathogenicity of BA.2.75 clinical isolates in Syrian hamsters. Although  
42 we found no substantial differences in weight change among hamsters infected with BA.2, BA.5,  
43 or BA.2.75, the replicative ability of BA.2.75 in the lungs was higher than that of BA.2 and  
44 BA.5. Of note, BA.2.75 caused focal viral pneumonia in hamsters, characterized by patchy  
45 inflammation interspersed in alveolar regions, which was not observed in BA.5-infected  
46 hamsters. Moreover, in competition assays, BA.2.75 replicated better than BA.5 in the lungs of  
47 hamsters. These results suggest that BA.2.75 can cause more severe respiratory disease than  
48 BA.5 and BA.2 and should be closely monitored.

49

50 **Key words**

51 BA.2.75, Omicron, Syrian hamster, hACE2-expressing hamster, lung inflammation

## 52 **Introduction**

53 Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), first detected in China  
54 at the end of 2019, is responsible for COVID-19, which is associated with mild to severe  
55 symptoms ranging from cough and fever to severe pneumonia and death. Over two years have  
56 passed since the World Health Organization (WHO) declared COVID-19 a pandemic  
57 (<https://covid19.who.int/>). Yet, SARS-CoV-2 still imposes huge public health and economic  
58 burdens worldwide. The currently circulating Omicron (B.1.1.529) variant emerged at the end  
59 of 2021 and has since evolved into complex sublineages; three of the major Omicron lineages  
60 have serially transitioned as globally dominant forms: first BA.1, then BA.2, and then BA.5  
61 (**Fig. 1a**). The BA.5 lineage is currently the dominant variant circulating globally  
62 (<https://covariants.org/per-variant>). BA.5 was just beginning to expand in India in May 2022,  
63 when BA.2.75 (a subvariant of the BA.2 sublineage) first emerged there. This subvariant  
64 appears to be more transmissible than BA.5 in India and Nepal, where it is gaining prevalence  
65 (<https://covariants.org/per-variant>). Recently, WHO has categorized BA.2.75 as a variant of  
66 concern (VOC) lineages under monitoring (VOC-LUM).

67 Compared with the original Wuhan Hu-1 strain, the Omicron BA.1 virus had more than  
68 30 amino acid differences in the spike protein of SARS-CoV-2 including insertions and  
69 deletions (**Fig. S1A**) (by comparison, Delta differed from the original Wuhan Hu-1 by only 11  
70 amino acids in its Spike)(Flemming, 2022). BA.2 differed from BA.1 at 27 Spike positions, and  
71 BA.5 differs from BA.2 by 5 amino acids in the S protein (**Fig. S1A**). We recently demonstrated  
72 that the pathogenicity of BA.1 and BA.2 sublineage viruses is comparable in animal models and  
73 attenuated compared with previously circulating variants of concern (VOCs), consistent with  
74 clinical data in humans (Halfmann et al., 2022; Uraki et al., 2022a). In addition, our recent data  
75 suggest that BA.4 and BA.5 have similar pathogenicity to that of BA.2 in rodent models  
76 ((Kawaoka et al., 2022): <https://www.researchsquare.com/article/rs-1820048/v1>). SARS-CoV-2  
77 initiates infection through the binding of the receptor-binding domain (RBD) of its spike protein  
78 to host cell surface receptors [i.e., human angiotensin-converting enzyme 2 (hACE2)]. BA.2.75  
79 differs from that of BA.2 by nine amino acids in the Spike, including four in the RBD (i.e.,  
80 G339H, G446S, N460K, and the wild-type amino acid at position Q493). Recent studies  
81 reported that the RBD of BA.2.75 has a higher binding affinity for hACE2 than that of BA.2  
82 ((Cao et al., 2022): <https://www.biorxiv.org/content/10.1101/2022.07.18.500332v1.full>, (Saito et  
83 al., 2022): <https://www.biorxiv.org/content/10.1101/2022.08.07.503115v1>), raising the  
84 possibility that this property may increase the replicative ability and/or pathogenicity of  
85 BA.2.75. Moreover, in addition to the substitutions in the RBD, there are several amino acid  
86 differences in the other viral proteins of BA.2.75, which may also alter its replicative capability  
87 and pathogenicity (**Fig. S1b**). Here, we assessed the replicative capacity and pathogenicity of

88 authentic BA.2.75 subvariants isolated from COVID-19 patients in established COVID-19  
89 animal models.  
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92 **Results.**

93 **Transitions in Omicron variant prevalence throughout 2022.**

94 SARS-CoV-2 has undergone a series of variant transitions since the Omicron lineage  
95 was first observed in November of 2021. The initial global transition from Delta to the Omicron  
96 BA.1 lineage was extremely swift and was followed successively by waves of BA.2 and BA.5,  
97 with each variant, essentially replacing the previous dominant form (**Fig. 1**). This may indicate  
98 that each variant has been more transmissible than the prior variant, particularly in settings with  
99 histories of prior infection and vaccination resulting in changes in immune status at the  
100 population level. BA.2.75, a BA.2 sublineage, was first detected in India in May of 2022, and  
101 since then has been rapid increasing in sampling frequency (**Fig.1, Fig. S2 and S3**). As of this  
102 writing, there are more than 3,000 sequences in GISAID (Elbe and Buckland-Merrett, 2017;  
103 Khare et al., 2021) with the Pango lineage designation BA.2.75, sampled in 35 nations.  
104 Although BA.2.75 is still rare outside of India and Nepal, it has been sampled at least 10 times  
105 in 10 nations, and in each of these 10 it is significantly increasing in sampling frequency (**Fig.**  
106 **S2**); the regularity of this pattern suggests that BA.2.75 may have a selective advantage over  
107 co-circulating variants. BA.2.75 is established in 12 states in India, and is significantly  
108 increasing in frequency throughout India, indicating that its increased prevalence in India is  
109 unlikely to be a founder effect or sampling issue (**Fig. S3**). BA.5 was just beginning to expand  
110 in India and when BA.2.75 was first detected (**Fig. 1**), and where BA.5 and BA.2.75 are  
111 co-circulating, the prevalence of BA.2.75 tends to be increasing faster (**Fig. 1b**). Therefore,  
112 BA.2.75 is a likely candidate for the next major transition to a more transmissible form, unless a  
113 novel variant emerges with an even greater selective advantage.

114

115 **BA.2.75 infection in hamsters**

116 To characterize BA.2.75 *in vivo*, we amplified three BA.2.75 clinical isolates in  
117 VeroE6/TMPRSS2 cells: hCoV-19/Japan/TY41-716/2022 (TY41-716)(Takashita et al., 2022),  
118 hCoV-19/Japan/UT-NCD1757-1N/2022 (NCD1757), and  
119 hCoV-19/Japan/UT-NCD1759-1N/2022 (NCD1759). We confirmed that the S protein of all  
120 three isolates contained the nine additional amino acid changes (i.e., K147E, W152R, F157L,  
121 I210V, G257S, D339H, G446S, N460K, and Q493 (reversion)) (**Fig. S1b**) that distinguish the  
122 consensus form of BA.2.75  
123 (<https://cov.lanl.gov/components/sequence/COV/pangocommonforms.comp>) from a BA.2 isolate  
124 (hCoV-19/Japan/UT-NCD1288-2N/2022; NCD1288), which carries the most common  
125 circulating form of BA.2 in Spike. However, two of the isolates (NCD1757 and NCD1759) had  
126 a D574V substitution in the subdomain (SD), in addition to the nine mutations; this and several  
127 other distinctive mutations found in other proteins are summarized in **Fig. S1b**.

128 We first evaluated the pathogenicity of the BA.2.75 isolates in wild-type Syrian  
129 hamsters, a well-established small animal model for the study of COVID-19 (Chan et al., 2020;  
130 Imai et al., 2020; Sia et al., 2020). Syrian hamsters were intranasally inoculated with  $10^5$   
131 plaque-forming units (PFU) of BA.2.75 (TY41-716, NCD1757, or NCD1759). For comparison,  
132 additional hamsters were infected with clinical isolates of BA.2 ( $10^5$  PFU of  
133 NCD1288)(Takashita et al., 2022; Uraki et al., 2022a), BA.5 [ $10^5$  PFU of  
134 hCoV-19/Japan/TY41-702/2022 (TY41-702)] (Kawaoka et al., 2022), or B.1.617.2 [ $10^5$  PFU of  
135 hCoV-19/USA/WI-UW-5250/2021 (Delta: UW5250)](Halfmann et al., 2022). Intranasal  
136 infection with B.1.617.2 resulted in significant body weight loss by 6 days post-infection (dpi)  
137 (-5.4%) (**Fig. 2a**), consistent with our previous observations (Halfmann et al., 2022; Kawaoka et  
138 al., 2022). By contrast, most of the animals infected with any of the three BA.2.75 isolates  
139 gained weight over the 6-day experiment, similar to BA.2-, BA.5-, or mock-infected animals.  
140 We also examined pulmonary functions in the infected hamsters by measuring Penh and Rpef,  
141 which are surrogate markers for bronchoconstriction and airway obstruction, respectively, by  
142 using a whole-body plethysmography system. Inoculation of hamsters with the BA.2, BA.5,  
143 BA.2.75 (NCD1757), or BA.2.75 (NCD1759) isolate did not cause substantial changes in either  
144 Penh or Rpef at any timepoint post-infection compared to the mock-infected group. Infection  
145 with BA.2.75 (TY41-716) caused a slight increase in Penh at 3 and 5 dpi, although no  
146 statistically significant differences in Penh values were observed among BA.2-, BA.5-, and  
147 BA.2.75 (TY41-716)-infected animals. Consistent with our previous data, infection with  
148 B.1.617.2 caused significant changes in Rpef in comparison with the five Omicron isolates (**Fig.**  
149 **2b**).

150 We next assessed levels of infection in the respiratory tract of wild-type Syrian  
151 hamsters (**Fig. 2c**). Hamsters were intranasally infected with  $10^5$  PFU of BA.2.75 (TY41-716),  
152 BA.2.75 (NCD1757), BA.2 (NCD1288), BA.5 (TY41-702), or B.1.617.2 (Delta: UW5250); at 3  
153 and 6 dpi, the animals were sacrificed, and their nasal turbinates and lungs were collected for  
154 virus titration. The virus titers were determined by performing plaque assays on Vero  
155 E6-TMPRSS2-T2A-ACE2 cells. BA.2 (NCD1288), BA.5 (TY41-702), BA.2.75 (TY41-716),  
156 and BA.2.75 (NCD1757) replicated in the nasal turbinates of the infected animals with no  
157 significant differences in viral titers at both timepoints examined. However, the virus titers in  
158 the nasal turbinates were significantly lower in the respiratory tract of animals infected with the  
159 BA.2, BA.5, BA.2.75 (TY41-716), or BA.2.75 (NCD1757) isolates, compared to animals  
160 infected with B.1.617.2 [mean differences in viral titer = 0.75, 0.75, 0.98, or 0.94 and 1.4, 1.6,  
161 1.8, or 1.5  $\log_{10}$  (PFU/g) at 3 and 6 dpi, respectively].

162 Consistent with our previous report (Kawaoka et al., 2022), the virus titers in the lungs  
163 of animals infected with BA.2 or BA.5 were lower than those in animals infected with

164 B.1.617.2 [mean differences in viral titer = 5.0 or 4.2 and 2.2 or 3.4 log<sub>10</sub> (PFU/g) at 3 and 6 dpi,  
165 respectively], although the difference was not statistically significant between the BA.2- and  
166 B.1.617.2-infected groups at 6 dpi. The lung titers in the BA.2.75 (TY41-716)-infected groups  
167 were also lower than those in the B.1.617.2-infected groups [mean difference in viral titer = 2.1  
168 and 1.7 log<sub>10</sub> (PFU/g) at 3 and 6 dpi, respectively], although these differences did not reach  
169 statistical significance. The viral titers in the lungs of another BA.2.75 strain  
170 (NCD1757)-infected groups were similarly lower than those in the B.1.617.2-infected group at  
171 3 dpi [mean differences in viral titer = 2.0 log<sub>10</sub> (PFU/g)]; however, animals infected with  
172 BA.2.75 or B.1.617.2 had similar titers in the lungs at 6 dpi. The lung titers in the BA.2.75  
173 (TY41-716)- and BA.2.75 (NCD1757)-infected groups were higher than those in BA.5-infected  
174 groups [for BA.2.75 (TY41-716), mean differences in viral titer = 2.1 and 1.7 log<sub>10</sub>(PFU/g), at 3  
175 and 6 dpi, respectively; for BA.2.75 (NCD1757), mean differences in viral titer = 2.2 and 2.8  
176 log<sub>10</sub> (PFU/g), at 3 and 6 dpi, respectively]; however, the differences were not statistically  
177 significant among the three groups. At 3 dpi, the virus titers in the lungs were significantly  
178 higher in the respiratory tract of animals infected with BA.2.75 (TY41-716), compared to  
179 animals infected with BA.2 (NCD1288) [mean difference in viral titer = 2.9 log<sub>10</sub> (PFU/g)];  
180 however, at 6 dpi, similar titers were detected in the lungs of animals inoculated with BA.2.75  
181 (TY41-716) or BA.2. The viral titers in the lungs of the BA.2.75 (NCD1757)-infected groups  
182 were also higher than those in the BA.2 (NCD1288)-infected groups [mean differences in viral  
183 titer = 3.0 and 1.6 log<sub>10</sub> (PFU/g), at 3 and 6 dpi, respectively], although the difference was not  
184 statistically significant between the BA.2.75 (NCD1757)- and BA.2-infected groups at 6 dpi.  
185 Taken together, these results suggest that the replicative ability of BA.2.75 in the lungs of  
186 wild-type hamsters is higher than that of previous Omicron variants, including BA.2 and BA.5.

187 We then investigated the infectivity of BA.2.75 in respiratory organs by using a more  
188 susceptible model, specifically transgenic hamsters expressing hACE2 (**Fig. 2d**). At 5 dpi, the  
189 virus titers in the lungs and nasal turbinates of hACE2-expressing hamsters infected with  
190 BA.2.75 (TY41-716) were lower than those in animals infected with B.1.617 (UW5250) [mean  
191 differences in viral titer = 2.7 and 1.1 log<sub>10</sub> (PFU/g), respectively], although the differences in  
192 the lungs were not statistically significant between the two groups. Similar titers were detected  
193 in the lungs of animals inoculated with BA.2.75 (TY41-716) or BA.5 (TY41-702); however, the  
194 virus titers in the nasal turbinates of the animals infected with BA.2.75 were slightly but  
195 significantly lower than in those infected with BA.5 [mean differences in viral titer = 0.98 log<sub>10</sub>  
196 (PFU/g)]. The virus titers in the lungs were substantially higher in the respiratory tract of  
197 animals infected with BA.2.75 (TY41-716) compared with animals infected with BA.2  
198 (NCD1288) [mean differences in viral titer = 2.6 log<sub>10</sub> (PFU/g)], although animals infected with  
199 BA.2.75 or BA.2 exhibited similar viral titers in nasal turbinates. These results suggest that



200 BA.2.75 may have a higher replicative ability than BA.2 in the lungs of hACE2 transgenic  
201 hamsters.

202

### 203 **Histopathological findings in the lungs of SARS-CoV-2 BA.2.75 virus-inoculated Syrian** 204 **hamsters**

205 The lungs of Syrian hamsters that were inoculated with BA.2.75, BA.5, or B.1.617.2  
206 were also analyzed histopathologically. Hamsters were intranasally inoculated with BA.2.75  
207 (TY41-716), BA.5 (TY41-702), or B.1.617.2 (Delta, UW5250) and euthanized at 3 and 6 dpi  
208 for histopathological evaluation; representative images are shown in Figure 3.

209 This examination revealed that inflammation was not obvious in the lungs of either  
210 BA.2.75 (TY41-716)- or BA.5-inoculated animals at 3 dpi; however, infiltration of  
211 inflammatory cells such as mononuclear cells and neutrophils was observed in peribronchial  
212 and peribronchiolar regions in these two groups at 6 dpi (**Fig. 3a, 3b, and S4**). It is noteworthy  
213 that focal pneumonia, characterized by patchy inflammation interspersed in alveolar regions,  
214 was observed in the lungs of BA.2.75 (TY41-716)-inoculated animals at 6 dpi. Similar  
215 histopathological findings (i.e., focal pneumonia) were observed in the lungs of animals  
216 inoculated with another BA.2.75 strain (NCD1757) at 6 dpi (**Fig. S5**). However, there was no  
217 obvious pneumonia in the lungs of BA.5-inoculated animals at the same timepoint. By contrast,  
218 in the lungs of the B.1.617.2-inoculated animals, peribronchial and peribronchiolar  
219 inflammation was prominent at 3 dpi, and extensive pneumonia with focal alveolar hemorrhage  
220 was observed in the alveolar regions at 6 dpi (**Fig. 3a, 3b and S4**). In addition, we detected viral  
221 RNA and protein in the lung tissue of BA.2.75 (TY41-716)-, BA.5- or B.1.617.2-infected  
222 hamsters by use of in situ hybridization and immunohistochemistry. These analyses revealed  
223 that viral RNA and antigen were readily detected on bronchial/bronchiolar epithelium in both  
224 BA.2.75 (TY41-716)- and BA.5-inoculated animals at 3 dpi with a clear decrease in positive  
225 cells over time (**Fig. 3a and 3b**). In the alveolar regions, a small number of cells were positive  
226 for viral RNA or antigen in the BA.2.75 (TY41-716)-inoculated group at both timepoints  
227 examined, and fewer cells were positive in the BA.5-inoculated group at the corresponding  
228 timepoints (**Fig. 3a and 3b**). Comparatively, at 3 dpi, the lungs of the B.1.617.2-inoculated  
229 hamsters had diffusely positive viral RNA and antigen in the bronchial/bronchiolar areas and  
230 patchily positive viral RNA and antigen in the alveolar regions (**Fig. 3a and 3b**). BA.2.75  
231 (TY41-716) thus produced mild viral pneumonia in the hamster model with attenuated  
232 pathogenicity compared with B.1.617.2, whereas BA.5 did not cause obvious viral pneumonia.  
233 In addition, the number of viral RNA/antigen-positive cells in the alveolar regions of the  
234 BA.2.75 (TY41-716)-inoculated animals was higher than that in the BA.5-inoculated animals,  
235 but lower than that in the B.1.617.2-inoculated ones.



236

237 **The replicative fitness of BA.2.75 compared with that of BA.5 in hamsters**

238 To further investigate the replicative fitness of BA.2.75, we compared the growth of  
239 BA.2.75 in wild-type hamsters with that of BA.5, which is currently the dominant variant  
240 circulating globally. Wild-type hamsters were intranasally inoculated with  $2 \times 10^5$  PFU of a  
241 mixture of BA.2.75 (TY41-716) and BA.5 (TY41-702) at ratios of 1:1, 1:3, 1:19, or 1:199. At 4  
242 dpi, the proportion of each virus in the nasal turbinates and lungs of the infected hamsters was  
243 determined by using Next Generation Sequencing (NGS). The proportion was calculated on the  
244 basis of the differences between these two viruses across 6 regions in the S protein.

245 NGS analysis revealed that the proportion of BA.2.75 had increased in the nasal  
246 turbinates and lungs of all infected animals compared to that in each inoculum for any ratio,  
247 except for the lung samples from hamsters 2, 10, and 19 (**Fig. 4**). For animals inoculated with a  
248 1:1 or 1:3 ratio of BA.2.75:BA.5, the lung and nasal turbinate samples showed a greater  
249 proportion of BA.2.75, except for the lung sample from hamsters 2, 9, and 10 (**Fig. 4a and 4b**).  
250 Of note, even though the proportion of BA.2.75 in the inoculum was much lower than that of  
251 BA.5 (i.e., a 1:19 or 1:199 mixture of BA.2.75:BA.5), BA.2.75 became dominant in the lungs of  
252 four (#s 11, 12, 15, and 20) of the ten animals (**Fig. 4c and 4d**). Taken together, these results  
253 suggest that BA.2.75 may have greater replicative fitness than BA.5, especially in the upper  
254 respiratory tract.

255 **Discussion**

256

257 We previously showed that Omicron sublineage BA.2 and BA.5 variants exhibit similar  
258 pathogenicity in rodent models by using several clinical isolates, and showed that both variants  
259 are significantly attenuated compared to previous circulating VOCs (Kawaoka et al., 2022;  
260 Uraki et al., 2022a). Here, we evaluated the replication and pathogenicity of Omicron  
261 sublineage BA.2.75 variants in hamsters. Our data show that there are no substantial differences  
262 in weight change among hamsters infected with BA.2.75, BA.2, or BA.5 (**Fig. 2a**);  
263 however, viral titers in the lungs of BA.2.75-infected hamsters were higher than those in the  
264 lungs of BA.2- or BA.5-infected hamsters (**Fig. 2c**). In addition, in competition assays, BA.2.75  
265 replicated better than BA.5 in the lungs (**Fig. 4**). Of note, in the lungs of BA.2.75-inoculated  
266 hamsters, we observed focal pneumonia, characterized by patchy inflammation interspersed in  
267 alveolar regions, indicating that BA.2.75 can cause mild pneumonia (**Fig. 3 and S5**). In contrast,  
268 BA.5 mainly affected the bronchi, resulting in bronchitis/bronchiolitis, and did not cause obvious  
269 pneumonia (**Fig. 3**). Similar results were observed with hamsters infected with BA.1, BA.2, or  
270 BA.4 ((Halfmann et al., 2022; Kawaoka et al., 2022; Uraki et al., 2022b)). These findings  
271 suggest that among the Omicron variants, the Omicron subvariant BA.2.75 causes the most  
272 severe tissue damage in the lungs of hamsters.

273 Omicron variants, including BA.1 or BA.2, are less likely than Delta variants to be  
274 associated with pneumonia in COVID-19 patients (Christensen et al., 2022; Kozlov, 2022; Li et  
275 al., 2022), consistent with our previous data obtained in a hamster model (Halfmann et al.,  
276 2022; Uraki et al., 2022a). However, in the present study, we found that BA.2.75 can cause focal  
277 viral pneumonia in hamsters, unlike the other Omicron variants (i.e., BA.1, BA.2, BA.4, and  
278 BA.5) (Halfmann et al., 2022; Kawaoka et al., 2022; Uraki et al., 2022a). The reason for this is  
279 unclear; however, it might be due to differences in the binding affinity of the S protein for  
280 hACE2 among BA.1, BA.2, BA.4, BA.5, and BA.2.75. Recent studies have reported that the  
281 RBD of BA.2.75 exhibits higher binding affinity for the hACE2 receptor than that of BA.2 and  
282 BA.4/5 (Cao et al., 2022; Saito et al., 2022). SARS-CoV-2 enters cells in two distinct ways: by  
283 fusion of the viral lipid envelope with the target cell plasma membrane or fusion of the  
284 viral envelope with the endosomal membrane after internalization through the endocytic  
285 pathway (Hoffmann et al., 2020; Jackson et al., 2022; Walls et al., 2020). The internalization of  
286 SARS-CoV-2 via the endocytic pathway is believed to be induced by the binding of the virus to  
287 ACE2 (Bayati et al., 2021; Inoue et al., 2007). The Omicron variants have been shown to  
288 preferentially utilize the endocytic pathway to enter cells (Hui et al., 2022; Meng et al., 2022).  
289 In addition, previous studies have demonstrated that the enhanced binding affinity between  
290 ACE2 and the RBD increases the efficiency of SARS-CoV-2 entry (Ou et al., 2021; Ozono et al.,

291 2021). Therefore, the higher ACE2 binding affinity of BA.2.75 may enhance its ability to infect  
292 the lungs, thereby allowing BA.2.75 to cause viral pneumonia in hamsters. Also, this higher  
293 ACE2 affinity of BA.2.75 may increase its competitive fitness compared to BA.5 in the  
294 respiratory tracts of hamsters, as observed in our *in vivo* competition assay (**Fig. 4**). Further  
295 investigations are required to determine whether ACE2 binding affinity truly influences  
296 Omicron infection.

297 We note two key limitations in this study: (1) although hamsters are one of the most  
298 widely used animals that are known to be susceptible to SARS-CoV-2, including mice and  
299 non-human primates (Chan et al., 2020; Imai et al., 2020; Sia et al., 2020), it is unclear whether  
300 the BA.2.75 variant causes more clinically severe respiratory disease than other Omicron  
301 variants in humans; and (2) our study was performed in immunologically naïve animals;  
302 however, many people have already acquired immunity to SARS-CoV-2 through natural  
303 infection and/or vaccination. Therefore, it remains unclear whether our data reflect the clinical  
304 outcome in patients with immunity against SARS-CoV-2. Clinical studies are needed to  
305 corroborate our findings in the hamster model.

306 In summary, the prevalence of BA.2.75 has increased throughout India, and has been  
307 increasing faster in regions where BA.5 and BA.2.75 are co-circulating, suggesting the potential  
308 for BA.2.75 to become the next globally dominant variant. Our data show that, compared to  
309 BA.5 and BA.2, BA.2.75 can replicate efficiently in the lungs of hamsters and cause more  
310 severe respiratory disease. This higher replicative ability of BA.2.75 in the lower respiratory  
311 tract may affect the clinical outcome in infected humans. Accordingly, the spread of this new  
312 variant should be monitored closely.

313

314 **Materials and Methods**

315

316 **Variant tracking strategies.**

317 Figures 1, S2, and S3 show transitions between variant forms, emphasizing the recent expansion  
318 of the BA.2.75 variant that is indicative of a possible selective advantage. Details of the  
319 methods used to make these figures are described in Korber et al. (Korber et al., 2020), and  
320 web-based updates of these figures based on recent GISAID data can be generated via the  
321 “Embers” and “Isotonic Regression” tools at the Los Alamos National Laboratory SARS-CoV-2  
322 variant analysis website (<https://cov.lanl.gov>). Figure 1 was created using Embers and displays  
323 running weekly counts and proportions of variants at different geographic levels. The Isotonic  
324 Regression analysis explores the dynamics of the transition towards higher frequencies of  
325 BA.2.75 over time, testing whether it is increasing in frequency relative to other variant forms at  
326 the country or state level, everywhere globally that BA.2.75 is well enough established to have  
327 been sampled ten or more times. A resampling statistic was used to evaluate whether the  
328 increasing sampling of BA.2.75 is significant (Elbe and Buckland-Merrett, 2017; Khare et al.,  
329 2021; Korber et al., 2020). The data sets for these figures were uploaded from GISAID  
330 2022-08-15 (Elbe and Buckland-Merrett, 2017; Khare et al., 2021).

331

332 **Cells.**

333 VeroE6/TMPRSS2 (JCRB 1819) cells (Matsuyama et al., 2020) were propagated in the  
334 presence of 1 mg/ml geneticin (G418; Invivogen) and 5 µg/ml plasmocin prophylactic  
335 (Invivogen) in Dulbecco’s modified Eagle’s medium (DMEM) containing 10% Fetal Calf  
336 Serum (FCS). Vero E6-TMPRSS2-T2A-ACE2 cells (provided by Dr. Barney Graham, NIAID  
337 Vaccine Research Center) were cultured in DMEM supplemented with 10% FCS, 10 mM  
338 HEPES pH 7.3, 100 U/mL penicillin-streptomycin, and 10 µg/mL puromycin.  
339 VeroE6/TMPRSS2 and Vero E6-TMPRSS2-T2A-ACE2 cells were maintained at 37 °C with 5%  
340 CO<sub>2</sub>. The cells were regularly tested for mycoplasma contamination by using PCR, and  
341 confirmed to be mycoplasma-free.

342

343 **Viruses.**

344 hCoV-19/Japan/UT-NCD1288-2N/2022 (BA.2; NCD1288, Accession ID; EPI\_ISL\_9595604)  
345 (Takashita et al., 2022; Uraki et al., 2022a), hCoV-19/Japan/TY41-716/2022 (BA.2.75;  
346 TY41-716, Accession ID; EPI\_ISL\_14011362)(Takashita et al., 2022),  
347 hCoV-19/Japan/UT-NCD1757-1N/2022 (BA.2.75; NCD1757, Accession ID;  
348 EPI\_ISL\_14321758 ), hCoV-19/Japan/UT-NCD1759-1N/2022 (BA.2.75; NCD1759, Accession  
349 ID; EPI\_ISL\_14321760), hCoV-19/Japan/TY41-702/2022 (BA.5; TY41-702, Accession ID;

350 EPI\_ISL\_13512581) (Kawaoka et al., 2022), and hCoV-19/USA/WI-UW-5250/2021  
351 (B.1.617.2; UW5250) (Gagne et al., 2022; Halfmann et al., 2022) were propagated in  
352 VeroE6/TMPRSS2 cells in VP-SFM (Thermo Fisher Scientific). BA.2.75 (NCD1757) and  
353 BA.2.75 (NCD1759) were subjected to next generation sequencing (NGS) (see Whole genome  
354 sequencing). All experiments with SARS-CoV-2 were performed in enhanced biosafety level 3  
355 (BSL3) containment laboratories at the University of Tokyo and the National Institute of  
356 Infectious Diseases, Japan, which are approved for such use by the Ministry of Agriculture,  
357 Forestry, and Fisheries, Japan, or in BSL3 agriculture containment laboratories at the University  
358 of Wisconsin-Madison, which are approved for such use by the Centers for Disease Control and  
359 Prevention and by the US Department of Agriculture.

360

#### 361 **Animal experiments and approvals.**

362 Animal studies were carried out in accordance with the recommendations in the Guide for the  
363 Care and Use of Laboratory Animals of the National Institutes of Health. The protocols were  
364 approved by the Animal Experiment Committee of the Institute of Medical Science, the  
365 University of Tokyo (approval number PA19-75) and the Institutional Animal Care and Use  
366 Committee at the University of Wisconsin, Madison (assurance number V006426). Virus  
367 inoculations were performed under isoflurane, and all efforts were made to minimize animal  
368 suffering. *In vivo* studies were not blinded, and animals were randomly assigned to infection  
369 groups. No sample-size calculations were performed to power each study. Instead, sample sizes  
370 were determined based on prior *in vivo* virus challenge experiments.

371

#### 372 **Experimental infection of Syrian hamsters.**

373 Six-week-old male wild-type Syrian hamsters (Japan SLC Inc., Shizuoka, Japan) were used in  
374 this study. Baseline body weights were measured before infection. Under isoflurane anesthesia,  
375 five hamsters per group were intranasally inoculated with  $10^5$  PFU (in 30  $\mu$ L) of BA.2  
376 (NCD1288), BA.2.75 (TY41-716), BA.2.75 (NCD1757), BA.2.75 (NCD1759), BA.5  
377 (TY41-702), or B.1.617.2 (UW5250). Body weight was monitored daily for 6 days. For  
378 virological and pathological examinations, ten hamsters per group were intranasally infected  
379 with  $10^5$  PFU (in 30  $\mu$ L) of BA.2 (NCD1288), BA.2.75 (TY41-716), BA.2.75 (NCD1757),  
380 BA.5(TY41-702), or B.1.617.2 (UW5250); 3 and 6 dpi, five animals were euthanized and nasal  
381 turbinates and lungs were collected. The virus titers in the nasal turbinates and lungs were  
382 determined by use of plaque assays on Vero E6-TMPRSS2-T2A-ACE2 cells.

383 For co-infection studies, BA.2.75 (TY41-716) was mixed with BA.5 (TY41-702) at a 1:1, 1:3,  
384 1:19, or 1:199 ratio on the basis of their titers, and each virus mixture (total  $2 \times 10^5$  PFU in 60  
385  $\mu$ L) was inoculated into five wild-type hamsters. At 4 dpi, five animals were euthanized and

386 nasal turbinates and lungs were collected to determine virus titers.  
387 The K18-hACE2 transgenic hamster line (line M41) were developed by using a  
388 piggyBac-mediated transgenic approach. The K18-hACE2 cassette from the pK18-hACE2  
389 plasmid was transferred into a piggyBac vector, pmhyGENIE-3, for pronuclear injection  
390 (Gilliland et al., 2021). Then, female 6-8-week-old K18-hACE2 homozygous transgenic  
391 hamsters, whose hACE2 expression was confirmed, were intranasally inoculated with  $10^5$  PFU  
392 (in 30  $\mu$ L) of BA.2 (NCD1288), BA.5 (TY41-702), BA.2.75 (TY41-716), or B.1.617.2  
393 (UW5250). At 5 dpi, the animals were euthanized and nasal turbinates and lungs were collected.  
394 The virus titers in the nasal turbinates and lungs were determined by use of plaque assays on  
395 Vero E6-TMPRSS2-T2A-ACE2 cells.

396

### 397 **Lung function.**

398 Respiratory parameters were measured by using a whole-body plethysmography system  
399 (PrimeBioscience) according to the manufacturer's instructions. In brief, infected hamsters were  
400 placed in the unrestrained plethysmography chambers and allowed to acclimate for 1 min before  
401 data were acquired over a 3-min period by using FinePointe software.

402

### 403 **Histopathology**

404 Histopathological examination was performed as previously described (Halfmann et al., 2022;  
405 Kawaoka et al., 2022; Uraki et al., 2022a). In brief, excised animal lungs were fixed in 4%  
406 paraformaldehyde in phosphate buffered saline (PBS) and processed for paraffin embedding.  
407 The paraffin blocks were sliced into 3 $\mu$ m-thick sections and mounted on silane-coated glass  
408 slides, followed by hematoxylin and eosin (H&E) stain for histopathological examination. To  
409 detect SARS-CoV-2 RNA, in situ hybridization was performed using an RNA scope 2.5 HD  
410 Red Detection kit (Advanced Cell Diagnostics, Newark, California) with an antisense probe  
411 targeting the nucleocapsid gene of SARS-CoV-2 (Advanced Cell Diagnostics) following  
412 manufacturer's instructions. Tissue sections were also processed for immunohistochemistry with  
413 a rabbit polyclonal antibody for SARS-CoV nucleocapsid protein (ProSpec; ANT-180, 1:500  
414 dilution, Rehovot, Israel), which cross-reacts with SARS-CoV-2 nucleocapsid protein. Specific  
415 antigen-antibody reactions were visualized by means of 3,3'-diaminobenzidine  
416 tetrahydrochloride staining using the Dako Envision system (Dako Cytomation; K4001, 1:1  
417 dilution, Glostrup, Denmark).

418

### 419 **Whole genome sequencing**

420 Viral RNA was extracted by using a QIAamp Viral RNA Mini Kit (QIAGEN). The whole  
421 genome of SARS-CoV-2 was amplified by using a modified ARTIC network protocol in which

422 some primers were replaced or added (Itokawa et al., 2020; Quick). Briefly, viral cDNA was  
423 synthesized from the extracted RNA by using a LunarScript RT SuperMix Kit (New England  
424 BioLabs). The DNA was then amplified by performing a multiplexed PCR in two pools using  
425 the ARTIC-N5 primers and the Q5 Hot Start DNA polymerase (New England BioLabs)  
426 (Itokawa et al.). The DNA libraries for Illumina NGS were prepared from pooled amplicons by  
427 using a QIAseq FX DNA Library Kit (QIAGEN) and were then analyzed by using the iSeq 100  
428 System (Illumina). To determine the sequences of BA.2.75 (NCD1757) and BA.2.75  
429 (NCD1759), the reads were assembled by the CLC Genomics Workbench (version 22, Qiagen)  
430 with the Wuhan/Hu-1/2019 sequence (GenBank accession no. MN908947) as a reference. The  
431 sequences of BA.2.75 (NCD1757) and BA.2.75 (NCD1759) were deposited in the Global  
432 Initiative on Sharing All Influenza Data (GISAID) database with Accession IDs:  
433 EPI\_ISL\_14321758, and EPI\_ISL\_14321760, respectively. For the analysis of the ratio of BA.5  
434 to BA.2.75 after co-infection, the ratio of BA.2.75 to BA.5 was calculated from the 6 amino  
435 acid differences in the S gene between the two viruses. Samples with more than 300 read-depths  
436 were analyzed.

437

#### 438 **Statistical analysis.**

439

440 GraphPad Prism software was used to analyze the data. Statistical analysis included the  
441 Kruskal-Wallis test followed by Dunn's test, and an ANOVA with post-hoc tests. Differences  
442 among groups were considered significant for  $P$  values  $< 0.05$ .

443

#### 444 **Data and code availability.**

445 All data supporting the findings of this study are available in the paper. There are no restrictions  
446 in obtaining access to the primary data. The source data are provided with this paper.

447 No novel code was used in the course of the data acquisition or analysis, the most representative  
448 forms of each virus, dynamics plots, and isotonic regression analyses are available at  
449 <https://cov.lanl.gov/content/index>.

450

451

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465

#### 466 **Author contributions**

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468 S.M., M.Imai, and T.S performed the hamster infection experiments, titrated virus in tissues,  
469 and/or analyzed pathology. S.Y. performed next generation sequencing. Z.W., R.L., Y.L., and  
470 D.L. generated human ACE2 hamsters. S.Y., Y.S.-T., N.I., S.F., S.W., K.M., and N.O.  
471 propagated and/or sequenced viruses. J.T. and B.K. analyzed variant dynamics and Spike  
472 genomes. W. F. processed viral sequence data for identifying variant-representative full-length  
473 genomes. R.U., S.I., P.J.H, S.Y., M.Imai, T.S. and Y.K. obtained funding, conceived the study,  
474 and/or supervised the research. R.U., M.Imai and Y.K. wrote the initial draft, with all other  
475 authors providing editorial comments.  
476

#### 477 **Competing interests**

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482

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487

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605

606 **Figure legends**

607 **Fig. 1. Pango lineage dynamics between 01-12-2021 and 08-08-2022.**

608 **a**, BA.2.75 frequencies over time globally and in India, where BA.2.75 is currently most  
609 commonly found. Omicron variants have been through waves of global dominance since  
610 Omicron began to spread in late 2021. BA.1 (and BA.1.1, both in red) very rapidly replaced  
611 Delta globally. BA.2 then replaced BA.1 as the globally dominant form. Within the BA.2  
612 lineage, BA.2.12.1 began to expand in North America, but BA.2, including the BA.2.12.1  
613 sublineage, is being replaced by BA.5, the currently globally dominant form. BA.2.75 is still  
614 very rare globally, at only 0.3% of the global sample in the 30 days ending on 08-08-2022, but  
615 is being increasingly sampled in India, representing 25% of the last 30-day sample. BA.5 was  
616 slower to begin its expansion in India than in most countries, but was still on a trajectory of  
617 increasing prevalence when BA.2.75 was first sampled in late May/early June; BA.2.75 is much  
618 more rapidly gaining in prevalence in India than BA.5, suggesting a possible selective  
619 advantage. **b**, Examples illustrating the increase in prevalence of BA.2.75 relative to BA.5,  
620 despite BA.5 being well-established prior to BA.2.75's introduction, at both the state level  
621 within India (left), and in countries outside of India (right). Singapore and Nepal were selected  
622 as examples from Fig. S2 as they were the two countries with the highest frequency of BA.2.75  
623 outside of India. Maharashtra and West Bengal were selected from Sup. Fig. S3 as they are the  
624 states within India that currently have the most available samples. All data are from GISAID;  
625 the illustrations we made with the “Embers” web-based tool at cov.lanl.gov (Korber et al.,  
626 2020).

627

628 **Figure 2. The infectivity and pathogenicity of BA.2.75 in hamsters.**

629 **a,b**, Wild-type Syrian hamsters were intranasally inoculated with  $10^5$  PFU in 30  $\mu$ L of BA.2  
630 (NCD1288) (n=9), BA.5 (TY41-702) (n=9), BA.2.75 (TY41-716) (n=5), BA.2.75 (NCD1757)  
631 (n=5), BA.2.75 (NCD1759) (n=5), B.1.617.2 (UW5250) (n=9), or PBS (mock) (n=8). **a**, Body  
632 weights of virus-infected and mock-infected hamsters were monitored daily for 6 days. Data are  
633 presented as the mean percentages of the starting weight ( $\pm$  s.e.m.). **b**, Pulmonary function  
634 analyses in virus-infected and mock-infected hamsters. Penh and Rpef were measured by using  
635 whole-body plethysmography. Mean  $\pm$  s.e.m. Data were analyzed by using a two-way ANOVA  
636 followed by Tukey's multiple comparisons test. **c**, Virus replication in infected Syrian hamsters.  
637 Hamsters (n =10) were intranasally inoculated with  $10^5$  PFU in 30  $\mu$ L of BA.2 (NCD1288),  
638 BA.5 (TY41-702), BA.2.75 (TY41-716), BA.2.75 (NCD1757), or B.1.617.2 (UW5250) and  
639 euthanized at 3 and 6 dpi for virus titration (n =5/day). Virus titers in the nasal turbinates and  
640 lungs were determined by performing plaque assays with Vero E6-TMPRSS2-T2A-ACE2 cells.  
641 Vertical bars show the mean  $\pm$  s.e.m. Points indicate data from individual hamsters. The lower

642 limit of detection is indicated by the horizontal dashed line. Data were analyzed by using a  
643 one-way ANOVA with Tukey's multiple comparisons test (titers in the lungs at 3 dpi and nasal  
644 turbinates at 3 and 6 dpi) or the Kruskal-Wallis test followed by Dunn's test (titers in the lungs  
645 at 6 dpi). **d**, hACE2-expressing Syrian hamsters ( $n = 4$ ) were intranasally inoculated with  $10^5$   
646 PFU in 30  $\mu$ L of BA.2 (NCD1288), BA.5 (TY41-702), BA.2.75 (TY41-716), or B.1.617.2  
647 (UW5250). Infected animals were euthanized at 5 dpi for virus titration ( $n = 4$ /group). Virus  
648 titers in the nasal turbinates and lungs were determined by performing plaque assays with Vero  
649 E6-TMPRSS2-T2A-ACE2 cells. Vertical bars show the mean  $\pm$  s.e.m. Points indicate data from  
650 individual animals. The lower limit of detection is indicated by the horizontal dashed line. Data  
651 were analyzed by using a one-way ANOVA with Tukey's multiple comparisons test (titers in the  
652 nasal turbinates) or the Kruskal-Wallis test followed by Dunn's test (titers in the lungs). *P* values  
653 of  $< 0.05$  were considered statistically significant.

654

655 **Figure 3. Histopathological findings in hamsters inoculated with BA.2.75.**

656 Syrian hamsters ( $n = 5$ , per group) were inoculated with  $10^5$  PFU of BA.2.75 (TY41-716), BA.5  
657 (TY41-702), or B.1.617.2 (UW5250) and sacrificed at 3 or 6 dpi for histopathological  
658 examinations. **a**, Representative images of the lungs at low magnification are shown. Left  
659 columns, hematoxylin and eosin staining. Right columns, in situ hybridization targeting the  
660 nucleocapsid gene of SARS-CoV-2. Scale bars, 1 mm. **b**, Representative images of the  
661 bronchi/bronchioles and alveoli at high magnification are shown. Upper rows, hematoxylin and  
662 eosin staining. Middle rows, in situ hybridization targeting the nucleocapsid gene of  
663 SARS-CoV-2. Lower rows: immunohistochemistry for the detection of SARS-CoV-2  
664 nucleocapsid protein by a rabbit polyclonal antibody. Scale bars, 100  $\mu$ m.

665

666 **Figure 4. Relative viral fitness of BA.2.75 and BA.5 in hamsters.**

667 BA.2.75 (TY41-716) and BA.5 (TY41-702) were mixed at a 1:1 (**a**), 1:3 (**b**), 1:19 (**c**), or 1:199  
668 (**d**) ratio on the basis of their infectious titers, and the virus mixture (total  $2 \times 10^5$  PFU in 60  $\mu$ L)  
669 was intranasally inoculated into wild-type hamsters ( $n = 5$ ). Nasal turbinates and lungs were  
670 collected from the infected animals at 4 dpi and analyzed using next generation sequencing  
671 (NGS). Shown are the relative proportions of BA.5 and BA.2.75 in the infected animals.

672

673 **Figure S1. Amino acid differences between representative forms of recently emerged**  
674 **Omicron variants.**

675 **a**, Amino acid differences in the Spike of Omicron variants. BA.1 was rapidly globally replaced  
676 by BA.2; here we used the most common form of BA.2 as the reference Omicron variant. Spike  
677 amino acid differences between the Wuhan reference strain WIV04/2019|EPI\_ISL\_402124|2019



678 and the baseline form of BA.2 are shown in black. When other Omicron variants share spike  
679 BA.2 defining mutations in a given position, they are noted in grey. When they differ, the amino  
680 acid change is highlighted in the color assigned to each variant (the same color as used in Figure  
681 1). Deletions are indicated by a dash (-), insertions by a plus sign (e.g., +214EPE means a three  
682 amino acid insertion of EPE after position 214). Reversions from BA.2 to the ancestral Wuhan  
683 form are indicated by an underscore ( \_ ). **b**, Highlighting amino acid differences between BA.2,  
684 BA.5, and BA.2.75 throughout the full proteome. Only amino differences from the most  
685 representative form of BA.2 are shown in this figure, illustrated as a tick mark. The grey line  
686 represents the full proteome. All changes in the most common forms of BA.5 and BA.2.75  
687 relative to BA.2 are noted, as these are candidates for contributing to a selective advantage of  
688 BA.5 over BA.2, and of BA.2.75 over BA.5 and BA.2. Because details for the spike protein are  
689 shown in **a**, they are not shown here. The amino acids that are distinctive in the three BA.2.75  
690 variants studied in this paper are highlighted at the bottom, BA.2.75 V1–V3. Full length  
691 representative forms of Pango lineages are defined as the most common circulating form of a  
692 given Pango lineage.

693

694 **Figure S2. Isotonic regression analysis showing BA.2.75 is increasingly sampled over time**  
695 **in countries where it has become established.**

696 The table provides summary statistics for all countries where BA.2.75 sequences have been  
697 sampled more than 10 times with a sampling date between 12-05-2022 and 08-08-2022. Four  
698 examples of the data over time are plotted to illustrate the increasing frequency of BA.2.75  
699 sampling. We show the countries with the most available data (India, Singapore, and the US) as  
700 well as Nepal, with a lower sampling frequency but higher fraction of BA.2.75 cases – these  
701 numbers are highlighted in red in the table. The proportion of BA.2.75 in the total sample  
702 (y-axis) is calculated each day samples are available (x-axis). The size of the dot reflects the  
703 relative sample size on a given day. The *p*-value is calculated based on a one-sided resampling  
704 test with 400 randomizations. All *p*-values in the right-hand column are highly significant,  
705 showing that the frequency of BA.2.75 is increasing in every country where it has been sampled  
706 more than ten times. The results can be updated using the Isotonic Regression tool at  
707 [cov.lanl.gov](https://cov.lanl.gov). ([https://cov.lanl.gov/content/sequence/ISORG/pango\\_isorg.html](https://cov.lanl.gov/content/sequence/ISORG/pango_isorg.html))(Korber et al.,  
708 2020). An additional analysis was performed comparing BA.2.75 to just BA.5 at the country  
709 level, and these direct comparisons also supported the conclusion that BA.2.75 is expanding  
710 significantly faster than BA.5 in regions where they are co-circulating.

711

712 **Figure S3. Isotonic regression analysis showing BA.2.75 is increasingly sampled over time**  
713 **in all states in India where it has been sampled more than 10 times.**

714 These figures follow the format of Figure S2, but at a more geographically restricted. The  
715 analysis establishes that BA.2.75 is increasing in frequency throughout India, providing  
716 evidence that the increase at the country level in India seen in Figs. 1 and S2 are not due to  
717 regional founder effects within India. The four Indian states with the highest number of samples  
718 (the values in red) were selected to illustrate the increases in the lower panels. BA.2.75  
719 prevalence is also increasing in the four US states and two Canadian provinces where it has  
720 been sampled more than 10 times, as well as in England and New South Wales, Australia. An  
721 additional analysis was performed where we compared BA.2.75 to *just* BA.5 at the state level,  
722 and these direct comparisons also supported the conclusion that BA.2.75 is expanding  
723 significantly faster than BA.5 in regions where they are co-circulating.

724

725 **Figure S4. Semi-macroscopic images of the lungs of hamsters inoculated with**  
726 **SARS-CoV-2.**

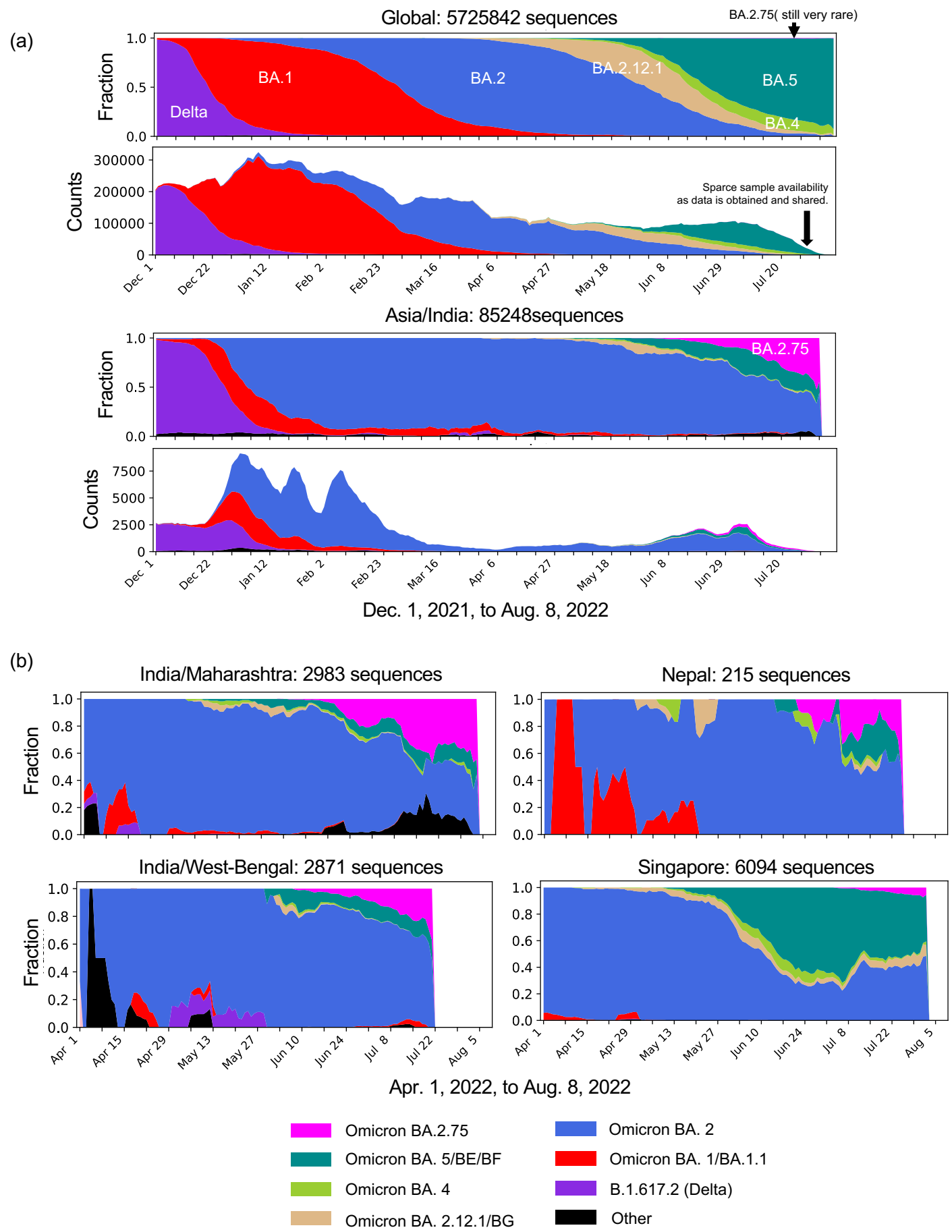
727 Syrian hamsters (n = 5, per group) were inoculated with 10<sup>5</sup> PFU of BA.2.75 (TY41-716), BA.5  
728 (TY41-702), or B.1.617.2 (UW5250) and sacrificed at 3 or 6 dpi for histopathological  
729 examinations. Semi-macroscopic images (hematoxylin and eosin staining) of the lungs from all  
730 animals examined are shown. Scale bars, 5 mm.

731

732 **Figure S5. Histopathological findings in hamsters inoculated with BA.2.75 (NCD1757).**

733 Syrian hamsters (n = 5) were inoculated with 10<sup>5</sup> PFU of BA.2.75 (Omicron, NCD1757) and  
734 sacrificed at 6 dpi for histopathological examinations. **a**, Semi-macroscopic images  
735 (hematoxylin and eosin staining) of the lungs from all animals examined are shown. Scale bars,  
736 5 mm. **b**, Representative images (hematoxylin and eosin staining) of the lungs at low  
737 magnification are shown. Scale bars, 1 mm. **c**, Representative images (hematoxylin and eosin  
738 staining) of the bronchi/bronchioles and alveoli at high magnification are shown. Scale bars,  
739 100 μm.

# Figure 1



# Figure 2

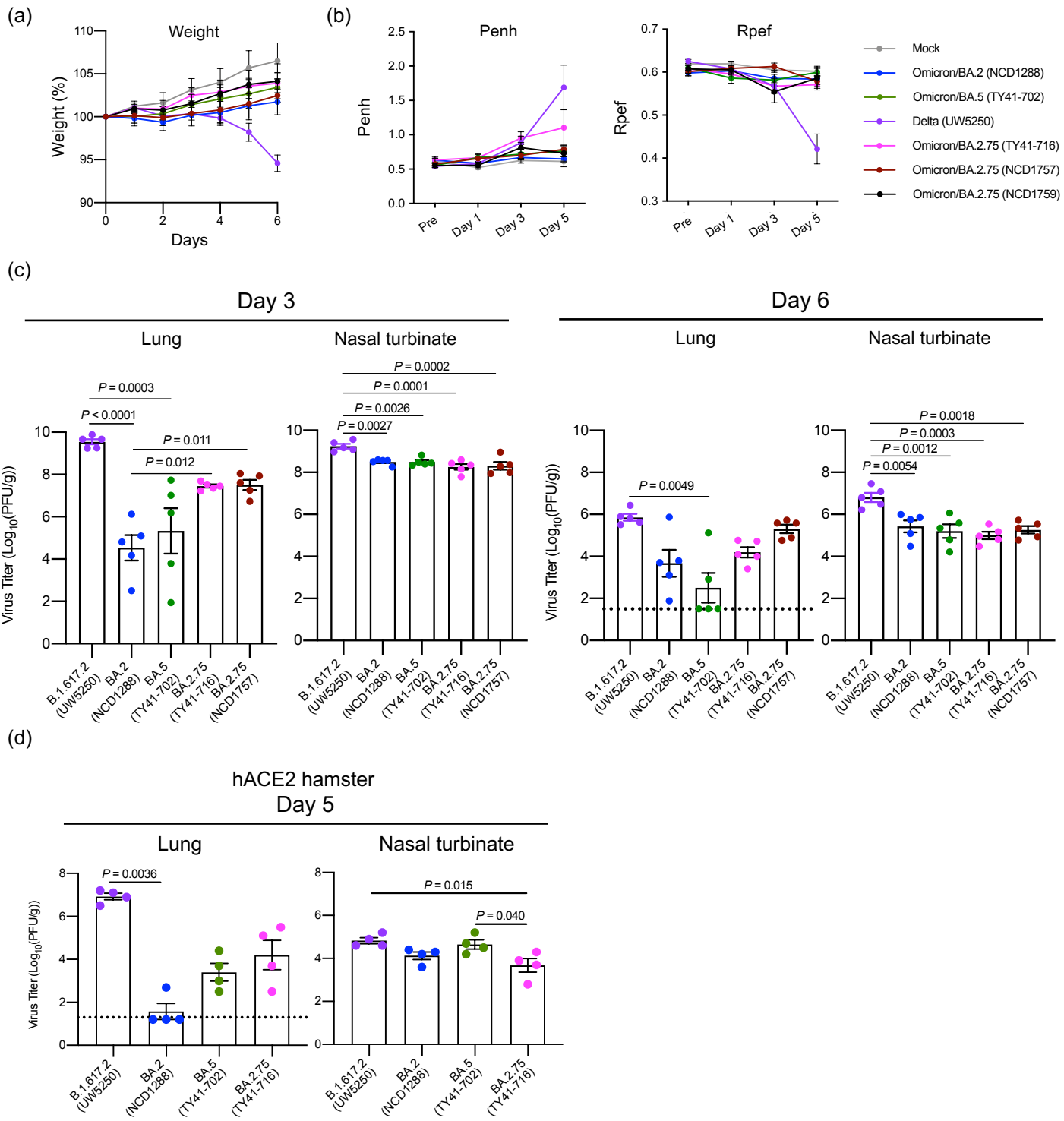




Figure 3 (a)

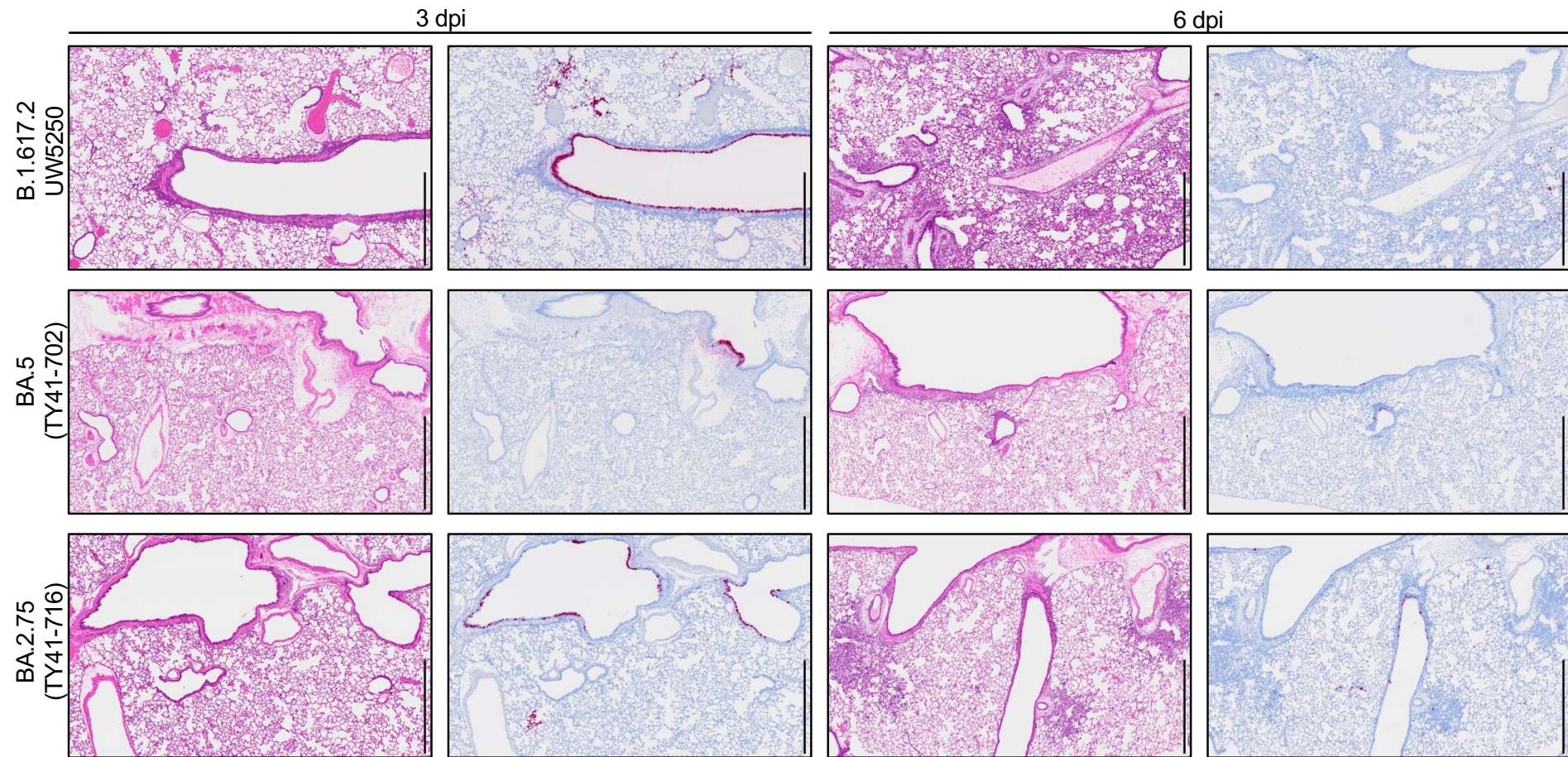
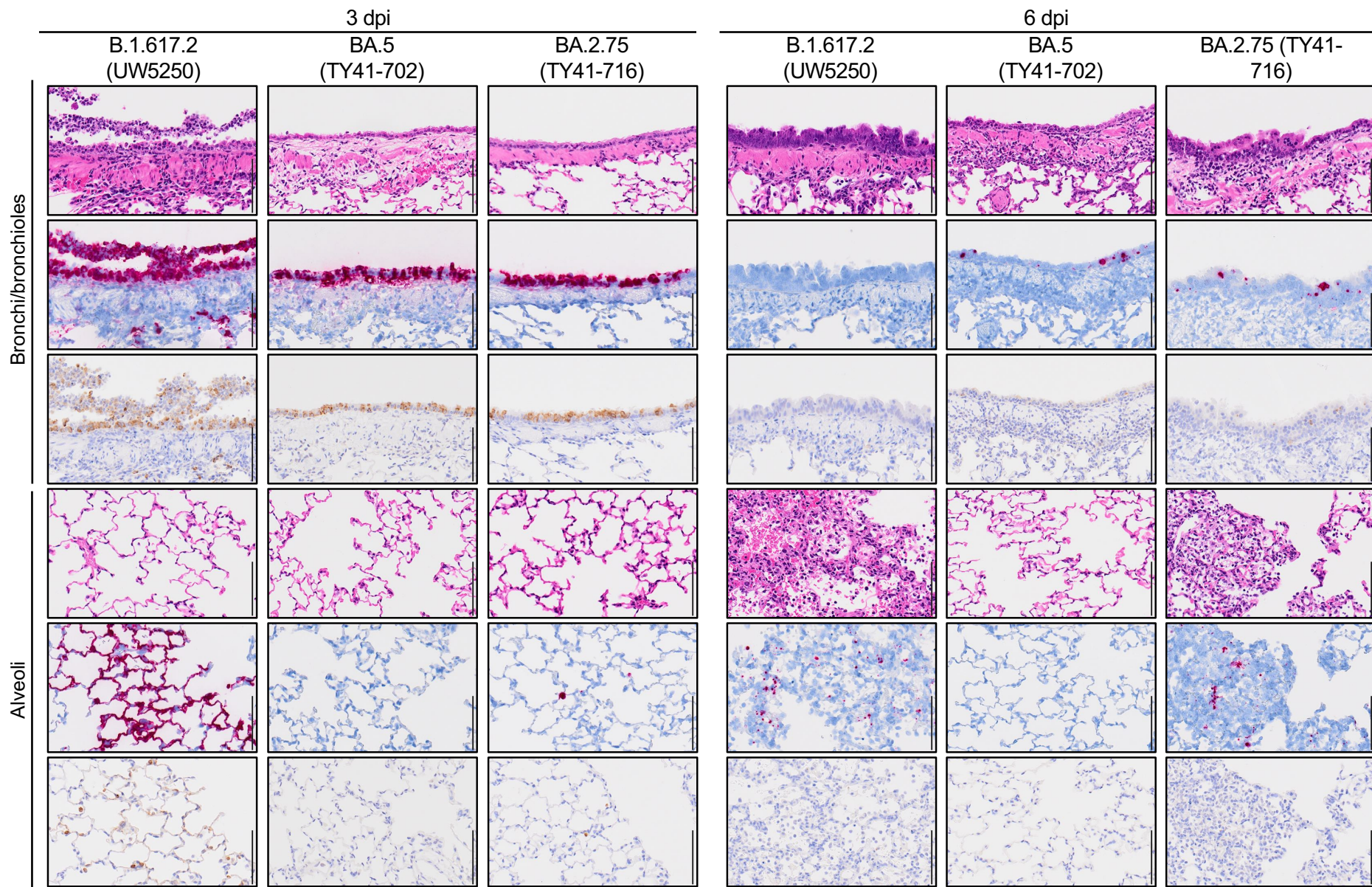




Figure 3 (b)



# Figure 4

BA.5 (TY41-702)  
BA.2.75 (TY41-716)

