

1 **Characterization of the SARS-CoV-2 BA.5.5 and BQ.1.1 Omicron Variants in**
2 **Mice and Hamsters.**

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23
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25 **ABSTRACT**

26 The continued evolution and emergence of novel SARS-CoV-2 variants has resulted in
27 challenges to vaccine and antibody efficacy. The emergence of each new variant necessitates the
28 need to re-evaluate and refine animal models used for countermeasure testing. Here, we tested a
29 currently circulating SARS-CoV-2 Omicron lineage variant, BQ.1.1, in multiple rodent models
30 including K18-hACE2 transgenic, C57BL/6J, and 129S2 mice, and Syrian golden hamsters. In
31 contrast to a previously dominant BA.5.5 Omicron variant, inoculation of K18-hACE2 mice with
32 BQ.1.1 resulted in a substantial weight loss, a characteristic seen in pre-Omicron variants.
33 BQ.1.1 also replicated to higher levels in the lungs of K18-hACE2 mice and caused greater lung
34 pathology than the BA.5.5 variant. However, C57BL/6J mice, 129S2 mice, and Syrian hamsters
35 inoculated with BQ.1.1 showed no differences in respiratory tract infection or disease compared
36 to animals administered BA.5.5. Airborne or direct contact transmission in hamsters was
37 observed more frequently after BQ.1.1 than BA.5.5 infection. Together, these data suggest that
38 the BQ.1.1 Omicron variant has increased virulence in some rodent species, possibly due to the
39 acquisition of unique spike mutations relative to other Omicron variants.

40

41 **IMPORTANCE**

42 As SARS-CoV-2 continues to evolve, there is a need to rapidly assess the efficacy of
43 vaccines and antiviral therapeutics against newly emergent variants. To do so, the commonly
44 used animal models must also be reevaluated. Here, we determined the pathogenicity of the
45 circulating BQ.1.1 SARS-CoV-2 variant in multiple SARS-CoV-2 animal models including
46 transgenic mice expressing human ACE2, two strains of conventional laboratory mice, and
47 Syrian hamsters. While BQ.1.1 infection resulted in similar levels of viral burden and clinical

48 disease in the conventional laboratory mice tested, increases in lung infection were detected in
49 human ACE2-expressing transgenic mice, which corresponded with greater levels of pro-
50 inflammatory cytokines and lung pathology. Moreover, we observed a trend towards greater
51 animal-to-animal transmission of BQ.1.1 than BA.5.5 in Syrian hamsters. Together, our data
52 highlight important differences in two closely related Omicron SARS-CoV-2 variant strains and
53 provide a foundation for evaluating countermeasures.

54 INTRODUCTION

55 The COVID-19 pandemic caused by severe acute respiratory syndrome coronavirus 2
56 (SARS-CoV-2) is now in its third year. While major advances have been made in understanding
57 the biology of SARS-CoV-2, the efficacy of existing vaccines and therapeutics has been
58 challenged by the continued evolution of the virus spike glycoprotein. In late 2021, the first
59 Omicron (BA.1) variant emerged, which encoded a greater number of spike mutations than
60 previously detected variants (> 30 mutations relative to WA1/2020). Despite the considerable
61 escape from natural, hybrid, and vaccine-induced immunity in humans, BA.1 caused attenuated
62 infection and disease in mice, hamsters, and non-human primates (1-3). Subsequently, the BA.1
63 variant split into multiple lineages (BA.2 – 2.75, BA.4, and BA.5), each encoding a unique set of
64 mutations. Since October of 2022, cases of the BQ.1.1 variant, a descendent of the BA.5 lineage,
65 have steadily increased. Relative to BA.5.5, BQ.1.1 encodes only four differences in the spike
66 protein (I76T, R346T, K444T, and N460K) (4).

67 Here, we evaluated the pathogenesis of the SARS-CoV-2 BQ.1.1 variant relative to the
68 closely related, but genetically distinct, BA.5.5 variant in commercially available mouse strains
69 and Syrian golden hamsters that have been used previously as models of SARS-CoV-2
70 pathogenesis. For each mouse model, we assessed clinical disease and the levels of viral
71 infection. Using a hamster model, we also evaluated the relative transmissibility of BA.5.5 and
72 BQ.1.1. Finally, we used *in vitro* protein-protein interaction assays to investigate whether the
73 disparities in pathogenesis we observed between variants might be explained by differences in
74 RBD-receptor interactions.

75 **RESULTS**

76 ***BQ.1.1 infection in C57BL/6J, 129S2, and K18-hACE2 mice.*** The naturally occurring
77 SARS-CoV-2 spike N501Y mutation enables engagement of endogenous murine ACE2 and
78 productive infection of wild-type C57BL/6J and 129S2 mice (5-9). Nonetheless, C57BL/6J,
79 129S2, and BALB/c mice inoculated with an earlier Omicron variant strain (BA.1) that has the
80 N501Y mutation sustained low levels of viral infection and no clinical disease (1). To determine
81 the pathogenicity of the newly emerged BQ.1.1 variant in these laboratory strains of mice, we
82 inoculated 8- to 10-week-old female C57BL/6J or 16-week-old male 129S2 mice with 10^4 FFU
83 of BA.5.5 or BQ.1.1. For both C57BL/6J and 129S2 mice, weight loss was not observed after
84 inoculation with BA.5.5 or BQ.1.1 over a four-day period (**Fig 1A-B**), consistent with
85 phenotypes observed with other Omicron strains (1). On day 4 post-infection (dpi), tissues were
86 collected, and the levels of viral infection were determined. In C57BL/6J mice, viral RNA levels
87 in the nasal washes, nasal turbinates, and lungs of animals infected with BA.5.5 or BQ.1.1 were
88 at or barely above the limit of detection, and infectious virus was not recovered from the lungs
89 (**Fig 1A**). Although viral RNA levels in the nasal washes of 129S2 mice were barely detectable
90 (**Fig 1B**), appreciable amounts of BA.5.5 and BQ.1.1 RNA were measured in the lungs and nasal
91 turbinates, with ~11-fold ($P < 0.0001$) higher amounts of viral RNA in the lung after BA.5.5
92 infection. Nonetheless, infectious virus recovered from the lungs of 129S2 mice was at or just
93 above the limit of detection.

94 We next tested the capacity of BQ.1.1 and BA.5.5 to infect K18-hACE2 transgenic mice,
95 which are a model of severe disease and pathogenesis for most SARS-CoV-2 strains (10, 11),
96 with the exception of some Omicron isolates (*e.g.*, BA.1 and BA.2) that are attenuated (1, 5, 10).
97 After inoculation with 10^4 FFU, BQ.1.1, but not BA.5.5, resulted in greater than 15% loss in

98 body-weight beginning at 3 dpi (**Fig 1C**). BQ.1.1 infection also resulted in a 3-fold ($P < 0.05$)
99 increase in viral RNA and 33-fold ($P < 0.0001$) increase in infectious virus in the lung compared
100 to the BA.5.5 strain. In the nasal washes and nasal turbinates, comparable levels of viral RNA
101 were observed for both strains. These data suggest the BQ.1.1 variant has an increased capacity
102 to infect the lungs of hACE2-expressing mice, and this correlates with clinical disease as
103 reflected by weight loss.

104 ***BQ.1.1 infection causes increased lung inflammation and pathology in K18-hACE2***
105 ***mice.*** Previous reports showed that infection of K18-hACE2 mice with the BA.1 strain did not
106 induce substantive pro-inflammatory cytokine/chemokine responses in the lung (1). Given the
107 capacity of BA.5.5 and BQ.1.1 to replicate in the lungs of K18-hACE2 mice, we quantified the
108 levels of pro-inflammatory cytokines and chemokines present at 6 dpi (**Fig 2A-B**). While
109 increased levels of pro-inflammatory cytokines and chemokines were present in lung
110 homogenates of BA.5.5 or BQ.1.1-infected compared to uninfected mice, many (*e.g.*, G-CSF,
111 IL-17, LIF, CCL2, CXCL9, CCL3, and CCL4) were expressed at higher levels in BQ.1.1- than
112 BA.5.5-infected mice. These data suggest that the increased viral replication in the lungs of
113 BQ.1.1-infected K18-hACE2 mice resulted in greater production of pro-inflammatory mediators.

114 To determine the impact of the differences in viral burden and cytokines on lung injury,
115 we performed histopathological analysis of lung tissues using hematoxylin and eosin staining
116 (**Fig 2C-F**). Lungs isolated from K18-hACE2 mice at 6 dpi with BA.5.5 showed limited immune
117 cell infiltration or lung injury. In contrast, BQ.1.1-infected lungs showed evidence of pneumonia
118 with increased numbers of inflammatory lesions characterized by immune cell infiltrates,
119 alveolar space consolidation, vascular congestion, and interstitial edema (**Fig 2C-D**). As

120 expected, lung tissues isolated from C57BL/6J and 129S2 mice infected with BA.5.5 or BQ.1.1
121 showed no evidence of lung pathology (**Fig 2E-F**).

122 ***BA.5.5. and BQ.1.1 infection and transmission in Syrian hamsters.*** We evaluated the
123 pathogenicity and transmissibility of BA.5.5 and BQ.1.1 in Syrian hamsters. Animals were
124 inoculated with 2.5×10^4 PFU of BA.5.5 or BQ.1.1, weights were measured daily, and at 3 or 6
125 dpi, tissues were collected to assess viral infection (**Fig 3A**). Compared to initial weights at the
126 time of inoculation, BA.5.5 and BQ.1.1 did not cause weight loss in hamsters over a 14-day
127 period and had only small, non-significant trends, in weight gain (**Fig 3B**). Viral RNA levels in
128 tissues were generally highest at 3 dpi (**Fig 3C**). Between the two variants, the amounts of viral
129 RNA were comparable with the exception of nasal turbinates at 3 dpi, which showed higher (7.5-
130 fold, $P < 0.01$) BA.5.5 levels than BQ.1.1. In a second analysis of infectious virus by plaque
131 assay, higher (81- and 51-fold, $P < 0.01$) levels of BA.5.5 were detected at 3 dpi in the nasal
132 washes and nasal turbinates but not in the lungs (**Fig 3D**). These data demonstrate that direct
133 inoculation of hamsters with BA5.5 or BQ.1.1 results in similar levels of infection and weight
134 loss, with BA.5.5 exhibiting slightly greater viral burden than BQ.1.1 in the upper respiratory
135 tract.

136 Unlike mice, the SARS-CoV-2 pathogenesis in Syrian hamsters also allows for
137 assessment of viral transmission from animal-to-animal (12, 13). We evaluated the capacity of
138 BA.5.5 and BQ.1.1 to transmit to naïve hamsters via direct contact or airborne transmission (**Fig**
139 **4A-B**). Donor hamsters were inoculated with 2.5×10^4 PFU of BA.5.5 or BQ.1.1. At 24 h post-
140 infection, donor animals were transferred to cages containing naïve animals for 8 h of either
141 direct contact or placed inside porous stainless-steel isolation canisters with directional airflow
142 from the donor animal to the naïve animal for airborne transmission. After the 8 h period, donor

143 and contact animals were separated, returned to individual caging, and respiratory tissues were
144 collected 4 days later for detection of infectious virus. Whereas BA.5.5 was transmitted
145 approximately 67% (4 of 6 animals) of the time under direct contact conditions, airborne
146 transmission was not observed (0 of 8 animals) for this variant (**Fig 4C-E**). In contrast, BQ.1.1
147 was transmitted to recipient animals at frequencies of 100% (6 of 6 animals) and 17% (1 of 6
148 animals) in direct contact and airborne settings, respectively (**Fig 4C-E**). The difference in direct
149 contact or airborne transmission between the two variants of SARS-CoV-2 was not statistically
150 significant, but trended toward greater transmission of BQ.1.1.

151 *Binding affinities of SARS-CoV-2 BA.5.5 and BQ.1.1 RBDs for human and mouse*
152 *ACE2*. As the SARS-CoV-2 spike protein has evolved in variant strains, differences in binding
153 affinity for hACE2 have been observed (14-16). Since we observed an elevated lung viral burden
154 for BQ.1.1 in K18-hACE2 mice, which express high levels of hACE2 in epithelial cells (10, 17),
155 but not in C57BL/6J or 129S2 mice, which express mouse ACE2 but not hACE2, we performed
156 biolayer interferometry (BLI) experiments to quantify RBD-ACE2 binding interactions (**Fig 5A-**
157 **C**). For BA.5.5 and BQ.1.1 RBDs, the affinities for hACE2 were similar in the low nanomolar-
158 range (6.06 and 4.4 nM, respectively). In contrast, the affinity of BQ.1.1 for mouse ACE2
159 (mACE2) was more than 5-fold higher than BA.5.5 (23 versus 121 nM, respectively). Thus, the
160 increased viral burden observed during BQ.1.1 infection in K18-hACE2, but not C57BL/6J or
161 129S2 mice, is likely not explained by altered affinity of binding to hACE2.

162 **DISCUSSION**

163 In this study, we compared the pathogenicity of a circulating SARS-CoV-2 Omicron
164 variant BQ.1.1 to the closely related BA.5.5 variant, the latter of which is rapidly diminishing in
165 prevalence (4). Our experiments highlight phenotypic differences among Omicron variant strains
166 in different animal models commonly used for countermeasure testing. For instance, infection
167 with BA.1 and BA.2 Omicron variant strains results in decreased infection and attenuated
168 disease in mice and hamsters (1, 14). Our studies showed that BQ.1.1 infection of K18-hACE2
169 transgenic mice resulted in weight loss that was similar to that seen with of pre-Omicron SARS-
170 CoV-2 variants including WA1/2020 D614G, B.1.1.7, and B.1.351 (5, 18), whereas, BA.5.5
171 infection did not result in weight loss regardless of the model tested. While the exact mechanism
172 of selective BQ.1.1-, but not BA5.5-induced weight loss in K18-hACE2 mice remains to be
173 determined, it may be due in part to the relatively higher levels of infection and inflammation,
174 which correlate with clinical disease progression in K18-hACE2 mice (11).

175 We observed increased viral burden and pathogenicity in the lungs of BQ.1.1-infected
176 K18-hACE2 mice compared to BA.5.5-infected animals. One potential mechanism that may, in
177 part, explain this observation is differences in mouse and human ACE2 receptor affinity due to
178 the mutations present in BQ.1.1, but not BA.5.5, spike proteins (I76T, R346T, K444T, and
179 N460K). Indeed, a recent report suggested that BQ.1.1 RBD had increased affinity for hACE2 in
180 a yeast surface display assay (1.5-fold), and this correlated with greater cell infectivity and
181 fusogenicity *in vitro* compared to BA.5 (19). In agreement with these data, our BLI results
182 showed that BQ.1.1 RBD had a 1.5-fold greater affinity for hACE2 than BA.5.5, suggesting a
183 mechanism that might contribute to and/or explain the phenotypic differences in K18-hACE2
184 mice. Reverse genetic experiments with amino acid substituted BA.5 RBDs suggest that the

185 R346T mutation alone is sufficient to enhance affinity for hACE2, whereas R346T and N460K
186 are required to increase *in vitro* cell infectivity (19). Nonetheless, amino acid differences in other
187 structural and nonstructural proteins apart from spike (*e.g.*, nsp2: Q376K, nsp6: L260F, nsp12:
188 Y273H, nsp13: M233I, N268S, and N: E136D) also might play a role. This hypothesis that
189 amino acid substitutions outside of spike may be important in distinguishing BA.5.5 and BQ.1.1
190 infectivity is supported by our observation that a 5-fold increase in BQ.1.1 RBD affinity for
191 mACE2 was insufficient to promote greater infection of C57BL/6J and 129S2 mice compared to
192 BA.5.5. Furthermore, the observation of a trend toward increased transmissibility for BQ.1.1
193 relative to BA.5.5 in hamsters suggests that more studies in animal models and humans are
194 needed to fully discern differences in pathogenesis for these and other SARS-CoV-2 variants.

195 **Limitations of study.** Several limitations exist in our study: (1) We evaluated three
196 mouse strains and a single Syrian hamster model of SARS-CoV-2 disease. Although these
197 animal models are used extensively in studying SARS-CoV-2 pathogenesis and
198 countermeasures, future testing of the virulence of BQ.1.1 and BA.5.5 in non-human primates
199 will be important; (2) While the hamster transmission model is useful, it will be important to
200 corroborate our BQ.1.1 transmission findings in humans as data becomes available; (3) All of
201 our studies were performed in naïve animals, which does not address the relative immune
202 evasive potential of BQ.1.1 and BA.5.5, a key question given that most of the global population
203 has been infected or immunized; and (4) We did not evaluate even newer strains of the XBB.1
204 lineage. During the latter stages of our study, cases of SARS-CoV-2 associated with the XBB
205 lineage have increased substantially. Future studies are needed to compare the pathogenicity,
206 transmissibility, and immune evasive potential of XBB and BQ.1.1 strains in animal models and
207 humans.

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216 were created using BioRender.com.

217

218 **AUTHOR CONTRIBUTIONS**

219 J.B.C. and S.M.S. performed mouse experiments and viral burden analyses. S.M.S.
220 propagated and validated SARS-CoV-2 viruses. T.L.D., T.L.B., H.H., and R.T. performed
221 hamster experiments. T.L.D., T.L.B., and H.H. performed hamster viral burden analyses. L.J.A.,
222 J.B.C., and S.S. performed BLI experiments. S.S. performed histopathology scoring. D.H.F.,
223 A.C.M.B, and M.S.D. obtained funding and supervised the research. J.B.C. and M.S.D. wrote the
224 initial draft, with the other authors providing editorial comments.

225

226 **COMPETING FINANCIAL INTERESTS**

227 M.S.D. is a consultant for Inbios, Vir Biotechnology, Ocugen, Topspin, Moderna, and
228 Immunome. The Diamond laboratory has received unrelated funding support in sponsored
229 research agreements from Vir Biotechnology, Emergent BioSolutions, Generate Biomedicines,
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232 declare no competing financial interests.

233

234 **FIGURE LEGENDS**

235 **Figure 1. BQ.1.1-infection results in increased infection and weight loss in K18-**
236 **hACE2 mice.** C57BL/6J (A), 129S2 (B), or K18-hACE2 (C) mice were inoculated intranasally
237 with 10^4 FFU of the indicated SARS-CoV-2 strain. Animals were monitored for weight loss (A-
238 C) daily (differences in area under the curves assessed by student's t-test with Welch's
239 correction; ** $P < 0.01$). At 4 or 6 dpi, the indicated tissues were collected. Viral RNA levels in
240 the lungs, nasal turbinates, and nasal washes were determined by RT-qPCR, and infectious virus
241 in the lungs were quantified by plaque assay (lines indicate median \pm SEM., dotted lines indicate
242 limits of detection; $n = 9-10$ mice per group, two experiments; Mann-Whitney test between
243 BA.5.5- and BQ.1.1-infected groups; * $P < 0.05$, ****, $P < 0.0001$).

244 **Figure 2. BQ.1.1 infection induces inflammatory cytokines and pathology in the**
245 **lungs of K18-hACE2 mice.** (A) Heat map of cytokine and chemokine protein expression levels
246 in lung homogenates. Data from BQ.1.1-infected mice are presented as \log_2 -transformed fold-
247 change compared to BA.5.5-infected mice. White, baseline; red, increase. (B) Graphs of cytokine
248 and chemokine protein levels in the lungs of naïve, BA.5.5, or BQ.1.1-infected lungs from K18-
249 hACE2 mice at 6 dpi (line indicates median, dotted lines indicate limits of detection; $n = 2-3$
250 naïve, $n = 9-10$ for all other groups (two-way ANOVA with Tukey's post-test with comparisons
251 between all groups: *, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$, ****, $P < 0.0001$). (C)
252 Inflammatory foci from mice in D-F were quantified blindly and plotted (Mann-Whitney test
253 between BA.5.5- and BQ.1.1-infected groups; * $P < 0.05$). (D-F) Hematoxylin and eosin staining
254 of lung sections from K18-hACE2, C57BL/6J, and 129S2 mice collected six days after intranasal
255 inoculation with 10^4 PFU of the indicated SARS-CoV-2 strain. Images show 2.5x (left), 5x
256 (middle), and 20x (right) power magnification. Scale bars indicate 500 μm , 500 μm , and 100 μm ,

257 left-to-right, respectively. Two representative images are shown from four mice per group
258 harvested from two experiments.

259 **Figure 3. BA.5.5 and BQ.1.1 infections of Syrian hamsters.** Hamsters were inoculated
260 intranasally with 2.5×10^4 PFU of the indicated SARS-CoV-2 strain (**A**). Naïve and BA.5.5 or
261 BQ.1.1-inoculated animals were monitored for weight change daily for 14 days (**B**). At 3 or 6
262 dpi, the nasal washes, nasal turbinates, and lungs, were collected from each animal and levels of
263 viral RNA and infectious virus were determined by RT-qPCR (**C**) or plaque assay (**D**),
264 respectively (lines indicate median \pm SEM., dotted lines indicate limits of detection; n = 6-
265 7 hamsters per group per timepoint, two experiments; Mann-Whitney test between BA.5.5- and
266 BQ.1.1-infected groups; * $P < 0.05$, ** $P < 0.01$).

267 **Figure 4. BA.5.5 and BQ.1.1 transmission in Syrian hamsters.** For transmission
268 studies, donor hamsters were inoculated intranasally with 2.5×10^4 PFU of the indicated SARS-
269 CoV-2 strain. At 24 h post-inoculation, animals were transferred to cages containing naïve
270 contact animals (direct contact; **A**) or porous canisters (airborne; **B**) upwind of naïve contact
271 animals for a total exposure time of 8 h. After exposure, animals were returned to individual
272 cages. At 4 days post-exposure, the percentage of SARS-CoV-2 positive contact hamsters were
273 quantified (**C** and **D**). Nasal washes, nasal turbinates, and lungs, were collected from contact
274 animals and levels of infectious virus were determined (**E**) (lines indicate median \pm SEM., dotted
275 lines indicate limits of detection; n = 6-7 hamsters per group, two experiments). Positive
276 transmission events were registered when infectious virus was detected above the limit of
277 detection within any tissue for a given animal.

278 **Figure 5. Determination of ACE2-BA.5.5/BQ.1.1 RBD binding affinity by BLI.**
279 Recombinant human (**A**) or mouse (**B**) ACE2-Fc proteins were loaded onto biolayer

280 interferometry (BLI) protein G pins at a concentration of 10 $\mu\text{g/mL}$ and dipped into the indicated
281 concentrations of BA.5.5 or BQ.1.1 RBDs. Samples were allowed to associate and dissociate for
282 300 s and 600 s, respectively. Dashed black curves show fits to a 1:1 binding model with a
283 drifting baseline. (C) Association rate (k_a), dissociation rate (k_d), and kinetic dissociation
284 constant (K_D) values were calculated and reported.
285

286 **METHODS**

287 **Cells.** Vero-TMPRSS2 (20) and Vero-hACE2-TMPRSS2 (21) cells were cultured at
288 37°C in Dulbecco's Modified Eagle medium (DMEM) supplemented with 10% fetal bovine
289 serum (FBS), 10 mM HEPES pH 7.3, 1 mM sodium pyruvate, 1× non-essential amino acids,
290 and 100 U/ml of penicillin–streptomycin. Vero-TMPRSS2 cells were supplemented with 5
291 µg/mL of blasticidin. Vero-hACE2-TMPRSS2 cells were supplemented with 10 µg/mL of
292 puromycin. All cells routinely tested negative for mycoplasma using a PCR-based assay.

293 **Viruses.** The BA.5.5 (hCoV-19/USA/COR-22-063113/2022) and BQ.1.1 strains (hCoV-
294 19/USA/CA-Stanford-79_S31/2022) were obtained from nasopharyngeal isolates as generous
295 gifts of Andrew Pekosz (Johns Hopkins School of Public Health) and Mehul Suthar (Emory
296 University), respectively. All virus stocks were generated in Vero-TMPRSS2 cells and subjected
297 to next-generation sequencing as described previously (21) to confirm the presence and stability
298 of expected substitutions. All virus experiments were performed in an approved biosafety level 3
299 (BSL-3) facility.

300 **Mouse experiments.** Animal studies were carried out in accordance with the
301 recommendations in the Guide for the Care and Use of Laboratory Animals of the National
302 Institutes of Health. The protocols were approved by the Institutional Animal Care and Use
303 Committee at the Washington University School of Medicine (assurance number A3381–01).
304 Virus inoculations were performed under anesthesia that was induced and maintained with
305 ketamine hydrochloride and xylazine, and all efforts were made to minimize animal suffering.

306 Heterozygous K18-hACE2 C57BL/6J mice (strain: 2B6.Cg-Tg(K18-ACE2)2PrImn/J),
307 and wild-type C57BL/6J (strain: 000664) were obtained from The Jackson Laboratory. 129S2
308 mice (strain: 129S2/SvPasCrl) were obtained from Charles River Laboratories. All animals were

309 housed in groups and fed standard chow diets. For mouse experiments, eight- to ten-week-old
310 female K18-hACE2 and C57BL/6J mice or 16-week-old male 129S2 mice were administered the
311 indicated doses of the respective SARS-CoV-2 strains by intranasal administration. *In vivo*
312 studies were not blinded, and mice were randomly assigned to treatment groups. No sample-size
313 calculations were performed to power each study. Instead, sample sizes were determined based
314 on prior *in vivo* virus challenge experiments.

315 **Measurement of viral RNA levels.** Tissues were weighed and homogenized with
316 zirconia beads in a MagNA Lyser instrument (Roche Life Science) in 1 mL of DMEM medium
317 supplemented with 2% heat-inactivated FBS. Tissue homogenates were clarified by
318 centrifugation at 10,000 rpm for 5 min and stored at -80°C . RNA was extracted using the
319 MagMax mirVana Total RNA isolation kit (Thermo Fisher Scientific) on the Kingfisher Flex
320 extraction robot (Thermo Fisher Scientific). RNA was reverse transcribed and amplified using
321 the TaqMan RNA-to-CT 1-Step Kit (Thermo Fisher Scientific). Reverse transcription was
322 carried out at 48°C for 15 min followed by 2 min at 95°C . Amplification was accomplished over
323 50 cycles as follows: 95°C for 15 s and 60°C for 1 min. Copies of SARS-CoV-2 *N* gene RNA in
324 samples were determined using a previously published assay (22). Briefly, a TaqMan assay was
325 designed to target a highly conserved region of the *N* gene (Forward primer:
326 ATGCTGCAATCGTGCTACAA; Reverse primer: GACTGCCGCCTCTGCTC; Probe: /56-
327 FAM/TCAAGGAAC/ZEN/AACATTGCCAA/3IABkFQ/). This region was included in an RNA
328 standard to allow for copy number determination down to 10 copies per reaction. The reaction
329 mixture contained final concentrations of primers and probe of 500 and 100 nM, respectively.

330 **Viral plaque assay.** Vero-TMPRSS2-hACE2 cells were seeded at a density of 1×10^5
331 cells per well in 24-well tissue culture plates. The following day, medium was removed and

332 replaced with 200 μ L of material to be titrated diluted serially in DMEM supplemented with 2%
333 FBS. One hour later, 1 mL of methylcellulose overlay was added. Plates were incubated for 72 h,
334 then fixed with 4% paraformaldehyde (final concentration) in PBS for 20 min. Plates were
335 stained with 0.05% (w/v) crystal violet in 20% methanol and washed twice with distilled,
336 deionized water.

337 **Hamster experiments.** Five-six-week-old male hamsters were obtained from Charles
338 River Laboratories and directly transferred into an enhanced animal biosafety level 3 laboratory
339 (ABSL-3+). Hamsters were challenged via the intranasal route with 2.5×10^4 PFU of SARS-
340 CoV-2 BA.5.5 or BQ.1.1. Twenty-four hours after challenge, naïve contact hamsters were
341 exposed to the directly inoculated (donor) hamsters. Potential virus exposure was either airborne,
342 wherein the donor and contact hamsters were placed in individual porous stainless-steel isolation
343 canisters with directional airflow coming from the donor cage, or through direct contact, wherein
344 the contact hamster was placed directly into the donor cage. Upon eight hours of exposure, the
345 contact hamsters were returned to their original cages. Hamsters directly inoculated were
346 weighed daily and harvested at three- or six-days post-inoculation. Contact hamsters were
347 harvested at four days post exposure. At time of harvest, the left lung lobe, nasal wash, and nasal
348 turbinate of each animal was collected. The nasal wash was collected with 1 mL of PBS
349 supplemented to contain 0.1% BSA and subsequently clarified at 1,200 rpm for ten minutes at
350 4°C. The left lung lobe was homogenized in 1.0 mL DMEM and clarified by centrifugation
351 1,000 x g for 5 min. Nasal turbinates were collected by removing the skin along the nose and
352 cheeks followed by cutting the jaw to expose the upper palate. A sagittal incision through the
353 palate exposed the nasal turbinates which were then removed using blunt forceps. The nasal
354 turbinates were homogenized in 1.0 mL of DMEM supplemented with 2% FBS, 10mM HEPES

355 (pH 7.3) and 2 mM L-glutamine and clarified by centrifugation 1,000 x g for 5 min. The nasal
356 wash, lung and nasal turbinates were used for viral titer analysis by quantitative RT-PCR using
357 primers and probes targeting the N gene, and by plaque assay. Infectious viral titer detected in
358 any of the contact hamster tissues was considered a positive transmission event.

359 **Cytokine and chemokine protein measurements.** Lung homogenates were incubated
360 with Triton-X-100 (1% final concentration) for 1 h at room temperature to inactivate SARS-
361 CoV-2. Homogenates were analyzed for cytokines and chemokines by Eve Technologies
362 Corporation (Calgary, AB, Canada) using their Mouse Cytokine Array/Chemokine Array
363 platform.

364 **Lung pathology.** Animals were euthanized before harvest and fixation of tissues. Briefly,
365 lungs were inflated with approximately 1.2 mL of 4% paraformaldehyde using a 3-mL syringe
366 and catheter inserted into the trachea. Tissues were allowed to fix for 24 h at room temperature,
367 embedded in paraffin, and sections were stained with hematoxylin and eosin. Slides were
368 scanned using a Hamamatsu NanoZoomer slide scanning system, and the images were viewed
369 using NDP view software (ver.1.2.46). Inflammatory lesions were quantified blindly.

370 **Binding analysis by biolayer interferometry.** BLI was used to quantify the binding of
371 BA.5.5 and BQ.1.1 SARS-CoV-2 RBDs to recombinant human or mouse ACE2 proteins. Fc
372 conjugated human and mouse ACE2 proteins were expressed and purified as previously
373 described (23). Subsequently, 10 µg/mL of each protein was immobilized onto protein G
374 biosensors (GatorBio) for 3 min. After a 30 s wash, the pins were submerged in running buffer
375 (10 mM HEPES, 150 mM NaCl, 3 mM EDTA, 0.05% P20 surfactant, and 1% BSA) containing
376 BA.5.5 or BQ.1.1 RBD protein (Sino Biologicals) ranging from 3.125 to 1,000 nM, followed by a

377 dissociation step in running buffer alone. The BLI signal was recorded and analyzed as a 1:1
378 binding model with a drifting baseline using BIAevaluation Software (Biacore).

379 **Data availability.** All data supporting the findings of this study are available within the
380 paper and are available from the corresponding author upon request.

381 **Statistical analysis.** All statistical tests were performed as described in the indicated
382 figure legends using Prism 9.4.1. Statistical significance was determined using an ANOVA when
383 comparing three or more groups. When comparing two groups, a Mann-Whitney test was
384 performed. The number of independent experiments performed are indicated in the relevant
385 figure legends.

386

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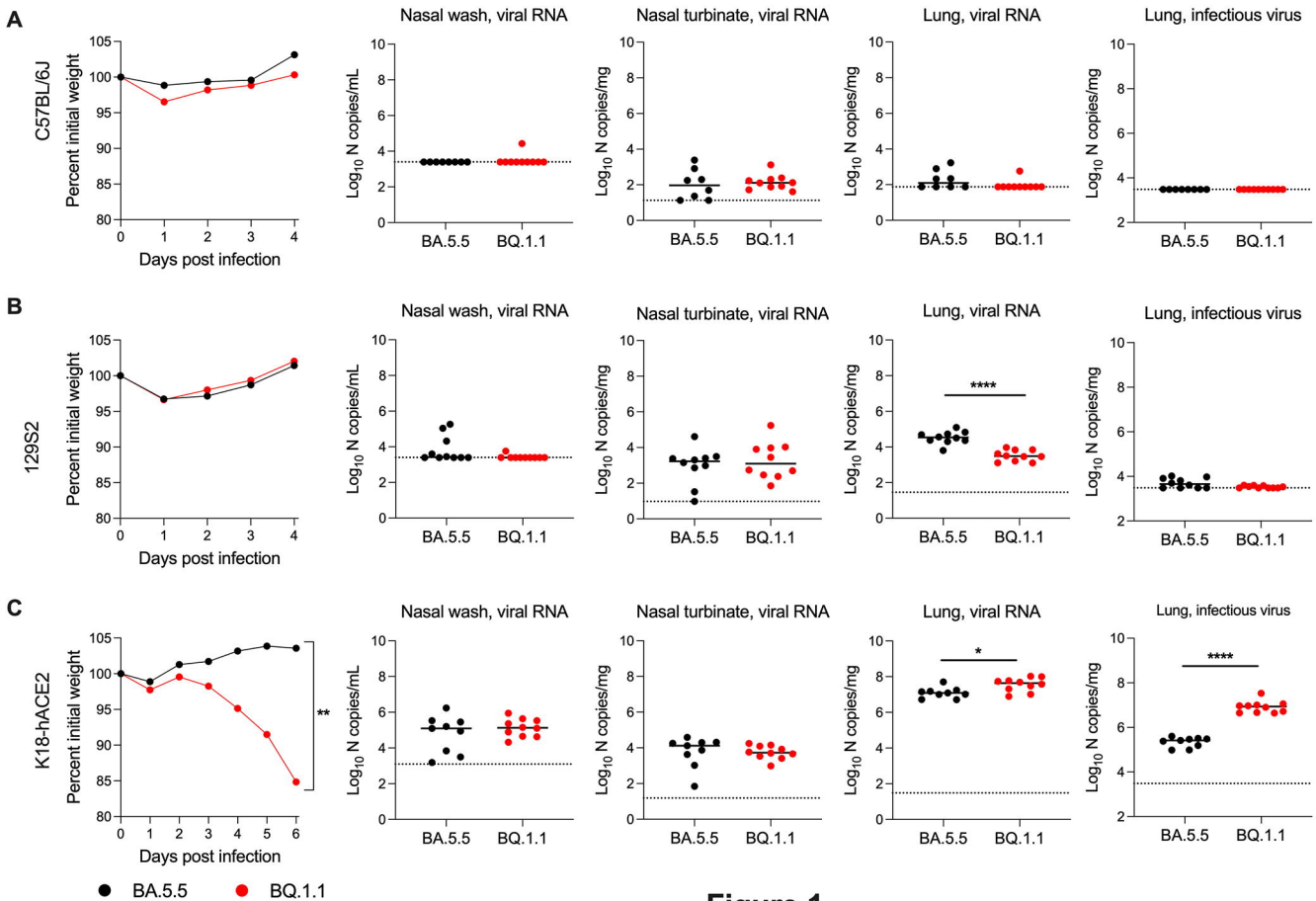


Figure 1

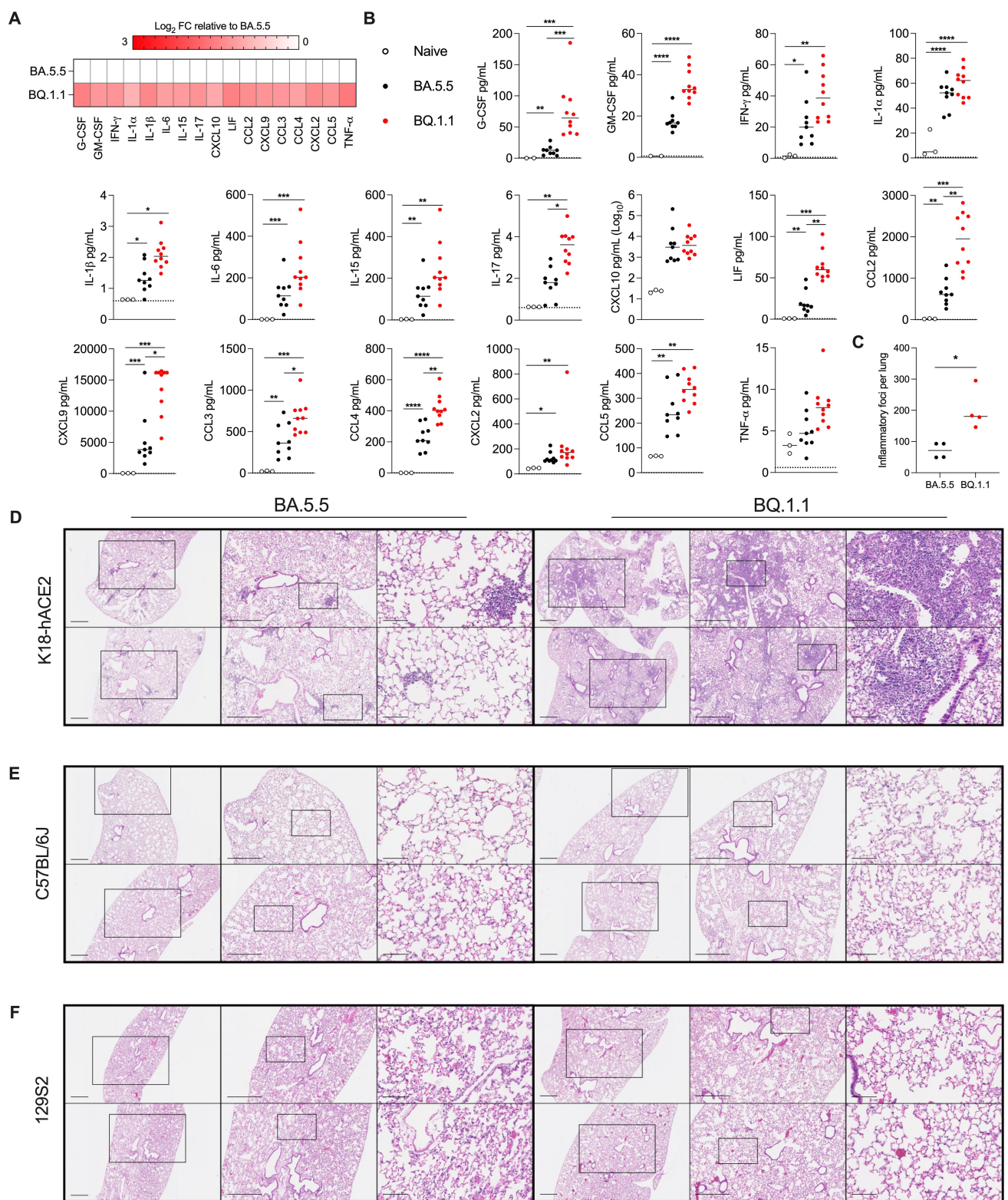


Figure 2

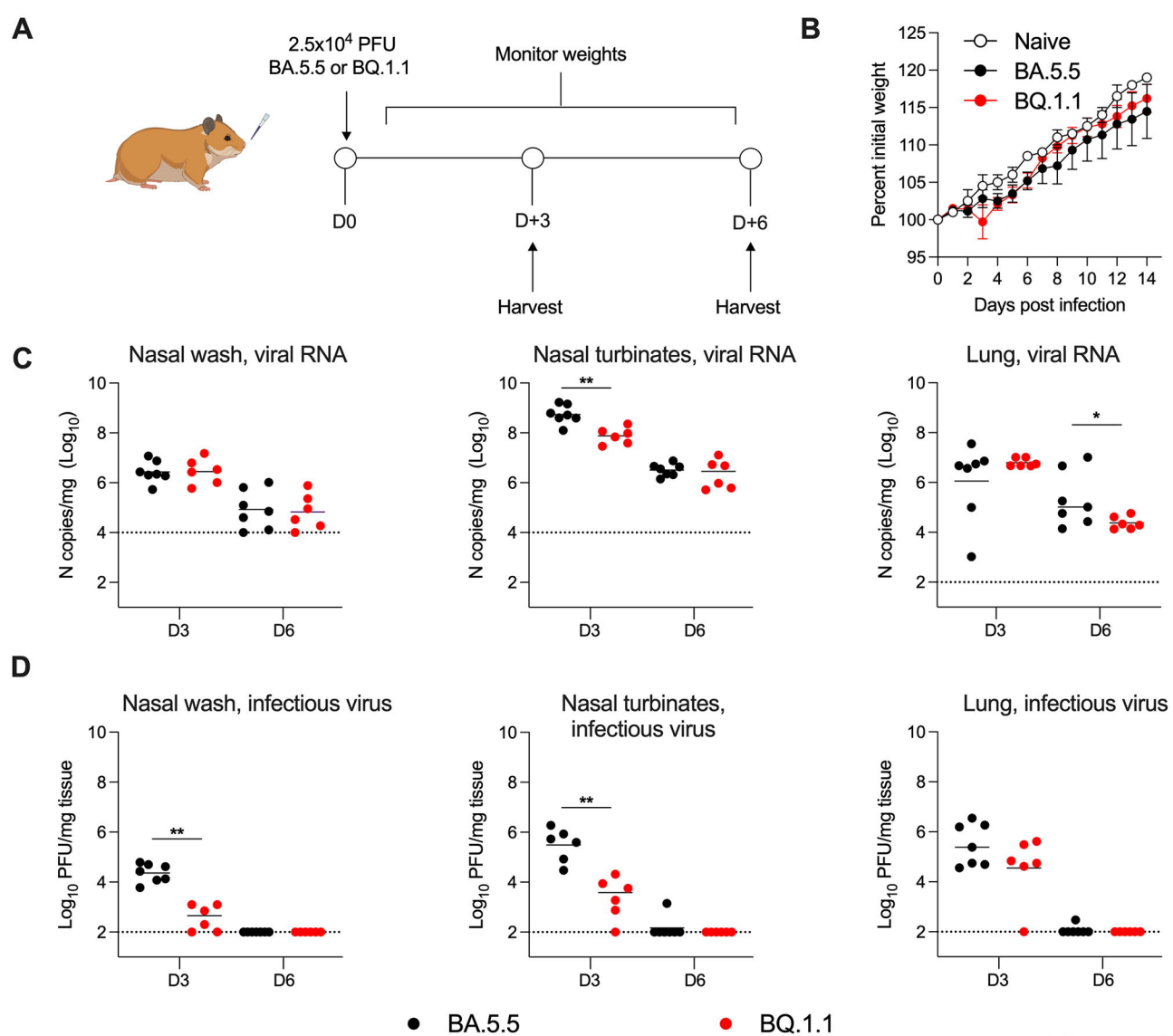


Figure 3

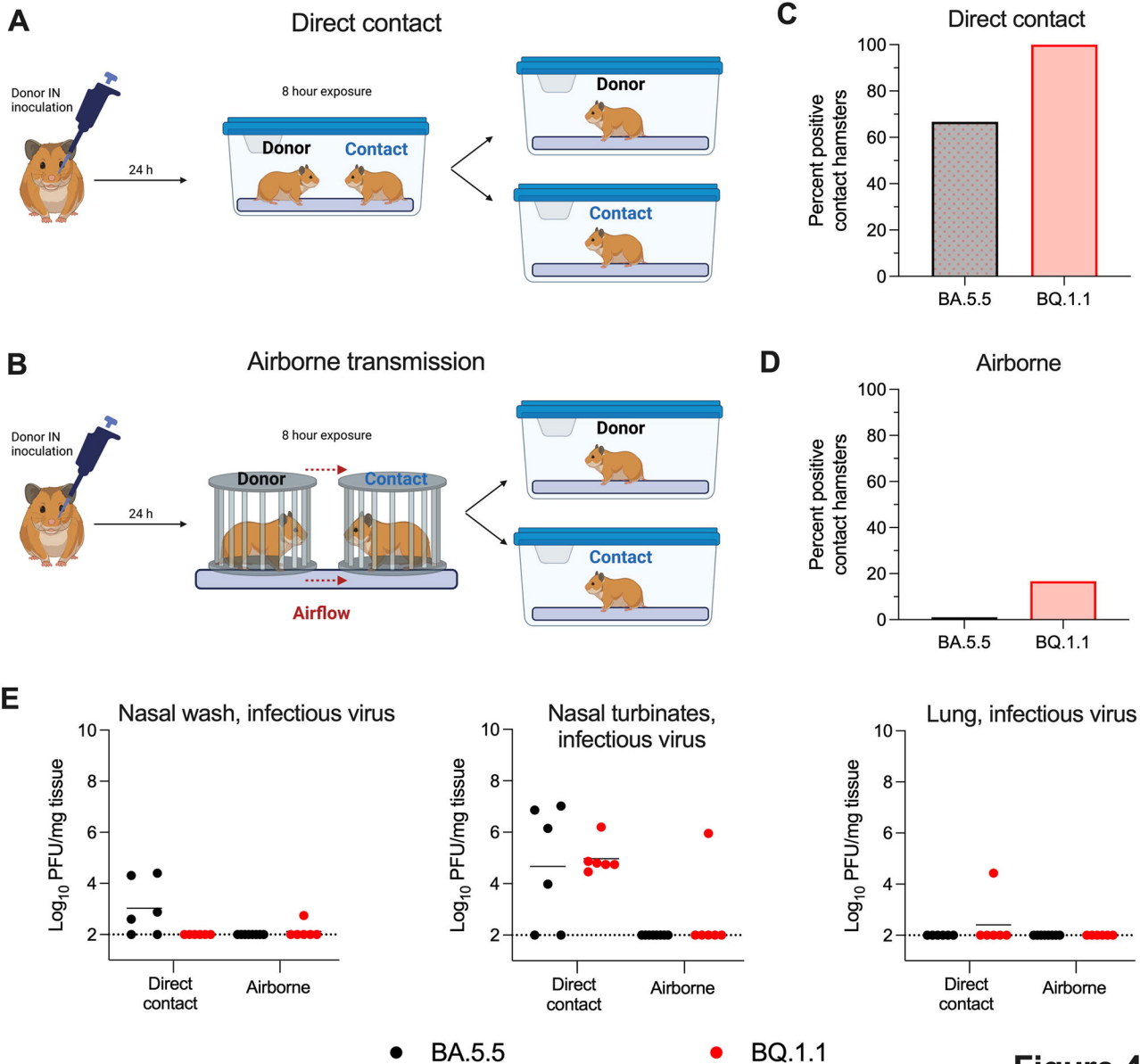
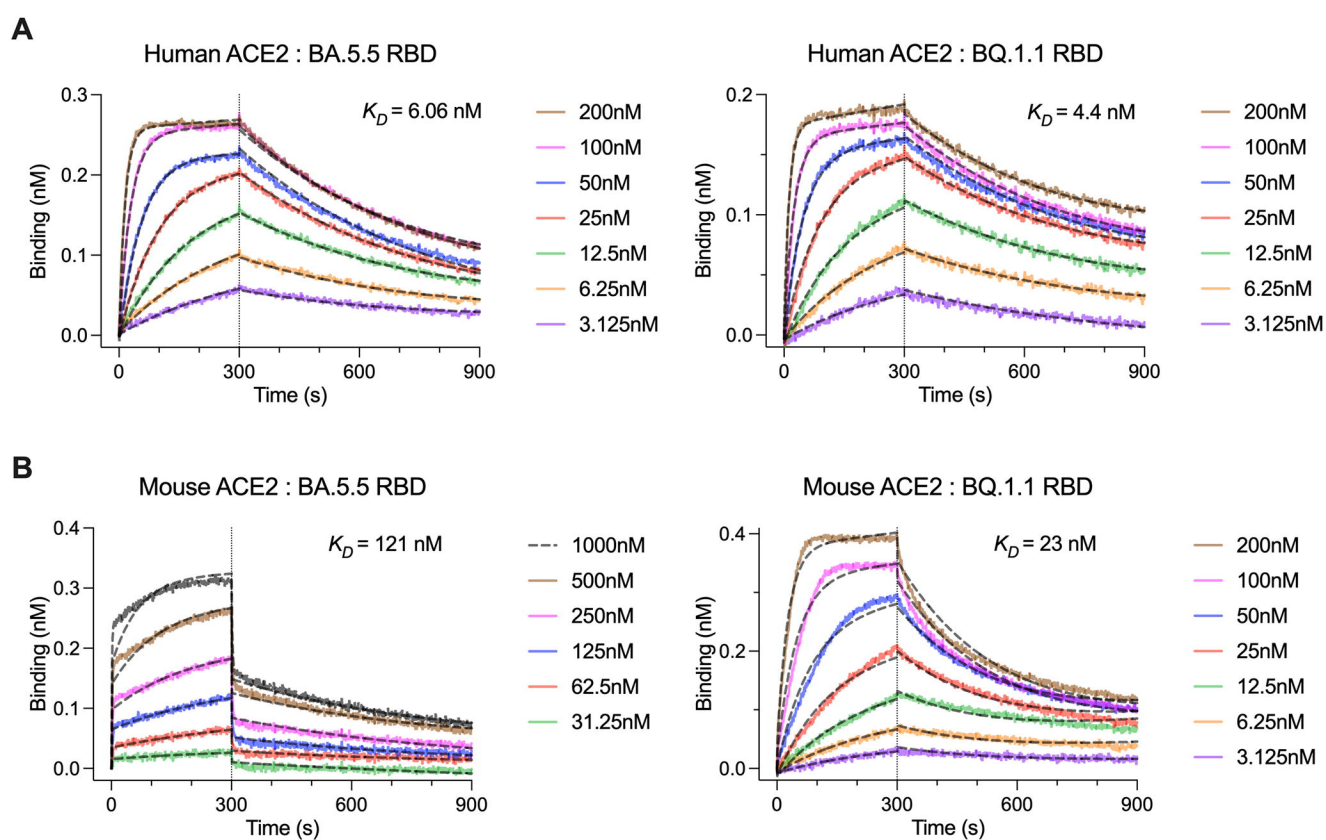


Figure 4



C

ACE2	RBD	k_a ($\times 10^4/\text{Ms}$)	k_d ($\times 10^{-3}/\text{s}$)	K_D (nM)
Human	BA.5.5	32.8 ± 0.05	1.99 ± 0.02	6.0 ± 0.06
Human	BQ.1.1	38.9 ± 1.0	1.72 ± 0.2	4.4 ± 0.75
Mouse	BA.5.5	1.44 ± 0.09	1.75 ± 0.09	121 ± 1.5
Mouse	BQ.1.1	17.0 ± 0.4	3.97 ± 0.2	23 ± 0.7

Figure 5