

1 **Distinct Neutralizing Antibody Escape of SARS-CoV-2 Omicron Subvariants BQ.1,**  
2 **BQ.1.1, BA.4.6, BF.7 and BA.2.75.2**

3  
4 Panke Qu<sup>1,2,#</sup>, John P. Evans<sup>1,2,3,#</sup>, Julia Faraone<sup>1,2,3</sup>, Yi-Min Zheng<sup>1,2</sup>, Claire Carlin<sup>4</sup>,  
5 Mirela Anghelina<sup>5</sup>, Patrick Stevens<sup>5</sup>, Soledad Fernandez<sup>5</sup>, Daniel Jones<sup>6</sup>, Gerard Lozanski<sup>6</sup>,  
6 Ashish Panchal<sup>7</sup>, Linda J. Saif<sup>8,9,10</sup>, Eugene M. Oltz<sup>11</sup>, Kai Xu<sup>1,2</sup>,  
7 Richard J. Gumina<sup>4,12,13</sup>, and Shan-Lu Liu<sup>1,2,10,11\*</sup>

8  
9 <sup>1</sup>Center for Retrovirus Research, The Ohio State University, Columbus, OH 43210, USA

10 <sup>2</sup>Department of Veterinary Biosciences, The Ohio State University, Columbus, OH 43210, USA

11 <sup>3</sup>Molecular, Cellular, and Developmental Biology Program,  
12 The Ohio State University, Columbus, OH 43210, USA

13 <sup>4</sup>Department of Internal Medicine, Division of Cardiovascular Medicine,  
14 The Ohio State University, Columbus, OH 43210, USA

15 <sup>5</sup>Department of Biomedical Informatics, College of Medicine, The Ohio State University,  
16 Columbus, OH, 43210, USA

17 <sup>6</sup>Department of Pathology, The Ohio State University Wexner Medical Center,  
18 Columbus, OH, USA.

19 <sup>7</sup>Department of Emergency Medicine, The Ohio State University Wexner Medical Center,  
20 Columbus, USA.

21 <sup>8</sup>Center for Food Animal Health, Animal Sciences Department, OARDC, College of Food,  
22 Agricultural and Environmental Sciences, The Ohio State University, Wooster, OH 44691, USA

23 <sup>9</sup>Veterinary Preventive Medicine Department, College of Veterinary Medicine,  
24 The Ohio State University, Wooster, OH 44691, USA

25 <sup>10</sup>Viruses and Emerging Pathogens Program, Infectious Diseases Institute,  
26 The Ohio State University, Columbus, OH 43210, USA

27 <sup>11</sup>Department of Microbial Infection and Immunity, The Ohio State University,  
28 Columbus, OH 43210, USA

29 <sup>12</sup>Dorothy M. Davis Heart and Lung Research Institute, The Ohio State University Wexner  
30 Medical Center, Columbus, OH 43210, USA

31 <sup>13</sup>Department of Physiology and Cell Biology, College of Medicine, The Ohio State University  
32 Wexner Medical Center, Columbus, OH  
33 43210, USA

34  
35  
36 #Authors contributed equally to this work

37 \*Corresponding Author: [liu.6244@osu.edu](mailto:liu.6244@osu.edu)

38

39 **Abstract**

40 Continued evolution of SARS-CoV-2 has led to the emergence of several new Omicron  
41 subvariants, including BQ.1, BQ.1.1, BA.4.6, BF.7 and BA.2.75.2. Here we examine the  
42 neutralization resistance of these subvariants, as well as their ancestral BA.4/5, BA.2.75 and  
43 D614G variants, against sera from 3-dose vaccinated health care workers, hospitalized BA.1-  
44 wave patients, and BA.5-wave patients. We found enhanced neutralization resistance in all new  
45 subvariants, especially the BQ.1 and BQ.1.1 subvariants driven by a key N460K mutation, and to  
46 a lesser extent, R346T and K444T mutations, as well as the BA.2.75.2 subvariant driven largely  
47 by its F486S mutation. The BQ.1 and BQ.1.1 subvariants also exhibited enhanced fusogenicity  
48 and S processing dictated by the N460K mutation. Interestingly, the BA.2.75.2 subvariant saw an  
49 enhancement by the F486S mutation and a reduction by the D1199N mutation to its fusogenicity  
50 and S processing, resulting in minimal overall change. Molecular modelling revealed the  
51 mechanisms of receptor-binding and non-receptor binding monoclonal antibody-mediated  
52 immune evasion by R346T, K444T, F486S and D1199N mutations. Altogether, these findings  
53 shed light on the concerning evolution of newly emerging SARS-CoV-2 Omicron subvariants.

## 54 Introduction

55 Since its emergence in late 2021, the Omicron variant of severe acute respiratory  
56 syndrome coronavirus 2 (SARS-CoV-2) has produced numerous subvariants that continue to  
57 erode vaccine- and infection-induced immunity, and alter virus biology (Evans et al., 2022;  
58 Kurhade et al., 2022; Qu et al., 2022a; Qu et al., 2022b; Xia et al., 2022b; Yamasoba et al., 2022;  
59 Yu et al., 2022). The initial BA.1 Omicron subvariant spurned a large wave of coronavirus disease  
60 2019 (COVID-19) cases and exhibited strong immune escape from 2-mRNA vaccine dose-  
61 induced immunity that was recovered by booster mRNA vaccine administration (Abu-Raddad et  
62 al., 2022; Cao et al., 2022b; Cerutti et al., 2022; Evans et al., 2022; Gruell et al., 2022; Liu et al.,  
63 2022; Xia et al., 2022a; Zou et al., 2022). In addition, the BA.1 subvariant exhibited reduced cell-  
64 cell fusogenicity, impaired replication in lower airway epithelial cells, as well as altered entry route  
65 preference (Barut et al., 2022; Cui et al., 2022; Meng et al., 2022; Shuai et al., 2022; Suzuki et  
66 al., 2022; Wang et al., 2022a). These features correlated with a reduced replication capacity of  
67 Omicron in lung tissues, enhanced nasopharyngeal tropism, and decreased pathogenicity *in vivo*  
68 (Barut et al., 2022; McMahan et al., 2022; Shuai et al., 2022; Su et al., 2022b; Suzuki et al., 2022).  
69 Importantly, these characteristics were largely maintained by subsequent Omicron subvariants.

70 The BA.2 subvariant overtook BA.1 due to its slightly enhanced transmissibility and  
71 immune evasion, with an ability to reinfect individuals who were previously infected with BA.1  
72 (Iketani et al., 2022; Lyngse et al., 2022; Stegger et al., 2022). From BA.2, several subvariants  
73 emerged in quick succession, often with concurrent circulations; these included the BA.4 and  
74 BA.5 subvariants (bearing identical S proteins, referred to as BA.4/5 hereafter) that next rose to  
75 dominance and exhibited further immune escape (Cao et al., 2022c; Hachmann et al., 2022; Khan  
76 et al., 2022; Kimura et al., 2022; Qu et al., 2022b; Tuekprakhon et al., 2022; Wang et al., 2022b).  
77 In addition, BA.2 gave rise to the BA.2.75 subvariant, which is currently increasing in proportion  
78 of COVID-19 cases (Centers for Disease Control and Prevention, 2022), but does not exhibit as  
79 substantial immune escape compared to BA.4/5 (Cao et al., 2022a; Qu et al., 2022a; Saito et al.;

80 Wang et al., 2022c). The BA.4/5 and BA.2.75 subvariants have driven further diversification of  
81 the circulating SARS-CoV-2, with the emergence of several additional subvariants including the  
82 BA.4.6, BF.7, BQ.1, and BQ.1.1 (derived from BA.4/5), as well as BA.2.75.2 (derived from  
83 BA.2.75). These new subvariants are currently increasing in frequency (Centers for Disease  
84 Control and Prevention, 2022; Iacobucci, 2022) and may be the next major dominant Omicron  
85 subvariant.

86 The extent of immune evasion and functional alterations to the spike protein (S) in these  
87 emerging Omicron subvariants remains unclear. To address this, we examine the resistance of  
88 the BA.4.6, BF.7, BQ.1, BQ.1.1, and BA.2.75.2 subvariants to neutralization by serum from  
89 recipients of 3 mRNA vaccine doses, as well as COVID-19 patients infected with the BA.1 or  
90 BA.4/5 variants. We observe strong neutralization resistance in the BQ.1 and BQ.1.1 subvariants  
91 driven largely by their key N460K mutation, as well as in the BA.2.75.2 subvariant driven largely  
92 by its signature F486S mutation. We further examine the fusogenicity and processing of the  
93 subvariant S proteins and observe enhanced fusogenicity and S processing in the BA.4/5-derived  
94 subvariants driven largely by the N460K mutation, but comparable fusogenicity and S processing  
95 in BA.2.75-derived BA.2.75.2 subvariant modulated by its F485S and D1199N mutations. Finally,  
96 structural modeling showed that the F486S mutation reduced binding affinity for both the ACE2  
97 receptor and class I and II antibodies, while the R346T and K444T mutations are likely responsible  
98 for evasion of class III antibody recognition.

99

## 100 **Results**

### 101 *BQ.1, BQ1.1, and BA.2.75.2 exhibit potent neutralization resistance*

102 To examine the neutralization resistance of emerging Omicron subvariants, we utilized  
103 our previously reported pseudotyped lentivirus neutralization assay (Zeng et al., 2020). Lentivirus  
104 pseudotyped with S from each of the critical Omicron subvariants (**Fig. 1A**), as well as from the  
105 prototype D614G variant were produced. All Omicron subvariant-pseudotyped viruses exhibited

106 modestly enhanced infectivity in HEK293T-ACE2 cells over the D614G variant, with the exception  
107 of BA.2.75.2 (**Fig. 1B**). Additionally, all Omicron subvariants exhibited comparably poor infectivity  
108 in lung-derived CaLu-3 cells (**Fig. 1C**) compared to D614G, consistent with prior Omicron  
109 subvariants, and the weak lung tropism observed for the Omicron variant (Barut et al., 2022; Meng  
110 et al., 2022; Shuai et al., 2022; Wang et al., 2022a).

111 We next examined the resistance of these emerging Omicron subvariants to sera from  
112 health care workers (HCWs) collected 2-13 weeks after vaccination with a homologous booster  
113 dose of monovalent Moderna mRNA-1273 (n =3) or Pfizer/BioNTech BNT162b2 vaccine (n =12).  
114 We observed potent neutralization resistance among all Omicron subvariants compared to  
115 ancestral D614G (**Fig. 1D** and **Fig. S1A**). Specifically, compared to D614G, the BA.4.6, BF.7,  
116 BQ.1, and BQ.1.1 subvariants exhibited a 10.6-fold ( $p < 0.0001$ ), 11.0-fold ( $p < 0.0001$ ), 18.7-fold  
117 ( $p < 0.0001$ ), and 22.9-fold ( $p < 0.0001$ ) lower neutralization sensitivity, respectively, while BA.4/5  
118 exhibited an 8.7-fold ( $p < 0.0001$ ) lower neutralization sensitivity than D614G (**Fig. 1D**). Similarly,  
119 compared to D614G, the BA.2.75.2 subvariant exhibited a 48.4-fold ( $p < 0.0001$ ) lower  
120 neutralization sensitivity while BA.2.75 exhibited a 4.4-fold ( $p < 0.0001$ ) lower neutralization  
121 sensitivity than D614G (**Fig. 1D**). These data indicate further neutralization escape in emerging  
122 Omicron subvariants, with BA.Q.1, BA.Q.1.1, and BA.2.75.2, especially the latter, showing the  
123 most substantial neutralization resistance.

124 We also examined the resistance of Omicron subvariants to neutralization by sera from  
125 hospitalized COVID-19 patients (n =15) infected during the BA.1 wave of the pandemic. Despite  
126 exposure to an Omicron antigen, these patients displayed a remarkably similar neutralization  
127 resistance pattern to the boosted HCWs. Specifically, compared to D614G, the BA.4.6, BF.7,  
128 BQ.1, and BQ.1.1 subvariants exhibited a 3.5-fold ( $p > 0.05$ ), 3.2-fold ( $p < 0.05$ ), 5.3-fold ( $p < 0.01$ ),  
129 and 5.0-fold ( $p < 0.05$ ) lower neutralization sensitivity, respectively, while BA.4/5 exhibited an 2.7-  
130 fold ( $p < 0.05$ ) lower neutralization sensitivity than D614G (**Fig. 1E**). Similarly, compared to D614G,  
131 the BA.2.75.2 subvariant exhibited a 6.3-fold ( $p < 0.01$ ) lower neutralization sensitivity, while

132 BA.2.75 exhibited a 3.9-fold ( $p < 0.01$ ) lower neutralization resistance (**Fig. 1E**). As would be  
133 expected, BA.1 patient sera neutralized BA.2 with a higher efficiency compared to these BA.2-  
134 derived subvariants (**Fig. 1E** and **Fig. S1B**).

135 To determine the breadth of immunity from individuals infected with more recent Omicron  
136 subvariants, we next examined sera from Columbus, Ohio first responders and household  
137 contacts testing positive for COVID-19 during the BA.4/5 wave of the pandemic, with 11/20  
138 subjects having the infecting variant confirmed as BA.4/5 or a derivative by sequencing. Notably,  
139 this cohort exhibited the weakest neutralizing antibody titers of the three groups examined,  
140 consistent with milder COVID-19 cases correlating with weaker neutralizing antibody responses  
141 (Zeng et al., 2020). Critically, this cohort again displayed a similar pattern of neutralization  
142 resistance. Specifically, compared to D614G, the BA.4.6, BF.7, BQ.1, and BQ.1.1 subvariants  
143 exhibited a 3.9-fold ( $p < 0.0001$ ), 4.4-fold ( $p < 0.0001$ ), 10.4-fold ( $p < 0.0001$ ), and 10.7-fold ( $p <$   
144  $0.0001$ ) lower neutralization sensitivity, respectively, while BA.4/5 exhibited a 3.7-fold ( $p < 0.0001$ )  
145 lower neutralization sensitivity than D614G (**Fig. 1F**). Additionally, compared to D614G, the  
146 BA.2.75.2 subvariant exhibited a 10.6-fold ( $p < 0.0001$ ) lower neutralization sensitivity whereas  
147 BA.2.75 showed a 3.4-fold ( $p < 0.0001$ ) decreased neutralization sensitivity (**Fig. 1F**). Interestingly,  
148 BA.2 exhibited less resistance to sera of BA.4/5-wave infection than BA.4/5, with 2.1-fold reduced  
149 neutralization sensitivity compared to D614G ( $p < 0.01$ ) (**Fig. 1F** and **Fig. S1C**). Together, these  
150 results showed that BQ.1, BQ.1.1, and BA.2.75.2 are strongly resistant to neutralization by sera  
151 from subjects infected with the recently dominant BA.4/5 variant and suggested that BA.4/5  
152 infection does not offer a broader protection against newly emerging subvariants.

153

154 *R346T, K444T, N460K, and F486S represent key neutralization escape mutations*

155 To determine the features governing neutralization resistance, we produced single and  
156 double mutant S constructs to probe the contributions of individual amino acid substitutions alone  
157 or in combination to neutralization resistance. The panel of mutants exhibited largely similar

158 infectivity compared to their parental BA.4/5 or BA.2.75 variants in HEK293T-ACE2 cells (**Fig.**  
159 **2A-B**) and Calu-3 cells (**Fig. S2A-B**), although F486S-containing, BA.2.75-derived mutants  
160 appear to have modestly reduced titers in Calu-3 cells (**Fig. 2A-B**, and **Fig. S2A-B**). For HCWs  
161 who received 3 mRNA vaccine doses introduction of the N460K mutation reduced neutralization  
162 sensitivity by an additional 2.6-fold ( $p < 0.0001$ ) compared to the parental BA.4/5 subvariant, with  
163 a similar 2.8-fold ( $p < 0.0001$ ) reduction for the R346T/N460K double mutant (**Fig. 2C**). Consistent  
164 with this finding, the N460K-bearing subvariants BQ.1 and BQ.1.1 showed the strongest  
165 neutralization resistance, with a 2.1-fold ( $p < 0.01$ ) and 2.6-fold ( $p < 0.01$ ) reduction in  
166 neutralization sensitivity compared to BA.4/5 (**Fig. 2C**, and **Fig. S3A**). Though not significant, the  
167 other key individual mutations, R346T and K444T, were associated with a milder 20.7% ( $p$   
168  $=0.0782$ ) and 29.3% ( $p = 0.1886$ ) reduction in neutralization sensitivity, respectively (**Fig. 2C**).  
169 Similarly, the N658S mutation did not appear to be strongly associated with neutralization  
170 resistance (**Fig. S3A**), with a 23.7 % ( $p > 0.05$ ) reduction compared to the parental BA.4/5 (**Fig.**  
171 **2C**). Altogether, these results showed that the N460K mutation, and to a lesser extent R346T,  
172 K444T and N658S, are critical for the enhanced resistance to mRNA booster-vaccinated sera in  
173 the BQ.1 and BQ.1.1 subvariants.

174 For BA.2.75-derived mutants, the introduction of the F486S, R346T/F486S, or  
175 F486S/D1199N mutations reduced neutralization sensitivity by 5.2-fold ( $p < 0.0001$ ), 12.8-fold ( $p$   
176  $< 0.0001$ ), 4.8-fold ( $p < 0.0001$ ), respectively, compared to the 10.9-fold ( $p < 0.0001$ ) reduction  
177 seen for the parental BA.2.75.2 subvariant (**Fig. 2D**). However, the introduction of the R346T,  
178 D1199N, or R346T/D1199N resulted in only a 26.3% ( $p = 0.1074$ ), 22.1% ( $p > 0.05$ ), and 31.4%  
179 ( $p < 0.05$ ) reduction in neutralization sensitivity, respectively (**Fig. 2** and **Fig. S3B**). These results  
180 indicated that the F486S mutation is the major driver of enhanced resistance to mRNA booster  
181 vaccinee sera in BA.2.75.2.

182 The pattern of neutralization resistance was similar for the BA.1-wave hospitalized  
183 COVID-19 patient sera. Introduction of the N460K and R346T/N460K mutations into BA.4.5



184 strongly reduced neutralization sensitivity by 2.2-fold ( $p < 0.01$ ) and 4.9-fold ( $p < 0.05$ ),  
185 respectively (**Fig. 2E** and **Fig.S3C**). Further, introduction of the R346T/F486S mutations into  
186 BA.2.75 reduced neutralization sensitivity by 4.0-fold ( $p < 0.01$ ) (**Fig. 2F** and **Fig.S3D**).  
187 Surprisingly, the F486S mutation alone only resulted in a 31.7% ( $p = 0.3698$ ) reduction in  
188 neutralization sensitivity (**Fig. 2F**).

189 Consistent with the patterns of HCWs and BA.1-wave patients, neutralization resistance  
190 from the BA.4/5-infected COVID-19 patient sera was again largely driven by N460K, F486S, and  
191 R346T. Introduction of the N460K and R346T/N460K mutations into BA.4.5 reduced  
192 neutralization sensitivity by 1.7-fold ( $p < 0.01$ ) and 1.8-fold ( $p < 0.01$ ), respectively (**Fig. 2G** and  
193 **Fig. S3E**). Similar to the BA.1-wave patients, introduction of the R346T/F486S mutations into  
194 BA.2.75 reduced neutralization sensitivity by 2.8-fold ( $p < 0.01$ ) (**Fig. 2H** and **Fig.S3F**). Thus, the  
195 N460K, F486S, and to a lesser extent R346T, mutations drive neutralization resistance to mRNA  
196 vaccinated and boosted, BA.1-infected, and BA.4/5-infected patient sera.

197

#### 198 *BQ.1, and BQ.1.1 exhibit enhanced fusogenicity and S processing*

199 To examine how alterations to the S protein in these new Omicron subvariants impact  
200 function, we examined the ability of the S from these subvariants to mediate cell-cell fusion.  
201 Consistent with prior Omicron subvariants (Qu et al., 2022a; Wang et al., 2022a; Zeng et al.,  
202 2021), all new subvariants exhibited diminished syncytia formation compared to ancestral D614G  
203 (**Fig. 3A-B**), despite comparable levels of cell surface expression of each S construct (**Fig. 3C-**  
204 **D**). Notably, the new Omicron subvariants BA.4.6, BQ.1, and BQ.1.1 exhibited enhanced  
205 fusogenicity compared to their parental variant BA.4/5, and similarly, BA.2.75.2 showed an  
206 increased fusogenicity relative to BA.2.75 (**Fig. 3C-D**).

207 Given the key role of cellular furin in processing viral S protein into S1 and S2 subunits for  
208 SARS-CoV-2 entry (Mykytyn et al., 2021; Peacock et al., 2021), we next examined the proteolytic  
209 processing of S in lentivirus producer cells. We saw an enhanced S2/S ratio compared to



210 ancestral D614G in BA.4.6, BF.7, BQ.1, and BQ.1.1 with a 1.8-fold, 2.2-fold, 3.6-fold, and 2.8-fold  
211 higher S2/S ratio, respectively, compared to the 1.6-fold higher S2/S ratio in BA.4/5 (**Fig. 3E**).  
212 However, compared to BA.4/5, the S1/S ratio of these BA.4/5-derived subvariants was  
213 comparable (BF.7 and BQ.1.1) or even reduced (BA.4.6 and BQ.1) (**Fig. 3E**), suggesting possible  
214 shedding of S1 into the culture media. BA.2.75 and BA.2.75.2 exhibited minimal difference in  
215 S2/S ratio compared to D614G (**Fig. 3E**), but once again, BA.2.75.2 exhibited a decreased S1/S  
216 ratio compared to the parental BA.2.75 (**Fig. 3E**), suggesting possibly increased S1 shedding for  
217 the BA.2.75.2 subvariant.

218

219 *The N460K and F486 mutations enhance fusogenicity while D1199N diminishes fusogenicity*

220 To identify the determinants of enhanced fusogenicity in new Omicron subvariants, we  
221 examined S-mediated syncytia formation for single and double S mutants. We observed that  
222 introduction of the N460K mutation in BA.4/5 enhanced mean syncytia size by 1.7-fold ( $p < 0.0001$ )  
223 (**Fig. 4A-B**). Notably all N460K-containing mutants and variants exhibited enhanced syncytia size  
224 over BA.4/5 (**Fig. 4A-B**). Further, while the single mutants had no effect, introduction of the  
225 R346T/K444T and R346T/N658S mutations slightly enhanced mean syncytia size by 1.4-fold ( $p$   
226  $< 0.001$ ) and 1.3-fold ( $p < 0.05$ ) over BA.4/5, potentially indicating a synergistic effect likely  
227 contributed by R346T (**Fig. 4A-B**). Additionally, while the BA.2.75.2 variant exhibited only  
228 modestly enhanced syncytia formation over BA.2.75, i.e., 33.2% ( $p < 0.0001$ ), the introduction of  
229 the F486S or R346T/F486S mutations into BA.2.75 enhanced mean syncytia size by 33.8% ( $p <$   
230  $0.0001$ ) and 58.1% ( $p < 0.0001$ ), respectively, suggesting a critical role of F486S in promoting  
231 fusion (**Fig. 4C-D**). Notably, the introduction of the D1199N mutation into BA.2.75 reduced the  
232 mean syncytia size by 17.2% ( $p < 0.05$ ), revealing opposing effects of the F486S and D1199N  
233 mutations on the fusogenicity of BA.2.75.2 (**Fig. 4C-D**). These differences in S-mediated cell-cell  
234 fusion occurred despite comparable or even somewhat reduced (especially for BA.2.75.2 mutants)  
235 levels of surface expression among these Omicron subvariant S constructs (**Fig. 4E-F, and Fig.**

236 **S4A-B).**

237 We next sought to determine the impact of the subvariant determining mutations on S  
238 processing. For the BA.4/5-derived subvariants, introduction of the N460K, N658S, R346T/K444T,  
239 and R346T/N460K mutations exhibited 2.2-fold, 1.7-fold, 1.5-fold, and 1.7-fold higher S2/S ratios,  
240 largely consistent with those mutants exhibiting enhanced fusogenicity and S processing,  
241 especially for the BQ.1 and BQ.1.1 subvariants (**Fig. 4G**). Remarkably, the S1/S ratio for almost  
242 all mutants examined, except the R346T mutation, was decreased compared to the BA.4/5  
243 subvariant (**Fig. 4G**), suggesting an enhanced S1 shedding in these BA.4/5 derived mutants,  
244 which is consistent with increased fusion. For BA.2.75-derived mutants, the impacts on S2/S and  
245 S1/S ratios were fairly modest, with most exhibiting reductions relative to the parental BA.2.75,  
246 consistent with the more modest impact on cell-cell fusion of these variants (**Fig. 4H**). Notably,  
247 the introduction of the F486S mutation increased the S2/S ratio by 1.2-fold but had no effect on  
248 S1/S ratio compared to BA.2.75 (**Fig. 4H**).

249

250 *Structural modeling reveals mechanism of mutation-mediated antibody evasion and alteration in*  
251 *receptor utilization*

252 To further understand the functional impact of the mutations in these Omicron subvariants, we  
253 performed homology modeling-based structural analyses. R346 and K444 are located outside of  
254 SARS-CoV2 receptor binding motif (RBM) and are within the epitope of class III neutralizing  
255 antibodies. Structural analysis indicated an interference of antibody recognition introduced by  
256 R346S (**Fig. 5A**) and K444T (**Fig. 5B**), where hydrogen bonds and a salt-bridge can be abolished.  
257 In contrast, F486 is located within the RBM and is a key residue for binding to both ACE2 receptor  
258 and neutralizing antibodies; F486 interacts hydrophobically with M82 and Y83 on ACE2 (**Fig. 5C**),  
259 whereas the F486S mutation negatively impacts the interaction with ACE2, as well as binding by  
260 some monoclonal antibodies in class I and III categories. Further structural analysis showed that  
261 residue D1199 is located in the heptad repeat 2 (HR2) domain on a solvent-accessible surface

262 close to the transmembrane domain or membrane (**Fig. 5D**). Electrostatic surface potential (**Fig.**  
263 **5C inset**) of this region reveals a strong negative surface charge, which repulses the negatively  
264 charged membrane and could help keep the spike in an up-right position. However, the D1199N  
265 mutation in the S2 subunit would reduce the electrostatic repulsion, resulting in a more tilted spike  
266 and rendering its less efficient processing and receptor utilization.

267

## 268 **Discussion**

269 We examined the neutralization resistance, fusogenicity, and S processing of the  
270 emerging BA.4.6, BF.7, BQ.1, BQ.1.1, and BA.2.75.2 Omicron subvariants, as well as their  
271 lineage-defining mutations. All of these subvariants exhibit some degree of enhanced  
272 neutralization resistance over their parental BA.4/5 or BA.2.75.2 subvariants, with BQ.1, BQ.1.1,  
273 and BA.2.75.2 exhibiting the strongest resistance. Notably, this pattern is consistent for sera  
274 collected from HCWs following a homologous mRNA booster vaccination, from BA.1-wave  
275 hospitalized COVID-19 patients, as well as from BA.4/5-wave SARS-CoV-2 COVID-19 positive  
276 first responders and household contacts. Critically, the neutralization resistance of BQ.1 and  
277 BQ.1.1 appears to be driven largely by their N460K mutation, while the neutralization resistance  
278 of BA.2.75.2 variant is largely determined by the F486S mutation. Additionally, we provide  
279 evidence that the N460K and F486S mutations dictate the enhanced fusogenicity and S  
280 processing in their respective subvariants, with the D1199N mutation negatively modulating the  
281 fusogenicity of the BA.2.75.2 subvariant. Structural modeling and additional analyses revealed  
282 crucial roles of residues R436, K444 and F486 in antibody recognition and the potential  
283 mechanism of immune evasion through R436S, K444T and F486S mutations present in Omicron  
284 subvariants. Intriguingly, our structural analyses also suggest that D1199N mutation located in  
285 the HR2 region of S2 could alter the spike position in either an upright or a tilted status by reducing  
286 repulsion to the cellular membrane. A tilted spike with less efficient processing and receptor  
287 utilization could explain the reduced fusogenicity observed in D1199N-bearing mutations.

288           The neutralization resistance of the BQ.1, BQ.1.1, and BA.2.75.2 variants has concerning  
289 implications for the persistence of vaccine- and infection-induced immunity. The strong resistance  
290 of these variants to neutralization by patient sera, regardless of the immunogen — mRNA  
291 vaccination, BA.1 infection, or BA.4/5 infection — is particularly striking. This finding may indicate  
292 selection for immune evasion of even broadly neutralizing antibodies induced by multiple  
293 vaccinations and SARS-CoV-2 infections, as is now common in the population. In particular, the  
294 strong evasion of BA.4/5 infection-induced sera is concerning, as the recently recommended  
295 bivalent mRNA vaccine boosters contain BA.4/5 S along with the prototype. This possibility,  
296 together with the emergence of more diverse SARS-CoV-2 variants necessitates the development  
297 of more broadly active, even pan-coronavirus, COVID-19 vaccines (Chen et al., 2022; Su et al.,  
298 2022a). As circulating SARS-CoV-2 diversifies, the ability to vaccinate against dominant  
299 circulating variants may be even more compromised. Hence, examination of the efficacy of  
300 bivalent mRNA vaccines against emerging variants is critical to control and end the global  
301 pandemic.

302           In addition to changing the neutralization resistance, these new Omicron subvariants also  
303 affect the fusogenicity of the S proteins. Similar to our previous finding that BA.4/5 and BA.2.75  
304 exhibit increased fusion capability (Qu et al., 2022a), here we also observed increased cell-cell  
305 fusion in several new Omicron subvariants compared to their respective parental subvariants,  
306 BA.4/5 or BA.2.75. These data may together indicate a continuing shift towards more efficient  
307 transmembrane protease, serine 2 (TMPRSS2) utilization to allow for plasma membrane-  
308 mediated viral fusion and entry pathway. Consistent with this notion, we observed an increased  
309 furin-cleavage efficiency in several S proteins, especially BQ.1, and BQ.1.1 subvariants, which  
310 could improve TMPRSS2 utilization (Mykytyn et al., 2021). This is concerning, because previously  
311 it has been shown that the shift in entry pathway from TMPRSS2-mediated plasma membrane  
312 fusion used by the prototype SARS-CoV-2 towards cathepsin B/L-mediated endosomal entry  
313 observed in the Omicron clade is associated with reduced lung tropism, increased

314 nasopharyngeal tropism, and reduced pathogenicity of the Omicron variant (Barut et al., 2022;  
315 McMahan et al., 2022; Meng et al., 2022; Mykytyn et al., 2021; Shuai et al., 2022; Suzuki et al.,  
316 2022; Wang et al., 2022a). That said, here we show new Omicron subvariants continue to exhibit  
317 weak infection efficiency in lung-derived CaLu-3 cells. Examination of tissue tropism and  
318 pathogenicity *in vivo* for these and future emerging Omicron subvariants is critical to tailor  
319 appropriate public health responses.

320 In this work, we observed altered S processing and fusogenicity in Omicron subvariants.  
321 Notably, we found that many mutations examined, including N460K, N658S, and F486S, enhance  
322 S processing, as evidenced by increased S2/S ratios in viral producer cells. However, they fail to  
323 enhance, and in most cases reduce, the S1/S ratios in virus producer cells. This may indicate that  
324 the mutations likely destabilize the S1-S2 interaction and trimer conformation, consistent with  
325 increased cell-cell fusion and likely resulting enhanced S1 shedding. Intriguingly, we found that  
326 the D1199N mutation reduced S-mediated fusion and S processing, which could suggest that this  
327 mutation emerged to compensate for alterations in S functionality introduced by the F486S  
328 mutation. Further structural analyses on the impact of these key residues on S trimer conformation  
329 are needed to determine if these immune escape mutants are negatively impacting S functionality  
330 — potentially limiting the fitness of further immune escape mutations to be introduced.

331 The perpetual emergence of SARS-CoV-2 variants with enhanced immune escape  
332 continues to threaten public health. Monitoring the immune escape of emerging variants will be  
333 critical to improving mRNA vaccine reformulation, assessing new broader coronavirus vaccine  
334 candidates, as well as directing ongoing public health measures. Further, emerging variants must  
335 be monitored closely for any indication of selective pressure in enhancing lung tropism and  
336 potentially pathogenicity to ensure that any highly transmissible and more pathogenic variants are  
337 better and more quickly contained.

338

339 **Acknowledgements**

340 We thank the NIH AIDS Reagent Program and BEI Resources for providing important reagents  
341 for this work. We also thank the Clinical Research Center/Center for Clinical Research  
342 Management of The Ohio State University Wexner Medical Center and The Ohio State University  
343 College of Medicine in Columbus, Ohio, specifically Francesca Madiari, Dina McGowan, Breona  
344 Edwards, Evan Long, and Trina Wemlinger, for logistics, collection and processing of samples. In  
345 addition, we thank Sarah Karow, Madison So, Preston So, Daniela Farkas, and Finny Johns in  
346 the clinical trials team of The Ohio State University for sample collection and other supports. This  
347 work was supported by a fund provided by an anonymous private donor to OSU. S.-L.L., F.S.,  
348 D. J., G.L., A.P., R.J.G., L.J.S. and E.M.O. were supported by the National Cancer  
349 Institute of the NIH under award no. U54CA260582. The content is solely the  
350 responsibility of the authors and does not necessarily represent the official views of the  
351 National Institutes of Health. J.P.E. was supported by Glenn Barber Fellowship from the  
352 Ohio State University College of Veterinary Medicine. R.J.G. was additionally supported  
353 by the Robert J. Anthony Fund for Cardiovascular Research and the JB Cardiovascular  
354 Research Fund, and L.J.S. was partially supported by NIH R01 HD095881. K.X. was  
355 supported by the Ohio State University James Cancer Center and a Path to K award from  
356 the Ohio State University Office of Health Sciences and the Center for Clinical &  
357 Translational Science. The content is solely the responsibility of the authors and does not  
358 necessarily represent the official views of the university, or the Center for Clinical &  
359 Translational Science.

360

361

362 **Figure 1: Omicron subvariants, especially BQ.1, BQ.1.1, and BA.2.75.2, exhibit strong**  
363 **neutralization resistance.**

364 (A) Displayed is a schematic of SARS-CoV-2 Omicron subvariant evolution indicating the  
365 mutations acquired by the BA.4.6, BF.7, BQ.1, BQ.1.1, and BA.2.75.2 subvariants. (B-C)  
366 Infectivity of lentivirus pseudotyped with the indicated S constructs in HEK293T-ACE2 cells, n =  
367 3 (B), or lung-derived CaLu-3 cells, n = 3 (C). Bars represent means +/- standard error.  
368 Significance relative to D614G was determined by one-way ANOVA with Bonferroni's multiple  
369 testing correction. P values are represented as ns for  $p \geq 0.05$ , \* $p < 0.05$ , and \*\*\*\* $p < 0.0001$ ,  
370 respectively. (D-F) Neutralizing antibody tiers against lentivirus pseudotyped with S from the  
371 indicated SARS-CoV-2 variants were determined for sera from health care workers (HCWs) (n =  
372 15) who received a single homologous monovalent Moderna mRNA-1273 (n =3) or  
373 Pfizer/BioNTech BNT162b2 (n =12) mRNA booster vaccination (D), for sera from BA.1-wave  
374 hospitalized COVID-19 patients (n = 15) (E), and for sera from BA.4/5-wave SARS-CoV-2 infected  
375 Columbus, Ohio first responders and household contacts (n = 20) (F). Bars represent geometric  
376 means with 95% confidence intervals. Significance relative to D614G was determined by one-  
377 way repeated measures ANOVA with Bonferroni's multiple testing correction. P values are  
378 displayed as ns for  $p \geq 0.05$ , \* $p < 0.05$ , \*\* $p < 0.01$ , and \*\*\*\* $p < 0.0001$ .

379

380 **Figure 2: Mutations N460K and F486S, and to a lesser extent, R346T and K444T, drive**  
381 **Omicron subvariant neutralization resistance.**

382 (A-B) Infectivity of lentivirus pseudotyped with the indicated BA.4/5-derived mutant S constructs,  
383 n = 3 (A) or BA.2.75-derived mutant S constructs, n = 3 (B) in HEK293T-ACE2 cells. Bars  
384 represent means +/- standard error. Significance relative to BA.4/5 or BA.2.75 was determined  
385 by one-way ANOVA with Bonferroni's multiple testing correction. P values are represented as ns  
386 for  $p \geq 0.05$ , \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , and \*\*\*\* $p < 0.0001$ , respectively. (C-H) Neutralizing  
387 antibody tiers were determined for sera from health care workers (HCWs) (n = 15) who received  
388 a single homologous monovalent Moderna mRNA-1273 (n = 3) or Pfizer/BioNTech BNT162b2 (n  
389 = 12) mRNA booster vaccination against lentivirus pseudotyped with S from the BA.4/5-derived



390 mutants (C) and BA.2.75-derived mutants (D); for sera from BA.1-wave hospitalized COVID-19  
391 patients (n = 15) against lentivirus pseudotyped with S from the BA.4/5 derived mutants (E) and  
392 BA.2.75 derived mutants (F); and for sera from BA.4/5-wave SARS-CoV-2 infected Columbus,  
393 Ohio first responders and household contacts (n = 20) against lentivirus pseudotyped with S from  
394 the BA.4/5-derived mutants (G) and BA.2.75-derived mutants (H). Bars represent geometric  
395 means with 95% confidence intervals. Significance relative to D614G was determined by one-  
396 way repeated measures ANOVA with Bonferroni's multiple testing correction. P values are  
397 displayed as ns for  $p \geq 0.05$ , \* $p < 0.05$ , \*\* $p < 0.01$ , and \*\*\*\* $p < 0.0001$ .

398

399 **Figure 3: Omicron subvariants, especially BA.4.6, BQ.1 and BQ.1.1, exhibit enhanced**  
400 **fusogenicity and S processing.**

401 (A) Representative images from syncytia formation assays for S constructs from each of the  
402 indicated variants are displayed. The scale bars represent 150  $\mu\text{m}$ . (B) Quantification of syncytia  
403 formation for the indicated S constructs, n = 6. Bars represent means +/- standard error.  
404 Significance relative to D614G was determined by one-way ANOVA with Bonferroni's multiple  
405 testing correction. P values are displayed as \*\*\*\* $p < 0.0001$ . (C) Representative histograms of  
406 anti-S surface staining in HEK293T cells transfected to express the indicated S constructs. (D)  
407 Quantification of S surface expression (mean fluorescence intensity) relative to the D614G  
408 construct is displayed, n = 3. Bars represent means +/- standard error. Significance relative to  
409 D614G was determined by one-way ANOVA with Bonferroni's multiple testing correction. P values  
410 are displayed as ns for  $p \geq 0.05$ . (E) S processing is displayed as determined by Western blot  
411 probing with anti-S1 and anti-S2 antibodies. The ratio of band intensities for S1 and S as well as  
412 S2 and S relative to D614G are displayed.

413

414 **Figure 4: Mutations N460K, N658S, F486S, and D1199N determine the fusogenicity and S**  
415 **processing of Omicron subvariants.**

416 (A) Representative images from syncytia formation assays for S constructs from each of the  
417 indicated BA.4/5-derived mutants are displayed. The scale bars represent 150  $\mu$ m. (B)  
418 Quantification of syncytia formation for the indicated BA.4/5 derived S mutants, n = 6. Bars  
419 represent means +/- standard error. Significance relative to BA.4/5 was determined by one-way  
420 ANOVA with Bonferroni's multiple testing correction. P values are displayed as ns for  $p \geq 0.05$ , \* $p$   
421  $< 0.05$ , \*\*\* $p < 0.001$ , and \*\*\*\* $p < 0.0001$ . (C) Representative images from syncytia formation  
422 assays for S constructs from each of the indicated BA.2.75-derived mutants are displayed. The  
423 scale bars represent 150  $\mu$ m. (D) Quantification of syncytia formation for the indicated BA.2.75-  
424 derived S mutants, n = 6. Bars represent means +/- standard error. Significance relative to  
425 BA.2.75 was determined by one-way ANOVA with Bonferroni's multiple testing correction. P  
426 values are displayed as ns for  $p \geq 0.05$ , \* $p < 0.05$ , and \*\*\*\* $p < 0.0001$ . (E) Quantification of S  
427 surface expression relative to the BA.4/5 construct for the BA.4/5-derived mutants is displayed, n  
428 = 3. Bars represent means +/- standard error. Significance relative to BA.4/5 was determined by  
429 one-way ANOVA with Bonferroni's multiple testing correction. P values are displayed as ns for  $p$   
430  $\geq 0.05$  and \* $p < 0.05$ . (F) Quantification of S surface expression relative to the BA.2.75 construct  
431 for the BA.2.75 derived mutants is displayed, n = 3. Bars represent means +/- standard error.  
432 Significance relative to BA.4/5 was determined by one-way ANOVA with Bonferroni's multiple  
433 testing correction. P values are displayed as ns for  $p \geq 0.05$ . (G) S processing for the BA.4/5-  
434 derived mutants as well as ancestral D614G is displayed, which was determined by Western blot  
435 probing with anti-S1, anti-S2, and anti- $\beta$ -actin antibodies. The ratio of band intensities for S1 and  
436 S as well as S2 and S relative to BA.4/5 are displayed. (H) S processing for the BA.2.75-derived  
437 mutants as well as ancestral D614G is displayed as determined by Western blot probing with anti-  
438 S1, anti-S2, and anti- $\beta$ -actin antibodies. The ratio of band intensities for S1 and S as well as S2  
439 and S relative to BA.2.75 are displayed.

440

441

442

**Figure 5: Homology modeling-based structural analysis for the mutations.**

443  
444 **(A)** and **(B)** Structures of spike-antibody binding interface shown as ribbons. Spike recognition of  
445 class III neutralizing antibodies C1365 **(A)** and SW186 **(B)** are interfered by R346S and K444T  
446 mutations, where multiple hydrogen bonds and salt-bridge (shown as yellow dot lines) are  
447 abolished. **(C)** Structure of spike-ACE2 binding interface shown as ribbon. F486 interacts  
448 hydrophobically with M82 and Y83 on ACE2, whereas F486S impedes this interaction. **(D)**  
449 Structural model of HR2 domain of SARS-CoV2 S. Inset: Electrostatic surface potential of HR2  
450 membrane proximal region. D1199 contributes to the overall negative charge of this region.

451

## 452 **Methods**

### 453 *Samples and Patient Information*

454 Sera samples were collected from HCWs at the Ohio State University Wexner Medical  
455 Center in Columbus, Ohio with approval from an institutional review board (Gordon et al.) (IRB  
456 2020H0228 and IRB 2020H0527). These HCWs samples were collected 2-13 weeks after  
457 vaccination with a third homologous dose of the monovalent Moderna mRNA-1273 (n = 3) or  
458 Pfizer BioNTech BNT162b2 (n =12) vaccines. HCWs included 10 male and 5 female subjects  
459 with ages ranging from 26 to 61 (median 33).

460 Sera from BA.1-wave COVID-19 patients hospitalized in Columbus, Ohio were collected  
461 with approval from an IRB (IRB 2020H0527). The patient samples were collected 1-7 days after  
462 hospitalization with COVID-19. Hospitalizations occurred between the end of January and the end  
463 of February of 2022, a BA.1 dominant period in Columbus, Ohio. Patients included 12 male and  
464 3 female patients with ages ranging from 29 to 78 (median 57). Patients included 6 unvaccinated  
465 patients, 5 patients vaccinated with 2 doses of Pfizer/BioNTech BNT162b2 (n = 2) or Moderna  
466 mRNA-1273 (n = 3), and 4 patients vaccinated and boosted with Pfizer/BioNTech BNT162b2.

467 Sera from BA.4/5-wave Columbus, Ohio first responders and household contacts who  
468 tested positive for SARS-CoV-2 infection were collected with IRB approval (IRB 2020H0527,

469 2020H0531, and 2020H0240). 11 patient nasal swab samples were sequenced to confirm  
470 infection with BA.4, BA.5, or a derivative variants, with 4 patients infected with BA.4, 7 with BA.5,  
471 and 9 patients could not have their variant determined. Of those who could not have the specific  
472 variant identified, their samples were collected between late July and late September of 2022, a  
473 BA.4/5 dominant period. Except for one patient whose gender and age are unknown, patients  
474 included 4 male and 15 female with ages ranging from 27 to 58 (median 44). Patients included  
475 17 unvaccinated, and 3 vaccinated and boosted with Pfizer/BioNTech BNT162b2 (n=1) or  
476 Moderna mRNA-1273 (n = 2).

477

#### 478 *Cell Lines and Maintenance*

479 Human female embryonic kidney cell lines HEK293T (ATCC CRL-11268, RRID:  
480 CVCL\_1926) and HEK293T cells stably expressing human ACE2 (BEI NR-52511, RRID:  
481 CVCL\_A7UK) were maintained in DMEM (Gibco, 11965-092) with 10% FBS (Sigma, F1051) and  
482 1% penicillin-streptomycin (HyClone, SV30010) added. Human male adenocarcinoma lung  
483 epithelial cell line Calu-3 (RRID:CVCL\_0609) were maintained in EMEM (ATCC, 30-2003) with  
484 10% FBS and 1% penicillin-streptomycin added. All cells were passaged first by washing with  
485 Dulbecco's phosphate buffered saline (Sigma, D5652-10X1L) then incubating in 0.05% Trypsin +  
486 0.53 mM EDTA (Corning, 25-052-CI) until cells were completely detached. Cells were maintained  
487 at 37°C and 5.0% CO<sub>2</sub> in 10 cm cell culture dishes (Greiner Bio-one, 664160).

488

#### 489 *Plasmids*

490 The pNL4-3 inGluc lentiviral vector has been reported on in our previous publications  
491 (Zeng et al., 2020). Briefly, the vector is in the HIV-1 pNL4-3 backbone with a deletion of Env and  
492 an addition of a *Gaussia* luciferase reported gene that is expressed in target cells without  
493 premature expression in producer cells. The S variant constructs were cloned into the pcDNA3.1  
494 vector by GenScript Biotech (Piscataway, NJ) using restriction enzyme cloning by KpnI and

495 BamHI; alternatively, they were produced by PCR mutagenesis. The constructs bear FLAG tags  
496 on the N- and C-terminal ends. All constructs were confirmed by sequencing.

497

#### 498 *Pseudotyped lentivirus production and infectivity*

499 Pseudotyped lentiviral vectors were produced as previously described (Zeng et al., 2020).  
500 HEK293T cells were transfected with the pNL4-3-inGluc and S constructs in a 2:1 ratio using  
501 polyethyleneimine transfection (Transporter 5 Transfection Reagent, Polysciences) in order to  
502 generate viral particles. Virus was harvested 24, 48, and 72 hours post-transfection. Relative  
503 infectivity was determined by infection of HEK293T-ACE2 cells and measurement of *Gaussia*  
504 luciferase activity 48 hours post-infection. *Gaussia* luciferase activity was measured by combining  
505 equal volumes of cell culture media and *Gaussia* luciferase substrate (0.1 M Tris pH 7.4, 0.3 M  
506 sodium ascorbate, 10  $\mu$ M coelenterazine) with luminescence measured immediately by a BioTek  
507 Cytation5 plate reader.

508

#### 509 *Lentivirus neutralization assay*

510 Neutralization assays with pseudotyped lentiviral vectors were performed as described  
511 previously (Zeng et al., 2020). HCW and COVID-19 patient samples were 4-fold serially diluted  
512 and equal amounts of SARS-CoV-2 pseudotyped virus were added to the diluted sera. Final  
513 dilutions were 1:80, 1:320, 1:1280, 1:5120, 1:20480, and no serum control. The virus and sera  
514 mixture was incubated for 1 hour at 37°C then added to HEK293T-ACE2 cells to allow for infection.  
515 *Gaussia* luciferase activity was measured at 48 and 72 hours post-infection by combining equal  
516 volumes of cell culture media and *Gaussia* luciferase substrate with luminescence measured  
517 immediately by a BioTek Cytation5 plate reader. The 50% neutralization titers (NT<sub>50</sub>) were  
518 determined by least-squares-fit, non-linear regression in GraphPad Prism 9 (San Diego, CA).

519

#### 520 *Spike surface expression*

521 HEK293T cells used to produce pseudotyped lentiviral vectors were harvested 72 hours  
522 post-transfection. The producer cells were incubated in PBS+5mM EDTA for 30 minutes at 37C  
523 to disassociate them. The cells were then fixed in 4% formaldehyde and stained with anti-SARS-  
524 CoV-2 polyclonal S1 antibody (Sino Biological, 40591-T62; RRID: AB\_2893171). Cells were then  
525 stained with anti-rabbit-IgG-FITC (Sigma, F9887, RRID: AB\_259816) and assayed with a Life  
526 Technologies Attune NxT flow cytometer. FlowJo v7.6.5 (Ashland, OR) was used to process flow  
527 cytometry data.

528

### 529 *Syncytia formation*

530 HEK293T-ACE2 cells were co-transfected with the S of interest alongside GFP. Then 24  
531 hours post transfection, the cells were imaged on Leica DMI8 confocal microscope to visualize  
532 syncytia. Representative images were selected. Mean syncytia size was determine using Leica  
533 X Applications Suite. The scale bars represent 150  $\mu$ m.

534

### 535 *Spike processing and incorporation*

536 Lysate was collected from virus producer cells through a 30-minute incubation on ice in  
537 RIPA lysis buffer (50 mM Tris pH 7.5, 150 mM NaCl, 1 mM EDTA, Nonidet P-40, 0.1% SDS)  
538 supplemented with protease inhibitor (Sigma, P8340). Samples were run on a 10% acrylamide  
539 SDS-PAGE gel and transferred to a PVDF membrane. Membranes were probed with anti-S1  
540 (Sino Biological, 40591-T62; RRID:AB\_2893171), anti-S2 (Sino Biological, 40590;  
541 RRID:AB\_2857932), and anti- $\beta$ -actin (ThermoFisher, MA5-15740; RRID:AB\_10983927).  
542 Secondary antibodies included Anti-mouse-IgG-Peroxidase (Sigma, A5278; RRID:AB\_258232)  
543 and Anti-rabbit-IgG-HRP (Sigma, A9169; RRID:AB\_258434). Blots were imaged using Immobilon  
544 Crescendo Western HRP substrate (Millipore, WBLUR0500) on a GE Amersham Imager 600.  
545 Band intensities were quantified using NIH ImageJ (Bethesda, MD) image analysis software.

546

547 *Structural modeling and analysis*

548 Homology modeling of Omicron spike protein complexes with either ACE2 receptor or  
549 neutralizing antibodies was performed on SWISS-MODEL server with published X-ray  
550 crystallography and cryo-EM structures as templates (PDB IDs: 7K8Z, 8DT3, 7XB0,  
551 2FXP). Molecular contacts of Omicron mutants were examined and illustrated with the  
552 programs PyMOL.

553

554 *Quantification and statistical analysis*

555 NT<sub>50</sub> values were determined by least-squares-fit, non-linear-regression in GraphPad Prism 9  
556 (San Diego, CA). NT<sub>50</sub> values were log<sub>10</sub> transformed for hypothesis testing to better approximate  
557 normality, and multiplicity was addressed by the use of Bonferroni corrections. The statistical  
558 analysis was performed using GraphPad Prism 9 and are referenced in the figure legends,  
559 including one-way ANOVA (Figs. 1B-C, 2A-B, 3B, 3D, 4B, 4D, 4E, 4F, and S2A-B) and one-way  
560 repeated measures ANOVA (Figs. 1D-F, and 2C-H). Syncytia sizes (Figs. 3B, 4B, and 4D) were  
561 quantified by Leica Applications Suit X (Wetzlar, Germany). Band intensities (Figures 3E, 4G, and  
562 4H) were quantified using NIH ImageJ (Bethesda, MD) analysis software.

563

564 **References**

565

566 Abu-Raddad, L.J., Chemaitelly, H., Ayoub, H.H., AlMukdad, S., Yassine, H.M., Al-  
567 Khatib, H.A., Smatti, M.K., Tang, P., Hasan, M.R., Coyle, P., *et al.* (2022). Effect of  
568 mRNA Vaccine Boosters against SARS-CoV-2 Omicron Infection in Qatar. *The New*  
569 *England journal of medicine* 386, 1804-1816.  
570 Barut, G.T., Halwe, N.J., Taddeo, A., Kelly, J.N., Schön, J., Ebert, N., Ulrich, L.,  
571 Devisme, C., Steiner, S., Trüeb, B.S., *et al.* (2022). The spike gene is a major  
572 determinant for the SARS-CoV-2 Omicron-BA.1 phenotype. *Nat Commun* 13, 5929.  
573 Cao, Y., Song, W., Wang, L., Liu, P., Yue, C., Jian, F., Yu, Y., Yisimayi, A., Wang, P.,  
574 Wang, Y., *et al.* (2022a). Characterization of the enhanced infectivity and antibody  
575 evasion of Omicron BA. 2.75. *Cell Host & Microbe*.



576 Cao, Y., Wang, J., Jian, F., Xiao, T., Song, W., Yisimayi, A., Huang, W., Li, Q., Wang,  
577 P., An, R., *et al.* (2022b). Omicron escapes the majority of existing SARS-CoV-2  
578 neutralizing antibodies. *Nature* 602, 657-663.

579 Cao, Y., Yisimayi, A., Jian, F., Song, W., Xiao, T., Wang, L., Du, S., Wang, J., Li, Q.,  
580 Chen, X., *et al.* (2022c). BA.2.12.1, BA.4 and BA.5 escape antibodies elicited by  
581 Omicron infection. *Nature* 608, 593-602.

582 Centers for Disease Control and Prevention (2022). COVID Data Tracker. Atlanta, GA:  
583 US Department of Health and Human Services, CDC; 2022, October 16.  
584 <https://covid.cdc.gov/covid-data-tracker>.

585 Cerutti, G., Guo, Y., Liu, L., Liu, L., Zhang, Z., Luo, Y., Huang, Y., Wang, H.H., Ho,  
586 D.D., Sheng, Z., *et al.* (2022). Cryo-EM structure of the SARS-CoV-2 Omicron spike.  
587 *Cell Rep* 38, 110428.

588 Chen, Y., Zhao, X., Zhou, H., Zhu, H., Jiang, S., and Wang, P. (2022). Broadly  
589 neutralizing antibodies to SARS-CoV-2 and other human coronaviruses. *Nature reviews*  
590 *Immunology*, 1-11.

591 Cui, Z., Liu, P., Wang, N., Wang, L., Fan, K., Zhu, Q., Wang, K., Chen, R., Feng, R., Jia,  
592 Z., *et al.* (2022). Structural and functional characterizations of infectivity and immune  
593 evasion of SARS-CoV-2 Omicron. *Cell* 185, 860-871.e813.

594 Evans, J.P., Zeng, C., Qu, P., Faraone, J., Zheng, Y.M., Carlin, C., Bednash, J.S.,  
595 Zhou, T., Lozanski, G., Mallampalli, R., *et al.* (2022). Neutralization of SARS-CoV-2  
596 Omicron sub-lineages BA.1, BA.1.1, and BA.2. *Cell Host Microbe* 30, 1093-1102.e1093.

597 Gordon, D.E., Jang, G.M., Bouhaddou, M., Xu, J., Obernier, K., White, K.M., O'Meara,  
598 M.J., Rezelj, V.V., Guo, J.Z., Swaney, D.L., *et al.* (2020). A SARS-CoV-2 protein  
599 interaction map reveals targets for drug repurposing. *Nature* 583, 459-468.

600 Gruell, H., Vanshylla, K., Tober-Lau, P., Hillus, D., Schommers, P., Lehmann, C., Kurth,  
601 F., Sander, L.E., and Klein, F. (2022). mRNA booster immunization elicits potent  
602 neutralizing serum activity against the SARS-CoV-2 Omicron variant. *Nat Med* 28, 477-  
603 480.

604 Hachmann, N.P., Miller, J., Collier, A.Y., Ventura, J.D., Yu, J., Rowe, M., Bondzie, E.A.,  
605 Powers, O., Surve, N., Hall, K., *et al.* (2022). Neutralization Escape by SARS-CoV-2  
606 Omicron Subvariants BA.2.12.1, BA.4, and BA.5. *The New England journal of medicine*  
607 387, 86-88.

608 Iacobucci, G. (2022). Covid-19: Hospital admissions rise in England as some trusts  
609 reinstate mask requirements. *Bmj* 379, o2440.

610 Iketani, S., Liu, L., Guo, Y., Liu, L., Chan, J.F., Huang, Y., Wang, M., Luo, Y., Yu, J.,  
611 Chu, H., *et al.* (2022). Antibody evasion properties of SARS-CoV-2 Omicron  
612 sublineages. *Nature* 604, 553-556.

613 Khan, K., Karim, F., Ganga, Y., Bernstein, M., Jule, Z., Reedoy, K., Cele, S., Lustig, G.,  
614 Amoako, D., Wolter, N., *et al.* (2022). Omicron BA.4/BA.5 escape neutralizing immunity  
615 elicited by BA.1 infection. *Nat Commun* 13, 4686.

616 Kimura, I., Yamasoba, D., Tamura, T., Nao, N., Suzuki, T., Oda, Y., Mitoma, S., Ito, J.,  
617 Nasser, H., Zahradnik, J., *et al.* (2022). Virological characteristics of the SARS-CoV-2  
618 Omicron BA.2 subvariants, including BA.4 and BA.5. *Cell* 185, 3992-4007.e3916.

619 Kurhade, C., Zou, J., Xia, H., Cai, H., Yang, Q., Cutler, M., Cooper, D., Muik, A.,  
620 Jansen, K.U., Xie, X., *et al.* (2022). Neutralization of Omicron BA.1, BA.2, and BA.3  
621 SARS-CoV-2 by 3 doses of BNT162b2 vaccine. *Nat Commun* 13, 3602.

622 Liu, L., Iketani, S., Guo, Y., Chan, J.F., Wang, M., Liu, L., Luo, Y., Chu, H., Huang, Y.,  
623 Nair, M.S., *et al.* (2022). Striking antibody evasion manifested by the Omicron variant of  
624 SARS-CoV-2. *Nature* 602, 676-681.

625 Lyngse, F.P., Kirkeby, C.T., Denwood, M., Christiansen, L.E., Mølbak, K., Møller, C.H.,  
626 Skov, R.L., Krause, T.G., Rasmussen, M., Sieber, R.N., *et al.* (2022). Transmission of  
627 sars-cov-2 omicron voc subvariants BA.1 and BA.2: Evidence from danish households.  
628 *MedRxiv*.

629 McMahan, K., Giffin, V., Tostanoski, L.H., Chung, B., Siamatu, M., Suthar, M.S.,  
630 Halfmann, P., Kawaoka, Y., Piedra-Mora, C., Jain, N., *et al.* (2022). Reduced  
631 pathogenicity of the SARS-CoV-2 omicron variant in hamsters. *Med (New York, NY)* 3,  
632 262-268.e264.

633 Meng, B., Abdullahi, A., Ferreira, I., Goonawardane, N., Saito, A., Kimura, I.,  
634 Yamasoba, D., Gerber, P.P., Fatihi, S., Rathore, S., *et al.* (2022). Altered TMPRSS2  
635 usage by SARS-CoV-2 Omicron impacts infectivity and fusogenicity. *Nature* 603, 706-  
636 714.

637 Mykytyn, A.Z., Breugem, T.I., Riesebosch, S., Schipper, D., van den Doel, P.B., Rottier,  
638 R.J., Lamers, M.M., and Haagmans, B.L. (2021). SARS-CoV-2 entry into human airway  
639 organoids is serine protease-mediated and facilitated by the multibasic cleavage site.  
640 *eLife* 10.

641 Peacock, T.P., Goldhill, D.H., Zhou, J., Baillon, L., Frise, R., Swann, O.C., Kugathasan,  
642 R., Penn, R., Brown, J.C., Sanchez-David, R.Y., *et al.* (2021). The furin cleavage site in  
643 the SARS-CoV-2 spike protein is required for transmission in ferrets. *Nat Microbiol* 6,  
644 899-909.

645 Qu, P., Evans, J.P., Zheng, Y.M., Carlin, C., Saif, L.J., Oltz, E.M., Xu, K., Gumina, R.J.,  
646 and Liu, S.L. (2022a). Evasion of neutralizing antibody responses by the SARS-CoV-2  
647 BA.2.75 variant. *Cell Host Microbe*.

648 Qu, P., Faraone, J., Evans, J.P., Zou, X., Zheng, Y.M., Carlin, C., Bednash, J.S.,  
649 Lozanski, G., Mallampalli, R.K., Saif, L.J., *et al.* (2022b). Neutralization of the SARS-  
650 CoV-2 Omicron BA.4/5 and BA.2.12.1 Subvariants. *N Engl J Med* 386, 2526-2528.

651 Saito, A., Tamura, T., Zahradnik, J., Deguchi, S., Tabata, K., Anraku, Y., Kimura, I., Ito,  
652 J., Yamasoba, D., Nasser, H., *et al.* Virological characteristics of the SARS-CoV-2  
653 Omicron BA. 2.75 variant. *Cell Host & Microbe*.

654 Shuai, H., Chan, J.F., Hu, B., Chai, Y., Yuen, T.T., Yin, F., Huang, X., Yoon, C., Hu,  
655 J.C., Liu, H., *et al.* (2022). Attenuated replication and pathogenicity of SARS-CoV-2  
656 B.1.1.529 Omicron. *Nature* 603, 693-699.

657 Stegger, M., Edslev, S.M., Sieber, R.N., Ingham, A.C., Ng, K.L., Tang, M.-H.E.,  
658 Alexandersen, S., Fonager, J., Legarth, R., Utko, M., *et al.* (2022). Occurrence and  
659 significance of Omicron BA. 1 infection followed by BA. 2 reinfection. *medRxiv*.

660 Su, S., Li, W., and Jiang, S. (2022a). Developing pan- $\beta$ -coronavirus vaccines against  
661 emerging SARS-CoV-2 variants of concern. *Trends Immunol* 43, 170-172.

662 Su, W., Choy, K.T., Gu, H., Sia, S.F., Cheng, K.M., Nizami, S.I.N., Krishnan, P., Ng,  
663 Y.M., Chang, L.D.J., Liu, Y., *et al.* (2022b). Omicron BA.1 and BA.2 sub-lineages show  
664 reduced pathogenicity and transmission potential than the early SARS-CoV-2 D614G  
665 variant in Syrian hamsters. *J Infect Dis*.

666 Suzuki, R., Yamasoba, D., Kimura, I., Wang, L., Kishimoto, M., Ito, J., Morioka, Y., Nao,  
667 N., Nasser, H., Uriu, K., *et al.* (2022). Attenuated fusogenicity and pathogenicity of  
668 SARS-CoV-2 Omicron variant. *Nature* 603, 700-705.

669 Tuekprakhon, A., Nutalai, R., Dijokaite-Guraliuc, A., Zhou, D., Ginn, H.M., Selvaraj, M.,  
670 Liu, C., Mentzer, A.J., Supasa, P., Duyvesteyn, H.M.E., *et al.* (2022). Antibody escape  
671 of SARS-CoV-2 Omicron BA.4 and BA.5 from vaccine and BA.1 serum. *Cell* 185, 2422-  
672 2433.e2413.

673 Wang, Q., Anang, S., Iketani, S., Guo, Y., Liu, L., Katsamba, P.S., Shapiro, L., Ho, D.D.,  
674 and Sodroski, J.G. (2022a). Functional properties of the spike glycoprotein of the  
675 emerging SARS-CoV-2 variant B.1.1.529. *Cell Rep* 39, 110924.

676 Wang, Q., Guo, Y., Iketani, S., Nair, M.S., Li, Z., Mohri, H., Wang, M., Yu, J., Bowen,  
677 A.D., Chang, J.Y., *et al.* (2022b). Antibody evasion by SARS-CoV-2 Omicron  
678 subvariants BA.2.12.1, BA.4 and BA.5. *Nature* 608, 603-608.

679 Wang, Q., Iketani, S., Li, Z., Guo, Y., Yeh, A.Y., Liu, M., Yu, J., Sheng, Z., Huang, Y.,  
680 Liu, L., *et al.* (2022c). Antigenic characterization of the SARS-CoV-2 Omicron  
681 subvariant BA.2.75. *Cell Host Microbe*.

682 Xia, H., Zou, J., Kurhade, C., Cai, H., Yang, Q., Cutler, M., Cooper, D., Muik, A.,  
683 Jansen, K.U., Xie, X., *et al.* (2022a). Neutralization and durability of 2 or 3 doses of the  
684 BNT162b2 vaccine against Omicron SARS-CoV-2. *Cell Host Microbe* 30, 485-  
685 488.e483.

686 Xia, S., Wang, L., Zhu, Y., Lu, L., and Jiang, S. (2022b). Origin, virological features,  
687 immune evasion and intervention of SARS-CoV-2 Omicron sublineages. *Signal*  
688 *Transduct Target Ther* 7, 241.

689 Yamasoba, D., Kimura, I., Nasser, H., Morioka, Y., Nao, N., Ito, J., Uriu, K., Tsuda, M.,  
690 Zahradnik, J., Shirakawa, K., *et al.* (2022). Virological characteristics of the SARS-CoV-  
691 2 Omicron BA.2 spike. *Cell* 185, 2103-2115.e2119.

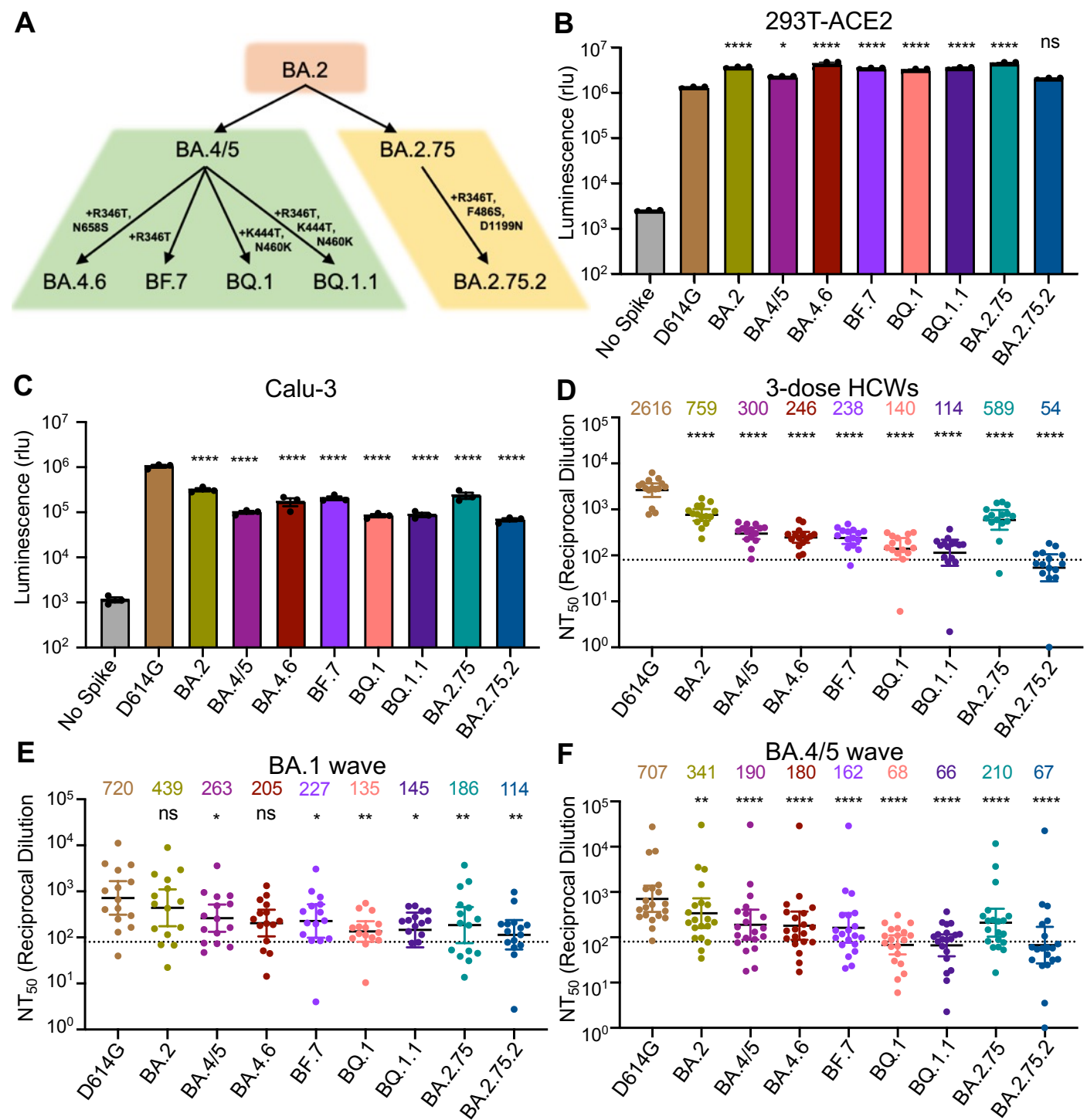
692 Yu, J., Collier, A.Y., Rowe, M., Mardas, F., Ventura, J.D., Wan, H., Miller, J., Powers,  
693 O., Chung, B., Siamatu, M., *et al.* (2022). Neutralization of the SARS-CoV-2 Omicron  
694 BA.1 and BA.2 Variants. *The New England journal of medicine* 386, 1579-1580.

695 Zeng, C., Evans, J.P., Pearson, R., Qu, P., Zheng, Y.M., Robinson, R.T., Hall-Stoodley,  
696 L., Yount, J., Pannu, S., Mallampalli, R.K., *et al.* (2020). Neutralizing antibody against  
697 SARS-CoV-2 spike in COVID-19 patients, health care workers, and convalescent  
698 plasma donors. *JCI Insight* 5.

699 Zeng, C., Evans, J.P., Qu, P., Faraone, J., Zheng, Y.M., Carlin, C., Bednash, J.S.,  
700 Zhou, T., Lozanski, G., Mallampalli, R., *et al.* (2021). Neutralization and Stability of  
701 SARS-CoV-2 Omicron Variant. *bioRxiv*.

702 Zou, J., Xia, H., Xie, X., Kurhade, C., Machado, R.R.G., Weaver, S.C., Ren, P., and Shi,  
703 P.Y. (2022). Neutralization against Omicron SARS-CoV-2 from previous non-Omicron  
704 infection. *Nat Commun* 13, 852.

705



**Figure 1**

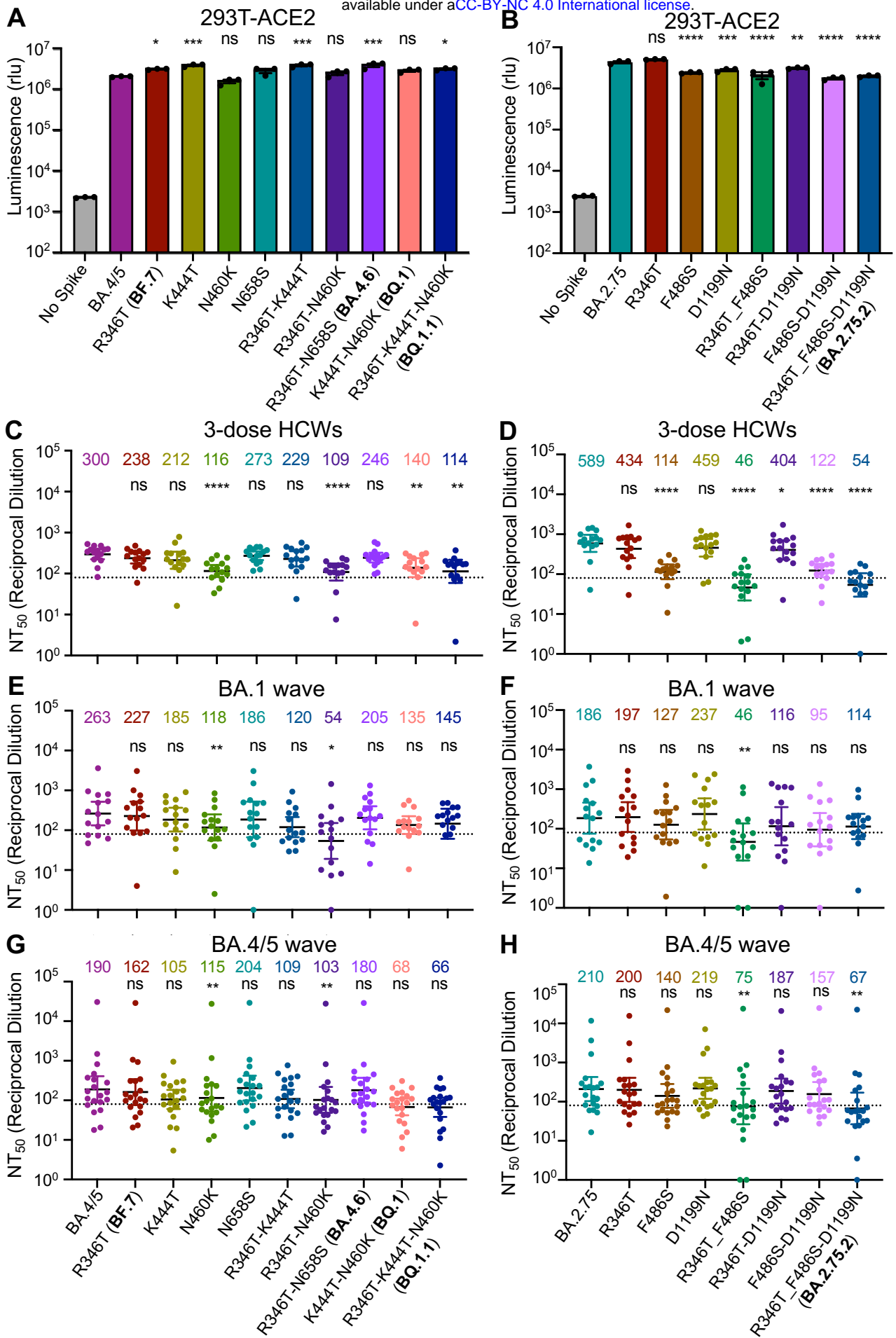
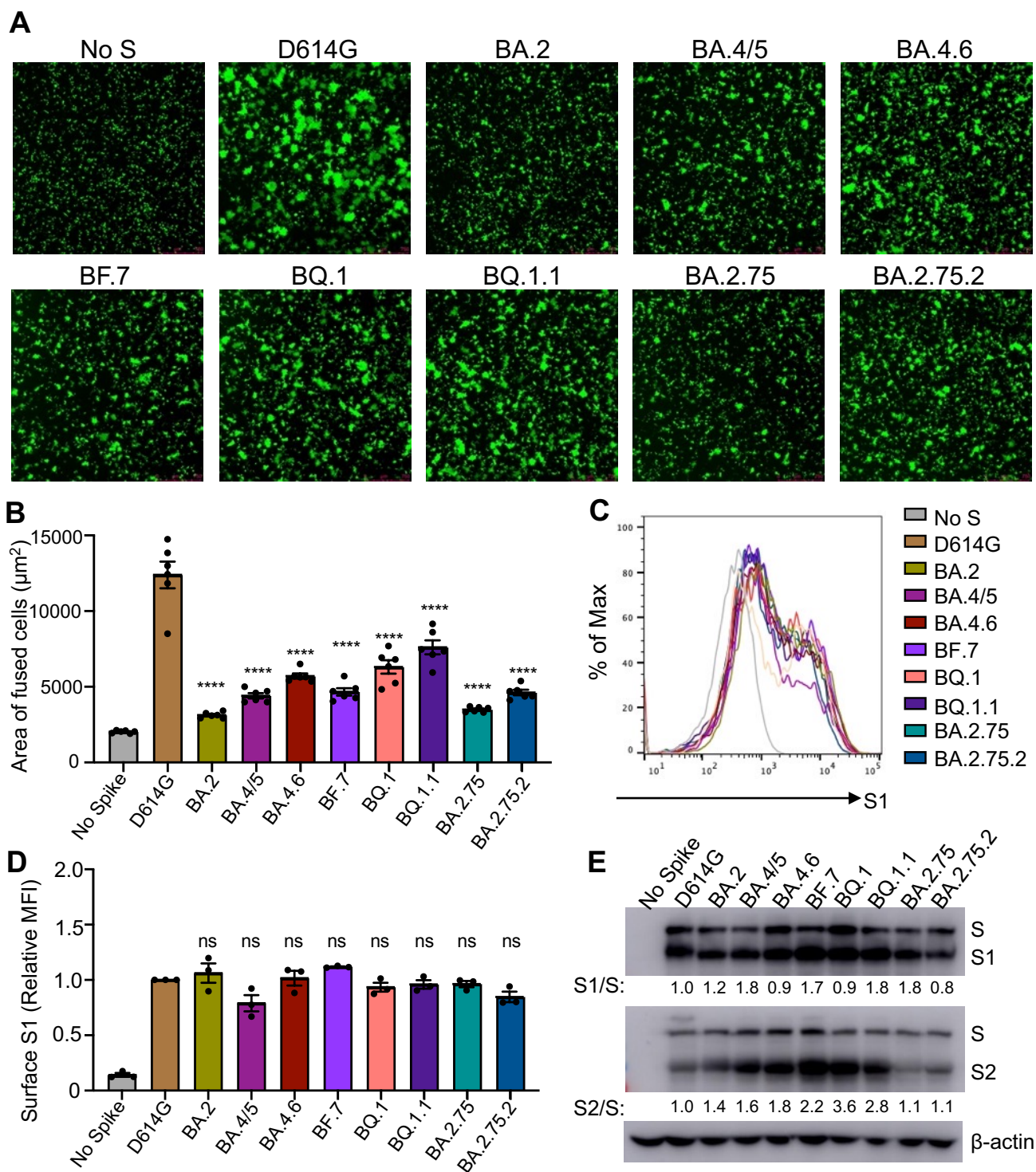


Figure 2





**Figure 3**

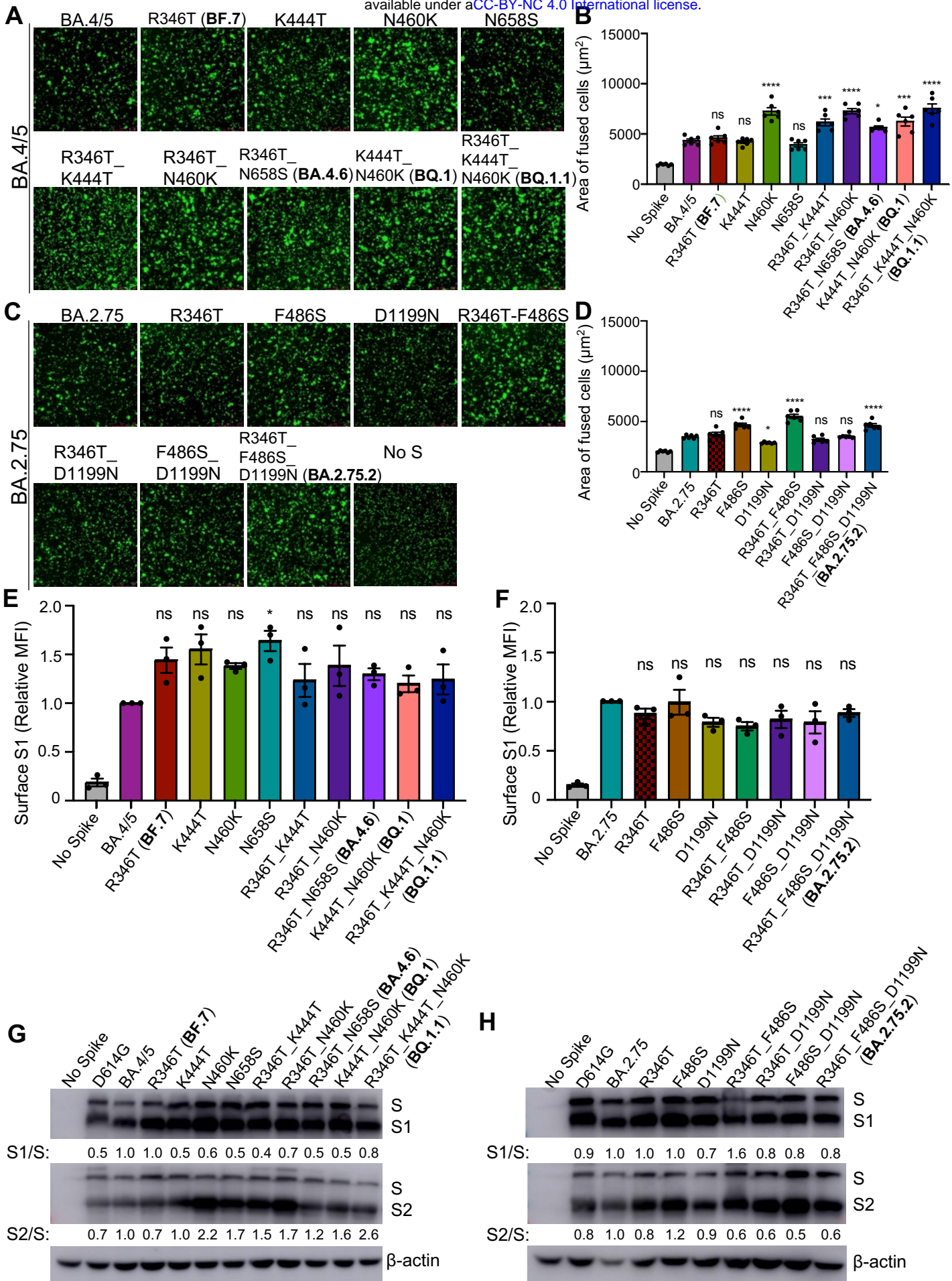


Figure 4



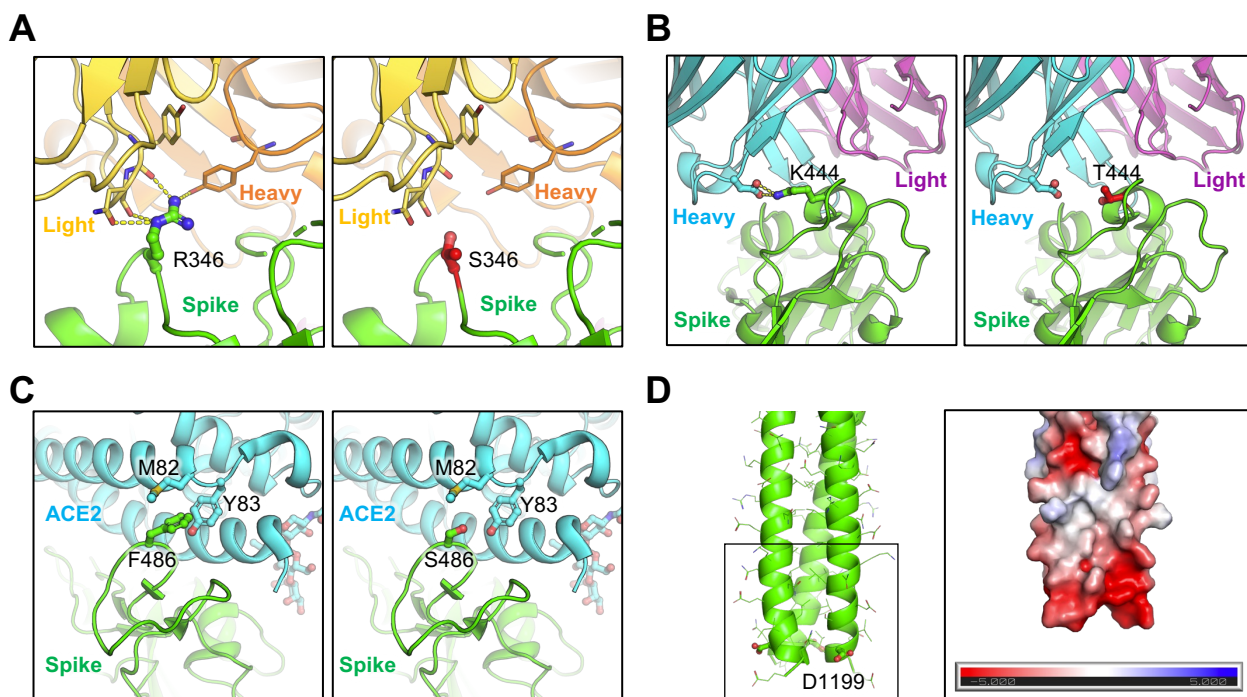


Figure 5