

Antibody evasion by SARS-CoV-2 Omicron subvariants BA.2.12.1, BA.4, and BA.5

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1 **Abstract**

2 SARS-CoV-2 Omicron subvariants BA.2.12.1 and BA.4/5 have surged dramatically to become
3 dominant in the United States and South Africa, respectively^{1,2}. These novel subvariants carrying
4 additional mutations in their spike proteins raise concerns that they may further evade neutralizing
5 antibodies, thereby further compromising the efficacy of COVID-19 vaccines and therapeutic
6 monoclonals. We now report findings from a systematic antigenic analysis of these surging
7 Omicron subvariants. BA.2.12.1 is only modestly (1.8-fold) more resistant to sera from vaccinated
8 and boosted individuals than BA.2. However, BA.4/5 is substantially (4.2-fold) more resistant
9 and thus more likely to lead to vaccine breakthrough infections. Mutation at spike residue L452
10 found in both BA.2.12.1 and BA.4/5 facilitates escape from some antibodies directed to the so-
11 called class 2 and 3 regions of the receptor-binding domain³. The F486V mutation found in BA.4/5
12 facilitates escape from certain class 1 and 2 antibodies but compromises the spike affinity for the
13 viral receptor. The R493Q reversion mutation, however, restores receptor affinity and
14 consequently the fitness of BA.4/5. Among therapeutic antibodies authorized for clinical use, only
15 bebtelovimab retains full potency against both BA.2.12.1 and BA.4/5. The Omicron lineage of
16 SARS-CoV-2 continues to evolve, successively yielding subvariants that are not only more
17 transmissible but also more evasive to antibodies.

18 **Main text**

19 Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) Omicron or B.1.1.529 variant
20 continues to dominate the coronavirus disease 2019 (COVID-19) pandemic. Globally, the BA.2
21 subvariant has rapidly replaced previous subvariants BA.1 and BA.1.1 (Fig. 1a). The recent
22 detection and dramatic expansion of three new Omicron subvariants have raised concerns.
23 BA.2.12.1 emerged in the United States in early February and expanded substantially (Fig. 1a),
24 now accounting for over 55% of all new SARS-CoV-2 infections in the country². BA.4 and BA.5
25 emerged in South Africa in January and rapidly became dominant there with a combined frequency
26 of over 88%⁴. These new Omicron subvariants have been detected worldwide, with a combined
27 frequency of over 50% in recent weeks. However, their growth trajectories in the U.S. and South
28 Africa indicate a significant transmission advantage that will likely result in further expansion, as
29 is being observed in countries such as the United Kingdom (Fig. 1a). Phylogenetically, these new
30 subvariants evolved independently from BA.2 (Fig. 1b). The spike protein of BA.2.12.1 contains
31 L452Q and S704L alterations in addition to the known mutations in BA.2, whereas the spike
32 proteins of BA.4 and BA.5 are identical, each with four additional alterations: Del69-70, L452R,
33 F486V, and R493Q, a reversion mutation (Fig. 1c). The location of several of these mutations
34 within RBD of the spike protein raises the specter that BA.2.12.1 and BA.4/5 may have evolved
35 to further escape from neutralizing antibodies.

36

37 **Neutralization by monoclonal antibodies**

38 To understand antigenic differences of BA.2.12.1 and BA.4/5 from previous Omicron subvariants
39 (BA.1, BA.1.1, and BA.2) and the wild-type SARS-CoV-2 (D614G), we produced each
40 pseudovirus and then assessed the sensitivity of each pseudovirus to neutralization by a panel of
41 21 monoclonal antibodies (mAbs) directed to known neutralizing epitopes on the viral spike.
42 Among these, 19 target the four epitope classes in the receptor binding domain (RBD)³, including
43 REGN10987 (imdevimab)⁵, REGN10933 (casirivimab)⁵, COV2-2196 (tixagevimab)⁶, COV2-
44 2130 (cilgavimab)⁶, LY-CoV555 (bamlanivimab)⁷, CB6 (etesevimab)⁸, Brii-196 (amubarvimab)⁹,
45 Brii-198 (romlusevimab)⁹, S309 (sotrovimab)¹⁰, LY-CoV1404 (bebtelovimab)¹¹, ADG-2¹²,
46 DH1047¹³, S2X259¹⁴, CAB-A17¹⁵ and ZCB11¹⁶, as well as 1-20, 2-15, 2-7¹⁷ and 10-40¹⁸ from our
47 group. Two other mAbs, 4-18 and 5-7¹⁷, target the N-terminal domain (NTD). Our findings are
48 shown in Fig. 2a, as well as in Extended Data Figs. 1 and 2. Overall, 18 and 19 mAbs lost

49 neutralizing activity completely or partially against BA.2.12.1 and BA.4/5, respectively.
50 Neutralization profiles were similar for BA.2 and BA.2.12.1 except for three class 3 RBD mAbs
51 (Brii-198, REGN10987, and COV2-2130) that were either inactive or further impaired against the
52 latter subvariant. Compared to BA.2 and BA.2.12.1, BA.4/5 showed substantially greater
53 neutralization resistance to two class 2 RBD mAbs (ZCB11 and COV2-2196) as well as modest
54 resistance to two class 3 RBD mAbs (REGN10987 and COV2-2130). Collectively, these
55 differences suggest that mutations in BA.2.12.1 confer greater evasion from antibodies to class 3
56 region of RBD, whereas mutations in BA.4/5 confer greater evasion from antibodies to class 2 and
57 class 3 regions. Only four RBD mAbs (CAB-A17, COV2-2130, 2-7, and LY-COV1404) retained
58 good in vitro potency against both BA.2.12.1 and BA.4/5 with IC₅₀ below 0.1 µg/mL. Importantly,
59 among these four mAbs, COV2-2130 (cilgavimab) is one component of a combination known as
60 Evusheld that is authorized for prevention of COVID-19, while only LY-COV1404 or
61 bebtelovimab is authorized for therapeutic use in the clinic. For antibody combinations previously
62 authorized or approved for clinical use, all showed a substantial loss of activity in vitro against
63 BA.2.12.1 and BA.4/5. As for a mAb directed to the antigenic supersite of N-terminal domain
64 (NTD)¹⁹, 4-18 lost neutralizing activity against all Omicron subvariants. A mAb to the NTD
65 alternate site, 5-7²⁰, was also inactive against BA.2.12.1 and BA.4/5 but retained modest activity
66 against BA.1 and BA.1.1 (Fig. 2a).

67
68 A subset of the pseudovirus neutralization data was confirmed for four monoclonal antibodies
69 (COV2-2196, ZCB11, REGN10987, and LY-CoV1404) in neutralization experiments using
70 authentic viruses BA.2 and BA.4 (Extended Data Figs. 1b and 2b). Similar neutralization patterns
71 were observed in the two assays, although the precise 50% neutralizing titers were different.

72
73 To identify the mutations in BA.2.12.1 and BA.4/5 that confer antibody resistance, we assessed
74 the neutralization sensitivity of pseudoviruses carrying each of the point mutations in the
75 background of D614G or BA.2 to the aforementioned panel of mAbs and combinations. Detailed
76 findings are presented in Extended Data Figs. 3, 4, and 5, and most salient results are highlighted
77 in Fig. 2b and discussed here. Substitutions (M, R, and Q) at residue L452, previously found in
78 the Delta and Lambda variants^{21,22}, conferred resistance largely to classes 2 and 3 RBD mAbs,
79 with L452R being the more detrimental mutation. F486V broadly impaired the neutralizing

80 activity of several class 1 and 2 RBD mAbs. Notably, this mutation decreased the potency of
81 ZCB11 by >2000-fold. In contrast, the reversion mutation R493Q sensitized BA.2 to
82 neutralization by several class 1 and 2 RBD mAbs. This finding is consistent with our previous
83 study²³ showing that Q493R found in the earlier Omicron subvariants mediated resistance to the
84 same set of mAbs. L452, F486, and Q493, situated at the top of RBD, are among the residues
85 most commonly targeted by SARS-CoV-2 neutralizing mAbs whose epitopes have been defined
86 (Fig. 2c). In silico structural analysis showed that both L452R and L452Q caused steric hindrance
87 to the binding by class 2 RBD mAbs. One such example is shown for LY-CoV555 (Fig. 2d),
88 demonstrating the greater clash because of the arginine substitution and explaining why this
89 particular mutation led to a larger loss of virus-neutralizing activity (Fig. 2b). Structural modeling
90 of the F486V again revealed steric hindrance to binding by class 2 RBD mAbs such as
91 REGN10933, LY-CoV555, and 2-15 caused by the valine substitution (Fig. 2e).

92

93 **Receptor affinity**

94 Epidemiological data clearly indicate that both BA.2.12.1 and BA.4/5 are very transmissible (Fig.
95 1a); however, the additional mutations at the top of RBD (Fig. 2c) of these subvariants raises the
96 possibility of a significant loss of affinity for the viral receptor, human angiotensin-converting
97 enzyme 2 (hACE2). We therefore measured the binding affinity of purified spike proteins of
98 D614G and major Omicron subvariants to dimeric hACE2 using surface plasmon resonance (SPR).
99 The spike proteins of the Omicron subvariants exhibited similar binding affinities to hACE2, with
100 K_D values ranging from 1.66 nM for BA.4/5 to 2.36 nM for BA.2.12.1 to 2.79 nM for BA.1.1 (Fig.
101 3a). Impressively, despite having ≥ 17 mutations in the RBD including some that mediate antibody
102 escape, BA.2.12.1 and BA.4/5 also evolved concurrently to gain a slightly higher affinity for the
103 receptor than an ancestral SARS-CoV-2, D614G (K_D 5.20 nM).

104

105 To support the findings by SPR and to probe the role of point mutations in hACE2 binding, we
106 tested BA.2, BA.2.12.1, and BA.4/5 pseudoviruses, as well as pseudoviruses containing key
107 mutations, to neutralization by dimeric hACE2 in vitro. The 50% inhibitory concentration (IC_{50})
108 values were lower for BA.4/5 and BA.2.12.1 than that of BA.2 (Fig. 3b), again indicating that
109 these two emerging Omicron subvariants have not lost receptor affinity. Our results also showed
110 that the F486V mutation compromised receptor affinity, as previously reported²⁴, while the R493Q

111 reversion mutation improved receptor affinity. To structurally interpret these results, we modeled
112 F486V and R493Q mutations based on the crystal structure of BA.1-RBD-hACE2 complex²⁵
113 overlaid with ligand-free BA.2 RBD (PDB: 7U0N and 7UB0). This analysis found that both R493
114 and F486 are conformationally similar between BA.1 and BA.2, and F486V led to a loss of
115 interaction with a hydrophobic pocket in hACE2 (Fig. 3c). On the other hand, the R493Q reversion
116 mutation restored a hydrogen bond with H34 and avoided the charge repulsion by K31, seemingly
117 having the opposite effect of F486V. Interestingly, the mutation frequency at F486 had been
118 exceedingly low (<10E-5) until the emergence of BA.4/5 (Extended Data Table 1), probably
119 because of a compromised receptor affinity. Taken together, our findings in Figs. 2 and 3 suggest
120 that F486V allowed BA.4 and BA.5 to extend antibody evasion while R493Q compensated to
121 regain fitness in receptor binding.

122

123 **Neutralization by polyclonal sera**

124 We next assessed the extent of BA.2.12.1 and BA.4/5 resistance to neutralization by sera from
125 four different clinical cohorts. Sera from persons immunized with only two doses of COVID-19
126 mRNA vaccines were not examined because most of them could not neutralize earlier Omicron
127 subvariants^{23,26}. Instead, we measured serum neutralizing activity for persons who received three
128 shots of mRNA vaccines (boosted), individuals who received mRNA vaccines before or after non-
129 Omicron infection, and patients with either BA.1 or BA.2 breakthrough infection after vaccination.
130 Their clinical information is described in Extended Data Table 2, and the serum neutralization
131 profiles are presented in Extended Data Fig. 6 and the 50% inhibitory dose (ID₅₀) titers are
132 summarized in Fig. 4a. For the “boosted” cohort, neutralization titers were noticeably lower (4.6-
133 fold to 6.2-fold) for BA.1, BA.1.1, and BA.2 compared to D614G (Fig. 4b), as previously
134 reported^{23,26}. Titers for BA.2.12.1 and BA.4/5 were even lower, by 8.1-fold and 19.2-fold,
135 respectively, relative to D614G, and by 1.8-fold and 4.2-fold, respectively, relative to BA.2. A
136 similar trend was observed for serum neutralization for the other cohorts, with the lowest titers
137 against BA.4/5, followed next by titers against BA.2.12.1. Relative to BA.2, BA.2.12.1 and
138 BA.4/5 showed 1.2-fold to 1.4-fold and 1.6-fold to 4.3-fold, respectively, greater resistance to
139 neutralization by sera from these individuals who had both mRNA vaccination and SARS-CoV-2
140 infection. In addition, sera from vaccinated and boosted individuals were assayed for

141 neutralization of authentic viruses (Extended Data Figs. 6e and 6f). Neutralization titers for BA.4
142 were 2.7-fold lower on average compared to titers for BA.2, in line with the pseudovirus results.

143

144 We also conducted serum neutralization assays on pseudoviruses containing point mutations found
145 in BA.2.12.1 or BA.4/5 in the background of BA.2. Del69-70, L452M/R/Q, and F486V each
146 modestly (1.1-fold to 2.4-fold) decreased the neutralizing activity of sera from all cohorts, while
147 the R493Q reversion mutation modestly (~1.5-fold) enhanced the neutralization (Fig. 4c and
148 Extended Data Fig. 7). S704L, a mutation close to the S1/S2 cleavage site, did not appreciably
149 alter the serum neutralization titers against BA.2. For “boosted” serum samples, the impact of
150 each point mutant on neutralization resistance was quantified and summarized in Fig. 4b.

151

152 Using these serum neutralization results, we then constructed a graphic display to map antigenic
153 distances among D614G, various Omicron subvariants, and individual point mutants using only
154 results from the “boosted” serum samples to avoid confounding effects from differences in clinical
155 histories in the other cohorts. Utilizing methods well established in influenza research²⁷, all virus
156 and serum positions on the antigenic map were optimized so that the distances between them
157 correspond to the fold drop in neutralizing ID₅₀ titer relative to the maximum titer for each serum.
158 Each unit of distance in any direction on the antigenic map corresponds to a two-fold change in
159 ID₅₀ titer. The resultant antigenic cartography (Fig. 4d) shows that BA.1, BA.1.1, and BA.2 are
160 approximately equidistant from the “boosted” sera, with each about 2-3 antigenic units away.
161 BA.2.12.1 is further away from BA.2 by about 1 antigenic unit. Most strikingly, BA.4/5 is 4.3
162 antigenic units further from “boosted” sera than D614G, and 2 antigenic units further than BA.2.
163 Each of the point mutants Del69-70, L452M/Q/R, and F486V adds antigenic distance from these
164 sera compared to BA.2 and D614G, whereas R493Q has the opposite effect. Overall, this map
165 makes clear that BA.4/5 is substantially more neutralization resistant to sera obtained from boosted
166 individuals, with several mutations contributing to the antibody evasion.

167

168 **Discussion**

169 We have systematically evaluated the antigenic properties of SARS-CoV-2 Omicron subvariants
170 BA.2.12.1 and BA.4/5, which are rapidly expanding globally (Fig. 1a). It is apparent that
171 BA.2.12.1 is only modestly (1.8-fold) more resistant to sera from vaccinated and boosted

172 individuals than the BA.2 subvariant that currently dominates the global pandemic (Figs. 4b). On
173 the other hand, BA.4/5 is substantially (4.2-fold) more resistant, a finding consistent with results
174 recently posted by other groups^{1,28}. This antigenic distance is similar to that between the Delta
175 variant and the ancestral virus²⁹ and thus is likely to lead to more breakthrough infections in the
176 coming months. A key question now is whether BA.4/5 would out-compete BA.2.12.1, which
177 poses less of an antigenic threat. This competition is now playing out in the United Kingdom.
178 These new Omicron subvariants were first detected there almost simultaneously in late March of
179 2022. However, BA.2.12.1 now accounts for 13% of new infections in the U.K., whereas the
180 frequency is over 50% for BA.4/5 (Fig. 1a), suggesting a transmission advantage for the latter.

181

182 Epidemiologically, since both of these two Omicron subvariants have a clear advantage in
183 transmission, it is therefore not surprising that their abilities to bind the hACE2 receptor remain
184 robust (Fig. 3a) despite numerous mutations in the spike protein. In fact, BA.4/5 may have slightly
185 higher affinity for the receptor, consistent with suggestions that it might be more fit³⁰. However,
186 assessment of transmissibility would be more revealing by conducting studies on BA.2.12.1 and
187 BA.4/5 in animal models³¹.

188

189 Our studies on the specific mutations found in BA.2.12.1 and BA.4/5 show that Del69-70,
190 L452M/R/Q, and F486V could individually contribute to antibody resistance, whereas R493Q
191 confers antibody sensitivity (Fig. 4b). Moreover, the data generated using SARS-CoV-2-
192 neutralizing mAbs suggest that a mutation at L452 allows escape from class 2 and class 3 RBD
193 antibodies and that the F486V mutation mediates escape from class 1 and class 2 RBD antibodies
194 (Fig. 2b). It is not clear how Del69-70, a mutation that might increase infectivity³² and previously
195 seen in the Alpha variant³³, contributes to antibody resistance except for the possible evasion from
196 certain neutralizing antibodies directed to the NTD. As for the use of clinically authorized mAbs
197 to treat or block infection by BA.2.12.1 or BA.4/5, only bebtelovimab (LY-COV1404)¹¹ retains
198 exquisite potency while the combination of tixagevimab and cilgavimab (COV2-2196 and COV2-
199 2130)⁶ shows a modest loss of activity (Fig. 2a).

200

201 As the Omicron lineage has evolved over the past few months (Fig. 1b), each successive subvariant
202 has seemingly become better and better at human transmission (Fig. 1a) as well as in antibody
203 evasion^{23,34}. It is only natural that scientific attention remains intently focused on each new
204 subvariant of Omicron. However, we must be mindful that each of the globally dominant variants
205 of SARS-CoV-2 (Alpha, Delta, and Omicron) emerged stochastically and unexpectedly.
206 Vigilance in our collective surveillance effort must be sustained.

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286 **Figure legends**

287 **Fig. 1 | Prevalence of SARS-CoV-2 Omicron subvariants.** **a**, Frequencies of BA.1, BA.1.1,
288 BA.2, BA.2.12.1, and BA.4/5 deposited in GISAID. The value in the upper right corner of each
289 box denotes the cumulative number of sequences for all circulating viruses in the denoted time
290 period. **b**, Unrooted phylogenetic tree of Omicron and its subvariants along with other major
291 SARS-CoV-2 variants. The scale bar indicates the genetic distance. **c**, Key spike mutations found
292 in BA.2, BA.2.12.1, BA.4, and BA.5. Del, deletion.

293 **Fig. 2 | Resistance of Omicron subvariants to neutralization by monoclonal antibodies.** **a**,
294 Neutralization of D614G and Omicron subvariants by RBD- and NTD-directed mAbs. Values
295 above the limit of detection of 10 $\mu\text{g}/\text{mL}$ (dotted line) are arbitrarily plotted to allow for
296 visualization of each sample. **b**, Fold change in IC_{50} values of point mutants relative to D614G or
297 BA.2, with resistance colored red and sensitization colored green. **c**, Location of F486V, L452R/Q,
298 and R493Q on D614G RBD, with the color indicating the per residue frequency recognized by
299 SARS-CoV-2 neutralizing antibodies. Modeling of L452R/Q (**d**) and F486V (**e**) affect class 2 mAb
300 neutralization. The clashes are shown in red plates; the hydrogen bonds are shown in dark dashed
301 lines. The results shown in **a** and **b** are representative of those obtained in two independent
302 experiments.

303 **Fig. 3 | Affinity of the spike proteins of SARS-CoV-2 Omicron subvariants to hACE2.** **a**,
304 Binding affinities of Omicron subvariant S2P spike proteins to hACE2 as measured by SPR. **b**,
305 Sensitivity of pseudotyped Omicron subvariants and the individual mutations in the background
306 of BA.2 to hACE2 inhibition. The hACE2 concentrations resulting in 50% inhibition of infectivity
307 (IC_{50}) are presented. Data are shown as mean \pm standard error of mean (SEM) for three technical
308 replicates. **c**, In silico analysis for how R493Q and F486V affect hACE2 binding. The hACE2
309 surface is shown with charge potential, with red and blue representing negative and positive
310 charges, respectively. Omicron BA.1 RBD in complex with hACE2 was downloaded from PDB
311 7U0N, and the ligand-free BA.2 RBD was downloaded from PDB 7UB0. The results shown in **a**
312 and **b** are representative of those obtained in two independent experiments.

313 **Fig. 4 | BA.2.12.1 and BA.4/5 exhibit greater serum neutralization resistance profiles relative**
314 **to BA.2.** **a**, Neutralization of pseudotyped D614G and Omicron subvariants by sera from 4
315 different clinical cohorts. **b**, Fold change in geometric mean ID_{50} titers of boosted vaccinee sera

316 relative to D614G and BA.2, with resistance colored red and sensitization colored green. **c**, Serum
317 neutralization of BA.2 pseudoviruses containing single mutations found within BA.2.12.1 and
318 BA.4/5. **d**, Antigenic map based on the neutralization data of boosted vaccinee sera. SARS-CoV-
319 2 variants are shown as colored circles and sera are shown as grey squares. The x-, y-, and z-axis
320 represent antigenic units (AU) with one grid corresponding to a two-fold serum dilution of the
321 neutralization titer. An interactive map is available online
322 (<https://figshare.com/articles/media/OmicronAntigenicMap/19854046>). The map orientation
323 within the x-, y-, and z-axis is free to rotate. For all the panels in **a** and **c**, values above the symbols
324 denote the geometric mean ID₅₀ values and values on the lower left show the sample size (n) for
325 each group. *P* values were determined by using two-tailed Wilcoxon matched-pairs signed-rank
326 tests. The results shown are representative of those obtained in two independent experiments.

327 **Methods**

328

329 **Data reporting**

330 No statistical methods were used to predetermine sample size. The experiments were not
331 randomized and the investigators were not blinded to allocation during experiments and outcome
332 assessment.

333

334 **Serum samples**

335 Sera from individuals who received three doses of the mRNA-1273 or BNT162b2 vaccine were
336 collected at Columbia University Irving Medical Center. Sera from individuals who were infected
337 by non-Omicron variants of SARS-CoV-2 in addition to vaccination were collected from January
338 2021 to September 2021 at Columbia University Irving Medical Center or at the Hackensack
339 Meridian Center for Discovery and Innovation (CDI). Sera from individuals who were infected by
340 Omicron (BA.1 or BA.2) following vaccinations were collected from December 2021 to May 2022
341 at Columbia University Irving Medical Center. All samples were confirmed for prior SARS-CoV-
342 2 infection status by anti-nucleoprotein (NP) ELISA. All collections were conducted under
343 protocols reviewed and approved by the Institutional Review Board of Columbia University or the
344 Hackensack Meridian Center for Discovery and Innovation. All participants provided written
345 informed consent. Clinical information on the different cohorts of study subjects is provided in
346 Extended Data Table 2.

347

348 **Monoclonal antibodies**

349 Antibodies were expressed as previously described¹⁷. Heavy chain variable (VH) and light chain
350 variable (VL) genes for each antibody were synthesized (GenScript), then transfected into Expi293
351 cells (Thermo Fisher Scientific), and purified from the supernatant by affinity purification using
352 rProtein A Sepharose (GE). REGN10987, REGN10933, COV2-2196, and COV2-2130 were
353 provided by Regeneron Pharmaceuticals; Brii-196 and Brii-198 were provided by Bii Biosciences;
354 CB6 was provided by B. Zhang and P. Kwong (NIH); and ZCB11 was provided by Z. Chen (HKU).

355

356 **Cell lines**

357 Expi293 cells were obtained from Thermo Fisher Scientific (A14527); Vero-E6 cells were
358 obtained from the ATCC (CRL-1586); HEK293T cells were obtained from the ATCC (CRL-3216).
359 Cells were purchased from authenticated vendors and morphology was confirmed visually before
360 use. All cell lines tested mycoplasma negative.

361

362 **Variant SARS-CoV-2 spike plasmid construction**

363 BA.1, BA.1.1, and BA.2 spike-expressing plasmids were generated as previously described^{23,26}.
364 Plasmids encoding the BA.2.12.1 and BA.4/5 spikes, as well as the individual and double
365 mutations found in BA.2.12.1 and BA.4/5, were generated using the QuikChange II XL site-
366 directed mutagenesis kit according to the manufacturer's instructions (Agilent). To make the
367 constructs for expression of stabilized soluble S2P spike trimer proteins, 2P substitutions (K986P
368 and V987P) and a "GSAS" substitution of the furin cleavage site (682-685aa in WA1) were
369 introduced into the spike-expressing plasmids³⁵, and then the ectodomain (1-1208aa in WA1) of
370 the spike was fused with a C-terminal 8x His-tag and cloned into the **paH** vector. All constructs
371 were confirmed by Sanger sequencing.

372

373 **Expression and purification of SARS-CoV-2 S2P spike proteins**

374 SARS-CoV-2 S2P spike trimer proteins of the D614G and Omicron subvariants were generated
375 by transfecting Expi293 cells with the S2P spike trimer-expressing constructs using 1 mg mL⁻¹
376 polyethylenimine (PEI) and then purifying from the supernatants five days post-transfection using
377 Ni-NTA resin (Invitrogen) according to the manufacturer's instructions¹⁷.

378

379 **Surface plasmon resonance**

380 Surface plasmon resonance (SPR) binding assays for hACE2 binding to SARS-CoV-2 S2P spike
381 were performed using a Biacore T200 biosensor equipped with a Series S CM5 chip (Cytiva), in a
382 running buffer of 10 mM HEPES pH 7.4, 150 mM NaCl, 3 mM EDTA, 0.05% P-20 (Cytiva) at
383 25 °C. Spike proteins were captured through their C-terminal His-tag over an anti-His antibody
384 surface. These surfaces were generated using the His-capture kit (Cytiva) according to the
385 manufacturer's instructions, resulting in approximately 10,000 RU of anti-His antibody over each
386 surface. An anti-His antibody surface without antigen was used as a reference flow cell to remove
387 bulk shift changes from the binding signal.

388 Binding of human ACE2-Fc protein (Sino Biological) was tested using a three-fold dilution
389 series with concentrations ranging from 2.46 nM to 200 nM. The association and dissociation rates
390 were each monitored for 60 s and 300 s respectively, at 30 $\mu\text{L}/\text{min}$. The bound spike/ACE2
391 complex was regenerated from the anti-His antibody surface using 10 mM glycine pH 1.5. Blank
392 buffer cycles were performed by injecting running buffer instead of human ACE2-Fc to remove
393 systematic noise from the binding signal. The resulting data was processed and fit to a 1:1 binding
394 model using Biacore Evaluation Software.

395

396 **Pseudovirus production**

397 Pseudoviruses were produced in the vesicular stomatitis virus (VSV) background, in which the
398 native glycoprotein was replaced by that of SARS-CoV-2 and its variants, as previously
399 described¹⁷. In brief, HEK293T cells were transfected with a spike expression construct with 1 mg
400 mL^{-1} polyethylenimine (PEI) and cultured overnight at 37 °C under 5% CO_2 , and then infected
401 with VSV-G pseudotyped ΔG -luciferase ($\text{G}^*\Delta\text{G}$ -luciferase, Kerafast) one day post-transfection.
402 After 2 h of infection, cells were washed three times, changed to fresh medium, and then cultured
403 for approximately another 24 h before the supernatants were collected, clarified by centrifugation,
404 and aliquoted and stored at -80 °C for further use.

405

406 **Pseudovirus neutralization assay**

407 All viruses were first titrated to normalize the viral input between assays. Heat-inactivated sera or
408 antibodies were first serially diluted (five-fold) in medium in 96-well plates in triplicate, starting
409 at 1:100 dilution for sera and 10 $\mu\text{g mL}^{-1}$ for antibodies. Pseudoviruses were then added and the
410 virus-sample mixture was incubated at 37 °C for 1 h. Vero-E6 cells were then added at a density
411 of 3×10^4 cells per well and the plates were incubated at 37 °C for approximately 10 h. Luciferase
412 activity was quantified using the Luciferase Assay System (Promega) according to the
413 manufacturer's instructions using SoftMax Pro v.7.0.2 (Molecular Devices). Neutralization curves
414 and IC_{50} values were derived by fitting a nonlinear five-parameter dose-response curve to the data
415 in GraphPad Prism v.9.2.

416

417 **Authentic virus neutralization assay**

418 The SARS-CoV-2 viruses hCoV-19/USA/CO-CDPHE-2102544747/2021 (BA.2) and hCoV-
419 19/USA/MD-HP30386/2022 (BA.4) were obtained from BEI Resources (NIAID, NIH) and
420 propagated by passaging in Vero-E6 cells. Virus infectious titers were determined by an end-point
421 dilution and cytopathogenic effect assay on Vero-E6 cells as previously described¹⁷.

422

423 An end-point dilution microplate neutralization assay was performed to measure the neutralization
424 activity of sera from vaccinated and boosted individuals as well as of purified monoclonal
425 antibodies. In brief, serum samples were subjected to successive five-fold dilutions starting from
426 1:100. Monoclonal antibodies were serially diluted (five-fold) starting at 5 µg/ml. Triplicates of
427 each dilution were incubated with SARS-CoV-2 at a multiplicity of infection of 0.1 in EMEM
428 with 7.5% inactivated fetal calf serum for 1 h at 37 °C. After incubation, the virus-antibody
429 mixture was transferred onto a monolayer of Vero-E6 cells grown overnight. The cells were
430 incubated with the mixture for around 70 h. Cytopathogenic effects of viral infection were visually
431 scored for each well in a blinded manner by two independent observers. The results were then
432 converted into the percentage of neutralization at a given sample dilution or monoclonal antibody
433 concentration, and the data (mean ± SEM) were plotted using a five-parameter dose-response curve
434 in GraphPad Prism v.9.2.

435

436 **Antibody targeting frequency and mutagenesis analysis for RBD**

437 The SARS-CoV-2 spike structure (6ZGE) used for displaying epitope footprints was downloaded
438 from the Protein Data Bank (PDB). Epitope residues were identified using PISA³⁶ with default
439 parameters, and the RBD residues with non-zero buried accessible surface area were considered
440 epitope residues. For each residue within the RBD, the frequency of antibody recognition was
441 calculated as the number of contact antibodies³⁷. The structures of antibody-spike complexes for
442 modeling were also obtained from PDB (7L5B (2-15), 6XDG (REGN10933), and 7KMG (LY-
443 CoV555)). Omicron BA.1 RBD in complex with hACE2 was downloaded from PDB 7U0N, and
444 the ligand-free BA.2 RBD was downloaded from PDB 7UB0. PyMOL v.2.3.2 was used to perform
445 mutagenesis and to generate structural plots (Schrödinger, LLC).

446

447 **Antigenic cartography**

448 The antigenic distances between SARS-CoV-2 variants were approximated by incorporating the
449 neutralization potency of each serum sample into a previously described antigenic cartography
450 approach²⁷. The map was generated by the Racmacs package (<https://acorg.github.io/Racmacs/>,
451 version 1.1.4) in R with the optimization steps set to 2000, and with the minimum column basis
452 parameter set to “none”.
453

454 **Acknowledgements**

455 This study was supported by funding from the Gates Foundation, JPB Foundation, Andrew and
456 Peggy Cherng, Samuel Yin, Carol Ludwig, David and Roger Wu, Regeneron Pharmaceuticals,
457 and the NIH SARS-CoV-2 Assessment of Viral Evolution (SAVE) Program. We acknowledge
458 David S. Perlin for providing serum samples from a few COVID-19 patients. We thank all who
459 contributed their data to GISIAD.

460

461 **Author contributions**

462 D.D.H. and L.L. conceived this project. Q.W. and L.L. conducted pseudovirus neutralization
463 experiments and purified SARS-CoV-2 spike proteins. Y.G. and Z.S. conducted bioinformatic
464 analyses. Q.W., L.L., and S.I. constructed the spike expression plasmids. Q.W. managed the
465 project. J.Y. M.W., and Z.C. expressed and purified antibodies. L.L. and Z.L. performed surface
466 plasmon resonance (SPR) assay. M.T.Y., M.E.S., J.Y.C., A.D.B. J.G.S., N.N., and K.M. provided
467 clinical samples. H.M. aided sample collections. M.S.N. and Y.H. performed infectious SARS-
468 CoV-2 neutralization assays. D.D.H. and L.L. directed and supervised the project. Q.W., Y.G.,
469 L.L., and D.D.H. analyzed the results and wrote the manuscript.

470

471 **Competing interests**

472 S.I, J.Y., Y.H., L.L., and D.D.H. are inventors on patent applications (WO2021236998) or
473 provisional patent applications (63/271,627) filed by Columbia University for a number of SARS-
474 CoV-2 neutralizing antibodies described in this manuscript. Both sets of applications are under
475 review. D.D.H. is a co-founder of TaiMed Biologics and RenBio, consultant to WuXi Biologics
476 and Brie Biosciences, and board director for Vicarious Surgical.

477

478 **Additional information**

479 Correspondence and requests for materials should be addressed to L.L. or D. D. H.

480 Reprints and permissions information is available at www.nature.com/reprints.

481

482 **Data availability**

483 All data are provided in the manuscript. Materials in this study will be made available under an
484 appropriate Materials Transfer Agreement. Sequences for Omicron prevalence analysis were

485 downloaded from GISAID (<https://www.gisaid.org/>). The structures used for analysis in this study
486 are available from PDB under IDs 6ZGE, 7L5B, 6XDG, 7U0N, 7UB0 and 7KMG. The interactive
487 antigenic map based on the neutralization data of boosted vaccine sera in Figure 4d is available
488 online (<https://figshare.com/articles/media/OmicronAntigenicMap/19854046>).
489

490 **Extended Data Figure Legends**

491

492 **Extended Data Fig. 1 | Pseudovirus (a) and authentic virus (b) neutralization curves of**
493 **D614G and Omicron subvariants by monoclonal antibodies.** Data are shown as mean \pm SEM
494 from three technical replicates and representative of those obtained in two independent
495 experiments.

496

497 **Extended Data Fig. 2 | Neutralization IC₅₀ values for indicated pseudoviruses (a) and**
498 **authentic viruses (b) by monoclonal antibodies.** Data are representative of those obtained in two
499 independent experiments.

500

501 **Extended Data Fig. 3 | Pseudovirus neutralization curves for monoclonal antibodies against**
502 **individual SARS-CoV-2 mutations in the background of D614G.** Data are shown as mean \pm
503 SEM from three technical replicates and representative of those obtained in two independent
504 experiments.

505

506 **Extended Data Fig. 4 | Pseudovirus neutralization curves for monoclonal antibodies against**
507 **individual SARS-CoV-2 mutations in the background of BA.2.** Data are shown as mean \pm SEM
508 from three technical replicates and representative of those obtained in two independent
509 experiments.

510

511 **Extended Data Fig. 5 | Pseudovirus neutralization IC₅₀ values for monoclonal antibodies.** IC₅₀
512 values of **a**, D614G carrying individual mutations; **b**, BA.2 carrying individual mutations. Data
513 are representative of those obtained in two independent experiments.

514

515 **Extended Data Fig. 6 | Neutralization curves of serum against D614G and Omicron**
516 **subvariants.** Neutralization by **a**, boosted vaccinee sera on pseudoviruses. **b**, non-Omicron
517 infection & vaccination sera on pseudoviruses. **c**, BA.1 breakthrough sera on pseudoviruses. **d**,
518 BA.2 breakthrough sera on pseudoviruses. **e**, boosted vaccinee sera on authentic viruses. **f**,
519 Neutralization ID₅₀ titers of authentic BA.2 and BA.4 by boosted vaccinee sera. Values above the
520 symbols denote the geometric mean ID₅₀ values and values on the lower left show the sample size

521 (n). *P* values were determined by using two-tailed Wilcoxon matched-pairs signed-rank tests.
522 Error bars in **a**, **b**, **c**, **d**, and **e** denote mean \pm SEM for three technical replicates. All data are
523 representative of those obtained in two independent experiments.

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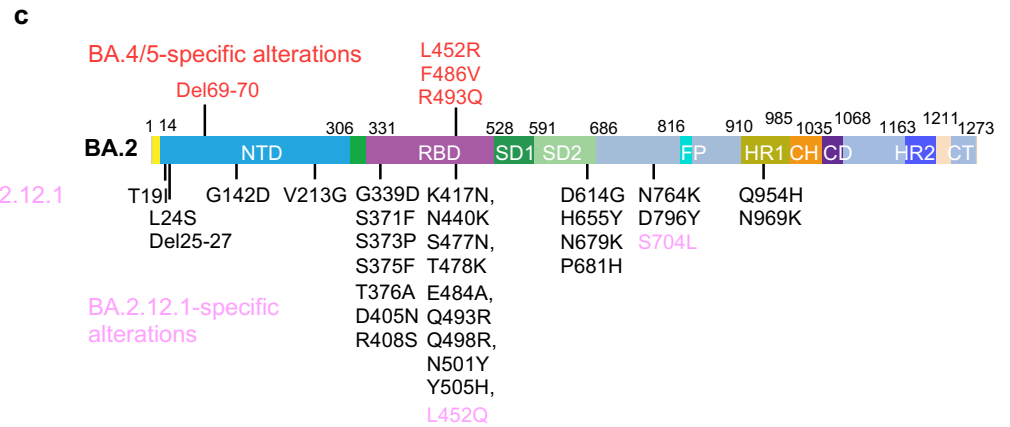
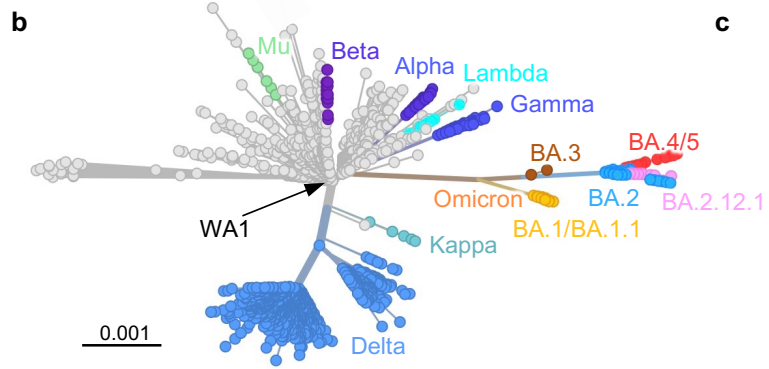
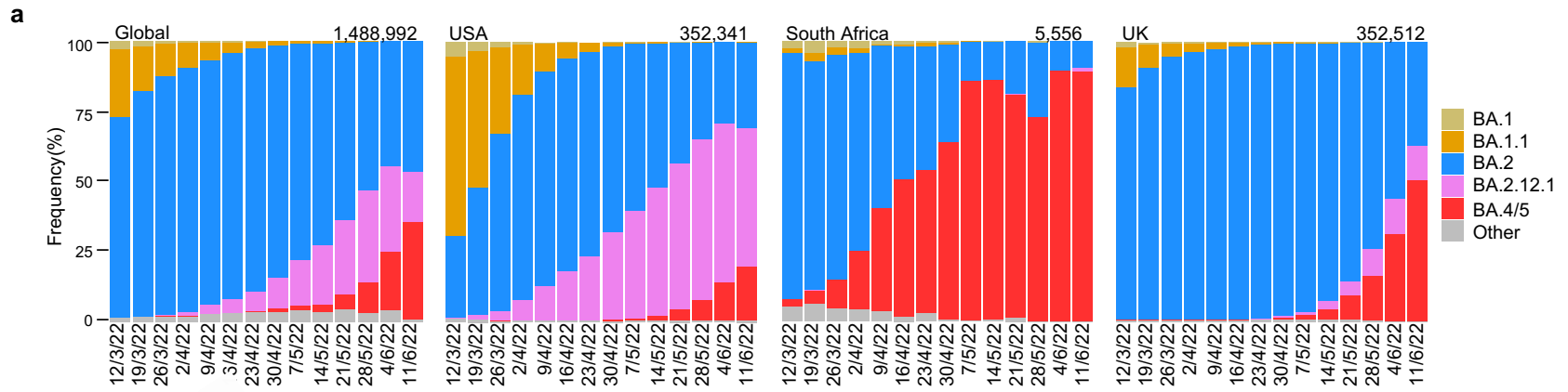
525 **Extended Data Fig. 7 | Pseudovirus neutralization curves of serum against BA.2 and BA.2**
526 **pseudovirus carrying individual mutations.** Neutralization by **a**, boosted vaccinee sera. **b**, non-
527 Omicron infection & vaccination sera. **c**, BA.1 breakthrough sera. **d**, BA.2 breakthrough sera.
528 Error bars denote mean \pm SEM for three technical replicates. Data are representative of those
529 obtained in two independent experiments.

530

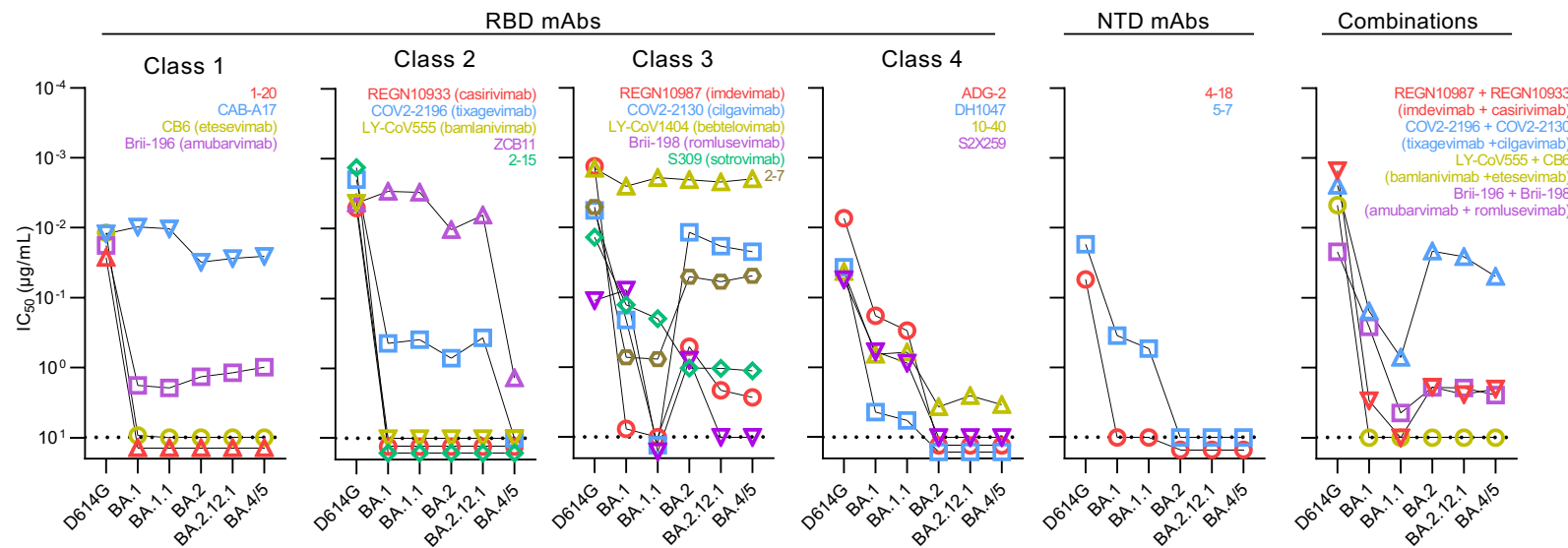
531 **Extended Data Table 1 | Mutation frequencies at position F486 within different SARS-CoV-**
532 **2 variants.**

533

534 **Extended Data Table 2 | Demographics on the clinical cohorts.**



a

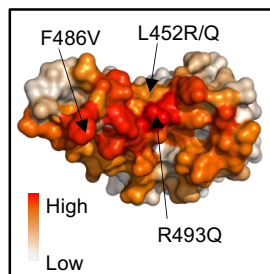


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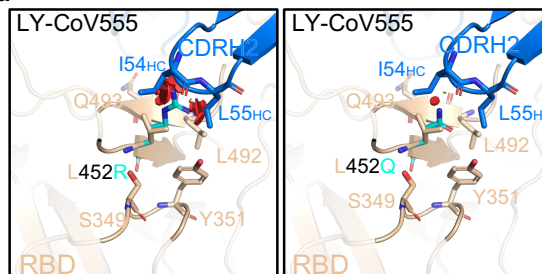
Fold change in IC ₅₀		RBD mAbs																		NTD mAbs		Combinations				
		Class 1				Class 2					Class 3					Class 4				4-18	5-7	REGN-10987 + REGN-10933	COV2-2196 + COV2-2130	LY-CoV555 + CB6	Brii-196 + Brii-198	
		1-20	CAB-A17	CB6	Brii-196	REGN10933	COV2-2196	LY-CoV555	ZCB11	2-15	REGN10987	COV2-2130	LY-CoV1404	Brii-198	S309	2-7	ADG-2	DH1047	10-40	S2X259						
Compared with D614G	D614G-Del69-70	1.3	1.1	1.3	-1.3	-1.0	-1.0	2.0	-1.4	1.3	-1.3	-2.1	-1.3	-1.1	-2.0	-1.0	-1.6	-1.3	-1.7	-1.6	-1.1	-4.6	-1.7	-1.5	-1.1	-1.0
	D614G-L452M	1.5	-1.0	1.5	-1.0	<-1.9	-1.0	-1.8	-1.0	-0.9	-1.5	-1.7	-1.0	-6.9	-2.0	-1.0	-1.5	1.1	1.3	-1.0	-1.1	-1.3	-1.5	-1.1	-1.0	
	D614G-L452R	-1.2	-1.3	-1.4	-1.5	<-2.0	-1.0	-2266	-1.1	-16	-2.1	-2.6	-1.2	-27	-1.6	-1.8	-1.3	-1.0	1.4	1.8	1.1	-1.1	-1.3	-1.0	-3.9	-1.3
	D614G-L452Q	-1.3	1.1	-1.0	-1.4	<-2.1	-1.0	-5.3	-1.0	-11	-3.7	-3.8	-1.2	-18	-2.7	-2.2	-1.5	-1.5	-1.8	-1.7	-2.0	-1.6	-2.5	-1.7	-3.0	-1.9
	D614G-F486V	-1.5	-1.2	-8.1	-14	<-10000	<-272	-886	-712	-24	-1.1	-1.4	-1.3	-1.4	-1.0	-0.8	-1.4	1.1	-1.7	-2.2	1.1	-1.0	-4.8	-10	-154	-11
	D614G-S704L	1.3	-1.0	-1.6	-1.2	-1.0	-1.0	-1.0	-1.0	-1.3	-1.6	-3.0	1.2	-1.5	-2.7	-1.6	-1.3	-1.7	-1.9	-1.9	-1.9	-1.1	-1.8	-1.5	-1.5	-1.2
D614G-L452Q/S704L	-1.3	-1.3	-1.9	-1.6	<-7.2	<-1.5	-8.5	-2.8	-32	-4.2	-4.9	-1.5	-48	-2.0	-3.1	-1.8	-1.4	-2.4	-3.0	-2.0	-2.1	-2.4	-2.1	-3.6	-2.2	
Compared with BA.2	BA.2-Del69-70	-1.0	-1.5	-1.0	-2.1	-1.0	1.3	-1.0	1.1	-1.0	1.7	1.1	-1.2	1.6	-1.2	-1.0	-1.0	-1.0	<-2.1	-1.0	-1.0	-1.0	-1.5	-1.0	-1.0	1.2
	BA.2-L452M	-1.0	-1.4	-1.0	1.5	-1.0	1.1	-1.0	-1.5	-1.0	-2.2	-2.8	-1.4	<-16	-1.4	-1.3	-1.0	-1.0	1.1	-1.0	-1.0	-1.0	1.2	-1.0	-1.0	1.4
	BA.2-L452R	-1.0	-1.8	-1.0	-5.1	-1.0	-1.2	-1.0	-1.8	-1.0	-5.7	-4.6	-1.1	<-16	-2.6	1.2	-1.0	-1.0	<-2.1	-1.0	-1.0	-1.0	1.3	-1.8	-1.0	<-6.3
	BA.2-L452Q	-1.0	-1.3	-1.0	-1.3	-1.0	1.6	-1.0	1.5	-1.0	-1.8	-1.9	-1.4	<-16	-1.4	-1.6	-1.0	-1.0	<-2.1	-1.0	-1.0	-1.0	-3.2	-1.5	-1.0	-3.5
	BA.2-F486V	-1.0	-8.6	-1.0	<-7.5	1.0	<-9.4	-1.0	<-2182	-1.0	-3.4	1.1	1.1	-1.4	-1.0	-1.7	-1.0	-1.0	-1.2	-1.0	-1.0	-5.0	-1.2	-1.0	-3.4	
	BA.2-R493Q	>5.0	2.7	-1.0	41.0	>10	22	-1.0	4.6	-1.0	1.1	-1.7	-1.7	-1.7	-1.2	-1.5	-1.0	-1.0	1.6	-1.0	-1.0	2.3	1.8	-1.0	76	
	BA.2-S704L	-1.0	-1.2	-1.0	-1.1	-1.0	1.5	-1.0	1.2	-1.0	1.9	-1.1	-1.3	-1.1	-1.4	1.1	-1.0	-1.0	1.9	-1.0	-1.0	1.2	1.7	-1.0	2.0	
BA.2-F486V/R493Q	-1.0	1.3	-1.0	3.4	-1.0	<-9.4	-1.0	-1694	-1.0	1.5	1.7	1.6	2.2	1.9	1.4	-1.0	-1.0	1.8	-1.0	-1.0	1.7	1.1	-1.0	2.7		

>10
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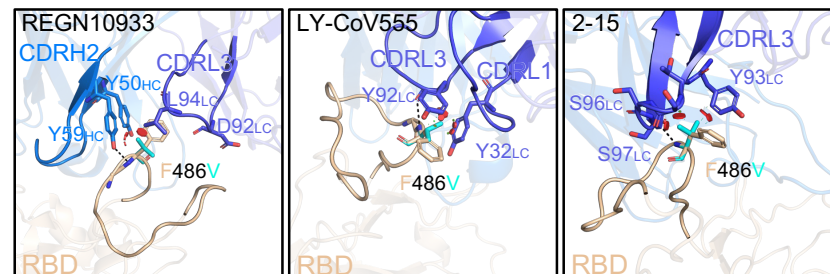
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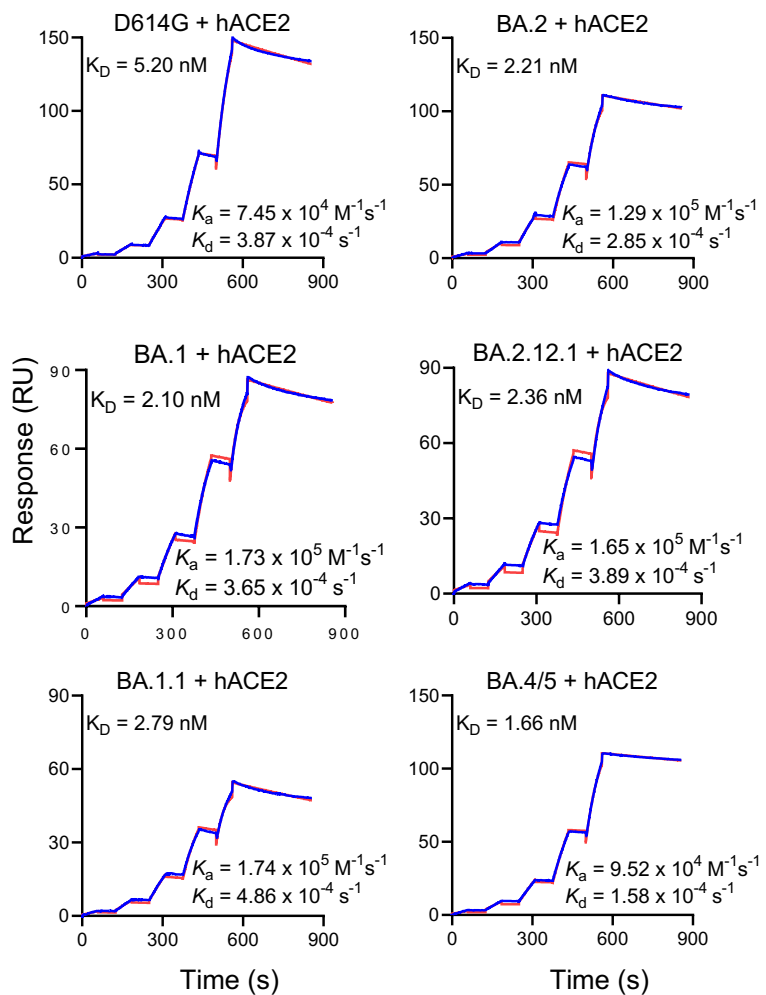
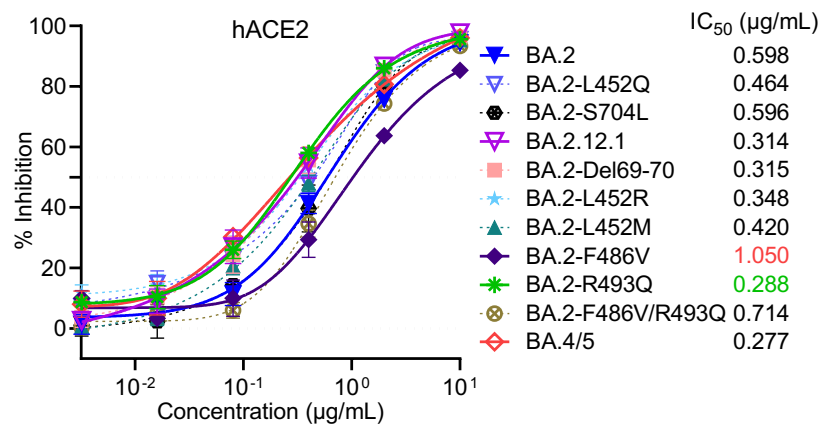
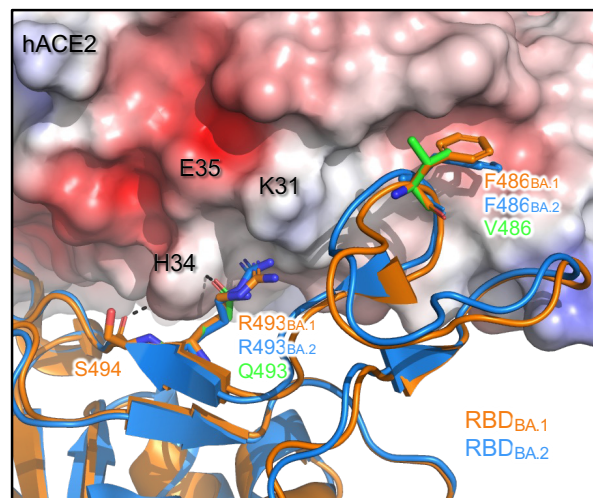


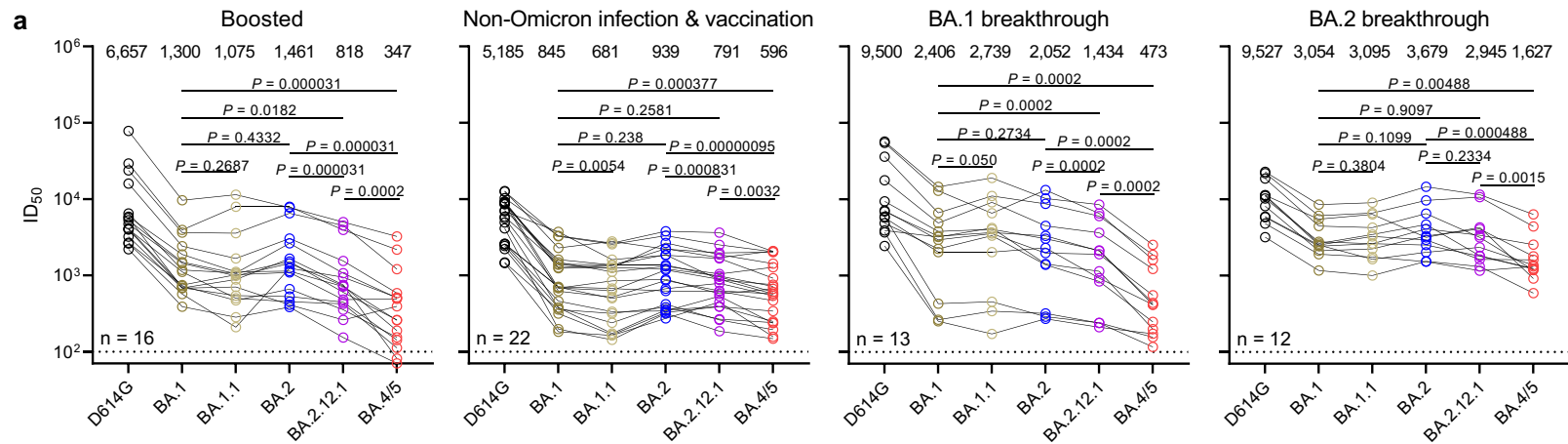
d



e



a**b****c**



b

Variant	Fold change in geometric mean titers	
	Compared with D614G	Compared with BA.2
D614G	1.0	4.6
BA.1	5.1	1.1
BA.1.1	6.2	1.4
BA.2	4.6	1.0
BA.2.12.1	8.1	1.8
BA.4/5	19.2	4.2
BA.2-Del69-70	9.6	2.1
BA.2-L452M	9.2	2.0
BA.2-L452R	11.0	2.4
BA.2-L452Q	10.1	2.2
BA.2-F486V	9.9	2.2
BA.2-R493Q	3.0	1.5
BA.2-S704L	5.5	1.2

Resistance

- >3.0
- 1.5-3.0
- 1-1.5
- 1.5-3.0
- >3.0

Sensitization

