

1 **Site of vulnerability on SARS-CoV-2 spike induces broadly protective**
2 **antibody to antigenically distinct omicron SARS-CoV-2 subvariants**

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34 **Summary**

35 The rapid evolution of SARS-CoV-2 Omicron variants has emphasized the need to
36 identify antibodies with broad neutralizing capabilities to inform future monoclonal
37 therapies and vaccination strategies. Herein, we identify S728-1157, a broadly
38 neutralizing antibody (bnAb) targeting the receptor-binding site (RBS) and derived from
39 an individual previously infected with SARS-CoV-2 prior to the spread of variants of
40 concern (VOCs). S728-1157 demonstrates broad cross-neutralization of all dominant
41 variants including D614G, Beta, Delta, Kappa, Mu, and Omicron
42 (BA.1/BA.2/BA.2.75/BA.4/BA.5/BL.1). Furthermore, it protected hamsters against *in vivo*
43 challenges with wildtype, Delta, and BA.1 viruses. Structural analysis reveals that this
44 antibody targets a class 1 epitope via multiple hydrophobic and polar interactions with its
45 CDR-H3, in addition to common class 1 motifs in CDR-H1/CDR-H2. Importantly, this
46 epitope is more readily accessible in the open and prefusion state, or in the hexaprotline
47 (6P)-stabilized spike constructs, as compared to diprotline (2P) constructs. Overall, S728-
48 1157 demonstrates broad therapeutic potential, and may inform target-driven vaccine
49 design against future SARS-CoV-2 variants.

50

51

52 **Introduction**

53 Since the start of the pandemic in December 2019, the severe acute respiratory syndrome
54 coronavirus 2 (SARS-CoV-2) virus has led to over 576 million cases of coronavirus
55 disease 2019 (COVID-19) and over six million deaths globally. Although the rapid
56 development and distribution of vaccines and therapeutics has curbed the impact of
57 COVID-19 to an extent, the emergence of circulating variants of concern (VOCs)
58 continues to represent a major threat due to the potential for further immune evasion and
59 enhanced pathogenicity. The D614G variant was the earliest variant to emerge and
60 became universally prevalent thereafter. In comparison to wildtype (WT), the D614G
61 variant exhibited increased transmissibility rather than increased pathogenicity and was
62 therefore unlikely to reduce efficacy of vaccines in clinical trials¹. Between the emergence
63 D614G and October 2021, four additional significant VOC evolved worldwide, including
64 Alpha, Beta, Gamma, and Delta. Among these variants, Delta became a serious global
65 threat as a result of its transmissibility, increased disease severity, and partial immune
66 evasion as shown by the reduced ability of polyclonal serum and monoclonal antibodies
67 (mAbs) to neutralize this strain²⁻⁶. Shortly afterwards, in November 2021, the Omicron
68 variant was identified and announced as a novel VOC. This variant possessed the largest
69 number of mutations to date and appeared to spread more rapidly than previous strains^{7,8}.
70 Currently, there are five major subvariant lineages of Omicron (BA.1, BA.2, BA.3, BA.4
71 and BA.5) leading to new COVID-19 cases, with BA.5 becoming dominant over BA.2 and
72 accounting for most new cases in the United States at the time of writing. The Omicron
73 variants can escape recognition by COVID-19 vaccine-associated immunity to varying
74 extents, thereby significantly reducing the neutralizing potency of serum antibodies from
75 convalescent and fully mRNA-vaccinated individuals⁹. Similarly, Omicron variants were
76 able to escape binding of several Emergency Use-Authorization (EUA) therapeutic mAbs
77 even though these had been previously shown to be effective against earlier VOCs^{10,11}.
78 Due to the lowered neutralization against Omicron and the continued threat of future
79 VOCs, there is an urgent need to identify broad and potent neutralizing antibodies that
80 can protect against diverse evolving SARS-CoV-2 lineages.

81 In this study, we identify a potent RBD-reactive monoclonal antibody from the
82 peripheral blood of SARS-CoV-2 convalescent individual that effectively neutralize Alpha,
83 Beta, Kappa, Delta, Mu, and Omicron variants (BA.1, BA.2, BA.2.75, BA.4, BA.5 and
84 BL.1). This mAb, S728-1157, can reduce BA.1 Omicron viral titer *in vivo* and significantly
85 reduced viral loads during wildtype and Delta infection. In terms of specificity, S728-1157
86 bound the receptor binding site (RBS) that is fully exposed when the RBD on the spike is
87 in the up conformation. S728-1157 binds using motifs found in the CDR-H1 and CDR-H2
88 domains that are common to IGHV3-53/3-66 class 1 antibodies but also via extensive
89 unique contacts with CDR-H3 to circumvent mutations in the variant virus spikes. This
90 suggests that the rational design of future vaccine boosts covering Omicron variants
91 should be modified to present stabilized spike in the up configuration to optimally induce
92 class 1 mAbs that have similar CDR-H3 features.

93

94 **Results**

95 **Isolation of RBD-reactive mAbs that exhibit diverse patterns of neutralization and** 96 **potency**

97 Before the spread of the Omicron variant, we previously characterized 43 mAbs targeting
98 distinct epitopes on the spike protein, including the N-terminal domain (NTD), RBD, and
99 subunit 2 (S2), although none were able to neutralize all existing SARS-CoV-2 variants
100 at that time¹². In the current study, an additional panel of RBD-reactive mAbs were
101 expressed from three high-responder subjects who mounted robust anti-spike IgG
102 responses, as defined previously ([Table S1 and Table S2](#))¹³. Although the proportion of
103 spike RBD-binding B cells was similar in high-responders as compared to mid- and low-
104 responders ([Figure 1a-c](#)), heavy chain somatic hypermutation rates were significantly
105 greater in the high-responder group ([Figure 1d](#)), suggesting that these subjects may have
106 the highest potential to generate potent cross-reactive mAbs¹³. These antibodies were
107 assayed for binding to key RBD mutants to identify their epitope classifications ([Table](#)
108 [S3](#))¹⁴. Among 14 RBD-reactive mAbs, we identified four class 2 mAbs, two class 3 mAbs,
109 and eight unclassified mAbs that showed little to no reduction of binding against any key
110 RBD mutants tested ([Figure 1f](#)). Class 2 and 3 RBD mAbs did not recognize a multivalent

111 RBD mutant containing K417N/E484K/L452R/N501Y substitutions, an artificially
112 designed RBD to include key mutations for virus escape^{14,15}, nor cross-reactivity to the
113 RBD of SARS-CoV-1 and Middle Eastern respiratory syndrome (MERS)-CoV (Figure 1f).
114 Functionally, class 2 and 3 RBD mAbs potently neutralized D614G and Delta but
115 neutralizing activity was limited against Beta, Kappa and Mu (Figure 1g). No class 2 or 3
116 antibodies assayed could neutralize any tested Omicron variant.

117 In contrast, the majority of unclassified mAbs bound to the RBD multivariant and
118 cross-reacted to the SARS-CoV-1 RBD (Figure 1f). Among these, we went on to identify
119 three bnAbs, S451-1140, S626-161 and S728-1157, that showed high neutralization
120 potency against D614G and could cross-neutralize Beta, Delta, Kappa, Mu and BA.1 with
121 99% inhibitory concentration (IC₉₉) in the range of 20-2500 ng/ml (Figure 1g). Given the
122 broad neutralization potency of these three mAbs, in addition of plaque assay platform,
123 we also performed the neutralization activity against authentic BA.2.75, BL.1
124 (BA.2.75+R346T), BA.4, and BA.5 viruses using focus reduction neutralization test
125 (FRNT) (Figure 1g). Of these, S728-1157 displayed high neutralizing activities against
126 the panel of Omicron variants including BA.1, BA.2, BA.4 and BA.5, with IC₉₉ up to 100
127 ng/ml as measured by plaque assay. A similar scenario was observed using FRNT, S728-
128 1157 maintains its high neutralization activity against BA.2.75, BL.1, BA.4 and BA.5 with
129 50% inhibitory concentration (IC₅₀) in the range of 8-16 ng/ml (Figure 1g). S451-1140
130 neutralized BA.1, BA.2, BA.2.75 and BL.1 potently, but not BA.4 and BA.5 as observed
131 in both neutralization assay platforms. On the other hand, S626-161 did not demonstrate
132 neutralizing activity against Omicron variants beyond the BA.1 variant (Figure 1g).
133 Although S626-161 had a lower neutralization potency against VOC than the other two,
134 it was the only mAb which showed cross-reactivity to SARS-CoV-1 RBD and was able to
135 neutralize bat coronaviruses WIV-1 and RsSHC014 (Figure 1f-g). These data suggest
136 that S626-161 recognizes a conserved epitope that is shared between these sarbecovirus
137 lineages, but is absent in BA.2. Additionally, compared to S728-1157 and S451-1140,
138 S626-161 has a longer CDR-H3 which could provide an enhanced capability to recognize
139 a highly conserved patch of residues shared across sarbecoviruses as described in a
140 previous study¹⁶ (Figure S1). When comparing immunoglobulin heavy (IGHV) and light

141 chain (IGLV or IGKV) variable genes of these three bnAbs with the available SARS-CoV-
142 2 neutralizing mAbs database^{12,17-25}, we found that heavy chain variable genes utilized
143 by S728-1157 (IGHV3-66), S451-1140 (IGHV3-23) and S626-161 (IGHV4-39) have been
144 previously reported to encode several potentially neutralizing SARS-CoV-2 antibodies
145 targeting the RBD^{18,19,26,27}. However, only S728-1157 had unique heavy and light chain
146 variable gene pairings that have not been reported in the database (Table S2), indicating
147 that it is not public clonotype.

148 These three bnAbs (S451-1140, S626-161 and S728-1157) were characterized
149 further to determine the binding breadth against SARS-CoV-2 VOCs (Figure 1h-k). The
150 prefusion-stabilized spike containing two-proline substitutions in the S2 subunit (2P;
151 diproline) has been shown to be a superior immunogen compared to the wildtype spike
152 and is the basis of several current SARS-CoV-2 vaccines, including current mRNA-based
153 vaccines^{28,29}. More recently, spike protein stabilized with six prolines (6P; hexaproline)
154 was shown to boost expression and be even more stable than the original diproline
155 construct; as a result, it has been proposed for use in improving the next-generation of
156 COVID-19 vaccines^{30,31}. To determine if there are antigenicity differences between the
157 diproline and hexaproline spike constructs, both immunogens were included in our test
158 panel. As measured by ELISA assay, we found that three bnAbs bound 6P-WT spike
159 antigen to a greater extent compared to WT-2P spike (Figure 1h-j). All three bnAbs
160 showed comparable binding to the spikes of Alpha, Beta, Gamma and Delta viruses,
161 relative to that of WT-2P (Figure 1h-j). However, the binding reactivity of these three
162 bnAbs were substantially reduced against a panel of Omicron-family antigens (Figure 1h-
163 k). S451-1140 binding was sensitive to mutations found in BA.1 and BA.2, resulting in
164 largely decrease in binding and a 31-fold decrease in neutralization against these variants
165 compared with WT-2P antigen and D614G virus, respectively (Figure 1g, i, k). The
166 sarbecovirus-cross neutralizing mAb, S626-161 also showed 1.7 to 3.9-fold reduced
167 binding to spike BA.1 antigens which may be affected in a 2-fold reduction in
168 neutralization activity against BA.1 (Figure 1g, j, k). For the most potent bnAb, S728-1157,
169 binding to Omicron antigens was substantially reduced by greater than 1.7-fold (range of
170 1.7- to 5.5-fold) compared with WT-2P spike but was unaffected in neutralizing activity

171 (Figure 1g, h, k). Notably, all three bnAbs showed over 3-fold increased binding to spike
172 BA.1-6P compared with the BA.1-2P version, suggesting a better accessibility of bnAbs
173 to the hexaproline spike BA.1 construct. In addition to ELISA, biolayer interferometry (BLI)
174 was used to quantify the binding rate and equilibrium constants (k_{on} , k_{off} , and K_D) of these
175 three bnAbs to a panel of spike antigens (Figure S2b-d). The recognition k_{on} rates of Fabs
176 were 1.5 to 3.3-fold faster to hexaproline spikes, showing that the antibodies bound to the
177 6P construct more rapidly than to 2P. This is expected if the epitopes are more exposed
178 on the RBD in the open state on the hexaproline spike (Figure S2c). Except for S626-
179 161, off-rate of the Fabs were also longer such that the overall K_D showed that S728-1157
180 and S451-1140 bound to the hexaproline spike with substantially greater affinity (Figure
181 S2c-d). The increased off rates further suggest partial occlusion of the binding site on
182 diproline spike. The improved binding to hexaproline spike was even more notable for
183 whole dimeric IgG by the 1:2 interaction model and for all three bnAbs, consistent with
184 exposure of multiple epitopes with 6P stabilization allowing improved avidity (Figure S2b-
185 d). Taken together, these results suggest that the epitopes targeted may be comparatively
186 more accessible on the 6P-stabilized spike with the RBD in the open state. Structural
187 analyses were next performed to verify this conjecture.

188

189 **Structural analysis of broadly neutralizing monoclonal antibodies**

190 As a first approximation of epitopes bound, an ELISA competition assay was used to
191 determine whether the three broadly-neutralizing mAbs shared any overlap with our
192 current panel of mAbs and a collection of mAbs with known epitope specificities from
193 previous studies^{12,32,33}, plus two other mAbs currently in clinical use (LY-CoV555 (Eli
194 Lilly)³⁴ and REGN10933 (Regeneron)³⁵). The binding sites of S451-1140 and S728-1157
195 partially overlapped with CC12.3^{33,36}, a class 1 neutralizing antibody, and most class 2
196 antibodies, including LY-CoV555 and REGN10933, but not with class 3 and class 4
197 antibodies (Figure 2a). S626-161 shared a notable overlap in binding region with class 1
198 CC12.3, several class 4 antibodies including CR3022, and other unclassified antibodies,
199 while having some partial overlap with several class 2 and one class 3 antibodies (Figure
200 2a). Analogously, competition BLI assay revealed that S451-1140 and S728-1157

201 strongly competed with one another for binding to spike WT-6P, whereas S626-161 did
202 not (Figure S3). Overall, these data suggest S451-1140 and S728-1157 recognize similar
203 epitopes that are distinct from S626-161.

204 S728-1157 was encoded by IGHV3-66 and possessed a short complementarity
205 determining region 3 (CDR-H3). Notably, mAbs that bind the receptor binding site (RBS)
206 in binding mode 1 (i.e. RBS-A or class 1 site), typified by CC12.1, CC12.3, B38, and
207 C105^{15,25,27,36-38}, tend to use IGHV3-53/3-66 and are sensitive to VOC mutations³⁹.
208 However, the CDR-H3 region of S728-1157 is highly distinct from other antibodies of this
209 class, potentially accounting for its broader activity. To understand the structural basis of
210 broad neutralization by S728-1157 at this epitope, we resolved a cryo-electron
211 microscopy (cryo-EM) structure (Figure 2b) of IgG S728-1157 in complex with spike WT-
212 6P-Mut7, a version of spike WT-6P possessing interprotomer disulfide bond at C705 and
213 C883, at ~3.3 Å global resolution (Figure S4e). Using symmetry expansion, focused
214 classification, and refinement methods, we achieved local resolution at the RBD-Fv
215 interface to ~4Å (Figure S4e and Table S6). A crystal structure of S728-1157 Fab was
216 determined at 3.1 Å resolution and used to build the atomic model at the RBD-Fv
217 interface. Our structures confirm that S728-1157 binds the RBS-A (or class 1) epitope in
218 the RBD-up conformation (Figure 2b and Figure S4e), similar to other IGHV3-53/3-66
219 antibodies (Figure 2c). Steric blockage of the angiotensin converting enzyme 2 (ACE2)
220 binding site by S728-1157 explains its high neutralization potency against SARS-CoV-2.
221 The ₃₂NY₃₃ motif and ₅₃SGGS₅₆ motif³⁶ in S728-1157 CDR-H1 and-H2 interact with the
222 RBD in almost the same way as CC12.3 (Figure S4b-c). However, V_H ₉₈DY₉₉ in S728-
223 1157 CDR-H3 forms more extensive interactions including both hydrophobic and polar
224 interactions with the RBD, compared to V_H ₉₈DF₉₉ in CC12.3 (Figure 2d and Table S5).
225 The diglycine V_H ₁₀₀GG₁₀₁ in S728-1157 CDR-H3 may also facilitate more extensive
226 binding compared to V_H Y₁₀₀ in CC12.3 likely due to the flexibility in the glycine residues
227 that lead to a different conformation of the tip of the CDR-H3 loop and a relative shift of
228 residues at ₉₈DY₉₉.

229 Although the Omicron VOCs have extensive mutations in the RBD (Figure 2c and
230 Figure S2a), most of these residues do not make interactions with or are dispensable for

231 binding to S728-1157, as binding is still observed (Figure S4a). From our spike WT-6P-
232 Mut7 + Fab S728-1157 model, Y505 to V_L Q31, and E484 to V_H Y99 are predicted to make
233 hydrogen bonds (Figure S4d and Table S5), which have the potential to be disrupted by
234 Omicron mutations Y505H and E484A. However, a Y505H mutation would still allow for
235 a hydrogen bond with V_L Q31 and an E484A mutation would add another hydrophobic
236 side chain near hydrophobic residues V_L Y99, F456, and Y489. These contacts may
237 explain the mechanism which enabled S728-1157 to retain neutralizing activity (Figure
238 1g), albeit reduced binding reactivity against spike BA.1 antigen, which is in turn possibly
239 due to the function of Omicron mutations in altering the conformational landscape of the
240 spike protein⁴⁰. Notably, while the variable genes were well-mutated, all but one of the
241 contact residues between the CDR-H3 of S728-1157 and the VOC were predicted to be
242 germline encoded and not introduced by somatic mutations, likely limiting the number of
243 existing memory B cells of this class that could be further adapted by somatic mutation to
244 protect against VOC strains (Figure S1, Table S5). Overall, our structural studies
245 revealed the basis of broad neutralization of S728-1157 that can accommodate most
246 mutations in the SARS-CoV-2 VOCs.

247

248 **S728-1157 reduces replication of SARS-CoV-2 Delta and Omicron variants in Syrian** 249 **hamsters**

250 To evaluate the protective efficacy of our broadly neutralizing mAbs, we utilized a golden
251 Syrian hamster infection model that has been widely used for SARS-CoV-2 infection.
252 Hamsters received 5 mg/kg of individual mAbs or an irrelevant antigen (ebolavirus
253 glycoprotein)-specific isotype control via intraperitoneal injection one day post-infection
254 with SARS-CoV-2 viruses. Lung and nasal tissues were collected at 4 days post-infection
255 (dpi) (Figure 3a). Therapeutic administration of S728-1157 resulted in reduced titers of
256 wildtype, BA.1 Omicron and Delta variants in both the nasal turbinates and lungs of
257 infected hamsters (Figure 3b-d). Interestingly, the effect of S728-1157 in the lungs was
258 dramatic, reducing wildtype viral loads by $\sim 10^4$ PFU, and BA.1 Omicron by $\sim 10^5$ PFU,
259 with the viral titers of the latter being completely abolished (Figure 3c). In contrast to *in*
260 *vitro* neutralization, S451-1140 did not reduce BA.1 Omicron viral replication in lung and

261 nasal turbinates, indicating the disconnect between *in vitro* neutralization and *in vivo*
262 protection for S451-1140 (Figure 3e). In comparison, S626-161 administration resulted in
263 significant but marginal reductions in lung viral titers following wildtype and BA.1
264 challenge (Figure 3f-g). These data underscore that to precisely define broadly protective
265 mAbs, evaluating protection efficacy in parallel with neutralization activity is required.
266 Overall, S728-1157 represents a promising mAb with broad neutralization efficacy against
267 SARS-CoV-2 variants that is capable of dramatically reducing wildtype, Delta and BA.1
268 replication *in vivo*.

269

270 **SARS-CoV-2 infection rarely elicits potent S728-1157-like cross-neutralizing mAbs**

271 Given the cross-neutralization and prophylactic potential of S728-1157, we sought to
272 evaluate whether S728-1157-like antibodies are commonly induced among polyclonal
273 responses in SARS-CoV-2 patients. To assess this, we performed competition ELISAs
274 using convalescent serum to detect anti-RBD antibody titers that could compete for
275 binding with S728-1157 (Figure 4a). Subjects were divided into three groups based on
276 their magnitude of antibody responses, as defined previously^{12,13}. Although high- and
277 moderate-responders had higher titers of S728-1157-competitive serum antibodies
278 compared to low-responders (Figure 4b), the titers were quite low across all groups
279 suggesting that it is uncommon to acquire high levels of S728-1157-like antibodies in
280 polyclonal serum following wildtype SARS-CoV-2 infection. In addition to S728-1157, we
281 tested the competition of convalescent serum with other mAbs, including S451-1140 and
282 S626-161, LY-CoV555, REGN10933, CR3022, and CC12.3. Similar to S728-1157, we
283 observed relatively low titers of antibodies competing with S451-1140, S626-161, LY-
284 CoV555, REGN10933 and CC12.3 in polyclonal serum from most of the convalescent
285 individuals (Figure 4c-f, h). Nonetheless, high-responders tended to have significantly
286 higher titers against those neutralizing mAbs than low-responders (Figure 4b-f, h). In
287 contrast, antibodies targeting the CR3022 epitope site were more pronounced in
288 convalescent individuals, suggesting the enrichment of class 4 RBD antibodies in
289 polyclonal serum (Figure 4g). Notably, there was no difference in titers of CR3022 across
290 the three responder groups, suggesting that CR3022-site antibodies were largely induced

291 during wildtype SARS-CoV-2 infection. Interestingly, as compared to CC12.3, S728-1157
292 was detected at 4-fold lower levels in the serum of high-responders. Thus, despite class
293 1 antibodies being frequently induced by natural infection and vaccination^{17,26,27,41-44}, our
294 data suggest that S728-1157-like antibodies represent a subset of this class that are
295 comparatively rare.

296 Lastly, we examined the difference in reactivity to 2P- versus 6P-stabilized spike
297 in our convalescent cohort sera (Figure 4i-k). We found that all three responder groups
298 mounted anti-spike reactive antibodies against 6P-stabilized spike wildtype to a greater
299 extent than 2P-stabilized spike wildtype, by a factor of 6 to 11-fold (Figure 4j), indicating
300 that the major antigenic epitopes were better exhibited or stabilized on 6P-stabilized
301 antigen. Using the same samples, high and moderate responders also had lower anti-
302 spike antibodies against BA.1-2P than BA.1-6P, by 4 to 5-fold (Figure 4k). Of note, low
303 responders had a smaller fold change in binding reactivity against spike BA.1 Omicron-
304 2P and 6P (2-fold reduction) compared to wildtype-2P and 6P spike (11-fold reduction)
305 (Figure 4j-k), suggesting that serum antibody against BA.1 Omicron-reactive epitopes
306 may be limited in low responder subjects. Overall, these data suggest that there is
307 improved polyclonal binding induced by natural infection to 6P-stabilized spike, both for
308 wildtype and Omicron viruses.

309

310 Discussion

311 In this study, we identify three potent bnAbs isolated from memory B cells of
312 individuals who had recovered from SARS-CoV-2 infection during the initial wave of the
313 COVID-19 pandemic. Among them, S728-1157 maintains substantial binding reactivity
314 and had consistent neutralizing activity against all tested SARS-CoV-2 VOC including
315 Omicron BA.1, BA.2, BA.2.75, BL.1 (BA.2.75+R346T), BA.4 and BA.5, and was able to
316 substantially reduce infectious viral titers following Delta and BA.1 infection in hamsters.

317 We found convalescent serum from our cohort contained low concentrations of
318 antibodies that compete with S728-1157 (a class 1 antibody) and class 2 epitope mAbs.
319 This suggests that S728-1157 is somewhat unique from other antibodies targeting class
320 1 epitopes and is infrequently induced in the RBD-specific memory B cells pool. Instead,

321 in our cohort natural infection preferably induced antibodies targeting the CR3022 (class
322 4) epitope; antibodies of this specificity are also often non-neutralizing or less potently
323 neutralizing than RBS-targeting antibodies. These data are complementary to our
324 previous findings that demonstrated that an abundance of class 3 antibodies in
325 convalescent sera may contribute to neutralizing activity against Alpha and Gamma
326 variants, whereas a lack of class 2 antibodies may account for reduced neutralization
327 capability against Delta¹². Notwithstanding, the breadth of activity against Omicron
328 variants of most of these RBS-targeting antibodies (RBS-A/class 1, RBS-B,C/class 2 and
329 RBS-D/class 3) is reported to be highly limited^{10,39,45}. This is consistent with experimental
330 evidence documenting that convalescent unvaccinated patients showed a marked
331 reduction of neutralizing activity against Omicron BA.1⁹. This phenomenon highlights the
332 need to shape the antibody repertoire toward broadly conserved, protective epitopes, as
333 typified by S728-1157.

334 The structures herein illustrated that S728-1157 bound the RBS-A/class 1 epitope
335 in the 'up' conformation RBD. This epitope is more readily accessible on 6P-stabilized
336 spikes, which present two RBDs in the 'up' state, as compared to 2P spikes which
337 presents only one^{28,31,46,47}. The S728-1157 was isolated after natural infection; in such
338 contexts, the odds of inducing S728-1157-like clones are likely higher given that the RBD
339 must be able to adopt an up conformation, even transiently, to bind to ACE2, thereby
340 exposing this epitope. Using extensive interactions between CDR-H3 and the RBD, S728-
341 1157 can accommodate key mutations in VOC spikes, indicating this antibody is unlike
342 majority of IGHV3-53/3-66 RBS-A/class 1 antibodies^{27,48,49,50}. S728-1157 also uses a
343 different light chain (IGLV3-9) compared to other non-broad antibodies such as CC12.3
344 (IGKV3-20), which may affect overall binding conformation; however, our analysis
345 indicates that there is limited direct interaction between the S728-1157 light chain and the
346 RBD. Most of the CDR-H3 contact residues critical for VOC cross-reactivity in this
347 interaction are germline-encoded and not introduced by somatic mutations, suggesting
348 that most memory B cells encoding IGHV3-53/66 class antibodies could not acquire this
349 degree of cross-reactivity by further affinity maturation, which is a consideration for
350 vaccines tailored to help induce such antibodies. While it may be challenging to design

351 vaccines that can specifically elicit S728-1157-like antibodies with select CDR-H3s
352 capable of overcoming the VOC mutations, it is encouraging that IGHV-gene restriction
353 is observed in other potent SARS-CoV-2 neutralizing mAbs studies^{12,17-25}. Ultimately,
354 such a vaccination approach may be feasible through iterative immunization with
355 optimized RBD immunogens, as has been previously reported in the influenza literature<sup>51-
356 55</sup>.

357 Although many mutations have been observed in the class 1 antigenic site¹⁵, with
358 regards to the S728-1157 epitope 13/15 total RBD contact residues, and 2/3 CDR-H3-
359 bound RBD contact residues, are conserved within Omicron and all other VOCs. This
360 suggests that the RBD region where the S728-1157 epitope is found may include
361 residues critical for its dynamic function and viral fitness and would therefore be less
362 tolerant of mutations and antigenic drift than surrounding class 1 site residues. If this is
363 the case, the tendency for this particular epitope to be lost as viral variants evolve should
364 be reduced, making characterization of S728-1157 and similar antibodies and epitopes
365 important for variant-resistant vaccines or mAb therapeutic development.

366 In summary, our study identifies broadly neutralizing antibodies that may inform
367 immunogen design for next-generation variant-proof coronavirus vaccines or serve as
368 mAb therapeutics that are resistant to SARS-CoV-2 evolution. In particular, in terms of
369 combined potency and breadth, S728-1157 appears to be the best-in-class antibody
370 isolated to date. Given that this antibody is predicted to be preferentially induced by 6P-
371 stabilized recombinant spike proteins or whole virus, these findings suggest that
372 hexaprolin modification could benefit future vaccine constructs to optimally protect
373 against future SARS-CoV-2 variants and other sarbecoviruses.

374

375 **Acknowledgements**

376 We thank the study participants for their willingness to enroll in this study. We are grateful
377 for the clinical staff at the University of Chicago Medicine Plasma Transfusion Program
378 for their assistance in collecting the sample and transfer to the lab. We also kindly thank
379 the University of Chicago CAT Facility (RRID SCR_017760) and the University of Chicago
380 Genomics Facility (RRID SCR_019196) for assisting in sorting and sequencing samples.

381 We thank Henry Tien for technical support with the crystallization robot, and Robyn
382 Stanfield for assistance in data collection. We thank Jeffrey Copps for producing the spike
383 proteins used for electron microscopy. We thank Bill Anderson and Charles Bowman for
384 maintaining the microscope facility and for technical assistance. We are grateful to the
385 staff of the Stanford Synchrotron Radiation Lightsource (SSRL) beamline 12-1 for
386 assistance. Use of resources of the SSRL, SLAC National Accelerator Laboratory is
387 supported by the U.S. Department of Energy, Office of Science, Office of Basic Energy
388 Sciences under Contract No. DE-AC02–76SF00515. Extraordinary facility operations
389 were supported in part by the DOE Office of Science through the National Virtual
390 Biotechnology Laboratory, a consortium of DOE national laboratories focused on the
391 response to COVID-19, with funding provided by the Coronavirus CARES Act. The SSRL
392 Structural Molecular Biology Program is supported by the DOE Office of Biological and
393 Environmental Research, and by the National Institutes of Health, National Institute of
394 General Medical Sciences (including P41GM103393).

395

396 **Author contributions**

397 Conceptualization, SC, PCW; Methodology, SC, PJH, HL, JLT, GO, JT, MH, SAE, YF,
398 DW; Sample Collection, NYZ, HLT; Resources, GS, FK, DNS, IAW, ABW, YK.
399 Investigation, SC, PJH, HL, JLT, JJM, LL, MK, TM; Writing – Original Draft, SC, HL, JLT;
400 Writing – Review and Editing, all authors; Funding, PCW, FK, DNS, IAW, ABW, YK.
401 Supervision, PCW, IAW, ABW, YK.

402

403 **Declaration of Interests**

404 The University of Chicago has filed a patent application on November 11, 2021, relating
405 to anti-SARS-CoV-2 antibodies with P.C.W. and S.C. as inventors. Some of mAbs in this
406 study are being considered for the development of therapeutic antibodies. The Icahn
407 School of Medicine at Mount Sinai has filed patent applications relating to SARS-CoV-2
408 serological assays and NDV-based SARS-CoV-2 vaccines, which list F.K. as a
409 coinventor. Mount Sinai has spun out a company, Kantaro, to market serological tests for
410 SARS-CoV-2. F.K. has consulted for Merck and Pfizer (before 2020) and is currently

411 consulting for Pfizer, Seqirus, Third Rock Ventures and Avimex. The Krammer laboratory
412 is also collaborating with Pfizer on animal models of SARS-CoV-2.

413

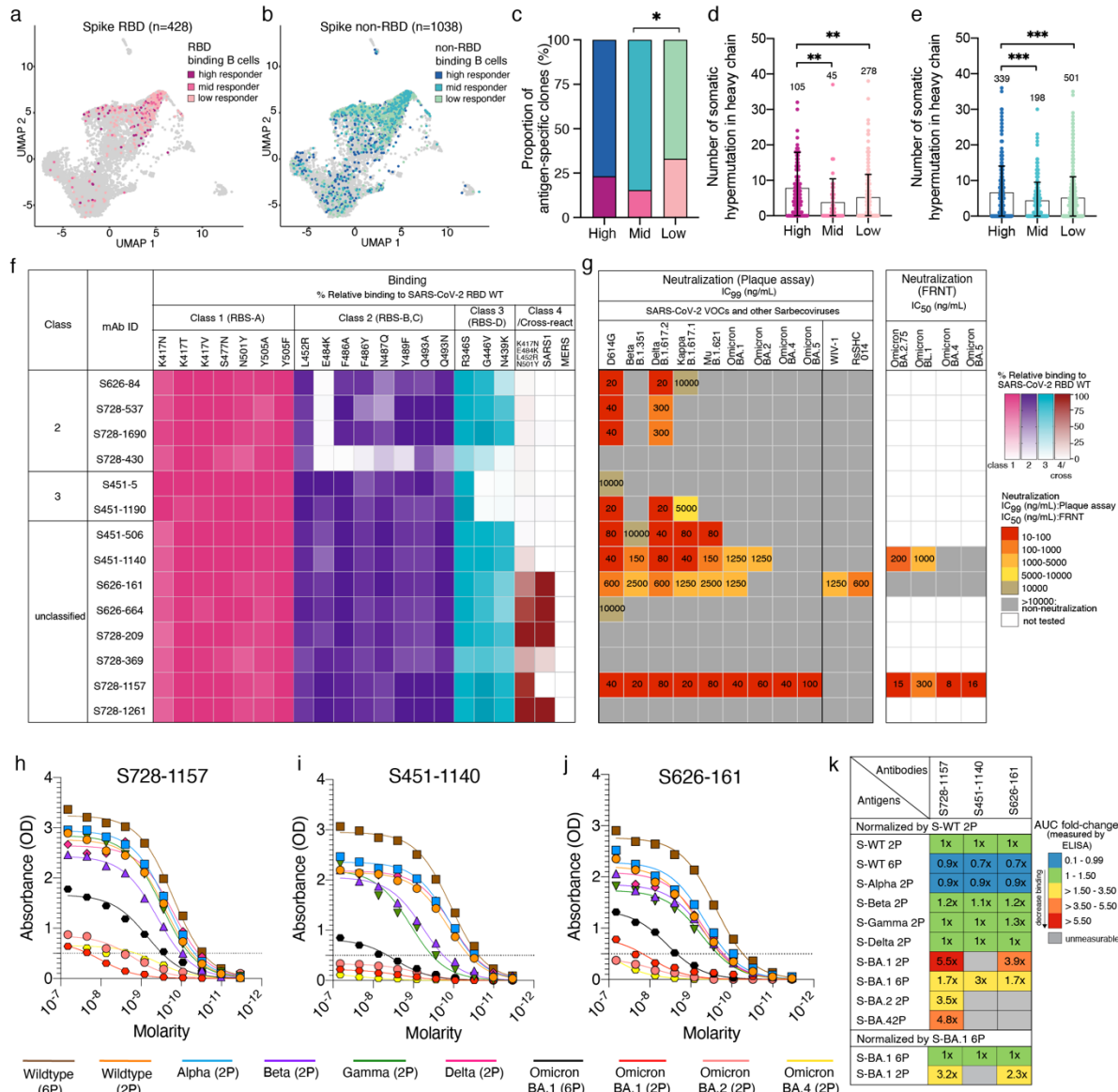
414 **Funding information**

415 This project was funded in part by the National Institute of Allergy and Infectious Diseases
416 (NIAID; National Institutes of Health grant numbers U19AI082724 (PCW), U19AI109946
417 (PCW), U19AI057266 (PCW), the NIAID Centers of Excellence for Influenza Research
418 and Surveillance (CEIRS) grant number HHSN272201400005C (PCW), and the NIAID
419 Centers of Excellence for Influenza Research and Response (CEIRR) grant number
420 75N93019R00028 (PCW). This work was also partially supported by the NIAID
421 Collaborative Influenza Vaccine Innovation Centers (CIVIC; 75N93019C00051, PCW).
422 Y.K. and P.C.W. were funded by NIAID's Pan-Coronavirus Vaccine Development
423 Program (P01AI165077). Y.K. was also funded by the Research Program on Emerging
424 and Re-emerging Infectious Diseases (JP19fk0108113, JP20fk0108272,
425 JP20fk0108301, and JP21fk0108586); the Japan Program for Infectious Diseases
426 Research and Infrastructure (JP20wm0125002) from the Japan Agency for Medical
427 Research and Development (AMED); NIAID CEIRS contract HHSN272201400008C.
428 D.N.S. was funded by BEI/NIAID contract HHSN272201600013C. I.A.W and A.B.W. were
429 supported by the Bill and Melinda Gates Foundation award INV-004923. Work in the
430 Krammer laboratory was funded by the NIAID Collaborative Influenza Vaccine Innovation
431 Centers (CIVIC) contract 75N93019C00051. In addition, this work was also partially
432 funded by the NIAID Centers of Excellence for Influenza Research and Surveillance
433 (CEIRS, contract # HHSN272201400008C), the NIAID Centers of Excellence for
434 Influenza Research and Response (CEIRR, contract# 75N93021C00014) and by
435 anonymous donors. The content is solely the responsibility of the authors and does not
436 necessarily represent the official views of the National Institutes of Health.

437

438

439 Main Figures and Legends

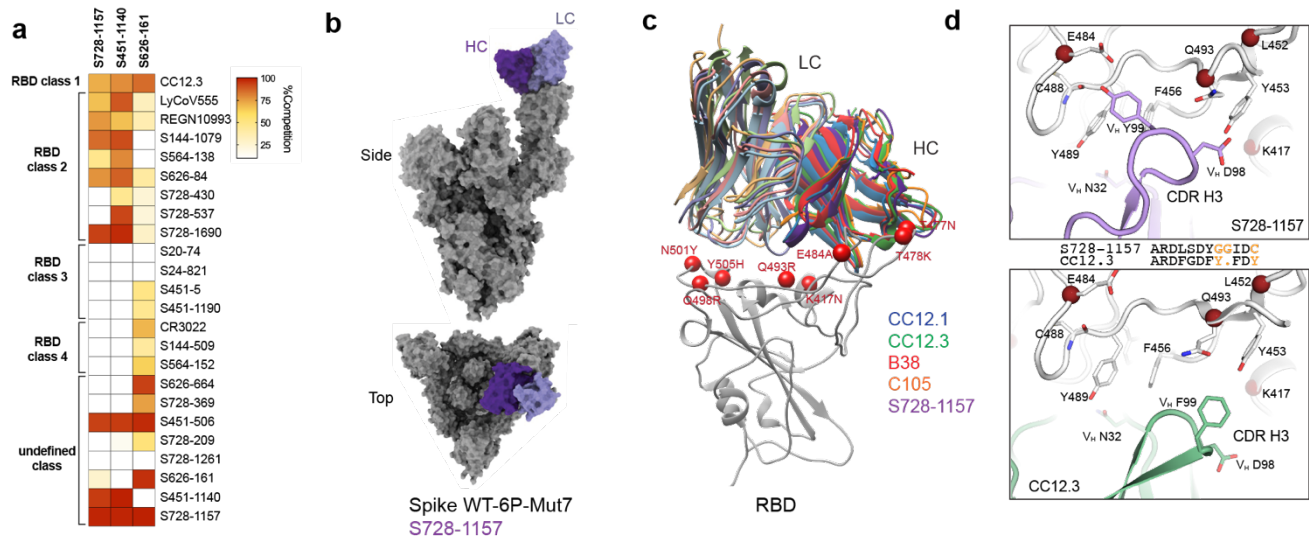


440

441 **Figure 1. Proportion of SARS-CoV-2-specific B cells and characterization of RBD-**
 442 **reactive mAbs isolated from COVID-19 convalescent individuals. a-b,** Uniform
 443 manifold approximation and projection (UMAP) of SARS-CoV-2 **(a)** spike RBD binding
 444 and **(b)** spike non-RBD binding B cells isolated from convalescent subjects that could be
 445 characterized into 3 groups (high, mid and low responder) based on their serological
 446 response against SARS-CoV-2 spike¹³. **c,** Proportion of spike non-RBD- and spike RBD-
 447 specific binding B cells representing in each responder group. Colors in **a** and **b** are
 448 representative of antigen-specific B cells from each responder group as follow: **(a)** RBD-

449 binding B cells; plum, high responder; pink, mid responder; pale-pink, low responder and
450 **(b)** non-RBD-binding B cells; navy, high responder; blue, mid responder; pale-green, low
451 responder. **d-e**, Number of somatic hypermutations in the IGHV in antibodies targeting
452 **(d)** RBD and **(e)** non-RBD. **f**, Binding profile of RBD-reactive mAbs against single RBD
453 mutants associated with different antibody classes, a combinatorial RBD mutant, and the
454 RBDs of SARS-CoV-1 and MERS-CoV. Color gradients indicate relative binding
455 percentage compared to RBD WT with the labeling color as follow: pink, class 1; purple,
456 class 2; teal, class 3; burgundy, class 4 and cross-reactive epitopes. **g**, Neutralization
457 potency measured by plaque assay (complete inhibitory concentration; IC₉₉) and focus
458 reduction neutralization test (FRNT; half inhibitory concentration; IC₅₀) of RBD-reactive
459 mAbs to SARS-CoV-2 variants and sarbecoviruses. Binding breadth against full-length
460 spike SARS-CoV-2 variants determined by ELISA is shown for **(h)** S728-1157, **(i)** S451-
461 1140, and **(j)** S626-161. Dashed line in **h-j** indicate the limit of detection. **k**, Heatmap
462 represents area under curve (AUC) fold-change of broadly neutralizing RBD-reactive
463 mAbs against ectodomain spike SARS-CoV-2 variants relative to WT-2P and the
464 differences of AUC fold-change between spike BA.1-2P relative to spike BA.1-6P. Colors
465 in **k** indicate range of fold-change from blue, 0.1 to 0.99-fold (increase affinity binding);
466 green, 1 to 1.5-fold (none to little reduction in affinity binding); yellow, >1.5 to 3.5-fold
467 (moderate reduction in affinity binding); orange, >3.5 to 5.5-fold (high reduction in affinity
468 binding); red, >5.5-fold (extreme reduction in affinity binding) and grey indicated
469 unmeasurable fold-change due to the absorbance values are below limit of detection. The
470 statistical analysis in **c** was determined using Tukey multiple pairwise-comparisons and
471 in **d-e** was determined using Kruskal-Wallis with Dunn's multiple comparison test. Data
472 in **f-g and h-j** are representative of two independent experiments performed in duplicate.
473 Genetic information for each antibody is in **Table S2**. The SARS-CoV-2 viruses used in
474 neutralization assay are indicated in **Table S4**.

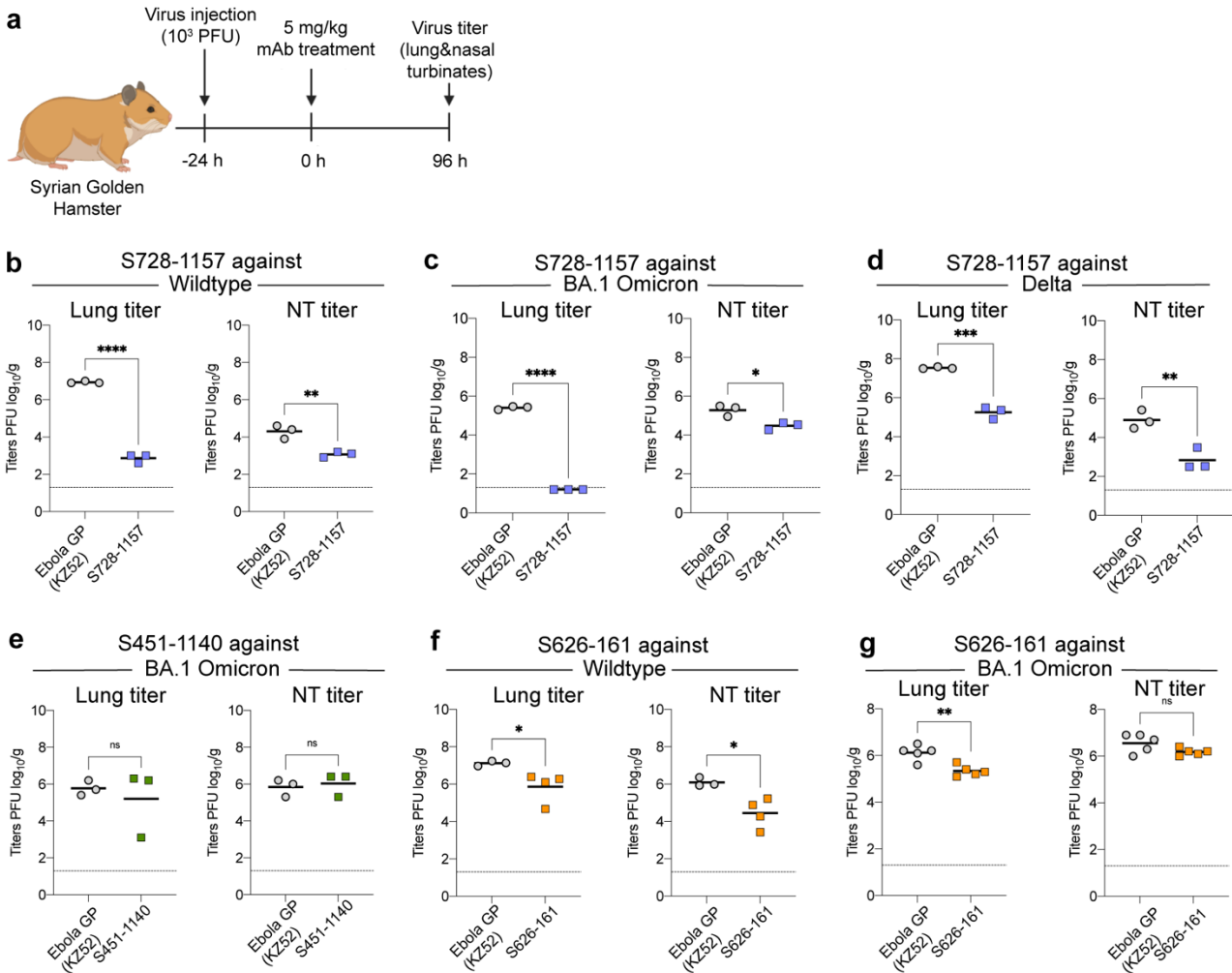
475



476

477 **Figure 2: Mechanism of broad neutralization of S728-1157.** (a) Epitope binning of
478 broadly neutralizing RBD-reactive mAbs. Heatmap demonstrating the percentage of
479 competition between each RBD-reactive mAb from previous studies^{12,20,33-35} with three
480 broadly neutralizing mAbs, S728-1157, S451-1140 and S626-161. Data are
481 representative of two independent experiments performed in triplicate. (b) Surface
482 representation of the model derived from the cryoEM map of spike WT-6P-Mut7 in
483 complex with IgG S728-1157. The heavy chain is shown in dark purple, light chain in light
484 purple, and the spike protein in gray. Although we observe full mAb occupancy in the
485 cryo-EM map, only one Fv is shown here. (c) Structural comparison of S728-1157 to other
486 RBS-A antibodies such as CC12.1 (PDB ID: 6XC2, blue), CC12.3 (PDB ID: 6XC4, green),
487 B38 (PDB ID: 7BZ5, red), and C105 (PDB ID: 6XCN, orange). The heavy chains are a
488 darker shade, and the light chains are a lighter shade of their respective colors. Omicron
489 BA.1 mutations near the epitope interface are shown as red spheres. (d) CDR-H3 forms
490 distinct interactions with SARS-CoV-2 RBD between S728-1157 and CC12.3. Sequence
491 alignment of CDR-H3 of the two antibodies are shown in the middle with non-conserved
492 residues shown in orange.

493



494

495 **Figure 3: Protective efficacy of broadly neutralizing antibodies against SARS-CoV-**

496 **2 infection in hamster.** Schematic illustrating the in vivo experiment schedule **(a)**. Lung

497 and nasal turbinate (NT) viral replication SARS-CoV-2 are shown for hamster treated

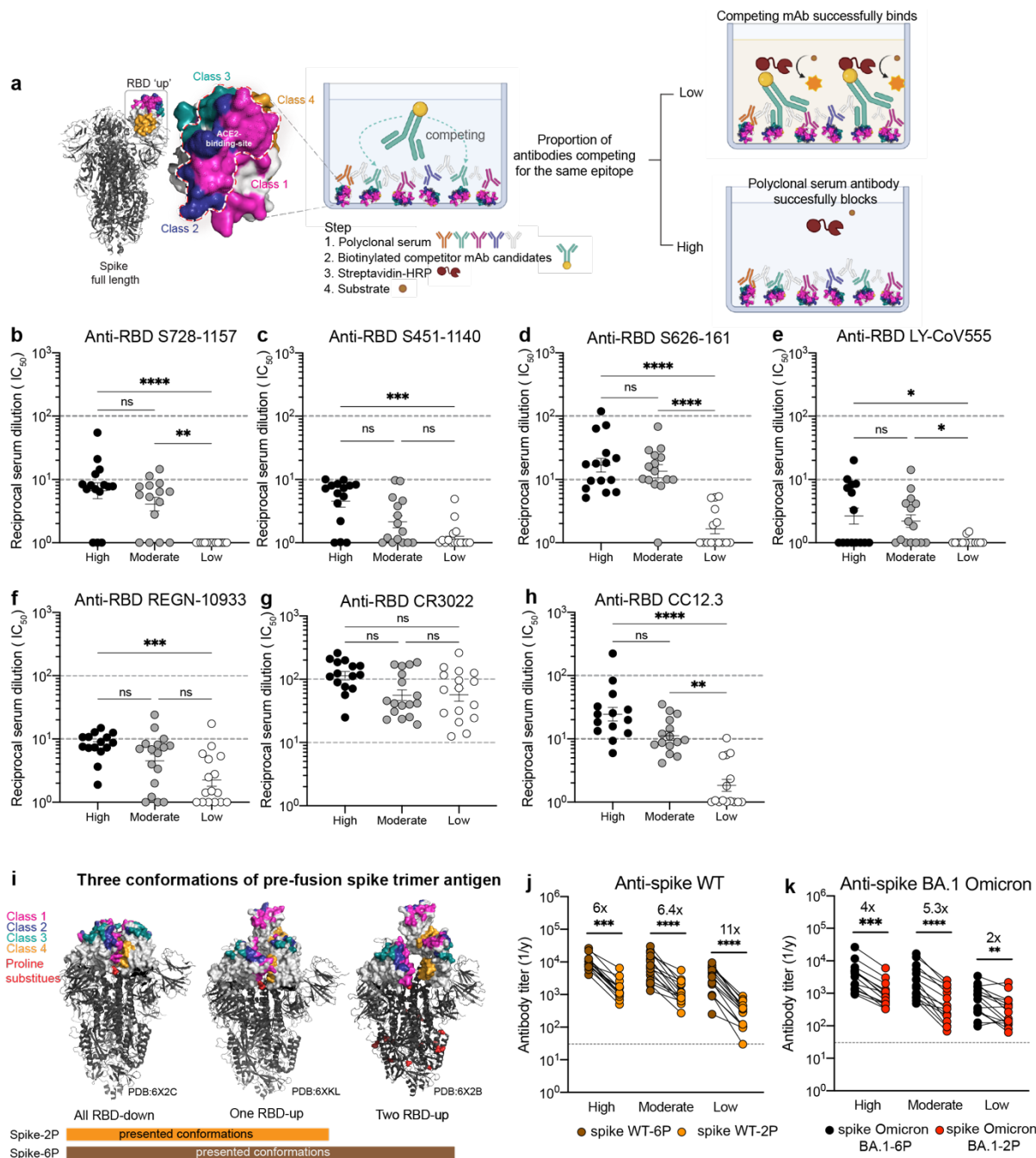
498 therapeutically with **(b-d)** S728-1157 (n=3) **(e)** S451-1140 (n=3) and **(f-g)** S626-161 (n=4)

499 at day 4 post-challenge with SARS-CoV-2 compared with control mAb, anti-Ebola surface

500 glycoprotein (KZ52) antibody. Dashed horizontal lines represent the limit of detection

501 (LOD) of the experiment. P-values in **(b-g)** were calculated using Unpaired t-test. The

502 infected SARS-CoV-2 viruses are detailed in **Table S4**.



503

504 **Figure 4: Convalescent serum antibody competition with broadly neutralizing RBD-**
 505 **reactive mAbs and comparison of serum antibody response against spike 6P-**
 506 **versus 2P-stabilized.** Schematic diagram for experimental procedure of serum
 507 competitive ELISA (a). Half-maximal inhibitory concentration (EC_{50}) of polyclonal
 508 antibody serum from convalescent individuals that could compete with broadly
 509 neutralizing mAbs (competitor mAb): S728-1157 (b), S451-1140 (c) and S626-161 (d),

510 therapeutic neutralizing mAbs LY-CoV555 **(e)**, REGN-10933 **(f)**, non-neutralizing mAb
511 CR3022 **(g)** and well-defined class 1 mAb CC12.3 **(h)**. The reciprocal serum dilutions in
512 **b-h** are showed as Log₁₀P of the IC₅₀ of serum dilution that can achieve 50% competition
513 with the competitor mAb of interest. The statistical analysis in **b-h** was determined using
514 Kruskal-Wallis with Dunn's multiple comparison test. Representative three conformations
515 of pre-fusion spike trimer antigen observed in the previous structural characterization of
516 SARS-CoV-2 stabilized by 2P and 6P^{31,47} **(i)**. Endpoint titer of convalescent sera against
517 SARS-CoV-2 spike wildtype (WT) **(j)** and Omicron BA.1 **(k)** in two versions of spike
518 substituted by 2P and 6P. Data in **b-h** and **j-k** are representative of two independent
519 experiments performed in duplicate. Wilcoxon matched-pairs signed rank test was used
520 to compare the anti-spike antibody titer against 2P and 6P in **j-k**. Fold change indicated
521 in **j-k** is defined as the mean fold change.
522
523

524 **Supplementary Figures and Tables**

525

S728-1157

Heavy chain	FR1	FR2	FR3	FR4
	<---CDR1--->	<---CDR2--->	<---CDR3--->	<---CDR4--->
Sequence	R N Y M N I I Y S G G S T F Y A D S V E G D L S D Y G G I D C			
V IGHV3-66*02	AGAAATTACATGAACATTATTATAGTGGTGGCAGCACATTCTACGCAGACTCCGTGGAGGGCGATCTCTCCGACTACGGTGGGATTGACTCC			
D IGHV4-23*01	.C.C.....G.G.....C.....T.....A.....A.....			
J IGHV4*02A.....			
Light chain	FR1	FR2	FR3	FR4
	<---CDR1--->	<---CDR2--->	<---CDR3--->	<---CDR4--->
Sequence	G G D N V G S Q N V H R D S N R P S Q V W D S S T V A			
V IGLV3-9*01	GGGGAGACACCTGGGAAGTCAAAATGTGCACAGGGATAGCAACCGGCCCTTCAGGTGTGGGACGACGACTGTGGCT			
J IGLV2*01A.....A.T.....A.....			

S451-1140

Heavy chain	FR1	FR2	FR3	FR4
	<---CDR1--->	<---CDR2--->	<---CDR3--->	<---CDR4--->
Sequence	N Y A M T A I S G G G S T D Y A D S V K G D L F G S G W S L F D N			
V IGHV3-23*04	AACATGCCATGACCGCTATTAGTGGTGGTGGGAGCACAGACTACGCAGACTCCGTGAAGGGCGATCTATTGGCAGTGGCTGGTCCCTCTTGACAAAC			
D IGHV6-19*01	.G.....G.....A.....T.....T.....			
J IGHV4*02T.....			
Light chain	FR1	FR2	FR3	FR4
	<---CDR1--->	<---CDR2--->	<---CDR3--->	<---CDR4--->
Sequence	K S S Q S V L Y S S N N K N Y L A W A S T R E S Q Q Y Y S P P I T			
V IGHV4-1*01	AAGTCCAGCCAGAGTGTCTTATACAGTCCCAACAATAAGAACTACTTAGCTTGGGCATCTACCCGGGAATCCAGCAATATTATAGTCCCTCTATCACT			
J IGHV3*01T.....			

S626-161

Heavy chain	FR1	FR2	FR3	FR4
	<---CDR1--->	<---CDR2--->	<---CDR3--->	<---CDR4--->
Sequence	T S N Y Y W G S I I Y R G G T H Y N P S L K T H T Y F Y D I V G A A V W E P F D I			
V IGHV4-39*01	ACTAGTAATTACTACTGGGGCAGTACTATATTATCGTGGGGCACCCTACAACCCCTCCCTCAAGACTCATACCTATTTCTATGATATCGTGGGGCAGCGTTTGGGAACCTTTGATATC			
D IGHV3-22*01	.G.....G.....A.....A.....T.....G.....A.....			
J IGHV3*02A.....			
Light chain	FR1	FR2	FR3	FR4
	<---CDR1--->	<---CDR2--->	<---CDR3--->	<---CDR4--->
Sequence	R A S Q S V S S S Y L A D A S S R A T Q Q Y G S S P P W T			
V IGHV3-20*01	AGGGCCAGTCAGAGTGTAGCAGCAGCTACTTAGCCGATGCATCCAGCAGGGCCACTCAGCAGTATGGTAGCTCACCTCCSTGGACG			
J IGHV1*01G.....			

526

527 **Figure S1: Amino acid and nucleotide sequences of complementarity-determining**
 528 **region (CDR) of heavy chain and light chain of the three bnAbs.** Contacting residues
 529 within CDR of S728-1157 and SARS-CoV-2 are highlighted as light purple. Genetic
 530 information for each antibody is in **Table S2**.

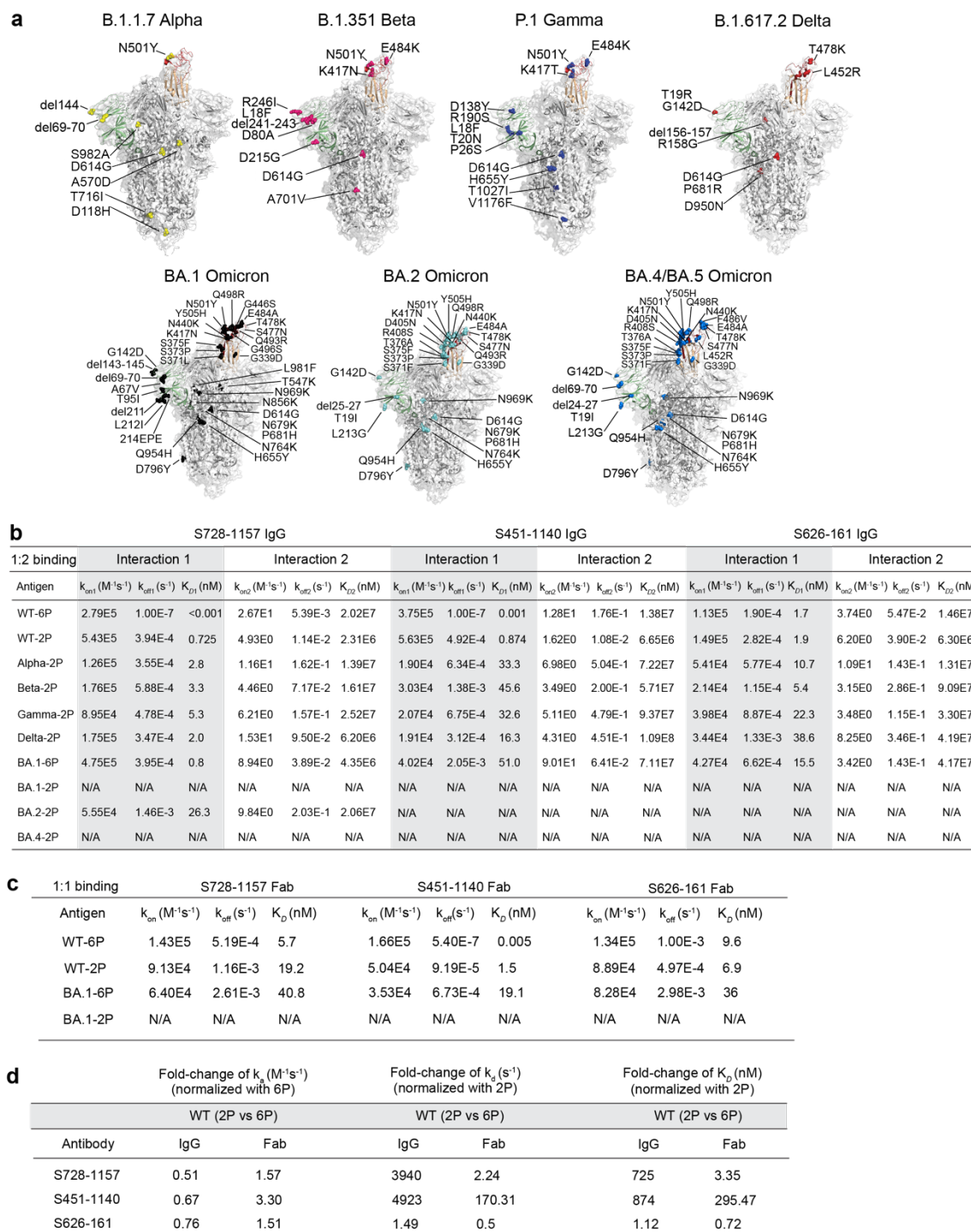


Figure S2: Broadly neutralizing RBD-reactive mAbs activity against SARS-CoV-2 and emerging variants. a, Structural models for the full-length spike protein variants and amino acid substitutions that encoded in B.1.1.7 Alpha, B.1.351 Beta, P.1 Gamma, B.1.617.2 Delta and Omicron, BA.1, BA.2 and BA.4. The structural models in **a** are modified from PDB ID: 6XM4. **b,** The table illustrating the binding rate and equilibrium

constants (k_{on} , k_{off} , and affinity binding K_D) measured by BLI of S728-1157, S451-1140 and S626-161 IgG in response to the panel of SARS-CoV-2 VOCs (either former or current VOCs). **c**, The binding rate comparison of Fabs of S728-1157, S451-1140 and S626-161 in responding to spike WT and BA.1-6P and 2P constructs. The binding traces of IgG and Fab analyzed by BLI were represented by the 1:2 and 1:1 interaction model, respectively. **d**, The fold-change of binding rate (K_{on} , K_{off}) and binding affinity (K_D) between spike WT-6P and spike WT-2P bound by neutralizing RBD-reactive mAbs, whole IgG form and Fab. Data in **c-d** are representative of two independent experiments, the data from experiments that have the best fit ($R^2 > 0.90$) are selected for analysis.

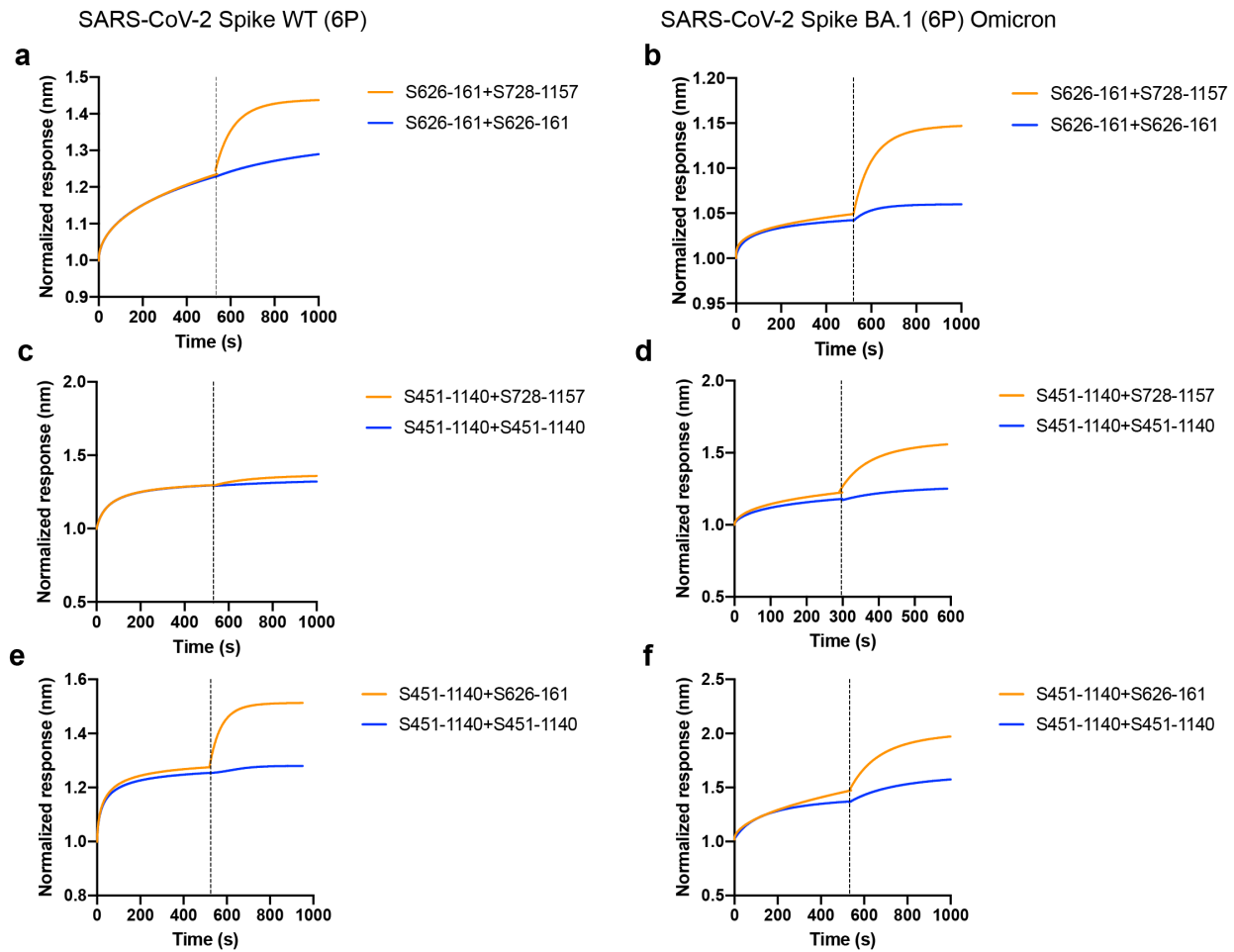


Figure S3: Bi-layer interferometry analysis demonstrates binding affinity curves of three broadly neutralizing mAbs competing with each other in response to biotinylated spike wildtype (WT)-6P (left panel) and spike BA.1 Omicron-6P (right panel). a-b, S626-161 was firstly bound, followed by S728-1157 mAb as competing mAb. c-d, S451-1140 was firstly bound and competed with S728-1157 and e-f, S626-161. The response curve was normalized in relation to its starting response value.

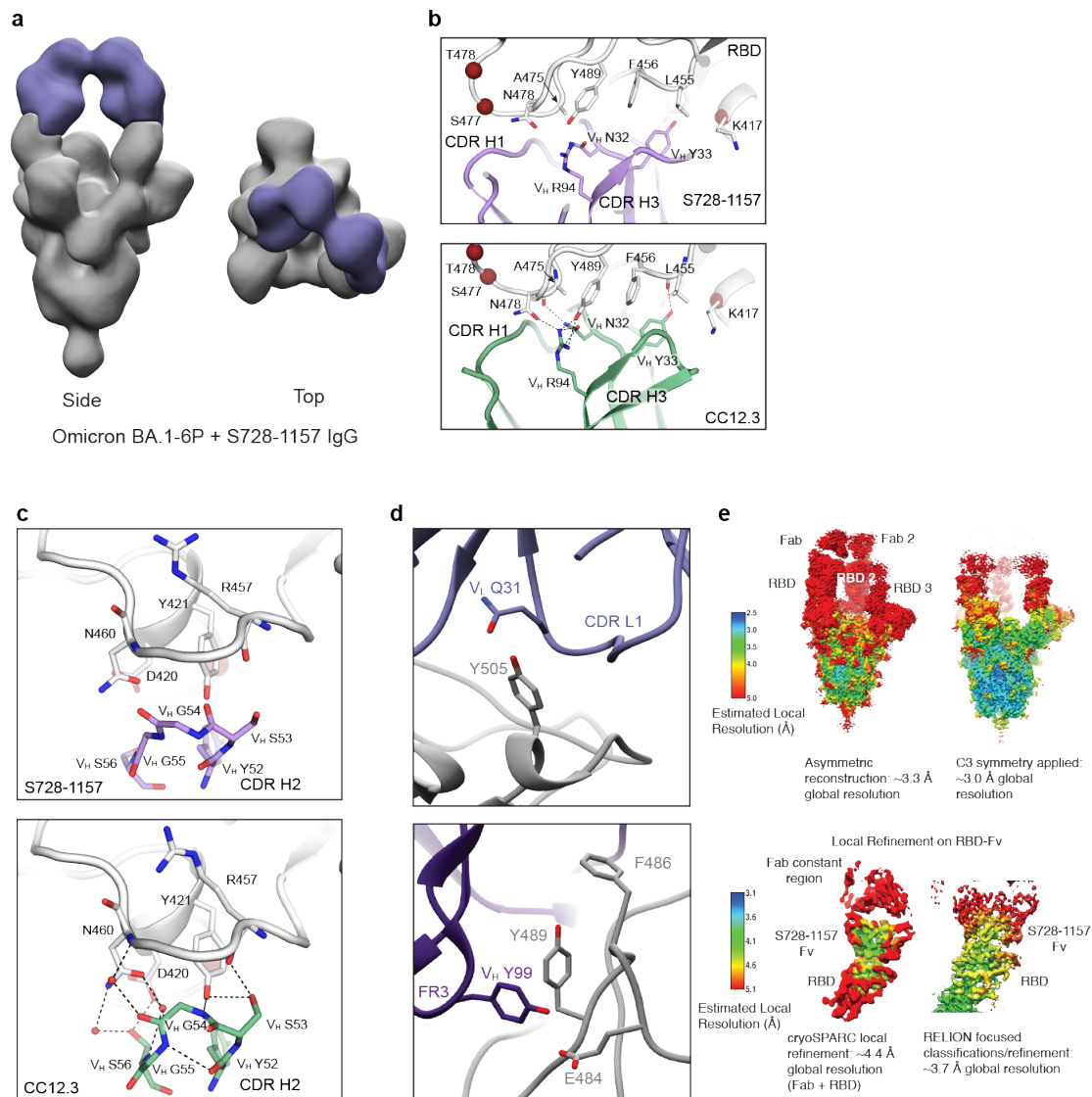


Figure S4. Structural analysis of S728-1157 binding to SARS-CoV-2 spike. (a) Three-dimensional (3D) reconstruction of Omicron BA.1-6P in complex with IgG S728-1157 shows binding by negative stain electron microscopy. The binding mode is the same as binding to spike WT-6P-Mut7 shown in **Figure 2b**. **(b)** CDR-H1 of S728-1157 forms similar interactions with SARS-CoV-2 RBD compared to another IGHV3-53 antibody CC12.3 (PDB ID: 6XC4). **(c)** CDR-H2 of S728-1157 forms similar interactions with the RBD compared to CC12.3 (PDB ID: 6XC4). **(d)** For spike WT-6P-Mut7 in complex with S728-1157, residues Y505 and V_L Q31, and E484 and V_L Y99 are predicted to make hydrogen bonds. Hydrophobic residues Y486 and Y489 are shown as well. Since S728-1157 binds spike Omicron BA.1-6P in the same way as to spike WT-6P-Mut7, it may

accommodate the E484A and Y505H mutations in Omicron. (e) Local resolution estimates of the cryo-EM map (upper panel) and local refinement on the RBD-Fv after symmetry expansion using RELION (lower panel).

Table S1: COVID-19 convalescent subjects. Related to Figure 1 and Figure 4. The mAbs from high responder subjects, S451, S626, S728 were characterized in this study. Responder group and severity were categorized in a previous study¹³. Serum antibody from each responder group were tested for competition ELISA with broad neutralizing mAbs, other therapeutic mAbs and non-neutralizing mAb.

Subject ID	Age	Sex	SARS-CoV-2 PCR Test	Duration of symptoms (days)	Symptom start to donation (days)	Responder Category ²⁶	Severity Category ²⁶
3	20	M	3/16/20	4	33	Low	Moderate
11	66	M	3/30/20	16	49	High	Severe (hospitalized)
17	42	M	3/21/20	17	55	High	Severe
19	55	F	3/15/20	14	44	Low	Moderate
20	31	M	3/31/20	19	48	High	Critical (hospitalized)
22	31	F	3/23/20	3	31	Mid	Moderate
24	34	M	3/23/20	12	41	High	Severe
42	30	M	3/18/20	11	39	Mid	Moderate
63	44	M	3/30/20	2	33	Low	Moderate
80	33	M	3/26/20	12	40	Mid	Moderate
89	64	M	3/19/20	13	43	High	Mild
108	58	M	3/15/20	11	39	High	Moderate
109	34	M	3/15/20	9	41	Low	Moderate
112	43	M	3/20/20	9	40	Low	Moderate
116	65	F	3/25/20	18	49	Low	Moderate
130	52	M	3/26/20	7	35	Mid	Mild
135	28	F	3/24/20	7	36	Low	Moderate
141	66	M	3/20/20	19	48	High	Moderate

144	56	M	3/16/20	23	54	Low	Moderate
156	50	F	3/23/20	11	41	High	Moderate
166	42	F	3/25/20	17	55	Low	Moderate
176	26	M	3/22/20	6	35	Low	Moderate
210	47	M	4/4/20	7	41	Low	Moderate
218	51	F	3/16/20	19	48	Mid	Severe
229	55	M	3/11/20	2	42	Low	Mild
251	53	M	3/18/20	22	51	Low	Severe
266	20	F	3/25/20	4	32	Low	Mild
270	50	M	3/18/20	9	39	Mid	Moderate
272	42	M	3/18/20	14	43	Mid	Moderate
277	65	M	3/18/20	13	45	High	Moderate
278	52	F	3/12/20	12	47	Mid	Moderate
293	72	M	3/8/20	17	63	High	Severe (hospitalized)
305	43	F	4/17/20	4	47	Low	Moderate
319	76	M	3/27/20	4	36	High	Mild
332	32	M	3/21/20	6	35	Mid	Moderate
346	30	M	3/16/20	11	39	Mid	Moderate
355	45	F	3/14/20	14	44	Low	Moderate
373	48	M	3/16/20	7	39	High	Moderate
377	44	M	3/14/20	9	41	High	Moderate
385	33	M	3/11/20	7	47	Mid	Moderate
407	34	M	4/1/20	11	43	Mid	Moderate
433	33	M	3/20/20	6	35	Low	Moderate
447	42	M	4/1/20	21	61	High	Severe
451	46	M	4/4/20	11	49	High	Severe (hospitalized)
537	36	M	3/23/20	14	59	Mid	Moderate
564	24	F	3/19/20	32	60	Low	Severe
573	25	M	3/20/20	17	56	High	Severe (hospitalized)
626	44	M	3/31/20	19	56	High	Moderate

728	62	F	3/15/20	53	130	High	Severe
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Table S2: Characteristics of SARS-CoV-2 RBD-reactive mAbs. Related to Figure

1. Cross-neutralizing mAbs against D614G and B.1.351 Beta, B.1.,617.2 Delta, B.1.617.1 Kappa, B.1.621 Mu, BA.1 Omicron are bolded.

mAb ID	Epitope specificity	VH gene	VL gene	# VH SHM	#VL SHM	CDR-H3 length	CDR-L3 length
S451-5	RBD Class 2	IGHV2-70*01	IGLV1-44*01	4	1	12	11
S451-506	RBD Class 3	IGHV3-53*02	IGKV1-9*01	9	4	12	10
S451-1140	RBD Unclassified	IGHV3-23*04	IGKV4-1*01	8	7	12	9
S451-1190	RBD Class 3	IGHV2-5*02	IGLV2-14*01	8	8	9	11
S626-84	RBD Class 2	IGHV1-2*02	IGLV2-23*02	7	9	16	10
S626-161	RBD Unclassified	IGHV4-39*01	IGKV3-20*01	8	2	18	10
S626-664	RBD Unclassified	IGHV4-39*01	IGLV1-51*02	8	5	19	10
S728-209	RBD Unclassified	IGHV2-5*04	IGKV1-12*01	14	12	12	9
S728-369	RBD Unclassified	IGHV4-31*03	IGKV1-5*03	18	13	23	8
S728-430	RBD Class 2	IGHV3-53*01	IGKV1-33*01	1	1	12	10
S728-537	RBD Class 2	IGHV1-2*02	IGKV1-12*01	15	9	17	9
S728-1157	RBD Unclassified	IGHV3-66*02	IGLV3-9*01	20	9	10	9
S728-1261	RBD Unclassified	IGHV4-4*02	IGKV3-20*01	8	12	13	10
S728-1690	RBD Class 2	IGHV1-69*04	IGKV3-20*01	19	8	15	9

Table S3: Antigen information and resource. Proline substitutions are indicated as *italic*. **Related to Figure 1 and Figure S2.**

Antigen	S1 NTD	RBD	S1 CTD	S2	Source
Spike FL, trimer					
Wildtype(WT)-2P	-	-	-	<i>K986P</i> , <i>V987P</i>	Krammer lab
Wildtype(WT)-6P	-	-	-	<i>F817P</i> , <i>A829P</i> , <i>A899P</i> , <i>A942P</i> , <i>K986P</i> , <i>V987P</i>	Krammer lab
B.1.1.7 Alpha-2P	del69-70, del144	N501Y	A570D, D614G, P681H	T716I, S982A, <i>K986P</i> , <i>V987P</i> , D1118H	Sather lab
B.1.351 Beta-2P	L18F, D80A, D215G, del241-243, R246I	K417N, E484K, N501Y	D614G	A701V, <i>K986P</i> , <i>V987P</i>	Sather lab
P.1 Gamma-2P	L18F, T20N, P26S, D138Y, R190S	K417T, E484K, N501Y	D614G, H655Y	<i>K986P</i> , <i>V987P</i> , T1027I, V1176F	Sather lab
B.1.617.2 Delta-2P	T19R, G142D, del156-157, R158G	L452R, T478K,	D614G, P681R	D950N, <i>K986P</i> , <i>V987P</i>	Sather lab
BA.1 Omicron-2P	A67V, H69del, V70del, T95I, G142D, V143del, Y144del, Y145del, N211del, L212I, insert214EPE	G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H	T547K, D614G, H655Y, N679K, P681H	N764K, D796Y, N856K, Q954H, N969K, L981F, <i>K986P</i> , <i>V987P</i>	Sather lab
BA.1 Omicron-6P	A67V, H69del, V70del, T95I, G142D, V143del, Y144del,	G339D, S371L, S373P, S375F, K417N, N440K, G446S,	T547K, D614G, H655Y, N679K, P681H	<i>V705C</i> , N764K, D796Y, <i>F817P</i> , <i>A829P</i> , N856K, <i>T883C</i> ,	Ward lab

	Y145del, N211del, insert214EPE	S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H		<i>A899P,</i> <i>A942P,</i> <i>Q954H,</i> <i>N969K,</i> <i>L981F,</i> <i>K986P,</i> <i>V987P</i>	
BA.2 Omicron-2P	T19I, L24del, P25del, P26del, A27S, G142D, V213G,	G339D, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, S477N, T478K, E484A, Q493R, Q498R, N501Y, Y505H	D614G, H655Y, N679K, P681H,	N764K, D796Y, Q954H, N969K, <i>K986P,</i> <i>V987P</i>	Sather lab
BA.4 Omicron-2P	T19I, L24del, P25del, P26del, A27S, H69del, V70del, G142D, V213G,	G339D, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, L452R, S477N, T478K, E484A, F486V, Q498R, N501Y, Y505H,	D614G, H655Y, N679K, P681H,	N764K, D796Y, Q954H, N969K, <i>K986P,</i> <i>V987P</i>	Sather lab
RBD					
WT	-	-	-	-	In-house
R346S	-	R346S	-	-	In-house
K417N	-	K417N	-	-	In-house
K417V	-	K417V	-	-	Krammer lab
K417T	-	K417T	-	-	In-house
G446V	-	G446V	-	-	In-house
N439K	-	N439K	-	-	Krammer lab
L452R	-	L452R	-	-	In house
S477N	-	S477N	-	-	In-house
E484K	-	E484K	-	-	Krammer lab
F486A	-	F486A	-	-	In-house

F486Y	-	F486Y	-	-	In-house
N487Q	-	N487Q	-	-	In-house
Y489F	-	Y489F	-	-	In-house
Q493A	-	Q493A	-	-	In-house
Q493N	-	Q493N	-	-	In-house
N501Y	-	N501Y	-	-	In-house
Y505A	-	Y505A	-	-	In-house
Y505F	-	Y505F	-	-	In-house
K417N/E484K/ L452R/N501Y	-	K417N/ E484K/ L452R/ N501Y	-	-	In-house
SARS-CoV-1 RBD WT					In-house
MERS-CoV RBD WT					In-house

Table S4: SARS-CoV-2 virus information and resource. Related to Figure 1 and 3.

Virus	S1 NTD	RBD	S1 CTD	S2	Source
D614G	-	-	D614G	-	2019-nCoV/USA-WA1/2020 D614G
B.1.351 Beta	L18F, D80A, D215G, L241del, L242del, A243del	K417N, E484K, N501Y	D614G	A701V	hCoV-19/USA/MD-HP01542/2021
P.1 Gamma	L18F, T20N, P26S, D138Y, G181V, R190S	K417T, E484K, N501Y	D614G, H655Y	T1027I, V1176F	hCoV-19/Japan/TY7-501/2021 from BEI
B.1.621 Mu	in3T, T95I, Y144S, Y145N,	R346K, E484K, N501Y	D614G, P681H	D950N	hCoV-19/USA/WI-UW-4340/2021
B.1.617.1 Kappa	G142D, E154K	L452R, E484Q	D614G, P681R	Q1071H, H1101D	hCoV-19/USA/CA-Stanford-15_S02/2021 from BEI
B.1.617.2 Delta	T19R, T95I, G142D, E156G, F157del, R158del	L452R, T478K	D614G, P681R	D950N	hCoV-19/USA/WI-UW-5250/2021
BA.1 Omicron	A67V, H69del, V70del, T95I, G142D, V143del, Y144del,	G339D, S371L, S373P, S375F, K417N, N440K, G446S,	T547K, D614G, H655Y, N679K, P681H	N764K, D796Y, N856K, Q954H, N969K, L981F	hCoV-19/USA/WI-WSLH-221686/2021

	Y145del, N211del, L212I, ins214EPE	S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H			
BA.2 Omicron	T19I, delL24, delP25, delP26, A27S, G142D, V213G	G339D, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, S477N, T478K, E484A, Q493R, Q498R, N501Y, Y505H	D614G, H655Y, N679K, P681H	N764K, D796Y, Q954H, N969K	hCoV-19/Japan/UT- NCD1288-2N/2022
BA.2.75 Omicron	T19I, delL24, delP25, delP26, A27S, G142D, K147E, W152R, F157L, I210V, V213G, G257S	G339H, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, G446S, N460K, S477N, T478K, E484A, Q498R, N501Y, Y505H	D614G, H655Y, N679K, P681H	N764K, D796Y, Q954H, N969K	hCoV-19/Japan/TY41- 716/2022
BA.4 Omicron	T19I, delL24, delP25, delP26, A27S, delH69, delV70, G142D, V213G	G339D, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, L452R, S477N, T478K,	D614G, H655Y, N679K, P681H,	N764K, D796Y, Q954H, N969K	hCoV-19/USA/MD- HP30386- PIDNBVCCQ/2022

		F486V, E484A, Q498R, N501Y, Y505H,			
BA.5 Omicron	T19I, delL24, delP25, delP26, A27S, delH69, delV70, G142D, V213G	G339D, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, L452R, S477N, T478K, F486V, E484A, Q498R, N501Y, Y505H,	D614G, H655Y, N679K, P681H,	N764K, D796Y, Q954H, N969K	SARS-CoV- 2/human/USA/COR-22- 063113/2022
BL.1 Omicron	T19I, delL24, delP25, delP26, A27S, G142D, K147E, W152R, F157L, I210V, V213G, G257S	G339H, R346T, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, G446S, N460K, S477N, T478K, E484A, Q498R, N501Y, Y505H	D574V, D614G, H655Y, N679K, P681H	N764K, D796Y, Q954H, N969K	SARS-CoV-2/human /USA/WI-UW-12980/2022

Table S5: Pairs of S728-1157 and spike-WT-6P-Mut7 residues within predicted hydrogen bonding distances. Calculated using EpitopeAnalyzer⁶³ using a cutoff distance of 3.4 Å. **Related to Figure 2 and Figure S4.**

#	RBD Residue [Atom]	Ab Residue [atom]	Antibody Region	Distance (Å)	RBD residue mutated in Omicron VOC	RBD residue conserved across all VOC's
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1	T415 [OG]	S56 [OG1]	CDRH2	2.78	No	Yes
2	Y421 [OH]	S53 [O]	CDRH2	2.73	No	Yes
3	Y453 [OH]	D98 [OD1]	CDRH3	3.5	No	Yes
4	L455 [O]	Y33 [OH]	CDRH1	3.29	No	Yes
5	R457 [O]	S53 [OG]	CDRH2	3.25	No	Yes
6	Y473 [OH]	R31 [O]	CDRH1	2.76	No	Yes
7	Y473 [OH]	S53 [OG]	CDRH2	3.26	No	Yes
8	Q474 [O]	R31 [NH1]	CDRH1	3.08	No	Yes
9	A475 [O]	L28 [N]	CDRH1	3.05	No	Yes
10	A475 [O]	N32 [ND2]	CDRH1	2.98	No	Yes
11	E484 [OE2]	Y99 [OH]	CDRH3	2.61	Yes	No
12	N487 [ND2]	G26 [O]	FR1	3.01	No	Yes
13	C488 [O]	Y99 [OH]	CDRH3	3.25	No	Yes
14	Y489 [OH]	R94 [NH1]	FR3	2.64	No	Yes
15	Y505 [OH]	Q31 [NE2]	CDRL1	2.62	Yes	No

Table S6. Cryo-EM data collection, refinement and model building statistics. Related to Figure 2 and Figure S4.

Map	S728-1157 + SARS-CoV-2-6P-Mut7 (global refinement)	S728-1157 + SARS-CoV-2-6P-Mut7 (focused refinement)
EMDB	EMD-27112	EMD-27113
Data collection		
Microscope	Thermo Fisher Titan Krios	
Voltage (kV)	300	
Detector	Gatan K2 Summit	
Recording mode	Counting	
Nominal magnification	130kx	
Movie micrograph pixelsize (Å)	1.045	
Dose rate (e ⁻ /[(camera pixel)*s])	6.017	
Number of frames per movie micrograph	36	
Frame exposure time (ms)	250	
Movie micrograph exposure time (s)	9	
Total dose (e ⁻ /Å ²)	50.0	
Defocus range (μm)	-0.8 to -1.5	
EM data processing		
Number of movie micrographs	1,718	1,718
Number of molecular projection images in map	151,948	29,595
Symmetry	C1	C1
Map resolution (FSC 0.143; Å)	3.3	3.7

Map sharpening B-factor (Å ²)	-85.3	-71.1
Structure Building and Validation		
<i>Number of atoms in deposited model</i>		
SARS-CoV-2-6P-Mut7	<i>n/a</i>	20,759
Fab Fv	<i>n/a</i>	1,653
Glycans	<i>n/a</i>	182
MolProbity score	<i>n/a</i>	1.07
Clashscore	<i>n/a</i>	1.66
Map correlation coefficient	<i>n/a</i>	0.75
EMRinger score	<i>n/a</i>	2.57
d FSC model (0.5; Å)	<i>n/a</i>	3.8
<i>RMSD from ideal</i>		
Bond length (Å)	<i>n/a</i>	0.021
Bond angles (°)	<i>n/a</i>	1.81
<i>Ramachandran plot</i>		
Favored (%)	<i>n/a</i>	97.13
Allowed (%)	<i>n/a</i>	2.87
Outliers (%)	<i>n/a</i>	0.00
Side chain rotamer outliers (%)	<i>n/a</i>	0.08
Cβ outliers (%)	<i>n/a</i>	0.00
PDB	<i>n/a</i>	8d0z

Materials and Methods

Monoclonal antibody isolation

We isolated a panel of RBD-reactive mAbs from peripheral blood mononuclear cells (PBMCs) of convalescent donors who previously had experienced symptomatic infection with SARS-CoV-2 (Table S1). The samples were collected during the first wave of the pandemic in May 2020, before other SARS-CoV-2 variants emerged. All studies were performed with the approval of the University of Chicago institutional review board (IRB20-0523). All participants provided prior written informed consent for the use of blood in research applications. This clinical trial was registered at ClinicalTrials.gov under identifier NCT04340050.

PBMCs were isolated from leukoreduction filters and frozen as described previously²¹. B cells were enriched from PBMCs via fluorescence-activated cell sorting (FACS). Cells were stained with CD19, CD3, and antigen probes conjugated oligo-fluorophore; cells of interest were identified as CD3⁻CD19⁺Antigen⁺. All mAbs were generated from oligo-tagged antigen bait-sorted cells identified through single-cell RNA sequencing (RNA-seq), as described previously^{12,21}.

Antigen-specific B cells were selected to generate mAbs based on antigen-probe intensity analyzed by JMP Pro 15. Antibody heavy and light chain genes were synthesized and cloned into human IgG1 and human kappa or lambda light chain expression vectors by Gibson assembly as previously described⁵⁶. The heavy and light chains of the corresponding mAb were transiently co-transfected into HEK293T cells. After transfection for 18 h, the transfected cells were supplemented with Protein-Free Hybridoma Medium Supernatant (PFHM-II, Gibco). The supernatant containing secreted mAb was harvested at day 4 and purified using protein A-agarose beads (Thermo Fisher) as detailed previously⁵⁶.

Recombinant spike protein expression

The recombinant D614G SARS-CoV-2 full-length (FL) spike, WT RBD, single RBD mutants (R346S, K417N, K417T, G446V, L452R, S477N, F486A, F486Y, N487Q, Y489F, Q493A, Q493N, N501Y, Y505A, Y505F), combination RBD mutant

(K417N/E484K/L452R/NN501Y), SARS-CoV-1 RBD and MERS-CoV RBD were generated in-house. Briefly, the recombinant antigens were expressed using Expi293F cells. The gene of interest was cloned into mammalian expression vector (in-house modified AbVec) and transfected using ExpiFectamine 293 kit according to the manufacturer's protocol. The supernatant was harvested at day 4 after transfection and incubated with Ni-nitrilotriacetic acid (Ni-NTA) agarose (Qiagen). The purification was carried out using gravity flow column and eluted with imidazole-containing buffer as previously described^{57,58}. The eluate was buffering-exchanged with PBS using Amicon centrifugal unit (Millipore). The recombinant FL spikes derived from variants B.1.1.7 Alpha, B.1.351 Beta, P.1 Gamma, B.1.617.2 Delta, BA.1, BA.2 and BA.4 Omicron were produced in the Sather Laboratory at Seattle Children's Research Institute. The K417V, N439K, E484K RBDs and recombinant FL spike WT-2P and 6P were produced in Krammer laboratory at the Icahn School of Medicine at Mount Sinai. The SARS-CoV-2-6P-Mut7 and spike BA.1 Omicron-6P were designed and produced as described in a previous study⁵⁹. The protein sequences and resources for each antigen are listed in [Table S3](#).

Enzyme-linked immunosorbent assay (ELISA)

Recombinant SARS-CoV-2 spike/RBD proteins were coated onto high protein-binding microtiter plates (Costar) at 2 µg/ml in phosphate buffered saline (PBS) at 50 µl/well, and kept overnight at 4°C. Plates were washed with PBS containing 0.05% Tween 20 (PBS-T) and blocked with 150 µl of PBS containing 20% fetal bovine serum (FBS) for 1 h at 37°C. Monoclonal antibodies were serially diluted 3-fold starting from 10 µg/ml in PBS and incubated in the wells for 1 h at 37°C. Plates were then washed and incubated with horseradish peroxidase (HRP)-conjugated goat anti-human IgG antibody (Jackson ImmunoResearch, 1:1000) for 1 h at 37°C. After washing, 100 µl of Super AquaBlue ELISA substrate (eBioscience) was added per well. Absorbance was measured at 405nm on a microplate spectrophotometer (Bio-Rad). The assays were standardized using control antibodies with known binding characteristics in every plate, and the plates were

developed until the absorbance of the control reached an optical density (OD) of 3.0. All mAbs were tested in duplicate, and each experiment was performed twice.

Serum ELISA

High protein-binding microtiter plates were coated with recombinant SARS-CoV-2 spike antigens at 2 µg/ml in PBS overnight at 4°C. Plates were washed with PBS 0.05% Tween and blocked with 200 µl PBS 0.1% Tween + 3% skim milk powder for 1 hour at room temperature (RT). Plasma samples were heat-inactivated for 1 hour at 56°C before perform serology experiment. Plasma were serially diluted 2-fold in PBS 0.1% Tween + 1% skim milk powder. Plates were incubated with serum dilutions for 2 hours at RT. The HRP-conjugated goat anti-human Ig secondary antibody diluted at 1:3000 with PBS 0.1% Tween + 1% skim milk powder was used to detect binding of antibodies. After 1-hour of incubation, plates were developed with 100 µl SigmaFast OPD solution (Sigma-Aldrich) for 10 minutes. Then, 50 µl 3M HCl was used to stop the development reaction. Absorbance was measured at 490 nm on a microplate spectrophotometer (BioRad). End point titers were extrapolated from sigmoidal 4PL (where X is log concentration) standard curve for each sample. Limit of detection (LOD) is defined as the mean plus 3 S.D. of the O.D. signal recorded using plasma from pre-SARS-CoV-2 subjects. All calculations were performed in GraphPad Prism software (version 9.0).

Competition ELISA

To determine the target epitope classification of RBD-reactive mAbs, competition ELISAs were performed using other mAbs with known epitope binding characteristics as competitor mAbs. Competitor mAbs were biotinylated using EZ-Link sulfo-NHS-biotin (Thermo Scientific) for 2h at room temperature (RT). The excess biotin of biotinylated mAbs was removed with 7k molecular weight-cutoff (MWCO) Zeba spin desalting columns (Thermo Scientific). Plates were coated with 2 µg/ml RBD antigen overnight at 4°C. Plates were blocked with PBS–20% FBS for 2h at RT, and the 2-fold dilution of the mAbs of an undetermined class, or serum, was added, starting at 20 µg/ml of mAbs and a 1:10 dilution of serum. After antibody incubation for 2h at RT, the biotinylated competitor

mAb was added at a concentration twice that of its dissociation constant (K_D) and incubated for another 2 h at RT together with the mAb or serum that was previously added. Plates were washed and incubated with 100 μ l HRP-conjugated streptavidin (Southern Biotech) at a dilution of 1:1000 for 1 h at 37°C. The plates were developed with the Super AquaBlue ELISA substrate (eBioscience). To normalize the assays, the competitor biotinylated mAb was added in a well without any competing mAbs or serum as a control. Data were recorded when the absorbance of the control well reached and OD of 1.0-1.5. The percent competition between mAbs was then calculated by dividing a sample's observed OD by the OD reached by the positive control, subtracting this value from 1, and multiplying by 100. For serum, ODs were \log_{10} -transformed and analyzed by nonlinear regression to determine the 50% inhibition concentration (IC_{50}) values using GraphPad Prism software (version 9.0). The data were transformed to $\text{Log}_{10}P$ and plotted into graph representative of reciprocal serum dilution of the IC_{50} of serum dilution that can achieve 50% competition with the competitor mAb of interest. All mAbs were tested in duplicate, each experiment was performed two times independently, and values from two independent experiments were averaged.

Plaque assays

Plaque assays were performed with SARS-CoV-2 variant viruses on Vero E6/TMPRSS2 cells (Table S4). Cells were cultured to achieve 90% confluency prior to being trypsinized and seeded at a density of 3×10^4 cells/well in 96-well plates. On the following day, 10^2 plaque-forming unit (PFU) of SARS-CoV-2 variant was incubated with 2-fold-diluted mAbs for 1h. The antibody-virus mixture was incubated with Vero E6/TMPRSS2 cells for 3 days at 37°C. Plates were fixed with 20% methanol and then stained with crystal violet solution. The complete inhibitory concentrations (IC_{99}) were calculated using the $\log(\text{inhibitor})$ versus normalized response (variable slope), performed in GraphPad Prism (version 9.0). All mAbs were tested in duplicate, and each experiment was performed twice.

Focus reduction neutralization test (FRNT)

Focus reduction neutralization test (FRNT) were used to determine neutralization activities as an additional platform beside plaque assay. Serial dilutions of serum starting at a final concentration of 1:20 will be mixed with 10^3 focus-forming units of virus per well and incubated for 1 h at 37 °C. A pooled pre-pandemic serum sample is served as a control. The antibody-virus mixture will be inoculated onto Vero E6/TMPRSS2 cells in 96-well plates and incubated for 1 h at 37 °C. An equal volume of methylcellulose solution was added to each well. The cells were incubated for 16 h at 37 °C and then fixed with formalin. After the formalin was removed, the cells were immunostained with a mouse monoclonal antibody against SARS-CoV-1/2 nucleoprotein [clone 1C7C7 (Sigma-Aldrich)], followed by a HRP-labeled goat anti-mouse immunoglobulin (SeraCare Life Sciences). The infected cells were stained with TrueBlue Substrate (SeraCare Life Sciences) and then washed with distilled water. After cell drying, the focus numbers were quantified by using an ImmunoSpot S6 Analyzer, ImmunoCapture software, and BioSpot software (Cellular Technology). The IC_{50} was calculated from the interpolated value from the log(inhibitor) versus normalized response, using variable slope (four parameters) nonlinear regression performed in GraphPad Prism (version 9.0).

Negative stain electron microscopy

Spike BA.1 Omicron-6P was complexed with a 0.5-fold molar excess of IgG S728-1157 and incubated for 30 mins at room temperature. The complex was diluted to 0.03 mg/ml and deposited on a glow-discharged carbon-coated copper mesh grid. 2% uranyl formate (w/v) was used to stain the sample for 90 seconds. The negative stain dataset was collected on a Thermo Fisher Tecnai T12 Spirit (120keV, 56,000x magnification, 2.06 apix) paired with a FEI Eagle 4k x 4k CCD camera. Legion⁶⁰ was used to automate the data collection and raw micrographs were store in the Appion database⁶¹. Dogpicker⁶² picked particles and the dataset was processed in RELION 3.0⁶². UCSF Chimera⁶³ was used for map segmentation and figure making.

Cryo-electron microscopy and model building

SARS-CoV-2-6P-Mut7 was complexed with a 0.5-fold molar excess of IgG S728-1157 and incubated for 30 mins at room temperature. Grids were prepared using a Thermo Fisher Vitrobot Mark IV set to 4°C and 100% humidity. The complex, at 0.7 mg/ml, was briefly incubated with lauryl maltose neopentyl glycol (final concentration of 0.005 mM; Anatrace), deposited on a glow-discharged Quantifoil 1.2/1.3-400 mesh grid, and blotted for 3 seconds. The grid was loaded into a Thermo Fisher Titan Krios (130,000x magnification, 300 kEV, 1.045-Å pixel size) paired with a Gatan 4k x 4k K2 Summit direct electron detector. The Leginon software was used for data collection automation and resulting images were stored in the Appion database. Initial data processing was performed with cryoSPARC v3.2⁶⁴, which included CTF correction using GCTF⁶⁵, template picking, and 2D and 3D classification and refinement methods leading to a ~3.3 Å C1 global reconstruction. The particles from this reconstruction were imported into Relion 3.1⁶⁶, subjected to C3 symmetry expansion, followed by focused 3D classifications without alignments using a mask around the antibody Fab and S-protein RBD regions of a single protomer. Classes with well-resolved density in this region were selected and subjected to additional rounds of focused classification. Refinements were performed with limited angular searches and a mask around the trimeric S-protein and a single Fab. The final set of particles reconstructed to ~3.7 Å global resolution.

Model building was initiated by rigid body docking of the x-ray structure of the Fab and a published cryo-EM model of the SARS-CoV-2 spike open state (PDB ID: 6VYB) into the cryo-EM map using UCSF Chimera⁶³. Manual building, mutagenesis and refinement were performed in Coot 0.9.6⁶⁷, followed by relaxed refinement using Rosetta Relax⁶⁸. Model manipulation and validation was also done using Phenix 1.20⁶⁹. More complete data collection, processing and model building statistics are summarized in [Table S6](#). Figures were generated using UCSF ChimeraX⁷⁰.

Crystallization and X-ray structure determination

384 conditions of the JCSG Core Suite (Qiagen) were used for crystal screening of S728-1157 Fab crystals on the robotic CrystalMation system (Rigaku) at Scripps Research. Crystallization trials were set-up by the vapor diffusion method in sitting drops containing 0.1 μ l of protein complex and 0.1 μ l of reservoir solution. Crystals appeared on day 14, were harvested on day 21, pre-equilibrated in cryoprotectant containing 15% ethylene glycol, and then flash cooled and stored in liquid nitrogen until data collection. Diffraction quality crystals were obtained in solution containing 0.2 M di-Ammonium tartrate, 20% (w/v) polyethylene glycol (PEG) 3350. Diffraction data were collected at cryogenic temperature (100 K) on Scripps/Stanford beamline 12-1 at the Stanford Synchrotron Radiation Lightsource (SSRL). The X-ray data were processed with HKL2000⁷¹. The X-ray structures were solved by molecular replacement (MR) using PHASER⁷² with MR models for the Fabs from PDB ID: 7KN4⁷³. Iterative model building and refinement were carried out in COOT⁷⁴ and PHENIX⁷⁵, respectively.

Animals and challenge viruses

To determine whether mAbs in the panel could reduce viral load *in vivo*, Syrian hamsters (females, 6-8 weeks old) were intraperitoneally administered 5 mg/kg of candidate mAb 1 day after intranasal infection with 10³ PFU of SARS-CoV-2 viruses (an early SARS-CoV-2 isolate, Delta or BA.1 Omicron). Control animals were treated with an Ebola-specific mAb (KZ52) of matched isotype. At day 4 post-infection, lung tissues and nasal turbinate were collected to evaluate viral titers by standard plaque assay on Vero E6/TMPRSS2 cells. The animal study was conducted in accordance with the recommendations for care and use of animals by the Institutional Animal Care and Use Committee at the University of Wisconsin under BSL-3 containment using approved protocols.

Biolayer interferometry (BLI)

To determine precise binding affinity, the dissociation constant (K_D) of each mAb was performed by biolayer interferometry (BLI) with an Octet K2 instrument (Forte Bio/Sartorius). The trimeric spike SARS-CoV-2 and its variants were biotinylated (EZ-

Link Sulfo-NHS-Biotin, ThermoFisher), desalted (Zeba Spike Desalting, ThermoFisher), and loaded at a concentration of 500 nM onto streptavidin (SA) biosensor (Forte Bio/Sartorius) for 300 s, followed by kinetic buffer (1x PBS containing 0.02% Tween-20 and 0.1% bovine serum albumin) for 60 s. The biosensor was then moved to associate with mAbs of interest (142 nM) for 300 s, followed by disassociation with the kinetic buffer for 300 s. On rate, off-rate, and K_D were evaluated with a global fit, the average of those values with high R-squared from two independent experiments were presented. Analysis was performed by Octet Data Analysis HT software (Forte Bio/Sartorius) with 1:1 fitting model for Fabs and 1:2 interacting model for IgG.

For competitive assay by BLI, streptavidin (SA) biosensor was pre-equilibrated in 1xPBS for at least 600s to bind with the biotinylated trimeric spike WT-6P and spike BA.1 Omicron-6P for 300s. The first mAb was associated on the loaded sensor for 300s, followed by the second mAb for another 300s. The final volume for all the solutions was 200 μ l/well. All of the assays were performed with kinetic buffer at 30°C. Data were analyzed by Octet Data Analysis HT software (Forte Bio/Sartorius) and plotted using GraphPad Prism.

Statistics

All statistical analyses were performed using GraphPad Prism software (version 9.0). The numbers of biological repeats for experiments and specific tests for statistical significance used are described in the corresponding figure legends. P values of ≤ 0.05 were considered significant [* , $P \leq 0.05$; ** , $P \leq 0.01$; *** , $P \leq 0.001$; **** , $P < 0.0001$), while P values of > 0.05 were considered as non-significant (ns)].

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