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## **Rapid Identification of Neutralizing Antibodies against SARS-CoV-2 Variants by mRNA Display** — [Source link](#)

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1                                   **Rapid Identification of Neutralizing Antibodies**  
2                                   **against SARS-CoV-2 Variants by mRNA Display**

3  
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22 **Abstract**

23       The increasing prevalence of SARS-CoV-2 variants with the ability to escape existing  
24 humoral protection conferred by previous infection and/or immunization necessitates the  
25 discovery of broadly-reactive neutralizing antibodies (nAbs). Utilizing mRNA display, we  
26 identified a set of antibodies against SARS-CoV-2 spike (S) proteins and characterized the  
27 structures of nAbs that recognized epitopes in the S1 subunit of the S glycoprotein. These  
28 structural studies revealed distinct binding modes for several antibodies, including targeting of  
29 rare cryptic epitopes in the receptor-binding domain (RBD) of S that interacts with angiotensin-  
30 converting enzyme 2 (ACE2) to initiate infection, as well as the S1 subdomain 1. A potent  
31 ACE2-blocking nAb was further engineered to sustain binding to S RBD with the E484K and  
32 L452R substitutions found in multiple SARS-CoV-2 variants. We demonstrate that mRNA  
33 display is a promising approach for the rapid identification of nAbs that can be used in  
34 combination to combat emerging SARS-CoV-2 variants.

35

36 **Keywords**

37 SARS-CoV-2, mRNA display, antibody, antibody design, neutralizing antibody, anti-spike  
38 antibody, SARS-CoV-2 variants

39

## 40 **Introduction**

41 The emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the  
42 causative agent of the respiratory disease COVID-19, has resulted in a pandemic that brought the  
43 world to a standstill (Zhou et al., 2020). Despite the rapid development and success of vaccines  
44 and antibody therapies, ongoing SARS-CoV-2 antigenic drift has resulted in the emergence of  
45 variants that pose new threats (Davies et al., 2021; Plante et al., 2021; Yurkovetskiy et al., 2020).  
46 Various studies have shown that several of these variants have the ability to escape antibody  
47 neutralization mediated by antisera from recovered COVID-19 patients/vaccinated individuals or  
48 recombinant neutralizing antibodies (nAbs) developed as therapeutics (Cerutti et al., 2021;  
49 McCallum et al., 2021a; Suryadevara et al., 2021). Thus, along with modified vaccines to combat  
50 variants, there is an urgent need for development of prophylactic and therapeutic anti-viral drugs,  
51 including biologics such as nAbs, with sustained efficacy against SARS-CoV-2 variants.

52 The trimeric SARS-CoV-2 spike (S) glycoprotein serves as the fusion machinery for viral  
53 entry, and therefore represents the main target of nAbs (Brouwer et al., 2020; Cao et al., 2020;  
54 Robbiani et al., 2020). The SARS-CoV-2 S trimer utilizes the angiotensin-converting enzyme 2  
55 (ACE2) as its host receptor (Hoffmann et al., 2020; Li et al., 2003; Zhou et al., 2020), through  
56 interactions with the receptor-binding domains (RBDs) located at the apex of the S trimer. The  
57 RBDs adopt either ‘down’ or ‘up’ conformations, with RBD binding to ACE2 facilitated only by  
58 the ‘up’ conformation (Kirchdoerfer et al., 2016; Li et al., 2019; Walls et al., 2016, 2020; Wrapp  
59 et al., 2020; Yuan et al., 2017). While the majority of potent anti-SARS-CoV-2 nAbs target the  
60 RBD and directly compete with ACE2 binding (Barnes et al., 2020a; Brouwer et al., 2020; Cao  
61 et al., 2020; Robbiani et al., 2020), recent studies have revealed nAbs that target the N-terminal  
62 domain (NTD) (Liu et al., 2020; McCallum et al., 2021b) and S2 stem helix (Zhou et al., 2021).

63 The structures of numerous monoclonal antibodies (mAbs) recognizing the RBD and NTD  
64 have been characterized (Barnes et al., 2020b, 2020a; Baum et al., 2020; Brouwer et al., 2020;  
65 Hansen et al., 2020; Pinto et al., 2020), enabling their classification based on shared epitopes and  
66 neutralizing properties (Barnes et al., 2020b; Dejnirattisai et al., 2021; McCallum et al., 2021b;  
67 Yuan et al., 2021). A subset of mAbs that recognize non-overlapping epitopes are in clinical  
68 trials or have received emergency use authorization from the US Food and Drug Administration  
69 (FDA) for the treatment and prevention of COVID-19 (Cathcart et al., 2021; Jones et al., 2021;  
70 Weinreich et al., 2021). However, ongoing viral evolution and genetic drift has resulted in an  
71 accumulation of mutations and/or deletions found in the S RBD and NTD that enhance affinity  
72 of ACE2 binding and allow some variants to evade existing immunity (Cele et al., 2021; Tegally  
73 et al., 2021). Thus, current emergency-authorized therapies developed early in the pandemic  
74 based on the first-wave or ‘A’ strain S sequence could potentially be less effective against  
75 emerging SARS-CoV-2 variants that harbor escape mutations mapped to their epitopes (Greaney  
76 et al., 2021a, 2021b; Starr et al., 2020, 2021; Weisblum et al., 2020).

77 Here, we report our identification via mRNA display (Newton et al., 2020; Olson et al.,  
78 2008; Roberts and Szostak, 1997; Takahashi et al., 2003) of a set of novel mAbs targeting  
79 SARS-CoV-2 S, which we demonstrate neutralize both authentic and pseudoviral SARS-CoV-2  
80 with  $IC_{50}$ s between 0.076 – 7.0  $\mu$ g/mL. Structural analysis revealed a subset of these nAbs  
81 recognize RBD and NTD epitopes, including a rare, cryptic, cross-reactive RBD epitope.  
82 Moreover, we characterize a weakly neutralizing antibody that recognizes the S1 subdomain 1  
83 (SD1), providing insight into a unique class of antibodies that are infrequently found among  
84 convalescent individuals (Zost et al., 2020a, 2020b) that can be utilized in the fight against  
85 COVID-19. Finally, we describe the utility of mRNA display for rapid identification of variant-

86 resistant antibody clones. This powerful technique enabled the rapid selection of a discovered  
87 SARS-CoV-2 nAb to extend its neutralizing capability to SARS-CoV-2 expressing the E484K  
88 and L452R S RBD mutations found in multiple SARS-CoV-2 variants.

## 89 **Results**

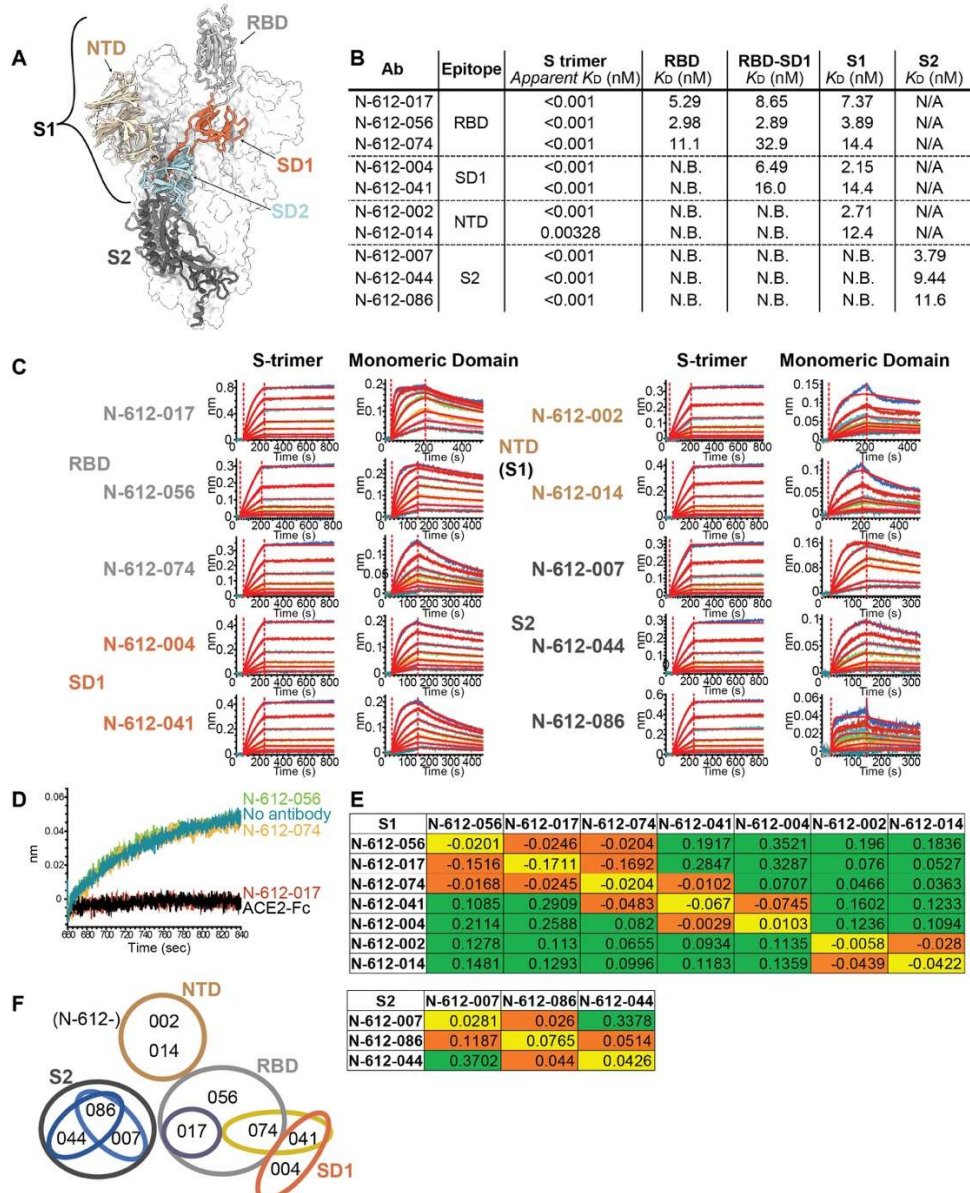
### 90 **Identification of Anti-SARS-CoV-2 Spike Antibodies by mRNA Display**

91 We utilized mRNA display to identify mAbs targeting the S protein of SARS-CoV-2 and  
92 discovered 10 novel VH/VL sequences that bind various domains on S (Table S1). S comprises  
93 an N-terminal fragment known as S1, which further divides into the NTD, ACE2 RBD, small C-  
94 terminal subdomains 1 and 2 (SD1 and SD2), and a C-terminal “S2” fragment (Figure 1A). Bio-  
95 layer interferometry (BLI) kinetic analysis using recombinant SARS-CoV-2 RBD (residue 319-  
96 541), RBD-SD1 (residue 319-591), S1 (residue 16-685), and S2 (residue 686-1213) proteins  
97 revealed 3 antibodies bind the RBD (N-612-017, N-612-056, and N-612-074), 2 antibodies bind  
98 the SD1 domain (N-612-004 and N-612-041), 2 antibodies bind the NTD (N-612-002 and N-  
99 612-014), and 3 antibodies bind the S2 domain (N-612-007, N-612-044, and N-612-086) (Figure  
100 1A-C). All 10 antibodies bind corresponding binding domains with low nM binding affinity ( $K_D$ )  
101 (Figure 1B and Table S2), but apparent affinities are far superior ( $K_D < 3$  pM) for S trimers  
102 (Figure 1B and 1C, and Table S3). Among the 3 RBD binders, only N-612-017 showed  
103 competition with ACE2 binding (Figure 1D).

104 To further map the binding regions of the 10 antibodies, we performed epitope binning  
105 experiments using S1 and S2 fragments separately (Figure 1E). Two NTD binders blocked each  
106 other but not RBD or SD1 binding antibodies. The 3 RBD binders competed with each other  
107 (Figure 1E and F) despite N-612-017 being the only ACE2 blocker (Figure 1D). SD1 binders N-  
108 612-004 and N-612-041 blocked each other, but only N-612-041 blocked N-612-074 (an RBD

109 binder) suggesting N-612-041 and N-612-074 have proximal or overlapping binding sites  
110 (Figure 1E and 1F). Epitope binning using the S2 domain revealed N-612-007 and N-612-044  
111 are non-competing whereas N-612-086 competes with both N-612-007 and N-612-044,  
112 suggesting they all bind distinct epitopes on S2 (Figure 1E and 1F).

113 In addition, multiple biophysical assays were carried out to determine the developability of  
114 all 10 antibodies (Table S4) (Jain et al., 2017). All 10 mAbs displayed low polyreactivity scores  
115 by meso scale diagnostic (MSD) analysis and low self-interaction scores by BLI-clone self-  
116 interaction (CSI) (Table S4). Eight of the mAb candidates exhibited low hydrophobicity in the  
117 hydrophobic interaction column (HIC) chromatography while higher hydrophobicity was  
118 observed for N-612-041 and N-612-074 (Table S4). N-612-041 also showed more rapid  
119 aggregation in an accelerated stability assay system while the other 9 mAbs demonstrated long-  
120 term stability (Table S4). Furthermore, all 10 mAbs exhibited desirable thermostability of Fab in  
121 differential scanning fluorimetry (DSF) melting temperature ( $T_m$ ) analysis, although N-612-044  
122 exhibited heterogeneous characteristics in thermostability and hydrophobic interaction column  
123 chromatography. Ultimately, 7 out of 10 antibodies displayed biophysical characteristics within  
124 the acceptance criteria, indicating antibodies engineered by mRNA display can have favorable  
125 developability.

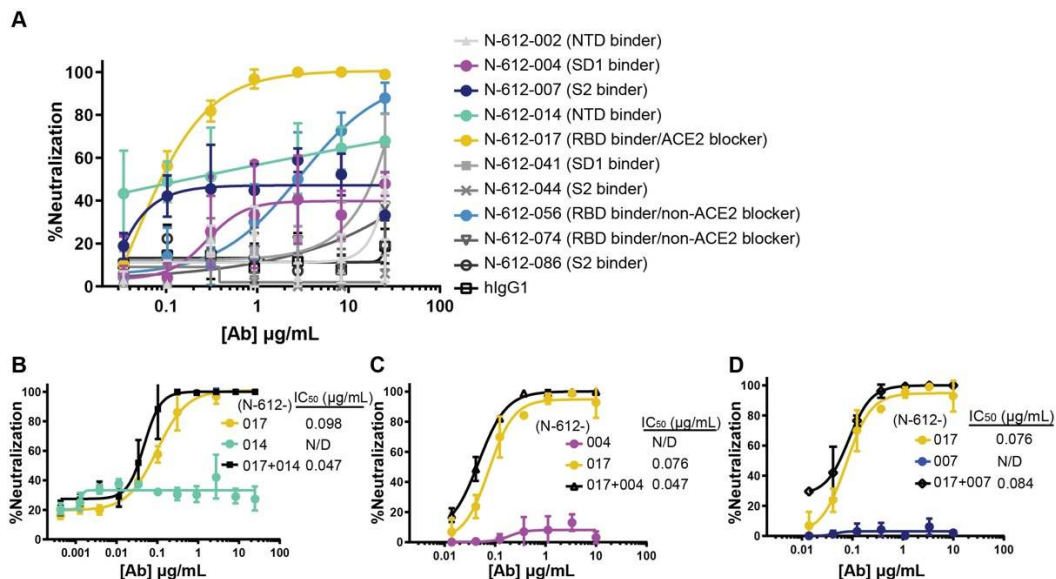


126  
 127 **Figure 1. Identification of SARS-CoV-2 Spike Targeting Monoclonal Antibodies.** (A) Model  
 128 of the SARS-CoV-2 spike trimer domains (PDB 6VYB); NTD (wheat), RBD (light gray), SD1  
 129 (coral), SD2 (powder blue), and S2 (dark blue). (B)  $K_D$  summary table from BLI kinetic analysis  
 130 of 10 antibodies against spike trimer and various domains used as analytes. N.B. indicates no  
 131 binding. N/A is untested. Apparent  $K_D$  values for S-trimer were obtained by curve fitting with a  
 132 bivalent model. (C) BLI kinetic analysis of 10 antibodies against the spike trimer (left) and each  
 133 corresponding domain (right). (D) BLI blocking assay: biosensors were coated with RBD and  
 134 subsequently all RBD binding antibodies (N-612-017, N-612-056, and N-612-074) and ACE2-  
 135 IgG1Fc were incubated with RBD coated biosensor. The recorded signal from ACE2-IgG1Fc  
 136 binding to the RBD on the biosensor indicates the RBD blocking capability of the test samples.  
 137 Both N-612-017 and ACE2-IgG1Fc completely blocked RBD and ACE2 interaction. (E) Epitope  
 138 binning data indicating competing antibody pairs in red and non-competing antibody pairs in  
 139 green. Self-blocking is in orange. (F) Epitope binning diagram mapping overlapping regions of  
 140 binding sites of 10 mAbs.



## 141 Neutralization Activity Assessment of Anti-SARS-CoV-2 Antibodies

142 Ten mAbs identified by mRNA display were assessed for neutralization activity against  
143 authentic SARS-CoV-2 virus in a Vero E6 cell neutralization assay. The ACE2-blocking anti-  
144 RBD antibody N-612-017 demonstrated the highest neutralization activity and the non-ACE2-  
145 blocking RBD binder N-612-056 showed weaker but nearly complete neutralization of ~87%.  
146 (Figure 2A). N-612-004 (SD1 binder), N-612-007 (S2 binder), and N-612-014 (NTD) antibodies  
147 all showed some neutralization activity that plateaued at 40~60% (Figure 2A), similar to  
148 previous observations made for anti-NTD antibodies (McCallum et al., 2021b). We next  
149 investigated the activity of N-612-017 in combination with N-612-004, N-612-007, and N-612-  
150 014. N-612-017 and respective partners were mixed in equal concentrations. All combinations  
151 tested showed slightly improved  $IC_{50}$  values compared to N-612-017 by itself, suggesting both  
152 antibodies present in mixture can bind to S simultaneously and in some cases non-RBD domain  
153 binders can enhance activity of RBD-binding nAb (Figure 2B, C and D).



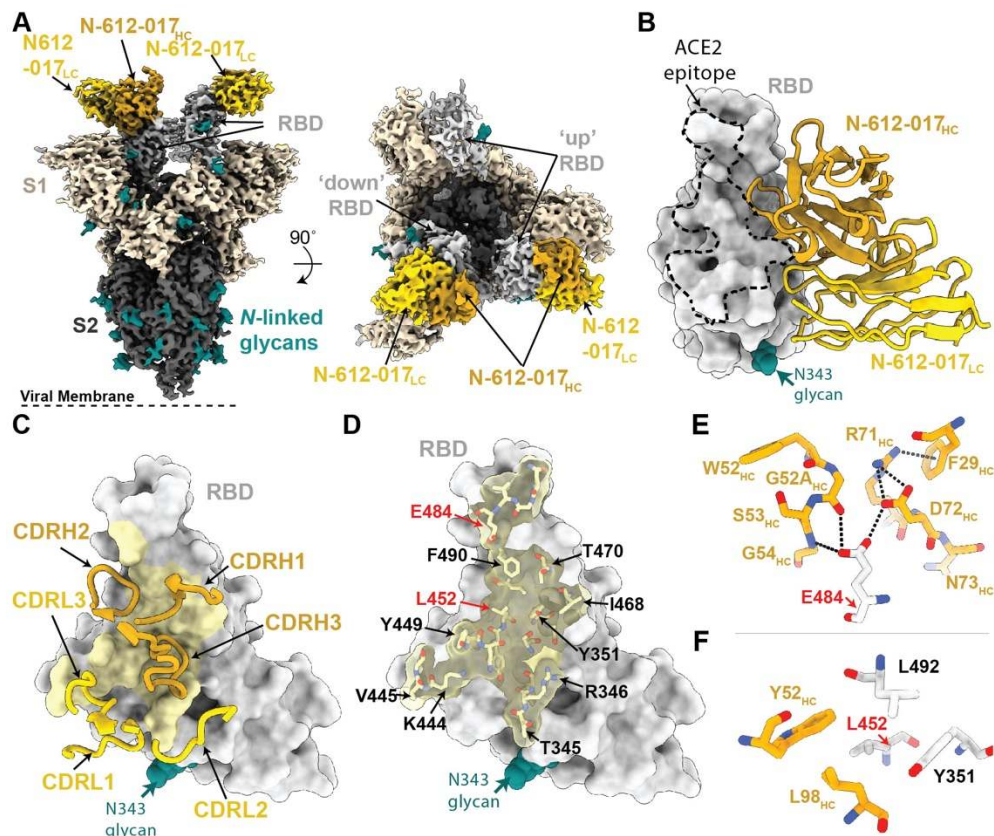
154  
155 **Figure 2. Neutralization Activity of mAbs in Vero E6 Live Virus Neutralization Assay.** (A)  
156 Dose-dependent neutralization of SARS-CoV-2 virus by 10 mAbs selected by mRNA library  
157 display. N-612-017 neutralization activity in combination with (B) N-612-014, (C) N-612-004,  
158 and (D) N-612-007. X-axis represents the concentration of antibodies when used alone; when  
159 antibodies were combined, an equal concentration of each antibody was used.

160 Neutralization activity of N-612-014 (NTD binder) showed variable saturation between  
161 assays and prevented accurate IC<sub>50</sub> determination (Figure S1A). To test whether activity of N-  
162 612-014 changes in time-dependent manner, we tested the effects of longer antibody-virus  
163 incubation times on neutralization potency. With a virus-antibody incubation time of ~30 min,  
164 neutralization activity plateaued between 20~90% (Figure S1A). In contrast, when virus was  
165 incubated with antibody for 24 hours, neutralization activity plateaued at ~90% with IC<sub>50</sub> values  
166 of 0.023-0.025 µg/mL (Figure S1B). Longer incubation also resulted in improved neutralization  
167 potencies for N-612-017, N-612-056, and positive control convalescent plasma serum (Figure  
168 S1C), suggesting a change in viral infectivity due to a time-dependent conformational change in  
169 spike (Huo et al., 2020; Wec et al., 2020).

#### 170 **Structural Characterization of RBD-Specific, ACE2 Blocking nAb N-612-017**

171 To investigate the specificity of RBD-targeting for nAbs N-612-017 and N-612-056, we  
172 determined a 3.2 Å single-particle cryo-electron microscopy (cryo-EM) structure of a complex  
173 between SARS-CoV-2 S trimer and the N-612-017 Fab (Figure 3, Figure S2 and Table S5), and  
174 a 2.9 Å X-ray crystal structure of a SARS-CoV-2 RBD – N-612-056 Fab complex (Figure 4 and  
175 Table S6). The N-612-017 – S trimer complex structure revealed N-612-017 Fab binding to both  
176 ‘up’ and ‘down’ RBD conformations, and recognition of an epitope that partially overlapped  
177 with the ACE2 receptor binding site (Figure 3A and 3B), consistent with BLI competition data  
178 (Figure 1D). N-612-017 uses five of its six complementarity-determining region (CDR) loops  
179 and HC framework region 3 (FWR3) to interact with an epitope focused on RBD residues  
180 adjacent to the ACE2 receptor binding ridge (Figure 3C and 3D), resulting in ~1018Å<sup>2</sup> buried  
181 surface area (BSA) on the epitope. The CDRH2 and CDRH3 loops mediate the majority of RBD  
182 contacts (~616Å<sup>2</sup> of ~1030Å<sup>2</sup> total paratope BSA), establishing hydrophobic and hydrogen bond

183 interactions at the Fab-RBD interface. Of note, N-612-017 CDRH2 loop residues contact RBD  
184 positions frequently mutated among circulating variants (Deng et al., 2021; Kuzmina et al., 2021;  
185 McCallum et al., 2021a; Wang et al., 2021). RBD residue E484<sub>RBD</sub> established hydrogen bond  
186 interactions with G52<sub>HC</sub> and G54<sub>HC</sub> in CDR2 and D72<sub>HC</sub> in FWR3 (Figure 3E), while L452<sub>RBD</sub>  
187 formed stacking interactions with CDR2 residue Y52<sub>HC</sub> (Figure 3F). Taken together, these data  
188 indicate nAb N-612-017 targets the RBD similarly to nAbs that belong to the class 2 binding  
189 mode, which is the predominant nAb class identified in convalescent and vaccinated donors  
190 (Barnes et al., 2020b; Wang et al., 2021).



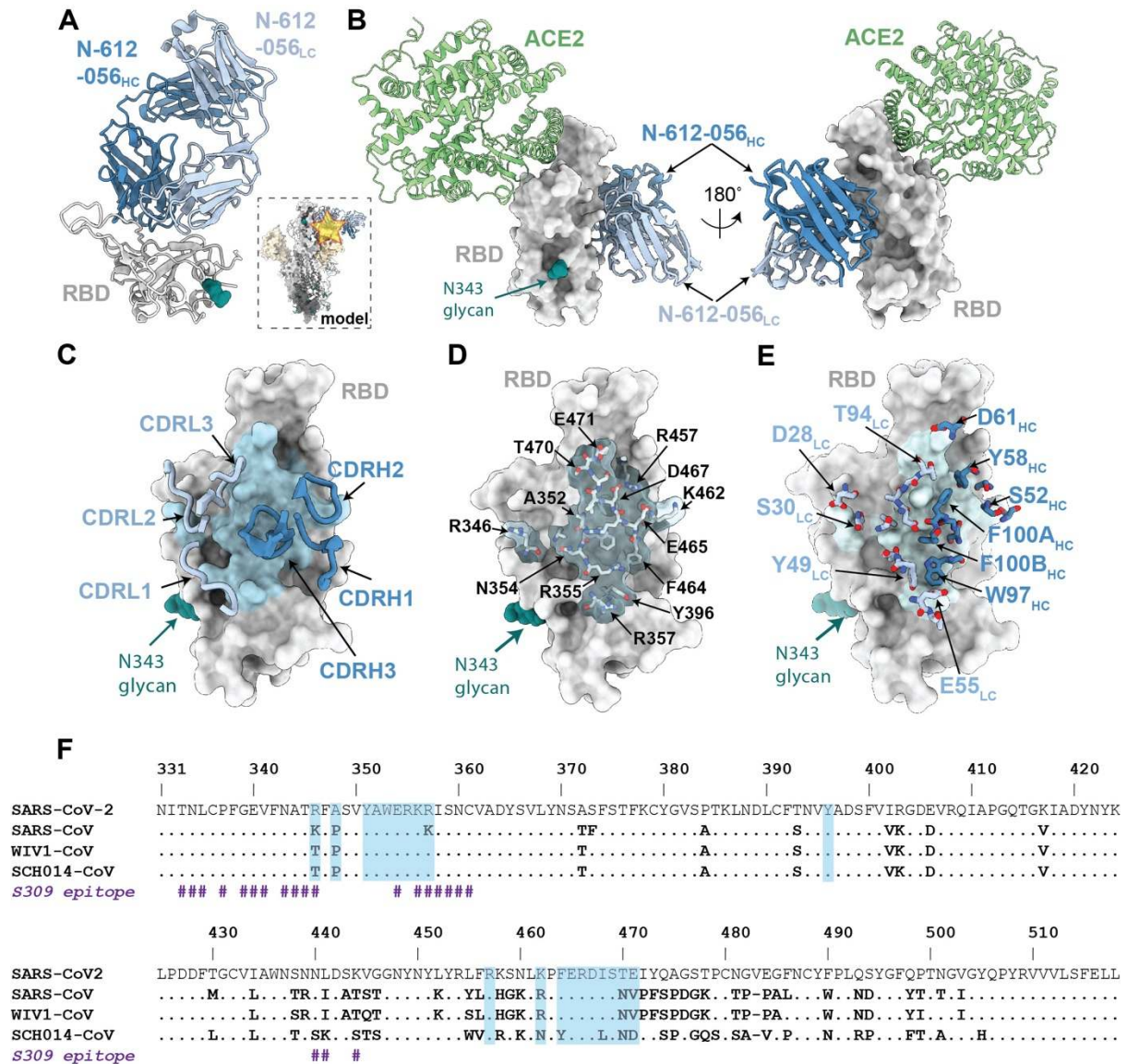
191  
192 **Figure 3. Cryo-EM Structure of the N-612-017 – S Complex.** (A) Cryo-EM density for the N-  
193 612-017- S trimer complex. Side view (left panel) illustrates orientation with respect to the viral  
194 membrane (dashed line). (B) Close-up view of N-612-017 variable domains (HC: gold, LC:  
195 yellow) bound to RBD (gray surface). ACE2 receptor binding site is shown as a dashed line. (C)  
196 N-612-017 CDR loops mapped on the RBD. (D) Surface and stick representation of N-612-017  
197 epitope (yellow) on RBD surface (gray). (E,F). Residue-level interactions between N-612-017  
198 (gold) and SARS-CoV-2 RBD (gray). Potential hydrogen bond interactions are illustrated by  
199 dashed black lines.

## 200 **Structural Characterization of RBD-Specific nAb N-612-056 Targeting Cryptic Site**

201       Next, we analyzed the high-resolution X-ray crystal structure of the SARS-CoV-2 RBD –N-  
202 612-056 Fab complex (Figure 4A). This method was used rather than cryo-EM due to N-612-  
203 056’s lack of binding to intact S trimers (Figure 4A; inset). Similar to the donor-derived antibody  
204 COVOX-45 (Dejnirattisai et al., 2021), N-612-056 binds a rare cryptic epitope that is not readily  
205 found in the repertoire of antibodies from convalescent donors (Figure S4). Consistent with  
206 observed binding to dissociated S1 protomers by single-particle cryo-EM (data not shown), the  
207 N-612-056 cryptic epitope is inaccessible on an S trimer due to steric clashes with the  
208 neighboring NTD, and does not overlap with the ACE2 binding site (Figure 4A and 4B). N-612-  
209 056 HC and LC CDR loops participate equally to bury  $\sim 890 \text{ \AA}^2$  of the RBD epitope surface area  
210 that comprises residues 352-357 in the  $\beta 1$  strand, which is part of a structurally conserved 5-  
211 stranded RBD  $\beta$ -sheet, and residues 457-471 that comprise a disordered loop directly beneath the  
212 ACE2 receptor binding ridge (Figure 4C and 4D).

213       N-612-056 establishes a network of hydrogen bond and hydrophobic interactions that  
214 include a stretch of hydrophobic residues in CDRH3 that mediate van der Waals interactions at  
215 the RBD interface, and the formation of salt bridges between N-612-056 residues D28<sub>LC</sub> and  
216 E55<sub>LC</sub> with R346<sub>RBD</sub> and R357<sub>RBD</sub>, respectively (Figure 4E). These structural data explain the  
217 observed cross-reactivity against SARS-CoV RBD (Figure S3A)(Cohen et al., 2021), as 14 of 20  
218 epitope residues are strictly conserved and three additional residues (R346, R357 and K462  
219 SARS-CoV-2 RBD numbering) are conservatively-substituted (K333, K344, and R449 SARS-  
220 CoV RBD numbering) in SARS-CoV and SARS-CoV-2. (Figure 4F). Overall, these structural  
221 data for the two RBD-targeting nAbs analyzed suggest comparable modes of recognition and

222 neutralization for antibodies N-612-017 and N-612-056, which were selected from mRNA  
 223 display, as those identified in convalescent or vaccinated donors.



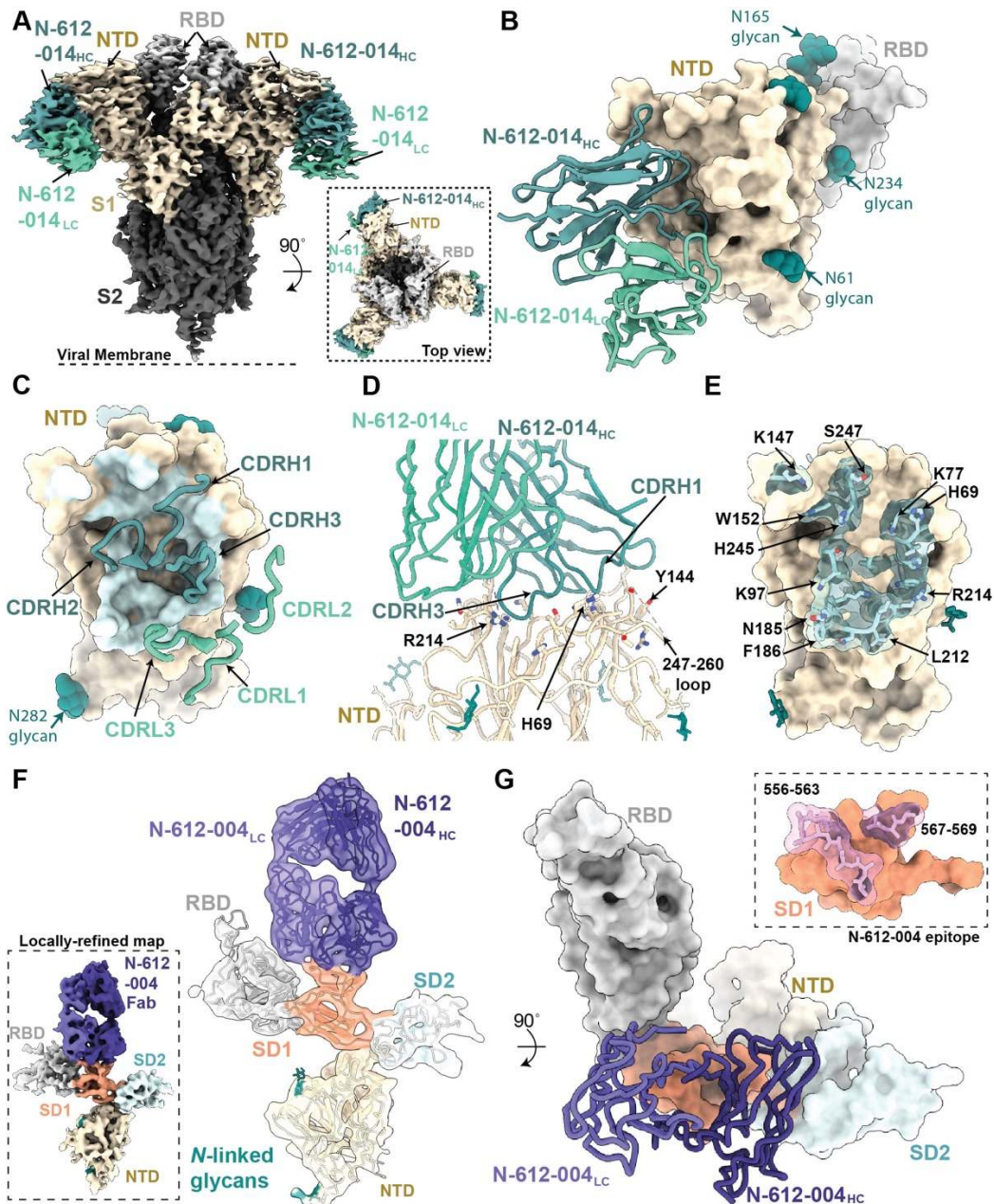
224 **Figure 4. X-ray Crystal Structure of SARS-CoV-2 RBD in Complex with the N-612-056**  
 225 **Fab.** (A) 2.9 Å X-ray crystal structure for the N-612-056 Fab – RBD complex. Inset: Overlay of  
 226 the N-612-056-RBD crystal structure on a S trimer with ‘up’ RBD conformation (PDB 6VYB).  
 227 (B) Composite model of N-612-056 – RBD (blue ribbon and gray surface, respectively) overlaid  
 228 with soluble ACE2 (green, PDB 6M0J). The model was generated by aligning RBDs on 191  
 229 matched C $\alpha$  atoms. (C) N-612-056 CDR loops (blue) mapped on the RBD surface (gray). The  
 230 N-612-056 epitope is shown as a light blue surface. (D) Surface and stick representation of N-  
 231 612-056 epitope. (E) N-612-056 paratope residues mapped on the RBD surface with epitope  
 232 residues shown in light blue. (F) Sequence alignment of SARS-CoV-2, SARS-CoV, WIV1-CoV,  
 233 and SCH014-CoV. N-612-056 epitope residues are shaded blue. S309 epitope residues are also  
 234 shown (# symbol).  
 235

## 236 **Structural Characterization of S1-Specific Antibodies N-612-014 and N-612-004**

237 The primary target of SARS-CoV-2 nAbs is the viral spike glycoprotein, with the majority  
238 of nAbs targeting the RBD (Greaney et al., 2021a; Piccoli et al., 2020). The S NTD represents a  
239 common site of antigenic drift (Cele et al., 2021; McCarthy et al., 2021; Ribes et al., 2021), and  
240 nAbs that bind to this region have recently been identified (Cerutti et al., 2021; McCallum et al.,  
241 2021b; Suryadevara et al., 2021). To understand the binding mode of the NTD-targeting  
242 antibody N-612-014 (Figure 1), we determined a 3.5Å cryo-EM structure of N-612-014 Fabs  
243 complexed with stabilized S trimers (Figure 5A and Figure S2). N-612-014 adopted a binding  
244 pose parallel to the viral membrane and primarily used HC CDR loops to recognize an epitope at  
245 the periphery of the NTD (Figure 5A-C). The NTD epitope recognized by N-612-014 closely  
246 resembles that recognized by the human-derived SARS-CoV-2 antibody S2X316 that targets  
247 NTD antigenic site v, which resides outside of the antigenic supersite (site i) that is the main  
248 target of neutralizing NTD antibodies (McCallum et al., 2021b). The N-612-014 epitope (~1070  
249 Å<sup>2</sup> epitope BSA) involves contacts with peripheral loops comprising NTD residues 68-78, 175-  
250 188, and 245-260, as well as contacts with the tip of the supersite β-hairpin (Figure 5D and 5E).  
251 Despite contacts with residues 69-70<sub>NTD</sub> and 144<sub>NTD</sub>, N-612-014 maintains binding to S trimers  
252 of the B.1.1.7 lineage sequence (Figure S3), which has deletions at these positions that allow  
253 escape from NTD supersite antibodies (McCallum et al., 2021b). These data suggest that N-612-  
254 014 retains NTD binding capability and may retain potency in the presence of NTD mutations  
255 commonly found in viral variants.

256 In addition to N-612-014, we also identified antibody N-612-004, an S1-specific antibody  
257 that was mapped to a domain outside of the NTD and RBD (Figure 1). Using single-particle  
258 cryo-EM, we determined a 4.8Å structure of N-612-004 bound to a dissociated S1 protomer,

259 which revealed recognition of a SD1 epitope (Figure 5F and Figure S2). Consistent with our  
260 library design that varied CDR loops H2, H3 and L3, N-612-004 contacts were solely mediated  
261 by these three regions, which led to recognition of loops 556-563 and 567-69 in the SD1 domain  
262 (Figure 5G). The epitope for N-612-004 is not accessible on S trimers, which likely explains the  
263 lack of N-612-004-like antibodies identified among a repertoire of antibodies found in  
264 convalescent plasma (Figure S4).



265

266 **Figure 5. Structures of S1-Specific Antibodies N-612-014 and N-612-004 Bound to SARS-**  
267 **CoV-2 Spike.** (A) Cryo-EM structure of the N-612-014 – S trimer complex. Inset: top down  
268 view of complex. (B) Close-up view of the N-612-014 variable domains (teal green) contacting  
269 the NTD (tan surface). The RBD (gray surface) of an adjacent protomer is shown as reference.  
270 (C) N-612-014 CDR loops (green ribbons) mapped onto the surface of the NTD (tan surface).  
271 (D) Cartoon representation of the N-612-014 – NTD interface. (E) Surface and stick  
272 representation of the N-612-014 epitope (light green surface). (F) Cryo-EM structure of the N-  
273 612-004 – S1 protomer (inset) rigid body fit with individual S1 domains (cartoon). (G) Cartoon  
274 and surface representation of N-612-004 (purple) recognition of the SD1 domain. Inset: N-612-  
275 004 epitope (pink sticks) highlighted on the SD1 surface (orange). Given the low resolution,  
276 epitope residues were assigned using a criterion of a distance of  $\leq 7$  Å between antibody-antigen  
277 C $\alpha$  atoms.

### 278 **Activity of Identified nAbs Against Variants**

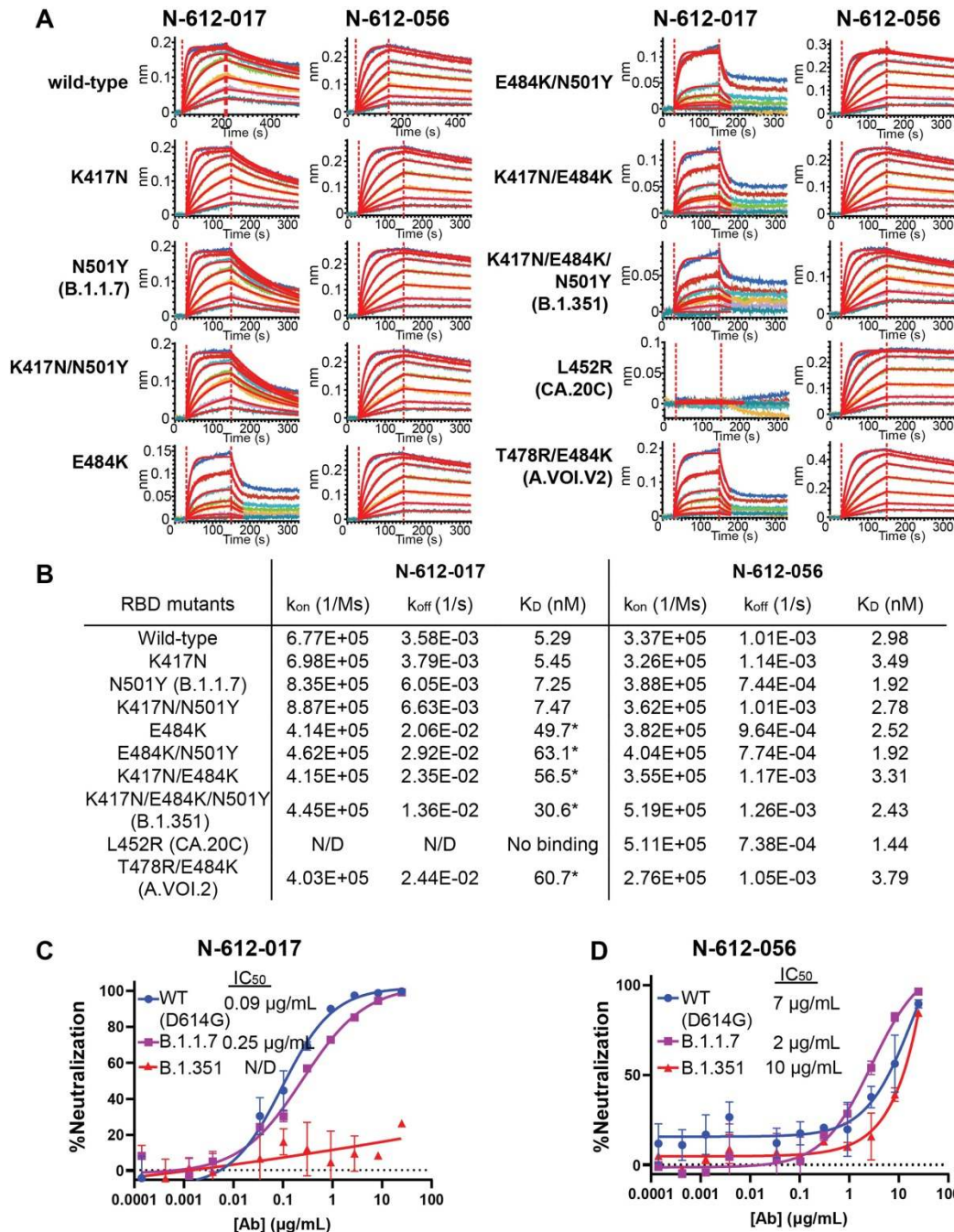
280 To assess the relative affinity of RBD-binding nAbs N-612-017 and N-612-056 against a  
281 series of variants, BLI was performed using RBD variants B.1.1.7 (N501Y), B.1.351  
282 (K417N/E484K/N501Y), CAL.20C (L452R), and A.VOI.V2(T478R/E484K) with single or  
283 combined mutations. N-612-056 binding affinity was not affected by any of the RBD mutations  
284 tested, which was anticipated based on structural characterization findings that indicate N-612-  
285 056 recognizes a more conserved epitope on the surface of RBD. Neither N501Y (the only RBD  
286 mutation in B.1.1.7 and one of 3 RBD mutations in B.1.351) nor K417N, (one of 3 RBD  
287 mutations in B.1.351) disrupted the binding affinity of N-612-017. E484K - an escape mutant  
288 found in many different variants, including B.1.351 and A.VOI.V2 (Oliveira et al., 2021; Tegally  
289 et al., 2021; Weisblum et al., 2020) - did, however, reduce binding affinity of N-612-017 by 6-10  
290 fold (Figure 6A and 6B). Furthermore, the L452R mutation found in CA.20C (also known as  
291 B.1.1.427 and B.1.1.429) completely abolished the binding of RBD by N-612-017.

292 N-612-017 and N-612-056 were then evaluated in a pseudovirus neutralization assay  
293 (Crawford et al., 2020) using wild-type (containing D614G), B.1.1.7, and B.1.351 pseudoviruses.  
294 N-612-017 neutralized wild-type (D614G) and B.1.1.7 pseudoviruses with  $IC_{50} = 0.09-0.25$



295  $\mu\text{g}/\text{mL}$  but failed to neutralize B.1.351. N-612-056 retained neutralization activity against all  
296 variants with  $\text{IC}_{50}$  of 2-10  $\mu\text{g}/\text{mL}$  as expected (Figure 6C).

297 The binding affinity of N-612-014 and N-612-004 against the recombinant S1 domain  
298 containing B.1.1.7 mutations was tested and it was determined that 69-70del and Y144del on  
299 NTD did not affect binding affinity of N-612-014 for S1 whereas these mutations moderately  
300 lowered (by about 3-fold) the binding affinity of N-612-004 for S1 (Figure S3B and C).



301  
 302 **Figure 6. Binding Affinity and Neutralization Activity of N-612-017 and N-612-056 Against**  
 303 **Known SARS-CoV-2 Variants.** (A) BLI kinetic analysis of N-612-017 and N-612-056 affinity  
 304 against various mutations found in SARS-CoV-2 variants alone or in combination. N-612-017  
 305 binding curves against RBD mutants containing E484K were fit with 1:1 binding model using a  
 306 shorter dissociation time (30 sec) to highlight weakened binding. (B) Table of BLI kinetic assay  
 307 values. SARS-CoV-2 pseudovirus neutralization assay of antibodies. Asterisk indicates  $K_D$   
 308 values obtained from processing the data with a shorter dissociation time to fit the curves to 1:1  
 309 binding and may not represent accurate  $K_D$ . (C) N-612-017 and (D) N-612-056 against wild-type  
 310 (D614G), B.1.1.7, and B.1.351 variants. Mean and standard deviation of duplicate experiments  
 311 (n=4), is shown.

## 312 **Generation of E484K and L452R-Resistant N-612-017**

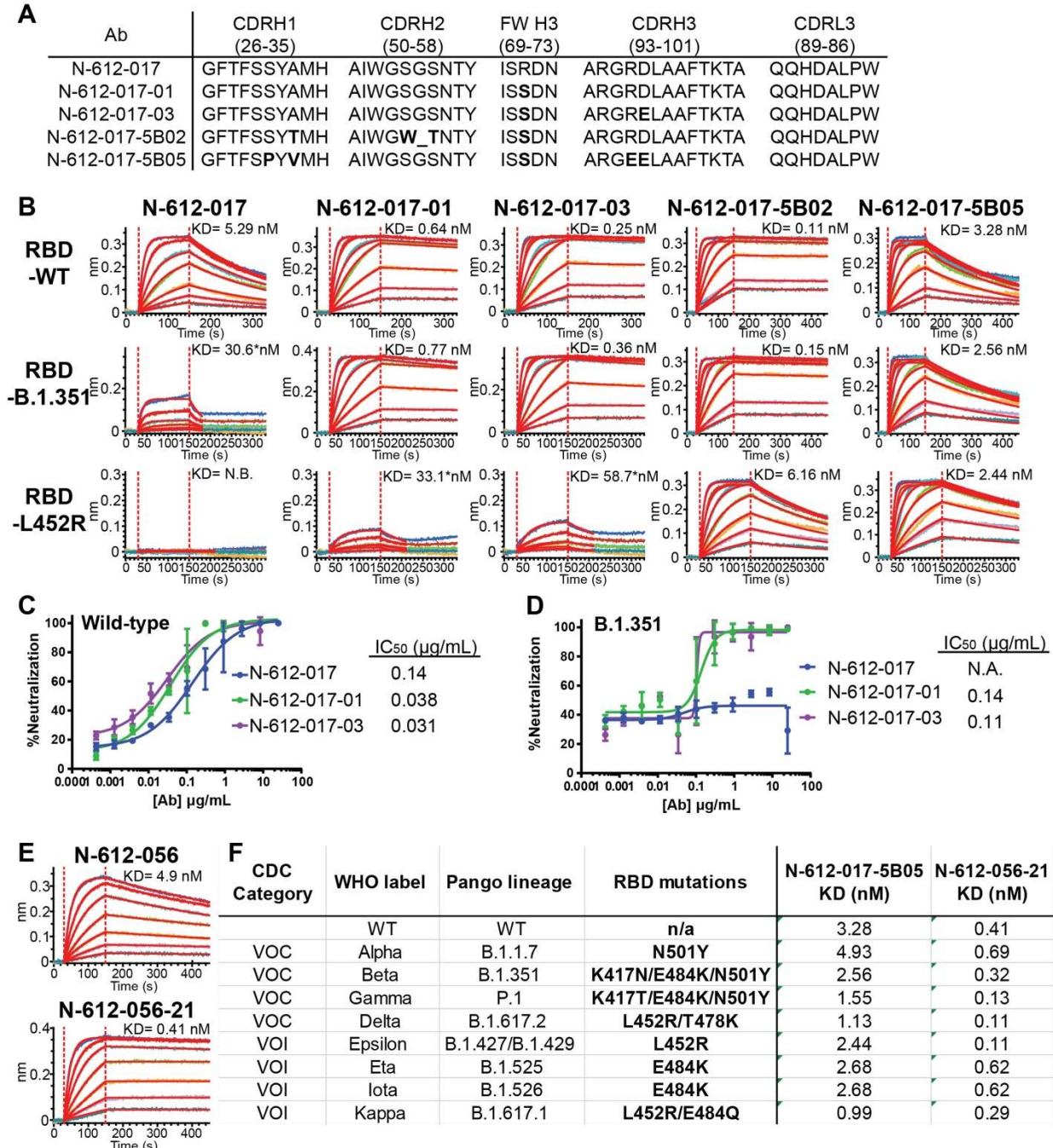
313 To recover N-612-017 binding against RBD with the E484K substitution (RBD-E484K), we  
314 used an mRNA-doped library for affinity maturation and identified mutations on VH framework  
315 3 (Arg 71 → Ser) and CDR H3 (Asp 97 → Glu) that restored binding affinity against RBD-  
316 E484K. The N-612-017 subclone N-612-017-01 containing a single VH:R71S mutation and  
317 subclone N-612-017-03 containing double VH:R71S/D97E mutations were tested by BLI for  
318 their binding of RBD-B.1.351 and RBD-L452R. Interestingly, both N-612-017-01 and N-612-  
319 017-03 subclones not only exhibited restored binding affinity against E484K-expressing RBD  
320 variants, they had 10- to 20-fold enhanced affinity against wild-type RBD (Figure 7A, 7B and  
321 Table S7). While neither subclone displayed complete recovery of binding affinity against RBD-  
322 L452R, affinity was relatively enhanced ( $K_D = 33.1\text{-}58.7$  nM). These subclones were then tested  
323 in a live virus neutralization assay against wild-type (D614G) and B.1.351 viruses and showed  
324 neutralization activity against both, whereas the parent N-612-017 did not show neutralization  
325 activity against B.1.351 (Figure 7C and 7D).

326 Subsequently, we used N-612-017-001 in affinity maturation against RBD-L452R and  
327 identified 2 clones with restored affinity against RBD-L452R. N-612-017-5B02 containing 4  
328 additional VH mutations (A33T/S54W/G54Δ/S55T) and N-612-017-5B05 containing 4  
329 additional VH mutations (S31P/A33V/R96E/D97E) were tested by BLI for their binding of  
330 B.1.351 and RBD-L452R. Both subclones showed complete recovery of binding affinity against  
331 RBD-B.1.351 and RBD-L452R (Figure 7A, 7B, and Table S7).

## 332 **Affinity Maturation of N-612-056**

333 To improve potency of N-612-056, we utilized mRNA display for affinity maturation and  
334 identified N-612-056-21 containing a single point mutation in VH CDR3 (Ser 99 → Pro) that  
335 resulted in a 10-fold improvement in binding affinity ( $K_D= 0.41$  nM) (Figure 7E).

336 Affinity matured N-612-017-5B05 and N-612-056-21 were tested against all the variants of  
337 concern (VOC) and variants of interest (VOI) using BLI. N-612-017-5B05 showed binding  
338 affinity to the variants that was similar to the parent molecule N-612-017, whereas N-612-056-21  
339 displayed binding affinities improved as much as ~10-fold compared to N-612-056 (Figure 7F).



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**Figure 7. Affinity Maturation of N-612-017 and N-612-056** (A) VH and VL sequences of N-612-017 affinity matured subclones. (B) BLI kinetic analysis of N-612-017 affinity matured subclones against RBD-wild-type, RBD-B.1.351, and RBD-L452R. Asterisk indicates  $K_D$  values obtained from processing the data with a shorter dissociation time to fit the curves to 1:1 binding model and may not represent accurate  $K_D$  values. SARS-CoV-2 live virus neutralization assay of N-612-017, N-617-017-01, and N-612-017-03 against (C) wild-type and (D) B.1.351 variant. Mean and standard deviation of duplicate experiments (n=3) (E) BLI kinetic analysis of N-612-056 and affinity matured N-612-056-21 against RBD-wild-type. (F) Table of binding affinity of N-612-017-5B05 and N-612-056-21 against RBD containing variant mutations from all the VOC and VOI listed by CDC.

351 **Discussion**

352 Our use of *in vitro* mRNA display facilitated our identification of novel antibody sequences  
353 and enabled us to enhance their binding affinity for mutated S of SARS-CoV-2 variants through  
354 affinity maturation. BLI and epitope binning analysis determined that 10 unique IgG1 antibody  
355 sequences identified here bind 7 distinct epitope regions on SARS-CoV-2 spike protein. Major  
356 sequence differences in CDRH3 and CDRL3 loops in combination with minor variation in  
357 CDRH1 and CDRH2 can drive the recognition of a broad spectrum of epitopes and create potent  
358 neutralizing interactions with SARS-CoV-2 spike protein. Previous IGHV (immunoglobulin  
359 heavy chain variable region) gene analysis identified distinct IGVH genes (e.g. 1-53) that were  
360 more likely to produce potent RBD-binding nAbs (Dejnirattisai et al., 2021; Robbiani et al.,  
361 2020; Yuan et al., 2020) within the human antibody repertoire. The nAb N-612-017 VH  
362 sequence is most similar to IGHV 3-23, which are most abundant in human antibodies, and  
363 shows potent neutralizing activity ( $< 0.1 \mu\text{g/mL}$ ), suggesting CDR sequence variation is essential  
364 in determining potency against antigen regardless of germline genes.

365 The majority of potent nAbs (~90%) are RBD targeting and the remainder target NTD of  
366 spike protein (Brouwer et al., 2020; Cao et al., 2020; Liu et al., 2020; McCallum et al., 2021b).  
367 Our most potent nAb N-612-017 is RBD targeting and categorized into Class 2 as characterized  
368 by cryo-EM. Though N-612-056 also neutralized SARS-CoV-2 by targeting RBD, albeit at  
369 lower potency, it lacks the ability to directly block ACE2 binding and binds to a cryptic epitope  
370 on RBD. Similar antibody binding to this cryptic epitope by a patient-derived antibody has been  
371 previously reported (Dejnirattisai et al., 2021). The rarity of this epitope is evident in the  
372 convalescent plasma blocking assay, in which convalescent plasma from 3 out of 4 patients failed  
373 to block N-612-056 from binding to spike protein. This cryptic interface is well conserved, and

374 although the potency of N-612-056 is relatively low, cross-reactivity with SARS-CoV RBD and  
375 sustained binding affinity for mutant SARS-CoV-2 RBD found in circulating variants suggests  
376 N-612-056 may be an attractive monoclonal antibody therapy candidate against novel variants.

377 The cryo-EM structural data presented here reveal that N-612-014 binds NTD at a site  
378 different from that of the majority of described antibodies (McCallum et al., 2021b), suggesting  
379 the presence of a second neutralization site on the NTD. Proposed mechanisms for nAbs  
380 targeting NTD include destabilization of S trimer by S1 shedding and blockage of cell-cell fusion  
381 auxiliary receptor binding, membrane fusion, or proteolytic activation (Huo et al., 2020; Walls et  
382 al., 2019; Wec et al., 2020; Wrobel et al., 2020). N-612-014 displayed neutralization activity in  
383 live virus assays whereas it lacked neutralization activity in a pseudovirus assay. Although this  
384 type of discrepancy is rare, such inter-assay discrepancies have been described previously (Liu et  
385 al., 2020). N-612-014 may require a longer incubation time to reach maximum neutralization  
386 because this allows the opportunity for the S trimer to adopt a conformation that is more  
387 susceptible to S1 shedding that is promoted by the antibody, thus destabilizing spike; this  
388 hypothesis awaits experimental confirmation.

389 The SD1-targeting antibody N-612-004 displayed partial neutralization activity and was  
390 only observed in complex with S1 domain dissociated from the spike trimer in cryo-EM. To our  
391 knowledge, there have been no reports on SD1-targeting antibodies that display neutralization  
392 activity. We also identified the S2-targeting antibody N-612-007 that displayed partial  
393 neutralization activity in a live virus neutralization assay and while structural analysis was  
394 attempted, we were unable to visualize/characterize an S trimer/N-612-007 complex. nAbs  
395 targeting S2 domain have been previously observed in MERS-CoV and SARS-CoV (Elshabrawy  
396 et al., 2012; Lai et al., 2005; Lip et al., 2006; Pallesen et al., 2017), and recently reported

397 structures of SARS-CoV-2 S2/antibody complex have also revealed antibody binding to the S2  
398 stem helix, which may interfere with membrane fusion machinery (Zhou et al., 2021). Other S2  
399 epitope regions identified for SARS-CoV nAb are two heptad repeats region essential in cell  
400 fusion during virus entry (Elshabrawy et al., 2012; Lip et al., 2006; Pallesen et al., 2017).  
401 Neutralization activities of these non-RBD binders (N-612-014, N-612-004, and N-612-007)  
402 were inconsistent between multiple assays and generally not very potent when tested  
403 individually. However, when tested in combination with N-612-017, all slightly enhanced the  
404 neutralization activity of N-612-017. This may suggest the role of non-RBD binding antibodies  
405 in neutralization.

406 Bamlanivimab is a Class 2 RBD binder that neutralizes wild-type SARS-CoV-2 and was the  
407 first antibody to attain emergency use authorization (EUA) by the FDA (Jones et al., 2021). This  
408 EUA was, however, recently revoked due to loss of potency against SARS-CoV-2 variants  
409 (Widera et al., 2021). Two alternate monoclonal antibody therapies remain available under EUA:  
410 a combination of casirivimab plus imdevimab (Baum et al., 2020; Pinto et al., 2020) and  
411 combination bamlanivimab plus etesevimab. In both cases, administering 2 monoclonal  
412 antibodies together is key to compounding potency and reducing the risk of variant virus escape  
413 from neutralization. Our N-612-017 antibody is a Class 2 RBD binder similar to bamlanivimab  
414 that also displays a loss of activity in the presence of the E484K mutation. To address this  
415 potential loss of efficacy, we used affinity maturation for N-612-017 and quickly identified  
416 subclones that restored affinity for both E484K and L452R. N-612-056 is resistant to all the  
417 RBD variants and N-612-014 is not affected by NTD mutation present in B.1.1.7. These nAbs  
418 are attractive candidates for the use in combination with N-612-017. N-612-056 was quickly



419 affinity matured to present an attractive combo approach in combination with N-612-017 to  
420 combat variants.

421 The recent emergence of more transmissible and infectious variants such as B.1.351 ('Beta')  
422 and B.1.617 ('Delta') highlights the need for a method to rapidly address mutations that  
423 overcome current therapies and existing immunity. The results described in this study  
424 demonstrate the utility of mRNA display-based nAb discovery in the identification of antiviral  
425 monoclonal antibodies against the rapidly evolving SARS-CoV-2 pathogen which should be  
426 applicable to other novel or seasonal pathogens.

427

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446

#### 447 **Author contributions**

448 Experimental designs: C.A.O., S.T., C.O.B, K.N., S.R., and P.S.S; mRNA library display:  
449 C.A.O; Cloning: C.A.O and W.H.; Protein Expression and Purification: C.A.O., S.T. and M.G.;  
450 Kinetic Analysis, ACE2 Blocking Assay, Epitope Binning, and ELISA ACE2 blocking assay:  
451 S.T., cryo-EM and X-ray crystallography: C.O.B and P.J.B.; Vero E6 Live Virus Neutralization  
452 Assay: J.T., A.R., M.M.F. and D.B.; pseudo-typed virus neutralization assay: P.G.. Manuscript  
453 preparation: S.T., C.O.B., C.A.O., and P.S..

454

#### 455 **Declaration of interests**

456 C.A.O., S.T., W.H., K.N., and P.S.S. are inventors for an international patent application with  
457 this work.

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## 695 **Materials Availability**

696 All expression plasmids generated in this study for CoV proteins, CoV pseudoviruses, human

697 Fabs and IgGs are available upon request through a MTA.

698

699 **Data availability**

700 The atomic model generated for the N-612-056 Fab complexed with SARS-CoV-2 RBD have been  
701 deposited in the Protein Data Bank (PDB, <http://www.rcsb.org/>) under accession code 7S0B. The  
702 atomic models and cryo-EM maps generated for the N-612-017, N-612-014, and N-612-004 Fabs  
703 complexed with SARS-CoV-2 S have been deposited at the PDB (<http://www.rcsb.org/>) and the  
704 Electron Microscopy Databank (EMDB) (<http://www.emdataresource.org/>) under accession codes  
705 7S0C, 7S0D, 7S0E and EMD-24786, EMD-24787, EMD-24788, respectively. All models and  
706 maps are publicly available as of the date of publication.

707

708 **Experimental Methods**

709 **mRNA display**

710 A synthetic VH3/Vk1 scFv library was transcribed by T7 run-off transcription (Thermo  
711 Fisher), followed by ligation to the pF30P linker (Liu et al., 2000) via a splint oligonucleotide by  
712 T4 DNA ligase (NEB). After lambda exonuclease digestion to remove splint and unincorporated  
713 linker, the ligated mRNA was purified by oligo(dT)<sub>25</sub> dynabeads (Thermo Fisher). The mRNA-  
714 puromycin template was translated (Purexpress, NEB) followed by incubation with KCl (550  
715 mM final) and MgCl<sub>2</sub> (60 mM final) for 1 hour at room temperature to enhance fusion formation  
716 (Liu et al., 2000). The mRNA-scFv fusions were then affinity-purified using M2 anti-Flag beads  
717 (Sigma-Aldrich) to remove non-fused template and sequences containing nonsense mutations  
718 (Liao et al., 2009; Olson et al., 2011). After elution with 3XFlag peptide (Sigma-Aldrich), the  
719 fusions were reverse transcribed with super script II (ThermoFisher). The pool was incubated  
720 with biotinylated SARS CoV2 Spike extracellular domain bound to 5 µL streptavidin M280  
721 dynabeads (ThermoFisher) for 1 hour at room temperature. After washing, the immobilized

722 fusion samples were eluted by heat (95°C) and PCR amplified with KOD hot start polymerase  
723 (EMD). Affinity maturation was performed by replacing the wild type CDRs H1, H2, H3, and  
724 L3 with synthetic DNA cassettes derived from oligonucleotides doped at 6% (Hutchison et al.,  
725 1986) and performing 3-5 rounds of mRNA display as described above.

## 726 **Production antibodies and recombinant SARS-CoV-2 S domains**

### 727 *Molecular cloning*

728 SARS-CoV-2 Spike ECD 1-1208 (682-GSAS-685; 986-PP-987) fused to the T4 fibrin  
729 trimerization domain with C-terminal Avi- and His-tag were synthesized with gene block (IDT)  
730 and cloned into pcDNA.3 vector. RBD-SD1, wild type RBD and mutant RBD domains were  
731 subcloned into pcDNA.3 vector with C-terminal His-tag.

732 VH and VL sequences of candidate sequences were cloned into a pcDNA.3 based vector  
733 with dual CMV promotor harboring IgG1 heavy chain and light chain backbone using  
734 NEBuilder Hifi DNA Assembly Master Mix (New England Biolabs).

### 735 *FectoPRO® transient transfection of antibodies*

736 For transient expression of antibodies by FectoPRO® transfection, CHO-S cells in  
737 suspension were cultured in CD-CHO media supplemented with 8 mM L-glutamine in shaker  
738 flasks at 37°C with 125 rpm rotation and 8 % CO<sub>2</sub>. One day before transfection, CHO-S cells  
739 were seeded at a density of 1 x 10<sup>6</sup> cells/mL in 45 mL culture flask. On the day of transfection,  
740 75 µL of FectoPRO® transfection reagent (PolyPlus-transfection®) was mixed with 5 mL of 15  
741 µg/mL pcDNA3 plasmid DNA harboring antibody encoding sequence in CD-CHO media and  
742 incubated for 10 min at room temperature. The DNA/transfection reagent mixture was added to  
743 45 mL of CHO-S culture and incubated at 37°C with 5% CO<sub>2</sub> and 125 rpm rotation. On Day 3,

744 50 mL of the CD-CHO media supplemented with 8 mM L-glutamine was added and the culture  
745 incubated for an additional 4 days.

746 *Lipofectamine® transient transfection of RBD constructs*

747 For transient expression of RBD-SD1, RBD wild-type and RBD mutants, 293T cells were  
748 cultured and incubated at 37°C with 5% CO<sub>2</sub>. Plasmid harboring RBD constructs were  
749 mixed with lipofectamin 2000 (Life Technology) with 1:1 (v:v) ratio and incubated for 20 min at  
750 room temperature. The mixture was then added to the culture and incubated for 3-4 days.

751 *Maxcyte® transient transfection of SARS-CoV-2-S ECD trimer*

752 For transient expression of SARS-CoV-2 S di-Pro ECD timer by Maxcyte® transfection,  
753 CHO-S cells were cultured in suspension in CD-CHO media supplemented with 8 mM L-  
754 glutamine in shaker flasks at 37°C with 125 rpm rotation and 8% CO<sub>2</sub>. For transfection, cells in  
755 the exponential growth stage were pelleted by centrifugation at 1,400 rpm for 10 min, re-  
756 suspended in 10 mL of electroporation buffer, and re-pelleted at 1,400 rpm for 5 min. The cell  
757 pellet was resuspended at a density of 2 x 10<sup>8</sup> cells/mL in electroporation buffer, mixed with the  
758 plasmid harboring SARS-CoV-2 S di-Pro ECD sequence at a concentration of 150 µg/mL, and  
759 transfected using OC-400 processing assemblies in a Maxcyte® ExPERT ATx Transfection  
760 System. Transfected cells were incubated for 30 min at 37°C, 5% CO<sub>2</sub> and then resuspended in  
761 Efficient Feed A Cocktail (CHO-CD EfficientFeed™ A + 0.2% Pluronic F-68 + 1% HT  
762 Supplement + 1% L-glutamine) at a density of ~4-6 x 10<sup>6</sup> cells/mL. This cell culture was  
763 incubated at 37°C with 5% CO<sub>2</sub> and 125 rpm rotation overnight, 1 mM sodium butyrate was  
764 added, and the culture was further incubated at 32°C with 3% CO<sub>2</sub> and 125 rpm for 13 more  
765 days; during this incubation period, Maxcyte® Feed Cocktail (13.9% CD Hydrolysate, 69.5%

766 CHO CD EfficientFeed™ A, 6.2% Glucose, 6.9% FunctionMax™ Titer Enhancer, 3.5% L-  
767 Glutamine) was added at 10% of the culture volume on Days 3 and Day 8.

#### 768 *Purification of IgGs*

769 FectoPRO® transfection cell culture medium was centrifuged and filtered through a 0.22 µm  
770 filter to remove cells and debris, then loaded onto a HiTrap™ MabSelect SuRe™ column (GE  
771 Healthcare Life Sciences) on the AKTA Pure system pre-equilibrated with 10 mM Na Phosphate  
772 and 150 mM NaCl at pH 7.0. After loading, the column was washed with 10 column volumes of  
773 the same buffer. The protein was eluted with 100 mM sodium acetate, pH 3.6, then immediately  
774 neutralized using 2 M Tris pH 8.0. The elution fractions were pooled and dialyzed into 10 mM  
775 Hepes and 150 mM sodium chloride at pH 7.4.

#### 776 *Purification of Fabs*

777 Fabs were generated by papain digestion using crystallized papain (Sigma-Aldrich) in 50  
778 mM sodium phosphate, 2 mM EDTA, 10 mM L-cysteine, pH 7.4 for 30-60 min at 37°C at a 1:100  
779 enzyme:IgG ratio. Fab and partially cleaved IgG were applied on 1-mL HiTrap Protein L column  
780 (GE Healthcare Life Science). After loading, the column was washed with 10 column volumes of  
781 10 mM Na Phosphate and 150 mM NaCl at pH 7.0. The protein was eluted with 100 mM sodium  
782 citrate, pH 2.5, then immediately neutralized using 2 M Tris pH 8.0. The elution fractions were  
783 pooled and dialyzed into 10 mM Hepes and 150 mM sodium chloride at pH 7.4. Fabs were  
784 further purified by SEC using a Superdex 200 10/300 GL column (GE Healthcare Life Sciences)  
785 in 10 mM Hepes and 150 mM sodium chloride at pH 7.4.

#### 786 *Purification of di-Pro S timer, RBD-SD1, RBD wild-type and RBD mutants*

787 The Lipofectamin transfection cell culture medium and Maxcyte transfection cell culture  
788 medium was centrifuged and filtered through a 0.22 µm filter or 0.45 µm, respectively, remove

789 cells and debris. 50 mM Tris, 100 mM sodium chloride, and 10 mM imidazole was added to the  
790 supernatant then loaded to a gravity column packed with Ni-NTA resins (Qiagen) pre-  
791 equilibrated with 20 mM Tris, 300 mM sodium chloride, and 10 mM imidazole, pH 8.0. After  
792 loading, the column was washed with 10 column volumes of the same buffer. The protein was  
793 eluted with 20 mM Tris, 150 mM sodium chloride, and 300 mM imidazole. The elution fractions  
794 were pooled and dialyzed into 10 mM Hepes and 150 mM sodium chloride, pH 7.4.

### 795 **Bio-Layer Interferometry (BLI) Kinetic Analysis of Antibodies**

796 BLI buffer used in all experiments was 10 mM Hepes, 150 mM NaCl, pH 7.4, with 0.02%  
797 Tween 20, and 0.1% BSA. Analytes used in kinetic analysis were uncleavable S trimer, RBD-  
798 SD1, RBD wild-type, RBD mutants, commercially purchased recombinant SARS-CoV-2 S2  
799 (SinoBiological), and SARS-CoV-2 S1 (SinoBiological). For determining binding affinities,  
800 IgGs were immobilized on Anti-hIgG Fc Capture (AHC) biosensors (Sartorius Corporation) and  
801 a concentration series of 200, 100, 50, 25, 12.5, 6.25, 3.125 nM was used to determine the  
802 equilibrium dissociation constants ( $K_D$  values) for RBD-SD1, RBD wild-type, RBD mutants, and  
803 S1 using 1:1 binding curve fit. For some RBD mutants that weakened binding and showed  
804 biphasic dissociation, only 30 seconds dissociation curves were used to fit 1:1 binding model. A  
805 concentration series of 20, 10, 5, 2.5, 1.25, 0.625, 0.3125 nM was used to determine apparent  $K_D$  for  
806 uncleavable S trimer using bivalent model on Octet HT software. For determining 1:1 binding  
807 affinity for S2, S2-His-tag was immobilized on Anti-Penta-His (HIS1K) biosensors (Sartorius  
808 Corporation), and a concentration series of S2 binding mAb Fab at 200, 100, 50, 25, 12.5, 6.25,  
809 3.125 nM was used.

### 810 **ACE2 blocking assay**

811 RBD-His-tag at 5  $\mu\text{g}/\text{mL}$  was first loaded on Ni-NTA (NTA) biosensors (Sartorius  
812 Corporation) for 15 sec and subsequently blocked with 5  $\mu\text{g}/\text{mL}$  mAbs or BLI assay buffer for 5  
813 min. BLI signal from ACE2 binding were measured by incubating RBD-coated/mAb blocked  
814 biosensors in 25 nM ACE2-IgG1Fc for 3 min.

### 815 **Epitope binning**

816 For epitope binning using S1 domain, biotinylated S1 binding mAbs at 25  $\mu\text{g}/\text{mL}$  were first  
817 loaded on High Precision Streptavidin SAX biosensors (Sartorius Corporation) for 10 sec. 3.75  
818  $\mu\text{g}/\text{mL}$  of recombinant SARS-CoV-2 S1 were used to bind mAb captured on biosensors for 3  
819 min and subsequently 10  $\mu\text{g}/\text{mL}$  S1 binding mAb were incubated with biosensors to observe  
820 binding competition and signal was recorded for 3 min. For epitope binning using S2 domain,  
821 recombinant SARS-CoV-2 S2-His-tag at 10  $\mu\text{g}/\text{mL}$  was loaded on Anti-Penta-HIS (HIS1K)  
822 biosensors (Sartorius Corporation) for 1 min. 10  $\mu\text{g}/\text{mL}$  of S2 binding mAbs were sequentially  
823 incubated with biosensors for 3-5 min to observe binding competition.

### 824 **Vero E6 neutralization assay**

825 All aspects of the assay utilizing virus were performed in a BSL3 containment facility  
826 according to the ISMMS Conventional Biocontainment Facility SOPs for SARS-CoV-2 cell  
827 culture studies. Vero E6 cells were seeded into 96-well plates at 20,000 cells/well and cultured  
828 overnight at 37°C. The next day, 3-fold serial dilutions of mAbs were prepared in DMEM  
829 containing 2% FBS, 1% NEAAs, and 1% Pen-Strep (vDMEM). SARS-CoV-2 virus stock was  
830 prepared in vDMEM at 10,000 TCID<sub>50</sub>/mL, mixed 1:1 (v:v) with the mAb dilutions, and  
831 incubated for 30 min or 24 hr at 37°C. Media was removed from the Vero E6 cells, mAb-virus  
832 complexes were added and incubated at 37°C for 48 hours before fixation with 4% PFA. Fixed  
833 cells were stained for SARS-CoV-2 nucleocapsid protein to measure infection. The percent

834 neutralization was calculated as  $100 - ((\text{sample of interest} - [\text{average of "no virus"}]) / [\text{average of}$   
835  $\text{"virus only"}]) * 100$ ).

### 836 **Pseudovirus neutralization assays**

837 Pseudoviruses based on HIV lentiviral particles were prepared as described (Robbiani et al.,  
838 2020). Three-fold serially diluted mAbs were incubated with SARS-CoV-2 pseudovirus for 1  
839 hour at 37°C. After incubation with 293TACE2 cells for 48 hours at 37°C, cells were washed  
840 twice with PBS, lysed with Luciferase Cell Culture Lysis 5x reagent (Promega), and NanoLuc  
841 Luciferase activity in lysates was measured using the Nano-Glo Luciferase Assay System  
842 (Promega). Relative luminescence units (RLUs) were normalized to values derived from cells  
843 infected with pseudovirus in the absence of mAbs. Half-maximal inhibitory concentrations (IC<sub>50</sub>  
844 values) for mAbs were determined using 4-parameter nonlinear regression (Prism, GraphPad).

### 845 **Cryo-EM sample preparation**

846 N-612-004, N-612-014 and N-612-017 Fab-S complexes were assembled by incubating  
847 purified SARS-CoV-2 S trimer at a 1.1:1 molar excess of purified Fab per S protomer at RT for  
848 20 min. Complex was mixed with F-octylmaltoside solution (Anatrace) to a final concentration  
849 of 0.02% w/v and then 3 μL were immediately applied to a 300 mesh, 1.2/1.3 QuantiFoil grid  
850 (Electron Microscopy Sciences) that had been freshly glow discharged for 30s at 20 mA using a  
851 PELCO easiGLOW (Ted Pella). The grid was blotted for 3s with Whatman No. 1 filter paper at  
852 22°C and 100% humidity then vitrified in 100% liquid ethane using a Mark IV Vitrobot (FEI)  
853 and stored under liquid nitrogen.

### 854 **Cryo-EM structure determination of N-612-004, N-612-014, and N-612-017 Fab in complex** 855 **with S-6P**



856 Single-particle cryo-EM data were collected for Fab-S trimer complexes as previously  
857 described (Barnes et al., 2020b) Briefly, movies were collected on a 200 kV Talos Arctica  
858 transmission electron microscope (Thermo Fisher) equipped with a Gatan K3 Summit direct  
859 electron detector operating in counting mode. Movies were collected using a 3x3 beam image  
860 shift pattern with SerialEM automated data collection software (Mastronarde, 2005) at a nominal  
861 magnification of 45,000x (super-resolution 0.4345 Å/pixel) using a defocus range of -0.7 to -2.0  
862 μm. An average dose rate of 13.5 e<sup>-</sup>/pix/s resulted in a total dose of ~60 e<sup>-</sup>/Å<sup>2</sup> over 40 frames for  
863 all datasets.

864 For all datasets, movies were patch motion corrected for beam-induced motion including  
865 dose-weighting within cryoSPARC v3.1 (Punjani et al., 2017) after binning super resolution  
866 movies by 2 (0.869 Å/pixel). The non-dose-weighted images were used to estimate CTF  
867 parameters using Patch CTF in cryoSPARC, and micrographs with poor CTF fits, signs of  
868 crystalline ice, and field of views that were majority carbon were discarded. Particles were  
869 picked in a reference-free manner using Gaussian blob picker in cryoSPARC (Punjani et al.,  
870 2017) Initial particle stacks were extracted, binned x4 (3.48 Å/pixel), and subjected to *ab initio*  
871 volume generation (4 classes) and subsequent heterogeneous refinement with all particles. The  
872 3D classes that showed features for a Fab-S trimer complex or Fab-S1 protomer were 2D  
873 classified to polish particle stacks. The resulting particle stacks were unbinned (0.869 Å/pixel)  
874 and re-extracted using a 432 box size, and moved to Relion v3.1 (Zivanov et al., 2018) for  
875 further 3D classification. Particles corresponding to distinct states were separately refined using  
876 non-uniform 3D refinement imposing C1 symmetry in cryoSPARC and final resolutions were  
877 estimated according to the gold-standard FSC (Bell et al., 2016).

878 To improve features at the Fab-RBD interface, focused, non-uniform 3D local refinement  
879 in cryoSPARC were performed by applying a soft mask around the Fab  $V_HV_L$  – RBD (N-612-  
880 017), NTD (N-612-014), or SD1 (N-612-004) domains. These efforts resulted in a modest  
881 improvement in the Fab-S interface, which helped accurate model building.

## 882 **X-ray crystallography structure determination of N-612-056 in complex with RBD**

883 The N-612-056-RBD complex was assembled by incubating the SARS-CoV-2 RBD with  
884 a 2x molar excess of Fab for 1 h on ice followed by size exclusion chromatography on an  
885 superdex200 10/300 increase column (Cytiva). Fractions containing complex were pooled and  
886 concentrated to 5-8 mg/mL. Crystallization trials using commercially-available screens  
887 (Hampton Research) were performed at room temperature using the sitting drop vapor diffusion  
888 method by mixing equal volumes of the Fab-RBD complex and reservoir using a TTP LabTech  
889 Mosquito instrument. Crystals were obtained for N-612-056-RBD complex in 0.2 M Lithium  
890 citrate tribasic tetrahydrate and 20% w/v polyethylene glycol 3,350, subsequently cryoprotected  
891 by adding glycerol directly to drops to a final concentration of 20% v/v and cryopreserved in  
892 liquid nitrogen.

893 X-ray diffraction data were collected at the Stanford Synchrotron Radiation Lightsource  
894 (SSRL) beamline 12-2 on a Pilatus 6M pixel detector (Dectris). Data from single crystals were  
895 indexed and integrated in XDS (Kabsch, 2010) and merged using AIMLESS in *CCP4* (Winn et  
896 al., 2011)(Table S6). The N-612-056-RBD structure was solved by molecular replacement in  
897 PHASER (McCoy et al., 2007) using unmodified RBD coordinates (PDB 7K8M) and  
898 coordinates from C002 Fab (PDB 7K8O) as search models, after removal of C002 heavy chain  
899 and light chain CDR loops. Coordinates were rigid body and B-factor refined in PHENIX v1.19

900 (Adams et al., 2010) followed by sequence matching and repeated cycles of *phenix.refine* and  
901 manual building in Coot (v0.9.3) (Emsley et al., 2010) (Table S6).

## 902 **Structure analyses**

903 Buried surface area estimates were made using PDBePISA with a 1.4Å probe (Krissinel and  
904 Henrick, 2007). Potential hydrogen bonds were assigned using a distance of <3.6Å and an A-D-  
905 H angle of >90°, and the maximum distance allowed for a van der Waals interaction was 4.0 Å.  
906 Structure figures were made using UCSF Chimera v1.14 (Goddard et al., 2018).

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923 **Supplementary Material for**  
924 **Rapid Identification of Neutralizing Antibodies**  
925 **against SARS-CoV-2 Variants by mRNA Display**

926 **Supplementary Methods**

927 **Developability assays**

928 *Meso Scale Diagnostics (MSD) Polyreactivity:* Six different antigens, cardiolipin (50  
929  $\mu\text{g/mL}$ , C0563; Sigma), KLH (5  $\mu\text{g/mL}$ , H8283; Sigma), LPS (10  $\mu\text{g/mL}$ , tlr1-eblps; InvivoGen),  
930 ssDNA (1  $\mu\text{g/mL}$ , D8899; Sigma), dsDNA (1  $\mu\text{g/mL}$ , D4522; Sigma), and insulin (5  $\mu\text{g/mL}$ ,  
931 I9278; Sigma) were coated onto MSD MULTI-Array 96-well plate (MSD) individually at 50  $\mu\text{L}$   
932 per well overnight at 4°C. Plates were blocked with PBS with 0.5% BSA at room temperature  
933 for 1 h, followed by three washes with PBST (PBS plus 0.05% Tween 20). Fifty microliters of  
934 100 nM testing antibody solution was added to each well and incubated at RT for 1 hour  
935 followed by six washes with 100  $\mu\text{L}$  of PBS. Twenty microliters of 0.25  $\mu\text{g/mL}$  SULFO-tag,  
936 anti-Human antibody was added to the wells and incubated for 1 hour followed by six washes as  
937 before. Finally, 150  $\mu\text{L}$  of 2X MSD Read Buffer T (MSD) was added to each well, and  
938 electrochemiluminescence signal was read by MSD Sector Imager. Polyreactivity score was  
939 determined by normalizing signal by control wells with no test antibody.

940 *Hydrophobic Interaction Column (HIC):* 5  $\mu\text{g}$  antibody samples (1 mg/mL) were spiked in  
941 with a mobile phase A solution (1.8 M ammonium sulfate and 0.1 M sodium phosphate at pH  
942 6.5) to achieve a final ammonium sulfate concentration of about 1 M before analysis. A Sepax  
943 Proteomix HIC butyl-NP5 column on Agilent 1100 HPLC was used with a liner gradient of  
944 mobile phase A and mobile phase B solution (0.1 M sodium phosphate, pH 6.5) over 20 min at a  
945 flow rate of 1 mL/min with UV absorbance monitoring at 280 nm. Elution time was recorded.

946 *Clone Self-interaction by Bio-layer Interferometry (CSI-BLI)*: Human IgG (Sigma) was  
947 loaded to an AHQ biosensor (ForteBio) to ~1 nm, followed by sensor blocking with human  
948 IgG1 Fc (R&D systems). The self-association was performed at 1  $\mu$ M solution concentration of  
949 antibodies for 300s on an Octet Red96e system (Sartorius Corporation). The binding response  
950 from the association step was subtracted from that of a reference IgG.

951 *Accelerated Stability Assay*: Antibody samples at 1 mg/mL were kept at 40°C for 30 days in  
952 10 mM Hepes and 150 mM sodium chloride, pH 7.4. 10  $\mu$ g of antibody was loaded onto Zenix-C  
953 SEC-300 size-exclusion column (Sepax) on HPLC at Day 0, 5, 20, and 30. A long-term stability  
954 slope (% aggregation/day) was calculated from the percent aggregated measured on the SEC-  
955 HPLC at each time-point.

956 *Differential Scanning Fluorimetry (DSF) Analysis of Melting Temperature ( $T_m$ )*: Twenty  
957 microliters of 1mg/mL antibody sample was mixed with 10  $\mu$ L of 20x SYPRO Orange  
958 (ThermoFisher) in a 96-well PCR plate (ThermoFisher). The plate was scanned from 40°C to  
959 95°C at a rate of 0.5°C/ 2 min in a CFX96 Real-Time PCR system (Bio-Rad). The Fab  $T_m$  was  
960 assigned using the first derivative of the raw data.

#### 961 **Convalescent plasma blocking assay**

962 Spike trimer with C-terminal biotin at 5  $\mu$ g/mL was first loaded on High Precision  
963 Streptavidin SAX biosensors (Sartorius Corporation) for 75 min. Spike coated biosensor was  
964 subsequently blocked with 10-fold diluted SARS-CoV-2 convalescent plasma for 15 min. BLI  
965 signal from 10 mAbs binding to available surface of spike timer were measure by incubating  
966 Spike-coated/plasma blocked biosensors in 10  $\mu$ g/mL of mAbs for 3 min. BLI signal was  
967 compared to self-blocking of N-612-017/N-612-017 and non-blocking pair of N-612-017/N-612-

968 004 to determine whether each mAb was completely blocked, partially blocked, or non-blocked  
 969 by convalescent plasma from 4 different patients.

970 **Supplementary Tables**

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972 **Table S1. Ten unique spike binding VH/VL sequences identified by mRNA display**

Ab	CDRH1 (26-35)	CDRH2 (50-58)	CDRH3 (93-101)	CDRL3 (89-86)	
N-612-017	GFTFSSYAMH	AIWGSGSNTY	ARGRDLAAFTKTA	QQHDALPW	RBD binder
N-612-056	GFTFSSYAMS	LISGSGGSTY	ARDLWGSGFFA	QQDAGTPL	
N-612-074	GFTFSAYAMH	AIWGSGGSTY	ARDLWMAMWFG	QQRSTYPL	
N-612-004	GFTFSSYMH	AISGSGGYTY	ARDRDHAYDWG	QQWADWPL	SD1 binder
N-612-041	GFTFSSYTMH	AISGSGGYTY	ARDRDLLWMGWA	QQYANWPL	
N-612-002	GFTFSSYTMH	AISGSGGSTY	ARDLFDWG	QQDYGFPL	NTD binder
N-612-014	GFTFSSYAMT	YISGSGGSTY	ARDRWASGWLA	QQAYAYPL	
N-612-007	GFTFSNYAMH	AISGNGGSTG	ARDRWYVKNA	QQLDGTPF	S2 binder
N-612-044	GFTFSNYAMH	AISGSGGSTY	ARDLSFWLTYHLASA	QQSYSDPL	
N-612-086	GFTFSSYAMH	AISWSGRSTY	ARDLSSNWGSG	QQSADTPF	

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974 **Table S2. BLI kinetic parameters obtained for various SARS-CoV-2 Spike domains (RBD,**

975 **RBD-SD1, S1, S2)**

Analyte	nAb	$k_{on}$ (1/Ms)	$k_{off}$ (1/s)	$K_D$ (nM)
RBD	N-612-017	6.77E+05	3.58E-03	5.29
	N-612-056	3.37E+05	1.01E-03	2.98
	N-612-074	1.33E+05	1.48E-03	11.1
RBD-SD1	N-612-017	4.56E+05	3.94E-03	8.65
	N-612-056	3.59E+05	1.04E-03	2.89
	N-612-074	1.21E+05	3.97E-03	32.9
	N-612-004	1.51E+05	9.79E-04	6.49
	N-612-041	2.10E+05	3.35E-03	16.0
S1	N-612-017	2.77E+05	2.04E-03	7.37
	N-612-056	1.51E+05	5.86E-04	3.89
	N-612-074	1.01E+05	1.46E-03	14.4
	N-612-004	9.92E+04	2.13E-04	2.15
	N-612-041	1.25E+05	1.83E-03	14.4
	N-612-002	2.68E+05	7.26E-04	2.71
	N-612-014	1.76E+05	2.18E-03	12.4
S2 (Ligand)	N-612-007 (Fab)	3.70E+05	1.40E-03	3.79
	N-612-044 (Fab)	1.70E+05	1.61E-03	9.44
	N-612-086 (Fab)	2.67E+05	0.96E-02	11.6

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977 **Table S3. BLI kinetic parameters obtained for Spike trimer using bivalent model fit**

Ab	$k_{on1}$ (1/Ms)	$k_{on2}$ (1/Ms)	$k_{off1}$ (1/s)	$k_{off2}$ (1/s)	Apparent $K_D$ (nM)
N-612-017	8.35E+04	1.55E+00	<1.0E-07	1.69E-02	<0.001
N-612-056	6.24E+04	1.94E+00	<1.0E-07	5.15E-02	<0.001
N-612-074	9.23E+04	8.12E-01	<1.0E-07	1.89E-01	<0.001
N-612-004	7.60E+04	4.24E+00	<1.0E-07	1.00E+00	<0.001
N-612-041	1.11E+05	4.06E-01	<1.0E-07	1.75E-01	<0.001
N-612-002	5.69E+04	6.82E-02	<1.0E-07	6.76E-02	<0.001
N-612-014	2.54E+04	3.82E-03	2.29E-07	2.82E+00	0.0033
N-612-007	5.78E+04	7.80E-02	<1.0E-07	2.92E-02	<0.001
N-612-044	7.15E+04	2.62E+00	<1.0E-07	7.14E-02	<0.001
N-612-086	6.11E+04	1.55E-01	<1.0E-07	5.53E-02	<0.001

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979 **Table S4. Developability assay summary table**

Ab	Polyreactivity MSD (Fold-over-PBS)	HIC (min)	BLI-CSI (nm)	Accelerated stability % monomer increase/day	Fab Tm (°C)
N-612-017	3	10.1	-0.06	0.09	85
N-612-056	7	11.5	0.00	0.10	81
N-612-074	31	<b>22.1</b>	-0.01	0.10	76
N-612-004	14	14.9	0.05	0.13	85
N-612-041	10	<b>20.4</b>	0.05	<b>0.37</b>	88
N-612-002	22	13.9	0.08	0.18	82
N-612-014	4	12.3	0.09	0.16	88
N-612-007	10	11.8	0.04	0.11	82
N-612-044	8	<b>13.4/15.4</b>	-0.06	0.10	88/91
N-612-086	10	13.2	0.07	0.11	88
Acceptance criteria	<50	<16	<0.2	<0.2	>65 °C

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982 **Table S5. Cryo-EM data collection and refinement statistics (related to Figures 3 and 5).**

	<b>N-612-017 Fab SARS-CoV-2 S 6P</b>	<b>N-612-014 Fab SARS-CoV-2 S 6P</b>	<b>N-612-004 Fab SARS-CoV-2 S 6P</b>
<b>PDB</b>	<b>7S0C</b>	<b>7S0D</b>	<b>7S0E</b>
<b>EMD</b>	<b>24786</b>	<b>24787</b>	<b>24788</b>
<b>Data collection conditions</b>			
Microscope	Talos Arctica	Talos Arctica	Talos Arctica
Camera	Gatan K3 Summit	Gatan K3 Summit	Gatan K3 Summit
Magnification	45,000x	45,000x	45,000x
Voltage (kV)	200	200	200
Recording mode	counting	counting	counting
Dose rate (e <sup>-</sup> /pixel/s)	13.5	13.3	13.8
Electron dose (e <sup>-</sup> /Å <sup>2</sup> )	60	60	60
Defocus range (µm)	0.7 – 2.0	0.7 – 2.0	0.7 – 2.0
Pixel size (Å)	0.8689	0.8689	0.8689
Micrographs collected	2,585	3,791	3,717
Micrographs used	2,132	3,211	2,047
Total extracted particles	282,890	505,695	595,163
Refined particles	175,986	389,223	115,068
Particles in final refinement	108,746	137,684	107,271
Symmetry imposed	C1	C1	C1
FSC 0.143 (unmasked/masked)			
unmasked	4.4 Å	6.4 Å	7.1 Å
masked	3.2 Å	3.5 Å	4.8 Å
<b>Refinement and Validation</b>			
Initial model used	6XKL	6XKL	6XKL
Number of atoms			
Protein	29,164	33,650	5,307
Ligand	434	873	28
MapCC (global/local)	0.81/0.78	0.79/0.76	0.87/0.69
Map sharpening B-factor	65.8	75.3	155
R.m.s. deviations			
Bond lengths (Å)	0.01	0.005	0.02
Bond angles (°)	0.9	0.89	1.5
MolProbity score	2	2.2	2.46
Clashscore (all atom)	13.4	17.7	7.9
Poor rotamers (%)	0.2	0.1	0
Ramachandran plot			
Favored (%)	94.7	93.4	92.8
Allowed (%)	5.1	6	6.3
Disallowed (%)	0.2	0.6	0.9

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986 **Table S6. X-ray crystallography data collection and refinement statistics (related to Figure**  
 987 **4).**

<b>PDB ID</b>	<b>N-612-056 - SARS2-RBD (12-2, SSRL) 7S0B</b>
<b>Data collection<sup>a</sup></b>	
Space group	P2 <sub>1</sub> 2 <sub>1</sub> 2
Unit cell (Å)	102.3, 153.7, 96.6
$\alpha, \beta, \gamma$ (°)	90, 90, 90
Wavelength (Å)	0.979
Resolution (Å)	38.9-29 (3.04-2.9)
Unique Reflections	34,420 (4,471)
Completeness (%)	99.8 (99.1)
Redundancy	6.6 (6.7)
CC <sub>1/2</sub> (%)	99.3 (72.8)
$\langle I/\sigma \rangle$	6.4 (1.5)
Mosaicity (°)	0.25
R <sub>merge</sub> (%)	18.7 (133)
R <sub>pim</sub> (%)	8.3 (59.4)
Wilson <i>B</i> -factor	56.6
<b>Refinement and Validation</b>	
Resolution (Å)	384 - 2.9
Number of atoms	
Protein	9,829
Ligand	28
Waters	0
R <sub>work</sub> /R <sub>free</sub> (%)	212/25.4
R.m.s. deviations	
Bond lengths (Å)	0.006
Bond angles (°)	1.2
MolProbity score	2.47
Clashscore (all atom)	11.6
Poor rotamers (%)	5
Ramachandran plot	
Favored (%)	94.8
Allowed (%)	5.2
Disallowed (%)	0
Average <i>B</i> -factor (Å)	73.5

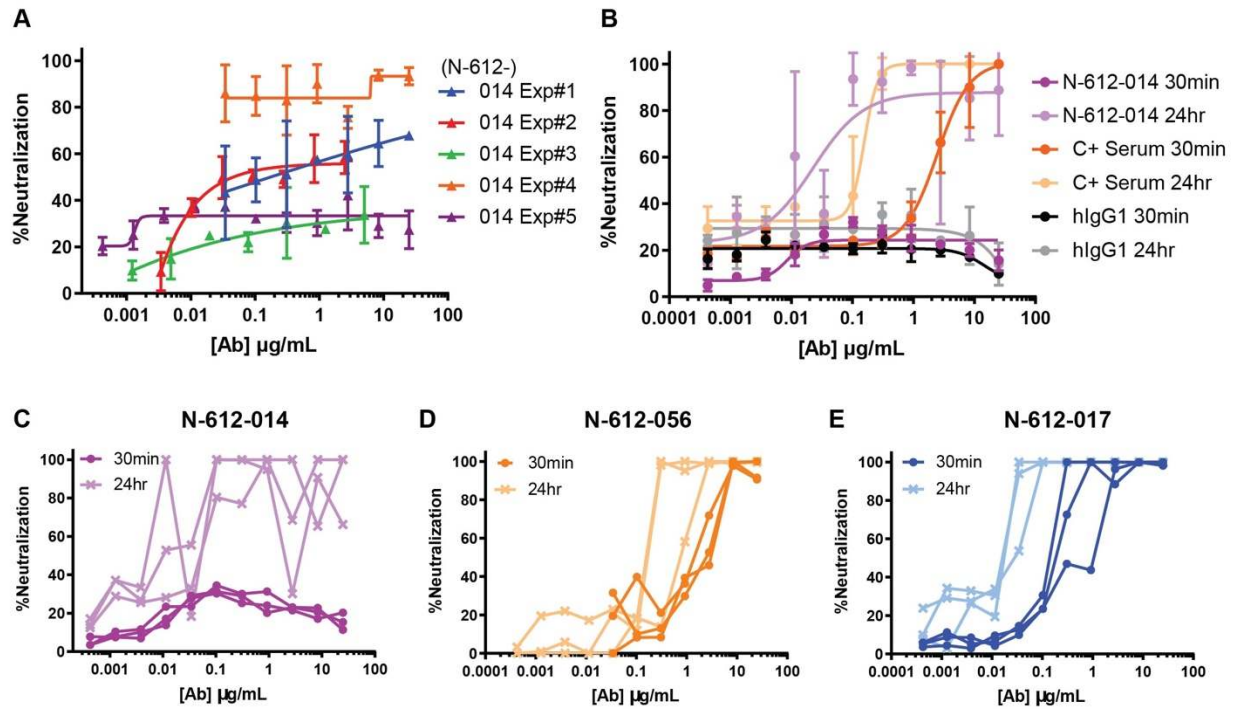
<sup>a</sup>Numbers in parentheses correspond to the highest resolution shell

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990 **Table S7. BLI kinetic analysis of N-612-017, N-612-017-01, N-612-017-03, N-612-017-5B02,**  
 991 **and N-612-017-5B05 against RBD-WT, RBD-B.1.351 (K417N/E484K/N501Y) and RBD-**  
 992 **L452R**

	RBD-WT			RBD-B.1.351			RBD-L452R		
	$k_{on}$ (1/Ms)	$k_{off}$ (1/s)	$K_D$ (nM)	$k_{on}$ (1/Ms)	$k_{off}$ (1/s)	$K_D$ (nM)	$k_{on}$ (1/Ms)	$k_{off}$ (1/s)	$K_D$ (nM)
N-612-017	6.77E+05	3.58E-03	5.29	4.45E+05	1.36E-02	30.6	--	--	N.B.
N-612-017-01	5.59E+05	3.59E-04	0.64	5.31E+05	4.06E-04	0.77	2.34E+05	7.75E-03	33.1
N-612-017-03	6.48E+05	1.60E-04	0.25	6.47E+05	2.33E-04	0.36	1.18E+05	6.90E-03	58.7
N-612-017-5B02	8.24E+05	9.26E-05	0.11	7.99E+05	1.20E-04	0.15	5.74E+05	3.54E-03	6.16
N-612-017-5B05	9.62E+05	3.16E-03	3.28	9.68E+05	2.48E-03	2.56	8.01E+05	1.96E-03	2.44

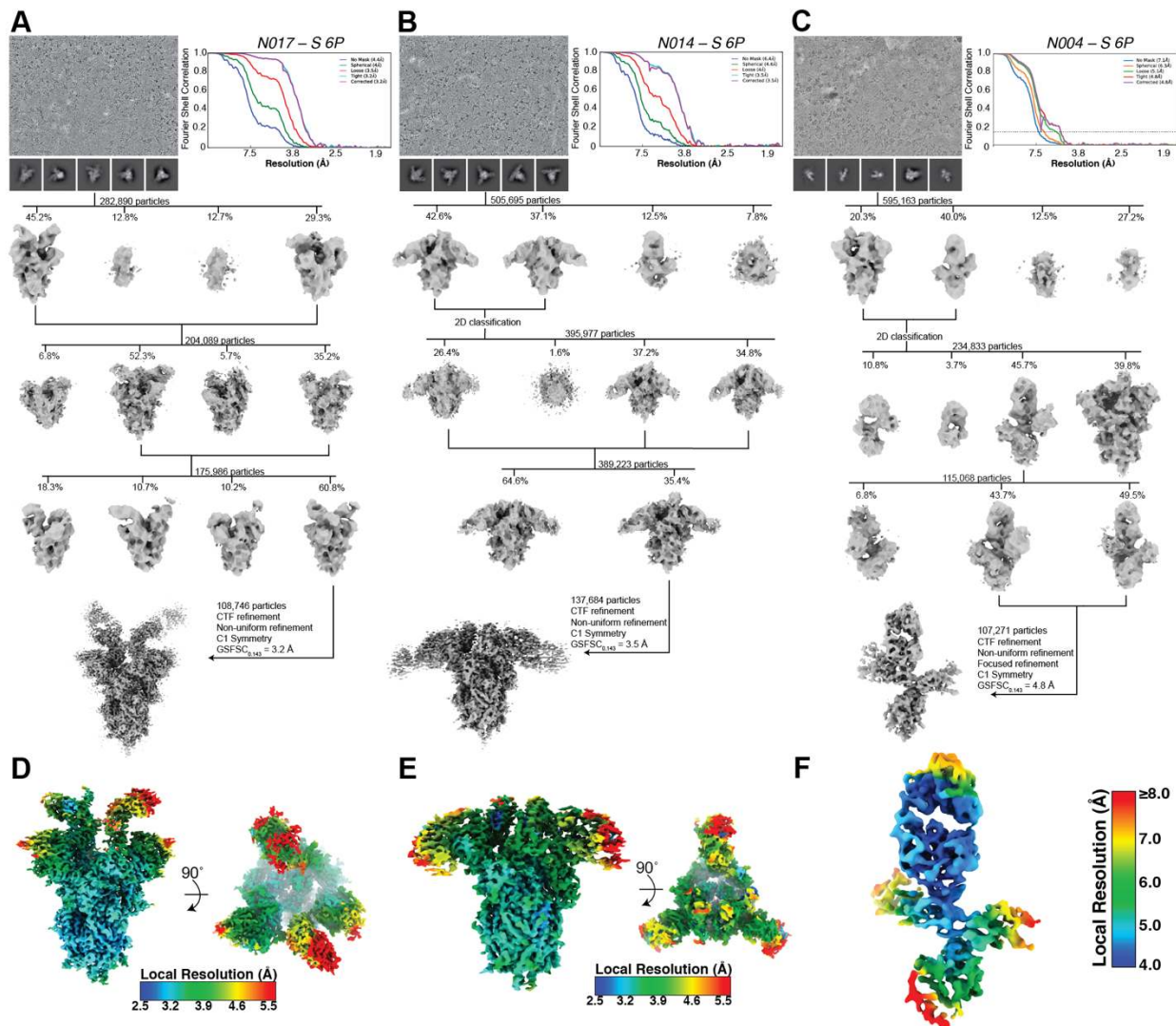
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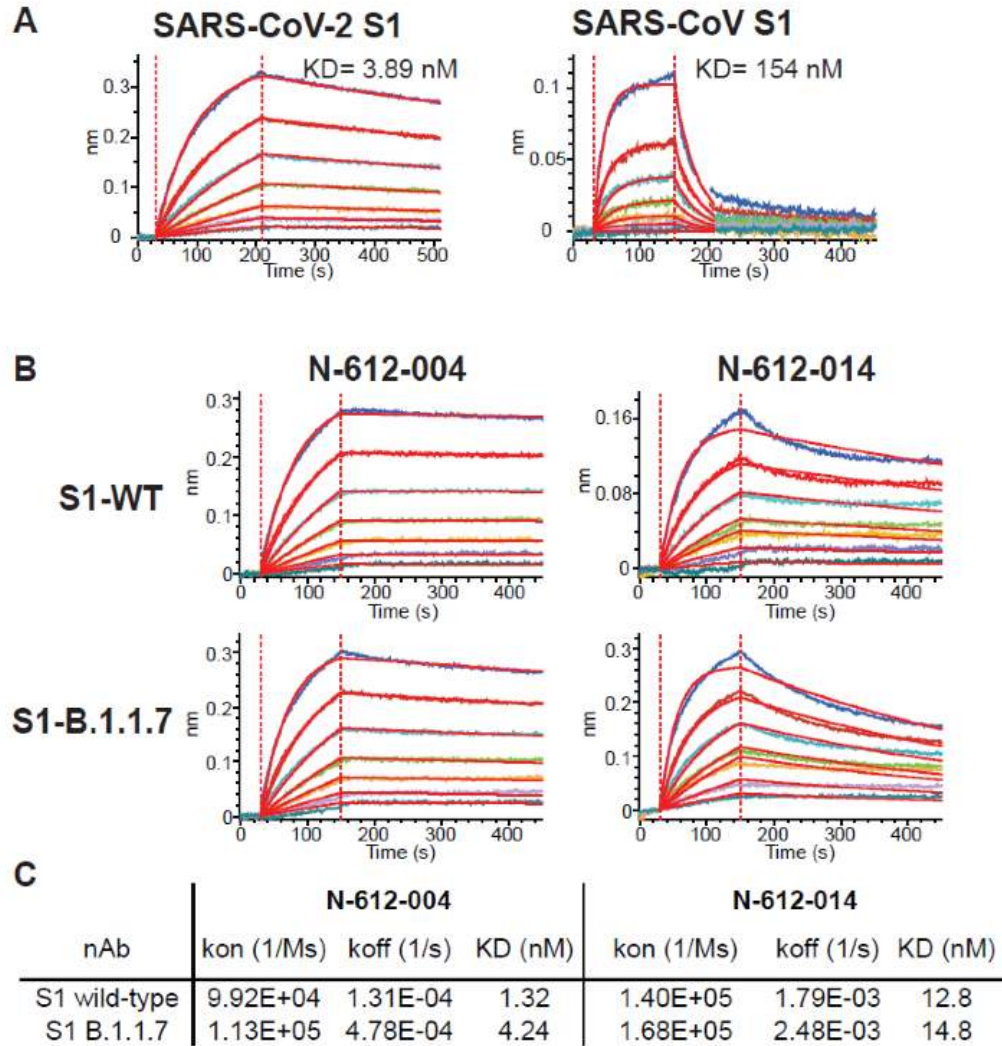
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**Figure S1. Vero E6 live virus neutralization assay** (A) Neutralization activity of N-612-014 in 5 separate experiments. (B) Comparison of neutralization activity of N-612-014, convalescent (C+) serum, and control human IgG with 30-min vs 24-hr antibody-virus incubation. (C, D, E) Change in neutralization potency by incubation time.

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 1037 **Figure S2. Cryo-EM data processing and validation (related to Figures 3 and 5).** (A-C)  
 1038 Representative micrograph, 2D class averages, data processing workflow, and Gold Standard  
 1039 FSC plots for the final reconstructions of (A) N-612-017 – S 6P, (B) N-612-014 – S 6P, and (C)  
 1040 N-612-056 – S 6P complexes. (D-F) Local resolution estimates calculated in cryoSPARC v3.1  
 1041 for (D) N-612-017 – S 6P, (E) N-612-014 – S 6P, and (F) N-612-056 – S 6P complexes.



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**Figure S3:** (A) Cross-reactivity of N-612-056 against SARS-CoV. BLI kinetic analysis of SARS-CoV-2 and SARS-CoV S1 domain binding to N-612-056. (B, C) BLI kinetic analysis of N-612-004 and N-612-014 against S1 domain from WT and B.1.1.7 variant of SARS-CoV-2 Spike protein.

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	EX1	EX2	EX3	EX4
<b>No blocking</b>	004(SD1)	004(SD1)	004(SD1)	004(SD1)
	041(SD1)	041(SD1)	041(SD1)	041(SD1)
	056(RBD)	056(RBD)	056(RBD)	
<b>Partial Blocking</b>	002(NTD)	002(NTD)	002(NTD)	002(NTD)
	014(NTD)	014(NTD)	014(NTD)	014(NTD)
	017(RBD)	017(RBD)	017(RBD)	017(RBD)
	074(RBD)	074(RBD)	074(RBD)	056(RBD)
<b>Blocking</b>	007(S2)	007(S2)	007(S2)	074(RBD)
	044(S2)	044(S2)	044(S2)	007(S2)
	086(S2)	086(S2)	086(S2)	044(S2)
				086(S2)

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1052 **Figure S4. Convalescent plasma blocking of 10 mAbs binding to SARS-CoV-2 Spike.**

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