

1 **Landscape analysis of escape variants identifies SARS-CoV-2 spike mutations that**  
2 **attenuate monoclonal and serum antibody neutralization**

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24 **Figures:7**

25

26 **ABSTRACT**

27           Although neutralizing antibodies against the SARS-CoV-2 spike (S) protein are a goal of  
28 COVID-19 vaccines and have received emergency use authorization as therapeutics, viral escape  
29 mutants could compromise their efficacy. To define the immune-selected mutational landscape in  
30 S protein, we used a VSV-eGFP-SARS-CoV-2-S chimeric virus and 19 neutralizing monoclonal  
31 antibodies (mAbs) against the receptor-binding domain (RBD) to generate 50 different escape  
32 mutants. The variants were mapped onto the RBD structure and evaluated for cross-resistance  
33 to mAbs and convalescent human sera. Each mAb had a unique resistance profile, although many  
34 shared residues within an epitope. Some variants (*e.g.*, S477N) were resistant to neutralization  
35 by multiple mAbs, whereas others (*e.g.*, E484K) escaped neutralization by convalescent sera,  
36 suggesting some humans induce a narrow repertoire of neutralizing antibodies. Comparing the  
37 antibody-mediated mutational landscape in S with sequence variation in circulating SARS-CoV-  
38 2, we define substitutions that may attenuate neutralizing immune responses in some humans.  
39

## 40 INTRODUCTION

41 Control of the ongoing SARS-CoV-2 pandemic will require deployment of multiple  
42 countermeasures including therapeutics and vaccines. Therapeutic candidates that have  
43 received emergency use authorization (EUA) or are in development include several monoclonal  
44 antibodies (mAbs) (Chen et al., 2020; Group et al., 2020; Weinreich et al., 2020) that recognize  
45 the SARS-CoV-2 spike (S) protein, which decorates the virion surface (Ke et al., 2020). The S  
46 protein is comprised of an N-terminal subunit (S1) that mediates receptor binding and a C-terminal  
47 subunit (S2) responsible for virus-cell membrane fusion (Wrapp et al., 2020). During viral entry  
48 into cells, the receptor-binding domain (RBD) of S1 engages the primary receptor, human  
49 angiotensin converting enzyme 2 (hACE2) (Letko et al., 2020). Processing of S by host cell  
50 proteases, typically TMPRSS2, TMPRSS4, or endosomal cathepsins, facilitates the S2-  
51 dependent fusion of viral and host-cell membranes (Hoffmann et al., 2020; Zang et al., 2020).  
52 Potently neutralizing antibodies against SARS-CoV-2 target the RBD (Brouwer et al., 2020; Group  
53 et al., 2020; Rogers et al., 2020; Wu et al., 2020b; Zost et al., 2020) with many inhibiting infection  
54 by blocking receptor engagement (Alsoussi et al., 2020; Wu et al., 2020b). Understanding the  
55 epitopes recognized by protective antibodies and whether natural variation in the S protein is  
56 associated with resistance to neutralization may predict the utility of antibody-based  
57 countermeasures.

58 RNA viruses exist as a swarm or “quasispecies” of genome sequences around a core  
59 consensus sequence (Dolan et al., 2018). Under conditions of selection, such as those imposed  
60 by neutralizing antibodies or drugs, variants of the swarm can escape genetically and become  
61 resistant. The relative fitness of escape mutants determines whether they are lost rapidly from  
62 the swarm or provide a competitive advantage. The intrinsically high error rates of viral RNA-  
63 dependent RNA polymerases (RdRp) result in the stochastic introduction of mutations during viral  
64 genome replication with substitutions approaching a nucleotide change per genome for each  
65 round of replication (Sanjuan et al., 2010). Coronaviruses, because of their large genome size,

66 encode a proofreading 3'-to-5' exoribonuclease (ExoN, nsp14) that helps to correct errors made  
67 by the RdRp during replication (Smith and Denison, 2013). As a result of ExoN activity, the  
68 frequency of escape from antibody neutralization by coronaviruses is less than for other RNA  
69 viruses lacking such an enzyme (Smith et al., 2013).

70 To date, 4150 mutations have been identified in the S gene of SARS-CoV-2 isolated from  
71 humans (CoV-GLUE, 2021; GISAID, 2021). These mutations give rise to 1,246 amino acid  
72 changes including 187 substitutions in the RBD. The abundance of many variants in the human  
73 population suggests they are not accompanied by a fitness loss. Multiple mechanisms likely  
74 account for the emergence of such substitutions including host adaptation, immune selection  
75 during natural infection, and possibly reinfection of individuals with incomplete or waning  
76 immunity. Convalescent plasma therapy, vaccination, and administration of therapeutic  
77 antibodies each could select for additional variants, and their effectiveness as countermeasures  
78 might be compromised by preexisting resistant mutants. Thus, as therapeutic antibodies and  
79 vaccines are deployed, it is increasingly important to define the patterns of antibody resistance  
80 that arise. The impact of SARS-CoV-2 adaptation for infection of other hosts including mice  
81 (Dinnon et al., 2020; Gu et al., 2020), mink (Oude Munnink et al., 2020) and domesticated animals  
82 (Halfmann et al., 2020; Shi et al., 2020) could also contribute to selection of new variants.

83 Here, we used a panel of antibodies including the previously reported 2B04, 1B07 and  
84 2H04 mAbs (Alsoussi et al., 2020) and newly-generated neutralizing mAbs against SARS-CoV-2  
85 RBD to select for escape variants and define the mutational landscape of resistance. To facilitate  
86 selection, we used a chimeric, infectious vesicular stomatitis virus (VSV) in which the endogenous  
87 glycoprotein was replaced with the S protein of SARS-CoV-2 (Case et al., 2020). VSV-eGFP-  
88 SARS-CoV-2-S<sub>Δ21</sub> (herein, VSV-SARS-CoV-2) replicates to high titer ( $10^7$ - $10^8$  plaque forming  
89 units ml<sup>-1</sup> within 48 hours), mimics the SARS-CoV-2 requirement for human ACE2 as a receptor,  
90 and is neutralized by SARS-CoV-2 S-specific mAbs (Case et al., 2020). In three selection  
91 campaigns using 19 different mAbs, we isolated 50 different escape mutants within the RBD.



92 Many escape mutations arose proximal to or within the ACE2 binding footprint suggesting that  
93 multiple neutralizing mAbs inhibit infection by interfering with receptor engagement. Cross-  
94 neutralization studies involving 29 of the escape mutants and 10 mAbs identified mutants that  
95 were resistant to multiple antibodies and also those with unique resistance profiles. Remarkably,  
96 substitutions at residue E484 of S protein were associated with resistance to neutralization by  
97 polyclonal human immune sera, suggesting that some individuals generate neutralizing  
98 antibodies recognizing a focused target on the RBD. Resistance to inhibition by soluble  
99 recombinant human ACE2, a candidate decoy molecule drug (Chan et al., 2020; Monteil et al.,  
100 2020) currently in clinical trials (NCT04375046, NCT04287686), was observed with an F486S  
101 substitution. Cross-referencing of our 50 resistant mutants with sequences of clinical isolates of  
102 SARS-CoV-2 demonstrates that some already circulating variants will be resistant to monoclonal  
103 and polyclonal antibodies. This data and functional approach may be useful for monitoring and  
104 evaluating the emergence of escape from antibody-based therapeutic and vaccine  
105 countermeasures as they are deployed.  
106

107 **RESULTS**

108 **Selection of mAb escape mutants in SARS-CoV-2 S.**

109 To select for SARS-CoV-2 S variants that escape neutralization, we used VSV-SARS-  
110 CoV-2 (Case et al., 2020) and mAb 2B04, which was generated from cloned murine B cells  
111 following immunization of C57BL/6 mice with recombinant RBD and boosted with recombinant S.  
112 Antibody neutralization resistant mutants were recovered by plaque isolation (**Fig 1A**), and their  
113 resistance was verified by subsequent virus infection in the presence or absence of antibody.  
114 Antibody 2B04 failed to inhibit VSV-SARS-CoV-2 resistant variants as judged by plaque number  
115 and size (**Fig 1B**). Sequence analysis identified the mutations E484A, E484K, and F486S (**Fig**  
116 **1B**), each of which fall within the RBD and map to residues involved in ACE2 binding (Lan et al.,  
117 2020) (**Fig 2**).

118 We extended this neutralization escape approach to nine additional inhibitory mAbs (**Fig**  
119 **S1, S2 and Table 1**). Sequence analysis of each isolated plaque identified multiple mutations  
120 within the RBD (**Table 2**), which we positioned on the reported crystal structure (PDB: 6M0J) (**Fig**  
121 **2**): 2B04 (green), 2H04 (lime), 1B07 (blue), SARS2-01 (yellow), SARS2-02 (teal), SARS2-07  
122 (tangerine), SARS2-16 (violet), SARS2-19 (red), SARS2-32 (fuschia), and SARS2-38 (magenta).  
123 Substitutions that led to resistance of mAbs 2B04, 1B07, SARS2-02, SARS2-07, SARS2-16,  
124 SARS2-32, and SARS2-38 cluster within and proximal to the ACE2 binding site. Resistance to  
125 antibodies SARS2-01 and SARS2-19 mapped to substitutions at sites on the side of the RBD (**Fig**  
126 **2**). MAb 2H04 gave rise to resistance mutations that map exclusively on the side and base of the  
127 RBD (**Fig 2**). The identification of resistance mutations at the side of the RBD suggest that the  
128 mechanism of virus neutralization may be allosteric or possibly through blocking interactions with  
129 alternative attachment factors. The presence of resistance mutations at the base of RBD, which  
130 lie outside the 2H04 binding footprints, suggests an allosteric mechanism of resistance, perhaps  
131 related to the ability of the RBD to adopt the 'up' conformation requisite for ACE2 binding.

132 From this panel of mAbs, we observed resistance substitutions at shared positions. Four  
133 mAbs yielded substitutions at E484 (2B04, 1B07, SARS2-02, and SARS2-32), three resulted in  
134 changes to residues G446 (SARS2-02, SARS2-32, and SARS2-38) and S477 (SARS2-07,  
135 SARS2-16 and SARS2-19), and two promoted escape substitutions at F486 (2B04 and 1B07),  
136 K444 (2H04 and SARS2-38), L452 (SARS2-01 and SARS2-32), and N450 (SARS2-07 and  
137 SARS2-32), and R346 (2H04 and SARS2-01). The overlapping nature of these epitopes suggests  
138 they represent major antigenic sites within the RBD. Although amino acid changes were selected  
139 at the same position, many of the substitutions were distinct, consistent with a unique mode of  
140 binding for each antibody.

141 Two mAbs gave rise to variants containing linked amino acid substitutions: 2H04 (T345A  
142 and L517R) and SARS2-19 (S477N and S514F). For 2H04, substitution T345A likely arose first,  
143 as we isolated this mutation alone, and acquisition of the L517R substitution appeared to enhance  
144 infectivity as judged by plaque morphology (**Fig S2**). For SARS2-19, S477N was isolated as a  
145 single variant suggesting that this substitution arose first, however acquisition of the S514F did  
146 not alter plaque morphology (**Fig S2**). As the L517R or S514F substitutions were not identified in  
147 isolation, it remains unclear whether they cause resistance to 2H04 or SARS2-19 respectively.  
148 Collectively, these results show that escape mutational profiling can identify key epitopes and  
149 dominant antigenic sites.

### 150 **Escape mutants confer cross-resistance to multiple mAbs.**

151 We next evaluated whether individual mutants could escape neutralization by the other  
152 inhibitory mAbs in the panel. We tested the 29 identified escape mutants for neutralization by ten  
153 different mAbs. We defined the degree of resistance as a percentage by expressing the number  
154 of plaques formed by each mutant in the presence or absence of antibody. We plotted the degree  
155 of resistance to neutralization as a heat map and arbitrarily set 50% as the cut-off value for  
156 defining resistance (**Fig 3A**). Substitutions at residues T345, R346, K444, G446, N450, L452,  
157 S477, T478, E484, F486 and P499 each were associated with resistance to more than one mAb,

158 with substitutions at S477 and E484 exhibiting broad resistance (**Fig 3A**). For residues at which  
159 multiple alternate amino acids with different side chains were selected, each particular substitution  
160 was associated with a unique resistance profile. For example, K444E was resistant to SARS2-38  
161 and 2H04 with some resistance to SARS2-1, SARS2-2 and SARS2-7, whereas K444N conferred  
162 complete resistance to SARS2-38, partial resistance to 2H04 and only weak resistance to SARS2-  
163 1 and SARS2-2. G446D was resistant to SARS2-2, SARS2-32 and SARS2-38, but G446V  
164 acquired resistance to SARS2-01. Substitutions N450K and N450Y were resistant to SARS2-01  
165 and SARS2-32, whereas N450D facilitated resistance to SARS2-07. Substitution L452R  
166 conferred resistance to SARS2-01, SARS2-02, and SARS2-32; S477N, S477G and S477R were  
167 each highly resistant to SARS2-07, SARS2-16, and SARS2-19, and S477N and S477G result in  
168 a degree of resistance across the entire panel of antibodies; and T478I yielded resistance to  
169 SARS2-16 and SARS2-19.

170 Escape variants at residue E484 were isolated using 2B04, 1B07, SARS2-02, and  
171 SARS2-32, and specific substitutions at this residue led to varying degrees of resistance across  
172 the entire panel of antibodies. E484A exhibited a high degree of resistance to 2B04, 1B07,  
173 SARS2-01, SARS2-07, SARS2-19, SARS2-32, SARS2-38; E484G exhibited resistance to 2B04,  
174 1B07, SARS2-01, SARS2-32 and SARS2-38; E484K was resistant to 2B04, 1B07, SARS2-01,  
175 SARS2-02, SARS2-16 and SARS2-32; and E484D was resistant only to 1B07 (**Fig 3A and S3**).  
176 Substitution F486S was resistant to 2B04, 1B07, SARS2-07, SARS2-16 and SARS2-19, whereas  
177 F486Y exhibited resistance only to 1B07 and SARS2-16. Finally, substitution P499L was resistant  
178 to SARS2-07, SARS2-16, and SARS2-19. In addition to demonstrating that some mutations  
179 confer resistance to multiple neutralizing mAbs, these data suggest that each mAb recognizes  
180 distinct yet partially overlapping epitopes.

### 181 **Soluble human ACE2-Fc receptor decoy inhibition of escape mutants.**

182 Soluble human ACE2 decoy receptors are under evaluation in clinical trials for treatment  
183 of COVID-19 (NCT04375046 and NCT04287686). As several of the escape mutants contain

184 substitutions within or proximal to the ACE2 binding site, we evaluated the ability of soluble  
185 recombinant ACE2 to inhibit infection of each variant. We incubated each VSV-SARS-CoV-2  
186 mutant with increasing concentrations of soluble human (h) or murine (m) ACE2-Fc for 1 h at  
187 37°C and measured residual infectivity on Vero E6 cells (**Fig 3B and S4**). As observed with  
188 chimeric viruses expressing the wild-type S protein, the escape mutants were inhibited by hACE2-  
189 Fc but not mACE2-Fc. However, the extent of neutralization by hACE2-Fc varied substantially  
190 (**Fig 3B**), with some mutants more sensitive to receptor inhibition and others exhibiting relative  
191 resistance. Substitutions at residues R346, A352, N450, S477, S494 and P499 were more  
192 sensitive to inhibition by soluble hACE2 than the wild-type S as evidenced by reduced IC<sub>50</sub> values  
193 (**Fig 3B**) and leftward shifts of the inhibition curves (**Fig S4**). This effect was substitution-  
194 dependent, as N450K was 6-fold more sensitive to hACE2 than N450Y ( $P < 0.001$ ). Several  
195 mutants required higher (3-5-fold) concentrations of hACE2 to block infection, including  
196 substitutions at T345A, T345N, G446D, G446V, E484D and F486Y. Again, the specific  
197 substitution of a given residue impacted the effect, as T345A and T345N required higher  
198 concentrations of hACE2 to inhibit infection, whereas T345S was similar to wild type. Of the 4  
199 substitutions observed at position E484, only E484D was less sensitive (4.6-fold,  $P < 0.0001$ ) to  
200 hACE2 inhibition. The most striking effect was observed for F486S, where we achieved only 38%  
201 inhibition at the highest concentration (20 µg/ml) of hACE2-Fc tested (**Fig 3B and C**). Residue  
202 F486 is located on the top of the hACE2 contact loop of RBD, and the presence of a large  
203 hydrophobic residue facilitates efficient receptor engagement (Lan et al., 2020; Shang et al.,  
204 2020). Although this substitution alters sensitivity to soluble ACE2 inhibition of infection, its impact  
205 on cell surface ACE2 engagement by virus was not examined.

#### 206 **Escape mutants exhibit resistance to neutralization by polyclonal human immune sera.**

207 We previously evaluated the ability of convalescent sera from SARS-CoV-2-infected  
208 humans to neutralize VSV-SARS-CoV-2 and defined a strong correlation with inhibition of a  
209 clinical isolate of SARS-CoV-2 using a focus reduction neutralization test (FRNT) (Case et al.,

210 2020). We tested four of the serum samples (13, 29, 35 and 37) from patients who had recovered  
211 from COVID-19 against our panel of escape mutants (**Table 3**). All four sera neutralized infection  
212 of VSV-SARS-CoV-2-S displaying the wild-type S protein as we previously demonstrated.  
213 Remarkably, several of the escape mutants were resistant to neutralization at the highest  
214 concentration (1:80 initial dilution) of sera tested. All four of the substitutions at residue E484 were  
215 resistant to each of the four sera, suggesting that this is part of a dominant neutralizing epitope.  
216 Indeed, change at E484 was the only position that led to escape from sera 29 (**Fig 4A-B, and**  
217 **S5A**). Four other substitutions (K444E, G446V, L452R and F490S) resulted in resistance to  
218 neutralization of sera 13, 35 and 37 (**Fig 4A and S5A**). Substitutions N450D and N450Y but not  
219 N450K were resistant to sera 13 and 35. Sera 13 and 35 also did not efficiently neutralize S477G,  
220 L441R, and T478I. All four sera neutralized the single substitution S477N as well as wild-type  
221 virus (**Fig 4A-B**). Substitution S477N was sensitive to neutralization by sera 13 and 35 except in  
222 the presence of a second S514F substitution (**Fig 4A and S5A**). Additional amino acid  
223 substitutions that conferred resistance to serum 13 include T345S and G446D. Substitution  
224 F486S, which altered sensitivity to soluble ACE2, escaped neutralization by serum 35 but not 13,  
225 29 or 37. We consistently noticed that some sera also led to an increase in infectivity of specific  
226 escape mutants (e.g., E484A) at some concentrations (**Fig 4B**). The significance of this increase  
227 is unclear, but was observed consistently across sera for several mutants (**Fig S5A**).

228 To extend these findings, we employed a higher throughput screening assay to test 16  
229 additional human sera (11, 15, 16, 18, 21, 23, 27, 28, 30, 31, 32, 34, 35, 37, 38 and 39) for their  
230 ability to neutralize the VSV-SARS-CoV-2 mutants N450Y, S477N, E484A, E484D and E484K  
231 (**Table 3**). Although we observed neutralization of the various mutants at the highest  
232 concentrations of human sera tested (1:10), VSV-SARS-CoV-2 variants with substitutions at  
233 residues 484 were consistently less sensitive to neutralization by all sera (**Fig 4C and S5B**). Thus,  
234 individual escape mutants can exhibit resistance to neutralization by polyclonal human  
235 convalescent sera. This observation suggests that the repertoire of antigenic sites on RBD that

236 bind neutralizing antibodies is limited in some humans. We again observed the increase in  
237 infectivity of substitutions at residue E484 in the presence of multiple human sera (**Fig S5B**).

### 238 **Comparison of escape mutants with S sequence variants isolated in humans.**

239 To broaden our analysis, we performed a second campaign of escape mutant selection  
240 using nine additional neutralizing mAbs generated against the RBD (**Fig 5, S6, S7 and Table 2**).  
241 This effort generated 19 additional escape mutants, bringing the total to 48. To determine whether  
242 any of the 48 escape mutants we isolated represent S protein variants circulating in humans, we  
243 compiled all publically available genome sequences of SARS-CoV-2. Using 323,183 genomes  
244 from GISAID, we calculated the substitution frequencies throughout RBD protein (**Fig 6A**) and  
245 mapped the identified residues onto RBD structure (**Fig 6B**). Of the 48 escape variants we  
246 selected, 32 are present in circulating human isolates of SARS-CoV-2 (**Fig 6A**). The most  
247 frequent S sequence variant seen in clinical isolates is D614G which is present in 69% of  
248 sequenced isolates. The fourth most frequent substitution is S477N, which is present in 4.6% of  
249 sequenced isolates and the dominant virus in Oceania. The penetrance of the remaining  
250 substitutions among clinical isolates is relatively low, with G446V, T478I, E484K, S477I and  
251 S494P ranking 79, 102, 123, 135 and 146 of the top 150 variants in S or roughly 0.05% of  
252 sequenced variants. Collectively, this analysis highlights that neutralizing mAbs against RBD can  
253 select for variants or changes at positions that already exist within the human population and  
254 establishes that some substitutions are present at high frequency.

### 255 **Sequential selection of 2B04 and 2H04 escape mutants**

256 To examine whether mutations resistant to antibody combinations could be isolated, we  
257 undertook a third selection campaign using a combination of 2B04 and 2H04. We were unable to  
258 isolate mutants resistant to the two antibodies when added concurrently. However, using the 2B04  
259 resistant-viruses E484A, E484K and F486S, we selected additional mutations by growth in the  
260 presence of 2H04 (**Fig 7 and Table 2**). These selected variants were resistant to both antibodies.  
261 For the 2B04 resistant mutant E484A, we selected T345A, R346G and K444E; for mutant E484K,

262 we isolated R346K, A372T and K444E; and for mutant F486S we selected T345S. Two of the  
263 mutants (R346K and A372T) were not seen in our prior selection campaigns with 2H04 alone,  
264 although both variants exist in human isolates (**Fig 6**). Taken together, this analysis suggests that  
265 cocktails of mAbs binding distinct epitopes on SARS-CoV-2 S protein pose an increased but not  
266 complete barrier to resistance, especially if circulating strains already encode substitutions that  
267 compromise effectiveness of one of the two mAbs.  
268



269 **DISCUSSION**

270 Therapeutic mAbs, convalescent plasma and vaccines are in clinical development as  
271 countermeasures against SARS-CoV-2. The efficacy of these strategies will be impacted by viral  
272 mutants that escape antibody binding and neutralization. To define the landscape of mutations in  
273 the RBD associated with resistance, we selected escape mutants to 19 neutralizing mAbs  
274 including some in clinical development. Characterization of escape mutants identified several that  
275 exhibit resistance to multiple antibodies, convalescent human sera, and soluble receptor decoys.  
276 Resistance to neutralization by serum from naturally-infected humans suggests that the  
277 neutralizing response to SARS-CoV-2 in some individuals may be dominated by antibodies that  
278 recognize relatively few epitopes. Many of the escape mutants we identified contain substitutions  
279 in residues at which variation is observed in circulating human isolates of SARS-CoV-2. If a similar  
280 limited polyclonal response occurs following S protein-based vaccination, escape variants could  
281 emerge in the human population and compromise the efficacy of such vaccines.

282 From 19 different mAbs that neutralize SARS-CoV-2, we isolated 50 viral mutants that  
283 escape neutralization. Selection of escape mutants was facilitated by the use of VSV-SARS-CoV-  
284 2, which we previously validated as an effective mimic of SARS-CoV-2 S protein mediated  
285 infection (Case et al., 2020). The mAbs were obtained following immunization with soluble RBD,  
286 and although some mice received a boost with stabilized S ectodomain protein, all escape  
287 substitutions map within the RBD. Multiple different mAbs led to resistance substitutions at K444,  
288 G446, N450, L452, S477, T478, P479, E484, F486 and P499, suggesting that they comprise  
289 major antigenic sites within the RBD. In earlier work, substitutions at residues K444, N450, E484  
290 and F486 were identified using two antibodies in clinical development (Group et al., 2020), and a  
291 separate study using three different antibodies defined resistance substitutions at R346, N440,  
292 E484, F490 and Q493 (Greaney et al., 2020; Weisblum et al., 2020).

293 The mutations we selected also inform the mechanism by which the different antibodies  
294 function. All of the resistance mutations we identified map within or proximal to the ACE2 binding

295 site. Likely, the majority of the antibodies we tested neutralize infection by interfering with receptor  
296 engagement. Antibodies from human survivors also interfere with receptor engagement (Wu et  
297 al., 2020b; Zost et al., 2020) suggesting a common mechanism of neutralization. Some of the  
298 resistance mutations from 2H04, SARS2-01 and SARS2-31 we identified map outside the ACE2  
299 binding site including at the side and the base of the RBD. Direct competition with ACE2 binding  
300 is consistent with the escape mutants we selected with 2B04, whereas an indirect mechanism of  
301 action fits with the escape mutants we identified to 2H04. Our finding of an escape mutant to 2H04  
302 located at the base of the RBD, outside the footprint of the antibody, suggests a possible allosteric  
303 mechanism of resistance. This mutation might affect the ability of the RBD to adopt the ‘up’  
304 conformation necessary for engagement of the cellular receptors perhaps by shielding the epitope  
305 or stabilizing the RBD in the ‘down’ conformation. Further structural and functional work is  
306 required to define how different mutations promote antibody resistance and determine the  
307 mechanisms by which specific antibodies inhibit SARS-CoV-2 infection.

308         The relatively low genetic barrier to resistance combined with knowledge of the presence  
309 of relevant substitutions in clinical isolates suggests that effective mAb therapy likely will require  
310 a combination of at least two neutralizing antibodies (Baum et al., 2020; Du et al., 2020; Greaney  
311 et al., 2020; Group et al., 2020; Li et al., 2020; Weisblum et al., 2020). Profiling whether different  
312 residues are associated with resistance to specific antibodies could facilitate the selection of  
313 combinations based on their non-overlapping resistance mutations. Although we isolated several  
314 escape mutants that exhibit cross-resistance to multiple antibodies, other antibodies are  
315 associated with unique and non-overlapping resistance. Resistance to such combinations could  
316 still arise through sequential escape whereby a resistant variant to one antibody acquires  
317 resistance to a second. Sequential escape could be favored *in vivo* for two antibodies with  
318 different half-lives, or when a pre-existing resistant variant to one antibody already is circulating.  
319 Indeed, while we could not generate escape mutants to the antibody cocktail of 2B04 and 2H04,  
320 we readily isolated escape mutants to both mAbs through sequential selection.

321 Substitution S477N, the fourth most abundant S protein sequence variant in circulating  
322 human isolates of SARS-CoV-2, led to a degree of resistance to all of the mAbs we profiled,  
323 including 2B04 and 2H04. How S477N could confer such broad resistance is of interest, given its  
324 penetrance among clinical isolates (6.5%). One possible explanation may relate to changes in  
325 glycosylation at this position. Additional analysis is required to determine how broad the  
326 resistance associated with S477N is, and to probe the mechanism by which it occurs. The broad  
327 mAb resistance observed here for S477N was not accompanied by resistance to neutralization  
328 by human convalescent sera, suggesting that other epitopes – such as those centered around  
329 E484 are more dominant in humans. Among our panel of mutants, we isolated a total of 14  
330 substitutions at sites of glycosylation, including 8 N-linked glycans site: T345N, K444N, S477N,  
331 L441R, L517R, L452R, S477R, K444R; and 6 O-linked glycans: F486S, T345S, F490S, P479S;  
332 F486Y, N450Y.

333 Substitutions at position E484 were associated with relative resistance to neutralization by  
334 several convalescent human sera. Four variants at this position (E484A, E484D, E484G and  
335 E484K) exhibited resistance to each of the human convalescent sera we tested. This suggests  
336 that in some humans, neutralizing antibodies may be directed toward a narrow repertoire of  
337 epitopes following natural infection. Substitution at position E484 has become increasingly  
338 common among clinical isolates. As of October 2020, just 0.03% of sequenced isolates exhibited  
339 variation at E484, which led us to suspect that variation at this position may come with an apparent  
340 fitness cost for viral replication. However, by January of 2021, the prevalence of substitutions at  
341 this position had increased to 0.09%. Substitution E484K is likely to increase in penetrance further  
342 as it linked together with N501Y and K417N changes that are present in variant 501.V2, which is  
343 believed to be more transmissible (Tegally et al., 2020). The relative resistance of the  
344 substitutions at E484 to the human sera tested highlight how variants at even a single position  
345 can affect neutralization. Given the apparent limited breadth of the human neutralizing antibody  
346 response to natural infection, it will be important to define the epitope repertoire following

347 vaccination and develop strategies that broaden neutralizing antibody responses. In this regard,  
348 the 50 viral mutants described here, combined with additional mutants reported in related studies  
349 (Greaney et al., 2020; Group et al., 2020; Li et al., 2020; Weisblum et al., 2020), provide a  
350 compendium of functionally relevant S protein variants that could be used to profile sera from  
351 vaccine recipients in existing clinical trials.

352         Among the escape variants we selected, there were several that altered susceptibility to  
353 neutralization by soluble ACE2. Substitution F486S was particularly notable, as we were unable  
354 to attain 50% neutralization at the highest concentrations of soluble hACE2 tested (>20 µg/ml).  
355 The finding of an antibody escape mutant mapping to a critical residue within the ACE2 binding  
356 site raises questions regarding possible receptor usage by viruses containing S proteins with  
357 F486S. Future studies that introduce F486S into an infectious cDNA clone of SARS-CoV-2 are  
358 needed to determine the significance of this change to hACE2 interactions *in vivo*. The escape  
359 variants we selected were also examined for their sensitivity to neutralization by soluble mACE2.  
360 For the wild type S sequence and some escape mutants (*e.g.*, L441R, K444N, L452R, and  
361 S477N), we observed a modest increase in infectivity at increasing concentration of soluble  
362 mACE2. Further studies using the infectious molecular clone of SARS-CoV-2 will be required to  
363 discern the significance of this observation.

364         We did not directly address the fitness of the mutants generated in this study, and any  
365 studies of the fitness of the VSV-SARS-CoV-2 variants would pertain to their relative replicative  
366 fitness measured in cell culture (Domingo et al., 2012). We can, however, make several  
367 inferences about fitness of specific viral mutants based on the prevalence of the corresponding  
368 mutants among circulating human isolates of SARS-CoV-2. Over 60% of the mutants we isolated  
369 in this study already circulate as natural viral variants. The escape mutants we isolated were  
370 based on single nucleotide changes starting from the sequence of the S protein of the SARS-  
371 CoV-2 Wuhan-Hu-1 strain (Wu et al., 2020a). In the context of VSV-SARS-CoV-2, the fitness of  
372 the variants in cell culture relates to their ability to resist neutralization by the indicated mAb and

373 infect Vero cells presumably through interactions with ACE2. Our functional screens complement  
374 other systematic mutational analyses of the amino acid residues of the RBD of the SARS-CoV-2  
375 S, such as those based on yeast display (Starr et al., 2020).

### 376 **Limitations of this study**

377           Use of chimeric VSV that depends on SARS-CoV-2 S protein for entry into cells enabled  
378 the selection of 50 escape mutants. Although chimeric VSV serves as an effective mimic of SARS-  
379 CoV-2 S protein-mediated entry and viral neutralization, sequence analysis of circulating human  
380 isolates revealed that 34 of those escape mutants are present in the context of infectious SARS-  
381 CoV-2. The remaining 16 variants may represent S sequences with compromised fitness in the  
382 background of SARS-CoV-2 highlighting one potential limitation of our work. Additional limitations  
383 of our study are the relatively limited number of polyclonal human sera that we profiled against  
384 the panel of escape mutants. Additional human sera samples at lower dilutions may help  
385 determine the extent to which serum-based neutralization of virus is affected by individual or  
386 combinations of escape mutants.

387

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391 Agency (HR001117S0019) and gifts from Washington University in Saint Louis.

392

393 **AUTHOR CONTRIBUTIONS**

394 Z.L designed and performed the experiments. L.A.V generated and validated all hybridoma-  
395 produced mAbs. P.W.R., L.M.B., R.E.C., S.S., B. A., provided experimental assistance. M.J.L and  
396 W.J.B set up the high-throughput assay, H.Z. and D.H.F. generated and provided purified ACE2  
397 proteins, J.M.E mapped escape mutant. E.S.T identified and provided the human immune serum.  
398 A.H.E, generated and provided cloned versions of mAbs. Z.L., M.S.D., and S.P.J.W. analyzed  
399 data. Z.L., L.A.V., M.S.D and S.P.J.W. wrote the initial draft, with the other authors providing  
400 editing comments.

401

402 **COMPETING FINANCIAL INTERESTS**

403 M.S.D. is a consultant for Inbios, Vir Biotechnology, NGM Biopharmaceuticals, and on the  
404 Scientific Advisory Board of Moderna and Immunome. The Diamond laboratory has received  
405 unrelated funding support in sponsored research agreements from Moderna, Vir Biotechnology,  
406 and Emergent BioSolutions. The Ellebedy laboratory has received unrelated funding support in  
407 sponsored research agreements from Emergent BioSolutions and funding support in sponsored  
408 research agreement from AbbVie to further develop 2B04 and 2H04 as therapeutic mAbs. A.H.E.  
409 and Washington University have filed a patent application that includes the SARS-CoV-2  
410 antibodies 2B04 and 2H04 for potential commercial development. S.P.J.W. and Z.H.L have filed  
411 a disclosure with Washington University for VSV-SARS-CoV-2 mutants to characterize antibody  
412 panels. S.P.J.W. and Washington University have filed a patent application on VSV-SARS-CoV-  
413 2. S.P.J.W has received unrelated funding support in sponsored research agreements with Vir

414 Biotechnology, AbbVie and sAB therapeutics.

415 **FIGURE LEGENDS**

416 **Figure 1. VSV-SARS-CoV-2 escape mutant isolation.** (A) Outline of escape mutant  
417 selection experiment. 2B04 and a control anti-influenza virus mAb were tested for neutralizing  
418 activity against VSV-SARS-CoV-2. The concentration of 2B04 added in the overlay completely  
419 inhibited viral infection (middle panel). Data are representative of two independent experiments.  
420 Plaque assays were performed to isolate the VSV-SARS-CoV-2 escape mutant on Vero E6  
421 TMPRSS2 cells (red arrow indicated). Plaque assays with 2B04 in the overlay (*Bottom plaque in*  
422 *the right panel*); plaque assays without Ab in the overlay (*Top plaque in the right panel*). Data are  
423 representative of three independent experiments. (B) Schematic of S gene, which underwent  
424 Sanger sequencing to identify mutations (*left panel*). For validation, each VSV-SARS-CoV-2  
425 mutant was tested in plaque assays with or without 2B04 in the overlay on Vero cells (*right panel*).  
426 Representative images of two independent experiments are shown.

427 **Figure 2. Mapping of escape mutations.** The surface model of RBD (from PDB 6M0J)  
428 is depicted, and contact residues of the SARS-CoV-2 RBD-hACE2 interfaces are colored in brown.  
429 Amino acids whose substitution confers resistance to each mAb in plaque assays are indicated  
430 for 2B04 (green), 2H04 (lemon), 1B07 (blue), SARS2-01 (yellow), SARS2-02 (teal), SARS2-07  
431 (tangerine), SARS2-16 (violet), SARS2-19 (red), SARS2-32 (fuschia), and SARS2-38 (magenta).  
432 See **Figure S1 and S2**.

433 **Figure 3. Map of cross-neutralizing activity of VSV-SARS-CoV-2 mutants and**  
434 **neutralization potency of hACE2 decoy receptors against each VSV-SARS-CoV-2 mutant.**  
435 (A) Neutralization of VSV-SARS-CoV-2 mutants was evaluated by plaque assays. Degree of  
436 resistance was defined as percentage by expressing the number of plaques formed by each  
437 mutant in the presence or absence of antibody versus and is represented as a heatmap from  
438 white (low degree of resistance) to red (high degree of resistance). Representative images of two  
439 independent experiments are shown in **Figure S3**. (B) Neutralization assay of VSV-SARS-CoV-  
440 2 mutants in the presence of hACE2-Fc. Virus was incubated with mACE2 or hACE2 at



441 concentrations ranging from 9 ng/ml to 20  $\mu$ g/ml for 1 h at 37°C and cells were scored for infection  
442 at 7.5 h post inoculation by automated microscopy. IC<sub>50</sub> values were calculated for each virus-  
443 hACE2 combination from three independent experiments. (\*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ ,  
444 \*\*\*\*  $P < 0.0001$ ; one-way ANOVA with Dunnett's post-test; error bars indicate standard error of  
445 the mean [SEM]). (C) Representative neutralization curves of wild-type and F486S mutant VSV-  
446 SARS-CoV-2 with hACE2-Fc and mACE2-Fc. Error bars represent the SEM. Data are  
447 representative of three independent experiments. Neutralization curves are provided in **Figure**  
448 **S4**.

449 **Figure 4. Neutralization potency of human serum against each VSV-SARS-CoV-2**  
450 **mutant.** (A) Neutralization potency of four human sera against VSV-SARS-CoV-2 mutants. IC<sub>50</sub>  
451 values were calculated from three independent experiments. Neutralization potency is  
452 represented as a rainbow color map from red (most potent with low IC<sub>50</sub>) to violet (less potent with  
453 high IC<sub>50</sub>). LOD indicates limit of detection (1:80) (B) Representative neutralization curves of wild-  
454 type, S477N and E484A mutant with four different human sera. Error bars represent the SEM.  
455 Data are representative of three independent experiments. (C) Neutralization potency of  
456 additional 16 human sera against VSV-SARS-CoV-2 mutants. IC<sub>50</sub> values were calculated from  
457 one independent experiment each. Neutralization potency is represented as a rainbow color map  
458 from red (most potent with low IC<sub>50</sub>) to violet (less potent with high IC<sub>50</sub>). Neutralization curves are  
459 provided in **Figure S5**.

460 **Figure 5. Mapping of additional VSV-SARS-CoV-2 escape mutants.** The surface  
461 model of RBD (from PDB 6M0J) is depicted, and contact residues of the SARS-CoV-2 RBD-  
462 hACE2 interfaces are colored in brown. Amino acids whose substitution confers resistance to  
463 each mAb in the plaque assays are indicated for SARS2-21 (lime), SARS2-22 (green), SARS2-  
464 23 (blue), SARS2-31 (yellow), SARS2-34 (cyan), SARS2-55 (orange), SARS2-58 (magenta),  
465 SARS2-66 (red), and SARS2-71 (pink). See **Figure S6 and S7**.

466 **Figure 6. Position and frequency of RBD amino acid substitutions in SARS-CoV-2.**

467 (A) RBD amino acid substitutions in currently circulating SARS-CoV-2 viruses isolated from  
468 humans. For each site of escape, we counted the sequences in GISAID with an amino acid  
469 change (323,183 total sequences at the time of the analysis). Variant circulating frequency is  
470 represented as a rainbow color map from red (less circulating with low frequency) to violet (most  
471 circulating with high frequency). A black cell indicates the variant has not yet been isolated from  
472 a patient. A rainbow cell with cross indicates the variant has been isolated from a patient, but not  
473 appear in those 48 escape mutants. (B) Location of natural sequence variation within the RBD.  
474 The RBD is modeled as a surface representation, Variant frequency is rainbow colored as in (A).  
475 Black coloration indicates variation at that residue has not yet been isolated.

476 **Figure 7. Sequential selection of 2B04 and 2H04 escape mutants.** (A) Plaque assays  
477 were performed to isolate the VSV-SARS-CoV-2-S wild-type, E484A, E484K, and F486S escape  
478 mutant on Vero E6 TMPRSS2 cells in the present of the indicated mAb in the overlay.  
479 Representative images of three independent experiments are shown. (B) The surface model of  
480 RBD (from PDB 6M0J) is depicted, and contact residues of the SARS-CoV-2 RBD-hACE2  
481 interfaces are colored in brown. 2B04 escape mutants including E484A, E484K, and F486S are  
482 indicated in green. Amino acids whose substitution confers resistance to 2H04 in the plaque  
483 assays are indicated in lemon. (C) Wild-type and sequentially-identified double mutants were  
484 tested for neutralizing activity using a plaque assay with the indicated mAb in the overlay. MAb  
485 concentrations added were the same as those used to select the escape mutants. Representative  
486 images of two independent experiments are shown.

487 **SUPPLEMENTARY FIGURE LEGENDS**

488 **Figure S1. Isolation of VSV-SARS-CoV-2 escape mutants by plaque assay. Related**  
489 **to Fig 2. (A)** RBD-specific antibodies were tested for neutralizing activity against VSV-SARS-  
490 CoV-2. MAbs in the left panel were purified from Expi293F cells transfected with antibody  
491 expression vector (pABVec6W) expressing heavy chain V-D-J and light Chain V-J cloned from  
492 single B cells. MAbs in the right panel were from hybridomas that bound to SARS-CoV-2-infected  
493 Vero CCL81 cells by flow cytometry. Data are representative of two independent experiments. **(B)**  
494 Plaque assays were performed to isolate the VSV-SARS-CoV-2-S escape mutant on Vero E6  
495 TMPRSS2 cells in the presence of the indicated mAb in the overlay. Representative images of two  
496 independent experiments are shown.

497 **Figure S2. Validation of selected VSV-SARS-CoV-2 mutants. Related to Fig 2.** Plaque  
498 assays were performed to validate the VSV-SARS-CoV-2 mutant on Vero cells in the presence  
499 and absence of the mAb in the overlay. MAb concentrations added in the overlay were the same  
500 as those used to select the escape mutants. Representative images of two independent  
501 experiments are shown.

502 **Figure S3. Plaque assay validation of cross-neutralization of VSV-SARS-CoV-2**  
503 **mutants. Related to Fig 3A.** Wild-type and identified VSV-SARS-CoV-2 mutants were tested for  
504 neutralizing activity using a plaque assay with the indicated mAb in the overlay. MAb  
505 concentrations added were the same as those used to select the escape mutants. Representative  
506 images of two independent experiments are shown.

507 **Figure S4. Neutralization of VSV-SARS-CoV-2 mutants by hACE2 decoy receptors.**  
508 **Related to Fig 3B and 3C.** hACE2-Fc or mACE2-Fc were tested for neutralizing activity against  
509 wild-type and mutant VSV-SARS-CoV-2 (n=3). Error bars represent the SEM. Data are  
510 representative of three independent experiments.

511 **Figure S5. Neutralization of VSV-SARS-CoV-2 mutants by human sera. Related to**  
512 **Fig 4. (A)** Four human sera were tested for neutralization of wild-type and mutant VSV-SARS-

513 CoV-2 (n = 3). Error bars represent the SEM. Data are representative of three independent  
514 experiments. Related to **Fig 4A and 4B**. **(B)** Sixteen human sera were tested for neutralization of  
515 wild-type and 5 mutant VSV-SARS-CoV-2 (n = 1). Related to **Fig 4C**.

516 **Figure S6. A second neutralization escape selection campaign with nine additional**  
517 **mAbs. Related to Fig 5.** **(A)** Nine additional RBD-specific antibodies were tested for  
518 neutralization activity against VSV-SARS-CoV-2. Data are representative of two independent  
519 experiments. **(B)** Plaque assays were performed to isolate the VSV-SARS-CoV-2 escape mutant  
520 on Vero E6 TMPRSS2 cells in the presence of the indicated mAb in the overlay. Representative  
521 images of six independent experiments are shown.

522 **Figure S7. Validation of selected VSV-SARS-CoV-2 mutants. Related to Fig 5.** Plaque  
523 assays were performed to validate the VSV-SARS-CoV-2 mutant on Vero cells in the presence  
524 and absence of mAb in the overlay. MAb concentration added in the overlay were the same as  
525 those used to select the escape mutants. Representative images of two independent experiments  
526 are shown.

527

528 **STAR METHODS**

529 **RESOURCE AVAILABILITY**

530 **Lead Contact.** Further information and requests for resources and reagents should be  
531 directed to and will be fulfilled by the Lead Contact, Sean P. J. Whelan ([spjwhelan@wustl.edu](mailto:spjwhelan@wustl.edu)).

532 **Materials Availability.** All requests for resources and reagents should be directed to and  
533 will be fulfilled by the Lead Contact author. This includes antibodies, hybridomas, viruses, and  
534 other proteins. All reagents will be made available on request after completion of a Materials  
535 Transfer Agreement.

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

538

539 **EXPERIMENTAL MODEL AND SUBJECT DETAILS**

540 **Cells.** Cells were cultured in humidified incubators at 34° or 37°C and 5% CO<sub>2</sub> in the  
541 indicated media. Vero CCL81, Vero E6 and Vero E6-TMPRSS2 were maintained in DMEM  
542 (Corning or VWR) supplemented with glucose, L-glutamine, sodium pyruvate, and 10% fetal  
543 bovine serum (FBS). MA104 cells were propagated in Medium 199 (Gibco) containing 10% FBS.  
544 Vero E6-TMPRSS2 cells were generated using a lentivirus vector described as previously (Case  
545 et al., 2020).

546 **VSV-SARS-CoV-2 mutants.** Plaque assays were performed to isolate the VSV-SARS-  
547 CoV-2 escape mutant on Vero E6-TMPRSS2 cells with the indicated mAb in the overlay. The  
548 concentration of mAb in the overlay was determined by neutralization assays at a multiplicity of  
549 infection (MOI) of 100. Escape clones were plaque-purified on Vero-E6 TMPRSS2 cells in the  
550 presence of mAb, and plaques in agarose plugs were amplified on MA104 cells with the mAb  
551 present in the medium. Viral stocks were amplified on MA104 cells at an MOI of 0.01 in Medium  
552 199 containing 2% FBS and 20 mM HEPES pH 7.7 (Millipore Sigma) at 34°C. Viral supernatants

553 were harvested upon extensive cytopathic effect and clarified of cell debris by centrifugation at  
554 1,000 x g for 5 min. Aliquots were maintained at -80°C.

555 **Mouse experiments.** Animal studies were carried out in accordance with the  
556 recommendations in the Guide for the Care and Use of Laboratory Animals of the National  
557 Institutes of Health. The protocols were approved by the Institutional Animal Care and Use  
558 Committee at the Washington University School of Medicine (Assurance number A3381-01).  
559 Virus inoculations were performed under anesthesia that was induced and maintained with  
560 ketamine hydrochloride and xylazine, and all efforts were made to minimize animal suffering.  
561 Female BALB/c mice (catalog 000651) were purchased from The Jackson Laboratory.

562

## 563 **METHOD DETAILS**

564 **Sequencing of the S gene.** Viral RNA was extracted from VSV-SARS-CoV-2 mutant  
565 viruses using RNeasy Mini kit (Qiagen), and S was amplified using OneStep RT-PCR Kit (Qiagen).  
566 The mutations were identified by Sanger sequencing (GENEWIZ).

567 **Plaque assays.** Plaque assays were performed on Vero and Vero E6-TMPRSS2 cells.  
568 Briefly, cells were seeded into 6 or 12 well plates for overnight. Virus was serially diluted using  
569 DMEM and cells were infected at 37°C for 1 h. Cells were cultured with an agarose overlay in the  
570 presence of Ab or absence of Ab at 34°C for 2 days. Plates were scanned on a biomolecular  
571 imager and expression of eGFP is show at 48 hours post-infection.

572 **Protein expression and purification.** Soluble hACE2-Fc and mACE2-Fc were  
573 generated and purified as described as previously (Case et al., 2020).

574 **Monoclonal antibodies.** mAbs 2B04, 1B07 and 2H04 were described previously  
575 (Alsoussi et al., 2020). Other mAbs (SARS2-01, SARS2-02, SARS2-07, SARS2-16, SARS2-19,  
576 SARS2-21, SARS2-22, SARS2-23, SARS2-31, SARS2-32, SARS2-34, SARS2-38, SARS2-55,  
577 SARS2-58, SARS2-66 and SARS2-71) were generated as follows. BALB/c mice were immunized  
578 and boosted twice (two and four weeks later) with 5-10 µg of RBD and S protein (twice)

579 sequentially, each adjuvanted with 50% AddaVax and given via an intramuscular route. Mice  
580 received a final, non-adjuvanted boost of 25 µg of SARS-CoV-2 S or RBD (25 µg split via  
581 intravenous and interperitoneal routes) 3 days prior to fusion of splenocytes with P3X63.Ag.6.5.3  
582 myeloma cells. Hybridomas producing antibodies were screened by ELISA with S protein, flow  
583 cytometry using SARS-CoV-2 infected cells, and single endpoint neutralization assays.

584 **Human immune sera.** The human sera samples 11, 13, 15, 16, 18, 21, 23, 27, 28, 29,  
585 30, 31, 32, 34, 35, 37, 38, 39 used in this study were previously reported (Case et al., 2020),  
586 Human donor samples were collected from PCR-confirmed COVID-19 patients. Sera samples  
587 were obtained by routine phlebotomy (Case et al., 2020). This study was approved by the Mayo  
588 Clinic Institutional Review Board.

589 **Neutralization assays using a recombinant VSV-SARS-CoV-2.** Briefly, serial dilutions  
590 of sera beginning with a 1:80 initial dilution were three-fold serially diluted in 96-well plate over  
591 eight dilutions. Indicated dilutions of human serum were incubated with 10<sup>2</sup> PFU of VSV-SARS-  
592 CoV-2 for 1 h at 37 °C. Human serum-virus complexes then were added to Vero E6 cells in 96-  
593 well plates and incubated at 37 °C for 7.5 h. Cells were fixed at room temperature in 2%  
594 formaldehyde containing 10 µg/mL of Hoechst 33342 nuclear stain for 45 min. Fixative was  
595 replaced with PBS prior to imaging. Images were acquired using an In Cell 2000 Analyzer  
596 automated microscope (GE Healthcare) in both the DAPI and FITC channels to visualize nuclei  
597 and infected cells (×4 objective, 4 fields per well). Images were analyzed using the Multi Target  
598 Analysis Module of the In Cell Analyzer 1000 Workstation Software (GE Healthcare). GFP-  
599 positive cells were identified using the top hat segmentation method and counted within the InCell  
600 Workstation software. ACE2 neutralization assays using VSV-SARS-CoV-2 were conducted  
601 similarly. The initial dilution started at 20 µg/mL and was three-fold serially diluted in 96-well plates  
602 over eight dilutions. mAb neutralization assays using VSV-SARS-CoV-2 were conducted similarly  
603 but using an MOI of 100.

604           **High-throughput assay using a recombinant VSV-SARS-CoV-2.** Serial dilutions of  
605 patient sera beginning with a 1:10 initial dilution were performed in 384-well plates and were  
606 incubated with  $10^4$  PFU of VSV-SARS-CoV-2 for 1 h at 37°C. Vero E6 cells then were added to  
607 the human serum-virus complexes in 384-well plates at  $3 \times 10^3$  cells per well and incubated at  
608 37°C for 16 h. Cells were fixed at room temperature in 4% formaldehyde and then rinsed with  
609 PBS. Cells were stained at room temperature with NucRed Live 647 (Invitrogen) for 30 min.  
610 Images were acquired using an InCell 6500 confocal imager (Cytiva) to visualize nuclei and  
611 infected cells (4X objective, 1 field per well). Images were segmented using InCarta (Cytiva).  
612 Virally-infected cells were identified by comparing to the uninfected threshold in Spotfire (Tibco).  
613 Cells were also quality-controlled (gated) based on nuclear parameters.

614

615

## 616   **QUANTIFICATION AND STATISTICAL ANALYSIS**

617           All statistical tests were performed as described in the indicated figure legends. Non-linear  
618 regression (curve fit) was performed to calculate  $IC_{50}$  values for **Fig 3C, 4B, S4, and S5A** using  
619 Prism 8.0 (GraphPad). Non-linear regression (curve fit) was performed for **Fig 1A, S1A, and S6A**  
620 using Prism 8.0. Non-linear regression (curve fit) was performed to calculate  $IC_{50}$  values for **Fig**  
621 **S5B** using Spotfire (Tibco) after adding additional baseline and plateau points. Statistical  
622 significance in data **Fig 3B** was calculated by one-way ANOVA with Dunnett's post-test using  
623 Prism 8.0. The number of independent experiments used are indicated in the relevant Figure  
624 legends.

625



626 **REFERENCES**

- 627 Alsoussi, W.B., Turner, J.S., Case, J.B., Zhao, H., Schmitz, A.J., Zhou, J.Q., Chen, R.E., Lei, T.,  
628 Rizk, A.A., McIntire, K.M., *et al.* (2020). A Potently Neutralizing Antibody Protects Mice against  
629 SARS-CoV-2 Infection. *J Immunol* 205, 915-922.
- 630 Baum, A., Fulton, B.O., Wloga, E., Copin, R., Pascal, K.E., Russo, V., Giordano, S., Lanza, K.,  
631 Negron, N., Ni, M., *et al.* (2020). Antibody cocktail to SARS-CoV-2 spike protein prevents rapid  
632 mutational escape seen with individual antibodies. *Science* 369, 1014-1018.
- 633 Brouwer, P.J.M., Caniels, T.G., van der Straten, K., Snitselaar, J.L., Aldon, Y., Bangaru, S.,  
634 Torres, J.L., Okba, N.M.A., Claireaux, M., Kerster, G., *et al.* (2020). Potent neutralizing antibodies  
635 from COVID-19 patients define multiple targets of vulnerability. *Science* 369, 643-650.
- 636 Case, J.B., Rothlauf, P.W., Chen, R.E., Liu, Z., Zhao, H., Kim, A.S., Bloyet, L.M., Zeng, Q., Tahan,  
637 S., Droit, L., *et al.* (2020). Neutralizing Antibody and Soluble ACE2 Inhibition of a Replication-  
638 Competent VSV-SARS-CoV-2 and a Clinical Isolate of SARS-CoV-2. *Cell Host Microbe*.
- 639 Chan, K.K., Dorosky, D., Sharma, P., Abbasi, S.A., Dye, J.M., Kranz, D.M., Herbert, A.S., and  
640 Procko, E. (2020). Engineering human ACE2 to optimize binding to the spike protein of SARS  
641 coronavirus 2. *Science* 369, 1261-1265.
- 642 Chen, P., Nirula, A., Heller, B., Gottlieb, R.L., Boscia, J., Morris, J., Huhn, G., Cardona, J.,  
643 Mocherla, B., Stosor, V., *et al.* (2020). SARS-CoV-2 Neutralizing Antibody LY-CoV555 in  
644 Outpatients with Covid-19. *N Engl J Med*.
- 645 CoV-GLUE (2021). <http://cov-glue.cvr.gla.ac.uk/#/home>.
- 646 Dinno, K.H., 3rd, Leist, S.R., Schafer, A., Edwards, C.E., Martinez, D.R., Montgomery, S.A.,  
647 West, A., Yount, B.L., Jr., Hou, Y.J., Adams, L.E., *et al.* (2020). A mouse-adapted model of SARS-  
648 CoV-2 to test COVID-19 countermeasures. *Nature* 586, 560-566.
- 649 Dolan, P.T., Whitfield, Z.J., and Andino, R. (2018). Mapping the Evolutionary Potential of RNA  
650 Viruses. *Cell Host Microbe* 23, 435-446.

651 Domingo, E., Sheldon, J., and Perales, C. (2012). Viral quasispecies evolution. *Microbiol Mol Biol*  
652 *Rev* 76, 159-216.

653 Du, S., Cao, Y., Zhu, Q., Yu, P., Qi, F., Wang, G., Du, X., Bao, L., Deng, W., Zhu, H., *et al.* (2020).  
654 Structurally Resolved SARS-CoV-2 Antibody Shows High Efficacy in Severely Infected Hamsters  
655 and Provides a Potent Cocktail Pairing Strategy. *Cell* 183, 1013-1023 e1013.

656 GISAID (2021). <https://www.gisaid.org/>.

657 Greaney, A.J., Starr, T.N., Gilchuk, P., Zost, S.J., Binshtein, E., Loes, A.N., Hilton, S.K.,  
658 Huddleston, J., Eguia, R., Crawford, K.H.D., *et al.* (2020). Complete Mapping of Mutations to the  
659 SARS-CoV-2 Spike Receptor-Binding Domain that Escape Antibody Recognition. *Cell Host*  
660 *Microbe*.

661 Group, A.-T.L.-C.S., Lundgren, J.D., Grund, B., Barkauskas, C.E., Holland, T.L., Gottlieb, R.L.,  
662 Sandkovsky, U., Brown, S.M., Knowlton, K.U., Self, W.H., *et al.* (2020). A Neutralizing Monoclonal  
663 Antibody for Hospitalized Patients with Covid-19. *N Engl J Med*.

664 Gu, H., Chen, Q., Yang, G., He, L., Fan, H., Deng, Y.Q., Wang, Y., Teng, Y., Zhao, Z., Cui, Y., *et*  
665 *al.* (2020). Adaptation of SARS-CoV-2 in BALB/c mice for testing vaccine efficacy. *Science* 369,  
666 1603-1607.

667 Halfmann, P.J., Hatta, M., Chiba, S., Maemura, T., Fan, S., Takeda, M., Kinoshita, N., Hattori,  
668 S.I., Sakai-Tagawa, Y., Iwatsuki-Horimoto, K., *et al.* (2020). Transmission of SARS-CoV-2 in  
669 Domestic Cats. *N Engl J Med* 383, 592-594.

670 Hoffmann, M., Kleine-Weber, H., Schroeder, S., Kruger, N., Herrler, T., Erichsen, S., Schiergens,  
671 T.S., Herrler, G., Wu, N.H., Nitsche, A., *et al.* (2020). SARS-CoV-2 Cell Entry Depends on ACE2  
672 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. *Cell* 181, 271-280 e278.

673 Ke, Z., Oton, J., Qu, K., Cortese, M., Zila, V., McKeane, L., Nakane, T., Zivanov, J., Neufeldt,  
674 C.J., Cerikan, B., *et al.* (2020). Structures and distributions of SARS-CoV-2 spike proteins on  
675 intact virions. *Nature*.

676 Lan, J., Ge, J., Yu, J., Shan, S., Zhou, H., Fan, S., Zhang, Q., Shi, X., Wang, Q., Zhang, L., *et al.*  
677 (2020). Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor.  
678 *Nature* 581, 215-220.

679 Letko, M., Marzi, A., and Munster, V. (2020). Functional assessment of cell entry and receptor  
680 usage for SARS-CoV-2 and other lineage B betacoronaviruses. *Nat Microbiol* 5, 562-569.

681 Li, Q., Wu, J., Nie, J., Zhang, L., Hao, H., Liu, S., Zhao, C., Zhang, Q., Liu, H., Nie, L., *et al.*  
682 (2020). The Impact of Mutations in SARS-CoV-2 Spike on Viral Infectivity and Antigenicity. *Cell*  
683 182, 1284-1294 e1289.

684 Monteil, V., Kwon, H., Prado, P., Hagelkruys, A., Wimmer, R.A., Stahl, M., Leopoldi, A., Garreta,  
685 E., Hurtado Del Pozo, C., Prosper, F., *et al.* (2020). Inhibition of SARS-CoV-2 Infections in  
686 Engineered Human Tissues Using Clinical-Grade Soluble Human ACE2. *Cell* 181, 905-913 e907.

687 Oude Munnink, B.B., Sikkema, R.S., Nieuwenhuijse, D.F., Molenaar, R.J., Munger, E.,  
688 Molenkamp, R., van der Spek, A., Tolsma, P., Rietveld, A., Brouwer, M., *et al.* (2020).  
689 Transmission of SARS-CoV-2 on mink farms between humans and mink and back to humans.  
690 *Science*.

691 Rogers, T.F., Zhao, F., Huang, D., Beutler, N., Burns, A., He, W.T., Limbo, O., Smith, C., Song,  
692 G., Woehl, J., *et al.* (2020). Isolation of potent SARS-CoV-2 neutralizing antibodies and protection  
693 from disease in a small animal model. *Science* 369, 956-963.

694 Sanjuan, R., Nebot, M.R., Chirico, N., Mansky, L.M., and Belshaw, R. (2010). Viral mutation rates.  
695 *J Virol* 84, 9733-9748.

696 Shang, J., Ye, G., Shi, K., Wan, Y., Luo, C., Aihara, H., Geng, Q., Auerbach, A., and Li, F. (2020).  
697 Structural basis of receptor recognition by SARS-CoV-2. *Nature* 581, 221-224.

698 Shi, J., Wen, Z., Zhong, G., Yang, H., Wang, C., Huang, B., Liu, R., He, X., Shuai, L., Sun, Z., *et*  
699 *al.* (2020). Susceptibility of ferrets, cats, dogs, and other domesticated animals to SARS-  
700 coronavirus 2. *Science* 368, 1016-1020.

701 Smith, E.C., Blanc, H., Surdel, M.C., Vignuzzi, M., and Denison, M.R. (2013). Coronaviruses  
702 lacking exoribonuclease activity are susceptible to lethal mutagenesis: evidence for proofreading  
703 and potential therapeutics. *PLoS Pathog* 9, e1003565.

704 Smith, E.C., and Denison, M.R. (2013). Coronaviruses as DNA wannabes: a new model for the  
705 regulation of RNA virus replication fidelity. *PLoS Pathog* 9, e1003760.

706 Stadlbauer, D., Zhu, X., McMahon, M., Turner, J.S., Wohlbold, T.J., Schmitz, A.J., Strohmeier,  
707 S., Yu, W., Nachbagauer, R., Mudd, P.A., *et al.* (2019). Broadly protective human antibodies that  
708 target the active site of influenza virus neuraminidase. *Science* 366, 499-504.

709 Starr, T.N., Greaney, A.J., Hilton, S.K., Ellis, D., Crawford, K.H.D., Dingens, A.S., Navarro, M.J.,  
710 Bowen, J.E., Tortorici, M.A., Walls, A.C., *et al.* (2020). Deep Mutational Scanning of SARS-CoV-  
711 2 Receptor Binding Domain Reveals Constraints on Folding and ACE2 Binding. *Cell* 182, 1295-  
712 1310 e1220.

713 Tegally, H., Wilkinson, E., Giovanetti, M., Iranzadeh, A., Fonseca, V., Giandhari, J., Doolabh, D.,  
714 Pillay, S., James San, E., Msomi, N., *et al.* (2020). Emergence and rapid spread of a new severe  
715 acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2) lineage with multiple spike  
716 mutations in South Africa. *medRxiv*.

717 Weinreich, D.M., Sivapalasingam, S., Norton, T., Ali, S., Gao, H., Bhore, R., Musser, B.J., Soo,  
718 Y., Rofail, D., Im, J., *et al.* (2020). REGN-COV2, a Neutralizing Antibody Cocktail, in Outpatients  
719 with Covid-19. *N Engl J Med*.

720 Weisblum, Y., Schmidt, F., Zhang, F., DaSilva, J., Poston, D., Lorenzi, J.C., Muecksch, F.,  
721 Rutkowska, M., Hoffmann, H.H., Michailidis, E., *et al.* (2020). Escape from neutralizing antibodies  
722 by SARS-CoV-2 spike protein variants. *Elife* 9.

723 Wrapp, D., Wang, N., Corbett, K.S., Goldsmith, J.A., Hsieh, C.L., Abiona, O., Graham, B.S., and  
724 McLellan, J.S. (2020). Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation.  
725 *Science* 367, 1260-1263.

726 Wu, F., Zhao, S., Yu, B., Chen, Y.M., Wang, W., Song, Z.G., Hu, Y., Tao, Z.W., Tian, J.H., Pei,  
727 Y.Y., *et al.* (2020a). A new coronavirus associated with human respiratory disease in China.  
728 *Nature* 579, 265-269.

729 Wu, Y., Wang, F., Shen, C., Peng, W., Li, D., Zhao, C., Li, Z., Li, S., Bi, Y., Yang, Y., *et al.* (2020b).  
730 A noncompeting pair of human neutralizing antibodies block COVID-19 virus binding to its  
731 receptor ACE2. *Science* 368, 1274-1278.

732 Zang, R., Gomez Castro, M.F., McCune, B.T., Zeng, Q., Rothlauf, P.W., Sonnek, N.M., Liu, Z.,  
733 Brulois, K.F., Wang, X., Greenberg, H.B., *et al.* (2020). TMPRSS2 and TMPRSS4 promote SARS-  
734 CoV-2 infection of human small intestinal enterocytes. *Sci Immunol* 5.

735 Zost, S.J., Gilchuk, P., Case, J.B., Binshtein, E., Chen, R.E., Nkolola, J.P., Schafer, A., Reidy,  
736 J.X., Trivette, A., Nargi, R.S., *et al.* (2020). Potently neutralizing and protective human antibodies  
737 against SARS-CoV-2. *Nature* 584, 443-449.

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## STAR METHODS

### KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
<b>Antibodies</b>		
2B04	(Alsoussi et al., 2020)	PDB: 7K9H
2H04	(Alsoussi et al., 2020)	PDB: 7K9J
1B07	(Alsoussi et al., 2020)	N/A
Anti-influenza NA antibody	(Stadlbauer et al., 2019)	N/A
SARS2 antibody set	This study	N/A
<b>Bacterial and Virus Strains</b>		
VSV-eGFP-SARS-CoV-2	(Case et al., 2020)	N/A
48 Escape mutants set	This study	N/A
<b>Biological Samples</b>		
Patient serum set	(Case et al., 2020)	N/A
<b>Chemicals, Peptides, and Recombinant Proteins</b>		
Formaldehyde Solution	Millipore Sigma	Cat# FX0410-5
HEPES, free acid	Millipore Sigma	Cat# 5310-OP
Hoechst 33342	Invitrogen/Thermo Fisher	Cat# H3570
NucRed Live 647	Invitrogen/Thermo Fisher	Cat# R37106
RNeasy® Mini Kit	QIAGEN	Cat# 74106
OneStep RT-PCR Kit	QIAGEN	Cat# 210212
Gel Extraction Kit	QIAGEN	Cat# 28706
Minimum Essential Medium	Gibco	Cat# 11700-077
L-Glutamine solution	Sigma	Cat# RNBG9716
Agarose I™	VWR Life Science	Cat# 0710-500G
Human ACE2-Fc	(Case et al., 2020)	GenBank: BAB40370.1 & AAC82527.1
Mouse ACE2-Fc	(Case et al., 2020)	NCBI Reference Sequence: NP_001123985.1
<b>Critical Commercial Assays</b>		
Sanger sequencing	Genewiz	N/A
<b>Deposited Data</b>		
Nucleotide sequence of VSV-eGFP-SARS-CoV-2-S <sub>Δ21</sub>	(Case et al., 2020)	BioProject: PRJNA635934; SRA: SRR11878607
<b>Experimental Models: Cell Lines</b>		
MA104	Gift from Harry Greenberg	N/A
Vero CCL81	ATCC	Cat# CCL-81; RRID: CVCL_0059
Vero E6	ATCC	Cat# CRL-1586; RPID: CVCL_0574
Vero E6-TMPRSS2	(Case et al., 2020)	N/A
<b>Experimental Models: Organisms/Strains</b>		
N/A	N/A	N/A
<b>Oligonucleotides</b>		
Reverse Primer 1 GTCTACAGCATCTGTAATGG	This study	N/A
Forward Primer 1 GATTCTTCTTCAGGTTGGACAG	This study	N/A

Reverse Primer 2 GAACAGCAACCTGGTTAGAAG	This study	N/A
Forward Primer 2 CAGAGACATTGCTGACACTAC	This study	N/A
Reverse Primer 3 CACTATTAATTGGTTGGCAATC	This study	N/A
Forward Primer 3 GTACAATCACTTCTGGTTGG	This study	N/A
Forward Primer 4 CACACTTTCCTCGTGAAGG	This study	N/A
Recombinant DNA		
pFM1.2-hACE2-Fc	(Case et al., 2020)	GenBank: AB046569.1
pFM1.2-mACE2-Fc	(Case et al., 2020)	NCBI Reference Sequence: NM_001130513.1
Software and Algorithms		
Image Analyses: GE InCell Analyzer 1000 Workstation	GE Life Sciences (now Cytiva)	N/A
InCell 6500 confocal imager	Cytiva	N/A
IN Carta image analysis software	Cytiva	N/A
Statistics: Prism 8.0	GraphPad	N/A; RRID: SCR_005375
Snapgene	GSL Biotech	N/A; RRID: SCR_015052
Spotfire	Tibco	N/A; PRID: SCR_008858
Other		
N/A	N/A	N/A

**Table 1. Neutralizing mAbs**

mAb	Immunogen <sup>a</sup>	Isotype	Concentration µg/mL	EC50 ng/mL <sup>b</sup>
2B04	RBD, S, S	Human IgG1 <sup>c</sup>	7,630	1.46
2H04	RBD, S, S	Human IgG1 <sup>c</sup>	6,700	279.3
1B07	RBD, S, S	Human IgG1 <sup>c</sup>	5,200	15
SARS2-01	RBD, S, RBD	mouse IgG1	44	86
SARS2-02	RBD, S, RBD	mouse IgG1	40	7
SARS2-07	RBD, S, RBD	mouse IgG1	87	65
SARS2-16	RBD, S, S	mouse IgG1	111	27
SARS2-19	RBD, S, S	mouse IgG1	31	15
SARS2-32	RBD, S, RBD	mouse IgG1	50	23
SARS2-38	RBD, S, S	mouse IgG1	49	8
SARS2-21	RBD, S, RBD	mouse IgA	42	24
SARS2-22	RBD, S, S	mouse IgG1	25	10
SARS2-23	RBD, S, S	mouse IgG1	126	65
SARS2-31	RBD, S, S	mouse IgG1	36	28
SARS2-34	RBD, S, S	mouse IgG1	40	19
SARS2-55	RBD, S, RBD	mouse IgG1	37	10
SARS2-58	RBD, S, S	mouse IgG1	27	6
SARS2-66	RBD, S, S	mouse IgG1	44	12
SARS2-71	RBD, S, S	mouse IgG1	49	11

<sup>a</sup> The order of immunogens used to immunize the mice, as described in the Methods.

<sup>b</sup> Neutralization of SARS-CoV-2 by each mAb was assessed by focus-reduction neutralization test. The half-maximal effective concentration (EC50 value) was determined by nonlinear regression. Results are the geometric mean from three to four independent experiments.

<sup>c</sup> mAb was identified as mouse IgG1 and expressed as human IgG1.



**Table 2. List of mutants**

Campaign 1

mAb	Nucleotide	Amino Acid
2B04	A1451C	E484A
	G1450A	E484K
	T1457C	F486S
2H04	A1033G	T345A
	C1034A	T345N
	A1033T	T345S
	A1036G	R346G
	T1322G	L441R
	A1330G	K444E
	A1033G	T345A
	/T1550G	/L517R
1B07	A1451C	E484A
	A1452C	E484D
	A1451G	E484G
	G1450A	E484K
SARS2-01	T1457A	F486Y
	A1036G	R346G
	C1055A	A352D
	T1355G	L452R
SARS2-02	T1480C	S494P
	G1337A	G446D
	G1337T	G446V
SARS2-07	G1450A	E484K
	A1348G	N450D
	A1429G	S477G
	G1430A	S477N
	C1431G	S477R
SARS2-16	C1496T	P499L
	A1429G	S477G
	G1430A	S477N
SARS2-19	C1431A	S477R
	A1429G	S477G
	G1430A	S477N
	C1433T	T478I
	G1430A	S477N
	/C1541T	/S514F
SARS2-32	G1337A	G446D
	T1350G	N450K
	A1348T	N450Y
	T1355G	L452R
	G1450A	E484K
	T1469C	F490S
SARS2-38	A1330G	K444E
	G1332T	K444N
	G1337A	G446D

Campaign 2

mAb	Nucleotide	Amino Acid
SARS2-21	G1427A	G476D
	G1426A	G476S
	C1433T	T478I
	C1435T	P479S
	T1456C	F486L
SARS2-22	T1457C	F486S
	A1330G	K444E
	G1332T	K444N
	A1331G	K444R
SARS2-23	T1334G	V445G
	G1337A	G446D
	C1431G	S477R
SARS2-31	G1447T	V483F
	T1448G	V483G
	A1452C	E484D
SARS2-34	A1132G	K378E
	A1132C	K378Q
	G1223A	R408K
	G1511A	G504D
SARS2-55	A1421C	Q474P
	G1427A	G476D
	A1429G	S477G
	G1430A	S477N
	A1429C	S477R
SARS2-58	C1436T	P479L
	G1450A	E484K
	A1429G	S477G
SARS2-66	G1430T	S477I
	G1430A	S477N
	T1456G	F486V
	A1372C	K458Q
SARS2-71	A1452C	E484D
	T1468C	F490L
	G1427A	G476D
	A1427G	S477G
	C1433T	T478I
SARS2-71	A1432C	T478P
	C1436T	P479L
	T1456G	F486V

### Campaign 3

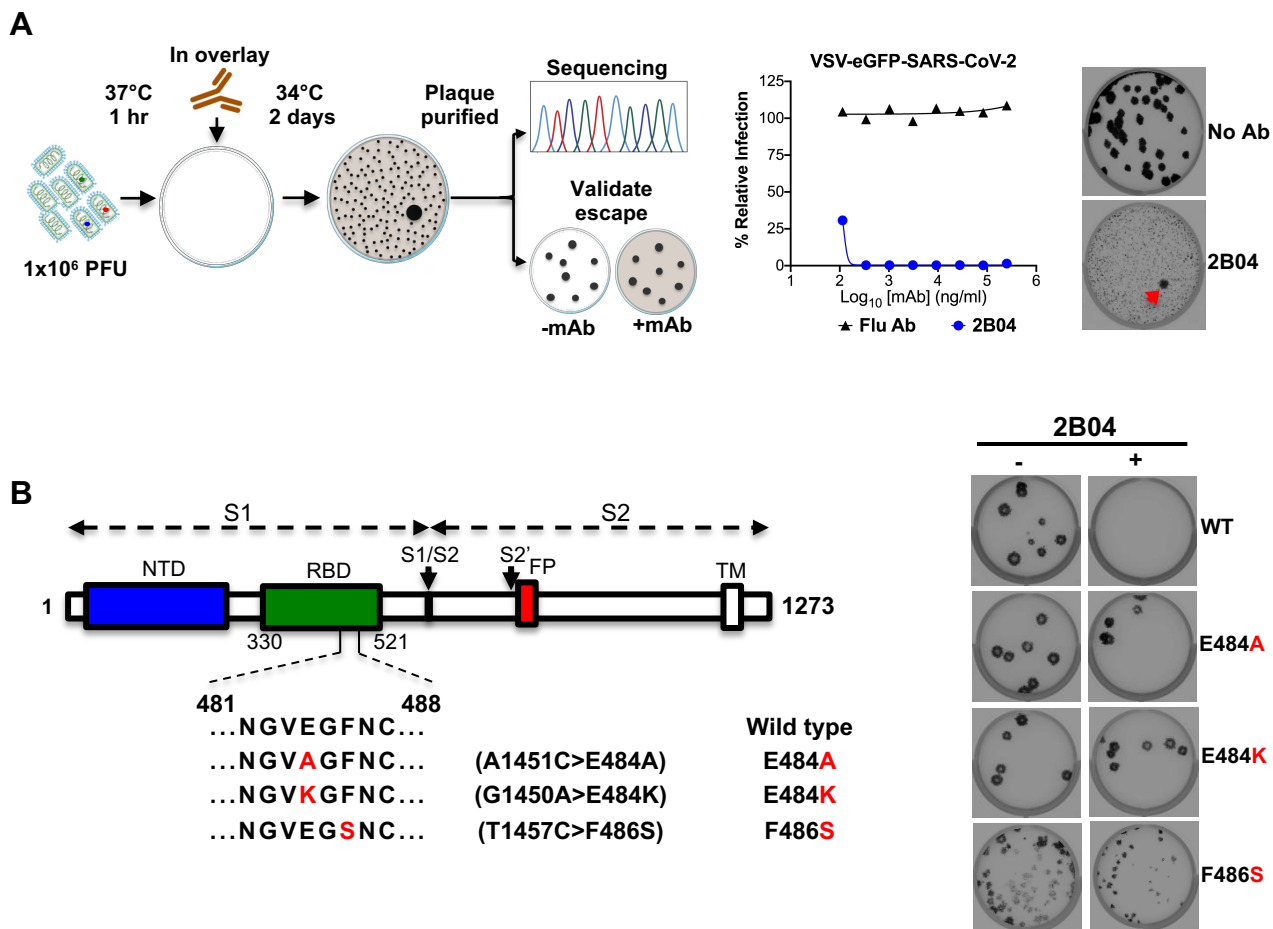
Virus	mAb	Nucleotide	Amino Acid
VSV-SARS-CoV-S E484A	2H04	A1033G/A1451C	T345A/E484A
		A1036G/A1451C	T346G/E484A
		A1330G/A1451C	K444E/E484A
VSV-SARS-CoV-S E484K	2H04	G1037A/G1450A	R346K/E484K
		G1114A/G1450A	A372T/E484K
		A1330G/G1450A	K444E/E484K
VSV-SARS-CoV-S F486S	2H04	A1033T/T1457C	T345S/F486S

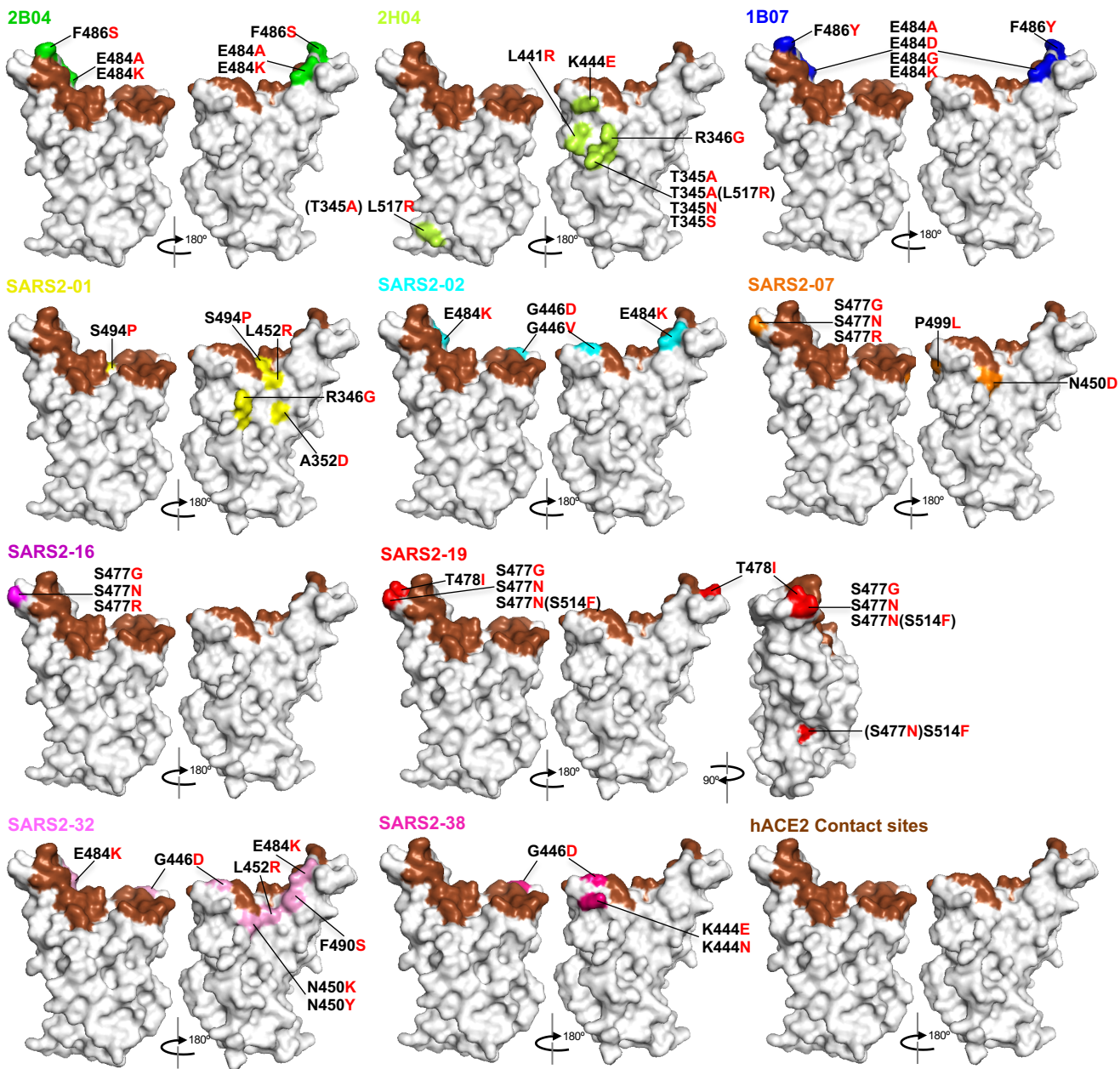
Sanger sequencing of isolated escape variants selected for by each mAb. The mutated nucleotides and residues in the RBD region of S are highlighted in red.

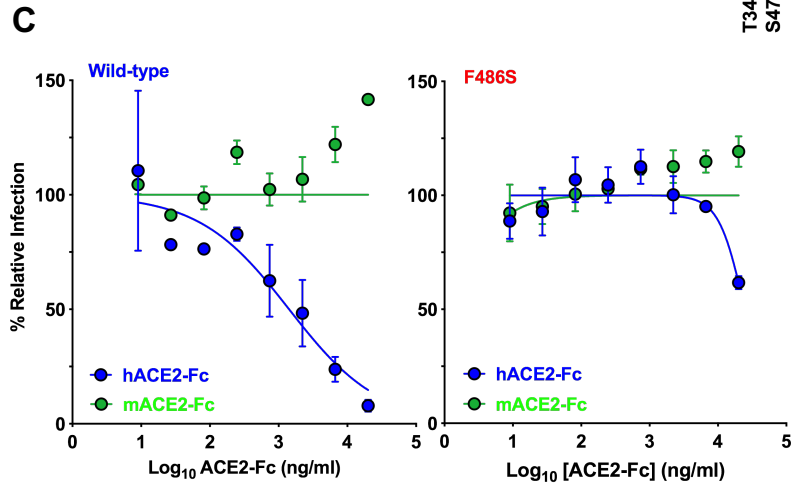
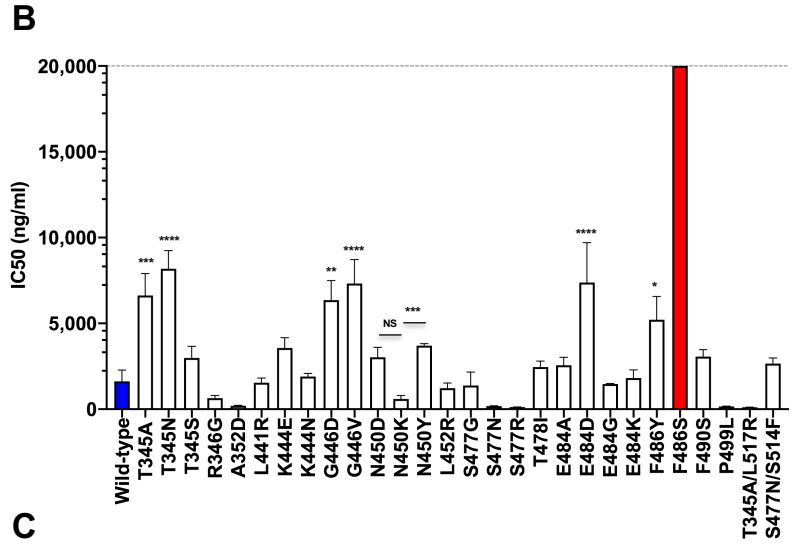
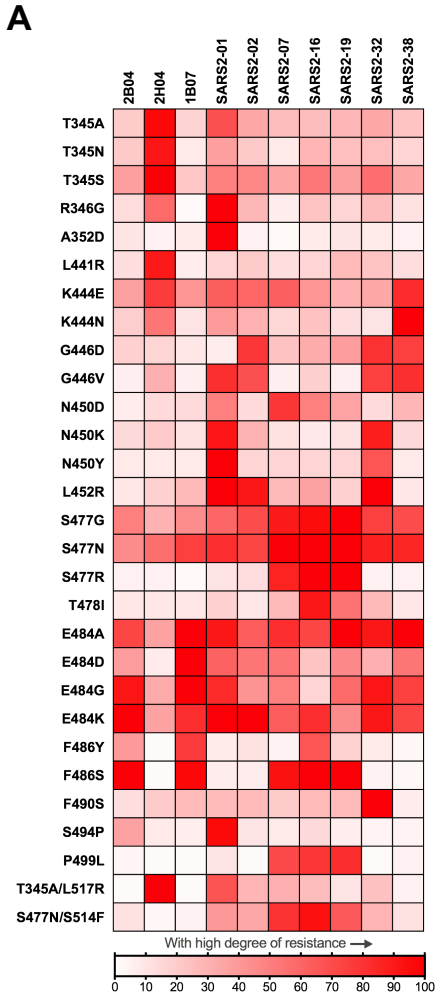
**Table 3. Human Serum IgG ELISA**

Serum	Days Post Symptom Onset	Euroimmun IgG		Epitope IgG	
		Index	Reactive	Index	Reactive
11	-	5.6	+	0.9	+/-
13	9	0.7	-	1.1	+
15	13	0.4	-	2.0	+
16	13	0.5	-	0.9	-
18	10	0.2	-	0.7	-
21	11	0.5	-	2.0	+
23	17	7.6	+	4.2	+
27	13	0.5	-	1.9	+
28	14	9.2	+	4.6	+
29	13	3.9	+	2.8	+
30	16	3.4	+	4.6	+
31	15	10.7	+	3.6	+
32	6	0.5	-	1.1	+
34	12	0.6	-	2.5	+
35	14	3.7	+	4.1	+
37	7	0.7	-	1.2	+
38	8	1.7	+	1.9	+
39	7	0.3	-	0.7	-

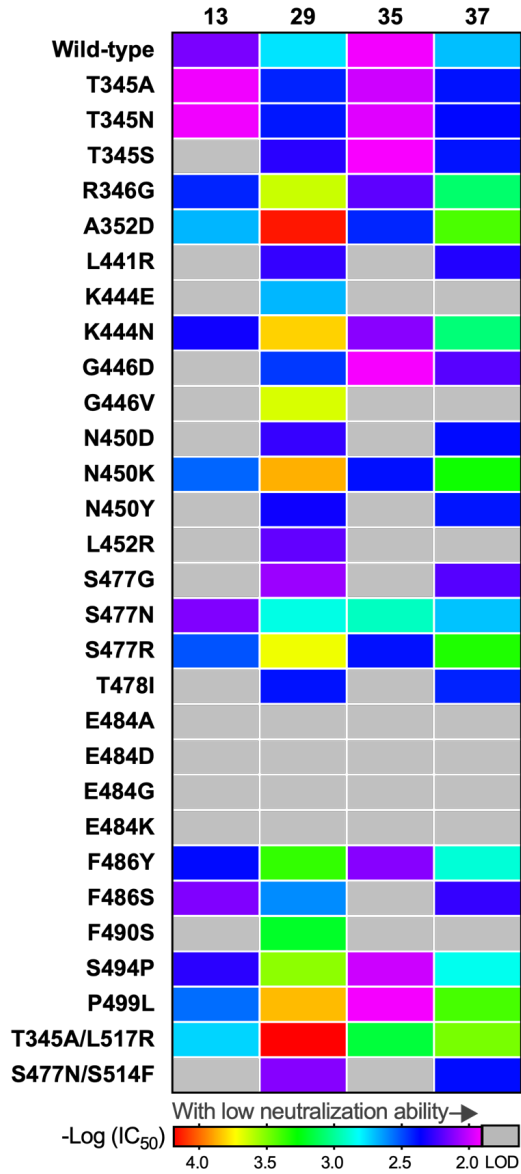
Serum samples from 18 individuals were collected at different time points post-onset of COVID-19 symptoms and screened using two ELISA assays (Euroimmun or Epitope). The serum identifier numbers in the first column correspond to those of **Fig 5 and S5**. IgG index values were calculated by dividing the optical density (O.D.) of the serum sample by a reference O.D. control, and ratios were interpreted using the following criteria as recommended by the manufacturer: Negative (-) < 0.8, Indeterminate (+/-) 0.8–1.1, Positive (+) ≥1.1



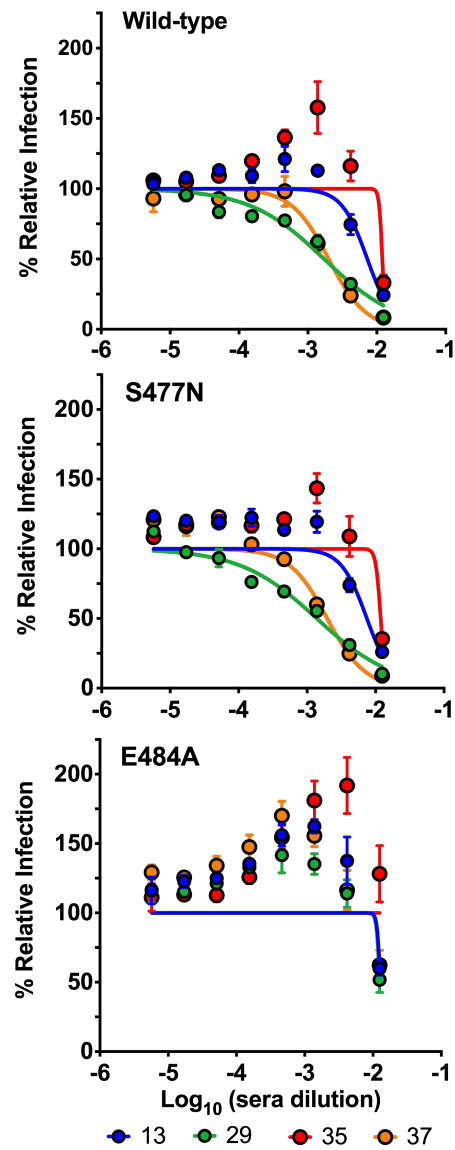




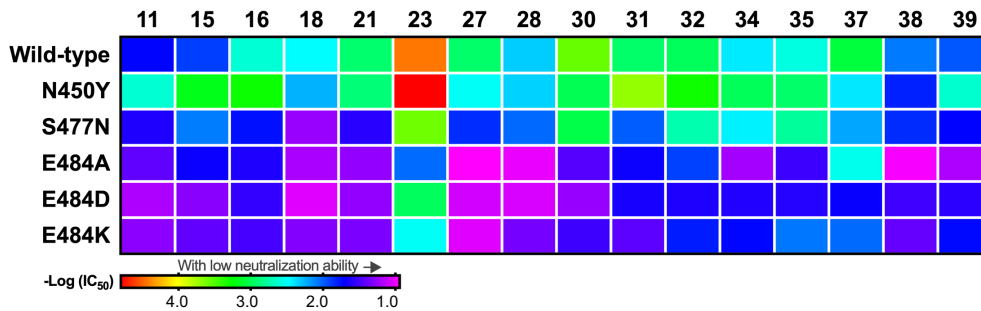
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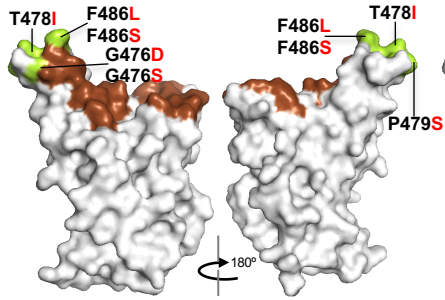
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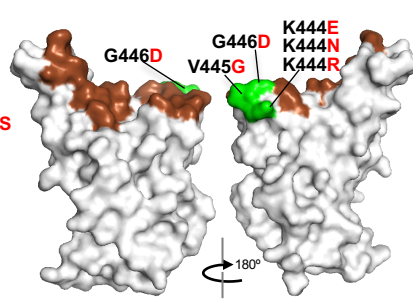
C



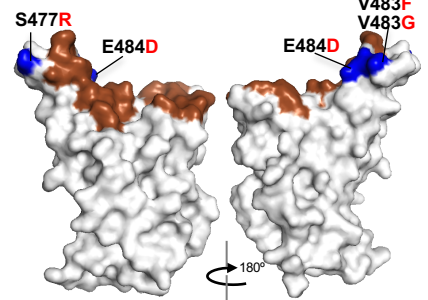
## SARS2-21



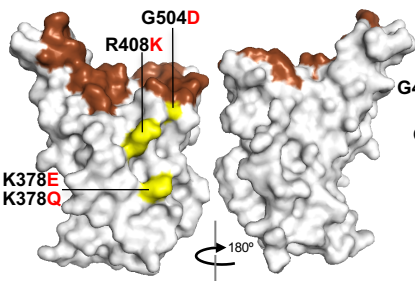
## SARS2-22



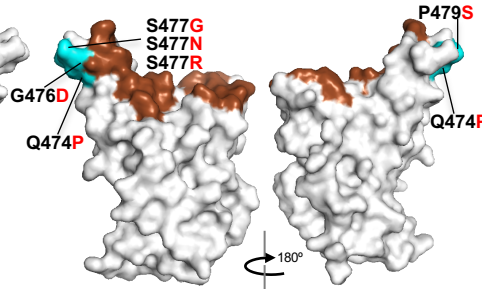
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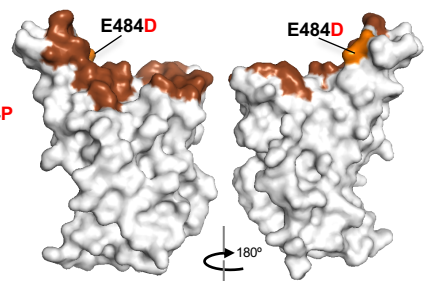
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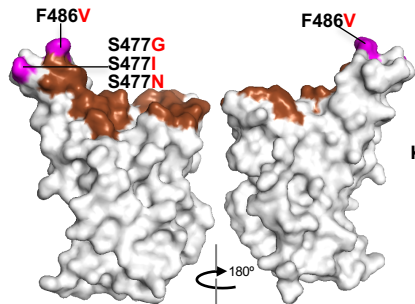
## SARS2-34



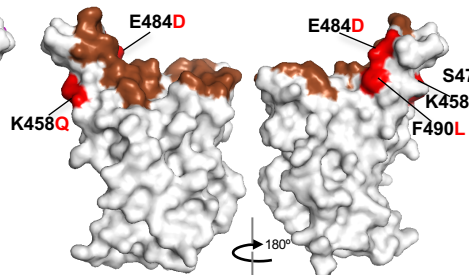
## SARS2-55



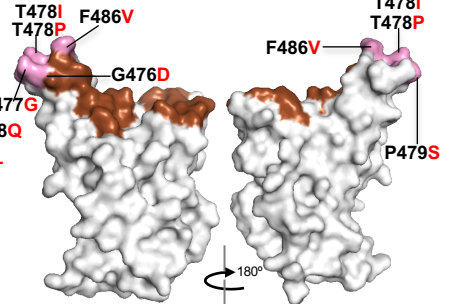
## SARS2-58



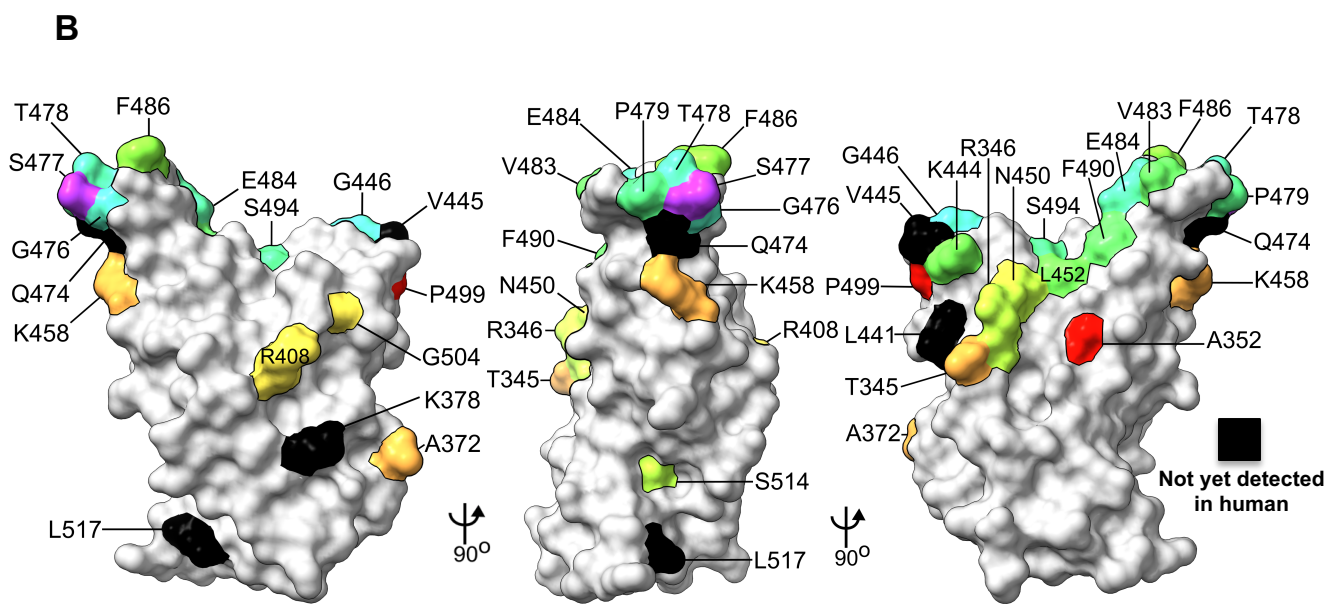
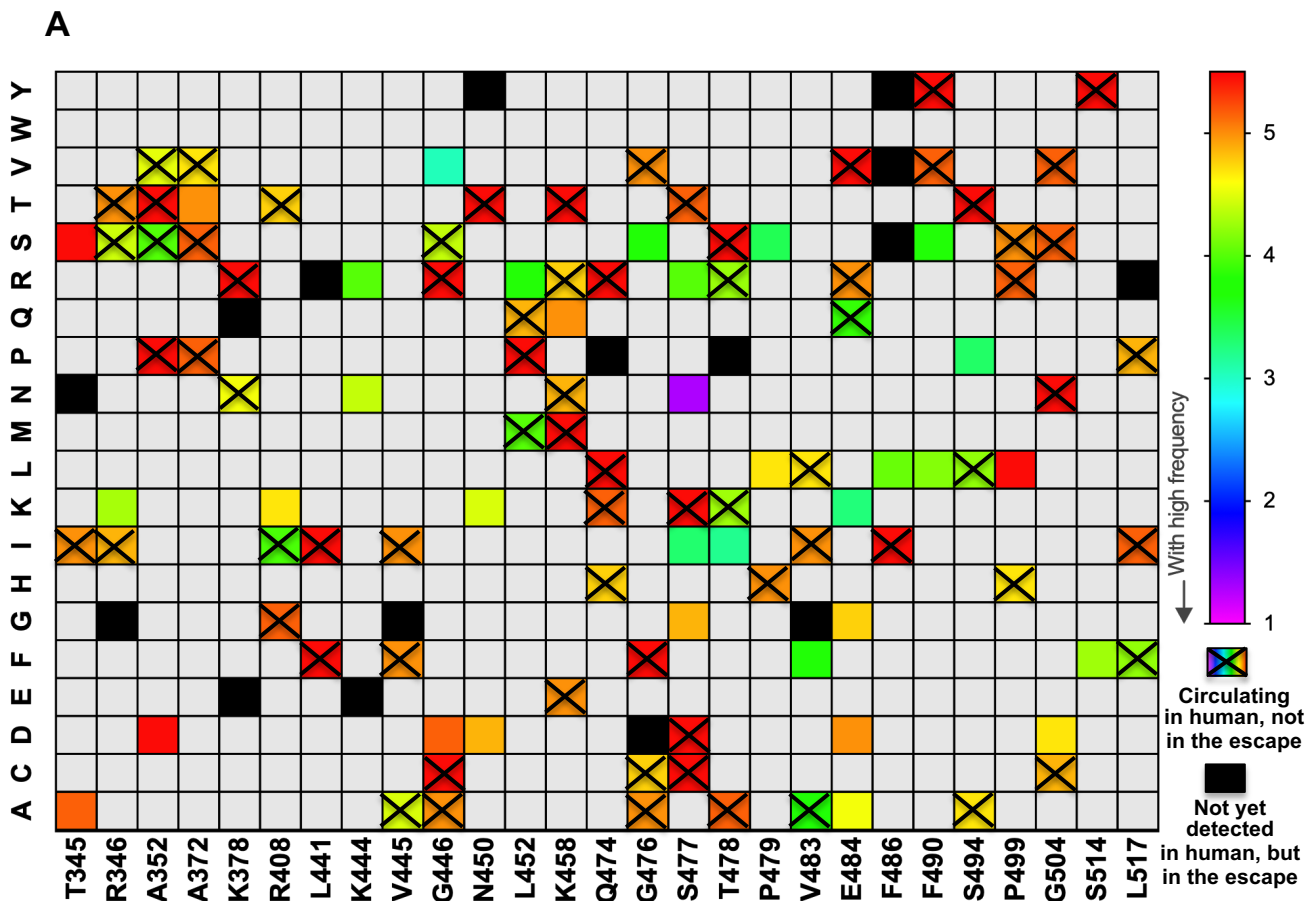
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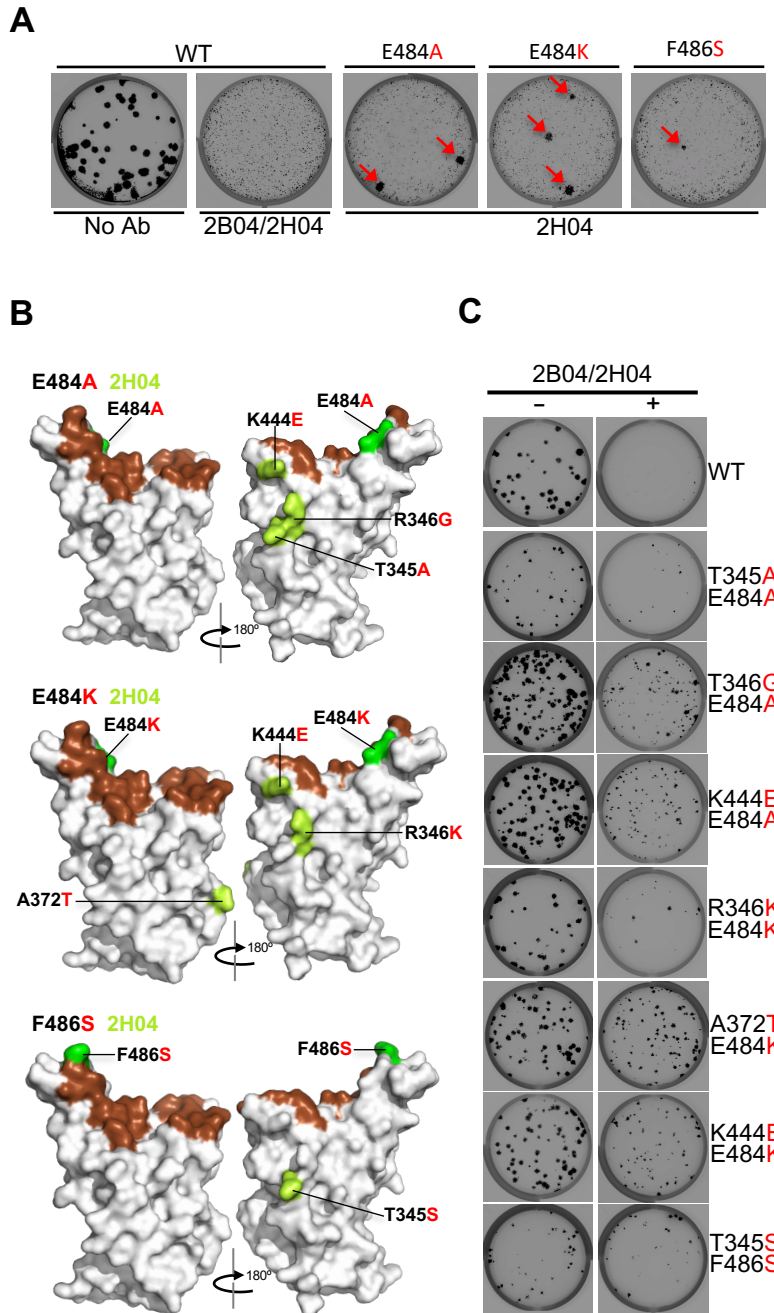
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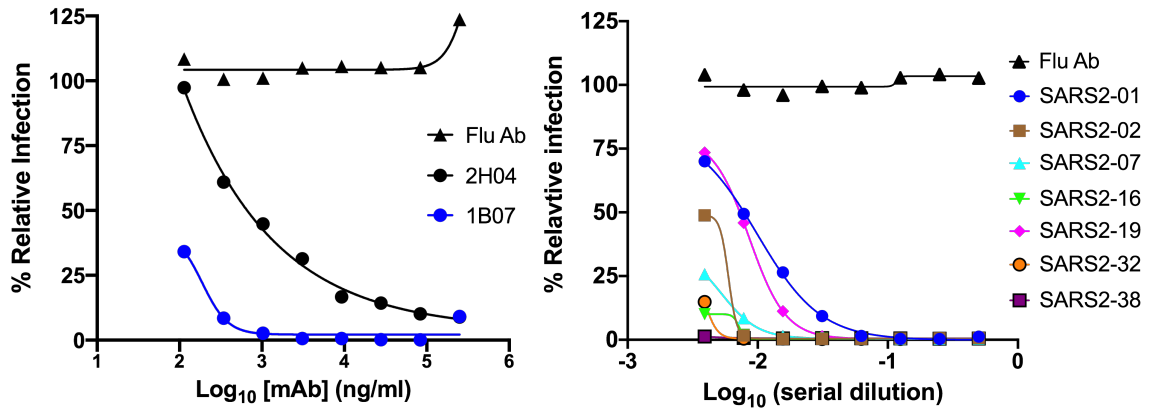




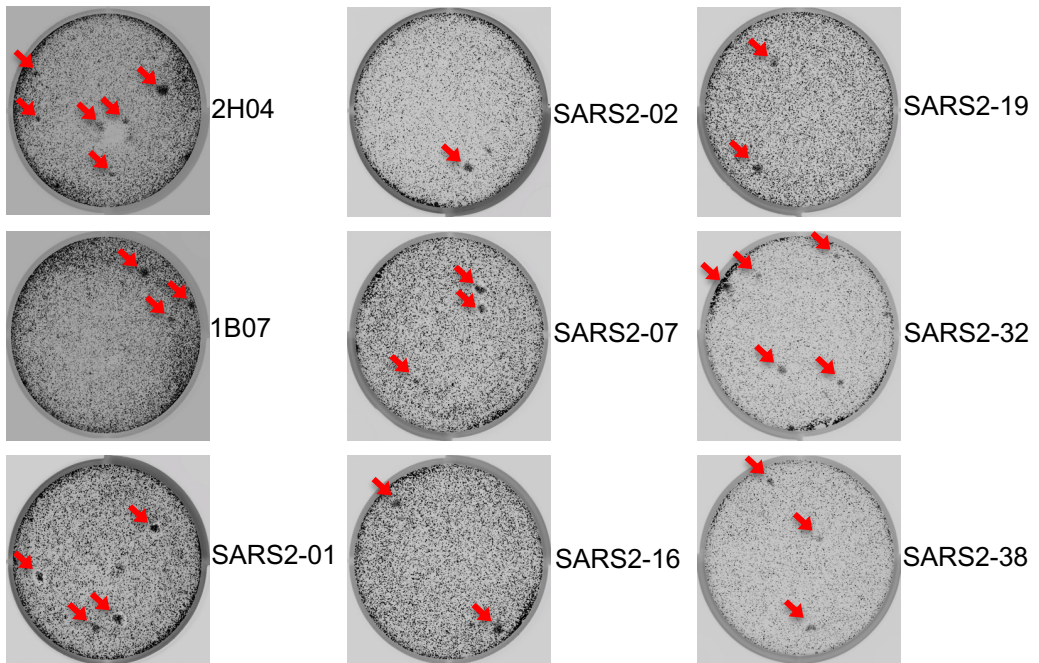


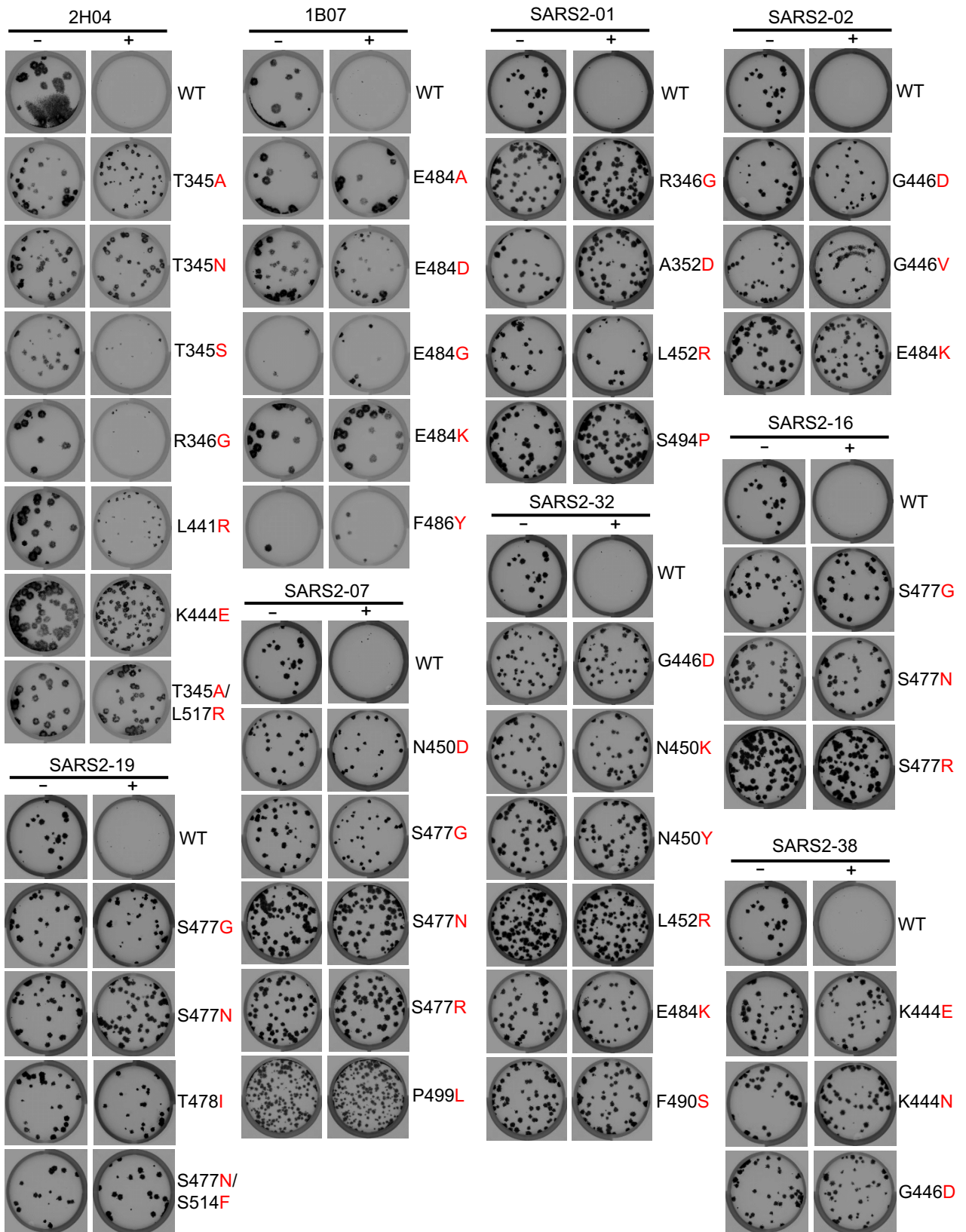


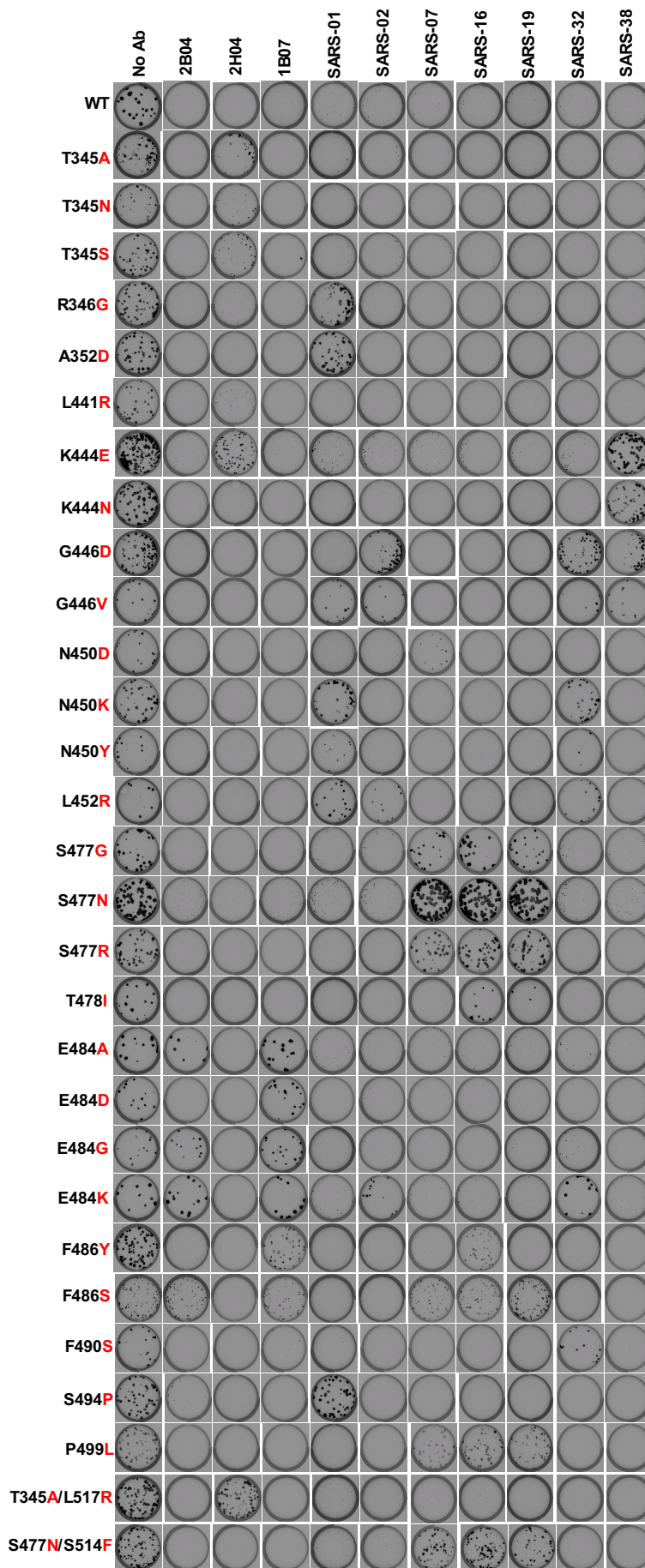
**A**



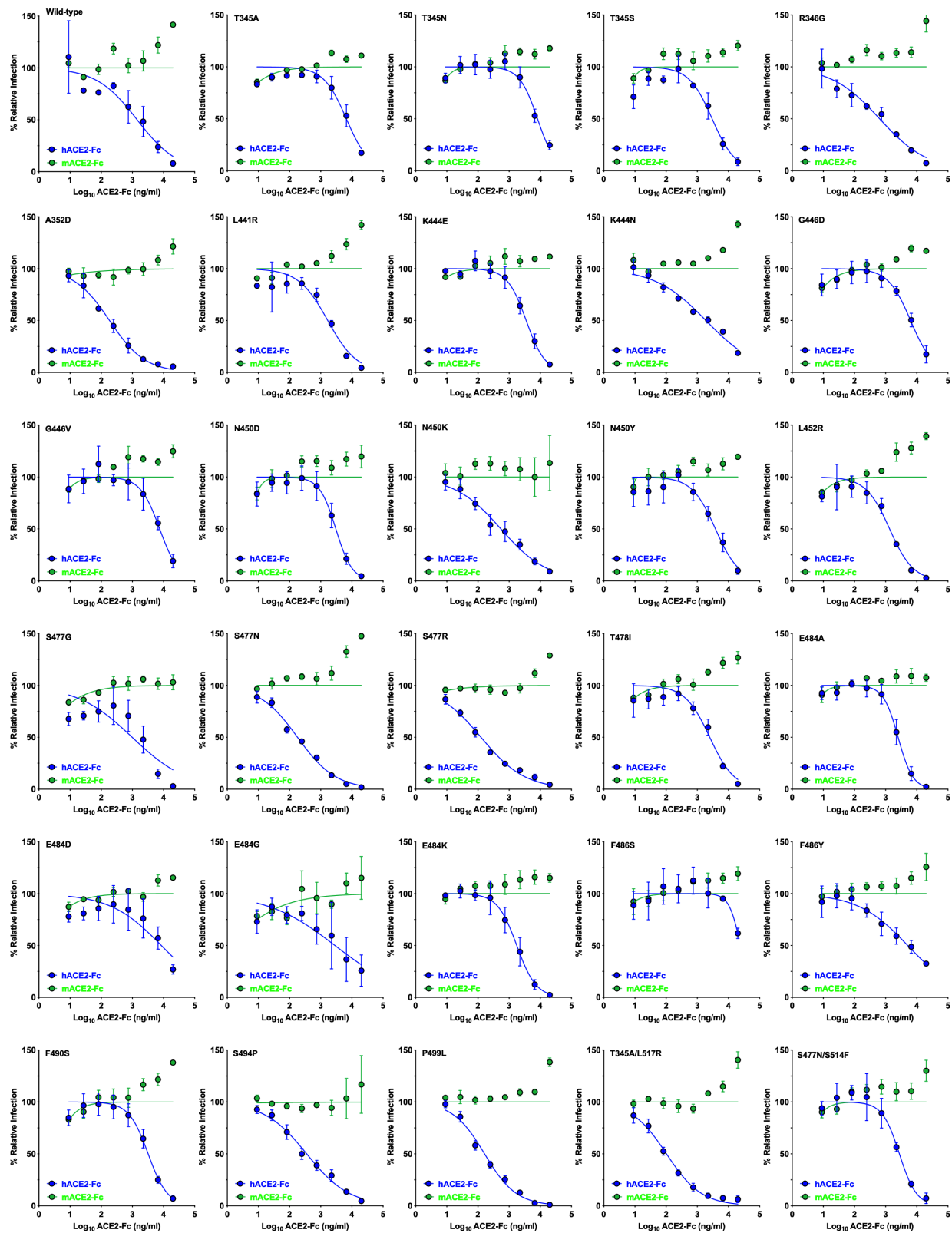
**B**



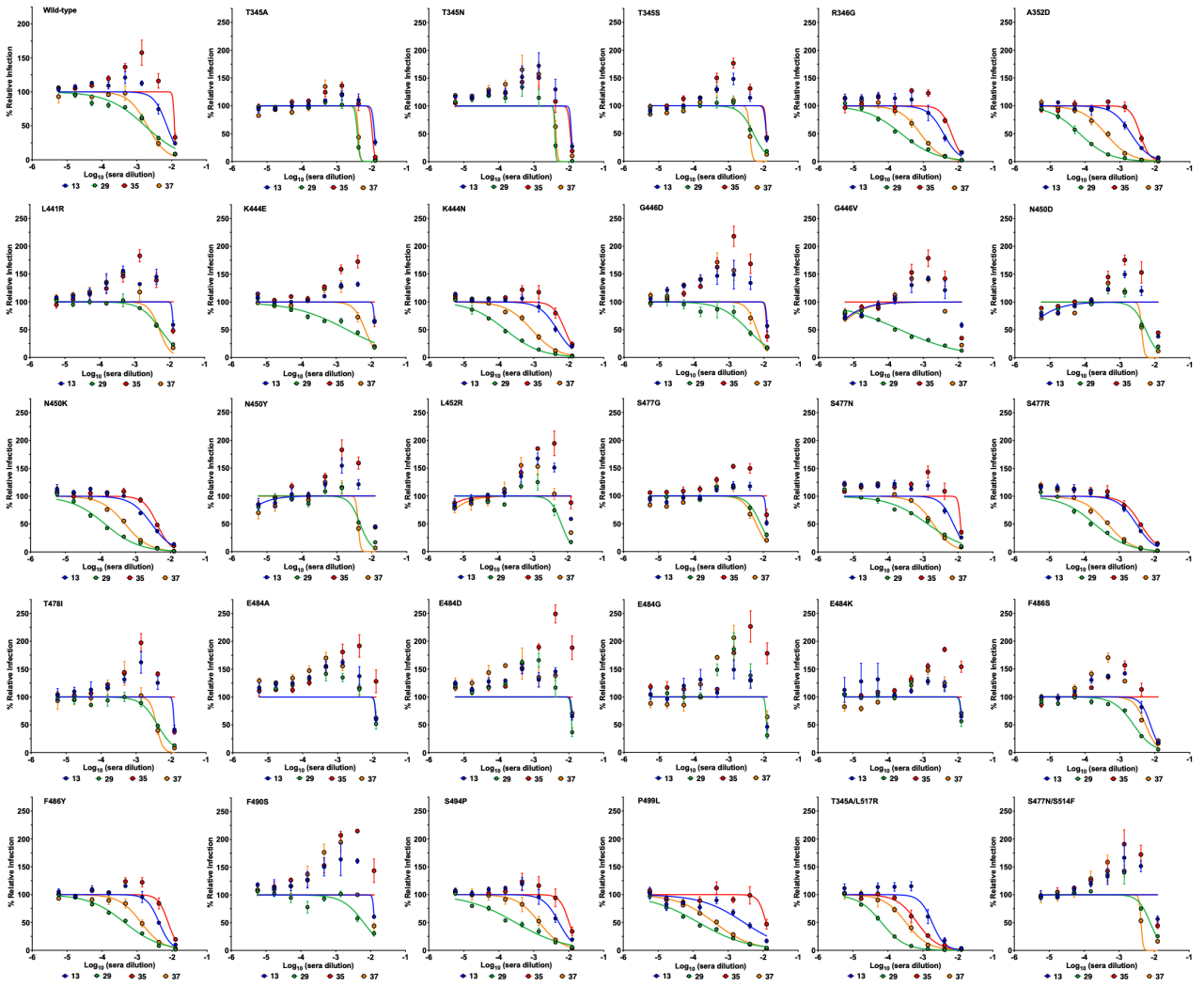
**Fig S-2**

**Fig S-3**

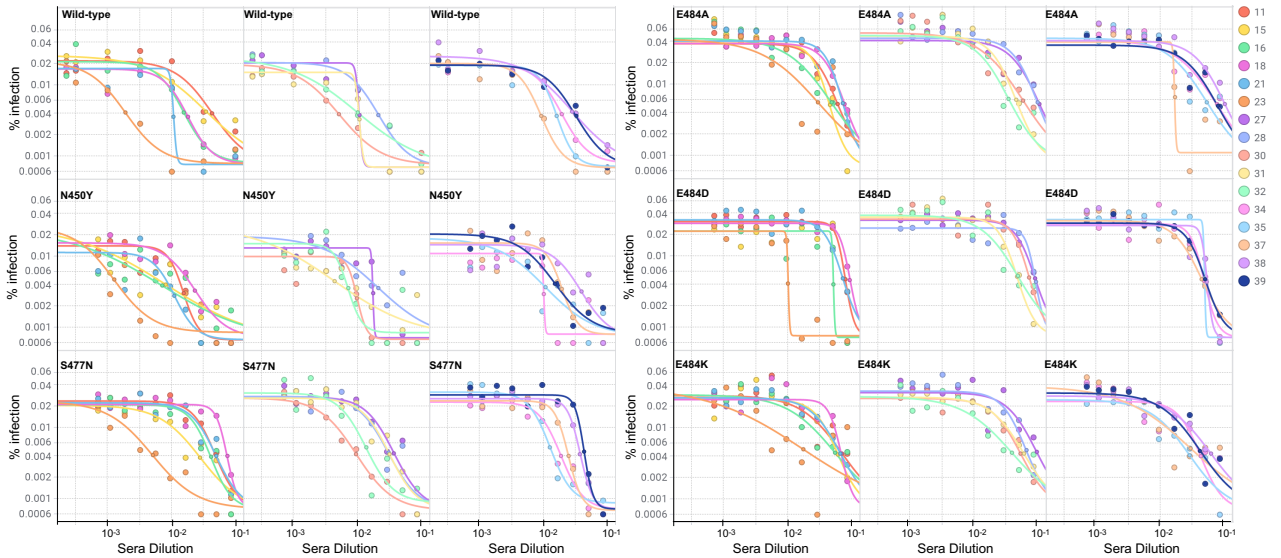




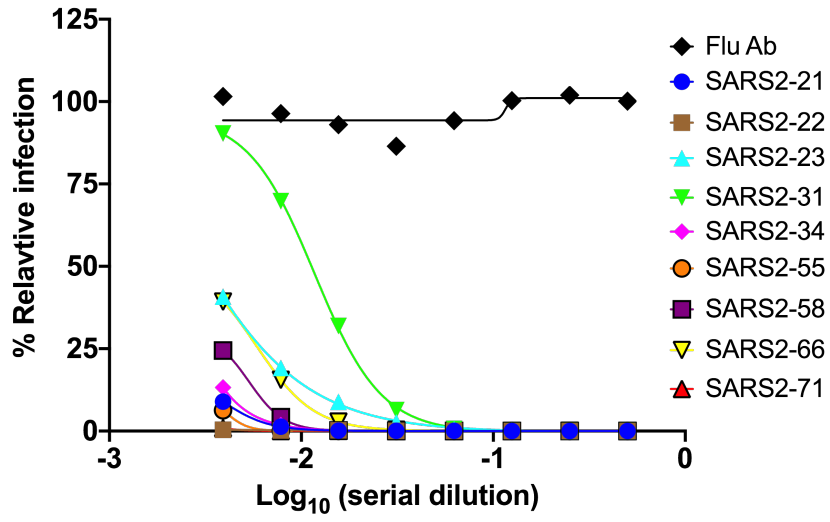
## A



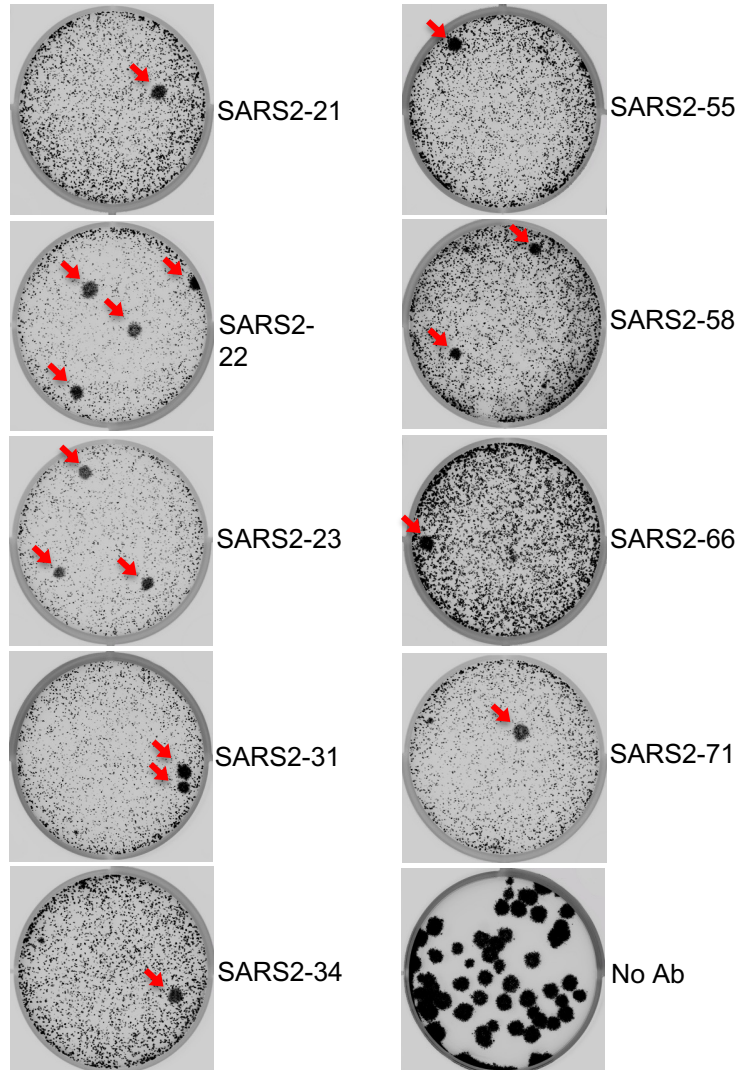
## B



A



B



**Fig S-7**