

1 **Characterization of neutralizing antibodies from a SARS-CoV-2 infected individual**

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22 **ABSTRACT**

23 B cells specific for the SARS-CoV-2 S envelope glycoprotein spike were isolated from a
24 COVID-19-infected subject using a stabilized spike-derived ectodomain (S2P) twenty-one
25 days post-infection. Forty-four S2P-specific monoclonal antibodies were generated, three
26 of which bound to the receptor binding domain (RBD). The antibodies were minimally
27 mutated from germline and were derived from different B cell lineages. Only two
28 antibodies displayed neutralizing activity against SARS-CoV-2 pseudo-virus. The most
29 potent antibody bound the RBD in a manner that prevented binding to the ACE2 receptor,
30 while the other bound outside the RBD. Our study indicates that the majority of antibodies
31 against the viral envelope spike that were generated during the first weeks of COVID-19
32 infection are non-neutralizing and target epitopes outside the RBD. Antibodies that disrupt
33 the SARS-CoV-2 spike-ACE2 interaction can potently neutralize the virus without
34 undergoing extensive maturation. Such antibodies have potential preventive/therapeutic
35 potential and can serve as templates for vaccine-design.

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46 **KEY WORDS**

47 COVID-19, SARS, SARS-CoV-2, antibody, B cells, spike protein, receptor binding
48 domain, neutralization

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50 **IN BRIEF**

51 SARS-CoV-2 infection leads to expansion of diverse B cells clones against the viral spike
52 glycoprotein (S). The antibodies bind S with high affinity despite being minimally mutated.
53 Thus, the development of neutralizing antibody responses by vaccination will require the
54 activation of certain naïve B cells without requiring extensive somatic mutation.

55 **Highlights**

- 56 • Analysis of early B cell response to SARS-CoV-2 spike protein
- 57 • Most antibodies target non-neutralizing epitopes
- 58 • Potent neutralizing mAb blocks the interaction of the S protein with ACE2
- 59 • Neutralizing antibodies are minimally mutated

60 INTRODUCTION

61 The WHO declared the 2020 COVID-19 to be a global pandemic on March 11th, 2020
62 (World Health Organization, 2020). There are currently 4.2 million documented cases of
63 COVID-19 and over 290 000 deaths (Dong et al., 2020). The infection is caused by SARS-
64 CoV-2, a beta coronavirus, closely related to SARS-CoV (Wan et al., 2020). Presently the
65 immune response to COVID-19 is not well understood and preventative measures, such
66 as vaccines, are not available. It is also unclear which immune responses are required to
67 prevent or control SARS CoV-2 infection.

68 High resolution structures of the SARS-CoV-2 prefusion-stabilized spike (S) ectodomain
69 revealed that it adopts multiple conformations with either one receptor binding domain
70 (RBD) in the “up” or “open” conformation or all RBDs in the “down” or “closed”
71 conformation, similar to previous reports on both SARS-CoV S and MERS-CoV S (Gui et
72 al., 2017; Kirchdoerfer et al., 2018; Pallesen et al., 2017; Song et al., 2018; Walls et al.,
73 2020; Walls et al., 2019; Wrapp et al., 2020; Yuan et al., 2017). Like SARS-CoV, SARS-
74 CoV-2 utilizes angiotensin-converting enzyme 2 (ACE2) as an entry receptor binding with
75 nM affinity (Li et al., 2003; Walls et al., 2020; Wrapp et al., 2020) (Hoffmann et al., 2020;
76 Letko et al., 2020; Ou et al., 2020). Indeed, the S proteins of the two viruses share a high
77 degree of amino acid sequence homology; 76% overall and 74% in RBD (Wan et al.,
78 2020).

79 Although binding and neutralizing antibody responses are known to develop following
80 SARS-CoV-2 infection (Ni et al.; Okba et al., 2020), no information is currently available
81 on the epitope specificities, clonality, binding affinities and neutralizing potentials of the
82 antibody response.

83 Monoclonal antibodies (mAbs) isolated from SARS-CoV-infected subjects can recognize
84 the SARS-CoV-2 S protein (Yuan et al., 2020) and immunization with SARS S protein
85 can elicit anti-SARS-CoV-2 neutralizing antibodies in wildtype, and humanized mice, as
86 well as llamas (Walls et al., 2020; Wang et al., 2020; Wrapp et al., 2020). However, SARS-
87 CoV-2 infection appears to not elicit strong anti-SARS-CoV neutralizing antibody
88 responses and vice versa (Ou et al., 2020).

89 Here, we employed diverse but complementary approaches to investigate the serum
90 binding and neutralizing antibody responses to a stabilized ectodomain variant of the
91 SARS-CoV-2 spike protein (S2P) as well as the frequency and clonality of S2P-specific B
92 cells in a SARS-CoV-2-infected individual 21 days post post the onset of respiratory
93 symptoms. We isolated anti-SARS-CoV-2 S mAbs and characterized their binding
94 properties and determined their neutralizing potencies. Among all B cells analyzed, no
95 particular VH or VL gene family was expanded and the isolated antibodies were minimally
96 mutated. Our analysis reveals that only a small fraction of S2P-specific B cells recognized
97 the RBD. Of the forty-four mAbs analyzed, only two displayed neutralizing activity. The
98 most potent mAb, CV30, bound the RBD in a manner that disrupted the spike-ACE2
99 interaction. The second mAb, CV1, bound to an epitope distinct from the RBD and was
100 much less potent.

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105 **RESULTS**

106 **Serology**

107 Serum and PBMC were collected twenty-one days after the onset of clinical disease. The
108 serum contained high titers of antibodies to the SARS-CoV-2 S2P (Fig. 1A). The
109 specificity of this response was confirmed by the absence of S2P reactivity by serum
110 antibodies isolated from donors collected prior to the SARS-CoV-2 pandemic, or donors
111 with confirmed infection by endemic coronaviruses. We also measured the serum
112 antibody response to RBD, and again observed specific high titers of binding antibodies
113 (Fig. 1B). Isotype-specific ELISA revealed that the IgG titers were higher than the IgA and
114 the IgM titers to both S2P and RBD, suggesting a significant portion of the antibody
115 responses to SARS-CoV-2 S are IgG (Fig. 1C and D). The serum from the SARS-CoV-2
116 infected donor displayed potent neutralizing activity (Reciprocal ID₅₀~3000) against a
117 pseudovirus expressing the S protein from SARS-CoV-2 isolate Wuhan-Hu-1 (Fig 1E).
118 We concluded that this donor had developed strong binding and neutralizing antibody
119 responses within three weeks of disease onset.

120 **B cell sorts and VH/VL sequencing**

121 Fluorescently labeled S2P and RBD probes were used as baits to identify B cells specific
122 to the SARS-CoV-2 S protein that were circulating at this timepoint. S2P was labeled with
123 either phycoerythrin (PE) or brilliant violet 711 (BV711) and used to stain B cells
124 concurrently. This double labeling strategy helps to discriminate between bona fide S2P-
125 specific B cells and non-specific background staining to the fluorophores. RBD was
126 labeled with alexa fluor 647 to identify B cells specific for that domain.

127 Approximately 0.65% of total CD19+ B cells were S2P positive compared to 0.07% of
128 total B cells from a naïve donor (Fig. S1A and B). The dominant responding B cells were
129 IgM+ IgD+ (49% of S2P+ B cells; Fig. S1C); 90% of which were CD27+ suggesting that
130 although these B cells have not class-switched, they were antigen-experienced memory
131 B cells. The second most prominent subset of S2P-specific B cells were class-switched
132 IgG+ IgD- B cells (27% of S2P+ B cells; Fig. S1C). In fact, 1.7% of the IgG+ B cells
133 stained with S2P (Fig. 2A) and of those ~7% (or 0.12% of total IgG+ B cells, Fig. 2B) were
134 also positive for RBD.

135 We hypothesized that the class-switched SARS-CoV-2-specific B cells were more likely
136 to have undergone some affinity maturation and contain antibodies capable of
137 neutralizing the virus. Thus, we focused on our B cell receptor (BCR) sequencing on S2P+
138 IgG+ B cells. 576 S2P+ B cells were single-cell sorted into individual wells of a 96 well
139 plate and the variable heavy and light chain regions of B cell receptor transcripts were
140 sequenced using nested RT-PCR. We successfully recovered 103 successful VH
141 sequences, and 187 successful VL sequences, 97 of which were kappa and 90 were
142 lambda. B cells specific for S2P+ were derived from diverse antibody heavy and light
143 chain genes (Fig. 2C-E) and had normal distributions of CDRH3 and CDRL3 lengths (Fig.
144 2 F-G). Consistent with the relatively short time of infection the majority of BCR sequences
145 showed low levels of somatic mutation (Fig. 2H).

146 **Antibody-binding**

147 Among all successfully sequenced VH and VL transcripts, we obtained paired sequences
148 from forty-four. These were produced as recombinant monoclonal antibodies (mAbs) of
149 the IgG1 isotype and tested for binding to recombinant S-derived proteins (Fig. 3).

150 All the mAbs bound to the stabilized SARS-CoV-2 ectodomain, S2P (Fig. 3A and E).
151 Consistent with the B cell staining that revealed very few RBD-specific B cells (Fig. 2B),
152 only three mAbs, CV5, CV30 and CV43, also bound the SARS-CoV-2 RBD (Fig. 3B and
153 E). The majority of S2P-specific mAbs also bound to full-length membrane-bound
154 wildtype SARS-CoV-2 S on the surface of 293 cells (Fig. 3C and Fig. S2). The observation
155 that some S2P-specific mAbs failed to bind to cell surface S indicates that there may be
156 conformational differences between the stabilized soluble ectodomain and cell surface S.
157 The fact that a subset of the mAbs bound to a stabilized ectodomain variant of the closely
158 related SARS-CoV S protein (Fig. 3D and E), demonstrates that there are conserved
159 epitopes among the two viruses. Consistent with the lower degree of conservation of the
160 S1 subunit between SARS-CoV and SARS-CoV-2, the anti-RBD mAbs CV30 and CV43
161 did not cross react with SARS-CoV S2P, while CV5 showed weak binding.

162 **Neutralizing activity**

163 The S2P-binding mAbs were evaluated for their ability to neutralize SARS CoV-2
164 pseudovirus infection of 293T cells stably expressing ACE2. All but two of the mAbs were
165 non-neutralizing (Fig. 4A and Table S1). Although it did not achieve 100% neutralization
166 at the highest concentration, CV1 which binds an epitope outside the RBD was weakly
167 neutralizing ($IC_{50}=15\mu\text{g/ml}$, Fig. 4A and Table S1). CV1 neutralized less potently than an
168 ACE2-Fc fusion protein which acts as a soluble competitor for the interaction between S

169 and the cell surface-expressed ACE2 ($IC_{50}=2.2 \mu\text{g/ml}$). In contrast, CV30 achieved 100%
170 neutralization and was ~480 times more potent than CV1 ($IC_{50}=0.03\mu\text{g/ml}$, Fig. 4A and
171 Table S1). CV1, CV30 and the ACE2-Fc fusion did not neutralize a murine leukemia virus
172 pseudovirus demonstrating their specificity for the SARS-CoV-2 S protein (Fig. 4B). CV30
173 is derived from a heavy chain utilizing an IGHV3-53*01 heavy chain and an IGKV3-30*01
174 light chain. CV1 binds an epitope outside the RBD and is derived from an IGHV4-38*02
175 heavy chain and an IGLV1-44*01 light chain. Both represent unique clones among all B
176 cells sequenced (Table S1).

177 Based on the observations that CV30 is potently neutralizing and it binds RBD with high
178 affinity, we investigated whether it would block the interaction between the SARS-CoV-2
179 protein and the ACE2 receptor. To this end we setup binding competition experiments
180 using BLI. Indeed, CV30 completely inhibited the RBD-ACE2 interaction. In contrast, CV5
181 and CV43, the other two anti-RBD mAbs and the CR3022 control, which binds the RBD
182 outside of the binding site, did not (Fig. 4C). We also measured the relative binding
183 affinities of CV30 and ACE2 to the SARS-CoV-2 RBD. ACE2 bound the RBD with an
184 affinity of 5.9 nM (Fig. 4D), while CV30 bound with a slightly higher affinity of 3.6 nM (Fig.
185 4E). The kinetics of the interactions were notably different, ACE2 had both a faster
186 association and dissociation rate than CV30 (Figs. 4D and E, and Table S2). Collectively
187 these results indicate that CV30 neutralizes SARS-CoV-2 infection by blocking the S-
188 ACE2 interaction through an interaction that is higher affinity.

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191 **DISCUSSION**

192 The development of therapeutic interventions, of immunoprophylaxis and of an effective
193 vaccine against SARS-CoV-2 will benefit from understanding the protective immune
194 responses elicited during infection. A recent report indicated that neutralizing antibodies
195 are present in the sera collected from convalescent COVID-19 patients (Ni et al.; Okba et
196 al., 2020). However, the kinetics of neutralizing antibody development as well as the
197 characteristics and epitope specificities of neutralizing antibodies generated during
198 SARS-CoV-2 infection are presently poorly understood.

199 Serological analysis revealed that this COVID-19-infected patient developed high titers of
200 binding and neutralizing antibody responses twenty-one days following infection. The
201 development of neutralizing antibody titers at this early timepoint has been reported for
202 other COVID patients (Ni et al.; Okba et al., 2020) and is consistent with the rapid
203 development of neutralizing responses to SARS-CoV infection (Corti et al., 2011). At this
204 time point, IgG constituted the major fraction of anti-S2P and anti-RBD serum antibodies,
205 although both IgM and IgA antibodies against these viral antigens were detected in the
206 serum as well. The S2P-specific, class switched B cells circulating at this time point were
207 not dominated by any particular clone. Rather, they were derived from a diverse VH/VL
208 gene repertoire, with frequencies similar to those reported in healthy uninfected
209 individuals (Briney et al., 2019; DeKosky et al., 2016; Soto et al., 2019; Vazquez Bernat
210 et al., 2019).

211 Although anti-S2P antibodies isolated at this time point could bind the S protein, the
212 majority lacked neutralizing activity. However, two, CV1 and CV30, were able to

213 neutralize SARS-CoV-2. CV1 and C30 were derived from unique rearrangements among
214 all antibodies we examined. Thus, although diverse B cell clones became activated during
215 infection, the serum neutralizing activity is due to a relatively small subset.

216 CV1 binds to an unknown epitope region outside of the RBD, but the more potent CV30
217 recognizes the RBD and likely neutralizes infection by directly inhibiting SARS-CoV-2 S
218 binding to the ACE2 receptor. The RBD is a major target of neutralizing antibodies in
219 SARS-CoV infection (Cao et al., 2010). Several neutralizing monoclonal antibodies that
220 block the interaction of SARS-CoV with the ACE2 receptor have been described (Hwang
221 et al., 2006; Prabakaran et al., 2006; Rockx et al., 2008; Sui et al., 2004; Walls et al.,
222 2019). Moreover, the neutralizing potency correlated with the degree of S-ACE2 inhibition
223 (Rockx et al., 2008). The RBD of MERS-CoV is also a target of potent neutralizing
224 antibodies (Jiang et al., 2014; Niu et al., 2018; Tang et al., 2014; Ying et al., 2014),
225 highlighting the importance of receptor blocking antibodies for coronavirus vaccine
226 development. Although SARS-CoV and SARS-CoV-2 share extensive amino acid
227 sequence in the receptor binding domain (74%) and both viruses utilize human ACE2 for
228 entry, the amino acid identity in the receptor binding motif is only ~50% (Wan et al., 2020).
229 In line with this, potent anti-SARS-CoV neutralizing monoclonal antibodies that bind RBD
230 fail to cross react with SARS-CoV-2 (Wrapp et al., 2020), similarly the anti-RBD mAb,
231 CV30 described herein fails to cross react with the SARS-CoV spike protein.

232 Consistent with the short time period post-infection, the majority of S-specific BCRs from
233 individual B cells were unmutated or had only accumulated very few mutations. This was
234 true for the neutralizing antibodies as well. CV1 was unmutated from germline, while
235 CV30 had 2 amino acid mutations in VH and none in VL. Largely unmutated antibodies

236 against SARS-CoV S (Prabakaran et al., 2006; Sui et al., 2004) and MERS-CoV S (Jiang
237 et al., 2014; Tang et al., 2014; Ying et al., 2014; Ying et al., 2015) have been isolated
238 from phage display libraries created from uninfected donors.

239 Potent anti-SARS-CoV neutralizing monoclonal antibodies that bind RBD are derived
240 from different VH genes (VH1-18, VH1-69, or VH3-30) than CV30 (Prabakaran et al.,
241 2006; Sui et al., 2004; Traggiai et al., 2004; Walls et al., 2019). Anti-RBD antibodies that
242 neutralize MERS are derived from diverse gene families (Jiang et al., 2014; Niu et al.,
243 2018; Tang et al., 2014).

244 Collectively these results indicate that high-affinity coronavirus-neutralizing antibodies
245 require a short developmental pathway. This suggests that a vaccine against this virus
246 may only need to activate a subset of B cells for potent neutralizing antibody responses
247 to be developed, and that potent neutralizing antibodies are not V-gene restricted.

248 In sum, we provide information on the characteristics of early antibody and B cell
249 responses to the SARS-CoV-2 spike protein during infection. Moreover, the neutralizing
250 antibodies discussed here can serve as templates for the design of immunogens and
251 potentially have utility as therapeutic and prophylactic agents to combat the SARS-CoV2
252 pandemic.

253

254 **ACKNOWLEDGMENTS**

255 This work was supported by generous donations to Fred Hutch COVID-19 Research
256 Fund. We thank Dr. McLellan for providing the SARS-CoV, and SARS-CoV-2 S2P and
257 RBD plasmids, Dr. Neil King for providing the ACE2-Fc fusion protein and the CR3022
258 mAb and Dr. M. Boeckh for providing sera from endemic coronavirus infected patients.
259 We thank Todd Haight for specimen processing.

260 **AUTHORS CONTRIBUTIONS**

261 L.S, M.P and A.M: designed the study, analyzed data and wrote the manuscript; A.B.S,
262 K. R. P, M. J, A. M, J. F, N. H, S. S, Y-H. W, L. H, E. S, N. A and K.W.C: performed
263 experiments and analyzed data; J. M: analyzed data; H. Y. C and J. E: designed the
264 clinical study and provided biospecimens.

265 **DATA AND REAGENT AVAILABILITY**

266 The sequences of monoclonal antibodies reported will be deposited on 05/11/2020 at
267 GenBank (submission ID: 2343258) Further information and requests for reagents should
268 be directed to and will be fulfilled by Leonidas Stamatatos (Istamata@fredhutch.org). All
269 reagents generated in this study can be made available upon request through Material
270 Transfer Agreements. pTT3-derived plasmids require a license from the National
271 Research Council (Canada)

272

273 **DECLARATION OF INTERESTS**

274 The authors declare no competing financial interests. A provisional patent application on
275 the antibodies discussed here has been filed.

276 HYC: Merck, Sanofi-Pasteur, GSK

277 **FIGURE LEGENDS**

278 **Figure 1. Serum antibody reactivity to the SARS-CoV-2 ecto- and receptor binding**

279 **domain.** Total antibody binding in serum from a donor with confirmed SARS-CoV-2
280 infection (COVID-19+), from two donors collected prior to the COVID-19 pandemic with
281 an unknown history of coronavirus infection (pre-pandemic), and from nine donors with
282 confirmed infection by endemic corona viruses (endemic), was tested for binding to the
283 SARS-CoV-2 S2P ectodomain (**A**) and the RBD (**B**) by ELISA. Serum from the donor in
284 SARS-CoV-2 infection in **A** was tested for binding to the SARS-CoV-2 S2P ectodomain
285 (**C**) and the RBD (**D**) using isotype-specific secondary antibodies by ELISA. (**E**) Serum
286 from donor with confirmed SARS-CoV-2 infection, and serum from a pre-pandemic donor
287 were evaluated for their ability to neutralize a SARS-CoV-2 pseudovirus.

288 **Figure 2. Early B cells response to SARS-CoV-2 is diverse and largely unmutated.**

289 (**A**) Class switched (IgM- IgG+) B cells were stained with SARS-CoV-2 S2P labeled with
290 BV710 or PE. (**B**) SARS-CoV-2 S2P+ IgG+ B cells were further analyzed for binding to
291 Alexafluor647-labeled SARS-CoV-2 RBD. (**C, D, E**) Individual SARS-CoV-2 S2P+ IgG+
292 B cells were sorted into separate wells of a 96 well plate and sequenced using RT-PCR.
293 VH (**C**), VK (**D**), and VL (**E**) gene usage of successfully sequenced S2P-specific B cells.
294 CDRH3 (**F**) and CDRL3 (**G**) length distributions of successfully sequenced S2P-specific
295 B cells. Number of amino acid substitutions from germline in S2P-specific heavy and light
296 chains (**H**).

297 **Figure 3. Sorted mAbs bind to SARS-CoV-2 and a subset cross-react with SARS-**
298 **CoV S.** mAbs isolated from SARS-CoV-2 S2P-specific B cells were tested for binding to
299 SARS-CoV-2 S2P (**A**) and to SARS-CoV-2 RBD (**B**) using BLI. (**C**) mAbs were labeled
300 with phycoerythrin (PE) and used to stain 293 cells transfected with wildtype SARS-Cov-
301 2 S by flow cytometry. Heatmap shows mean fluorescence intensity of PE+ cells at
302 2.5µg/ml. Titration curves are shown in Fig. S2. (**D**) mAbs were tested for binding to
303 SARS-CoV S2P by BLI (**D**). (**E**) Heatmap shows maximum binding response (average
304 nm shift of the last 5 seconds of association phase) of binding data in **A**, **B** and **D**.

305 **Figure 4. The RBD-specific mAb CV30 neutralizes SARS CoV-2 by blocking the**
306 **ACE2- SARS-CoV-2 S interaction.** (**A**) CV1 and CV30 were serially diluted and tested
307 for their ability to neutralize SARS-CoV-2 pseudovirus infection of 293T cells stably
308 expressing ACE2. An ACE2-FC fusion and the anti-EBV mAb AMMO1 were included as
309 positive and negative controls. Data are representative of 6 independent experiments
310 (see Table S1 for details). (**B**) The same mAbs were tested for neutralization of an MLV
311 pseudovirus. (**C**) Biotinlyated ACE2-Fc was immobilized on streptavidin biosensors and
312 then tested for binding to SARS-CoV-2 RBD in the absence and presence of the indicated
313 mAbs using BLI. (**D**) ACE2-Fc was immobilized Protein A biosensors and binding to the
314 indicated serial dilutions of SARS-CoV-2 RBD were measured by BLI and used to
315 determine the binding constant (kD). Red lines represent the measured data and black
316 lines indicate the theoretical fit. (**E**) CV30 was immobilized onto anti-human Fc biosensors
317 and binding to the indicated serial dilutions of SARS-CoV-2 RBD were measured by BLI
318 and used to determine the binding constant (kD). Blue lines represent the measured data

319 and black lines indicate the theoretical fit. Kinetic measurements from **D** and **E** are
320 summarized in Table S2.

321 **METHODS**

322 **Human Subjects**

323 Peripheral blood mononuclear cells (PBMC) and serum were collected from a SARS-
324 CoV-2 positive donor as part of the Hospitalized and Ambulatory Adults with Respiratory
325 Viral Infections (HAARVI) study. All participants signed informed consent, and the
326 following institutional human subjects review committee approved the protocol prior to
327 study initiation: University of Washington IRB (Seattle, Washington, USA).

328 **Recombinant Coronavirus Protein Expression and Purification**

329 pαH-derived plasmids encoding a stabilized His- and strep-tagged SARS-CoV-2
330 ectodomain (pαH-SARS-CoV-2 S2P), SARS-CoV S2P (pαH-SARS-CoV S2P), and the
331 SARS-CoV-2 receptor binding domain fused to a monomeric Fc (pαH-RBD-Fc) have
332 been previously described and were a kind gift from Dr. Jason McLellan (Pallesen et al.,
333 2017; Wrapp et al., 2020).

334 1L of 293 EBNA cells were cultured to a density of 1 million cells/ml and were transfected
335 with 500µg of pαH-SARS-CoV-2 S2P, pαH-SARS-CoV S2P, or pαH-SARS-CoV-2 RBD-
336 Fc using 2 mg polyethylenimine (Polysciences, Cat# 24765). 6 days after transfection,
337 supernatants were harvested by centrifugation and passed through a 0.22µm filter.
338 Supernatant from cells transfected with SARS-CoV-2 S2P, or SARS-CoV S2P, was
339 passed over a HisTrap FF affinity column (GE Healthcare, Cat# 17-5255-01) pre-
340 equilibrated in HisTrap binding buffer (20mM sodium Phosphate, 0.5M NaCl, 10mM
341 Imidazole HCl, pH 7.4) and then washed with HisTrap binding buffer until a baseline A280

342 absorbance was reached and then eluted with 20mM sodium Phosphate, 0.5M NaCl,
343 500mM Imidazole HCl, pH 7.4). SARS-CoV S2P was further purified using a 2ml Strep-
344 Tactin sepharose column (IBA Lifesciences Cat# 2-1201-002) and Strep-Tactin
345 Purification Buffer Set (IBA Lifesciences Cat # 2-1002-001) according to the
346 manufacturer's instructions. The S2P variants were then further purified using a Superose
347 6 10/300 GL column pre-equilibrated in 1XPBS or 2mM Tris 200mM NaCl, pH 8.0.
348 Supernatant containing RBD-Fc was purified over protein A agarose resin (Goldbio, Cat#
349 P-400), cleaved with HRV3C protease (made in house) on-column. The eluate containing
350 the RBD was further purified by SEC using HiLoad 16/600 Superdex 200 pg column (GE
351 Healthcare) pre-equilibrated in 2mM Tris-HCl, 200mM NaCl, pH 8.0. Proteins were
352 directly used for subsequent assays or aliquoted, flash frozen and kept at -80C until
353 further use.

354 **Protein biotinylation**

355 Purified recombinant S2P or RBD were biotinylated at a theoretical 1:1 ratio using the
356 Easylink NHS-biotin kit (Thermofisher) according to the manufacturer's instructions.
357 Excess biotin was removed via size exclusion chromatography using an ENrich SEC 650
358 10 x 300 mm column (Bio-Rad).

359 **ELISA**

360 Immulon 2HB microtiter plates (Thermo Scientific) were coated with 50ng/well of RBD or
361 S2P overnight at room temperature. Plates were washed 4X with PBS with 0.02% Tween-
362 20 (wash buffer). Plates were blocked with 250 μ L of 10% non-fat milk and 0.02% Tween-
363 20 in PBS (blocking buffer) for 1 hr at 37°C. After washing 4X with wash buffer, plasma
364 was prepared at 1:50 dilution in blocking buffer and diluted in three-, four-, or fivefold

365 serial dilutions in plate and incubated for 1 hr at 37°C. Plates were washed 4X in wash
366 buffer and the secondary antibody Goat anti-Human Ig-HRP (Southern Biotech, Cat#
367 2010-05), Peroxidase-conjugated AffiniPure Donkey Anti-Human IgG, Fcγ fragment
368 specific (Jackson ImmunoResearch, Cat#709-035-098), Mouse anti-Human IgM-HRP
369 (Southern Biotech, Cat# 9022-05), or Mouse anti-Human IgA-HRP (Southern Biotech,
370 Cat# 9130-05) was added and incubated at 37°C for 1 hr. After a final 4X wash, 50μL of
371 SureBlue Reserve TMB Peroxidase Substrate (Seracare KPL, Cat# 5120-0080) was
372 added and incubated for 4 min followed by addition of 100μL of 1 N H₂SO₄ to stop the
373 reaction. The optical density at 450nm was measured using a SpectraMax M2 plate
374 reader (Molecular Devices). All wash steps were performed using a BioTek 405 Select
375 Microplate Washer.

376 **B cell sorting**

377 Fluorescent SARS-CoV-2-specific S2P and RBD probes were made by combining
378 biotinylated protein with fluorescently labeled streptavidin (SA). The S2P probes were
379 made at a ratio of 2 moles of trimer to 1 mole SA. Two S2P probes, one labeled with
380 phycoerythrin (PE) (Invitrogen), one labeled with brilliant violet (BV) 711 (Biolegend),
381 were used in this panel in order to increase specificity of the detection of SARS-CoV-2-
382 specific B cells. The RBD probe was prepared at a molar ratio of 4 to 1 of protein to SA,
383 labeled with alexa fluor 647 (Invitrogen). Cryopreserved PBMC from the SARS-CoV-2-
384 infected participant and a SARS-naïve donor were thawed at 37°C and stained for SARS-
385 CoV-2-specific memory B cells with a flow cytometry panel consisting of: a viability dye
386 (7AAD, Invitrogen), CD14 PE-Cy5, CD69 APC-Fire750, CD8a alexa fluor 700, CD3
387 BV510, CD27 BV605, IgM PE-Dazzle594 (BioLegend), CD4 brilliant blue 515 (BB515),

388 IgD BV650, IgG BV786, CD56 PE-Cy5, CD19 PE-Cy7, and CD38 PerCP-Cy5.5 (BD
389 Biosciences). Cells were stained first with the cocktail of the three SARS-CoV-2 probes
390 for 30 min at 4°C, then washed with 2% FBS/PBS and stained with the remaining antibody
391 panel and incubated for 30 min at 4°C. The cells were washed two times and
392 resuspended for sorting in 10% FBS/RPMI media containing 7AAD. The sample was
393 sorted on a FACS Aria II instrument (BD Biosciences) using the following gating strategy:
394 singlets, lymphocytes, live, CD3-, CD14-, CD4-, CD19+, IgD-, IgG+, S2P-PE+ and S2P-
395 BV711+. Two plates of S2P double positive IgD- B cells were single-cell index-sorted
396 into 96-well plates containing 16µl lysis buffer ((3.90% IGEPAL, 7.81mM DTT, 1250
397 units/ml RNase Out (ThermoFisher)). 4 additional plates of the S2P double positive IgD-
398 IgG+ B cell population were single-cell index-sorted into dry 96-well plates and flash
399 frozen on dry ice. The RBD+ frequency of sorted B cells was analyzed post-sort using
400 the index file data in Flow Jo version 9.9.4 (Becton, Dickinson and Company).

401 **B cell sequencing**

402 cDNA was generated from sorted B cells by adding 4µl of iScript (Bio-Rad Cat# 1708891)
403 and cycling according to the manufacturer's instructions. The VH and VL sequences were
404 recovered using gene specific primers and cycling conditions previously described (Tiller
405 et al., 2008). VH or VL amplicons were sanger sequenced (Genewiz). The antibody gene
406 usage was assigned using IMGT/V-QUEST (Brochet et al., 2008). Sequences were
407 included in sequence analysis if a V and J gene identity could be assigned and the
408 sequence contained an in-frame CDR3. Paired VH and VL sequences from S2P positive
409 B cells were codon optimized for human expression using the Integrated DNA
410 Technologies (IDT) codon optimization tool, synthesized as eBlocks (IDT) and cloned into

411 full-length pTT3 derived IgL and IgK expression vectors (Snijder et al., 2018) or subcloned
412 into the pT4-341 HC vector (Mouquet et al., 2010) using inFusion cloning (Clontech).

413 **Antibody purification**

414 Antibody expression plasmids were co-transfected into 293E cells at a density of 106
415 cells/ml in Freestyle 293 media using the 293Free transfection reagent according to the
416 manufacturer's instructions. Expression was carried out in Freestyle 293 media for 6
417 days, after which cells and cellular debris were removed by centrifugation at 4,000 × g
418 followed by filtration through a 0.22 µm filter. Clarified cell supernatant containing
419 recombinant antibodies was passed over Protein A Agarose (Goldbio Cat# P-400-5),
420 followed by extensive washing with PBS, and then eluted with 1 ml of Pierce IgG Elution
421 Buffer, pH 2.0, into 0.1 ml of Tris HCl, pH 8.0. Purified antibodies were then dialyzed
422 overnight into PBS, passed through a 0.2µM filter under sterile conditions and stored at -
423 80°C until use.

424 **Quantification and Statistical methods**

425 Amino acid mutations were identified by aligning the VH/VL gene sequences to the
426 corresponding germline genes (IMGT Repertoire) using the Geneious Software (Version
427 8.1.9). Mutations were counted beginning at the 5' end of the V-gene to the 3' end of the
428 FW3. To quantify the number of amino acid mutations, the sequence alignments were
429 exported from Geneious and imported into R (Version 3.4.1) for analysis (R Core Team,
430 2017) (R Core Team, 2018). This analysis uses the packages Biostrings (Pages H, 2018),
431 seqinr (Charif D, 2007), and tidyverse (Wickham, 2017) in R and GraphPad Prism were
432 used to create graphs.

433 **Biolayer Interferometry (BLI) (Anna and Maddy will double check)**

434 BLI assays were performed on the Octet Red instrument at 30°C with shaking at 500-
435 1,000 RPM.

436 *mAb binding screen:*

437 mAbs were diluted in PBS to a concentration of 20µg/ml and captured using Anti-Human
438 IgG Fc capture (AHC) biosensors (Fortebio) for 240s. After loading, the baseline signal
439 was then recorded for 60s in KB. The sensors were then immersed in PBS containing
440 0.5-2µM of purified SARS CoV-2 S2P, SARS CoV-2 RBD, or SARS-CoV S2P for a 300s
441 association step. The dissociation was then measured for 300s by immersing sensors in
442 kinetics buffer (KB: 1X PBS, 0.01% BSA, 0.02% Tween 20, and 0.005% NaN₃, pH 7.4).
443 As a control for non-specific binding the background signal of VRC01 binding to S2P or
444 RBD was subtracted at each time point.

445 *Kinetic analyses:*

446 For kinetic analyses CV30 was captured on anti-Human IgG Fc capture (AHC) sensors,
447 and ACE-2 Fc was captured on protein A biosensors. ligands were diluted to 10 µg/ml in
448 PBS and loaded for 100s. After loading, the baseline signal was then recorded for 1min
449 inKB. The sensors were immersed into wells containing serial dilutions of purified SARS-
450 CoV-2 RBD in KB for 150s (association phase), followed by immersion in KB for an
451 additional 600s (dissociation phase). The background signal from each analyte-
452 containing well was measured using empty reference sensors and subtracted from the
453 signal obtained with each corresponding mAb loaded sensor. Kinetic analyses were
454 performed at least twice with an independently prepared analyte dilution series. Curve
455 fitting was performed using a 1:1 binding model and the ForteBio data analysis software.

456 Mean k_{on} , k_{off} values were determined by averaging all binding curves that matched the
457 theoretical fit with an R^2 value of ≥ 0.98 .

458 *Antibody competition binding assays*

459 ACE2-Fc was biotinylated with EZ-Link NHS-PEG4-Biotin (Thermo scientific) at a molar
460 ratio of 1:2. Free biotin was removed using a Zeba desalting spin column (Thermo
461 Scientific). Biotinylated ACE2-FC was diluted to $1\mu\text{M}$ in PBS and captured onto
462 streptavidin biosensors (Forte Bio) for 240s. The baseline interference was then read for
463 60s in KB buffer, followed by immersion in a $0.5\mu\text{M}$ solution of recombinant SARS CoV-
464 2 RBD or $0.5\mu\text{M}$ solution of recombinant SARS CoV-2 RBD plus $0.5\mu\text{M}$ of mAb for the
465 300 second association phase. The dissociation was then measured for 300 seconds by
466 immersing sensors in KB. As a control for non-specific binding the background signal of
467 binding of RBD and mAb to uncoated biosensors was subtracted at each time point.

468 *Cell surface SARS-CoV-2 S binding assay.*

469 cDNA for the full-length SARS CoV-2 S isolate USA-WA1/2020 was codon optimized and
470 synthesized by Twist Biosciences and cloned into the pTT3 vector using InFusion cloning
471 (Clontech). pTT3-SARS-CoV-2-S was transfected into 293E cells using 293 Free
472 transfection reagent (EMD Millipore Cat # 72181) according to the manufacturer's
473 instructions. Transfected cells were incubated for 24h at 37°C with shaking.

474 The next day, $1\mu\text{g}$ of each mAb was complexed with $3\mu\text{g}$ of PE-conjugated AffiniPure Fab
475 fragment goat anti-human IgG (Jackson Immunoresearch Cat #109-117-008), and the
476 labeled mAb was incubated for 30 min at RT prior to dilution to $5\mu\text{g}/\text{mL}$ in Freestyle
477 medium containing 10% FBS and 1% Pen/Strep. mAbs were then diluted 2-fold over 8
478 points in 96 well round bottom plates, and an equal volume containing 5×10^5 293E cells

479 expressing SARS-CoV-2 spike proteins was added to each well. The mAb-cells mixture
480 was incubated for 30 min at 37°C. Controls included cells treated with a mAb neutralizing
481 SARS-CoV (CR3022) or with an unrelated mAb (AMMO1, specific for EBV), and
482 untreated with mAb (cells only). The plates were then washed with FACS buffer (PBS +
483 2% FBS + 1mM EDTA) and fixed with 10% formalin. The mean fluorescence intensity
484 (MFI) for PE+ cells was measured on an X-50 flow cytometer (BD Biosciences) and the
485 data analyzed using FlowJo (Tree Star).

486 **Neutralization Assay**

487 HIV-1 derived viral particles were pseudotyped with full length wildtype SARS CoV-2 S
488 (Crawford et al., 2020). Briefly, plasmids expressing the HIV-1 Gag and pol (pHDM-
489 Hgpm2, BEI resources Cat# NR-52517), HIV-1Rev (pRC-CMV-rev1b, BEI resources
490 Cat# NR-52519), HIV-1 Tat (pHDM-tat1b, BEI resources Cat# NR-52518), the SARS
491 CoV2 spike (pHDM-SARS-CoV-2 Spike, BEI resources Cat# NR-52514) and a
492 luciferase/GFP reporter (pHAGE-CMV-Luc2-IRES-ZsGreen-W, BEI resources Cat# NR-
493 52516) were co-transfected into 293T cells at a 1:1:1:1.63:4.63 ratio using 293 Free
494 transfection reagent (EMD Millipore Cat# 72181) according to the manufacturer's
495 instructions. 72 hours later the culture supernatant was harvested, clarified by
496 centrifugation and frozen at -80°C.

497 293 cells stably expressing ACE2 (BEI resources Cat# NR-5251) were seeded at a
498 density of 4×10^3 cells/well in a 100µl volume in 96 well flat bottom tissue culture plates.
499 The next day, mAbs were initially diluted to 100µg/ml in 30µl of cDMEM in 96 well round
500 bottom plates in triplicate. An equal volume of viral supernatant diluted to result in $2 \times$
501 10^5 luciferase units was added to each well and incubated for 60 min at 37 °C. Meanwhile

502 50ul of cDMEM containing 6µg/ml polybrene was added to each well of 293T-ACE2 cells
503 (2µg/ml final concentration) and incubated for 30 min. The media was aspirated from
504 293T-ACE2 cells and 100µl of the virus-antibody mixture was added. The plates were
505 incubated at 37°C for 72 hours. The supernatant was aspirated and replaced with 100ul
506 of Steadyglo luciferase reagent (Promega). 75µl was then transferred to an opaque, white
507 bottom plate and read on a Fluorskan Ascent Fluorimeter. Control wells containing virus
508 but no antibody (cells + virus) and no virus or antibody (cells only) were included on each
509 plate.

510 % neutralization for each well was calculated as the RLU of the average of the cells +
511 virus wells, minus test wells (cells +mAb + virus), and dividing this result difference by
512 the average RLU between virus control (cells+ virus) and average RLU between wells
513 containing cells alone, multiplied by 100.

514 mAbs that showed >50% neutralization at 50µg/ml, or plasma were further analyzed to
515 determine neutralizing potency, by preparing serial dilutions and conducting the
516 neutralization assay as described above. The antibody concentration or plasma dilution
517 that neutralized 50% of infectivity (IC₅₀ or ID₅₀, respectively) was interpolated from the
518 neutralization curves determined using the log(inhibitor) vs. response -- Variable slope
519 (four parameters) fit using automatic outlier detection in Graphpad Prism Software. As a
520 control for specificity SARS CoV2-mAbs were tested for neutralizing activity against HIV-
521 1 derived virions pseudotyped with murine leukemia virus envelope (MLV).

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670

Figure 1

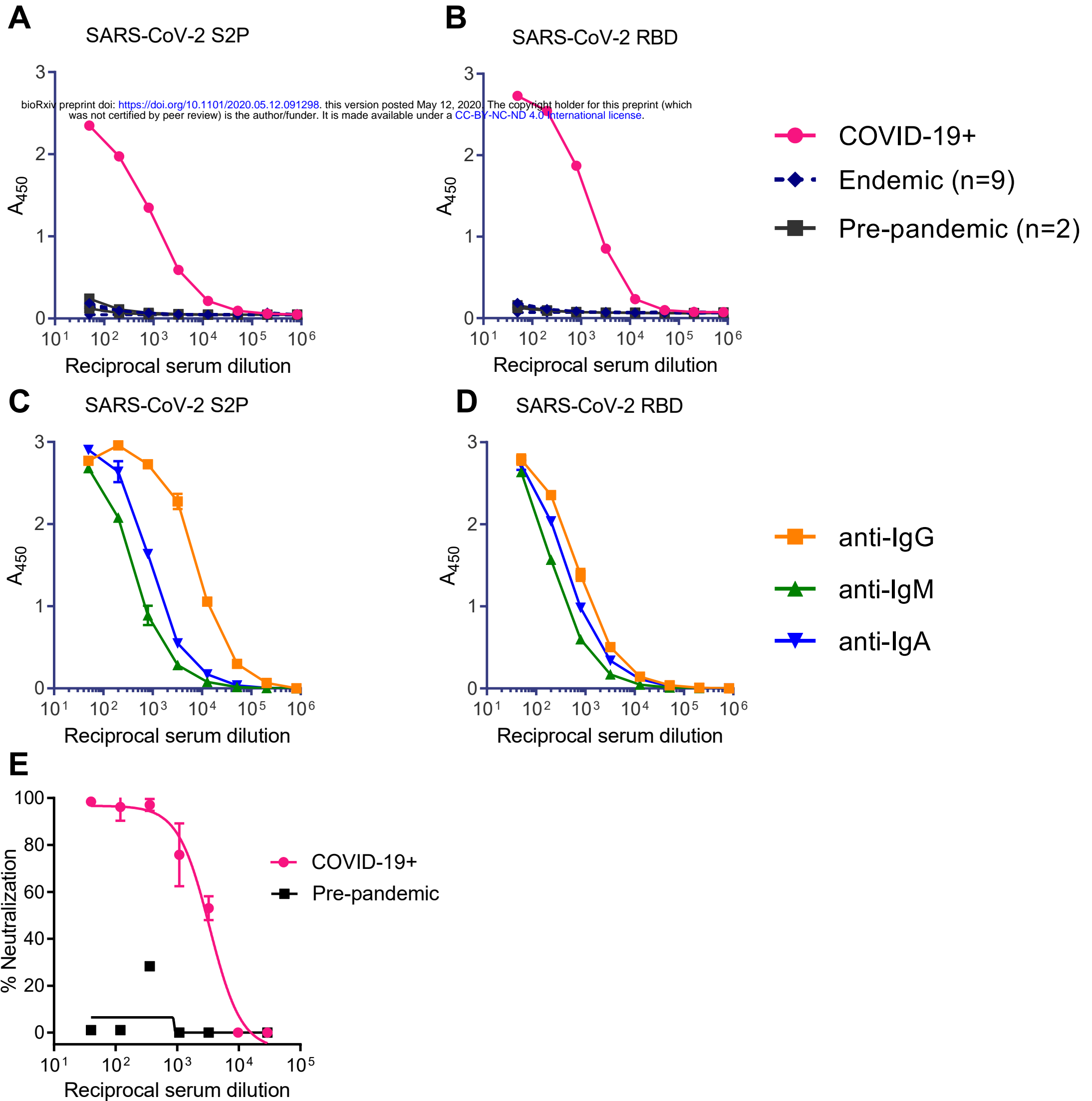


Figure 2

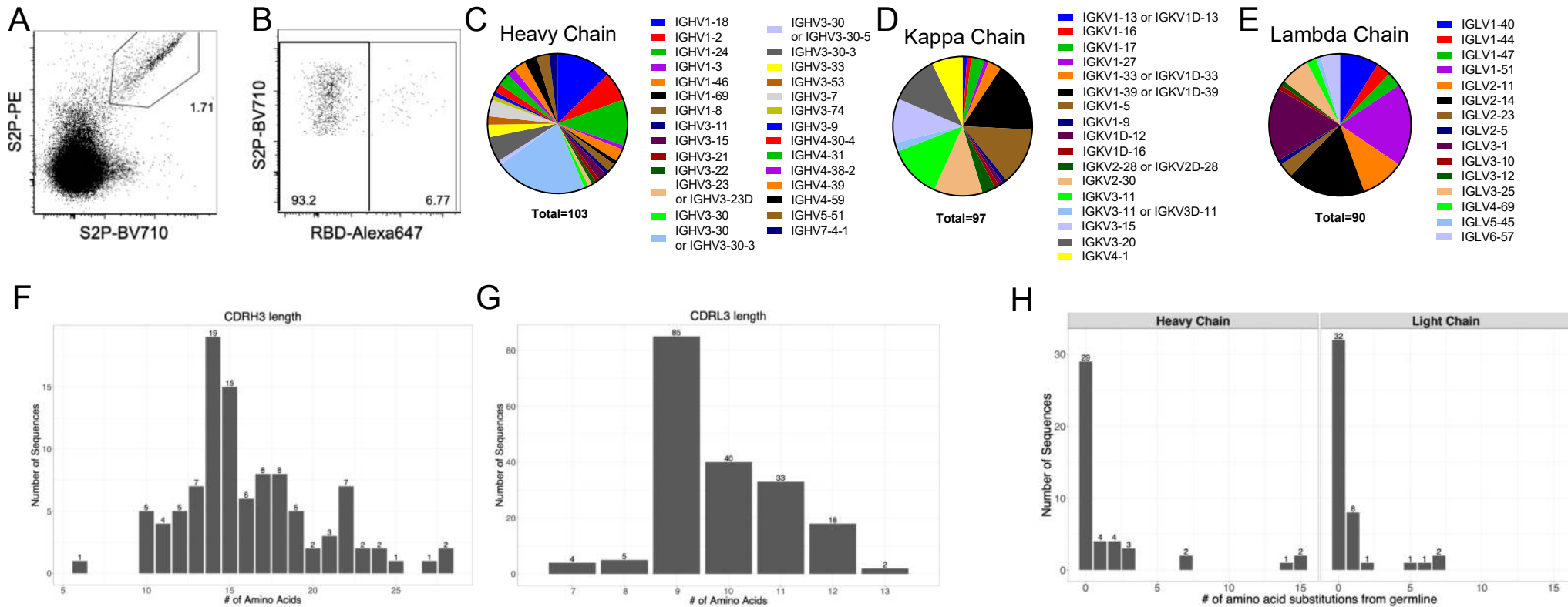


Figure 3

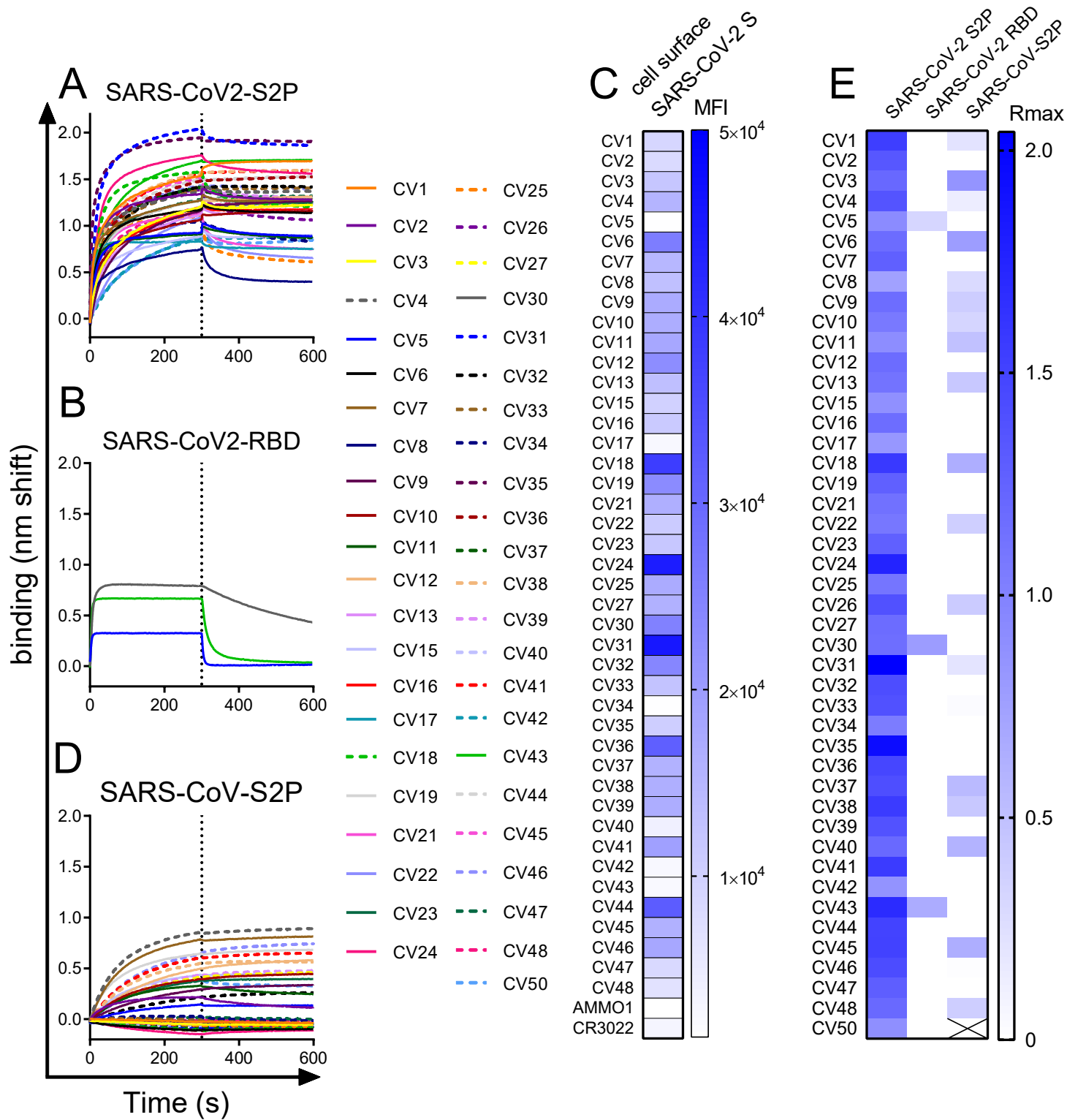
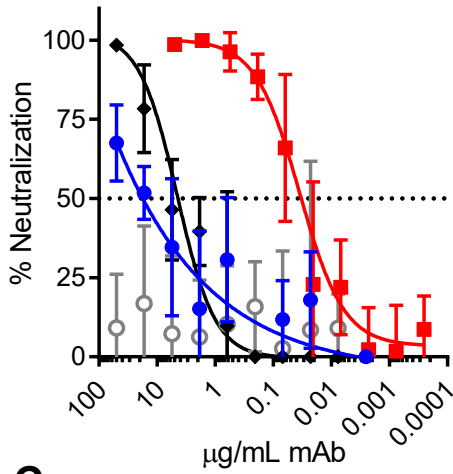
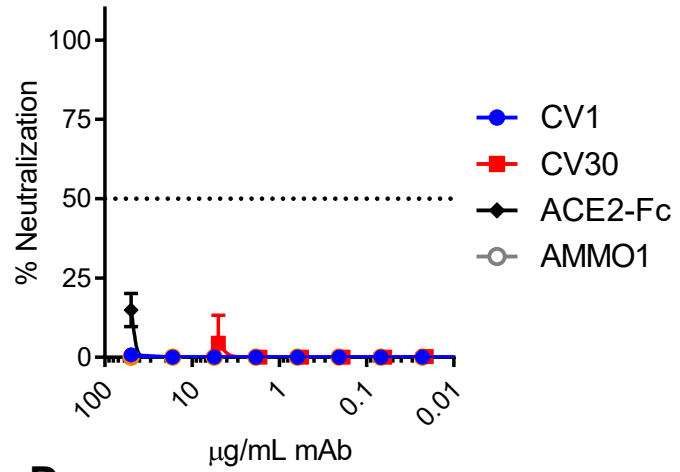


Figure 4

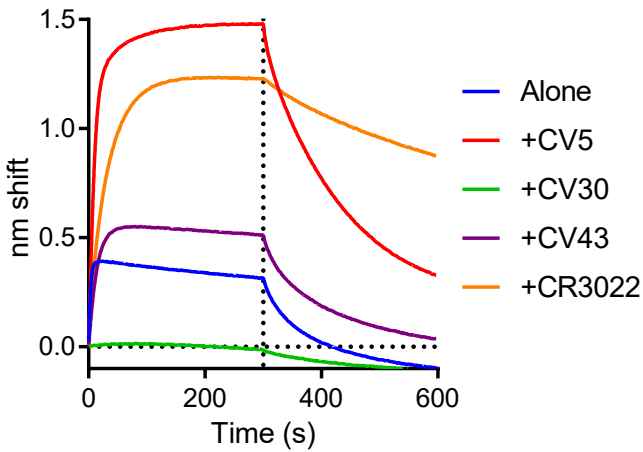
A SARS-CoV-2 Pseudovirus



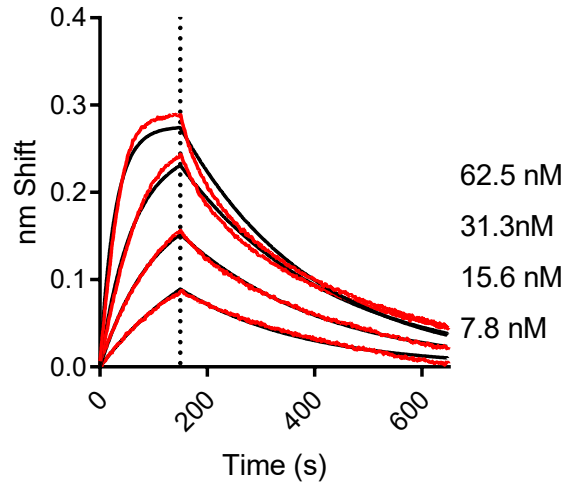
B MLV Pseudovirus



C ACE2-Fc Binding to SARS-CoV-2 RBD



D ACE2-Fc binding to SARS-CoV-2 RBD



E CV30 binding to SARS-CoV-2 RBD

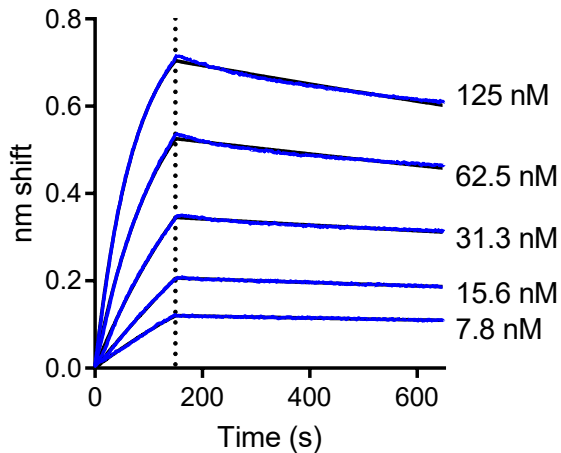


Figure S1

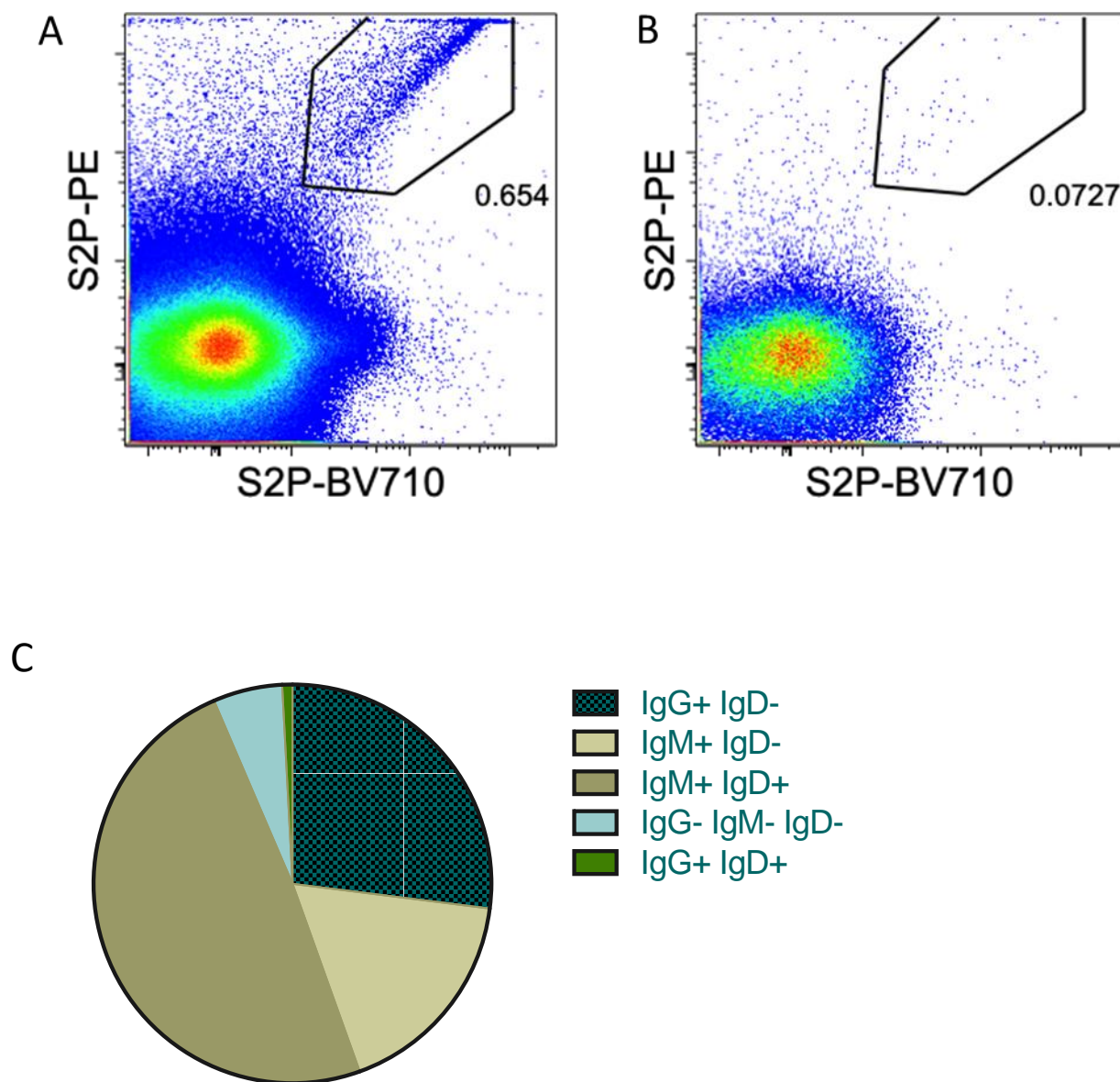


Figure S1. Identification and isolation of SARS-CoV-2 specific B cells by flow cytometry. Staining of PBMCs with S2P-probes gated on total live CD3⁻ CD19⁺ B cells indicating the frequency of S2P⁺ B cells for the (A) confirmed SARS-CoV-2 donor ~3 weeks post-infection and (B) a pre-pandemic control subject. (C) the proportion of S2P⁺ B cells analyzed from the SARS-CoV-2⁺ participant by isotype expression.

Figure S2

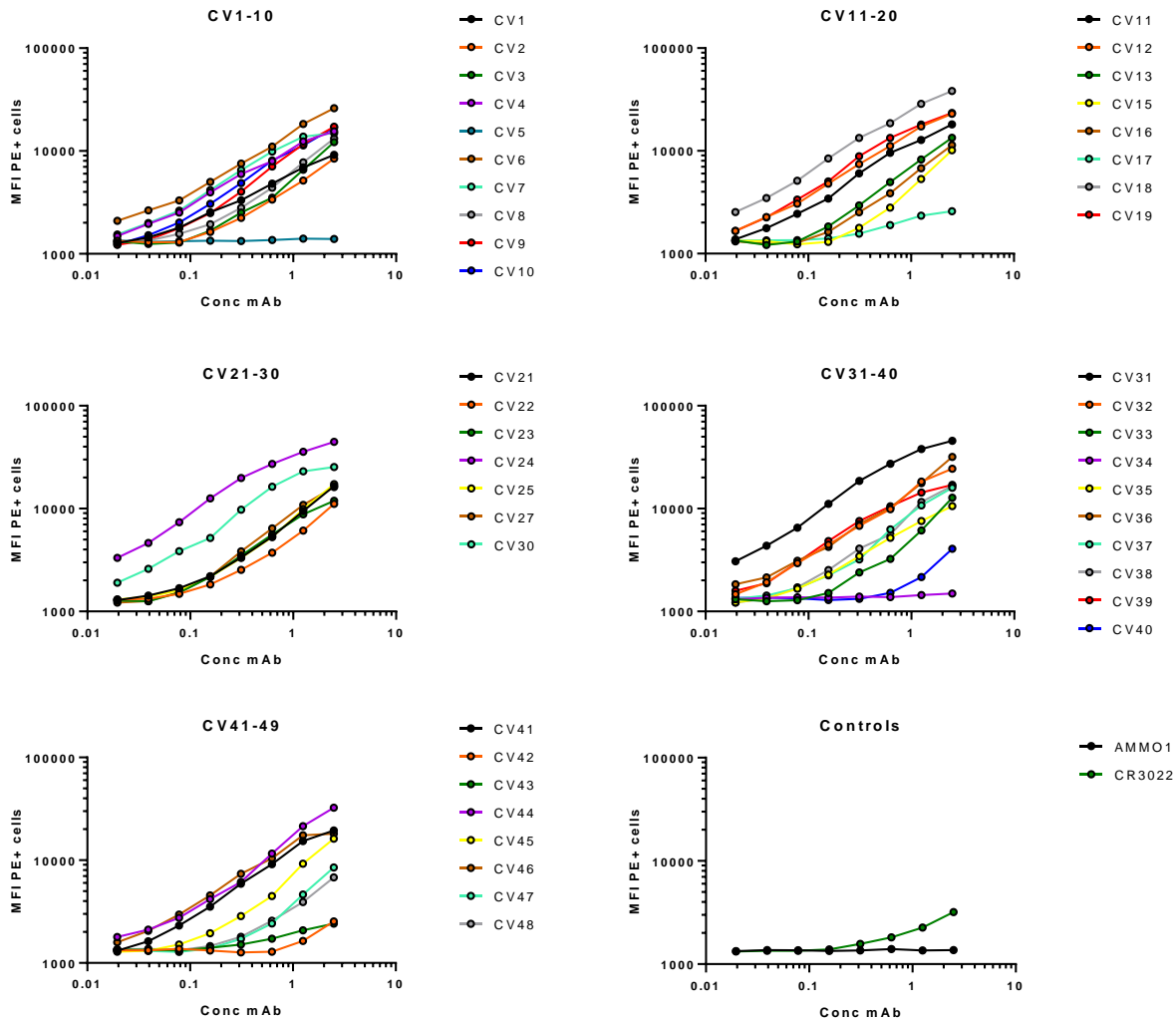


Figure S2. Staining of cell-surface expressed SARS-CoV-2 S. The indicated mAbs were labeled with phycoerythrin (PE) and used to stain 293E cells transfected with wildtype SARS-Cov-2 S by flow cytometry at the indicated dilutions. The mean fluorescence intensity (MFI) of PE+ cells is shown.

Table S1. Neutralizing activity and gene usage of cloned mAbs

mAb	IC50 (µg/ml)	Isotype	VH gene	AA mutations	VH/VL gene	AA mutations
CV1	15±6.4 n=6	IgG	IGHV4-38-2	0	IGLV1-44*01	0
CV2	>50	IgG	IGHV3-30*04	0	IGKV3-15*01	0
CV3	>50	IgG	IGHV7-4-1*02	7	IGKV1-39*01	7
CV4	>50	IgG	IGHV3-30*01	0	IGKV1-5*03	0
CV5	>50	N/D	IGHV1-46*01	3	IGKV4-1*01	1
CV7	>50	IgG	IGH3-30*01	0	IGKV1-5*03	0
CV8	>50	IgG	IGHV1-18*01	0	IGKV3-20*01	0
CV9	>50	IgG	IGHV4-39*01	0	IGLV2-14*01	1
CV10	>50	IgG	IGHV4-59*01	1	IGKV3-20*01	1
CV11	>50	IgG	IGHV4-31*03	0	IGKV3-11*01	0
CV12	>50	IgG	IGHV3-30*04	15	IGKV2-30*01	6
CV13	>50	IgG	IGH7-4-1*02	7	IGKV1-39*01	7
CV15	>50	IgG	IGHV3-7*01	0	IGLV2-11*01	0
CV16	>50	IgG	IGHV5-51*01	0	IGKV3-20*01	0
CV17	>50	N/D	IGHV1-2*02	0	IGLV2-23*01	0
CV18	>50	IgG	IGHV1-24*01	0	IGLV1-51*01	0
CV19	>50	N/D	IGHV1-2*02	0	IGKV3-20*01	0
CV21	>50	IgG	IGHV3-15*01	0	IGKV3-11*01	0
CV22	>50	I N/D	IGHV3-21*01	0	IGLV4-69*01	0
CV23	>50	IgG	IGHV1-3*01	0	IGLV3-25*03	0
CV24	>50	IgG	IGHV1-24*01	0	IGLV1-51*01	0
CV25	>50	IgG	IGHV4-30-4*01	0	IGKV3-15*01	0
CV26	>50	IgG	IGHV3-30-3*01	0	IGKV1-17*01	0
CV27	>50	IgG	IGHV3-30*04	1	IGLV2-14*01	1
CV30	0.03±0.02 n=6	IgG	IGHV3-53*01	2	IGKV3-20*01	0
CV31	>50	IgG	IGHV1-24*01	0	IGLV1-51*01	0
CV32	>50	IgG	IGHV1-2*02	2	IGLV1-51*01	0
CV33	>50	IgG	IGHV1-18*01	1	IGLV1-40*1	0
CV34	>50	IgG	IGHV3-30-3*01	0	IGLV3-12*02	0
CV35	>50	IgG	IGHV4-38*02	0	IGLV1-44*01	0
CV36	>50	IgG	IGHV1-2*02	3	IGLV3-25*03	1
CV37	>50	IgG	IGHV1-18*01	0	IGKV1-33*01	0
CV38	>50	IgG	IGHV3-30*04	0	IGKV3-11*01	0
CV39	>50	IgG	IGHV3-30*04	14	IGKV2-30*01	2
CV40	>50	IgG	IGHV1-18*01	2	IGKV1-17*01	0
CV41	>50	IgG	IGHV3-30*04	0	IGKV3-15*01	0
CV42	>50	IgG	IGHV1-18*01	1	IGKV1-39*01	1
CV43	>50	IgG	IGHV3-30*04	0	IGLV6-57*02	0

CV44	>50	IgG	IGHV1-46*01	0	IGLV3-25*03	1
CV45	>50	IgG	IGHV1-18*01	0	IGLV1-40*01	0
CV46	>50	IgG	IGHV3-30*04	15	IGKV2-30*01	5
CV47	>50	IgG	IGHV1-18*01	2	IGKV1-17*01	0
CV48	>50	IgG	IGHV1-69*09	3	IGKV2-30*01	1
CV50	N/D	IgG	IGHV3-33*01	0	IGLV3-10*01	0

*N/D: not determined

Table S2. Kinetic analysis of ACE2-Fc and CV30 IgG interaction with SARS-CoV-2 RBD

Ligand	Analyte	K_D (M X10 ⁻⁹)	k_{on} (1/Ms)X10 ⁴	K_{on} errorX10 ³	k_{off} (1/s)X10 ⁻³	k_{off} error X 10 ⁻⁵
ACE2-FC	SARS-CoV -2 RBD	5.97	54.8	19.7	3.27	2.58
CV30 IgG	SARS-CoV -2 RBD	3.63	8.36	2.97	0.30	0.30