

26 **Abstract**

27 The World Health Organization has recently declared the ongoing outbreak of COVID-
28 19, which is caused by a novel coronavirus SARS-CoV-2, as pandemic. There is
29 currently a lack of knowledge in the antibody response elicited from SARS-CoV-2
30 infection. One major immunological question is concerning the antigenic differences
31 between SARS-CoV-2 and SARS-CoV. We address this question by using plasma from
32 patients infected by SARS-CoV-2 or SARS-CoV, and plasma obtained from infected or
33 immunized mice. Our results show that while cross-reactivity in antibody binding to the
34 spike protein is common, cross-neutralization of the live viruses is rare, indicating the
35 presence of non-neutralizing antibody response to conserved epitopes in the spike.
36 Whether these non-neutralizing antibody responses will lead to antibody-dependent
37 disease enhancement needs to be addressed in the future. Overall, this study not only
38 addresses a fundamental question regarding the antigenicity differences between
39 SARS-CoV-2 and SARS-CoV, but also has important implications in vaccine
40 development.

41 **Introduction**

42 The emergence of spread of a novel coronavirus SARS-CoV-2 causing severe
43 respiratory disease (COVID-19) has now led to a pandemic with major impact on global
44 health, economy and societal behavior (Coronaviridae Study Group of the International
45 Committee on Taxonomy of, 2020; Poon and Peiris, 2020; Zhu et al., 2020). As of 2020
46 March 15, over 150,000 confirmed cases of SARS-CoV-2 have been reported with close
47 to 6,000 deaths. Phylogenetic analysis has demonstrated that SARS-CoV-2 and SARS-
48 CoV, a coronavirus that also caused a global outbreak in 2003, are closely related
49 phylogenetically, with genomic nucleotide sequence identity of around 80% (Wu et al.,
50 2020; Zhou et al., 2020). Moreover, it has been shown that both viruses use the
51 angiotensin-converting enzyme 2 (ACE2) as the receptor for cell entry and infection
52 (Letko et al., 2020; Li et al., 2003).

53

54 The spike glycoprotein (S) on the surface of coronaviruses is essential for virus entry
55 through binding to the ACE2 receptor and viral fusion with the host cell. The S protein
56 forms a homotrimer in which each protomer is composed of two subunits, S1 and S2
57 (Figure 1A). Binding between the receptor-binding domain (RBD) in the S1 subunit and
58 the ACE2 receptor triggers a conformational change in the S protein that subsequently
59 initiates membrane fusion events with the host cell. The RBD is also a primary target of
60 the antibody response in humoral immunity and is believed to be the major protective
61 antigen (Chen et al., 2005). The prefusion structure of the S protein of SARS-CoV-2 has
62 been recently determined by cryo-EM (Wrapp et al., 2020), and revealed overall
63 structural similarity to that of SARS-CoV. However, most monoclonal antibodies tested
64 to date that target the RBD of SARS-CoV have failed to bind to the RBD of SARS-CoV-2
65 (Tian et al., 2020; Wrapp et al., 2020), suggesting that the antigenicity of these two
66 viruses to the RBD is quite distinct. So far, data have not yet been reported from

67 polyclonal human sera from patients to evaluate the antibody response elicited by
68 SARS-CoV-2 infection and to determine whether cross-reactive antibody responses
69 between SARS-CoV-2 and SARS-CoV can be generated. In this study, we examined the
70 antibody responses in 15 patients from Hong Kong who were infected by SARS-CoV-2,
71 and seven by SARS-CoV. Mice infected or immunized with SARS-CoV-2 or SARS-CoV
72 were also used to investigate cross-reactivity of antibody responses between SARS-
73 CoV-2 and SARS-CoV.

74

75 **Results**

76 **Patient samples show cross-reactivity in binding**

77 Fifteen heparin anticoagulated plasma samples (from day 2 to 22 post-symptom onset)
78 from SARS-CoV-2 infected patients were analyzed (Table S1). Binding of plasma to the
79 S ectodomain and RBD of both SARS-CoV-2 and SARS-CoV (see Methods) was
80 measured by ELISA (Figure 1B, Figure S1). Plasma samples from healthy donors
81 collected from the Hong Kong Red Cross served as controls. As compared to the
82 plasma from healthy donors, plasma from patients from day 10 post-symptom onward
83 reacted strongly in ELISA binding assays to the S ectodomain (p -value $< 2e-16$, two-
84 tailed t-test) and RBD (p -value = $2e-13$, two-tailed t-test) of SARS-CoV-2. Interestingly,
85 the plasma from SARS-CoV-2-infected patients could also cross-react, although less
86 strongly, with the SARS-CoV S ectodomain (p -value = $8e-06$, two-tailed t-test) and the
87 SARS-CoV RBD (p -value = 0.048, two-tailed t-test) (Figure 1B). Nevertheless, only five
88 of the samples from the SARS-CoV-2-infected patients had convincing antibody binding
89 responses to the SARS-CoV RBD. The other plasma reacted more weakly or not at all
90 with the SARS-CoV RBD (Figure 1B). This result indicates that the cross-reactive
91 antibody response to the S protein after SARS-CoV-2 infection mostly targets non-RBD
92 regions. Consistent with that observation, reactivity of the plasma from SARS-CoV-2-

93 infected patients could be detected with the S2 subunit of SARS-CoV-2 (p -value = $2e-4$,
94 two-tailed t -test, Figure 1B).

95

96 We also analyzed seven heparin anticoagulated convalescent (3-6 months post
97 infection) plasma samples from SARS-CoV infected patients. Similar to that observed in
98 plasma samples from SARS-CoV-2-infected patients, cross-reactivity in binding could be
99 detected (Figure 1B). As compared to the plasma from healthy donors, SARS-CoV-
100 infected patients have significant cross-reactivity in binding to SARS-CoV-2 spike (p -
101 value = 0.03, two-tailed t -test), RBD (p -value = 0.03, two-tailed t -test), and S2 subunit (p -
102 value = 0.007, two-tailed t -test). These results show that cross-reactivity in binding is
103 common between SARS-CoV and SARS-CoV-2 infections in both directions.

104

105 **Patient samples show limited cross-neutralization**

106 We next tested the neutralization activity of these plasma samples from SARS-CoV-2-
107 infected patients. Except for four plasma samples that came from patients with less than
108 12 days post-symptom onset with concomitantly low reactivity to both SARS-CoV-2 S
109 ectodomain and RBD, all other plasma samples could neutralize the SARS-CoV-2 virus
110 with titers ranging from 1:40 to 1:640 (Figure 1C, Table S1). However, only one plasma
111 sample could cross-neutralize SARS-CoV, with very low neutralization activity (1:10). In
112 fact, that cross-neutralizing plasma sample had the strongest reactivity in binding against
113 SARS-CoV S ectodomain among all 15 patient samples, although its binding activity
114 against SARS-CoV RBD is not particularly strong (Table S1).

115

116 Similarly, while five of the seven plasma samples from SARS-CoV-convalescent patients
117 could neutralize SARS-CoV with titers ranging from 1:40 to 1:320, none can cross-
118 neutralize SARS-CoV-2 (Figure 1C). These results show that although cross-reactivity in

119 binding is common between plasma from SARS-CoV-2 and SARS-CoV infected
120 patients, cross-neutralization activity is rare.

121

122 **Cross-reactivity in mouse infection and vaccination**

123 To further investigate the cross-reactivity of antibody responses to SARS-CoV-2 and
124 SARS-CoV, we analyzed the antibody response of plasma collected from mice infected
125 or immunized with SARS-CoV-2 or SARS-CoV ($n = 5$ or 6 per experimental and control
126 groups). Plasma from mice with mock immunization with a genetically more distant
127 betacoronavirus coronavirus OC43-CoV, PBS or adjuvant were used as negative
128 controls (Figure 2A-D). As compared to controls, plasma from mice immunized with
129 SARS-CoV-2 virus reacted strongly to its autologous S ectodomain (p -value < 0.002 ,
130 two-tailed t -test, Figure 2A) and RBD (p -value $< 1e-4$, two-tailed t -test, Figure 2B).
131 Similarly, plasma from mice immunized with SARS-CoV virus reacted strongly to its
132 autologous S ectodomain (p -value $< 2e-7$, two-tailed t -test, Figure 2C) and RBD (p -value
133 $< 6e-6$, two-tailed t -test, Figure 2D). In addition, plasma from mice immunized with
134 SARS-CoV S ectodomain could react to its autologous RBD (p -value < 0.02 , two-tailed t -
135 test, Figure 2D). However, while plasma from mice infected with SARS-CoV virus could
136 react with its autologous S ectodomain (p -value $< 8e-6$, two-tailed t -test, Figure 2C) and
137 RBD (p -value $< 2e-5$, two-tailed t -test, Figure 2D), the reactivity of plasma from mice
138 infected with SARS-CoV-2 virus to its autologous S ectodomain and RBD could not be
139 observed in this assay (p -value > 0.28 , two-tailed t -test, Figure 2A-B). Unlike SARS-CoV
140 virus, which can replicate in wild-type mice (Yang et al., 2004), it has been recently
141 shown that SARS-CoV-2 is only able to replicate in human ACE2-expression transgenic
142 mice but not wild-type mice (Bao et al., 2020), which then can explain the weak immune
143 response from SARS-CoV-2-infected wild-type mice in this study.

144

145 Interestingly, we observed some cross-reactivity of plasma from SARS-CoV-2-
146 immunized mice to the SARS-CoV S ectodomain (p -value $< 4e-5$, two-tailed t-test,
147 Figure 2C) and less so to SARS-CoV RBD (p -value < 0.006 , two-tailed t-test, Figure 2D),
148 as well as plasma from SARS-CoV-infected mice to the SARS-CoV-2 S ectodomain (p -
149 value < 0.005 , two-tailed t-test, Figure 2A). The conclusion that the cross-reactive
150 antibodies mostly target non-RBD regions is supported by the stronger reactivity of the
151 antibody responses from SARS-CoV-2 immunization with the SARS-CoV S ectodomain
152 than to its RBD, and that plasma from SARS-CoV-infected mice did not react at all with
153 SARS-CoV-2 RBD (p -value > 0.5 , two-tailed t-test, Figure 2B). Despite the presence of
154 cross-reactivity in binding, cross-neutralization activity was not detected in any of the
155 mouse plasma samples (Figure 2E-F), corroborating with our findings from human
156 patients.

157

158 **Discussion**

159 The work here shows that antibody responses in the SARS-CoV-2 infected patient
160 cohort are generated to both S protein and RBD in the majority of the patients.
161 Furthermore, cross-reactivity with SARS-CoV can be detected in these plasma samples
162 as well as in mice studies. These cross-reactive antibody responses mostly target non-
163 RBD regions. Consistently, higher sequence conservation is found between the S2
164 subunits of SARS-CoV-2 and SARS-CoV (90% amino-acid sequence identity) as
165 compared to that of their RBDs (73% amino-acid sequence identity). Nonetheless, some
166 SARS-CoV-2-infected patients were able to produce cross-reactive antibody responses
167 to SARS-CoV RBD. Consistent with these findings, a human antibody CR3022 that
168 neutralizes SARS-CoV (ter Meulen et al., 2006) has recently been reported to also bind
169 to the RBD of SARS-CoV-2 (Tian et al., 2020).

170

171 While cross-reactive antibody binding responses to both SARS-CoV-2 and SARS-CoV S
172 proteins appears to be relatively common in this cohort, cross-neutralizing responses are
173 rare. Only one out of 15 SARS-CoV-2-infected patients was able to generate a cross-
174 neutralizing response to both SARS-CoV-2 and SARS-CoV viruses, and this cross-
175 reactive response was very weak. Therefore, it is possible that only a subset of the
176 cross-reactive binding epitopes is a *bona fide* neutralizing epitope. This notion is also
177 supported by our recent study, which showed that the cross-reactive antibody CR3022
178 could not neutralize SARS-CoV-2 despite its strong binding (Yuan et al., 2020). Future
179 studies need to investigate whether these non-neutralizing antibody responses can
180 confer *in vivo* protections despite the lack of *in vitro* neutralization activity, which have
181 been observed in some non-neutralizing antibodies to other viruses (Bajic et al., 2019;
182 Bangaru et al., 2019; Bootz et al., 2017; Burke et al., 2018; Dreyfus et al., 2012; Henschel
183 et al., 1988; Lee et al., 2016; Petro et al., 2015; Watanabe et al., 2019). On the contrary,
184 non-neutralizing antibody responses can also lead to antibody-dependent enhancement
185 (ADE) of infection as reported in other coronaviruses (Tseng et al., 2012; Wang et al.,
186 2014; Weiss and Scott, 1981). Whether ADE plays a role in SARS-CoV-2 infection will
187 need to be carefully examined, due to its potential adverse effect in vaccination (Tseng
188 et al., 2012).

189

190 SARS-CoV-2 is the third newly emerged coronavirus to cause outbreaks (along with
191 SARS-CoV and MERS-CoV) in the past two decades. Since Coronavirus outbreak are
192 likely to continue to pose global health risks in the future (Menachery et al., 2015;
193 Menachery et al., 2016), the possibility of developing a cross-protective vaccine against
194 multiple coronaviruses has been considered. Identification of cross-protective epitopes
195 on the coronavirus S protein will be important for the development of a more universal
196 coronavirus vaccine analogous to those currently in development for influenza virus. Our

197 findings suggest that such broadly cross-protective epitopes are not common in the
198 human immune repertoire. Moving forward, monoclonal clonal antibodies discovery and
199 characterization will be crucial to the development of a SARS-CoV-2 vaccine in short-
200 term, as well as a cross-protective coronavirus vaccine in long-term.

201

202 **Methods**

203 **Recruitment of patients and specimen collections**

204 Patients with RT-PCR confirmed COVID-19 disease at the Infectious Disease Centre of
205 the Princess Margaret Hospital, Hong Kong, were invited to participate in the study after
206 providing informed consent. The study was approved the institutional review board of the
207 Hong Kong West Cluster of the Hospital Authority of Hong Kong (approval number:
208 UW20-169). Specimens of heparinized blood were collected from the patients, and the
209 plasma were separated and stored at -80°C until use. The plasma was heat inactivated
210 at 56°C for 30 minutes before use. The plasma samples from patients with SARS-CoV
211 infection were obtained from the bio-repository of specimens stored from patients
212 following the SARS outbreak in 2003.

213

214 **Protein expression and purification**

215 Ectodomain (residues 14-1195) with K968P/V969P mutations and RBD (residues: 306-
216 527) of the SARS-CoV spike (S) protein (GenBank: ABF65836.1), as well as the
217 ectodomain (residues 14-1213) with R682G/R683G/R685G/K986P/V987P mutations
218 and RBD (residues 319-541) of the SARS-CoV-2 spike protein (GenBank: QHD43416.1)
219 were cloned into a customized pFastBac vector (Ekiert et al., 2011). K968P/V969P were
220 stabilizing mutations in the SARS-CoV spike protein (Kirchdoerfer et al., 2018) and the
221 corresponding K986P/V987P mutations in the SARS-CoV-2 spike protein should have
222 the same stabilizing effect due to sequence similarity. R682G/R683G/R685G mutations

223 in the SARS-CoV-2 spike protein were designed to knock-out the furin cleavage site that
224 is a novel addition to this coronavirus compared to related sequences in bats and
225 pangolins (Wong et al., 2020). The spike ectodomain constructs were fused with an N-
226 terminal gp67 signal peptide and a C-terminal BirA biotinylation site, thrombin cleavage
227 site, trimerization domain, and His₆ tag. The RBD constructs were fused with an N-
228 terminal gp67 signal peptide and a C-terminal His₆ tag. Recombinant bacmid DNA was
229 generated using the Bac-to-Bac system (Life Technologies). Baculovirus was generated
230 by transfecting purified bacmid DNA into Sf9 cells using FuGENE HD (Promega), and
231 subsequently used to infect suspension cultures of High Five cells (Life Technologies) at
232 an MOI of 5 to 10. Infected High Five cells were incubated at 28 °C with shaking at
233 110 r.p.m. for 72 h for protein expression. The supernatant was then concentrated using
234 a Centrimate cassette (10 kDa MW cutoff for RBD and 30 kDa MW cutoff for spike
235 protein, Pall Corporation). Spike ectodomain and RBD proteins were purified by Ni-NTA
236 (Figure S2), followed by size exclusion chromatography, and then buffer exchanged into
237 PBS. The S2 extracellular domain of SARS-CoV-2 was purchased from Sino Biological,
238 China.

239

240 **Mouse immunization**

241 6-8 weeks Balb/c mice were immunized with 10⁵ pfu of SARS-CoV, SARS-CoV-2,
242 HCoV-OC43 or 15 µg of SARS-CoV spike protein in 150 µL PBS together with 50 µL
243 Addavax (MF59-like squalene adjuvant from InvivoGen) through intraperitoneally
244 injection (i.p.). For the control group, Balb/c mice were injected intraperitoneally (i.p.)
245 with 50 µL Addavax plus 150 µL PBS, or 200 µL PBS only. The plasma samples were
246 collected on day 14 post-vaccination using heparin tubes. The experiments were
247 conducted in The University of Hong Kong Biosafety Level 3 (BSL3) facility. This study
248 protocol was carried out in strict accordance with the recommendations and was

249 approved by the Committee on the Use of Live Animals in Teaching and Research of the
250 University of Hong Kong (CULATR 4533-17).

251

252 **Mouse infection**

253 6-8 weeks Balb/c mice were anesthetized with Ketamine and Xylazine, and infected
254 intranasally (i.n.) with 10^5 pfu of SARS-CoV or SARS-CoV-2 diluted in 25 μ L PBS.

255 Mouse plasma samples were collected on day 14 post-infection using heparin tubes.

256 The experiments were conducted in the University of Hong Kong Biosafety Level 3
257 (BSL3) facility.

258

259 **ELISA binding assay**

260 A 96-well enzyme-linked immunosorbent assay (ELISA) plate (Nunc MaxiSorp, Thermo
261 Fisher Scientific) was first coated overnight with 100 ng per well of purified recombinant
262 protein in PBS buffer. To substrate the background noise caused by the unspecific

263 binding of antibodies from the samples, serum-specific background noise (SSBN)

264 normalization approach was used (Moritz et al., 2019). In brief, an additional plate was

265 coated overnight with PBS buffer only. The plates coated with either purified

266 recombinant protein or PBS were then blocked with PBS containing 5% non-fat milk

267 powder at room temperature for 2 hours. Each mouse plasma sample was 1:10 diluted

268 and human sample was serially diluted from 1:100 to 1:12800 in PBS. Each sample was

269 then added into the ELISA plates that were coated with purified recombinant protein or

270 PBS buffer respectively for 2-hour incubation at 37°C. After extensive washing with PBS

271 containing 0.1% Tween 20, each well in the plate was further incubated with the HRP-

272 sheep anti-mouse or anti-human secondary antibody (1:5000, GE Healthcare) for 1 hour

273 at 37°C. The ELISA plates were then washed five times with PBS containing 0.1%

274 Tween 20. Subsequently, 50 μ L of each solution A and B (R&D Systems) was added

275 into each well. After 15 minutes incubation, the reaction was stopped by adding 50 μ L of
276 2 M H₂SO₄ solution and analyzed on a Sunrise (Tecan) absorbance microplate reader at
277 450 nm wavelength. The normalized results were obtained by the calculating the
278 difference between the OD of the purified recombinant protein-coated well and the PBS-
279 coated well.

280

281 **Microneutralization assay**

282 Plasma samples were diluted in serial two-fold dilutions commencing with a dilution of
283 1:10, and mixed with equal volumes of SARS-CoV or SARS-CoV-2 at a dose of 200
284 tissue culture infective doses 50% (TCID₅₀) determined by Vero and Vero E6 cells
285 respectively. After 1 h of incubation at 37°C, 35 μ l of the virus-serum mixture was added
286 in quadruplicate to Vero or Vero E6 cell monolayers in 96-well microtiter plates. After 1 h
287 of adsorption, the virus-serum mixture was removed and replaced with 150ul of virus
288 growth medium in each well. The plates were incubated for 3 days at 37°C in 5% CO₂ in
289 a humidified incubator. Cytopathic effect was observed at day 3 post-inoculation. The
290 highest plasma dilution protecting 50% of the replicate wells was denoted as the
291 neutralizing antibody titer. A virus back-titration of the input virus was included in each
292 batch of tests.

293

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404 **Acknowledgements**

405 This work was supported by Calmette and Yersin scholarship (H.L.), NIH K99 AI139445
406 (N.C.W.), Bill and Melinda Gates Foundation OPP1170236 (I.A.W.), Guangzhou Medical
407 University High-level University Innovation Team Training Program (Guangzhou Medical
408 University released [2017] No.159) (C.K.P.M. and J.S.M.P), the US National Institutes of
409 Health (contract no. HHSN272201400006C) (J.S.M.P), National Natural Science
410 Foundation of China (NSFC)/Research Grants Council (RGC) Joint Research Scheme
411 (N_HKU737/18) (C.K.P.M. and J.S.M.P), and International Cooperation and Exchange
412 of the National Natural Science Foundation of China (Grant No. 8181101118) (J.Z.). We
413 acknowledge the support of the clinicians who facilitated this study including Drs John
414 Yu Hong Chan, Daphne Pui-Lin Lau, and Ying Man Ho and the clinical team at

415 Infectious Diseases Centre, Princess Margaret Hospital, Hospital Authority of Hong
416 Kong and the patients who consented to participate in this investigation.

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418 **Author contributions**

419 H.L., N.C.W., J.S.M.P., I.A.W., C.K.P.M and O.T.Y.T conceived and designed the study.

420 N.C.W. and M.Y. expressed and purified the proteins. H.L., R.T.Y.S., W.W.N., G.K.Y.,

421 Y.L., Y.W. and R.A.P.M.P performed the experiments. O.T.Y.T, W.S.L, J.M.C.C,

422 T.S.H.C, and C.Y.C.C. organized patient recruitment, data collection and sampling. H.L.,

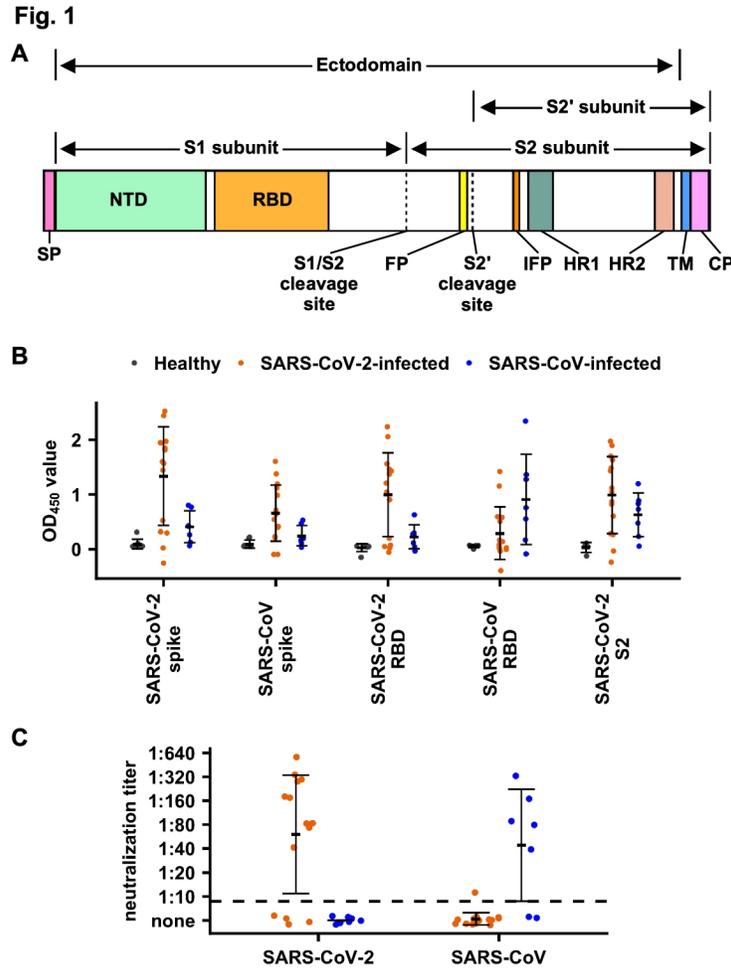
423 N.C.W., J.Z. L.L.M.P, and C.K.P.M. analyzed the data. H.L., N.C.W., J.S.M.P., I.A.W.

424 and C.K.P.M. wrote the paper and all authors reviewed and edited the paper.

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426 **Competing interests**

427 The authors declare no competing interests.

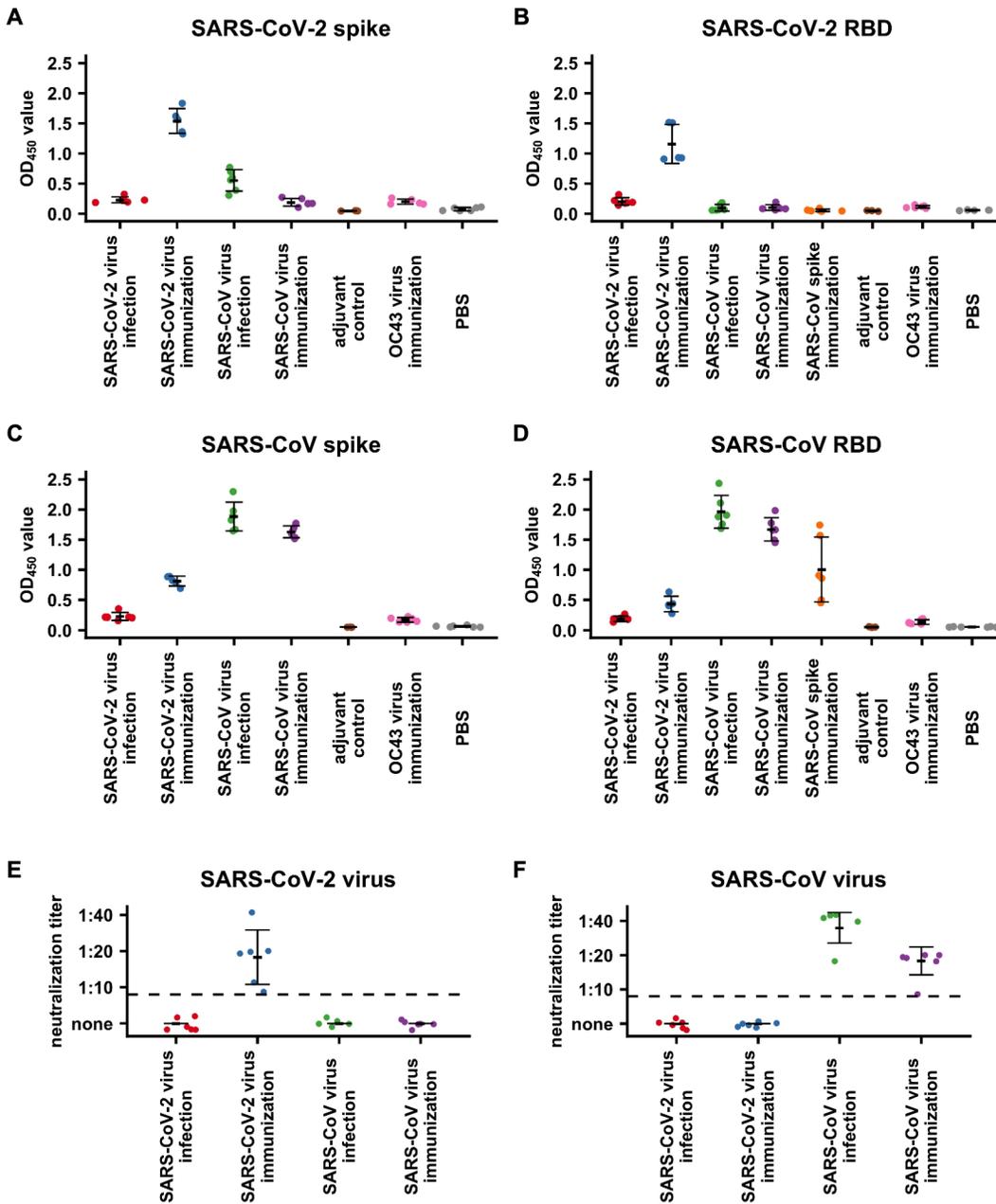


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429 **Figure 1. Human serological responses to SARS-CoV-2.** (A) Schematic diagram of
430 the SARS-CoV-2 spike protein. Locations of secretion signal peptide (SP), N-terminal
431 domain (NTD), receptor-binding domain (RBD), S1/S2 cleavage site, fusion peptide
432 (FP), S2' cleavage site, internal fusion peptide (IFP), heptad repeat 1 (HR1), heptad
433 repeat 1 (HR2), transmembrane domain (TM), and cytoplasmic domain (CP) are
434 indicated. Regions corresponding to the S1, S2, S2' subunits, and ectodomain are also
435 indicated. (B) Binding of plasma from healthy donors and SARS-CoV-2 infected patients
436 to SARS-CoV-2 spike protein, SARS-CoV-2 RBD protein, SARS-CoV-2 S2 subunit,
437 SARS-CoV spike protein and SARS-CoV RBD protein were measured by ELISA. The
438 mean OD₄₅₀ values calculated after testing each plasma sample in triplicate are shown.

439 **(C)** Neutralization activities of plasma from SARS-CoV-2 infected patients to SARS-CoV-
440 2 and SARS-CoV viruses were measured. Dashed line represents the lower detection
441 limit. Black lines indicate mean +/- standard deviation. **(B-C)** Grey: plasma samples from
442 healthy donors. Orange: plasma samples from SARS-CoV-2-infected patients. Blue:
443 plasma samples from SARS-CoV-infected patients.

Fig. 2



444

445 **Figure 2. Mouse serological response to SARS-CoV-2 and SARS-CoV. (A-D)**

446 Binding of plasma from OC43-CoV-immunized mice, SARS-CoV-immunized mice,

447 SARS-CoV-infected mice and mock-immunized mice against **(A)** SARS-CoV-2 spike

448 protein, **(B)** SARS-CoV-2 RBD protein, **(C)** SARS-CoV spike protein and **(D)** SARS-CoV

449 RBD protein were measured by ELISA. Since both SARS-CoV spike protein and SARS-

450 CoV-2 spike contained a C-terminal foldon domain, binding of plasma from mice
451 immunized with SARS-CoV spike protein plasma was not tested against spike proteins
452 from SARS-CoV and SARS-CoV-2. The mean OD₄₅₀ values calculated after testing each
453 plasma sample in triplicate are shown. **(E-F)** Neutralization activities of plasma from
454 mice infected or immunized by SARS-CoV-2 or SARS-CoV to **(E)** SARS-CoV-2 virus or
455 **(F)** SARS-CoV virus were measured. Dashed line represents the lower detection limit.
456 Black lines indicate mean +/- standard deviation.