

1 **Plasmablast-derived antibody response to acute SARS-CoV-**  
2 **2 infection in humans**

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## 34 **Abstract**

35 Plasmablast responses and derived IgG monoclonal antibodies (MAbs) have been  
36 analysed in three COVID-19 patients. An average of 13.7% and 13.0% of  
37 plasmablast-derived IgG MAbs were reactive with virus spike glycoprotein or  
38 nucleocapsid, respectively. Of thirty-two antibodies specific for the spike  
39 glycoprotein, ten recognised the receptor-binding domain (RBD), thirteen were  
40 specific for non-RBD epitopes on the S1 subunit, and nine recognised the S2 subunit.  
41 A subset of anti-spike antibodies (10 of 32) cross-reacted with other  
42 betacoronaviruses tested, five targeted the non-RBD S1, and five targeted the S2  
43 subunit. Of the plasmablast-derived MAbs reacting with nucleocapsid, over half of  
44 them (19 of 35) cross-reacted with other betacoronaviruses tested. The cross-reactive  
45 plasmablast-derived antibodies harboured extensive somatic mutations, indicative of  
46 an expansion of memory B cells upon SARS-CoV-2 infection. We identified 14 of 32  
47 anti-spike MAbs that neutralised SARS-CoV-2 in independent assays at  $\leq 133$  nM (20  
48  $\mu\text{g/ml}$ ) (five of 10 anti-RBD, three of 13 anti-non-RBD S1 subunit, six of nine anti-S2  
49 subunit). Six of 10 anti-RBD MAbs showed evidence of blockade of ACE2 binding to  
50 RBD, and five of six of these were neutralising. Non-competing pairs of neutralising  
51 antibodies were identified, which offer potential templates for the development of  
52 prophylactic and therapeutic agents against SARS-CoV-2.

## 53 **Introduction**

54 In late 2019, a novel coronavirus emerged and was identified as the cause of a cluster  
55 of respiratory infection cases in Wuhan, China. It spread quickly around the world. In  
56 March of 2020 a pandemic was declared by the World Health Organization , the virus  
57 was formally named as Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-  
58 CoV-2) and the resulting disease was named COVID-19. As of 12 August 2020, there  
59 have been over 20 million confirmed cases of SARS-CoV-2 infection with 737,417  
60 deaths (World Health Organization, <https://covid19.who.int/>).

61 There is no fully effective drug or licenced vaccine for COVID-19. Antibodies  
62 neutralise SARS-CoV-2 *in vitro*, offering hope that a vaccine may induce a protective  
63 response, and antibodies may be an effective treatment for COVID-19 in clinical  
64 practice. Convalescent plasma is being tested in ongoing clinical trials as a therapy for  
65 COVID-19 (1, 2), and was previously used in the treatment of SARS (3). The virus  
66 spike glycoprotein is a target of neutralising antibodies, which makes it a key  
67 candidate for both vaccine development and immunotherapy (4). B cell responses in  
68 COVID-19 patients have been detected concomitantly with follicular helper T cell  
69 responses from week one after illness onset (5). In SARS patients, B cell responses  
70 typically arise first against the nucleocapsid protein then, within four to eight days  
71 after symptom onset, antibody responses to spike glycoprotein have been found;  
72 neutralising antibody responses begin to develop by week two, and most patients  
73 develop neutralising antibodies by week three (6). Two serological studies of COVID-  
74 19 patients showed anti-SARS-CoV-2 IgG seroconversion at week three after onset  
75 and some cross-reactivity to nucleocapsid of SARS (7, 8).

76 Antibodies may play a role in protection against SARS-CoV-2 infection. The  
77 underlying B-cell response leading to the rapid production of plasmablasts (antibody-  
78 secreting cells) that secrete antibodies upon natural SARS-CoV-2 exposure/infection  
79 is only beginning to be understood (5). Here, we characterised the infection-induced  
80 plasmablast response and the derived IgG anti-SARS-CoV-2 spike glycoprotein and  
81 nucleocapsid monoclonal antibodies (MAbs) from adult human patients with  
82 laboratory-confirmed COVID-19. The antigenic specificity and breadth of antibodies  
83 and the sequence of their variable domains have been characterised in detail. Virus  
84 neutralising antibodies were detected that bound epitopes on receptor-binding domain  
85 (RBD), non-RBD regions of the S1 polypeptide, and the S2 polypeptide of the spike  
86 glycoprotein.

## 87 **Results**

### 88 *Isolation of anti-spike glycoprotein and anti-nucleocapsid IgG antibodies from* 89 *circulating plasmablasts*

90 Serum IgG antibodies to the virus spike glycoprotein and the isolated RBD were  
91 analysed by indirect enzyme-linked immunosorbent and flow cytometry assays in  
92 three patients with laboratory-confirmed COVID-19. The clinical characteristics of  
93 the three patients studied are shown in Supplementary Table 1. Antibodies to spike  
94 glycoprotein and RBD were detected in all three patients after week 3 of illness onset  
95 (Figure 1a). Case A showed a robust response to spike glycoprotein and the isolated  
96 RBD by day (D) 22 (D22). Longitudinal sera from case B and C showed lower anti-  
97 spike glycoprotein and RBD IgG titres at week 1 or the beginning of week 2 and an  
98 elevated titre that peaked at the end of week 2 through week 3 (a peak 50% effective  
99 dilution (ED<sub>50</sub>) titre to RBD 1:1,051 at D18 in case B and 1:588 at D14 in case C).  
100 Case B had prolonged fever and developed pneumonia at the end of week 2, which  
101 was followed by a robust increase of anti-spike glycoprotein and RBD IgG titres at  
102 week 3 (Figure 1a, Supplementary Table 1). By contrast, case C experienced a two-  
103 day course of febrile illness and reduction of all symptoms within the first week,  
104 followed by anti-spike glycoprotein and RBD IgG titres that peaked earlier at the end  
105 of week 2 (Figure 1a, Supplementary Table 1).

106 An increased frequency of circulating plasmablasts was detected in all three patients  
107 (healthy adults baseline less than 1%) (5) (Figure 1b). In case A, a plasmablast  
108 response containing a substantial IgG subset was detected at the end of week 2 (D14),  
109 followed by a high serological titre at week 3 (D22). Case B had a robust plasmablast  
110 response at the end of week 2 (D14) but the IgG plasmablast subset continued to rise,

111 dominated at week 3 (D18), but then subsided at the beginning of week 4 (D22),  
112 which is compatible with high anti-spike glycoprotein and RBD IgG serological titres  
113 at week 3. Case C produced a significant early plasmablast response at the end of  
114 week 1 (D6), the IgG plasmablast subset dominated at the same time, and both the  
115 plasmablast response and its IgG subsets subsided at the end of week 2 (D14).

116 Circulating plasmablasts were identified and used to generate human IgG monoclonal  
117 antibodies (MAbs) from the three patients (Figure 2a). A total of 219 plasmablast-  
118 derived IgG MAbs were produced, of which 67 (10 of 50 from case A, 48 of 131 from  
119 case B, nine of 38 from case C) were shown to bind spike glycoprotein or  
120 nucleocapsid antigens of SARS-CoV-2 by one or more of the following: staining of  
121 spike glycoprotein and RBD-expressing cells, ELISA, or immunofluorescence and  
122 specific virus neutralisation. Averages of  $13.7\pm 6.8\%$  (6.0-18.4%) and  $13.0\pm 7.3\%$  (5.3-  
123 19.9%) of plasmablast-derived IgG MAbs were reactive with virus spike glycoprotein  
124 and nucleocapsid, respectively.

### 125 ***Genetic and phenotypic characteristics of anti-spike glycoprotein antibodies***

126 Among 32 MAbs, 10 bound to the RBD, 13 to non-RBD S1, and the other 9 to the S2  
127 subunit of the SARS-CoV-2 spike glycoprotein (Figure 2a and Table 1). Twenty-four  
128 of these MAbs bound to virus antigens as assessed by immunofluorescence of SARS-  
129 CoV-2-infected Vero E6 cells (Supplementary Figure 1), suggesting that the majority  
130 of anti-spike glycoprotein human antibodies recognise complex conformational  
131 epitopes on the virus glycoprotein. Weak binding was observed for a subset (4 of 9) of  
132 anti-S2 MAbs with full-length spike glycoprotein ectodomain in the indirect ELISA  
133 but bound strongly to the isolated S2 subunit (Figure 2b).

134 Ten of 32 anti-SARS-CoV-2 spike glycoprotein MAbs cross-reacted with the  
135 glycoproteins of other betacoronaviruses, including SARS, MERS or human common  
136 cold coronavirus OC43 in ELISA (Table 1), suggesting the presence of conserved  
137 epitopes on the spike glycoproteins of betacoronaviruses.

138 Each of 32 anti-spike glycoprotein MAbs was encoded by a unique set of heavy chain  
139 VDJ and light chain VJ rearrangements in the variable domain (Supplementary Table  
140 2). Fourteen of 32 SARS-CoV-2 spike-reactive Mab genes possessed low numbers of  
141 somatic mutations resulting in 0 or 1 amino acid substitutions suggesting a *de-novo* B  
142 cell response to the SARS-CoV-2 virus in humans. Six of the 32 Mab genes possessed  
143  $\geq 20$  nucleotide mutations, and these cross-reacted on other beta-coronaviruses,  
144 including OC43. Of the nine anti-S2 antibodies five cross-reacted on OC43 virus and  
145 three of these also cross-reacted on MERS (Table 1). All five cross-reactive anti-S2  
146 antibodies had high rates of somatic mutation ( $25\pm 5$ ), indicating a memory  
147 phenotype, and three of the five were neutralising to a moderate level (half maximal  
148 effective concentration,  $EC_{50}$ , 36-133.33 nM, Table 1).

149 The CDR3 length varied among anti-spike glycoprotein antibodies (Supplementary  
150 Table 2). No significant differences were found between anti-S2 and anti-S1 or anti-  
151 RBD subsets. Among anti-S2 MAbs, a significantly longer heavy chain CDR3 length  
152 was found in the cross-reactive group compared to the specific group (Cross-reactive  
153  $20\pm 2$  versus Specific  $12\pm 4$ ,  $p= 0.02$ ; Figure 2c), indicating that a long CDR3 may  
154 play a role in antigen binding, which is also found in several broadly reactive human  
155 MAbs against human immunodeficiency virus and influenza virus (9, 10).

156 ***Characterisation of anti-RBD antibodies***



157 The binding sites of 10 anti-RBD MAbs were further characterised in detail. Using  
158 MDCK-SIAT1 cells transduced to express the RBD and flow cytometry, binding  
159 activities of the anti-RBD MAbs were shown to vary with 50% binding concentration  
160 from 0.10 to 1.83  $\mu\text{g/ml}$  (see methods; Supplementary Figure 2). The MAbs with  
161 strong anti-RBD binding have a relatively long heavy chain CDR3 length (50%  
162 binding concentration  $<0.5 \mu\text{g/ml}$  versus  $>0.5 \mu\text{g/ml}$ ,  $p=0.03$ ; Supplementary Figure  
163 3).

#### 164 ***Division of anti-RBD antibodies into cross-inhibiting groups***

165 The ten anti-RBD IgG MAbs were divided into cross-inhibiting groups as described  
166 for human MAbs to Ebola (11) by assessing competition of unlabelled antibodies at  
167 10-fold (or greater) excess over a biotin labelled target antibody by ELISA. Included  
168 as controls were the VHH72-Fc (12) and H11-H4-Fc (13) nanobodies linked to the  
169 hinge and Fc region of human IgG1, and CR3022 human MAb (14) reconstituted as  
170 an IgG1 antibody. These three control molecules have characterised binding footprints  
171 on the RBD defined by crystal structures (12, 13, 15), as does EY 6A (16). Also  
172 included was the protease domain (residues 18-615) of ACE2 linked to the Fc region  
173 of human IgG1 (ACE2-Fc dimer).

174 When optimising the binding assay we found that soluble RBD bound to a standard  
175 ELISA plate showed reduced binding to a soluble ACE2-Fc molecule (Supplementary  
176 Figure 4) compared to RBD displayed on a mi3 Virus Like Particle (RBD-VLP) (17).  
177 Therefore, in competition experiments we used the RBD-VLP molecule bound to the  
178 plate to display RBD to antibodies or ACE2-Fc.

179 The ten antibodies formed four cross-inhibiting clusters (Table 2), represented by  
180 antibodies EY 6A (cluster 1, which included CR3022), FI 3A (cluster 2), FD 11A

181 (cluster 3) and FJ 10B (cluster 4). The strongest inhibitors of ACE2-Fc binding were  
182 in clusters 2 and 3 (Tables 1 and 2, Supplementary Figure 5). Neutralising antibodies  
183 were detected in clusters 1, 2 and 3, with the strongest antibodies FI 3A and FD 11A  
184 being in clusters 2 and 3 (Tables 1 and 2).

### 185 *ACE2 blockade by anti-RBD antibodies*

186 We compared two assays designed to detect antibody-blocking of the ACE2-RBD  
187 interaction (Supplementary Figure 5, Table 1), including an assay that we reported  
188 previously (13, 16). In the first assay unlabelled MAbs in at least 10-fold excess were  
189 mixed with biotin labelled ACE2-Fc and binding to the RBD, displayed on RBD-VLP  
190 bound to an ELISA plate, was detected with Streptavidin-HRP (Supplementary Figure  
191 5a). The definition of the antibody clusters in Table 2 was based on this assay.

192 We noted that when unlabelled ACE2-Fc was used to compete with labelled MAbs a  
193 slightly different pattern was detected: for example, the unlabelled nanobody VHH72-  
194 Fc in excess strongly inhibited the binding of labelled ACE2-Fc to RBD, but not *vice-*  
195 *versa*. The structure of VHH72-Fc bound to RBD is known (12) and its footprint on  
196 the RBD does not overlap that of ACE2, so inhibition is thought to occur by steric  
197 hindrance. The second example is the pair FD 5D and FD 11A in cluster 3. These  
198 antibodies showed no inhibition of labelled ACE2-Fc, but the biotinylated versions  
199 were strongly competed by unlabelled ACE2-Fc (Table 2).

200 In the second assay, we employed MDCK-SIAT1 cells overexpressing full-length  
201 human ACE2 as a transmembrane protein. Unlabelled antibodies or ACE2-Fc were  
202 mixed in excess with biotinylated RBD, and binding of RBD was detected with  
203 Streptavidin-HRP in ELISA (Supplementary Figure 5b). The results of this assay  
204 mostly mirrored those of the first assay and confirmed that in this orientation cluster 3

205 antibodies FD 5D and FD 11A competed in excess with soluble RBD for binding to  
206 ACE2 (Supplementary Figure 5). In addition, antibodies in cluster 1 showed partial  
207 (CR3022) or near complete (EY 6A) competition. These two antibodies are known to  
208 bind to the same region of RBD away from the ACE2 binding site, but they influence  
209 the binding kinetics of RBD to ACE2, presumably through steric effects (16).

### 210 *Neutralisation by anti-spike glycoprotein antibodies*

211 The optimum method for detecting neutralisation of SARS-CoV-2 has not been  
212 established. For example, different laboratories have obtained varying results for the  
213 well-defined monoclonal antibody CR3022 (14, 15, 18). We have therefore compared  
214 results from neutralisation assays performed in four independent laboratories: a  
215 systematic survey of the thirty two anti-Spike MAbs at the Francis Crick Institute  
216 (London), with follow up for a selection of MAbs at the Sir William Dunn School  
217 (Oxford), Public Health England (Porton Down) and Chang Gung Memorial Hospital  
218 (Taiwan).

219 The 32 anti-spike glycoprotein MAbs were systematically screened at the Francis  
220 Crick Institute by plaque reduction neutralisation (PRNT) assay for neutralisation of  
221 wild type SARS-CoV-2 virus (see methods; summarised in Table 1). A total of 14  
222 neutralising antibodies distributed between different regions of the spike glycoprotein  
223 were identified: three of 13 to S1 (non-RBD), six of nine to S2, five of 10 to RBD.  
224 The EC<sub>50</sub> concentrations, as a measure of potency, ranged from 0.05 nM to ~133.33  
225 nM (8 ng/ml - ~20 µg/ml). Corroboration for a selection of these antibodies was then  
226 sought.

227 Neutralisation was corroborated by a microneutralisation test at Oxford (see  
228 methods), that measured a reduction in fluorescent focus-forming units, summarised

229 in Table 1 and Figure 3. EW 9C and EY 6A inhibited wild-type viruses by PRNT at  
230 PHE Porton Down (see methods, Supplementary Figure 6). MAbs were also screened  
231 by a PCR-based neutralisation assay at Chang Gung Memorial Hospital (Taiwan) (see  
232 methods): inhibition of virus replication was measured by quantitative PCR in the  
233 supernatant bathing the infected cells. This assay confirmed that at least five anti-  
234 spike glycoprotein antibodies (EW 9C, S2; EY 6A, RBD; FD 5D, RBD; FD 11A,  
235 RBD; FI 3A, RBD), as crude culture supernatants from transfected ExpiCHO cells,  
236 reduced the virus signal from ~56- to ~10,085-fold (Supplementary Figure 7).

### 237 *Neutralising antibodies to RBD and relationship to ACE2 blockade*

238 Five neutralising MAbs target the RBD and all of these partially or completely  
239 blocked the interaction between RBD and ACE2 in one or the other type of assay  
240 (Tables 1 and 2, Supplementary Figure 5). The most potent neutralising antibodies  
241 were ACE2 blockers (FI 3A in cluster 2, and FD 11A in cluster 3), and bound  
242 independently of each other to the RBD. MAb EY 6A has been shown to alter the  
243 binding kinetics of the interaction without full inhibition (16) and it had a moderate  
244 effect on ACE2 binding here in the assay where ACE2 was expressed at the cell  
245 surface. These three MAbs bound independently of each other indicating the existence  
246 of at least three neutralisation-sensitive epitopes within the RBD. All five neutralising  
247 MAbs to the RBD (EY 6A, FI 3A, FI 1C, FD 11A, FD 5A) had V gene sequences  
248 close to germline (Supplementary Table 2).

### 249 *Neutralising antibodies to S2*

250 Six of nine MAbs specific for S2 showed moderate neutralisation in the PRNT assay  
251 (Table 1). The antibodies FB 1E, FJ 4E and EW 9C, are moderately neutralising ( $EC_{50}$   
252 36-133.33 nM), cross-react on the spike glycoprotein from the common cold

253 betacoronavirus OC43, and show sequence characteristics of memory cells with high  
254 numbers of somatic mutations. This indicates that memory B cells, likely primed by  
255 an endemic or epidemic betacoronavirus related to OC43, can give rise to antibodies  
256 that neutralise SARS-CoV-2, albeit modestly. The other three neutralising antibodies  
257 specific for S2, FD 10A, FG 7A and FM 1A were close to germline in sequence  
258 (Supplementary Table 2) and did not cross-react strongly with other betacoronaviruses  
259 (Table 1). FD 10A exhibits the most potent neutralising activity in the PRNT assay  
260 and completely inhibits SARS-CoV-2-induced cytopathic effect (see methods) at 8.33  
261 nM.

#### 262 *Neutralising antibodies to non-RBD S1*

263 Thirteen MAbs were defined that bound the S1 region and three, close to germline in  
264 sequence, were neutralising. FJ 1C showed strong neutralisation ( $EC_{50}$  55.5 nM),  
265 whilst FD 11E ( $EC_{50}$  70 nM) and FD 1E ( $EC_{50}$  110 nM) were moderately neutralising  
266 (Table 1). We investigated the specificity of these antibodies using flow cytometry  
267 with MDCK-SIAT1 cells expressing the N-terminal domain (NTD) of spike  
268 glycoprotein linked to the transmembrane and cytoplasmic region of influenza  
269 haemagglutinin (see methods). FJ 1C (neutralising) and FD 7C bound strongly to this  
270 NTD construct defining them as NTD specific (Table 1, Supplementary Figure 8).

271 In Table 1 we have summarised all of the results from the Francis Crick institute and  
272 the Sir William Dunn School. It will be noted that not all of the results agree,  
273 particularly for MAbs directed at epitopes other than the RBD. The results in the two  
274 laboratories were reproducible.

#### 275 *Characterisation of anti-nucleocapsid antibodies*

276 A set of 35 anti-SARS-CoV-2 nucleocapsid MAbs were derived from circulating  
277 plasmablasts in the naturally infected subjects (Table 3, Supplementary Figure 9); 19  
278 of these strongly cross-react with at least one of the betacoronaviruses tested, 17 with  
279 SARS CoV, 14 with OC43 virus, and 13 with MERS CoV in ELISA. This suggested  
280 extensive cross-reactivity of the antibody response to epitopes in nucleocapsids of  
281 betacoronaviruses following natural infection with SARS-CoV-2.

282 The 35 MAbs were evolved from 33 clonal groups defined by their heavy chain VDJ  
283 and light chain VJ rearrangements (Supplementary Table 3). Those cross-reactive  
284 with other betacoronaviruses carried considerably more amino acid substitutions than  
285 SARS-CoV-1-specific MAbs in their variable domains (CR heavy  $22\pm 15$  versus  
286 specific heavy  $9\pm 11$ ,  $p=0.008$ ; Figure 2d), supporting the interpretation that  
287 approximately 50% of the anti-nucleocapsid antibody response in these three patients  
288 was derived from memory B cells, presumably primed by betacoronaviruses endemic  
289 to humans and related to OC43.

290 Antibodies EW 4C, EY 2A and EY 3B bound to paraformaldehyde-fixed and Triton  
291 X-100-permeabilised SARS-CoV-2 infected cells by immunofluorescence. These  
292 antibodies were screened for binding to fixed and permeabilised infected cells for use  
293 in scoring wells in microneutralisation assays. Antibody EY 2A performed well for  
294 this purpose.

## 295 **Discussion**

### 296 *Plasmablast responses*

297 We detected a robust and rapid plasmablast response encoding diverse anti-spike  
298 glycoprotein and anti-nucleocapsid antibody populations within 3 weeks of onset of  
299 illness in COVID-19 patients. The concomitant serologic response occurred as early  
300 as the first week after illness onset, with gradually increasing levels of SARS-CoV-2  
301 spike glycoprotein- and RBD-binding IgG antibodies from the second to third week  
302 after symptom onset. The kinetics of plasmablast and virus-specific serologic  
303 responses was observed to vary between subjects. Consecutive samples from donor B  
304 showed a highly expanded plasmablast subset at the time of progression into  
305 pneumonia, followed by class-switching to an IgG-predominant plasmablast response  
306 until week three of illness. On the other hand, donor C presented with mild symptoms  
307 and produced an early plasmablast response with class-switching at the end of first  
308 week of symptom onset. Thevarajan et al demonstrated a similar kinetic of B cell  
309 response in a mild COVID-19 case, in whom the peripheral plasmablast response and  
310 the SARS-CoV-2-binding IgG antibodies peaked at day eight, soon after the  
311 disappearance of fever (5). An early class-switching phenotype of the humoral  
312 response was also noted within the first week of onset in paediatric patients who  
313 mainly experienced mild illness after SARS-CoV-2 infection, although the  
314 immunological basis for this phenotype is unclear (19). Both peak plasmablast  
315 frequency and serologic titre for spike glycoprotein were higher in donor B, who  
316 presented with severe symptoms, than in donor C. Similar observations on the  
317 serologic titre and clinical severity have been reported by others (7, 20).

### 318 *Cross-reactivity and somatic hypermutation*

319 In this study, a substantial subset of plasmablast-derived anti-S2 (five of nine, 56%)  
320 and anti-nucleocapsid antibodies (14 of 35, 40%) cross-reacted with human  
321 betacoronavirus OC43. Cross-reactivity among betacoronaviruses has also been  
322 reported in polyclonal sera (21, 22). Human coronavirus OC43, discovered in the  
323 1960s, is one of the major betacoronaviruses that cause common colds in the  
324 community (23, 24), and severe respiratory infections in elderly and  
325 immunocompromised individuals (22, 25). Epidemiologic surveys reveal that OC43  
326 infection can occur in early childhood, and that OC43 seropositivity reaches nearly  
327 90% in adults (26, 27). The S2 component of SARS-CoV-2 spike glycoprotein shares  
328 43~89% amino acid identity with SARS, MERS and OC43, and, similarly, the amino  
329 acid sequence of SARS-CoV-2 nucleocapsid protein of is 34~90% homologous to  
330 those of other betacoronaviruses (Supplementary Figures 10 and 11), suggesting the  
331 presence of conserved epitopes on these antigens.

332 The presence of pre-existing immune memory to betacoronavirus that cross-react with  
333 SARS-CoV-2 is supported by the accumulation of somatic mutations in the genes  
334 encoding cross-reactive antibodies isolated from COVID-19 patients (Figures 2c and  
335 2d, Supplementary Tables 2 and 3). This situation is reminiscent of re-exposure to  
336 immunogenic epitopes shared by closely related viruses leading to induction of  
337 broadly cross-reactive antibodies in patients infected with influenza, dengue or Zika  
338 viruses (28-30).

339 Our results have significance for serologic tests employing the N and S2 antigens of  
340 SARS-CoV-2. Serologic surveys, some of which are based on these antigens, with  
341 sera from donors infected with SARS-COV-2 during the spring and summer months  
342 have shown very high specificity (as judged by comparing convalescent sera from



343 COVID-19 patients versus sera collected in the pre-COVID-19  
344 period)([www.gov.uk/government/publications/covid-19-laboratory-evaluations-of-](http://www.gov.uk/government/publications/covid-19-laboratory-evaluations-of-serological-assays)  
345 [serological-assays](http://www.gov.uk/government/publications/covid-19-laboratory-evaluations-of-serological-assays)). However, if many individuals have memory B cells to S2 and N  
346 antigens of circulating betacoronaviruses that cross-react with SARS-CoV-2,  
347 concurrent winter infections with these viruses might erode the specificity of  
348 serologic tests for SARS-CoV-2 based on these two antigens. Cross-reactivity of  
349 antibodies targeting the RBD is much lower, so using this antigen is more likely to  
350 give a true indication of SARS-CoV-2 specificity.

### 351 *Neutralising antibodies*

352 The 32 MAbs that bound to the spike glycoprotein were systematically tested for  
353 neutralisation in two laboratories (summarised in Table 1). Results established that  
354 neutralising epitopes were present on the RBD, S1-NTD, S1-non NTD/RBD, and S2  
355 regions of the spike glycoprotein. The range of neutralisation EC<sub>50</sub> titres against live  
356 SARS-CoV-2 reported in the literature for human and murine monoclonal antibodies  
357 span at least three orders of magnitude, from ng/ml to µg/ml (31, 32). Our results  
358 reflect this range. The relationship between EC<sub>50</sub> titre in neutralisation assays and  
359 therapeutic potential is not established, although antibodies to the RBD with EC<sub>50</sub> in  
360 the nanogram range have shown therapeutic activity in small animal models (33-35).  
361 The majority of the most strongly neutralising antibodies were close to germline in  
362 sequence, showing that full affinity maturation is not required in order to achieve  
363 SARS-CoV-2 virus neutralisation. We made a similar observation for antibodies  
364 induced by an Ebola vaccine (11), and this has been observed in other collections of  
365 Monoclonal antibodies to SARS-CoV-2 (4,32-39).

366 The RBDs of SARS and SARS-CoV-2 are known to contain neutralising epitopes (16,  
367 31-42), and vaccines based on the RBD of SARS and SARS-CoV-2 induce strong  
368 neutralising antibodies that are protective in animal models (43-45). There is a  
369 tendency for the most potent neutralising antibodies to be those that block the binding  
370 of the RBD to its receptor ACE2 (31-42, 46-48). Occasional antibodies that do not  
371 occupy the ACE2 footprint and fail to block ACE2 binding, can be almost as powerful  
372 (40), perhaps through triggering a conformational change in the spike glycoprotein  
373 that renders it non-functional (4, 16).

374 We describe ten MAbs targeting the RBD that can be arranged into four cross-  
375 inhibiting clusters (Table 2). Three of these Mab clusters (represented by EY 6A, FI  
376 3A and FD 11A) demonstrate neutralisation of SARS-CoV-2 to some level, while  
377 those in the fourth (represented by FM 7B) did not neutralise. This suggests that three  
378 neutralising antibodies may be able to bind to the RBD simultaneously. The most  
379 potent neutralising MAbs fall in two clusters and interfere strongly with ACE2  
380 binding, something which may be exploited therapeutically. For instance, antibodies  
381 FI 3A in cluster 2 ( $EC_{50}$  8.67 nM) and FD 11A in cluster 3 ( $EC_{50}$  0.05 nM) could be  
382 combined to limit possible selection of neutralisation-resistant variants. This principle  
383 has been demonstrated recently (35, 37, 49).

384 Neutralisation by human MAbs to the S1-NTD of SARS-CoV-2 has been described  
385 (34, 50), but their mechanism of action is not known. The range of neutralising  $EC_{50}$   
386 titres (0.09-51.1 nM) was overlapping with those targeting the RBD, similar to our  
387 NTD-specific antibody FJ 1C. Cocktails of antibodies that include a representative to  
388 the NTD would further reduce the likelihood of selecting neutralisation-resistant  
389 viruses. A second strong binder to the NTD, FD 7C, was not strongly neutralising.

390 Structural comparisons of these two antibodies bound to spike glycoprotein may  
391 provide insight into the neutralising action of FJ 1C.

392 We detected a subset of six MAbs to the S2 region of the spike glycoprotein that  
393 neutralised moderately ( $EC_{50}$  36-133 nM). Three of these were clearly derived from a  
394 memory population; showing significant accumulation of somatic mutations in the  
395 MAb encoding genes and cross-reactivity for the OC43 common cold virus spike  
396 glycoprotein (Figure 2c). Further investigation is required to ascertain whether such  
397 antibodies, that may be weak- or non-neutralising and cross-reactive with common  
398 cold viruses, are beneficial or detrimental with respect to COVID-19 disease.

399 In summary, COVID-19 patients developed strong anti-SARS-CoV-2 spike  
400 glycoprotein and nucleocapsid plasmablast responses. A panel of 32 IgG MAbs  
401 targeted a diverse spectrum of epitopes on the RBD, S1-NTD, non-NTD/RBD S1 and  
402 S2 regions of the spike glycoprotein, of which 14 neutralised wild type virus with  
403  $EC_{50}$ s in the range 0.05 to ~133 nM. Neutralising activities of the majority of anti-  
404 RBD MAbs were linked to ACE2-binding blockade, and non-competing pairs of such  
405 MAbs, perhaps combined with a neutralising MAb to the NTD, offer potential  
406 formulations for the development of prophylactic and therapeutic agents against  
407 SARS-CoV-2. Antibody responses to nucleocapsid and the S2 component of spike  
408 glycoprotein confirm marked cross-reactivity with a common cold virus.

## 409 **Materials and Methods**

### 410 **Study design**

411 This study was designed to isolate SARS-CoV-2 antigen-specific MAbs from  
412 peripheral plasmablasts of humans infected with SARS-CoV-2 and to characterise the  
413 antigenic specificity and phenotypic activities of the MAbs. Diagnosis of SARS-CoV-  
414 2 infection was based on positive real-time reverse transcriptase polymerase chain  
415 reaction results of respiratory samples. The study protocol and informed consent were  
416 approved by the ethics committee at the Chang Gung Medical Foundation and the  
417 Taoyuan General Hospital, Ministry of Health and Welfare. Each patient provided  
418 signed informed consent. The study and all associated methods were carried out in  
419 accordance with the approved protocol, the Declaration of Helsinki and Good Clinical  
420 Practice guidelines.

### 421 **Staining and sorting of plasmablasts**

422 Freshly separated peripheral blood mononuclear cells (PBMCs) or thawed PBMCs  
423 were stained with fluorescent-labelled antibodies to cell surface markers purchased  
424 from BD Biosciences, USA; Pacific blue anti-CD3 (clone UCHT1, Cat. No. 558117,  
425 BD), Fluorescein isothiocyanate anti-CD19 (clone HIB19, Cat. No. 555412, BD),  
426 Phycoerythrin-Cy7 anti-CD27 (clone M-T271, Cat. No. 560609, BD),  
427 Allophycocyanin-H7 anti-CD20 (clone L27, Cat. No. 641396, BD), Phycoerythrin-  
428 Cy5 anti-CD38 (clone HIT2, Cat. No. 555461, BD) and Phycoerythrin anti-human  
429 IgG (clone G18-145, Cat. No. 555787, BD). The  
430 CD3<sup>neg</sup>CD19<sup>pos</sup>CD20<sup>neg</sup>CD27<sup>hi</sup>CD38<sup>hi</sup>IgG<sup>pos</sup> plasmablasts were gated and isolated in  
431 chamber as single cells as previously described (51).

432 **Production of human IgG 1 monoclonal antibodies**

433 Sorted single cells were used to produce human IgG MAbs as previously described  
434 (51). Briefly, the variable region genes from each single cell were amplified in a  
435 reverse transcriptase polymerase chain reaction (RT-PCR: QIAGEN, Germany) using  
436 a cocktail of sense primers specific for the leader region and antisense primers to the  
437 C $\gamma$  constant region for heavy chain and C $\kappa$  and C $\lambda$  for light chain. The RT-PCR  
438 products were amplified in separate polymerase chain reactions for the individual  
439 heavy and light chain gene families using nested primers to incorporate restriction  
440 sites at the ends of the variable gene as previously described (51). These variable  
441 genes were then cloned into expression vectors for the heavy and light chains.  
442 Plasmids were transfected into the HEK293T cell line for expression of recombinant  
443 full-length human IgG MAbs in serum-free transfection medium. A selected panel of  
444 MAbs were further expanded and purified.

445 To determine the individual gene segments employed by VDJ and VJ rearrangements  
446 and the number of nucleotide mutations and amino acid replacements, the variable  
447 domain sequences were aligned with germline gene segments using the international  
448 ImMunoGeneTics (IMGT) alignment tool ([http://www.imgt.org/IMGT\\_vquest/input](http://www.imgt.org/IMGT_vquest/input)).

449 **Enzyme-linked immunosorbent assay (ELISA)**

450 ELISA plates (Corning® 96-well Clear Polystyrene High Bind Stripwell™  
451 Microplate, USA) were coated with 8  $\mu$ g/ml SARS-CoV-2 antigens (spike  
452 glycoprotein extracellular or receptor-binding domains, or nucleocapsid: Sino  
453 Biological, China) or SARS antigen (spike glycoprotein S1 subunit: Sino Biological,  
454 China) or Middle East Respiratory Syndrome coronavirus (MERS) antigen (spike  
455 glycoprotein extracellular domain: Sino Biological, China) or human coronavirus

456 OC43 antigen (spike glycoprotein extracellular domain: Sino Biological, China) at  
457 4°C overnight. Plates were washed with phosphate-buffered saline containing 0.05%  
458 Tween-20 and blocked with 3% bovine serum albumin (BSA) at room temperature for  
459 1 hour on a shaker. Serial dilutions of MAb-containing cell culture supernatant or  
460 purified MAb were added and plates were incubated at 37°C for 1 hour. Plates were  
461 washed and incubated with horseradish peroxidase-conjugated rabbit anti-human IgG  
462 secondary antibody (Rockland Immunochemicals, USA). Plates were washed and  
463 developed with TMB substrate reagent (BD Biosciences, USA). Reactions were  
464 stopped with 0.5M hydrochloric acid and absorbances was measured at 450nm on a  
465 microplate reader. Non-transfected cell culture supernatant, anti-influenza H3 human  
466 IgG MAb BS 1A (in house), anti-SARS spike glycoprotein MAb CR3022 and  
467 convalescent serum were used as controls for each experiment. Reaction yielding an  
468 absorbance value above three times the mean absorbance of the negative control BS  
469 1A were considered positive.

#### 470 **Flow-cytometry based binding assay**

471 MDCK-Spike cells were produced by stably transfecting parental MDCK-SIAT1 cells  
472 with cDNA expressing full-length SARS-CoV-2 spike glycoprotein. MDCK-RBD  
473 cells were produced by, stably transducing MDCK-SIAT1 cells with a Lentiviral  
474 vector encoding a cDNA expressing RBD amino acids 340-538 (NITN.GPKK  
475 underlined) fused via a short linker to the transmembrane domain of haemagglutinin  
476 H7 (A/Hong Kong/125/2017) (EPI977395) at the C-terminus for surface expression  
477 (sequence:

478 MNTQILVFALIAIPTNA/DKIGSGSNITNLCPFGEVFNATRFASVYAWNKRKRISN

479 CVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVQRQIAPG

480 QTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYNLYRLFRKSNLKPFE

481 RDISTEIYQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVVLSELL

482 HAPATVCGPKKTGSGGSGKLSSGYKDVILWFSFGASCFILLAIVMGLVFICVKN

483 GNMRICTICI\*). MDCK-NTD cells were produced by stably transfecting parental

484 MDCK-SIAT1 cells with cDNA expressing SARS-CoV-2 NTD.

485 Both MDCK-Spike and MDCK-RBD cells were then FACS sorted for highly

486 expressing cells using the CR3022 antibody. MDCK-Spike or MDCK-RBD cells

487 were prepared and resuspended. Cells were probed with purified MAbs in 3% BSA.

488 Bound primary antibodies were detected with FITC-conjugated anti-IgG secondary.

489 The binding activities were analyzed by BD FACSCanto™ II flow cytometer (BD

490 Biosciences, USA).

#### 491 **Immunofluorescence assay**

492 Under biosafety level 3 (BSL-3) conditions, Vero E6 cells were infected with 100

493 TCID<sub>50</sub> (median tissue culture infectious dose) SARS-CoV-2 (hCoV-

494 19/Taiwan/CGMH-CGU-01/2020, EPI\_ISL\_411915). Infected cells were placed on

495 coverslips and, and fixed with acetone at room temperature for 10 minutes. After

496 blocking with 1% BSA at room temperature for 1 hour and washing, fixed cells were

497 incubated with MAb-containing cell culture supernatant. The anti-influenza human

498 monoclonal antibody BS 1A, anti-SARS spike glycoprotein MAb CR3022 and

499 convalescent serum were used as antibody controls for each experiment. Following

500 incubation and wash, cells were stained with FITC-conjugated anti-human IgG

501 secondary antibody and Evans blue dye as counterstain. Antibody-bound infected

502 cells demonstrated an apple-green fluorescence against a background of red

503 fluorescing material stained by the Evans Blue counterstain. Images were acquired

504 with original magnification 40x, scale bar 20 μm.

505 **Plaque reduction neutralisation assay (Francis Crick Institute)**

506 Confluent monolayers of Vero E6 cells in 96-well plates were incubated with ~14  
507 plaque forming units (PFU) of SARS CoV-2 (hCoV-19/England/02/2020,  
508 EPI\_ISL\_407073) and antibodies in a 2-fold dilution series (triplicates) for 3 hours at  
509 room temperature. Inoculum was then removed, and cells were overlaid with plaque  
510 assay overlay. Cells were incubated at 37°C, 5% CO<sub>2</sub> for 24 hours prior to fixation  
511 with 4% paraformaldehyde at 4°C for 30 minutes. Fixed cells were then  
512 permeabilised with 0.2% Triton-X-100 and stained with a horseradish peroxidase  
513 conjugated-antibody against virus protein for 1 hour at room temperature. TMB  
514 substrate was then added to visualise virus plaques as described previously for  
515 influenza virus (52). Convalescent serum from COVID-19 patients was used as a  
516 control.

517 **Fluorescent focus-forming units microneutralisation assay (FMNT) (Oxford)**

518 In brief, this rapid, high-throughput assay determines the concentration of antibody  
519 that produces a 50% reduction in infectious focus-forming units of authentic SARS-  
520 CoV-2 in Vero cells, as follows. Triplicate serial dilutions of antibody are pre-  
521 incubated with a fixed dose of SARS-CoV-2 (Australia/VIC01/2020, GenBank  
522 MT007544) (53) in triplicate before incubation with Vero cells. A carboxymethyl  
523 cellulose-containing overlay is used to prevent satellite focus formation. Twenty hours  
524 post-infection, the monolayers are fixed with paraformaldehyde and stained for N  
525 antigen using MAb EY 2A. After development with a peroxidase-conjugated antibody  
526 and substrate, foci are enumerated by enzyme-linked immune absorbent spot reader.  
527 Data are analysed using four-parameter logistic regression (Hill equation) in  
528 GraphPad Prism 8.3.



529 **Plaque reduction neutralisation assay (Porton Down)**

530 SARS-CoV-2 (Australia/VIC01/2020, GenBank MT007544) (52) was diluted to a  
531 concentration of 933 PFU/ml (70 PFU/75  $\mu$ l) and mixed 50:50 in minimal essential  
532 medium (MEM) (Life Technologies, California, USA) containing 1% foetal bovine  
533 serum (FBS: Life Technologies) and 25 mM HEPES buffer (Sigma, Dorset, UK) with  
534 doubling antibody dilutions in a 96-well V bottomed plate. The plate was incubated at  
535 37°C in a humidified incubator for 1 hour, to allow neutralisation to take place, before  
536 the virus-antibody mixture was transferred into the wells of a twice Dulbecco's PBS-  
537 washed 24-well plate containing confluent monolayers of Vero E6 cells (ECACC  
538 85020206; PHE, UK) that had been cultured in MEM containing 10% (v/v) FBS.  
539 Virus was allowed to adsorb onto cells at 37°C for a further hour in a humidified box  
540 and then overlaid with MEM containing 1.5% carboxymethylcellulose (Sigma), 4%  
541 (v/v) FBS and 25mM HEPES buffer. After 5 days incubation at 37°C in a humidified  
542 box, the plates were fixed overnight with 20% formalin/PBS (v/v), washed with tap  
543 water and then stained with 0.2% crystal violet solution (Sigma) and plaques were  
544 counted. A mid-point probit analysis (written in R programming language for  
545 statistical computing and graphics) was used to determine the dilution of antibody  
546 required to reduce numbers of SARS-CoV-2 virus plaques by 50% ( $ND_{50}$ ) compared  
547 with the virus-only control ( $n=5$ ). The script used in R was based on a previously  
548 reported source script (54). Antibody dilutions were run in duplicate and an internal  
549 positive control for the PRNT assay was also run in duplicate using a sample of heat-  
550 inactivated (56°C for 30 minutes) human MERS convalescent serum known to  
551 neutralise SARS-CoV-2 (National Institute for Biological Standards and Control,  
552 UK).

553 **Quantitative PCR-based neutralisation assay**

554 Neutralisation activity of MAb-containing supernatant was measured using SARS-  
555 CoV-2 (hCoV-19/Taiwan/CGMH-CGU-01/2020, EPI\_ISL\_411915) infected Vero E6  
556 cells. Briefly, Vero E6 cells were pre-seeded in a 96 well plate at a concentration of  
557  $10^4$  cells per well. The following day, MAb-containing supernatants were mixed with  
558 equal volumes of 100 TCID<sub>50</sub> virus preparation and incubated at 37°C for 1 hour, then  
559 mixtures were added to seeded Vero E6 cells and incubated at 37°C for 5 days. Cell,  
560 virus and virus back-titration controls were setup for each experiment. At day 5 the  
561 culture supernatant was harvested from each well, and virus RNA was extracted and  
562 quantified by real-time RT-PCR targeting the E gene of SARS-CoV-2 as previously  
563 described. The cycle threshold values of real-time RT-PCR were used as indicators of  
564 the copy number of SARS-CoV-2 RNA in samples with lower cycle threshold values  
565 corresponding to higher virus copy numbers.

566 **CPE-based neutralisation assay**

567 Vero E6 cells in Dulbecco's Modified Eagle's Medium containing 10% FBS were  
568 added into 96-well plates and incubated at 37°C with 5% CO<sub>2</sub> overnight to reach  
569 confluence. After washing with virus growth medium (VGM: Dulbecco's Modified  
570 Eagle's Medium containing 2% FBS), two-fold serially diluted MAbs in VGM  
571 starting at 100 µg/ml were added to each duplicated well. The plates were  
572 immediately transferred to a BSL-3 laboratory and 100 TCID<sub>50</sub> SARS-CoV-2 (hCoV-  
573 19/Taiwan/4/2020, EPI\_ISL\_411927) in VGM was added. The plates were further  
574 incubated at 37°C with 5% CO<sub>2</sub> for three days and the cytopathic morphology of the  
575 cells was recorded using an ImageXpress Nano Automated Cellular Imaging System.

576 **Competitive binding assays**

577 Competitive binding assays were performed as described previously (11) with slight  
578 modifications for epitope mapping of the anti-RBD MAbs. Briefly, 0.5 µg/ml of  
579 RBD-VLP were coated on NUNC plates (50 µl per well) overnight at 4°C, washed  
580 and blocked with 300 µl of 5% (w/v) dried skimmed milk in PBS for 1 hour at room  
581 temperature prior to the assays. Antibody was biotinylated using EZ-Link Sulfo-NHS-  
582 LC-biotin (21237; Life Technologies) and then mixed with competing MAb (in at  
583 least 10-fold excess) and transferred to the blocked NUNC plates for 1 hour. A second  
584 layer Streptavidin-HRP (S911, Life Technologies) diluted 1:1,600 in PBS/0.1% BSA  
585 (37525; Thermo Fisher Scientific) was then added and incubated for another 1 hour.  
586 Plates were then washed, and signal was developed by adding POD substrate  
587 (11484281001, Roche) for 5 minutes before stopping the reaction with 1 M H<sub>2</sub>SO<sub>4</sub>.  
588 Absorbance (OD<sub>450</sub>) was measured using a Clariostar plate reader (BMG, Labtech).  
589 Mean and 95% confidence interval of 4 replicate measurements were calculated.  
590 Competition was measured as:  $(X - \text{minimum binding}) / (\text{maximum binding} - \text{minimum}$   
591  $\text{binding})$ , where X is the binding of the biotinylated MAb in the presence of  
592 competing MAb. Minimum binding is the self-blocking of the biotinylated MAb or  
593 background binding. Maximum binding is binding of biotinylated MAb in the  
594 presence of non-competing MAb (anti-influenza N1 neuraminidase MAb).

### 595 **ACE2 blocking assays**

596 Two assays were used to determine the blocking of binding of ACE2 to RBD by  
597 MAbs. RBD was anchored on the plate in the first assay whereas ACE2 was anchored  
598 for the second assay.

599 In the first ACE2 blocking assay, RBD-VLP (Spycatcher-mi3 VLP-particles  
600 conjugated with Spytagged-RBD recombinant protein) (17) was coated on ELISA

601 plates as described for the competitive binding assay. Recombinant ACE2-Fc (18-615)  
602 protein expressed in Expi293F (Life Technologies) cells was chemically biotinylated  
603 using EZ-link Sulfo-NHS-Biotin (A39256; Life Technologies) and buffer exchanged  
604 to PBS using a Zebaspin desalting column (Thermo Fischer). MAbs were titrated in  
605 duplicate or triplicate as half-log serial dilution, 8-point series starting at 1  $\mu$ M in 30  
606  $\mu$ l volume with PBS/0.1% BSA buffer. 30  $\mu$ l of biotinylated ACE2-Fc at approx. 0.2  
607 nM (40 ng/ml) was added to the antibodies. 50  $\mu$ l of the mixture was transferred to the  
608 PBS-washed RBD-VLP coated plates and incubated for 1 hour at room temperature.  
609 Secondary Streptavidin-HRP antibody (S911, Life Technologies) diluted to 1:1600  
610 was then added to the PBS-washed plates and incubated for 1 h at room temperature.  
611 Plates were then washed four times with PBS and signal was developed by adding  
612 POD substrate (11484281001, Roche) for 5 minutes before stopping with 1 M H<sub>2</sub>SO<sub>4</sub>.  
613 OD450 was measured using a Clariostar plate reader (BMG, Labtech). The control  
614 antibody (a non-blocking anti-influenza N1 MAb) or ACE2-Fc without antibody used  
615 to obtain the maximum signal and wells with PBS/BSA buffer only were used to  
616 determine the minimum signal. Graphs were plotted as % binding of biotinylated  
617 ACE2 to RBD. Binding % =  $\{(X - \text{Min})/(\text{Max} - \text{Min})\} * 100$  where X = measurement  
618 of the antibody, Min = buffer only, Max = biotinylated ACE2-Fc alone. 50%  
619 inhibitory concentrations of the antibodies against ACE2 was determined using non-  
620 linear regression curve fit using GraphPad Prism 8.

621 The second ACE2 blocking assay was performed as described previously (13, 16).  
622 Briefly, MDCK-SIAT1 cells were stably transfected to overexpress codon-optimised  
623 human ACE2 cDNA (NM\_021804.1) using lentiviral vector and FACS sorted  
624 (MDCK-ACE2). Cells ( $3 \times 10^4$  per well) were seeded on a flat-bottomed 96-well plate  
625 the day before the assay. RBD-6H (340-538; NITN.GPKK) was chemically

626 biotinylated using EZ-link Sulfo-NHS-Biotin (A39256; Life Technologies). Serial  
627 half-log dilutions (starting at 1  $\mu$ M) of antibodies and controls were performed in a U-  
628 bottomed 96 well plate in 30  $\mu$ l volume. 30  $\mu$ l of biotinylated RBD (25 nM) were  
629 mixed and 50  $\mu$ l of the mixture was then transferred to the MDCK-ACE2 cells. After  
630 1 hour a second layer Streptavidin-HRP antibody (S911, Life Technologies) diluted  
631 1:1,600 in PBS/0.1% BSA (37525; Thermo Fisher Scientific) was added and  
632 incubated for another 1 hour. Plates were then washed four times with PBS and signal  
633 was developed by adding POD substrate (11484281001, Roche) before stopping with  
634 1 M H<sub>2</sub>SO<sub>4</sub> after 5 minutes. OD450 was measured using a Clariostar plate reader  
635 (BMG, Labtech). The control antibody (a non-blocking anti-influenza N1 antibody)  
636 was used to obtain maximum signal and PBS only wells were used to determine  
637 background. Graphs were plotted as % binding of biotinylated RBD to ACE2. The  
638 50% inhibitory concentration of the blocking antibody was determined as described  
639 above.

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## 784 **Contributions**

785 K.-Y.A.H. conceived and designed the study of MAb isolation and characterization.  
786 K.-Y.A.H. produced and characterised plasmablast-derived MAbs. A.R.T. conceived

787 and designed the study of MAb characterization at Oxford. P.R, T.K.T, L.S generated  
788 cell lines, expressed proteins and antibodies, and performed experiments. S.H., R.H.  
789 R.S.D and J.W.M. designed and performed neutralisation assay at Crick Institute. A.  
790 H., J. G-J., X. L., M. K. and W.J. designed and performed neutralisation assay at  
791 Oxford. T-H.C., C-G.H., C-P.C., S-R.S, Y-C.L., C-Y.C., S-H.C., Y-C.H., T-Y.L., J-T.J.  
792 and C.M helped prepare materials, perform experiments and analyse data. All authors  
793 read and approved the manuscript.

#### 794 **Competing interests**

795 K-Y.A.H. has filed a provisional patent application on anti-spike glycoprotein and  
796 anti-nucleocapsid antibodies. Other authors declare no competing interest.

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**Table 1. The antigenic specificity, cross-reactivity and function of 32 anti-SARS-CoV-2 spike antibodies derived from COVID-19 patients.**

RBD-specific													
Antibody	Case	Domain	SARS-CoV-2 <sup>a</sup>				Cross-reactivity <sup>a</sup>			PRNT <sup>b</sup>	FMNT <sup>c</sup>	ACE2 Block <sup>d</sup>	
			IFA	Spike	RBD	S2	SARS	MERS	OC43	EC <sub>50</sub> (nM)	EC <sub>50</sub> (nM)	RBD Anchored	ACE2 Anchored
FD 11A	B	RBD	pos	1.64	1.17	0.13	0.10	0.10	0.12	0.05	3.68	+	+++
FI 3A	B	RBD	pos	1.60	1.12	0.12	0.08	0.08	0.09	8.67	0.51	++++	++++
FI 1C	B	RBD	pos	1.88	1.22	0.16	0.15	0.15	0.18	16.67	2.24	++	+++
FD 5D	B	RBD	pos	1.83	1.19	0.13	0.09	0.10	0.09	133.33	partial	+	+++
EY 6A	A	RBD	pos	1.75	1.18	0.13	1.82	0.12	0.11	133.33	22.50	-ve	++
EZ 7A	B	RBD	pos	1.66	1.12	0.33	2.05	1.00	1.05	-ve	-ve	-ve	-ve
FI 4A	B	RBD	pos	1.68	1.12	0.12	0.08	0.09	0.10	-ve	-ve	+	-ve
FJ 10B	B	RBD	-ve	1.48	1.22	0.13	1.26	0.10	0.14	-ve	-ve	-ve	-ve
FM 7B	C	RBD	pos	1.80	1.27	0.12	2.25	0.09	0.10	-ve	-ve	-ve	-ve
FN 12A	C	RBD	pos	2.24	1.33	0.30	0.36	0.29	0.38	-ve	-ve	-ve	-ve
S1-non-RBD													
Antibody	Case	Domain	SARS-CoV-2 <sup>a</sup>				Cross-reactivity <sup>a</sup>			PRNT <sup>b</sup>	FMNT <sup>c</sup>		
			IFA	Spike	RBD	S2	SARS	MERS	OC43	EC <sub>50</sub> (nM)	EC <sub>50</sub> (nM)		
FJ 1C	B	NTD	pos	1.61	0.27	0.15	0.23	0.15	0.16	55.50	partial		
FD 11E	B	non-NTD S1	pos	1.45	0.11	0.12	0.11	0.12	0.14	70.00	-ve		
FD 1E	B	non-NTD S1	pos	1.45	0.11	0.13	0.12	0.12	0.14	110.00	-ve		
EW 8B	B	non-NTD S1	-ve	1.61	0.11	0.13	0.11	0.11	0.12	-ve	-ve		
FD 11D	B	NTD	pos	1.42	0.18	0.22	0.42	0.25	0.29	-ve	partial		
FD 11C	B	non-NTD S1	pos	1.20	0.14	0.20	0.11	0.11	0.12	-ve	partial		
FD 7D	B	non-NTD S1	-ve	1.44	0.12	0.14	0.11	0.11	0.12	-ve	-ve		
FD 8B	B	non-NTD S1	-ve	1.10	0.14	0.12	0.08	0.10	0.09	-ve	-ve		
FD 7C	B	NTD	pos	1.90	0.15	0.15	0.13	0.12	0.13	-ve	35.60		
FG 12C	A	non-NTD S1	pos	1.74	0.14	0.11	0.08	0.08	0.10	-ve	-ve		
FN 8C	C	non-NTD S1	-ve	0.54	0.16	0.16	0.11	0.09	0.12	-ve	-ve		
FD 5E	B	non-NTD S1	pos	0.34	0.16	0.16	0.13	0.11	0.11	-ve	-ve		
EW 9B	B	non-NTD S1	-ve	0.22	0.17	0.11	0.09	0.10	0.09	-ve	-ve		
S2-specific													
Antibody	Case	Domain	SARS-CoV-2 <sup>a</sup>				Cross-reactivity <sup>a</sup>			PRNT <sup>b</sup>	FMNT <sup>c</sup>		
			IFA	Spike	RBD	S2	SARS	MERS	OC43	EC <sub>50</sub> (nM)	EC <sub>50</sub> (nM)		
FD 10A	B	S2	pos	1.78	0.15	1.32	0.22	0.36	0.39	111.13	-ve		
FB 1E*	C	S2	pos	1.40	0.11	1.20	0.12	1.01	2.34	36.00	-ve		
FJ 4E*	B	S2	-ve	0.26	0.18	0.91	0.18	1.08	1.68	75.33	-ve		
EW 9C*	B	S2	pos	1.22	0.18	1.17	0.14	0.26	1.82	133.33	-ve		
FG 7A	A	S2	pos	0.28	0.17	1.05	0.12	0.13	0.16	133.33	-ve		
FM 1A	C	S2	-ve	0.25	0.17	0.88	0.12	0.09	0.14	133.33	-ve		
FB 9D*	C	S2	pos	1.43	0.12	1.27	0.10	1.50	2.13	-ve	-ve		
FD 1D	B	S2	pos	0.40	0.15	1.06	0.16	0.22	0.25	-ve	-ve		
FN 2C*	C	S2	pos	1.77	0.14	1.15	0.09	0.10	2.20	-ve	-ve		
Controls													
CR3022		RBD	pos	1.08	1.31	0.10	2.24	0.11	0.11	42.00		-ve	-ve
BS 1A		Flu H3	-	0.07	0.09	0.11	0.10	0.09	0.11	-ve		-ve	-ve

799 <sup>a</sup> A sample (10 µg/ml) was considered positive when the measured extinction is at least 3 times the OD value of the  
800 negative control in the ELISA. CR3022 is an anti-SARS RBD human MAb and BS-1A is an anti-influenza H3  
801 human MAb. The OD value ≥ 1.00, 0.50-0.99, or ≤ 0.49 is highlighted in deep green, green and light green,  
802 respectively.

803 <sup>b</sup> The PRNT assay was performed with wild type SARS-CoV-2 at Francis Crick Institute (see methods) and the  
804 half maximal effective concentration (EC<sub>50</sub>) was determined using linear regression analysis.

805 <sup>c</sup> The FMNT assay was performed with wild type SARS-CoV-2 at Oxford (see methods) and the half maximal  
806 effective concentration (EC<sub>50</sub>) was determined using logistic regression model. Partial: MAb neutralises at least  
807 ~40% viruses at 100 nM (highest concentration tested).

808 <sup>d</sup> ACE2 blocking activity of anti-RBD antibody compared to ACE2-Fc (see methods): +, partial; ++, IC<sub>50</sub> > ACE2-  
809 Fc; +++, IC<sub>50</sub> ~ ACE2-Fc; +++++, IC<sub>50</sub> < ACE2-Fc.

810 \* Memory phenotype.

811 Abbreviations: IFA, immunofluorescence; RBD, receptor-binding domain; PRNT, plaque reduction neutralisation  
812 assay; FMNT, fluorescent focus-forming units microneutralisation test; ACE2, Angiotensin-Converting Enzyme 2.

**Table 2. Competitive binding analysis of anti-SARS-CoV-2 RBD antibodies.<sup>a</sup>**

Antibody	Vh	Neut <sup>b</sup>	Cluster	FI 4A-Bio	EY 6A-Bio	CR3022-Bio	VHH72-Fc-Bio	ACE2-Fc-Bio	FI 3A-Bio	FI 1C-Bio	H11-H4-Fc-Bio	FN 12A-Bio	FD 5D-Bio	FD 11A-Bio	FJ 10B-Bio	FM 7B-Bio	EZ 7A-Bio	
FI 4A	3-21	-	1	100	13	-3	27	28	2	2	-1	14	-12	-7	13	4	2	
EY 6A	3-30	+	1	103	100	103	108	-13	1	-2	3	4	22	-5	8	0	-3	
CR3022*		+	1	103	95	100	107	-1	-3	5	0	-6	45	0	15	10	-1	
VHH72-Fc*		+	1	103	63	50	100	129	-1	8	-6	-5	15	15	13	2	-4	
ACE2-Fc*		+		100	-4	-5	60	100	40	31	8	10	85	67	3	6	0	
FI 3A	3-53	+	2	-27	-1	-3	-20	141	100	97	85	194	-3	-19	21	3	-1	
FI 1C	3-11	+	2	-2	2	4	-17	119	76	100	95	223	20	-4	7	5	1	
H11-H4-Fc*		+	2	-9	-6	-3	-18	143	66	105	100	217	110	-12	7	0	1	
FN 12A	1-69	-	3	-7	-4	0	-1	4	7	29	-5	100	118	71	7	65	-1	
FD 5D	3-33	+	3	0	0	-6	35	10	2	6	-8	38	100	100	91	58	15	
FD 11A	3-48	+	3	-19	-4	-7	21	5	2	-3	-11	28	114	77	-2	3	7	
FJ 10B	5-10	-	4	15	15	8	-2	6	1	24	16	3	86	12	100	94	98	
FM 7B	1-3	-	4	16	14	5	-19	-5	-5	37	32	40	116	6	103	100	99	
EZ 7A	5-51	-	4	0	2	-3	-2	7	4	3	-8	3	16	4	101	63	100	
<i>-ve controls</i>																		
EW 8B		-						0					0	0				
EW 9C		+						0					0	0				
Z3B2*		-		0	0	0	0	0	0	0	0	0	0	0	0	0	0	

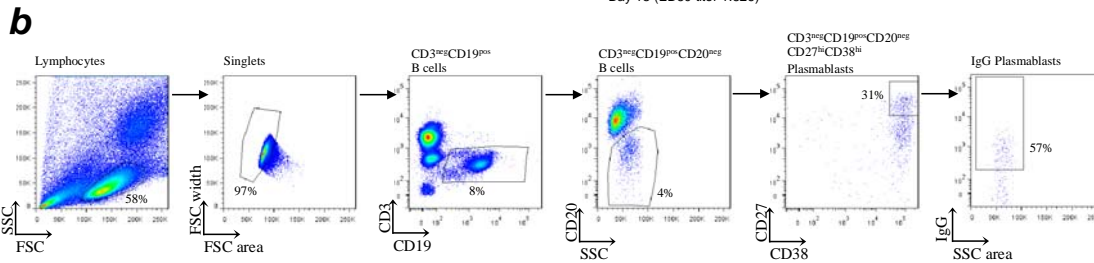
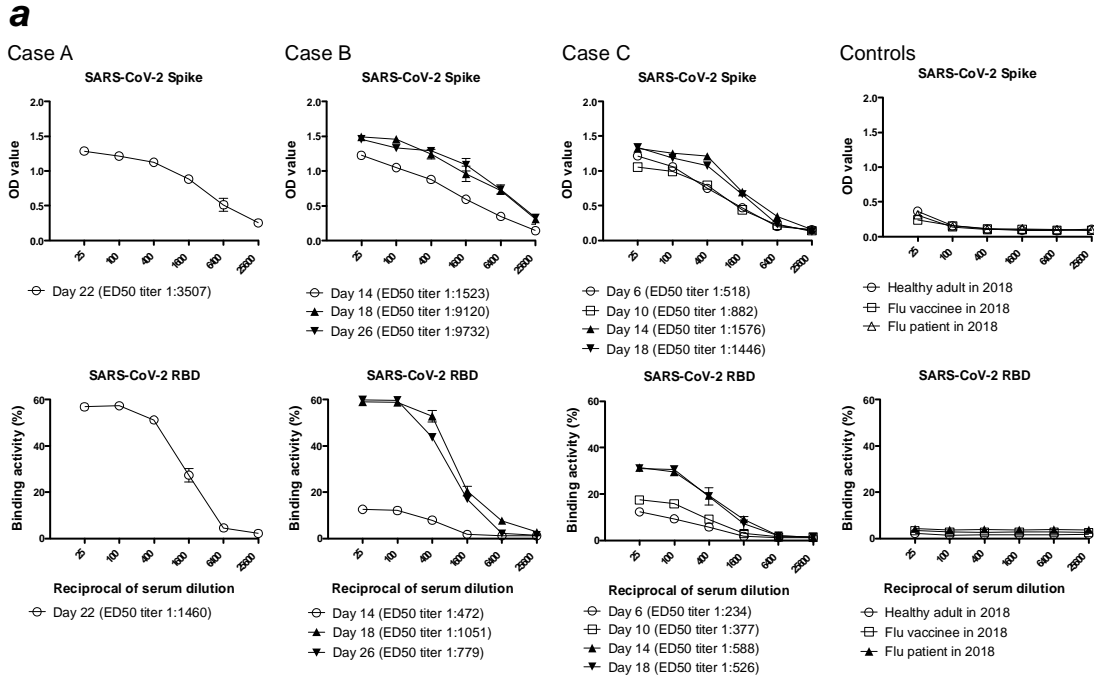
813 <sup>a</sup> Competitive inhibition: values are shown for percentage inhibition and those with  $\geq 75\%$  blocking, 50-  
814 74% blocking, and  $< 50\%$  blocking are highlighted in red, orange and green, respectively.  
815 <sup>b</sup> Neutralisation of antibody against wild type SARS-CoV-2 was analysed in the PRNT assay (+ =  
816 positive, - = negative).  
817 \* SARS and SARS-CoV-2 cross-reactive anti-RBD MAb CR3022 was included as a positive control.  
818 SARS and SARS-CoV-2 cross-reactive anti-RBD nanobodies VHH72 and H11-H4 linked to the hinge  
819 and Fc region of human IgG1 were included as positive controls. ACE2-Fc was included as a positive  
820 control. Anti-influenza MAb Z3B2 was included as a negative control.



**Table 3. The antigenic specificity and cross-reactivity of 35 anti-SARS-CoV-2 nucleocapsid antibodies derived from COVID-19 patients.**

Antibody	Case	SARS-CoV-2 <sup>a</sup>	Cross-reactivity <sup>a</sup>			Antibody	Case	SARS-CoV-2 <sup>a</sup>	Cross-reactivity <sup>a</sup>		
			SARS	MERS	OC43				SARS	MERS	OC43
EY 12B	A	1.23	1.24	1.10	1.24	EZ 9B	B	1.28	0.66	0.72	0.91
EY 9C	A	1.42	1.12	1.07	1.39	FD 9B	B	1.11	0.66	0.74	0.47
EY 5A	A	1.40	1.30	1.32	1.32	FD 3E	B	1.14	0.69	0.75	0.81
EY 12A	A	1.34	1.37	1.39	1.45	FD 5B	B	1.06	0.28	0.58	0.42
EY 2A	A	1.28	1.13	0.31	0.33	EZ 7B	B	1.01	0.29	0.29	0.33
EY 3B	A	1.22	1.00	0.22	0.26	EZ 4A	B	1.03	0.37	0.36	0.45
EY 8A	A	0.65	0.63	0.66	0.57	FJ 3C	B	0.98	1.28	1.16	0.86
						FD 4B	B	0.98	0.43	0.44	0.46
EZ 8C	B	1.34	1.11	1.07	1.44	FD 4E	B	0.93	0.53	0.63	0.65
EZ 8B	B	1.38	1.28	1.24	1.12	FD 4C	B	0.84	0.47	0.59	0.54
FJ 6D	B	1.30	1.30	1.24	1.05	FD 8C	B	0.84	0.46	0.47	0.56
EW 1A	B	1.37	1.36	1.31	1.28	EZ 4C-1	B	0.60	0.47	0.26	0.33
EW 9A	B	1.45	1.35	1.24	1.23	EZ 4C-2	B	0.71	0.59	0.58	0.34
EW 5A	B	1.37	1.12	1.04	1.30	EW 10C	B	0.56	0.62	0.57	0.59
EZ 9A	B	1.51	1.28	1.29	1.13	EZ 11A	B	0.53	0.46	0.46	0.23
EW 4C	B	1.24	1.23	1.24	1.31						
FD 6D	B	1.73	1.19	0.12	0.21	FB 9B	C	1.27	1.28	0.13	0.21
EZ 11C	B	1.46	0.73	0.72	1.16	FL 9B	C	0.92	0.60	0.68	0.94
EZ 9C	B	1.53	0.87	0.87	1.27						
<b>Controls</b>											
CR3022		0.07	0.06	0.07	0.17						
BS-1A		0.08	0.07	0.07	0.15						

821 <sup>a</sup> A sample (10 µg/ml) is considered positive when the measured extinction is at least 3 times the OD<sub>450</sub> value of  
822 the negative control in the ELISA. CR3022 is an anti-SARS RBD human MAbs and BS-1A is an anti-influenza H3  
823 human MAbs. OD values of ≥ 1.00 or 0.50-0.99 or ≤ 0.49 are highlighted in deep orange, orange and light orange,  
824 respectively.



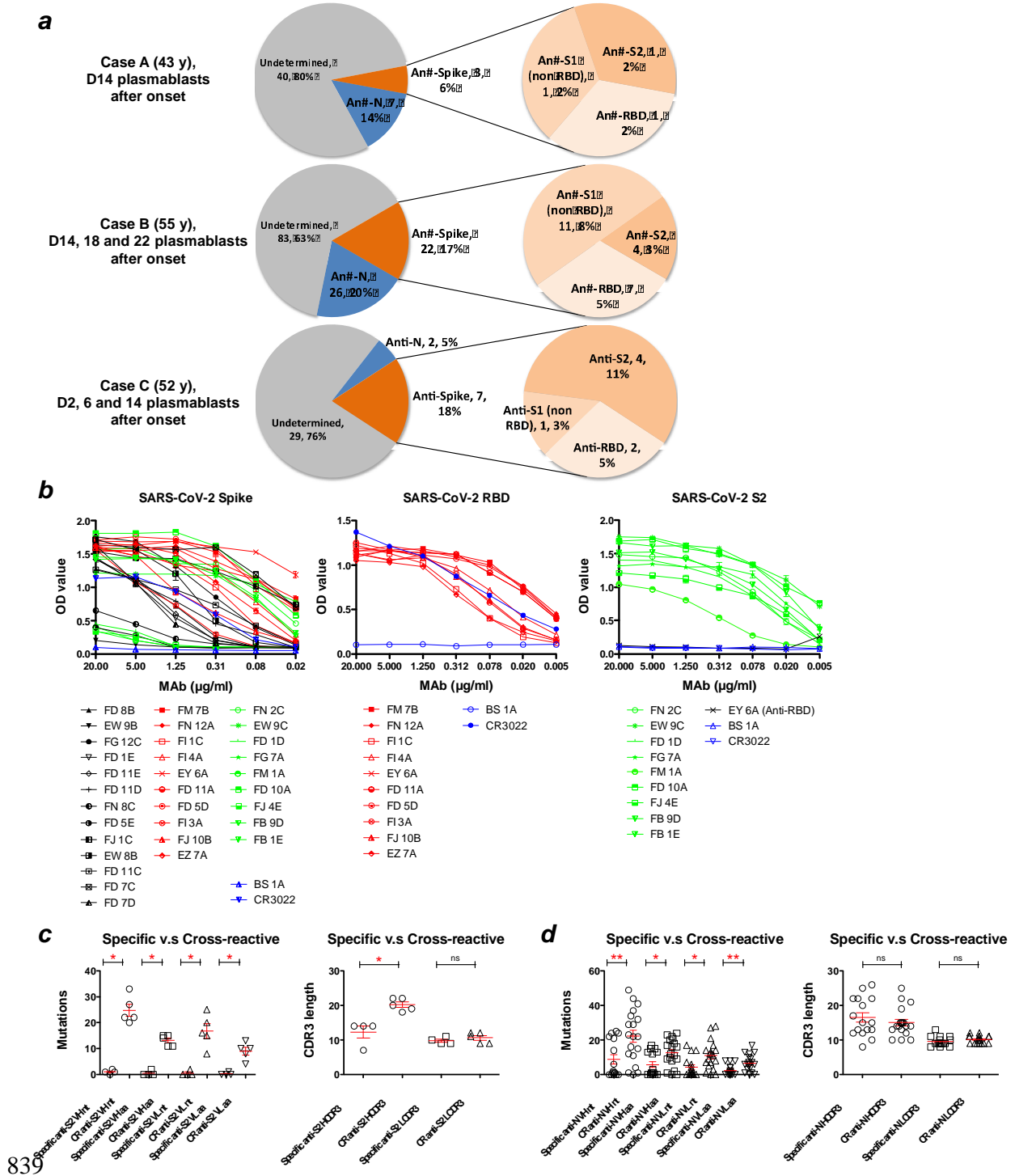
Case	Onset date	IgG Plasmablasts		
		Plasmablasts of CD3 <sup>neg</sup> CD19 <sup>pos</sup> B cells (%)	of B cells (%)	of Plasmablasts (%)
A	D14	1.99	0.34	16.95
	B			
	D18	37.48	1.67	4.47
B	D18	1.13	0.64	56.93
	D22	0.20	0.05	23.33
	C			
C	D2	2.56	0.11	4.38
	D6	2.09	0.97	46.39
	D14	0.83	0.09	11.11

825

826

827 **Figure 1. The IgG serology and plasmablast response to acute SARS-CoV-2**  
 828 **infection among enrolled patients. (a) The binding activity of post-infection sera**  
 829 **IgG with SARS-CoV-2 spike glycoprotein in an ELISA and SARS-CoV-2 RBD**  
 830 **assessed by flow cytometry on transfected cells, among enrolled patients. Each**

831 experiment was repeated twice. Values are presented as mean  $\pm$  standard error of the  
832 mean. Two sera from healthy adults (one collected at day 28 post 2018-19 influenza  
833 vaccination and one collected from an influenza-infected patient 9 days after  
834 symptom onset) in 2018 were included as controls. Linear regression was used to  
835 determine the 50% end-point dilution (ED<sub>50</sub>). **(b)** The gating strategy used for  
836 peripheral total B cells, plasmablasts and IgG plasmablasts in flow cytometry. The  
837 frequency of circulating plasmablasts (percentage of total B cells) among enrolled  
838 cases was measured by flow cytometry. Onset date (D = Day).



839

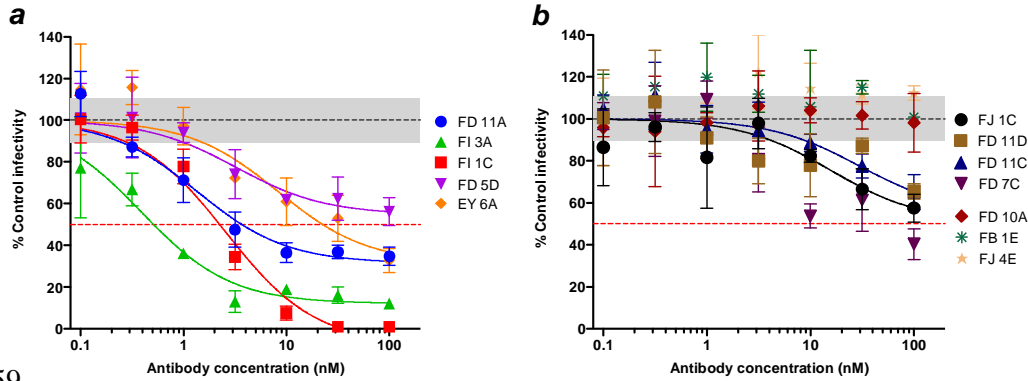
840

841 **Figure 2. Plasmablast-derived IgG monoclonal antibodies from three COVID-19**

842 **patients. (a)** A total of 219 IgG monoclonal antibodies were produced from COVID-

843 19 patients (50 from case A, 131 from case B, 38 from case C). An average of

844 13.7±6.8% and 13.0±7.3% of antibodies were reactive with spike glycoprotein (S)  
845 and nucleocapsid (N) antigens of SARS-CoV-2, respectively. The data are presented  
846 as specificity, number of antibodies, and the percentage of total antibodies isolated  
847 from each patient. **(b)** The binding activity of anti-SARS-CoV-2 MAbs with spike  
848 glycoprotein, RBD and the S2 subunit in ELISA. Anti-influenza H3 MAb BS-1A and  
849 anti-SARS RBD CR3022 were included as controls. Each experiment was repeated  
850 twice. The OD<sub>450</sub> values are presented as mean ± standard error of the mean. Panels  
851 **(c)** and **(d)** show numbers of variable domain mutations in MAb genes and variation  
852 in MAb CDR3 lengths among anti-S2 and anti-N MAbs, respectively. Antibodies that  
853 strongly cross-react with at least one betacoronavirus (SARS or MERS or OC43)  
854 were defined as cross-reactive MAbs. CDR3 length and mutation numbers are  
855 presented as mean ± standard error of the mean (anti-S2, specific, n=4 versus cross-  
856 reactive, n=5; anti-N, specific, n=16 versus cross-reactive, n=19). The two-tailed  
857 Mann-Whitney test was performed to compare the mutations between two groups. \* p  
858 < 0.05, \*\* p < 0.01 ; D, =Day ; ns, non-significant ; CR, cross-reactive.



859

860

861 **Figure 3. Neutralisation of wild type SARS-CoV-2 by (a) anti-RBD, (b) anti-S1-**  
862 **non-RBD and -S2 monoclonal antibodies.** Neutralisation assays were performed on  
863 the indicated antibodies according to the fluorescent focus-forming units  
864 microneutralisation method (see methods). Data were normalized to control (no  
865 antibody) values of foci, and the grey region comprises  $\pm 1$  standard deviation the  
866 mean control values. Individual points are displayed  $\pm 1$  standard deviation of  
867 technical, and curves are shown only where the data for a particular antibody fitted  
868 the standard dose-response (Hill) equation (n=3).