

1 **Neutralizing antibody responses to SARS-CoV-2 in a COVID-19 recovered**  
2 **patient cohort and their implications**

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4 Fan Wu\*, Aojie Wang, Mei Liu, Qimin Wang, Jun Chen, Shuai Xia, Yun Ling, Yuling  
5 Zhang, Jingna Xun, Lu Lu, Shibo Jiang, Hongzhou Lu, Yumei Wen, Jinghe Huang\*

6  
7 Shanghai Public Health Clinical Center and Key Laboratory of Medical Molecular  
8 Virology (MOE/NHC/CAMS), School of Basic Medical Sciences, Fudan University,  
9 Shanghai, China

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11 \*Correspondence to: Dr. Jinghe Huang, 86-21-37990333-7326, Email:  
12 [Jinghehuang@fudan.edu.cn](mailto:Jinghehuang@fudan.edu.cn); or Dr. Fan Wu, 86-21-37990333-5295, Email:  
13 [wufan@fudan.edu.cn](mailto:wufan@fudan.edu.cn);

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## 24 **Background**

25 The COVID-19 pandemic caused by SARS-CoV-2 coronavirus threatens global public  
26 health. Currently, neutralizing antibodies (NAbs) versus this virus are expected to  
27 correlate with recovery and protection of this disease. However, the characteristics of  
28 these antibodies have not been well studied in association with the clinical  
29 manifestations in patients.

## 31 **Methods**

32 Plasma collected from 175 COVID-19 recovered patients with mild symptoms were  
33 screened using a safe and sensitive pseudotyped-lentiviral-vector-based neutralization  
34 assay. Spike-binding antibody in plasma were determined by ELISA using RBD, S1,  
35 and S2 proteins of SARS-CoV-2. The levels and the time course of SARS-CoV-2-  
36 specific NAbs and the spike-binding antibodies were monitored at the same time.

## 38 **Findings**

39 SARS-CoV-2 NAbs were unable to cross-reactive with SARS-CoV virus. SARS-CoV-  
40 2-specific NAbs were detected in patients from day 10-15 after the onset of the disease  
41 and remained thereafter. The titers of NAb among these patients correlated with the  
42 spike-binding antibodies targeting S1, RBD, and S2 regions. The titers of NAbs were  
43 variable in different patients. Elderly and middle-age patients had significantly higher  
44 plasma NAb titers ( $P < 0.0001$ ) and spike-binding antibodies ( $P = 0.0003$ ) than young  
45 patients. Notably, among these patients, there were ten patients whose NAb titers were  
46 under the detectable level of our assay ( $ID_{50} < 40$ ); while in contrast, two patients,  
47 showed very high titers of NAb, with  $ID_{50} : 15989$  and  $21567$  respectively. The NAb  
48 titers were positive correlated with plasma CRP levels but negative correlated with the  
49 lymphocyte counts of patients at the time of admission, indicating an association  
50 between humoral response and cellular immune response.

## 52 **Interpretation**

53 The variations of SARS-CoV-2 specific NAbs in recovered COVID-19 patients may  
54 raise the concern about the role of NAbs on disease progression. The correlation of  
55 NAb titers with age, lymphocyte counts, and blood CRP levels suggested that the  
56 interplay between virus and host immune response in coronavirus infections should be  
57 further explored for the development of effective vaccine against SARS-CoV-2 virus.  
58 Furthermore, titration of NAb is helpful prior to the use of convalescent plasma for  
59 prevention or treatment.

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64 Sciences

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67

## 68 **Introduction**

69 The outbreak of coronavirus disease 2019 (COVID-19) in December 2019 has spread  
70 around the world and become a global pandemic.<sup>1</sup> The etiological agent of COVID-19  
71 was identified as a SARS-related coronavirus designated as SARS-CoV-2  
72 coronavirus.<sup>2,3</sup> As of March 27, 2020, it had caused a total of 509,164 cases of infection  
73 and resulted in 23,335 deaths worldwide.<sup>1</sup> About 81% of infected patients showed only  
74 mild symptoms, but 14% of them had severe symptoms such as dyspnea, high  
75 respiratory frequency and low blood oxygen saturation. Another 5% of patients,  
76 especially those over 60, or with comorbidities, progressed to critical condition. About  
77 3.4% of patients died from respiratory failure or multiple organ failure.<sup>4</sup> Although the  
78 estimated mortality rate of COVID-19 was lower than SARS and MERS, the number  
79 of deaths associated with COVID-19 has already surpassed those of SARS and MERS  
80 owing to the extremely high transmissibility of SARS-CoV-2 coronavirus. Currently,  
81 no licensed vaccine or drugs are available to prevent or treat COVID-19 infection, and  
82 most infected patients have been treated with supportive care.

83

84 Neutralizing antibodies (NAbs) play important roles in virus clearance and have been  
85 considered as a key immune product for protection or treatment against viral diseases.  
86 Virus-specific NAbs, induced through either infection or vaccination, have the ability  
87 to block viral infection. The level of NAbs has been used as a gold standard to evaluate  
88 the efficacy of vaccines against smallpox, polio and influenza viruses.<sup>5</sup> Passive  
89 antibody therapy, such as plasma fusion, was successfully used to treat infectious viral  
90 diseases, including SARS-CoV virus,<sup>6</sup> influenza viruses,<sup>7</sup> and Ebola virus.<sup>8</sup> The  
91 efficacy of passive antibody therapy was associated with the concentration of NAbs in  
92 plasma or antibodies of recovered donors.<sup>8</sup> As the global pandemic of COVID-19  
93 proceeds, transfusion of convalescent plasma or serum from recovered patients was also  
94 considered as a promising therapy for prophylaxis of infection or treatment of disease.<sup>9</sup>  
95 However, the levels and roles of SARS-CoV-2-specific NAbs in patients with COVID-  
96 19 have not been reported.

97

98 Here, we used a pseudotyped-lentiviral-vector-based neutralization assay to measure  
99 SARS-Cov-2-specific NAbs in plasma from recovered COVID-19 patients with mild  
100 symptoms. The pseudovirus (PsV) neutralization assay is a sensitive and reproducible  
101 assay. It does not produce any highly pathogenic virus, and it can be safely handled in  
102 a biosafety level 2 facility. Herein, we aimed to explore the clinical characteristics  
103 associated with the level of NAbs in recovered patients, the outcome of which may  
104 provide useful information for the development of vaccines and passive antibody  
105 therapy for the prevention and treatment of SARS-CoV-2.

106

## 107 **Methods**

## 108 **Study design and participants**

109 The study included a cohort of 175 adult COVID-19 patients admitted to Shanghai  
110 Public Health Clinical Center. The study was conducted under a clinical protocol  
111 approved by the Investigational Review Board in the Shanghai Public Health Clinical  
112 Center (Study number: YJ-2020-S021-01). All participants signed an informed consent  
113 approved by the IRB. All patients were diagnosed with laboratory-confirmed COVID-  
114 19 and discharged after meeting effective national treatment standards. Clinical  
115 information, including complete blood counts, blood biochemistry was collected at the  
116 time of admission.

117

## 118 **Materials**

119 293T cells expressing human angiotensin converting enzyme II (ACE2) (293 T/ACE2)  
120 were obtained from the American Type Culture Collection (ATCC; Manassas, VA, USA)  
121 and were cultured in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal  
122 bovine serum (FBS). The three domains of SARS-CoV-2 spike (S) protein, including  
123 S1 and S2 subunits, as well as RBD, were purchased from Sino Biological Company  
124 (Beijing, China). The expression plasmids for SARS S protein pcDNA3.1-SARS-S  
125 (ABD72979.1) and SARS-CoV-2 S protein pcDNA3.1-SARS-CoV-2-S (NC\_045512)  
126 were synthesized by Genscript. The VSV-G envelope eukaryotic expression vector  
127 pHEF-VSVG and the HIV-1 Env-deficient luciferase reporter vector pNL4-3. Luc. R-  
128 E- were obtained through the NIH AIDS Reagent Program.

129

## 130 **Neutralization assay**

131 Neutralization activity of plasma from COVID-19 patients was measured using a  
132 single-round PsV infection of 293 T/ACE2 cells. PsVs of SARS-CoV-2, SARS-CoV  
133 and VSV-G virus were generated by co-transfection of 293T cells with pNL4-  
134 3.Luc.R-E- backbone and viral envelope protein expression plasmids pcDNA3.1-  
135 SARS-CoV-2-S, pcDNA3.1-SARS-S or pHEF-VSVG. PsVs could infect the same cells  
136 as those infected by SARS-CoV-2 or SARS-CoV viruses.<sup>10,11</sup> The neutralization assay  
137 was performed in accordance with the following steps. First, 293 T/ACE2 cells were  
138 seeded in a 96-well plate at a concentration of  $10^4$  cells per well and cultured for 12  
139 hours. Then, ten  $\mu$ l heat-inactivated plasma were five-fold serially diluted with DMEM  
140 with 10% FBS and mixed with 40  $\mu$ l of PsV. The mixture was added to cultured  
141 293 T/ACE2 for infection. The culture medium was refreshed after 12 hours and  
142 incubated for an additional 48 hours. Assays were developed with a luciferase assay  
143 system (Promega), and the relative light units (RLU) were read on a luminometer  
144 (Perkin Elmer). The titers of NAbS were calculated as 50% inhibitory dose (ID<sub>50</sub>),  
145 expressed as the highest dilution of plasma which resulted in a 50% reduction of  
146 luciferase luminescence compared with virus control.

147

## 148 **ELISA**

149 SARS-CoV-2 RBD, S1, or S2 protein and SARS-CoV RBD or S1 protein (1 µg/ml)  
150 was coated on a MaxiSorp Nunc-immuno 96-well plate overnight at 4 °C. Wells were  
151 blocked with 5% nonfat milk in PBS for 1 hour at room temperature, followed by  
152 incubation with 1:400 diluted sera or serially diluted sera in disruption buffer (PBS, 5%  
153 FBS, 2% BSA, and 1% Tween-20) for 1 hour at room temperature. A 1:2500 dilution  
154 of horseradish peroxidase (HRP)-conjugated goat anti-human IgG antibody was added  
155 for 1 hour at room temperature. Wells were washed five times between each step with  
156 0.2% Tween-20 in PBS. Wells were developed using ABST (Thermo) and read at 405  
157 nm.

158

## 159 **Statistical analysis**

160 Statistical analyses were carried out using GraphPad Prism 7.0. Data are indicated as  
161 medians. Differences between nominal data were tested for statistical significance by  
162 use of paired or unpaired *t* test. Correlations were calculated using standard Pearson  
163 correlation.

164

## 165 **Role of the funding source**

166 The funders of the study had no role in study design, data collection, data analysis, data  
167 interpretation, or writing of the report. The corresponding author had full access to all  
168 the data in the study and had final responsibility for the decision to submit for  
169 publication.

170

## 171 **Results**

### 172 **Clinical Characteristics**

173 A total of 175 COVID-19 patients had recovered and were discharged from the  
174 Shanghai Public Health Clinical Center as of February 26, 2020. Their symptoms were  
175 common or mild, and none of them was admitted to the ICU. The median age of the  
176 patients was 50 years (ranging from 16 to 85 years); 53 % of the patients were female.  
177 The median length of hospital stay was 16 days (ranging from 7 to 30 days), and the  
178 median disease duration was 21 days (9 to 34 days).

179

### 180 **Convalescent plasma from COVID-19 patients specifically inhibited SARS-CoV- 181 2, but not SARS-CoV infection**

182 We collected five plasma samples from COVID-19 patients at the time of discharge and  
183 measured their neutralizing titers against SARS-CoV-2 infection of 293T/ACE2 cells.  
184 All five plasma showed a concentration-dependent inhibition of SARS-CoV-2 PsV

185 infection of 293T/ACE2 cells (Figure 1A). Plasma with high titers of NAb showed  
186 higher titers of SARS-CoV-2 RBD, S1, and S2-specific binding antibodies (Figure 1B).  
187 Moreover, plasma from these patients also showed cross-binding to SRAS-CoV RBD  
188 and S1 regions (Figure 1C), but the binding to SARS-CoV S protein was not consistent  
189 with that to SARS-CoV-2 S protein. Furthermore, plasma from COVID-19 patients  
190 could not inhibit SARS-CoV infection in PsV neutralization assay. 26 plasma samples  
191 from COVID-19 patients, which showed strong SARS-CoV-2 neutralizing activities  
192 (Figure 1D), could neither neutralize SARS-CoV PsV infection nor the control VSV-G  
193 PsV infection (Figure 1E). These results suggest that SARS-CoV-2 was able to  
194 stimulate SARS-CoV cross-binding antibodies. However, it was unable to induce the  
195 cross-neutralizing antibodies against SARS-CoV. These results suggested that the  
196 epitope or immunogenicity between SARS-CoV-2 and SARS-CoV were different.

197

### 198 **COVID-19 patients generated SARS-CoV-2-specific NAb and spike-binding** 199 **antibodies concurrently from day 10 to 15 after infection**

200 We monitored the kinetics of SARS-CoV-2-specific NAb development during the  
201 course of disease. The titers of NAb were evaluated in plasma collected from six  
202 patients at different time points after the disease onset. The kinetics of NAb  
203 development were similar among patients. The titers of NAb in all patients were low  
204 (ID<sub>50</sub>: < 200) before day 10 post-disease onset and then increased at day 10 to 15 post-  
205 disease onset, remaining stable thereafter (Figure 2A). We also measured the binding  
206 antibodies to the different domains (RBD, S1, and S2) of SARS-CoV-2 spike protein  
207 in the plasma of these six patients. The kinetics of NAb (right Y axis) and binding  
208 antibodies targeting RBD, S1, and S2 domains (left Y axis) were aligned with individual  
209 patients (Figure 2B). We evaluated the SARS-CoV-2-specific NAb titers and the spike-  
210 binding antibody levels in the plasma of 175 recovered patients on the day of discharge.  
211 We observed that SARS-CoV-2-specific NAb titers moderately correlated with spike-  
212 binding antibodies targeting RBD ( $r=0.51$ ,  $p<0.0001$ ), S1 ( $r=0.42$ ,  $p<0.0001$ ), and S2  
213 ( $r=0.435$ ,  $p<0.0001$ ) (Figure 2C). These results suggested that humoral immune  
214 responses of COVID-19 patients against SARS-CoV-2 occurred on day 10 to 15 after  
215 infection. Besides RBD region, S2 domain might be the target of SARS-CoV-2-NAb.  
216 Since binding antibodies may also play a role in viral clearance through antibody-  
217 dependent phagocytosis or antibody-dependent cellular cytotoxicity, the effect of NAb  
218 and binding antibodies on disease progression is worth comprehensive evaluation in  
219 further study.

220

### 221 **About 30% of recovered patients generated very low titers of SARS-CoV-2-** 222 **specific NAb**

223 We observed that NAb titers were variable in the plasma of 175 recovered patients.  
224 ID<sub>50</sub>s ranged from below detection limit (<40) to 21567 (Figure 3A). About 30% of  
225 recovered patients generated a very low level of NAb titers (ID<sub>50</sub>: < 500) (Figure 3A,

226 3B, and Supplementary Table 1), and NAb titers in ten of them were below the limit of  
227 detection (ID50: <40), though all of them were lab confirmed infected with SARS-  
228 CoV-2 (Supplementary Table 2). About 17%, 39%, and 14% showed medium-low  
229 (ID50: 500-999), medium-high (ID50: 1000-2500), and high (ID50: > 2500) NAb titers,  
230 respectively (Figure 3B). We also collected and measured the levels of NAb in plasma  
231 from 47 of the 175 patients during the follow-up examination two weeks after discharge.  
232 As shown in Figure 3C, NAb plasma titers collected at the time of follow-up  
233 examinations did not significantly differ from those collected at the time of discharge  
234 ( $P=0.250$ , paired- $t$  test). Patients who did not generate NAb at the time of discharge  
235 did not develop NAb thereafter. These results revealed that a proportion of patients  
236 infected with SARS-CoV-2 would recover without developing high titers of virus-  
237 specific NAb. How these patients recovered without the help of NAb and whether  
238 they were at risk of re-infection of SARS-CoV-2 should be further explored. Titration  
239 of NAb is helpful prior to the use of convalescent plasma for prevention or treatment.

240

#### 241 **Elderly and middle-age recovered COVID-19 patients developed higher levels of** 242 **SARS-CoV-2-specific NAb**

243 We observed that elderly patients were more likely to induce higher titers of NAb than  
244 younger patients. As shown in Figure 4A, the patients were divided into three groups  
245 based on their age, young (15-39 years), middle-age (40-59 years) and elderly (60-85  
246 years). Patient numbers from each group were similar (55, 64 and 56) (Supplementary  
247 Table 3). NAb titers of elderly and middle-age recovered patients were significantly  
248 higher than those of young recovered patients ( $p<0.0001$  and  $p<0.0001$ ,  $t$  test) (Figure  
249 4A), and the corresponding median ID50s were 1537, 1255, and 488, respectively  
250 (Figure 4A). A moderate positive correlation was also observed between age and NAb  
251 titers ( $r=0.436$ ,  $P<0.001$ , Pearson) (Figure 4C), confirming the important role of age in  
252 the generation of NAb. Elderly and middle-age recovered patients had significantly  
253 higher levels of spike-binding antibodies, targeting RBD ( $p<0.0001$  and  $p=0.0094$ ,  $t$   
254 test), S1 ( $p=0.0003$  and  $p=0.0035$ ,  $t$  test), and S2 ( $p=0.0003$  and  $p=0.0019$ ,  $t$  test) than  
255 those of young recovered patients (Figure 4C). However, no difference was observed  
256 between patients' ages and the length of stay in hospital (Figure 4D). These results  
257 indicated that high level of NAb might be useful to clear the viruses and helpful for  
258 the recovery of elderly and middle-age patients.

259

#### 260 **COVID-19 recovered patients age and SARS-CoV-2-specific NAb titers** 261 **negatively correlated with lymphocyte count and positively correlated with CRP** 262 **levels on admission**

263 Older age was usually associated with poor outcome among COVID-19 patients<sup>12</sup>.  
264 Consistent with the previous reports, the elderly and middle-age patients in this cohort  
265 had lower lymphocyte counts ( $r= -0.389$ ,  $p<0.0001$ , Figure 5A left) and higher CRP  
266 level ( $r= -0.432$ ,  $p<0.0001$ , Figure 5A right) than young patients on admission (Figure

267 5A left and right). However, none of the patient progressed into severe conditions, and  
268 no significant difference was observed between age and length of hospital stay among  
269 these patients (Figure 4D). Interestingly, we observed that the NAb titers negatively  
270 correlated with blood lymphocyte counts ( $r = -0.44$ ,  $p < 0.0001$ , Figure 5B left) and  
271 positively correlated with blood CRP levels ( $r = 0.5$ ,  $p < 0.0001$ , Figure 5B right),  
272 suggesting that the humoral response might play an important role when cellular  
273 response was dysfunction or impaired.

274

## 275 **Discussion**

276 Spread of the COVID-19 global pandemic highlights the urgent need to develop  
277 effective treatments or vaccines against SARS-CoV-2 infection. NABs have been  
278 considered as an effective drug to treat or prevent virus infection. Here we evaluated  
279 the level of NABs in recovered patients of COVID-19 by using a PsVs neutralization  
280 assay, which has been extensively used for the evaluation of NABs for many highly  
281 pathogenic viruses, including Ebola,<sup>13</sup> highly pathogenic influenza virus,<sup>14,15</sup> SARS-  
282 CoV,<sup>16</sup> and MERS-CoV.<sup>17</sup> The PsVs neutralization assay was also used for the  
283 evaluation of NABs for SARS-CoV-2 in some recent reports,<sup>11,18,19</sup> generating  
284 consistent results compared with traditional plaque reduction neutralization assay.<sup>18</sup>

285 We found that most COVID-19 patients developed SARS-CoV-2-specific NABs at the  
286 convalescent phase of infection. The titers of NABs reached their peak at 10 to 15 days  
287 after disease onset and remained stable thereafter in patients. Antibodies targeting on  
288 different domains of S protein, including S1, RBD and S2, may all contribute to the  
289 neutralization.

290 Conserved epitopes may exist between SARS-CoV-2 and SARS-CoV since they share  
291 77.2% identical amino acids in their spike proteins.<sup>2</sup> Few reports have demonstrated  
292 that SARS-CoV-specific monoclonal NABs could cross-neutralize SARS-CoV-2 PsV  
293 infection,<sup>3,11,18</sup> Even though plasma from COVID-19 patients showed cross-binding to  
294 SARS-Cov, they did not neutralize SARS-CoV, indicating that the antigenicity of  
295 SARS-CoV-2 is different from that of SARS-CoV. Evidence deduced from this study  
296 only suggested that cross-neutralizing antibodies targeted the conserved epitopes of  
297 SARS-CoV and SARS-CoV-2 may not be easily elicited during the infection of  
298 COVID-19, making this a potential line of advanced study.

299 It is also noteworthy that the levels of NABs in patients were variable. About 30% of  
300 patients failed to develop high titers of NABs after COVID-19 infection. However, the  
301 disease duration of these patients compared to others was similar. Notably, there were  
302 ten recovered patients whose NAB titers were very low, under the detectable level of  
303 this study (ID50:  $< 40$ ), suggesting that other immune responses, including T cells or  
304 cytokines, may contribute to the recovery of these patients. Whether these patients were  
305 at high risk of rebound or reinfection should be explored in further studies. On the other  
306 hand, two patients had very high titer of NABs, which were over ID50: 15989 and 21567  
307 respectively, but did not show any antibody-related adverse reactions.



308 The NAbs titers in patients were also observed to be correlated with the age of the  
309 patients. Elderly patients had significantly higher titers of NAbs than younger patients.  
310 Age has been reported as an important predictor of adverse disease outcome after  
311 infection with coronavirus, including SARS-CoV<sup>20</sup>, MERS-CoV<sup>21</sup> and SARS-CoV<sup>22</sup>.  
312 Previous studies in SARS-CoV-infected macaques revealed that aged macaques  
313 induced elevated innate immune response, resulting in more severe pathology than  
314 young adult macaques<sup>22</sup>. The elderly patients in this cohort also had higher blood CRP  
315 level and lower lymphocyte counts at the time of admission, indicating the induction of  
316 stronger innate immune response than younger patients. High level of NAbs may be a  
317 result of strong immune response in these elderly patients. Whether the high level of  
318 NAbs protect these patients from progression into severe and critical conditions is  
319 worthy of comprehensive evaluation. Further study of the immunological  
320 characteristics of COVID-19 patients may reveal key determinants in the generation of  
321 NAbs and effective cell-mediated immune responses, which is important for the  
322 development of an effective vaccine against SARS-CoV-2 virus.

323 This study is preliminary and has several limitations. First, viral RNA was not  
324 detectable in patients' blood. Owing to the lack of respiratory specimens, information  
325 about the kinetics of viral loads was not available. Second, patients in severe and critical  
326 condition were excluded from the study because they received passive antibody  
327 treatment before sample collection. Thus, we were not able to directly evaluate the  
328 effect of NAbs on virus clearance or disease progression of COVID-19 patients in this  
329 study. A further comprehensive study should be made to address the question.

330 To the best of our knowledge, this is the first report about NAbs drawn from the plasma  
331 of a COVID-19 recovered patient cohort, potentially providing useful information for  
332 passive antibody therapy and vaccine development against SARS-CoV-2 virus. The  
333 highly variable levels of NAbs in the patients of COVID-19 indicated that convalescent  
334 plasma and serum from recovered donors should be titrated before use in passive  
335 antibody therapy, an easy task that can be performed using the PsV neutralization assay.  
336 Correlation of NAbs titers with the age, lymphocyte counts and blood CRP levels of  
337 patients also lays the groundwork for further study to explore the mechanism of NAbs  
338 development in COVID-19 patients.

339

#### 340 **Declaration of interests**

341 We declare no competing interests.

342

343

#### 344 **Contributions**

345 JH, FW, and YW conceived and designed the experiments. JH, FW, AW, ML, QW, and  
346 YZ performed the experiments. JH, FW, SX, and LL constructed the SARS-CoV-2 PsV  
347 plasmid. FW, JH, HL, JC, YL, QW, and JX collected the samples of recovered patient  
348 and clinical information. JH, FW, YW, and SJ analyzed the data and wrote the  
349 manuscript.

350

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## 421 **Figure legends**

422 **Figure 1. Plasma from COVID-19 recovered patients specifically inhibited SARS-CoV-2 infection**  
423 **but not SARS-CoV virus.** (A) Plasma from five COVID-19 recovered patients inhibited infection of  
424 SARS-CoV-2. Plasma from a healthy donor was used as a negative control. The assay was performed in  
425 duplicate and the median percentage of neutralization is shown. (B) Biding of COVID-19 recovered  
426 patient plasma to SARS-CoV-2 RBD, S1, and S2 proteins. (C) Biding of COVID-19 recovered patient  
427 plasma to SARS-CoV RBD and S1 proteins. (D) The SARS-CoV-2 NAbs titers of 26 plasma from  
428 COVID-19 recovered patients were compared with 13 plasma from healthy donors. P value was  
429 calculated using *t* test. (E)The titers of NAbs against VSV, SARS-CoV, and SARS-CoV-2 PsV in 26  
430 COVID-19 recovered patient plasma were compared. P values were calculated using *t* test.

431  
432 **Figure 2. SARS-CoV-2-specific NAbs and spike-binding antibodies emerged concurrently on day**  
433 **10-15 during the COVID-19 disease progression and shown correlation.** (A) Kinetics of SARS-CoV-  
434 2 NAbs titers in six COVID-19 patients are shown. Plasma were collected at different time points post  
435 syndrome onset. (B) Kinetics of spike binding antibodies (left Y axis), targeting RBD (blue), S1 (green),

436 and S2 (brown), in six COVID-19 patient plasma are shown and compared with the kinetics of NAb  
437 titers (right Y axis, red) in the same patient. (C) The correlations between the SARS-CoV-2 NAb titers  
438 and RBD, S1, or S2 binding antibodies levels of patients were analyzed by Pearson correlation test. 1:400  
439 diluted plasma was incubate with RBD, S1, or S2 protein.

440

441 **Figure 3. COVID-19 recovered patients developed variable levels of SARS-CoV-2 specific NAb.**

442 (A) SARS-CoV-2 NAb titers (ID50) of 175 COVID-19 recovered patient plasma collected on the day of  
443 discharge were measured in a PsV neutralization assay. (B) Percentages of patients with low (ID50: <500),  
444 medium-low (ID50: 500-999), medium-high (ID50: 1000-2500), and high (ID50: >2500) titers of SARS-  
445 CoV-2-specific NAb are shown. (C) NAb titers of 47 COVID-19 recovered patient plasma collected  
446 on the day of discharge and the subsequent visit in two weeks were compared. P value was calculated  
447 using *t* test.

448

449 **Figure 4. Elderly and middle-age recovered COVID-19 patients developed higher levels of SARS-**

450 **CoV-2-specific NAb than young recovered patients.** (A) NAb titers of young (15-39 years), middle-  
451 age (40-59 years), and elderly (60-85 years) patients were compared. P values were calculated using *t*  
452 test. (B) The correlation between ages of patients and the titers of SARS-CoV-2-specific NAb was  
453 analyzed by Pearson correlation test. (C) RBD, S1, or S2 binding antibodies levels of young, middle-age,  
454 and elderly recovered COVID-19 patients were compared. P values were calculated using *t* test.

455

456 **Figure 5. Age and SARS-CoV-2-specific NAb levels negatively correlated with lymphocyte count**  
457 **and positively correlated with CRP levels of patients on the time admission.**

458 (A) The correlations between patient age and lymphocyte counts (left) or C-reactive protein (CRP) level  
459 (right) on admission were analyzed by Pearson correlation test. (B) Correlations between SARS-CoV-2-  
460 specific NAb titers and lymphocyte count (left) or CRP level (right) of patients were analyzed by Pearson  
461 correlation tests. The reference range for lymphocyte counts is  $1.1-3.2 \times 10^9$  /L and for blood CRP is less  
462 than 3mg/L.

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Figure 1

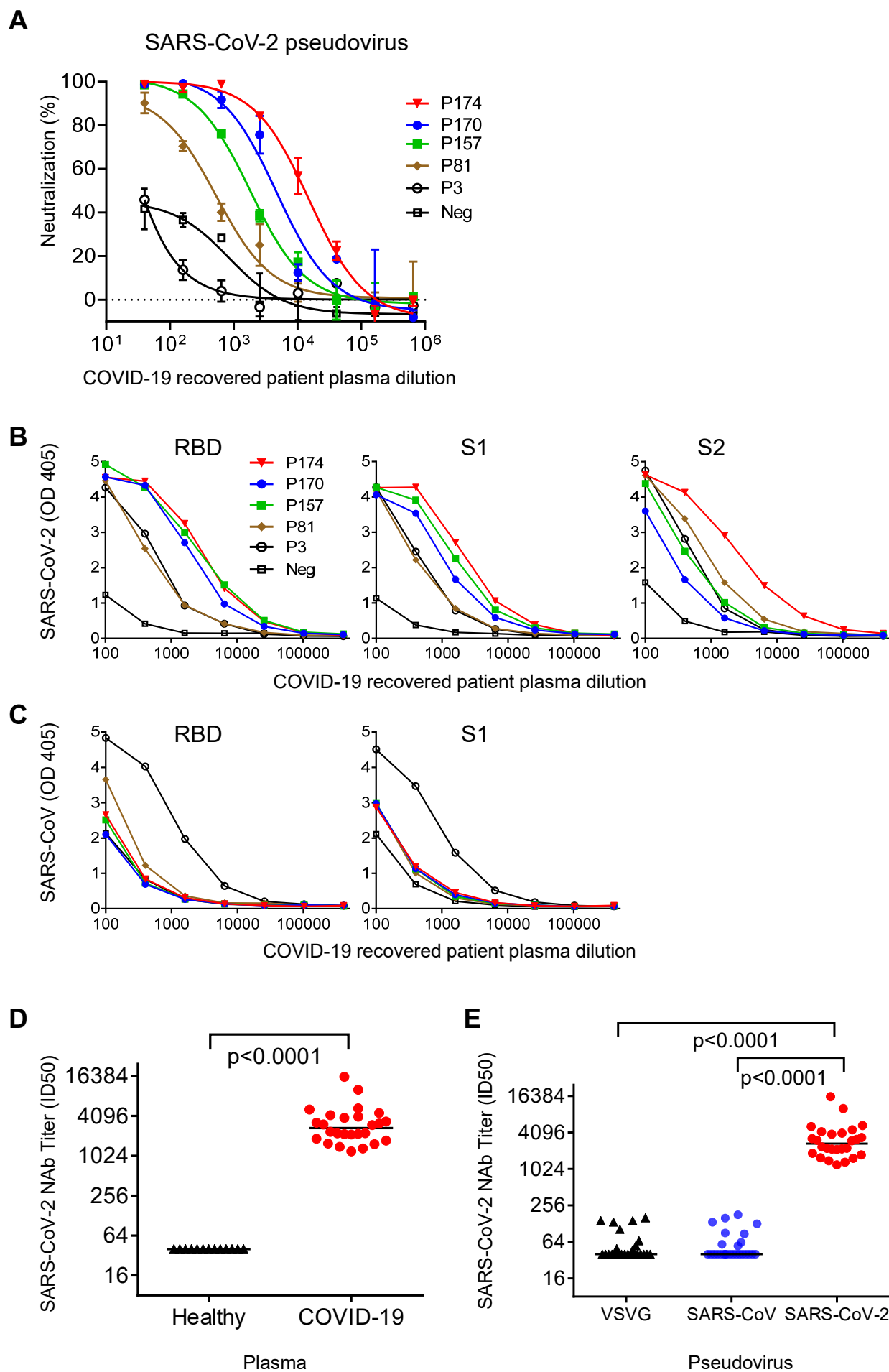
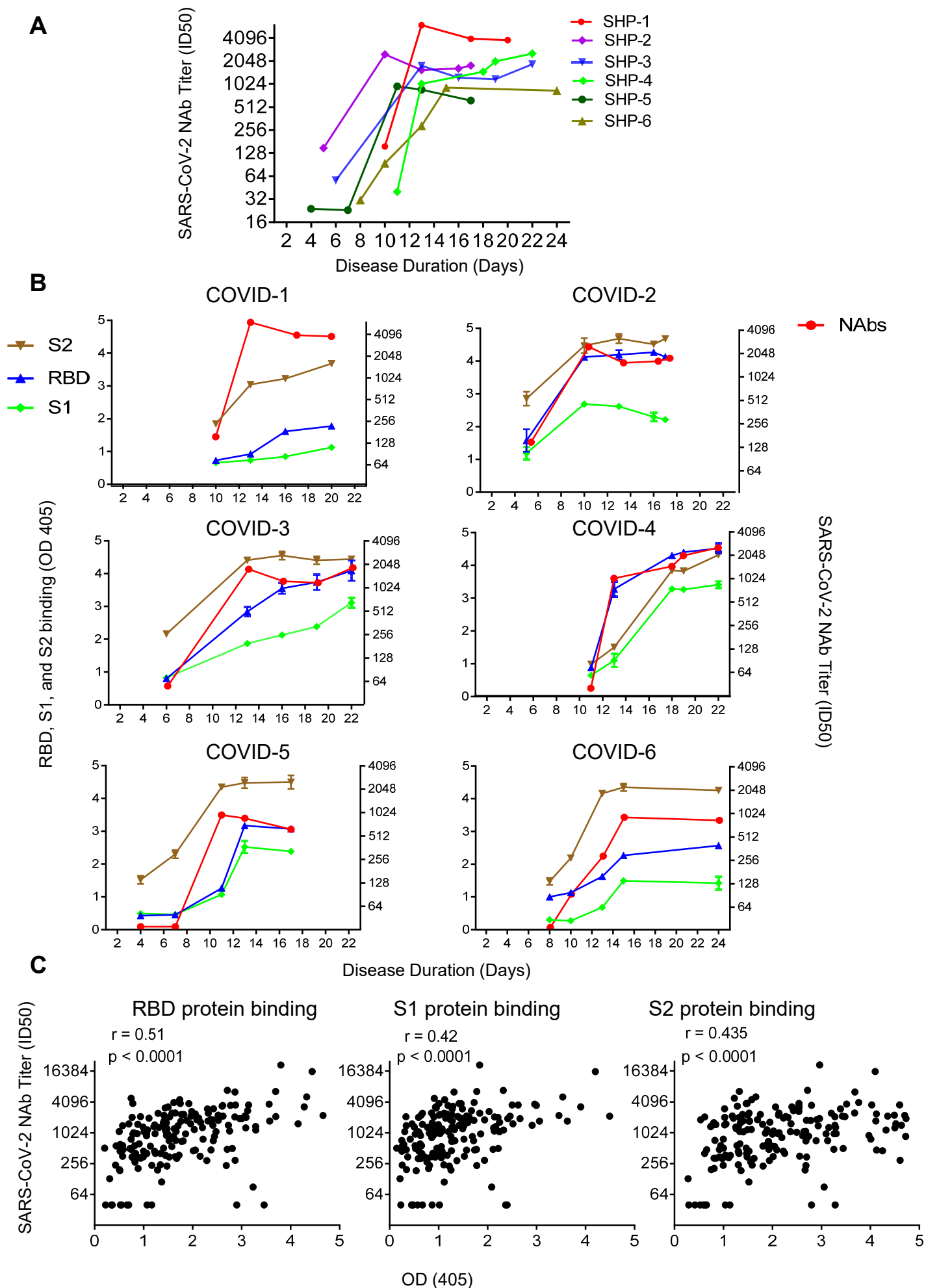


Figure 2



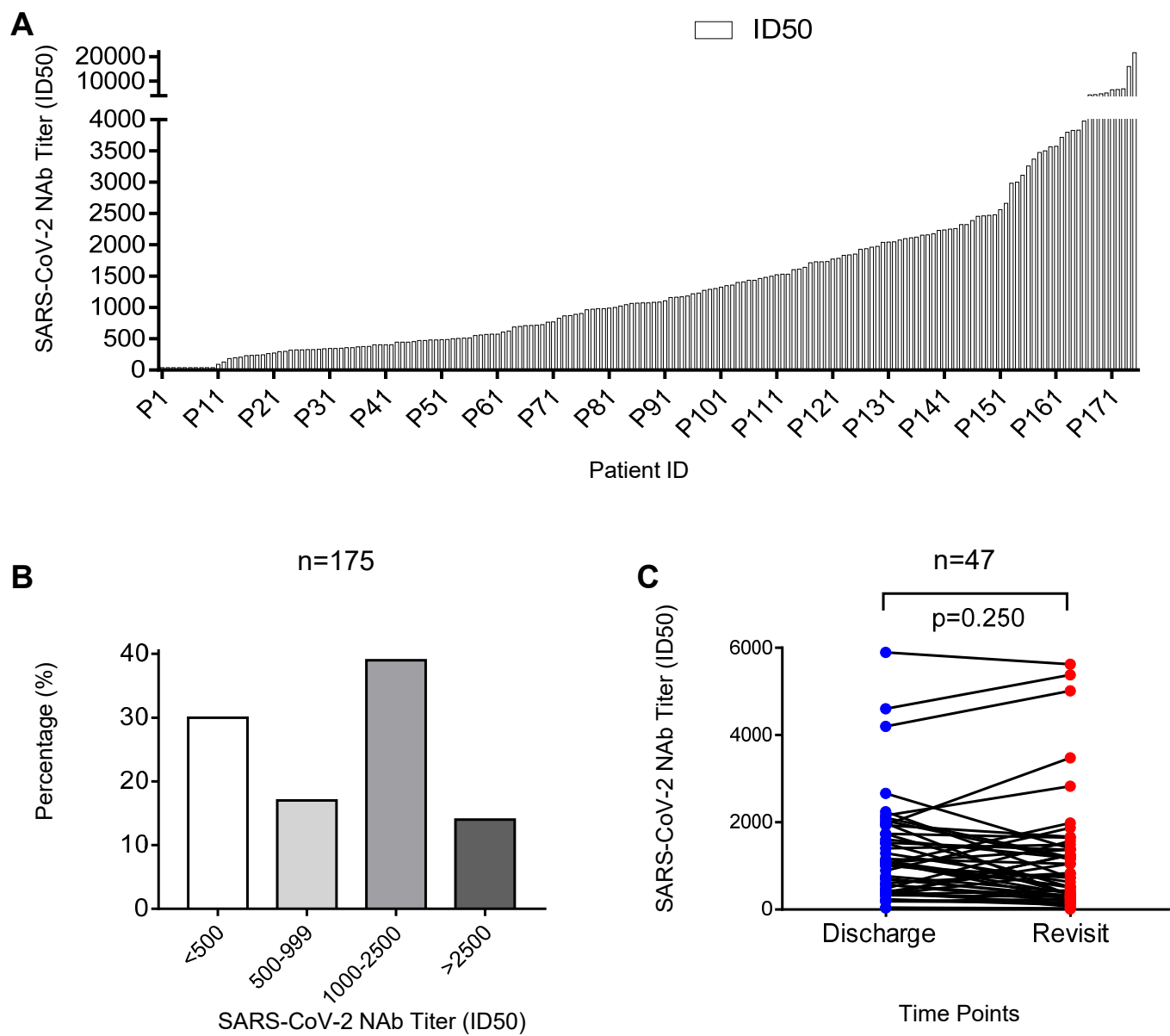
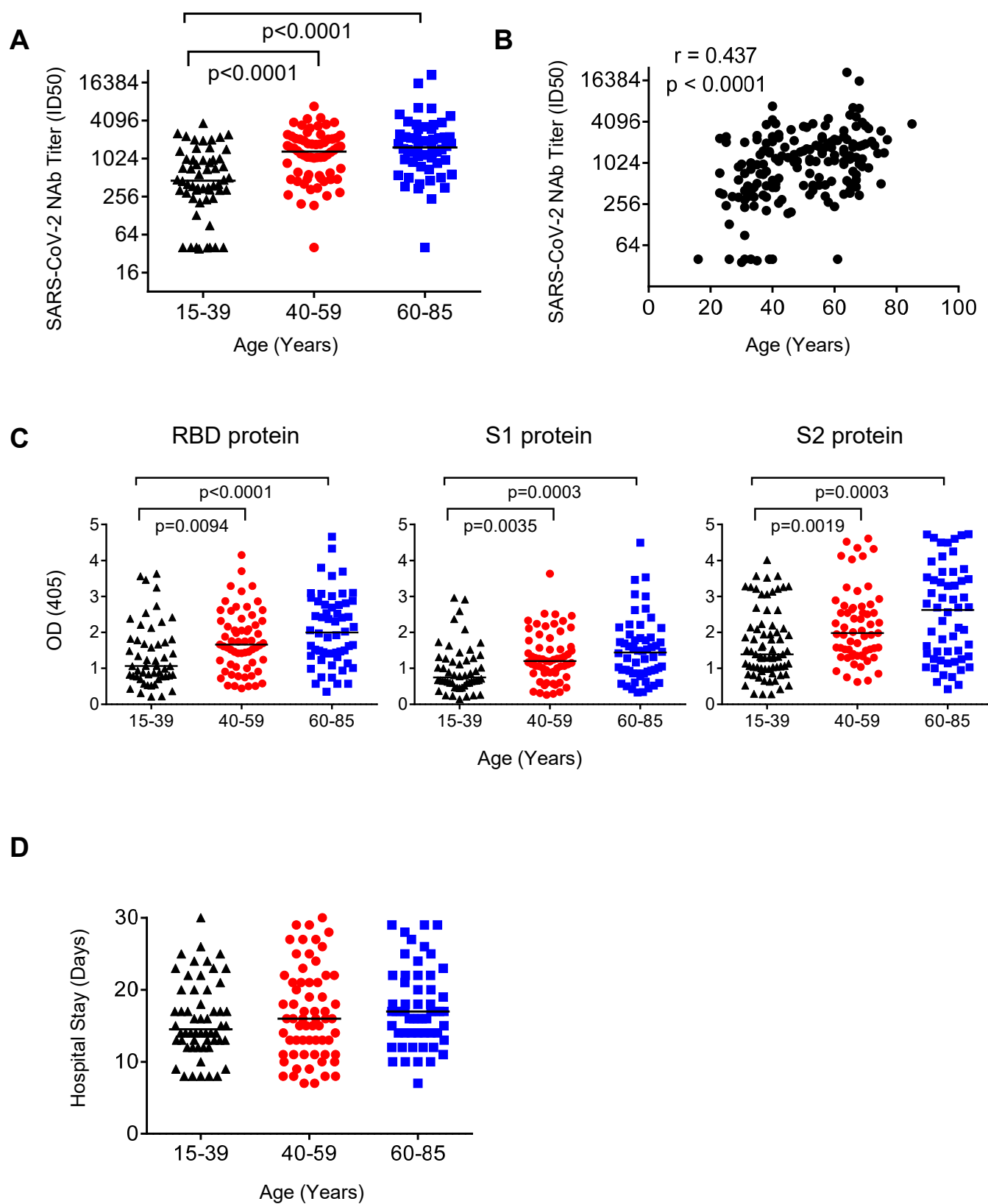
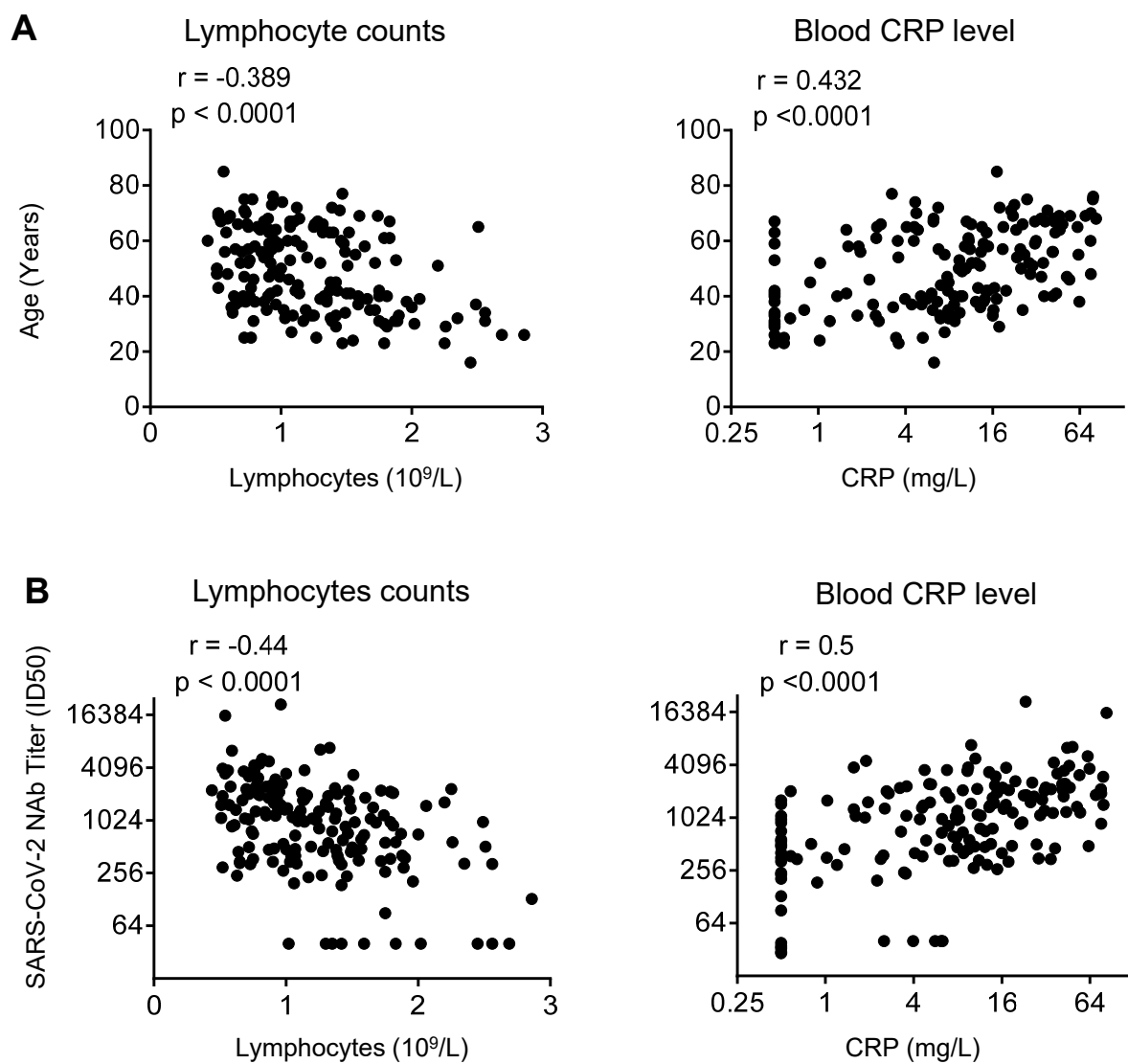


Figure 4







**Supplementary Table 1. Clinical characteristics of COVID-19 recovered patients with low, medium- low, medium-high, and high titers of SARS-CoV-2-specific NAb**

<b>Patient Information</b>	<b>Low<sup>a</sup></b>	<b>Medium-low<sup>a</sup></b>	<b>Medium-high<sup>a</sup></b>	<b>High<sup>a</sup></b>
	<b>&lt;500</b>	<b>500-999</b>	<b>1000-2500</b>	<b>&gt;2500</b>
Recovered Patient No.	52 (30%)	29 (17%)	69 (39%)	25 (14%)
Male	19 (23%)	13 (16%)	36 (44%)	14 (17%)
Female	33 (35%)	16 (17%)	33 (35%)	11 (12%)
Median Age (Years)	38 (16-68)	42 (23-75)	56 (23-77)	63 (35-85)
Length of Stay (Days)	14.5 (8-29)	15 (7-28)	16 (8-30)	18 (10-29)
Disease Duration (Days)	20 (9-33)	21 (16-31)	22 (11-34)	23 (13-32)
Median NAb titers (ID50)	327 (40-488)	715 (504-989)	1642 (1004-2482)	3800 (2560-21567)

<sup>a</sup> SARS-CoV-2-specific NAb titer (ID50) values < 500 were defined as low levels, values between 500 and 999 were defined as medium-low levels, values between 1000 and 2500 were defined as medium-high levels, and values >2500 were defined as high levels.

**Supplementary Table 2. Clinical characteristics of ten COVID-19 recovered patients with undetectable level of SARS-CoV-2 specific NABs.**

ID	Age (Years)	Gender	ID50 <sup>a</sup>	ID80 <sup>a</sup>	Length of Hospital (Days)	Disease Duration (Days)	Temp (°C)	Viral RNA tests	Symptoms
P1	30	F	<40	<40	22	31	37.8	+	fever and stuffy nose
P2	35	F	<40	<40	17	22	37.6	+	Cough, sore muscles, and stuffy nose
P3	16	M	<40	<40	9	12	37.7	+	Stuffy nose, runny nose, and cough
P4	39	F	<40	<40	8	12	38.1	+	Cough
P5	40	M	<40	<40	13	14	37.9	+	Cough and chest pain
P6	33	F	<40	<40	13	15	37.4	+	Fatigue
P7	61	F	<40	<40	18	22	37.2	+	Chill
P8	39	F	<40	<40	21	23	38.1	+	Sore throat, cough, and fatigue
P9	26	F	<40	<40	8	9	38	+	Cough
P10	31	F	<40	<40	12	23	38.4	+	Cough and dizziness

<sup>a</sup> ID50, ID80: < 40 represents the NAb titers were under the detectable level in neutralization assay.

**Supplementary Table 3. Clinical characteristics and SARS-CoV-2-specific NAb titers of young, middle-age, and elderly COVID-19 recovered patients**

<b>Patient Information</b>	<b>Age Distribution (Years)</b>		
	<b>15-39</b>	<b>40-59</b>	<b>60-85</b>
Recovered Patient No.	55 (31%)	64 (37%)	56 (32%)
Male	27 (33%)	33 (40%)	22 (27%)
Female	28 (30%)	31 (33%)	34 (37%)
Length of stay (days)	14 (8-26)	16 (7-30)	17 (7-29)
Disease duration (days)	21 (9-32)	21 (11-34)	22 (15-33)
Median NAb titers (ID50)	448 (40-3717)	1255 (40-6888)	1537 (40-21576)