

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32

A SARS-CoV-2 neutralizing antibody with exceptional spike binding coverage and optimized therapeutic potentials

Yu Guo^{1,7,12,*}, Lisu Huang², Guangshun Zhang^{1,4}, Yanfeng Yao⁵, He Zhou³, Shu Shen⁷, Bingqing Shen³, Bo Li^{1,4}, Xin Li^{1,4}, Mingjie Chen³, Da Chen^{1,4}, Jia Wu³, Dan Fu¹, Xinxin Zeng², Mingfang Feng³, Chunjiang Pi³, Yuan Wang^{1,4}, Xingdong Zhou^{1,4}, Minmin Lu³, Yaohui Fang⁷, Yun-Yueh Lu³, Xue Hu⁷, Shanshan Wang³, Wanju Zhang², Qian Zhang³, Ge Gao⁵, Francisco Adrian³, Qisheng Wang¹⁰, Feng Yu¹⁰, Yun Peng⁵, Alexander G. Gabibov¹¹, Juan Min⁵, Yuhui Wang^{1,4}, Heyu Huang², Alexey Stepanov¹¹, Wei Zhang^{1,4}, Yan Cai⁶, Junwei Liu⁶, Zhiming Yuan⁵, Chen Zhang¹, Zhiyong Lou^{8,*}, Fei Deng^{7,*}, Hongkai Zhang^{1,4,9,12*}, Chao Shan^{7,*}, Liang Schweizer^{3,*}, Kun Sun^{2,*}, Zihao Rao^{1,4,*}

¹ State Key Laboratory of Medicinal Chemical Biology and College of Pharmacy, Nankai University, 38 Tongyan Road, Tianjin, 300071, China

² Xinhua Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China.

³ HiFiBio (Hong Kong) Limited, 2 Ice House Street, Central, Hong Kong

⁴ College of Life Science, Nankai University, 94 Weijin Road, Tianjin, 300071, China

⁵ Center for Biosafety Mega-Science, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan, Hubei 430071, China.

⁶ Tianjin International Joint Academy of Biotechnology & Medicine, Tianjin 300457, People's Republic of China

⁷ State Key Laboratory of Virology and National Virus Resource Center, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan, Hubei 430071, China

⁸ MOE Key Laboratory of Protein Science & Collaborative Innovation Center of Biotherapy, School of Medicine, Tsinghua University, Beijing, China

⁹ Shanghai Institute for Advanced Immunochemical Studies, ShanghaiTech University, Shanghai, 201210, P.R. China

¹⁰ Shanghai Synchrotron Radiation Facility, Shanghai Advanced Research Institute, Chinese Academy of Sciences, Shanghai 201204, China

33 ¹¹ Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of
34 Sciences, 117997 Moscow, Russia

35 ¹² Frontiers Science Center for Cell Responses, Nankai University, 94 Weijin Road,
36 Tianjin, 300071, China

37

38

39 § These authors contribute equally to this work

40 * Correspondence should be sent to: Z.R (raozh@mail.tsinghua.edu.cn),

41 K.S(sunkun@xinhumed.com.cn), L.S (l.schweizer@hifibio.com);

42 C.S(shanchao@wh.iov.cn), H.Z(hongkai@nankai.edu.cn), , F.D(df@wh.iov.cn),

43 Z.L(louzy@mail.tsinghua.edu.cn) and Y.G(guoyu@nankai.edu.cn)

44 Abstract

45 The Coronavirus Disease of 2019 (COVID-19) pandemic caused by severe acute
46 respiratory syndrome coronavirus 2 (SARS-CoV-2) threatens global public health and
47 economy. Therapeutic options such as monoclonal antibodies (mAbs) against SARS-
48 CoV-2 are in urgent need. We have identified potent monoclonal antibodies binding to
49 SARS-CoV-2 Spike protein from COVID-19 convalescent patients and one of these
50 antibodies, P4A1, interacts directly and covers the majority of the Receptor Binding
51 Motif (RBM) of Spike receptor-binding domain (RBD), shown by high-resolution
52 complex structure analysis. We further demonstrated P4A1 binding and neutralizing
53 activities against wild type and mutant spike proteins. P4A1 was subsequently
54 engineered to reduce the potential risk for antibody-dependent enhancement (ADE)
55 of infection and to extend its half-life. The engineered mAb exhibits optimized
56 pharmacokinetic and safety profile, and results in complete viral clearance in a rhesus
57 monkey model of COVID-19 following a single injection.

58 Keywords:

59 SARS-CoV-2, spike glycoprotein, neutralizing antibody, antibody engineering,
60 structure analysis, in vivo efficacy

61 **Introduction**

62 COVID-19 has recently emerged throughout the world as the largest pandemic
63 of the 21st century, with over 35 million confirmed cases and over 1 million deaths
64 worldwide as of Oct. 7th, 2020, which requires urgent prophylactic and treatment
65 options. Like other Class I fusion proteins, the spike (S) glycoprotein forms
66 homotrimers on the surface of SARS-CoV-2 virus particle, mediates the recognition
67 and binding to the human receptor, angiotensin-converting enzyme 2 (hACE2),
68 through its receptor-binding domain (RBD), and induces the virus-host cell membrane
69 fusion, and serves as the primary target for interfering the virus entry process(1). A
70 number of SARS-CoV-2 vaccines currently under development have shown
71 successful induction of anti-viral antibodies in clinical trials (2-4). The final success of
72 anti-SARS-CoV-2 vaccines may be depending on the quality of immune responses
73 induced, particularly in elderly or individuals with pre-existing conditions, or the
74 willingness to accept vaccination. Recent reports of SARS-CoV-2 reinfection in
75 patients brought new challenges to the vaccine approaches.

76 To date, many efforts have been devoted to the discovery and development of
77 SARS-CoV-2 neutralizing antibodies using various platforms, including antibody
78 isolation and screening from SARS-CoV(5, 6) or SARS-CoV-2 convalescent
79 patients(7-16), humanized mice(17, 18), and phage libraries(19, 20). Among them,
80 several leading antibodies have entered the clinical trial stage. Of note, a major

81 concern for the development of vaccine or neutralizing antibody therapies is the
82 potential risk of antibody-dependent enhancement (ADE) of infection (21, 22), which
83 also previously reported in infections of viruses such as dengue(23) and SARS-
84 CoV(24), and raised as a concern for SARS-CoV-2 countermeasures (15, 25). Another
85 aspect to consider is the ability of the evolving virus to mutate and escape from
86 treatments. To date, about 19 mutation sites in the Spike protein have been described
87 from an online database tracing ~65k documented sequences
88 (<https://cov.lanl.gov/content/index>), of which D614G has become the most prevalent
89 form in the global pandemic(26), the development of potent treatments effective to
90 different SARS-CoV-2 mutants is urgently needed.

91 **Results**

92 To identify SARS-CoV-2 neutralizing antibodies from convalescent patients, we
93 utilized a high-throughput single-cell B-cell receptor sequencing approach. SARS-
94 CoV-2 neutralizing antibodies were isolated from the two convalescent patients with
95 the highest titer against S protein (patient #4 and patient # 20) among 23 patients
96 studied (Fig. S1A). SARS-CoV-2 S protein binding B cells from PBMCs were enriched
97 with biotinylated S protein conjugated magnetic beads as probes as described (Fig.
98 S1C and S1D). Among the sequenced antibodies, P4A1, P20A2 and P20A3 were
99 shown to bind to the S1 subunit with sub-to-nM EC_{50} s, but not to the S2 subunit, and
100 blocked the binding of the S1 subunit to hACE2 with IC_{50} values in the sub-to-nM range

101 (Fig.1A). P4A1, P20A2, P20A3 neutralized the live SARS-CoV-2 infection of Vero-E6
102 cells with ND₅₀ values of 5.212, 43.79, and 29.03 nM, respectively (Fig. 1B). According
103 to the ImmunoGenetics (IMGT) database-based analysis, IGHV3-53 was assigned as
104 the antibody P4A1's IGHV germline origin, which has been proved to be one of the
105 most frequent used germlines after SARS-CoV-2 infection in patients(16) (Fig. S3 and
106 S4A).

107 To gain further insight into the structural basis for the blocking and neutralizing
108 mechanism of P4A1, the structure of the antibody Fab/Spike-RBD complex was
109 resolved by X-ray crystallography at the resolution of 2.1 Å (Fig.2A, Table.S1). Three
110 heavy chain complementarity-determining regions (HCDRs), two light chain
111 complementarity-determining regions (LCDRs), together with weak interaction from
112 the light chain framework region 3 (LFR3) constitute the interaction network with
113 SARS-CoV-2 RBD (Fig.2B). The buried surface area (BSA) from the heavy-chain
114 interaction in P4A1 (781 Å²) is relatively larger compared to reported neutralizing
115 antibodies including CC12.1 (723 Å²), CC12.3 (698 Å²)(16), CB6(734 Å²)(14) and B38
116 (713 Å²)(14) (Fig.S3); However, the BSA from the light chain varies significantly, with
117 P4A1 (577 Å²) being the largest compared to CC12.1 (566 Å²), CC12.3 (176 Å²)(16),
118 CB6 (334 Å²)(15) and B38 (495 Å²)(14). Specifically, the interaction network is mainly
119 contributed by hydrogen bond and hydrophobic interaction. For the heavy chain, P4A1
120 HCDR1 and HCDR2 shares similar interaction pattern with SARS-CoV-2 Spike RBD
121 as CC12.1 and CC12.3, while these antibodies differ in their HCDR3 sequence, with

122 Q100, E101 forming hydrogen bond with K417 and Y453, and the side chain of L102
123 inserts into the hydrophobic cavity formed by L485, F456 and Y489 (Fig. 2C, upper
124 panel); For the light chain, the hydrogen bond network is mediated by G28, S30, S31
125 W32 in LCDR1 with T500, N501, G502, Q498, Y449, and G496; and E90, N92, S93
126 in LCDR3 with Y505, and R403(Fig. 2C, lower panel). It is worth noting that the water
127 molecule plays more important role for light chain than it does for the heavy chain.

128 P4A1 Fab locates directly at the Receptor Binding Motif (RBM) region of SARS-
129 CoV-2 Spike RBD, hence inducing steric hindrance to the binding of receptor hACE2
130 (Fig. 2A). Except for residues G446 and Y449, 15 of the 17 residues identified as
131 SARS-CoV-2 Spike RBD-hACE2 binding sites(1) are within P4A1 epitope using the
132 distance of $<4 \text{ \AA}$ as the cutoff, while Y449 is exactly 4 \AA from P4A1 (Fig. 2B, Table S2).
133 This represents one of the most extensive coverages of residues involved in the Spike-
134 hACE2 interaction to date and may explain potent neutralization activity for P4A1. This
135 broad coverage may suggest effectiveness against viral mutants as mutations to
136 hACE2 epitopes on Spike protein are rare in clinical isolates because of their
137 importance in viral infectivity. Structure analysis showed that all 8 residues with
138 reported mutations in the Spike RBD of clinical isolates (27), are mostly located far
139 away from P4A1 epitope (Fig. S4B), are expected to have no or minimal effects on the
140 binding and neutralization activity of P4A1. This was supported by the test of P4A1
141 binding ability to wild type, different RBD mutants and D614G variants of S1 protein
142 by SPR (Fig. 3A) or ELISA (Fig. S5), as well as neutralization of pseudoviruses

143 expressing wildtype or D614G, V367F, D264Y or W436R variants of Spike protein with
144 similar IC₅₀s (Fig. 3B).

145 To facilitate its clinical applications, P4A1 was further engineered as an IgG4
146 antibody (named as P4A1-2A) to reduce the risks of Fcγ- or complement-receptor-
147 mediated ADE with modifications to reduce Fab-arm exchange(28), and to increase
148 antibody blood half-life in primates. Consistent with its isotype and Fc engineering,
149 P4A1-2A displayed 4-81 fold decrease in binding affinity to different FcγRs, no binding
150 to complement C1q, and enhanced affinity for FcRn when compared to its IgG1 form
151 P4A1 (Fig. 3C and S6). The effect of enhanced FcRn binding is further validated by
152 the longer serum half-life in cynomolgus monkey (Fig. S6). The prolonged half-life is
153 highly desired as it will be beneficial for long-term protection in individuals at high risk
154 of contracting SARS-CoV-2 infection during the pandemic. Furthermore, the safety of
155 P4A1-2A was demonstrated in a GLP Toxicity study where cynomolgus monkeys were
156 treated with 2 weekly i.v infusions of up to 300 mg/kg/dose and no treatment-related
157 findings were observed in any of the tests performed, including cytokine levels, such
158 as IL-2, IL-6, IL-10, TNF-α and IFN-γ at different timepoints. Besides, no tissue cross-
159 reactivity was observed for 37 different normal human tissues.

160 The effectiveness of the engineered antibody was examined in a SARS-CoV-2
161 infection model in rhesus macaques (Figure 4A). Isotype control or P4A1-2A were
162 administered in a single intravenous (i.v.) infusion 1 day after intra-tracheal virus
163 inoculation at 1×10^5 TCID₅₀. Consistent with the previous report(29), in the isotype

164 control (50 mg/kg) group, viral load using oropharyngeal swabs was at high level 1
165 day post-infection (d.p.i.), showing the colonization of virus, decreased 2 d.p.i.,
166 suggesting viral distribution, increased 3-4 d.p.i. and maintained at high level through
167 7 d.p.i indicating viral replication. Importantly, in 3 out of 3 (50 mg/kg) and 2 out the 3
168 (10mg/kg) P4A1-2A-treated animals, the viral load dropped continuously to a level
169 near or below the detection limit, and viral elimination occurred on 3-4 d.p.i., indicating
170 effective blocking of viral propagation by P4A1-2A (Fig. 4B).

171 At necropsy, trachea, left and right bronchus and different lung lobes were
172 isolated, homogenized and tested for viral load. Substantial viral load was detected in
173 5 or 6 out of the 9 tissues examined (including consistent viral presence in trachea,
174 left and right bronchus) with moderate to severe lung lesions similar to previous report
175 (29) in animals in the isotype control treatment group. In contrast, no viral load was
176 detected 6 or 7 d.p.i in any of these tissues examined and with to mild-moderate lung
177 lesions in the 50 mg/kg P4A1-2A-treated animals. In the 10 mg/kg P4A1-2A-treated
178 animals, virus was detected in only 1 of the 9 tissues examined 7 d.p.i and no viral
179 RNA detected in any of the lung tissues in all 3 animals (Fig. 4C, 4D and Fig S8).

180 These results represent similar or better anti-viral efficacy in the respiratory
181 system than the currently reported antibodies(15) with lower i.v. dose of neutralizing
182 antibody used in similar NHP SARS-CoV-2 infection models. In addition to the potency
183 of P4A1-2A, a potentially better distribution to the respiratory tract associated with the
184 engineered IgG4 antibodies by previous work(30) may contribute to the total viral

185 clearance in some of the animals. These data demonstrated that P4A1-2A was
186 effective despite reduced FcγR effector functions.

187 **Discussion**

188 High affinity neutralizing antibodies from convalescent patients may represent
189 some of the most efficient anti-viral weapons contributing to a patient recovery from
190 viral infection. Given that not every COVID-19 patient is able to produce high quality
191 antibodies such as P4A1, this antibody along with antibody engineering to reduce
192 potential safety concerns and to prolong the serum half-life, may provide durable
193 protection to every patient and, especially, to individuals at high risk of viral infection.
194 The structural analysis demonstrates the molecular basis for neutralizing mechanism
195 of P4A1, whose epitope include most of the binding residues of hACE2 and exhibited
196 potent antiviral activities *in vitro* and *in vivo*. The broad coverage of hACE2 epitopes
197 may contribute to effectiveness to viral mutants, supported by activities against all
198 spike protein mutants tested, including the most prevalent variant D614G, with similar
199 EC_{50}/IC_{50} s compared to wild type protein.

200 With prolonged half-life, potential for a broad spectrum of viral neutralization,
201 P4A1-2A represents not only optimized therapeutic potency, but also an important
202 prophylactic option to safeguard the vulnerable or aged populations, complementary
203 to vaccine approaches. The comprehensive characterization of this antibody provides

204 strong evidence for its promising potential to effectively treat and prevent COVID-19
205 in humans.

206 **References**

- 207 1. J. Shang *et al.*, Structural basis of receptor recognition by SARS-CoV-2. *Nature*
208 **581**, 221-224 (2020).
- 209 2. H. Wang *et al.*, Development of an Inactivated Vaccine Candidate, BBIBP-CorV,
210 with Potent Protection against SARS-CoV-2. *Cell* **182**, 713-721 e719 (2020).
- 211 3. F. C. Zhu *et al.*, Immunogenicity and safety of a recombinant adenovirus type-
212 5-vectored COVID-19 vaccine in healthy adults aged 18 years or older: a
213 randomised, double-blind, placebo-controlled, phase 2 trial. *Lancet* **396**, 479-
214 488 (2020).
- 215 4. P. M. Folegatti *et al.*, Safety and immunogenicity of the ChAdOx1 nCoV-19
216 vaccine against SARS-CoV-2: a preliminary report of a phase 1/2, single-blind,
217 randomised controlled trial. *Lancet* **396**, 467-478 (2020).
- 218 5. D. Pinto *et al.*, Cross-neutralization of SARS-CoV-2 by a human monoclonal
219 SARS-CoV antibody. *Nature* **583**, 290-295 (2020).
- 220 6. A. Z. Wec *et al.*, Broad neutralization of SARS-related viruses by human
221 monoclonal antibodies. *Science* **369**, 731-736 (2020).
- 222 7. D. Zhou *et al.*, Structural basis for the neutralization of SARS-CoV-2 by an
223 antibody from a convalescent patient. *Nat Struct Mol Biol*, (2020).
- 224 8. P. J. M. Brouwer *et al.*, Potent neutralizing antibodies from COVID-19 patients
225 define multiple targets of vulnerability. *Science* **369**, 643-650 (2020).
- 226 9. Y. Cao *et al.*, Potent Neutralizing Antibodies against SARS-CoV-2 Identified by
227 High-Throughput Single-Cell Sequencing of Convalescent Patients' B Cells.
228 *Cell* **182**, 73-84 e16 (2020).
- 229 10. X. Chi *et al.*, A neutralizing human antibody binds to the N-terminal domain of
230 the Spike protein of SARS-CoV-2. *Science* **369**, 650-655 (2020).
- 231 11. C. Kreer *et al.*, Longitudinal Isolation of Potent Near-Germline SARS-CoV-2-
232 Neutralizing Antibodies from COVID-19 Patients. *Cell* **182**, 843-854 e812
233 (2020).
- 234 12. D. F. Robbiani *et al.*, Convergent antibody responses to SARS-CoV-2 in
235 convalescent individuals. *Nature* **584**, 437-442 (2020).
- 236 13. T. F. Rogers *et al.*, Isolation of potent SARS-CoV-2 neutralizing antibodies and
237 protection from disease in a small animal model. *Science* **369**, 956-963 (2020).
- 238 14. Y. Wu *et al.*, A noncompeting pair of human neutralizing antibodies block
239 COVID-19 virus binding to its receptor ACE2. *Science* **368**, 1274-1278 (2020).
- 240 15. R. Shi *et al.*, A human neutralizing antibody targets the receptor-binding site of
241 SARS-CoV-2. *Nature* **584**, 120-124 (2020).
- 242 16. M. Yuan *et al.*, Structural basis of a shared antibody response to SARS-CoV-2.
243 *Science* **369**, 1119-1123 (2020).
- 244 17. J. Hansen *et al.*, Studies in humanized mice and convalescent humans yield a
245 SARS-CoV-2 antibody cocktail. *Science* **369**, 1010-1014 (2020).

- 246 18. Z. Lv *et al.*, Structural basis for neutralization of SARS-CoV-2 and SARS-CoV
247 by a potent therapeutic antibody. *Science*, (2020).
- 248 19. H. A. Parray *et al.*, Identification of an anti-SARS-CoV-2 receptor binding
249 domain directed human monoclonal antibody from a naive semi-synthetic
250 library. *J Biol Chem*, (2020).
- 251 20. Y. Wu *et al.*, Identification of Human Single-Domain Antibodies against SARS-
252 CoV-2. *Cell Host Microbe* **27**, 891-898 e895 (2020).
- 253 21. A. M. Arvin *et al.*, A perspective on potential antibody-dependent enhancement
254 of SARS-CoV-2. *Nature* **584**, 353-363 (2020).
- 255 22. W. Hoepel *et al.*, Anti-SARS-CoV-2 IgG from severely ill COVID-19 patients
256 promotes macrophage hyper-inflammatory responses. *bioRxiv*, (2020).
- 257 23. L. C. Katzelnick *et al.*, Antibody-dependent enhancement of severe dengue
258 disease in humans. *Science* **358**, 929-932 (2017).
- 259 24. M. S. Yip *et al.*, Antibody-dependent infection of human macrophages by severe
260 acute respiratory syndrome coronavirus. *Virology* **11**, 82 (2014).
- 261 25. L. M. Walker, D. R. Burton, Passive immunotherapy of viral infections: 'super-
262 antibodies' enter the fray. *Nat Rev Immunol* **18**, 297-308 (2018).
- 263 26. B. Korber *et al.*, Tracking Changes in SARS-CoV-2 Spike: Evidence that D614G
264 Increases Infectivity of the COVID-19 Virus. *Cell* **182**, 812-827 e819 (2020).
- 265 27. J. Ou *et al.*, Emergence of RBD mutations in circulating SARS-CoV-2 strains
266 enhancing the structural stability and human ACE2 receptor affinity of the spike
267 protein. 2020.2003.2015.991844 (2020).
- 268 28. A. F. Labrijn *et al.*, Therapeutic IgG4 antibodies engage in Fab-arm exchange
269 with endogenous human IgG4 in vivo. *Nat Biotechnol* **27**, 767-771 (2009).
- 270 29. C. Shan *et al.*, Infection with novel coronavirus (SARS-CoV-2) causes
271 pneumonia in Rhesus macaques. *Cell Res* **30**, 670-677 (2020).
- 272 30. W. F. Dall'Acqua, P. A. Kiener, H. Wu, Properties of human IgG1s engineered
273 for enhanced binding to the neonatal Fc receptor (FcRn). *J Biol Chem* **281**,
274 23514-23524 (2006).

276

277

278 Correspondence

279 Z.R (raozh@mail.tsinghua.edu.cn), K.S(sunkun@xinhumed.com.cn), L.S
280 (l.schweizer@hifibio.com); C.S(shanchao@wh.iov.cn), H.Z(hongkai@nankai.edu.cn),
281 F.D(df@wh.iov.cn), Z.L(louzy@mail.tsinghua.edu.cn) and Y.G(guoyu@nankai.edu.cn)

282 Acknowledgments

283 We thank the running team of National Biosafety Laboratory, staffs from Center for
284 Instrumental Analysis and Metrology, Wuhan, Chinese Academy of Sciences, and staffs
285 from BL-17U1, Shanghai Synchrotron Radiation Facility. We thank Cheryl Cui and Jeff He
286 for their critical role in setting up collaboration among different institutions, Ning Zhang from
287 Red Avenue Foundation for her support.

288 This work was supported by the National Program on Key Research Project of China
289 (2018YFE0200400, 2018YFA0507203, 2017YFC840300, 2017YFA0504801 and
290 2020YFC0845801), the National Natural Science Foundation of China (NSFC) (31670731,
291 31870733), Projects of International Cooperation and Exchanges NSFC (grant
292 no.81520108019), Medical and Industrial Cross Research Foundation of Shanghai Jiao
293 Tong University (YG2020YQ27), and Science and Technology Innovation Achievements and
294 Team Building Foundation of Nankai University (grant no. ZB19500403, ZB19100123 and
295 63201101).

296 Author contributions

297 Z. R., K.S., L.S., H.-K.Z., Z.-Y. L., and Y.G. conceived the project. L.-S.H. X.-X.Z., W.-
298 J.Z., H.-Y.H. and K.S. collected the convalescent PBMC and serum and take responsibility
299 for the human ethic issue. Y.G., L.-S.H., G.-S.Z., B.-Q.S., M.-J.C., J.W., M.-F.F., B.L., D.F.,
300 W.Z., Y.-H.W., Y.C., J.-W.L. and C.Z. performed gene construction, protein expression and
301 purification, and crystal screening and optimization. Y.G., X.L., Q.-S.W., Q.Z., F.Y., A.S.,
302 and A.G.G. performed the collection of X-ray diffraction data and structure determination.
303 L.-S.H., G.-S.Z., B.-Q.S., S.-S.W., Y.-Y.L., M.-M.L., C.-J.P., D.C., Y.W., and X.-D.Z.
304 performed the in vitro binding assays and analysis data. S.S., Y.-H.F., and F.D. performed
305 live virus neutralization assays. C.S., Y.F.Y., X.H., G.G., Y.P., J.M., and Z.-M.Y. performed
306 animal assays. Y.G., H.-K.Z., L.S., H.Z., Z. R., K.S., and F.A. designed the experiments
307 and wrote the manuscript. All authors read and approved the contents of the manuscript.

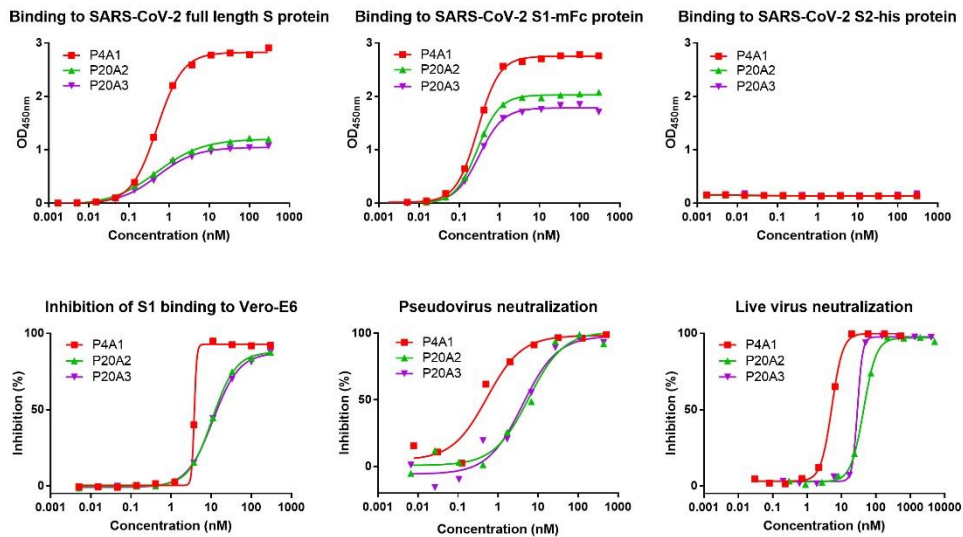
308 Competing interests

309 L.S., B.-Q.S., H.Z., M.-J.C., Y.-Y.L.; L.S.H.; K.S. are the inventors of the pending patent
310 application filed on the reported antibodies. L.S., B.-Q.S., H.Z., M.-J.C., J.W., M.-F.F., C.-
311 J.P., M.-M.L., Y.-Y.L.; S.-S.W., Q.Z., F.A. employees of HiFiBio (Hong Kong) Ltd. Other
312 authors declare no competing interests. All reagents and information presented in this study
313 are available from corresponding authors upon reasonable request.

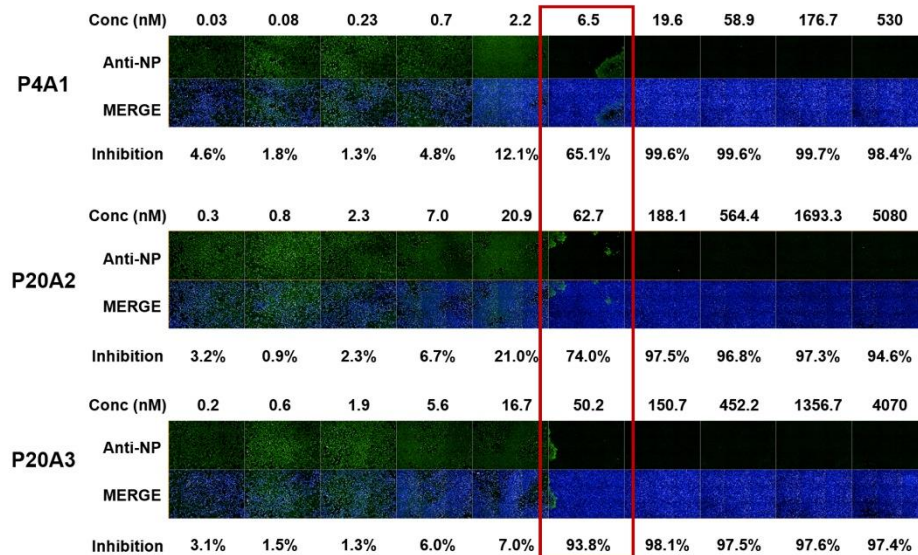
314 **Figures and Figure Legends**

315 **Figure 1**

A



B



316

317 **Figure 1. Characterization of neutralizing antibodies from convalescent patients. (A)**

318 Characterization of SARS-CoV-2 S protein specific antibodies. (upper panels) Binding of

319 antibodies to the full-length S protein, S1 protein and S2 protein were evaluated by ELISA.

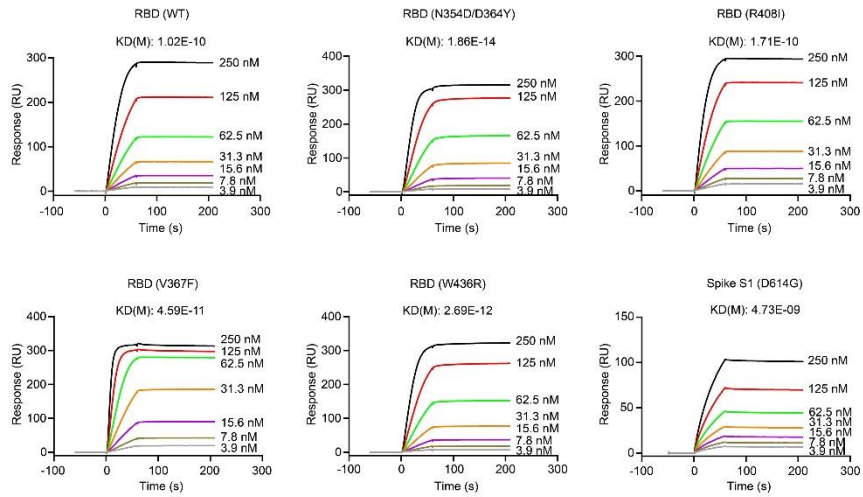
320 (lower left panel) Blockage of the binding of SARS-CoV-2 spike S1 protein to Vero E6 cell

321 line by antibodies evaluated by flow cytometry. (Lower middle panel) Pseudovirus
322 neutralization assay in Huh-7 cell line. (lower right panel and B) SARS-CoV-2 live virus
323 neutralization assay. (B) Images of Vero E6 cells infected SARS-CoV-2 treated with
324 antibodies of different concentrations.
325

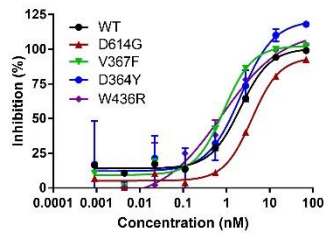
338 labelled in red. The residues are numbered according to SARS-CoV-2 RBD. (C) The detailed
339 interactions between SARS-CoV-2 RBD with HCDR, LCDR and LFR3. The residues are
340 shown in sticks with identical colors to (B).
341

342 **Figure 3**

A

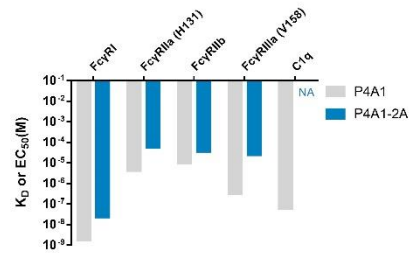


B



	WT	D614G	V367F	D364Y	W436R
IC ₅₀ (nM)	2.077	3.869	0.871	2.147	0.787

C



343

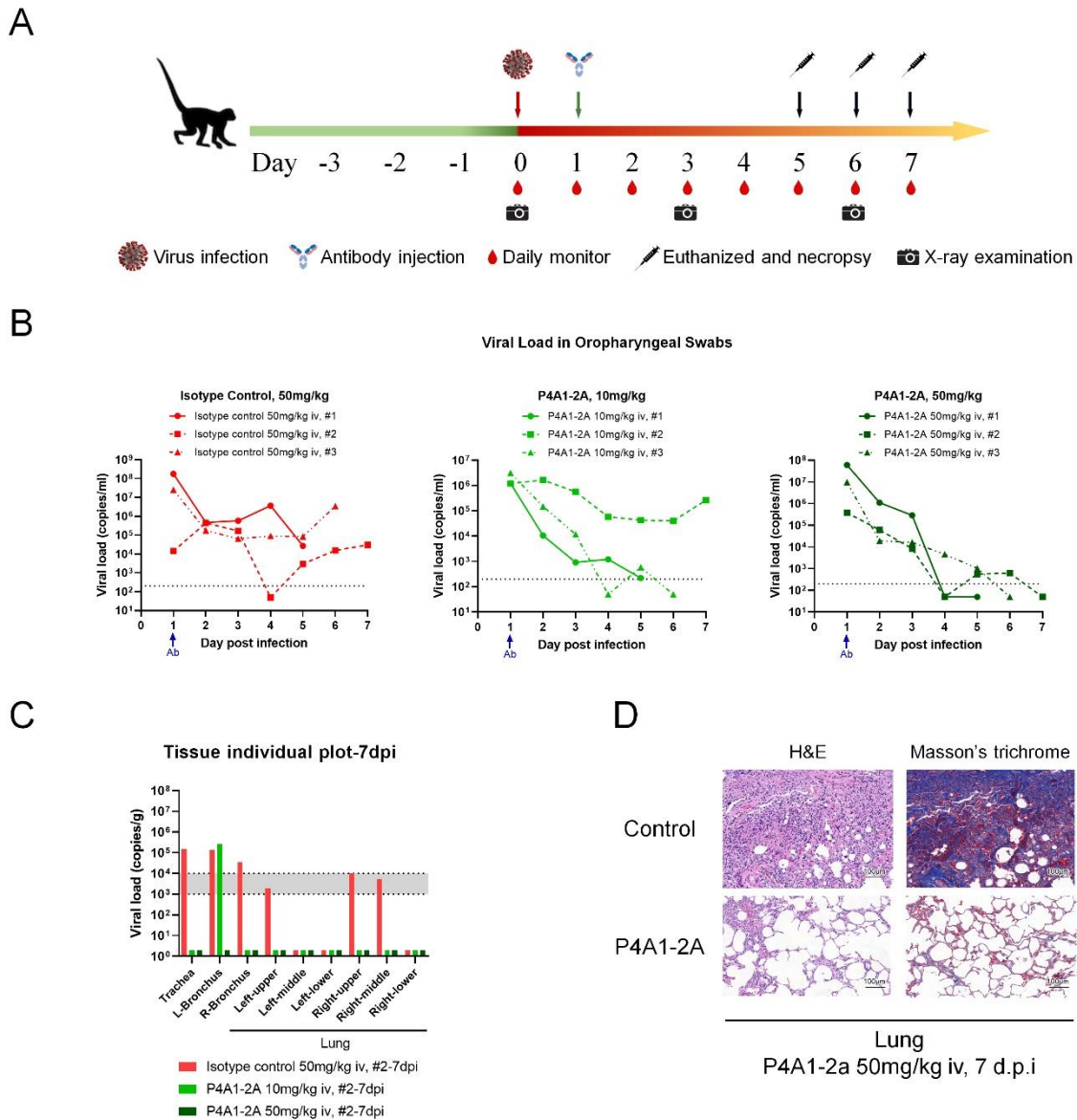
344

345 **Figure 3. The activities of IgG4 antibody P4A1-2A to different SARS-CoV-2 S protein**
 346 **mutants, FcγRs and C1q. (A) Binding of antibody P4A1 to SARS-CoV-2 S protein**
 347 **N354D/D364Y, R408I, W436R, V367F or D614G mutants determined by SPR. (B) Pseudo**
 348 **virus neutralization assay in hACE2 overexpressing HEK293 cells. (C) The binding affinity**
 349 **of P4A1 and P4A1-2A for different human FcγRs and complement C1q.**

350

351

352

353 **Figure 4**

354

355 **Figure 4. Therapeutic efficacy of in the rhesus macaque model of SARS-CoV-2**356 **infection.** (A) Experimental design for therapeutic testing of P4A1-2A in the rhesus

357 macaque. (B) Viral load in oropharyngeal swabs tested by RT-qPCR were monitored for 7

358 days. (C) Viral load in the respiratory tissues (including trachea, left and right bronchus, and

359 all six lung lobes) collected at necropsy on 7 days post-infection (d.p.i) were tested by RT-

360 qPCR. (D) Representative images of histopathology in lung tissue from isotype control or

361 P4A1-2A 50 mg/kg treated animals (collected at 7 d.p.i).

362

363

364

365

366

367

368

369

370

371

372

1 **Methods**

2 **Ethics statements**

3 The study was approved by the Ethics Committee of Xinhua Hospital affiliated to Shanghai
4 Jiao Tong University (approval #XHEC-C-2020-006-2), Yongjia People's Hospital and
5 Yongjia Center for Disease Control and Prevention in Zhejiang Province. Blood samples
6 were collected from convalescent COVID-19 patients and healthy volunteers with signed
7 informed consent forms according to study protocol approved by IRBs

8 All animal experiments were approved by the Institutional Animal Care and Use Committee
9 (IACUC) of Wuhan Institute of Virology, Chinese Academy of Sciences (Ethics number:
10 WIVA42202001) or Wuxi AppTec (Number: SZ20200529-Monkeys for PK study and
11 SZ20200608-Monkeys for GLP Toxicology study). Rhesus macaque studies were
12 conducted within the animal biosafety level 4 (ABSL- 4) facility in the National Biosafety
13 Laboratory (Wuhan), Chinese Academy of Sciences.

14
15 **Study design and participants**

16 Convalescent COVID-19 patients were recruited between February 11th, 2020 and March
17 1st, 2020 at Yongjia People's Hospital and Yongjia Center for Disease Control and
18 Prevention in Zhejiang Province, China. All the patients with COVID-19 were clinically
19 diagnosed and laboratory-confirmed. A laboratory-confirmed case of COVID-19 was defined
20 as a positive result on high-throughput sequencing or real-time reverse-transcriptase

21 polymerase-chain-reaction (RT-PCR) assay of nasal and pharyngeal swab specimens
22 based on the WHO interim guidance (WHO, 2020). The criteria of recovery were normal
23 temperature for at least 3 days, obvious improvement in clinical symptoms, significant
24 absorption of pulmonary inflammation on computer tomography scan and negative tests for
25 SARS-CoV-2 two times in a row with a test interval for at least one day. Individuals tested
26 positive for HIV, HBV, HAV, HCV or syphilis were excluded. All the clinical data were
27 reviewed by a team of physicians from departments of respiratory, intensive care and
28 infectious diseases. Patients without the baseline assessment test were excluded. 23
29 convalescent COVID-19 patients and one healthy volunteer were included in the study.

30

31 **PBMC and serum sample preparation**

32 Blood samples were collected using heparin as anticoagulant 3-4 days after donors were
33 discharged from the hospital and separated into plasma and peripheral blood mononuclear
34 cells (PBMCs) by Ficoll-Hypaque gradient centrifugation. Plasmas and PBMCs in freezing
35 media were stored at -80°C. The plasma was heat-inactivated at 56°C for 1h before use.

36

37 **Titer measurements by ELISA**

38 384 well plates were coated overnight at 4°C with PBS containing 1 µg/mL of SARS-CoV-2
39 Spike receptor-binding domain (RBD)-mFc recombinant protein (SinoBiological, catalog #
40 40592-V05H) or full-length S-his (SinoBiological, catalog # 40589-V08B1) or S1-mFc
41 (SinoBiological, catalog # 40591-V05H1) or S1-his (Kactus, catalog # COV-VM4S1). The

42 next day the plate was washed 4 times with washing buffer (PBS and 0.05% Tween) and
43 then incubated 1 hour at 37°C in blocking buffer (PBS with 2% BSA). After two washes the
44 plate was incubated for 1 hour at 37 °C with the serum or the positive control ACE2 protein
45 (SinoBiological, catalog # 10108-H08H). The human serum samples were diluted to 1:100
46 in PBS + 2% BSA followed by 5-fold serial dilutions. The plates were then washed 4 times
47 and incubated for 1 hour in blocking buffer (PBS with 0.05% Tween and 1% BSA) containing
48 diluted (1:5000) secondary antibody (HRP labelled mouse anti-human IgG Fc antibody,
49 Thermo Fisher, catalog # 05-4220, clone name: HP6017) for 1 hour at room temperature.
50 Following this the plate was washed again 4 times and developed in TMB substrate
51 (Biolegend, catalog # 421101) for 5 min before stopping the reaction with the stop solution
52 (Solarbio, catalog # C1058).

53

54 **Flow cytometry for B cell immune profiling**

55 PBMCs was thawed at 37°C and then centrifuged at 450x g for 8 min. The supernatant was
56 discarded, and the cells resuspended in 200 µL of DMEM (Gibco, catalog # 11995-065).
57 Following the addition of 1µL of Dnase I (Qiagen, catalog # 79254), cells were incubated for
58 3 min and spun down again. The pellet was resuspended in 20 µL of FcR Blocking Reagent
59 (Miltenyi Biotec, catalog # 130-059-901), incubated for 10 mins and centrifuged. The cells
60 were suspended in 200 µL PBS. 3 µL (1:70 dilution) of anti-CD19 (FITC labeled, eBioscience,
61 catalog # 11-0199-42, clone name: HIB19), anti-CD27 (APC labeled, eBiosciences, catalog
62 # 17-0279-42, clone name: O323), anti-CD38 (PE labeled, eBioscience, catalog # 12-0388-

63 42, clone name: HB7) or its isotype PE Mouse isotype control (BioLegend, catalog # 400114,
 64 clone name: MOPC-21) and FITC labeled mouse IgG1 isotype control (ebioscience, catalog
 65 # 11-4714-41, clone name: P3.6.2.8.1) and APC labeled mouse IgG1 isotype control (BD
 66 Biosciences, catalog#550854, clone name: MOPC-21) was then added, and incubated for
 67 30 min at room temperature. Following centrifugation, cells were resuspended in a 100 μ L
 68 of 4% Paraformaldehyde fix solution (PFA; Beyotime, catalog # P0099-500ml). After 10 min
 69 the cells were washed twice and finally resuspended in PBS and analyzed using a Cytoflex
 70 (Beckman Coulter) flow cytometer. The median fluorescence intensity (MFI) was calculated
 71 with FlowJo (version 10.6.0).

72

73 Patient sample information for FACS

	Patient #	Cell Number	Viability (%)	Gender
PBMC	Patient 4	2.54×10^5 /mL, 200 μ L	84	Female
PBMC	Patient 20	7.1×10^5 /mL, 200 μ L	90	Male
PBMC	Healthy donor	7.0×10^5 /mL, 200 μ L	92	Male

74

75 **Isolation of S protein specific B cells**

76 Avi tag and His tag SARS-CoV-2 S protein was expressed in human embryonic kidney cell
 77 293-F (ThermoFisher, catalog# R79007. The cell line was not authenticated.) which was
 78 tested negative for mycoplasma contamination. The S protein was purified using HISTRAP
 79 HP column (GE, catalog # 17-5248-01) and biotinylated using Biotin-Protein Ligase
 80 (GeneCopoeia, catalog # BI001). The B cells were stained with biotinylated S protein and
 81 incubated at 4°C for 1 hour. After incubation, the cells were washed three times with PBS.

82 Subsequently, the cells were labelled with Streptavidin microbeads (Miltenyi Biotec, catalog
83 # 130-048-101) at 4°C for 1 hour. After the incubation, the cell suspension was loaded onto
84 a MACS column which is placed on a magnetic field of a MACS separator. The column was
85 washed three times so the magnetically labelled B cells were retained in the column and
86 unlabeled cells passed through. After removing from the MACS separator, the magnetically
87 labelled B cells were eluted. The isolated B cells were counted by using 0.4% (w/v) Trypan
88 blue stain.

89

90 **Single-cell BCR sequencing**

91 Enriched S protein specific B cells were individually co-compartmentalized in droplets with
92 single barcoded hydrogel beads and lysis and reverse transcription reagents using a
93 microfluidic device as described¹⁵. Droplets of ~1 nL volume were formed at 250 s⁻¹. The
94 droplets were collected in a 1.5mL tube containing HFE-7500 (3MTM, catalog # Novec 7500)
95 and 0.1% surfactant (RanBio, catalog # 008-FluoroSurfactant), UV photo-cleaved for 90
96 seconds (OmniCure ac475-365) and incubated at 50°C for cell lysis and cDNA synthesis.
97 Reverse transcription (RT) of VH and VL mRNAs from single B cells took place in droplet
98 using barcoded primers carrying the T7-SBS12 sequences followed by barcode and gene-
99 specific primer sequences complementary to heavy chain J genes and light chain constant
100 region sequences.

101 The emulsion containing the barcoded cDNA was broken by adding one volume of
102 1H,1H,2H,2H-Perfluoro-1-octanol (Sigma-Aldrich, catalog # 370533-25G) after the droplet

103 RT reaction finished. The pooled, barcoded cDNAs were purified with Agencourt RNA
104 CleanUp beads (Beckman, catalog # A63987) at a 1:1 ratio (vol/vol) twice and eluted in 60
105 μ L DNase- and RNase-free H₂O. The sequencing library was generated by two-step nested
106 PCR using GoTaq Polymerase (Promega, catalog#M7406). In the first PCR, forward primers
107 were priming on the T7 and with reverse primers priming on the VH, V λ and Vk leader and
108 framework 1 sequenced of the V genes. In the second PCR, the forward primer appends
109 the Illumina P7 and Illumina index sequences by priming on the SBS12 sequence and the
110 reverse primer appends the Illumina P5 and SBS3 sequence. The approximately 550 bp
111 final PCR products were extracted by agarose gel electrophoresis (Qiagen, catalog#28606).
112 The constructed NGS libraries were sequenced using Illumina MiSeq PE300 which allows
113 sequencing of the entire VH and VL domain as well as the barcode sequence (GeneScript
114 sequencing service supplier) with data varying from 6-12 million reads per samples. The
115 resulting FASTQ data was analyzed by a bioinformatics pipeline enable trimming, merging,
116 barcode extraction and clustering as described(1). Briefly, paired-end reads were first
117 trimmed at the 3' end to remove low quality score bases then merged using the program
118 FLASH requiring at least 10bp overlap. The barcodes were extracted from merged reads
119 followed by clustering requiring the DNA sharing at least 93% identify. The consensus
120 sequence was created from clusters by aligning up to 200 sequencings using
121 ClustalO(version1.2), and each antibody sequence was characterized for immunoglobulin
122 content using VDJFasta(version 2.0). We also applied a minimum number of reads 10 for
123 VH and VL. VH-VL pairing was carried out by identifying the most abundant VH and VL

124 consensus sequence (by the number of reads that contributed to that in each barcode
125 cluster).

126

127 **Production of recombinant antibody**

128 The DNA of P4A1, P20A2 and P20A3 variable regions of the heavy and light chains were
129 synthesized and cloned into expression plasmids containing the human IgG1 heavy chain
130 and kappa light chain constant regions. The antibodies were expressed in ExpiCHO cell for
131 8 days after the co-transfection of both heavy and light chain expression plasmids. P4A1-
132 2A was produced by cloning of P4A1 variable regions into expression plasmid containing
133 human IgG4 heavy chain with Fc modifications and expressed in CHO.K1 cells. Antibodies
134 were purified from cell culture supernatants using Protein-A affinity chromatography.

135

136 **Antibody binding and competition with receptor ACE2**

137 The binding affinity of antibodies to S protein was analyzed by ELISA. 384 well plate
138 (Corning, catalog # 3700), was coated overnight at 4°C with PBS containing 30 µL 20nM of
139 the SARS-CoV-2 Spike S1+S2 ECD, his Tag protein (SinoBiological, catalog # 40589-
140 V08B1). The next day the plate was washed 5 times with washing buffer (PBS and 0.05%
141 Tween) and then incubated 1 hour at room temperature in blocking buffer (PBS with 2%
142 BSA). After 5 washes the plate was incubated with a serial dilution of purified antibodies for
143 1 hour at room temperature. The plates were then washed 5 times and incubated for 1 hour
144 in blocking buffer (PBS with 0.05% Tween and 1% BSA) containing Mouse anti-Human IgG

145 Fc HRP labelled (Thermo Fisher, catalog # 05-4220, clone name: HP6017, dilution 1:5000)
146 for 1 hour at room temperature. The plate was then washed again 5 times and incubated
147 with TMB substrate (Biolegend, catalog # 421101) for 5 min before adding the stop solution
148 (Solarbio, catalog # C1058). The OD values at 450nm wavelength were determined using
149 Thermo MultiSkan or MD SpectraMax i3X, data were analyzed with GraphPad Prism
150 (version 8.0.1).

151 The blocking S1 binding with Vero-E6 cell surface ACE2 receptor was performed using flow
152 cytometry analysis. Vero-E6 cell line (ATCC, CRL-1587) was validated by CoBIOER
153 (<http://www.cobioer.com/>) and tested negative for mycoplasma contamination. 10 nM SARS-
154 CoV-2 spike S1, mFc tag protein (SinoBiological, catalog # 40591-V05H1) was incubated
155 with a serial dilution of purified antibodies in duplicates at room temperature for 1 hour and
156 then added to Vero E6 cells (105 cells per well). Rabbit anti-mouse IgG Fc-AF647 (Jackson
157 ImmunoResearch, catalog # 315-606-046, 1:800 dilution) was then added before final wash
158 and data acquisition with a Cytoflex flow cytometer (Beckman) and data analysis using
159 FlowJo software (version 10.6.0). Non-linear regression was used to calculate the IC₅₀ of
160 the evaluated antibodies with GraphPad Prism (version 8.0.1).

161

162 **Antibody neutralization activity against pseudovirus**

163 Huh-7 cell line, which expresses ACE2 receptor, was infected with pseudo-virus expressing
164 the full length of SARS-CoV-2 Spike protein and luciferase reporter gene in the presence
165 and absence of serial dilutions of testing antibodies. Viral entry to the cells was quantified

166 using Britelite™ plus Reporter Gene Assay System. In separate studies, pseudoviruses
167 encoding wild type or different mutant S protein (GenScript, SARS-CoV-2/Wild-type (WT),
168 SARS-CoV-2/D614G, SARS-CoV-2/V367F, SARS-CoV-2/W436R and SARS-CoV-2/D364Y)
169 were incubated with a serial dilution of purified antibodies (starting from 100µg/ml, 3 folds
170 dilution for 8 points) at room temperature for 1 hour. The mixture was then added to ACE2
171 overexpressing HEK293 cells (2×10^4 per well) cultured in DMEM containing 10% FBS in
172 triplicate. Following infection at 37°C 5% CO₂ for 48 hours, luciferase activity was determined
173 using the Promega Bio-Glo luciferase assay (Promega, catalog # G7491) system. The dose-
174 response curves were plotted with the relative luminescence unit against the sample
175 concentration. Non-linear regression was used to calculate IC₅₀ using GraphPad Prism 6.

176

177 **Live virus assay**

178 Microneutralization assays towards live virus were performed as previously described(2)
179 with slight modifications. The Vero E6 cell line (ATCC; CRL-1586, Lot#: 60526234; this clone
180 was not authenticated) was tested negative for mycoplasma contamination using a
181 commercial EZ-PCRTM Mycoplasma Test kit (Biological Industries, Beit-Haemek, Israel; 20-
182 700-20; Lot#: 1251719) (data not shown). Briefly, SARS-CoV-2 (strain
183 BetaCoV/Wuhan/WIV04/2019 preserved in National Virus Resource Center under the
184 accession number: IVCAS 6.7512) was mixed with the equivalent volume of culture medium
185 containing serially diluted antibodies and incubated at 37°C for 1 hour. Then, Vero E6 cells
186 seeded in 96-well plates were incubated with a pre-incubated mixture containing virus at

187 100 50% tissue-culture infectious doses (TCID₅₀) per well and diluted antibodies and were
188 further sustained at 37°C for 48 hours. Subsequently, cells were fixed with 4%
189 paraformaldehyde diluted in PBS for 15 minutes and penetrated by 0.25% Triton-X 100.
190 After three washes with PBS, cells were blocked at 37°C for 1 hour using PBS containing 5%
191 BSA, then incubated with in-house prepared anti-SARS-CoV-2 nucleocapsid protein (NP)
192 rabbit serum produced internally at 1:1000 dilution as primary antibody and Goat Anti-Rabbit
193 IgG H&L (Alexa Fluor® 488) (1:500 dilution, Abcam, Cambridge, UK; ab150077; Lot#:
194 GR3244688-2) as the secondary antibody. Cell nuclei were stained using Hoechst 33258
195 (Beyotime, Shanghai, China; C1018). Images were taken, and numbers of nuclei and cells
196 infected with viruses were counted, respectively, using an Operetta CLSTM system
197 (PerkinElmer, Waltham, USA). Inhibition was calculated by (total nuclei-infected cells)/ total
198 nuclei x 100%. Fifty percent neutralization dose (ND₅₀) were calculated with GraphPad
199 Prism 8.0.

200

201 **Surface plasmon resonance analysis of wild-type or mutant RBD/S1 binding.**

202 SPR experiments were performed using Biacore T200 system (GE Healthcare). In brief,
203 experiments were performed at 25°C in HBS-EP+ buffer. The antibody was immobilized onto
204 a protein A sensor chip (GE healthcare, catalog#29139131-AA). Serially diluted SARS-CoV-
205 2 RBD (WT, AcroBiosystems, catalog#SPD-C52H3), RBD (N354D/D364Y, AcroBiosystems,
206 catalog#SPD-S52H3), RBD (R408I, AcroBiosystems, catalog#SPD-S52H8), RBD (W436R,
207 AcroBiosystems, catalog#SPD-S52H7), RBD (V367F, AcroBiosystems, catalog#SPD-

229 Healthcare). The resin was washed 5-6 times with 30 mL of wash buffer (25 mM Tris, 150
230 mM NaCl, 40 mM imidazole, pH 7.5), the target protein was eluted with elution buffer
231 containing 25 mM Tris, 150 mM NaCl, 500 mM imidazole, pH 7.5. The protein was further
232 purified on a Superdex S75 (GE Healthcare) column equilibrated with 25 mM Tris, 150 mM
233 NaCl, pH 7.5. SDS-PAGE analysis revealed over 95% purity of the final purified recombinant
234 protein. Fractions from the single major peak were pooled and concentrated to 15 mg/mL
235 RBD were collected.

236

237 **Crystallization**

238 The SARS-CoV-2 RBD protein and P4A1-Fab fragment were mixed at a molar ratio of 1.5:1.
239 The mixture was incubated on ice for 1 h and further purified by Superdex S75 (GE
240 Healthcare). 7 mg/mL and 10 mg/mL of SARS-CoV-2 RBD/Fab proteins were used for
241 crystal screening by vapour-diffusion sitting-drop method at 16 °C, including the Index,
242 Crystal Screen, PEG/Ion, SaltRX from Hampton Research and wizard I -IV from Emerald
243 BioSystems.

244 The rode-like crystals appeared after two days at the mother liquid containing 20% w/v PEG
245 3350, 0.2 M potassium citrate tribasic. Further optimization with additive and hanging-drop
246 vapour-diffusion method was performed, the final optimized diffraction crystals at the mother
247 liquid containing 20% w/v PEG 3350, 0.2 M potassium citrate tribasic by the hanging-drop
248 vapour-diffusion method. Crystals were dehydrated and cryo-protected in 4M Sodium
249 formate solution and cooled in a dry nitrogen stream at 100 K for X-ray data collection.

250

251 **X-ray data collection, processing, and structure determination**

252 Diffraction data were collected at the Shanghai Synchrotron Radiation Facility (SSRF)
253 BL17U1 (wavelength, 0.97915 Å) at 100K. All data sets were processed using the HKL3000
254 package(3). Structures were solved by molecular replacement using PHASER(4) with the
255 SARS-CoV-2 RBD structure (PDB ID: 6M0J) (5) and the structures of the Fab fragment
256 available in the PDB with the highest sequence identities. The initial model was built into the
257 modified experimental electron density using COOT (6) and further refined in PHENIX(7).
258 Model geometry was verified using the program MolProbity. Structural figures were drawn
259 using the program PyMOL(8) (<http://www.pymol.org>). Epitope and paratope residues, as
260 well as their interactions, were identified by accessing PISA
261 (http://www.ebi.ac.uk/pdbe/prot_int/pistart.html) at the European Bioinformatics Institute.

262

263 **Binding affinity to FcγRs and C1q**

264 The binding affinity of P4A1-2A as well as its IgG1 form for different human FcγRs, FcRn
265 was tested by SPR using Biacore 8K (GE Healthcare). FcγRI, FcγRIIIa (H131), FcγRIIb,
266 FcγRIIIa (V158) (all from AcrobioSystems) were captured on the activated CM5 sensor chips,
267 followed by flow-through of 10 concentrations of serial diluted P4A1 (IgG1) or P4A1-2A
268 (IgG4) antibodies. For FcRn binding, P4A1 or P4A1-2A were captured on the activated CM5
269 sensor chips followed by flow-through of 10 concentrations of serial diluted FcRn
270 (AcrobioSystems). The sensor chips were regenerated by flow-through of 10 mM glycine

271 (pH 1.5).

272 Antibody binding to C1q was tested by ELISA, with P4A1 or P4A1-2A antibodies to coat the
273 plate overnight at 4°C, followed by incubation with 11 concentration of half-log titrated human
274 C1q (Complement Technology), then incubation with secondary antibody Sheep anti-human
275 C1q Ab-HRP (Complement Technology), before TMB substrate was added and absorbance
276 at 450 nm was determined using a SpectraMax Plus384 microplate reader.

277

278 **RT-PCR**

279 Total RNA was extracted from organs with an RNeasy Mini Kit (Qiagen, USA) and
280 PrimerScript RT Reagent Kit (TaKaRa, Japan). The forward and reverse primers targeting
281 the SARS-CoV-2 NP gene for RT-PCR were 5'-GGGGAACTTCTCCTGCTAGAAT-3' and 5'-
282 CAGACATTTTGCTCTCAAGCTG-3', respectively. RT-PCR was performed under the
283 following reaction conditions: 42°C for 5 min, 95°C for 10 s, and 40 cycles of 95°C for 10 s
284 and 60°C for 30 s.

285

286 **Pharmacokinetic (PK) analysis**

287 Six (6, 3/sex) naïve cynomolgus monkeys were enrolled in the study and administered with
288 P4A1-2A at 10 mg/kg by single intravenous infusion. Blood samples PK analysis were
289 collected at pre-dose, and different time-points after the treatment. Serum concentrations of
290 P4A1-2A were determined by ELISA.

291

292 **Safety evaluation**

293 Cynomolgus monkeys (3-5 years old, 2.2 to 3.6 kg for females and 2.3 to 5.2 kg for males,
294 sourced from GuangDong Blooming-Spring Biological Technology Development Co., Ltd.)
295 were randomly assigned to 3 groups of 5/sex/group and given P4A1-2A at 0, 50, or 300
296 mg/kg/dose on Day 1 and Day 8 by intravenous (IV) infusion. Animals were monitored daily
297 with blood samples collected at different time-point for various testing. Animals were
298 euthanized on Day 15 to examine thoroughly for any potential toxicity.

299

300 **In vivo efficacy in SARS-CoV-2 infection model in rhesus monkey**

301 Nine rhesus monkeys (RMs, three males, six females; 6–7 years of age, 5.3-7.3 kg, sourced
302 from Hubei Tianqin Biotech Company), were inoculated with SARS-CoV-2 virus
303 (BetaCoV/Wuhan/WIV04/2019)(2) at 1×10^5 TCID₅₀ intratracheally under anesthesia on
304 Day 0. Animals were randomized into 3 groups (1 male and 2 females in each group) and
305 receive a single intravenous (i.v.) treatment of Isotype control W332-1.80.12.xAb.hlgG4
306 (sourced from Wuxi Biologics, Co. Ltd.) at 50 mg/kg, or P4A1-2A at 10 or 50 mg/kg,
307 respectively, one day after viral infection. The RMs were observed twice daily with detailed
308 recording of clinical signs. Swab samples of the oropharyngeal, nasal turbinate, and rectal
309 regions, as well as whole blood (in K2EDTA tubes), were collected at 0-7 days post-infection
310 (d.p.i.). To confirm the pathogenesis and injury in the respiratory system, one animal from
311 each treatment groups were sacrificed at 5-7 d.p.i., respectively. The trachea, right bronchus,
312 left bronchus, all six lung lobes and other tissue organs were collected on the day of

313 euthanization for various pathological, virological analyses. Viral loads in swabs and tissues
314 were determined and pathological examination was conducted as previously described (9).

315

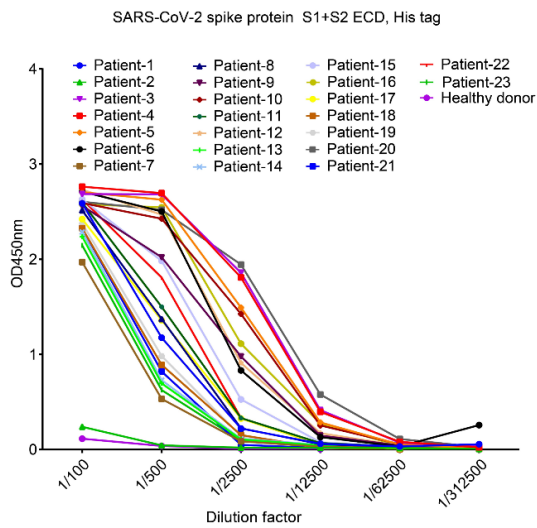
316 **Data availability**

317 Protein coordinate and structure factors are deposited in the RCSB Protein Data Bank
318 under ID 7CJF. All data is available in the main text or the supplementary materials.

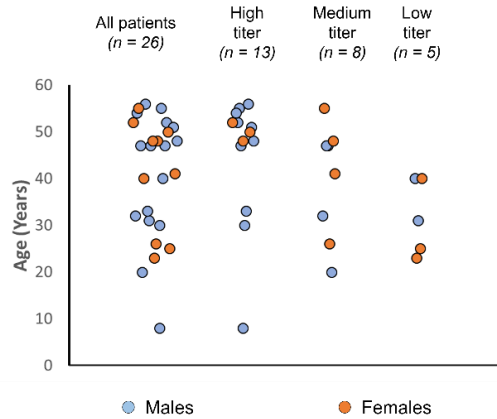
319 **Extended Figures and Tables**

320 **Figure S1**

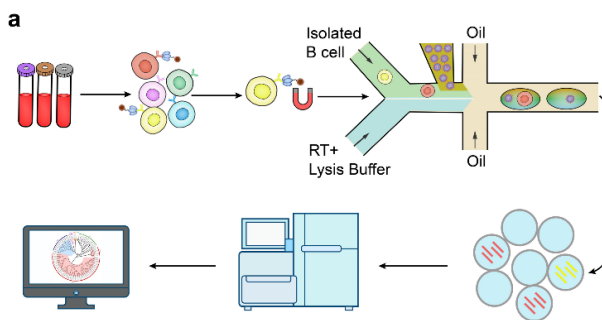
A



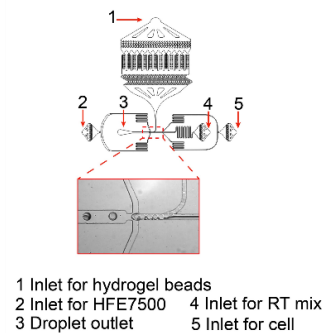
B



C



D



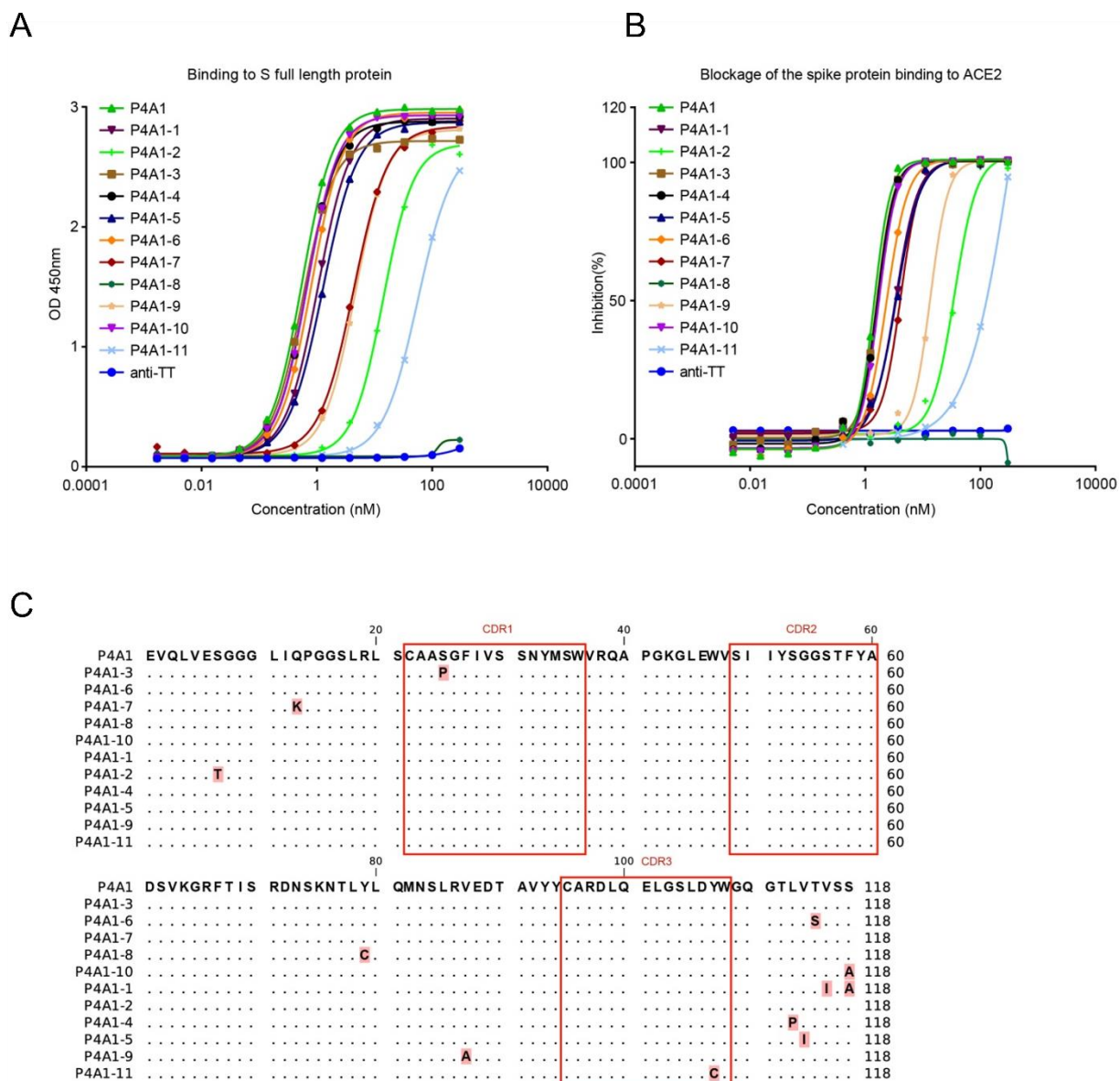
321

322

323 **Fig.S1. Analyses of plasma responses to SARS-CoV-2 proteins and antibody**
 324 **identification from convalescent patients using single cell sequencing.**

325 (A) Serums from 23 convalescent patients and one healthy donor were analyzed for their
 326 binding abilities to the SARS-CoV-2 spike protein using ELISA. (B) Classification of patient
 327 samples into high (>2500), medium (500-2500), and low (<500) titer categories. (C)

328 Schematic diagram of the antibody identification from convalescent patients using single cell
329 sequencing. SARS-CoV-2 S protein binding B cells were isolated from PBMC of
330 convalescent patients with magnetic beads that conjugated with biotinylated S protein as
331 probes. The isolated cells were individually co-compartmentalized in droplets along with
332 lysis buffer, reverse transcriptase and one hydrogel bead. Each hydrogel bead carried VH
333 and VL specific oligos tagged with a unique barcode. The resulting cDNAs from one cell
334 carried an identical barcode. The barcoded cDNAs were sequenced to identify cognate
335 heavy and light chain pairs. (D) The design of the microfluidics chip for co-
336 compartmentalization of single cells and single hydrogel bead in droplets.
337



339

340 **Fig.S2. Identification and characterization of somatic variants of antibody P4A1.**

341 Eleven heavy chain sequences closely related to P4A1 were bioinformatically identified from
 342 NGS results. These P4A1-class heavy chains were reconstituted with the light chain from
 343 P4A1 and numbered P4A1-1 to P4A1-11. (A) Binding of the antibodies to the full-length S
 344 protein was evaluated by ELISA. (B) Blocking the binding of S1 protein to Vero E6 cell line

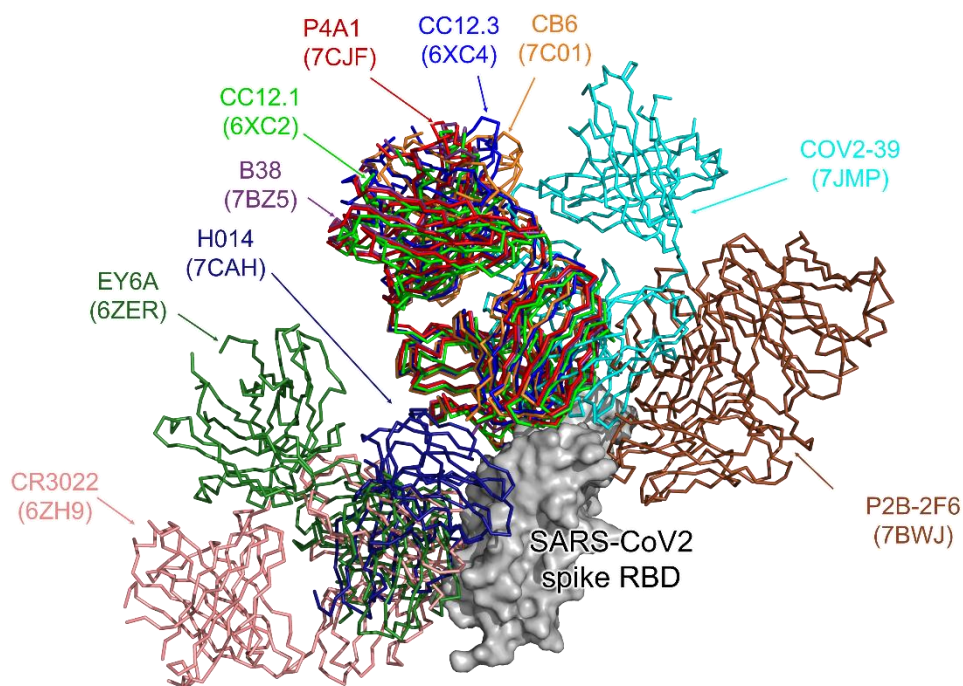
345 by antibodies was evaluated by flow cytometry. (C) The amino acid sequences of the P4A1-

346 class antibodies were aligned.

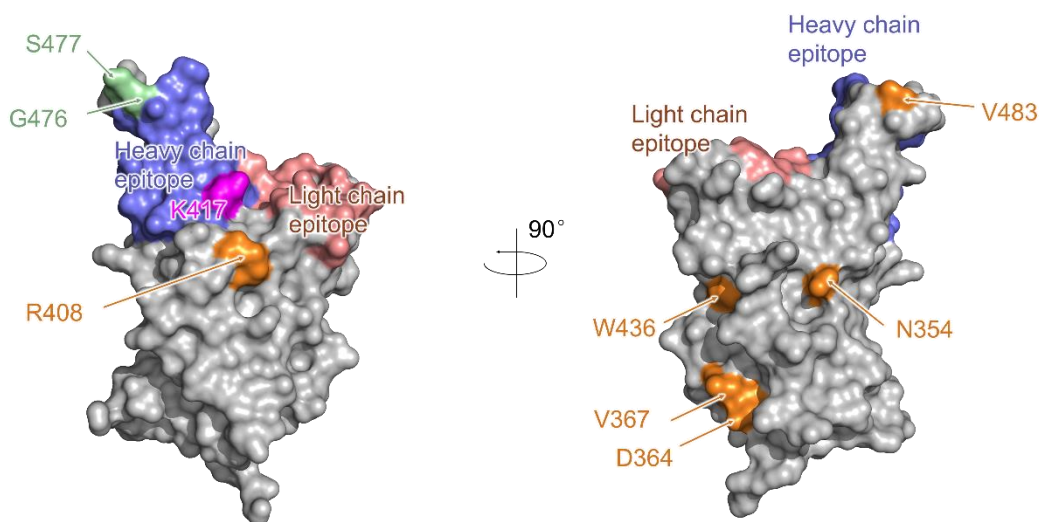
347

348

A



B



357

358 **Fig.S4. Structural comparison of the binding mode among several reported RBD-**

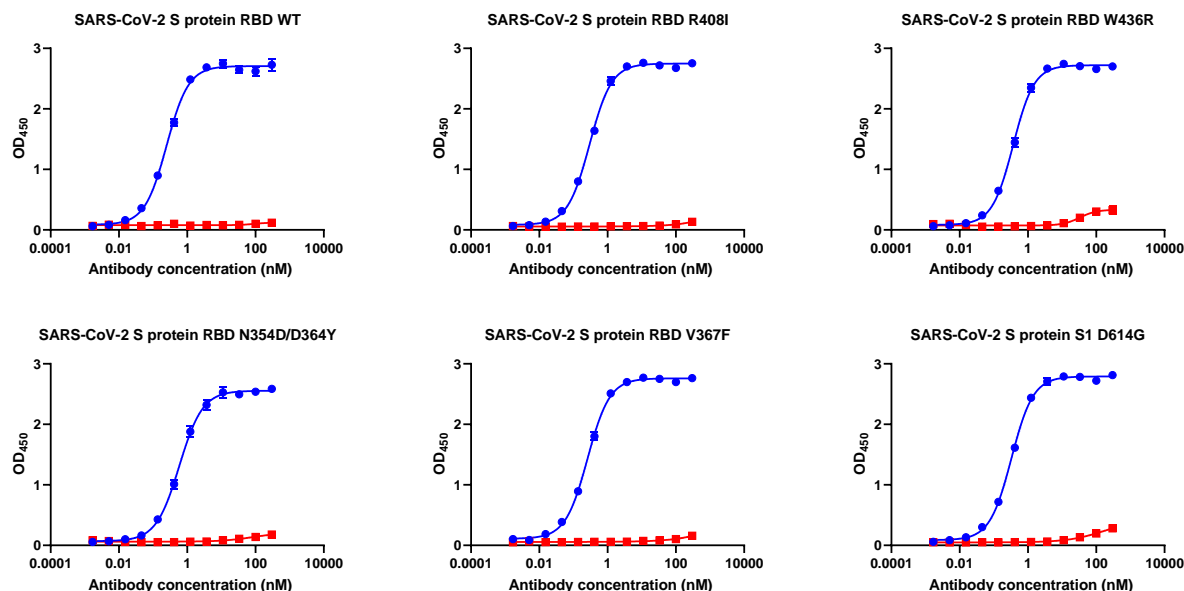
359 **specific neutralizing antibodies from various germ lines. (A) Superimpose of P4A1 (red,**

360 **PDB 7CJF), CC12.1 (green, PDB 6XC2), CC12.3 (blue, PDB 6XC4), B38 (purple, PDB**

361 **7BZ5), CB6 (orange, PDB 7C01), COV2-39 (cyan, PDB 7JMP), H014 (deep blue, PDB**

362 7CAH), EY6A (forest green, PDB 6ZER), CR3022 (salmon red, PDB 6ZH9), P2B-2F6
363 (brown, PDB 7BWJ), to SARS-CoV-2 spike glycoprotein RBD (gray). (B) Surface
364 representation of several clinical isolates with Spike RBD mutations. The SARS-CoV-2 RBD
365 is colored in gray and displayed in surface representation. The epitope of P4A1 heavy chain
366 (slate blue), light chain (salmon red), residue K417 (pink) are displayed and colored as
367 Figure2. The clinic mutations G476 and S477, which located at the edge of the P4A1 epitope
368 are colored in pale green. The clinic mutations N354, D364, V367, R408, W436, and V483,
369 which are adjacent to the epitope residues or on the opposite side of the P4A1 epitope, are
370 colored in orange.
371

372 **Figure S5**



373

374

	WT	R408I	W436R	N354D/D364Y	V367F	D614G
EC ₅₀ (nM)	0.250	0.299	0.369	0.587	0.259	0.329

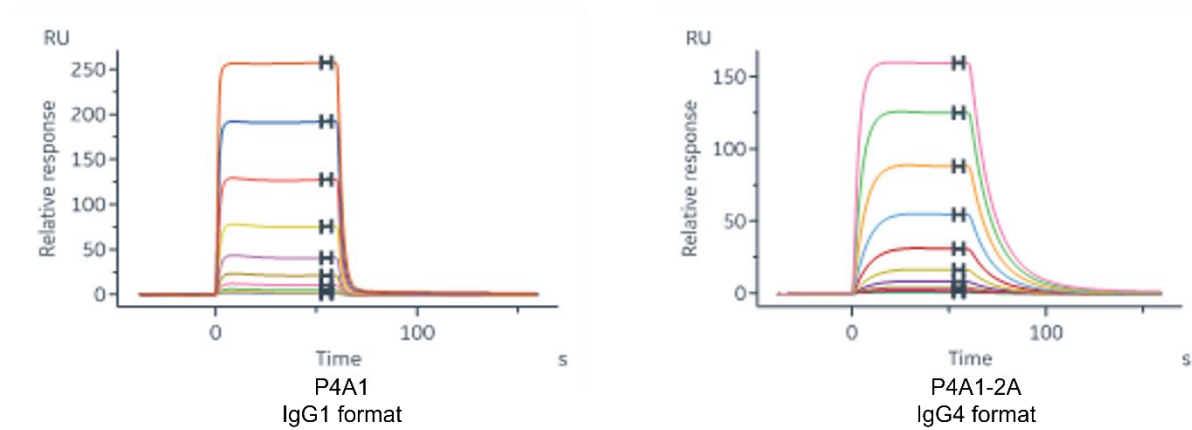
375

376 **Fig. S5. The binding of the P4A1-2A (blue) or isotype control (red) antibodies to the**
 377 **SARS-CoV-2 S protein RBD or S1 variants was determined by ELISA.** The 384-well
 378 plates were coated with 20 nM of the respective SARS-CoV-2 S protein RBD/S1 variants.
 379 The binding of P4A1-2A or isotype control antibodies (12 concentrations obtained by 3-fold
 380 serial dilutions of a 300 nM antibody stock solution, in triplicate) was detected by goat F(ab')₂
 381 anti-human IgG (H+L)-HRP. Table: summary of EC₅₀s calculated by non-linear regression of
 382 the data.

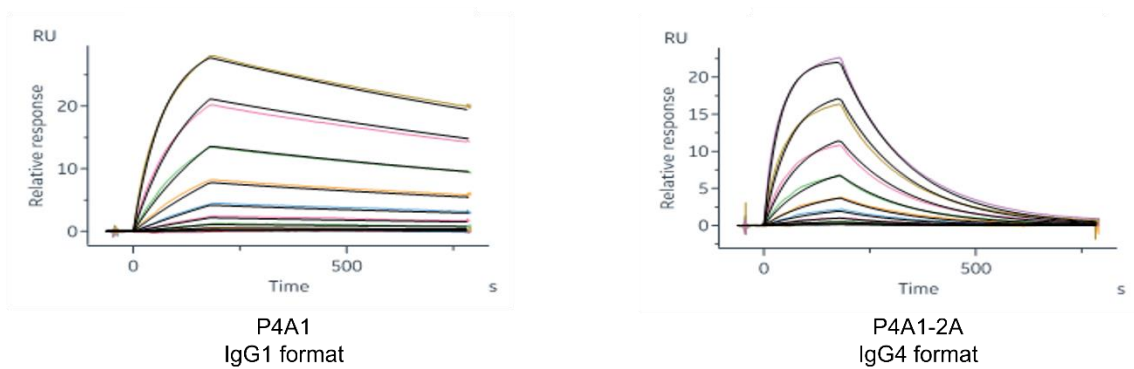
383

384 **Figure S6**

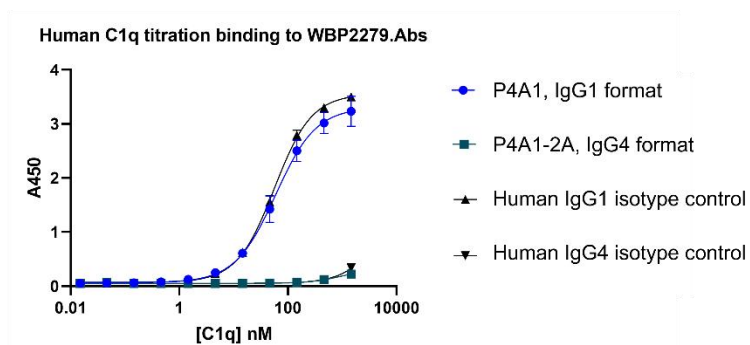
A



B



C



385

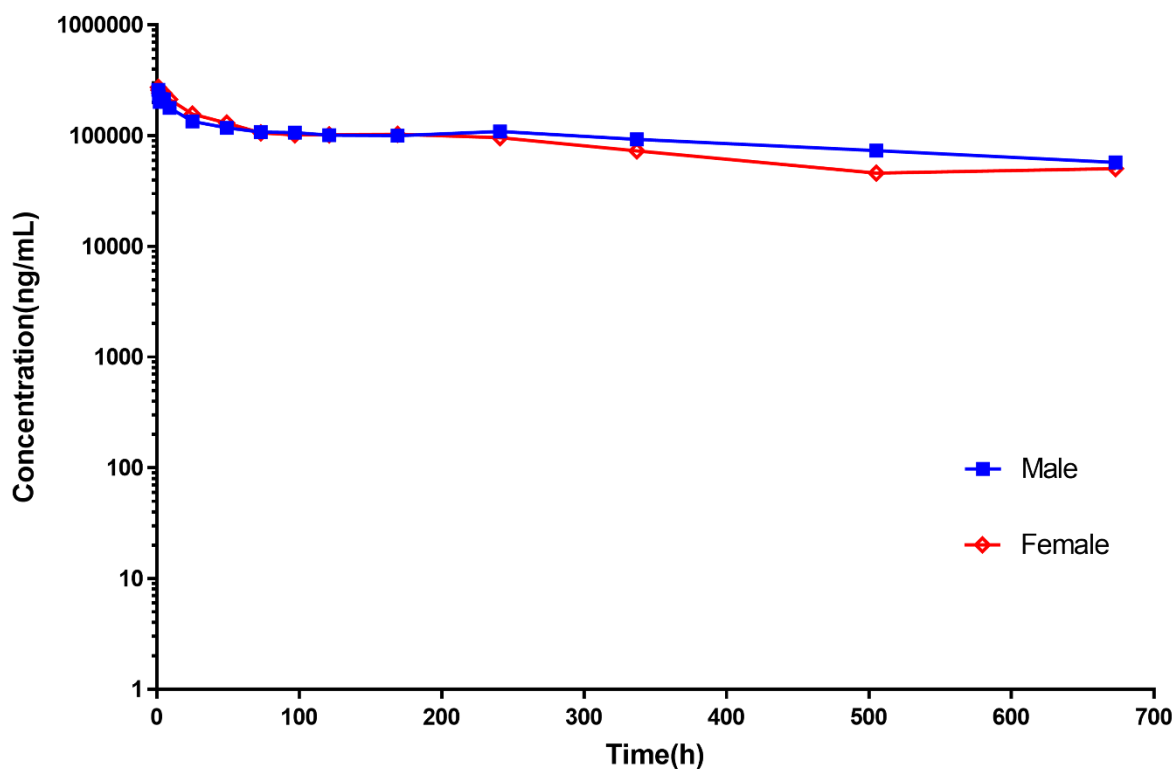
386 **Fig.S6. Binding of P4A1 (IgG1 form) and P4A1-2A (IgG4 form) to FcRn (A), FcγRI (B)**

387 **by SPR using Biacore 8K and C1q (C) by ELISA.** Assays are detailed in material and

388 methods.

389

390 **Figure S7**



391
392

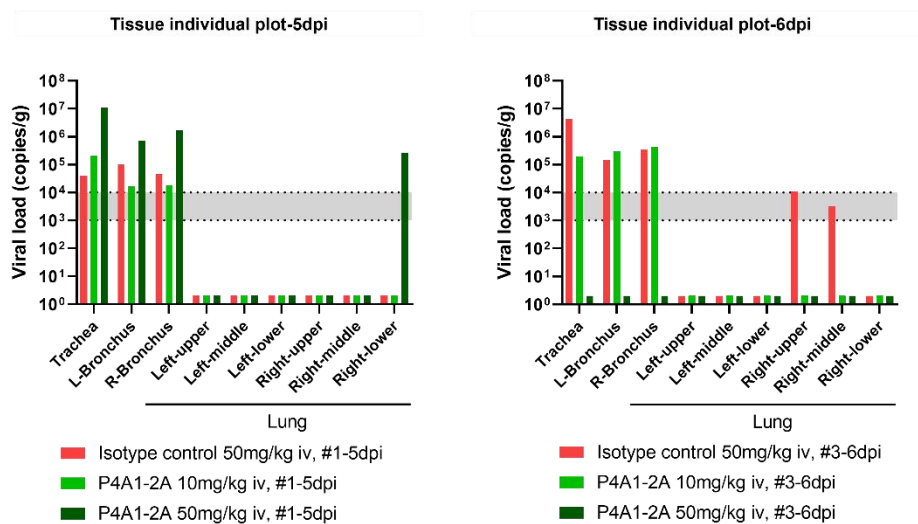
PK Parameter	Mean (Male)	Mean (Female)
C _{max} (ng/mL)	259000	273000
C _{last} (ng/mL)	27700	13000
T _{max} (h)	1.0833	1.0833
T _{1/2} (h)	586	332
AUC _{0-last} (ng.h/mL)	88200000	66700000

393
394

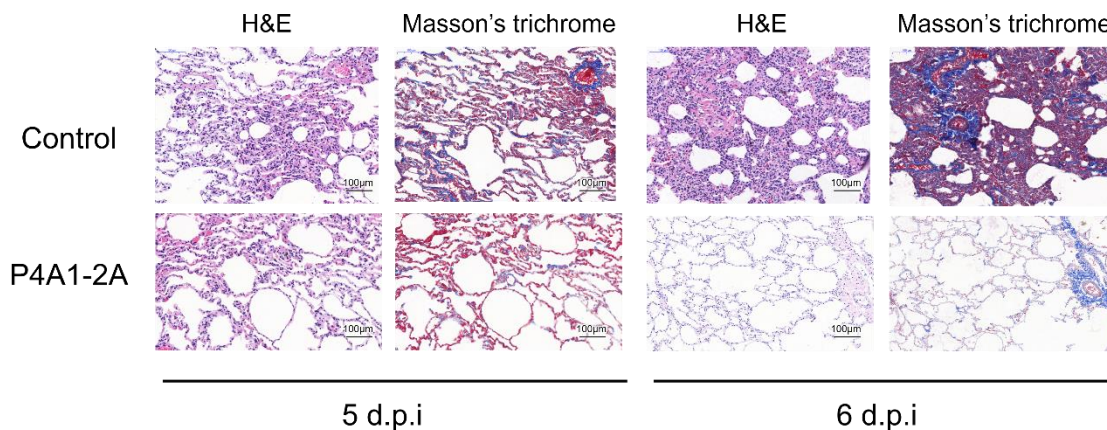
395 **Fig.S7. Mean serum P4A1-2A concentration in cynomolgus monkeys.** Male and female
 396 cynomolgus monkeys (N=3/sex) were treated with a single i.v. infusion administration at 10
 397 mg/kg P4A1-2A in 60 mins infusion time. Blood samples collected at different timepoints
 398 and serum concentrations of P4A1-2A were determined with ELISA. PK parameters derived
 399 are listed in the lower panel.

400
401

A



B



403
404

405 **Fig.S8. Viral load and histopathology in lung tissue in the rhesus macaque model of**
 406 **SARS-CoV-2 infection 5 and 6 d.p.i.** (A) Viral load in the respiratory tissues (including
 407 trachea, left and right bronchus, and all six lung lobes) collected at necropsy on 5 (left) or 6
 408 (right) days post-infection (d.p.i) were tested by RT-qPCR. (B) Representative images of
 409 histopathology in lung tissue from isotype control or P4A1-2A 50 mg/kg treated animals
 410 (collected at 5 and 6 d.p.i).

411 **Table S1. Data collection and refinement statistics.**

Wavelength	0.97915
Resolution range	36.16 - 2.108 (2.183 - 2.108)
Space group	C 2 2 21
Unit cell	85.958 148.575 144.655 90 90 90
Total reflections	475973
Unique reflections	48014 (4316)
Multiplicity	9.6 (9.7)
Completeness (%)	89.21 (81.11)
Mean I/sigma(I)	23.5 (2.3)
Wilson B-factor	32.62
R-merge	0.091 (0.800)
R-meas	0.097 (0.844)
R-pim	0.031 (0.264)
CC1/2	0.996 (0.895)
Reflections used in refinement	47909 (4316)
Reflections used for R-free	1991 (185)
R-work	0.1858 (0.2309)
R-free	0.2284 (0.2616)
Number of non-hydrogen atoms	5466
macromolecules	4861
ligands	14
solvent	591
Protein residues	793
RMS(bonds)	0.007
RMS(angles)	0.95
Ramachandran favored (%)	97.1
Ramachandran allowed (%)	2.9
Ramachandran outliers (%)	0
Rotamer outliers (%)	0.55
Clashscore	4.8
Average B-factor	39.13
macromolecules	37.67
ligands	82.89
solvent	50.16
Number of TLS groups	1

412 Statistics for the highest-resolution shell are shown in parentheses.

413

414 **Table S2. Residues contributed to interaction between P4A1/SARS-CoV-2-RBD.**

SARS-CoV-2 RBD	Distance (Å)			P4A1 Antibody
Hydrogen Bonds				
SER 477 [N]	3.1			VH:GLY 26 [O]
LYS 458 [NZ]	2.7			VH:SER 30 [O]
TYR 473 [OH]	2.8			VH:SER 31 [O]
TYR 449[OH]	4.0			VH: SER 31[OG]
LYS 458 [NZ]	3.1			VH:SER 53 [O]
TYR 421 [OH]	3.4			VH:SER 53 [OG]
LYS 417 [NZ]	2.7			VH: GLN 100 [OE1]
TYR 453 [OH]	2.4			VH: GLU 101 [OE1]
ASP 420 [OD2]	2.5			VH:SER 56 [OG]
TYR 421 [OH]	3.6			VH:SER 53 [N]
TYR 421 [OH]	2.9			VH:GLY 54 [N]
LEU 455 [O]	2.8			VH:TYR 33 [OH]
ARG 457 [O]	2.6			VH:SER 53 [OG]
ALA 475 [O]	3.2			VH:ILE 28 [N]
ALA 475 [O]	2.9			VH:ASN 32 [ND2]
ASN 487 [OD1]	2.9			VH:ARG 97 [NH1]
TYR 489 [OH]	3.4			VH:ARG 97 [NH2]
GLY 502 [N]	3.0			VL:GLY 28 [O]
ARG 403 [NH1]	2.9			VL:ASN 92 [O]
ARG 403 [NH2]	3.1			VL:ASN 92 [O]
TYR 505 [OH]	2.7			VL:SER 93 [OG]
ASN 501 [OD1]	3.2			VL:SER 30 [N]
GLY 496 [O]	3.0			VL:SER 30 [OG]
ASN 501 [OD1]	3.0			VL:SER 30 [OG]
GLN 498 [OE1]	3.4			VL:SER 67 [OG]
TYR 505 [OH]	3.6			VL:SER 93 [N]
Salt Bridge				
LYS 417 [NZ]	3.1			VH: GLU 101 [OE2]
Solvent Hydrogen Bond Bridge				
GLY 476 [O]	3.0	W740	3.0	VH: SER 31 [OG]
TYR 505 [OH]	2.8	W388	2.8	VL: TRP 32[O]
TYR 505 [OH]	2.8	W388	3.1	VL: ALA 91 [N]
TYR 505 [OH]	2.8	W388	2.9	VL: ASN 92 [N]

415

416

417 **Reference.**

- 418 1. A. Gérard *et al.*, High-throughput single-cell activity-based screening and sequencing
419 of antibodies using droplet microfluidics. *Nature Biotechnology* **38**, 715-721 (2020).
- 420 2. P. Zhou *et al.*, A pneumonia outbreak associated with a new coronavirus of probable
421 bat origin. *Nature* **579**, 270-273 (2020).
- 422 3. W. Minor, M. Cymborowski, Z. Otwinowski, M. Chruszcz, HKL-3000: the integration
423 of data reduction and structure solution--from diffraction images to an initial model in
424 minutes. *Acta Crystallogr D Biol Crystallogr* **62**, 859-866 (2006).
- 425 4. A. J. McCoy *et al.*, Phaser crystallographic software. *J Appl Crystallogr* **40**, 658-674
426 (2007).
- 427 5. J. Lan *et al.*, Structure of the SARS-CoV-2 spike receptor-binding domain bound to
428 the ACE2 receptor. *Nature* **581**, 215-220 (2020).
- 429 6. P. Emsley, B. Lohkamp, W. G. Scott, K. Cowtan, Features and development of Coot.
430 *Acta Crystallogr D Biol Crystallogr* **66**, 486-501 (2010).
- 431 7. P. D. Adams *et al.*, PHENIX: a comprehensive Python-based system for
432 macromolecular structure solution. *Acta Crystallogr D Biol Crystallogr* **66**, 213-221
433 (2010).
- 434 8. Schrodinger, LLC. (2015).
- 435 9. C. Shan *et al.*, Infection with novel coronavirus (SARS-CoV-2) causes pneumonia in
436 Rhesus macaques. *Cell Res* **30**, 670-677 (2020).

437