Supplemental Information

Human-IgG-Neutralizing Monoclonal Antibodies

Block the SARS-CoV-2 Infection

Jinkai Wan, Shenghui Xing, Longfei Ding, Yongheng Wang, Chenjian Gu, Yanling Wu, Bowen Rong, Cheng Li, Siqing Wang, Kun Chen, Chenxi He, Dandan Zhu, Songhua Yuan, Chengli Qiu, Chen Zhao, Lei Nie, Zhangzhao Gao, Jingyu Jiao, Xiaoyan Zhang, Xiangxi Wang, Tianlei Ying, Haibin Wang, Youhua Xie, Yanan Lu, Jianqing Xu, and Fei Lan

Supplemental Information

Table S1. Summary of the numbers of obtained B cells and antibody clones from 11 convalescent patients. Related to Figure 1 and 2

Table S2. Summary of the characteristics and symptoms of the 11 COVID-19 patients. Related to Figure 1

Figure S1. Related to Figure 1

(A) Schematic depicting the screening strategy used to sort B cells from SARS-CoV-2 convalescent patients and antibody identification. (B) Flow cytometry B cell sorting from PBMCs of 10 convalescent patients (data of 505 sorting not shown).

Figure S2. Related to Figure 2

(A) Summary of ELISA binding data of the selected SARS-CoV-2 specific monoclonal antibodies. (B) Flow cytometry analysis (FCA) of representative antibodies binding to SARS-CoV-2 S protein expressing on A549 cell membrane, 10 thousand cells in 100 μl were incubated with antibodies 10nM. IgG-Fc-PE antibody was used as control. (C) Results of binding ELISA to SARS-CoV-2 Spike NTD.

Figure S3. Related to Figure 3

(A) ACE2 binding to SARS-CoV-2 Spike protein expressed in A549 detected by flow cytometry. (B) ACE2 competition assay of the indicated antibodies using FCA. Indicated concentrations of antibodies were used to compete ACE2 binding to S protein expressed on A549 and HEK293T cell membrane. 10 thousand A549 cells in 100 μl were used. (C) Mutagenesis analyses of the critical amino acid residues of RBD in mediating the interaction with the indicated antibodies. Interaction strengths were reflected by ELISA values

shown as the distances from the center, the closer to the center the more affected of the interaction by the mutation. (D) Structure views of 4 recently reported antibodies. Note, the RBD binding surfaces of all reported antibodies with structure information substantially overlap with the ACE2 binding surface.

Figure S4. Related to Figure 4

ACE2 binding to S proteins of SARS-CoV-2 and SARS-CoV expressed on HEK293T membrane. Non-transfected HEK293T were used as controls.

Figure S5. Related to Figure 3

Performance of the indicated neutralizing antibodies in the various assays.

Table S1

Donor	B cells counts	Clones
71	192	16
105	192	42
413	192	35
414	192	38
415	192	22
501	192	87
505	192	74
509	192	36
507	384	146
515	192	81
553	384	152
Total	2304	729

Table S1. Summary of the numbers of obtained B cells and antibody clones from 11 convalescent patients. Related to Figure 1 and 2

Table S2

Patient Information

Patient number	71	105	413	414	415	501	505	507	509	515	553
Gender	Female	Male	Female	Male	Female	Male	Female	Male	Male	Female	Male
Age (years)	35	30	48	56	65	29	60	15	54	59	40
Sampling time point (after hospitalization)	10 days	18 days	19 days	16 days	16 days	12 days	15 days	9 days	1 days	9 days	25 days
Fever	Yes	Yes	Yes	Yes	No data	No data	Yes	Yes	No data	Yes	Yes (with myaligia)
Other information	Traveled in Shanghai from Wuhan at Jan 21st	Jan 13rd to 17th, spent time with a confirmed patient	Been to Wuhan at Jan 15th	Passed by Wuhan at Feb 23rd	Spent time with a low fever friend at Jan 19th	No data	Passing by Wuhan at Jan 17th for 1 hour	No data	Been close with a confirmed patient at Jan 14th	No data	Been to Wuhan for working at Jan 11st
Admission time	2020/1/27	2020/1/20	2020/1/26	2020/1/29	2020/1/29	2020/2/3	2020/1/31	2020/2/6	2020/2/14	2020/2/6	2020/1/21
Discharge time	2020/2/8	2020/2/8	2020/2/15	2020/2/14	2020/2/14	2020/2/15	2020/2/15	2020/2/15	2020/2/29	2020/2/15	2020/2/15

Table S2. Summary of the characteristics and symptoms of the 11 COVID-19 patients. Related to Figure 1

Figure S1

A

Immunization | FACS sorting single B cell | RT-PCR and sequencing analysis | Antibody expression and verification
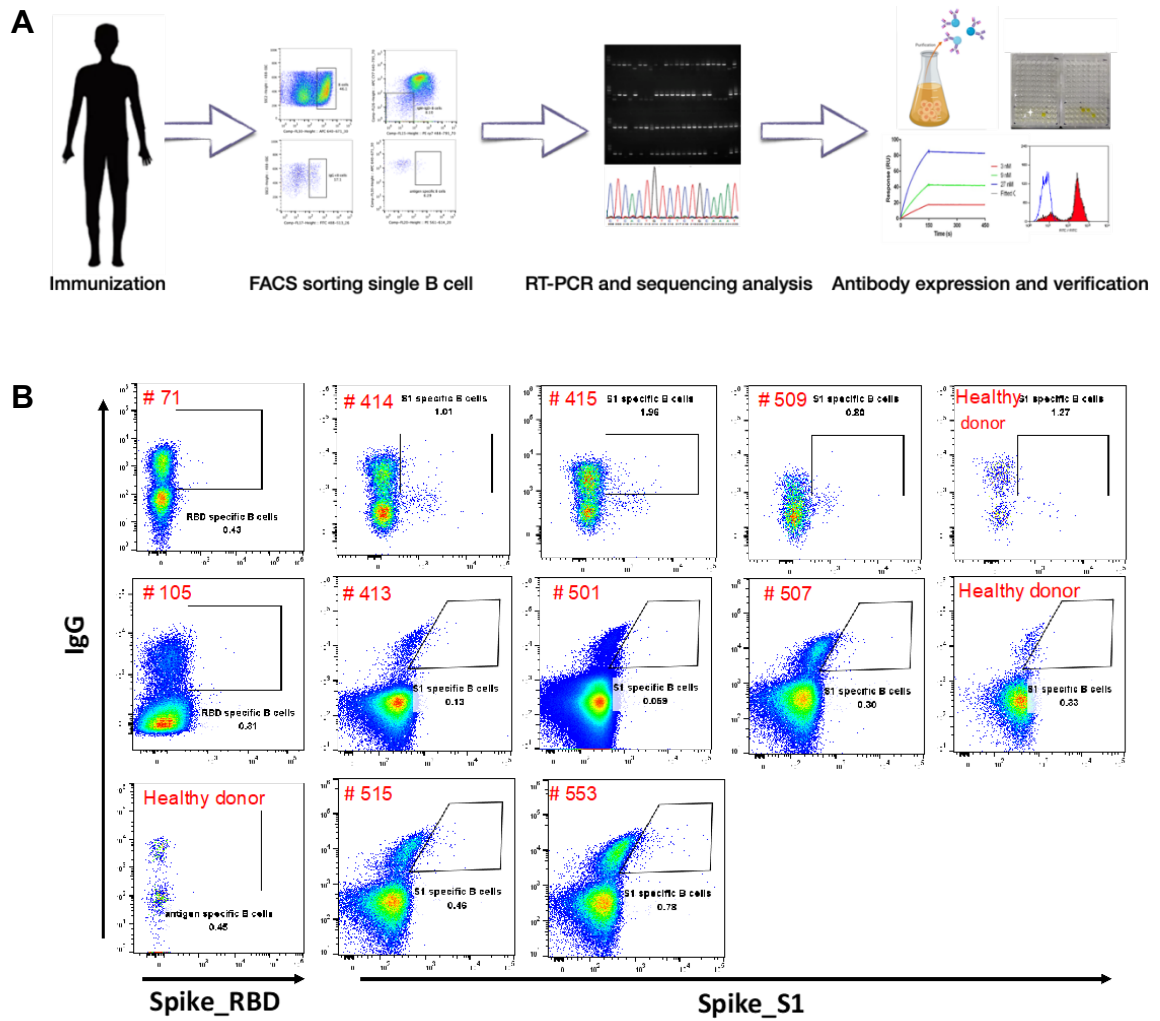
B

Figure S1. Related to Figure 1

(A) Schematic depicting the screening strategy used to sort B cells from SARS-CoV-2 convalescent patients and antibody identification. (B) Flow cytometry B cell sorting from PBMCs of 10 convalescent patients (data of 505 sorting not shown).
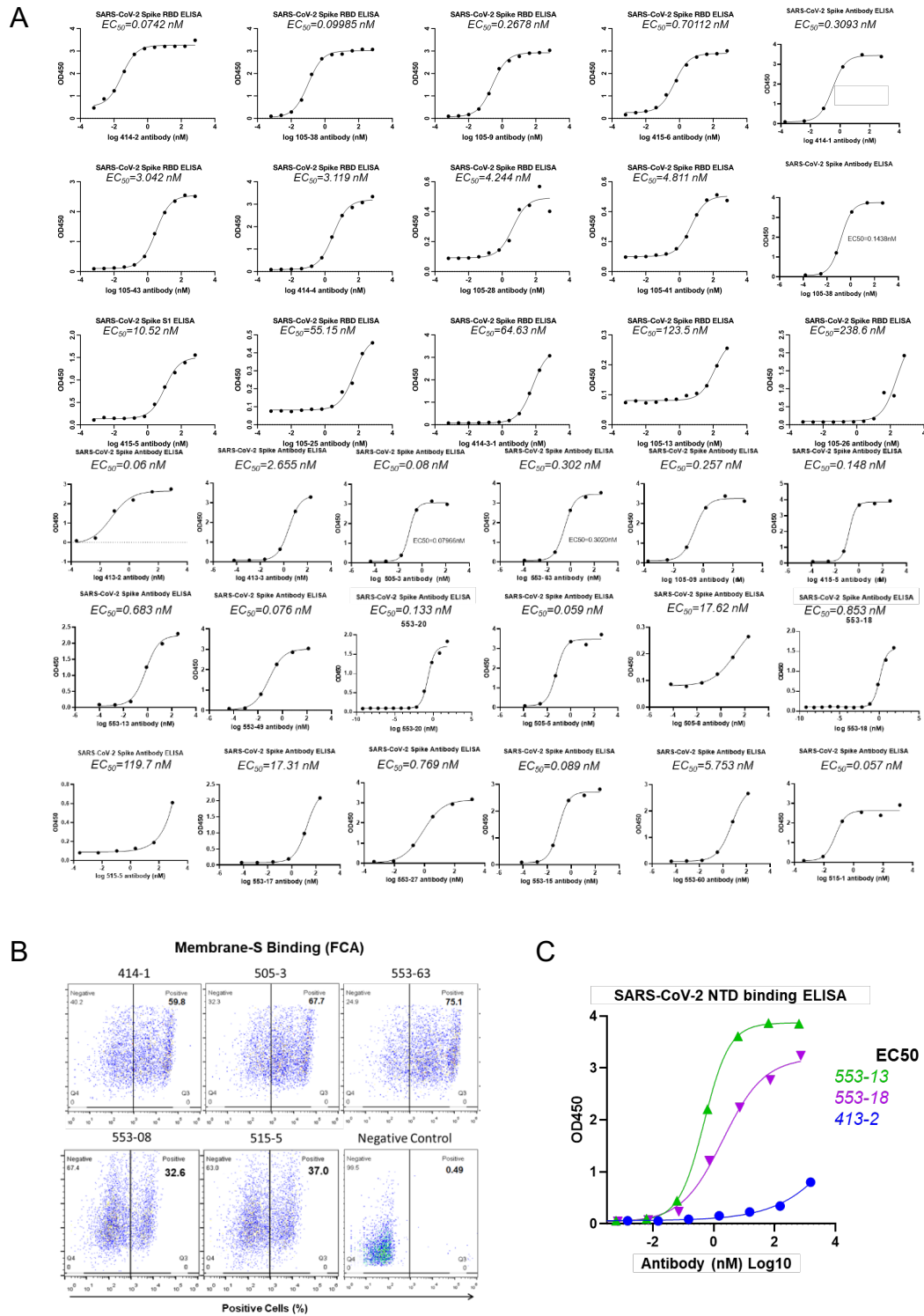
Figure S2

Figure S2. Related to Figure 2

(A) Summary of ELISA binding data of the selected SARS-CoV-2 specific monoclonal antibodies. (B) Flow cytometry analysis (FCA) of representative antibodies binding to SARS-CoV-2 S protein expressing on A549 cell membrane, 10 thousand cells in 100 µl were incubated with antibodies 10nM. IgG-Fc-PE antibody was used as control. (C) Results of binding ELISA to SARS-CoV-2 Spike NTD.
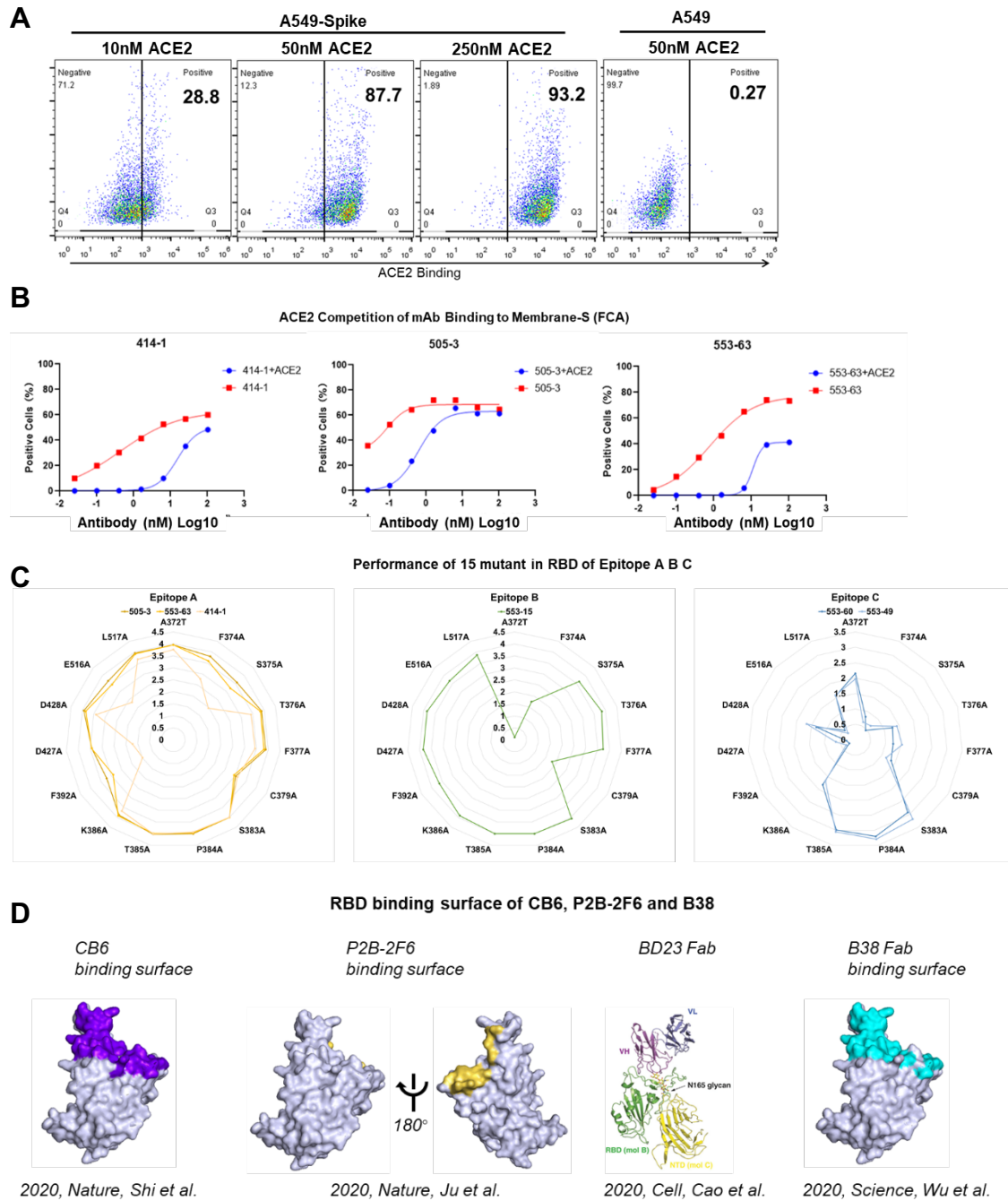
Figure S3

A

B

ACE2 Competition of mAb Binding to Membrane-S (FCA)

C

Performance of 15 mutant in RBD of Epitope A B C

D

RBD binding surface of CB6, P2B-2F6 and B38

CB6 binding surface — 2020, Nature, Shi et al.

P2B-2F6 binding surface — 2020, Nature, Ju et al.

BD23 Fab — 2020, Cell, Cao et al.

B38 Fab binding surface — 2020, Science, Wu et al.

Figure S3. Related to Figure 3

(A) ACE2 binding to SARS-CoV-2 Spike protein expressed in A549 detected by flow cytometry. (B) ACE2 competition assay of the indicated antibodies using FCA. Indicated concentrations of antibodies were used to compete ACE2 binding to S protein expressed on A549 and HEK293T cell membrane. 10 thousand A549 cells in 100 μl were used. (C) Mutagenesis analyses of the critical amino acid residues of RBD in mediating the interaction with the indicated antibodies. Interaction strengths were reflected by ELISA values shown as the distances from the center, the closer to the center the more affected of the interaction by the mutation. (D) Structure views of 4 recently reported antibodies. Note, the RBD binding surfaces of all reported antibodies with structure information substantially overlap with the ACE2 binding surface.
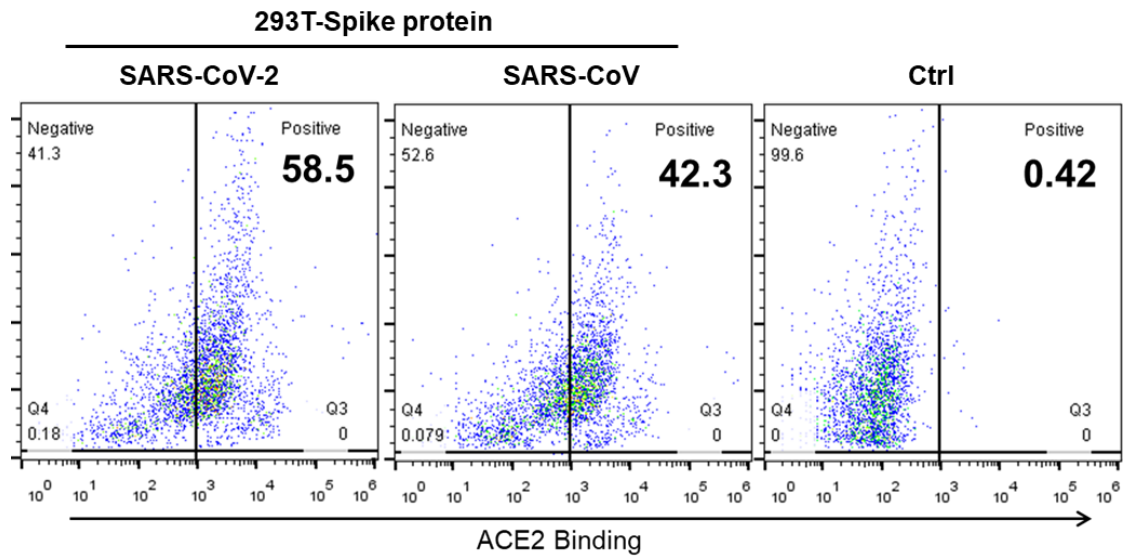
Figure S4

Figure S4. Related to Figure 4

ACE2 binding to S proteins of SARS-CoV-2 and SARS-CoV expressed on HEK293T membrane.
Non-transfected HEK293T were used as controls.
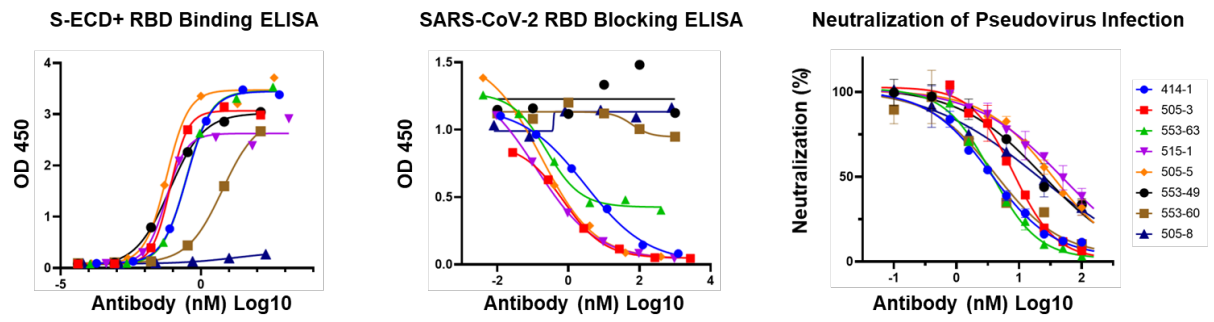
Figure S5

Figure S5. Related to Figure 3

Performance of the indicated neutralizing antibodies in the various assays.