Human IgG cell neutralizing monoclonal antibodies block SARS-CoV-2 infection

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Abstract

The coronavirus induced disease 19 (COVID-19) caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has become a worldwide threat to human lives, and neutralization antibodies present a great therapeutic potential in curing affected patients. We purified more than one thousand memory B cells specific to SARS-CoV-2 recombinant S1 or RBD antigens from 11 convalescent COVID-19 patients, and a total of 729 naturally paired heavy and light chain fragments were obtained by single B cell cloning technology. Among these, 178 recombinant monoclonal antibodies were tested positive for antigen binding, and 17 strong binders to S1 or RBD were identified with K_d(EC50) below 1 nM. Importantly, 12 antibodies could block pseudoviral entry into HEK293T cells overexpressing ACE2, with the best ones showing IC50 around 2-3 nM. From these 12 antibodies, we had tested two in authentic virus infection assay, and found one was able to effectively block live viral entry with IC50 around or below 15 nM. Interestingly, we

also found a substantial portion of these antibodies crossreacting with the SARS-CoV spike protein.

Altogether, our study provided potent neutralization antibodies as clinical therapeutics candidates for further development.

Introduction

Over the last two decades in 21th century, the outbreaks of several viral infectious diseases affected millions of people. Among these, 3 coronaviruses, SARS-CoV, MERS and SARS-CoV-2, have received significant attention due to current outbreak of COVID-19 caused by SARS-CoV-2, and high mortality rates of the infected individuals. Most patients died due to severe pneumonia and multi-organ failure [1]. Despite of rare exceptions, such as asymptomatic carriers, exist, it is generally believed if the infected individuals could not develop effective adaptive immune responses for viral clearance to prevent sustained infection, there are high chances for transformation into severe acute respiratory infection. Supporting this idea, treatment with convalescent plasma to COVID-19 patients showed significant clinical improvement and decreased viral load within days[2]. However, the sources of convalescent plasma are limited and could not be amplified, therefore, effective and scalable treatments are still urgently needed.

Recently, owing to rapid development of single cell cloning technology, the process of antibody identification has been much shortened, from years to even less than 1 month. Therefore, full human and humanized neutralization antibodies represent as great hopes for a prompt development of therapeutics in treating infectious diseases. In support of this, cocktail treatment of 3 mixed antibodies recognizing different epitopes, with one of them able to neutralize, was successfully used in the curation of a British Ebola patient (Role of the Ebola membrane in the protection conferred by the three-mAb cocktail MIL77[3]). Regarding coronaviruses, neutralization antibodies against MERS were tested effective in animals[4]. While SARS-CoV neutralization antibodies did not meet human due to lack of patients after development, one of them, CR3022, was shown to be able to cross-react with and to weakly neutralize SARS-CoV-2[5]. However, the RBD regions (key targets for viral neutralization) only share 74% sequence identity between the two SARS viruses, raising concerns about the effectiveness of SARS-CoV neutralization antibodies against SARS-CoV-2.

The spike proteins of coronaviruses play essential role in viral entry to human target cells. The S1 region, especially the RBD domain, primes the viral particle to human cell surface through the interaction with the receptor protein Angiotensin I Converting Enzyme 2 (ACE2), which then triggers infusion process primarily mediated by S2 region[6]. The primary amino acid sequences of the spike proteins of SARS-CoV and SARS-CoV-2 share 76 % identity throughout the full coding regions, with 79.59 % similarity and 74% identity in RBD domains[5]. Structure analyses revealed high similarity between the spike proteins of the two viruses, and both form trimerization and interact with ACE2 through the RBD domains. In addition, recent research reported that it is difficult to distinguish exposure to SARS-CoV-2 from SARS-CoV in serological studies using S ectodomain trimer, and they elicits neutralizing antibodies against SARS-CoV-2 from SARS-CoV. Importantly, the interaction between the SARS-CoV-2 RBD and ACE2 was assessed at around 1.2nM[6], 4 folds stronger than the SARS-CoV RBD. While this enhanced affinity may explain a much stronger spreading ability of SARS-CoV-2, it also suggests that finding potent neutralization antibodies targeting SARS-CoV-2 RBD could be much more challenging.

While this manuscript was under preparation, a few studies from George Fu Gao[7], Wei Chen[8] and Berend-Jan Bosch[9] were published in Science, bioRxiv and Nature Communications. While these groups and ours all employed similar approaches and obtained live viral neutralization antibodies, the performances of these antibodies displayed large differences in various assays, i.e. binding affinity, pseudoviral neutralization abilities. Nevertheless, more human antibodies could either directly neutralize SARS-CoV-2 entry to human cells or opsonize free viral particles for rapid immune clearances are still needed.

Here, we report the identification of 178 S1 and RBD binding full human monoclonal antibodies from the memory B cells of 11 recently recovered patients. The CDR3 sequences of the majority of these antibodies are different, indicating they are developed from different B cell clones. Among the stronger binders, we found 28 RBD binders and 7 non-RBD binders. A total of 17 antibodies showed binding affinities lower than 1 nM, and 12 antibodies showed IC50 with best one was 2 nM for pseudoviral neutralization ability. Two of the leading antibodies were tested for

blocking the live SARS-CoV-2 infection, and one of them, 414-1, could effectively block live viral infection at around of below 15 nM. Moreover, among the 24 best binders, we found 3 antibodies could cross-react with SARS-CoV spike protein with similar affinity, but none could bind MERS spike protein.

Results

Serologic Responses and single B cell isolation

We screened 11 patients recently recovered from COVID-19, and identified 9 out of 11 individuals with strong serological responses to SARS-CoV-2 Spike RBD and S1 protein (Figure 1B), and 7 sera showed neutralization abilities for pseudoviral infection of HEK293T cells stably expressing human ACE2. We also noticed that sera from different individuals displayed a wide range of antibody responses to SARS-CoV-2 infection in our assays.

The RBD domain in S1 region of SARS-Cov-2 spike protein is the critical region that mediates viral entry though host receptor ACE2. Using recombinant viral antigens, we then isolated RBD and S1 binding memory B cells for antibody identification from 11 individuals by flow cytometry based sorting technology (Figure 1A). Each individual exhibited different frequencies of viral antigen specific memory B cells (Figure 1C and Table S1).

Sequences encoding antibody heavy (IGH) and light (IGL) chains were amplified from single B cell cDNAs after reverse transcription and then cloned through homologous recombination into mammalian expressing vectors[10]. Overall, 729 naturally paired antibody genes were obtained from the 11 individuals, of which the No.71 was failed to give any positive antibody. However, no strong correlation was found between sera binding capacity and the number of acquired SARS-CoV-2 S-specific antibodies. Unlike other samples, No.509 blood sample was obtained at the second day after hospitalizing (Table S2), but the sera already had certain S-specific affinity and pseudoviral neutralizing capacity. However, we did not get good antibodies from No.509 sample.

Identification and binding characterization of human monoclonal antibodies to SARS-CoV-2

All the 729 antibodies were further expressed in HEK293E cells and the supernatants were tested in ELISA for S1 or RBD binding (Figure 2C, Figure S1). Among these, 178 antibody supernatants showed RBD or S1 binding positivity. We then purify all these antibodies in a larger quantity to measure the precise values of K_d (EC50), and found the values varied broadly, with the most potent one at 57 pm (8.55 pg/µl), and 17 strongest ones having K_d (EC50) below 1 nM (Figure 3A).

All the positive clones were then sequenced. Notably, most （98.6%） of the sequences obtained were unique ones(Figure 1D), unlikely what were previously reported for HIV-1, influenza and ZIKV[10] .

As the spike S1 protein of SARS-CoV-2 tends to undergo conformation changes during storage, we also performed flow cytometry analyses for all 729 purified antibodies or supernatants for the binding ability of the freshly expressed spike protein in the membrane bound form using HEK293T cells (Figure 2B). Among these 729 antibodies, 58 were obtained from B cells purified by recombinant RBD domain, and 671 were from B cells purified by recombinant S1 protein. From the latter expression supernatant, 21 are able to bind S-ECD while showed low RBD affinity, tested by ELISA. The result indicates that RBD regions are the primary antigen inducing antibody generation and recognition. (Figure 2C)

We found that the results from the flow cytometry and ELISA assays were largely consist among the robust binding of 17 antibodies (K_d<1 nM) detected by ELISA, only two could not bind SARS-CoV-2 Spike protein on cell membrane by flow cytometry. However, a total of 49 high-affinity antibodies screened by flow cytometry or ELISA have 21 have overlap (Figure 2D), indicating that the cell membrane-bound and soluble recombinant S proteins might undergo certain conformation alterations.

Identification of potent neutralizing antibodies by pseudoviral and live viral infection assays

To identify neutralization antibodies, we first employed pseudoviral infection assays using HEK293T-ACE2 cells and screened 135 antibodies. From all the antibodies tested, we found a total of 12 pseudoviral neutralization antibodies, with IC50 from 2.3 – 50 nM. The best 3 antibodies in neutralizing pseudoviruses are 414-1, 505-3 and 553-63 (Figure 3B, IC50 from 2.3 to 3.6 nM), all of which also showed strong affinities towards RBD domain (Figure 2A, K_d from 0.079 nM to 0.31 nM) to the receptor binding domain (RBD) of Spike (S) protein, indicating certain level of correlation between the two abilities. Subsequently 414-1 was tested in pH5.0 binding buffer by ELISA (Figure S4), the result showed a good affinity performance of 414-1 in low pH which indicating a good performance to SARS-CoV-2 Spike protein in lysosome. Consistent with this idea, the majority (10) of these 12 antibodies indeed recognized RBD domain in ELISA. However, to our surprise, two of them, 413-2 and 505-8, did not. Instead, these latter two were able to recognize membrane bound spike protein overexpressing in HEK293T cells robustly, indicating there is an alternative neutralization mechanism of non-RBD binders.

Among 12 pseudoviral neutralization antibodies, only 414-4 cannot bind S protein expressed in cell membrane detected by flow cytometry analysis, and all of those 11 neutralization antibodies can competed by 50nM ACE2. We confirmed that over-dose ACE2(50nM) will binding most of cells(100000/ml) expressing Spike protein .(Fig S2) However, the affinity of antibodies binding S protein have no significant correlation with neutralization ability, for example, 414-1, 505-3 and 553-63 with the strongest neutralizing activity can be completed by 5, 50, 500 times concentration of ACE2 respectively. (Fig 3C, Fig S3)

We then performed live viral neutralization assay of 414-1 and 414-2, and found 414-1 could effectively block live viral infection into Vero-E6 cells overexpressing ACE2 at an IC50 estimated around or below 15 nM (Figure 3D).

Cross-reactivity of with SARS-CoV Spike protein

The spike proteins of SARS-CoV-2 share about 76% and 35% of amino acid identities with SARS-CoV and MERS-CoV, therefore, we tested whether these

antibodies recognize the S protein of these two coronaviruses. We overexpressed S protein of SARS-CoV-2, SARS-CoV and MERS-CoV in HEK293T freshly, and tested binding ability by flow cytometry analysis. Considering that some antibodies bind non-specifically with HEK293T, we screened 31 antibodies with robust S protein binding ability (Fig 4A). And found that only 3 (415-5, 415-6, 515-5) can recognized S protein of SARS-CoV and no one can recognized MERS-CoV(Fig 4B).Interestingly, 415-5 and 515-5 share same S protein affinity between SARS-CoV-2 and SARS-CoV, but 415-6 have lower S protein affinity in SARS-CoV(Fig 4C).

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Author contributions:

F.L., J.X., Y.L. and X.W. conceived the project. J.W., S.X., L.D. and, Y.W. S.Y., C.Z. and C.Q. did the experiments. All authors contributed to data analysis. F.L., J.W., S.X., L.D. wrote the manuscript.

Competing interests:

Fei Lan hold the share of Active Motif China Inc.

Materials and methods

Ethics statement

The experiments involving authentic COVID-19 virus were performed in Fudan University biosafety level 3 (BSL-3) facility. The overall study was reviewed and approved by the SHAPHC Ethics Committee (approval no. 2020-Y008-01)

Cell and Viruses

HEK293E cell line was a gift from Yanhui Xu lab, Fudan University. Vero E6, A549-Spike, and A549-ACE2 cell lines were supplied by Shanghai Public Health Clinical Center, Fudan University. 293T-ACE2 cell line was provided by Lu Lu from Fudan University. Pseudovirus was provided by Shanghai Public Health Clinical Center, and Fudan University and SARS-CoV-2-SH01 was from BSL-3 of Fudan University

B cell sorting and single-cell RT-PCR

Samples of peripheral blood for serum or mononuclear cells (PBMCs) isolation were obtained from Shanghai Public Health Clinical Center， per 5 mL blood. PBMCs were purified using the gradient centrifugation method with Ficoll and cryopreserved in 90% heat-inactivated fetal bovine serum (FBS) supplemented with 10% dimethylsulfoxide (DMSO), storage in liquid nitrogen.

The fluorescently labeled S1 bait was previously prepared by incubating 5 ug of His tag-S1 protein with Anti His tag antibody-PE for at least 1 hr at 4C in the dark. PBMCs were stained using 7AAD, anti-human CD19 (APC), IgM [PE-Cy7], IgG (fluorescein isothiocyanate (FITC)), PE labeled Antigen. Single antigen specific memory B cells were sorted on BD FACSAria II into 96-well PCR plates (Axygen) containing 10 µl per well of lysis buffer [10 mM DPBS, 4 U Mouse RNase Inhibitor (NEB)]. Plates were immediately frozen on dry ice and stored at 80℃ or processed for cDNA synthesis.

Reverse transcription and subsequent PCR amplification of heavy and light chain variable genes were performed using SuperScript III (Life Technologies). First and second PCR reactions were performed in 50 ml volume with 5ul of reaction product using PCR mixture (SMART-Lifesciences). PCR products were then purified using DNA FragSelect XP Magnetic Beads (SMART-Lifesciences) and cloned into human IgG1, lambda or kappa expression plasmids for antibody expression by seamless cloning method. After transformation, individual colonies were picked for sequencing and characterization. Sequences were analyzed using IMGT/ V-QUEST (http://www.imgt.org/IMGT_vquest) and IgBlast (IgBLAST, http:// www.ncbi.nlm.nih.gov/igblast)

Expression and purification of human monoclonal antibodies

The antibody VH/VL and constant region genes were then amplified and cloned into expression vector pcDNA3.4 using SMART Assembly Cloning Kit (SMART-Lifesciences), subsequently antibodies plasmids were amplified in competent cells (SMART-Lifesciences). Expressing in HEK293E with transfecting by polyethylenimine (PEI) (Sigma), after 3days cell culture， antibodies purification was processing in medium supernatant. Purified antibodies was binding with ProteinA magnetic beads (SMART-Lifesciences) 30min at room temperature， then eluted in 100mM Glycine pH3.0 and neutralized with Tris-Cl 7.4.

ELISA analysis of antibody binding to CoV spike antigens

ELISA analysis 96-well plates (Falcon and MATRIX) were coated overnight at 4℃ with 0.5 μg/mL SARS-CoV-2 RBD-mFC (Novoprotein Scientific Inc.), and 0.6 ug/mL SARS-CoV-2 S-ECD (GenScript). After washing with PBS/T (SMART-Lifesciences), the plates were blocked using 3% non-fat milk in PBS/T for 1 h at 37℃. Washing with PBST, gradient dilutions in PBS/T of antibodies were added to each well and incubated at 37℃ for 1 h. Washing with PBS/T three times, HRP-conjugated anti-human IgG Fab antibody (Sigma) was added at the dilution of 1:10000 in PBS/T containing 3%BSA (Sangon Biotech) and incubated at 37℃ for 0.5 h. After washing with PBS/T three times, TMB solution (SMART-Lifesciences) was added to the microplate and incubated at room temperature for 5-10 min, followed by adding 1M HCl to terminate the reaction. The OD450 absorbance was detected by Synergy HT Microplate Reader（Bio-Tek）. The curves and EC50 were analyzed by GraphPad Prism 8.0.

Sensor preparation for surface plasmon resonance（SPR）

Kinetic screening of the mAb panel was performed in the IBIS MX96 by injecting the S protein RBD in a 2-fold dilution series from 50 to 3.125 nM in running buffer (PBS with 0.075% Tween80). After each antigen injection, the sensor surface was regenerated twice with 20 mM H3PO4 pH 2.0 for 16 s. IBIS SPRintX software was

used to process the data. Scrubber2 software (BioLogic Software) was used to analyze the data and obtain kinetic information.

Flow cytometry-based receptor-binding inhibition assay

Flow cytometry analysis was performed to detect the binding ability of antibodies to Spike protein in HEK293T cells freshly expressing of SARS-CoV-2 SARS-CoV and MERS-CoV. HEK293T without transfection were used as controls. Briefly, 10 thousand cells in 100 μl were incubated with antibodies for 30 min at room temperature, after washed twice incubated with PE-labeled goat anti-human IgG-Fc antibody (1:5000; ABcam) for 30 min and analyzed by flow cytometry.

Flow cytometry analysis was also performed to detect the ACE2-binding inhibition of SARS-CoV-2 S protein in A549 cells stably expressing SARS-CoV-2 S Protein. Briefly, 10 thousand cells in 100 μl were incubated with ACE2 at 50nM for 30 min at room temperature, then put in antibody incubated for 30 min, finally incubated with PE-labeled goat anti-human IgG-Fc antibody (1:5000; ABcam) for 30 min and analyzed by flow cytometry. The ACE2 binding assay was performed by incubation of soluble human PE-Labeled ACE2 50nM with 10 thousand cells in 100 μl for 10 min at room temperature, then washed twice and analyzed by flow cytometry. PE-labeled ACE2 was performed by ACE2-Cter-6XHis incubated with rabbit anti-His-PE antibody in 1.2:1(*n*:*n*) for 30 min at room temperature.

Virus neutralization assay (pseudotyped and authentic)

Genes of SARS-CoV-2(YP_009724390.1), SARS-CoV (NP_828851.1)and MERS (AFS88936.1)Spike proteins were synthesized (GenScript) and cloned into pcDNA3.1 . SARS-CoV-2, SARS-CoV and MERS pseudo-typed viruses were produced as previously described[11]. Briefly, pseudovirus were generated by co-transfection of 293T cells with pNL4-3.Luc.R-E- backbone and the SARS-CoV-2 spike protein expression plasmid in 10cm cell culture dishes and the supernatants were harvested after 48 h, and followed by centrifuge at 2000rpm 5 mins and stored pseudovirus in -80℃.

The neutralization assay was performed as the following steps: Pseudovirus was diluted in complete DMEM mixed with or without an equal volume (50 μl) of diluted serum or antibody and then incubated at 37 °C for 1 h. The mixtures were then

transferred to 96-well plate seeding with 20,000 293T-ACE2 cells for 12h and incubated at 37 °C for additional 48 h. Assays were developed with bright glo luciferase assay system (Promega), and the relative light units (RLU) were read on a luminometer (Promega GloMax 96). The titers of neutralizing antibodies were calculated as 50% inhibitory dose (ID50), expressed as the highest dilution of plasma which resulted in a 50% reduction of luciferase luminescence compared with virus control.

All experiments about authentic virus were done in BSL-3. The cell culture medium contained a series of gradient of human monoclonal antibodies to SARS-CoV-2. After incubating with 200 PFU SARS-CoV-2 SH01 at 37℃ for 1 hour, it is added to VERO E6 cell line (96-well plate, $2x10^4$ cells per well), and the cells were continued to be cultured for 24-72h with observing the cytopathic phenotype every day.

Figure legends

Fig.1 Isolation of antigen-specific monoclonal antibodies from convalescent patients of SARS-CoV-2.

(A) Schematic depicting the screening strategy that was used to sorting B cells from SARS-CoV-2 patient and antibodies expression. (B) Spike protein binding and pseudoviral neutralizing of donors plasma. RBD (receptor binding domain) and S1, were used in ELISA to test the binding of plasma. Plasma of heathy donors were used as control. Neutralization of pseudotyped virus by 11 patients' sera The mean values and standard deviations of two technical replicates are shown (C) Flow cytometry sorting from PBMCs of 11 convalescent patients. (D) Maximum-likelihood phylogenetic tree of our sequenced monoclonal antibodies' heavy chains. Different color represents the sequence isolated from different patient serum.

Fig.2 Binding profiles of Spike protein-specific antibodies.

(A) ELISA and SPR binding curves of 414-1, 505-3 and 553-63 to RBD of SARS-CoV-2 coated at 96-wells microplate with the concentration of 0.5 ug/mL. （B） Flow cytometry analysis of representative antibodies binding to SARS-CoV-2 S protein expressing at cell membrane of A549 cell lines. Incubated only with IgG-Fc-PE antibody cells were as control. (C) Characteristic of antibodies binding with Spike protein RBD and S-ECD. RBD and S-ECD double binding positive antibodies are within red circle, and only S-ECD positive antibodies are within purple circle. (D)

Overlapping of ELISA binding positive (K_D <10nM, 27 antibodies) and flow cytometry (gated rate >10%, 43 antibodies). The red area represents ELISA assay positive, and the green area represents flow cytometry assay positive.

Fig.3 Neutralizing capacities (pseudotyped virus and authentic virus) of SARS-CoV-2 specific mAbs.

(A) Summary of phenotypical characterization of partial isolated monoclonal antibodies of 11 patients. Red color highlights the good characteristics of mAbs, and green color (flow cytometry) represents binding positive with SARS-CoV-2 Spike on cell membrane. (B) Curves of pseudoviral neutralizing capacity and blocking ELISA of 414-1, 505-3 and 553-63. The mean values and standard deviations of two technical replicates are shown in pseudovial neutralizing assay. (C) Binding competition assay of 414-1, 505-3 and 553-63 using flow cytometry. The blue dots represent A549-Spike treated with mAbs, and the red dots represent A549-Spike with mAbs and 50nM human ACE2. (D) Neutralization of authentic virus assay in VERO-E6 with infecting by SARS-CoV-2-SH01.

Fig.4 Cross-reactivity with SARS-CoV.

(A) Heatmap of representative antibodies cross-reactivity with SARS-CoV and MERS-CoV. Flow cytometry analysis of antibodies binding to S protein of SARS-CoV-2, SARS-CoV and MERS-CoV in 293T cells freshly expressing. Non-transfection 293T were used as controls. Antibodies (50nM) incubated with cells. (B) Flow cytometry analysis of 3 antibodies binding to S protein of SARS-CoV-2, SARS-CoV and MERS-CoV in 293T. (C) Binding ability of cross-reactive with SARS-CoV. Binding ability of antibodies detected by flow cytometry.

Supplementary Figure legends

Tab.S1

Summary of numbers of obtained B cells and antibody clones from 11 patients.

Tab.S2

Summary of characteristics and symptoms of 11 COVID-19 patients.

Fig. S1

Summary of ELISA binding curves of partial SARS-CoV-2 specific monoclonal antibodies.

Fig. S2

ACE2 binding with Spike protein expressed in A549. Flow cytometry analysis of ACE2 binding to Spike protein in A549 stable expression cells. Non-expressing Spike protein A549 were used as controls. ACE2 protein (50nM) labeled-PE incubated with 10 thousand cells for 30min.

Fig. S3

Three representative antibodies binding ability of Spike protein expressed in cell membrane. Antibodies incubated with 10 thousand cells for 30min and detected by flow cytometry analysis. Non-expressing Spike protein A549 were used as controls.

Fig. S4

The binding performance of 414-1 antibody to RBD protein of SARS-CoV-2 in pH7.4 (shown in blue) and pH5.0 (shown in red).

References and notes:

1 Chen, N. *et al.* Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *Lancet* **395**, 507-513, doi:10.1016/S0140-6736(20)30211-7 (2020).

2 Chen, L., Xiong, J., Bao, L. & Shi, Y. Convalescent plasma as a potential therapy for COVID-19. *The Lancet Infectious Diseases* **20**, 398-400, doi:10.1016/s1473-3099(20)30141-9 (2020).

3 Group, P. I. W. *et al.* A Randomized, Controlled Trial of ZMapp for Ebola Virus Infection. *N Engl J Med* **375**, 1448-1456, doi:10.1056/NEJMoa1604330 (2016).

4 Stalin Raj, V. *et al.* Chimeric camel/human heavy-chain antibodies protect against MERS-CoV infection. *Sci Adv* **4**, eaas9667, doi:10.1126/sciadv.aas9667 (2018).

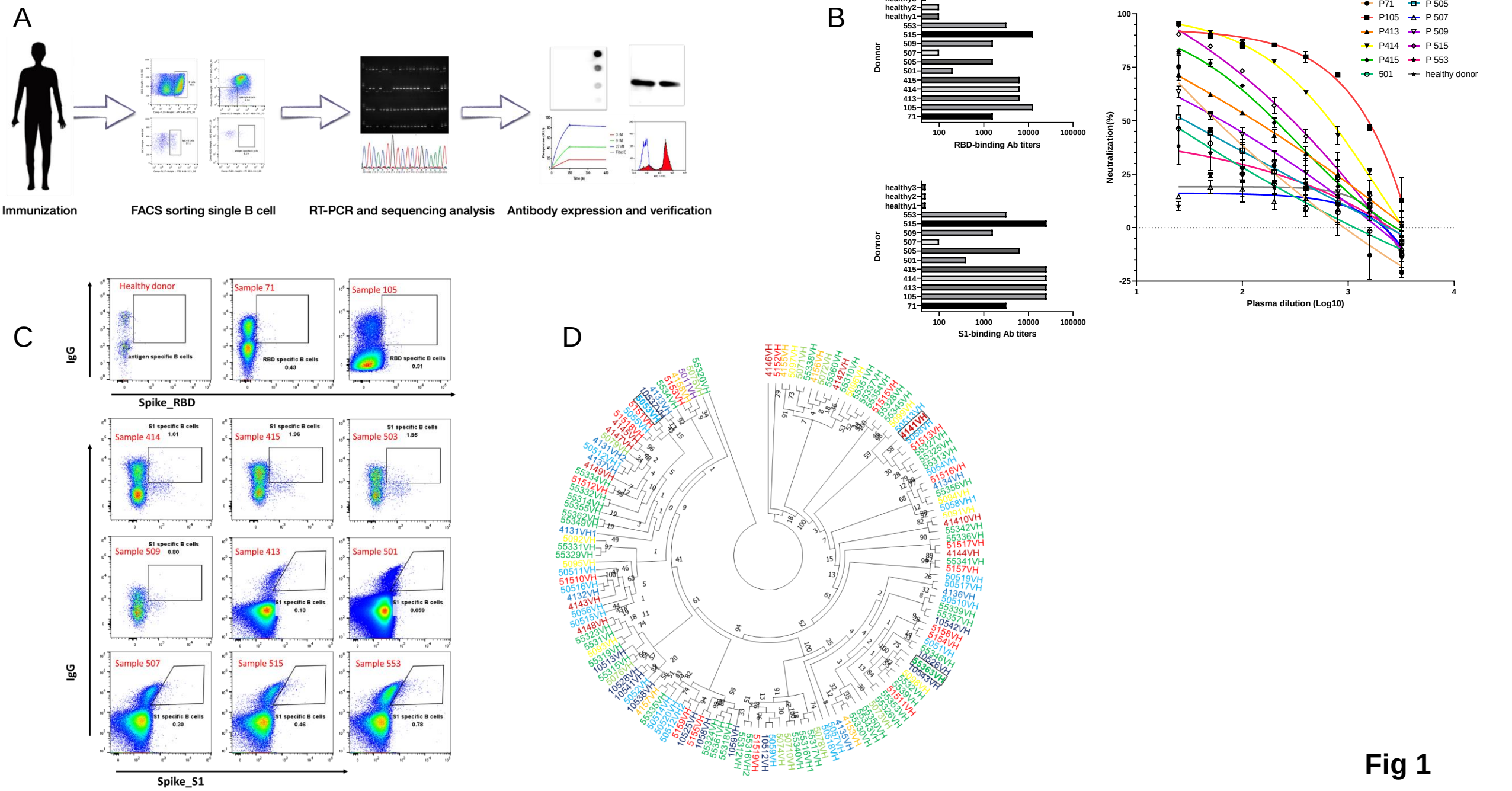
5 Ou, X. *et al.* Characterization of spike glycoprotein of SARS-CoV-2 on virus entry and its immune cross-reactivity with SARS-CoV. *Nat Commun* **11**, 1620, doi:10.1038/s41467-020-15562-9 (2020).

6 Walls, A. C. *et al.* Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein. *Cell* **181**, 281-292 e286, doi:10.1016/j.cell.2020.02.058 (2020).

7 Wu, Y. *et al.* A noncompeting pair of human neutralizing antibodies block COVID-19 virus binding to its receptor ACE2. *Science*, doi:10.1126/science.abc2241 (2020).

8 Chi, X. *et al. A potent neutralizing human antibody reveals the N-terminal domain of the Spike protein of SARS-CoV-2 as a site of vulnerability* (Cold Spring Harbor Laboratory, 2020).

9 Wang, C. *et al.* A human monoclonal antibody blocking SARS-CoV-2 infection. *Nat Commun* **11**, 2251, doi:10.1038/s41467-020-16256-y (2020).
10 Robbiani, D. F. *et al.* Recurrent Potent Human Neutralizing Antibodies to Zika Virus in Brazil and Mexico. *Cell* **169**, 597-609 e511, doi:10.1016/j.cell.2017.04.024 (2017).
11 Qiu, C. *et al.* Safe pseudovirus-based assay for neutralization antibodies against influenza A(H7N9) virus. *Emerg Infect Dis* **19**, 1685-1687, doi:10.3201/eid1910.130728 (2013).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Antibody expression and verification
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healthy1
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415
414
413
105
71
Donnor
RBD-binding Ab titers
S1-binding Ab titers
100
1000
10000
100000
P71
P105
P413
P414
P415
501
P 505
P 507
P 509
P 515
P 553
healthy donor
Neutralization(%)
Plasma dilution (Log10)
C
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Sample 71
RBD specific B cells
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Sample 105
RBD specific B cells
0.31
IgG
Spike_RBD
Sample 414
S1 specific B cells
1.01
Sample 415
1.96
Sample 503
1.95
Sample 509
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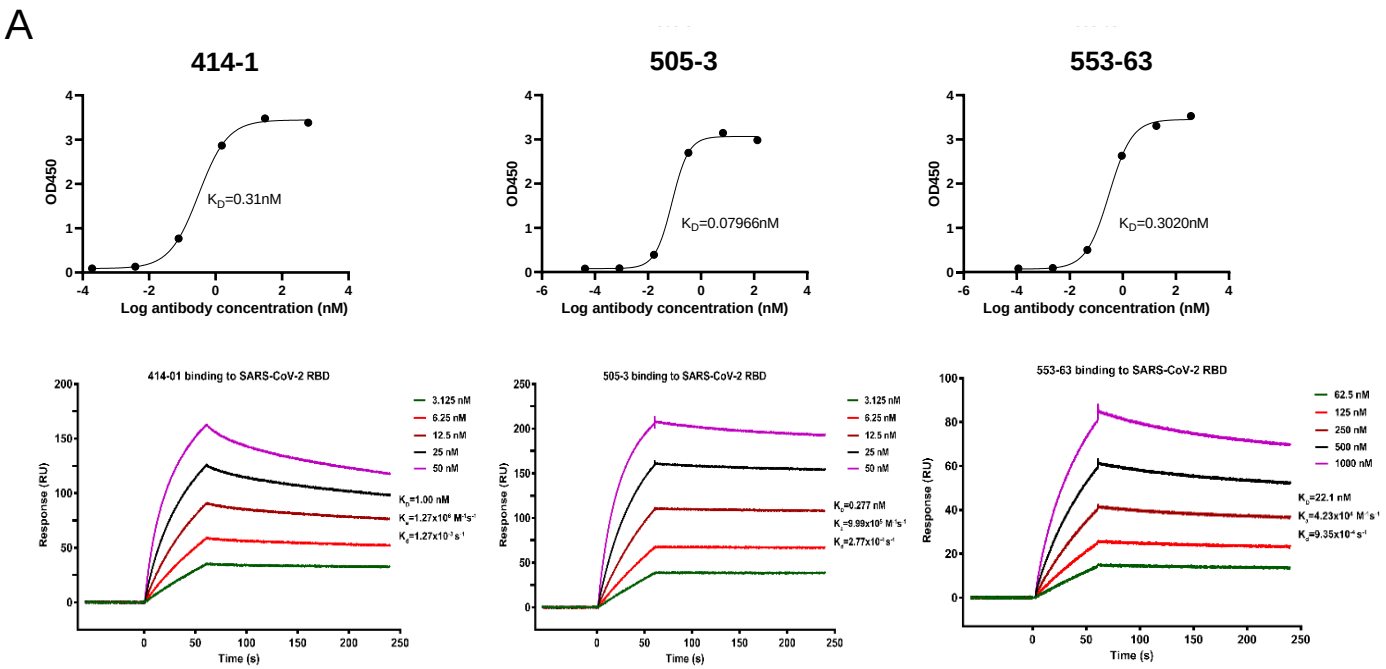
Spike_S1
D

Fig 1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414-1
505-3
553-63
OD450
Log antibody concentration (nM)
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K_D=0.07966nM
K_D=0.3020nM
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505-3 binding to SARS-CoV-2 RBD
553-63 binding to SARS-CoV-2 RBD
Response (RU)
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25 nM
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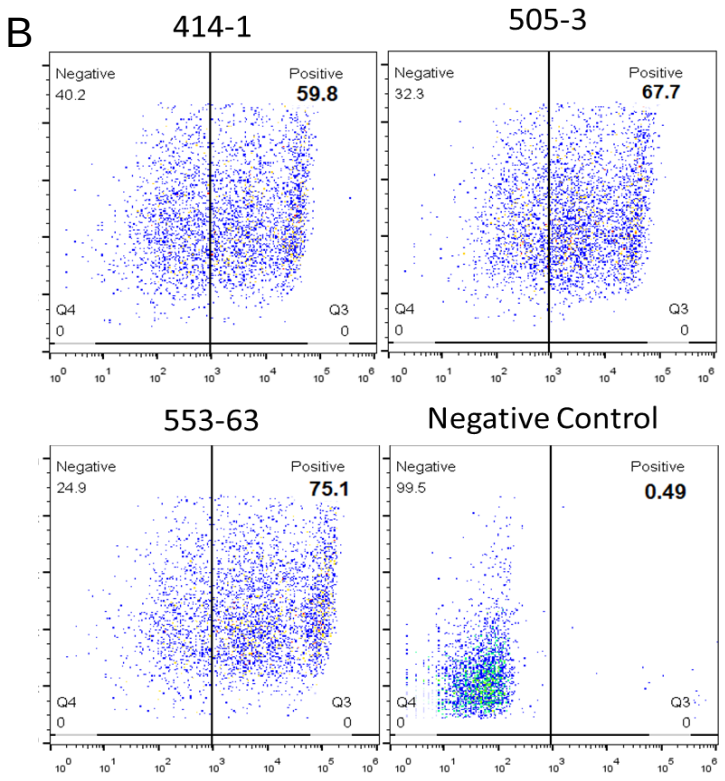
125 nM
250 nM
500 nM
1000 nM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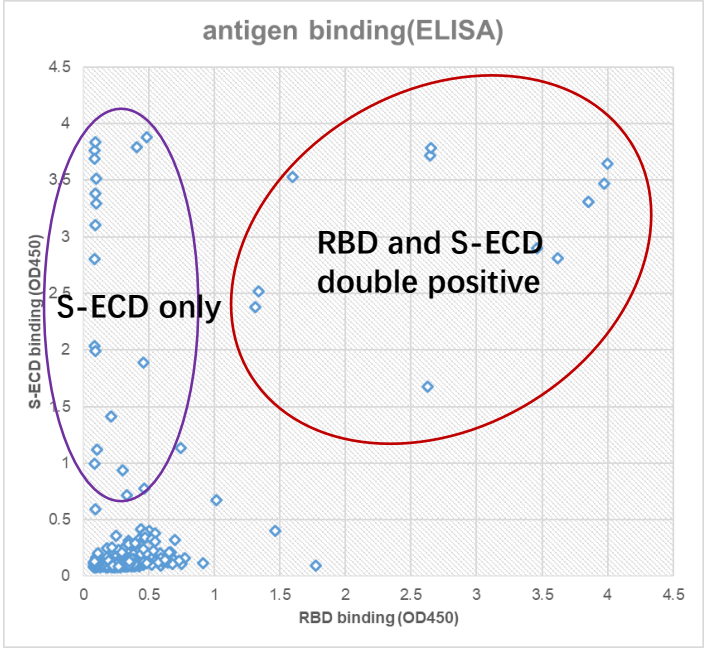
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RBD binding (OD450)
S-ECD only
RBD and S-ECD
double positive

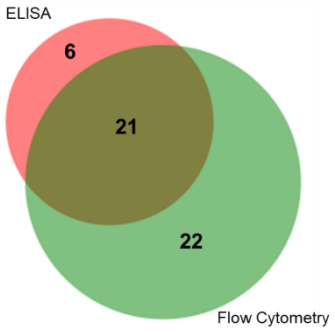

D
ELISA
Flow Cytometry
6
21
22

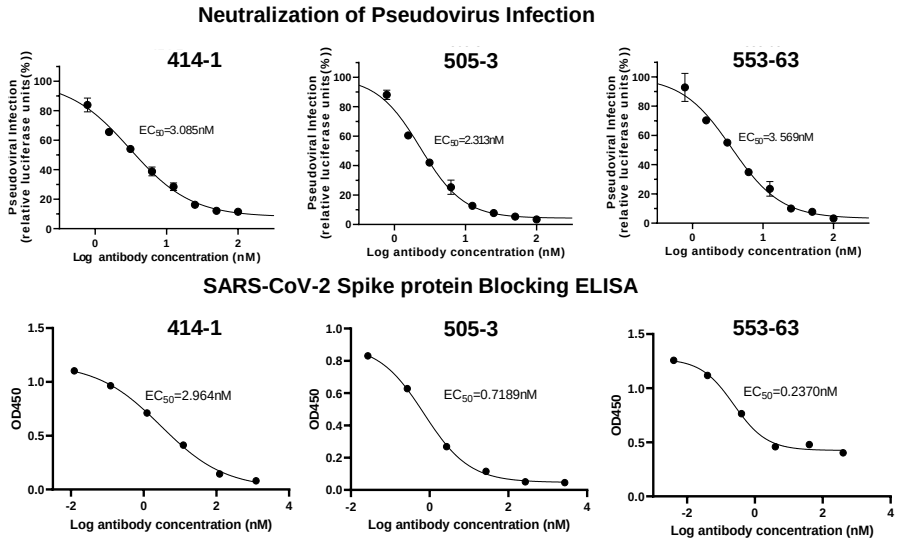
Fig 2

A

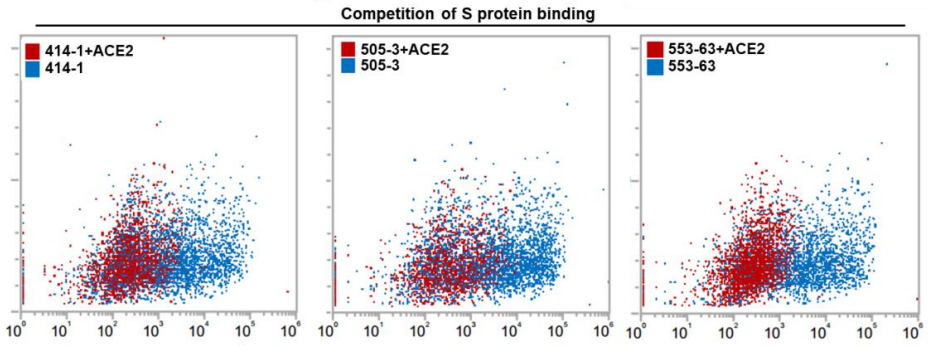
Antibody	Binding ELISA Kd (nM)	Pseudoviral IC50(nM)	Blocking ELISA IC50(nM)	SARS-CoV-2 positivity (%)	50nM ACE2 10nM	50nM ACE2 1nM	50nM ACE2 0.1nM
105-9	0.257	>100	4.663	56.260			
105-25	55.150	non	non	<1			
105-28	4.244	non	non	<1			
105-38	0.099	>100	0.122	33.110			
105-41	4.811	non	non	<1			
105-43	3.042	non	non	34.580			
413-2	0.060	non	non	39.320			
413-3	2.655	non	non	<1			
414-1	0.560	3.085	2.964	58.530			
414-2	0.074	12.721	non	64.160			
414-3-1	64.630	non	non	17.960			
414-3-2	6.650	non	non	30.680			
414-4	3.119	>100	non	<1			
414-5	0.148	non	12.030	<1			
415-5	10.520	>100	non	44.530			
415-6	0.701	non	non	17.580			
505-3	0.080	2.313	0.719	61.640			
505-5	0.059	26.370	0.175	56.630			
505-8	17.620	>100	non	30.440			
505-17	78.630	non	non	29.760			
515-1	0.057	26.110	0.719	53.800			
515-5	119.700	non	non	34.080			
553-13	0.683	non	non	77.000			
553-15	0.089	non	0.325	41.680			
553-17	17.310	non	non	42.770			
553-18	0.853	non	non	61.040			
553-20	0.133	non	non	63.450			
553-27	0.769	non	non	41.630			
553-49	0.076	>100	non	32.561			
553-60	5.753	non	non	36.750			
553-63	0.302	3.569	0.237	73.730			

5 times Competition
50 times Competition
500 times Competition

B



C



D

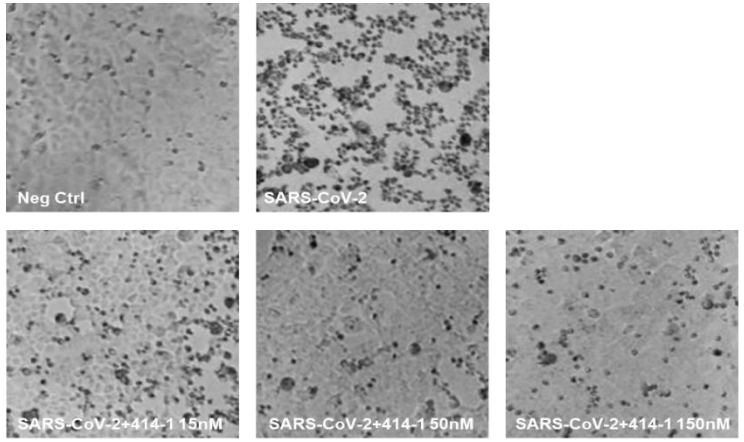


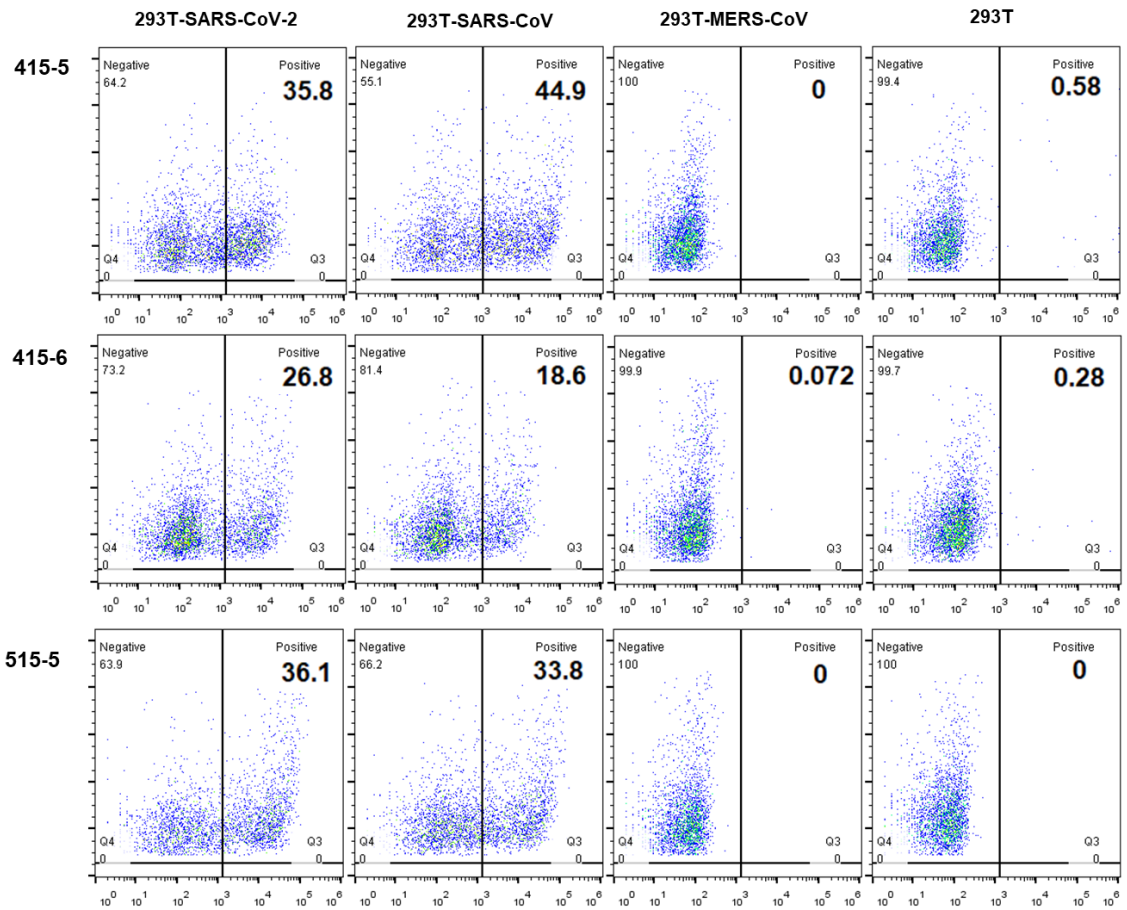
Fig 3

A

Antibody	SARS-CoV2	SARS-CoV	MERS-CoV	Neg Ctrl
105-9	■			
105-25				
105-28				
105-38	■			
105-41				
105-43	■			
413-2	■			
413-3				
414-1	■			
414-2	■			
414-3-1	■			
414-3-2	■			
414-4				
414-5				
415-5	■	■		
415-6	■	■		
505-3	■			
505-5	■			
505-8	■			
505-17	■			
515-1	■			
515-5	■	■		
553-13	■			
553-15	■			
553-17	■			
553-18	■			
553-20	■			
553-27	■			
553-49				
553-60	■			
553-63	■			

■ S protein Binding

B



C

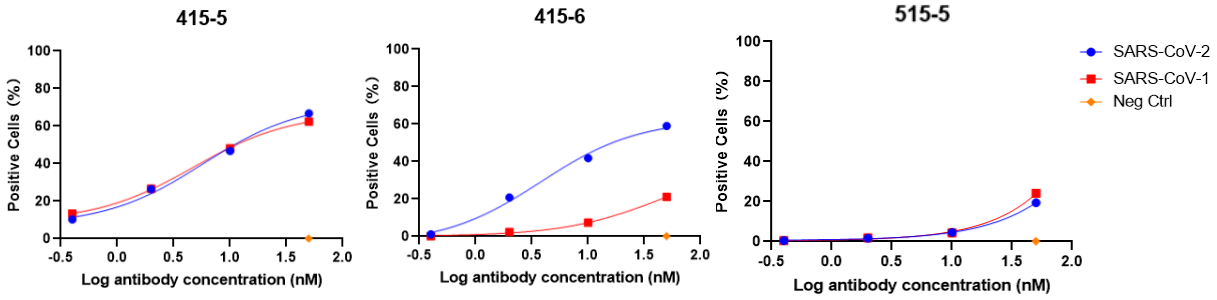


Fig 4

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

Patient characteristics

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	Yes	Yes	Yes	No data	Yes	Yes(with myalgia)
Other infomations	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

Table.S2

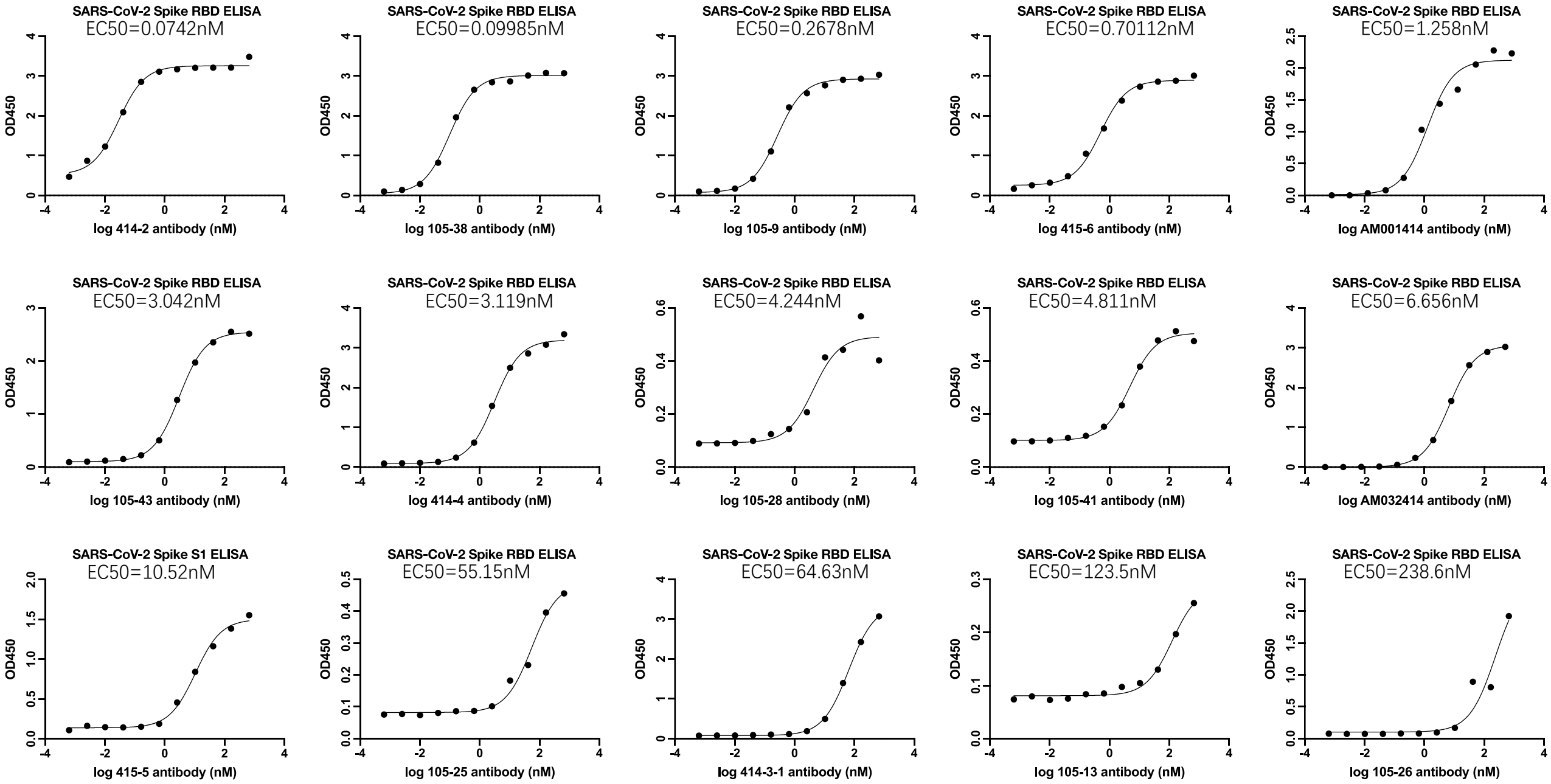

SARS-CoV-2 Spike RBD ELISA
EC50=0.0742nM
OD450
log 414-2 antibody (nM)
SARS-CoV-2 Spike RBD ELISA
EC50=0.09985nM
OD450
log 105-38 antibody (nM)
SARS-CoV-2 Spike RBD ELISA
EC50=0.2678nM
OD450
log 105-9 antibody (nM)
SARS-CoV-2 Spike RBD ELISA
EC50=0.70112nM
OD450
log 415-6 antibody (nM)
SARS-CoV-2 Spike RBD ELISA
EC50=1.258nM
OD450
log AM001414 antibody (nM)
SARS-CoV-2 Spike RBD ELISA
EC50=3.042nM
OD450
log 105-43 antibody (nM)
SARS-CoV-2 Spike RBD ELISA
EC50=3.119nM
OD450
log 414-4 antibody (nM)
SARS-CoV-2 Spike RBD ELISA
EC50=4.244nM
OD450
log 105-28 antibody (nM)
SARS-CoV-2 Spike RBD ELISA
EC50=4.811nM
OD450
log 105-41 antibody (nM)
SARS-CoV-2 Spike RBD ELISA
EC50=6.656nM
OD450
log AM032414 antibody (nM)
SARS-CoV-2 Spike S1 ELISA
EC50=10.52nM
OD450
log 415-5 antibody (nM)
SARS-CoV-2 Spike RBD ELISA
EC50=55.15nM
OD450
log 105-25 antibody (nM)
SARS-CoV-2 Spike RBD ELISA
EC50=64.63nM
OD450
log 414-3-1 antibody (nM)
SARS-CoV-2 Spike RBD ELISA
EC50=123.5nM
OD450
log 105-13 antibody (nM)
SARS-CoV-2 Spike RBD ELISA
EC50=238.6nM
OD450
log 105-26 antibody (nM)

Fig S1

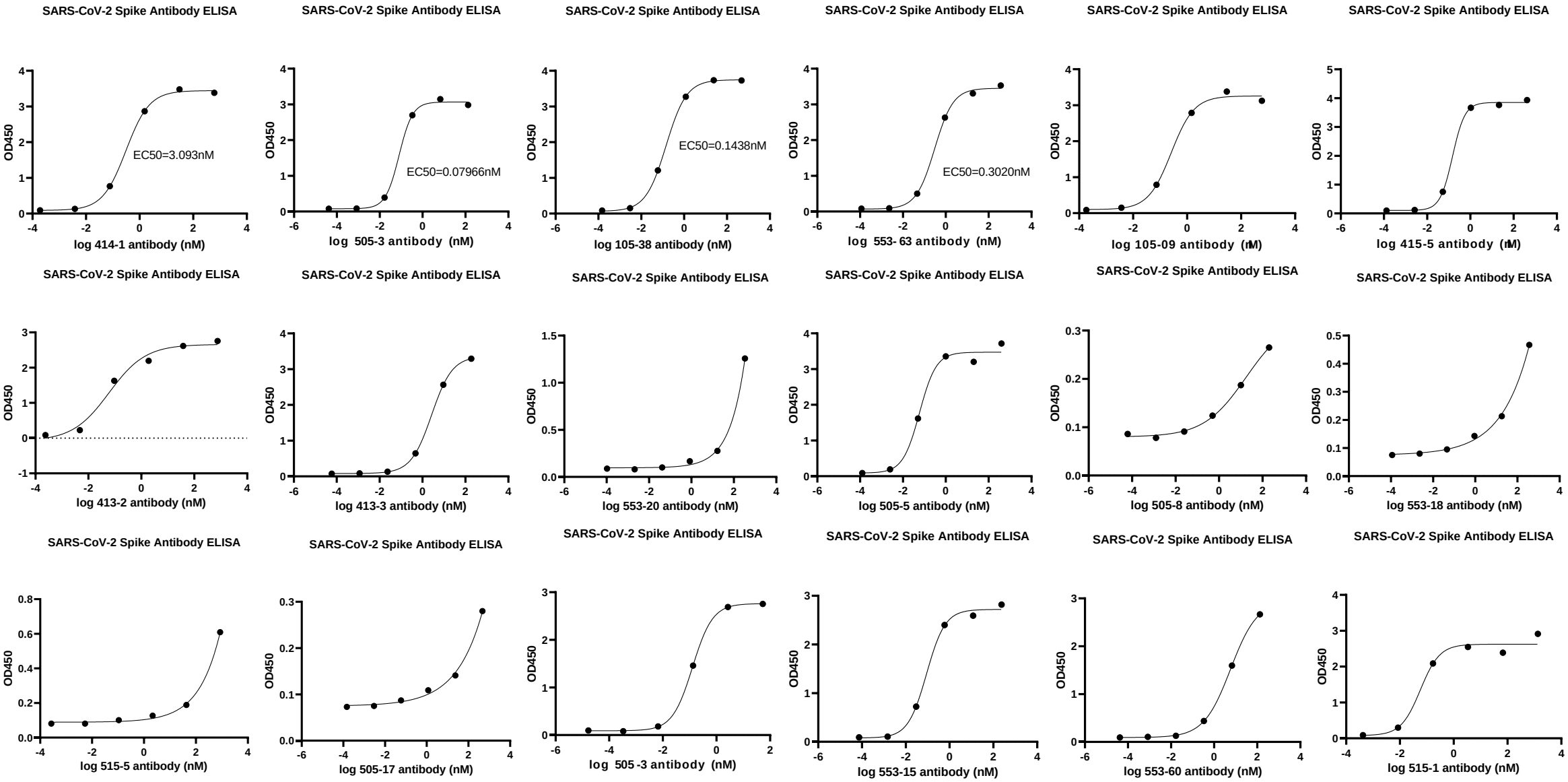
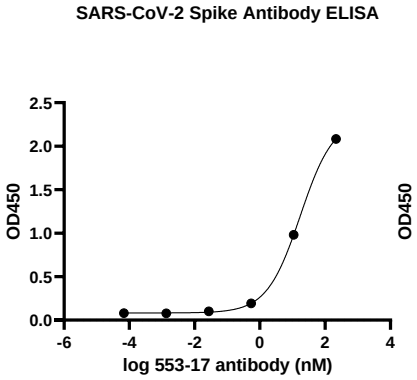
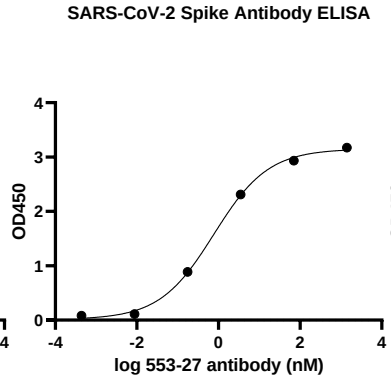
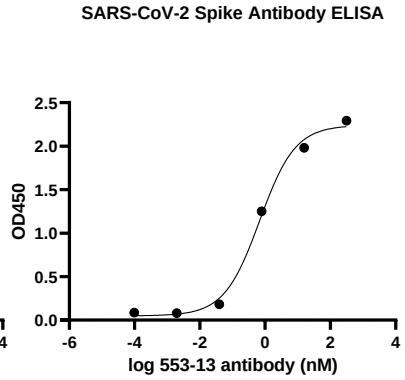
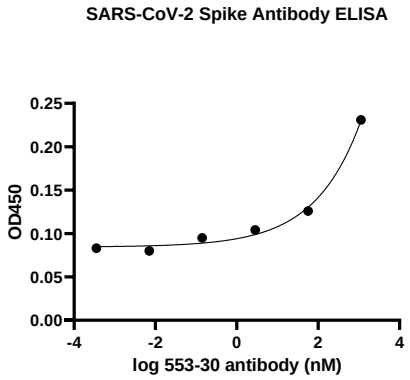

SARS-CoV-2 Spike Antibody ELISA
OD450
EC50=3.093nM
log 414-1 antibody (nM)
EC50=0.07966nM
log 505-3 antibody (nM)
EC50=0.1438nM
log 105-38 antibody (nM)
EC50=0.3020nM
log 553-63 antibody (nM)
log 105-09 antibody (nM)
log 415-5 antibody (nM)
log 413-2 antibody (nM)
log 413-3 antibody (nM)
log 553-20 antibody (nM)
log 505-5 antibody (nM)
log 505-8 antibody (nM)
log 553-18 antibody (nM)
log 515-5 antibody (nM)
log 505-17 antibody (nM)
log 505-3 antibody (nM)
log 553-15 antibody (nM)
log 553-60 antibody (nM)
log 515-1 antibody (nM)

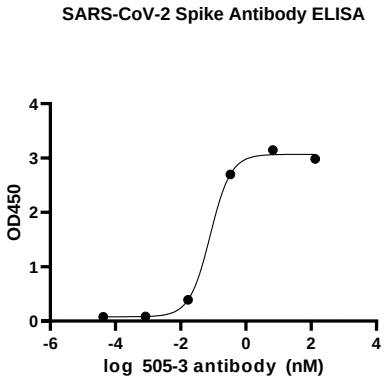
Fig S1

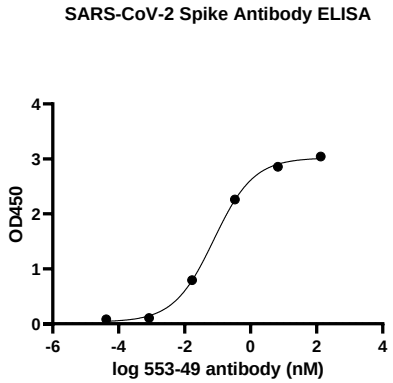

SARS-CoV-2 Spike Antibody ELISA
OD450
log 553-17 antibody (nM)


SARS-CoV-2 Spike Antibody ELISA
OD450
log 553-27 antibody (nM)


SARS-CoV-2 Spike Antibody ELISA
OD450
log 553-13 antibody (nM)


SARS-CoV-2 Spike Antibody ELISA
OD450
log 553-30 antibody (nM)


SARS-CoV-2 Spike Antibody ELISA
OD450
log 505-3 antibody (nM)


SARS-CoV-2 Spike Antibody ELISA
OD450
log 553-49 antibody (nM)

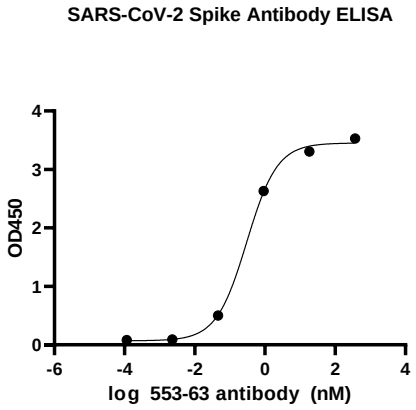

SARS-CoV-2 Spike Antibody ELISA
OD450
log 553-63 antibody (nM)

Fig S1

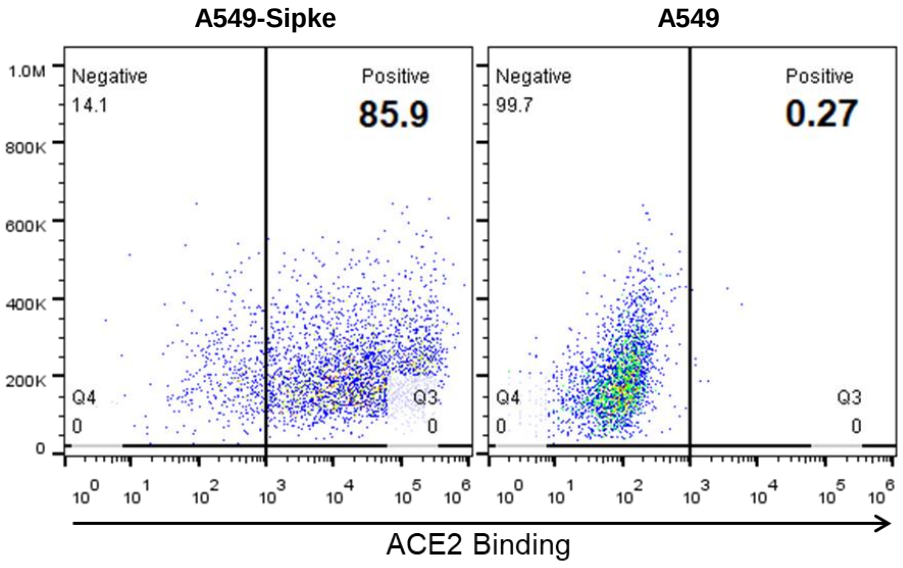

A549-Sipke
A549
Negative
14.1
Positive
85.9
Negative
99.7
Positive
0.27
Q4
0
Q3
0
Q4
0
Q3
0
1.0M
800K
600K
400K
200K
0
ACE2 Binding

Fig S2

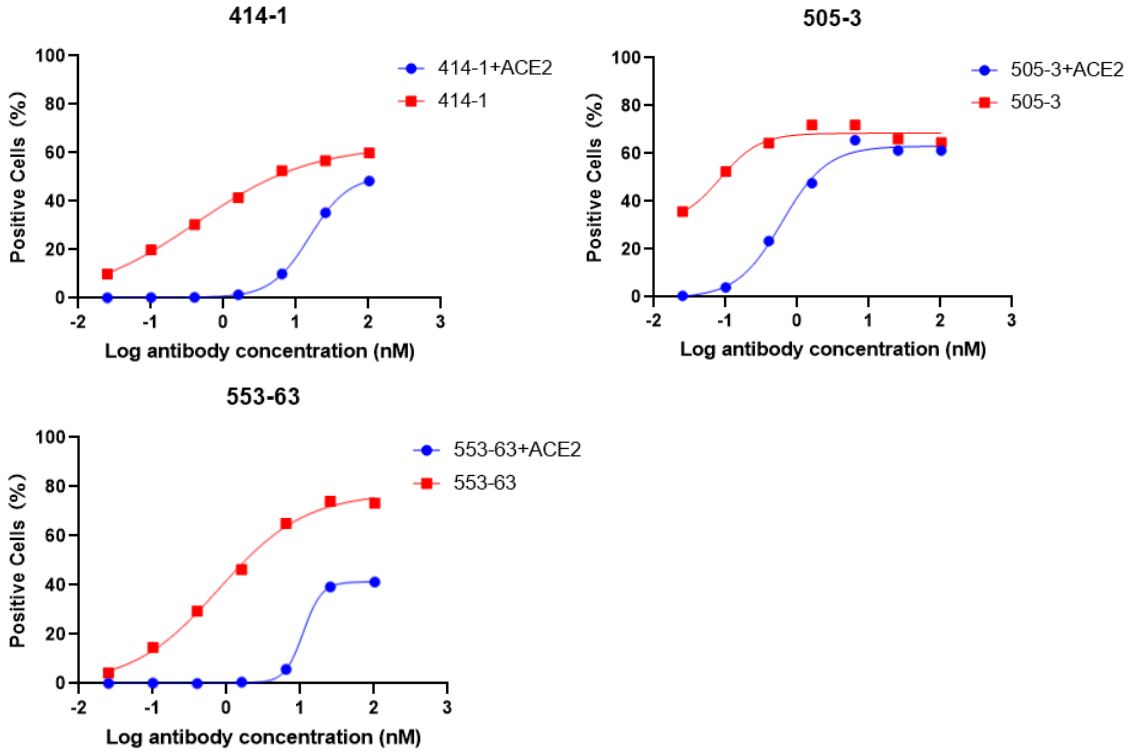

414-1
414-1+ACE2
414-1
Positive Cells (%)
Log antibody concentration (nM)
505-3
505-3+ACE2
505-3
Positive Cells (%)
Log antibody concentration (nM)
553-63
553-63+ACE2
553-63
Positive Cells (%)
Log antibody concentration (nM)

Fig S3

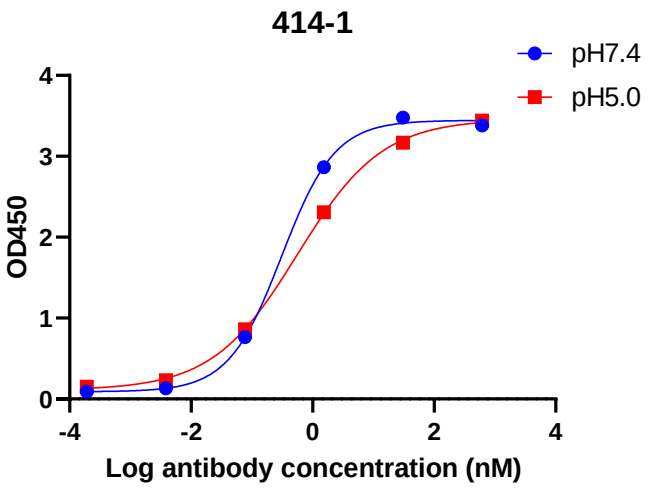

414-1
pH7.4
pH5.0
OD450
4
3
2
1
0
-4
-2
0
2
4
Log antibody concentration (nM)

Fig S4