

A potent neutralizing human antibody reveals the N-terminal domain of the Spike protein of SARS-CoV-2 as a site of vulnerability

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Abstract

The pandemic of coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) presents a global public health threat. Most research on therapeutics against SARS-CoV-2 focused on the receptor binding domain (RBD) of the Spike (S) protein, whereas the vulnerable epitopes and functional mechanism of non-RBD regions are poorly understood. Here we isolated and characterized monoclonal antibodies (mAbs) derived from convalescent COVID-19 patients. An mAb targeting the N-terminal domain (NTD) of the SARS-CoV-2 S protein, named 4A8, exhibits high neutralization potency against both authentic and pseudotyped SARS-CoV-2, although it does not block the interaction between angiotensin-converting enzyme 2 (ACE2) receptor and S protein. The cryo-EM structure of the SARS-CoV-2 S protein in complex with 4A8 has been determined to an overall resolution of 3.1 Angstrom and local resolution of 3.4 Angstrom for the 4A8-NTD interface, revealing detailed interactions between the NTD and 4A8. Our functional and structural characterizations discover a new vulnerable epitope of the S protein and identify promising neutralizing mAbs as potential clinical therapy for COVID-19.

The global outbreak of COVID-19 has emerged as a severe threat to human health (1-4) , claiming over two hundred and sixty thousand lives as 7 May, 2020 (5). COVID-19 is caused by a novel coronavirus, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which is an enveloped, positive-strand RNA virus that causes upper respiratory diseases, fever and severe pneumonia in humans (2, 3, 6).

SARS-CoV-2 is a new member of the β coronavirus genus, which also contains SARS-CoV and MERS-CoV that caused epidemic in 2002 and 2012, respectively (7, 8). SARS-CoV-2 shares about 80% sequence identity to SARS-CoV, implying similar infection mechanism (2). Indeed, SARS-CoV-2, same as SARS-CoV, hijacks angiotensin-converting enzyme 2 (ACE2) as cellular receptor (9-19).

The trimeric S protein covers the surface of coronavirus and plays a pivotal role during viral entry (20, 21). The S protein is cleaved into the N-terminal S1 subunit and C-terminal S2 subunit by host proteases such as TMPRSS2 (21, 22) and undergoes conformational change from prefusion to postfusion state during infection (23). S1 and S2 mediate receptor binding and membrane fusion, respectively (18). S1, consisting of the N-terminal domain (NTD) and the receptor binding domain (RBD), is critical in determining tissue tropism and host ranges (24, 25). The RBD is responsible for binding to ACE2, while the function of NTD is not well understood. In some coronaviruses, the NTD may recognize specific sugar moieties upon initial attachment and might play an important role in the prefusion to postfusion transition of the S protein (26-29). The NTD of the MERS-CoV S protein can serve as a critical epitope for antibody neutralizing (29).

The SARS-CoV-2 S protein-targeting monoclonal antibodies (mAbs) with potent neutralizing activity have become the focus of therapeutic interventions for COVID-19 (30-32). Most SARS-CoV-2 antibodies reported previously were sorted to target the RBD in order to inhibit the association between the S protein and ACE2 (31-34). The RBD-targeting antibodies, when applied individually, may induce viral escaping mutations, thus fostering evolution of new viral strains that are insensitive to these antibodies (29). Antibodies targeting non-RBD regions may serve as “cocktail” therapeutics for SARS-CoV-2.

Here we report the isolation and characterization of S protein-specific monoclonal antibodies derived from memory B or plasma B cells of COVID-19 survivors. We also determined the structure of the complex between the SARS-CoV-2 S protein and 4A8, one of the isolated mAbs, at an overall resolution of 3.1 Å and local resolution of 3.4 Å for the interfaces between 4A8 and the S protein. 4A8 targets the S-NTD with potent neutralizing activity. These findings reveal a new epitope of the SARS-CoV-2 S protein for antibody neutralizing, which may lay the foundation for new prophylactic and therapeutic interventions for SARS-CoV-2.

Results

Isolation of human mAbs from memory B cells and plasma B cells

To isolate monoclonal antibodies and analyze the humoral antibody responses to SARS-CoV-2, we collected plasma and peripheral blood mononuclear cells (PBMCs) from 10 patients recovered from SARS-CoV-2 infection. The age of donors ranges from 25 to 53 years. The interval from blood collection date to disease confirmation date ranged from 23 to 29 days for

No. 1-5 patients and 10 to 15 days for No. 6-10 patients (Table S1). We evaluated the titers of binding antibodies in plasma to different fragments of the SARS-CoV-2 S protein, including the extracellular domain (S-ECD), S1, S2, and RBD, and the Nucleocapsid (N) Protein. Plasma from all the patients except donor No. 2 bound to all 5 SARS-CoV-2 protein segments, while that from donor No. 2 recognized S-ECD and S2 only (Fig. 1A). The neutralizing capacities of plasma against live SARS-CoV-2 and HIV-vectored pseudotyped SARS-CoV-2 are correlated ($r=0.6868$, $p<0.05$) (Fig. 1B). These results indicate that humoral immune responses were specifically elicited for all of the 10 patients during the natural infection of SARS-CoV-2.

To isolate S protein-specific monoclonal antibodies, we first sorted the IgG⁺ memory B cells from peripheral blood mononuclear cells (PBMCs) of the No. 1-5 convalescent patients with flow cytometry using S-ECD as probe (Fig. 1C). The percentage of S-ECD-reactive IgG⁺ B cells ranges from 0.56% to 11% as revealed by fluorescence activating cell sorter (FACS). To avoid bias introduced by S-ECD, we sorted plasma B cells from mixed PBMCs derived from another five convalescent patients (No. 6-10) without any antigen-specific probes. The percentage of plasma B cells in CD3-CD19⁺ B cells was 12.8%, higher than that of memory B cells (Fig. 1C).

From the sorted B cells, we identified 9, 286, 43, 12 and 26 clones of single B cell from patients No. 1 to 5, respectively, and 23 clones of single B cell from the mixed PBMCs of patients No. 6 to 10 (Fig. 1D). The distribution of the sequenced heavy (IgH) gene families was comparable among the 10 donors, with VH3 being the most commonly used VH gene, while different donors displayed variable preferences for the light chain (IgL) gene families (Fig. 1D). The combination of V3 and J4, V3 and D3, and D3 and J4 were the most common usage for the IgH gene family

(Fig. S1). The plasma B cells have less mutations than memory B cells in both heavy variable chains (VH) and light variable chains (VL) (Fig. 1E), suggesting lower levels of somatic hypermutation (SHM) in plasma B cells than memory B cells after SARS-CoV-2 infection. The lengths of complementarity-determining region (CDR) 3 for antibodies were similar among the donors, ranging from 13.9 to 17.7 for VH and 9.3 to 10.1 for VL (Fig. 1F).

Binding profiles of SARS-CoV-2 S protein-specific human mAbs

To screen S protein-specific antibodies, we determined the binding specificity using ELISA for the 399 human mAbs sorted above. 1, 16, 1, 3, and 9 S-ECD-specific mAbs were identified from donors No. 1-5, respectively. A total of 5 mAbs were identified from donors No. 6 -10 (Fig. 2A). The sequence identities of CDRH3 in the 35 S-ECD-specific mAb ranged from 40.9% to 97.6% (Fig. S2 and Table S2). We further characterized domain specificities of the 35 mAbs with different fragments of the S protein, including S1, S2 and RBD (Fig. 2A). The S-reactive mAbs are classified into 4 major groups based on their EC_{50} values (Fig. 2A). Group 1 recognizes only S-ECD. Group 2 recognizes S-ECD and S1, with subgroup 2A binding S-ECD and S1 and subgroup 2B binding S-ECD, S1, and RBD. Group 3 interacts with both S1 and S2, with subgroup 3A targeting RBD and subgroup 3B fails to bind RBD. Group 4 recognizes S-ECD and S2. Remarkably, only 4 mAbs recognize RBD among the 35 S-specific mAbs (Figure 2A and 2B).

A competition-binding assay using ELISA were performed for several representative mAbs to determine if there are any overlapping antigenic sites within distinct binding groups or between different mAbs, with CR3022 being used as a control that cross-reacts with the RBD of both SARS-CoV and SARS-CoV-2 (35) (Fig. 2C). Among these mAbs, 4A8 competed with 1D2.

Another RBD-reactive mAb, 2M-10B11, competed with CR3022, suggesting overlapped epitopes on RBD for these two mAbs. These results indicate that the S-specific antibodies elicited by SARS-CoV-2 infection target at least four antigenic regions on the S protein of SARS-CoV-2.

To characterize the diversity in gene usage and affinity maturation, the phylogenetic trees of these S-specific mAbs were analyzed based on the amino acid sequences of VHDJH and VLJL using a neighbor-joining method in MEGA7 Software. Results indicate that the VH gene usage is very diverse among the 35 mAbs from 10 donors, with VH 3-30 being the most frequently used germline gene. Moreover, there was no particularly favored VH gene identified among S1, S2, or RBD-reactive mAbs (Fig. 2D).

Neutralizing activities of SARS-CoV-2 S-specific human mAbs

We first performed *in vitro* neutralization studies of the 35 S-specific mAbs using live SARS-CoV-2 virus in Vero-E6 cells (Fig. 3A). 1M-1D2 and 4A8 exhibited medium to high neutralizing capacity with EC_{50} of 23.99 and 0.607 μ g/ml, respectively, while the neutralizing curves of 0304-3H3 cannot be fit well. As expected, the RBD-targeting control mAb, CR3022, failed to neutralize live SARS-CoV-2 (35). Moreover, while the CR3022-competing mAb, 10B11, bound

to the SARS-CoV-2 RBD with EC_{50} of 5 ng/ml, it also failed to neutralize live SARS-CoV-2.

These results suggest that binding affinities of mAbs against RBD did not correlate fully with the neutralizing abilities of mAbs. To further investigate the inhibitory activity of 4A8, 0304-3H3, and 1M-1D2 to live virus, we tested the RNA load of live viruses in Vero-E6 cells treated with each mAb using real time qPCR (Fig. 3B). Consistent with the cytopathic effect (CPE) assay results (Fig. 3A), 4A8 displayed higher inhibitory capacities than 1M-1D2.

We next performed luciferase reporter gene assays for all 35 S-binding mAbs using HIV-vectored pseudotyped SARS-CoV-2, among which 3 mAbs exhibited neutralizing activity against the pseudotyped virus (Fig. 3C). 4A8 protected ACE2-293T cells with EC_{50} of 52.03 μ g/ml, while neutralization of 0304-3H3 and 1M-1D2 were not observed. 10B11 and 9A1 showed a minor level of inhibition. These findings suggest that the results of pseudotyped SARS-CoV-2 were not entirely consistent with that of live SARS-CoV-2. This difference is likely explained by the insusceptibility of pseudotyped SARS-CoV-2 to some mAbs with specific neutralizing mechanisms. 4A8 is likely to be a potential candidate for the treatment of SARS-CoV-2, since 4A8 displayed high levels neutralizing capacities against both authentic and pseudotyped SARS-CoV-2.

Binding characterization of candidate mAbs

To determine the possible neutralizing mechanism of the mAbs, we first determined the binding affinities of mAbs to different segments of the S protein, including S-ECD, S1, S2, and RBD using bio-layer interferometry. All 5 tested mAbs bound to S-ECD with high affinity, with the

equilibrium dissociation constant (K_D) less than 2.14 nM (Fig. 4A). 4A8, 1M-1D2, and 10B11 bound to S1 with similar K_D ranging from 92.7 to 159 nM, whereas 0304-3H3 and 9A1 targeted S2. Additionally, 10B11 were shown to bind RBD with K_D of 26.8 nM.

To further investigate whether these mAbs block the binding of S protein to ACE2, we performed flow cytometry using HEK 293T cells expressing human ACE2. As expected, only 10B11 among the 5 mAbs prevented S protein from binding to ACE2, with percentage of 0.52% for IgG and S protein double-positive cell (Fig 4B). However, CR3022, which competes with 10B11, did not interfere with the binding of S to ACE2 with double positive cell percentage of 31.7%. 4A8 also failed to interfere with binding of the S protein to ACE2.

Cryo-EM structure of the complex between 4A8 and S-ECD

The mAb 4A8 was overexpressed and purified by Protein A resin from the B memory cells and S-ECD of SARS-CoV-2 was purified through M2 affinity resin and size exclusion chromatography. 4A8 and S-ECD protein were mixed and incubated at a stoichiometric ratio of ~ 1.2 to 1 for 1 hour and applied to size exclusion chromatography to remove excess proteins. The fraction containing the complex was concentrated for cryo-EM sample preparation.

To investigate the interactions between 4A8 and the S protein, we solved the cryo-EM structure of the complex at an overall resolution of 3.1 Å (Fig. 5, Movie S1). Details of cryo-EM sample preparation, data collection and processing, and model building can be found in Materials and Methods and supplementary materials (Figs. S3-S5). The S protein exhibits asymmetric

conformations similar to the previously reported structures (24, 25), with one RBD “up” and the other two “down” (Fig. 5, fig. S3I).

Recognition of the NTD by 4A8

Three 4A8 molecules bind to one trimeric S protein, each interacting with one NTD domain. Despite the different conformations of the three S protein protomers, the interface between 4A8 and each NTD is identical (Fig. 5). The map quality at the NTD-4A8 region was improved by focused refinement with a local resolution of 3.4 Å, enabling reliable analysis of the interactions between the NTD and the 4A8.

Association with 4A8 appears to stabilize the NTD epitope, which is invisible in the reported S protein structure alone (24, 25). Supported by the high resolution of NTD, we were able to build the structural model for three new loops for NTD, designated N1 (residues 67-79), N2 (residues 141-156), and N3 (residues 246-260), among which N2 and N3 loops mediate the interaction with 4A8.

Only the heavy chain of 4A8 participates in binding to the NTD mainly through three loops, named L1, L2 and L3 (Fig. 6A). The interface is constituted by extensive hydrophilic interaction network. R246 on the N3 loop of the NTD represents one docking site, simultaneously interacting with Glu1, Tyr27 and Glu31 of 4A8 (Fig. 6B). On the N2 loop of the NTD, Lys150 and Lys147 respectively form salt bridge with Glu54 and Glu72 of 4A8 (Fig. 6C). Lys150 is also hydrogen bonded (H-bond) with 4A8-Tyr111, while His146 forms a H-bond with 4A8-Thr30 (Fig. 6C). In addition to the hydrophilic interactions, Trp152 and Tyr145 on the N2 loop of the

NTD also interact with Val102, Pro106, and Phe109 on the L3 loop of the mAb through hydrophobic and/or π - π interactions (Fig. 6D).

Discussion

There is an urgent need for prophylactic and therapeutic interventions for SARS-CoV-2 infections given the ongoing COVID-19 pandemic. No fully human neutralizing Abs have been reported, and little is known about the structural determinants of neutralization on which to base the rational selection of antibodies. Our work reveals that naturally occurring human SARS-CoV-2 mAbs isolated from the B cells of 10 recovered donors are diverse in gene usage and epitope recognition of S protein. Remarkably, the majority of the isolated mAbs did not recognize the RBD, and all the mAbs that neutralize live SARS-CoV-2 failed to inhibit the binding of S protein to ACE2. These unexpected results suggest the presence of other important mechanisms for SARS-CoV-2 neutralization in addition to suppressing the viral interaction with the receptor.

The S1-targeting mAb 4A8 does not block the interaction between ACE2 and S protein, but exhibits high levels of neutralization against both authentic and pseudotyped SARS-CoV-2 *in vitro*. Many neutralizing antibodies against the SARS-CoV-2 were reported to target the RBD of the S protein and block the binding between RBD and ACE2. Our results show that 4A8 bind to the NTD of S protein with potent neutralizing activity. Previous study showed that mAb 7D10 could bind to the NTD of S protein of MERS-CoV probably by inhibiting the RBD-ACE2 binding and the prefusion to postfusion conformational change of S protein (29). We aligned the crystal structure of 7D10 in complex with the NTD of S protein of MERS-CoV with our

complex structure and found that the interfaces between the mAb and the NTDs are partially overlapped (Fig. S6). 7D10 may inhibit the interaction between MERS-CoV and ACE2 through its light chain that is close to the RBD. In our complex, the light chain of 4A8 is away from the RBD (Fig. S6). Therefore, we speculate that 4A8 may neutralize SARS-CoV-2 by restraining the conformational changes of the S protein. Furthermore, sequences alignment of the S proteins from SARS-CoV-2, SARS-CoV, and MERS-CoV revealed varied NTD surface sequences that are respectively recognized by different mAbs (Fig. S7).

Overall, this work reports a fully human neutralizing mAb recognizing a vulnerable epitope of NTD on S protein of SARS-CoV-2, functioning with a mechanism that is independent of receptor binding inhibition. Combination of 4A8 with RBD-targeting antibodies may avoid the escaping mutations of virus and serve as promising “cocktail” therapeutics. The information obtained from these studies can be used for development of the structure-based vaccine design against SARS-CoV-2.

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the experiments. All authors contributed to data analysis. X.C., R.Y., J.L., Q.Z. and W.C. wrote
the manuscript.

Competing interests: Authors declare no competing interests.

Data and materials availability: Atomic coordinates and cryo EM density maps of the S protein of SARS-CoV-2 in complex bound with 4A8 (PDB: 7C2L; whole map: EMD-30276, antibody-epitope interface-focused refined map: EMD-30277) have been deposited to the Protein Data Bank (<http://www.rcsb.org>) and the Electron Microscopy Data Bank (<https://www.ebi.ac.uk/pdbe/emdb/>), respectively.

Supplementary Materials:

Materials and Methods

Figures S1-S7

Tables S1-S3

Movie S1

Figure legends

Fig. 1. Isolation of antigen-specific monoclonal antibodies from convalescent patients of SARS-CoV-2. (A) Reactions of plasma to SARS-CoV-2 proteins. S-ECD (extracellular domain of S protein), S1, S2, RBD (receptor binding domain) and N (nucleotide protein) were used in ELISA to test the binding of plasma. Plasma of healthy donors were used as control, and cut-off values were calculated as O.D. 450 of control $\times$ 2.1. **(B)** The correlations between the authentic SARS-CoV-2 neutralizing antibody titers and the pseudotyped SARS-CoV-2 neutralizing antibody titers in plasma. Neutralizing assays of plasma against authentic SARS-CoV-2 were

performed using Vero E6 cells, and neutralization against pseudotyped SARS-CoV-2 were determined using ACE2-293T cells. The correlations were calculated by Pearson correlation test in Graphpad 7.0. **(C)** Flow cytometry sorting from PBMCs of 10 convalescent patients. **(D)** Distribution of V gene families in heavy and light chains of all unique clones (the total number is shown in the center of the pie charts) for each donor. **(E)** The number of mutations from the germline of all clonal sequences identified in **(D)** was shown. **(F)** CDR3 amino acid lengths of VH and VL of all clonal sequences identified in **(D)**.

Fig. 2. Binding profiles of Spike protein-specific mAbs. **(A)** Heatmap showing the binding of mAbs to different types of spike proteins determined using ELISA. The EC₅₀ value for each S-mAb combination is shown, with dark red, orange, yellow, or white shading indicating high, intermediate, low, or no detectable binding, respectively. EC₅₀ values greater than 10,000 ng/ml are indicated (>). **(B)** Binding curves of representative mAbs. CR3022 is a control that was reported to bind SARS-CoV and SARS-CoV-2 RBD. **(C)** Heatmap showing the competing binding of some representative S-reactive mAbs assayed in ELISA. Numbers in the box indicate the percentage binding of detecting mAb in the presence of the blocking antibody compared to the binding of detecting mAb in the absence of the blocking antibody. The mAbs were considered competing if the inhibiting percentage is <30% (black boxes with white numbers). The mAbs were judged to non-compete for the same site if the percentage is >70% (white boxes with red numbers). Gray boxes with black numbers indicate an intermediate phenotype (30%~70%). **(D)** Phylogenetic trees of all the S-specific mAbs.

Fig. 3. Neutralizing capacities of S-reactive mAbs. **(A)** Neutralization of S-reactive mAbs to authentic SARS-CoV-2 in Vero-E6 cells. **(B)** The authentic SARS-CoV-2 virus RNA load was determined in Vero-E6 cells treated with S-reactive mAbs using qPCR. Percent infection was

calculated as the ratio of RNA load in mAb-treated wells to that in wells containing virus only.

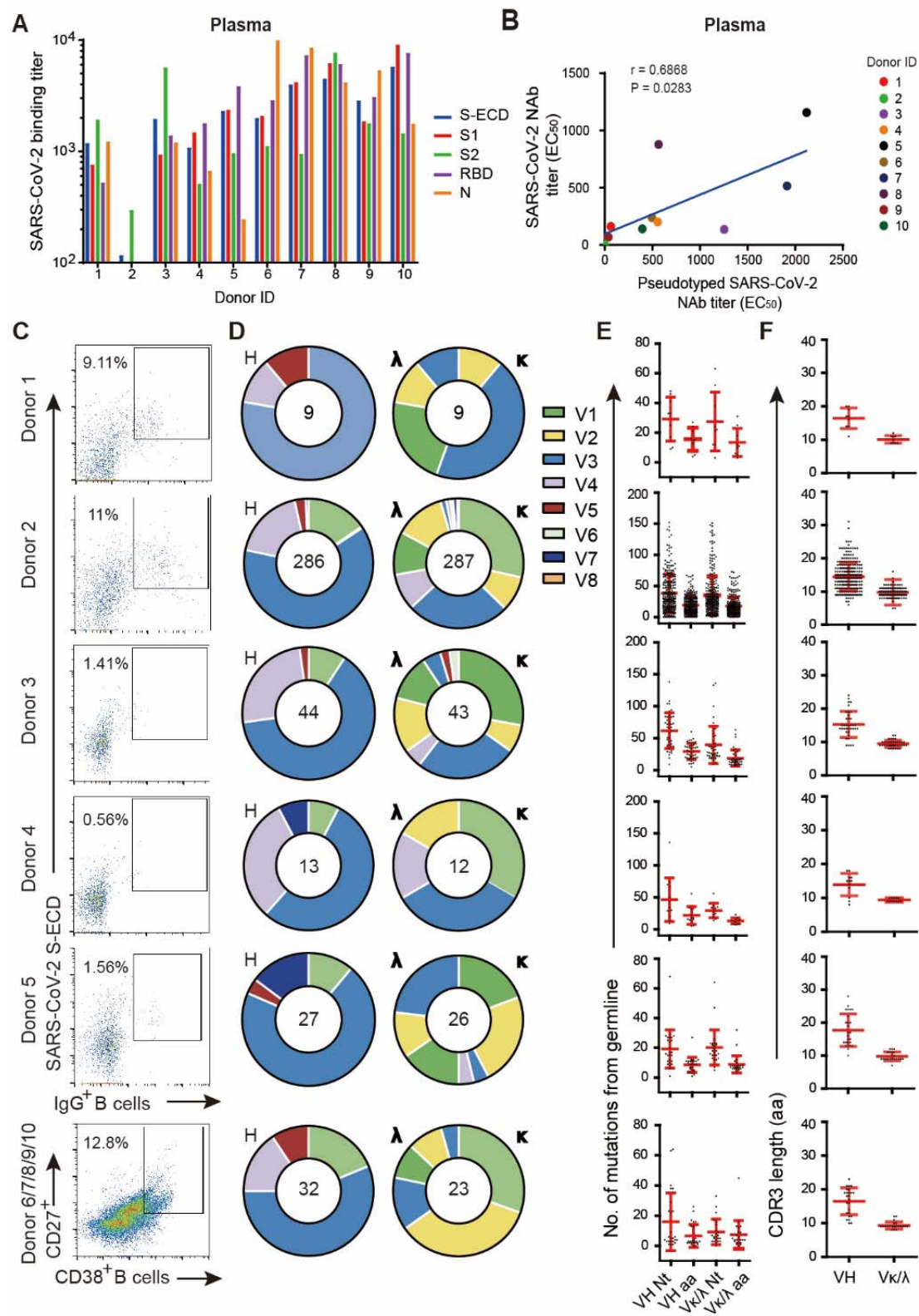
(C) Neutralization of S-reactive mAbs against HIV-vectored pseudotyped SARS-CoV-2 in ACE2-293T cells. data were shown as mean $\pm$ SD of a representative experiments.

Fig. 4. 4A8 did not block the binding of Spike protein to ACE2 receptor. **(A)** BLI sensorgrams and kinetics of mAbs binding to S proteins. **(B)** The binding of S protein to human ACE2 overexpressing 293T cells were determined by flow cytometry. Following the preincubation of S protein with each indicated mAb, the mAb-S mixtures were added to the ACE2-expressing cells. cells were stained with anti-human IgG FITC (mAb binding, x-axis) and anti-His (S binding, y-axis). Percentages of double positive cells were shown. Control mAb CR3022 and 1A8 were previously reported to bind SARS-CoV RBD and Marburg glycoprotein, respectively, and ACE2-Fc protein was a human ACE2 protein conjugated with human Fc.

Fig. 5. Cryo-EM structure of the 4A8 and S-ECD complex. The domain-colored cryo-EM map of the complex is shown on the right, and two perpendicular views of the overall structure are shown on the right. The heavy and light chains of 4A8 are colored blue and magenta, respectively. The NTDs of the trimeric S protein are colored orange. The one “up” RBD and two “down” RBDs of trimeric S protein are colored green and cyan, respectively.

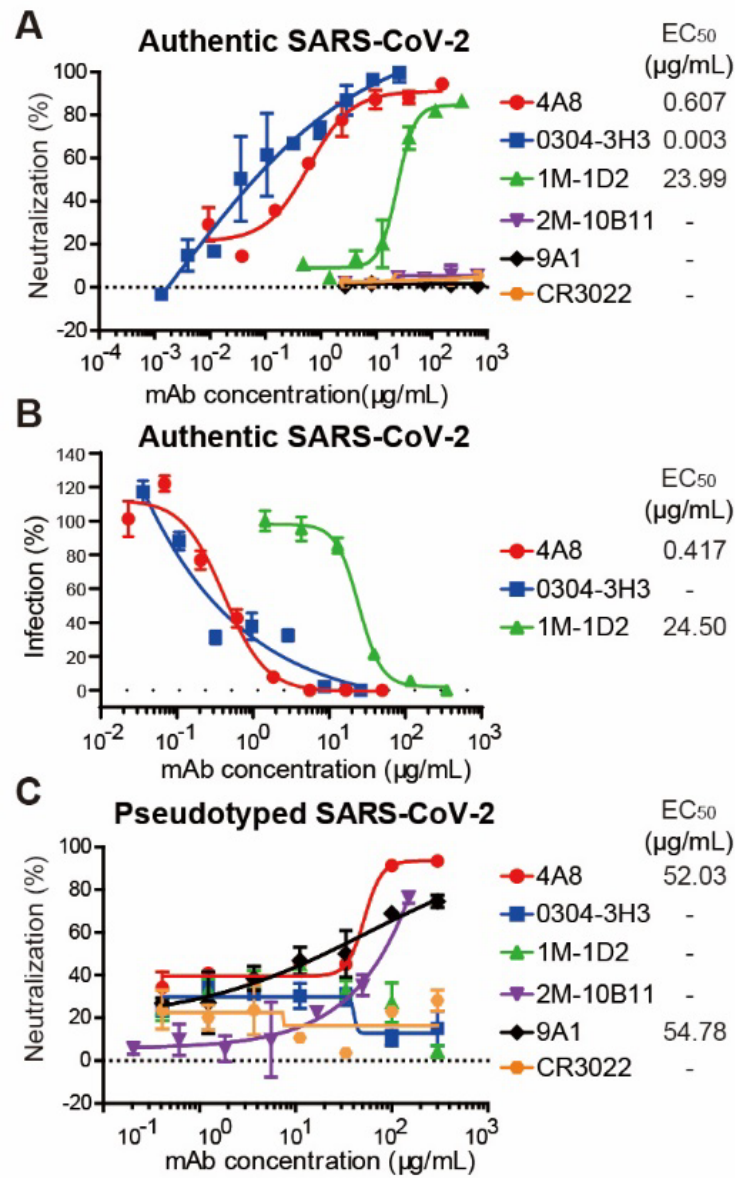
Fig. 6. Interactions between the NTD and 4A8. **(A)** Extensive hydrophilic interactions on the interface between NTD and 4A8. Only one NTD-4A8 is shown. **(B-D)** Detailed analysis of the interface between NTD and 4A8. Polar interactions are indicated by red, dashed lines. The residues involved in hydrophobic interactions are presented as spheres.

Fig. 1



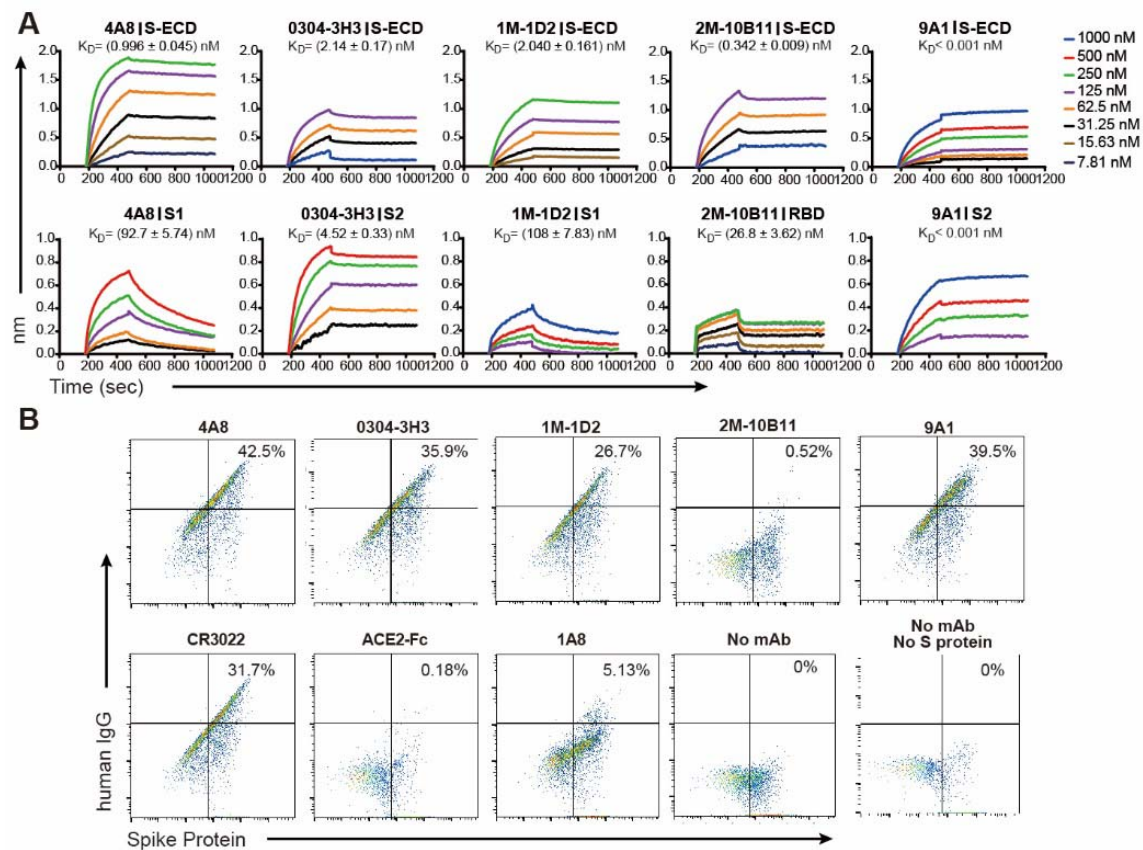
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Figure 3



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Figure 4



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Figure 5

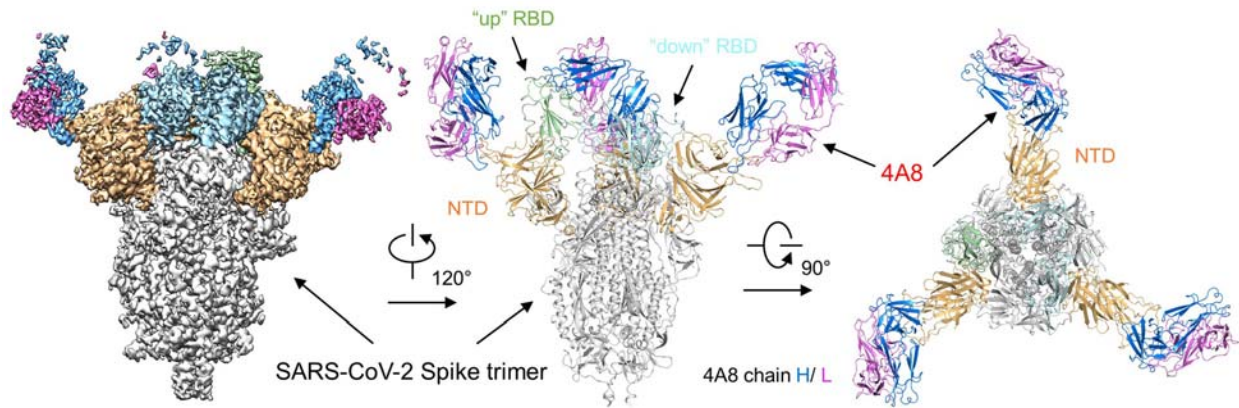
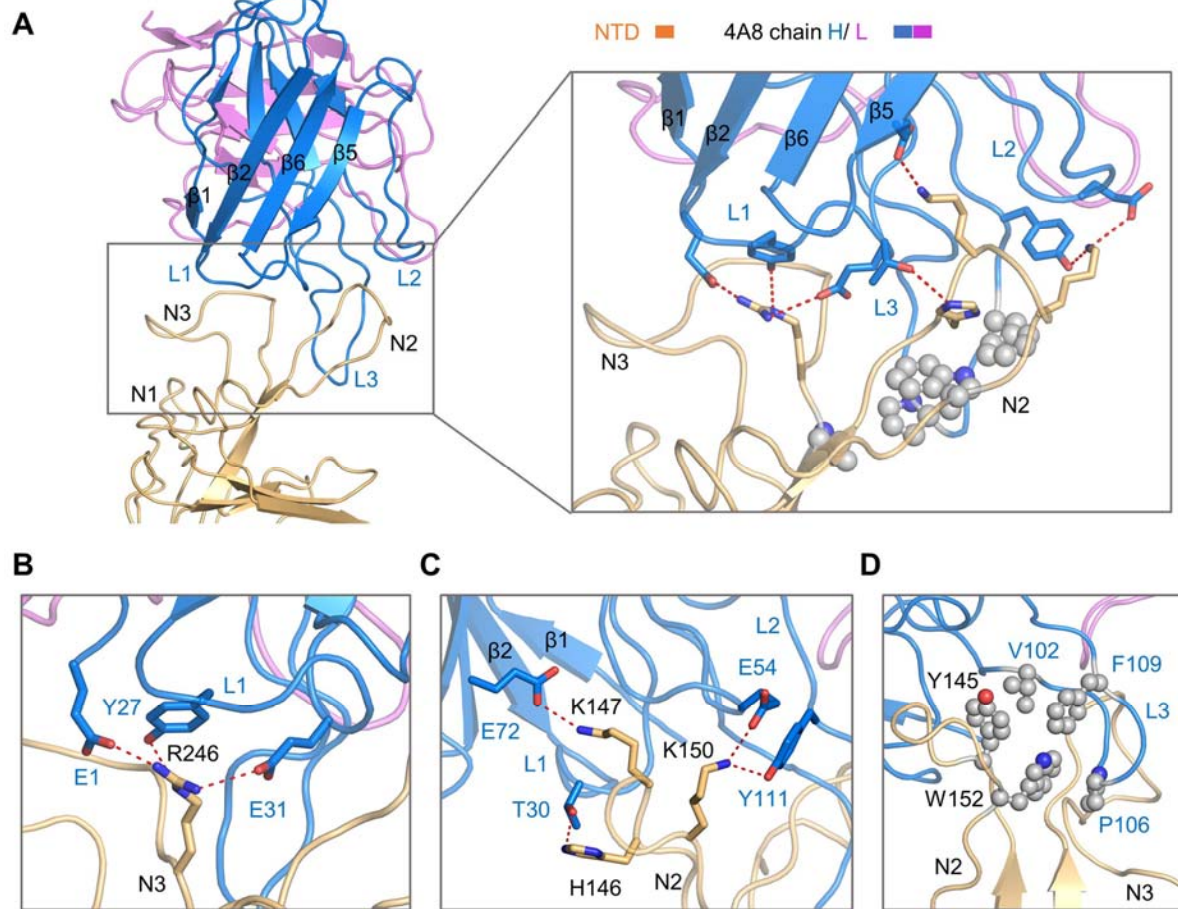


Figure 6



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Supplementary Materials for

A potent neutralizing human antibody reveals the N-terminal domain of the Spike protein of SARS-CoV-2 as a site of vulnerability

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Materials and Methods

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Movie. S1

Supplemental References

Materials and methods

Ethic Statement

A written informed consent was regularly obtained from all patients. The study was approved by the Ethics Committee of Wuhan Infectious Disease Hospital, Hubei Province, China.

Viruses and cells culture

SARS-CoV-2 was isolated from the lung lavage fluid of an infected patient. All work with infectious SARS-CoV-2 was performed in the biosafety level 3 (BSL-3) facility of Beijing Institute of Microbiology and Epidemiology, Academy of Military Medical Sciences, China. Vero E6 cells, HEK293T cells and ACE2-293T cells were grown in Dulbecco's modified Eagle's medium (DMEM) (Gibco) supplemented with 10% fetal bovine serum (Gibco), penicillin (100 IU/mL) and streptomycin (100 IU/mL). ACE2-293T cells, which are HEK293T cells expressing ACE2 receptor, were produced and kept in our laboratory. Expi293F cells (Thermo Scientific) were grown in Expi293™ Expression Medium (Thermo Scientific). HEK293F cells were grown in SMM 293T-II medium (Sino Biological Inc.).

Human subjects

After informed consent was obtained, peripheral blood samples were collected from ten survivors of SARS-CoV-2. Peripheral blood mononuclear cells (PBMCs) were isolated using human lymphocyte separation medium (Dakewe Biotech) according to the manufacturer's protocol. Briefly, peripheral blood was diluted with phosphate buffered saline (PBS) (Gibco). The mixture was then slowly transferred above lymphocyte separation medium. The volume of blood, PBS and the separation medium were the same. After centrifuging at room temperature at 800 g for 20 minutes, PBMCs were separated and collected to a new centrifuge tube. Following two steps of washing with PBS, the PBMCs were resuspended with Cell Freezing Medium (Thermo Scientific) and stored at -80C° until use.

Fluorescent Cell sorting and single-cell PCR

To sort antibody secreting cells (ASCs), PBMCs were stained with anti-CD3-PerCP (BD), anti-CD20-PerCP (BD), anti-CD19-Alexa Fluor700 (Beckman Coulter), anti-CD27-PE-Cy7 (Beckman Coulter), and anti-CD38-FITC (Stem Cell). ASCs were gated as CD19⁺CD3⁻CD20⁻CD27^{High}CD38^{High}. To sort SARS-CoV-2 S protein specific memory B cells, PBMCs were stained with anti-CD3-PerCP (BD), anti-CD19-Alexa Fluor700 (Beckman Coulter), anti-IgG-PE (BD), and SARS-CoV-2 S protein (His tag) (Sino Biological), followed by washing with PBS and staining with anti-His tag-Alexa Fluor647 (Thermo Scientific). The SARS-CoV-2 S protein specific memory B cells were defined as CD3⁻CD19⁺IgG⁺S-ECD⁺. Single gated cells were sorted into 96-well plates (Bio-Rad) containing 20 μ L RNase-free-water (TransGen Biotech) and 20 U RNase inhibitor (Promega) in each well. Double-stranded cDNA was produced using One-Step reverse transcription PCR (RT-PCR) kit (Qiagen). Antibody VH and VL genes were amplified through nested PCR using TransStart Taq DNA polymerase (TransGen Biotech) as described previously (1). The PCR products were sequenced by Sangon Biotech. Sequences were analyzed using IMGT/ V-QUEST (http://www.imgt.org/IMGT_vquest).

Protein expression and purification

The full-length Ig heavy- and light-chain linear expression cassettes were produced by assembling the cytomegalovirus (CMV) promoter, Ig leader sequence, VH/VL gene, Ig constant region (IgG1), and poly(A) sequence fragment together using overlapping PCR as previously described (2). The antibody VH/VL and constant region genes were then amplified and cloned into expression vector pcDNA3.4 using NEBuilder® HiFi DNA Assembly Cloning Kit (NEB). The plasmids of paired Ig H and L genes were co-transfected into Expi293F expression system (Thermo Scientific) following the manufacturer's protocol to produce recombinant mAbs. HiTrap rProtein A column (GE Healthcare) was used to purify antibodies from cell culture supernatants.

The extracellular domain (ECD) (1-1208 a.a) was cloned into the pCAG vector (Invitrogen) with two proline substitutions at residues 986 and 987, a "GSAS" substitution at residues 682 to 685 and a C-terminal T4 fibrin trimerization motif followed by one Flag tag. The mutants were generated with a standard two-step PCR-based strategy.

The recombinant S-ECD protein was overexpressed using the HEK 293F mammalian cells (Invitrogen) at 37°C under 5% CO₂ in a Multitron-Pro shaker (Infors, 130 rpm). When the cell density reached 2.0×10^6 cells/mL, the plasmid was transiently transfected into the cells. To transfect one liter of cell culture, about 1.5 mg of the plasmid was premixed with 3 mg of polyethylenimines (PEIs) (Polysciences) in 50 mL of fresh medium for 15 mins before adding to cell culture. Cells were removed by centrifugation at 4000×g for 15 mins after sixty hours transfection. The secreted S-ECD proteins were purified by anti-FLAG M2 affinity resin (Sigma Aldrich). After loading two times, the anti-FLAG M2 resin was washed with the wash buffer containing 25 mM Tris (pH 8.0), 150 mM NaCl. The protein was eluted with the wash buffer plus 0.2 mg/mL flag peptide. The eluent was then concentrated and subjected to size-exclusion chromatography (Superose 6 Increase 10/300 GL, GE Healthcare) in buffer containing 25 mM Tris (pH 8.0), 150 mM NaCl. The peak fractions were collected and concentrated to incubate with mAb 4A8. The purified S-ECD was mixed with the mAb 4A8 at a molar ratio of about 1:1.2 for one hour. Then the mixture was subjected to size-exclusion chromatography (Superose 6 Increase 10/300 GL, GE Healthcare) in buffer containing 25 mM Tris (pH 8.0), 150 mM NaCl. The peak fractions were collected for EM analysis.

Enzyme-linked immunosorbent assay (ELISA)

Polystyrene microplates (Corning) were coated overnight with 2 µg/mL SARS-CoV-2 S, S1, S2 or RBD protein (Sino Biological). After washing with PBS containing 0.2% Tween 20 (Solarbio Life Sciences), the plates were blocked using 2% BSA (Sigma Aldrich) in PBST for 1 h at 37°C. Following washing with PBST, serial dilutions of testing antibodies were added to each well and incubated at 37°C for 1 h. After washing with PBST, horseradish peroxidase (HRP)-conjugated anti-human IgG antibody (Abcam) was added at the dilution of 1:10000 and incubated at 37°C for 1 h. After washing, TMB single-component substrate solution (Solarbio Life Sciences) was added to the microplate and incubated at room temperature for 6 min, followed by adding 2M H₂SO₄ to stop the reaction. The absorbance was detected at 450 nm/630 nm. The data was analyzed using GraphPad Prism 7.0.

Competition binding analysis

Detecting antibodies were conjugated with biotin using EZ-Link™ Sulfo-NHS-Biotin (Thermo Scientific) following the manufacturer's protocol. Microplates were coated with 2 µg/mL SARS-CoV-2 S protein (Sino Biological) overnight at 4 °C. Detecting antibodies at the final concentration of 1 µg/mL and blocking antibodies at the final concentration of 100 µg/mL were added to the same well and incubated for 1 h at 37 °C. After washing with PBST, streptavidin conjugated with HRP (Thermo Scientific) was added at the concentration 1 µg/mL and incubated for 1 h at 37 °C. Following washing with PBST, TMB single-component substrate solution (Solarbio Life Sciences) was added and incubated at room temperature for 6 min. 2 M H₂SO₄ was then added to stop the reaction. The absorbance was detected at 450nm/630nm. The competition value was determined by comparing the binding value of detecting antibody in the presence of blocking antibody divided by the binding value of detecting antibody alone.

Sequence analysis of antibody variable region genes

Antibody sequences were annotated with igBLAST (3) and IMGT/V-QUEST (4) and then trimmed to extract only the variable region from FWR1 to the end of the J gene. Based on the gene annotation results of igBLAST, the count and relative abundance of V(D)J alleles, genes or families were determined by R package 'Alakazam' in Change-O toolkit (5). R package 'ggpubr' and 'ggplot2' were used for pie chart. Circos plots indicating the combination of V/D/J alleles were generated by self-written Perl scripts and R scripts. R package 'circlize' was used for data visualization. Antibody amino acid sequence alignments and evolutionary analyses were conducted in MEGA7 (6). The evolutionary history was inferred using the Neighbor-Joining method (7). The bootstrap consensus tree inferred from 500 replicates (8) was taken to represent the evolutionary history of the taxa analyzed (8). Branches corresponding to partitions reproduced in less than 50% bootstrap replicates were collapsed. The evolutionary distances were computed using the Maximum Composite Likelihood method (9). To analyze sequence similarity, antibody DNA sequences were compared to each other (pairwise alignments) by ClustalW (10), a multiple alignment program. The scores were calculated from separate pairwise alignments by the

method of Wilbur and Lipman method (11) with the default DNA weight matrix (IUB). Data format conversion were completed by self-written Perl scripts and R scripts. R package ‘corrplot’ was used for data visualization.

Neutralization experiment

Vero-E6 cells were inoculated in 96-well cell culture plates (20,000 cells per well) with DMEM (Gibco) supplemented with 10% fetal bovine serum and grown overnight at 37 °C. Sera or antibodies were serially diluted and then mixed with 100 TCID₅₀ SARS-CoV-2. The mixture was moved to the wells containing Vero-E6 cells and incubated at 37 °C for 1 h. Following removing the supernatants, 200 µL cell culture medium were added and the plates were then incubated at 37°C° with 5% CO₂ for 3 days. Cells were stained with crystal violet and absorbance at 570nm/630nm were measured. Neutralization was defined as percentage reduction compared to positive controls. Neutralization titers of two replicates were calculated using a non-linear regression analysis in GraphPad Prism 7.

Supernatants at 3 days post infection were harvested and tested using quantitative real-time PCR to determine the number of viral genomes. RNA extraction was performed using RNAeasy Plus mini kit (Qiagen) following the manufacturer’s protocol. Eluted RNA was used for reverse transcription using Maxima First Strand cDNA Synthesis Kit for RT-qPCR (Thermo Scientific) according to the manufacturer’s instructions with oligo(dT). Subsequently, quantitative real-time PCR was performed using TaqMan Universal Master Mix II with UNG (Thermo Scientific) according to the manufacturer’s instructions, with the primer CoV-RT-F3 5’- GGGGAAGTCTCTCCTGCTAGAAT-3’, primer CoV-RT-R3 5’- CAGACATTTTGCTCTCAAGCTG-3’ and a probe CoV-RT-P3 5’- FAM-TTGCTGCTGCTTGACAGATT-3’. The primers and probe were purchased from Sangon Biotech. The IC₅₀ was calculated using a non-linear regression analysis in GraphPad Prism 7.

Pseudotyped virus packaging and neutralizing

Gene encoding full-length SARS-CoV-2 S protein (Genebank ID: QHD43416.1) was inserted into the pDC316 vector, resulting in plasmid pDC316- SARS-CoV-2-S. A total of 7.0×10^6 ACE2-293T cells were inoculated in a 10 cm cell culture dish and grown

overnight at 37°C with 5% CO₂. The pDC316-SARS-CoV-2-S and HIV backbone vector pNL4-3.Luc.R-E- were co-transfected into 293T cells with Lipofectamine3000 transfection reagent (Invitrogen). The culture medium was replaced after 6 hours. The supernatants containing HIV-pseudotyped virus with S protein were collected 48 h post-transfection and filtered through a 0.45 µm filter. The supernatants were then aliquoted and stored at -80 °C. To determine the neutralization ability of sera or antibodies, pseudotyped virus was incubated with serially diluted sera or antibodies at 37 °C for 1 h and then added to 96-well culture plates containing 2×10^4 ACE2-293T cells with two replicates. The cells were then maintained at 37 °C with 5% CO₂ for 48 h. The cells were lysed and luciferase activity was measured using firefly luciferase assay system (Promega). Neutralization was calculated as $(1 - \text{Lucmeasured} / \text{Lucvirus control}) \times 100\%$. Neutralization titers were calculated using a non-linear regression analysis in GraphPad Prism 7.

Biolayer interferometry

Antibodies to be tested were diluted to the concentration of 5 µg/mL with PBS containing 0.05% Tween 20 (Solarbio Life Sciences) and then immobilized onto Anti-hIgG Fc Capture (AHC) biosensors (Sartorius AG). After a 60-seconds washing step with PBST, biosensor tips were immersed into the wells containing SARS-CoV-2 S-ECD/S1/S2/RBD protein (Sino Biological) of serial dilutions and allowed to associate for 300 seconds, followed by a dissociation step of 600 seconds. The K_d value for each pair of antibody and antigen was calculated using Data Analysis Software 9.0 (Sartorius AG).

Flow cytometry receptor binding inhibition assay

Antibodies to be tested were incubated at the concentration of 400 µg/mL with 40 µg/mL SARS-CoV-2 S protein (Sino Biological) in 50 µL PBS for 1 h at 37 °C. The mAb-S protein mixture was then incubated with 5×10^5 ACE2-293T cells for 30 min at room temperature. After washing twice with PBS containing 2% FBS (Gibco), cells were resuspended and incubated with anti-His tag antibody conjugated with Alexa Fluor 647 (Thermo Scientific) and anti-human IgG antibody conjugated with FITC (Abcam) for 30 min at room temperature. Cells were washed twice and resuspended with PBS containing 2% FBS

before analyzed using BD FACSCanto II. ACE2-Fc (Novoprotein) was used as positive control. Data in FCS format were analyzed using FlowJo X 10.0.7r2.

Cryo-EM sample preparation

The peak fractions of S-ECD and mAb 4A8 complex was concentrated to about 1.5 mg/mL and applied to the grids. Aliquots (3.3 μ L) of the protein complex were placed on glow-discharged holey carbon grids (Quantifoil Au R1.2/1.3). The grids were blotted for 2.5 s or 3.0 s and flash-frozen in liquid ethane cooled by liquid nitrogen with Vitrobot (Mark IV, Thermo Scientific). The cryo-EM samples were transferred to a Titan Krios operating at 300 kV equipped with Cs corrector, Gatan K3 Summit detector and GIF Quantum energy filter. Movie stacks were automatically collected using AutoEMation (*12*), with a slit width of 20 eV on the energy filter and a defocus range from -1.2 μ m to -2.2 μ m in super-resolution mode at a nominal magnification of 81,000 $\times$. Each stack was exposed for 2.56 s with an exposure time of 0.08 s per frame, resulting in a total of 32 frames per stack. The total dose rate was approximately 50 e $^-$ /Å² for each stack. The stacks were motion corrected with MotionCor2 (*13*) and binned 2-fold, resulting in a pixel size of 1.087 Å/pixel. Meanwhile, dose weighting was performed (*14*). The defocus values were estimated with Gctf (*15*).

Data processing

Particles were automatically picked using Relion 3.0.6 (*16-19*) from manually selected micrographs. After 2D classification with Relion, good particles were selected and subject to one cycle of heterogeneous refinement without symmetry using cryoSPARC (*20*). The good particles were selected and subjected to homogeneous refinement with C1 symmetry, resulting in the 3D reconstruction for the whole structures, which was further subject to 3D auto-refinement and post-processing with Relion. For interface between SARS-CoV-2 S protein and mAb 4A8, the dataset were subject to focused refinement with adapted mask on each NTD-4A8 sub-complex to improve the map quality. Then the dataset of three NTD-4A8 sub-complexes were combined and subject to focused refinement with Relion, resulting in the 3D reconstruction of better quality on the interface between S protein and mAb 4A8.

The resolution was estimated with the gold-standard Fourier shell correlation 0.143 criterion (21) with high-resolution noise substitution (22). Refer to Supplemental Figures S4-S5 and Supplemental Table S3 for details of data collection and processing.

Model building and structure refinement

For model building of the complex of S protein of SARS-CoV-2 with mAb 4A8, the atomic model of S protein (PDB ID: 6VSB) and an mAb molecule (PDB ID: 5FUZ) were used as templates, which were molecular dynamics flexible fitted (23) into the whole cryo-EM map of the complex and the focused-refined cryo-EM map of the NTD-4A8 sub-complex, respectively. And the fitted atomic models were further manually adjusted with Coot (24). Each residue was manually checked with the chemical properties taken into consideration during model building. Several segments, whose corresponding densities were invisible, were not modeled. Structural refinement was performed in Phenix (25) with secondary structure and geometry restraints to prevent overfitting. To monitor the potential overfitting, the model was refined against one of the two independent half maps from the gold-standard 3D refinement approach. Then, the refined model was tested against the other map. Statistics associated with data collection, 3D reconstruction and model building were summarized in Table S3.

Supplementary Figures and Legends

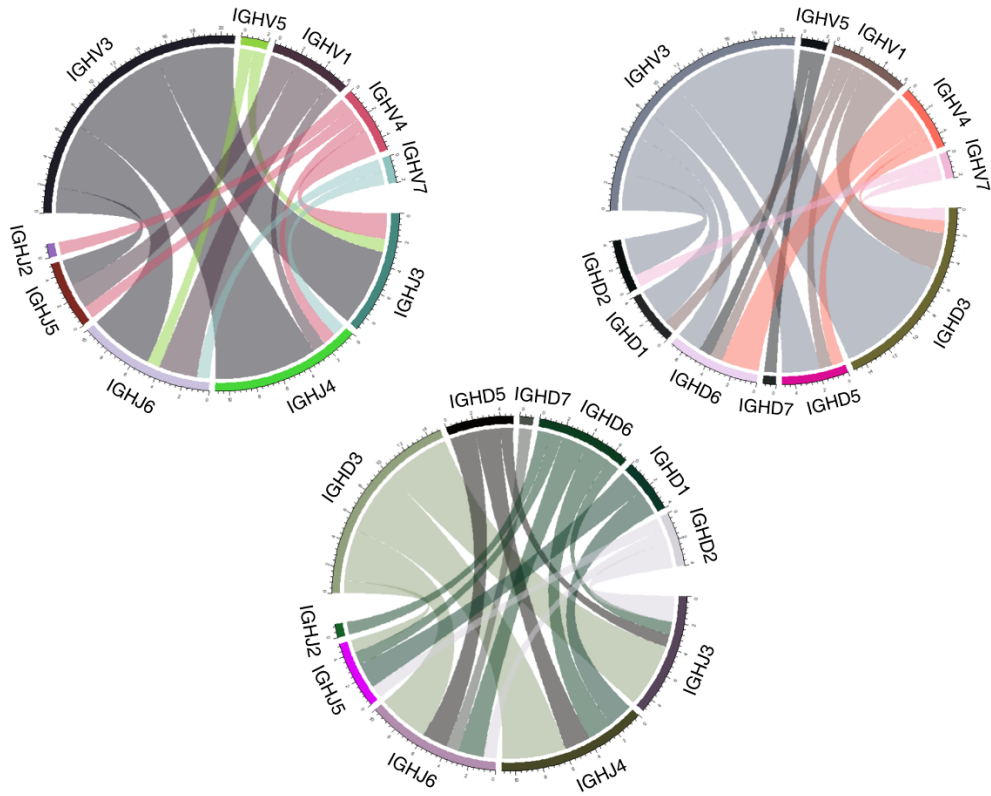


Fig. S1

Combination of heavy chain V/D/J genes of the thirty-five spike protein-specific antibodies. Each arc of the circle represents a family of IGH V/D/J gene.

The bands between two families represent the combination of V and D, V and J or D and J genes. The width of bands represents the frequency of combination.

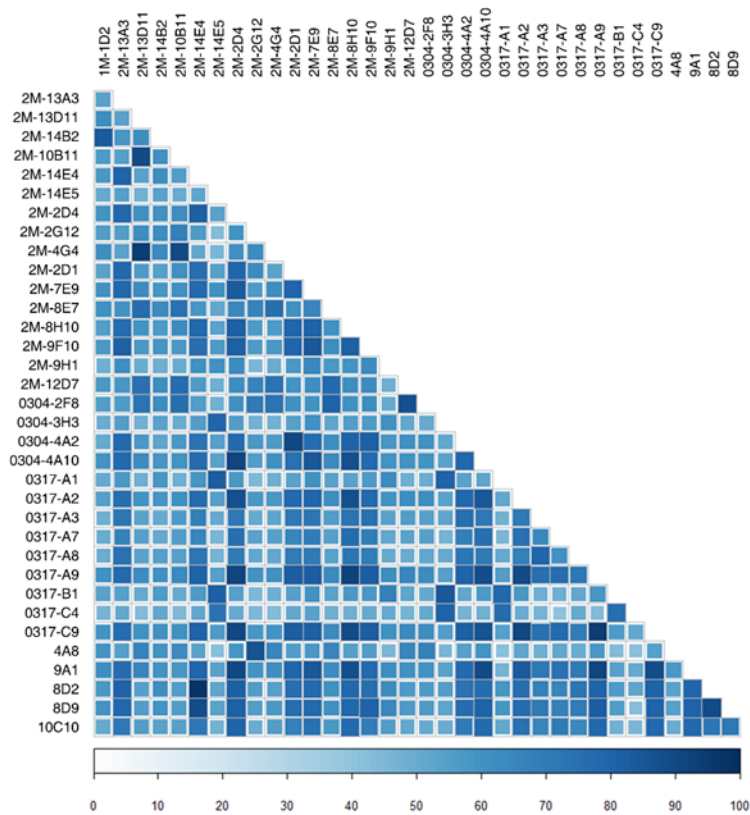


Fig. S2

Quantification of CDRH3 sequence identities of the thirty-five spike protein-specific antibodies by similarity index.

The index was determined using BLOSUM (Blocks Substitution Matrix).

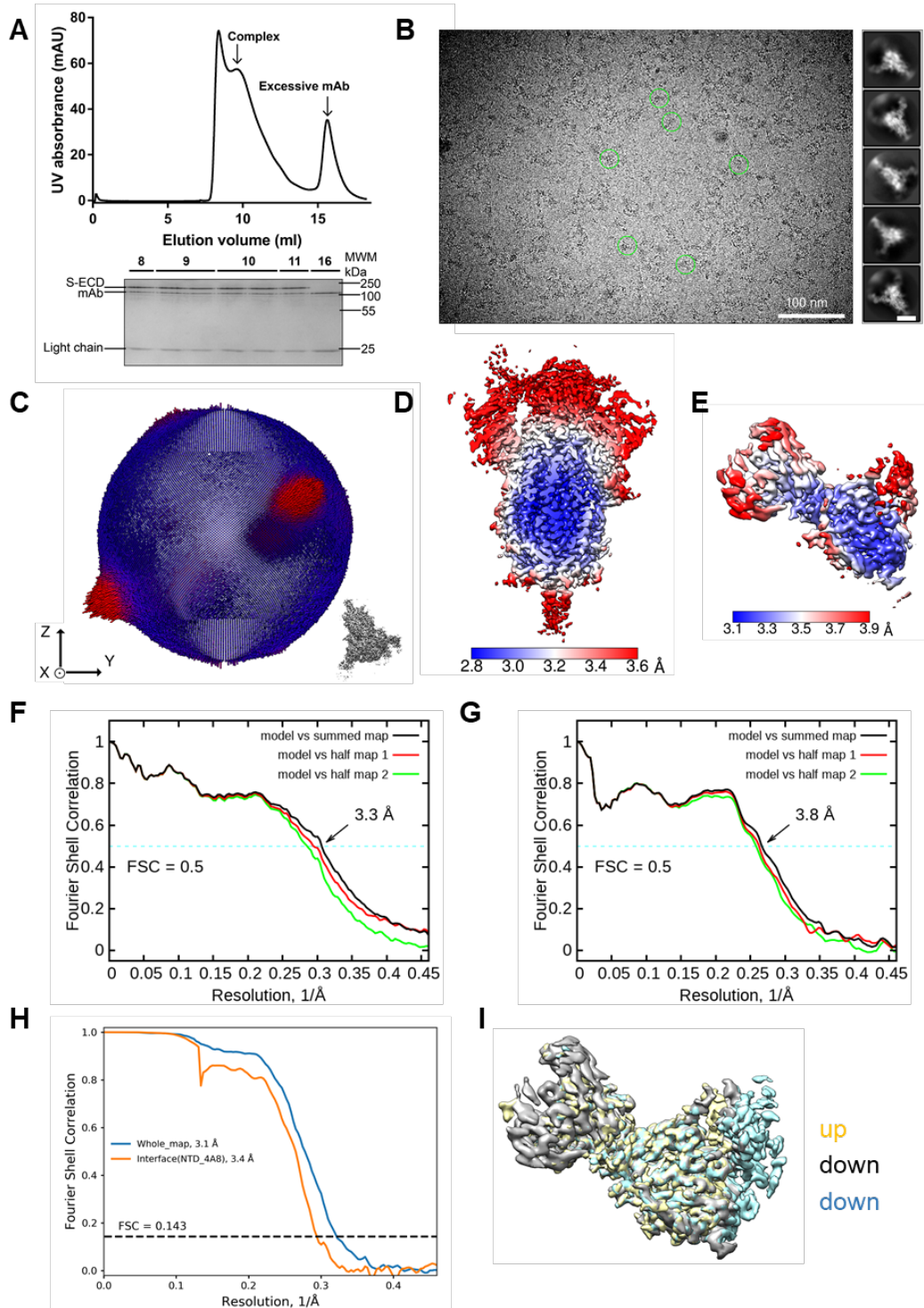


Fig. S3

Cryo-EM analysis of S-ECD of SARS-CoV-2 bound with 4A8 complex.

(A) Representative SEC purification profile of the S-ECD of SARS-CoV-2 in complex with mAb 4A8. **(B)** Representative cryo-EM micrograph and 2D class averages of cryo-

EM particle images. The scale bar in 2D class averages is 10 nm. **(C)** Euler angle distribution in the final 3D reconstruction of S-ECD of SARS-CoV-2 bound with 4A8 complex. **(D)** and **(E)** Local resolution maps for the 3D reconstruction of the overall structure and interface between NTD of S-ECD and 4A8, respectively. **(F)** FSC curve of the refined model of S-ECD of SARS-CoV-2 bound with 4A8 complex versus the overall structure that it is refined against (black); of the model refined against the first half map versus the same map (red); and of the model refined against the first half map versus the second half map (green). The small difference between the red and green curves indicates that the refinement of the atomic coordinates is not enough overfitting. **(G)** FSC curve of the refined model of interface between NTD of S-ECD and 4A8, which is the same as the (F). **(H)** FSC curve of the overall structure (blue) and interface between NTD of S-ECD and 4A8 (orange). **(I)** Superposition in local map of interface between NTD of S-ECD and 4A8 for different conformation of SARS-CoV-2 S protein trimer in “up” or “down” RBD, which has no difference among three maps. The maps for one “up” RBD and two “down” RBDs are colored yellow and cyan, respectively.

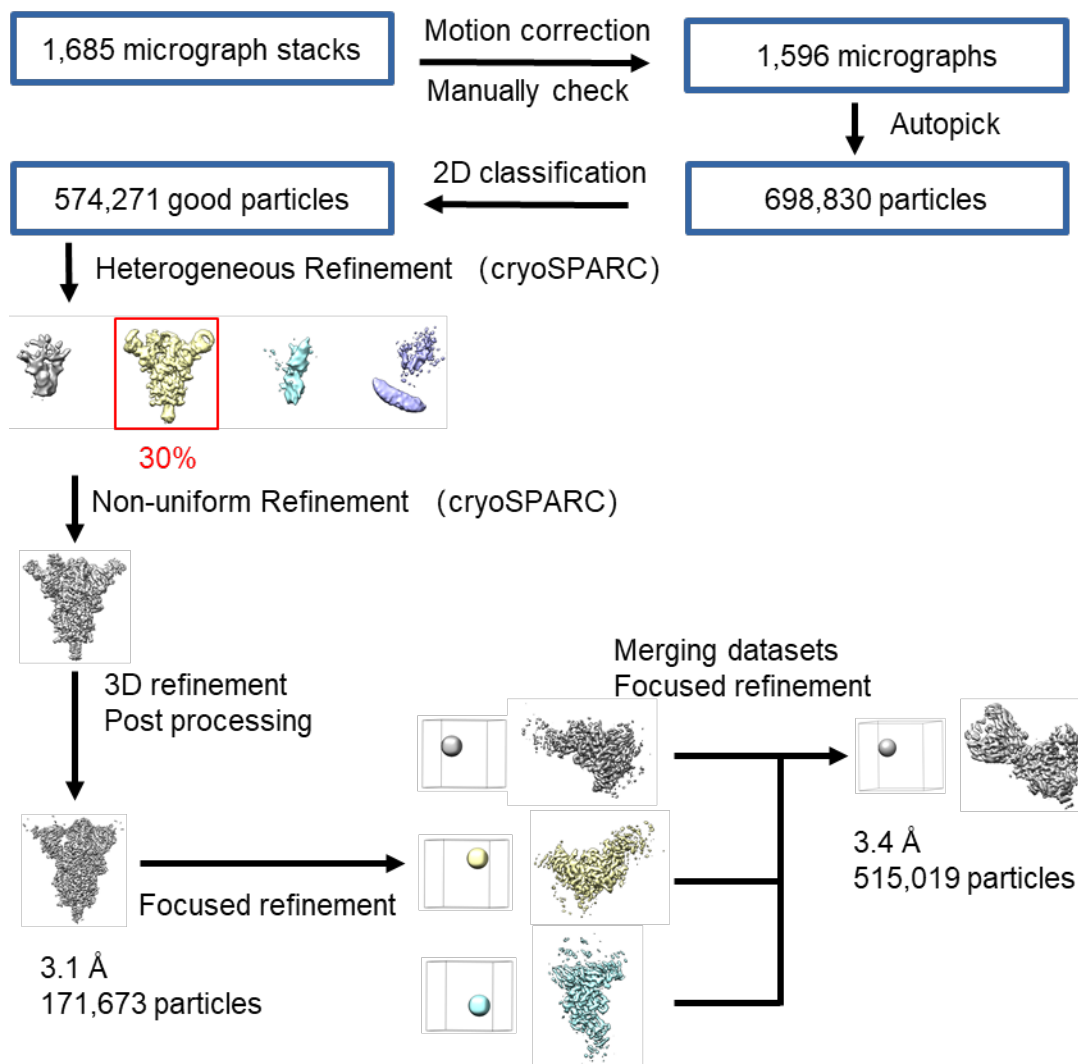


Fig. S4

Flowchart for cryo-EM data processing of S-ECD of SARS-CoV-2 bound with 4A8 complex.

Please refer to the ‘Data Processing’ section in Methods for details.

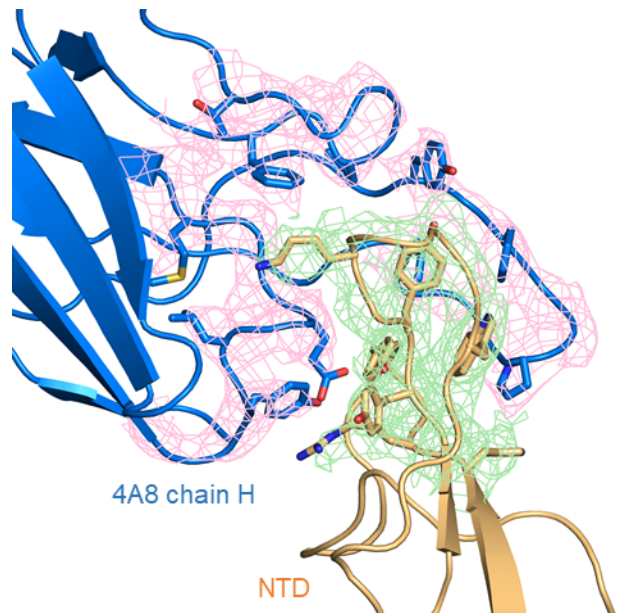


Fig. S5

Cryo-EM density of the interface between NTD of S-ECD and 4A8.

The density is contoured at 12σ , which is shown as pink meshes in chain H of 4A8 and green meshes in NTD of S-ECD, respectively.

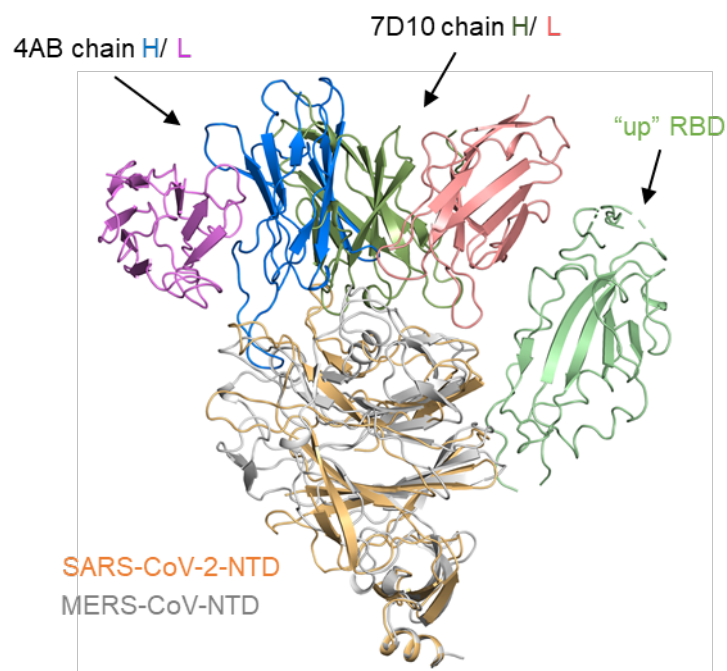


Fig. S6

Structural comparison of SARS-CoV-2 bound with 4A8 complex and MERS-CoV bound with 7D10 complex (PDB ID: 6J11).

Shown here is the NTD of MERS-CoV superimposing with the NTD of SARS-CoV-2, the NTD can be aligned with root mean squared deviation of ~ 8.6 Å over ~ 193 pairs of C α atoms, which is overlapping in heavy chain between 4A8 and 7D10.

SARS-CoV-2	----MFVFLVLPVSSQCVN--LITRTQLPQATN--SFTRGVYYPDKVFRSSVLHSTQDLFLP----FSNVIVFHAHVSGTNGTKR	78
SARS-CoV	----MFIPLLFTLTSGDLDRCTIFDDVQAPNYTHTSMRGVYYPDEIFRSDTLYLTQDLFLP----FYSNVIVGHTINHT-----	75
MERS-CoV	MIHSVELLMFLLTPTESYVDVGPDSVKSAACI EVDI DQTFDKTWRPPI DVS KADGI I YPDQRTYSNI TI TYGQLFPYQGDHGMVYVYSAG	90
SARS-CoV-2	FDNPVLPFNDGVYFASTEKSNIRGWFGITLQSKQSLI VNNATNVVI KVCEQFCNDFFLGVIYHKNNKSWMESEFRVYSS--ANN	165
SARS-CoV	FGNPVLPFKDGIYFAATEKSNVVRGWVFGSTIMNKSQSVII I NNSTNVVI RACNEELGDNFFAVSKPMG--TQHTMI FDN--AFN	158
MERS-CoV	HATGTPQLFLFANYSDQVKQFANGFVRI GAAANSTGTVII SPSTSATI RKI YPAIMLGSSVGNFSDGKMGRFFNHTLVLLPDGCGTLL	180
SARS-CoV-2	CTFEYVSQPFLMDLEGKQGNFKNLRREFVFKNI DG YFKI YSKHTPI NLVRDLP-----DQFSALESLV	227
SARS-CoV	CTFEYISDAFSLDVSEKSGNFKHLREFVFKNKDGLFYVYKGYQPI DVVRDLP-----SGFNTLKDI F	220
MERS-CoV	RAIYICLERPSGNHCPAGNSYTSFATYHTPATDCSDGNIYRNASLNSFKEYFNLRNCTFMYTYNI TEDEI LEWFGI TQTAQGVHLFSSRY	270
SARS-CoV-2	DLPIGINITRFOILLALHRSYLT PGDSSGWTAG----AAAYYVGYLQFRTFLKKVNGTITDAVDCALDPLSETKCTLSKSTVEKGI	312
SARS-CoV	KLQLGGINIINFRATITAFS-----PAQDWGTS-----AAAYFVOYLKHTIFMLKYDENGTITDAVDCSQNPLALCKSVASPEIDAGI	299
MERS-CoV	VDLYSGNMFGQATLPVYDTIKYYSIIPHSTSRISQSDRKAAWAFYVYKLPPLTFLDFSDVGYRRATDCGFNDLSQLHCSVSESDVSGY	360
SARS-CoV-2	YQTSNFRVQPTESVRFPNITNLCPFGVEFNATRFASVYAWNKRISNOCVADSVLYNSASFSTFKCYGVSPTKLNDLCFTNVYADSVF	402
SARS-CoV	YQTSNFRVVPSPDVRFPNITNLCPFGVEFNATKFPSSVYAWERKKISNOCVADSVLYNSTFFSTFKCYGVSAIKLNDLCFSNVYADSVFV	389
MERS-CoV	YVSSEAEKPSGSVSEAEQ-VEDEPSPLLSGI-PPQVNFKELVFTNQNYNLTKLKLSLFSVNDFTSQISAAALASNGYSLLIDVYSY	448
SARS-CoV-2	RGDEVROIAPGQTGKADYNYKLDDFTGCVI AWNSNNLDSKYGGNYNYLYRLFKNLKFPERDI STEI YQAGSTPONGVEGFNCY---	489
SARS-CoV	KGDDVROIAPGQTGVADYNYKLDDFMGCVLAWNTRNI DAISTGNINYKYRYLRHGKLRFFERDISNVPFSPDGKPGT-PPALNCY---	475
MERS-CoV	PLSMKSDLVSSAGPIISGFNYKQSFNPTCLLATVPHNLTTITKPLKYSVINKCSRFLSDDRTEVPQLVNANQYSPPVSI V PSTVWEDG	538
SARS-CoV-2	-----FPLQSYGFQPTNGVGYQPYRVVLSFELLHAPATVCGPKKSTNLVKNI-----KCVNFNFNGLTGTGVLTESNKKFLPFQ	563
SARS-CoV	-----WPLNDVGYFTTTGIGYQPYRVVLSFELLHAPATVCGPKLSTDLIKNI-----QCVNFNFNGLTGTGVLTPSSKRFQDFQ	549
MERS-CoV	DYYRKQLSPLLEGGGWLVASGSTVAMTEQLQMGIGITVQYGTDTNSVCPKLEFADTKIASQLGNQVEYSLYSVSRGVFNCTAVGVRQD	628
SARS-CoV-2	QFGRDADITDAVRDPTLEI LDITPCSFGGVSVITPGTINQVAVLYQDVNCTEVPVAIHADQLTPTWRVYSGNSVVFQTRAGCLIGA	653
SARS-CoV	QFGRDVSDFDSDVRDPKSEILDISPCSFGGVSVITPGTINQVAVLYQDVNCTDSTAIHADQLTPAWRIYSTGNKVSFQTRAGCLIGA	639
MERS-CoV	RIFYDQAYQNLVGYYS-DDGNYYCLRAVSVVPVSVIYDKETKTHATLFGSVACEHI SSTMSQYSRSTRSMLKRRDSTYGPLDTPTGCLQL	717
SARS-CoV-2	EHYNN-SYECDDIPAGICASYQTQTSNPRARSVASQEI IAYTMSLGAENSVAYSNN--SI AIPTNFIVSVTTEI LPVSWTKTSVDCT	739
SARS-CoV	EHYDT-SYECDDIPAGICASYHTVG---LLRSTSQEKI VAYTMSLGAQSSIAYSNN--TIAIPTNFSISITTEVMFVSMAKTSVDCCN	721
MERS-CoV	VNSSLFVEDQKPLQGSLCALPDTPSTLTPSVRSVPGEMRLASIAFNHPI QDQLNSSYFKLSIPTNFSFGVTCQYIQTTLQKVTVDOCK	807
SARS-CoV-2	MYICGDSTEQSNLLQYGSFCTQLNRALTGIAVEODKNTQEVFAQVKQIKYTPPIKDEGG-FNFSQILPDPSKP--SKRSFIEDLLFNK	825
SARS-CoV	MYICGDSTEQANLLQYGSFCTQLNRALSGIAAEODRNTREVFQVKQIKYTPPIKYFGG-FNFSQILPDLPKP--TKRSFIEDLLFNK	807
MERS-CoV	QYVNGFGKQEDLLREYGFQCSKINQALHGANLRQDSDVNLFAVSKSSQSSPIIPFGGDFNLTLLERVSI STGSRARSALIEDLLFDK	897
SARS-CoV-2	VTLDAGFIKQYGDCLG-DIAARDLI CAQKFNGLTVLPLLTDEMI AQYTSALLAGTITSGWTFGAGAAALQIPFAMQMAYRFNGI GVTQ	913
SARS-CoV	VTLDAGFGMKYQYEGCLG-DINARDLI CAQKFNGLTVLPLLTDDMI AAYTAALYSOTATAGWTFGAGAAALQIPFAMQMAYRFNGI GVTQ	895
MERS-CoV	VTADPGYVQGDQCQGGPASARDLI CAQYVASYKVLPLLMOVNMEAAYSLLLSGIAGVGTWAGLSFFAAIPFAQSI FYRLNGVGTITQ	987
SARS-CoV-2	NVLYENQKLI ANQFNSAIGKI QDSLSSITASALGKLQDVVNQNAQALNTLVKQLSSNFGAISVVLNDILSRLDKVEAEVQIDRLITGRLQS	1003
SARS-CoV	NVLYENQKLI ANQFNKALISQIQESLTTTSTALGKLQDVVNQNAQALNTLVKQLSSNFGAISVVLNDILSRLDKVEAEVQIDRLITGRLQS	985
MERS-CoV	QVISENQKLI ANKFNAALGAMGTGFTTTNEAFHVQDAVNNAQALSKELELNTFGAISASIGDIQLQSLVLEQDAQIDRLINGRLLT	1077
SARS-CoV-2	LQTYVTQQLI RAAELRASANLAATKMSECVLGQSKRVDFCGGYHLMSPFQSAAPHGVVFLHVTYVPADEKNFTTAPAI CHDQ---KAHFP	1090
SARS-CoV	LQTYVTQQLI RAAELRASANLAATKMSECVLGQSKRVDFCGGYHLMSPFQSAAPHGVVFLHVTYVPSDERNETTAPAI CHES---KAYFP	1072
MERS-CoV	LNAFVADQLVRGSEAAALSAQLAKDKVNECVKAQSKRSGFCGGSTHVSFVVNAPNGLYFMHVGYPNSHI EVVSAVGLQDAANPTNCIAP	1167
SARS-CoV-2	REGVYFVSNGTHWFVTQRNFYEPQIITDNTFVSGNCDVVI GIVNNTVYDPLQPELDS-----FKEELDKEYFKNHTSPDVLDGDISGINA	1174
SARS-CoV	REGVYFVNGTSWFI TQRNFYSPQIITDNTFVSGNCDVVI GIVNNTVYDPLQPELDS-----FKEELDKEYFKNHTSPDVLDGDISGINA	1156
MERS-CoV	VNGYFVKTNTNRI VDEWSYTGSSFYAPEPI TSLNTKYVAPQVYQNI STNLPPPI LGNSTGI DEQDELDEFKNVSTGISIPNFGSLTQINT	1257
SARS-CoV-2	SVVNI KQEI DRLNEVAKNLESLL DLQELGKYEQYI KWPWYI WL GFIAGLI AIVMVTI LCCMTSCCSCCLKGACSCGSCCK-FDEDDSEP	1263
SARS-CoV	SVVNI KQEI DRLNEVAKNLESLL DLQELGKYEQYI KWPWYI WL GFIAGLI AIVMVTI LCCMTSCCSCCLKGACSCGSCCK-FDEDDSEP	1245
MERS-CoV	TLLDLTYEMLSLQGVVKALNESYI DLKELGNYTYNKPWYI WL GFIAGLI AIVMVTI LCCMTSCCSCCLKGACSCGSCCK-FDEDDSEP	1347
SARS-CoV-2	VLKGVKLHYT	1273
SARS-CoV	VLKGVKLHYT	1255
MERS-CoV	HKVHV-----	1353

Fig. S7

Sequence alignment for S protein of SARS-CoV-2, SARS-CoV and MERS-CoV.

The sequences were aligned using ClustalX. The aligned sequences are S protein of SARS-CoV-2, SARS-CoV and MERS-CoV. Amino acids that are identical or conserved in at least 2 sequences are colored red or yellow. The amino acid residues located in the N-terminal domain are lined out by black box. Key residues involved in interaction with mAb 4A8 are marked with black circles. Uniprot IDs are listed below: **S protein of SARS-CoV-2**: P0DTC2; **S protein of SARS-CoV**: P59594; **S protein of MERS-CoV**: K9N5Q8.

Table S1**Clinical characteristics of COVID-19 recovered patients**

ID	Age (Years)	Gender	Duration from collection date confirmation date (Days)	blood to disease	First Symptoms
Pt 1	43	M	29		Cough, fever, and sore muscle
Pt 2	25	M	29		Fever and fatigue
Pt 3	30	M	31		Fever and chest tightness
Pt 4	35	F	29		Fever
Pt 5	50	M	23		Fever and cough
Pt 6	32	F	11		Fever, cough, and fatigue
Pt 7	53	M	15		Fever and cough
Pt 8	28	F	15		Fever and sore muscle
Pt 9	43	M	12		Fever
Pt 10	36	F	10		Fever

Table S2

Origination, germline sequence and variable region CDR3 sequence of the 35 S protein -specific antibodies.

Sequence ID	Donor	IGHV	VH-CDR3	IGLV	VL-CDR3
1M-1D2	1	IGHV3-64*01	ARGAEYYDFWSG YYSAYFDY	IGLV1-47*01	SMGAQPDLC A
2M-13A3	2	IGHV3-30*04	ARGGGSYYYWFD P	IGKV4-1*01	QQYYST
2M-13D11	2	IGHV4-34*01	ARAGYSSSWYGV RGVDP	IGKV3-20*01	QQYGSSRSWT
2M-14B2	2	IGHV3-30*18	AKGSDIVVVPVGN WFDP	IGKV1-39*01	QQSYSTFTLY T
2M-10B11	2	IGHV3-66*02	ARATWLRGVMDV	IGLV6-57*02	QSYDSSNHW V
2M-14E4	2	IGHV4-61*01	ARVQRYYPDSSGF YGRRFDI	IGKV1-39*01	QQSHSFPT
2M-14E5	2	IGHV3-30*04	ARSGGGSYRGPF DY	IGKV1-9*01	QQLNSYVT
2M-2D4	2	IGHV3-23*04	AKIGLGLGGLLR YFDY	IGKV3-11*01	QQR TNWPL
2M-2G12	2	IGHV3-11*04	DANYDYVVAQT GYGR	IGKV1-39*01	QQNYSTWT
2M-4G4	2	IGHV1-46*01	ARERGDSSGYEII TTANRRFGMDV	IGLV2-23*01	CSYAVSSTWV
2M-2D1	2	IGHV4-39*01	ARGDRIQLWLLD AFDI	IGLV1-47*01	CSTGAQPEWL G
2M-7E9	2	IGHV1-69*01	ARIPGWDRGTDR NWNDD	IGKV3-11*01	QQR SNWPPAF T
2M-8E7	2	IGHV1-69*01	ARTYSFDSSGY DY	IGKV3-11*01	QQHSNWPPKI T
2M-8H10	2	IGHV3-30*04	ARAFYDSNWSVG SYFDS	IGKV4-1*01	QQYYNNQWT
2M-9F10	2	IGHV3-9*01	AKDSVRREYTHAR VPFDN	IGKV1-39*01	QQSFVSPRT
2M-9H1	2	IGHV3-30*18	AKSSKIFYLGES REVDY	IGKV1-17*01	LQHKSYP LT
2M-12D7	2	IGHV3-9*01	AKDVRYCSSTSC YFSAFDI	IGKV1-39*01	QQSYSTPRT
0304-2F8	3	IGHV5-51*01	ARRGDGLYYYGM DV	IGKV2-28*01	MQALQTPQT
0304-3H3	4	IGHV4-59*01	ARDRIAPVGKFF GWYFDL	IGKV3-15*01	QQYNKWPPW T

0304-4A2	4	IGHV4-39*07	ARELFTAVAGKGG IDY	IGKV4-1*01	HQYYNTPRT
0304-4A10	4	IGHV3-64*01	ARSSSRGFDY	IGKV4-1*01	QQYYSSPYA
0317-A1	5	IGHV3-30*18	AKDFKGGSSSWYT PEIEYGMDV	IGKV3-11*01	QQRSNWPPT
0317-A2	5	IGHV7-4-1*02	ARLIRHEAHTYCS GGSCYSPDYYYG MDV	IGKV1-39*01	QQSYSTPPT
0317-A3	5	IGHV3-48*03	ASNPLGEPYFDI	IGKV1-39*01	QQTYRPPWT
0317-A7	5	IGHV3-30-3*01	ARWGGGMQYLDV	IGKV2-28*01	MQTLQTPYT
0317-A8	5	IGHV7-4-1*02	ARAGPNYDFWSG YYQTFDY	IGKV1-33*01	QQYDNLPLT
0317-A9	5	IGHV1-24*01	ATATAMDGYYYY YGMDV	IGKV2-24*01	MQATQFPYT
0317-B1	5	IGHV5-51*01	ASAGSSWYGDAFD I	IGKV4-1*01	QQYYSTYGS
0317-C4	5	IGHV1-24*01	ATATIFGVANNWF DP	IGLV1-51*01	GTWDSSLSVV V
0317-C9	5	IGHV3-30*18	AKDLGYDILTQG LGGYYYYYGMDV	IGLV1-44*01	AAWDDSLNG VV
4A8	6/7/8/9/ 10	IGHV1-24*01	ATSTAVAGTPDLF DYYYGMDV	IGKV2-24*01	TQATQFPYT
9A1	6/7/8/9/ 10	IGHV3-30*18	AKVSAIFWLQQL SPIDV	IGKV3-15*01	HQYSKWVPT
8D2	6/7/8/9/ 10	IGHV3-7*01	ARDWDYDILTGS WFGAFDI	IGKV1-17*01	LQHNSYPLT
8D9	6/7/8/9/ 10	IGHV3-7*01	ARPTIGYSYGSY	IGLV3-1*01	QAWDSSTGV
10C10	6/7/8/9/ 10	IGHV3-7*01	ARDWDYDILTGS WFGAFDI	IGKV1-17*01	LQHNNYPLT

Table S3**Cryo-EM data collection and refinement statistics.**

Data collection		
EM equipment	Titan Krios (Thermo Fisher Scientific)	
Voltage (kV)	300	
Detector	Gatan K3 Summit	
Energy filter	Gatan GIF Quantum, 20 eV slit	
Pixel size (Å)	1.087	
Electron dose (e-/Å2)	50	
Defocus range (µm)	-1.2 ~ -2.2	
Number of collected micrographs	1,685	
Number of selected micrographs	1,596	
Sample	S-ECD of SARS-CoV-2 bound with 4A8 complex	
3D Reconstruction		
	Overall	Interface between NTD and 4A8
Software	cryoSPARC/ Relion	Relion
Number of used particles	171,673	515,019
Resolution (Å)	3.1	3.4
Symmetry	C1	
Map sharpening B factor (Å²)	-90	
Refinement		
Software	Phenix	
Cell dimensions (Å)	313.056	
Model composition		
Protein residues	4,420	
Side chains assigned	4,420	
Sugar	74	
R.m.s deviations		
Bonds length (Å)	0.008	
Bonds Angle (°)	1.032	
Ramachandran plot statistics (%)		
Preferred	90.98	
Allowed	8.62	
Outlier	0.40	

Movie S1.

Cryo-EM structure of the 4A8 and S-ECD complex revealing the recognition of the NTD by monoclonal antibody.

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