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## **Cross-neutralization of a SARS-CoV-2 antibody to a functionally conserved site is mediated by avidity**

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26 **ABSTRACT**

27 Most antibodies isolated from COVID-19 patients are specific to SARS-CoV-2. COVA1-  
28 16 is a relatively rare antibody that also cross-neutralizes SARS-CoV. Here we determined  
29 a crystal structure of COVA1-16 Fab with the SARS-CoV-2 RBD, and a negative-stain EM  
30 reconstruction with the spike glycoprotein trimer, to elucidate the structural basis of its  
31 cross-reactivity. COVA1-16 binds a highly conserved epitope on the SARS-CoV-2 RBD,  
32 mainly through a long CDR H3, and competes with ACE2 binding due to steric hindrance  
33 rather than epitope overlap. COVA1-16 binds to a flexible up conformation of the RBD on  
34 the spike and relies on antibody avidity for neutralization. These findings, along with  
35 structural and functional rationale for the epitope conservation, provide a blueprint for  
36 development of more universal SARS-like coronavirus vaccines and therapies.

37

38 **MAIN**

39 The ongoing coronavirus infectious disease 2019 (COVID-19) pandemic of severe acute  
40 respiratory syndrome coronavirus 2 (SARS-CoV-2) [1] is unlikely to end anytime soon [2].  
41 Given the current lack of protective vaccines and antivirals, virus clearance and recovery  
42 of SARS-CoV-2 patients have to rely mainly on the generation of a neutralizing antibody  
43 response. To date, most neutralizing antibodies from convalescent patients target the  
44 receptor-binding domain (RBD) on the trimeric spike (S) glycoprotein [3-7], whose natural  
45 function is to mediate viral entry by first attaching to the human receptor angiotensin-  
46 converting enzyme 2 (ACE2) and then fusing its viral membrane with the host cell [1, 8-  
47 11]. SARS-CoV-2 is phylogenetically closely related to SARS-CoV [1], which caused the  
48 2002-2003 human epidemic. However, SARS-CoV-2 and SARS-CoV only share 73%  
49 amino-acid sequence identity in their RBD, compared to 90% in their S2 fusion domain.  
50 Nevertheless, a highly conserved epitope on the SARS-CoV-2 RBD was previously  
51 identified from studies of a SARS-CoV neutralizing antibody CR3022 [12, 13], which was  
52 originally isolated almost 15 years ago [14]. Many human monoclonal antibodies have  
53 now been shown to target the SARS-CoV-2 S protein [3-7, 13, 15-24], but cross-  
54 neutralizing antibodies are relatively uncommon in COVID-19 patients [5, 6, 19, 25]. To  
55 date, the only structurally characterized cross-neutralizing human antibodies are S309 [18]  
56 and ADI-56046 [17] from SARS-CoV survivors, as well as EY6A from a COVID-19 patient  
57 [26]. Such structural and molecular characterization of cross-neutralizing antibodies is  
58 extremely valuable for therapeutic and vaccine design to confer broader protection against  
59 human SARS-like viruses that include the extensive reservoir of zoonotic coronaviruses  
60 in bats, camels, pangolins etc.

61

62 Antibody COVA1-16 was recently isolated from a convalescent COVID-19 patient and can  
63 cross-neutralize both SARS-CoV-2 (IC<sub>50</sub> 0.13 µg/mL) and SARS-CoV (IC<sub>50</sub> 2.5 µg/mL)

64 pseudovirus [6]. The heavy and light chains of COVA1-16 are encoded by IGHV1-46,  
65 IGHD3-22, IGHJ1, and by IGKV1-33, IGKJ4, with a relatively long complementarity  
66 determining region (CDR) H3 of 20 amino acids (Figure S1). IGHV of COVA1-16 is only  
67 1% somatically mutated at the nucleotide sequence level (one amino-acid change) from  
68 the germline gene, whereas its IGKV is 1.4% somatically mutated (three amino-acid  
69 changes). Here we determined the crystal structure of COVA1-16 in complex with SARS-  
70 CoV-2 RBD at 2.89 Å resolution to identify its binding site (epitope) and mechanism of  
71 cross-neutralization (Figure 1A, Table S1). The epitope of COVA1-16 overlaps extensively  
72 with that of CR3022, but also extends towards the periphery of the ACE2 binding site  
73 (Figure 1B) [13]. Seventeen out of 25 residues in the COVA1-16 epitope overlap with the  
74 CR3022 binding site (17 of 28 residues) (Figure 1C). Consistent with structural  
75 identification of its epitope, COVA1-16 can compete with CR3022 for RBD binding (Figure  
76 S2). COVA1-16 appears to have some resemblance to SARS-CoV cross-neutralizing  
77 antibody ADI-56046, whose epitope appears to span both the CR3022 epitope and ACE2-  
78 binding site, as indicated by negative-stain electron microscopy (nsEM) [17]. Interestingly,  
79 COVA1-16 also competes with ACE2 for RBD binding (Figure S2) [6], although its epitope  
80 does not overlap the ACE2 binding site (Figure 1B). Therefore, COVA1-16 inhibits ACE2  
81 binding due to steric hindrance with its light chain rather than by direct interaction with the  
82 receptor binding site (Figure 1D).

83

84 The RBD can adopt up and down conformations on the S trimer [27, 28]. While the ACE2  
85 receptor only binds the RBD in the up conformation [9], previously characterized cross-  
86 neutralizing antibodies S309 from a convalescent SARS-CoV patient and COVA2-15 from  
87 a SARS-CoV-2 patient [6], can bind the RBD in both up and down conformations [18, 27].  
88 However, unlike S309, the COVA1-16 epitope is completely buried when the RBD is in  
89 the down conformation (Figure 2A), akin to the CR3022 epitope [13]. Even in the up

90 conformation of the RBD on an unliganded SARS-CoV-2 S trimer [27], the epitope of  
91 COVA1-16 would not be fully exposed (Figure 2A). We thus performed nsEM analysis of  
92 COVA1-16 in complex with the SARS-CoV-2 S trimer (Figure 2B). Three-dimensional (3D)  
93 reconstructions revealed that COVA1-16 can bind to a range of RBD orientations on the  
94 S protein when in the up position, indicating its rotational flexibility (Figure 2C). COVA1-  
95 16 can bind the S trimer either from the top (i.e. perpendicular to the trimer apex, Figure  
96 2C, yellow, blue and pink) or from the side (i.e. more tilted, Figure 2C, brown). Model fitting  
97 of the COVA1-19/RBD crystal structure into the nsEM map indicates that the RBD on the  
98 S trimer is more open around the apex when COVA1-16 binds compared to unliganded  
99 trimers (Figure S3A-B). Bivalent binding of the COVA1-16 IgG between adjacent S trimers  
100 also appears to be plausible (Figure S3C). A recent cryo-electron tomography (cryo-ET)  
101 analysis demonstrated that the average distance between prefusion S on the viral surface  
102 is around 150 Å [29], which is comparable to the distance spanned between the tip of the  
103 two Fabs on an IgG (typically around 100 Å to 150 Å, although longer distances have been  
104 observed) [30]. Indeed, COVA1-16 IgG binds much more tightly than Fab to SARS-CoV-  
105 2 RBD, with dissociation constants ( $K_D$ ) of 0.2 nM and 46 nM, respectively (Figure S4A),  
106 reflecting bivalent binding in the assay format. Similarly, COVA1-16 IgG binds more  
107 strongly than Fab to SARS-CoV RBD ( $K_D$  of 125 nM vs 405 nM) (Figure S4B). Moreover,  
108 the apparent affinity of COVA1-16 IgG decreased to approximately the Fab value when  
109 the amount of SARS-CoV-2 RBD loaded on the biosensor was decreased, substantiating  
110 the notion that COVA1-16 can bind bivalently in this assay (Figure S4C).

111

112 Bivalent IgG binding is also important for the neutralization activity of COVA1-16 (Figure  
113 2D-E). COVA1-16 IgG neutralizes SARS-CoV-2 pseudovirus with a half maximal inhibitory  
114 concentration ( $IC_{50}$ ) of 0.02 µg/mL, which is similar to that previously measured for SARS-  
115 CoV-2 pseudovirus ( $IC_{50}$  of 0.13 µg/mL) [6]. In contrast, COVA1-16 Fab does not

116 neutralize SARS-CoV-2 pseudovirus even up to 13  $\mu\text{g}/\text{mL}$ . A similar effect is also  
117 observed for SARS-CoV pseudovirus, which is neutralized by COVA1-16 IgG at an  $\text{IC}_{50}$   
118 of 29  $\mu\text{g}/\text{mL}$ , but not by COVA1-16 Fab even up to 67  $\mu\text{g}/\text{mL}$  (Figure 2E). Of note, COVA1-  
119 16 is less potent against authentic SARS-CoV-2 ( $\text{IC}_{50} = 0.75 \mu\text{g}/\text{mL}$ ) [6]. Whether such a  
120 difference is due to variation in S protein density on the viral surface versus pseudovirus  
121 or to other factors deserves future investigation. It will also be informative to compare the  
122 number, density and conformational states of the S proteins on SARS-CoV-2 and SARS-  
123 CoV virions. Overall, our findings support the importance of bivalent binding for SARS-  
124 CoV-2 neutralizing antibodies, and especially for cross-neutralization of SARS-CoV. Such  
125 a contribution of bivalent IgG (avidity) to SARS-CoV-2 neutralization has also been  
126 suggested in a recent study that compared binding of polyclonal IgGs and Fabs [24].  
127 Furthermore, a single-domain camelid antibody VHH-72 dramatically improved its  
128 neutralization activity to SARS-CoV-2 when expressed as a bivalent Fc-fusion [31]. These  
129 observations are similar to some influenza broadly neutralizing antibodies to the  
130 hemagglutinin (HA) receptor binding site, where bivalent binding can increase avidity and  
131 neutralization breadth [32, 33].

132

133 Next we examined the molecular details of the interactions between COVA1-16 and  
134 SARS-CoV-2. COVA1-16 binding to the RBD is dominated by the heavy chain, which  
135 accounts for 82% of its total buried surface area (BSA, 694  $\text{\AA}^2$  out of a total of 844  $\text{\AA}^2$ ).  
136 Most of the interactions are mediated by CDR H3 (Figure 3A), which contributes 70% (594  
137  $\text{\AA}^2$ ) of the total BSA. CDR H3 forms a beta-hairpin with a type I beta-turn at its tip and is  
138 largely encoded by IGHD3-22 (from  $V_H$  N98 to  $V_H$  Y100f, Figure S1C and Figure 3B). The  
139 beta-hairpin conformation is stabilized by four main chain-main chain hydrogen-bonds (H-  
140 bonds) and a side chain-side chain H-bond between  $V_H$  N98 and  $V_H$  Y100f at either end  
141 of the IGHD3-22-encoded region (Figure 3B). Four H-bonds between the tip of CDR H3

142 and the RBD are formed from two main chain-main chain interactions with RBD C379,  
143 and two with V<sub>H</sub> R100b (Table S2). The positively charged guanidinium of V<sub>H</sub> R100b also  
144 interacts with the negative dipole at the C-terminus of a short  $\alpha$ -helix in the RBD (residues  
145 Y365 to Y369). Interestingly, V<sub>H</sub> R100b is a somatically mutated residue (codon = AGG in  
146 the IGHD3-22-encoded region, where the germline residue is a Ser (codon = AGT, Figure  
147 S1C). The short Ser side chain would likely not contact the RBD nor provide electrostatic  
148 complementarity. Interestingly, a somatic revertant V<sub>H</sub> R100bS actually improved binding  
149 affinity of COVA1-16 to the RBD, mostly due to an increased on-rate (Figure S5).  
150 Nevertheless, COVA1-16 has a much slower off-rate than its V<sub>H</sub> R100bS mutant, which  
151 may have led to its selection. The CDR H3 tip also interacts with the RBD through  
152 hydrophobic interactions between V<sub>H</sub> Y99 and the aliphatic portion of RBD K378, as well  
153 as a  $\pi$ - $\pi$  interaction between V<sub>H</sub> Y100 and the RBD V382-S383 peptide backbone (Figure  
154 3B). CDR H3 forms an additional four H-bonds with the RBD, involving the side chains of  
155 V<sub>H</sub> R97 and Q101 (Figure 3B). We further determined the unliganded structure of COVA1-  
156 16 Fab to 2.53 Å resolution and found that the CDR H3 distal region was not resolved due  
157 to lack of electron density indicating its inherent flexibility (Figure S6). CDR H1 and CDR  
158 L2 of COVA1-16 also interact with the RBD, but much less so compared to CDR H3. The  
159 V<sub>H</sub> T28 main chain and V<sub>H</sub> Y32 side chain in CDR H1 H-bond with D427 (Figure 3C, Table  
160 S2), whereas V<sub>L</sub> N53 in CDR L2 H-bonds with RBD R408 (Figure 3D, Table S2).

161

162 CDR H3-dominant antibodies have been seen in the human immune response to other  
163 viral pathogens. Striking examples are antibodies PG9 and PG16, whose CDR H3s  
164 interact extensively along their length with the apex of the HIV-1 Envelope protein [34, 35].  
165 Another example is C05, which is essentially a single loop binder that inserts its very long  
166 CDR H3 (24 residues) into the RBD of influenza HA [32], thereby providing a template for  
167 design of a high-avidity protein inhibitor of influenza virus, where the H3 loop was fused

168 to a scaffold protein [36]. The long CDR H3 of COVA1-16 may similarly facilitate  
169 therapeutic designs that could also include peptide-based antivirals, as exemplified by a  
170 potent cyclic peptide fusion inhibitor of influenza HA [37, 38].

171

172 Compared to the ACE2-binding site, the COVA1-16 epitope is much more highly  
173 conserved among SARS-CoV-2, SARS-CoV, and other SARS-related coronaviruses  
174 (SARSr-CoV) (Figure 4A-D, Figure S7 and Figure S8) [6]. To investigate possible  
175 structural and functional reasons for this sequence conservation, we analyzed the epitope  
176 location in the context of the SARS-CoV-2 trimeric S protein with all RBDs in the “down”  
177 conformation [39] (Figure 4E and Figure S7). The COVA1-16 epitope is completely buried  
178 at the center of the trimer in the interface between the S1 and S2 domains and is largely  
179 hydrophilic (Figure S9). The polar side chains of K378, Q414, R408, and D427, which are  
180 involved in binding to COVA1-16, are all very close to the interface with adjacent  
181 protomers in the S trimer. Interestingly, the R408 side chain, which is positioned by Q414  
182 via a H-bond, points towards a region in the adjacent protomer 2 with a positive  
183 electrostatic potential. Similarly, D427 is juxtaposed to a region in protomer 2 with a  
184 negative electrostatic potential. These repulsive charges would help favor the metastability  
185 required for transient opening and closing of the RBD in “up” and “down” conformations  
186 prior to ACE2 receptor binding. In contrast, the K378 side chain points towards a region  
187 in protomer 3 with negative electrostatic potential, thus favoring the “down” RBD  
188 conformation. Furthermore, in the “down” conformation, part of the COVA1-16 epitope  
189 interacts with the long helices formed from the heptad repeat motifs of S2 fusion domain  
190 (Figure 4E-F). Notably, S383 and T385 in the COVA1-16 epitope make three H-bonds  
191 with the tops of the helices and their connecting regions (Figure 4F). This mixture of  
192 attractive and repulsive forces would seem to be important for control of the dynamics of  
193 the RBD and, hence, for the biological function of the metastable pre-fusion S protein in



194 receptor binding and fusion. The complementarity of fit of the epitope interface with the  
195 other RBDs and the S2 domain in the S trimer further explains the epitope conservation  
196 (Figure S10). Therefore, the high sequence conservation of the COVA1-16 epitope  
197 appears related to the functional requirement for this component of the RBD surface to be  
198 deeply buried within the S trimer in the "down" conformation.

199

200 From the SARS-CoV-2 RBD/antibody complex structures to date, a significant portion of  
201 the RBD surface can be targeted by antibodies (Figure 5). One surface not yet observed  
202 to be targeted is partially covered by N-glycans at residues N165 on the N-terminal domain  
203 (NTD) and N343 on the RBD, which may hinder B cell receptor access and create a "silent  
204 face" (Figure S11), although the N343 glycan is incorporated in the S309 epitope [18].  
205 While antibodies that target the ACE2-binding site, such as BD23 [7], CB6 [23], B38 [20],  
206 P2B-2F6 [19], CC12.1 [40], and CC12.3 [40], do not show cross-neutralization activity to  
207 SARS-CoV, the conserved epitopes further from the ACE2-binding site seem to be more  
208 able to support cross-neutralization [13, 18, 26]. It is also interesting that these so far rare  
209 cross-neutralizing antibodies, including COVA1-16, often seem to bind to epitopes that  
210 are not readily accessible in the pre-fusion native structure [17, 26]. This finding is similar  
211 to a recent discovery in influenza virus, where a class of cross-protective antibodies target  
212 a conserved epitope in the trimeric interface of the HA [41-43]. Due to the inaccessibility  
213 of the COVA1-16 epitope on the S protein, it is possible that an RBD-based rather than S-  
214 based immunogen can elicit larger numbers of COVA1-16-like antibodies. Cross-  
215 neutralizing antibodies have also provided important insights into therapeutic and vaccine  
216 design, as for influenza virus [44] and HIV [45]. As SARS-CoV-2 continues to circulate in  
217 the human population and other zoonotic coronaviruses constitute future pandemic  
218 threats [46], it is certainly worth considering the development of more universal  
219 coronavirus vaccines and therapeutics that can cross-neutralize antigenically drifted

220 SARS-CoV-2 viruses, as well as zoonotic SARS-like coronaviruses.

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464

## 465 **MATERIALS AND METHODS**

### 466 **Expression and purification of SARS-CoV-2 RBD**

467 The receptor-binding domain (RBD) (residues 319-541) of the SARS-CoV-2 spike (S)  
468 protein (GenBank: QHD43416.1), and the RBD (residues 306-527) of the SARS-CoV S  
469 protein (GenBank: ABF65836.1), were cloned into a customized pFastBac vector [47],  
470 and fused with an N-terminal gp67 signal peptide and C-terminal His<sub>6</sub> tag [13]. For each  
471 RBD, we further cloned a construct with an AviTag inserted in front of the His<sub>6</sub> tag. To  
472 express the RBD, a recombinant bacmid DNA was generated using the Bac-to-Bac  
473 system (Life Technologies). Baculovirus was generated by transfecting purified bacmid  
474 DNA into Sf9 cells using FuGENE HD (Promega), and subsequently used to infect  
475 suspension cultures of High Five cells (Life Technologies) at an MOI of 5 to 10. Infected  
476 High Five cells were incubated at 28 °C with shaking at 110 r.p.m. for 72 h for protein  
477 expression. The supernatant was then concentrated using a 10 kDa MW cutoff  
478 Centrimate cassette (Pall Corporation). The RBD protein was purified by Ni-NTA,  
479 followed by size exclusion chromatography, and buffer exchanged into 20 mM Tris-HCl  
480 pH 7.4 and 150 mM NaCl. For binding experiments, RBD with AviTag was biotinylated as  
481 described previously [32] and purified by size exclusion chromatography on a Hiload 16/90  
482 Superdex 200 column (GE Healthcare) in 20 mM Tris-HCl pH 7.4 and 150 mM NaCl.

483

### 484 **Expression and purification of Fabs**

485 Expression plasmids encoding the heavy and light chains of the COVA1-16 Fab were  
486 transiently co-transfected into ExpiCHO cells at a ratio of 2:1 (HC:LC) using



487 ExpiFectamine™ CHO Reagent (Thermo Fisher Scientific) according to the  
488 manufacturer's instructions. The supernatant was collected at 10 days post-transfection.  
489 The Fabs were purified with a CaptureSelect™ CH1-XL Affinity Matrix (Thermo Fisher  
490 Scientific) followed by size exclusion chromatography.

491

## 492 **Expression and purification of ACE2**

493 The N-terminal peptidase domain of human ACE2 (residues 19 to 615, GenBank:  
494 BAB40370.1) was cloned into phCMV3 vector and fused with a C-terminal Fc tag. The  
495 plasmids were transiently transfected into Expi293F cells using ExpiFectamine™  
496 Reagent (Thermo Fisher Scientific) according to the manufacturer's instructions. The  
497 supernatant was collected at 7 days post-transfection. Fc-tagged ACE2 protein was then  
498 purified with a Protein A column (GE Healthcare) followed by size exclusion  
499 chromatography.

500

## 501 **Crystallization and x-ray structure determination**

502 The COVA1-16 Fab complex with RBD was formed by mixing each of the protein  
503 components in an equimolar ratio and incubating overnight at 4°C. The COVA1-16  
504 Fab/RBD complex and COVA1-16 Fab apo (unliganded) protein were adjusted to around  
505 11 mg/mL and screened for crystallization using the 384 conditions of the JCSG Core  
506 Suite (Qiagen) on our custom-designed robotic CrystalMation system (Rigaku) at Scripps  
507 Research. Crystallization trials were set-up by the vapor diffusion method in sitting drops  
508 containing 0.1 µl of protein and 0.1 µl of reservoir solution. Crystals used for x-ray data  
509 collection were harvested from drops containing 0.2 M sodium iodide and 20% (w/v)  
510 polyethylene glycol 3350 for the COVA1-16 Fab/RBD complex and from drops containing  
511 0.08 M acetate pH 4.6, 20% (w/v) polyethylene glycol 4000, 0.16 M ammonium sulfate  
512 and 20% (v/v) glycerol for the COVA1-16 Fab. Crystals appeared on day 3, were

513 harvested on day 7, pre-equilibrated in cryoprotectant containing 20% glycerol, and then  
514 flash cooled and stored in liquid nitrogen until data collection. Diffraction data were  
515 collected at cryogenic temperature (100 K) at Stanford Synchrotron Radiation Lightsource  
516 (SSRL) on the new Scripps/Stanford beamline 12-1 with a beam wavelength of 0.97946  
517 Å, and processed with HKL2000 [48]. Structures were solved by molecular replacement  
518 using PHASER [49]. The models for molecular replacement of RBD and COVA1-16 were  
519 from PDB 6XC4 [40], 4IMK [50] and 2Q20 [51]. Iterative model building and refinement  
520 were carried out in COOT [52] and PHENIX [53], respectively. Epitope and paratope  
521 residues, as well as their interactions, were identified by accessing PISA at the European  
522 Bioinformatics Institute ([http://www.ebi.ac.uk/pdbe/prot\\_int/pistart.html](http://www.ebi.ac.uk/pdbe/prot_int/pistart.html)) [54].

523

#### 524 **Expression and purification of recombinant S proteins**

525 The SARS-CoV-2 S construct used for negative stain EM contains the mammalian-codon-  
526 optimized gene encoding residues 1-1208 of the S protein (GenBank: QHD43416.1),  
527 followed by a C-terminal T4 fibritin trimerization domain, an HRV3C cleavage site, 8x-His  
528 tag and a Twin-strep tags subcloned into the eukaryotic-expression vector pcDNA3.4.  
529 Three amino-acid mutations were introduced into the S1/S2 cleavage site (RRAR to  
530 GSAS) to prevent cleavage and two stabilizing proline mutations (K986P and V987P) to  
531 the HR1 domain. For additional S stabilization, residues T883 and V705 were mutated to  
532 cysteines to introduce a disulphide bond. The S plasmid was transfected into 293F cells  
533 and supernatant was harvested at 6 days post transfection. S protein was purified by  
534 running the supernatant through a streptactin column and then by size exclusion  
535 chromatography using a Superose 6 increase 10/300 column (GE Healthcare  
536 Biosciences). Protein fractions corresponding to the trimeric S protein were collected and  
537 concentrated.

538

539 **ns-EM sample preparation and data collection**

540 SARS-COV-2 S protein was complexed with 3x molar excess of Fab at 30 minutes prior  
541 to direct deposition onto carbon-coated 400-mesh copper grids. The grids were stained  
542 with 2 % (w/v) uranyl-formate for 90 seconds immediately following sample application.  
543 Grids were either imaged at 200 KeV or at 120 KeV on a Tecnai T12 Spirit using a 4kx4k  
544 Eagle CCD. Micrographs were collected using Legikon [55] and the images were  
545 transferred to Appion for processing. Particle stacks were generated in Appion [56] with  
546 particles picked using a difference-of-Gaussians picker (DoG-picker) [57]. Particle stacks  
547 were then transferred to Relion [58] for 2D classification followed by 3D classification to  
548 sort well-behaved classes. Selected 3D classes were auto-refined on Relion and used to  
549 make figures with UCSF Chimera.

550

551 **Protein expression and purification for antibody binding studies**

552 All constructs were expressed transiently in HEK293F (Invitrogen, cat no. R79009) cells  
553 maintained in Freestyle medium (Life Technologies). For soluble RBD proteins, cells were  
554 transfected at a density of 0.8-1.2 million cells/mL by addition of a mix of PEI<sub>max</sub> (1 µg/µL)  
555 with expression plasmids (312.5 µg/L) in a 3:1 ratio in OptiMEM. Supernatants of the  
556 soluble RBD proteins were harvested six days post transfection, centrifuged for 30 min at  
557 4000 rpm and filtered using 0.22 µm Steritop filters (Merck Millipore). Constructs with a  
558 His<sub>6</sub>-tag were purified by affinity purification using Ni-NTA agarose beads. Protein eluates  
559 were concentrated, and buffer exchanged to PBS using Vivaspin filters with a 10 kDa  
560 molecular weight cutoff (GE Healthcare). Protein concentrations were determined by  
561 Nanodrop using the proteins peptidic molecular weight and extinction coefficient as  
562 determined by the online ExPASy software (ProtParam). For the COVA1-16 IgG1  
563 antibody, suspension HEK293F cells (Invitrogen, cat no. R79007) were cultured in  
564 FreeStyle medium (Gibco) and co-transfected with the two IgG plasmids expressing the

565 corresponding HC and LC in a 1:1 ratio at a density of 0.8-1.2 million cells/mL in a 1:3  
566 ratio with 1 mg/L PEI<sub>max</sub> (Polysciences). The recombinant IgG antibodies were isolated  
567 from the cell supernatant after five days as described previously (20, 48). In short, the cell  
568 suspension was centrifuged 25 min at 4000 rpm, and the supernatant was filtered using  
569 0.22 µm pore size SteriTop filters (Millipore). The filtered supernatant was run over a 10  
570 mL protein A/G column (Pierce) followed by two column volumes of PBS wash. The  
571 antibodies were eluted with 0.1 M glycine pH 2.5, into the neutralization buffer of 1 M TRIS  
572 pH 8.7 in a 1:9 ratio. The purified antibodies were buffer exchanged to PBS using 100 kDa  
573 VivaSpin20 columns (Sartorius). The IgG concentration was determined on the NanoDrop  
574 2000 and the antibodies were stored at 4°C until further analyses.

575

#### 576 **Measurement of binding affinities using biolayer interferometry**

577 To determine the binding affinity of COVA1-16 IgG and His-tagged Fabs, 20 µg/mL of His-  
578 tagged SARS-CoV or SARS-CoV-2 RBD protein in running buffer (PBS, 0.02% Tween-  
579 20, 0.1% BSA) was loaded on Ni-NTA biosensors (ForteBio) for 300 s. Streptavidin  
580 biosensors (ForteBio) were used if the RBD was biotinylated. Next, the biosensors were  
581 transferred to running buffer containing IgG or Fab to determine the association rate, after  
582 which the sensor was transferred to a well containing running buffer to allow dissociation.  
583 As negative control, an anti-HIV-1 His-tagged Fab was tested at the highest concentration  
584 used for COVA1-16 Fab (400 nM). After each cycle, the sensors were regenerated by  
585 alternating 20 mM glycine in PBS and running buffer three times, followed by reactivation  
586 in 20 mM NiCl<sub>2</sub> for 120 s. All steps were performed at 1000 rpm shaking speed. K<sub>D</sub>s were  
587 determined using ForteBio Octet CFR software. The avidity effects of IgG were  
588 investigated by titrating the SARS-CoV-2 RBD concentration (5, 1, 0.2 and 0.04 µg/mL)  
589 followed by loading on Ni-NTA biosensors for 480 s with an additional loading step with

590 His-tagged HIV-1 gp41 for 480 s to minimize background binding of His-tagged Fabs to  
591 the biosensor. All other steps were performed as described above.

592

### 593 **Competition studies of antibodies with ACE-2 receptor**

594 For competition assays, COVA1-16 IgG, CR3022 IgG, and human ACE2-Fc were all  
595 diluted to 250 nM. Ni-NTA biosensors were used. In brief, the assay has five steps: 1)  
596 baseline: 60 s with 1x kinetics buffer; 2) loading: 180 s with 20 µg/mL, His<sub>6</sub>-tagged SARS-  
597 CoV-2 RBD proteins; 3) baseline: 150 s with 1x kinetics buffer; 4) first association: 300 s  
598 with CR3022 IgG or human ACE2-Fc; and 5) second association: 300 s with human ACE2-  
599 Fc, CR3022 IgG, or COVA1-16 IgG.

600

### 601 **Pseudovirus neutralization assay**

602 Neutralization assays were performed using SARS-CoV and SARS-CoV-2 S-  
603 pseudotyped HIV-1 virus and HEK-293T/ACE2 cells as described previously [59]. In brief,  
604 pseudotyped virus was produced by co-transfecting expression plasmids of SARS-CoV S  
605 and SARS-CoV-2<sub>Δ19</sub> S proteins (GenBank; AAP33697.1 and MT449663.1, respectively)  
606 with an HIV backbone expressing NanoLuc luciferase (pHIV-1<sub>NL4-3</sub> ΔEnv-NanoLuc) in  
607 HEK293T cells (ATCC, CRL-11268). After 3 days, the cell culture supernatants containing  
608 SARS-CoV and SARS-CoV-2 S-pseudotyped HIV-1 viruses were stored at -80°C. HEK-  
609 293T/ACE2 cells were seeded 10,000 cells/well in a 96-well plate one day prior to the start  
610 of the neutralization assay. To determine the neutralizing capacity of COVA1-16 IgG and  
611 His<sub>6</sub>-tagged Fab, 20 or 100 µg/mL COVA1-16 IgG and equal molar of COVA1-16 Fab  
612 were serially diluted in 3-fold steps and mixed with SARS-CoV or SARS-CoV-2  
613 pseudotyped virus and incubated for 1 h at 37°C. The pseudotyped virus and COVA1-16  
614 IgG/Fab mix were then added to the HEK-293T/ACE2 cells and incubated at 37°C. After  
615 48 h, cells were washed twice with PBS (Dulbecco's Phosphate-Buffered Saline,

616 eBiosciences) and lysis buffer was added. Luciferase activity of cell lysate was measured  
617 using the Nano-Glo Luciferase Assay System (Promega) and GloMax Discover System.  
618 The inhibitory concentration (IC<sub>50</sub>) was determined as the concentration of IgG or Fab that  
619 neutralized 50% of the pseudotyped virus using GraphPad Prism software (version 8.3.0).

620

### 621 **Sequence conservation analysis**

622 RBD protein sequences from SARS-CoV and SARS-related coronavirus (SARSr-CoV)  
623 strains were retrieved from the following accession codes:

- 624 • GenBank ABF65836.1 (SARS-CoV)
- 625 • GenBank ALK02457.1 (Bat SARSr-CoV WIV16)
- 626 • GenBank AGZ48828.1 (Bat SARSr-CoV WIV1)
- 627 • GenBank ACU31032.1 (Bat SARSr-CoV Rs672)
- 628 • GenBank AIA62320.1 (Bat SARSr-CoV GX2013)
- 629 • GenBank AAZ67052.1 (Bat SARSr-CoV Rp3)
- 630 • GenBank AIA62300.1 (Bat SARSr-CoV SX2013)
- 631 • GenBank ABD75323.1 (Bat SARSr-CoV Rf1)
- 632 • GenBank AIA62310.1 (Bat SARSr-CoV HuB2013)
- 633 • GenBank AAY88866.1 (Bat SARSr-CoV HKU3-1)
- 634 • GenBank AID16716.1 (Bat SARSr-CoV Longquan-140)
- 635 • GenBank AVP78031.1 (Bat SARSr-CoV ZC45)
- 636 • GenBank AVP78042.1 (Bat SARSr-CoV ZXC21)
- 637 • GenBank QHR63300.2 (Bat CoV RaTG13)
- 638 • NCBI Reference Sequence YP\_003858584.1 (Bat SARSr-CoV BM48-31)
- 639 • GISAID EPI\_ISL\_410721 (Pangolin BetaCoV Guandong2019)

640 Multiple sequence alignment of the RBD sequences was performed by MUSCLE version  
641 3.8.31 [60]. Sequence logos were generated by WebLogo [61]. The conservation score  
642 of each RBD residue was calculated and mapped onto the SARS-CoV-2 RBD x-ray  
643 structure with ConSurf [62].

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662 P41GM103393).

663

664 **AUTHOR CONTRIBUTIONS**

665 H.L., N.C.W., M.Y. and I.A.W. conceived and designed the study. H.L., N.C.W., M.Y., and  
666 C.C.D.L. expressed and purified the proteins for crystallization. T.G.C., P.J.M.B., M.J.v.G.  
667 and R.W.S. provided antibody clones and sequences. T.G.C. performed binding analyses  
668 and J.v.S. provided neutralization data. H.L., N.C.W., M.Y. and X.Z. crystallized and  
669 determined the X-ray structures. S.B., J.L.T., and A.B.W. provided nsEM data and  
670 reconstructions. H.L., N.C.W., M.Y., and I.A.W. wrote the paper and all authors reviewed  
671 and/or edited the paper.

672

673 **COMPETING INTERESTS**

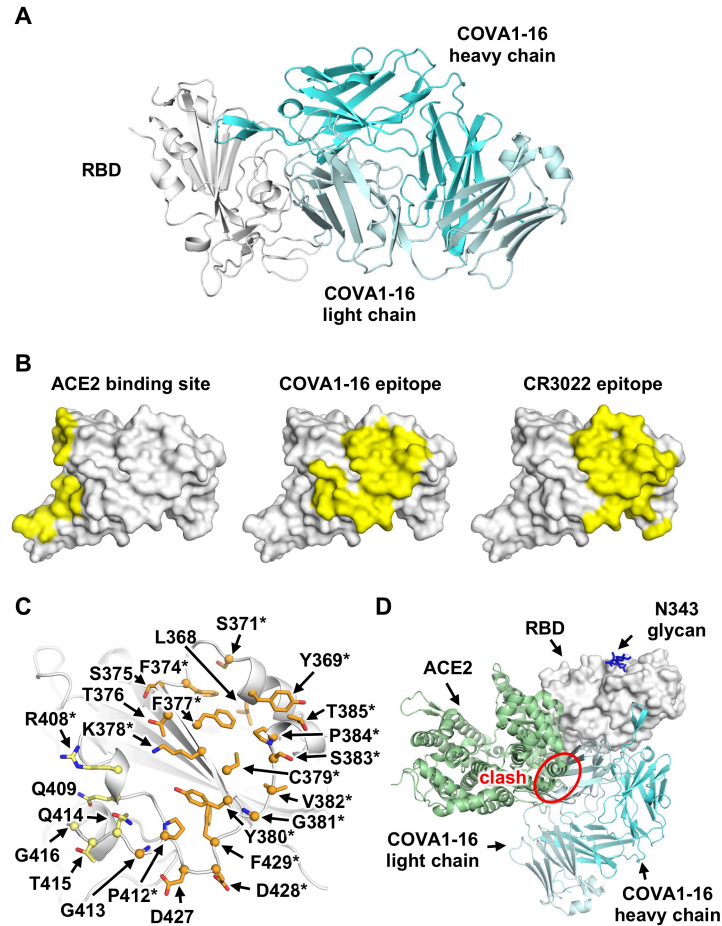
674 Amsterdam UMC previously filed a patent application on the SARS-CoV-2 antibody  
675 COVA1-16 described here [6].

676

677 **DATA AVAILABILITY**

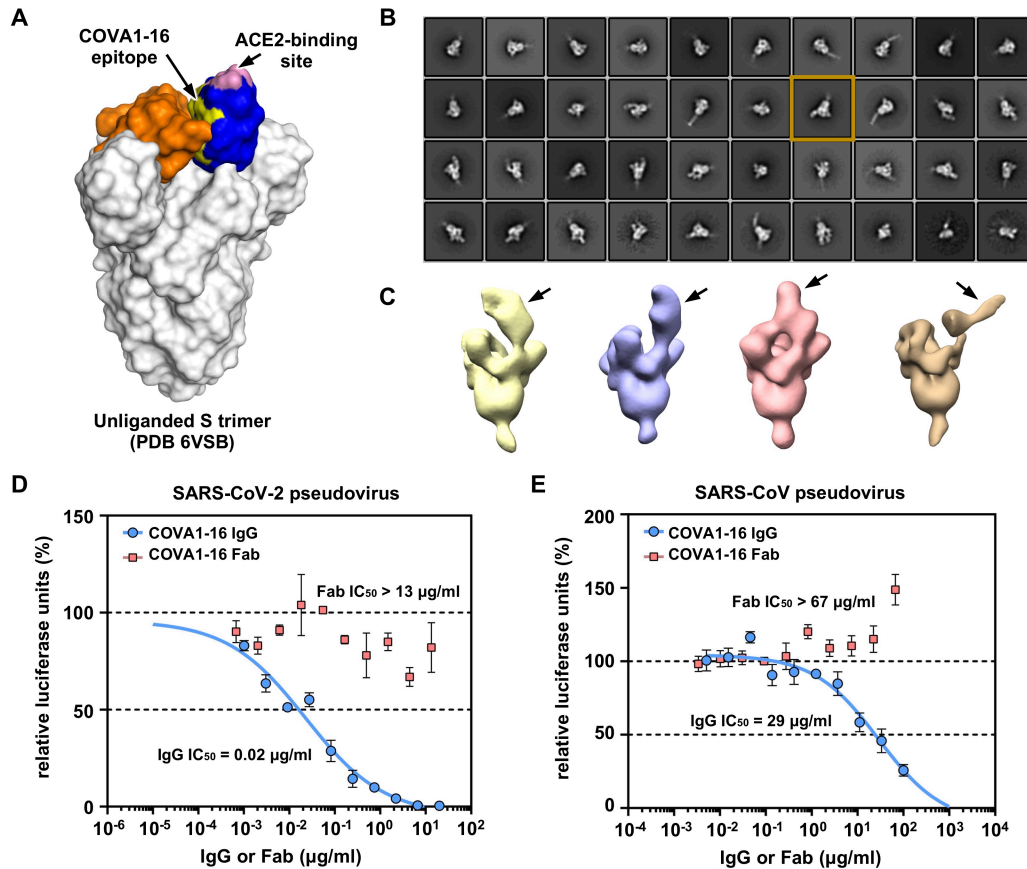
678 X-ray coordinates and structure factors are being deposited to the RCSB Protein Data  
679 Bank. The COVA1-16 IGVH and IGVK sequences are available in GenBank: MT599919  
680 and MT599835. The plasmids encoding the COVA1-16 IgG and Fab will be available from  
681 M.J.v.G. and R.W.S. under an MTA with the Amsterdam UMC. Other materials related to  
682 this paper will be available on request from the corresponding author.





683

684 **Figure 1. Comparison of COVA1-16 binding mode with CR3022 and ACE2. (A)** Crystal  
685 structure of COVA1-16/RBD complex with RBD in grey and COVA1-16 Fab in cyan (heavy  
686 chain) and greyish blue (light chain). **(B)** ACE2-binding site (PDB 6M0J, left) [10], COVA1-  
687 16 epitope (this study, middle), and CR3022 epitope (PDB 6W41, right) [13] are  
688 highlighted in yellow. **(C)** RBD residues in the COVA1-16 epitope are shown. Epitope  
689 residues contacting the heavy chain are in orange and light chain in yellow. Representative  
690 epitope residues are labeled. Residues that are also part of CR3022 epitope are indicated  
691 with asterisks. **(D)** The ACE2/RBD complex structure is aligned in the same orientation as  
692 the COVA1-16/RBD complex. COVA1-16 (cyan) would clash with ACE2 (green) if they  
693 were to approach their respective RBD binding sites at the same time (indicated by red  
694 circle).



695

696 **Figure 2. Negative-stain electron microscopy analysis and IgG avidity effect of**

697 **COVA1-16. (A)** The COVA1-16 epitope on the unliganded SARS-CoV-2 S trimer with one

698 RBD in the “up” conformation (blue) and two in the “down” conformation (orange) (PDB

699 6VSB) [27]. COVA1-16 epitope is in yellow and ACE2-binding site in pink. **(B)**

700 Representative 2D class averages from negative-stain EM analysis of SARS-CoV-2 S

701 trimer complexed with COVA1-16 Fab. The 2D class corresponding to the most outward

702 conformation of COVA-16 Fab in complex with S trimer is highlighted in a mustard box.

703 **(C)** Various conformations of COVA1-16 Fab in complex with the S trimer is revealed by

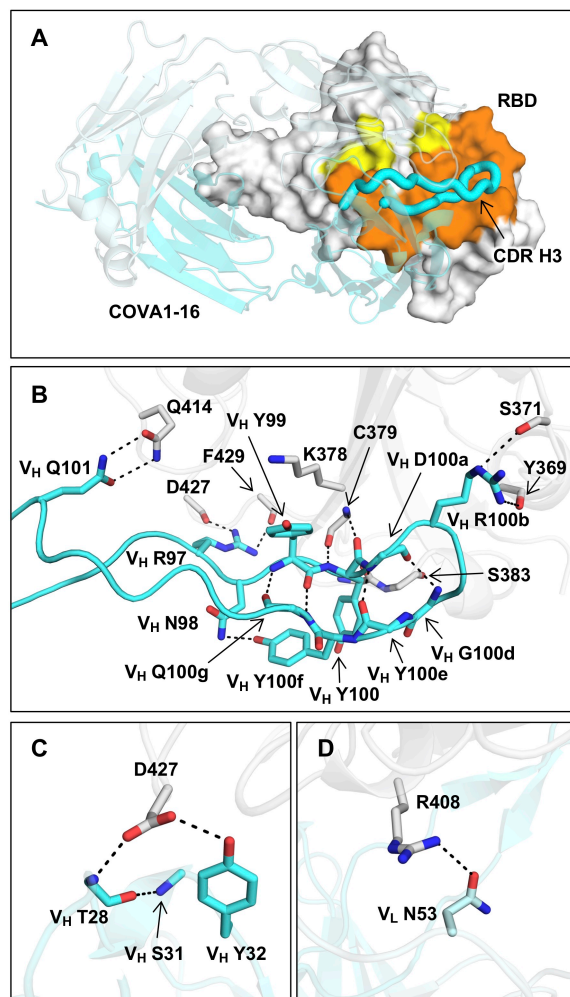
704 3D reconstructions. The location of COVA1-16 Fab is indicated by an arrow. **(D-E)**

705 Neutralization activities of COVA1-16 IgG (blue) and Fab (red) against **(D)** SARS-CoV-2

706 and **(E)** SARS-CoV are measured in a luciferase-based pseudovirus assay. The half

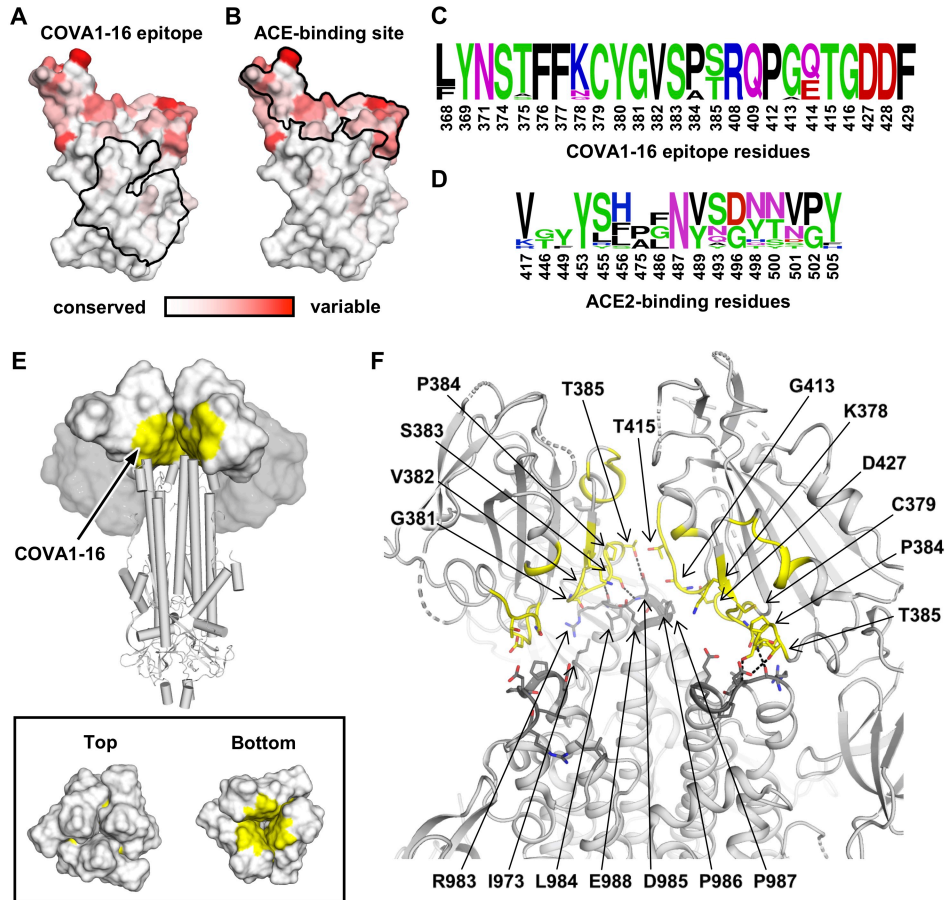
707 maximal inhibitory concentrations (IC<sub>50</sub>s) for IgG and Fab are indicated in parenthesis. Of

708 note, neutralization for the IgG ( $IC_{50} = 0.08 \mu\text{g/mL}$ ) against SARS-CoV-2 pseudovirus  
709 infecting 293T/ACE2 cells is comparable to that measured in Huh7 cells ( $IC_{50} = 0.13$   
710  $\mu\text{g/mL}$ ) as reported previously [6].



711

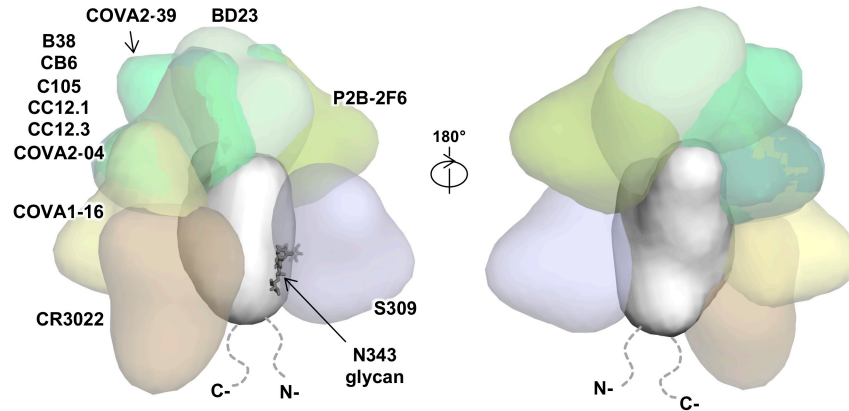
712 **Figure 3. Interaction between SARS-CoV-2 RBD and COVA1-16.** (A) The epitope of  
713 COVA1-16 is highlighted in yellow and orange. Epitope residues that are in contact with  
714 CDR H3 are in orange, and yellow otherwise. COVA1-16 (cyan) is in cartoon  
715 representation with CDR H3 depicted in a thick tube. The RBD (white) is in a surface  
716 representation. The BSA on COVA1-16 and RBD are  $844 \text{ \AA}^2$  and  $779 \text{ \AA}^2$ , respectively. (B)  
717 Interactions of SARS-CoV-2 RBD (white) with (B) CDR H3, (C) CDR H1, and (D) CDR L2  
718 of COVA1-16 (cyan) are shown. Hydrogen bonds are represented by dashed lines. In (C),  
719 a  $3_{10}$  turn is observed in CDR H1 for residues  $V_H$  T28 to  $V_H$  S31.



720

721 **Figure 4. Sequence conservation of COVA1-16 epitope and ACE2-binding site. (A-**  
 722 **B)** Sequence conservation of the RBD among 17 SARS-like CoVs (Figure S7) is  
 723 highlighted on the RBD structure with the **(A)** COVA1-16 epitope and **(B)** ACE2-binding  
 724 site indicated by the black outline. The backside of this view is shown in Figure S8. **(C-D)**  
 725 Sequence conservation of **(C)** COVA1-16 epitope and **(D)** ACE2-binding site is shown as  
 726 a sequence logo. **(E)** Location of COVA1-16 epitope (yellow) on the SARS-CoV-2 S trimer  
 727 when all three RBDs are in the down conformation (PDB 6VXX) [39]. RBDs are  
 728 represented as a white surface, N-terminal domains (NTDs) as a grey surface, and the S2  
 729 domain in a cartoon representation. Top panel: for visualization of the COVA1-16 epitope,  
 730 the RBD and NTD from one of the three protomers was removed. Bottom panel: top and  
 731 bottom views of the COVA1-16 epitopes on the three RBDs in the “down” conformation.  
 732 **(F)** COVA1-16 epitope is shown in yellow on a ribbon representation of a SARS-CoV-2 S

733 trimer (PDB 6VXX) [39]. Epitope residues in the RBD involved in interaction with the S2  
734 domain are shown in yellow sticks, and S2 domain interacting residues in dark grey sticks.  
735 Dashed lines indicate hydrogen bonds. Interface residues are calculated using PISA [54].  
736 The S1 segment from the third protomer is omitted to clarify the view of the interfaces that  
737 the COVA1-16 epitope makes with the S2 domain.



738

739 **Figure 5. Interaction between SARS-CoV-2 RBD and structurally characterized**

740 **antibodies.** The binding of known SARS-CoV-2 RBD-targeting antibodies to the RBD is

741 compared. The ACE2-binding site overlaps with epitopes of B38 (PDB 7BZ5) [20], C105

742 (6XCM) [24], CB6 (7C01) [23], CC12.1 (6XC3) [40], CC12.3 (6XC4) [40], BD23 (7BYR)

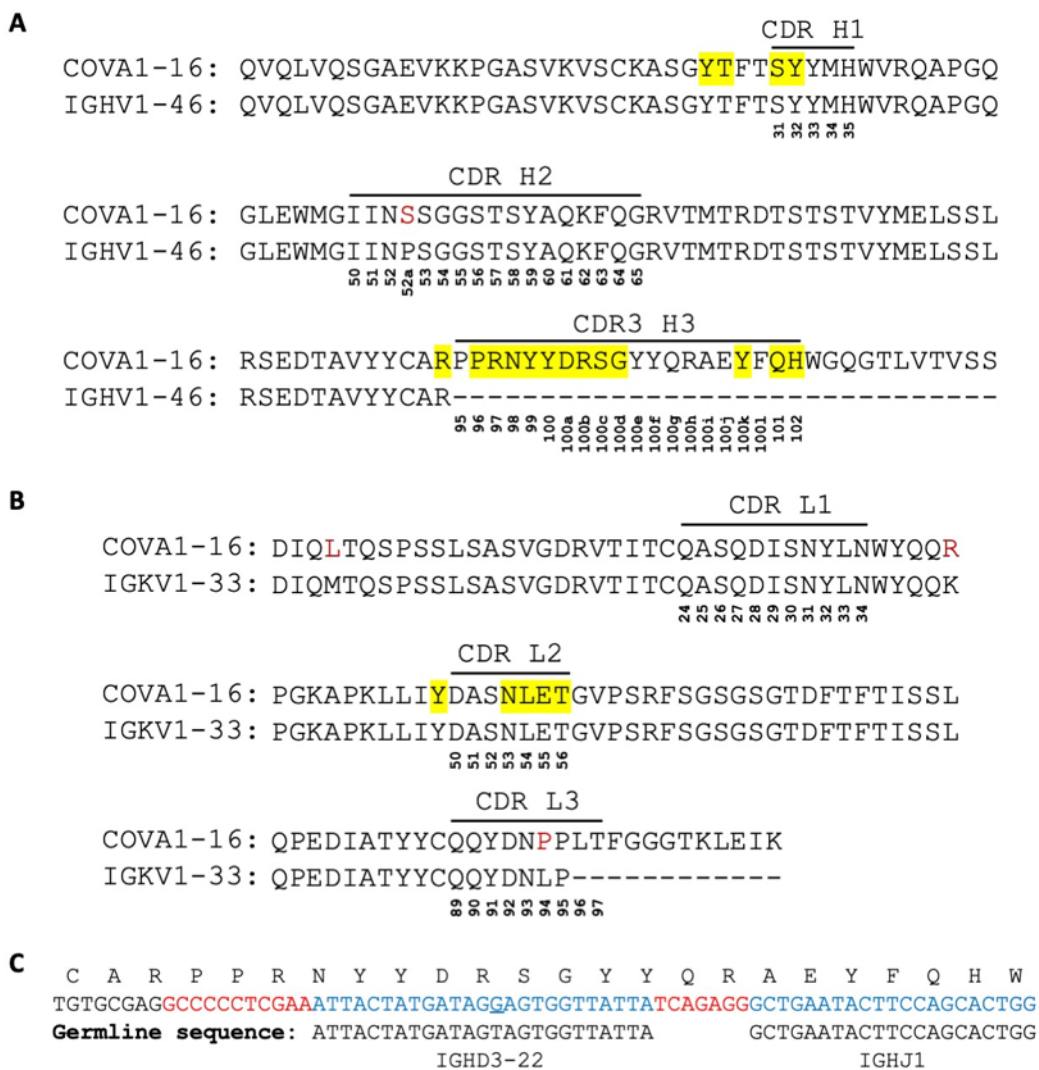
743 [7], and P2B-2F6 (7BWJ) [19], but not the epitopes of COVA1-16 (this study), CR3022

744 (PDB 6W41) [13], COVA2-04 [63], COVA2-39 [63], and S309 (PDB 6WPS) [18]. Of note,

745 while CR3022 only neutralizes SARS-CoV but not SARS-CoV-2 in *in vitro* assays [13], a

746 recent study isolated an antibody (EY6A) that binds to a similar epitope as CR3022 and

747 cross-neutralizes SARS-CoV-2 and SARS-CoV [26].



Total gene-derived nucleotides: 46

Total non-gene-derived nucleotides: 18

1

2 **Supplementary Figure 1. Comparison of COVA1-16 and putative germline**

3 **sequences.** Alignment of COVA1-16 Fab amino-acid sequence with (A) germline IGHV1-

4 46 sequence, and (B) germline IGKV1-33 sequence. The regions that correspond to CDR

5 H1, H2, H3, L1, L2, and L3 are indicated. Residues that differ from germline are highlighted

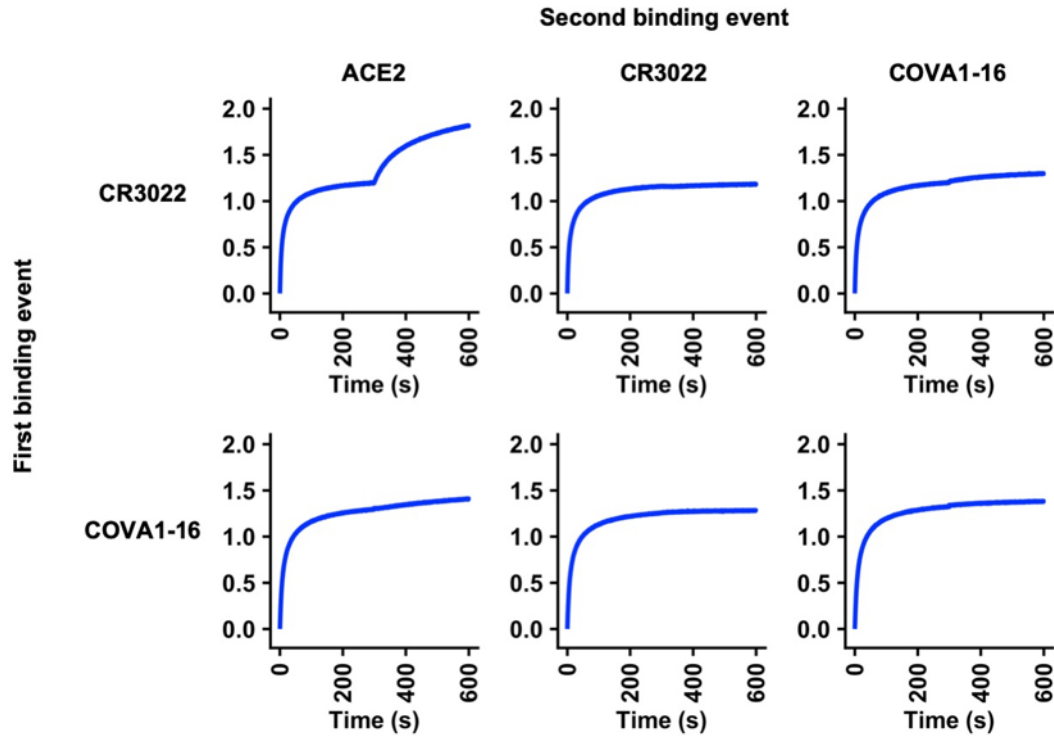
6 in red. COVA1-16 Fab residues that interact with the RBD are highlighted in yellow.

7 Residue positions in the CDRs are labeled according to the Kabat numbering scheme. (C)

8 Amino acid and nucleotide sequences of the V-D-J junction of COVA1-16, with putative



9 gene segments (blue) and N-regions (red), are indicate. The germline sequences of  
10 IGHD3-22 and IGJ1 are also shown. The only somatically mutated nucleotide in the D  
11 region is underlined.



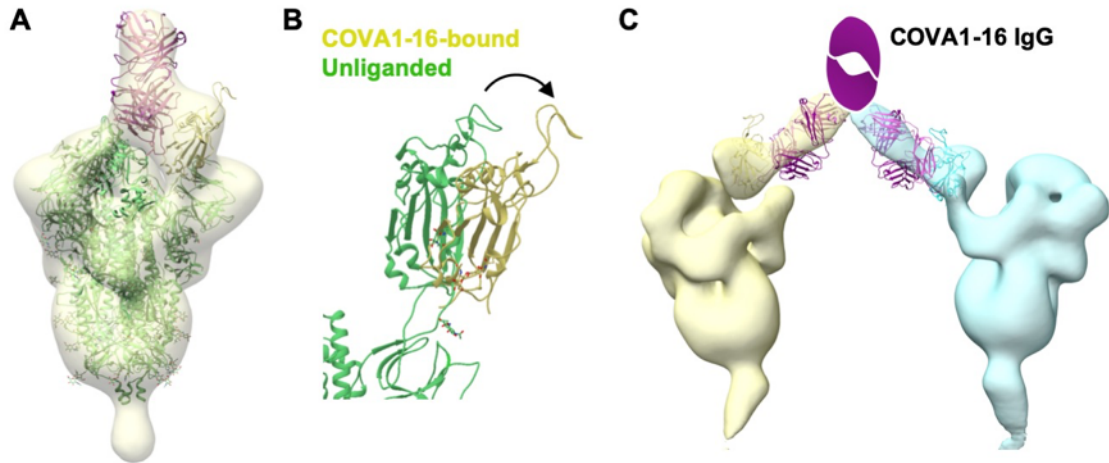
12

13 **Supplementary Figure 2. Competition assay between different IgGs and ACE2.**

14 Competition between COVA1-16 IgG, CR3022 IgG, and Fc-tagged ACE2 was measured  
15 by biolayer interferometry (BLI). Y-axis represents the response. The biosensor was first  
16 loaded with SARS-CoV-2 RBD, followed by two binding events: 1) CR3022 IgG or  
17 COVA1-16 IgG, and 2) ACE2, CR3022 IgG, or COVA1-16 IgG. A period of 300 s was  
18 used for each binding event. A further increase in signal during the second binding event  
19 (starting at 300 s time point) indicates lack of competition with the first ligand.

20

21



22

23 **Supplementary Figure 3. Negative-stain EM analysis of COVA1-16 binding to SARS-**

24 **CoV-2 S trimer. (A)** An atomic model from the crystal structure of SARS-CoV-2 RBD

25 bound to COVA1-16 Fab was fit into the negative-stain EM reconstruction of the SARS-

26 CoV-2 spike bound to COVA1-16 Fab. The COVA1-16 Fab approaches the apex of the S

27 trimer in a perpendicular orientation. A secondary structure backbone representation of

28 the prefusion spike model (PDB: 6Z97, green) [1] was also fit into the EM density with

29 RBD residues (334-528) removed from one of the protomers here for clarity. The COVA1-

30 16 heavy and light chains are in magenta and pink, respectively, and COVA1-16-bound

31 RBD in yellow. **(B)** Conformation of RBD in an up conformation from an unliganded SARS-

32 CoV-2 S trimer (PDB: 6Z97, green) [1] is compared to that of the RBD (yellow) bound by

33 COVA1-16 Fab. The arrow indicates that the RBD further rotates and opens up when

34 bound to COVA1-16, thereby moving further away from the trimer threefold axis. **(C)** An

35 atomic model of the spike RBD bound to COVA1-16 Fab is fit into a negative-stain EM

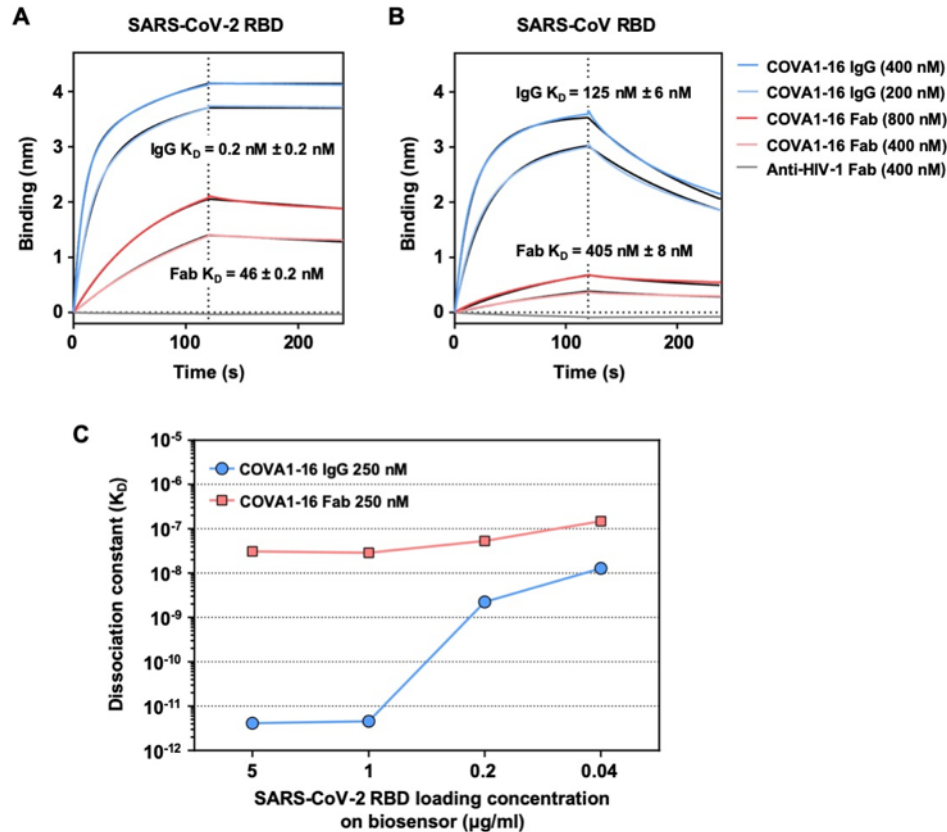
36 reconstruction, where COVA1-16 Fab approaches the SARS-CoV-2 S trimer from the

37 side. COVA1-16 is modelled as an IgG to illustrate the feasibility of bivalent binding to

38 adjacent spike proteins on the virus surface. The Fab heavy and light chains are shown in

39 magenta and pink. A schematic representation of the Fc domain of the IgG is shown in

40 magenta. The RBD model and spike density for each trimer is shown in yellow and cyan.



41

42 **Supplementary Figure 4. Sensorgrams for binding of COVA1-16 to SARS-CoV-2**

43 **RBD and SARS-CoV RBD. (A-B)** Binding kinetics of COVA1-16 Fab and IgG to (A)

44 SARS-CoV-2 RBD and (B) SARS-CoV RBD were measured by biolayer interferometry

45 (BLI) with RBD on the biosensor and antibody in solution. Y-axis represents the response.

46 An anti-HIV His-tagged Fab (4E1) was used as a negative control. Dissociation constants

47 ( $K_D$ ) for IgG and Fab were obtained using a 1:2 bivalent model and 1:1 binding model,

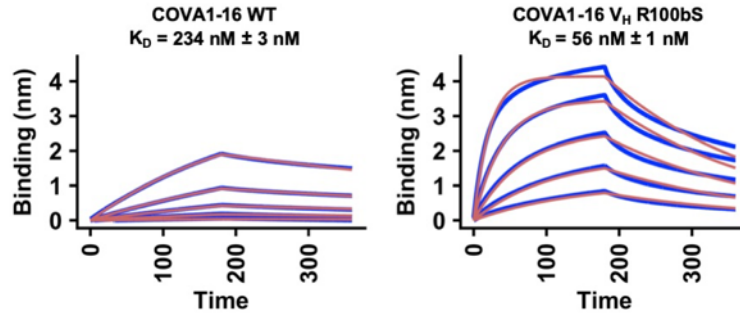
48 respectively, which are represented by the black lines. Representative results of two

49 replicates for each experiment are shown. (C) The relationship between SARS-CoV-2

50 RBD loading concentration on the biosensor and the dissociation constant of COVA1-16

51 IgG is shown.

52



53

54 **Supplementary Figure 5. Sensorgrams for binding of COVA1-16 wild-type and V<sub>H</sub>**

55 **R100bS mutant Fabs to SARS-CoV-2 RBD.** Binding kinetics of COVA1-16 wild-type and

56 V<sub>H</sub> R100bS mutant Fab to SARS-CoV-2 RBD were measured by biolayer interferometry

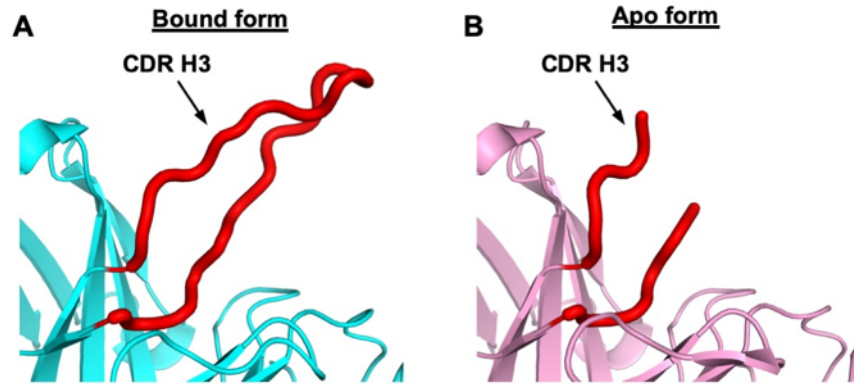
57 (BLI) with RBD on the biosensor and antibody in solution. Y-axis represents the response.

58 Dissociation constants ( $K_D$ ) for Fabs were obtained using a 1:1 binding model, which are

59 represented by the red lines. Representative results of two replicates for each experiment

60 are shown. Unlike Figure S4, which used HEK293F-expressed SARS-CoV-2, the

61 experiment here used insect cell-expressed SARS-CoV-2.



62

63 **Supplementary Figure 6. CDR H3 of COVA1-16 Fab is disordered in its unliganded**

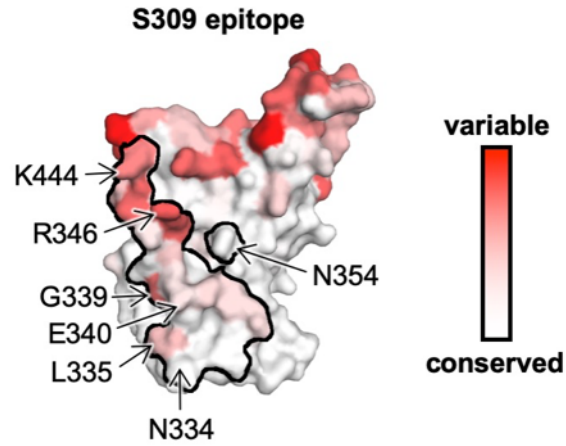
64 **apo form. (A)** In the crystal structure of the RBD-bound form of COVA1-16 Fab, the

65 CDR H3 loop is completely ordered (red). **(B)** In the crystal structure of the apo form of

66 COVA1-16, the distal end of the CDR H3 loop is intrinsically disordered or flexible (red).





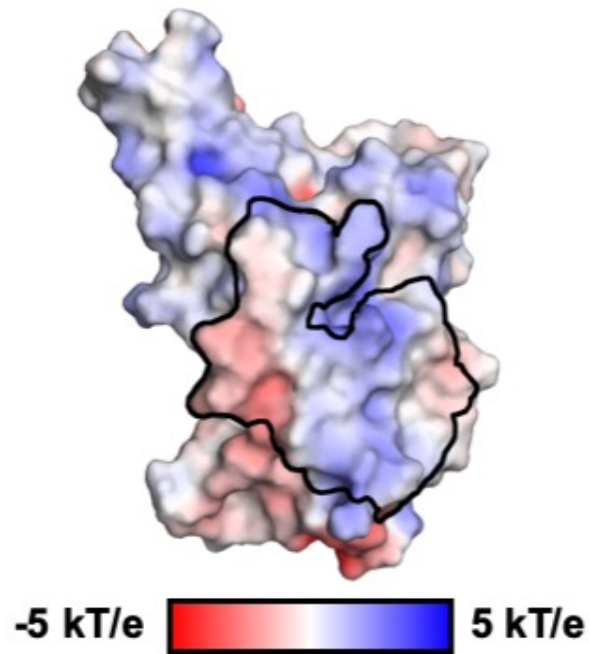


73

74 **Supplementary Figure 8. Sequence conservation of S309 epitope.** Sequence  
75 conservation of the RBD is highlighted on the structure for S309 epitope [2]. This view  
76 corresponds to the opposite side (rotated 180 degrees along the vertical axis) from that  
77 shown in Figure 4A-B.

78

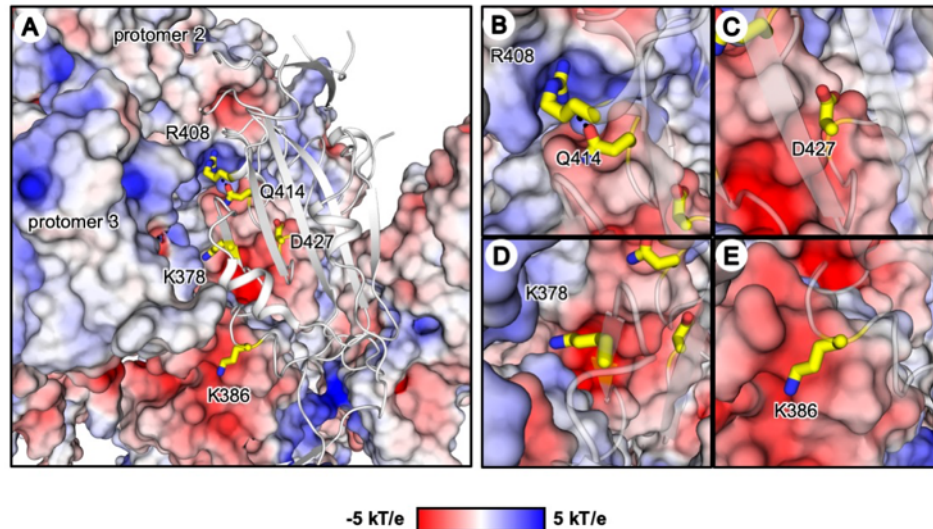
## COVA1-16 epitope



79

80 **Supplementary Figure 9. COVA1-16 epitope in electrostatic surface representation.**

81 The epitope of COVA1-16 is outlined and shows its largely polar nature.



82

83 **Supplementary Figure 10. Location of residues of interest in the COVA1-16 epitope**

84 **when all three RBDs are in the “down” conformation. (A)** The RBD of one of the three

85 protomers is shown as a gray cartoon with the side chains of five residues of interest

86 shown in yellow stick representation. RBD residues K378, R408, Q414, and D427 are

87 within the COVA1-16 epitope, whereas K386 is not a COVA1-16 epitope residue. The

88 other two protomers (protomers 2 and 3) are shown in a surface electrostatic

89 representation. **(B-E)** Zoomed-in views for the regions surrounding residues **(B)** R408 and

90 Q414, **(C)** D427, **(D)** K378, and **(E)** K386. A hydrogen bond in **(B)** is represented by a

91 dashed line. Due to charge difference or similarity between the side chain and the proximal

92 region of the neighboring protomer, either repulsive (same charge) or attractive (opposite

93 charge) environments are found and visualized here. PDB 6VXX is used to represent the

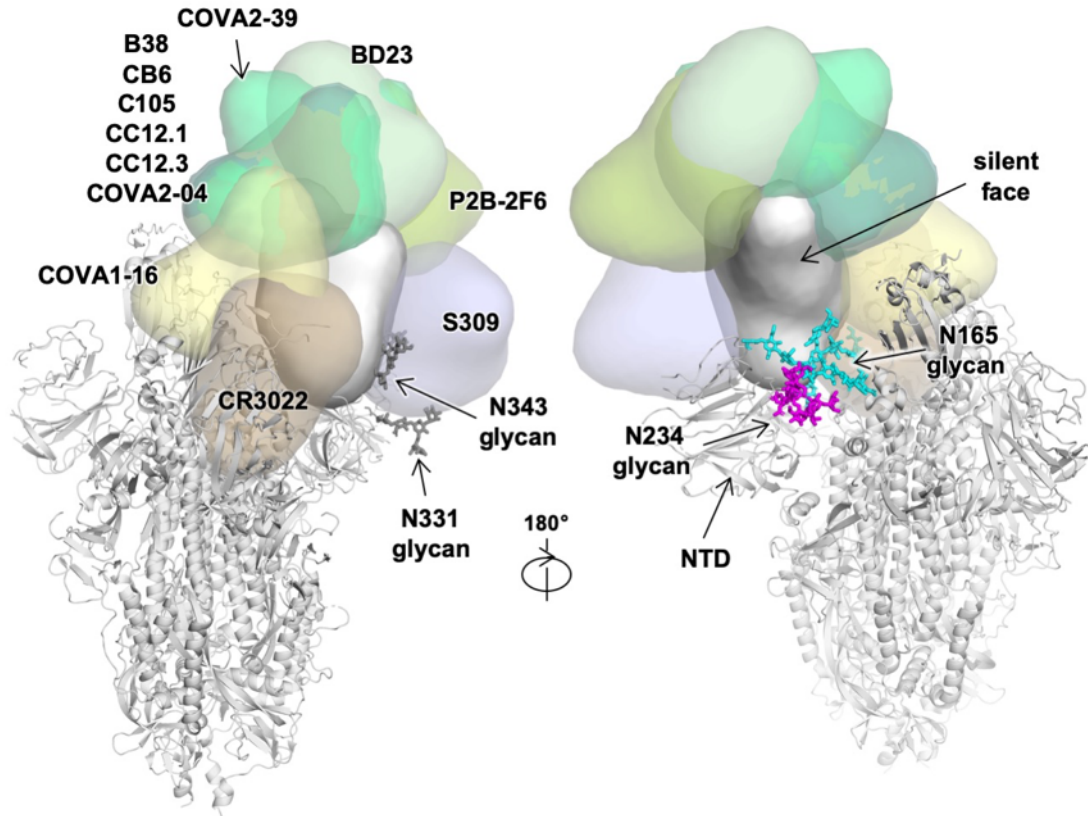
94 spike protein [3]. Of note, the shape complementarity values ( $Sc$ ) [4] of the COVA1-16

95 epitope/RBD interface, COVA1-16 epitope/S2 interface, and COVA1-16 epitope/COVA1-

96 16 interface are 0.53, 0.75, and 0.74, respectively, indicating good complementary and

97 tight fit of the COVA1-16 epitope surface with the rest of the trimer in the RBD down

98 conformation. Sc values can range from 0 to 1, with a larger Sc value represents higher  
99 shape complementarity.  
100



101

102 **Supplementary Figure 11. The N-glycan on the N-terminal domain (NTD) also**

103 **shields part of the RBD.** The antibody-bound RBD, which is displayed and colored as in

104 Figure 5, is shown in the up conformation on the S protein (PDB 6VSB) [5]. N-glycans on

105 N165 (NTD), N234, N331, and N343 (RBD) are modelled according to the main glycoform

106 observed at these sites in [6], and shown in stick representation. Antibody Fabs from

107 published crystal and cryo-EM structures are represented as globular outlines in different

108 colors as outlined in Figure 5. B38, CB6, C105, CC12.1, CC12.3, COVA2-04, COVA2-39,

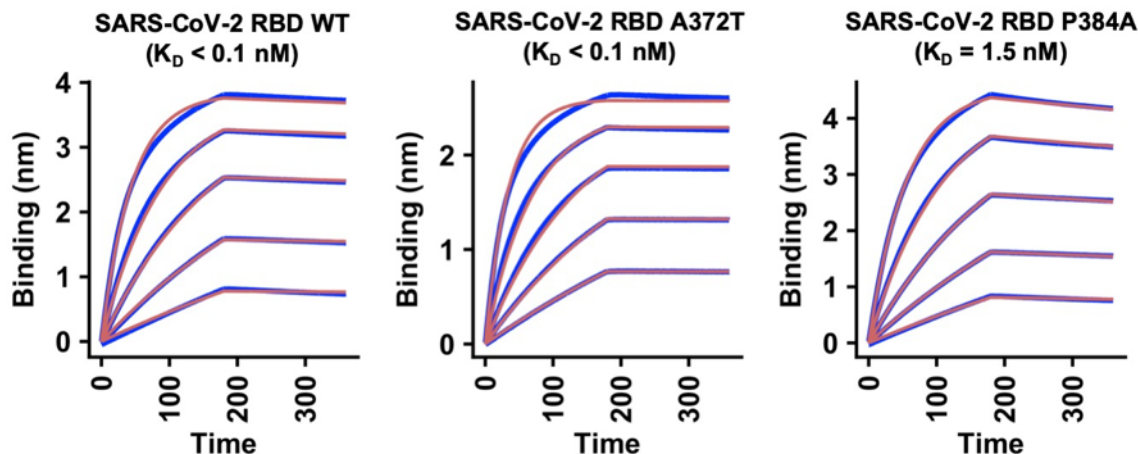
109 BD23, P2B-2F6 all bind at or around the receptor binding site. S309 binds to the elongated

110 accessible face of the RBD in both up and down conformations, and CR3022 binds to the

111 opposite face that is exposed in the RBD up conformation, but buried in the RBD down

112 conformation.

113



114

115 **Supplementary Figure 12. Sensorgrams for binding of COVA1-16 IgG to SARS-CoV-**

116 **2 RBD WT or mutants.** Binding kinetics of COVA1-16 IgG to SARS-CoV-2 RBD WT,

117 A372T, and P384A were measured by biolayer interferometry (BLI) with RBD on the

118 biosensor and antibody in solution. Y-axis represents the response. Dissociation

119 constants (K<sub>D</sub>) for Fabs were obtained using a 1:1 binding model, which are represented

120 by the red lines. Representative results of two replicates for each experiment are shown.

121 A372T and P384A are the only two mutations that differ between the SARS-CoV-2 and

122 SARS-CoV sequences in COVA1-16 epitope. The affinity of COVA1-16 IgG to the A372T

123 mutant did not show any detectable difference from WT. Although the affinity (K<sub>D</sub>) of

124 COVA1-16 IgG to the P384A mutant decreases, the binding is still 100 times tighter than

125 that measured between COVA1-16 IgG and SARS-CoV RBD (Figure S4B). As a result,

126 the binding affinity of COVA1-16 to the RBD may be influenced by residues outside of the

127 epitope as well as the dynamics of the RBD fluctuations between up and down

128 conformations.

129  
130

## Supplementary Table 1. X-ray data collection and refinement statistics

<b>Data collection</b>		
	COVA1-16 Fab + SARS-CoV-2 RBD	COVA1-16 Fab
Beamline	SSRL 12-1	SSRL 12-1
Wavelength (Å)	0.97946	0.97946
Space group	<i>P</i> 1 2 <sub>1</sub> 1	<i>P</i> 4 <sub>1</sub> 3 2
Unit cell parameters		
<i>a</i> , <i>b</i> , <i>c</i> (Å)	57.4, 124.9, 57.6	156.3, 156.3, 156.3
$\alpha$ , $\beta$ , $\gamma$ (°)	90, 96.1, 90	90, 90, 90
Resolution (Å) <sup>a</sup>	50.0-2.89 (2.95-2.89)	50.0-2.53 (2.58-2.53)
Unique reflections <sup>a</sup>	17,656 (845)	22,357 (1,084)
Redundancy <sup>a</sup>	3.7 (3.2)	37.0 (14.1)
Completeness (%) <sup>a</sup>	97.9 (93.9)	100.0 (100.0)
$\langle I/\sigma_I \rangle$ <sup>a</sup>	7.4 (1.2)	21.5 (1.3)
$R_{\text{sym}}^b$ (%) <sup>a</sup>	15.3 (69.1)	23.6 (>100)
$R_{\text{pim}}^b$ (%) <sup>a</sup>	9.0 (42.9)	3.8 (54.3)
CC <sub>1/2</sub> <sup>c</sup> (%) <sup>a</sup>	96.3 (66.8)	99.6 (52.1)
<b>Refinement statistics</b>		
Resolution (Å)	42.8-2.89	34.1-2.53
Reflections (work)	17,632	21,872
Reflections (test)	948	1,069
$R_{\text{cryst}}^d / R_{\text{free}}^e$ (%)	23.7/29.4	21.2/24.4
No. of atoms	4,873	3,284
Macromolecules	4,845	3,223
Glycans	28	-
Average <i>B</i> -values (Å <sup>2</sup> )	49	43
Macromolecules	49	43
Fab	45	43
RBD	56	-
Glycans	89	-
Wilson <i>B</i> -value (Å <sup>2</sup> )	43	40
<b>RMSD from ideal geometry</b>		
Bond length (Å)	0.004	0.007
Bond angle (°)	0.74	1.02
<b>Ramachandran statistics (%)<sup>f</sup></b>		
Favored	95.9	96.7
Outliers	0.16	0.0
<b>PDB code</b>		
	pending	pending

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<sup>a</sup> Numbers in parentheses refer to the highest resolution shell.

<sup>b</sup>  $R_{\text{sym}} = \sum_{hkl} \sum_i |I_{hkl,i} - \langle I_{hkl} \rangle| / \sum_{hkl} \sum_i I_{hkl,i}$  and  $R_{\text{pim}} = \sum_{hkl} (1/(n-1))^{1/2} \sum_i |I_{hkl,i} - \langle I_{hkl} \rangle| / \sum_{hkl} \sum_i I_{hkl,i}$ , where  $I_{hkl,i}$  is the scaled intensity of the *i*<sup>th</sup> measurement of reflection *h*, *k*, *l*,  $\langle I_{hkl} \rangle$  is the average intensity for that reflection, and *n* is the redundancy.

<sup>c</sup> CC<sub>1/2</sub> = Pearson correlation coefficient between two random half datasets.

<sup>d</sup>  $R_{\text{cryst}} = \sum_{hkl} |F_o - F_c| / \sum_{hkl} |F_o| \times 100$ , where  $F_o$  and  $F_c$  are the observed and calculated structure factors, respectively.

<sup>e</sup>  $R_{\text{free}}$  was calculated as for  $R_{\text{cryst}}$ , but on a test set comprising 5% of the data excluded from refinement.

<sup>f</sup> From MolProbity [7].

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**Supplementary Table 2. Hydrogen bonds identified in the antibody-RBD interface using the PISA program**

<b>COVA1-16 Fab</b>	<b>Distance [Å]</b>	<b>SARS-CoV-2 RBD</b>
H:ARG100b[NH2]	3.3	A:TYR369[O]
H:ARG100b[NE]	3.9	A:SER371[O]
H:ARG100b[N]	3.8	A:PHE377[O]
H:TYR100[N]	2.6	A:CYS379[O]
H:GLN101[NE2]	3.1	A:GLN414[OE1]
H:ARG97[NH1]	2.5	A:ASP427[O]
H:TYR32[OH]	3.1	A:ASP427[OD1]
H:THR28[ N]	3.2	A:ASP427[OD2]
H:ARG97[NH1]	3.0	A:PHE429[O]
H:TYR100[O]	2.9	A:CYS379[N]
H:SER100c[O]	3.3	A:THR385[OG1]
H:GLN101[OE1]	3.8	A:GLN414[NE2]
L:ASN53[OD1]	3.2	A:ARG408[NH2]
L:LEU54[O]	3.7	A:ARG408[NE]



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