

1 **An Engineered Antibody with Broad Protective Efficacy in Murine Models of SARS and**  
2 **COVID-19**

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31 **The recurrent zoonotic spillover of coronaviruses (CoVs) into the human population**  
32 **underscores the need for broadly active countermeasures. Here, we employed a directed**  
33 **evolution approach to engineer three SARS-CoV-2 antibodies for enhanced neutralization**  
34 **breadth and potency. One of the affinity-matured variants, ADG-2, displays strong binding**  
35 **activity to a large panel of sarbecovirus receptor binding domains (RBDs) and neutralizes**  
36 **representative epidemic sarbecoviruses with remarkable potency. Structural and**  
37 **biochemical studies demonstrate that ADG-2 employs a unique angle of approach to**  
38 **recognize a highly conserved epitope overlapping the receptor binding site. In murine**  
39 **models of SARS-CoV and SARS-CoV-2 infection, passive transfer of ADG-2 provided**

40 **complete protection against respiratory burden, viral replication in the lungs, and lung**  
41 **pathology. Altogether, ADG-2 represents a promising broad-spectrum therapeutic**  
42 **candidate for the treatment and prevention of SARS-CoV-2 and future emerging SARS-**  
43 **like CoVs.**

44 Over the past two decades, three pathogenic CoVs have emerged from zoonotic  
45 reservoirs to cause outbreaks of deadly pneumonia in humans: severe acute respiratory syndrome  
46 coronavirus (SARS-CoV), Middle-East respiratory syndrome coronavirus (MERS-CoV), and  
47 severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) (1). SARS-CoV emerged in  
48 2002 in the Guangdong province of China and infected ~8000 people with a case fatality rate of  
49 ~10% before being contained by public health measures (2). MERS-CoV emerged in the human  
50 population in 2012 and is still a significant public health threat in the Middle East (3, 4). In late  
51 2019, SARS-CoV-2 emerged in the city of Wuhan in China's Hubei province and rapidly caused  
52 an ongoing pandemic that has resulted in over a million deaths while disrupting the global  
53 economy (1, 5). Currently, there are no approved vaccines to prevent SARS-CoV-2 infection and  
54 only one antiviral drug has been approved to treat SARS-CoV-2 associated disease (i.e. COVID-  
55 19). Furthermore, the recurrent zoonotic spillover of CoVs into the human population, along with  
56 the broad diversity of SARS-like CoVs circulating in animal reservoirs (6), suggests that novel  
57 pathogenic CoVs are likely to emerge in the future and underscores the need for broadly active  
58 countermeasures.

59 Similar to other CoVs, the SARS-CoV-2 spike (S) protein mediates viral entry and is the  
60 only known target for neutralizing antibodies (nAbs). The S glycoprotein consists of two  
61 functional subunits, S1 and S2, that mediate receptor binding and viral fusion, respectively.  
62 Previous studies have shown that the vast majority of potent neutralizing antibodies induced by

63 natural CoV infection target the RBD on the S1 subunit (7-10). Although SARS-CoV and  
64 SARS-CoV-2 both belong to the sarbecovirus subgenus and their S glycoproteins share 76%  
65 amino acid identity, only a handful of cross-neutralizing antibodies have been described to date  
66 (11-13). These rare broadly neutralizing antibodies (bnAbs) represent an attractive opportunity  
67 for therapeutic drug stockpiling to prevent or mitigate future outbreaks of SARS-related CoVs,  
68 but their limited neutralization potency may translate into suboptimal protective efficacy or  
69 impractical dosing regimens. Here, we show that such bnAbs can be engineered for improved  
70 neutralization potency while retaining neutralization breadth, and we demonstrate that these  
71 bnAbs can provide broad protection *in vivo*.

72         We recently isolated several antibodies from the memory B cells of a 2003 SARS  
73 survivor that cross-neutralize multiple SARS-related viruses with relatively modest potency (11).  
74 Although breadth and potency are often opposing characteristics, we sought to engineer these  
75 bnAbs for improved neutralization potency against SARS-CoV-2, while also maintaining or  
76 improving neutralization breadth and potency against other SARS-related viruses. Because  
77 binding affinity and neutralization potency are generally well-correlated (14), we employed  
78 yeast-surface display technology to improve the binding affinities of three of the bnAbs (ADI-  
79 55688, ADI-55689, and ADI-56046) for a prefusion-stabilized SARS-CoV-2 S protein (11, 15-  
80 17).

81         Yeast display libraries were generated by introducing diversity into the heavy (HC)- and  
82 light-chain (LC) variable genes of ADI-55688, ADI-55689, and ADI-56046 through  
83 oligonucleotide-based mutagenesis and transformation into *Saccharomyces cerevisiae* by  
84 homologous recombination (15). Following four rounds of selection with a recombinant SARS-  
85 CoV-2 S1 protein, improved binding populations were sorted, and between 20 and 50 unique

86 clones from each lineage were screened for binding to SARS-CoV-2 S (17) (Fig. 1A, B and Fig.  
87 S1). The highest affinity binders from each of the three lineages bound to the SARS-CoV-2 S  
88 protein with monovalent equilibrium dissociation constants ( $K_{Ds}$ ) in the picomolar range,  
89 representing 25 to 630-fold improvements in binding relative to their respective parental clones  
90 (Fig. 1B and Fig. S2). To determine whether the improvements in SARS-CoV-2 S binding  
91 affinity translated into enhanced neutralization potency, we selected between 9 and 14 affinity-  
92 matured progeny from each lineage, and evaluated them for SARS-CoV-2 neutralizing activity  
93 in a murine leukemia virus (MLV) pseudovirus assay (18). We also measured the neutralizing  
94 activities of several clinical-stage neutralizing antibodies (nAbs) (S309, REGN10933,  
95 REGN10987, and CB6/LY-CoV016) as benchmarks (12, 19, 20). All of the affinity-matured  
96 antibodies showed improved neutralizing activity relative to their parental clones, and the most  
97 potent neutralizers from each lineage (ADG-1, ADG-2, and ADG-3) displayed neutralization  
98  $IC_{50}$ s that were comparable to or lower than those observed for the clinical SARS-CoV-2 nAb  
99 controls (Fig. 1B).

100       Because *in vitro* engineering can lead to polyspecificity with potential risks of off-target  
101 binding and accelerated clearance *in vivo* (21), we assessed the polyspecificity of ADG-1, ADG-  
102 2, and ADG-3 using a previously described assay that has been shown to be predictive of serum  
103 half-life in humans (22). All three antibodies lacked polyreactivity in this assay, indicating a low  
104 risk for poor pharmacokinetic behavior (Fig. S3). The three antibodies also showed low  
105 hydrophobicity, a low propensity for self-interaction, and thermal stabilities within the range  
106 observed for clinically approved antibodies (Fig. S3). In summary, the process of *in vitro*  
107 engineering did not negatively impact biophysical properties that are often linked to down-

108 stream behaviors such as serum half-life, ease of manufacturing, ability to formulate to high  
109 concentrations, and long-term stability.

110 To determine whether the process of SARS-CoV-2 affinity engineering impacted  
111 neutralization breadth, we evaluated ADG-1, ADG-2, and ADG-3, as well as their respective  
112 parental antibodies, for neutralizing activity against a panel of representative authentic clade I  
113 sarbecoviruses (SARS-CoV, SHC014, SARS-CoV-2, and WIV-1). Consistent with the MLV-  
114 SARS-CoV-2 assay results, ADG-2 displayed highly potent neutralizing activity against  
115 authentic SARS-CoV-2, with an  $IC_{50}$  comparable to or lower than that observed for the  
116 benchmark SARS-CoV-2 nAbs (Fig. 1C and Fig. S4). Furthermore, in contrast to the benchmark  
117 nAbs, ADG-2 displayed high neutralization potency against SARS-CoV and the two SARS-  
118 related bat viruses, with  $IC_{50}$ s between 4 and 8 ng/mL (Fig. 1C and Fig. S4). ADG-3 and the  
119 clinical nAb S309 also cross-neutralized all four sarbecoviruses, but with markedly lower  
120 potency than ADG-2. Finally, ADG-1 potently neutralized SARS-CoV-2, SARS-CoV, and  
121 WIV1, but it lacked activity against SHC014.

122 Based on its potent cross-neutralization and favorable biophysical properties, we selected  
123 ADG-2 as a lead therapeutic candidate and confirmed its potent neutralizing activity in two  
124 alternative authentic SARS-CoV-2 neutralization assays ( $IC_{50} \sim 1$  ng/mL) (Fig. 1C, D and Fig.  
125 S4). Interestingly, ADG-2, CB6/LY-CoV016, REGN10987 and REGN10933 reached 100%  
126 neutralization on both Vero and HeLa-hACE2 target cells in this assay, whereas S309 showed  
127 complete neutralization on Vero target cells but plateaued at approximately 40% neutralization  
128 on HeLa-hACE2 target cells (Fig. 1D). S309 also failed to neutralize MLV-SARS-CoV-2 on  
129 HeLa-hACE2 target cells (Fig. 1B). The reason for this is unclear but may relate to glycan  
130 heterogeneity within the S309 epitope (12) coupled with differences in receptor expression or

131 protease cleavage efficiency between the two types of target cells (23). Because SARS-CoV-2  
132 D614G has emerged as the dominant pandemic strain (24), we also evaluated ADG-2 for  
133 neutralizing activity against this variant in the MLV pseudovirus assay. As expected, based on  
134 the location of the D614G substitution outside of the RBD, ADG-2 neutralized the D614G  
135 variant with equivalent potency as wild-type (WT) SARS-CoV-2 (Fig. S5).

136 We further assessed the breadth of sarbecovirus recognition by ADG-2 by measuring its  
137 apparent binding affinity ( $K_D^{APP}$ ) to a panel of 17 representative sarbecovirus RBDs expressed on  
138 the surface of yeast (25). Thirteen viruses were selected from clade I — representing the closest  
139 known relatives of SARS-CoV-2 (GD-Pangolin and RaTG13) to the most divergent (SHC014  
140 and Rs4231) — as well as four viruses from the distantly related clades 2 and 3, which do not  
141 utilize ACE2 as a host receptor (26) (Fig. 2A). Recombinant hACE2-Fc and the benchmark  
142 SARS-CoV-2 nAbs described above were also included as controls. Consistent with previous  
143 reports (17, 25), hACE2 only recognized clade I RBDs and bound with higher affinity to SARS-  
144 CoV-2 than SARS-CoV (Fig. 2B). In addition, the benchmark SARS-CoV-2 nAbs CB6/LY-  
145 CoV016, REGN10987, and REGN10933 bound to the SARS-CoV-2 RBD with  $K_D^{APPS}$   
146 comparable to published reports (Fig. 2B) (19, 20). Notably, S309 displayed diminished binding  
147 in this expression platform, likely due to recognition of an epitope containing an N-glycan that  
148 may be hyper-mannosylated in yeast (12).

149 Consistent with their broadly neutralizing activities, S309, ADG-2, and ADG-3 displayed  
150 remarkably broad binding reactivity to clade I sarbecovirus RBDs, with ADG-2 and ADG-3  
151 strongly binding 12/13 viruses and S309 binding all 13 (Fig. 2B). In contrast, ADG-1 only bound  
152 to 9/13 viruses and CB6/LY-CoV016, REGN10987, and REGN10933 bound only the closest  
153 evolutionary neighbor(s) of SARS-CoV-2, consistent with their narrow neutralization profiles

154 (Fig. 2B and Fig. 1C). Importantly, ADG-2 bound with high affinity ( $K_D^{APP}$  0.24-1.12 nM) to  
155 every clade I sarbecovirus RBD that exhibited detectable hACE2 binding in our assay. This  
156 finding supports the high degree of ADG-2 epitope conservation among sarbecoviruses that use  
157 hACE2 as a receptor.

158         Several recent studies have shown that RBD mutants that are resistant to commonly  
159 elicited SARS-CoV-2 nAbs are circulating at low levels in the human population (24, 27). We  
160 therefore sought to assess the breadth of ADG-2 binding to naturally circulating SARS-CoV-2  
161 variants that contain single point mutations in the RBD. ADG-1, ADG-3, and the benchmark  
162 SARS-CoV-2 nAbs were also included as comparators. Using the yeast surface-display platform  
163 described above, we expressed the 30 most frequently observed SARS-CoV-2 RBD variants  
164 reported in the GISAID database as well as six naturally circulating SARS-CoV-2 variants that  
165 have been shown to be resistant to previously described SARS-CoV-2 nAbs (24, 27, 28). One or  
166 more of the 36 SARS-CoV-2 variants exhibited loss of binding to ADG-1, CB6/LY-CoV016,  
167 REGN10987, and REGN10933, as defined by >75% loss relative to the WT construct (Fig. 2C).  
168 Notably, the loss-of-binding variants identified for REGN10987 and REGN10933 partially  
169 overlapped with those identified in previous *in vitro* neutralization escape studies, validating the  
170 use of RBD display for the prediction of antibody escape mutations (29). In contrast, ADG-2,  
171 ADG-3, and S309 bound to all 36 variants at levels  $\geq 50\%$  of WT SARS-CoV-2 (Fig. 2C). This  
172 result, combined with the remarkable neutralization breadth observed for these three mAbs (Fig.  
173 1C and Fig. 2B, D), suggests a potential link between epitope conservation and resistance to viral  
174 escape.

175         To gain further insight into the antigenic surface recognized by ADG-2, we generated a  
176 mutagenized yeast surface-display RBD library and performed rounds of selection to identify



177 RBD variants that exhibited loss of binding to ADG-2 relative to the WT construct (Fig. 3A, Fig.  
178 S6A, B). To exclude mutations that globally disrupt the conformation of the RBD, a final round  
179 of positive selection was performed using a mixture of recombinant hACE2 and two RBD-  
180 directed mAbs (S309 and CR3022) that target non-overlapping epitopes distinct from the ADG-2  
181 binding site (12, 30) (Fig. S6B, and Fig. S7). Selected RBD mutants encoding single amino acid  
182 substitutions were individually tested for binding to ADG-2, recombinant hACE2, CR3022, and  
183 S309 to confirm site-specific knock-down mutations (Fig. S6C). Substitutions at only four RBD  
184 positions specifically abrogated ADG-2 binding: D405E, G502E/R/V, G504A/D/R/S/V and  
185 Y505C/N/S (Fig. 3B). These four residues are highly conserved among the clade I sarbecovirus  
186 subgenus and invariant among SARS-CoV-1, SARS-CoV-2, SHC014 and WIV1 viruses (Fig.  
187 3C), providing a molecular explanation for the breadth of binding and neutralization exhibited by  
188 ADG-2. Consistent with the conservation of these residues among clade I sarbecoviruses, none  
189 of the substitutions that impacted ADG-2 binding were present in full-length SARS-CoV-2  
190 sequences deposited in the GISAID database as of October 19, 2020. In addition, 3 of the 4  
191 identified mutations that abrogate ADG-2 binding lie within the hACE2 binding site (31) and at  
192 least one mutation at each position (G502E/R/V, G504V and Y505C/N/S) also abrogated hACE2  
193 binding (Fig. 3B), likely accounting for their absence among circulating SARS-CoV-2 isolates.  
194 These results suggest that the evolutionary conservation of the ADG-2 epitope is likely directly  
195 linked to ACE2 binding.

196 To support the results of this experiment, we performed low-resolution cryogenic  
197 electron microscopy (cryo-EM) of the complex of ADG-2 bound to prefusion-stabilized SARS-  
198 CoV-2 S. This yielded a  $\sim 6\text{\AA}$  resolution 3D reconstruction that clearly had at least one ADG-2  
199 Fab bound to an RBD in the up conformation and allowed us to unambiguously dock in

200 previously determined high-resolution models of the SARS-CoV-2 spike and a homologous Fab  
201 (Fig. 3D, Fig. S8A-D, Table S1). Consistent with our fine epitope mapping and competitive  
202 binding experiments (Fig. 3B and Fig. S7C), the epitope recognized by ADG-2 overlaps with the  
203 hACE2-binding site and each position identified by epitope mapping clustered to the cleft  
204 between the heavy and light chains of ADG-2 (Fig. 3D). This epitope also partially overlaps with  
205 those recognized by frequently observed “class 1” SARS-CoV-2 nAbs, which are exemplified by  
206 *VH3-53* antibodies with short CDRH3s and compete with hACE2 in the RBD “up” conformation  
207 (32) (Fig. 3E). However, in contrast to previously reported nAbs in this class, ADG-2 binds with  
208 a divergent angle of approach and displays broadly neutralizing activity (32) (Fig. 3E, Fig. 1C,  
209 and Fig. S8E). Thus, ADG-2 binds to a highly conserved motif via a unique angle of approach,  
210 providing additional structural insight into its broad recognition of SARS-like CoVs.

211         Because Fc-mediated effector functions can contribute to protection independently of  
212 viral neutralization, we next assessed the ability of ADG-2 to induce antibody-dependent natural  
213 killer cell activation and degranulation (ADNKDA), antibody-dependent cellular phagocytosis  
214 (ADCP) mediated by monocytes and neutrophils, and antibody-mediated complement deposition  
215 (ADCD) using previously described *in vitro* assays (33). Benchmark SARS-CoV-2 nAbs S309  
216 and REGN10987 were also included as comparators. ADG-2 displayed a highly polyfunctional  
217 profile, resulting in the induction of phagocytosis by monocytes and neutrophils, deposition of  
218 the complement component C3, and induction of NK cell degranulation (a surrogate marker of  
219 ADCC) and activation (Fig. 4). Interestingly, while ADG-2, S309, and REGN10957 showed  
220 comparable recruitment of phagocytosis (Fig. 4B), these antibodies differed with respect to  
221 complement deposition and NK cell activation (Fig. 4A, C); S309 showed reduced complement  
222 deposition compared with ADG-2 and REGN10987, and ADG-2 showed superior NK cell

223 activation over both S309 and REGN10987 (Fig.4). In summary, ADG-2 robustly triggers  
224 diverse Fc-mediated effector activities with potencies comparable or superior to those of current  
225 lead SARS-CoV-2 clinical antibodies. However, it should be noted that the contribution of extra-  
226 neutralizing activities to protection against SARS-CoV-2 is currently unknown and may vary  
227 among different antibody specificities.

228 Finally, we tested the ability of ADG-2 to provide broad *in vivo* protection in  
229 immunocompetent mouse models of SARS and COVID-19 using mouse-adapted SARS-CoV  
230 (MA15)- and SARS-CoV-2 (MA10), respectively (34, 35). Balb/c mice were prophylactically  
231 treated with either 200  $\mu$ g of ADG-2 or PBS via IP injection 12 hours prior to intranasal  
232 challenge with a  $10^3$  PFU dose of MA15 or MA10. All mice were monitored daily for weight  
233 loss and changes in respiratory function and groups of mice were euthanized at day two or four  
234 post-infection to allow for measurement of virus replication in the lung and analysis of lung  
235 histopathology. We observed substantial, progressive weight loss in sham-treated mice infected  
236 with both viruses along with increases in Penh, a calculated measure of airway resistance (35). In  
237 contrast, mice treated prophylactically with ADG-2 demonstrated minimal weight loss, no  
238 change in Penh and no signs of gross pathology at the time of harvest (Fig. 5A, B). Furthermore,  
239 prophylactic antibody treatment prevented viral replication in the lungs at both two and four days  
240 post-infection (dpi). We next investigated the ability of ADG-2 to act anti-virally against SARS-  
241 CoV-2 MA10 in a therapeutic setting. Mice were treated with 200  $\mu$ g of ADG-2 or PBS 12 hours  
242 following intranasal challenge with a  $10^3$  PFU dose of MA10. Mice given therapeutic ADG-2  
243 had intermediate levels of weight loss, moderate respiratory function changes and some gross  
244 lung pathology; significantly more than prophylactically-treated mice but significantly less than  
245 sham-treated mice (Fig. 5C). Therapeutic antibody treatment also resulted in a significant

246 reduction in lung viral loads at four dpi, but not at two dpi, relative to sham-treated mice. We  
247 conclude that ADG-2 treatment can reduce disease burden in mice infected with both SARS-  
248 CoV MA15 and SARS-CoV-2 MA10.

249         Since the beginning of the COVID-19 pandemic, a plethora of potentially neutralizing  
250 SARS-CoV-2 antibodies have been isolated, and some have rapidly advanced into clinical trials  
251 (36). However, the epitopes recognized by most of these nAbs are highly variable among other  
252 clade 1a and 1b sarbecoviruses, hence limiting their neutralization breadth and increasing their  
253 susceptibility to antibody escape mutations (27). Here, we described an engineered antibody that  
254 neutralizes SARS-CoV-2 with a potency that rivals current lead SARS-CoV-2 clinical nAbs, but  
255 also broadly neutralizes other clade I sarbecoviruses, potentially triggers Fc-mediated effector  
256 functions, and provides significant protection against SARS and COVID-19 disease in mouse  
257 models. Thus, ADG-2 represents a promising candidate for the prevention and treatment of not  
258 only COVID-19 but also future respiratory diseases caused by pre-emergent SARS-related  
259 CoVs. Furthermore, our fine epitope mapping and structural studies demonstrate that ADG-2  
260 employs a unique angle of approach to recognize a highly conserved epitope overlapping the  
261 receptor binding site. This epitope represents an Achilles' heel for clade 1a and 1b  
262 sarbecoviruses and hence an attractive target for the rational design of “pan-SARS” vaccines that  
263 aim to elicit similar broadly protective antibodies.

## 264 **References and Notes**

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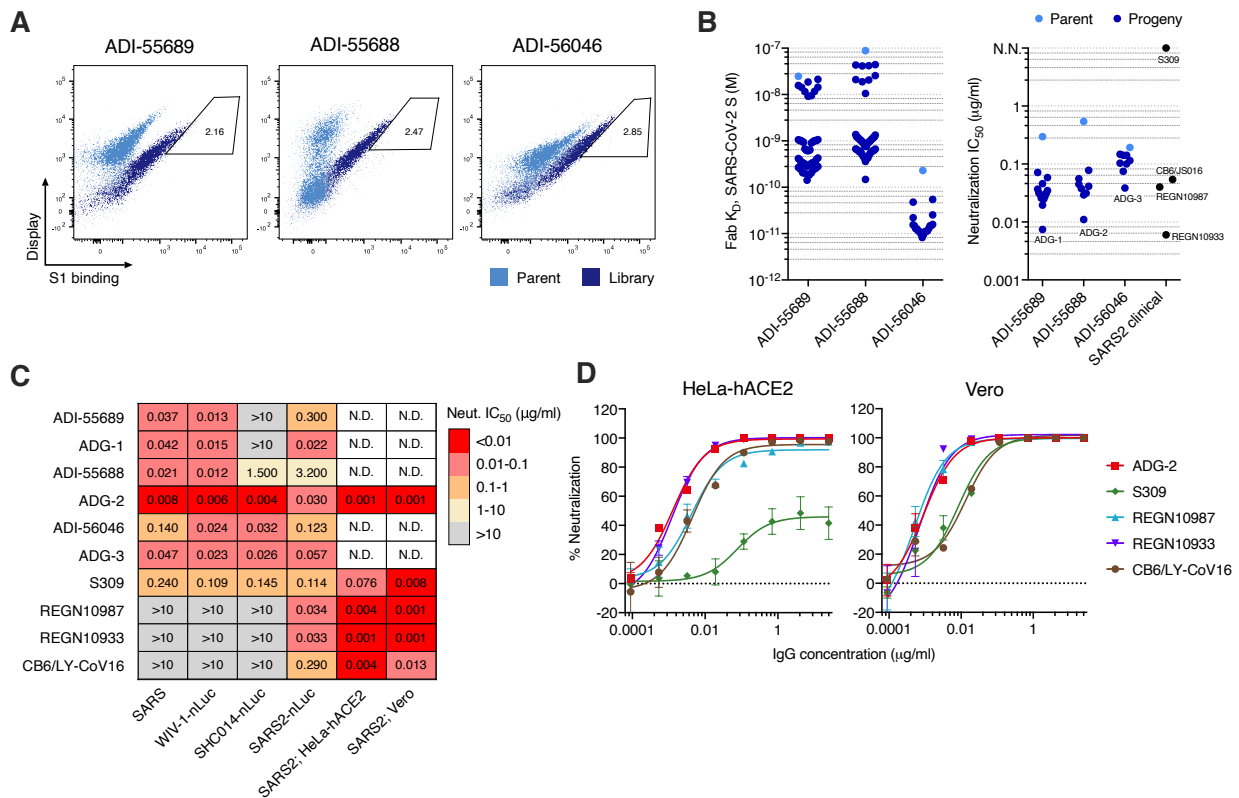
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378

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393 designed the study. L.M.D. and J.B. performed the directed evolution experiments. L.V.T., D.H.,  
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395 and performed neutralization assays. M.E.B. and J.C.G. designed and supervised developability  
396 and biolayer interferometry assays. C.G.R., C.I.K., M.S., and M.B.B. designed and performed  
397 the yeast surface-display RBD experiments. D.W. and J.S.M. designed and performed Biacore  
398 SPR and structural assays. T.J.Y. and B.M.G. designed and performed Fc-effector functional  
399 assays. L.E.G. designed and performed the animal challenge studies. C.G.R., L.V.T., C.I.K.,  
400 D.W., M.S., D.H., L.M.D., A.S.H., M.B.B., B.M.G., L.E.G., and L.M.W. analyzed the data.  
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405 Therapeutics Inc. D.R.B. is on the SAB of Adimab, LLC and Adagio Therapeutics Inc. and holds  
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407 corresponding author under MTA from Adagio Therapeutics, Inc.

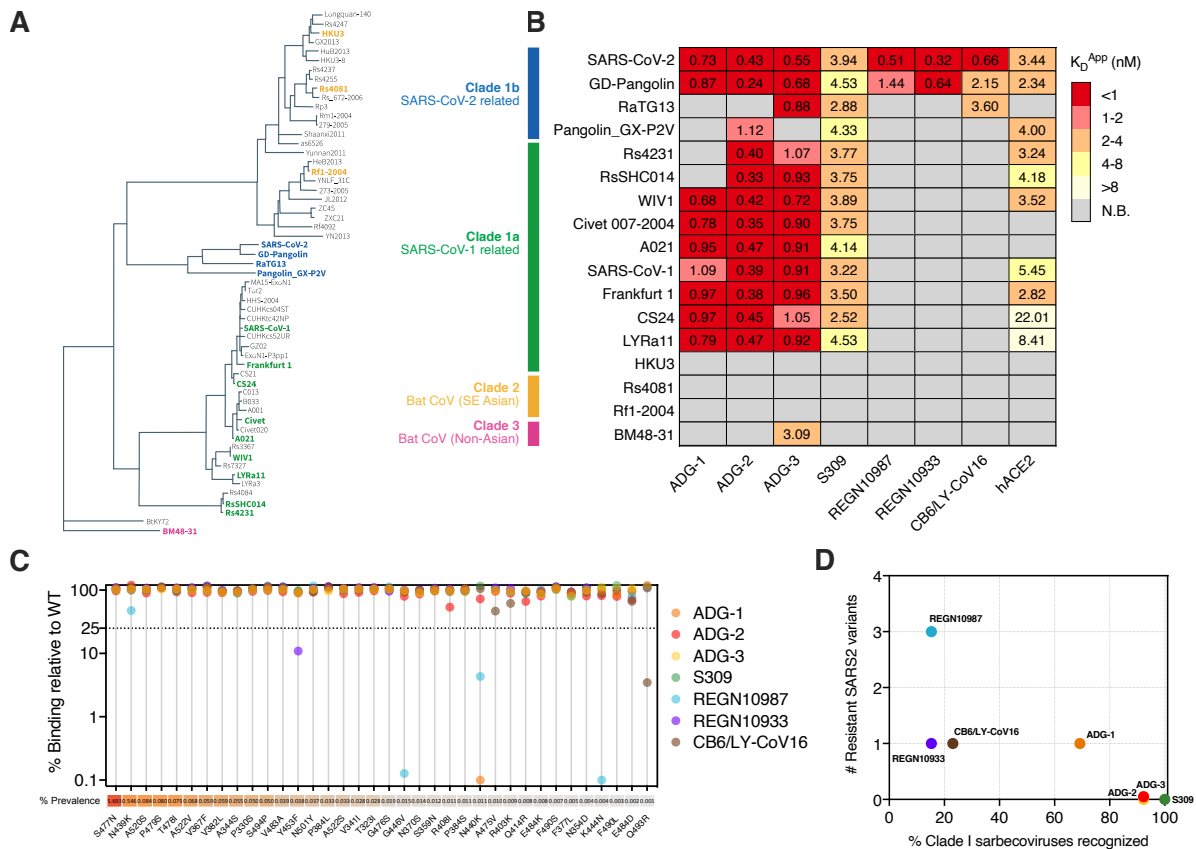
408 Main Text Figures



409

410 **Figure 1.** Engineering of SARS-CoV-2 antibodies for enhanced neutralization breadth and  
 411 potency. **(A)** Flow cytometry plots from the terminal round of selection showing binding of  
 412 parental antibodies (light blue) and affinity maturation library antibodies (dark blue) to the  
 413 SARS-CoV-2 S1 protein at 1 nM. Gates indicate the yeast populations sorted for antibody  
 414 sequencing and characterization. **(B)** Dot plots of Fab binding affinities (left) and MLV-SARS-  
 415 CoV-2 pseudovirus neutralization IC<sub>50</sub>s (right) of parental antibodies and affinity matured  
 416 progeny. SARS-CoV-2 clinical antibodies are shown for comparison. **(C)** Heat map showing the  
 417 neutralization IC<sub>50</sub>s of the indicated antibodies against authentic SARS-CoV, WIV-1-nLuc,  
 418 SHC014-nLuc, SARS-CoV-2-nLuc, and SARS-CoV-2 using either HeLa-hACE2 or Vero target  
 419 cells. SARS-CoV assays were performed on Vero cells. WIV-1-nLuc, SHCO14-nLuc, and

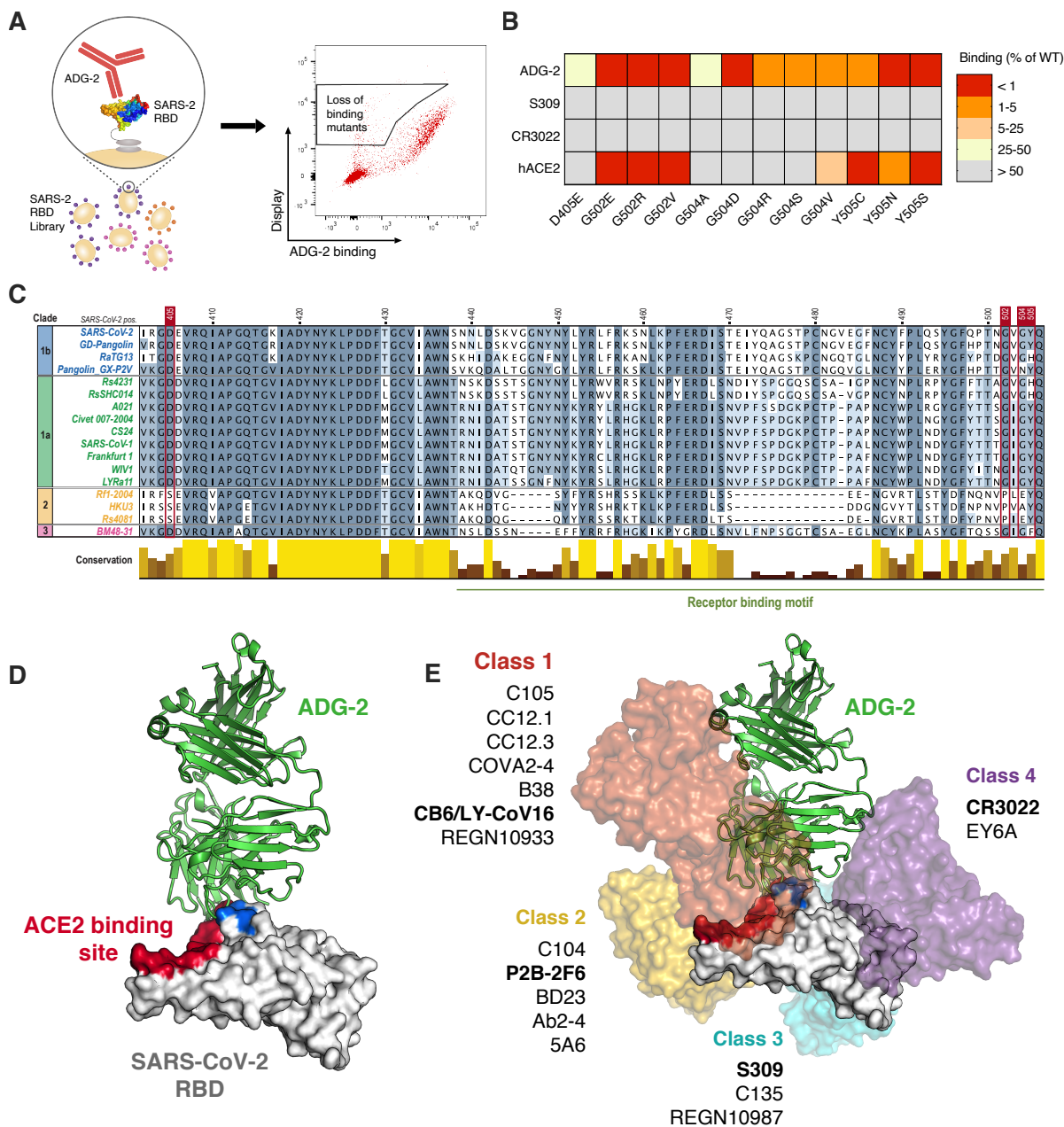
420 SARS-CoV-2 nLuc assays were performed on Vero cells with recombinant, reverse genetics-  
421 derived viruses encoding a nano-luciferase reporter gene. **(D)** Authentic SARS-CoV-2  
422 neutralization titrations performed using either HeLa-hACE2 (left) or Vero (right) target cells.  
423 The curves were fit by nonlinear regression. Error bars represent standard deviation. N.D., not  
424 determined; N.N., non-neutralizing.



425

426 **Figure 2.** Breadth of antibody binding to diverse sarbecoviruses and circulating SARS-CoV-2  
 427 variants. **(A)** Phylogenetic tree of 57 sarbecoviruses constructed via MAFFT and maximum  
 428 likelihood analysis of RBD-SD1 amino acid sequences extracted from the European Nucleotide  
 429 Archive and GISAID database. Representative sarbecovirus RBDs selected for further study are  
 430 denoted in bold and colored according to their canonical phylogenetic lineages. **(B)** Heat map of  
 431 antibody and recombinant hACE2 binding to yeast-displayed RBDs from 17 representative  
 432 sarbecoviruses, grouped by phylogenetic lineages.  $K_D^{APP}$  values were calculated by normalized  
 433 nonlinear regression fitting. **(C)** Antibody binding to naturally-occurring SARS-CoV-2 RBD  
 434 variants displayed on the surface of yeast. SARS-CoV-2 sequences were retrieved from the  
 435 GISAID database on July 14, 2020 ( $n = 63551$ ). Antibody binding signal was normalized to  
 436 RBD expression and calculated as percent binding of the variant relative to the WT SARS-CoV-

437 2 RBD, assessed at their respective  $K_D^{APP}$  concentrations for the WT construct. The prevalence of  
438 each variant, calculated from deposited sequences on October 19, 2020 ( $n = 148115$ ), is shown  
439 as a percentage of the total number of sequences analyzed. **(D)** Correlation between the number  
440 of resistant SARS-CoV-2 variants and percentage of clade I sarbecovirus RBDs recognized.  
441 N.B., non-binder.

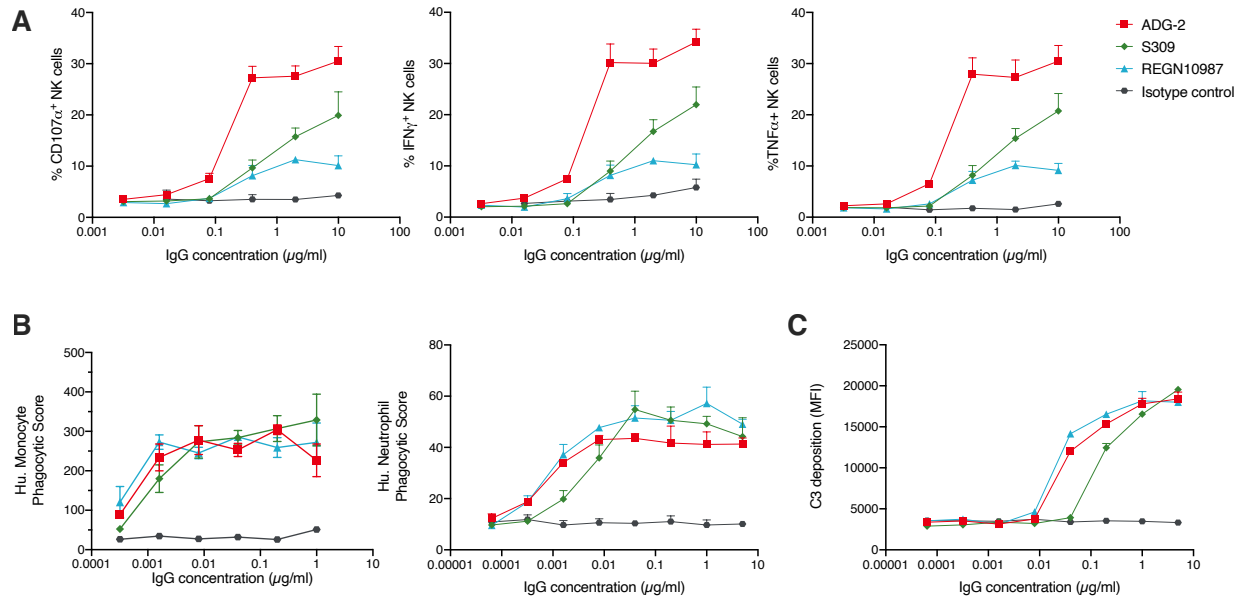


442

443 **Figure 3.** ADG-2 binds to an evolutionarily conserved epitope on the SARS-CoV-2 RBD  
 444 overlapping with the hACE2 binding site. (A) Schematic showing the generation and selection of  
 445 a mutagenized, yeast surface-displayed SARS-CoV-2 RBD library to identify mutations that  
 446 knock-down ADG-2 binding. (B) Heat map showing mutations that abrogate binding of ADG-2  
 447 to the SARS-CoV-2 RBD. S309 and CR3022, which bind non-overlapping epitopes distinct from

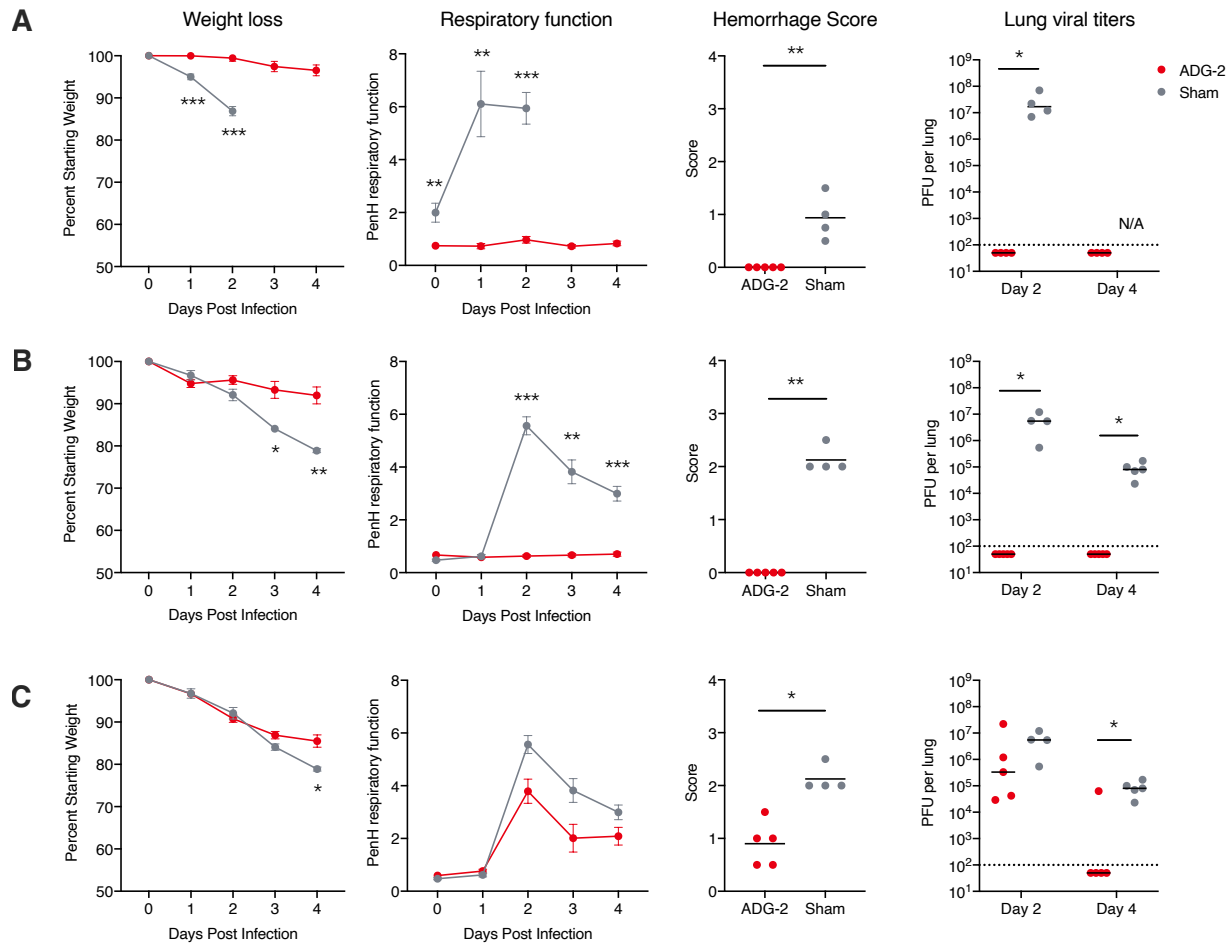


448 the ADG-2 binding site, are included to control for mutations that globally disrupt the  
449 conformation of the RBD. Values indicate percent antibody or recombinant hACE2-Fc binding  
450 to the mutant SARS-CoV-2 RBD relative to the WT SARS-CoV-2 RBD, assessed at their  
451 respective EC<sub>80</sub> concentrations for the WT RBD construct. **(C)** Protein sequence alignment of  
452 representative sarbecovirus RBDs with sequences colored by percentage sequence identity and  
453 conservation shown as a bar plot. Positions delineating the receptor binding motif are based on  
454 the SARS-CoV-2 RBD. Residues determined to be important for ADG-2 binding based on the  
455 data shown in (B) are denoted in red. **(D)** Cryo-EM reconstruction of the SARS-CoV-2 RBD  
456 bound by ADG-2, with ADG-2 knock-down mutations and the hACE2 binding site highlighted  
457 in blue and red, respectively. **(E)** Structures of previously reported antibodies (bold) representing  
458 frequently observed SARS-CoV-2 nAb classes 1-4 overlaid on the ADG-2 structure (D), with  
459 additional representative SARS-CoV-2 nAbs listed.



460

461 **Figure 4.** ADG-2 triggers Fc-mediated effector functions. The indicated antibodies were  
462 assessed for the ability to induce Fc-mediated effector functions against RBD-coated targets at  
463 varying concentrations. **(A)** Primary human NK cells were analyzed for surface expression of  
464 CD107a, indicating degranulation (left), and the production of IFN $\gamma$  (middle) or TNF $\alpha$  (right)  
465 following incubation with antibody-RBD immune complexes for 5 hours. **(B)** Antibody-  
466 mediated phagocytosis of RBD-coated fluorescent beads by differentiated HL-60 neutrophils  
467 (left) or THP-1 monocytes (right) was measured following incubation with immune complexes  
468 for 18 hours. **(C)** Antibody-mediated complement deposition was measured by detection of  
469 complement component C3 onto RBD-coated fluorescent beads following incubation of guinea  
470 pig complement with immune complexes for 20 minutes.

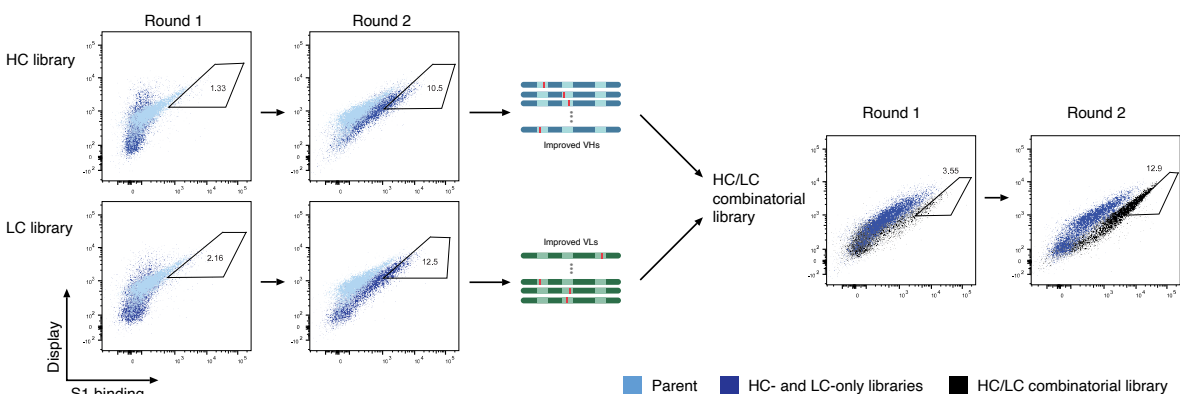


471

472 **Figure 5.** Prophylactic and therapeutic administration of ADG-2 protects mice from SARS-CoV-  
 473 and SARS-CoV-2-associated viral diseases. Efficacy of prophylactic treatment with ADG-2 in  
 474 (A) SARS-CoV-MA15 and (B) SARS-CoV-2-MA10 challenge models. A single dose of ADG-2  
 475 or sham treatment were delivered intraperitoneally 12 hours prior to infection. Mouse body  
 476 weight and respiratory function were monitored for 4 days. Gross lung hemorrhage scores were  
 477 determined on day 2 (MA15) or day 4 (MA10) post-infection and lung viral titers were measured  
 478 on day 2 and day 4 post-infection. (C) Therapeutic treatment with ADG-2 or sham treatment at  
 479 12 hours post-SARS-CoV-2-MA10-infection. Mouse body weight, respiratory function, gross  
 480 hemorrhage scores (day 2), and lung viral titers (days 2 and 4) were assessed as described above.

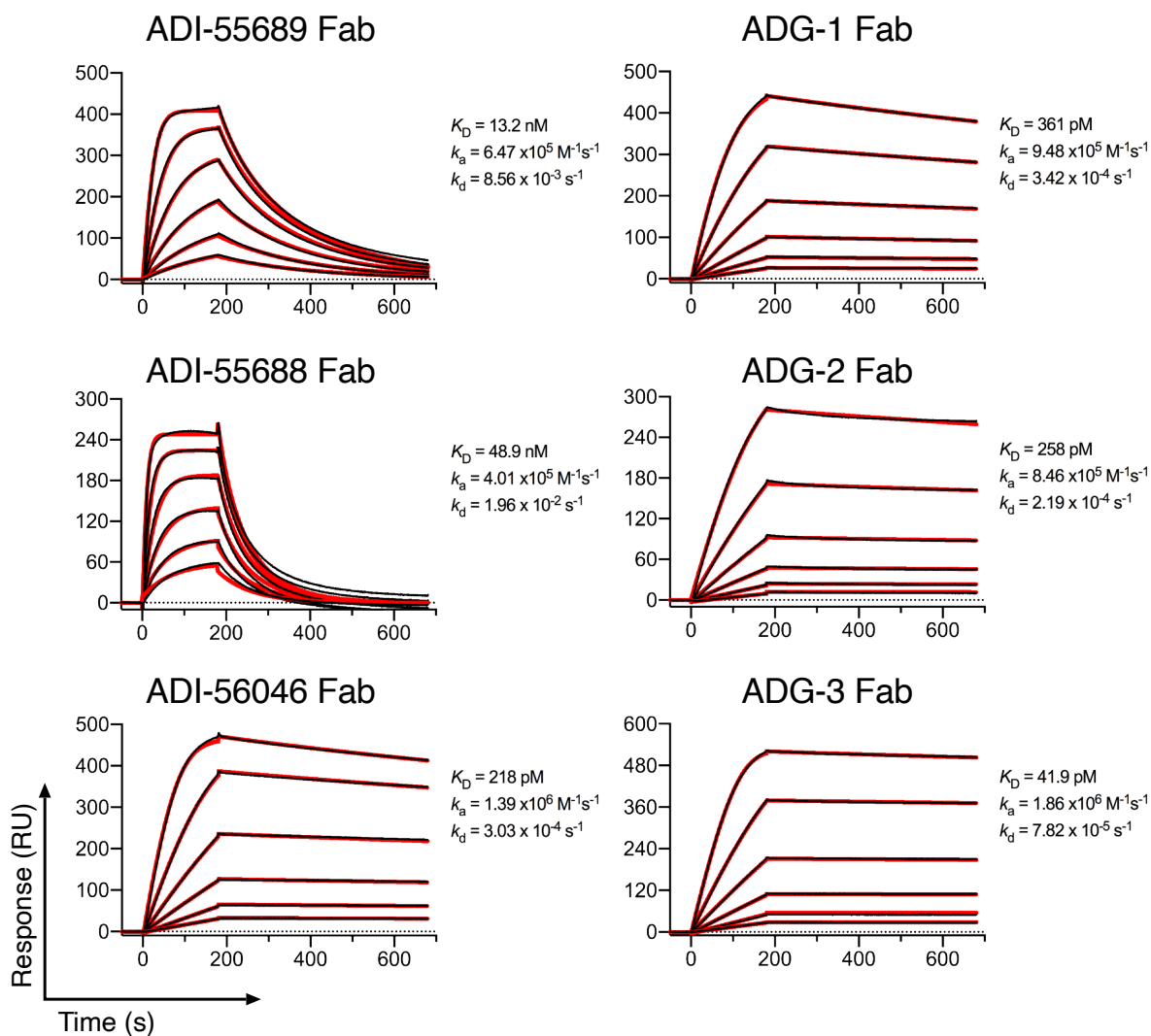
481 Statistical comparisons were made using Mann-Whitney U tests or two-sided t-tests with Holm-  
482 Sidak corrections for multiple comparisons (\*P < 0.05, \*\*P < 0.01; \*\*\*P < 0.001). Dotted lines  
483 indicate the limit of detection.

## 484 Supplementary Figures



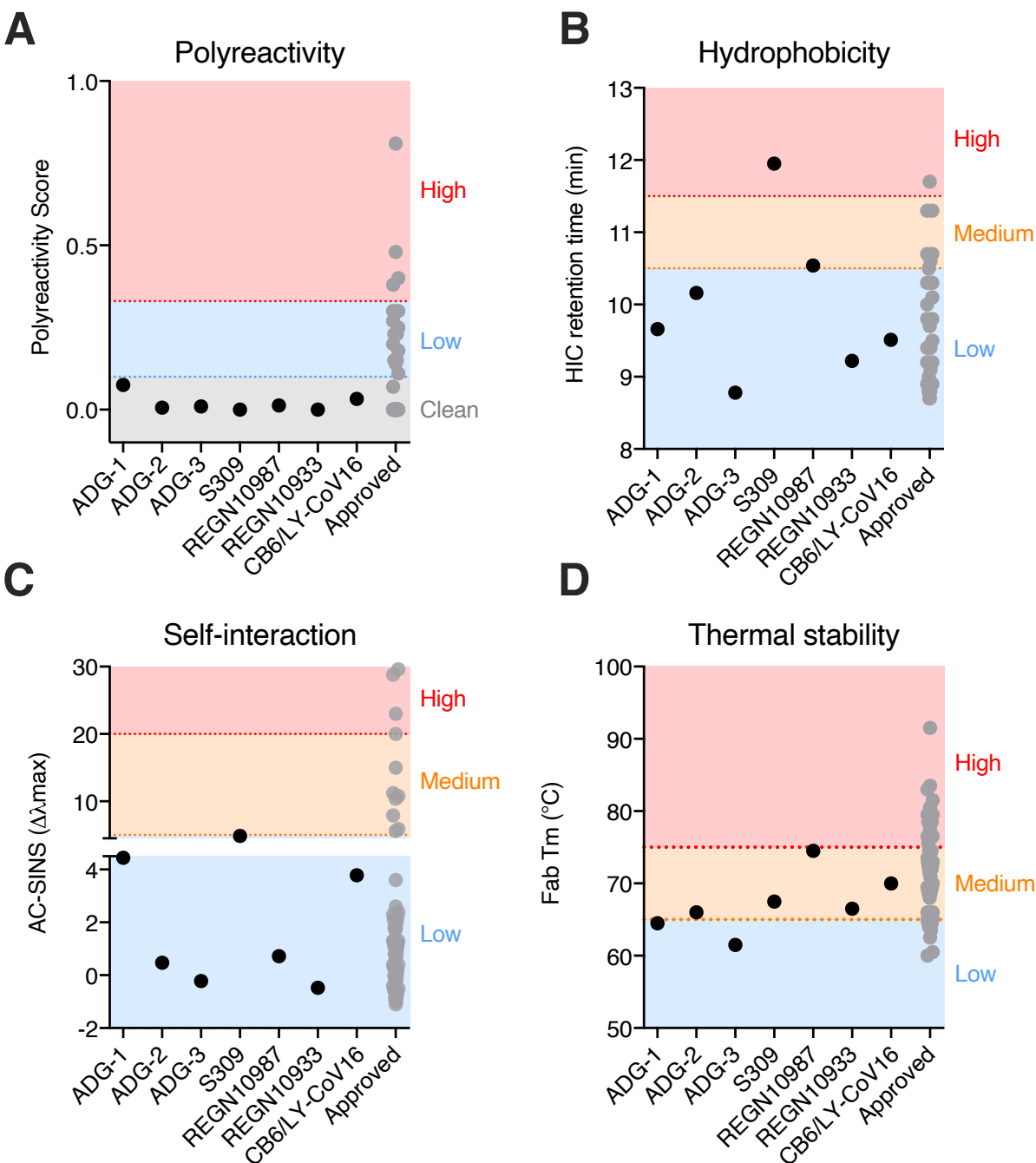
485

486 **Figure S1.** Representative selection strategy for affinity maturation libraries. **(A)** Flow  
487 cytometric sorting of libraries containing diversity in the HC (top) or LC (bottom) of ADI-  
488 55688. Libraries (dark blue) were sorted for improved binding to the SARS-CoV-2 S1 protein  
489 relative to the parent clone (light blue). Round 1 gates indicate the yeast populations that were  
490 sorted for a second round of selection, and round 2 gates indicate the yeast populations that were  
491 sorted for amplification of heavy- or light-chain variable region genes and subsequent  
492 transformation into yeast to generate a HC/LC combinatorial library. **(B)** Flow cytometric sorting  
493 of the HC/LC combinatorial library (black) for improved binding to the SARS-CoV-2 S1 protein  
494 relative to the round 2 output of the HC diversity libraries (dark blue). The round 1 gate indicates  
495 the yeast population that was sorted for a second round of selection and the round 2 gate  
496 indicates the yeast population that was sorted for individual colony isolation and sequencing.



497

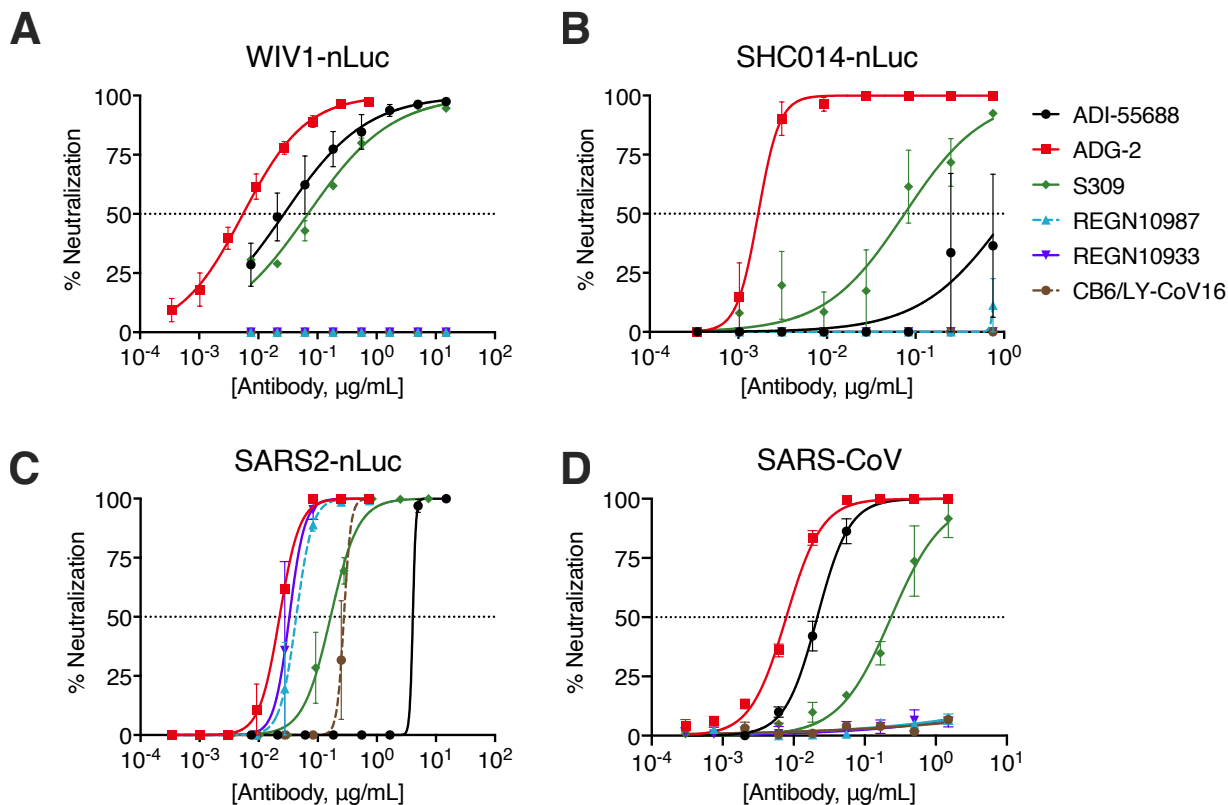
498 **Figure S2.** Binding kinetics of progenitor and affinity-matured Fabs. SPR sensorgrams showing  
499 binding of each Fab to the SARS-CoV-2 RBD-SD1 protein. Binding data are shown as black  
500 lines, and the best fits of a 1:1 binding model are shown as red lines.



501  
502 **Figure S3.** Biophysical properties of SARS-CoV-2 antibodies. (A) Antibody polyreactivity, as  
503 assessed based on binding to a previously described polyspecificity reagent (37). Binding was  
504 assessed by flow cytometry. The thresholds for high, low, and “clean” polyreactivity were  
505 defined based on a previously reported correlation between polyreactivity in this assay and

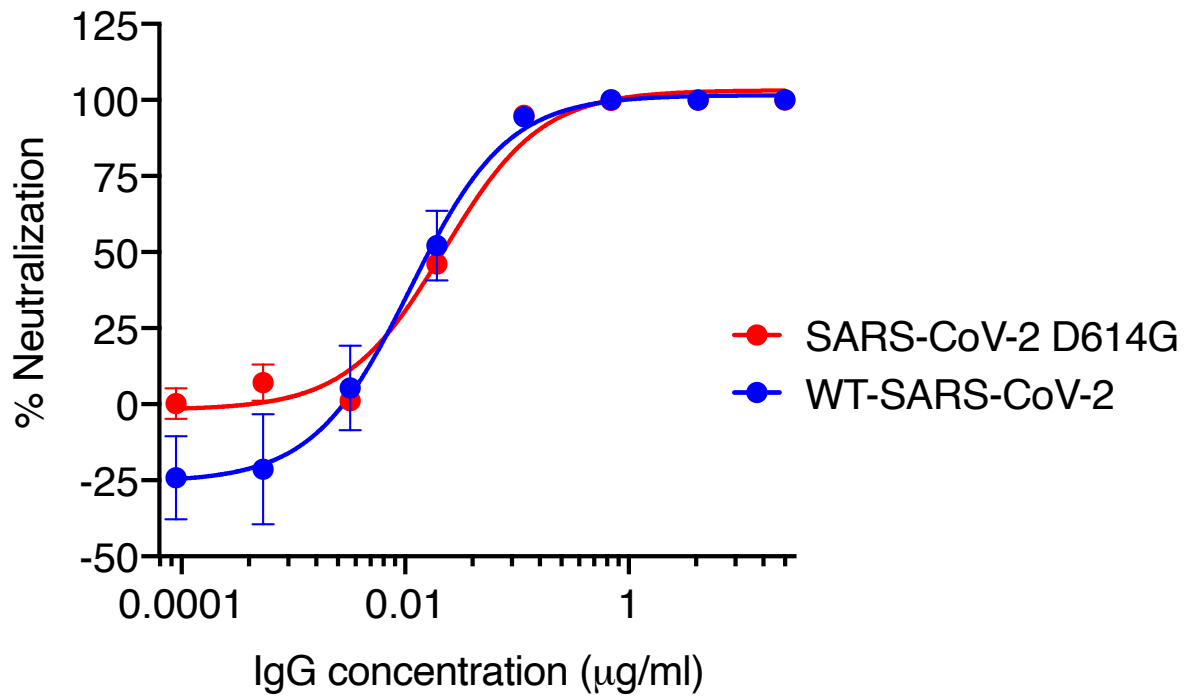
506 serum half-life in humans (22). **(B)** Antibody hydrophobicity, as determined by hydrophobic  
507 interaction chromatography. **(C)** Antibody self-association propensity, as determined by affinity-  
508 capture self-interaction nanoparticle spectroscopy (AC-SINS). **(D)** Fab thermal stability, as  
509 determined by differential scanning fluorimetry (DSF). Forty-two clinically approved antibodies  
510 (38) were included in each assay as comparators and used to determine the thresholds for  
511 high/medium/low hydrophobicity, self-interaction propensity and thermal stability.





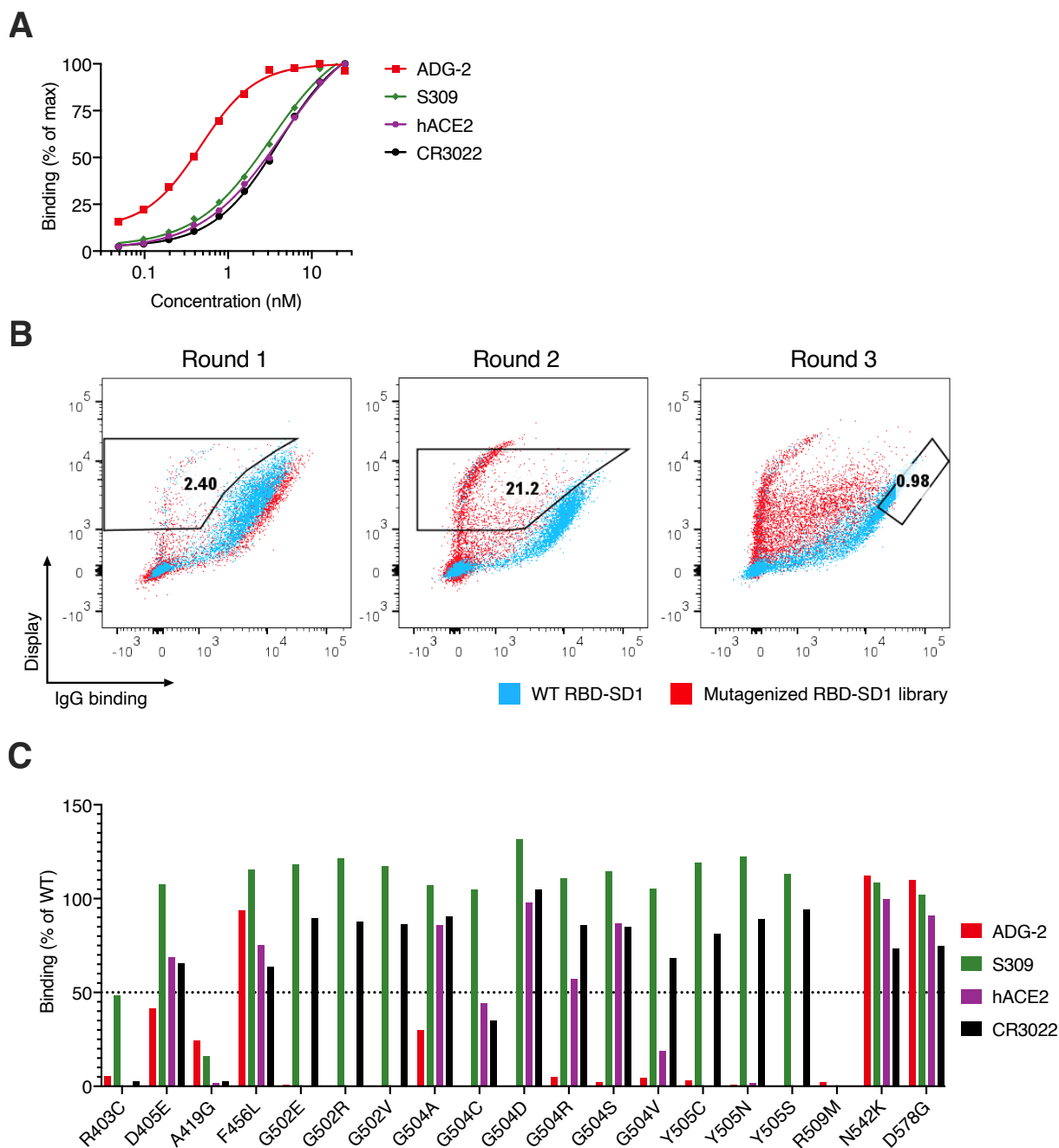
512

513 **Figure S4.** Representative neutralization curves for ADG-2 and SARS-CoV-2 clinical antibodies  
514 against authentic WIV-1 (**A**), SHC014 (**B**), SARS-CoV-2 (**C**), or SARS-CoV (**D**) on Vero target  
515 cells. Error bars represent standard deviations.



516

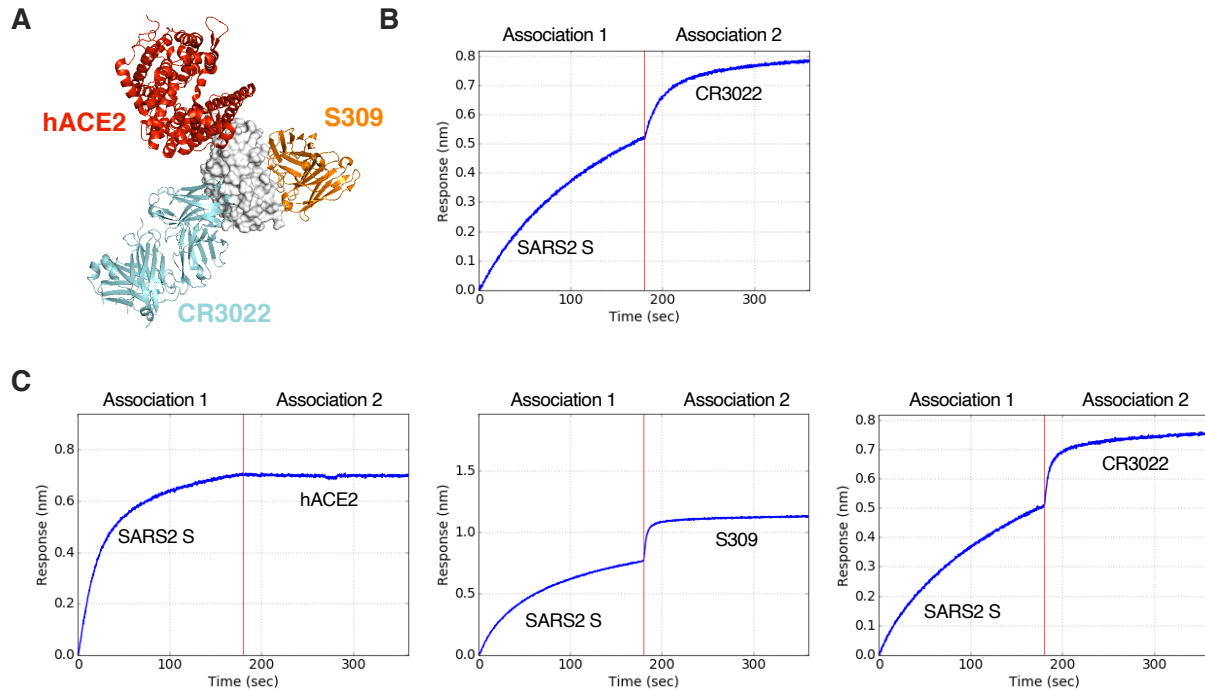
517 **Figure S5.** ADG-2 neutralization of SARS-CoV-2 D614G. Neutralizing activity of ADG-2  
518 against WA1-SARS-CoV-2 and WA1-SARS-CoV-2 D614G viruses was assessed using a  
519 murine leukemia virus (MLV)-based pseudovirus assay and HeLa-hACE2 target cells.



520

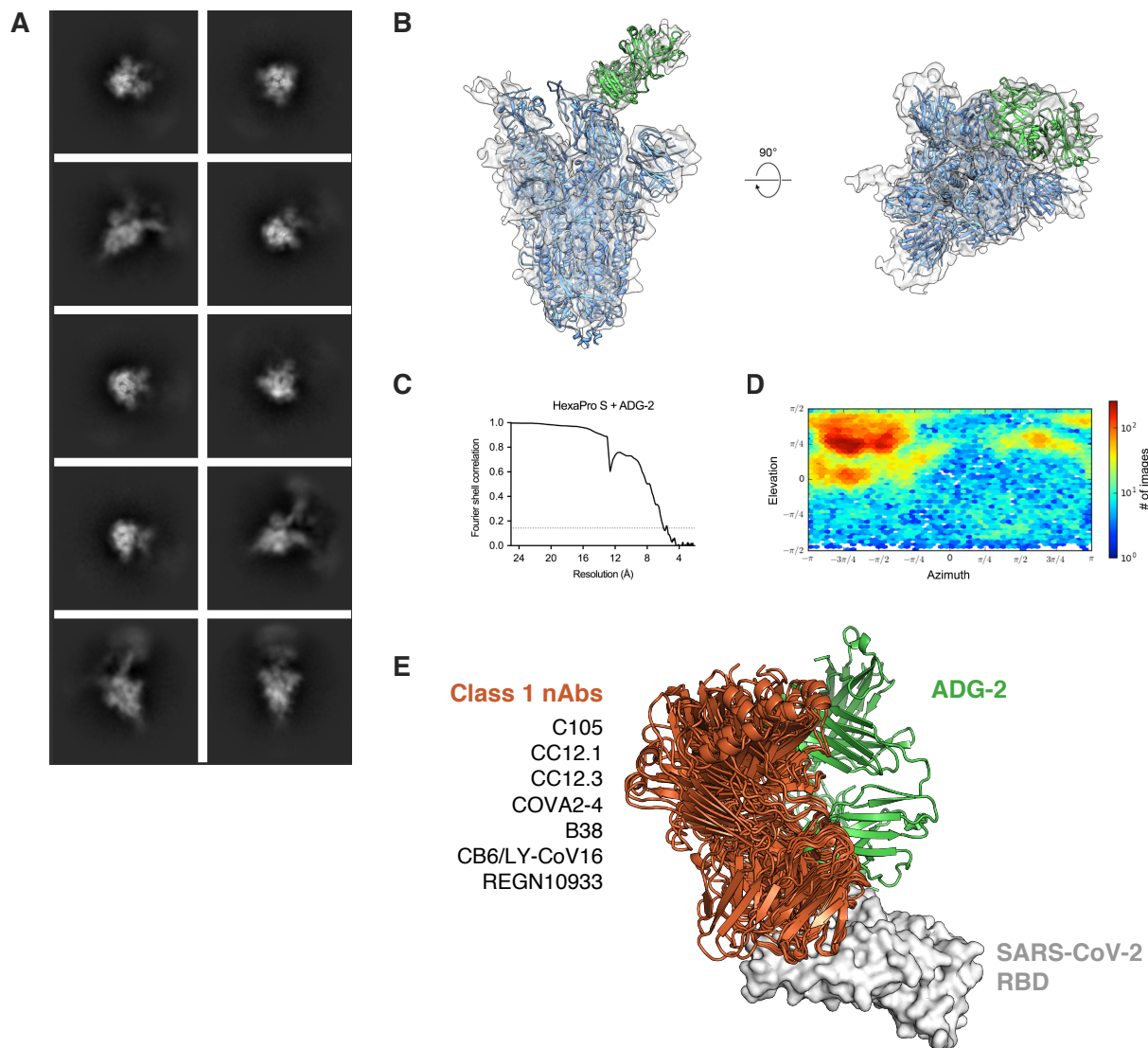
521 **Figure S6.** Fine epitope mapping using an RBD display library. (A) Binding titration curves of  
 522 ADG-2, S309, CR3022, and recombinant hACE2-Fc protein on the WT yeast surface-displayed  
 523 SARS-CoV-2 RBD, used to calculate  $K_D^{app}$  and  $EC_{80}$  concentrations. (B) Flow cytometric  
 524 selection of a mutagenized, yeast surface-displayed RBD library (red) to enrich for variants that  
 525 display loss of binding to ADG-2 (round 1 and 2) but retain binding to antibodies (S309 and

526 CR3022) that bind to epitopes distinct from the ADG-2 binding site (round 3). The round 1 and 2  
527 gates indicate the yeast populations that were sorted for sequential rounds of selection and the  
528 round 3 gate indicates the yeast population that was sorted for individual colony isolation and  
529 sequencing. (C) Percent antibody or recombinant hACE2-Fc binding to yeast surface-displayed  
530 SARS-CoV-2 RBD variants relative to the WT SARS-CoV-2 RBD. Antibody binding was  
531 assessed at their respective  $EC_{80}$  concentrations for the WT RBD construct.



532

533 **Figure S7.** Competitive binding assays. **(A)** Structure of the SARS-CoV-2 RBD (white) bound  
534 to hACE2 (red) (PDB ID: 6M0J) docked with SARS-CoV-2 mAbs S309 (orange, PDB ID:  
535 6WPS) and CR3022 (cyan, PDB ID: 6W41). **(B)** S309 and CR3022 sandwich binning  
536 sensorgram. The traces depict the association (0-180 seconds) of SARS-CoV-2 S to S309  
537 captured on the probe followed by exposure (180-360 seconds) to CR3022. **(C)** ADG-2 and  
538 hACE2-Fc (left), S309 (middle), and CR3022 (right) sandwich binning sensorgrams. The traces  
539 depict the association (0-180 seconds) of SARS-CoV-2 S to ADG-2 captured on the probe  
540 followed by exposure (180-360 seconds) to hACE2-Fc, S309 IgG, or CR3022 IgG. Additional  
541 binding of the competitor protein indicates an unoccupied epitope (non-competitor), while no  
542 binding by the competitor protein indicates blocking (competitor) of the epitope by the IgG.



543

544 **Figure S8.** Cryo-EM validation and structural analysis of ADG-2 bound to the SARS-CoV-2  
545 RBD. **(A)** Two-dimensional class averages of ADG-2 Fab bound to the SARS-CoV-2 spike. **(B)**  
546 Side and top views of the 5.94 Å cryo-EM reconstruction, with the map displayed as a  
547 transparent surface and high-resolution models of the SARS-CoV-2 spike (PDB ID: 6XKL) and  
548 a homologous Fab (PDB ID: 6APC) displayed as blue and green ribbons, respectively. **(C)**  
549 Fourier shell correlation (FSC) curve for the 3D reconstruction. The dashed line corresponds to  
550 an FSC value of 0.143. **(D)** The viewing distribution plot for the 3D reconstruction, calculated in

551 cryoSPARC. **(E)** Cryo-EM reconstruction of the SARS-CoV-2 RBD (white) bound by ADG-2  
552 Fab overlaid with high-resolution structures of several Class I SARS-CoV-2 neutralizing  
553 antibody Fabs (PDB IDs: 6XC4, 6XC2, 6XCN, 7JMO, 7BZ5, 6XDG and 7C01), shown as green  
554 and orange structures, respectively.

**Cryo-EM data collection and reconstruction statistics**

---

Protein	SARS-CoV-2 HexaPro S + ADG-2
EMDB	EMD-XXXXX
Microscope	FEI Titan Krios
Voltage (kV)	300
Detector	Gatan K3
Magnification (nominal)	22,500
Pixel size (Å/pix)	1.073
Flux (e <sup>-</sup> /pix/sec)	8.0
Frames per exposure	30
Exposure (e <sup>-</sup> /Å <sup>2</sup> )	36
Defocus range (μm)	0.6-3.5
Micrographs collected	4,748
Particles extracted/final	267,247/57,078
Symmetry imposed	n/a (C1)
Map sharpening B-factor	344.6
Masked resolution at 0.143 FSC (Å)	5.94

555

556 **Table S1.** Cryo-EM data collection and reconstruction statistics.



## 557 **Methods and Materials**

### 558 **HeLa-hACE2 stable cell line**

559 Stable human ACE2 (hACE2)-expressing HeLa cells for authentic SARS-CoV-2 neutralization  
560 assays were generated as previously described (11). Briefly, hACE2 (NM\_001371415) was  
561 cloned into the pBOB vector and co-transfected with lentiviral vectors pMDL (Addgene  
562 #12251), pREV (Addgene #12253), and pVSV-G (Addgene #8454) into HEK293T cells using  
563 Lipofectamine 2000 (Thermo Fisher Scientific) according to the manufacturer's protocol.  
564 Culture media was exchanged 16 hours post-transfection, and supernatant was harvested 32  
565 hours post-transfection. Pre-seeded HeLa cells were transduced using harvested supernatant with  
566 10 µg/mL polybrene (Sigma). At 12 hours post-transduction, cell surface expression of hACE2  
567 was confirmed by flow cytometry.

### 568 **Authentic SARS-CoV neutralization assay**

569 To generate authentic SARS-CoV, Vero African grivet monkey kidney cells (Vero E6, ATCC-  
570 CRL1586) were grown in Dulbecco's Modified Eagle Medium (DMEM high glucose; Gibco,  
571 Cat # 11995065), 2% heat-inactivated fetal bovine serum (FBS, Atlanta Biologicals), 0.05%  
572 Trypsin-EDTA solution (Gibco), 1% Pen/Strep (Gibco), and 1% GlutaMAX (Gibco). Cells were  
573 infected with SARS-CoV/Urbani at a multiplicity of infection (MOI) of 0.01 and incubated at 37  
574 °C with 5% CO<sub>2</sub> and 80% relative humidity (RH). 50 hours post-infection, cells were frozen at -  
575 80 °C for 1 hour and then thawed at room temperature (RT). The supernatant was collected and  
576 clarified by centrifugation at 2500 x g for 10 minutes before aliquoting for storage at -80 °C.

577 Virus neutralization was assessed as previously described (11). Briefly, SARS-CoV/Urbani  
578 (MOI = 0.2) was added to serial dilutions of antibodies and incubated for 1 hour at RT. The  
579 antibody-virus mixture was applied to monolayers of Vero E6 cells in a 96-well plate and  
580 incubated for 1 hour at 37 °C, 5% CO<sub>2</sub> and 80% RH. Next, media was exchanged by washing  
581 cells once with 1x PBS and adding fresh cell culture media. At 24-hour post-infection, cells were  
582 washed out of media with 1x PBS to then be treated with formalin fixing solution, permeabilized  
583 with 0.2% Triton-X for 10 minutes at RT, and finally treated with blocking solution. Fixed and  
584 permeabilized cells were first stained with a primary antibody recognizing SARS-CoV  
585 nucleocapsid protein (Sino Biological), followed by secondary antibody staining with  
586 AlexaFluor 488-conjugated goat anti-rabbit antibody. Infected cells were enumerated by an  
587 Operetta high content imaging instrument, and data was analyzed using Harmony software  
588 (Perkin Elmer).

### 589 **MLV-SARS-CoV-2 pseudovirus neutralization assay**

590 To generate the MLV pseudoviruses, pCDNA3.3 plasmids (ThermoFisher) encoding the WT  
591 (NC\_045512) or D614G-variant SARS-CoV-2 spike gene with a 28 amino acid deletion at its C-  
592 terminus (IDT); a luciferase reporter gene plasmid (Addgene # 18760) modified with a  
593 cytomegalovirus (CMV) promoter to replace the internal ribosome entry site (IRES); and a  
594 murine leukemia virus (MLV) Gag-Pol plasmid (Addgene # 14887) were purified using the  
595 Endo-Free Plasmid Maxi Kit (Qiagen) according to manufacturer's instructions. To generate  
596 single-round infection competent pseudoviruses, HEK293T cells were co-transfected with 2 µg  
597 of MLV Gag-Pol-, 2 µg of MLV luciferase-, and 0.5 µg of either SARS-CoV-2 WT S or SARS-  
598 CoV-2 D614G S-encoding plasmids in 6-well plates using Lipofectamine 2000 (Thermo  
599 FisherScientific), according to the manufacturer's directions. Cell culture media was exchanged

600 16 hours post-transfection. At 48 hours post-transfection, the supernatant containing SARS-CoV-  
601 2 S-pseudotyped viral particles was harvested, aliquoted, and frozen at -80 °C.

602 Antibody neutralization of pseudoviruses was assessed via a luminescence-based assay on HeLa-  
603 hACE2 cells as previously described (39). The SARS-CoV-2 WT or D614G pseudovirus was  
604 mixed with serially diluted antibodies, incubated for 1 hour at 37 °C, and applied to 10,000  
605 HeLa-hACE2 cells. Following infection for 42-48 hours at 37 °C, HeLa-hACE2 cells were lysed  
606 with 1x luciferase lysis buffer (25 mM Gly-Gly pH 7.8, 15 mM MgSO<sub>4</sub>, 4 mM EGTA, 1%  
607 Triton X-100). Luciferase intensity was measured on a luminometer using Bright-Glo luciferase  
608 substrate (Promega, PR-E2620) following manufacturer's directions. Percentage of  
609 neutralization was calculated from sample and control relative units of light (RUL) according to  
610 the formula:  $100 * (1 - [RUL_{\text{sample}} - RUL_{\text{background}}] / [RUL_{\text{virus-only}} - RUL_{\text{background}}])$ .

#### 611 **Authentic WIV-1, SHC014 and SARS-CoV-2 nano-luciferase (nLuc) neutralization assays**

612 Mouse-adapted SARS-CoV (MA15), mouse adapted SARS-CoV-2 (MA2) and WT SARS-CoV-  
613 2 nano-luciferase (nLuc) viruses were generated by CoV reverse genetics as described previously  
614 (40). WIV-1-nLuc and SHC014-nLuc were generated by replacing the CoV ORF7 and ORF8  
615 regions with nano-luciferase. All nLuc viral assays were performed with Vero E6 cells, which  
616 were grown in DMEM high glucose media (Gibco, Cat # 11995065) supplemented with 10%  
617 fetal clone II (GE, Cat # SH3006603HI), 1% non-essential amino acids, and 1% Pen/Strep at  
618 37°C and 5% CO<sub>2</sub>. Vero E6 cells were seeded at  $2 \times 10^4$  cells/well in a black-wall, tissue culture  
619 treated, 96-well plate (Corning, Cat # 3603) 24 hours prior to pseudovirus assays.

620 Antibodies were serially diluted in growth media and mixed at a 1:1 ratio with either 75 plaque  
621 forming units (PFU)/well SARS-CoV-MA15-nLuc, 100 PFU/well SARS-CoV-2-nLuc, 85

622 PFU/well SARS-CoV-2-MA2-nLuc, 20 PFU/well SHC014-nLuc, or 250 PFU/well WIV1-nLuc  
623 viruses and incubated at 37 °C for 1 hour. Virus and antibody mixture was then added to Vero  
624 E6 cells and incubated at 37 °C with 5% CO<sub>2</sub> for 48 hours (SARS-CoV-MA15, SARS-CoV-2-  
625 MA2 and SARS-CoV-2-nLuc) or 24 hours (SHC014-nLuc and WIV1-nLuc). Luciferase  
626 activities were measured by the Nano-Glo Luciferase Assay System (Promega Cat. #N1130),  
627 following the manufacturer's protocol, using a SpectraMax M3 luminometer (Molecular  
628 Devices). Percent inhibition was calculated by the following equation:  $100 \times (1 - [\text{RLU}_{\text{sample}} /$   
629  $\text{RLU}_{\text{mock-treatment}}])$ . Half-maximal inhibitory concentrations (IC<sub>50</sub>) were calculated for each  
630 condition by curve-fitting with non-linear regression.

#### 631 **Authentic SARS-CoV-2 neutralization assay**

632 Authentic SARS-CoV-2 virus was produced in Vero E6 cells as described previously (23). Vero  
633 E6 cells were grown overnight in complete DMEM (Corning, Cat # 15-013-CV) supplemented  
634 with 10% FBS, 1x Pen/Strep (Corning, Cat # C20-002-CL), and 2 mM L-Glutamine (Corning,  
635 Cat # 25-005-CL) at 37°C and 5% CO<sub>2</sub>. Cells were incubated with 2 mL of SARS-CoV-2 strain  
636 USA-WA1/2020 (BEI Resources, Cat # NR-52281) at MOI of 0.5 for 30 minutes at 34°C and  
637 5% CO<sub>2</sub>, followed by direct addition of 30 mL of complete DMEM. At 5 days post-infection, the  
638 supernatant was collected and centrifuged at 1000 x g for 5 minutes, passed through 0.22 μM  
639 filters, and frozen at -80 °C for future use.

640 Antibody neutralization against authentic SARS-CoV-2 was assessed using both Vero E6 and  
641 HeLa-hACE2 target cells. Both types of target cells were grown in complete DMEM at 37°C and  
642 5% CO<sub>2</sub>. For neutralization assays, HeLa-hACE2 or Vero E6 target cells were seeded in a 96-  
643 well half-well plate at approximately 8000 cells/well suspended in 50 μL complete DMEM and

644 grown overnight. 1,000 plaque forming units (PFU)/well of SARS-CoV-2 was added to titrating  
645 amounts of antibody and incubated for 30 minutes. The virus-antibody mixture was subsequently  
646 incubated with either HeLa-hACE2 or Vero E6 cells for 24 hours at 37°C and 5% CO<sub>2</sub>.  
647 Following incubation, the infection media was removed. Cells were submerged in 4%  
648 formaldehyde for 1 hour, followed by three cycles of washing with PBS, and incubated with 100  
649 µL/well of permeabilization buffer (1x PBS with 1% Triton-X) with gentle shaking. The plates  
650 were then blocked with 100 µL of 3% [w/v] bovine serum albumin (BSA) for 2 hours at room  
651 temperature (RT) and subsequently washed out of blocking solution with wash buffer (1x PBS  
652 with 0.1% Tween-20).

653 SARS-CoV-2 viruses were detected with a mixture of CC6.29, CC6.33, L25-dP06E11, CC12.23,  
654 and CC12.25 antibodies, previously derived from a cohort of convalescent SARS-CoV-2 donors  
655 (23). Pooled antibodies were added to wells at a concentration of 2 µg/mL (50 µL/well) and  
656 incubated for 2 hours at RT. Cells were subsequently washed 3 times with wash buffer, stained  
657 with 0.5 µg/mL peroxidase-conjugated AffiniPure goat anti-human IgG (Jackson  
658 ImmunoResearch Laboratories, Inc, Cat # 109-035-088) for 2 hours at RT, and followed by 6  
659 washes with wash buffer. Freshly prepared HRP substrate (Roche, Ca # 11582950001) at a 100:1  
660 volume ratio of Solution A:B was added to each well. Chemiluminescence was measured using a  
661 microplate luminescence reader (BioTek, Synergy 2).

662 A standard curve of serially diluted virus from 3000 to 1 PFU was plotted against relative light  
663 units (RLU) using a 4-parameter logistic regression as follows:  $y = a + (b - a) / (1 + (x / x_0)^c)$ ,  
664 where  $y$  = variable in RLU,  $x$  = variable in PFU and  $a$ ,  $b$ ,  $c$  and  $x_0$  are parameters fit by the  
665 standard curve. Using parameters generated by the standard curve, sample RLU values were  
666 converted into PFU values ( $x = x_0 \times \log_c [(b - y) / (y - a)]$ ), and percentage neutralization was

667 calculated with the following equation: % Neutralization =  $100 \times [(VC - ADG-2 \text{ treated}) / (VC -$   
668  $CC)]$ , where VC = average of vehicle-treated control and CC = average of cell only control, both  
669 variables in PFU values. Half maximal inhibitory concentration ( $IC_{50}$ ) values were determined  
670 using curve fitting using non-linear regression.

### 671 **Mammalian expression and purification of recombinant SARS-CoV-2 S antigens**

672 Plasmids encoding residues 319–591 of SARS-CoV-2 S with a C-terminal monomeric human  
673 IgG Fc-tag and an 8x HisTag (SARS-CoV-2 RBD-SD1); residues 1–1208 of the SARS-CoV-2  
674 spike with a mutated S1/S2 cleavage site, proline substitutions at positions 986 and 987, and a C-  
675 terminal T4-fibrin trimerization motif, an 8x HisTag, and a TwinStrepTag (SARS-CoV-2 S-  
676 2P); or residues 1–1208 of the SARS-CoV-2 spike with a mutated S1/S2 cleavage site, proline  
677 substitutions at positions 817, 892, 899, 942, 986, and 987, a C-terminal T4-fibrin trimerization  
678 motif, an 8x HisTag, and a TwinStrepTag (SARS-CoV-2 HexaPro S) were transiently  
679 transfected into FreeStyle293F cells (Thermo Fisher) using polyethylenimine. Two hours post-  
680 transfection, cells were treated with kifunensine to ensure uniform, high-mannose glycosylation.  
681 Cell supernatants were harvested after 6 days of protein expression. SARS-CoV-2 RBD-SD1  
682 was purified using Protein A resin (Pierce) and SARS-CoV-2 S-2P and SARS-Cov-2 HexaPro S  
683 were purified using StrepTactin resin (IBA). Affinity-purified SARS-CoV-2 RBD-SD1 was  
684 further purified over a Superdex75 column (GE Life Sciences). SARS-CoV-2 S-2P and SARS-  
685 CoV-2 HexaPro S were purified over a Superose6 Increase column (GE Life Sciences).

### 686 **In vitro affinity maturation of ADI-55688, ADI-55689, and ADI-56046**

687 For each antibody, the complementarity-determining regions (CDRs) 1, 2, and 3 of the heavy-  
688 and light-chains were diversified separately via oligo-based mutagenesis using NNK-randomized

689 oligos spanning CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, and CDRL3 (IDT). Overlap-  
690 extension PCR was used to assemble and amplify forward-priming NNK oligos and reverse-  
691 priming oligo pools covering framework regions 1-4 with added homology to the CDR oligos to  
692 generate full-length variable regions. For the CDRH1/CDRH2/CDRH3 selections, heavy-chain  
693 variable regions (HCFR1-HCFR4) and the unmutated light-chain variable regions of the  
694 nominated parent were recombined *in situ* by homologous recombination with linearized vector  
695 to create a yeast library of  $1 \times 10^7$  diversity via electroporation.

696 Heavy- and light-chain libraries of each parent antibody (ADI-55688, ADI-55689, and ADI-  
697 56046) were subject to two rounds of selection for binding to a recombinant SARS-CoV-2 S1  
698 protein (Sino Biological, Cat # 40591-V08H). Induced yeast libraries covering at least 10-fold of  
699 their respective diversities were incubated with 10 or 1 nM biotinylated SARS-CoV-2 S1 protein  
700 under equilibrium conditions. Yeast was washed twice in PBSF (1x PBS, 0.1% [w/v] BSA),  
701 stained with anti-human LC-FITC (Southern Biotech), Streptavidin 633 (Invitrogen, Cat #  
702 S21375), and propidium iodide (Invitrogen, Cat # P1304MP) for 15 minutes on ice. Labeled  
703 cells were subsequently washed twice and resuspended in PBSF before sorting on a BD FACS  
704 Aria II (Becton Dickerson). Gates were drawn for cells with improved S1 binding over parental  
705 clones.

706 Following two rounds of sorting, the variable heavy and light regions of enriched output clones  
707 were recombined to generate new CDRH1/CDRH2/CDRH3/CDRL1/CDRL2/CDRL3 libraries.  
708 An additional two rounds of selections were performed as described above. Sorted yeast from the  
709 final round of selection were resuspended in SDCAA media and plated on SDCAA agar plates  
710 for single colony isolation and sequencing.

## 711 **Expression and purification of IgGs and Fab fragments**

712 Monoclonal antibodies ADI-55688, ADI-55689, and ADI-56046, as well as their progeny, were  
713 produced as full-length IgG<sub>1</sub> proteins in *S. cerevisiae* cultures, as previously described (11).  
714 Briefly, yeast cultures were incubated in 24-well plates placed in Infors Multitron shaking  
715 incubators at 30 °C, 650 rpm, and 80% relative humidity. After 6 days, the supernatants  
716 containing the IgGs were harvested by centrifugation and purified by protein A-affinity  
717 chromatography. The bound IgGs were eluted with 200 mM acetic acid with 50 mM NaCl (pH  
718 3.5) into 1/8 [v/v] 2 M HEPES (pH 8.0) and buffer-exchanged into PBS (pH 7.0).

719 ADG1-3 and benchmark SARS-CoV-2 mAbs REGN10933, REGN10987, CB6/LY-CoV016,  
720 and S309 were expressed in CHO cells as full-length IgG<sub>1</sub> proteins. The VH- and VL-encoding  
721 gene fragments were subcloned into heavy- and light-chain vectors and transiently transfected  
722 into CHO cells. After 6 days, the supernatants containing the IgGs were harvested by  
723 centrifugation and purified by protein A-affinity chromatography. Bound IgGs were eluted and  
724 further purified by size exclusion chromatography (SEC) to at least 95% purity, then buffer-  
725 exchanged into 150 mM NaCl with 20 mM histidine, pH 6.0.

726 Fab fragments for structural studies were generated by digestion with papain for 2 hours at 30  
727 °C, followed by the addition of iodoacetamide to terminate the reaction. To remove the Fc  
728 fragments and any undigested IgG fractions, the mixtures were passed over Protein A agarose.  
729 The flow-through of the Protein A resin was then passed over CaptureSelect™ IgG-CH1 affinity  
730 resin (ThermoFisher Scientific) and the captured Fabs were eluted with 200 mM acetic acid with  
731 50 mM NaCl (pH 3.5) into 1/8 [v/v] 2 M HEPES (pH 8.0), followed by buffer exchange into  
732 PBS (pH 7.0).



733 **Surface plasmon resonance Fab kinetic binding measurements**

734 SEC-purified SARS-CoV-2 RBD-SD1 was immobilized to a Ni-NTA sensor chip in a Biacore  
735 X100 (GE Life Sciences) to a response level of ~500 RUs. Fabs were then injected at increasing  
736 concentrations, ranging from 18.75-300 nM (ADI-55688), 1.56-25 nM (ADI-56046), 6.25-100  
737 nM (ADI-55689), or 1.25-20 nM (ADG-1, ADG-2, ADG-1). The sensor chip was doubly  
738 regenerated between cycles using 0.35 M EDTA and 0.1 M NaOH. The resulting data were  
739 double-reference subtracted and fit to a 1:1 binding model using Biacore Evaluation Software.

740 **Competitive binding experiments using biolayer-interferometry**

741 Competition of ADG-2 with recombinant hACE2-Fc protein (Sino Biological, Cat # 10108-  
742 H02H), CR3022, and S309 for binding to soluble SARS-CoV-2 S trimer was assessed using the  
743 ForteBio Octet HTX (Sartorius Bioanalytical Instruments). All reagents were diluted to 100 nM  
744 in PBSF. Anti-heavy-chain (AHC) sensor tips were loaded with S309 or ADG-2 IgG, followed  
745 by exposure to an inert IgG to block any remaining Fc capture sites. Tips were subsequently  
746 equilibrated in PBSF for 30 minutes. IgG-loaded sensor tips were transferred to wells containing  
747 hACE2, CR3022, or S309 to check for any interaction with the loaded IgG. Sensor tips were  
748 then loaded in wells containing fresh PBSF buffer (60 seconds), followed by exposure to SARS-  
749 CoV-2 S protein (180 seconds), and lastly, exposure to hACE2, CR3022, or S309 (180 seconds).  
750 Data were cropped to include only SARS-CoV-2 S protein and hACE2, CR3022, or S309  
751 exposure steps and aligned by x- and y-axes using ForteBio Data Analysis software version  
752 11.1.3.10.

753 **Antibody-dependent natural killer cell activation and degranulation (ADNKDA)**

754 Primary human NK cells were enriched from the peripheral blood of human donors using  
755 RosetteSep Human NK cell Enrichment Cocktail (Stem Cell Technologies, Cat #15065) and  
756 cultured overnight in RPMI-1640 (Corning, Cat # 15-040-CV) supplemented with 10% FBS  
757 (Hyclone, Cat # SH30071.03), 1% Pen/Strep (Gibco, Cat # 15070-063), 1% L-Glutamine  
758 (Corning, Cat # 25-005-CI), 1% HEPES (Corning, Cat # 25-060-CI) and 5 ng/mL recombinant  
759 human IL-15 (StemCell Technologies, Cat # 78031). Recombinant SARS-CoV-2 receptor  
760 binding domain was coated onto MaxiSorp 96-well plates (Thermo Scientific, Cat # 442404) at  
761 200 ng/well at 4 °C overnight. Wells were washed with PBS and blocked with 5% BSA prior to  
762 addition of antibodies that were diluted in a five-fold dilution series in PBS (10 µg/mL - 0.32  
763 ng/mL) and incubation for 2 h at 37 °C. Unbound antibodies were removed by washing with  
764 PBS were added at 5 x 10<sup>4</sup> cells/well in the presence of 4 µg/mL brefeldin A (Biolegend, Cat #  
765 420601), 5 µg/mL GolgiStop (BD Biosciences, Cat # 554724) and anti-CD107a antibody (Clone  
766 H4A3 PE-Cy7, Biolegend, Cat # 328618) for 5 hours. Cells were stained for surface expression  
767 of CD16 (Clone 3G8 Pacific Blue, Biolegend, Cat # 302032), CD56 (clone 5.1H11  
768 AlexaFluor488, Biolegend, Cat # 362518) and CD3 (clone UCHT1 Alexa Fluor700, Biolegend,  
769 Cat # 300424). Cells were fixed and permeabilized with Fix/Perm (Biolegend, Cat # 421002)  
770 according to the manufacturer's instructions to stain for intracellular IFN $\gamma$  (Clone B27 PE,  
771 Biolegend, Cat # 506507) and TNF $\alpha$  (clone Mab11 APC, Biolegend, Cat # 502912). Cells were  
772 analyzed on a Cytex Aurora spectral flow cytometer.

### 773 **Antibody-dependent cellular phagocytosis (ADCP) with monocytes and neutrophils**

774 For ADCP assays with neutrophils, HL-60 promyeloblast cells (ATCC, Cat # CCL-240) were  
775 maintained in Iscove's Modified Dulbecco's Medium (ATCC, Cat # 30-2005) with 20% fetal  
776 bovine serum and 1% Pen/Strep. HL-60 cells were differentiated into neutrophils by growth for 5

777 days in the presence of 1.3% DMSO. Recombinant SARS-CoV-2 RBD protein was coupled to  
778 fluorescent beads (Thermo Scientific, Cat # F8819) by carbodiimide coupling. Antibodies were  
779 diluted in a five-fold dilution curve in HL-60 culture medium (1000 - 0.32 ng/mL) and incubated  
780 with RBD-coated beads for 2 hours at 37 °C. Cells ( $5 \times 10^4$ /well) were incubated for 18 hours at  
781 37 °C. Cells were then stained for CD11b (Clone M1/70 APC-Fire750, Biolegend, Cat #  
782 101262) and CD16 (Clone 3G8 Pacific Blue, Biolegend, Cat # 302032), fixed with 4%  
783 paraformaldehyde, and analyzed by flow cytometry. CD11b<sup>+</sup> and CD16<sup>+</sup> cells were analyzed  
784 for uptake of fluorescent beads. A phagocytic score was determined using the following formula:  
785 (percentage of FITC<sup>+</sup> cells)\*(geometric mean fluorescent intensity (gMFI) of the FITC<sup>+</sup>  
786 cells)/100,000.

787 For ADCP assays with monocytes, THP-1 monocytes were maintained in RPMI-1640  
788 supplemented with 10% FBS, 1% Pen/Strep, 1% L-glutamine, and  $\beta$ -mercaptoethanol.  
789 Recombinant SARS-CoV-2 RBD-coated beads were generated as described above. Antibodies  
790 were diluted in a five-fold dilution curve in THP-1 culture medium to (5000–0.064 ng/mL) and  
791 incubated with RBD-coated beads for 2 h at 37 °C. Unbound antibodies were removed by  
792 centrifugation prior to the addition of THP-1 cells at  $2.5 \times 10^4$  cells/well. Cells were fixed with  
793 4% paraformaldehyde and analyzed by flow cytometry. A phagocytic score was determined as  
794 described above.

### 795 **Antibody-mediated complement deposition (ADCD)**

796 Recombinant SARS-CoV-2 receptor binding domain-coated beads were generated as described  
797 for ADCP assays. Antibodies were diluted in a five-fold dilution series in RPMI-1640 (5000 -  
798 0.064 ng/mL) and incubated with RBD-coated beads for 2 hours at 37 °C. Unbound antibodies

799 were removed by centrifugation prior to the addition of reconstituted guinea pig complement  
800 (Cedarlane Labs, Cat # CL4051) and diluted in veronal buffer supplemented with calcium and  
801 magnesium (Boston Bioproducts, Cat # IBB-300) for 20 minutes at 37 °C. Beads were washed  
802 with PBS containing 15 mM EDTA, and stained with an FITC-conjugated anti-guinea pig C3  
803 antibody (MP Biomedicals, Cat # 855385). C3 deposition onto beads was analyzed by flow  
804 cytometry. The gMFI of FITC for all beads was measured.

### 805 **Polyreactivity assay**

806 Polyspecificity reagent binding of antibodies was performed as described previously (37).  
807 Briefly, soluble membrane protein (SMP) and soluble cytosolic protein (SCP) fractions were  
808 extracted from Chinese hamster ovary (CHO) cells and biotinylated using NHS-LC-Biotin  
809 (Thermo Fisher Scientific) reagent. Yeast-presented IgGs were incubated with 1:10 diluted stock  
810 of biotinylated SMP and SCP for 20 minutes on ice, followed by two washes with PBSF, and  
811 stained with 50 µL of a secondary labeling mix containing ExtrAvidin-R-PE (Sigma-Aldrich),  
812 anti-human LC-FITC (Southern Biotech), and propidium iodide (Invitrogen) for 15 minutes on  
813 ice. Cells were subsequently washed with PBSF and resuspended in PBSF for flow cytometric  
814 analysis on a BD FACS Canto II (BD Biosciences). Polyreactivity scores were also reported for  
815 42 previously described clinical antibodies for comparison (38).

### 816 **Affinity-capture self-interaction nanoparticle spectroscopy (AC-SINS)**

817 To measure the propensity for antibodies to self-associate, AC-SINS was performed as  
818 previously described (41). Briefly, polyclonal goat anti-human IgG Fc antibodies (capture;  
819 Jackson ImmunoResearch Laboratories) and polyclonal goat non-specific antibodies (non-  
820 capture; Jackson ImmunoResearch Laboratories) were buffer exchanged into 20 mM sodium

821 acetate (pH 4.3) and concentrated to 0.4 mg/mL. A 4:1 volume ratio of capture:non-capture was  
822 prepared and further incubated at a 1:9 volume ratio with 20 nm gold nanoparticles (AuNP; Ted  
823 Pella Inc.) for 1 hour at room temperature (RT). Thiolated PEG (Sigma-Aldrich) was then used  
824 to block empty sites on the AuNP and filtered via a 0.22  $\mu\text{m}$  PVDF membrane (Millipore).  
825 Coated particles were subsequently added to the test antibody solution and incubated for 2 hours  
826 at RT before measuring absorbance from 510 to 570 nm on a plate reader. Data points were fit  
827 with a second-order polynomial in Excel to obtain wavelengths at maximum absorbance. Values  
828 are reported as the difference between plasmon wavelengths of the sample and background  
829 ( $\Delta\lambda_{\text{max}}$ ). AC-SINS values were also reported for 42 previously described clinical antibodies for  
830 comparison (38).

### 831 **Fab thermal stability**

832 Apparent melting temperatures ( $T_m^{\text{APP}}$ ) of Fab fragments were obtained as previously described  
833 (42). Briefly, 20  $\mu\text{l}$  of test antibody solution at 1 mg/mL was mixed with 10  $\mu\text{l}$  of 20  $\times$  SYPRO  
834 orange. The plate was scanned with a CFX96 Real-Time System (BioRad) from 40  $^{\circ}\text{C}$  to 95  $^{\circ}\text{C}$   
835 at a rate of 0.25  $^{\circ}\text{C}/\text{minute}$ .  $T_m^{\text{APP}}$  was calculated from the primary derivative of the raw data via  
836 the BioRad analysis software. Melting temperatures were also reported for 42 previously  
837 described clinical antibodies for comparison (38).

### 838 **Hydrophobic interaction chromatography (HIC)**

839 Antibody hydrophobicity was evaluated using HIC as previously described (43). Briefly, test  
840 antibody samples were diluted in phase A solution (1.8 M ammonium sulfate and 0.1 M pH 6.5  
841 sodium phosphate) to a final concentration of 1.0 M ammonium sulfate. A linear gradient from  
842 phase A solution to phase B solution (0.1 M pH 6.5 sodium phosphate) was run for 20 minutes at

843 a flow rate of 1.0 mL/minute using the Sepax Proteomix HIC butyl-NP5 column. Peak retention  
844 times were obtained from monitoring UV absorbance at 280 nm. Hydrophobicity values were  
845 also reported for 42 previously described clinical antibodies for comparison (38).

#### 846 **Sarbecovirus phylogeny and alignment**

847 Representative sarbecovirus RBD-SD1 sequences were selected based on previously curated  
848 sequence sets (25, 26). Four additional ACE2-utilizing clade I sarbecoviruses (Frankfurt 1,  
849 CS24, Civet 007-2004, and A021) not represented in these curated sets were included for added  
850 diversity at the RBD-ACE2 interface. A limited set of clade 2 and clade 3 viruses, which do not  
851 utilize ACE2 as a target receptor (26), were included as controls. A phylogram of sarbecoviruses  
852 was generated using maximum likelihood analysis of MAFFT-aligned RBD-SD1 sequences.  
853 Multiple sequence alignment of sarbecovirus RBD sequences was visualized in Jalview. Amino  
854 acid sequences for each sarbecovirus were colored by percentage sequence identity and the  
855 overall degree of conservation per residue was calculated as a numerical index weighted by  
856 physio-chemical properties of amino acids (44).

#### 857 **GISAID analysis of circulating SARS-CoV-2 variants**

858 Genome sequences were downloaded from the GISAID database (28) and aligned pairwise  
859 against the reference Wuhan-Hu-1 sequence (ENA QHD43416.1) via an internal implementation  
860 of the Needleman–Wunsch algorithm to extract all RBD-SD1 sequences using amino acid  
861 residues 319 to 591 of the Wuhan-Hu-1 spike sequence. Incomplete RBD-SD1 nucleotide  
862 sequences and those containing ambiguous (“n”) base calls, plus translated sequences including  
863 “X”, “\*”, or “-,” were excluded from further analysis. RBD-SD1 sequence variants observed at  
864 least 6 times out of 63551 sequences analyzed as of July 14, 2020, as well as several literature

865 controls and antibody escape mutants (24, 27) observed in the GISAID database, were compiled  
866 as a panel 36 variants to assess antibody binding. Sequence frequencies were updated October  
867 19, 2020 and used to calculate each percent prevalence.

868 **Cloning and expression of SARS-CoV-2 variant and homologous sarbecovirus RBD**  
869 **constructs**

870 The spike RBD-SD1 of SARS-CoV-2 (residues 319 to 591, as defined by Uniprot: P0DTC2) and  
871 additional related sarbecoviruses (HKU3, ENA AAY88866.1; Rf1-2004, ENA ABD75323.1;  
872 BM48-31, ENA ADK66841; Pangolin\_GX-P2V GISAID MT072864.1; RaTG13, ENA  
873 QHR63300.2; SARS-CoV-2, ENA QHD43416.1; GD-Pangolin, ENA MT121216.1; Rs4231,  
874 ENA ATO98157.1; WIV1, ENA AGZ48831.1; Civet 007-2004, ENA AAU04646.1; A021,  
875 ENA AAV97986.1; Frankfurt 1, ENA BAE93401.1; SARS-CoV-1, ENA AAP13441; CS24,  
876 ENA ABF68959; LYRa11, ENA AHX37558.1; Rs4081, ENA KY417143.1) were obtained as  
877 gBlocks (IDT) and cloned into a yeast surface-display expression vector encoding a flexible  
878 Gly4Ser linker and a hemagglutinin (HA) epitope tag at its N-terminus. Two consecutive  
879 Gly4Ser linkers connect RBD-SD1 to Aga2p at the C-terminus. Circulating SARS-CoV-2  
880 variant sequences (described above) were cloned into the same expression vector. The A352S  
881 variant was excluded due to an error present in the provided gBlock. Plasmids were transformed  
882 into *S. cerevisiae* (EBY100) using the Frozen-EZ Yeast Transformation II Kit (Zymo Research)  
883 following the manufacturer's protocol and recovered in selective SDCAA media.

884 For induction of RBD expression, fresh yeast cultures were inoculated at 0.2 OD<sub>600</sub> in selective  
885 SDCAA media and grown at 30 °C and 180 rpm until cultures reached an 0.8-1.0 OD<sub>600</sub>. Cells  
886 were centrifuged at 2,400 x g for 3 minutes, resuspended in an equal volume of SGCAA (6.7 g/L

887 Yeast Nitrogen Base, 4.0 g/L drop out amino acid mix, 0.46 g/L NaH<sub>2</sub>PO<sub>4</sub>, 0.88 g/L Na<sub>2</sub>HPO<sub>4</sub>,  
888 7.7 g/L NaCl, 2% galactose, 2% raffinose), and incubated for 16 to 20 hours at 20 °C and 200  
889 rpm.

### 890 **Antibody binding to yeast surface-displayed RBD variants**

891 To assess binding breadth, IgGs and recombinant hACE2 (expressed in a bivalent format as a C-  
892 terminal IgG1 Fc conjugate; Sino Biological, Cat # 10108-H02H) were tested against the panel  
893 of 17 sarbecovirus RBDs. Initially, binding was determined at a single 100 nM concentration of  
894 IgG or hACE2. Induced cells (0.2 OD<sub>600</sub> / well) were aliquoted into 96-well plates and washed  
895 out of SGCAA media with PBSF. Cells were resuspended in 100 µL of 100 nM IgG or hACE2  
896 and incubated at room temperature for 30 minutes. Cells were subsequently washed twice with  
897 PBSF and labeled with 50 µL of APC-conjugated monoclonal mouse anti-hemagglutinin tag  
898 (HA).11 antibody (BioLegend, Cat # 901524), PE-conjugated goat anti-human IgG polyclonal  
899 antibodies (Southern Biotech, Cat # 2040-09), and propidium iodide (Invitrogen, Cat #  
900 P1304MP) for 20 minutes on ice. For each sarbecovirus RBD, a secondary reagent control was  
901 included. Cells were washed twice with PBSF before analyzing via flow cytometry on a BD  
902 FACS Canto II (BD Biosciences).

903 To account for differences in RBD expression across sarbecoviruses, binding signal was  
904 normalized to HA-tag signals ( $MFI_{\text{anti-human IgG PE}}/MFI_{\text{anti-HA APC}}$ ). Binding with normalized ratios  
905 below 1.0 were considered non-binding (NB) at the concentration tested. Those with ratios above  
906 1.0 were titrated between 100 nM to 0.048 nM to calculate their apparent binding affinity  
907 ( $K_D^{\text{APP}}$ ). Mean anti-human IgG PE MFI signal was normalized according to the formula:  
908  $(MFI_{\text{sample}} - MFI_{\text{minimum}}) * 100 / (1 - MFI_{\text{minimum}})$  and fitted as nonlinear regression curves in



909 GraphPad Prism using the following equation:  $Y = Y_{x=\text{minimum}} + X * (Y_{x=\text{max}} - Y_{x=\text{minimum}}) / (K_D^{\text{APP}} +$   
910  $X)$ , where X is the IgG or hACE2 concentration and Y is the normalized binding signal.  
911 Concentrations displaying hook effects, defined as concentrations higher than those generating  
912 the maximum PE MFI signal, were excluded from analysis. To maximize the dynamic range of  
913 potential differences in binding affinity to SARS-CoV-2 variants, binding experiments were  
914 conducted at each antibody's respective SARS-CoV-2  $K_D^{\text{APP}}$  concentration. Binding signal was  
915 normalized using the following equation:  $(\text{MFI}_{\text{anti-hu IgG PE}} / \text{MFI}_{\text{anti-HA APC}}) - (\text{MFI}_{\text{background anti-hu IgG}}$   
916  $\text{PE} / \text{MFI}_{\text{background anti-HA APC}})$ , and calculated as a percentage of normalized signal of the reference  
917 WT SARS-CoV-2 strain RBD-SD1.

#### 918 **ePCR library construction and selection of RBD mutants**

919 SARS-COV-2 RBD-SD1 gBlock (IDT) was amplified by polymerase chain reaction (PCR) with  
920 iProof High-Fidelity PCR system (Bio-Rad, Cat # 1725310) following the manufacturer's  
921 recommendations. The amplified DNA was purified (Nucleospin Gel and PCR Clean-up Kit,  
922 Macherey-Nagel, Cat # 740609.250) and subsequently mutagenized by error-prone PCR (ePCR)  
923 via the GeneMorph II Random Mutagenesis Kit (Agilent Technologies, Cat # 200550) with a  
924 target nucleotide mutation frequency of 0–4.5 mutations per kilobase of DNA. The mutagenized  
925 DNA product was cloned into yeast via electroporation as described earlier. The ePCR library  
926 was validated by plating a subset of the transformed ePCR yeast library on tryptophan dropout  
927 agar plates (Teknova, Cat # C6099) and sequencing single colonies. Prior to performing FACS  
928 selection, the ePCR RBD-SD1 library and WT RBD-SD1 yeast were induced as described  
929 above.

930 To select for mutants with diminished binding to ADG-2, induced cells were incubated for 30  
931 minutes on ice with ADG-2 IgG diluted in PBSF to its EC<sub>80</sub> concentration, which was calculated  
932 by titration on the yeast surface-displayed WT RBD-SD1 construct. Cells were washed twice in  
933 PBSF, stained in a secondary staining mixture, and analyzed on a BD FACS Aria II (Becton  
934 Dickerson), as described above. A subset of yeast population exhibiting HA-tag expression and  
935 reduced ADG-2 binding relative to the WT RBD-SD1 construct were sorted and propagated in  
936 SDCAA media for 48 hours at 30 °C. Selection was repeated for a second round to further enrich  
937 yeast encoding ADG-2 binding knock-down mutations. In the final round of selection, the  
938 induced library was stained with a mixture of recombinant hACE2-Fc, and S309 and CR3022  
939 IgGs at their respective EC<sub>80</sub> concentrations. The subset of the stained population that mirrored  
940 the binding profile of WT RBD-SD1-stained yeast was sorted and plated on agar plates for  
941 isolation and sequencing of single colonies. Clones possessing single amino acid substitutions  
942 identified from sequencing were cultured, induced, and evaluated for binding to ADG-2, S309,  
943 CR3022 IgGs and recombinant hACE2-Fc at their respective EC<sub>80</sub> concentrations through flow  
944 cytometric analysis on the BD FACS Canto II (BD Biosciences). Binding signal was normalized  
945 and calculated as a percentage of the binding signal to reference WT RBD-SD1, as described  
946 above.

#### 947 **Cryo-EM studies**

948 SEC-purified SARS-CoV-2 HexaPro S was diluted to a concentration of 0.35mg/mL in a buffer  
949 composed of 2 mM Tris pH 8.0, 200 mM NaCl and 0.02% NaN<sub>3</sub>. Diluted spike was mixed with  
950 a two-fold molar excess of ADG-2 Fab and allowed to bind on ice for 5 minutes before the  
951 mixture was applied to a plasma-cleaned CF-1.2/1.3 grid. Excess liquid was blotted away using a  
952 Vitrobot Mark IV (Thermo Fisher) and the grid was vitrified by rapid plunging into liquid

953 ethane. 4,748 micrographs were collected using Legikon (45) in a Titan Krios (Thermo Fisher)  
954 equipped with a K3 direct electron detector (Gatan). Motion correction, CTF-estimation and  
955 particle picking were performed in Warp (46) and extracted particles were imported into  
956 cryoSPARC v2.15.0 (47). 2D and 3D classification resulted in a final stack of 57,078 particles,  
957 which was used to calculate a 5.94 Å 3D reconstruction using non-uniform refinement (48).  
958 High-resolution crystallographic models of the SARS-CoV-2 RBD (PDB ID: 6M0J) (31) and a  
959 homologous Fab (PDB ID: 6APC) (49) were docked into the density using Chimera (50). A full  
960 description of the data collection and processing parameters can be found in Table S1.

#### 961 **Animal studies**

962 Twelve-month old female Balb/c mice (Envigo, strain 047) were treated with 200 µg of ADG-2  
963 IgG via intraperitoneal (IP) injection at either 12 hours prior to infection (prophylactic) or 12  
964 hours post-infection (therapeutic). Mice were anesthetized with ketamine/xylazine before being  
965 challenged with 1000 PFU of either SARS-CoV-MA15 or SARS2-CoV-2-MA10 (34, 35) via  
966 intranasal inoculation. Mouse body weights and respiratory function were monitored daily for 4  
967 days. Respiratory function was monitored by whole body plethysmography (DSI) with a 30-  
968 minute acclimation period and a 5-minute measurement window as previously described (51).  
969 Viral lung titer was measured by plaque assay, assessing the lower lobe of the right lung. Gross  
970 pathology was performed on mice sacrificed on day 2 and day 4 post-infection. Gross pathology  
971 in the lung scored using a 4-point system, in which 0 represents no hemorrhage and 4 represents  
972 complete and total hemorrhage. All animal husbandry and experiments were performed at BSL3  
973 and in accordance with all University of North Carolina at Chapel Hill Institutional Animal Care  
974 and Use Committee guidelines (AAALAC Institutional Number 329).