

1 **TITLE: Targeted isolation of panels of diverse human protective broadly**  
2 **neutralizing antibodies against SARS-like viruses**

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4 **AUTHORS:**

5 Wan-ting He<sup>1,2,3†</sup>, Rami Musharrafieh<sup>1,2,3†</sup>, Ge Song<sup>1,2,3†</sup>, Katharina Dueker<sup>1,2,3†</sup>, Longping  
6 V. Tse<sup>4</sup>, David R. Martinez<sup>4</sup>, Alexandra Schäfer<sup>4</sup>, Sean Callaghan<sup>1,2,3</sup>, Peter Yong<sup>1,2,3</sup>,  
7 Nathan Beutler<sup>1</sup>, Jonathan L. Torres<sup>5</sup>, Reid M. Volk<sup>5</sup>, Panpan Zhou<sup>1,2,3</sup>, Meng Yuan<sup>5</sup>,  
8 Hejun Liu<sup>5</sup>, Fabio Anzanello<sup>1,2,3</sup>, Tazio Capozzola<sup>1,2,3</sup>, Mara Parren<sup>1</sup>, Elijah Garcia<sup>1</sup>,  
9 Stephen A. Rawlings<sup>6</sup>, Davey M. Smith<sup>6</sup>, Ian A. Wilson<sup>2,3,5,7</sup>, Yana Safonova<sup>8</sup>, Andrew B.  
10 Ward<sup>2,3,5</sup>, Thomas F. Rogers<sup>1,6</sup>, Ralph S. Baric<sup>4,9,\*</sup>, Lisa E. Gralinski<sup>4,\*</sup>, Dennis R.  
11 Burton<sup>1,2,3,10,\*</sup>, Raiees Andrabi<sup>1,2,3,\*</sup>

12  
13 **AUTHOR AFFILIATIONS:**

14 <sup>1</sup>Department of Immunology and Microbiology, The Scripps Research Institute, La Jolla,  
15 CA 92037, USA.

16 <sup>2</sup>IAVI Neutralizing Antibody Center, The Scripps Research Institute, La Jolla, CA 92037,  
17 USA

18 <sup>3</sup>Consortium for HIV/AIDS Vaccine Development (CHAVID), The Scripps Research  
19 Institute, La Jolla, CA 92037, USA.

20 <sup>4</sup>Department of Epidemiology, The University of North Carolina at Chapel Hill, Chapel  
21 Hill, NC 27599, USA.

22 <sup>5</sup>Department of Integrative Structural and Computational Biology, The Scripps Research  
23 Institute, La Jolla, CA 92037, USA.

24 <sup>6</sup>Division of Infectious Diseases, Department of Medicine, University of California, San  
25 Diego, La Jolla, CA 92037, USA.

26 <sup>7</sup>Skaggs Institute for Chemical Biology, The Scripps Research Institute, La Jolla, CA  
27 9203

28 <sup>8</sup>Department of Computer Science, Johns Hopkins University, Baltimore, MD 21218,  
29 USA

30 <sup>9</sup>Departments of Microbiology and Immunology, The University of North Carolina at  
31 Chapel Hill, Chapel Hill, NC 27599, USA.

32 <sup>10</sup>Ragon Institute of Massachusetts General Hospital, Massachusetts Institute of  
33 Technology, and Harvard University, Cambridge, MA 02139, USA.

34 †These authors contributed equally to this work.

35 \*Corresponding author. Email: [rbaric@email.unc.edu](mailto:rbaric@email.unc.edu) (R.S.B.); [lgralins@email.unc.edu](mailto:lgralins@email.unc.edu)  
36 (L.E.G.); [burton@scripps.edu](mailto:burton@scripps.edu) (D.R.B.); [andrabi@scripps.edu](mailto:andrabi@scripps.edu) (R.A.).

37

38 **SUMMARY**

39 The emergence of current SARS-CoV-2 variants of concern (VOCs) and potential future  
40 spillovers of SARS-like coronaviruses into humans pose a major threat to human health  
41 and the global economy <sup>1-7</sup>. Development of broadly effective coronavirus vaccines that  
42 can mitigate these threats is needed <sup>8,9</sup>. Notably, several recent studies have revealed  
43 that vaccination of recovered COVID-19 donors results in enhanced nAb responses  
44 compared to SARS-CoV-2 infection or vaccination alone <sup>10-13</sup>. Here, we utilized a targeted  
45 donor selection strategy to isolate a large panel of broadly neutralizing antibodies (bnAbs)  
46 to sarbecoviruses from two such donors. Many of the bnAbs are remarkably effective in  
47 neutralization against sarbecoviruses that use ACE2 for viral entry and a substantial  
48 fraction also show notable binding to non-ACE2-using sarbecoviruses. The bnAbs are  
49 equally effective against most SARS-CoV-2 VOCs and many neutralize the Omicron  
50 variant. Neutralization breadth is achieved by bnAb binding to epitopes on a relatively  
51 conserved face of the receptor binding domain (RBD) as opposed to strain-specific nAbs  
52 to the receptor binding site that are commonly elicited in SARS-CoV-2 infection and  
53 vaccination <sup>14-18</sup>. Consistent with targeting of conserved sites, select RBD bnAbs  
54 exhibited *in vivo* protective efficacy against diverse SARS-like coronaviruses in a  
55 prophylaxis challenge model. The generation of a large panel of potent bnAbs provides  
56 new opportunities and choices for next-generation antibody prophylactic and therapeutic  
57 applications and, importantly, provides a molecular basis for effective design of pan-  
58 sarbecovirus vaccines.

59

## 60 **Introduction**

61  
62 Relatively early in the COVID-19 pandemic, it appeared that SARS-CoV-2 was a virus  
63 that might be particularly amenable to control by vaccination. Many different vaccine  
64 modalities, most notably mRNA vaccination, showed spectacular success in phase 3  
65 protection studies <sup>19,20</sup>. The success was attributed at least in part to the ability of the  
66 different modalities to induce robust neutralizing antibody (nAb) responses <sup>21-23</sup>. However,  
67 as the virus has now infected hundreds of millions worldwide, variants have arisen  
68 (variants of concern, VOCs), some of which show notable resistance to neutralization by  
69 immunodominant nAb responses induced through infection and vaccination <sup>1,3-5,24</sup>.  
70 Current vaccines are still apparently largely effective in preventing hospitalization and  
71 death caused by VOCs <sup>25,26</sup>. However, as vaccine-induced nAb responses naturally  
72 decline, breakthrough infections are on the increase and there are concerns that these  
73 may become more prevalent and perhaps more clinically serious, and that more  
74 pathogenic and resistant VOCs may appear. There are also concerns that emerging  
75 SARS-like viruses may seed new pandemics from spillover events <sup>2,6</sup>.

76  
77 These concerns drive a search for nAbs and vaccines that are effective against a greater  
78 diversity of sarbecoviruses. Indeed, several individual broadly neutralizing antibodies  
79 (bnAbs) have now been generated either by direct isolation of bnAbs from convalescent  
80 donors or from antibody engineering of more strain-specific nAbs to generate breadth <sup>27-</sup>  
81 <sup>35</sup>. Ideally, large panels of bnAbs would provide more options in the use of such Abs for  
82 prophylaxis and therapy <sup>36</sup>. Importantly, a range of bnAbs would allow for better definition

83 of the requirements for neutralization breadth and more rational effective design of  
84 appropriate immunogens<sup>37,38</sup>. A range of bnAbs has been crucial in germline targeting  
85 approaches to HIV vaccine design<sup>37-41</sup>. Inspired by the demonstration<sup>10-13,30,42-48</sup> of the  
86 strong serum nAb responses in individuals who are infected with SARS-CoV-2 and then  
87 receive an mRNA vaccine, we isolated and characterized 40 bnAbs from two COVID-19  
88 convalescent donors who were recently vaccinated, many of which combine excellent  
89 potency and breadth to sarbecoviruses. *In vivo* evaluation of select RBD bnAbs in a  
90 prophylaxis challenge model showed robust protection against diverse ACE2-utilizing  
91 sarbecoviruses.

92

## 93 **Results**

94

### 95 **Donors for bnAb isolation**

96 To identify donors for bnAb isolation, we first screened sera from 3 different groups for  
97 SARS-CoV-2 neutralization. The groups were: i) COVID-19 convalescent donors (n = 21);  
98 ii) spike-mRNA vaccinated (2X) donors (n = 10) and iii) COVID-19 convalescent donors  
99 (n = 15) who had subsequently been mRNA vaccinated (1X) (Fig. 1a, Extended Data Fig.  
100 1). Consistent with earlier studies, we observed significantly higher levels of plasma nAbs  
101 in donors who were previously infected and then vaccinated (“recovered-vaccinated”)  
102 compared to donors who were only infected or only vaccinated (Fig. 1a, Extended Data  
103 Fig. 1). To examine the breadth of nAb responses across these 3 groups, we tested sera  
104 for neutralization against ACE2 receptor-utilizing sarbecoviruses (Pang17, SARS-CoV-1  
105 and WIV1) and against SARS-CoV-2 VOCs (B.1.1.7 (Alpha), B.1.351 (Beta), P.1

106 (Gamma) and B.1.617.2 (Delta) and B.1.1.529 (Omicron)) (Fig. 1b-c, Extended Data Fig.  
107 1). Sera from recovered-vaccinated donors showed greater breadth of neutralization and  
108 more effective neutralization of VOCs than sera from donors who were only previously  
109 infected or only vaccinated. Consistent with previous studies, neutralization efficacy of  
110 recovered-vaccinated sera against VOCs was similar to that against the ancestral strain  
111 of SARS-CoV-2 <sup>12,44,49,50</sup> (Fig. 1c, Extended Data Fig. 1). Neutralization of SARS-CoV-1,  
112 whose spike is phylogenetically distinct (~15% divergent at the amino acid level) from  
113 SARS-CoV-2 (Extended Data Fig. 1) <sup>2,51</sup>, was relatively low but was clearly above  
114 background for about half of the recovered-vaccinated donors (Extended Data Fig. 1).  
115 None of the convalescent-only or vaccinated-only donor sera could neutralize SARS-  
116 CoV-1, as also noted by us earlier <sup>52</sup>. Of note, many of the existing SARS-CoV-2 cross-  
117 reactive or cross-neutralizing antibodies were isolated from SARS-CoV-1 convalescent  
118 donors <sup>27,32,53-56</sup> and only more recently from SARS-CoV-2 infected donors <sup>30,31,34,35,57</sup>.  
119 BnAbs have also been isolated from SARS-CoV-2 S-protein vaccinated macaques that  
120 show serum cross-neutralizing activity against SARS-CoV-1 <sup>52,58-60</sup>. Hence, SARS-CoV-  
121 1/2 cross-neutralization appears to be a good indicator of the presence of pan-  
122 sarbecovirus activity and greater CoV neutralization breadth <sup>61</sup>. Accordingly, for isolation  
123 of bnAbs in this study, we focused on two SARS-CoV-2 infection recovered-vaccinated  
124 donors (CC25 and CC84) with the most potent SARS-CoV-1 cross-neutralizing antibody  
125 titers.

126

127 **Isolation and characterization of a large panel of sarbecovirus bnAbs**

128 Using SARS-CoV-1 and SARS-CoV-2 S-proteins as baits, we sorted antigen-specific  
129 single B cells to isolate 107 mAbs from two COVID-19 recovered donors who had been  
130 recently vaccinated with the Moderna mRNA-1273 vaccine (CC25 (n = 56) and CC84 (n  
131 = 51)) (Extended Data Fig. 2) <sup>62</sup>. Briefly, from the peripheral blood mononuclear cells  
132 (PBMCs) of the donors, we sorted CD19<sup>+</sup>CD20<sup>+</sup> IgG<sup>+</sup> IgM<sup>-</sup> B cells that bound to both  
133 SARS-CoV-2 and SARS-CoV-1 S-proteins (Fig. 1d, Extended Data Fig. 2). Flow  
134 cytometry profiling of pre-vaccination (post-infection) PMBCs of CC25 and CC84 donors  
135 revealed that SARS-CoV-1/2 cross-reactive IgG<sup>+</sup> B cells were likely seeded after infection  
136 and were recalled upon vaccination (Fig. 1d-e, Extended Data Fig. 2), as also observed  
137 in other studies <sup>44,63</sup>. Heavy (HC) and light (LC) chain sequences from 107 S-protein  
138 sorted single B cells were recovered and expressed as IgGs (Extended Data Fig. 3).

139  
140 All 107 mAbs exhibited cross-reactive ELISA binding to SARS-CoV-2 and SARS-CoV-1  
141 S-proteins (Fig. 2a, Extended Data Fig. 3). Very few of the mAbs showed weak but  
142 detectable binding to  $\beta$ -HCoV (MERS-CoV HCoV-HKU1 and HCoV-OC43) and  $\alpha$ -HCoV  
143 (HCoV-NL63 and HCoV-229E)-derived S-proteins (Fig. 2a, Extended Data Fig. 3). To  
144 determine the epitope specificities of the mAbs, we tested ELISA binding with SARS-  
145 CoV-2 S1 subunit domains and observed that the vast majority of the mAbs (>80%)  
146 displayed RBD-specific binding (Fig. 2a). To determine the cross-reactivity of RBD  
147 binding, we investigated 12 diverse RBDs representing all the 4 major sarbecovirus  
148 clades: clades 1a, 1b, 2 and 3 <sup>2,27,51</sup> (Fig. 2b, Extended Data Fig. 3). The mAbs showed  
149 the greatest degree of cross-reactivity with clades 1a and 1b and the least with clade 2.

150 Approximately a third of the mAbs (31%) showed cross-reactivity with all 12 RBDs derived  
151 from all 4 sarbecovirus clades (Fig. 2b, Extended Data Fig. 3).

152

153 Next, we evaluated cross-clade neutralization with mAb supernatants on a panel of clade  
154 1a (SARS-CoV-1 and WIV-1) and clade 1b (SARS-CoV-2 and Pang17) pseudoviruses of  
155 ACE2-utilizing sarbecoviruses <sup>2</sup>. Two-thirds of mAbs neutralized both SARS-CoV-1 and  
156 SARS-CoV-2 and 43% (40 out of 93 mAbs) neutralized all 4 sarbecoviruses in the panel  
157 and are categorized as bnAbs in this study (Fig. 2b, Extended Data Fig. 3). More  
158 comprehensive and quantitative cross-neutralization is described with a smaller panel of  
159 mAbs below.

160

161 In terms of antibody sequences, of the 107 isolated mAbs, 93 were encoded by unique  
162 immunoglobulin germline gene combinations and 11 were expanded lineages (CC25 [n  
163 = 6] and CC84 [n = 5]) that had 2 or more clonal members (Fig. 2a, Extended Data Fig.  
164 3). There was a notable enrichment of IGHV3-30, particularly, and also IGHV1-46 and  
165 IGHV1-69 germline gene families for both donors as compared to human baseline  
166 germline frequencies (Fig. 2a, c-d, Extended Data Fig. 4) <sup>64,65</sup>. Light chains of certain  
167 germline-gene families (IGKV1-33, IGKV2-30, IGLV1-40, IGLV3-21) were also modestly  
168 enriched in the isolated mAbs (Extended Data Fig. 4). Interestingly, the mAbs showed  
169 modest levels of V-gene nucleotide somatic hypermutation (SHM): for VH, median = 5.0%  
170 and for VL, median = 4.0% (Extended Data Fig. 3). As multiple studies have shown that  
171 heavy chains dominate the epitope interaction by RBD nAb <sup>5,16,17,66</sup>, we sought to  
172 determine whether the IGHV germline gene usage and/or VH SHM levels were correlated

173 with the extent of neutralization breadth (Fig. 2a, c-d). We observed enrichment of IGHV3-  
174 30 in mAbs that bind to clade 2 RBDs and all 12 sarbecovirus RBDs, but otherwise no  
175 notable trends (Fig. 2c-d). VH-gene SHM levels did not distinguish potent broadly  
176 neutralizing from less broad or non-neutralizing mAbs. Overall, we observed that some  
177 IGHV or IGLV genes were enriched in bnAbs, and several human immunoglobulin gene  
178 combinations could encode for sarbecovirus bnAbs.

179

180 To further investigate the potential contribution of SHM to broad reactivity to  
181 sarbecoviruses, we tested the binding of mAbs to SARS-CoV-2 S-protein and to  
182 monomeric RBD by BLI (Extended Data Fig. 5). We found no association of SHM with S-  
183 protein binding and a weak correlation with binding to RBD. Consistent with this lack of  
184 correlation, we did not observe any correlation of SARS-CoV-2 RBD mAb binding with  
185 sarbecovirus neutralization breadth or binding breadth, although some modest correlation  
186 was observed for S-protein binding (Extended Data Fig. 5). These results suggest that  
187 critical antibody paratope features for sarbecovirus breadth when targeting the sites  
188 described below may be germline-encoded and limited affinity maturation is needed.  
189 While recent findings demonstrate that the accumulation of SHM may increase potency  
190 and breadth <sup>13,67</sup>, this may not be a requisite feature of sarbecovirus bnAbs, as noted by  
191 others <sup>68</sup>. The predominant use of certain germline gene segments in bnAbs suggests  
192 that a germline-targeting approach <sup>39-41</sup> to pan-sarbecovirus vaccines may be rewarding  
193 and the relatively low levels of SHM are promising for successful vaccine deployment  
194 provided appropriate immunogens can be designed.

195



196 Next, we examined the CDRH3 loop lengths of the isolated Abs and observed a strong  
197 enrichment for 20- and 21-residue long CDRH3s compared to the human baseline  
198 reference database (Fig. 2e)<sup>64,65</sup>. These long CDRH3s were found to contain high  
199 proportions of two D genes, IGH D2-15 and D3-22, that were notably enriched in bnAbs  
200 (Fig. 2g). Notably, 71% (12/17) of mAbs with 20 amino acid CDRH3s utilized the germline  
201 IGHD2-15 D-gene, and the majority of mAbs bearing 21-amino acid-CDRH3s utilized  
202 either the IGHD2-15 or IGHD3-22 germline D-genes (Fig. 2f, Extended Data Figs. 3 and  
203 4). We noted that the D3-22 D-gene was also selected in bnAbs isolated in other studies  
204 <sup>31,57,69,70</sup>. Therefore, vaccine design strategies will likely need to take these germline  
205 features into consideration <sup>39-41</sup>.

206

207 Altogether, we have isolated a large panel of human sarbecovirus bnAbs. The isolated  
208 bnAbs, although encoded by several immunoglobulin germline gene families, are strongly  
209 enriched for certain germline gene features that will inform pan-sarbecovirus vaccine  
210 strategies.

211

## 212 **Detailed binding and neutralization characteristics of a smaller panel of bnAbs**

213

214 We selected 30 SARS-CoV-1/SARS-CoV-2 RBD cross-reactive mAbs for more detailed  
215 characterization (Fig. 3a). Selection of mAbs was made based on a high degree of cross-  
216 reactive binding with RBDs of multiple sarbecovirus clades. The large panel above  
217 included nAbs that likely had more potent neutralization of SARS-CoV-1 and/or SARS-  
218 CoV-2 individually but lacked broad binding activity (Fig. 2a). To determine sarbecovirus

219 binding cross-reactivity more extensively, we evaluated 12 soluble monomeric RBDs  
220 representing the major sarbecovirus clades, as above in Figure 2. Almost all mAbs bind  
221 SARS-CoV-2 and other clade 1b-derived RBDs, with most binding in a nanomolar (nM)  
222 to picomolar (pM)  $K_D$  affinity range (Fig. 3b, Extended Data Fig. 6). The mAbs that bound  
223 most effectively to clade 1b RBDs tended to also bind well to clade 1a and clade 3 RBDs,  
224 albeit with somewhat lower affinities, yet still in the nM-pM  $K_D$  affinity range. Cross-  
225 reactive binding was least to the clade 2 RBDs, although there was generally some level  
226 of reactivity and some mAbs did show high affinity binding to clade 2 RBDs. Remarkably,  
227 several mAbs showed consistently high affinity binding to RBDs from all 4 sarbecovirus  
228 clades (Fig. 3b, Extended Data Fig. 6).

229

230 Neutralization was investigated only for clade 1a and 1b ACE2-utilizing viruses, since  
231 neutralization assays were not available for clade 2 and 3 viruses. 22 of 30 mAbs  
232 neutralized SARS-CoV-2 with a range of  $IC_{50}$  neutralization titers ( $IC_{50}$  range = 0.05-4.9  
233  $\mu\text{g}/\text{mL}$ ) (Fig. 3c) and 28 of 30 mAbs neutralized SARS-CoV-1, including all mAbs that  
234 neutralized SARS-CoV-2. Neutralization potency was typically stronger against SARS-  
235 CoV-1 than SARS-CoV-2. All mAbs showed neutralization against WIV1, while a majority  
236 exhibited cross-neutralization with Pang17, and to a lesser degree with SHC014. 13 out  
237 of 30 mAbs neutralized all 5 ACE2-utilizing sarbecoviruses tested with a geomean  $IC_{50}$   
238 potency of 0.12  $\mu\text{g}/\text{ml}$ . The three most potent SARS-CoV-2 bnAbs, CC25.52, CC25.54  
239 and CC25.3, neutralized all 5 ACE2-utilizing sarbecoviruses with geomean potencies of  
240 0.03, 0.04 and 0.04  $\mu\text{g}/\text{ml}$ , respectively. Although neutralization assays differ, this

241 suggests they are amongst the most potent and broad individual nAbs described to date  
242 (compare also control nAbs in Fig. 3c).

243

244 We tested neutralization of SARS-CoV-2 VOCs by 20 select SARS-CoV-2 bnAbs.  
245 Consistent with the donor CC25 and CC84 sera neutralization above, the bnAbs were  
246 consistently effective against SARS-CoV-2 VOCs tested (Fig. 3d, Extended Data Fig. 7).  
247 The IC<sub>50</sub> neutralization titers of bnAbs remained largely unchanged against the Alpha,  
248 Beta, Gamma and Delta SARS-CoV-2 VOCs but were more affected by substitutions in  
249 the Omicron variant (Fig. 3d, Extended Data Fig. 7). Nevertheless, IC<sub>50</sub> neutralization  
250 titers for many bnAbs were unchanged or minimally affected for the Omicron variant and  
251 remarkably 14 of 20 bnAbs retained significant neutralization against this highly evolved  
252 SARS-CoV-2 variant (Fig. 3d, Extended Data Fig. 7). In comparison, SARS-CoV-2 strain-  
253 specific nAb, CC12.1 showed substantial or complete loss of neutralization with VOCs.  
254 The results suggest that these bnAbs target more conserved RBD epitopes that are likely  
255 more resistant to SARS-CoV-2 escape mutations. Overall, we have identified multiple  
256 potent sarbecoviruses bnAbs that exhibit broad reactivity to SARS-CoV-2 variants and  
257 diverse sarbecovirus lineages.

258

### 259 **Epitope specificity of sarbecovirus bnAbs**

260 To help map the epitopes recognized by the sarbecovirus bnAbs, we first epitope binned  
261 them using BLI competition with RBD nAbs of known specificities (Fig. 4a, Extended Data  
262 Fig. 8), including 5 human nAbs: (1) CC12.1, an RBS-A or class 1 nAb targeting the ACE2  
263 binding site<sup>5,14,17</sup>; (2) CC12.19, which is thought to recognize a complex RBD epitope

264 and competes with some non-RBD Abs <sup>15</sup>; (3) CR3022, which recognizes the class 4  
265 epitope site <sup>5,14</sup>; (4) S309, which recognizes the class 3 epitope site <sup>5,14</sup>; and (5) DH1047,  
266 which recognizes a conserved site and is class 4 <sup>32</sup>. In addition, we included K398.22, a  
267 macaque bnAb <sup>52</sup>, which targets an RBD bnAb epitope distinct from that recognized by  
268 human bnAbs characterized to date but has features characteristic of class 4 bnAbs (Fig.  
269 4a-b). The bnAbs we describe here can be clustered for convenience into two major  
270 groups. Group-1 bnAbs strongly competed with SARS-CoV-2 class 4 human bnAbs,  
271 CR3022 and DH1047, and macaque bnAb K398.22, showed more sporadic competition  
272 with CC12.1 and did not compete with CC12.19 or S309. Group-2 mAbs competed  
273 strongly with CC12.19, weakly with macaque K398.22, and only infrequently and/or  
274 weakly with any of the other bnAbs. Group-1 bnAbs were potent and broad in  
275 neutralization against ACE2-utilizing sarbecoviruses, but many lineage members  
276 displayed limited binding reactivity with clade 2 sarbecovirus RBDs. The group-2 mAbs  
277 showed broader binding reactivity with sarbecoviruses but were relatively less potent  
278 compared to group-1 bnAbs (Fig. 4a). Notably, one group-2 bnAb, CC25.11 showed  
279 strong competition with human class 3 RBD bnAb, S309 <sup>53</sup>, and the macaque bnAb,  
280 K398.22 <sup>52</sup>. The findings suggest that both group-1 and 2 bnAbs target more conserved  
281 RBD epitopes but group-1 bnAbs are overall more potent but less broad against clade 2  
282 sarbecoviruses, with some exceptions.

283

284 To further investigate the epitopes recognized by the bnAbs, we utilized single-particle,  
285 negative-stain electron microscopy (nsEM) and confirmed that the 9 Group-1 and 2  
286 Group-2 bnAb Fabs bound to the RBD of SARS-CoV-2 S-protein (Fig. 4c, Extended Data

287 Fig. 9). The binding modes of bnAbs to SARS-CoV-2 S-protein were largely similar with  
288 some differences in the angles of approaches, but not distinct enough to clearly segregate  
289 group-1 epitope bnAbs. Further, structural studies that reveal molecular details of the  
290 antibody-antigen interactions contributing to the differences in the epitope recognition are  
291 important. The group-2 bnAb reconstructions are consistent with an epitope that spans  
292 the RBD, and other parts of the S-protein as described for the competitive Ab CC12.19  
293 <sup>15</sup>. These bnAb Fabs showed binding to S-protein with all three stoichiometries (Fab:  
294 trimer; 1:1, 1:2 and 1:3) with some of the Fabs exhibiting destabilizing effects on the S-  
295 trimer (Fig. 4c, Extended Data Fig. 9). This destabilization is seen as dimers and flexible  
296 densities in the 2D class averages (Extended Data Fig. 9).

297

### 298 **Immunogenetics of group 1 and 2 RBD bnAbs and vaccine targeting**

299 To further understand the differences between group 1 and 2 RBD bnAbs and to  
300 determine if germline gene features can differentiate their epitope properties, we  
301 performed detailed antibody immunogenetic analysis. Both groups of RBD bnAbs were  
302 encoded by a number of IGHV germline gene families (Fig. 5a). The average CDRH3  
303 loop lengths were significantly longer ( $p < 0.05$ ) in group 1 compared to group 2 RBD  
304 bnAbs (Fig. 5b). Notably, group 1 bnAbs strongly enriched (60%: 9 out of 15 group 1 RBD  
305 bnAbs) for IGHD3-22 germline D-gene-encoded CDRH3 “YYDxxG” motifs and  
306 possessed significantly longer CDRH3 loops ( $p < 0.005$ ) compared to the other mAbs  
307 (Fig. 5c-d). The IGHD3-22 germline D-gene “YYDxxG” motif-bearing group 1 RBD bnAbs  
308 utilized several IGHV germline gene combinations and the D-gene motifs were either  
309 retained in a germline configuration (YYDSSG: CC25.48 and CC84.2) or one or both “x”

310 residues were mutated (Fig. 5c-d). The most common mutation was the substitution of  
311 YYD-proceeding x-residue, Serine-(S) to an Arginine-(R), which recurrently appeared in  
312 multiple YYDxxG motif bearing RBD bnAbs from both donors suggesting common B cell  
313 affinity maturation pathways. Interestingly, the S-R somatic mutation in the CDRH3  
314 YYDxxG motif appeared to be important for resisting Omicron neutralization escape, as  
315 the non-mutated YYDxxG motif bearing group 1 RBD bnAbs failed or weakly neutralized  
316 this variant (Fig. 5d, Extended Data Fig. 7). Consistent with this observation, recent  
317 studies provide evidence for how YYDRxG RBD bnAbs can effectively bind to the  
318 conserved face of SARS-CoV-2 spike RBD <sup>71,72</sup>. The findings reveal that RBD bnAbs with  
319 certain recurrent germline features can effectively resist SARS-CoV-2 Omicron escape  
320 (Extended Data Fig. 7), but several antibody solutions can counter this extreme antigenic  
321 shift and should be considered for vaccine targeting. Remarkably, in our study genetically  
322 diverse RBD bnAbs in both groups 1 and 2 were capable of effectively neutralizing the  
323 Omicron variant (Extended Data Fig. 7) suggesting that several human antibody solutions  
324 can counter SARS-CoV-2 antigenic shift.

325

326 The association of a germline D-gene encoded motif, provides an opportunity for broad  
327 vaccine targeting, as has been described for HIV <sup>39,41,73</sup>. For SARS-like coronaviruses,  
328 the YYDxxG motif <sup>71,72</sup> appears promising for vaccine targeting. Encouragingly, human  
329 naïve B cell repertoires encode a sizable fraction of IGHD3-22 germline D-gene-encoded  
330 YYDxxG motif bearing B cells with desired CDRH3 lengths (Fig. 5g) that could be targeted  
331 by rationally designed vaccines <sup>74</sup>.

332

### 333 **RBD bnAbs protect against challenge with diverse sarbecoviruses**

334 To determine the protective efficacy of the RBD bnAbs, we conducted passive antibody  
335 transfer followed by challenge with sarbecoviruses in aged mice <sup>75</sup>. We selected 3 of the  
336 broadest group-1 bnAbs, CC25.36, CC25.53 and CC25.54 and investigated their *in vivo*  
337 protective efficacy against SARS-CoV-2, SARS-CoV-1 and SHC014 sarbecoviruses in  
338 mice. SHC014 was chosen as it encodes extensive heterogeneity in the spike RBD,  
339 reduces mRNA SARS-CoV-2 polyclonal neutralization sera titers by ~300-fold <sup>76</sup> and  
340 replicates efficiently in mice <sup>77</sup>. Prior to the protection studies, we compared neutralization  
341 by RBD bnAbs of replication-competent viruses with that of pseudoviruses (Extended  
342 Data Fig. S10). Neutralization of replication-competent SARS-CoV-1 and SARS-CoV-2  
343 by the bnAbs was more effective (lower IC<sub>50</sub> values) than the corresponding  
344 pseudoviruses. Neutralization of replication-competent and pseudovirus versions of  
345 SHC014 by the bnAbs was approximately equivalent. The 3 RBD bnAbs, individually, or  
346 a DEN3 control antibody were administered intra-peritoneally (i.p.) at 300µg/animal into  
347 12 groups of 10 animals (3 groups per antibody; Fig. 6a). Each group was challenged  
348 with one of 3 mouse-adapted (MA) sarbecoviruses, (MA10 = SARS-CoV-2, MA15 =  
349 SARS-CoV or MA15-SHC = SARS-CoV MA15 - SHC014 chimera), by intranasal (i.n.)  
350 administration of virus 12h post-antibody infusion (Fig. 6a). The animals were monitored  
351 for signs of clinical disease due to infection, including daily weight changes, and  
352 pulmonary function. Animals in each group were euthanized at day 2 or day 4 post  
353 infection and lung tissues were collected to determine virus titers by plaque assay. Gross  
354 pathology was also assessed at the time of tissue harvest. The RBD bnAb-treated  
355 animals in all 3 sarbecoviruses challenge experiments showed significantly reduced

356 weight loss (Fig. 6b, f, j), reduced hemorrhage (Fig. 6c, g, k), and largely unaffected  
357 pulmonary function (Fig. 6d, h, l), as compared to the DEN3-treated control group  
358 animals, suggesting a protective role for bnAbs. We also examined virus load in the lungs  
359 at day 2 and day 4 post infection and, consistent with the above results, both the day 2  
360 and day 4 viral titers in RBD bnAb-treated animals were substantially reduced compared  
361 to the DEN3-treated control group animals (Fig. 6e, i, m). Overall, all 3 RBD bnAbs  
362 protected against severe sarbecoviruses disease, CC25.54 and CC25.36 bnAbs being  
363 relatively more protective than CC25.53 bnAb. The animal data suggest potential  
364 utilization of the bnAbs in intervention strategies against diverse sarbecoviruses.

365

## 366 **Discussion**

367 Here, we characterized a large panel of sarbecovirus bnAbs isolated from two SARS-  
368 CoV-2 recovered-vaccinated donors. Select bnAbs showed robust *in vivo* protection  
369 against diverse SARS-like viruses, including SARS-CoV-1, SARS-CoV-2 and SHC014,  
370 in a prophylaxis challenge model. The bnAbs are potent and show neutralization of a  
371 range of VOCs, and many are effective against Omicron. The bnAbs recognize a  
372 relatively conserved face of the RBD that overlaps with the footprint of a number of  
373 antibodies including ADG 61123, DH1047 and CR3022<sup>32,54,72</sup> and broadly the face  
374 designated as that recognized by class 4 antibodies<sup>5,66</sup>. However, as illustrated in Fig 4,  
375 the panel of bnAbs differ in many details of recognition, for instance some compete with  
376 ACE2, others do not, and these differences are important in resistance to mutations. As  
377 variants such as Omicron emerge during this and future CoV pandemics, the availability



378 of a selection of potent bnAbs provides choice of optimal reagents for antibody-based  
379 interventions to respond to the viral threats.

380

381 In terms of vaccine design, the generation of HIV immunogens typically draws heavily on  
382 the availability of multiple bnAbs to a given site to provide the best input for design  
383 strategies <sup>37,78</sup>. The same consideration is likely to apply to pan-sarbecovirus vaccine  
384 design. Further, although the bnAbs that we isolated were encoded by several gene  
385 families, certain V and D gene families were highly enriched. We confirmed and identified  
386 specific antibody germline gene features associated with broad activity against diverse  
387 sarbecoviruses and vaccine design strategies may seek to target these genetic features  
388 by rationally designed prophylactic vaccines <sup>37,39-41,74</sup>. Some of the most potent bnAbs  
389 compete with the immunodominant human SARS-CoV-2 RBS-A/class 1 nAb CC12.1 that  
390 shows relatively low cross-reactivity. Elicitation of nAbs like CC12.1 may then reduce the  
391 elicitation of bnAbs, and rational vaccine design modalities may need to mask RBS-  
392 A/class 1 immunodominant sites <sup>79-81</sup> whilst leaving the bnAb sites intact. Resurfaced  
393 RBD-based immunogens in various flavors <sup>51,58-60</sup> may achieve a similar goal.

394

395 Given the strong bnAb responses induced through infection-vaccination as indicated from  
396 serum studies and by our mAbs, are there lessons here for vaccine design? The higher  
397 frequency of bnAbs in infection-vaccination may have a number of causes. First, the spike  
398 S-protein may have subtle conformational differences, particularly in the sites targeted by  
399 bnAbs, between the native structure on virions and the stabilized form presented by  
400 mRNA immunization. This may favor the activation of bnAbs in the infection step followed

401 by recall during mRNA boosting. Second, the long time-lag between infection and  
402 vaccination may have favored the accumulation of key mutations associated with bnAbs.  
403 There is evidence that intact HIV virions can be maintained on follicular dendritic cells in  
404 germinal centers over long time-periods in a mouse model <sup>82</sup>. Third, T cell help provided  
405 by the infection may be superior to that provided by mRNA vaccination alone. Overall,  
406 there is an intriguing possibility that pan-sarbecovirus nAb activity may be best achieved  
407 by a hybrid approach <sup>43</sup> to immunization that seeks to mimic infection-vaccination, once  
408 the key contributing factors to breadth development in that approach can be determined.  
409 However, we also note that a very recent report, published as a resubmission of this  
410 manuscript was prepared, describes bnAbs arising from a third immunization with an  
411 inactivated vaccine <sup>83</sup>.

412

413 In summary, we isolated multiple potent sarbecovirus protective cross-neutralizing human  
414 antibodies and provide a molecular basis for broad neutralization. The bnAbs identified  
415 may themselves have prophylactic utility and the bnAb panel delineates the boundaries  
416 and requirements for broad neutralization and will be an important contributor to rational  
417 vaccine design.

418

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430 Institute.

431

432 **Author contributions**

433 W.H., R.M., G.S., K.D., D.R.B. and R.A. conceived and designed the study. N.B., M.P.,  
434 E.G., S.A.R., D.M.S., and T.F.R. recruited donors and collected and processed plasma  
435 and PBMC samples. W.H., R.M., G.S., K.D., S.C., P.Y. and F.A. performed BLI, ELISA,  
436 virus preparation, neutralization and isolation and characterization of monoclonal  
437 antibodies. Y.S. performed immunogenetic analysis of antibodies. P.Z. prepared virus  
438 mutant plasmids. J.L.T. and R.M.V. conducted negative stain electron microscopy  
439 studies. M.Y. and H.L. generated antibody-antigen structural models. L.V.T. performed  
440 live virus neutralizations assays and L.V.T., D.R.M., A.S., and L.E.G. conducted *in vivo*  
441 animal protection studies. W.H., R.M., G.S., K.D., L.V.T., D.R.M., A.S., S.C., P.Y., N.B.,

442 J.L.T., R.M.V., P.Z., M.Y. H.L., F.A., M.P., E.G., I.A.W., A.B.W., T.F.R., R.S.B., L.E.G.,  
443 D.R.B. and R.A. designed the experiments and/or analyzed the data. W.H., R.M., D.R.B.  
444 and R.A. wrote the paper, and all authors reviewed and edited the paper.

445

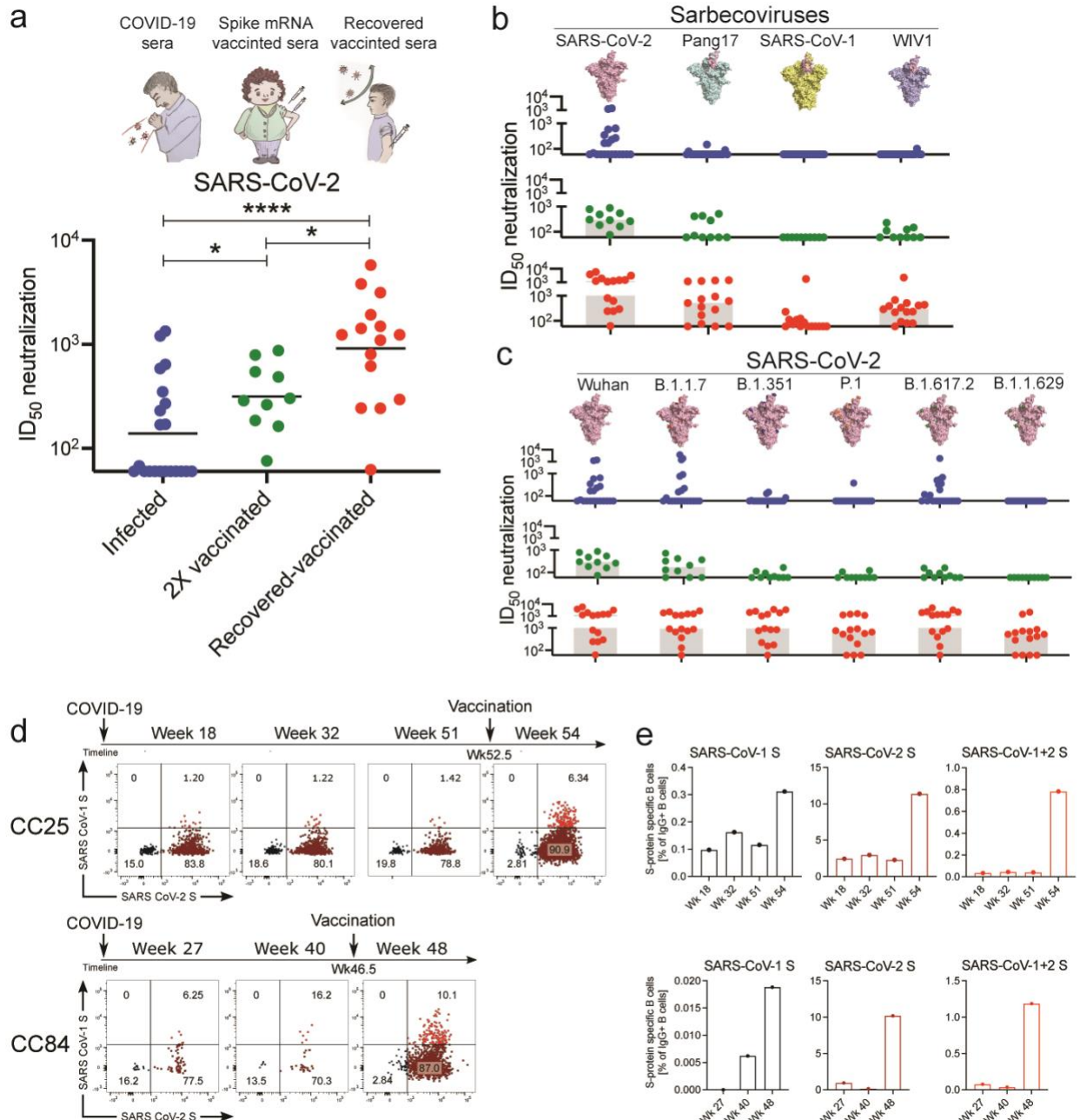
#### 446 **Competing interests**

447 Competing interests: W.H., R.M., G.S., K.D., T.F.R., D.R.B. and R.A. are listed as  
448 inventors on pending patent applications describing the sarbecovirus broadly neutralizing  
449 antibodies isolated in this study. A.B.W, I.A.W and D.R.B. receive research funding from  
450 Adagio. RSB and LEG have ongoing collaborations with Adagio. All other authors have  
451 no competing interests to declare.

452

453

454 **Figures and legends**  
455



**Fig. 1. Plasma neutralization and B cell responses in convalescent recovered, vaccinated, and recovered-vaccinated donors.**

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457  
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459 **a.** SARS-CoV-2 pseudovirus neutralization of plasma samples from COVID-19  
460 convalescent recovered donors (blue: recovered), vaccinated donors (green: 2X  
461 vaccinated) or vaccinated donors with a prior history of SARS-CoV-2 infection (red:  
462 recovered-vaccinated). Statistical comparisons between the two groups were performed  
463 using a Mann-Whitney two-tailed test, (\* $p < 0.05$ , \*\*\*\* $p < 0.0001$ ).

464 **b.** Plasma neutralization for all three groups against distantly related sarbecoviruses.  
465 Pang17 (cyan), SARS-CoV-1 (yellow), and WIV1 (violet) are shown. RBDs are colored

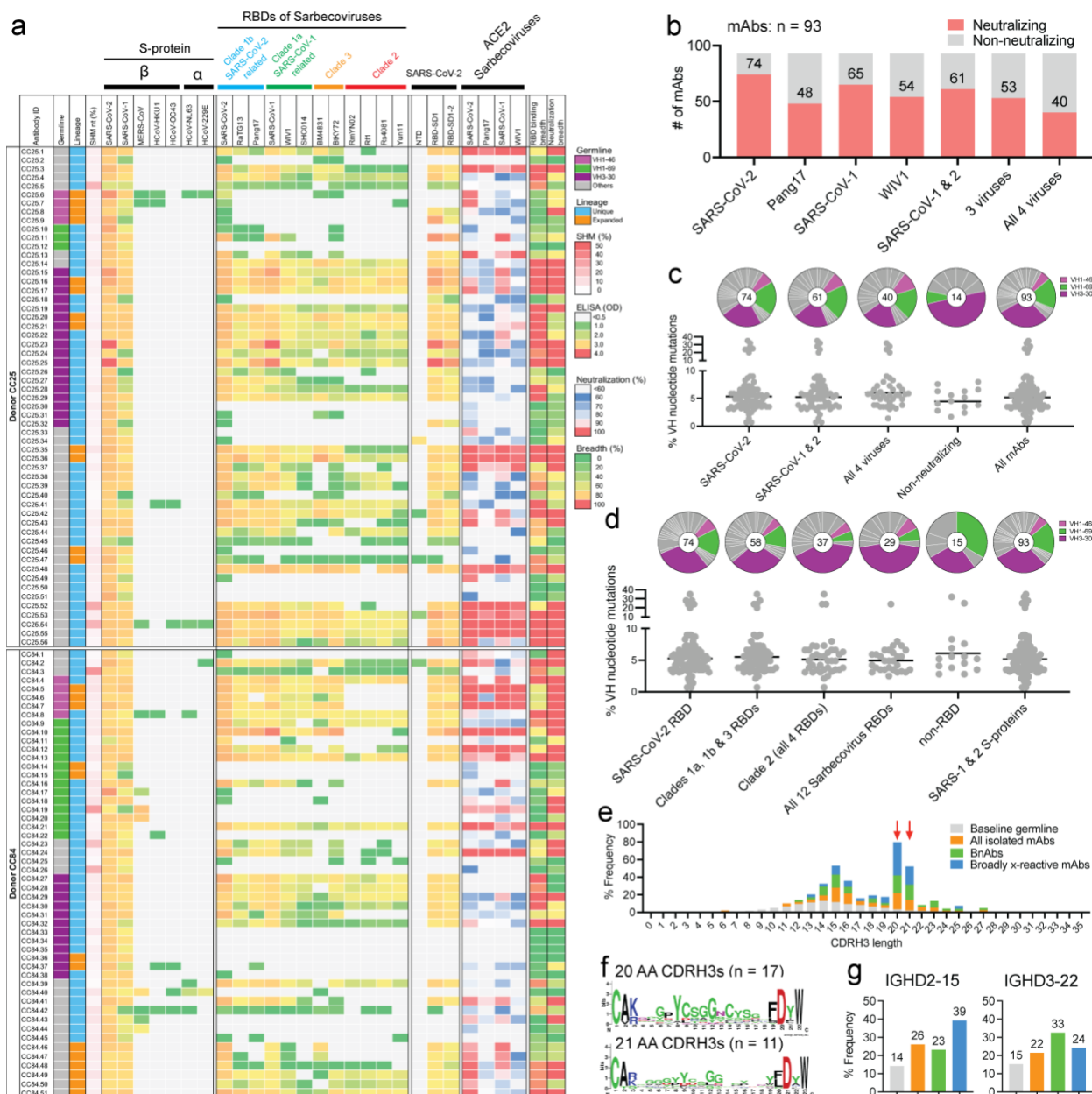
466 pink for all spikes. In contrast to infected-only and vaccinated-only donors, approximately  
467 half of the recovered-vaccinated donors have neutralizing titers against SARS-CoV-1  
468 above background (Extended Data Fig. 1).

469 **c.** Plasma neutralizing activity against SARS-CoV-2 (Wuhan) and SARS-CoV-2 variants  
470 of concern (B.1.1.7 (Alpha), B.1.351 (Beta), P.1 (Gamma), B.1.617.2 (Delta) and  
471 B.1.1.529 (Omicron)).

472 **d.** Flow cytometric analysis of IgG<sup>+</sup> B cells from PBMCs of human donors CC25 and CC84  
473 isolated at the indicated timepoints (see Extended Data Fig. 2 for gating strategy).  
474 Numbers indicate percentage of cells binding to SARS-CoV-1 and SARS-CoV-2 spike  
475 proteins, respectively.

476 **e.** Quantification of SARS-CoV-1-specific, SARS-CoV-2-specific, and cross-reactive IgG<sup>+</sup>  
477 B cells from donor CC25 (top) and CC84 (bottom) donors.

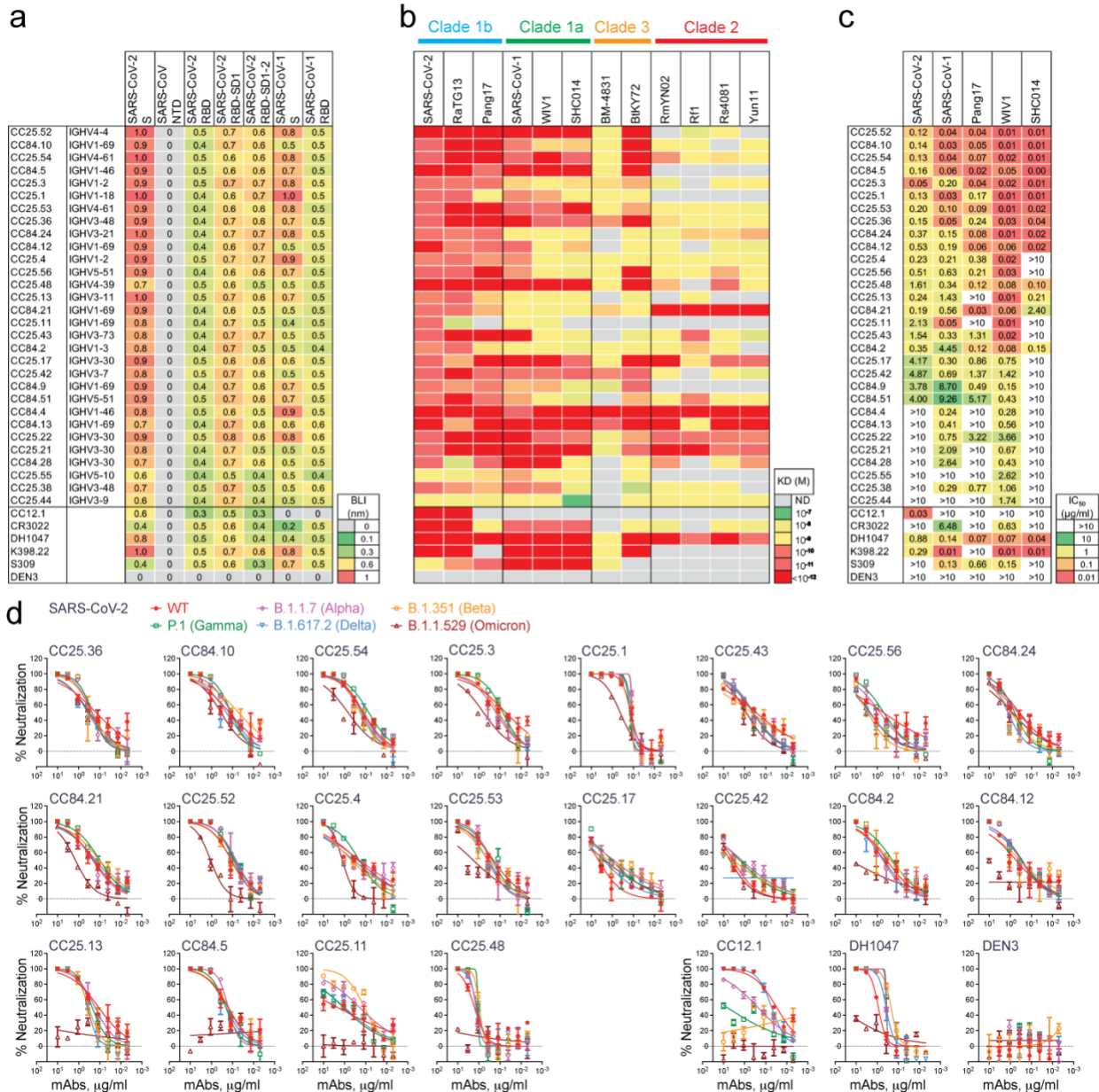
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479  
 480 **Fig. 2. Binding, neutralization and immunogenetic properties of sarbecovirus**  
 481 **mAbs.** A total of 107 mAbs were isolated, 56 mAbs from donor CC25 and 51 mAbs from  
 482 donor CC84. MAbs were isolated by single B cell sorting using SARS-CoV-1 and SARS-  
 483 CoV-2 S-proteins as baits.  
 484 **a.** Heatmap showing IGHV germline gene usage (colored: VH1-46 (magenta), VH1-69  
 485 (spring) and VH3-30 (plum) and other V-genes (grey)), lineage information (unique (sky)  
 486 and expanded (tangerine) lineages) and V-gene nucleotide somatic mutations (SHMs).  
 487 ELISA binding activity of mAbs with SARS-CoV-2, SARS-CoV-1 and other  $\beta$ - and  $\alpha$ -HCoV  
 488 derived S-proteins and domains of SARS-CoV-2 S-protein (NTD, RBD-SD1, RBD-SD1-  
 489 2) (LOD <0.5 OD<sub>405</sub>). Binding of mAbs with clade 1a (SARS-CoV-2 related: SARS-CoV-  
 490 2, RatG13, Pang17), clade 1b (SARS-CoV-1 related: SARS-CoV-1, WIV1, SHC014),  
 491 clade 2 (RmYN02, Rf1, Rs4081, Yun11) and clade 3 (BM4831, BtKY72) sarbecovirus S-  
 492 protein derived monomeric RBDs. Percent neutralization of ACE2-utilizing

493 sarbecoviruses (SARS-CoV-2, Pang17, SARS-CoV-1 and WIV1) by mAb supernatants  
494 (cut-off <60%). Breadth (%) of binding to 12 sarbecovirus RBDs and breadth (%) of  
495 neutralization with 4 ACE2 sarbecoviruses is indicated for each mAb. MAb expression  
496 levels in the supernatants were quantified by total IgG ELISA and the concentrations were  
497 approximately comparable overall. For reproducibility, the binding and neutralization  
498 assays were all performed twice with mAb supernatants expressed independently twice.  
499 **b.** Number of mAbs (unique clones) neutralizing SARS-CoV-2 and other sarbecoviruses.  
500 40 mAbs neutralized all 4 ACE2 sarbecoviruses tested.  
501 **c.** Pie plots showing IGHV gene usage distribution of isolated mAbs with enriched gene  
502 families colored, VH1-46 (magenta) VH1-69 (spring) and VH3-30 (plum). Dot plots  
503 showing % nucleotide mutations (SHMs) in the heavy chain (VH) of isolated mAbs. The  
504 mAbs are grouped by neutralization with sarbecoviruses.  
505 **d.** Pie and dot plots depicting IGHV gene distribution and VH nucleotide SHMs  
506 respectively. The mAbs are grouped by binding to sarbecovirus-derived RBDs.  
507 **e.** CDRH3 length distributions of isolated mAbs across broadly neutralizing and broadly  
508 cross-reactive mAb groups compared to human baseline germline reference. MAbs with  
509 20- and 21- amino acid-CDRH3s are highly enriched.  
510 **f.** Sequence conservation logos of 20 (n = 17) and 21 (n = 11) amino acid long CDRH3-  
511 bearing mAbs show enrichment of D-gene derived residues, including IGHD2-15 germline  
512 D-gene encoded two cysteines in 20 amino acid long CDRH3-bearing mAbs that may  
513 potentially form a disulfide bond.  
514 **g.** Enrichment of IGHD2-15 and IGHD3-22 germline D-genes in sarbecovirus broadly  
515 neutralizing or broadly cross-reactive mAbs compared to corresponding human baseline  
516 germlines.  
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519 **Fig. 3. Binding and neutralization of mAbs in terms of affinity/potency and breadth.**

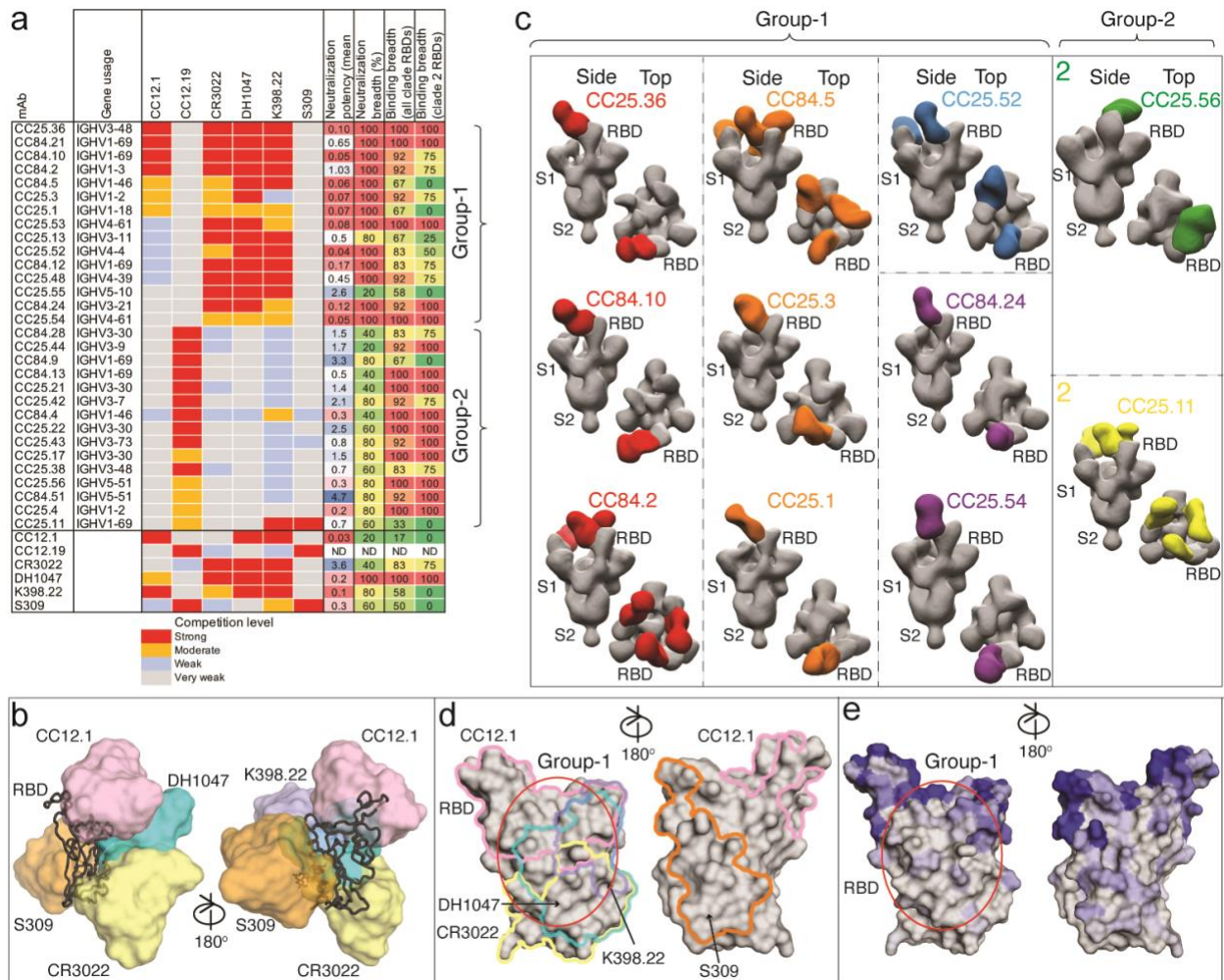
520 A total of 19 mAbs from donor CC25 and 11 mAbs from donor CC84 were selected to  
 521 determine specificity, relative affinities and neutralization of sarbecoviruses and SARS-  
 522 CoV-2 VOCs.

523 **a.** Heatmap of binding responses (nm) determined by BLI using SARS-CoV-1 and SARS-  
 524 CoV-2 S and S-protein domains and subdomains with IGHV gene usage for each mAb  
 525 indicated.

526 **b.** Heatmap of dissociation constants ( $K_D$  (M)) values for mAb binding to spike-derived  
 527 monomeric RBDs from four sarbecovirus clades: clade 1b (n = 3); clade 1a (n = 3); clade  
 528 2 (n = 4); clade 3 (n = 2). Binding kinetics were obtained using the 1:1 binding kinetics  
 529 fitting model on ForteBio Data Analysis software.

530 **c.** IC<sub>50</sub> neutralization of mAbs against SARS-CoV-2, SARS-CoV-1, Pang17, WIV1, and  
 531 SHC014 determined using pseudotyped viruses.

532 **d**, Neutralization of 20 bnAbs against SARS-CoV-2 (Wuhan) and five major SARS-CoV-  
533 2 variants of concern (B.1.1.7 (Alpha), B.1.351 (Beta), P.1 (Gamma), B.1.617.2 (Delta)  
534 and B.1.1.529 (Omicron)). SARS-CoV-2 Abs, CC12.1, DH1047, and Dengue Ab, DEN3  
535 were used as controls.



**Fig. 4. Epitope specificities of sarbecovirus bnAbs.**

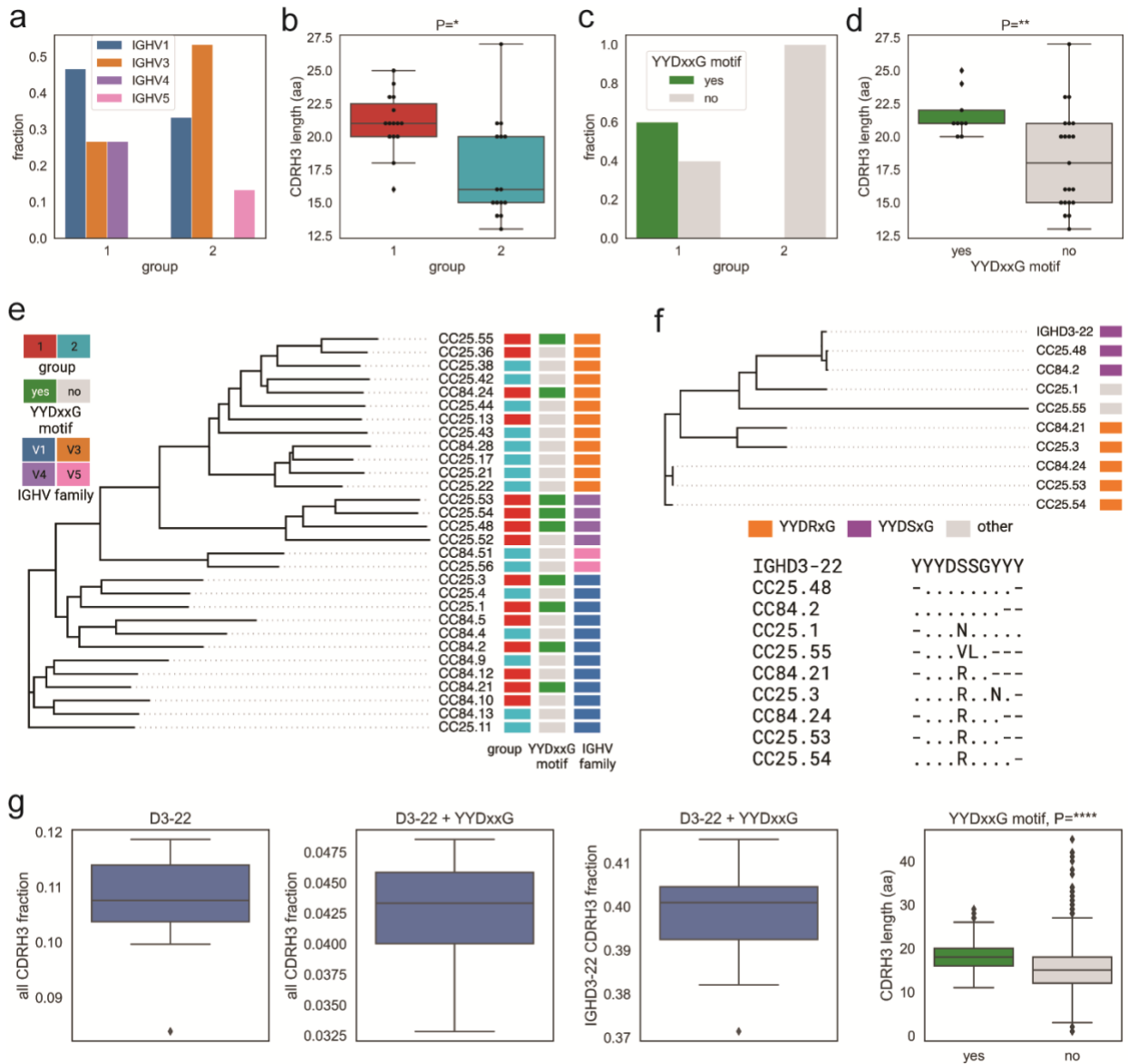
**a.** Heatmap summary of epitope binning of sarbecovirus bnAbs based on BLI competition of bnAbs with human (CC12.1, CC12.19, CR3022, DH1047 and S309) and macaque (K398.22) RBD-specific nAbs. IGHV gene usage for each mAb is indicated. Geomean neutralization potency and breadth (calculated from Fig. 3c) and RBD binding breadth with clade 2 or all clade sarbecoviruses (calculated from Fig. 3b) for each mAb are indicated. The BLI competition was performed with monomeric SARS-CoV-2 RBD, and the competition levels are indicated as red (strong), orange (moderate), light blue (weak) and grey (very weak) competition. Based on competition with human and one macaque nAb of known specificities, the sarbecovirus bnAbs were divided into groups-1 and -2.

**b.** Binding of human nAbs to SARS-CoV-2 RBD. The RBD is shown as a black chain trace, whereas antibodies are represented by solid surfaces in different colors: CC12.1 (pink, PDB 6XC2), CR3022 (yellow, PDB 6W41), S309 (orange, PDB 7R6W), DH1047 (cyan, 7LD1), and K398.22 (blue, PDB submitted)<sup>52</sup>.

**c.** Electron microscopy 3D reconstructions of sarbecovirus bnAb Fabs with SARS-CoV-2 S-protein. Fabs of group-1 (n = 9) and group-2 (n = 2) were complexed with SARS-CoV-2 S-protein and 3D reconstructions were generated from 2D class averages. Fabs are shown in different colors and the spike S1 and S2 subunits (grey) and the RBD sites are labelled.

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556 **d.** The epitope of each antibody is outlined in different colors corresponding to panel b.  
557 Epitope residues are defined by buried surface area (BSA)  $> 0 \text{ \AA}^2$  as calculated by  
558 PDBePISA ([https://www.ebi.ac.uk/msd-srv/prot\\_int/pistart.html](https://www.ebi.ac.uk/msd-srv/prot_int/pistart.html)). Putative epitope regions  
559 of group-1 bnAbs based on the competitive binding assay are indicated by red circles.  
560 **e.** Conservation of 12 sarbecovirus RBDs. Gray surface represents conserved regions,  
561 whereas blue represents variable regions. The conservation was calculated by ConSurf  
562 (<https://consurf.tau.ac.il/>). The putative epitope region targeted by group-1 bnAbs is  
563 circled as in panel d.  
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**Fig. 5. Immunogenetic properties of Group 1 and 2 RBD bnAbs.**

**a.** VH gene family usage: IGHV1 (blue), IGHV3 (orange), IGHV4 (violet), and IGHV5 (red).

**b.** CDRH3 length distribution (amino acids) in Groups 1 (red) and 2 (cyan). P-values computed using the Kruskal-Wallis test and denoted as follows:  $P < 0.05$ :\*,  $P < 0.005$ :\*\*,  $P < 0.00005$ :\*\*\*\*.

**c.** CDRH3 use of YYDxxG motif in group 1 and 2 RBD bnAbs: with (green) and without (gray).

**d.** CDRH3 length distribution in RBD bnAbs with (green) and without (gray) YYDxxG motifs.

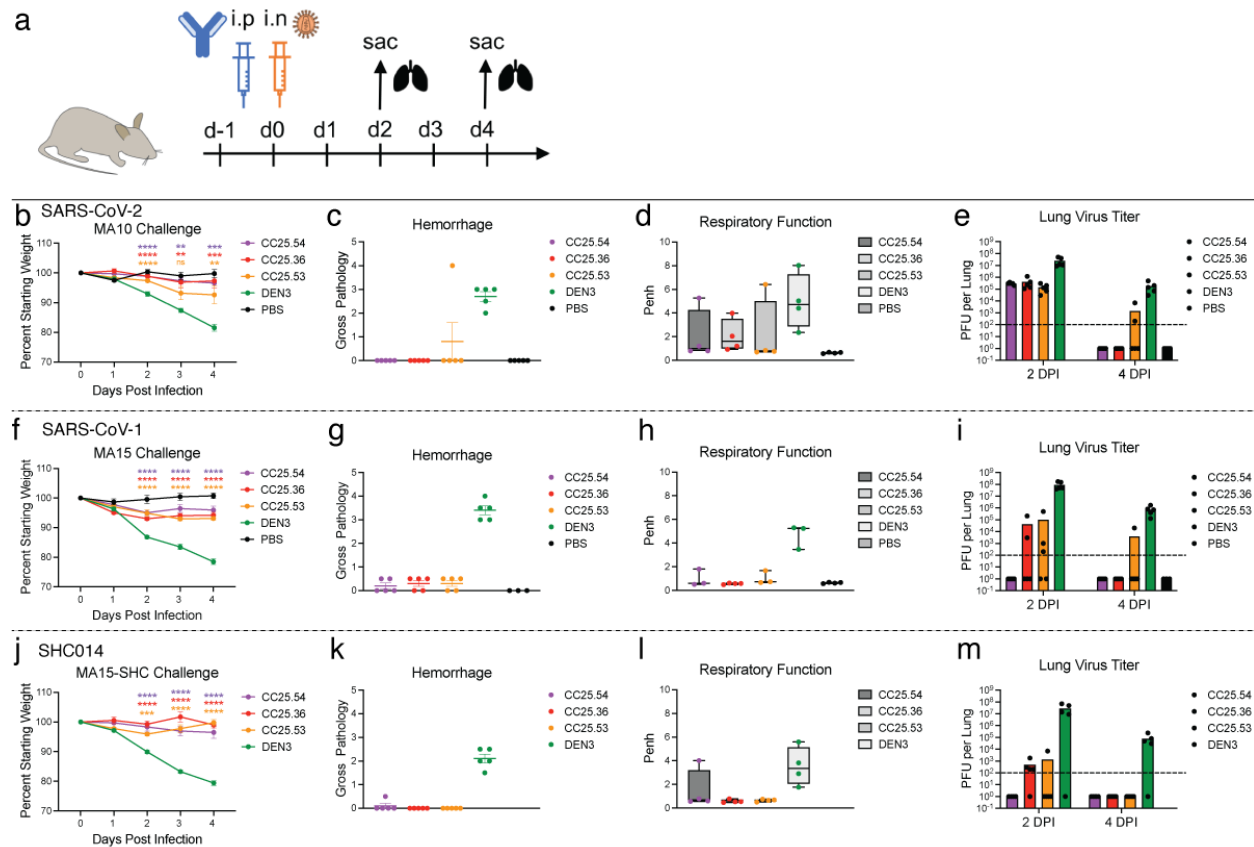
**e.** Phylogenetic tree of heavy chain sequences of 30 RBD bnAbs. Each sequence is colored according to its group (left bar), IGHV gene family (middle bar), and the presence of the YYDxxD motif in the HCDR3 (right bar). Colors of heavy chain characteristics are consistent with panels **a-d**. Here and further, the phylogenetic tree is computed using Clustal Omega<sup>84</sup>.

581 **f.** Phylogenetic tree combining IGHD gene fragments of CDRH3s of nine mAbs with  
582 YYDxxG motifs and the amino acid translation of the germline sequence of IGHD3-22-  
583 containing YYGSSG. Each sequence is colored according to the amino acid following  
584 YYD: S (violet), R (orange), or others (gray). The alignment corresponding to the tree is  
585 shown below. Dots represent amino acids matching the germline amino acids. Germline  
586 amino acids truncated in CDRH3s are shown by dashes.

587 **g.** Frequencies of IGHD3-22 germline genes with differing characteristics in naive heavy  
588 chain repertoires. From left to right: all IGHD3-22 in all CDRH3s; IGHD3-22 with YYDxxG  
589 motif in all CDRH3s; IGHD3-22 with YYDxxG motif in CDRH3s derived from IGHD3-22;  
590 distribution of lengths (in amino acids) of CDRH3s with (green) and without (gray)  
591 YYDxxG motifs. The fraction statistics were computed using ten Rep-seq libraries  
592 representing ten donors from the study by <sup>85</sup>: ERR2567178–ERR2567187. The  
593 distribution of CDRH3 lengths was computed for library ERR2567178.

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**Fig. 6. Prophylactic treatment of aged mice with RBD bnAbs protects against challenge with diverse SARS-like viruses.**

**a.** Three RBD bnAbs (CC25.54, CC25.36 and CC25.53) individually, or a dengue DEN3 control antibody were administered intra-peritoneally (i.p.) at 300 $\mu$ g per animal into 12 groups of aged mice (10 animals per group). Each group of animals was challenged intranasally (i.n.) 12h after antibody infusion with one of 3 mouse-adapted (MA) sarbecoviruses, (MA10 = SARS-CoV-2;  $1 \times 10^3$  plaque forming units (PFU), MA15 = SARS-CoV-1;  $1 \times 10^3$  PFU or MA15-SHC = SARS-CoV MA15-SHC014 chimera;  $1 \times 10^5$  PFU). As a control, groups of mice were exposed only to PBS in the absence of virus.

**b,f,j.** Percent weight change in RBD bnAbs or DEN3 control antibody-treated animals after challenge with mouse-adapted sarbecoviruses. Percent weight change was calculated from day 0 starting weight for all animals.

**c,g,k.** Day 2 post-infection Hemorrhage (Gross Pathology score) scored at tissue harvest in mice prophylactically treated with RBD bnAbs or DEN3 control mAb.

**d,h,i.** Day 2 post-infection pulmonary function (shown as Penh score) was measured by whole body plethysmography in mice prophylactically treated with RBD bnAbs or DEN3 control mAb.

**e,i,m.** Lung virus titers (PFU per Lung) were determined by plaque assay of lung tissues collected at day 2 or day 4 after infection.

Statistical significance was calculated with Dunnett's multiple comparisons test between each experimental group and the DEN3 control Ab group. (\*\*p < 0.01, \*\*\*p < 0.001; \*\*\*\*p < 0.0001; ns- p > 0.05).

## 620 **Methods**

621  
622 **Convalescent COVID-19 and human vaccinee sera**  
623 Sera from convalescent COVID-19 donors<sup>29</sup>, spike-mRNA-vaccinated humans, and from  
624 COVID-19-recovered vaccinated donors, were provided through the “Collection of  
625 Biospecimens from Persons Under Investigation for 2019–Novel Coronavirus Infection to  
626 Understand Viral Shedding and Immune Response Study” UCSD IRB# 200236. The  
627 protocol was approved by the UCSD Human Research Protection Program.  
628 Convalescent serum samples were collected based on COVID-19 diagnosis regardless  
629 of gender, race, ethnicity, disease severity, or other medical conditions. All human donors  
630 were assessed for medical decision-making capacity using a standardized, approved  
631 assessment, and voluntarily gave informed consent prior to being enrolled in the study.  
632 The summary of the demographic information of the COVID-19 convalescent and  
633 vaccinated donors is listed in Table S1.

634  
635 **Plasmid construction**  
636 To generate soluble S ectodomain proteins from SARS-CoV-1 (residues 1-1190;  
637 GenBank: AAP13567) and SARS-CoV-2 (residues 1-1208; GenBank: MN908947), we  
638 constructed the expression plasmids by synthesizing the DNA fragments from GeneArt  
639 (Life Technologies) and cloned them into the pHCMV3 vector (Genlantis, USA). To keep  
640 the soluble S proteins in a stable trimeric prefusion state, the following changes in the  
641 constructs were made: double proline substitutions (2P) were introduced in the S2  
642 subunit; the furin cleavage sites (in SARS-CoV-2 residues 682–685, and in SARS-CoV-  
643 1 residues 664–667) were replaced by “GSAS” linker; the trimerization motif T4 fibrin  
644 was incorporated at the C-terminus of the S proteins. To purify and biotinylate the spike  
645 proteins, the HRV-3C protease cleavage site, 6x HisTag, and AviTag spaced by GS-  
646 linkers were added to the C-terminus after the trimerization motif. To produce truncated  
647 proteins of SARS-CoV-1 and SARS-CoV-2 spike, the PCR amplifications of the gene  
648 fragments encoding SARS-CoV-1 RBD (residue 307-513), SARS-CoV-2 NTD (residue 1-  
649 290), RBD (residue 320-527), RBD-SD1 (residue 320-591), and RBD-SD1-2 (residue  
650 320-681) subdomains were carried out using the SARS-CoV-1 and SARS-CoV-2  
651 plasmids as templates. To generate pseudoviruses of non-human sarbecoviruses, the  
652 DNA fragments encoding the spikes of the sarbecoviruses without the ER retrieval signal  
653 were codon-optimized and synthesized at GeneArt (Life Technologies). The spike  
654 encoding genes of Pang17 (residues 1-1249, GenBank: QIA48632.1), WIV1 (residues 1-  
655 1238, GenBank: KF367457) and SHC014 (residue 1-1238, GenBank: AGZ48806.1) were  
656 constructed into the pHCMV3 vector (Genlantis, USA) using the Gibson assembly (NEB,  
657 E2621L) according to the manufacturer’s instructions. To express the monomeric RBDs  
658 of sarbecovirus clades (clades, 1b, 1a, 2 and 3), the conserved region aligning to SARS-  
659 CoV-2 RBD (residue 320-527) were constructed into pHCMV3 vector with 6x HisTag, and  
660 AviTag spaced by GS-linkers on C-terminus. The sarbecovirus RBD genes encoding  
661 RaTG13 (residues 320-527, GenBank: QHR63300.2), Pang17 (residues 318-525,  
662 GenBank: QIA48632.1), WIV1 (residues 308-514, GenBank: KF367457), RsSHC014  
663 (residues 308-514, GenBank: AGZ48806.1), BM-4831 (residues 311-514, NCBI  
664 Reference Sequence: NC\_014470.1), BtKY72 (residues 310-516, GenBank: KY352407),  
665 RmYN02 (residues 299-487, GSAID EPI\_ISL\_412977), Rf1 (residues 311-499,



666 GenBank: DQ412042.1), Rs4081 (residues 311-499, GenBank: KY417143.1) and Yun11  
667 (residues 311-499, GenBank: JX993988) were synthesized at GeneArt (Life  
668 Technologies) and constructed using the Gibson assembly (NEB, E2621L).

669

### 670 **Cell lines**

671 HEK293F cells (Life Technologies) and Expi293F cells (Life Technologies) were  
672 maintained using 293FreeStyle expression medium (Life Technologies) and Expi293  
673 Expression Medium (Life Technologies), respectively. HEK293F and Expi293F cell  
674 suspensions were maintained in a shaker at 150 rpm, 37°C with 8% CO<sub>2</sub>. Adherent  
675 HEK293T cells were grown in DMEM supplemented with 10% FBS and 1% penicillin-  
676 streptomycin and maintained in an incubator at 37°C with 8% CO<sub>2</sub>. A stable hACE2-  
677 expressing HeLa cell line was generated using an ACE2 lentivirus protocol previously  
678 described. Briefly, the pBOB-hACE2 plasmid and lentiviral packaging plasmids (pMDL,  
679 pREV, and pVSV-G (Addgene #12251, #12253, #8454)) were co-transfected into  
680 HEK293T cells using the Lipofectamine 2000 reagent (ThermoFisher Scientific,  
681 11668019).

682

### 683 **Transfection for protein expression**

684 For expression of mAbs, HC and LC gene segments that were cloned into corresponding  
685 expression vectors were transfected into Expi293 cells (Life Technologies) (2-3 million  
686 cells/mL) using FectoPRO PolyPlus reagent (Polyplus Cat # 116-040) for a final  
687 expression volume of 2, 4 or 50 mL. After approximately 24 hours, sodium valproic acid  
688 and glucose were added to the cells at a final concentration of 300 mM each. Cells were  
689 allowed to incubate for an additional 4 days to allow for mAb expression. For expression  
690 of spike proteins, RBDs, and NTDs, cloned plasmids (350 µg) were transfected into  
691 HEK293F cells (Life Technologies) (1 million cells/mL) using Transfectagro reagent  
692 (Corning) and 40K PEI (1 mg/mL) in a final expression volume of 1 L as previously  
693 described. Briefly, plasmid and transfection reagents were combined and filtered  
694 preceding PEI addition. The combined transfection solution was allowed to incubate at  
695 room temperature for 30 mins before being gently added to cells. After 5 days,  
696 supernatant was centrifuged and filtered.

697

### 698 **Protein purification**

699 For mAb purification, a 1:1 solution of Protein A Sepharose (GE Healthcare) and Protein  
700 G Sepharose (GE Healthcare) was added to Expi293 supernatant for 2h at room  
701 temperature or overnight at 4°C. The solution was then loaded into an Econo-Pac column  
702 (BioRad #7321010), washed with 1 column volume of PBS, and mAbs were eluted with  
703 0.2 M citric acid (pH 2.67). The elution was collected into a tube containing 2 M Tris Base.  
704 Buffer was exchanged with PBS using 30K Amicon centrifugal filters (Millipore,  
705 UFC903008). His-tagged proteins were purified using HisPur Ni-NTA Resin (Thermo  
706 Fisher). Resin-bound proteins were washed (25 mM Imidazole, pH 7.4) and slowly eluted  
707 (250 mM Imidazole, pH 7.4) with 25 mL elution buffer. Eluted proteins were buffer-  
708 exchanged with PBS, and further purified using size-exclusion chromatography using  
709 Superdex 200 (GE Healthcare).

710

### 711 **ELISA**

712 ELISAs were performed on 96-well half-area microplates (ThermoFisher Scientific) as  
713 described previously<sup>15</sup>. The plate was coated with 2 µg/mL mouse anti-His antibody  
714 (Invitrogen cat. #MA1-21315-1MG, ThermoFisher Scientific) overnight at 4°C. The  
715 following day, plates were washed three times with PBST (PBS + 0.05% Tween20) and  
716 incubated for 1h with blocking buffer (3% bovine serum albumin (BSA)). Following  
717 removal of blocking buffer, plates were treated with His-tagged proteins (5 µg/mL in PBST  
718 + 1% BSA) for 1.5h at room temperature. Plates were washed and serum was added at  
719 threefold dilutions (beginning at 1:30) and allowed to incubate for 1.5h. Following washes,  
720 secondary antibody (AffiniPure Goat anti-human IgG Fc fragment specific, Jackson  
721 ImmunoResearch Laboratories cat. #109-055-008) was added for an additional 1h.  
722 Secondary antibody was washed, and staining substrate (alkaline phosphatase substrate  
723 pNPP tablets, Sigma) was added. Absorbance at 405 nm was measured after 8, 20, and  
724 30 min using VersaMax microplate reader (Molecular Devices) and analyzed using  
725 SoftMax version 5.4 (Molecular Devices).

726

### 727 **Biotinylation of proteins**

728 To randomly biotinylate the proteins described in this paper, we used an EZ-Link NHS-  
729 PEG Solid-Phase Biotinylation Kit (Thermo Scientific #21440). To dissolve the reagents  
730 supplied in the kit for stock solutions, 10 µL DMSO was added into each tube. To make  
731 a working solution, 1 µL stock solution was diluted by 170 µL water freshly before use.  
732 To concentrate the proteins before biotinylation, the proper sized filter Amicon tubes were  
733 used. The proteins were adjusted to 7-9 mg/mL in PBS. For each 30 µL aliquoted protein,  
734 3 µL of working solution was added and mixed thoroughly following by a 3h incubation on  
735 ice. To stop the reaction and remove the free NHS-PEG4-Biotin, the protein solution was  
736 buffer exchanged into PBS using Amicon tubes. All proteins were evaluated by BioLayer  
737 Interferometry after biotinylation.

738

### 739 **BirA biotinylation of proteins for B cell sorting**

740 For B cell sorting, the spike probes with the His and Avi-tag at the C-terminus were  
741 biotinylated by the intracellular biotinylating reaction during transfection step. To  
742 biotinylate the recombinant Avi-tagged spike probes, the BirA biotin-protein ligase  
743 encoding plasmid was co-transfected with the spike probe-Avi-tag encoding plasmids in  
744 the FreeStyle™ 293-F cell. 150ug BirA plasmid and 300ug spike probe plasmids were  
745 transfected with PEI reagent as described in the Transient transfection section. The spike  
746 probes were purified with HisPur Ni-NTA Resin (Thermo Fisher) as described in the  
747 Protein purification section. After the purification, the biotinylated proteins were evaluated  
748 by BioLayer Interferometry.

749

### 750 **BioLayer Interferometry (BLI)**

751 Binding assays were performed on an Octet RED384 instrument using Anti-Human IgG  
752 Fc Capture (AHC) biosensors (ForteBio). All samples were diluted in Octet buffer (PBS  
753 with 0.1% Tween 20) for a final concentration of 10 µg/mL for mAbs and 200 nM for viral  
754 proteins. For supernatant mAb binding screening, 125 µL of expression supernatant was  
755 used. For binding assays, antibodies were captured for 60 s and transferred to buffer for  
756 an additional 60 s. Captured antibodies were dipped into viral proteins for 120 s in order  
757 to obtain an association signal. For dissociation, biosensors were moved to Octet buffer

758 only for an additional 240 s for the dissociation step. The data generated was analyzed  
759 using the ForteBio Data Analysis software for correction, and the kinetic curves were fit  
760 to 1:1 binding mode. Note that the IgG: spike protomer binding can be a mixed population  
761 of 2:1 and 1:1, such that the term 'apparent affinity' dissociation constants ( $K_D^{App}$ ) are  
762 shown to reflect the binding affinity between IgGs and spike trimers tested.

763

### 764 **Isolation of monoclonal antibodies (mAbs)**

765 To isolate antigen-specific memory B cells, we used SARS-CoV-1 and SARS-CoV-2  
766 spike proteins as probes to perform single cell sorting in a 96-well format. PBMCs from  
767 post-infection vaccinated human donors were stained with fluorophore labeled antibodies  
768 and spike proteins. To generate spike probes, streptavidin-AF647 (Thermo Fisher  
769 S32357) was coupled to BirA biotinylated SARS-CoV-1 spike. Streptavidin-AF488  
770 (Thermo Fisher S32354) and streptavidin-BV421 (BD Biosciences 563259) were coupled  
771 to BirA biotinylated SARS-CoV-2 spike separately. The conjugation reaction was carried  
772 freshly before use with spike protein versus streptavidin-fluorophores at 2:1 or 4:1  
773 molecular ratio. After 30 min incubation at room temperature, the conjugated spike  
774 proteins were stored on ice or at 4 °C for up to 1 week. To prepare PBMCs, the frozen  
775 PBMCs were thawed in 10mL recover medium (RPMI 1640 medium containing 50% FBS)  
776 immediately before staining. The cells were washed with 10mL FACS buffer (PBS, 2%  
777 FBS, 2 mM EDTA) and each 10 million cells were resuspended in 100 $\mu$ L of FACS buffer.  
778 To isolate SARS-CoV-1 and SARS-CoV-2 cross-reactive IgG+ B cells, PBMCs were  
779 stained for CD3 (APC Cy7, BD Pharmingen #557757), CD4 (APC-Cy7, Biolegend,  
780 #317418), CD8 (APC-Cy7, BD Pharmingen #557760), CD14 (APC-H7, BD Pharmingen  
781 #561384, clone M5E2), CD19 (PerCP-Cy5.5, Biolegend, #302230, clone HIB19), CD20  
782 (PerCP-Cy5.5, Biolegend, #302326, clone 2H7), IgG (BV786, BD Horizon, #564230,  
783 Clone G18-145) and IgM (PE, Biolegend, #314508, clone MHM-88). Antibodies were  
784 incubated with PBMCs on ice for 15 min. After the 15 min staining, SARS-CoV-1- S-  
785 AF647, SARS-CoV-2-S-AF488, and SARS-CoV-2-S-BV421 were added to the PBMC  
786 solution incubating on ice. After another 30 min incubation, FVS510 Live/Dead stain  
787 (Thermo Fisher Scientific, #L34966) 1:1000 diluted with FACS buffer was added to the  
788 PBMC solution for 15 min. Subsequently, cells were washed with 10 mL ice cold FACS  
789 buffer. Each 10 million cells were resuspended with 500 $\mu$ L FACS buffer and then filtered  
790 through 70 $\mu$ m nylon mesh FACS tube caps (Fisher Scientific, #08-771-23). A BD  
791 FACSMelody sorter (BRV 9 Color Plate 4way) was used for the single cell sorting  
792 process. To isolate cross-reactive B cells, the gating strategy was set as follows:  
793 lymphocytes (SSC-A vs. FSC-A) and singlets (FSC-H vs. FSC-A) were gated first, and  
794 then live cells were selected by FVS510 Live/Dead negative gating. B cells were identified  
795 as CD19+CD20+CD3-CD4-CD8-CD14-IgM-IgG+ live singlets. Cross-reactive S-protein  
796 specific B cells were sequentially selected for SARS-CoV-2-S-BV421/SARS-CoV-2-S-  
797 AF488 double positivity and SARS-CoV-1-S-AF647/SARS-CoV-2-S-AF488 double  
798 positivity. Single cells were sorted into 96-well plates on a cooling platform. To prevent  
799 degradation of mRNA, plates were moved onto dry ice immediately after sorting. Reverse  
800 transcription was done right after. Superscript IV Reverse Transcriptase (Thermo Fisher),  
801 dNTPs (Thermo Fisher), random hexamers (Gene Link), Ig gene-specific primers, DTT,  
802 and RNaseOUT (Thermo Fisher), and Igepal (Sigma) were used in the reverse  
803 transcription PCR reaction as described previously<sup>86,87</sup>. To amplify IgG heavy and light

804 chain variable regions, two rounds of nested PCR reactions were carried out using the  
805 cDNAs as template and Hot Start DNA Polymerases (Qiagen, Thermo Fisher) and  
806 specific primer sets described previously<sup>86,87</sup>. The PCR products of the heavy and light  
807 chain variable regions were purified with SPRI beads according to the manufacturer's  
808 instructions (Beckman Coulter). Then, the purified DNA fragments were constructed into  
809 expression vectors encoding human IgG1, and Ig kappa/lambda constant domains,  
810 respectively. Gibson assembly (NEB, E2621L) was used according to the manufacturer's  
811 instructions in the construction step. To produce mAbs, the paired heavy and light chain  
812 were co-transfected into 293Expi cells.

813

### 814 **Immunogenetics analysis**

815 Heavy and light chain sequences of monoclonal antibodies as well as ten Rep-seq  
816 libraries representing naive heavy chain repertoires of ten donors (ERR2567178–  
817 ERR2567187) from BioProject PRJEB26509<sup>85</sup> were processed using the  
818 DiversityAnalyzer tool<sup>88</sup>. Clonal lineages for mAbs were computed in three steps. The  
819 first step was applied to heavy chain sequences following the procedures described  
820 previously<sup>89</sup>. Briefly, heavy chain sequences were combined into the same clonal lineage  
821 if (i) they share V and J germline genes, (ii) their CDRH3s have the same lengths, and  
822 (iii) their CDRH3s share at least 90% nucleotide identity. At the second step, the same  
823 procedure was applied to light chain sequences. Finally, each heavy chain clonal lineage  
824 was split according to the clonal lineage assignments of corresponding light chain  
825 sequences. Phylogenetic trees derived from heavy chain sequences and IGHD gene  
826 segments of mAbs were constructed using the ClusterW2 tool<sup>90</sup> and visualized using the  
827 Iroki tool<sup>91</sup>.

828

### 829 **Pseudovirus production**

830 To generate pseudoviruses, plasmids encoding the SARS-CoV-1, SARS-CoV-2 or other  
831 variants spike proteins with the ER retrieval signal removed were co-transfected with  
832 MLV-gag/pol and MLV-CMV-Luciferase plasmids into HEK293T cells. Lipofectamine  
833 2000 (Thermo Fisher Scientific, 11668019) was used according to the manufacturer's  
834 instructions. 48 hours post transfection, supernatants containing pseudoviruses were  
835 collected and filtered through a 0.22 µm membrane to remove debris. Pseudoviruses  
836 could be stored at -80°C prior to use.

837

### 838 **Pseudovirus entry and serum neutralization assays**

839 To generate hACE2-expressing stable cell lines for the pseudovirus infection test, we  
840 used lentivirus to transduce the hACE2 into HeLa cells. Stable cell lines with consistent  
841 and high hACE2 expression levels were established as HeLa-hACE2 and used in the  
842 pseudovirus neutralization assay. To calculate the neutralization efficiency of the sera or  
843 mAbs, the samples were 3-fold serially diluted and 25 µL of each dilution was incubated  
844 with 25 µL of pseudovirus at 37 °C for 1 h in 96-half area well plates (Corning, 3688). Just  
845 before the end of the incubation, HeLa-hACE2 cells were suspended with culture medium  
846 at a concentration of 2 x 10<sup>5</sup>/mL. The DEAE-dextran (Sigma, # 93556-1G) was added to  
847 the cell solutions at 20 µg/mL. 50 µL of the cell solution was distributed into each well.  
848 The plates were incubated at 37 °C for 2 days and the neutralization efficiency was  
849 calculated by measuring the luciferase levels in the HeLa-hACE2 cells. After removal of

850 the supernatant, the HeLa-hACE2 cells were lysed by luciferase lysis buffer (25 mM Gly-  
851 Gly pH 7.8, 15 mM MgSO<sub>4</sub>, 4 mM EGTA, 1% Triton X-100) at room temperature for 10-  
852 20 mins. After adding Bright-Glo (Promega, PRE2620) to each well, luciferase activity  
853 was inspected by a luminometer. Each experiment was carried out with duplicate samples  
854 and repeated independently at least twice. Percentage of neutralization was calculated  
855 according to the equation:

856

$$857 \quad \% \text{ Neutralization} = 100 * \left( 1 - \frac{(RLU \text{ of sample}) - (Average RLU \text{ of CC})}{(Average RLU \text{ of VC}) - (Average RLU \text{ of CC})} \right)$$

858

859 The neutralization percentage was calculated and plots against antibody concentrations  
860 or sera dilution ratio were made in Graph Pad Prism. The curves were fitted by nonlinear  
861 regression and the 50% pseudovirus neutralizing (IC<sub>50</sub>) or binding (ID<sub>50</sub>) antibody titer was  
862 calculated.

### 863

#### 864 **Neutralization assay of replication competent sarbecoviruses**

865 Vero E6 cells (ATCC-C1008) were seeded at 2 x 10<sup>4</sup> cells/well in a black-well, black-wall,  
866 tissue culture treated, 96-well plate (Corning Cat. #3916) 24 h before the assay. MAb  
867 were diluted in MEM supplemented with 5%FBS and 1%Pen/Strep media to obtain an 8-  
868 point, 3-fold dilution curve with starting concentration at 20 µg/ml. Eight hundred PFU of  
869 SARS1-nLuc, SARS2-D614G-nLuc and SHC014-nLuc replication competent viruses  
870 were mixed with mAbs at a 1:1 ratio and incubated at 37°C for 1 h. One-hundred  
871 microliters of virus and mAb mix was added to each well and incubated at 37°C + 5% CO<sub>2</sub>  
872 for 20 to 22 h. Luciferase activities were measured by the Nano-Glo Luciferase Assay  
873 System (Promega Cat. #N1130) following the manufacturer's protocol using a GloMax  
874 luminometer (Promega). Percent inhibition and IC<sub>50</sub> were calculated as pseudovirus  
875 neutralization assay described above. All experiments were performed as duplicate and  
876 independent repeated for three times. All the live virus experiments were performed under  
877 biosafety level 3 (BSL-3) conditions at negative pressure, by operators in Tyvek suits  
878 wearing personal powered-air purifying respirators.

### 879

#### 880 **Competition BLI**

881 To determine the binding epitopes of the isolated mAbs compared with known human  
882 SARS-CoV-2 mAbs, we did in-tandem epitope binning experiments using the Octet  
883 RED384 system. 200 nM of randomly biotinylated SARS-CoV-2 S or RBD protein antigen  
884 was captured using SA biosensors (18-5019, Sartorius). The biosensor was loaded with  
885 antigen for 5 min and then moved into the saturating mAbs at a concentration of 100  
886 µg/mL for 10 min. The biosensors were then moved into bnAb solution for 5 min to  
887 measure binding in the presence of saturating antibodies. As control, biosensors loaded  
888 with antigen were directly moved into bnAb solution. The percent (%) inhibition in binding  
889 is calculated with the formula: [Percent (%) binding inhibition = 1 - (bnAb binding response  
890 in presence of the competitor antibody / binding response of the corresponding control  
891 bnAb without the competitor antibody)]

### 892

#### 893 **Fab production**

894 To generate the Fab from the IgG, a stop codon was inserted in the heavy chain constant  
895 region at "KSCDK". The truncated heavy chains were co-transfected with the

896 corresponding light chains in 293Expi cells to produce the Fabs. The supernatants were  
897 harvested 4 days post transfection. Fabs were purified with CaptureSelect™ CH1-XL  
898 MiniChrom Columns (#5943462005). Supernatants were loaded onto columns using an  
899 Econo Gradient Pump (Bio-Rad #7319001). Following a wash with 1x PBS, Fabs were  
900 eluted with 25 mL of 50 mM acetate (pH 4.0) and neutralized with 2 M Tris Base. The  
901 eluate was buffer exchanged with 1x PBS in 10K Amicon tubes (Millipore, UFC901008)  
902 and filtered with a 0.22 µm spin filter.

903

#### 904 **Negative stain electron microscopy**

905 S-protein was complexed with Fab at three times molar excess per trimer and incubated  
906 at room temperature for 30 mins. Complexes were diluted to 0.03mg/ml in 1x Tris-  
907 buffered saline and 3µl applied to a 400mesh Cu grid, blotted with filter paper, and stained  
908 with 2% uranyl formate. Micrographs were collected on a Thermo Fisher Tecnai Spirit  
909 microscope operating at 120kV with an FEI Eagle CCD (4k x 4k) camera at 52,000 X  
910 magnification using Legikon automated image collection software<sup>92</sup>. Particles were  
911 picked using DogPicker<sup>93</sup> and data was processed using Relion 3.0<sup>94</sup>. Map segmentation  
912 was performed in UCSF Chimera<sup>95</sup>.

913

#### 914 **In vivo infections**

915 All mouse experiments were performed at the University of North Carolina, NIH/PHS  
916 Animal Welfare Assurance Number: D16-00256 (A3410-01), under approved IACUC  
917 protocols. All animal manipulation and virus work was performed in a Class 2A biological  
918 safety cabinet. 12 month old female Balb/c mice (strain 047) were purchased from Envigo.  
919 Mice were housed in individually ventilated Seal-Safe cages, provided food and water ad  
920 libitum and allowed to acclimate at least seven days before experimental use. Twelve  
921 hours prior to infection, mice were intraperitoneally injected with 300µg of antibody.  
922 Immediately prior to infection, mice were anesthetized by intraperitoneal injection of  
923 ketamine and xylazine and weighed. Virus was diluted in 50µL of sterile PBS and  
924 administered intranasally. Mice were weighed daily and observed for signs of disease. At  
925 the designated timepoint, mice were euthanized via isoflurane overdose, gross lung  
926 pathology was assessed, and the inferior lobe was collected for virus titration. Respiratory  
927 function was measured at day two post infection via Buxco whole body plethysmography,  
928 as previously described<sup>96</sup>.

929

#### 930 **Virus titration**

931 SARS-CoV-2 MA10, SARS-CoV-1 MA15 and chimeric SARS-CoV-1 MA15-SHC014  
932 were grown and titered using VeroE6 cells as previously described<sup>97</sup>. Briefly, lung tissue  
933 was homogenized in 1mL sterile PBS via Magnalyser (Roche), centrifuged to pellet  
934 debris, plated in 10-fold serial dilutions on VeroE6 cells on a 6-well plate and covered with  
935 a 1:1 mixture of 1.6% agarose and media. At two (SARS-CoV-1) or three (SARS-CoV-2)  
936 days post plating, cells were stained with neutral red and plaques counted.

937

#### 938 **Statistical Analysis**

939 Statistical analysis was performed using Graph Pad Prism 8 for Mac, Graph Pad  
940 Software, San Diego, California, USA. Statistical comparisons between the two groups  
941 were performed using a Mann-Whitney two-tailed test. The correlation between two

942 groups was determined by Spearman rank test. Groups of data were compared using the  
943 Kruskal-Wallis non-parametric test. Dunnett's multiple comparisons test were also  
944 performed between experimental groups. Data were considered statistically significant at  
945 \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ , and \*\*\*\*  $p < 0.0001$ .

946

947 **Data availability**

948 The authors declare that the data supporting the findings of this study are available within  
949 the paper and its supplementary information files or from the corresponding author upon  
950 reasonable request. Antibody sequences have been deposited in GenBank under  
951 accession numbers OM467906 - OM468119. Antibody plasmids are available from  
952 Raiees Andrabi or Dennis Burton under an MTA from The Scripps Research Institute.

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1204 **Table S1. Demographic information of human donors**

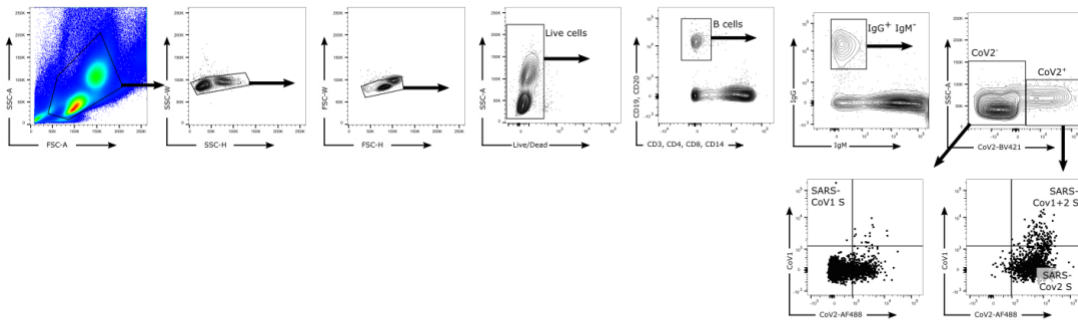
	<b>COVID convalescent donors (n = 21)</b>	<b>COVID convalescent donors vaccinated (n = 15)</b>	<b>Healthy donors vaccinated (n=10)</b>
<b>Age</b> (years)	28–72 (median = 42)	28–72 (median = 42)	25–69 (median = 42)
<b>Gender</b>			
Male	57% (12/21)	66% (10/15)	60% (6/10)
Female	43% (9/21)	33% (5/15)	40% (4/10)
<b>Race/Ethnicity</b>			
White, non-Hispanic	76% (16/21)	80% (12/15)	70% (7/10)
Hispanic	14% (3/21)	7% (1/15)	20% (2/10)
Black, non-Hispanic	5% (1/21)	7% (1/15)	0% (0/10)
Asian, non-Hispanic	5% (1/21)	7% (1/15)	10% (1/10)
<b>SARS-CoV-2 PCR</b>			
Positivity	90% (19/21)	93% (14/15)	n/a
Lateral Flow Positivity	81% (17/21)	73% (11/15)	n/a
<b>Disease Severity</b>			
Asymptomatic	5% (1/21)	7% (1/15)	n/a
Mild	57% (12/21)	60% (9/15)	n/a
Mild to Moderate	5% (1/21)	7% (1/15)	n/a
Moderate	14% (3/21)	13% (2/15)	n/a
Moderate to Severe	10% (2/21)	0% (0/15)	n/a
Severe	10% (2/21)	13% (2/15)	n/a
<b>Symptoms</b>			
Fever	57% (12/21)	47% (7/15)	n/a
Cough	52% (11/21)	40% (6/15)	n/a
Fatigue	48% (10/21)	33% (5/15)	n/a
Anosmia	43% (9/21)	40% (6/15)	n/a
Headache	38% (8/21)	47% (7/15)	n/a
Myalgia	33% (7/21)	33% (5/15)	n/a
Dyspnea	24% (5/21)	20% (3/15)	n/a
Diarrhea	19% (4/21)	20% (3/15)	n/a
Anorexia	14% (3/21)	0% (0/15)	n/a
Tachycardia	10% (2/21)	13% (2/15)	n/a
Days Post Symptom Onset at Collection	21–111 (median = 35)	21–111 (median = 75)	n/a

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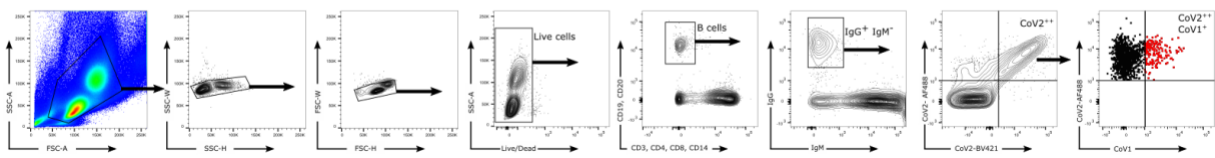
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A) Figure 1 gating strategy:



B) Single cell sort gating strategy:



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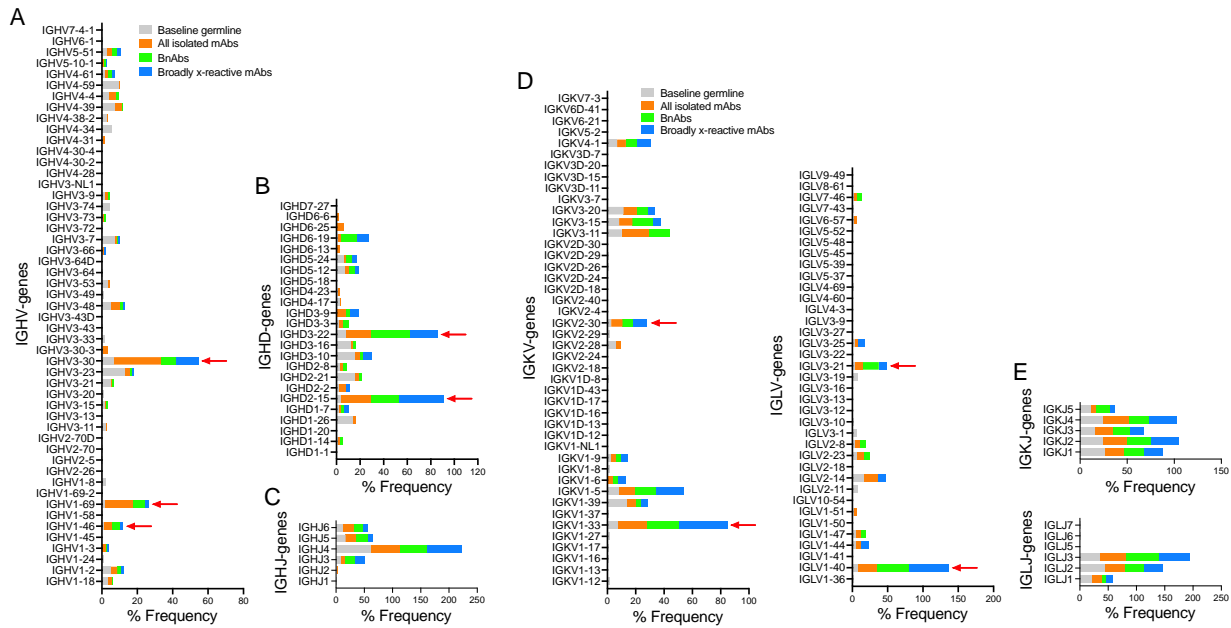
**Extended Data Fig. 2. Flow cytometry B cell profiling and sorting strategies.**

**a.** Gating strategy for analysis of IgG<sup>+</sup> B cell populations that bind SARS-CoV-1 S-protein only (CD19<sup>+</sup>CD20<sup>+</sup>CD3<sup>+</sup>CD4<sup>+</sup>CD8<sup>+</sup>CD14<sup>+</sup>IgM<sup>-</sup>IgG<sup>+</sup>CoV2BV421<sup>-</sup>CoV2AF488<sup>+</sup>CoV1<sup>+</sup>), SARS-CoV-2 S-protein only (CD19<sup>+</sup>CD20<sup>+</sup>CD3<sup>+</sup>CD4<sup>+</sup>CD8<sup>+</sup>CD14<sup>+</sup>IgM<sup>-</sup>IgG<sup>+</sup>CoV2BV421<sup>+</sup>CoV2AF488<sup>+</sup>CoV1<sup>-</sup>), or both SARS-CoV-1 and SARS-CoV-2 S-proteins (CD19<sup>+</sup>CD20<sup>+</sup>CD3<sup>+</sup>CD4<sup>+</sup>CD8<sup>+</sup>CD14<sup>+</sup>IgM<sup>-</sup>IgG<sup>+</sup>CoV2BV421<sup>+</sup>CoV2AF488<sup>+</sup>CoV1<sup>+</sup>).

**b.** Gating strategy used to isolate single cross-reactive IgG<sup>+</sup> B cells (indicated in red).

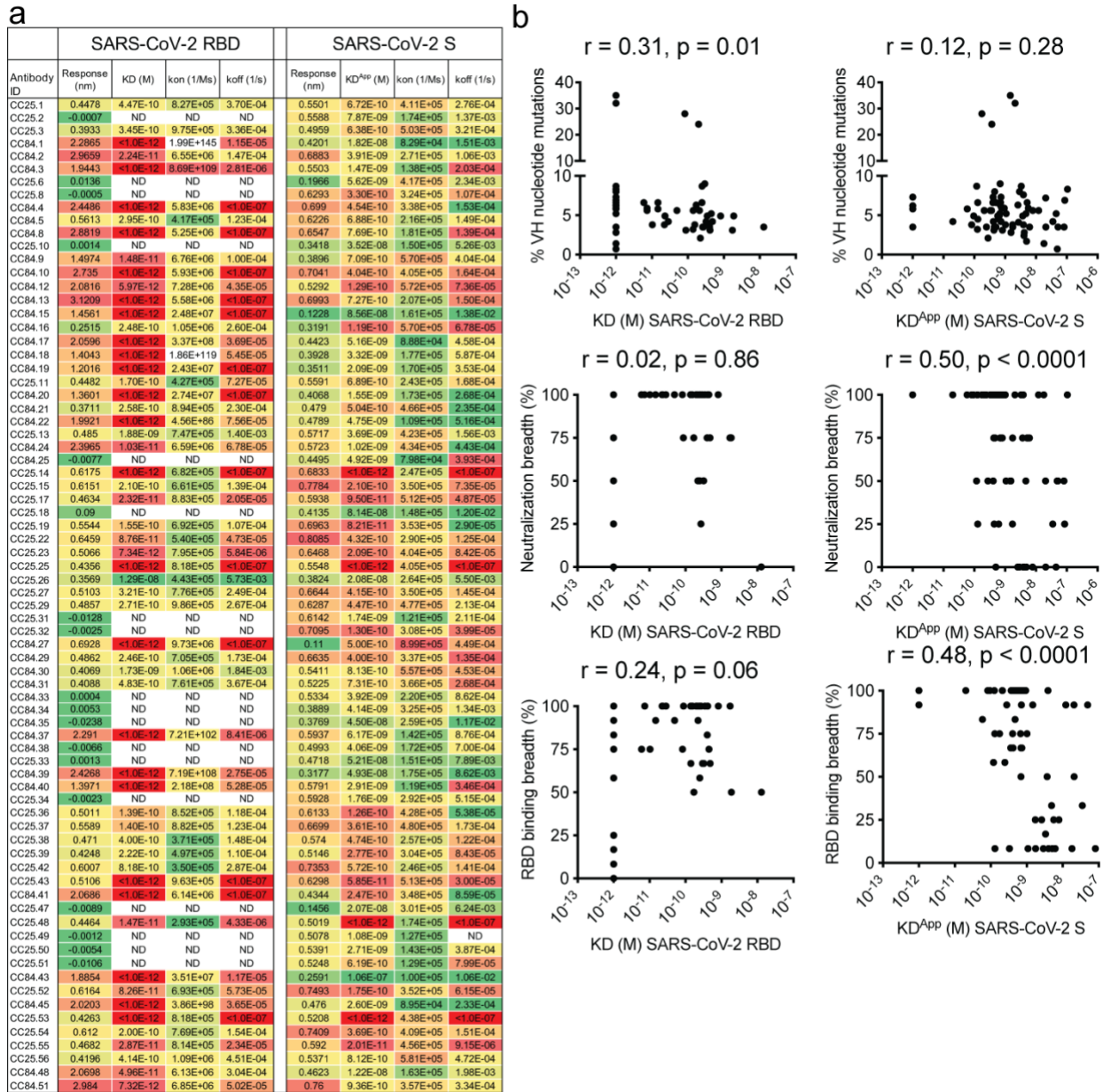






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**Extended Data Fig. 4. Immunoglobulin heavy and light chain germline gene enrichments in isolated RBD mAbs compared to a reference human germline database.** Baseline germline frequencies of heavy chain genes (IGHV (a), IGHD (b) and IGHJ (c) genes) and light chain genes (IGKV and IGLV (d), IGKJ and IGLJ (e) genes) are shown in grey, and mAb, bnAbs and cross-reactive mAbs in a-e panels are colored according to the key in (a and d). Arrows indicate gene enrichments compared to human baseline germline frequencies. The gene usage enrichments in panels a-e are shown for all unique clone mAbs isolated from CC25 and CC84 donors.



**Extended Data Fig. 5. mAb supernatant binding to SARS-CoV-2 RBD and SARS-CoV-2 S and association with SHM, binding and neutralization breadth.**

**a.** Supernatants from Expi293F cell-expressed mAbs were screened for BLI binding with SARS-CoV-2 RBD and SARS-CoV-2 S-protein. Binding kinetics ( $K_D$  (monomeric SARS-CoV-2 RBD),  $K_D^{APP}$  (SARS-CoV-2 S-protein),  $K_{on}$  and  $K_{off}$  constants) of antibodies with human proteins are shown. Binding kinetics were obtained using the 1:1 binding kinetics fitting model on ForteBio Data Analysis software.

**b.** Correlations of mAb binding ( $K_D$  or  $K_D^{APP}$  (M) values) to SARS-CoV-2 RBD or S-protein with heavy chain SHMs, neutralization breadth (neutralization against 4 ACE2-using-sarbecovirus panel), and sarbecovirus RBD breadth (binding against all 12 RBDs of clades 1a, 1b, 2 and 3) are determined by nonparametric Spearman correlation two-tailed test with 95% confidence interval. The Spearman correlation coefficient ( $r$ ) and  $p$ -value are indicated.

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SARS-CoV-2	Maximum binding response (nm)											
	Clade 1b			Clade 1a			Clade 3		Clade 2			
	RatG13	Pang17	SARS-CoV-1	WIV1	SHC014	BM-4831	BtKY72	RmYN02	Rf1	Rs4081	Yun11	
CC25.52	0.50	0.55	0.51	0.51	0.54	0.50	0.26	0.46	0.00	0.17	0.07	0.01
CC84.10	0.41	0.54	0.49	0.53	0.50	0.47	0.39	0.43	0.07	0.35	0.02	0.07
CC25.54	0.49	0.56	0.51	0.49	0.56	0.51	0.47	0.44	0.50	0.46	0.47	0.43
CC84.5	0.46	0.53	0.53	0.47	0.42	0.35	0.37	0.45	0.01	0.00	0.00	0.00
CC25.3	0.46	0.52	0.51	0.54	0.52	0.49	0.23	0.43	0.17	0.03	0.10	0.07
CC25.1	0.45	0.54	0.42	0.55	0.54	0.48	0.43	0.40	0.02	0.00	0.02	0.03
CC25.53	0.43	0.50	0.50	0.45	0.51	0.49	0.29	0.43	0.44	0.40	0.39	0.29
CC25.36	0.43	0.51	0.49	0.51	0.46	0.48	0.43	0.45	0.45	0.34	0.34	0.40
CC84.24	0.43	0.64	0.55	0.52	0.60	0.59	0.02	0.32	0.20	0.14	0.18	0.12
CC84.12	0.43	0.52	0.49	0.50	0.49	0.46	0.00	0.41	0.10	0.03	0.26	0.26
CC25.4	0.48	0.57	0.56	0.51	0.42	0.25	0.25	0.34	0.46	0.40	0.33	0.38
CC25.56	0.42	0.53	0.51	0.46	0.44	0.28	0.27	0.46	0.41	0.40	0.41	0.39
CC25.48	0.45	0.47	0.47	0.47	0.45	0.43	0.37	0.38	0.37	0.04	0.36	0.35
CC25.13	0.48	0.55	0.08	0.47	0.45	0.41	0.01	0.30	0.00	0.42	0.00	0.01
CC84.21	0.43	0.45	0.44	0.46	0.45	0.18	0.25	0.37	0.39	0.35	0.33	0.39
CC25.11	0.43	0.16	0.00	0.46	0.28	-0.02	0.00	-0.02	-0.01	-0.01	0.00	0.00
CC25.43	0.42	0.51	0.53	0.49	0.51	0.32	0.01	0.22	0.10	0.43	0.05	0.09
CC84.2	0.43	0.56	0.53	0.44	0.39	0.23	0.33	0.49	0.15	0.09	0.06	0.04
CC25.17	0.47	0.44	0.48	0.51	0.50	0.46	0.48	0.41	0.42	0.36	0.44	0.42
CC25.42	0.52	0.50	0.52	0.54	0.52	0.51	0.45	0.43	0.45	0.01	0.40	0.18
CC84.9	0.44	0.50	0.49	0.55	0.50	0.48	0.14	0.41	-0.02	0.00	0.01	0.00
CC84.51	0.47	0.57	0.51	0.51	0.50	0.19	0.04	0.42	0.39	0.50	0.43	0.40
CC84.4	0.46	0.59	0.52	0.52	0.61	0.52	0.47	0.50	0.53	0.49	0.51	0.49
CC84.13	0.42	0.50	0.50	0.57	0.52	0.50	0.41	0.42	0.48	0.39	0.43	0.43
CC25.22	0.49	0.56	0.55	0.59	0.56	0.53	0.31	0.45	0.51	0.50	0.51	0.43
CC25.21	0.43	0.49	0.54	0.54	0.54	0.49	0.24	0.43	0.47	0.44	0.45	0.48
CC84.28	0.41	0.16	0.49	0.51	0.51	0.45	0.04	0.39	0.47	0.00	0.43	0.43
CC25.55	0.36	0.26	0.17	0.37	0.28	0.24	0.00	0.33	0.00	-0.01	0.01	0.00
CC25.38	0.46	0.49	0.49	0.57	0.53	0.27	-0.01	0.11	0.38	0.42	0.46	0.04
CC25.44	0.43	0.42	0.30	0.52	0.46	0.10	0.01	0.36	0.39	0.40	0.38	0.36
CC12.1	0.30	0.22	0.00	0.00	0.00	-0.01	0.00	0.00	-0.01	0.00	-0.01	0.00
CR3022	0.40	0.37	0.47	0.51	0.48	0.43	-0.01	0.38	0.12	0.00	0.15	0.21
DH1074	0.33	0.43	0.50	0.43	0.54	0.47	0.23	0.43	0.44	0.27	0.30	0.30
K398.22	0.46	0.54	0.03	0.54	0.56	0.52	0.25	0.49	0.00	0.00	0.01	0.01
S309	0.29	0.38	0.40	0.52	0.41	0.43	0.06	0.00	0.00	0.00	0.00	0.00
DEN3	0.00	0.00	-0.01	0.00	0.00	0.00	0.00	-0.01	0.00	0.00	0.00	0.00

SARS-CoV-2	KD (M) values											
	Clade 1b			Clade 1a			Clade 3		Clade 2			
	RatG13	Pang17	SARS-CoV-1	WIV1	SHC014	BM-4831	BtKY72	RmYN02	Rf1	Rs4081	Yun11	
CC25.52	<1.0E-12	<1.0E-12	<1.0E-12	<1.0E-12	<1.0E-12	<1.0E-12	4.1E-08	<1.0E-12	ND	1.46E-07	7.96E-09	ND
CC84.10	7.97E-11	<1.0E-12	<1.0E-12	6.63E-10	3.04E-09	2.07E-09	2.07E-08	<1.0E-12	3.90E-09	1.77E-08	ND	6.79E-09
CC25.54	1.91E-11	<1.0E-12	<1.0E-12	1.23E-10	<1.0E-12	<1.0E-12	6.91E-12	6.91E-09	1.20E-09	4.19E-09	3.17E-09	8.32E-09
CC84.5	<1.0E-12	<1.0E-12	3.23E-10	<1.0E-12	<1.0E-12	<1.0E-12	9.98E-09	ND	ND	ND	ND	ND
CC25.3	3.98E-10	4.69E-10	3.74E-10	2.91E-09	6.88E-09	3.21E-09	3.16E-09	2.05E-09	8.46E-08	ND	3.76E-09	3.82E-09
CC25.1	2.05E-10	3.11E-11	1.75E-08	1.06E-09	1.79E-09	5.06E-09	1.36E-08	1.01E-08	ND	ND	ND	ND
CC25.53	1.58E-10	<1.0E-12	<1.0E-12	5.68E-11	2.92E-10	<1.0E-12	8.70E-09	1.76E-09	5.43E-09	9.23E-09	1.48E-08	6.08E-08
CC25.36	5.23E-11	<1.0E-12	<1.0E-12	<1.0E-12	8.88E-11	5.10E-11	1.29E-09	<1.0E-12	3.08E-09	1.52E-08	1.20E-08	9.30E-09
CC84.24	3.70E-10	7.36E-10	3.21E-10	3.57E-09	1.37E-08	5.44E-09	ND	2.53E-08	6.21E-08	2.69E-09	2.91E-09	2.03E-09
CC84.12	<1.0E-12	8.26E-10	2.84E-10	1.69E-10	3.59E-09	2.49E-09	ND	7.51E-09	1.34E-08	ND	6.24E-08	8.46E-08
CC25.4	1.74E-10	4.00E-10	1.93E-09	1.12E-09	1.93E-08	6.98E-08	9.82E-09	2.19E-08	7.34E-09	1.30E-08	1.94E-08	2.46E-08
CC25.56	2.41E-11	9.54E-11	<1.0E-12	1.37E-09	5.47E-09	7.44E-08	4.92E-09	<1.0E-12	7.11E-09	5.85E-09	2.00E-09	1.52E-08
CC25.48	<1.0E-12	<1.0E-12	<1.0E-12	<1.0E-12	<1.0E-12	<1.0E-12	5.54E-09	<1.0E-12	2.29E-09	ND	2.04E-10	6.25E-09
CC25.13	8.30E-10	1.46E-12	2.74E-09	5.42E-09	1.95E-08	2.44E-08	ND	4.12E-08	ND	1.13E-08	ND	ND
CC84.21	3.62E-10	3.25E-10	2.11E-09	6.86E-09	2.98E-08	4.34E-08	2.71E-09	8.40E-09	<1.0E-12	<1.0E-12	<1.0E-12	<1.0E-12
CC25.11	2.27E-10	2.84E-07	ND	1.03E-08	6.04E-08	ND	ND	ND	ND	ND	ND	ND
CC25.43	3.49E-11	<1.0E-12	<1.0E-12	<1.0E-12	4.70E-09	6.50E-08	ND	9.04E-08	7.77E-09	2.75E-11	6.52E-09	1.90E-07
CC84.2	1.08E-09	2.73E-10	3.21E-09	9.70E-09	3.79E-08	7.75E-08	2.05E-08	3.55E-09	1.29E-08	2.46E-09	8.00E-09	ND
CC25.17	32.67E-11	2.01E-09	<1.0E-12	<1.0E-12	<1.0E-12	2.19E-10	1.20E-08	<1.0E-12	1.1E-12	1.0E-12	3.29E-09	2.43E-10
CC25.42	2.41E-11	<1.0E-12	2.75E-10	3.03E-10	4.62E-10	2.09E-11	8.57E-10	1.47E-11	1.05E-08	ND	1.26E-08	1.46E-07
CC84.9	5.50E-10	1.50E-09	7.36E-10	3.32E-09	2.08E-09	1.18E-07	2.07E-07	1.00E-10	ND	ND	ND	ND
CC84.51	6.15E-10	<1.0E-12	4.58E-10	1.75E-09	7.22E-09	1.55E-07	ND	2.19E-09	1.21E-08	7.59E-10	1.07E-08	1.60E-08
CC84.4	<1.0E-12	<1.0E-12	<1.0E-12	3.98E-11	<1.0E-12	<1.0E-12	<1.0E-12	<1.0E-12	<1.0E-12	<1.0E-12	<1.0E-12	<1.0E-12
CC84.13	<1.0E-12	1.88E-09	<1.0E-12	<1.0E-12	<1.0E-12	<1.0E-12	8.41E-11	<1.0E-12	8.17E-09	<1.0E-12	<1.0E-12	<1.0E-12
CC25.22	1.25E-10	3.03E-09	3.03E-12	3.03E-12	1.02E-10	6.83E-08	6.89E-08	1.76E-09	1.61E-10	4.24E-10	6.11E-10	8.83E-10
CC25.21	3.31E-11	<1.0E-12	<1.0E-12	<1.0E-12	<1.0E-12	<1.0E-12	9.39E-08	2.35E-08	<1.0E-12	<1.0E-12	<1.0E-12	3.31E-10
CC84.28	1.50E-10	2.37E-07	7.70E-11	<1.0E-12	<1.0E-12	4.91E-09	ND	7.06E-09	1.38E-09	ND	3.42E-09	1.44E-09
CC25.55	1.39E-08	7.02E-08	3.05E-09	1.70E-09	2.06E-08	1.33E-08	ND	1.91E-08	ND	ND	ND	ND
CC25.38	2.53E-10	1.42E-09	2.27E-09	6.27E-10	9.45E-10	7.98E-08	ND	3.63E-07	1.22E-08	5.74E-09	6.51E-09	ND
CC25.44	1.57E-08	3.41E-08	6.66E-08	6.10E-09	2.04E-08	9.19E-07	ND	1.41E-08	3.30E-08	1.02E-08	3.44E-08	3.58E-08
CC12.1	<1.0E-12	<1.0E-12	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
CR3022	<1.0E-12	<1.0E-12	6.25E-09	8.00E-11	1.77E-10	1.81E-11	ND	1.96E-08	7.95E-08	ND	7.23E-09	8.35E-08
DH1074	<1.0E-12	<1.0E-12	<1.0E-12	<1.0E-12	<1.0E-12	<1.0E-12	1.50E-07	1.50E-07	4.19E-07	4.19E-07	4.19E-07	3.31E-10
K398.22	9.02E-09	1.36E-09	1.61E-10	<1.0E-12	<1.0E-12	<1.0E-12	6.63E-09	ND	ND	ND	ND	ND
S309	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
DEN3	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

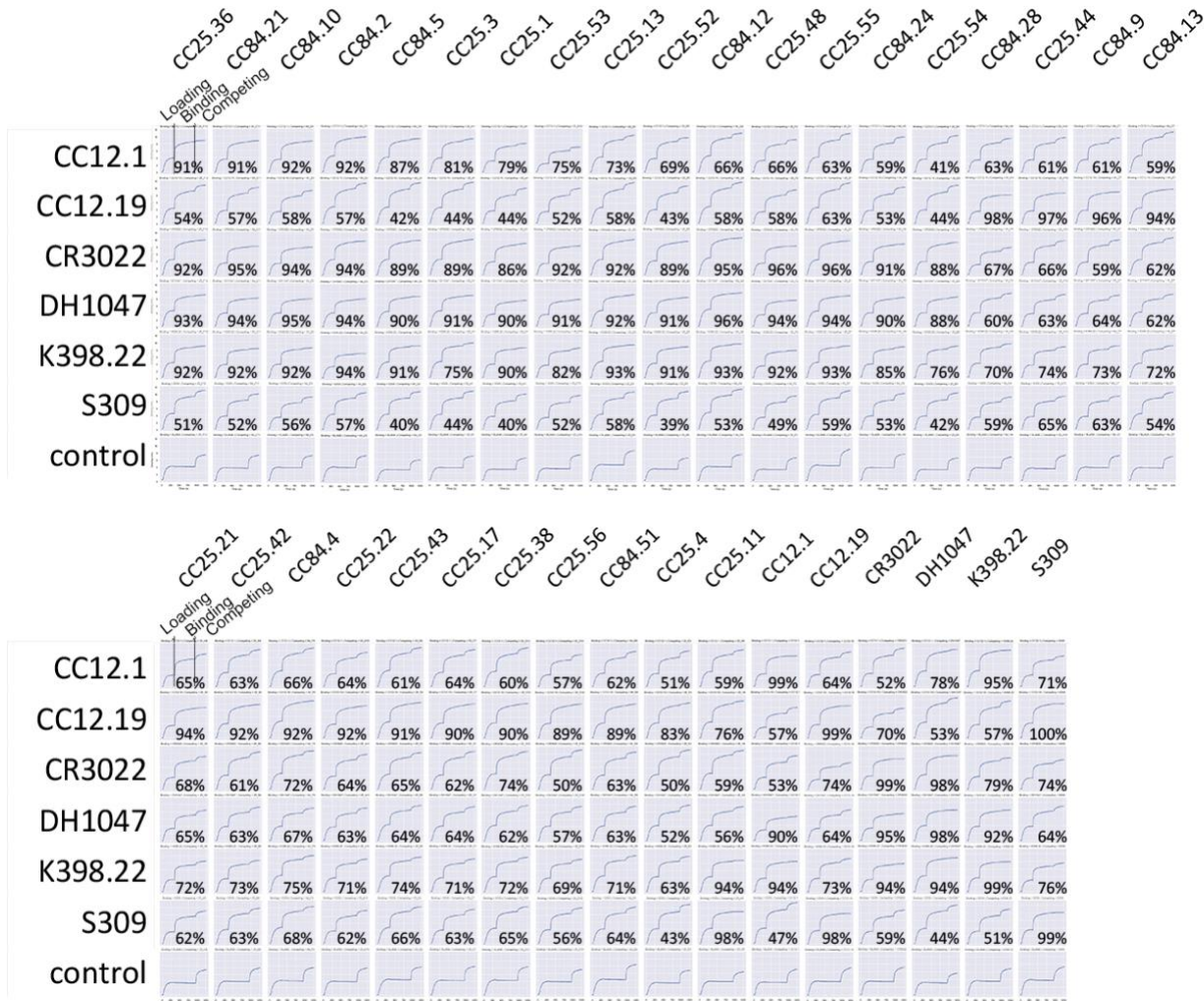
SARS-CoV-2	Kon values											
	Clade 1b			Clade 1a			Clade 3		Clade 2			
	RatG13	Pang17	SARS-CoV-1	WIV1	SHC014	BM-4831	BtKY72	RmYN02	Rf1	Rs4081	Yun11	
CC25.52	6.71E+05	2.49E+05	3.10E+05	6.98E+05	2.69E+05	3.36E+05	1.78E+05	2.34E+05	ND	1.89E+05	8.01E+05	ND
CC84.10	9.59E+05	3.89E+05	5.71E+05	1.26E+06	4.05E+05	5.89E+05	4.40E+05	5.31E+05	2.18E+06	3.79E+05	ND	1.73E+06
CC25.54	1.95E+05	3.94E+05	5.36E+05	3.81E+05	3.83E+05	4.55E+05	4.29E+05	4.29E+05	4.29E+05	4.09E+05	3.46E+05	3.99E+05
CC84.5	4.23E+05	1.81E+05	2.16E+05	5.19E+05	2.02E+05	2.06E+05	1.31E+05	1.81E+05	ND	ND	ND	ND
CC25.3	8.15E+05	3.80E+05	5.57E+05	1.30E+06	4.23E+05	5.43E+05	7.24E+05	5.76E+05	1.01E+06	ND	1.05E+06	1.27E+06
CC25.1	8.37E+05	3.53E+05	7.11E+05	1.23E+06	4.49E+05	4.06E+05	3.96E+05	4.63E+05	ND	ND	ND	ND
CC25.53	7.73E+05	3.52E+05	4.82E+05	7.47E+05	2.98E+05	3.70E+05	7.67E+05	4.11E+05	3.88E+05	3.84E+05	3.40E+05	3.41E+05
CC25.36	8.03E+05	4.09E+05	4.97E+05	1.19E+06	3.39E+05	5.40E+05	2.67E+05	4.95E+0				

	SARS-CoV-2						IC <sub>50</sub> (µg/ml)	SARS-CoV-2						IC <sub>50</sub> Fold-change
	WT	B.1.1.7	B.1.351	P.1	B.1.617.2	B.1.1.529		WT	B.1.1.7	B.1.351	P.1	B.1.617.2	B.1.1.529	
CC25.52	0.12	0.07	0.11	0.09	0.08	1.10		1.0	0.6	1.0	0.8	0.7	9.4	
CC84.10	0.14	0.12	0.05	0.12	0.20	0.35		1.0	0.8	0.4	0.8	1.4	2.5	
CC25.54	0.13	0.13	0.18	0.07	0.15	0.69		1.0	1.0	1.5	0.6	1.2	5.4	
CC84.5	0.16	0.16	0.15	0.21	0.20	>10		1.0	1.0	0.9	1.3	1.2	61	
CC25.3	0.05	0.15	0.12	0.07	0.12	0.60		1.0	3.0	2.3	1.4	2.2	11	
CC25.1	0.13	0.11	0.12	0.15	0.12	0.42		1.0	0.8	0.9	1.1	0.9	3.1	
CC25.53	0.20	0.30	0.38	0.23	0.40	1.85		1.0	1.5	1.9	1.2	2.0	9.1	
CC25.36	0.15	0.24	0.22	0.23	0.37	0.31		1.0	1.6	1.5	1.5	2.5	2.1	
CC84.24	0.37	0.44	0.90	0.63	1.30	0.80		1.0	1.2	2.4	1.7	3.5	2.2	
CC84.12	0.53	0.31	0.35	0.28	0.29	>10		1.0	0.6	0.6	0.5	0.5	19	
CC25.4	0.23	0.19	0.46	0.20	0.37	1.27		1.0	0.8	2.0	0.9	1.6	5.4	
CC25.56	0.51	0.74	2.74	0.49	2.33	2.18		1.0	1.5	5.4	1.0	4.6	4.3	
CC25.48	1.61	1.38	0.95	1.10	1.19	>10		1.0	0.9	0.6	0.7	0.7	6.2	
CC25.13	0.24	0.14	0.33	0.09	0.02	>10		1.0	0.6	1.4	0.4	0.1	42	
CC84.21	0.19	0.22	0.10	0.10	0.16	1.43		1.0	1.1	0.5	0.5	0.8	7.4	
CC25.11	2.13	0.19	0.11	1.14	1.02	>10		1.0	0.1	0.1	0.5	0.5	4.7	
CC25.43	1.54	0.37	0.48	0.26	0.07	0.83		1.0	0.2	0.3	0.2	0.0	0.5	
CC84.2	0.35	0.54	0.24	0.19	0.47	>10		1.0	1.6	0.7	0.5	1.4	29	
CC25.17	4.17	0.82	1.46	0.22	0.21	2.24		1.0	0.2	0.3	0.1	0.1	0.5	>100
CC25.42	4.87	1.49	3.59	1.25	1.14	4.95		1.0	0.3	0.7	0.3	0.2	1.0	100
CC12.1	0.03	0.24	>10	6.82	0.05	>10		1.0	7.8	333	227	1.7	333	50
DH1047	0.88	0.37	0.22	0.36	0.38	>10		1.0	0.4	0.3	0.4	0.4	11	10
DEN3	>10	>10	>10	>10	>10	>10		1.0	1.0	1.0	1.0	1.0	1.0	3

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**Extended Data Fig. 7. Neutralization of RBD bnAbs against SARS-CoV-2 and major variants of concern (VOCs).**

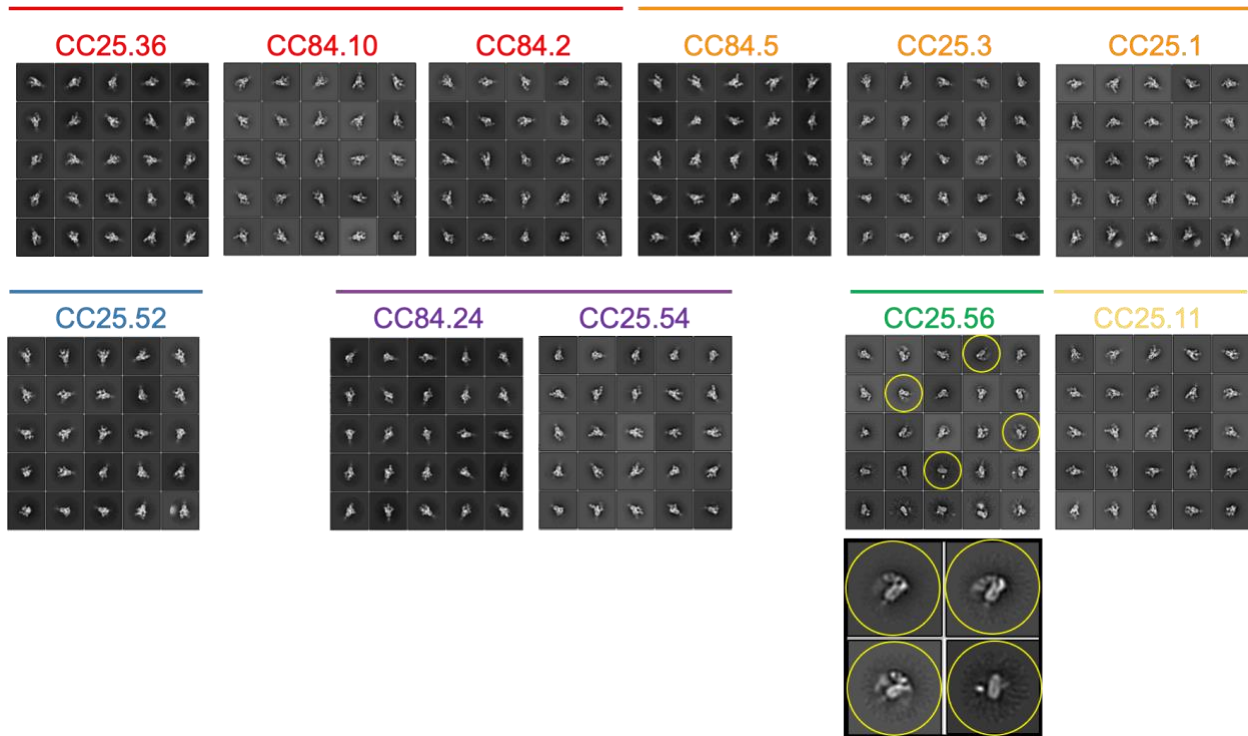
IC<sub>50</sub> neutralization titers of RBD bnAbs against SARS-CoV-2 (WT) and five major SARS-CoV-2 VOCs: (B.1.1.7 (Alpha), B.1.351 (Beta), P.1 (Gamma), B.1.617.2 (Delta) and B.1.1.529 (Omicron)). The IC<sub>50</sub> neutralization fold-change of RBD bnAbs with SARS-CoV-2 VOCs compared to the WT virus. CC12.1, DH1047 and DEN mAbs were used as controls.



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**Extended Data Fig. 8. Epitope binning of mAbs using a competition assay.**

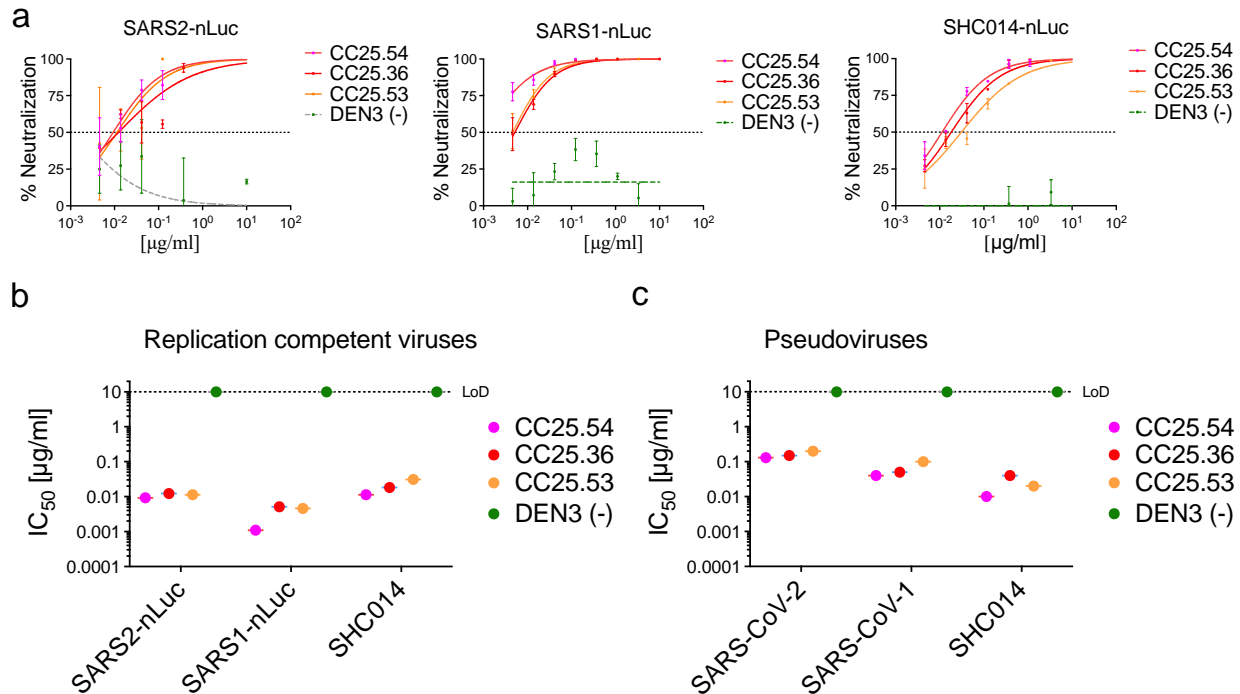
30 select mAbs (19 mAbs from donor CC25 and 11 mAbs from donor CC84) were assayed in BLI competition binning to evaluate epitope properties shared with previously isolated human (CC12.1, CC12.19, CR3022, DH1047 and S309) and macaque (K398.22) mAbs with known epitope specificities. His-tagged SARS-CoV-2 RBD protein (200nM) was captured on anti-His biosensors and incubated with the indicated mAbs at a saturating concentration of 100µg/mL for 10 mins and followed by nAb incubation for 5 min at a concentration of 25µg/mL. All BLI measurements were performed on an Octet RED384 system. BLI traces are shown for each binding. The binding inhibition % is calculated with the formula: percent (%) of inhibition in the BLI binding response = 1- (response in presence of the competitor antibody / response of the corresponding control antibody without the competitor antibody).



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**Extended Data Fig. 9. Epitope mapping of bnAbs using negative stain Electron Microscopy (ns-EM).**

Electron microscopy (EM) images of sarbecovirus cross-neutralizing antibody Fabs with SARS-CoV-2 S-protein. 2D class averages of S-protein bound Fabs for each mAbs are shown. One of the group 2 bnAb Fabs, CC25.56, had some destabilizing effect on the S-protein trimer (indicated in yellow circles).



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**Extended Data Fig. 10. Neutralization of replication competent sarbecoviruses by select RBD bnAbs.**

**a.** Neutralization of replication competent viruses encoding SARS-CoV-2 (SARS2-nLuc), SARS-CoV-1 (SARS1-nLuc) and SHC014 (SHC014-nLuc) by 3 select RBD bnAbs, CC25.54, CC25.36 and CC25.53. DEN3 antibody was a negative control for the neutralization assay.

**b-c.** Comparison of IC<sub>50</sub> neutralization titers of 3 RBD bnAbs with replication-competent (**b**) and pseudoviruses (**c**) of SARS-CoV-2, SARS-CoV-1 and SHC014 ACE2-utilizing sarbecoviruses.