



26 **Summary**

27 **SARS-CoV-2 infection or vaccination produces neutralizing antibody responses that**  
28 **contribute to better clinical outcomes. The receptor binding domain (RBD) and the N-**  
29 **terminal domain (NTD) of the spike trimer (S) constitute the two major neutralizing**  
30 **targets for the antibody system. Neutralizing antibodies targeting the RBD bind to several**  
31 **different sites on this domain. In contrast, most neutralizing antibodies to NTD**  
32 **characterized to date bind to a single supersite, however these antibodies were obtained by**  
33 **methods that were not NTD specific. Here we use NTD specific probes to focus on anti-**  
34 **NTD memory B cells in a cohort of pre-omicron infected individuals some of which were**  
35 **also vaccinated. Of 275 NTD binding antibodies tested 103 neutralized at least one of three**  
36 **tested strains: Wuhan-Hu-1, Gamma, or PMS20, a synthetic variant which is extensively**  
37 **mutated in the NTD supersite. Among the 43 neutralizing antibodies that were further**  
38 **characterized, we found 6 complementation groups based on competition binding**  
39 **experiments. 58% targeted epitopes outside the NTD supersite, and 58% neutralized either**  
40 **Gamma or Omicron, but only 14% were broad neutralizers. Three of the broad**  
41 **neutralizers were characterized structurally. C1520 and C1791 recognize epitopes on**  
42 **opposite faces of the NTD with a distinct binding pose relative to previously described**  
43 **antibodies allowing for greater potency and cross-reactivity with 7 different variants**  
44 **including Beta, Delta, Gamma and Omicron. Antibody C1717 represents a previously**  
45 **uncharacterized class of NTD-directed antibodies that recognizes the viral membrane**  
46 **proximal side of the NTD and SD2 domain, leading to cross-neutralization of Beta, Gamma**  
47 **and Omicron. We conclude SARS-CoV-2 infection and/or Wuhan-Hu-1 mRNA vaccination**  
48 **produces a diverse collection of memory B cells that produce anti-NTD antibodies some of**

49 **which can neutralize variants of concern. Rapid recruitment of these cells into the antibody**  
50 **secreting plasma cell compartment upon re-infection likely contributes to the relatively**  
51 **benign course of subsequent infections with SARS-CoV-2 variants including omicron.**

52

53

## 54 **Introduction**

55 Several independent studies purified anti-SARS-CoV-2 specific B cells from infected or  
56 vaccinated individuals using soluble spike (S) protein as a bait. In all cases the neutralizing  
57 antibodies obtained by this method targeted the RBD most frequently and were generally more  
58 potent than those targeting the NTD (Kreer et al., 2020; Liu et al., 2020; Zost et al., 2020b).

59

60 Further characterization of the neutralizing antibodies to RBD showed that infected or vaccinated  
61 humans produce a convergent set of neutralizing antibodies dominated by specific Ig heavy  
62 chain variable (IGHV) regions (Barnes et al., 2020b; Brouwer et al., 2020; Robbiani et al., 2020;  
63 Wang et al., 2021d; Yuan et al., 2020). Structural analysis of the interaction between these  
64 antibodies and the RBD revealed four classes of anti-RBD antibodies each of which can interfere  
65 with the interaction between this domain and ACE2 the cellular receptor for the SARS-CoV-2 S  
66 protein (Barnes et al., 2020a; Yuan et al., 2020). The most frequently occurring anti-RBD  
67 antibodies select for resistance mutations in *in vitro* experiments that are also found in variants of  
68 concern (Baum et al., 2020; Greaney et al., 2021; Wang et al., 2021d; Weisblum et al., 2020).

69 These include the K417N, E484A and N501Y changes that are found in Omicron, the most  
70 recently emerging variant of concern (Callaway, 2021). However, anti-RBD antibodies that  
71 remain resistant to these changes evolve over time in the memory B cell compartment of both

72 naturally infected and vaccinated individuals over time (Cho et al., 2021; Muecksch et al., 2021;  
73 Wang et al., 2021c).  
74  
75 Less is known about anti-NTD antibody responses. In contrast to RBD, the anti-NTD  
76 neutralizing antibodies obtained to date primarily target a single supersite consisting of variable  
77 loops flanked by glycans (Amanat et al., 2021; Cerutti et al., 2021b; Chi et al., 2020; Dussupt et  
78 al., 2021; Haslwanter et al., 2021; Li et al., 2021; Liu et al., 2021; McCallum et al., 2021a;  
79 Planas et al., 2021b; Suryadevara et al., 2021b; Voss et al., 2021). Residues in the supersite are  
80 mutated in several variants of concern including Beta, Gamma and Omicron, the latter of which  
81 carries 3 deletions, 4 amino acid substitutions and an insertion in the NTD that render antibodies  
82 to the supersite infective (Callaway, 2021; Faria et al., 2021; Tegally et al., 2021). Residues in  
83 this site are also mutated in the S protein of PMS20, a synthetic construct that is highly antibody  
84 resistant and chimeric proteins build from WT and PMS20 proteins show that NTD-specific  
85 antibodies are an important component of the neutralizing activity in convalescent and vaccine  
86 recipient plasma (Schmidt et al., 2021c). NTD supersite mutations are therefore likely to  
87 contribute to the poor plasma neutralizing activity against the Omicron variant in individuals that  
88 received 2 doses of currently available vaccines or convalescent individuals exposed to pre-  
89 Omicron variants of SARS-CoV-2. Nevertheless, boosting with currently available mRNA  
90 vaccines elicits high levels of plasma Omicron neutralizing antibodies(Dejnirattisai et al., 2022;  
91 Gruell et al., 2022; Rossler et al., 2022; Schmidt et al., 2021b; Wu et al., 2022), and vaccinated  
92 individuals are protected from serious disease following Omicron infection. Whether additional  
93 anti-NTD omicron neutralizing epitopes exist, and how memory B cell anti-NTD neutralizing  
94 responses evolve over time is poorly understood.



95

96 To focus on the development and evolution of the human antibody response to NTD we studied a  
97 cohort of SARS-CoV-2 convalescent and/or mRNA vaccinated individuals using the isolated  
98 NTD domain as a probe to capture memory B cells producing antibodies specific to this domain.

99

## 100 **Results**

101 To examine the development of anti-NTD antibodies we studied a previously described  
102 longitudinal cohort of individuals sampled 1.3 and 12 months after infection, some of whom  
103 received an mRNA vaccine approximately 40 days before the 12-month study visit ((Wang et al.,  
104 2021c) and Table S1). All individuals were infected between 1 April and 8 May of 2020, before  
105 the emergence of Omicron. Antibody reactivity in plasma to the isolated Wuhan-Hu-1 and  
106 Omicron NTD was measured by ELISA (Figure S1). There was no association between IgM,  
107 IgG or IgA anti-NTD Wuhan-Hu-1 or Omicron ELISA titers and age, sex, symptom severity,  
108 duration of symptoms, persistence of symptoms, or time between symptom onset and the first  
109 clinic visit (Figure S2).

110

111 In convalescent individuals that had not been vaccinated anti-Wuhan-Hu-1 NTD IgG reactivity  
112 was not significantly different between the 1.3- and 12-month time points (Figure S1A). In  
113 contrast, IgG reactivity to the Omicron NTD dropped significantly between the 2 time points  
114 (Figure S1B). Notably, there was no correlation between NTD and RBD IgG ELISA reactivity or  
115 NTD IgG ELISA reactivity and plasma geometric mean half-maximal neutralizing titers (NT<sub>50s</sub>,  
116 see below) in convalescent individuals that had not been vaccinated (Figure S3). Vaccination of  
117 convalescent individuals resulted in significantly increased IgG ELISA reactivity to both

118 Wuhan-Hu-1 and Omicron NTDs with a positive correlation between NTD and RBD ELISA,  
119 and NTD and plasma NT<sub>50</sub> (Figures S3A and S3B, (Schmidt et al., 2021a)). IgM and IgA anti-  
120 NTD reactivity in plasma was relatively low in all individuals tested, and only IgA was boosted  
121 with vaccination (Figures S1C and S1D). We conclude that anti-NTD ELISA reactivity to both  
122 Wuhan-Hu-1 and Omicron is enhanced by vaccination in convalescent individuals.

123

### 124 **Memory anti-NTD Antibodies**

125 Nearly all anti-NTD antibodies characterized to date were obtained using the intact S protein to  
126 capture specific memory B cells. Neutralizing anti-NTD antibodies obtained by this method  
127 represent a small subset of the total anti-S antibodies and in most cases, they target a single  
128 carbohydrate flanked epitope that faces away from the cell membrane and is mutated in several  
129 variants of concern including Omicron (Amanat et al., 2021; Cerutti et al., 2021b; Chi et al.,  
130 2020; Dussupt et al., 2021; Haslwanter et al., 2021; Li et al., 2021; Liu et al., 2021; McCallum et  
131 al., 2021a; Planas et al., 2021b; Suryadevara et al., 2021b; Voss et al., 2021). To focus on NTD  
132 we used a combination of soluble Wuhan-Hu-1 and Gamma NTD proteins to identify memory B  
133 cells producing antibodies specific for this domain.

134

135 Convalescent individuals that had not been vaccinated showed a small but significant decrease in  
136 the number of anti-NTD memory B cells between the 1.3- and 12-month time points, however, it  
137 was boosted by vaccination (Figures 1A and S4). We obtained 914 anti-NTD antibody sequences  
138 from 3 non-vaccinated and 3 vaccinated convalescent individuals assayed at the 2 time points  
139 (Figure 1B and Table S2). Like the anti-RBD response, different individuals made closely

140 related antibodies to NTD among which antibodies encoded by VH1-24, VH3-30, VH3-33,  
141 VH4-4, and VH4-39 were over-represented (Figures 1C and S5).

142

143 Expanded clones of memory B cells were found at both time points. On average expanded clones  
144 accounted for 22% and 27% of all antibodies at the 1.3- and 12-month time points respectively,  
145 and 30 out of 90 such clones were conserved between time points (Figure 1B and Table S2).

146 Thus, most of the clones were unique to one of the two time points indicating that the antibody  
147 response continued to evolve with persisting clonal expansion. B cell clonal evolution is

148 associated with accumulation of somatic hypermutation (Elsner and Shlomchik, 2020; Victora  
149 and Nussenzweig, 2012). Consistent with the notion that the anti-NTD antibody response

150 continues to evolve there was a significant increase in Ig heavy and/or light chain somatic

151 mutation between the 2 time points in all 6 individuals examined (Figure 1D). CDR3 length was

152 unchanged overtime and hydrophobicity was slightly lower for anti-NTD antibodies than control

153 (Figure S6).

154

155 We expressed 275 antibodies from the 6 individuals (124 and 151 from 1.3- and 12-month

156 timepoint, respectively) including: 1) 158 that were randomly selected from those that appeared

157 only once evenly divided between 1.3- and 12-months; 2) 29 that appeared as expanded clones

158 that were conserved at the two time points; 3) 16 and 43 newly arising expanded clones present

159 at either the 1.3- or 12-month time points (Table S3). Each of the antibodies was tested for

160 binding to Wuhan-Hu-1, Delta, Gamma, and Omicron NTDs by ELISA (Figure 2A). 97% of the

161 antibodies cloned from the 1.3-month time point bound to the Gamma NTD, and 82%, 69% and

162 52% to Wuhan-Hu-1, Delta, and Omicron respectively. The fraction of binding antibodies to

163 Wuhan-Hu-1 and Delta NTD improved significantly after 12 months (Figures 2A and S7A). In  
164 addition, the geometric mean ELISA half-maximal concentration ( $EC_{50}$ ) decreased significantly  
165 for all the variant NTDs after 12 months suggesting an increase in overall binding affinity over  
166 time (Figure 2A and Table S3).

167

168 To determine whether antibody affinity increased between the 2 time points we performed  
169 biolayer interferometry experiments (Figures 2A and S7B). Between 1.3- and 12-months affinity  
170 to the Wuhan-Hu-1 NTD increased among clonal pairs (28.0nM to 5.6nM) and unique antibodies  
171 (40.3nM to 8.8nM in unvaccinated or to 10.3nM in vaccinated individuals) (Figure 2B). We  
172 conclude that anti-NTD antibodies evolve to higher affinity during the 12 months following  
173 infection irrespective of subsequent vaccination.

174

### 175 **Anti-NTD Antibody Neutralizing Activity**

176 Antibodies that showed binding to NTD by ELISA were tested for neutralizing activity in a  
177 SARS-CoV-2 neutralization assay using pseudotyped viruses encoding Wuhan-Hu-1, Gamma  
178 and PMS20 S proteins (Table S3). Among 275 NTD-binding antibodies, 103 neutralized at least  
179 one of the pseudoviruses with an  $IC_{50}$  of less than 1000 ng/ml (Table S3). The fraction of  
180 Wuhan-Hu-1-neutralizers among the anti-NTD antibodies obtained from convalescent  
181 individuals was 22%, 28%, and 24% at 1.3-, 12-month and 12-month vaccinated individuals,  
182 respectively. In contrast, the overall neutralizing activity among anti-RBD antibodies obtained  
183 from the same time points from this cohort was significantly higher in all cases (58%, 68%, 75%  
184 respectively) (Figure 2C, (Robbiani et al., 2020; Wang et al., 2021c)). Anti-NTD neutralizing  
185 antibodies were significantly enriched in IGVH1-24, IGVH3-33 and IGVH3-30 all of which are

186 enriched among neutralizing antibodies that target the NTD supersite (Figure 2D and Table S4,  
187 (Amanat et al., 2021; Cerutti et al., 2021b; Chi et al., 2020; Dussupt et al., 2021; Haslwanter et  
188 al., 2021; Li et al., 2021; Liu et al., 2021; McCallum et al., 2021a; Planas et al., 2021b;  
189 Suryadevara et al., 2021b; Voss et al., 2021)). IGVH1-24 was frequently associated with IGVL1-  
190 51 which is also over-represented among neutralizers compared to the database (Table S4). The  
191 neutralizers obtained by this method were also enriched in VH4-39 which has not been  
192 associated with NTD supersite neutralizing activity (Figure 2D).

193

194 Among the antibodies tested the geometric mean  $IC_{50}$  against Wuhan-Hu-1, Gamma, PMS20  
195 was similar at 1.3- and 12-months (Figures 3A and S8A, Table S3). Vaccination was associated  
196 with increased neutralizing activity at 12 months against Gamma but not the other 2  
197 pseudoviruses tested (Figure 3A). There was also no change in geometric mean  $IC_{50}$  when only  
198 the conserved clones were considered (Figure S8B). Therefore, there is no general increase in  
199 anti-NTD antibody neutralizing activity over time despite the relative increase in affinity.

200

201 Although the neutralizing activity of the NTD antibodies was generally lower than RBD  
202 antibodies cloned from the same time points from this cohort (Figures 3A and S8A, (Robbiani et  
203 al., 2020; Wang et al., 2021c)), some anti-NTD antibodies were very potent with  $IC_{50}$  values as  
204 low as 0.17 nanograms per milliliter (Figure 3A). Of the 103 neutralizing anti-NTD antibodies  
205 with demonstrable neutralizing activity 14 were specific for Wuhan-Hu-1, 20 were limited to  
206 Gamma, and 13 were PMS20- specific (Figure 3B). The remaining 56 antibodies neutralized 2 or  
207 more viruses (Figure 3B). Antibodies targeting the NTD supersite are enriched in VH1-24, VH3-  
208 30 and VH3-33 and these 3 VH genes account for 59 of the 103 antibodies tested (Figures 3B

209 and S8C, Table S4). Notably, despite its mutations in the NTD supersite, 24 antibodies  
210 neutralized PMS20 and 6 neutralized all 3 viruses suggesting that some of these antibodies might  
211 bind to epitopes outside of the supersite.

212

213 To document the neutralizing breadth of the 6 broadest antibodies, we tested them against  
214 viruses pseudotyped with SARS-CoV-2 Alpha, Beta, Delta, Iota and Omicron and SARS-CoV S  
215 proteins (Figure 3C). Although none of the antibodies neutralized SARS-CoV, 4 of the 6  
216 antibodies neutralized all strains tested albeit at relatively high neutralizing concentrations.  
217 However, pseudovirus neutralization was incomplete even at very high antibody concentrations  
218 for 2 of the more potent antibodies tested (Figure 3D).

219

220 To determine whether intact virus neutralization resembles pseudovirus neutralization we  
221 performed microneutralization experiments using authentic SARS-CoV-2-WA1/2020 (Robbiani  
222 et al., 2020) and -Beta. In contrast to the pseudovirus, the two antibodies that showed the most  
223 incomplete neutralization profiles against pseudovirus, C1520 and C1565, reached complete  
224 neutralization and were exquisitely potent with  $IC_{50}$ s in the low nanogram per milliliter range  
225 against both strains (Figures 3C and 3E). We conclude that some naturally arising memory anti-  
226 NTD antibodies produced in response to Wuhan-Hu-1 infection and immunization are  
227 insensitive to the mutations found in Omicron and other variants of concern.

228

229 To determine whether our collection of anti-NTD neutralizing antibodies target overlapping  
230 epitopes we performed biolayer interferometry competition experiments (Figures 4A and 4B).  
231 Among the 43 antibodies with the highest neutralizing activity tested there were 6 discernible

232 complementation groups. Groups I and II were overlapping and highly enriched in VH3-30,  
233 VH3-33 and VH1-24 respectively accounting for nearly 90% of the antibodies in these 2 groups  
234 (Figure 4B). These antibodies neutralized either or both Wuhan-Hu-1 and Gamma, but none  
235 neutralized PMS20 or omicron that are mutated in the NTD supersite. Thus, these 2 groups of  
236 antibodies appear to target the previously defined supersite on NTD (Amanat et al., 2021; Cerutti  
237 et al., 2021b; Chi et al., 2020; Dussupt et al., 2021; Haslwanter et al., 2021; Li et al., 2021; Liu et  
238 al., 2021; McCallum et al., 2021a; Planas et al., 2021b; Suryadevara et al., 2021b; Voss et al.,  
239 2021). Group III and IV are also overlapping but in contrast, groups III and IV only 3 of the 19  
240 neutralize Wuhan-Hu-1 and those that do are broad. Notably, the remaining antibodies fail to  
241 measurably neutralize Wuhan-Hu-1 but neutralize PMS20 and/or Omicron (Figure 4B and Table  
242 S4). Group V contains 4 members, 3 of which are broad. The final group VI contains 2 members,  
243 C1621 only neutralizes Wuhan-Hu-1, and C1554 neutralized broadly but not potently. The  
244 broadest neutralizing anti-NTD antibodies appear to recognize sites outside of the supersite and  
245 are not dominated by VH1-24 and VH3-30/3-33. Altogether 16 out of the 43 anti-NTD  
246 neutralizing antibodies tested neutralized PMS20 and/or Omicron but not Wuhan-Hu-1. Thus,  
247 the B cell memory compartment produced in response to infection with Wuhan-Hu-1 contains  
248 antibodies that bind to this strain with high affinity, but do not neutralize it and instead neutralize  
249 PMS20 and/or Omicron.

250

### 251 **Neutralizing epitopes on NTD outside the supersite**

252 To delineate the structural basis for broad-recognition of NTD-directed antibodies, we  
253 determined structures of WT SARS-CoV-2 S 6P(Hsieh et al., 2020) bound to Fab fragments of  
254 C1717 (group III), C1520 (group IV), and C1791 (group V) using single-particle cryo-electron

255 microscopy (cryo-EM) (Figure S9 and Table S5). Global refinements yielded maps at 2.8Å  
256 (C1520-S), 3.5Å (C1717-S), and 4.5Å (C1791-S) resolutions, revealing Fab fragments bound to  
257 NTD epitopes on all three protomers within a trimer irrespective of ‘up’/‘down’ RBD  
258 conformations for all three Fab-S complexes. C1520 and C1791 Fabs recognize epitopes on  
259 opposite faces of the NTD, with binding poses orthogonal to the site i antigenic supersite and  
260 distinct from C1717 pose, consistent with BLI mapping data (Figures 4b and S9J). A 4.5Å  
261 resolution structure of antibody C1791 (*VH3-23\*01/VK1-17\*01*) bound to a S trimer revealed a  
262 glycopeptidic NTD epitope wedged between the N61<sub>NTD</sub>- and N234<sub>NTD</sub>-glycans, engaging  
263 several N-terminal regions including the N1-, N2- and b8-b9 hairpin loops (Figure S9K). The  
264 binding pose of C1791 is similar to the cross-reactive antibody S2L20, which was shown to  
265 maintain binding against single NTD mutations and several VOCs but was non-  
266 neutralizing(McCallum et al., 2021a; McCallum et al., 2021b).

267  
268 Antibody C1520 (*VH3-48\*03/VL4-68\*02*) recognizes the NTD b-sandwich fold, with a distinct  
269 pose relative to similar cross-reactive NTD mAbs (Figures 5A, 5B and S10A)(Cerutti et al.,  
270 2021a; McCallum et al., 2021b). The C1520 epitope comprises residues along the supersite b-  
271 hairpin (residues 152-158), the b8-strand (residue 97-102), N4-loop (residues 178-188), and N-  
272 linked glycans at positions N122 and N149 (Figure 5C). Targeting of the NTD epitope was  
273 driven primarily by heavy chain contacts (the buried surface area (BSA) of NTD epitope on the  
274 C1520 HC represented ~915Å<sup>2</sup> of ~1150Å<sup>2</sup> total BSA), mediated by the 20-residue long CDRH3  
275 that contributed 55% of the antibody paratope (Figures 5C and 5D). Previous studies have shown  
276 that CDRH3 loops of antibodies targeting this face of the NTD disrupt a conserved binding  
277 pocket that accommodates hydrophobic ligands, including polysorbate 80 and biliverdin, which



278 have the potential to prevent binding of supersite antibodies(Cerutti et al., 2021a; Rosa et al.,  
279 2021). The 20-residue long CDRH3 of C1520 displaces the supersite b-hairpin and N4 loops,  
280 which acts as a gate for the hydrophobic pocket (Figure 5E), in a manner similar to antibody  
281 P008-056 ( Figure S10B). This contrasts Ab5-7 that directly buries the tip of its CDRH3 into the  
282 hydrophobic pocket, and S2X303 that maintains a closed gate and partially-overlaps with the  
283 supersite b-hairpin ( Figure S10B). Thus, C1520's increased cross-reactivity and neutralization  
284 breadth relative to Ab5-7 and S2X303 is likely mediated by displacement of the supersite b-  
285 hairpin and N4-loops, which harbor escape mutations found in several SARS-CoV-2 VOCs and  
286 can undergo structural remodeling to escape antibody pressure (Figure 5E)(McCallum et al.,  
287 2021b).

288

289 We next investigated the group III antibody, C1717, that showed neutralization breadth against  
290 SARS-CoV-2 VOCs and distinct binding properties from group IV and V antibodies (Figures  
291 3C, 4B and S9J). Antibody C1717 (*VH1-69\*04/VL1-44\*01*) recognizes the viral membrane  
292 proximal side of the NTD (Figure 5F), similar to S2M24(McCallum et al., 2021a), DH1052(Li et  
293 al., 2021), and polyclonal Fabs from donor COV57(Barnes et al., 2020b). However, C1717's  
294 pose is unique and represents a new antigenic site that recognizes a glycopeptide epitope flanked  
295 by the N282<sub>NTD</sub>- and N603<sub>SD2</sub>-glycans and is positioned in close proximity (<12 Å) to the S2-  
296 fusion peptide region (Figures 5F and 5G). All six CDR loops contribute to an epitope that spans  
297 both the NTD and SD2 regions (residues 600-606) with a spike epitope BSA of ~1325 Å<sup>2</sup>  
298 (Figure 5G). CDRH1 and CDRH3 loops mediate extensive hydrogen bond and van der Waals  
299 contacts with the C-terminus of the NTD (residues 286-303) and SD2 loop (residues 600-606),  
300 whereas CDRH2 engages N-terminal regions (residues 27-32, 57-60) and NTD loop residues

301 210-218 (Figures 5H-5K). C1717 light chain CDRL2 and CDRL3 loops contact the C-terminus  
302 of the NTD (residues 286-303) and the NTD loop 210-218, respectively (Figures 5I and 5J). In  
303 addition, light chain FWR3 buries against the N282<sub>NTD</sub>-glycan, a complex-type N-glycan(Wang  
304 et al., 2021a) that wedges against the SD1 domain of the adjacent protomer (Figures 5F-G, I).  
305 Modeling of Omicron mutations found in NTD loop 210-218 shows accommodation of the 214-  
306 insertion and potential establishment of backbone contacts with the NTD loop and R95<sub>LC</sub> in  
307 CDRL3 (Figure 5K). Moreover, stabilization of the N282<sub>NTD</sub>-glycan against the adjacent  
308 protomer and the proximity of the C1717 LC to the S2 fusion machinery potentially contributes  
309 to C1717's neutralization mechanism by preventing access to the S2' cleavage site or  
310 destabilization of S1. Such a mechanism could explain the lack of Delta VOC neutralization, as  
311 this variant has been shown to have enhanced cell-cell fusion activity relative to all other  
312 VOCs(McCallum et al., 2021b; Zhang et al., 2021).

313

## 314 **Discussion**

315 In animal models, passive antibody infusion early in the course of infection accelerates viral  
316 clearance and protects against disease (Hansen et al., 2020; Hassan et al., 2020; Rogers et al.,  
317 2020; Schmitz et al., 2021; Tortorici et al., 2020; Zost et al., 2020a). In humans, rapid  
318 development of neutralizing antibodies to SARS-CoV-2 is associated with better clinical  
319 outcomes (Khoury et al., 2021). Finally, early passive transfer of neutralizing antibodies to high-  
320 risk individuals alters the course of the infection and can prevent hospitalization and death  
321 (Dougan et al., 2021; Gupta et al., 2021; Weinreich et al., 2021). Thus, antibodies play an  
322 essential role in both protection against SARS-CoV-2 infection and serious disease.

323

324 The RBD and the NTD are the two dominant targets of neutralizing antibodies on the SARS-  
325 CoV-2 S protein. When the memory B cell compartment is probed using intact S the majority of  
326 the neutralizing antibodies obtained target the RBD and only a small number that are usually less  
327 potent or broad target the NTD (Kreer et al., 2020; Liu et al., 2020; Zost et al., 2020b). Among  
328 the neutralizers that recognize the NTD, the majority target the site i supersite and these  
329 antibodies are enriched in IGVH3-33 and IGVH1-24 (Amanat et al., 2021; Cerutti et al., 2021b;  
330 Chi et al., 2020; Dussupt et al., 2021; Haslwanter et al., 2021; Li et al., 2021; Liu et al., 2021;  
331 McCallum et al., 2021a; Planas et al., 2021b; Suryadevara et al., 2021b; Voss et al., 2021). The  
332 few existing exceptions target a region orthogonal to the supersite featuring a pocket that  
333 accommodates hydrophobic ligands, such as polysorbate 80 and biliverdin (Cerutti et al., 2021a;  
334 Rosa et al., 2021). Focusing on the NTD and using complementary probes revealed 6 partially  
335 overlapping complementary groups of NTD neutralizing antibodies.

336

337 Groups I and II are partially overlapping and appear to target the supersite on the target cell  
338 facing surface of the NTD. Group I is enriched in IGVH3-33 and IGVH3-30, while group II is  
339 enriched in IGVH1-24. Together, these two groups make up 42% of all the neutralizers  
340 characterized, none of which are broad or able to neutralize Omicron or PMS20 that carry  
341 extensive site i supersite mutations. Groups I and II differ in their ability to inhibit each other's  
342 binding to NTD likely because they assume different binding poses. Group III is enriched in  
343 IGVH4-39 and is unusual in that all the antibodies in this group neutralize the synthetic PMS20  
344 variant, but only 24% neutralize Gamma, and only 1 of 18 tested is broad. The antibodies in this  
345 group target a novel site of neutralization on the membrane proximal side of the NTD, bridging  
346 the SD2 domain on the same protomer, and potentially infringe on the S2 domain to impede

347 spike function in viral-host membrane fusion(Suryadevara et al., 2021a). Groups IV, V and VI  
348 comprise only 19% of all the NTD neutralizers tested but are highly enriched in the broad  
349 neutralizers, which have the potential to provide universal protection against SARS-CoV-2  
350 variants infection. Group IV and V antibodies target opposite sides of the NTD orthogonal to the  
351 site i supersite, recognizing epitopes that are non- or only partially-overlapping with VOC  
352 mutations and are not significantly impacted by conformational changes in the supersite b-  
353 hairpin(McCallum et al., 2021b). While structurally unique in their binding pose, antibodies  
354 Group IV and V antibodies likely fail to inhibit ACE2 interactions suggesting a potent  
355 neutralization mechanism that stabilizes the prefusion spike glycoprotein. Such mechanisms  
356 have been suggested for similar NTD-directed antibodies(Cerutti et al., 2021a; McCallum et al.,  
357 2021a; Suryadevara et al., 2021b), and remains to be explored.

358  
359 Although a great number of SARS-CoV-2 neutralizing antibodies have been identified, Omicron  
360 escapes the majority of them, including most antibodies in clinical use (Cameroni et al., 2021;  
361 Cao et al., 2021; Planas et al., 2021a). Identifying new epitopes that are targeted by the broad  
362 neutralizers and are conserved among SARS-CoV-2 variants would be ideal targets for future  
363 development of broadly active sarbecovirus vaccines and antibody drugs to overcome antigenic  
364 drift.

365  
366 Immune responses selectively expand two independent groups of B lymphocytes, plasma cells  
367 and memory B cells. Plasma cells are end stage cells that are selected based on affinity. They are  
368 home to the bone marrow where they reside for variable periods of time and secrete specific  
369 antibodies found in plasma. An initial contraction in the number of these cells in the first 6

370 months after SARS-CoV-2 infection or vaccination likely accounts for the decrease in plasma  
371 neutralizing activity and subsequent loss of protection from infection(Cho et al., 2021; Gaebler et  
372 al., 2021).

373

374 Memory cells are found in circulation and in lymphoid organs where they are relatively  
375 quiescent. B cells selected into this compartment are far more diverse than those that become  
376 plasma cells and include cells with somatically mutated receptors that have variable affinities for  
377 the immunogen (Viant et al., 2020; Viant et al., 2021). This includes cells producing antibodies  
378 with relatively high affinities that are highly specific for the antigen as well as cells producing  
379 antibodies with affinity for the initial immunogen and the ability to bind to closely related  
380 antigens. Production of a pool of long lived but quiescent memory cells expressing a diverse  
381 collection of closely related antibodies, some of which can recognize pathogen variants, favors  
382 rapid secondary responses upon SARS-CoV-2 variant exposure in pre-exposed and vaccinated  
383 individuals.

384

385 SARS-CoV-2 infection or vaccination with Wuhan-Hu-1 produces plasma antibody responses  
386 that are relatively specific for Wuhan-Hu-1 and are sub-optimally protective against infection  
387 with variant strains such as Omicron. In contrast, memory B cell populations produced in  
388 response to infection or vaccination are sufficiently diversified to contain high affinity antibodies  
389 that can potently neutralize numerous different variants including Omicron. This diverse  
390 collection of memory cells and their cognate memory T cells are likely to make a significant  
391 contribution to the generally milder course of infection in individuals that have been infected  
392 and/or vaccinated with SARS-CoV-2.

393

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410

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412 analyzed the experiments. M. Caskey and C.G. designed clinical protocols. Z.W., F.M., A.C., H.-  
413 H.H., S.Z., F.S., J. D.S, E.B., T.B.T., R.R., K.Y., Y.L., T.C., and C.O.B., carried out  
414 experiments. M. Cipolla., B.J. and A.G., produced antibodies. M.T., K.G.M., J. D., A.K., C.G.  
415 and M. Caskey recruited participants, executed clinical protocols and processed samples. T.Y.O.

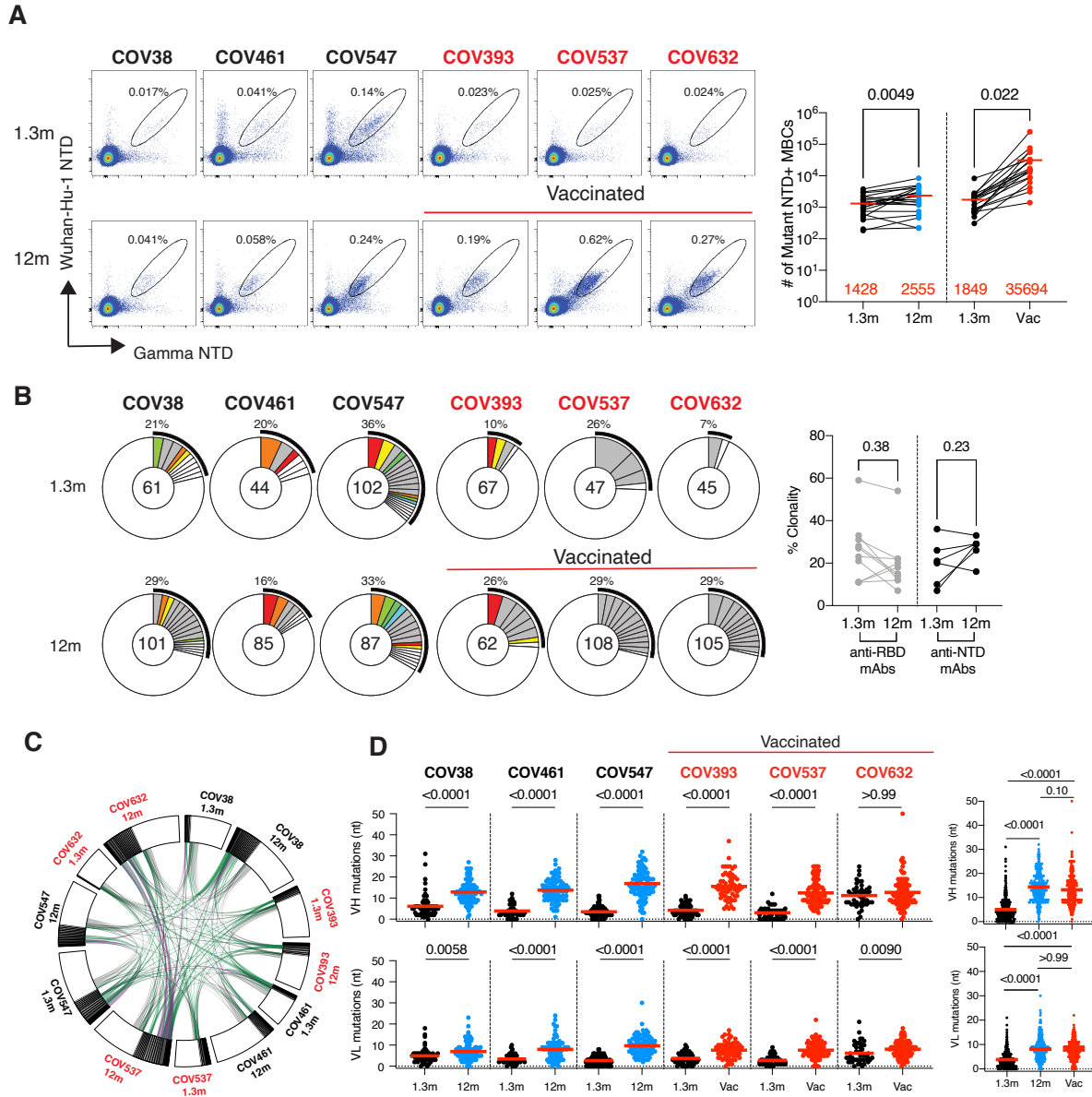
416 and V.R. performed bioinformatic analysis. Z.W., F.M., P.D.B., T.H., C.O.B and M.C.N. wrote  
417 the manuscript with input from all co-authors.

418

419 **Declaration of interests:** All authors declare no conflict of interest.

420

421 **FIGURES**

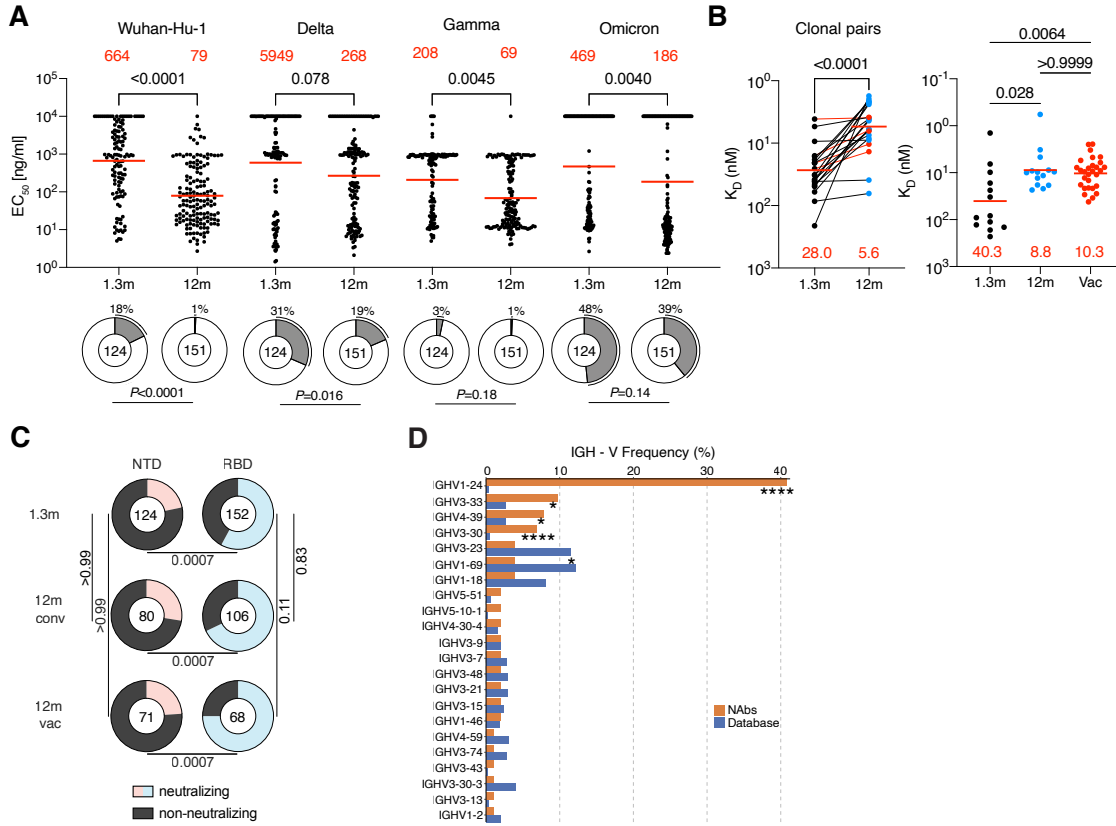


422

423 **Figure 1 Anti-SARS-CoV-2 NTD memory B cells**



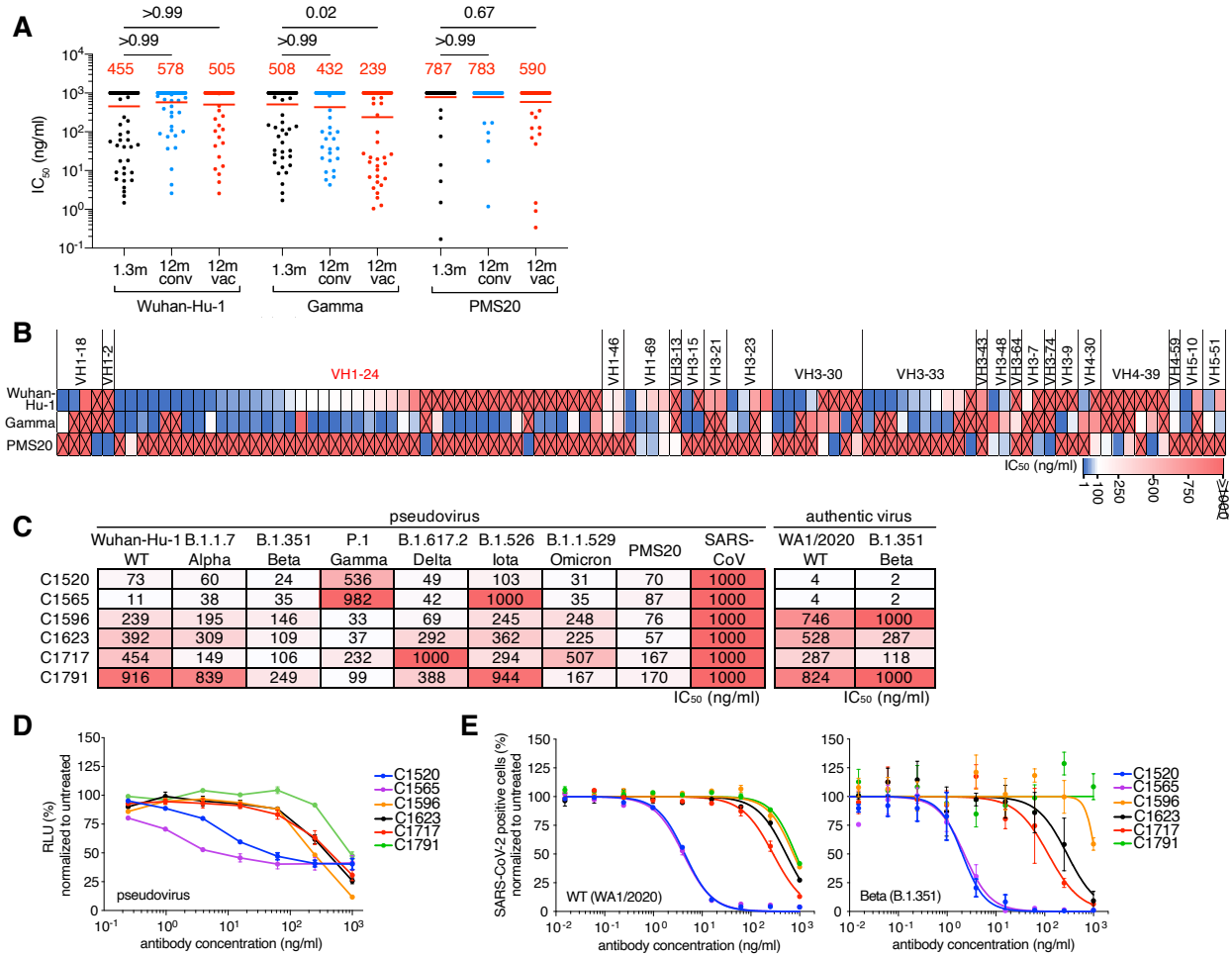
424 **A**, Representative flow cytometry plots showing dual PE-Wuhan-Hu-1 and AlexaFluor-647-  
425 Gamma NTD binding B cells for 6 individuals (vaccinees, n=3, non-vaccinees, n=3) at 1.3  
426 month(Robbiani et al., 2020) and 12 months(Wang et al., 2021c). Gating strategy is found in  
427 **Figure S4**. Right panel shows number of NTD/Mutant NTD positive B cells per 10 million B  
428 cells (also see in **Figure S4B and 4C**) obtained at 1.3 month and 12 months from 39 randomly  
429 selected individuals (vaccinees n=18, and non-vaccinees, n=21). Graphs showing dot plots from  
430 individuals 1.3 months after infection (black), non-vaccinated convalescents (blue) or vaccinated  
431 convalescents (red) 12m after infection. Each dot is one individual. Red horizontal bars indicate  
432 mean values. Statistical significance was determined using two-tailed Mann–Whitney U-tests. **B**,  
433 Pie charts show the distribution of antibody sequences from 6 individuals after 1.3 month (upper  
434 panel) or 12 months (lower panel). The number in the inner circle indicates the number of  
435 sequences analyzed for the individual denoted above the circle. Pie slice size is proportional to  
436 the number of clonally related sequences. The black outline indicates the frequency of clonally  
437 expanded sequences detected. Colored slices indicate persisting clones (same IGHV and IGLV  
438 genes, with highly similar CDR3s) found at both timepoints in the same patient. Grey slices  
439 indicate clones unique to the timepoint. The percentage of BCR clonality from each individual  
440 was summarized in the right panel. Statistical significance was determined using two tailed-  
441 Wilcoxon matched-pairs signed rank tests. **C**, Circus plot depicts the relationship between  
442 antibodies that share V and J gene segment sequences at both IGH and IGL. Purple, green, and  
443 grey lines connect related clones, clones and singles, and singles to each other, respectively.  
444 Vaccinees are marked in red. **D**, Number of somatic nucleotide mutations in the IGVH and  
445 IGVL in antibodies obtained after 1.3 or 12 months from 6 donors. Right panel showing dot plots  
446 from individuals 1.3 months after infection (black), non-vaccinated convalescents (blue) or  
447 vaccinated convalescents (red) 12m after infection. Red horizontal bars indicate mean values.  
448 Statistical significance was determined using Kruskal Wallis test with subsequent Dunn’s  
449 multiple comparisons.



450

451 **Figure 2 Binding and neutralizing activity of anti-SARS-CoV-2 NTD antibodies**

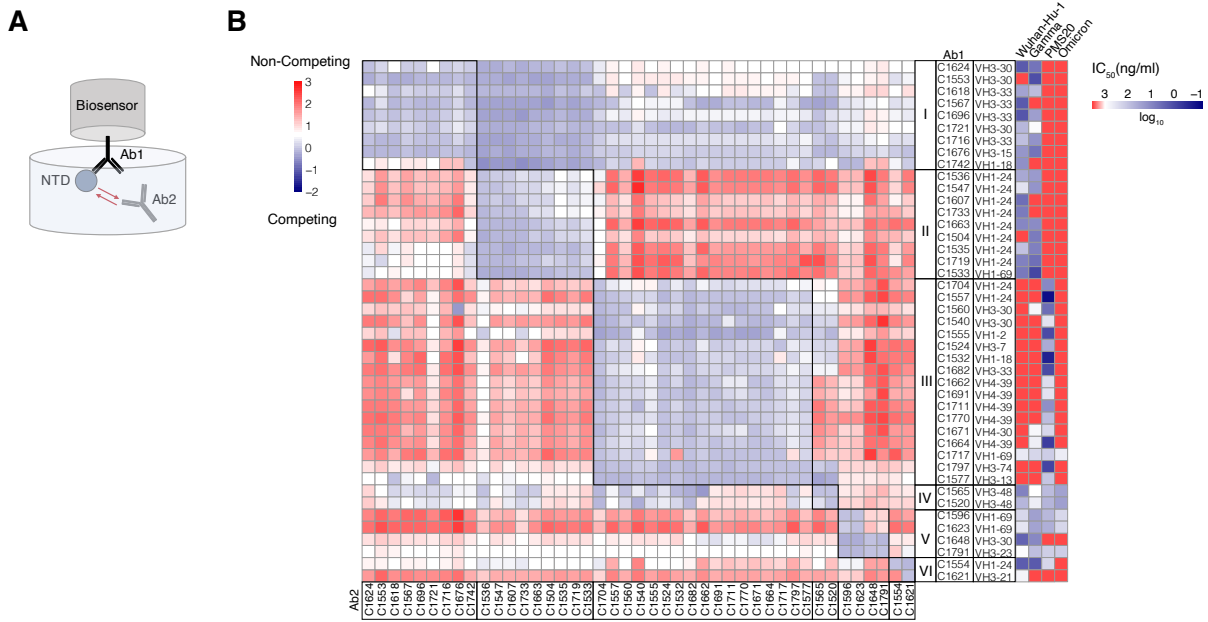
452 **A**, Dot plots show  $EC_{50}$ s against SARS-CoV-2 Wuhan-Hu-1-, Delta-, Gamma-, or Omicron-NTD  
453 for mAbs isolated from convalescents individuals at 1.3- and 12-months after infection. Each dot  
454 represents an individual antibody. Red horizontal bars indicate geometric mean values. Statistical  
455 significance was determined through the two-sided Kruskal Wallis test with subsequent Dunn's  
456 multiple comparisons. Pie charts illustrate the fraction of binders ( $EC_{50}$  0-10000 ng/ml, white  
457 slices), and non-binders ( $EC_{50}$  = 10000 ng/ml, grey slices). Inner circle shows the number of  
458 antibodies tested per group. The black outline indicates the percentage of non-binders. Statistical  
459 significance was determined with two-sided Fisher's exact test. **B**, Graphs depict affinity  
460 measurements for mAbs obtained 1.3- and 12-months after infection. Left panel shows  
461 dissociation constants  $K_D$  values for 22 clonally paired antibodies. Right panel shows  $K_D$  values  
462 for randomly selected antibodies. Antibodies from individuals 1.3 months after infection are in  
463 black, from convalescents 12 m after infection non-vaccinated are in blue or vaccinated in red.  
464 Statistical significance was determined with two-sided Kruskal-Wallis test with subsequent  
465 Dunn's multiple comparisons. Horizontal bars indicate geometric mean values. Statistical  
466 significance was determined using Wilcoxon matched-pairs signed rank tests. **C**, Pie charts  
467 illustrate the fraction of neutralizing (colored slices) and non-neutralizing ( $IC_{50}$  > 1000 ng/ml, grey  
468 slices) anti-NTD (left) and anti-RBD (Wang et al., 2021c) (right) monoclonal antibodies, inner  
469 circle shows the number of antibodies tested per group. Statistical significance was determined  
470 with Fisher's exact test with subsequent Bonferroni-Dunn correction. **D**, Graph shows comparison  
471 of the frequency distributions of human IGH V genes of anti-SARS-CoV-2 NTD neutralizing  
472 antibodies from donors at 1.3 month (Robbiani et al., 2020) and 12 months(Wang et al., 2021c)  
473 after infection. Statistical significance was determined by two-sided binomial test.



474

475 **Figure 3 Neutralizing breath of anti-SARS-CoV-2 NTD antibodies**

476 **A**, Graph shows anti-SARS-CoV-2 neutralizing activity of monoclonal antibodies measured by a  
477 SARS-CoV-2 pseudovirus neutralization assay (Robbiani et al., 2020; Schmidt et al., 2020). Half-  
478 maximal inhibitory concentration ( $IC_{50}$ ) values for antibodies isolated at 1.3 and 12 months after  
479 infection in non-vaccinated (1.3m/12m) and convalescent vaccinated (12m vac) participants.  $IC_{50}$ s  
480 against Wuhan-Hu-1, gamma and PMS20 (Schmidt et al., 2021c) SARS-CoV-2. Spike plasmids  
481 are based on Wuhan-Hu-1 strain containing the R683G substitution. Each dot represents one  
482 antibody from individuals 1.3 months after infection (black), non-vaccinated convalescents (blue)  
483 or vaccinated convalescents (red) 12m after infection. Statistical significance was determined by  
484 two-sided Kruskal Wallis test with subsequent Dunn's multiple comparisons. Horizontal bars and  
485 red numbers indicate geometric mean values. **B**, Heatmap showing neutralizing activity and VH  
486 groups of 103 neutralizing antibodies against Wuhan-Hu-1, Gamma and PMS20 SARS-CoV-2  
487 pseudovirus.  $IC_{50}$  values are indicated by color (from blue to red, most potent to least potent) with  
488 non-neutralizing antibodies ( $IC_{50}$  values of  $>1000$  ng/ml) marked by crossed red tiles. **C**,  $IC_{50}$   
489 values for  $n=6$  broadly neutralizing NTD antibodies against the indicated variant SARS-CoV-2  
490 pseudoviruses, PMS20 (Schmidt et al., 2021c) and SARS-CoV pseudovirus (Muecksch et al., 2021)  
491 (left) as well as WT (WA1/2020) (Robbiani et al., 2020) and Beta (B.1.351) authentic virus. **D**,  
492 Neutralization curves for the antibodies in **C**, against Wuhan-Hu-1 pseudovirus. **E**, Neutralization  
493 curves for the antibodies in **D**, against WT (WA1/2020) and Beta (B.1.351) authentic virus.  
494

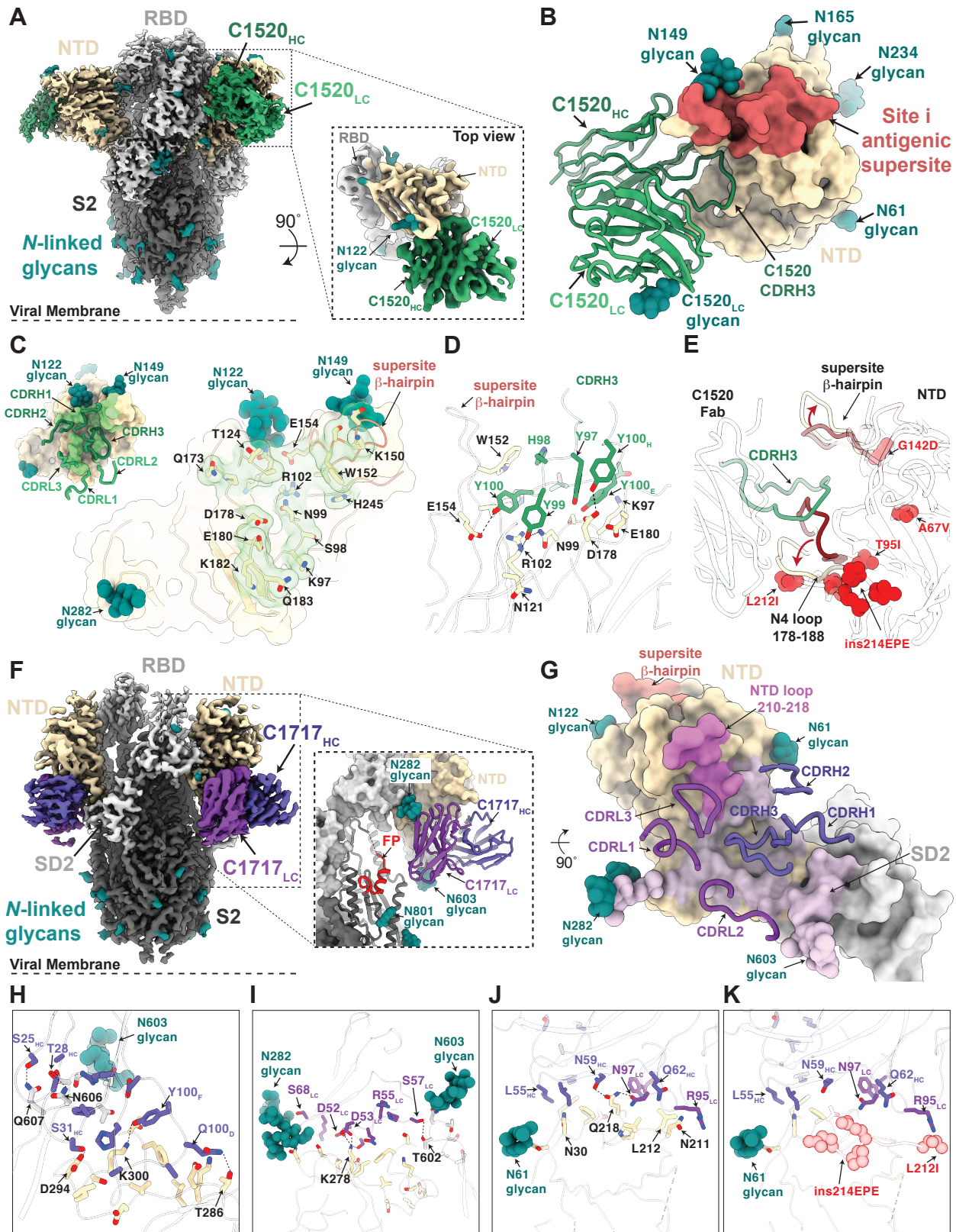


495

496 **Figure 4 Neutralizing epitope mapping of anti-SARS-CoV-2 NTD antibodies**

497 **A**, Diagram of the biolayer interferometry experiment. **B**, Biolayer interferometry results presented  
498 as a heat-map of relative inhibition of Ab2 binding to the preformed Ab1-NTD complexes (from  
499 red to blue, non-competing to competing). Values are normalized through the subtraction of the  
500 autologous antibody control. VH gene usage and IC<sub>50</sub> values of Wuhan-Hu-1, Gamma, PMS20  
501 and Omicron (heat map, from blue to red, most potent to least potent) for each antibody are shown.  
502 The average of two experiments is shown.







504 **Figure 5 Cryo-EM structures of C1520 and C1717 bound to the SARS-CoV-2 S 6P**  
505 **ectodomain**

506 **A**, 2.8Å cryo-EM density for C1520-S 6P trimer complex. Inset: 3.1Å locally-refined cryo-EM  
507 density for C1520 variable domains (shades of green) bound to NTD (wheat). **B**, Close-up view  
508 of C1520 variable domains (green ribbon) binding to NTD (surface rendering, wheat). Site i  
509 antigenic supersite is highlighted on NTD for reference (salmon). **C**, Surface rendering of C1520  
510 epitope (green) with CDR loops shown (inset). NTD epitope residues (defined as residues  
511 containing atom(s) within 4Å of a Fab atom) are shown as sticks. **D**, CDRH3-mediated contacts  
512 on the NTD. Potential hydrogen bonds are shown as black dashed lines. **E**, Overlay of WT  
513 (WA1/2020) NTD (this study) and unliganded Omicron NTD (PDB 7TB4) with mutations and  
514 loop conformational changes highlighted in red. **F**, 3.5Å cryo-EM density for C1717-S 6P trimer  
515 complex. Inset: C1717 variable domains (shades of purple) binding to a S1 protomer (surface  
516 rendering). The fusion peptide (FP, red) in the S2 domain is shown. **G**, Surface rendering of C1717  
517 epitope (thistle) with C1717 CDR loops shown as ribbon. **H-K**, Residue-level contacts between  
518 C1717 (purple), NTD (wheat) and the SD2 domain (gray). Omicron mutations in the N5 loop are  
519 shown as red spheres in panel **K**.

520

521 **METHODS**

522

523 **Study participants.**

524 Samples were obtained from 62 individuals under a study protocol approved by the Rockefeller  
525 University in New York from April 1, 2020 to March 26, 2021 as described in (Robbiani et al.,  
526 2020; Wang et al., 2021c). All participants provided written informed consent before participation  
527 in the study, and the study was conducted in accordance with Good Clinical Practice. For detailed  
528 participant characteristics see Table S1.

529

530 **Blood samples processing and storage.**

531 Peripheral Blood Mononuclear Cells (PBMCs) obtained from samples collected at Rockefeller  
532 University were purified as previously reported by gradient centrifugation and stored in liquid  
533 nitrogen in the presence of FCS and DMSO (Gaebler et al., 2021; Robbiani et al., 2020).  
534 Heparinized plasma and serum samples were aliquoted and stored at -20°C or less. Prior to  
535 experiments, aliquots of plasma samples were heat-inactivated (56°C for 1 hour) and then stored  
536 at 4°C.

537

### 538 **ELISAs**

539 ELISAs (Amanat et al., 2020; Grifoni et al., 2020) to evaluate antibodies binding to SARS-CoV-  
540 2 Wuhan-Hu-1-, Delta-, Gamma and Omicron-NTD were performed by coating of high-binding  
541 96-half-well plates (Corning 3690) with 50 µl per well of a 1 µg/ml protein solution in PBS  
542 overnight at 4 °C. Plates were washed 6 times with washing buffer (1× PBS with 0.05% Tween-  
543 20 (Sigma-Aldrich)) and incubated with 170 µl per well blocking buffer (1× PBS with 2% BSA  
544 and 0.05% Tween-20 (Sigma)) for 1 h at room temperature. Immediately after blocking,  
545 monoclonal antibodies or plasma samples were added in PBS and incubated for 1 h at room  
546 temperature. Plasma samples were assayed at a 1:66 starting dilution and 7 additional threefold  
547 serial dilutions. Monoclonal antibodies were tested at 10 µg/ml starting concentration and 10  
548 additional fourfold serial dilutions. Plates were washed 6 times with washing buffer and then  
549 incubated with anti-human IgG, IgM or IgA secondary antibody conjugated to horseradish  
550 peroxidase (HRP) (Jackson Immuno Research 109-036-088 109-035-129 and Sigma A0295) in  
551 blocking buffer at a 1:5,000 dilution (IgM and IgG) or 1:3,000 dilution (IgA). Plates were  
552 developed by addition of the HRP substrate, TMB (ThermoFisher) for 10 min (plasma samples)  
553 or 4 minutes (monoclonal antibodies), then the developing reaction was stopped by adding 50 µl

554 1 M H<sub>2</sub>SO<sub>4</sub> and absorbance was measured at 450 nm with an ELISA microplate reader (FluoStar  
555 Omega, BMG Labtech) with Omega and Omega MARS software for analysis. For plasma  
556 samples, a positive control (plasma from participant COV57, diluted 66.6-fold and seven  
557 additional threefold serial dilutions in PBS) was added to every assay plate for validation. The  
558 average of its signal was used for normalization of all of the other values on the same plate with  
559 Excel software before calculating the area under the curve using Prism V9.1(GraphPad). For  
560 monoclonal antibodies, the EC<sub>50</sub> was determined using four-parameter nonlinear regression  
561 (GraphPad Prism V9.1).

562

### 563 **Expression of NTD proteins**

564 Mammalian expression vectors encoding the SARS-CoV-2 Wuhan-Hu-1- NTD (GenBank  
565 MN985325.1; S protein residues 14-307), or Delta, Gamma and Omicron NTD mutants with an  
566 N-terminal human IL-2 or Mu phosphatase signal peptide and a C-terminal polyhistidine tag  
567 followed by an AviTag were used to express soluble NTD proteins by transiently-transfecting  
568 Expi293F cells (GIBCO). After four days, NTD proteins were purified from the supernatants by  
569 nickel affinity and size-exclusion chromatography. Peak fractions were identified by SDS-  
570 PAGE, and fractions corresponding to monomeric NTDs were pooled and stored at 4°C.

571

### 572 **Cell Lines**

573 293T cells (Homo sapiens; sex: female, embryonic kidney) obtained from the ATCC (CRL-3216)  
574 and HT1080Ace2 cl14 cells (parental HT1080: homo sapiens; sex: male, fibrosarcoma) (Schmidt  
575 et al., 2020) were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with

576 10% fetal bovine serum (FBS) at 37 °C and 5% CO<sub>2</sub>. VeroE6 cells (*Chlorocebus sabaues*; sex:  
577 female, kidney epithelial) obtained from the ATCC (CRL-1586™) and from Ralph Baric  
578 (University of North Carolina at Chapel Hill), and Caco-2 cells (*Homo sapiens*; sex: male, colon  
579 epithelial) obtained from the ATCC (HTB-37™) were cultured in DMEM supplemented with 1%  
580 nonessential amino acids (NEAA) and 10% FBS at 37 °C and 5% CO<sub>2</sub>. Expi293F cells (GIBCO)  
581 for protein expression were maintained at 37°C and 8% CO<sub>2</sub> in Expi293 Expression medium  
582 (GIBCO), transfected using Expi293 Expression System Kit (GIBCO) and maintained under  
583 shaking at 130 rpm. All cell lines have been tested negative for contamination with mycoplasma.

584

#### 585 **SARS-CoV-2 and sarbecovirus spike protein pseudotyped reporter virus**

586 Plasmids pSARS-CoV-2-SΔ19(R683G) and pSARS-CoV-SΔ19 expressing C-terminally  
587 truncated SARS-CoV-2 and SARS-CoV spike proteins and the polymutant PMS20 spike were as  
588 described before (Schmidt et al., 2021c). A panel of plasmids expressing spike proteins from  
589 SARS-CoV-2 variants were based on pSARS-CoV-2-SΔ19(R683G) and contain the following  
590 substitutions/deletions: Alpha (B.1.1.7): ΔH69/V70, ΔY144, N501Y, A470D, D614G, P681H,  
591 T761I, S982A, D118H; Beta (B.1.351): D80A, D215G, L242H, R246I, K417N, E484K, N501Y,  
592 D614G, A701V; Gamma (P.1): L18F, T20N, P26S, D138Y, R190S, K417T, E484K, N501Y,  
593 D614G, H655Y, T1027I, V1167F; Delta (B.1.617.2): T19R, Δ156-158, L452R, T478K, D614G,  
594 P681R, D950N; Iota (B.1.526): L5F, T95I, D253G, E484K, D614G, A701V (Cho et al., 2021);  
595 Omicron (B.1.1.529)<sup>8</sup>: A67V, Δ69-70, T95I, G142D, Δ143-145, Δ211, L212I, ins214EPE,  
596 G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493K,  
597 G496S, Q498R, N501Y, Y505H, T547K, D614G, H655Y, H679K, P681H, N764K, D796Y,  
598 N856K, Q954H, N969H, N969K, L981F. All SARS-CoV-2 spike proteins including variants and

599 the polymutant spike protein PMS20 included the R683G substitution, which disrupts the furin  
600 cleavage site and generates higher titer virus stocks without significant effects on pseudotyped  
601 virus neutralization sensitivity (Schmidt et al., 2021c). SARS-CoV-2 pseudotyped particles were  
602 generated as previously described (Robbiani et al., 2020; Schmidt et al., 2020). Briefly, 293T  
603 cells were transfected with pNL4-3ΔEnv-nanoluc(Robbiani et al., 2020; Schmidt et al., 2020)  
604 and either spike plasmid. Particles were harvested 48 hpt, filtered and stored at -80C.

605

### 606 **Pseudotyped virus neutralization assay**

607 Monoclonal antibodies were initially screened at a concentration of 1000 ng/ml to identify  
608 those that show >40% neutralization at this concentration. Antibodies were incubated with  
609 SARS-CoV-2 Wuhan-Hu-1(Robbiani et al., 2020) or SARS-CoV pseudotyped virus for 1 h at 37  
610 °C. The mixture was subsequently incubated with HT1080Ace2 c114 cells (Schmidt et al., 2020)  
611 for 48 h after which cells were washed with PBS and lysed with Luciferase Cell Culture Lysis 5×  
612 reagent (Promega). Nanoluc Luciferase activity in lysates was measured using the Nano-Glo  
613 Luciferase Assay System (Promega) with the Glomax Navigator (Promega). The obtained  
614 relative luminescence units were normalized to those derived from cells infected with  
615 pseudotyped virus in the absence of monoclonal antibodies. Antibodies that showed >40%  
616 neutralization at a concentration of 1000 ng/ml were subjected to further titration experiments to  
617 determine their IC<sub>50</sub>s. Antibodies were 4-fold serially diluted and tested against pseudoviruses as  
618 detailed above. IC<sub>50</sub>s were determined using four-parameter nonlinear regression (least squares  
619 regression method without weighting; constraints: top=1, bottom=0) (GraphPad Prism).

### 620 **Virus and Virus Titration**

621 SARS-CoV-2 strains USA-WA1/2020 and the South African beta variant B.1.351 were obtained  
622 from BEI Resources (catalog no. NR-52281 and NR-54008, respectively). The original WT virus  
623 was amplified in Caco-2 cells, which were infected at a multiplicity of infection (MOI) of 0.05  
624 plaque forming units (PFU)/cell and incubated for 6 days at 37 °C. The B.1.351 variant was  
625 amplified in VeroE6 cells obtained from the ATCC that were engineered to stably express  
626 TMPRSS2 (VeroE6<sub>TMPrSS2</sub>). VeroE6<sub>TMPrSS2</sub> cells were infected at a MOI = 0.1 PFU/cell and  
627 incubated for 4 days at 33 °C. Virus-containing supernatants were subsequently harvested,  
628 clarified by centrifugation (3,000 g × 10 min), filtered using a disposable vacuum filter system  
629 with a 0.22 µm membrane and stored at -80 °C. Virus stock titers were measured by standard  
630 plaque assay (PA) on VeroE6 cells obtained from Ralph Baric (referred to as VeroE6<sub>UNC</sub>). Briefly,  
631 500 µL of serial 10-fold virus dilutions in Opti-MEM were used to infect 4x10<sup>5</sup> cells seeded the  
632 day prior into wells of a 6-well plate. After 1.5 h adsorption, the virus inoculum was removed, and  
633 cells were overlaid with DMEM containing 10% FBS with 1.2% microcrystalline cellulose  
634 (Avicel). Cells were incubated for 4 days at 33 °C, followed by fixation with 7% formaldehyde  
635 and crystal violet staining for plaque enumeration. All SARS-CoV-2 experiments were performed  
636 in a biosafety level 3 laboratory.

637 To confirm virus identity and evaluate for unwanted mutations that were acquired during the  
638 amplification process, RNA from virus stocks was purified using TRIzol Reagent (ThermoFisher  
639 Scientific, catalog no. 15596026). Brief, 200 µL of each virus stock was added to 800 µL TRIzol  
640 Reagent, followed by 200 µL chloroform, which was then centrifuged at 12,000 g x 5 min. The  
641 upper aqueous phase was moved to a new tube, mixed with an equal volume of isopropanol, and  
642 then added to a RNeasy Mini Kit column (QIAGEN, catalog no. 74014) to be further purified

643 following the manufacturer's instructions. Viral stocks were subsequently confirmed via next  
644 generation sequencing using libraries for Illumina MiSeq.

#### 645 **Microscopy-Based Neutralization Assay**

646 The day prior to infection VeroE6<sub>UNC</sub> cells were seeded at  $1 \times 10^4$  cells/well into 96-well plates.  
647 Antibodies were serially diluted (4-fold) in BA-1, consisting of medium 199 (Lonza, Inc.)  
648 supplemented with 1% bovine serum albumin (BSA) and 1x penicillin/streptomycin. Next, the  
649 diluted samples were mixed with a constant amount of SARS-CoV-2 and incubated for 1 h at 37  
650 °C. The antibody-virus-mix was then directly applied to each well ( $n = 3$  per dilution) and  
651 incubated for 24 h at 37 °C. The infectious dose for each virus was pre-determined on VeroE6<sub>UNC</sub>  
652 cells to yield 50-60% antigen-positive cells upon this incubation period (USA-WA1/2020: 1,250  
653 PFU/well and B.1.351: 175 PFU/well). Cells were subsequently fixed by adding an equal volume  
654 of 7% formaldehyde to the wells, followed by permeabilization with 0.1% Triton X-100 for 10  
655 min. After extensive washing, cells were incubated for 1h at RT with blocking solution of 5% goat  
656 serum in PBS (Jackson ImmunoResearch, catalog no. 005–000-121). A rabbit polyclonal anti-  
657 SARS-CoV-2 nucleocapsid antibody (GeneTex, catalog no. GTX135357) was added to the cells  
658 at 1:1,000 dilution in blocking solution and incubated overnight at 4 °C. Next, goat anti-rabbit  
659 AlexaFluor 594 (Life Technologies, catalog no. A-11012) was used as a secondary antibody at a  
660 dilution of 1:2,000 and incubated overnight at 4 °C. Nuclei were stained with Hoechst 33342  
661 (ThermoFisher Scientific, catalog no. 62249) at a  $1 \mu\text{g/mL}$ . Images were acquired with a  
662 fluorescence microscope and analyzed using ImageXpress Micro XLS (Molecular Devices,  
663 Sunnyvale, CA). All statistical analyses were done using Prism 8 software (Graphpad).

664

665 **Biotinylation of viral protein for use in flow cytometry**

666 Purified and Avi-tagged SARS-CoV-2 NTD was biotinylated using the Biotin-Protein Ligase-  
667 BIRA kit according to manufacturer's instructions (Avidity) as described before (Robbiani et al.,  
668 2020). Ovalbumin (Sigma, A5503-1G) was biotinylated using the EZ-Link Sulfo-NHS-LC-  
669 Biotinylation kit according to the manufacturer's instructions (Thermo Scientific). Biotinylated  
670 ovalbumin was conjugated to streptavidin-BV711 (BD biosciences, 563262), Gamma NTD to  
671 streptavidin-PE (BD Biosciences, 554061) and Wuhan-Hu-1 NTD to streptavidin-AF647  
672 (Biolegend, 405237).

673

674 **Flow cytometry and single cell sorting**

675 Single cell sorting by flow cytometry was performed as described (Robbiani et al., 2020).  
676 Briefly, peripheral blood mononuclear cells were enriched for B cells by negative selection using  
677 a pan-B-cell isolation kit according to the manufacturer's instructions (Miltenyi Biotec, 130-101-  
678 638). The enriched B cells were incubated in FACS buffer (1× PBS, 2% FCS, 1 mM EDTA)  
679 with the following anti-human antibodies (all at 1:200 dilution): anti-CD20-PECy7 (BD  
680 Biosciences, 335793), anti-CD3-APC-eFluor 780 (Invitrogen, 47-0037-41), anti-CD8-APC-  
681 eFluor 780 (Invitrogen, 47-0086-42), anti-CD16-APC-eFluor 780 (Invitrogen, 47-0168-41), anti-  
682 CD14-APC-eFluor 780 (Invitrogen, 47-0149-42), as well as Zombie NIR (BioLegend, 423105)  
683 and fluorophore-labelled RBD and ovalbumin (Ova) for 30 min on ice. Single  
684 CD3<sup>-</sup>CD8<sup>-</sup>CD14<sup>-</sup>CD16<sup>-</sup>CD20<sup>+</sup>Ova<sup>-</sup>Gamma NTD-PE<sup>+</sup>Wuhan-Hu-1 NTD-AF647<sup>+</sup> B cells  
685 were sorted into individual wells of 96-well plates containing 4 µl of lysis buffer (0.5× PBS, 10  
686 mM DTT, 3,000 units/ml RNasin Ribonuclease Inhibitors (Promega, N2615) per well using a  
687 FACS Aria III and FACSDiva software (Becton Dickinson) for acquisition and FlowJo for



688 analysis. The sorted cells were frozen on dry ice, and then stored at  $-80^{\circ}\text{C}$  or immediately used  
689 for subsequent RNA reverse transcription.

690

### 691 **Antibody sequencing, cloning and expression**

692 Antibodies were identified and sequenced as described previously (Robbiani et al., 2020). In  
693 brief, RNA from single cells was reverse-transcribed (SuperScript III Reverse Transcriptase,  
694 Invitrogen, 18080-044) and the cDNA stored at  $-20^{\circ}\text{C}$  or used for subsequent amplification of  
695 the variable IGH, IGL and IGK genes by nested PCR and Sanger sequencing. Sequence analysis  
696 was performed using MacVector. Amplicons from the first PCR reaction were used as templates  
697 for sequence- and ligation-independent cloning into antibody expression vectors. Recombinant  
698 monoclonal antibodies and Fabs were produced and purified as previously described (Robbiani  
699 et al., 2020).

700

### 701 **Biolayer interferometry**

702 BLI assays were performed on the Octet Red instrument (ForteBio) at  $30^{\circ}\text{C}$  with shaking at  
703 1,000 r.p.m. Epitope binding assays were performed with protein A biosensor (ForteBio 18-  
704 5010), following the manufacturer's protocol "classical sandwich assay" as follows: (1) Sensor  
705 check: sensors immersed 30 sec in buffer alone (buffer ForteBio 18-1105), (2) Capture 1st Ab:  
706 sensors immersed 10 min with Ab1 at  $10\ \mu\text{g}/\text{mL}$ , (3) Baseline: sensors immersed 30 sec in buffer  
707 alone, (4) Blocking: sensors immersed 5 min with IgG isotype control at  $10\ \mu\text{g}/\text{mL}$ . (5) Baseline:  
708 sensors immersed 30 sec in buffer alone, (6) Antigen association: sensors immersed 5 min with  
709 NTD at  $10\ \mu\text{g}/\text{mL}$ . (7) Baseline: sensors immersed 30 sec in buffer alone. (8) Association Ab2:  
710 sensors immersed 5 min with Ab2 at  $10\ \mu\text{g}/\text{mL}$ . Curve fitting was performed using the Fortebio

711 Octet Data analysis software (ForteBio). Affinity measurement of anti-SARS-CoV-2 IgGs  
712 binding were corrected by subtracting the signal obtained from traces performed with IgGs in the  
713 absence of WT NTD. The kinetic analysis using protein A biosensor (ForteBio 18-5010) was  
714 performed as follows: (1) baseline: 60sec immersion in buffer. (2) loading: 200sec immersion in  
715 a solution with IgGs 10 µg/ml. (3) baseline: 200sec immersion in buffer. (4) Association: 300sec  
716 immersion in solution with WT NTD at 200, 100, 50 or 25 µg/ml (5) dissociation: 600sec  
717 immersion in buffer. Curve fitting was performed using a fast 1:1 binding model and the Data  
718 analysis software (ForteBio). Mean  $K_D$  values were determined by averaging all binding curves  
719 that matched the theoretical fit with an  $R^2$  value  $\geq 0.8$ .

720

#### 721 **Recombinant protein expression for structural studies**

722 Expression and purification of stabilized SARS-CoV-2 6P ectodomain was conducted as  
723 previously described(Barnes et al., 2020a). Briefly, constructs encoding the SARS-CoV-2 S  
724 ectodomain (residues 16-1206 with 6P stabilizing mutations(Hsieh et al., 2020), a mutated furin  
725 cleavage site, and C-terminal foldon trimerization motif followed by hexa-His tag) were used to  
726 transiently transfect Expi293F cells (Gibco). Four days after transfection, supernatants were  
727 harvested and S 6P proteins were purified by nickel affinity and size-exclusion chromatography.  
728 Peak fractions from size-exclusion chromatography were identified by SDS-PAGE, and fractions  
729 corresponding to spike trimers were pooled and stored at 4°C. Fabs and IgGs were expressed,  
730 purified, and stored as previously described(Barnes et al., 2020a).

731

#### 732 **Cryo-EM sample preparation**

733 Purified Fabs were mixed with SARS-CoV-2 S 6P trimer at a 1.1:1 molar ratio of Fab-to-  
734 protomer for 30 minutes at room temperature. Fab-S complexes were concentrated to 3-4 mg/mL  
735 prior to deposition on a freshly glow-discharged 300 mesh, 1.2/1.3 Quantifoil grid (Electron  
736 Microscopy Sciences). Immediately prior to deposition of 3  $\mu$ L of complex onto grid, fluorinated  
737 octyl-maltoside (Anatrace) was added to the sample to a final concentration of 0.02% w/v.  
738 Samples were vitrified in 100% liquid ethane using a Mark IV Vitrobot (Thermo Fisher) after  
739 blotting at 22°C and 100% humidity for 3s with Whatman No. 1 filter paper.

740

#### 741 **Cryo-EM data collection and processing**

742 Single-particle cryo-EM data were collected on a Titan Krios transmission electron microscope  
743 (Thermo Fisher) equipped with a Gatan K3 direct detector, operating at 300 kV and controlled  
744 using SerialEM automated data collection software(Mastronarde, 2005). A total dose of  $\sim 60$  e<sup>-</sup>  
745 / $\text{\AA}^2$  was accumulated on each movie comprising 40 frames with a pixel size of 0.515  $\text{\AA}$  (C1520-S  
746 dataset) or 0.852  $\text{\AA}$  (C1717-S and C1791-S) and a defocus range of -1.0 and -2.5  $\mu$ m. Further  
747 data collection parameters are summarized in Table S5.

748

749 Movie frame alignment, CTF estimation, particle-picking and extraction were carried out using  
750 cryoSPARC v3.1(Punjani et al., 2017). Reference-free particle picking and extraction were  
751 performed on dose-weighted micrographs curated to remove images with poor CTF fits or signs  
752 of crystalline ice. A subset of 4x-downsampled particles were used to generate *ab initio* models,  
753 which were then used for heterogeneous refinement of the entire dataset in cryoSPARC. Particles  
754 belonging to classes that resembled Fab-S structures were extracted, downsampled x2 and  
755 subjected to 2D classification to select well-defined particle images. 3D classifications (k=6,

756 tau\_fudge=4) were carried out using Relion v3.1.1(Fernandez-Leiro and Scheres, 2017) without  
757 imposing symmetry and a soft mask. Particles corresponding to selected classes were re-  
758 extracted without binning and 3D refinements were carried out using non-uniform refinement in  
759 cryoSPARC. Particle stacks were split into individual exposure groups based on the beamtilt  
760 angle used for data collection and subjected to per particle CTF refinement and aberration  
761 corrections. Another round of non-uniform refinement in cryoSPARC was then performed. For  
762 focused classification and local refinements of the Fab V<sub>H</sub>V<sub>L</sub>-NTD interface, particles were 3D  
763 classified in Relion without alignment using a mask that encompassed the Fab-NTD region.  
764 Particles in good 3D classes were then used for local refinement in cryoSPARC. Details of  
765 overall resolution and locally-refined resolutions according to the gold-standard Fourier shell  
766 correlation of 0.143 criterion(Bell et al., 2016) can be found in Table S5.

767

### 768 **Cryo-EM structure modeling, refinement, and analyses**

769 Coordinates for initial complexes were generated by docking individual chains from reference  
770 structures (see Table S5) into cryo-EM density using UCSF Chimera(Goddard et al., 2007).  
771 Initial models for Fabs were generated from coordinates from PDB 6RCO (for C1717 Fab) or  
772 PDB 7RKS (for C5120). Models were refined using one round of rigid body refinement followed  
773 by real space refinement in Phenix(Adams et al., 2010). Sequence-updated models were built  
774 manually in Coot(Emsley et al., 2010) and then refined using iterative rounds of refinement in  
775 Coot and Phenix. Glycans were modeled at potential *N*-linked glycosylation sites (PNGSs) in  
776 Coot. Validation of model coordinates was performed using MolProbity(Chen et al., 2010).

777

778 Structure figures were made with UCSF ChimeraX(Goddard et al., 2018). Local resolution maps  
779 were calculated using cryoSPARC v3.1(Punjani et al., 2017). Buried surface areas were  
780 calculated using PDBePISA(Krissinel and Henrick, 2007) and a 1.4Å probe. Potential hydrogen  
781 bonds were assigned as interactions that were <4.0Å and with A-D-H angle >90°. Potential van  
782 der Waals interactions between atoms were assigned as interactions that were <4.0Å. Hydrogen  
783 bond and van der Waals interaction assignments are tentative due to resolution limitations. Spike  
784 epitope residues were defined as residues containing atom(s) within 4Å of a Fab atom for the  
785 C1520-S and C1717-S complexes, and defined as spike Cα atom within 7Å of a Fab Cα atom  
786 for the C1791-S complex.

787

## 788 **Computational analyses of antibody sequences**

789 Antibody sequences were trimmed based on quality and annotated using Igblastn v.1.14. with  
790 IMGT domain delineation system. Annotation was performed systematically using Change-O  
791 toolkit v.0.4.540 (Gupta et al., 2015). Heavy and light chains derived from the same cell were  
792 paired, and clonotypes were assigned based on their V and J genes using in-house R and Perl  
793 scripts (Figure S4). All scripts and the data used to process antibody sequences are publicly  
794 available on GitHub ([https://github.com/stratust/igpipeline/tree/igpipeline2\\_timepoint\\_v2](https://github.com/stratust/igpipeline/tree/igpipeline2_timepoint_v2)).

795

796 The frequency distributions of human V genes in anti-SARS-CoV-2 antibodies from this study  
797 was compared to 131,284,220 IgH and IgL sequences generated by (Soto et al., 2019) and  
798 downloaded from cAb-Rep(Guo et al., 2019), a database of human shared BCR clonotypes  
799 available at <https://cab-rep.c2b2.columbia.edu/>. Based on the 174 distinct V genes that make up  
800 the 3085 analyzed sequences from Ig repertoire of the 6 participants present in this study, we

801 selected the IgH and IgL sequences from the database that are partially coded by the same V  
802 genes and counted them according to the constant region. The frequencies shown in (Figures 2D  
803 and S5) are relative to the source and isotype analyzed. We used the two-sided binomial test to  
804 check whether the number of sequences belonging to a specific IgHV or IgLV gene in the  
805 repertoire is different according to the frequency of the same IgV gene in the database. Adjusted  
806 p-values were calculated using the false discovery rate (FDR) correction. Significant differences  
807 are denoted with stars.

808

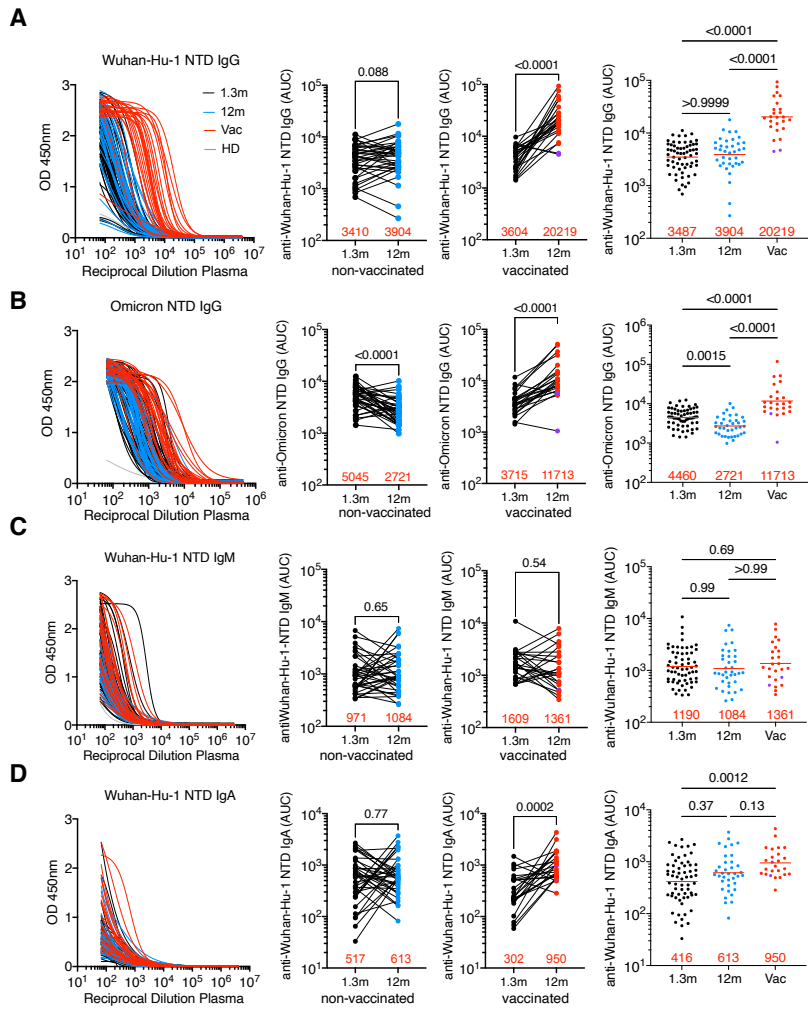
809 Nucleotide somatic hypermutation and CDR3 length were determined using in-house R and Perl  
810 scripts. For somatic hypermutations, IGHV and IGLV nucleotide sequences were aligned against  
811 their closest germlines using Igblastn and the number of differences were considered nucleotide  
812 mutations. The average mutations for V genes were calculated by dividing the sum of all  
813 nucleotide mutations across all participants by the number of sequences used for the analysis. To  
814 calculate the GRAVY scores of hydrophobicity (Kyte and Doolittle, 1982) we used Guy H.R.  
815 Hydrophobicity scale based on free energy of transfer (kcal/mole) (Guy, 1985) implemented by  
816 the R package Peptides (the Comprehensive R Archive Network repository; [https://journal.r-  
817 project.org/archive/2015/RJ-2015-001/RJ-2015-001.pdf](https://journal.r-project.org/archive/2015/RJ-2015-001/RJ-2015-001.pdf)). We used heavy chain CDR3 amino  
818 acid sequences from this study and 22,654,256 IGH CDR3 sequences from the public database  
819 of memory B cell receptor sequences (DeWitt et al., 2016). The two-tailed Wilcoxon  
820 nonparametric test was used to test whether there is a difference in hydrophobicity distribution.

821

822

823

824 **SUPPLEMENTARY FIGURES AND TABLES**

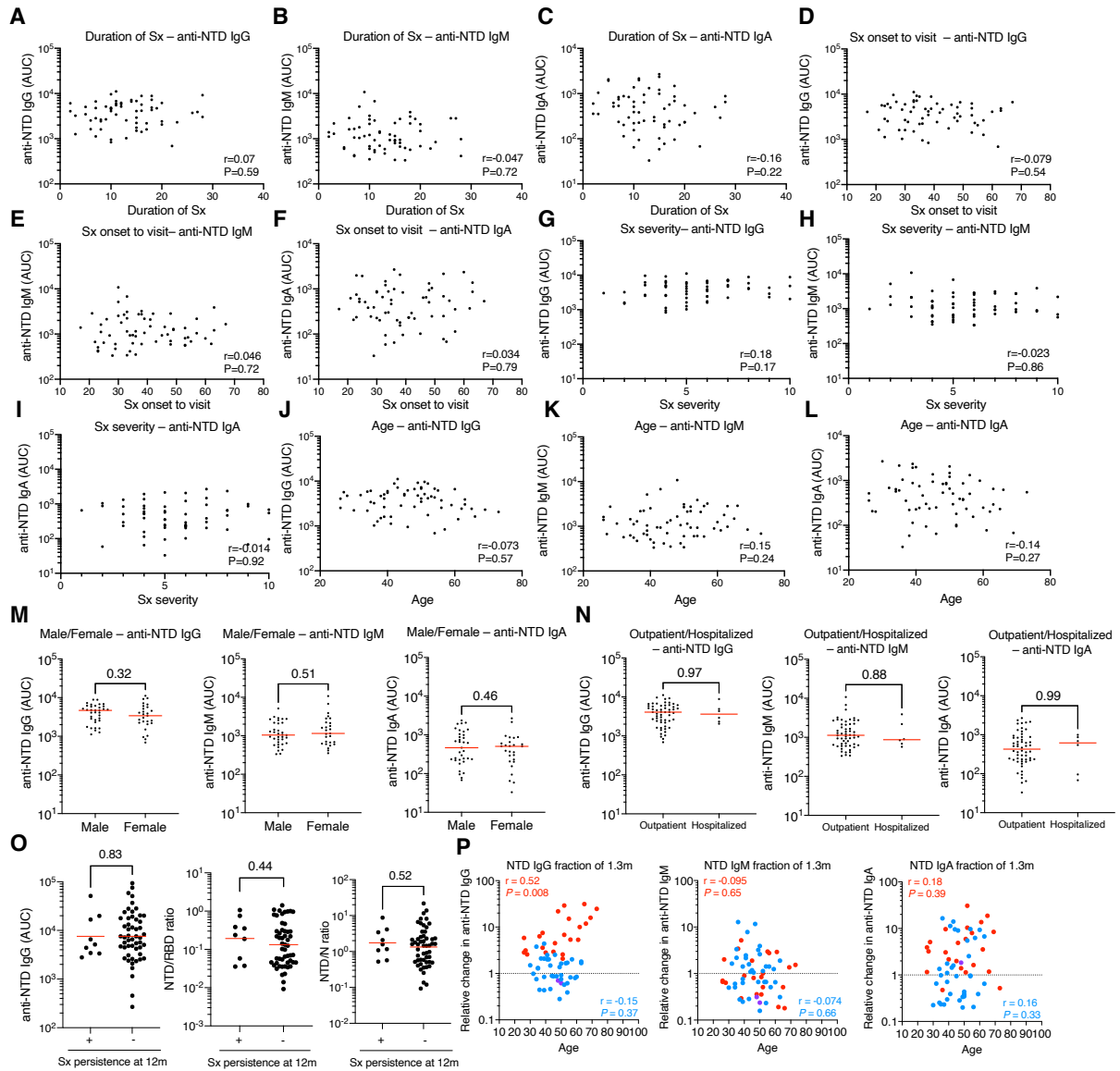


825

826 **Figure S1 Plasma anti-SARS-CoV-2 NTD reactivity**

827 **A-B**, Plasma IgG antibody binding to Wuhan-Hu-1 NTD (**A**) and Omicron NTD protein (**B**) in  
828 unvaccinated and vaccinated (vac) convalescent individuals at 1.3 month (Robbiani et al., 2020)  
829 and 12 months (Wang et al., 2021c) after infection (n=62). Graphs showing ELISA curves from  
830 individuals 1.3 months after infection (black lines), from individuals 12m after infection  
831 unvaccinated (blue lines) and vaccinated (red lines) (left panels). Area under the curve (AUC)  
832 over time in non-vaccinated and vaccinated individuals, as indicated (middle panels). Lines  
833 connect longitudinal samples. Two outliers who received their first dose of vaccine 24-48 hours  
834 before sample collection are depicted in purple. Numbers in red indicate geometric mean AUC at  
835 the indicated timepoint. **C-D**, same as **A**, shown as IgM (**C**) and IgA (**D**) antibody binding to  
836 SARS-CoV-2 NTD 1.3- and 12-months after infection. Statistical significance was determined  
837 using Wilcoxon matched-pairs signed rank tests. Right panel shows combined values as a dot  
838 plot for all individuals. Statistical significance was determined through the Kruskal Wallis test  
839 with subsequent Dunn's multiple comparisons.

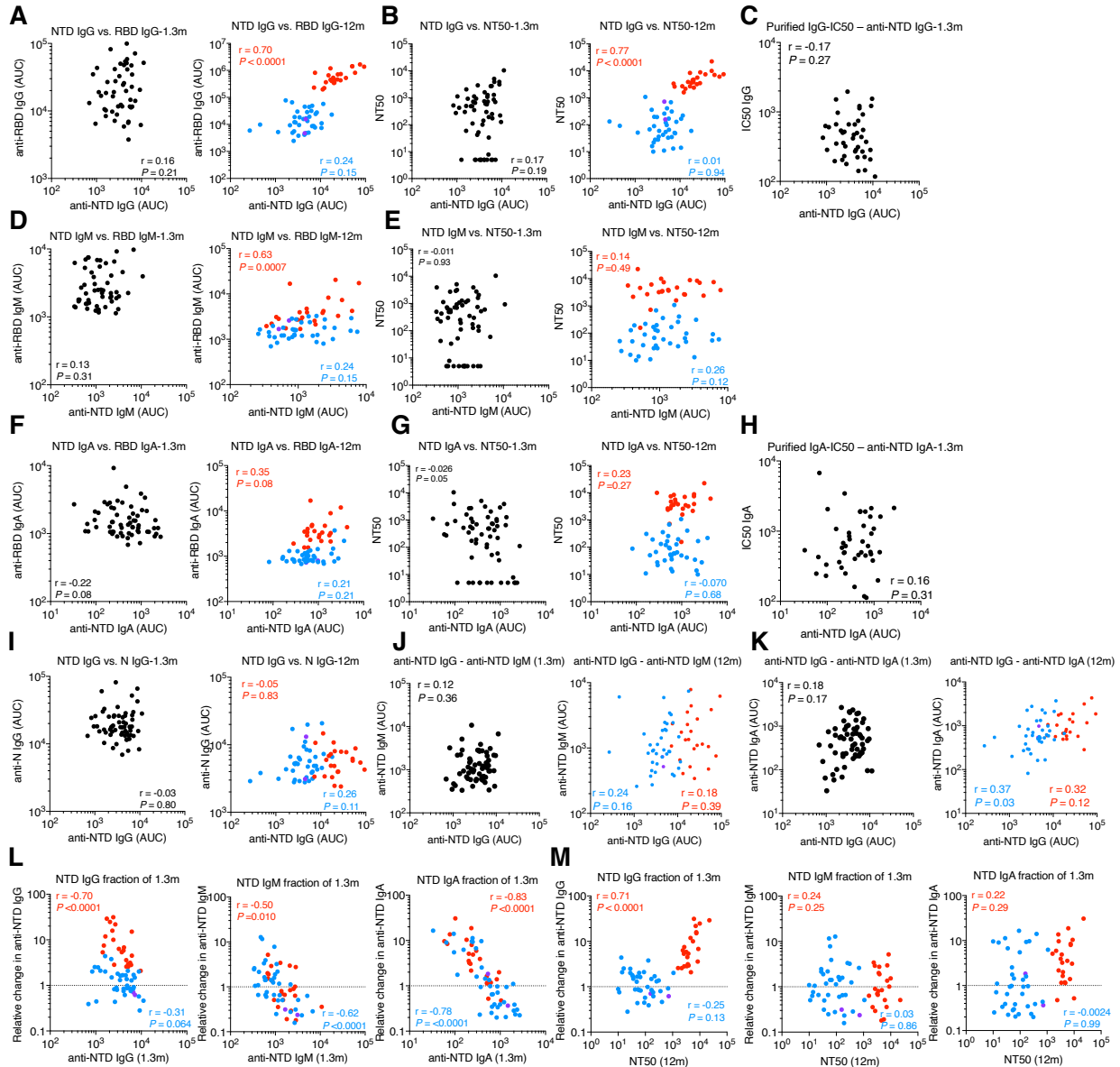




840

841 **Figure S2 Clinical correlations of plasma anti-SARS-CoV-2 NTD antibody titers**

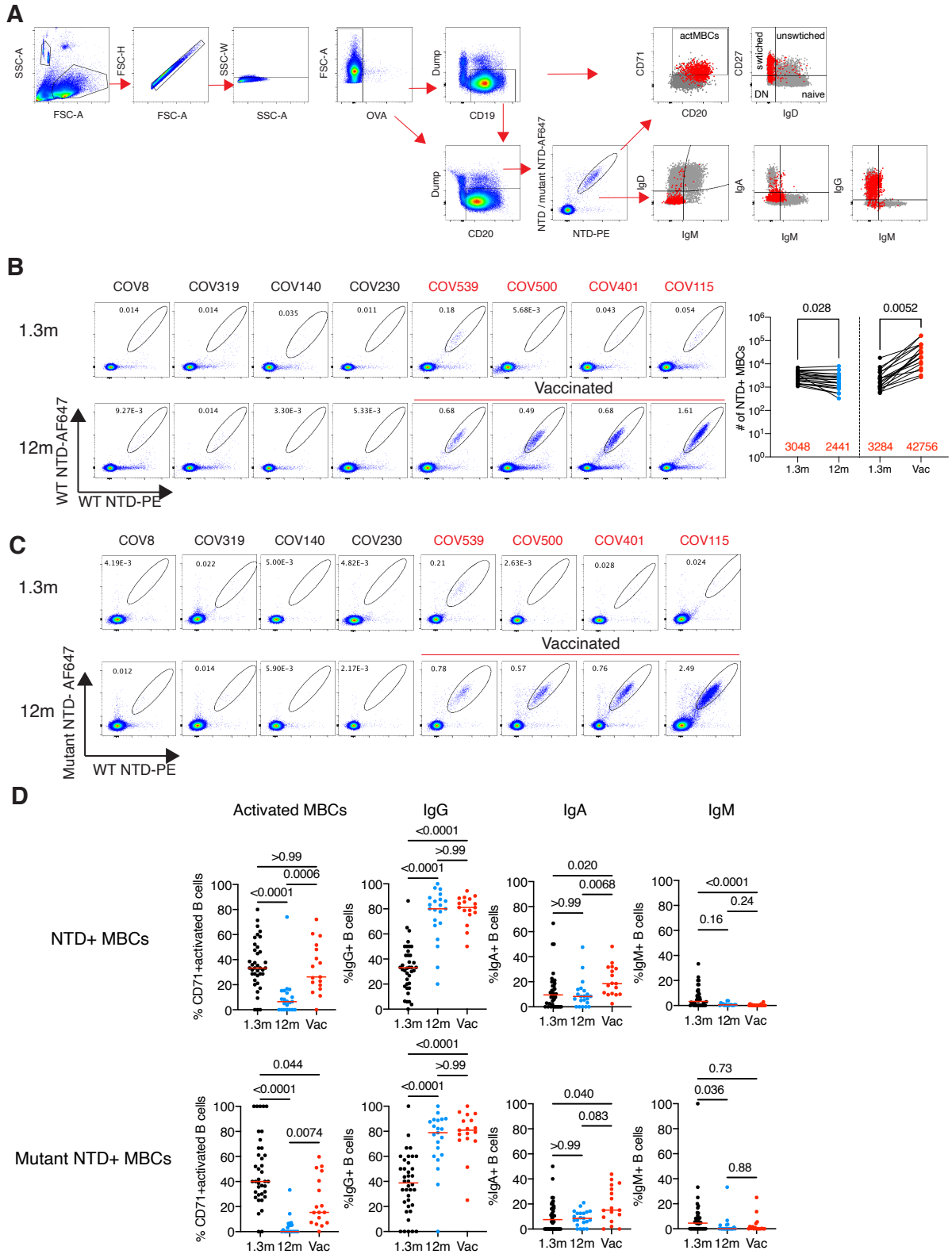
842 **A-C**, Clinical correlations of plasma anti-NTD IgG titers at 1.3month after infection(Robbiani et  
843 al., 2020). **A-C**, Duration of Symptom (Sx) in days (X axis) plotted against normalized AUC for  
844 plasma IgG (**A**), IgM (**B**) and IgA (**C**) binding to NTD (Y axis). **D-F**, Sx onset to time of sample  
845 collection in days plotted against normalized AUC for plasma IgG (**D**), IgM (**E**) and IgA (**F**) anti-  
846 NTD. **G-I**, Sx severity plotted against normalized AUC for plasma IgG (**G**), IgM (**H**) and IgA (**I**)  
847 anti-NTD. **J-L**, Age plotted against normalized AUC for plasma IgG (**J**), IgM (**K**) and IgA(**L**)  
848 anti-NTD. **M**. Normalized plasma IgG, IgM and IgA AUC for males (n=34) and females (n=28).  
849 **N**, Normalized plasma IgG, IgM and IgA AUC for outpatient (n = 56) and hospitalized (n = 6). **O**,  
850 Anti-NTD IgG, NTD/RBD IgG ratio and NTD/N IgG ratio at 12 months post-infection in  
851 individuals reporting persistent symptoms (+) compared to individuals who are symptom-free (-)  
852 12 months post-infection. **P**, Correlation of remaining plasma titers at 12 months (expressed as the  
853 fraction of 1.3-month titers on the Y axis) and participant age for anti-NTD IgG (left), anti-NTD  
854 IgM (middle), anti-NTD IgA (right). For **A-L**, **P**, the correlations were analyzed by two-tailed  
855 Spearman's tests; For **M-N**, Statistical significance was determined using two-tailed Mann-  
856 Whitney U-tests.



857

858 **Figure S3 Plasma activity**

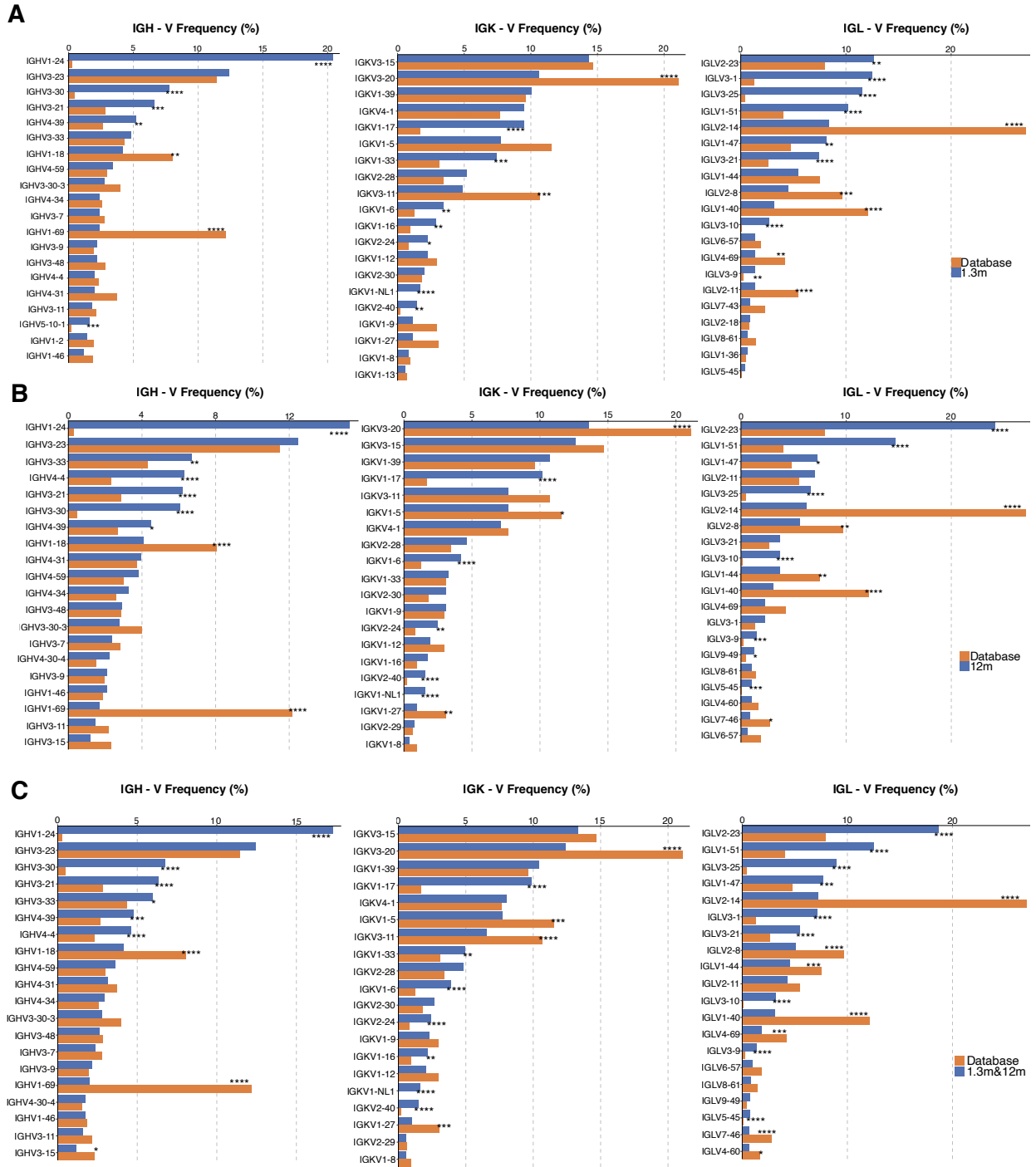
859 **A**, Correlation of 1.3- (left) and 12-month (right) titers of anti- NTD IgG and anti-RBD IgG at  
860 each timepoint, separately (Robbiani et al., 2020; Wang et al., 2021c). **B**, Correlation of 1.3- (left)  
861 and 12-month (right) titers of anti- NTD IgG and NT<sub>50</sub> at each timepoint, separately (Robbiani et  
862 al., 2020; Wang et al., 2021c). **C**, Correlation of 1.3-month plasma titers of anti-NTD IgG and  
863 purified plasma IgG IC<sub>50</sub> (Wang et al., 2021b). **D**, Correlation of 1.3-(left) and 12-month (right)  
864 plasma titers of anti-NTD IgM and anti-RBD IgM (Robbiani et al., 2020; Wang et al., 2021c). **E**,  
865 Correlation of 1.3-(left) and 12-month (right) plasma titers of anti-NTD IgM and NT<sub>50</sub> (Robbiani  
866 et al., 2020; Wang et al., 2021c). **F**, Correlation of 1.3-(left) and 12-month (right) plasma titers of  
867 anti-NTD IgA and anti-RBD IgA (Robbiani et al., 2020; Wang et al., 2021c). **G**, Correlation of  
868 1.3- (left) and 12-month (right) plasma titers of anti-NTD IgA and NT<sub>50</sub> (Robbiani et al., 2020;  
869 Wang et al., 2021c). **H**, Correlation of 1.3-month plasma titers of anti-NTD IgA and purified  
870 plasma IgA IC<sub>50</sub> (Wang et al., 2021b). **I**, Correlation of 1.3- (left) and 12-month (right) plasma  
871 titers of anti-NTD IgG and anti-N IgG (Robbiani et al., 2020; Wang et al., 2021c). **J**, Correlation  
872 of 1.3- (left) and 12-month (right) plasma titers of anti-NTD IgG and anti-NTD IgM. **K**,  
873 Correlation of 1.3- (left) and 12-month (right) plasma titers of anti-NTD IgG and anti-NTD IgA.  
874 **L**, Correlation of remaining plasma titers at 12 months (expressed as the fraction of 1.3-month  
875 titers on the Y axis) and 1.3- month plasma titers of anti-NTD IgG (left), anti-NTD IgM (middle),  
876 anti-NTD IgA (right) (Robbiani et al., 2020; Wang et al., 2021c). **M**, Correlation of remaining  
877 plasma titers of anti-NTD IgG (left), anti-NTD IgM (middle), anti-NTD IgA (right) (expressed as  
878 the fraction of 1.3-month titers on the Y axis) and plasma NT<sub>50</sub> at 12 months (Wang et al., 2021c).  
879 For **A**, **B**, **D**, **E**, **F**, **G**, **I-M**, graphs showing dot plots from individuals 1.3 months after infection  
880 (black), non-vaccinated convalescents (blue) or vaccinated convalescents (red) 12m after  
881 infection. Statistical significance was determined using the Spearman correlation test for the non-  
882 vaccinated and vaccinated subgroups independently. All experiments were performed at least in  
883 duplicate.



884

885 **Figure S4 Flow cytometry.**

886 **A**, Gating strategy. Gating was on singlets that were CD20<sup>+</sup> or CD19<sup>+</sup> and CD3<sup>-</sup>CD8<sup>-</sup>CD16<sup>-</sup>Ova<sup>-</sup>.  
887 Anti-IgG, IgM, IgA, IgD, CD71 and CD27 antibodies were used for B cell phenotype analysis. **B**  
888 and **C**, Flow cytometry showing the percentage of NTD-double positive (**B**) and AF647-mutant  
889 NTD cross-reactive (**C**) memory B cells from 1.3- and 12-months post-infection in 39 selected  
890 patients. **D**. B cell phenotyping. Expression of CD71, IgG, IgA, IgM in antigen-specific memory  
891 B cells. Each dot is one individual. Red horizontal bars indicate mean values. Statistical  
892 significance was determined using two-tailed Mann–Whitney U-tests (**B**) and Kruskal Wallis test  
893 with subsequent Dunn’s multiple comparisons (**D**).



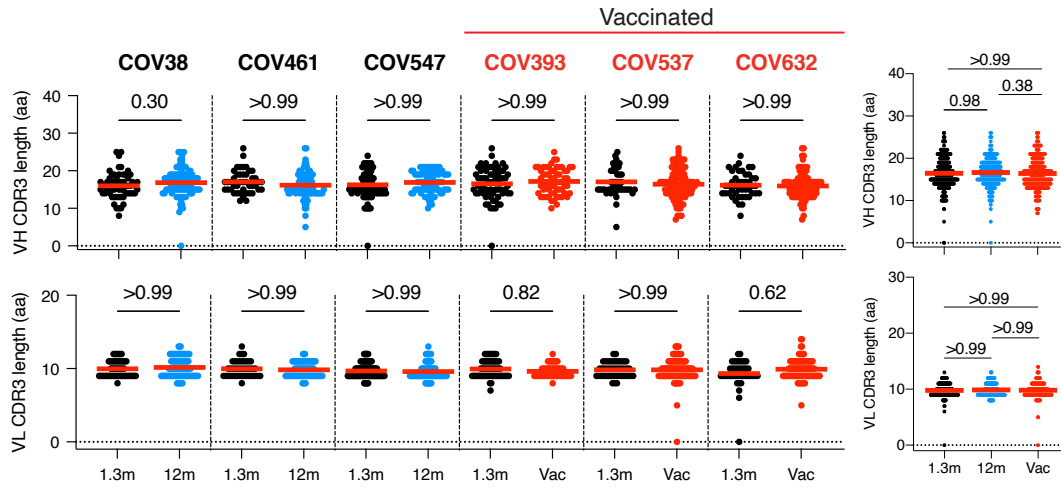
894

895 **Figure S5 Frequency distribution of human V genes.**

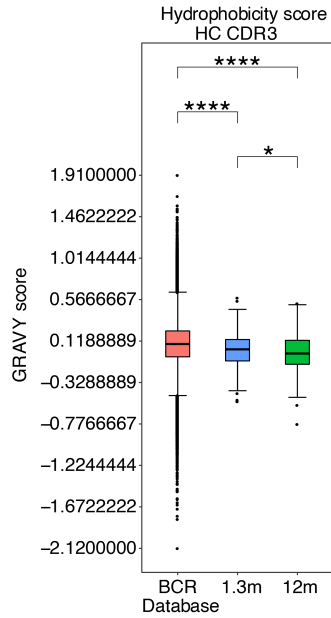
896 Comparison of the frequency distribution of human V genes for heavy chain and light chains of  
897 anti-NTD antibodies from this study and from a database of shared clonotypes of human B cell  
898 receptor generated by Cinque Soto et al(Soto et al., 2019). **A** and **B**, Graph shows relative  
899 abundance of human IGVH, IGVK and IGVL genes Sequence Read Archive accession  
900 SRP010970 (orange), 1.3- (**A**), and 12-month antibodies (**B**). **C**, Same as **A** and **B** but shows the  
901 comparison between Sequence Read Archive accession SRP010970 (orange) and combined (1.3m  
902 and 12m) anti-NTD antibodies (blue). Statistical significance was determined by two-sided  
903 binomial test. Significant differences are denoted with stars.



**A**



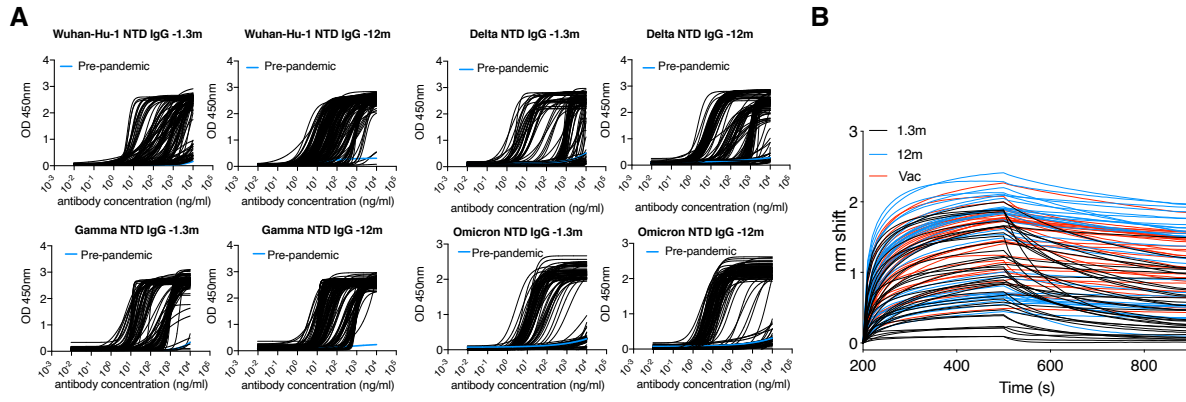
**B**



904

905 **Figure S6 CDR3 length and hydrophobicity of anti-SARS-CoV-2 NTD antibodies**

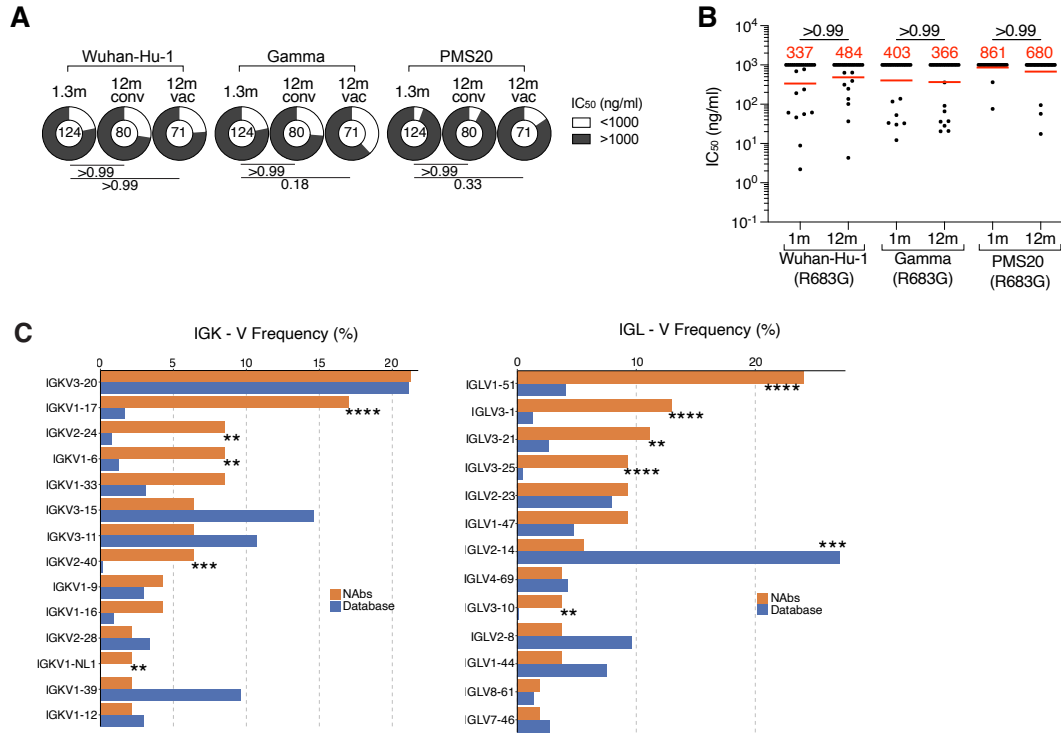
906 **A**, IGVH and IGVL CDR3s length (Y axis) for anti-SARS-CoV-2 NTD antibodies isolated from  
907 6 individuals 1.3- and 12-months after infection. Vaccinees are marked in red. Each dot represents  
908 one antibody from individuals 1.3 months after infection (black), non-vaccinated convalescents  
909 (blue) or vaccinated convalescents (red) 12m after infection. Statistical significance was  
910 determined using Kruskal Wallis test with subsequent Dunn's multiple comparisons. **B**,  
911 Distribution of the hydrophobicity GRAVY scores at the IGH CDR3 in antibody sequences from  
912 this study compared to a public database (see Methods for statistical analysis). The box limits are  
913 at the lower and upper quartiles, the center line indicates the median, the whiskers are 1.5x  
914 interquartile range and the dots represent outliers.  
915



916

917 **Figure S7 Binding curves of anti-SARS-CoV-2 NTD monoclonal antibodies**

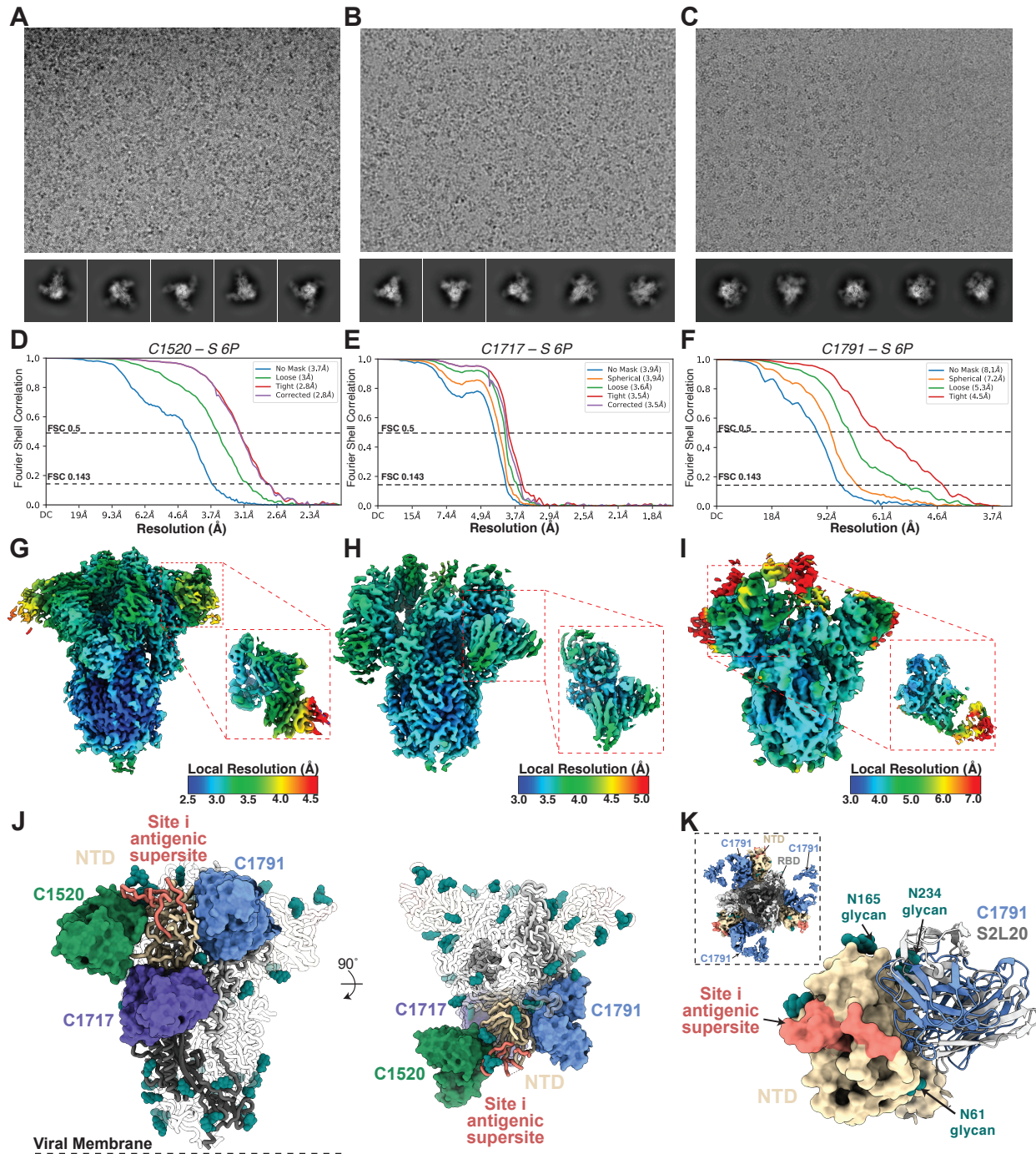
918 **A**, ELISA binding curves of mAbs isolated from convalescents individuals at 1.3- and 12 months  
919 after infection, EC<sub>50</sub>s against SARS-CoV-2 Wuhan-Hu-1-, Delta-, Gama- or Omicron-NTD were  
920 shown. **B**, Biolayer interferometry association and dissociation curves for 1.3- (black), 12-month  
921 convalescents (blue lines), and 12-month convalescent vaccinees (red lines).



922

923 **Figure S8 anti-SARS-CoV-2 NTD monoclonal antibodies**

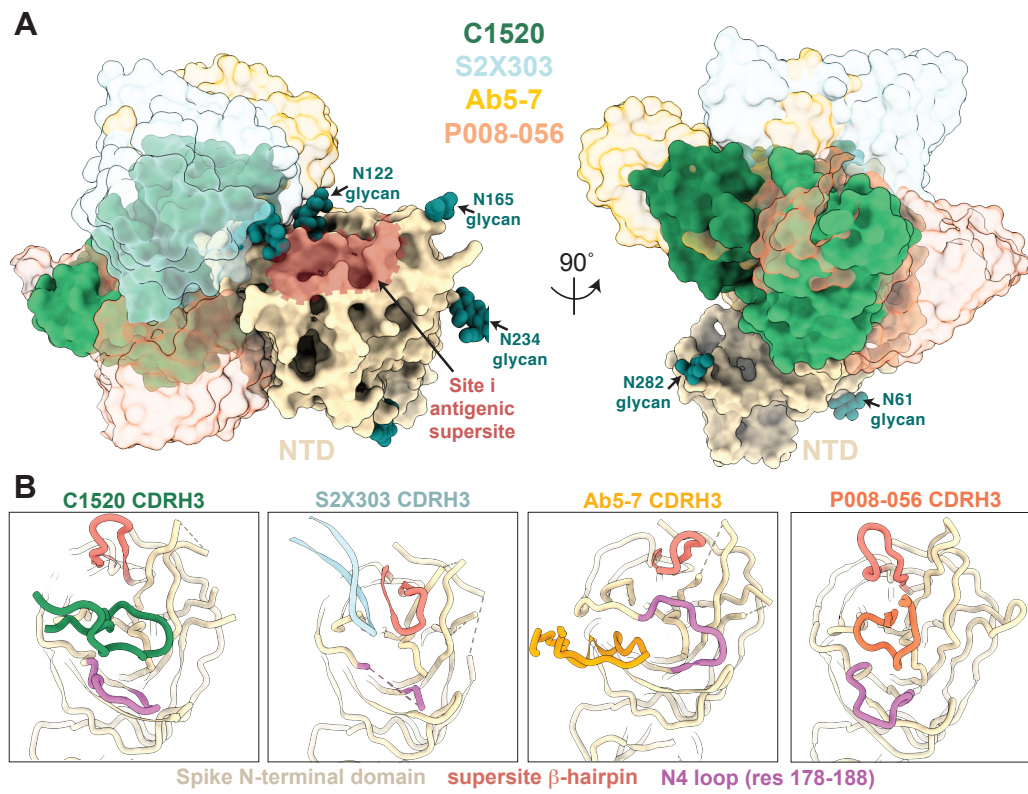
924 **A**, Corresponding to Figure 3A, Pie charts illustrate the fraction of neutralizing (white slices) and  
925 non-neutralizing (dark grey slices) antibodies, inner circle shows the number of antibodies tested  
926 per group. Statistical significance was determined with Fisher's exact test with subsequent  
927 Bonferroni-Dunn correction. **B**, Graph shows anti-SARS-CoV-2 neutralizing activity of shared  
928 clones of antibodies isolated 1.3- and 12-months after infection, measured by a SARS-CoV-2  
929 pseudovirus neutralization assay (Robbiani et al., 2020; Schmidt et al., 2020). Statistical  
930 significance was determined through Friedman test with subsequent Dunn's multiple comparisons.  
931 Horizontal bars and red numbers indicate geometric mean values. **C**, Graph shows comparison of  
932 the frequency distributions of human IGK and IGL V genes of anti-SARS-CoV-2 NTD  
933 neutralizing antibodies from donors at 1.3 month (Robbiani et al., 2020) and 12 months (Wang et  
934 al., 2021c) after infection. Statistical significance was determined by two-sided binomial test.



936 **Figure S9 Cryo-EM data processing and validation**

937 **A-F**, Representative micrographs selected from total datasets (see Table S5), 2D class averages,  
938 and gold-standard FSC plots for **A,D** C1520-S 6P; **B,E** C1717-S 6P; and **C,F** C1791-S 6P. **G-I**,  
939 Local resolution estimations for **G**, C1520-S, **H**, C1717-S, and **I**, C1791-S complexes. Insets show  
940 resolution estimates for Fab-NTD focused refinements. **J**, Composite model illustrating non-  
941 overlapping epitopes of NTD-targeting mAbs (C1520 – green; C1717 – purple; C1791 – slate  
942 blue) bound to a single NTD (wheat) on a spike trimer. The site i antigenic supersite (coral) is  
943 shown as reference. **K**, Structural superposition of NTD-targeting antibodies C1791 (slate blue)  
944 and S2L20 (gray – PDB 7N8I) on a NTD (wheat surface rendering). The NTD site i antigenic  
945 supersite is depicted as in panel **J**. Cryo-EM density for the C1791-S is also shown (inset).





947 **Figure S10 Comparison of C1520-like NTD neutralizing antibodies**

948 **A**, Overlay of V<sub>H</sub> and V<sub>L</sub> domains of C1520 (green), S2X303 (PDB 7SOF; powder blue), Ab5-7  
949 (PDB 7RW2; gold), and P008-056 (PDB 7NTC; orange) after alignment on NTD residues 27-303,  
950 illustrating distinct binding poses to NTD epitopes adjacent to the site i supersite (coral). **B**,  
951 Comparison of CDRH3 loop position, the NTD supersite b-hairpin (coral), and NTD N4-loop  
952 (orchid) for antibodies shown in panel **A**.

953

954

955

956 **SUPPLEMENTARY TABLES**

957 **Table S1: Individual participant characteristics**

958 **Table S2: Antibody sequences from patients is provided as a separate Excel file.**

959 **Table S3: Sequences, half maximal effective concentrations (EC50s) and inhibitory**

960 **concentrations (IC50s) of the cloned monoclonal antibodies is provided as a separate Excel**  
961 **file.**

962 **Table S4: V gene usage and neutralization activity of anti-NTD nAbs.**

963 **Table S5: Cryo-EM data collection and processing statistics**

964

965

966 **Reference**

967 Adams, P.D., Afonine, P.V., Bunkoczi, G., Chen, V.B., Davis, I.W., Echols, N., Headd, J.J., Hung,  
968 L.W., Kapral, G.J., Grosse-Kunstleve, R.W., *et al.* (2010). PHENIX: a comprehensive Python-based  
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