

1 **Antibody feedback regulation of memory B cell development in SARS-CoV-2 mRNA**  
2 **vaccination**

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7 Dennis Schaefer-Babajew<sup>1\*</sup>, Zijun Wang<sup>1\*</sup>, Frauke Muecksch<sup>2\*</sup>, Alice Cho<sup>1\*</sup>, Raphael Raspe<sup>1</sup>,  
8 Brianna Johnson<sup>1</sup>, Marie Canis<sup>2</sup>, Justin DaSilva<sup>2</sup>, Victor Ramos<sup>1</sup>, Martina Turroja<sup>1</sup>, Katrina G.  
9 Millard<sup>1</sup>, Fabian Schmidt<sup>2</sup>, Juan Dizon<sup>1</sup>, Irina Shimelovich<sup>1</sup>, Kai-Hui Yao<sup>1</sup>, Thiago Y. Oliveira<sup>1</sup>,  
10 Anna Gazumyan<sup>1,4</sup>, Christian Gaebler<sup>1</sup>, Paul D. Bieniasz<sup>2,4</sup>, Theodora Hatziioannou<sup>2</sup>, Marina  
11 Caskey<sup>1</sup> and Michel C. Nussenzweig<sup>1,4</sup>

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15 <sup>1</sup>Laboratory of Molecular Immunology, The Rockefeller University, New York, NY 10065, USA

16 <sup>2</sup>Laboratory of Retrovirology, The Rockefeller University, New York, NY 10065, USA

17 <sup>4</sup>Howard Hughes Medical Institute, New York, NY, USA  
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21

22 \*equal contribution

23 Address correspondence to: Paul D. Bieniasz [pbieniasz@rockefeller.edu](mailto:pbieniasz@rockefeller.edu); Theodora

24 Hatziioannou [thatzio@rockefeller.edu](mailto:thatzio@rockefeller.edu); Marina Caskey [mcaskey@rockefeller.edu](mailto:mcaskey@rockefeller.edu); or Michel C.

25 Nussenzweig [nussen@rockefeller.edu](mailto:nussen@rockefeller.edu)

## 26 Abstract

27

28 **Feedback inhibition of humoral immunity by antibodies was initially documented in guinea**  
29 **pigs by Theobald Smith in 1909, who showed that passive administration of excess anti-**  
30 **Diphtheria toxin inhibited immune responses<sup>1</sup>. Subsequent work documented that**  
31 **antibodies can enhance or inhibit immune responses depending on antibody isotype, affinity,**  
32 **the physical nature of the antigen, and engagement of immunoglobulin (Fc) and complement**  
33 **(C') receptors<sup>2,3</sup>. However, little is known about how pre-existing antibodies might influence**  
34 **the subsequent development of memory B cells. Here we examined the memory B cell**  
35 **response in individuals who received two high-affinity IgG1 anti-SARS-CoV-2 receptor**  
36 **binding domain (RBD)-specific monoclonal antibodies, C144-LS and C135-LS, and**  
37 **subsequently two doses of a SARS-CoV-2 mRNA vaccine. The two antibodies target Class 2**  
38 **and 3 epitopes that dominate the initial immune response to SARS-CoV-2 infection and**  
39 **mRNA vaccination<sup>4-8</sup>. Antibody responses to the vaccine in C144-LS and C135-LS recipients**  
40 **produced plasma antigen binding and neutralizing titers that were fractionally lower but not**  
41 **statistically different to controls. In contrast, memory B cells enumerated by flow cytometry**  
42 **after the second vaccine dose were present in higher numbers than in controls. However, the**  
43 **memory B cells that developed in antibody recipients differed from controls in that they were**  
44 **not enriched in VH3-53, VH1-46 and VH3-66 genes and predominantly expressed low-**  
45 **affinity IgM antibodies that carried small numbers of somatic mutations. These antibodies**  
46 **showed altered RBD target specificity consistent with epitope masking, and only 1 out of 77**  
47 **anti-RBD memory antibodies tested neutralized the virus. The results indicate that pre-**  
48 **existing high-affinity antibodies bias memory B cell selection and have a profound effect on**  
49 **the development of immunological memory in humans that may in part explain the shifting**  
50 **target profile of memory antibodies elicited by the 3<sup>rd</sup> mRNA vaccine dose.**

51

## 52 Results

### 53 Study design & cohorts

54 To examine how passive administration of monoclonal antibodies (mAbs) might influence  
55 subsequent humoral responses to vaccination, we studied a group of 18 healthy volunteers who  
56 received a single dose of the combination of two long-acting monoclonal antibodies to SARS-

57 CoV-2 and subsequently received 2 doses of a SARS-CoV-2 mRNA vaccine (Fig 1a). The 2  
58 antibodies, C144-LS and C135-LS, bind Class 2 and 3 epitopes on the receptor binding domain  
59 (RBD) of the SARS-CoV-2 spike (S) protein with high affinity ( $K_D = 18$  nM and  $K_D = 6$  nM,  
60 respectively) and neutralize the virus with IC50s of 2.55 and 2.98 ng/ml, respectively<sup>5,8</sup>.

61  
62 Between January 13 and March 3, 2021, 23 SARS-CoV-2-naïve individuals received C144-LS  
63 and C135-LS (n = 21) or placebo (n = 2), in a first-in-human, phase 1 clinical trial at the Rockefeller  
64 University Hospital (NCT04700163). The antibodies were modified to extend their half-life by  
65 introducing the M428L and N343S mutations into their Fc domains<sup>9</sup> (LS). Individuals received a  
66 single dose of C144-LS and C135-LS IgG1 antibodies at a 1:1 ratio, starting with 100 mg of each  
67 subcutaneously (s.c.) in the lowest- and up to 15 mg/kg intravenously (i.v.) in the highest-dose  
68 group. Participants were followed longitudinally to assess the safety and tolerability of the infused  
69 mAbs and determine their pharmacokinetic properties.

70  
71 Eighteen of the 21 phase 1 study participants who had received the monoclonal antibodies elected  
72 to receive SARS-CoV-2 mRNA vaccination and volunteered to enroll in a parallel observational  
73 study assessing their immune responses to SARS-CoV-2 vaccination (Ext. Data Tables S1 and  
74 S2). The first and second vaccine doses were administered a median of 82 (range 42-110) and 103  
75 (range 70-131) days after antibody administration (Ext. Data Tables S1 and S2). At the time of  
76 vaccination, the plasma levels of C144-LS and C135-LS were between 5 and 100 µg/ml depending  
77 on the dosing group (Fig 1b). The estimated half-lives of C144-LS and C135-LS were 69-99 days  
78 and 73-95 days, respectively.

79  
80 The 18 vaccinated antibody recipients were compared to a cohort of 31 randomly selected mRNA  
81 vaccine recipients with no prior history of infection (Fig 1a and Ext. Data Tables S1, also see<sup>10,11</sup>).  
82 Both groups were sampled between 13-28 (median 19) and 15-91 (median 29) days after their first  
83 and second vaccine doses, respectively. The two cohorts were relatively matched for demographic  
84 characteristics and vaccine formulation (for details see Ext. Data Tables S1 and S2) and none of  
85 the individuals included in the study seroconverted to nucleocapsid (N) at any time during  
86 observation period suggesting that they remained infection naïve.

87

## 88 **Plasma antibody reactivity**

89 Plasma IgM and IgG antibody binding activity against RBD were measured by ELISA using  
90 Wuhan-Hu-1 (WT) and mutant forms of RBD (R346S/E484K and N440K/E484K) that eliminate  
91 binding by C144 and C135 but not Class 1 or 4, or some affinity-matured Class 2 or 3 antibodies  
92 (Ext. Data Fig 1, also see<sup>11,12</sup>). When measured for WT RBD binding after one or two vaccine  
93 doses, the IgM titers in mAb recipients were not significantly different from controls (Fig. 1c). In  
94 contrast, IgG anti-WT-RBD titers were significantly higher in mAb recipients than in controls after  
95 one vaccine dose but equalized following the second dose (Fig. 1d,  $p < 0.0001$  and  $p = 0.93$ ,  
96 respectively). The initial difference was attributed to the infused monoclonals because when the  
97 same samples were tested against either R346S/E484K or N440K/E484K mutant RBDs that  
98 interfere with C144 and C135 binding, plasma IgG antibody levels in the mAb recipient samples  
99 were slightly but not significantly lower than the controls (Fig. 1e).

100

101 To determine whether the pre-existing antibodies to RBD interfered with humoral immunity to an  
102 independent domain of the SARS-CoV-2 S protein, the same plasma samples were tested for  
103 binding to the N-terminal domain (NTD). IgG titers to NTD were similar in mAb recipients and  
104 controls (Fig. 1f). We conclude that high circulating levels of C144-LS and C135-LS do not  
105 interfere with IgM anti-RBD antibody responses and have only a small effect on IgG responses.  
106 Thus, the infused antibodies do not clear the vaccine antigen or measurably interfere with its  
107 overall ability to produce an immune response<sup>13</sup>.

108

## 109 **Neutralization**

110 To assess plasma neutralizing activity, we used HIV-1 pseudotyped with WT or mutant S proteins  
111 that carry a furin-cleavage site mutation (R683G)<sup>14</sup>. As expected, based on the amount of C144-  
112 LS and C135-LS in circulation, neutralizing titers against WT were significantly higher in mAb  
113 recipients than in controls at both timepoints (Fig. 1g,  $p < 0.0001$ ). To determine the contribution  
114 of the endogenous neutralizing response to epitopes outside of the C144-LS and C135-LS target  
115 sites, we used viruses pseudotyped with R346S/Q493K and R346S/N440K/E484K mutations that  
116 abolish the neutralizing activity of the 2 infused mAbs (Ext. Data Fig. 2). Despite the initial  
117 dominance of Class 1-2 epitopes among neutralizing antibodies elicited by mRNA vaccination<sup>6</sup>,  
118 the neutralizing titers of the control plasmas against the 2 mutant pseudoviruses were comparable

119 to those against WT (Fig. 1h-i), suggesting that a significant proportion of circulating endogenous  
120 neutralizing antibodies are unaffected by the R346S/Q493K and R346S/N440K/E484K mutations.  
121 After the first vaccine dose, mAb recipients showed significantly lower neutralizing titers against  
122 the mutant pseudoviruses than controls (2.7- and 3.5-fold for R346S/Q493K and  
123 R346S/N440K/E484K; Fig. 1h and i,  $p=0.0015$  and  $p=0.014$ , respectively). Consistent with a  
124 recent report<sup>13</sup>, neutralizing activity improved and was no longer significantly different from  
125 controls after the second vaccine dose (Fig 1h and i).

126  
127 In conclusion, recipients of C144-LS and C135-LS had high initial levels of serum neutralizing  
128 activity due to the passively administered antibodies and they developed their own neutralizing  
129 antibodies that were not sensitive to RBD mutations in the C144/C135 target sites after mRNA  
130 vaccination.

131  
132 **Memory B cells**

133 In addition to the plasma cells that produce circulating antibodies, vaccination also elicits memory  
134 B cells that contribute to protection upon re-exposure to the pathogen. Although the feedback  
135 effects of antibodies on humoral responses have been investigated extensively beginning in  
136 1909<sup>1,2</sup>, little is known about their effects on the development of memory B cells. To investigate  
137 the effects of passive mAb administration on B cell memory responses, we used flow cytometry  
138 to enumerate and purify circulating memory B cells binding to phycoerythrin (PE) and Alexa-  
139 Fluor-647 (AF647) labeled RBDs (Ext. Data Fig 3a-c)<sup>5</sup>. mRNA vaccination elicited robust RBD-  
140 specific memory B cell responses in mAb recipients that were approximately 4- and 3-fold higher  
141 than in controls after the first and second vaccine doses, respectively (Fig 2a;  $p<0.0001$  and  
142  $p<0.0001$ , respectively). Thus, C144-LS and C135-LS administration increases the magnitude of  
143 the anti-RBD memory B cell response when compared to controls.

144  
145 Human memory B cells represent a diverse pool of cells that can develop in germinal centers  
146 (GCs) or through an extrafollicular GC-independent pathway<sup>15-17</sup>. Memory B cells expressing  
147 class-switched and highly somatically mutated antibodies are primarily of germinal center  
148 origin<sup>16,18</sup>. IgM-expressing memory B cells, that express antibodies that carry only small  
149 numbers of mutations typically develop by a germinal center independent pathway<sup>19-22</sup>. In

150 control individuals, IgG-expressing RBD-specific memory cells comprised the majority of the  
151 memory B cell pool at both time points assayed with many fewer such cells found in mAb  
152 recipients ( $p=0.0038$  and  $p<0.0001$ , Fig 2b). Consistent with the relative decrease in IgG<sup>+</sup>  
153 memory B cells, nearly two thirds (65%) of the RBD-specific memory B cells from mAb  
154 recipients were cell surface IgM<sup>+</sup> after the first vaccine dose and this decreased only slightly to  
155 54% after the second vaccine dose (Fig 2c). We conclude that pre-existing high-affinity anti-  
156 RBD antibodies alter the immune response to SARS-CoV-2 mRNA vaccination to favor the  
157 development of IgM-expressing memory B cells.

158

### 159 **Memory B cell Antibodies**

160 To gain further insight into the effects of pre-existing antibodies on the human memory response  
161 to SARS-CoV-2 mRNA vaccination, we purified RBD-specific memory B cells from 5 mAb  
162 recipients after the second vaccine dose. A total of 353 and 856 paired antibody sequences from  
163 mAb recipients and previously characterized controls were examined, respectively (Fig 2d, Ext.  
164 Data Fig. 3d-f and Ext. Data Table 3, also see<sup>10</sup>). IgM transcripts accounted for 70-94% of  
165 sequences recovered from mAb recipients with an average of 9% belonging to expanded clones  
166 (Fig 2d, upper panel and Fig 2e). In contrast, IgG transcripts accounted for >90% of the  
167 immunoglobulin sequences isolated from controls (Fig 2e and Ext. Data Fig 3f). IgM memory cells  
168 originating from the extrafollicular non-GC pathway are generally less somatically mutated than  
169 IgG memory cells because they undergo fewer divisions<sup>16,22</sup>. Consistent with this idea, and the  
170 reversed ratio of IgM:IgG memory B cells in mAb recipients, the antibodies obtained from these  
171 individuals showed significantly fewer somatic mutations than controls (Fig. 2f,  $p<0.0001$ ).  
172 However, when comparing IgM or IgG cells independently, the average mutational burden was  
173 not significantly different between mAb recipients and controls (Fig 2g,  $p>0.99$  and  $p=0.40$  for  
174 IgM and IgG, respectively). Thus, IgM- and IgG-expressing B cells in vaccinated individuals who  
175 had received C144-LS and C135-LS carry normal numbers of somatic mutations, but the relative  
176 ratio of the two memory cell types is reversed, which accounts for the overall lower level of  
177 mutation in the memory compartment. Finally, in contrast to controls there was no enrichment for  
178 VH3-53, VH1-69, VH1-46 and VH3-66 heavy chains, which often target Class 1 and 2 epitopes.  
179 Instead, there was relative enrichment for VH3-9, VH5-51, VH4-39 and VH1-8 genes (Ext Data  
180 Fig. 4a). In summary, the data suggest that pre-existing antibodies can alter the cellular and

181 molecular composition of the RBD-specific MBC compartment that develops in response to  
182 mRNA vaccination.

183  
184 To examine the binding and neutralizing activity of the memory antibodies elicited by mRNA  
185 vaccination in C144-LS and C135-LS recipients, we produced 178 representative monoclonals  
186 obtained from 5 individuals as IgGs and tested them for binding to the WT SARS-CoV-2 RBD by  
187 ELISA (Fig. 3a-c and Ext. Data Table 4). In contrast to controls, where over 95% of the memory  
188 antibodies bound strongly to RBD, monoclonal antibodies isolated from volunteers that received  
189 C144-LS and C135-LS showed diverse levels of binding activity. Approximately one quarter  
190 (24%) of the antibodies displayed relatively poor binding with ELISA half-maximal effective  
191 concentrations (EC50s) that were only slightly above our limit of detection, and a little over one  
192 third (38%) showed no detectable binding above background (Fig. 3a-b). Accordingly, the median  
193 (EC50) of antibodies isolated from mAb recipients was significantly higher than in controls (Fig.  
194 3b,  $p < 0.0001$ ). Notably, this difference remained significant when the monoclonals isolated from  
195 IgM and IgG memory cells were analyzed independently (Fig 3c,  $p = 0.0005$  and  $p < 0.0001$ ,  
196 respectively).

197  
198 Memory antibodies obtained from C144-LS and C135-LS recipients that bound to WT SARS-  
199 CoV-2 RBD with EC50s  $< 10 \mu\text{g/ml}$  were tested for neutralizing activity against viruses  
200 pseudotyped with WT spike. Whereas almost two thirds (63%) of the IgG and 17% of the IgM  
201 antibodies isolated from controls showed measurable neutralizing activity, only 1 out of 45 IgG  
202 and none of the 32 IgM antibodies obtained from C144-LS and C135-LS recipients neutralized  
203 SARS-CoV-2 (Fig. 3d-e). Thus, the antibodies isolated from the RBD-specific memory B cell  
204 compartment of vaccinated mAb recipients show significantly less neutralizing activity than  
205 controls.

206  
207 To examine the affinity of the antibodies, we performed biolayer interferometry experiments (BLI)  
208 in which monoclonal antibodies were immobilized on the biosensor chip and exposed to WT RBD  
209 monomers<sup>5</sup> (Fig. 4a and b). In contrast to controls, where 96% of the antibodies tested displayed  
210 measurable affinities, only two thirds (67%) of the antibodies derived from mAb recipients did so  
211 (Fig. 4b, d and f,  $p < 0.0001$ ). When all antibodies were considered together, the median affinity



212 (Kd) differed by nearly one order of magnitude between mAb recipients and controls (Fig. 4f,  
213  $p < 0.0001$ ). Moreover, this difference remained significant when IgM and IgG monoclonals were  
214 considered independently (Fig 4g,  $p = 0.0058$  and  $p < 0.0001$ , respectively), indicating that the lower  
215 affinities observed in the memory compartment of mAb recipients cannot solely be explained by  
216 the preponderance of IgM.

217  
218 C144-LS and C135-LS have the potential to form immune complexes with the vaccine antigen *in*  
219 *vivo* and present it as a multimer that could increase the apparent affinity of a B cell for the  
220 multimerized antigen by avidity effects. To determine whether memory cell-derived antibodies  
221 from mAb recipients with no apparent affinity to monomeric antigen would show binding under  
222 higher valency conditions, we exposed the immobilized monoclonals to biotin-streptavidin  
223 tetramerized trimers of S (Fig. 4a, c and e). Of the 25 antibodies with no apparent monomeric  
224 binding tested, 23 (92%) bound to multimerized S (Fig. 4c and e). We conclude that most of the  
225 anti-RBD antibodies isolated from mAb recipients that failed to show detectable binding to RBD  
226 monomers bind to multimerized antigen.

227  
228 To examine the epitopes targeted by the vaccine-elicited anti-RBD antibodies produced by  
229 memory B cells of C144-LS and C135-LS recipients, we performed BLI experiments in which a  
230 pre-formed antibody-RBD complex composed of one of 4 structurally characterized  
231 antibodies<sup>8,23,24</sup> was exposed to a second monoclonal targeting an unknown epitope (Fig 4h and  
232 Ext. Data Fig. 5). 49% of the anti-RBD memory antibodies obtained from vaccinated controls  
233 target Class 1, 2 or 3 epitopes or combinations thereof (Fig. 4i, Ext. Data Fig 5a-e, and <sup>10,11</sup>). In  
234 contrast, only 20% of the memory antibodies obtained from mAb recipients targeted Class 1 or 2  
235 epitopes, and none were Class 3-specific. Instead, we found that 78% of these antibodies targeted  
236 either Class 4-containing epitopes or epitopes that could not be classified by our method (Fig. 4i  
237 and Ext. Data Fig 5a-e). Thus, there was a significant shift in the distribution of epitopes targeted  
238 by memory antibodies isolated from mAb recipients compared to controls (Fig. 4i,  $p = 0.0089$ ). In  
239 conclusion, C144-LS and C135-LS alter the development of memory B cells expressing anti-RBD  
240 antibodies and their epitope target preference.

241



242

## 243 **Discussion**

244 Our experiments show that pre-existing antibodies alter the development of memory B cells in  
245 response to SARS-CoV-2 mRNA vaccination in humans. Consistent with a recent report, C144-  
246 LS and C-135-LS did not significantly interfere with the development of circulating antibodies  
247 that bind to epitopes outside of the target sites of the two monoclonal antibodies<sup>13</sup>. And while we  
248 found that neutralizing responses were reduced in mAb recipients after one dose, this difference  
249 was no longer significant after two doses of mRNA vaccination. In contrast, anti-RBD specific  
250 memory B cell development was profoundly altered. Memory cells expressing IgG antibodies  
251 specific to Class 1, 2 or 3 epitopes normally dominate the anti-RBD response after 2 doses of  
252 mRNA vaccination<sup>4-8</sup>. In contrast, mAb recipients develop increased numbers of anti-RBD  
253 memory cells that express low-affinity IgM antibodies with altered epitope specificity.

254

255 Beginning with experiments on anti-Diphtheria toxin antibodies in the early part of the 20<sup>th</sup> century,  
256 extensive work in experimental animals showed that passive transfer of polyclonal immune serum  
257 or monoclonal antibodies can alter subsequent humoral immune responses in an epitope-specific  
258 manner<sup>1,2</sup>. However, the effect of pre-existing antibodies on the development of memory B cells  
259 is not well understood. C144 and C135 bind to Class 2 and 3 epitopes on the RBD and interfere  
260 with the development of memory responses to the normally dominant classes of antibodies by  
261 what appears to be epitope masking. Epitope masking may also explain the shift in memory B cell  
262 specificity away from Class 1 and 2 after the 3<sup>rd</sup> dose of the SARS-CoV-2 mRNA vaccines that  
263 increases the breadth of the neutralizing response<sup>11</sup>. In chronic infections, such as HIV-1, masking  
264 could account for the shift from initial anti-gp41 responses to other parts of the trimer<sup>25,26</sup> and it  
265 could interfere with the development of broad neutralizing responses to HIV-1 or influenza by  
266 shifting memory responses away from conserved epitopes<sup>27</sup>.

267

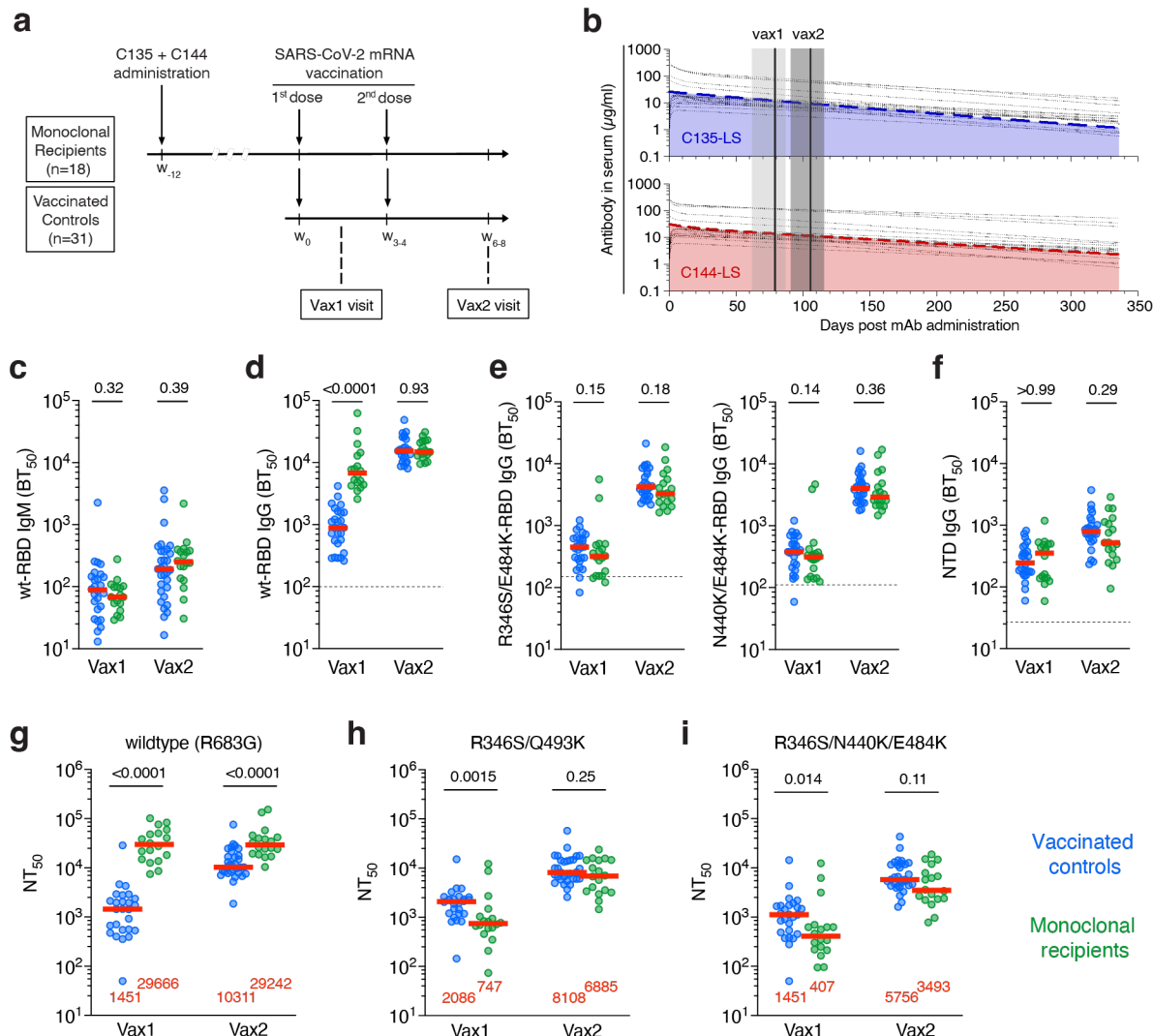
268 Memory B cells can develop by 2 different pathways<sup>15,16</sup>. Class-switched memory B cells that  
269 carry relatively higher-affinity antibodies with large numbers of somatic mutations develop in  
270 germinal centers (GCs). In contrast, IgM-expressing memory B cells that carry lower-affinity  
271 antibodies and only small numbers of mutations develop by a GC-independent pathway<sup>16,17</sup>.  
272 Passive transfer of C144-LS and C135-LS may favor the GC-independent pathway by creating

273 immune complexes that contain multiple copies of the antigen in a form that increases apparent  
274 affinity by avidity effects, thereby enabling recruitment of B cells with very low affinity receptors  
275 into the immune response. A reduction in selection stringency may also explain the greater total  
276 number of RBD-binding cells found in mAb recipients. In addition, pre-existing high affinity  
277 antibodies may alter secondary GC responses by favoring entry of cells expressing lower-affinity  
278 antibodies that target a further diversified group of epitopes. In contrast, low-affinity polyclonal  
279 antibodies emerging after vaccine priming may enhance vaccine booster responses by mechanisms  
280 that remain to be fully elucidated<sup>2,3</sup>.

281  
282 In conclusion, the development of memory in response to vaccination is influenced by pre-existing  
283 antibodies that can alter the antibody target profile, affinity and isotype of the responding cells.  
284 Diversification of the antibody response by this mechanism may help increase the breadth of  
285 vaccines like the SARS-CoV-2 vaccine but interfere with the development of breadth in others,  
286 like HIV-1 or influenza, by diverting immunity away from broadly neutralizing to strain-specific  
287 epitopes.

288 **Figures**

289



290

291 **Fig. 1: Study design and plasma antibody activity.** **a**, Schematic overview of the study design with

292 markers (w) denoting weeks relative to the time of the first vaccine dose. **b**, Serum levels of C135-LS

293 (upper panel, in blue) and C144-LS (lower panel, in red) over time are shown. The thick colored dashed

294 lines indicate the median serum concentrations among mAb recipients (n=18), while the thin dotted black

295 lines represent individual participants. The two solid vertical lines indicate the median and the grey shaded

296 areas the range of time from mAb administration.

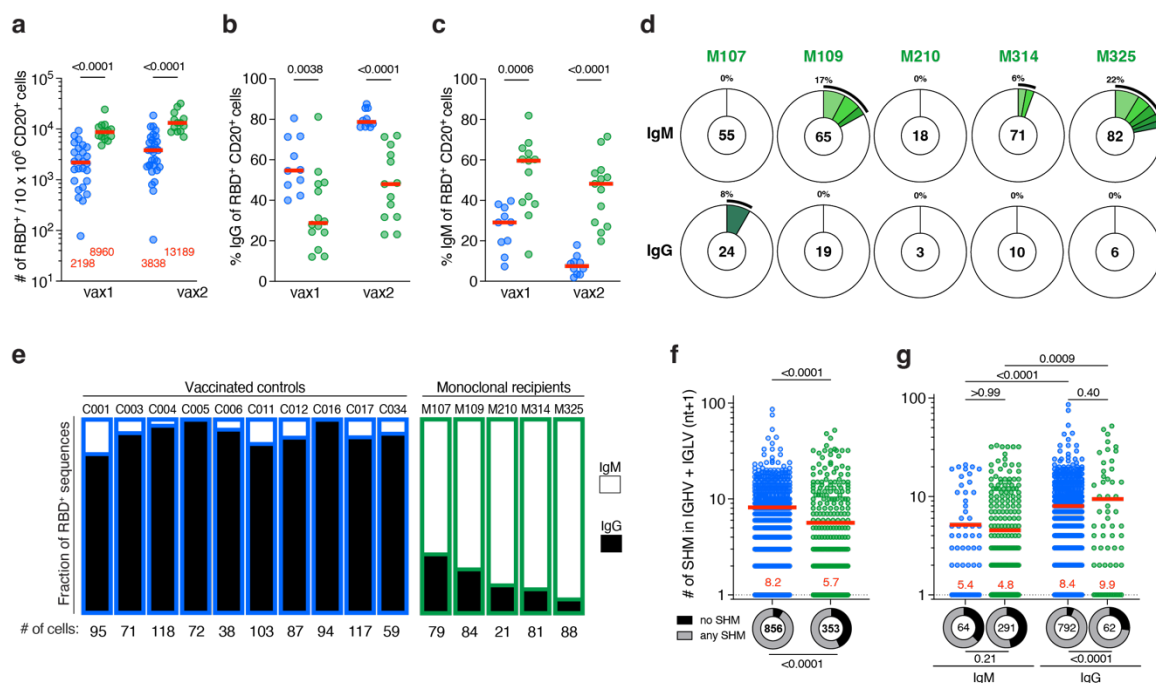
297 **c-f**, Half-maximal plasma binding titers (BT<sub>50</sub>) to RBD after one (vax1) and two doses (vax2) mRNA vaccinations for monoclonal antibody recipients (n=18, in

298 green) and controls (n=26, in blue). Each dot represents one individual. Dashed horizontal lines represent

299 the median binding activity of healthy pre-pandemic plasma samples, which served as negative controls.

300 **c,d**, IgM (**c**) and IgG (**d**) binding titers to WT RBD. **e**, IgG binding to R346S/E484K (left panel) and

301 N440K/E484K RBDs, see also Ext. Data Fig 1. **f**, IgG binding to the NTD. **g-i**, Plasma half-maximal  
302 neutralizing titers (NT50s) against HIV-1 pseudotyped with **g**, SARS-CoV-2 WT S. **h**, R346S/Q493K  
303 mutant S. **(i)** R346S/N440K/E484K mutant S (see also Ext. Data Fig 2). The S protein in the pseudoviruses  
304 in **g-i** contained an R683G substitution. Red horizontal bars in **c-i** and red numbers in **g-i** represent median  
305 values. Statistical significance in **c-i** was determined using the two-tailed Mann-Whitney test comparing  
306 differences between monoclonal recipients and controls for each time point independently. All experiments  
307 were performed at least in duplicate.  
308

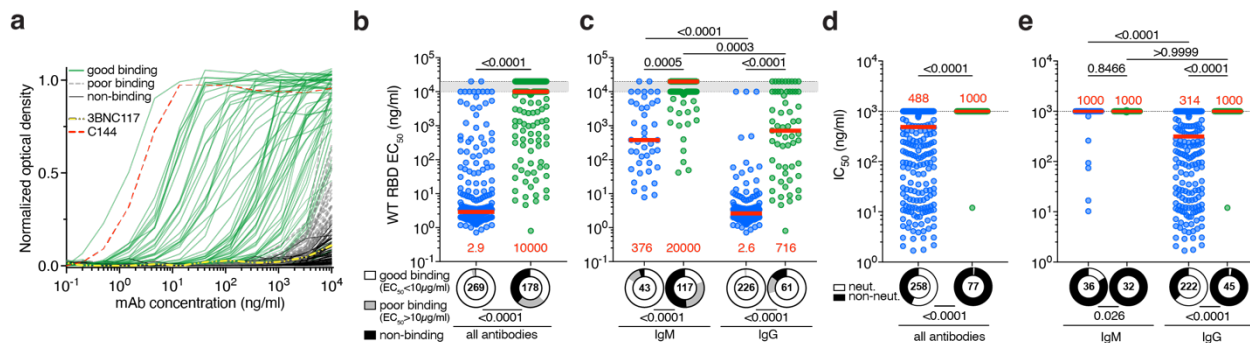


309

310 **Fig. 2: Anti-SARS-CoV-2 RBD memory B cells from vaccinated monoclonal recipients.**

311 **a-c**, Flow-cytometric enumeration and surface immunoglobulin expression of SARS-CoV-2 RBD-specific  
 312 memory B cells after vax1 and vax2. mAb recipients (green, n=18 in panel **a**, n=13 in panels **b** and **c**) and  
 313 controls<sup>10,11</sup> (blue, n=26 for vax1 and n=31 for vax2 in panel **a**, and n=10 in panels **b,c**). Each dot represents  
 314 one individual and red horizontal bars (and numbers in panel **a**) depict median values. **a**, Number of WT  
 315 RBD-specific memory B cells per 10 million CD20<sup>+</sup> B cells (see also Ext. Data Fig 3a and b). **b,c**,  
 316 Percentage of cells among WT RBD-binding CD20<sup>+</sup> B cells that express cell surface IgG (**b**) or IgM (**c**). **d**,  
 317 Pie charts show the distribution of antibody sequences derived from cells isolated from 5 vaccinated  
 318 monoclonal recipients after vax2 (see also Ext. Data Fig 3d and e). The upper panel shows IgM, and the  
 319 lower panel depicts IgG. The number in the inner circle indicates the number of sequences analyzed for the  
 320 individual denoted above the circle. Slices colored in shades of green indicate cells that are clonally  
 321 expanded (same IGHV and IGLV genes, with highly similar CDR3s) within an individual. Pie slice size is  
 322 proportional to the number of clonally related sequences. The black outline and % value indicate the  
 323 frequency of clonally expanded sequences detected within an individual. White pie areas indicate the  
 324 proportion of sequences isolated only once. **e**, Fraction of cells harboring IgG (black) vs IgM (white)  
 325 transcripts from the indicated individuals (mAb recipients outlined in green, and controls in blue). See also  
 326 Ext Data Fig. 3f and <sup>10,11</sup>). **f,g**, Somatic hypermutation (SHM) shown as combined heavy- and light-chain  
 327 variable region nucleotide substitutions plus one (IGHV+IGVL+1), with each dot representing one  
 328 sequence from mAb recipients (green) or controls (blue). Ring plots below each column show the fraction  
 329 of sequences with no (IGHV+IGVL+1 = 1) vs. any (IGHV+IGVL+1 > 1) SHM, and the number in the

330 circle indicates the number of sequences analyzed, (**f**) for all cells irrespective of isotype, (**g**) IgM and IgG  
331 analyzed independently. Red horizontal bars and numbers in **f** and **g** indicate mean values. Statistical  
332 significance was determined using the two-tailed Mann-Whitney test comparing differences between  
333 monoclonal recipients and controls for **a-c** and **f**, the Kruskal-Wallis test with subsequent Dunn's correction  
334 for multiple comparisons was used for **g**, and Fisher's exact test was used to compare fractions in **f** and **g**.  
335



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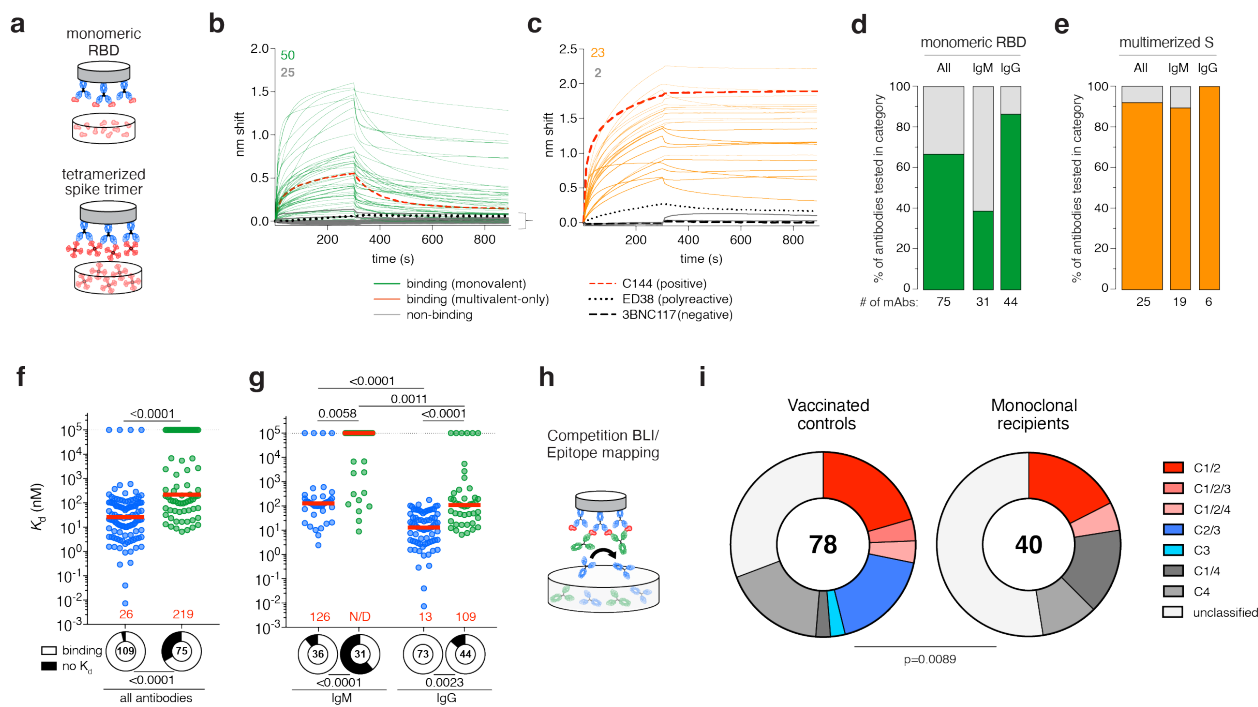
337 **Fig. 3: Functional characterization of anti-SARS-CoV-2 RBD memory antibodies from vaccinated**  
 338 **mAb recipients.**

339 **a-c**, Monoclonal antibody binding to WT RBD. **a**, Graph shows ELISA binding monoclonal antibodies  
 340 derived from mAb recipients after serial dilution. Each curve represents one antibody. Green curves show  
 341 EC50s <10  $\mu\text{g/ml}$ , grey dashed lines EC50s >10  $\mu\text{g/ml}$ , solid black lines are antibodies that were below or  
 342 equal to a negative control anti-HIV1 antibody 3BNC117 (thick, white-dashed line). C144 (thick, red-  
 343 dashed line) is positive control. **b** Summary of EC50s derived from (**a**) mAb recipients in green, and  
 344 controls in blue for all antibodies irrespective of isotype. **c**, as in (**b**) but IgM and IgG analyzed  
 345 independently. Grey shaded area between horizontal dotted lines indicates antibodies with EC50s >10  
 346  $\mu\text{g/ml}$  (poor binding) and non-binding antibodies arbitrarily grouped at 10 and 20  $\mu\text{g/ml}$ , respectively. Ring  
 347 plots summarize the fraction of all antibodies tested for the respective groups (encircled number). **d**, Plots  
 348 show IC50s for all monoclonal antibodies isolated from vaccinated mAb recipients (green) or controls  
 349 (blue). Ring plots illustrate the fraction of non-neutralizing (IC50 > 1000 ng/ml) antibodies (black slices)  
 350 among all antibodies tested for the respective group (encircled number). **e**, as in (**d**) but IgM and IgG  
 351 antibodies analyzed independently. For panels **b-e**, red horizontal bars and numbers represent median  
 352 values. Statistical significance was determined using the two-tailed Mann-Whitney test for **b** and **d**, whereas  
 353 the Kruskal-Wallis test with subsequent Dunn's correction for multiple comparisons was used for **c** and **e**.  
 354 To compare fractions from ring plots, the Chi-squared contingency statistic was used in **b** and **c**, and  
 355 Fisher's exact test for **d** and **e**. All experiments were performed at least in duplicate.

356

357





358  
359 **Fig. 4: Affinities and epitope distribution of anti-SARS-CoV-2 RBD memory antibodies from**  
360 **vaccinated mAb recipients.**

361 **a-g**, Monoclonal antibody binding to monomeric and multimerized antigen by BLI. **a**, Schematic  
362 representation for monomeric binding measurements where IgG is immobilized on the biosensor chip and  
363 subsequently exposed to monomeric RBD (upper panel), and multimeric binding using 6P-stabilized WT  
364 SARS-CoV-2 S protein trimers that had been tetramerized using streptavidin (lower panel). **b**, Graphs show  
365 BLI traces obtained under monovalent conditions. Each curve represents one antibody. Colored solid lines  
366 denote binding above background represented by polyreactive antibody ED38<sup>28</sup> (dotted black line) and anti-  
367 HIV-1 antibody 3BNC117 (dashed black line). Grey lines show non-binding antibodies. C144 (thick, red-  
368 dashed line) is a positive control. Colored and grey numbers in upper left of each panel indicate the number  
369 of binding and non-binding antibodies, respectively. **c**, As in **(b)** for antibodies that showed no measurable  
370 binding in **(b)** and were subsequently tested for binding under polyvalent conditions. **d**, Bar charts show  
371 the percentage of binding antibodies under monovalent conditions for all antibodies and by isotype. Values  
372 below bars indicate the number of antibodies tested. **e**, as in **(d)** for antibodies shown in **(c)**. **f**, Graphs show  
373 affinity constants ( $K_d$ ) derived under monomeric binding conditions **(b)** for mAb recipients (green) and  
374 controls (blue) irrespective of isotype. Ring plots illustrate the fraction of antibodies tested for the respective  
375 group (encircled number) that measurably bound to monomeric RBD (“binding”, in white) and those for  
376 which a  $K_d$  value could not be established (“no  $K_d$ ”, black). Red horizontal bars and numbers represent  
377 median values (N/D, not determined). **g**, as in **(f)** analyzed independently for IgM and IgG. **h**, Schematic  
378 representation of BLI competition experiment in which a capture antibody of known epitope-specificity

379 (class-reference antibody) is bound to the biosensor chip and exposed to antigen. In a second step, the  
380 antibody of interested is added to the chip. **i**, Pie charts show the distribution of epitopes targeted. The  
381 number in the center is the number of antibodies tested. Slices colored in shades of red and blue represent  
382 Class 1, 2 and 3 or combined epitopes, shades of grey represent Class 4-containing epitopes or epitopes that  
383 could not be classified by this method. Statistical significance was determined using the two-tailed Mann-  
384 Whitney test for **f**, and the Kruskal-Wallis test with subsequent Dunn's correction for multiple comparisons  
385 for **g**. To compare categories and distributions from ring plots, Fisher's exact test was used for **f** and **g**, and  
386 the Chi-squared contingency statistic was used for panel **i**.  
387

388

## 389 **Methods**

390

### 391 **Study participants.**

392 Participants in the “monoclonal antibody recipient” group were healthy SARS-CoV-2-naïve  
393 volunteers who were enrolled in a first-in-human phase 1 study at the Rockefeller University  
394 Hospital in New York and received single doses of two anti-SARS-CoV-2 RBD monoclonal  
395 antibodies (mAbs), C144-LS and C135-LS, between January 13 and March 3, 2021  
396 (NCT04700163). The phase 1 clinical trial had a dose escalation design and evaluated the safety  
397 and tolerability, and pharmacokinetics of the two mAbs. The mAb cocktail (1:1 ratio of C144-LS  
398 and C135-LS) was administered to 21 out of the 23 enrolled individuals (n=2 receiving placebo),  
399 allowing for multiple interim safety analyses. The mAbs were administered at 100 or 200 mg each,  
400 subcutaneously, or at 1, 5 or 15 mg/kg each, intravenously (see Ext. Data. Table S2). Eligible  
401 participants for the phase 1 study were healthy adults with no history of SARS-CoV-2 infection or  
402 vaccination, or prior receipt of any SARS-CoV-2 therapeutics, including other monoclonal  
403 antibodies or convalescent plasma. Further details on inclusion and exclusion criteria, study design  
404 and endpoints of the phase 1 study can be found on [clinicaltrials.gov](https://clinicaltrials.gov) (NCT04700163). Of the 21  
405 individuals who received the mAbs in the phase 1 study, 18 co-enrolled in a parallel observational  
406 study to assess their immune responses to subsequent SARS-CoV-2 mRNA vaccination. One  
407 individual chose to receive the Janssen (Ad26.COV2.S) vaccine, and another individual (a placebo  
408 recipient) displayed Nucleocapsid (N) titer changes prior to enrollment in the observational study  
409 that were compatible with a recent SARS-CoV-2 infection, making them ineligible for inclusion  
410 in this study. The remaining phase 1 study participant chose not to enroll in the parallel study of  
411 immune responses. The 18 mAb-recipients included in this observational study received either the  
412 Moderna (*Spikevax*, mRNA-1273) or Pfizer-BioNTech (*Comirnaty*, BNT162b2) mRNA vaccines  
413 against the wildtype (Wuhan-Hu-1) strain of the severe acute respiratory syndrome coronavirus 2  
414 (SARS-CoV-2). Participants in the “vaccinated control” group were healthy SARS-CoV-2-naïve  
415 volunteers who had received two doses of one of the two currently approved SARS-CoV-2 mRNA  
416 vaccines, Moderna (*Spikevax*, mRNA-1273) or Pfizer-BioNTech (*Comirnaty*, BNT162b2) mRNA  
417 vaccines. These control individuals had been recruited to the Rockefeller University Hospital for  
418 serial blood donations to longitudinally assess their immune responses to SARS-CoV-2 mRNA

419 vaccination<sup>10,11</sup>. We previously reported the findings obtained from this group and refer to Cho et  
420 al.<sup>10</sup> and Muecksch et al.<sup>11</sup> for further details on participant recruitment, inclusion and exclusion  
421 criteria, and demographic characteristics (also see Ext. Data Tables S1 and S2). At each sample  
422 collection visit, participants of either group presented to the Rockefeller University Hospital for  
423 blood sample collection and were asked to provide details of their vaccination regimen, possible  
424 side effects, comorbidities, and possible COVID-19 history. Vaccinations were administered  
425 outside of the study, at the discretion of the individual and their health care provider consistent  
426 with existing guidelines and, as such, not influenced by their participation in our study. Baseline  
427 and longitudinal plasma samples were tested for binding activity toward the nucleocapsid protein  
428 (N) (Sino Biological, 40588-V08B) of SARS-CoV-2. Absence of seroconversion toward N during  
429 the study interval was used to exclude SARS-CoV-2 infection, in addition to participants' reported  
430 history. Clinical data collection and management were carried out using the software iRIS by  
431 iMedRIS (v. 11.02). All participants provided written informed consent before participation in the  
432 study, which was conducted in accordance with Good Clinical Practice. The study was performed  
433 in compliance with all relevant ethical regulations, and the clinical protocols (CGA-1015 and  
434 DRO-1006) for studies with human participants were approved by the Institutional Review Board  
435 of the Rockefeller University. For detailed participant characteristics see Supplementary Tables 1  
436 and 2 and <sup>10,11</sup>

437

#### 438 **Blood samples processing and storage.**

439 Peripheral Blood Mononuclear Cells (PBMCs) obtained from samples collected at Rockefeller  
440 University were purified as previously reported by gradient centrifugation and stored in liquid  
441 nitrogen in the presence of Fetal Calf Serum (FCS) and Dimethylsulfoxide (DMSO)<sup>5</sup>. Heparinized  
442 plasma and serum samples were aliquoted and stored at -20°C or less. Prior to experiments,  
443 aliquots of plasma samples were heat-inactivated (56°C for 1 hour) and then stored at 4°C.

444

445

#### 446 **Pharmacokinetics of C144-LS and C135-LS**

447 To evaluate the pharmacokinetic (PK) properties of the passively administered antibodies, C144-  
448 LS and C135-LS, their serum antibody levels were measured on the day of antibody  
449 administration (day 0) at 1, 3, 6, 9 and 12 hours after infusion, and on days 1, 3, 7, 14, 21, 28, 56,

450 84, 126, 168, 252, and 336. C144-LS and C135-LS levels in serum were measured by mass-  
451 spectrometry (MS/MS). Briefly, analytes were isolated from serum samples through  
452 immunocapture using streptavidin beads and biotinylated RBD protein. The isolated proteins  
453 were denatured with dithiothreitol, alkylated with iodoacetamide, and digested with trypsin. The  
454 final extract was analyzed via high-performance liquid chromatography (HPLC) with column-  
455 switching and MS/MS detection using positive ion electrospray. A linear,  $1/\text{concentration}^2$   
456 weighted, least-squares regression algorithm was used for quantification. Noncompartmental  
457 analysis (NCA) was used to estimate PK parameters from measured serum levels of C144-LS  
458 and C135-LS. Phoenix WinNonlin® (v8.2) was used for the NCA. Actual sample time post  
459 administration of each mAb was used for the estimation of serum PK parameters instead of  
460 nominal time. Half-life estimates were similar between administration routes for both C144-LS  
461 and C135-LS, indicating a half-life of 2-3 months for both mAbs by either administration route  
462 (C144-LS: 68.9 to 99.3 days for s.c. groups and 86.9 to 92.3 days for i.v. groups; C135-LS: 72.7  
463 to 77.9 days for s.c. groups and 70.5 to 94.7 days for i.v. groups). Visualization of the PK data  
464 was performed in GraphPad Prism, using the three-phase decay model.

465

466

#### 467 **ELISAs**

468 Enzyme-Linked Immunosorbent Assays (ELISAs)<sup>29,30</sup> to evaluate antibodies binding to SARS-  
469 CoV-2 Wuhan-Hu-1 RBD, NTD or S were performed by coating of high-binding 96-half-well  
470 plates (Corning 3690) with 50  $\mu\text{l}$  per well of a 1  $\mu\text{g}/\text{ml}$  protein solution in Phosphate-buffered Saline  
471 (PBS) overnight at 4°C. Plates were washed 6 times with washing buffer (1 $\times$  PBS with 0.05%  
472 Tween-20 (Sigma-Aldrich)) and incubated with 170  $\mu\text{l}$  per well blocking buffer (1 $\times$  PBS with 2%  
473 BSA and 0.05% Tween-20 (Sigma)) for 1 hour at room temperature. Immediately after blocking,  
474 monoclonal antibodies or plasma samples were added in PBS and incubated for 1 hour at room  
475 temperature. Plasma samples were assayed at a 1:66 starting dilution and serially diluted by either  
476 three- or fourfold. Monoclonal antibodies were tested at 10  $\mu\text{g}/\text{ml}$  starting concentration and 11  
477 additional threefold serial dilutions. Plates were washed 6 times with washing buffer and then  
478 incubated with anti-human IgG or IgM secondary antibody conjugated to horseradish peroxidase  
479 (HRP) (Jackson Immuno Research 109-036-088 and 109-035-129) in blocking buffer at a 1:5,000  
480 dilution (IgM and IgG). Plates were developed by addition of the HRP substrate, 3,3',5,5'-

481 Tetramethylbenzidine (TMB) (ThermoFisher) for 6 minutes. The developing reaction was stopped  
482 by adding 50  $\mu$ l of 1 M H<sub>2</sub>SO<sub>4</sub> and absorbance was measured at 450 nm with an ELISA microplate  
483 reader (FluoStar Omega, BMG Labtech) with Omega and Omega MARS software for analysis.  
484 Normalizer control samples were included on each plate. For plasma samples and monoclonal  
485 antibodies half-maximal binding titers (BT50s) and half-maximal effective concentrations  
486 (EC50s), respectively, were calculated using four-parameter nonlinear regression (GraphPad  
487 Prism V9.3, with the following settings: [Agonist] vs. response -- Variable slope (four parameters),  
488 bottom=0, Hillslope>0, Top=plate/experiment-specific upper plateau of the normalizer control  
489 antibody/plasma reaching saturation for at least 3-consecutive dilution steps. The curve-fit was  
490 constrained to an upper limit that corresponds to the maximal optical density achieved by the  
491 known normalizer control to limit inter-plate-/experiment variability (batch effects).  
492 Pre-pandemic plasma samples from healthy donors and isotype control monoclonal antibodies  
493 served as negative controls as indicated and were used for validation (for more details see <sup>5</sup>). All  
494 reported EC50 and BT50 values are the average of at least 2 independent experiments.

495

## 496 **Proteins**

497 The mammalian expression vector encoding the Receptor Binding-Domain (RBD) of SARS-CoV-  
498 2 (GenBank MN985325.1; Spike (S) protein residues 319-539) was previously described<sup>23</sup>.  
499 Plasmids encoding the R346S/E484K and N440K/E484K substitutions, were generated using site-  
500 directed mutagenesis kit according to the manufacturer's instructions (New England Biolabs  
501 (NEB), E0554S). All constructs were confirmed by Sanger sequencing and used to express soluble  
502 proteins by transiently transfecting Expi293F cells (GIBCO). Supernatants were harvested after  
503 four days, and RBD proteins were purified by nickel affinity chromatography. S 6P proteins were  
504 purified by nickel affinity following with size-exclusion chromatography. Peak fractions from  
505 size-exclusion chromatography were identified by native gel electrophoresis, and peak fractions  
506 corresponding to monomeric RBDs or spike trimers were pooled and stored at 4°C.

507

508

## 509 **SARS-CoV-2 pseudotyped reporter virus**

510 A plasmid expressing SARS-CoV-2 spike in the context of pSARS-CoV-2-S<sub>Δ19</sub> (Wuhan-Hu-1)  
511 has been described<sup>6</sup>. Two plasmids containing C135/C144 antibody escape mutations were

512 constructed based on pSARS-CoV-2-S<sub>Δ19</sub> by overlap extension PCR-mediated mutagenesis and  
513 Gibson assembly. Specifically, the substitutions introduced were: R346S/Q493K and  
514 R346S/N440K/E484K. Those substitutions were incorporated into a spike protein that also  
515 includes the R683G substitution, which disrupts the furin cleavage site and increases particle  
516 infectivity. Neutralizing activity against mutant pseudoviruses were compared to a wildtype (WT)  
517 SARS-CoV-2 spike sequence (NC\_045512), carrying R683G where appropriate, as indicated.  
518 SARS-CoV-2 pseudotyped particles were generated as previously described<sup>5,31</sup>. Briefly, 293T  
519 cells were transfected with pNL4-3ΔEnv-nanoluc and pSARS-CoV-2-S<sub>Δ19</sub>, particles were  
520 harvested 48 hours post-transfection, filtered and stored at -80°C.

521

### 522 **Pseudotyped virus neutralization assay**

523 Fivefold serially diluted pre-pandemic negative control plasma from healthy donors (technical  
524 negative controls, data not shown), plasma from vaccinated mAb recipients and mRNA vaccinated  
525 controls or monoclonal antibodies were incubated with SARS-CoV-2 pseudotyped virus for 1 hour  
526 at 37 °C. The mixture was subsequently incubated with 293T<sub>Ace2</sub> cells<sup>5</sup> (for all monoclonal  
527 antibody WT neutralization assays) or HT1080Ace2 c114 cells<sup>32</sup> (for all plasma neutralization  
528 assays) for 48 hours after which cells were washed with PBS and lysed with Luciferase Cell  
529 Culture Lysis 5× reagent (Promega). Nanoluc Luciferase activity in lysates was measured using  
530 the Nano-Glo Luciferase Assay System (Promega) with the ClarioStar Multimode reader (BMG).  
531 The relative luminescence units were normalized to those derived from cells infected with SARS-  
532 CoV-2 pseudotyped virus in the absence of plasma or monoclonal antibodies. The half-maximal  
533 neutralization titers for plasma (NT<sub>50</sub>) or half-maximal and 90% inhibitory concentrations for  
534 monoclonal antibodies (IC<sub>50</sub> and IC<sub>90</sub>) were determined using four-parameter nonlinear regression  
535 (least squares regression method without weighting; constraints: top=1, bottom=0) (GraphPad  
536 Prism).

537

### 538 **Biotinylation of viral protein for use in flow cytometry and biolayer interferometry**

539 Purified and Avi-tagged SARS-CoV-2 Wuhan-Hu-1 RBD or S were biotinylated using the Biotin-  
540 Protein Ligase-BIRA kit according to manufacturer's instructions (Avidity) as described before<sup>5</sup>.  
541 Ovalbumin (Sigma, A5503-1G) was biotinylated using the EZ-Link Sulfo-NHS-LC-Biotinylation  
542 kit according to the manufacturer's instructions (Thermo Scientific). Biotinylated ovalbumin was



543 conjugated to streptavidin-BV711 for single-cell sorts (BD biosciences, 563262) or to streptavidin-  
544 BB515 for phenotyping panel (BD biosciences, 564453). RBD was conjugated to streptavidin-PE  
545 (BD Biosciences, 554061) and streptavidin-AF647 (Biolegend, 405237)<sup>5,11</sup>.

546

#### 547 **Flow cytometry and single-cell sorting**

548 Single-cell sorting by flow cytometry was described previously<sup>5</sup>. Briefly, peripheral blood  
549 mononuclear cells were enriched for B cells by negative selection using a pan-B-cell isolation kit  
550 according to the manufacturer's instructions (Miltenyi Biotec, 130-101-638). The enriched B cells  
551 were incubated in Fluorescence-Activated Cell-sorting (FACS) buffer (1× PBS, 2% FCS, 1 mM  
552 ethylenediaminetetraacetic acid (EDTA)) with the following anti-human antibodies (all at 1:200  
553 dilution): anti-CD20-PECy7 (BD Biosciences, 335793), anti-CD3-APC-eFluor 780 (Invitrogen,  
554 47-0037-41), anti-CD8-APC-eFluor 780 (Invitrogen, 47-0086-42), anti-CD16-APC-eFluor 780  
555 (Invitrogen, 47-0168-41), anti-CD14-APC-eFluor 780 (Invitrogen, 47-0149-42), as well as  
556 Zombie NIR (BioLegend, 423105) and fluorophore-labeled RBD and ovalbumin (Ova) for 30 min  
557 on ice. AccuCheck Counting Beads (Life Technologies, PCB100) were added as indicated to each  
558 sample according to manufacturer's instructions. Single CD3-CD8-CD14-  
559 CD16-CD20+Ova-RBD-PE+RBD-AF647+ B cells were sorted into individual wells of 96-well  
560 plates containing 4 µl of lysis buffer (0.5× PBS, 10 mM Dithiothreitol (DTT), 3,000 units/ml  
561 RNasin Ribonuclease Inhibitors (Promega, N2615) per well using a FACS Aria III and FACSDiva  
562 software (Becton Dickinson) for acquisition and FlowJo for analysis. The sorted cells were frozen  
563 on dry ice, and then stored at -80 °C for subsequent RNA reverse transcription. For B cell isotype  
564 analysis by flow-cytometry, in addition to above antibodies, B cells were also stained with  
565 following anti-human antibodies (all at 1:200 dilution): anti-CD19-BV605 (Biolegend, 302244),  
566 anti- IgG-PECF594 (BD, 562538), anti-IgM-AF700 (Biolegend, 314538), and anti-CD38-BV421  
567 (Biolegend, 303526).

568

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

570 Antibodies were identified and sequenced as described previously<sup>5,33</sup>. In brief, RNA from single  
571 cells was reverse-transcribed (SuperScript III Reverse Transcriptase, Invitrogen, 18080-044) and  
572 the cDNA was stored at -20 °C or used for subsequent amplification of the variable IGH, IGL and  
573 IGK genes by nested PCR and Sanger sequencing. Sequence analysis was performed using

574 MacVector and Geneious Prime (version 2022.1.1). Amplicons from the first PCR reaction were  
575 used as templates for sequence- and ligation-independent cloning into antibody expression vectors.  
576 Recombinant monoclonal antibodies were produced and purified as previously described<sup>5</sup>.

577

### 578 **Biolayer interferometry**

579 Biolayer interferometry assays were performed as previously described<sup>5</sup>, with minor modifications  
580 as below. Briefly, we used the Octet Red instrument (ForteBio) at 30 °C with shaking at 1,000  
581 r.p.m. Monomeric affinities of anti-SARS-CoV-2 RBD IgG binding were derived by subtracting  
582 the signal obtained from traces performed with IgGs in the absence of WT RBD. Kinetic analysis  
583 using protein A biosensor (ForteBio, 18-5010) was performed as follows: (1) baseline: immersion  
584 for 60 s in buffer; (2) loading: immersion for 200 s in a solution with IgGs at 10 µg ml<sup>-1</sup>; (3)  
585 baseline: immersion for 200 s in buffer; (4) association: immersion for 300 s in solution with WT  
586 RBD at three different concentrations ranging from 200 to 5 µg ml<sup>-1</sup>; (5) dissociation: immersion  
587 for 600 s in buffer. Curve fitting was performed using a fast 1:1 binding model and the data analysis  
588 software from ForteBio. Mean equilibrium dissociation constants (K<sub>d</sub>) were determined by  
589 averaging all binding curves that matched the theoretical fit with an R<sup>2</sup> value ≥0.8. To establish  
590 binding of low-affinity antibodies to multimerized antigen, 6P-stabilized and biotinylated S trimers  
591 were incubated with recombinant Streptavidin (ACROBiosystems, STN-N5116) for 30 min at RT,  
592 resulting in up to 12 RBD-binding moieties per molecule and assayed on the Octet Red instrument  
593 (ForteBio) as above, with the following modification: Association step (4) was performed with the  
594 S-multimer at 430 µg ml<sup>-1</sup>. Epitope mapping assays were performed with protein A biosensor  
595 (ForteBio 18-5010), following the manufacturer's protocol "classical sandwich assay" as follows:  
596 (1) Sensor check: sensors immersed 30 sec in buffer alone (buffer ForteBio 18-1105), (2) Capture  
597 1st Ab: sensors immersed 10 min with Ab1 at 10 µg/mL, (3) Baseline: sensors immersed 30 sec  
598 in buffer alone, (4) Blocking: sensors immersed 5 min with IgG isotype control (3BNC117) at 20  
599 µg/mL. (5) Baseline: sensors immersed 30 sec in buffer alone, (6) Antigen association: sensors  
600 immersed 5 min with RBD at 20 µg/mL. (7) Baseline: sensors immersed 30 sec in buffer alone.  
601 (8) Association Ab2: sensors immersed 5 min with Ab2 at 10 µg/mL. Curve fitting was performed  
602 using the Fortebio Octet Data analysis software (ForteBio).

603

604

## 605 **Computational analyses of antibody sequences**

606 Antibody sequences were trimmed based on quality and annotated using Igbblastn v.1.14. with  
607 IMGT domain delineation system. Annotation was performed systematically using Change-O  
608 toolkit v.0.4.540<sup>34</sup>. Clonality of heavy and light chain was determined using DefineClones.py  
609 implemented by Change-O v0.4.5<sup>34</sup>. The script calculates the Hamming distance between each  
610 sequence in the data set and its nearest neighbor. Distances are subsequently normalized and to  
611 account for differences in junction sequence length, and clonality is determined based on a cut-off  
612 threshold of 0.15. Heavy and light chains derived from the same cell were subsequently paired,  
613 and clonotypes were assigned based on their V and J genes using in-house R and Perl scripts. All  
614 scripts and the data used to process antibody sequences are publicly available on GitHub  
615 ([https://github.com/stratust/igpipeline/tree/igpipeline2\\_timepoint\\_v2](https://github.com/stratust/igpipeline/tree/igpipeline2_timepoint_v2)).

616 The frequency distributions of human V genes in anti-SARS-CoV-2 antibodies from this study  
617 was compared to 131,284,220 IgH and IGL sequences generated by Soto et al.<sup>35</sup> and downloaded  
618 from cAb-Rep<sup>36</sup>, a database of human shared BCR clonotypes available at [https://cab-](https://cab-rep.c2b2.columbia.edu/)  
619 [rep.c2b2.columbia.edu/](https://cab-rep.c2b2.columbia.edu/). Based on the 108 distinct V genes that make up the 417 analyzed  
620 sequences from the Ig repertoire of the individuals described in this study (353 sequences isolated  
621 from 5 monoclonal antibody recipients and 65 IgM sequences isolated from 9 vaccinated control  
622 individuals (for IgG sequences isolated from controls see <sup>10,11</sup>), we selected the IgH and IGL  
623 sequences from the database that are partially coded by the same V genes and counted them  
624 according to the constant region. The frequencies shown in Extended Data Fig. 4 are relative to  
625 the source and isotype analyzed. We used the two-sided binomial test to check whether the number  
626 of sequences belonging to a specific IGHV or IGLV gene in the repertoire is different according  
627 to the frequency of the same IgV gene in the database. Adjusted p-values were calculated using  
628 the false discovery rate (FDR) correction. Significant differences are denoted with stars.

629  
630 Nucleotide somatic mutations and Complementarity-Determining Region (CDR3) length were  
631 determined using in-house R and Perl scripts. For quantification of somatic mutations, *IGHV* and  
632 *IGLV* nucleotide sequences were aligned against their closest germlines using Igbblastn and the  
633 number of differences were considered nucleotide mutations.

634

635

636 **Data presentation**

637 Figures arranged in Adobe Illustrator 2022.

638

639 **Data availability statement:** Data are provided in Supplementary Tables 1-4. The raw sequencing  
640 data and computer scripts associated with Figure 2 and Ext. Data Fig. 3 have been deposited at  
641 Github ([https://github.com/stratust/igpipeline/tree/igpipeline2\\_timepoint\\_v2](https://github.com/stratust/igpipeline/tree/igpipeline2_timepoint_v2)). This study also  
642 uses data from “A Public Database of Memory and Naive B-Cell Receptor Sequences”  
643 (<https://doi.org/10.5061/dryad.35ks2>), PDB (6VYB and 6NB6), cAb-Rep ([https://cab-](https://cab-rep.c2b2.columbia.edu/)  
644 [rep.c2b2.columbia.edu/](https://cab-rep.c2b2.columbia.edu/)), Sequence Read Archive (accession SRP010970), and from “High  
645 frequency of shared clonotypes in human B cell receptor repertoires”  
646 (<https://doi.org/10.1038/s41586-019-0934-8>).

647

648 **Code availability statement:** Computer code to process the antibody sequences is available at  
649 GitHub ([https://github.com/stratust/igpipeline/tree/igpipeline2\\_timepoint\\_v2](https://github.com/stratust/igpipeline/tree/igpipeline2_timepoint_v2)).

650

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666

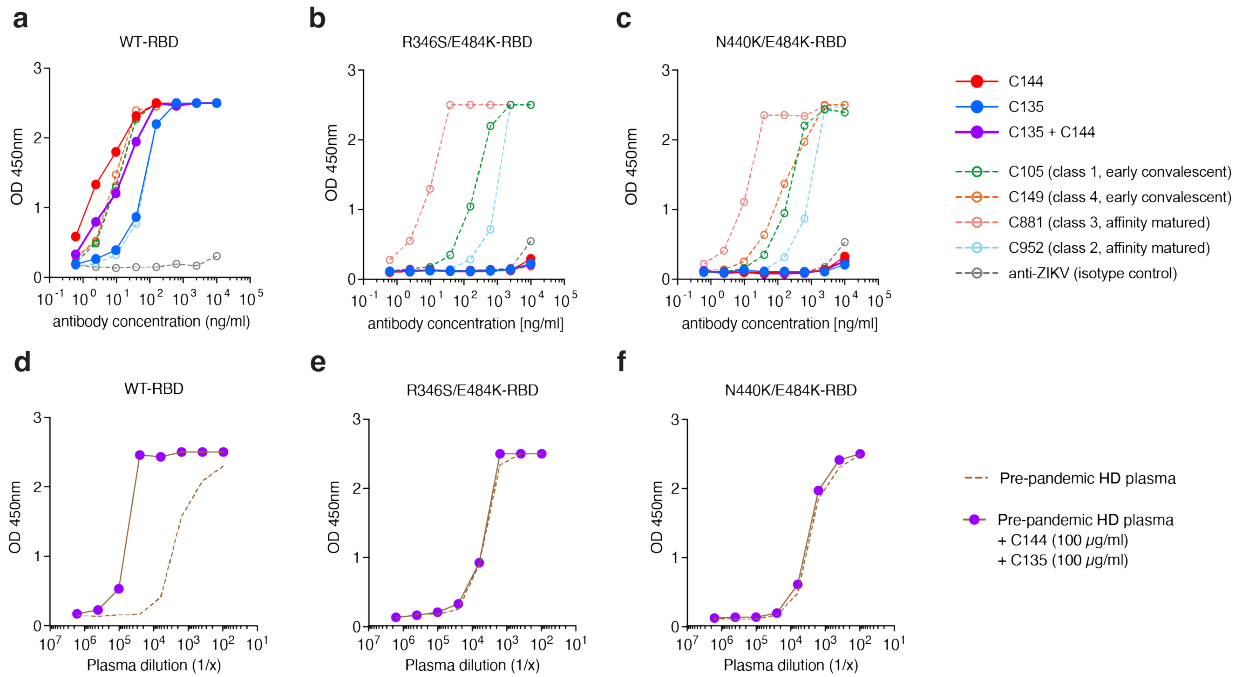
667 **Author Contributions:**

668 D.S.-B. and M.C.N. conceptualized the study. D.S.-B., F.M., Z.W., A.C., P.D.B., T.H., and M.C.N.  
669 conceived, designed and analyzed experiments. D.S.-B., C.G., and M. Caskey designed clinical  
670 protocols. D.S.-B., Z.W., F.M., A.C., R.R., M. Canis, J.DaSilva., F.S. and K.Y. carried out  
671 experiments. B.J. and A.G. produced antibodies. M.T., K.G.M., I.S., J.Dizon, C.G. and M. Caskey  
672 recruited participants, executed clinical protocols, and processed samples. T.Y.O. and V.R.  
673 performed bioinformatic analysis. D.S.-B. and M.C.N. wrote the manuscript with input from all  
674 co-authors.

675 **Extended Data Figures**

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680 **Extended Data Fig. 1: Mutant RBDs selectively abrogate binding by C135 and C144.** Monoclonal

681 antibody binding to mutant forms of RBD. **a-c**, Graphs show concentration-dependent antibody binding to

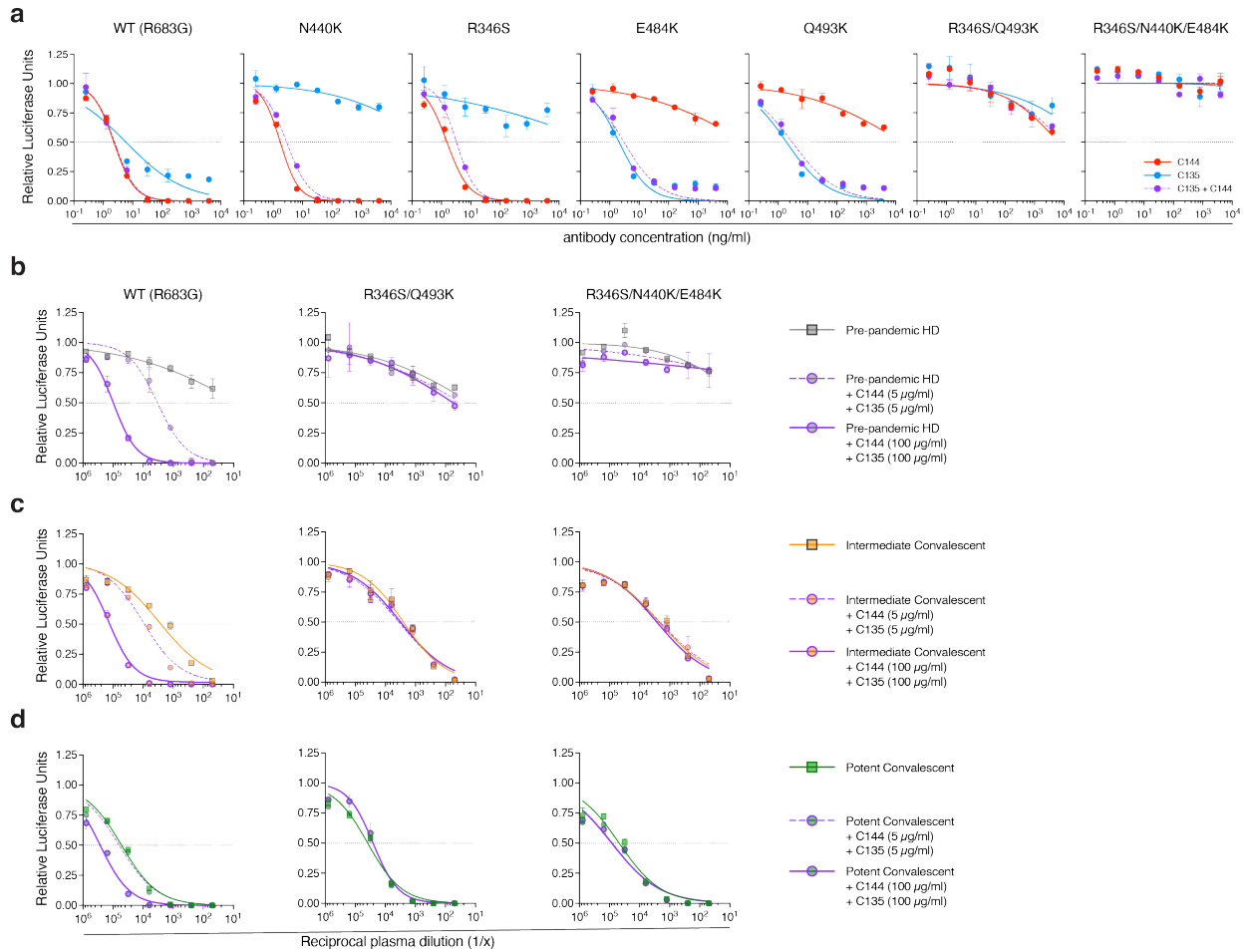
682 **(a)** WT, **(b)** R436S/E484K, and **(c)** N440K/E484K RBDs by C144, C135, and Class 1 (C105), Class 2

683 (C952), Class 3 (C881), and Class 4 (C149)<sup>5,6,8,23</sup>. **d-f**, Graphs show concentration dependent pre-pandemic

684 healthy donor plasma binding to **(d)** WT, **(e)** R436S/E484K, and **(e)** N440K/E484K RBDs in the presence

685 (purple) or absence (dotted lines) of 100μg/ml of C135 and C144. Addition of C144 and C135 to plasma

686 increases the binding activity of plasma against the WT but not the 2 mutant RBDs.



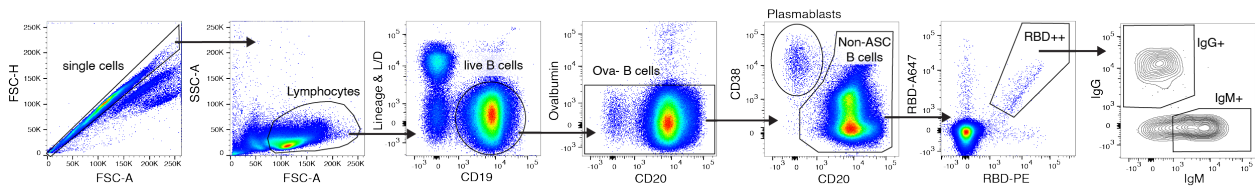
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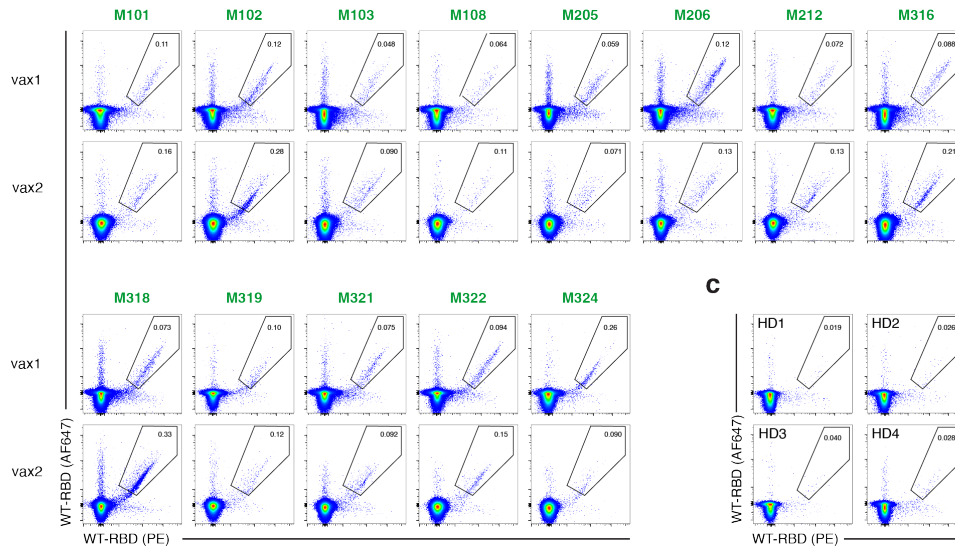
689 **Extended Data Fig. 2: SARS-CoV-2 R346S/Q493K and R346S/N440K/E484K pseudotype virus**  
 690 **neutralization by C135-LS and C144-LS. a,** Graphs show concentration-dependent neutralization curves  
 691 for SARS-CoV-2 pseudoviruses by monoclonal antibodies. C144 (red), C135 (blue), and their equimolar  
 692 combination (purple). **b,** Pre-pandemic plasma (squares) neutralization of WT or R346S/Q493K or  
 693 R346S/N440K/E484K pseudoviruses in the absence or presence of 5 (purple dashed circles) or 100 µg/ml  
 694 (purple solid circles) of C135 and C144<sup>14</sup>. **c-d,** As in (b) but for convalescent plasma with intermediate (c,  
 695 COV157)<sup>12</sup> or strong (d, COV31)<sup>12</sup> neutralizing activity. The horizontal lines in all panels indicate half-  
 696 maximal neutralization.



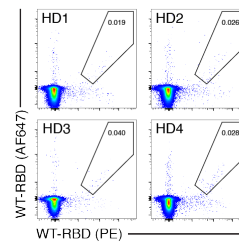
**a**



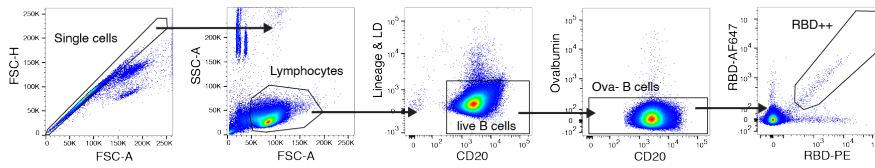
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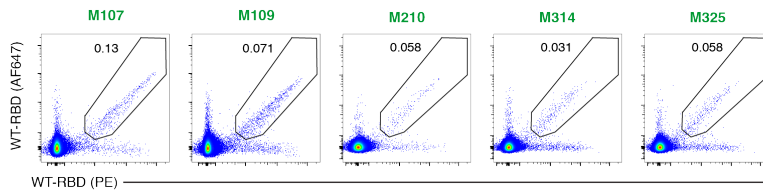
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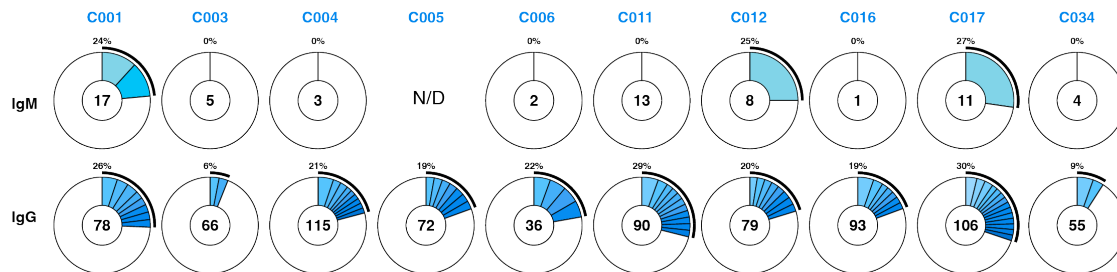
**d**



**e**



**f**



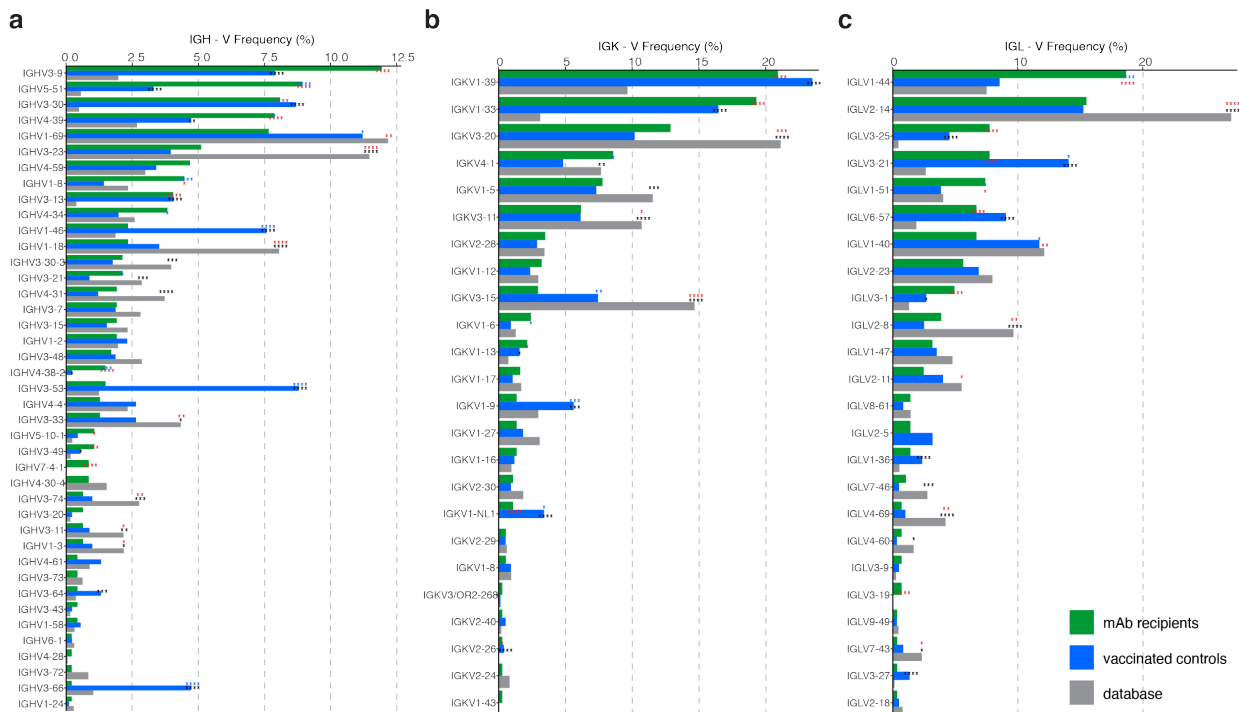
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700 **Extended Data Fig. 3: Flow cytometry, single-cell sorting and BCR sequencing**

701 **a**, Gating strategy for flow-cytometry phenotyping. Gating was on single lymphocytes that were CD19<sup>+</sup>  
702 and CD20<sup>+</sup>, and CD3<sup>-</sup> CD8<sup>-</sup> CD16<sup>-</sup> Ova<sup>-</sup> without uptake of live-dead dye (L/D). Antigen-specific cells  
703 were those with dual binding to Wuhan-Hu1 RBD-PE and RBD-AF647. Anti-IgG, -IgM were used to  
704 phenotype dual RBD-labelled B cells. **b,c**, Representative flow cytometry plots of Wuhan Hu-1 RBD-  
705 binding memory B cells from 13 mAb recipients after one and two doses of vaccination (**b**) and pre-  
706 pandemic health donors (**c**) serving as negative controls. Numbers in RBD-gate denote percentage of  
707 RBD dual-labelled cells of parent gate (see **a**). Corresponding flow-cytometry plots and gating strategy  
708 for vaccinated controls can be found in <sup>10</sup> **d**, Gating strategy for single-cell sorting of RBD-specific  
709 memory B cells. Dual-labelled (RBD-PE<sup>+</sup>/-AF647<sup>+</sup>) CD20<sup>+</sup> CD3<sup>-</sup> CD8<sup>-</sup> CD16<sup>-</sup> Ova<sup>-</sup> cells were sorted. **e**,  
710 Representative flow cytometry plots show RBD-binding cells that were sorted from 5 mAb recipients  
711 (Fig. 2g). **f**, Pie charts show the distribution of antibody sequences derived from cells isolated from 10  
712 vaccinated control individual after vax2 (Fig 2h). The upper panel shows IgM, and the lower panel  
713 depicts IgG sequences<sup>10,11</sup>. The number in the inner circle indicates the number of sequences analyzed for  
714 the individual denoted above the circle. Slices colored in shades of blue indicate cells that are clonally  
715 expanded (same IGHV and IGLV genes, with highly similar CDR3s). Pie slice size is proportional to the  
716 number of clonally related sequences. The black outline and % value indicate the frequency of clonally  
717 expanded sequences detected within an individual. White pie areas indicate the proportion of sequences  
718 isolated only once. For C005, there were no IgM transcripts amplified at the timepoint assayed.



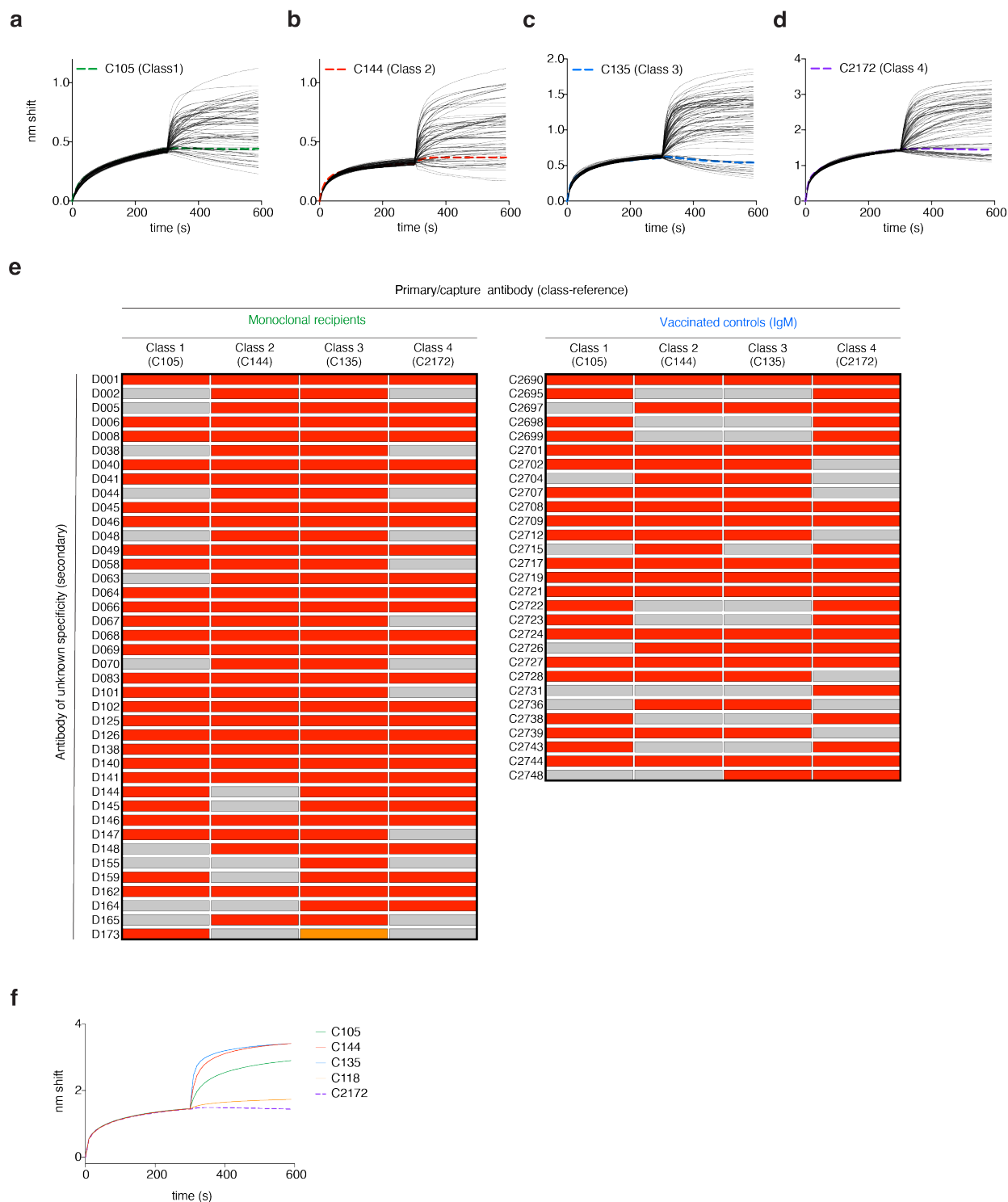
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721 **Extended Data Fig. 4: Frequency distribution of human V genes**

722 **a-c**, Comparison of the frequency distribution of V gene usage for the IgH and IgL among antibodies  
 723 isolated from mRNA-vaccinated mAb recipients (this study) and controls<sup>10,11</sup>, after vax2, and from  
 724 database of shared clonotypes of human antibodies from Soto et al<sup>35</sup>. Graphs show relative abundance of  
 725 human IGHV (**a**), IGKV (**b**), and IGLV (**c**) genes within the human V gene database (in grey, Sequence  
 726 Read Archive accession SRP010970), antibodies isolated from mAb recipients (in green) or vaccinated  
 727 controls (in blue). Colors of stars indicate levels of statistical significance for the following frequency  
 728 comparisons: black – vaccinated controls vs. database; red – mAb recipients vs. database; blue – mAb  
 729 recipients vs. vaccinated controls.

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733 **Extended Data Fig. 5: Competition BLI**

734 **a-d**, BLI traces of antibodies assayed for competition with class-reference antibodies. Traces show initial

735 association curve (antigen capture phase of the primary antibody) and subsequent addition of secondary

736 antibodies of unknown class. Thin solid black lines represent antibodies isolated from mAb recipients or  
737 vaccinated controls. Thick dashed lines are self-competition traces of C105 (green in **a**), C144 (red in **b**),  
738 C135 (blue in **c**) and C2172 (purple in **d**) for classes 1-4, respectively. **e**, Heat-map of relative inhibition of  
739 secondary antibody binding to the preformed capture antibody-RBD complexes (grey=no binding,  
740 red=unimpaired binding, orange=indeterminate). The left panel shows antibodies from mAb recipients,  
741 while the right panel shows IgM antibodies from vaccinated controls isolated in this study (both after vax2).  
742 Details on IgG antibodies isolated from vaccinated controls can be found in <sup>10,11</sup>. **f**, BLI traces defining  
743 C2172 as Class 4. C2172 is the primary/capture antibody (in dashed purple). The addition of known class-  
744 defining antibodies C105 (in green, Class 1<sup>8</sup>), C144 (in red, Class 2<sup>8</sup>), C135 (in blue, Class 3<sup>8</sup>), and C118  
745 (in orange, Class 1/4<sup>24</sup>) establish C2172 as a bona fide Class 4 antibody.

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