

1 **The plasmablast response to SARS-CoV-2 mRNA vaccination is dominated by non-neutralizing**  
2 **antibodies and targets both the NTD and the RBD**

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43 **Summary**

44 In this study we profiled vaccine-induced polyclonal antibodies as well as plasmablast derived mAbs from  
45 individuals who received SARS-CoV-2 spike mRNA vaccine. Polyclonal antibody responses in vaccinees  
46 were robust and comparable to or exceeded those seen after natural infection. However, the ratio of  
47 binding to neutralizing antibodies after vaccination was greater than that after natural infection and, at  
48 the monoclonal level, we found that the majority of vaccine-induced antibodies did not have neutralizing  
49 activity. We also found a co-dominance of mAbs targeting the NTD and RBD of SARS-CoV-2 spike and an  
50 original antigenic-sin like backboost to seasonal human coronaviruses OC43 and HKU1. Neutralizing  
51 activity of NTD mAbs but not RBD mAbs against a clinical viral isolate carrying E484K as well as extensive  
52 changes in the NTD was abolished, suggesting that a proportion of vaccine induced RBD binding antibodies  
53 may provide substantial protection against viral variants carrying single E484K RBD mutations.

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## 64 Introduction

65 Understanding of the innate and adaptive immune responses to severe acute respiratory  
66 syndrome coronavirus 2 (SARS-CoV-2) has progressed rapidly since the beginning of the coronavirus  
67 disease 2019 (COVID-19) pandemic (Carvalho et al., 2021). Polyclonal antibody responses against the spike  
68 protein of the virus in serum, and to a lesser degree also at mucosal surfaces, have been well characterized  
69 with respect to their kinetics, binding capacity and functionality (Grandjean et al., 2020; Isho et al., 2020;  
70 Iyer et al., 2020; Ripperger et al., 2020; Seow et al., 2020; Wajnberg et al., 2020). Similarly, encouraging  
71 data have been published on both the plasmablast response and the memory B-cell response induced by  
72 SARS-CoV-2 infection (Dan et al., 2021; Gaebler et al., 2020; Guthmiller et al., 2021; Huang et al., 2021;  
73 Robbiani et al., 2020; Rodda et al., 2021; Wilson et al., 2020). The immune responses to SARS-CoV-2  
74 vaccination, including to mRNA-based vaccines, are less well studied since these vaccines have only  
75 become available in the last months of 2020 (Baden et al., 2020; Polack et al., 2020). However,  
76 understanding vaccine-induced immunity is of high importance given the goal to achieve immunity for  
77 most people through vaccination, rather than as a consequence of infection.

78 The receptor binding domain (RBD) of the SARS-CoV-2 spike is an important target for serological  
79 and B-cell studies because it directly interacts with the cellular receptor angiotensin converting enzyme 2  
80 (ACE2) mediating host cell entry (Letko et al., 2020; Wrapp et al., 2020). Antibodies binding to the RBD  
81 can potently block attachment of the virus to ACE2 and thereby neutralize the virus (Barnes et al., 2020).  
82 As a consequence, RBD-based vaccines are in development in addition to full length spike-based vaccines  
83 (Krammer, 2020). Analyses of the B-cell responses to the spike generally focus on the RBD and on cells  
84 sorted with RBD baits introducing an inherent bias by omitting non-RBD targets (Cao et al., 2020; Gaebler  
85 *et al.*, 2020; Robbiani *et al.*, 2020; Weisblum et al., 2020). This is also true for B cells and monoclonal  
86 antibodies (mAbs) isolated from vaccinated individuals (Wang et al., 2021). However, other epitopes  
87 within the spike protein, notably the N-terminal domain (NTD) but also S2, do harbor neutralizing epitopes  
88 (Chi et al., 2020; Liu et al., 2020; McCallum et al., 2021b; Song et al., 2020). In fact, the NTD is heavily  
89 mutated in the three most prominent variants of concern (VOCs, B.1.1.7, B.1.351 and P.1 (Davies et al.,  
90 2021; Faria et al., 2021; Tegally et al., 2020)). Here, we studied the unbiased plasmablast response to  
91 SARS-CoV-2 mRNA-based vaccination and report several new findings. First, we document that RBD and  
92 NTD co-dominate as B-cell targets on the viral spike protein, highlighting the importance of the NTD. We  
93 also report the first vaccine-induced NTD mAbs. In addition, we show that the majority of mAbs isolated  
94 are non-neutralizing, which is reflective of the higher binding to neutralization ratios found in serum after  
95 vaccination compared to natural infection. Finally, data from plasmablasts suggest that, at least, some of  
96 the vaccine-induced response is biased by pre-existing immunity to human  $\beta$ -coronaviruses.

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## 98 Results

### 99 The polyclonal antibody response to mRNA vaccination exceeds titers seen in convalescent individuals 100 but is characterized by a high ratio of non-neutralizing antibodies

101 In late 2020, six adult participants of an ongoing observational study received mRNA-based SARS-  
102 CoV-2 vaccines (**Suppl. Table 1**). Blood from these individuals (termed V1-V6) was collected at several  
103 time points including before vaccination (for 4/6), after the first vaccination and at several time points  
104 after the second vaccination. We examined their immune responses to recombinant spike protein and  
105 RBD in enzyme-linked immunosorbent assays (ELISA), in comparison to those of 30 COVID-19 survivors  
106 (**Figure 1A and 1B, Suppl. Table 1**). The sera from convalescent individuals were selected based on their  
107 anti-spike titers and grouped into three groups (low +: n=8; moderate ++: n=11; and high +++: n=11, based  
108 on the antibody titer measured in the Mount Sinai's CLIA laboratory (Wajnberg *et al.*, 2020)), in order to  
109 facilitate identifying different features that may track with the strength of the antibody response. Five out  
110 of six vaccinees produced anti-spike and anti-RBD responses that were, at the peak, markedly higher than  
111 responses observed even in the high titer convalescent group while one vaccinee (V4) produced titers  
112 comparable to the high titer group. Notably, the antibody response peaked one week after the second  
113 vaccine dose, followed by a decline in titers over the following weeks as expected from an antibody  
114 response to vaccination. Interestingly, anti-RBD antibody titers seemed to decline faster than anti-spike  
115 antibody titers, which appeared to be more stable over time. We also measured neutralizing antibody  
116 titers using authentic SARS-CoV-2 and found a similar trend with all vaccinees displaying high titers, even  
117 though V4 responded with delayed kinetics (**Figure 1C**). Importantly, although at the peak response, the  
118 vaccine group mounted neutralization titers that fell in the upper range for the high convalescent group,  
119 they did not exceed that group markedly. This finding prompted us to calculate the proportions of spike  
120 binding to neutralizing antibodies. For the convalescent group, we found that individuals with lower titers  
121 had a higher proportion of binding to neutralizing antibodies than high responding convalescent  
122 individuals (**Figure 1D**). When determined at the time of peak response, the vaccinees had the highest  
123 proportion of binding to neutralizing antibody titers, indicating an immune response focused on non-  
124 neutralizing antibodies or an induction of less potent neutralizing antibodies in general (or both). These  
125 proportions remained stable over time with the ratio of binding to neutralizing antibodies in vaccinated  
126 individuals being significantly higher than those observed for any of the three convalescent groups ( $p =$   
127 0.0004, 0.0002 and 0.0041 for the three groups respectively; **Suppl. Figure 1**). We also investigated the  
128 spike binding to RBD binding ratio and found no difference to convalescent individuals except a general  
129 trend towards proportionally less RBD binding over time in the vaccinees (**Suppl. Figure 1**).

### 130 mRNA vaccination induces a modest but measurable immune response to seasonal $\beta$ -coronavirus 131 spike proteins

132 It has been reported that SARS-CoV-2 infection induces an original antigenic sin-type immune  
133 response against human coronaviruses (hCoVs) to which the majority of the human population has pre-  
134 existing immunity (Aydillo *et al.*, 2020; Song *et al.*, 2020). Here, we explored whether this phenomenon is  
135 also induced by SARS-CoV-2 mRNA vaccination. Antibody titers in four vaccinees against spike protein  
136 from  $\alpha$ -coronaviruses 229E and NL63 were detectable at the pre-vaccination time point, but did not  
137 increase substantially post-vaccination (**Figure 1E-F**; for V5 and V6 no pre-vaccination serum was  
138 available). However, titers against the spike proteins of  $\beta$ -coronaviruses OC43 and HKU1 increased  
139 substantially in these four vaccinees after vaccination (**Figure 1G-H**). Thus, vaccination with mRNA SARS-

140 CoV-2 spike also boosts immune responses against seasonal  $\beta$ -coronavirus spike proteins in a manner  
141 reminiscent of that reported for natural infection with SARS-CoV-2.

#### 142 **Plasmablast response to SARS-CoV-2 mRNA vaccination targets both the RBD and the NTD**

143 In order to characterize the B-cell response to vaccination in an unbiased manner, plasmablasts  
144 were single-cell sorted from blood specimens obtained from three individuals (V3, V5 and V6) one week  
145 after the booster immunization (**Suppl. Figure 2**). All mAbs were generated from single-cell sorted  
146 plasmablasts and probed for binding to recombinant SARS-CoV-2 spike protein. Twenty-one (40 mAbs  
147 were screened, with 28 being clonally unique, **Suppl. Table 2**) spike-reactive mAbs were isolated from V3,  
148 six (82 screened, 20 unique) from V5 and fifteen (84 screened, 24 unique) from V6 (**Figure 2A**). Using  
149 recombinant spike, RBD, NTD and S2 proteins, we mapped the domains to which these mAbs bind.  
150 Interestingly, only a minority of these antibodies recognized RBD (24% for V3, 47% for V6 and no RBD  
151 binders were identified for V5) (**Figure 2B and 2E**). A substantial number of the isolated mAbs bound to  
152 NTD including 14% for V3, 33% for V5 and 33% for V6 (**Figure 2C and 2E**). These data indicate that RBD  
153 and NTD are co-dominant in the context of mRNA-induced plasmablast response. The epitopes for the  
154 majority of the remaining spike binding mAbs, 52% for V3, 50% for V5 and 20% for V6, mapped to S2  
155 (**Figure 2D and 2E**). Only three mAbs were not accounted for in terms of binding target (two for V3 and  
156 one for V5, **Figure 2E**).

#### 157 **The majority of isolated mAbs from SARS-CoV2 vaccinees are non-neutralizing**

158 All antibodies were tested for neutralizing activity against the USA-WA1/2020 strain of SARS-CoV-  
159 2. Only a minority of the binding antibodies, even those targeting the RBD, showed neutralizing activity  
160 (**Figure 2F and 2G**). For V3, only one (an RBD binder) out of 21 mAbs (5%) displayed neutralizing activity  
161 (**Figure 2G**). For V5, a single NTD antibody neutralized authentic SARS-CoV-2 (17%) (**Figure 2G**). The  
162 highest frequency of neutralizing antibodies was found in V6 (34%) with one RBD neutralizer and four NTD  
163 neutralizers (**Figure 2G**). Interestingly, the highest neutralizing potency was found in mAb PVI.V5-6, an  
164 NTD binder followed by PVI.V6-4, an RBD binder.

165 We also tested all the antibodies for reactivity to the spike proteins of the four hCoVs 229E, NL63,  
166 HKU1 and OC43. No antibody binding to the spike proteins of  $\alpha$ -coronaviruses 229E and NL63 was found  
167 but we identified five mAbs (including three from V3, one from V5 and one from V6) that bound, to varying  
168 degrees, to the spike of OC43, which, like SARS-CoV-2, is a  $\beta$ -coronavirus (**Figure 2H**). Three mAbs showed  
169 strong binding (PVI.V3-8, PVI.V3-12 and PVI.V6-1), while PVI.V3-17 showed an intermediate binding  
170 phenotype and PVI.V5-1 bound very weakly. Three of these mAbs also showed binding to the spike of  
171 HKU1, another  $\beta$ -coronavirus. Of these, PVI.V6-1 showed only very weak binding while PVI.3-8 and PVI.3-  
172 12 had low minimal binding concentrations (MBCs) indicating higher affinity (**Figure 2I**).

#### 173 **The spike-reactive plasmablast response is dominated by IgG1+ cells and is comprised of a mixture of** 174 **cells with low and high levels of somatic hypermutation (SHM)**

175 Single-cell RNA sequencing (scRNAseq) was performed on bulk sorted plasmablasts from the  
176 three vaccinees (V3, V5, V6) to comprehensively examine the transcriptional profile, isotype distribution  
177 and somatic hypermutation (SHM) of vaccine-induced plasmablasts. We analyzed 4,584, 3,523 and 4,461  
178 single cells from subjects V3, V5, and V6, respectively. We first verified the identity of sequenced cells as  
179 plasmablasts through the combined expression of B cell receptors (BCRs) (**Figure 3A**) and that of the

180 canonical transcription as well as other factors essential for plasma cell differentiation, such as *PRDM1*,  
181 *XBP1* and *MZB1* (**Figure 3B**). To identify vaccine-responding B cell clones among the analyzed  
182 plasmablasts, we used scRNAseq to also analyze gene expression and V(D)J libraries from the sorted  
183 plasmablasts and clonally matched the BCR sequences to those from which spike-specific mAbs had been  
184 made. Using this method, we recovered 332, 7 and 1,384 BCR sequences from the scRNAseq data that are  
185 clonally related to the spike-binding mAbs derived from subjects V3, V5 and V6, respectively (**Figure 3C**).  
186 It is important to note here that we were not able to recover clonally related sequences for all of the mAbs  
187 that we cloned and expressed from each of the three vaccinees.

188 We next examined the isotype and IgG subclass distribution among the recovered sequences.  
189 IgG1 was by far the most dominant isotype in the three vaccinees (**Figure 3D**). Finally, we assessed the  
190 level of somatic hypermutation (SHM) among the mAbs-related sequences from the three subjects. We  
191 used the SHM levels observed in human naïve B cells and seasonal influenza virus vaccination-induced  
192 plasmablasts that were previously published for comparison (Turner et al., 2020). Spike-reactive  
193 plasmablasts from V3 and V6 but not V5 had accumulated SHM at levels that are significantly greater than  
194 those observed with naïve B cells (**Figure 3E, left panel**). Strikingly, the SHM among V6 plasmablasts was  
195 equivalent to those observed after seasonal influenza virus vaccination (**Figure 3E, left panel**). We  
196 reasoned that the high level of SHM among spike-reactive plasmablasts may be derived from those  
197 targeting conserved epitopes that are shared with human  $\beta$ -coronaviruses. Indeed, we found that the  
198 SHM level among clones that are related to cross-reactive mAbs was significantly higher than their non-  
199 cross-reactive counterparts (**Figure 3E, right panel**).

## 200 **Competition of RBD binding neutralizing mAbs with ACE2 and affinity of variant RBDs for human ACE2**

201 Two mAbs were identified as neutralizing and binding to RBD. We wanted, therefore, to test if  
202 they competed with ACE2 for RBD binding. Concentration-dependent competition was indeed observed  
203 for both mAbs demonstrating that inhibition of ACE2 binding is the mechanism of action of the two mAbs  
204 (**Figure 4**). Since we prepared RBD proteins of viral variants of concern for analysis of antibody binding  
205 (see below), we also wanted to assess the affinity of each variant RBD for human ACE2. Using biolayer  
206 interferometry (BLI), we measured association and dissociation rates of the N501Y RBD mutant (B.1.1.7  
207 carries that mutation as its sole RBD mutation), Y453F, as found in mink isolates (Larsen et al., 2021),  
208 N439K, which is found in some European clades (Thomson et al., 2021), a combination of Y453F and  
209 N439K, E484K (part of B.1.351 and P.1) as well as for the B.1.351 and the P.1 RBDs for a recombinant  
210 version of human ACE2 (**Figure 4A, 4B and 4D**). Almost all of the single and double mutations in RBD tested  
211 increased affinity to human ACE2. Specifically, N501Y and Y453F combined with N439K increased affinity  
212 for human ACE2 by 5-fold (**Figure 4D, Suppl. Figure 3**). In contrast, E484K on its own decreased affinity by  
213 4-fold. Of note, the B.1.351 RBD affinity for ACE2 was comparable to that of the wild-type RBD. These data  
214 were confirmed using an ELISA-based method which showed the same trends (**Suppl. Figure 4**).

## 215 **Binding profiles of polyclonal serum and mAbs to RBDs carrying mutations found in viral variants of concern**

217 Next, we assessed binding of sera from vaccinated individuals, COVID-19 survivors and mAbs  
218 derived from plasmablasts to variant RBDs. Our panel of RBDs includes published mAb escape mutants,  
219 RBD mutants detected by the Mount Sinai Hospital's Pathogen Surveillance Program in patients seeking  
220 care at the Mount Sinai Health System in NYC as well as mutations found in viral variants of interest and  
221 variants of concern (Baum et al., 2020; Greaney et al., 2021; Larsen et al., 2021; Thomson et al., 2021;

222 Weisblum *et al.*, 2020). Serum from convalescent individuals showed strong fluctuations depending on  
223 the viral variant (**Figure 5A**). In general, single mutants E406Q, E484K and F490K exerted the biggest  
224 impact on binding. However, complete loss of binding was rare and 2-4-fold reduction in binding was more  
225 common. Interestingly, almost all sera bound better to N501Y RBD (B.1.1.7) than to wild-type RBD  
226 (average 129% compared to wild type). Conversely, the B.1.351 RBD caused, on average, a 39% reduction  
227 in binding. The impact was slightly lower for the P.1 RBD (average 70% binding compared to wild-type).  
228 For sera from the six vaccinated individuals, however, the highest reduction seen was only two-fold for  
229 E406Q, N440K, E484K and F490K (**Figure 5B**). Of note, the vaccinees' later samples (V1=d89, V2=d102,  
230 V3=d47, V4=d48, V5=49 and V6=48) were assayed to allow for some affinity maturation. The highest  
231 reduction observed for E484K, F484A, B.1.351 and P.1 were also approximately two-fold but this did not  
232 apply to all six vaccinees. Some vaccinees maintained binding levels against these RBDs at levels  
233 comparable to wild-type RBD.

234 RBD binding mAbs were also tested for binding to the same variants. In general, mAbs maintained  
235 binding levels within 2-fold of the binding seen with the wild-type RBD with some exceptions. In fact, for  
236 most mAbs, no impact on binding was observed (**Figure 5C**) with the exception of PVI.V3-9, which lost  
237 binding to the RBD carrying F486A. Although there was a negative impact on binding of several mAbs to  
238 the B.1.351 variant, binding was almost unaffected by the mutations in the P.1 variant RBD. Only one  
239 mAb, PVI.V6-4, showed a drop in binding to P.1.

#### 240 **Escape of an NTD and E484K mutant virus from polyclonal post-vaccination serum is negligible but** 241 **NTD mutations significantly impact the neutralizing activity of NTD binding mAbs**

242 Through the Mount Sinai Hospital's Pathogen Surveillance Program, we had access to the SARS-  
243 CoV-2 isolate PV14252 (Clade 20C, Pango lineage B.1) that featured two mutations (W64R, L141Y) and  
244 one deletion ( $\Delta$ 142-145) in the NTD as well as the E484K mutation in the RBD (**Figure 5D**). To determine  
245 the susceptibility of this virus variant to neutralization by post-vaccination serum, we performed  
246 microneutralization assays. Wild-type SARS-CoV-2 and PV14252 were tested in parallel to ensure that the  
247 assay setup for both viruses allowed comparison. We found a relatively minor impact when testing  
248 polyclonal sera from vaccinees for neutralizing activity (**Figure 5E**). The activity of sera from V2, V5 and V6  
249 slightly increased while the activity for V1, V3 and V4 decreased. Next, we tested the seven neutralizing  
250 mAbs that we isolated from plasmablasts. Consistent with their binding profiles in the variant RBD ELISA,  
251 the two RBD mAbs neutralized both viruses with comparable efficiency (**Figure 5F**). In fact, the activity of  
252 PVI.V3-9 increased slightly (**Figure 5F**). In stark contrast, all five anti-NTD antibodies completely lost  
253 neutralizing activity against PV14252 due to mutations present in the NTD of this viral isolate.

#### 254 **B.1.1.7 and B.1.351 partially escape from plasmablast derived neutralizing antibodies**

255 We also tested the neutralizing activity of the two RBD and the five NTD antibodies against the  
256 variants of concern B.1.1.7 and B.1.351 (**Figure 5G**). Both variants contain deletions as well as mutations  
257 in the NTD. In addition, B.1.1.7 carries the N501Y RBD mutation and B.1.351 carries N417K, E484K and  
258 N501Y mutations in the RBD (**Figure 4 A and B**). The two RBD binding antibodies lost no (PVI.V6-4) or little  
259 (PVI.V3-9) neutralizing activity against B.1.1.7. However, PVI.V3-9 lost all activity against B.1.351 and the  
260 remaining neutralizing activity of PVI.V6-4 was low (but measurable). All but one (PVI.V6-11) NTD mAbs  
261 lost neutralizing activity against B.1.1.7 and all of them lost neutralizing activity against B.1.351 once more  
262 highlighting the importance of changes in the NTD on the antibody activity.

263

## 264 Discussion

265 Our knowledge of B-cell responses to SARS-CoV-2 mRNA vaccination remains incomplete. We  
266 urgently need information about the nature of polyclonal vaccine-induced responses as well as unbiased,  
267 in depth analyses of plasmablast responses. Our data provide important new insights into these responses  
268 in comparison with immune responses to natural infection. Indeed, SARS-CoV-2 infection results in a very  
269 heterogeneous antibody response to the spike protein in terms of antibody quantity. In contrast, mRNA  
270 vaccination appears to induce a high antibody response of relatively homogenous titers. However, we  
271 also found that vaccinees generate more non-neutralizing antibodies than COVID-19 survivors resulting in  
272 a lower ratio of neutralizing to binding antibodies. These data were already apparent in the early phase  
273 clinical trials but remained unrecognized at the time (Walsh et al., 2020). Interestingly, low titer  
274 convalescent serum had the highest relative amount of neutralizing antibodies, whereas the proportion  
275 of binding antibodies was increased in sera with higher measured antibody titers. The majority of  
276 plasmablasts sampled after vaccination do, in fact, produce non-neutralizing antibodies. Two recent  
277 studies have performed a similarly unbiased plasmablast analysis for individuals naturally infected with  
278 SARS-CoV-2 (Cho et al., 2021; Huang *et al.*, 2021). Of course, the antibody response after SARS-CoV-2  
279 infection is not only targeting the spike protein but several other proteins expressed by the virus. When  
280 accounting for spike binding only, these studies report proportions of 44% and 25% neutralizing antibodies  
281 (Cho *et al.*, 2021; Huang *et al.*, 2021). While plasmablast analysis is in general not quantitative (e.g. one  
282 clone per clonotype is selected etc.) our analysis of post-vaccination plasmablasts found a lower number  
283 of neutralizing antibodies (17%).

284 Future studies are needed to reveal the role of non-neutralizing antibodies in SARS-CoV-2 immune  
285 protection. Indeed, antibody functions other than neutralization have been shown to correlate with  
286 protection (Bartsch et al., 2021; Gorman et al., 2021; Schäfer et al., 2021). The importance of absolute  
287 antibody titers and not ratios is underscored by the fact that post-vaccination neutralization titers were  
288 equal to or exceeded the titers found in the high responder convalescent group.

289 Of the four seasonal CoVs that are widely circulating in humans,  $\beta$ -coronaviruses OC43 and HKU1 have  
290 higher homology to SARS-CoV-2 spike. Vaccinated individuals mounted a response to spike proteins from  
291 OC43 and HKU1 but not to  $\alpha$ -coronaviruses 229E and NL63. This phenomenon resembles the immune  
292 imprinting described in influenza virus immunology and has already been shown for natural infection with  
293 SARS-CoV-2 where a ‘backboost’ to  $\beta$ -coronaviruses was also found (Aydillo *et al.*, 2020; Song *et al.*, 2020).  
294 A few of the mAbs isolated in our study had, indeed, such a cross-reactive phenotype. It remains unclear  
295 whether these antibodies, which target mostly S2 epitopes, contribute to protection against SARS-CoV-2,  
296 OC43 or HKU1 infection. However, the cross-reactive epitopes of mAbs that do bind SARS-CoV-2, HKU1  
297 and OC43 spikes could provide the basis for future pan- $\beta$ -coronavirus vaccines. While it is likely the case  
298 that the B-cells producing these mAbs come from recall responses and were initially induced by human  $\beta$ -  
299 coronaviruses (which is supported by serology and of course the extensive SHM that the mAbs show),  
300 they could hypothetically also be *de novo* induced antibodies. While this is probably not the case, we  
301 cannot exclude this possibility with our current data.

302

303 Another interesting point we noted is the co-dominance of RBD and NTD. Previous analyses of B-  
304 cell responses to SARS-CoV-2 mRNA vaccination focused on cells baited by labeled RBD (Wang *et al.*,  
305 2021). We, in contrast, took an unbiased approach to sort and clone plasmablasts in an antigen-agnostic



306 manner. We found similar levels of NTD and RBD binders with many mAbs binding to epitopes outside the  
307 RBD and the NTD. In one vaccinee not a single RBD binding mAbs was isolated with the caveats that the  
308 overall number of mAbs derived from that individual were low and their polyclonal serum antibody  
309 responses included RBD recognition. These data suggest that the NTD, which also harbors neutralizing  
310 epitopes, is - at least - as important as the RBD and warrants as much attention. In fact, five out of seven  
311 neutralizing antibodies isolated in this study bound to the NTD and only two targeted the RBD. Recent  
312 studies analyzing the plasmablast response after natural infection have found a similar co-dominance of  
313 RBD and NTD (Cho *et al.*, 2021; Huang *et al.*, 2021) with one study reporting 59 mAbs targeting the RBD,  
314 64 targeting the NTD and 46 binding outside of RBD and NTD and the second study finding 10 RBD mAbs,  
315 13 non-RBD S1 binding mAbs (strongly suggesting NTD binding) and 9 mAbs targeting S2. Further  
316 characterization of the mAbs obtained in our study showed a complete loss of neutralization against an  
317 authentic, replication-competent variant virus that harbored extensive changes in the NTD. All NTD mAbs  
318 also lost neutralizing activity against B.1.351 and all but one lost activity against B.1.1.7. These  
319 observations may explain why a reduction in neutralization against the viral variant of concern B.1.1.7 is  
320 seen in some studies despite the fact the N501Y substitution in the RBD of this variant does not  
321 significantly impact binding and neutralizing activity (Emary *et al.*, 2021). The key role of NTD as target for  
322 antibodies has recently also been shown using memory B cell derived mAbs (McCallum *et al.*, 2021a).

323 In addition, we assessed the impact of different RBD mutations on affinity towards human ACE2.  
324 Interestingly, N501Y increased the affinity by five-fold. This increase in receptor binding affinity may  
325 contribute to the higher infectivity of B.1.1.7, which carries this mutation in its RBD. In contrast,  
326 introduction of E484K reduced the affinity by 4-fold which may explain why virus variants carrying only  
327 the E484K mutation have rarely spread efficiently, although viruses carrying E484K have been detected  
328 since the fall of 2020 in a handful of patients receiving care at the Mount Sinai Health System and have  
329 also been reported in immunocompromised patients (Choi *et al.*, 2020). It is tempting to speculate that  
330 the N501Y mutation enables the acquisition of E484K without a fitness loss. In fact, the B.1.351 RBD, which  
331 carries N501Y and E484K (as well as N417K) showed binding to hACE2 that was similar to wild- type RBD.  
332 Recently, B.1.1.7 variant strains carrying E484K, in addition to N501Y, have been isolated in the UK (PHE,  
333 2021), providing evidence for the hypothesis that N501Y enables acquisition of mutations in the RBD that  
334 may be detrimental to receptor binding. However, recent expansion of B.1.526, a lineage also featuring  
335 E484K but without N501Y in New York City, suggests that this fitness loss may be overcome by other, yet  
336 uncharacterized, changes in the virus as well (Annavajhala *et al.*, 2021; Lasek-Nesselquist *et al.*, 2021).  
337 Interestingly, binding of convalescent sera to the N501Y RBD was also increased, suggesting that changes  
338 that increase affinity for the receptor may also increase affinity of a set of antibodies that may mimic the  
339 receptor.

340 We also noted that the two neutralizing antibodies against the RBD showed some reduced binding  
341 to a mutant RBD carrying the E484K mutation while having similar or even increased neutralizing potency  
342 against a variant virus carrying the E484K mutation as the only change in its RBD. The reduced affinity of  
343 the E484K variant RBD for hACE2 could render the virus more susceptible to RBD binding mAbs. Thus, an  
344 antibody binding to the RBD may just be more effective in interfering with a low affinity as compared to  
345 a high affinity RBD-hACE2 interaction. Increased affinity as an escape mechanism for viruses has been  
346 described in the past (Hensley *et al.*, 2009; O'Donnell *et al.*, 2012) and the converse mechanism could be  
347 at play here.

348 Whether or not the current vaccines will provide effective protection against circulating and  
349 emerging viral variants of concern is an important question which has gathered a lot of attention in early  
350 2021. Our data indicate that reduction in binding to the E484K and B.1.351 variant RBDs was minor (often  
351 only 2-fold) compared to reported reduction in neutralization (which ranges from 6-8 fold to complete  
352 loss of neutralization (Cele et al., 2021; Wibmer et al., 2021; Wu et al., 2021)). Although not tested here,  
353 it is likely that the reduction in binding to full length spike is even lower, given the many epitopes on the  
354 spike other than NTD and RBD. The maintenance of binding to a large degree observed in this study  
355 suggests that viral variants will have a minor impact on serological assays which are currently in wide use  
356 for medical, scientific and public health reasons. Binding, non-neutralizing antibodies have also been  
357 shown to have a protective effect in many viral infections (Asthagiri Arunkumar et al., 2019; Dilillo et al.,  
358 2014; Saphire et al., 2018) and may be a factor in the substantial residual protection seen in the Johnson  
359 & Johnson and Novavax vaccine trials against B.1.351 in South Africa (Shinde et al., 2021). Production of  
360 non-neutralizing antibodies may also play a role in protection by mRNA vaccines after the first dose, as it  
361 is substantial and occurs during a time when neutralizing antibody titers are either very low or absent  
362 (Baden et al., 2020; Dagan et al., 2021; Polack et al., 2020). Finally, although some antibodies may lose  
363 neutralizing activity due to reduced affinity, they do still bind. Furthermore, B cells with these specificities  
364 potentially could undergo affinity maturation after exposure to a variant virus or a variant spike-  
365 containing vaccine, leading to high affinity antibodies to variant viruses of concern.

366 In summary, we demonstrate that the antibody responses to SARS-CoV-2 mRNA vaccination comprise a  
367 large proportion of non-neutralizing antibodies and are co-dominated by NTD and RBD antibodies. The  
368 NTD portion of the spike represents, thus, an important vaccine target. Since all viral variants of concern  
369 are heavily mutated in this region, these observations warrant further attention to optimize SARS-CoV-2  
370 vaccines. Finally, broadly cross-reactive mAbs to  $\beta$ -coronavirus spike proteins are induced after  
371 vaccination, and suggest a potential development path for a pan-  $\beta$ -coronavirus vaccine.

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## 388 **Conflict of interest statement**

389 The Icahn School of Medicine at Mount Sinai has filed patent applications relating to SARS-CoV-2  
390 serological assays and NDV-based SARS-CoV-2 vaccines which list Florian Krammer as co-inventor. Viviana  
391 Simon is also listed on the serological assay patent application as co-inventors. Mount Sinai has spun out  
392 a company, Kantaro, to market serological tests for SARS-CoV-2. Florian Krammer has consulted for Merck  
393 and Pfizer (before 2020), and is currently consulting for Pfizer, Seqirus and Avimex. The Krammer  
394 laboratory is also collaborating with Pfizer on animal models of SARS-CoV-2. Ali Ellebedy has consulted for  
395 InBios and Fimbrion Therapeutics (before 2021) and is currently a consultant for Mubadala Investment  
396 Company. The Ellebedy laboratory received funding under sponsored research agreements that are  
397 unrelated to the data presented in the current study from Emergent BioSolutions and from AbbVie.  
398

## 399 **Materials and methods.**

400 **Human subjects and specimen collection.** The study protocols for the collection of clinical specimens from  
401 individuals with and without SARS-CoV-2 infection by the Personalized Virology Initiative were reviewed  
402 and approved by the Mount Sinai Hospital Institutional Review Board (IRB-16-16772; IRB-16-00791; IRB-  
403 20-03374). All participants provided written informed consent prior to collection of specimen and clinical  
404 information. All specimens were coded prior to processing and analysis. An overview of the characteristics  
405 of the vaccinees as well as the study participants with and without COVID-19 is provided in **Suppl. Table**  
406 **1**. The vaccinees received two doses of the Pfizer mRNA vaccine.

407 Whole blood was collected via phlebotomy in serum separator tubes (SST) or ethylenediaminetetraacetic  
408 acid (EDTA) tubes. Serum was collected after centrifugation as per manufacturers' instructions. Peripheral  
409 blood mononuclear cells (PBMCs) isolation was performed by density gradient centrifugation using  
410 SepMate tubes (Stemcell) according to manufacturers' instructions. PBMCs were cryo-preserved and  
411 stored in liquid nitrogen until analysis.

412 **Recombinant proteins.** All recombinant proteins were produced using Expi293F cells (Life Technologies).  
413 Receptor binding domain (RBD) and spike protein of SARS-CoV-2 (GenBank: MN908947.3) was cloned into  
414 a mammalian expression vector, pCAGGS as described earlier (Amanat et al., 2020b; Stadlbauer et al.,  
415 2020). RBD mutants were generated in the pCAGGS RBD construct by changing single residues using  
416 mutagenesis primers. All proteins were purified after transient transfections with each respective plasmid.  
417 Six-hundred million Expi293F cells were transfected using the ExpiFectamine 293 Transfection Kit and  
418 purified DNA. Supernatants were collected on day four post transfection, centrifuged at 4,000 g for 20  
419 minutes and finally, the supernatant was filtered using a 0.22  $\mu$ m filter. Ni-NTA agarose (Qiagen) was used  
420 to purify the protein via gravity flow and proteins were eluted as previously described (Amanat *et al.*,  
421 2020b; Stadlbauer *et al.*, 2020). The buffer was exchanged using Amicon centrifugal units (EMD Millipore)  
422 and all recombinant proteins were finally re-suspended in phosphate buffered saline (PBS). Proteins were  
423 also run on a sodium dodecyl sulphate (SDS) polyacrylamide gels (5–20% gradient; Bio-Rad) to check for  
424 purity (Amanat et al., 2018; Margine et al., 2013). Plasmids to express recombinant spike proteins of 229E,  
425 HKU1, NL63 and OC43 were generously provided by Dr. Barney Graham (Pallesen et al., 2017). NTD and  
426 S2 proteins were purchased from SinoBiologics.

427  
428 **ELISA.** Ninety-six well plates (Immulon 4 HBX; Thermo Scientific) were coated overnight at 4°C with  
429 recombinant proteins at a concentration of 2  $\mu$ g/ml in PBS (Gibco; Life Technologies) and 50  $\mu$ l/well. The  
430 next day, the coating solution was discarded. One hundred  $\mu$ l per well of 3% non-fat milk prepared in PBS

431 (Life Technologies) containing 0.01% Tween-20 (TPBS; Fisher Scientific) was added to the plates to block  
432 the plates for 1 hour at room temperature (RT). All serum dilutions were prepared in 1% non-fat milk  
433 prepared in TPBS. All serum samples were diluted 3-fold starting at a dilution of 1:50. After the blocking  
434 step, serum dilutions were added to the respective plates for two hours at RT. Next, plates were washed  
435 thrice with 250  $\mu$ l/well of TPBS to remove any residual primary antibody. Secondary antibody solution  
436 was prepared in 1% non-fat milk in TPBS as well and 100  $\mu$ l/well was added to the plates for 1 hour at  
437 RT. For human samples, anti-human IgG conjugated to horseradish peroxidase (HRP) was used at a  
438 dilution of 1:3000 (Millipore Sigma; catalog #A0293). For mouse samples, anti-mouse IgG conjugated to  
439 HRP was used at the same dilution (Rockland antibodies and assays; catalog #610-4302). Specifically, a  
440 mouse anti-histidine antibody (Takara; catalog #631212) was used as a positive control to detect proteins  
441 with a hexa-histidine tag. Once the secondary incubation was done, plates were again washed thrice with  
442 250  $\mu$ l/well of TPBS. Developing solution was made in 0.05M phosphate-citrate buffer at pH 5 using o-  
443 phenylenediamine dihydrochloride tablets (Sigma-Aldrich; OPD) at a final concentration of 0.04 mg/ml.  
444 One hundred  $\mu$ l/well of developing solution was added to each plate for exactly 10 minutes after which  
445 the reaction was halted with addition of 50  $\mu$ l/well of 3M hydrochloric acid (HCl). Plates were read at an  
446 optical density of 490 nanometers using a Synergy 4 (BioTek) plate reader. Eight wells on each plate  
447 received no primary antibody (blank wells) and the optical density in those wells was used to assess  
448 background. Area under the curve was calculated by deducting the average of blank values plus 3 times  
449 standard deviation of the blank values. All data was analyzed in Graphpad Prism 7. This protocol has been  
450 described in detail earlier (Bailey et al., 2019; Wohlbold et al., 2015).

451 Purified monoclonal antibodies were used at a concentration of 30  $\mu$ g/ml and then subsequently diluted  
452 3-fold. Purified monoclonal antibodies were only incubated on the coated plates for an hour. The  
453 remaining part of the protocol was the same as above (Amanat et al., 2020a; Wohlbold et al., 2016).

454 **Bio-layer Interferometry Binding Experiments.** Bio-layer Interferometry (BLI) experiments were  
455 performed using the BLItz system (fortéBIO, Pall Corporation). Recombinant human Fc fusion ACE2  
456 (SinoBiological) was immobilized on an anti-human IgG Fc biosensor, and RBDs were then applied to  
457 obtain binding affinities. Single-hit concentrations were tested at 5.8  $\mu$ M for binding. All measurements  
458 were repeated in subsequent independent experiments.  $K_D$  values were obtained through local fit of the  
459 curves by applying a 1:1 binding isotherm model using vendor-supplied software. All experiments were  
460 performed in PBS pH 7.4 and at room temperature.

461  
462 **hACE2 competition interferometry experiments,** Interferometry experiments were performed using a  
463 BLItz instrument (fortéBIO, Sartorius). Polyhistidine-tagged Fabs were immobilized on Ni-NTA biosensors  
464 at 10  $\mu$ g/ml and SARS-CoV-2 RBD was supplied as analyte at 5  $\mu$ M alone or pre-mixed with hACE2-Fc at  
465 different concentrations. Maximal signal at association ( $R_{max}$ ) was used to plot the concentration-  
466 dependent competition with hACE2. All experiments were performed in PBS at pH 7.4 and at room  
467 temperature.

468  
469 **RBD-hACE2 ELISA.** 25ng of hACE2-Fc fusion protein expressed in HEK293 cells were adhered to high-  
470 capacity binding, 96 well-plates (Corning) overnight in PBS. Plates were blocked with 5% BSA in PBS  
471 containing Tween-20 (PBS-T) for 1hr at room temperature (RT). Blocking solution was discarded and 5-  
472 fold dilutions of 6xHis-tagged RBDs in PBS were added to wells and incubated for 1hr at RT. Plates were  
473 then washed three times with PBS-T. Anti-polyhistidine IgG-Biotin (Abcam) in PBS-T was added to each  
474 and incubated for 1hr at RT. Plates were then washed three times with PBS-T. Streptavidin-HRP (Abcam)  
475 in PBS-T was added to each and incubated for 1hr at RT. Plates were then washed three times with PBS-T

476 Plates were developed using 1-Step Ultra TMB substrate (ThermoFisher), stopped with sulfuric acid and  
477 immediately read using a plate reader at 450nm. Data were plotted using Prism 9 (GraphPad Software)  
478 and affinities determined by applying a nonlinear regression model.

479  
480 **Viruses and cells.** Vero.E6 cells (ATCC #CRL-1586) cells were maintained in culture using Dulbecco's  
481 Modified Eagles Medium (DMEM, Gibco) which was supplemented with 10% fetal bovine serum (FBS,  
482 Corning) and antibiotics solution containing 10,000 units/mL of penicillin and 10,000 µg/mL of  
483 streptomycin (Pen Strep, Gibco)(10). Wild type SARS-CoV-2 (isolate USA-WA1/2020), hCoV-19/South  
484 Africa/KRISP-K005325/2020 (B.1.351, BEI Resources NR-54009) and hCoV-19/England/204820464/2020  
485 (B.1.1.7, BEI Resources NR-54000) were grown in cells for 3 days, the supernatant was clarified by  
486 centrifugation at 4,000 g for 5 minutes and aliquots were frozen at -80°C for long term use. The viruses  
487 were subjected to deep sequencing to ensure that no mutations had taken place in culture. The polybasic  
488 cleavage site changed to WRAR in the B.1.351 variant virus during cultivation in cell culture (as known for  
489 this virus at BEI Resources) and no other unexpected mutations occurred. A primary virus isolate,  
490 PV14252, bearing mutations and deletions in the spike was obtained by incubating 200 uls of viral  
491 transport media from the nasopharyngeal swab with Vero.E6 cells. The sequence of the passage 2 viral  
492 isolate was identical to the sequence obtained directly from the clinical specimen. Sequencing was  
493 performed on the Illumina platform as described previously (Gonzalez-Reiche et al., 2020). Both  
494 replication competent viruses were used to test serum from study participants and antibodies for  
495 neutralization activity.

496  
497 **Neutralization assay.** Twenty-thousand cells in 100 uls per well were seeded on sterile 96-well cell culture  
498 plates one day prior to the neutralization assay. In general, cells were used at 90% confluency to perform  
499 the assay. All serum samples were heat-inactivated to eliminate any complement activity. Serial dilutions  
500 of serum samples were made in 1X minimal essential medium (MEM; Life Technologies) starting at a  
501 dilution of 1:20. All work with authentic SARS-CoV-2 (isolate USA-WA1/2020 and PV14252) was done in a  
502 biosafety level 3 (BSL3) laboratory following institutional biosafety guidelines and has been described in  
503 much greater detail earlier (Amanat *et al.*, 2020b; Amanat et al., 2020c). Six hundred median cell culture  
504 infectious doses (TCID<sub>50</sub>s) of authentic virus (USA-WA1/2020 and PV14252) was added to each serum  
505 dilution and virus-serum mixture was incubated together for 1 hour inside the biosafety cabinet. Media  
506 from the cells was removed and 120 uls of the virus-serum mixture was added onto the cells for 1 hour at  
507 37°C. After one hour, the virus-serum mixture was removed and 100 uls of each corresponding dilution  
508 was added to every well. In addition, 100ul of 1X MEM was also added to every well. Cells were incubated  
509 for 48 hours at 37°C after which the media was removed and 150 uls of 10% formaldehyde (Polysciences)  
510 was added to inactivate the virus. For assay control, remdesivir was used against both the wild type virus  
511 as well as the patient isolate. After 24 hours, cells were permeabilized and stained using an anti-  
512 nucleoprotein antibody 1C7 as discussed in detail earlier (Amanat *et al.*, 2020b; Sun et al., 2020).

513 **Cell sorting and flow cytometry.** Staining for sorting was performed using cryo-preserved PBMCs in 2%  
514 FBS and 2 mM ethylenediaminetetraacetic acid (EDTA) in PBS (P2). Cells were stained for 30 min on ice  
515 with CD20-Pacific Blue (2H7, 1:400), Zombie Aqua, CD71-FITC (CY1G4, 1:200), IgD-PerCP-Cy5.5 (IA6-2,  
516 1:200), CD19-PE (HIB19, 1:200), CD38-PE-Cy7 (HIT2, 1:200), and CD3-Alexa 700 (HIT3a, 1:200), all  
517 BioLegend. Cells were washed twice, and single plasmablasts (live singlet CD19<sup>+</sup> CD3<sup>-</sup> IgD<sup>lo</sup> CD38<sup>+</sup> CD20<sup>-</sup>  
518 CD71<sup>+</sup>) were sorted using a FACS Aria II into 96-well plates containing 2 µL Lysis Buffer (Clontech)  
519 supplemented with 1 U/µL RNase inhibitor (NEB) and immediately frozen on dry ice, or bulk sorted into  
520 PBS supplemented with 0.05% BSA and processed for single cell RNAseq.

521 **Monoclonal antibody (mAb) generation.** Antibodies were cloned as described previously (Wrammert et  
522 al., 2011). Briefly, VH, Vk, and Vλ genes were amplified by reverse transcription-PCR and nested PCR

523 reactions from singly sorted plasmablasts using primer combinations specific for IgG, IgM/A, Igκ, and Igλ  
524 from previously described primer sets (Smith et al., 2009) and then sequenced. To generate recombinant  
525 antibodies, restriction sites were incorporated via PCR with primers to the corresponding heavy and light  
526 chain V and J genes. The amplified VH, Vk, and Vλ genes were cloned into IgG1 and Igκ expression vectors,  
527 respectively, as described previously (Nachbagauer et al., 2018; Wrammert et al., 2008). Heavy and light  
528 chain plasmids were co-transfected into Expi293F cells (Gibco) for expression, and antibody was purified  
529 with protein A agarose (Invitrogen).

530 **Single-cell RNAseq library preparation and sequencing.** Bulk-sorted plasmablasts were processed using  
531 the following 10× Genomics kits: Chromium Next GEM Single Cell 5' Kit v2 (PN-1000263); Library  
532 Construction Kit (PN-1000190); Chromium Next GEM Chip K Single Cell Kit (PN-1000286); Chromium Single  
533 Cell Human BCR Amplification Kit (PN-1000253), and Dual Index Kit TT Set A (PN-1000215). The cDNAs  
534 were prepared after GEM generation and barcoding, followed by GEM RT reaction and bead cleanup  
535 steps. Purified cDNA was amplified for 10–14 cycles before cleaning with SPRIselect beads. Then, samples  
536 were evaluated on a 4200 TapeStation (Agilent) to determine cDNA concentration. B-cell receptor (BCR)  
537 target enrichments were performed on full-length cDNA. Gene expression and enriched BCR libraries were  
538 prepared as recommended by the Chromium Next GEM Single Cell 5' Reagent Kits v2 (Dual Index) user  
539 guide, with appropriate modifications to the PCR cycles based on the calculated cDNA concentration. The  
540 cDNA libraries were sequenced on Novaseq S4 (Illumina), targeting a median sequencing depth of 50,000  
541 and 5,000 read pairs per cell for gene expression and BCR libraries, respectively.

542 **Single cell RNAseq analysis.** Single-cell RNA sequencing and BCR sequencing data was processed using  
543 Cell Ranger v5.0 and the GRCh38-2020 version of the human genome provided by the manufacturer. Total  
544 recovered cells by RNA sequencing were V3: 6,608, V5: 5,256, and V6: 6,325 with a mean of 90.64% read  
545 mapped to the genome. Count matrices were processed in R (v4.0.2) using the Seurat (v3.2.2) R package  
546 (Stuart et al., 2019). Cells were filtered for percentage of mitochondrial genes less than 15% and number  
547 features less than 4,000. The three specimen sequencing runs were integrated using log-normalized count  
548 values and canonical correlation approach (Stuart *et al.*, 2019) with 2,000 variable features. The resulting  
549 single-cell object underwent principal component analysis and the top 30 principal components were used  
550 for uniform manifold approximation and projection and identifying neighbors. Clustering was performed  
551 using a resolution of 0.6. The integrated RNA sequencing object included 12,568 cells with V3: 4,584, V5:  
552 3,523, and V6: 4,461 cells. The filtered contig annotation output of Cell Ranger v2 were loaded into R and  
553 processed using the scRepertoire (v1.1.3) R package (Borcherding et al., 2020). Clonotypes were assigned  
554 using igrph (v1.2.6) network analysis of components generated from CDR3 sequences greater than or  
555 equal to 0.85 normalized Levenshtein distance. Percent of cells expressing genes along the UMAP  
556 embedding was visualized using the schex (v1.3.0) R package. For mutation analysis, heavy chains of mAbs  
557 and single-cell BCRs first underwent V(D)J gene annotation using IgBLAST (v1.14.0) (Ye et al., 2013) with  
558 human reference (release 201931-4) from the international ImMunoGeneTics information system (IMGT)  
559 (Giudicelli et al., 2005) and then parsing using Change-O (v0.4.6) (Gupta et al., 2015). Mutation frequency  
560 was calculated, as described in (Turner *et al.*, 2020), using the "calcObservedMutations" function from  
561 SHazaM (v.1.0.2) (Gupta *et al.*, 2015) and by counting the number of nucleotide mismatches from the  
562 germline sequence in the heavy chain variable segment leading up to the complementary-determining  
563 region 3 (CDR3), while excluding the first 18 positions that could be error-prone due to the primers used  
564 for generating the mAb sequences.

565

566 **Structure visualization and statistical analysis.** Structural figures were modeled and rendered in Pymol  
567 (The PyMOL Molecular Graphics System, Version 2.4 Schrödinger, LLC). Statistical analysis was performed  
568 in GraphPad Prism using a one-way ANOVA with correction for multiple comparisons.

569

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822

## 823 **Figure Legends**

824 **Figure 1: Antibody responses in individuals vaccinated with mRNA-based SARS-CoV-2 vaccines.**  
825 Antibody responses of convalescent individuals and vaccinees to full length spike protein (A) and RBD (B)  
826 as measured by ELISA and neutralizing activity of the sera of the same individuals in a microneutralization  
827 assay against authentic SARS-CoV-2 (C). Convalescent individuals were grouped based on their initial  
828 antibody response (measured in a CLIA laboratory) to spike protein into +, ++, and +++. D shows ratios  
829 between binding and neutralizing antibody levels in vaccinees and convalescent individuals. Higher ratios  
830 indicate a bias towards non-neutralizing antibodies. E, F, G and H show antibody responses against  $\alpha$ -  
831 coronavirus 229E and NL63 and  $\beta$ -coronavirus OC43 and HKU1 spike proteins over time.

832

833 **Figure 2. Characterization of mAbs derived from vaccine plasmablasts.** Binding of plasmablasts derived  
834 from three vaccinees (V3, V5 and V6) against full length spike (A), RBD (B), NTD (C) and S2 (D). E shows  
835 the percentages of the respective antibodies per subject. F shows neutralizing activity of the mAbs against  
836 authentic SARS-CoV-2 and the proportion of neutralizing antibodies per subject is shown in G. H and I  
837 show reactivity of mAbs to spike protein of human  $\beta$ -coronaviruses OC43 and HKU1. MBC = minimal  
838 binding concentration.

839

840 **Figure 3. Characterization of bulk sorted plasmablasts via single-cell RNA sequencing. (A)** Uniform  
841 manifold approximation and projection (UMAP) of scRNAseq from bulk plasmablast with recovered BCR  
842 sequences (purple) or unrecovered (grey). **(B)** UMAP overlay of percent of cellular population expressing  
843 *MZB1*, *PRDM1*, and *XPB1*. Hexbin equals 80 individual cells. **(C)** UMAP overlay of BCR sequences with  
844 confirmed spike binding activity. **(D)** Proportional composition of heavy chains genes in the spike binding  
845 sequences broken down by sample. **(E)** Comparison of nucleotide-level mutation frequency in  
846 immunoglobulin heavy chain variable (IGHV) genes between plasmablasts clonally related to spike binding  
847 mAbs from SARS-CoV-2 vaccinees, plasmablasts sorted from PBMCs one week after seasonal influenza  
848 vaccination and found in vaccine-responding B cell clones, and naïve B cells found in blood of an influenza  
849 vaccinee (left panel); and between plasmablasts from SARS-CoV-2 vaccinees found to be clonally related  
850 to spike-binding mAbs that were, respectively, cross-reactive and non-cross-reactive to human  $\beta$ -  
851 coronaviruses spike proteins (right panel).

852

853 **Figure 4. Mapping of the amino-acid substitutions and deletions onto the structure of the SARS-CoV-2**  
854 **spike glycoprotein. A** lists mutations of the three major variants of concern B.1.17, B.1.315 and P.1. **B**  
855 shows these mutations mapped onto the structure of the spike glycoprotein (model generated by  
856 superposition of PDB 6M0j and 7C2L (Chi *et al.*, 2020; Lan *et al.*, 2020)). One RBD in the up conformation  
857 (red) is bound with ACE2 receptor (pink). The NTD is colored blue and the various amino-acid substitutions  
858 are shown as yellow spheres. One spike protomer is shown in bold colors while the other two are colored  
859 white. **C** shows competition between ACE2 and neutralizing RBD targeting mAbs PVI.V3-9 and PVI.V6-4  
860 for binding to RBD. **D** BLI- measured binding affinities of the RBD mutants to ACE2, as well as the calculated  
861 fold change, are shown in the table on the right.

862

863 **Figure 5. Binding and neutralization of SARS-CoV-2 variants.** Binding of serum samples from convalescent  
864 individuals, vaccinees and vaccine derived mAbs to a panel of RBD mutants is shown in **A, B and C**  
865 respectively. The red line in A indicates the average reduction. Dotted lines in A and B indicate 100%, the  
866 line with smaller dots in C indicated reactivity of the anti-his coating control. For vaccinees late samples  
867 (V1=d89, V2=d102, V3=d47, V4=d48, V5=49 and V6=48) were assayed. **D** shows the spike mutations of  
868 virus isolate PVI14252 modelled on a co-crystal structure of the SARS-CoV-2 spike protein with ACE2  
869 (model generated by superposition of PDB 6M0j and 7C2L (Chi *et al.*, 2020; Lan *et al.*, 2020)). **E and F** show  
870 the inhibitory effect of vaccine serum and vaccine derived neutralizing antibodies on both wild type SARS-  
871 CoV-2 and PV14252. **G** shows neutralizing activity of the plasmablast derived neutralizing antibodies  
872 against wild type, B.1.1.7 and B.1.351 virus isolates. Of note, these comparative assays were always  
873 performed side by side but sets are run by different operators and on a different Vero cell clone as the  
874 neutralization assays shown in Figure 2.

875

876

877 **Supplemental Table 1: Study participant and biospecimen information**

Vaccinees	Spike IgG response <sup>1*</sup>	Sex	Age group (yrs)	Specimen tested
-----------	----------------------------------	-----	-----------------	-----------------

V1	Strong positive	F	>60	several longitudinal time points including days 34, 68, 89 and 188 post vaccination
V2	Strong positive	M	30-40	several longitudinal time points including days 19, 47, 74, 102, 129, 157 and 186 post vaccination
V3	Strong positive	F	50-60	several longitudinal time points including days 19, 27, 47, 77 and 102 post vaccination
V4	Strong positive	M	>60	several longitudinal time points including days 27, 48 and 89 post vaccination
V5	Strong positive	F	40-50	several longitudinal time points including 27, 49 and 83 days post vaccination
V6	Strong positive	F	30-40	several longitudinal time points including 27, 48 and 83 days post vaccination
<b>Seronegative, post pandemic</b>		<b>Sex</b>	<b>Age group (yrs)</b>	<b>Days from last negative serology test when the sample was taken</b>
N1	Negative	F	40-50	23
N2	Negative	F	20-29	24
N3	Negative	F	20-29	23
N4	Negative	F	30-35	22
<b>Seropositive, natural infection</b>		<b>Sex</b>	<b>Age group (yrs)</b>	<b>Days post onset of COVID-19 symptoms when the sample was taken</b>
P1	Weak positive	M	20-29	260
P2	Weak positive	M	50-59	no data available
P3	Weak positive	F	30-39	111
P4	Weak positive	F	30-39	221

P5	Weak positive	F	30-39	254
P6	Weak positive	F	20-29	247
P7	Weak positive	M	30-39	220
P8	Weak positive	F	20-29	Asymptomatic
P9	Moderate positive	M	30-39	no data available
P10	Moderate positive	F	30-39	197
P11	Moderate positive	F	50-59	Asymptomatic
P12	Moderate positive	F	30-39	Asymptomatic
P13	Moderate positive	M	30-39	234
P14	Moderate positive	F	20-29	273
P15	Moderate positive	M	30-39	Asymptomatic
P16	Moderate positive	F	20-29	258
P17	Moderate positive	F	20-29	246
P18	Moderate positive	M	20-29	Asymptomatic
P19	Moderate positive	F	50-59	204
P20	Strong positive	F	50-59	no data available
P21	Strong positive	F	30-39	245
P22	Strong positive	M	NA	170
P23	Strong positive	F	>60	Asymptomatic
P24	Strong positive	F	40-49	no data available
P25	Strong positive	F	50-59	191
P26	Strong positive	F	30-39	no data available
P27	Strong positive	F	50-59	113
P28	Strong positive	M	>60	Asymptomatic
P29	Strong positive	M	18-19	218
P30	Strong positive	M	50-59	219

878 <sup>1</sup>Samples were categorized based on initial titers obtained from Mount Sinai's CLIA laboratory test. Weak  
879 positive: 1:80 – 1:160 weak positive; 1:320-1:960 moderate positive; 1:960-1: ≥2880 strong positive

880 \* All six vaccinees were sero-negative for SARS-CoV-2 without clinical evidence of COVID10 prior to SARS-  
881 CoV-2 spike mRNA vaccination.

882

883 **Supplemental Table 2: Immunoglobulin gene usage of the spike-mAbs**

Name	Native isotype	Heavy chain		Light chain	
		Gene usage	HCDR3 AA sequence	Gene usage	LCDR3 AA sequence
PVI.V5-1	IgG1	VH3-23 DH5-18 JH4	CAPHRGQLWFDYW	VK3-20 JK4	CQQYGSSPPTF
PVI.V5-2	IgG1	VH3-21 DH2-2 JH4	CARDLKLSPAAIGWDYFDYW	VK3-15 JK2	CQQYNNWPRSF
PVI.V5-3	IgG1	VH3-7 DH6-13 JH4	CAIFGAAGTDYW	VL3-16 JL3	CLSADSSGTYWVF
PVI.V5-4	IgG1	VH3-30 DH3-22 JH4	CARENYYDSSGYSYFDYW	VK3-20 JK2	CQQYGSSPMCSF
PVI.V5-5	IgA1	VH1-69 DH5-24 JH4	CARDFGREWLQYFYFDCW	VK3-20 JK4	CQQYGSSPPTF

PVI.V5-6	IgG1	VH3-7 DH3-3 JH4	CARDNNDFWSGYLYFDYW	VL3-10 JL2	CYSTDSSGNHRGVF
PVI.V6-1	IgG1	VH3-30 DH6-19 JH4	CARGAVAGQHSFDNW	VK2-30 JK4	CMQGTHWPPPTF
PVI.V6-2	IgG1	VH3-33 DH6-13 JH4	CARDKRGSSSWLDQYFDYW	VL3-21 JL2	CQVWDSSSDHVVF
PVI.V6-3	IgG1	VH4-31 DH3-22 JH5	CARDMISGRGLFDPW	VK1-33 JK2	CQQYDNLPTF
PVI.V6-4	IgG1	VH1-69 DH4-17 JH3	CARGNYDYGDYLLKGSADFIDW	VK4-1 JK2	CQQYYSTPPNTF
PVI.V6-5	IgG1	VH3-30 DH3-10 JH4	CAKDGGYYYGSGSYPSYFDYW	VK2D-29 JK4	CMQSIQLPLTF
PVI.V6-6	IgG1	VH4-31 DH3-10 JH6	CASEKFLWGQGYGMDVW	VL2-14 JL2	CSSYTSSSTLVF
PVI.V6-7	IgG1	VH4-39 DH3-22 JH4	CATQSDYDSSGLEFDYW	VL2-14 JL3	CSSYTSSSSWVF
PVI.V6-8	IgG1	VH4-31 DH3-22 JH3	CARGREEPIVVTDADFIDW	VK3-11 JK2	CQQRSNWPPMYTF
PVI.V6-9	IgG1	VH3-30 DH2-15 JH4	CAKSGYPYCGGGTCYSGWFDYW	VK1-33 JK2	CQRYDNPPYTF
PVI.V6-10	IgG1	VH1-2 DH6-19 JH6	CAREIAVAGNDYSYGLDVW	VK3-20 JK4	CQQYGSSLLTF
PVI.V6-11	IgG1	VH1-46 DH6-19 JH6	CASQSHWQWLGGDSYGMVDVW	VK1-9 JK2	CQQLNSYPYTF
PVI.V6-12	IgG1	VH5-51 DH1-26 JH4	CARRFGSYPYFDYW	VL3-21 JL1	CQVWDSNSDLYVVF
PVI.V6-13	IgG1	VH3-30 DH5-18 JH4	CAKAGYSYAYGDFYFDYW	VK1-33 JK3	CQHYDNLPPAVTF
PVI.V6-14	IgG1	VH4-39 DH3-10 JH4	CACRPEYFYFGSGYLDFDYW	VK1-12 JK4	CQQYNSFPLTF
PVI.V6-15	IgG1	VH3-30 DH5-18 JH4	CAKDWGWIQLWGLDYW	VL2-18 JL3	CSSYTSSSTWVF
PVI.V3-1	IgG1	VH4-4 DH1-26 JH4	CVSRGVGATREKDYW	VK3-15 JK4	CQQYNNWPPDLTF
PVI.V3-2	IgG1	VH4-39 DH3-10 JH4	CASLDYYGSGSGPGYFDYW	VK3-11 JK4	CQQRSNWLTF
PVI.V3-3	IgG3	VH3-33 DH6-19 JH4	CASDSSGWYFDYW	VL3-9 JL2	CQVWDSSTVVF
PVI.V3-4	IgG1	VH3-21 DH2-21 JH3	CAVTLTPTYCGGEWCAFDIW	VK3-15 JK2	CQQYNNWPPYTF
PVI.V3-5	IgG1	VH1-69 DH3-22 JH4	CARNYDSSGSQGMIDYW	VK3-11 JK4	CQQRSNWPPVLTF
PVI.V3-6	IgA1	VH3-66 DH3-3 JH4	CARHLGVVI <sup>1</sup>	VK4-1 JK1	CQQYYSTLWTF
PVI.V3-7	IgG3	VH4-39 DH1-26 JH4	CAKPSGSYLGFDYW	VK1-39 JK3	CQQSYSTPFTF
PVI.V3-8	IgG1	VH4-38 DH3-3 JH4	CARSDFSVRVGFDCW	VK4-1 JK2	CQQSYTTNTF
PVI.V3-9	IgG1	VH3-53 DH3-16 JH6	CARDLMEGGGMDVW	VK3-20 JK1	CQQYGSSLGTF
PVI.V3-10	IgG1	VH4-34 DH3-22 JH4	CARSQPLLWSSGYCCDYW	VL2-11 JL2	CCSYAGSYTLVF
PVI.V3-11	IgG1	VH3-64D DH4-11 JH4	CVRGPTVTTENDFDSW	VK2-30 JK2	CMQGTHSYTF
PVI.V3-12	IgG2	VH1-46 DH5-24 JH4	CASDPNRDGLALDSW	VK3-20 JK2	CQQYGTSPLYTF
PVI.V3-13	IgG1	VH1-69 DH1-14 JH4	CARDRYHGSPVDYW	VK3-11 JK4	CQQRSNWPPSLTF
PVI.V3-14	IgG1	VH4-31 DH5-12 JH4	CARARYSGSARGPPKQYYFDYW	VK3-20 JK1	CQHLVTF
PVI.V3-15	IgG1	VH3-21 DH3-3 JH3	CARDGGRGYDFWSGYYIGAFDIW	VK3-15 JK4	CQQYNDWPLPLTF
PVI.V3-16	IgG1	VH3-33 DH2-15 JH6	CARGLGWDIVVVVSGEMDGMVDVW	VL1-40 JL1	CQSYDSSLSGPPYVF
PVI.V3-17	IgA1	VH3-20 DH2-2 JH4	CARGESSDYW	VK3-20 JK1	CQQYGSSPKTF
PVI.V3-18	IgG1	VH3-30 DH2-15 JH4	CAKASGLYCSGGNCLVADFIDW	VL1-39 JL4	CQQSYSTPLSF
PVI.V3-19	IgG1	VH5-51 DH6-19 JH6	CARRNTSAQYSSGWYVHYYYGMDVW	VK2-28 JK3	CMQALQTPGFTF
PVI.V3-20	IgG1	VH3-30 DH3-3 JH6	CAKQDLGAIFAHYYYGMDVW	VL1-40 JL1	CQSYDSSLSGYVVF
PVI.V3-21	IgG1	VH5-51 DH5-24 JH4	CAKLSRDAYRGPFDYW	VL6-57 JL2	CQSYDSSNPDVVF

884 <sup>1</sup>Indicates absence of the W118 residue from the junction of the CDR3.

885

886 **Supplementary Figure 1. Full length spike to RBD ratios (A) and comparison of binding to neutralizing**  
 887 **titer ratios between naturally infected and vaccinated individuals (B).**

888

889 **Supplementary Figure 2. Gating strategy for sorting plasmablasts from total PBMCs isolated one week**  
 890 **after second immunization.**

891

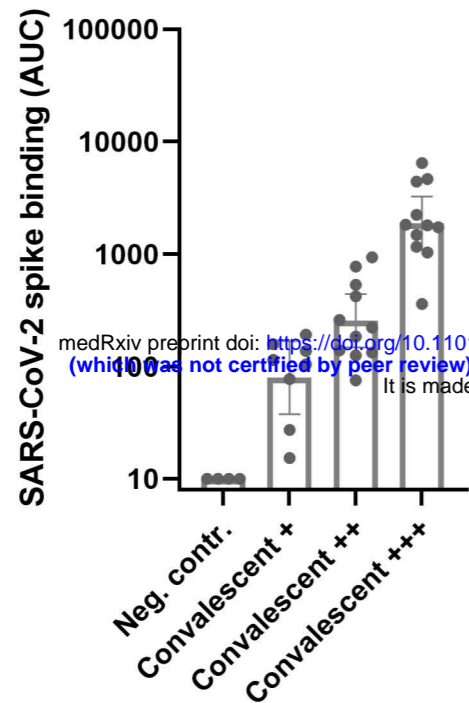
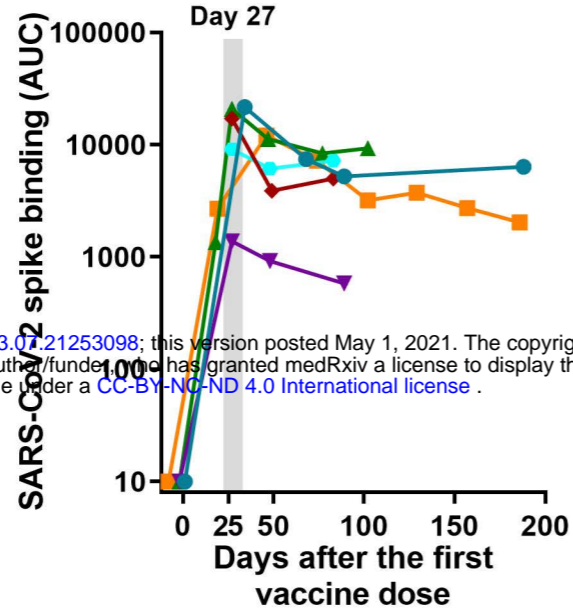
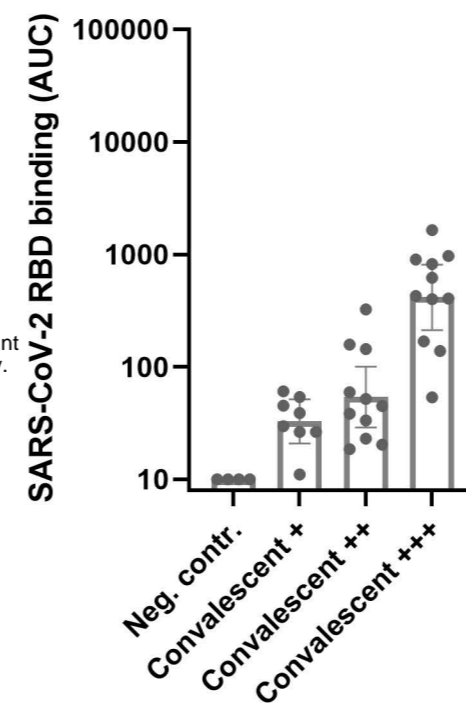
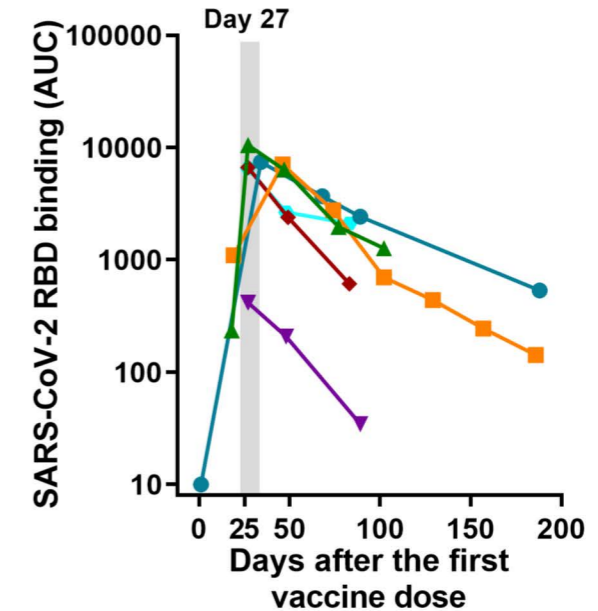
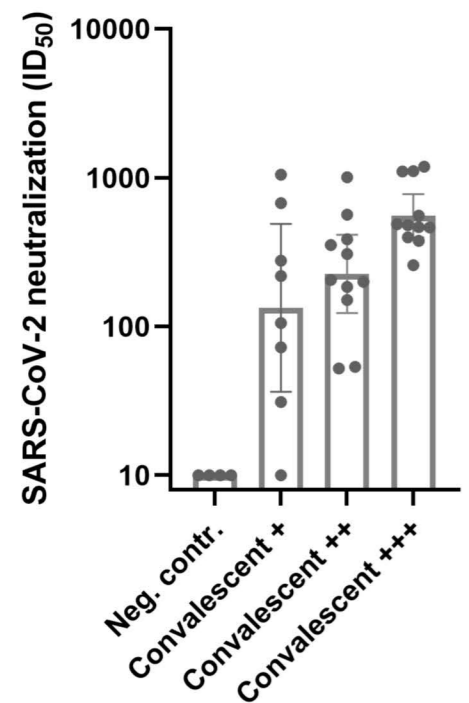
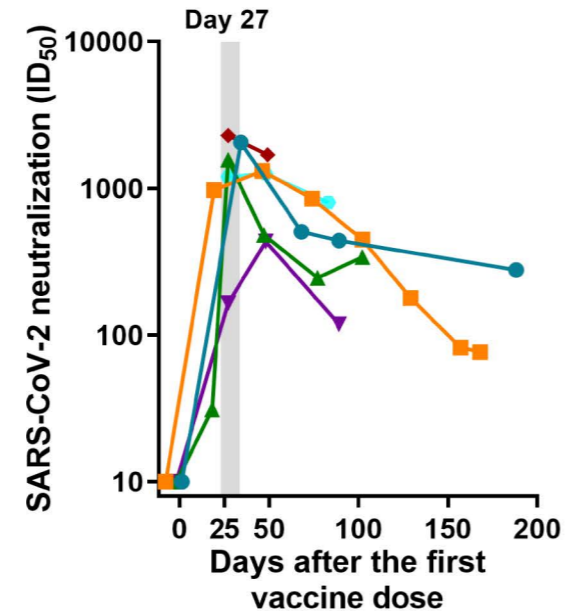
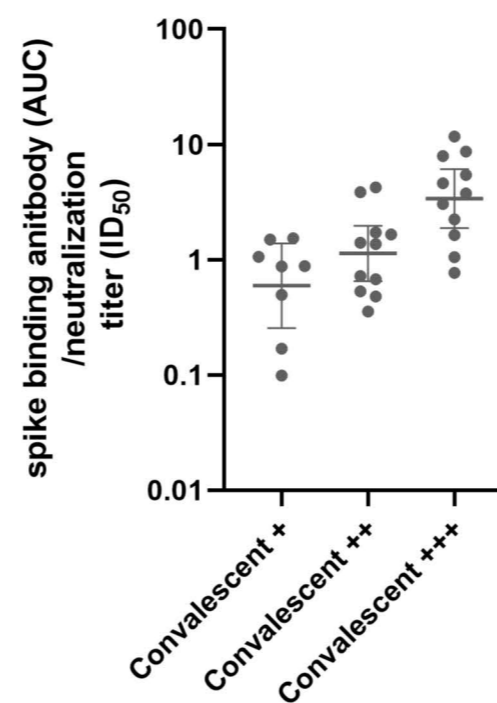
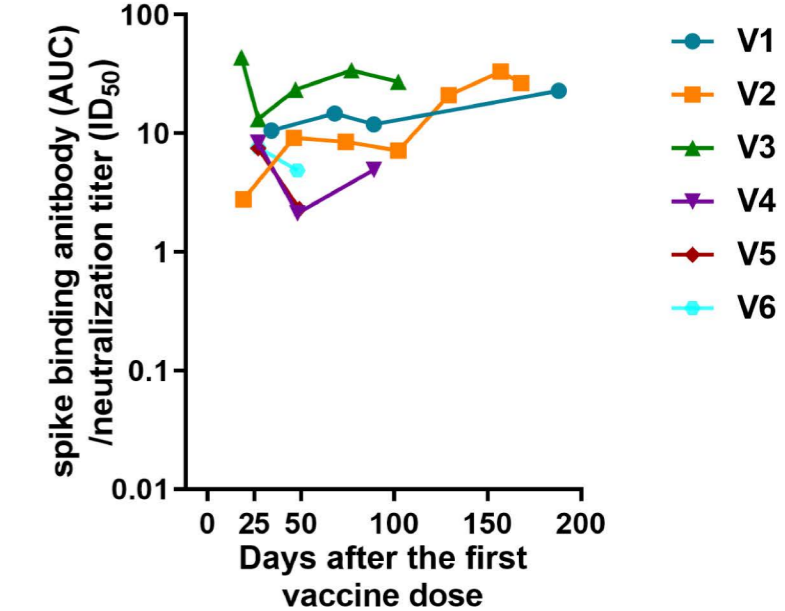
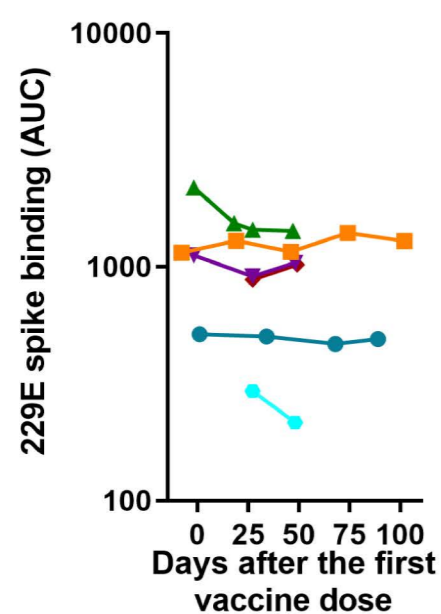
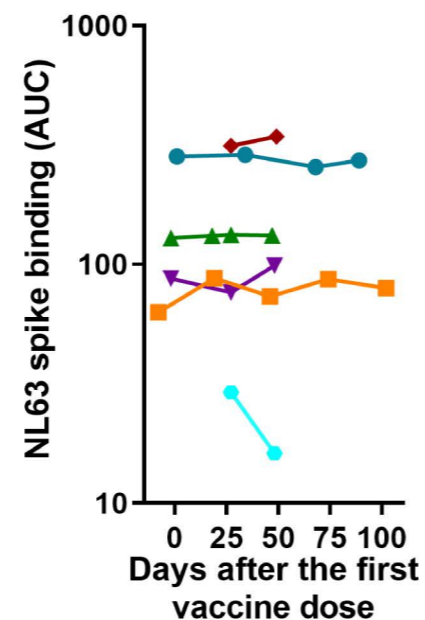
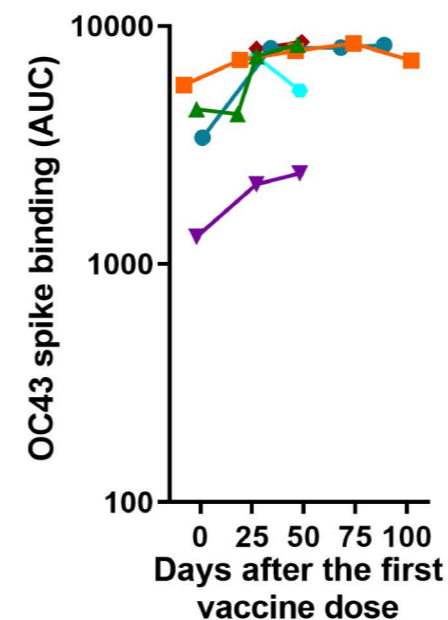
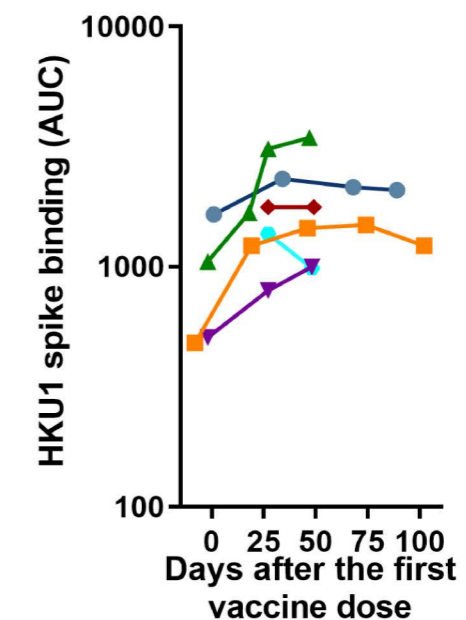
892 **Supplementary Figure 3. Representative Biolayer Interferometry binding isotherms from two**  
 893 **independent experiments. The raw data are show in pink and the Langmuir 1:1 kinetics fit is show in**  
 894 **black.**



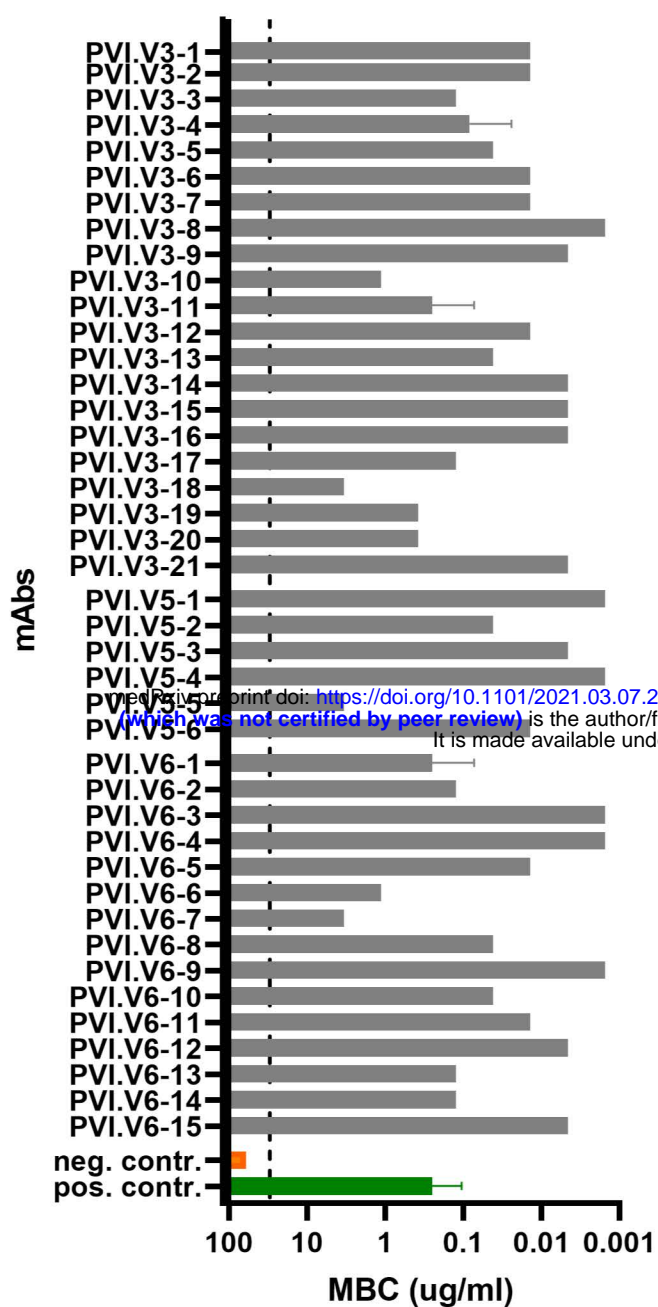
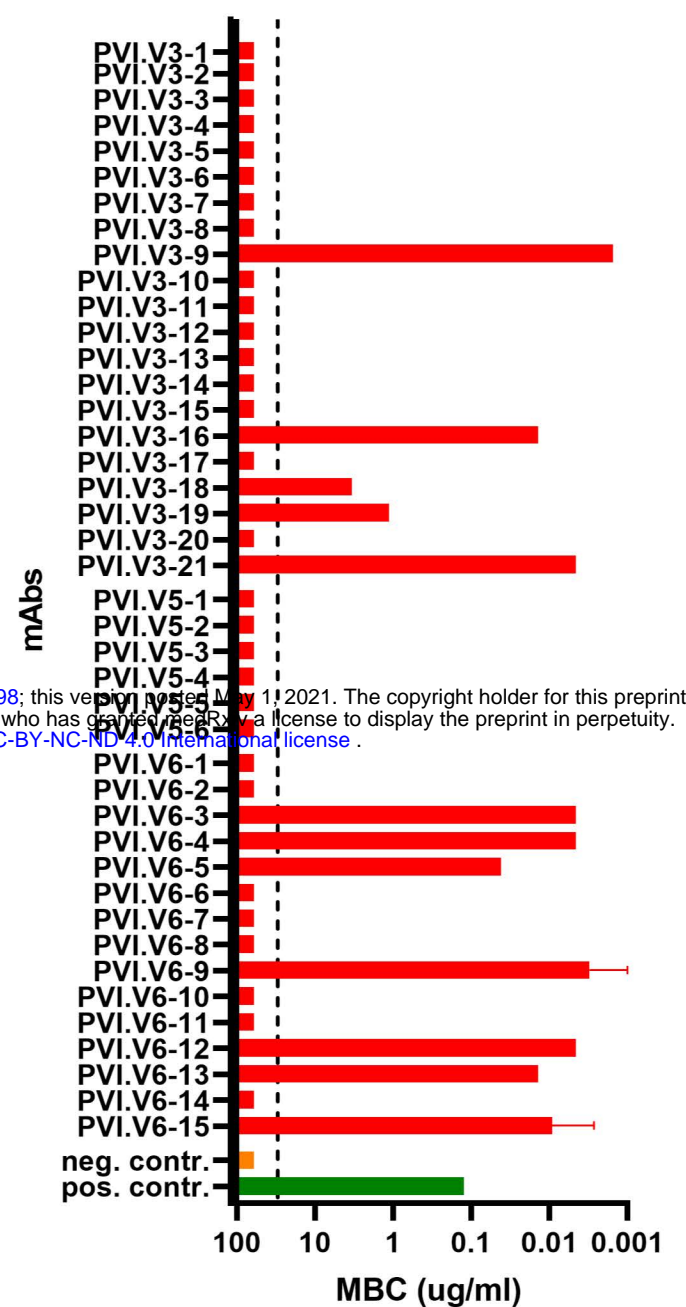
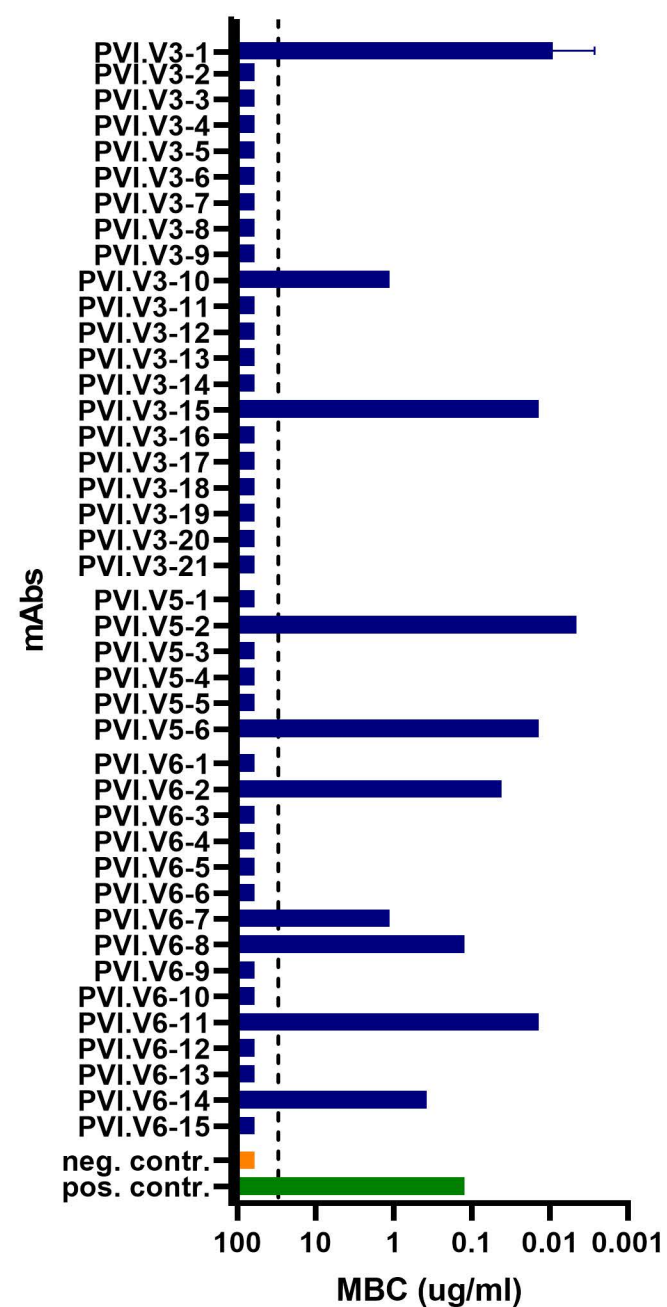
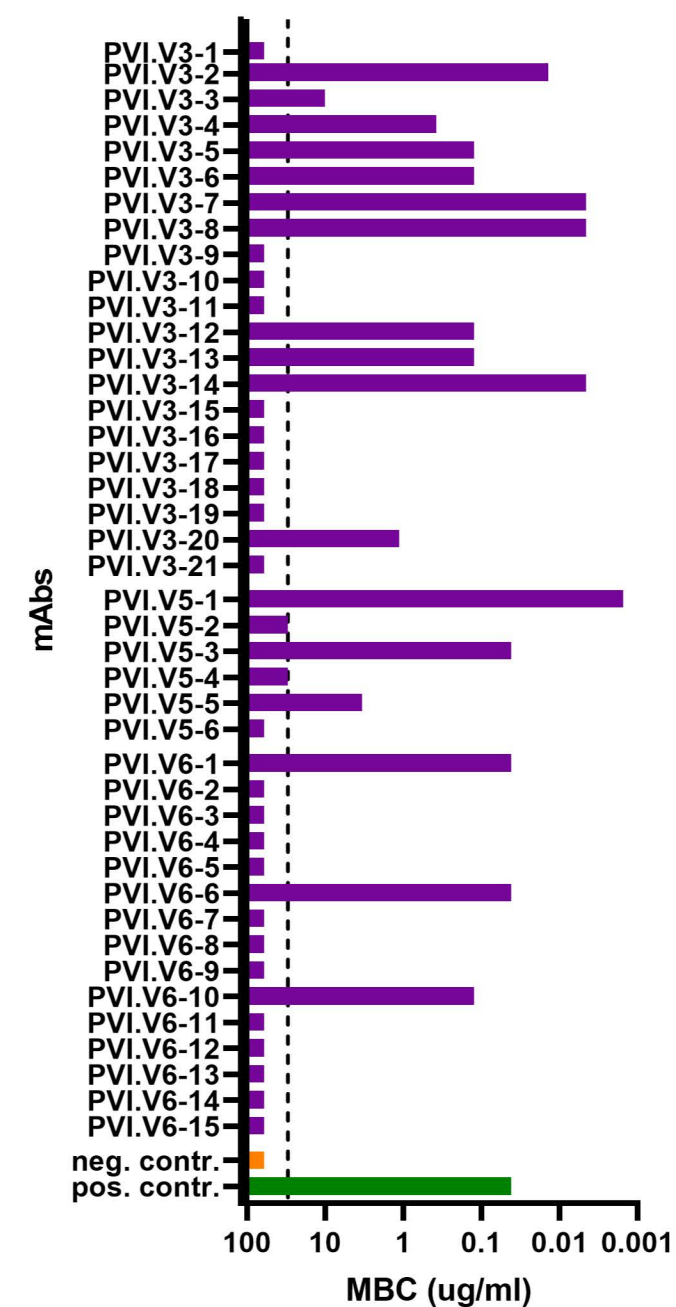
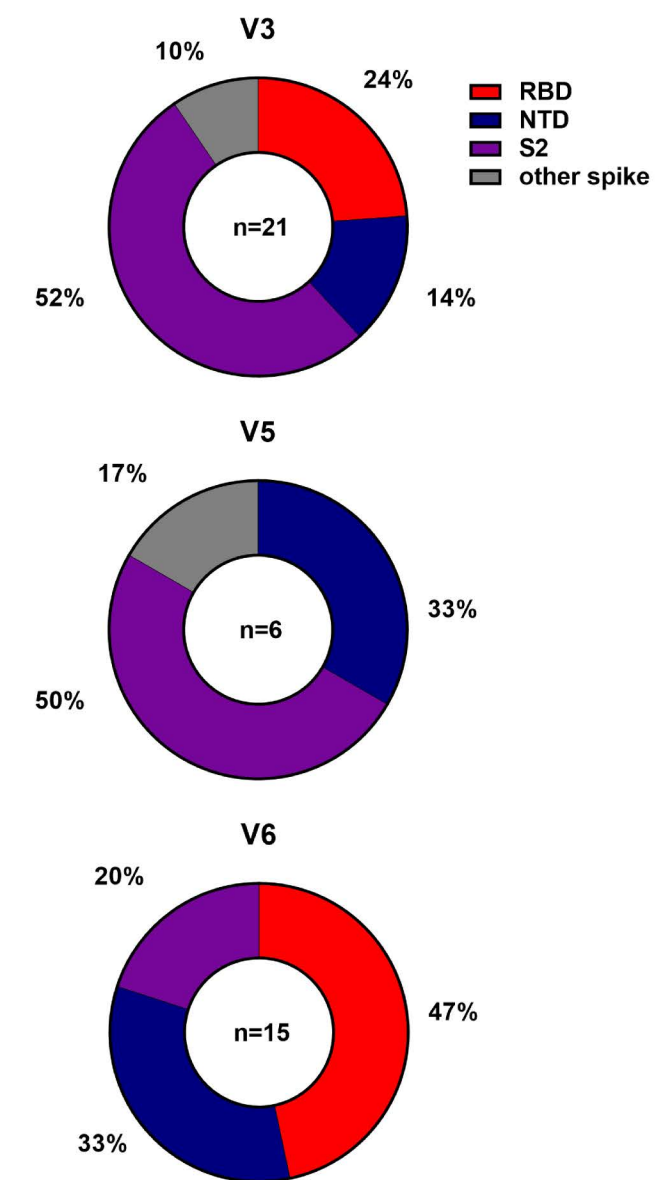
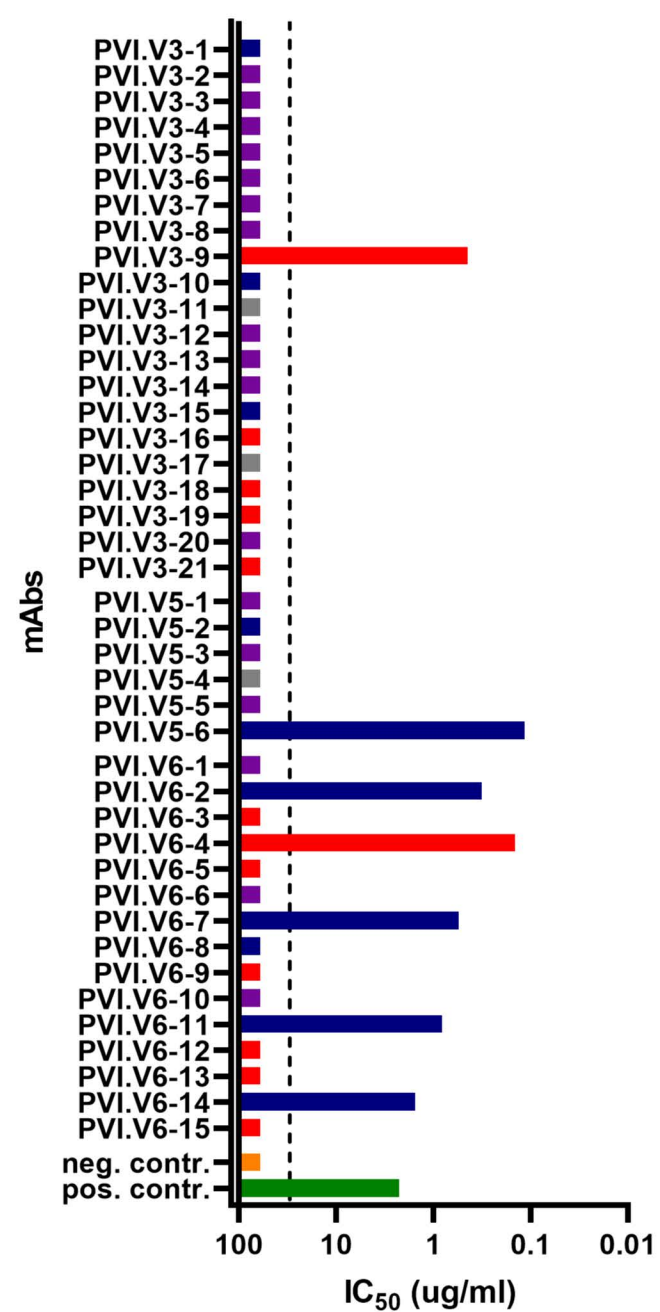
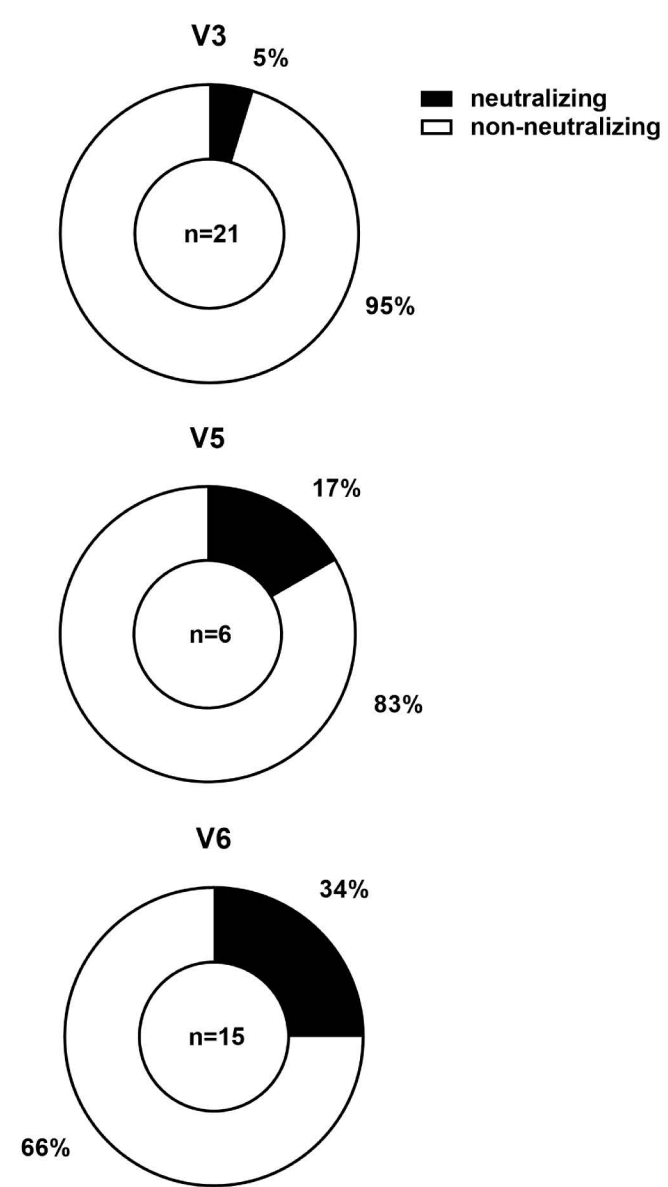
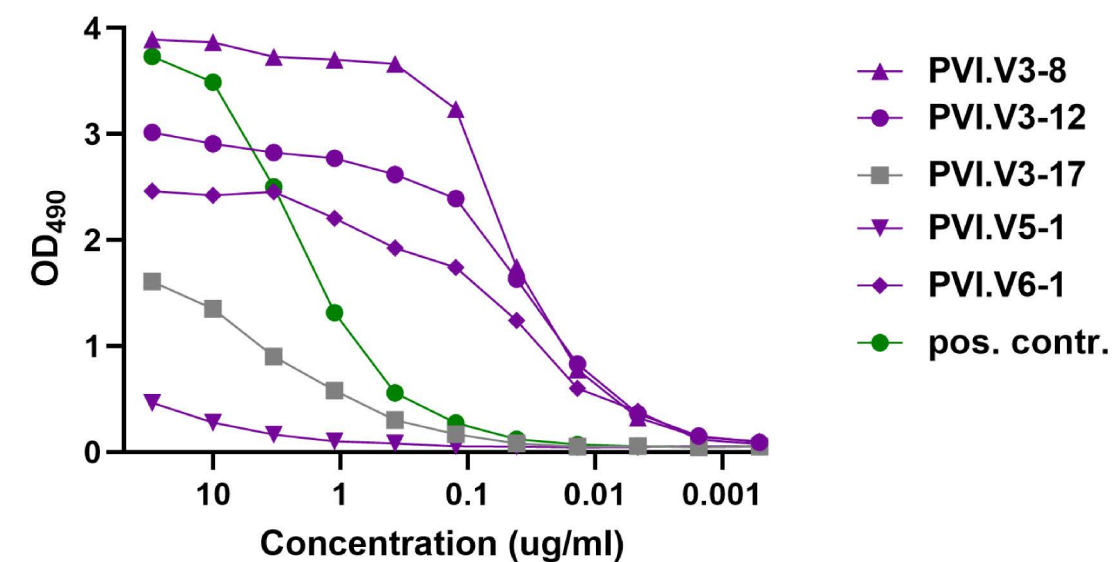
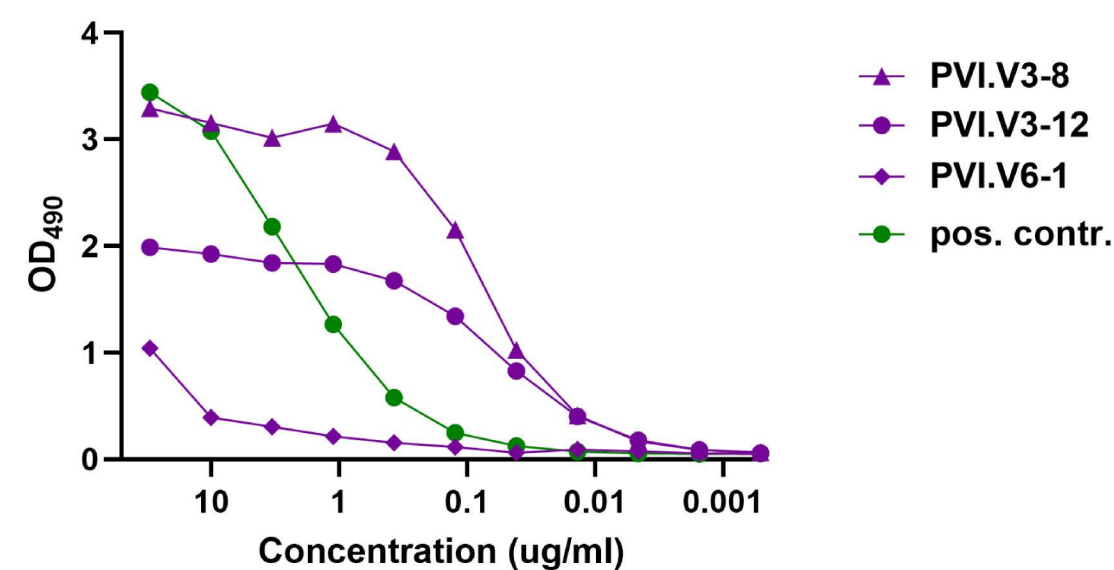
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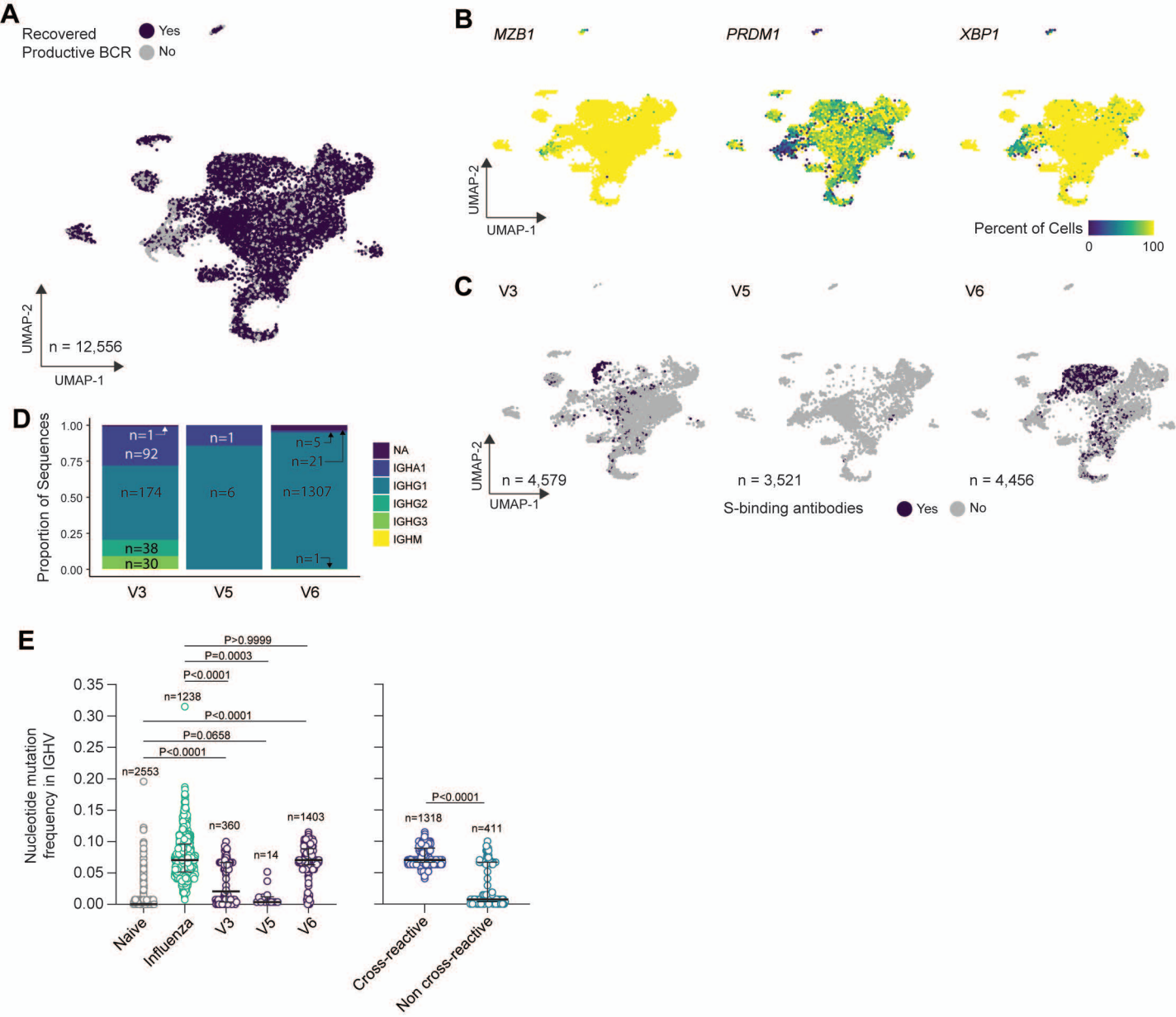
896 **Supplementary Figure 4. Binding of SARS-CoV-2 variant RBDs to ACE2. A.** ELISA curves of the RBD variants  
897 binding to human ACE2. Shown are the binding curves calculated with nonlinear regression to the  
898 arithmetic mean values from eight replicates  $\pm$  SEM. The calculated steady-state  $K_D$  values  $\pm$  SEM from  
899 end-point ELISA measurements and the fold-change in comparison to wild type RBD are reported in **B**.

900

**A****Spike binding  
convalescent individuals****SARS-CoV-2 spike binding  
vaccinated individuals****B****RBD binding  
convalescent individuals****RBD binding  
vaccinated individuals****C****SARS-CoV2 neutralization  
convalescent individuals****SARS-CoV2 neutralization  
vaccinated individuals****D****Spike binding/neutralizing activity ratio  
convalescent individuals****Spike binding/neutralizing activity ratio  
vaccinated individuals****E****229E (α-CoV)****F****NL63 (α-CoV)****G****OC43 (β-CoV)****H****HKU1 (β-CoV)**

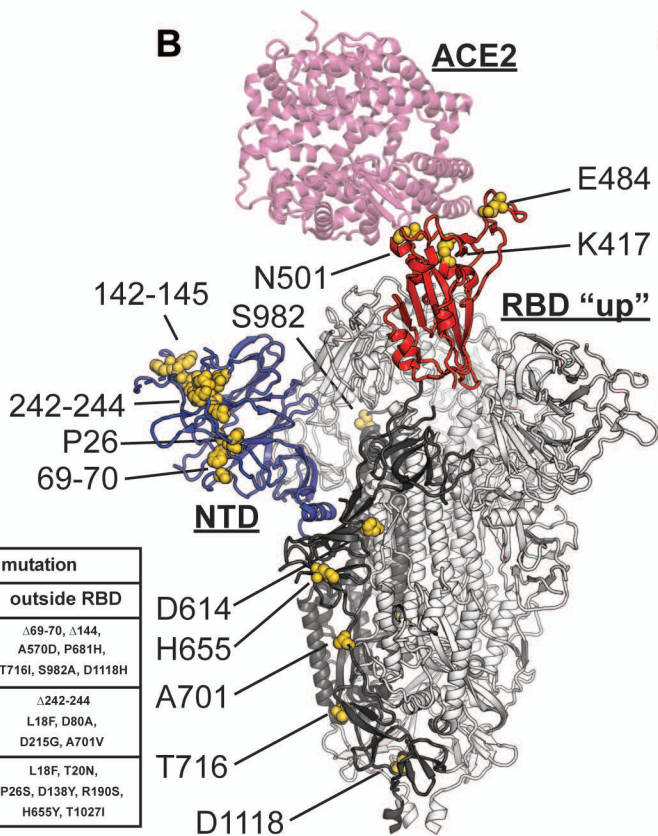
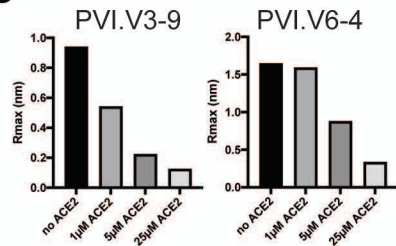
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**A****Spike binding****B****RBD binding****C****NTD binding****D****S2 binding****E****F****Neutralization****G****H****OC43 binding****I****HKU1 binding**

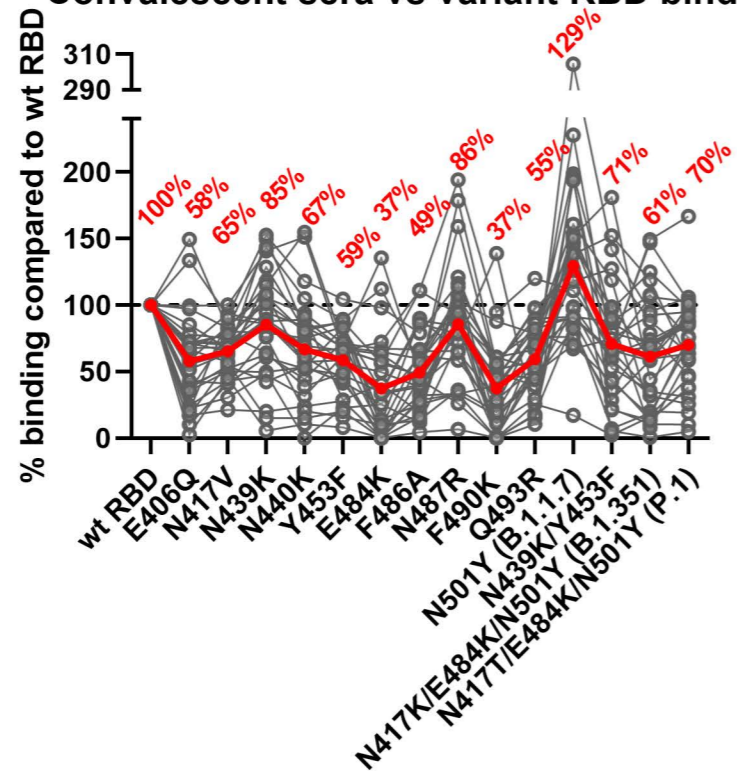
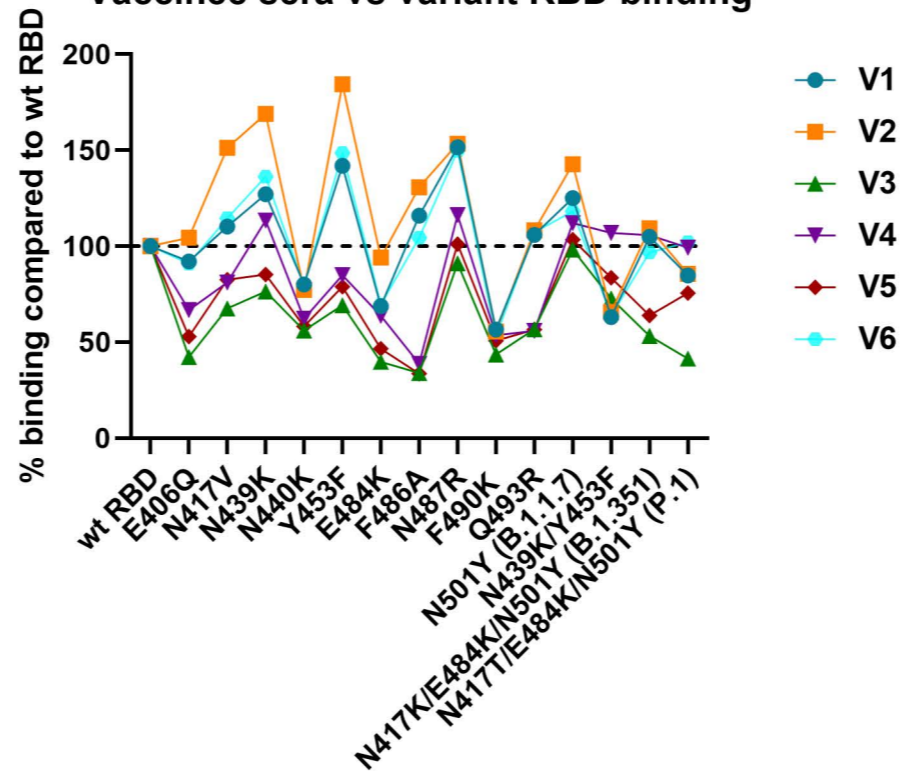
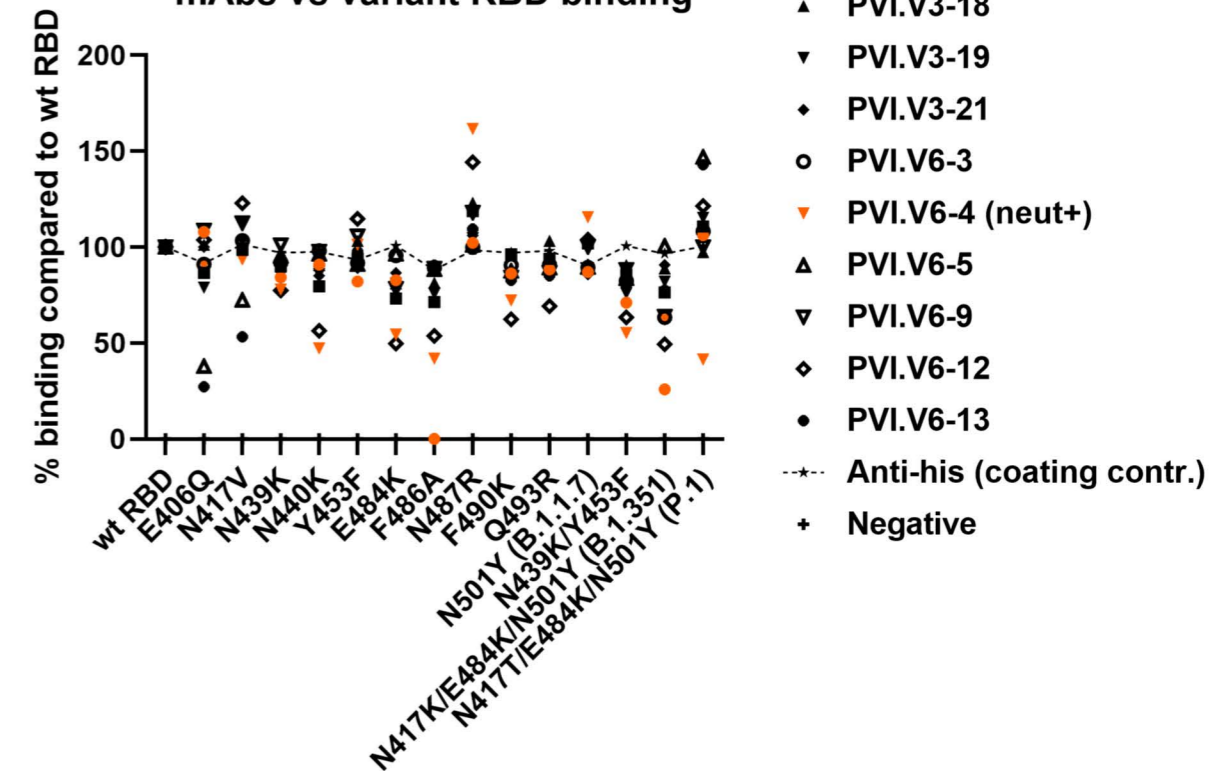
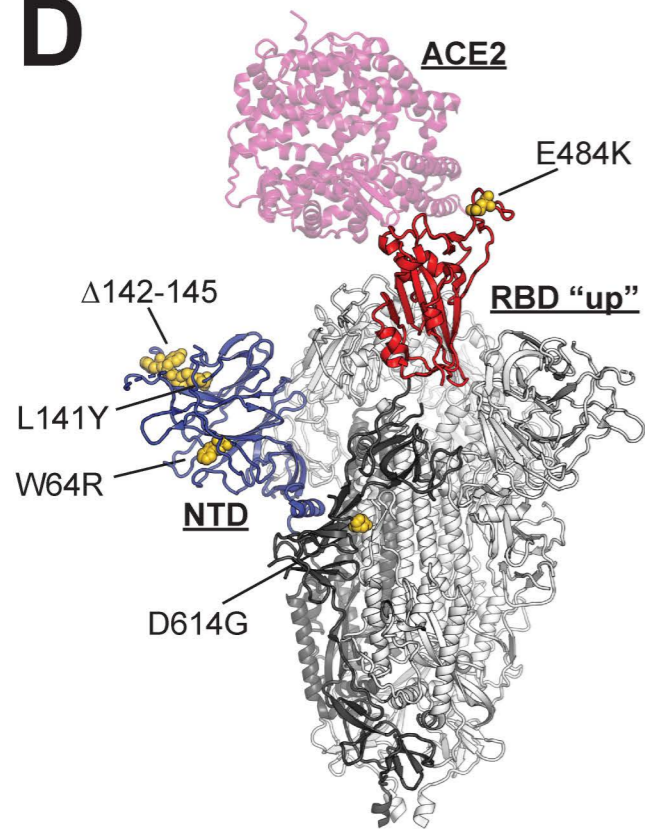
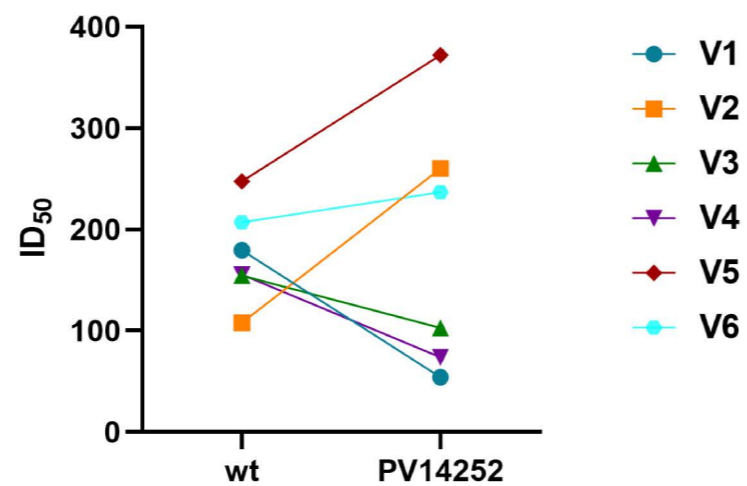
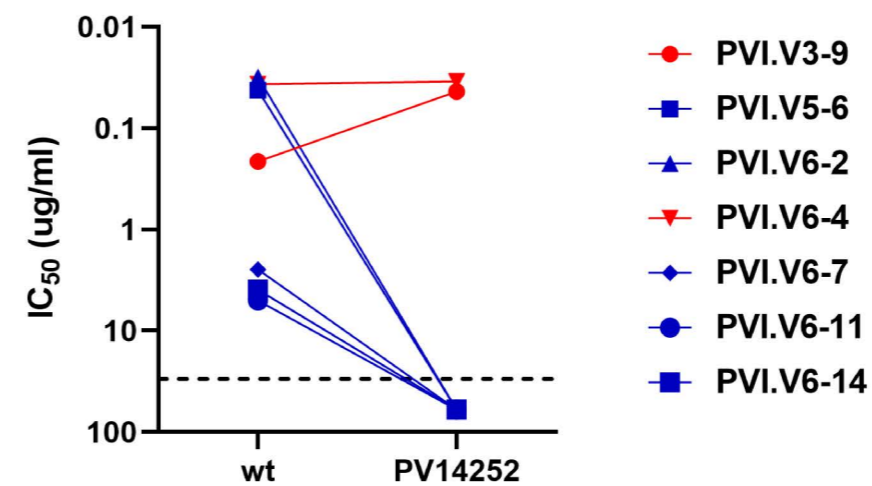
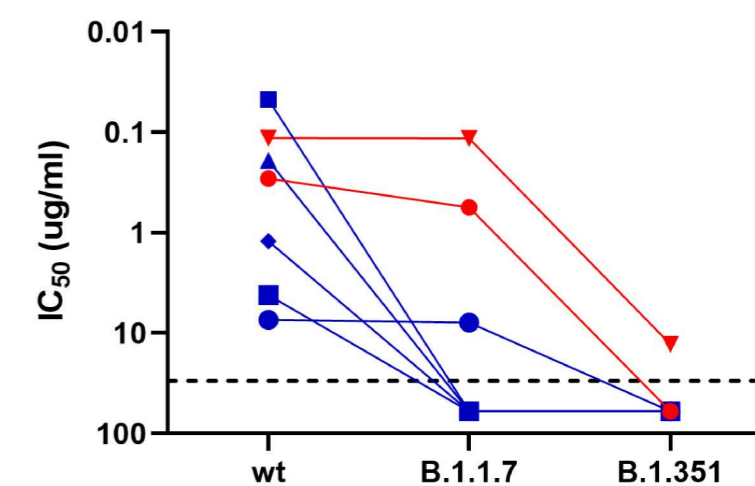


**A**

variant	spike mutation	
	RBD	outside RBD
B.1.1.7	N501Y	$\Delta$ 69-70, $\Delta$ 144, A570D, P681H, T716I, S982A, D1118H
B.1.351	K417N, E484K, N501Y	$\Delta$ 242-244, L18F, D80A, D215G, A701V
P.1	K417T, E484K, N501Y	L18F, T20N, P26S, D138Y, R190S, H655Y, T1027I

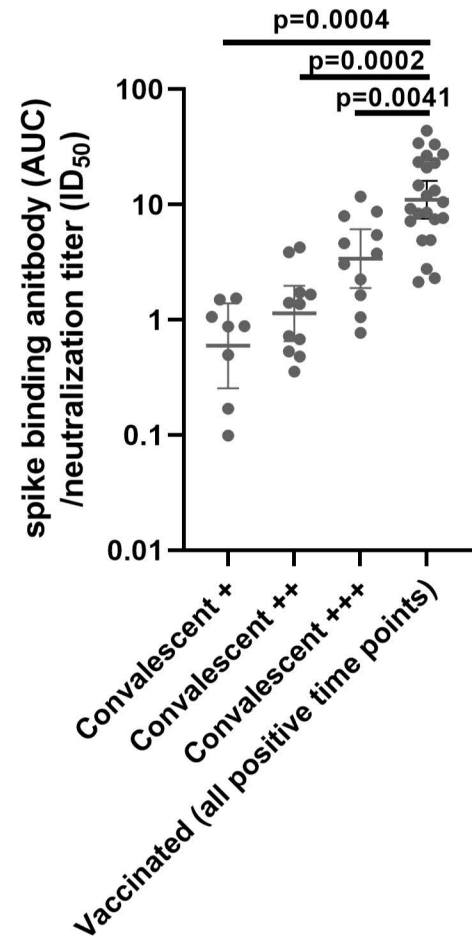
**C****D**

RBD variant	$K_D$ (nM)	Fold change
wild-type	35.4 $\pm$ 5.6	1
N501Y	7.0 $\pm$ 1.1	0.2
Y453F	29.9 $\pm$ 18.1	0.8
N439K	15.7 $\pm$ 3.8	0.4
Y453F/N439K	7.2 $\pm$ 0.1	0.2
E484K	138.3 $\pm$ 30.9	4
K417N/E484K/N501Y	34.0 $\pm$ 4.6	1
K417T/E484K/N501Y	4.0 $\pm$ 1.7	0.1

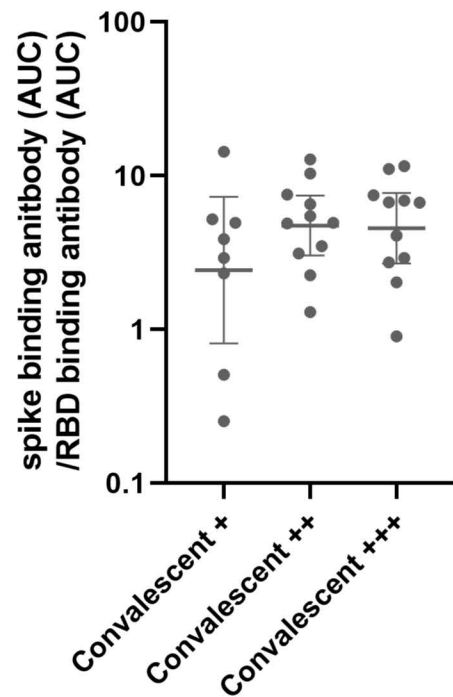
**A****Convalescent sera vs variant RBD binding****B****Vaccinee sera vs variant RBD binding****C****mAbs vs variant RBD binding****D****PV14252 spike****E****Vaccinee serum wt versus PV14252 (W64R, L141Y, E484K, D614G, Δ142-145) neutralization****F****mAb wt versus PV14252 (W64R, L141Y, E484K, D614G, Δ142-145) neutralization****G****mAb wt versus B.1.1.7 and B.1.351 neutralization**

**A**

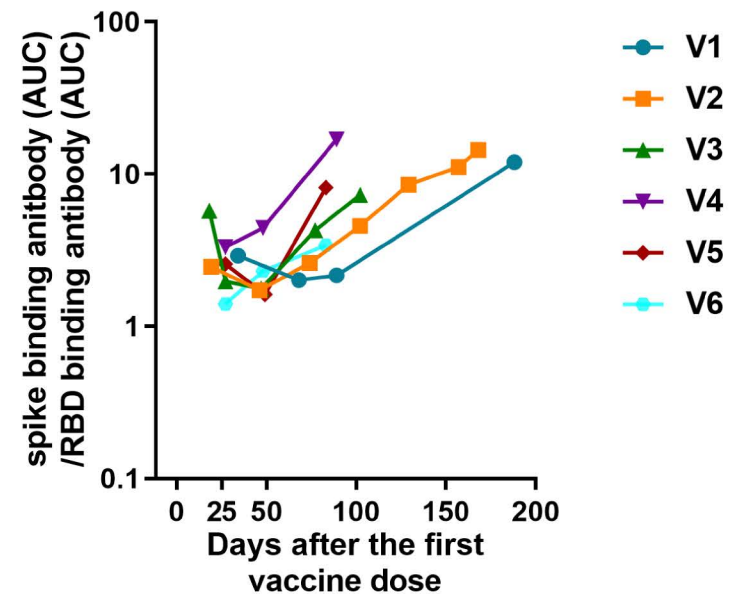
### Spike binding/neutralizing activity ratio convalescent individuals

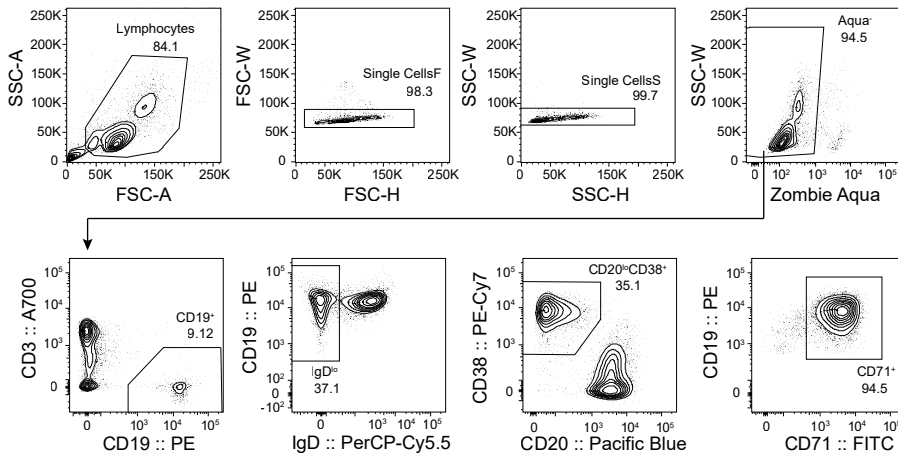
**B**

### Ratio spike binding/RBD binding antibodies convalescent individuals



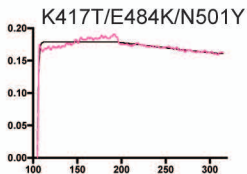
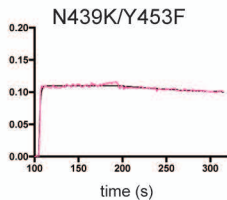
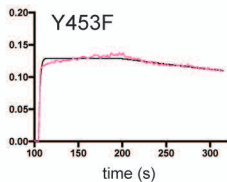
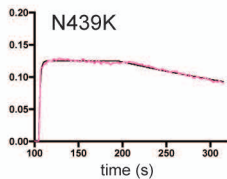
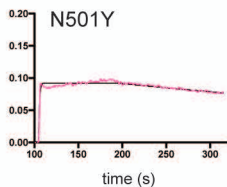
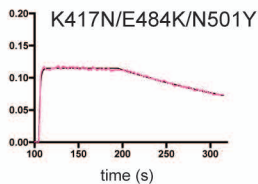
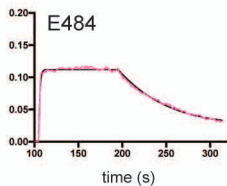
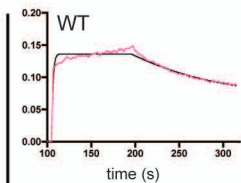
### Ratio spike binding/RBD binding antibodies vaccinated individuals



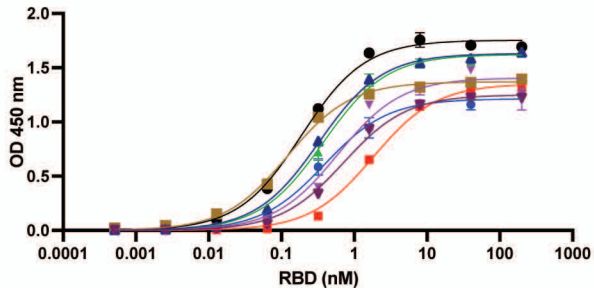




RU (nm)



— data  
— fit

**A**

- Wild Type
- E484K
- ▲ Y453F/N439K
- ▼ N439K
- Y453F
- N501Y
- ▲ K417T/E484K/N501Y
- ▼ K417N/E484K/N501Y

**B**

RBD variant	$K_D$ ( $10^{-11}$ M)	Fold change
wild-type	38.8±0.5	1
N501Y	12±0.1	0.3
Y453F	18.9±0.1	0.5
N439K	59.8±9.3	1.5
Y453F/N439K	37.4±0.3	1
E484K	181.9±14.5	4.7
K417N/E484K/N501Y	71.3±3.7	1.8
K417T/E484K/N501Y	32.9±1.3	0.8