

1 **Title:** Convergent epitope specificities, V gene usage and public clones elicited by primary  
2 exposure to SARS-CoV-2 variants

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29  
30 **Abstract:**

31 While humoral immune responses to infection or vaccination with ancestral SARS-CoV-2 have  
32 been well-characterized, responses elicited by infection with variants are less understood. Here  
33 we characterized the repertoire, epitope specificity, and cross-reactivity of antibodies elicited by  
34 Beta and Gamma variant infection compared to ancestral virus. We developed a high-throughput  
35 approach to obtain single-cell immunoglobulin sequences and isolate monoclonal antibodies for  
36 functional assessment. Spike-, RBD- and NTD-specific antibodies elicited by Beta- or Gamma-  
37 infection exhibited a remarkably similar hierarchy of epitope immunodominance for RBD and  
38 convergent V gene usage when compared to ancestral virus infection. Additionally, similar public  
39 B cell clones were elicited regardless of infecting variant. These convergent responses may  
40 account for the broad cross-reactivity and continued efficacy of vaccines based on a single  
41 ancestral variant.

42  
43 **One Sentence Summary:** WA1, Beta and Gamma variants of SARS-CoV-2 all elicit antibody  
44 responses targeting similar RBD epitopes; public and cross-reactive clones are common.

46 **Main Text:**

47

48 **INTRODUCTION**

49

50 The sustained spread of SARS-CoV-2 infection has resulted in the emergence of virus  
51 variants characterized by the accumulation of multiple sequence mutations. Variants that acquired  
52 enhanced transmissibility, pathogenicity or a mechanism of immune escape are considered  
53 “variants of concern” (VOC), and include Alpha (PANGO lineage B.1.1.7), Beta (B.1.351), Gamma  
54 (P.1), Delta (B.1.617.2), and Omicron (B.1.1.529) (1). Their ability to escape immune responses  
55 elicited by vaccines based on the first reported sequence from Wuhan (PANGO lineage B.1, here  
56 called “WA1”), or by previous infection with a different variant, is a major obstacle to efforts to  
57 control the pandemic. A better understanding of the similarities and differences in the immune  
58 responses induced by each variant will help guide vaccine design strategies to overcome immune  
59 evasion by the virus.

60 Early in the pandemic, D614G was the first amino acid substitution in the Spike protein to  
61 become dominant, and it was shown to confer increased infectivity due to improved Spike stability,  
62 albeit with higher sensitivity to antibody neutralization (2, 3). Over the course of the pandemic,  
63 selective immune pressure is proposed to have led to the accumulation of changes in residues  
64 targeted for antibody recognition and neutralization, most importantly in the receptor binding  
65 domain (RBD) (4, 5). N501Y and other substitutions in RBD increase binding affinity to ACE2 and  
66 can compensate for affinity-lowering substitutions that are selected by immune pressure (6-9).  
67 The combination of K417N/T, E484K/A and N501Y substitutions arose independently in the Beta,  
68 Gamma and Omicron variants (10, 11), with N501Y also present in the Alpha, Theta, and Mu  
69 variants, showing convergent viral evolution along these pathways. Changes in the N-terminal  
70 domain (NTD), by contrast, are less often convergent across variants, but may also account for  
71 epitope disruption (12). Although neutralizing antibodies that recognize NTD are less frequent  
72 than those binding to RBD (13-15), this domain is a major target for non-neutralizing antibodies  
73 that can elicit Fc effector functions (16, 17). Therefore, neutralizing capacity and Fc-mediated  
74 functionality of antibodies induced by WA1 are significantly reduced against these variants. In  
75 addition, CD4 and CD8 T cell responses do not seem to be substantially impacted by variant  
76 substitutions (18-22).

77 The epitopes on the ancestral virus targeted by the humoral response have been well  
78 characterized (15, 23-31) and conservation of these regions among VOCs and across a broad  
79 range of sarbecoviruses can be readily assessed. Previous studies of the SARS-CoV-2-specific

80 antibody repertoire have revealed polarization toward usage of specific  $V_H$  genes including  
81 IGHV3-53, IGHV3-66, IGHV3-30 and IGHV1-2 (28, 32-36). In many cases, convergent V(D)J  
82 rearrangements (so-called “public clones”) have been found in multiple individuals and several  
83 structural classes have been identified (37) which comprise antibodies derived from the same  
84 genetic elements with a shared binding mode (13, 27, 30, 33, 38-41). Many of the most common  
85 neutralizing antibodies from these classes make contact with residues such as K417 and E484  
86 and thus lose potency against VOCs (6, 42), although some can maintain potency through specific  
87 substitutions that adjust the binding conformation to avoid variable residues (43, 44). However,  
88 neutralizing antibodies make up only a minority of the total binding repertoire, which has not been  
89 well characterized in VOC infections compared to WA1.

90 In-depth characterization of antigen-specific B cell repertoires requires rapid, high-  
91 throughput monoclonal antibody (mAb) discovery and functional testing. Further, high-resolution  
92 observations of differences in immune responses to SARS-CoV-2 variants can be leveraged to  
93 inform future rational vaccine design for boosters and an efficacious pan-coronavirus vaccine.  
94 Using a novel method for high-throughput, cloning-free recombinant mAb synthesis and  
95 sequencing, we investigated the differences in epitope targeting,  $V_H$  gene usage, and B cell clonal  
96 repertoires from convalescent individuals infected with WA1, Beta, or Gamma variants.

97

## 98 **RESULTS**

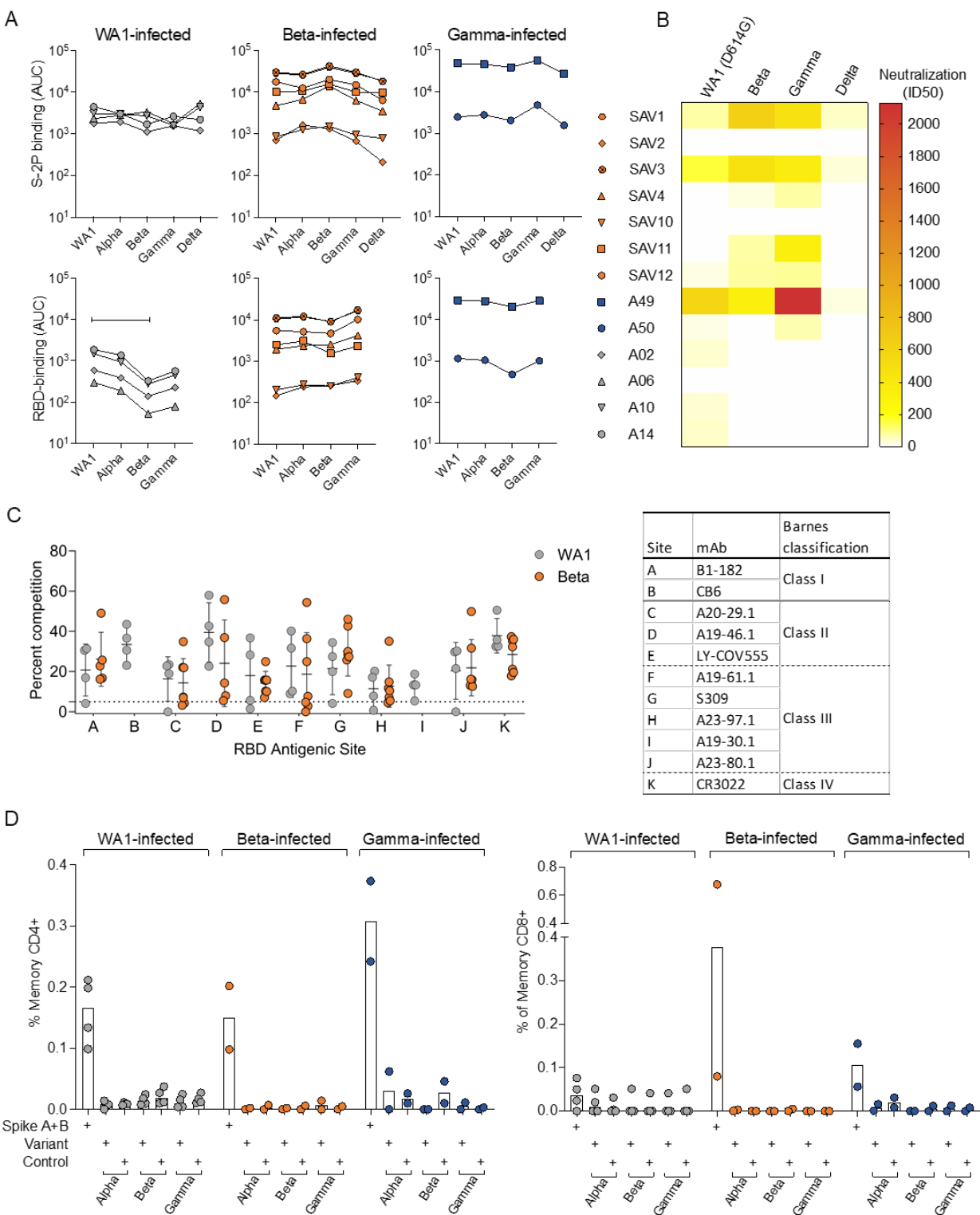
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### 100 **Spike binding antibody titers in WA1-, Beta-, and Gamma-infected individuals**

101 We collected serum or plasma and PBMC from individuals infected with WA1, Beta, or  
102 Gamma variants at 17-38 days after symptom onset (Table S1) to compare antibody and B cell  
103 responses. The infecting variant for Beta and Gamma cases was confirmed by sequencing; WA1  
104 cases were from early in the pandemic, prior to the rise of VOCs. All individuals were previously  
105 naïve to SARS-CoV-2. As we were interested in studying the total antigen-specific B cell  
106 repertoire, we did not select individuals based solely on high neutralization titers, but rather  
107 focused on the time post-infection when frequencies of B and T cells are typically high.

108 We measured serum binding titers to stabilized Spike trimer (S-2P) from WA1, Alpha,  
109 Beta, Gamma, and Delta variants, and to RBD from WA1, Alpha, Beta, and Gamma variants using  
110 a Meso Scale Discovery electrochemiluminescence immunoassay (MSD-ECLIA) (Fig. 1A).  
111 Additionally, we assessed binding titers to Spike from WA1 (with and without D614G), Alpha,  
112 Beta, Gamma, Delta, or Omicron variants expressed on the surface of HEK293T cells (Fig. S1A).

113



114 **Figure 1:** Homologous and cross-reactive antibodies induced by WA1 and variant infections. **(A)** Binding  
 115 antibody titers to spike (top panels) and RBD (bottom panels) from different variants indicated on the x-  
 116 axis. **(B)** Heatmap showing neutralizing antibody titers (reciprocal 50% inhibitory dilution) for each individual  
 117 labeled on the left against each variant indicated on the top. **(C)** Epitope mapping on homologous spike by  
 118 competition assay using surface plasmon resonance. Antibodies CB6 (RBD-B epitope) and A19-30.1  
 119 (RBD-I) do not bind to Beta and competition is not measured at these sites. **(D)** CD4 (left) and CD8 (right)  
 120 T cell responses to WA1 spike peptide pools A+B, selected pools containing altered variant peptides and  
 121 control pool containing correspondent peptides for each variant pool.

122 Both assays showed that all convalescent individuals had antibodies against the homologous  
123 Spike as well as cross-reactive antibodies to Spike from other variants. The WA1-infected  
124 individuals showed a significant reduction in antibody titers against beta RBD, but variant-infected  
125 individuals recognized WA1 RBD at similar levels as the homologous RBD (Fig. 1A), consistent  
126 with previous reports (45, 46). Individuals with the highest serum binding titers (SAV1, SAV3 and  
127 A49) could cross-neutralize WA1, Beta, Gamma, and, with lower potency, Delta variants,  
128 however, low levels of neutralization were detected in the other serum samples (Fig. 1B).

129

### 130 **VOC infection does not alter B or T cell immunodominance profiles**

131 We next used a surface plasmon resonance (SPR)-based competition assay (47, 48) to  
132 characterize epitopes targeted by serum antibodies. We individually blocked specific RBD  
133 epitopes on S-2P using structurally validated mAbs (Figure S1B) and measured the fraction of  
134 polyclonal serum binding activity remaining compared to unblocked trimer. Notably, when the  
135 binding activity of each serum was characterized against the homologous Spike, the patterns of  
136 reactivity were comparable between individuals infected either with WA1 or Beta (Fig 1C),  
137 revealing a similar immunodominance hierarchy across variants. Likewise, there were no  
138 differences in competition at each epitope when sera from Beta- or Gamma-infected individuals  
139 were mapped against WA1, Beta, or Delta Spike (Fig. S1, C and D). Only one of the WA1-infected  
140 individuals produced sufficiently high binding titers against variant Spike to enable epitope  
141 mapping by competition (Table S2).

142 We evaluated the ability of T cells elicited by Beta and Gamma infections to recognize  
143 WA1 Spike peptides by measuring upregulation of CD69 and CD154 on CD4 T cells, and  
144 production of IFN- $\gamma$ , TNF, or IL-2 by CD8 T cells (Fig. S1E). Due to PBMC availability, the Beta-  
145 infected individuals included in this analysis were from a different cohort. CD4 and CD8 T cell  
146 responses to WA1 Spike peptides were similar in Beta- and Gamma-infected individuals  
147 compared to WA1-infected individuals (Fig. 1D). When stimulated with selected peptides covering  
148 only regions containing substitutions in each variant, CD4 and CD8 T cell responses were  
149 minimal, suggesting that the substituted residues are not included within immunodominant T cell  
150 epitopes (Fig. 1D).

151

### 152 **High-throughput mAb production and repertoire characterization**

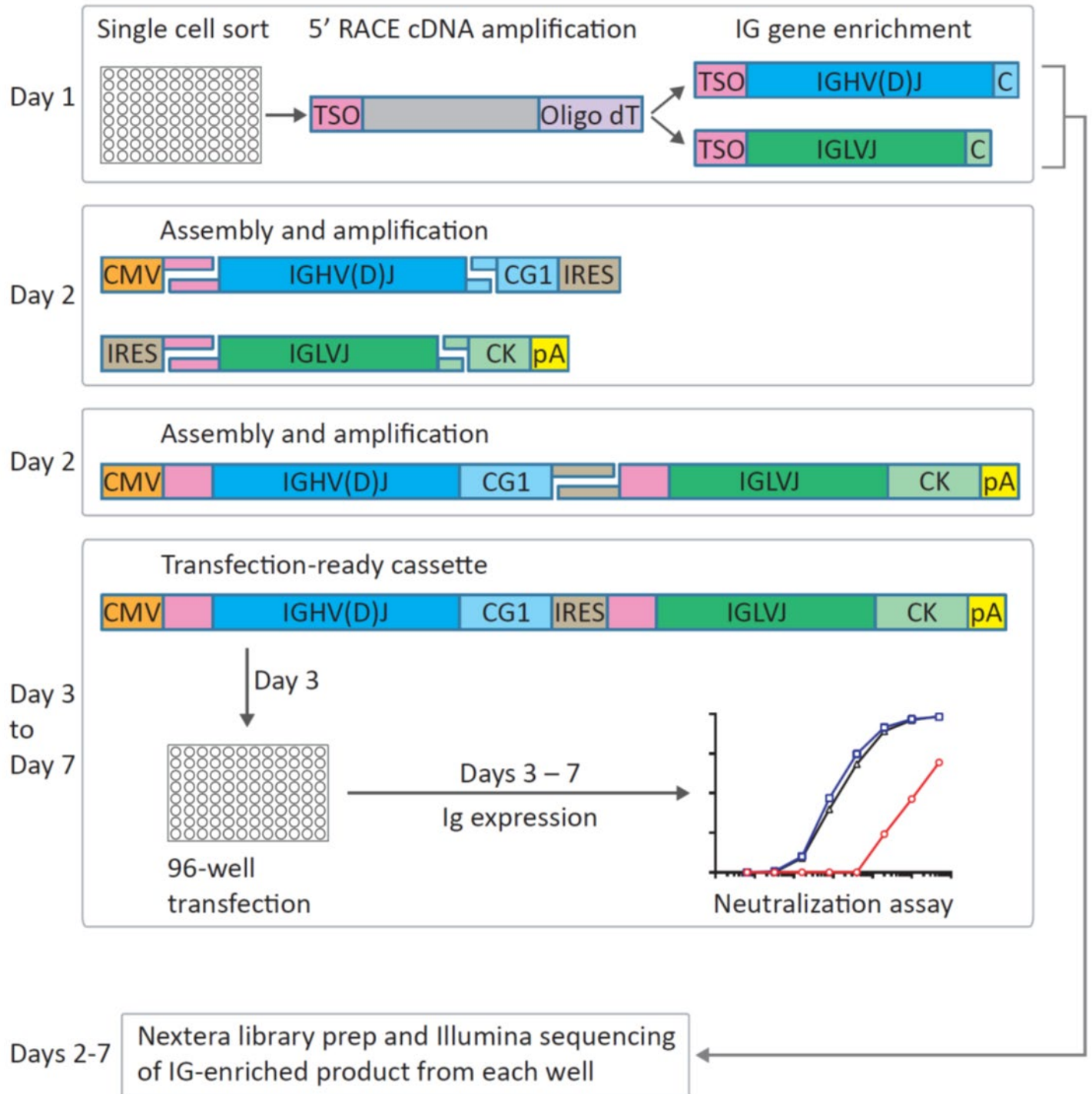
153 The three individuals in our cohort with the highest binding titers (Fig 1A) were selected  
154 for in-depth characterization of the antibody repertoire and identification of mAb binding patterns.  
155 Two individuals (SAV1 and SAV3) had been infected with the Beta variant, and the third (A49)

156 with Gamma (Fig S2A). To swiftly characterize antibodies from single B cells, we developed a  
157 method for rapid assembly, transfection, and production of immunoglobulins (abbreviated to  
158 R ATP-Ig) that enables high-throughput discovery of mAbs from single-sorted B cells. R ATP-Ig  
159 relies on 5'-RACE and high-fidelity DNA assembly to produce recombinant heavy and light chain-  
160 expressing linear DNA cassettes. These cassettes can be synthesized within two days after  
161 single-cell sorting and can be directly transfected into 96-well microtiter mammalian cell cultures.  
162 Resulting culture supernatants containing the expressed mAbs can then be tested for functionality  
163 (Fig. 2). We sorted cross-reactive WA1<sup>+</sup>Beta<sup>+</sup> B cells using S-2P, RBD, or NTD probes (Fig. S2B)  
164 from the three selected individuals, resulting in a total of 509 single cells for analysis (Fig. 3A).  
165 We recovered paired heavy and light chain sequences from 355 (70%) of cells (Fig. 3A). In  
166 parallel, we screened the R ATP-Ig supernatants by ELISA for binding to Spike, RBD, and NTD  
167 derived from each of WA1, Beta, and Gamma variants. IgG binding at least one antigen was  
168 produced in 240 (47%) of wells with a single sorted B cell (Fig. 3, A and B). All three individuals  
169 yielded high levels of cross-reactive antibodies to Spike, NTD, and RBD (Fig. 3B and Tables S3-  
170 S5). Antibodies isolated from Beta-infected individuals SAV1 and SAV3 showed similar binding  
171 profiles dominated by cross-reactive mAbs among WA1, Beta, and Gamma variants (Fig. 3B).  
172 While the majority of antibodies isolated from individuals A49 were also cross-reactive, we  
173 isolated a large population of Gamma-specific S-2P binding mAbs and another population whose  
174 epitope specificity was indeterminate and appeared to bind both RBD and NTD (Fig. 3B and Table  
175 S5), perhaps due to high background ELISA signal.

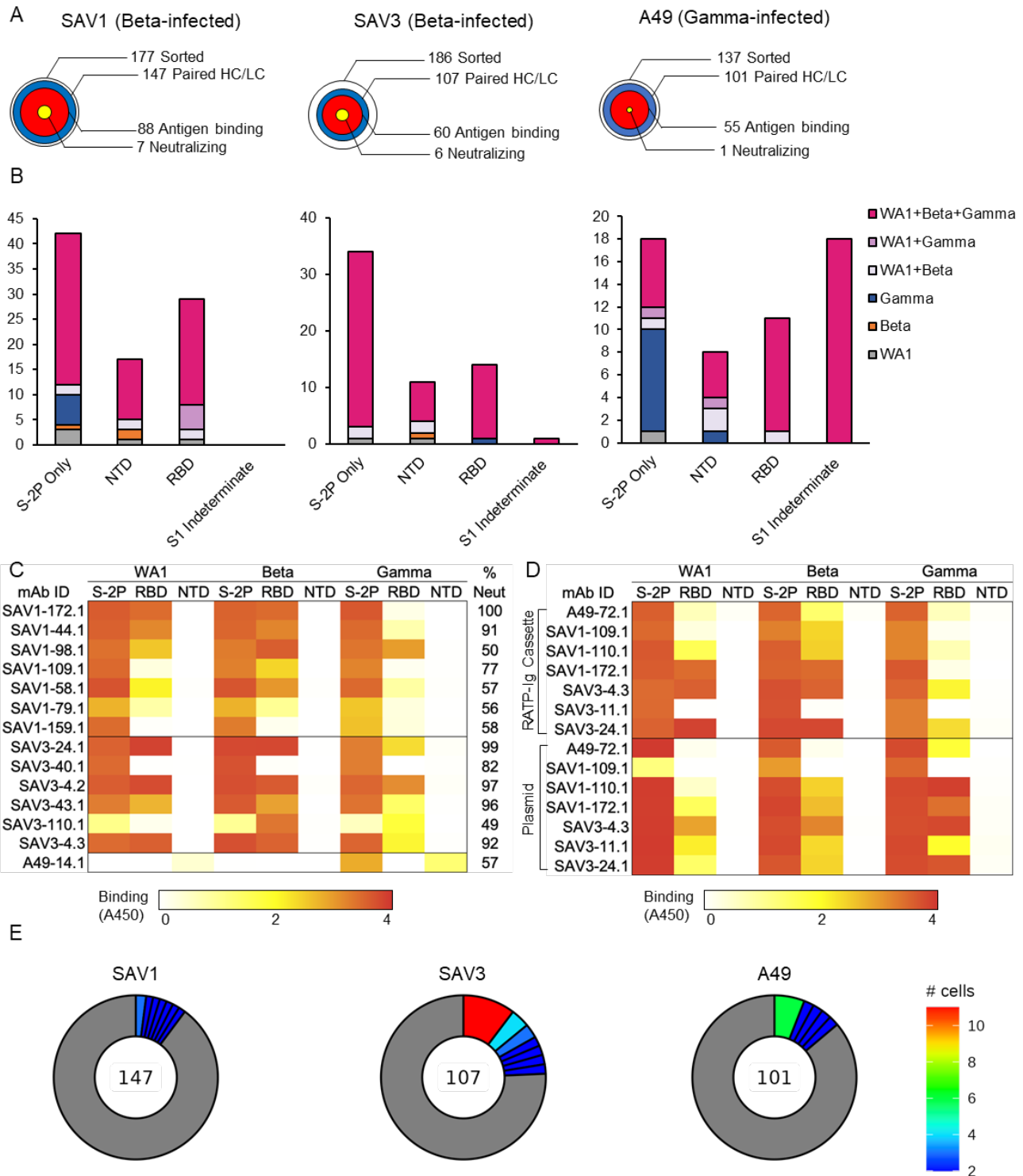
176 We next performed D614G pseudovirus neutralization screening for all supernatants at a  
177 4-fold dilution. This assay identified 7, 6, and 1 neutralizing antibodies from individuals SAV1,  
178 SAV3, and A49, respectively (Fig. 3C). Neutralizing antibodies were predominately cross-reactive  
179 and RBD-specific, except for two which bound to S-2P only and a single NTD-specific antibody  
180 (Fig. 3C). RBD-specific neutralizing antibodies were also the most potent of those isolated, with  
181 6/12 neutralizing >90% of pseudovirus at 4-fold dilution. It is important to note that supernatant  
182 IgG titers were not calculated but were only verified to reach a minimum cutoff value for functional  
183 assays, limiting our ability to compare potency between antibodies. Overall, we found that  
184 infection with Beta or Gamma variants elicited robust B-cell responses with cross-reactive binding  
185 and neutralizing mAbs.

186 To validate our results from supernatants produced by R ATP-Ig, we selected seven  
187 antibodies for heavy and light chain synthesis and expression. After performing antigen-specific  
188 ELISA on the plasmid-transfected supernatants, we found R ATP-Ig screening to be reliably  
189





190 **Figure 2:** Rapid assembly, transfection and production of immunoglobulin (RATP-Ig) workflow. 5'-RACE is  
191 used to generate total cDNA. Full-length heavy and light chain immunoglobulin V genes are enriched by  
192 PCR and assembled into recombinant mAb linear expression cassettes. In parallel, V gene libraries are  
193 synthesized and sequenced by NGS. Final cassettes are transfected into 96-well Expi293 microtiter  
194 cultures, and culture supernatants are collected up to 7 days after initial sort for functional screening.



195 **Figure 3:** Functional Characterization of RATP-Ig Isolated mAbs. (A) RATP-Ig screening overviews for  
 196 three individuals, represented as bullseyes. The area of each circle is proportional to the number of  
 197 antibodies. (B) Supernatants were screened for antigen-specific binding by single-point ELISA for WA1,  
 198 Beta, and Gamma S2P, RBD, and NTD. (C) Neutralization screening of isolated antibodies at 4-fold  
 199 supernatant dilutions using a D614G pseudovirus luciferase reporter assay, reported as % virus neutralized  
 200 derived from reduction in luminescence. Associated ELISA heatmap reported as absorbance at 450nm. (D)  
 201 Validation of RATP-Ig screening with synthesized plasmids. (E) Clonal expansion in each individual.  
 202 Expanded clones are colored by the number of cells in each clone as shown; singleton clones are shown  
 203 in gray.



204 predictive of mAb functionality, with 59/63 (94%) of functional interactions being reproduced (Fig.  
205 3D).

206 While all three individuals had polyclonal antigen-specific repertoires (Fig. 3E), SAV3 and  
207 A49 had highly expanded clones matching a widely reported public clone using IGHV1-69 and  
208 IGKV3-11 (28, 34, 49-53). Members of this public clone were also recovered from SAV1, although  
209 they were not greatly expanded. RATP-Ig ELISA data indicated that these antibodies bound a  
210 non-RBD, non-NTD epitope on Spike, consistent with available data for previously described  
211 members of this public clone. In addition, most antibodies from this public clone have been  
212 reported to bind SARS-CoV-1 (28, 34, 49, 50, 52), and one, mAb-123 (50), weakly binds endemic  
213 human coronaviruses HKU1 and 229E.

214 We also found 2 antibodies, SAV1-109.1 and SAV1-168.1, with a YYDRxG motif that can  
215 target the epitope of mAb CR3022 on RBD and produce broad and potent neutralization of a  
216 variety of sarbecoviruses (54). While SAV1-168.1 was cross-reactive but non-neutralizing (Table  
217 S3), SAV1-109.1 showed good neutralization potency and bound to all three variants tested when  
218 expressed both via RATP-Ig and from a plasmid (Fig. 3, C and D).

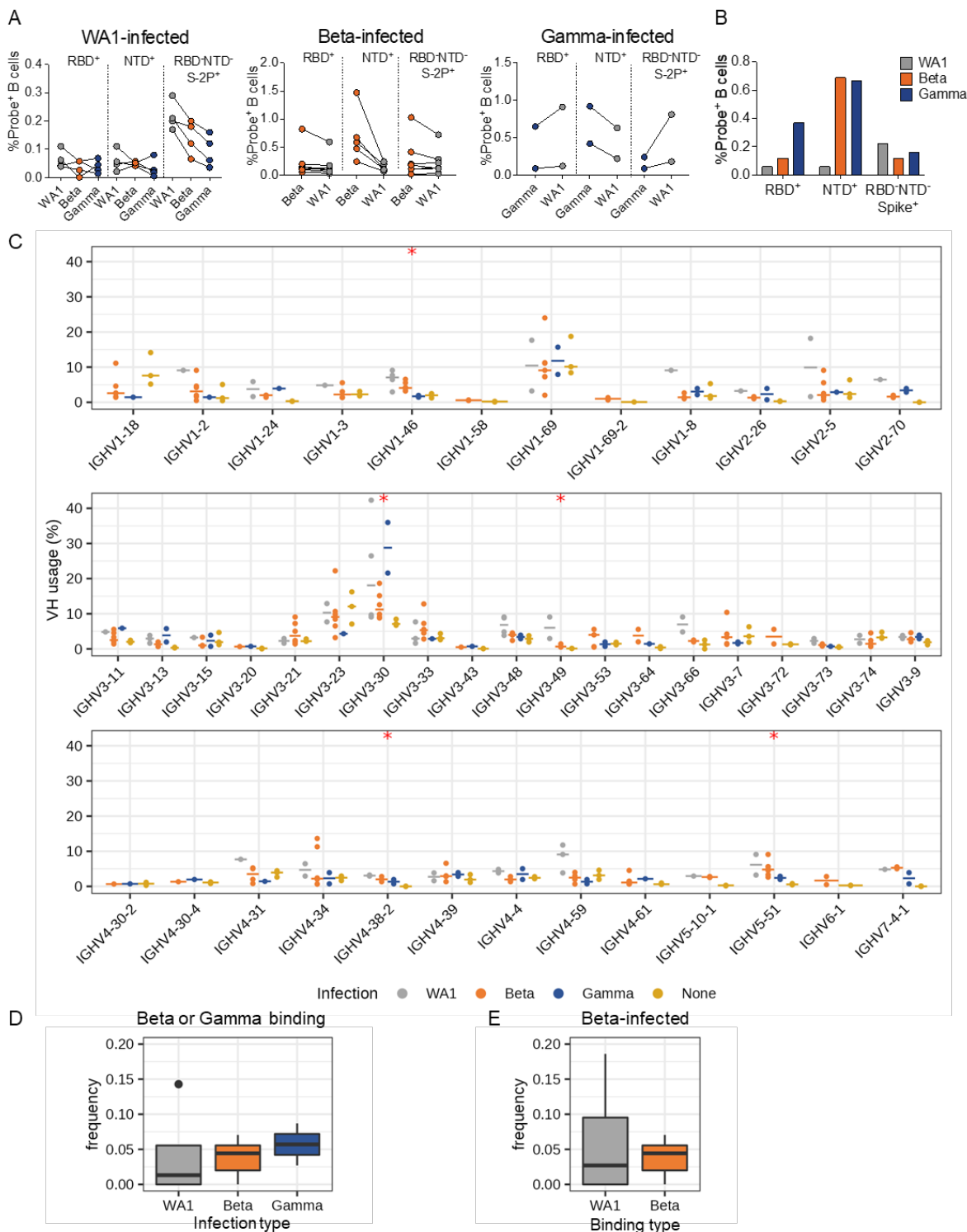
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## 220 **Sequence analysis of SARS-CoV-2 B cell repertoires elicited by different infecting** 221 **variants**

222 To investigate possible differences in targeting of domains outside of RBD, we stained  
223 memory B cells with fluorescently labeled S-2P and individual RBD and NTD probes and  
224 examined the specificities by flow cytometry (Fig. S2B). Cells from WA1-infected individuals were  
225 stained separately with WA1-, Beta-, and Gamma-based probes, while Beta- and Gamma-  
226 infected samples were stained for WA1 and the infecting variant probes (Fig. S2A). As expected,  
227 the frequency of antigen-specific cells was generally higher in individuals who had higher serum  
228 binding titers, and cells capable of binding to heterologous variants were typically less frequent  
229 than those binding the infecting variant (Fig. 4A). In addition, both Beta- and Gamma-infected  
230 individuals showed higher frequencies of NTD-binding B cells against the homologous virus when  
231 compared to WA1-infected individuals (Fig. 4B).

232 To analyze the SARS-CoV-2 spike-specific B cell repertoire elicited by each variant in  
233 more depth, we generated libraries from sorted antigen-specific single cells using the 10x  
234 Genomics Chromium platform. After sequencing, we recovered a total of 162, 319, and 107 paired  
235 heavy and light chain sequences from WA1-, Beta-, and Gamma-infected groups, respectively  
236 (Table S6). As observed in the sequences isolated via RATP-Ig, all three SARS-CoV-2 infected

237

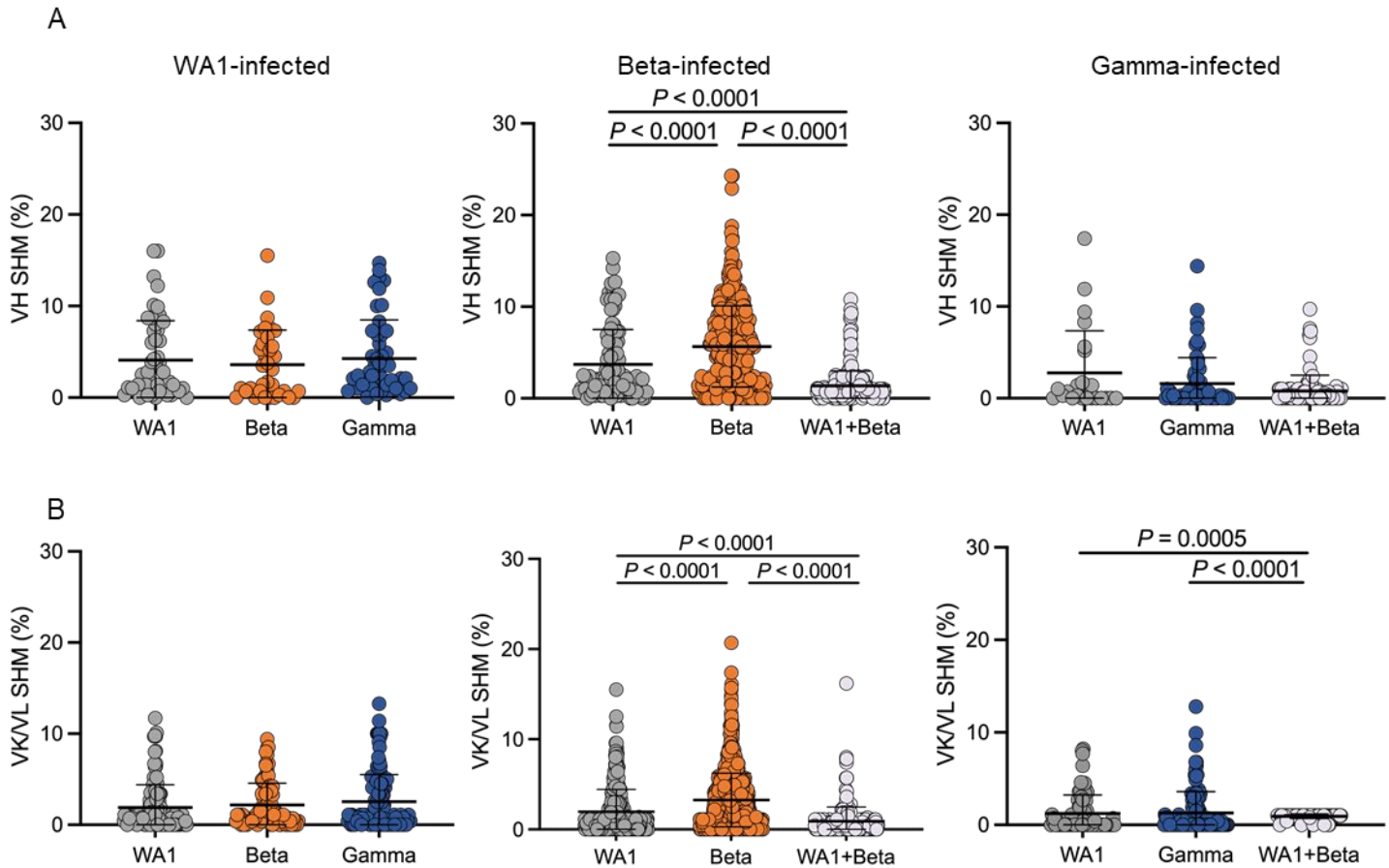


238 **Figure 4:** Anti-SARS-CoV-2 Ig repertoires. **(A)** Frequencies of probe<sup>+</sup> B cells sorted for IG repertoire  
 239 analysis. **(B)** Proportion of probe<sup>+</sup> B cells binding to each domain. **(C)** SARS-CoV-2-specific VH repertoire  
 240 analysis by infecting variant WA1, Beta and Gamma shown in grey, orange and blue, respectively, with  
 241 data from pre-pandemic controls in yellow. X-axis shows all germline genes used; y-axis represents percent  
 242 of individual gene usage. Stars indicate genes with at least one significant difference between groups;  
 243 pairwise comparisons are in Table S4. **(D)** and **(E)** Combined frequency of VH genes capable of giving rise  
 244 to stereotypical Y501-dependent antibodies (IGHV4-30, IGHV4-31, IGHV4-39, and IGHV4-61) in **(D)** Beta-  
 245 or Gamma-binding B cells from individuals infected with each variant or **(E)** B cells from Beta-infected  
 246 individuals sorted with either WA1- or Beta-derived probes.

247 IG repertoires showed little clonal expansion. We then combined these data with the sequences  
248 generated by RAMP-Ig for downstream analysis. Antigen-specific V gene usage was highly similar  
249 across all three infection types (Figs. 4C and S3), with differences noted only for IGHV1-46 and  
250 IGLV1-47 (Table S7). However, when we compared these specific repertoires to the total memory  
251 B cell repertoire in pre-pandemic controls (55), we observed significant enrichment for several  
252 genes (Figs. 4C and S3; Table S7). For example, IGHV1-46, IGHV5-51, and IGLV3-19 were all  
253 used at higher levels in both WA1- and Beta-elicited repertoires compared to the total memory  
254 pool, while IGHV3-30 was enriched in both WA1- and Gamma-infected individuals compared to  
255 controls (adjusted  $P$ -value  $\leq 0.05$ , Table S7). This highlights the convergence in responses to all  
256 SARS-CoV-2 variants we investigated.

257 Recent studies have shown that Y501-dependent mAbs derived from IGHV4-39 and  
258 related genes are overrepresented among neutralizing antibodies isolated from Beta-infected  
259 individuals (56, 57). Structural evidence suggests that bias toward these genes may in part be  
260 due to germline-encoded residues Y35 and Y54 in complementarity-determining region (CDR)  
261 H1 and H2, respectively (56). We therefore analyzed the observed frequency of germline genes  
262 encoding these residues (IGHV4-30, IGHV4-31, IGHV4-39, and IGHV4-61) among Beta- and  
263 Gamma-binding B cells but found no significant differences based on infecting variant (Fig. 4D).  
264 Furthermore, we compared the frequency of sequences using these germline genes for WA1-  
265 versus Beta-binding B cells among Beta-infected individuals (excluding cross-reactive B cells  
266 isolated by RAMP-Ig), and again found no difference in usage (Fig 4E). In addition, in many of the  
267 sequences we observed from these germline genes, Y35 and/or Y54 had been substituted due  
268 to somatic hypermutation (SHM), indicating that they likely are not members of the neutralizing  
269 class. This suggests that differences in the neutralization sensitivity of variants are not reflected  
270 in the overall binding patterns or sequences of specific mAbs, which instead remain highly  
271 consistent among individuals infected with different variants.

272 We next investigated SHM levels in these repertoires. The median  $V_H$  SHM levels among  
273 individuals ranged between 0.3% to 6.6% in  $V_H$  and 0.0 to 3.0% in  $V_L$ , compared to 6.7% and  
274 2.4%, respectively, in the control repertoires. We then further examined SHM by both infecting  
275 variant and the probes used to isolate each cell. We found no differences in SHM in single probe-  
276 binding repertoires for either WA1- or Gamma-infected individuals (Fig. 5). Surprisingly, cross-  
277 reactive (WA1 and Beta) cells sorted for RAMP-Ig had lower SHM than the single probe-binding  
278 repertoires sorted for 10x Genomics and sequencing. Moreover, single probe-binding Beta-  
279 specific B cells from Beta-infected individuals had significantly higher SHM (median of 4.9% in  $V_H$



280

281 **Figure 5:** Somatic hypermutation (SHM) levels of SARS-CoV-2 specific B cells (unpaired sequences). SHM  
282 percent in variable heavy ( $V_H$ ) (A) or variable kappa/lambda ( $V_K/V_L$ ) (B) regions. Error bars indicate the  
283 average number of nucleotide substitutions  $\pm$  standard deviation. Statistical significance was determined  
284 by the Mann-Whitney t-test.

285 and 2.7% in  $V_L$ ) compared to single probe WA1-binding cells from the same individuals (2.1% and  
286 0.8%, respectively) (Fig. 5). Overall, the low levels of SHM across all the SARS-CoV-2-specific B  
287 cells that we isolated is consistent with prior reports (32, 34, 36, 58-61). This further demonstrates  
288 that the human immune system can readily generate antibodies capable of cross-binding multiple  
289 variants, regardless of infecting variant.

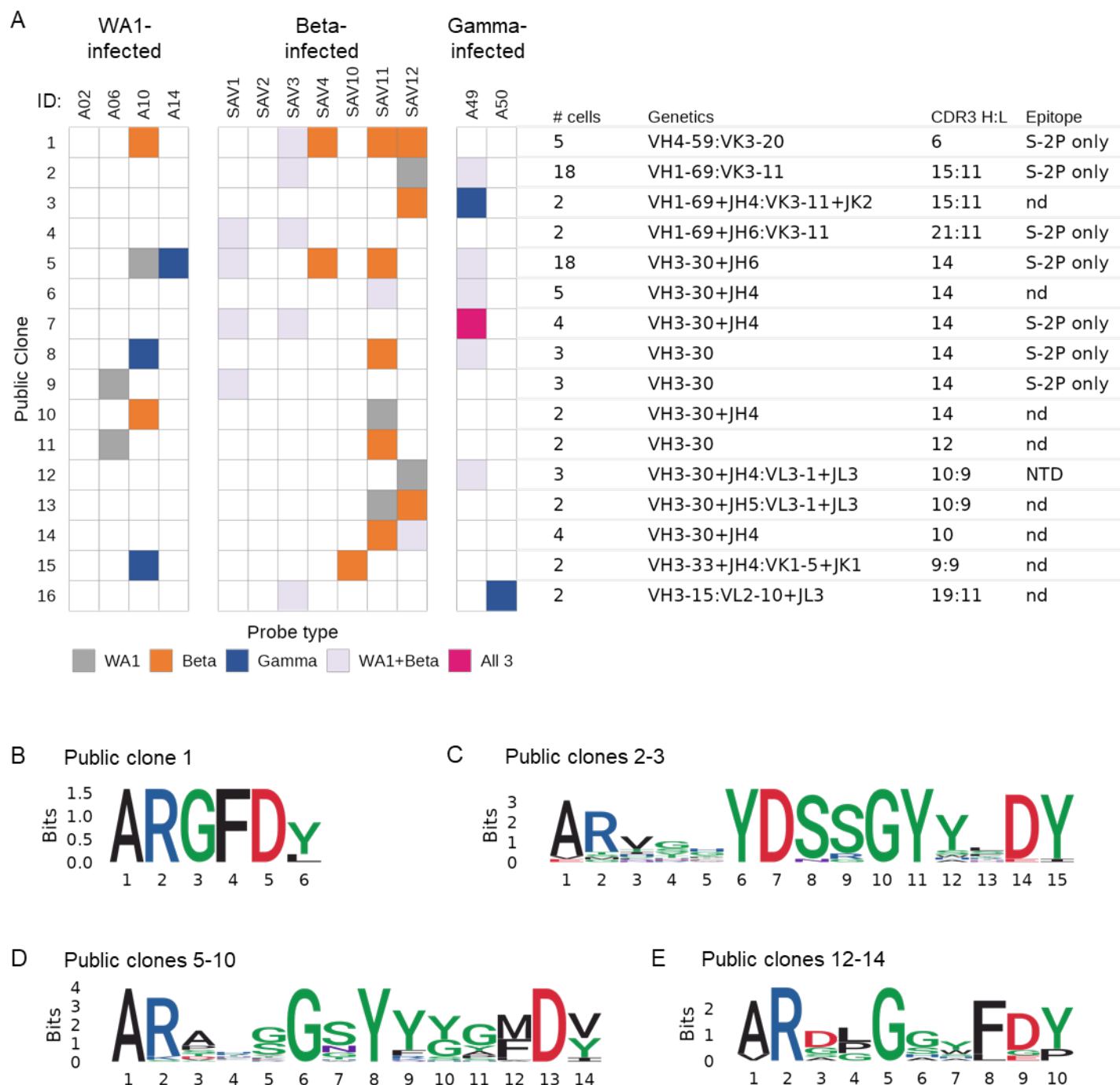
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### 291 **Identification of public clones**

292 We next identified public clones in the SARS-CoV-2-specific repertoires elicited after  
293 infection with different variants. We defined public clones as antibodies from multiple individuals  
294 using the same  $V_H$  gene and having at least 80% amino acid sequence identity in CDR H3. In  
295 total, 16 public clones were identified from 11 of the 13 infected individuals distributed across  
296 infection with all three variants (Fig. 6A). Notably, the two people for whom we failed to observe  
297 public clones were the least sampled individuals, with only 10 and 16 cells recovered, respectively  
298 (Table S6). While light chain V genes and CDR3 were not used to define public clones, they are  
299 reported when we found a consistent signature within a public clone.

300 One public clone, found in 5 individuals, uses IGHV4-59 with a short 6 amino acid CDR  
301 H3 and IGKV3-20. This public clone comprises B cells from a WA1-infected individual and 4 Beta-  
302 infected individuals which bound to both WA1 and Beta probes (Fig. 6A) and has a strongly  
303 conserved CDR H3 (Fig. 6B). Antibodies matching the signature of public clone 1 have been  
304 previously published (28, 49, 59, 62-64); notably, they have been characterized as binding the S2  
305 domain of Spike and are generally cross-reactive with SARS-CoV-1. Indeed, one member of this  
306 public clone, H712427+K711927, was isolated from an individual who was infected with SARS-  
307 CoV-1 (49). This suggests that the convergent immune responses we observe may not be limited  
308 only to variants of SARS-CoV-2 but may even extend to a broader range of sarbecoviruses.

309 Public clone 2 contains the expanded clones identified by RATP-Ig in SAV3 and A49,  
310 discussed above, as well as cells from SAV12. Public clone 3 includes sequences from two  
311 individuals that bound to either Beta or Gamma probes. Notably, both public clones 2 and 3 use  
312 the same heavy and light chain germline genes with the same CDR H3 and L3 lengths, though  
313 they fall outside of the 80% amino acid identity threshold. Combining sequences from both public  
314 clones revealed a strongly conserved IGHD3-22-encoded YDSSGY motif at positions 6-11 of  
315 CDR H3 (Fig. 6C). Strikingly, this is the same D gene implicated in targeting a Class IV RBD  
316 epitope (54), although public clones 2 and 3 instead target an epitope in S2 and appear to be  
317 restricted to IGHV1-69 and IGKV3-11 V genes.



318 **Figure 6:** Public and cross-reactive clones. **(A)** Sixteen public clones were identified. Public clones are  
 319 numbered 1-16 by row, as shown on the far left. Each column of boxes in the middle panel represents a  
 320 single individual, as labeled at top, and is colored by probe(s) used, as shown at bottom. Right panel shows  
 321 additional information about each public clone. Light chain information is provided after a colon if a  
 322 consistent signature was found. Epitopes are inferred from ELISA of RATP-Ig supernatants of at least 1  
 323 public clone member; nd, not determined. **(B)** CDR H3 logogram for the top public clone, found in 5 of 13  
 324 individuals. **(C)-(E)** Combined CDR H3 logograms for **(C)** 2 public clones using IGHV1-69 and IGKV3-11  
 325 with a 15 amino acid CDR H3 length. **(D)** 6 public clones using IGHV3-30 with a 14 amino acid CDR H3  
 326 length. **(E)** 3 public clones using IGHV3-30 with a 10 amino acid CDR H3 length.



327 We also observed the repeated use of IGHV3-30 with a 14 amino acid CDR H3 in six  
328 public clones which together comprise 35 cells from 8 different individuals. Each of these public  
329 clones included cells that bound to at least two variants, and all six were identified in individuals  
330 from at least two of our three variant-infected cohorts, suggesting a common, cross-reactive  
331 binding mode. When we combined CDR H3 sequences from all 6 public clones in this group, we  
332 found a consistent small-G-polar-Y-aromatic motif spanning positions 5-9 of CDR H3 (Fig. 6D). A  
333 large number of antibodies matching this signature have been previously described (25, 28, 34,  
334 49-52, 59, 60, 62-66). Similar to the above public clones, the epitope targeted by these antibodies  
335 has generally been reported as being in the S2 domain of Spike, and approximately one third of  
336 them have been shown to also bind SARS-CoV-1.

337 We identified only one public clone, 12, that we were able to verify bound to either RBD  
338 or NTD, although public clones 13 and 14 also have highly similar V genes and CDR lengths (Fig.  
339 6, A and E). These public clones were identified in both Beta- and Gamma-infected individuals  
340 from cells isolated with WA1 and/or Beta probes. Two previously reported antibodies, WRAIR-  
341 2038 (16) and COV-2307 (34), match the signature of these public clones and are also confirmed  
342 to bind NTD. The identification of a cross-reactive public clone is remarkable given deletions in  
343 Beta that disrupt the main NTD supersite for neutralizing antibodies (12). This again highlights  
344 the discordance between neutralization, which is variant-restricted, and reproducible binding  
345 modes which show a consensus in the face of differences among variants.

346

## 347 **DISCUSSION**

348 The rise of novel, antigenically distinct SARS-CoV-2 variants both threatens the efficacy  
349 of lifesaving mAb therapies and emphasizes the need for continued therapeutic mAb discovery  
350 (67). Moreover, although T cell responses are predominantly directed toward conserved epitopes  
351 and hence are broadly cross-reactive (18-22), Spike-binding IgG antibodies and serum  
352 neutralization have been identified as key correlates of protection for SARS-CoV-2 infection (68-  
353 70). Thus, a deep understanding of the IG repertoires that generate these protective responses  
354 will be critical for predicting the impact of infections with different variants.

355 In this study, we used rapid mAb production and functional analysis and single cell  
356 sequencing using the 10x Genomics platform to conduct an in-depth, unbiased characterization  
357 of total antigen-specific B cell repertoires from people infected with the ancestral WA1, Beta, or  
358 Gamma variants of SARS-CoV-2. Our principal findings were: 1) infection with any of these  
359 variants elicited antibodies targeting the same immunodominant epitopes in RBD; 2) antigen-  
360 specific memory B cells elicited by SARS-CoV-2 are polyclonal and use similar patterns of heavy

361 and light chain V genes, irrespective of the infecting variant; and 3) public clones and other cross-  
362 reactive antibodies are common among responses to all infecting variants. Our results  
363 demonstrate a fundamentally convergent humoral immune response across different SARS-CoV-  
364 2 variants.

365 To date, most analyses of SARS-CoV-2-specific B cells have focused on neutralizing  
366 antibodies with potential therapeutic applications. Those which have investigated the total binding  
367 repertoire have used samples from people infected with the ancestral WA1 variant (9, 25, 31);  
368 here we complement these studies with new data from Beta- and Gamma-infected individuals  
369 and show that the hierarchy of immunodominant epitopes remains unchanged. Indeed, while a  
370 recent report found that serum antibodies elicited by Beta infection were less likely to contact  
371 Spike residue F456 compared to antibodies elicited by WA1 infection (71), we found no changes  
372 in targeting of the RBD-A epitope, which includes this residue. Combined with the fact that Spike  
373 F456 is unchanged between WA1 and Beta, this suggests that the difference in escape is likely  
374 due to a shifted binding conformation (43, 44). Thus, just as binding epitope immunodominance  
375 is known to be consistent in response to WA1, Beta, or Omicron mRNA immunization (47, 48),  
376 we now demonstrate here similar immunodominance after variant infection. This insight will be  
377 helpful for understanding and predicting the burdens of serial infections with different variants.

378 In addition to concordant epitope targeting, we also found consistent V gene usage in the  
379 antibody response to all three variants we investigated. Although some recent studies have noted  
380 enrichment for IGHV4-39 and closely related V<sub>H</sub> genes in Beta-infection (56, 57) we did not  
381 observe any differences in the usage of these genes. Our findings likely highlight the difference  
382 between the neutralizing antibody repertoires investigated in prior studies compared to the total  
383 binding repertoires examined here and emphasize the insights to be gleaned by taking a broader  
384 perspective. However, despite this consistent epitope immunodominance and convergent V gene  
385 usage, we observed an excess of SHM in homologous Beta-binding B cells isolated from Beta-  
386 infected individuals, an effect we did not observe in WA1- or Gamma-infected individuals. This is  
387 even more unexpected in light of the lower overall SHM in cross-binding B cells sorted for mAb  
388 isolation by RATP-Ig. Other studies have also suggested the possibility that Beta may be  
389 somewhat distinct from other SARS-CoV-2 variants, inducing neutralization that appears to wane  
390 more slowly and that can be boosted to higher levels by additional vaccine doses (47, 48). In this  
391 light, it is perhaps even more important that we identified the same cross-reactive public clones  
392 induced by Beta infection as by WA1 and Gamma, suggesting that key elements for protection  
393 against other variants are likely to be maintained.

394 Furthermore, the genetic convergence among IG repertoires was not limited to V gene  
395 usage but extended to the public clones we identified. These were reliably observed irrespective  
396 of the infecting variant and were consistently identified as cross-reactive to multiple variants.  
397 Additionally, members of public clones 1 and 2 have been reported in the literature as having  
398 cross-reactivity extending to SARS-CoV-1. While they appear to be non-neutralizing and S2  
399 domain-binding, they may yet be important for Fc-dependent functions (16, 17) and thus their  
400 elicitation by different variants may contribute to protection from future infection with other  
401 variants. Overall, more than 8% of the cells that we sequenced belong to a public clone,  
402 highlighting again the extraordinary convergence of the antibody response across variants of  
403 SARS-CoV-2.

404 Importantly, we also observed convergences that are not encompassed within the  
405 standard definition of a public clone, consistent with structural modeling and clustering  
406 demonstrating that high CDR H3 sequence similarity and even convergent V genes are not  
407 required for antibodies to target overlapping epitopes using comparable binding conformations  
408 (72). As a specific example, we identified antibodies with a previously described IGHD3-22-  
409 encoded YYDRxG motif that can result in broad neutralization of divergent sarbecoviruses (54).  
410 Furthermore, we also observed three sets of multiple public clones with overlapping gene usage  
411 and CDR H3 lengths. Despite the low CDR H3 sequence homology between public clones, we  
412 found conserved motifs which are likely to drive functional convergence. These findings further  
413 highlight the capability of the human immune system to respond to SARS-CoV-2 in a manner that  
414 is largely consistent yet tolerant of differences between variants.

415 In summary, our data reveal marked convergence that defines multiple aspects of the  
416 humoral immune response to different SARS-CoV-2 variants. Despite the emergence of key  
417 escape mutations which have pronounced impact on neutralizing antibody function, first-  
418 generation vaccine designs using the ancestral Spike protein sequence have demonstrated the  
419 capacity to generate a cross-reactive anamnestic response that can be mobilized upon infection  
420 with novel variants (47, 48, 73, 74). Our observations show that this phenomenon may be  
421 explained in part by convergent V-gene usage and epitope specificities elicited by primary  
422 exposure to SARS-CoV-2 variant Spikes.

423

## 424 **LIMITATIONS OF THE STUDY**

425 Our study is limited by sampling of paired heavy and light chain sequences from fewer  
426 than 1,000 SARS-CoV-2-specific B cells across 13 individuals. This scale is small in comparison  
427 to bulk IG sequencing studies (32, 61) and even a few single-cell studies (58, 60, 75). We are

428 also limited in our ability to make functional repertoire comparisons due to varied sorting strategies  
429 and differences in functional assays used to assess isolated mAbs. Moreover, our cohort was  
430 sampled only at a single time point early in convalescence and included only one individual with  
431 high serum neutralization titers. It will be important to verify that our findings extend to later time  
432 points when the antibody repertoire has matured. In addition, further studies are needed to  
433 examine the response elicited by more recent SARS-CoV-2 variants such as Delta and Omicron.

434

## 435 **MATERIALS AND METHODS**

### 436 **Study design**

437 We selected 13 convalescent individuals that had experienced symptomatic Covid-19  
438 infection with either WA1 virus or the Beta or Gamma variants. Serum, plasma and PBMC were  
439 isolated at each respective clinical center. The selection of individuals was based on the  
440 availability of samples collected at similar time-points (between 17 and 38 days after symptoms  
441 onset), rather than the severity of disease or neutralizing antibody titers (Table S1). Seven  
442 individuals were infected with the Beta variant and recruited at the Sheba Medical Center, Tel  
443 HaShomer, Israel. Two individuals were infected with the Gamma variant and recruited at the  
444 University of Minnesota Hospital, USA. The samples from four WA1-infected individuals, collected  
445 early in the pandemic, as well as the two additional beta-infected individuals used for T cell  
446 analysis were collected under the Vaccine Research Center's (VRC), National Institute of Allergy  
447 and Infectious Diseases (NIAID), National Institutes of Health protocol VRC 200 (NCT00067054)  
448 in compliance with the NIH Institutional Review Board (IRB) approved protocol and procedures.  
449 All subjects met protocol eligibility criteria and agreed to participate in the study by signing the  
450 NIH IRB approved informed consent. Research studies with these samples were conducted by  
451 protecting the rights and privacy of the study participants. All participants provided informed  
452 consent in accordance with protocols approved by the respective institutional review boards and  
453 the Helsinki Declaration.

454

### 455 **Serology**

456 Antibody binding was measured by 10-plex Meso Scale Discovery  
457 Electrochemiluminescence immunoassay (MSD-ECLIA) as previously described (76). Cell-  
458 surface spike binding was assessed as previously described (76). Serum neutralization titers for  
459 either WA1-D614G, Beta, Gamma or Delta pseudotyped virus particles were obtained as  
460 previously described (76).

461

## 462 **Antigen-specific ELISA**

463           Reacti-Bind 96-well polystyrene plates (Pierce) were coated with 100  $\mu$ l of affinity purified  
464 goat anti-human IgG Fc (Rockland) at 1:20,000 in PBS, or 2  $\mu$ g/ml SARS-CoV-2 recombinant  
465 protein in PBS overnight at 4°C. Plates were washed in PBS-T (500ml 10XPBS + 0.05% Tween-  
466 20 + 4.5L H<sub>2</sub>O) and blocked for 1 h at 37°C with 200  $\mu$ L/well of B3T buffer: 8.8 g/liter NaCl, 7.87  
467 g/liter Tris-HCl, 334.7 mg/liter EDTA, 20 g BSA Fraction V, 33.3 ml/liter fetal calf serum, 666  
468 ml/liter Tween-20, and 0.02% Thimerosal, pH 7.4). Diluted antibody samples were applied and  
469 incubated 1 hr at 37°C followed by 6 washes with PBS-T; plates were the incubated with HRP-  
470 conjugated anti-human IgG (Jackson ImmunoResearch) diluted 1:10,000 in B3T buffer for 1 h at  
471 37°C. After 6 washes with PBS-T, SureBlue TMB Substrate (KPL) was added, incubated for 10  
472 min, and the reaction was stopped with 1N H<sub>2</sub>SO<sub>4</sub> before measuring optical densities at 450nm  
473 (Molecular Devices, SpectraMax using SoftMax Pro 5 software). For single-point assays,  
474 supernatants from transfected cells were diluted 1:10 in B3T and added to the blocked plates.  
475 Purified monoclonal antibodies were assessed using 5-fold serial dilutions starting at 10 $\mu$ g/ml. To  
476 assess the levels of IgG in supernatants, standard curves were run on the same plates as  
477 supernatants, using threefold serial dilutions of human IgG (Sigma) starting at 100ng/ml IgG.

478

## 479 **Intracellular cytokine staining**

480           The T cell staining panel used in this study was modified from a panel developed by the  
481 laboratory of Dr. Steven De Rosa (Fred Hutchinson Cancer Research Center). Directly  
482 conjugated antibodies purchased from BD Biosciences include CD19 PE-Cy5 (Clone HIB19; cat.  
483 302210), CD14 BB660 (Clone M0P9; cat. 624925), CD3 BUV395 (Clone UCHT1; cat. 563546),  
484 CD4 BV480 (Clone SK3; cat. 566104), CD8a BUV805 (Clone SK1; cat. 612889), CD45RA  
485 BUV496 (Clone H100; cat. 750258), CD154 PE (Clone TRAP1; cat. 555700), IFN $\gamma$  V450 (Clone  
486 B27; cat. 560371 and IL-2 BB700 (Clone MQ1-17H12; cat. 566404). Antibodies from Biolegend  
487 include CD16 BV570 (Clone 3G8; cat. 302036), CD56 BV750 (Clone 5.1H11; cat. 362556), CCR7  
488 BV605 (Clone G043H7; cat. 353244) and CD69 APC-Fire750 (Clone FN50; cat. 310946). TNF  
489 FITC (Clone Mab11; cat. 11-7349-82) and the LIVE/DEAD Fixable Blue Dead Cell Stain (cat.  
490 L34962) were purchased from Invitrogen.

491           Cryopreserved PBMC were thawed into pre-warmed R10 media (RPMI 1640, 10% FBS,  
492 2 mM L-glutamine, 100 U/ml penicillin, and 100  $\mu$ g/ml streptomycin) containing DNase and rested  
493 for 1 hour at 37°C/5% CO<sub>2</sub>. For stimulation, 1 – 1.5 million cells were plated into 96-well V-bottom  
494 plates in 200mL R10 and stimulated with SARS-CoV-2 peptide pools (2 $\mu$ g/mL for each peptide)  
495 for 6 hours at 37°C/5%CO<sub>2</sub>. A DMSO-only condition was used to determine background

496 responses. Following stimulation samples were stained with LIVE/DEAD Fixable Blue Dead Cell  
497 Stain for 10 minutes at room temperature and surface stained with titrated amounts of anti-CD19,  
498 anti-CD14, anti-CD16, anti-CD56, anti-CD4, anti-CD8, anti-CCR7 and anti-CD45RA for 20  
499 minutes at room temperature. Cells were washed in FACS Buffer (PBS + 2% FBS), and fixed and  
500 permeabilized (Cytotfix/Cytoperm, BD Biosciences) for 20 minutes at room temperature.  
501 Following fixation, cells were washed with Perm/Wash buffer (BD Biosciences) and stained  
502 intracellularly with anti-CD3, anti-CD154, anti-CD69, anti-IFN $\gamma$ , anti-IL-2 and anti-TNF for 20  
503 minutes at room temperature. Cells were subsequently washed with Perm/Wash buffer and fixed  
504 with 1% paraformaldehyde. Data were acquired on a modified BD FACSymphony and analyzed  
505 using FlowJo software (version 10.7.1). Cytokine frequencies were background subtracted and  
506 negative values were set to zero.

507 Synthetic peptides (>75% purity by HPLC; 15 amino acids in length overlapping by 11  
508 amino acids) were synthesized by GenScript. To measure T cell responses to the full-length WA-  
509 1 Spike glycoprotein (YP\_009724390.1), 2 peptide pools were utilized, Spike pool A (peptides 1-  
510 160; residues 1-651) and Spike pool B (peptides 161-316; residues 641-1273) (Table S8).  
511 Peptides were 15 amino acids in length and overlapped by 11 amino acids. Spike pool A  
512 contained peptides for both D614 and the G614 mutation. Responses to full-length Spike were  
513 calculated by summing the responses to both pools after background subtraction. Select peptide  
514 pools were used to measure T cell responses to mutated regions of the Spike glycoproteins of  
515 the Alpha, Beta and Gamma SARS-CoV-2 variants along with control pools corresponding to the  
516 same regions within the WA-1 Spike glycoprotein (Table S9).

517

### 518 **Epitope mapping by Surface Plasmon Resonance (SPR)**

519 Serum epitope mapping competition assays were performed, as previously described (47,  
520 48), using the Biacore 8K+ surface plasmon resonance system (Cytiva). Anti-histidine antibody  
521 was immobilized on Series S Sensor Chip CM5 (Cytiva) through primary amine coupling using a  
522 His capture kit (Cytiva). Following this, his-tagged SARS-CoV-2 S protein containing 2 proline  
523 stabilization mutations (S-2P) was captured on the active sensor surface.

524 Human IgG monoclonal antibodies (mAb) used for these analyses include: B1-182, CB6,  
525 A20-29.1, A19-46.1, LY-COV555, A19-61.1, S309, A23-97.1, A19-30.1, A23-80.1, and CR3022.  
526 Either competitor or negative control mAb was injected over both active and reference surfaces.  
527 Human sera were then flowed over both active and reference sensor surfaces, at a dilution of  
528 1:50. Following the association phase, active and reference sensor surfaces were regenerated  
529 between each analysis cycle.



530 Prior to analysis, sensorgrams were aligned to Y (Response Units) = 0, using Biacore 8K  
531 Insights Evaluation Software (Cytiva), at the beginning of the serum association phase. Relative  
532 “analyte binding late” report points (RU) were collected and used to calculate percent competition  
533 (% C) using the following formula:  $\% C = [1 - (100 * (RU \text{ in presence of competitor mAb} / (RU$   
534  $\text{ in presence of negative control mAb}))]$ . Results are reported as percent competition and statistical  
535 analysis was performed using unpaired, two-tailed t-test (Graphpad Prism v.8.3.1). All assays  
536 were performed in duplicate and averaged.

537

### 538 **Production of antigen-specific probes**

539 Biotinylated probes for S-2P, NTD and RBD were produced as described previously (77,  
540 78). Briefly, single-chain Fc and AVI-tagged proteins were expressed transiently for 6 days. After  
541 harvest, the soluble proteins were purified and biotinylated in a single protein A column followed  
542 by final purification on a Superdex 200 16/600 gel filtration column. Biotinylated proteins were  
543 then conjugated to fluorescent streptavidin.

544

### 545 **Antigen-specific B cell sorting**

546 PBMC vials containing approximately  $10^7$  cells were thawed and stained with Live/Dead  
547 Fixable Blue Dead Cell Stain Kit (Invitrogen, cat# L23105) for 10 min at room temperature,  
548 followed by incubation for 20 min with the staining cocktail consisting of antibodies and probes.  
549 The antibodies used in the staining cocktail were: CD8-BV510 (Biolegend, clone RPA-T8, cat#  
550 301048), CD56-BV510 (Biolegend, clone HCD56, cat# 318340), CD14-BV510 (Biolegend, clone  
551 M5E2, cat# 301842), CD16-BUV496 (BD Biosciences, clone 3G8, cat# 612944), CD3-APC-Cy7  
552 (BD Biosciences, clone SP34-2, cat# 557757), CD19-PECy7 (Beckmann Coulter, clone J3-119,  
553 cat# IM36284), CD20 (BD Biosciences, clone 2H7, cat# 564917), IgG-FITC (BD Biosciences,  
554 clone G18-145, cat# 555786), IgA-FITC (Miltenyi Biotech, clone IS11-8E10, cat# 130-114-001)  
555 and IgM-PECF594 (BD Biosciences, clone G20-127, cat# 562539). For each variant, a set of two  
556 spike probes S-2P-APC and S-2P-BUV737, in addition to RBD-BV421 and NTD-BV711 were  
557 included in the staining cocktail for flow cytometry sorting.

558 For RATP-Ig, single-cells were sorted in 96-well plates containing 5  $\mu$ L of TCL buffer  
559 (Qiagen) with 1%  $\beta$ -mercaptoethanol according to the gating strategy shown in Fig. S2B. Samples  
560 sorted for 10x Genomics single-cell RNAseq were individually labelled with an oligonucleotide-  
561 linked hashing antibody (TotalSeq-C, Biolegend) in addition to the staining cocktail and sorted into  
562 a single tube according to the gating strategy shown in Fig. S2B. All cell sorts were performed

563 using a BD FACSAria II instrument (BD Biosciences). Frequency of antigen-specific B cells were  
564 analyzed using FlowJo 10.8.1 (BD Biosciences).

565

### 566 **Monoclonal antibody isolation and characterization by RATP-Ig**

567 **cDNA synthesis:** Variable heavy and light chains were synthesized using a modified  
568 SMARTSeq-V4 protocol by 5' RACE. Single-cell RNA was first purified with RNAClean beads  
569 (Beckman Coulter). cDNA was then synthesized using 5' RACE reverse-transcription, adding  
570 distinct 3' and 5' template switch oligo adapters to total cDNA. cDNA was subsequently amplified  
571 with TSO\_FWD and TS\_Oligo\_2\_REV primers. Excess oligos and dNTPs were removed from  
572 amplified cDNA with EXO-CIP cleanup kit (New England BioLabs).

573 **Immunoglobulin enrichment and sequencing:** Heavy and light chain variable regions were  
574 enriched by amplifying cDNA with TSO\_FWD and IgA/IgG\_REV or IgK/IgL\_REV primer pools. An  
575 aliquot of enriched product was used to prepare Nextera libraries with Unique Dual Indices  
576 (Illumina) and sequenced using 2x150 paired-end reads on an Illumina MiSeq. Separate aliquots  
577 were used for IG production; RATP-Ig is a modular system and can produce single combined or  
578 separate HC/LC cassettes.

579 **Cassette fragment synthesis:** Final cassettes include CMV, and HC/LC-TBGH polyA fragments.  
580 To isolate these fragments, amplicons were first synthesized by PCR. PCR products were run on  
581 a 1% agarose gel and fragments of the correct length were extracted with Thermo gel extraction  
582 and PCR cleanup kit (ThermoFisher Scientific). Gel-extracted products were digested with DpnI  
583 (New England Biolabs) to further remove any possible contaminating plasmid. These fragment  
584 templates were then further amplified to create final stocks of cassette fragments.

585 **Cassette assembly:** Enriched variable regions were assembled into linear expression cassettes  
586 in two sequential ligation reactions. The first reaction assembles CMV-TSO, TSO-V-LC, and KC-  
587 IRES fragments into part 1 and IRES-TSO, TSO-V-HC, and IgGC-TBGH fragments into part 2  
588 using NEBuilder HIFI DNA Assembly Mastermix (New England BioLabs). Following reaction 1,  
589 parts 1 and 2 were combined into a single reaction 2 and ligated into a single cassette.

590 **Separate cassettes:** Enriched variable regions were assembled into linear expression  
591 cassettes by ligating CMV-TSO, TSO-V-C, and C-TBGH fragments using NEBuilder HIFI DNA  
592 Assembly Mastermix (New England BioLabs). Assembled cassettes were amplified using  
593 CMV\_FWD and TBGH\_REV primers. Amplified linear DNA cassettes encoding monoclonal heavy  
594 and light chain genes were co-transfected into Expi293 cells in 96-well deep-well plates using the  
595 Expi293 Transfection Kit (ThermoFisher Scientific) according to the manufacturer's protocol.

596 Microtiter cultures were incubated at 37 degrees and 8% CO<sub>2</sub> with shaking at 1100 RPM for 5-7  
597 days before supernatants were clarified by centrifugation and harvested.

598

### 599 **Droplet-based single cell isolation and sequencing**

600 Antigen-specific memory B cells were sorted as described above. Cells from two separate  
601 sorts were pooled in a single suspension and loaded on the 10x Genomics Chromium instrument  
602 with reagents from the Next GEM Single Cell 5' Kit v1.1 following the manufacturer's protocol to  
603 generate total cDNA. Heavy and light chains were amplified from the cDNA using custom 3'  
604 primers specific for IgG, IgA, IgK or IgL with the addition of Illumina sequences (79). The Illumina-  
605 ready libraries were sequenced using 2x300 paired-end reads on an Illumina MiSeq. Hashing  
606 oligonucleotides were amplified and sequenced from the total cDNA according to the 10x  
607 Genomics protocol.

608

### 609 **V(D)J sequence analysis**

610 For cells processed via RATP-Ig, reads were demultiplexed using a custom script and  
611 candidate V(D)J sequences were generated using BALDR (80) and filtered for quality using a  
612 custom script. The resulting sequences were annotated using SONAR v4.2 (81) in single-cell  
613 mode.

614 For cells processed via the 10x Genomics Chromium device, reads from the hashing  
615 libraries were processed using cellranger (10x Genomics). The resulting count matrix was  
616 imported into Seurat (82) and the sample of origin called using the HTODemux function. Paired-  
617 end reads from V(D)J libraries were merged and annotated using SONAR in single-cell mode with  
618 UMI detection and processing.

619 For all datasets, nonproductive rearrangements were discarded, as were any cells with  
620 more than one productive heavy or light chain. Cells with an unpaired heavy or light chain were  
621 included in calculations of SHM and gene usage statistics, but were excluded from assessments  
622 of clonality and determination of public clones. Public clones were determined by using the  
623 clusterfast algorithm in vsearch (83) to cluster CDR H3 amino acid sequences at 80% identity.  
624 Where relevant, all clonally related B cells in a single individual were included in a public clone,  
625 even if not all were directly clustered together in the vsearch analysis.

626

### 627 **Supplementary Materials:**

628 **Fig. S1:** Additional serology and epitope mapping data. **A)** Antibody binding titers against multiple  
629 variants assessed by cell surface binding assay; **B)** Structural schematic of spike protein showing

630 epitopes from monoclonal antibodies used for RBD epitope mapping by competition assay; **C)**  
631 Epitope mapping of Beta-infected individuals on WA1, Beta and Delta spike proteins; **D)** Epitope  
632 mapping of Gamma-infected individuals on WA1, Beta and Delta spike proteins; **E)** Gating  
633 strategy for T cell response analysis.

634 **Fig. S2:** Antigen-specific B cell sorting. **(A)** Arrows indicate probes used for sorting antigen-  
635 specific B cells from each group of convalescent individuals. The individual marked with a star  
636 was used for both RATP-Ig and total BCR repertoire sequencing. **(B)** Flow cytometry  
637 representative plots and gating strategies for B cell sorting and analysis; final sort gates are shown  
638 in blue.

639 **Fig. S3:** SARS-CoV-2-specific light chain V gene usage frequencies. **(A)** Kappa and **(B)** Lambda  
640 chain V gene repertoire analysis by infecting variant, with WA1, Beta and Gamma shown in grey,  
641 orange and blue, respectively, and data from pre-pandemic controls in yellow. The x-axis shows  
642 all germline genes used; the y-axis represents the percent of individual gene usage. Stars indicate  
643 genes with at least one significant difference between groups; pairwise comparisons using the  
644 Dunn test are in Table S7.

645

646 **Table S1:** Details of the study cohort.

647 **Table S2:** Serum epitope competition

648 **Table S3:** Heatmaps of complete RATP-Ig ELISA results for SAV1. Values are reported as  
649 absorbance at 450nm wavelength.

650 **Table S4:** Heatmaps of complete RATP-Ig ELISA results for SAV3. Values are reported as  
651 absorbance at 450nm wavelength.

652 **Table S5:** Heatmaps of complete RATP-Ig ELISA results for A49. Values are reported as  
653 absorbance at 450nm wavelength.

654 **Table S6:** Sample recovery from 10x Genomics-based single cell isolation and sequencing.

655 **Table S7:** Significant differences in gene-usage. For genes with a significant difference detected  
656 by the Kruskal-Wallis test (Figs. 4B and S3), the Dunn test was used to find significant pairwise  
657 difference. P values were adjusted for multiple testing using the Benjami-Hochberg procedure.

658 **Table S8:** Sequences of peptides included in Spike pools A and B used for T cell stimulation.  
659 Highlighted peptides did not meet >75% purity and were not included in the pool.

660 **Table S9:** Sequences of peptides included in selected peptide pools for each variant used for T  
661 cell stimulation.

662

663

664 **References and Notes**

665

- 666 1. WHO, in *Tracking SARS-CoV-2 variants*, [https://www.who.int/en/activities/tracking-](https://www.who.int/en/activities/tracking-SARS-CoV-2-variants)  
667 [SARS-CoV-2-variants](https://www.who.int/en/activities/tracking-SARS-CoV-2-variants), Ed. (2022).
- 668 2. B. Korber, W. M. Fischer, S. Gnanakaran, H. Yoon, J. Theiler, W. Abfalterer, N.  
669 Hengartner, E. E. Giorgi, T. Bhattacharya, B. Foley, K. M. Hastie, M. D. Parker, D. G.  
670 Partridge, C. M. Evans, T. M. Freeman, T. I. de Silva, C.-G. G. Sheffield, C. McDanal, L.  
671 G. Perez, H. Tang, A. Moon-Walker, S. P. Whelan, C. C. LaBranche, E. O. Saphire, D. C.  
672 Montefiori, Tracking Changes in SARS-CoV-2 Spike: Evidence that D614G Increases  
673 Infectivity of the COVID-19 Virus. *Cell* **182**, 812-827 e819 (2020).
- 674 3. D. Weissman, M. G. Alameh, T. de Silva, P. Collini, H. Hornsby, R. Brown, C. C.  
675 LaBranche, R. J. Edwards, L. Sutherland, S. Santra, K. Mansouri, S. Gobeil, C. McDanal,  
676 N. Pardi, N. Hengartner, P. J. C. Lin, Y. Tam, P. A. Shaw, M. G. Lewis, C. Boesler, U.  
677 Sahin, P. Acharya, B. F. Haynes, B. Korber, D. C. Montefiori, D614G Spike Mutation  
678 Increases SARS CoV-2 Susceptibility to Neutralization. *Cell Host Microbe* **29**, 23-31 e24  
679 (2021).
- 680 4. Y. Weisblum, F. Schmidt, F. Zhang, J. DaSilva, D. Poston, J. C. Lorenzi, F. Muecksch, M.  
681 Rutkowska, H. H. Hoffmann, E. Michailidis, C. Gaebler, M. Agudelo, A. Cho, Z. Wang,  
682 A. Gazumyan, M. Cipolla, L. Luchsinger, C. D. Hillyer, M. Caskey, D. F. Robbiani, C. M.  
683 Rice, M. C. Nussenzweig, T. Hatziioannou, P. D. Bieniasz, Escape from neutralizing  
684 antibodies by SARS-CoV-2 spike protein variants. *Elife* **9**, (2020).
- 685 5. F. Schmidt, Y. Weisblum, M. Rutkowska, D. Poston, J. DaSilva, F. Zhang, E. Bednarski,  
686 A. Cho, D. J. Schaefer-Babajew, C. Gaebler, M. Caskey, M. C. Nussenzweig, T.  
687 Hatziioannou, P. D. Bieniasz, High genetic barrier to SARS-CoV-2 polyclonal neutralizing  
688 antibody escape. *Nature* **600**, 512-516 (2021).
- 689 6. W. Dejnirattisai, D. Zhou, P. Supasa, C. Liu, A. J. Mentzer, H. M. Ginn, Y. Zhao, H. M.  
690 E. Duyvesteyn, A. Tuekprakhon, R. Nutalai, B. Wang, C. Lopez-Camacho, J. Slon-  
691 Campos, T. S. Walter, D. Skelly, S. A. Costa Clemens, F. G. Naveca, V. Nascimento, F.  
692 Nascimento, C. Fernandes da Costa, P. C. Resende, A. Pauvolid-Correa, M. M. Siqueira,  
693 C. Dold, R. Levin, T. Dong, A. J. Pollard, J. C. Knight, D. Crook, T. Lambe, E. Clutterbuck,  
694 S. Bibi, A. Flaxman, M. Bittaye, S. Belij-Rammerstorfer, S. C. Gilbert, M. W. Carroll, P.  
695 Klenerman, E. Barnes, S. J. Dunachie, N. G. Paterson, M. A. Williams, D. R. Hall, R. J. G.  
696 Hulswit, T. A. Bowden, E. E. Fry, J. Mongkolsapaya, J. Ren, D. I. Stuart, G. R. Screaton,  
697 Antibody evasion by the P.1 strain of SARS-CoV-2. *Cell* **184**, 2939-2954 e2939 (2021).
- 698 7. W. Dejnirattisai, J. Huo, D. Zhou, J. Zahradnik, P. Supasa, C. Liu, H. M. E. Duyvesteyn,  
699 H. M. Ginn, A. J. Mentzer, A. Tuekprakhon, R. Nutalai, B. Wang, A. Djokaite, S. Khan,  
700 O. Avinoam, M. Bahar, D. Skelly, S. Adele, S. A. Johnson, A. Amini, T. G. Ritter, C.  
701 Mason, C. Dold, D. Pan, S. Assadi, A. Bellass, N. Omo-Dare, D. Koeckerling, A. Flaxman,  
702 D. Jenkin, P. K. Aley, M. Voysey, S. A. Costa Clemens, F. G. Naveca, V. Nascimento, F.  
703 Nascimento, C. Fernandes da Costa, P. C. Resende, A. Pauvolid-Correa, M. M. Siqueira,  
704 V. Baillie, N. Serafin, G. Kwatra, K. Da Silva, S. A. Madhi, M. C. Nunes, T. Malik, P. J.  
705 M. Openshaw, J. K. Baillie, M. G. Semple, A. R. Townsend, K. A. Huang, T. K. Tan, M.  
706 W. Carroll, P. Klenerman, E. Barnes, S. J. Dunachie, B. Constantinides, H. Webster, D.  
707 Crook, A. J. Pollard, T. Lambe, O. Consortium, I. C. Consortium, N. G. Paterson, M. A.  
708 Williams, D. R. Hall, E. E. Fry, J. Mongkolsapaya, J. Ren, G. Schreiber, D. I. Stuart, G. R.



- 709 Screaton, SARS-CoV-2 Omicron-B.1.1.529 leads to widespread escape from neutralizing  
710 antibody responses. *Cell* **185**, 467-484 e415 (2022).
- 711 8. F. Amanat, M. Thapa, T. Lei, S. M. S. Ahmed, D. C. Adelsberg, J. M. Carreno, S.  
712 Strohmeier, A. J. Schmitz, S. Zafar, J. Q. Zhou, W. Rijnink, H. Alshammary, N.  
713 Borchering, A. G. Reiche, K. Srivastava, E. M. Sordillo, H. van Bakel, I. Personalized  
714 Virology, J. S. Turner, G. Bajic, V. Simon, A. H. Ellebedy, F. Krammer, SARS-CoV-2  
715 mRNA vaccination induces functionally diverse antibodies to NTD, RBD, and S2. *Cell*  
716 **184**, 3936-3948 e3910 (2021).
- 717 9. T. N. Starr, A. J. Greaney, S. K. Hilton, D. Ellis, K. H. D. Crawford, A. S. Dingens, M. J.  
718 Navarro, J. E. Bowen, M. A. Tortorici, A. C. Walls, N. P. King, D. Veelsler, J. D. Bloom,  
719 Deep Mutational Scanning of SARS-CoV-2 Receptor Binding Domain Reveals  
720 Constraints on Folding and ACE2 Binding. *Cell* **182**, 1295-1310 e1220 (2020).
- 721 10. M. Kandeel, M. E. M. Mohamed, H. M. Abd El-Lateef, K. N. Venugopala, H. S. El-Beltagi,  
722 Omicron variant genome evolution and phylogenetics. *J Med Virol* **94**, 1627-1632 (2022).
- 723 11. E. Boehm, I. Kronig, R. A. Neher, I. Eckerle, P. Vetter, L. Kaiser, D. Geneva Centre for  
724 Emerging Viral, Novel SARS-CoV-2 variants: the pandemics within the pandemic. *Clin*  
725 *Microbiol Infect* **27**, 1109-1117 (2021).
- 726 12. P. Wang, M. S. Nair, L. Liu, S. Iketani, Y. Luo, Y. Guo, M. Wang, J. Yu, B. Zhang, P. D.  
727 Kwong, B. S. Graham, J. R. Mascola, J. Y. Chang, M. T. Yin, M. Sobieszczyk, C. A.  
728 Kyratsous, L. Shapiro, Z. Sheng, Y. Huang, D. D. Ho, Antibody resistance of SARS-CoV-  
729 2 variants B.1.351 and B.1.1.7. *Nature* **593**, 130-135 (2021).
- 730 13. L. Wang, T. Zhou, Y. Zhang, E. S. Yang, C. A. Schramm, W. Shi, A. Pegu, O. K. Oloniyi,  
731 A. R. Henry, S. Darko, S. R. Narpala, C. Hatcher, D. R. Martinez, Y. Tsybovsky, E. Phung,  
732 O. M. Abiona, A. Antia, E. M. Cale, L. A. Chang, M. Choe, K. S. Corbett, R. L. Davis, A.  
733 T. DiPiazza, I. J. Gordon, S. H. Hait, T. Hermanus, P. Kgagudi, F. Laboune, K. Leung, T.  
734 Liu, R. D. Mason, A. F. Nazzari, L. Novik, S. O'Connell, S. O'Dell, A. S. Olia, S. D.  
735 Schmidt, T. Stephens, C. D. Stringham, C. A. Talana, I. T. Teng, D. A. Wagner, A. T.  
736 Widge, B. Zhang, M. Roederer, J. E. Ledgerwood, T. J. Ruckwardt, M. R. Gaudinski, P.  
737 L. Moore, N. A. Doria-Rose, R. S. Baric, B. S. Graham, A. B. McDermott, D. C. Douek,  
738 P. D. Kwong, J. R. Mascola, N. J. Sullivan, J. Misasi, Ultrapotent antibodies against diverse  
739 and highly transmissible SARS-CoV-2 variants. *Science* **373**, (2021).
- 740 14. S. Changrob, Y. Fu, J. J. Guthmiller, P. J. Halfmann, L. Li, C. T. Stamper, H. L. Dugan,  
741 M. Accola, W. Rehrauer, N. Y. Zheng, M. Huang, J. Wang, S. A. Erickson, H. A. Utset,  
742 H. M. Graves, F. Amanat, D. N. Sather, F. Krammer, Y. Kawaoka, P. C. Wilson, Cross-  
743 Neutralization of Emerging SARS-CoV-2 Variants of Concern by Antibodies Targeting  
744 Distinct Epitopes on Spike. *mBio* **12**, e0297521 (2021).
- 745 15. L. Piccoli, Y. J. Park, M. A. Tortorici, N. Czudnochowski, A. C. Walls, M. Beltramello,  
746 C. Silacci-Fregni, D. Pinto, L. E. Rosen, J. E. Bowen, O. J. Acton, S. Jaconi, B. Guarino,  
747 A. Minola, F. Zatta, N. Sprugasci, J. Bassi, A. Peter, A. De Marco, J. C. Nix, F. Mele, S.  
748 Jovic, B. F. Rodriguez, S. V. Gupta, F. Jin, G. Piumatti, G. Lo Presti, A. F. Pellanda, M.  
749 Biggiogero, M. Tarkowski, M. S. Pizzuto, E. Cameroni, C. Havenar-Daughton, M.  
750 Smithey, D. Hong, V. Lepori, E. Albanese, A. Ceschi, E. Bernasconi, L. Elzi, P. Ferrari,  
751 C. Garzoni, A. Riva, G. Snell, F. Sallusto, K. Fink, H. W. Virgin, A. Lanzavecchia, D.  
752 Corti, D. Veelsler, Mapping Neutralizing and Immunodominant Sites on the SARS-CoV-2  
753 Spike Receptor-Binding Domain by Structure-Guided High-Resolution Serology. *Cell*  
754 **183**, 1024-1042 e1021 (2020).



- 755 16. V. Dussupt, R. S. Sankhala, L. Mendez-Rivera, S. M. Townsley, F. Schmidt, L. Wieczorek,  
756 K. G. Lal, G. C. Donofrio, U. Tran, N. D. Jackson, W. I. Zaky, M. Zemil, S. R. Tritsch, W.  
757 H. Chen, E. J. Martinez, A. Ahmed, M. Choe, W. C. Chang, A. Hajduczki, N. Jian, C. E.  
758 Peterson, P. A. Rees, M. Rutkowska, B. M. Slike, C. N. Selverian, I. Swafford, I. T. Teng,  
759 P. V. Thomas, T. Zhou, C. J. Smith, J. R. Currier, P. D. Kwong, M. Rolland, E. Davidson,  
760 B. J. Doranz, C. N. Mores, T. Hatzioannou, W. W. Reiley, P. D. Bieniasz, D. Paquin-  
761 Proulx, G. D. Gromowski, V. R. Polonis, N. L. Michael, K. Modjarrad, M. G. Joyce, S. J.  
762 Krebs, Low-dose in vivo protection and neutralization across SARS-CoV-2 variants by  
763 monoclonal antibody combinations. *Nat Immunol* **22**, 1503-1514 (2021).
- 764 17. G. Beaudoin-Bussieres, Y. Chen, I. Ullah, J. Prevost, W. D. Tolbert, K. Symmes, S. Ding,  
765 M. Benlarbi, S. Y. Gong, A. Tauzin, R. Gasser, D. Chatterjee, D. Vezina, G. Goyette, J.  
766 Richard, F. Zhou, L. Stamatatos, A. T. McGuire, H. Charest, M. Roger, E. Pozharski, P.  
767 Kumar, W. Mothes, P. D. Uchil, M. Pazgier, A. Finzi, A Fc-enhanced NTD-binding non-  
768 neutralizing antibody delays virus spread and synergizes with a nAb to protect mice from  
769 lethal SARS-CoV-2 infection. *Cell Rep* **38**, 110368 (2022).
- 770 18. D. Geers, M. C. Shamier, S. Bogers, G. den Hartog, L. Gommers, N. N. Nieuwkoop, K. S.  
771 Schmitz, L. C. Rijsbergen, J. A. T. van Osch, E. Dijkhuizen, G. Smits, A. Comvalius, D.  
772 van Mourik, T. G. Caniels, M. J. van Gils, R. W. Sanders, B. B. Oude Munnink, R.  
773 Molenkamp, H. J. de Jager, B. L. Haagmans, R. L. de Swart, M. P. G. Koopmans, R. S.  
774 van Binnendijk, R. D. de Vries, C. H. GeurtsvanKessel, SARS-CoV-2 variants of concern  
775 partially escape humoral but not T-cell responses in COVID-19 convalescent donors and  
776 vaccinees. *Sci Immunol* **6**, (2021).
- 777 19. A. Tarke, J. Sidney, N. Methot, E. D. Yu, Y. Zhang, J. M. Dan, B. Goodwin, P. Rubiro, A.  
778 Sutherland, E. Wang, A. Frazier, S. I. Ramirez, S. A. Rawlings, D. M. Smith, R. da Silva  
779 Antunes, B. Peters, R. H. Scheuermann, D. Weiskopf, S. Crotty, A. Grifoni, A. Sette,  
780 Impact of SARS-CoV-2 variants on the total CD4(+) and CD8(+) T cell reactivity in  
781 infected or vaccinated individuals. *Cell Rep Med* **2**, 100355 (2021).
- 782 20. Y. Gao, C. Cai, A. Grifoni, T. R. Muller, J. Niessl, A. Olofsson, M. Humbert, L. Hansson,  
783 A. Osterborg, P. Bergman, P. Chen, A. Olsson, J. K. Sandberg, D. Weiskopf, D. A. Price,  
784 H. G. Ljunggren, A. C. Karlsson, A. Sette, S. Aleman, M. Buggert, Ancestral SARS-CoV-  
785 2-specific T cells cross-recognize the Omicron variant. *Nat Med*, (2022).
- 786 21. A. D. Redd, A. Nardin, H. Kared, E. M. Bloch, A. Pekosz, O. Laeyendecker, B. Abel, M.  
787 Fehlings, T. C. Quinn, A. A. R. Tobian, CD8+ T-Cell Responses in COVID-19  
788 Convalescent Individuals Target Conserved Epitopes From Multiple Prominent SARS-  
789 CoV-2 Circulating Variants. *Open Forum Infect Dis* **8**, ofab143 (2021).
- 790 22. A. Tarke, C. H. Coelho, Z. Zhang, J. M. Dan, E. D. Yu, N. Methot, N. I. Bloom, B.  
791 Goodwin, E. Phillips, S. Mallal, J. Sidney, G. Filaci, D. Weiskopf, R. da Silva Antunes, S.  
792 Crotty, A. Grifoni, A. Sette, SARS-CoV-2 vaccination induces immunological T cell  
793 memory able to cross-recognize variants from Alpha to Omicron. *Cell* **185**, 847-859 e811  
794 (2022).
- 795 23. C. O. Barnes, C. A. Jette, M. E. Abernathy, K. A. Dam, S. R. Esswein, H. B. Gristick, A.  
796 G. Malyutin, N. G. Sharaf, K. E. Huey-Tubman, Y. E. Lee, D. F. Robbani, M. C.  
797 Nussenzweig, A. P. West, Jr., P. J. Bjorkman, SARS-CoV-2 neutralizing antibody  
798 structures inform therapeutic strategies. *Nature* **588**, 682-687 (2020).
- 799 24. H. L. Dugan, C. T. Stamper, L. Li, S. Changrob, N. W. Asby, P. J. Halfmann, N. Y. Zheng,  
800 M. Huang, D. G. Shaw, M. S. Cobb, S. A. Erickson, J. J. Guthmiller, O. Stovicek, J. Wang,

- 801 E. S. Winkler, M. L. Madariaga, K. Shanmugarajah, M. O. Jansen, F. Amanat, I. Stewart,  
802 H. A. Utset, J. Huang, C. A. Nelson, Y. N. Dai, P. D. Hall, R. P. Jedrzejczak, A.  
803 Joachimiak, F. Krammer, M. S. Diamond, D. H. Fremont, Y. Kawaoka, P. C. Wilson,  
804 Profiling B cell immunodominance after SARS-CoV-2 infection reveals antibody  
805 evolution to non-neutralizing viral targets. *Immunity* **54**, 1290-1303 e1297 (2021).
- 806 25. P. Tong, A. Gautam, I. W. Windsor, M. Travers, Y. Chen, N. Garcia, N. B. Whiteman, L.  
807 G. A. McKay, N. Storm, L. E. Malsick, A. N. Honko, F. J. N. Lelis, S. Habibi, S. Jenni, Y.  
808 Cai, L. J. Rennick, W. P. Duprex, K. R. McCarthy, C. L. Lavine, T. Zuo, J. Lin, A. Zuiani,  
809 J. Feldman, E. A. MacDonald, B. M. Hauser, A. Griffiths, M. S. Seaman, A. G. Schmidt,  
810 B. Chen, D. Neuberg, G. Bajic, S. C. Harrison, D. R. Wesemann, Memory B cell repertoire  
811 for recognition of evolving SARS-CoV-2 spike. *Cell* **184**, 4969-4980 e4915 (2021).
- 812 26. A. J. Greaney, T. N. Starr, P. Gilchuk, S. J. Zost, E. Binshtein, A. N. Loes, S. K. Hilton, J.  
813 Huddleston, R. Eguia, K. H. D. Crawford, A. S. Dingens, R. S. Nargi, R. E. Sutton, N.  
814 Suryadevara, P. W. Rothlauf, Z. Liu, S. P. J. Whelan, R. H. Carnahan, J. E. Crowe, Jr., J.  
815 D. Bloom, Complete Mapping of Mutations to the SARS-CoV-2 Spike Receptor-Binding  
816 Domain that Escape Antibody Recognition. *Cell Host Microbe* **29**, 44-57 e49 (2021).
- 817 27. M. McCallum, A. De Marco, F. A. Lempp, M. A. Tortorici, D. Pinto, A. C. Walls, M.  
818 Beltramello, A. Chen, Z. Liu, F. Zatta, S. Zepeda, J. di Iulio, J. E. Bowen, M. Montiel-  
819 Ruiz, J. Zhou, L. E. Rosen, S. Bianchi, B. Guarino, C. S. Fregni, R. Abdelnabi, S. C. Foo,  
820 P. W. Rothlauf, L. M. Bloyet, F. Benigni, E. Cameroni, J. Neyts, A. Riva, G. Snell, A.  
821 Telenti, S. P. J. Whelan, H. W. Virgin, D. Corti, M. S. Pizzuto, D. Veessler, N-terminal  
822 domain antigenic mapping reveals a site of vulnerability for SARS-CoV-2. *Cell* **184**, 2332-  
823 2347 e2316 (2021).
- 824 28. P. J. M. Brouwer, T. G. Caniels, K. van der Straten, J. L. Snitselaar, Y. Aldon, S. Bangaru,  
825 J. L. Torres, N. M. A. Okba, M. Claireaux, G. Kerster, A. E. H. Bentlage, M. M. van  
826 Haaren, D. Guerra, J. A. Burger, E. E. Schermer, K. D. Verheul, N. van der Velde, A. van  
827 der Kooi, J. van Schooten, M. J. van Breemen, T. P. L. Bijl, K. Slieden, A. Aartse, R.  
828 Derking, I. Bontjer, N. A. Kootstra, W. J. Wiersinga, G. Vidarsson, B. L. Haagmans, A. B.  
829 Ward, G. J. de Bree, R. W. Sanders, M. J. van Gils, Potent neutralizing antibodies from  
830 COVID-19 patients define multiple targets of vulnerability. *Science* **369**, 643-650 (2020).
- 831 29. W. Dejnirattisai, D. Zhou, H. M. Ginn, H. M. E. Duyvesteyn, P. Supasa, J. B. Case, Y.  
832 Zhao, T. S. Walter, A. J. Mentzer, C. Liu, B. Wang, G. C. Paesen, J. Slon-Campos, C.  
833 Lopez-Camacho, N. M. Kafai, A. L. Bailey, R. E. Chen, B. Ying, C. Thompson, J. Bolton,  
834 A. Fyfe, S. Gupta, T. K. Tan, J. Gilbert-Jaramillo, W. James, M. Knight, M. W. Carroll, D.  
835 Skelly, C. Dold, Y. Peng, R. Levin, T. Dong, A. J. Pollard, J. C. Knight, P. Klenerman, N.  
836 Temperton, D. R. Hall, M. A. Williams, N. G. Paterson, F. K. R. Bertram, C. A. Siebert,  
837 D. K. Clare, A. Howe, J. Radecke, Y. Song, A. R. Townsend, K. A. Huang, E. E. Fry, J.  
838 Mongkolsapaya, M. S. Diamond, J. Ren, D. I. Stuart, G. R. Screaton, The antigenic  
839 anatomy of SARS-CoV-2 receptor binding domain. *Cell* **184**, 2183-2200 e2122 (2021).
- 840 30. G. Cerutti, Y. Guo, T. Zhou, J. Gorman, M. Lee, M. Rapp, E. R. Reddem, J. Yu, F. Bahna,  
841 J. Bimela, Y. Huang, P. S. Katsamba, L. Liu, M. S. Nair, R. Rawi, A. S. Olia, P. Wang, B.  
842 Zhang, G. Y. Chuang, D. D. Ho, Z. Sheng, P. D. Kwong, L. Shapiro, Potent SARS-CoV-  
843 2 neutralizing antibodies directed against spike N-terminal domain target a single supersite.  
844 *Cell Host Microbe* **29**, 819-833 e817 (2021).
- 845 31. L. Liu, P. Wang, M. S. Nair, J. Yu, M. Rapp, Q. Wang, Y. Luo, J. F. Chan, V. Sahi, A.  
846 Figueroa, X. V. Guo, G. Cerutti, J. Bimela, J. Gorman, T. Zhou, Z. Chen, K. Y. Yuen, P.

- 847 D. Kwong, J. G. Sodroski, M. T. Yin, Z. Sheng, Y. Huang, L. Shapiro, D. D. Ho, Potent  
848 neutralizing antibodies against multiple epitopes on SARS-CoV-2 spike. *Nature* **584**, 450-  
849 456 (2020).
- 850 32. J. D. Galson, S. Schaetzle, R. J. M. Bashford-Rogers, M. I. J. Raybould, A. Kovaltsuk, G.  
851 J. Kilpatrick, R. Minter, D. K. Finch, J. Dias, L. K. James, G. Thomas, W. J. Lee, J. Betley,  
852 O. Cavlan, A. Leech, C. M. Deane, J. Seoane, C. Caldas, D. J. Pennington, P. Pfeffer, J.  
853 Osbourn, Deep Sequencing of B Cell Receptor Repertoires From COVID-19 Patients  
854 Reveals Strong Convergent Immune Signatures. *Front Immunol* **11**, 605170 (2020).
- 855 33. M. Rapp, Y. Guo, E. R. Reddem, J. Yu, L. Liu, P. Wang, G. Cerutti, P. Katsamba, J. S.  
856 Bimela, F. A. Bahna, S. M. Manneppalli, B. Zhang, P. D. Kwong, Y. Huang, D. D. Ho, L.  
857 Shapiro, Z. Sheng, Modular basis for potent SARS-CoV-2 neutralization by a prevalent  
858 VH1-2-derived antibody class. *Cell Rep* **35**, 108950 (2021).
- 859 34. S. J. Zost, P. Gilchuk, R. E. Chen, J. B. Case, J. X. Reidy, A. Trivette, R. S. Nargi, R. E.  
860 Sutton, N. Suryadevara, E. C. Chen, E. Binshtein, S. Shrihari, M. Ostrowski, H. Y. Chu, J.  
861 E. Didier, K. W. MacRenaris, T. Jones, S. Day, L. Myers, F. Eun-Hyung Lee, D. C.  
862 Nguyen, I. Sanz, D. R. Martinez, P. W. Rothlauf, L. M. Bloyet, S. P. J. Whelan, R. S. Baric,  
863 L. B. Thackray, M. S. Diamond, R. H. Carnahan, J. E. Crowe, Jr., Rapid isolation and  
864 profiling of a diverse panel of human monoclonal antibodies targeting the SARS-CoV-2  
865 spike protein. *Nat Med* **26**, 1422-1427 (2020).
- 866 35. Y. Cao, B. Su, X. Guo, W. Sun, Y. Deng, L. Bao, Q. Zhu, X. Zhang, Y. Zheng, C. Geng,  
867 X. Chai, R. He, X. Li, Q. Lv, H. Zhu, W. Deng, Y. Xu, Y. Wang, L. Qiao, Y. Tan, L. Song,  
868 G. Wang, X. Du, N. Gao, J. Liu, J. Xiao, X. D. Su, Z. Du, Y. Feng, C. Qin, C. Qin, R. Jin,  
869 X. S. Xie, Potent Neutralizing Antibodies against SARS-CoV-2 Identified by High-  
870 Throughput Single-Cell Sequencing of Convalescent Patients' B Cells. *Cell* **182**, 73-84 e16  
871 (2020).
- 872 36. D. F. Robbiani, C. Gaebler, F. Muecksch, J. C. C. Lorenzi, Z. Wang, A. Cho, M. Agudelo,  
873 C. O. Barnes, A. Gazumyan, S. Finkin, T. Hagglof, T. Y. Oliveira, C. Viant, A. Hurley, H.  
874 H. Hoffmann, K. G. Millard, R. G. Kost, M. Cipolla, K. Gordon, F. Bianchini, S. T. Chen,  
875 V. Ramos, R. Patel, J. Dizon, I. Shimeliovich, P. Mendoza, H. Hartweger, L. Nogueira, M.  
876 Pack, J. Horowitz, F. Schmidt, Y. Weisblum, E. Michailidis, A. W. Ashbrook, E. Waltari,  
877 J. E. Pak, K. E. Huey-Tubman, N. Koranda, P. R. Hoffman, A. P. West, Jr., C. M. Rice, T.  
878 Hatzioannou, P. J. Bjorkman, P. D. Bieniasz, M. Caskey, M. C. Nussenzweig, Convergent  
879 antibody responses to SARS-CoV-2 in convalescent individuals. *Nature* **584**, 437-442  
880 (2020).
- 881 37. P. D. Kwong, J. R. Mascola, HIV-1 Vaccines Based on Antibody Identification, B Cell  
882 Ontogeny, and Epitope Structure. *Immunity* **48**, 855-871 (2018).
- 883 38. M. Yuan, H. Liu, N. C. Wu, C. D. Lee, X. Zhu, F. Zhao, D. Huang, W. Yu, Y. Hua, H.  
884 Tien, T. F. Rogers, E. Landais, D. Sok, J. G. Jardine, D. R. Burton, I. A. Wilson, Structural  
885 basis of a shared antibody response to SARS-CoV-2. *Science* **369**, 1119-1123 (2020).
- 886 39. N. C. Wu, M. Yuan, H. Liu, C. D. Lee, X. Zhu, S. Bangaru, J. L. Torres, T. G. Caniels, P.  
887 J. M. Brouwer, M. J. van Gils, R. W. Sanders, A. B. Ward, I. A. Wilson, An Alternative  
888 Binding Mode of IGHV3-53 Antibodies to the SARS-CoV-2 Receptor Binding Domain.  
889 *Cell Rep* **33**, 108274 (2020).
- 890 40. C. O. Barnes, A. P. West, Jr., K. E. Huey-Tubman, M. A. G. Hoffmann, N. G. Sharaf, P.  
891 R. Hoffman, N. Koranda, H. B. Gristick, C. Gaebler, F. Muecksch, J. C. C. Lorenzi, S.  
892 Finkin, T. Hagglof, A. Hurley, K. G. Millard, Y. Weisblum, F. Schmidt, T. Hatzioannou,

- 893 P. D. Bieniasz, M. Caskey, D. F. Robbiani, M. C. Nussenzweig, P. J. Bjorkman, Structures  
894 of Human Antibodies Bound to SARS-CoV-2 Spike Reveal Common Epitopes and  
895 Recurrent Features of Antibodies. *Cell* **182**, 828-842 e816 (2020).
- 896 41. J. Dong, S. J. Zost, A. J. Greaney, T. N. Starr, A. S. Dingens, E. C. Chen, R. E. Chen, J. B.  
897 Case, R. E. Sutton, P. Gilchuk, J. Rodriguez, E. Armstrong, C. Gainza, R. S. Nargi, E.  
898 Binshtein, X. Xie, X. Zhang, P. Y. Shi, J. Logue, S. Weston, M. E. McGrath, M. B.  
899 Frieman, T. Brady, K. M. Tuffy, H. Bright, Y. M. Loo, P. M. McTamney, M. T. Esser, R.  
900 H. Carnahan, M. S. Diamond, J. D. Bloom, J. E. Crowe, Jr., Genetic and structural basis  
901 for SARS-CoV-2 variant neutralization by a two-antibody cocktail. *Nat Microbiol* **6**, 1233-  
902 1244 (2021).
- 903 42. D. Zhou, W. Dejnirattisai, P. Supasa, C. Liu, A. J. Mentzer, H. M. Ginn, Y. Zhao, H. M.  
904 E. Duyvesteyn, A. Tuekprakhon, R. Nutalai, B. Wang, G. C. Paesen, C. Lopez-Camacho,  
905 J. Slon-Campos, B. Hallis, N. Coombes, K. Bewley, S. Charlton, T. S. Walter, D. Skelly,  
906 S. F. Lumley, C. Dold, R. Levin, T. Dong, A. J. Pollard, J. C. Knight, D. Crook, T. Lambe,  
907 E. Clutterbuck, S. Bibi, A. Flaxman, M. Bittaye, S. Belij-Rammerstorfer, S. Gilbert, W.  
908 James, M. W. Carroll, P. Klenerman, E. Barnes, S. J. Dunachie, E. E. Fry, J.  
909 Mongkolsapaya, J. Ren, D. I. Stuart, G. R. Screaton, Evidence of escape of SARS-CoV-2  
910 variant B.1.351 from natural and vaccine-induced sera. *Cell* **184**, 2348-2361 e2346 (2021).
- 911 43. K. Vanshylla, V. Di Cristanziano, F. Kleipass, F. Dewald, P. Schommers, L. Gieselmann,  
912 H. Gruell, M. Schlotz, M. S. Ercanoglu, R. Stumpf, P. Mayer, M. Zehner, E. Heger, W.  
913 Johannis, C. Horn, I. Suarez, N. Jung, S. Salomon, K. A. Eberhardt, B. Gathof, G.  
914 Fatkenheuer, N. Pfeifer, R. Eggeling, M. Augustin, C. Lehmann, F. Klein, Kinetics and  
915 correlates of the neutralizing antibody response to SARS-CoV-2 infection in humans. *Cell*  
916 *Host Microbe* **29**, 917-929 e914 (2021).
- 917 44. T. Zhou, L. Wang, J. Misasi, A. Pegu, Y. Zhang, D. R. Harris, A. S. Olia, C. A. Talana, E.  
918 S. Yang, M. Chen, M. Choe, W. Shi, I. T. Teng, A. Creanga, C. Jenkins, K. Leung, T. Liu,  
919 E. D. Stancofski, T. Stephens, B. Zhang, Y. Tsybovsky, B. S. Graham, J. R. Mascola, N.  
920 J. Sullivan, P. D. Kwong, Structural basis for potent antibody neutralization of SARS-CoV-  
921 2 variants including B.1.1.529. *Science*, eabn8897 (2022).
- 922 45. T. Moyo-Gwete, M. Madzivhandila, Z. Makhado, F. Ayres, D. Mhlanga, B. Oosthuysen,  
923 B. E. Lambson, P. Kgagudi, H. Tegally, A. Iranzadeh, D. Doolabh, L. Tyers, L. R.  
924 Chinhoyi, M. Mennen, S. Skelem, G. Marais, C. K. Wibmer, J. N. Bhiman, V.  
925 Ueckermann, T. Rossouw, M. Boswell, T. de Oliveira, C. Williamson, W. A. Burgers, N.  
926 Ntusi, L. Morris, P. L. Moore, Cross-Reactive Neutralizing Antibody Responses Elicited  
927 by SARS-CoV-2 501Y.V2 (B.1.351). *N Engl J Med* **384**, 2161-2163 (2021).
- 928 46. C. Liu, H. M. Ginn, W. Dejnirattisai, P. Supasa, B. Wang, A. Tuekprakhon, R. Nutalai, D.  
929 Zhou, A. J. Mentzer, Y. Zhao, H. M. E. Duyvesteyn, C. Lopez-Camacho, J. Slon-Campos,  
930 T. S. Walter, D. Skelly, S. A. Johnson, T. G. Ritter, C. Mason, S. A. Costa Clemens, F.  
931 Gomes Naveca, V. Nascimento, F. Nascimento, C. Fernandes da Costa, P. C. Resende, A.  
932 Pauvolid-Correa, M. M. Siqueira, C. Dold, N. Temperton, T. Dong, A. J. Pollard, J. C.  
933 Knight, D. Crook, T. Lambe, E. Clutterbuck, S. Bibi, A. Flaxman, M. Bittaye, S. Belij-  
934 Rammerstorfer, S. C. Gilbert, T. Malik, M. W. Carroll, P. Klenerman, E. Barnes, S. J.  
935 Dunachie, V. Baillie, N. Serafin, Z. Ditse, K. Da Silva, N. G. Paterson, M. A. Williams, D.  
936 R. Hall, S. Madhi, M. C. Nunes, P. Goulder, E. E. Fry, J. Mongkolsapaya, J. Ren, D. I.  
937 Stuart, G. R. Screaton, Reduced neutralization of SARS-CoV-2 B.1.617 by vaccine and  
938 convalescent serum. *Cell* **184**, 4220-4236 e4213 (2021).



- 939 47. K. S. Corbett, M. Gagne, D. A. Wagner, O. C. S, S. R. Narpala, D. R. Flebbe, S. F. Andrew,  
940 R. L. Davis, B. Flynn, T. S. Johnston, C. D. Stringham, L. Lai, D. Valentin, A. Van Ry, Z.  
941 Flinchbaugh, A. P. Werner, J. I. Moliva, M. Sriparna, S. O'Dell, S. D. Schmidt, C. Tucker,  
942 A. Choi, M. Koch, K. W. Bock, M. Minai, B. M. Nagata, G. S. Alvarado, A. R. Henry, F.  
943 Laboune, C. A. Schramm, Y. Zhang, E. S. Yang, L. Wang, M. Choe, S. Boyoglu-Barnum,  
944 S. Wei, E. Lamb, S. T. Nurmukhambetova, S. J. Provost, M. M. Donaldson, J. Marquez, J.  
945 M. Todd, A. Cook, A. Dodson, A. Pekosz, E. Boritz, A. Ploquin, N. Doria-Rose, L.  
946 Pessaint, H. Andersen, K. E. Foulds, J. Misasi, K. Wu, A. Carfi, M. C. Nason, J. Mascola,  
947 I. N. Moore, D. K. Edwards, M. G. Lewis, M. S. Suthar, M. Roederer, A. McDermott, D.  
948 C. Douek, N. J. Sullivan, B. S. Graham, R. A. Seder, Protection against SARS-CoV-2 Beta  
949 variant in mRNA-1273 vaccine-boosted nonhuman primates. *Science* **374**, 1343-1353  
950 (2021).
- 951 48. M. M. Gagne, J.I.; Foulds, K.E.; Andrew, S.F.; Flynn, B.J.; Werner, A.P.; Wagner, D.A.;  
952 Teng, I.-T.; Lin, B.C.; Moore, C.; Jean-Baptiste, N.; Carroll, R.; Foster, S.L.; Patel, M.;  
953 Ellis, M.; Edara, V.-V.; Maldonado, N.V.; Minai, M.; McCormick, L.; Honeycutt, C.C.;  
954 Nagata, B.M.; Bock, K.W.; Dulan, C.N.M.; Cordon, J.; Flebbe, D.R.; Todd, J.-P.M.;  
955 McCarthy, E.; Pessaint, L.; Van Ry, A.; Narvaez, B.; Valentin, D.; Cook, A.; Dodson, A.;  
956 Steingrebe, K.; Nurmukhambetova, S.T.; S. H. Godbole, A.R.; Laboune, F.; Roberts-  
957 Torres, J.; Lorang, C.G.; Amin, S.; Trost, J.; Naisan, M.; Basappa, M.; Willis, J.; Wang,  
958 L.; Shi, W.; Doria-Rose, N.A.; Zhang, Y.; Yang, E.S.; Leung, K.; O'Dell, S.; Schmidt,  
959 S.D.; OIia, A.S.; Liu, C.; Harris, D.R.; Chuang, G.-Y.; Stewart-Jones, G.; Renzi, I.; Lai,  
960 Y.- T.; Malinowski, A.; Wu, K.; Mascola, J.R.; Carfi, A.; Kwong, P.D.; Edwards, D.K.;  
961 Lewis, M.G.; Andersen, H.; Corbett, K.S.; Nason, M.C.; McDermott, A.B.; Suthar, M.S.;  
962 Moore, I.N.; Roederer, M.; Sullivan, N.J.; Douek, D.C.; Seder, R.A., mRNA-1273 or  
963 mRNA-Omicron boost in vaccinated macaques elicits comparable B cell expansion,  
964 neutralizing antibodies and protection against Omicron. *Cell*, (2022).
- 965 49. D. Li, R. J. Edwards, K. Manne, D. R. Martinez, A. Schafer, S. M. Alam, K. Wiehe, X. Lu,  
966 R. Parks, L. L. Sutherland, T. H. Oguin, 3rd, C. McDanal, L. G. Perez, K. Mansouri, S. M.  
967 C. Gobeil, K. Janowska, V. Stalls, M. Kopp, F. Cai, E. Lee, A. Foulger, G. E. Hernandez,  
968 A. Sanzone, K. Tilahun, C. Jiang, L. V. Tse, K. W. Bock, M. Minai, B. M. Nagata, K.  
969 Cronin, V. Gee-Lai, M. Deyton, M. Barr, T. Von Holle, A. N. Macintyre, E. Stover, J.  
970 Feldman, B. M. Hauser, T. M. Caradonna, T. D. Scobey, W. Rountree, Y. Wang, M. A.  
971 Moody, D. W. Cain, C. T. DeMarco, T. N. Denny, C. W. Woods, E. W. Petzold, A. G.  
972 Schmidt, I. T. Teng, T. Zhou, P. D. Kwong, J. R. Mascola, B. S. Graham, I. N. Moore, R.  
973 Seder, H. Andersen, M. G. Lewis, D. C. Montefiori, G. D. Sempowski, R. S. Baric, P.  
974 Acharya, B. F. Haynes, K. O. Saunders, In vitro and in vivo functions of SARS-CoV-2  
975 infection-enhancing and neutralizing antibodies. *Cell* **184**, 4203-4219 e4232 (2021).
- 976 50. A. Z. Wec, D. Wrapp, A. S. Herbert, D. P. Maurer, D. Haslwanter, M. Sakharkar, R. K.  
977 Jangra, M. E. Dieterle, A. Lilov, D. Huang, L. V. Tse, N. V. Johnson, C. L. Hsieh, N.  
978 Wang, J. H. Nett, E. Champney, I. Burnina, M. Brown, S. Lin, M. Sinclair, C. Johnson, S.  
979 Pudi, R. Bortz, 3rd, A. S. Wirchnianski, E. Laudermitch, C. Florez, J. M. Fels, C. M.  
980 O'Brien, B. S. Graham, D. Nemazee, D. R. Burton, R. S. Baric, J. E. Voss, K. Chandran,  
981 J. M. Dye, J. S. McLellan, L. M. Walker, Broad neutralization of SARS-related viruses by  
982 human monoclonal antibodies. *Science* **369**, 731-736 (2020).
- 983 51. X. Chi, R. Yan, J. Zhang, G. Zhang, Y. Zhang, M. Hao, Z. Zhang, P. Fan, Y. Dong, Y.  
984 Yang, Z. Chen, Y. Guo, J. Zhang, Y. Li, X. Song, Y. Chen, L. Xia, L. Fu, L. Hou, J. Xu,

- 985 C. Yu, J. Li, Q. Zhou, W. Chen, A neutralizing human antibody binds to the N-terminal  
986 domain of the Spike protein of SARS-CoV-2. *Science* **369**, 650-655 (2020).
- 987 52. G. Song, W. T. He, S. Callaghan, F. Anzanello, D. Huang, J. Ricketts, J. L. Torres, N.  
988 Beutler, L. Peng, S. Vargas, J. Cassell, M. Parren, L. Yang, C. Ignacio, D. M. Smith, J. E.  
989 Voss, D. Nemazee, A. B. Ward, T. Rogers, D. R. Burton, R. Andrabi, Cross-reactive serum  
990 and memory B-cell responses to spike protein in SARS-CoV-2 and endemic coronavirus  
991 infection. *Nat Commun* **12**, 2938 (2021).
- 992 53. E. Andreano, E. Nicastri, I. Paciello, P. Pileri, N. Manganaro, G. Piccini, A. Manenti, E.  
993 Pantano, A. Kabanova, M. Troisi, F. Vacca, D. Cardamone, C. De Santi, J. L. Torres, G.  
994 Ozorowski, L. Benincasa, H. Jang, C. Di Genova, L. Depau, J. Brunetti, C. Agrati, M. R.  
995 Capobianchi, C. Castilletti, A. Emiliozzi, M. Fabbiani, F. Montagnani, L. Bracci, G. Sautto,  
996 T. M. Ross, E. Montomoli, N. Temperton, A. B. Ward, C. Sala, G. Ippolito, R. Rappuoli,  
997 Extremely potent human monoclonal antibodies from COVID-19 convalescent patients.  
998 *Cell* **184**, 1821-1835 e1816 (2021).
- 999 54. H. Liu, C. I. Kaku, G. Song, M. Yuan, R. Andrabi, D. R. Burton, L. M. Walker, I. A.  
1000 Wilson, A recurring YYDRxG pattern in broadly neutralizing antibodies to a conserved  
1001 site on SARS-CoV-2, variants of concern, and related viruses. *bioRxiv*,  
1002 2021.2012.2015.472864 (2021).
- 1003 55. C. Soto, R. G. Bombardi, A. Branchizio, N. Kose, P. Matta, A. M. Sevy, R. S. Sinkovits,  
1004 P. Gilchuk, J. A. Finn, J. E. Crowe, Jr., High frequency of shared clonotypes in human B  
1005 cell receptor repertoires. *Nature* **566**, 398-402 (2019).
- 1006 56. C. Liu, D. Zhou, R. Nutalai, H. M. E. Duyvesteyn, A. Tuekprakhon, H. M. Ginn, W.  
1007 Dejnirattisai, P. Supasa, A. J. Mentzer, B. Wang, J. B. Case, Y. Zhao, D. T. Skelly, R. E.  
1008 Chen, S. A. Johnson, T. G. Ritter, C. Mason, T. Malik, N. Temperton, N. G. Paterson, M.  
1009 A. Williams, D. R. Hall, D. K. Clare, A. Howe, P. J. R. Goulder, E. E. Fry, M. S. Diamond,  
1010 J. Mongkolsapaya, J. Ren, D. I. Stuart, G. R. Screaton, The antibody response to SARS-  
1011 CoV-2 Beta underscores the antigenic distance to other variants. *Cell Host Microbe* **30**, 53-  
1012 68 e12 (2022).
- 1013 57. S. M. Reincke, M. Yuan, H. C. Kornau, V. M. Corman, S. van Hoof, E. Sanchez-Sendin,  
1014 M. Ramberger, W. Yu, Y. Hua, H. Tien, M. L. Schmidt, T. Schwarz, L. M. Jeworowski,  
1015 S. E. Brandl, H. F. Rasmussen, M. A. Homeyer, L. Stoffler, M. Barner, D. Kunkel, S. Huo,  
1016 J. Horler, N. von Wardenburg, I. Kroidl, T. M. Eser, A. Wieser, C. Geldmacher, M.  
1017 Hoelscher, H. Ganzer, G. Weiss, D. Schmitz, C. Drosten, H. Pruss, I. A. Wilson, J. Kreye,  
1018 SARS-CoV-2 Beta variant infection elicits potent lineage-specific and cross-reactive  
1019 antibodies. *Science* **375**, 782-787 (2022).
- 1020 58. C. Kreer, M. Zehner, T. Weber, M. S. Ercanoglu, L. Gieselmann, C. Rohde, S. Halwe, M.  
1021 Korenkov, P. Schommers, K. Vanshylla, V. Di Cristanziano, H. Janicki, R. Brinker, A.  
1022 Ashurov, V. Krahling, A. Kupke, H. Cohen-Dvashi, M. Koch, J. M. Eckert, S. Lederer, N.  
1023 Pfeifer, T. Wolf, M. Vehreschild, C. Wendtner, R. Diskin, H. Gruell, S. Becker, F. Klein,  
1024 Longitudinal Isolation of Potent Near-Germline SARS-CoV-2-Neutralizing Antibodies  
1025 from COVID-19 Patients. *Cell* **182**, 843-854 e812 (2020).
- 1026 59. E. Seydoux, L. J. Homad, A. J. MacCamy, K. R. Parks, N. K. Hurlburt, M. F. Jennewein,  
1027 N. R. Akins, A. B. Stuart, Y. H. Wan, J. Feng, R. E. Whaley, S. Singh, M. Boeckh, K. W.  
1028 Cohen, M. J. McElrath, J. A. Englund, H. Y. Chu, M. Pancera, A. T. McGuire, L.  
1029 Stamatatos, Analysis of a SARS-CoV-2-Infected Individual Reveals Development of



- 1030 Potent Neutralizing Antibodies with Limited Somatic Mutation. *Immunity* **53**, 98-105 e105  
1031 (2020).
- 1032 60. T. F. Rogers, F. Zhao, D. Huang, N. Beutler, A. Burns, W. T. He, O. Limbo, C. Smith, G.  
1033 Song, J. Woehl, L. Yang, R. K. Abbott, S. Callaghan, E. Garcia, J. Hurtado, M. Parren, L.  
1034 Peng, S. Ramirez, J. Ricketts, M. J. Ricciardi, S. A. Rawlings, N. C. Wu, M. Yuan, D. M.  
1035 Smith, D. Nemazee, J. R. Tejjaro, J. E. Voss, I. A. Wilson, R. Andrabi, B. Briney, E.  
1036 Landais, D. Sok, J. G. Jardine, D. R. Burton, Isolation of potent SARS-CoV-2 neutralizing  
1037 antibodies and protection from disease in a small animal model. *Science* **369**, 956-963  
1038 (2020).
- 1039 61. S. C. A. Nielsen, F. Yang, K. J. L. Jackson, R. A. Hoh, K. Roltgen, G. H. Jean, B. A.  
1040 Stevens, J. Y. Lee, A. Rustagi, A. J. Rogers, A. E. Powell, M. Hunter, J. Najeeb, A. R.  
1041 Otrelo-Cardoso, K. E. Yost, B. Daniel, K. C. Nadeau, H. Y. Chang, A. T. Satpathy, T. S.  
1042 Jardetzky, P. S. Kim, T. T. Wang, B. A. Pinsky, C. A. Blish, S. D. Boyd, Human B Cell  
1043 Clonal Expansion and Convergent Antibody Responses to SARS-CoV-2. *Cell Host*  
1044 *Microbe* **28**, 516-525 e515 (2020).
- 1045 62. A. K. Wheatley, P. Pymm, R. Esterbauer, M. H. Dietrich, W. S. Lee, D. Drew, H. G. Kelly,  
1046 L. J. Chan, F. L. Mordant, K. A. Black, A. Adair, H. X. Tan, J. A. Juno, K. M. Wragg, T.  
1047 Amarasena, E. Lopez, K. J. Selva, E. R. Haycroft, J. P. Cooney, H. Venugopal, L. L. Tan,  
1048 O. N. MT, C. C. Allison, D. Cromer, M. P. Davenport, R. A. Bowen, A. W. Chung, M.  
1049 Pellegrini, M. T. Liddament, A. Glukhova, K. Subbarao, S. J. Kent, W. H. Tham,  
1050 Landscape of human antibody recognition of the SARS-CoV-2 receptor binding domain.  
1051 *Cell Rep* **37**, 109822 (2021).
- 1052 63. Y. Zhou, Z. Liu, S. Li, W. Xu, Q. Zhang, I. T. Silva, C. Li, Y. Wu, Q. Jiang, Z. Liu, Q.  
1053 Wang, Y. Guo, J. Wu, C. Gu, X. Cai, D. Qu, C. T. Mayer, X. Wang, S. Jiang, T. Ying, Z.  
1054 Yuan, Y. Xie, Y. Wen, L. Lu, Q. Wang, Enhancement versus neutralization by SARS-  
1055 CoV-2 antibodies from a convalescent donor associates with distinct epitopes on the RBD.  
1056 *Cell Rep* **34**, 108699 (2021).
- 1057 64. A. R. Shiakolas, K. J. Kramer, N. V. Johnson, S. C. Wall, N. Suryadevara, D. Wrapp, S.  
1058 Periasamy, K. A. Pilewski, N. Raju, R. Nargi, R. E. Sutton, L. M. Walker, I. Setliff, J. E.  
1059 Crowe, Jr., A. Bukreyev, R. H. Carnahan, J. S. McLellan, I. S. Georgiev, Efficient  
1060 discovery of SARS-CoV-2-neutralizing antibodies via B cell receptor sequencing and  
1061 ligand blocking. *Nat Biotechnol*, (2022).
- 1062 65. J. S. Turner, J. A. O'Halloran, E. Kalaidina, W. Kim, A. J. Schmitz, J. Q. Zhou, T. Lei, M.  
1063 Thapa, R. E. Chen, J. B. Case, F. Amanat, A. M. Rauseo, A. Haile, X. Xie, M. K. Klebert,  
1064 T. Suessen, W. D. Middleton, P. Y. Shi, F. Krammer, S. A. Teefey, M. S. Diamond, R. M.  
1065 Presti, A. H. Ellebedy, SARS-CoV-2 mRNA vaccines induce persistent human germinal  
1066 centre responses. *Nature* **596**, 109-113 (2021).
- 1067 66. C. Graham, J. Seow, I. Huettner, H. Khan, N. Kouphou, S. Acors, H. Winstone, S.  
1068 Pickering, R. P. Galao, L. Dupont, M. J. Lista, J. M. Jimenez-Guardeno, A. G. Laing, Y.  
1069 Wu, M. Joseph, L. Muir, M. J. van Gils, W. M. Ng, H. M. E. Duyvesteyn, Y. Zhao, T. A.  
1070 Bowden, M. Shankar-Hari, A. Rosa, P. Cherepanov, L. E. McCoy, A. C. Hayday, S. J. D.  
1071 Neil, M. H. Malim, K. J. Doores, Neutralization potency of monoclonal antibodies  
1072 recognizing dominant and subdominant epitopes on SARS-CoV-2 Spike is impacted by  
1073 the B.1.1.7 variant. *Immunity* **54**, 1276-1289 e1276 (2021).
- 1074 67. L. A. VanBlargan, J. M. Errico, P. J. Halfmann, S. J. Zost, J. E. Crowe, Jr., L. A. Purcell,  
1075 Y. Kawaoka, D. Corti, D. H. Fremont, M. S. Diamond, An infectious SARS-CoV-2

- 1076 B.1.1.529 Omicron virus escapes neutralization by therapeutic monoclonal antibodies. *Nat*  
1077 *Med*, (2022).
- 1078 68. K. S. Corbett, M. C. Nason, B. Flach, M. Gagne, S. O'Connell, T. S. Johnston, S. N. Shah,  
1079 V. V. Edara, K. Floyd, L. Lai, C. McDanal, J. R. Francica, B. Flynn, K. Wu, A. Choi, M.  
1080 Koch, O. M. Abiona, A. P. Werner, J. I. Moliva, S. F. Andrew, M. M. Donaldson, J. Fintzi,  
1081 D. R. Flebbe, E. Lamb, A. T. Noe, S. T. Nurmukhambetova, S. J. Provost, A. Cook, A.  
1082 Dodson, A. Faudree, J. Greenhouse, S. Kar, L. Pessaint, M. Porto, K. Steingrebe, D.  
1083 Valentin, S. Zouantcha, K. W. Bock, M. Minai, B. M. Nagata, R. van de Wetering, S.  
1084 Boyoglu-Barnum, K. Leung, W. Shi, E. S. Yang, Y. Zhang, J. M. Todd, L. Wang, G. S.  
1085 Alvarado, H. Andersen, K. E. Foulds, D. K. Edwards, J. R. Mascola, I. N. Moore, M. G.  
1086 Lewis, A. Carfi, D. Montefiori, M. S. Suthar, A. McDermott, M. Roederer, N. J. Sullivan,  
1087 D. C. Douek, B. S. Graham, R. A. Seder, Immune correlates of protection by mRNA-1273  
1088 vaccine against SARS-CoV-2 in nonhuman primates. *Science* **373**, eabj0299 (2021).
- 1089 69. P. B. Gilbert, D. C. Montefiori, A. B. McDermott, Y. Fong, D. Benkeser, W. Deng, H.  
1090 Zhou, C. R. Houchens, K. Martins, L. Jayashankar, F. Castellino, B. Flach, B. C. Lin, S.  
1091 O'Connell, C. McDanal, A. Eaton, M. Sarzotti-Kelsoe, Y. Lu, C. Yu, B. Borate, L. W. P.  
1092 van der Laan, N. S. Hejazi, C. Huynh, J. Miller, H. M. El Sahly, L. R. Baden, M. Baron,  
1093 L. De La Cruz, C. Gay, S. Kalams, C. F. Kelley, M. P. Andrasik, J. G. Kublin, L. Corey,  
1094 K. M. Neuzil, L. N. Carpp, R. Pajon, D. Follmann, R. O. Donis, R. A. Koup, Immune  
1095 correlates analysis of the mRNA-1273 COVID-19 vaccine efficacy clinical trial. *Science*  
1096 **375**, 43-50 (2022).
- 1097 70. D. Cromer, M. Steain, A. Reynaldi, T. E. Schlub, A. K. Wheatley, J. A. Juno, S. J. Kent, J.  
1098 A. Triccas, D. S. Houry, M. P. Davenport, Neutralising antibody titres as predictors of  
1099 protection against SARS-CoV-2 variants and the impact of boosting: a meta-analysis.  
1100 *Lancet Microbe* **3**, e52-e61 (2022).
- 1101 71. A. J. Greaney, T. N. Starr, R. T. Eguia, A. N. Loes, K. Khan, F. Karim, S. Cele, J. E.  
1102 Bowen, J. K. Logue, D. Corti, D. Veessler, H. Y. Chu, A. Sigal, J. D. Bloom, A SARS-  
1103 CoV-2 variant elicits an antibody response with a shifted immunodominance hierarchy.  
1104 *PLoS Pathog* **18**, e1010248 (2022).
- 1105 72. S. A. Robinson, M. I. J. Raybould, C. Schneider, W. K. Wong, C. Marks, C. M. Deane,  
1106 Epitope profiling using computational structural modelling demonstrated on coronavirus-  
1107 binding antibodies. *PLoS Comput Biol* **17**, e1009675 (2021).
- 1108 73. K. S. Corbett, A. P. Werner, S. O. Connell, M. Gagne, L. Lai, J. I. Moliva, B. Flynn, A.  
1109 Choi, M. Koch, K. E. Foulds, S. F. Andrew, D. R. Flebbe, E. Lamb, S. T.  
1110 Nurmukhambetova, S. J. Provost, K. W. Bock, M. Minai, B. M. Nagata, A. V. Ry, Z.  
1111 Flinchbaugh, T. S. Johnston, E. B. Mokhtari, P. Mudvari, A. R. Henry, F. Laboune, B.  
1112 Chang, M. Porto, J. Wear, G. S. Alvarado, S. Boyoglu-Barnum, J. M. Todd, B. Bart, A.  
1113 Cook, A. Dodson, L. Pessaint, K. Steingrebe, S. Elbashir, M. Sriparna, A. Pekosz, H.  
1114 Andersen, K. Wu, D. K. Edwards, S. Kar, M. G. Lewis, E. Boritz, I. N. Moore, A. Carfi,  
1115 M. S. Suthar, A. McDermott, M. Roederer, M. C. Nason, N. J. Sullivan, D. C. Douek, B.  
1116 S. Graham, R. A. Seder, mRNA-1273 protects against SARS-CoV-2 beta infection in  
1117 nonhuman primates. *Nat Immunol* **22**, 1306-1315 (2021).
- 1118 74. M. Gagne, K. S. Corbett, B. J. Flynn, K. E. Foulds, D. A. Wagner, S. F. Andrew, J. M.  
1119 Todd, C. C. Honeycutt, L. McCormick, S. T. Nurmukhambetova, M. E. Davis-Gardner, L.  
1120 Pessaint, K. W. Bock, B. M. Nagata, M. Minai, A. P. Werner, J. I. Moliva, C. Tucker, C.  
1121 G. Lorang, B. Zhao, E. McCarthy, A. Cook, A. Dodson, I. T. Teng, P. Mudvari, J. Roberts-

- 1122 Torres, F. Laboune, L. Wang, A. Goode, S. Kar, S. Boyoglu-Barnum, E. S. Yang, W. Shi,  
1123 A. Ploquin, N. Doria-Rose, A. Carfi, J. R. Mascola, E. A. Boritz, D. K. Edwards, H.  
1124 Andersen, M. G. Lewis, M. S. Suthar, B. S. Graham, M. Roederer, I. N. Moore, M. C.  
1125 Nason, N. J. Sullivan, D. C. Douek, R. A. Seder, Protection from SARS-CoV-2 Delta one  
1126 year after mRNA-1273 vaccination in rhesus macaques coincides with anamnestic  
1127 antibody response in the lung. *Cell* **185**, 113-130 e115 (2022).
- 1128 75. J. F. Scheid, C. O. Barnes, B. Eraslan, A. Hudak, J. R. Keeffe, L. A. Cosimi, E. M. Brown,  
1129 F. Muecksch, Y. Weisblum, S. Zhang, T. Delorey, A. E. Woolley, F. Ghantous, S. M. Park,  
1130 D. Phillips, B. Tusi, K. E. Huey-Tubman, A. A. Cohen, P. N. P. Gnanapragasam, K. Rzasz,  
1131 T. Hatzioanno, M. A. Durney, X. Gu, T. Tada, N. R. Landau, A. P. West, Jr., O.  
1132 Rozenblatt-Rosen, M. S. Seaman, L. R. Baden, D. B. Graham, J. Deguine, P. D. Bieniasz,  
1133 A. Regev, D. Hung, P. J. Bjorkman, R. J. Xavier, B cell genomics behind cross-  
1134 neutralization of SARS-CoV-2 variants and SARS-CoV. *Cell* **184**, 3205-3221 e3224  
1135 (2021).
- 1136 76. A. Pegu, S. E. O'Connell, S. D. Schmidt, S. O'Dell, C. A. Talana, L. Lai, J. Albert, E.  
1137 Anderson, H. Bennett, K. S. Corbett, B. Flach, L. Jackson, B. Leav, J. E. Ledgerwood, C.  
1138 J. Luke, M. Makowski, M. C. Nason, P. C. Roberts, M. Roederer, P. A. Rebolledo, C. A.  
1139 Rostad, N. G. Roupheal, W. Shi, L. Wang, A. T. Widge, E. S. Yang, R. N. A. S. G. s. s. m,  
1140 J. H. Beigel, B. S. Graham, J. R. Mascola, M. S. Suthar, A. B. McDermott, N. A. Doria-  
1141 Rose, J. Arega, J. H. Beigel, W. Buchanan, M. Elsfay, B. Hoang, R. Lampley, A. Kolhekar,  
1142 H. Koo, C. Luke, M. Makhene, S. Nayak, R. Pikaart-Tautges, P. C. Roberts, J. Russell, E.  
1143 Sindall, J. Albert, P. Kunwar, M. Makowski, E. J. Anderson, A. Bechnak, M. Bower, A. F.  
1144 Camacho-Gonzalez, M. Collins, A. Drobeniuc, V. V. Edara, S. Edupuganti, K. Floyd, T.  
1145 Gibson, C. M. G. Ackerley, B. Johnson, S. Kamidani, C. Kao, C. Kelley, L. Lai, H.  
1146 Macenczak, M. P. McCullough, E. Peters, V. K. Phadke, P. A. Rebolledo, C. A. Rostad,  
1147 N. Roupheal, E. Scherer, A. Sherman, K. Stephens, M. S. Suthar, M. Teherani, J.  
1148 Traenkner, J. Winston, I. Yildirim, L. Barr, J. Benoit, B. Carste, J. Choe, M. Dunstan, R.  
1149 Erolin, J. Ffitch, C. Fields, L. A. Jackson, E. Kiniry, S. Lasicka, S. Lee, M. Nguyen, S.  
1150 Pimienta, J. Suyehira, M. Witte, H. Bennett, N. E. Altaras, A. Carfi, M. Hurley, B. Leav,  
1151 R. Pajon, W. Sun, T. Zaks, R. N. Coler, S. E. Larsen, K. M. Neuzil, L. C. Lindesmith, D.  
1152 R. Martinez, J. Munt, M. Mallory, C. Edwards, R. S. Baric, N. M. Berkowitz, E. A. Boritz,  
1153 K. Carlton, K. S. Corbett, P. Costner, A. Creanga, N. A. Doria-Rose, D. C. Douek, B.  
1154 Flach, M. Gaudinski, I. Gordon, B. S. Graham, L. Holman, J. E. Ledgerwood, K. Leung,  
1155 B. C. Lin, M. K. Louder, J. R. Mascola, A. B. McDermott, K. M. Morabito, L. Novik, S.  
1156 O'Connell, S. O'Dell, M. Padilla, A. Pegu, S. D. Schmidt, W. Shi, P. A. Swanson, 2nd, C.  
1157 A. Talana, L. Wang, A. T. Widge, E. S. Yang, Y. Zhang, J. D. Chappell, M. R. Denison,  
1158 T. Hughes, X. Lu, A. J. Pruijssers, L. J. Stevens, C. M. Posavad, M. Gale, Jr., V.  
1159 Menachery, P. Y. Shi, Durability of mRNA-1273 vaccine-induced antibodies against  
1160 SARS-CoV-2 variants. *Science* **373**, 1372-1377 (2021).
- 1161 77. T. Zhou, I. T. Teng, A. S. Olia, G. Cerutti, J. Gorman, A. Nazzari, W. Shi, Y. Tsybovsky,  
1162 L. Wang, S. Wang, B. Zhang, Y. Zhang, P. S. Katsamba, Y. Petrova, B. B. Banach, A. S.  
1163 Fahad, L. Liu, S. N. Lopez Acevedo, B. Madan, M. Oliveira de Souza, X. Pan, P. Wang,  
1164 J. R. Wolfe, M. Yin, D. D. Ho, E. Phung, A. DiPiazza, L. A. Chang, O. M. Abiona, K. S.  
1165 Corbett, B. J. DeKosky, B. S. Graham, J. R. Mascola, J. Misasi, T. Ruckwardt, N. J.  
1166 Sullivan, L. Shapiro, P. D. Kwong, Structure-Based Design with Tag-Based Purification

- 1167 and In-Process Biotinylation Enable Streamlined Development of SARS-CoV-2 Spike  
1168 Molecular Probes. *Cell Rep* **33**, 108322 (2020).
- 1169 78. I. T. Teng, A. F. Nazzari, M. Choe, T. Liu, M. O. de Souza, Y. Petrova, Y. Tsybovsky, S.  
1170 Wang, B. Zhang, M. Artamonov, B. Madan, A. Huang, S. N. Lopez Acevedo, X. Pan, T.  
1171 J. Ruckwardt, B. J. DeKosky, J. R. Mascola, J. Misasi, N. J. Sullivan, T. Zhou, P. D.  
1172 Kwong, Molecular probes of spike ectodomain and its subdomains for SARS-CoV-2  
1173 variants, Alpha through Omicron. *bioRxiv*, (2021).
- 1174 79. S. J. Krebs, Y. D. Kwon, C. A. Schramm, W. H. Law, G. Donofrio, K. H. Zhou, S. Gift,  
1175 V. Dussupt, I. S. Georgiev, S. Schatzle, J. R. McDaniel, Y. T. Lai, M. Sastry, B. Zhang,  
1176 M. C. Jarosinski, A. Ransier, A. L. Chenine, M. Asokan, R. T. Bailer, M. Bose, A. Cagigi,  
1177 E. M. Cale, G. Y. Chuang, S. Darko, J. I. Driscoll, A. Druz, J. Gorman, F. Laboune, M. K.  
1178 Louder, K. McKee, L. Mendez, M. A. Moody, A. M. O'Sullivan, C. Owen, D. Peng, R.  
1179 Rawi, E. Sanders-Buell, C. H. Shen, A. R. Shiakolas, T. Stephens, Y. Tsybovsky, C.  
1180 Tucker, R. Verardi, K. Wang, J. Zhou, T. Zhou, G. Georgiou, S. M. Alam, B. F. Haynes,  
1181 M. Rolland, G. R. Matyas, V. R. Polonis, A. B. McDermott, D. C. Douek, L. Shapiro, S.  
1182 Tovanabutra, N. L. Michael, J. R. Mascola, M. L. Robb, P. D. Kwong, N. A. Doria-Rose,  
1183 Longitudinal Analysis Reveals Early Development of Three MPER-Directed Neutralizing  
1184 Antibody Lineages from an HIV-1-Infected Individual. *Immunity* **50**, 677-691 e613 (2019).
- 1185 80. A. A. Upadhyay, R. C. Kauffman, A. N. Wolabaugh, A. Cho, N. B. Patel, S. M. Reiss, C.  
1186 Havenar-Daughton, R. A. Dawoud, G. K. Tharp, I. Sanz, B. Pulendran, S. Crotty, F. E.  
1187 Lee, J. Wrammert, S. E. Bosinger, BALDR: a computational pipeline for paired heavy and  
1188 light chain immunoglobulin reconstruction in single-cell RNA-seq data. *Genome Med* **10**,  
1189 20 (2018).
- 1190 81. C. A. Schramm, Z. Sheng, Z. Zhang, J. R. Mascola, P. D. Kwong, L. Shapiro, SONAR: A  
1191 High-Throughput Pipeline for Inferring Antibody Ontogenies from Longitudinal  
1192 Sequencing of B Cell Transcripts. *Front Immunol* **7**, 372 (2016).
- 1193 82. Y. Hao, S. Hao, E. Andersen-Nissen, W. M. Mauck, 3rd, S. Zheng, A. Butler, M. J. Lee,  
1194 A. J. Wilk, C. Darby, M. Zager, P. Hoffman, M. Stoeckius, E. Papalex, E. P. Mimitou, J.  
1195 Jain, A. Srivastava, T. Stuart, L. M. Fleming, B. Yeung, A. J. Rogers, J. M. McElrath, C.  
1196 A. Blish, R. Gottardo, P. Smibert, R. Satija, Integrated analysis of multimodal single-cell  
1197 data. *Cell* **184**, 3573-3587 e3529 (2021).
- 1198 83. T. Rognes, T. Flouri, B. Nichols, C. Quince, F. Mahe, VSEARCH: a versatile open source  
1199 tool for metagenomics. *PeerJ* **4**, e2584 (2016).

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1217

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1219 Conceptualization: NSL, CAS, DCD

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1231

### 1232 **Competing interests**

1233 None declared.

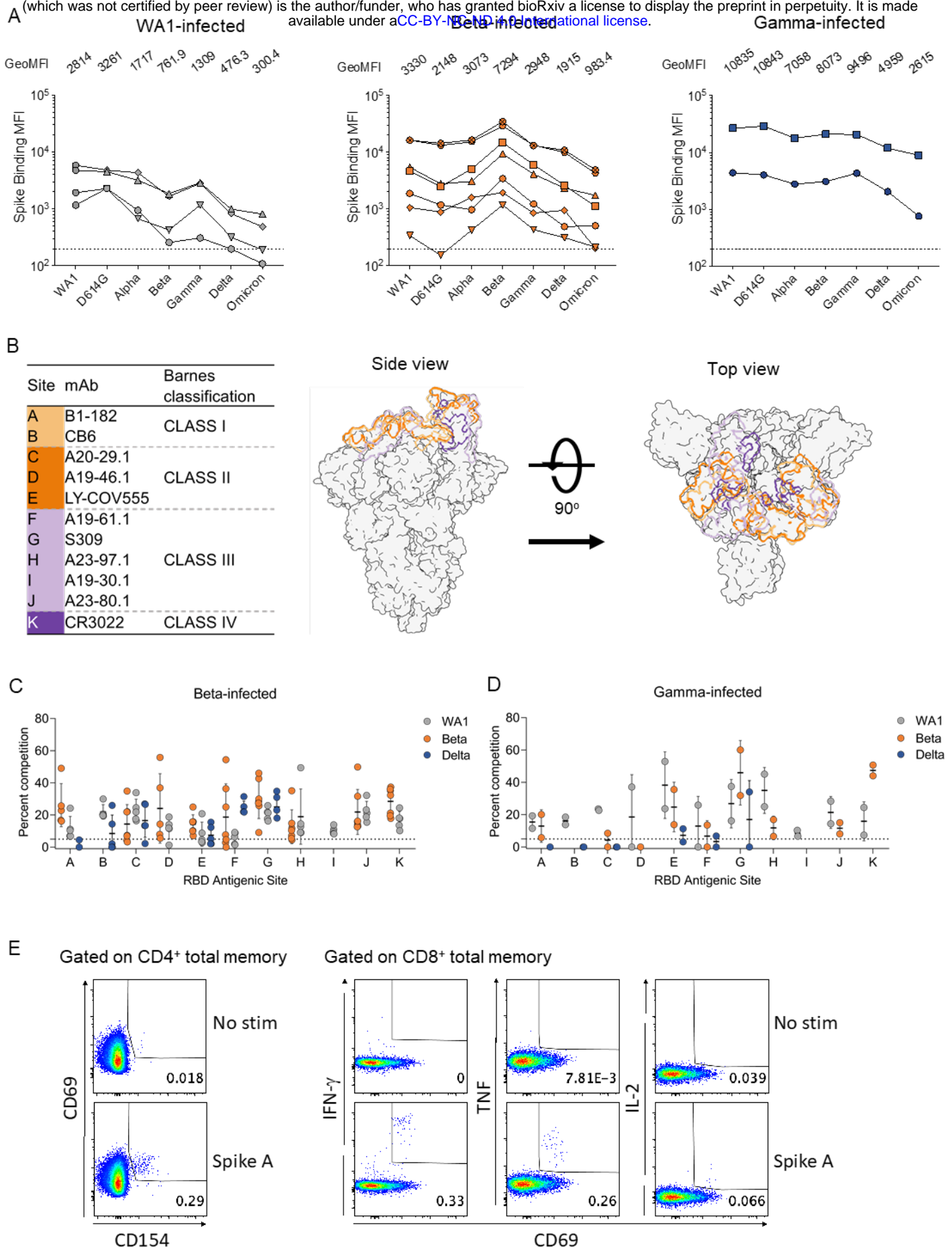
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### 1235 **Data and materials availability**

1236 All data and materials are available upon request.

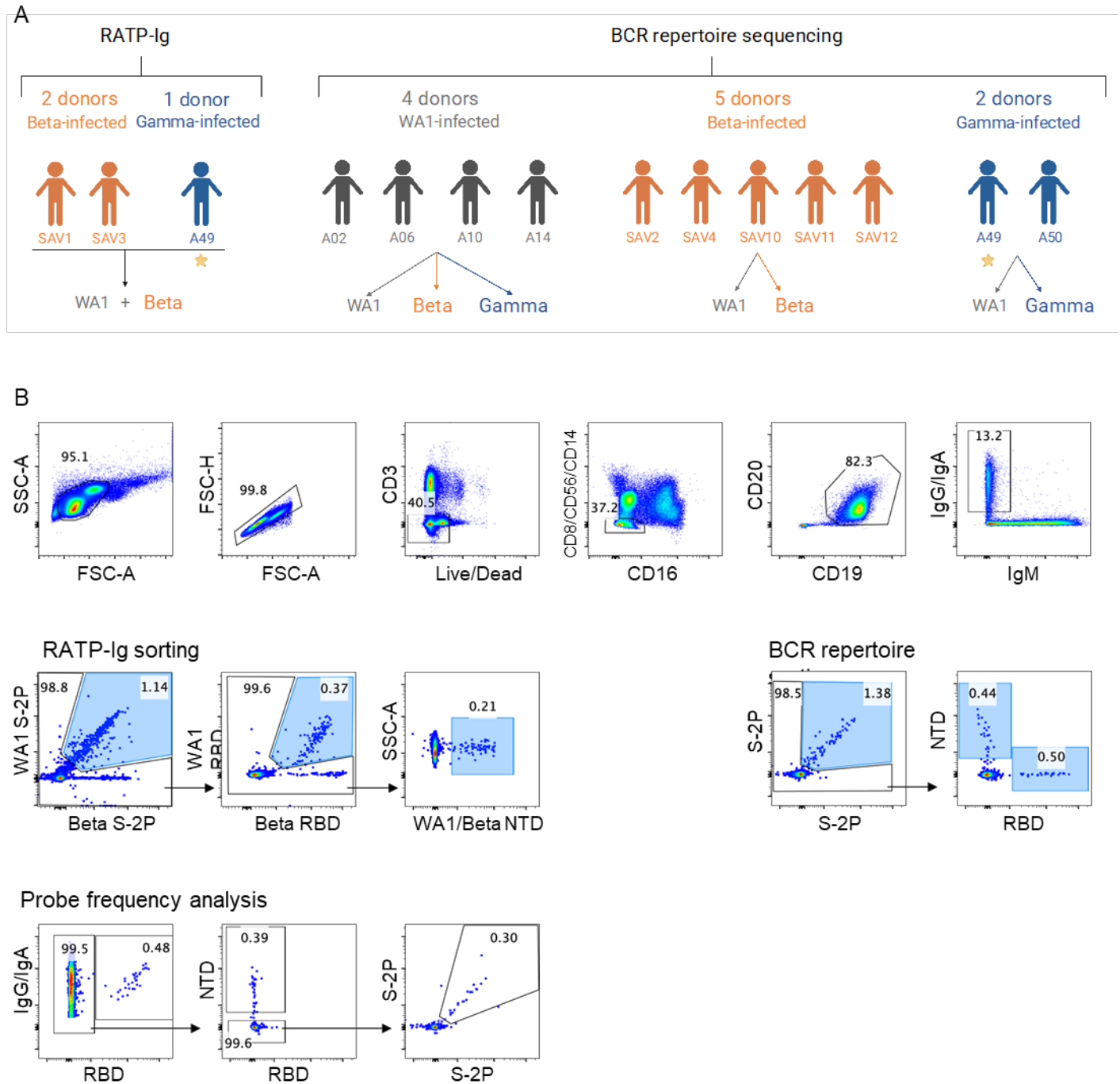
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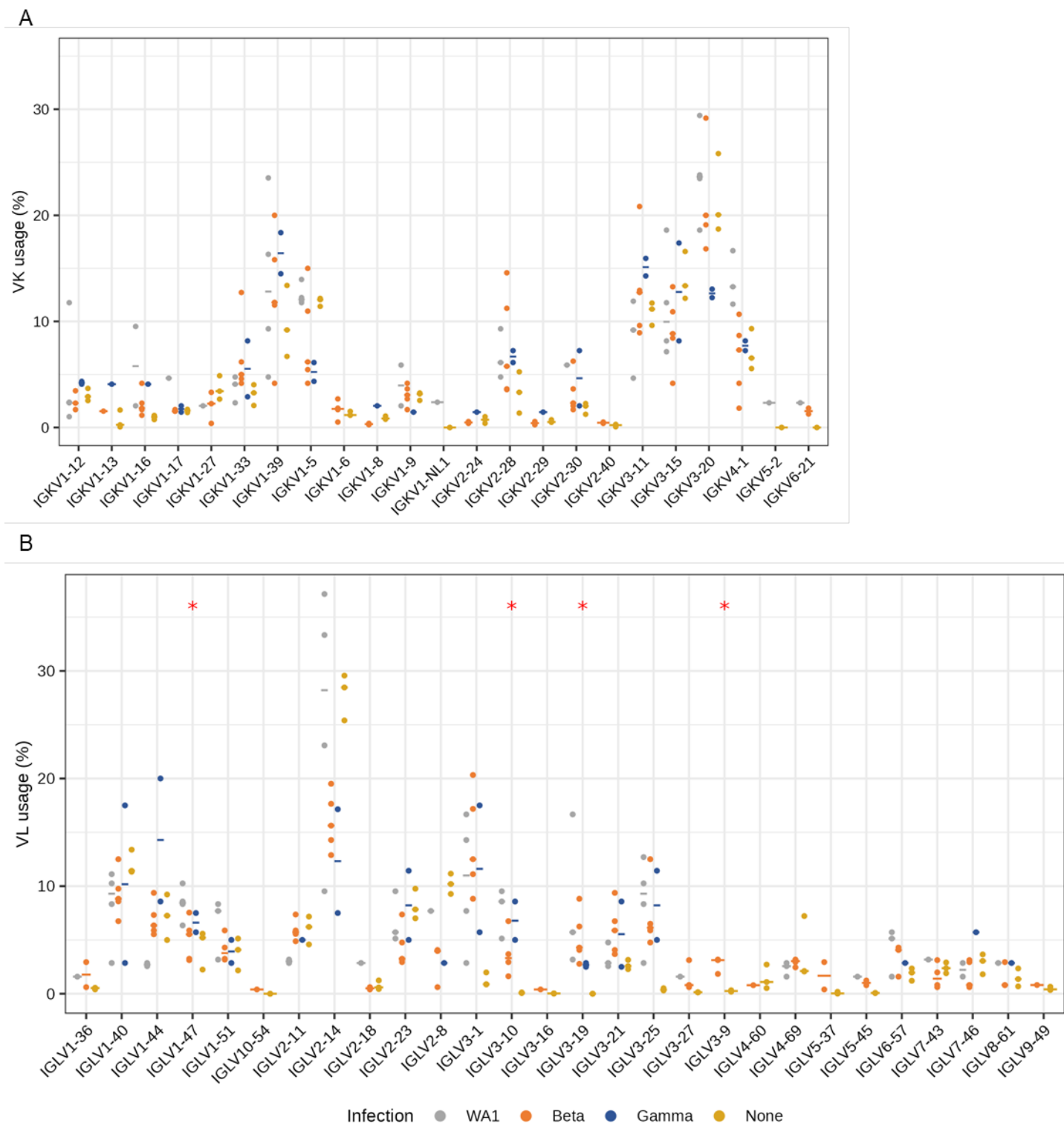


**Supplemental Figure 1:** Additional serology and epitope mapping data. **A)** Antibody binding titers against multiple Variants assessed by cell surface binding assay; **B)** Structural schematic of spike protein showing epitopes from monoclonal antibodies used for RBD epitope mapping by competition assay; **C)** Epitope mapping of Beta-infected individuals on WA1, Beta and Delta spike proteins; **D)** Epitope mapping of Gamma-infected individuals on WA1, Beta and Delta spike proteins; **E)** Gating strategy for T cell response analysis.





**Supplemental Figure 2: Antigen-specific B cell sorting.** (A) Arrows indicate probes used for sorting antigen-specific B cells from each group of convalescent individuals. The individual marked with a star was used for both RATP-Ig and total BCR repertoire sequencing. (B) Flow cytometry representative plots and gating strategies for B cell sorting and analysis; final sort gates are shown in blue.



**Supplementary Figure 3: SARS-CoV-2-specific light chain V gene usage frequencies.** (A) Kappa and (B) Lambda chain V gene repertoire analysis by infecting variant, with WA1, Beta and Gamma shown in grey, orange and blue, respectively, and data from pre-pandemic controls in yellow. The x-axis shows all germline genes used; the y-axis represents the percent of individual gene usage. Stars indicate genes with at least one significant difference between groups; pairwise comparisons using the Dunn test are in Table S4.

	<b>Infecting virus</b>	<b>Days after symptoms</b>	<b>Disease severity</b>	<b>Date of collection</b>	<b>Gender</b>	<b>Age</b>
<b>A02</b>	WA1	28	Mild	Mar-20	Male	39
<b>A06</b>	WA1	34	Mild	Apr-20	Female	59
<b>A10</b>	WA1	33	Moderate	Apr-20	Female	67
<b>A14</b>	WA1	34	Mild	Apr-20	Male	27
<b>SAV1</b>	Beta	33	Severe	Jan-21	Male	60
<b>SAV2</b>	Beta	33	Mild	Jan-21	Male	35
<b>SAV3</b>	Beta	30	Mild	Jan-21	Female	58
<b>SAV4</b>	Beta	28	Mild	Jan-21	Female	30
<b>SAV10</b>	Beta	38	Mild	Feb-21	Female	43
<b>SAV11</b>	Beta	37	Mild	Feb-21	Female	52
<b>SAV12</b>	Beta	35	Mild	Feb-21	Male	44
<b>A49</b>	Gamma	24	Moderate	Jan-21	Female	53
<b>A50</b>	Gamma	17	Mild	Jan-21	Male	32

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**Supplementary Table 1:** Details of the study cohort.

Sample ID	Infected with:	Spike variant		
		WA1	Beta	Delta
A02	WA1		below LLOQ	below LLOQ
A06	WA1		below LLOQ	below LLOQ
A10	WA1		below LLOQ	below LLOQ
A14	WA1		below LLOQ	
SAV1	Beta			
SAV2	Beta	below LLOQ		below LLOQ
SAV3	Beta			
SAV4	Beta			
SAV10	Beta	below LLOQ		below LLOQ
SAV11	Beta			
SAV12	Beta			
A49	Gamma			
A50	Gamma			

1244

1245 **Supplementary Table 2:** Serum epitope competition

1246

		SARS-CoV-2 Probe:						Total paired sequences by subject:
		WA1		Beta		Gamma		
WA1-infected	A02	3/23	(13%)	3/8	(28%)	4/13	(31%)	10
	A06	11/140	(8%)	4/74	(5%)	9/62	(15%)	24
	A10	9/87	(10%)	10/46	(22%)	11/34	(32%)	30
	A14	20/205	(10%)	14/76	(18%)	23/79	(29%)	57
Beta-Infected	SAV2	2/104	(2%)	14/214	(7%)	N/A		16
	SAV4	16/328	(5%)	40/630	(6%)	N/A		56
	SAV10	6/102	(6%)	12/131	(9%)	N/A		18
	SAV11	39/645	(6%)	125/2028	(6%)	N/A		164
	SAV12	32/306	(10%)	97/1318	(7%)	N/A		129
Gamma-Infected	A49	10/129	(8%)	N/A		23/148	(16%)	33
	A50	14/89	(16%)	N/A		37/128	(29%)	51
Total paired sequences by probe:		162		319		107		

1247

1248 **Supplementary Table 6:** Sample recovery from 10x Genomics-based single cell isolation and  
 1249 sequencing.  
 1250

Gene	Enriched Group	Median usage frequency, enriched	Depleted Group	Median usage frequency, depleted	Adjusted P-value
IGHV1-46	Beta-infected	4.1%	Gamma-infected	1.7%	0.037
IGHV1-46	Beta-infected	4.1%	Control	2.0%	0.045
IGHV1-46	WA1-infected	7.1%	Gamma-infected	1.7%	0.025
IGHV1-46	WA1-infected	7.1%	Control	2.0%	0.041
IGHV3-30	Gamma-infected	29%	Control	7.1%	0.021
IGHV3-30	WA1-infected	18%	Control	7.1%	0.026
IGHV3-49	WA1-infected	6.0%	Control	0.13%	0.021
IGHV4-38-2	WA1-infected	3.1%	Control	0.00%	0.020
IGHV5-51	Beta-infected	4.8%	Control	0.57%	0.021
IGHV5-51	WA1-infected	6.2%	Control	0.57%	0.046
IGLV1-47	WA1-infected	8.5%	Control	5.2%	0.027
IGLV1-47	WA1-infected	8.5%	Beta-infected	5.5%	0.041
IGLV3-9	Beta-infected	3.1%	Control	0.25%	0.050
IGLV3-10	WA1-infected	8.6%	Control	0.07%	0.016
IGLV3-19	Beta-infected	4.3%	Control	0.00%	0.021
IGLV3-19	WA1-infected	5.7%	Control	0.00%	0.036

1251  
1252 **Supplementary Table 7:** Significant differences in gene-usage. For genes with a significant difference  
1253 detected by the Kruskal-Wallis test (Figs. 4B and S5), the Dunn test was used to find significant pairwise  
1254 difference. P values were adjusted for multiple testing using the Benjami-Hochberg procedure.  
1255