

1 **Longitudinal analysis shows durable and broad immune memory after SARS-**
2 **CoV-2 infection with persisting antibody responses and memory B and T cells**

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48

49 **SUMMARY**

50 Ending the COVID-19 pandemic will require long-lived immunity to SARS-CoV-2. Here,
51 we evaluate 254 COVID-19 patients longitudinally up to eight months and find durable
52 broad-based immune responses. SARS-CoV-2 spike binding and neutralizing
53 antibodies exhibit a bi-phasic decay with an extended half-life of >200 days suggesting
54 the generation of longer-lived plasma cells. SARS-CoV-2 infection also boosts antibody
55 titers to SARS-CoV-1 and common betacoronaviruses. In addition, spike-specific IgG+
56 memory B cells persist, which bodes well for a rapid antibody response upon virus re-
57 exposure or vaccination. Virus-specific CD4+ and CD8+ T cells are polyfunctional and
58 maintained with an estimated half-life of 200 days. Interestingly, CD4+ T cell responses
59 equally target several SARS-CoV-2 proteins, whereas the CD8+ T cell responses
60 preferentially target the nucleoprotein, highlighting the potential importance of including
61 the nucleoprotein in future vaccines. Taken together, these results suggest that broad
62 and effective immunity may persist long-term in recovered COVID-19 patients.

63

64 **Keywords:**

65 SARS-CoV-2, COVID-19, Immune memory, Antibody, Kinetics, Neutralization, CD4+ T
66 cells, CD8+ T cells, B cells, Spike, RBD, Endemic coronaviruses

67

68 INTRODUCTION

69 The COVID-19 pandemic caused by the rapid spread of SARS-CoV-2, a novel
70 betacoronavirus, continues to cause significant morbidity and mortality. The induction of
71 effective early immune control of SARS-CoV-2 and durable immune memory is critical
72 to prevent severe disease and to protect upon re-exposure. SARS-CoV-2 infection
73 induces polyclonal humoral and cellular responses targeting multiple viral proteins
74 described in cross-sectional and longitudinal studies.¹ More comprehensive,
75 quantitative analyses with extensive serial sampling in larger numbers of COVID-19
76 patients are limited, and could resolve some conflicting views about the durability of
77 humoral immunity. Importantly, defining the frequency, immune function and specificity
78 of the antibodies, memory B and T cell responses among COVID-19 patients, and
79 identifying when they appear and how long they persist can provide understanding of
80 the integral components for long-lived immunity to SARS-CoV-2 and potentially other
81 human coronaviruses that emerge in the future.²

82 We initiated two prospective COVID-19 patient cohorts in Seattle and Atlanta during
83 the first surge of the pandemic to investigate long-term immunity to SARS-CoV-2.
84 Among 254 COVID-19 patients enrolled and frequently sampled, we identify binding
85 and neutralizing antibodies to SARS-CoV-2 as well as antigen-specific B and T cells
86 elicited early after infection, define their specificities, quantify the extent of antibody
87 boosting of cross-reactive responses to other coronaviruses, and further characterize
88 the decay rate and durability of these immune parameters over 250 days. We employ
89 highly standardized or validated assays that are also being used to evaluate immunity in

90 recent and ongoing clinical vaccine trials.³⁻⁵ This indepth longitudinal study
91 demonstrates that durable immune memory persists in most COVID-19 patients,
92 including those with mild disease, and serves as a framework to define and predict long-
93 lived immunity to SARS-CoV-2 after natural infection. This investigation will also serve
94 as a benchmark for immune memory induced in humans by SARS-CoV-2 vaccines.

95

96 **RESULTS**

97 **COVID-19 study population**

98 COVID-19-confirmed patients were recruited into our longitudinal study of SARS-
99 CoV-2 specific B and T cell memory after infection. A total of 254 patients enrolled at
100 two sites, Atlanta and Seattle, starting in April 2020 and returned for follow up visits over
101 a period of 250 days. We were able to collect blood samples at 2-3 time points from 165
102 patients and at 4-7 time points from another 80 patients, which allowed us to perform a
103 longitudinal analysis of SARS-CoV-2-specific B and T cell responses on a large number
104 of infected patients. The demographics and baseline characteristics of this cohort are
105 described in Table S1. The study group was 55% female and 45% male and between
106 18-82 years old (median, 48.5 years). Based on World Health Organization (WHO)
107 guidelines of disease severity, 71% of study participants exhibited mild disease, 24%
108 had moderate disease, and 5% experienced severe disease.

109

110 **Antibody responses to SARS-CoV-2 spike protein show a bi-phasic decay with an**
111 **extended half-life**

112 Binding antibodies to the SARS-CoV-2 full length spike protein, to the receptor
113 binding domain (RBD) and to the N terminal domain (NTD) of the spike protein were
114 assessed in COVID-19 patients (n=222) over a period of 8 months post symptom onset.
115 We included healthy individuals age 18-42 years as negative controls whose
116 longitudinal blood samples were collected before the emergence of the COVID-19
117 pandemic. These pre-pandemic samples (n=51) were from recipients of either the
118 seasonal inactivated influenza vaccine (n=27, collected from 2014-2018) or the live
119 yellow fever virus (YFV-17D) vaccine (n=24, collected from 2005-2007). The Mesoscale
120 multiplex assay was used to measure IgG, IgA, and IgM antibody responses to SARS-
121 CoV-2 proteins in the COVID-19 patients and in the pre-pandemic healthy controls.

122 The magnitude of serum IgG antibodies binding to the SARS-CoV-2 spike protein
123 increased in 92% of COVID-19 convalescent participants (n=222) relative to pre-
124 pandemic controls (Figure 1A). The IgG responses to SARS-CoV-2 spike, RBD, and
125 NTD declined over time with half-lives of 126 (95% confidence interval [95%CI] [107,
126 154]), 116 (95%CI [97, 144]), and 130 (95%CI [110, 158]) days, respectively, as
127 estimated by an exponential decay model (Figure 1A-C and S1A). We also estimated
128 antibody waning using a power law model, which models a scenario in which the rate of
129 antibody decay slows over time. The power law model produced a better fit for the
130 decay of the SARS-CoV-2 spike, RBD, and NTD binding IgG antibodies ($\Delta AICs > 10$),
131 suggesting that spike-specific antibodies plateau over time. Because the decay rate
132 changes over time, the half-life is predicted to change over time as well; therefore, we
133 used the power law model to estimate the half-lives at 120 days after symptom onset.

134 The power law estimated half-lives for the IgG antibody responses to spike ($t_{1/2}$ =238
135 days), RBD ($t_{1/2}$ =209 days), and NTD ($t_{1/2}$ =244 days) were longer than those estimated
136 by the exponential decay model (Figure S1A, C), indicating that the concentration of
137 these IgG antibodies may be starting to stabilize. IgA (Figure 1D-F) and IgM (Figure 1G-
138 I) antibodies reactive to the SARS-CoV-2 spike also increased after SARS-CoV-2
139 infection but were detected at lower levels and declined faster than the SARS-CoV-2-
140 reactive IgG antibodies. As expected, spike-binding IgM decayed more rapidly than
141 spike-binding IgA and IgG. Taken together these results show that antibody responses,
142 especially IgG antibody, were not only durable in the vast majority of patients in the 250
143 day period but also that the bi-phasic decay curve suggests the generation of longer
144 lived plasma cells producing antibody to the SARS-CoV-2 spike protein.

145 We also examined the antibody response to the SARS-CoV-2 nucleocapsid protein
146 in these infected patients. As expected, the COVID-19 patients showed higher levels of
147 antibody to the nucleocapsid protein compared to the pre-pandemic healthy controls
148 (Figure S2). However, the nucleocapsid-specific antibodies declined with a much
149 shorter half-life of 63 days (95%CI [58, 70]) compared to the spike protein antibodies
150 (Figure S1A, C). Also, the nucleocapsid reactive IgG decay rate was best fit by the
151 exponential model and not the power law model in contrast to what we observed with
152 the spike IgG antibody decay rate (Figure S1A). Thus, the nucleocapsid reactive IgG
153 not only declined much faster but also showed less evidence of stabilizing antibody
154 levels consistent with a response driven disproportionately by short-lived antibody
155 secreting cells – at least at this stage of the immune response.

156

157 **Stable and long-lived antibody responses to common human alpha- and**

158 **betacoronaviruses in pre-pandemic healthy controls**

159 We were interested in determining if SARS-CoV-2 infection had any effect on the
160 levels of antibody to the circulating human alpha- and betacoronaviruses. As a prelude
161 to this question, we first examined antibody levels to the spike protein of the two
162 circulating alphacoronaviruses (229E and NL63) and the two betacoronaviruses (HKU1
163 and OC43) in our pre-pandemic samples. As shown in Figure 2, all 51 pre-pandemic
164 samples had clearly detectable levels of IgG and IgA antibodies to the spike proteins of
165 the four human coronaviruses. This is the expected result since seropositivity to these
166 coronaviruses is very high in the adult population, but what was quite interesting was
167 the remarkable stability of these antibody responses over a 200-day period in the pre-
168 pandemic serum samples (shown as red lines in Figure 2). These were essentially flat
169 lines with no decline in the antibody levels and question the prevailing belief that
170 antibody responses to the endemic coronaviruses are short-lived.⁶⁻⁸ While some
171 occasional boosting of these childhood-acquired coronavirus infections cannot be ruled
172 out, these data showing such stable antibody titers are best explained by the
173 persistence of long-lived plasma cells in the bone marrow many years after infection.⁹⁻¹³

174

175 **COVID-19 infection results in increased levels of antibodies to two common**

176 **human betacoronaviruses (HKU1 and OC43) and to SARS-CoV-1**

177 We next examined if SARS-CoV-2 infection had any impact on the levels of
178 antibodies to the other human coronaviruses. We measured IgG, IgA, and IgM antibody
179 binding to the spike proteins of other known human coronaviruses in the COVID-19
180 patients (n=222 for IgG and n=190 for IgA and IgM) and compared these data to the 51
181 pre-pandemic healthy donor samples. In the COVID-19 patients, IgG and IgA antibodies
182 to the alphacoronaviruses, 229E and NL63, did not show any significant changes
183 compared to the antibody levels in the pre-pandemic healthy controls (Figure 2A, B, F,
184 G; Figure S1C, D). In contrast, the IgG and IgA antibodies to betacoronaviruses HKU1
185 and OC43 were substantially elevated in COVID-19 patients relative to pre-pandemic
186 controls (Figure 2C, D, H, I; Figure S1C, D; $p < 0.0001$). After this boost, HKU1 and
187 OC43 IgG antibody levels declined with estimated half-lives of 288 (95%CI [235, 372])
188 and 212 (95%CI [176, 268]) days, respectively (exponential decay model). IgM levels to
189 common betacoronaviruses HKU1 and OC43 were low in both pre-pandemic controls
190 and COVID-19 patients (Figure 2M, N). While pre-existing exposure and antibodies
191 against HKU1 and OC43 betacoronaviruses are common in adults, pre-existing SARS-
192 CoV-1 exposure is rare and antibody levels to SARS-CoV-1 spike protein were very low
193 (essentially negative) in the pre-pandemic healthy controls. However, SARS-CoV-1
194 spike-reactive antibodies increased significantly after SARS-CoV-2 infection. These
195 increases were quite striking for IgG ($p = 0.0038$) and also IgA ($p = 0.0084$) and most likely
196 represent cross-reactive antibodies directed to SARS-CoV-2 spike epitopes that are
197 conserved between SARS-CoV-2 and SARS CoV-1¹⁴. These newly induced cross-
198 reactive IgG antibodies generated after COVID-19 infection declined with an estimated

199 half-life of 215 days (95%CI [168, 298]) (exponential decay model) (Figure 2). Taken
200 together, these results show that people infected with SARS-CoV-2 may have also have
201 some heightened immunity against the common human betacoronaviruses and more
202 importantly against SARS-CoV-1.

203

204 **Durable neutralizing antibody responses to SARS-CoV-2 in infected patients**

205 Neutralizing antibodies were measured with a live virus focus reduction
206 neutralization test that uses a recombinant SARS-CoV-2 virus expressing the
207 fluorescent reporter gene mNeonGreen (FRNT-mNG) (Figure 3A). During the first 250
208 days post-symptom onset, FRNT₅₀ titers varied considerably between individuals and
209 ranged from <20 to 3726 (Figure 3A). Of the 183 individuals for whom longitudinal
210 neutralization titers were assayed, 140 (77%) had at least one timepoint with
211 neutralization titers above the limit of detection (>20). Seventy-five percent (43/57) of
212 COVID-19 patients generated serum neutralizing antibodies between 30 – 50 days after
213 symptom onset, and similarly 72% (48/67) had measurable titers between 180 – 263
214 days after symptom onset. Using an exponential decay model, we evaluated the
215 kinetics of neutralizing antibody titers after day 42 and estimated a half-life of 150 days
216 (95%CI [124, 226]). However, similar to the spike reactive IgG binding antibodies, we
217 hypothesized that the neutralizing antibody rate of decay may actually slow over time
218 during the recovery period. To address this, we fit a power law to the data. The power
219 law model fit significantly better than the exponential decay model ($\Delta AIC=9$) and

220 estimated the half-life of neutralizing antibody responses at 120 days post-symptom
221 onset to be 254 days (95%CI [183, 400]).

222 Next, we assessed the relationship between the levels of spike and RBD binding
223 antibodies and SARS-CoV-2 neutralization. Figures 3B and C show the SARS-CoV-2
224 spike and RBD binding antibody response kinetics of the 183 participants for whom
225 neutralization titers were assessed. These exhibited a wide range of antibody binding
226 levels ranging from non-responders (n=11) who did not elicit antibody titers above those
227 of pre-pandemic controls (defined as a COVID-19 patient titer below the mean pre-
228 pandemic antibody titer plus 3 standard deviations, see dashed line on Figure 3B, C) to
229 those with IgG levels >200,000 AU/ml. Spike and RBD binding IgG levels correlated
230 significantly with the neutralization titers (Figure 3D, E; $p < 0.0001$).

231 Taken together, our findings show that induction of neutralizing antibodies occurs in
232 the majority of COVID-19 patients. These neutralizing antibodies can persist over the 8-
233 9 month period following infection, and show a correlation with spike and RBD binding
234 IgG.

235

236 **SARS-CoV-2 spike and RBD-specific memory B cells increase for several months** 237 **after infection and then plateau over 8 months**

238 Memory B cells (MBC) are an important component of humoral immunity and
239 contribute to viral control by generating antibody responses upon re-exposure to the
240 pathogen. We used full-length spike and RBD antigen probes to quantify the
241 frequencies of SARS-CoV-2 spike- and RBD-specific MBC in longitudinal PBMC

242 samples from 111 COVID-19 patients (Figure 4) and from 29 pre-pandemic controls
243 (Figure S3A, B). Our flow cytometric gating strategy to identify SARS-CoV-2 specific
244 MBC and classify them as IgG, IgM and IgA MBC isotypes is shown in Figure 4A.

245 Among the total MBC, the spike IgG+ MBCs were significantly increased in COVID-
246 19 patients (n=111; Figure 4B) in comparison to pre-pandemic controls (n=29; Figure
247 S3A) (median increase, 0.73% vs. 0.02%; $p < 0.0001$). After a steep early expansion
248 over the first 2-3 months, the spike IgG+ MBC persisted in COVID-19 patients with no
249 decline out to 250 days post symptom onset. These findings (Figure 4B) are supported
250 by a positive slope (0.004) from the model of the longitudinal spike IgG+ MBC
251 responses after day 30 (95%CI [0.002, 0.006], $p < 0.001$; Figures S4A, B).

252 The spike IgM+ MBC appeared within the first two weeks post-symptom onset and
253 quickly declined (Figure 4C, D). The decay continued after day 30 (slope=-0.007,
254 95%CI [-0.010, -0.005], $p < 0.001$). One month after symptom onset, 56% of spike MBC
255 were IgG+, which increased to a peak of 80% at 5-6 months (Figure 4D). Circulating
256 spike IgA+ MBC were also detectable in many subjects at low frequencies and without
257 significant change over time (day 30 to 250: slope=0.000, 95%CI [-0.002, 0.002],
258 $p = 0.91$, Figure 4D).

259 Since the RBD contains the primary neutralizing epitopes on the spike, we also
260 used an RBD-specific probe to characterize this subset of spike-specific memory B
261 cells. Overall, approximately 20% of the spike IgG+ memory B cells targeted the RBD,
262 which was consistent across subjects and time (Figure 4E, F). As expected, RBD+ IgM+
263 MBC emerged early in infection and subsequently switched to RBD+ IgG+ MBCs, which

264 gradually increased during follow-up (day 30 to 250: slope=0.004, 95%CI [0.002, 0.005],
265 p<0.001, Figure 4E). Thus, the maintenance of circulating spike- and RBD-specific IgG
266 memory B cells suggests that these cells could be recruited for a rapid secondary
267 response following re-exposure or vaccination.

268

269 **Induction of durable and polyfunctional virus specific memory CD4+ and CD8+ T** 270 **cells in infected patients**

271 CD4+ T cells are critical for generation of high affinity antibody responses and can
272 also have anti-viral effects. In addition, they provide help for CD8+ T cell responses,
273 which are vital for killing infected cells and mediating viral clearance. Thus, we next
274 examined virus-specific CD4+ and CD8+ T cell responses longitudinally in COVID-19
275 patients and uninfected controls using a high-dimensional, multi-parameter *ex vivo*
276 intracellular cytokine staining (ICS) assay. The assay is sensitive, precise and specific
277 for detection of antigen-specific T cells expressing multiple cytokines and effector
278 molecules following a short-term (6 hours) stimulation with peptide pools. Our lab
279 developed and validated the assay, and we are currently using the method to quantitate
280 Th1/Th2 CD4+ and CD8+ T cell responses in SARS-CoV-2 vaccine trials. Here, we
281 assessed T cell responses to the SARS-CoV-2 structural (S, E, M and N) and
282 accessory proteins (ORF 3a, 6, 7a, 7b, and 8) using overlapping peptide pools that
283 span the sequences of these proteins.

284 Among COVID-19 patients, 89% (102/113) mounted CD4+ T cell responses (Figure
285 5A) recognizing at least one SARS-CoV-2 structural protein that was detectable at one

286 or more visits. By contrast, SARS-CoV-2 specific CD4+ T cells were rarely detected in
287 the uninfected control group using this assay (Figure S3C). Antigen-specific CD4+ T
288 cells expanded over the first month after infection and then gradually declined over
289 subsequent months. Their estimated half-life was 207 days (95%CI [104, 211]) as
290 shown in Figure 5A, and these findings are supported by the individual CD4+ T cell
291 response levels and slopes after day 30 (slope= -0.0033, 95%CI [-0.0017, -0.0066],
292 $p < 0.0001$) (Figure S4C, D). Of note, we observed a wide range in the total magnitude of
293 responses, some reaching >1% of circulating CD4+ T cells, and an overall median
294 frequency of 0.51% (Figures 5A and S5).

295 To better characterize the development of T cell memory in SARS-CoV-2 infection,
296 we examined the differentiation profiles of virus-specific T cells longitudinally in COVID-
297 19 patients. Based on CD45RA and CCR7 expression, SARS-CoV-2-specific CD4+ T
298 cells were primarily central memory phenotype (CD45RA- CCR7+) and to a lesser
299 extent effector memory (CCR4-CCR7-); this profile of the memory T cell subsets was
300 very consistent between subjects and stable over time (Figure 5B). The antigen-specific
301 CD4+ T cells were Th1-biased with a predominant CXCR3+CCR6- phenotype, and
302 highly polyfunctional, with simultaneous detection of antigen-specific CD154, IFN- γ , IL-
303 2, TNF- α and less frequently granzyme B in the early expansion phase (21-60 days post
304 symptom onset; median, 30 days) (Figure 5C). Interestingly, many of the virus-specific
305 CD4+ T cells also exhibited this polyfunctionality at the memory time point (>180 days
306 post symptom onset; median, 203 days) (Figure 5D). Circulating SARS-CoV-2-specific

307 Th2 (IL-4, IL-5 and IL-13), Th17 (IL-17) or perforin-expressing subsets were not
308 detected (Figure 5C and D).

309 Next, we examined the CD8+ T cell responses in COVID-19 patients and found that
310 69% generated CD8+ T cells recognizing at least one SARS-CoV-2 structural protein
311 that were detectable at one or more visits (Figure 6A), in contrast to infrequent to rare,
312 low-level antigen specific responses in the uninfected control donors (Figure S3D).
313 Expansion of CD8+ T cells occurred over the first month and then frequencies gradually
314 declined, with a half-life of 196 days (95%CI [92, 417]) and a negative estimated slope
315 after 30 days of symptom onset (slope=-0.004, 95%CI [-0.002, -0.008], $p < 0.0001$)
316 (Figure 6A). The median frequency of SARS-CoV-2-specific CD8+ T cells was 0.2%,
317 indicating a lower overall response magnitude than observed for CD4+ T cells.
318 However, like the CD4+ T cells, a wide range in magnitudes was observed with many
319 SARS-CoV-2-specific CD8+ T cell frequencies above 1% and even up to 12% (Figure
320 6A).

321 A very different pattern of phenotypic changes were observed with virus-specific
322 CD8+ T cells compared to what we saw with the CD4+ T cells (Figure 6B vs. Figure
323 Figure 5B). In contrast to the dominance of the central memory subset with SARS-CoV-
324 2-specific CD4+ T cells, the vast majority of the virus-specific CD8+ T cells showed an
325 effector memory phenotype during the early phase of the response. However, this
326 population of SARS-CoV-2-specific effector memory (CD45RA-CCR7-) contracted over
327 time (slope=-0.904, $p < 0.0001$; Figure 6B) and simultaneously there was an increase in
328 the proportion of the TEMRA (CD45RA+CCR7-) subset of virus-specific CD8+ T cells

329 (slope=0.075, $p < 0.0001$; Figure 6B). A small but stable fraction of SARS-CoV-2-specific
330 CD8+ T cells expressed a central memory phenotype (slope=0.024, $p = \text{ns}$; Figure 6B).

331 The SARS-CoV-2-specific CD8+ T cells were highly polyfunctional with the highest
332 magnitude populations secreting IFN- γ , TNF- α and granzyme B; other dominant
333 subsets also expressed IL-2 or perforin (Figure 6C, D). This polyfunctional profile was
334 seen in the expansion phase (median 30 days; Figure 6C) and also at the later time
335 points (>180 days post symptom onset; median 203 days; Figure 6D). It is important to
336 note that this pattern of CD8+ T cell differentiation has been described in detail after
337 vaccination in humans with the live attenuated yellow fever virus vaccine (YFV-17D).¹⁵
338 This YFV-17D vaccine generates long-lived and functional virus specific memory CD8+
339 T cells that persist in humans for decades.^{15,16} That the CD8+ T cell differentiation
340 program after COVID-19 infection resembles what is seen after YFV infection of human
341 suggests that COVID-19 patients may also generate long-lived CD8+ T cell memory.

342

343 **CD4+ and CD8+ cells target different SARS-CoV-2 antigen specificities**

344 The majority of COVID-19 patients generated CD4+ T cells that recognized most
345 SARS-CoV-2 viral structural and accessory proteins, with the highest percentage
346 responding to S2 (78%) and S1 (69%) (Figure 5E, F). Among the COVID-19 subjects
347 with positive responses, the proportion of SARS-CoV-2-specific CD4+ T cells reacting to
348 each peptide pool was evenly distributed (Figure 5F). Thus, CD4+ T cells equally
349 targeted multiple SARS-CoV-2 proteins.

350 In contrast to the results seen with CD4+ T cells, SARS-CoV-2-specific CD8+ T
351 cells showed preferential recognition of the nucleocapsid protein. The dominant CD8+ T
352 cell response rate was directed to the nucleocapsid (57%), followed by ORFs 7a, 7b,
353 and/or 8 (25%), S1 (25%), ORFs 3a and/or 6 (16%), S2 (12%) and E and/or M (9%)
354 (Figure 6E). Also, among the COVID-19 patients with CD8+ T cell responses, there was
355 a bias with the largest percentage (median, 43%) reacting to the nucleoprocapsid
356 protein (Figure 6F). While SARS-CoV-2 CD8+ T cell responses rates were much lower
357 in uninfected controls, when present in a few control donors with lower frequencies,
358 these were also targeted to the nucleocapsid protein (Figure S3D). A likely explanation
359 for these findings is that in SARS-CoV-2 infection, antigen-presenting cells *in vivo* may
360 display a higher proportion of peptides derived from the nucleocapsid protein and hence
361 more nucleocapsid-specific CD8+ T cells are generated during infection. This has
362 interesting implications suggesting that nucleocapsid-specific CD8+ T cells might be
363 more efficient in recognizing virally infected cells.

364

365 **Age and disease severity are significantly associated with magnitude of SARS-** 366 **CoV-2 immune responses**

367 We evaluated whether COVID-19 patient age, disease severity, or gender could
368 account in part for the heterogeneity observed among the SARS-CoV-2-specific
369 immune responses as estimated from the individual models (post day 30 for cellular and
370 post day 42 for antibody responses). We observed that age was significantly associated
371 with higher immune responses to SARS-CoV-2, independently of any covariation with

372 disease severity (Figure 7A). Neutralizing antibody titers and IgG antibody responses to
373 nucleocapsid increased 1.35-fold and 1.25-fold, respectively, with each decade of age
374 and the same disease severity (95%CI [1.19, 1.54] and [1.08, 1.43], p values<0.003).
375 Similarly, increased age positively correlated with increased frequencies of spike and
376 RBD-specific IgG+ memory B cells, with 1.19 to 1.24-fold higher responses per decade
377 of age (p values<0.02; Figure 7A), accounting for disease severity. Increased age also
378 correlated with higher SARS-CoV-2 and S1-specific CD4+ T cell responses (1.16-1.20-
379 fold increase by decade of age, p values<0.02) and N-specific CD8+ T cell responses
380 (1.24-fold increase by decade of age, p=0.039) accounting for disease severity (Figure
381 7A).

382 Since the cohort included primarily persons with mild to moderate COVID-19, we
383 had limited ability to assess the relationship of severe disease and SARS-CoV-2
384 immune responses, especially among the cellular responses. However, we found that
385 after accounting for age, severe disease (WHO score >4) was associated with higher
386 IgG antibodies to nucleocapsid, spike, RBD, and NTD (Figure 7B, C), and SARS-CoV-2
387 neutralization titers (Figure 7D). Severe disease was also associated with 2.30 to 2.46-
388 fold higher S1, E and/or M, and nucleocapsid-specific CD4+ T cells (all p values<0.05;
389 Figure 7B). We found no significant relationships between gender and the immune
390 responses evaluated, apart from 1.66-fold higher IgG NTD responses antibodies among
391 males compared to females, after accounting for age and disease severity (95%CI
392 [1.08, 2.55], p=0.022). In all, our analyses suggest that there are synergistic, but also

393 independent mechanisms driving higher adaptive immune responses in COVID-19
394 patients who are older and/or who experienced more severe disease.

395

396 **Early SARS-CoV-2 B and T cell responses correlated with durable spike and RBD**
397 **IgG antibody binding and neutralization titers**

398 We assessed correlations between SARS-CoV-2-specific immune responses using
399 the individual-level models to interpolate the magnitude of responses for each COVID-
400 19 patient at early (day 30) or later (day 180) convalescent time points (Figure 7E). We
401 found that durable serum neutralization titers correlated with the magnitude of IgG+
402 binding antibodies to spike, NTD and RBD at day 180 each (day 180; Spearman
403 $R=0.62$, 0.61 , and 0.61 , respectively; all p values <0.0001). Similarly, the frequency of
404 RBD+ IgG+ memory B cells at day 30 correlated with the maintenance of RBD+ IgG
405 antibodies (day 180; Spearman $R=0.53$, $p<0.0001$) and neutralization antibody titers
406 (day 180; Spearman $R=0.48$, $p<0.0001$). We also observed that the magnitude of S1-
407 specific CD4+ T cells at day 30 correlated with durable IgG antibodies against spike
408 (day 180; Spearman $R=0.56$, $p<0.0001$), NTD (Spearman $R=0.62$, $p<0.0001$), and RBD
409 (Spearman $R=0.47$, $p=0.0002$) (Figure 7E). These findings are consistent with early
410 SARS-CoV-2 memory B cells and CD4+ T cells supporting the generation of durable
411 antibody responses.

412

413 **DISCUSSION**

414 Establishing immune memory is essential in the defense against SARS-CoV-2
415 infection. To end the COVID-19 pandemic, it is critical to know how long immunity
416 against SARS-CoV-2 will persist after infection and whether it will be sufficient to
417 prevent new infections and severe disease in years to come. Identifying in depth the
418 adaptive immune components leading to recovery and modeling the trends of each
419 response was enabled by the longitudinal sampling of a large number of COVID-19
420 patients. Here, we show that most convalescent COVID-19 patients mount durable
421 antibody, B and T cells specific for SARS-CoV-2 up to 250 days, and the kinetics of
422 these responses provide an early indication for a favorable course ahead to achieve
423 long-lived immunity. Because the cohort will be followed for 2-3 more years, we can
424 build on these results to define the progression to long-lived immunity against this novel
425 human coronavirus, which can guide rational responses when future outbreaks occur.

426 The hallmark of the initial immune defense against SARS-CoV-2 is the emergence
427 of antibodies recognizing the SARS-CoV-2 spike protein, including the RBD and NTD
428 components of the S1 subunit, during the early phase of viral replication. These
429 antibodies are likely secreted from plasmablasts rapidly generated from B cells that are
430 activated upon their first encounter with the pathogen spike antigen. The brisk rise over
431 the first month of infection, followed by a fast decline of the circulating spike IgG and IgA
432 antibodies, is a consistent finding and likely explained by the disappearance of the
433 short-lived plasmablasts. These events occur even sooner for the spike IgM and
434 nucleocapsid antibodies.

435 Some antibodies that bind to specific epitopes on the spike RBD and NTD can block
436 SARS-CoV-2 infection of respiratory epithelial cells by inhibiting the interactions of the
437 viral spike with the ACE2 receptor.¹⁷⁻²⁰ Thus, as expected, the early rise and decline of
438 antibodies neutralizing live SARS-CoV-2 were similar to the kinetics of antibodies
439 binding the spike and RBD protein. The striking finding is the bi-phasic curve of the
440 spike-specific binding and neutralizing antibody responses when analyzed with the
441 power law model, which provides a better fit for the antibody kinetics after the peak
442 response.²¹ This bi-phasic decline accords with other recently published observations
443 on SARS-CoV-2 serological kinetics.^{22,23} With sampling data extended to 250 days, we
444 were able to detect a slowing of the decay of these functional antibodies toward a
445 plateau level, suggestive of the generation of longer-lived plasma cells, and durable
446 antibody responses. The importance of these observations is that following recovery,
447 neutralizing antibodies may persist, albeit at low levels, and may act as the first line of
448 defense against future encounters of SARS-CoV-2 and possibly related human
449 coronaviruses.

450 Another interesting finding of this investigation is the remarkably stable antibody
451 responses among the pre-pandemic and COVID-19 patients to the common human
452 coronaviruses that are acquired in children and adults. These data are most consistent
453 with the generation of long-lived plasma cells and refute the current notion that these
454 antibody responses to human coronaviruses are short-lived. Moreover, the COVID-19
455 patients mounted increased IgG antibody responses to SARS-CoV-1, a related
456 pathogen that none likely had experienced previous exposure. This finding is consistent

457 with the booster response of SARS-CoV-1 neutralizing antibodies that we recently
458 observed following SARS-CoV-2 mRNA vaccination.^{3,24} Taken together, these results
459 may have implications for a broader strategy for vaccines targeting multiple
460 betacoronaviruses.

461 The durable antibody responses in the COVID-19 recovery period are further
462 substantiated by the ongoing rise in both the spike and RBD memory B cell responses
463 after over 3-5 months before entering a plateau phase over 6-8 months. Persistence of
464 RBD memory B cell has been noted.²⁵⁻²⁷ We presume this may be explained by
465 sustained production of memory B cells in germinal centers of lymph nodes draining the
466 respiratory tract in the early months, followed by the memory B cell redistribution into
467 the circulation as the germinal centers begin to recede. Thus, the induction and
468 maintenance of memory B cells and, over time, long-lived plasma cells, will continue to
469 furnish higher affinity antibodies if re-exposures occur.

470 In contrast to spike memory B cell kinetics, SARS-CoV-2-specific CD4+ and CD8+
471 memory T cells each peak early, within the first month, but then slowly decline over the
472 next 6-7 months. Central memory Th1-type CD4+ T cells dominate throughout the early
473 infection and recovery period. However, the CD8+ T cells exhibit a predominant
474 effector memory phenotype early that transitions to those effector memory cells re-
475 expressing CD45RA, maintaining expression of antiviral cytokines and effector functions
476 which have been shown to provide protective immunity against other viral pathogens.
477 We also provide clear evidence that the CD4+ T cells mount a broader antigen-specific
478 response across the structural and accessory gene products, whereas the CD8+ T cells

479 are predominantly nucleocapsid specific and spike-specific responses are substantially
480 lower in frequency.

481 Our study demonstrates the considerable immune heterogeneity in the generation
482 of potentially protective response against SARS-CoV-2, and by focusing on the
483 dynamics and maintenance of B and T cell memory responses, we were able to identify
484 features of these early cellular responses that can forecast the durability of a potentially
485 effective antibody response. The ability to mount higher frequencies of RBD-specific
486 memory IgG+ B cells early in infection was the best indicator for a durable RBD-specific
487 IgG antibody and neutralizing antibody response. In addition, higher frequency CD4+ T
488 cells were associated with stronger spike IgG and neutralizing antibody responses.
489 However, the induction and peak response of SARS-CoV-2-specific CD8+ T cells
490 occurs independently to these antibody responses. Interestingly, while it has been
491 widely reported that age correlates with COVID-19 disease severity, we found that age
492 and disease severity were independent co-variables associated with the magnitude of
493 both SARS-CoV-2-specific CD4+ T cell and humoral SARS-CoV-2 immunity, but not
494 with the magnitude of CD8+ T cell responses. In the case of T cells, whether the T cell
495 differences are related to the frequencies or specificities of pre-existing coronavirus
496 CD4+ and CD8+ T cell immunity will require additional future analysis.

497 The COVID-19 pandemic remains a global public health threat after one year of
498 overwhelming disruption and loss. Overcoming the challenges to end the pandemic is
499 accentuated by the recognition that SARS-CoV-2 can undergo rapid antigenic variation
500 that may lower vaccine effectiveness in preventing new cases and progression to

501 severe disease.^{24,28,29} Our findings show that most COVID-19 patients induce a wide-
502 ranging immune defense against SARS-CoV-2 infection, encompassing antibodies and
503 memory B cells recognizing both the RBD and other regions of the spike, broadly-
504 specific and polyfunctional CD4+ T cells, and polyfunctional CD8+ T cells. The immune
505 response to natural infection is likely to provide some degree of protective immunity
506 even against SARS-CoV-2 variants because the CD4+ and CD8+ T cell epitopes will
507 likely be conserved. Thus, vaccine induction of CD8+ T cells to more conserved
508 antigens such as the nucleocapsid, rather than just to SARS-CoV-2 spike antigens, may
509 add benefit to more rapid containment of infection as SARS-CoV-2 variants overtake
510 the prevailing strains.

511

512 **Limitations of study**

513 Our study evaluates COVID-19 patients only up to 8 months and requires models to
514 estimate immune response half-lives thereafter. Because our longitudinal study will
515 extend beyond two years, we can corroborate our models with subsequent experimental
516 data on the persistence of immune memory. Our study population was primarily
517 outpatients with mild to moderate COVID-19 and thus we were unable to evaluate
518 immune memory in those with the extreme presentations, both asymptomatic and
519 severe COVID-19. However, mild-moderate illness accounts for nearly all cases of
520 COVID-19 and highlights the relevance of our findings over time.

521

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535

536 **AUTHOR CONTRIBUTIONS**

537 M.J.M and R. Ahmed conceived the study. M.J.M., S.E., J.C., E.J.A., A.K.M, N.R. and
538 J.O.K. established the cohort and recruited the participants. S.L.L., M.P.L., C.W.D.,
539 M.P.G., S.G., K.A.S., G.M., C.N., V.V.E., L.L. and D.S.S. conducted serological assays
540 and related analyses. H.A., V.I.Z., B.P. and Z.M. conducted formal statistical analyses
541 and modeling. K.W.C., R.W. and L.E.N. planned, performed and analyzed antigen-
542 specific B cell flow cytometry. S.C.D., K.W.C. and S.F. conceived, supervised,
543 performed, and analyzed T cell experiments. V.E.E, K.F., and L.L. performed FRNT
544 assays. K.W.C., S.L.L. and Z.M. drafted the original manuscript; M.J.M., M.S.S. and

545 R.Ahmed edited the manuscript. All authors read and approved the manuscript. M.J.M.,

546 R.A, J.W. and M.S.S. secured funds and supervised the project.

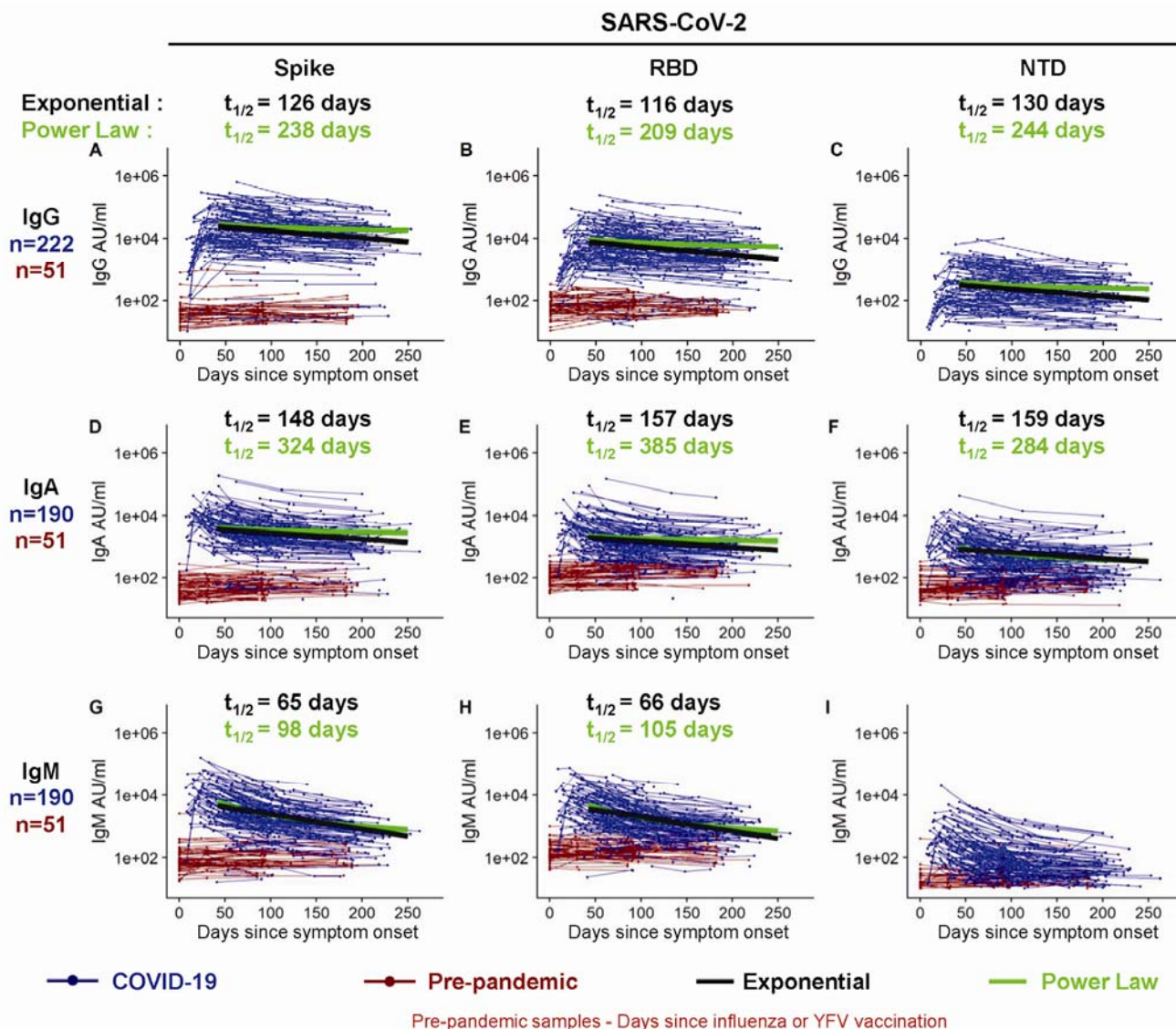
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548 **DECLARATION OF INTERESTS**

549 The authors declare no competing interests.

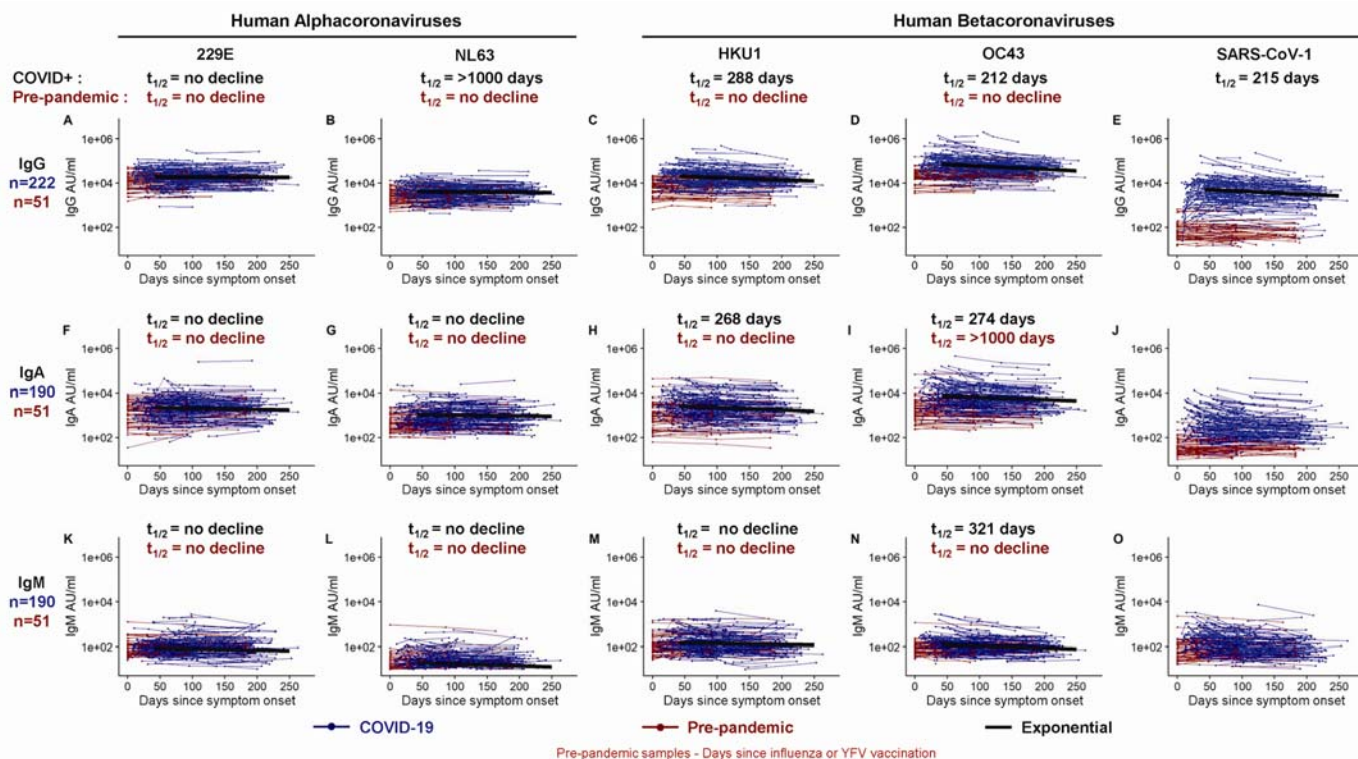
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551 **FIGURES**



552

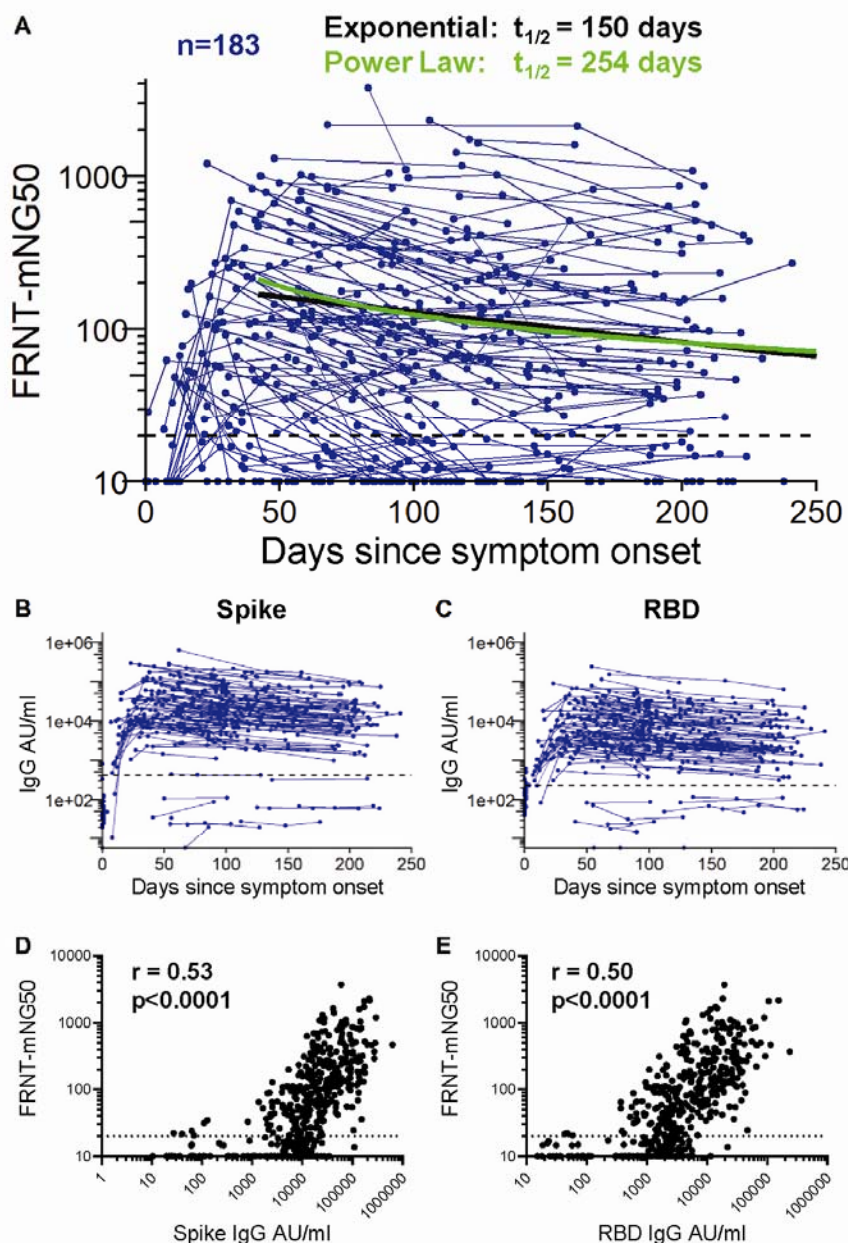
553 **Figure 1. Longitudinal SARS-CoV-2 spike binding antibody responses.** IgG (A-C), IgA (D-
554 F), and IgM (G-I) antibodies reactive to SARS-CoV-2 spike (A, D, G), spike receptor binding
555 domain (RBD; B, E, H), and the spike N terminal domain (NTD; C, F, I) were measured in
556 triplicate by an electrochemiluminescent multiplex immunoassay and reported as arbitrary units
557 per ml (AU/ml) as normalized by a standard curve. Longitudinal antibody titers of COVID-19
558 patients (in blue, n=222 COVID-19+ for IgG; n=190 COVID-19+ for IgA and for IgM) are plotted
559 over days since symptom onset, whereas longitudinal pre-pandemic donor samples (in red,
560 n=51 for IgG, IgA and IgM) were collected in the course of a non-SARS-CoV-2 vaccine study
561 before 2019 and plotted over days since immunization. IgG decay curves and half-lives
562 estimated by an exponential decay model are shown in black, and the decay curves and half-
563 lives at day 120 post symptom onset estimated by a power law model are shown in green.
564



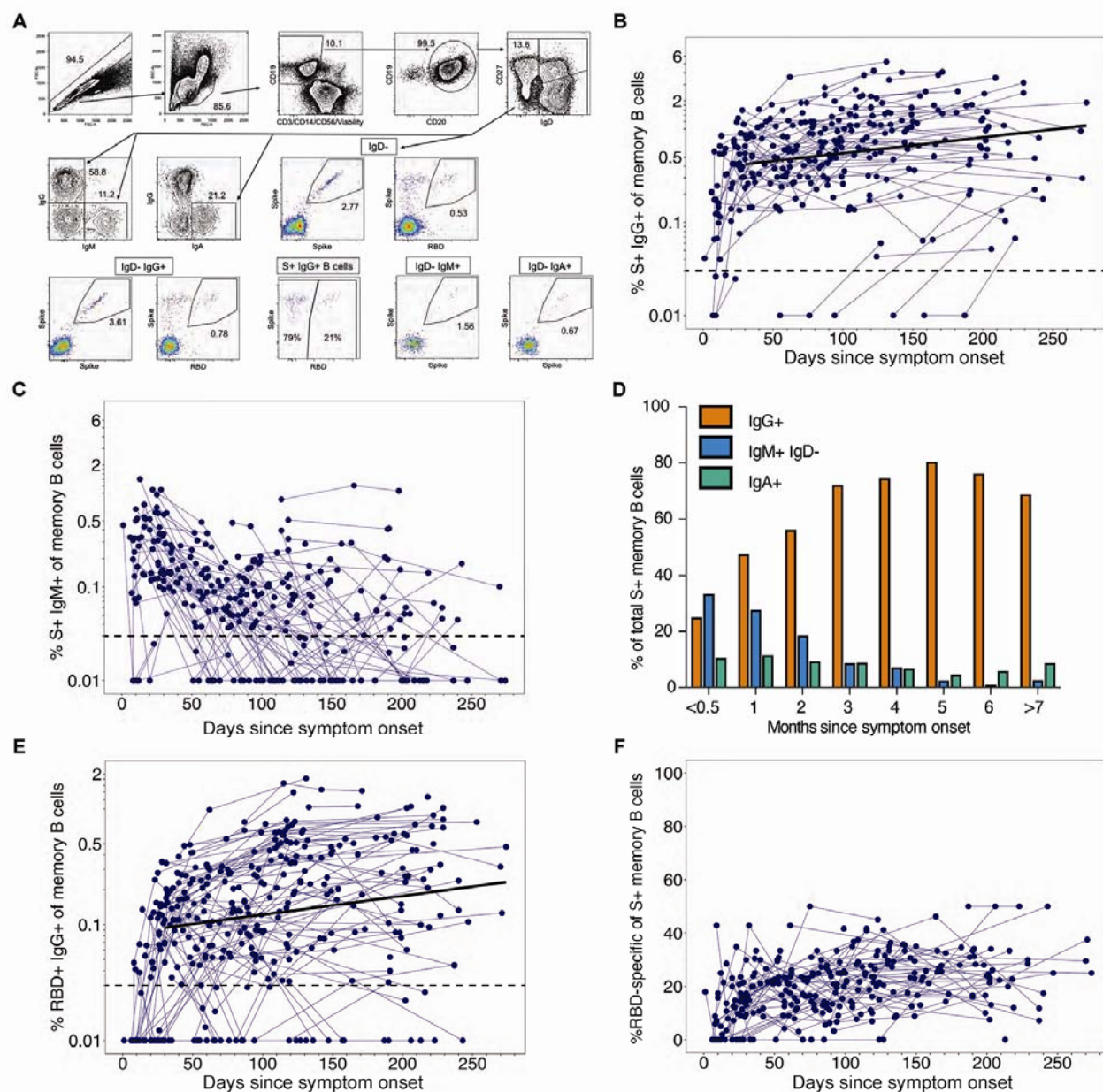
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566 **Figure 2. Longitudinal binding antibody responses to other coronavirus spike proteins.**
 567 IgG (A-E), IgA (F-J), and IgM (K-O) antibody responses in sera collected from COVID-19+
 568 patients (in blue, n=222 for IgG; n=190 for IgA and IgM) and pre-pandemic donors (in red, n=51
 569 for IgG, IgA and IgM) that were measured to 229E spike (A, F, K), NL63 spike (B, G, L), HKU1
 570 spike (C, H, M), OC43 spike (D, I, N), and the SARS-CoV-1 spike protein (E, J, O) in triplicate.
 571 Longitudinal antibody titers of COVID-19 patients are plotted over days since symptom onset,
 572 whereas longitudinal pre-pandemic donor samples were collected in the course of a non-SARS-
 573 CoV-2 vaccine study before 2019 and plotted over days since immunization. Antibody
 574 responses were measured by an electrochemiluminescent multiplex immunoassay and reported
 575 as arbitrary units per ml (AU/ml) as normalized by a standard curve. IgG decay curves and half-
 576 lives estimated by an exponential decay model are shown in black. There was no significant
 577 decline in IgG reactive to endemic alpha and betacoronaviruses in longitudinal samples
 578 collected in healthy donors before the pandemic (red, A-D).
 579

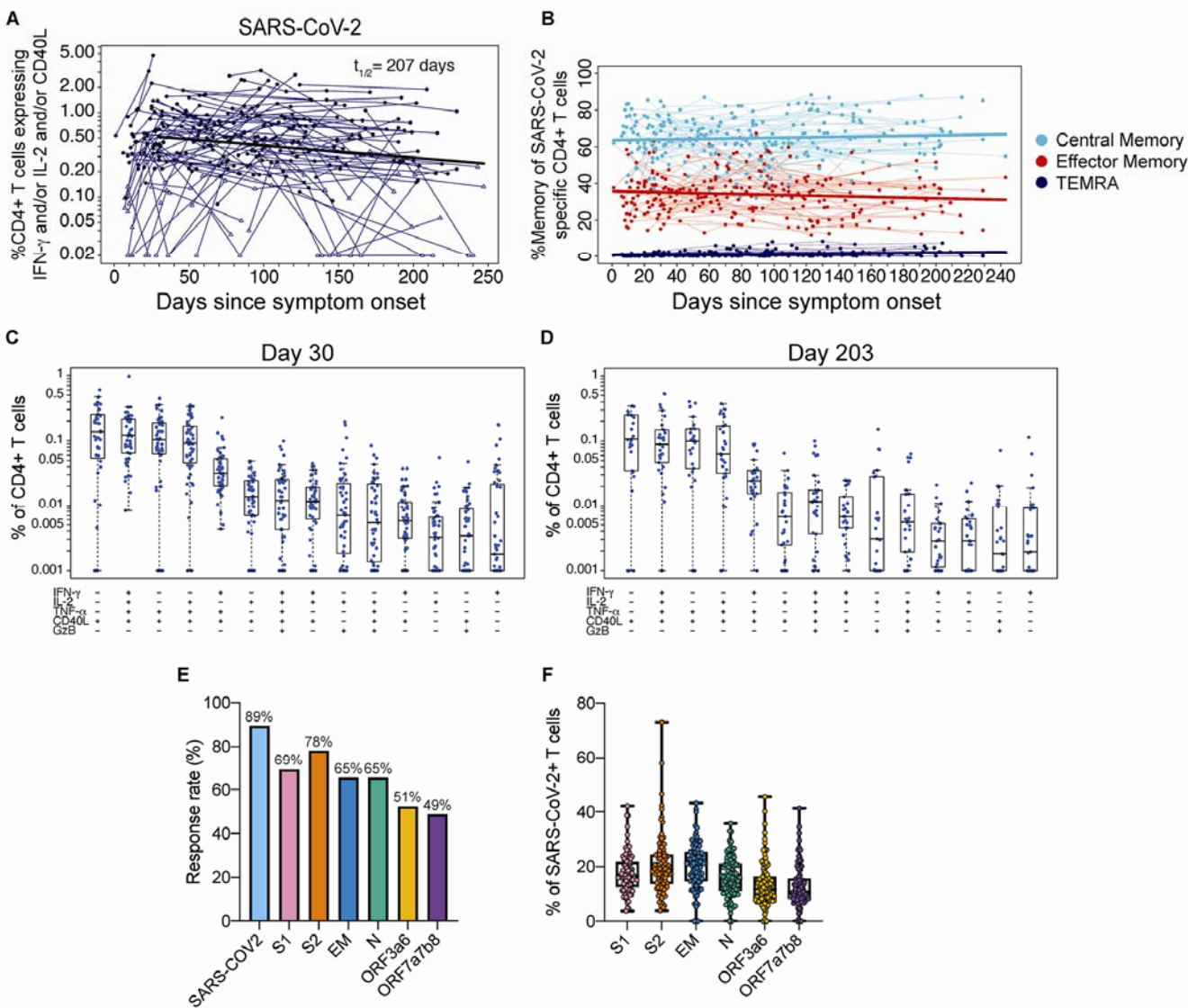
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581
582 **Figure 3. Neutralizing antibody responses to SARS-CoV-2** (A) *In vitro* serum neutralization
583 antibody titers to SAR-CoV-2 were measured in duplicate by focus-reduction neutralization
584 assay COVID-19 patients ($n=183$). The limit of detection is indicated with a dashed line at
585 $FRNT-mNG_{50} = 20$. The half-life estimated by the exponential decay model (black) is 150 days,
586 whereas the half-life estimated at day 120 using the power law model (green) is 254 days. IgG
587 antibody titers reactive to SARS-CoV-2 spike (B) and RBD (C) of the matched 183 COVID-19
588 for whom neutralization titers were assessed. The geometric mean titer plus 3 standard
589 deviations of pre-pandemic samples is indicated by a dashed line (B and C). SARS-CoV-2 spike
590 (D) and RBD (E) reactive IgG levels correlated with neutralization titers at the matched time
591 point (repeated measures correlation, $p < 0.0001$). The limit of detection is indicated with a
592 dashed line at $FRNT-mNG_{50} = 20$.

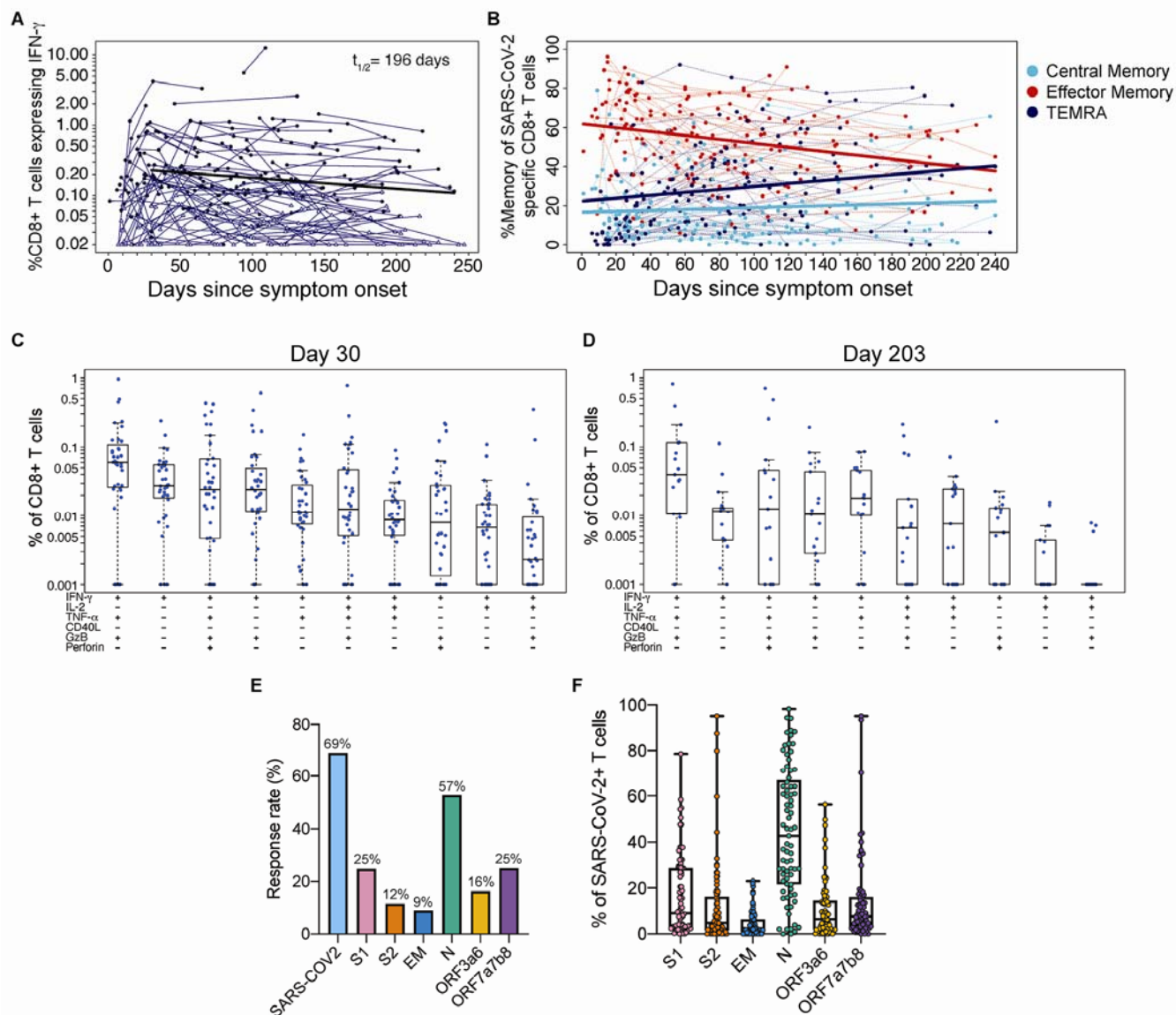


593
 594 **Figure 4. SARS-CoV-2 spike and RBD specific memory B cells.** (A) Representative memory
 595 B cell gating strategy is shown for identification of SARS-CoV-2 spike and RBD-specific IgD-
 596 IgG+, IgD- IgM+ and IgD- IgA+ memory B cells in PBMCs from a SARS-CoV-2 convalescent
 597 participant. The frequency of spike+ (B) IgG+ and (C) IgM+ memory B cells out of memory B
 598 cells (IgD- CD19+ CD20+) is displayed over time from initial symptom onset among SARS-CoV-2
 599 infected subjects (n=105 subjects; measured in singlet replicates). The dashed line indicates
 600 the limit of detection. The bold line represents the median fitted curve from a linear mixed
 601 effects model of post-day 30 responses. (D) The median percent of spike+ memory B cells
 602 expressing IgG, IgM or IgA isotypes was assessed at monthly intervals post-symptom onset. (E)
 603 The frequency of RBD+ IgG+ of memory B cells over time (n=141). (F) The proportion of S+
 604 IgG+ memory B cells that are specific for the receptor binding domain are depicted over time.



605
 606 **Figure 5. CD4+ T cell responses to SARS-CoV-2 antigens.** (A) The sum of background-
 607 subtracted CD4+ T cells expressing *ex vivo* IFN- γ , IL-2 and/or CD40L to peptide pools spanning
 608 SARS-CoV-2 structural proteins: S1, S2, envelope (E), membrane (M), nucleocapsid (N), and
 609 the following ORFs: 3a, 3b, 6, 7a, 7b, and 8 (n=114; tested in singlets) for each
 610 individual/timepoint. Each sample that is 'positive' (by MIMOSA) for at least one SARS-CoV-2
 611 antigen is indicated by a solid circle, whereas samples that are 'negative' for all of the SARS-
 612 CoV-2 antigens at that timepoint are indicated by open triangles. The bold line represents the
 613 median fitted curve from a nonlinear mixed effects model of post-day 30 responses among
 614 those with a positive response at ≥ 1 time point; $t_{1/2}$ is the median half-life estimated from the
 615 median slope, with 95%CI [104, 411]. (B) The proportion of SARS-CoV-2-specific CD4+ T cells
 616 expressing a specific memory phenotype over time: central memory (CCR7+ CD45RA-),
 617 effector memory (CCR7- CD45RA-) or T_{EMRA} (CCR7- CD45RA+); restricted to 'positive'
 618 responders. Polyfunctionality of SARS-CoV-2-specific CD4+ T cells are shown at (C) 21-60
 619 days since symptom onset (median, 30 days) and (D) >180 days median post symptom onset

620 (median, 203 days). Percentages of cytokine-expressing CD4+ T cells are background
621 subtracted and only subsets with detectable T cells are displayed. Data shown were restricted
622 to 'positive' responders and a single data point per individual per time frame. All subsets were
623 also evaluated for expression of IL-4, IL-5, IL-13, IL-17 and perforin, and were found to be
624 negative. (E) Bar graphs indicate the proportion of COVID-19 convalescent patients who had a
625 positive CD4+ T cell response to the individual SARS-CoV-2. peptide pool *ex vivo* stimulations.
626 Some antigens were combined for stimulation as indicated. (F) For each subject with positive
627 SARS-CoV-2- specific CD4+ T cells, the proportion of the total SARS-CoV-2 responding CD4+
628 T cells that are specific for each stimulation.
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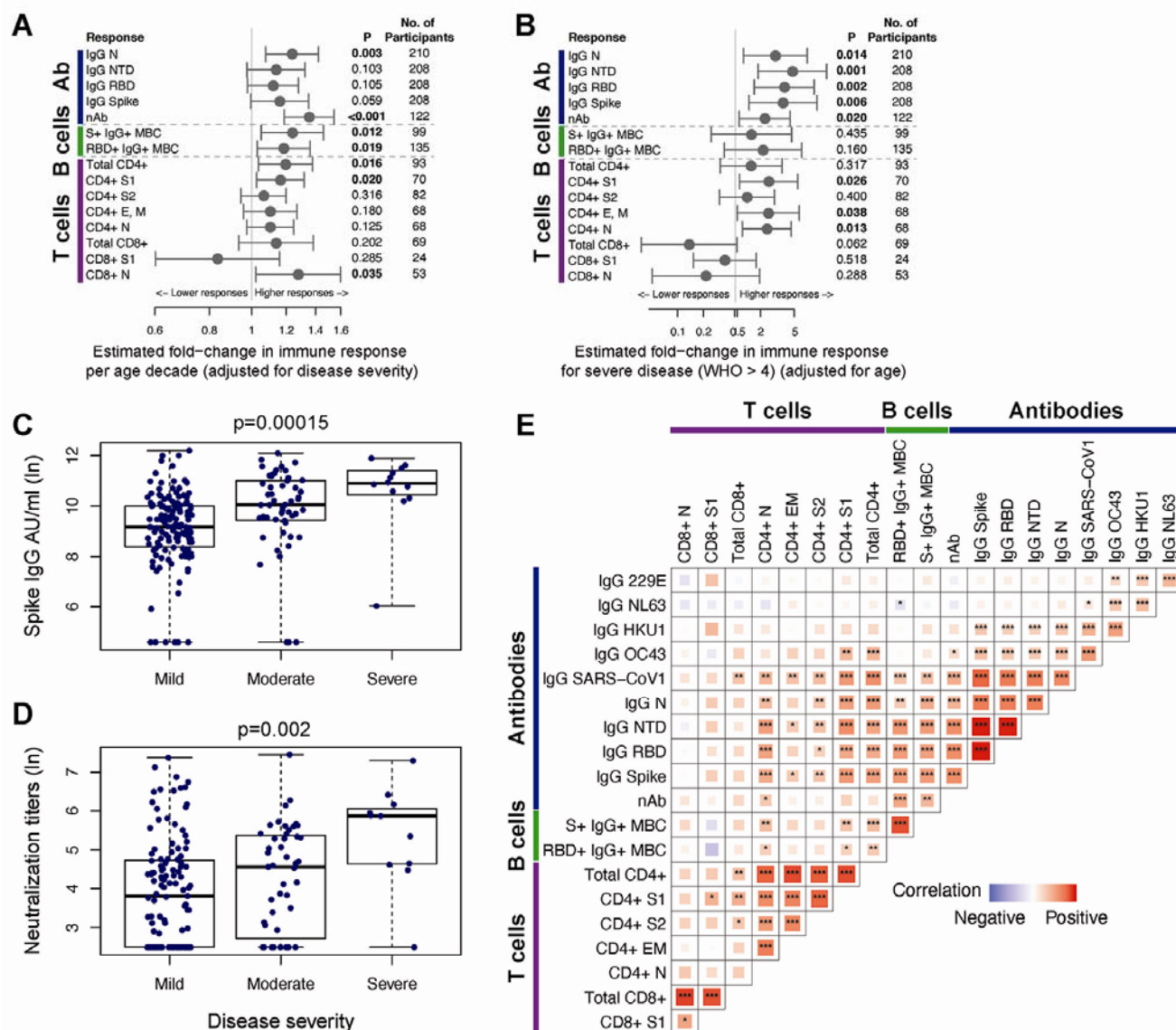


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Figure 6. CD8+ T cell responses to SARS-CoV-2 antigens.

(A) The sum of background-subtracted CD8+ T cells expressing IFN- γ (with or without other cytokines), in response to peptide pools covering SARS-CoV-2 structural proteins: S1, S2, envelope (E), membrane (M), nucleocapsid (N), and the following ORFs: 3a, 3b, 6, 7a, 7b, and 8 (n=114; tested in singlets) for each individual/timepoint. Each sample that is ‘positive’ (MIMOSA) for at least 1 SARS-CoV-2 antigen is indicated by a solid circle, whereas samples that are ‘negative’ for all of the SARS-CoV-2 antigens at that timepoint are indicated by open triangles. The bold black line represents the median fitted curve from a nonlinear mixed effects model of post-day 30 responses among those with a positive response to the antigen(s) under consideration at ≥ 1 time point; $t_{1/2}$ shown is the median half-life estimated from the median slope, with 95%CI [92, 417]. (B) The proportion of SARS-CoV-2-specific CD8+ T cells by memory phenotype over time: effector memory (EM; CCR7- CD45RA-), T_{EMRA} (CCR7- CD45RA+) and central memory (CM; CCR7+ CD45RA-). Analyses were restricted to ‘positive’

644 responders. Polyfunctionality of SARS-CoV-2-specific CD8 T cells at (C) 21-60 days post
645 symptom onset (median, 30 days) and (D) >180 days median post symptom onset (median, 203
646 days). Percentages of cytokine expressing CD8+ T cells are background subtracted and only
647 subsets with detectable T cells are displayed. Data shown were restricted to positive responders
648 and a single data point per individual per time frame. All CD8+ T cell subsets were also
649 evaluated for expression of IL-4, IL-5, IL-13, and IL-17 and were found to be negative. (E) The
650 bar graphs indicate the proportion of COVID-19 convalescent patients who had a 'positive'
651 CD8+ T cell response to the individual SARS-CoV-2 stimulations. (F) The fraction of the total
652 SARS-CoV-2 responding CD8+ T cells per subject that are specific for each peptide pool.
653



654
655 **Figure 7. Correlations between SARS-CoV-2-specific immune responses and assessment**
656 **of covariates.**

657 (A) The forest plot depicts the estimated fold-change in the level of each immune response per
658 decade of age, with 95% Wald-based CIs and p-values. (B) The forest plot shows the
659 estimated fold-change in the level of each immune response for severe (WHO score > 4) vs.
660 non-severe (WHO score ≤ 4) disease, with 95% Wald-based CIs and p-values. S1 CD8+ T cell
661 responses compared moderate-severe (WHO score > 2) to mild (WHO score ≤ 2) disease as
662 there were no participants with severe disease with at least one positive S1 CD8+ T cell
663 response post-day 30. Estimates in (A) and (B) are from mixed effects models of post-day 30 (B
664 and T cell responses) or post-day 42 (antibody responses) among responders that account for
665 fixed effects of age and disease severity on the level of immune response. Univariate
666 assessment of disease severity on the magnitude of (C) spike IgG antibodies and (D) SARS-
667 CoV-2 neutralizing antibodies at day 120 is shown for mild (WHO score: 0-2), moderate (WHO

668 score: 3-4), and severe disease (WHO score: 5+); p-values from one-way ANOVA. (E) The
669 heatmap shows Spearman correlations between critical SARS-CoV-2 memory immune
670 responses (day 30 B and T cell responses and day 180 antibody responses) with significance
671 levels: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. The tile size and color intensity correspond to the
672 absolute value of the Spearman rank correlation coefficient, with red or blue indicating a positive
673 or negative correlation, respectively. Day 30, 42 and 180 immune responses were estimated
674 from mixed effects models of the longitudinal SARS-CoV-2 binding antibodies, SARS-CoV-2
675 neutralizing antibodies, CD4+ and CD8+ T cell responses, and B cell responses.
676
677

678 RESOURCE AVAILABILITY

679 **Lead contact**

680 Further information and requests for resources and reagents should be directed to and
681 will be fulfilled by the lead contacts: M. Juliana McElrath (jmcelrat@fredhutch.org); and
682 Rafi Ahmed (rahmed@emory.edu).

683 **Materials Availability**

684 This study did not generate new unique reagents.

685 **Data and code availability**

686 All data and code reported in this paper will be shared by the lead contact upon request.

687

688 EXPERIMENTAL MODEL AND SUBJECT DETAILS

689 **Study Populations**

690 Two longitudinal COVID-19 cohort studies at Fred Hutchinson Cancer Research
691 Center (Seattle, Washington) and Emory University (Atlanta, Georgia) began after
692 receiving institutional review board approvals (IRB 10440, IRB 00001080 and
693 IRB00022371). Adults ≥ 18 years were enrolled who met eligibility criteria for SARS-
694 CoV-2 infection and provided informed consent. Study participants provided medical
695 history of co-morbidities, presentation of SARS-CoV-2 infection onset and disease
696 course, and peripheral blood at initial and follow up visits for analysis of serum antibody
697 and cellular immune responses. Additional longitudinal archived sera and PBMC from
698 pre-pandemic study populations from Emory and Seattle served as controls for the
699 immune assays.

700 The Atlanta study population included adult volunteers over the age of 18 who were
701 diagnosed with COVID-19 by a commercially available SARS CoV-2 PCR assay, rapid
702 antigen test, or clinical syndrome only (later confirmed with serology) due to limited
703 SARS-CoV-2 testing during the early period of the pandemic. Ambulatory participants
704 were recruited through local advertisements, internet-based avenues (such as social
705 media, listserves), COVID-19 testing sites, and primary care clinics. Hospitalized
706 patients were identified through SARS-CoV-2 testing. Informed consent was obtained
707 from all participants prior to conduct of study procedures. Initial acute peripheral blood
708 samples were collected from hospitalized patients at the time of enrollment.
709 Convalescent samples from hospitalized patients were collected when the patients were
710 able to return for a visit to the clinical research site at the next study visit. Serial
711 peripheral blood samples were collected starting at about 30 days after the onset of
712 COVID-19 symptoms and/or after PCR positivity for SARS-CoV-2. Thereafter, samples
713 were collected at 3, 6, and 9 months. The study is ongoing with expected completion of
714 sample collection from participants in February 2023. Participants were excluded if they
715 were immunocompromised, HIV positive, had active hepatitis B or C virus infection,
716 used immunosuppressive drugs for 2 weeks or more in the preceding 3 months,
717 received blood products or immune globulin 42 days prior to enrollment, received
718 convalescent COVID-19 plasma, or were pregnant or breast feeding. We report on 110
719 participants to date, of which 73% were diagnosed by SARS-CoV-2 PCR, the remaining
720 were diagnosed by rapid antigen test or serology. Demographic features of the
721 participants are as follows: median age was 48; 45% were male; the majority (80%)

722 were white, 11% Black/African American, 6% Asian, and 8% were Hispanic/Latinx
723 ethnicity. The most frequent co-morbid conditions were hypertension, obesity, heart
724 disease and diabetes mellitus. The most frequent COVID-19 symptoms were
725 myalgia/fatigue, fever, cough, headache, loss of smell and taste (Table S1).
726 Hospitalized patients were older, with a median age of 56; a higher percentage were
727 Black/African American (27%); and 100% had fever.

728 Longitudinal pre-pandemic sera samples from Emory were collected from
729 individuals participating in a yellow fever vaccine study from 2014-2016 or an influenza
730 vaccine study from 2015-2018^{15,30}. Data were included for analysis of binding antibody
731 responses and are presented as days post-irrelevant (yellow fever) vaccination.

732 The Seattle COVID-19 Cohort study participants were recruited from the Seattle
733 metropolitan area by social media advertisements, partnership with the local emergency
734 medical service and by word of mouth. Study participants were screened and enrolled
735 by the Seattle Vaccine Trials Unit staff. Eligibility criteria included adults at risk for
736 SARS-CoV-2 infection or those diagnosed with COVID-19 by a commercially available
737 SARS-CoV-2 PCR assay or blood antibody test and willing to have at least four blood
738 draws collected over one year. Exclusion criteria included pregnancy and inability to
739 donate blood.

740 Informed electronic consent was obtained from all Seattle participants during a
741 screening phone call with study clinical staff. Interested participants were screened,
742 consented and medical history and COVID-19 illness onset date and symptoms
743 collected. Participants undiagnosed with COVID-19 had a nasopharyngeal (NP) swab

744 collected and tested for SARS-CoV-2 via an FDA-approved PCR test and blood was
745 collected for SARS-CoV-2 antibody (Abbott) and study assays. Those with either a
746 positive PCR or antibody test were asked to return for future blood draws. Those who
747 tested negative were asked to return as controls for the positive cohort and in case they
748 tested positive in the future. Participants with a positive test prior to study enrollment or
749 those diagnosed in study were asked to provide blood donation at approximately 7
750 days, 2 weeks, 1, 2, 3, 4, 6, 9- and 12-months post symptom onset. After completing
751 one year of study, participants will be given the option of continuing the longitudinal
752 study for up to two or more years. At each study visit, participant symptoms and medical
753 history is updated. Those with COVID-19 symptoms after enrollment in all groups are
754 offered a nasopharyngeal swab PCR SARS-CoV-2 test.

755 As of October 2020, 805 individuals have contacted the Seattle COVID-19 cohort
756 study and 425 have enrolled. This includes 281 negative and 144 SARS-CoV-2 positive
757 participants. Reasons for not enrolling include lack of interest, not meeting the eligibility
758 criteria, inability to travel to blood draw location and inability to collect study blood. No
759 participants have terminated from the study. Study enrollment and follow-up remains
760 ongoing. Samples from SARS-CoV-2 negative subjects were included in B and T cell
761 assays as ‘contemporaneous’ negative controls.

762 Peripheral blood mononuclear cells (PBMC) were obtained from HIV-1 seronegative
763 donors who were recruited at the Seattle Vaccine Trials Unit before 2019 as part of the
764 study “Establishing Immunologic Assays for Determining HIV-1 Prevention and Control”.
765 All participants signed informed consent, and the Fred Hutchinson Cancer Research

766 Center IRB (Seattle, WA, USA) institutional human subjects review committee approved
767 the protocol prior to study initiation. Pre-pandemic samples from this cohort were used
768 as assay controls in B and T cell assays.

769

770 METHOD DETAILS

771 **PBMC processing**

772 PBMC for cellular assays were isolated by density centrifugation and cryopreserved
773 from ACD-anticoagulated whole blood within eight hours of venipuncture, as described
774 previously³¹. Sera were also processed and cryopreserved within 4 hours after
775 collection.

776

777 **Antibody binding assay**

778 Antibody binding titers were measured using a multiplex plate coated with the SARS-
779 CoV-2 spike, SARS-CoV-2 spike receptor binding domain, SARS-CoV-2 spike N
780 terminal domain, SARS-CoV-2 nucleocapsid, SARS-CoV-1 spike, 229E spike, NL63
781 spike, HKU1 spike, and OC43 spike proteins (Mesoscale Discovery). Plates were
782 blocked with 150µl/well with 5% bovine serum albumin in phosphate buffered saline
783 (PBS) and shaken at 700 RPM at room temperature for at least 30 min. Plates were
784 washed 3 times with 150µl/well 0.05% Tween-20 in PBS. Serum and plasma samples
785 were added to the plate at dilutions between 1:500 and 1:50,000 and shaken at 700
786 RPM at room temperature for 2 hr. Following a wash, plates were incubated with
787 50ul/well of Sulfo-Tag anti-human IgG, IgA, or IgM detection antibody and shaken at

788 700RPM at room temperature for 1 hr. After a subsequent wash, 150µl/well of MSD
789 GOLD read buffer was added to the plate and plates were immediately read on the
790 MSD instrument to measure light intensity. Antibody levels are reported as arbitrary
791 units/ml (AU/ml) based on normalization to a standard curve.

792

793 **Viruses and cells**

794 VeroE6 cells were obtained from ATCC (clone E6, ATCC, #CRL-1586) and cultured in
795 complete DMEM medium consisting of 1× DMEM (VWR, #45000-304), 10% FBS,
796 25mM HEPES Buffer (Corning Cellgro), 2mM L-glutamine, 1mM sodium pyruvate, 1×
797 Non-essential Amino Acids, and 1× antibiotics. The infectious clone SARS-CoV-2
798 (icSARS-CoV-2-mNG), derived from the 2019-nCoV/USA_WA1/2020 strain, was
799 propagated in VeroE6 cells (ATCC) and sequenced ³².

800

801 **Focus reduction neutralization test**

802 Neutralization assays with SARS-CoV-2 virus were performed as previously described
803 ³²⁻³⁴. Plasma/serum were serially diluted (three-fold) in serum-free Dulbecco's modified
804 Eagle's medium (DMEM) in duplicate wells and incubated with 100–200 FFU infectious
805 clone derived SARS-CoV-2-mNG virus at 37°C for 1 hr ³². The antibody-virus mixture
806 was added to VeroE6 cell (C1008, ATCC, #CRL-1586) monolayers seeded in 96-well
807 blackout plates and incubated at 37°C for 1 hr. Post-incubation, the inoculum was
808 removed and replaced with pre-warmed complete DMEM containing 0.85%
809 methylcellulose. Plates were incubated at 37°C for 24 hr. After 24 hr, methylcellulose

810 overlay was removed, cells were washed twice with PBS and fixed with 2%
811 paraformaldehyde in PBS for 30 min at room temperature. Following fixation, plates
812 were washed twice with PBS and foci were visualized on a fluorescence ELISPOT
813 reader (CTL ImmunoSpot S6 Universal Analyzer) and enumerated using Viridot³⁵. The
814 neutralization titers were calculated as follows: $1 - (\text{ratio of the mean number of foci in}$
815 $\text{the presence of sera and foci at the highest dilution of respective sera sample})$. Each
816 specimen was tested in two independent assays performed at different times. The
817 FRNT-mNG₅₀ titers were interpolated using a 4-parameter nonlinear regression in
818 GraphPad Prism 8.4.3. Samples with an FRNT-mNG₅₀ value that was below the limit of
819 detection were plotted at 20.

820

821 **Spike and RBD memory B cell flow cytometry assays**

822 Fluorescent SARS-CoV-2-specific S6P³⁶ (provided by Roland Strong, Fred Hutchinson
823 Cancer Research Center, Seattle, WA) and RBD (provided by Leonidas Stamatatos,
824 Fred Hutchinson Cancer Research Center, Seattle, WA) probes were made by
825 combining biotinylated protein with fluorescently labeled streptavidin (SA). The S6P
826 probes were made at a ratio of 1:1 molar ratio of trimer to SA. Two S6P probes, one
827 labeled with AlexaFluor488 (Invitrogen), one labeled with AlexaFluor647 (Invitrogen),
828 were used in this panel in order to increase specificity of the detection of SARS-CoV-2-
829 specific B cells. The RBD probe was prepared at a 4:1 molar ratio of RBD monomers to
830 SA, labeled with R-phycoerythrin (Invitrogen). Cryopreserved PBMCs from SARS-CoV-
831 2-convalescent participants and a pre-pandemic SARS-CoV-2-naïve donor were

832 thawed at 37°C and stained for SARS-CoV-2-specific memory B cells as described
833 previously¹⁹ with a panel of fluorescently-labeled antibodies (see Key Resource Table).
834 Cells were stained first with the viability stain (Invitrogen) in PBS for 15 min at 4°C. Cells
835 were then washed with 2% FBS/PBS and stained with a cocktail of the three probes for
836 30 min at 4°C. The probe cocktail was washed off with 2% FBS/PBS and the samples
837 were stained with the remaining antibody panel and incubated for 25 min at 4°C. The
838 cells were washed two times and resuspended in 1% paraformaldehyde/1× PBS for
839 collection on a LSR II or FACSymphony flow cytometer (BD Biosciences). Data was
840 analyzed in Flow Jo version 9.9.4.

841

842 **Intracellular cytokine staining (ICS) assay**

843 Flow cytometry was used to examine SARS-CoV-2-specific CD4+ and CD8+ T-cell
844 responses using a validated ICS assay. The assay was similar to a published report
845 ^{5,37,38} and the details of the staining panel are included in the Key Resource Table.
846 Peptide pools covering the structural proteins of SARS-CoV-2 were used for the six-
847 hour stimulation. Peptides matching the SARS-CoV-2 spike sequence (316 peptides,
848 plus 4 peptides covering the G614 variant) were synthesized as 15 amino acids long
849 with 11 amino acids overlap and pooled in 2 pools (S1 and S2) for testing
850 (BioSynthesis). All other peptides were 13 amino acids overlapping by 11 amino acids
851 and were synthesized by GenScript. The peptides covering the envelope (E),
852 membrane (M) and nucleocapsid (N) were initially combined into one peptide pool, but
853 the majority of the assays were performed using a separate pool for N and one that

854 combined only E and M. Several of the open reading frame (ORF) peptides were
855 combined into two pools: ORF 3a and 6, and ORF 7a, 7b and 8. All peptide pools were
856 used at a final concentration of 1 µg/ml for each peptide. As a negative control, cells
857 were not stimulated, only the peptide diluent (DMSO) was included. As a positive
858 control, cells were stimulated with a polyclonal stimulant, staphylococcal enterotoxin B
859 (SEB). Cells expressing IFN-γ and/or IL-2 and/or CD154 was the primary
860 immunogenicity endpoint for CD4+ T cells and cells expressing IFN-γ was the primary
861 immunogenicity endpoint for CD8+ T cells. The overall response to SARS-CoV-2 was
862 defined as the sum of the background-subtracted responses to each of the individual
863 pools. A sample was considered positive for CD4+ or CD8+ T cell responses to SARS-
864 CoV-2 if any of the CD4+ or CD8+ T cell responses to the individual peptide pool
865 stimulations was positive. Positivity was determined using MIMOSA³⁹. The total number
866 of CD4+ T cells must have exceeded 10,000 and the total number of CD8+ T cells must
867 have exceeded 5,000 for the assay data to be included in the analysis.

868

869 QUANTIFICATION AND STATISTICAL ANALYSIS

870 *Binding and neutralizing antibody responses*

871 Mixed effects exponential and power law models were used to analyze waning of
872 antibody (day 42 to day 263 post symptom onset). For binding antibody analyses,
873 antibody (Ab) was natural log transformed, yielding linear equations of the form
874 $\ln(\text{Ab})=a+b*(\text{day}-42)$ and $\ln(\text{Ab})=a+b*\ln(\text{day}/42)$ for the exponential and power law
875 models, respectively, and fit using the lmer function (lme4 package) in R. Models

876 included population level fixed effects and individual level random effects for intercept
877 and slope and covariance between the random effects. Simplified models – with random
878 effects only for intercept – were also fit. Neutralization antibody data were analyzed in
879 Monolix (Lixoft). For analysis in Monolix, the exponential and power law models were
880 formulated as ordinary differential equations, $dAb/dt=k*Ab$ and $dAb/dt=k*Ab/t$,
881 respectively, with antibody at day 42 lognormally distributed and lognormal multiplicative
882 error. Neutralization titers < 20 were treated as left censored. For comparison of
883 models, difference in Akaike information criterion (ΔAIC) > 4 was considered statistically
884 significant. Models (in R and Monolix) were fit using maximum likelihood. To account for
885 repeated measures, correlations between antibody binding levels and neutralization
886 titers were calculated using a repeated measures correlation (rmcorr package) in R⁴⁰.

887

888 *B cell responses*

889 We considered linear mixed effects models for B cell response, \mathcal{Y}_{ij} , as a function of t_{ij} ,
890 the j^{th} time since symptom onset for the i^{th} individual, with random effects for intercept
891 and slope and $t_{ij} > 30$ days for all i, j :

$$\log_e \mathcal{Y}_{ij} = \beta_{0i} + \beta_{1i} t_{ij} + \epsilon_{ij}$$

892 where $\beta_{0i} = \beta_0 + b_i$ and $\beta_{1i} = \beta_1 + c_i$ with (b_i, c_i) iid $\sim N_2(\mathbf{0}, \Sigma)$, with

893

$$\Sigma = \begin{bmatrix} \sigma_b^2 & Cov(b, c) \\ Cov(b, c) & \sigma_c^2 \end{bmatrix}$$

894 and σ_b^2 and σ_c^2 are the between-person variation in the intercept and slope of log B cell
895 responses respectively, $Cov(b, c)$ is the covariance between the intercept and slope,
896 and ϵ_{ij} iid $\sim N(0, \sigma^2)$. The random effects, b_i and c_i , are each assumed to be
897 independent for different individuals and the within-individual errors ϵ_{ij} are assumed to
898 be independent for different i, j and to be independent of the random effects. The
899 function `lme` from the R package `nlme` was used to fit the models.

900

901 *T cell responses*

902 Longitudinal analyses of CD4+ and CD8+ T cell responses were performed for
903 individuals with a positive response for at least one time point 30 days after symptom
904 onset. The MIMOSA (Mixture Models for Single-Cell Assays)³⁹ model incorporated cell
905 count and cell proportion information to define a positive CD4+/CD8+ T cell response by
906 ICS by comparing peptide pools stimulated cells and unstimulated negative controls.
907 This method assumed a common distribution for cytokine positive CD4+/CD8+ T cells in
908 stimulated and unstimulated samples in non-responders, resulting in paired differences
909 that were zero on average. In contrast, for responders, the distribution of the proportion
910 of cytokine positive cells for stimulated samples was assumed to be greater than for
911 unstimulated samples, resulting in paired differences that were greater than zero on
912 average. The MIMOSA method modeled this structure through a Bayesian hierarchical
913 mixture model framework. One component (or distribution) of the model represented the
914 responders, and the other component modeled the non-responders. The parameters
915 defining these distributions, as well as the probabilities that each ICS response was

916 either a responder or non-responder, were estimated from the observed data. This
917 sharing of information across SARS-CoV-2 responders and non-responders increased
918 the sensitivity and specificity to make positivity calls⁴¹. Responses with probability of
919 response > 0.999 were considered positive responders.
920 We considered nonlinear mixed effects models for T cell response, \mathcal{Y}_{ij} , as a function of
921 t_{ij} , the j^{th} time since symptom onset for the i^{th} individual, with random effects for
922 intercept and slope and $t_{ij} > 30$ days for all i, j :

$$\log_e \mathcal{Y}_{ij} = \beta_{0i} - \exp(\beta_{1i}) t_{ij} + \epsilon_{ij}$$

923 where $\beta_{0i} = \beta_0 + b_i$ and $\exp(\beta_{1i}) = \exp(\beta_1 + c_i)$ with (b_i, c_i) iid $\sim N_2(\mathbf{0}, \Sigma)$, with

$$\Sigma = \begin{bmatrix} \sigma_b^2 & 0 \\ 0 & \sigma_c^2 \end{bmatrix}$$

924 and σ_b^2 and σ_c^2 are the between-person variation in the intercept and slope of log T cell
925 responses respectively, and ϵ_{ij} iid $\sim \logNormal(0, \sigma^2)$. The random effects, b_i and c_i ,
926 are each assumed to be independent for different individuals and the within-individual
927 errors ϵ_{ij} are assumed to be independent for different i, j and to be independent of the
928 random effects. The function nlme from the R package nlme was used to fit the models.
929 Diagnostic plots of residuals were examined to assess validity of the model
930 assumptions.

931 Age at enrollment, gender, and disease severity (WHO score > 4) were included as
932 covariates in the mixed effects models to assess their association with each immune
933 response.

934

935 Individual-level estimates at days 30 (T and B cell responses), day 42 (binding and
936 neutralizing antibody responses) and day 180 (all responses) were obtained from the
937 mixed effects models described above. Spearman rank correlations, Wald-based two-
938 sided 95% confidence intervals and p-values were reported.

939 Generalized estimating equations (GEE), with an independence working covariance
940 matrix, were used to confirm the results of the covariate assessments for B and T cell
941 responses from the mixed effects models. Two-tailed P-values based on the robust
942 standard error estimates for the covariate coefficients were consistent with the
943 corresponding two-tailed P-values for the covariate associations from the mixed effects
944 models.

945 All tests were two-sided and P-values < 0.05 were considered statistically significant
946 unless otherwise noted. Details of specific statistical analyses can be found in the
947 Results section and in the Figure legends.

948

949 KEY RESOURCES TABLE

950

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Mouse Anti-Human CD3/BV510	BD Biosciences	564713; RRID:AB_2738909
Mouse Anti-Human CD14/BV510	BD Biosciences	563079; RRID:AB_2737993
Mouse Anti-Human CD56/BV510	BD Biosciences	563041; RRID:AB_2732786
Mouse Anti-Human CD19/BUV395	BD Biosciences	563549; RRID:AB_2738272
Mouse Anti-Human CD20/BUV737	BD Biosciences	612849; RRID:AB_2870169
Mouse Anti-Human CD21/PE-Cy7	BD Biosciences	561374; RRID:AB_10681717
Mouse Anti-Human CD27/BV605	BD Biosciences	302830; RRID: AB_2561450
Mouse Anti-Human CD38/BB700	BioLegend	566445; RRID:AB_2744375
Mouse Anti-Human IgA/VioBlue	Miltenyi Biotec	130-114-005; RRID:AB_2733958
Mouse Anti-Human IgD/BV650	BD Biosciences	740594; RRID:AB_2740295
Mouse Anti-Human IgG/BV786	BD Biosciences	564230; RRID:AB_2738684
Mouse Anti-Human IgM/PE-Dazzle 594	BioLegend	314530; RRID:AB_2566483

Streptavidin (PE)	Invitrogen	S21388
Streptavidin (AF488)	Invitrogen	S32354; RRID:AB_2315383
Streptavidin (AF647)	Invitrogen	S32357
Live/Dead Fixable Aqua Stain	Invitrogen	L34957
Fixable Viability Dye/eFluor 450	Invitrogen	65-0863
Mouse Anti-Human CD14/BUV661	BD Biosciences	741684; RRID:AB_2868407
Mouse Anti-Human CD19/BUV563	BD Biosciences	612916; RRID:AB_2870201
Mouse Anti-Human CD16/BV570	BioLegend	302036; RRID:AB_2632790
Mouse Anti-Human CD56/BV750	BioLegend	362556; RRID:AB_2801001
Mouse Anti-Human CD3/APC-Fire750	BioLegend	300470; RRID:AB_2629689
Mouse Anti-Human CD4/BV480	BD Biosciences	566104; RRID:AB_2739506
Mouse Anti-Human CD8/BUV805	BD Biosciences	612889; RRID:AB_2833078
Mouse Anti-Human CD197(CCR7)/BV605	BioLegend	353224; RRID:AB_2561753
Mouse Anti-Human CD45RA/BUV496	BD Biosciences	750258; RRID:AB_2874456
Mouse Anti-Human CD25/BV650	BD Biosciences	563719; RRID: AB2744337
Rat Anti-Human FOXP3/PE-Cy5.5	Invitrogen	35-4776-42; RRID:AB_11218682
Mouse Anti-Human CD32/PE-Dazzle	BioLegend	303218; RRID:AB_2716072
Mouse Anti-Human CD65/BV711	BioLegend	305042; RRID:AB_2800778
Mouse Anti-Human CD183/PE-Cy5	BD Biosciences	551128; RRID:AB_394061
Mouse Anti-Human CD196 (CCR6)/BV786	BD Biosciences	563704; RRID:AB_2738381
Rat Anti-Human CD294 (CRTH2)/PE	BioLegend	350106; RRID:AB_10900060
Mouse Anti-Human IFN-g/V450	BD Biosciences	560371; RRID:AB_1645594
Rat Anti-Human IL-2/APC	BioLegend	500310; RRID:AB_315097
Mouse Anti-Human TNF/BUV395	BD Biosciences	563996; RRID:AB_2738533
Mouse Anti-Human IL-17A/PE-Cy7	BioLegend	512315; RRID:AB_2295923
Rat Anti-Human IL-4/BB700	BD Biosciences	Custom
Rat Anti-Human/Anti-Mouse IL-5/BB630	BD Biosciences	Custom
Rat Anti-Human IL-13/BV421	BD Biosciences	Custom
Mouse Anti-Human CD154 (BUV737)	BD Biosciences	748983; RRID:AB_2873383
Mouse Anti-Human Granzyme B/AF700	BD Biosciences	560213; RRID:AB_1645453
Mouse Anti-Human Perforin/FITC	BD Biosciences	353310; RRID:AB_2571967
Mouse Anti-Human Ki-67/BB660	BD Biosciences	Custom
Bacterial and virus strains		
icSARS-CoV-2-mNG	Xie et al.	N/A
Chemicals, peptides, and recombinant proteins		
SARS-CoV-2 Spike peptides	Biosynthesis	Custom
SARS-CoV-2 E, M, N and ORF peptides	Genscript	Custom
SARS-CoV-2 Spike protein (S6P)	Fred Hutchinson Cancer Research Center	Custom
SARS-CoV-2 RBD protein	Fred Hutchinson Cancer Research Center	Custom
Methylcellulose	Sigma-Aldrich	M0512-250G
TrueBlue Peroxidase Substrate	KPL	5510-0050
Critical commercial assays		

V-PLEX COVID-19 Coronavirus Panel 2 (IgG) Kit	Meso Scale Discovery	K15369U
V-PLEX COVID-19 Coronavirus Panel 2 (IgA) Kit	Meso Scale Discovery	K15371U
V-PLEX COVID-19 Coronavirus Panel 2 (IgM) Kit	Meso Scale Discovery	K15370U
Experimental models: Cell lines		
VeroE6 C1008 cells	ATCC	Cat# CRL-1586; RRID:CVCL_0574
Software and algorithms		
FlowJo	BD Biosciences	V9.9.4
R	R Foundation for Statistical Computing	V3.6.1
GraphPad Prism	GraphPad	V7, 8 and 9
Viridot	Katzelnick et al.	https://github.com/leahkatzelnick/Viridot
Monolix	Lixoft	MonolixSuite2019R1
Other		
ELISPOT reader	Immunospot	CTL ImmunoSpot S6 Universal Analyzer

951

952

953 References

- 954 1. Sette, A., and Crotty, S. (2021). Adaptive immunity to SARS-CoV-2 and COVID-
955 19. *Cell* 184, 861-880. [10.1016/j.cell.2021.01.007](https://doi.org/10.1016/j.cell.2021.01.007).
- 956 2. Stephens, D.S., and McElrath, M.J. (2020). COVID-19 and the Path to Immunity.
957 *JAMA* 324, 1279-1281. [10.1001/jama.2020.16656](https://doi.org/10.1001/jama.2020.16656).
- 958 3. Doria-Rose, N., Suthar, M.S., Makowski, M., O'Connell, S., McDermott, A.B.,
959 Flach, B., Ledgerwood, J.E., Mascola, J.R., Graham, B.S., Lin, B.C., et al.
960 (2021). Antibody Persistence through 6 Months after the Second Dose of mRNA-
961 1273 Vaccine for Covid-19. *N Engl J Med*. [10.1056/NEJMc2103916](https://doi.org/10.1056/NEJMc2103916).
- 962 4. Anderson, E.J., Roupheal, N.G., Widge, A.T., Jackson, L.A., Roberts, P.C.,
963 Makhene, M., Chappell, J.D., Denison, M.R., Stevens, L.J., Pruijssers, A.J., et al.
964 (2020). Safety and Immunogenicity of SARS-CoV-2 mRNA-1273 Vaccine in
965 Older Adults. *N Engl J Med* 383, 2427-2438. [10.1056/NEJMoa2028436](https://doi.org/10.1056/NEJMoa2028436).
- 966 5. Sadoff, J., Le Gars, M., Shukarev, G., Heerwegh, D., Truyers, C., de Groot, A.M.,
967 Stoop, J., Tete, S., Van Damme, W., Leroux-Roels, I., et al. (2021). Interim
968 Results of a Phase 1-2a Trial of Ad26.COV2.S Covid-19 Vaccine. *N Engl J Med*
969 384, 1824-1835. [10.1056/NEJMoa2034201](https://doi.org/10.1056/NEJMoa2034201).
- 970 6. Callow, K.A., Parry, H.F., Sergeant, M., and Tyrrell, D.A. (1990). The time course
971 of the immune response to experimental coronavirus infection of man. *Epidemiol
972 Infect* 105, 435-446. [10.1017/s0950268800048019](https://doi.org/10.1017/s0950268800048019).
- 973 7. Edridge, A.W.D., Kaczorowska, J., Hoste, A.C.R., Bakker, M., Klein, M., Loens,
974 K., Jebbink, M.F., Matser, A., Kinsella, C.M., Rueda, P., et al. (2020). Seasonal
975 coronavirus protective immunity is short-lasting. *Nat Med* 26, 1691-1693.
976 [10.1038/s41591-020-1083-1](https://doi.org/10.1038/s41591-020-1083-1).
- 977 8. Lavine, J.S., Bjornstad, O.N., and Antia, R. (2021). Immunological characteristics
978 govern the transition of COVID-19 to endemicity. *Science* 371, 741-745.
979 [10.1126/science.abe6522](https://doi.org/10.1126/science.abe6522).
- 980 9. Slifka, M.K., Antia, R., Whitmire, J.K., and Ahmed, R. (1998). Humoral Immunity
981 Due to Long-Lived Plasma Cells. *Immunity* 8, 363-372. [10.1016/s1074-
982 7613\(00\)80541-5](https://doi.org/10.1016/s1074-7613(00)80541-5).
- 983 10. Hammarlund, E., Lewis, M.W., Hansen, S.G., Strelow, L.I., Nelson, J.A., Sexton,
984 G.J., Hanifin, J.M., and Slifka, M.K. (2003). Duration of antiviral immunity after
985 smallpox vaccination. *Nat Med* 9, 1131-1137. [10.1038/nm917](https://doi.org/10.1038/nm917).
- 986 11. Manz, R.A., Thiel, A., and Radbruch, A. (1997). Lifetime of plasma cells in the
987 bone marrow. *Nature* 388, 133-134. [Doi 10.1038/40540](https://doi.org/10.1038/40540).
- 988 12. Amanna, I.J., Carlson, N.E., and Slifka, M.K. (2007). Duration of humoral
989 immunity to common viral and vaccine antigens. *N Engl J Med* 357, 1903-1915.
990 [10.1056/NEJMoa066092](https://doi.org/10.1056/NEJMoa066092).
- 991 13. Davis, C.W., Jackson, K.J.L., McCausland, M.M., Darce, J., Chang, C.,
992 Linderman, S.L., Chennareddy, C., Gerkin, R., Brown, S.J., Wrammert, J., et al.
993 (2020). Influenza vaccine-induced human bone marrow plasma cells decline
994 within a year after vaccination. *Science* 370, 237-241. [10.1126/science.aaz8432](https://doi.org/10.1126/science.aaz8432).

- 995 14. Ellis, P., Somogyvari, F., Virok, D.P., Nosedá, M., and McLean, G.R. (2021).
996 Decoding Covid-19 with the SARS-CoV-2 Genome. *Curr Genet Med Rep*, 1-12.
997 10.1007/s40142-020-00197-5.
- 998 15. Akondy, R.S., Fitch, M., Edupuganti, S., Yang, S., Kissick, H.T., Li, K.W.,
999 Youngblood, B.A., Abdelsamed, H.A., McGuire, D.J., Cohen, K.W., et al. (2017).
1000 Origin and differentiation of human memory CD8 T cells after vaccination. *Nature*
1001 552, 362-367. 10.1038/nature24633.
- 1002 16. Veit, O., Domingo, C., Niedrig, M., Staehelin, C., Sonderegger, B., Hequet, D.,
1003 Stoeckle, M., Calmy, A., Schiffer, V., Bernasconi, E., et al. (2018). Long-term
1004 Immune Response to Yellow Fever Vaccination in Human Immunodeficiency
1005 Virus (HIV)-Infected Individuals Depends on HIV RNA Suppression Status:
1006 Implications for Vaccination Schedule. *Clinical infectious diseases : an official*
1007 *publication of the Infectious Diseases Society of America* 66, 1099-1108.
1008 10.1093/cid/cix960.
- 1009 17. Walls, A.C., Park, Y.J., Tortorici, M.A., Wall, A., McGuire, A.T., and Veesler, D.
1010 (2020). Structure, Function, and Antigenicity of the SARS-CoV-2 Spike
1011 Glycoprotein. *Cell* 181, 281-292 e286. 10.1016/j.cell.2020.02.058.
- 1012 18. Ju, B., Zhang, Q., Ge, J., Wang, R., Sun, J., Ge, X., Yu, J., Shan, S., Zhou, B.,
1013 Song, S., et al. (2020). Human neutralizing antibodies elicited by SARS-CoV-2
1014 infection. *Nature* 584, 115-119. 10.1038/s41586-020-2380-z.
- 1015 19. Seydoux, E., Homad, L.J., MacCamy, A.J., Parks, K.R., Hurlburt, N.K.,
1016 Jennewein, M.F., Akins, N.R., Stuart, A.B., Wan, Y.H., Feng, J., et al. (2020).
1017 Analysis of a SARS-CoV-2-Infected Individual Reveals Development of Potent
1018 Neutralizing Antibodies with Limited Somatic Mutation. *Immunity* 53, 98-105
1019 e105. 10.1016/j.immuni.2020.06.001.
- 1020 20. Zost, S.J., Gilchuk, P., Case, J.B., Binshtein, E., Chen, R.E., Nkolola, J.P.,
1021 Schafer, A., Reidy, J.X., Trivette, A., Nargi, R.S., et al. (2020). Potently
1022 neutralizing and protective human antibodies against SARS-CoV-2. *Nature* 584,
1023 443-449. 10.1038/s41586-020-2548-6.
- 1024 21. Zarnitsyna, V.I., Akondy, R.S., Ahmed, H., McGuire, D.J., Zarnitsyn, V.G., Moore,
1025 M., Johnson, P.L.F., Ahmed, R., Li, K., Hellerstein, M., and Antia, R. (2021).
1026 Dynamics and turnover of memory CD8 T cell responses following yellow fever
1027 vaccination. *bioRxiv*, 2021.2001.2023.427919. 10.1101/2021.01.23.427919.
- 1028 22. Wheatley, A.K., Juno, J.A., Wang, J.J., Selva, K.J., Reynaldi, A., Tan, H.X., Lee,
1029 W.S., Wragg, K.M., Kelly, H.G., Esterbauer, R., et al. (2021). Evolution of
1030 immune responses to SARS-CoV-2 in mild-moderate COVID-19. *Nat Commun*
1031 12, 1162. 10.1038/s41467-021-21444-5.
- 1032 23. Turner, J.S., Kim, W., Kalaidina, E., Goss, C.W., Rauseo, A.M., Schmitz, A.J.,
1033 Hansen, L., Haile, A., Klebert, M.K., Pusic, I., et al. (2021). SARS-CoV-2 infection
1034 induces long-lived bone marrow plasma cells in humans. *Nature*.
1035 10.1038/s41586-021-03647-4.
- 1036 24. Stamatatos, L., Czartoski, J., Wan, Y.H., Homad, L.J., Rubin, V., Glantz, H.,
1037 Neradilek, M., Seydoux, E., Jennewein, M.F., MacCamy, A.J., et al. (2021).

- 1038 mRNA vaccination boosts cross-variant neutralizing antibodies elicited by SARS-
1039 CoV-2 infection. *Science*. 10.1126/science.abg9175.
- 1040 25. Gaebler, C., Wang, Z., Lorenzi, J.C.C., Muecksch, F., Finkin, S., Tokuyama, M.,
1041 Cho, A., Jankovic, M., Schaefer-Babajew, D., Oliveira, T.Y., et al. (2021).
1042 Evolution of antibody immunity to SARS-CoV-2. *Nature* 591, 639-644.
1043 10.1038/s41586-021-03207-w.
- 1044 26. Dan, J.M., Mateus, J., Kato, Y., Hastie, K.M., Yu, E.D., Faliti, C.E., Grifoni, A.,
1045 Ramirez, S.I., Haupt, S., Frazier, A., et al. (2021). Immunological memory to
1046 SARS-CoV-2 assessed for up to 8 months after infection. *Science* 371.
1047 10.1126/science.abf4063.
- 1048 27. Rodda, L.B., Netland, J., Shehata, L., Pruner, K.B., Morawski, P.A., Thouvenel,
1049 C.D., Takehara, K.K., Eggenberger, J., Hemann, E.A., Waterman, H.R., et al.
1050 (2021). Functional SARS-CoV-2-Specific Immune Memory Persists after Mild
1051 COVID-19. *Cell* 184, 169-183 e117. 10.1016/j.cell.2020.11.029.
- 1052 28. Mascola, J.R., Graham, B.S., and Fauci, A.S. (2021). SARS-CoV-2 Viral
1053 Variants-Tackling a Moving Target. *JAMA* 325, 1261-1262.
1054 10.1001/jama.2021.2088.
- 1055 29. Edara, V.V., Norwood, C., Floyd, K., Lai, L., Davis-Gardner, M.E., Hudson, W.H.,
1056 Mantus, G., Nyhoff, L.E., Adelman, M.W., Fineman, R., et al. (2021). Infection-
1057 and vaccine-induced antibody binding and neutralization of the B.1.351 SARS-
1058 CoV-2 variant. *Cell Host Microbe* 29, 516-521 e513.
1059 10.1016/j.chom.2021.03.009.
- 1060 30. Ellebedy, A.H., Jackson, K.J., Kissick, H.T., Nakaya, H.I., Davis, C.W., Roskin,
1061 K.M., McElroy, A.K., Oshansky, C.M., Elbein, R., Thomas, S., et al. (2016).
1062 Defining antigen-specific plasmablast and memory B cell subsets in human blood
1063 after viral infection or vaccination. *Nat Immunol* 17, 1226-1234. 10.1038/ni.3533.
- 1064 31. Cross-Network PBMC SOP Working Group (2018). Cross-Network PBMC
1065 Processing SOP v6.0. HIV/AIDS Network Coordination (HANC)
1066 [https://www.hanc.info/labs/Documents/PBMC%20Documents/HANC-LAB-](https://www.hanc.info/labs/Documents/PBMC%20Documents/HANC-LAB-P0001_v6.0_2018-04-26_PBMC_SOP.pdf)
1067 [P0001_v6.0_2018-04-26_PBMC_SOP.pdf](https://www.hanc.info/labs/Documents/PBMC%20Documents/HANC-LAB-P0001_v6.0_2018-04-26_PBMC_SOP.pdf).
1068 <https://doi.org/10.1016/j.jim.2014.03.024>.
- 1069 32. Xie, X., Muruato, A., Lokugamage, K.G., Narayanan, K., Zhang, X., Zou, J., Liu,
1070 J., Schindewolf, C., Bopp, N.E., Aguilar, P.V., et al. (2020). An Infectious cDNA
1071 Clone of SARS-CoV-2. *Cell Host Microbe* 27, 841-848 e843.
1072 10.1016/j.chom.2020.04.004.
- 1073 33. Vanderheiden, A., Edara, V.V., Floyd, K., Kauffman, R.C., Mantus, G., Anderson,
1074 E., Roupheal, N., Edupuganti, S., Shi, P.Y., Menachery, V.D., et al. (2020).
1075 Development of a Rapid Focus Reduction Neutralization Test Assay for
1076 Measuring SARS-CoV-2 Neutralizing Antibodies. *Curr Protoc Immunol* 131,
1077 e116. 10.1002/cpim.116.
- 1078 34. Suthar, M.S., Zimmerman, M.G., Kauffman, R.C., Mantus, G., Linderman, S.L.,
1079 Hudson, W.H., Vanderheiden, A., Nyhoff, L., Davis, C.W., Adekunle, O., et al.
1080 (2020). Rapid Generation of Neutralizing Antibody Responses in COVID-19
1081 Patients. *Cell Rep Med* 1, 100040. 10.1016/j.xcrm.2020.100040.

- 1082 35. Katzelnick, L.C., Coello Escoto, A., McElvany, B.D., Chavez, C., Salje, H., Luo,
1083 W., Rodriguez-Barraquer, I., Jarman, R., Durbin, A.P., Diehl, S.A., et al. (2018).
1084 Viridot: An automated virus plaque (immunofocus) counter for the measurement
1085 of serological neutralizing responses with application to dengue virus. *PLoS Negl*
1086 *Trop Dis* 12, e0006862. [10.1371/journal.pntd.0006862](https://doi.org/10.1371/journal.pntd.0006862).
- 1087 36. Hsieh, C.L., Goldsmith, J.A., Schaub, J.M., DiVenere, A.M., Kuo, H.C.,
1088 Javanmardi, K., Le, K.C., Wrapp, D., Lee, A.G., Liu, Y., et al. (2020). Structure-
1089 based design of prefusion-stabilized SARS-CoV-2 spikes. *Science* 369, 1501-
1090 1505. [10.1126/science.abd0826](https://doi.org/10.1126/science.abd0826).
- 1091 37. Dintwe, O., Rohith, S., Schwedhelm, K.V., McElrath, M.J., Andersen-Nissen, E.,
1092 and De Rosa, S.C. (2019). OMIP-056: Evaluation of Human Conventional T
1093 Cells, Donor-Unrestricted T Cells, and NK Cells Including Memory Phenotype by
1094 Intracellular Cytokine Staining. *Cytometry A* 95, 722-725. [10.1002/cyto.a.23753](https://doi.org/10.1002/cyto.a.23753).
- 1095 38. Horton, H., Thomas, E.P., Stucky, J.A., Frank, I., Moodie, Z., Huang, Y., Chiu,
1096 Y.L., McElrath, M.J., and De Rosa, S.C. (2007). Optimization and validation of an
1097 8-color intracellular cytokine staining (ICS) assay to quantify antigen-specific T
1098 cells induced by vaccination. *J Immunol Methods* 323, 39-54.
1099 [10.1016/j.jim.2007.03.002](https://doi.org/10.1016/j.jim.2007.03.002).
- 1100 39. Finak, G., McDavid, A., Chattopadhyay, P., Dominguez, M., De Rosa, S.,
1101 Roederer, M., and Gottardo, R. (2014). Mixture models for single-cell assays with
1102 applications to vaccine studies. *Biostatistics* 15, 87-101.
1103 [10.1093/biostatistics/kxt024](https://doi.org/10.1093/biostatistics/kxt024).
- 1104 40. Bakdash, J.Z., and Marusich, L.R. (2017). Repeated Measures Correlation. *Front*
1105 *Psychol* 8, 456. [10.3389/fpsyg.2017.00456](https://doi.org/10.3389/fpsyg.2017.00456).
- 1106 41. Newton, M.A., Noueiry, A., Sarkar, D., and Ahlquist, P. (2004). Detecting
1107 differential gene expression with a semiparametric hierarchical mixture method.
1108 *Biostatistics* 5, 155-176. [10.1093/biostatistics/5.2.155](https://doi.org/10.1093/biostatistics/5.2.155).
- 1109