

1 Longitudinal Analysis Reveals Distinct Antibody and Memory 2 B Cell Responses in SARS-CoV2 Naïve and Recovered 3 Individuals Following mRNA Vaccination

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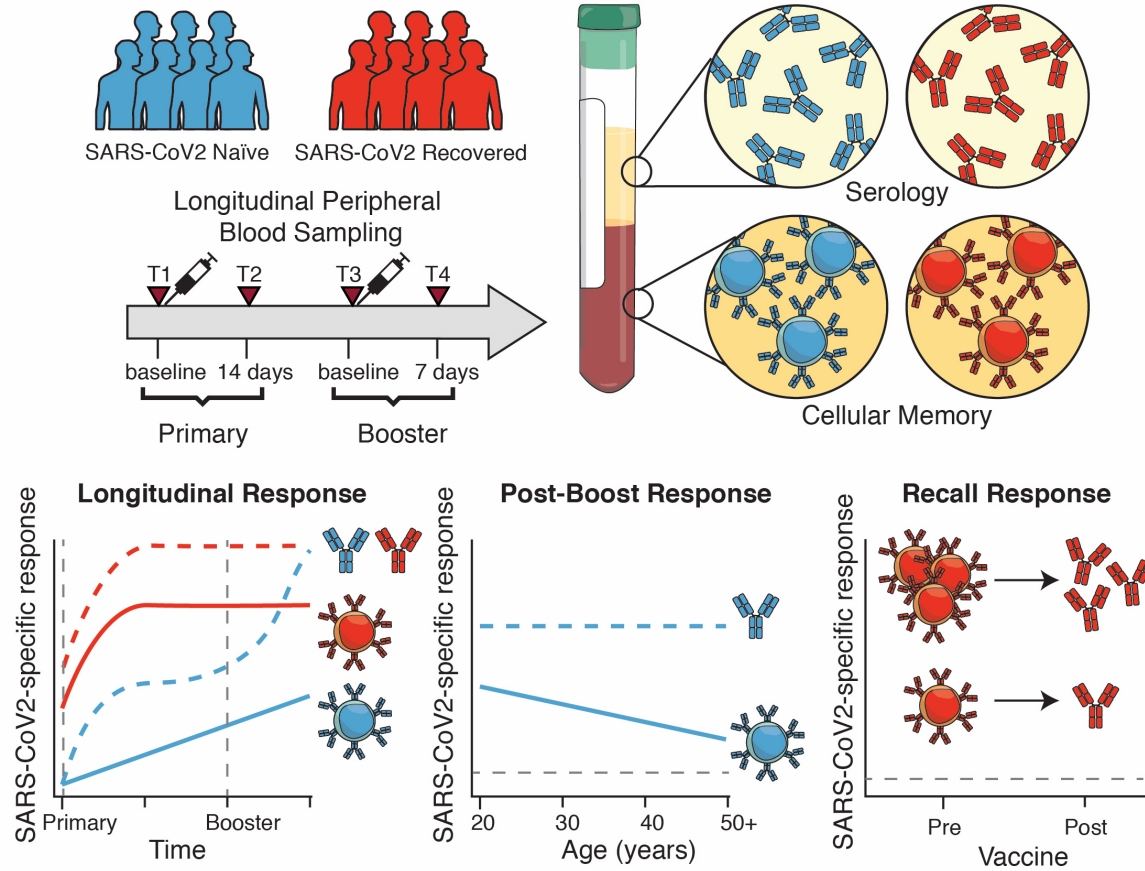
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30 **ABSTRACT**

31

32 Novel mRNA vaccines for SARS-CoV2 have been authorized for emergency use and are
33 currently being administered to millions of individuals worldwide. Despite their efficacy in
34 clinical trials, there is limited data on vaccine-induced immune responses in individuals with
35 a prior SARS-CoV2 infection compared to SARS-CoV2 naïve subjects. Moreover, how mRNA
36 vaccines impact the development of antibodies as well as memory B cells in COVID-19
37 experienced versus COVID-19 naïve subjects remains poorly understood. In this study, we
38 evaluated antibody responses and antigen-specific memory B cell responses over time in 33
39 SARS-CoV2 naïve and 11 SARS-CoV2 recovered subjects. mRNA vaccination induced
40 significant antibody and memory B cell responses against full-length SARS-CoV2 spike
41 protein and the spike receptor binding domain (RBD). SARS-CoV2 naïve individuals
42 benefitted from both doses of mRNA vaccine with additional increases in antibodies and
43 memory B cells following booster immunization. In contrast, SARS-CoV2 recovered
44 individuals had a significant immune response after the first dose with no increase in
45 circulating antibodies or antigen-specific memory B cells after the second dose. Moreover,
46 the magnitude of the memory B cell response induced by vaccination was lower in older
47 individuals, revealing an age-dependence to mRNA vaccine-induced B cell memory. Side
48 effects also tended to associate with post-boost antibody levels, but not with post-boost
49 memory B cells, suggesting that side effect severity may be a surrogate of short-term antibody
50 responses. The frequency of pre-vaccine antigen-specific memory B cells in SARS-CoV2
51 recovered individuals strongly correlated with post-vaccine antibody levels, supporting a key
52 role for memory B cells in humoral recall responses to SARS-CoV2. This observation may
53 have relevance for future booster vaccines and for responses to viral variants that partially
54 escape pre-existing antibodies and require new humoral responses to be generated from
55 memory B cells. Finally, post-boost antibody levels were not correlated with post-boost
56 memory responses in SARS-CoV2 naïve individuals, indicating that short-term antibody
57 levels and memory B cells are complementary immunological endpoints that should be
58 examined in tandem when evaluating vaccine response. Together, our data provide evidence
59 of both serological response and immunological memory following mRNA vaccination that is
60 distinct based on prior SARS-CoV2 exposure. These findings may inform vaccine distribution
61 in a resource-limited setting.



62

63 INTRODUCTION

64

65 The COVID-19 pandemic has resulted in more than 100 million infections and 2.5 million
66 deaths worldwide. Novel vaccines have recently been issued emergency use
67 authorization by the FDA and are currently being administered to front-line workers and
68 at-risk individuals. Early data from clinical trials suggest that these vaccines are safe and
69 effective^{1,2}, however there is still a paucity of information interrogating how these novel
70 mRNA vaccines elicit immune responses at the cellular and molecular level.

71

72 The humoral immune response to infection or vaccination results in two major outcomes:
73 the production of antibodies by antibody secreting cells (ASC), which can provide rapid
74 protective immunity, and the generation of long-lived memory B cells capable of mounting
75 recall responses³. If circulating antibodies fail to confer protection to a future exposure,
76 memory B cells drive the recall response by producing new antibodies through formation
77 of new ASC or re-initiating germinal center reactions to generate new high-affinity B cell
78 clones through additional rounds of somatic hypermutation. In the context of acute SARS-
79 CoV2 infection, immunological memory in the form of antibodies and memory B cells has
80 been shown to be durable for over 8 months post-symptom onset⁴⁻⁶. However, studies
81 on vaccinated individuals have largely focused on measuring binding and/or neutralizing
82 antibodies as primary endpoints^{7,8}. Although antibodies are a central component of
83 vaccine efficacy, immunological memory in the form of memory B cells may be important
84 for long-term protection, responses to subsequent infection, and the ability to respond to
85 emerging variant strains. The induction of memory B cells by mRNA vaccines remains
86 poorly understood. Furthermore, it is unclear how memory B cell responses relate to
87 serological responses, and how both antibody and memory B cell responses differ in
88 subjects who previously experienced SARS-CoV2 infection versus those who are SARS-
89 CoV2 naïve.

90

91 A related question is whether individuals who experienced prior SARS-CoV2 infection
92 require a second dose of mRNA vaccine. This question is particularly important given the
93 currently limited vaccine supply and challenges with deployment. As these individuals

94 have already generated a primary immune response to SARS-CoV2 during their natural
95 infection, it is possible that a single dose of vaccine could be enough to sufficiently boost
96 their antibody and memory B cell responses. Indeed, several recent studies have
97 indicated that antibody responses can be robustly induced in SARS-CoV2 experienced
98 individuals, consistent with an anamnestic response⁹⁻¹². Although one study suggests
99 that memory B cells might also be boosted after a single vaccine dose¹³, it remains
100 unclear how well memory B cell responses are induced in SARS-CoV2 naïve versus
101 SARS-CoV2 experienced subjects after one versus two doses of mRNA vaccine.
102 Moreover, how antibody levels predict or relate to memory B cell responses following
103 mRNA vaccination remains to be determined. These key gaps in our understanding
104 require longitudinal analysis of antibodies together with memory B cell responses after
105 the first and second dose of mRNA vaccine in SARS-CoV2 naïve and experienced
106 subjects.

107
108 In this study, we established a longitudinal cohort of SARS-CoV2 naïve and SARS-CoV2
109 recovered individuals who received mRNA vaccines at the University of Pennsylvania
110 Health System. From these longitudinal samples, we assessed both circulating antibodies
111 and antigen-specific memory B cells over the course of first and second immunization.
112 We further integrated these serologic and cellular assays with clinical metadata and
113 compared these immune responses with those from non-vaccinated SARS-CoV2
114 recovered subjects. These studies revealed several key findings. First, as others have
115 reported, vaccination boosts antibody levels more quickly in SARS-CoV2 recovered
116 versus naïve subjects. Second, memory B cell responses are also robustly induced by
117 the first dose of vaccine in SARS-CoV2 recovered subjects, but no additional boosting is
118 observed after the second vaccine dose. In contrast, memory B cell responses continue
119 to improve after the second vaccination in SARS-CoV2 naïve subjects. Third, although
120 subjects of all ages benefit from induction of serological and cellular immunity, vaccine
121 induction of memory B cells declines with age. Fourth, there was a trend for mRNA
122 vaccine-induced antibody levels to be higher in subjects with more systemic side effects,
123 but side effects had no relation to memory B cell responses. Finally, there was no
124 relationship between post-vaccination serum antibody and memory B cells in SARS-

125 CoV2 naïve subjects, indicating that measuring short-term antibody titers alone may fail
126 to predict long-term immunity elicited by the vaccine. Pre-existing memory B cells did
127 strongly correlate with post-vaccination antibody responses in SARS-CoV2 recovered
128 subjects, further emphasizing the importance of measuring these cells. These data
129 highlight the efficacy of SARS-CoV2 mRNA vaccines and support a single-dose vaccine
130 regimen in SARS-CoV2 recovered subjects, which may allow more effective distribution
131 of vaccines to the general population.

132 **METHODS**

133

134 **Recruitment and Clinical Sample Collection**

135 44 individuals (33 SARS-CoV2 naïve, 11 SARS-CoV2 recovered) were consented and
136 enrolled in the study with approval from the University of Pennsylvania Institutional
137 Review Board (IRB# 844642). All subjects received either Pfizer (BNT162b2) or Moderna
138 (mRNA-1273) mRNA vaccines. Samples were collected at 4 timepoints: baseline, 2
139 weeks post-primary immunization, day of booster immunization, and 1 week post-booster
140 immunization. 80-100mL of peripheral blood samples and clinical questionnaire data were
141 collected at each study visit. Full cohort and demographic information is provided in **figure**
142 **S1**. Non-vaccinated recovered COVID-19 donors (RD) were adults with a prior positive
143 COVID-19 PCR test by self-report who met the definition of recovery by the Centers for
144 Disease Control¹⁴.

145

146 **Sample Processing**

147 Venous blood was collected into sodium heparin and EDTA tubes by standard
148 phlebotomy. Blood tubes were centrifuged at 3000rpm for 15 minutes to separate plasma.
149 Heparin and EDTA plasma were stored at -80C for downstream antibody analysis.
150 Remaining whole blood was diluted 1:1 with RPMI + 1% FBS and layered onto SEPMATE
151 tubes (STEMCELL Technologies) containing lymphoprep gradient (STEMCELL
152 Technologies). SEPMATE tubes were centrifuged at 1200g for 10 minutes and the PBMC
153 fraction was collected into new tubes. PBMCs were then washed with RPMI + 1% FBS
154 and treated with ACK lysis buffer (Thermo Fisher) for 5 minutes. Samples were washed
155 again with RPMI + 1% FBS, filtered with a 70um filter, and counted using a Countess
156 automated cell counter (Thermo Fisher). Aliquots containing 5×10^6 PBMCs were
157 cryopreserved in 90% FBS 10% DMSO.

158

159 **Detection of SARS-CoV2-Specific Antibodies**

160 Plasma samples were tested for SARS-CoV2-specific antibody by enzyme-linked
161 immunosorbent assay (ELISA) as previously described¹⁵. Plasmids encoding the
162 recombinant full-length spike protein and the receptor binding domain (RBD) were

163 provided by F. Krammer (Mt. Sinai) and purified by nickel-nitrilotriacetic acid resin
164 (Qiagen). ELISA plates (Immulon 4 HBX, Thermo Fisher Scientific) were coated with PBS
165 or 2 ug/mL recombinant protein and stored overnight at 4C. The next day, plates were
166 washed with phosphate-buffered saline containing 0.1% Tween-20 (PBS-T) and blocked
167 for 1 hour with PBS-T supplemented with 3% non-fat milk powder. Samples were heat-
168 inactivated for 1 hour at 56C and diluted in PBS-T supplemented with 1% non-fat milk
169 powder. After washing the plates with PBS-T, 50 uL diluted sample was added to each
170 well. Plates were incubated for 2 hours and washed with PBS-T. Next, 50 uL of 1:5000
171 diluted goat anti-human IgG-HRP (Jackson ImmunoResearch Laboratories) or 1:1000
172 diluted goat anti-human IgM-HRP (SouthernBiotech) was added to each well and plates
173 were incubated for 1 hour. Plates were washed with PBS-T before 50 uL SureBlue
174 3,3',5,5'-tetramethylbenzidine substrate (KPL) was added to each well. After 5 minutes
175 incubation, 25 uL of 250 mM hydrochloric acid was added to each well to stop the
176 reaction. Plates were read with the SpectraMax 190 microplate reader (Molecular
177 Devices) at an optical density (OD) of 450 nm. Monoclonal antibody CR3022 was
178 included on each plate to convert OD values into relative antibody concentrations.
179 Plasmids to express CR3022 were provided by I. Wilson (Scripps).

180

181 **Detection of SARS-CoV2-Specific Memory B Cells**

182 Antigen-specific B cells were detected using biotinylated proteins in combination with
183 different streptavidin (SA)-fluorophore conjugates^{4,6}. Biotinylated proteins were
184 multimerized with fluorescently labeled SA for 1 hour at 4C. Full-length spike protein (R&D
185 Systems) was mixed with SA-BV421 (Biolegend) at a 10:1 mass ratio (e.g., 200ng spike
186 with 20ng SA; ~4:1 molar ratio). Spike RBD (R&D Systems) was mixed with SA-APC
187 (Biolegend) at a 2:1 mass ratio (e.g., 25ng RBD with 12.5ng SA; ~4:1 molar ratio).
188 Biotinylated influenza HA pools were mixed with SA-PE (Biolegend) at a 6.25:1 mass
189 ratio (e.g., 100ng HA pool with 16ng SA; ~6:1 molar ratio). Individual influenza HA
190 antigens corresponding with the 2019 trivalent vaccine (A/Brisbane/02/2018/H1N1,
191 B/Colorado/06/2017; Immune Technology) were biotinylated using an EZ-Link Micro
192 NHS-PEG4 Biotinylation Kit (Thermo Fisher) according to the manufacturer's instructions.
193 Excess biotin was subsequently removed using Zebra Spin Desalting Columns 7K

194 MWCO (Thermo Fisher) and protein was quantified with a Pierce BCA Assay (Thermo
195 Fisher). SA-BV711 (BD Bioscience) was used as a decoy probe without biotinylated
196 protein to gate out cells that non-specifically bind streptavidin. All experimental steps were
197 performed in a 50/50 mixture of PBS + 2% FBS and Brilliant Buffer (BD Bioscience).
198 Antigen probes for spike, RBD, and HA were prepared individually and mixed together
199 after multimerization with 5uM free D-biotin (Avidity LLC) to minimize potential cross-
200 reactivity between probes. For staining, 5×10^6 cryopreserved PBMC samples were
201 prepared in a 96-well U-bottom plate. Cells were first stained with Fc block (Biolegend,
202 1:200) and Ghost 510 Viability Dye (Tonbo Biosciences, 1:600) for 15 minutes at 4C.
203 Cells were then washed and stained with 50uL antigen probe master mix containing
204 200ng spike-BV421, 25ng RBD-APC, 100ng HA-PE, and 20ng SA-BV711 decoy for 1
205 hour at 4C. Following incubation with antigen probe, cells were washed again and stained
206 with anti-CD3 (BD Bioscience, 1:200), anti-CD19 (Biolegend, 1:100), anti-CD20 (BD
207 Bioscience, 1:500), anti-CD27 (BD Bioscience, 1:200), anti-CD38 (BD Bioscience,
208 1:200), anti-CD71 (BD Bioscience, 1:50), anti-IgD (BD Bioscience, 1:50), anti-IgM
209 (Biolegend, 1:200), and anti-IgG (Biolegend, 1:400). After surface stain, cells were
210 washed and fixed in 1% PFA overnight at 4C.

211

212 **Flow Cytometry**

213 Samples were acquired on a BD Symphony A5 instrument. Standardized SPHERO
214 rainbow beads (Spherotech) were used to track and adjust photomultiplier tubes over
215 time. UltraComp eBeads (Thermo Fisher) were used for compensation. Up to 5×10^6 cells
216 were acquired per sample. Data were analyzed using FlowJo v10 (BD Bioscience). Full
217 gating strategy is shown in **figure S2**.

218

219 **Data Visualization and Statistics**

220 All data were analyzed using custom R scripts. Statistical tests are indicated in the
221 corresponding figure legends. * indicates $p < 0.05$, ** indicates $p < 0.01$, *** indicates $p <$
222 0.001 , **** indicates $p < 0.0001$. Source code and data files are available upon request
223 from the authors.

224 RESULTS

225

226 For this study, we recruited 44 individuals who received SARS-CoV2 mRNA vaccines
227 (Pfizer BNT162b2 or Moderna mRNA-1273) at the University of Pennsylvania Health
228 System. Of this cohort, 11 individuals had a prior SARS-CoV2 infection. Peripheral blood
229 samples were collected for immunological analysis at 4 key timepoints (**figure 1A**): pre-
230 vaccine baseline (timepoint 1), 2 weeks following the first dose (timepoint 2), the day of
231 second dose (timepoint 3), and 1 week following the second dose (timepoint 4). This study
232 design allowed us to investigate the kinetics of immune responses following both primary
233 and secondary immunizations.

234

235 We first measured circulating antibody responses in longitudinal serum samples by
236 ELISA. At baseline, SARS-CoV2 naïve individuals had undetectable levels of IgG
237 antibodies specific for either full-length spike protein or the spike receptor binding domain
238 (RBD) (**figure 1B**). Primary vaccination induced a significant increase in SARS-CoV2-
239 specific antibodies, that was further enhanced by the booster dose (**figure 1B**). In
240 contrast, all SARS-CoV2 recovered individuals had detectable levels of anti-spike and
241 anti-RBD IgG at baseline and these antibody responses were significantly increased after
242 the first dose of vaccine (**figure 1B**). However, in SARS-CoV2 recovered subjects, there
243 was no additional increase in antibody levels following the second vaccine dose (**figure**
244 **1B**). Notably, the levels of anti-RBD IgG were similar in the SARS-CoV2 naïve and SARS-
245 CoV2 recovered individuals at 1 week post-boost (timepoint 4) (**figure 1B**).

246

247 We next asked how mRNA vaccination impacted the responses of memory B cells
248 specific for SARS-CoV2. To address this question, we developed a flow cytometric assay
249 using fluorescently labeled antigens as probes to track induction of virus-specific memory
250 B cells in longitudinal PBMC samples (**figure 2A**). Consistent with the antibody data,
251 SARS-CoV2 naïve individuals had minimal spike-specific memory B cells at baseline,
252 whereas SARS-CoV2 recovered individuals had a significant population of spike-specific
253 memory B cells ranging from ~0.15-0.8% of total memory B cells (**figure 2B**). The
254 frequency of these antigen-specific memory B cells was comparable to a separate cohort

255 of non-vaccinated SARS-CoV2 recovered donors (**figure 2B**). Similar trends were
256 observed for memory B cells targeting the spike RBD (**figure 2B**). After primary
257 immunization, SARS-CoV2 naïve individuals had a significant increase in spike-specific
258 and RBD-specific memory B cells over baseline (**figure 2B**). These memory B cells were
259 also significantly boosted after administration of the second dose, approaching the levels
260 of memory B cells observed in non-vaccinated SARS-CoV2 recovered donors (**figure**
261 **2B**). In contrast, SARS-CoV2 recovered individuals had a robust expansion of spike- and
262 RBD-specific memory B cells following primary immunization, but had no additional
263 boosting after the second vaccine dose (**figure 2B**), suggesting minimal benefit of the
264 second dose in these recovered subjects. As a control we also examined the frequency
265 of influenza hemagglutinin (HA)-specific memory B cells in both SARS-CoV2 naïve and
266 recovered individuals following SARS-CoV2 vaccination. The frequency of these antigen-
267 unrelated memory B cells remained stable throughout the mRNA vaccination timecourse
268 (**figure 2B**), confirming the specificity of this memory B cell assay. Together, these results
269 demonstrate robust induction of SARS-CoV2-specific memory B cells by two doses of
270 mRNA vaccine in SARS-CoV2 naïve subjects. Alternatively, a single dose of mRNA
271 vaccine amplified pre-existing antigen-specific memory B cells in SARS-CoV2 recovered
272 subjects, with no additional quantitative benefit after the second vaccine dose.

273
274 We further analyzed the immunoglobulin isotype of SARS-CoV2 specific memory B cells.
275 On day 15 after primary immunization, ~25-30% of responding spike-specific memory B
276 cells were IgG⁺ and ~40-50% were IgM⁺ in SARS-CoV2 naïve individuals (**figure 2C**).
277 The frequency of IgG⁺ memory B cells increased to >50% following the second dose of
278 vaccine in these subjects (**figure 2C-D**), consistent with a qualitative improvement in B
279 cell memory formation after the boost. Conversely, in SARS-CoV2 recovered individuals,
280 ~60-70% of spike-specific memory B cells detected prior to vaccination were IgG⁺ (**figure**
281 **2C-D**). Although the frequency of IgG⁺ memory B cells increased slightly to ~75%
282 following the first dose of vaccine, further increases were not observed after the second
283 immunization (**figure 2C-D**). A similar pattern of IgG frequency was observed for RBD-
284 specific memory B cells (**figure 2C-D**). In addition, the fraction of spike-specific memory
285 B cells that recognized RBD remained stable over time in SARS-CoV2 recovered

286 individuals. In SARS-CoV2 naïve subjects, the fraction of the overall spike-specific
287 memory B cell response that was focused on RBD increased over time, becoming
288 equivalent to that observed in SARS-CoV2 recovered individuals after the second vaccine
289 dose (**figure S2**). Overall, these data indicate a qualitative benefit to the virus-specific
290 memory B cell response following both doses of vaccine in SARS-CoV2 naïve individuals,
291 and limited qualitative improvement following the first but not the second vaccine dose in
292 SARS-CoV2 recovered subjects.

293
294 Several previous studies have reported a negative association between age and vaccine-
295 induced antibody titers after a single dose of mRNA vaccines^{16,17}. We therefore
296 investigated potential relationships between sex or age and B cell responses after one or
297 two doses of vaccine. In our cohort of SARS-CoV2 naïve vaccinees, there were no
298 associations between sex and either antibody or memory B cell responses (**figure 3A,**
299 **3C**). Although there was no association between age and spike-specific IgG after the first
300 immunization (i.e. pre-boost), there was a modest trend towards a negative relationship
301 between RBD-specific IgG titers and age after the first vaccine dose (**figure 3B**). There
302 was no significant correlation between age and either spike- or RBD-specific serum IgG
303 after the second dose (**figure 3B**). In contrast, there was a clear negative correlation
304 between the post-boost frequency of antigen-specific memory B cells and age (**figure**
305 **3D**). Although this relationship represented weaker induction of memory B cells with older
306 age, all age groups still displayed an increase in the frequency of SARS-CoV2 specific
307 memory B cells compared to pre-vaccine baseline (**figure S3A-D**). There was also no
308 change in the frequency of total memory B cells by sex or age, indicating the antigen-
309 specific nature of this effect (**figure S3E**). While our cohort is not extensively enriched in
310 those over 50 years old, and does not directly address elderly vaccinees, age
311 associations with weaker vaccine-induced antibody responses appeared to normalize
312 following the second dose of vaccine. Conversely, the effect of age on memory B cell
313 responses was more prominent after the second immunization. These data point to
314 potentially relevant age-related changes in immune response to vaccination.

315

316 An additional question is whether vaccine-induced side effects have any relationship to
317 immune responses⁹. We addressed this question by comparing vaccine-induced antibody
318 and memory B cell responses in subjects with or without self-reported systemic side
319 effects (i.e. fever, chills, headache, fatigue, myalgia). In SARS-CoV2 naïve vaccinees
320 with systemic side-effects following the second dose, there was a trend towards increase
321 in antibody responses at the post-boost timepoint (**figure S4**). Such a trend was not
322 observed for the memory B cell response. Although these data only represent a statistical
323 trend, they do provoke questions about potential relationships between early vaccine-
324 induced inflammation and the induction of antibody responses, which should be
325 addressed in future studies.

326
327 Finally, we investigated the potential relationships between antibody and memory B cell
328 responses. In SARS-CoV2 naïve subjects, we examined the relationship between
329 circulating antibody responses and memory B responses after two doses of vaccine.
330 Despite strong induction of both spike- and RBD-specific antibody and memory B cells in
331 these subjects, there was no association between the levels of post-boost antibodies and
332 B cell memory (**figure 4A**), indicating that short-term serologies and cellular memory are
333 distinct immunological measures of vaccine efficacy. Similarly, pre-vaccine baseline
334 antibody levels did not correlate with baseline memory B cell frequencies in SARS-CoV2
335 recovered individuals (**figure 4B**). We next asked which measure of humoral immunity
336 predicted antibody recall responses post-vaccination. Interestingly, the baseline levels of
337 SARS-CoV2-specific antibody did not correlate with the level of antibody achieved after
338 primary vaccine (timepoint 2) in SARS-CoV2 recovered donors (**figure 4C**). However, the
339 baseline frequency of antigen-specific memory B cells (timepoint 1) strongly correlated
340 with post-primary vaccine antibody levels (timepoint 2, **figure 4D**), consistent with the
341 notion that these pre-vaccination memory B cells are major contributors to the SARS-
342 CoV2 antibody recall response. Overall, these data highlight the importance of measuring
343 antigen-specific memory B cells in addition to more conventional serologic approaches
344 as an immunological correlate of vaccine-induced immunity.

345 DISCUSSION

346

347 Our data indicate that mRNA vaccines induce significant antibody and memory B cell
348 responses to full-length spike and the RBD. These results are encouraging for both short-
349 and long-term vaccine efficacy. Overall, these data also add to our understanding of
350 SARS-CoV2 mRNA vaccine-induced immune responses in several ways. First, our
351 serological data is consistent with several other recent studies^{9,10,12,13,16,17} indicating
352 robust boosting of antibody responses in SARS-CoV2 recovered subjects after the first
353 vaccine dose, but little benefit to antibody titers after the second vaccine dose. Moreover,
354 we identified a similar effect for virus-specific memory B cells, demonstrating that both a
355 quantitative and qualitative plateau in vaccine-induced memory B cells is achieved
356 following the first dose of vaccine with little additional change to the memory B cell
357 response following booster vaccination. These data advocate for only a single vaccine
358 dose in individuals confirmed to have previously been infected with SARS-CoV2. It is
359 important, however, to point out that our cohort consisted of individuals who were not
360 hospitalized during their SARS-CoV2 infections, and it may be necessary to address this
361 question in individuals who experienced more severe COVID-19.

362

363 It remains unclear if the second vaccine dose in recovered individuals has other
364 immunological effects not reflected in overall antibody titers or memory B cell frequencies,
365 such as expansion of specific, high-affinity B cell clones. Additional analysis of BCR
366 sequences and memory B cell differentiation states is necessary to fully address these
367 questions. It is also possible that booster vaccination has some beneficial effects on virus-
368 specific T cell responses in SARS-CoV2 recovered individuals, and this topic should also
369 be investigated. A second related point is that in SARS-CoV2 naïve individuals, both the
370 antibody response and the memory B cell response displayed considerable benefit from
371 the second dose of mRNA vaccine. It is possible that some of this serological and memory
372 B cell maturation would occur over time in the absence of a booster vaccination, but the
373 spike- and RBD-specific antibody titers appeared to plateau between the first and second
374 doses of vaccine. Moreover, the frequency of the memory B cell response that was IgG⁺
375 and the fraction of the overall spike-specific memory B cell response that was focused on

376 RBD both improved after booster vaccination, arguing strongly for the benefit of a two
377 dose mRNA vaccine schedule in SARS-CoV2 naïve individuals.

378
379 In this cohort we also observed a negative association of age with induction of B cell
380 memory. Although others have reported a negative association between age and serum
381 antibody titers after a single mRNA vaccine dose^{16,17}, we found that this relationship was
382 not significant following two doses of mRNA vaccination. However, we observed that the
383 magnitude of the memory B cell response following the second dose was lower with
384 increased age, confirming age as a key variable in mRNA vaccine induced immunity. It
385 remains unclear if the age-associated effect on memory B cell induction represents a true
386 difference in the magnitude of response or a difference in kinetics that will resolve at later
387 timepoints. It is also challenging to define an exact threshold for how much immunological
388 memory is sufficient to provide long-term protection. Although all subjects, regardless of
389 age, had significant humoral and memory B cell responses to vaccination, these data
390 highlight a need to further understand the age-related changes in responses to mRNA
391 vaccination. In examining correlates of vaccine-induced immune responses, we also
392 uncovered a trend suggesting that vaccine-induced side effects may be related to post-
393 vaccination serum antibodies, but not memory B cells. While more data are needed, it is
394 possible that systemic inflammation early after vaccination could contribute to an initial
395 induction of antibody with less of an impact on the development of memory B cells. Larger
396 cohorts and more quantitative measures of vaccine-induced side effects may further
397 clarify these relationships.

398
399 Finally, our data demonstrate the importance of interrogating vaccine-induced memory B
400 cell responses. The strong correlation of pre-existing antigen-specific memory B cells with
401 post-vaccination serum antibody underscores the immunological connection between
402 memory B cells and recall antibody responses. This relationship likely indicates a role for
403 antigen-specific memory B cells as a source of new antibody secreting cells, as well as
404 potentially contributing to new germinal center responses¹⁸. Although high circulating
405 titers of neutralizing antibodies are common surrogates of protective immunity, there are
406 many scenarios where circulating antibodies may not achieve sterilizing immunity and

407 additional immune responses will be necessary¹⁹. For example, high dose viral
408 inoculums may require rapid generation of additional antibody from memory B cells. In
409 addition, if circulating antibodies wane over time, durable memory B cells are likely to
410 provide a rapid source of protective antibody upon antigen re-exposure. Future booster
411 vaccinations, if needed, will focus at least partly on reactivating these antigen-specific
412 memory B cells. Lastly, infection with variant strains that partially escape neutralization
413 by existing circulating antibodies might require strong memory B cell populations that can
414 rapidly re-seed germinal centers and diversify to respond to novel spike antigens²⁰. Thus,
415 including analysis of B cell memory in our monitoring of vaccine-induced immune
416 responses not only provides insight into immunological mechanisms of immunity to
417 SARS-CoV2, but may also be useful to inform vaccine implementation decisions across
418 different populations.

419 **ACKNOWLEDGEMENTS**

420 We would like to thank all the subjects who participated in our studies. We also thank the
421 Wherry Lab for helpful discussions.

422

423 **CONTRIBUTIONS**

424 RRG, SAA, MMP, DM, ARG, and EJW conceived the study. RRG, SAA, MMP, DM, AP,
425 and SG carried out experiments. RRG, SAA and OK were involved in clinical recruitment
426 and sample acquisition. All authors participated in data analysis and interpretation. RRG
427 and EJW wrote the manuscript.

428

429 **FUNDING**

430 This work was supported by grants from NIH: AI105343 and AI082630 (EJW); T32
431 AR076951-01 (SAA); HL143613, and T32 CA009140 (JRG). This work was also
432 supported by the University of Pennsylvania Perelman School of Medicine COVID fund;
433 the Allen Institute for Immunology (SAA, EJW); the Chen Family Research Fund (SAA);
434 and a Cancer Research Institute-Mark Foundation Fellowship (JRG). RRG was supported
435 through a Raffensperger 21st Century Scholar Award from the University of Pennsylvania.
436 JRG and EJW are also funded by the Parker Institute for Cancer Immunotherapy which
437 supports the Cancer Immunology Program at the University of Pennsylvania. We further
438 thank Jeffrey Lurie and Joel Embiid, Josh Harris, and David Blitzer for philanthropic
439 support.

440

441 **COMPETING INTERESTS**

442 EJW is consulting or is an advisor for Merck, Elstar, Janssen, Related Sciences,
443 Synthekine and Surface Oncology. EJW is a founder of Surface Oncology and Arsenal
444 Biosciences. EJW is an inventor on a patent (US Patent number 10,370,446) submitted
445 by Emory University that covers the use of PD-1 blockade to treat infections and cancer.

446 **Figure 1. Antibody responses following mRNA vaccination in SARS-CoV2 naïve**
447 **and SARS-CoV2 recovered individuals. A)** UPenn Immune Health COVID vaccine
448 study design. **B)** Concentration of anti-spike and anti-RBD IgG antibodies in vaccinated
449 individuals over time. Dotted lines indicate the limit of detection for the assay. Blue =
450 SARS-CoV2 naïve + mRNA vaccine, red = SARS-CoV2 recovered + mRNA vaccine.
451 Statistics were calculated using unpaired Kruskal-Wallis or Wilcoxon test with adjustment
452 for multiple comparisons.

453
454 **Figure 2. Antigen-specific memory B cell responses following mRNA vaccination**
455 **in SARS-CoV2 naïve and SARS-CoV2 recovered individuals. A)** Gating strategy and
456 representative plots for flow cytometric analysis of SARS-CoV2-specific B cells. Cells
457 were stained with fluorescently labeled SARS-CoV2 full-length spike protein, SARS-
458 CoV2 spike receptor binding domain (RBD), and influenza hemagglutinin (HA). Memory
459 B cells were identified as live, CD3⁻, CD19⁺, non-naïve (\neq IgD⁺ CD27⁻), CD20⁺ CD38^{lo/int},
460 decoy⁻ cells. Spike⁺ HA⁻ cells were subsequently analyzed for binding to RBD, as well as
461 immunoglobulin class (IgG vs. IgM). **B)** Frequency of spike⁺, spike⁺/RBD⁺, and HA⁺
462 memory B cells over time in vaccinated individuals. Data are represented as frequency
463 of antigen-specific cells in the total memory B cell compartment. **C)** Frequency of antigen-
464 specific IgG⁺ memory B cells over time in vaccinated individuals. Data are represented
465 as frequency of antigen-specific IgG⁺ cells in the total memory B cell compartment. **D)**
466 Frequency of IgG and IgM isotypes over time in the antigen-specific memory cell
467 compartments. RD = non-vaccinated, SARS-CoV2 recovered donors. Dotted lines
468 indicate the mean at baseline in SARS-CoV2 naïve and SARS-CoV2 recovered
469 individuals. Blue = SARS-CoV2 naïve + mRNA vaccine, red = SARS-CoV2 recovered +
470 mRNA vaccine, purple = SARS-CoV2 recovered. Statistics were calculated using
471 unpaired Kruskal-Wallis or Wilcoxon test with adjustment for multiple comparisons.

472
473 **Figure 3. Age-associated decreases in antigen-specific B cell responses following**
474 **mRNA vaccination. A, B)** Concentration of anti-spike and anti-RBD IgG antibodies over
475 time compared with sex and age in SARS-CoV2 naïve individuals. Dotted lines indicate
476 the limit of detection for the assay. **C, D)** Frequency of spike⁺ and spike⁺/RBD⁺ memory

477 B cells over time compared with sex and age in SARS-CoV2 naïve individuals. Data are
478 represented as frequency of spike⁺ and spike⁺/RBD⁺ cells in the total memory B cell
479 compartment. Pre-boost indicates samples collected at timepoint 2 (~15 days post-
480 primary vaccination). Post-boost indicates samples collected at timepoint 4 (~7 days post-
481 secondary vaccination). Dotted lines indicate the mean frequency of cells at baseline.
482 Statistics for sex were calculated using Wilcoxon test. Associations with age were
483 calculated using Spearman correlation.

484

485 **Figure 4. Antigen-specific memory cells are a distinct measure of vaccine efficacy**
486 **and correlate with antibody recall responses. A)** Association of post-boost (timepoint
487 4) antibody levels with post-boost (timepoint 4) antigen-specific memory cell frequencies
488 in SARS-CoV2 naïve individuals. **B)** Association of baseline (timepoint 1) antibody levels
489 with baseline (timepoint 1) antigen-specific memory cell frequencies in SARS-CoV2
490 recovered individuals. **C)** Association of baseline (timepoint 1) antibody levels with post-
491 primary vaccination (timepoint 2) antibody levels in SARS-CoV2 recovered individuals.
492 **D)** Association of baseline (timepoint 1) antigen-specific memory cell frequencies with
493 post-primary vaccination (timepoint 2) antibody levels in SARS-CoV2 recovered
494 individuals. Associations between immunological parameters were calculated using
495 Pearson correlation.

		SARS-CoV2 Naïve	SARS-CoV2 Recovered
N	Number of Individuals	33 (75%)	11 (25%) ⁴⁹⁸
	Average	37.3	34.7 ⁴⁹⁹
Age	20-30	11 (33.3%)	4 (36.4%) ⁵⁰⁰
	30-40	9 (27.3%)	4 (36.4%) ⁵⁰¹
	40-50	8 (24.2%)	1 (9.1%) ⁵⁰²
	50+	5 (15.2%)	2 (18.2%) ⁵⁰³
Sex	Male	15 (45.5%)	7 (63.6%) ⁵⁰⁴
	Female	18 (54.5%)	4 (36.4%)
Race/ Ethnicity	White - Non-Hispanic/Latino	19 (57.6%)	7 (63.6%)
	White - Hispanic/Latino	5 (15.2%)	1 (9.1%)
	Asian	6 (18.2%)	2 (18.2%)
	Black	2 (6.1%)	0 (0%)
	Native	0 (0%)	1 (9.1%)
	Other	1 (3%)	0 (0%)
Vaccine Type	Pfizer	32 (97%)	8 (72.7%)
	Moderna	1 (3%)	3 (27.3%)

505

506 **Table 1. Clinical Characteristics of Individuals Enrolled in the UPenn COVID**

507 **Vaccine Study.**

508 **Supplemental Figure 1. Gating strategy for antigen-specific B cells.** Lymphocytes
509 were gated by FSC vs. SSC. Doublets were then excluded by FSC-A vs. FSC-H and
510 FSC-A vs. FSC-W. Live cells were identified as Ghost 510⁻ and total B cells were identified
511 as CD3⁻ CD19⁺. Naïve B cells were then identified as IgD⁺ CD27⁻ and excluded with a
512 boolean not gate. Memory B cells were identified as CD20⁺ CD38^{lo/int} non-naïve B cells.
513 A decoy SA-BV711 probe was used to gate out cells that non-specifically bind
514 streptavidin. Spike-and hemagglutinin-specific B cells were then identified based on their
515 binding to fluorescent probes. Spike⁺ cells were further analyzed for binding to fluorescent
516 RBD probe. Both spike⁺ and spike⁺/RBD⁺ cells were analyzed for IgG vs. IgM expression.
517

518 **Supplemental Figure 2. RBD-specificity of spike⁺ memory B cells.** Frequency of
519 RBD⁺ memory B cells over time in vaccinated individuals. Data are represented as
520 frequency of RBD-specific cells in the spike⁺ memory B cell compartment. RD = non-
521 vaccinated, SARS-CoV2 recovered donors. Dotted lines indicate the mean at baseline in
522 SARS-CoV2 naïve and SARS-CoV2 recovered individuals. Blue = SARS-CoV2 naïve +
523 mRNA vaccine, red = SARS-CoV2 recovered + mRNA vaccine, purple = SARS-CoV2
524 recovered. Statistics were calculated using unpaired Kruskal-Wallis or Wilcoxon test with
525 adjustment for multiple comparisons.
526

527 **Supplemental Figure 3. Sex and age subgroups have increased B cell responses**
528 **over pre-vaccine baseline. A, B)** Concentration of anti-spike and anti-RBD IgG
529 antibodies at baseline and post-boost compared with sex and age in SARS-CoV2 naïve
530 individuals. Dotted lines indicate the limit of detection for the assay. **C, D)** Frequency of
531 spike⁺ and spike⁺/RBD⁺ memory B cells at baseline and post-boost compared with sex
532 and age in SARS-CoV2 naïve individuals. Data are represented as frequency of spike⁺
533 and spike⁺/RBD⁺ cells in the total memory B cell compartment. Dotted lines indicate the
534 mean frequency of cells at baseline. **E)** Frequencies of total naïve B, non-naïve B, and
535 memory B cell populations compared with sex and age in SARS-CoV2 naïve individuals.
536

537 **Supplemental Figure 4. Association between vaccine-induced side effects and**
538 **short-term antibody responses. A)** Frequency of self-reported side effects in SARS-

539 CoV2 naïve individuals after the first and second dose of mRNA vaccine. Local side
540 effects include injection site pain, swelling, and redness. Systemic side effects include
541 fever, chills, headache, fatigue, and myalgia. **B)** Concentration of anti-spike and anti-RBD
542 IgG antibodies over time compared with self-reported side effects in SARS-CoV2 naïve
543 individuals. Dotted lines indicate the limit of detection for the assay. **C)** Frequency of
544 spike⁺ and spike⁺/RBD⁺ memory B cells over time compared with self-reported side
545 effects in SARS-CoV2 naïve individuals. Data are represented as frequency of spike⁺ and
546 spike⁺/RBD⁺ cells in the total memory B cell compartment. Post-boost indicates samples
547 collected at timepoint 4 (~7 days post-secondary vaccination). Statistics were calculated
548 using Wilcoxon test.

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596

Figure 1

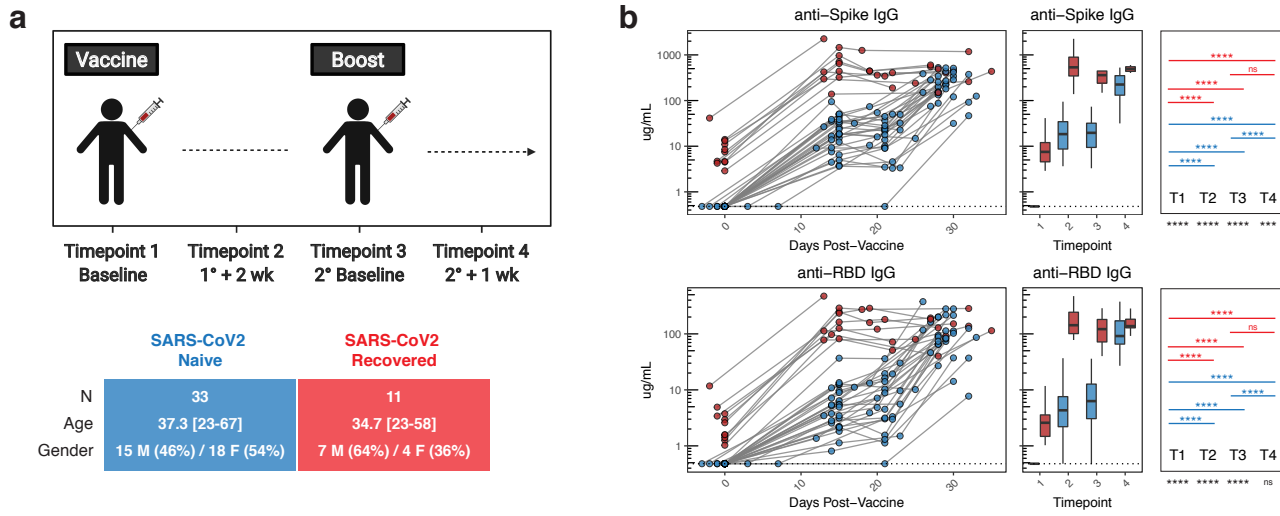


Figure 2

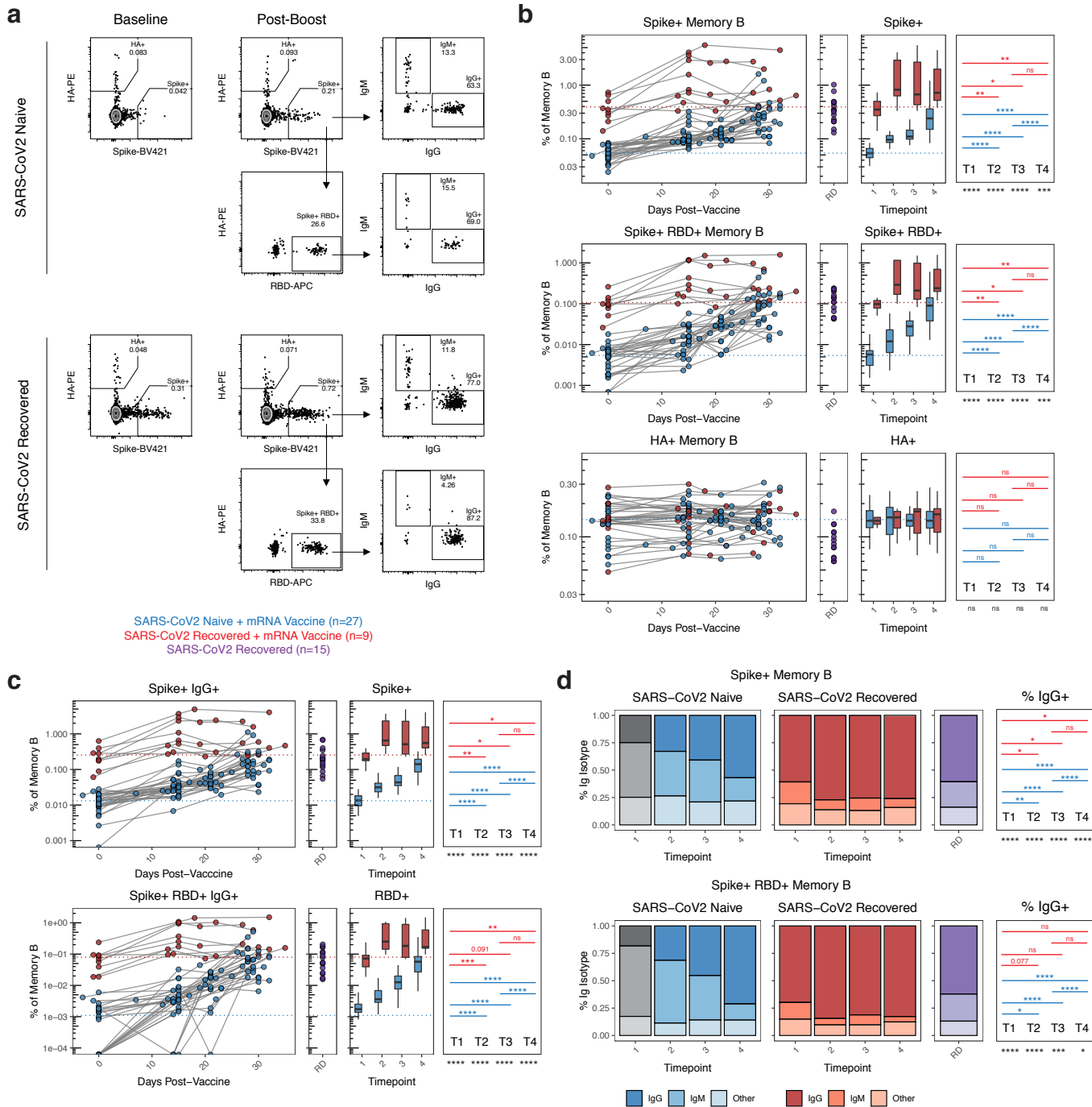


Figure 3

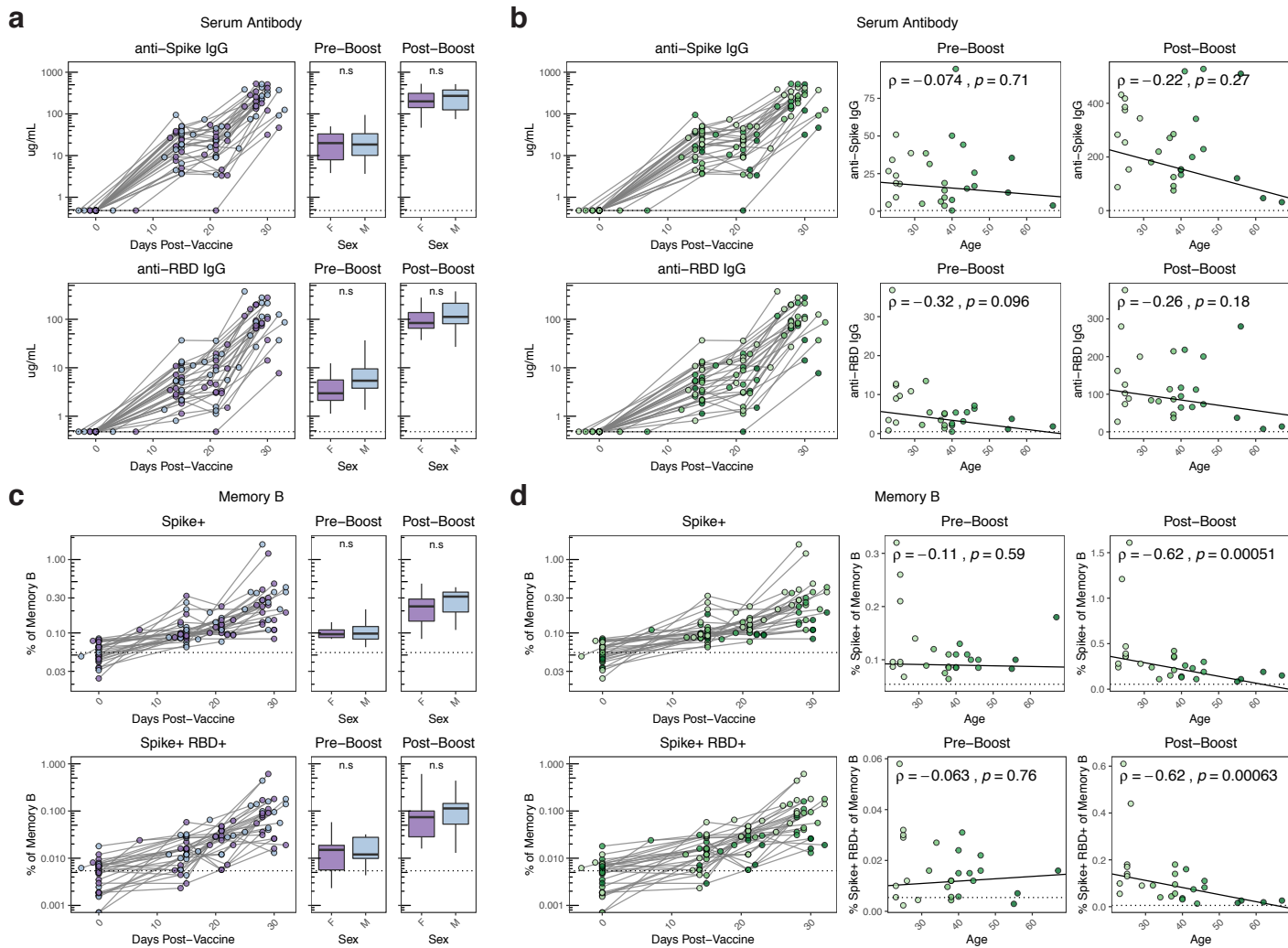


Figure 4

