Competent immune responses to SARS-CoV-2 variants in older adults following mRNA vaccination

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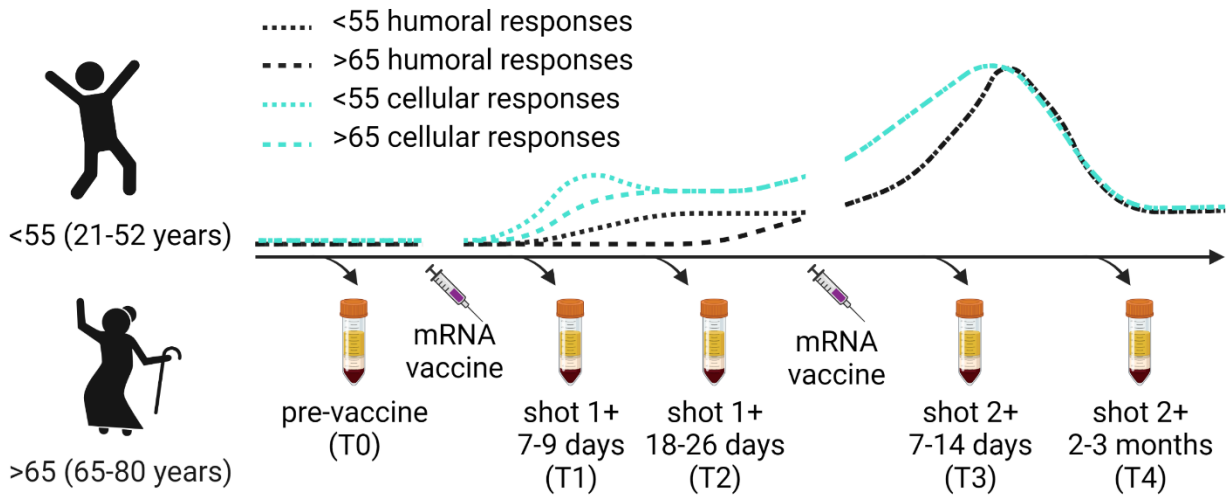
25 **Graphical Abstract**



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Abstract

Aging is associated with a reduced magnitude of primary immune responses to vaccination and constriction of immune receptor repertoire diversity. Clinical trials demonstrate high efficacy of mRNA based SARS-CoV-2 vaccines in older adults but concerns about virus variant escape have not been well addressed. We have conducted an in-depth analysis of humoral and cellular immunity against an early-pandemic viral isolate and compared that to the P.1. (Gamma) and B.1.617.2 (Delta) variants, as well to a B.1.595 SARS-CoV-2 isolate bearing Spike mutation E484Q, in <55 and >65 age cohorts of mRNA vaccine recipients. As reported, robust immunity required the second dose of vaccine. Older vaccine recipients exhibited an expected 3-5x reduction (but not a complete loss) in neutralizing antibody titers against both P.1. (Gamma) and the B.1.595 virus at the peak of the boosted response. However, older vaccinees manifest robust cellular immunity against early-pandemic SARS-CoV-2 and more recent variants, which remained statistically comparable to the adult group. While the duration of these immune responses remains to be determined over longer periods of time, these results provide reasons for optimism regarding vaccine protection of older adults against SARS-CoV-2 variants and inform thinking about boost vaccination with variant vaccines.

eTOC summary: Vaccine responses are often diminished with aging, but we found strong responses to SARS-CoV-2 in older adults following mRNA vaccination. T cell responses were not diminished when confronted by SARS-CoV-2 variants. Neutralizing Ab were reduced but not more than those in adults.

Introduction

RNA viruses have a high mutation rate resulting in diverse viral populations (Robson *et al.*, 2020). Since its emergence in the human population in late 2019, SARS-CoV-2 has infected more than 180 million people globally leading to the appearance of multiple new variants. Several variants of concern (VOC) have quickly become dominant in their countries of identification, spreading across the globe. B.1.1.7 (Alpha) was identified in the UK in December 2020 and spread to more than 90 other countries (Karim, 2021). Other identified VOC with broad spread include B.1.351 (Beta), P.1. (Gamma) and B.1.617.2 (Delta). These lineages are transmitted more efficiently, conferring evolutionary advantage over wild-type virus (Jewell, 2021). Two mRNA vaccines based on the first published genome sequence of SARS-CoV-2 (Wuhan/Hu-1/2019), BNT162b2 (Pfizer) and mRNA-1273 (Moderna), are being deployed to reduce COVID-19 disease severity and transmission, raising the obvious question of whether they will afford similar protection against variants of concern. Reduced plasma antibody (Ab) neutralization titers against pseudovirus in mRNA vaccine recipients has been reported for multiple variants (Garcia-Beltran *et al.*, 2021). Another report showed that B.1.351 (Beta) variant might be more effective in escaping humoral immunity than B.1.1.7 (Alpha) variant (Planas *et al.*, 2021), as one may expect from the location of its mutations to Ab-binding regions. Despite reduced humoral immunity, T cell responses to Alpha and Beta variants seem to be preserved in adult mRNA vaccine recipients (Geers *et al.*, 2021). Similar results were obtained with adenovirus vaccine recipients, which showed 3-5 fold lower neutralizing antibody titers against Beta and Gamma variants but uncompromised T cell responses (Alter *et al.*, 2021).

Efficacy and immunogenicity of many vaccines are known to be decreased in advanced age (Schmader *et al.*, 2012; Young *et al.*, 2017; Crooke *et al.*, 2019). Fortunately, SARS-CoV-2 mRNA based vaccines were shown to be both well tolerated and highly effective in older adults (Anderson *et al.*, 2020; Spector *et al.*, 2020; Li *et al.*, 2021). It remains unknown whether the breadth of the immune response to these mRNA vaccines will remain sufficient for protection against new viral variants.

85 To answer this, we have performed comprehensive analysis of antigen specific humoral
86 and cellular immune responses in adult mRNA vaccinees of different ages. For humoral
87 responses we compared a viral isolate representative of the early pandemic
88 (USA/WA1/2020, hereafter 'WA1') to the P.1. (Gamma) and a local Arizona virus variant
89 within lineage B.1.595 but, uniquely for that lineage, bearing Spike mutation E484Q
90 (USA/AZ-WL-CVG00126, hereafter 'AZ-E484Q'). For T cell responses, we compared
91 WA1 with both the P.1 (Gamma) and B.1.617.2 (Delta) variants. We show that the
92 magnitude and neutralization capacity of the overall antibody response to these
93 vaccines is not reduced in our older adult cohort (>65 years) as might have been
94 expected based on historic vaccine studies. Regarding variants, we confirm lower
95 neutralizing antibody titers against the P.1. (Gamma) variant and AZ-E484Q, with the
96 reduction more pronounced in our older cohort than in younger adults. Importantly,
97 robust cellular immunity against WA1, P.1. (Gamma), and B.1.617.2 (Delta) was
98 preserved in both adult and old mRNA vaccine recipients. Overall, the effect of age on
99 the immune response to the COVID mRNA vaccines was measurable, but smaller than
100 expected for humoral immunity, and equal to that found in adults for cellular immunity,
101 suggesting that these vaccines will provide considerable protection even as variants
102 continue to emerge.

103

104 **Results and Discussion**

105 A total of 25 participants were enrolled in our study before receiving either the
106 BNT162b2 (Pfizer) or mRNA-1273 (Moderna) COVID vaccine. Blood draws were
107 collected prior to initial dose of vaccine, 7-9, and 18-26 days after first vaccine dose,
108 and 7-14 days and 2-3 months after their second dose. These time points are
109 designated as T0, T1, T2, T3, and T4 on all graphs. One participant was excluded from
110 the study because they had a high neutralizing antibody titer and a strong antigen (Ag)
111 specific T cell response before vaccination, indicating prior infection. Our final cohort
112 included 15 participants aged 53 and younger (<55) and 9 participants over the age of
113 65 (>65).

114 Antibody ELISA assays for the receptor-binding domain (RBD) and S2 region of Spike
115 protein on plasma samples at each time point show similar rates and levels of
116 seroconversion between cohorts (Figure 1A, B). Area under curve (AUC) values confirm
117 that participants over age 65 have robust and comparable antibody responses to the
118 younger cohort with no significant differences measured at any time point (Figure
119 1C). Neutralizing antibody test titers utilizing WA1 were also comparable between both
120 cohorts across time points (Figure 2A). These data taken together are important
121 because they demonstrate that the humoral immune response in advanced age is
122 robust in response to COVID mRNA vaccines. We found a diminished capacity to
123 neutralize the P.1. (Gamma) variant and AZ-E484Q, with both being less well
124 neutralized at the third time point in the older cohort but only AZ-E484Q being less well
125 neutralized by the adult cohort (Figure 2A). E484K, a mutation in the receptor binding
126 domain (RBD) of Spike protein, is present in both B.1.351 (Beta) and P.1. (Gamma) and
127 has already been demonstrated to impact neutralization capacity (Wang *et al.*, 2021).
128 We chose AZ-E484Q to test against vaccine induced antibodies because it has Spike
129 E484Q and D614G mutations in common with B.1.617.1 (Kappa) but not L452R. L452R
130 has already been shown by others to impact neutralization in pseudotyped virus
131 systems (Li *et al.*, 2020) but it remained an open question how E484Q impacts
132 neutralization capacity (Verghese *et al.*, 2021). Our use of authentic B.1.595 SARS-
133 CoV-2, which in Spike bears only E484Q and D614G, demonstrates that E484Q also
134 affords the virus an opportunity to escape neutralization.

135 Antibody assays are considered the gold-standard when assessing the quality of
136 immunity after vaccination because antibodies can provide sterilizing immunity.
137 However, the establishment of memory B cell populations is also critical to lasting
138 vaccine efficacy as these are the resources available for response to the virus, or a
139 variant, upon next encounter. To assess whether there is an impact of age on the
140 formation of memory B cell populations we measured the frequency and number of
141 circulating memory B cells specific for SARS-CoV-2 pre-vaccination and one week after
142 booster dose by dual staining with tetramers specific for RBD and S1 using flow
143 cytometry (Shroff *et al.*, 2021) (gating strategy in Figure 3A). Both groups of participants
144 had a small (0.1-0.3% of all B cells) but detectable population of antigen specific cells

145 double positive for S1 and RBD tetramer (Figure 3A). This population increased as a
146 percentage of B cells post vaccination in all but two (one from each cohort) participants
147 with no difference between groups (Figure 3B). Similar results were obtained with S1
148 single-positive cells (Figure 3D) again with no difference between adult and older
149 participants post vaccination. By multiplying percentages of tetramer positive B cells
150 with total B cell percentage and lymphocyte counts we have calculated the absolute
151 numbers of SARS-CoV-2 specific B cells in circulation and again observed no difference
152 between cohorts for S1+RBD+ double positive cells (Figure 3C) or S1 single positive
153 cells (Figure 3E). Next, we examined the phenotype of the SARS-CoV-2 specific B cells
154 (gating strategy in Figure S1) with respect to class switching, as it has been previously
155 reported that aging is associated with a decline in the percentage and numbers of
156 switched memory B cells (Frasca *et al.*, 2008). To investigate this possibility, we
157 examined differentiation and class switching of total tetramer (S1+) positive B cells by
158 flow cytometric staining for CD27, IgM, IgD, CD21 and CD11c (representative flow
159 cytometric gating in Figure S1A). Ag-specific B cells from adult and older participants
160 expressed CD27 at identical levels (Figure S1B) and equal numbers of both CD27
161 positive and negative cells were class switched (Figure S1C,D). Adult and older
162 participants also displayed no difference in classical memory (CD21+) phenotype
163 among the class switched tetramer-positive cells (Figure S1E). Thus overall, we
164 conclude that induction and differentiation of SARS-CoV-2 specific B cells through
165 vaccination was not compromised by aging.

166 Finally, we measured antigen-specific T cells prior to, and at all time points available
167 post, vaccination. Given that ELISpot was previously reported to be a highly sensitive
168 method for detection of rare antigen specific T cells (Chauvat *et al.*, 2014) we
169 simultaneously measured the number of T cells expressing costimulatory molecules
170 CD137 and OX-40 by flow cytometry (used in several reports examining SARS-Cov-2
171 specific T cell immunity - (Grifoni *et al.*, 2020; Rydyznski Moderbacher *et al.*, 2020)
172 (representative flow cytometry in Figure S2A) and performed IFN-γ ELISpot on PBMCs
173 from select participants stimulated with peptide pools corresponding to the Spike protein
174 of WA1. Confirming prior data, ELISpot proved to be a much more sensitive method for
175 enumeration of Ag-specific T cells. We detected a statistically significant increase in

176 ELISpots per 10^6 PBMC's after vaccination analyzing just 5 samples, whereas parallel
177 flow cytometry samples showed no significant differences (Figure S2B-E). The only
178 limitation of ELISpot, as compared to flow cytometry based enumeration, is that total T
179 cell responses are measured without separate quantification of CD4 and CD8
180 responses.

181 We also analyzed Ag-specific T cell responses to stimulation with Spike protein peptide
182 pools from WA1 versus two variants of concern by ELISpot. Participant PBMC's were
183 stimulated with 16-mer overlapping peptide pools corresponding to the spike protein of
184 WA1, P.1. (Gamma) and B.1.617.2 (Delta). In accordance with previously published
185 results (Sahin *et al.*, 2020), mRNA vaccines induced a robust T cell response to WA1,
186 an early 2020 virus, which did not differ for Gamma and Delta variants, as evidenced by
187 a 10-fold increase in ELISpots from post-vaccination samples stimulated by S peptide
188 pools compared to unstimulated wells (Figure 4A). Next, we analyzed T cell responses
189 of both groups at different time points. Given that some samples had high responses in
190 the unstimulated wells we subtracted the number of ELISpots from the unstimulated
191 well to calculate the number of Ag-specific ELISpots for each time point. Data from aged
192 mice (Brien *et al.*, 2009; Fulton *et al.*, 2013; Jergović *et al.*, 2019) showed that induction
193 of Ag-specific T cell responses becomes delayed and decreased with age. In our data,
194 there was a slightly lower response in the older cohort at day 7 post first dose with all
195 three variants but it was not statistically significant (Figure 4B). However, after a second
196 (booster) dose both groups had a very robust T cell response against all three viral
197 variants examined (Figures 4C, D, E). Therefore, while we acknowledge the possibility
198 that the initiation of the Ag-specific T cell response may be delayed in some of our older
199 participants, we conclude that the outcome after booster dose is competent T cell
200 mediated immunity against WA1 and variants of concern virus in all mRNA vaccine
201 recipients in our study.

202 It is well established that primary immune responses wane with age and contribute to
203 the increased susceptibility to infection experienced by older adults (reviewed in
204 Nikolich-Žugich, 2018). There is also evidence that generation of immune memory in
205 older adults is reduced, but not maintenance of memory. Studies with multiple

206 pathogens have shown decreased T cell receptor repertoire with age (Yager *et al.*,
207 2008; Smithey *et al.*, 2018) which could mean easier escape from existing immunity for
208 pathogen variants. All of the above-mentioned findings warrant an extensive and long
209 term monitoring of immunity in SARS-CoV-2 vaccinees, especially those over age 65.

210 The emergence of SARS-CoV-2 effected older adults especially hard with more than
211 80% of deaths in those over age 65 (Lithander *et al.*, 2020). Vaccination reduces these
212 rates dramatically (Haas *et al.*, 2021) demonstrating that the principles of immunology
213 hold true for this virus and that immune memory is what our species is lacking. Well
214 tolerated and effective, SARS-CoV-2 mRNA vaccines induce potent humoral and
215 cellular immune responses (Anderson *et al.*, 2020; Sahin *et al.*, 2020). Their deployment
216 also offers an opportunity to establish, in a truly immune naive population, correlates,
217 and contours, of protective immunity. Recent elegant studies in rhesus macaques have
218 shown that even sub-sterilizing neutralizing antibody titers are protective and decrease
219 SARS-CoV-2 severity (McMahan *et al.*, 2021). The same research study demonstrated
220 a protective role for T cell memory responses and this is supported by human research
221 showing that accumulation of oligoclonal CD8 T cells in bronchoalveolar lavage fluid
222 inversely correlated with disease severity (Liao *et al.*, 2020). Recently, Collier et al.
223 analyzed 102 partially and 38 fully vaccinated subjects and concluded that subjects >80
224 years of age produced lower primary, but not secondary (boosted) antibody neutralizing
225 responses, including those against variants (Collier, Ferreira *et al.* 2021). They did not
226 analyze T cell immunity against variants and, somewhat curiously, did not observe
227 increased T cell responses following boosting in their older groups (>80). These authors
228 argue that older adults – those over 80 – remain vulnerable at least until they receive
229 the second vaccine dose. While this study with larger cohorts highlights the potential
230 importance of boosting the antibody response in the oldest adults, the key issue
231 remains that of biological relevance of the totality of measured differences. Given the
232 published and above-cited literature, we suggest that a decrease in neutralizing titer to
233 viral variants, accompanied by robust immune memory responses to both WA1 and the
234 variants, shown in this study for both B and T cells, gives reasons for optimism relative
235 to protection of older adults. This is supported by the efficacy of the mRNA vaccines in
236 protecting this vulnerable group. The decrease in antibody titer when challenged with

more recent SARS-CoV-2 variants does suggest that the breadth of immunity may be more narrow in advanced age; a challenge that can be met with booster doses of heterologous sequence virus. Further studies on the durability and breadth of protection by current and future heterologous vaccines in older adults will be necessary to answer all the germane questions on their immunity and SARS-CoV-2 protection.

Materials and methods

Study participants

This study was approved by the University of Arizona IRB (Protocol#2102460536). We collected blood from 16 adults <55 years old (mean age 31.3 ± 6.2) and 9 above 65 years old (71 ± 5.2). Demographics are provided in Table I. Samples for all participants were collected prior to vaccination, one week after first dose (mRNA vaccine: Pfizer N=15; Moderna N=10), day before booster dose and 7-10 days after booster. A smaller number of participants was sampled 3 months following first dose. The time points are labeled T0, T1, T2, T3, and T4 in all graphs. Blood for complete blood count was collected in BD vacutainer with EDTA and submitted to Sonora Quest Laboratories (Arizona). Blood for peripheral blood mononuclear cells (PBMCs) and plasma was collected in BD Vacutainer with sodium heparin. Plasma was separated by centrifugation at 1100 rpm for 10 minutes and PBMC was isolated from the buffy coat by Ficoll-Paque PLUS (GE Healthcare) and cryopreserved in fetal calf serum + 10% DMSO.

Virus

SARS-Related Coronavirus 2 (SARS-CoV-2), Isolate USA-WA1/2020, was deposited by Dr. Natalie J. Thornburg at the Centers for Disease Control and Prevention and obtained from the World Reference Center for Emerging Viruses and Arboviruses (WRCEVA). P.1. (Gamma) variant was obtained through BEI Resources, NIAID, NIH: SARS-Related Coronavirus 2, Isolate hCoV-19/Japan/TY7-503/2021, NR-54982, contributed by National Institute of Infectious Diseases. AZ-E484Q, a B.1.595 virus (GISAID: EPI_ISL_765942) was grown from a nasopharyngeal swab on Calu-3 cells for 48 hours. AZ-E484Q has Spike D614G and E484Q mutations but has not emerged as a variant of concern. Stocks of SARS-CoV-2 were generated as a single passage from received stock vial (USA-WA1/2020 and P.1.) or primary culture (B.1.595) on mycoplasma negative Vero cells (ATCC #CCL-81) at a MOI of 0.005 for 48 h. Supernatant and cell lysate were combined, subjected to a single freeze-thaw, and then centrifuged at 3000RPM for 10 min to remove cell debris.

273

274 **Plaque Reduction Neutralization Test**

275 A plaque reduction neutralization test (PRNT) for WA1 and SARS-CoV-2 variants was
276 performed as described in Ripperger *et al.*, 2020. Briefly, mycoplasma negative Vero
277 cells (ATCC # CCL-81) were plated in flat bottom, 96 well, tissue culture plates and
278 grown overnight. Three-fold serial dilutions (1:20 - 1:43,740) of plasma/serum samples
279 were incubated with 100 plaque forming units of virus for 1 h at 37°C. Plasma/serum
280 dilutions plus virus were transferred to the cell plates and incubated for 2 h at 37°C, 5%
281 CO2 then overlayed with 1% methylcellulose in media. After 96 hours, plates were fixed
282 with 10% Neutral Buffered Formalin for 30 min and stained with 1% crystal violet.
283 Plaques were imaged using an ImmunoSpot Versa (Cellular Technology Limited) plate
284 reader. The serum/plasma dilution that contained 10 or less plaques was designated as
285 the NT90 titer.

286 **ELISpot assays**

287 T cell specific immunity to peptide pools corresponding to Spike, Nucleocapsid, and
288 Matrix proteins were measured as previously described (Shroff *et al.*, 2021). Briefly,
289 frozen PBMCs were thawed and rested overnight incubated in 24-well plates overnight
290 at 37°C with 5% CO2. Following day cells were stimulated in X-VIVO 15 media with 5%
291 male human AB serum containing ~1 nmol of peptide pool corresponding of SARS-
292 CoV-2 Prot S of US-WA (wild-type), Gamma (P.1./B1.1.28) and Delta (B.1.617.2)
293 variants or positive control anti-CD3 mAb CD3-2 from or blank media as negative
294 control. Overlapping 16mer peptide pools were purchased from 21st century
295 Biochemicals,Inc. Cell suspensions were transferred to pre-coated Human IFN-γ
296 ELISpot plus kit (Mabtech) and developed after 20h according to manufacturer
297 instructions. Spots were imaged and counted using an ImmunoSpot Versa (Cellular
298 Technology Limited).

299 **Antibody ELISA**

300 Serological assays were performed exactly as previously described in Shroff *et al.*,
301 2021). Area under the curve (AUC) values were calculated using R version 4.0.3 (R
302 Core Team, 2020) and the DescTools package (Signorelli, 2021).

303 **Flow cytometry**

304 Cryopreserved PBMC (2-5 x 10^6/sample) were thawed in prewarmed RPMI-1640 with L-
305 glutamine (Lonza) + 10% FCS. Thawed PBMCS were rested overnight at 37 ^{o}C in X-
306 VIVO 15 Serum-free Hematopoietic Cell Medium (Lonza) supplemented with 5% human
307 Ab serum. Cells were stained with surface antibodies in PBS (Lonza) + 2% FCS, and
308 then stained with the live dead fixable blue dye (Thermofisher). Samples were acquired
309 using a Cytek Aurora cytometer (Cytek) and analyzed by FlowJo software (Tree Star).

310 **Statistical analysis**

311 SPSS and Graph Pad Prism were used for statistical analysis. Upon inspection of data
312 distribution by group differences were calculated either by Mann Whitney U-test, one-
313 way ANOVA or Kruskal Wallis test with post hoc correction. For all statistical differences
314 *$p<0.05$, **$p<0.01$, ***$p<0.001$. ****$p<0.0001$.

315

316 **Competing Interest Statement**

317 J.N.Ž. is co-chair of the scientific advisory board of and receives research funding from
318 Young Blood Institute, Inc. Sana Biotechnology has licensed intellectual property of D.B.
319 and Washington University in St. Louis. D.B. is a co-founder of Clade Therapeutics.
320 B.J.L. has a financial interest in Cofactor Genomics, Inc. and Iron Horse Dx.

321

322

Table I – Demographic characteristics of the cohort

	<55 yrs (N=16)	>65 yrs (N=9)
Gender [N (%)]		
Male	5 (31.3)	3 (33.3)
Female	11 (68.8)	6 (66.7)
Age [mean (range)]	33.3 (24-52)	71 (65-80)
Race [N (%)]		
White	8 (50)	7 (77.8)
Asian	8 (50)	1 (11.1)
Unknown	0	1 (11.1)
Vaccine Type [N (%)]		
Pfizer	9 (56.3)	3 (33.3)
Moderna	7 (43.8)	6 (66.7)

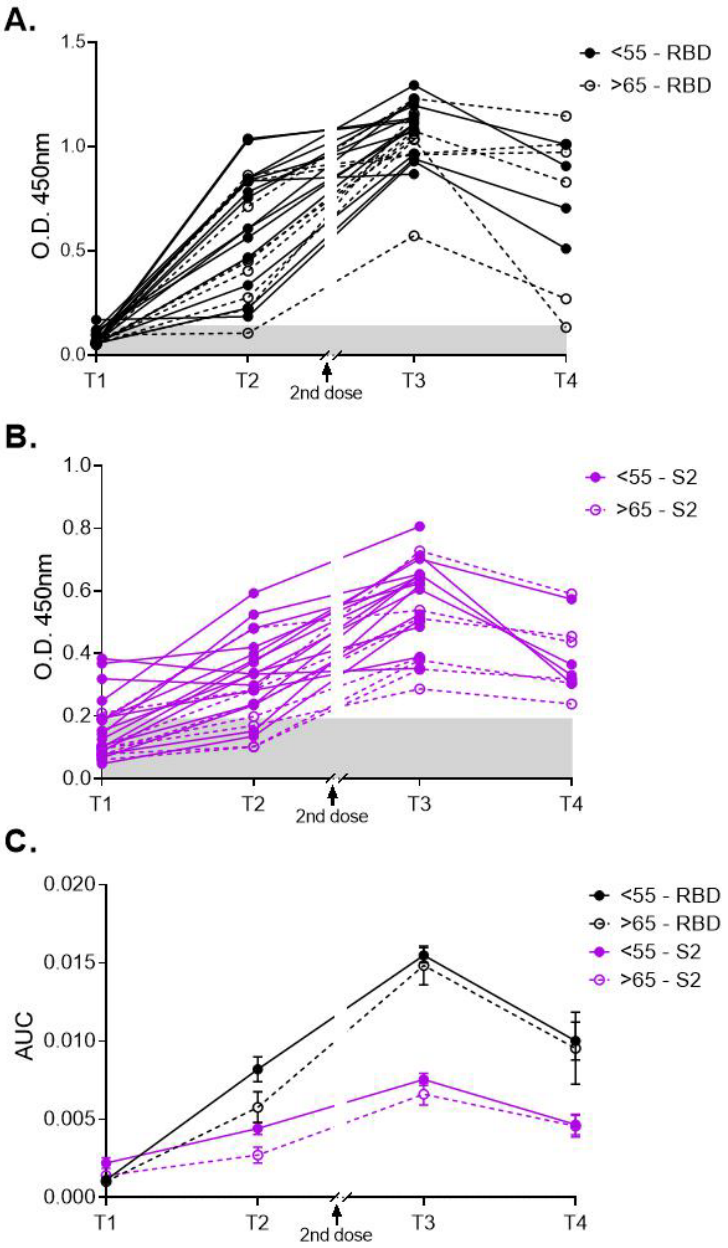
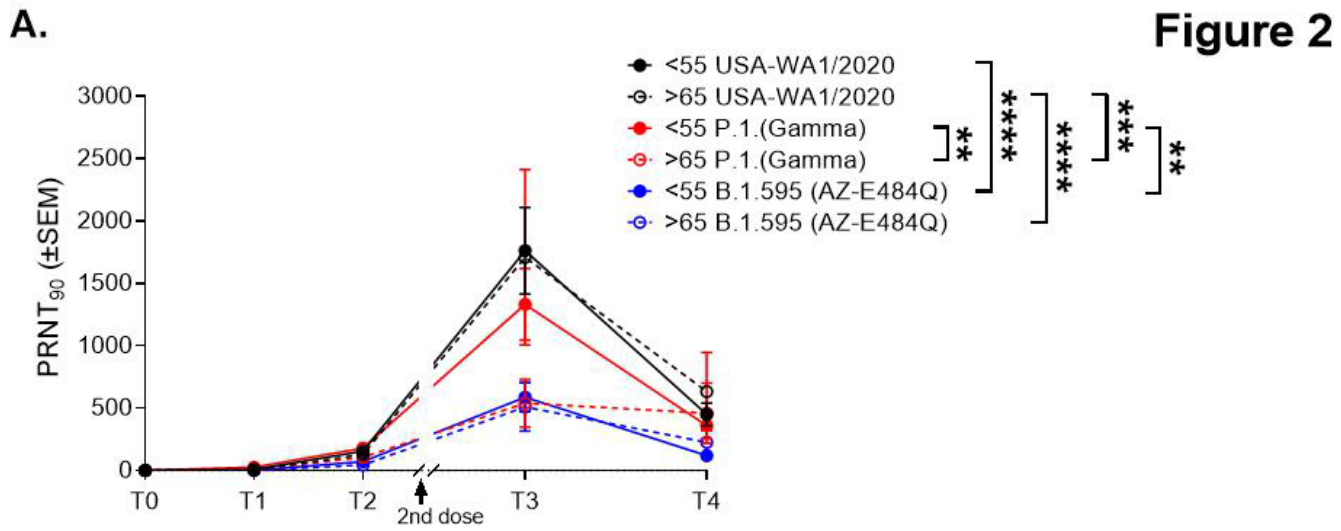


Figure 1. Antibody responses to mRNA vaccines are comparable regardless of age.

Time points denote days 7-9 (T1) and 18-26 (T2) days post first and 7-14 days (T3) and 2-3 months (T4) post second vaccine dose. Semi-quantitative 1:60 serum dilution ELISA results for reactivity to RBD **(A)** and S2 **(B)** of SARS-CoV-2 Spike protein. Individuals are connected across time points. Gray shaded area indicates positivity threshold for each assay. There is no statistical difference (Two-way ANOVA with Šídák's multiple comparisons test) between age groups at any time point measured. **C)**

342 Quantitative titers for RBD and S2 were calculated for each individual at each time point
343 and shown as area under the curve (AUC) values. There is no statistical difference
344 (Two-way ANOVA with Šídák's multiple comparisons test) between age groups at any
345 time point measured.
346



347

348 **Figure 2**

349 **mRNA vaccines induce robust neutralization titers in adults under 55 and over 65**
350 **that is variably affected, but not abolished, by SARS-CoV-2 variants.**

351 Time points are pre-bleed (T0); days 7-9 (T1) and 18-26 (T2) days post first dose of
352 vaccine; and 7-14 days (T3) and 2-3 months (T4) after second dose of vaccine. Virus
353 neutralization assays were performed using the USA-WA1/2020, P.1. (Gamma), and
354 AZ-E484Q isolates of SARS-CoV-2. Serial 1:3 dilutions of plasma were assayed for
355 neutralization capacity on Vero cells. The highest dilution capable of preventing >90% of
356 plaques was considered to be the PRNT90 value. Statistical significance was assessed
357 by Two-way ANOVA with Tukey's multiple comparisons test and determined significant
358 only at time point 3 as represented to the right of the legend. Neutralizing titers were
359 comparable between the <55 and > 65 cohorts to USA-WA1/2020; reduced against P.1.
360 (Gamma) as compared to WA1 for only the >65 cohort; and reduced in both cohorts
361 against B.1.595.

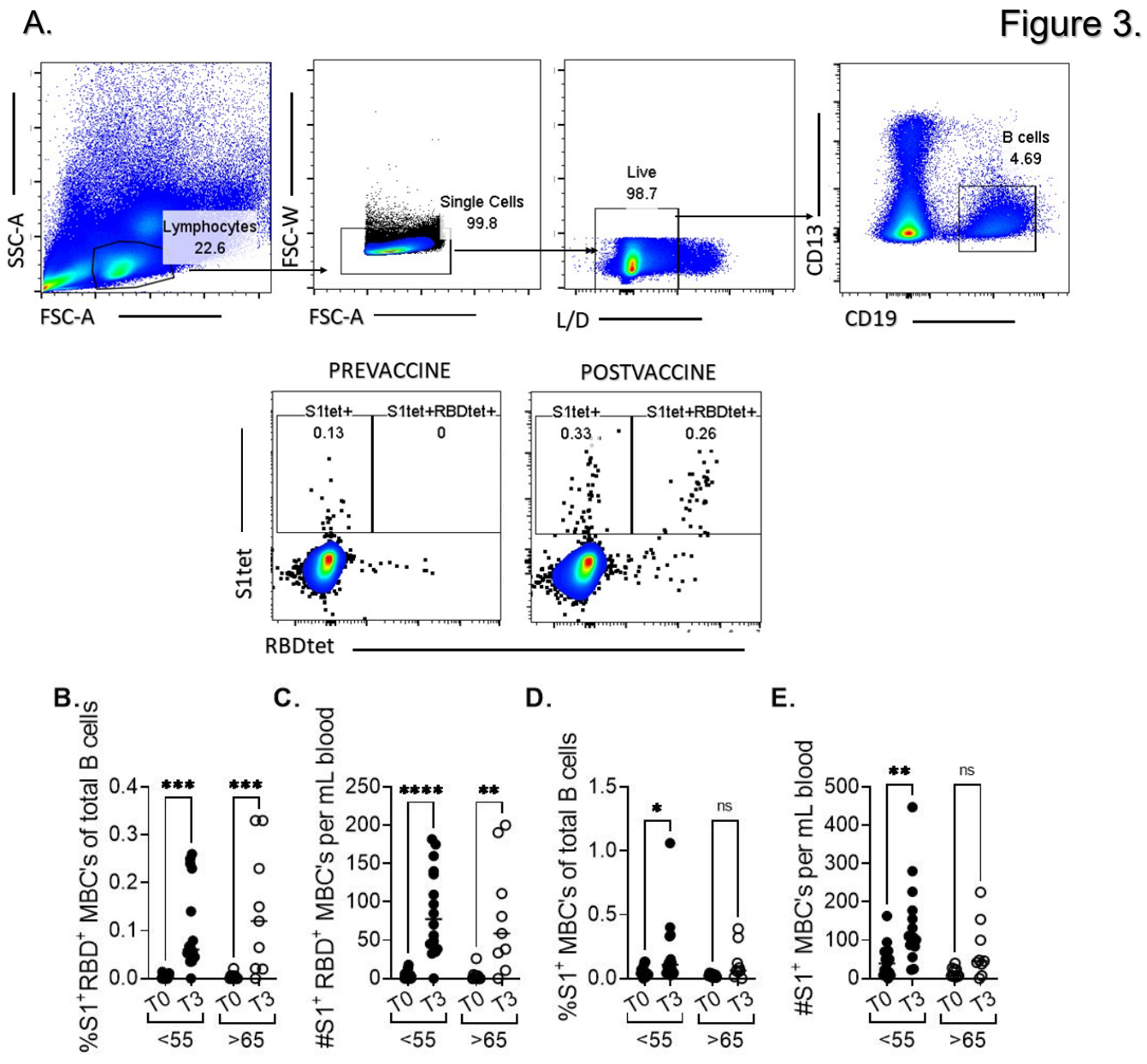
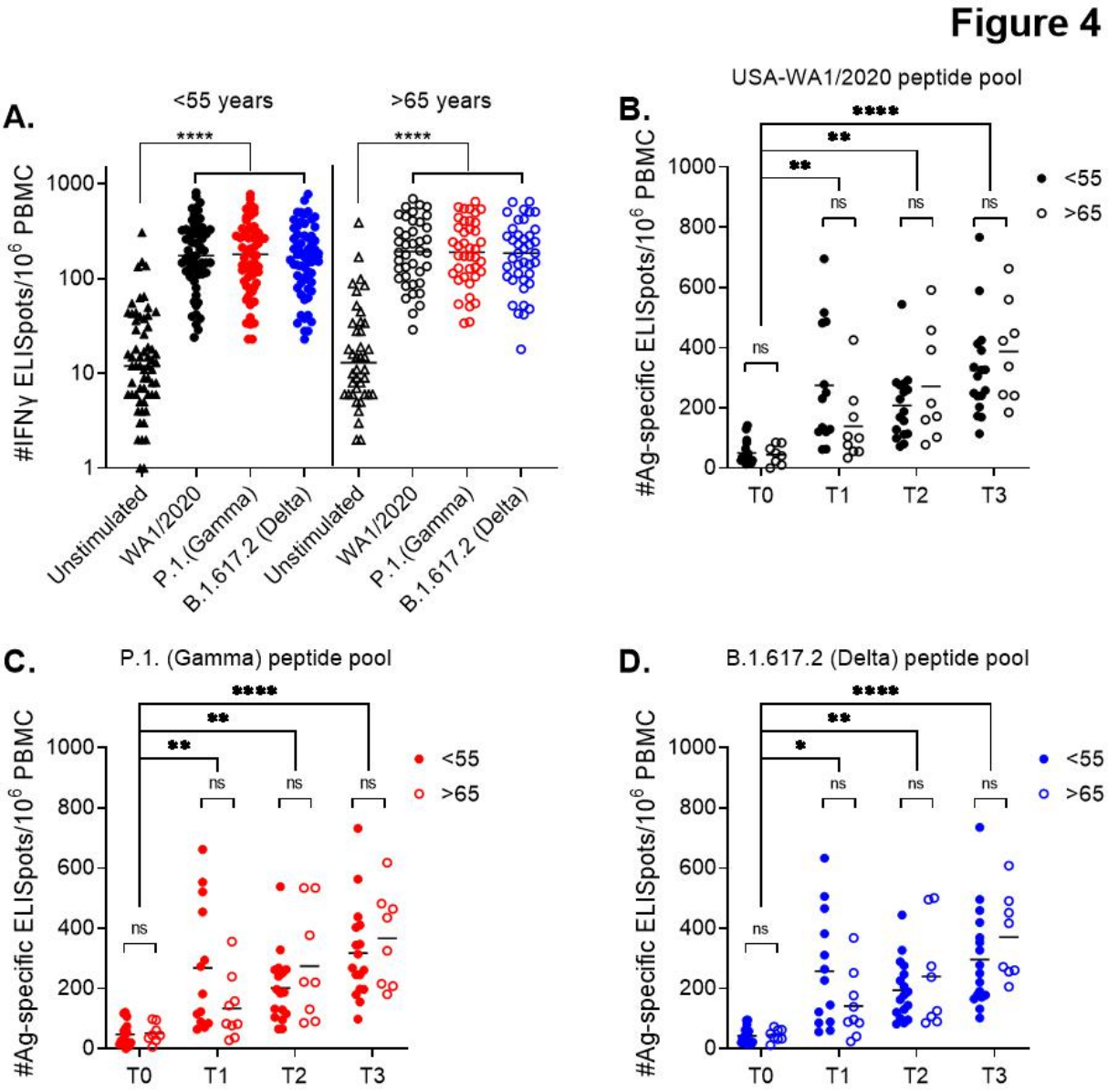


Figure 3. mRNA vaccines induce SARS-CoV-2 specific memory B cells in adult and old vaccinee cohorts. A) Representative flow cytometric gating after gating on total lymphocytes. Doublets and dead cells were excluded and percentage of S1 and RBD tetramer positive cells was analyzed on total CD19+ B cells; **B)** Percentage of tetramer double positive (S1+RBD+) B cells was increased after vaccine booster dose (T3) compared to pre-vaccination (T0) and to an equal extent in adult and old groups **C)** absolute numbers of S1+RBD+ B cells were also equally increased in <55 and >65 cohorts. **D)** Percentage of S1+ tetramer single positive B cells was equally increased in

372 <55 and >65 cohorts; **E)** as was their absolute number per ml of blood. Mann-Whitney U
373 test, for all statistical differences *p<0.05, **p<0.01, ***p<0.001. ****p<0.0001.



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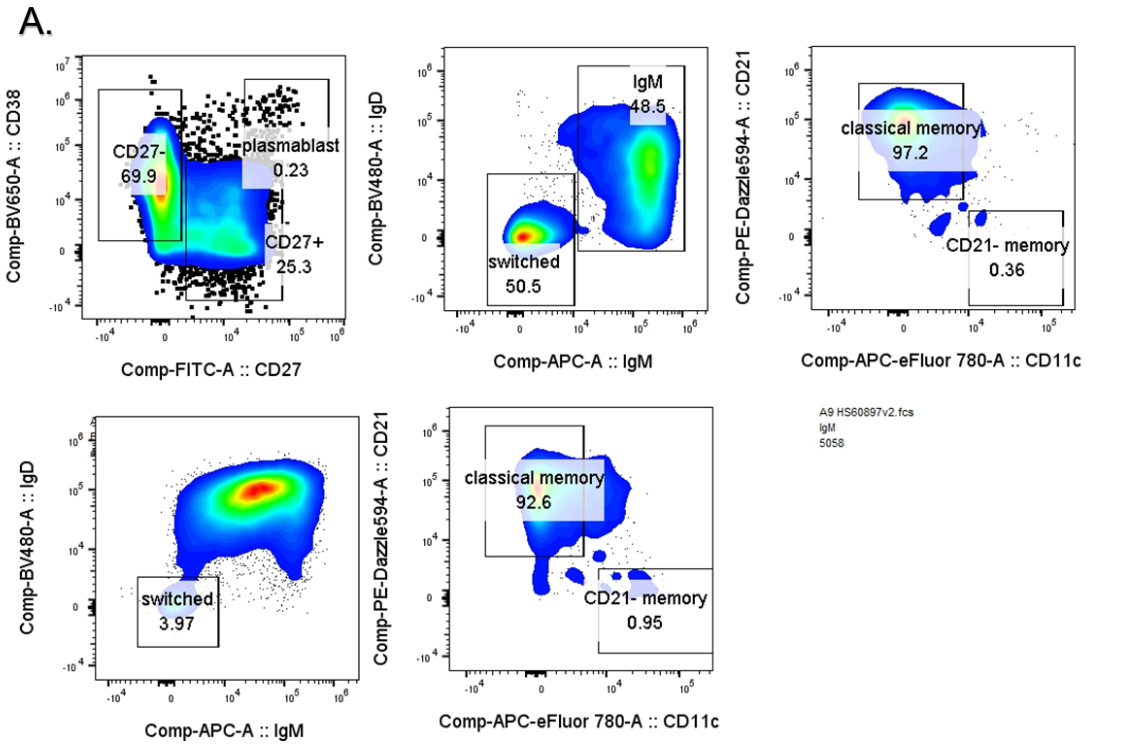
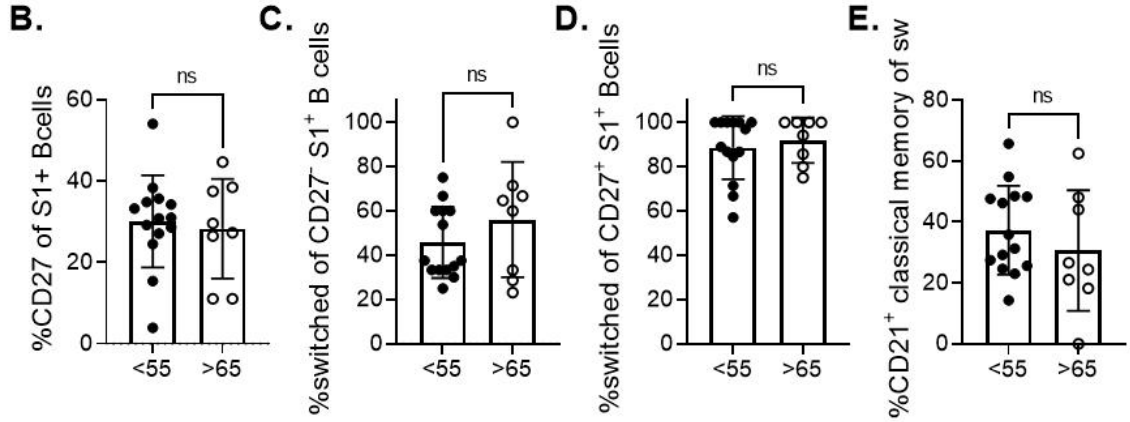
375 **Figure 4. mRNA vaccines induce SARS-CoV-2 specific T cells reactive to Spike**
376 **peptide pools from all three variants A)** 10^6 PBMCs per well were stimulated with
377 Spike peptide pools from USA-WA1/2020, P.1 (Gamma), and B.1.617.2 (Delta) cultured
378 overnight in pre-coated IFN-γ ELISpot plates. All three peptide pools induced an equally
379 strong IFN-γ response compared to unstimulated wells **B)** Ag-specific ELISpot numbers

were calculated by subtracting the unstimulated wells of each participant from the peptide stimulated wells. USA-WA1/2020 induced an equal number of ELIspots in <55 and >65 cohorts at each time point. **C)** Similarly, P.1 (Gamma) peptide pool induced a strong response at all time points post-vaccination which did not differ between <55 and >65 cohorts. **D)** Identical results were obtained with stimulation with B.1.617.2 (Delta) pool. Kruskal Wallis test with Dunn's post hoc correction. For all statistical differences *p<0.05, **p<0.01, ***p<0.001. ****p<0.0001.

Figure S1.





S1. SARS-CoV-2 specific memory B cells differentiate equally in <55 and >65 cohorts. A) Representative flow cytometric gating to examine B cell differentiation. **B)** Percentage of CD27 expressing B cells among tetramer positive cells was equal between cohorts. **C & D)** Equal percentage of SARS-CoV-2 specific B cells were class switched among CD27 positive and negative cells between <55 and >65 cohorts. **E)** Expression of CD21 (Classical memory) was also equal between <55 and >65 cohorts. Mann-Whitney U test, for all statistical differences *p<0.05, **p<0.01, ***p<0.001. ****p<0.0001.

Figure S2.

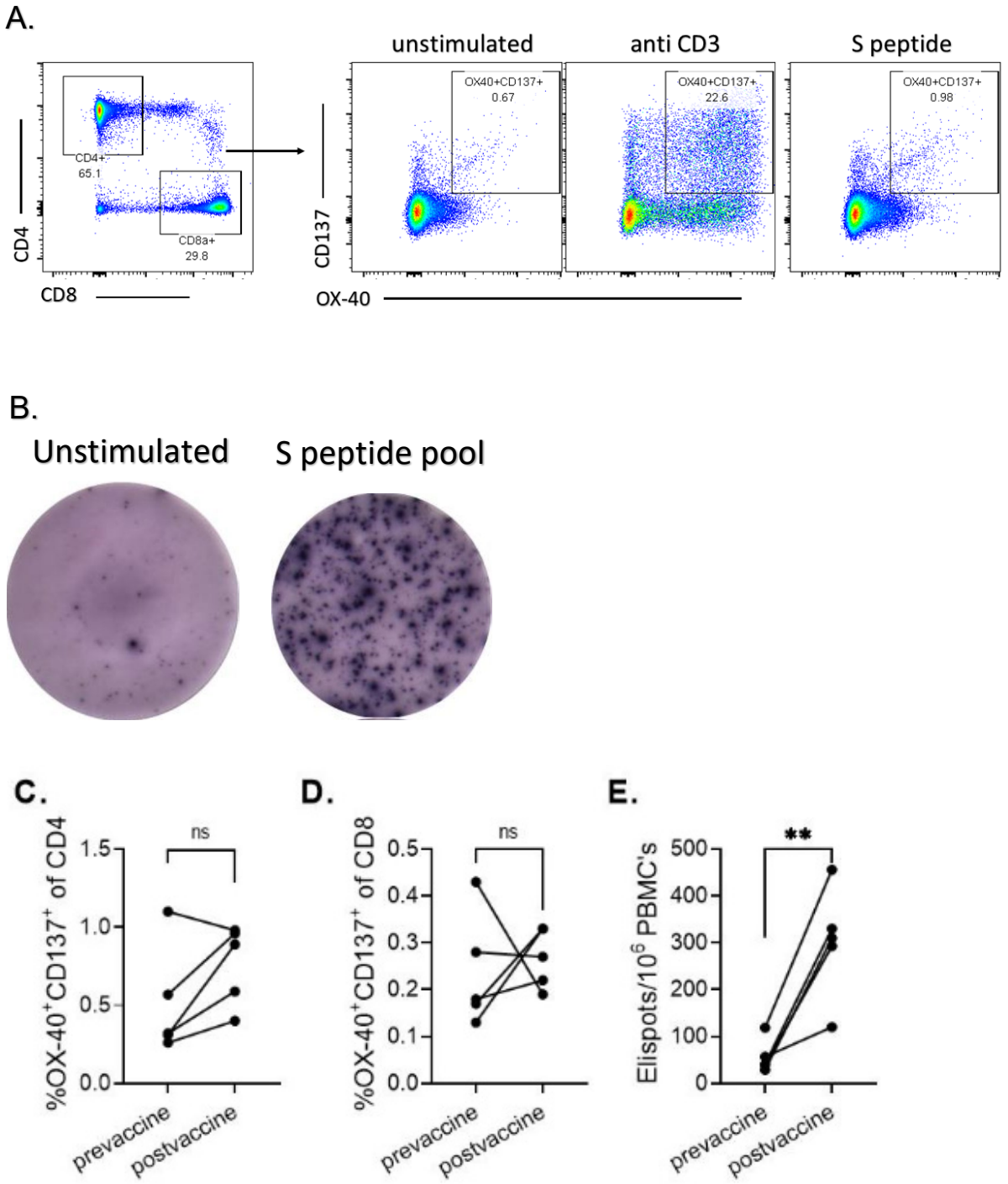


Figure S2. ELISpot is more specific method for enumeration of SARS-CoV-2 specific T cells. A) Representative flow cytometric gating for identifying CD137+OX-

422 40+ double positive CD4 and CD8 T cells. **B)** Representative unstimulated and Spike
423 protein peptide pool stimulated ELISpot wells. PBMCS from 5 participants were
424 stimulated overnight with USA-WA1/2020 spike peptide pool. SARS-CoV-2 specific T
425 cells were enumerated by either flow cytometry **(C,D)** or ELISpot **(E)**. ELISpot proved to
426 be a more sensitive method with statistically significant difference before and after
427 vaccination with N=5. Mann-Whitney U test, for all statistical differences *$p<0.05$,
428 **$p<0.01$, ***$p<0.001$. ****$p<0.0001$.

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