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

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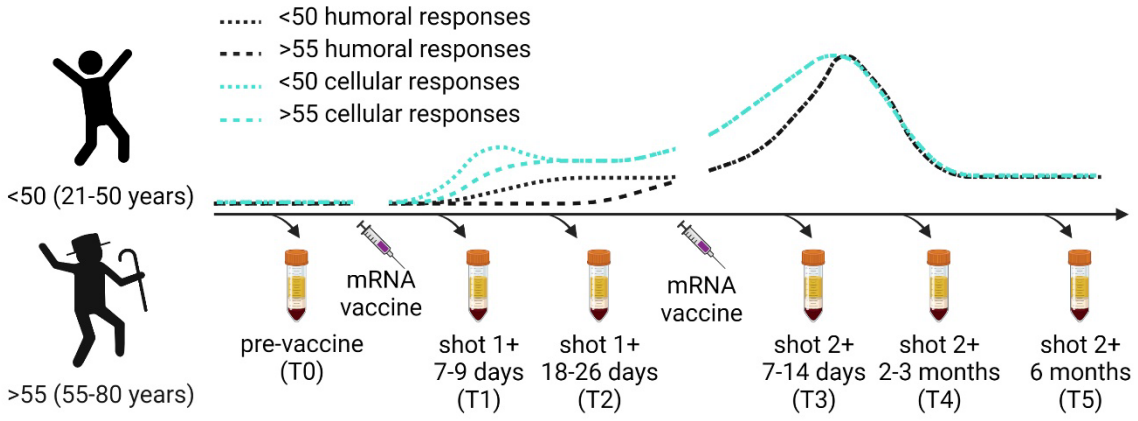
25 **Graphical Abstract**



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28

Abstract

Aging is associated with a reduced magnitude of primary immune responses to vaccination and constriction of immune receptor repertoire diversity. Clinical trials demonstrated 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 in <50 and >55 age cohorts of mRNA vaccine recipients. We have further measured neutralizing antibody titers for B.1.617.1 (Kappa) and B.1.595; a SARS-CoV-2 isolate bearing Spike mutation E484Q. As reported, robust immunity required the second dose of vaccine. Older vaccinees manifested robust cellular immunity against early-pandemic SARS-CoV-2 and more recent variants, which remained statistically comparable to the adult group. The older cohort had lower neutralizing capacity at the first time point following the second dose, but at later time points immunity was indistinguishable between them. 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 295 million people globally leading to the appearance of multiple new variants. Several variants of concern (VOC) quickly became 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 ancestral 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 were 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 help answer this, we have performed comprehensive analysis of antigen specific
86 humoral and cellular immune responses in adult mRNA vaccinees of different ages. For
87 humoral responses we compared a viral isolate representative of the ancestral virus
88 (USA/WA1/2020, hereafter 'WA1') to P.1. (Gamma), B.1.617.1 (Kappa), and B.1.617.2
89 (Delta) and an additional local Arizona virus variant within lineage B.1.595 but, uniquely
90 for that lineage, bearing Spike mutation E484Q (USA/AZ-WL-CVG00126, hereafter 'AZ-
91 E484Q'). For T cell responses, we compared WA1 with both the P.1 (Gamma) and
92 B.1.617.2 (Delta) variants. We show that the magnitude and neutralization capacity of
93 humoral memory to these vaccines is not reduced in our older adult cohort (>55 years)
94 as might have been expected based on historic vaccine studies. Regarding variants, we
95 confirm lower neutralizing antibody titers against P.1. (Gamma), B.1.617.1 (Kappa), and
96 AZ-E484Q, but not B.1.617.2 (Delta), in our older cohort. Importantly, robust cellular
97 immunity against WA1, P.1. (Gamma), and B.1.617.2 (Delta) was preserved in both
98 cohorts of mRNA vaccine recipients. Overall, the effect of age on the immune response
99 to the COVID mRNA vaccines was measurable, but minimal. It was most evident in the
100 antibody response at the first time point after the second dose. For cellular immunity
101 responses were essentially equivalent between the two cohorts. These results
102 demonstrate that effective immunity in older adults is attainable with mRNA vaccines.

103

104 **Results and Discussion**

105 A total of 40 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, 2-3 months, and 6 months after their second dose. These time points
109 are designated as T0, T1, T2, T3, T4, and T5 on the graphs. One participant was
110 excluded from the study because they had a high neutralizing antibody titer and a
111 strong antigen (Ag) specific T cell response before vaccination, indicating prior infection.
112 Our final cohort included 23 participants under 50 years of age (<50) and 17 participants
113 over the age of 55 (>55).

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 55 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 older adults is
122 preserved to these mRNA vaccines when tested with the virus they were generated
123 against. We found a diminished capacity to neutralize the P.1. (Gamma), AZ-E484Q,
124 and B.1.617.1 (Kappa) but not to B.1.617.2 (Delta) at the third time point with equivalent
125 neutralization in memory (Figure 2A and Table 1). Our <50 cohort demonstrated
126 reduced neutralization against AZ-E484Q, B.1.617.1 (Kappa), and B.1.617.2 (Delta)
127 (Figure 2A and Table 1). E484Q, a mutation in the receptor binding domain (RBD) of
128 Spike protein, is present in both B.1.351 (Beta) and P.1. (Gamma) and has already
129 been demonstrated to impact neutralization capacity (Wang *et al.*, 2021). We chose
130 AZ-E484Q to test against vaccine induced antibodies because it has Spike E484Q and
131 D614G mutations in common with B.1.617.1 (Kappa) but not L452R. L452R has already
132 been shown by others to impact neutralization in pseudotyped virus systems (Li *et al.*,
133 2020) but it remained an open question how E484Q impacts neutralization capacity
134 (Verghese *et al.*, 2021). Our use of authentic B.1.595 SARS-CoV-2, which in Spike
135 bears only E484Q and D614G, demonstrates that E484Q also affords the virus an
136 opportunity to escape neutralization. Understanding how this particular mutation
137 impacts neutralization may be important during the emergence of future variants.
138 Finally, we compared neutralizing antibody titers between vaccine brands (n=16
139 Moderna, n=21 Pfizer) at T3 and T4 and determined that antibody responses to the
140 Pfizer vaccine were lower than for Moderna when tested against WA but were
141 statistically indistinguishable when evaluated against the variant viruses (Supp. Fig.1A).

142 Antibody assays are considered the gold-standard when assessing the quality of
143 immunity after vaccination because antibodies can provide sterilizing immunity.
144 However, the establishment of memory B cell populations is also critical to lasting

145 vaccine efficacy as these are the resources available for response to the virus, or a
146 variant, upon next encounter. To assess whether there is an impact of age on the
147 formation of memory B cell populations we measured the frequency and number of
148 circulating memory B cells specific for SARS-CoV-2 pre-vaccination and one week after
149 booster dose by dual staining with tetramers specific for RBD and S1 using flow
150 cytometry (Shroff *et al.*, 2021) (gating strategy in Figure 3A). Both groups of participants
151 had a small (0.1-0.3% of all B cells) but detectable population of antigen specific cells
152 double positive for S1 and RBD tetramer (Figure 3A). This population increased as a
153 percentage of B cells post vaccination in all but two (one from each cohort) participants
154 with no difference between groups (Figure 3B). Similar results were obtained with S1
155 single-positive cells (Figure 3D) again with no difference between adult and older
156 participants post vaccination. By multiplying percentages of tetramer positive B cells
157 with total B cell percentage and lymphocyte counts we have calculated the absolute
158 numbers of SARS-CoV-2 specific B cells in circulation and again observed no difference
159 between cohorts for S1+RBD+ double positive cells (Figure 3C) or S1 single positive
160 cells (Figure 3E). Next, we examined the phenotype of the SARS-CoV-2 specific B cells
161 (gating strategy in Figure S1B) with respect to class switching, as it has been previously
162 reported that aging is associated with a decline in the percentage and numbers of
163 switched memory B cells (Frasca *et al.*, 2008). To investigate this possibility, we
164 examined differentiation and class switching of total tetramer (S1+) positive B cells by
165 flow cytometric staining for CD27, IgM, IgD, CD21 and CD11c (representative flow
166 cytometric gating in Figure S1A). Ag-specific B cells from adult and older participants
167 expressed CD27 at identical levels (Figure S1C) and equal numbers of both CD27
168 positive and negative cells were class switched (Figure S1D,E). Adult and older
169 participants also displayed no difference in classical memory (CD21+) phenotype
170 among the class switched tetramer-positive cells (Figure S1F). Thus overall, we
171 conclude that induction and differentiation of SARS-CoV-2 specific B cells through
172 vaccination was not compromised by aging.

173 Finally, we measured antigen-specific T cells elicited by vaccinated. Given that ELISpot
174 was previously reported to be a highly sensitive method for detection of rare antigen
175 specific T cells (Chauvat *et al.*, 2014) we simultaneously measured the number of T

176 cells expressing costimulatory molecules CD137 and OX-40 by flow cytometry (used in
177 several reports examining SARS-CoV-2 specific T cell immunity - (Grifoni *et al.*, 2020;
178 Rydyznski Moderbacher *et al.*, 2020) (representative flow cytometry in Figure S2A) and
179 performed IFN-γ ELISpot on PBMCs from select participants stimulated with peptide
180 pools corresponding to the Spike protein of WA1. Confirming prior data, ELISpot proved
181 to be a much more sensitive method for enumeration of Ag-specific T cells. We
182 detected a statistically significant increase in ELISpots per 10^6 PBMC's after vaccination
183 analyzing just 5 samples, whereas parallel flow cytometry samples showed no
184 significant differences (Figure S2B-E). The only limitation of ELISpot, as compared to
185 flow cytometry based enumeration, is that total T cell responses are measured without
186 separate quantification of CD4 and CD8 responses.

187 We also analyzed Ag-specific T cell responses to stimulation with Spike protein peptide
188 pools from WA1 versus two VOC by ELISpot. Participant PBMC's were stimulated with
189 16-mer overlapping peptide pools corresponding to the spike protein of WA1, P.1.
190 (Gamma) and B.1.617.2 (Delta). In accordance with previously published results (Sahin
191 *et al.*, 2020), mRNA vaccines induced a robust T cell response to WA1, the ancestral
192 strain of SARS-CoV-2, which did not differ for Gamma and Delta variants, as evidenced
193 by a 10-fold increase in ELISpots from post-vaccination samples stimulated by S
194 peptide pools compared to unstimulated wells (Figure 4A). Of note, the data
195 represented in Figure 4A shows concatenated time points for each peptide pool to
196 demonstrate the resolution of this assay. Next, we parsed that data to compare T cell
197 responses of both cohorts at different time points post-vaccination and subtracted the
198 number of spots in the unstimulated wells for each sample to properly calculate the
199 number of Ag-specific ELISpots. Data from old mice (Brien *et al.*, 2009; Fulton *et al.*,
200 2013; Jergović *et al.*, 2019) showed that induction of Ag-specific T cell responses
201 becomes delayed and decreased with age. In our data, there was a slightly lower
202 response in the older cohort at day 7 post first dose with all three variants which was
203 statistically significant only with the WA1 peptide pool (Figure 4B). However, after a
204 second dose both groups had a very robust T cell response against all three viral
205 variants examined (Figures 4C, D, E). Therefore, while we acknowledge that the
206 primary Ag-specific T cell response is lower, and likely delayed, in some of our older

207 participants, we conclude that the outcome after booster dose is competent T cell
208 mediated immunity against WA1 and tested VOC in all mRNA vaccine recipients in our
209 study. This is in agreement with our previous studies of vaccination in aged mice which
210 showed that at least two cycles of in vivo restimulation are required for adequate ag-
211 specific T cell response in aged animals (Uhrlaub *et al.*, 2011). All of these data taken
212 together demonstrate the rather expected blunted primary response in older adults and
213 the improvement of this response to the levels seen in adults following a second
214 vaccine dose. Since IFN-γ is not the only effector cytokine produced by T cells
215 following antigen stimulation, we have additionally measured polyfunctional responses.
216 Spike peptide pools induce a dramatic number of IFN-γ spots in comparison to
217 unstimulated wells, but also an increase in IL-2 and GrB spots (Figure 5A). We
218 observed no difference between the age groups in IL-2 or GrB spots (Figure 5B) in
219 response to WA/2020 or Delta peptide pools at post-second dose time points (T3 and
220 T4). Similarly, there was no difference between the number of polyfunctional double
221 positive (Figure 5C) or triple positive cells (Figure 5D). We also analyzed FLUORISpot
222 responses in recipients of mRNA vaccines from different manufacturers. We did not
223 measure any difference in IFN-γ, IL-2 or GrB T cell responses between recipients of
224 Moderna vs. Pfizer mRNA vaccine (Figure S3A and Figure S3B).

225 It is well established that primary immune responses wane with age and contribute to
226 the increased susceptibility to infection experienced by older adults (reviewed in
227 Nikolich-Žugich, 2018). There is also evidence that generation of immune memory in
228 older adults is reduced, but not maintenance of memory. Studies with multiple
229 pathogens have shown decreased T cell receptor repertoire with age (Yager *et al.*,
230 2008; Smithey *et al.*, 2018) which could mean easier escape from existing immunity for
231 pathogen variants. All of the above-mentioned findings warrant an extensive and long-
232 term monitoring of immunity in SARS-CoV-2 vaccinees, especially those over age 55.

233 The emergence of SARS-CoV-2 impacted older adults especially hard with more than
234 80% of deaths in those over age 65 (Lithander *et al.*, 2020) and mortality rates rising
235 sharply above the age of 55 (Yanez *et al.*, 2020). Vaccination reduces these rates
236 dramatically (Haas *et al.*, 2021) demonstrating that the principles of immunology hold

237 true for this virus and that immune memory is what our species is lacking. Well
238 tolerated and effective, SARS-CoV-2 mRNA vaccines induce potent humoral and
239 cellular immune responses (Anderson *et al.*, 2020; Sahin *et al.*, 2020). Their deployment
240 also offers an opportunity to establish, in a truly immune naive population, correlates,
241 and contours, of protective immunity. Recent elegant studies in rhesus macaques have
242 shown that even sub-sterilizing neutralizing antibody titers are protective and decrease
243 SARS-CoV-2 severity (McMahan *et al.*, 2021). The same research study demonstrated
244 a protective role for T cell memory responses and this is supported by human research
245 showing that accumulation of oligoclonal CD8 T cells in bronchoalveolar lavage fluid
246 inversely correlated with disease severity (Liao *et al.*, 2020). Recently, Collier et al.
247 analyzed 102 partially and 38 fully vaccinated subjects and concluded that subjects >80
248 years of age produced lower primary, but not secondary antibody neutralizing
249 responses, including those against variants (Collier *et al.*, 2021). They did not analyze T
250 cell immunity against variants and, somewhat curiously, did not observe increased T
251 cell responses following the second dose in their older groups (>80). These authors
252 argue that older adults – those over 80 – remain vulnerable at least until they receive
253 the second vaccine dose. Our results agree with these conclusions with regard to
254 antibody immunity, and suggest that T cell immunity in response to mRNA vaccines is
255 robust in older adults and against variants, even though we did not analyze subjects in
256 the octogenarian bracket. Clinical efficacy of the mRNA vaccines in protecting older
257 adults has been strong, consistent with both our data and those by Collier et al (Collier
258 *et al.*, 2021). The decrease in antibody titer when challenged with more recent SARS-
259 CoV-2 variants does suggest that the breadth of immunity may be narrower in advanced
260 age; a challenge that can be met with booster doses of heterologous sequence
261 virus. Further studies on the durability and breadth of protection by current and future
262 heterologous vaccines in older adults will be necessary to answer these and other
263 germane questions on their immunity and SARS-CoV-2 protection in older adults.

264

Materials and methods

Study participants

This study was approved by the University of Arizona IRB (Protocol#2102460536). We collected blood from 23 adults <50 years old and 17 above 55 years old. Demographics are provided in Table II. Samples for all participants were collected prior to vaccination, one week after first dose (mRNA vaccine: Pfizer N=23; Moderna N=17), day before booster dose and 7-10 days after booster. Many participants were also sampled at 3 or 6-9 months following first dose. The time points are labeled T0, T1, T2, T3, T4, and T5 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. B.1.617.1 was received from BEI (NR-55486 SARS-CoV-2, Isolate hCoV-19/USA/CA-Stanford-15_S02/2021 (Kappa Variant) B.1.617.1 G EPI_ISL_1675223). B.1.617.2 was received from WRCEVA. Documentation says it was isolated from a patient at a Galveston County (UTMB) hospital; collected 7/6/2021 and passaged once on Vero cells. Strain designation GNL-1205. It has silent mutation C24208T by Sanger sequencing. 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

295 passage from received stock vial (USA-WA1/2020, P.1., B.1.617.1, B.1.617.2) or
296 primary culture (B.1.595) on mycoplasma negative Vero cells (ATCC #CCL-81) at a
297 MOI of 0.005 for 48 h. Supernatant and cell lysate were combined, subjected to a single
298 freeze-thaw, and then centrifuged at 3000RPM for 10 min to remove cell debris.

299

300 **Plaque Reduction Neutralization Test**

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

312 **ELISpot assays**

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

325 **Antibody ELISA**

326 Serological assays were performed exactly as previously described in Shroff *et al.*,
327 2021). Area under the curve (AUC) values were calculated using R version 4.0.3 (R
328 Core Team, 2020) and the DescTools package (Signorelli, 2021).

329 **Flow cytometry**

330 Cryopreserved PBMC (2-5 x 10^6/sample) were thawed in prewarmed RPMI-1640 with L-
331 glutamine (Lonza) + 10% FCS. Thawed PBMCS were rested overnight at 37 ^{o}C in X-
332 VIVO 15 Serum-free Hematopoietic Cell Medium (Lonza) supplemented with 5% human
333 Ab serum. Cells were stained with surface antibodies in PBS (Lonza) + 2% FCS, and
334 then stained with the live dead fixable blue dye (Thermofisher). B cell tetramers were
335 assembled by mixing 100 µg ml−1 of C-terminal AviTagged RBD or S1
336 (ACROBiosystems) with 100 µg ml−1 of streptavidin-PE (eBiosciences) or streptavidin-
337 BV421 (BioLegend), respectively, at a 5:1 molar ratio in which 1/10 of the final volume
338 of streptavidin was added every 5 min. Samples were stained for 1h at 4^0 C. Samples
339 were acquired using a Cytek Aurora cytometer (Cytek) and analyzed by FlowJo
340 software (Tree Star).

341 **FLUORISpot assays**

342 Cryopreserved PBMC (5 x 10^6/sample) were thawed in prewarmed RPMI-1640 with L-
343 glutamine (Lonza, Basel, Switzerland) + 10% FCS. Thawed PBMCS were rested for 3-
344 4h at 37°C in X-VIVO 15 Serum-free Hematopoietic Cell Medium (Lonza) supplemented
345 with 5% human Ab serum. Cells were then stimulated with ~1 nmol of peptide pool
346 corresponding to SARS-CoV-2 spike (S) of US-WA (ancestral), P.1. (Gamma), or
347 B.1.617.2 (Delta). Peptides were 16mer peptide pools, overlapping by 10 amino acids,
348 purchased from 21st century Biochemicals Inc. Cell suspensions were transferred to
349 pre-coated Human IFN-γ, IL-2, Granzyme-B (Gz-B) FLUORISpot kit plates (Mabtech,
350 Inc.) and developed after 48h according to manufacturer instructions. Spots were
351 imaged and counted using a Mabtech Iris Fluorispot reader (Mabtech).

352 **Statistical analysis**

SPSS and Graph Pad Prism were used for statistical analysis. Upon inspection of data distribution by Shapiro-Wilks normality test group differences were calculated by Mann Whitney U-test or Kruskal-Wallis test with Dunn's post-hoc correction. For comparisons where groups means were compared over differing time points two-way ANOVA with Tukey post hoc correction for multiple comparisons. For all statistical differences *$p<0.05$, **$p<0.01$, ***$p<0.001$. ****$p<0.0001$.

Competing Interest Statement

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

Data Availability

All data is available upon request.

Figure 1

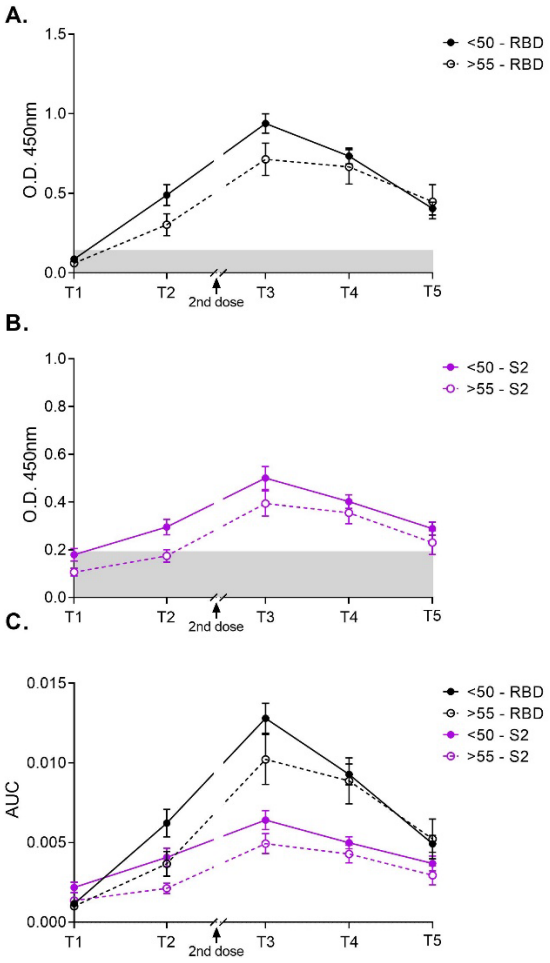

Figure 1. Antibody responses to mRNA vaccines are comparable regardless of age.

Time points denote days 7-9 (T1) and 18-26 (T2) days post first and 7-14 days (T3), 2-3 months (T4), and 6-9 months (T5) 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. Mean ± SEM is shown. Gray shaded area indicates positivity threshold for each assay. There is no statistical difference (Two-way ANOVA with Tukey's multiple comparisons test) between age groups at any time point measured. **C)** Quantitative titers for RBD and S2 were calculated for each individual at each time point and are shown as area under the curve (AUC) values. There is no statistical difference (Two-way ANOVA with Tukey's multiple comparisons test) between age groups at any time point measured.

Figure 2

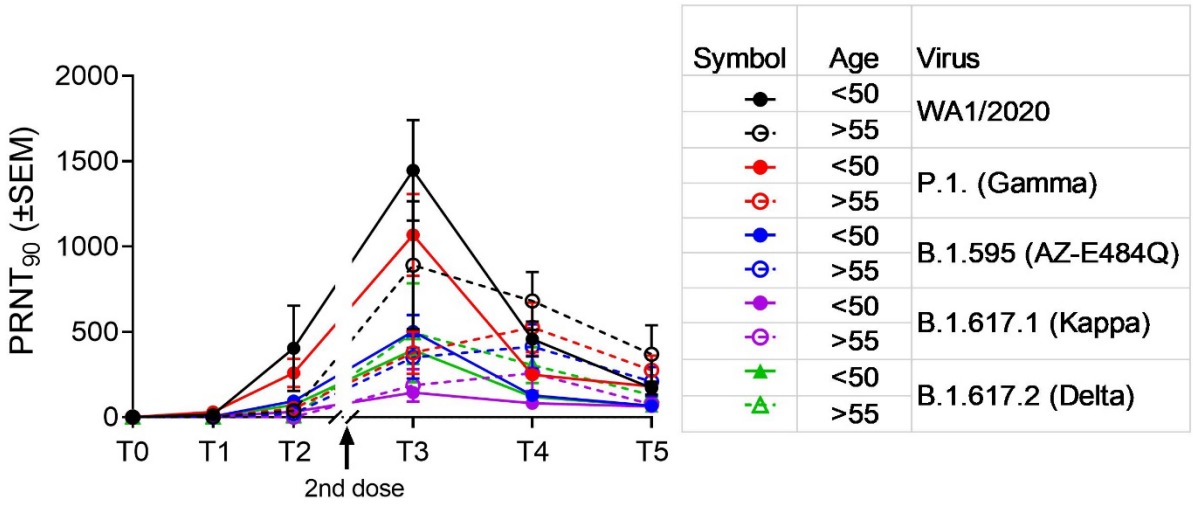

Figure 2. mRNA vaccines induce robust neutralization titers in adults under 50 and over 55 that is variably affected, but not abolished, by SARS-CoV-2 variants.

Time points are pre-bleed (T0); days 7-9 (T1) and 18-26 (T2) days post first dose of vaccine; and 7-14 days (T3), 2-3 months (T4), and more than 6 months (T5) after second dose of vaccine. Virus neutralization assays were performed using the USA-WA1/2020, P.1. (Gamma), AZ-E484Q, B.1.617.1 (Kappa), and B.1.617.2 (Delta) isolates of SARS-CoV-2. Serial 1:3 dilutions of plasma were assayed for neutralization capacity on Vero cells. The highest dilution capable of preventing >90% of plaques was considered to be the PRNT90 value. Statistical significance was assessed by Two-way ANOVA with Tukey's multiple comparisons test and determined significant only at time point 3 as represented in Table II.

395 **Table I – Descriptive statistics of T3 for Figure 2**

		Summary	Adjusted P Value
WA1/2020	<50 vs. >55	*	0.0104
P.1. (Gamma)	<50 vs. >55	***	0.0003
B.1.595 (AZ-E484Q)	<50 vs. >55	ns	0.9926
B.1.617.1 (Kappa)	<50 vs. >55	ns	>0.9999
B.1.617.2 (Delta)	<50 vs. >55	ns	0.9997
<50	WA1/2020 vs. P.1. (Gamma)	ns	0.2237
<50	WA1/2020 vs. B.1.595 (AZ-E484Q)	****	<0.0001
<50	WA1/2020 vs. B.1.617.1 (Kappa)	****	<0.0001
<50	WA1/2020 vs. B.1.617.2 (Delta)	****	<0.0001
>55	WA1/2020 vs. P.1. (Gamma)	*	0.0406
>55	WA1/2020 vs. B.1.595 (AZ-E484Q)	*	0.0235
>55	WA1/2020 vs. B.1.617.1 (Kappa)	***	0.0004
>55	WA1/2020 vs. B.1.617.2 (Delta)	ns	0.2711
<50	P.1. (Gamma) vs. B.1.595 (AZ-E484Q)	**	0.0044
<50	P.1. (Gamma) vs. B.1.617.1 (Kappa)	****	<0.0001
<50	P.1. (Gamma) vs. B.1.617.2 (Delta)	***	0.0002

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Figure 3.

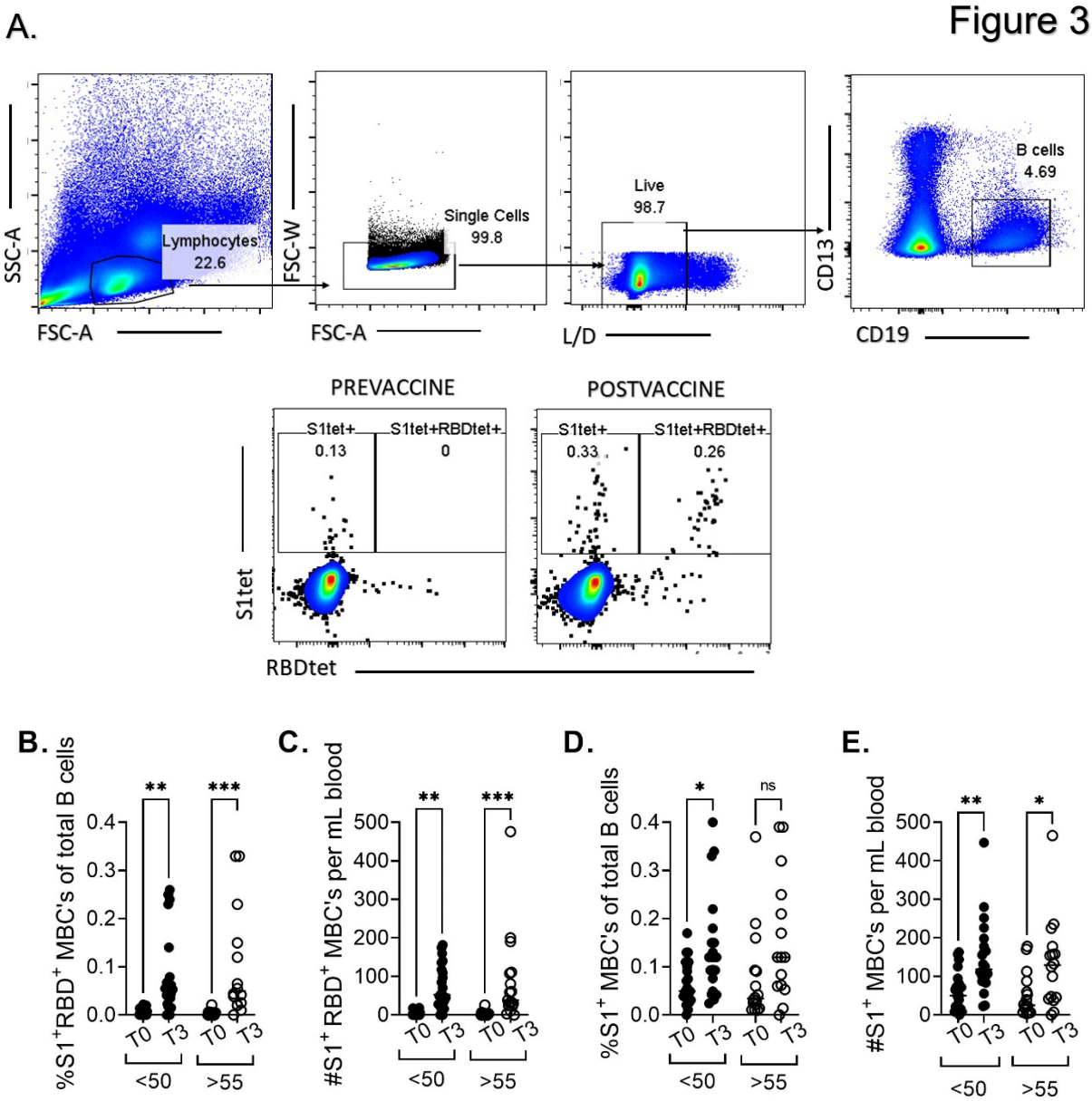

A.
SSC-A
FSC-A
Lymphocytes
22.6
FSC-W
FSC-A
Single Cells
99.8
Live
98.7
L/D
CD13
CD19
B cells
4.69
PREVACCINE
POSTVACCINE
S1tet+
0.13
S1tet+RBDtet+
0
S1tet+
0.33
S1tet+RBDtet+
0.26
S1tet
RBDtet
B.
%S1+RBD+ MBC's of total B cells
C.
#S1+ RBD+ MBC's per mL blood
D.
%S1+ MBC's of total B cells
E.
#S1+ MBC's per mL blood
T0
T3
<50
>55
ns

Figure 4

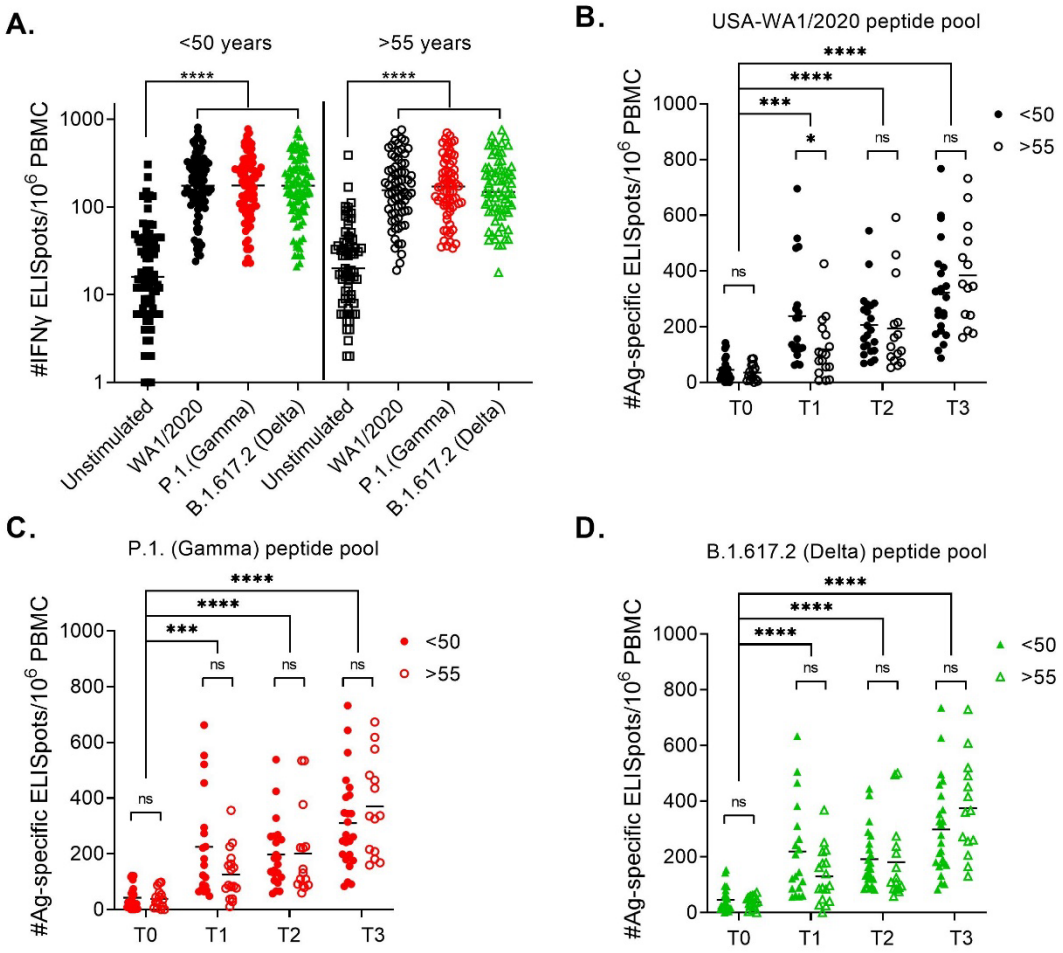
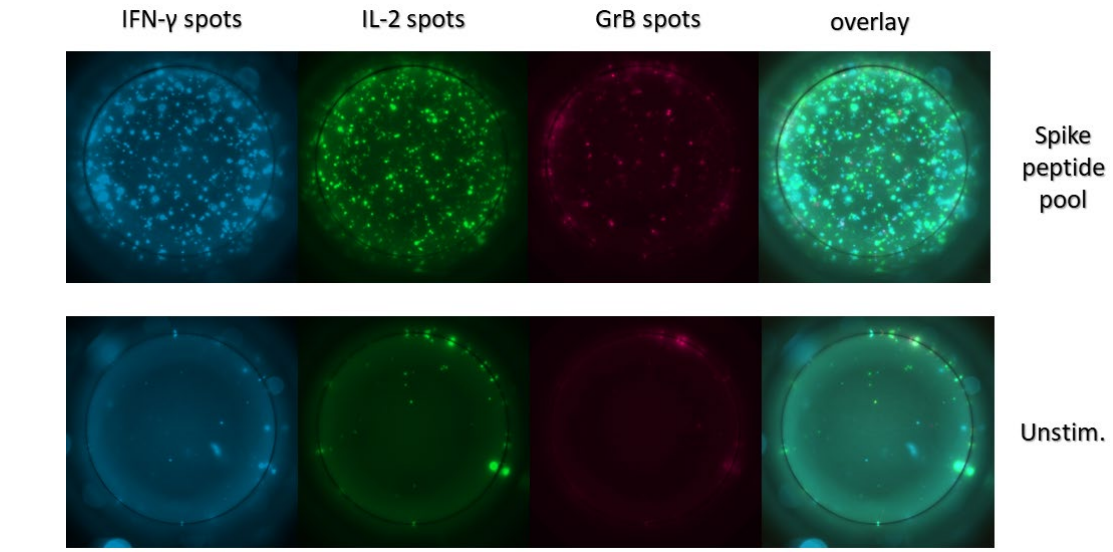


Figure 4. mRNA vaccines induce SARS-CoV-2 specific T cells reactive to Spike peptide pools from all three variants A) 10^6 PBMCs per well were stimulated with Spike peptide pools from USA-WA1/2020, P.1 (Gamma), and B.1.617.2 (Delta) cultured overnight in pre-coated IFN-γ ELISpot plates. All three peptide pools induced an equally strong IFN-γ response compared to unstimulated wells when all post-vaccination time points were pooled together **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 <50 and >55 cohorts at later time points (T2 and T3) but the response was lower in individuals >55 yo after the first dose (T1). **C)** P.1 (Gamma) peptide pool induced a strong response at all time points post-vaccination which did not differ between <50 and >55 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 5.



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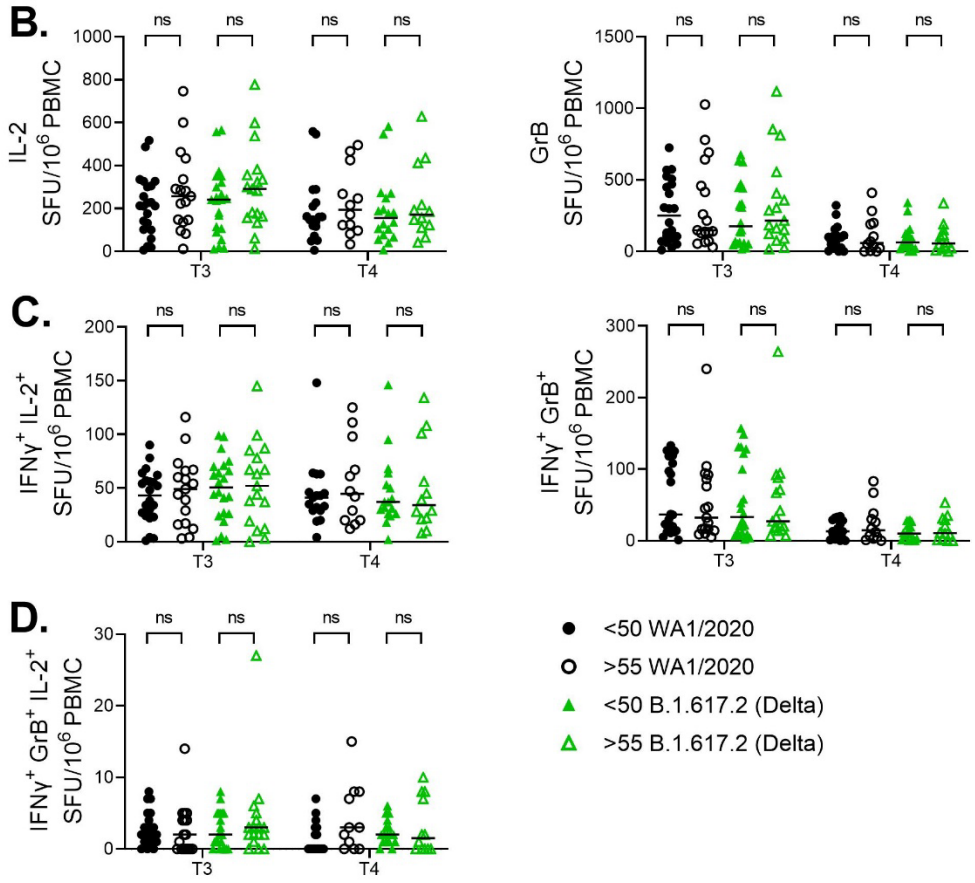

440

441 **Figure 5. mRNA vaccines induce polyfunctional SARS-CoV-2 specific T cells in**
442 **adult and older adult vaccine recipients A)** 10^6 PBMCs per well were stimulated with
443 Spike peptide pools from USA-WA1/2020 and B.1.617.2 (Delta) cultured overnight in
444 pre-coated IFN-γ, IL-2 and GrB FLUORISpot plates. **B)** Ag-specific FLUORISpot
445 numbers were calculated by subtracting the unstimulated wells of each participant from
446 the peptide stimulated wells. USA-WA1/2020 and B.1.617.2 (Delta) pools induced an
447 equal number of IL-2 and GrB spots in <50 and >55 cohorts at each time point. **C)**
448 Similarly, numbers of double positive spots (IFN-γ+IL-2+ or IFN-γ+GrB+) did not differ
449 between <50 and >55 cohorts with both peptide pools. **D)** Number of triple positive
450 spots (IFN-γ+IL-2+GrB+) were also not different between the age groups and the
451 variant peptide pools. Kruskal Wallis test with Dunn's post hoc correction. For all
452 statistical differences *p<0.05, **p<0.01, ***p<0.001. ****p<0.0001.

453

Table II – Demographic characteristics of the cohort

	<50 yrs (N=23)	>55 yrs (N=17)
Gender [N (%)]		
Male	7 (30%)	5 (29%)
Female	16 (70%)	12 (71%)
Age [mean (range)]	31 (20-47)	67 (56-80)
Race [N (%)]		
White	13 (57%)	15 (88%)
Asian	6 (26%)	1 (6%)
Unknown	4 (17%)	1 (6%)
Vaccine Type [N (%)]		
Pfizer	15 (65%)	8 (47%)
Moderna	8 (35%)	9 (53%)

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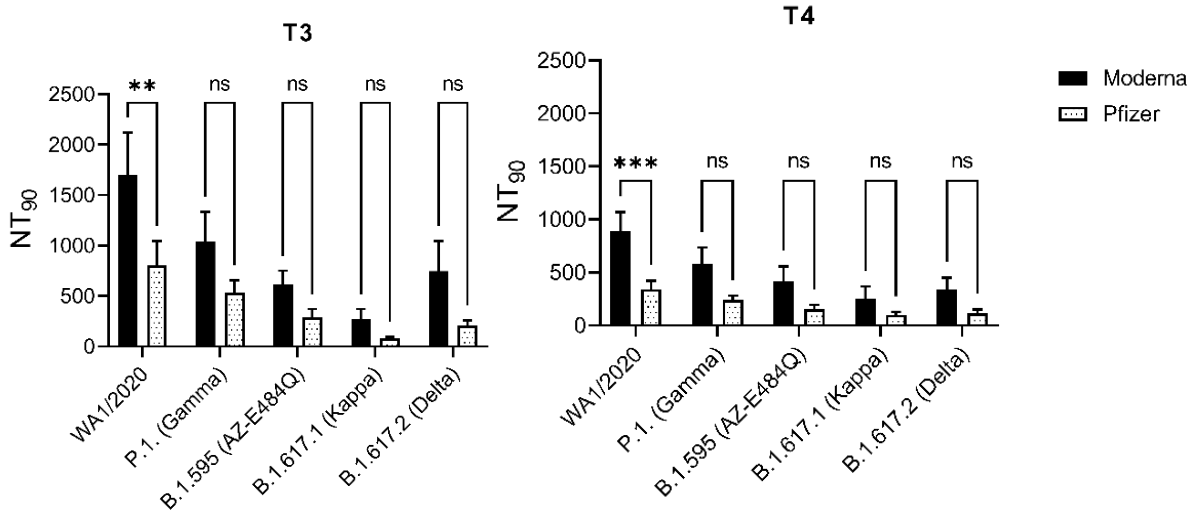
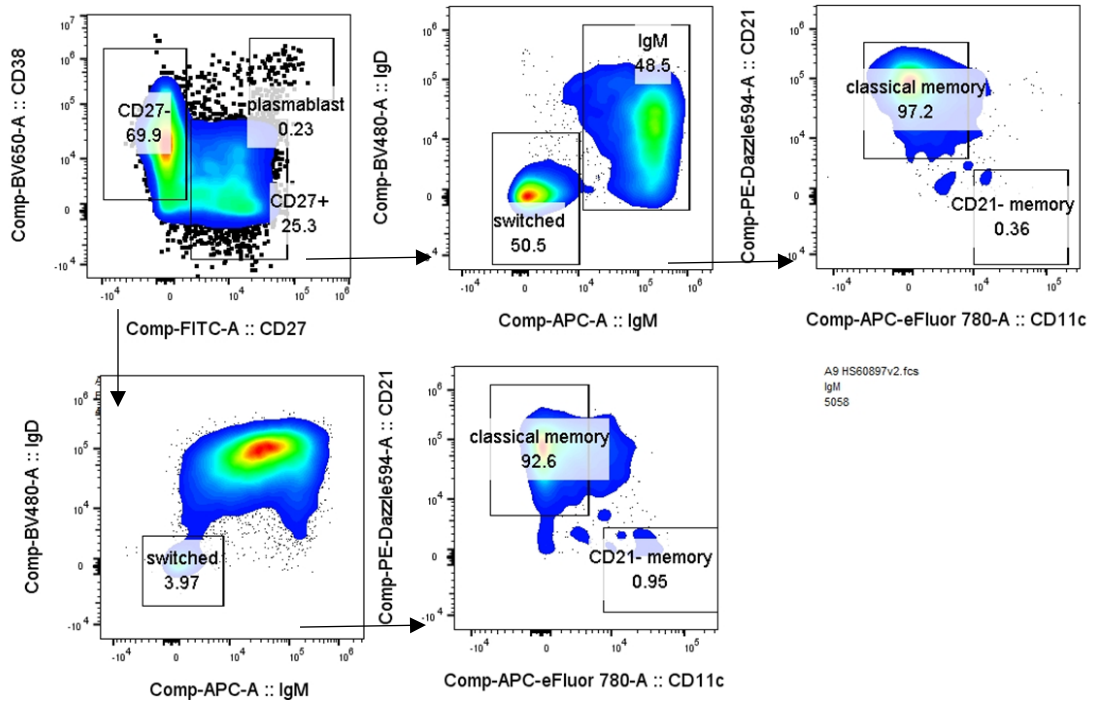
550

Figure S1.

A.


T3
T4
Moderna
Pfizer
NT90
2500
2000
1500
1000
500
0
**
***
ns
WA1/2020
P.1. (Gamma)
B.1.595 (AZ-E484Q)
B.1.617.1 (Kappa)
B.1.617.2 (Delta)

B.


Comp-BV650-A :: CD38
CD27-
69.9
plasmablast
0.23
CD27+
25.3
Comp-FITC-A :: CD27
Comp-BV480-A :: IgD
IgM
48.5
switched
50.5
Comp-APC-A :: IgM
Comp-PE-Dazzle594-A :: CD21
classical memory
97.2
CD21- memory
0.36
Comp-APC-eFluor 780-A :: CD11c
A9 HS60897v2.fcs
IgM
5058
switched
3.97
classical memory
92.6
CD21- memory
0.95

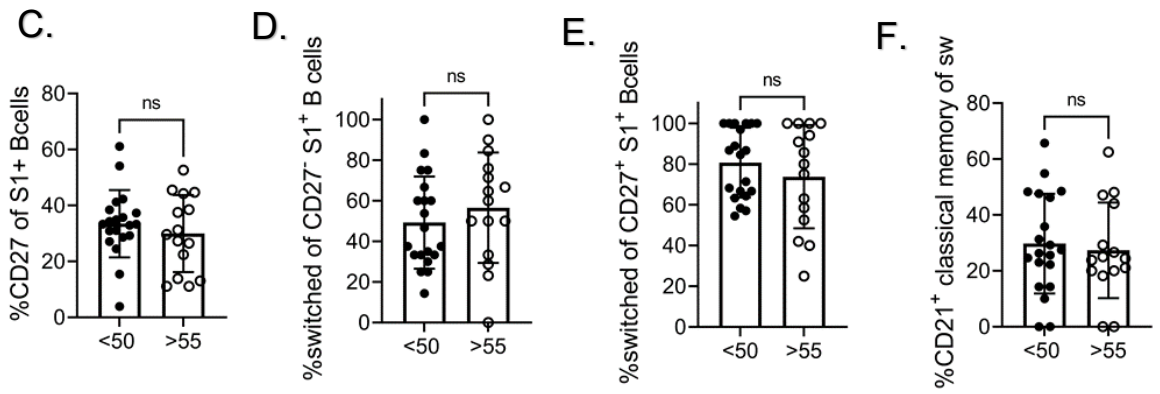


Figure S1. SARS-CoV-2 specific memory B cells differentiate equally in <50 and >55 cohorts. A) Lower neutralization titers in recipients of Pfizer's mRNA vaccine**. B)** Representative flow cytometric gating to examine B cell differentiation. **C)** Percentage of CD27 expressing B cells among tetramer positive cells was equal between cohorts. **D & E)** Equal percentage of SARS-CoV-2 specific B cells were class switched among CD27 positive and negative cells between <50 and >55 cohorts. **F)** Expression of CD21 (Classical memory) was also equal between <50 and >55 cohorts. Mann-Whitney U test, for all statistical differences *p<0.05, **p<0.01, ***p<0.001. ****p<0.0001.

Figure S2.

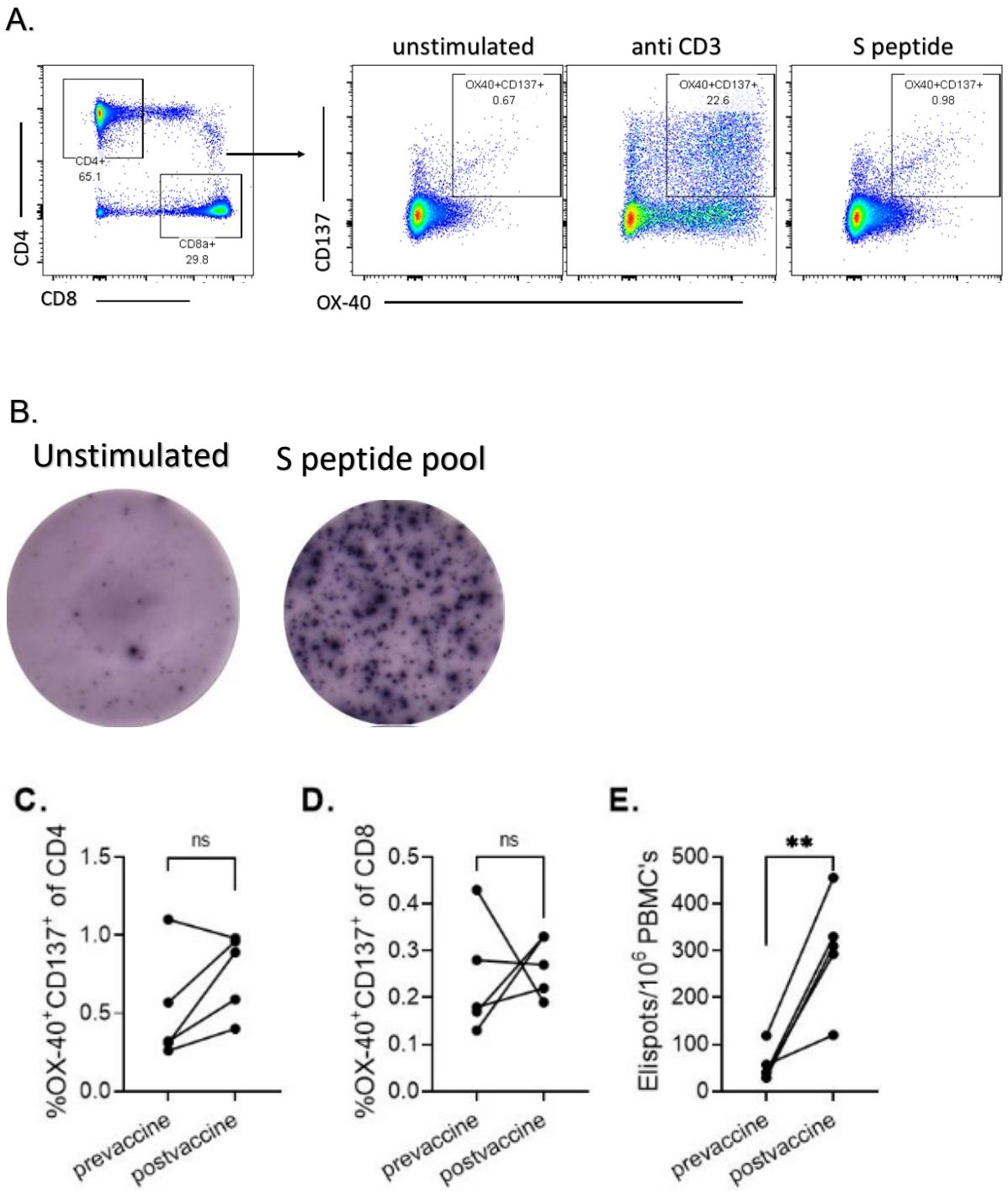
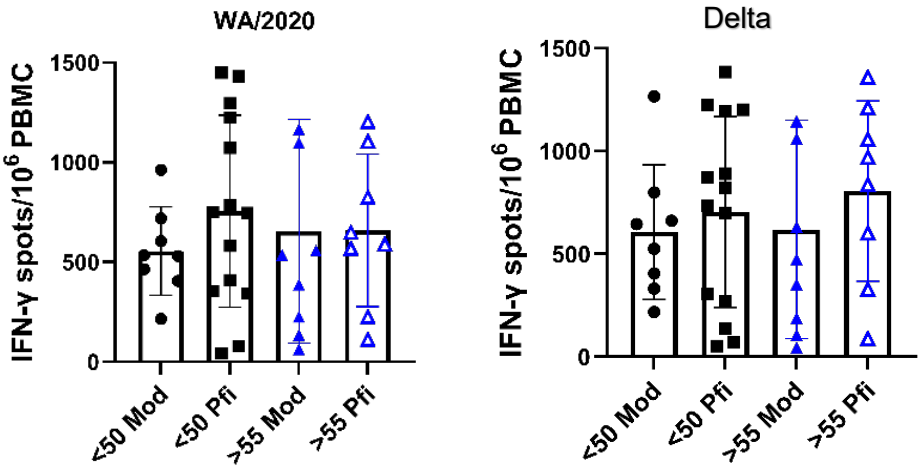


Figure S2. ELISpot is more specific method for enumeration of SARS-CoV-2 specific T cells. A) Representative flow cytometric gating for identifying CD137+OX-

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

Figure S3.

A.



B.

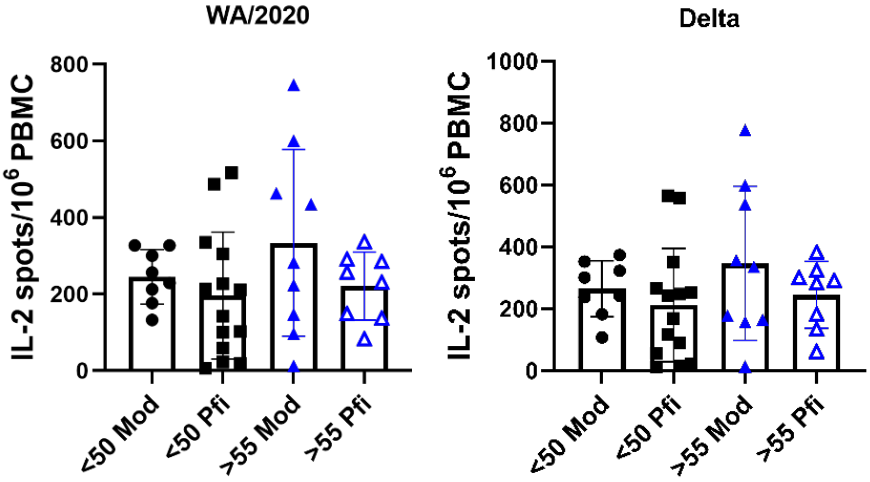


Figure S3. No difference in T cell responses between recipients of different mRNA vaccine brands. **A)** Number of IFN-γ spots was the same between recipients of Moderna and Pfizer vaccines at T4 time point **B)** Number of IL-2 spots was the same between recipients of Moderna and Pfizer vaccines at T4 time point for. 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.