Age-related heterogeneity in immune responses to SARS-CoV-2 following BNT162b2 vaccination

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Age-related heterogeneity in immune responses to SARS-CoV-2 vaccine BNT162b2

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Key words: SARS-CoV-2; COVID-19; immune response; vaccine; neutralising antibodies, T cell, B cell repertoire.

Abstract

Background

Vaccines remain the cornerstone for containing the SARS-CoV-2 pandemic. mRNA vaccines provide protection in clinical trials using a two-dose approach, separated by a three to four week gap. UK policy in 2021 is to extend the dosing interval from three to twelve weeks and other countries are likely to follow suit given the demand for mRNA vaccines and ongoing uncontrolled transmission. There is a paucity of data in the elderly, even though these individuals are the first to receive vaccines due to risk of severe disease. Here we assessed real world immune responses following vaccination with mRNA-based vaccine BNT162b2. Median age was 81 years amongst 101 participants after the first dose of the BNT162b2 vaccine. Geometric mean neutralisation titres in participants over 80 years old after the first dose were lower than in younger individuals [83.4 (95% CI 52.0-133.7) vs 46.6 (95% CI 33.5-64.8) p 0.01]. A lower proportion of participants 80 years and older achieved adequate neutralisation titre of >1:20 for 50% neutralisation as compared to those under 80 (21% vs 51%, p 0.003). Binding IgG responses correlated with neutralisation. Sera from participants in both age groups showed significantly lower neutralisation potency against B.1.1.7 Spike pseudotyped viruses as compared to wild type. The adjusted ORs for inadequate neutralisation in the 80 years and above age group were 3.7 (95% CI 1.2-11.2) and 4.4 (95% CI 1.5-12.6) against wild type and B.1.1.7 pseudotyped viruses. We observed a trend towards lower somatic hypermutation in participants with suboptimal neutralisation, and elderly

participants demonstrated clear reduction in class switched somatic hypermutation, driven by the IgA1/2 isotype. SARS-CoV-2 Spike specific T- cell IFNγ and IL-2 responses were impaired in the older age group after 1 dose and although IFNγ increased between vaccine doses, IL-2 responses did not significantly increase. There was a significantly higher risk of suboptimal neutralising antibody and T cell response following first dose vaccination with BNT162b2 in half of participants above the age of 80, persisting up to 12 weeks. These high risk populations warrant specific measures in order to mitigate against vaccine failure, particularly where SARS-CoV-2 variants of concern are circulating.

Background

Vaccines designed to elicit protective immune responses remain the key hope for containing the SARS-CoV-2 pandemic. In particular, mRNA vaccines have shown promise in clinical trials and have used a two-dose approach, separated by a three or four week gap[1,2]. However, duration of protection is not known and clinical trials provide few data on neutralising responses or efficacy in individuals above the age of 80. For example there were only twelve patients above the age of 65 evaluated for neutralising responses in a Pfizer vaccine study[3]. This is pertinent for multiple settings where a dosing interval of twelve instead of three weeks is currently being considered/used to maximise first dose administration[4]. Data on vaccine responses are vital in order to understand the efficacy of vaccination using this regime, particularly in groups under-represented in clinical trials, for example those aged above 80 years who are at greatest risk of severe disease and death[5]. In the UK, the government targeted this age group for access to the first vaccine available, BNT162b2. However, some weeks later the decision was made to extend the dosing schedule from 3 to 12 weeks, in order to vaccinate a greater proportion of the population during a period of substantial transmission. Other countries have been considering similar strategies given vaccine shortages and safety concerns regarding adenovirus vectored vaccines.

Here we assessed real world immune responses following vaccination with mRNA-based vaccine BNT162b2[1] in unselected elderly participants from the community and younger health care workers. During the study, the second dose of vaccination was initially given under the manufacturer recommended schedule based on two-doses with a three week interval. This was then changed following recommendation by the UK Joint Committee on Vaccination and Immunisation (JCVI) to a dosing interval of 12 weeks[6].

Results

Neutralisation of SARS-CoV-2 following first and second dose mRNA vaccination

One hundred and one participants received at least one vaccination, with median age 81 years (IQR 70-84) and 39% of participants female, with study flow shown in Supplementary Figure 1. Sera from vaccinated individuals exhibited an increase in neutralising titres against pseudotyped viruses expressing wild type (WT) SARS-CoV-2 Spike protein between the first and second doses (Figure 1A). Geometric mean neutralisation titre after the first dose was lower in participants 80 years and older than in younger individuals [83.4 (95% CI 52.0-133.7) vs 46.6 (95% CI 33.5-64.8) $p<0.01$ Table 1, Figure 1B]. A lower proportion of participants over 80 years old achieved adequate neutralisation titre of >1:20 for 50% neutralisation as compared to those under 80 (49.1% versus 78.6%, $p<0.001$). Between 3 to 8 weeks after the first dose, sera from 57.7% of those aged 80 years and older failed to neutralise against WT compared to 20.8% of those younger 80 years. We were interested to know if the poorer responses persisted up to 12 weeks to reflect with the extended dosing interval. We found that just before the second dose at 12 weeks, sera from 55% of those aged 80 years and older failed to neutralise against WT, compared to 20% in the <80 age group, Figure 1C).

Age showed statistically significant correlation with serum neutralisation of WT virus after the first but not second dose (Figure 1D, $p=0.0036$). In those participants with suboptimal neutralisation who received second doses within the study period (examples shown in Figure 1F), testing after the second dose showed that all responded, with neutralisation activity comparable to those responding well to the first dose (Table 1 and Figure 1B).

Given our observation that the participants 80 years old and older had lower neutralisation responses following first dose, we hypothesised that this would be exacerbated by the B.1.1.7 variant. We therefore examined serum neutralisation by age group against WT or the B.1.1.7 spike variant after the first dose (Figure 1E). The adjusted odds ratio for achieving inadequate neutralisation against WT was 3.7 (1.2-11.2, $p=0.02$) for participants 80 years old and older versus those younger than 80 years (Table 2). The adjusted odds ratio for inadequate neutralisation activity against the B.1.1.7 variant in the older age group was 4.4 (1.5-12.6, $p<0.007$) (Table 2).

Binding Antibody responses and B cell Repertoire Analyses

Binding antibody responses to the WT Spike were comprehensively measured using a particle based assay. IgA responses were detected both in convalescent sera and after both doses, with an increase between the two time points (Figure 2A). IgA concentrations was significantly correlated with age (Figure 2B). All IgG subclasses against Spike and RBD increased between vaccine doses (Figure 2E), as is observed in cases of natural infection. The concentration of total and subclass anti-Spike IgGs were significantly lower in the 80 years and older age group (Figure 2F) and showed significant correlation with age (Supplementary Figure 2A). Spike specific IgA, total IgG and IgG1-4 levels after dose 1 correlated strongly with neutralisation (Figure 2C, D, Supplementary Figure 2B).

Next the B cell Repertoire (BCR) isotype proportion was determined per patient using bulk BCR sequencing. We looked at isotype usage in patients who had been vaccinated at a minimum 17 days prior. There were no differences in isotype usage between the two age groups (Supplementary Figure 3A), or by neutralisation (Figure 3A). We next looked for skewing in V gene usage across the two age groups (Figure 3B) and association with neutralisation (Supplementary Figure 3B). We found an increase in usage in the 80 years and older age group with an increased proportion of IGHV4.34, IGHV4.39, IGHV4.59 and IGHV4.61 (Figure 3B). In the under 80 year age group there was an increase in Vgene usage in IGHV1 family with increases in IGHV1.18 and IGHV1.69D. We did not find any significant differences in V gene usage with neutralisation (Figure 3B).

Differences in somatic hypermutation could impact neutralisation through antibody affinity maturation. We found that participants 80 years and older had a lower level of somatic hypermutation compared with those younger than 80 years in class-switched BCRs, which was driven by the IgA1/2 isotype (Figure 4A). We did not observe a relationship between titres of IgA or IgG and mutation in BCRs for IgA1/2 and IgG1/2 respectively (Figure 4C, D). There was a trend towards correlation between IC50 and somatic hypermutation in class-switched and in IGHA BCR (Figure 4B, E).

To assess whether there was higher clonal expansion in those younger than 80 years old to explain higher neutralising responses, we calculated richness, using the Chaol1 measure and diversity using D50, Simpson's and Shannon-weiner indices. We did not find any significant differences between age groups and relationship between measures of diversity and neutralisation potency (Figure 4F, Supplementary Figure 3C).

CD4 and CD8 T cell responses to spike following mRNA vaccination

Next we determined the T cell response to SARS-CoV-2 spike protein by stimulating PBMC with overlapping peptide pools to the wild type SARS-CoV-2 spike or as a virus specific control, a peptides pool including Cytomegalovirus, EBV and Flu (CEF+) specific peptides, using IFNγ and IL-2 FluoroSpot assay to enumerate spike specific T cells. We also stimulated PBMC that had been collected and biobanked between 2014-2016 which represented a healthy SARS-CoV-2 unexposed population to provide a background control for SARS-CoV-2 spike protein responses. The results show that following the first dose of vaccine in those younger than 80 years old, the frequency of IFNγ spike specific T cells was significantly larger than then responses seen in an unexposed population. However, in the 80 years and older participants, the IFNγ spike specific T cell responses were not different from the unexposed controls following first dose (Figure 5A). However, spike specific IL-2 T cell frequencies were significantly greater than unexposed control in both age groups. There was also a difference between those younger than 80 and those 80 years and older with the latter having significantly lower IL-2 responses (Figure 5B).

We also analysed spike specific T cell responses for IFNγ and IL2 production from individuals that had been infected with SARS-CoV-2 and compared these to the responses observed in vaccinees post first and second dose (Figure 5 C, D). As might be expected IFNγ and IL2 responses in infected individuals were significantly greater than unexposed donors. IFNγ and IL2 responses increased following the second dose however only the IFNγ response was statistically significant (Figure 5 C, D).

We compared the IFNγ and IL-2 vaccine induced spike T cell responses between those younger than 80 years and those 80 years and older for paired first and second vaccine doses samples. For spike specific IFNγ responses there was a significant increase for both age groups following the second vaccine dose. For spike specific IL-2 responses overall, the second dose did not further increase the robust response induced by the first dose of vaccine. The IL-2 response, however, was significantly lower in the younger than 80 years group than the 80 years and older group following the first dose of the vaccine (Figure 5 E, F). IFNγ and IL-2 vaccine induced spike T cell responses did not correlate with ID50 against WT Spike pseudotyped virus after first or second dose vaccine (Supplementary Figures 4A-D).

Responses to CEF+ peptides were comparable across age groups, indicating that differences in observed responses were likely vaccine specific and unlikely due to generalised suboptimal T cell responses/immune paresis (Supplementary Figure 5).

We then tested CMV serostatus and related this to T cell responses and serum neutralisation. As expected, CMV IgG positivity was higher in the 80 years and older age group (Supplementary Figure 6A). CMV positive individuals in the 80 years and older group had significantly higher IFNγ, but not IL2, responses to SARS-CoV-2 spike peptides compared to the under 80 years old group (Supplementary Figure 6B, C). There was a trend towards a lower ID50 in HCMV positive individuals although this was not significant (Supplementary Figure 6D). HCMV seropositive individuals have a greater response to CEF peptides than HCMV seronegative, as might be expected, as the seropositive group can responded to the HCMV peptide components in the CEF pool, while the HCMV seronegatives can only respond to the EBV and Flu components, this is irrespective of age (Supplementary Figure 6E).

Immune senescence, autoantibodies and responses to mRNA vaccination

Finally, we investigated possible interplay between senescence and mRNA vaccine responses. Autoantibodies are associated with immune senescence[7]. We measured a panel of autoantibodies in the sera of 101 participants following the first dose of the BNT162b2 vaccine. 8 participants had positive autoantibodies for anti-myeloperoxidase (anti-MPO), 2 for anti-fibrillarin and 1 for anti-cardiolipin antibodies (Figure *6A*). As expected, all but one of the participants with anti-myeloperoxidase autoantibody was over the age of 80 years (Figure 6B). There was a trend towards reduced anti-Spike IgG levels and serum neutralisation against wild type and B.1.17 Spike mutant in participants with positive autoantibodies, although this did not reach statistical significance, likely due to small numbers (Figure 6C, D).

Discussion

Neutralising antibodies dominate protection from SARS-CoV-2 infection [8]. In further support for the role of neutralising antibodies in protection from infection are two clinical studies: (i) use of early convalescent sera in COVID-19 disease within elderly patients demonstrating improved clinical outcomes[9] and (ii) the recent report of very low efficacy of

the ChAdOx nCov-19 vaccine against prevention of mild to moderate COVID-19 in the context of the antibody escape variant B.1.351/501Y.V2[10]. However, studies in rhesus macaques also show that CD8 T cells may play a role in contributing to protection from SARS-CoV-2 disease when neutralising antibody levels are low[11]. This may explain why recent observational studies have demonstrated some effect against hospitalisation after 1 dose of the BNT162b2 or ChAdOx1nCoV-19[12-14] in settings where B.1.1.7 dominates. It is unclear what the impact of single dose would be in the context of a more vaccine resistant variant such as B.1.351, though poorer clinical outcomes would be expected in the elderly.

Here we have shown that around half of individuals above the age of 80 have a suboptimal neutralising antibody response after vaccination with BNT162b2. This is associated with lower spike specific IgA and IgG antibody concentrations. The suboptimal lower response persists in individuals until at least 11 weeks post first dose. The second dose is associated with robust neutralising responses across all age groups albeit in a limited sample number. As expected from previous studies[15,16], sera from participants in both age groups indicated modestly lower neutralisation potency against B.1.1.7 Spike pseudotyped viruses as compared to wild type. As a result, when B.1.1.7 was considered, a greater proportion of individuals in the over 80 age group moved into the poor neutralisation category following first dose when considering B.1.1.7 (72.0% versus 57.7%). Binding IgA antibodies to Spike and RBD increased following the first and second doses, mirroring levels seen in natural infection. IgG3 responses to Spike and RBD increased predominantly after the second dose. B.1.351 would be predicted to reduce adequate neutralisation responses to below 10%[17,18].

In a clinical study specifically looking at older adults vaccinated with BNT162b2 the GMT (geometric mean titre) after first dose was 12 in a set of 12 subjects between ages of 65 and 85 years, rising to 149 seven days after the second dose [3]. Furthermore, in the Moderna 1273 mRNA vaccine study in older individuals (above 55 years), neutralisation was only detectable after the second dose, whilst binding antibodies were detectable after both doses[19]. As such therefore it is not surprising that while a single dose of BNT162b2 failed to induce neutralizing antibodies in a proportion of participants, a second dose 3 weeks later resulted in all participants mounting a neutralizing antibody response. Important parallels have been reported in aged mice where ChAdOx nCov-19 vaccine responses were reported as being lower as compared to younger mice, and this was overcome by booster dosing[20].

Importantly the UK REACT study, a large, observational community based study, has shown that the prevalence of IgG positivity was 34.7% 21 days after the first dose of BNT162b2 in those over 80 years[21]. Similarly, REACT reported that IgG positivity increased to 87.8% after the second dose. Although it remains unclear how this will translate into protection from COVID-19, it raises concerns about if those over 80 years will be adequately protected with one dose.

Spike specific IFNγ T cell responses to vaccine were impaired in those over 80. IL-2 secreting CD4 T cell responses were also significantly lower in the over 80 age group. As expected, CMV IgG prevalence was higher in the over 80 group. Surprisingly, in IFNγ but not IL2 T cell responses were greater in the HCMV seropositive >80 year old participants, in contrast to literature on vaccine responses in HCMV elderly individuals. A number of longitudinal and population cohort studies have suggested that HCMV seropositivity is linked to age-related poor response to vaccinations. However, other studies have shown no such age-related correlation between HCMV seropositivity and declines in immune responses to vaccination with some showing enhanced responses to Influenza vaccination[22].In the context of BNT162b2 vaccination, vaccinees >80 years old and HCMV seropositive had significantly higher IFNγ responses and no effect on the distribution of SARS-CoV-2 neutralizing antibody responses.

The T cell response following infection with SARS-CoV-2 has been studied by numerous groups using peptide stimulated PBMC and analysed by both EliSpot for IFNγ and intracellular cytokine staining (ICS) for IL2 and IFNγ. The results show that infection generates robust IFNγ T cell responses to spike protein in the majority of individuals post infection. In addition, ICS analysis for IFNγ and IL2 production by spike specific CD4 and CD8 T cells demonstrated that the IL2 response was predominantly produced by CD4+ T cells and that IFNγ production was seen in both spike specific CD4 and CD8+ T cell subsets[23,24]. Phase I/II clinical trials of the mRNA and Adenovirus vectored SARS-COV-2 vaccines have similarly showed a Th1 skewed response with elevated TNFα, IL2 and IFNγ being secreted by both CD4+ and CD8+ T cells[19,25-28].

Immune senescence is a well described phenomenon whereby responses to pathogens[29] and indeed vaccines are impaired/dysregulated with age[30]. As an example, effective seasonal influenza vaccination of the elderly is a significant public health challenge due to greater morbidity and mortality in this group. Lower neutralizing antibody titres using standard dose influenza vaccines in elderly individuals has been addressed by using higher dose vaccine [31].

Vaccines against RNA viruses such as influenza[32] and HIV-1[33] are thought to rely on the generation of neutralising antibodies through interactions between B-cells and T follicular helper (T_{FH}) in the germinal centre (GC) that are needed for long-lived memory B cells/ plasma cells and high-affinity, class-switched antibodies[34,35]. Indeed a recent report suggested SARS-CoV-2 mRNA vaccine generated potent T_{FH} and GC responses, correlating with neutralisation[36]. In our B cell repertoire analyses we explored correlates for the impaired virus neutralisation by antibodies observed in a high proportion of older individuals. We found that participants >80 had a lower mean somatic hypermutation in class-switched BCRs, driven by the IgA1/2 isotype. There was a trend towards significant correlation between IC50 and somatic hypermutation in class-switched and in IGHA BCR. As a caveat we did not look at SARS-CoV-2 specific B cells in this analysis and circulating antigen specific B cells are likely to be at relatively low prevalence. Our data hint towards a mechanism whereby impaired T cell responses, as indicated by our data showing lower SARS-CoV-2 spike induced T cell cytokine production in the elderly, could impair generation of high affinity, class-switched, potently neutralising antibodies.

The proportion of individuals over 80 years old with poor neutralisation responses did not change between 3 and 12 weeks after the first dose. This argues against increased responses after 3 weeks for the Pfizer vaccine and indicates prolonged increased risk for infection in this group[8,37]. Although the second dose was able to boost neutralising antibodies, SARS-CoV-2 infection during an enlarged window period between doses in the presence of only partially protective antibody titres could also lead to favourable conditions for selecting escape mutations in populations such as the elderly who are also clinically vulnerable to severe disease [38,39]. Given the thrombotic events associated with adenovirus-vectored vaccines AZ ChadOx1[40] and more recently the J&J vaccine, there has been interruption in their use in Europe and the US. There is now intense pressure on mRNA vaccines and extension of the dosing window to 12 weeks is likely to be applied across a number of

countries due to the ongoing pandemic. Indeed Canada has implemented a 16 week gap between doses of mRNA vaccination (https://bcmj.org/articles/what-evidence-extending-sars-cov-2-covid-19-vaccine-dosing-schedule). South Africa, where the B.1.351 variant with reduced sensitivity to Pfizer vaccine is circulating, has also halted use of both vaccines (https://www.bbc.co.uk/news/world-us-canada-56733715). Our data indicate that such a policy of dose widening should not be applied to persons over the age of 80, and possibly for those with compromised immunity.

It will be important to follow our participants over the following months to measure the kinetics of neutralisation activity as well as to gather data on re-infection. We speculate that those with suboptimal responses to the first dose may experience loss of immunity faster following the second dose. Waning of immunity coupled with arrival of new variants with the potential to compromise vaccines[41-44] may mandate regular re-vaccination with modified vaccine preparations.

Limitations

We were unable to adjust for confounders such as immune suppression and comorbidities as these data were not collected. They may also be other unmeasured confounders that may modify the association between age and neutralisation status. However, consecutive participants were recruited without exclusion. Although participant numbers were modest our sample size estimation suggest we recruited sufficient participants to limit type I error. In addition, the numbers of elderly participants undergoing assessment for neutralisation and T cell responses was greater than manufacturer-sponsored vaccine studies[3, 9].

Conclusion

Whilst significant public health impact of vaccines is anticipated, and indeed has been demonstrated[45], a significant proportion of individuals above 80 appear to require the second dose to achieve in vitro virus neutralisation. Our data caution against extending the dosing interval of BNT162b2 in the elderly population, particularly during periods of high transmission, where there is the added risk from variants that are less susceptible to vaccine-elicited neutralising antibodies[15,41,42,44]. If rapid antibody tests are able to differentiate those in risk groups who have satisfactory neutralisation responses then a widened dosing interval may be appropriate under certain circumstances.

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Methods

Study Design

Community participants or health care workers receiving the first dose of the BNT162b2 vaccine between the 14th of December 2020 to the 29th of January 2021 were consecutively recruited at Addenbrookes Hospital into the COVID-19 cohort of the NIHR Bioresource. Participants were followed up for up to 3 weeks after receiving their second dose of the BNT162b2 vaccine. They provided blood samples 3 to 8 weeks after their first dose and again 3 weeks after the second dose of the vaccine. Consecutive participants were eligible without exclusion. An additional group of participants who were 80 years and above were recruited at between 9 to 12 weeks after receiving the first dose of the vaccine. The exposure of interest was age, categorised into 2 exposure levels- < 80 and ≥ 80 years. The outcome of interest was inadequate vaccine-elicited serum antibody neutralisation activity at least 3 weeks after the first dose. This was measured as the dilution of serum required to inhibit infection by 50% (ID50) in an *in vitro* neutralisation assay. An ID50 of 20 or below was deemed as inadequate neutralisation. Binding antibody responses to Spike protein were measured by multiplex particle-based flow cytometry and T cell responses measured by IFNγ and IL-2 FLUOROSPOT assays. Serum autoantibodies and characterising the B cell repertoire (BCR) following the first vaccine dose were exploratory outcomes.

We assumed a risk ratio of non-neutralisation in the ≥80 years group compared with <80 years group of 5. Using an alpha of 0.05 and power of 90% required a sample size of 50 with a 1:1 ratio in each group.

Ethical approval

The study was approved by the East of England – Cambridge Central Research Ethics Committee (17/EE/0025). PBMC from unexposed volunteers previously recruited by the NIHR BioResource Centre Cambridge through the ARIA study (2014-2016), with ethical approval from the Cambridge Human Biology Research Ethics Committee (HBREC.2014.07) and currently North of Scotland Research Ethics Committee 1 (NS/17/0110).

Statistical Analyses

Descriptive analyses of demographic and clinical data are presented as median and interquartile range (IQR) when continuous and as frequency and proportion (%) when categorical. The difference in continuous and categorical data were tested using Wilcoxon rank sum and Chi-square test respectively. Logistic regression was used to model the association between age and the neutralisation by vaccine-elicited antibodies after the first dose of the BNT162b2 vaccine. The effect of sex and time interval from vaccination to sampling as confounders were adjusted for. Linear regression was also used to explore the association between age and log transformed ID50, binding antibody levels, antibody subclass levels and T cell response after dose 1 and dose 2 of the BNT162b2 vaccine. The time interval from vaccination to sampling was adjusted for. Bonferroni adjustment was made for multiple comparisons. The Pearson correlation coefficient was reported. Statistical analyses were done using Stata v13, Prism v9 and R (version 3.5.1).

Generation of Mutants and pseudotyped viruses

Wild-type (WT) bearing 614G and B.1.1.7 bearing mutations del-69/70, del-144, N501Y, A570D, D614G, P681H, S982A, T716I and D1118H or K417N, E484K and N501Y pseudotyped viruses were generated as previously described[15]. In brief, amino acid substitutions were introduced into the D614G pCDNA_SARS-CoV-2_S plasmid as previously described[38] using the QuikChange Lightening Site-Directed Mutagenesis kit, following the manufacturer's instructions (Agilent Technologies, Inc., Santa Clara, CA). Sequences were verified by Sanger sequencing. The pseudoviruses were generated in a triple plasmid

transfection system whereby the Spike expressing plasmid along with a lentviral packaging vector- p8.9 and luciferase expression vector- psCSFLW where transfected into 293T cells with Fugene HD transfection reagent (Promega). The viruses were harvested after 48 hours and stored at -80oC. TCID50 was determined by titration of the viruses on 293Ts expressing ACE-2 and TMPRSS2.

Neutralisation assays

Spike pseudotype assays have been shown to have similar characteristics as neutralisation testing using fully infectious wild type SARS-CoV-2[46]. Virus neutralisation assays were performed on 293T cell transiently transfected with ACE2 and TMPRSS2 using SARS-CoV-2 Spike pseudotyped virus expressing luciferase[47]. Pseudotyped virus was incubated with serial dilution of heat inactivated human serum samples or sera from vaccinees in duplicate for 1h at 37˚C. Virus and cell only controls were also included. Then, freshly trypsinized 293T ACE2/TMPRSS2 expressing cells were added to each well. Following 48h incubation in a 5% CO_2 environment at 37°C, luminescence was measured using the Steady-Glo Luciferase assay system (Promega). Neutralization was calculated relative to virus only controls. Dilution curves were presented as a mean neutralization with standard error of the mean (SEM). 50% neutralization- ID50 values were calculated in GraphPad Prism. The limit of detection for 50% neutralisation was set at an ID50 of 20. The ID50 within groups were summarised as a geometric mean titre (GMT) and statistical comparison between groups were made with Mann-Whitney or Wilxocon ranked sign test.

SARS-CoV-2 serology by multiplex particle-based flow cytometry (Luminex):

Recombinant SARS-CoV-2 N, S and RBD were covalently coupled to distinct carboxylated bead sets (Luminex; Netherlands) to form a 3-plex and analyzed as previously described[48]. Specific binding was reported as mean fluorescence intensities (MFI).

CMV serology:

HCMV IgG levels determined using an IgG enzyme-linked immunosorbent (EIA) assay, HCMV Captia (Trinity Biotech, Didcot, UK) following manufacturer's instructions, on plasma derived from clotted blood samples.

Serum autoantibodies:

Serum was screened for the presence of autoantibodies using the ProtoPlexTM autoimmune panel (Life Technologies) according to the manufacturer's instructions. Briefly, 2.5µl of serum was incubated with Luminex MagPlex magnetic microspheres in a multiplex format conjugated to 19 full length human autoantigens (Cardiolipin, CENP B, H2a(F2A2) & H4 (F2A1), Jo-1, La/SS-B, Mi-2b, myeloperoxidase, proteinase-3, pyruvate dehydrogenase, RNP complex, Ro52/SS-A, Scl-34, Scl-70, Smith antigen, Thyroglobulin, Thyroid peroxidase, transglutaminase, U1-snRNP 68, whole histone) along with bovine serum albumin (BSA). Detection was undertaken using goat-anti-human IgG-RPE in a 96 well flat-bottomed plate and the plate was read in a Luminex xMAP 200 system. Raw fluorescence intensities (FI) were further processed in R (version 3.5.1) Non-specific BSA-bound FI was subtracted from background-corrected total FI for each antigen before $\log_2$ transformation and thresholding. Outlier values (Q3+1.5*IQR) in each distribution were defined as positive.

B Cell Receptor Repertoire Library Preparation

PBMC were lysed and RNA extracted using Qiagen AllPrep® DNA/RNA mini kits and Allprep® DNA/RNA Micro kits according to the manufactures protocol. The RNA was quantified using a Qubit. B cell receptor repertoire libraries were generated for 52 COVID-19 patients (58 samples) using as follows: 200ng of total RNA from PAXgenes (14ul volume) was combined with 1uL 10mM dNTP and 10uM reverse primer mix (2uL) and incubated for 5 min at 70°C. The mixture was immediately placed on ice for 1 minute and then subsequently combined with 1uL DTT (0.1 M), 1uL SuperScriptIV (Thermo Fisher Scientific), 4ul SSIV Buffer (Thermo Fisher Scientific) and 1uL RNAse inhibitor. The solution was incubated at 50 °C for 60 min followed by 15 min inactivation at 70 °C. cDNA was cleaned with AMPure XP beads and PCR-amplified with a 5′ V-gene multiplex primer mix and 3′ universal reverse primer using the KAPA protocol and the following thermal cycling conditions: 1cycle (95°C, 5min); 5cycles (98°C, 20s; 72°C, 30s); 5cycles (98°C, 15s; 65°C, 30s; 72°C, 30s); 19cycles (98 °C, 15s; 60°C, 30s; 72°C, 30s); 1 step (72°C, 5 min). Sequencing libraries were prepared using Illumina protocols and sequenced using 300-bp paired-end sequencing on a MiSeq machine.

Sequence analysis

Raw reads were filtered for base quality using a median Phred score of $\geq$32 (http://sourceforge.net/projects/quasr/). Forward and reverse reads were merged where a

minimum 20bp identical overlapping region was present. Sequences were retained where over 80% base sequence similarity was present between all sequences with the same barcode. The constant-region allele with highest sequence similarity was identified by 10-mer matching to the reference constant-region genes from the IMGT database. Sequences without complete reading frames and non-immunoglobulin sequences were removed and only reads with significant similarity to reference IGHV and J genes from the IMGT database using BLAST were retained. Immunoglobulin gene use and sequence annotation were performed in IMGT V-QUEST, and repertoire differences were performed by custom scripts in Python.

IFNγ and IL2 FLUOROSPOT T cell assays

Peripheral blood mononuclear cells (PBMC) were isolated from the heparinized blood samples using Histopaque-1077 (Sigma-Aldrich) and SepMate-50 tubes (Stemcell Technologies). Frozen PBMCs were rapidly thawed and diluted into 10ml of TexMACS media (Miltenyi Biotech), centrifuged and resuspended in 10ml of fresh media with 10U/ml DNase (Benzonase, Merck-Millipore via Sigma-Aldrich), PBMCs were then incubated at 37°C for 1h, followed by centrifugation and resuspension in fresh media supplemented with 5% Human AB serum (Sigma Aldrich) before being counted. PBMCs were stained with 2ul of LIVE/DEAD Fixable Far Red Dead Cell Stain Kit (Thermo Fisher Scientific) and live PBMC enumerated on the BD Accuri C6 flow cytometer.

1.0 to 2.5 x 10^5 PBMCs were incubated in pre-coated FluoroSpot^{FLEX} plates (anti IFNg and IL2 capture antibodies Mabtech AB, Nacka Strand, Sweden)) in duplicate with either peptide mixes specific for Wuhan-1(QHD43416.1) Spike SARS-CoV-2 protein (Miltenyi Biotech) or a mixture of peptides specific for Cytomegalovirus, Epstein Barr virus and Influenza virus (CEF+) (final peptide concentration 1µg/ml/peptide, Miltenyi Biotech) in addition to an unstimulated (media only) and positive control mix (containing anti-CD3 (Mabtech AB) and Staphylococcus Enterotoxin B (SEB), (Sigma Aldrich)) at 37ºC in a humidified CO2 atmosphere for 42 hours. The cells and medium were then decanted from the plate and the assay developed following the manufacturer's instructions. Developed plates were read using an AID iSpot reader (Oxford Biosystems, Oxford, UK) and counted using AID EliSpot v7 software (Autoimmun Diagnostika GmbH, Strasberg, Germany). Peptide specific

frequencies were calculated by subtracting for background cytokine specific spots (unstimulated control) and expressed as SFU/Million PBMC.

Table 1: Characteristics of study participants

	<80 years (N=42 or n/N)	**≥80 years (N=60 or n/N)**	**P value**
Female %	40.5 (17)	38.3 (23)	0.83[a]
Median age (IQR) *years*	62.5 (47.0-71.0)	83.0 (81.0-85.5)	-
Sera GMT WT (95% CI)			
dose 1	83.4 (52.0-133.7)	46.6 (33.5-64.8)[c]	0.01[b]
dose 2	651.0 (155.6-2722.9)[d]	555.1 (351.5-876.5)[e]	ns[b]
Serum ID50<20 for WT %			
dose 1	21.4 (9)	50.9 (30/59)[c]	0.003[a]
dose 2	0 (0/5)	0 (0/16)	-

[a] Chi-square test, [b] Mann-Whitney test, [c]neutralisation data unavailable for one individual, [d]neutralisation data available for 5 of 42, [e]neutralisation data available for 16 of 60, GMT- geometric mean titre, WT- wild type, ID50- (Inhibitory dilution) – the serum dilution achieving 50% neutralisation, ns- non-significant, CI-confidence interval.

Table 2: Neutralisation in participants after the first dose of BNT162b2 vaccine against wild type and B.1.1.7 spike mutant pseudotyped viruses.

	Number	Risk ID50<20	Unadjusted OR (95% CI)	P value	Adjusted OR* (95% CI)	P value
WT						
Age group *years*						
<80	42	19.1 (8/42)	1		1	
≥ 80	59	50.9 (30/59)	4.4 (1.7-11.1)	0.002	3.7 (1.2-11.2)	0.02
Sex						
Male	61	36.1 (22/61)	1		1	
Female	40	40.0 (16/40)	1.2 (0.5- 2.7)	0.59	1.2 (0.5-2.9)	0.72
Time since dose 1 *weeks*						
3-8	68	29.4 (20/68)	1		1	
9-12	33	54.6 (18/33)	2.88 (1.2-6.8)	0.02	1.4 (0.5-3.9)	0.57
B.1.1.7						
Age group *years*						
<80	41	29.3 (12/41)	1		1	
≥ 80	58	60.3 (35/58)	3.7 (1.6-8.6)	0.003	4.4 (1.5-12.6)	0.007
Sex						
Male	60	46.7 (28/60)	1		1	
Female	39	48.7 (19/39)	1.1 (0.5-2.4)	0.84	1.1 (0.5-2.7)	0.78
Time since dose 1 *weeks*						
3-8	66	42.4 (28/66)	1		1	
9-12	33	57.6 (19/33)	1.8 (0.8-4.3)	0.16	0.74 (0.3-2.2)	0.59

* Mutually adjusted for other variables in the table. WT- wild type, B.1.1.7- Spike mutant with N501Y, A570D, **Δ**H69/V70, **Δ**144/145, P681H, T716I, S982A and D1118H, ID50- (Inhibitory dilution) – the serum dilution achieving 50% neutralisation, ns- non-significant, CI-confidence interval.

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Figure 1

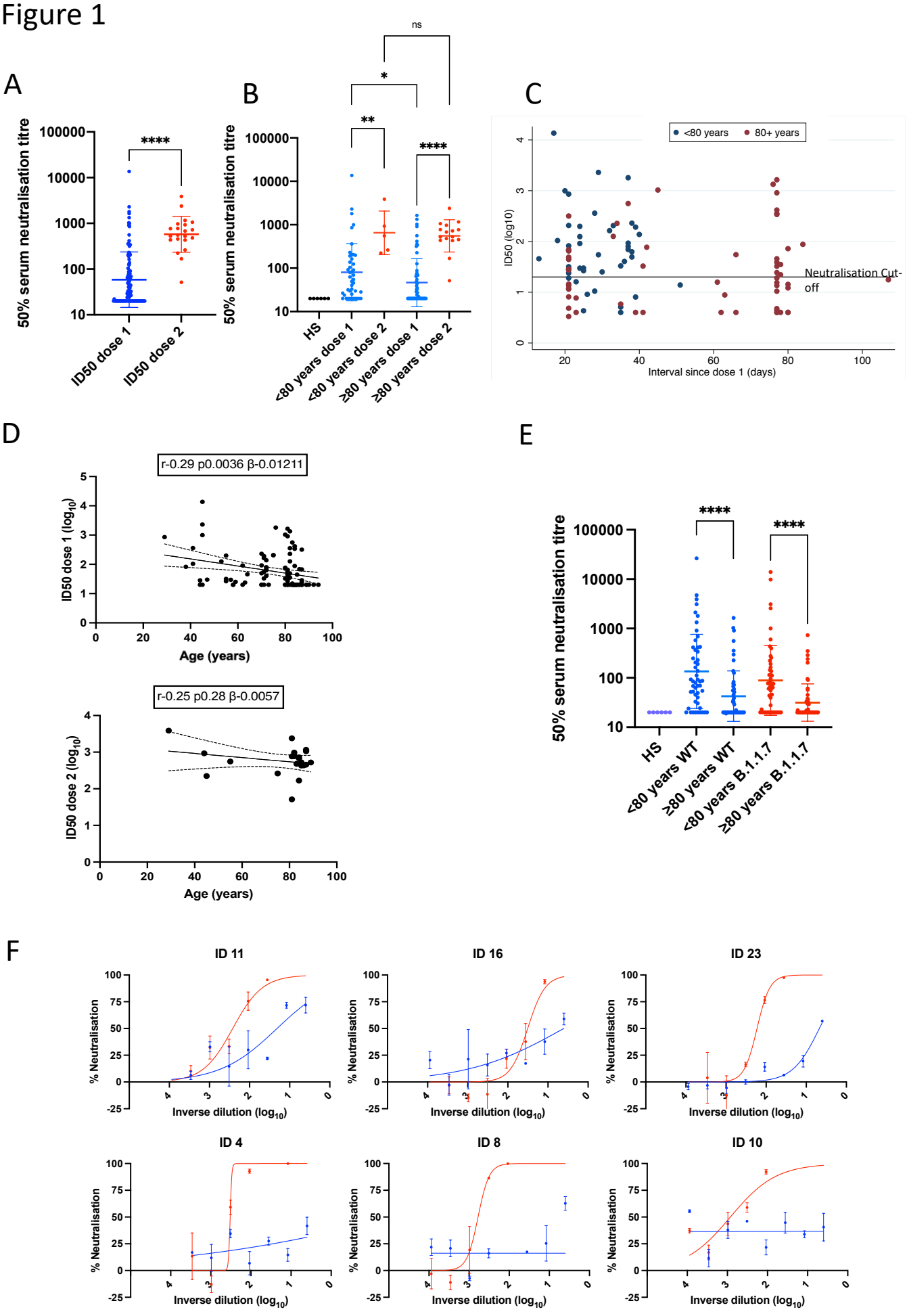


Figure 1. A, B. Neutralisation by Pfizer BNT162b2 vaccine sera against SARS-CoV-2 in a Spike lentiviral pseudotyping assay expressing wild type Spike (D614G). Data are shown as mean ID50 values for individuals after Dose 1 (n=101) and after Dose 2 (n=21). Geometric mean with s.d is shown. Each point is a mean of technical replicates from two experiment repeats. **C.** Serum neutralisation of Spike (D614G) pseudotyped lentiviral particles (inhibitory dilution at which 50% inhibition of infection is achieved, ID50) after Dose 1 by age-group <80 years (blue n=42), ≥80 years (red n=59). Line is the neutralization cut off at ID50 of 20. **D.** Correlation between serum neutralisation of Spike (D614G) pseudotyped lentiviral particles (inhibitory dilution at which 50% inhibition of infection is achieved, ID50)and age after the first dose n=101 and the second dose n=21 of Pfizer BNT162b2 vaccine. **E**. GMT with s.d of first dose Pfizer BNT162b2 vaccine sera against wild type (blue) and B.1.1.7 Spike mutant (red) SARS-CoV-2 pseudotyped viruses by age-group. **F.** Neutralisation curves for serum from six individuals with reduced responses after first dose of Pfizer BNT162b2 vaccine against pseudovirus expressing wild type Spike (D614G). B.1.1.7 Spike mutant with N501Y, A570D, **Δ**H69/V70, **Δ**144/145, P681H, T716I, S982A and D1118H. GMT with s.d are of two independent experiments each with two technical repeats. Neutralisation cut off for 50% neutralisation set at 20. Neutralisation are means of technical replicates, plotted with error bars representing standard error of mean. Data are representative of 2 independent experiments. Mann-Whitney test was used for unpaired comparisons and Wilcoxon matched-pairs signed rank test for paired comparisons. p-values * <0.05, ** <0.01, **** <0.0001, ns not significant, HS – human AB serum control, r– Pearson's correlation coefficient, β slope/regression coefficient, p p-value.

Figure 2

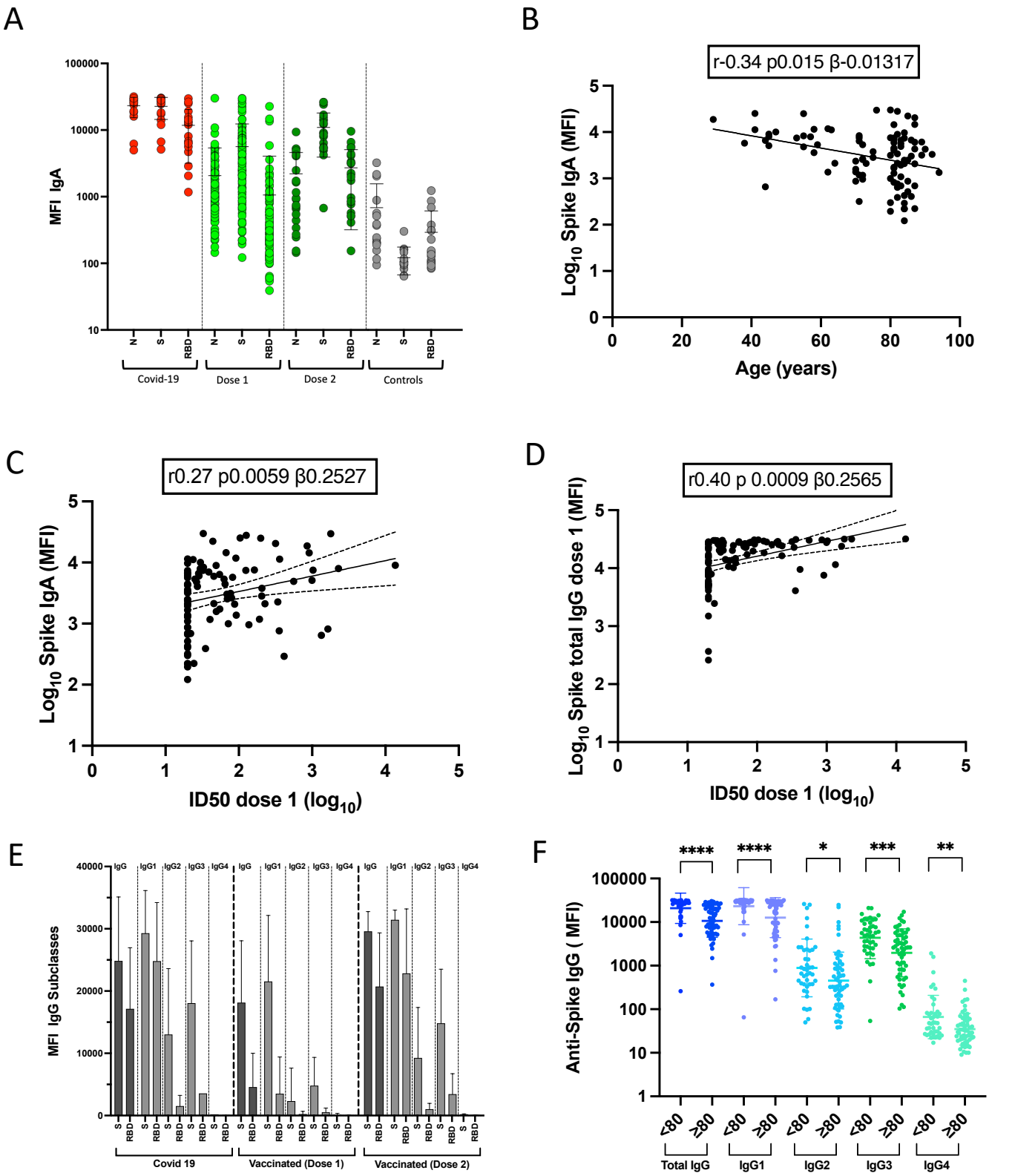
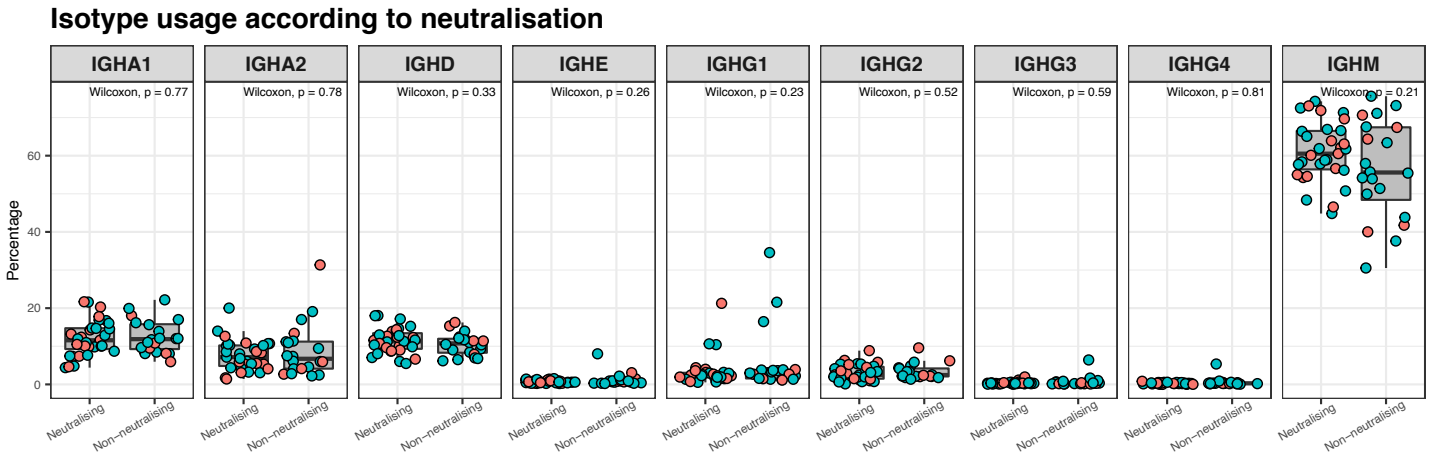


Figure 2: Binding antibody responses following vaccination with Pfizer BNT162b2 vaccine. A. IgA responses to S, N, RBD post first dose (light green, n=99) and second dose (dark green, n=21) compared to individuals with prior infection (red, n=18) and negative controls (grey, n=18) at serum dilutions 1 in 100. B. Correlation between anti-Spike IgA binding antibody responses and age. **C, D.** Correlations between anti-Spike IgA (n=99) (C) anti-Spike IgG (n=100) (D) binding antibody responses and neutralisation by vaccine sera against SARS-CoV-2 in a Spike lentiviral pseudotyping assay expressing wild type Spike (D614G) **E.** IgG subclass responses to S, N, RBD post first and second dose compared to individuals with prior infection. **F.** Anti-Spike IgG- total and subclasses after first dose of vaccine stratified by age < and >80 years old. MFI – mean fluorescence intensity. S – Spike, N – nucleocapsid, RBD – Spike receptor binding domain. Mann-whitney test was used for unpaired comparisons. p-values * <0.05, **<0.01, *** <0.001, ****<0.0001, ns- not significant HS – human AB serum control, r– Pearson's correlation coefficient, p- P value, β slope/regression coefficient.

Figure 3

A



B

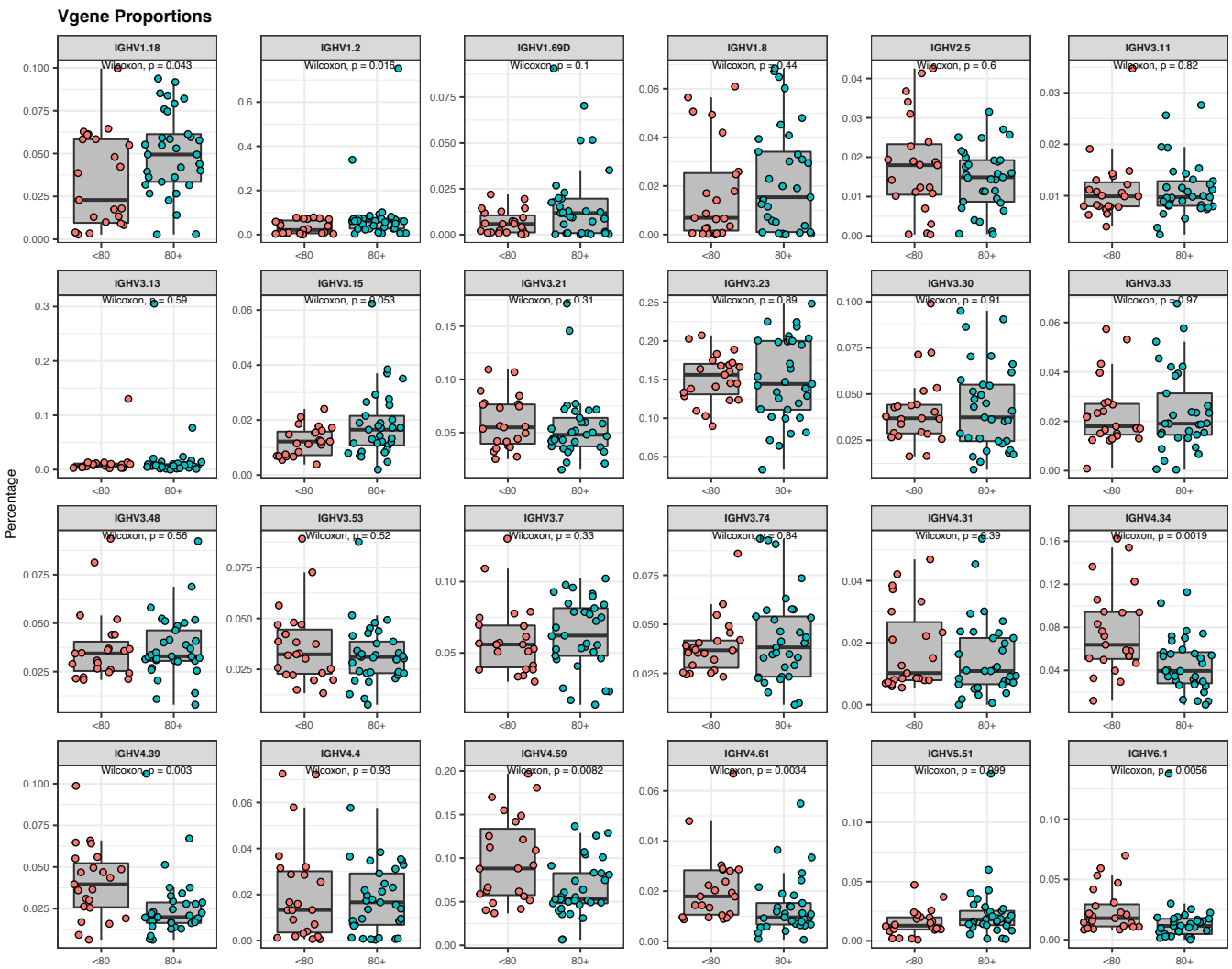


Figure 3: B cell repertoire following vaccination with first dose of Pfizer BNT162b2 vaccine. A. Boxplots showing Isotype usage according to unique VDJ sequence comparing participants <80 vs > 80 years old and association with neutralisation of spike pseudotyped virus. Neutralisation cut-off for 50% neutralisation was set at 20. **B.** Boxplots showing V gene usage as a proportion, comparing under 80 year olds with 80 year olds and older.

Figure 4

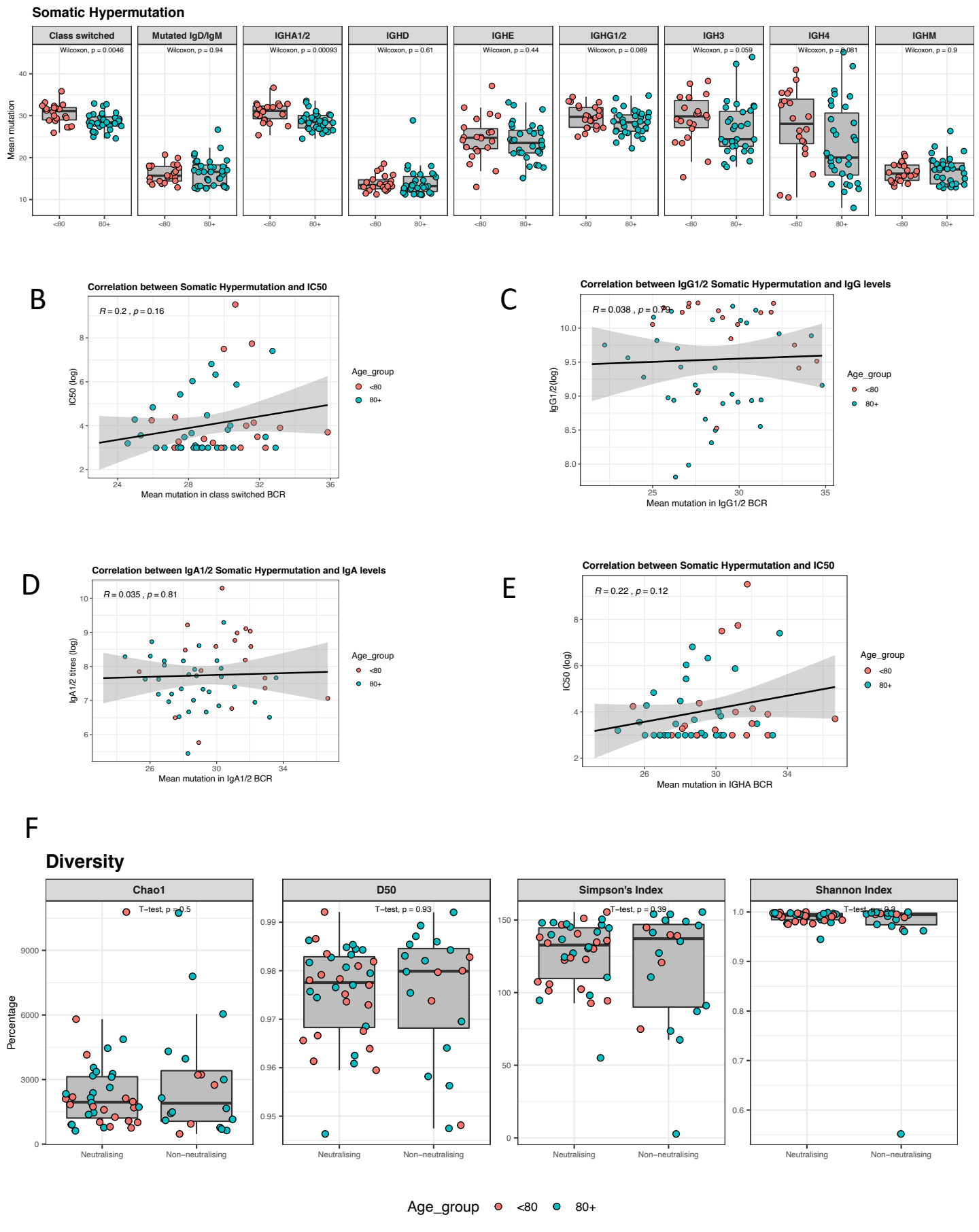


Figure 4: B cell somatic hypermutation and BCR diversity following vaccination with first dose of Pfizer BNT162b2 vaccine. A. Boxplots showing mean somatic hypermutation comparing <80 year olds with > 80 year old participants, grouped according to isotype class **B.** Correlation between somatic hypermutation in class-switched isotypes and IC50 **C.** Correlation between somatic hypermutation in IgG BCRs and total IgG. **D.** Correlation between somatic hypermutation in IgA BCRs and total IgA. **E.** Correlation between somatic hypermutation in IgHA BCR and IC50**. F.** Diversity Indices. The inverse is depicted for the Simpson's index and the Shannon-Weiner index is normalised.

Figure 5

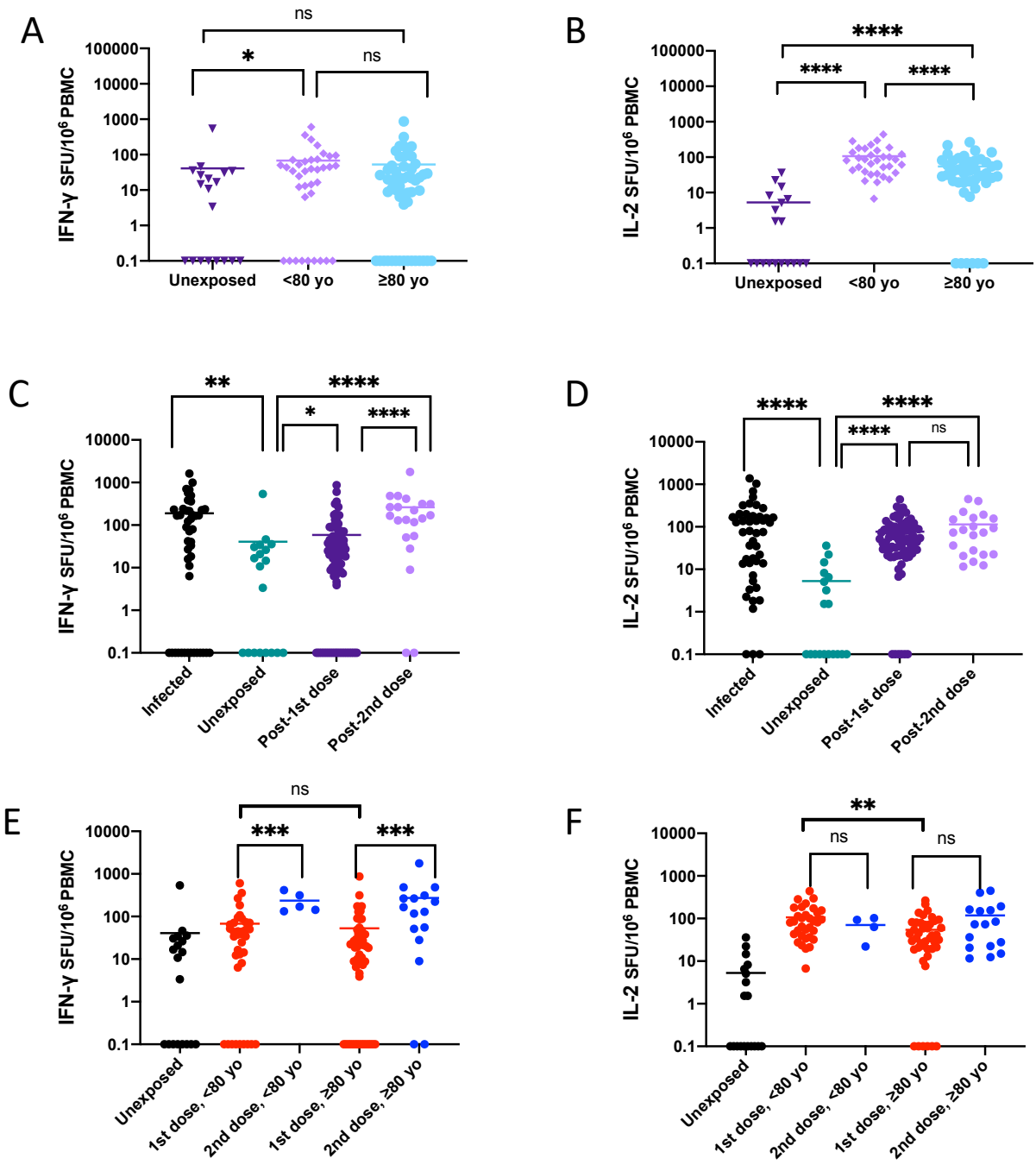
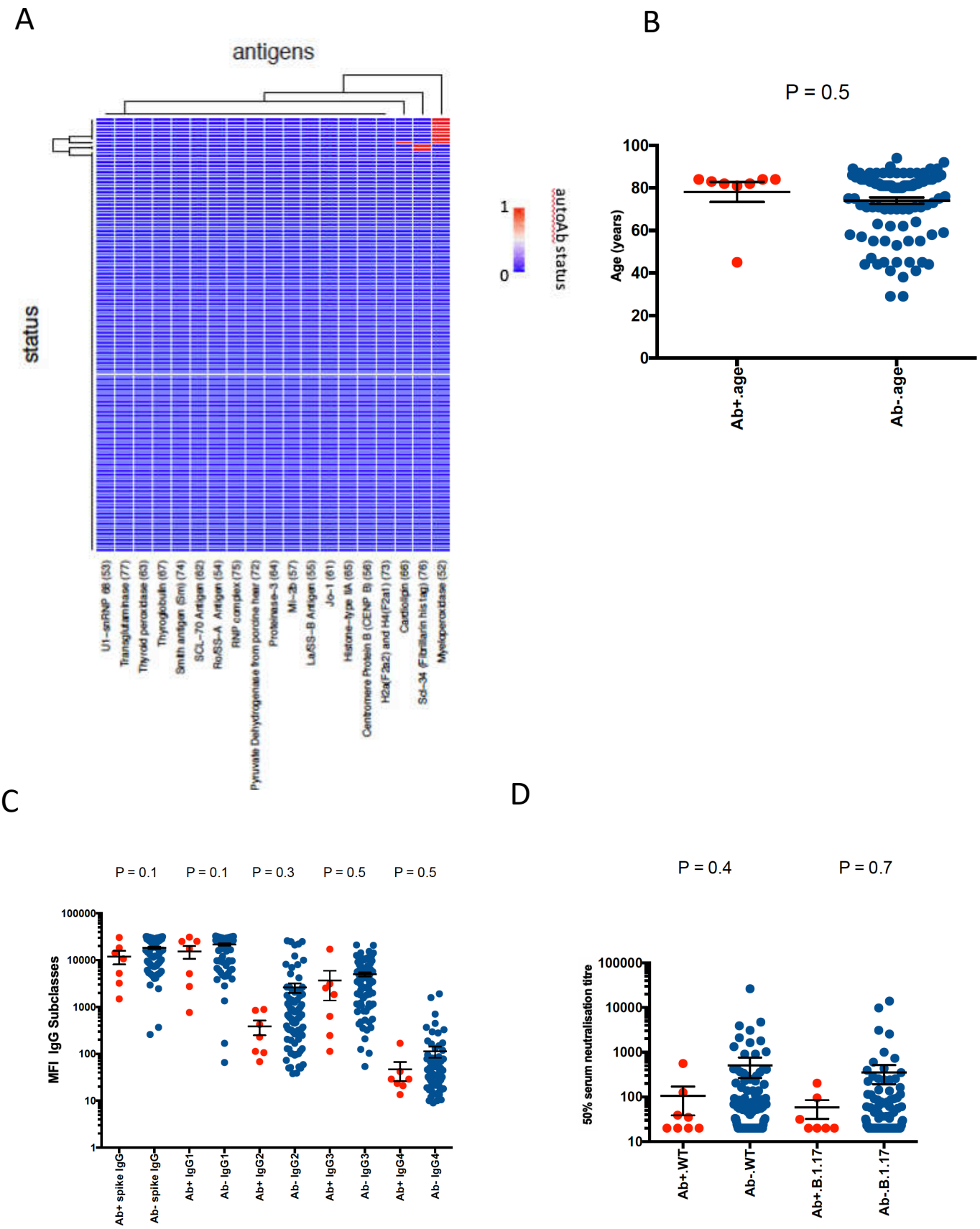


Figure 5: T cell responses to Pfizer BNT162b2 vaccine after the first and second doses of vaccine. Dose 1 n=91, dose 2 n=21. A. FluoroSpot analysis for IFNγ and **B**. IL-2 T cell responses specific to SARS-CoV-2 Spike protein peptide pool following PBMC stimulation of a cohort of unexposed (stored PBMC 2014-2016 n=20) and vaccinees <80yo IFNγ (n=37), IL-2 (n= 33) and >80yo IFNγ (n=54), IL-2 (n=43) three weeks or more after the first doses of Pfizer BNT162b2 vaccine. **C.** FluoroSpot analysis for IFNγ and **D**. IL-2 T cell responses specific to SARS-CoV-2 Spike protein peptide pool following PBMC stimulation of a cohort of infected (n=46), unexposed (n=20) and all vaccinees three weeks or more after the first doses IFNγ (n=92), IL-2 n=77 and three weeks after second IFNγ and IL-2 (n=21) of Pfizer BNT162b2 vaccine. **E.** FluoroSpot analysis for IFNγ and **F**. IL-2 T cell responses specific to SARS-CoV-2 Spike protein peptide pool following PBMC stimulation of a cohort of unexposed (n=20) and vaccinees <80yo IFNγ (n=37), IL-2 n=33 and >80yo IFNγ (n=54), IL-2 (n=43) three weeks after the first doses and <80yo IFNγ (n=5), IL-2 n=4 and >80yo IFNγ (n=16), IL-2 (n=16) three weeks after second dose of Pfizer BNT162b2 vaccine. Mann-whitney test was used for unpaired comparisons and Wilcoxon matched-pairs signed rank test for paired comparisons. p-values ** <0.01, *** <0.001, ns not significant.

Figure 6



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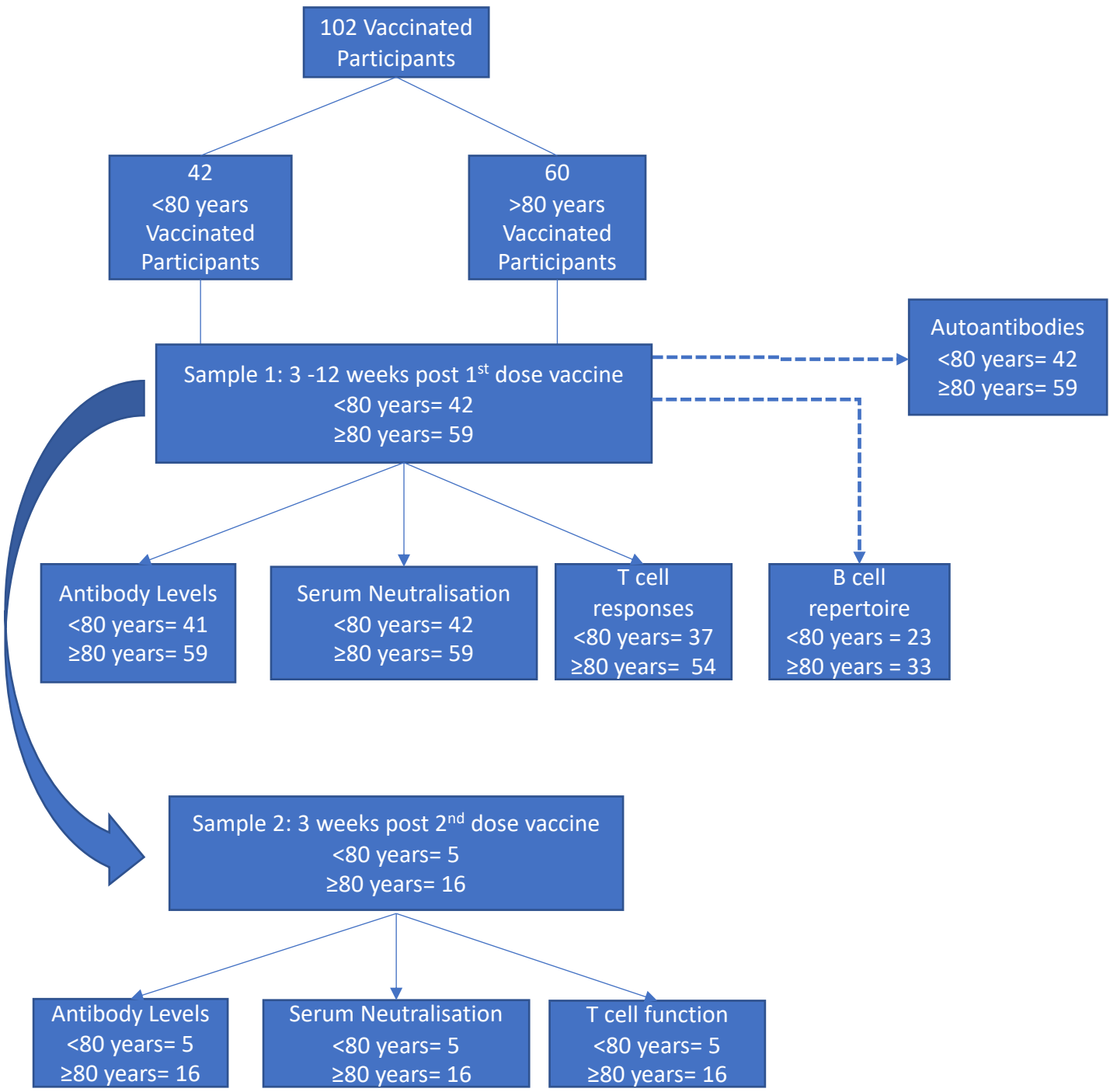
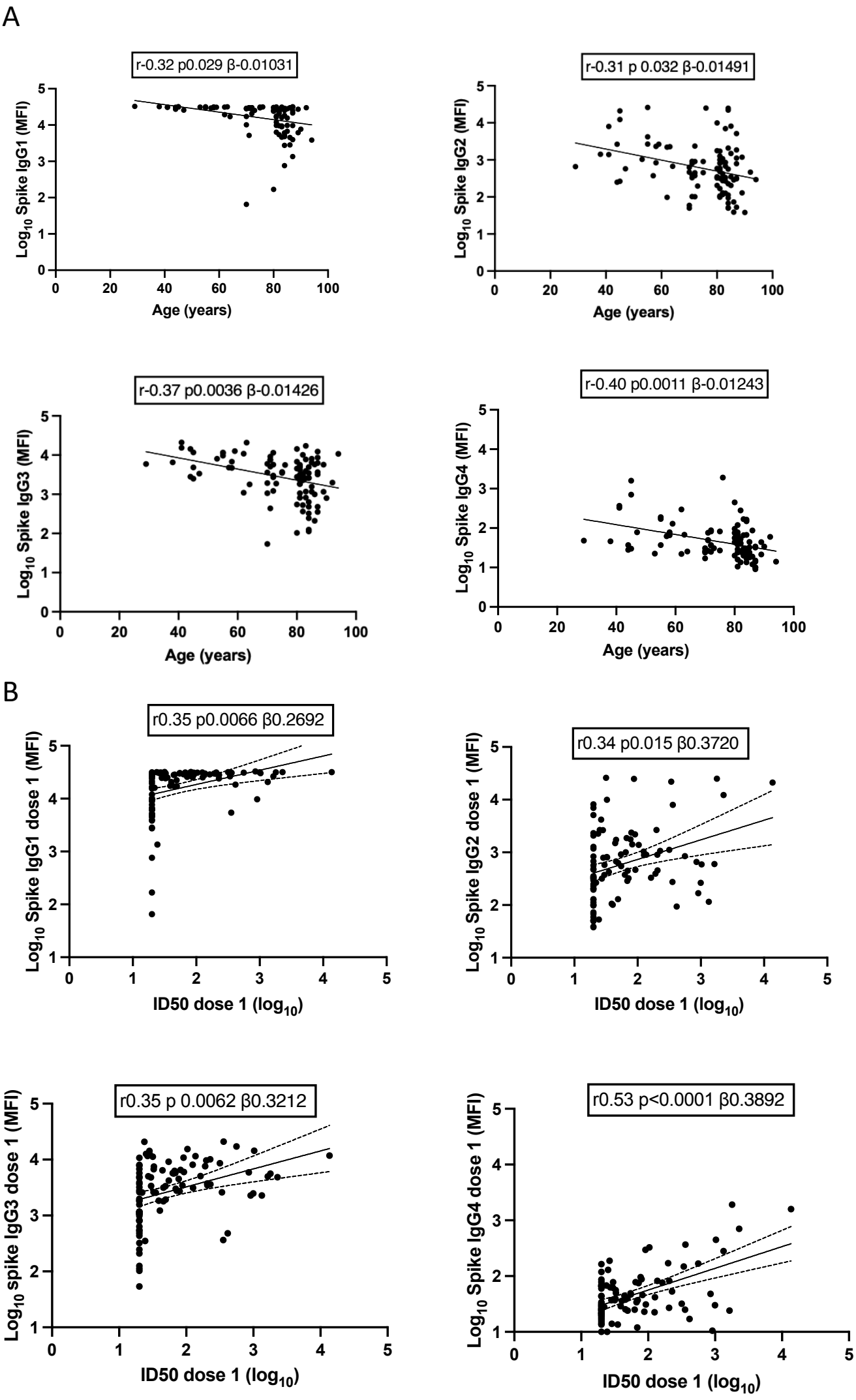
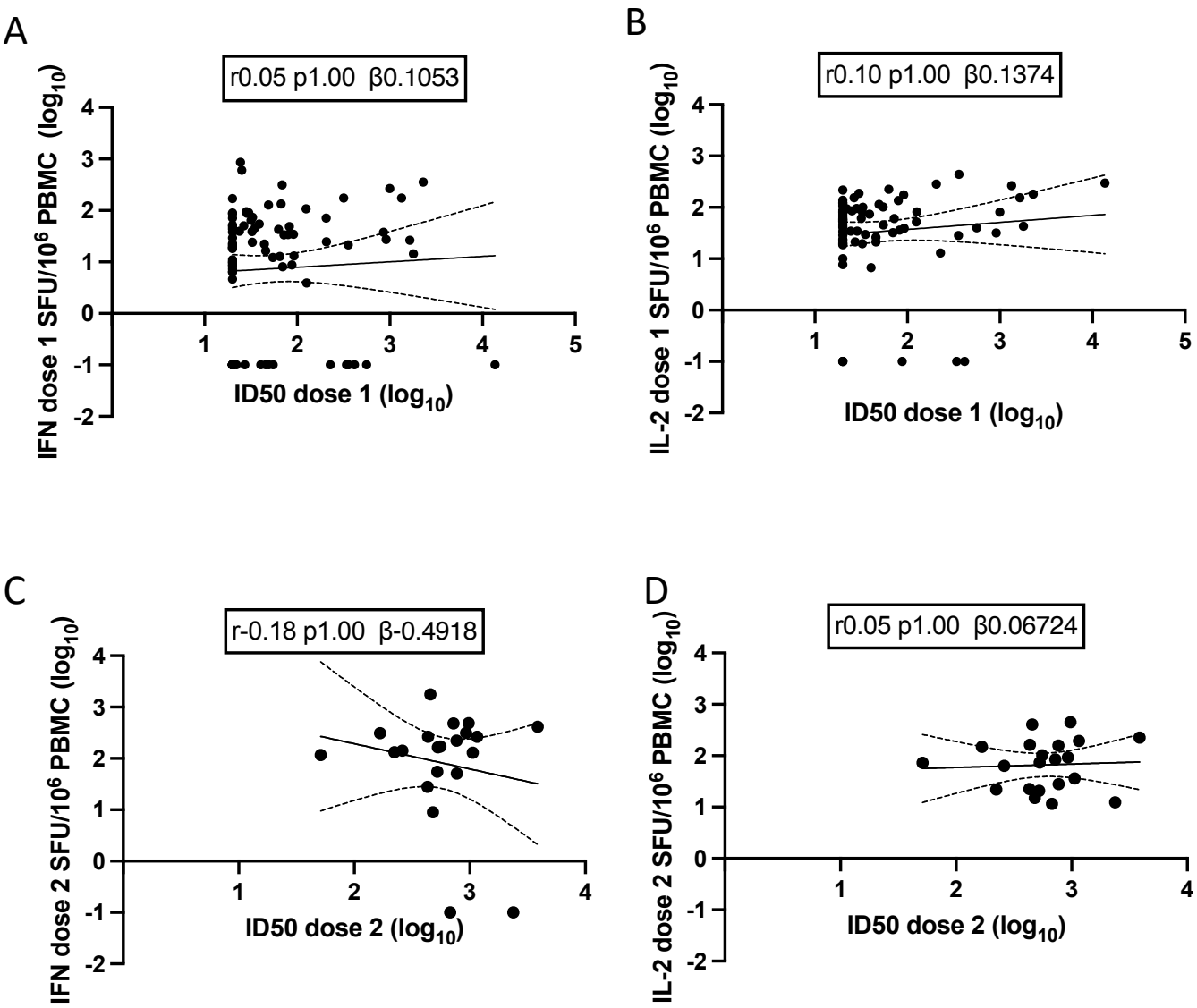


Supplementary figure 1: study flow diagram for samples and analyses

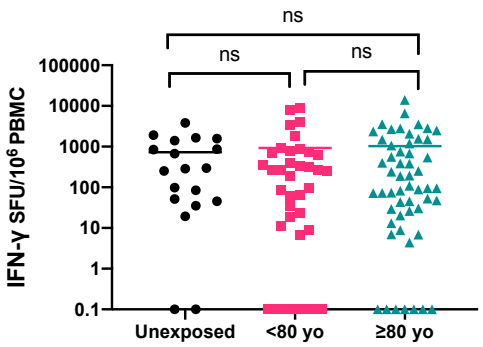


Supplementary Figure 2. A. Correlations between serum binding IgG subclass 1-4 antibody responses following vaccination with first dose of Pfizer BNT162b2 vaccine and age in years. **B.** Correlations between serum binding IgG subclass 1-4 antibody responses following vaccination with first dose of Pfizer BNT162b2 vaccine and serum neutralization using a pseudotyped viral system. MFI- mean fluorescence intensity ID50 – inhibitory dilution required to achieve 50% inhibition of viral infection. r pearson's correlation coefficient, β slope/regression coefficient, p p-value. Bonferroni adjustment was made for multiple comparisons.

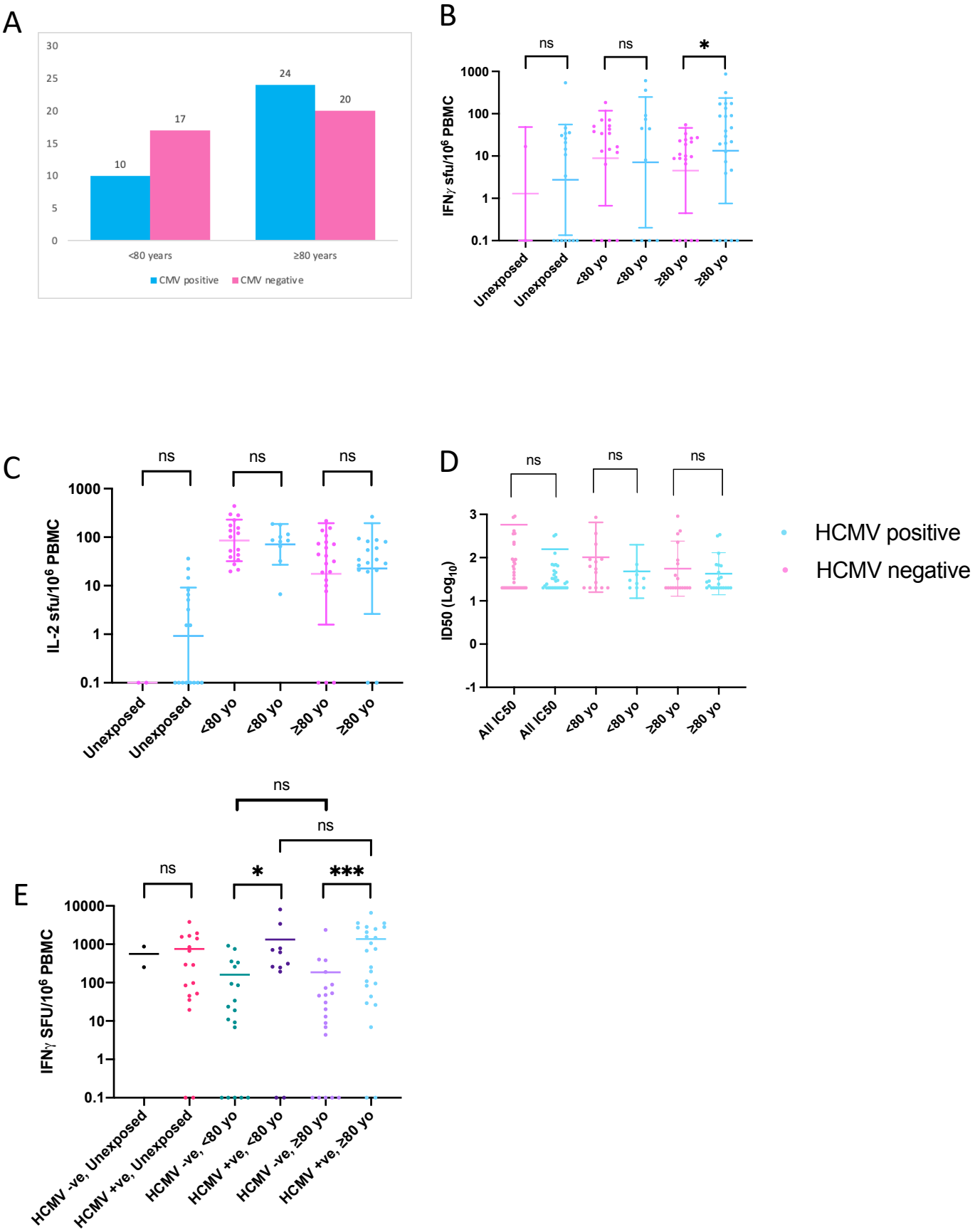
Supplementary Figure 3. B cell repertoire following vaccination with first dose of Pfizer BNT162b2 vaccine. A. Isotype usage according to unique VDJ sequence comparing under 80 year olds with 80 year olds and older. **B.** Boxplots showing V gene usage as a proportion, comparing neutralisation of spike pseudotyped virus. Neutralisation cut-off for 50% neutralisation was set at 20. C. Diversity Indices comparing under 80 year olds with 80 year olds and older. The inverse is depicted for the Simpson's index and the Shannon-Weiner index is normalised.



Supplementary Figure 4. Correlation between serum neutralisation of Spike (D614G) pseudotyped lentiviral particles (inhibitory dilution at which 50% inhibition of infection is achieved, ID50) and T cell responses against SARS-CoV-2 Spike peptide pool. A,B. IFNγ (n=89) and IL2 (n=74) FluoroSpot after first. C,D. IFNγ (n=21) and IL2 (n=21) FluoroSpot after second dose. SFU: spot forming units. r: pearson correlation coefficient with p value indicated and b the slope or coefficient. Bonferroni adjustment was made for multiple comparisons.



Supplementary Figure 5. FluoroSpot interferon gamma PBMC responses to peptide pool of Cytomegalovirus, Epstein Barr virus and Influenza virus (CEF) A. Response from unexposed stored PBMC 2014-2016 n=20), <80yo (n=37) and >80yo (n=54) three weeks after the first doses of Pfizer BNT162b2 vaccine. Mann-whitney test was used for unpaired comparisons and Wilcoxon matched-pairs signed rank test for paired comparisons. p-values * <0.05, ns not significant



Supplementary Figure 6. Human cytomegalovirus serostatus, T cell responses and serum neutralisation of Spike (D614G) pseudotyped lentiviral particles (inhibitory dilution at which 50% inhibition of infection is achieved, ID50) to Pfizer BNT162b2 vaccine after the first dose of vaccine. Dose 1. A. (n=72) HCMV serostatus by <80 and ≥80 year age groups, HCMV positive (blue), HCMV negative (pink). **B** IFNγ (n=72) and **C.** IL2 (n=64) FluoroSpot response after the first dose. **D.** Inhibitory dilution at which 50% inhibition of infection after the first dose. **E**. IFN*γ* FluoroSpot response to CEF peptides after the first dose of BNT162b2. SFU- spot forming units. ID50-inhibitory dilution at which 50% inhibition of infection is achieved. HCMC- Human cytomegalovirus., CEF- Cytomegalovirus Epstein Barr virus, Influenza virus

Figures

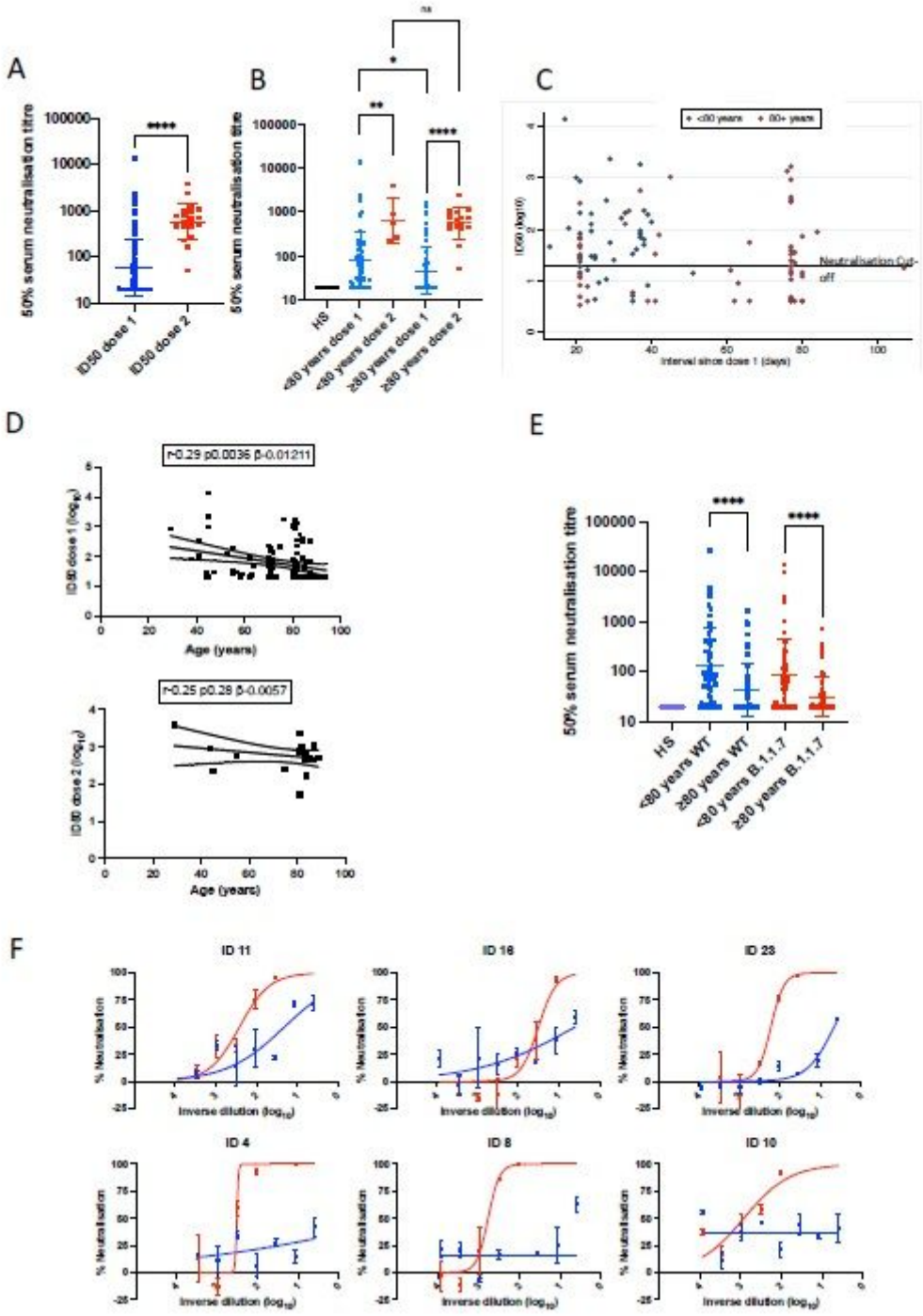
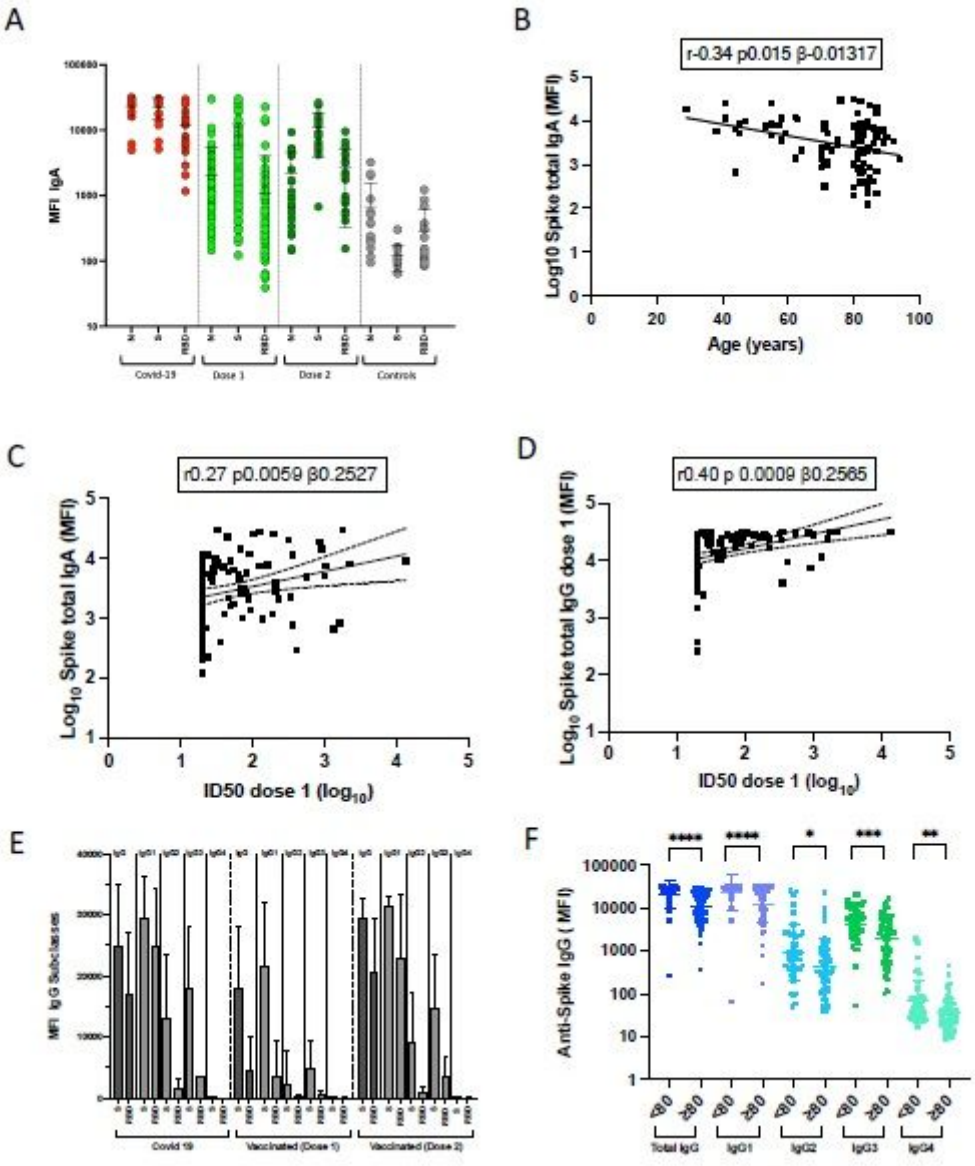


Figure 1

A, B. Neutralisation by Pfizer BNT162b2 vaccine sera against SARS-CoV-2 in a Spike lentiviral pseudotyping assay expressing wild type Spike (D614G). Data are shown as mean ID50 values for individuals after Dose 1 (n=101) and after Dose 2 (n=21). Geometric mean with s.d is shown. Each point is a mean of technical replicates from two experiment repeats. C. Serum neutralisation of Spike (D614G) pseudotyped lentiviral particles (inhibitory dilution at which 50% inhibition of infection is achieved, ID50)

after Dose 1 by age-group <80 years (blue n=42), ≥80 years (red n=59). Line is the neutralization cut off at ID50 of 20. D. Correlation between serum neutralisation of Spike (D614G) pseudotyped lentiviral particles (inhibitory dilution at which 50% inhibition of infection is achieved, ID50)and age after the first dose n=101 and the second dose n=21 of Pfizer BNT162b2 vaccine. E. GMT with s.d of first dose Pfizer BNT162b2 vaccine sera against wild type (blue) and B.1.1.7 Spike mutant (red) SARS-CoV-2 pseudotyped viruses by age-group. F. Neutralisation curves for serum from six individuals with reduced responses after first dose of Pfizer BNT162b2 vaccine against pseudovirus expressing wild type Spike (D614G). B.1.1.7 Spike mutant with N501Y, A570D, ΔH69/V70, Δ144/145, P681H, T716I, S982A and D1118H. GMT with s.d are of two independent experiments each with two technical repeats. Neutralisation cut off for 50% neutralisation set at 20. Neutralisation are means of technical replicates, plotted with error bars representing standard error of mean. Data are representative of 2 independent experiments. Mann-Whitney test was used for unpaired comparisons and Wilcoxon matchedpairs signed rank test for paired comparisons. p-values * <0.05, ** <0.01, **** <0.0001, ns not significant, HS – human AB serum control, r– Pearson's correlation coefficient, β slope/regression coefficient, p p-value.



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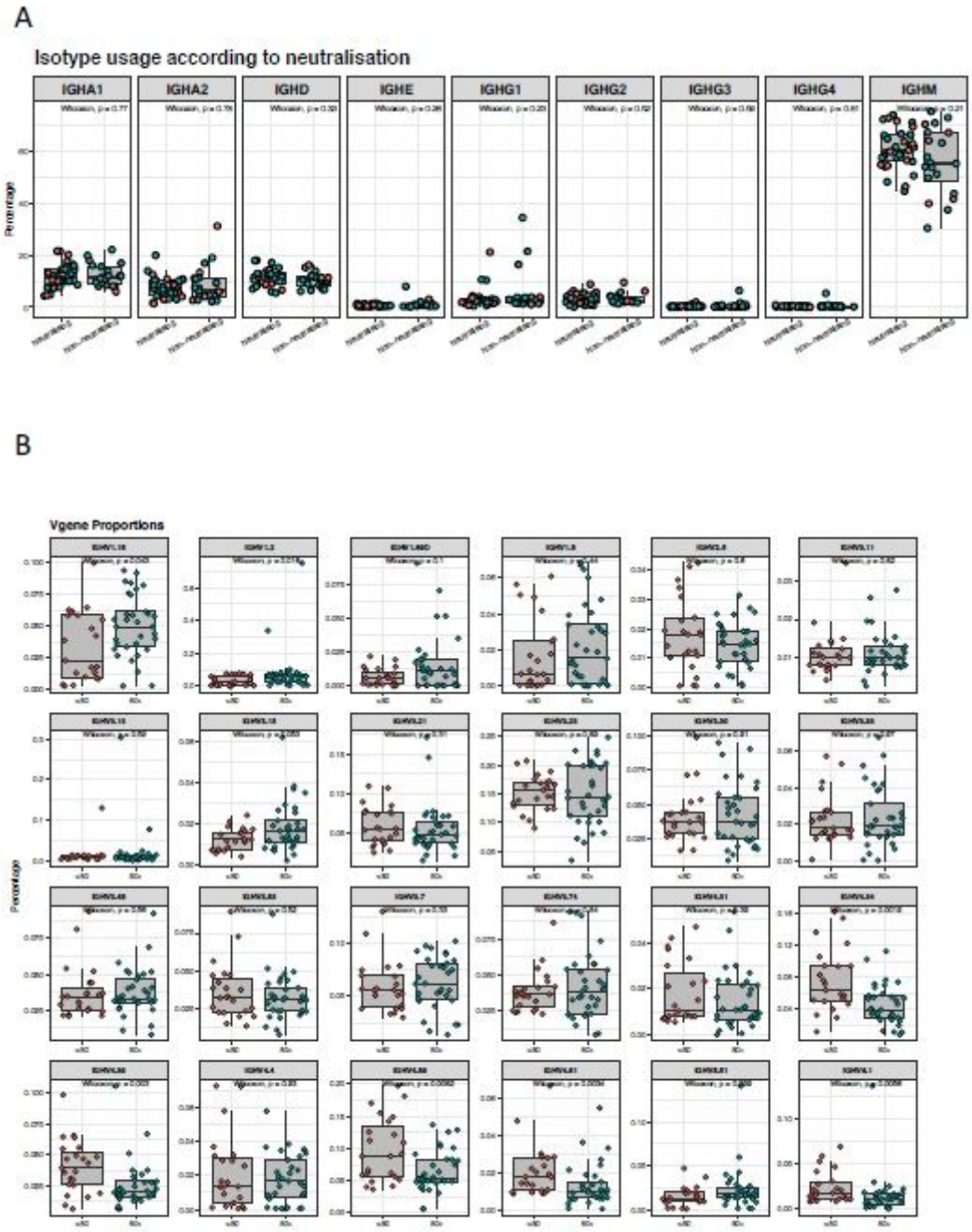

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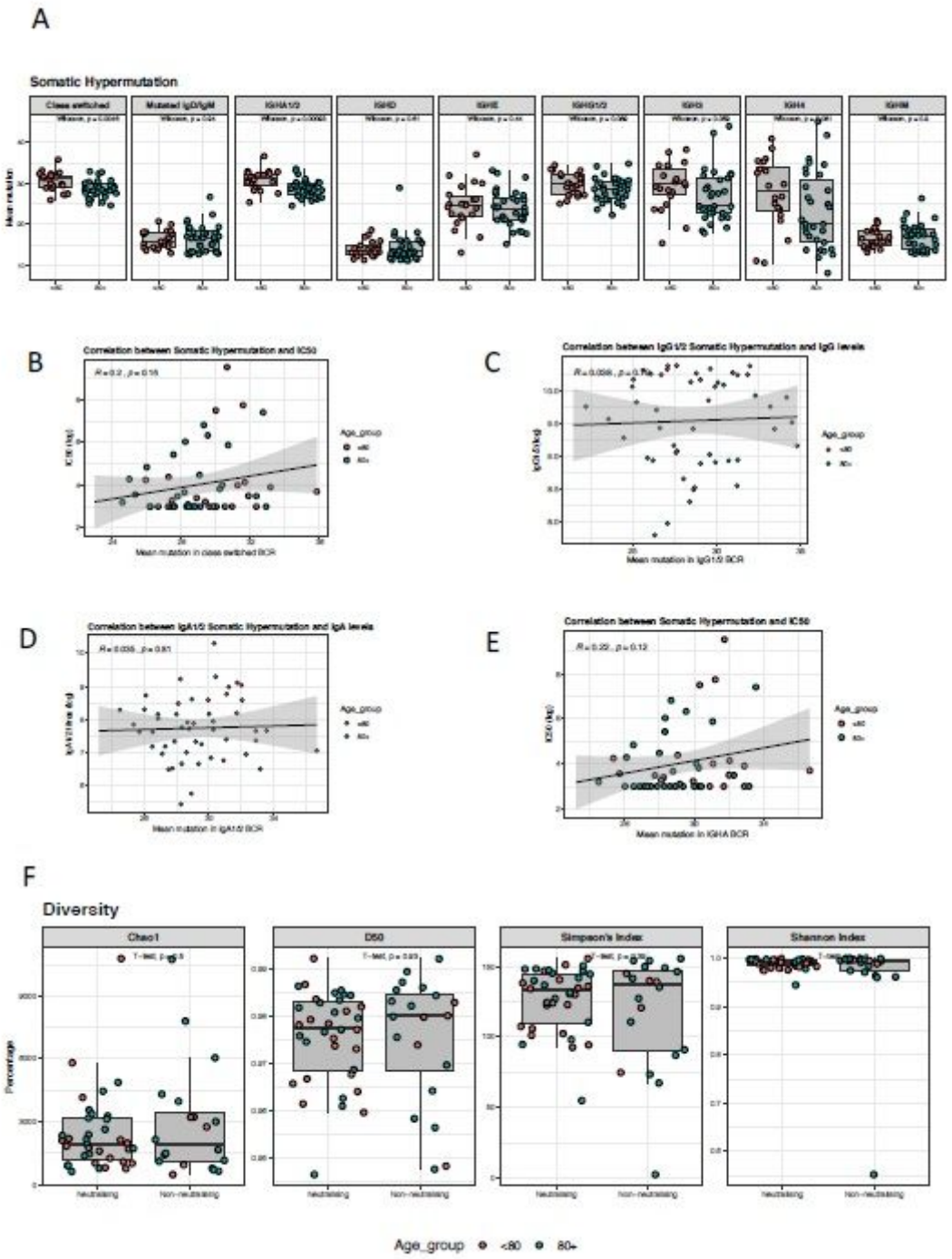

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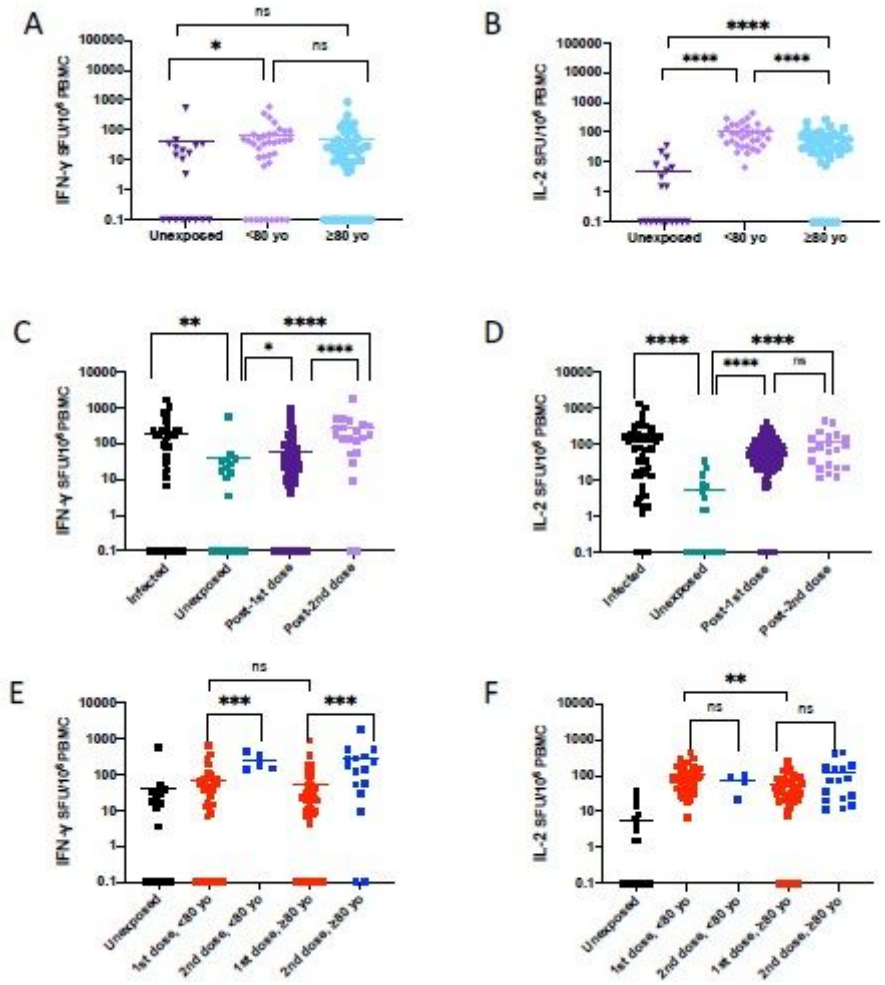
B cell somatic hypermutation and BCR diversity following vaccination with first dose of Pfizer BNT162b2 vaccine. A. Boxplots showing mean somatic hypermutation comparing <80 year olds with > 80 year old participants, grouped according to isotype class B. Correlation between somatic hypermutation in class-switched isotypes and IC50 C. Correlation between somatic hypermutation in IgG BCRs and total IgG. D. Correlation between somatic hypermutation in IgA BCRs and total IgA. E. Correlation between somatic hypermutation in IgHA BCR and IC50. F. Diversity Indices. The inverse is depicted for the Simpson's index and the Shannon-Weiner index is normalised.



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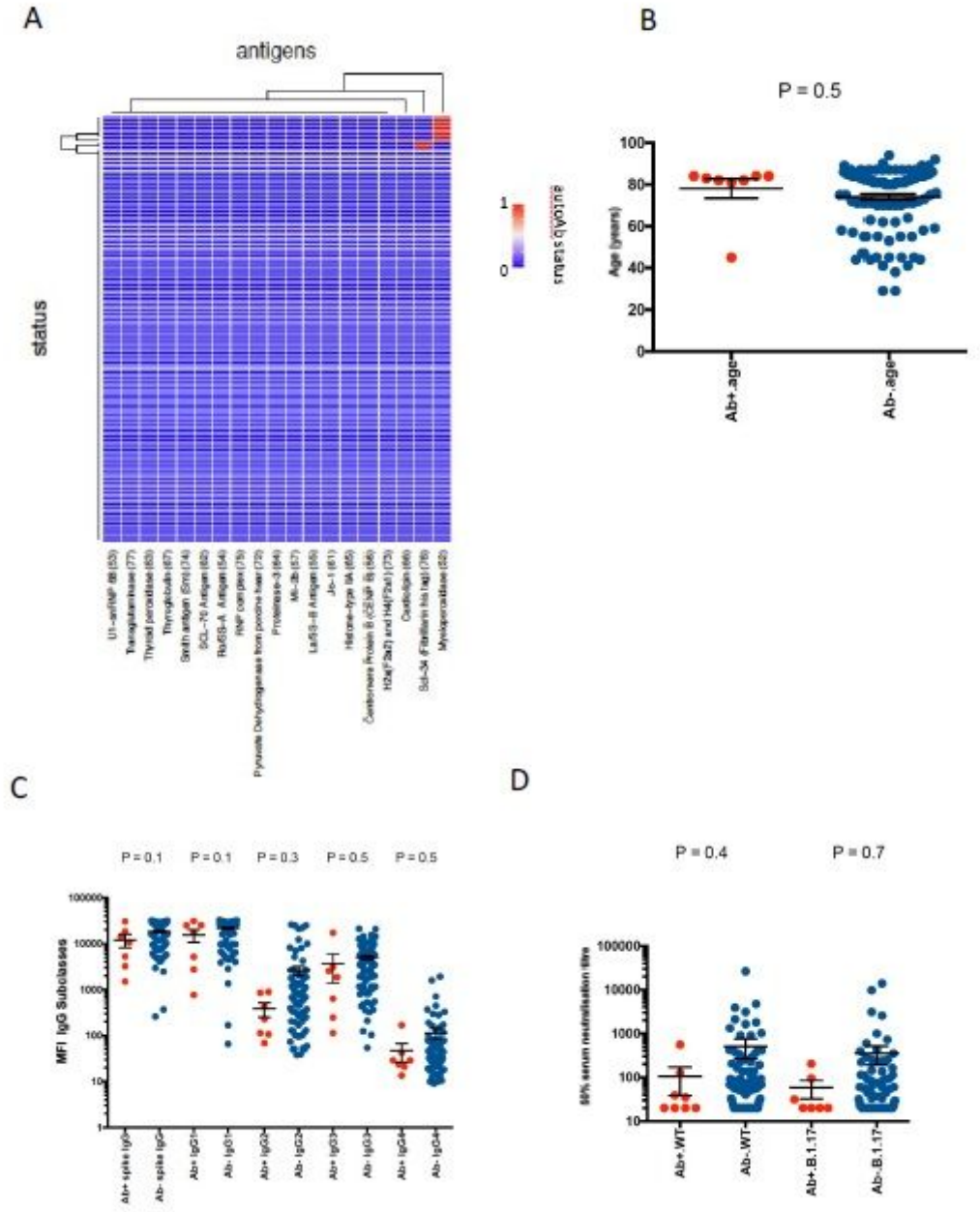
T cell responses to Pfizer BNT162b2 vaccine after the first and second doses of vaccine. Dose 1 n=91, dose 2 n=21. A. FluoroSpot analysis for IFNg and B. IL-2 T cell responses specific to SARS-CoV-2 Spike protein peptide pool following PBMC stimulation of a cohort of unexposed (stored PBMC 2014-2016 n=20) and vaccinees <80yo IFNg (n=37), IL-2 (n= 33) and >80yo IFNg (n=54), IL-2 (n=43) three weeks or more after the first doses of Pfizer BNT162b2 vaccine. C. FluoroSpot analysis for IFNg and D. IL-2 T cell responses specific to SARS-CoV-2 Spike protein peptide pool following PBMC stimulation of a cohort of infected (n=46), unexposed (n=20) and all vaccinees three weeks or more after the first doses IFNg (n=92), IL-2 n=77 and three weeks after second IFNg and IL-2 (n=21) of Pfizer BNT162b2 vaccine. E. FluoroSpot analysis for IFNg and F. IL-2 T cell responses specific to SARS-CoV-2 Spike protein peptide pool following PBMC stimulation of a cohort of unexposed (n=20) and vaccinees <80yo IFNg (n=37), IL-2 n=33 and >80yo IFNg (n=54), IL-2 (n=43) three weeks after the first doses and <80yo IFNg (n=5), IL-2 n=4 and >80yo IFNg (n=16), IL-2 (n=16) three weeks after second dose of Pfizer BNT162b2 vaccine. Mann-whitney test was used for unpaired comparisons and Wilcoxon matched-pairs signed rank test for paired comparisons. p-values ** <0.01, *** <0.001, ns not significant.



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Autoantibodies in participants receiving at least one dose of the Pfizer BNT162b2 vaccine. (n=101). A. Heatmap of log2 transformed fluorescence intensity(FI) of 19 autoantibodies, positive (red), negative (blue). B. Age in years by anti-MPO antibody positive (red) or negative (blue) status. Plotted is the mean age and s.d. C. (n=100) IgG subclass responses to Spike post first dose Pfizer BNT162b2 vaccine comparing individuals with anti-MPO antibody positive (red) or negative (blue) status. D. GMT with s.d of first dose Pfizer BNT162b2 vaccine sera against wild type and B.1.1.7 Spike mutant SARS-CoV-2 pseudotyped viruses by anti-MPO antibody positive (red) or negative (blue) status. Ab+ antibody positive, Ab- antibody negative, MPO- myeloperoxidase, P – pvalue, WT- wild type, B.1.1.7 Spike mutant with N501Y, A570D, ΔH69/V70, Δ144/145, P681H, T716I, S982A and D1118H.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryFigures.pdf