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Supplementary appendix 2

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Vinh DC, Gouin J-P, Cruz-Santiago D, et al. Real-world serological responses to extended-interval and heterologous COVID-19 mRNA vaccination in frail, older people (UNCoVER): an interim report from a prospective observational cohort study. *Lancet Healthy Longev* 2022; published online Feb 21. https://doi.org/10.1016/S2666-7568(22)00012-5.

Research Protocol

Réponse immunitaire aux vaccins contre la COVID-19 chez la personne âgée

Background

On January 20th, 2020, the World Health Organization declared the SARS-coV-2 outbreak a pandemic. In Quebec, physical distancing and confinement directives were put in place on March 13th, 2020. Despite these public health measures, there was a significant spread of COVID-19 within the population, resulting in over 7,867 deaths to date. In Quebec, most of the individuals who died from COVID-19 were older adults aged 70 and over. In particular, residents in CHSLD were over-represented among the patients deceased following a SARS-coV-2 infection (Gouvernement du Quebec, 2020).

Vaccines are thought to be a key strategy for the long-term management of COVID-19. In a phase II-III clinical trial, the Pfizer-BioNTech *COVID*-19 mRNA vaccine was 95.0% effective in preventing laboratory-confirmed COVID-19 in persons without evidence of previous SARS-CoV-2 infection (Polack et al., 2020). This vaccine, administered in two doses given 21 days apart, was tested on individuals aged 16 years or older who were healthy or had stable chronic medical conditions. Although preliminary data suggest that the vaccine is effective among healthy adults aged 65 years and older, it is not clear whether the same efficacy rate would be observed among older adults with multiple comorbidities. Indeed, in this large trial, there was a limited number of COVID-19-related hospitalizations and deaths. Furthermore, it is currently unknown how long the immune response to the vaccine last and what is the stability of the immune response after one dose of the vaccine when the second dose is delayed. Similar issues have been reported for the Moderna COVID-19 vaccine (Widge et al., 2020). Furthermore, there is ongoing debate about whether a third dose of the mRNA vaccines is needed among frail elderly.

Past research indicates that elderly individuals have blunted immune responses to many vaccines (Chen et al., 2009). Socio-demographic and psychosocial factors are known to influence the stability of antibody response to vaccines in the elderly (Ayling et al., 2019; Glaser et al., 2000). There is thus a need to identify factors that may predict poorer responses to the COVID-19 vaccines among older adults.

Objectives and Research Questions

The goals of this observational study are 1) to examine the evolution of the immune response to the vaccine over a 24-month period, 2) to examine the stability of the immune response after one, two, and three doses of the vaccine, 3) to examine the prevalence of COVID-19 and COVID-19-related hospitalizations and deaths following a COVID vaccine, and 4) to identify sociodemographic, clinical, and psychosocial predictors of poor immune responses to the vaccine among older adults in long-term care. The first 3 objectives are descriptive in nature; therefore no hypothesis is formulated. For the fourth research questions, we hypothesize that older, frailer, and more distressed individuals will exhibit a lower peak antibody levels as well as a larger decrease in antibodies over time.

Methodology

Research Design:

This observational, longitudinal study will include venipunctures and telephone-based interviews with patients, their caregivers, and their health care professionals at up to 10 occasions over a 24-month period. The first blood draw will be taken prior to vaccination for participants who have not been vaccinated already. The second blood draw will be taken about one month after the first dose. The third blood draw will be between 6 and 10 weeks after the first dose of the vaccine. This blood draw will be done only if the second dose has not been administered by that time. The fourth blood draw will be right before receiving the second dose of the vaccine. The fifth blood draw will be about one month after the second dose of the vaccine. The sixth blood draw will be at 6 months after the second dose. The seventh blood draw will be done before the third dose of the vaccine. This blood draw will be done only if the 3rd dose of the vaccine is administered after November 2021. The eighth, ninth, and tenth blood draws will be done respectively at 1, 4, and 12 months after the third dose of the vaccine. Participants or their caregiver as well as their health care professionals will complete a 5-minute phone-based interview once per year.

Participants:

This study will enroll residents in long-term care facility who have agreed to receive a COVID-19 vaccine. A minimum of 200 patients may be enrolled. Patients who are SARS-COV-2 negative as well as SARS-COV-2 positive recovered patients will be enrolled. There are no exclusion criteria.

Procedures:

The nurse who obtains consent for COVID-19 vaccination from the patient or their caregiver will inform them of the possibility of participating in the current study. If the patient or their caregiver agreed to receive the vaccine and agreed to be contacted about the study, our research assistant will call them to explain the study. Oral consent will be obtained via telephone. The research assistant will read the consent form to the participant or their caregiver and answer any questions that they may have. If the participant or their caregiver gives oral consent to participate in the study, the research assistant will indicate the date and time that oral consent was received and sign the consent form confirming the oral consent. When possible, the research assistant will keep a copy of the phone record of their call with the participant or their caregivers.

About 8 ml of blood will be collected per venipuncture at each assessment. The first blood draw will be taken via antecubital venipuncture the day of the vaccination by a registered nurse to obtain the pre-vaccine serology. The following blood draws will be conducted one month after the first dose, between 6 and 10 weeks after the first dose, at the time of the second dose, one month after the second dose, and 6, months after the second dose as well as 1, 4, and 12 months after the 3rd dose of the COVID-19 vaccine. Once per year, a 5-minute telephone-based psychosocial assessment with the resident or their caregiver and their health care professional will be conducted.

At each time point, samples will be transported to Dr Vinh's laboratory at the Glen (MUHC) by a certified carrier. There, they will be processed and stored at -80°C. Samples will be later analysed in a COVID-19 Immunity Task Force (CITF)-associated laboratory.

Measures:

The telephone-based assessment will assess current social and emotional functioning as well as sleep. This short questionnaire will be administered in about 5 minutes.

Blood samples will be assessed for IgG antibodies against spike protein, receptor binding domain, nucleocapsid protein as well as neutralizing antibodies to quantify the immune response to the vaccine. Other immune analysis maybe conducted pending budget availability.

Medical chart review will be used to extract information on sociodemographic characteristics, current chronic medical conditions, frailty level, and medication use as well as COVID-19 related medical complications.

Statistical analysis:

Hierarchical linear modeling will be used to describe the trajectory of change in immune response over time. Logistic regressions will be used to predict dichotomous outcomes, including COVID-19-related hospitalizations and deaths.

Ethical considerations

Participants may experience some transient pain from the venipuncture procedure. There is also a minor risk of bruising, infection, or fainting following the blood draw. The venipuncture will be conducted by trained nurses to minimize these risks. Nurses performing the venipuncture will have to have a SARS-coV-2 negative test prior to interacting with participants. The nurse will also wear the personal protective equipment, per institutional policies. If the patient experiences some medical complications due to the study procedure, Dr. Cruz-Santiago will inform the treatment team. All data will be coded to protect the participant's confidentiality. Data will be stored in a secured server in Dr. Gouin's laboratory.

Implications

This study will provide key information on older adults' responses to the COVID-19 vaccines.

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Supplementary Material

Real-world serologic responses to Extended-interval and Heterologous COVID-19 mRNA vaccination in Frail Elderly - Interim report from a prospective observational cohort study

Methods:

Chemiluminescent automated ELISA

Automated chemiluminescent ELISAs were based upon and optimized from assays first described by Isho et al[1]. Hamilton MicroLab Star robotic liquid handlers and plate washer (Biotek 405 TS/LS LHC2, all washes were performed 4x in 100 µl PBS + 0·1% Tween 20 (PBST)) were used for all steps at the University of Ottawa, Faculty of Medicine. All incubation times on the platform were done at room temperature shaking at 500-700 rpm. COVID-19 antigens (Spike trimer, RBD and N), as well as the anti-hIgG#5-HRP fusion antibody were generously provided by Dr. Yves Durocher, National Research Council of Canada (NRC), Montreal. Antigen production was described previously[1]. Antigens (spike trimer, RBD, N) were diluted in Phosphate Buffered Saline (PBS) and dispensed into wells of a 384-well high-binding polystyrene Nunc plate (ThermoFisher, #460372) at a final amount of 50 ng/well. The plates were centrifuged in a plate spinner for 1 minute to ensure even coating, incubated overnight rocking at 4°C and washed. Wells were blocked with 80 µl of 3% w/v skim milk powder dissolved in PBST for one hour and then washed. Samples were diluted at 1:100 (for Spike, RBD and NP analysis) and 1:10000 (for analysis of Spike and RBD in vaccinated individuals) in 1% w/v skim milk powder dissolved in PBST and 10 µL was added to each well from a 96-well source plate. Standard curves and controls were diluted as per Supplemental Table 1 in 1% w/v skim milk powder dissolved in PBST and 10 µL was added to each plate. Plates were incubated for 2 hours and wells were washed with PBST. Secondary antibodies (anti-human IgG-HRP (NRC anti-hIgG#5-HRP fusion), anti-human IgA-HRP (Jackson ImmunoResearch, 109-035-011), anti-human IgM-HRP (Jackson ImmunoResearch, 109-035-129)) were diluted at 1:5400, 1:8000 and 1:9600, respectively, in 1% w/v skim milk powder in PBST and 10 µl was added to each well. After incubation for 1 hour, the wells were washed and 10 µl of ELISA Pico substrate (ThermoFisher Scientific, #37069, diluted 1:2 in MilliQ H_2O) was dispensed to each well. After a 5 min incubation, plates were read on an NEO2 (Bio-Tek) plate reader at 20 ms/well at a read height of 1·0 mm.

Data Processing

For consistency in data processing, a constant plate layout containing all of the controls and standard curves in quadruplicates was established. Luminescence values obtained from the antigen-specific standard curve were modelized using a four-parameter log-logistic function to identify the inflection point. Blank-subtracted luminescence values for samples were scaled in relation to the luminescence value corresponding to the inflection point to allow data normalization for subsequent processing. Normalized luminescence values were then converted into Binding Antibody Units (BAU) using titration curves derived from the WHO International Standard (IS, National Institute for Biological Standards and Control (NIBSC), Code 20/136) at different dilutions, as described by Colwill et al[2]. BAU/ml values were calculated by accounting for the dilution factors. All data processing was performed in R. Standard curve processing was determined using "LL.4" four-parameter log-logistic self-starter function from the "drc" package in R used with default parameters.

Supplemental Material References

1. Isho B, Abe KT, Zuo M, et al. Persistence of serum and saliva antibody responses to SARS-CoV-2 spike antigens in COVID-19 patients. Science immunology. 2020 Oct 8;5(52).
2. Colwill K, Galipeau Y, Stuible M, et al. A" Made-in-Canada" serology solution for profiling humoral immune responses to SARS-CoV-2 infection and vaccination. medRxiv. doi:10.1101/2021.10.25.21265476

Supplemental Table 1: Standard curves and controls for automated chemiluminescent ELISA:

			Concentrations (ng/µL)							
	Reagent	**Source**	STD1	STD2	STD3	STD4	STD5	STD6	STD7	STD8
Standard Curves	anti-Spike, anti-RBD	Absolute Antibody CR3022 (Ab01680-10.0)	0·5	0·25	0·125	0·0625	0·03125	0·015625	0·00390625	0·000976563
	anti-NP	GenScript HC2003 (A02039)	0·5	0·25	0·125	0·0625	0·03125	0·015625	0·00390625	0·000976563

			Concentrations							
Controls	Pooled Positive Serum	In House	1:50	1:100	1:250					
	Pooled Negative Serum	in House	1:50	1:100						
	IgG from Human Serum	Sigma (I4506)	1.0 µg/mL							

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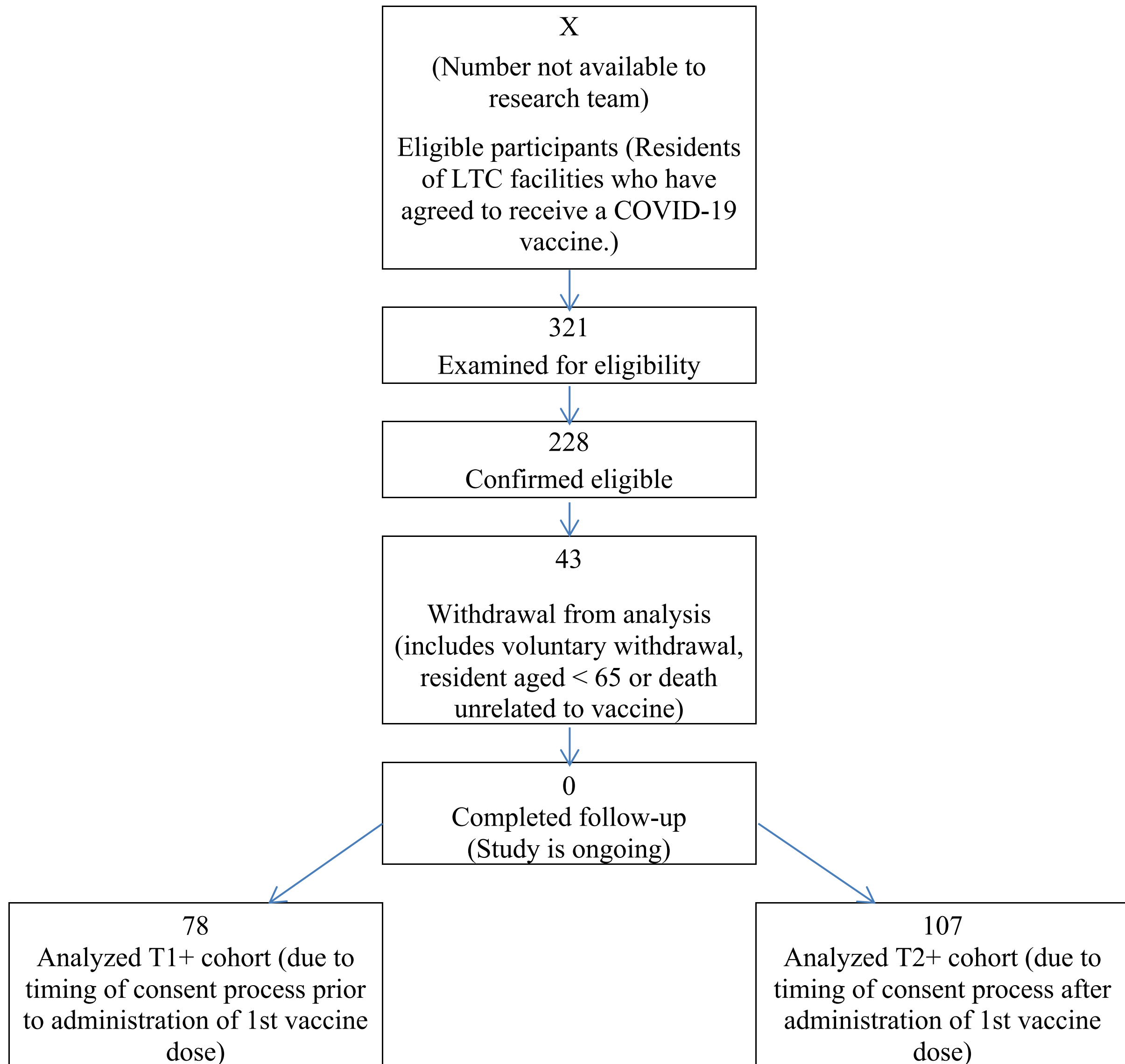
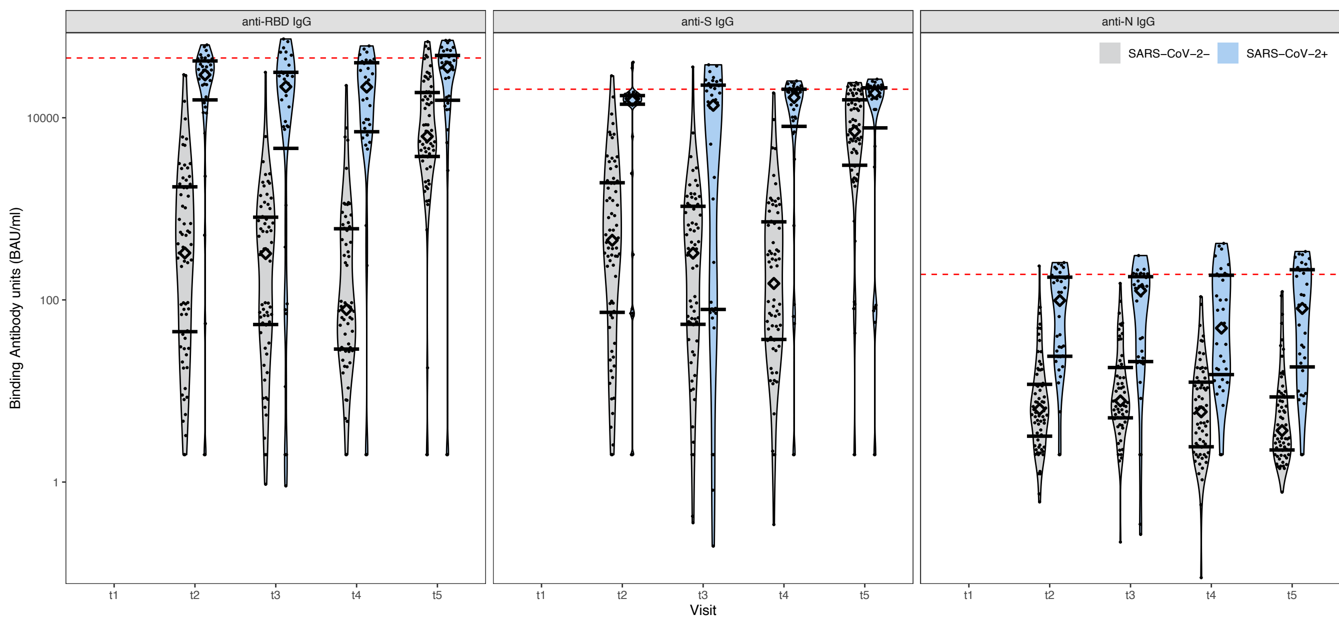
Supplementary Figure 1. Flow-chart of participants in the UNCoVER project.

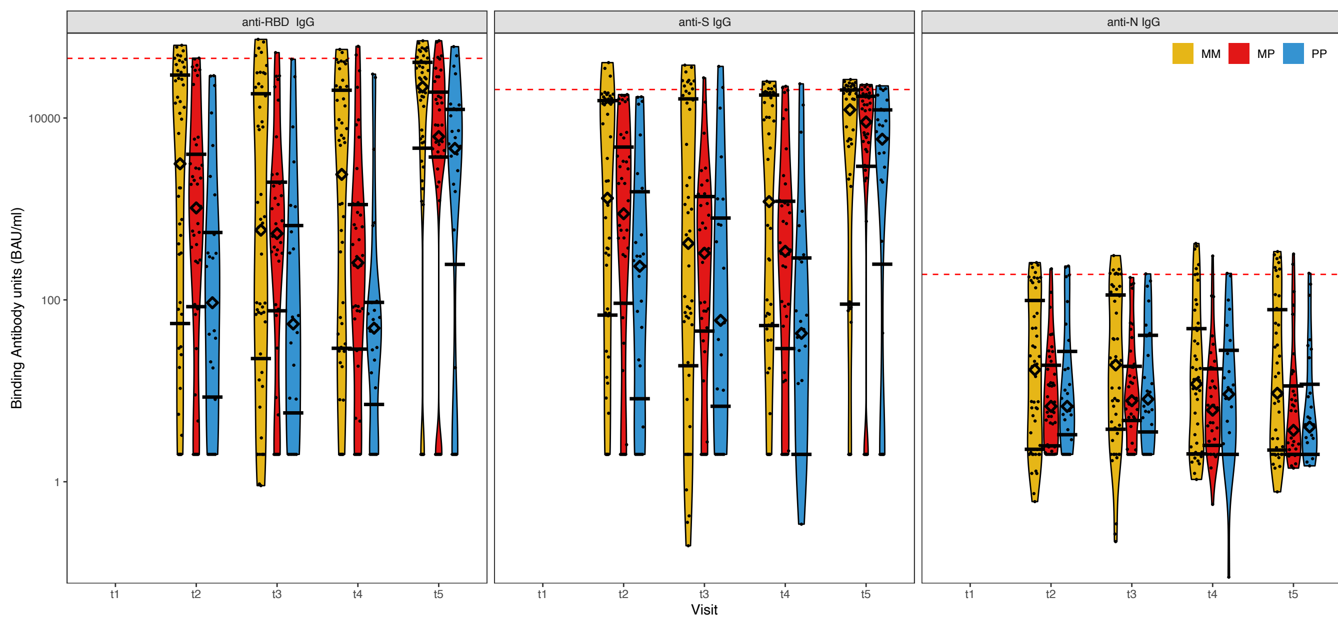


Supplemental Figure 2. Antibody responses based on previous infection with SARS-CoV-2 in the confirmatory (T2+) cohort



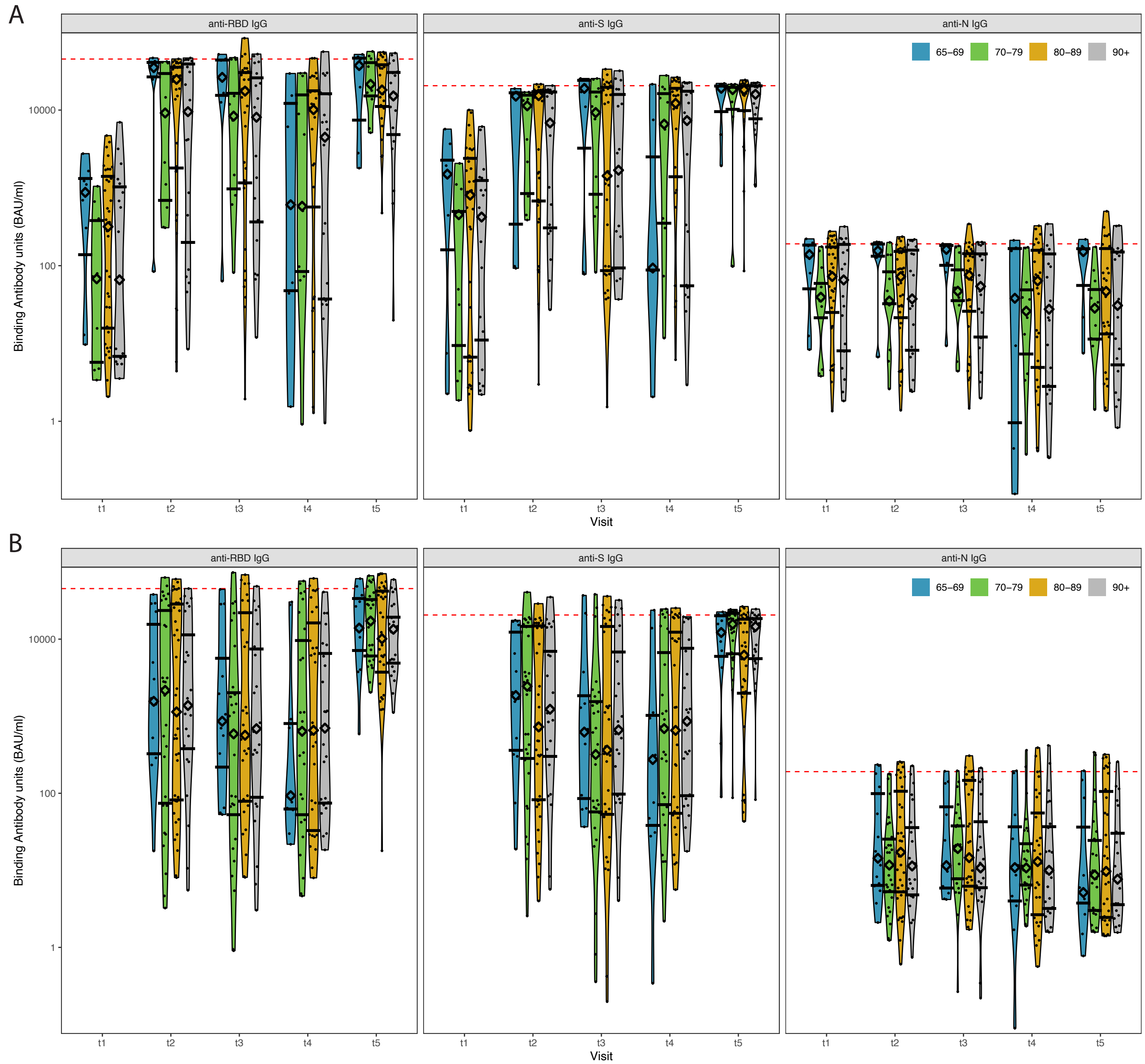
Antibody responses to RBD, S and N antigens, based on previous infection with SARS-CoV-2.

Supplemental Figure 3. Antibody responses based on homologous vs. heterologous use of mRNA vaccines in the confirmatory (T2+) cohort



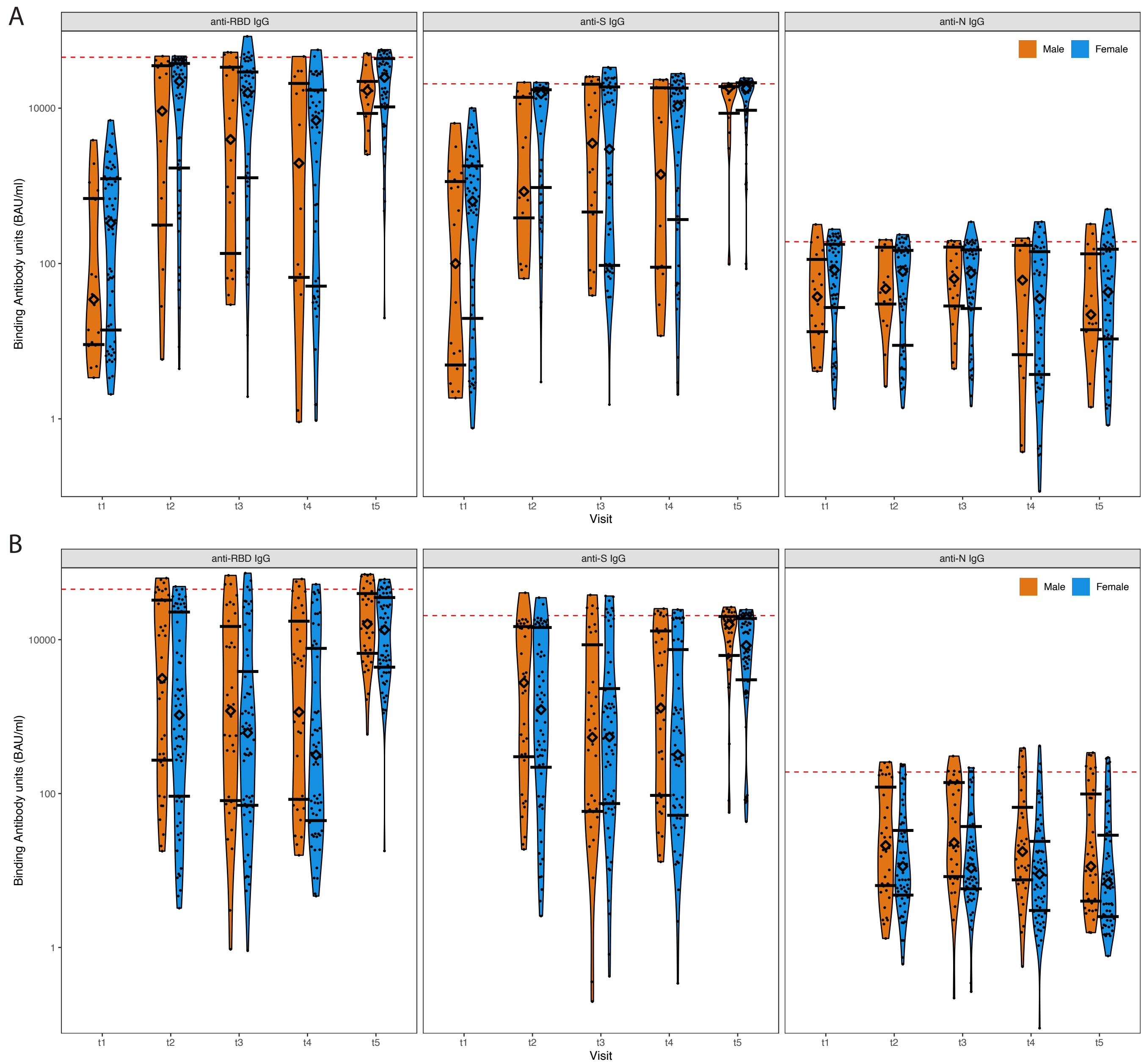
Antibody responses to RBD, S and N antigens, based on homologous vs. heterologous use of mRNA vaccines. Homologous vaccination consisted of both doses being either mRNA-1273 (MM) or BNT162b2 (PP), while heterologous vaccination consisted of mRNA-1273 followed by BNT162b2 (MP).

Supplemental Figure 4a. Antibody responses based on age



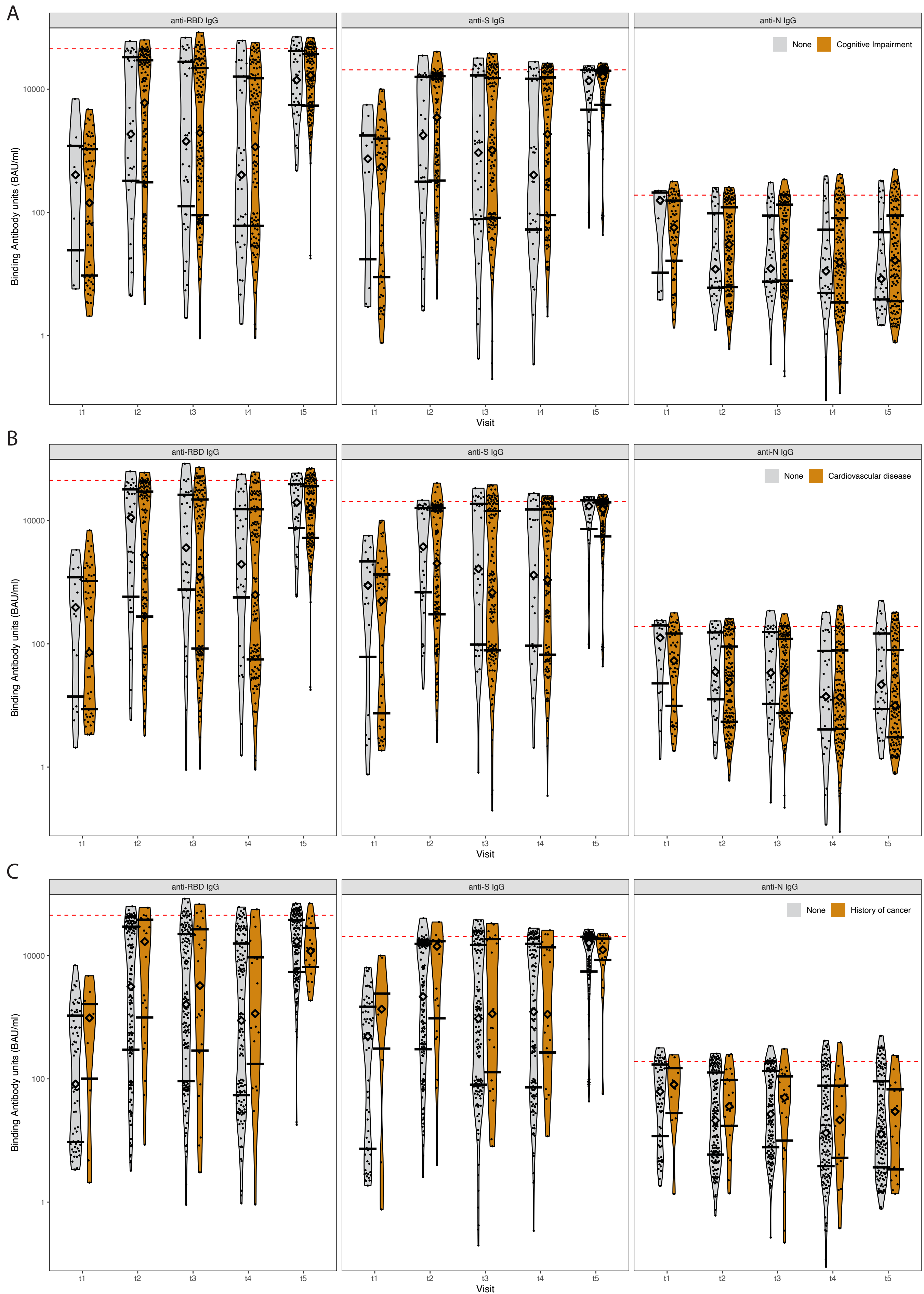
Antibody responses to RBD, S and N antigens, based on age group in T1+ (A) and T2+ (B) cohorts.

Supplemental Figure 4b. Antibody responses based on sex

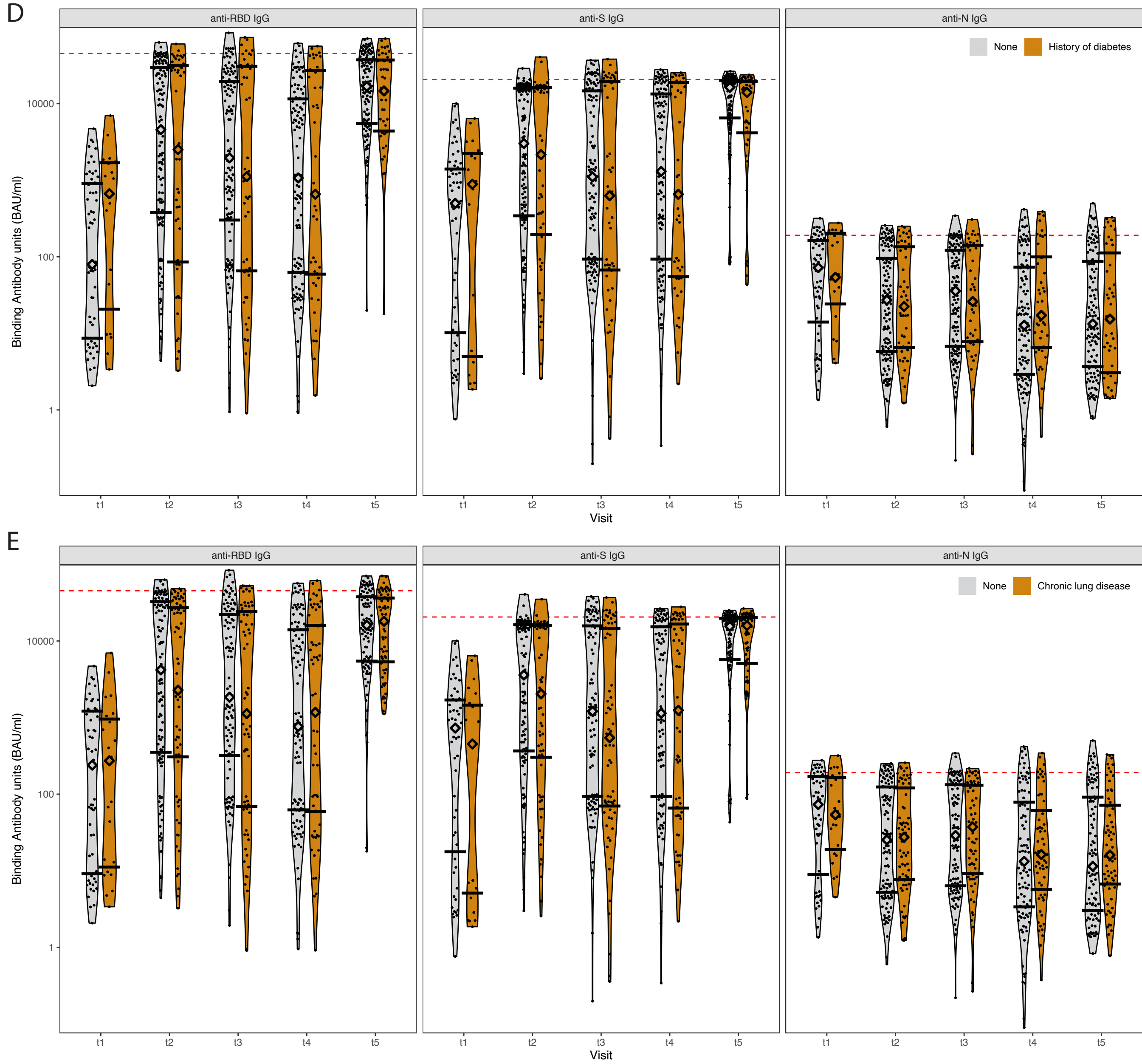


Antibody responses to RBD, S and N antigens, based on sex in T1+ (A) and T2+ (B) cohorts.

Supplemental Figure 4c. Antibody responses based on comorbidity

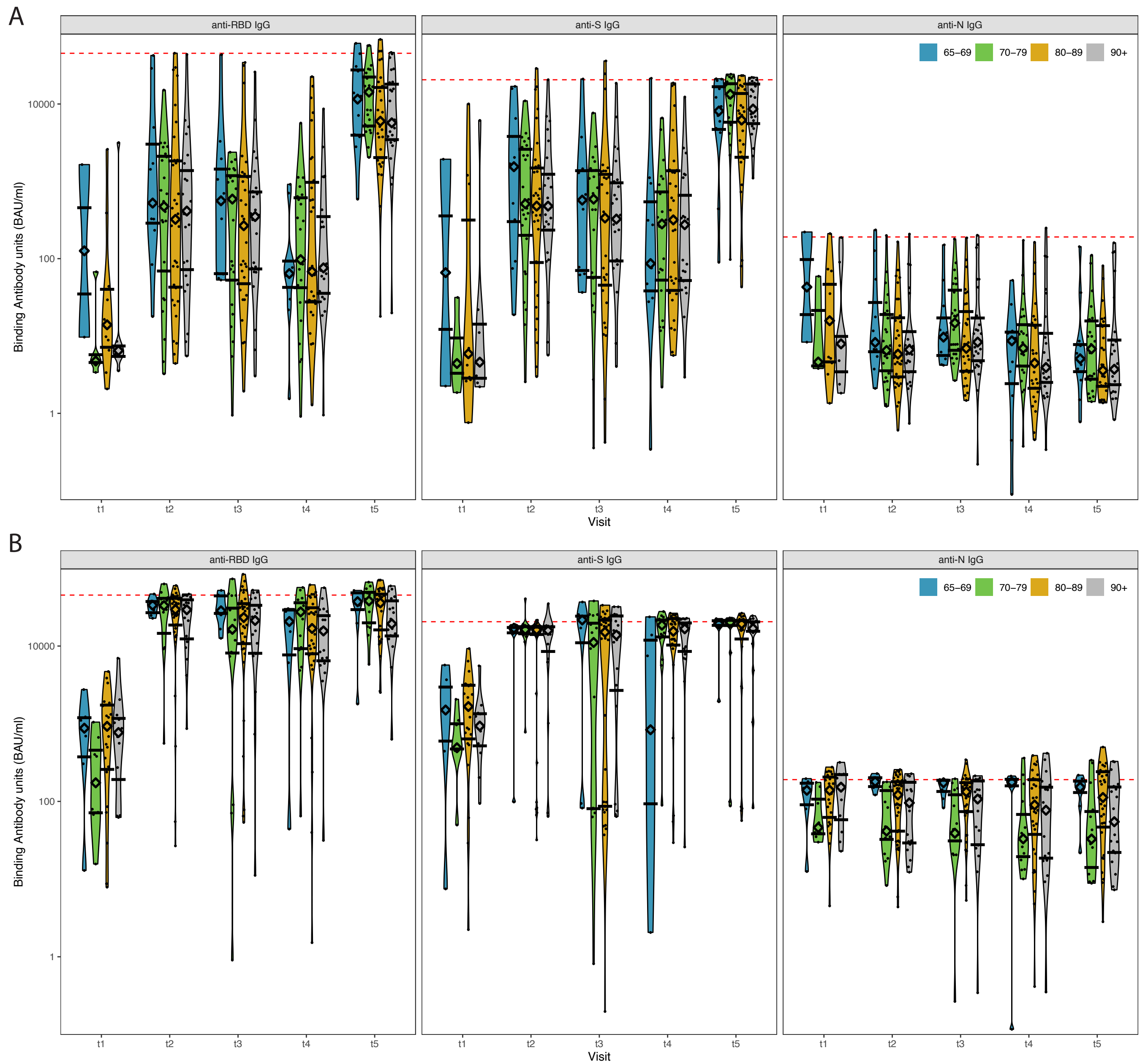


Supplemental Figure 4c. Antibody responses based on comorbidity (cont'd)



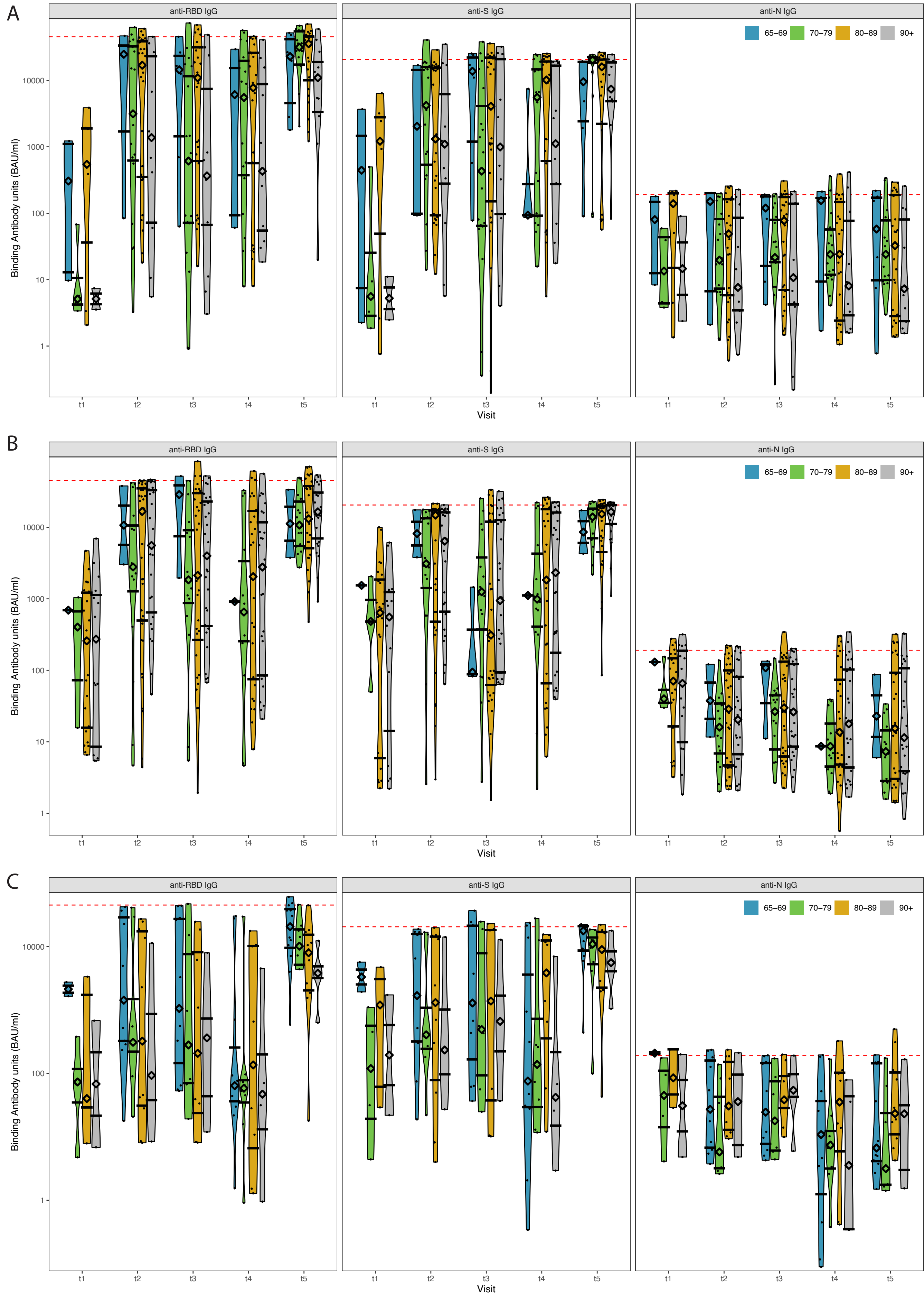
Antibody responses to RBD, S and N antigens, based on comorbidity. Cognitive impairement (A); Cardiovascular disease (B); History of cancer (C); Diabetes (D); Chronic lung disease (E).

Supplemental Figure 5. Antibody responses based on age stratified by previous infection status



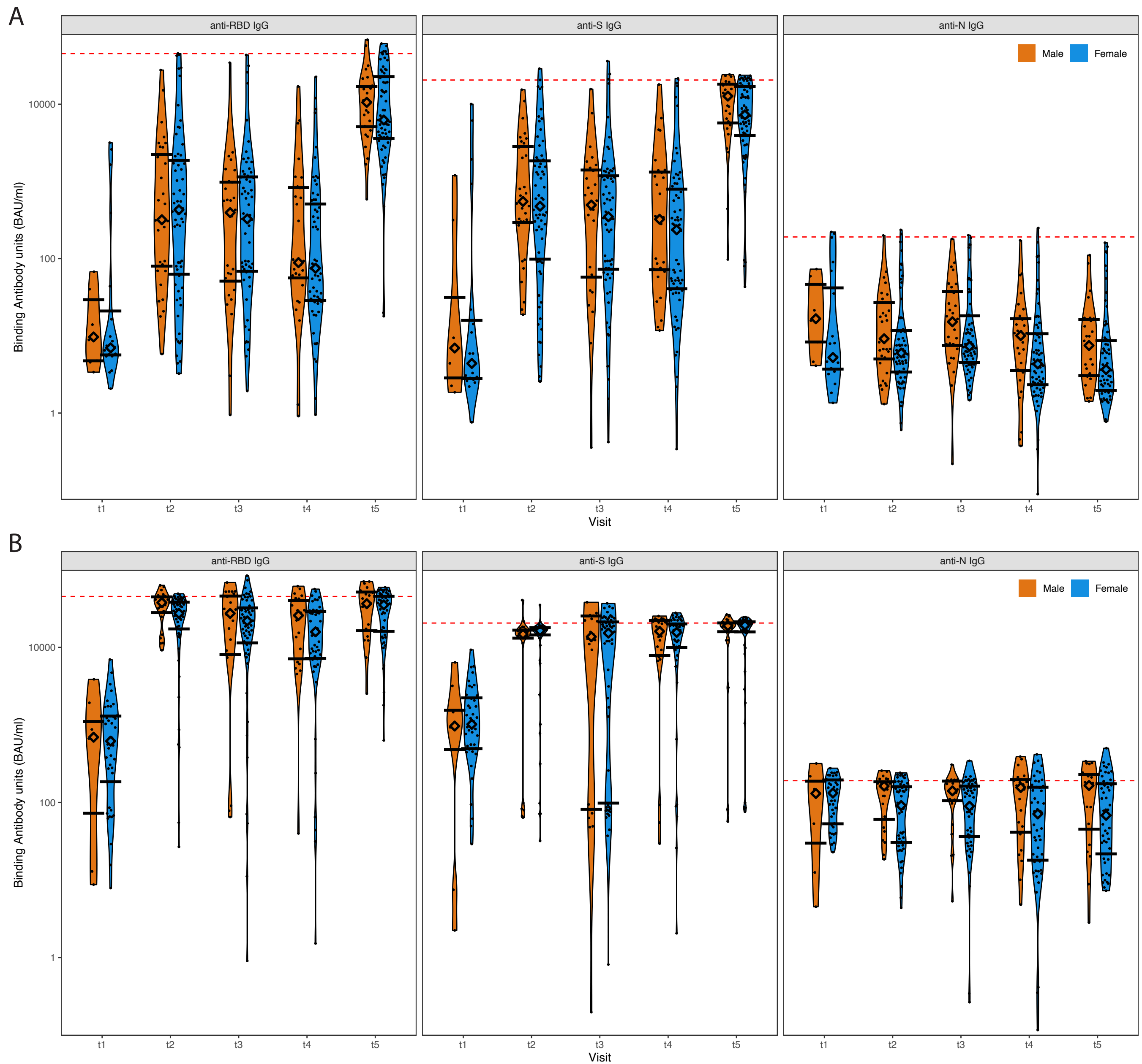
Antibody responses to RBD, S and N antigens, based on age group, stratified by absence (A) or presence (B) of previous SARS-CoV2 infection status.

Supplemental Figure 6. Antibody responses based on age stratified by homologous vs. heterologous use of mRNA vaccines



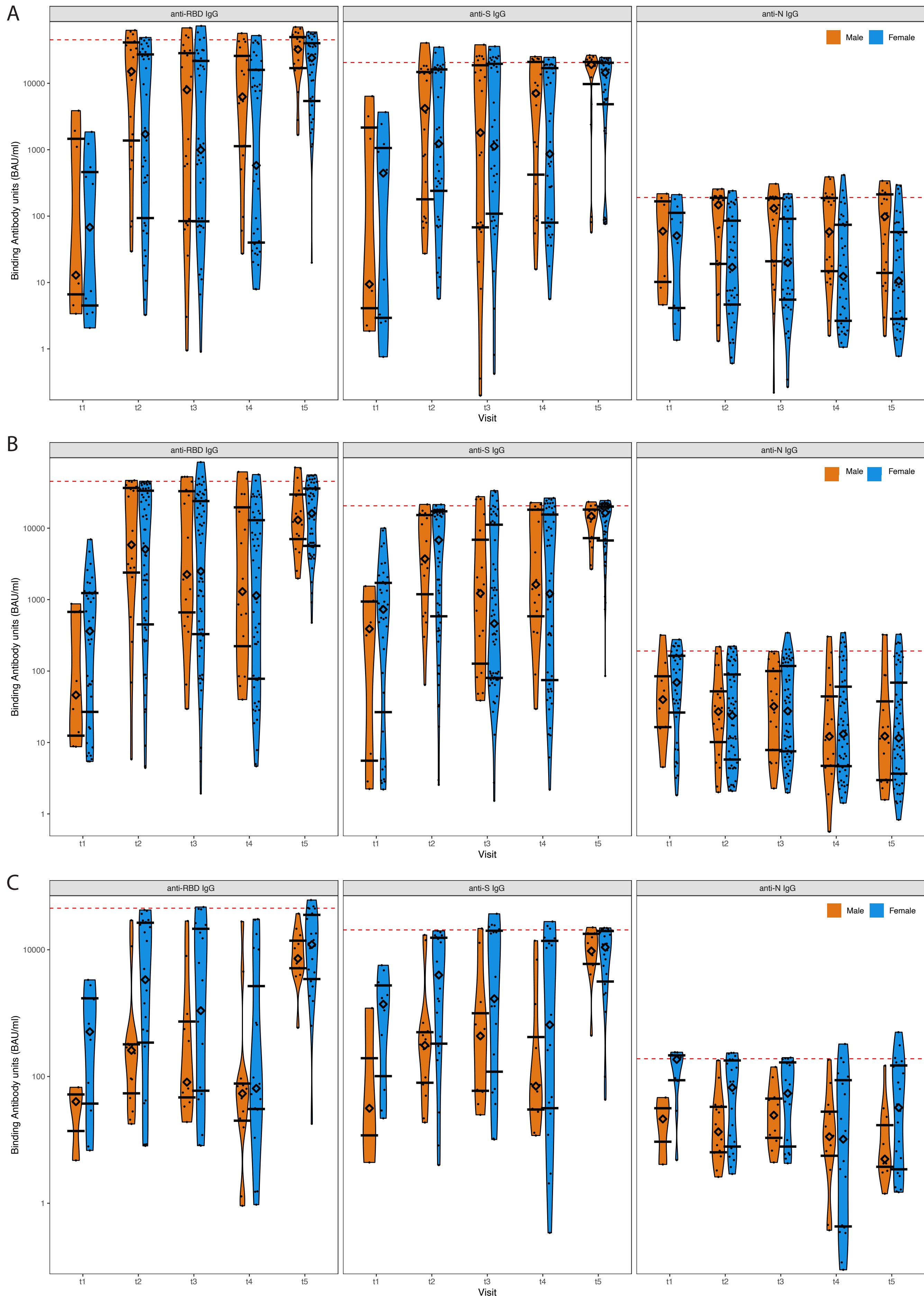
Antibody responses to RBD, S and N antigens, based on age group, stratified by homologous vs. heterologous use of mRNA vaccines. Homologous vaccination consisted of both doses being either mRNA-1273 (A) or BNT162b2 (C), while heterologous vaccination consisted of mRNA-1273 followed by BNT162b2 (B).

Supplemental Figure 7. Antibody responses based on sex stratified by previous infection status



Antibody responses to RBD, S and N antigens, based on sex, stratified by absence (A) or presence (B) of previous SARS-CoV2 infection status.

Supplemental Figure 8. Antibody responses based on sex stratified by homologous vs. heterologous use of mRNA vaccines



Antibody responses to RBD, S and N antigens, based on sex, stratified by homologous vs. heterologous use of mRNA vaccines. Homologous vaccination consisted of both doses being either mRNA-1273 (A) or BNT162b2 (C), while heterologous vaccination consisted of mRNA-1273 followed by BNT162b2 (B).