Older adults mount less durable humoral responses to two doses of COVID-19 mRNA vaccine, but strong initial responses to a third dose

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ABSTRACT

Background: Third COVID-19 vaccine doses are broadly recommended, but immunogenicity data remain limited, particularly in older adults.

Methods: We measured circulating antibodies against the SARS-CoV-2 spike protein receptor-binding domain, ACE2 displacement, and virus neutralization against ancestral and Omicron (BA.1) strains from pre-vaccine up to one month following the third dose, in 151 adults aged 24-98 years who received COVID-19 mRNA vaccines.

Results: Following two vaccine doses, humoral immunity was weaker, less functional and less durable in older adults, where a higher number of chronic health conditions was a key correlate of weaker responses and poorer durability. Third doses boosted antibody binding and function to higher levels than second-doses, and induced responses in older adults that were comparable in magnitude to those in younger adults. Humoral responses against Omicron were universally weaker than against the ancestral strain after both second and third doses; nevertheless, after three doses, anti-Omicron responses in older adults reached equivalence to those in younger adults. After three vaccine doses, the number of chronic health conditions, but not age per se, was the strongest consistent correlate of weaker humoral responses.

Conclusion: Results underscore the immune benefits of third COVID-19 vaccine doses, particularly in older adults.

Key words: COVID-19, mRNA vaccine, SARS-CoV-2, humoral immunity, older adults, binding antibodies, ACE2 displacement, viral neutralization, Omicron

INTRODUCTION

Older adults are at increased risk of lethal COVID-19 following SARS-CoV-2 infection (SARS-CoV-2) [1-3]. While two doses of a COVID-19 mRNA vaccine broadly protects against hospitalization and death [4-6], weaker vaccine-induced immunity observed in the elderly and certain other groups [7-12] has led to their prioritization to receive third doses [13-16]. Vaccine-induced antibodies also decline over time, which can increase the risk of breakthrough infections [17-19], particularly with the more transmissible and immune evasive Omicron variant (B.1.1.529) [20-22].

We and others have shown that older age is associated with weaker antibody responses to COVID-19 mRNA vaccines, Comirnaty (Pfizer/BioNTech) and Spikevax (Moderna) [10-12]. We previously characterized longitudinal humoral responses up to three months after the second vaccine dose in a cohort of 151 adults 24 to 98 years of age that includes COVID-19 naïve and convalescent individuals [12]. Here, we examine binding and neutralizing antibody responses up to six months following the second vaccine dose, as well as one month following the third vaccine dose. We also evaluate binding antibodies, ACE2 displacement, and virus neutralization against Omicron (BA.1) one month following the second and third doses.

METHODS

Study design. We conducted a prospective longitudinal cohort study in British Columbia, Canada, to examine SARS-CoV-2 specific humoral responses following vaccination with Comirnaty or Spikevax. Our cohort of 151 individuals included 81 healthcare workers (HCW) and 56 older adults (including 18 residents of long-term care or assisted living facilities) who were COVID-19 naive at study entry, and 14 COVID-19 convalescent individuals with anti-SARS-CoV-2 N antibodies at study entry (including 8 HCW and 6 older adults) [12]. Serum and plasma were collected prior to vaccination; one month after the first dose; one, three and six months after the second dose; and one month following the third dose. Specimens were processed same-day and frozen until analysis.

Ethics approval. Written informed consent was obtained from all participants or their authorized decision makers. This study was approved by the University of British Columbia/Providence Health Care and Simon Fraser University Research Ethics Boards.

Data sources. Sociodemographic, health and vaccine information was collected by self-report and confirmed through medical records where available. Chronic health conditions were defined as hypertension, diabetes, asthma, obesity (body mass index ≥30), chronic diseases of lung, liver, kidney, heart or blood, cancer, and immunosuppression due to chronic conditions or medication, to generate a score ranging from 0-11 per participant [12].

Binding antibody assays. We measured total binding antibodies against SARS-CoV-2 nucleocapsid (N) and spike (S) receptor binding domain (RBD) in serum using the Roche Elecsys Anti-SARS-CoV-2 and Anti-SARS-CoV-2 S assays, respectively, on a Cobas e601 module analyzer (Roche

Diagnostics). Following SARS-CoV-2 infection, both assays should be positive, whereas post-vaccination only the S assay should be positive, allowing identification of convalescent individuals. Both tests are electro-chemiluminescence sandwich immunoassays, and report results in Arbitrary Units (AU)/mL, calibrated against an external standard. For the S assay, the manufacturer indicates that AU values can be considered equivalent to international binding antibody units (BAU) as defined by the World Health Organization [23]. For the S assay, sera were tested undiluted, with samples above the upper limit of quantification (ULOQ) re-tested at 1:100 dilution, allowing a measurement range of 0.4 - 25,000 U/mL. We also quantified plasma IgG binding antibodies against RBD using the V-plex SARS-CoV-2 (IgG) Panel 22 ELISA kit (Meso Scale Diagnostics), which features the ancestral (Wuhan) and Omicron RBD antigens, on a Meso QuickPlex SQ120 instrument. Plasma samples were diluted 1:10000 as directed by the manufacturer, with results reported in Arbitrary Units (AU)/mL.

ACE2 competition assay. We assessed the ability of plasma antibodies to block the RBD-ACE2 receptor interaction by competition ELISA (Panel 22 V-plex SARS-CoV-2 [ACE2]; Meso Scale Diagnostics) on a Meso QuickPlex SQ120 instrument. Plasma was diluted 1:20 as directed by the manufacturer and results reported as % ACE2 displacement.

Live virus neutralization. Neutralizing activity in plasma was examined using a live SARS-CoV-2 infectivity assay in a Containment Level 3 facility. Assays were performed using isolate USA-WA1/2020 (BEI Resources) and a local Omicron isolate (BA.1 strain; GISAID Accession # EPI_ISL_9805779) on VeroE6-TMPRSS2 (JCRB-1819) target cells. Viral stock was adjusted to 50 $TCID_{50}$/200 µl in Dulbecco's Modified Eagle Medium in the presence of serial 2-fold dilutions of plasma (from 1/20 to 1/2560), incubated at 4°C for 1 hour and then added to target cells in 96-well

plates in triplicate. Cultures were maintained at 37°C with 5% CO_2 and the appearance of viral cytopathic effect (CPE) was recorded three days post-infection. Neutralizing activity is reported as the highest reciprocal plasma dilution able to prevent CPE in all three replicate wells. Samples exhibiting only partial or no neutralization at the lowest dilution of 1/20 were coded as having a reciprocal dilution of "10", defined as below the limit of quantification (BLOQ) in this assay.

Statistical analysis. Comparisons of binary variables were performed using Fisher's exact test. Comparisons of continuous variables were performed using the Mann-Whitney U-test (for unpaired data) or Wilcoxon test (for paired data). Multiple linear regression was used to investigate the relationship between sociodemographic, health and vaccine-related variables and humoral outcomes. Variables included age (per year increment), sex at birth (female as reference group), ethnicity (non-white as reference), number of chronic health conditions (per number increment), mRNA vaccine received (Comirnaty as reference), interval between doses (per day increment), sampling date following the most recent dose (per day increment), and convalescent status (COVID-19 naive as reference). Binding antibody half-lives in serum were calculated by fitting exponential decay curves to antibody concentrations at one, three and six months after the second dose. All tests were two-tailed, with $p<0.05$ considered statistically significant. Analyses were conducted using Microsoft Excel and Prism v9.2.0 (GraphPad).

RESULTS

Participant characteristics

As described previously [12], the cohort is predominantly female (**Table 1**). HCW, older adults and COVID-19 convalescent individuals at study entry were a median of 41, 79 and 48 years old, respectively. Older adults were predominantly (77%) of white ethnicity (compared to 46% of HCW) and had a higher burden of chronic health conditions (a median of 1, interquartile range [IQR] 0-2, range 0-5, vs. a median of 1, IQR 0-0, range 0-3 in HCW). All participants received two COVID-19 mRNA vaccine doses between December 2020-July 2021, where the dose interval was up to 112 days as per national guidelines to delay second doses due to initially limited vaccine supply. A total of 141 (93%) and 138 (91%) of participants received Comirnaty as their first and second dose, respectively. At the time of writing, 114 participants had received a third dose between October-December 2021, on average 7 months following their second dose. For participants whose third dose was Spikevax (53% of the cohort), those aged ≥70 years received a full dose, whereas those <70 years received a half-dose, as per national guidelines. An additional six (7.4%) HCW and two (3.6%) older adults developed anti-N antibodies during follow-up, reflecting breakthrough infections. Three of these infections, all in HCW, occurred between December 2021-Jan 2022 and are likely Omicron. In longitudinal analyses that span the entire study, participants with a post-vaccination SARS-CoV-2 infection are retained in their original "COVID-19 naive at study entry" groups but identified in the Figures, while in analyses that focus on third dose responses, they are grouped in a single "prior COVID-19" group.

After two-dose vaccination, lower binding antibodies are associated with older age and burden of chronic health conditions, but older adults mount strong responses after a third dose.

We measured total anti-RBD binding antibody concentrations in serum before and after immunization (**Figure 1A)**. As reported previously [12], antibody concentrations in older adults were significantly lower than those in HCW one month after the first dose (a median of 2.00 [IQR 1.75-2.25] $\log_{10}$ U/mL in HCW versus a median of 1.50 [IQR 1.05-1.99] in older adults), as well as one month after the second dose (a median of 4.02 [IQR 3.88-4.25] in HCW versus a median of 3.74 [IQR 3.49-3.91] in older adults) (Mann-Whitney; both $p<0.0001$). Three months following the second dose, antibody concentrations had declined by ~0.4 $\log_{10}$ on average, to a median of 3.63 [IQR 3.44-3.83] in HCW versus a median 3.32 [IQR 3.04-3.56] in older adults) (Mann-Whitney $p<0.0001$ for comparison between groups). Six months following the second dose, antibody concentrations had declined by a further ~0.3 $\log_{10}$ on average, to a median of 3.30 [IQR 3.09-3.47] in HCW versus a median 2.96 [IQR 2.68-3.20] in older adults ($p<0.0001$). This confirms that, following two-dose COVID-19 mRNA vaccination, antibody concentrations remain consistently and significantly lower in older compared to younger adults. By contrast, antibody concentrations in COVID-19 convalescent individuals remained consistently higher than COVID-19 naive individuals at all time points after two doses. Six months after the second dose for example, convalescent individuals maintained median responses of 3.50 (IQR 3.40-3.71) $\log_{10}$ U/mL ($p=0.027$ compared to HCW; $p<0.0001$ compared to older adults).

Multivariable analyses of antibody concentrations after two doses, that adjusted for sex , ethnicity, number of chronic health conditions, first-dose vaccine brand, dosing interval and day of specimen collection post-immunization confirmed that older age remained independently associated with lower antibody concentrations at one and three months after the second dose (**Table S1**). One month following the second dose for example, each decade of older age was associated with an ~0.06 $\log_{10}$ lower antibody concentration ($p=0.0067$). A higher number of chronic conditions was also independently associated with lower antibody concentrations at both these time points. Six months

following the second dose, a higher number of chronic health conditions remained the strongest independent correlate of lower responses, with each additional condition associated with an 0.14 $\log_{10}$ lower antibody concentration (p=0.0001). A longer dose interval was also associated with higher antibody concentrations at all time points after the second dose (all p<0.05), consistent with previous reports [24-26]. COVID-19 convalescent status was also associated with maintaining 0.26 $\log_{10}$ higher antibody concentrations at three and six months following the second dose (both p<0.05), consistent with superior durability of "hybrid" immunity induced by infection followed by vaccination [27-29].

In both HCW and older adults, the third dose boosted antibody concentrations at least ~0.3-0.4 $\log_{10}$ higher than peak values observed after two doses (Wilcoxon paired test p<0.0001 for both groups). Binding antibodies in HCW rose to a median of 4.31 (IQR 4.13 to upper limit of quantification [ULOQ]) whereas those in older adults rose to a median of 4.33 (4.14 to ULOQ) (p=0.33), indicating that older and younger adults mounted comparable initial binding antibody responses following a third dose. In multivariable analyses of third-dose responses, a higher number of chronic health conditions was the sole significant correlate of lower antibody concentrations (p=0.0078), while having received Spikevax as the third dose was associated with higher antibody concentrations (p=0.0091) (**Table S2**).

After two-dose vaccination, weaker virus neutralizing activity is associated with age and chronic health conditions, but older adults mount strong responses after a third dose.

We performed live SARS-CoV-2 neutralization assays to quantify the ability of plasma to block virus infection of target cells (**Figure 1B**). Neutralizing activity is reported as the highest reciprocal plasma dilution capable of preventing viral cytopathic effects in all wells of a triplicate assay, where a reciprocal dilution of "10" indicates no or limited neutralization. As previously reported [12], one vaccine dose largely failed to induce neutralizing activity in COVID-19 naïve individuals, though two

doses induced this activity in most participants, albeit at consistently lower levels in older compared to younger adults. One month after the second dose for example, the median reciprocal dilution was 160 [IQR 80-160] in HCW versus 40 [IQR 20-80] in older adults ($p<0.0001$). Three months after the second dose, neutralizing activity had declined by more than two-fold on average, to a median reciprocal dilution of 40 (IQR 20-80) in HCW versus a median of 20 (IQR BLOQ-40) in older adults ($p<0.0001$). Six months after the second dose, neutralizing activity had declined to below the limit of quantification (BLOQ) in 58% of HCW and 83% of older adults (Mann-Whitney $p=0.0048$ for comparison between groups). COVID-19 convalescent individuals by contrast maintained significantly higher neutralizing activity compared to naive individuals at all time points following two-dose vaccination. Multivariable analyses confirmed that older age remained significantly associated with weaker neutralizing activity at one and three months after two-dose vaccination, while COVID-19 convalescent status was associated with superior neutralizing activity at all time points following two-dose vaccination (all $p\leq0.0002$) (**Table S1**).

A third vaccine dose boosted neutralizing activity in both HCW and older adults, achieving responses that were two-fold and eight-fold higher than peak values after two doses, respectively (Wilcoxon paired test $p\leq0.006$ for both groups; **Figure 1B**). Specifically, the median reciprocal dilution in HCW and older adults rose to 320 [IQR 160-320] and 320 [IQR 80-320], respectively ($p=0.6$), indicating that older adults mounted comparable neutralizing responses to younger adults after three doses. A multivariable analysis identified prior COVID-19 as the strongest independent predictor of higher neutralizing activity after a third vaccine dose ($p=0.0044$; **Table S2**).

After two-dose vaccination, binding antibody responses decline faster in those with a higher burden of chronic conditions.

We next assessed temporal reductions in antibody concentrations after two-dose vaccination (**Figure 2A**). Assuming exponential decay and restricting the analysis to participants with a complete longitudinal data series with no values above the ULOQ, we estimated antibody concentration half-lives to be a median of 59 [IQR 52-75] days in HCW versus a median of 52 [IQR 45-65] days in older adults (p=0.016; **Figure 2B**). This suggests that, in addition to mounting overall weaker responses to two-dose vaccination compared to younger adults, antibody concentrations in older adults also decline more rapidly. In multivariable analyses however, a higher number of chronic health conditions emerged as the sole independent correlate of antibody decline, with each additional condition associated with a 5-day shorter half-life (p=0.017; **Table 2**). Furthermore, COVID-19 convalescent status was associated with a 14-day longer antibody half-life after adjustment for other factors (p=0.056), consistent with improved durability of hybrid immunity [27-29].

Humoral responses against Omicron following two and three vaccine doses

Given the rapid rise of the Omicron variant, we compared peak antibody responses against this strain in plasma collected at one month after the second and third vaccine doses. Here, we grouped all participants with prior COVID-19, regardless of infection timing, in the convalescent category. Overall, IgG binding antibodies against the Omicron RBD, measured using the Meso Scale Diagnostics V-Plex assay, were on average 0.4 to 0.5 $\log_{10}$ U/mL lower than those against the wild type (WT; ancestral Wuhan strain) RBD antigen after two and three doses (all within-group comparisons $p \leq 0.0002$; **Figure 3A**). Nevertheless, the third dose universally boosted anti-Omicron IgG concentrations to an average of 0.5 $\log_{10}$ higher than levels induced by two doses (all within-group comparisons $p<0.05$). Consistent with total binding antibody concentrations quantified using the Roche assay (**Figure 1A**), binding IgG concentrations against the WT RBD were significantly higher in HCW compared to older adults after

two doses ($p<0.0001$) but reached equivalence after three doses ($p=0.4$). IgG concentrations capable of binding Omicron followed a similar pattern, with HCW showing marginally higher anti-Omicron IgG levels compared to older adults after two doses ($p=0.09$), but equivalent levels after three doses ($p=0.49$). A multivariable analysis of Omicron-specific IgG concentrations after three doses identified a higher number of chronic health conditions as the strongest correlate of poorer responses, with each additional condition associated with a 0.12 $\log_{10}$ reduction in Omicron binding IgG ($p=0.0033$; **Table 3**). A longer interval between the first and second vaccine doses was marginally associated with a lower third dose response ($p=0.02$).

We also assessed the ability of plasma to block the interaction between WT and Omicron RBD and the cellular ACE2 receptor, which represents a higher throughput approach to estimate potential virus neutralizing activity (also referred to as a surrogate virus neutralization test [30]). This activity was significantly weaker against Omicron compared to WT RBD after both two and three doses in all groups (all within-group comparisons $p\leq0.0002$; **Figure 3B**), though the discrepancy was most pronounced for older adults after two doses (where median activity against WT was 90% compared to only 23% against Omicron). The third dose universally boosted anti-Omicron activity (all within-group comparisons $p<0.05$), with, for example, median anti-Omicron activity in older adults rising from 23% after two doses to 66% after three. Consistent with results for binding IgG antibodies, surrogate neutralization of WT RBD was significantly higher in HCW compared to older adults after two doses ($p<0.0001$), but reached equivalence after three doses (in fact, activities in older adults were slightly higher at this time point; $p=0.08$). Surrogate neutralization of Omicron RBD followed a similar pattern, with HCW exhibiting significantly higher activity compared to older adults after two doses ($p<0.0001$), but equivalent levels after three doses ($p=0.2$). In multivariable analyses, a higher number of chronic health conditions was the strongest correlate of poorer surrogate neutralizing activity against Omicron

after three vaccine doses, with each additional condition associated with a ~6% reduction in this activity ($p=0.0046$; **Table 3**). Male sex, a longer interval between the first and second doses, and the number of days elapsed since the third dose also correlated with weaker responses after three doses (all $p<0.05$).

Finally, we assessed plasma neutralizing activity against WT (ancestral USA-WA1/2020 strain) and Omicron using a live virus assay in a subset of 20 HCW and 21 older adults who remained COVID-19 negative throughout the study (**Figure 4**). Neutralizing activity against Omicron was significantly weaker compared to WT following two and three doses in both groups (all $p<0.0001$). The third dose nevertheless boosted anti-Omicron activity in both groups, where the increase in older adults was particularly pronounced (from a median of BLOQ after the second dose to a median reciprocal dilution of 40 after the third; $p<0.0001$). Consistent with binding IgG and surrogate neutralization results, anti-Omicron neutralizing activity was significantly lower in older adults compared to HCW after two vaccine doses ($p=0.0003$) but reached equivalence after the third dose ($p=0.79$).

DISCUSSION

At every time point following two doses of COVID-19 mRNA vaccine, antibody binding and neutralizing activity were significantly weaker in older compared to younger adults. Antibody concentrations were also less durable in older adults , though responses declined substantially in all groups over time (*e.g.* by six months after the second dose, neutralizing activities had declined to BLOQ in almost 60% of HCW and >80% of older adults). In multivariable analyses adjusting for sociodemographic, health and vaccine-related variables, a higher number of chronic health conditions remained consistently and independently associated with weaker and less durable binding antibody responses, while a longer interval between first and second doses was consistently associated with higher binding antibody responses after the second dose, as previously reported [24-26]. These findings support public health decisions to provide third doses on or before the six-month mark, with older adults receiving priority.

Third doses of COVID-19 vaccine increased antibody binding and neutralizing function to levels that were significantly higher than those achieved by two doses, where the magnitude of boosting in older adults was particularly prominent. Indeed, antibody binding, surrogate neutralization and live virus neutralization activities in older adults were equivalent to those observed in younger adults after three doses. Consistent with recent evidence [20, 21, 31-37], antibody responses against Omicron were universally weaker than those against the ancestral strain after both two and three vaccine doses; nevertheless, anti-Omicron responses in older adults reached equivalence to those observed in younger adults after three doses. Notably, the number of chronic health conditions persisted as an independent correlate of weaker anti-Omicron responses, even after three doses.

Similar to other reports [27-29], our findings indicate that individuals who have contracted COVID-19 are likely to benefit from vaccination. Compared to naïve participants, convalescent

individuals displayed a slower rate of antibody decline, and multivariable analyses demonstrated that binding and neutralization activity was higher in this group at six months after the second dose .

Our study has several limitations. As the precise immune correlates of protection for SARS-CoV-2 transmission and disease severity remain incompletely characterized [38], the implications of our results on individual-level protection from SARS-CoV-2 infection and COVID-19 remain uncertain. We did not investigate T-cell responses, which may play critical roles in protection against severe COVID-19, particularly in the context of variants [39-46]. Our study was not powered to investigate potential differences in immune responses between the two mRNA vaccines [47, 48], nor differences in full vs. half-doses of Spikevax when administered as third doses to individuals ≥70 versus <70 years old, respectively, in Canada. Third dose responses were measured at a single time point, so durability assessments are needed. Nevertheless, results provide additional insight into COVID-19 mRNA vaccine immunogenicity in the elderly and in the context of an extended interval between first and second doses (of up to 112 days).

In conclusion, while the observation of strong binding and neutralizing antibody responses to third COVID-19 vaccine doses in older adults, including to Omicron, are encouraging, it will be important to closely monitor the durability of these responses over time in this population.

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341 government.
342
343 **FIGURE LEGENDS**
344 **Figure 1. Longitudinal antibody binding and neutralization responses to spike RBD following**
345 **one, two and three COVID-19 vaccine doses.** *Panel A:* Binding antibody responses to the SARS-
346 CoV-2 spike RBD in serum, in HCW (blue circles) and older adults (orange circles) who were COVID-
347 19 naive at study entry, as well as COVID-19 convalescent individuals (black circles) at six timepoints:
348 prior to vaccination (pre-vax), one month following the first dose, one, three and six months following
349 the second dose, and one month following the third vaccine dose. Individuals with post-vaccination
350 infections are indicated by red dots at their first N seropositive time point. Participant Ns are provided
351 at the bottom of the plot. A thick horizontal red bar represents the median; thinner horizontal red bars
352 represent the IQR. P-values were computed using the Mann-Whitney U-test (for comparisons between
353 groups) or the Wilcoxon matched pairs test (for comparisons across time points within a group) and are
354 uncorrected for multiple comparisons. ULOQ/LLOQ: upper/lower limit of quantification. *Panel B:*
355 same as A, but for virus neutralization activity, defined as the lowest reciprocal plasma dilution at
356 which neutralization was observed in all wells of a triplicate assay. Plasma samples showing
357 neutralization in fewer than three wells at a 1/20 dilution were coded as having a reciprocal dilution of
358 10, corresponding to the LLOQ in this assay. The highest dilution tested was 1/2560, which
359 corresponds to the ULOQ. Note that only a subset of pre-vaccine plasma samples was assayed for this
360 activity.
361

Figure 2: Decay rates of serum binding antibody responses to spike RBD following two COVID-19 vaccine doses. *Panel A:* Temporal declines in serum binding antibody responses to spike RBD following two vaccine doses in HCW (blue) and older adults (orange) who were COVID-19 naive at study entry, as well as COVID-19 convalescent participants (black circles). ULOQ: upper limit of quantification. Only participants with a complete longitudinal data series with no values above the ULOQ are shown. *Panel B*: Binding antibody half-lives following two COVID-19 vaccine doses, calculated by fitting an exponential curve to each participant's data shown in panel A. Participant Ns are indicated at the bottom of the plot. Red bars and whiskers represent the median and IQR. P-values were computed using the Mann-Whitney U-test and are uncorrected for multiple comparisons.

Figure 3: Anti-Omicron IgG binding and ACE2 displacement activities one month after the second and third COVID-19 vaccine doses. *Panel A:* Binding IgG responses in plasma to the wild-type (WT, ancestral Wuhan strain) and Omicron (OM) S-RBD, measured using the Meso Scale Diagnostics (MSD) V-Plex assay, in HCW (blue circles) and older adults (orange circles) who remained COVID-19 naive throughout the study, as well as individuals with prior COVID-19 regardless of infection timing (COVID-19 convalescent; black circles) at one month after the second and third COVID-19 vaccine doses. Participant Ns are shown at the bottom of the plot. A thick horizontal red bar represents the median; thinner horizontal red bars represent the IQR. P-values were computed using the Wilcoxon matched pairs test (for all within-group comparisons) or the Mann-Whitney U-test (for between-group comparisons) and are uncorrected for multiple comparisons. *Panel B:* same as A, but for ACE2 displacement activity, measured using the V-plex SARS-CoV-2 (ACE2) assay, where results are reported in terms of % ACE2 displacement.

Figure 4: Anti-Omicron neutralization activities one month after the second and third COVID-19 vaccine doses. Neutralization activities, reported as the lowest reciprocal plasma dilution at which neutralization was observed in all wells of a triplicate assay, against the wild-type (WT, ancestral WA1/2020 strain) and Omicron (OM) virus isolates a subset of HCW (blue circles) and older adults (orange circles) who remained COVID-19 naive throughout the study. Participant Ns are shown at the bottom of the plot. A thick horizontal red bar represents the median; thinner horizontal red bars represent the IQR. P-values were computed using the Wilcoxon matched pairs test (for within-group comparisons) or the Mann-Whitney U-test (for between-group comparisons) and are uncorrected for multiple comparisons.

REFERENCES

1. Fisman DN, Bogoch I, Lapointe-Shaw L, McCready J, Tuite AR. Risk Factors Associated With Mortality Among Residents With Coronavirus Disease 2019 (COVID-19) in Long-term Care Facilities in Ontario, Canada. JAMA Netw Open **2020**; 3:e2015957.

2. Chinnadurai R, Ogedengbe O, Agarwal P, et al. Older age and frailty are the chief predictors of mortality in COVID-19 patients admitted to an acute medical unit in a secondary care setting- a cohort study. BMC Geriatr **2020**; 20:409.

3. Banerjee A, Pasea L, Harris S, et al. Estimating excess 1-year mortality associated with the COVID-19 pandemic according to underlying conditions and age: a population-based cohort study. Lancet **2020**; 395:1715-25.

4. Baden LR, El Sahly HM, Essink B, et al. Efficacy and Safety of the mRNA-1273 SARS-CoV-2 Vaccine. N Engl J Med **2021**; 384:403-16.

5. Polack FP, Thomas SJ, Kitchin N, et al. Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine. N Engl J Med **2020**; 383:2603-15.

6. Dagan N, Barda N, Kepten E, et al. BNT162b2 mRNA Covid-19 Vaccine in a Nationwide Mass Vaccination Setting. N Engl J Med **2021**; 384:1412-23.

7. Grupper A, Rabinowich L, Schwartz D, et al. Reduced humoral response to mRNA SARS-CoV-2 BNT162b2 vaccine in kidney transplant recipients without prior exposure to the virus. Am J Transplant **2021**; 21:2719-26.

8. Massarweh A, Eliakim-Raz N, Stemmer A, et al. Evaluation of Seropositivity Following BNT162b2 Messenger RNA Vaccination for SARS-CoV-2 in Patients Undergoing Treatment for Cancer. JAMA Oncol **2021**; 7:1133-40.

9. Monin L, Laing AG, Munoz-Ruiz M, et al. Safety and immunogenicity of one versus two doses of the COVID-19 vaccine BNT162b2 for patients with cancer: interim analysis of a prospective observational study. Lancet Oncol **2021**; 22:765-78.

10. Collier DA, Ferreira I, Kotagiri P, et al. Age-related immune response heterogeneity to SARS-CoV-2 vaccine BNT162b2. Nature **2021**; 596:417-22.

11. Muller L, Andree M, Moskorz W, et al. Age-dependent Immune Response to the Biontech/Pfizer BNT162b2 Coronavirus Disease 2019 Vaccination. Clin Infect Dis **2021**; 73:2065-72.

12. Brockman MA, Mwimanzi F, Lapointe HR, et al. Reduced magnitude and durability of humoral immune responses to COVID-19 mRNA vaccines among older adults. J Infect Dis **2021**; Online ahead of print. Accepted 9 Dec 2021. doi: 10.1093/infdis/jiab592.

13. Barda N, Dagan N, Cohen C, et al. Effectiveness of a third dose of the BNT162b2 mRNA COVID-19 vaccine for preventing severe outcomes in Israel: an observational study. Lancet **2021**; 398:2093-100.

14. Gilboa M, Mandelboim M, Indenbaum V, et al. Early Immunogenicity and safety of the third dose of BNT162b2 mRNA Covid-19 vaccine among adults older than 60 years; real world experience. J Infect Dis **2021**; Online ahead of print. Accepted, 29 Nov 2021. doi: 10.1093/infdis/jiab584.

15. Bar-On YM, Goldberg Y, Mandel M, et al. Protection of BNT162b2 Vaccine Booster against Covid-19 in Israel. N Engl J Med **2021**; 385:1393-400.

16. Falsey AR, Frenck RW, Jr., Walsh EE, et al. SARS-CoV-2 Neutralization with BNT162b2 Vaccine Dose 3. N Engl J Med **2021**; 385:1627-9.

17. Mizrahi B, Lotan R, Kalkstein N, et al. Correlation of SARS-CoV-2-breakthrough infections to time-from-vaccine. Nat Commun **2021**; 12:6379.

18. Goldberg Y, Mandel M, Bar-On YM, et al. Waning Immunity after the BNT162b2 Vaccine in Israel. N Engl J Med **2021**; 385:e85.

19. Levin EG, Lustig Y, Cohen C, et al. Waning Immune Humoral Response to BNT162b2 Covid-19 Vaccine over 6 Months. N Engl J Med **2021**; 385:e84.

20. Collie S, Champion J, Moultrie H, Bekker LG, Gray G. Effectiveness of BNT162b2 Vaccine against Omicron Variant in South Africa. N Engl J Med **2022**; 386:494-6.

21. Cele S, Jackson L, Khoury DS, et al. Omicron extensively but incompletely escapes Pfizer BNT162b2 neutralization. Nature **2021**; Online ahead of print. Accepted, 23 Dec 2021. doi: 10.1038/s41586-021-04387-1.

22. Song JS, Lee J, Kim M, et al. Serial Intervals and Household Transmission of SARS-CoV-2 Omicron Variant, South Korea, 2021. Emerg Infect Dis **2022**; 28.

23. Bentley E, Mee ET, Routley S, et al. Collaborative Study for the Establishment of a WHO International Standard for SARS-CoV-2 RNA. World Health Organization, Expert Committee on Biological Standardization **2020**:WHO/BS/2020.402.

24. Amirthalingam G, Bernal JL, Andrews NJ, et al. Serological responses and vaccine effectiveness for extended COVID-19 vaccine schedules in England. Nat Commun **2021**; 12:7217.

25. Parry H, Bruton R, Stephens C, et al. Differential immunogenicity of BNT162b2 or ChAdOx1 vaccines after extended-interval homologous dual vaccination in older people. Immun Ageing **2021**; 18:34.

26. Payne RP, Longet S, Austin JA, et al. Immunogenicity of standard and extended dosing intervals of BNT162b2 mRNA vaccine. Cell **2021**; 184:5699-714 e11.

27. Cho A, Muecksch F, Schaefer-Babajew D, et al. Anti-SARS-CoV-2 receptor-binding domain antibody evolution after mRNA vaccination. Nature **2021**; 600:517-22.

28. Stamatatos L, Czartoski J, Wan YH, et al. mRNA vaccination boosts cross-variant neutralizing antibodies elicited by SARS-CoV-2 infection. Science **2021**; 372:1413-8.

29. Wang Z, Muecksch F, Schaefer-Babajew D, et al. Naturally enhanced neutralizing breadth against SARS-CoV-2 one year after infection. Nature **2021**; 595:426-31.

30. Tan CW, Chia WN, Qin X, et al. A SARS-CoV-2 surrogate virus neutralization test based on antibody-mediated blockage of ACE2-spike protein-protein interaction. Nat Biotechnol **2020**; 38:1073-8.

31. Doria-Rose NA, Shen X, Schmidt SD, et al. Booster of mRNA-1273 Strengthens SARS-CoV-2 Omicron Neutralization. medRxiv **2021**; Posted 20 Dec 2021. doi: 10.1101/2021.12.15.21267805. Preprint.

471 32. Schmidt F, Muecksch F, Weisblum Y, et al. Plasma neutralization properties of the SARS-CoV-2
472 Omicron variant. medRxiv **2021**; Posted 13 Dec 2021. doi: 10.1101/2021.12.12.21267646.
473 Preprint.

474 33. Garcia-Beltran WF, St Denis KJ, Hoelzemer A, et al. mRNA-based COVID-19 vaccine boosters
475 induce neutralizing immunity against SARS-CoV-2 Omicron variant. Cell **2022**; 185:457-66 e4.

476 34. Muik A, Lui BG, Wallisch AK, et al. Neutralization of SARS-CoV-2 Omicron by BNT162b2
477 mRNA vaccine-elicited human sera. Science **2022**; 375:678-80.

478 35. Debes AK, Xiao S, Egbert ER, et al. Comparison of total and neutralizing SARS-CoV-2 spike
479 antibodies against omicron and other variants in paired samples after two or three doses of
480 mRNA vaccine. medRxiv **2022**; Posted 31 Jan 2022. doi: 10.1101/2022.01.26.22269819.
481 Preprint.

482 36. Ai J, Zhang H, Zhang Y, et al. Omicron variant showed lower neutralizing sensitivity than other
483 SARS-CoV-2 variants to immune sera elicited by vaccines after boost. Emerg Microbes Infect
484 **2022**; 11:337-43.

485 37. Muecksch F, Wang Z, Cho A, et al. Increased Potency and Breadth of SARS-CoV-2 Neutralizing
486 Antibodies After a Third mRNA Vaccine Dose. bioRxiv **2022**; Posted 15 Jan 2022. doi:
487 10.1101/2022.02.14.480394. Preprint.:2022.02.14.480394.

488 38. Feng S, Phillips DJ, White T, et al. Correlates of protection against symptomatic and asymptomatic
489 SARS-CoV-2 infection. Nat Med **2021**; 27:2032-40.

39. Redd AD, Nardin A, Kared H, et al. Minimal cross-over between mutations associated with Omicron variant of SARS-CoV-2 and CD8+ T cell epitopes identified in COVID-19 convalescent individuals. bioRxiv **2021**; Posted 9 Dec 2021. doi: 10.1101/2021.12.06.471446. Preprint.

40. Peng Y, Felce SL, Dong D, et al. An immunodominant NP105-113-B*07:02 cytotoxic T cell response controls viral replication and is associated with less severe COVID-19 disease. Nat Immunol **2022**; 23:50-61.

41. Swadling L, Diniz MO, Schmidt NM, et al. Pre-existing polymerase-specific T cells expand in abortive seronegative SARS-CoV-2. Nature **2022**; 601:110-7.

42. Grifoni A, Sidney J, Vita R, et al. SARS-CoV-2 human T cell epitopes: Adaptive immune response against COVID-19. Cell Host Microbe **2021**; 29:1076-92.

43. Dan JM, Mateus J, Kato Y, et al. Immunological memory to SARS-CoV-2 assessed for up to 8 months after infection. Science **2021**; 371:eabf4063.

44. Riou C, Keeton R, Moyo-Gwete T, et al. Escape from recognition of SARS-CoV-2 variant spike epitopes but overall preservation of T cell immunity. Sci Transl Med **2022**; 14:eabj6824.

45. Keeton R, Tincho MB, Ngomti A, et al. T cell responses to SARS-CoV-2 spike cross-recognize Omicron. Nature **2022**; Online ahead of print. Accepted, 31 Jan 2022. doi: 10.1038/s41586-022-04460-3.

508 46. Liu J, Chandrashekar A, Sellers D, et al. Vaccines Elicit Highly Conserved Cellular Immunity to
509 SARS-CoV-2 Omicron. Nature; Online ahead of print, 31 Jan 2022. doi: 10.1038/s41586-022-
510 04465-y.

511 47. Steensels D, Pierlet N, Penders J, Mesotten D, Heylen L. Comparison of SARS-CoV-2 Antibody
512 Response Following Vaccination With BNT162b2 and mRNA-1273. JAMA **2021**; 326:1533-5.

513 48. Nanduri S, Pilishvili T, Derado G, et al. Effectiveness of Pfizer-BioNTech and Moderna Vaccines
514 in Preventing SARS-CoV-2 Infection Among Nursing Home Residents Before and During
515 Widespread Circulation of the SARS-CoV-2 B.1.617.2 (Delta) Variant - National Healthcare
516 Safety Network, March 1-August 1, 2021. MMWR Morb Mortal Wkly Rep **2021**; 70:1163-6.

517

Table 1: Participant characteristics and sampling information

Variable category	Characteristic	Healthcare Workers (n=81)	Older Adults (n=56)	COVID-19 Convalescent at study entry (n=14)
Sociodemographic/health	Age in years, median [IQR][a]	41 [35-51]	78 [73-83]	48 [36-87]
	Female sex, n (%)	61 (75%)	38 (68%)	10 (71%)
	White/Caucasian ethnicity, n (%)	37 (46%)	43 (77%)	7 (50%)
	Chronic health or immunosuppressive conditions, median [IQR]	0 [0-0]	1 [0-2]	0 [0-1]
Vaccine information	Comirnaty, First mRNA Vaccine, n (%)	80 (99%)	48 (86%)	13 (93%)
	Comirnaty, Second mRNA Vaccine, n (%)	79 (98%)	46 (82%)	13 (93%)
	Time between first and second doses in days, median [IQR]	97 [91-102]	76 [45-85]	112 [87-118]
	Comirnaty, Third mRNA Vaccine, n (%)[b]	32/61 (52%)	19/47 (40%)	3/6 (50%)
	Time between second and third dose in days, median [IQR]	210 [200-241]	169 [160-231]	189 [170-194]
Specimen collection	Specimens collected pre-vaccine, n (%)	80 (99%)	49 (88%)	13 (93%)
	Specimens collected one month after first dose, n (%)	79 (98%)	49 (88%)	13 (93%)
	Day of specimen collection one month after first dose, median [IQR] days	28 [27-30]	30 [28-32]	31 [28-32]
	Specimens collected one month after second dose, n (%)	81 (100%)	55 (98%)	14 (100%)
	Day of specimen collection one month after second dose, median [IQR] days	29 [29-32]	29 [29-31]	32 [30-36]
	Specimens collected three months after second dose, n (%)	79 (98%)	53 (95%)	13 (93%)
	Day of specimen collection three months after second dose, median [IQR] days	90 [90-91]	90 [89-92]	90 [87-91]
	Specimens collected six months after second dose, n (%)	78 (96%)	40 (71%)	10 (71%)
	Day of specimen collection six months after second dose, median [IQR] days	181 [179-182]	176 [167-182]	180 [179-181]
	Specimens collected one month after third dose, n (%)	61 (75%)	47 (84%)	6 (38%)
	Day of specimen collection one month after third dose, median [IQR] days	30 [29-31]	32 [29-33]	30 [29-30]
COVID-19 post-vax	Anti-N seroconversion during study follow-up	6 (7.4%)	2 (3.6%)	-

[a] interquartile range

[b] denominators are the n of specimens collected one month after third dose

Table 2: Multivariable analysis of the relationship between sociodemographic, health and vaccine-related variables on serum antibody half-life following two-dose COVID-19 mRNA vaccination

Outcome measure	Variable	Estimate	95% CI	p-value
Ab half-life after two vaccine doses	Age (per year)	0.058	-0.17 to 0.29	0.61
	Male sex	5.31	-2.57 to 13.18	0.18
	White ethnicity	3.11	-4.67 to 10.88	0.43
	# chronic conditions (per add'l)	-4.62	-8.39 to -0.85	**0.017**
	Spikevax as first dose	3.37	-13.26 to 20.00	0.69
	Dose interval (per day)	0.00014	-0.16 to 0.16	0.99
	COVID-19 convalescent[a]	13.78	-0.37 to 27.93	0.056

[a] participants with positive anti-N serology at study entry

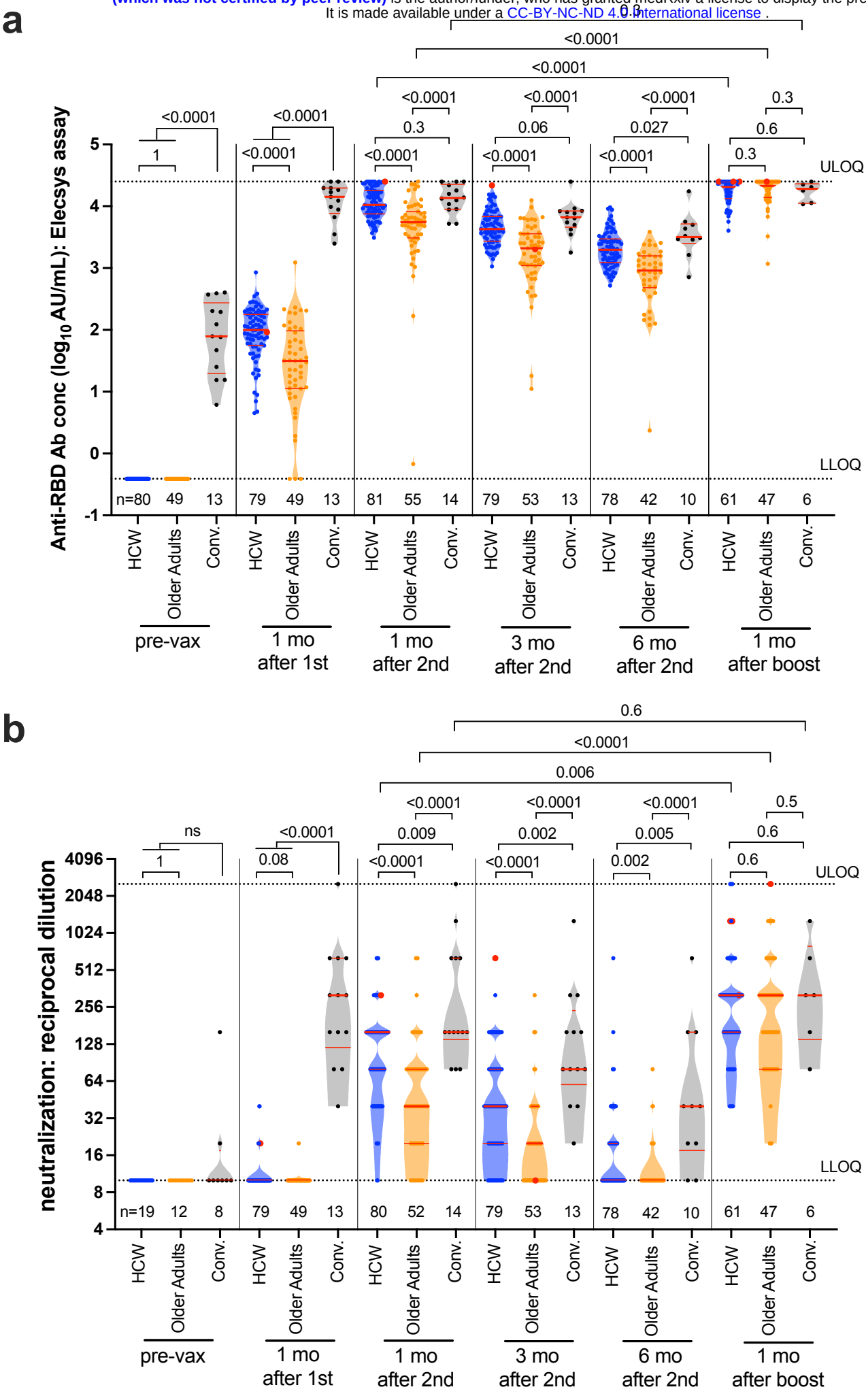
Table 3: Multivariable analyses of the relationship between sociodemographic, health and vaccine-related variables on Omicron-specific humoral immunogenicity measures following three-dose COVID-19 mRNA vaccination

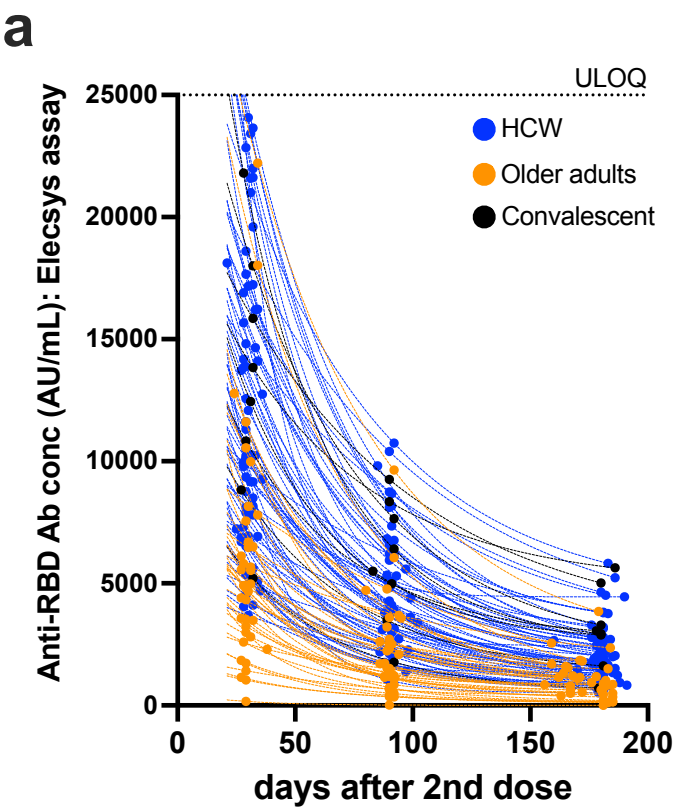
Humoral measure	Variable	1 mo after 3rd dose		
		Estimate	**95% CI**	**p-value**
anti-Omicron RBD IgG (log10)[a]	Age (per year)	0.0035	-0.0027 to 0.0097	0.26
	Male sex	-0.14	-0.34 to 0.054	0.15
	White ethnicity	-0.018	-0.21 to 0.17	0.85
	# chronic conditions (per add'l)	-0.12	-0.20 to -0.041	**0.0033**
	Spikevax as third dose (vs. Comirnaty)	0.15	-0.039 to 0.34	0.12
	Interval between 1st and 2nd dose (per day)	-0.0066	-0.012 to -0.0011	**0.020**
	Interval between 2nd and 3rd dose (per day)	0.00043	-0.0034 to 0.0042	0.83
	Days since 3rd vaccine dose	-0.0086	-0.038 to 0.021	0.56
	Prior COVID-19[b]	0.1	-0.16 to 0.37	0.43
anti-Omicron ACE2 % displacement[a]	Age (per year)	0.29	-0.046 to 0.63	0.090
	Male sex	-12.38	-23.16 to -1.60	**0.025**
	White Ethnicity	-2.36	-12.74 to 8.01	0.65
	# chronic conditions (per add'l)	-6.41	-10.80 to -2.03	**0.0046**
	Spikevax as third dose (vs. Comirnaty)	1.69	-8.58 to 11.97	0.74
	Interval between 1st and 2nd dose (per day)	-0.41	-0.71 to -0.11	**0.0079**
	Interval between 2nd and 3rd dose (per day)	-0.038	-0.25 to 0.17	0.72
	Days since 3rd vaccine dose	-1.82	-3.43 to -0.21	**0.027**
	Prior COVID-19[b]	12.28	-2.11 to 26.67	0.094

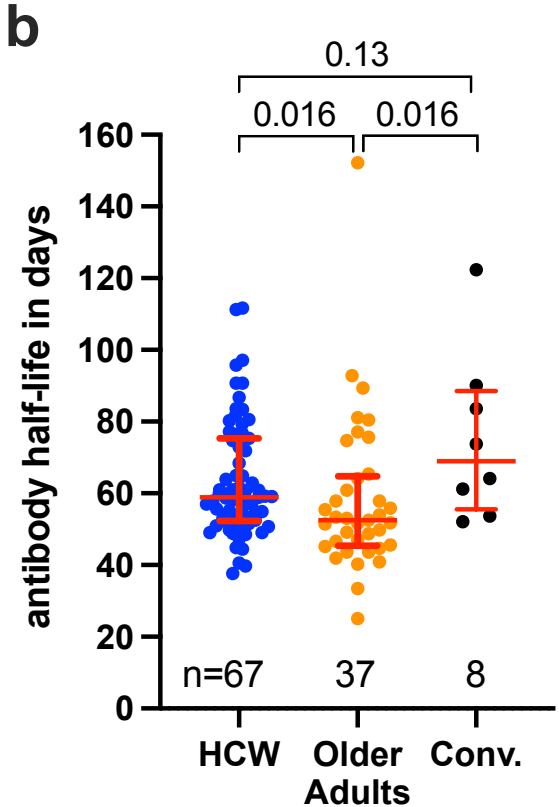
[a] Measured using the Meso Scale Diagnostics (MSD) V-plex assay system

[b] Includes all participants with positive anti-N serology at any time during the study (*i.e.* both pre- and post-vaccine COVID-19 cases)

Figure 1




a
ULOQ
HCW
Older adults
Convalescent
Anti-RBD Ab conc (AU/mL): Elecsys assay
25000
20000
15000
10000
5000
0
0
50
100
150
200
days after 2nd dose


b
0.13
0.016
0.016
antibody half-life in days
160
140
120
100
80
60
40
20
0
n=67
37
8
HCW
Older Adults
Conv.

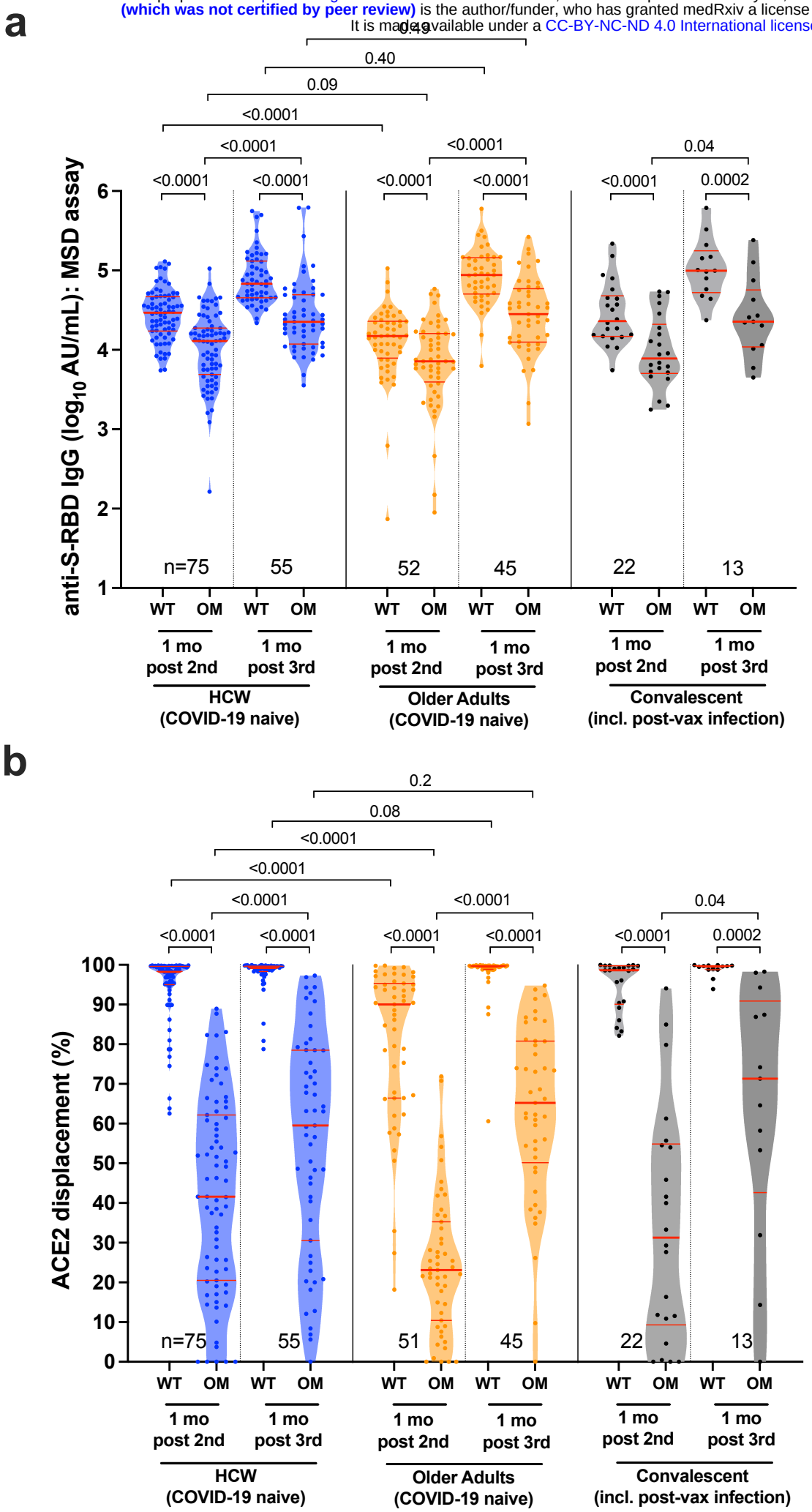


Figure 4

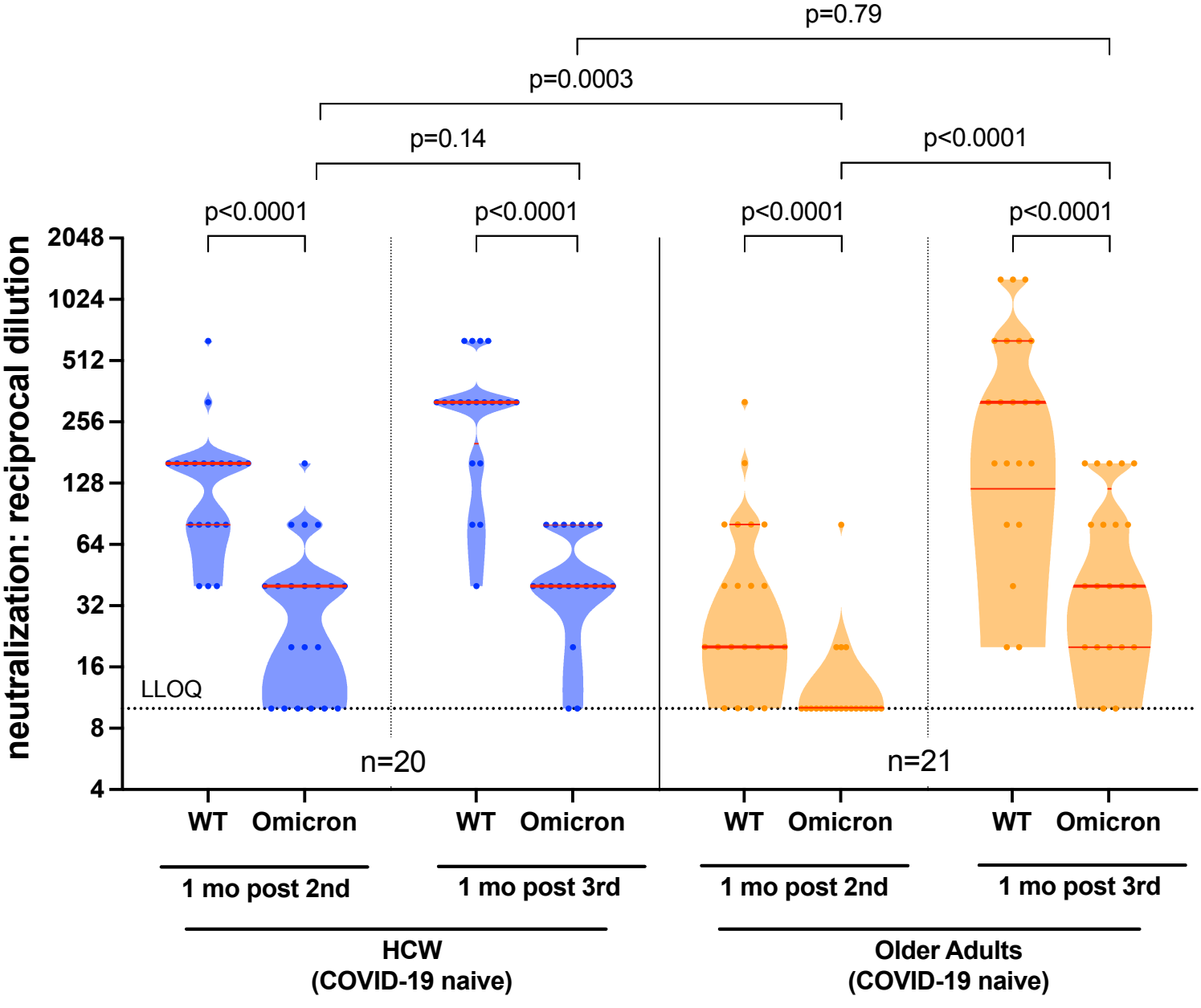

p=0.79
p=0.0003
p=0.14
p<0.0001
p<0.0001
p<0.0001
p<0.0001
p<0.0001
neutralization: reciprocal dilution
2048
1024
512
256
128
64
32
16
8
4
LLOQ
n=20
n=21
WT
Omicron
WT
Omicron
WT
Omicron
WT
Omicron
1 mo post 2nd
1 mo post 3rd
1 mo post 2nd
1 mo post 3rd
HCW
(COVID-19 naive)
Older Adults
(COVID-19 naive)

Table S1: Multivariable analyses of the relationship between sociodemographic, health and vaccine-related variables on immunogenicity measures following two-dose COVID-19 mRNA vaccination

Humoral measure	Variable	1 mo after 2nd dose			3 mo after 2nd dose			6 mo after 2nd dose		
		Estimate	95% CI	p-value	Estimate	95% CI	p-value	Estimate	95% CI	p-value
anti-RBD	Age (per year)	-0.0061	-0.010 to -0.0017	**0.0067**	-0.0047	-0.0085 to -0.00077	**0.019**	-0.0029	-0.0076 to 0.0018	0.22
Abs	Male sex	0.012	-0.14 to 0.17	0.88	0.089	-0.052 to 0.23	0.21	0.062	-0.083 to 0.21	0.40
(log_{10})[a]	White ethnicity	0.098	-0.056 to 0.25	0.21	0.15	0.012 to 0.29	**0.033**	0.089	-0.055 to 0.23	0.22
	# chronic cond. (per add'l)	-0.096	-0.17 to -0.022	**0.011**	-0.11	-0.18 to -0.047	**0.001**	-0.14	-0.21 to -0.068	**0.0001**
	Spikevax as first dose	0.25	-0.038 to 0.54	0.088	0.32	0.054 to 0.59	**0.019**	0.26	-0.039 to 0.57	0.087
	Dose interval (per day)	0.0034	0.00011 to 0.0067	**0.043**	0.0054	0.0025 to 0.0083	**0.0003**	0.0049	0.00194 to 0.0079	**0.0014**
	Days since 2nd dose	0.0039	-0.026 to 0.033	0.80	0.015	-0.0098 to 0.040	0.24	0.00095	-0.0088 to 0.010	0.85
	COVID-19 convalescent[c]	0.16	-0.087 to 0.42	0.20	0.23	0.0081 to 0.46	**0.042**	0.26	0.00938 to 0.51	**0.042**
Viral	Age (per year)	-0.018	-0.033 to -0.0030	**0.019**	-0.020	-0.034 to -0.0052	**0.008**	-0.0022	-0.016 to 0.012	0.75
neut	Male sex	-0.33	-0.85 to 0.18	0.21	0.22	-0.30 to 0.75	0.40	0.14	-0.29 to 0.57	0.53
(log_2)[b]	White ethnicity	-0.075	-0.59 to 0.43	0.77	0.27	-0.24 to 0.78	0.30	0.067	-0.36 to 0.49	0.76
	# chronic cond. (per add'l)	-0.10	-0.34 to 0.14	0.42	-0.16	-0.40 to 0.095	0.22	-0.0080	-0.21 to 0.20	0.94
	Spikevax as first dose	0.86	-0.098 to 1.82	0.078	0.71	-0.30 to 1.7	0.17	0.81	-0.088 to 1.71	0.077
	Dose interval (per day)	0.0066	-0.0044 to 0.018	0.24	-0.00046	-0.011 to 0.010	0.93	0.0074	-0.0015 to 0.016	0.10
	Days since 2nd dose	0.0040	-0.094 to 0.10	0.94	-0.066	-0.16 to 0.026	0.16	0.010	-0.019 to 0.039	0.48
	COVID-19 convalescent	1.64	0.81 to 2.47	**0.0001**	1.84	1.0 to 2.7	**<0.0001**	1.46	0.72 to 2.19	**0.0002**

measured using the Elecsys Anti-SARS-CoV-2 S assay

for viral neutralization, reciprocal plasma dilutions were log_2 transformed prior to multivariable analysis.

participants with positive anti-N serology at study entry

Table S2: Multivariable analyses of the relationship between sociodemographic, health and vaccine-related variables on humoral responses following three-dose COVID-19 mRNA vaccination

Humoral measure	Variable	1 mo after 3rd dose		
		Estimate	**95% CI**	**p-value**
anti-RBD Abs (log_{10})[a]	Age (per year)	0.0018	-0.0011 to 0.0048	0.22
	Male sex	0.0080	-0.086 to 0.10	0.87
	White ethnicity	0.0089	-0.082 to 0.10	0.85
	# chronic conditions (per add'l)	-0.053	-0.091 to -0.014	**0.0078**
	Spikevax as third dose (vs. Comirnaty)	0.12	0.030 to 0.21	**0.0091**
	Interval between 1st and 2nd dose (per day)	-0.00064	-0.0033 to 0.0020	0.63
	Interval between 2nd and 3rd dose (per day)	0.00037	-0.0014 to 0.0022	0.69
	Days since 3rd vaccine dose	0.0016	-0.013 to 0.016	0.82
	Prior COVID-19[c]	0.070	-0.057 to 0.20	0.28
Viral neut. (log_2) [b]	Age (per year)	0.022	0.0025 to 0.042	**0.028**
	Male sex	-0.075	-0.70 to 0.55	0.81
	White ethnicity	-0.28	-0.88 to 0.33	0.37
	# chronic conditions (per add'l)	-0.17	-0.43 to 0.081	0.18
	Spikevax as third dose (vs. Comirnaty)	0.70	0.11 to 1.29	**0.021**
	Interval between 1st and 2nd dose (per day)	0.012	-0.0056 to 0.029	0.18
	Interval between 2nd and 3rd dose (per day)	0.013	0.00097 to 0.025	**0.035**
	Days since 3rd vaccine dose	-0.028	-0.12 to 0.065	0.55
	Prior COVID-19[c]	1.23	0.39 to 2.06	**0.0044**

[a] Measured using the Elecsys Anti-SARS-CoV-2 S assay

[b] Viral neutralization results were log_2-transformed prior to multivariable analysis

[c] Includes all participants with positive anti-N serology at any time during the study (*i.e.* both pre- and post-vaccine COVID-19 cases)