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Neutralizing antibodies against the SARS-CoV-2 Delta and Omicron variants following heterologous CoronaVac plus BNT162b2 booster vaccination

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Abstract

The recent emergence of the SARS-CoV-2 Omicron variant is raising concerns because of its increased transmissibility and by its numerous spike mutations with potential to evade neutralizing antibodies elicited by COVID-19 vaccines. Here, we evaluated the effects of a heterologous BNT162b2 mRNA vaccine booster on the humoral immunity of participants that had received a two-dose regimen of CoronaVac, an inactivated vaccine used globally. We found that heterologous CoronaVac prime followed by BNT162b2 booster regimen induces elevated virus-specific antibody levels and potent neutralization activity against the ancestral virus and Delta variant, resembling the titers obtained after two-doses of mRNA vaccines. While neutralization of Omicron was undetectable in participants that had received a two-dose regimen of CoronaVac vaccine, BNT162b2 booster resulted in a 1.4-fold increase in neutralization activity against Omicron, compared to two-dose mRNA vaccine. Despite this increase, neutralizing antibody titers were reduced by 7.1-fold and 3.6-fold for Omicron compared to ancestral and Delta variant, respectively. Our findings have immediate implications for multiples countries that previously used a CoronaVac regimen and reinforce the notion that the Omicron variant is associated with immune escape from vaccines or infection-induced immunity, highlighting the global need for vaccine boosters to combat the impact of emerging variants.

Main

The ongoing evolution of SARS-CoV-2 and recent emergence of Omicron variant raise concerns about its increased transmissibility and vaccine effectiveness. CoronaVac is a 2-dose β-propiolactone-inactivated, aluminium hydroxide-adjuvanted COVID-19 vaccine. CoronaVac is widely used globally and has been authorized in 48 countries, with 85% and 80% of effectiveness against hospital admission and death, respectively (World Health Organization, https://www.who.int/). However, with the emergence of new SARS-CoV-2 variants and the waning immunity of vaccines over time, multiple countries initiated the administration of booster doses [1–3]. The Dominican Republic was among the first countries to recommend the administration of a third booster dose to address potential waning immunity and reduced effectiveness against variants.

The Omicron variant contains up to 36 mutations in spike protein [4] rendering many vaccines less effective [5,6]. Omicron variant is also highly transmissible, overtaking Delta as the dominant variant in many countries. To assess the potential risk of vaccine immune evasion we assembled a cohort of CoronaVac-vaccinated individuals that received a heterologous BNT162b2 mRNA vaccine boost. We investigated vaccine-induced neutralizing antibody titers against the Delta (B.1.617.2) and Omicron (BA.1 sublineage of B.1.1.529) variants and compared them to the ancestral A lineage, using authentic SARS-CoV-2 isolates.

To characterize SARS-CoV-2-specific adaptive immune responses, we analyzed plasma samples from 101 non-hospitalized adult participants who received the BNT162b2 booster dose at least four weeks after the second dose of CoronaVac vaccine between July 30 and August 27, 2021. Plasma samples were collected longitudinally at Departamento de Investigaciones Biomedicas, Clinica Evangelina Rodriguez, Profamilia, Santo Domingo, Dominican Republic, at baseline (prior to booster), 7 and 28 days after de booster (third dose) administration and were subjected to ELISA and neutralization assays using authentic virus. Data from a previous cohort, composed of healthcare workers (HCWs) from the Yale-New Haven Hospital (YNHH) that received two doses of mRNA COVID-19 vaccines (mRNA-1273, Moderna or BNT162b2, Pfizer-BioNTech) were used as reference[7]. The mean ages of the participants (majority females, 70%) was 40.4 $\pm$ 13.4 and their body mass index was 27.5 $\pm$ 5.5. Cohort demographics, vaccination and previous infection status are summarized in the Extended Data Table 1 and Extended Data Table 2.

Plasma antibody reactivity to Spike protein and receptor binding domain (RBD) of SARS-CoV-2 were measured at baseline, 7 and 28 days post BNT162b2 booster. Virus-specific IgG titers increased at 7 days

over baseline, and were further elevated on day 28 post booster shot (Figure 1a,b). Individuals that received CoronaVac prime followed by BNT162b2 booster regimen developed high anti-RBD IgG titers that reached equivalent levels as the HCW who received 2 doses of mRNA vaccines[7].

We then measured the ability of serum samples to neutralize SARS-CoV-2, lineage A (ancestral strain, USA-WA1/2020) and B.1.617.2 (Delta variant). Individuals who were fully vaccinated with CoronaVac and received BNT162b2 booster displayed a 10.1- and 6.3-fold increase in neutralization activity against ancestral and delta variant, respectively, 28 days after the booster shot. No statistical differences were observed in neutralization titers between the CoronaVac/BNT162b2 versus two-dose mRNA vaccinated individuals, 28 days after the last shot (Figure 1c, d).

Next, we extended our analysis to investigate potential neutralizing antibody (NAb) escape against the Omicron variant post vaccination. Within our HCW cohort, we observed a 14.5-fold reduction in neutralization titers against the Omicron variant (Extended Data Figure 1), at 28 days post second vaccination dose, the peak of neutralization titers[7], measured by half-maximal plaque reduction neutralizing assays (PRNT50).

Recent studies demonstrate that booster doses of homologous mRNA vaccines can enhance NAb response against the Omicron variant [8–13]. To further assess neutralization activity in the heterologous vaccination regimen, we compared neutralizations titers against the ancestral virus, Delta and Omicron variant, previously and post BNT162b2 booster. Omicron was 7.1-fold less sensitive to neutralization than ancestral virus and 3.6-fold less sensitive than Delta variant when assayed with plasma samples obtained 28 days post BNT162b2 booster (Figure 1e). Notably, plasma from those who received 2 doses of CoronaVac had no neutralizing antibodies to Omicron prior to the BNT162b2 booster (Figure 1f). After the booster, 80% of Coronavac/BNT162b2 recipients developed neutralizing antibody titers against the Omicron variant. BNT162b2 booster resulted in a 1.4-fold increase in neutralization activity against Omicron, compared to two-dose mRNA vaccine, 28 days after the last shot (Figure 1f). Despite this increase, PRNT50 values and neutralizing antibody titers were reduced (IC50 mean, 1.4; 7.1-and -3.6 fold reduction) for Omicron compared to ancestral and Delta SARS-CoV-2, respectively.

Next, we separated individuals by their previous SARS-CoV-2 infection status (i.e., previously infected vs. non-previously infected) and determined their neutralization titers against Omicron post vaccination. The NAb titers against VOCs for previously infected individuals were higher when compared to non-previously infected individuals that received 2x mRNA vaccines, 28 days after the last shot, as previously

described[7]. Neutralization titers against Omicron decreased by 17.3-fold (compared to lineage A) in non-previously infected and by 10.7-fold in previously infected individuals who received 2 mRNA vaccine doses (Figure 2a). Additionally, in both cohorts, individuals that received 2x mRNA vaccines or the heterologous CoronaVac/BNT162b2 booster regimen, the NAb titers were elevated for the Delta variant in previously infected individuals but not for Omicron (Figure 2b-d).

Finally, we investigated whether the timing interval between vaccination doses and SARS-CoV-2 infection correlates with vaccine-induced IgG and PRNT50 levels. As previously described[1] anti-RBD IgG titers and neutralizing antibodies waned over time post CoronaVac vaccination (Extended Data Fig. 2a,b). Importantly, the time interval between the second vaccination dose and the booster shot did not affect neutralization titers against ancestral and SARS-CoV-2 variants, measured 28 days post BNT162b2 booster (Figure 2e). However, the time interval between previous SARS-CoV-2 infection and vaccination correlated with neutralization titers against ancestral and Delta variant: the furthest from the infection, the higher the neutralization responses (Figure 2f,g). Notably, our temporal analysis revealed that neutralization titers against Omicron do not show such correlation, even post booster shot (Figure 2g). Multivariable linear regression model results show that previous COVID-19 infection increased log PRNT50 by 0.17 (95%CI: 0.02-0.33; p-value: 0.03) for the ancestral strain, by 0.23 (95%CI: 0.08-0.39; p-value: 0.003) for the Delta variant, and by 0.15 (95%CI: (-)0.01-0.30; p-value:0.06) for the omicron variant after controlling for time since second dose of the vaccine to the booster dose and sex. It should be noted that stratification on previously infection status had limited our sample size, which could affect the precision of our estimates for temporal analysis. Together, these data suggested that previous SARS-CoV-2 exposure improves neutralizing antibodies production in vaccinated individuals for ancestral and Delta variant.

We found that a regimen of heterologous two-dose CoronaVac prime followed by a single BNT162b2 booster induces elevated virus-specific antibody levels and potent neutralization activity against ancestral and Delta SARS-CoV-2 strains, resembling the titers obtained after two doses of mRNA vaccines. After the BNT162b2 booster shot, 80% of participants developed NAbs against Omicron and the levels were 1.4-fold higher than participants that received two-doses of mRNA vaccines. However, the neutralization titers against Omicron were significantly reduced compared to NAbs levels against ancestral virus and Delta variant post booster, suggesting a greater risk of vaccine breakthrough infections. Clinical follow-up is needed to assess risk of serious disease in these individuals.

In agreement with previous reports, our data show that Omicron variant escapes neutralizing antibodies elicited by two mRNA vaccine doses or two CoronaVac vaccine doses[8,11,13–15]. Notably, none of the individuals fully vaccinated with CoronaVac had detectable neutralizing antibody against SARS-CoV-2 Omicron variant. Preliminary mRNA vaccine studies suggests that booster doses can enhance NAb response against the Omicron variant and therefore booster doses should be recommended [11,16]. However, our data suggest that the Omicron variant may be associated with lower COVID-19 vaccine effectiveness against infection, even post a single heterologous booster (CoronaVac followed by BNT162b2) and even among previously infected individuals. Our findings have immediate implications for multiple countries that previously used a two-dose regimen of CoronaVac and are of particular importance considering the increasing global need for heterologous vaccine boosters as a relevant future strategy to combat the impact of emerging variants in countries where inactivated vaccines have been the dominant product used.

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Author contributions E.P.T., C.L., V.S.M., M.M, VB, S.V., N.D.G, and A.I. conceived the study. S.O., A.K., and IY designed and implemented HCW cohort study. V.B., L.C., E.C., A.J., M.S., I.Yand P.L collected and processed plasma samples. C.L. and V.M.S. performed SARS-CoV-2 specific antibody ELISAs and the neutralization assays. VB, LC, M.C. and I.Y., collected epidemiological and clinical data. C.B.F.V., M.I.B., K.B., C.P., R.D., E.G., A.M., J.R., surveilled, detected and performed virus sequencing. C.L and V.S.M., isolated SARS-CoV-2 variants. VB, I.Y., and M.C., assisted volunteers' identification and enrolment. C.L., and V.M.S. analyzed the data. EPT and MM designed, managed and control the quality of epidemiological data collected at REDCap database. C.L., E.P.T., and A.I. drafted the manuscript. All authors reviewed and approved the manuscript. E.P.T and A.I. secured funds and supervised the project.

Competing interests: AI served as a consultant for RIGImmune, Xanadu and Revelar Biotherapeutics. IY reported being a member of the mRNA-1273 Study Group and has received funding to her institution to conduct clinical research from BioFire, MedImmune, Regeneron, PaxVax, Pfizer, GSK, Merck, Novavax, Sanofi-Pasteur, and Micron. NDG is a consultant for Tempus Labs to develop infectious disease diagnostic assays. A.I.K serves as an expert panel member for Reckitt Global Hygiene Institute, scientific advisory board member for Revelar Biotherapeutics and a consultant for Tata Medical and Diagnostics and Regeneron Pharmaceuticals, and has received grants from Merck, Regeneron

Pharmaceuticals and Tata Medical and Diagnostics for research related to COVID-19 but are outside the submitted work. All other authors declare no competing interests.

Figure Legends

Figure 1 | Characterization of vaccine induced immunity post heterologous CoronaVac/ BNT162b2 vaccination. Dominican Republic participants received 2 doses of CoronaVac vaccine followed by a heterologous booster with BNT162b2 mRNA vaccine. Plasma samples were collected as indicated: Baseline, prior to vaccination booster (CoronaVac (2x) DR), -7 and -28 days post 3 dose (CoronaVac (2x) + Pfizer (1x). HCW participants received 2 doses of the mRNA vaccine and plasma samples were used for comparison (mRNAVac (2x) Yale). **a, b,** Plasma reactivity to S protein and RBD in vaccinated participants after heterologous booster, measured over time by ELISA. S, spike. RBD, receptor binding domain. n=101 in each respective time point. **c-f,** Neutralization assay using wild-type SARS-CoV-2. Plasma neutralization capacity against ancestral strain (WA1, USA) (**c**) and Delta variant) (**d)** in vaccinated participants at the baseline (purple) or 28 days post 3 dose (blue). HCW participants received 2 doses of the mRNA vaccine and plasma samples were used for comparison (green). n=101 in each group. The numbers in parentheses indicate the median fold change in neutralization resistance for the indicated variants compared to ancestral. **e**, Plasma neutralization capacity against ancestral strain, Delta and Omicron variants at baseline (left panel) and -28 days post 3 dose (right panel). The numbers in parentheses indicate the median fold change in neutralization resistance for the indicated variants compared to ancestral. n=101 in each group. **f,** Plasma neutralization capacity against Omicron in vaccinated participants at the baseline (purple) or 28 days post 3 dose (blue). HCW participants received 2x mRNA vaccine and plasma samples were used for comparison (green). The numbers in parentheses indicate the median fold change in neutralization resistance for the indicated variants for participants post booster vaccination. n=64 CoronaVac (2x); n=101 CoronaVac (2x) + Pfizer (1x) DR; n=32 mRNAVac (2x) Yale. Significance was assessed by One-way ANOVA corrected for multiple comparisons using Tukey's method. Violin plots represent mean values ± standard deviations. Horizontal lines indicate mean values and were coloured accordingly. Each dot represents a single individual. ****p < .0001 ***p < .001 **p < .01*p < .05.

Figure 2 | Neutralizing activity comparison in CoronaVac/BNT162b2-vaccinated participants previously infected or not to SARS-CoV-2. a, Plasma neutralization titers against ancestral lineage A virus and VOCs from HCWs that received 2x mRNA vaccine, 28 days post second dose. Significance, One-way ANOVA corrected using Dunnett's. **b**, Neutralization titers comparison among participants previously infected or not to SARS-CoV-2. Significance, One-way ANOVA corrected using Tukey's. (-) Vaccinated-uninfected, n=16; (+) Vaccinated-Previously infected, n=14. **c-g,** Plasma neutralization titers from Dominican Republic participants that received 2x of CoronaVac followed by BNT162b2 booster. **c,** Neutralization titers against ancestral virus, Delta and Omicron at -28 days post 3 dose in non-previously infected (left) and SARS-CoV-2 previously infected participants, right. Significance, One-way ANOVA corrected using Tukey's. Violin plots represent mean values ± standard deviations. (Non-previously infected; n=75 (Ancestral); n=75 (Delta); n=75 (Omicron); (Previously infected; n=26 (Ancestral); n=26 (Delta); n=26 (Omicron). **d,** Neutralization titers comparison among previously infected or not to SARS-CoV-2. Significance, One-way ANOVA corrected using Tukey's. (-) Vaccinated-uninfected, n=57; (+) Vaccinated-Previously infected, n=24. **a,** Plasma neutralization titers measured 28 days post booster dose. Regression lines are shown over time as days post 2 CoronaVac dose vaccination, as purple (ancestral), dark green (Delta) and light green (Omicron). n=101in each group. Ancestral p= 0.41; Delta p= 0.27; Omicron p= 0.96. Plasma neutralization titers measured at baseline **(b)** and 28 days post booster dose **(c).** Regression lines are shown over time as days post SARS-CoV-2 infection, as purple (ancestral), dark green (Delta) and light green (Omicron). n=26 (Ancestral); n=26 (Delta); n=26 (Omicron). Ancestral p= 0.0072; Delta p= 0.0062; Omicron p= 0.89. Lines indicates cross-sectional averages from each group,

with shading representing 95% CI. Each dot represents a single individual. Boxes represent mean ± standard deviations. Numbers in parentheses indicate the median fold change in neutralization resistance for the indicated variants compared to ancestral strain. ****p < .0001 ***p < .001 **p < .01*p < .05.

References

Naaber, P. *et al.* Dynamics of antibody response to BNT162b2 vaccine after six months: a longitudinal prospective study. *The Lancet Regional Health - Europe* **10**, 100208 (2021).

Pegu, A. *et al.* Durability of mRNA-1273 vaccine–induced antibodies against SARS-CoV-2 variants. *Science* **373**, 1372–1377 (2021).

Goldberg, Y. *et al.* Waning Immunity after the BNT162b2 Vaccine in Israel. *New England Journal of Medicine* **385**, e85 (2021).

Pulliam, J. R. C. *et al.* Increased risk of SARS-CoV-2 reinfection associated with emergence of the Omicron variant in South Africa. *medRxiv* (2021) doi:10.1101/2021.11.11.21266068.

Andrews, N. *et al.* Effectiveness of COVID-19 vaccines against the Omicron (B.1.1.529) variant of concern. *medRxiv* 2021.12.14.21267615 (2021) doi:10.1101/2021.12.14.21267615.

Collie, S., Champion, J., Moultrie, H., Bekker, L.-G. & Gray, G. Effectiveness of BNT162b2 Vaccine against Omicron Variant in South Africa. *New England Journal of Medicine* (2021) doi:10.1056/NEJMc2119270.

Lucas, C. *et al.* Impact of circulating SARS-CoV-2 variants on mRNA vaccine-induced immunity. *Nature* **600**, 523–529 (2021).

Doria-Rose, N. A. *et al.* Booster of mRNA-1273 Vaccine Reduces SARS-CoV-2 Omicron Escape from Neutralizing Antibodies. *medRxiv* (2021) doi:10.1101/2021.12.15.21267805.

Gruell, H. *et al.* mRNA booster immunization elicits potent neutralizing serum activity against the SARS-CoV-2 Omicron variant. *medRxiv* (2021) doi:10.1101/2021.12.14.21267769.

Basile, K. *et al.* Improved neutralization of the SARS-CoV-2 Omicron variant after Pfizer-BioNTech BNT162b2 COVID-19 vaccine boosting. *bioRxiv* (2021) doi:10.1101/2021.12.12.472252.

Garcia-Beltran, W. F. *et al.* mRNA-based COVID-19 vaccine boosters induce neutralizing immunity against SARS-CoV-2 Omicron variant. *Cell* (2022) doi:10.1016/j.cell.2021.12.033.

Schmidt, F. *et al.* Plasma neutralization properties of the SARS-CoV-2 Omicron variant. doi:10.1101/2021.12.12.21267646.

Cele, S. *et al.* Omicron extensively but incompletely escapes Pfizer BNT162b2 neutralization. *Nature* (2021) doi:10.1038/s41586-021-04387-1.

Nemet, I. *et al.* Third BNT162b2 vaccination neutralization of SARS-CoV-2 Omicron infection. *medRxiv* (2021) doi:10.1101/2021.12.13.21267670.

Lu, L. *et al.* Neutralization of SARS-CoV-2 Omicron variant by sera from BNT162b2 or Coronavac vaccine recipients. *Clinical Infectious Diseases* ciab1041 (2021) doi:10.1093/cid/ciab1041.

Kalinich, C. C. *et al.* Real-time public health communication of local SARS-CoV-2 genomic epidemiology. *PLoS Biology* **18**, (2020).

Mao, T. *et al.* A stem-loop RNA RIG-I agonist protects against acute and chronic SARS-CoV-2 infection in mice. *Journal of Experimental Medicine* **219**, (2022).

METHODS

Ethics statement

This study was approved by the National Bioethics Committee of the Dominican Republic (CONABIOS). The participants received two doses of the inactivated whole-virion vaccine CoronaVac followed by a BNT162b2 booster dose at least four weeks after the second dose of CoronaVac. Republic initiated the COVID-19 vaccination and booster campaigns in February and July 2021, respectively. All DR participants were consented to enroll in this observational study. mRNA vaccine BNT162b2 booster were administrated between July 30 and August 27, 2021. Health care worker (HCW) volunteers from the Yale New Haven Hospital (YNHH) were enrolled and included in this study (IRB Protocol ID 2000028924, approved by the Yale Human Research Protection Program Institutional Review Board, by the Yale School of Medicine. The HCWs volunteers received the mRNA vaccine (100 micrograms mRNA-1273, Moderna or 20 micrograms, BNT162b2, Pfizer-BioNTech) between November 2020 and January 2021. Informed consent was obtained from all enrolled. None of the participants experienced serious adverse effects after vaccination.

Vaccinated volunteers

One hundred and one volunteers from the Dominican Republic and forty HCW participants from the YNHH were followed serially post-vaccination. For the Dominican Republic cohort, plasma samples were collected at baseline (prior to booster, after two doses), 7 and 28 days after the booster (third dose) administration. Plasma from HCWs included on this study were collected 28- days post second vaccination dose. Demographic information was aggregated through a systematic review and was used to construct Extended Data Table 1 and Extended Data Table 2. The clinical data were collected using REDCap (v5.19.15 @2021 Vanderbilt University) software. Blood acquisition was performed and recorded by a separate team. Vaccinated clinical information and time points of collection information was not available until after processing and analyzing raw dat. ELISA and neutralizations were performed blinded. Prior SARS-CoV-2 infection was confirmed by ELISA and/or PCR. Documented history of vaccination and vaccination date was evidenced by the official vaccination card. Previous COVID infections time window is based on participant`s reports. This information of time window post CoronaVac vaccination and previous infection are available in the Source Data 1.

Plasma isolation and storage

Whole blood was collected in heparinized CPT blood vacutainers (BD; # BDAM362780) and kept on gentle agitation until processing. All blood was processed on the day of collection in a single step standardized method. Plasma samples were collected after centrifugation of whole blood at 600 g for 20 min at room temperature (RT) without brake. The undiluted plasma was transferred to 15-ml polypropylene conical tubes, and aliquoted and stored at −80 °C for subsequent shipping and analysis. Plasma samples were sourced from Dominican Republican participants and were shipped to Yale University. The plasma was aliquoted and heat-inactivated at 56°C for 30 min to inactivate complement prior to micro-neutralization.

SARS-CoV-2 culture

TMPRSS2-VeroE6 kidney epithelial cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 1% sodium pyruvate (NEAA) and 10% fetal bovine serum (FBS) at 37°C and 5% CO2. The cell line has been tested negative for contamination with mycoplasma. SARS-CoV-2 lineage A (USA-WA1/2020), was obtained from BEI Resources (#NR-52281). Alpha, Beta, Gamma, Delta and Omicron variants were isolated from nasopharyngeal specimens. Alpha, Beta, Gamma, and Delta SARS-CoV-2 samples were sequenced as part of the Yale Genomic Surveillance Initiative's weekly surveillance program in Connecticut, United States [16]. Omicron (Lineage BA.1) was sequenced by the Connecticut State Department of Public Health (GISAID Accession: EPI_ISL_7313633). The isolates were cultured

and resequenced as previously described [7,17]. In brief, samples were filtered through a 45μM filter and serially diluted from 1:50 to 1:19,531,250. The dilution was subsequently incubated with TMPRSS2-Vero E6 in a 96 well plate and adsorbed for 1 hour at 37°C. After adsorption, replacement medium was added, and cells were incubated at 37°C for up to 5 days. Supernatants from cell cultures with cytopathic effect (CPE) were collected, frozen, thawed and subjected to RT- qPCR. Fresh cultures were inoculated with the lysates as described above for viral expansion. Nucleic acid was extracted using the ThermoFisher MagMAX viral/pathogen nucleic acid isolation kit and libraries were prepared using the Illumina COVIDSeq Test RUO version. Pooled libraries were sequenced on the Illumina NovaSeq (paired-end 150) by the Yale Center for Genome Analysis. Data was analyzed and consensus genomes were generated using iVar (version 1.3.1). The resequenced genomes were submitted to NCBI (GenBank Accession numbers: ancestral lineage A = MZ468053, Alpha = MZ202178, Beta = MZ468007, Gamma = MZ202306, Delta = MZ468047, Omicron = OL965559). The pelleted virus was then resuspended in PBS and aliquoted for storage at −80°C. Viral titers were measured by standard plaque assay using TMPRSS2-VeroE6. Briefly, 300 μl of serial fold virus dilutions were used to infect Vero E6 cells in MEM supplemented NaHCO3, 4% FBS 0.6% Avicel RC-581. Plaques were resolved at 48h post-infection by fixing in 10% formaldehyde for 1h followed by 0.5% crystal violet in 20% ethanol staining. Plates were rinsed in water to plaques enumeration. All experiments were performed in a biosafety level 3 laboratory with approval from the Yale Environmental Health and Safety office.

SARS-CoV-2 specific-antibody measurements

ELISAs were performed as previously described[7]. Briefly, Triton X-100 and RNase A were added to serum samples at final concentrations of 0.5% and 0.5mg/ml respectively and incubated at room temperature (RT) for 30 minutes before use, to reduce risk from any potential virus in serum. 96-well MaxiSorp plates (Thermo Scientific #442404) were coated with 50 μl/well of recombinant SARS Cov-2 STotal (ACROBiosystems #SPN-C52H9-100ug), or RBD (ACROBiosystems #SPD-C52H3-100ug) at a concentration of 2 μg/ml in PBS and were incubated overnight at 4°C. The coating buffer was removed, and plates were incubated for 1 h at RT with 200 μl of blocking solution (PBS with 0.1% Tween-20, 3% milk powder). Plasma was diluted serially 1:100, 1:200, 1:400 and 1:800 in dilution solution (PBS with 0.1% Tween-20, 1% milk powder) and 100 μl of diluted serum was added for two hours at RT. Human Anti-Spike (SARS-CoV-2 Human Anti-Spike (AM006415) (Active Motif #91351) was serially diluted to generate a standard curve, starting at the concentration of 1000ng/ml. Plates were washed three times with PBS-T (PBS with 0.1% Tween-20) and 50 μl of HRP anti-Human IgG Antibody (GenScript #A00166, 1:5,000) diluted in dilution solution added to each well. After 1 h of incubation at RT, plates were washed six times with PBS-T. Plates were developed with 100 μl of TMB Substrate Reagent Set (BD Biosciences #555214) and the reaction was stopped after 5 min by the addition of 2 N sulfuric acid. Plates were then read at a wavelength of 450 nm and 570nm.

Neutralization assay

Sera from vaccinated individuals were heat treated for 30 min at 56°C. Sixfold serially diluted plasma, from 1:10 to 1:2430 were incubated with SARS-CoV-2 variants, for 1 h at 37 °C. The mixture was subsequently incubated with TMPRSS2-VeroE6 in a 12-well plate for 1h, for adsorption. Then, cells were overlayed with MEM supplemented NaHCO3, 4% FBS 0.6% Avicel mixture. Plaques were resolved at 40 h post infection by fixing in 10% formaldehyde for 1 h followed by staining in 0.5% crystal violet. All experiments were performed in parallel with baseline controls sera, in an established viral concentration to generate 60-120 plaques/well.

Statistical analysis

All analyses of patient samples were conducted using GraphPad Prism 8.4.3 and JMP 15. Multiple group comparisons were analyzed by running parametric (ANOVA) statistical tests. Multiple comparisons were corrected using Tukey's and Dunnett's test as indicated in figure legends. We used a multivariable linear regression model with log PRNT50 as the dependent variable and previous COVID-19 infection, time

since second dose of the vaccine to the booster dose (in days) as explanatory variables to determine the effect of previous COVID-19 infection.

Data availability

All the background information of participants and data generated in this study are included in Source Data1. The genome information and aligned consensus genomes for SARS-CoV-2 variants used in this study are available on NCBI (GenBank Accession numbers: ancestral lineage A = MZ468053, Alpha = MZ202178, Beta = MZ468007, Gamma = MZ202306, Delta = MZ468047, Omicron = OL965559). Additional correspondence and requests for materials should be addressed to the corresponding authors.

Extended Data Table 1 | SARS-CoV-2 Dominican Republic-Vaccinated Cohort. Demographic data SARS-CoV-2 Vaccinated Cohort from Dominican Republic. Exact counts for each demographic category are displayed in N cell. Percentages of total, where applicable, are displayed in (%) cell. The mean with accompanying standard deviations for each measurement are displayed in Mean and SD cells respectively.

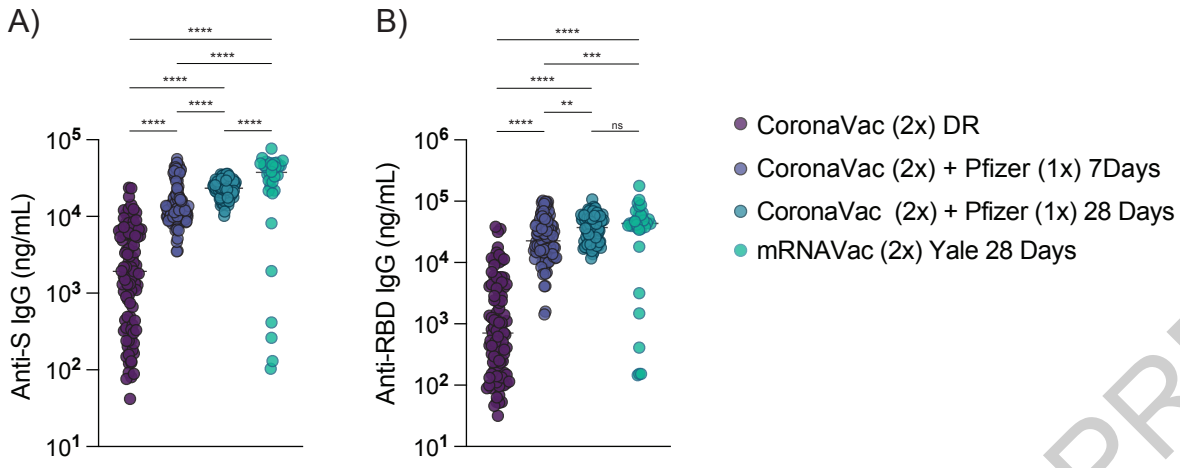
Extended Data Table 2 | SARS-CoV-2 Yale Healthcare workers-Vaccinated Cohort. Demographic data SARS-CoV-2 Vaccinated Cohort from Yale healthcare workers. Exact counts for each demographic category are displayed in N cell. Percentages of total, where applicable, are displayed in (%) cell. The mean with accompanying standard deviations for each measurement are displayed in Mean and SD cells respectively.

Extended Data Fig.1 | Impact of SARS-CoV-2 Omicron on neutralization capacity of in mRNA-vaccinated participants. a, Plasma neutralization titers against ancestral lineage A virus, (WA1, USA) and VOCs: Alpha, Beta, Delta and Omicron. SARS-CoV-2 variants were isolated from nasopharyngeal swabs of infected individuals and ancestral (WA1, USA) isolate was obtained from BEI. Neutralization capacity was accessed using plasma samples from HCW participants that received 2 doses of the mRNA vaccine (mRNAVac (2x) Yale), 28 days post second vaccination dose at the experimental sixfold serial dilutions (from 1:3 to 1:2430). Significance was assessed by One-way ANOVA corrected for multiple comparisons using Dunnett's method. Neutralization capacity to the variants was compared to neutralization capacity against the ancestral strain. Boxes represent mean values ± standard deviations. The numbers in parentheses indicate the median fold change in neutralization resistance for the indicated variants compared to ancestral strain. n=32/group Each dot represents a single individual. ****$p < .0001$ **$p < .005$.

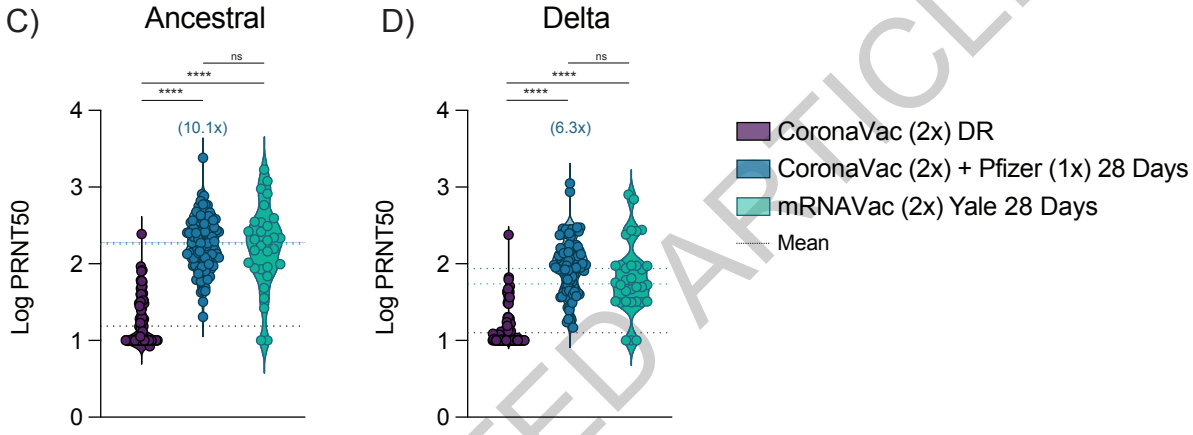
Extended Data Fig.2 | Plasma antibody kinetics post CoronaVac vaccination. a, Vaccinated participants plasma reactivity to RBD measured by ELISA**. b,** Plasma neutralization titers measured at baseline, previously to BNT162b2 booster shot against ancestral virus, Delta and Omicron variants. Regression lines are shown over time as days post 2 CoronaVac dose vaccination, as purple (ancestral), dark green (Delta) and light green (Omicron). Lines indicates cross-sectional averages from each group, with shading representing 95% CI and colored accordingly. Each dot represents a single individual. n=101 (Ancestral); n=101 (Delta); n=80 (Omicron).

Source Data Figure 1: Detailed clinical and immunological data for each patient. Clinical information, demographics and exact counts for immunological data.

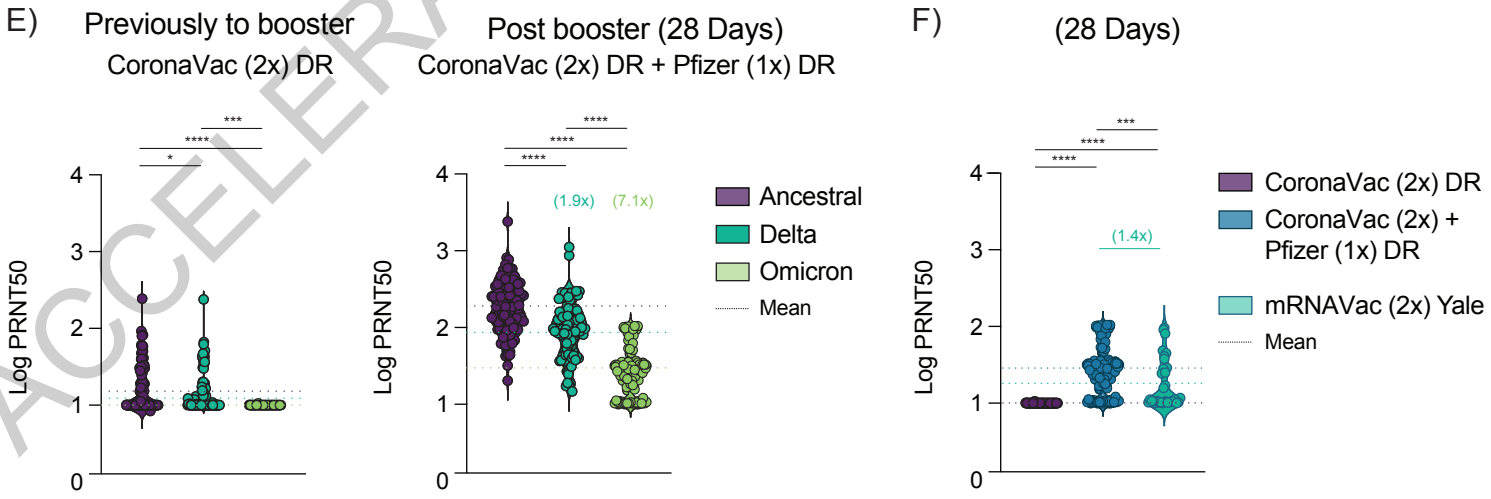
Plasma reactivity to S protein and RBD in CoVac/BNT vaccinated individuals

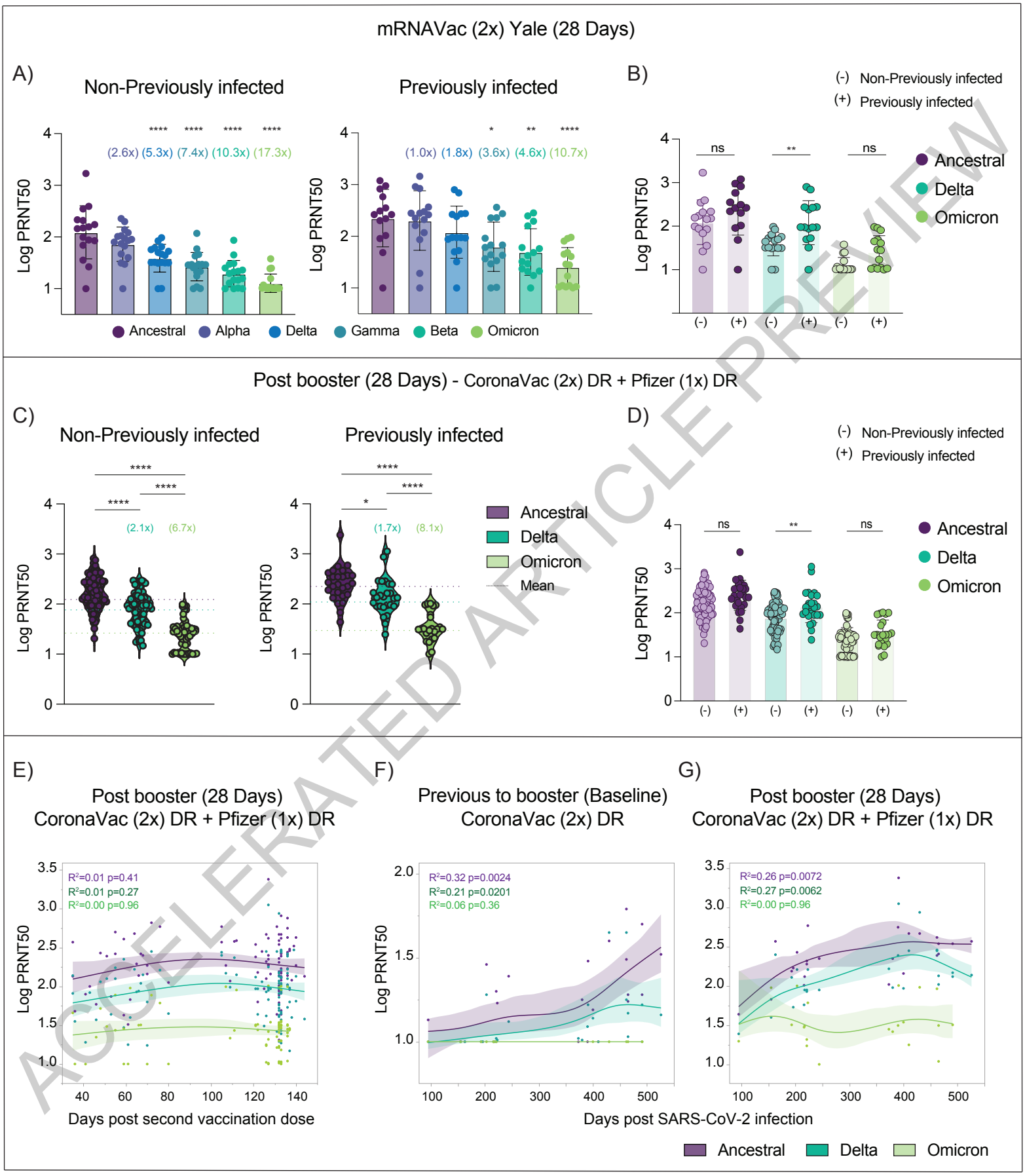


Plaque reduction half-maximal neutralizing titer (PRNT50) against ancestral and delta variant.



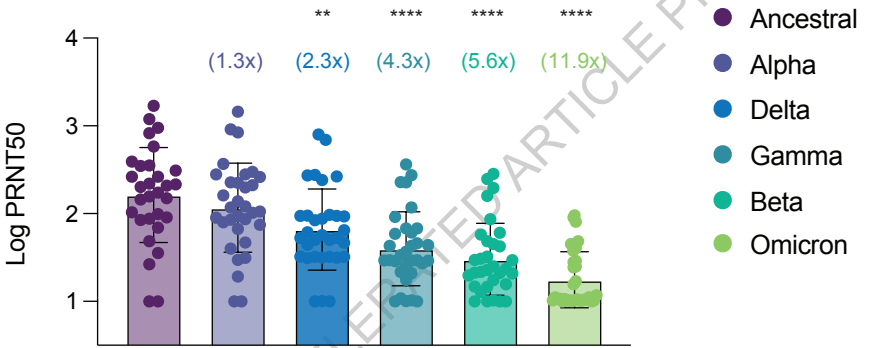
Plaque reduction half-maximal neutralizing titer (PRNT50) against Omicro variant





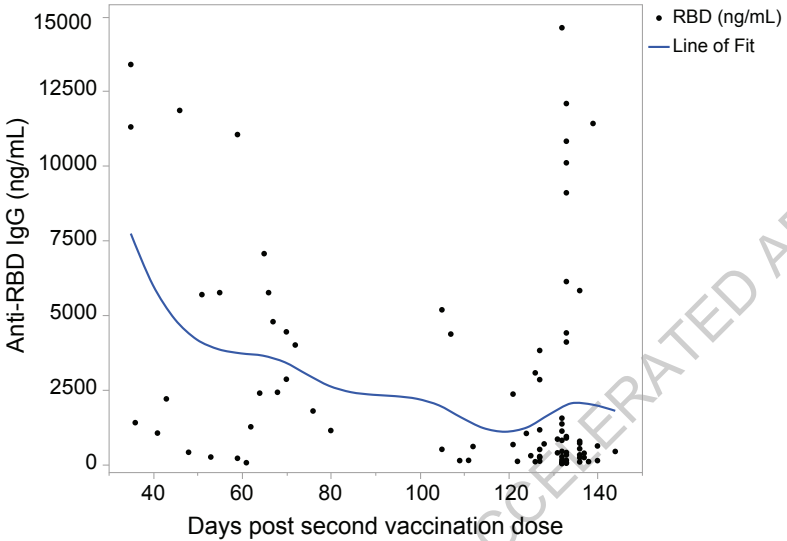
Plaque reduction half-maximal neutralizing titer (PRNT50) against VOCs

A) mRNAVac (2x) Yale (28 Days)

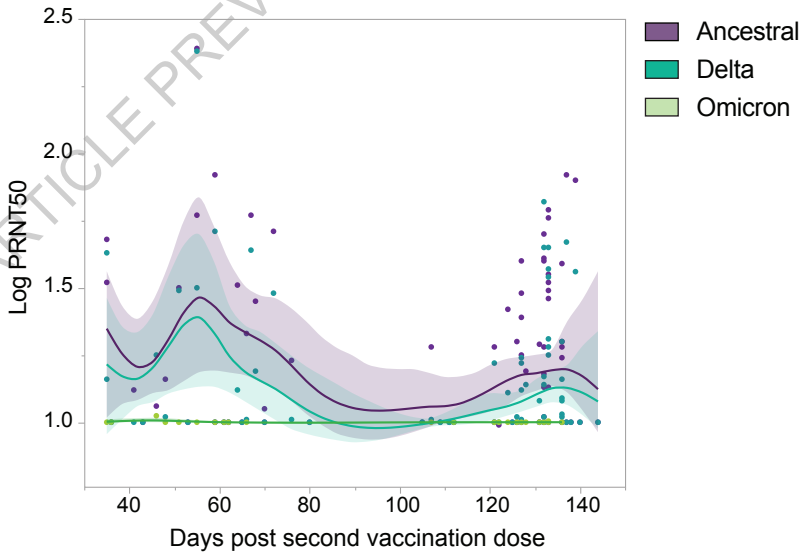


Previous to booster (Baseline) - CoronaVac (2x) DR

A)



B)



Extended Data Table 1. Patient's demographic data Dominican Republic

Dominican Republic Cohort

Individual variables	N	(%)	Mean	SD
Age	101		40.4	13.4
Gender				
Male	31	30.7		
Female	70	69.3		
BMI	101		27.63	5.53
Infection Status				
Non-previously infected	75	74.25		
Previously infected	26	25.74		
Days since last infection			326.26	131.01
Days since last SinoVac dose	101		113	33

Extended Data Table 2. Patient's demographic data HCWs-Yale Cohort

HCWs-Yale Cohort

Individual variables	N	(%)	Mean	SD
Age	37		44.9	12.5
Gender				
Male	7	18.9		
Female	30	81.1		
Infection Status				
Non-previously infected	18	48.65		
Previously infected	19	51.35		
Vaccine	101		113	33
BNT162b2	7	18.91		
mRNA-1273	30	81.09		

nature portfolio

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Last updated by author(s): Jan 13, 2022

Reporting Summary

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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☒	☐ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒ A description of all covariates tested
☐	☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒ For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐ Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	REDCap (v5.19.15 @2021 Vanderbilt University) (clinical data aggregation).
Data analysis	Jmp Pro 15.0.0 (SAS Institute) (graphs/statistics), and GraphPad Prism 8.4.3(graphs/statistics).

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

All the background information of participants and data generated in this study are included in Source Data1. The genome information and aligned consensus genomes for SARS-CoV-2 variants used in this study are available on NCBI (GenBank Accession numbers: ancestral lineage A = MZ468053, Alpha = MZ202178, Beta = MZ468007, Gamma = MZ202306, Delta = MZ468047, Omicron = OL965559). Additional correspondence and requests for materials should be addressed to the corresponding authors.

Field-specific reporting

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☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	No statistical methods were used to calculate the sample size. Sample size was determined based on the number of adults (≥ 18 years old) from the Dominican Republic that received the mRNA vaccine, Pfizer as a booster and consent in participating in the current study. Republic initiated the COVID-19 vaccination and booster campaigns in February and July 2021, respectively. mRNA vaccine BNT162b2 booster were administrated between July 30 and August 27, 2021. This study enrolled 103 volunteers and was approved by the National Bioethics Committee of the Dominican Republic (CONABIOS). Informed consent was obtained by trained staff and sample collection commenced immediately upon study enrollment. Participants were followed serially post-vaccination. Clinical specimens were collected at baseline (previous to booster), 7- and 28- post third vaccination dose. Alpha, Beta, Gamma, Delta and Omicron variants were isolated from nasopharyngeal specimens from the Yale SARS-CoV-2 Genomic Surveillance Initiative's weekly surveillance program in Connecticut, US.
Data exclusions	Twp participant that received an adenovirus- based vaccine were excluded from this study. For the current study we had only included participants that received CoronaVac and Pfizer vaccines as booster shot.
Replication	ELISAs were done in duplicate for each sample. Neutralization assays were done with 6 fold dilution for each sample to generate a curve and access the IC50 values. For all assays, longitudinal analyses were performed from human individuals samples. All replications were successful.
Randomization	Vaccinated donors were stratified in two major groups, previously infected with SARS-CoV2 (recovered) on uninfected (naive), confirmed by serology.
Blinding	The clinical data were collected using REDCap software. Blood acquisition was performed and recorded by a separate team. Clinical information and time points of collection information was not available until after processing and analyzing raw data. ELISA and neutralizations were blinded.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☐	☒ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	All antibodies used in this study are against human proteins: Human Anti-Spike (SARS-CoV-2 Human Anti-Spike (AM006415) (Active Motif #91351) was serially diluted to generate a standard curve. HRP anti-Human IgG Antibody (GenScript #A00166, 1:5,000.
Validation	The antibodies used in this study are commercially available, and all have been validated by the manufacturers and used by other publications. Likewise, we titrated these antibodies according to our own our staining conditions. Human Anti-Spike (SARS-CoV-2 Human Anti-Spike (Clone: AM006415, 415-6)) (Active Motif #91351) : this antibody has been tested by ELISA and is specific for SARS-CoV-2 Spike protein S1 Subunit of Receptor Binding Domain (RBD). Details on the development and characterization of this clone are available here: Wan J., et. al. Human IgG neutralizing monoclonal antibodies block SARS-CoV-2 infection. Cell Reports, 32: 107918, July 21, 2020. HRP anti-Human IgG Antibody: this antibody is purified by immunoaffinity chromatography and then conjugated to horseradish peroxidase. Species Reactivity: human

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	TMPRSS2-VeroE6 kidney epithelial cell line was obtained from the ATCC
Authentication	TMPRSS2-VeroE6 was obtained from ATCC, tested and authenticated by morphology, karyotyping, and PCR based approaches.
Mycoplasma contamination	The cell line tested negative for contamination with mycoplasma.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used in the study

Human research participants

Policy information about studies involving human research participants

Population characteristics	Cohort characteristics are available in Table 1.
Recruitment	Participants were recruited between February and July 2021. mRNA vaccine BNT162b2 booster were administrated between July 30 and August 27, 2021 as part of the Republic Dominican COVID-19 vaccination booster campaigns Participants were enrollment with no self selection. Informed consent was obtained by trained staff and sample collection commenced immediately upon study enrollment. Plasma samples were collected at baseline (prior to booster, after two doses), 7 and 28 days after the booster (third dose) administration. Health care worker (HCW) volunteers from the Yale New Haven Hospital (YNHH) were enrolled and included in this study. The HCWs volunteers received the mRNA vaccine (100 micrograms mRNA-1273, Moderna or 20 micrograms, BNT162b2, Pfizer-BioNTech) between November 2020 and January 2021. Plasma samples were collected at baseline (prior to vaccination, 7 and 28 days after the first and second vaccination dose. Participants were enrollment with no self selection. Informed consent was obtained by trained staff .
Ethics oversight	Yale Human Research Protection Program Institutional Review Boards. Informed consents were obtained from all enrolled healthcare workers. • Our research protocol was reviewed and approved by the Yale School of Medicine IRB Protocol ID 2000028924. This study was approved by the National Bioethics Committee of the Dominican Republic (CONABIOS). Informed consent was obtained by trained staff and records maintained in our research database for the duration of our study. There were no minors included on this study.

Note that full information on the approval of the study protocol must also be provided in the manuscript.