nature

https://doi.org/10.1038/d41586-021-03846-z

Accelerated Article Preview

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Received: 20 December 2021

Accepted: 31 December 2021

Accelerated Article Preview Published online: 31 December 2021

Cite this article as: Carreño, J. M. et al. Activity of convalescent and vaccine serum against SARS-CoV-2 Omicron. *Nature* https://doi.org/10.1038/d41586-021-03846-z (2021).

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Activity of convalescent and vaccine serum against SARS-CoV-2 Omicron

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The Omicron (B.1.1.529) variant of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was initially identified in November of 2021 in South Africa and Botswana as well as in a sample from a traveler from South Africa in Hong Kong.[1,2] Since then, B.1.1.529 has been detected globally. This variant seems to be at least equally infectious than B.1.617.2 (Delta), has already caused super spreader events[3] and has outcompeted Delta within weeks in several countries and metropolitan areas. B.1.1.529 hosts an unprecedented number of mutations in its spike gene and early reports have provided evidence for extensive immune escape and reduced vaccine effectiveness.[2,4-6] Here, we investigated the neutralizing and binding activity of sera from convalescent, mRNA double vaccinated, mRNA boosted, convalescent double vaccinated, and convalescent boosted individuals against wild type, B.1.351 and B.1.1.529 SARS-CoV-2 isolates. Neutralizing activity of sera from convalescent and double vaccinated participants was undetectable to very low against B.1.1.529 while neutralizing activity of sera from individuals who had been exposed to spike three or four times was maintained, albeit at significantly reduced levels. Binding to the B.1.1.529 receptor binding domain (RBD) and N-terminal domain (NTD) was reduced in convalescent not vaccinated individuals, but was mostly retained in vaccinated individuals.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged in late 2019 in Wuhan, China and has since then caused the coronavirus disease 2019 (COVID-19) pandemic. Although SARS-CoV-2 was antigenically relatively stable during its first few months of circulation, the first antigenically distinct variants, Alpha (B.1.1.7), (Beta) B.1.351 and Gamma (P.1), emerged in late 2020. Other variants of interest (VOI) and variants of concern (VOCs) followed. So far, Beta has shown the most antigenic drift in terms of reduction of *in vitro* neutralization, rivaled only by Mu (B.1.621).[7] Delta (B.1.617.2), which emerged in early 2021 has been the most consequential variant since it is more infectious than the viruses circulating in the beginning of the pandemic and also partially escapes neutralization *in vitro*.[8] Omicron (B.1.1.529) was first detected in South Africa, Botswana and in a traveler from South Africa in Hong Kong.[1,2] The variant hosts a large number of mutations in its spike protein including at least 15 amino acid changes in the receptor binding domain (RBD) and extensive changes in the N-terminal domain (NTD). These mutations are predicted to affect most neutralizing antibody epitopes. In addition, Omicron seems to be fit and highly transmissible[3] and has spread rapidly across the globe, outcompeting Delta within weeks to become the dominant circulating variant in several countries and urban areas.

Immunity to SARS-CoV-2 in human populations is highly variable and likely differs in individuals with infection induced immunity, double vaccinated individuals, boosted individuals, and individuals with hybrid immunity due to the combination of infection followed by vaccination. Understanding residual neutralizing and binding activity against highly antigenically distinct viral variants such as B.1.1.529 in these distinct groups is essential to gauge the level of protection that a specific community has against infection, mild or severe COVID-19.

Results

Neutralization of Omicron

To address these questions, we determined the loss of *in vitro* neutralizing and binding activity for Omicron (B.1.1.529, BA.1) in sera from individuals with different levels of immunity (infection, vaccine, hybrid). We included samples from convalescent individuals (N=15), individuals vaccinated twice with BNT162b2 (Pfizer/BioNTech mRNA vaccine, N=10), individuals vaccinated twice with mRNA-1273 (Moderna mRNA vaccine, N=10), individuals vaccinated three times with BNT162b2 (boosted, N=10), individuals vaccinated three times with mRNA-1273 (boosted, N=10), convalescent individuals who received 2 doses of BNT162b2 (N=10), convalescent individuals who received 2 doses of mRNA-1273 (N=10) and finally, convalescent individuals who received 3 doses of BNT162b2 (boosted, N=10) (**Figure 1A** and **Extended Data Tables 1 and 2**). First, we tested the *in vitro* neutralizing activity of the sera against wild type SARS-CoV-2 (USA-WA1/2020 as a reference for ancestral strains), Beta (as a reference for the most pronounced *in vitro* escape phenotype) and Omicron (isolated from one of the first cases identified in New York City in late November 2021, **Extended Data Table 3**). The neutralization assay used mimics physiological conditions, since it is performed with authentic SARS-CoV-2 in a multicycle replication setting in which serum/antibody is present at all times akin to the situation in a seropositive individual. Across all 85 samples, the reduction in neutralization for Omicron was greater than 14.5-fold (the actual fold reduction could not be calculated since many samples were below the limit of detection) (**Figure 1B**). In comparison, there was “only” a four-fold reduction against Beta in the

same sample set. In fact, 16.5% of samples lost all neutralizing activity against Omicron. When looking at the different groups, we noted that convalescent individuals had lower titers against wild type and Beta with the majority (73.3%) of samples having no measurable neutralizing activity for Omicron (**Figure 1C**). For samples from individuals double vaccinated with BNT162b2 and mRNA-1273, we observed a more than 23-fold and a 42-fold reduction in neutralizing activity respectively (**Figure 1D and E**). However, most individuals retained low but detectable neutralizing activity. Boosted individuals experienced lower reduction with a 7.5-fold drop in neutralization for BNT162b2 boosted individuals and a 16.7-fold reduction in mRNA-1273 boosted individuals (**Figure 1F and G**). Of note, the lower fold change and the higher starting neutralization titers led to considerable residual neutralizing activity in those groups. Convalescent individuals who received two BNT162b2, two mRNA-1272 or three BNT162b2 vaccine doses showed 14-fold, 11-fold and 13-fold drops in Omicron neutralization, respectively (**Figure 1H, I and J**). However, all individuals in these groups maintained relatively robust neutralization activity. These data indicate that convalescent individuals greatly benefit from vaccination, an observation that is of significant public health importance.

Binding to RBD, NTD and spike

While *in vitro* neutralization is an important antibody function, antibody binding – even in the absence of detectible neutralizing activity – can provide protection through Fc-mediated effector functions. This type of protection has been described in detail for influenza virus[9-11] but binding antibody titers also represent a correlate of protection for SARS-CoV-2.[12,13] Furthermore, retained binding to a highly mutated RBD or NTD, even if reduced, indicates that cognate B cells are present. These B cells could likely be rapidly recalled during variant infection or variant specific vaccination producing a strong plasmablast response leading to rapid control of viral spread. In addition, B cells with low affinity binding to antigenically drifted variant proteins may enter lymph nodes and engage in germinal center reactions leading to antibodies that may regain neutralizing activity through affinity maturation.

To investigate the reduction in binding, we expressed a recombinant RBD of Omicron and compared binding of this RBD with binding to wild type (Wuhan-1) and B.1.351 RBD (**Figure 2A**). Overall, reduction in binding to Omicron RBD was much less pronounced than reduction in neutralization (**Figure 2B**). However, this reduction was significantly greater than the one observed here and previously to Beta.[8] Reduction in binding was most pronounced for convalescent individuals (**Figure 2C**) with a drop of more than 7.5-fold and undetectable reactivity by enzyme linked immunosorbent assay (ELISA) in two thirds of the convalescent individuals who were infected early in the pandemic prior to the circulation of viral variants of concern. In all other groups binding was relatively well maintained with a reduction in binding ranging from a 2.9-fold drop in individuals who had received two vaccinations with mRNA-1273 to a 1.5-fold drop in individuals boosted with BNT162b2 (**Figures 2D to 2J**).

In addition to the RBD, the NTD is a prime target for B cells after COVID-19 mRNA vaccination.[14] The NTD also hosts neutralizing epitopes within and outside of the immunodominant 'super site'.[15-18] The NTD of Omicron carries a large number of amino acid substitutions, three deletions and one three amino acid long insertion (**Figure 2A** and **Extended Data Table 4**) which are,

collectively, predicted to significantly change the 'super site' as well as neutralizing epitopes outside of the 'super site'. To determine whether infection induced and vaccine induced antibodies retain binding to the B.1.1.529 NTD, we expressed both the wild type and variant NTDs to probe by ELISA using the same 85 samples tested for neutralization. Surprisingly, binding to the NTD was maintained with relatively minor reductions (maximum 1.9-fold), suggesting either maintained binding (e.g. at lower affinity) to the 'super site' or the presence of a large number of unchanged epitopes within this domain (**Figure 3**).

Finally, we also measured antibody binding to the wild type (Wuhan-1), Beta and Omicron spike protein ectodomains. Overall, the drop in binding to Omicron was 5.2-fold as compared to a 2.7-fold drop against Beta (**Figure 4A**). All convalescent individuals maintained binding to Omicron and binding levels between Beta and Omicron were not significantly different with some low titer sera even showing better binding to Omicron than to Beta (**Figure 4B**). However, in sera from vaccinated and convalescent plus vaccinated individuals who typically showed strong binding to spike protein, the reduction in binding to Omicron (ranging from 4.4 to 8.3-fold) was consistently higher than for Beta (ranging from 2-3.8-fold) (**Figure 4C-I**). Of note, all proteins used were his tagged, allowing to control for coating concentration. When probed with an anti-his antibody, binding was similar across the variants (**Extended Data Figure 1**).

Discussion

Our data align well with initial reports on the impact of Omicron on *in vitro* neutralizing activity of convalescent and vaccine serum and expand on these initial reports by inclusion of subcohorts with divergent SARS-CoV-2 exposure history including infection induced, primary vaccine regimen as well as booster induced and hybrid immunity.[2,5,6] We found that neutralizing activity against Omicron is most impacted in unvaccinated, convalescent individuals and in naive individuals who acquired immunity through two mRNA COVID-19 vaccine doses. Our findings support recent reports describing significantly reduced protection from reinfection[19] and almost non-existant vaccine effectiveness against symptomatic disease after two BNT162b2 vaccinations.[4] However, boosted individuals had, at least within the short time after the booster dose, significant protection against symptomatic disease in the range of 75%.[4] Although it is unclear how long this protection lasts, we observe titers similar to those in boosted individuals in convalescent vaccinated individuals, suggesting that those individuals may experience significant protection. With regard to neutralization, we made some interesting additional observations. It has been reported that in some vaccine effectiveness studies protection from infection is better maintained after mRNA-1273 vaccination as compared to BNT162b2 vaccination.[20] When looking at residual neutralizing activity to Omicron, we did not observe obvious differences between the two vaccines in naïve individuals who were vaccinated twice or three times while there was a trend towards higher titers in convalescent individuals after mRNA-1273 vaccination as compared to BNT162b2 vaccination. However, the failure to observe differences may be due to the small sample size per group, which is a major limitation of our study. Another interesting point is, that individuals with low neutralizing activity against wild type SARS-CoV-2 often only showed neutralization against Omicron in the first well, resulting in titers just above the limit of detection. Whether this is an assay artifact or bona fide neutralization is unclear. However, recent preliminary data shows that fetal bovine serum concentrations

typically used in cell culture can inhibit Omicron growth and serum concentrations are of course highest in the first well of a dilution series in a neutralization assay. It is currently unclear which epitopes are targeted by the antibodies responsible for the residual neutralizing activity against Omicron. Based on recent reports with data for monoclonal antibodies (mAbs) it is likely that most of the residual activity comes from antibodies binding to epitopes outside the receptor binding motif (site IV and V) but more rare antibodies to site I and II (complete and partial overlap with the receptor binding motif) may contribute as well.[21,22]

This study also provides first insights into Omicron RBD, NTD and spike specific binding changes. Compared to the changes in neutralizing activities, binding was surprisingly well preserved especially against NTD, in general, and against the RBD in vaccinated, boosted and convalescent vaccinated individuals. Interestingly, drops in binding to the full spike ectodomain where somewhat higher than against the RBD and the NTD, despite fewer mutations outside these two domains. A possible explanation for this finding could be that more – and more conserved – epitopes are accessible in recombinant RBD and NTD while mostly the mutated epitopes are accessible on the full length ectodomain. It is also curious, that in some instances in low titer convalescent samples the reactivity to Omicron spike was better than to Beta spike. While changes in binding at this very low reactivity should not be over-interpreted, this phenomenon could be driven by slight differences in spike conformation which could lead to exposure of additional epitopes, either due to sequence differences or differences in the spike preparations. However, no such phenomenon was seen in the other groups which had higher titers to wild type spike. Based on conservation, we assume that most of the crossreactive anti-spike antibodies do in fact bind to the S2 subunit.[14]

It is conceivable that these binding antibodies, which often have non-neutralizing phenotypes in cell culture, contribute to protection from disease as has been seen for other viral infections. [9-11] In concert with T cell based immunity,[23] these non-neutralizing but binding antibodies – which frequently target S2 but also the RBD and NTD[14] – could be responsible for the protection from severe disease that has been observed against Omicron in individuals with pre-existing immunity. In addition, the presence of strong binding antibodies suggests that, while some antibodies may have lost affinity for the drifted epitopes, B cells may be recalled when encountering Omicron spike through infection or vaccination. This could lead to a strong anamnestic response, which could positively impact COVID-19 progression. It could also lead to the recruitment of these B cells into germinal centers for further affinity maturation resulting in potent, high affinity neutralizing antibodies against Omicron.[24] Importantly, our data add to the growing body of evidence suggesting that Omicron specific vaccines are urgently needed.

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Materials and methods

Human Serum Samples: Convalescent and post-vaccine sera were collected from participants in the longitudinal observational PARIS (Protection Associated with Rapid Immunity to SARS-CoV-2) study.[8,25] This cohort follows health care workers longitudinally since April 2020. The study was reviewed and approved by the Mount Sinai Hospital Institutional Review Board (IRB-20-03374). All participants signed written consent forms prior to sample and data collection. All participants provided permission for sample banking and sharing. Serum samples from the PARIS cohort are unique to this study and are not publicly available.

For the antigenic characterization of the antigenically diverse B.1.1.529 variant, we selected 85 serum samples from 54 participants. 20/54 participants were seronegative prior to vaccination

while 34/54 had COVID-19 prior to vaccination (see **Supplemental Tables 1 and 2** for demographics and vaccine information). All participants with pre-vaccination immunity were infected in 2020 when only ancestral SARS-CoV-2 strains circulated in the New York metropolitan area. Convalescent samples (N=15) were obtained within three months of SARS-CoV-2 infection (average: 58 days, range: 23-87 days) while the post vaccinations samples were collected, on average, 23 days (range: 14-39 days) after the second dose (N= 40, 20 Pfizer 2x and 20 Moderna 2x) or 19 days (range: 14-33 days) after the third booster (N= 30, 20 Pfizer 3x and 10 Moderna 3x) vaccine dose.

Cells: Vero-E6 cells expressing TMPRSS2 (BPS Biosciences, catalog #78081) were cultured in Dulbecco's modified Eagles medium (DMEM; Corning, #10-013-CV) containing 10% heat-inactivated fetal bovine serum (FBS; GeminiBio, #100-106) and 1% minimum essential medium (MEM) amino acids solution (Gibco, #11130051), supplemented with 100 U/ml penicillin and 100 µg/ml streptomycin (Gibco, #15140122), 100 µg/ml normocin (InvivoGen, #ant-nr), and 3 µg/ml puromycin (InvivoGen, #ant-pr). FreeStyle™ 293-F cells (Gibco, #R79007) were cultured in ESF-SFM medium (Expression Systems, cat. no. 98-001) supplemented with 100 U/ml penicillin and 100 µg/ml streptomycin (Gibco, #15140122). Expi293F™ Cells (Gibco, #A14527) were cultured in Expi293™ Expression Medium (Gibco, #A1435102) supplemented with 100 U/ml penicillin and 100 µg/ml streptomycin (Gibco, #15140122). Cell lines were authenticated by supplier. No other authentication at the lab level was performed. Cell lines are tested on a regular basis for mycoplasma and are mycoplasma free.

Selection and culture of replication competent SARS-CoV-2 isolates. The Mount Sinai Pathogen Surveillance program (IRB approved, HS#13-00981) actively screens nasopharyngeal swab specimens from patients seeking care at the Mount Sinai Health System for emerging viral variants. After completion of the diagnostics, de-identified biospecimen were sequenced either using an established complete virus genome sequencing approach[26] (e.g., Beta isolate USA/NY-MSHSPSP-PV27007/2021) or based on the spike S1 mutational profile determined by Spike-ID (Omicron, manuscript in preparation). The B.1.1.529 isolate USA/NY-MSHSPSP-PV44488/2021 represents one of the first cases diagnosed in New York State (female, age bracket: 30-40 years, mild COVID-19 symptoms, vaccinated and boosted) in late November 2021. The SARS-CoV-2 virus USA-WA1/2020 was used as wild-type reference (BEI Resources, NR-52281). **Supplemental Table 3** summarizes the amino acid substitutions, insertions and deletions in the spike region of each of the three viral isolates

Viruses were grown by adding 200ul of viral transport media from the nasopharyngeal swabs to Vero-E6-TMPRSS2 cells in culture media supplemented with 0.5 µg/ml amphotericin B (Gibco, # 15290-018). Cytopathic effects (CPE) appears within 4-6 days at which point the culture supernatants was clarified by centrifugation at 4,000 g for 5 minutes. Expanded viral stocks used were sequence-verified and titered by the 50% tissue culture infectious dose ($TCID_{50}$) method on Vero-E6-TMPRSS2 cells prior to use in micro neutralization assays.

Generation of recombinant variant RBD, NTD and spike proteins: The recombinant RBD proteins were produced using Expi293F cells (Life Technologies). The proteins were cloned into a mammalian expression vector, pCAGGS as described earlier[27,28] and purified after transient

transfections with each respective plasmid. Six-hundred million Expi293F cells were transfected using the ExpiFectamine 293 Transfection Kit and purified DNA. Supernatants were collected on day four post transfection, centrifuged at 4,000 g for 20 minutes and finally filtered using a 0.22 µm filter. Ni-nitrilotriacetic acid (Ni-NTA) agarose (Qiagen) was used to purify the proteins via gravity flow and proteins were eluted as previously described.[27,28] The buffer was exchanged using Amicon centrifugal units (EMD Millipore) and all recombinant proteins were finally re-suspended in phosphate buffered saline (PBS). Proteins were also run on a sodium dodecyl sulphate (SDS) polyacrylamide gels (5–20% gradient; Bio-Rad) to check for purity.[29,30] The NTD protein constructs (residues 1-306) were cloned into pVRC8400 expression vector between *Sal*I and *Not*I endonuclease restriction sites yielding an NTD with an human rhinovirus (HRV) 3C protease-cleavable C-terminal hexahistidine and a streptavidin-binding protein tags. The NTDs were transiently expressed in FreeStyle™ 293-F cells. Four days post-transfection, supernatants were harvested by centrifugation and further purified using immobilized metal affinity chromatography (IMAC) with cobalt-TALON® resin (Takara) followed by Superdex 200 Increase 10/300 GL size exclusion column (GE Healthcare). Spike proteins were expressed as described before.[8]

Enzyme linked immunosorbent assay (ELISA): Antibody titers in sera from convalescent individuals and vaccinees were measured by a research grade ELISA using recombinant versions of the RBD and NTD of wild type SARS-CoV-2 as well as the B.1.351 (Beta), and B.1.1.529 (Omicron) (see **Supplemental Table 4** for specific substitutions in each variant). All samples were analyzed in a blinded manner. Briefly, 96-well microtiter plates (Corning, #353227) were coated with 50 µl/well of recombinant protein (2 µg/ml) overnight at 4 °C. Plates were washed three times with phosphate-buffered saline (PBS; Gibco, #10010-031) supplemented with 0.1% Tween-20 (PBS-T; Fisher Scientific ref. 202666) using an automatic plate washer (BioTek 405TS microplate washer). For blocking, PBS-T containing 3% milk powder (American Bio, # AB1010901000) was used. After 1-hour incubation at room temperature (RT), blocking solution was removed and initial dilutions (1:100) of heat-inactivated sera (in PBS-T 1%-milk powder) were added to the plates, followed by 2-fold serial dilutions. After 2-hour incubation, plates were washed three times with PBS-T and 50 µl/well of the pre-diluted secondary antibody anti-human IgG (Fab-specific) horseradish peroxidase (HRP) antibody (produced in goat; Sigma-Aldrich, Cat# A0293, RRID: AB_257875) diluted 1:3,000 in PBS-T containing 1% milk powder were added. After 1-hour incubation at RT, plates were washed three times with PBS-T and SigmaFast o-phenylenediamine dihydrochloride (Sigmafast OPD; Sigma-Aldrich, Ref. P9187-50SET) was added (100 µl/well) for 10min, followed by addition of 50 µl/well of 3 M hydrochloric acid (Thermo Fisher, Ref. S25856) to stop the reaction. Optical density was measured at a wavelength of 490 nm using a plate reader (BioTek, SYNERGY H1 microplate reader). The area under the curve (AUC) values were calculated and plotted using Prism 9 software (GraphPad).

SARS-CoV-2 multi-cycle microneutralization assay: Sera from vaccinees were used to assess the neutralization of wild type (WA1), B.1.351 (Beta) and B.1.1.529 (Omicron) SARS-CoV-2 isolates (**Supplementary Table 3**). All procedures were performed in a biosafety level 3 (BSL-3) facility at the Icahn School of Medicine at Mount Sinai following standard safety guidelines. Vero-E6-

TMPRSS2 cells were seeded in 96-well high binding cell culture plates (Costar, #07620009) at a density of 20,000 cells/well in complete Dulbecco's modified Eagle medium (cDMEM) one day prior to the infection. Heat inactivated serum samples (56°C for 1 hour) were serially diluted (3-fold) in minimum essential media (MEM; Gibco, #11430-030) supplemented with 2 mM L-glutamine (Gibco, #25030081), 0.1% sodium bicarbonate (w/v, HyClone, #SH30033.01), 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES; Gibco, #15630080), 100 U/ml penicillin, 100 µg/ml streptomycin (Gibco, #15140122) and 0.2% bovine serum albumin (BSA, MP Biomedicals, Cat#. 810063) starting at 1:10. Remdesivir (Medkoo Bioscience inc., #329511) was included to monitor assay variation. Serially diluted sera were incubated with 10,000 $TCID_{50}$ of WT USA-WA1/2020 SARS-CoV-2, MSHSPSP-PV27007/2021 (B.1.351, Beta) or USA/NY-MSHSPSP-PV44488/2021 (B.1.1.529, Omicron) for one hour at RT, followed by the transfer of 120µl of the virus-sera mix to Vero-E6-TMPRSS2 plates. Infection proceeded for one hour at 37°C and inoculum was removed. 100 µl/well of the corresponding antibody dilutions plus 100µl/well of infection media supplemented with 2% fetal bovine serum (FBS; Gibco, #10082-147) were added to the cells. Plates were incubated for 48h at 37°C followed by fixation overnight at 4°C in 200 µl/well of a 10% formaldehyde solution. For staining of the nucleoprotein, formaldehyde solution was removed, and cells were washed with PBS (pH 7.4) (Gibco, #10010-031) and permeabilized by adding 150 µl/well of PBS, 0.1% Triton X-100 (Fisher Bioreagents, #BP151-100) for 15 min at RT. Permeabilization solution was removed, plates were washed with 200 µl/well of PBS (Gibco, #10010-031) twice and blocked with PBS, 3% BSA for 1 hour at RT. During this time the primary antibody was biotinylated according to manufacturer protocol (Thermo Scientific EZ-Link NHS-PEG4-Biotin). Blocking solution was removed and 100 µl/well of biotinylated mAb 1C7C7[31], a mouse anti-SARS nucleoprotein monoclonal antibody generated at the Center for Therapeutic Antibody Development at The Icahn School of Medicine at Mount Sinai ISMMS (Millipore Sigma, Cat# ZMS1075) at a concentration of 1µg/ml in PBS, 1% BSA was added for 1 hour at RT. Cells were washed with 200 µl/well of PBS twice and 100 µl/well of HRP-conjugated streptavidin (Thermo Fisher Scientific) diluted in PBS, 1% BSA were added at a 1:2,000 dilution for 1 hour at RT. Cells were washed twice with PBS, and 100 µl/well of o-phenylenediamine dihydrochloride (Sigmafast OPD; Sigma-Aldrich) were added for 10 min at RT, followed by addition of 50 µl/well of a 3 M HCl solution (Thermo Fisher Scientific). Optical density (OD) was measured (490 nm) using a microplate reader (Synergy H1; Biotek). Analysis was performed using Prism 7 software (GraphPad). After subtraction of background and calculation of the percentage of neutralization with respect to the "virus only" control, a nonlinear regression curve fit analysis was performed to calculate the 50% inhibitory dilution (ID_{50}), with top and bottom constraints set to 100% and 0% respectively. All samples were analyzed in a blinded manner.

Statistics: A one-way ANOVA with Tukey's multiple comparisons test was used to compare the neutralization and RBD binding antibody titers. The exception is the 2x BNT162b2 RBD ELISA group where a mixed effects model had to be used due to a missing data point. A student's t test was used for comparing wild type and B.1.1.529 NTD binding data. Statistical analyses were performed using Prism 9 software (GraphPad).

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Acknowledgements

We thank the study participants for their generosity and willingness to participate in longitudinal COVID-19 research studies. None of this work would be possible without their contributions. We also are grateful to the Rapid Response Laboratories and the Molecular Microbiology Laboratory of the Mount Sinai Health System for their assistance in the expeditious transfer of SARS-CoV-2 positive diagnostic specimen.

We would like to thank Dr. Randy A. Albrecht for oversight of the conventional BSL3 biocontainment facility, which makes our work with live SARS-CoV-2 possible. Furthermore, we would like to thank Drs. Janine Kimpel and Dorothee von Laer for their willingness to speedily share B.1.1.529 virus isolates (even though they could not included in this work). We are also grateful for Mount Sinai's leadership during the COVID-19 pandemic. We want to especially thank Drs. Peter Palese, Carlos Cordon-Cardo, Dennis Charney, David Reich and Kenneth Davis for their support and Daniel Caughey for expert administrative assistance.

This work is part of the PARIS/SPARTA studies funded by the NIAID Collaborative Influenza Vaccine Innovation Centers (CIVIC) contract 75N93019C00051. In addition, this work was also partially funded by the NIAID Centers of Excellence for Influenza Research and Response (CEIRR) contract and 75N93021C00014 by anonymous donors. The Mount Sinai Pathogen Surveillance Program is supported by institutional School of Medicine and Hospital funds. This work is part of the NIAID SARS-CoV-2 Assessment of Viral Evolution (SAVE) program.

Author contribution statement

Conceptualization: JMC, HA, EMS, GB, HvB, VS, FK. Methodology: JMC, HA, DCA, ASGR. Investigation: JMC, HA, JT, GS, AR, HK, LS, JC, DCA, DB, ASGR, ND, VV, PSP-PARIS Study Group Visualization: GB, FK. FundingAcquisition: VS, FK. Project administration: KS, DNS, EMS, GB, HvB, VS, FK. Supervision: DNS, EMS, GB, HvB, VS, FK. Writing – first draft: FK Writing – review and editing: JMC, HA, JT, GS, AR, HK, LS, JC, DCA, DB, ASGR, ND, VV, PSP-PARIS Study Group, KS, DNS, EMS, GB, HvB, VS, FK.

Competing interest statement

The Icahn School of Medicine at Mount Sinai has filed patent applications relating to SARS-CoV-2 serological assays (U.S. Provisional Application Numbers: 62/994,252, 63/018,457, 63/020,503 and 63/024,436) and NDV-based SARS-CoV-2 vaccines (U.S. Provisional Application Number: 63/251,020) which list Florian Krammer as co-inventor. Viviana Simon is also listed on the serological assay patent application as co-inventor. Patent applications were submitted by the Icahn School of Medicine at Mount Sinai. Mount Sinai has spun out a company, Kantaro, to market serological tests for SARS-CoV-2. Florian Krammer has consulted for Merck and Pfizer (before 2020), and is currently consulting for Pfizer, Third Rock Ventures, Seqirus and Avimex. The Krammer laboratory is also collaborating with Pfizer on animal models of SARS-CoV-2.

Additional information statement

Correspondence and requests for materials should be addressed to Viviana Simon and Florian Krammer.

Data and code availability statement

Datasets (raw data) underlying the figures have been provided as Source Data. No custom code was used in this study. Complete genome sequences for the viral isolates cultured from nasal swabs (B.1.351 and B.1.1.529) were deposited to GISAID. The mutations included in the recombinant proteins are listed in the manuscript and source data are provided.

Fig. 1

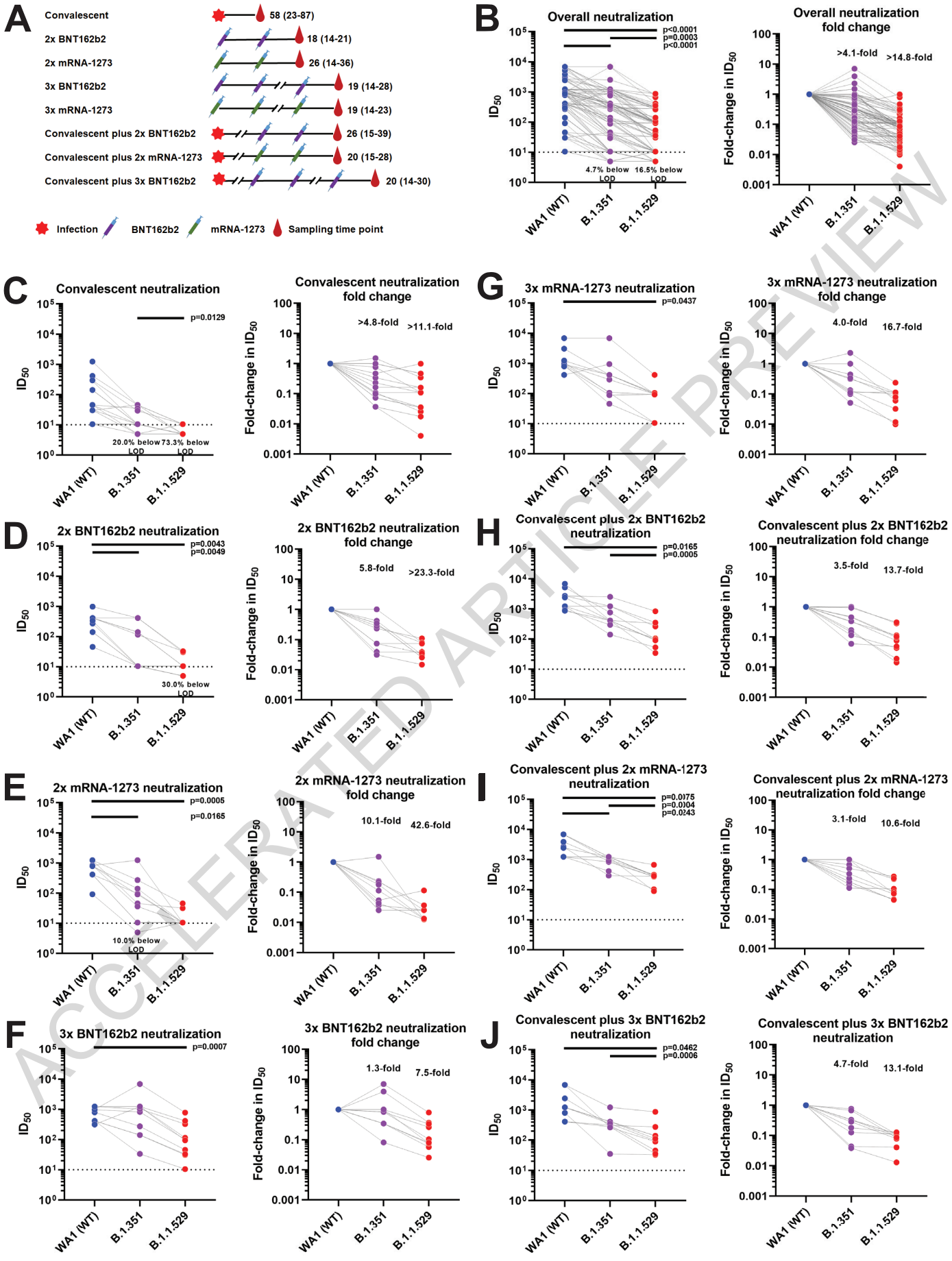


Figure Legends

Figure 1: Sera of convalescent and vaccinated individuals have strongly reduced neutralizing activity against Omicron as compared to wild type SARS-CoV-2. A Overview of different exposure groups from whom samples were obtained. Further details are provided in **Supplemental Table 1 and 2**. **B** shows absolute titers (left) and fold reduction (right) for the combined samples, **C** to **J** shows the different groups. A one-way ANOVA with Tukey's multiple comparisons test was used to compare the neutralization titers and significant p values (<0.05) are indicated in the figure. Data in panel B is based on 85 samples, data in panel C is based on 15 samples and data in all other panels is based on 10 samples each. The dotted line represents the limit of detection (10), negative samples were assigned half the limit of detection (5). Each dot represents a biological replicate and the assays were performed once. Fold change is defined as geometric mean fold change.

Fig. 2

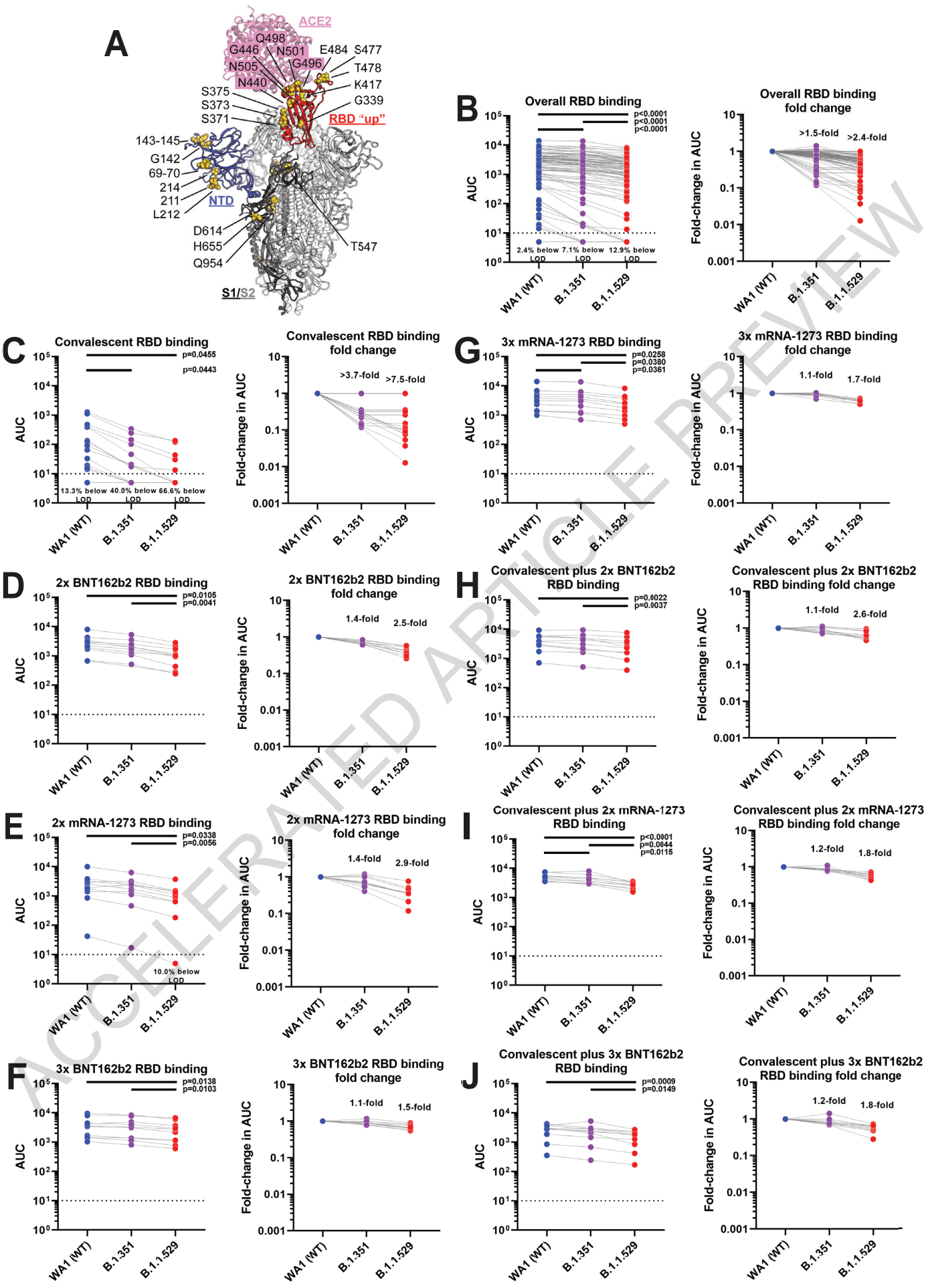


Figure 2: Sera of vaccinated individuals mostly maintain binding to the Omicron RBD. A shows a model of the B.1.1.529 spike protein in complex with the angiotensin converting enzyme 2 (ACE2) receptor with B.1.1.529 specific mutations indicated. The model is based on PDB 6M0J[32] and 7C2L[15] and the figure was made in PyMOL. **B** shows absolute titers (left) and fold reduction (right) for the combined samples, **C** to **J** shows the different groups. A one-way ANOVA with Tukey's multiple comparisons test was used to compare the neutralization titers and significant p values (<0.05) are indicated in the figure. The exception are panel **B** and **D** where a mixed effects model had to be used due to a missing data point. Data in panel B is based on 85 samples, data in panel C is based on 15 samples and data in all other panels is based on 10 samples each. The exception is D where one data point for Beta is missing. The dotted line represents the limit of detection (10), negative samples were assigned half the limit of detection (5). Each dot represents a biological replicate and the assays were performed twice. Fold change is defined as geometric mean fold change.

Fig. 3

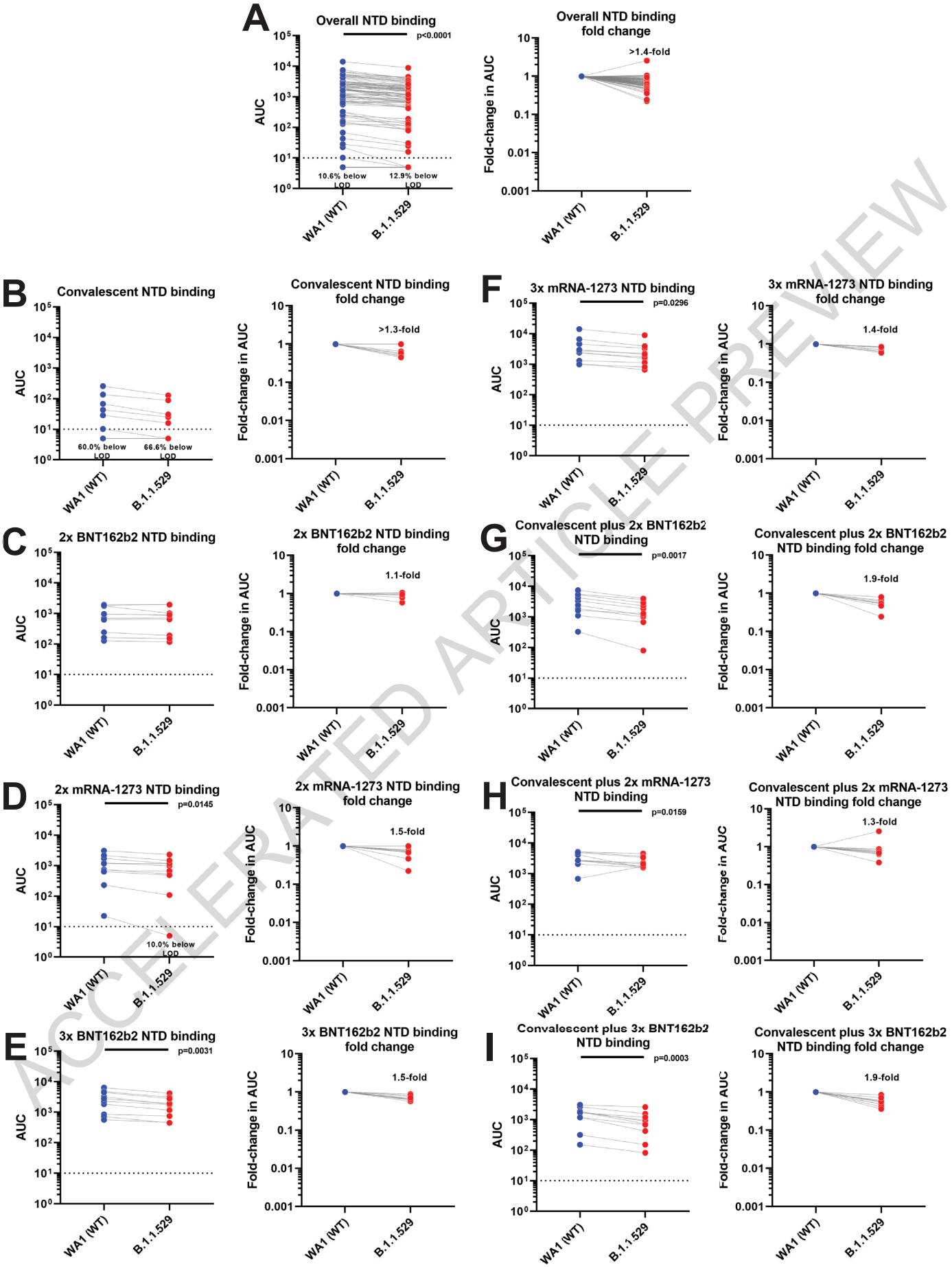


Figure 3: Serum of vaccinated individuals maintains binding to the Omicron NTD. A shows absolute titers (left) and fold reduction (right) for the combined samples, **B** to **I** shows the different groups. A student's t test was used for comparing wild type and B.1.1.529 NTD binding data and significant p values (<0.05) are indicated in the figure. Data in panel A is based on 85 samples, data in panel B is based on 15 samples and data in all other panels is based on 10 samples each. The dotted line represents the limit of detection (10), negative samples were assigned half the limit of detection (5). The assays were performed once. Each dot represents a biological replicate and the assays were performed once. Fold change is defined as geometric mean fold change.

Fig. 4

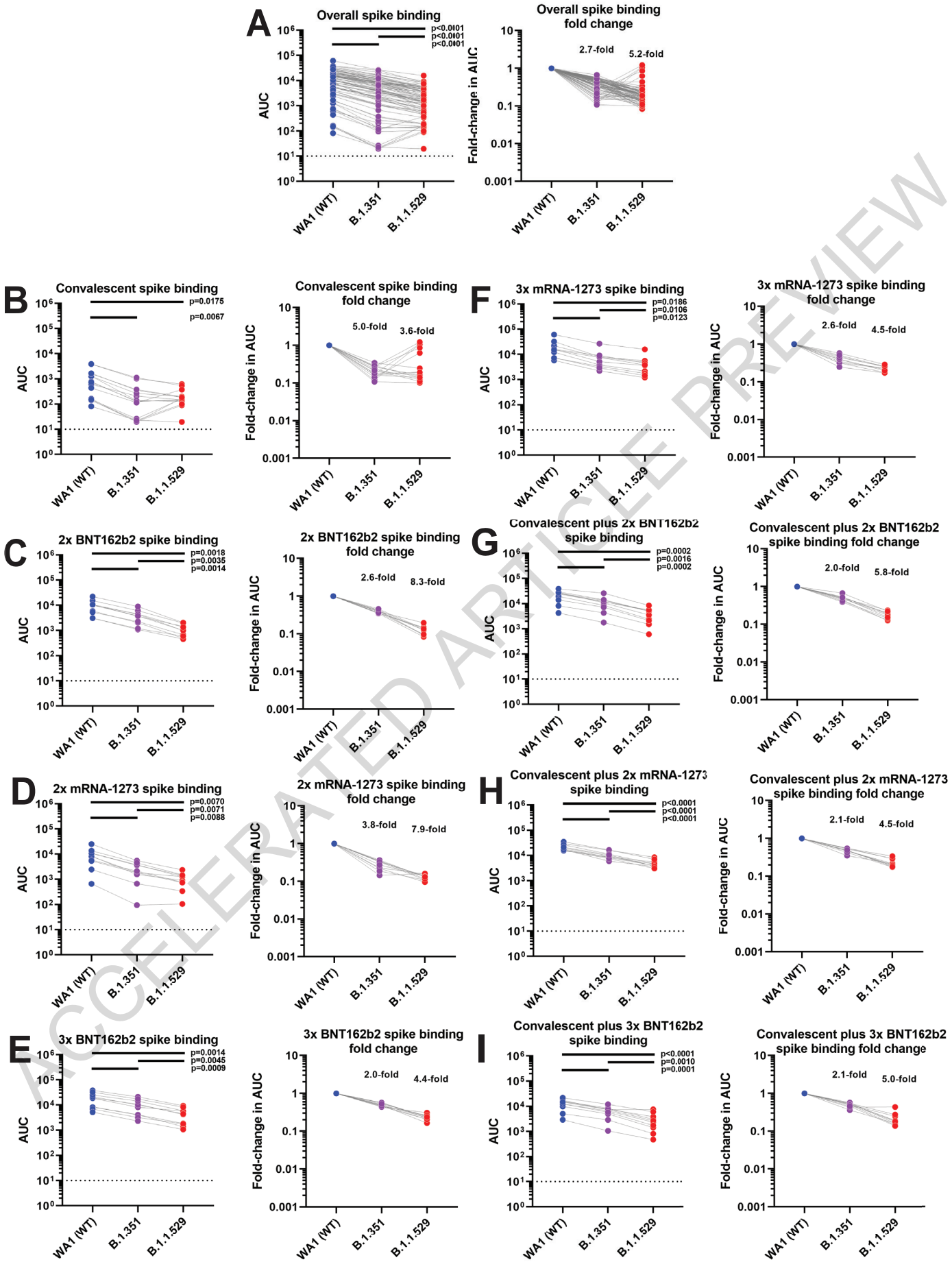
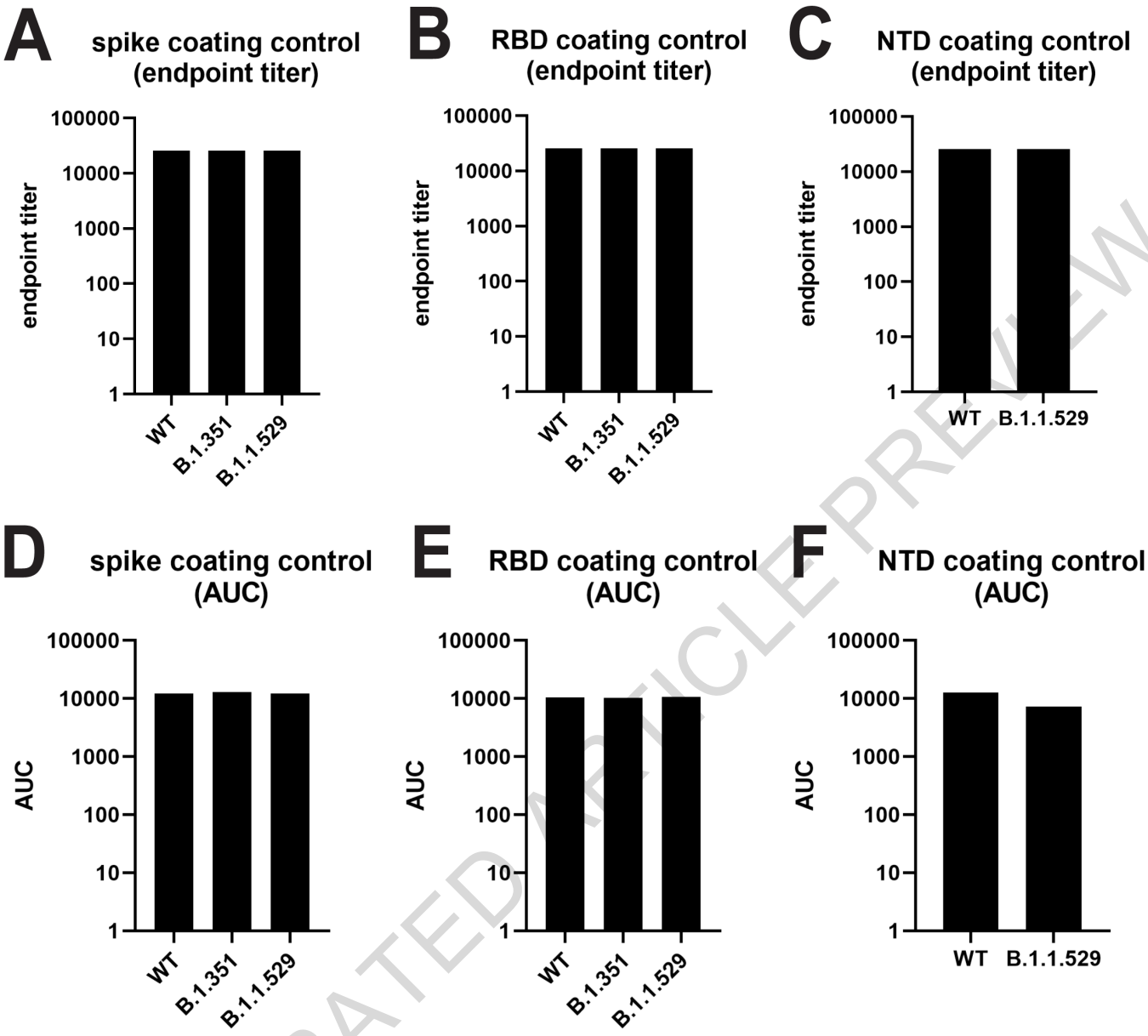


Figure 4: Sera of vaccinated individuals mostly maintain binding to the Omicron. A shows absolute titers (left) and fold reduction (right) for the combined samples, **B** to **I** shows the different groups. A one-way ANOVA with Tukey's multiple comparisons test was used to compare the neutralization titers and significant p values (<0.05) are indicated in the figure. Data in panel A is based on 85 samples, data in panel B is based on 15 samples and data in all other panels is based on 10 samples each. The dotted line represents the limit of detection (10), negative samples were assigned half the limit of detection (5). The assays were performed once. Each dot represents a biological replicate and the assays were performed once. Fold change is defined as geometric mean fold change.

Extended Data Fig. 1



Extended Data Figure 1: ELISA coating control data. All recombinant proteins used were his tagged which allows to control for coating efficiency by using an anti-his antibody. **A** shows endpoint titers of an anti-his mouse antibody to wild type, B.1.351 and B.1.1.529 spike. **B** shows the same for RBD. **C** shows binding of the anti-his antibody to NTDs from wild type and B.1.1529. **D**, **E** and **F** shows the same graphed as AUC. The assays were performed once. Data shown is based on three technical replicates.

535 **Extended Data Tables**

536

Extended Data Table 1

	COVID-19	Two vaccine doses				Three vaccine doses		
	Convalescent	**BNT16 2b2**		**mRNA-1273**		**BNT16 2b2**		**mRNA-1273**
		naïve	convalescent	naïve	convalescent	naïve	convalescent	naïve
Total N	15	10	10	10	10	10	10	10
Sex								
Female	10	8	7	8	5	8	7	8
Male	5	2	3	2	5	2	3	2
Days since infection								
Average (days)	58	no infection		no infection		no infection		no infection
Range (days)	23-87	no infection		no infection		no infection		no infection
Days since vaccine dose								
Average (days)	no vaccine	18	26	26	20	19	20	19
Range (days)	no vaccine	14-21	15-39	14-36	15-28	14-28	14-30	14-33

Extended Data Table 1: Overall description of samples used.

Extended Data Table 1 Legend: Overview of samples and subjects used for the analysis including time points post infection/time points post vaccination, sex distribution and number of samples.

Extended Data Table 2

Participant ID	Age Bracket	Sex	Ancestry	Time points included in this study	SARS-CoV-2 infection prior to vaccination	Vaccine Type
OMI-001	30-39	Female	Caucasian	Post-Vax, Post-Boost	No	Moderna
OMI-002	18-29	Female	Caucasian	Post-Vax, Post-Boost	No	Moderna
OMI-003	40-49	Male	Caucasian	Post-Vax, Post-Boost	No	Moderna
OMI-004	40-49	Female	Caucasian	Post-Vax, Post-Boost	No	Moderna
OMI-005	40-49	Female	Caucasian	Post-Vax, Post-Boost	No	Moderna
OMI-006	40-49	Male	Caucasian	Post-Vax, Post-Boost	No	Moderna
OMI-007	50-59	Female	Caucasian	Post-Vax, Post-Boost	No	Moderna
OMI-008	60-69	Female	Asian	Post-Vax, Post-Boost	No	Moderna
OMI-009	30-39	Female	Caucasian	Post-Vax, Post-Boost	No	Moderna
OMI-010	18-29	Female	Caucasian	Post-Vax, Post-Boost	No	Moderna
OMI-011	40-49	Female	Other	Post-Vax, Post-Boost	No	Pfizer
OMI-012	18-29	Female	Asian	Post-Vax, Post-Boost	No	Pfizer
OMI-013	60-69	Female	Caucasian	Post-Vax, Post-Boost	No	Pfizer
OMI-014	50-59	Female	Caucasian	Post-Vax, Post-Boost	No	Pfizer
OMI-015	18-29	Female	Caucasian	Post-Vax, Post-Boost	No	Pfizer
OMI-016	70-79	Male	Caucasian	Post-Vax, Post-Boost	No	Pfizer
OMI-017	30-39	Female	Asian Indian	Post-Vax, Post-Boost	No	Pfizer
OMI-018	30-39	Female	Caucasian	Post-Vax, Post-Boost	No	Pfizer
OMI-019	40-49	Male	Caucasian	Post-Vax, Post-Boost	No	Pfizer
OMI-020	40-49	Female	n.a.	Post-Vax, Post-Boost	No	Pfizer
OMI-021	40-49	Female	Asian	Post-Infection, Post-Vax	Yes	Pfizer
OMI-022	30-39	Female	Caucasian	Post-Infection, Post-Vax	Yes	Pfizer
OMI-023	30-39	Female	Asian	Post-Infection, Post-Vax	Yes	Pfizer
OMI-024	30-39	Male	Caucasian	Post-Infection, Post-Vax	Yes	Pfizer
OMI-028	18-29	Female	Caucasian	Post-Infection, Post-Vax	Yes	Moderna
OMI-029	30-39	Female	Caucasian	Post-Infection, Post-Vax	Yes	Pfizer
OMI-032	50-59	Male	Caucasian	Post-Infection, Post-Vax	Yes	Pfizer
OMI-027	40-49	Female	Caucasian	Post-Infection, Post-Vax	Yes	Pfizer
OMI-033	30-39	Female	Caucasian	Post-Infection, Post-Vax	Yes	Pfizer
OMI-034	40-49	Male	Latino	Post-Infection, Post-Vax	Yes	Pfizer
OMI-035	30-39	Female	Caucasian	Post-Infection, Post-Vax	Yes	Pfizer
OMI-025	50-59	Male	Latino	Post-Infection	Yes	no vax
OMI-026	30-39	Male	Caucasian	Post-Infection	Yes	no vax
OMI-030	40-49	Female	Other	Post-Infection	Yes	no vax
OMI-031	30-39	Female	Mexican	Post-Infection	Yes	no vax
OMI-036	50-59	Male	Asian	Post-Vax	Yes	Moderna
OMI-037	30-39	Male	Caucasian	Post-Vax	Yes	Moderna
OMI-038	18-29	Male	Caucasian	Post-Vax	Yes	Moderna
OMI-039	30-39	Female	Caucasian	Post-Vax	Yes	Moderna
OMI-040	60-69	Female	African American	Post-Vax	Yes	Moderna
OMI-041	40-49	Female	Caucasian	Post-Vax	Yes	Moderna
OMI-042	40-49	Female	Caucasian	Post-Vax	Yes	Moderna
OMI-043	60-69	Male	Caucasian	Post-Vax	Yes	Moderna
OMI-044	40-49	Male	n.a.	Post-Vax	Yes	Moderna
OMI-045	30-39	Male	Caucasian	Post-Boost	Yes	Pfizer
OMI-046	30-39	Female	Caucasian	Post-Boost	Yes	Pfizer
OMI-047	50-59	Female	n.a.	Post-Boost	Yes	Pfizer
OMI-048	60-69	Male	Caucasian	Post-Boost	Yes	Pfizer
OMI-049	60-69	Male	Caucasian	Post-Boost	Yes	Pfizer
OMI-050	30-39	Female	African American	Post-Boost	Yes	Pfizer
OMI-051	50-59	Female	Caucasian	Post-Boost	Yes	Pfizer
OMI-052	50-59	Female	Caucasian	Post-Boost	Yes	Pfizer
OMI-053	18-29	Female	Caucasian	Post-Boost	Yes	Pfizer
OMI-054	30-39	Female	Caucasian	Post-Boost	Yes	Pfizer

Extended Data Table 2: Detailed description of samples used.

Extended Data Table 2 Legend: Summary of the metadata of the 54 PARIS participants from whom a total of 85 serum samples were analyzed. For 31 PARIS participants, samples from two different time points were included (Post-Vax, Post-Boost or Post-Infection, Post-Vax).

The following abbreviations are used in the table:

Post-infection: serum collected 23 to 87 days after SARS-CoV-2 diagnosis

Post-Vax: serum collected 14-39 days after the second dose of mRNA vaccine

Post-Boost: serum collected 14-30 days after the booster vaccine dose

no vax: participant was not vaccinated at the time of sample collection

n.a: data not available

Extended Data Table 3

Isolate #	GISAID ID	Isolate ID	Lineage	RBD substitutions	NTD deletion/insertion	NTD substitutions	Spike substitutions
NR-52281	EPI_ISL_404895	USA-WA1/2020	A	None	None	None	None
PV27007	EPI_ISL_1708926	USA/NY-MSHSPSP-PV27007/2021	B.1.351	K417N, E484K, N501Y	ΔL241-A243	D80A, D215G	D80A, D215G, K417N, E484K, N501Y, D614G, A701V
PV44488	EPI_ISL_7908059	USA/NY-MSHSPSP-PV44488/2021	B.1.1.529 (BA.1)	G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H	ΔH69-V70, ΔG142-Y144, ΔN211, ins214EPE	A67V, T95I, Y145D, L212I	G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H, T547K, D614G, H655Y, N679K, P681H, N764K, D796Y, N856K, Q954H, N969K, L981F

Extended Data Table 3: Information on the viral isolates used in neutralization assays.

Extended Data Table 3 Legend: GISAID entry numbers, lineage information, isolate names and mutations found in the used B.1.351 and B.1.1.529 isolates as compared to wild type SARS-CoV-2.

Extended Data Table 4

Mutations in the sequence of recombinant RBD and NTD proteins generated for this study					
Protein	Lineage	RBD substitutions	NTD deletions	NTD insertions	NTD substitutions
RBD	Wuhan-1	None	NA	NA	NA
RBD	B.1.351	K417K, E484K, N501Y	NA	NA	NA
RBD	B.1.1.529	G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, G496S, Q498R, N501Y, Y505H	NA	NA	NA
NTD	Wuhan-1	NA	None	None	None
NTD	B.1.1.529	NA	69-70, 143-145, 211	214EPE	A67V, T95I, G142D, L212I

Extended Data Table 4: Overview of the mutations encoded in the RBD and NTD proteins used for the binding assays.

Extended Data Table 4 Legend: Mutations found in the used B.1.351 and B.1.1.529 RBD and NTD proteins as compared to wild type SARS-CoV-2.

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Corresponding author(s): Emilia Mia Sordillo, Goran Bajic, Harm van Bakel, Viviana Simon, Florian Krammer

Last updated by author(s): Dec 30, 2021

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☐	☒	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	N/A
Data analysis	Prism 9 (GraphPad)

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

Datasets (raw data) underlying the figures have been provided as Source Data. No custom code was used in this study. Complete genome sequences for the viral isolates cultured from nasal swabs (B.1.351 and B.1.1.529) were deposited to GISAID. The mutations included in the recombinant proteins are listed in the manuscript and source data are provided.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ 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	At least N=10 samples per group were included (males and females combined). The number of samples was determined based on an amount that allowed to perform robust statistical analyses, the number of donors and ability to process samples.
Data exclusions	No data were excluded. One data point from Figure 2A and D is missing due to a technical issue with the assay (as described in the figure legend).
Replication	RBD binding ELISAs were performed twice with the same results. All other assays were performed once.
Randomization	Samples were assigned to different groups based on the previous history of SARS-CoV-2 infection and vaccination.
Blinding	No blinding was performed.

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	IgG (Fab-specific) horseradish peroxidase (HRP) antibody (produced in goat; Sigma-Aldrich, Cat#A0293, RRID: AB_257875) mAb 1C7C7 Center for Therapeutic Antibody Development at The Icahn School of Medicine at Mount Sinai ISMMS (Millipore Sigma, Cat# ZMS1075) HRP-conjugated streptavidin (Thermo Fisher Scientific, Cat# N100)
Validation	All commercial antibodies were validated by their manufacturers and were titrated in the lab to determine optimal concentration for experimentation. In-house biotinylated 1C7C7 monoclonal antibody was validated in cells infected with WT SARS-CoV-2, B.1.351 and B.1.1.529 viral isolates. MAb concentrations were standardized based on the assay and starting concentration is described in methods section.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	Vero-E6-TMPRSS2 Cells (BPS Biosciences, catalog #78081) Expi293F™ Cells (Gibco, #A14527)
Authentication	Cell lines were authenticated by supplier. No other authentication at the lab level was performed.
Mycoplasma contamination	Mycoplasma free.

Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used in this study.

Human research participants

Policy information about studies involving human research participants

Population characteristics	85 serum samples from 54 participants were selected. 20/54 participants were seronegative prior to vaccination while 34/54 had COVID-19 prior to vaccination (see Supplemental Tables 1 and 2 for demographics and vaccine information). All participants with pre-vaccination immunity were infected in 2020 when only ancestral SARS-CoV-2 strains circulated in the New York metropolitan area. Convalescent samples (N=15) were obtained within three months of SARS-CoV-2 infection (average: 58 days, range: 23-87 days) while the post vaccinations samples were collected, on average, 23 days (range: 14-39 days) after the second dose (N= 40, 20 Pfizer 2x and 20 Moderna 2x) or 19 days (range: 14-33 days) after the third booster (N= 30, 20 Pfizer 3x and 10 Moderna 3x) vaccine dose.
Recruitment	Sera were collected from participants in the longitudinal observational PARIS (Protection Associated with Rapid Immunity to SARS-CoV-2) study. This cohort follows health care workers longitudinally since April 2020. All participants signed written consent forms prior to sample and data collection. All participants provided permission for sample banking and sharing.
Ethics oversight	The study was reviewed and approved by the Mount Sinai Hospital Institutional Review Board (IRB-20-03374).

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