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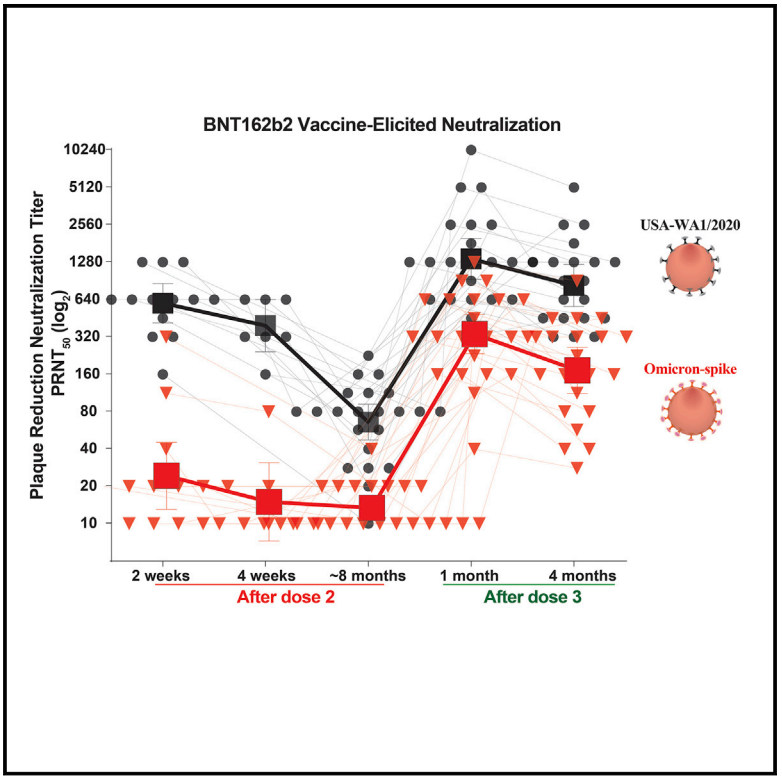
Brief Report

Cell Host & Microbe

Neutralization and durability of 2 or 3 doses of the BNT162b2 vaccine against Omicron SARS-CoV-2

Graphical abstract



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In brief

Through serially testing BNT162b2-vaccinated human sera against SARS-CoV-2 and its Omicron variant, Xia et al. found that 2 doses of BNT162b2 are insufficient to elicit robust neutralization against Omicron. Three doses increase the magnitude and breadth of neutralization against Omicron and remains robust for up to 4 months post dose 3.

Highlights

- Two doses of BNT162b2 are not sufficient to elicit robust neutralization against Omicron
- Three doses of BNT162b2 confer substantial neutralization against Omicron
- Neutralization against Omicron remains robust at 4 months after dose 3 of BNT162b2

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Brief Report

Neutralization and durability of 2 or 3 doses of the BNT162b2 vaccine against Omicron SARS-CoV-2

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SUMMARY

Two doses of the BNT162b2 mRNA vaccine are highly effective against SARS-CoV-2. Here, we tested the antibody neutralization against Omicron SARS-CoV-2 after 2 and 3 doses of BNT162b2. Serum from vaccinated individuals was serially tested for its ability to neutralize wild-type SARS-CoV-2 (USA-WA1/2020) and an engineered USA-WA1/2020 bearing the Omicron spike glycoprotein. At 2 or 4 weeks post dose 2, the neutralization geometric mean titers (GMTs) against the wild-type and Omicron-spike viruses were 511 and 20, respectively; at 1 month post dose 3, the neutralization GMTs increased to 1,342 and 336; and at 4 months post dose 3, the neutralization GMTs decreased to 820 and 171. The data support a 3-dose vaccination strategy and provide a glimpse into the durability of the neutralization response against Omicron.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) continues to evolve and generate new variants of concern (VOCs), including Alpha, Beta, Gamma, Delta, and Omicron. Variant mutations, particularly those in the spike glycoprotein, can alter viral transmission, disease severity, therapeutic antibody efficacy, and potentially vaccine effectiveness. The newly emerged Omicron variant (B.1.1.529 lineage) was first detected in South Africa; reported to the World Health Organization on November 24, 2021; and designated as a VOC 2 days later. It is rapidly spreading around the world and causing increased breakthrough infections in previously infected and vaccinated individuals (UK Health Security Agency, 2021b). Laboratory results are urgently needed to examine vaccine-elicited neutralization against the Omicron variant and the duration for which this activity persists.

BNT162b2, an mRNA vaccine that encodes the prefusion-stabilized full spike glycoprotein of SARS-CoV-2 isolate Wuhan-Hu-1, has received regulatory approval and/or authorization around the world, including approval for vaccination of people 16 years of age and older and authorization under emergency use provisions for immunization of individuals 5–15 years old by the US Food and Drug Administration. We formerly reported that recombinant SARS-CoV-2 bearing spike genes from previous VOCs, variants of interest, and variants under monitoring remained susceptible to BNT162b2 vaccine-elicited neutralization. While the Beta variant exhibited the neutralization reduction among all tested variants (Liu et al., 2021a; Liu et al., 2021b; Liu et al., 2021c), high efficacy against Beta was observed (Thomas et al., 2021).

To determine the susceptibility of the Omicron variant to BNT162b2-elicited neutralization, we engineered the complete Omicron sublineage BA.1 *spike* into the USA-WA1/2020 backbone (Figure S1A). The resulting recombinant Omicron-spike SARS-CoV-2 contained spike mutations A67V, H69-V70 deletion (Δ69–70), T95I, G142D, V143-Y145 deletion (Δ143–145), N211 deletion (Δ211), L212I, L214 insertion EPE (ins214EPE), 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, and L981F (*GISAID EPI_ISL_6640916*). The recombinant Omicron-spike SARS-CoV-2 and wild-type USA-WA1/2020 produced infectious titers of greater than 10^7 plaque-forming units (PFUs) per milliliter. The recombinant viruses for all experiments were sequenced to ensure no undesired mutations. Although Omicron-spike virus formed smaller plaques than the wild-type USA-WA1/2020 (Figure 1B), both viruses showed equivalent viral RNA genome/PFU ratios when analyzed on Vero E6 cells (Figure S1C), suggesting comparable specific infectivities of the viral stocks.

We determined 50% plaque reduction neutralization titers ($PRNT_{50}$) against recombinant USA-WA1/2020 and Omicron-spike SARS-CoV-2 using 4 serum panels from BNT162b2-vaccinated participants of the phase 1 portion of study C4591001. Figure 1A depicts the 4 serum panels used in this study. The first

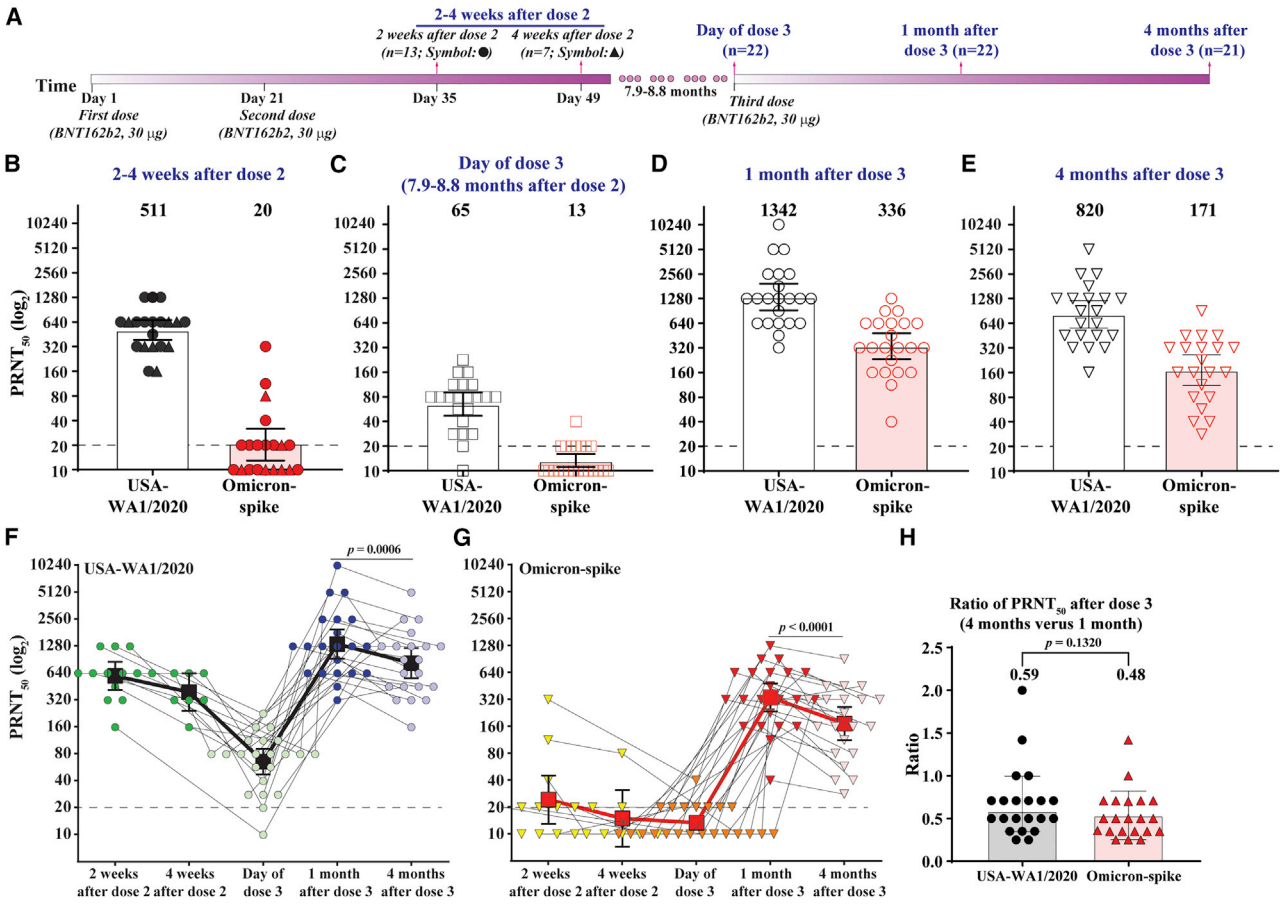

Figure 1. Serum neutralization of USA-WA1/2020 and Omicron-spike SARS-CoV-2 after 2 and 3 doses of BNT162b2

(A) BNT162b2-vaccinated serum collection. Four BNT162b2-vaccinated serum panels were used. The first panel had 20 samples obtained at 2 weeks (circles) or 4 weeks (triangles) after the second dose. The second panel (n = 22) was collected on the day of the third dose. The third (n = 22) and fourth (n = 21) panels were collected at 1 and 4 months after the third dose, respectively.

(B–E) $PRNT_{50}$ data for the 4 panels of BNT162b2-vaccinated sera collected at 2 or 4 weeks after the second dose (B), the day of the third dose (C), 1 month after the third dose (D), and 4 months after the third dose (E). Each data point represents the geometric mean $PRNT_{50}$ obtained with a serum specimen against the indicated virus, as detailed in Table S1. The neutralization titer was determined in duplicate assays, and the geometric mean was presented. The bar heights and the numbers above indicate geometric mean titers. The whiskers indicate 95% confidence intervals (CI). The dotted line indicates the limit of detection of $PRNT_{50}$. $PRNT_{50}$ of <20 was treated as 10 for plot purposes and statistical analysis. Statistical analysis was performed using the Wilcoxon matched-pairs signed-rank test. The statistical significance of the difference between geometric mean titers in the USA-WA1/2020 and Omicron-spike SARS-CoV-2 is $p < 0.0001$ for each panel of (B)–(E).

(F and G) $PRNT_{50}$ data from individual subjects against USA-WA1/2020 (F) and Omicron-spike SARS-CoV-2 (G). The $PRNT_{50}$ values from individual subjects are connected with lines. The GMTs for each time point are presented as squares. Error bars indicate the 95% CI of the GMTs. The dotted lines indicate the limit of detection of $PRNT_{50}$.

(H) Ratio $PRNT_{50}$ after dose 3 (month 4 $PRNT_{50}$ versus month 1 $PRNT_{50}$). Statistical analysis was performed using the Wilcoxon matched-pairs signed-rank test.

panel contained 20 sera, collected 2 or 4 weeks after the second dose of 30 μg of BNT162b2, which was administered 3 weeks after the first immunization. These post-dose-2 (PD2) sera neutralized USA-WA1/2020 and Omicron-spike SARS-CoV-2 with geometric mean titers (GMTs) of 511 and 20, respectively. All PD2 sera had neutralization titers of 160 or higher against the wild-type USA-WA1/2020, whereas only 11 of the 20 sera had neutralization titers of 20 (limit of detection) or higher against the Omicron-spike virus (Figure 1B). The second serum panel contained 22 specimens, collected on the day immediately before the third 30 μg BNT162b2 dose, which was administered 7.9 to 8.8 months after dose 2 (Falsey et al., 2021). The GMTs against USA-WA1/2020 decreased to 65, while the GMTs against Omicron remained low at 13, with only 8 of the 22 sera exhibiting neutralization titers of ≥20 (Figure 1C). The third and fourth serum panels were collected 1 and 4 months after the third dose, respectively (Figure 1A). The 1-month-post-dose-3 (1MPD3) sera neutralized USA-WA1/2020 and Omicron-spike SARS-CoV-2 with GMTs of 1,342 and 336, respectively (Figure 1D). The 4-month-post-dose-3 (4MPD3) sera neutralized USA-WA1/2020 and Omicron-spike SARS-CoV-2 with GMTs of 820 and 171, respectively; all of the 4MPD3 sera neutralized Omicron-spike virus at titers of 28 or higher (Figure 1E). Figures 1F and 1G summarize the $PRNT_{50}$ curves for individual subjects

against USA-WA1/2020 and Omicron, respectively. Collectively, the data support 3 conclusions. First, at 2 or 4 weeks PD2, the neutralization GMT against Omicron-spike SARS-CoV-2 was 25.6 times lower than that against USA-WA1/2020 and in a similar range of reduction noted in some preliminary and peer-reviewed reports (Cele et al., 2021). Differences in Omicron neutralization reduction compared to wild-type USA-WA1/2020 across studies are likely attributed to different serum samples, time of sampling, assay formats, and assay protocols but nonetheless support a substantial decrease in neutralization activity against Omicron after 2 doses of BNT162b2. Second, by 1MPD3, neutralization GMTs against wild-type USA-WA1/2020 and Omicron-spike virus increased by 2.6 and 16.8 times, respectively, when compared to the corresponding GMTs by 2 to 4 weeks PD2. This result agrees with our recent findings that a third dose of BNT162b2 increased the magnitude and breadth of neutralization against both Delta and Beta variants (Falsey et al., 2021). The same serum panel was used in our Delta, Beta, and Omicron neutralization studies. Third, from 1 to 4 months PD3, neutralization GMTs against USA-WA1/2020 and Omicron decreased by 1.6 and 2 times, respectively. The 4 month versus 1 month $PRNT_{50}$ ratios were not statistically significant between the USA-WA1/2020 and Omicron groups (Figure 1H), suggesting similar antibody decay kinetics for both variants. A more accurate estimation of neutralization decay will require a larger sample size and data on longer time points beyond 4 months after dose 3.

Our data demonstrate that 3 doses of BNT162b2 elicited substantial neutralization activity against Omicron-spike SARS-CoV-2, while 2 doses showed significantly reduced neutralization titers. The neutralization GMT against Omicron-spike virus after dose 3 (336) was close to the neutralization GMT against wild-type USA-WA1/2020 after dose 2 (511), which was previously associated with high efficacy in the pivotal efficacy study (C4591001) (Polack et al., 2020). These data suggest that a 3-dose vaccination strategy could minimize the health impact of Omicron. This is supported by initial reports demonstrating that while vaccine effectiveness against symptomatic infection due to Omicron may wane following a third dose of BNT162b2, the effectiveness against hospitalization remains high (UK Health Security Agency, 2021a).

We use a reverse genetic system to generate USA-WA1/2020 bearing variant spike glycoprotein. This approach allows us to rapidly examine the effect of variant spike on vaccine-elicited neutralization using synthetic DNA or site-directed mutagenesis shortly after variant sequences become available. Compared with waiting for clinical isolates, our approach provides faster response and guidance for potential vaccine adjustments. Because BNT162b2 encodes the full-length spike glycoprotein as the sole vaccine antigen, our recombinant viruses have a common USA/WA1-2020 backbone and differ from each other only in the spike sequence. This approach focuses the analysis on the impact of a variant spike on neutralization for decision making on potential vaccine updates. The method eliminates assay variation caused by viral mutations outside the spike gene, which are relevant for many viral phenotypes, but not for selection of vaccine spike sequences. The approach is analogous to the well-established practice of comparing the antigenicity of influenza A viral variants in the hemagglutinin and neuraminidase on a common A/Puerto Rico 8/1934 backbone (Dormitzer et al., 2013).

The current study has 3 limitations. First, the durability of the neutralization against Omicron variant beyond 4MPD3 remains to be determined. Second, the small serum specimen size makes it challenging for robust statistical analysis. Third, other immune effectors, such as T cells and non-neutralizing antibodies that mediate antibody-dependent cytotoxicity, have not been examined. CD8+ T cell responses likely play an important role in protection against severe COVID-19; despite the significant number of mutations in the Omicron spike, the majority of T cell epitopes of spike glycoprotein are preserved (Redd et al., 2021). Taken together, the current *in vitro* study suggests that 2 doses of BNT162b2 may remain effective against severe disease but that a third dose of BNT162b2 is likely required to maintain effectiveness against COVID-19 caused by Omicron. Additional real world effectiveness data and laboratory investigations will further inform the duration of protection and potential need for an additional dose at a later time and whether an Omicron modified vaccine is required.

STAR★METHODS

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.chom.2022.02.015.

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AUTHOR CONTRIBUTIONS

A.M., K.U.J., X.X., K.A.S., and P.-Y.S. conceived the study. H.X., J.Z., C.K., H.C., Q.Y., M.C., D.C., X.X., and K.A.S. performed the experiments. H.X., J.Z., M.C., D.C., A.M., K.U.J., X.X., K.A.S., and P.-Y.S. analyzed the results.

H.X., J.Z., X.X., K.A.S., and P.-Y.S. wrote the manuscript. M.C., D.C., K.U.J., X.X., K.A.S., and P.-Y.S. supervised the project.

DECLARATION OF INTERESTS

X.X. and P.-Y.S. have filed a patent on the reverse genetic system of SARS-CoV-2. H.X., J.Z., C.K., X.X., and P.-Y.S. received compensation from Pfizer to perform the project. H.C., Q.Y., M.C., D.C., K.U.J., and K.A.S. are employees of Pfizer and may hold stock options. A.M. is an employee of BioNTech and may hold stock options.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Bacterial and virus strains		
E. coli strain Top10	ThermoFisher Scientific	Cat# C404006
TransforMax™ EPI300™ Chemically Competent *E. coli*	Lucigen Corporation	Cat# C300C105
icSARS-CoV-2 virus	Xie et al., 2020	N/A
Biological samples		
Human serum	Falsey et al., 2021; Liu et al., 2021b	N/A
Chemicals, peptides, and recombinant proteins		
TRIzol™ LS Reagent	ThermoFisher Scientific	Cat# 10296028
Critical commercial assays		
T7 mMessage mMachine kit	Thermo Fisher Scientific	Cat# AM1344
Ingenio® Electroporation solution	Mirus Bio LLC	Cat# MIR 50117
Direct-zol RNA Miniprep Plus kit	Zymo Research	Cat# R2072
iTaq SYBR Green One-Step Kit	Bio Rad	Cat# 1725151
NEBuilder HiFi DNA Assembly kit	New England Biolabs	Cat#E5520S
Experimental models: Cell lines		
Vero E6 cells	ATCC	Cat# CRL-1586, RRID:CVCL_0574
Experimental models: Organisms/Ssrains		
Omicron variant sublineage BA.1	GISAID EPI_ISL_6640916	N/A
Oligonucleotides		
Primer CoV19-N2-F (TTACAAACATTGGCCGCAAA)	Plante et al., 2021	N/A
Primer CoV19-N2-R (GCGCGACATTCCGAAGAA)	Plante et al., 2021	N/A
Primers for Omicron-spike mutagenesis	Zou et al., 2022	NA
Recombinant DNA		
pUC57-CoV2-F1	Xie et al., 2020	N/A
pCC1-CoV2-F2	Xie et al., 2020	N/A
pCC1-CoV2-F3	Xie et al., 2020	N/A
pUC57-CoV2-F4	Xie et al., 2020	N/A
pCC1-F567-Omicron spike	This paper	N/A
Software and algorithms		
Prism 9.0 software	GraphPad	N/A
Illustrator CC	Adobe	N/A

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Pei-Yong Shi (peshi@utmb.edu).

Materials availability

The Omicron-spikeSARS-CoV-2 has been deposited to the World Reference Center for Emerging Viruses and Arboviruses (https://www.utmb.edu/wrceva) at UTMB for distribution. All reagents generated in this study are generated in this study are available from the lead contact with a completed Materials Transfer Agreement.

Date and code availablity

This study did not generate any code. Any additional information required to reanalyze the data reported in this work paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Cells

Vero E6 (ATCC® CRL-1586) were purchased from the American Type Culture Collection (ATCC, Bethesda, MD), and maintained in a high-glucose Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS; HyClone Laboratories, South Logan, UT) and 1% penicillin/streptomycin at 37°C with 5% CO_2. All culture medium and antibiotics were purchased from ThermoFisher Scientific (Waltham, MA). The cell line was tested negative for mycoplasma.

Recombinant SARS-CoV-2

Viruses were recovered from infectious cDNA clone of SARS-CoV-2 and propagated on the Vero cells in the DMEM medium (GIBCO) supplemented with 2% fetal bovine serum (FBS) and 1%penecillin/streptomycin (GIBCO).

Human serum

Serum samples were collected from BNT162b2 vaccinees participating in the phase 1 portion of the ongoing phase 1/2/3 clinical trial (https://clinicaltrials.gov/; identifier: NCT04368728). The protocol and informed consent were approved by institutional review boards for each of the investigational centers participating in the study. The study was conducted in compliance with all International Council for Harmonisation Good Clinical Practice guidelines and the ethical principles of the Declaration of Helsinki. Four BNT162b2-vaccinated serum panels were used in the study. Figure 1A summarizes the collection time of serum panels. The first panel (n = 20) was collected at 2 weeks (circles) or 4 weeks (triangles) after the second dose of BNT162b2. The second panel (n = 22) was collected on the day of the third dose of BNT162b2, which was administered at 7.9 to 8.8 months after the second dose. The third (n = 22) and fourth (n = 21) panels were collected at 1 and 4 months after the third dose, respectively. Table S1 summarizes the patient information (e.g., age and gender) and sample collection time points. The information for the first and third serum panels were reported previously (Falsey et al., 2021; Liu et al., 2021b).

METHOD DETAILS

Construction and characterization of recombinant Omicron-spike SARS-CoV-2

Recombinant Omicron-spike SARS-CoV-2 was constructed by engineering the complete spike gene from Omicron variant sublineage BA.1 (GISAID EPI_ISL_6640916) into an infectious cDNA clone of clinical isolate USA-WA1/2020 (Figure S1) (Xie et al., 2020). All spike mutations were introduced into the infectious cDNA clone of USA-WA1/2020 using PCR-based mutagenesis as previously described (Xie et al., 2021a; Zou et al., 2022). The full-length cDNA of viral genome containing the complete Omicron spike mutations was assembled via *in vitro* ligation. The resulting full-length cDNA was used as a template for *in vitro* transcription of genome-length viral RNA. The *in vitro* transcribed genome-length viral RNA was electroporated into Vero E6 cells. On day 3 post electroporation, the original viral stock (P0) was harvested from the electroporated cells. The P0 virus was propagated for another round on Vero E6 cells to produce the P1 stock for neutralization testing. The infectious titer of the P1 virus was quantified by plaque assay on Vero E6 cells (Figure S2A). The complete spike gene of the P1 virus was sequenced to ensure no undesired mutations. The protocols for the mutagenesis of SARS-CoV-2 and virus production were reported previously (Xie et al., 2021b). For determining the specific infectivity of each virus, the P1 virus stock was quantified for its plaque-forming unit (PFU) and genomic RNA content by plaque assay on Vero E6 cells and RT-qPCR, respectively. The methods for plaque assay and RT-qPCR were reported previously (Plante et al., 2021). The specific infectivity of each virus was indicated by the genomic RNA-to-PFU ratios (genome/PFU) in Figure S2B.

Plaque reduction neutralization testing

The 50% plaque-reduction neutralization titer ($PRNT_{50}$) was measured for each serum as previously reported (Muruato et al., 2020; Walsh et al., 2020). Individual sera were 2-fold serially diluted in culture medium with a starting dilution of 1:20. The diluted sera were incubated with 100 PFU of Omicron-spike SARS-CoV-2 or USA-WA1/2020. After 1 h incubation at 37°C, the serum-virus mixtures were inoculated onto 6-well plates with a monolayer of Vero E6 cells pre-seeded on the previous day. The $PRNT_{50}$ value was defined as the minimal serum dilution that suppressed > 50% of viral plaques. The neutralization titer was determined in duplicate assays, and the geometric mean was taken. Table S1 summarizes the $PRNT_{50}$ results for all serum samples.

RNA extraction and RT-qPCR

Culture supernatants were mixed with a five-fold volume of TRIzol LS Reagent (Thermo Fisher Scientific). Viral RNAs were extracted by using the Direct-zol RNA Miniprep Plus kit (ZYMO RESEARCH) according to the manufacturer's instructions. The extracted RNAs were eluted in 50 μL nuclease-free water. RT-qPCR were performed to quantify the viral RNA copies. Briefly, 2 μl of RNA samples were used in a 20-μl reaction system with iTaq SYBR Green One-Step Kit (Bio-Rad). A standard RT-qPCR were performed on the Applied Biosystems QuantStudio 7 system (ThermoFisher Scientific) following the manufacturers' instructions. An *in vitro* transcribed

RNA (3,839bp, genomic nucleotide positions 26,044 to 29,883 of SARS-CoV-2 genome) was used as a standard to determine the RNA copy numbers.

QUANTIFICATION AND STATISTICAL ANALYSIS

Statistical analysis

Data were plotted as scatted dots. The geometric mean with 95% confidence intervals were presented. Wilcoxon matched-pairs signed-rank test was used to compare the statistical differences in Figure 1.