Neutralization against Omicron SARS-CoV-2 from previous non-Omicron infection

Jing Zou[1,*], Hongjie Xia[1,*], Xuping Xie[1,*], Chaitanya Kurhade[1], Rafael R. G. Machado[2], Scott C. Weaver[2,3], Ping Ren[4,#], Pei-Yong Shi[1,3,5,6,7,8,#]

[1]Department of Biochemistry and Molecular Biology, University of Texas Medical Branch, Galveston TX, U.S.A.

[2]Departments of Microbiology and Immunology, University of Texas Medical Branch, Galveston TX, U.S.A.

[3]Institute for Human Infection and Immunity, University of Texas Medical Branch, Galveston, TX, U.S.A.

[4]Department of Pathology, University of Texas Medical Branch, Galveston TX, U.S.A.

[5]Sealy Institute for Drug Discovery, University of Texas Medical Branch, Galveston, TX, U.S.A.

[6]Institute for Translational Sciences, University of Texas Medical Branch, Galveston, TX, U.S.A.

[7]Sealy Institute for Vaccine Sciences, University of Texas Medical Branch, Galveston, TX, U.S.A.

[8]Sealy Center for Structural Biology & Molecular Biophysics, University of Texas Medical Branch, Galveston, TX, U.S.A.

*J.Z., H.X., and X.X. contributed equally to this study.

[#]Correspondence: P.R. (piren@utmb.edu) or P.-Y.S. (peshi@UTMB.edu)

Abstract

The explosive spread of the Omicron SARS-CoV-2 variant underscores the importance of analyzing the cross-protection from previous non-Omicron infection. We developed a high-throughput neutralization assay for Omicron SARS-CoV-2 by engineering the Omicron spike gene into an mNeonGreen USA-WA1/2020 SARS-CoV-2 (isolated in January 2020). Using this assay, we determined the neutralization titers of patient sera collected at 1- or 6-months after infection with non-Omicron SARS-CoV-2. From 1- to 6-month post-infection, the neutralization titers against USA-WA1/2020 decreased from 601 to 142 (a 4.2-fold reduction), while the neutralization titers against Omicron-spike SARS-CoV-2 remained low at 38 and 32, respectively. Thus, at 1- and 6-months after non-Omicron SARS-CoV-2 infection, the neutralization titers against Omicron were 15.8- and 4.4-fold lower than those against USA-WA1/2020, respectively.

The low cross-neutralization against Omicron from previous non-Omicron infection supports vaccination of formerly infected individuals to mitigate the health impact of the ongoing Omicron surge.

Main

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) continues to evolve, leading to the emergence of variants of concern (VoC), variants of interest, and variants of monitoring. These variants can increase viral transmission, immune evasion, and/or disease severity.[1-3] The recently emerged Omicron variant (B.1.1.529) was first identified in South Africa on November 2, 2021, and was designated as a new VoC on November 26, along with the four previous VoCs: Alpha, Beta, Gamma, and Delta.[4] Since its emergence, Omicron has rapidly spread to over 89 countries, with case doubling in as little as 1.5 to 3 days, leading to global surges of COVID-19 cases.[5] Compared to prior variants, the Omicron spike glycoprotein has accumulated more spike mutations, with over 34 mutations, many of which are known to evade antibody neutralization (*e.g.*, K417N, N440K, S477N, E484A and Q493R) or to enhance spike/hACE2 receptor binding (*e.g.*, Q498R, N501Y, and D614G).[1,3,6,7] The high number of spike mutations is associated with decreased potency of antibody therapy and increased breakthrough Omicron infections in vaccinated and previously infected individuals[5]. Laboratory studies are urgently needed to examine the susceptibility of Omicron SARS-CoV-2 to vaccine- and infection-elicited neutralization. This study aimed to examine the cross-neutralization of Omicron by antibodies derived from previous non-Omicron infection.

To measure neutralization of the Omicron variant, we developed a high-throughput assay. Using a previously established mNeonGreen (mNG) reporter USA-WA1/2020 SARS-CoV-2,[8] we swapped the original spike gene with an Omicron spike (BA.1 lineage; GISAID

EPI_ISL_6640916), resulting in recombinant mNG Omicron-spike SARS-CoV-2 (**Extended Data Fig. 1**). The mNG gene was placed at the open-reading-frame-7 (ORF7) of the viral genome.[9] The engineered Omicron spike contained 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 (**Extended Data Fig. 1**). The mNG Omicron-spike virus was sequenced to ensure no undesired mutations. After infecting Vero E6 cells, parental mNG USA-WA1/2020 developed larger fluorescent foci than Omicron-spike SARS-CoV-2 (**Extended Data Fig. 2**); however, comparable infectious titers of $>10^6$ focus-forming units per milliliter (FFU/ml) were obtained for both viruses. The mNG viruses were used to develop a fluorescent focus reduction neutralization test (FFRNT) as depicted in **Extended Data Fig. 3**.

We examined the cross-neutralization of non-Omicron SARS-CoV-2-infected patient sera against Omicron virus. Two panels of COVID-19 patient sera, one collected at 1-month post-infection (n=64) and another collected at 6-month post-infection (N=36), were measured for their 50% fluorescent focus reduction neutralization titers ($FFRNT_{50,}$ defined as the maximal dilution that neutralized 50% of foci) against both USA-WA1/2020 and Omicron-spike SARS-CoV-2. **Extended Data Tables 1** and **2** summarize the patient information (*e.g.*, age, gender, race, date of positive viral test, symptom, and hospitalization) for the 1- and 6-month post-infection serum panels**.** All patients were infected before February 2021, prior to the emergence of the Omicron variant. The 1-month post-infection sera neutralized USA-WA1/2020 and Omicron-spike SARS-CoV-2 with geometric mean titers (GMTs) of 601 and 38, respectively (**Fig. 1a** and **Extended Data Fig. 4a**). Only one serum had a neutralization titer of <20 against USA-WA1/2020, whereas 23 of 64 sera had neutralization titers of <20 against Omicron-spike SARS-CoV-2 (**Fig. 1a**). Sera with high neutralization titers against USA-WA1/2020 were from

symptomatic patients, most were hospitalized (**Extended Data Table 1**), confirming that neutralizing antibody levels are associated with COVID-19 disease severity.[10] Notably, many of the sera with the highest neutralization titers of >3,450 against USA-WA1/2020 were from patients who had received convalescent plasma treatment (**Extended Data Table 1**).

The 6-month post-infection sera neutralized USA-WA1/2020 and Omicron-spike SARS-CoV-2 with GMTs of 142 and 32, respectively (**Fig. 1b and Extended Data Fig. 4b**). Consistent with the 1-month post-infection results, symptomatic hospitalized patients tended to have higher neutralization titers against USA-WA1/2020 than asymptomatic individuals (**Extended Data Table 2**)**.** Thus, from 1- to 6-months post-infection, the mean neutralization titers against USA-WA1/2020 waned from 601 to 142 (a 4.2-fold decrease), while the neutralization titers against Omicron-spike virus remained low and nearly unchanged at 38 and 32, respectively. Consistent with our results, the waning neutralization overtime against non-Omicron SARS-CoV-2 was previously reported in naturally infected or vaccinated individuals.[11-13] Our data showed that 1- and 6-months after non-Omicron SARS-CoV-2 infections, the neutralization titers against Omicron were 15.8- and 4.4-fold lower than those against USA-WA1/2020, respectively. A similar range of neutralization reduction against the Omicron virus was reported for two-dose mRNA-vaccinated sera.[14-16] Collectively, these results demonstrate low cross-neutralization against the Omicron variant from previous non-Omicron viral infection or two-dose mRNA vaccination. The low cross-neutralization against the Omicron variant strongly suggests that individuals previously infected with SARS-CoV-2 should be vaccinated to mitigate Omicron-mediated infection, disease, and potential death.

Among all tested sera, only 6 pairs of 1- and 6-month samples were collected from same individuals (**Extended Data Tables 1** and **2**). Their neutralization patterns (**Extended Data Fig. 5**) were similar to those observed with the means from complete 1- and 6-month serum panels.

Our study has several limitations. First, we have not defined the contributions of individual spike mutations to Omicron neutralization evasion. The constellation of Omicron spike mutations may result from selection for either viral transmission, immune escape, or both. The emergence of the Omicron variant in South Africa, where herd immunity is believed to be high, is consistent with evolutionary pressure for immune escape as suggested by our data and others.[17] Second, the genotypes of viruses that infected the patients whose sera were analyzed in this study were not defined, although the timing suggests the Alpha variant. Third, we have not analyzed other immune modulators. $CD8^+$ T cells and non-neutralizing antibodies that can mediate antibody-dependent cytotoxicity are known to protect patients from severe disease. The Omicron spike mutations may not dramatically affect T cell epitopes.[18]

The rapid evolution of SARS-CoV-2 underscores the importance of surveillance for new variants and their impact on viral transmission, disease severity, and immune evasion. Surveillance, laboratory investigation, and real-world vaccine effectiveness are essential to guide if and when an Omicron-specific vaccine or booster is needed. Currently, vaccination with booster shots,[19,20] together with masking and social distance, remain to be the most effective means to mitigate the health impact of Omicron surge. Finally, the high-throughput fluorescent neutralization assay reported in this study can expedite therapeutic antibody screening, neutralization testing, and modified vaccine development.

Methods

Construction of recombinant viruses. The recombinant mNeoGreen (mNG) Omicron-spike SARS-CoV-2 was constructed on the genetic background of an infectious cDNA clone derived from clinical strain WA1 (2019-nCoV/USA_WA1/2020) containing an *mNG* reporter gene.[9] The Omicron spike mutations, including A67V, Δ69-70, T95I, G142D, Δ143-145, Δ211,

L212I, 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, were engineered using a PCR-based mutagenesis protocol as reported previously.[1] The full-length genomic cDNAs were *in vitro* ligated and used as templates to transcribe full-length viral RNA. Mutant viruses were recovered on day 3 after Vero E6 cells were electroporated with the *in vitro* RNA transcripts. The harvested virus stocks were quantified for their infectious titers (fluorescent focus units) by titrating the viruseson Vero E6 cells in a 96-well plate after 16 h of infection. The genome sequences of the virus stocks were confirmed to have no undesired mutations by Sanger sequencing. The detailed protocol of genome sequencing was recently reported.[21]

Serum specimens. The research protocol regarding the use of human serum specimens was reviewed and approved by the University of Texas Medical Branch (UTMB) Institutional Review Board (IRB#: 20-0070). The de-identified convalescent sera from COVID-19 patients (confirmed by the molecular tests with FDA's Emergency Use Authorization) were heat-inactivated at 56°C for 30 min before testing.

Fluorescent focus reduction neutralization test. Neutralization titers of human sera were measured by a fluorescent focus reduction neutralization test (FFRNT) using the mNG reporter SARS-CoV-2. Briefly, Vero E6 cells (2.5×10^4) were seeded in each well of black μCLEAR flat-bottom 96-well plate (Greiner Bio-one™). The cells were incubated overnight at 37°C with 5% CO_2. On the following day, each serum was 2-fold serially diluted in the culture medium with the first dilution of 1:20. The diluted serum was incubated with 100-150 fluorescent focus units (FFU) of mNG SARS-CoV-2 at 37°C for 1 h (final dilution range of 1:20 to 1:20,480), after which the serum-virus mixtures were inoculated onto the pre-seeded Vero E6 cell monolayer in 96-well plates. After 1 h infection, the inoculum was removed and 100 μl of overlay medium (DMEM supplemented with 0.8% methylcellulose, 2% FBS, and 1% P/S) was added to

each well. After incubating the plates at 37°C for 16 h, raw images of mNG fluorescent foci were acquired using Cytation™ 7 (BioTek) armed with 2.5× objective and processed using the default software setting. The foci in each well were counted and normalized to the non-serum-treated controls to calculate the relative infectivities. The curves of the relative infectivity versus the serum dilutions (log_{10} values) were plotted using Prism 9 (GraphPad). A nonlinear regression method was used to determine the dilution fold that neutralized 50% of mNG SARS-CoV-2 (defined as $FFRNT_{50}$). Each serum was tested in duplicates.

Statistics

The nonparametric Wilcoxon matched-pairs signed rank test was used to analyze the statistical significance in **Figure 1**.

Data availability

The data that support the findings of this study are available from the corresponding authors upon request.

References

1 Plante, J. A. *et al.* Spike mutation D614G alters SARS-CoV-2 fitness. *Nature*, doi:10.1038/s41586-020-2895-3 (2020).

2 Xie, X. *et al.* Neutralization of SARS-CoV-2 spike 69/70 deletion, E484K and N501Y variants by BNT162b2 vaccine-elicited sera. *Nat Med* **27**, 620-621, doi:10.1038/s41591-021-01270-4 (2021).

3 Liu, Y. *et al.* The N501Y spike substitution enhances SARS-CoV-2 transmission. *Nature*, In press, doi:10.1101/2021.03.08.434499 (2021).

4 WHO. Classification of Omicron (B.1.1.529): SARS-CoV-2 Variant of Concern. *https://www.who.int/news/item/26-11-2021-classification-of-omicron-(b.1.1.529)-sars-cov-2-variant-of-concern* (2021).

5 Agency, U. H. S. SARS-CoV-2 variants of concern and variants under investigation in England. *Technical brief 31* (2021).

6 Chen, R. E. *et al.* Resistance of SARS-CoV-2 variants to neutralization by monoclonal and serum-derived polyclonal antibodies. *Nat Med* **27**, 717-726, doi:10.1038/s41591-021-01294-w (2021).

7 Ku, Z. *et al.* Molecular determinants and mechanism for antibody cocktail preventing SARS-CoV-2 escape. *Nature Communications*, https://doi.org/10.1038/s41467-41020-20789-41467 (2021).

8 Muruato, A. E. *et al.* A high-throughput neutralizing antibody assay for COVID-19 diagnosis and vaccine evaluation. *Nat Commun* **11**, 4059, doi:10.1038/s41467-020-17892-0 (2020).

9 Xie, X. *et al.* An Infectious cDNA Clone of SARS-CoV-2. *Cell Host Microbe* **27**, 841-848 e843, doi:10.1016/j.chom.2020.04.004 (2020).

10 Gudbjartsson, D. F. *et al.* Humoral Immune Response to SARS-CoV-2 in Iceland. *N Engl J Med* **383**, 1724-1734, doi:10.1056/NEJMoa2026116 (2020).

11 Falsey, A. R. *et al.* SARS-CoV-2 Neutralization with BNT162b2 Vaccine Dose 3. *N Engl J Med*, doi:10.1056/NEJMc2113468 (2021).

12 Chia, W. N. *et al.* Dynamics of SARS-CoV-2 neutralising antibody responses and duration of immunity: a longitudinal study. *Lancet Microbe* **2**, e240-e249, doi:10.1016/S2666-5247(21)00025-2 (2021).

13 Widge, A. T. *et al.* Durability of Responses after SARS-CoV-2 mRNA-1273 Vaccination. *N Engl J Med* **384**, 80-82, doi:10.1056/NEJMc2032195 (2021).

14 Sandile Cele, L. J., Khadija Khan, David S Khoury, Thandeka Moyo-Gwete, Houriiyah Tegally, Cathrine Scheepers, Daniel Amoako, Farina Karim, Mallory Bernstein, Gila Lustig, Derseree Archary, Muneerah Smith, Yashica Ganga, Zesuliwe Jule, Kajal Reedoy, Deborah Cromer, James Emmanuel San, Shi-Hsia Hwa, Jennifer Giandhari, Jonathan M Blackburn, Bernadett I Gosnell, Salim S Abdool Karim, Willem Hanekom, NGS-SA; COMMIT-KZN Team; Anne von Gottberg, Jinal Bhiman, Richard J Lessells, Mahomed-Yunus S Moosa, Miles P Davenport, Tulio de Oliveira, Penny L Moore, Alex Sigal. SARS-CoV-2 Omicron has extensive but incomplete escape of Pfizer BNT162b2 elicited neutralization and requires ACE2 for infection. *medRxiv*, doi:10.1101/2021.12.08.21267417 (2021).

15 Alexander Wilhelm, M. W., Katharina Grikscheit, Tuna Toptan, Barbara Schenk, Christiane Pallas, Melinda Metzler, Niko Kohmer, Sebastian Hoehl, Fabian A. Helfritz, Timo Wolf, Udo Goetsch, Sandra Ciesek. Reduced Neutralization of SARS-CoV-2 Omicron Variant by Vaccine Sera and monoclonal antibodies. *MedRiv*, doi:https://doi.org/10.1101/2021.12.07.21267432 (2021).

16 Wanwisa Dejnirattisai, R. H. S., Piyada Supasa, Chang Liu, Arabella SV Stuart, Andrew J Pollard, Xinxue Liu, Teresa Lambe, Derrick Crook, Dave I Stuart, Juthathip Mongkolsapaya, Jonathan S Nguyen-Van-Tam, Matthew D Snape, Gavin R Screaton, the Com-COV2 study group. Reduced neutralisation of SARS-COV-2 Omicron-B.1.1.529 variant by post-immunisation serum. *MedRxiv*, doi:https://doi.org/10.1101/2021.12.10.21267534.

17 Guardian, T. South Africa: previous infections may explain Omicron hospitalisation rate. https://www.theguardian.com/world/2021/dec/2014/south-africa-previous-infections-may-explain-omicron-hospitalisation-rate?fr=operanews (2021).

18 Andrew D Redd, A. N., Hassen Kared, Evan M Bloch, Brian Abel, Andrew Pekosz, Oliver Laeyendecker, Michael Fehlings, Thomas C Quinn, Aaron AR Tobian. Minimal cross-over between mutations associated with Omicron variant of SARS-CoV-2 and CD8+ T cell epitopes identified in COVID-19 convalescent individuals. *BioRxiv*, doi:https://doi.org/10.1101/2021.12.06.471446 (2021).

19 Henning Gruell, K. V., Pinkus Tober-Lau, David Hillus, Philipp Schommers, Clara Lehmann, Florian Kurth, Leif E. Sander, Florian Klein. mRNA booster immunization elicits potent neutralizing serum activity against the SARS-CoV-2 Omicron variant. *MedRiv*, doi:https://doi.org/10.1101/2021.12.14.21267769 (2021).

20 Pfizer. Pfizer and BioNTech Provide Update on Omicron Variant. *https://www.pfizer.com/news/press-release/press-release-detail/pfizer-and-biontech-provide-update-omicron-variant* (2021).

21 Xie, X. *et al.* Engineering SARS-CoV-2 using a reverse genetic system. *Nature Protocols* **16**, 1761-1784, doi:10.1038/s41596-021-00491-8 (2021).

Acknowledgments

We thank colleagues at Pfizer and the University of Texas Medical Branch for helpful discussion. P.-Y.S. was supported by NIH grants HHSN272201600013C, AI134907, AI145617, and UL1TR001439, and awards from the Sealy & Smith Foundation, the Kleberg Foundation, the John S. Dunn Foundation, the Amon G. Carter Foundation, the Gilson Longenbaugh Foundation, and the Summerfield Robert Foundation. S.C.W. was supported by NIH grant R24 AI120942.

Author contributions

Conceptualization, X.X., P.R., P.-Y.S.; Methodology, J.Z., H.X., X.X., C.K., R.R.G.M., S.C.W., P.R., P.-Y.S; Investigation, J.Z., H.X., X.X., C.K., R.R.G.M., S.C.W., P.R., P.-Y.S; Resources, H.X., S.C.W., P.R., P.-Y.S; Data Curation, J.Z., H.X., X.X., P.R., P.-Y.S.; Writing-Original Draft, J.Z., H.X., X.X., R.R., P.-Y.S; Writing-Review & Editing, X.X., S.C.W., P.R., P.-Y.S.; Supervision, X.X., S.C.W., P.R., P.-Y.S.; Funding Acquisition, S.C.W., P.-Y.S.

Ethics declarations

Competing interests

The authors declare competing interests. X.X. and P.-Y.S. have filed a patent on the reverse genetic system. J.Z., H.X., X.X., and P.-Y.S. received compensation from Pfizer for COVID-19 vaccine development. Other authors declare no competing interests.

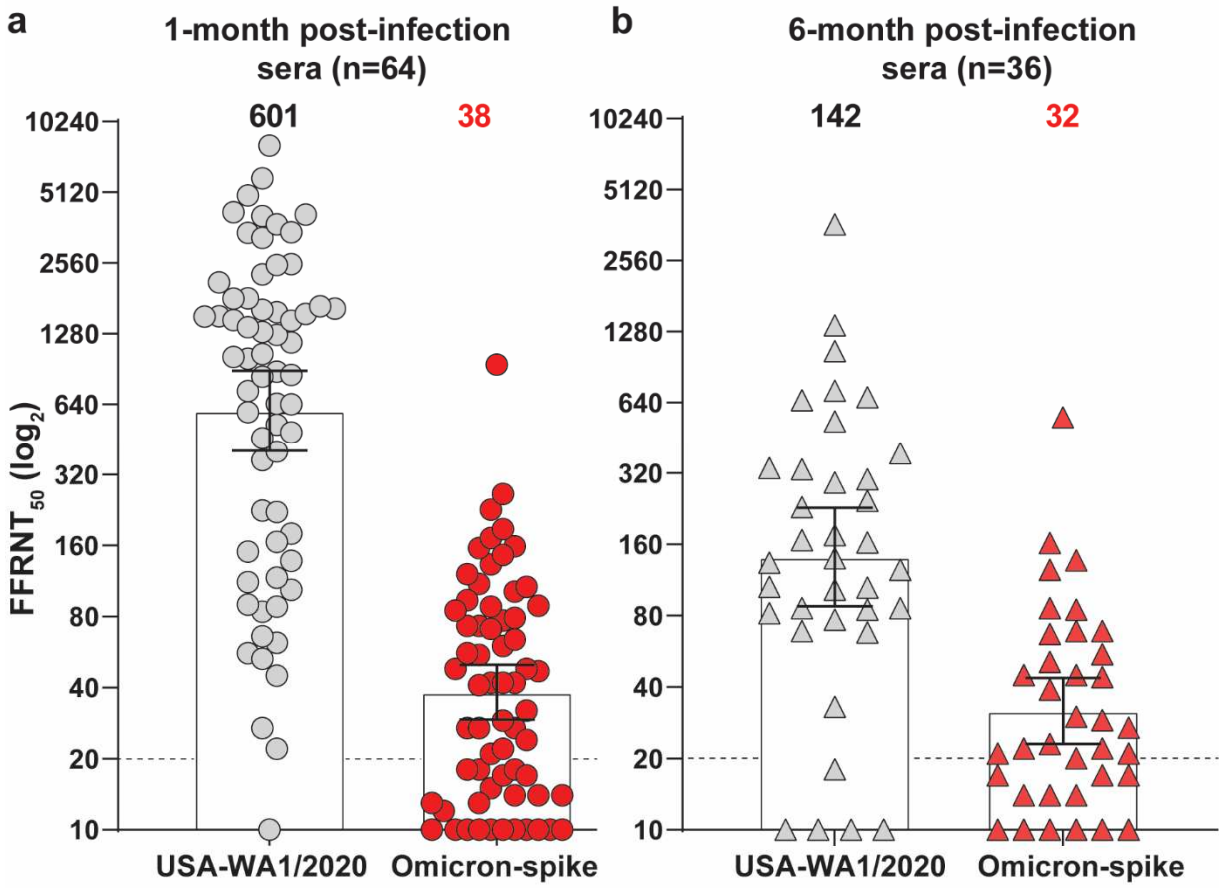
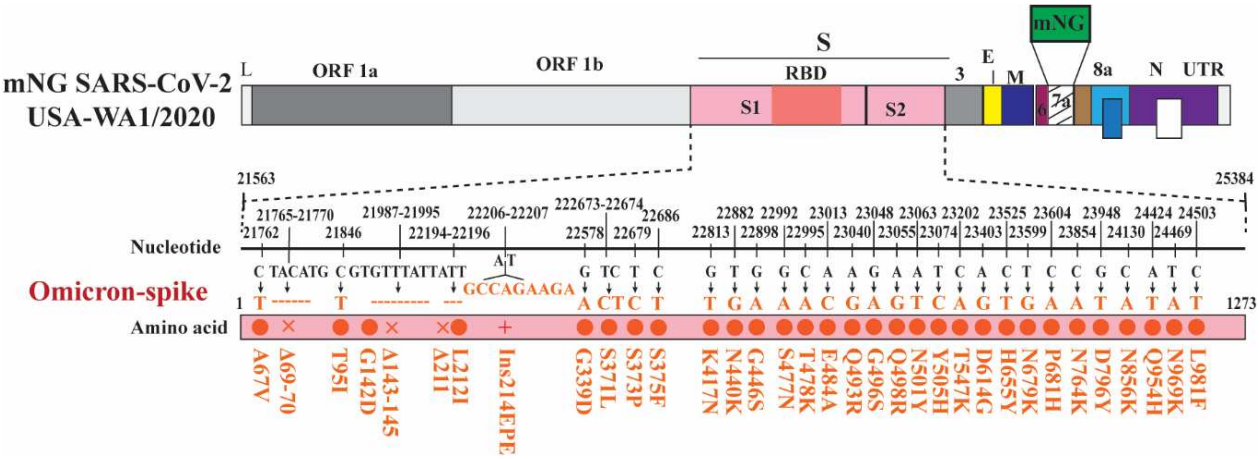
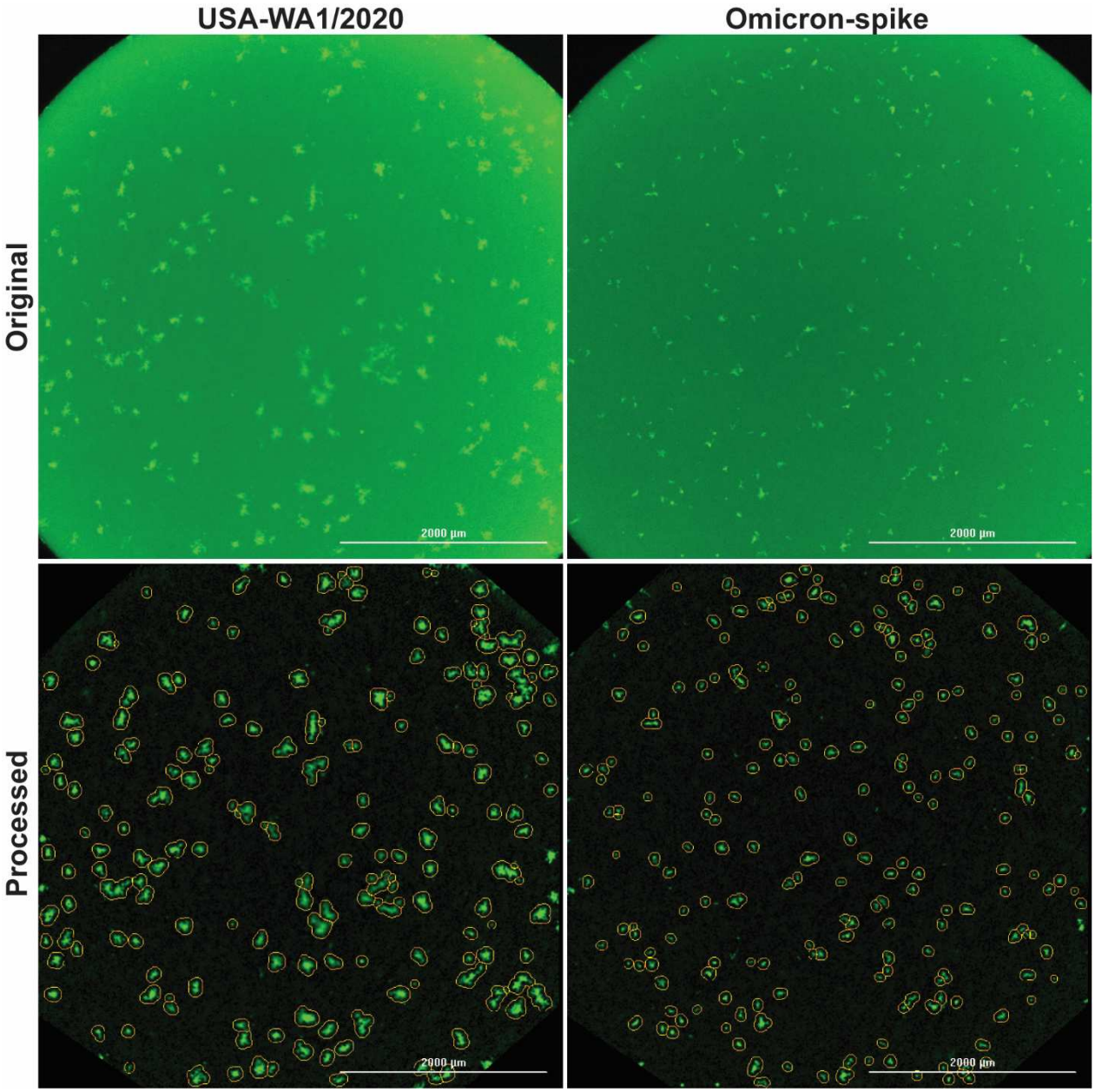


Figure 1. Reduced neutralization of Omicron SARS-CoV-2 by previous non-Omicron viral infection. 50% fluorescent focus reduction neutralization titers ($FFRNT_{50}$) were measured for two serum panels from patients previously infected with non-Omicron SARS-CoV-2. The first serum panel was collected at 1-month post-infection (n=64) and the second panel collected at 6-months post-infection (n=36). For each serum, $FFRNT_{50}$ values were determined against mNG USA-WA1/2020 and Omicron-spike SARS-CoV-2. **a,** $FFRNT_{50}$s of 1-month post-infection sera. **b,** $FFRNT_{50}$s of 6-month post-infection sera. **Extended Data Tables 1** and **2** summarize the $FFRNT_{50}$ values and serum information for (**a**) and (**b**), respectively. Each symbol of dots (**a**) and triangles (**b**) represents one serum specimen. The $FFRNT_{50}$ value for each serum was determined in duplicate assays and is presented as the geometric mean. The bar heights and the numbers above each set of data indicate geometric mean titers. The whiskers indicate 95% confidence intervals. The dotted line indicates the first serum dilution (1:20) of the FFRNT assay. The $FFRNT_{50}$ values of sera that did not show any inhibition of viral infection are presented 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 the geometric mean titers against USA-WA1/2020 and Omicron-spike SARS-CoV-2 is $p < 0.0001$ in both (**a**) and (**b**).

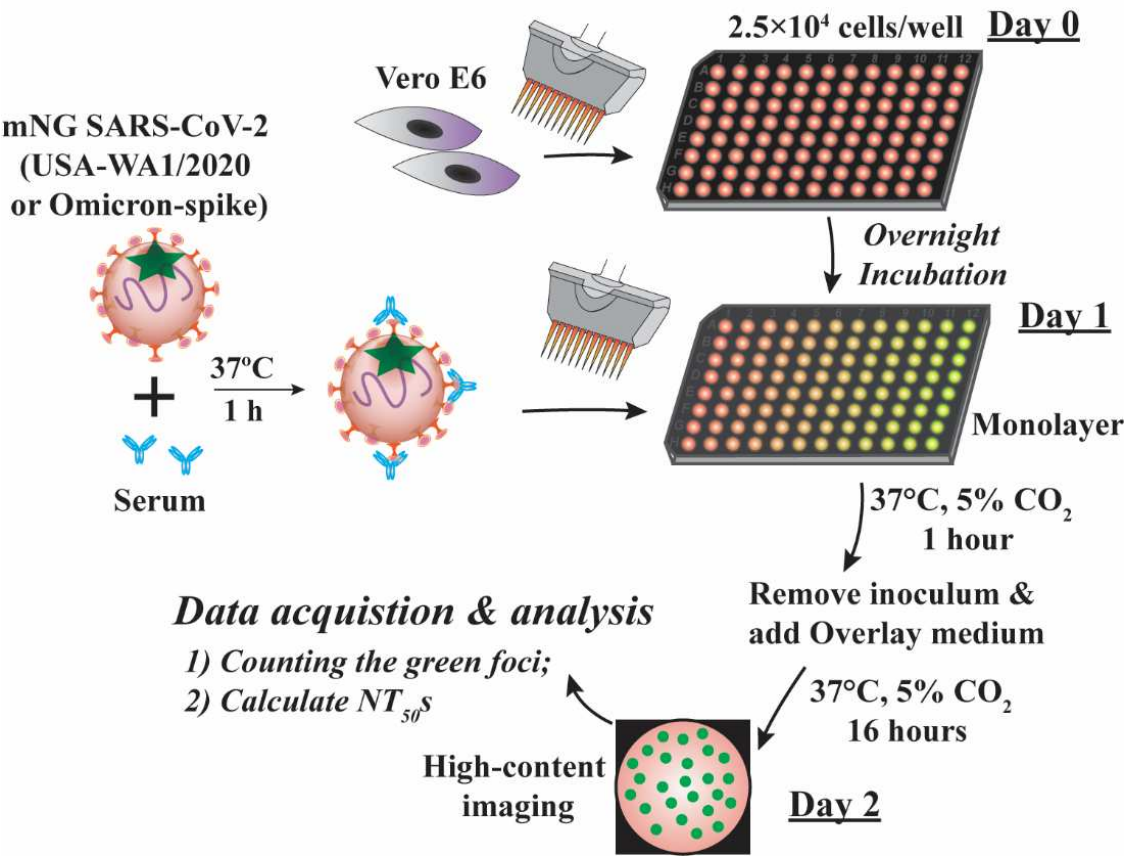


277

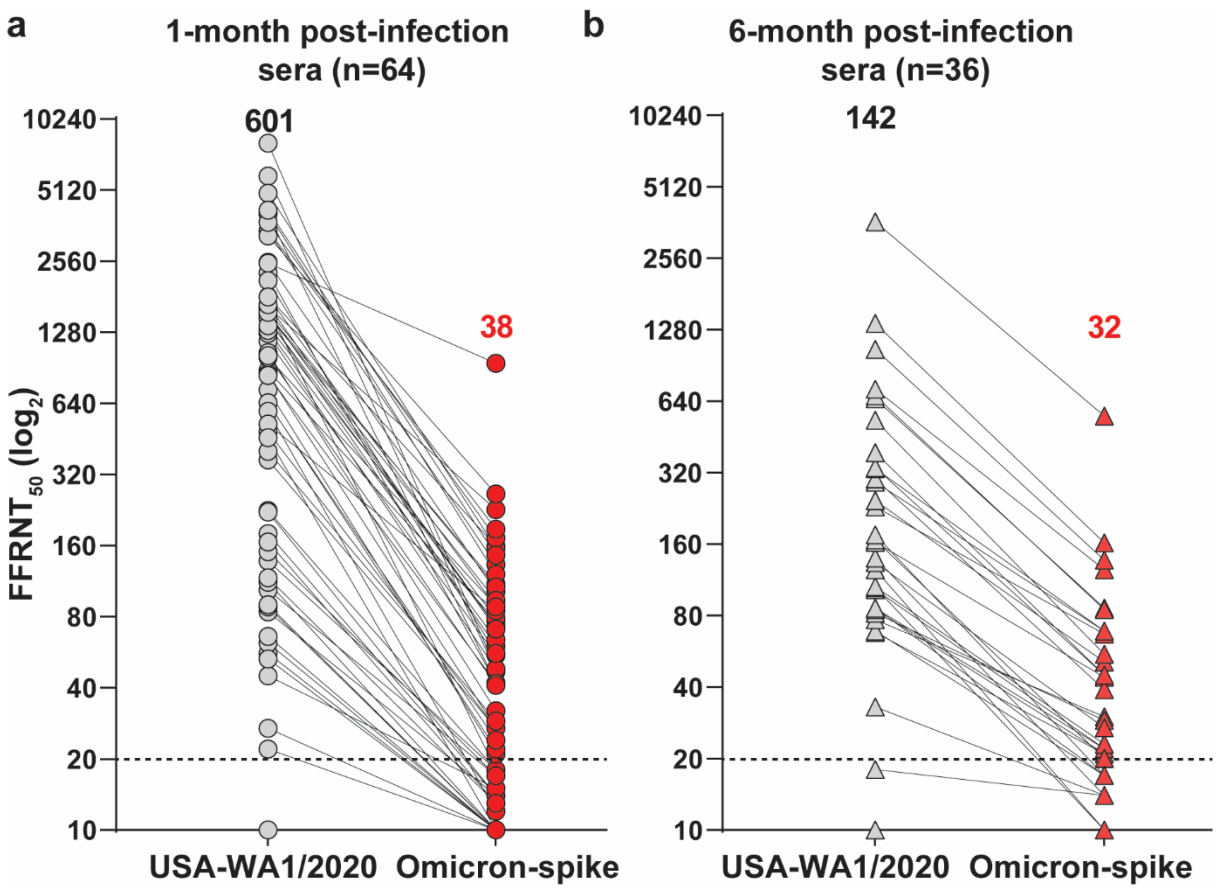
278 **Extended Data Figure 1**. Construction of mNeonGreen (mNG) Omicron-spike SARS-CoV-2.
279 mNG USA-WA1/2020 was used to engineer the complete *spike* gene from the Omicron variant,
280 resulting in mNG Omicron-spike SARS-CoV-2. Mutations (red circle), deletions (x), and
281 insertions (+) are indicated. Nucleotide and amino acid positions are depicted. L: leader
282 sequence; ORF: open reading frame; RBD: receptor binding domain; S: spike glycoprotein; S1:
283 N-terminal furin cleavage fragment of S; S2: C-terminal furin cleavage fragment of S; E:
284 envelope protein; M: membrane protein; N: nucleoprotein; UTR: untranslated region.



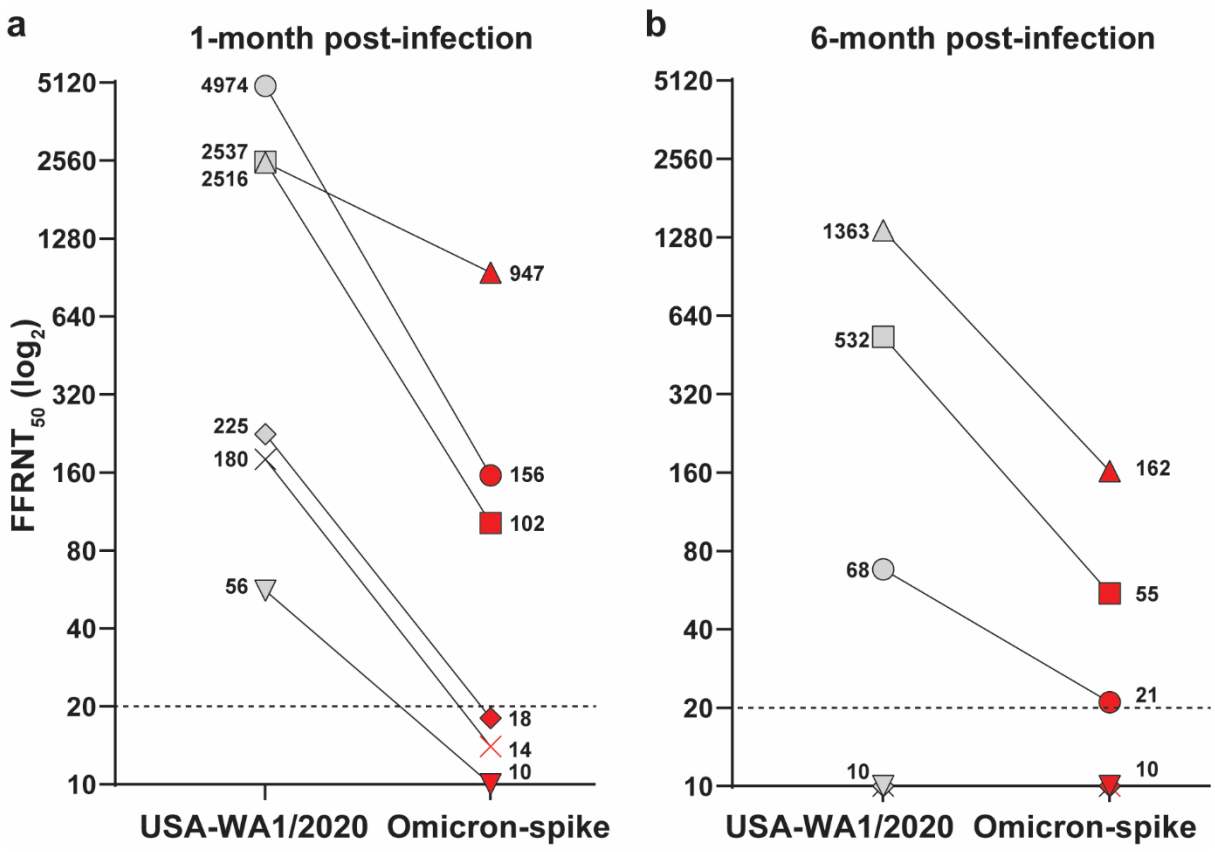
Extended Data Figure 2. Fluorescent foci of mNG USA-WA1/2020 and mNG Omicron-spike SARS-CoV-2 on Vero E6 cells. Original and processed images were collected by high-content imaging. The protocol of the fluorescent focus reduction neutralization test (FFRNT) is described in Methods. See **Extended Data Figure 3** for the experimental scheme of FFRNT.



Extended Data Figure 3. Experimental scheme of fluorescent focus reduction neutralization test (FFRNT). The FFRNT protocol is described in Methods.



Extended Data Figure 4. Reduced neutralization against Omicron SARS-CoV-2 by previous non-Omicron viral infection. 50% fluorescent focus reduction neutralization titers ($FFRNT_{50}$) were measured for two serum panels from COVID-19 patients previously infected with non-Omicron SARS-CoV-2. The first serum panel was collected at 1-month post-infection (n=64) and the second panel collected at 6-month post-infection (n=36). For each serum, two $FFRNT_{50}$ values against mNG USA-WA1/2020 and Omicron-spike SARS-CoV-2 are connected by a line. **a,** $FFRNT_{50}$s of 1-month post-infection sera. **b,** $FFRNT_{50}$s of 6-month post-infection sera. **Extended Data Tables 1** and **2** summarize the $FFRNT_{50}$ values and serum information for (**a**) and (**b**), respectively. This figure is a reformat of **Figure 1**.



Extended Data Figure 5. $FFRNT_{50}$ of 6 pairs of 1- and 6-month post-infection sera from same patients. **a,** $FFRNT_{50}$s of 1-month post-infection sera against mNG USA-WA1/2020 and Omicron-spike SARS-CoV-2. **b,** $FFRNT_{50}$s of 6-month post-infection sera against mNG USA-WA1/2020 and Omicron-spike SARS-CoV-2.

Extended Data Table 1. $FFRNT_{50}$ values of 1-month post-infection sera against mNG USA-WA1/2020 and Omicron-spike SARS-CoV-2

Serum ID	Age	Gender	Race and Ethnicity	Sample collection date yielding positive viral test	Symptomatic	Hospitalized	$FFRNT_{50}$	
							USA-WA1/2020	Omicron-spike
1	21	F	Hispanic or Latino	7/22/2020	No	No	10	10
2	38	F	White	11/27/2020	No	No	22	10
3	17	M	Hispanic or Latino	6/1/2020	Yes	No	27	10
4	18	F	Hispanic or Latino	7/11/2020	Yes	No	45	15
5	26	F	Hispanic or Latino	11/11/2020	Yes	No	53	10
▿6	24	F	Hispanic or Latino	6/24/2020	No	No	56	10
7	24	F	Hispanic or Latino	7/27/2020	Yes	No	62	10
8	35	F	Hispanic or Latino	1/5/2021	No	No	66	10
9	23	F	Hispanic or Latino	6/25/2020	No	No	84	14
10	24	F	Hispanic or Latino	7/2/2020	Yes	No	88	10
11	33	F	Black or African American	11/21/2020	Yes	No	90	10
12	26	F	Hispanic or Latino	7/2/2020	Yes	No	104	17
13	60	M	White	9/26/2020	Yes	Yes	112	10
14	67	M	Caucasian/White	11/16/2020	Yes	No	117	13
15	22	M	Hispanic or Latino	9/24/2020	No	No	138	18
16	69	M	White	12/28/2020	Yes	No	151	17
17	73	M	White	5/27/2020	Yes	No	166	13
× 18	17	F	Hispanic or Latino	6/22/2020	Yes	No	180	14
19	45	M	Hispanic or Latino	5/2/2020	Yes	Yes	222	14
◇20	24	F	Hispanic or Latino	6/24/2020	Yes	No	225	18
21	25	F	Black or African American	7/16/2020	No	No	369	27
22	75	F	Hispanic or Latino	9/14/2020	Yes	No	402	29
*23	80	F	White	11/9/2020	Yes	Yes	459	10
24	61	F	White	11/3/2020	Yes	No	486	27
25	78	M	White	12/15/2020	Yes	No	524	85
26	66	M	White	5/30/2020	Yes	Yes	592	12
27	38	M	Hispanic or Latino	6/27/2020	No	No	640	24
28	34	M	Hispanic or Latino	11/11/2020	Yes	No	645	22
29	25	F	Hispanic or Latino	7/17/2020	No	No	730	21
30	66	M	Black or African American	4/27/2020	Yes	Yes	840	42
31	39	M	Black or African American	4/9/2020	Yes	Yes	856	27
32	35	F	Hispanic or Latino	10/15/2020	Yes	Yes	882	77

33	55	M	Hispanic or Latino	5/6/2020	Yes	Yes	1003	89
*34	67	F	Hispanic or Latino	1/4/2021	Yes	Yes	1020	41
35	55	F	White	9/28/2020	Yes	No	1050	32
36	40	F	Hispanic or Latino	12/20/2020	Yes	No	1174	55
37	60	F	Hispanic or Latino	11/27/2020	Yes	Yes	1268	48
38	65	M	Hispanic or Latino	5/7/2020	Yes	Yes	1306	64
*39	69	M	White	11/22/2020	Yes	Yes	1365	79
40	68	M	Caucasian/White	5/10/2020	Yes	Yes	1454	73
41	50	M	Black or African American	4/8/2020	Yes	Yes	1465	60
42	63	M	Hispanic or Latino	1/23/2021	Yes	Yes	1517	94
43	39	M	Black or African American	3/31/2020	Yes	Yes	1519	110
44	72	M	White	12/23/2020	Yes	Yes	1555	73
45	55	F	White	10/6/2020	Yes	Yes	1584	107
46	57	M	White	7/5/2020	Yes	No	1618	18
47	1	F	Hispanic or Latino	1/18/2021	Yes	Yes	1638	227
48	87	M	White	1/5/2021	Yes	Yes	1679	71
49	96	F	White	12/30/2020	Yes	Yes	1807	88
50	66	M	Hispanic or Latino	12/19/2020	Yes	No	1814	159
51	75	M	Hispanic or Latino	10/27/2020	Yes	No	2119	56
52	63	F	Hispanic or Latino	12/12/2020	Yes	Yes	2289	42
△53	66	M	White	12/27/2020	Yes	Yes	2516	947
□54	49	M	Black or African American	1/3/2021	No	Yes	2537	102
55	56	M	Hispanic or Latino	7/13/2020	Yes	Yes	3277	265
56	44	F	Black or African American	8/20/20/	Yes	Yes	3443	133
*57	83	M	Hispanic or Latino	11/22/2020	Yes	Yes	3464	188
*58	75	M	White	12/27/2020	Yes	Yes	3741	172
*59	74	M	White	12/21/2020	Yes	Yes	4055	42
60	48	F	Hispanic or Latino	6/21/2020	Yes	Yes	4116	121
61	78	F	Hispanic or Latino	12/16/2020	Yes	Yes	4216	146
○*62	70	M	Hispanic or Latino	12/12/2020	Yes	Yes	4974	156
*63	49	M	White	12/29/2020	Yes	Yes	5876	47
64	50	F	Hispanic or Latino	11/9/2020	Yes	Yes	8088	48
GMT	44	-	-	-	-	-	601	38
95%CI	37-52	-	-	-	-	-	405-891	29-50

* Patients received convalescent plasma treatment.

▽×◇△□○ Patients who gave both 1- and 6-month post-infection sera.

Extended Data Table 2. $FFRNT_{50}$ values of 6-month post-infection sera against mNG USA-WA1/2020 and Omicron-spike SARS-CoV-2

Serum ID	Age	Gender	Race and Ethnicity	Sample collection date yielding positive viral test	Symptomatic	Hospitalized	$FFRNT_{50}$	
							USA-WA1/2020	Omicron-spike
▽1	17	F	Hispanic or Latino	6/22/2020	Yes	No	10	10
× 2	24	F	Hispanic or Latino	6/24/2020	No	No	10	10
◇3	24	F	Hispanic or Latino	6/24/2020	Yes	No	10	10
4	70	M	White	7/26/2020	No	No	10	10
5	29	F	Black or African American	8/3/2020	No	No	18	14
6	21	F	Hispanic or Latino	6/26/2020	No	No	33	14
○*7	70	M	Hispanic or Latino	12/12/2020	Yes	Yes	68	21
8	27	F	Hispanic or Latino	8/9/2020	No	No	69	17
9	22	F	Hispanic or Latino	10/1/2020	No	No	77	30
10	61	F	White	8/24/2020	No	No	82	29
11	40	F	Hispanic or Latino	7/31/2020	Yes	No	85	21
12	22	F	Hispanic or Latino	7/13/2020	No	No	86	22
13	50	F	White	11/25/2020	Yes	Yes	86	17
14	26	F	Hispanic or Latino	9/10/2020	No	No	103	22
15	21	F	Hispanic or Latino	6/2/2020	Yes	No	105	10
16	26	F	Hispanic or Latino	9/10/2020	No	No	106	23
17	73	F	White	10/14/2020	Yes	Yes	125	10
18	22	M	Hispanic or Latino	9/24/2020	Yes	Yes	134	27
19	47	M	Black or African American	4/23/2020	No	No	140	14
20	79	M	White	5/4/2020	Yes	Yes	163	44
**21	77	F	Black or African American	12/7/2020	Yes	No	167	20
22	57	M	White	5/13/2020	Yes	Yes	175	17
23	23	F	Hispanic or Latino	12/25/2020	Yes	No	230	67
24	40	F	Hispanic or Latino	3/16/2020	Yes	No	244	51
25	22	F	Hispanic or Latino	8/11/2020	Yes	No	292	69
26	54	M	White	4/10/2020	Yes	Yes	302	39
27	64	M	White	1/3/2021	Yes	Yes	333	69
28	39	F	Black or African American	7/9/2020	Yes	No	337	45
29	69	M	White	8/14/2020	Yes	No	389	45
□30	49	M	Black or African American	1/3/2021	Yes	Yes	532	55
31	96	F	White	12/30/2020	Yes	Yes	655	86
32	80	F	Hispanic or Latino	6/20/2020	Yes	No	675	85

33	49	F	Hispanic or Latino	3/26/2020	Yes	No	719	125
34	48	F	Hispanic or Latino	6/21/2020	Yes	Yes	1059	137
△35	66	M	White	12/27/2020	Yes	Yes	1363	162
36	70	M	Hispanic or Latino	11/19/2020	Yes	Yes	3648	554
GMT	41	-	-	-	-	-	142	32
95% CI	35-49	-	-	-	-	-	88-229	23-44

* Patients received convalescent plasma treatment.

** Patient received therapeutic antibody treatment.

▿×◇△□○ Patients who gave both 1- and 6-month post-infection sera.