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Low neutralization of SARS-CoV-2 Omicron BA.2.75.2, BQ.1.1 and XBB.1 by parental mRNA vaccine or a BA.5 bivalent booster

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The newly emerged severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) Omicron sublineages, including the BA.2-derived BA.2.75.2 and the BA.5-derived BQ.1.1 and XBB.1, have accumulated additional spike mutations that may affect vaccine effectiveness. Here we report neutralizing activities of three human serum panels collected from individuals 23–94 days after dose 4 of a parental mRNA vaccine; 14–32 days after a BA.5 bivalent booster from individuals with 2–4 previous doses of parental mRNA vaccine; or 14–32 days after a BA.5 bivalent booster from individuals with previous SARS-CoV-2 infection and 2–4 doses of parental mRNA vaccine. The results showed that a BA.5 bivalent booster elicited a high neutralizing titer against BA.4/5 measured at 14–32 days after boost; however, the BA.5 bivalent booster did not produce robust neutralization against the newly emerged BA.2.75.2, BQ.1.1 or XBB.1. Previous infection substantially enhanced the magnitude and breadth of BA.5 bivalent booster-elicited neutralization. Our data support a vaccine update strategy that future boosters should match newly emerged circulating SARS-CoV-2 variants.

The continuous emergence of new variants of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has caused successive global waves of infection. Since its first report in November 2021 in South Africa, Omicron has become the dominating variant due to its high transmissibility and immune evasion[1,2], with many Omicron sublineages emerging over time. The initial Omicron BA.1 was displaced by BA.2, which has further evolved to sublineages BA.2.12.1, BA.2.75, BA.2.75.2, BA.4 and BA.5, among which BA.5 is currently dominant in many countries. BA.4 and BA.5 have an identical spike sequence (defined as BA.4/5 hereafter), and their offspring, BA.4.6, BF.7 and BQ.1.1, are expanding in prevalence. As of 19 November 2022, the BA.2-derived sublineage BA.2.75.2 accounted for 0.8% of the total SARS-CoV-2 infection in the United States, whereas the BA.4/5-derived sublineages BA.4.6, BF.7, BQ.1 and BQ.1.1 accounted for 4.4%, 7.8%, 25.5% and 24.2% of total cases, respectively. In addition, another BA.5-derived sublineage, XBB, first identified in India in August 2022, is rapidly spreading in Europe and has been detected in the United States. XBB was predominant in Singapore, accounting for 54% of SARS-CoV-2 infections during the week of 3–9 October 2022 (Ministry of Health, Singapore; https://www.moh.gov.sg/).

SARS-CoV-2 spike mutations often contribute to immune evasion and/or transmission efficiency[3–9]. Previous studies showed that three or four doses of parental mRNA vaccine did not elicit robust neutralization against BA.4/5, supporting the development of bivalent vaccines that target both the ancestral spike and the BA.4/5 spike protein[10–12]. Because the newly emerged Omicron sublineages have accumulated additional spike mutations (Fig. 1a), it is important to examine the vaccine-elicited

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neutralization against these new sublineages. The goal of this study was to compare the neutralizing activities against six newly emerged Omicron sublineages (BA.5, BF.7, BA.4.6, BA.2.75.2, BQ.1.1 and XBB.1) using human sera collected from individuals who received four doses of parental mRNA vaccine or a BA.5 bivalent booster after 2–4 doses of parental mRNA vaccine.

To facilitate neutralization measurement, we engineered the complete *spike* gene from Omicron sublineage BA.4/5, BF.7, BA.4.6, BA.2.75.2, BQ.1.1 or XBB.1 into the backbone of mNeonGreen (mNG) reporter USA-WA1/2020 SARS-CoV-2 (Fig. 1a). Compared with wild-type USA-WA1/2020 (a strain isolated in January 2020), insertion of *mNG* gene at open reading frame 7 of the viral genome attenuated the virus in vivo[13]. So, the engineered live-attenuated mNG viruses can be used safely in a Biosafety Level 3 (BSL-3) facility with the correct procedures for neutralization and antiviral testing[14]. Passage 1 of recombinant BA.4/5-spike, BF.7-spike, BA.4.6-spike, BA.2.75.2-spike, BQ.1.1-spike and XBB.1-spike mNG USA-WA1/2020 viruses were sequenced to ensure no undesired mutations. Only the passage 1 virus stocks were used to determine the 50% fluorescent focus-reduction neutralization titers ($FFRNT_{50}$) of vaccinated human sera, to ensure no additional spike mutations in the tested recombinant viruses.

Three human serum panels with distinct vaccination and/or SARS-CoV-2 infection history were analyzed. The first panel consisted of 25 sera obtained from individuals 23–94 days (median 47 days) after dose 4 of parental monovalent mRNA-1273 or BNT162b2 vaccine (post-dose-4 sera); these sera were collected from 16 March to 30 June 2022 (Extended Data Table 1). The second panel consisted of 29 sera collected from individuals 14–32 days (median 22 days) after BA.5 bivalent booster (BA.5 bivalent booster sera); these specimens were collected from 30 September to 22 October 2022 (Extended Data Table 2). All sera from the first and second panels tested negative against viral nucleocapsid protein (Extended Data Fig. 1), suggesting no previous or recent SARS-CoV-2 infection. The third panel consisted of 23 sera collected from individuals who were previously infected by SARS-CoV-2 (nucleocapsid antibody positive; Extended Data Fig. 1) and received a BA.5 bivalent booster 14–32 days (median 21 days) ago (BA.5 bivalent booster infection sera); the viral infection time and genotype could not be determined because most infections were asymptomatic; these samples were collected from 4 to 22 October 2022 (Extended Data Table 3). All participants from the second and third panels had also received two, three or four doses of parental monovalent mRNA vaccine before receiving the BA.5 bivalent booster. Extended Data Tables 1–3 summarize the serum information and neutralization for each serum panel.

Post-dose-4 sera neutralized USA-WA1/2020-spike, BA.4/5-spike, BF.7-spike, BA.4.6-spike, BA.2.75.2-spike, BQ.1.1-spike and XBB.1-spike mNG SARS-CoV-2 with geometric mean titers (GMTs) of 1,533, 95, 69, 62, 26, 22 and 15, respectively (Fig. 1b and Extended Data Table 1). The neutralizing GMTs against BA.4/5-spike, BF.7-spike, BA.4.6-spike, BA.2.75.2-spike, BQ.1.1-spike and XBB.1-spike viruses were 16.1-fold, 22.2-fold, 24.7-fold, 59-fold, 69.7-fold and 102-fold lower than the GMT against the USA-WA1/2020-spike virus, respectively (Fig. 1b). Compared with the GMT against the current dominant BA.4/5, the neutralizing GMTs against BF.7-spike, BA.4.6-spike, BA.2.75.2-spike, BQ.1.1-spike and XBB.1-spike viruses were reduced by 1.4-fold, 1.5-fold, 3.7-fold, 4.3-fold and 6.3-fold, respectively. The GMTs against BA.2.75.2 (26) and BQ.1.1 (22) were barely above 20, the detection limit of FFRNT, whereas the GMT against XBB.1 (15) was below the FFRNT detection limit. These results indicate that (1) four doses of parental mRNA vaccine do not elicit robust neutralization against the newly emerged Omicron sublineages when measured at 23–94 days (median 47 days) after dose 4, and (2) the rank of neutralization evasion is in the order of BA.4/5 < BF.7 ≤ BA.4.6 < BA.2.75.2 ≤ BQ.1.1 < XBB.1.

BA.5 bivalent booster sera, collected at 14–32 days (median 22 days) after boost, neutralized USA-WA1/2020-spike, BA.4/5-spike, BF.7-spike, BA.4.6-spike, BA.2.75.2-spike, BQ.1.1-spike and XBB.1-spike SARS-CoV-2s with GMTs of 3,620, 298, 305, 183, 98, 73 and 35, respectively (Fig. 1c and Extended Data Table 2). The neutralizing GMTs against BA.4/5-spike, BF.7-spike, BA.4.6-spike, BA.2.75.2-spike, BQ.1.1-spike and XBB.1-spike viruses were 12.1-fold, 11.9-fold, 19.8-fold, 36.9-fold, 49.6-fold and 103-fold lower than the GMT against the USA-WA1/2020, respectively (Fig. 1c). The data indicate that, although BA.5 bivalent booster elicits high neutralizing titers against BA.4/5 measured at 14–32 days after boost, the neutralization against BA.2.75.2 (98), BQ.1.1 (73) and XBB.1 (35) remains low after BA.5 bivalent booster.

BA.5 bivalent booster infection sera, collected at 14–32 days (median 21 days) after boost, neutralized USA-WA1/2020-spike, BA.4/5-spike, BF.7-spike, BA.4.6-spike, BA.2.75.2-spike, BQ.1.1-spike and XBB.1-spike SARS-CoV-2s with GMTs of 5,776, 1,558, 1,223, 744, 367, 267 and 103, respectively (Fig. 1d and Extended Data Table 3). The neutralizing GMTs against BA.4/5-spike, BF.7-spike, BA.4.6-spike, BA.2.75.2-spike, BQ.1.1-spike and XBB.1-spike viruses were 3.7-fold, 4.7-fold, 7.8-fold, 15.7-fold, 21.6-fold and 56.1-fold lower than the GMT against the USA-WA1/2020-spike SARS-CoV-2, respectively (Fig. 1d). Compared with BA.5 bivalent booster sera without infection history, BA.5 bivalent booster infection sera increased the neutralizing GMTs against USA-WA1/2020-spike, BA.4/5-spike, BF.7-spike, BA.4.6-spike, BA.2.75.2-spike, BQ.1.1-spike and XBB.1-spike viruses by 1.6-fold, 5.2-fold, 4.0-fold, 4.1-fold, 3.7-fold, 3.7-fold and 2.9-fold, respectively (compare Fig. 1c,d). The results suggest that (1) previous infection substantially increases the magnitude and breadth of neutralization for BA.5 bivalent booster, and (2) among the tested Omicron sublineages, XBB.1 exhibits the highest level of immune evasion.

Fig. 1 | Neutralization against Omicron sublineages. a, Construction of Omicron sublineage-spike mNG SARS-CoV-2. Amino acid mutations, deletions (Δ) and insertions (Ins) are indicated in reference to the USA-WA1/2020-spike. L, leader sequence; ORF, open reading frame; NTD, N-terminal domain of S1; RBD, receptor binding domain; S, spike glycoprotein; S1, N-terminal furin cleavage fragment of S; S2, C-terminal furin cleavage fragment of S; E, envelope protein; M, membrane protein; N, nucleocapsid; UTR, untranslated region. **b**, $FFRNT_{50}$ values of human sera after dose 4 parental mRNA vaccine. The *P* values (two-sided) for group comparison of GMTs are the following. USA-WA1/2020 versus others: <0.0001; BA.4/5-spike versus BF.7-spike, BA.4.6-spike, BA.2.75.2-spike, BQ.1.1-spike and XBB.1-spike: 0.029, 0.001, <0.0001, <0.0001 and <0.0001; BF.7-spike versus BA.4.6-spike, BA.2.75.2-spike, BQ.1.1-spike and XBB.1-spike: 0.103, <0.0001, <0.0001 and <0.0001; BA.4.6-spike versus BA.2.75.2-spike, BQ.1.1-spike and XBB.1-spike: 0.0001, <0.0001 and <0.0001; BA.2.75.2-spike versus BQ.1.1-spike and XBB.1-spike: 0.24 and <0.0001; BQ.1.1-spike versus XBB.1-spike: 0.0028. The $FFRNT_{50}$ values against BA.4/5-spike were reported previously[11]. **c**, $FFRNT_{50}$ of 29 sera collected after BA.5 bivalent booster from individuals without infection history. The *P* values (two-sided) for group comparison of GMTs are the following. USA-WA1/2020 versus others: <0.0001; BA.4/5-spike versus BF.7-spike, BA.4.6-spike, BA.2.75.2-spike, BQ.1.1-spike and XBB.1-spike: 0.844, <0.0001, <0.0001, <0.0001 and <0.0001; BF.7-spike versus BA.4.6-spike, BA.2.75.2-spike, BQ.1.1-spike and XBB.1-spike: all <0.0001; BA.4.6-spike versus BA.2.75.2-spike, BQ.1.1-spike and XBB.1-spike: all <0.0001; BA.2.75.2-spike versus BQ.1.1-spike and XBB.1-spike: 0.69 and <0.0001; BQ.1.1-spike versus XBB.1-spike: <0.0001. **d**, $FFRNT_{50}$ of 23 sera collected after BA.5 bivalent booster from individuals with infection history. The *P* values (two-sided) for group comparison of GMTs are the following. USA-WA1/2020 versus others: <0.0001; BA.4/5-spike versus BF.7-spike, BA.4.6-spike, BA.2.75.2-spike, BQ.1.1-spike and XBB.1-spike: 0.0049, <0.0001, <0.0001, <0.0001 and <0.0001; BF.7-spike versus BA.4.6-spike, BA.2.75.2-spike, BQ.1.1-spike and XBB.1-spike: all <0.0001; BA.4.6-spike versus BA.2.75.2-spike, BQ.1.1-spike and XBB.1-spike: 0.0005, <0.0001 and <0.0001; BA.2.75.2-spike versus BQ.1.1-spike and XBB.1-spike: 0.114 and <0.0001; BQ.1.1-spike versus XBB.1-spike: <0.0001. For **b**–**d**, bar heights and the numbers above indicate GMTs. Error bars indicate 95% confidence interval. The fold of GMT reduction against each Omicron sublineage, compared with the GMT against USA-WA1/2020, is shown in italic font. The dotted line indicates the limit of detection of $FFRNT_{50}$. Statistical analyses were performed using the Wilcoxon matched-pairs signed-rank test for group comparison of GMTs.

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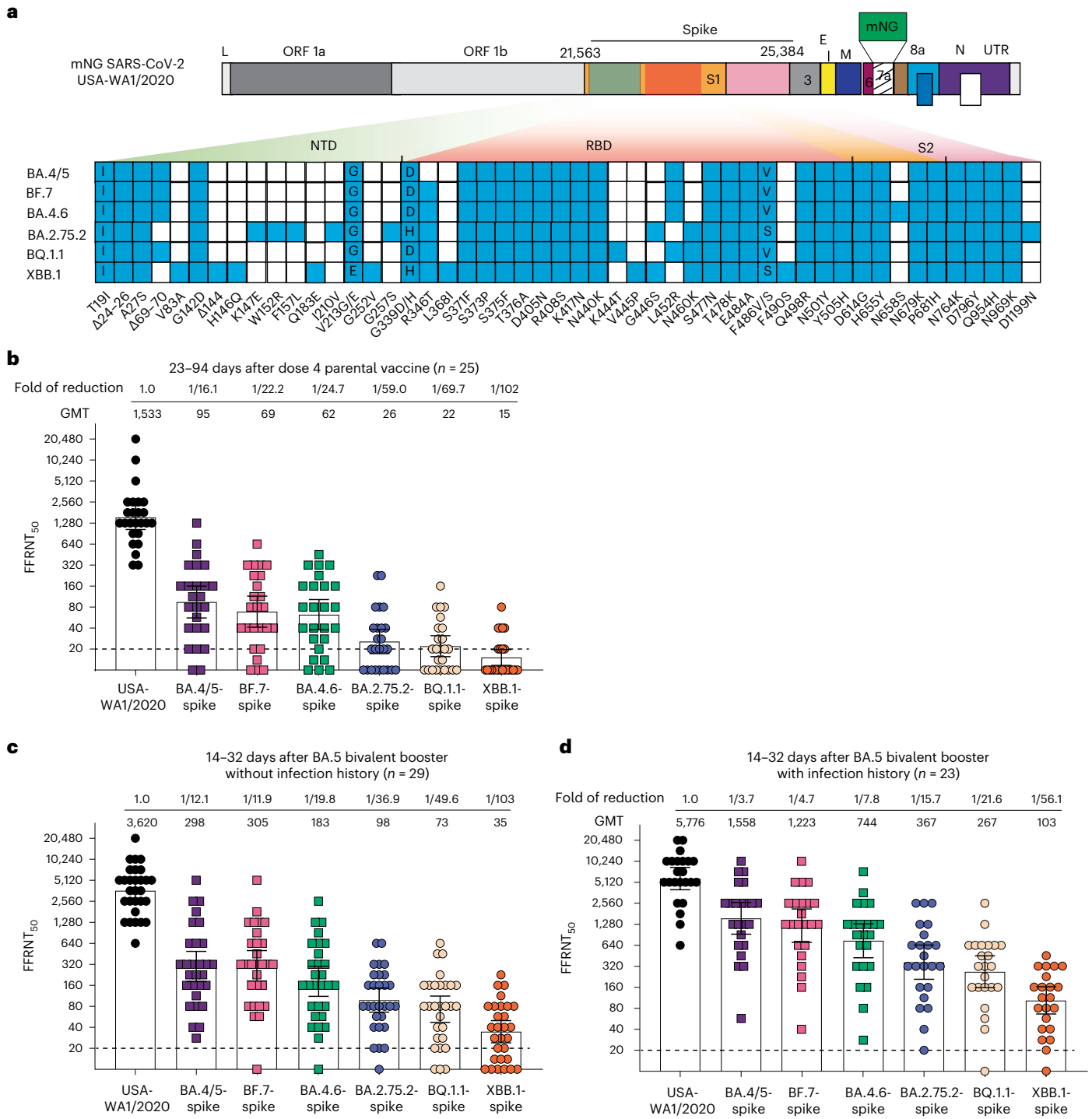
Collectively, our neutralization results support two conclusions. First, the newly emerged Omicron sublineages continue to increase their immune evasion of vaccine-elicited and/or infection-elicited neutralization. Among tested Omicron sublineages, BA.2.75.2, BQ.1.1 and XBB.1 exhibit the greatest evasion against vaccine-elicited neutralization, suggesting the potential of these new sublineages to dethrone BA.5 as the dominant lineage in circulation. Second, individuals with SARS-CoV-2 infection history develop higher and broader neutralization against the current circulating Omicron sublineages after the BA.5 bivalent booster.

This study has several limitations. First, we have not examined the antiviral roles of non-neutralizing antibodies and cell-mediated immunity. These two immune components, together with neutralizing antibodies, protect patients from severe disease and death[15,16]. Unlike neutralizing antibodies, many T cell epitopes after vaccination or natural infection are preserved in Omicron spikes[17]. However, robust antibody neutralization is critical to prevent viral infection[18]. Second, we have not defined the spike mutations that contribute to the observed immune evasion of the newly emerged Omicron sublineages. Spike mutation F486V was previously shown to drive the immune evasion of BA.4/5 (ref.10). The new Omicron sublineages BA.2.75.2, BA.4.6, BF.7, BQ.1.1 and XBB.1 share the spike R346T mutation that was reported to confer higher neutralization evasion[19]. Third, the current results do not allow a direct comparison of neutralization between parental mRNA vaccine and BA.5 bivalent booster because of the differences in individuals' demographics (for example, age), numbers of vaccine doses



and serum collection time. Fourth, we do not know (1) how neutralizing titers related to protection against infection, severe disease or death; (2) when and which variants infected individuals from the BA.5 bivalent booster infection cohort; and (3) the insight related to the differences in vaccine dose for Moderna's bivalent (Original and Omicron BA.4/BA.5) versus Pfizer/BioNTech's BA.4/BA.5 adapted bivalent booster, and (4) the baseline of the neutralization titers before boost were not determined due to sample unavailability.

Our laboratory investigation, along with the recent real-world effectiveness of BA.5 bivalent booster[20], supports a vaccine update strategy that future boosters should match new circulating SARS-CoV-2 variants. Given the advantage of mRNA vaccine platform that can rapidly adapt to new antigen sequences, the key challenge is to determine the future booster sequence before new variants become prevalent in circulation.

Online content

Any methods, additional references, Nature Portfolio reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41591-022-02162-x.

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Methods

Ethics statement

All virus work was performed in a BSL-3 laboratory with redundant fans in the biosafety cabinets at The University of Texas Medical Branch (UTMB) at Galveston. All personnel wore powered air-purifying respirators (Breathe Easy, 3M) with Tyvek suits, aprons, booties and double gloves. The research protocol regarding the use of human serum specimens was reviewed and approved by the UTMB institutional review board (IRB) (no. 20-0070). No informed consent was required because these de-identified sera were leftover specimens from routine standard of care and diagnostics before being discarded. No diagnosis or treatment was involved either. The use of human serum specimens in this study was reviewed and approved by the UTMB IRB.

Cells

Vero E6 (American Type Culture Collection (ATCC), CRL-1586) cells were purchased from ATCC, and Vero E6 cells expressing TMPRSS2 (JCRB1819) purchased from SEKISUI XenoTech were maintained in a high-glucose DMEM containing 10% FBS (HyClone Laboratories) and 1% penicillin–streptomycin at 37 °C with 5% CO_2. Culture media and antibiotics were purchased from Thermo Fisher Scientific. Both cell lines were tested negative for *Mycoplasma*.

Human serum

Three panels of human sera collected at UTMB were used in the study. Samples were collected based on availability. Varied ages with both genders are included. The population contains varied races or ethnicity, including White, Hispanic, Black and Asian. Individuals have received at least two doses of the Coronavirus Disease 2019 (COVID-19) vaccine with or without evidence of SARS-CoV-2 infection. The first panel consisted of 25 sera collected from individuals 23–94 days (median 47 days) after receiving dose 4 of parental vaccine mRNA-1273 or BNT162b2. This panel had been tested negative for SARS-CoV-2 nucleocapsid protein expression using Bio-Plex Pro Human IgG SARS-CoV-2 N/RBD/S1/S2 4-Plex Panel (Bio-Rad). The second panel consisted of 29 sera collected from individuals 14–32 days (median 22 days) after BA.5 bivalent booster from Pfizer (BA.4/BA.5 adapted bivalent booster) or Moderna (bivalent booster). All sera from this panel were tested negative for antibodies against SARS-CoV-2 nucleocapsid protein. The third panel consisted of 23 sera from individuals who were previously infected by SARS-CoV-2 (as determined by SARS-CoV-2 nucleocapsid ELISA), who were vaccinated with 2–4 doses of parental mRNA vaccine and who received a BA.5 bivalent booster 14–32 days (median 21 days) before serum collection. The genotypes of the infecting SARS-CoV-2 variants could not be determined for the third serum panel. Patient information was completely de-identified from all specimens. No informed consent was required because these de-identified sera were leftover specimens from standard of care and diagnostics before being discarded. The use of human sera for this study was reviewed and approved by the UTMB IRB (no. 20-0070). The de-identified human sera were heat-inactivated at 56 °C for 30 minutes before the neutralization test. The serum information is presented in Extended Data Tables 1–3.

Generation of recombinant Omicron sublineages mNG SARS CoV-2

Recombinant Omicron sublineage BA.4/5-spike, BF.7-spike, BA.4.6-spike, BA.2.75.2-spike, BQ.1.1-spike and XBB.1-spike mNG SARS-CoV-2s was constructed by engineering the complete *spike* gene from the indicated variants into an infectious cDNA clone of mNG USA-WA1/2020, as reported previously[21,22]. Spike sequences were based on BA.4/5 (BA.4: GISAID EPI_ISL_11 542270; BA.5: GISAID EPI_ISL_11542604; BA.4 and BA.5 have the identical spike sequence), BA.4.6 (GISAID EPI_ISL_15380489), BA.2.75.2 (GISAID EPI_ISL_14458978), BF.7 (GISAID EPI_ISL_14425795), BQ.1.1 (GISAID EPI_ISL_15542649) and XBB.1 (GISAID EPI_ISL_15232105). The full-length infectious cDNA clone of SARS-CoV-2 was assembled by in vitro ligation followed by in vitro transcription to synthesize the viral genomic RNA. The full-length RNA transcripts were electroporated in Vero E6-TMPRSS2 cells to recover the viruses. Viruses were rescued 2–3 days after electroporation and served as P0 stock. P0 stock was further passaged once on Vero E6 cells to produce P1 stock. The reason for using Vero E6 cells (rather than using Vero E6-TMPRSS2) to prepare the P1 virus is that the infectivity of the P1 virus can be affected by the cell types; because our established FFRNT assay uses Vero E6 cells, we chose to prepare the P1 viruses using the same Vero E6 cells. The *spike* gene was sequenced from all P1 stock viruses to ensure no undesired mutation. The infectious titer of the P1 virus was quantified by fluorescent focus assay on Vero E6 cells. The P1 virus was used for the neutralization test. The protocols for the mutagenesis of mNG SARS-CoV-2 and virus production were reported previously[11]. All virus preparation and neutralization assays were carried out at the BSL-3 facility at UTMB at Galveston.

FFRNT

Neutralization titers of human sera were measured by FFRNT using the USA-WA1/2020-spike, BA.4/5-spike, BF.7-spike, BA.4.6-spike, BA.2.75.2-spike, BQ.1.1-spike and XBB.1-spike mNG SARS-CoV-2s at BSL-3. The details of the FFRNT protocol were reported previously[11]. In brief, 2.5×10^4 Vero E6 cells per well were seeded in 96-well plates (Greiner Bio-One). The cells were incubated overnight. On the next day, each serum was two-fold serially diluted in the culture medium with the first dilution of 1:20 (final dilution range of 1:20 to 1:20,480). The diluted serum was incubated with 100–150 FFUs of mNG SARS-CoV-2 at 37 °C for 1 hour, after which the serum virus mixtures were loaded onto the pre-seeded Vero E6 cell monolayer in 96-well plates. After 1-hour infection, the inoculum was removed, and 100 µl of overlay medium (supplemented with 0.8% methylcellulose) was added to each well. After incubating the plates at 37 °C for 16 hours, raw images of mNG foci were acquired using Cytation 7 (BioTek) armed with a ×2.5 FL Zeiss objective with a wide field of view and processed using the Gen 5 software settings (GFP (469,525) threshold 4,000 and object selection size 50–1,000 µm). The foci in each well were counted using the Gen5 software and normalized to the non-serum-treated controls to calculate the relative infectivities. The $FFRNT_{50}$ value was defined as the minimal serum dilution that suppressed >50% of fluorescent foci. The neutralization titer of each serum was determined in duplicate assays, and the geometric mean was taken. Extended Data Tables 1–3 summarize the $FFRNT_{50}$ results. Data were initially plotted in GraphPad Prism 9 software and assembled in Adobe Illustrator. $FFRNT_{50}$ of <20 was treated as 10 for plot purposes and statistical analysis. The above $FFRNT_{50}$ protocol has been reliably used to support the clinical development of COVID-19 vaccines[23]. Thus, we applied the same FFRNT protocol to the current study.

Statistics and reproducibility

No statistical method was used to predetermine the sample size. The samples were collected based on availability. No data were excluded from the analyses. The experiments were not randomized. Patient information was blinded in the study. The investigators were blinded to sample identity during data collection and/or analysis. The experiments were performed in duplication. All attempts at replication were successful.

Continuous variables were summarized as the geometric mean with 95% confidence intervals or median. Sera with undetectable (<20) antibody titers were assigned an antibody titer of 10, for purposes of GMT calculations or statistical comparisons. Comparison between neutralization titers was performed using a Wilcoxon matched-pairs signed-rank test using GraphPad Prism 9.0. Absolute P values were provided. $P < 0.05$ was considered statistically significant. Images were assembled using Adobe Illustrator.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

The raw data that support the findings of this study are shown in the Source Data files. The sequence of SARS-CoV-2 variants can be accessed through GISAID (https://gisaid.org) with the following codes: BA.4/5 (BA.4: GISAID EPI_ISL_11542270; BA.5: GISAID EPI_ISL_11542604; BA.4 and BA.5 have the identical spike sequence), BA.4.6 (GISAID EPI_ISL_15380489), BA.2.75.2 (GISAID EPI_ISL_14458978), BF.7 (GISAID EPI_ISL_14425795), BQ.1.1 (GISAID EPI_ISL_15542649) and XBB.1 (GISAID EPI_ISL_15232105). The sequence of SARS-CoV-2 mNG can be found in Supplementary Information of our previous study[14]. Source data are provided with this paper.

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Author contributions

Conceptualization: P.R., X.X. and P.-Y.S. Methodology: C.K., J.Z., H.X., M.L., H.C.C., P.R., X.X. and P.-Y.S. Investigation: C.K., J.Z., H.X., M.L., H.C.C., P.R., X.X. and P.-Y.S. Resources: P.R., X.X. and P.-Y.S. Data curation: C.K., J.Z., P.R. and X.X. Writing—original draft: P.R., X.X. and P.-Y.S. Writing—review and editing: C.K., J.Z., H.X., M.L., H.C.C., P.R., X.X. and P.-Y.S. Supervision: P.R., X.X. and P.-Y.S. Funding acquisition: P.R., X.X. and P.-Y.S.

Competing interests

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

Additional information

Extended data is available for this paper at https://doi.org/10.1038/s41591-022-02162-x.

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41591-022-02162-x.

Correspondence and requests for materials should be addressed to Ping Ren, Xuping Xie or Pei-Yong Shi.

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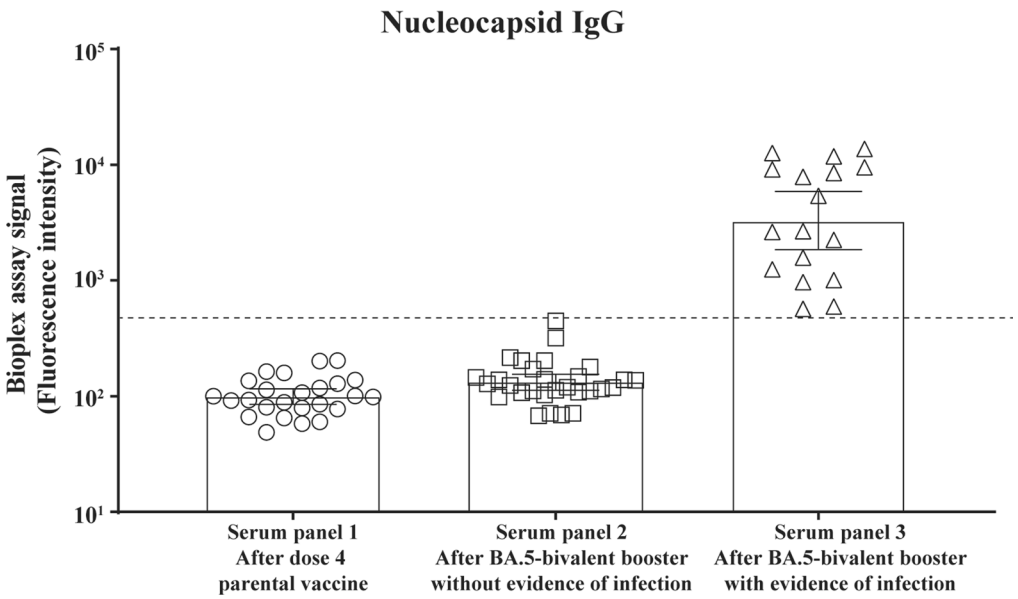

Extended Data Fig. 1 | Nucleocapsid IgG as detected by Bioplex assay. Luciferase signals indicated the IgG levels of SARS-CoV-2 nucleocapsid in serum samples. Dash line shows the limit of dection as suggested by the manufacturer. Bar height indicates the geometric mean; whiskers indicate the 95% CI.

https://doi.org/10.1038/s41591-022-02162-x

Extended Data Table 1 | Twenty-five human serum samples collected after dose 4 of the parental mRNA vaccine

Serum ID	Age (year)	Gender (F/M)	Race or Ethnicity	Serum collection time (days post-dose 4 vaccination)	Serum collection date	mRNA Vaccine type	*$FFRNT_{50}$						
							USA-WA1/2020	$BA.4/5-spike	BF.7-spike	BA.4.6-spike	BA.2.75.2-spike	BQ.1.1-spike	XBB.1-spike
1	62	F	White	37	3/16/2022	BTN162b	320	^10	10	10	10	10	10
2	80	M	Hispanic	30	4/7/2022	BTN162b	10240	320	80	80	40	20	20
3	84	F	White	55	5/11/2022	BTN162b	1280	80	14	10	10	10	10
4	92	F	White	27	5/12/2022	BTN162b	2560	113	40	40	28	10	10
5	78	M	White	56	5/17/2022	BTN162b	320	10	10	14	10	10	10
6	71	F	White	27	5/17/2022	mRNA-1273 (dose 1-3), BTN162b (dose 4)	2560	320	320	320	80	80	40
7	83	F	White	23	5/18/2022	BTN162b	1280	160	113	80	20	20	10
8	87	M	White	24	5/19/2022	BTN162b	1280	113	226	160	10	57	10
9	80	M	White	52	5/23/2022	BTN162b	5120	640	320	226	80	80	40
10	84	M	White	26	5/24/2022	BTN162b	640	20	10	10	10	10	10
11	75	M	Black	47	5/25/2022	BTN162b	1810	57	40	40	226	20	40
12	90	M	Black	34	5/25/2022	BTN162b	2560	160	80	80	40	40	20
13	59	F	Hispanic	27	5/25/2022	BTN162b (dose 1-3), mRNA-1273 (dose 4)	1810	160	113	160	10	28	10
14	72	F	White	52	6/2/2022	BTN162b	1810	40	40	40	20	14	10
15	73	M	White	94	6/3/2022	mRNA-1273	640	40	40	28	10	10	10
16	67	F	Black	50	6/7/2022	BTN162b	1280	80	80	80	20	40	10
17	75	F	White	78	6/8/2022	BTN162b	2560	453	320	320	80	80	40
18	86	M	White	49	6/9/2022	BTN162b	1810	320	226	320	40	20	20
19	66	F	White	48	6/9/2022	BTN162b	453	20	20	14	10	10	10
20	80	M	Black	44	6/10/2022	BTN162b	1280	160	160	160	40	28	20
21	78	M	White	73	6/13/2022	BTN162b	905	40	40	28	20	10	10
22	86	M	White	51	6/16/2022	BTN162b	1280	20	20	20	10	10	10
23	84	F	White	35	6/20/2022	BTN162b	20480	1280	640	453	226	160	80
24	94	F	White	47	6/26/2022	BTN162b	1280	80	40	40	20	20	10
25	87	F	Hispanic	43	6/30/2022	BTN162b	905	160	320	160	28	14	10
Median	80	-	-	47	-	-	-	-	-	-	-	-	-
#GMT	-	-	-	-	-	-	1533	95	69	62	26	22	15
†95% CI	-	-	-	-	-	-	1036-2268	56-160	41-115	38-103	17-38	16-31	12-20

*Individual $FFRNT_{50}$ value is the geometric mean of duplicate FFRNT results.
^$FFRNT_{50}$ of <20 was treated as 10 for plot purposes and statistical analysis.
#Geometric mean neutralizing titers (GMT).
†95% confidence interval (95% CI) for the GMT.
$This data set was reported previously[5].

https://doi.org/10.1038/s41591-022-02162-x

Extended Data Table 2 | Twenty-nine human serum samples collected 14–32 days after BA.5 bivalent booster without SARS-CoV-2 infection history

Serum ID	Age (year)	Gender (F/M)	Race or Ethnicity	Serum collection time (days post-BA.5-bivalent booster)	Serum collection date	Last dose of mRNA vaccine before BA.5-bivalent booster	*$FFRNT_{50}$						
							USA-WA1/2020	BA.4/5-spike	BF.7-spike	BA.4.6-spike	BA.2.75.2-spike	BQ.1.1-spike	XBB.1-spike
1	34	F	Black	15	9/30/2022	Dose 3	5120	640	1280	640	640	226	160
2	78	F	White	17	10/3/2022	Dose 4	5120	640	640	320	320	160	80
3	86	M	White	21	10/4/2002	Dose 4	1280	28	10	10	20	10	10
4	31	M	Asian	20	10/6/2022	Dose 2	5120	1280	1280	905	160	160	80
5	61	F	White	15	10/11/2022	Dose 4	5120	320	453	320	160	80	57
6	58	F	Black	14	10/11/2022	Dose 3	7241	1810	1810	1280	320	640	113
7	69	M	White	22	10/11/2022	Dose 4	2560	80	80	57	57	20	14
8	67	F	Asian	21	10/12/2022	Dose 4	20480	2560	1280	640	160	160	40
9	77	M	Asian	27	10/13/2022	Dose 4	2560	226	320	160	80	40	40
10	39	F	White	15	10/13/2022	Dose 3	5120	320	320	226	80	160	20
11	73	M	White	24	10/13/2022	Dose 4	1810	160	160	80	80	40	28
12	83	F	White	17	10/13/2022	Dose 4	1280	40	80	40	40	10	10
13	79	F	White	26	10/16/2022	Dose 3	3620	320	640	320	226	57	57
14	35	F	Asian	29	10/21/2022	Dose 3	5120	160	320	226	80	80	40
15	73	M	White	26	10/17/2022	Dose 3	7241	320	320	160	80	80	28
16	76	M	White	32	10/17/2022	Dose 4	7241	1280	905	640	226	160	160
17	71	M	White	28	10/18/2022	Dose 4	1280	40	57	40	40	20	10
18	22	M	Hispanic	19	10/18/2022	Dose 3	10240	2560	1280	1280	320	453	80
19	61	F	White	30	10/19/2022	Dose 4	640	80	80	57	20	28	14
20	56	M	White	21	10/19/2022	Dose 3	10240	640	640	453	160	160	80
21	66	F	White	26	10/19/2022	Dose 4	3620	320	320	160	80	80	40
22	76	F	White	30	10/20/2022	Dose 4	3620	226	226	160	10	80	10
23	61	F	Asian	31	10/20/2022	Dose 3	10240	5120	5120	2560	640	453	226
24	77	F	Black	31	10/20/2022	Dose 4	5120	80	80	40	40	28	10
25	59	F	unknown	28	10/21/2022	Dose 4	2560	320	320	160	160	80	28
26	71	M	Hispanic	22	10/21/2022	Dose 4	1280	160	160	80	80	80	14
27	70	F	White	22	10/21/2022	Dose 3	1280	113	57	28	20	20	10
28	79	F	White	25	10/22/2022	Dose 4	2560	226	160	80	57	10	20
29	79	M	White	18	10/22/2022	Dose 4	2560	160	320	160	226	160	80
Median	70	-	-	22	-	-	-	-	-	-	-	-	-
#GMT	-	-	-	-	-	-	3620	298	305	183	98	73	35
†95% CI	-	-	-	-	-	-	2668-4912	181-490	185-503	111-299	66-146	47-112	24-50

*Individual $FFRNT_{50}$ value is the geometric mean of duplicate FFRNT results.
#Geometric mean neutralizing titers (GMT).
†95% confidence interval (95% CI) for the GMT.

https://doi.org/10.1038/s41591-022-02162-x

Extended Data Table 3 | Twenty-three human serum samples collected 14–32 days after BA.5 bivalent booster with SARS-CoV-2 infection history

Serum ID	Age (year)	Gender (F/M)	Race or Ethnicity	Serum collection time (days post-BA.5-bivalent booster)	Serum collection date	Last dose of mRNA vaccine before BA.5-bivalent booster	*$FFRNT_{50}$						
							USA-WA1/2020	BA.4/5-spike	BF.7-spike	BA.4.6-spike	BA.2.75.2-spike	BQ.1.1-spike	XBB.1-spike
1	19	F	White	19	10/4/2022	Dose 3	10240	1280	1280	640	640	160	113
2	69	F	White	15	10/6/2022	Dose 4	10240	5120	5120	2560	905	1280	320
3	59	F	White	15	10/7/2022	Dose 3	20480	10240	10240	7241	2560	2560	453
4	46	F	White	18	10/11/2022	Dose 2	5120	2560	2560	640	320	320	160
$5	80	F	Black	22	10/11/2022	Dose 3	10240	7241	5120	3620	2560	640	320
6	76	F	Hispanic	21	10/12/2022	Dose 3	5120	1280	1280	905	453	160	113
7	67	M	White	15	10/12/2022	Dose 4	5120	2560	1280	1280	320	320	80
8	52	F	White	30	10/13/2022	Dose 4	5120	1280	1280	905	160	640	160
9	48	F	Asian	24	10/13/2022	Dose 3	14482	7241	5120	2560	1280	640	320
10	58	F	White	28	10/13/2022	Dose 2	5120	640	640	320	160	226	40
11	67	F	Black	25	10/14/2022	Dose 2	5120	2560	2560	1280	640	640	160
12	20	F	Hispanic	21	10/14/2022	Dose 2	1810	320	453	160	80	160	28
$13	75	M	White	21	10/14/2022	Dose 3	20480	2560	2560	1280	1280	453	320
14	64	F	White	19	10/4/2022	Dose 4	7241	2560	2560	1280	2560	453	320
15	90	F	White	22	10/18/2022	Dose 3	10240	1280	1280	1280	320	640	160
16	35	F	Hispanic	32	10/18/2022	Dose 2	1280	320	226	160	80	80	28
$17	39	M	Asian	31	10/19/2022	Dose 3	10240	5120	2560	2560	640	320	80
$18	67	M	White	17	10/19/2022	Dose 4	10240	2560	1810	1280	640	640	226
$19	68	M	Hispanic	14	10/19/2022	Dose 3	5120	2560	1280	905	320	160	80
20	51	M	White	28	10/21/2022	Dose 3	7241	905	320	320	320	160	57
21	65	M	Asian	29	10/20/2022	Dose 4	2560	453	160	80	113	57	40
22	64	M	White	16	10/21/2022	Dose 3	2560	640	640	320	40	40	20
$23	73	M	Black	31	10/22/2022	Dose 4	640	57	40	28	20	10	10
Median	64	-	-	21	-	-	-	-	-	-	-	-	-
#GMT	-	-	-	-	-	-	5776	1558	1223	744	367	267	103
†95% CI	-	-	-	-	-	-	3994-8352	922-2631	704-2125	425-1301	209-644	158-452	66-162

*Individual $FFRNT_{50}$ value is the geometric mean of duplicate FFRNT results.
#Geometric mean neutralizing titers (GMT).
†95% confidence interval (95% CI) for the GMT.
$Reported as SARS-CoV-2 RT-PCR positive. Nucleocapsid testing was not performed.

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☒	☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
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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	Cytation 7 with Gen5 software
Data analysis	GraphPad Prism 9.0, Adobe Illustrator

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.

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The raw data that support the findings of this study are shown in the Source data files. The sequence of SARS-CoV-2 variants can be accessed through GISAID (https://gisaid.org) with the following codes: BA.4/5 (BA.4: GISAID EPI_ISL_11 542270; BA.5: GISAID EPI_ISL_11542604; BA.4 and BA.5 have the identical spike sequence), BA.4.6 (GISAID EPI_ISL_15380489), BA.2.75.2 (GISAID EPI_ISL_14458978), BF.7 (GISAID EPI_ISL_14425795), BQ.1.1 (GISAID EPI_ISL_15542649) and XBB.1 (GISAID EPI_ISL_15232105). The sequence of SARS-CoV-2 mNG can be found in the supplementary information of our previous study.

Human research participants

Policy information about studies involving human research participants and Sex and Gender in Research.

Reporting on sex and gender	Human serum samples were collected based on availability. Both gender are included.
Population characteristics	Samples were collected based on availability. Varied ages with both genders are included. The population contains varied races or ethnicity, including white, Hispanic, black, and Asian. Subjects have received at least two doses of the COVID-19 vaccine with or without evidence of SARS-CoV-2 infection.
Recruitment	No patients were recruited in this study. No informed consent was required, because these deidentified sera were leftover specimens before being discarded. No diagnoses or treatment was involved either.
Ethics oversight	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).

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

Field-specific reporting

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Life sciences study design

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

Sample size	No sample size calculation was performed. Samples were collected based on the availability.
Data exclusions	No data was excluded in the study.
Replication	Each human serum sample was analyzed in duplication. The averaged results from the duplication were reported in this study. All attempts at replication were successful.
Randomization	This is no randomization in this study. All samples available were analyzed for the neutralizing activities against WT SARS-CoV-2 and variants in the same experimental settings.
Blinding	Patient information was blinded in the study. The investigators were blinded to sample identity during data collection and/or analysis.

Reporting for specific materials, systems and methods

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☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

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

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	Vero E6 cells (ATCC® CRL-1586) were obtained from ATCC; VeroE6-TMPRSS2 cells were Vero E6 cells expressing TMPRSS2 purchased from SEKISUI XenoTech, LLC.
Authentication	VeroE6 Cells have been authenticated by ATCC using morphologies and other groups using STR profiling (reference: Almeida JL, Hill CR, Cole KD. VeroE6-TMPRSS2 cells have been authenticated by the vendor. Authentication of African green monkey cell lines using human short tandem repeat markers. BMC Biotechnol. 2011;11:102. Published 2011 Nov 7. doi:10.1186/1472-6750-11-102).
Mycoplasma contamination	All cell lines were tested negative for mycoplasma.
Commonly misidentified lines (See ICLAC register)	None.