Title: Exposure to BA.4/BA.5 Spike glycoprotein drives pan-Omicron neutralization in vaccine-experienced humans and mice

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Abstract

The SARS-CoV-2 Omicron variant and its sublineages show pronounced viral escape from neutralizing antibodies elicited by vaccination or prior SARS-CoV-2 variant infection owing to over 30 amino acid alterations within the spike (S) glycoprotein. We and others have recently reported that breakthrough infection of vaccinated individuals with Omicron sublineages BA.1 and BA.2 are associated with distinct patterns of cross-neutralizing activity against SARS-CoV-2 variants of concern (VOCs). BA.2 breakthrough infection mediated overall stronger cross-neutralization of BA.2 and its descendants (BA.2.12.1, BA.4, and BA.5) compared to BA.1 breakthrough infection. Here we characterized the effect of Omicron BA.4/BA.5 S glycoprotein exposure on the magnitude and breadth of the neutralizing antibody response upon breakthrough infection in vaccinated individuals and in mice upon booster vaccination. We show that immune sera from triple mRNA-vaccinated individuals with subsequent Omicron BA.4/BA.5 breakthrough infection display broad and robust neutralizing activity against Omicron BA.1, BA.2, BA.2.12.1, and BA.4/BA.5. Administration of a prototypic BA.4/BA.5-adapted mRNA booster vaccine to mice following SARS-CoV-2 wild-type strain-based primary immunization is associated with similarly broad neutralizing activity. Immunization of naïve mice with a bivalent mRNA vaccine (wild-type + Omicron BA.4/BA.5) induces strong and broad neutralizing activity against Omicron VOCs and previous variants. These findings suggest that when administered as boosters, mono- and bivalent Omicron BA.4/BA.5-adapted vaccines may enhance neutralization breadth, and in a bivalent format may also have the potential to confer protection to individuals with no pre-existing immunity against SARS-CoV-2.

Introduction

45   SARS-CoV-2 Omicron and its sublineages have had a major impact on the epidemiological

landscape of the COVID-19 pandemic since initial emergence in November 2021 (*1, 2*).

Significant alterations in the spike (S) glycoprotein of the first Omicron variant BA.1 leading to

the loss of many neutralizing antibody epitopes (*3*) rendered BA.1 capable of partially escaping

previously established SARS-CoV-2 wild-type strain (Wuhan-Hu-1)-based immunity (*4-6*).

50   Hence, breakthrough infection of vaccinated individuals with Omicron are more common than

with previous VOCs. While Omicron BA.1 was displaced by the BA.2 variant in many countries

around the globe, other variants such as BA.1.1 and BA.3 temporarily and/or locally gained

momentum but did not become globally dominant (*7-9*). Omicron BA.2.12.1 subsequently

displaced BA.2 to become dominant in the United States, whereas BA.4 and BA.5 displaced

55   BA.2 in Europe, parts of Africa, and Asia/Pacific (*8, 10-12*). Currently, Omicron BA.5 is

dominant globally, including in the United States (*13*).

Omicron has acquired numerous alterations (amino acid exchanges, insertions, or deletions) in

the S glycoprotein, among which some are shared between all Omicron VOCs while others are

specific to one or more VOCs (fig S1). Antigenically, BA.2.12.1 exhibits high similarity with

60   BA.2 but not BA.1, whereas BA.4 and BA.5 differ considerably from their ancestor BA.2 and

even more so from BA.1, in line with their genealogy (*14*). Major differences of BA.1 from the

remaining Omicron VOCs include Δ143-145, L212I, or ins214EPE in the S glycoprotein N-

terminal domain and G446S or G496S in the receptor binding domain (RBD). Amino acid

changes T376A, D405N, and R408S in the RBD are in turn common to BA.2 and its descendants

65   but not found in BA.1. In addition, some alterations are specific for certain BA.2-descendant

VOCs, including L452Q for BA.2.12.1 or L452R and F486V for BA.4 and BA.5 (BA.4 and

BA.5 encode for the same spike sequence). Most of these shared and VOC-specific alterations were shown to play an important role in immune escape from monoclonal antibodies and polyclonal sera raised against the wild-type S glycoprotein. The BA.4/BA.5-specific alterations

70 in particular are strongly implicated in immune escape of these VOCs (*15, 16*).

We and others have previously reported that Omicron BA.1 and BA.2 breakthrough infection of individuals previously immunized with wild-type strain-based mRNA vaccines or an inactivated virus vaccine was associated with potent neutralizing activity against Omicron BA.1, BA.2 and previous VOCs (*16-21*). A considerable boost of Omicron BA.2.12.1 and BA.4/BA.5

75 neutralization was only evident in BA.2 breakthrough cases, albeit with titers against BA.4/BA.5 remaining lower than titers against previous Omicron VOCs (*16, 20-22*). Analyses of immune sera from clinical trial participants showed that sera from SARS-CoV-2 naïve individuals vaccinated with an Omicron BA.1-adapted mRNA vaccine as a 4th dose showed superior neutralizing responses against BA.1 compared to sera from individuals boosted with the

80 respective wild-type strain-based mRNA vaccine BNT162b2 (*23*) or mRNA-1273 (*24*). However, the BA.1-adapted booster was not associated with increased BA.4/BA.5 cross-neutralization, as responses against BA.4/BA.5 were approximately three-fold and six-fold lower than responses against BA.1 and the wild-type strain, respectively, in sera from both BA.1 and wild-type-boosted individuals (*24*).

85 Given the current global predominance of the highly contagious Omicron BA.5, we investigated whether exposure to Omicron BA.4/BA.5 S glycoprotein would trigger a broader neutralizing antibody response against relevant Omicron VOCs. We assessed the breadth of neutralizing activity against Omicron VOCs in immune sera from vaccinated individuals with Omicron BA.4/BA.5 breakthrough infection and in sera from mice that received Omicron BA.4/BA.5-

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90   adapted booster vaccines following primary immunization with BNT162b2 (Fig. 1a, b). We also

evaluated the breadth of neutralizing activity following immunization of SARS-CoV-2 naïve

mice with Omicron BA.4/BA.5-adapted vaccines (Fig 1c). We found that exposure to Omicron

BA.4/BA.5 S glycoprotein mediates pan-Omicron neutralization in humans and mice, with

robust neutralization of all currently or previously predominant Omicron VOCs. We believe our

95   data increases current understanding on Omicron immune escape mechanisms and the effects of

immunization on variant cross-neutralization, and may guide selection of vaccination strategies.

Results

Omicron BA.4/BA.5 breakthrough infection of triple mRNA-vaccinated individuals results in pan-Omicron neutralizing activity

We investigated the effect of Omicron BA.4/BA.5 breakthrough infection of individuals vaccinated with three prior doses of mRNA COVID-19 vaccine (BNT162b2/mRNA-1273 homologous or heterologous regimens) on serum neutralizing activity against SARS-CoV-2 variants (mRNA-Vax[3] + BA.4/BA.5, n=17, Fig. 1a and S2, Tables S1 and S2). Three cohorts were included for reference: triple mRNA vaccinated individuals with an Omicron BA.2 breakthrough infection (mRNA-Vax[3] + BA.2, n=19) or with an Omicron BA.1 breakthrough infection (mRNA-Vax[3] + BA.1, n=14), and BNT162b2 triple-vaccinated individuals who were SARS-CoV-2-naïve at the time of sampling (BNT162b2[3], n=18, fig. S2 and Table S1). Sera were obtained from a non-interventional study researching vaccinated patients that had experienced Omicron breakthrough infection, and from the biosample collections of BNT162b2 vaccine trials. Data for the reference cohorts were previously published and are used here for benchmarking (*17, 21*).

Serum neutralizing activity was tested in a well-characterized pseudovirus neutralization test (pVNT) (*21, 25, 26*) by determining 50% pseudovirus neutralization (pVN_{50}) geometric mean titers (GMTs) with pseudoviruses bearing the S glycoproteins of the SARS-CoV-2 wild-type strain or Omicron BA.1, BA.2, and the BA.2-derived sublineages BA.2.12.1, and BA.4/BA.5 (BA.4 and BA.5 are identical in their S glycoprotein sequence). In addition, we assayed SARS-CoV (herein referred to as SARS-CoV-1) to detect potential pan-Sarbecovirus neutralizing activity (*27*). As an orthogonal test system, we used a live SARS-CoV-2 neutralization test (VNT) that analyzes neutralization during multicycle replication of authentic virus (SARS-CoV-

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2 wild-type strain and Omicron VOCs BA.1, BA.2, and BA.4) with immune serum present during the entire test period.

In the pVNT, sera from the Omicron BA.4/BA.5 breakthrough infection cohort (mRNA-Vax[3] + BA.4/BA.5) robustly neutralized the wild-type strain and all tested Omicron VOCs (Fig. 2a). The pVN$_{50}$ GMTs against Omicron BA.2 and BA.2.12.1 pseudoviruses were within a 2-fold range of the GMT against the wild-type strain (GMTs 613 against Omicron vs. GMT 1085 against wild-type). Neutralization of BA.1 and BA.4/5 (GMTs 500-521) was broadly similar to that of BA.2, and the reduction relative to the wild-type strain significant (p<0.05) yet also within a ~2-fold range. The GMT against SARS-CoV-1 was significantly lower (p<0.0001; >50-fold lower than wild-type).

To compare mRNA-Vax[3] + BA.4/BA.5 to the reference cohorts with Omicron BA.1 or BA.2 breakthrough infection (mRNA-Vax[3] + BA.1 and mRNA-Vax[3] + BA.2) and triple BNT162b2-vaccinated SARS-CoV-2 naïve individuals (BNT162b2[3]), we normalized the VOC pVN$_{50}$ GMTs against the wild-type strain to allow for assessment of neutralization breadth irrespective of the magnitude of antibody titers, which expectedly differs between triple-vaccinated individuals with a breakthrough infection and triple-vaccinated individuals without infection (*17, 21*). While BNT162b2[3] sera mediated considerable cross-neutralization of Omicron BA.1 and BA.2, breakthrough infection with Omicron BA.1 was associated with significantly (p<0.05) higher neutralization of the homologous strain (Fig 2b). A similar trend only short of statistical significance (p=0.06) was observed for BA.2 neutralization after BA.2 breakthrough infection. Importantly, cross-neutralization of BA.4/5 was significantly (p<0.01) stronger in the mRNA-Vax[3] + BA.4/5 cohort (GMT ratio 0.48) compared to BNT162b2[3] and to mRNA-Vax[3] + BA.1 (GMT ratios 0.17 and 0.18, respectively). Cross-neutralization of BA.4/5 was also stronger than

in the mRNA-Vax^3 + BA.2 cohort but the difference was less pronounced (GMT ratios 0.48

145   versus 0.37, respectively) and not statistically significant. Cross-neutralization of Omicron BA.1

and BA.2 was maintained at comparatively higher levels in the mRNA-Vax^3 + BA.4/BA.5

cohort (GMT ratios 0.46 and 0.57, respectively). In conclusion, BA.4/BA.5 breakthrough

infection resulted in the most efficient cross-neutralization across all tested VOCs (GMT ratios

≥0.46) of all cohorts evaluated.

150   The authentic live SARS-CoV-2 virus neutralization assay largely confirmed the pVNT assay

findings shown in Fig. 2a-b. 50% virus neutralization (VN_{50}) GMT in Omicron BA.4/BA.5

breakthrough sera against BA.2 was comparable to that against the wild-type strain (Fig 2c).

BA.4 and BA.1 neutralization were significantly reduced yet also within a 2 to 2.5-fold range.

The GMTs normalized against the wild-type strain showed robust cross-neutralization of

155   Omicron BA.1, BA.2, and BA.4 by mRNA-Vax^3 + BA.4/BA.5 sera (GMT ratios 0.39, 0.73, and

0.52, respectively), whereas BA.4 cross-neutralization was considerably less efficient in mRNA-

Vax^3 + BA.1 (GMT ratio 0.20) and mRNA-Vax^3 + BA.2 (GMT ratio 0.39) sera (Fig 2d) (*17,*

21). In summary, our findings in both the pVNT and the VNT assay system showed that

Omicron BA.4/BA.5 breakthrough infection was associated with broad neutralizing activity

160   against all Omicron VOCs tested.

Booster immunization with an Omicron BA.4/BA.5 S glycoprotein adapted mRNA vaccine

drives pan-Omicron neutralization in BNT162b2 double-vaccinated mice

The heightened neutralization breadth seen after Omicron BA.4/BA.5 breakthrough infection

suggested that variant-adapted vaccines based on the Omicron BA.4/5 S glycoprotein sequence

165   elicit a recall response with broader cross-neutralization than those based on Omicron BA.1. To

test this hypothesis, we performed booster studies in BNT162b2-preimmunized mice (Fig. 1b).

Mice were administered a primary 2-dose series of BNT162b2 on days 0 and 21 and a third dose of either BNT162b2, or a variant-adapted prototypic vaccine encoding BA.4/BA.5 S glycoprotein (fig S3) on day 104 (3.5 months after dose 2). A variant-adapted vaccine encoding

170   Omicron BA.1 S glycoprotein was included for comparison. The variant adapted vaccines were either monovalent encoding Omicron BA.1 or BA.4/5 S glycoprotein (1 µg), or bivalent combining either of these Omicron variant S glycoprotein sequences with BNT162b2 (0.5 µg each). Neutralizing titers against pseudoviruses expressing the wild-type strain, Omicron BA.1, BA.2, BA.2.12.1, or BA.4/5 S glycoprotein were determined in pVNT assays using sera drawn

175   before the booster (day 104, pre-dose 3[D3]) and on days 7, 21, and 35 after the 3rd dose (d7D3, d21D3 and d35D3). The live SARS-CoV-2 neutralization test was used as an orthogonal test system to confirm the observed pseudovirus neutralizing activity post-boost on d35D3.

In sera drawn on pre-D3, pVN_{50} GMTs of the groups dedicated for the various boosters against all tested variants were largely comparable (fig. S5a). pVN_{50} GMTs against Omicron BA.1 and

180   BA.2 were 3 to 11-fold lower than those against the wild-type strain (GMT ratios ≤ 0.32, fig. S5b). GMTs against BA.2.12.1 and BA.4/5 were 10 to 25-fold lower than those against wild-type (GMT ratios ≤ 0.10).

On d7D3, neutralizing GMTs had increased substantially across groups and against all tested variants (fig. S6) with peak titers reached on d21D3 (Fig. 3). In sera from BNT162b2 boosted

185   mice, strong neutralizing activity against the wild-type strain was observed, whereas pVN_{50} GMTs against Omicron variants were substantially lower (Fig 3a). The Omicron BA.1 booster substantially increased neutralization titers against BA.1 and the wild-type strain, while the pVN_{50} GMTs against the remaining VOCs were considerably lower. In particular, GMTs against BA.4/5 were reduced 13-fold compared to those against wild-type. In contrast, administration of

190 the BA.4/5 booster elicited broad neutralizing activity against all Omicron variants and the wild-type strain. Sera from mice that received the BNT162b2/BA.1 bivalent booster had a high pVN_{50} GMT against the wild-type strain, and robust neutralization of Omicron BA.1, whereas the GMTs against BA.2 and its descendants were slightly lower. The BNT162b2/Omicron BA.4/5 bivalent booster gave rise to high titers against the wild-type strain, comparable to the

195 BNT162b2 monovalent booster. Omicron neutralization was broadly comparable across all VOCs, with pVN_{50} GMTs modestly below that against the wild-type strain.

We evaluated the fold-changes in pVN_{50} GMTs detected on d21D3 relative to the baseline GMTs determined before administration of the third dose. BNT162b2 comparably increased neutralization of all tested variants (pVN_{50} GMTs 6 to 10-fold higher than at baseline), whereas

200 the most pronounced effect of the BA.1 booster was detected for the neutralization of the homologous VOC (26-fold increase) (Fig 3b). The BA.4/5 booster strongly potentiated neutralizing activity against BA.2.12.1 and BA.4/5 (>120-fold) and had a substantial effect on BA.1 and BA.2 neutralization (increases of 34 and 37-fold, respectively). The bivalent vaccines (BNT162b2/BA.1 and BNT162b2/BA.4/5) showed similar response patterns as the BA.1 and

205 BA.4/5 monovalent vaccines, respectively, albeit with less focused increases in neutralization against the homologous VOCs.

To compare the groups with regard to neutralization breadth irrespective of the magnitude, we normalized the VOC pVN_{50} GMTs against the wild-type strain and showed that the Omicron BA.4/5 booster vaccine mediated pan-Omicron neutralization (≥0.65 for all tested variants) (Fig.

210 3c). In contrast, the BA.1 booster vaccine robustly neutralized BA.1 (GMT ratio 0.77), whereas ratios for BA.2 (0.39) and especially for BA.2.12.1 and BA.4/5 (≤0.16) were substantially lower. The bivalent BNT162b2/BA.4/5 vaccine also mediated broad neutralizing activity with enhanced

cross-neutralization of BA.2, BA.2.12.1 and BA.4/5 compared to the BNT162b2/BA.1 bivalent

vaccine, albeit with lower GMT ratios (between 0.27 and 0.46) than the BA.4/5 monovalent

215   vaccine.

While absolute titers were slightly lower on d7D3 compared to d21D3, the Omicron BA.4/5

vaccine mediated a similarly broad neutralizing activity against all variants at this early time

point (fig. S6). BNT162b2/BA.4/5 bivalent booster mediated comparable neutralization breadth

(GMT ratios ≥0.35), whereas sera from all other booster groups exhibited considerably lower

220   cross-neutralization of BA.2, BA.2.12.1 and BA.4/5. Similarly, d35D3 sera showed substantial

increases in neutralizing activity against Omicron variants by the BA.4/5 vaccine booster

compared to baseline, resulting in pan-Omicron neutralization (fig S7a-c). The authentic live

SARS-CoV-2 virus neutralization assay largely confirmed the major d35D3 pVNT assay

findings. Sera from both groups boosted with BA.4/5-containing vaccines exhibited substantially

225   stronger cross-neutralization of all tested Omicron variants compared to the BA.1-containing

vaccines and BNT162b2 (fig S7d-e).

Taken together, these findings suggest that a booster with an Omicron BA.4/5 S glycoprotein

adapted vaccine following primary immunization with a wild-type strain-based vaccine may

elicit superior pan-Omicron neutralizing activity compared to BA.1 S glycoprotein-based

230   booster.

Immunization with an Omicron BA.4/BA.5 S glycoprotein adapted mRNA vaccine drives pan-Omicron neutralization in previously unvaccinated mice

We sought to understand the impact of immunization with the Omicron-adapted vaccine on the

serum neutralizing capacity of mice with no pre-existing immune response against SARS-CoV-

235   2. Naïve mice were immunized twice, at day 0 and day 21 with either BNT162b2, with BA.1 or

BA.4/5 S glycoprotein adapted monovalent vaccines, or the bivalent BNT162b2/BA.4/5 vaccine (Fig. 1c). Neutralizing titers against pseudoviruses expressing the wild-type strain S glycoprotein, Alpha or Delta, or Omicron BA.1, BA.2, BA.2.12.1, or BA.4/5 were determined in pVNT assays using sera drawn 14 days after the second immunization (d14D2). In sera from

240 BNT162b2 immunized mice, robust neutralizing activity against the wild-type strain, Alpha, and Delta was observed, whereas pVN_{50} GMTs against Omicron variants were substantially (14 to 37-fold) lower than against wild-type (Fig. 4). Immunization with the Omicron BA.1 monovalent vaccine led to high neutralization of Omicron BA.1. In these sera, robust neutralization of Omicron BA.2 and BA.2.12.1 was also detected, while the pVN_{50} GMTs against the wild-type

245 strain and remaining VOCs were considerably (7 to 32-fold) lower than BA.1. Immunization with the Omicron BA.4/5 monovalent vaccine led to high neutralization of BA.4/5. In these sera, robust neutralization of Omicron BA.2 and BA.2.12.1 was also detected, while the pVN_{50} GMTs against the wild-type strain and previous VOCs were considerably (14 to >42-fold) lower than BA.4/5. Immunization with the BNT162b2/BA.4/5 bivalent vaccine resulted in high neutralizing

250 activity not only against BA.4/5, but also against the wild-type strain and all remaining VOCs (within a 6-fold range of BA.4/5).

These results show that a monovalent vaccine in naïve animals induces a high neutralizing antibody response mostly in a variant-specific manner, whereas a bivalent vaccine provided enhanced neutralization breadth across the wild-type strain and VOCs.

Discussion

Here, we report that BA.4/BA.5 breakthrough infection of triple-mRNA vaccinated individuals is associated with robust neutralization of all currently or previously predominant Omicron VOCs, i.e., pan-Omicron neutralization. Our findings are consistent with a recent report showing strong cross-neutralization of Omicron BA.1, BA.2, Beta and Delta in sera from individuals vaccinated with BNT162b2 or an adenovirus-based vaccine and subsequent BA.4 breakthrough infection (*28*). In line with those observations in humans, we also found pan-Omicron neutralizing activity in sera of mice that received an Omicron BA.4/5 booster vaccine following primary immunization with BNT162b2, whereas an Omicron BA.1 boost induced lower levels of neutralizing antibody titers against BA.4/BA.5. A bivalent BNT162b2/BA.4/5 boost elicited broad Omicron neutralization, albeit less pronounced than the BA.4/5 monovalent booster. Together these findings add to our current understanding on how breakthrough infections or vaccine boosters adapted to Omicron VOCs in a mono- or bivalent format shape immunity and suggest that exposure to Omicron BA.4/5 S glycoprotein may confer heightened protection against the currently circulating and potential future Omicron VOCs. Our finding that immunization of naïve mice with the BNT162b2/BA.4/5 bivalent vaccine elicits strong neutralizing antibody responses against the wild-type strain as well as Omicron and non-Omicron VOCs suggest that this bivalent approach may confer broad protection to unvaccinated individuals who have not been previously infected with SARS-CoV-2. These findings could inform future considerations for pediatric COVID-19 vaccination strategies.

BA.4/BA.5 breakthrough infection data sets are based on retrospective analyses using relatively small sample sizes and cohorts that are not fully aligned in terms of intervals between vaccine doses, intervals between the most recent vaccine dose and infection, and demographic

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characteristics such as age and sex of individuals. We used GMT ratios to report on

neutralization breadth to allow for interpretation independently of the magnitude of neutralizing

280   antibody titers, which could be affected by factors such as vaccination/infection intervals. In the

authentic live SARS-CoV-2 neutralization assay setting, such inter-assay response comparisons

across viral variants may fail to account for variant-intrinsic differences in cell entry or

replication. However, our interpretation of neutralization breadth is based on findings that were

consistent also in the pseudovirus neutralization assay, where the variant S glycoprotein used for

285   pseudotyping, i.e. the primary target of neutralizing antibodies, is the only variable. While the

mouse studies were conducted with a relatively small sample size and showed moderate

variability in some analyses, the main results of the booster study in mice were again consistent

across pseudovirus and live SARS-CoV-2 neutralization assay platforms. Further studies

investigating long-lived plasma cell, memory B cell, and T cell immunity could provide further

290   insights into the mechanisms underlying neutralization breadth of monovalent and bivalent

Omicron BA.4/5 vaccines.

While currently approved SARS-CoV-2 wild-type strain-based vaccines such as BNT162b2 have

proven effective at protecting from severe disease (*29-31*), prevention of transmission remains a

significant challenge as new variants continue to emerge that are antigenically distant from the

295   wild-type virus (*16-19*). Our data suggest that a mono- or bivalent BA.4/BA.5 S glycoprotein

adapted booster vaccine may confer higher benefit against the highly prevalent BA.4 and BA.5

VOCs than a vaccine based on a previously dominant Omicron variant such as BA.1. Given the

global predominance and high transmissibility of Omicron BA.5 (*8, 10, 11, 13*), it is possible that

new variants with further growth advantage emerge that may retain partial or full susceptibility

300   to an Omicron BA.4/BA.5-adapted vaccine. Boosting with an Omicron BA.4/5 S glycoprotein-

based adapted vaccine could represent a suitable strategy to address both current circulating

SARS-CoV-2 and potential future variants. A bivalent wild-type/Omicron BA.4/5-adapted

booster may at the same time confer broader neutralization of potential new variants that are

antigenically distinct from Omicron but closer to the wild-type strain. Close monitoring of virus

305   evolution and epidemiological landscapes remains instrumental for guidance on the potential

need for further vaccine adaptations in response to emerging threats.

Materials and Methods

Human study design, recruitment of participants and sample collection

310   The objective of this study was to investigate the effect of Omicron BA.4/BA.5 breakthrough

infection on the cross-variant neutralization capacity of human sera. We assessed neutralizing

activity in immune sera from triple-mRNA (BNT162b2/mRNA-1273)-vaccinated individuals

with a confirmed subsequent SARS-CoV-2 breakthrough infection, which either occurred in a

period of Omicron BA.4/BA.5 lineage-dominance in Germany (mid-June to mid-July 2022;) or

315   was variant-confirmed (BA.4 or BA.5) by genome sequencing (mRNA-Vax[3] + BA.4/5) (Fig. S2

and tables S1 and S2). We compared the neutralizing activity in immune sera of mRNA-Vax[3] +

BA.4/5 to neutralizing activity in immune sera from of triple mRNA vaccinated individuals with

a confirmed subsequent SARS-CoV-2 breakthrough infection in a period of Omicron BA.2

lineage-dominance (March to May 2022; mRNA-Vax[3] + BA.2), a period of Omicron BA.1

320   lineage-dominance in Germany (November 2021 to mid-January 2022; mRNA-Vax[3] + BA.1) (*1,*

2), and triple-BNT162b2-vaccinated individuals that were SARS-CoV-2-naïve (nucleocapsid

seronegative) at the time of sample collection (BNT162b2[3]). Serum neutralizing capability was

characterized using pseudovirus and live SARS-CoV-2 neutralization assays. Data for the

reference cohorts mRNA-Vax[3] + BA.2, mRNA-Vax[3] + BA.1, and BNT162b2[3] were previously

325   published (*17, 21*).

Participants from the mRNA-Vax[3] + Omi BA.4/5, mRNA-Vax[3] + Omi BA.2, and mRNA-Vax[3]

+ BA.1 cohorts were recruited from University Hospital, Goethe University Frankfurt as part of a

non-interventional study (protocol approved by the Ethics Board of the University Hospital [No.

2021-560]) researching patients that had experienced Omicron breakthrough infection following

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330   vaccination for COVID-19. Individuals from the BNT162b2[3] cohort provided informed consent

as part of their participation in the Phase 2 trial BNT162-17 (NCT05004181).

The infections of 5 BA.4/5 convalescent, 1 BA.2 convalescent, and 4 BA.1 convalescent

participants in this study were confirmed by genome sequencing (Table S2 and (*17, 21*)).

All participants had no documented history of SARS-CoV-2 infection prior to vaccination.

335   Participants were free of symptoms at the time of blood collection.

Serum was isolated by centrifugation of drawn blood at 2000 x g for 10 minutes and

cryopreserved until use.

In vitro transcription and lipid-nanoparticle (LNP) formulation of the RNA

340   The BNT162b2 vaccine was designed on a background of S sequences from SARS-CoV-2

isolate Wuhan-Hu-1 (GenBank: MN908947.3) with pre-fusion conformation-stabilizing K986P

and V987P mutations. Omicron BA.1 and Omicron BA.4/5 vaccine candidates were designed

based on BNT162b2 including sequence changes as shown in fig. S3. RNA production as well as

formulation were performed as described elsewhere (*32*). In brief, DNA templates were cloned

345   into a plasmid vector with backbone sequence elements (T7 promoter, 5′ and 3′ UTR, 100

nucleotide poly(A) tail) interrupted by a linker (A30LA70, 10 nucleotides) for improved RNA

stability and translational efficiency (*33, 34*), amplified via PCR and purified. RNA was *in vitro*

transcribed by T7 RNA polymerase in the presence of a trinucleotide cap1 analogue ((m27,3'-

O)Gppp(m2'-O)ApG; TriLink) and with N1-methylpseudouridine-5'-triphosphate (m1ΨTP;

350   Thermo Fisher Scientific) replacing uridine-5'-triphosphate (UTP) (*35*). RNA was purified using

magnetic particles (*36*). RNA integrity was assessed by microfluidic capillary electrophoresis

(Agilent 2100 Bioanalyzer) and all three RNAs show single sharp peaks demonstrating

comparable and high purity as well as integrity (fig. S4a). In addition, the concentration, pH, osmolality, endotoxin level and bioburden of the solution were determined.

355　One ionizable lipid ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate)), two structural lipids (1,2-distearoyl-sn-glycero-3-phosphocholine [DSPC] and cholesterol) and one PEGylated lipid (2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide) were used for formulation of RNA. After transfer into an aqueous buffer system via diafiltration, the LNP compositions were analyzed to ensure high RNA integrity and encapsulation efficacy, as well as

360　a particle size below 100 nm. The vaccine candidates were stored at -70 to -80 °C at a concentration of 0.5 mg/mL until time of use.

In vitro expression of RNAs and vaccines

HEK293T cells were transfected with 0.15 μg BNT162b2 or Omicron-adapted vaccines (lipid-

365　nanoparticle-formulated), or with vaccine RNAs using RiboJuice™ mRNA Transfection Kit (Merck Millipore) according to the manufacturer's instructions and incubated for 18 h. Transfected HEK293T cells were stained with Fixable Viability Dye (eBioscience) and incubated with mouse Fc-tagged recombinant human ACE-2 (Sino Biological). A secondary donkey anti-mouse antibody conjugated with AF647 was used for detection of surface

370　expression. Cells were fixed (Fixation Buffer, Biolegend) prior to flow cytometry analysis using a FACSCelesta flow cytometer (BD Biosciences, BD FACSDiva software version 8.0.1) and FlowJo software version 10.6.2 (FlowJo, BD Biosciences).

Mouse studies

375   All mouse studies were performed at BioNTech SE, and protocols were approved by the local

authorities (local welfare committee) and conducted according to Federation of European

Laboratory Animal Science Associations recommendations. Study execution and housing were

in compliance with the German Animal Welfare Act and Directive 2010/63/EU. Mice were kept

in individually ventilated cages with a 12-h light/dark cycle, controlled environmental conditions

380   (22 ± 2 °C, 45% to 65% relative humidity) and under specific-pathogen-free conditions. Food

and water were available ad libitum. Only mice with an unobjectionable health status were

selected for testing procedures.

For immunization, female BALB/c mice (Janvier) (9–21 weeks old) were randomly allocated to

groups. BNT162b2 and Omicron-based vaccines candidates were diluted in 0.9% NaCl and 1 µg

385   of the vaccine candidate was injected into the gastrocnemius muscle at a volume of 20 µl under

isoflurane anesthesia. For the mouse booster study, mice were immunized twice (day 0 and 21)

with BNT162b2. Third immunization with BNT162b2 and Omicron-based vaccine candidates

occurred at day 104 after study start. Peripheral blood was collected from the vena facialis

without anesthesia for interim bleedings shortly before (same day) the third immunization, and 7

390   and 21 days after the third immunization. Final bleeding was performed under isoflurane

anesthesia from the retro-orbital venous plexus at day 35 after the third immunization. For the

naïve mouse study, animals were immunized at day 0 and 21 with BNT162b2 and Omicron-

based vaccines candidates. 14 days after second immunization, blood was drawn. For serum

generation, blood was centrifuged for 5 min at 16,000xg and the serum was immediately used for

395   downstream assays or stored at −20 °C until time of use.

19

VSV-SARS-CoV-2 S variant pseudovirus generation

A recombinant replication-deficient vesicular stomatitis virus (VSV) vector that encodes green fluorescent protein (GFP) and luciferase instead of the VSV-glycoprotein (VSV-G) was

400 pseudotyped with SARS-CoV-1 S glycoprotein (UniProt Ref: P59594) or with SARS-CoV-2 S glycoprotein derived from either the wild-type strain (Wuhan-Hu-1, NCBI Ref: 43740568), the Alpha variant (alterations: Δ69/70, Δ144, N501Y, A570D, D614G, P681H, T716I, S982A, D1118H), the Delta variant (alterations: T19R, G142D, E156G, Δ157/158, K417N, L452R, T478K, D614G, P681R, D950N), the Omicron BA.1 variant (alterations: A67V, Δ69/70, T95I,

405 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, L981F), the Omicron BA.2 variant (alterations: T19I, Δ24-26, A27S, G142D, V213G, G339D, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, S477N, T478K, E484A, Q493R, Q498R, N501Y,

410 Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K), the Omicron BA.2.12.1 variant (alterations: T19I, Δ24-26, A27S, G142D, V213G, G339D, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, L452Q, S477N, T478K, E484A, Q493R, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, S704L, N764K, D796Y, Q954H, N969K), and the Omicron BA.4/5 variant (alterations: T19I, Δ24-26, A27S, Δ69/70, G142D,

415 V213G, G339D, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, L452R, S477N, T478K, E484A, F486V, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K) according to published pseudotyping protocols (*3*). A diagram of SARS-CoV-2 S glycoprotein alterations is shown in fig. S8a and a separate alignment of S glycoprotein alterations in Omicron VOCs is displayed in fig. S1.

420   In brief, HEK293T/17 monolayers (ATCC® CRL-11268™) cultured in Dulbecco's modified

Eagle's medium (DMEM) with GlutaMAX™ (Gibco) supplemented with 10% heat-inactivated

fetal bovine serum (FBS [Sigma-Aldrich]) (referred to as medium) were transfected with Sanger

sequencing-verified SARS-CoV-1 or variant-specific SARS-CoV-2 S expression plasmid with

Lipofectamine LTX (Life Technologies) following the manufacturer's instructions. At 24 hours

425   after transfection, the cells were infected at a multiplicity of infection (MOI) of three with VSV-

G complemented VSVΔG vector. After incubation for 2 hours at 37 °C with 7.5% CO_2, cells

were washed twice with phosphate buffered saline (PBS) before medium supplemented with

anti-VSV-G antibody (clone 8G5F11, Kerafast Inc.) was added to neutralize residual VSV-G-

complemented input virus. VSV-SARS-CoV-2-S pseudotype-containing medium was harvested

430   20 hours after inoculation, passed through a 0.2 µm filter (Nalgene) and stored at -80 °C. The

pseudovirus batches were titrated on Vero 76 cells (ATCC® CRL-1587™) cultured in medium.

The relative luciferase units induced by a defined volume of a SARS-CoV-2 wild-type strain S

glycoprotein pseudovirus reference batch previously described in Muik et al., 2021 (37), that

corresponds to an infectious titer of 200 transducing units (TU) per mL, was used as a

435   comparator. Input volumes for the SARS-CoV-2 variant pseudovirus batches were calculated to

normalize the infectious titer based on the relative luciferase units relative to the reference.

Pseudovirus neutralization assay

Vero 76 cells were seeded in 96-well white, flat-bottom plates (Thermo Scientific) at 40,000

440   cells/well in medium 4 hours prior to the assay and cultured at 37 °C with 7.5% CO_2. Human and

mouse serum samples were 2-fold serially diluted in medium with dilutions ranging from 1:5 to

1:30,720 (human sera), from 1:40 to 1:102,400 for mouse booster study (mouse sera; starting

dilution was 1:40 [pre-D3], 1:200 [d7D3]) as well as 1:100 [d21D3, d35D3]) and from 1:100 to

1:15,360 in the naïve setting (mouse sera; starting dilution was 1:120 [monovalent vaccinated

445   groups] and 1:100 [bivalent vaccinated groups]). VSV-SARS-CoV-2-S/VSV-SARS-CoV-1-S

particles were diluted in medium to obtain 200 TU in the assay. Serum dilutions were mixed 1:1

with pseudovirus (n=2 technical replicates per serum per pseudovirus) for 30 minutes at room

temperature before being added to Vero 76 cell monolayers and incubated at 37°C with 7.5%

CO_2 for 24 hours. Supernatants were removed and the cells were lysed with luciferase reagent

450   (Promega). Luminescence was recorded on a CLARIOstar® Plus microplate reader (BMG

Labtech), and neutralization titers were calculated as the reciprocal of the highest serum dilution

that still resulted in 50% reduction in luminescence. Results for all pseudovirus neutralization

experiments were expressed as geometric mean titers (GMT) of duplicates. If no neutralization

was observed, an arbitrary titer value of half of the limit of detection [LOD] was reported.

455   Neutralization titers in human sera are shown in Tables S3 to S6.

Live SARS-CoV-2 neutralization assay

SARS-CoV-2 virus neutralization titers were determined by a microneutralization assay based on

cytopathic effect (CPE) at VisMederi S.r.l., Siena, Italy. In brief, human and mouse serum

460   samples were serially diluted 1:2 (n=2 technical replicates per serum per virus; starting at 1:10

for human samples and starting at 1:50 [post-boost, day 139] for murine samples) and incubated

for 1 hour at 37 °C with 100 $TCID_{50}$ of the live wild-type-like SARS-CoV-2 virus strain 2019-

nCOV/ITALY-INMI1 (GenBank: MT066156), Omicron BA.1 strain hCoV-19/Belgium/rega-

20174/2021 (alterations: A67V, Δ69/70, T95I, G142D, Δ143-145, Δ211, L212I, ins214EPE,

465   G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R,

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G496S, Q498R, N501Y, Y505H, T547K, D614G, H655Y, N679K, P681H, N764K, D796Y, N856K, Q954H, N969K, L981F), sequence-verified Omicron BA.2 strain (alterations:T19I, Δ24-26, A27S, V213G, G339D, S371F, S373P, S375F, T376A, D405N, R408S, K417N, S477N, T478K, E484A, Q493R, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, R682W,

470   N764K, D796Y, Q954H, N969K), or sequence-verified Omicron BA.4 strain (alterations: V3G, T19I, Δ24-26, A27S, Δ69/70, G142D, V213G, G339D, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, L452R, S477N, T478K, E484A, F486V, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K) to allow any antigen-specific antibodies to bind to the virus. A diagram of S glycoprotein alterations is shown in Fig.

475   S8b. The 2019-nCOV/ITALY-INMI1 strain S glycoprotein is identical in sequence to the wild-type SARS-CoV-2 S (Wuhan-Hu-1 isolate). Vero E6 (ATCC® CRL-1586™) cell monolayers were inoculated with the serum/virus mix in 96-well plates and incubated for 3 days (2019-nCOV/ITALY-INMI1 strain) or 4 days (Omicron BA.1, BA.2 and BA.4 variant strain) to allow infection by non-neutralized virus. The plates were observed under an inverted light microscope

480   and the wells were scored as positive for SARS-CoV-2 infection (i.e., showing CPE) or negative for SARS-CoV-2 infection (i.e., cells were alive without CPE). The neutralization titer was determined as the reciprocal of the highest serum dilution that protected more than 50% of cells from CPE and reported as GMT of duplicates. If no neutralization was observed, an arbitrary titer value of 5 (half of the LOD) was reported. Neutralization titers in human sera are shown in

485   Tables S7 to S10.

Statistical analysis

The statistical method of aggregation used for the analysis of antibody titers is the geometric mean and for the ratio of SARS-CoV-2 VOC titer and wild-type strain titer the geometric mean

490   and the corresponding 95% confidence interval. The use of the geometric mean accounts for the non-normal distribution of antibody titers, which span several orders of magnitude. The Friedman test with Dunn's correction for multiple comparisons was used to conduct pairwise signed-rank tests of group geometric mean neutralizing antibody titers with a common control group. The Kruskal-Wallis test with Dunn's correction for multiple comparisons was used to

495   conduct unpaired signed-rank tests of group GMT ratios. Spearman correlation was used to evaluate the monotonic relationship between non-normally distributed datasets. All statistical analyses were performed using GraphPad Prism software version 9.

Fig.1

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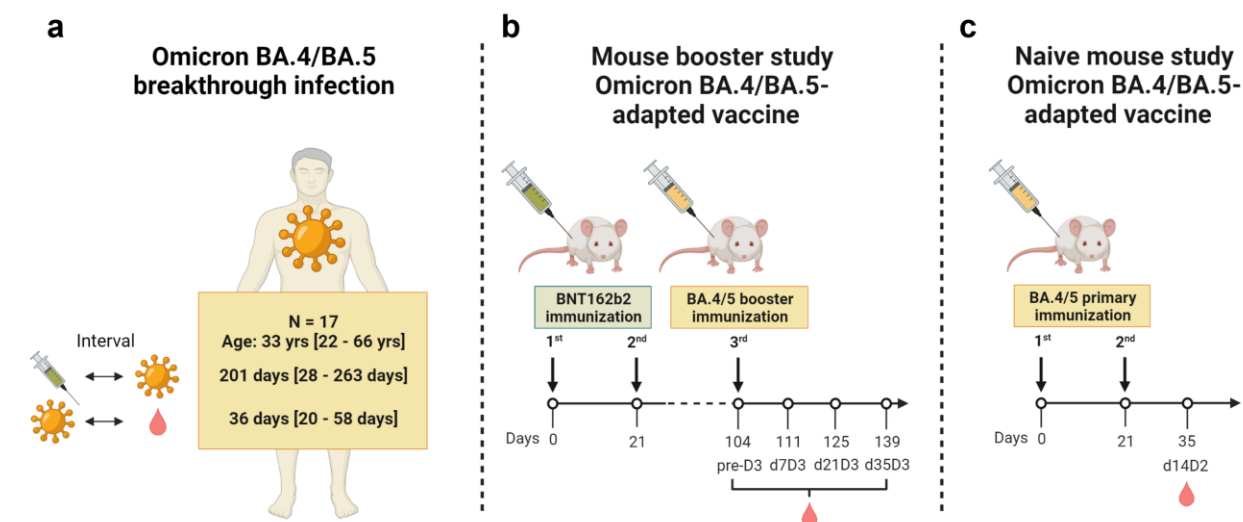
Fig. 1. Study design

(a) The effect of Omicron BA.4/BA.5 breakthrough infection on the serum neutralizing activity was evaluated in individuals vaccinated with three doses of mRNA COVID-19 vaccine (BNT162b2/mRNA-1273 homologous or heterologous regimens) who subsequently experienced an infection with Omicron BA.4 or BA.5. The intervals between vaccination, breakthrough infection and sampling are indicated as median/range. **(b)** Effects of prototypic Omicron BA.4/BA.5-adapted booster vaccines on serum neutralizing activity were investigated in mice vaccinated twice 21-days apart with BNT162b2, followed by a booster dose of BA.4/BA.5-adapted vaccines 3.5 months later. Neutralizing activity was assessed before (pre-D3) and 7, 21, and 35 days after the booster (d7D3, d21D3, d35D3, respectively). **(c)** Effects of prototypic Omicron BA.4/BA.5-adapted vaccines on serum neutralizing activity were investigated in naïve mice vaccinated twice 21-days apart with BA.4/BA.5-adapted vaccines. Neutralizing activity was assessed 14 days after administration of the second dose (d14D2). Comparably high RNA purity and integrity, and expression of antigens *in vitro* were confirmed for BNT162b2 and Omicron-adapted vaccines (fig S4a-b).

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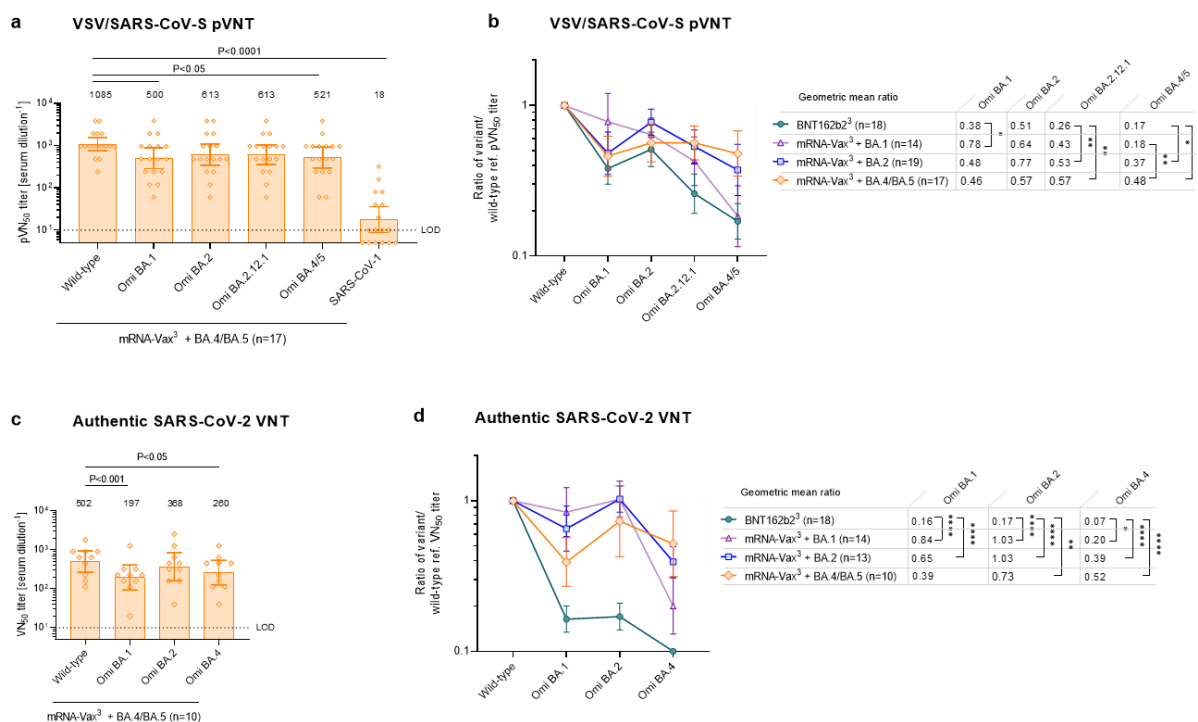
Fig. 2.

Fig. 2. Omicron BA.4/BA.5 breakthrough infection of triple mRNA vaccinated individuals mediates pan-Omicron neutralization

Cohorts and serum sampling as described in Fig. S2. **(a)** 50% pseudovirus neutralization (pVN_{50}) geometric mean titers (GMTs) in sera of mRNA-Vax[3] + BA.4/BA.5 against the indicated SARS-CoV-2 variants of concern (VOCs) or SARS-CoV-1 pseudoviruses. Values above bars represent group GMTs. The non-parametric Friedman test with Dunn's multiple comparisons correction was used to compare the wild-type strain neutralizing group GMTs with titers against the indicated variants and SARS-CoV-1. Multiplicity-adjusted p values are shown. **(b)** SARS-CoV-2 VOC pVN_{50} GMTs normalized against the wild-type strain pVN_{50} GMT (ratio VOC to wild-type) of mRNA-Vax[3] + BA.4/BA.5 and the reference cohorts as outlined in Fig S2. Group geometric mean ratios with 95% confidence intervals are shown. The non-parametric Kruskal-Wallis test with Dunn's multiple comparisons correction was used to compare the VOC GMT

ratios between cohorts. ****, $P>0.0001$; **, $P<0.01$; *, $P<0.05$. Data for the reference cohorts were previously published (*17, 21*). **(c)** 50% virus neutralization (VN_{50}) GMTs in sera of mRNA-Vax^3 + BA.4/BA.5 against the indicated SARS-CoV-2 VOCs. Statistical analysis was conducted as in (a). **(d)** SARS-CoV-2 VOC VN_{50} GMTs normalized against the wild-type strain VN_{50} GMT (ratio VOC to wild-type). Statistical analysis was conducted as in (b). Serum was tested in duplicate. For titer values below the limit of detection (LOD), LOD/2 values were plotted.
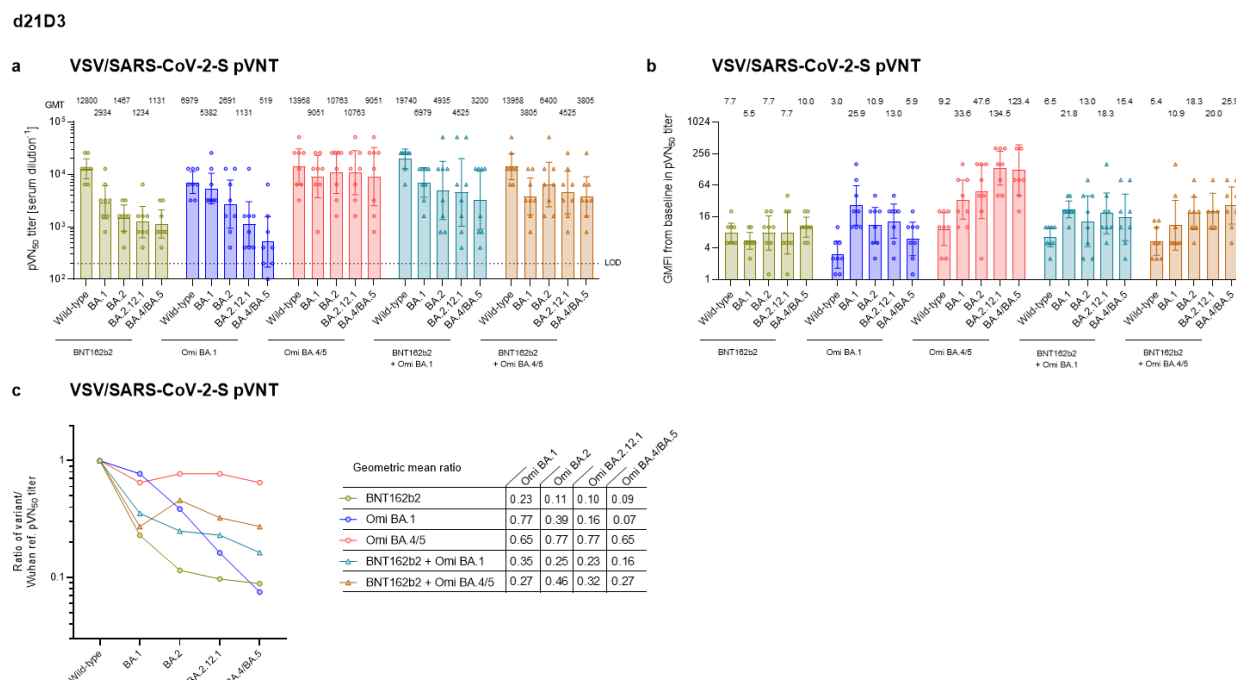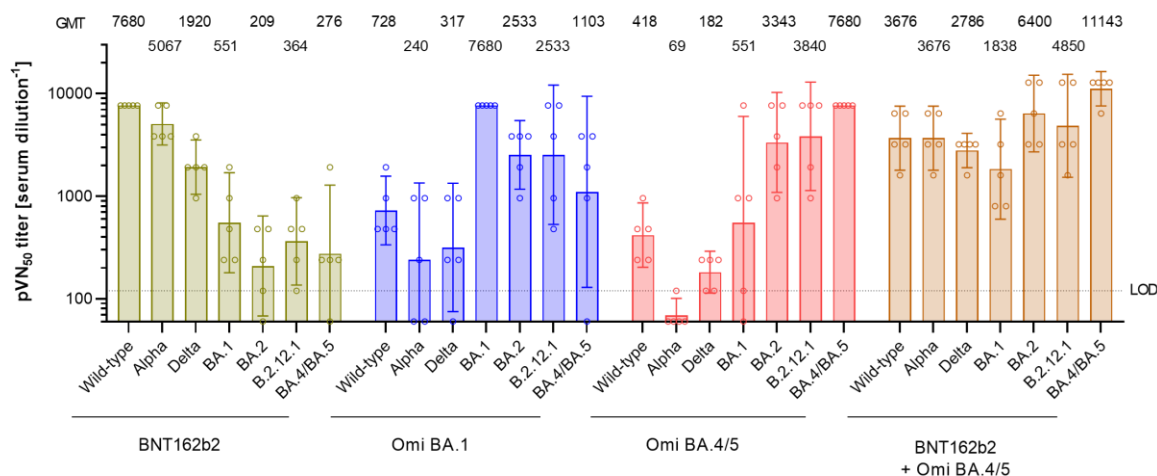
Fig. 3.

Fig. 3. Booster immunization with an Omicron BA.4/BA.5 S glycoprotein adapted RNA-vaccine mediates pan-Omicron neutralization in double-vaccinated mice

BALB/c mice (n=8) were injected intramuscularly with two doses of 1 µg BNT162b2 21 days apart, and a third dose of either BNT162b2 (1 µg) or the indicated monovalent (1 µg) or bivalent (0.5 µg of each component) Omicron BA.1 or BA.4/5-adapted vaccines 104 days after the first vaccination. **(a)** 50% pseudovirus neutralization (pVN_{50}) geometric mean titers (GMTs) against the indicated SARS-CoV-2 variants of concern (VOCs) in sera collected 21 days after the third vaccination (d21D3). Values above bars represent group GMTs. **(b)** Geometric mean fold-increase (GMFI) of pVN_{50} titers on d21D3 relative to baseline titers before the third vaccination. Values above bars represent group GMFIs. (c) SARS-CoV-2 VOC pVN50 GMTs normalized against the wild-type strain pVN_{50} GMT (ratio VOC to wild-type). Group geometric mean ratios

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are shown. Serum was tested in duplicate. For titer values below the limit of detection (LOD),

LOD/2 values were plotted. Error bars represent 95% confidence intervals.

Fig. 4.

Fig. 4. Immunization with an Omicron BA.4/BA.5 S glycoprotein supplemented BNT162b2 mRNA vaccine drives pan-Omicron neutralization in previously unvaccinated mice

Naïve BALB/c mice (n=5) were injected intramuscularly with two doses of either BNT162b2 (1 µg) or the indicated monovalent (1 µg) or bivalent (0.5 µg of each component) Omicron BA.1 or BA.4/5-adapted vaccines, 21 days apart. 50% pseudovirus neutralization (pVN_{50}) geometric mean titers (GMTs) against the indicated SARS-CoV-2 variants of concern (VOCs) in sera collected 14 days after the second vaccination (d14D2). Values above bars represent group GMTs. Error bars represent 95% confidence interval. Serum was tested in duplicate. For titer values below the limit of detection (LOD), LOD/2 values were plotted.

Author contributions:

U.S., Ö.T., K.A.S. and A.M. conceived and conceptualized the work. A.M, A.B.V., B.G.L., S.F, J.R., K.C.W. and H.H. planned and supervised experiments. K.K., O.O., J.G., K.G., N.K. and S.C. coordinated and conducted sample collection. K.G. coordinated sample shipments and clinical data transfer. A.M., B.G.L., M.B., A.W., A.G., C.C., V.M., G.J.S., J.M., S.S., F.W., T.Z., K.S. performed experiments. A.M., and B.G.L., A.G., A.B.V. analyzed data. Q.Y. and H.C. interpreted data. U.S., Ö.T., A.M., A.T., A.B.V. and A.F. interpreted data and wrote the manuscript. All authors supported the review of the manuscript.

Competing interests:

590   U.S. and Ö.T. are management board members and employees at BioNTech SE. A.M., B.G.L.,
M.B., A.W., A.T., A.G., C.C., V.M., G.J.V., J.M., T.Z., S.F., J.R., F.W., K.S., H.H., K.C.W.,
J.G., S.S., A.F., K.K., O.O., and A.B.V. are employees at BioNTech SE. K.G., N.K. and S.C. are
employees at the University Hospital, Goethe University Frankfurt. Q.Y., H.C., and K.A.S. are
employees of Pfizer and may hold stock options. U.S., Ö.T. A.B.V., A.G., T.Z., J.R., K.C.W. and

595   A.M. are inventors on patents and patent applications related to RNA technology and COVID-19
vaccines. Q.Y, H.C., and K.A.S. are inventors on a patent application related to RNA-based
COVID-19 vaccines. U.S., Ö.T., A.M., B.G.L., K.K., A.W., M.B., A.F., A.T., A.G., V.M.,
G.J.S., A.B.V. and O.O. have securities from BioNTech SE. S.C. has received an honorarium for
serving on a clinical advisory board for BioNTech.

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Data and materials availability:

Materials are available from the authors under a material transfer agreement with BioNTech.

Supplementary Materials:

605   Figs. S1-S8

Tables S1-S10

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Supplementary Materials for

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Exposure to BA.4/BA.5 Spike glycoprotein drives pan-Omicron neutralization in vaccine experienced humans and mice

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770   Cadima Couto, Alptekin Güler, Veena Mampilli, Geneva J. Schmitt, Jonathan Mottl, Thomas

Ziegenhals, Stephanie Fesser, Jonas Reinholz, Florian Wernig, Karla-Gerlinde Schraut, Hossam

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This PDF file includes:

Fig. S1 to S8

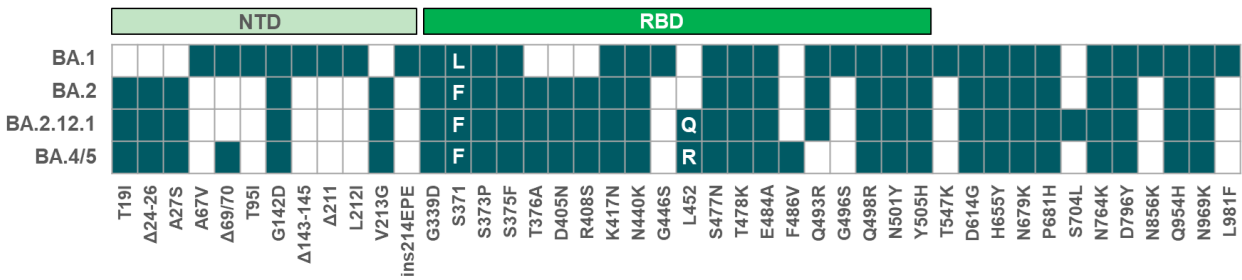780   Tables S1 to S10

fig. S1

Fig. S1. Alterations of the spike glycoprotein amino acid sequence of SARS-CoV-2

Omicron sub-lineages.

White letters in boxes indicate the amino acid substitution per sub-lineage; Δ, deletion; ins, insertion; NTD, N-terminal domain; RBD, receptor-binding domain

fig. S2

		SARS-CoV-2 Omicron Convalescent		SARS-CoV-2 Naïve
Interval	Triple-vaccinated BA.4/5 infected (mRNA-Vax[3] + BA.4/5) N = 17 Age: 33 yrs [22-66 yrs]	Triple-vaccinated BA.2 infected (mRNA-Vax[3] + BA.2) N = 19 Age: 30 yrs [25-74 yrs]	Triple-vaccinated BA.1 infected (mRNA-Vax[3] + BA.1) N = 14 Age: 32 yrs [23-60 yrs]	Triple BNT162b2 vaccinated (BNT162b2[3]) N = 18 Age: 38 yrs [23-54 yrs]
Vaccine ↔ 🟠	N/A	N/A	N/A	28 days [26-30 days]
↔ 🦠	201 days [28-263 days]	141 days [36-200 days]	22 days [3-112 days]	N/A
🦠 ↔ 🟠	36 days [20-58 days]	43 days [28-99 days]	43 days [25-55 days]	N/A

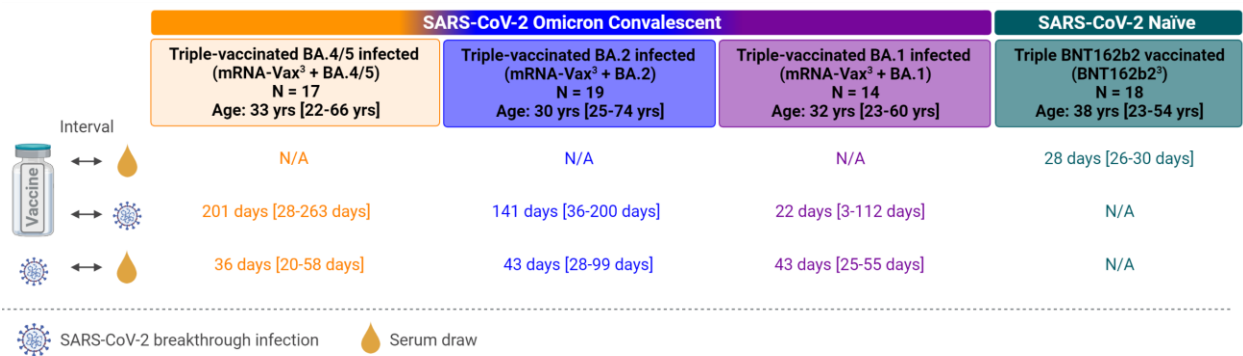🦠 SARS-CoV-2 breakthrough infection   🟠 Serum draw

Fig. S2. Cohorts and sampling

Serum samples were drawn from four cohorts: individuals vaccinated with three doses of mRNA COVID-19 vaccine (BNT162b2/mRNA-1273 homologous or heterologous regimens) who subsequently had a breakthrough infection with Omicron BA.4/BA.5 (mRNA-Vax3 + BA.4/BA.5, orange). Three cohorts were included as reference: Triple-mRNA vaccinated individuals who experienced breakthrough infection either at a time of BA.2 dominance (March to May 2022; mRNA-Vax3 + BA.2, blue), or at a time of BA.1 dominance (November 2021 to January 2022; mRNA-Vax3 + BA.1, purple), or individuals triple-vaccinated with BNT162b2 that were SARS-CoV-2-naïve at the time of sampling (BNT162b2[3], green). Breakthrough infections occurred at a time of respective VOC dominance (BA.4/BA.5: mid-June to mid-July 2022, BA.2: March to May 2022, BA.1: November 2021 to January 2021) and/or were variant confirmed by genome sequencing. For convalescent cohorts, relevant intervals between key events such as the most recent vaccination, SARS-CoV-2 infection, and serum isolation are indicated. All values specified as median-range. The age/gender composition of the cohorts is further detailed in Table S1. Participant-level information is provided for the mRNA-Vax[3] +

38

BA.4/BA.5 in Table S2. Data for the reference cohorts mRNA-Vax[3] + BA.2, mRNA-Vax[3] +

BA.1, and BNT162b2[3] were previously published (*1, 2*).

N/A, not applicable; Schematic was created with BioRender.com

810 **fig. S3**
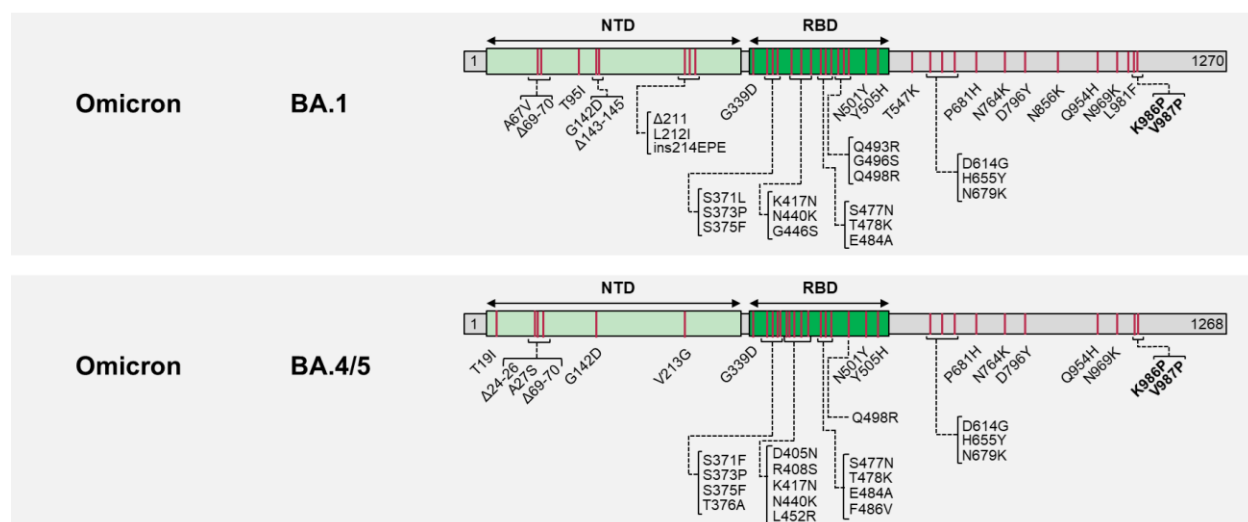
Fig. S3. Design of Omicron-adapted vaccines

The sequence of the Wuhan-Hu-1 isolate SARS-CoV-2 S glycoprotein (GenBank: QHD43416.1) was used as reference. Amino acid positions, amino acid descriptions (one letter code) and type

815 of alterations (substitutions, deletions, insertions) are indicated. Pre-fusion stabilizing mutations are highlighted in bold. NTD, N-terminal domain; RBD, Receptor-binding domain, Δ, deletion; ins, insertion

fig. S4

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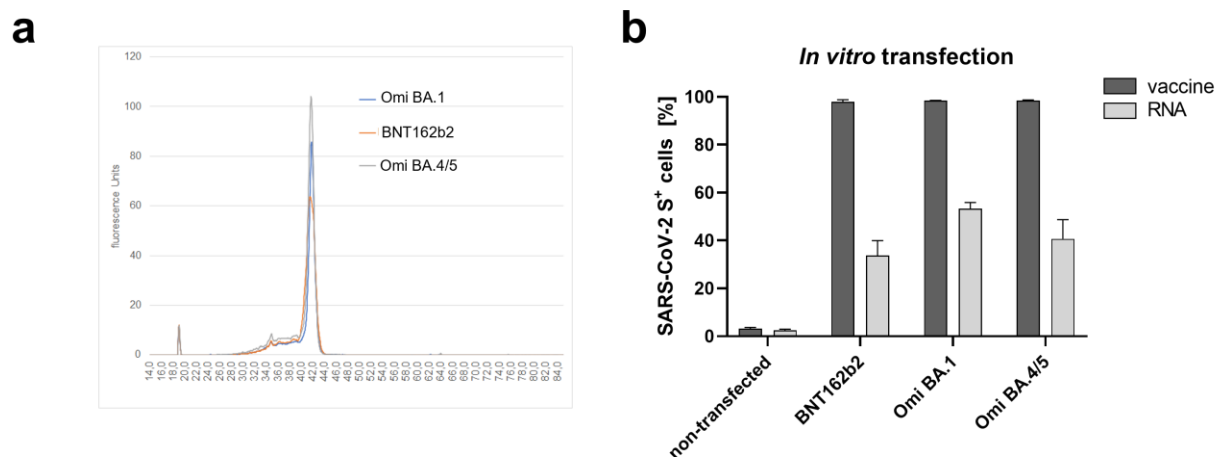
Fig. S4. Comparably high RNA purity and integrity, and *in vitro* expression of antigens encoded by Omicron-adapted vaccines

(a) Liquid capillary electropherograms of *in vitro* transcribed RNA samples merged into one

825   graph. (b) Surface expression of SARS-CoV-2 Spike (S) glycoprotein on HEK293T cells upon

transfection. HEK293T cells were transfected with S glycoprotein encoding mRNAs formulated

as lipid nanoparticles (vaccine) or mixed with a commercial transfection reagent (RNA), or no

vaccine/RNA (non-transfected). Surface expression was analyzed by flow cytometry using mFc-

tagged human ACE-2 as a detection reagent. Heights of bars indicate the means of n= 3 technical

830   replicates.

fig. S5
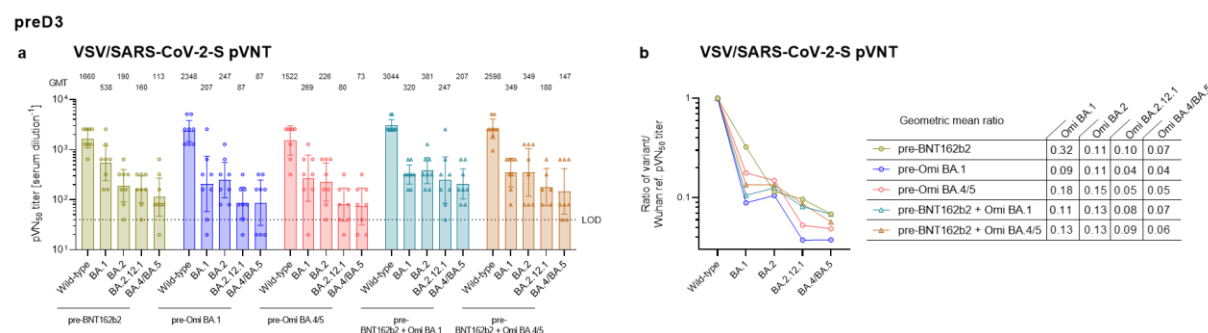
Fig. S5. Baseline magnitude and breadth of neutralizing activity against SARS-CoV-2 variants are comparable in BNT162b2-vaccinated mice prior to booster vaccination

BALB/c mice were injected intramuscularly with two doses of 1 µg BNT162b2 at a 21-day interval. Mice were allocated to groups (n=8) in preparation of administration of the booster dose of the indicated vaccines to be tested. Serum was collected from the mice on day 104 after the first vaccination, before booster vaccines were injected. **(a)** 50% pseudovirus neutralization (pVN_{50}) geometric mean titers (GMTs) against the indicated SARS-CoV-2 variants of concern (VOCs). Values above bars represent group GMTs. Error bars represent 95% confidence intervals. **(b)** SARS-CoV-2 VOC pVN_{50} GMTs normalized against the wild-type strain pVN_{50} GMT (ratio VOC to wild-type). Group geometric mean ratios are shown. Serum was tested in duplicate. For titer values below the limit of detection (LOD), LOD/2 values were plotted.

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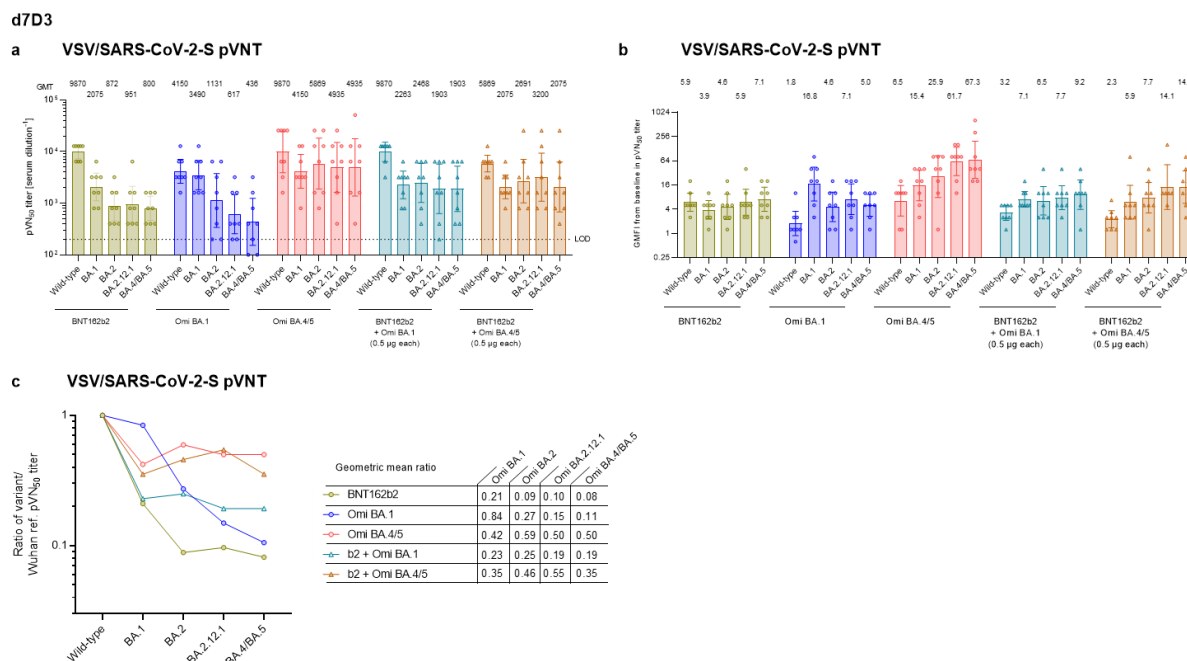
fig. S6

Fig. S6. Booster immunization with an Omicron BA.4/BA.5 S glycoprotein adapted mRNA vaccine mediates pan-Omicron neutralization in mice 7 days after the booster

BALB/c mice (n=8) were injected intramuscularly with two doses of 1 µg BNT162b2 at a 21-day interval, and a third dose of either BNT162b2 (1 µg) or the indicated monovalent (1 µg) or bivalent (0.5 µg of each component) Omicron BA.1 or BA.4/5-adapted vaccines 104 days after the first vaccination. **(a)** 50% pseudovirus neutralization (pVN_{50}) geometric mean titers (GMTs) against the indicated SARS-CoV-2 variants of concern (VOCs) in sera collected 7 days after the third vaccination (d7D3). Values above bars represent group GMTs. **(b)** Geometric mean fold-increase (GMFI) of pVN_{50} titers on d7D3 relative to baseline titers before the third vaccination. Values above bars represent group GMFIs. **(c)** SARS-CoV-2 VOC pVN_{50} GMTs normalized against the wild-type strain pVN_{50} GMT (ratio VOC to wild-type). Group geometric mean ratios

860   are shown. Serum was tested in duplicate. For titer values below the limit of detection (LOD),

LOD/2 values were plotted. Error bars represent 95% confidence intervals.

fig. S7
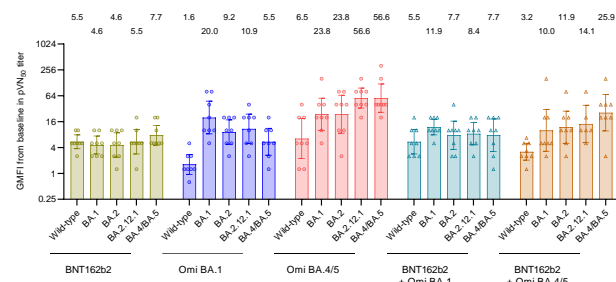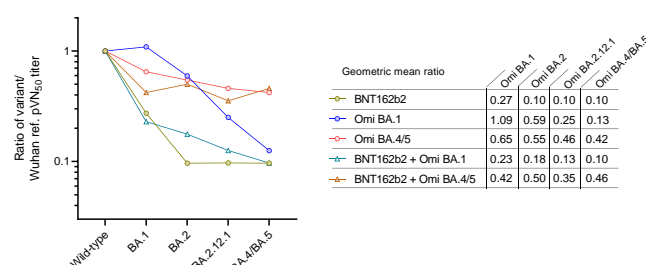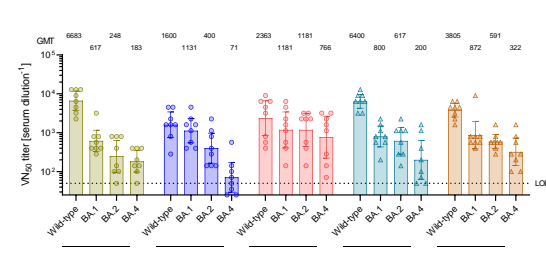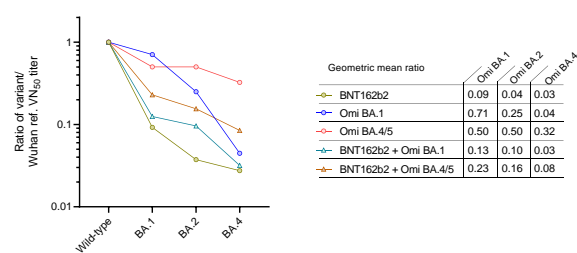
Fig. S7. Booster immunization with an Omicron BA.4/BA.5 S glycoprotein adapted vaccine mediates pan-Omicron neutralization in mice 35 days after the booster
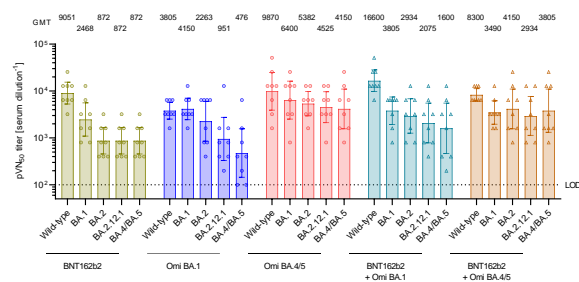
BALB/c mice (n=8) were injected intramuscularly with two doses of 1 µg BNT162b2 at a 21-day interval, and a third dose of either BNT162b2 (1 µg) or the indicated monovalent (1 µg) or bivalent (0.5 µg of each component) Omicron BA.1 or BA.4/5-adapted vaccines 104 days after the first vaccination. **(a)** 50% pseudovirus neutralization (pVN$_{50}$) geometric mean titers (GMTs) against the indicated SARS-CoV-2 variants of concern (VOCs) in sera collected 35 days after the third vaccination (d35D3). Values above bars represent group GMTs. **(b)** Geometric mean fold-

increase (GMFI) of pVN_{50} titers on d35D3 relative to baseline titers before the third vaccination. Values above bars represent group GMFIs. **(c)** SARS-CoV-2 VOC pVN50 GMTs normalized

875 against the wild-type strain pVN_{50} GMT (ratio VOC to wild-type). Group geometric mean ratios are shown. **(d)** 50% virus neutralization (VN_{50}) GMTs against the indicated SARS-CoV-2 VOCs on d35D3. Values above bars represent group GMTs. Error bars represent 95% confidence interval. **(e)** SARS-CoV-2 VOC VN_{50} GMTs normalized against the wild-type strain VN_{50} GMT (ratio VOC to wild-type). Group geometric mean ratios are shown. Serum was tested in

880 duplicate. For titer values below the limit of detection (LOD), LOD/2 values were plotted. Error bars represent 95% confidence intervals.

fig. S8

a

b

885
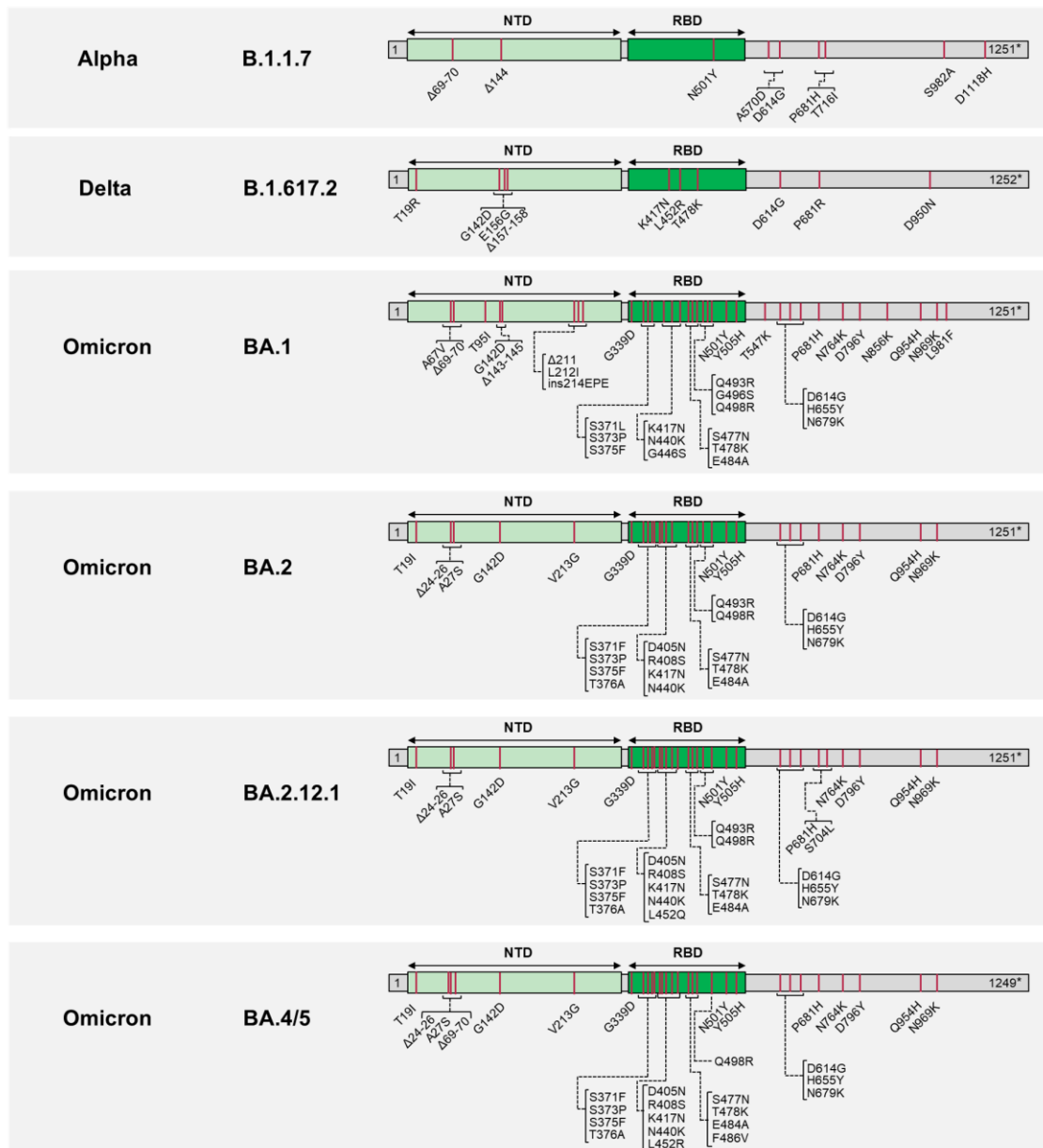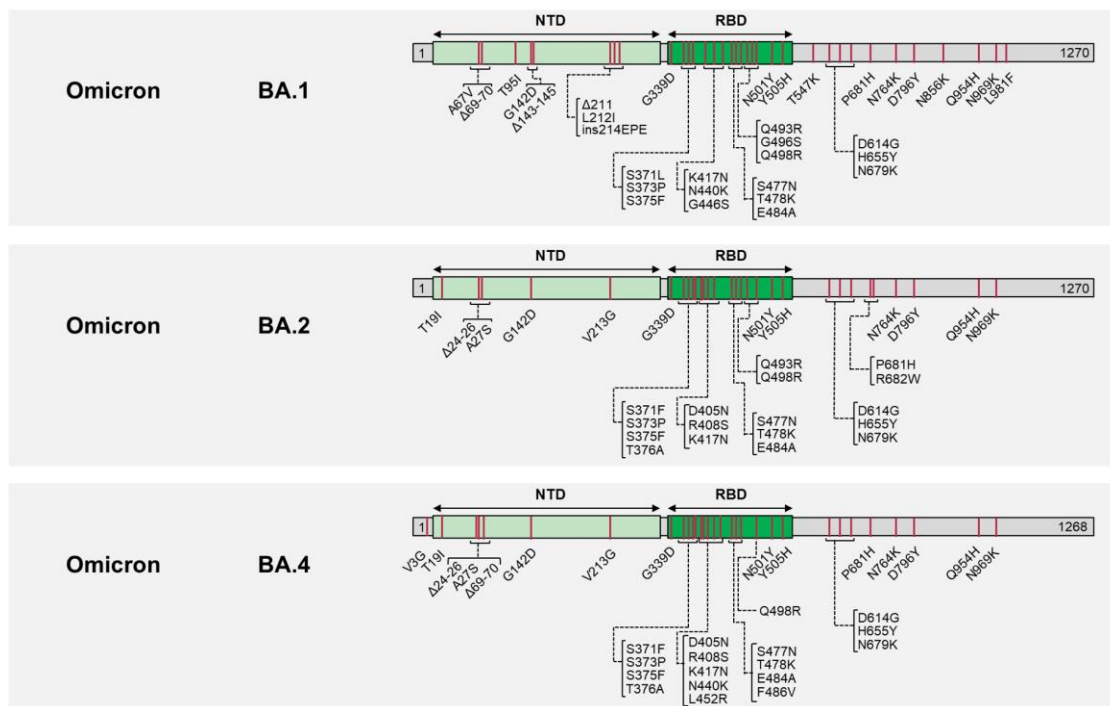
**Fig. S8. Characterization of SARS-CoV-2 S glycoproteins used in the assays based on (a) VSV-SARS-CoV-2 variant pseudoviruses and (b) live authentic SARS-CoV-2.**

The sequence of the Wuhan-Hu-1 isolate SARS-CoV-2 S glycoprotein (GenBank: QHD43416.1) was used as reference. Amino acid positions, amino acid descriptions (one letter code) and type

890 of alterations (substitutions, deletions, insertions) are indicated. NTD, N-terminal domain; RBD, Receptor-binding domain, Δ, deletion; ins, insertion; *, Cytoplasmic domain truncated for the C-terminal 19 amino acids.

Table S1. Vaccinated individuals analyzed for neutralizing antibody responses.

Characteristic	mRNA-Vax[3] + BA.4/BA.5 (n=17)	mRNA-Vax[3] + BA.2 (n=19)	mRNA-Vax[3] + BA.1 (n=14)	BNT162b2[3] (n=18)
Sex, n (%)				
Male	7 (41)	7 (37)	11 (79)	9 (50)
Female	10 (59)	12 (63)	3 (21)	9 (50)
Age, median (range)	33 (22-66)	30 (25-74)	32 (23-60)	38 (23-54)
Age group at vaccination, n (%)				
18-55 yrs	15 (88)	15 (79)	12 (86)	18 (100)
56-85 yrs	2 (12)	4 (21)	2 (14)	0 (0)
SARS-CoV-2 status, n (%)				
Positive	17 (100)*	19 (100)**	14 (100)#	0 (0)
Negative	0 (0)	0 (0)	0 (0)	18 (100)†
Unknown	0 (0)	0 (0)	0 (0)	0 (0)
Interval, median (range)				
Days between D1/D2	35 (21-45)	42 (15-43)	38 (20-92)	‡
Days between D2/D3	193 (156-305)	184 (152-259)	192 (154-256)	202 (181-266)
Days until serum draw after D3	N/A	N/A	N/A	28 (26-30)
Days between last dose/infection	201 (28-263)	141 (36-200)	25 (3-112)	N/A
Days until serum draw after infection	36 (20-58)	43 (28-99)	43 (25-55)	N/A

895 N/A, not applicable; D, dose; yrs, years; n, number.
*, 5 cases were sequence verified BA.5 infections, 2 cases were family members of individuals with sequence-verified BA.5 infections, 8 individuals experienced SARS-CoV-2 breakthrough infection between June-July 2022, at which time the BA.4 or BA.5 lineage was dominant in Germany
**, Individuals experienced SARS-CoV-2 breakthrough infections between March and May 2022, during which period
900 the BA.2 lineage was dominant in Germany. In two cases, BA.2 variant infection was confirmed by sequencing.
#, Omicron infection PCR-confirmed at time of recruitment to the research study. Individuals experienced SARS-CoV-2 breakthrough infections between November 2021 and January 2022, during which period the BA.1 lineage was dominant in Germany
†, No evidence of prior SARS-CoV-2 infection (based on COVID-19 symptoms/signs and SARS-CoV-2 PCR test)
905 ‡, Participants received the primary 2-dose series of BNT162b2 vaccine as part of a governmental vaccination program and the interval between doses was not recorded

Table S2. Individuals triple vaccinated with mRNA COVID-19 vaccine and subsequently infected with Omicron BA.4/BA.5 (mRNA-Vax^3 + BA.4/BA.5).

Participant ID	Age	Sex	Vaccination	Date positive test	Omicron subtype	Dose 1-2 interval (days)	Dose 2-3 interval (days)	Positive test after last vaccination (days)	Blood draw after positive test (days)	Severity (WHO grade)
1	32	f	BNT^2/MOD	MAY2022	N/A$^\#$	45	157	203	35	1-2
2	30	f	BNT^3	JUN2022	BA.5*	42	163	159	36	1-2
3	29	m	BNT^3	MAY2022	BA.5*	42	157	194	30	1-2
4	66	m	BNT^2/MOD	JUN2022	N/A$^\#$	42	183	178	41	1-2
5	57	m	BNT^3	JUL2022	BA.5*	24	283	246	20	1-2
6	36	m	BNT^2/MOD	JUN2022	N/A	35	198	201	27	1-2
7	22	f	BNT^3	JUL2022	BA.5*	42	189	187	26	1-2
8	33	f	BNT^2/MOD	JUN2022	N/A	42	156	194	47	1-2
9	32	f	BNT^3	JUL2022	N/A	21	305	214	35	1-2
10	25	f	MOD^2/BNT	JUL2022	BA.5*	42	198	180	37	1-2
11	30	m	BNT^3	JUN2022	N/A	21	269	211	57	1-2
12	28	f	BNT^3	JUL2022	N/A	21	193	216	28	1-2
13	32	f	BNT/MOD^2	JUN2022	N/A	42	177	189	50	1-2
14	37	m	BNT^3	JUN2022	N/A	21	274	233	50	1-2
15	49	m	BNT^3	JUL2022	N/A	21	178	203	39	1-2
16	30	f	BNT^3	JUN2022	N/A	21	294	199	58	1-2
17	31	f	BNT^3	JUL2022	N/A	21	276	263	29	1-2
Median	**32**	**N/A**	**N/A**	**N/A**	**N/A**	**35**	**193**	**201**	**36**	**N/A**

*Sequence-verified Omicron variant. $^\#$Family member of individual infected with sequence-verified Omicron variant.

m, male; f, female; n/a, not available; N/A, not applicable

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BNT, BioNTech/Pfizer BNT162b2; MOD, Moderna mRNA-1273; BNT^3, BNT162b2 three-dose series; MOD^2, mRNA-1273 two-dose series; MOD^3, mRNA-1273 three-dose series

Table S3. pVN$_{50}$ values of sera collected from individuals with Omicron BA.4/BA.5 breakthrough infection (mRNA-Vax^3 + BA.4/BA.5)

Participant ID	pVN$_{50}$					
	Wild-type	Omicron BA.1	Omicron BA.2	Omicron BA.2.12.1	Omicron BA.4/5	SARS-CoV-1
1	480	120	240	240	480	40
2	1920	480	960	960	960	80
3	960	960	480	480	480	160
4	960	960	480	960	960	5
5	240	60	60	60	60	5
6	1920	960	960	960	480	320
7	960	240	480	480	240	10
8	960	240	240	240	240	5
9	3840	3840	3840	3840	1920	40
10	960	480	480	960	480	5
11	1920	1920	1920	1920	960	10
12	960	480	1920	480	960	10
13	960	960	960	960	960	20
14	960	480	480	960	240	10
15	3840	1920	3840	1920	3840	80
16	480	120	120	120	60	5
17	960	240	480	480	960	5

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Table S4. pVN$_{50}$ values of sera collected from SARS-CoV-2-naïve triple-vaccinated individuals (BNT162b2[3])

Participant ID	pVN$_{50}$					
	Wild-type	Omicron BA.1	Omicron BA.2	Omicron BA.2.12.1	Omicron BA.4/5	SARS-CoV-1
18	160	80	160	80	40	5
19	640	160	320	160	40	40
20	5120	1280	1280	640	640	40
21	320	160	160	80	40	20
22	640	320	160	40	40	20
23	320	160	160	80	40	10
24	320	160	160	80	80	10
25	320	160	160	160	80	20
26	160	40	80	40	40	20
27	320	160	60	160	80	20
28	1280	640	640	640	320	80
29	40	5	40	10	5	5
30	320	80	160	80	40	20
31	160	80	160	80	40	20
32	320	320	320	160	160	20
33	640	160	320	80	80	40
34	2560	640	640	320	320	80
35	320	160	320	80	80	20

Table S5. pVN$_{50}$ values of sera collected from individuals with Omicron BA.1

920 **breakthrough infection (mRNA-Vax^3 + BA.1)**

Participant ID	pVN$_{50}$					
	Wild-type	Omicron BA.1	Omicron BA.2	Omicron BA.2.12.1	Omicron BA.4/5	SARS-CoV-1
36	1920	1920	960	640	320	120
37	960	480	480	640	160	120
38	3840	1920	1920	1280	640	960
39	960	960	960	640	160	120
40	480	480	480	640	40	20
41	1920	960	1920	640	320	40
42	960	1920	480	320	80	80
43	960	480	480	320	80	5
44	480	480	480	320	80	60
45	1920	3840	1920	1280	2560	120
46	3840	3840	3840	2560	2560	120
47	7680	15360	3840	2560	1280	480
48	480	60	120	40	40	30
49	1920	960	960	640	640	60

Table S6. pVN$_{50}$ values of sera collected from individuals with Omicron BA.2

breakthrough infection (mRNA-Vax^3 + BA.2)

Participant ID	pVN$_{50}$					
	Wild-type	Omicron BA.1	Omicron BA.2	Omicron BA.2.12.1	Omicron BA.4/5	SARS-CoV-1
50	480	240	480	160	120	5
51	3840	3840	3840	2560	960	40
52	480	240	240	240	120	10
53	240	120	240	120	120	5
54	1920	240	480	480	240	20
55	480	240	480	240	240	5
56	960	960	960	480	480	5
57	960	960	960	960	480	20
58	1920	960	1920	1920	480	20
59	960	240	480	240	120	10
60	960	240	480	480	480	10
61	1920	240	1920	1920	1920	5
62	960	960	960	480	1920	20
63	240	120	240	240	120	40
64	960	960	960	960	960	80
65	3840	1920	3840	960	480	640
66	960	240	480	480	240	20
67	960	960	960	960	960	160
68	3840	1920	1920	960	480	160

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Table S7. VN$_{50}$ values of sera collected from individuals with Omicron BA.4/BA.5 breakthrough infection (mRNA-Vax^3 + BA.4/BA.5)

Participant ID	VN$_{50}$			
	Wild-type	Omicron BA.1	Omicron BA.2	Omicron BA.4
1	160	113	160	113
2	905	320	640	453
3	226	160	320	453
4	905	320	453	453
5	113	20	40	40
6	640	226	2560	640
12	905	160	320	160
13	640	226	453	226
14	453	226	160	113
15	1810	1280	1280	1280

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Table S8. VN$_{50}$ values of sera collected from SARS-CoV-2-naïve triple-vaccinated individuals (BNT162b2[3])

Participant ID	VN$_{50}$			
	Wild-type	Omicron BA.1	Omicron BA.2	Omicron BA.4
18	226	40	40	28
19	640	160	80	28
20	3620	453	905	226
21	226	40	80	28
22	226	57	40	20
23	640	80	57	40
24	453	80	80	28
25	640	160	113	40
26	160	28	40	20
27	640	80	113	40
28	3620	453	453	160
29	28	5	10	5
30	453	40	80	20
31	226	57	57	28
32	905	160	113	40
33	1280	113	113	40
34	3620	1280	453	160
35	905	80	113	40

Table S9. VN_{50} values of sera collected from individuals with Omicron BA.1 breakthrough infection (mRNA-Vax^3 + BA.1)

Participant ID	VN_{50}			
	Wild-type	Omicron BA.1	Omicron BA.2	Omicron BA.4
36	453	640	905	226
37	453	453	453	80
38	1810	1810	1280	453
39	453	453	640	160
40	453	640	320	57
41	640	640	905	160
42	320	453	640	160
43	905	320	453	80
44	320	640	453	80
45	1280	1280	2560	640
46	5120	1280	3620	640
47	5120	5120	3620	453
48	160	40	113	7
49	1280	905	905	320

Table S10. VN$_{50}$ values of sera collected from individuals with Omicron BA.2

940 **breakthrough infection (mRNA-Vax^3 + BA.2)**

Participant ID	VN$_{50}$			
	Wild-type	Omicron BA.1	Omicron BA.2	Omicron BA.4
50	320	226	320	113
51	3620	3620	3620	905
52	320	160	226	113
53	160	80	226	40
54	905	226	640	226
55	160	226	160	113
56	640	640	640	226
57	640	905	453	226
58	905	640	1280	320
59	453	226	320	160
60	320	160	640	226
61	1280	320	1280	640
62	453	453	640	320
63	453	113	640	226
64	453	1280	640	226
65	2560	640	1810	640
66	226	226	453	113
67	640	226	453	226
68	1810	640	1810	320

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References

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2.   J. Quandt *et al.*, Omicron BA.1 breakthrough infection drives cross-variant neutralization and memory B cell formation against conserved epitopes. *Sci Immunol*, eabq2427 (2022).

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