Omicron BA.2 breakthrough infection enhances cross-neutralization of BA.2.12.1 and BA.4/BA.5

Authors: Alexander Muik[1], Bonny Gaby Lui[1], Maren Bacher[1], Ann-Kathrin Wallisch[1], Aras Toker[1], Andrew Finlayson[1], Kimberly Krüger[1], Orkun Ozhelvaci[1], Katharina Grikscheit[2], Sebastian Hoehl[2], Sandra Ciesek[2,3], Özlem Türeci[1,4], Ugur Sahin[1,5]*

Affiliations

[1]BioNTech, An der Goldgrube 12, 55131 Mainz, Germany.

[2]Institute for Medical Virology, University Hospital, Goethe University Frankfurt, 60596 Frankfurt am Main, Germany.

[3]DZIF – German Centre for Infection Research, External Partner Site, 60596 Frankfurt, Germany.

[4]HI-TRON – Helmholtz Institute for Translational Oncology Mainz by DKFZ, Obere Zahlbacherstr. 63, 55131 Mainz, Germany.

[5]TRON gGmbH – Translational Oncology at the University Medical Center of the Johannes Gutenberg, University Freiligrathstraße 12, 55131 Mainz, Germany.

*Correspondence to: Ugur.Sahin@biontech.de

Abstract

Recently, we reported that BNT162b2-vaccinated individuals after Omicron BA.1 breakthrough infection have strong serum neutralizing activity against Omicron BA.1, BA.2, and previous SARS-CoV-2 variants of concern (VOCs), yet less against the highly contagious Omicron sublineages BA.4 and BA.5 that have displaced previous variants. As the latter sublineages are derived from Omicron BA.2, we characterized serum neutralizing activity of COVID-19 mRNA vaccine triple-immunized individuals who experienced BA.2 breakthrough infection. We demonstrate that sera of these individuals have broadly neutralizing activity against previous VOCs as well as all tested Omicron sublineages, including BA.2 derived variants BA.2.12.1, BA.4/BA.5. Furthermore, applying antibody depletion we showed that neutralization of BA.2 and BA.4/BA.5 sublineages by BA.2 convalescent sera is driven to a significant extent by antibodies targeting the N-terminal domain (NTD) of the spike glycoprotein, whereas their neutralization by Omicron BA.1 convalescent sera depends exclusively on antibodies targeting the receptor binding domain (RBD). These findings suggest that exposure to Omicron BA.2, in contrast to BA.1 spike glycoprotein, triggers significant NTD specific recall responses in vaccinated individuals and thereby enhances the neutralization of BA.4/BA.5 sublineages. Given the current epidemiology with a predominance of BA.2 derived sublineages like BA.4/BA.5 and rapidly ongoing evolution, these findings are of high relevance for the development of Omicron adapted vaccines.

Introduction

Emergence of the SARS-CoV-2 Omicron variant of concern (VOC) in November 2021 (*1*) can be considered a turning point in the COVID-19 pandemic. Omicron BA.1, which is significantly altered in the spike (S) glycoprotein receptor binding domain (RBD) and N-terminal domain (NTD), partially escapes previously established immunity (*2*). The loss of many epitopes (*3, 4*) drastically impaired susceptibility to neutralizing antibodies induced by wild-type strain (Wuhan-Hu-1) S glycoprotein-based vaccines or by infection with previous strains (*5-7*), necessitating a third vaccine dose to establish full immunity (*8-10*). Omicron BA.1 was displaced by the BA.2 variant, which in turn was displaced by its descendants BA.2.12.1, BA.4 and BA.5 that in the meantime dominate in many regions (*11-14*).

Antigenically, BA.2.12.1 exhibits high similarity with BA.2 but not BA.1, whereas BA.4 and BA.5 differ considerably from BA.2 and even more so from BA.1, in line with their genealogy (*15, 16*). While some amino acid changes in the RBD are shared between all Omicron sub-lineages, the alteration L452Q is only found in BA.2.12.1 and is the only residue which distinguishes its RBD from that of the BA.2 variant. The L452R and F486V alterations are BA.4/BA.5-specific, whereas S371F, T376A, D405N, and R408S are shared by BA.2 and its descendants BA.2.12.1 and BA.4/BA.5, but not BA.1 (fig. S1). These amino acid exchanges are associated with further escape from vaccine-induced neutralizing antibodies and therapeutic antibody drugs targeting the wild-type S glycoprotein (*6, 15, 17-20*). The NTDs of BA.2 and its descendants are antigenically closer to the wild-type strain and lack several amino acid changes, insertions, and deletions that occurred in BA.1 (fig S1). For instance, Δ143-145, L212I, or ins214EPE, which rendered the BA.1 variant resistant to a panel of NTD-directed monoclonal

antibodies raised against the wild-type S glycoprotein, are not found in BA.2 and descendants (*21*, *22*).

We and others (*10*, *23*) have recently shown that Omicron BA.1 breakthrough infection of BNT162b2 vaccinated individuals augments broadly neutralizing activity against Omicron BA.1, BA.2 and previous VOCs at levels similar to those observed against SARS-CoV-2 wild-type. We showed that BA.1 breakthrough infection of triple BNT162b2-vaccinated individuals induced a robust recall response, primarily expanding memory B cells against epitopes shared broadly amongst variants, rather than inducing B cells specific to BA.1 only. Neutralization of the latest Omicron sublineages BA.4 and BA.5 was not enhanced, and geometric mean titers were rather comparable to those against the phylogenetically more distant SARS-CoV-1.

Given that Omicron BA.2 is more closely related to BA.4/BA.5 than to BA.1, we asked if BA.2 breakthrough infection would shift cross-neutralization activity more towards these most recent Omicron sublineages. We compared the neutralization of different Omicron sublineages by serum samples from three different cohorts of individuals triple-vaccinated with mRNA COVID-19 vaccines, namely from individuals with no history of SARS-CoV-2 infection and individuals that experienced breakthrough infection with either BA.1 or BA.2. In addition, we characterized the contribution of serum antibodies targeting the S glycoprotein RBD versus the NTD to Omicron sublineage neutralization. Our data will increase current understanding on Omicron immune escape mechanisms and the effects of immunization on variant cross-neutralization, and thus help guide further vaccine development.

Results

Cohorts and sampling

This study investigated serum samples from three cohorts: from BNT162b2 triple-vaccinated individuals who were SARS-CoV-2-naïve at the time of sampling (BNT162b2^3, n=18), from 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 at a time of BA.1 dominance (mRNA-Vax^3 + BA.1, n=14), or from triple mRNA vaccinated individuals with a breakthrough infection at a time of BA.2 dominance (mRNA-Vax^3 + BA.2, n=13). For convalescent cohorts, relevant intervals between key events such as the most recent vaccination and infection are provided in Fig. 1 and Table S1 to S3. Sera were derived from the biosample collections of BNT162b2 vaccine trials and from a non-interventional study researching vaccinated patients that had experienced Omicron breakthrough infection.

A subset of the samples included in this study had also been used in our previous investigation of the effect of BA.1 breakthrough infection on serum neutralizing activity and memory B cell repertoire (Quandt et al. (*10*)).

Omicron BA.2 breakthrough infection of triple mRNA-vaccinated individuals induces broad neutralization of VOCs including Omicron BA.4/BA.5

Neutralizing activity of immune sera was tested in a well-characterized pseudovirus neutralization test (pVNT) (*24*, *25*) 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 Alpha, Beta, Delta, Omicron BA.1, BA.2, and the BA.2-derived

sublineages BA.2.12.1, BA.4 and BA.5. As BA.4 and BA.5 share an identical S glycoprotein sequence, we refer to them as BA.4/5 in the context of the pVNT. In addition, we assayed SARS-CoV (herein referred to as SARS-CoV-1) to detect potential pan-Sarbecovirus neutralizing activity (*26*). 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-2 wild-type strain and VOCs including BA.4, except Omicron BA.2.12.1) with the antibodies present during the entire test period.

In the pVNT, sera from all three cohorts robustly neutralized the wild-type strain, Alpha, Beta, Delta VOCs as well as Omicron BA.1 and BA.2 lineages with neutralization activity being more pronounced in the breakthrough infected individuals, particularly in the BA.1 breakthrough infection cohort (mRNA-Vax^3 + BA.1). However, serum neutralizing activity of BNT162b2 triple-vaccinated SARS-CoV-2 naïve (BNT162b2^3) and mRNA-Vax^3 + BA.1 individuals against BA.2.12.1 was significantly reduced compared to wild-type ($p<0.05$) and even more so for BA.4/5 ($p<0.001$; >5-fold compared to the wild-type strain) (Fig. 2a, Table S4 and S5). In contrast, sera from triple mRNA-vaccinated individuals with Omicron BA.2 breakthrough infection (mRNA-Vax^3 + BA.2) neutralized the BA.2.12.1 pseudovirus as robustly as the wild-type strain. Neutralization of BA.4/5 was broadly similar to that of BA.2.12.1, and the reduction relative to the wild-type strain significant ($p<0.05$) yet less pronounced (~2.5-fold) as compared to the two other cohorts.

To compare the cohorts with regard to neutralization breadth irrespective of the magnitude of antibody titers, we normalized the VOC pVN_{50} GMTs against the wild-type strain. The ratios showed that BA.4/5 cross-neutralization was substantially stronger in mRNA-Vax^3 + BA.2 (GMT ratio 0.38) as compared to mRNA-Vax^3 + BA.1 and BNT162b2^3 sera (GMT ratios 0.18

and 0.17) (Fig. 2b). Similarly, cross-neutralization of Omicron BA.2.12.1 by mRNA-Vax^3 + BA.2 sera (GMT ratio 0.52) was stronger than by mRNA-Vax^3 + BA.1 sera (GMT ratio 0.43), and even more so than by BNT162b2^3 sera (GMT ratio 0.26).

A separate analysis including only the BNT162b2 vaccinated individuals within those three cohorts confirmed that BA.2 breakthrough infection is associated with considerable BA.4/5 cross-neutralization (BA.4/5 to wild-type GMT ratio 0.42), whereas after BA.1 breakthrough infection pVN_{50} GMTs against BA.4/5 were ~6-fold lower than those against wild-type (i.e., GMT ratio 0.17) (fig. S2a-c). Cross-neutralization of BA.2 and BA.2.12.1 by sera of the BA.1 or BA.2 convalescents was superior to that of BNT162b2 triple-vaccinated SARS-CoV-2 naïve individuals.

The authentic live SARS-CoV-2 virus neutralization assay provided VOC neutralizing titers that strongly correlated with those from the pVNT assay (fig. S3) and largely confirmed the major findings in Fig. 2. Again in this assay, 50% virus neutralization (VN_{50}) GMT against Omicron BA.2 in BNT162b2^3 sera was strongly reduced compared to that against wild-type ($p<0.0001$), whereas sera from both convalescent groups exhibited strong neutralizing activity, with VN_{50} GMTs comparable to those against the wild-type strain (Fig. 3a). Reduction of neutralizing activity against Omicron BA.4 was less pronounced in the BA.2 convalescent cohort as compared to BNT162b2^3 and mRNA-Vax^3 + BA.1 cohorts (VN_{50} GMTs ~2.5-fold as compared to ~15-fold and 5-fold lower than against the wild-type strain, respectively).

In line with the pVNT data, magnitude-independent analyses via the calculated ratios of VOC VN_{50} GMTs against the wild-type strain showed that BA.4 cross-neutralization was stronger in the mRNA-Vax^3 + BA.2 cohort (GMT ratio 0.39) as compared to the mRNA-Vax^3 + BA.1

(GMT ratio 0.20) and BNT162b2[3] (GMT ratio 0.07) cohorts (Fig. 3b) and similarly so within the sub-cohort of BNT162b2 triple-vaccinated individuals (fig. S2d-f).

In aggregate, these data demonstrate that Omicron BA.2 breakthrough infection of vaccinated individuals is associated with broad neutralizing activity against all tested Omicron-sublineages and previous SARS-CoV-2 VOCs. In particular, our data indicate that breakthrough infection with BA.2 is more effective (~2-fold higher cross neutralization) than that with BA.1 at refocusing neutralizing antibody responses towards the BA.4/BA.5 S glycoprotein.

Neutralization of Omicron BA.2 and BA.4/5 by sera of triple mRNA vaccinated BA.2 convalescent individuals is mediated to a large extent by NTD-targeting antibodies

To dissect the role of serum antibodies binding either to the RBD or the NTD of the S glycoprotein for neutralization of SARS-CoV-2 wild-type, Omicron BA.1, BA.2, and BA.4/5, we depleted those antibody fractions separately from sera of the three cohorts (n=6 each, fig. S4a, Table S10). We used the SARS-CoV-2 wild-type strain S glycoprotein RBD and NTD baits for depletion, as VOC breakthrough infections have been demonstrated to predominantly elicit recall responses recognizing epitopes conserved across known VOCs (*10*, *23*, *27*).

The depletion experiments removed >97% of all RBD-binding antibodies and >74% of all NTD-binding antibodies (fig. S4b). Depleted sera were subsequently tested in pVNT assays. RBD-antibody depletion strongly diminished neutralizing activity against the wild-type strain in sera from all cohorts, whereas neutralizing activity was mostly retained (>80% remaining activity) upon depletion of NTD-binding antibodies (Fig. 4a, Table S11). Neutralization of Omicron BA.1 was completely abrogated upon depletion of RBD-binding antibodies and largely unaffected by

NTD-binding antibody depletion. For neutralization of BA.2, RBD-antibody depletion almost completely abolished neutralizing activity of mRNA-Vax^3 + BA.1 sera (about 2% residual neutralization activity). The reduction of neutralizing titers for BNT162b2^3 and particularly mRNA-Vax^3 + BA.2 sera was less severe with ~12 and ~24% remaining neutralizing activity, respectively. In contrast, depletion of NTD-binding antibodies did not considerably impact the neutralizing activity of BNT162b2^3 and mRNA-Vax^3 + BA.1 sera (~91 and ~99% of undepleted control, respectively), while neutralizing activity of mRNA-Vax^3 + BA.2 sera was reduced to ~50%. A similar pattern was seen following RBD-antibody depletion for neutralization of BA.4/5, with strongly reduced neutralizing activity of mRNA-Vax^3 + BA.1 sera (~3% residual activity) versus less severe reductions for BNT162b2^3 and mRNA-Vax^3 + BA.2 sera (~20 and ~26% remaining activity, respectively). Depletion of NTD-binding antibodies had a larger impact for BA.4/5 neutralization compared to BA.2, with remaining neutralizing activity of BNT162b2^3 and mRNA-Vax^3 + BA.1 sera of ~70 and ~90% respectively, again with the strongest effect (~48% of undepleted control) of mRNA-Vax^3 + BA.2 sera.

As an orthogonal approach we assessed the neutralizing activity of sera from those 3 cohorts of vaccinated individuals against a pseudovirus harboring an engineered hybrid S glycoprotein consisting of the Omicron BA.1 N-terminus including the NTD (amino acids 1-338) and the BA.4/5 C terminus including the RBD.

The pVN_{50} GMT against the Omicron BA.1-BA.4/5 hybrid pseudovirus in sera from BNT162b2^3 was moderately below (1.86-fold) the GMT for the BA.4/5 pseudovirus, and in the BA.1 convalescents the GMT was only marginally affected (<1.5-fold reduction) (Fig. 4b, Tables S4 and S5). In contrast, in BA.2 convalescent sera titers against the hybrid pseudovirus were considerably lower than those against the BA.4/5 pseudovirus (>3-fold reduction of GMT) (Fig.

4b and Table S6), suggesting that substantial neutralizing activity can be attributed to NTD epitopes that are shared between Omicron BA.2 and BA.4/5.

In aggregate the data obtained in both experiments indicate that across all these VOCs RBD-binding antibodies have a major contribution to neutralization. Another key finding is that exposure to BA.1 (that differs substantially from previous VOCs in its NTD; fig. S1) boosts recall responses of vaccine-induced neutralizing antibodies that primarily bind the RBD, whereas exposure to BA.2 S glycoprotein (with an NTD closer related to previous VOCs) can build on existing memory and elicits a considerable recall of NTD-targeting antibodies that in turn contributes substantially to the neutralization of BA.2 and BA.4/5.

Discussion

Recent studies have demonstrated that Omicron BA.1 breakthrough infection in individuals vaccinated with mRNA vaccines BNT162b2 or mRNA-1273 or an inactivated virus vaccine boosts serum neutralizing titers against VOCs including BA.2 (*10, 15, 23*), but not against BA.2.12.1 or BA.4/BA.5. The immune escape has been attributed to boosting of pre-existing neutralizing antibody responses that recognize epitopes shared between the SARS-CoV-2 wild-type strain and Omicron BA.1 but are in part absent in BA.2.12.1, BA.4, and BA.5 due to alterations at key residues including L452Q/L452R, and F486V (*15*).

In our current study, we report that BA.2 breakthrough infection is associated with broadly neutralizing activity including BA.2 and its descendants BA.2.12.1, BA.4 and BA.5. These findings are in agreement with recent publications (*19, 28*) and suggest that the higher sequence similarity of BA.2 with BA.2.12.1 and BA.4/5 in the S glycoprotein RBD as well as the NTD drives more efficient cross-neutralization as compared to breakthrough infections with the

antigenically more distant BA.1 variant. In particular, BA.1 breakthrough infection may not elicit a strong recall of NTD-specific memory B cells owing to the substantial alterations within the BA.1 NTD (fig. S1) given that breakthrough infection with heterologous SARS-CoV-2 strains primarily expands a memory B cell repertoire against conserved S glycoprotein epitopes (*10, 23*). Our data obtained in antibody-depletion and hybrid pseudovirus experiments show that NTD-binding antibodies have a substantial contribution to neutralizing activity against Omicron BA.4/5 in triple-vaccinated BA.2 convalescent sera, whereas neutralizing activity in BA.1 convalescent sera largely relies on RBD-binding antibodies. This finding is consistent with the observation that NTD-binding antibodies isolated from BA.2 breakthrough infected individuals do not neutralize BA.1 (*29*). Together these important findings extend our knowledge on how vaccinations and boosters with the current wild-type strain-based vaccines together with breakthrough infections with the various VOCs shape the immunity patterns within the population and are material to inform further vaccine development and adaptation in response to current and emerging VOCs.

Our findings are based on retrospective analyses of samples derived from different studies, using relatively small samples 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 characteristics such as age and sex of individuals. While the SARS-CoV-2 naïve cohort was triple-vaccinated with BNT162b2, and the Omicron breakthrough cohorts were triple-vaccinated with BNT162b2 or mRNA-1273, or a heterologous regimen of the mRNA COVID-19 vaccines, key findings held true when only looking at a BNT162b2-vaccinated subset. Studies investigating long-lived plasma cell, memory B cell, and T cell immunity in cohorts with additional subjects could provide further insights into the mechanisms underlying the broad

neutralizing activity associated with Omicron BA.2 breakthrough infection and corroborate our findings.

Notwithstanding the importance of vaccination with currently approved wild-type-strain based vaccines such as BNT162b2 that offer effective protection from severe disease by current VOCs including Omicron BA.1 and BA.2 (*30, 31*), our findings highlight that consideration of rapidly evolving epidemiological landscapes and newly emerging SARS-CoV-2 variants is of high importance for guiding vaccine adaptation programs. For instance, while the efficacy of vaccine adaptation to the BA.1 strain S glycoprotein sequence is currently under investigation in clinical trials, our data suggest that further benefit may be derived from a vaccine adapted to the sequence of BA.2 or descendants.

Materials and Methods

Study design, recruitment of participants and sample collection

The objective of this study was to investigate the effect of Omicron BA.2 breakthrough infection on the cross-variant neutralization capacity of human sera. We compared immune responses in triple-mRNA (BNT162b2/mRNA-1273)-vaccinated individuals with a confirmed subsequent SARS-CoV-2 breakthrough infection in a period of Omicron BA.2 lineage-dominance in Germany (March to May 2022; mRNA-Vax^3 + BA.2), to that of triple-mRNA-vaccinated individuals with a confirmed subsequent SARS-CoV-2 breakthrough infection in a period of Omicron BA.1 lineage-dominance (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 BNT162b2[3] and mRNA-Vax[3] + BA.1 were previously published (*10*), except for newly generated BA.2.12.1 neutralization data. Cross-neutralization of variants was further characterized in smaller sub-cohorts after depletion of either wild-type S glycoprotein NTD- or RBD-targeted neutralizing antibodies.

Individuals from the BNT162b2[3] cohort provided informed consent as part of their participation in the Phase 2 trial BNT162-17 (NCT05004181). Participants from the mRNA-Vax[3] + Omi BA.1 and mRNA-Vax[3] + BA.2 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 vaccination for COVID-19. Omicron BA.1 infections were confirmed with variant-specific PCR. The infections of 4 BA.1 convalescent participants in this study were further characterized by genome sequencing. In all 4 cases, genome sequencing confirmed Omicron BA.1 infection (Table S3).

Demographic and clinical data for all participants and sampling timepoints are provided (Tables S1 to S3, and Fig. 1). All participants had no documented history of SARS-CoV-2 infection prior to vaccination. 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.

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 pseudotyped with SARS-CoV-1 S glycoprotein (UniProt Ref: P59594) and with SARS-CoV-2 S

glycoprotein derived from either the Wuhan-Hu-1 reference strain (NCBI Ref: 43740568), the Alpha variant (alterations: Δ69/70, Δ144, N501Y, A570D, D614G, P681H, T716I, S982A, D1118H), the Beta variant (alterations: L18F, D80A, D215G, Δ242–244, R246I, K417N, E484K, N501Y, D614G, A701V), 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, 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, 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), the Omicron BA.4/5 variant (alterations: 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), or an artificial Omicron BA.1-BA.4/5 hybrid S glycoprotein (alterations: A67V, Δ69/70, T95I, G142D, Δ143-145, Δ211, L212I, ins214EPE, 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. S5a and a separate alignment of S glycoprotein alterations in Omicron sub-lineages is displayed in fig. S1.

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 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 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 (*4*), that corresponds to an infectious titer of 200 transducing units (TU) per mL, was used as a 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 cells/well in medium 4 hours prior to the assay and cultured at 37 °C with 7.5% CO_2. Each

individual serum was serially diluted 2-fold in medium with the first dilution being 1:5 (SARS-CoV-2-naïve triple BNT162b2 vaccinated; dilution range of 1:5 to 1:5,120) or 1:30 (triple vaccinated after subsequent Omicron BA.1 or BA.2 breakthrough infection; dilution range of 1:30 to 1:30,720). In the case of the SARS-CoV-1 pseudovirus assay, the serum of all individuals was initially diluted 1:5 (dilution range of 1:5 to 1:5,120). 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 (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. For depletion studies resolution with regards to neutralization titers was increased, in order to discriminate smaller than 2-fold differences on an individual serum level. Neutralization titers were determined by generating a 4-parameter logistical (4PL) fit of the percent neutralization at each serial serum dilution. The 50% pseudovirus neutralization (pVN_{50}) titer was reported as the interpolated reciprocal of the dilution yielding a 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. Tables of the neutralization titers are provided (Tables S4 to S6, Table S11). SARS-CoV-2 wild-type strain, and Alpha, Beta, Delta, BA.1, BA.4/5 VOC, as well as SARS-CoV-1 pseudovirus neutralizing GMTs for the SARS-CoV-2 naïve BNT162b2 triple-vaccinated cohort and the

triple-vaccinated BA.1 convalescent cohort were previously reported in Quandt. et al. (*10*). Only the BA.2.12.1 neutralization data was newly generated from serum samples for this study.

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, heat-inactivated serum samples from individuals were serially diluted 1:2 (starting at 1:10; n=2 technical replicates per serum per virus) 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), Alpha virus strain nCoV19 isolate/England/MIG457/2020 (alterations: Δ69/70, Δ144, N501Y, A570D, D614G, P681H, T716I, S982A, D1118H), Beta virus strain nCoV19 isolate/England ex-SA/HCM002/2021 (alterations: D80A, D215G, Δ242–244, K417N, E484K, N501Y, D614G, A701V), sequence-verified Delta strain isolated from a nasopharyngeal swab (alterations: T19R, G142D, E156G, Δ157/158, L452R, T478K, D614G, P681R, R682Q, D950N), Omicron BA.1 strain hCoV-19/Belgium/rega-20174/2021 (alterations: A67V, Δ69/70, T95I, G142D, Δ143-145, Δ211, L212I, ins214EPE, G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H, T547K, D614G, H655Y, N679K, P681H, N764K, D796Y, N856K, Q954H, N969K, 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, 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. S5b. 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 (Alpha, Beta, Delta, 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 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. Tables of the neutralization titers are provided (Tables S7 to S9).

Depletion of RBD- or NTD-binding antibodies from human sera

SARS-CoV-2 wild-type strain S glycoprotein RBD- and NTD-coupled magnetic beads (Acro Biosystems, Cat.no. MBS-K002 and MBS-K019; 40 µg RBD/mg beads and 38 µg NTD/mg beads, respectively) were prepared according to the manufacturer's instructions. Beads were resuspended in ultrapure water at 1 mg beads/mL and a magnet was used to collect and wash the beads with PBS. Beads were resuspended in serum to obtain 20 µg RBD- or NTD-bait per 100 µL serum. A mock depletion (undepleted control) was performed for each serum by adding 0.5 mg Biotin-saturated MyOne™ Streptavidin T1 Dynabeads™ (ThermoFisher, Cat.no. 65601) per 100 µL serum. Beads were incubated with human sera for 1 hour with gentle rotation. A

magnet was used to separate bead-bound antibodies from the depleted supernatant. Depleted and undepleted sera were analyzed for cross-neutralization capacity using pseudovirus neutralization assays. Depletion efficacy for both RBD- and NTD-binding antibodies was determined by a multiplexed electrochemiluminescence immunoassay (Meso Scale Discovery, V-Plex SARS-CoV-2 Panel 1 Kit, Cat. No. K15359U-2). Table of the neutralization titers is provided (Table S11).

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 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. 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.

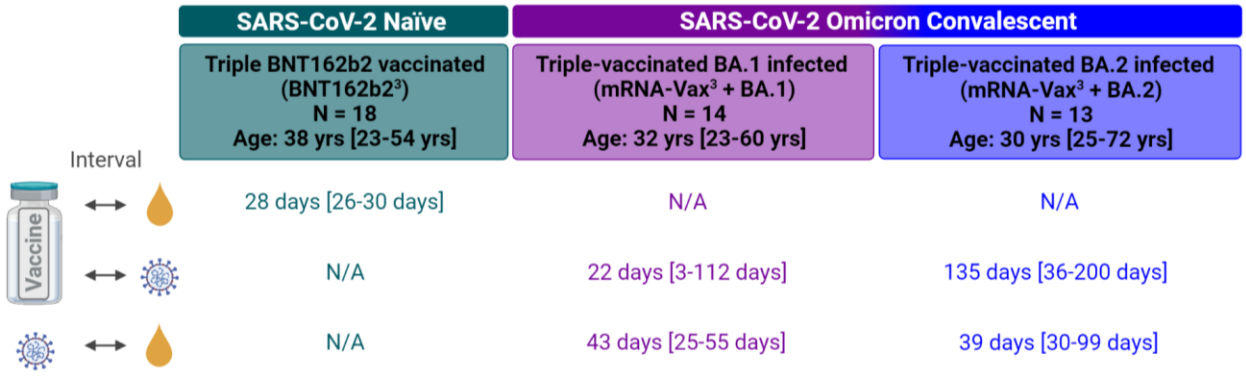


Fig. 1. Cohorts and sampling.

Serum samples (yellow droplet) were drawn from three cohorts: individuals triple-vaccinated with BNT162b2 that were SARS-CoV-2-naïve at the time of sampling (BNT162b2[3], green), and from 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 either at a time of BA.1 dominance (November 2021 to January 2022; mRNA-Vax[3] + BA.1, purple) or at a time of BA.2 dominance (March to May 2022; mRNA-Vax[3] + BA.2, blue). 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 cohorts is further detailed in Tables S1 to S3. Data for the reference cohorts BNT162b2[3] and mRNA-Vax[3] + BA.1 were previously published (*10*), except for newly generated BA.2.12.1 neutralization data.

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

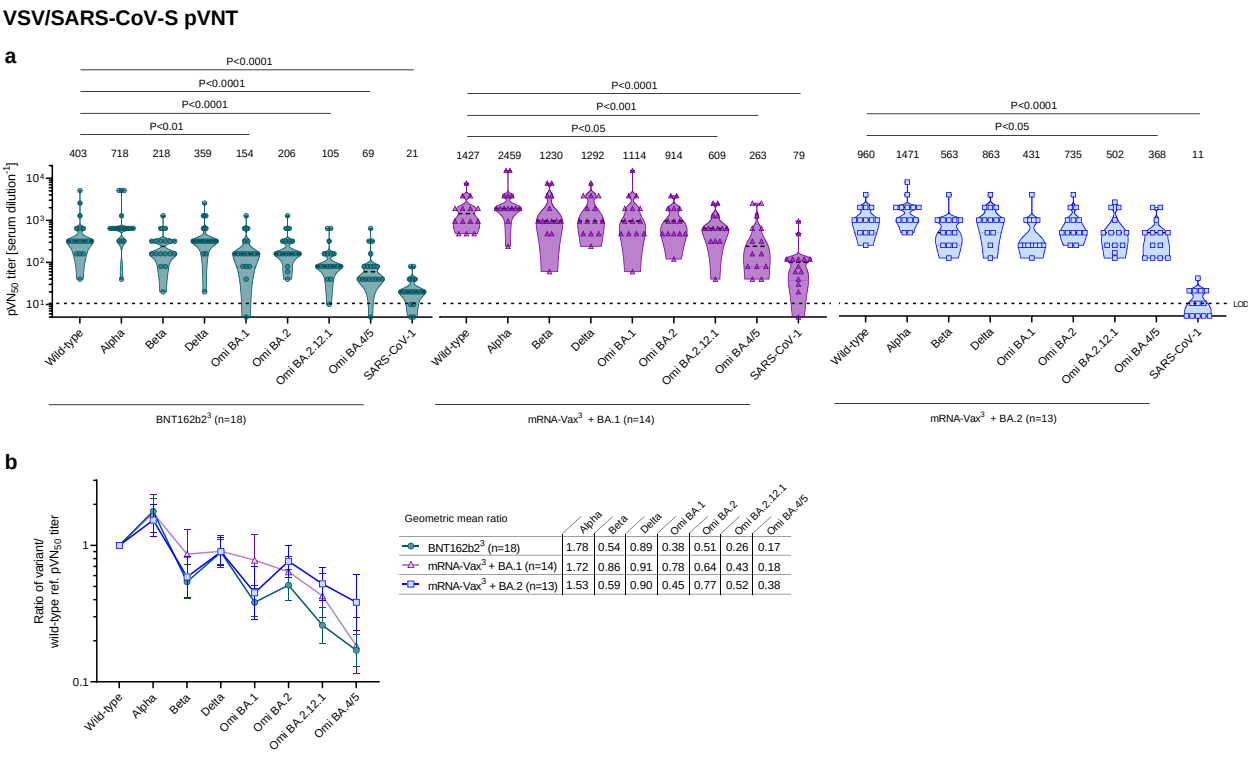


Fig. 2. Omicron BA.2 breakthrough infection of triple mRNA vaccinated individuals induces broad neutralization of SARS-CoV-2 variant pseudoviruses including Omicron BA.4/5.

Cohorts and serum sampling as described in Fig. 1. (a) 50% pseudovirus neutralization (pVN_{50}) geometric mean titers (GMTs) against the indicated SARS-CoV-2 variants of concern (VOCs) or SARS-CoV-1 pseudoviruses. Data for the reference cohorts BNT162b2[3] and mRNA-Vax[3] + BA.1 were previously published (*10*), except for newly generated BA.2.12.1 neutralization data. Values above violin plots represent group GMTs. (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 with 95% confidence intervals are shown. Serum was tested in duplicate. For titer values below the limit of detection (LOD), LOD/2 values were plotted. 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.

445

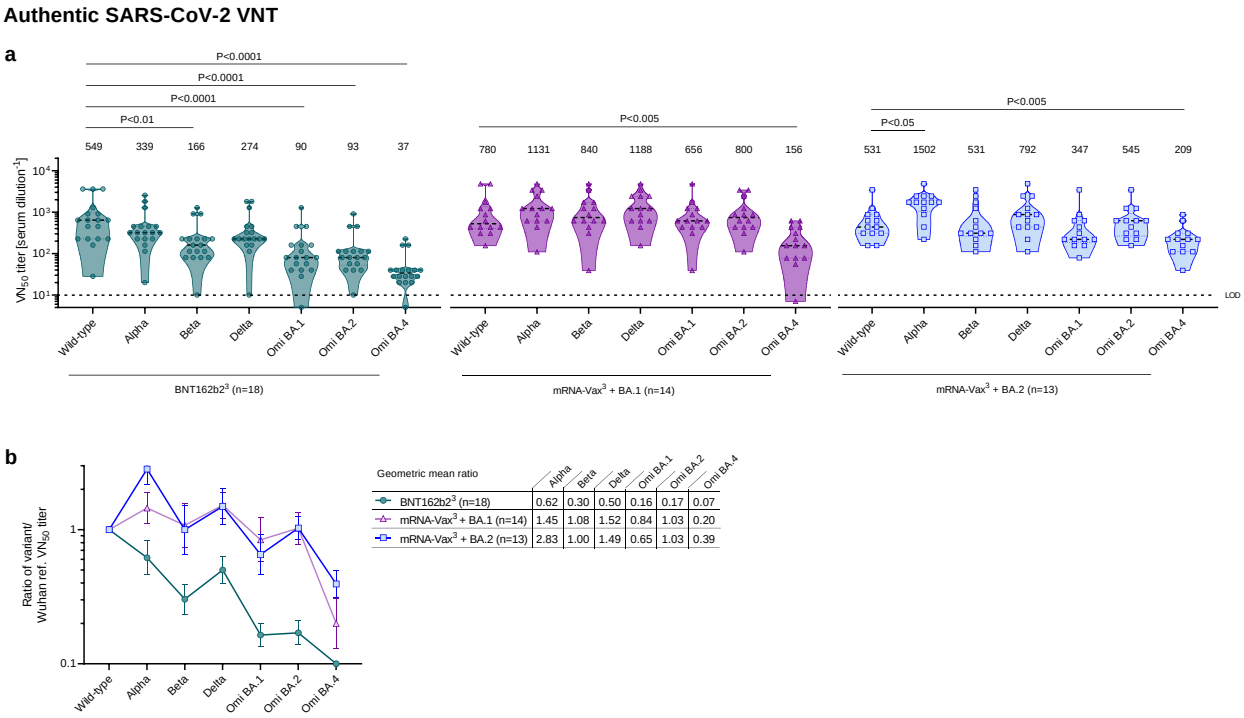


Fig. 3. Omicron BA.2 breakthrough infection of previously vaccinated individuals induces broad neutralization of authentic live SARS-CoV-2 variants including Omicron BA.4/5.

Cohorts and serum sampling as described in Fig. 1. (a) 50% virus neutralization (VN_{50}) geometric mean titers (GMTs) against the indicated SARS-CoV-2 variants of concern (VOCs). Data for the reference cohorts BNT162b2[3] and mRNA-Vax[3] + BA.1 were previously published (*10*). Values above violin plots represent group GMTs. (b) 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 with 95% confidence intervals are shown. Serum was tested in duplicate. For titer values below the limit of detection (LOD), LOD/2 values were plotted. 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.

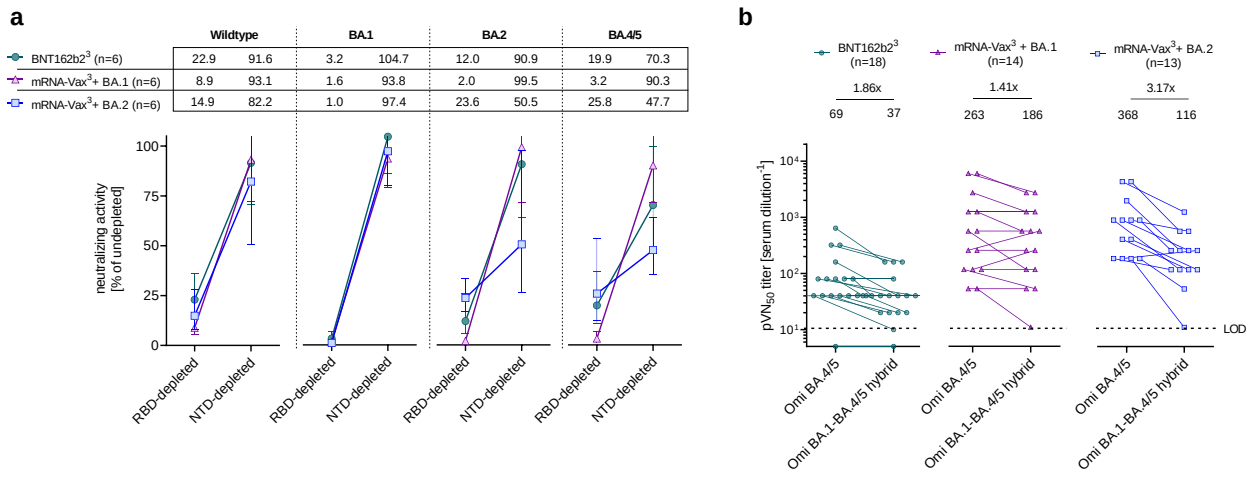


Fig. 4. Neutralization of Omicron BA.2 and BA.4/5 by sera of triple mRNA vaccinated BA.2 convalescent individuals is mediated to a large extent by NTD-targeting antibodies.

Cohorts and serum sampling as described in Fig. 1. (a) Serum samples (n=6 per cohort) were depleted of RBD- or NTD-binding antibodies. Relative neutralizing activity of RBD- and NTD-depleted sera (pVN_{50} titers of undepleted control sera were set to 100%) against the wild-type strain, BA.1, BA.2, and BA.4/5 was calculated and group geometric mean with 95% confidence intervals are shown. (b) 50% pseudovirus neutralization (pVN_{50}) geometric mean titers (GMTs) against Omicron BA.4/5 and Omicron BA.1-BA.4/5 hybrid pseudoviruses. Numbers above plots indicate group geometric mean titers (GMTs) and fold-change in GMTs between BA.4/5 and the hybrid pseudovirus. For titer values below the limit of detection (LOD), LOD/2 values are plotted.

Acknowledgments:

We thank the BioNTech global clinical Phase 2 trial (NCT04380701) participants, and the Omicron BA.1 and BA.2 convalescent research study participants from whom the post-immunization human sera were obtained. We thank the many colleagues at BioNTech and Pfizer who developed and produced the BNT162b2 vaccine candidate. We thank S. Jägle and N. Beckmann for logistical support; S. Shpyro, S. Nadim, C. Heiser, A. Telorman, C. Müller, A. Wanamaker, N. Williams, and J. VanCamp for sample demographics support; and the VisMederi team for work on live virus–neutralizing antibody assays.

Funding:

This work was supported by BioNTech.

Author contributions:

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

Competing interests:

U.S. and Ö.T. are management board members and employees at BioNTech SE. A.M., B.G.L., K.K., A.W., M.B., A.F., A.T., and O.O. are employees at BioNTech SE. K.G., S.H. and S.C. are employees at University Hospital, Goethe University Frankfurt. U.G. is an employee at the Health Protection Authority, City of Frankfurt am Main. U.S., Ö.T. and A.M. are inventors on patents and patent applications related to RNA technology and COVID-19 vaccines. U.S., Ö.T., A.M., B.G.L., K.K., A.W., M.B., A.F., A.T., and O.O. have securities from BioNTech SE. S.C. has received honorarium for serving on a clinical advisory board for BioNTech.

Data and materials availability:

Participant baseline characteristics are provided in Table S1 to Table S3 and Table S10. The neutralization titers are provided in Tables S4 to S9 and Table S11.

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

References

1. WHO Technical Advisory Group on SARS-CoV-2 Virus Evolution (TAG-VE): Classification of Omicron (B.1.1.259): SARS-CoV-2 Variant of Concern. (2021).
2. WHO Headquarters (HQ), WHO Health Emergencies Programme, Enhancing Response to Omicron SARS-CoV-2 variant: Technical brief and priority actions for Member States. (2022).
3. M. Hoffmann *et al.*, The Omicron variant is highly resistant against antibody-mediated neutralization: Implications for control of the COVID-19 pandemic. *Cell* **185**, 447-456 e411 (2022).
4. W. Dejnirattisai *et al.*, SARS-CoV-2 Omicron-B.1.1.529 leads to widespread escape from neutralizing antibody responses. *Cell* **185**, 467-484 e415 (2022).
5. V. Servellita *et al.*, Neutralizing immunity in vaccine breakthrough infections from the SARS-CoV-2 Omicron and Delta variants. *Cell* **185**, 1539-1548 e1535 (2022).
6. C. Kurhade *et al.*, Neutralization of Omicron BA.1, BA.2, and BA.3 SARS-CoV-2 by 3 doses of BNT162b2 vaccine. *Nat Commun* **13**, 3602 (2022).
7. Y. Cao *et al.*, Omicron escapes the majority of existing SARS-CoV-2 neutralizing antibodies. *Nature* **602**, 657-663 (2022).
8. WHO Technical Advisory Group on COVID-19 Vaccine Composition (TAG-CO-VAC): Interim statement on the composition of current COVID-19 vaccines. (2022).
9. M. E. McMenamin *et al.*, Vaccine effectiveness of one, two, and three doses of BNT162b2 and CoronaVac against COVID-19 in Hong Kong: a population-based observational study. *Lancet Infect Dis*, (2022).
10. 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).
11. E. Callaway, What Omicron's BA.4 and BA.5 variants mean for the pandemic. *Nature* **606**, 848-849 (2022).
12. H. Tegally *et al.*, Emergence of SARS-CoV-2 Omicron lineages BA.4 and BA.5 in South Africa. *Nat Med*, (2022).
13. S. Xia, L. Wang, Y. Zhu, L. Lu, S. Jiang, Origin, virological features, immune evasion and intervention of SARS-CoV-2 Omicron sublineages. *Signal Transduct Target Ther* **7**, 241 (2022).
14. European Centre for Disease Prevention and Control, Weekly COVID-19 country overview - Country overview report: week 27 2022 (2022).
15. Y. Cao *et al.*, BA.2.12.1, BA.4 and BA.5 escape antibodies elicited by Omicron infection. *Nature*, (2022).
16. N. P. Hachmann *et al.*, Neutralization Escape by SARS-CoV-2 Omicron Subvariants BA.2.12.1, BA.4, and BA.5. *N Engl J Med* **387**, 86-88 (2022).
17. T. N. Starr, A. J. Greaney, A. S. Dingens, J. D. Bloom, Complete map of SARS-CoV-2 RBD mutations that escape the monoclonal antibody LY-CoV555 and its cocktail with LY-CoV016. *Cell Rep Med* **2**, 100255 (2021).
18. A. Tuekprakhon *et al.*, Antibody escape of SARS-CoV-2 Omicron BA.4 and BA.5 from vaccine and BA.1 serum. *Cell* **185**, 2422-2433 e2413 (2022).
19. Q. Wang *et al.*, Antibody evasion by SARS-CoV-2 Omicron subvariants BA.2.12.1, BA.4, & BA.5. *Nature*, (2022).
20. Y. Wang *et al.*, Structural basis for SARS-CoV-2 Delta variant recognition of ACE2 receptor and broadly neutralizing antibodies. *Nat Commun* **13**, 871 (2022).
21. P. Wang *et al.*, Antibody resistance of SARS-CoV-2 variants B.1.351 and B.1.1.7. *Nature* **593**, 130-135 (2021).
22. L. Liu *et al.*, Striking antibody evasion manifested by the Omicron variant of SARS-CoV-2. *Nature* **602**, 676-681 (2022).
23. C. I. Kaku *et al.*, Recall of pre-existing cross-reactive B cell memory following Omicron BA.1 breakthrough infection. *Sci Immunol*, eabq3511 (2022).
24. A. Muik *et al.*, Neutralization of SARS-CoV-2 Omicron by BNT162b2 mRNA vaccine-elicited human sera. *Science* **375**, 678-680 (2022).
25. A. Muik *et al.*, Neutralization of SARS-CoV-2 lineage B.1.1.7 pseudovirus by BNT162b2 vaccine-elicited human sera. *Science* **371**, 1152-1153 (2021).
26. C. W. Tan *et al.*, Pan-Sarbecovirus Neutralizing Antibodies in BNT162b2-Immunized SARS-CoV-1 Survivors. *N Engl J Med* **385**, 1401-1406 (2021).

27. R. Nutalai *et al.*, Potent cross-reactive antibodies following Omicron breakthrough in vaccinees. *Cell* **185**, 2116-2131 e2118 (2022).
28. P. Arora *et al.*, Augmented neutralisation resistance of emerging omicron subvariants BA.2.12.1, BA.4, and BA.5. *Lancet Infect Dis*, (2022).
29. E. Andreano *et al.*, Anatomy of Omicron BA.1 and BA.2 neutralizing antibodies in COVID-19 mRNA vaccinees. *Nat Commun* **13**, 3375 (2022).
30. N. Andrews *et al.*, Covid-19 Vaccine Effectiveness against the Omicron (B.1.1.529) Variant. *N Engl J Med* **386**, 1532-1546 (2022).
31. S. Y. Tartof *et al.*, Immunocompromise and durability of BNT162b2 vaccine against severe outcomes due to omicron and delta variants. *Lancet Respir Med* **10**, e61-e62 (2022).

Supplementary Materials:

figs. S1-S5

Tables S1-S11

Supplementary Materials for

Omicron BA.2 breakthrough infection enhances cross-neutralization of BA.2.12.1 and BA.4/BA.5

Alexander Muik, Bonny Gaby Lui, Maren Bacher, Ann-Kathrin Wallisch, Aras Toker, Andrew Finlayson, Kimberly Krüger, Orkun Ozhelvaci, Katharina Grikscheit, Sebastian Hoehl, Sandra Ciesek, Özlem Türeci, Ugur Sahin

Correspondence to: Ugur.Sahin@biontech.de

This PDF file includes:

fig. S1 to S5

Tables S1 to S11

595 **fig. S1**

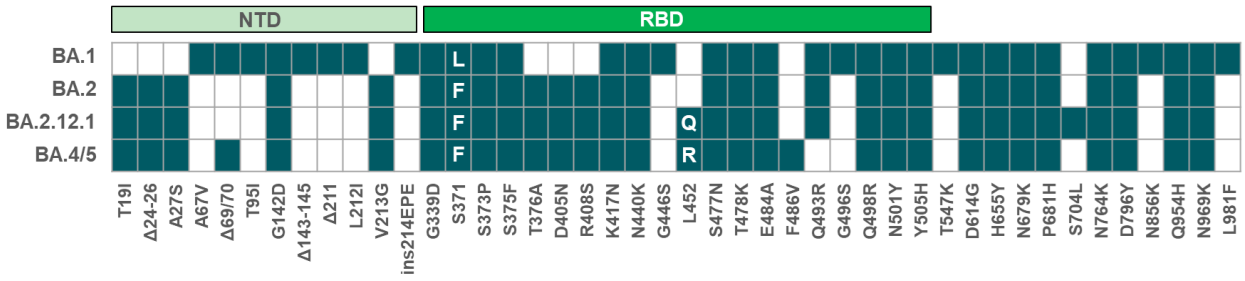
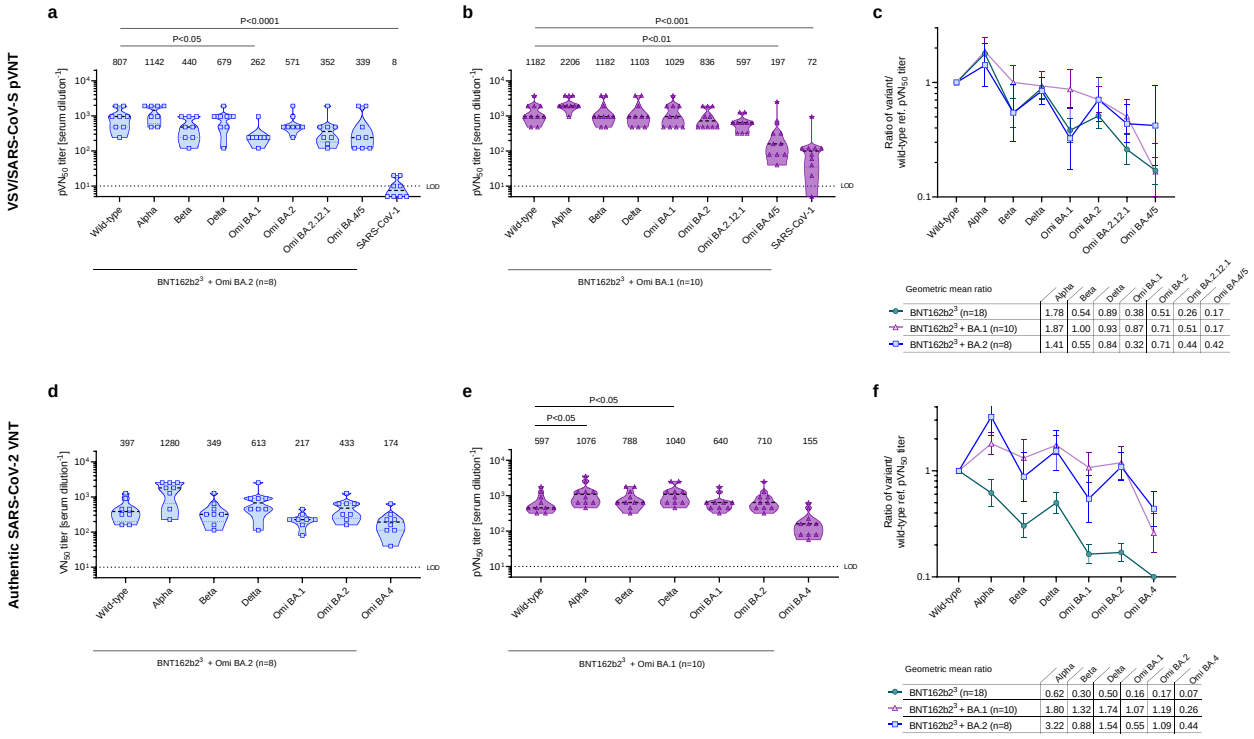


Fig. S1. Alterations of the spike glycoprotein amino acid sequence of SARS-CoV-2 Omicron sub-lineages.

Amino acid exchange and mutation type (substitutions, deletions, insertions) are indicated. White

600 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



605 **Fig. S2. Omicron BA.2 breakthrough infection of BNT162b2 triple-vaccinated individuals induces broad neutralization of VOCs including Omicron BA.4/BA.5.**

Cohorts and serum sampling as described in Fig. 1. (a-b) 50% pseudovirus neutralization (pVN_{50}) geometric mean titers (GMTs) against the indicated SARS-CoV-2 variants of concern (VOCs) or SARS-CoV-1 pseudoviruses. Values above violin plots represent group GMTs. (c) 610 The ratio of SARS-CoV-2 VOC pVN_{50} GMTs normalized against the wild-type strain pVN_{50} GMT. Geometric mean ratios for the Omicron BA.2 breakthrough infected cohort were compared to data previously published in Quandt et al. (*1*) for BNT162b2^3 and BNT162b2^3 + BA.1, except for newly generated BA.2.12.1 neutralization data. Group geometric mean ratios with 95% confidence intervals are shown. (d-e) 50% virus neutralization (VN_{50}) GMTs for 615 BNT162b2^3 + BA.1 and BNT162b2^3 + BA.2. Values above violin plots represent group GMTs.

(f) The ratio of SARS-CoV-2 VOC GMTs normalized against the wild-type strain VN_{50} GMT. Serum was tested in duplicate. For titer values below the limit of detection (LOD), LOD/2 values are plotted. The non-parametric Friedman test with Dunn's multiple comparisons correction was used to compare the group GMT against the wild-type strain with group GMTs against the indicated variants and SARS-CoV-1. Multiplicity-adjusted p values are shown.

fig. S3

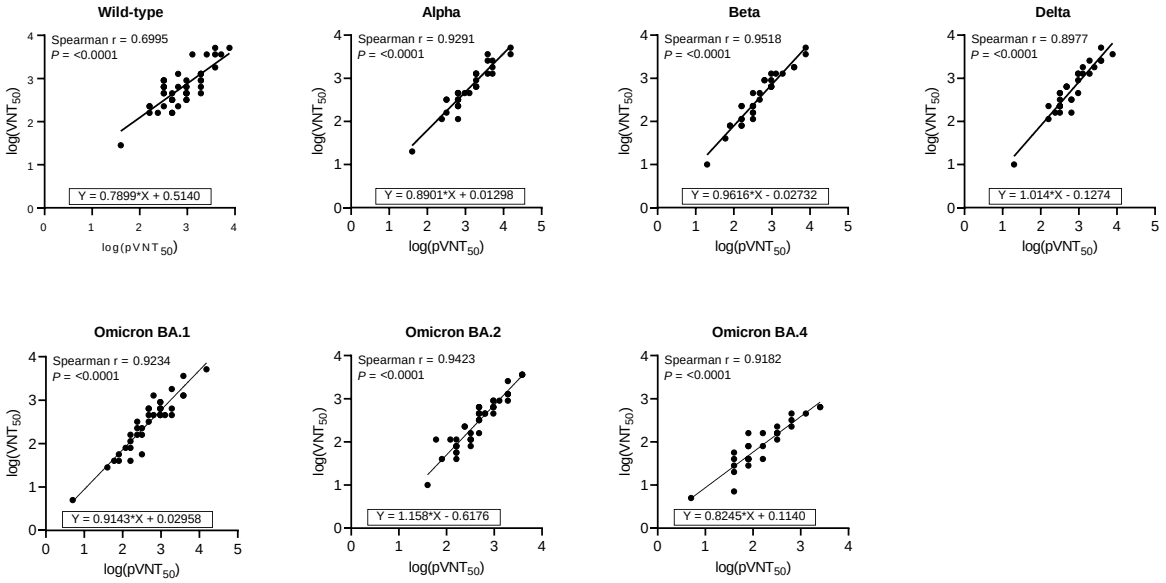


Fig. S3. 50% pseudovirus neutralization (pVN_{50}) correlates with 50% live SARS-CoV-2 neutralization (VN_{50}) titer data

Nonparametric Spearman correlation of VSV-SARS-CoV-2 pVN_{50} with live SARS-CoV-2 VN_{50} titers for n=45 serum samples drawn from SARS-CoV-2-naïve BNT162b2 triple-vaccinated individuals (BNT162b2[3]; n=18) after the third dose, from triple mRNA vaccinated individuals with subsequent Omicron BA.1 breakthrough infection (mRNA-Vax[3] + BA.1; n=14) post-infection, and from triple mRNA vaccinated individuals with subsequent Omicron BA.2 breakthrough infection (mRNA-Vax[3] + BA.2; n=13) post-infection. Correlations are plotted per SARS-CoV-2 variant. Correlation coefficient r, two-tailed P values and the linear equation are given.

.

fig. S4

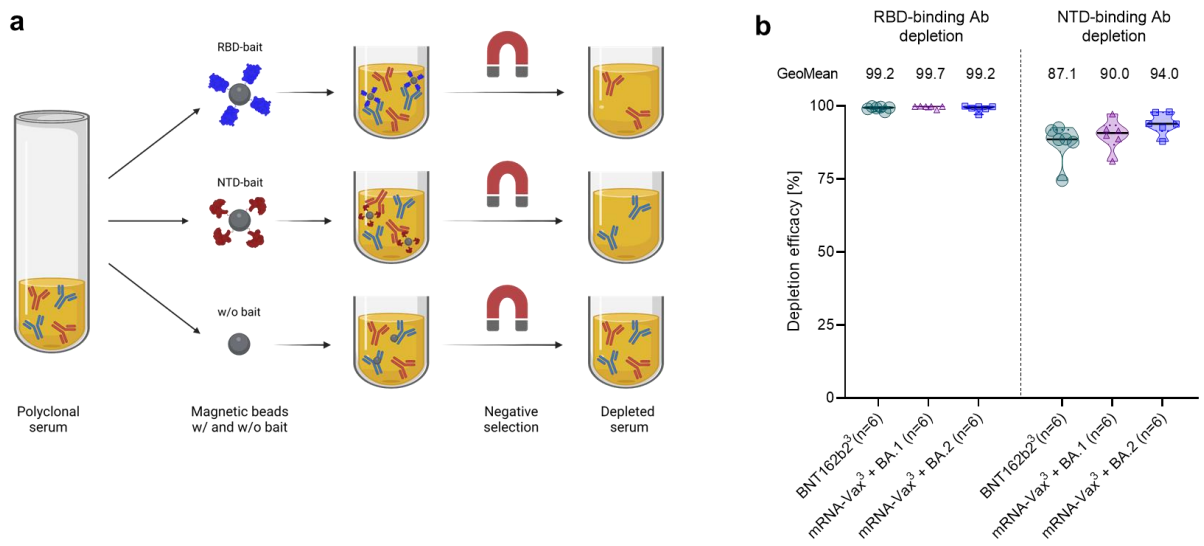
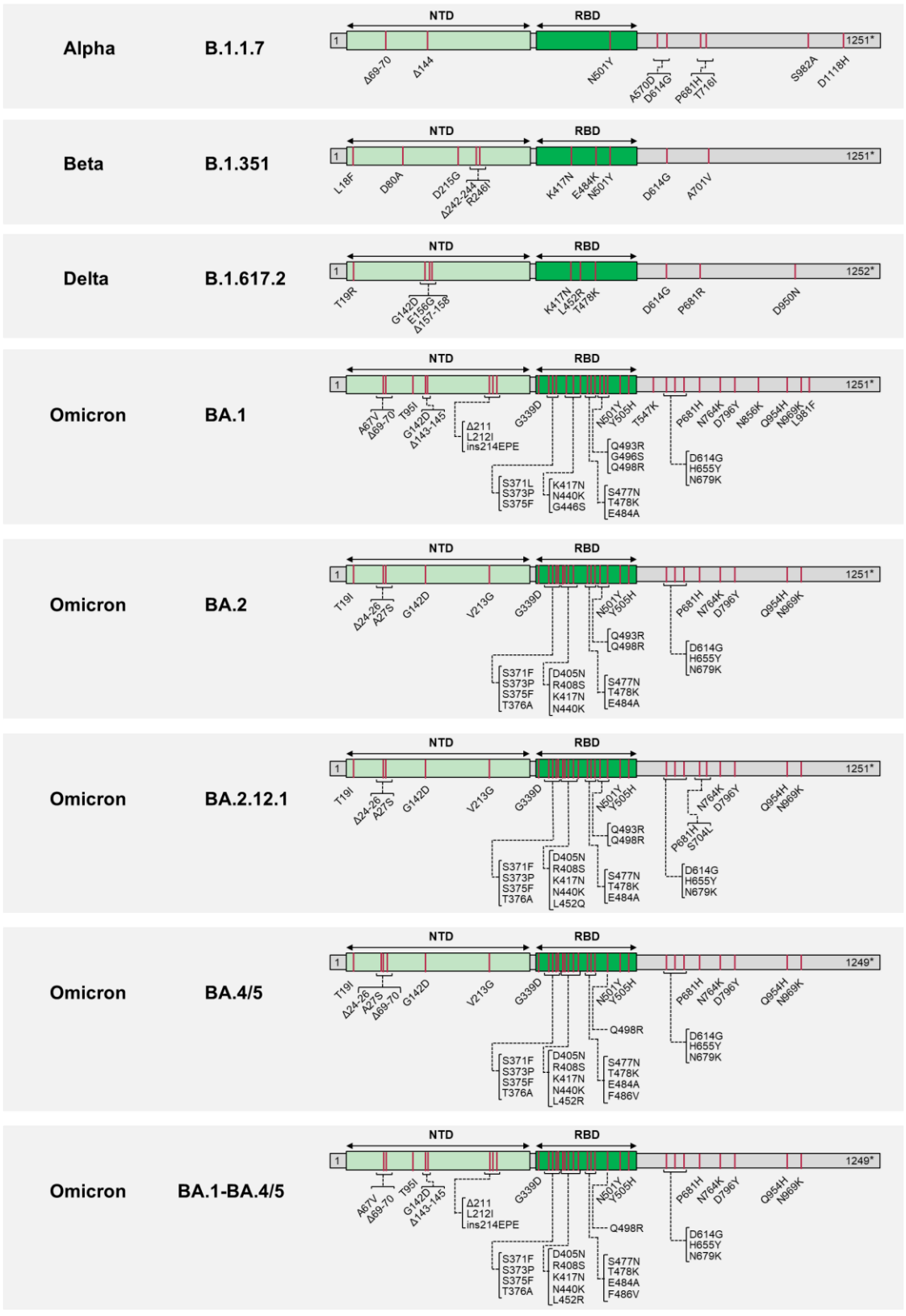


Fig. S4. RBD-binding and NTD-binding antibodies can be depleted from human serum

Serum was drawn from SARS-CoV-2-naïve BNT162b2 triple-vaccinated individuals (BNT162b2^3; n=6), and from triple mRNA vaccinated individuals with Omicron BA.1 (mRNA-Vax^3 + BA.1; n=6) or Omicron BA.2 breakthrough infection (mRNA-Vax^3 + BA.2; n=6). Magnetic bead technology was used for depleting serum of RBD- or NTD-binding antibodies, or for mock depleting. (a) Schematic of antibody depletion from serum. (b) The relative concentration of RBD-binding and NTD-binding antibodies was determined by a multiplexed electrochemiluminescence immunoassay. The relative decrease in antibody concentrations in depleted compared to mock-depleted sera are shown. Numbers above graph depict geometric mean reduction within groups.

Fig. S5

a



650

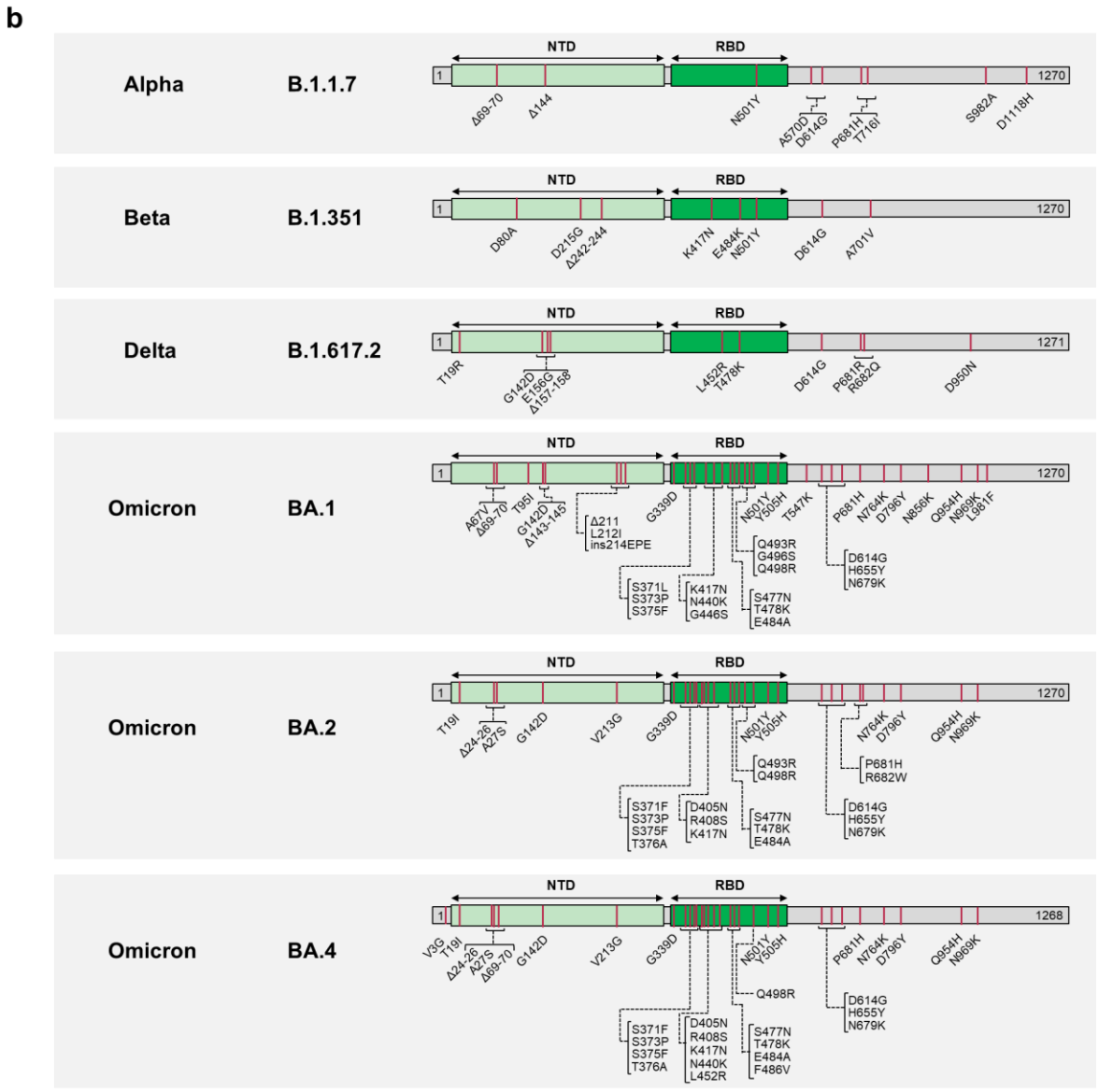


Fig. S5. Characterization of SARS-CoV-2 S glycoproteins used in the assays based on (a) VSV-SARS-CoV-2 variant pseudoviruses and (b) live authentic SARS-CoV-2.

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 kind 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	BNT162b2[3] (n=18)	mRNA-Vax[3] + BA.1 (n=14)	mRNA-Vax[3] + BA.2 (n=13)
Sex, n (%)			
Male	9 (50)	11 (79)	4 (31)
Female	9 (50)	3 (21)	9 (69)
Age, median (range)	38 (23-54)	32 (23-60)	30 (25-72)
Age group at vaccination, n (%)			
18-55 yrs	18 (100)	12 (86)	11 (85)
56-85 yrs	0 (0)	2 (14)	2 (15)
SARS-CoV-2 status, n (%)			
Positive	0 (0)	14 (100)[#]	13 (100)*
Negative	18 (100)†	0 (0)	0 (0)
Unknown	0 (0)	0 (0)	0 (0)
Interval, median (range)			
Days between D1/D2	‡	38 (20-92)	42 (15-43)
Days between D2/D3	202 (181-266)	192 (154-256)	184 (152-259)
Days until serum draw after D3	28 (26-30)	N/A	N/A
Days between last dose/infection	N/A	25 (3-112)	135 (36-200)
Days until serum draw after infection	N/A	43 (25-55)	39 (30-99)

N/A, not applicable; D, dose; yrs, years; n, number.
*, Individuals experienced SARS-CoV-2 breakthrough infections between March and May 2022, during which period the BA.2 lineage was dominant in Germany
[#], 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)
‡, 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.1 (mRNA-Vax^3 + BA.1).

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)
14	32	f	BNT^3	NOV2021	BA.1	24	243	64	55	1-2
15	32	m	BNT^3	NOV2021	BA.1	20	233	66	53	1-2
16	28	m	BNT^3	DEC2021	n/a	35	213	10	47	1-2
17	29	f	BNT^3	DEC2021	BA.1	36	189	3	46	1-2
18	23	m	BNT^3	DEC2021	n/a	42	159	27	44	1-2
19	31	m	BNT^3	DEC2021	n/a	42	166	20	43	1-2
20	53	m	BNT^3	JAN2022	n/a	39	194	22	25	1-2
21	50	f	BNT^3	JAN2022	n/a	92	169	35	28	1-2
22	50	m	BNT^3	JAN2022	n/a	42	169	44	31	1-2
23	60	m	BNT^3	JAN2022	n/a	26	236	112	43	1-2
24	28	m	MOD^2/BNT	DEC2021	n/a	28	252	22	40	1-2
25	32	m	MOD^2/BNT	DEC2021	n/a	42	154	13	42	1-2
26	50	m	MOD^2/BNT	JAN2022	n/a	28	256	45	31	1-2
27	60	m	MOD^3	DEC2021	BA.1	42	172	3	35	1-2
Median	**32**	**N/A**	**N/A**	**N/A**	**N/A**	**38**	**192**	**25**	**43**	**N/A**

m, male; f, female; n/a, not available; N/A, not applicable
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. Individuals triple vaccinated with mRNA COVID-19 vaccine and subsequently infected with Omicron BA.2 (mRNA-Vax^3 + BA.2).

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	42	F	BNT^3	APR2022	n/a	42	158	135	36	1-2
2	44	M	BNT^2/MOD	APR2022	n/a	42	164	129	36	1-2
3	28	f	MOD^2/BNT	MAR2022	n/a	42	163	36	99	1-2
4	26	f	BNT^3	APR2022	n/a	43	152	104	58	1-2
5	34	m	BNT^3	MAY2022	n/a	40	154	141	37	1-2
6	29	m	BNT^3	APR2022	n/a	42	165	89	63	1-2
7	57	f	MOD^2/BNT	APR2022	n/a	15	244	153	46	1-2
8	25	m	MOD/BNT^2	MAY2022	n/a	28	217	188	34	1-2
9	30	f	MOD^2/BNT	APR2022	n/a	42	195	129	66	1-2
10	28	f	BNT^2/MOD	MAY2022	n/a	21	224	64	39	1-2
11	29	f	BNT^3	APR2022	n/a	22	249	149	64	1-2
12	53	f	BNT^3	MAY2022	n/a	29	259	200	30	1-2
13	72	f	BNT^3	MAY2022	n/a	42	184	157	30	1-2
Median	**30**	**N/A**	**N/A**	**N/A**	**N/A**	**42**	**184**	**135**	**39**	**N/A**

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

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

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

Participant ID	pVN_{50}									
	Wild-type	Alpha	Beta	Delta	Omicron BA.1	Omicron BA.2	Omicron BA.2.12.1	Omicron BA.4/5	Omicron BA.1-BA.4/5	SARS-CoV-1
28	160	320	160	160	80	160	80	40	40	5
29	640	640	320	320	160	320	160	40	40	40
30	5120	5120	1280	2560	1280	1280	640	640	160	40
31	320	640	160	320	160	160	80	40	40	20
32	640	640	80	640	320	160	40	40	20	20
33	320	640	160	320	160	160	80	40	20	10
34	320	640	320	320	160	160	80	80	40	10
35	320	640	320	320	160	160	160	80	40	20
36	160	320	80	160	40	80	40	40	10	20
37	320	1280	160	320	160	60	160	80	40	20
38	1280	5120	640	1280	640	640	640	320	160	80
39	40	40	20	20	5	40	10	5	5	5
40	320	640	320	320	80	160	80	40	20	20
41	160	320	160	320	80	160	80	40	20	20
42	320	640	320	320	320	320	160	160	40	20
43	640	640	320	320	160	320	80	80	40	40
44	2560	5120	640	1280	640	640	320	320	160	80
45	320	640	160	640	160	320	80	80	80	20

Table S5. pVN_{50} values of sera collected from individuals with Omicron BA.1 breakthrough infection (mRNA-Vax^3 + BA.1)

Participant ID	pVN_{50}									
	Wild-type	Alpha	Beta	Delta	Omicron BA.1	Omicron BA.2	Omicron BA.2.12.1	Omicron BA.4/5	Omicron BA.1-BA.4/5	SARS-CoV-1
14	1920	3840	1920	1920	1920	960	640	320	80	120
15	960	1920	960	480	480	480	640	160	320	120
16	3840	3840	3840	3840	1920	1920	1280	640	640	960
17	960	1920	960	960	960	960	640	160	160	120
18	480	1920	960	480	480	480	640	40	40	20
19	1920	1920	960	960	960	1920	640	320	320	40
20	960	1920	960	960	1920	480	320	80	160	80
21	960	1920	480	960	480	480	320	80	80	5
22	480	960	480	480	480	480	320	80	40	60
23	1920	3840	3840	3840	3840	1920	1280	2560	1280	120
24	3840	15360	7680	7680	3840	3840	2560	2560	1280	120
25	7680	15360	7680	3840	15360	3840	2560	1280	640	480
26	480	240	60	240	60	120	40	40	10	30
27	1920	1920	960	1920	960	960	640	640	320	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	Alpha	Beta	Delta	Omicron BA.1	Omicron BA.2	Omicron BA.2.12.1	Omicron BA.4/5	Omicron BA.1-BA.4/5	SARS-CoV-1
1	480	1920	960	480	240	480	160	120	80	5
2	3840	7680	3840	3840	3840	3840	2560	960	160	40
3	480	960	240	240	240	240	240	120	40	10
4	240	480	120	120	120	240	120	120	10	5
5	1920	1920	480	960	240	480	480	240	80	20
6	480	480	240	480	240	480	240	240	80	5
7	960	960	480	960	960	960	480	480	160	5
8	960	1920	960	1920	960	960	960	480	320	20
9	1920	3840	960	1920	960	1920	1920	480	160	20
10	960	960	240	960	240	480	240	120	160	10
11	960	960	480	960	240	480	480	480	80	10
12	1920	1920	960	1920	240	1920	1920	1920	640	5
13	960	1920	960	960	960	960	480	1920	320	20

685

Table S7. VN_{50} values of sera collected from SARS-CoV-2-naïve triple-vaccinated individuals (BNT162b2[3])

Participant ID	VN_{50}						
	Wild-type	Alpha	Beta	Delta	Omicron BA.1	Omicron BA.2	Omicron BA.4
28	226	320	80	226	40	40	28
29	640	320	226	226	160	80	28
30	3620	2560	1280	1810	453	905	226
31	226	113	226	160	40	80	28
32	226	453	80	160	57	40	20
33	640	453	113	226	80	57	40
34	453	226	226	453	80	80	28
35	640	320	226	226	160	113	40
36	160	160	80	113	28	40	20
37	640	453	80	320	80	113	40
38	3620	1280	905	1280	453	453	160
38	28	20	10	10	5	10	5
39	453	320	113	226	40	80	20
40	226	320	113	226	57	57	28
41	905	226	160	453	160	113	40
42	1280	226	160	226	113	113	40
43	3620	1810	905	1810	1280	453	160
44	905	453	226	320	80	113	40
45	226	320	80	226	40	40	28

Table S8. 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	Alpha	Beta	Delta	Omicron BA.1	Omicron BA.2	Omicron BA.4
14	453	1280	1280	1280	640	905	226
15	453	640	640	640	453	453	80
16	1810	3620	1810	2560	1810	1280	453
17	453	1280	905	1280	453	640	160
18	453	640	640	640	640	320	57
19	640	1280	640	905	640	905	160
20	320	640	640	453	453	640	160
21	905	905	320	1280	320	453	80
22	320	453	453	640	640	453	80
23	1280	2560	1810	2560	1280	2560	640
24	5120	3620	3620	3620	1280	3620	640
25	5120	5120	5120	5120	5120	3620	453
26	160	113	40	160	40	113	7
27	1280	1280	1280	2560	905	905	320

690

Table S9. VN_{50} values of sera collected from individuals with Omicron BA.2 breakthrough infection (mRNA-Vax^3 + BA.2)

Participant ID	VN_{50}						
	Wild-type	Alpha	Beta	Delta	Omicron BA.1	Omicron BA.2	Omicron BA.4
1	320	1810	320	453	226	320	113
2	3620	5120	3620	5120	3620	3620	905
3	320	905	226	226	160	226	113
4	160	226	113	113	80	226	40
5	905	2560	320	905	226	640	226
6	160	453	160	453	226	160	113
7	640	1810	320	640	640	640	226
8	640	1810	1810	1280	905	453	226
9	905	1810	2560	2560	640	1280	320
10	453	1810	320	453	226	320	160
11	320	1280	453	905	160	640	226
12	1280	2560	640	2560	320	1280	640
13	453	2560	1280	905	453	640	320

.

Table S10. Vaccinated individuals analyzed for neutralizing antibody responses after depletion of RBD-/NTD-binding antibodies.

Characteristic	mRNA-Vax^3 + BA.2 (n=6)	mRNA-Vax^3 + BA.1 (n=6)	BNT162b2^3 (n=6)
Sex, n (%)			
Male	1 (17)	4 (67)	4 (67)
Female	5 (83)	2 (33)	2 (33)
Age, median (range)	41.5 (25-72)	32 (29-53)	36 (23-49)
Age group at vaccination, n (%)			
18-55 yrs	4 (67)	6 (100)	6 (100)
56-85 yrs	2 (33)	0 (0)	0 (0)
SARS-CoV-2 status, n (%)			
Positive	6 (100)*	6 (100)#	0 (0)
Negative	0 (0)	0 (0)	6 (100)†
Unknown	0 (0)	0 (0)	0 (0)
Interval, median (range)			
Days between D1/D2	28.5 (15-42)	39 (20-92)	‡
Days between D2/D3	230.5 (184-259)	189 (154-233)	219 (181-266)
Days until serum draw after D3	N/A	N/A	27.5 (26-30)
Days between last dose/infection	155 (129-200)	20 (3-66)	N/A
Days until serum draw after infection	40 (30-66)	43 (25-53)	N/A

N/A: not applicable; D, Dose; Yrs, Years; n, Number.
*, Individuals experienced SARS-CoV-2 breakthrough infections between March and May 2022, during which period the BA.2 lineage was dominant in Germany
#, 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)
‡, 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 S11. pVN_{50} values of sera depleted of NTD or RBD-binding antibodies

Participant ID	Cohort	pVN_{50}											
		Wild-type			Omicron BA.1			Omicron BA.2			Omicron BA.4/5		
		Mock	NTD	RBD	Mock	NTD	RBD	Mock	NTD	RBD	Mock	NTD	RBD
7	mRNA-Vax^3+BA.2	1179	1021	200	996	1143	10	880	668	219	494	252	193
8	mRNA-Vax^3+BA.2	2039	1187	158	1276	1491	10	981	730	162	1148	525	123
9	mRNA-Vax^3+BA.2	2226	1607	673	1224	1276	10	1217	738	460	1139	420	535
11	mRNA-Vax^3+BA.2	1435	939	251	650	641	10	537	346	169	538	273	97
12	mRNA-Vax^3+BA.2	3562	2287	834	674	479	10	5562	805	1129	1797	626	1043
13	mRNA-Vax^3+BA.2	1463	2956	98	1401	1219	10	1403	732	247	821	634	118
15	mRNA-Vax^3+BA.1	1037	953	103	601	623	10	359	354	10	239	232	10
17	mRNA-Vax^3+BA.1	1401	1681	61	1525	1379	10	973	740	25	492	430	45
19	mRNA-Vax^3+BA.1	1992	1589	235	1754	1363	54	990	923	10	573	438	10
20	mRNA-Vax^3+BA.1	842	766	127	789	836	10	825	557	10	241	319	10
21	mRNA-Vax^3+BA.1	1404	1751	77	697	546	10	388	556	10	277	254	10
25	mRNA-Vax^3+BA.1	1870	1224	219	1439	1613	46	849	1216	27	886	612	10
30	BNT162b2^3	3818	2279	497	746	923	10	1349	644	463	620	238	278
32	BNT162b2^3	521	537	160	143	173	10	98	644	10	59	238	10
38	BNT162b2^3	2085	2332	348	1014	1147	39	844	931	38	364	276	56
43	BNT162b2^3	631	671	281	237	265	10	187	164	40	108	101	36
44	BNT162b2^3	1985	2071	463	745	591	10	525	648	61	247	222	47
45	BNT162b2^3	631	487	133	191	167	10	131	130	10	119	97	10

References

1. 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).