THE LANCET
Infectious Diseases

Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Blom K, Marking U, Havervall S, et al. Immune responses after omicron infection in triple-vaccinated health-care workers with and without previous SARS-CoV-2 infection. *Lancet Infect Dis* 2022; published online June 9. https://doi.org/10.1016/S1473-3099(22)00362-0.

Supplementary Appendix

Methods

Study population

The COMMUNITY study enrolled 2149 healthcare workers employed at Danderyd Hospital, Stockholm, Sweden, between April and May, 2020. Study participants are followed every four months since study inclusion [1-6]. All healthcare workers were offered primary vaccination (BNT162b2 (BNT) or ChAdOx1 nCoV-19 (ChAd) depending on availability), starting in January 2021, and a booster dose (BNT or mRNA-1273 (MOD) starting December 2021. Data on vaccination status was obtained through the Swedish vaccination register (VAL Vaccinera). SARS-CoV-2 infection prior to vaccination was determined by either seroconversion at any of the follow-up visits and/or positive PCR test through the national communicable diseases register SmiNet (Public Health Agency of Sweden). All SARS-CoV-2 infections prior to the Omicron breakthrough infections in this study occurred prior to the first vaccine dose and prior to the circulation of SARS-CoV-2 variants in Sweden (before end of January 2021) and are therefore assumed to be WT infections.

A 4-week (January 19th to February 21st 2022) twice weekly qPCR screening study [7] of 375 healthcare workers was conducted, initiated 5 weeks (median 34 days, IQR 31-35 days) after a first booster vaccine dose. Blood samples were first collected at inclusion. Participants testing positive following a negative sample were enrolled in an extended study protocol with additional naso-oropharyngeal/saliva swabs every other day for 15 days (median 7 (IQR 7-7) samples collected) for investigation of viral characteristics[7], and blood samples 1, 2, 3, 5 weeks (n=30) and 7 weeks (n=56) after the first positive qPCR test. As reference, blood samples were collected at the end of the study period from 69 participants who remained negative throughout the screening period. qPCR, whole genome sequencing (WGS), and virus isolation was performed as previously described [8].

The study was approved by the Swedish Ethical Review Authority (dnr 2020-01653) and conducted in accordance with the declaration of Helsinki. Written informed consent was obtained from all study participants.

Serological responses

Anti-spike IgG, surrogate virus neutralizing titers (antibodies capable of blocking spike-ACE2 binding) and percent inhibition against SARS-CoV-2 wild type, delta, BA.1 and BA.2 were quantified by V-PLEX SARS-CoV-2 (Panel 25, Meso Scale Diagnostics, Maryland, USA). The assays were performed according to the manufacturer´s instructions and as previously described [9]. Antibody titers are expressed as arbitrary units (AU)/ml. An in-house calibration curve (starting dilution 1:2) was generated for the ACE2 competitive binding assay using a serum sample obtained from a previously omicron-infected study participant.

Live microneutralization assay based on cytopathic effects (CPE) was performed as previously described for wild-type SARS-CoV-2 and the omicron variant BA.1 on Vero E6 cells using heat-inactivated serum [10,11]. After 5 days of incubation, wells were inspected for signs of CPE by optical microscopy. Each well was scored as either neutralizing (if no signs of CPE was observed) or non-neutralizing (if any CPE was observed). The arithmetic mean neutralization titer of the reciprocals of the highest neutralizing dilutions from the two duplicates for each sample was then calculated to determine the 100% inhibitory dilution (ID_{100}).

T-cell responses

Peripheral blood mononuclear cells (PBMCs) were isolated from whole blood and quantified as previously described [12]. T-SPOT Discovery SARS-CoV-2 kits (Oxford Immunotec, Oxfordshire, UK) were performed according to the manufacturer´s instructions. Briefly, after dilution of recovered cells, 250,000 PBMCs were plated per well, stimulated with S1, N and M protein peptides, and 20 hours later interferon-γ secreting T-cells were revealed and counted.

T-cell responses were analyzed in samples collected 7 (median 6.7, IQR 6-7.4) weeks post omicron breakthrough infection, and in samples collected from participants who remained SARS-CoV-2 negative throughout the screening period.

Statistics

Participants were stratified according to SARS-CoV-2 infection prior to vaccination and according to occurrence of omicron BA.1 or BA.2 breakthrough infection during the

screening period. Comparisons of antibody titres and T-cell responses between groups and between time points were performed using Wilcoxon matched-pairs signed rank or Mann-Whitney U tests. Significance of correlation was analysed with Spearman's rank correlation. All statistical analyses were performed in GraphPad Prism version 9.2.0 (GraphPad Software, San Diego, California, USA).

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The funders had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

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Supplementary tables and figures

	qPCR neg			qPCR pos		
	All	No prior infection	Prior infection*	All	No prior infection	Prior infection*
n	69	44	25	56	40	16
Age, median (IQR)	55 (50-59)	57 (54-59)	52 (47-56)	52 (41-59)	53 (45-59)	48 (41-60)
Female, n (%)	61 (94)	40 (91)	21 (84)	52 (93)	38 (95)	14 (88)
Days booster vaccine to study inclusion, median (IQR)	34 (32-36)	34 (33-36)	34 (32-36)	34 (32-35)	35 (32-36)	31 (33-34)
Primary vaccine regimen						
BNT x 2, n (%)	26 (38)	18 (40)	8 (32)	32 (57)	26 (65)	6 (38)
ChAd x 2, n (%)	22 (32)	13 (30)	9 (36)	8 (14)	3 (8)	5 (31)
ChAd + BNT, n (%)	21 (30)	13 (30)	8 (32)	16 (29)	11 (27)	5 (31)
Booster vaccine						
BNT, n (%)	3 (4)	3 (7)	0 (0)	14 (25)	11 (28)	3 (19)
MOD, n (%)	66 (96)	41 (93)	25 (100)	42 (75)	29 (72)	13 (81)
Whole genome sequencing						
BA.1, n (%)	-	-	-	29 (52)	20 (50)	9 (56)
BA.2, n (%)	-	-	-	21 (37)	15 (37)	6 (38)
Undetermined, n (%)	-	-	-	6 (11)	5 (13)	1 (6)

Table S1. Demographics and vaccine regimens of study cohort, and sublineage if breakthrough infection. m; median, IQR; interquartile range; BNT; BNT162b2 mRNA vaccine, ChAd; ChAdox1 nCoV-19 vaccine, MOD; mRNA-1273 vaccine.

* All participants with prior infection had a confirmed SARS-CoV-2 infection prior to primary vaccination and prior to the circulation of SARS-CoV-2 variants in Sweden (before end of January 2021) and are therefore assumed to be WT infections.

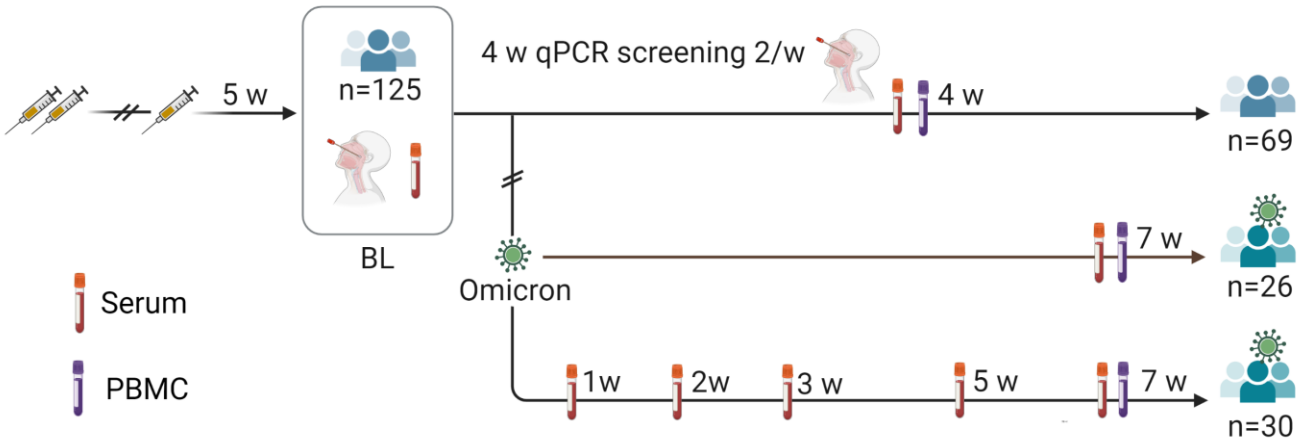

Figure S1. Study protocol. Blood samples were collected from 125 healthcare workers 5 weeks post a first mRNA booster vaccine dose (baseline; BL) at inclusion of a 4-week screening study where self-administered naso-oropharyngeal/saliva swabs were obtained twice weekly. Blood samples were collected 1, 2, 3 and 5 weeks after the first positive qPCR test in 30 participants who tested positive following a negative qPCR test. Blood samples were collected 7 weeks post infection in an additional 26 participants who tested positive following a negative study qPCR. As reference, blood samples were collected at the end of the study period from 69 participants who remained negative throughout the screening period.

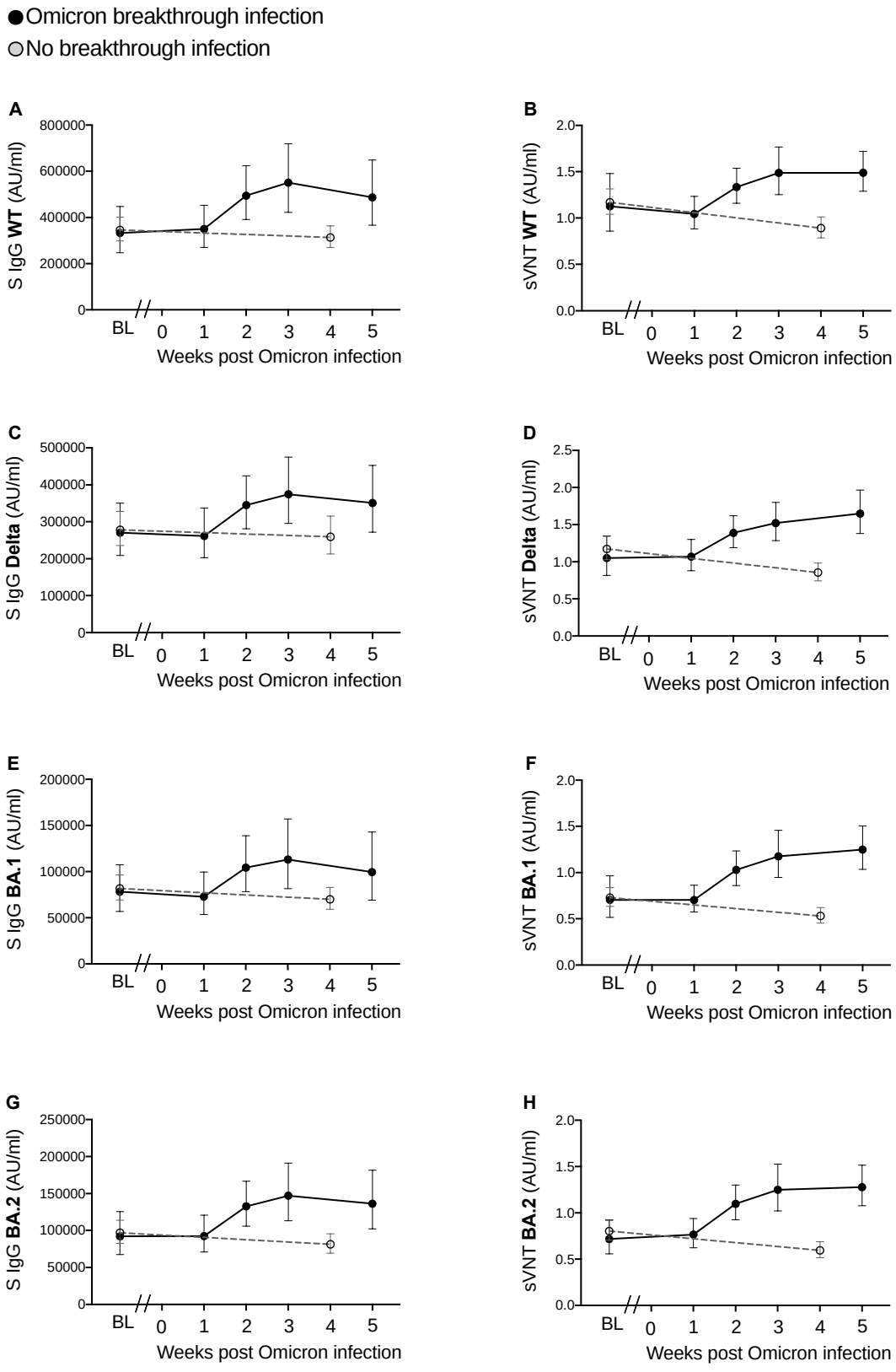

Figure S2. Antibody responses following omicron breakthrough infection in triple vaccinated healthcare workers. Blood samples were collected from 125 healthcare workers 5 weeks post a first mRNA booster vaccine dose (baseline; BL) at inclusion of a 4-week screening study where self-administered naso-

oropharyngeal/saliva swabs were obtained twice weekly. Blood samples were collected 1, 2, 3 and 5 weeks after the first positive qPCR test in 30 participants who tested positive following a negative qPCR. Blood samples were collected 7 weeks post infection in an additional 26 participants who tested positive following a negative study qPCR. As reference, blood samples were collected at the end of the study period from 69 participants who remained negative throughout the screening period. **A-H)** Geometric mean titers (GMT) of anti-spike IgG and surrogate virus neutralizing titers against WT (A-B), delta (C-D), and omicron BA.1 (E-F) and BA.2 (G-H). S; spike, sVNT; surrogate virus neutralization test, WT; wild type, BL; baseline.

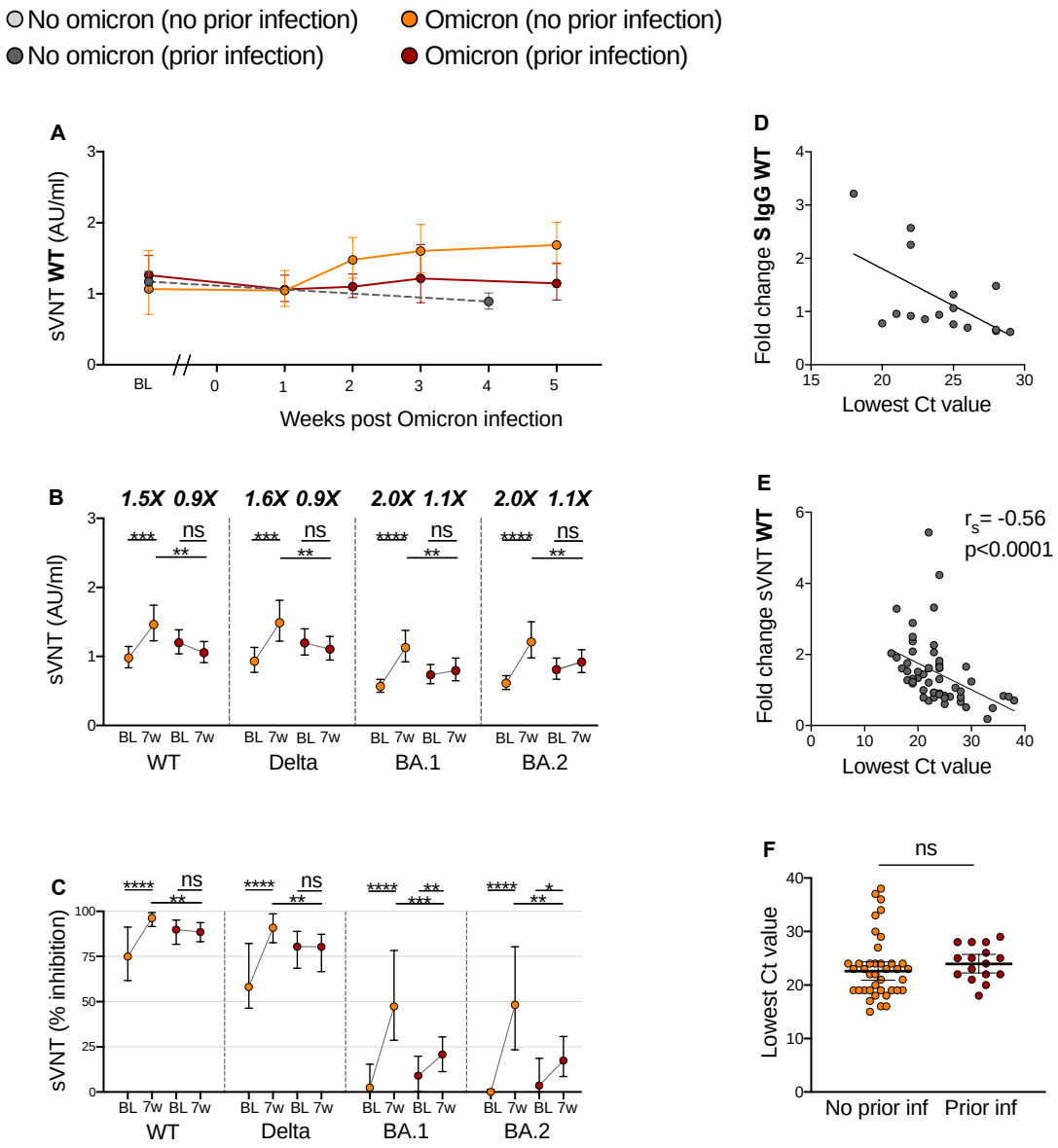

Figure S3. Antibody responses following omicron breakthrough infection in triple vaccinated healthcare workers with and without prior SARS-CoV-2 infection. A) Surrogate virus neutralizing titers against WT at BL and 1, 2, 3 and 5 weeks post omicron breakthrough infection in participants without (orange, n=20) and with (red, n=10) previous SARS-CoV-2 infection. Grey dots and dashed line represent participants who remained qPCR negative throughout the study period (n=69). **B)** Surrogate virus neutralizing titers against WT, delta, and omicron BA.1 and BA.2 variants at BL and 7 weeks after omicron breakthrough infection in participants without (orange, n=40) and with (red, n=16) previous SARS-CoV-2 infection. Numbers in bold italic depict fold change between BL and 7 weeks post omicron breakthrough infection. **C)** % inhibition of ACE2-binding to the WT, delta, BA.1 and BA.2 spike protein, respectively, at 5 weeks post a first mRNA booster

vaccine dose (baseline; BL) and 7 weeks post omicron breakthrough infection in participants without (orange, n=40) and with (red, n=16) previous SARS-CoV-2 infection. Serum was diluted 100 fold, allowing for calculations of % inhibition of ACE2-binding to the different Spike-variants. Data presented as median (IQR) **D-E)** Correlation between lowest detected Ct value during omicron breakthrough infection (n=56) and the fold change in anti-WT spike IgG (D) and surrogate neutralizing titers (E) between BL and 7 weeks post omicron breakthrough infection. **F)** Lowest Ct values during omicron breakthrough infection in participants without (orange, n=39) and with (red, n=16) prior SARS-CoV-2 infection. S; spike, sVNT; surrogate virus neutralization test, WT; wild type, BL; baseline, Ct; cycle threshold, inf; infection, **; $p<0.01$, ***; $p<0.001$, ****; $p<0.0001$, ns; not significant.

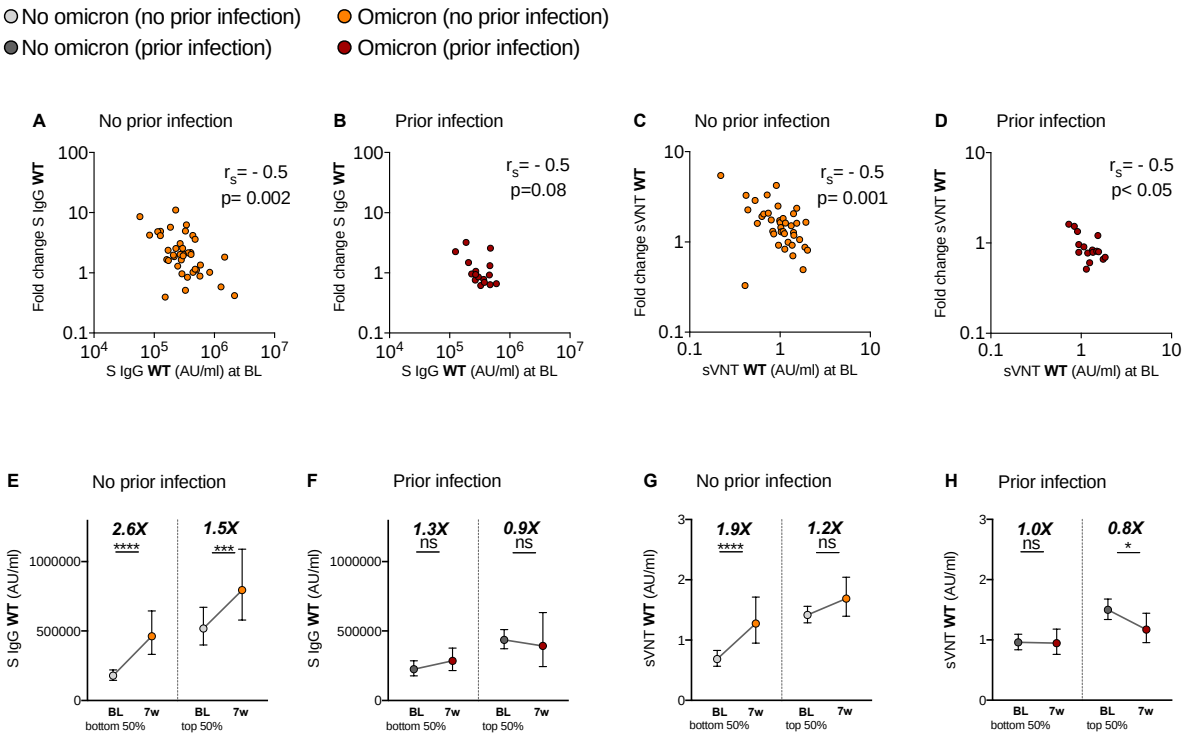

Figure S4. Correlations between pre-infection antibody titers and magnitude of immune responses following omicron breakthrough infection in triple vaccinated participants with and without previous SARS-CoV-2 infection. A-B) Correlations between pre-infection (baseline; BL, 5 weeks post mRNA booster dose) anti-spike WT IgG titers and fold change in anti-spike WT IgG titers 7 weeks after omicron breakthrough infection in participants without previous SARS-CoV-2 infection (A) and with previous SARS-CoV-2 infection (B). C-D) Correlations between pre-infection (baseline; BL, 5 weeks post mRNA booster dose) WT surrogate virus neutralizing titers and fold change in WT surrogate virus neutralizing titers 7 weeks after omicron breakthrough infection in participants without previous SARS-CoV-2 infection (C) and with previous SARS-CoV-2 infection (D). E-F) Anti-spike WT IgG titers pre-infection (5 weeks post mRNA booster dose) and 7 weeks after omicron breakthrough infection in participants with pre-infection titers above and below median. E) Participants without previous SARS-CoV-2 infection and F) participants with previous SARS-CoV-2 infection. G-H) WT surrogate virus neutralizing titers pre-infection (5 weeks post mRNA booster dose) and 7 weeks after omicron breakthrough infection in participants with pre-infection WT surrogate virus neutralizing titers above and below median. G) participants without previous SARS-

CoV-2 infection and H) participants with previous SARS-CoV-2 infection. sVNT; surrogate virus neutralization test, WT; wild type, BL; baseline.
*,p<0.05;****,p<0.0001.

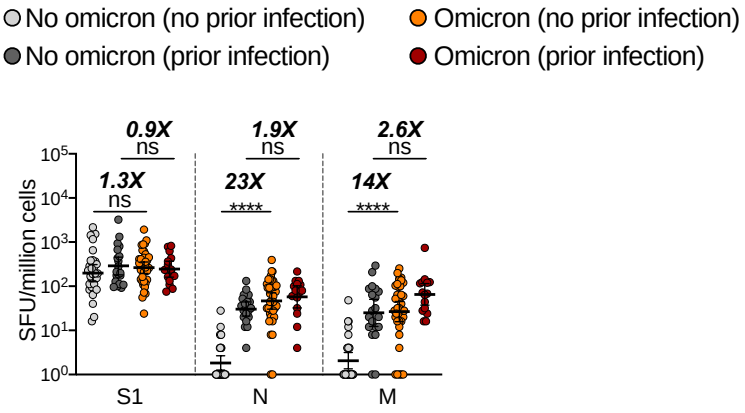

Figure S5. T-cell responses against S1, N and M in participants without and with breakthrough infection, stratified according to previous SARS-CoV-2 infection. Light grey; participants without previous SARS-CoV-2 infection who remained negative throughout the screening program, dark grey; participants with previous SARS-CoV-2 infection who remained negative throughout the screening program, orange; participants without previous SARS-CoV-2 infection with omicron breakthrough infection, red; participants with previous SARS-CoV-2 infection with omicron breakthrough infection. Numbers in bold italic depict ratio of SFU/million cells between participants without and with breakthrough infection. Post omicron infection (orange and red) PBMCs were collected 7 weeks after omicron breakthrough infection. S, spike; N, nucleocapsid; M, membrane;****,p<0.0001

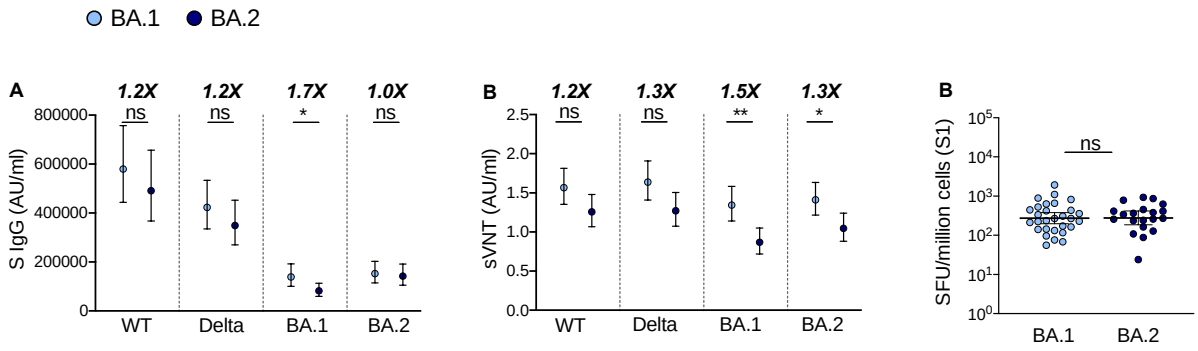

Figure S6. Antibody and T-cell responses following omicron BA.1 and BA.2 infection. A) Anti-spike WT IgG titers against WT, delta, and omicron BA.1 and BA.2 at baseline and 7 weeks after omicron BA.1 (light blue, n=29) and BA.2 (dark blue, n=21) breakthrough infection. B) Surrogate virus neutralizing titers against WT, delta, and omicron BA.1 and BA.2 at baseline and 7 weeks after omicron BA.1 (light blue, n=29) and BA.2 (dark blue, n=21) breakthrough infection. B) T-cell responses against WT S1 7 weeks after BA.1 (light blue, n=18) and BA.2 (dark blue, n=14) omicron breakthrough infection. sVNT; surrogate virus neutralization titers, WT; wild type. , SFU; spot forming units. *; p<0.05; **; p<0.01.

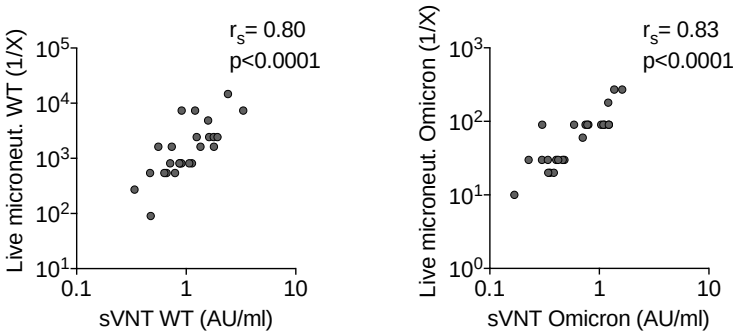

Figure S7. Correlations between live virus microneutralizing titers and surrogate virus neutralizing titers against SARS-CoV-2 wild type and Omicron BA.1 in samples from triple vaccinated healthcare workers (n=25). WT; wild type, sVNT; surrogate virus neutralization test.