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Supplementary appendix

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Supplement to: Yao L, Zhu K-L, Jiang X-L, et al. Omicron subvariants escape antibodies elicited by vaccination and BA.2.2 infection. *Lancet Infect Dis* 2022; published online June 20. https://doi.org/10.1016/S1473-3099(22)00410-8.

Supplemental Appendix

Omicron subvariants escape antibodies elicited by vaccination and BA.2.2 infection

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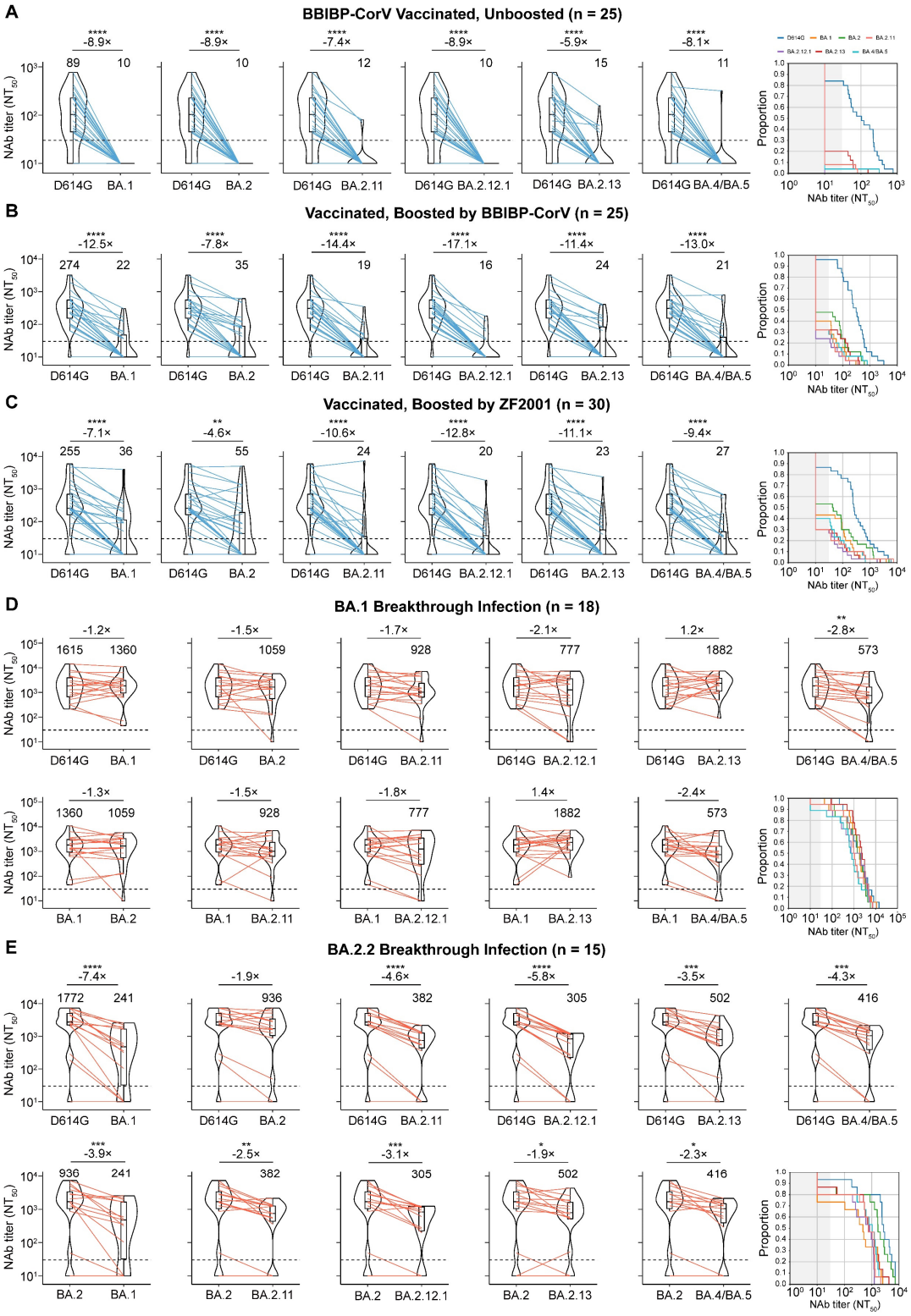


Figure 1. Neutralizing antibody levels in vaccinated individuals with or without booster vaccination and Omicron breakthrough infections. (**A-C**) Box-violin plots show the neutralizing antibody titers against the SARS-CoV-2 D614G and Omicron subvariants in two BBIBP-CoV doses vaccinated (**A**), BBIBP-CoV booster vaccinated (**B**) and ZF2001 booster

vaccinated (**C**) individuals (left), along with cumulative distribution function plots of titers against D614G and Omicron subvariant (right), showing the proportion of samples at or above a given titer. (**D**) Box-violin plots show the neutralizing antibody titers against Omicron subvariants compared to D614G and the neutralizing antibody titers against other Omicron subvariants compared to BA.1, along with cumulative distribution function plots of titers against D614G and Omicron subvariant (lower right), showing the proportion of samples at or above a given titer, in BA.1 breakthrough infections. (**E**) Box-violin plots show the neutralizing antibody titers against Omicron subvariants compared to D614G and the neutralizing antibody titers against other Omicron subvariants compared to BA.2, along with cumulative distribution function plots of titers against D614G and Omicron subvariant (lower right), showing the proportion of samples at or above a given titer, in BA.2.2 breakthrough infection. For the box-violin plots, the median is represented by the thick black line inside the box. The geomatic mean titers (GMTs) are shown above each column. The fold-change of GMT is displayed. The horizontal dotted line represents the limit of detection of 30. A two-tailed Friedman test with a false discovery rate for multiple comparisons was performed to compare Omicron subvariants to the D614G or BA.1 and BA.2. *p*-values are represented as ***p*<0.01, ****p*<0.001, and *****p*<0.0001. No asterisk indicates no statistical significance.

Methods

Human subjects

Sera for primary vaccinees with two doses of BBIBP-CorV were collected from SARS-CoV-2 naïve individuals 5 weeks after the second dose. Sera for booster vaccinees with the third dose of BBIBP-CorV or ZF2001 (an RBD-based protein subunit vaccine by Anhui Zhifei Longcom) were collected from previously vaccinated individuals with two BBIBP-CorV doses approximately one month after the third dose vaccination. Sera for Omicron BA.1 or Omicron BA.2.2 breakthrough infections were collected from patients when discharged from the hospital. The breakthrough infection was defined as fully vaccinated individuals are being diagnosed with SARS-CoV-2 infection[1,2]. Fully vaccination was defined as when the second or third shot of the BBIBP-CorV vaccination or the third shot of the ZF2001 vaccination was administered at least 14 days before symptom onset or a positive PCR test for SARS-CoV-2[1,2]. All individuals with breakthrough infection had sequence confirmed Omicron infection or PCR-confirmed symptomatic disease occurring whilst in isolation and direct contact with Omicron sequence-confirmed cases. The study was approved by the Institutional Review Board of the Beijing Institute of Microbiology and Epidemiology (IRB number: AF/SC-08/02.60 and AF/SC-08/02.124). Written informed consent was obtained from all participants.

Spike plasmid pseudovirus production

The SARS-CoV-2 Wuhan-Hu-1 spike, cloned into pCDNA3.1 was mutated using the QuikChange Lightning Site-Directed Mutagenesis kit (Agilent Technologies) and NEBuilder HiFi DNA Assembly Master Mix (NEB) to include D614G (original), Omicron BA.1 (A67V, Δ69-70, T95I, G142D, Δ143-145, Δ211, L212I, 214EPE, 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), Omicron BA.2 (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), Omicron BA.2.11 (BA.2+L452R), Omicron BA.2.12.1 (BA.2+L452Q+S704L), Omicron BA.2.13 (BA.2+L452M), and Omicron BA.4/BA.5 (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). Pseudovirus particles were generated by co-transfecting HEK-293T cells (ATCC) with human immunodeficiency virus backbones expressing firefly luciferase (pNL4-3-R-E-luciferase) and pcDNA3.1 vector encoding either D614G or mutated S proteins (Omicron subvariants) plasmid. The medium was replaced with fresh medium at 24 h, and supernatants were harvested at 48 h post-transfection and clarified by centrifugation at 300 *g* for 10 min before aliquoting and storing at -80°C until using.

Pseudovirus neutralization assay

SARS-CoV-2 pseudovirus neutralization assay (pVNT) was performed as described[3], with target cell line HeLa cells expressing ACE2 orthologs. All viruses were first titrated to normalize the viral input between assays. Duplicate 3-fold 8-point serial dilutions of heat-inactivated sera (starting at 1:30) were incubated with 500-1000 $TCID_{50}$ of SARS-CoV-2 pseudotyped virus for 1 h at 37°C, 5% CO_2. Subsequently, $1x10^4$ HeLa-ACE2 cells per well were added and incubated at 37°C, 5% CO_2 for 48 h. Afterward, the supernatant was removed, and cells were lysed using a passive lysis buffer (Vazyme) for 3 minutes at room temperature. The lysates were transferred to an opaque white 96-well plate, and reconstituted luciferase assay buffer (Vazyme) was added and mixed with each lysate. Luminescence was measured immediately after mixing using GloMax 96 Microplate Luminometer (Promega). Neutralization titer (NT_{50}) was determined by luciferase activity with a four-parameter non-linear regression inhibitor curve in GraphPad Prism 8.4.2 (GraphPad Software). NT_{50} was defined as the highest reciprocal serum dilution causing a 50% reduction of relative light units. A sample with NT_{50} values no more than 30 (the detectable limit) was considered negative for neutralizing antibodies and was assigned a nominal value of 10 in geometric mean titer (GMT) calculations, which is the lowest serum dilution factor used in the pseudovirus neutralization assay.

Statistical analysis

Data and statistical analyses were performed using GraphPad Prism 8.4.2 (La Jolla,

California, USA) and R v4.0.5. Fold changes in serum neutralizing activity were measured by comparing GMT. The Friedman test with false discovery rate method was used for multiple comparisons for paired groups, while the Kruskal-Wallis with false discovery rate method was used for multiple comparisons for unpaired groups. The Wilcoxon rank-sum test was used for unpaired comparisons between two groups. All statistical tests were 2-sided with a significance level of 0.05.

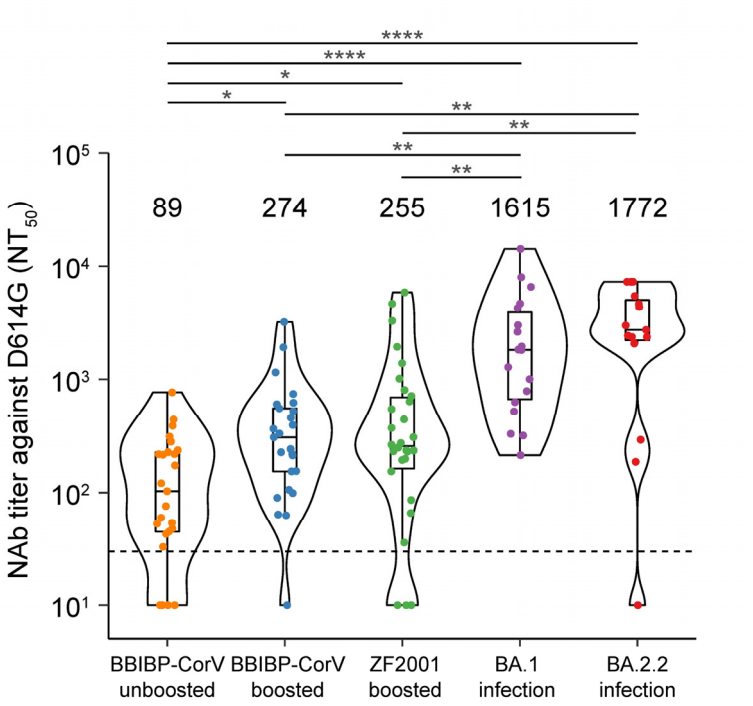
Supplementary references

1. Hacisuleyman E, Hale C, Saito Y, et al. Vaccine Breakthrough Infections with SARS-CoV-2 Variants. *N Engl J Med* 2021; **384**(23): 2212-8.
2. Juthani PV, Gupta A, Borges KA, et al. Hospitalisation among vaccine breakthrough COVID-19 infections. *The Lancet Infect Dis* 2021; **21**(11): 1485-6.
3. Nie J, Li Q, Wu J, et al. Establishment and validation of a pseudovirus neutralization assay for SARS-CoV-2. *Emerg Microbes Infect* 2020; **9**(1): 680-6.

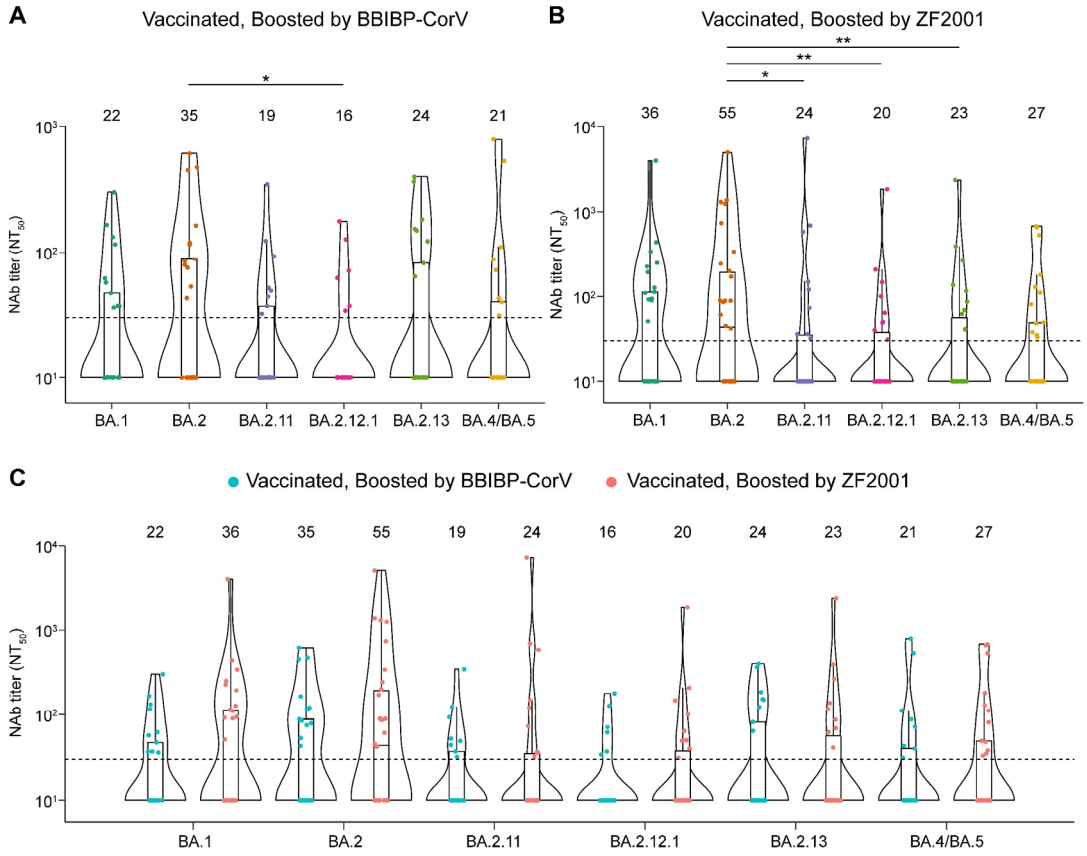
Supplementary Table S1. Characteristics of the study participants.

Characteristics	BBIBP-CorV Vaccinated, Unboosted	Vaccinated, Boosted		Omicron breakthrough infection	
		BBIBP-CorV	ZF2001	BA.1	BA.2.2
No. of subjects	25	25	30	18	15
Age (median, IQR)	41.0 (30.5-56.0)	46.0 (38.5-52.0)	39.0 (34.3-47.3)	16.5 (16.0-37.0)	48.0 (34.0-52.0)
Sex (%)					
Male	9 (36.0)	13 (52.0)	11 (36.7)	14 (77.8)	7 (46.7)
Female	16 (64.0)	12 (48.0)	19 (63.3)	4 (22.2)	8 (53.3)
Disease severity (%)					
Asymptomatic	NA	NA	NA	5 (27.8)	9 (60.0)
Mild	NA	NA	NA	11 (61.1)	4 (26.7)
Moderate	NA	NA	NA	2 (11.1)	2 (13.3)
Vaccination status (%)					
2 doses vaccination					
BBIBP-CorV	NA	NA	NA	13 (72.2)	2 (13.3)
3 doses vaccination					
BBIBP-CorV	NA	NA	NA	3 (16.7)	11 (73.3)
ZF2001	NA	NA	NA	2 (11.1)	2 (13.3)
Interval between last dose and symptom onset or rRT-PCR positive (median, IQR)	NA	NA	NA	191.5 (98.0-198.3)	132.0 (120.0-206.0)
Interval between 2nd or 3rd dose and sampling (median, IQR)	39.0 (27.5-41.0)	21.0 (18.5-28.0)	28.0 (28.0-28.0)	NA	NA
Interval between symptom onset or rRT-PCR positive and sampling (median, IQR)	NA	NA	NA	15.0 (12.8-17.0)	33.0 (32.0-34.0)

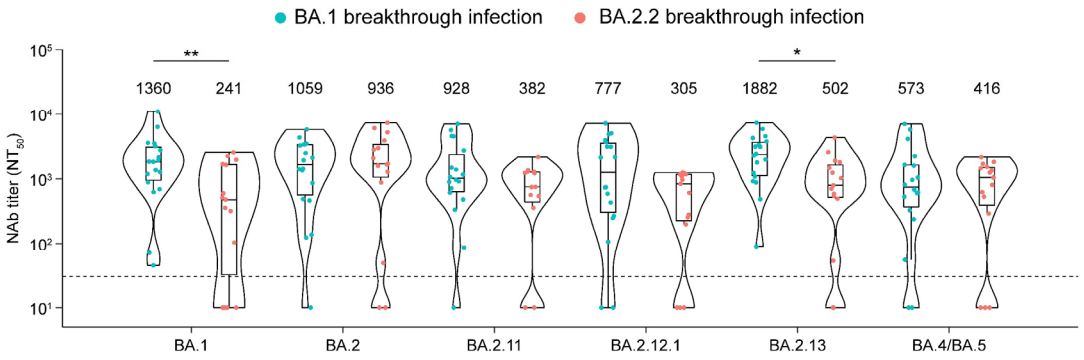
IQR, interquartile range; NA, not available; rRT-PCR, real-time reverse transcription-polymerase chain reaction.



Supplementary Figure S1. Neutralizing antibody titers against D614G between vaccinated individuals and Omicron breakthrough infections. Neutralizing antibody titers against D614G in two BBIBP-CorV doses vaccinated individuals, BBIBP-CorV and ZF2001 boosted individuals, and Omicron BA.1 and BA.2.2 breakthrough infections. The geomatic mean titers (GMTs) are shown above each column. The horizontal dotted line represents the limit of detection of 30. A two-tailed Kruskal-Wallis test with a false discovery rate was performed for multiple comparisons. *p*-values are represented as *p<0.05, **p<0.01, and ****p<0.0001. No asterisk indicates no statistical significance.



Supplementary Figure S2. Neutralizing antibody titers against Omicron subvariants in boosted individuals. (**A** and **B**) Neutralizing antibody titers against BA.1, BA.2, BA.2.11, BA.2.12.1, BA.2.13, and BA.4/BA.5 in BBIBP-CorV (**A**) and ZF2001 (**B**) boosted individuals. (**C**) Comparisons of neutralizing antibody titers against BA.1, BA.2, BA.2.11, BA.2.12.1, BA.2.13, and BA.4/BA.5 between BBIBP-CorV and ZF2001 boosted individuals. The geomatic mean titers (GMTs) are shown above each column. The horizontal dotted line represents the limit of detection of 30. A two-tailed Friedman test with a false discovery rate was performed for multiple comparisons in **A** and **B**, and a two-tailed Wilcoxon rank-sum test was used in **C**. *p*-values are represented as *p<0.05 and **p<0.01. No asterisk indicates no statistical significance.



Supplementary Figure S3. Neutralizing antibody titers against Omicron subvariants between BA.1 and BA.2.2 breakthrough infections. Comparisons of neutralizing antibody titers against BA.1, BA.2, BA.2.11, BA.2.12.1, BA.2.13, and BA.4/BA.5 between BA.1 and BA.2.2 breakthrough infections. The geomatic mean titers (GMTs) are shown above each column. The horizontal dotted line represents the limit of detection of 30. A two-tailed Wilcoxon rank-sum test was used. *p*-values are represented as *p<0.05 and **p<0.01. No asterisk indicates no statistical significance.