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Supplementary appendix

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APPENDIX

Finite Neutralisation Breadth of Omicron After Repeated Vaccination

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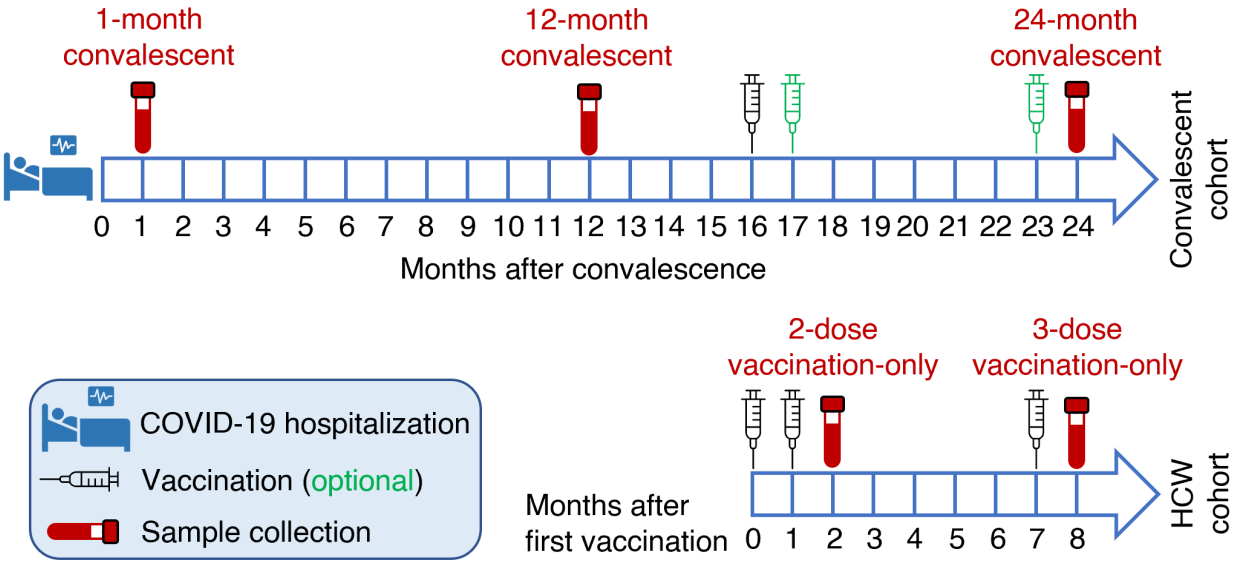

Figure S1. Schedules of sample collection for convalescent and HCW cohorts.

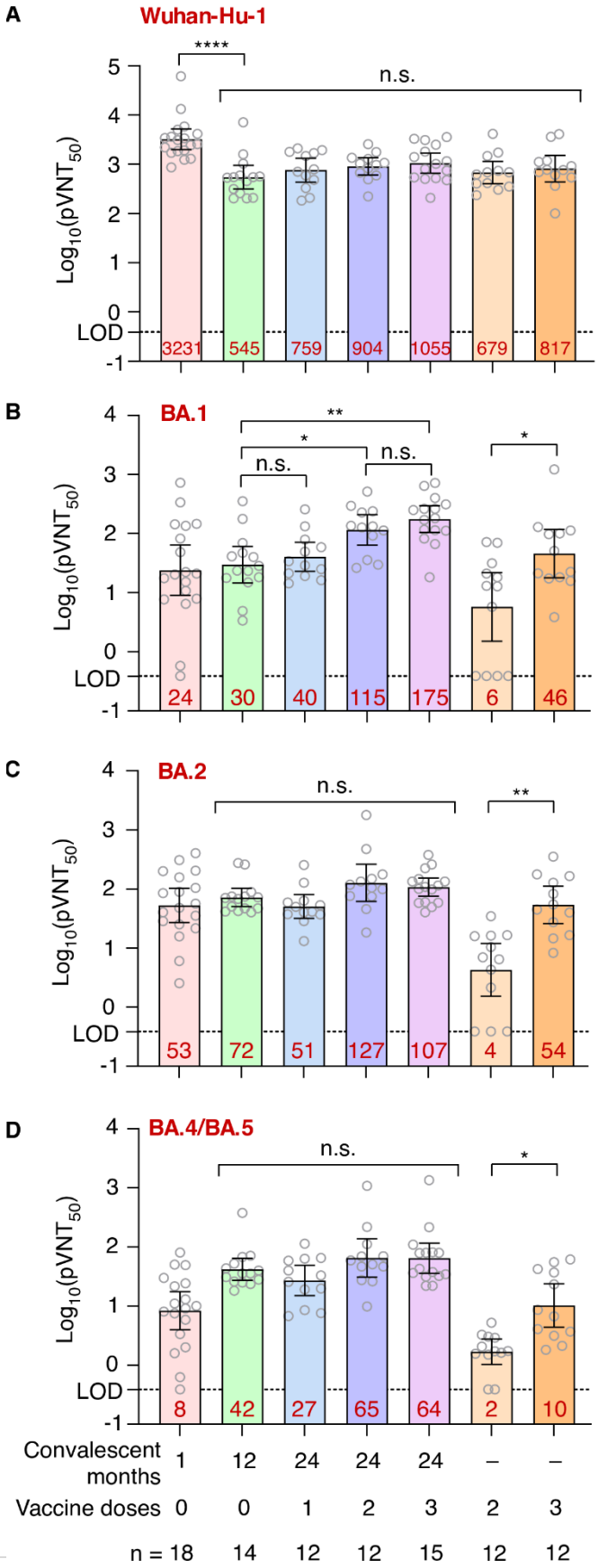
A
Wuhan-Hu-1
****
n.s.
Log10(pVNT50)
LOD
3231
545
759
904
1055
679
817
B
BA.1
**
*
n.s.
n.s.
*
24
30
40
115
175
6
46
C
BA.2
n.s.
**
53
72
51
127
107
4
54
D
BA.4/BA.5
n.s.
*
8
42
27
65
64
2
10
Convalescent months 1 12 24 24 24 – –
Vaccine doses 0 0 1 2 3 2 3
n = 18 14 12 12 15 12 12

Figure S2. Neutralisation of omicron sub-lineages and ancestral Wuhan-Hu-1 in uninfected and convalescent individuals after repeated vaccination.

Serum neutralisation of omicron sub-lineages and ancestral Wuhan-Hu-1 was assessed by pseudovirus neutralisation assays and titers of convalescent (left 5 groups with labels at the bottom of **Panel D**, see Table S1 for demographic and serological characteristics) and HCW cohorts (right 2 groups, see Table S2) were expressed as $pVNT_{50}$ in **Panel A to D**, which showed titers for each omicron sub-lineages and Wuhan-Hu-1 as indicated. The Wuhan-Hu-1 $pVNT_{50}$ of 1- and 12-month convalescent samples (left 2 groups) have been reported in our prior study,[1] and 4 of the 12-month convalescent samples were excluded due to insufficient volumes for omicron neutralisation assays in this study. Bars represent geometric mean ± 95% confidence interval. Red numbers are geometric means of $pVNT_{50}$ of the indicated bar. Blue numbers are geometric means of fold increase of resistance to neutralisation by omicron sub-lineages comparing to Wuhan-Hu-1, which are inversely associated with neutralisation breadth. Sample number of each group was indicated at the bottom of Panel E. Statistical significance was assessed by Kruskal-Wallis test followed by uncorrected Dunn's test and *p* value was indicated as n.s. (≥·05), * (<·05), ** (<·01), *** (<·001), **** (<·0001). Groups within the n.s. brackets in Panel C to E have no statistical difference with each other. HCW = healthcare workers, LOD = limit of detection, n.s. =

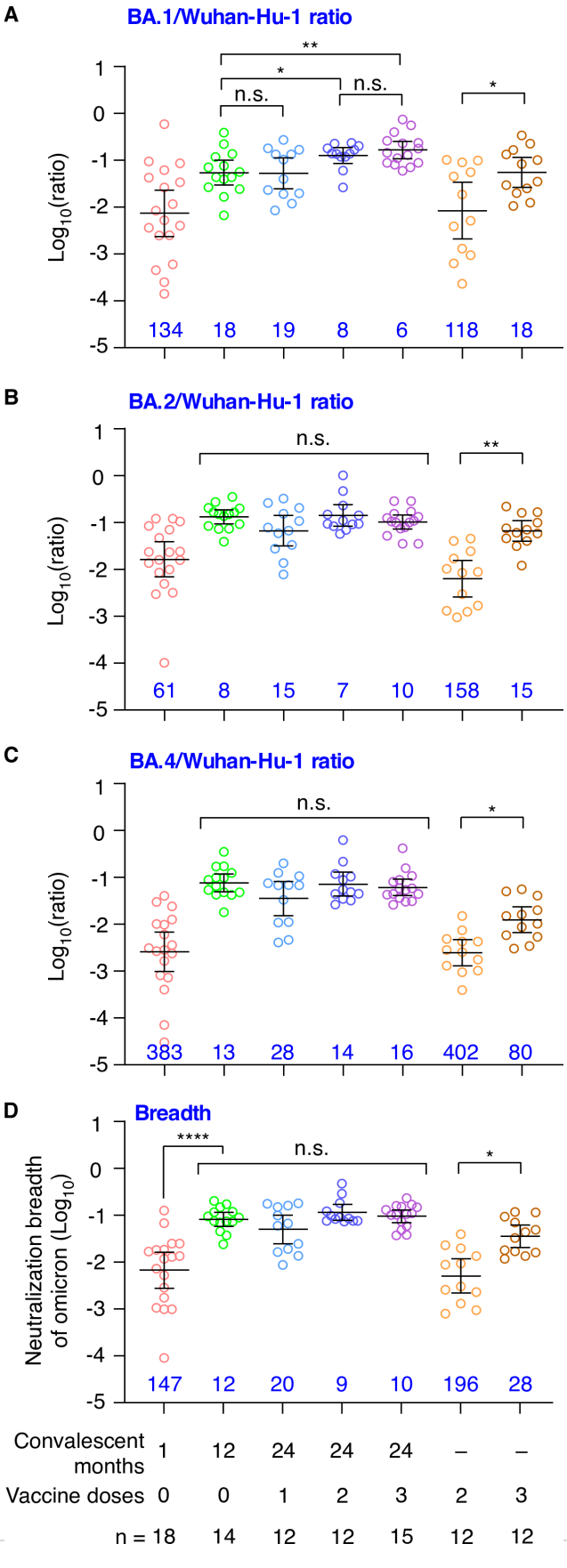
A
BA.1/Wuhan-Hu-1 ratio
B
BA.2/Wuhan-Hu-1 ratio
C
BA.4/Wuhan-Hu-1 ratio
D
Breadth
Log10(ratio)
Neutralization breadth of omicron (Log10)
n.s.
134 18 19 8 6 118 18
61 8 15 7 10 158 15
383 13 28 14 16 402 80
147 12 20 9 10 196 28
Convalescent months 1 12 24 24 24 – –
Vaccine doses 0 0 1 2 3 2 3
n = 18 14 12 12 15 12 12

Figure S3. Omicron sub-lineage vs Wuhan-Hu-1 ratios of neutralisation titers and estimated neutralisation breadth.

$pVNT_{50}$ ratios between each omicron sub-lineage and Wuhan-Hu-1 were shown in **Panel A-C**. Neutralisation breadth against omicron, which was calculated as the geometric mean of $pVNT_{50}$ ratios between each omicron sub-lineage and Wuhan-Hu-1, was shown in **Panel D**. Bars represent geometric mean ± 95% confidence interval. Blue numbers are geometric means of fold increase of resistance to neutralisation by omicron sub-lineages comparing to Wuhan-Hu-1. Sample number of each group was indicated at the bottom of Panel D. Statistical significance was assessed by Kruskal-Wallis test followed by uncorrected Dunn's test and *p* value was indicated as n.s. (≥.05), * (<.05), ** (<.01), **** (<·0001). Groups within the n.s. brackets in Panel B-D have no statistical difference with each other. n.s. = not significant, $pVNT_{50}$ = 50% pseudovirus neutralisation titer.

SUPPLEMENTARY TABLES

Table S1. Demographic and serological characteristics of the convalescent cohort.

	1-month convalescent (n=18)	12-month convalescent (n=14)	24-month convalescent			
			CoronaVac 1-dose (n=12)	CoronaVac 2-dose (n=12)	CoronaVac 3-dose (n=15)	*P* (24-month only)*
Age at sample collection, years median (IQR)	56.5 (43-61)	53.5 (43-62)	58.5 (51-60)	57 (51.5-59)	55.5 (51.5-64)	.915
Sex at birth						
Female, n (%)	9 (50)	9 (64)	6 (50)	7 (58)	8 (53)	.918
Male, n (%)	9 (50)	5 (36)	6 (50)	5 (42)	7 (47)	
COVID-19 severity						
Severe, n (%)	11 (61)	10 (71)	11 (92)	10 (83)	13 (87)	.828
Critical, n (%)	7 (39)	4 (29)	1 (8)	2 (17)	2 (13)	
Length of hospitalization, days, median (IQR)	22.5 (19-28.5)	22.5 (20-26.5)	23.5 (16.5-36)	26 (23-33)	27 (20.5-30)	.730
Timing of vaccination						
Hospitalization to 1st vaccination, days, median (IQR)	/	/	497.5 (488-526)	513 (498-547)	470 (457.5-491)	/
Hospitalization to 2nd vaccination, days, median (IQR)	/	/	/	537.5 (519.5-552)	500 (497.5-514)	
Hospitalization to 3rd vaccination, days, median (IQR)	/	/	/	/	688 (683-699.5)	
Last vaccination to sample collection, days, median (IQR)	/	/	237 (231-241)	201.5 (196-218)	34 (26.5-40)	
SARS-CoV-2 serology						

Positive sVNT, n (%)	18 (100)	14 (100)	12 (100)	12 (100)	15 (100)	/
Total anti-spike antibodies, S/CO, geometric mean (95% CI)	159 (100-252)	419 (287-613)	275 (149-505)	406 (251-657)	522 (358-760)	.192

*Categorical variables were assessed by Chi-square test and continuous variables were assessed by Kruskal-Wallis test. Tests were performed only between 24-month convalescent groups.

Abbreviations: CI, confidence interval; IQR, interquartile range; S/CO, signal/cut-off; sVNT, surrogate virus neutralisation test.

Table S2. Demographic and serological characteristics of the HCW cohort.

	CoronaVac vaccinated HCW (n=12)
Age at last sample collection, years median (IQR)	42.5 (32-50)
Sex at birth	
Female, n (%)	7 (58)
Male, n (%)	5 (42)
Allergy history, n (%)	0 (0)
Comorbidities, n (%)	2 (17)
Dosing interval of 1st and 2nd doses, days, median (IQR)	30.5 (26.5-35)
Dosing interval of 2nd and 3rd doses, days, median (IQR)	203 (187-225)
Samples after 2nd vaccination	
Last vaccination to sample collection, days, median (IQR)	30.5 (23.5-36)
Positive sVNT, n (%)	12 (100)
Total anti-spike antibodies, S/CO, geometric mean (95% CI)	144 (68-308)
Samples after 3rd vaccination	
Last vaccination to sample collection, days, median (IQR)	31.5 (28.5-34.5)
Positive sVNT, n (%)	12 (100)
Total anti-spike antibodies, S/CO, geometric mean (95% CI)	426 (278-653)

Abbreviations: CI, confidence interval; IQR, interquartile range; S/CO, signal/cut-off; sVNT, surrogate virus neutralisation test.

METHODS

Study subjects

This study was based on two prospective cohorts of convalescent COVID-19 patients or vaccinated healthcare workers (HCW) in Xiangyang, China. Enrollment requirements, exclusion criteria and data collection methods of the convalescent cohort have been published and current study included additional samples from the second annual follow-up.[2] Enrollment requirements, exclusion criteria and data collection methods of the vaccinated HCW cohort have also been published and current study included additional samples from the post-booster follow-up.[3] The human study protocols (#2021-003 for the convalescent cohort, and #2021-034 for the HCW cohort) are approved by the Medical Ethics Committee of Xiangyang Central Hospital.

SARS-CoV-2 serology

SARS-CoV-2 serology was determined by quantitative assays. Prescence of neutralising antibodies was screened by a surrogate virus neutralisation assay kit (2019-nCoV neutralising antibody detection kit, InnoDx) according to manufacturer's instructions. Results were expressed as inhibition percentages calculated by the following formula: inhibition percentage = (negative control value – sample value) × 100% / negative control value. Samples with inhibition percentages equal to or higher than 50% were deemed positive. Total anti-RBD antibodies were determined by quantitative chemiluminescent microparticle immunoassay (CMIA) (2019-nCoV antibody detection kit [CMIA], InnoDx). CMIA was performed on a Caris200 analyzer (UMIC Medical Instrument) following manufacturer's instructions. Cut-off value was calculated according to manufacturer's instructions. The assay sensitivity and specificity were 94.8% and 99·7% according to the manufacturer, and 90.8% and 98.9% in an independent study,[4] respectively. Assay linear range was determined by analysis of serial dilution of CR3022 monoclonal antibodies

and serologically positive samples as described previously (manuscript under consideration). Signal/cut-off (S/CO) values less than 0.01 were recorded as 0.01 for all analyses. Quality control checks of all serological assays were conducted according to manufacturer's instructions. Samples failing any checks were re-tested after necessary procedures to improve quality. Samples repeatedly failing checks or without sufficient volume for further re-test were excluded from the analysis.

Pseudovirus neutralisation assays (PNA)

Neutralisation titers of pseudotyped vesicular stomatitis virus (Vazyme Biotech) with spike sequence from Wuhan-Hu-1, B.1.1.529.1 (omicron BA.1) with A67V, Δ69-70, T95I, Δ142-144, Y145D, 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 and L981F mutations,[5] B.1.1.529.2 (omicron BA.2) with T19I, L24S, del25-27, G142D, V213G, G339D, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H and N969K mutations,[5] B.1.1.529.4 (omicron BA.4, shared spike sequence with BA.5) with T19I, L24S, del25-27, del69-70, G142D, V213G, G339D, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, G446S, L452R, S477N, T478K, E484A, F486V, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H and N969K mutations were measured by PNA.[6] All PNA procedures were performed in a Biosafety Level 2 laboratory and validated by serial dilution of CR3022 monoclonal antibodies (Abcam). In brief, pseudovirus carrying a luciferase reporter and encapsulated in ancestral or variant spike proteins was incubated with eight 4-fold serial dilutions of the serum sample by Opti-MEM (Gibco) for 1 h at room temperature. The mixture was then

added to the culture of replication-deficient HEK293-ACE2 cells in 96-well plates with DMEM (Gibco)/10% FBS (Gibco)/1× antibiotics (Gibco) and incubated in a humidified cell culture chamber at 37°C with 5% CO2 for 48 hours. Medium was removed at the end of incubation, and 50 μl one-step luciferase detection reagent (Vazyme Biotech) was added to each well. Luminescence in relative light units (RLUs) was measured by a luminometer (Synergy H1, BioTek Instruments) after 3 minutes of incubation at room temperature. Serum samples may be diluted to meet the initial volume requirement. Samples without maximum RLUs equal to 100 times of cell-only controls were tested again with dilution of the initial sample when necessary. Samples failed to yield meaningful results due to quality issues or limited volumes were excluded from analyses. RLUs of sample wells were normalized with positive control wells and pNT_{50} was calculated as EC_{50} by normalized four-parameter sigmoid curve fit with constrains of EC50 > 0 and hillslope > 0 in Prism 9 (GraphPad). pNT_{50} was arbitrarily set to 0.39, the limit of detection (LOD) of PNA, when EC_{50} was lower than the LOD or not computable due to low neutralisation activity. The LOD was calculated as mean + 1.96×SD of the Wuhan-Hu-1 pNT_{50} of 12 serologically negative samples from healthy donors.

Statistical analysis

Participant characteristics and serological data were assessed with Chi-square tests for categorical variables or Kruskal-Wallis tests for continuous variables. Post hoc comparison methods were detailed in figure legends. Analyses were performed using Prism 9 (GraphPad). Missing data were excluded pairwise from analyses. Significance was evaluated at $\alpha = .05$ and all tests were 2-sided.

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6. Cao Y, Yisimayi A, Jian F, et al. BA.2.12.1, BA.4 and BA.5 escape antibodies elicited by Omicron infection. *Nature* 2022.