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Supplementary appendix

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APPENDIX

Post-recovery enhancement of anti-variant neutralisation after severe COVID-19

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MATERIALS AND METHODS

Study subjects

This study was based on two prospective cohorts of recovered COVID-19 patients or vaccinated healthcare workers (HCW) in Xiangyang, China. Enrollment requirements, exclusion criteria and data collection methods of the recovered patient cohort have been published.[1] The vaccinated HCW cohort included participants 18 years of age or older who had received at least two doses of COVID-19 vaccines at least 7 days before the date of blood collection. Participants with the following conditions were excluded: (a) prior COVID-19 diagnosis or positive serological tests, (b) under immune-modulatory medication, (c) active infection or any inflammatory disease, (d) coagulation disorder or other conditions precluding safe blood collection, (e) physician assessment, laboratory examination, or any other conditions making the subject unsuitable for the study. The recruitment of participants began on June 8th, 2021 and is ongoing as of December 21st, 2021.

Age, gender, dates of vaccination and manufacturers of vaccines were provided by participants after signing the informed consent. The participants were asked for the history of allergy and chronic disease and medication taken since the first vaccine dose. Adverse effects potentially related to vaccination were also recorded. After confirming the eligibility of participation, 5 mL of venous blood was collected from the participant and proceeded to serum isolation immediately. Serum samples were aliquoted for each assay to minimize freeze-thaw cycles. The human study protocol (#2021-034) is approved by the Medical Ethics Committee of Xiangyang Central Hospital.

SARS-CoV-2 serology

SARS-CoV-2 serology was determined by both qualitative and quantitative assays. Prescence of anti-spike total and IgG antibodies were separately screened by qualitative ELISA kits (2019-nCoV antibody detection kit [ELISA], InnoDx; 2019-nCoV IgG antibody detection kit [ELISA], InnoDx) according to manufacturer's instructions. Cutoff value was calculated as 0·16 + the average of negative control readouts. Samples with readouts equal to or higher than the cutoff value were deemed positive. Prescence of neutralising antibodies were 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 CMIA (2019-nCoV antibody detection kit [CMIA], InnoDx). CMIA was performed on a Caris200 analyzer (UMIC Medical Instrument) following manufacturer's instructions. Cutoff 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,[2] 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). 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 Wuhan-Hu-1, P.1 (gamma) with L18F, T20N, P26S, D138Y, R190S, K417T, E484K, N501Y, D614G, H655Y and T1027I mutations (Vazyme Biotech), B.1.617.2 (delta) with T19R, Δ156-158, L452R, T478K, D614G, P681R and D950N mutations (GenScript), C.37 (lambda) with Δ246-252, G75V, T76I, L452Q, F490S, D614G and T859N mutations (GenScript), B.1.621 (mu) with T95I, Y144S, Y145N, R346K, E484K, N501Y, D614G, P681H and D950N mutation (GenScript), and B.1.1.529 (omicron) 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 (Vazyme Biotech) were measured by PNA. 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 (GenScript) 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.

Epidemiologic data

The data of biweekly confirmed COVID-19 cases, variant percentages, vaccine doses administered, and Stringency Index values were downloaded from Our World in Data (https://ourworldindata.org/explorers/coronavirus-data-explorer) on December 20th, 2021.

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 $\alpha = \cdot 05$ and all tests were 2-sided.

SUPPLEMENTAL REFERENCES

1. Zhan Y, Zhu Y, Wang S, et al. SARS-CoV-2 immunity and functional recovery of COVID-19 patients 1-year after infection. *Signal Transduct Target Ther* 2021; **6**(1): 368.

2. Liu W, Kou G, Dong Y, et al. Clinical application of Chemiluminescence Microparticle Immunoassay for SARS-CoV-2 infection diagnosis. *J Clin Virol* 2020; **130**: 104576.

SUPPLEMENTAL TABLE

Table S1 Demographic and serological characteristics of participants.

	Convalescent, (n=18)	CoronaVac (n=12)	BBIBP-CorV (n=12)
Age, years (IQR)	56·5 (43-61)	25 (23-27)	34 (23-37)
Female, n (%)	9 (50)	8 (75)	11 (92)
Interval between vaccine doses, days, median (IQR)	N/A	41(32-48)	50 (40-61)
Positive for anti-spike antibodies, n (%), early/late samples	18/18 (100/100)	12/12 (100/100)	12/12 (100/100)
Positive for anti-spike IgG, n (%), early/late samples	18/18 (100/100)	12/12 (100/100)	12/12 (100/100)
Positive for neutralising antibodies, n (%), early/late samples	18/18 (100/100)	12/12 (100/100)	12/12 (100/100)

Abbreviations: IQR, interquartile range

SUPPLEMENTAL FIGURES AND LEGENDS

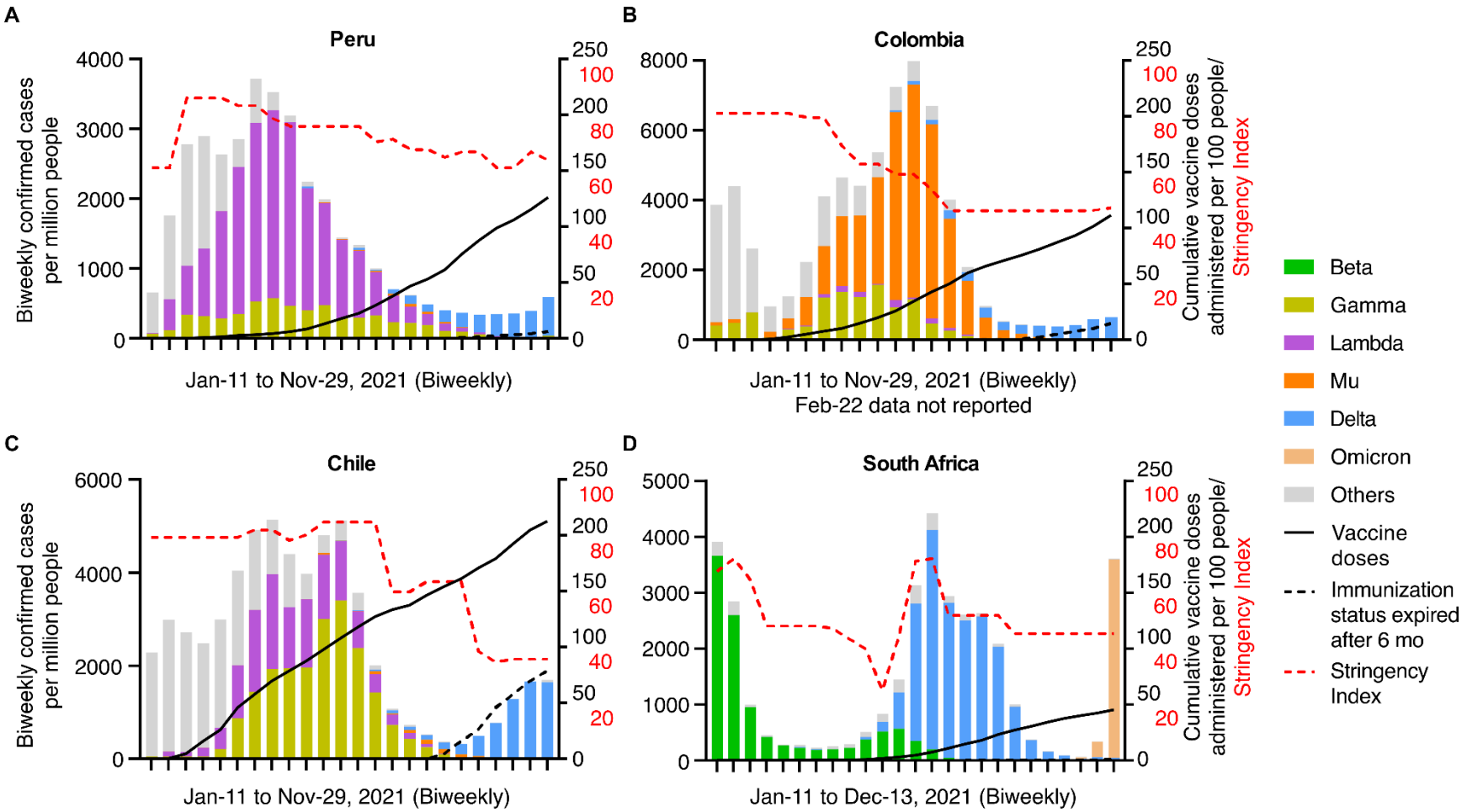

Figure S1 Epidemiologic data of recent outbreaks of SARS-CoV-2 variants.

Composite plots of biweekly epidemiological data of recent SARS-CoV-2 variant outbreaks in Peru-lambda (A), Colombia-mu (B), Chile-gamma (C) and South Africa-omicron (D) from January to December 2021 as indicated below each panel. The bar graphs represent biweekly confirmed COVID-19 cases per million people and are color-coded to each variant, which is calculated according to the share of analyzed sequences in the preceding two weeks that correspond to each variant group. The case numbers are plotted to the left y-axis. Black continuous lines indicate total number of doses administered, divided by the total population of the country. Black dotted lines indicate vaccine recipients that, assuming they received second dose 1 month after the first dose, have passed the 6-month mark since receiving the second dose of vaccine. Both black lines were plotted to the right Y-axis in black numbers. Red dotted lines indicate the trend of the Stringency Index in each country that are plotted to the right y-axis in red numbers. All source data in this figure are downloaded from Our World in Data website.

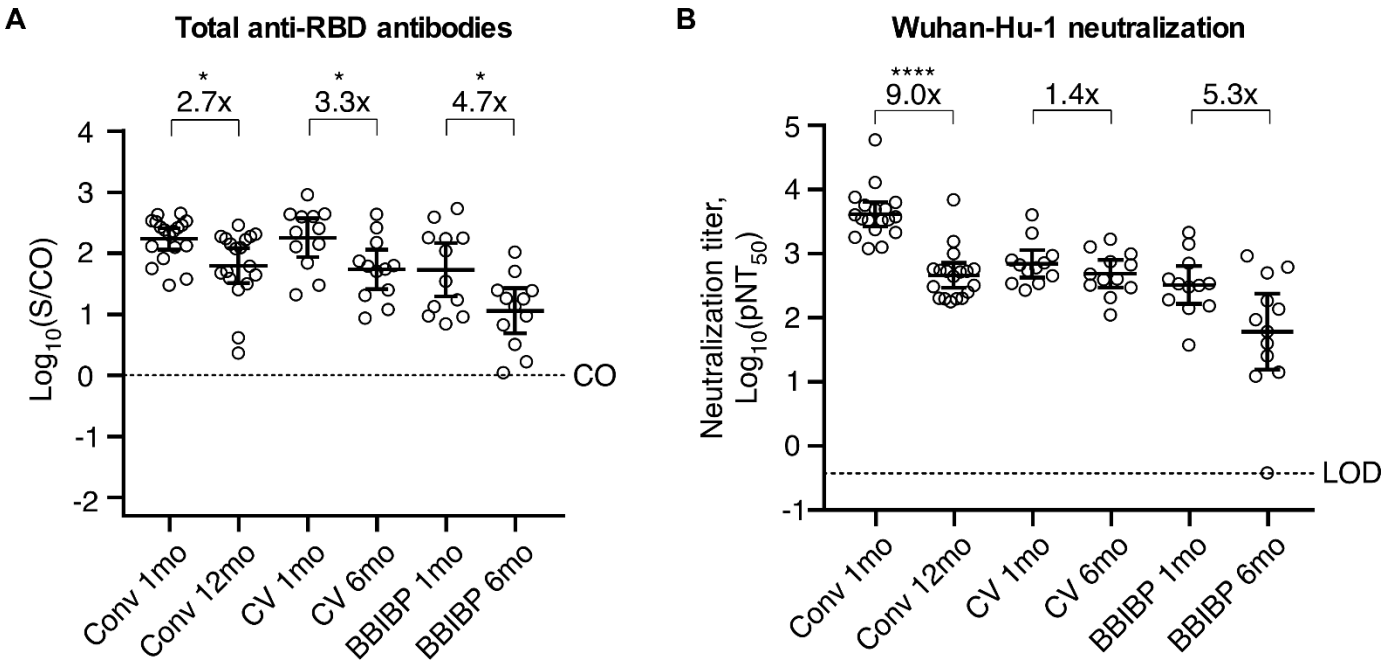

Figure S2 SARS-CoV-2 antibodies and neutralisation of Wuhan-Hu-1 pseudovirus.

Scatter plots of total anti-RBD antibody levels (A) and serum neutralisation titers of longitudinally paired samples against Wuhan-Hu-1 pseudovirus (B). Dotted line indicates the assay cut-off (CO) at 1·0 (A) or the assay limit of detection at 0·39 (B). Fold reduction of geometric means are listed above each pair of groups. Bar = geometric mean ± 95% CI. n = 18 (convalescent), 12 (CoronaVac) and 12 (BBIBP-CorV). Statistical significance was determined by Kruskal-Wallis test followed by uncorrected Dunn's test and indicated as *, $p < ·05$; ****, $p < ·0001$. Conv = convalescent sera, CV = CoronaVac vaccinated sera, BBIBP = BBIBP-CorV vaccinated sera, ZF = ZF2001 vaccinated sera.

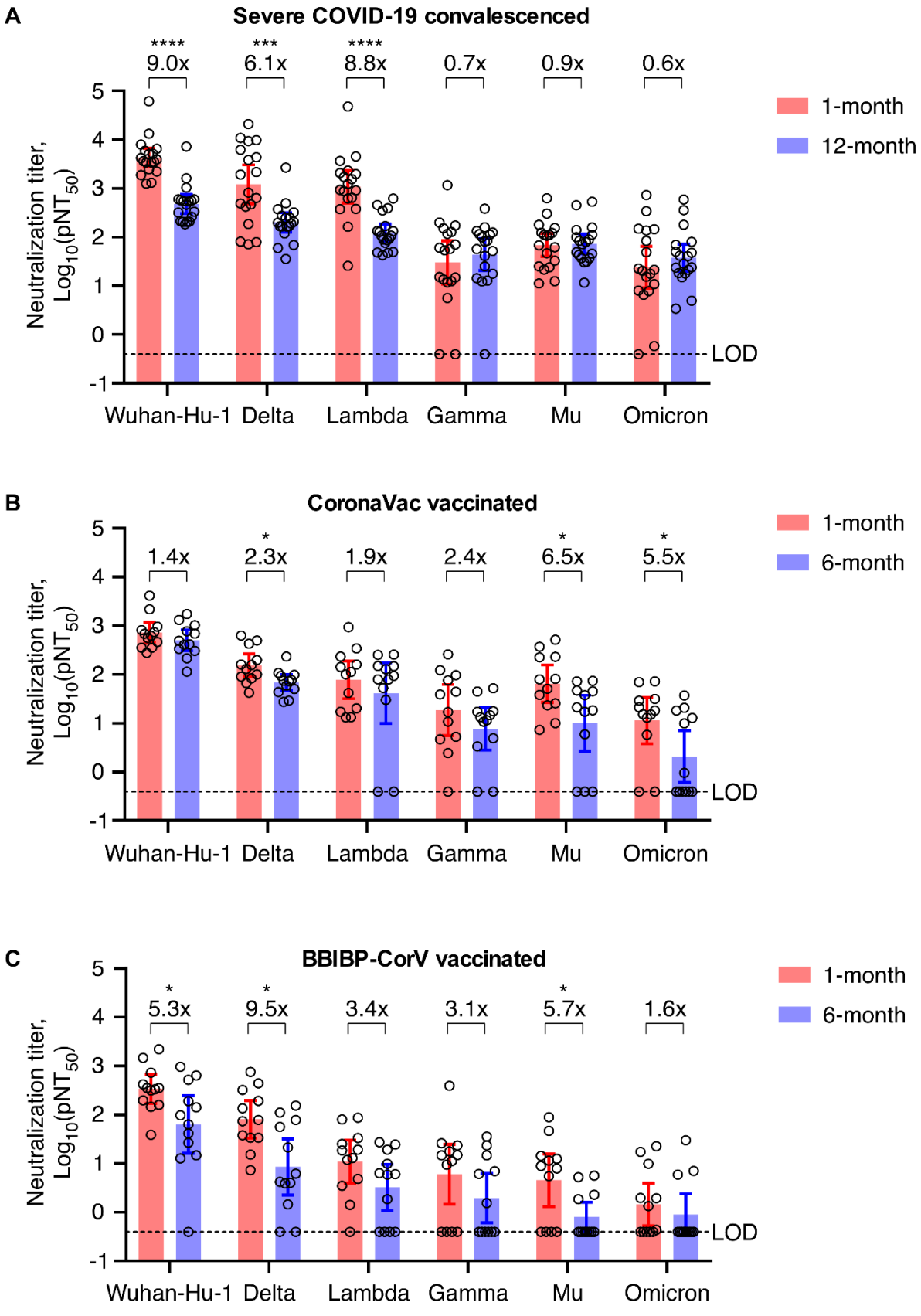
A
Severe COVID-19 convalescenced
****
9.0x
***
6.1x
****
8.8x
0.7x
0.9x
0.6x
Neutralization titer, Log10(pNT50)
5
4
3
2
1
0
-1
LOD
Wuhan-Hu-1
Delta
Lambda
Gamma
Mu
Omicron
1-month
12-month
B
CoronaVac vaccinated
1.4x
*
2.3x
1.9x
2.4x
*
6.5x
*
5.5x
1-month
6-month
C
BBIBP-CorV vaccinated
*
5.3x
*
9.5x
3.4x
3.1x
*
5.7x
1.6x
1-month
6-month

Figure S3 Durability of infection- or vaccine-induced neutralisation against SARS-CoV-2 and variants.

Longitudinally paired bar graphs of serum neutralisation titers of indicated pseudovirus by convalescent (A), CoronaVac (B) and BBIBP-CorV sera (C). Dotted line indicates the assay limit of detection at 0·39. Fold reduction of geometric means are listed above each pair of groups. Bar = geometric mean ± 95% CI. n = 18 (convalescent), 12 (CoronaVac) and 12 (BBIBP-CorV). Statistical significance between paired groups was determined by independent Mann-Whitney tests and indicated as *, $p < ·05$; ***, $p < ·001$; ****, $p < ·0001$.

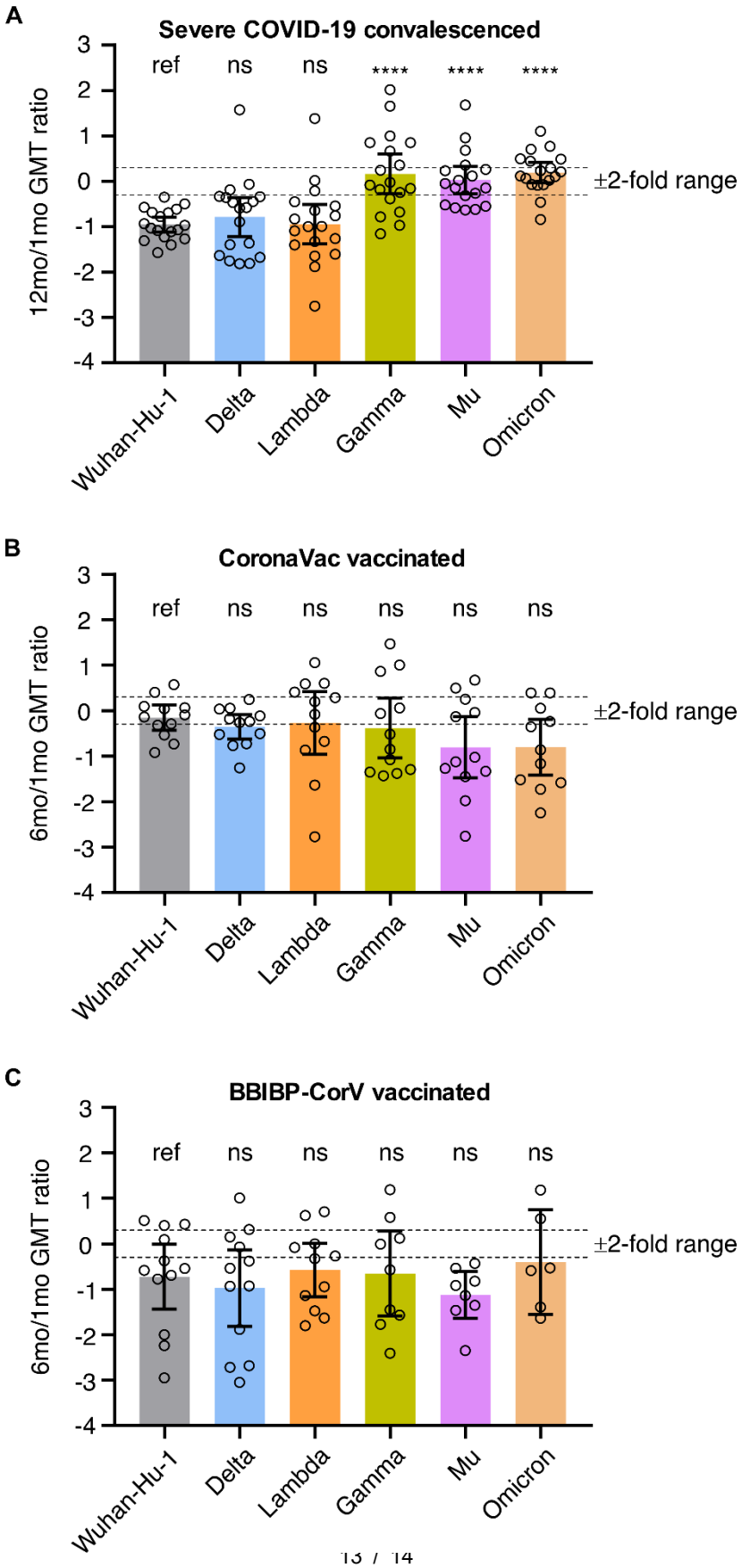
A
Severe COVID-19 convalescenced
12mo/1mo GMT ratio
ref
ns
ns
****
****
****
±2-fold range
Wuhan-Hu-1
Delta
Lambda
Gamma
Mu
Omicron
B
CoronaVac vaccinated
6mo/1mo GMT ratio
ref
ns
ns
ns
ns
ns
±2-fold range
Wuhan-Hu-1
Delta
Lambda
Gamma
Mu
Omicron
C
BBIBP-CorV vaccinated
6mo/1mo GMT ratio
ref
ns
ns
ns
ns
ns
±2-fold range
Wuhan-Hu-1
Delta
Lambda
Gamma
Mu
Omicron

Figure S4 Decay pattern of infection- or vaccine-induced neutralisation against SARS-CoV-2 and variants.

Bar graphs of late versus early ratios of serum neutralisation titers of indicated pseudovirus by convalescent (A), CoronaVac (B) and BBIBP-CorV sera (C). Dotted lines indicates the ±2-fold range that arbitrarily defined as consistent early and late GMT. Values above the upper line would indicate higher GMT in the late sample, while values below the lower line would indicate the opposite. Bar = geometric mean ± 95% CI. n = 18 (convalescent), 12 (CoronaVac) and 12 (BBIBP-CorV). Statistical significance between Wuhan-Hu-1 and each variant was determined by Kruskal-Wallis test followed by uncorrected Dunn's test and indicated as ns, $p > ·05$; ****, $p < ·0001$. ref = reference group.