nature portfolio

Corresponding author(s): Hin Chu

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Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☒	☐	A description of all covariates tested
☒	☐	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	Olympus cellSens Dimension (version 1.17),Olympus BX53 light microscope, Olympus BX73 light microscope, and ZEISS ZEN 2) microscope software were used to collect microscope data. Flow - Cell Analyzer BD LSR Fortessa. ImageJ was used to collect fusion assay signal. LightCycler96 (version SW1.1) was used to collect qPCR data. Alliance Q9 software (v17-02) was used to collect the Western blot data. TITAN Krios G4 transmission electron microscope (Thermo Fisher Scientific) was used to collect Cryo-EM data. GISAID Epicov database (https://gisaid.org/) was used to collect SARS-CoV-2 genome sequences.
Data analysis	GraphPad Prism (version 9.0) was used for analysis and plotting the figures.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

The data that support the findings of this study are available from the corresponding authors upon reasonable request.
Coordinates and maps associated with data reported in this manuscript was deposited to the Electron Microscopy Data Bank (EMDB) and Protein Data Bank (PDB) with accession numbers EMD-39689 [https://www.ebi.ac.uk/emdb/EMD-39689] and PDB 8YZC [https://www.rcsb.org/structure/unreleased/8YZC] (BA.2.86 spike-ACE2), EMD-39688 [https://www.ebi.ac.uk/emdb/EMD-39688] and PDB 8YZB [https://www.rcsb.org/structure/unreleased/8YZB] (BA.2.86 NTD-RBD-ACE2 local refinement), EMD-39691 [https://www.ebi.ac.uk/emdb/EMD-39691] and PDB 8YZE [https://www.rcsb.org/structure/unreleased/8YZE] (JN.1 spike-ACE2), EMD-39690 [https://www.ebi.ac.uk/emdb/EMD-39690] and PDB 8YZD [https://www.rcsb.org/structure/unreleased/8YZD] (JN.1 NTD-RBD-ACE2 local refinement). Source data are provided with this paper.

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	Gender was collected based on self-reporting. Sex or gender analysis was not performed due to the limited number of participants.
Reporting on race, ethnicity, or other socially relevant groupings	Given the design of the study, race, ethnicity and socially grouping were not pre-established and not reported.
Population characteristics	Given the exploratory design of the study, the characteristics of participants were not pre-established when entering the cohorts. Relevant covariates (age, sex, BMI, Breakthrough infections days after the last Coronavirus vaccines, vaccinations and previous COVID-19) are provided in the corresponding supplementary tables 1.
Recruitment	Individuals were recruted during their visit at the hospitals.
Ethics oversight	All collections were conducted according to the guidelines of the Declaration of Helsinki and approved by the Institutional Review Board of the Ethics Committee of Nanjing Hospital of Chinese Medicine Affiliated to Nanjing University of Chinese Medicine (KY2023073). All participants provided written informed consents. Background information of the convalescent donors is summarized in Supplementary Table 1.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	All experiments were repeated at least three times to give a n number of 3 or above. A n number equals to 3 is the the standard of biological experiments. No sample size calculation was performed. Sample size is chosen based on the standard of the corresponding field.
Data exclusions	No data were excluded.
Replication	All experiments were repeated at least three times on three different days. Similar findings were obtained from all repeats.
Randomization	Randomization is not relevant to the in vitro studies. In the studies, the experiments are well-controlled and the same number of cells are used for comparison so that there is no background difference between experimental groups. In the in vivo studies, animals were randomized into different groups.
Blinding	Blinding is not relevant to the in vitro and in vivo experiments of the study. The experiments for different groups are carried out in parallel using the same set of protocols and the experimental results are quantitative.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
[ ]	[x] Antibodies
[ ]	[x] Eukaryotic cell lines
[x]	[ ] Palaeontology and archaeology
[ ]	[x] Animals and other organisms
[ ]	[x] Clinical data
[x]	[ ] Dual use research of concern
[x]	[ ] Plants

Methods

n/a	Involved in the study
[x]	[ ] ChIP-seq
[ ]	[x] Flow cytometry
[x]	[ ] MRI-based neuroimaging

Antibodies

Antibodies used

Anti-VSV-G (CRL-2700, ATCC) for pseudoviruses assay (1:5000);
Mouse anti-beta-actin (clone AC-74) (A5316, Sigma) for Western blots (1:5000);
Rabbit anti-SARS-CoV-2 S1 (Sino Biological, 40591-T62) for Flow cytometry (1:200);
Mouse anti-beta-tubulin (T7941, Sigma-Aldrich) for immunofluorescence(1:500);
Rabbit anti-SARS-CoV-2 N immune serum (In house) for Western blots and immunofluorescence (1:5000);
Rabbit anti-SARS-CoV-2 spike S2 antibody (40590-T62, Sino Biological, China) for Western blots (1:5000);
Horseradish peroxidase (HRP) conjugated secondary antibodies (31460, Thermo Fisher Scientific) for Western blots(1:2000);
Alexa Fluor 594-conjugated goat anti-mouse secondary antibody (A-11005, Thermo Fisher Scientific, USA) for immunofluorescence (1:2000);
Alexa Fluor 488-conjugated goat anti-rabbit secondary antibody (A-11034, Thermo Fisher Scientific) for immunofluorescence (1:2000);
Mounting with ProLong™ Diamond Antifade Mountant with DAPI (P36962, Thermo Fisher Scientific) for immunofluorescence (1:2000).

Validation

Commercial primary antibodies were validated by the manufacturers and validation statements are available on the manufacturer's website.
The in-house anti-SARS-CoV-2-N and anti-SARS-CoV-N immune serum were validated with ELISA, Western blots, and immunofluorescence staining in our previous publication (PMIDs: 35922005 and 35062016).

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

293T, Caco2, Calu3, Huh7, VeroE6, A549 were obtained from ATCC. VeroE6-TMPRSS2 cells were obtained from Japanese Collection of Research Bioresources (JCRB) Cell Bank.

Authentication

The cell lines were not authenticated.

Mycoplasma contamination

All cell lines have been recently tested negative for mycoplasma contamination.

Commonly misidentified lines
(See ICLAC register)

No commonly misidentified cell lines were used in this study.

Animals and other research organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research

Laboratory animals

The golden Syrian hamsters (4-6 weeks old male hamsters);
Heterozygous K18-hACE2 C57BL/6J mice (2B6.Cg-Tg(K18-ACE2)2Prlmn/J) (6-8 weeks old, male and female);
C57BL/6J mice (8-10 weeks old, male and female);

Wild animals

The study did not involve wild animals.

Reporting on sex

The golden Syrian hamsters (4-6 weeks old male hamsters);
Heterozygous K18-hACE2 C57BL/6J mice (2B6.Cg-Tg(K18-ACE2)2Prlmn/J) (6-8 weeks old, male and female);

	C57BL/6J mice (8-10 weeks old, male and female);
Field-collected samples	The study did not involve samples collected from the field.
Ethics oversight	The use of animals has complied with all relevant ethical regulations and was approved by the Committee on the Use of Live Animals in Teaching and Research of The University of Hong Kong (CULATR #22-397).

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJEguidelines for publication of clinical research and a completedCONSORT checklist must be included with all submissions.

Clinical trial registration	KY2023073
Study protocol	Peripheral blood samples were collected from individuals who had previously experienced BA.5 infection and XBB or JN.1 reinfection after receiving two-to-three doses of inactivated vaccine. Sera were isolated from centrifuged blood samples and then stored at -80° C.
Data collection	A total of 16 patient donors with BA.5+XBB individual infections were included in this study. These included 7 males and 9 females with a mean age of 33.62 years (range, 18-69 years) A total of 16 patient donors with BA.5+JN.1 individual infections were included in this study. These included 8 males and 8 females with a mean age of 37.13 years (range, 23-60 years).
Outcomes	The patient sera were used to perform in vitro neutralization assays.

Plants

Seed stocks	The study did not involve plants.
Novel plant genotypes	Not relevant for this study.
Authentication	Not relevant for this study.

Flow Cytometry

Plots

Confirm that:

☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☒ All plots are contour plots with outliers or pseudocolor plots.

☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	Transfected 293T cells were detached from the culture plate with 10mM EDTA and fixed in 4% paraformaldehyde.
Instrument	BD FACSCanto II cell analyzer was used for data collection.
Software	FlowJo X 10.0.7 was used for data analysis.
Cell population abundance	At least 10,000 cells were acquired for each condition.
Gating strategy	The 293T cell population was gated with SSC-A vs FSC-A. A mock-transfected sample with the Empty Vector was used as the gating control for spike expression positive cells.

☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.