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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	Krios G4 (Thermo Fisher Scientific), EPU v3.2 (Thermo Fisher Scientific), Biacore T200 (Cytiva), Fluent Control (Tecan), Infinite200Pro (TECAN), Fluent 780 (TECAN), Magellan (Tecan), GloMax explorer multimode microplate reader 3500 (Promega)
Data analysis	NextClade v2.14.0, ggplot2 v3.4.4, ViralMSA v1.1.24, TrimAl v1.4.rev22, IQ-TREE v2.2.0, R v4.2.2, EnvStats v2.7.0, ggtree v3.6.2, Biacore T200 Evaluation software (Cytiva), cryoSPARC v4.3.1 (Structura Biotechnology), UCSF Chimera v1.16, UCSF ChimeraX v1.4, Coot v0.9.8.7, fastp v0.21.0, BWA-MEM v0.7.17, SAMtools v1.9, snpEff v5.0e, Pymol v2.5.0 (Schrodinger), Phenix v1.20, Molprobity v4.5.2, , Python v3.7 and 3.11.5, numpy v1.24.3, pandas v2.0.3, scipy v1.11.1, scikit-posthocs v0.9.0, matplotlib v3.7.2, seaborn v0.12.2, MAFFT v7.511, Modeller 10.5, Rosetta release 3.13, Prism 9 software (GraphPad Software), SnapGene v6.1.1 (SnapGene) The computational codes used in phylogenetic tree reconstruction and detection frequency calculation are available in the GitHub repository (https://github.com/TheSatoLab/BA.2.86_RBD).

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 atomic coordinates and cryo-EM maps of the structures of BA.2.86S-protein closed state (PDB: 8WXL [https://www.rcsb.org/structure/8WXL], EMD-37910 [https://www.ebi.ac.uk/emdb/EMD-37910]), RBD 1-up state (PDB: 8XUX [https://www.rcsb.org/structure/8XUX], EMD-38459 [https://www.ebi.ac.uk/emdb/EMD-38459]), RBD 2-up state in complex with ACE2 (PDB: 8XUY [https://www.rcsb.org/structure/8XUY], EMD-38686 [https://www.ebi.ac.uk/emdb/EMD-38686]), RBD 3-up state in complex with ACE2 (PDB: 8XVM [https://www.rcsb.org/structure/8XVM], EMD-38690 [https://www.ebi.ac.uk/emdb/EMD-38690]), RBD 2-up and 1-down state in complex with ACE2 (PDB: 8XUZ [https://www.rcsb.org/structure/8XUZ], EMD-38687 [https://www.ebi.ac.uk/emdb/EMD-38687]), up-RBD and ACE2 interface (PDB: 8XV0 [https://www.rcsb.org/structure/8XV0], EMD-38688 [https://www.ebi.ac.uk/emdb/EMD-38688]), and down-RBD and ACE2 interface (PDB: 8XV1 [https://www.rcsb.org/structure/8XV1], EMD-38689 [https://www.ebi.ac.uk/emdb/EMD-38689]), treated at 42 °C (EMD-60905 [https://www.ebi.ac.uk/emdb/EMD-60905]) and JN.1 S-protein RBD 2-up state in complex with ACE2 (EMD-60904 [https://www.ebi.ac.uk/emdb/EMD-60904]), RBD 2-up and 1-down state in complex with ACE2 (EMD-60906 [https://www.ebi.ac.uk/emdb/EMD-60906]) and up-RBD and ACE2 interface (PDB: 9IU1 [https://www.rcsb.org/structure/9IU1], EMD-60886 [https://www.ebi.ac.uk/emdb/EMD-60886]) generated in this study have been deposited in the Protein Data Bank (www.rcsb.org), and Electron Microscopy Data Bank (www.ebi.ac.uk/emdb/).

SARS-CoV-2 genomic sequences and their related surveillance data are available from GISAID database (https://www.gisaid.org). The supplemental tables for the GISAID datasets (EPI_SET_240301bk and EPI_SET_240301rn) are deposited in the GitHub repository (https://github.com/TheSatoLab/BA.2.86_RBD).

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	The sex of the participants in this study is determined by biological attribute. Convalescent sera were collected from fully vaccinated individuals who had been infected with XBB.1.5 (four 3-dose vaccinated, three 4-dose vaccinated, two 5-dose vaccinated, and one 6-dose vaccinated; the time interval between the last vaccination and infection, 44–435 days; 15–46 days after testing. n=10 in total; average age: 50.4 years, range: 18–74 years, 30% male). The SARS-CoV-2 variants were identified as previously described2, 18, 41 ,42. Sera were inactivated at 56 °C for 30 minutes and stored at –80 °C until use. Details of the convalescent sera are summarized in Supplementary Table 3.
Reporting on race, ethnicity, or other socially relevant groupings	The race of almost all of the participants in this study are Asian. Convalescent sera were collected from fully vaccinated individuals who had been infected with XBB.1.5 (four 3-dose vaccinated, three 4-dose vaccinated, two 5-dose vaccinated, and one 6-dose vaccinated; the time interval between the last vaccination and infection, 44–435 days; 15–46 days after testing. n=10 in total; average age: 50.4 years, range: 18–74 years, 30% male). The SARS-CoV-2 variants were identified as previously described2, 18, 41 ,42. Sera were inactivated at 56 °C for 30 minutes and stored at –80 °C until use. Details of the convalescent sera are summarized in Supplementary Table 3.
Population characteristics	Samples are collected from outpatients visiting Interpark Kuramochi Clinic in Japan for testing or treatments for COVID-19. Convalescent sera were collected from fully vaccinated individuals who had been infected with XBB.1.5 (four 3-dose vaccinated, three 4-dose vaccinated, two 5-dose vaccinated, and one 6-dose vaccinated; the time interval between the last vaccination and infection, 44–435 days; 15–46 days after testing. n=10 in total; average age: 50.4 years, range: 18–74 years, 30% male). The SARS-CoV-2 variants were identified as previously described2, 18, 41 ,42. Sera were inactivated at 56 °C for 30 minutes and stored at –80 °C until use. Details of the convalescent sera are summarized in Supplementary Table 3.
Recruitment	All protocols involving specimens from human subjects recruited at Interpark Kuramochi Clinic were reviewed and approved by the Institutional Review Board of Interpark Kuramochi Clinic (approval ID: G2021-004). All human subjects provided written informed consent. All protocols for the use of human specimens were reviewed and approved by the Institutional Review Boards of The Institute of Medical Science, The University of Tokyo (approval IDs: 2021-1-0416 and 2021-18-0617).
Ethics oversight	All protocols involving specimens from human subjects recruited at Interpark Kuramochi Clinic was reviewed and approved by the Institutional Review Board of Interpark Kuramochi Clinic (approval ID: G2021-004). All human subjects provided written informed consent. All protocols for the use of human specimens were reviewed and approved by the Institutional Review Boards of The Institute of Medical Science, The University of Tokyo (approval IDs: 2021-1-0416 and 2021-18-0617).

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	The sample sizes (n > 3) for cell culture experiments were chosen for applying statistical tests. For cell-based assays, no sample size calculation was performed. All experiments were performed with at least three biological replicates because they are sufficient to to evaluate a significant difference. The sample sizes (n > 10) for the neutralization studies were chosen because they have previously been shown to be sufficient to evaluate a significant difference among groups (Kaku, Lancet Infectious Diseases2024; Yisimayi, Nature2023; Park, Science2022; Tada, eBiomedicine2022; Chan, Cell2021; Saito, Nature2021;).
Data exclusions	No data were excluded from the analyses.
Replication	In vitro experiments representative of at least 2 experiments with multiple samples per time point. All attempts at replication were successful.
Randomization	For experiments, randomization is not applicable because homogenous materials (i.e., cell lines) were used.
Blinding	No blinding was carried out, because these are not relevant for an observational study.

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
☒	☐ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	1) LentiX-293T cell (Takara, Cat# 632180) 2) HEK293S GnTI(-) cells (Reeves PJ et al., PNAS 2002) 3) HOS-ACE2/TMPRSS2 cells (a derivative of HOS cells (a human osteosarcoma cell line; ATCC CRL-1543) stably expressing human ACE2 and TMPRSS2) (Ozono et al., Nat Commun, 2021; Ferreira et al., J Infect Dis, 2021)
Authentication	None of the cells used were authenticated.
Mycoplasma contamination	All cell lines were regularly tested for mycoplasma contamination by using PCR and were confirmed to be mycoplasma-free.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used.

Plants

Seed stocks	*Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.*
Novel plant genotypes	*Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.*
Authentication	*Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosiacism, off-target gene editing) were examined.*