

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
<i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i> |
| ☒ | ☐ | 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
<i>Give P values as exact values whenever suitable.</i> |
| ☒ | ☐ | 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 Shanghai Synchrotron Radiation Facility beamlines BL02U1, BL10U2, BL18U1, BL19U1; EnVision Manager (v1.13.3009.1409); Celigo Image Cytometer (Celigo v5.4)

Data analysis Coot (v0.8.9.2); Phenix (v1.20.1-4487); XDS (BUILT=20220110); GraphPad Prism (v9.4); PyMOL (v2.5.4)

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](#)

All experimental data are provided in the manuscript. The structures determined in this study have been deposited to the Protein Data Bank (PDB) under accession codes: 8HEI (CTSB-E64d), 8HE9 (CTSB-K777), 8HEN (CTSB-212-148), 8HET (CTSL-E64d), 8HFV (CTSL-K777), 7XYD (TMPRSS2-nafamostat), 7YOE (TMPRSS2-camostat), 7YOF (TMPRSS2-UK-371804), 8HD8 (TMPRSS2-212-148). 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 N/A, this study did not involve human research participants.

Reporting on race, ethnicity, or other socially relevant groupings N/A, this study did not involve human research participants.

Population characteristics N/A, this study did not involve human research participants.

Recruitment N/A, this study did not involve human research participants.

Ethics oversight N/A, this study did not involve human research participants.

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](https://www.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 For all enzymatic and antiviral assays, no statistical approaches were used to predetermine the sample size. As is commonly done in the field to achieve reproducible results, we used sample sizes of four independent biological replicates for enzymatic assays and at least two independent experiments for antiviral assays (Jin, Zhenming et al. Nature, PMID: 32272481; Iketani, Sho et al. Nature, PMID: 36351451; Duan, Yinkai et al. Nature, PMID: 37696289).

Data exclusions No data were excluded.

Replication The enzymatic assays were repeated independently four times. For cell experiments, at least two independent experiments were performed and were indicated in figure legends to ensure statistical power. All attempts at replication were successful.

Randomization Randomization was not used for the enzymatic and antiviral experiments reported here because no grouping was needed.

Blinding Blinding was not relevant to our study as no measure was subjective.

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)	Vero E6 cells (ATCC, CRL-1586), Huh-7 (JCRB, 0403), Human Calu-3 (ATCC, HTB-55), and 293T (ATCC, CRL-3216). All cells were cultured at 37°C in a fully humidified atmosphere containing 5% CO2, and have been tested negative for mycoplasma infection.
Authentication	Cell lines were purchased from authenticated vendors, and morphology was also confirmed visually prior to use.
Mycoplasma contamination	Cell lines tested mycoplasma negative.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used in this study.