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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	Amber24 was used to MD analysis of NSP14-C10 interaction. Schrödinger Suite 2021-2, DeepDock and MaSIF were used to the virtual screening of hit compounds targeting NSP14.
Data analysis	The IC50, EC50, Km values and statistical tests were determined by nonlinear regression using GraphPad Prism 9. SPR data were calculated using the Biacore 8K Evaluation software (1.1). DSF data were fitted with the Boltzmann equation in GraphPad software to calculate the melting temperature (Tm).HDX-MS data were analyzed with BioPharma Finder 2.0 software and HDExaminer 3.0 software. For Next-Generation Sequencing, BWA software was used. Cell cycle data analysis was carried out using ModFit LT 5.0. Pharmacokinetic parameters were calculated usingnon-compartmental pharmacokinetic analysis supported by the PK solver Excel Add in. For lC/Ms data analysis, Skyine Daily v22 and Analystsoftware iversion 1.6.2; For LC/MS data analysis, MassHunter Workstation and MassHunter Quantitative Analysis were used.

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 data supporting the findings of this study are available within the Article, its Supplementary Information, or the Source Data file. The RNA seq data generated in this study have been deposited in the NCBI database under accession code GSE290079 (https://www.ncbi.nlm.nih.gov/gds/?term=GSE290079). The NGS data generated in this study have been deposited in the NCBI database under accession code GSE304814 (https://www.ncbi.nlm.nih.gov/gds/?term=GSE304814) and PRJNA1321334 (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1321334). HDX-MS data are downloadable in the Open Science Framework (https://osf.io/6dcz4/?view_only=6073972cfa2e4740a06f7693aaa56553). The source data underlying Figs. 1b, d-j; 2a-j; 4b, c, e; 5d-k; 6a-c, e, 7g-j; 8a, c-d, f, i-j; and Supplementary Figs. 2a-c, e-g; 3a-d; 4a-c; 5a, b; 6a, b; 8; 10a-c; 11d-f; 12; 13b, c; 15b; and 16a are provided in the Source Data file. All genes shown in Fig. 7j are listed in the Source Data. 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 subjects.
Reporting on race, ethnicity, or other socially relevant groupings	N/A, this study did not involve human research subjects.
Population characteristics	N/A, this study did not involve human research subjects.
Recruitment	N/A, this study did not involve human research subjects.
Ethics oversight	N/A, this study did not involve human research subjects.

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	No sample size calculation was performed. We utilized sample sized as used in similar studies.(Meyer C et al.,2025)
Data exclusions	For our in-vivo efficacy study, when compounds were dosed 1 hour after infection, one animal from 15mg/kg Remdesivir group was euthanized early (Day 2) due to meeting humane euthanasia criteria. Animals were ethareic folowing dosing and blood collection ikely dueto the volume of blood colected and potential stress from handling as these mice were on the smaer side. That animal has been excluded from any analysis.
Replication	Unless otherwise stated, all assays were performed at least in triplicates. We confirm that all repeated experiments were successful, except for subjective operational errors during the experiments.
Randomization	Mice were assigned to experimental groups randomly, Besides animal work, randomization was not relevant to this study.
Blinding	As this is an observational study, investigators were not blinded. 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

Antibodies

Antibodies used: anti-HA: CST3724 1:1000; GAPDH: Santa Cruz sc-32233 1:10,000; AURKA: Proteintech 82906-1-RR 1:1000; Anti-SARS-COV 2 Spike glycoprotein S1 (RBD): GB12869-50 1:1000;

Validation: anti-HA:Epitope tags are useful for the labeling and detection of proteins using immunoblotting, immunoprecipitation, and immunostaining techniques. Because of their small size, they are unlikely to affect the tagged protein's biochemical properties. Antibody validated for IP, WB, IHC/IF;
GAPDH: antibody (6C5) is a mouse monoclonal IgG1 kappa light chain antibody that targets GAPDH purified from rabbit muscle. In applications such as Western blotting (WB), immunoprecipitation (IP), and immunofluorescence (IF), it is reactive to mouse, rat, human, and rabbit species. Antibody validated for WB, IF;
AURKA: 82906-1-RR targets AURKA in WB, IF/ICC, ELISA applications and shows reactivity with human samples. Antibody validated for WB, IHC/IF, ELISA;
Anti-SARS-COV 2 Spike glycoprotein S1 (RBD): SARS-COV-2 S protein S1 is a powerful weapon of coronavirus SARS-COV-2 to infect human normal cell. Because the receptor binding domain (RBD) lies in S spike protein S1 subunit, which exclusively binds to human ACES2 protein, followed by SARS-COV-2 nuclear materials entering into human cells cytoplasm and starting replication. Antibody validated for WB;

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s): HEK293T cells (Homo sapiens; sex: male; human embryonic kidney cells, ATCC #CRL-3216), HeLa cells (Homo sapiens; sex: female; human uterus; ATCC #CCL-2), A549 cells (Homo sapiens; sex: male; lung epithelial; ATCC #CCL-185) and Vero E6 cells (chlorocebus sabaeus; sex: female, kidney epithelial, ATCC #CRL-1586) were obatinded from the American Type Culture Collection (ATCC).

Authentication: Cell lines were all purchased from commercial vendors, were regularly visually checked for morphology.

Mycoplasma contamination: All cell lines have regularly been tested negative for contamination with mycoplasma.

Commonly misidentified lines (See ICLAC register): The study does not involve any cell lines that are prone to misidentification.

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: Male ICR mice (6–8 weeks, 18–20 g) obtained from Hangzhou Hangsi Biotechnology Limited Company were used to evaluate the PK. Male C57BL/6 mice (6–8 weeks, 18–20 g) was from Hangzhou Hangsi Biotechnology Limited Company designed for toxicity study. Male K18-hACE2 transgenic mice from Jiangsu GemPharmatech Limited Company (6–8 weeks, 18–20 g) were used in the study for antiviral research.

Wild animals: The study did not involve wild animals.

Reporting on sex: Mice were selected because they represent the lowest ordered species that are routinely used for efficacy evaluation of antiviral compounds against coronavirus infection. Only male mice were be used for PK profling until confirmation of the eficacy PK. For in vivo mouse efficacy studies, male (6-8 week old) transgenic K18-hACE2 mice were used.

Field-collected samples: This study did not involve samples collected from the field.

Ethics oversight: All animal studies were ethically reviewed and carried out in accordance with European Directive 2010/63/EU and the Gsk Policy onthe Care, Welfare and Treatment of Animals. The experimental protocol has been approved by the Animal Ethics Committee of the School of Basic Medical Sciences at Fudan University.

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

Plants

Seed stocks	Does not apply to this study.
Novel plant genotypes	Does not apply to this study.
Authentication	Does not apply to 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	Cells were synchronized using to arrest populations at specific cycle stages, followed by release into fresh medium for defined intervals. Harvested cells were fixed in 70% ice-cold ethanol for a minimum of 24 hours at −20°C, then treated with a staining solution containing propidium iodide (50 µg/mL) and RNase A (100 µg/mL) to label DNA content while eliminating RNA interference. Prior to analysis, samples were passed through a 40-µm cell strainer to ensure single-cell suspensions and minimize aggregate artifacts.
Instrument	The cell cycle distribution was analyzed using a CytoFLEX LX flow cytometer.
Software	Data analysis was carried out using ModFit LT 5.0.
Cell population abundance	Quantification of cell cycle distribution revealed a baseline population abundance of [39.15]% in G0/G1, [56.67]% in S phase, and [4.32]% in G2/M under control conditions, with notable shifts upon treatment.
Gating strategy	Initial gating excluded debris and doublets by sequential selection of single cells using forward scatter area (FSC-A) versus height (FSC-H) and side scatter (SSC-A) parameters. DNA content was analyzed by plotting propidium iodide intensity (PI-A) against cell counts, with cell cycle phases delineated using ModFit LT 5.0's computational mode. Doublet discrimination was confirmed via PI width (PI-W) versus area (PI-A) analysis, ensuring accurate phase assignment.

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