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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	In Fig. 3, for indirect immunofluorescence assay (cell line) and average infection ratio were measured by Celigo Image Cytometer (Nexcelom Bioscience). In Fig. 4 and 5, the copy numbers of SARS-CoV-2, MERS-CoV, 229E, NL63, OC43, HKU1 were measured by QuantStudio 6 (ABI), and images were captured using the Zeiss LSM880 High-Speed Super Resolution Laser Scanning Microscope (ZEISS). In Fig. 5,The numbers of SARS-CoV-2 and MERS-CoV foci were calculated using an EliSpot reader (Cellular Technology Ltd.). In Fig. 6, average infection ratio were measured by Celigo Image Cytometer (Nexcelom Bioscience). In Supplementary Fig. 3, average infection ratio were measured by Perkin Elmer Operetta High Content Imaging System. luminescence was measured by GloMax 20/20 (TurnerBio Systems). Sequencing data were collected by Illumina Novaseq 6000 system.
Data analysis	CLC Genomics Workbench Version 22 was used for mutation analysis. H&E imagine were process by CaseViewer_2.4 software. Raw data were analyzed using Microsoft Excel software(Microsoft 365), GraphPad PRISM (Versions 9)

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 included in this study have been deposited in the PDB with the accession codes: 8WTS [https://www.rcsb.org/structure/unreleased/8WTS]. NGS raw data have been deposited into SRA database (PRJNA1154822). All data supporting the results of this manuscript are available in the article or Supplementary Information. 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	Participants were not excluded based on sex or gender.
Reporting on race, ethnicity, or other socially relevant groupings	No socially constructed or socially relevant categorization variables were used in this manuscript.
Population characteristics	Lung tissue samples were obtained from adult patients undergoing thoracic surgery for non-infectious, non-malignant indications at the First Affiliated Hospital of Guangzhou Medical University. All tissue samples used for organoid derivation were confirmed to be from macroscopically and histologically normal regions of the lung. Both male and female patients were included. No identifiable personal information was collected. All participants provided written informed consent.
Recruitment	Residual normal lung tissues were collected from consenting patients undergoing clinically indicated thoracic surgery. No active recruitment was conducted beyond the standard clinical workflow.
Ethics oversight	This study involving human lung tissues was reviewed and approved by the Ethics Review Committee of the First Affiliated Hospital of Guangzhou Medical University (approval number: ES-2023-193-02). All participants provided written informed consent prior to sample collection, and all samples were anonymized before analysis.

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	In fig 5, for SARS-CoV-2, six- to eight-week old H11-K18-hACE2 C57BL/6 mice were administered intragastrically with vehicle, Nirmatrelvir (300 mpk) or ISM3312 (75, 150, 300 mpk) twice a day from the day of SARS-CoV-2 infection. For survival curve of Nirmatrelvir and ISM3312 against ancestral SARS-CoV-2 using H11-K18-hACE2 C57BL/6 mice (Mock, n=4; other groups, n = 8 mice). Virus titers in ancestral SARS-CoV-2 infected lungs and brains (Mock, n=4; other groups, n = 8 mice). For BA.2.3, Survival curve of Nirmatrelvir and ISM3312 against SARS-CoV-2 BA.2.3 using H11-K18-hACE2 C57BL/6 mice (n = 8), and virus titers in the SARS-CoV-2 BA.2.3 infected lungs and brains (Mock, n=4; other groups, n = 8 mice). For SARS-CoV-2 XBB.1 n = 5 per groups. For MERS-CoV infeciton, hDPP4 knockin C57BL/6 mice were used (n = 6) for survival curve. Viral titers in MERS-CoV MA30 infected lungs (n=4-5). Viral titers in 229E infected lungs from hAPN KI BALB/c mice (n=5). Viral titers in NL63 infected lungs from Ad5-hACE2 sensitized IFNARKO BALB/c mice (n=4-5). Survival curve of ISM3312 against OC43 using WT C57BL/6 mice (n =5-6). Virus titers in the OC43 infected brains and spinal cords at 5 dpi (n = 4). The determination on the sample sizes in in vivo study is based on the expected variations in the disease model and 3R principles (Replacement, Reduction and Refinement). In vitro: no special considerations.
Data exclusions	No data were excluded
Replication	All experiments were repeated in at least duplicates except indicated. Similar findings were obtained from all repeats.
Randomization	There was no allocation except for the grouping of the animals. Animals were randomly allocated to the groups.

Blinding	No blinding was done. Blinding was not relevant to the study because the results are quantitative and objective, and does not require a subjective judgment.

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	SARS-CoV / SARS-CoV-2 Nucleocapsid Antibody (Cat. No.: 40143-R004, Sino Biological, Inc. Beijing) 1:2000 HCoV-229E Nucleocapsid Antibody, Rabbit PAb (Cat. No.: 40640-T62, Sino Biological, Inc. Beijing) 1:2000 HCoV-OC43 Nucleocapsid Antibody, Rabbit PAb (Cat. No.: 40643-T62, Sino Biological, Inc. Beijing) 1:2000 HCoV-NL63 Nucleocapsid Antibody, Rabbit PAb (Cat. No.: 40641-T62, Sino Biological, Inc. Beijing) 1:2000 HCoV-HKU1 Nucleocapsid Antibody, Rabbit PAb (Cat. No.: 40642-T62, Sino Biological, Inc. Beijing) 1:2000 MERS Nucleocapsid Antibody, Rabbit PAb (Cat. No.: 40068-RP02, Sino Biological, Inc. Beijing) 1:2000 HRP-labelled goat anti-rabbit secondary antibody (Cat. No.: 111-035-144, Jackson ImmunoResearch Laboratories 1:5000 Goat anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ Plus 488 (Thwemo Fisher Scientific, A32731TR) 1:1000
Validation	All primary and secondary antibodies used are commercial antibodies reported by the manufacturer to be validated for use. https://www.sinobiological.com/

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	VeroE6 (ATCC, CRL-1586), Vero81 (ATCC, CCL-81), Huh7 (JCRB0403), and Huh7-hACE2 (In-house, constructed from Huh7)
Authentication	All cell lines were frequently checked for cellular morphologies, growth rates and functions. All purchased cell lines were available in commercial company.
Mycoplasma contamination	All cell lines used were tested (by PCR) and were mycoplasma free.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified 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	For SARS-CoV-2 infection, specific pathogen-free 6-8-week-old H11-K18-hACE2 and OC43 infection used WT C57BL/6 mice were purchased from GemPharmatech Co., Ltd. (Jiangsu, China). For MERS-CoV infection, hDPP4 knockin C57BL/6 mice were donated by Professor Stanley Perlman.For 229E infection, hAPN KI BALB/c mice were prepared by GemPharmatech Co., Ltd. For NL63 infection, IFNAR-/- BALB/c mice were generated by backcrossing IFNAR−/− C57BL/6 with WT BALB/c mice for at least ten generations. For in vivo PK, male CD-1 mice (purchased from Sino-British SIPPR/BK Lab Animal Ltd, Shanghai.), Female C57BL/6J mice (purchased from Sino-British SIPPR/BK Lab Animal Ltd, Shanghai.), non-naïve male and female Beagle dogs were used.
Wild animals	No wild animals were used in the study.
Reporting on sex	Male 6-8-week-old H11-K18-hACE2, male WT C57BL/6 mice, and female or male hDPP4 knockin C57BL/6 mice, hAPN KI BALB/c mice and IFNAR-/- BALB/c mice were used in this study. Male 7-9-week-old CD-1 mice, Female 7-9-week-old C57BL/6J mice and non-naïve male and female Beagle dogs were used in PK study.

Field-collected samples	No field collected samples were used in the study.
Ethics oversight	All animal experiments were complied with relevant ethical regulations regarding animal research. The animal study was reviewed and approved by Institutional Animal Care and Use Committees of the First Affiliated Hospital of Guangzhou Medical University (2022276) and Guangzhou Customs Inspection and Quarantine Technology Center (IQTC202314). Human proximal airway organoids were derived from biopsied normal human lung tissues from patients who underwent surgical operations at the First Affiliated Hospital of Guangzhou Medical University, and approved by Ethics Review Committee of the First Affiliated Hospital of Guangzhou Medical University (ES-2023-193-02). For in vivo PK, The protocol and any amendment(s) or procedures involving the care and use of animals in this study will be reviewed and approved by the Preclinical R&D Unit of Medicilon Institutional Animal Care and Use Committee (IACUC) and WuXi AppTec (Shanghai) Co., Ltd IACUC. IACUC no.: PK01-001-2019v1.0 for compound 1A CD-1 mice PK; IACUC no.: 09013-21049 for compound 1B CD-1 mice PK; IACUC no.: 09013-21161 and 09013-21182 for compound 2 CD-1 mice PK; 09013-21357 for compound 3 CD-1 mice PK; IACUC no.: 09013-22371 for compound 3 C57BL/6J mice PK; IACUC no.: NJ-20241025-Dogs for compound 3 Beagle dog PK.

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

Plants

Seed stocks	no
Novel plant genotypes	no
Authentication	no