

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 (<i>n</i>) 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. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> 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 <i>d</i> , Pearson's <i>r</i>), 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	1. Confocal imaging was performed using confocal microscope (FV-3000; Olympus, Japan). 2. Quantitative RT-qPCR was performed using TB Green Premix Ex Taq II (Takara Bio Inc.) in an Applied biosystems Quantstudio 5. 3. Before sequencing, the RNA quality was analyzed using the 2100 Bioanalyzer (Agilent Technologies). Subsequently, each library was prepared following the manufacturer's guidelines with the TruSeq Stranded mRNA Sample Prep Kit (Illumina). Sequencing was conducted using NextSeq2000(Illumina), and FASTQ files were generated via bcl2fastq-2.20.
Data analysis	1. Image processing was performed by FV31S-SW (2.4.1.198), imageJ(1.54f), and CellPathfinder (4.2.5). 2. GraphPad Prism (8.4.3(686), GraphPad software, USA) was used to make graphs. 3. RNA sequence analysis: To prepare the data for analysis, adapter sequences, and low-quality bases were trimmed from the raw reads using Cutadapt ver. 4.1. The trimmed reads were then mapped to the human reference genome sequences (hg38), SARS-CoV-2 genome (https://www.ncbi.nlm.nih.gov/nuccore/LC528233.1), and IAV genome (Jusing STAR version 2.7.10a and a GENCODE (release 32, GRCh38.p13) GTF file. Raw counts were calculated with htseq-count ver. 0.13.5, employing the GENCODE GTF file. DESeq2 in R studio (R version 4.4.1) was used for DEGs and Database for Annotation, Visualization, and Integrated Discovery (DAVID 2021) was used for GO. GO plots was achieved using the ggplot2 R package (version 3.4.2).

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 raw sequencing data files generated in the experiments can be found on NCBI Gene Expression Omnibus (GSE247677). <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE247677>

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
Reporting on race, ethnicity, or other socially relevant groupings	N/A
Population characteristics	N/A
Recruitment	N/A
Ethics oversight	N/A

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	No statistical method was used to predetermine sample size. Sample size was decided based on previously published paper related to MPS. For all the RT-PCR experiment, 3 or more chips were used unless specified otherwise in the figure caption/legend for each group. For image quantification, three or more fields of view from 3 or 4 chips (total n = 9 or more) were used for each group unless specified otherwise in the figure caption/legend for each group.
Data exclusions	SARS-CoV-2 viral titer did not increase in microfluidic chips on some occasions, such chips were excluded from further experiments. IFNB1 experiment: one chip without IFNB1 pretreatment (control) infected with SARS-CoV-2 was excluded owing to the failure in virus replication.
Replication	All attempts of replication were successful. Occasionally, some chips failed to show increase in viral titer and were excluded from further analysis. Chips that initially failed to form confluent layers and differentiation were discarded.
Randomization	On-chip samples were enumerated 1,2,3,.. and were randomly distributed into the groups for further experiments.
Blinding	Researchers from multiple laboratories were involved in each experiment, hence blinding was not necessary. All samples were under control of the authors.

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

Primary antibodies:

Anti-Acetylated Tubulin (Sigma-Aldrich T7451); Anti-MUC5B (Santa Cruz sc-21768); Alexa Fluor® Cyt5-647 (Antibody Abcam ab193895); Anti-ZO-1 (ThermoFisher 33-9100); Anti-VE-Cadherin (Abcam ab33168); Anti-GFP (Aveslabs GFP-1020); Anti-CPM (Gotoh lab, Kyoto University 43A1); Anti-SLC34A2 (A kind gift from Dr. Ritter Gerd MX35, <https://pubmed.ncbi.nlm.nih.gov/30773483/>); Anti-HT1-56 (Terrace Biotech TB29 AHT1-56); Anti-IAV NP (Genetex GTX125989); Anti-FOXJ1 (Abcam ab235445).

Secondary antibodies:

Alexa Fluor 488 Donkey Anti-mouse IgG (H+L) #A21202 Thermo Fisher Scientific; Alexa Fluor 568 Donkey Anti-Rabbit IgG (H+L) #A10042 Thermo Fisher Scientific; Alexa Fluor 647 Donkey Anti-mouse IgG (H+L) #A31571 Thermo Fisher Scientific; Alexa Fluor 568 Donkey Anti-mouse IgG (H+L) #A31570 Thermo Fisher Scientific; Microbeads-conjugated Anti-rat immunoglobulin kappa light chain antibody (130-047-401, Miltenyi Biotec); Alexa Fluor 488 donkey anti-chicken #703-485-155 Jackson ImmunoResearch.

Validation

We relied on information mentioned by the manufacturers in the data sheets provided for all antibodies were obtained from commercial vendors.

Anti-Acetylated Tubulin (Sigma-Aldrich T7451): Host Mouse, Used for IFA

Anti-MUC5B (Santa Cruz sc-21768): Host Mouse, Used for IFA

Alexa Fluor® Cyt5-647 (Antibody Abcam ab193895): Host Mouse, Used for IFA

Anti-ZO-1 (ThermoFisher 33-9100): Host Rabbit, Used for IFA

Anti-VE-Cadherin (Abcam ab33168): Host Rabbit, Used for IFA

Anti-GFP (Aveslabs GFP-1020): Host Chicken, Used for IFA

Anti-SLC34A2 (A kind gift from Dr. Ritter Gerd MX35): Host Mouse, Used for IFA

Anti-HT1-56 (Terrace Biotech TB29 AHT1-56): Host Mouse, Used for IFA

Anti-IAV NP (Genetex GTX125989): Host Rabbit, Used for IFA

Anti-FOXJ1 (Abcam ab235445): Host Rabbit, Used for IFA

Anti-CPM (Gotoh lab, Kyoto University 43A1): Hosokawa M. et al Cryptotanshinone is a candidate therapeutic agent for interstitial lung disease associated with a BRICHOS-domain mutation of SFTPC. iScience. 2023, used for Auto-MACS.

Microbeads-conjugated Anti-rat immunoglobulin kappa light chain antibody (Miltenyi Biotec 130-047-401) 20µl microbeads-conjugated anti-rat immunoglobulin kappa light chain antibody plus 30µl MACS buffer per 1x10⁷ cells.

Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

Cell line source(s)

SFTPC-GFP knockin reporter iPSCs (B2-3) cell line was previously established by Dr. Shimpei Gotoh and its parental hiPSC line is 201B7 (RRID:CVCL_A324). 201B7 is one of the first human iPSC lines reported by Prof. Yamanaka and can be obtained by Riken Cell Bank.

HUVEC (cAP-0001, Angio Proteomie, USA) were used to establish endothelial layer in both airway and alveolus epithelium. VeroE6/TMPRSS2 cell line was purchased from JCRB Cell Bank. MDCK cell line was originally sourced from the ATCC.

Authentication

- SFTPC-GFP knockin reporter iPSCs can be confirmed through GFP expression.

- HUVEC (cAP-0001, Angio Proteomie, USA) were authenticated by the supplier using following methods:

1. Cytoplasmic VWF/Factor VIII >95% positive by immunofluorescence
2. Cytoplasmic uptake of Di-I-Ac-LDL >95% positive by immunofluorescence
3. Cytoplasmic PECAM1 >95% positive by immunofluorescence

- VeroE6/TMPRSS2 were not authenticated authenticated.

Mycoplasma contamination

HUVEC (cAP-0001, Angio Proteomie, USA) cell lines were tested negative for Mycoplasma contamination by the supplier.

SFTPC-GFP knockin reporter iPSCs (B2-3) were routinely tested for mycoplasma contamination using the MycoAlert mycoplasma detection kit (Lonza, LT07-118) and we detected no contamination.

VeroE6/TMPRSS2 and MDCK cell lines tested negative for mycoplasma contamination.

Commonly misidentified lines
(See [ICLAC](#) register)

No commonly misidentified cell lines were used.

Plants

Seed stocks	N/A
Novel plant genotypes	N/A
Authentication	N/A