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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	LAS X (Leica Application Suite X), version 3.10.2.30243 (Leica Microsystems); SpectroFlo (Cytek Biosciences Version 3.4.0);ZetaView (Version 8.05.14 SP7)
Data analysis	ImageJ, version 1.54d (National Institutes of Health); GraphPad Prism, version 11 (GraphPad Software); De Novo Software Version 7.28.0035; SpectroFlo (Cytek Biosciences Version 3.4.0);ZetaView (Version 8.05.14 SP7)

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

Raw sequencing data and processed single-cell datasets that support the findings in this study have been deposited in the Gene Expression Omnibus (GEO) under accession GSE276140 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc= GSE276140). The hg38 reference genome was used for mapping human-derived

sequencing reads. Reanalyses of previously published datasets were also deposited and under accession GSE276140. The original datasets were obtained from GEO under accession numbers GSE13589346 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE135893) and GSE13683145 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE136831). Proteomics datasets generated in this study have been deposited in the ProteomeXchange Consortium under accession number PXD075505 (http://proteomecentral.proteomexchange.org/cgi/GetDataset?ID=PXD075505). The dataset PXD075507 contains data related to ongoing work and is therefore not publicly available at this time; access can be provided by the corresponding author upon reasonable request. Additional data supporting the findings of this study are available within the paper and its 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	Sex and gender were not considered in the study design. Human samples were obtained as de-identified specimens from clinical procedures, and information on participant sex or gender was not available to the investigators. No sex- or gender-based analyses were performed.
Reporting on race, ethnicity, or other socially relevant groupings	Race, ethnicity, or other socially relevant groupings were not considered in the study design. Human samples were obtained as de-identified clinical specimens, and demographic information including race or ethnicity was not available to the investigators. No analyses based on race, ethnicity, or other socially relevant variables were performed.
Population characteristics	Population characteristics such as age, sex, genotype, diagnosis, or treatment history were not analyzed in this study. Human lung tissue and bronchoalveolar lavage fluid (BALF) samples were obtained as de-identified clinical specimens, and covariate-relevant participant information was not available to the investigators.
Recruitment	Participants were not recruited for this study. Human lung tissue samples were obtained from explanted lungs removed during lung transplantation procedures, and bronchoalveolar lavage fluid (BALF) samples were obtained as de-identified specimens from clinically indicated procedures. Investigators had no access to participant identifiers or recruitment information.
Ethics oversight	The study protocol was approved by the Institutional Review Board (IRB) of the Icahn School of Medicine at Mount Sinai (STUDY-23-00129) and the Stanford University Institutional Review Board (protocol 68051). All procedures involving human samples were conducted in accordance with relevant institutional guidelines and regulations.

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 statistical methods were used to predetermine sample size. Sample sizes were chosen based on standard practice in the field and previous studies using similar experimental systems, and were sufficient to detect reproducible effects across independent biological replicates. No covariates were included in the statistical analyses. Experiments were performed under controlled experimental conditions, and comparisons were made directly between predefined experimental groups.
Data exclusions	No data points were excluded.
Replication	Individual experiments were replicated at least 2-3 times (n is indicated for all experiments in the figure legends). All attempts at replication were successful.
Randomization	No randomization was performed in this study.
Blinding	Investigators were not blinded during data collection or analysis because experiments involved predefined experimental conditions and objective quantitative measurements.

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

The following antibodies were used:
Antibody name applicable supplier name catalog number clone name lot number dilution
TMPRSS2 WB; IF; FC;EM N/A AL20 2 ug/ml
TMPRSS2 WB; IF; FC;EM EMD Millipore MABF2158 P5H9-A3 3584853 2 ug/ml
ACE2 WB;EM R&D Systems AF933 N/A(polyclonal) HOK0320061 1 ug/ml
TSG101 WB Thermo Fisher Scientific MA1-23296 4A10 WL3453886G 1.8 ug/ml
CD63 WB Thermo Fisher Scientific 10628D Ts63 00998136 2 ug/ml
HSP90 WB R&D Systems MAB3286 341320 XKB0221011 0.2 ug/ml
ZO-1 WB Cell Signaling Technology 13663S D6L1E 3 0.1 ug/ml
Calnexin WB Cell Signaling Technology 2433S N/A(polyclonal) 5 4 ng/ml
β-actin WB Santa Cruz sc-47778 HRP C4 G03919 40 ng/ml
anti-mouse-HRP-conjugated antibody WB SeraCare 5220-0341 N/A(polyclonal) 100 ng/ml
anti-rabbit-HRP-conjugated antibody WB SeraCare 5220-0336 N/A(polyclonal) 10440068 100 ng/ml
anti-goat-HRP-conjugated antibody WB SeraCare 5220-0362 N/A(polyclonal) 10422112 100 ng/ml
EPCAM IF Cell Signaling Technology 36746S D4K8R 1 0.5 ug/ml
Purified anti-human CD326 (EpCAM) IF BioLegend 312502 W18060B B383643 0.5 ug/ml
Sars nucleocapsid protein IF;EM Rockland 200-401-A50 N/A(polyclonal) 44838 10 ug/ml
Alexa Fluor 488-conjugated donkey anti-mouse IgG IF Invitrogen A21202 N/A(polyclonal) 1:500
Alexa Fluor 488-conjugated donkey anti-rat IgG IF Invitrogen A21208 N/A(polyclonal) 1:500
Alexa Fluor 555-conjugated donkey anti-mouse IgG IF Invitrogen A31570 N/A(polyclonal) 1:500
Alexa Fluor 555-conjugated donkey anti-rabbit IgG IF Invitrogen A31572 N/A(polyclonal) 1:500
Alexa Fluor 594-conjugated donkey anti-rat IgG IF Invitrogen A21209 N/A(polyclonal) 1:500
Alexa Fluor 647-conjugated donkey anti-rabbit IgG IF Invitrogen A31573 N/A(polyclonal) 1:500
APC/Fire™ 750 anti-human CD326 (EpCAM) FC BioLegend 324234 9C4 B308895 2 ug/ml
APC anti-human CD31 FC BioLegend 303116 WM59 B380800 1 ug/ml
APC/Cyanine7 anti-human CD11b FC BioLegend 301342 ICRF44 B374482 2 ug/ml
PE/Cyanine7 anti-human CD206 (MMR) FC BioLegend 321124 15-2 B388203 2 ug/ml
Alexa Fluor® 488 anti-human HLA-DR FC BioLegend 307620 L243 B394852 1 ug/ml
Alexa Fluor® 700 anti-human CD15 (SSEA-1) FC BioLegend 323026 W6D3 B360514 2 ug/ml
APC anti-human CD169 (Sialoadhesin, Siglec-1) FC BioLegend 346008 7-239 B364487 5 ug/ml
PerCP/Cyanine5.5 anti-human CD45 FC BioLegend 304028 HI30 B368349 1 ug/ml
PE Goat anti-mouse IgG (minimal x-reactivity) FC BioLegend 405307 Poly4053 B324932 1 ug/ml
APC anti-human CD184 (CXCR4) FC BioLegend 306510 12G5 B261520 2 ug/ml
PE anti-human CD117 (c-kit) FC BioLegend 313204 104D2 B257667 1 ug/ml
BV605 anti-human CD31 FC BioLegend 303122 WM59 B381683 1 ug/ml
APC anti-human EPCAM FC Abcam ab237398 EPR20532-225 GR3281794-1 0.5 mg/ml
Biotin anti-human CD9 antibody IF Biolegend 312112 HI9a B428718 0.5 ug/ml
Biotin anti-human CD63 IF Biolegend 353017 H5C6 B437774 2.5 ug/ml
Biotin anti-human CD81 (TAPA-1) IF Biolegend 349514 5A6 B439207 0.5 ug/ml
Alexa Fluor 555-conjugated Streptavidin IF Invitrogen S21381 2652988 1 ug/mL
4',6-diamidino-2-phenylindole, dihydrochloride (DAPI) IF; FC Thermo Fisher Scientific D1306 IF 1 ug/ml; FC 0.1 uM

Validation

All primary antibodies used in this study were validated by the manufacturers for the reported applications, with validation data available on the respective manufacturer websites. Antibody specificity was further confirmed in our laboratory by immunostaining of human adult lung tissue samples used as positive controls.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

The Rockefeller University Embryonic Stem Cell Line 2 (RUES2; NIH approval number NIHhESC-09-0013, registration number 0013) was obtained from Dr. Ali Brivanlou (Rockefeller University). Human induced pluripotent stem cell (hiPSC) lines generated using Sendai virus (SV) or modified mRNA (mRNA) reprogramming from healthy human dermal fibroblasts were obtained from the Mount Sinai Stem Cell Core facility. Irradiated CF1 mouse embryonic fibroblasts (MEFs; Gibco, A34181, Thermo Fisher Scientific) were used as feeder cells. A549 (ATCC CCL-185), Calu-3 (ATCC HTB-55), and LNCaP (ATCC CRL-1740) cell lines were obtained from the American Type Culture Collection (ATCC).

Authentication	Cell lines were authenticated in the lab by analysis of pluripotency markers and karyotyping every 6 months.
Mycoplasma contamination	Cells were routinely tested for mycoplasma contamination using the MycoProbe Mycoplasma Detection Kit (R&D Systems, CUL001B) every six months, and all cultures tested negative.
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.*

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	Lung organoids and cell lines were dissociated into single-cell suspensions using TrypLE™ Express Enzyme or 0.05% Trypsin/EDTA. Human lung tissue cells were digested using Dissociation Buffer (containing 1.6 U/mL Dispase (Corning, 354235), 100 U/mL Collagenase type I (Gibco, 17100017), and 40 U/mL DNase I (Bioland, EPD01-01) in PBS). For intracellular staining, the cells were fixed using the Fixation Buffer (BioLegend, 420801) and permeabilized with an Intracellular Staining Permeabilization Wash Buffer (BioLegend, 421002) according to the manufacturer's protocol. For surface staining, cells were skipped from the fixation and permeabilization steps. After resuspension in FACS buffer (0.1% Sodium Azide (Acros Organics, 190381000), 0.1% BSA, 1 mM EDTA (ThermoFisher Scientific, 15575020), and 25 µg/mL DNase I in Hanks' Balanced Salt solution (HBSS; ThermoFisher Scientific, 14185052)), cells were stained with desired anti-human antibodies. The antibodies used in this study were: APC/FireTM 750-conjugated EPCAM (2 µg/ml, clone 9C4, BioLegend, 324234), APC-conjugated CD31 (1 µg/ml, clone WM59, BioLegend, 303116), APC/Cy7-conjugated CD11b (2 µg/ml, clone ICRF44, BioLegend, 301342), PE/Cy7-conjugated CD206 (2 µg/ml, clone 15-2, BioLegend, 321124), AF488-conjugated HLA-DR (1 µg/ml, clone L243, BioLegend, 307620), AF700-conjugated CD15 (2 µg/ml, clone W6D3, BioLegend, 323026), APC-conjugated CD169 (5 µg/ml, clone 7-239, BioLegend, 346008), PerCP/Cyanine5.5-conjugated CD45 (1 µg/ml, clone HI30, BioLegend, 304028), APC-conjugated CD184 (CXCR4) (2 ug/ml, clone 12G5, BioLegend, 306510), PE-conjugated CD117 (c-kit) (1 ug/ml, BioLegend, clone 104D2, 313204), BV605-conjugated CD31 (1 ug/ml, clone WM59, BioLegend, 303122), APC-conjugated EPCAM (1/2500, clone EPR20532-225, Abcam, ab237398), 4',6-diamidino-2-phenylindole, dihydrochloride (DAPI) (0.1 uM, Thermo Fisher Scientific, D1306), and unconjugated TMPRSS2 (2 µg/ml, clone AL20). PE goat anti-mouse IgG (1 µg/ml, BioLegend, 405307) were used as secondary antibodies for AL20. Data acquisition was performed using a BD LSRFortessa and a Northern Lights™ Flow Cytometry (Cytek Biosciences) and analyzed with FCS Express (De Novo Software) and SpectroFlo (Cytek Biosciences).
Instrument	BD LSR Fortessa and Cytek Bioscience Northern Lights Flow Cytometry
Software	FCS express (De Novo Software) and SpectroFlo (Cytek Biosciences)
Cell population abundance	The frequency for all cell populations is shown in manuscript figures.
Gating strategy	Representative gating strategies are shown in Supplementary Fig. 7, 9, and 11.

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