

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	STAR; CellRanger v6.1.0; Euroset Trilly membranous oxygenator; Zeiss Lumar V12; Invitrogen EVOS M5000; Zeiss Zen 3.1; Olympus FV3000 or Zeiss LSM 980 Confocal Microscope; Affinity Designer v2.5.0; R v4.3.1; Python v3.11; Seurat v5.0.2; Fiji 2.0; CellProfiler v4.2.6.
Data analysis	No custom codes were developed in this study. For single cell RNA-seq data analysis the CellRanger v6.1.0 software with default settings was used for de-multiplexing, aligning reads with STAR software to human genome GRCh38, and counting unique molecular identifiers (UMIs). Downstream data analysis was conducted with Seurat v5.0.2 in R v4.3.1. RNA velocity was calculated using the scVelo v0.3.1 package in Python v3.11 and velocity calculations were overlaid on UMAP projections calculated in Seurat. The lists of DEGs were identified with a Model-based Analysis of Single-cell Transcriptomics (MAST) test. Pathway analysis of gene lists containing significantly differentially expressed genes were done with Ingenuity Pathway Analysis (Qiagen). Image analysis and quantification was performed using Fiji 2.0 and CellProfiler v4.2.6. FlowJo v10 was used for flow cytometry analysis. GraphPad Prism 9.0 was used for all statistical testing.

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 sequencing data reported in this study were deposited in Genome Sequence Archive for Human (<https://ngdc.cncb.ac.cn/gsa-human/>) under the accession numbers: HRA008155.

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	Samples from both male and female donors were used.
Reporting on race, ethnicity, or other socially relevant groupings	We don't have such information.
Population characteristics	For the donor lung tissue section studies: The lung tissue sample collection for this study was reviewed and approved by the affiliated Wuxi People's Hospital of Nanjing Medical University Institutional Review Board, and complied with all relevant ethical regulations. The control lung samples were from 5 brain-dead donors that were rejected for clinical use and IPF lungs were taken from the 7 patients undergoing lung transplantation for pulmonary fibrosis. Donors were 43 to 68 years of age.
Recruitment	Human lung samples were obtained from brain-dead donors and IPF patients undergoing lung transplantation.
Ethics oversight	The studies involving human tissue were approved by the affiliated Wuxi People's Hospital of Nanjing Medical University Institutional Review Board, under the approval number 2020(374).

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	Sample size was determined based on the number of biological replicates available, which were strictly controlled for sex and age between experimental groups to minimize confounding factors in our analysis. No statistical test was performed to determine sample size. For other experiments, no prior calculations were performed to determine sample size. Samples size were determined by availability of tissue specimens (in case of disease lungs). Significance was calculated based on pairwise comparisons which required a minimum of three samples. We had at least three samples in all experiments.
Data exclusions	No data was excluded from the analysis presented in this paper
Replication	Three or more biological replicates were used for every study. All in vitro experiments were performed at least two times to confirm reproducibility and all attempts at replications were successful.
Randomization	This study employed observational human samples only; no experimental intervention or animal randomization was performed. Sample allocation into disease/control groups was based on clinical diagnosis.
Blinding	For RNA and histologic quantification, performer was blinded to specimen during data collection and analysis. For in silico analyses of scRNA-seq the performer was not blinded because the coding required explicit labeling of different specimens.

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-CD44 (1:200, Biolegend, 103001), Anti-CD45 (1:200, Biolegend, 304058), Anti-CD3 (1:200, Abcam, ab16669), AntiCD8 (1:200, SinoBiological, 10980-MM07), Anti-CD4 (1:200, Biolynx, 110301A), Anti-CD20 (1:200, Abcam, ab64088), AntiCD138 (1:200, Sino Biological, 11429-R017-P), Anti-IgG (1:500, Sino Biological, SSA016), Anti-COL1A1 (1:2500, Proteintec h, 67288-1-Ig), Anti-GZMK(1:200, ThermoFisher, PA5-50908)
Validation	All antibodies have been validated by the manufacturer using specific assay for human cells and have multiple citations for the relevant cell type. Investigators independently validated antibodies by reproducing findings in published work. Primary and secondary antibody controls were used for immunofluorescence. FMO or IgG controls were used for flow cytometry.

Plants

Seed stocks	<i>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.</i>
Novel plant genotypes	<i>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.</i>
Authentication	<i>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, mosaicism, off-target gene editing) were examined.</i>

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	The donor lung was prepared and connected with EVLP device with the pulmonary vein (PV) left open. The EVLP circuit was driven by a centrifugal pump, perfusate from the PV gathered in the perfusate pool, and was driven through a leukocyte filter to a Euroset® Trilly membranous oxygenator (Italy, EUROSET) which connected to balanced gas (6% oxygen, 8% carbon dioxide). After deoxygenation and heated, perfusate was driven to the pulmonary artery (PA). Two liters of Steen® solution were used to prime the circuit. The lungs were ventilated using a protective ventilation mode with an effective tidal volume of 4.8 ml/kg body weight for the right lobes or 3.2 ml/kg for the left lobes at 10 times/min. The time of starting ventilation was set as 0 h. One-hour EVLP time was adopted for each lung. Before injecting antibodies, the leukocyte filter was bypassed. Then, 1.5 liters of 37°C perfusate were replaced in the circuit. To labeling circulating immune cells, 12.5 µg of anti-CD45-AF647 antibody (BioLegend, 304018) was diluted into 10 ml PBS and injected through PA. The labeling process was 30 min. After that, the lungs were disconnected from the EVLP and re-flushed using cold sterile low-potassium dextran fluid. After EVLP, the distal regions of the lung tissues were collected for tissue dissociation and flow cytometry. After washed in PBS (2 X times) and HBSS for 15 min in total and compressed to remove liquid, the pieces were further diced with razor blades. The HBSS containing Dispase II (15 U/ml; Thermo Fisher, 17105041), 225 U/ml collagenase type I (Thermo Fisher, 17100017), 100
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	U/ml Dnase I (Sigma, DN25), and 1% Pen/Strep/ Fungizone were used to digest the pieces for 2 h at 37°C undergoing gentle rocking. The digested suspension was continuously filtered through gauze and 100 µm, 70 µm and 40 µm strainers. Red blood cells were removed using red blood cell lysis buffer (Sigma). For distinguishing resident and circulating immune cells, single cell suspensions were incubated with anti-CD45-AF488 (BioLegend, 304017) for 30 min at 4°C.
Instrument	Sony MA900 Multi-Application Cell Sorter
Software	Sony SH800S Software
Cell population abundance	Circulating immune cells were sorted as live/CD45-AF488+/ CD45-AF647+ cells. Resident immune cells were sorted as live/ CD45-AF488+/ CD45-AF647- cells.
Gating strategy	Cells were selected using FSC/SSC scatter profile to remove debris and doublets were excluded using FSC-A/FSC-H to select for single cells. Doublets and dead cells were excluded based on forward and side scatters and 7-AAD (BioLegend, 420404) or DAPI fluorescence. Circulating immune cells were sorted as live/CD45-AF488+/ CD45-AF647+ cells. Resident immune cells were sorted as live/CD45-AF488+/ CD45-AF647- cells.

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