nature portfolio

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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	Leica LAS-X and FACS Diva software version 8.0, was used for confocal image and flow cytometry data collection.
Data analysis	Software used in this study are details in methods and include CellRanger 3.1.0 pipeline (ref 65), R package Seurat v.4.2.2 (2022-10-31 for normalization, scaling, integration, multi-modal reference mapping, Louvain clustering, dimensionality reduction, differential expression analysis, and visualization (ref 66), DoubletFinder (ref 67), EnhancedVolcano (version 1.16.0), Azimuth app reference mapping tool (ref 68), Connectome toolkit (version 1.0.1)(ref 31), MATLAB version 2021b, Autotube – Github (ref 69), OxyGEN by Fluigent Smart Microfluidics™, Leica LAS-X, ImageJ (http://imagej.nih.gov/ij/), GraphPad PRISM v9.2, FACS Diva software version 8.0, FlowJo v10

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

Single-cell RNA-Seq data presented in this study can be accessed via NCBI GEO (GSE237911, private token: yxupquwcntqzbut for review, data will be made publicly

available upon manuscript acceptance).

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 are important as all humans are impacted by lung diseases with differences in lung disease states. This study integrated male primary human lung fibroblasts (Lonza), endothelial cells (10-donor pooled umbilical vein endothelial cells, male and female donors but exact breakdown not detailed, Lonza) and male lung epithelial cell lines (H441 and A549 cells). While this limits generalization, we anticipate models developed with female cells will yield similar results but this will require future study to confirm.
Reporting on race, ethnicity, or other socially relevant groupings	Human lung fibroblasts, A549 and H441 cell lines were from Caucasian donors. Race and ethnicity were not reported reported for pooled umbilical vein endothelial cells.
Population characteristics	NA
Recruitment	NA
Ethics oversight	NA

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	Sample size was determined in all cases to be a minimum of 3 independent biological replicates. For all lung on a chip experiments a minimum of 4 to 6 independent biological replicates for each condition were utilized and sample sized determined based on higher biological variability of this platform.
Data exclusions	No data was excluded.
Replication	All attempts at replicates were successful. Note: only lung on a chip devices with fully formed and perfusable microvessels were used at days 7-10 for experiments.
Randomization	Randomization was not applicable as all platforms were inherently designed to have minimal variation in baseline characteristics.
Blinding	Blinding was performed where any possibility of subjective judgement was required for quantification. This is only notable for leukocyte lung on a chip image analysis that was not automated by software. This was done by image acquisition with an alphanumeric experiment key, quantification, and then unblinding for data analysis.

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	This information is provided within a detailed table in supplementary methods
Validation	All antibodies were used by the manufacturer and validated for use by manufacturer.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	A549 and H441 epithelial cells were from ATCC.
Authentication	All cell lines were purchased directly from ATCC and used within first 5-10 passages.
Mycoplasma contamination	All cell lines were confirmed negative by mycoplasma by commercial source (Lonza and ATCC) and further tested if used beyond passage 5.
Commonly misidentified lines (See ICLAC register)	None.

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	This study utilized wildt-type specific pathogen free C57BL/6 female mice all at 6-8 weeks of age and bred at the University of Calgary.
Wild animals	This study did not involve wild animals.
Reporting on sex	Animal studies on mice only apply to female mice used in this study due to availability. As with most animal models of pneumonia, there is a sex difference with male mice being more susceptible than female mice (eg Kadioglu et al JID, 2011) which is why we utilized a higher (10^8 CFU/mouse) dose in females than is typically used in male (10^6-7 CFU/mouse) to ensure our results represent early stages of a severe bacterial pneumonia.
Field-collected samples	This study did not involve samples collected from the field.
Ethics oversight	Animal studies were approved by the Animal Care Committee (Animal Protocol: AC22 0042).

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

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	Sample preparation is outlined in detail on lines 624-636 and in supplementary.
Instrument	BD FACSAria III (cell culture experiments) and BD FACSCanto (supplementary animal experiments) were used.
Software	Data collection was done using FACS Diva software version 8.0. Analysis was performed using FlowJo v10.
Cell population abundance	No sorting was performed. Cellular abundance was indicated where appropriate in graphs (eg Figure 1, S11, S13).
Gating strategy	Gating strategy is outlined in methods and demonstrated in Figures 1 and S10.

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