

## 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  |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> The exact sample size ( $n$ ) for each experimental group/condition, given as a discrete number and unit of measurement  |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly  |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> The statistical test(s) used AND whether they are one- or two-sided<br><i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>   |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> A description of all covariates tested  |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons   |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> 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) |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> For null hypothesis testing, the test statistic (e.g. $F$ , $t$ , $r$ ) with confidence intervals, effect sizes, degrees of freedom and $P$ value noted<br><i>Give <math>P</math> values as exact values whenever suitable.</i>                            |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings  |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes  |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> 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** BD Aria Cell Sorter, BD Symphony A4 Flow cytometer — for single-cell sorting and acquisition of flow cytometry data  
Leica SP5 Confocal Microscope — for whole-slide Z-stack image acquisition  
Vevo Imaging Systems — for subcutaneous LLC tumor vascularization imaging  
MACSima Imaging System (Miltenyi Biotec) — for high-content multiplexed protein imaging  
HiSeq2500, NovaSeq 6000, and NovaSeq X Plus (Illumina) — for RNA-seq, ATAC-seq, and spatial transcriptomic sequencing  
Visium CytAssist HD slides (10x Genomics) — for spatial transcriptomics sample preparation  
Rhapsody single cell platform (BD) and Chromium Next GEM Single Cell Multiome ATAC + Gene Expression platform (10x Genomics) for multiomic library generation.

**Data analysis** -ImageJ (v 2.3.0/153t, NIH) and Imaris (v.9.5, Bitplane) for imaging analysis  
-MACS iQ View Analysis Software (Version 1.3.2) (Miltenyi Biotec) — for MACSima image analysis  
-FlowJo v10 (BD Biosciences) — for analysis of flow cytometry data acquired on the Symphony A4 Flow Cytometer  
-GraphPad Prism v9 and 10.10— for data visualization and statistical tests  
  
-R v4.0.3 and R v5.2.1 — core statistical and computational environment  
-Seurat v4.0.5 and v4.3 — for single-cell and spatial transcriptomic analysis  
-CASAVA v1.8 for the processing and analysis of sequencing data generated by Illumina's next-generation sequencing platforms.  
-RTA v1.18.66.3 to perform image analysis and base calling on HiSeq sequencing Illumina's instruments.  
-bcl2fastq v2.20.0.422 to convert bcl files to FASTQ.

-BCL Convert v3.6.3 to convert the Binary Base Call (BCL) files produced by Illumina™ sequencing systems to FASTQ files.  
 - Cutadapt v4.9 to remove unwanted sequences.  
 -SpaceRanger v1.3.0 to detect Dual Index Kit TS Set A with spaceranger mkfastq  
 -Rhapsody analysis pipeline v1.9.1 for the processing and analysis of sequencing data generated by the Rhapsody BD system.

R packages:

- limma v3.32.2 and EdgeR v3.20.1 for processing of count data and differential gene expression analysis
- Batchelor (1.20.0) for single-cell batch correction
- RANN v.2.6.2-To find the k nearest neighbours
- SingleR v2.8.0 for cell annotation using the ImmGen database
- Monocle3 v.1.3.7 for pseudotime and trajectory inference
- SPOTlight v1.0.3 for deconvolution of spatial transcriptomic data using seeded NMF regression
- decoupleR v2.8.0 for gene signature enrichment scoring
- Signac 1.14.0 for single-cell ATAC-seq analysis
- chromVAR v1.28.0 for transcription factor motif deviation analysis
- FlowSOM, and MASS v7.3.61 & 7.3.64, graphics v4.4.3, stats v4.0.3 & v4.4.3., and vegas v2.1.4 for additional statistical and graphical analysis
- Igraph (2.1.4) for Network Analysis and Visualization.
- RSEM v1.3.1 to extract reference transcripts from a genome.
- DESeq2 v1.30.1 to perform differential gene expression analysis based on the negative binomial distribution
- TCGAbiolinks v2.34.1 for accessing, analyzing, and integrating data from The Cancer Genome Atlas (TCGA)
- GenomicRanges v1.58.0 to define general purpose containers for storing and manipulating genomic intervals and variables defined along a genome
- MACS2 v2.2.9.1 for Model-Based Analysis for ChIP-Seq data
- JASPAR2020 v.99.10 a database of curated, non-redundant transcription factor (TF)-binding profiles stored as position frequency matrices (PFMs) for TFs across multiple species in six taxonomic groups.
- chromVAR v1.28.0 to determine variation in chromatin accessibility across sets of annotations or peaks.
- cellidex v1.14.0 a collection of reference cell type expression datasets for single-cell and bulk RNA-seq data analysis.
- concaveman v1.1.0 to compute the concave polygon for one set of points.
- h5ad2sce v0.0.1
- scuttle v1.16.0, msigdb v7.5.1
- AUCell v1.28.0 AUCell to identify cells with active gene sets
- BSgenome.Mmusculus.UCSC.mm10 v1.4.3 Full genome sequences for Mus musculus (Mouse) as provided by UCSC

Python libraries:

- TBin2cell v0.3.2
- pandas v2.2.3
- velocity v0.17.17 for estimating RNA velocity in single-cell RNA sequencing data.

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](#)

Processed data and interactive visualization of the NeuMAP atlas are accessible through the scDAVIS (Single-Cell Data Analysis and VISualization) platform at: <https://bioinfo.cnic.es/scdavis/>

Data that support the findings of the present manuscript are available from the corresponding authors upon reasonable request. All the omics data used for the mNeuMap is available at Gene Expression Omnibus (GEO) as a Super-series GSE266680.

Data used for the hNeuMap is available at the Chinese Academy of Sciences, with accession number HRA013413.

For spatial transcriptomic of mouse PDAC and MI, we used a publicly available dataset GSE141017 and GSE176092, respectively.

## 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 or gender-specific analyses were not conducted for these datasets.](#)

Reporting on race, ethnicity, or other socially relevant groupings

[All samples were de-identified prior to analysis, and no clinical or demographic identifiers were used in downstream research.](#)  
 N/A

Population characteristics	<p>Formalin-fixed paraffin-embedded (FFPE) samples were collected from eight patients diagnosed with pulmonary invasive adenocarcinoma. Tissue microarrays (TMAs) were generated by a certified pathologist based on intratumoural neutrophil abundance.</p> <p>Bone marrow samples were obtained from healthy adult donors following written informed consent. All procedures were approved by the ethics committee of the University Hospital Tübingen. Human participants were not compensated. Samples were anonymized prior to processing, and no identifiable information was retained.</p> <p>Samples used to generate the human NeuMap come from healthy individuals or from anonymous acute-death donors without chronic inflammation. Additionally, SLE patient samples (umbilical cord/peripheral blood) come from pregnant patients with an active disease state (SLEPDAI &gt;5) and without other chronic inflammatory comorbidities.</p>
Recruitment	<p>Tumor specimens were obtained from our institutional biobank, which prospectively accrues samples from all eligible patients under approved protocols. For this study, a small subset of cases was randomly selected from the biobank. Because the biobank aims to systematically include all patients, no self-selection or recruitment bias is expected, and results are unlikely to be affected by sampling bias. Human bone marrow cells were obtained from healthy, anonymous adult donors.</p> <p>Human samples were collected from Renji Hospital, Shanghai, China. Healthy donor samples (bone marrow, peripheral blood, umbilical cord blood) were randomly collected without self-selection or recruitment bias. Other healthy tissues were obtained from anonymous acute-death donors without chronic inflammation to minimize the confounding effects of death shock on the organs. SLE patient samples (umbilical cord/peripheral blood) were randomly selected from pregnant patients with an active disease state (SLEPDAI &gt;5) and without other chronic inflammatory co-morbidities.</p>
Ethics oversight	<p>Human tissue microarray (TMA) samples were used under protocol #2019-5253, which was reviewed and approved by the McGill University Health Centre (MUHC) Research Ethics Board, specifically by the MUHC co-Chair of the Comité d'éthique de la recherche du CTGQ panel. Human lung tissue specimens were obtained through protocols approved by the McGill University Health Centre Institutional Review Board (IRB #2014-1119).</p> <p>Bone marrow samples were obtained from healthy adult donors following written informed consent. All procedures were approved by the ethics committee of the University Hospital Tübingen.</p> <p>Human samples were collected from Renji Hospital, Shanghai, China, with ethical approval (Renji Hospital Ethics Committee Protocol KY2024-090-B) and in accordance with the Declaration of Helsinki, following informed consent from all participants.</p>

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	For each experiment, a sample size was chosen to obtain sufficient number of experiments and samples to calculate statistical significance. No statistical test was performed to predetermine sample size. They were determined on pilot experiments or prior experience.
Data exclusions	No collected data were excluded from the analysis.
Replication	Data presented were replicated in at least 3 independent experiments
Randomization	Allocation to samples or mice to the experimental groups was random.
Blinding	Due to practical constraints, investigators were not blinded to treatment administration during in vivo mouse experiments. However, all subsequent analyses were performed on samples labeled with anonymized ID codes that did not reveal treatment group, ensuring blinding during data analysis. Mice were randomly assigned to treatment groups. Data collection for automated experiments (e.g., flow cytometry, sequencing) was conducted using standardized protocols, and data interpretation was guided by appropriate experimental controls.

# 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
<input type="checkbox"/>	<input checked="" type="checkbox"/> Antibodies
<input type="checkbox"/>	<input checked="" type="checkbox"/> Eukaryotic cell lines
<input checked="" type="checkbox"/>	<input type="checkbox"/> Palaeontology and archaeology
<input type="checkbox"/>	<input checked="" type="checkbox"/> Animals and other organisms
<input checked="" type="checkbox"/>	<input type="checkbox"/> Clinical data
<input checked="" type="checkbox"/>	<input type="checkbox"/> Dual use research of concern
<input checked="" type="checkbox"/>	<input type="checkbox"/> Plants

## Methods

n/a	Involved in the study
<input checked="" type="checkbox"/>	<input type="checkbox"/> ChIP-seq
<input type="checkbox"/>	<input checked="" type="checkbox"/> Flow cytometry
<input checked="" type="checkbox"/>	<input type="checkbox"/> MRI-based neuroimaging

## Antibodies

### Antibodies used

Species | Antibody | Supplier | Cat | Clone | Lot | Dilution

Human | CD19-PE-CY7 | Biolegend | 302216 | HiB19 | B368441 | 1:200

Human | CD3-PE-CY7 | Biolegend | 300316 | HIT3a | B368934 | 1:200

Human | CD45-APC-CY7 | Biolegend | 304014 | Hi30 | B355047 | 1:200

Human | CD56 PE-CY7 | Biolegend | 362510 | 5.1H11 | B369452 | 1:200

Mouse | CCR5-BUV615-P | BD Biosciences | 752321 | C34-3448 | 5023291 | 1:200

Mouse | CD101-PE-Cy7 | eBioscience | 25-1011-82 | MOUSHI 101 | 3023969 | 1:200

Mouse | CD106-BUV563 | BD Biosciences | 741246 | 429 | 5199058 | 1:200

Mouse | CD115-BUV737 | BD Biosciences | 750948 | AFS98 | 4096153 | 1:200

Mouse | CD11b-APC | Miltenyi Biotec | 130-113-239 | M1/70.15.11.5 | 5250304726 | 1:50

Mouse | CD11b-BV510 | Biolegend | 101263 | M1/70 | B448781 | 1:200

Mouse | CD11b-PE | Biolegend | 101208 | M1/70 | B338668 | 1:200

Mouse | CD14-APC-Cy7 | Biolegend | 123318 | Sa14-2 | B422513 | 1:200

Mouse | CD14-PE | Biolegend | 150106 | Sa14-2 | B339202 | 1:50

Mouse | CD150-PE-Cy5 | Biolegend | 115911 | TC15-12F12.2 | B448511 | 1:200

Mouse | CD16/32-PerCP-Cy5.5 | Biolegend | 101324 | 93 | B465412 | 1:200

Mouse | CD274-BV421 | Biolegend | 124315 | 10F-9G2 | B447175 | 1:200

Mouse | CD44-BV570 | Biolegend | 103037 | IM7 | B446242 | 1:200

Mouse | CD45-APC | Biolegend | 103112 | 30F11 | B417515 | 1:200

Mouse | CD45-FITC | Miltenyi Biotec | 130-110-658 | REA737 | 5241106535 | 1:50

Mouse | CD74-BUV661 | BD Biosciences | 741572 | In-1 | 5034863 | 1:200

Mouse | cKit-BV605 | Biolegend | 135121 | ACK2 | B430106 | 1:200

Mouse | Cx3Cr1-FITC | Biolegend | 149020 | SA011F11 | B464771 | 1:200

Mouse | CXCR2-PE | Biolegend | 149303 | SA044G4 | B374879 | 1:50

Mouse | DC-Trail-R1 biotinylated | R&D Systems | BAF2378 | Polyclonal | UOS0124061 | 1:200

Mouse | Goat anti-rabbit-FITC | Sigma-Aldrich | F9887 | Polyclonal | SLCN0577 | 1:100

Mouse | I-A/I-E-BUV496 | BD Biosciences | 750281 | M5/114.15.2 | 4260638 | 1:200

Mouse | ICAM1-PE-Dazzle 594 | Biolegend | 1161130 | YN1/1.7.4 | B438642 | 1:200

Mouse | Ly6C-BV711 | Biolegend | 128037 | HK1.4 | B425511 | 1:200

Mouse | Ly6C-PE | Miltenyi Biotec | 130-111-916 | REA796 | 5221102758 | 1:50

Mouse | Ly6G-PE | Biolegend | 127608 | 1A8 | B360223 | 1:200

Mouse | MHC-II-APC | Miltenyi Biotec | 130-112-388 | REA813 | 5221103758 | 1:50

Mouse | PD-L1-APC | Biolegend | 124312 | 10F.9G2 | B277024 | 1:50

Mouse | Podoplanin-PE | Biolegend | 127408 | 8.1.1 | B394979 | 1:50

Mouse | Sca1-BUV395 | BD Biosciences | 563990 | D7 | 3319689 | 1:200

Mouse | Streptavidin-BV650 | Biolegend | 405231 | 2514174 | 1:500

Mouse | TLR4-BV786 | BD Biosciences | 741015 | MTS510 | 5034859 | 1:200

### Validation

CD19-PE-CY7 302216 Validated for Human. Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis. Additional reported applications (for the relevant formats) include: immunohistochemical staining of acetone-fixed frozen tissue sections and blocking of B cell proliferation.

CD3-PE-CY7 300316 Validated for Human. Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis. Additional reported (for the relevant formats) applications include: immunohistochemical staining of acetone-fixed frozen sections, immunoprecipitation, and activation of T lymphocytes.

CD45-APC-CY7 304014 Validated for Human. Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis. Additional reported applications (for the relevant formats) include: immunohistochemical staining of acetone-fixed frozen tissue sections and formalin-fixed paraffin-embedded tissue sections, inhibition of CD45 functions, immunofluorescence, Western blotting, and spatial biology (IBEX).

CD56 PE-CY7 362510 Validated for Human. Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis.

CCR5-BUV615-P 752321 CD195 (CCR5) Monoclonal antibody specifically detects CD195 (CCR5) in Mouse samples. It is validated for Flow Cytometry.

CD101-PE-Cy7 25-1011-82 This Moushi101 antibody has been tested by flow cytometric analysis of mouse bone marrow cells.

CD106-BUV563 741246 This antibody was developed for use in flow cytometry. The production process underwent stringent testing and validation to assure that it generates a high-quality conjugate with consistent performance and specific binding activity. However, verification testing has not been performed on all conjugate lots.

CD115-BUV737 750948 This antibody was developed for use in flow cytometry. The production process underwent stringent testing and validation to assure that it generates a high-quality conjugate with consistent performance and specific binding activity. However, verification testing has not been performed on all conjugate lots.

CD11b-APC 130-113-239 Validated for mouse. Validated for Flow cytometry and MACSima Imaging Cyclic Staining.

CD11b-BV510 101263 Validated for mouse. Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis. Clone M1/70 has been verified for immunocytochemistry (ICC) and frozen immunohistochemistry (IHC-F).

CD11b-PE 101208 Validated for mouse. Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis. Clone M1/70 has been verified for immunocytochemistry (ICC) and frozen immunohistochemistry (IHC-F).

CD14-APC-Cy7 123318 Validated for mouse. Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis.

CD14-PE 150106 Validated for mouse. Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis.

CD150-PE-Cy5 115911 Validated for mouse. Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis. The TC15-12F12.2 antibody has been reported to enhance the production of IFN- $\gamma$  by Th1 cells stimulated through TCR. Additional reported applications (for the relevant formats) include: immunoprecipitation, enhancing IFN- $\gamma$  production by Th1 cells when stimulated with CD31, and inhibiting CD3 induced T cell proliferation.

CD16/32-PerCP-Cy5.5 101324 Validated for mouse. Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis. The 93 mAb is specific to the common epitope of CD16/CD32. Additional reported applications (for the relevant formats) include: immunoprecipitation and blocking of Fc-mediated reactions in functional studies. It is useful for blocking non-specific binding of immunoglobulin to Fc receptors. For blocking of Fc receptors in flow cytometric analysis, pre-incubate the cells with purified anti-CD16/CD32 antibody ( $\approx 1.0 \mu\text{g}$  per  $10^6$  cells in  $100 \mu\text{L}$  volume) for 5-10 minutes on ice prior to immunostaining.

CD274-BV421 124315 Validated for mouse. Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis. Additional reported applications (for the relevant formats) include: immunofluorescence, blocking, and immunohistochemistry of acetone-fixed frozen sections.

CD44-BV570 103037 Validated for mouse. Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis. Clone IM7 has been reported to recognize an epitope common to alloantigens and all isoforms of CD44 that is located between amino acids 145 and 18620. This clone has been verified for immunocytochemistry (ICC) and frozen immunohistochemistry (IHC-F). Additional reported applications (for the relevant formats) include: immunohistochemistry of acetone-fixed frozen sections and formalin-fixed paraffin-embedded sections, complement-mediated cytotoxicity, immunoprecipitation, in vivo inhibition of DTH, and spatial biology (IBEX).

CD45-APC 103112 Validated for mouse. Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis. Additional reported applications (for relevant formats) include: immunoprecipitation, complement-dependent cytotoxicity, immunohistochemistry (acetone-fixed frozen sections, zinc-fixed paraffin-embedded sections and formalin-fixed paraffin-embedded sections), Western blotting, and spatial biology (IBEX).

CD45-FITC 130-110-658 Validated for mouse. Validated for Flow cytometry, MACSima Imaging Cyclic Staining, and 3D immunofluorescence.

CD74-BUV661 741572 Validated for mouse. Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis.

cKit-BV605 135121 Validated for mouse. Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis. ACK2 has been reported to block c-Kit function. The LEAF<sup>TM</sup> purified antibody (Endotoxin  $<0.1 \text{ EU}/\mu\text{g}$ , Azide-Free,  $0.2 \mu\text{m}$  filtered) is recommended for functional assays (Cat. No. 135103).

Cx3Cr1-FITC 149020 Validated for mouse. Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis.

CXCR2-PE 149303 Validated for mouse. Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis.

DC-Trail-R1 biotinylated BAF2378 Detects mouse DcTRAIL R1/TNFRSF23 in Western blots. In Western blots, approximately 25% cross-reactivity with recombinant mouse (rm) DcTRAIL R2 is observed and less than 1% cross-reactivity with rmTRAIL R2, recombinant human (rh) TRAIL R3, and rhTRAIL R4 is observed.

Goat anti-rabbit-FITC F9887 Anti-Rabbit IgG (whole molecule)-FITC antibody produced in goat has been used in immunohistochemistry and indirect immunofluorescence.

I-A/I-E-BUV496 750281 Validated for mouse. This antibody was developed for use in flow cytometry. The production process underwent stringent testing and validation to assure that it generates a high-quality conjugate with consistent performance and specific binding activity. However, verification testing has not been performed on all conjugate lots.

ICAM1-PE-Dazzle 594 1161130 Validated for mouse. Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis. Additional reported applications (for the relevant formats) include: in vitro and in vivo blocking of cell-cell adhesion and CD54 functions, immunohistochemical staining of acetone-fixed frozen sections, immunoprecipitation, and Western blotting (non-reducing).

Ly6C-BV711 128037 Validated for mouse. Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis. Additional reported applications (for relevant formats of this clone) include: in vitro activation of T cells and immunohistochemistry of frozen sections.

Ly6C-PE 130-111-916 Validated for mouse. Validated for Flow cytometry and MACSima Imaging Cyclic Staining.

Ly6G-PE 127608 Validated for mouse. Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis. Additional reported applications (for the relevant formats) include: immunohistochemistry of frozen sections and paraffin-embedded sections, depletion, and spatial biology (IBEX).

MHC-II-APC 130-112-388 Validated for mouse. Validated for Flow cytometry and MACSima Imaging Cyclic Staining.

PD-L1-APC 124312 Validated for mouse. Validated for Flow cytometry, immunofluorescence, blocking, and immunohistochemistry of acetone-fixed frozen sections.

Podoplanin-PE 127408 Validated for mouse. Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis. Additional reported applications (for the relevant formats) include: immunohistochemistry, and spatial biology (IBEX).

Sca1-BUV395 563990 Validated for mouse. Flow cytometry Routinely Tested.  
 Streptavidin-BV650 405231 Each lot of this Streptavidin-Brilliant Violet 650™ is quality control tested by immunofluorescent staining with flow cytometric analysis.  
 TLR4-BV786 741015 Validated for mouse. This antibody was developed for use in flow cytometry. The production process underwent stringent testing and validation to assure that it generates a high-quality conjugate with consistent performance and specific binding activity. However, verification testing has not been performed on all conjugate lots.

## Eukaryotic cell lines

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

Cell line source(s)	The C57BL/6J-derived Lewis Lung Carcinoma (LLC), E0771 (luminal B breast cancer), and BALB/c-derived 4T1 (triple-negative breast cancer) cell lines were obtained from the American Type Culture Collection (ATCC). The FC1242 pancreatic adenocarcinoma cell line was a kind gift from Dr. Dannielle Engle (Tuveson Lab, Cold Spring Harbor Laboratory) and was originally derived from Pdx1-Cre; Kras <sup>G12D/+</sup> ; p53 <sup>null/+</sup> (KPC) mice. B16-OVA cells were provided by D. Sancho Lab, (CNIC, Madrid, Spain)(doi:10.1158/2159-8290.CD-15-0510). HoxB8-immortalized myeloid progenitor cells were used for in vitro differentiation studies. CRISPR-Cas9-mediated knockout of selected transcription factors in HoxB8 progenitors was performed as previously described (doi: 10.1038/s41590-021-00968-4).
Authentication	HoxB8 were validated by morphology and surface marker phenotype. Lewis Lung Carcinoma (LLC), E0771 (luminal B breast cancer), and BALB/c-derived 4T1 (triple-negative breast cancer) were authenticated using GenePrint 10 Loci Service. The FC1242 pancreatic adenocarcinoma and B16-OVA melanoma cell lines were not further verified.
Mycoplasma contamination	All the cell lines were routinely tested for mycoplasma contamination by qPCR. All the results were always negative.
Commonly misidentified lines (See <a href="#">ICLAC</a> register)	No commonly misidentified cell lines were used in the 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	<p>All experiments were performed in male and female C57BL/6 mice aged 6–24 weeks. Young mice were defined as 8–12 weeks old, and old mice as 22–24 months at the time of analysis. Mice were maintained under specific pathogen-free conditions with chow and water provided ad libitum. mouse lines used were on the C57BL/6J background and housed under specific pathogen-free conditions at the CNIC, SigN or Yale University. All mouse husbandry and experimentation was conducted using protocols approved by local animal ethics committees and authorities. Mice (<i>mus musculus</i>) were maintained in racks with individual ventilation cages according to current Spanish, Singapore and US legislation (RD 53/2013 and EU Directive 63/2010, respectively). Rodents have a dust/pathogen-free bedding, sufficient nesting and environmental enrichment material for the development of species-specific behavior. All the animals have food and water "ad libitum" in environmental conditions of 45-65% of relative humidity, temperature of 21-24C and a light/ dark cycle of 12:12 hours. In addition and in order to preserve animal welfare, an animal health surveillance program is applied for health monitoring, which follows FELASA recommendations for Specific Pathogen Free facilities. All mice strains used are listed below and in the Mice section of the Methods.</p> <p>Neutrophil-specific Tgfb2-deficient mice (TGFβR ΔN) were generated by crossing MRP8 CRE mice with Tgfb2 fl/fl mice. Similarly, Junbfl/fl, Csf2rfl/fl, and Ifnar1fl/fl mice were crossed with MRP8CRE to generate neutrophil-specific knockouts. Apoe<sup>-/-</sup> mice (B6.129P2-Apoe tm1Unc, Taconic M&amp;B) were used in atherosclerosis models.</p> <p>Ly6GCREERT2 mice were crossed with Rosa26tdTomato reporter mice to generate iLy6G tdTom mice for fate-mapping experiments. JAXBoy mice (Ptprc K302E) from Jackson Laboratories and Tet2<sup>-/-</sup> mice were used for adoptive bone marrow transfer experiments. Germ-free male C57BL/6 mice (8 weeks old) were kindly provided by Noah Palm's lab (Yale University) for microbiota-dependent studies.</p> <p>For rewilding experiments, litters from multiple breeding pairs were randomly assigned to remain in the institutional vivarium (laboratory group) or released into controlled outdoor enclosures (rewilded group) to ensure microbiota equivalence at baseline.</p>
Wild animals	No wild animals were used in these studies
Reporting on sex	For all single-cell sequencing experiments, the sex of the animals used is explicitly reported in the main text. Where applicable, computational analyses were performed to assess potential sex-biased differences in gene expression and cellular composition. For all other in vivo experiments, both male and female mice were included, and data from both sexes were pooled and analyzed together unless otherwise specified.
Field-collected samples	For the rewilding experiments, litters of mice were generated from multiple breeding pairs and randomly assigned to either remain in the institutional vivarium (laboratory mice) or be released into the outdoor enclosures (rewilded mice) to control for the microbiota at the onset of the experiment.
Ethics oversight	All protocols were reviewed and approved by the corresponding local authorities of Madrid, Singapore, Rutgers, Princeton, and Yale University. The protocols for releasing laboratory mice into the outdoor enclosure facility and then returning them to vivaria were approved by Princeton University (protocol #1982) and Rutgers University (protocol # PROTO999900794).

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, mosaicism, 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

##### Mice

Mice were euthanized, and blood was collected via cardiac puncture using a 1 mL syringe with a 26-gauge needle preloaded with 50  $\mu$ L of 0.5 M EDTA. After blood collection, mice were perfused via the right ventricle with 10 mL PBS to flush circulating cells. Tissues (lung, tumors, muscle, heart, placenta, pancreas) were minced and digested with Liberase TM (Sigma) and DNase I (Sigma) at 37°C for 30 minutes. Digests were filtered through 70  $\mu$ m nylon mesh sieves using syringe plungers to obtain single-cell suspensions.

Bone marrow was flushed from femurs using PBS with 2 mM EDTA and 2% FBS. Spleens were mechanically dissociated through 70  $\mu$ m filters. Colon tissues were cleaned and incubated in 100 mM EDTA for 30 minutes at 37°C to remove epithelium, then enzymatically digested as above. Ear skin was separated into dorsal and ventral layers, chopped, and digested for 90 minutes at 37°C. For peritoneal lavage, 10 mL of cold PBS was injected into the cavity, followed by gentle massage and aspiration.

Meninges were isolated post-mortem by removing the brain and peeling the dura with fine forceps. Brain infarct tissue was dissected, digested with a cocktail (collagenase, dispase, TLCK, DNase I), ground in a glass-glass grinder, and filtered through 70  $\mu$ m mesh. Leukocytes were isolated via 35% Percoll density centrifugation. All single-cell suspensions were treated with RBC lysis buffer (eBioscience), washed, and stained with antibody cocktails.

##### Primary Neutrophils and HoxB8 Cell Cultures

Primary mouse neutrophils were obtained from the femurs and tibias of C57BL/6J mice or relevant genetically modified strains. Bone marrow was centrifuged and erythrocytes were lysed using Red Blood Cell Lysis Solution (Qiagen, 79217). Cells were filtered and sorted on a BD Aria Cell Sorter into mature (DAPI<sup>-</sup> CD45<sup>+</sup> CD11b<sup>+</sup> Ly6G<sup>+</sup> CD101<sup>+</sup>) and immature (DAPI<sup>-</sup> CD45<sup>+</sup> CD11b<sup>+</sup> Ly6G<sup>+</sup> CD101<sup>-</sup>) neutrophils. Sorted cells were seeded into 96-well plates (50,000 cells/well) in complete DMEM and cultured under vehicle or treatment conditions.

HoxB8-immortalized myeloid progenitors were routinely screened for mycoplasma and cultured in RPMI 1640 supplemented with 10% fetal calf serum (FCS), 10  $\mu$ M  $\beta$ -mercaptoethanol, 4% SCF-producing CHO cell supernatant, 1% penicillin/streptomycin, and 1  $\mu$ M  $\beta$ -estradiol to maintain the progenitor state. Neutrophil differentiation was induced by  $\beta$ -estradiol withdrawal and continued in media with 4% SCF and 20 ng/mL G-CSF.

Human samples were collected in Renji Hospital, Shanghai, China, under the Renji Hospital Ethics Committee protocol KY2024-090-B, in accordance with the Declaration of Helsinki. Samples were collected from healthy donors, patients or perfused organ donors. Blood and bone marrow were collected in BD vacutainer K2E (EDTA) tubes (BD Healthcare, 367525) to prevent coagulation. Erythrocytes were lysed in 5-10 mL 1x red blood cells (RBC) lysis buffer (diluted from 10x BD Pharm Lyse, 555899) for 5 min for twice to deplete erythrocytes and then washed and re-suspension. Spleen, lung, omentum, mesentary fat, perirenal fat, liver, colon and rectum tissues were minced into small pieces and digested for 30 min at 37 °C in a mixture of collagenase IV (385U mL<sup>-1</sup>, Sigma) and DNaseI (2.5mg mL<sup>-1</sup>, Sigma) and the samples were homogenized into single-cell suspension using syringe plungers and passed through 70  $\mu$ m cell strainers (15-1070, BIOLOGIX). Then the samples were lysed in 2 mL 1x RBC lysis buffer (diluted from 10x BD Pharm Lyse, 555899) for 3 min to deplete erythrocytes and then washed and resuspended. Endometrium was cut into small pieces and enzymatically digested with the Tumor Dissociation Kit (130-095-929, Miltenyi Biotec). After digestion, the cell suspension was filtered through 70  $\mu$ m cell strainers and subjected to a 3-min erythrocyte lysis with 2 mL 1x RBC lysis buffer, followed by washing and re-suspension. All single-cell suspensions were

incubated with Fc-blocker (Human TruStain FcXTM, 422302, Biolegend) for 30 min on ice, then stained for 30 min at 4 °C in the dark with Fixable Viability Stain 700 (564997, BD Biosciences) (1:1000), CD45 APC-CY7 (304014, Biolegend) (1:200), CD3 PE-CY7 (300316, Biolegend) (1:200), CD19 PE-CY7 (302216, Biolegend) (1:200) or CD56 PE-CY7 (362510, Biolegend) (1:200). After washing with FACS buffer, cells were sorted on a FACS Aria III cell sorter (BD Biosciences).

Instrument

Samples were acquired on a BD Symphony A4 Flow Cytometer

Software

Data were analyzed with FlowJo software (BD). FlowAI (DOI: 10.1093/bioinformatics/btw191) was used for quality control. Dimensionality reduction was performed using the UMAP\_R plugin. Clustering was conducted using FlowSOM and ClusterExplorer. UMAP parameters were embedded per sample to enable statistical comparisons of neutrophil phenotypes.

Cell population abundance

Typical post-sorting purity >98%

Gating strategy

Neutrophils were sorted as live (DAPI-negative), CD11b<sup>+</sup> Ly6G<sup>+</sup> cells using a FACS Aria sorter (BD Biosciences) at the CNIC Cytometry Unit. Bone marrow neutrophils were captured as Lineage negative (B220, CD18, NK.1.1, Ter119, CD3). Human leukocytes were sorted as CD45+CD3-CD19-CD56- cells

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

Signed by BALLESTEROS MARTIN, IVAN  
JOSE (FIRMA) date 09/10/2025 certified  
by AC DNIE 004