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Corresponding author(s): Bin Zhou

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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<br>*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<br>*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 | Immunofluorescent images were acquired using Olympus FV1200, FV4000 confocal system, and Zeiss 880 confocal system. Quantitative RT-PCR data was acquired using QuantStudio 6 Real-Time PCR System (Applied Biosystems, ThermoFisher). FACS analysis was acquired using Sony MA900 and BD FACS Aria. Pulmonary function and airway responsivess measurement data were acquired using a whole-body plethysmograph (EMMS, Hants, UK) with the Resistance and Compliance System and the Flow Measurement system.Immunoelectron microscopy images were acquired using a Tecnai G2 Spirit transmission electron microscope (FEI). |
| Data analysis | Immunofluorescent images analysis using ImageJ (NIH, version: 2.3.0/1.53q) and Imaris (version: 9.5.1) software. FACS data analysis using FlowJo version 10.4. Statistical analysis used GraphPad Prism (v9.3.1) and R. Adaptor sequences and low-quality bases were trimmed from the raw data using Trim Galore version 0.6.7. The custom reference generation and alignment for single cell RNA-seq were performed using Cell Ranger version 6.1.1. The downstream analysis were conducted in R version 4.4.2.<br>The following R packages were used for data analysis.<br>Seurat_4.1.2 DBSCAN_1.1-10 destiny_3.20.0 limma_3.62.1 clusterProfiler_4.14.4<br>CellChat_1.6.1<br>Python (v3.8.18)<br>eDacq (v1.9.9) software<br>Signac (v.1.16.0) and Seurat (v.5.3.0) Cell Ranger ARC (v.2.1.0) SAW (v.8.2.2) Stereopy (v.1.6.2)<br>Zenodo web-link: https://zenodo.org/records/15333044<br>FACS data were analyzed using FlowJo 10.4 (FlowJo LLC) |

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 generated in this study are deposited in Genome Sequence Archive (GSA; https://ngdc.cncb.ac.cn/gsa/) under accession PRJCA039649. Source data are provided with this paper.
The codes executing the analysis workflow are available on Zenodo with persistent access through DOI: https://doi.org/10.5281/zenodo.21768825.

## 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 | NA |
| Reporting on race, ethnicity, or other socially relevant groupings | NA |
| 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 | No statistical method was used to predetermine the sample size. Sample sizes were chosen based on prior experience and pilot experiments for detecting statistically significant differences between conditions. For all in vivo and in vitro experiments, a minimum of four biological replicates ($n \geq 4$) was used. In compliance with IACUC guidelines, for in vivo experiments, a minimal number of animals with balanced sex was used to achieve statistically meaningful results. All specific sample sizes are indicated in the corresponding figure legends. |
| Data exclusions | No data was excluded from the analyses. |
| Replication | All in vivo mouse experiments and in vitro cell culture experiments included a minimum of three biological replicates, whereas single-cell transcriptomics and spatial transcriptomics analyses were carried out with one biological replicate per condition. Sample size is indicated in each figure and figure legend. |
| Randomization | Both adult male and female mice were randomly assigned to in vivo experimental groups. For in vitro experiments, random allocation was not applicable. Samples were equally distributed from a single homogenized pool, and all culture conditions (passage number, medium batch, seeding density, temperature, treatment duration) were standardized across groups. Thus, no additional covariate control was required, and randomization was not performed as it was not relevant to these assays. |
| Blinding | No blinding was applied as the same investigators performed the experiments and analyzed the data. The injury groups compared to uninjured groups are readily apparent. |

# 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 species, application, manufacturer, catalog number, dilution ratio, and official validation website links for the primary antibodies used in immunofluorescence staining are as follows:
Goat, anti-E-cadherin (R&D system, AF748, 1:500), https://www.rndsystems.com/cn/products/human-mouse-e-cadherin-antibody_af748
Goat, anti-Scgb1a1 (Santa Cruz, SC-9772, 1:200), https://www.scbt.com/p/cc10-antibody-t-18
Rabbit, anti-Scgb1a1 (Abcam, ab213203, 1:300), https://www.abcam.cn/uteroglobin-antibody-epr19846-ab213203.html
Goat, anti-Scgb3a2 (R&D system, AF3545-SP, 1:500), https://www.rndsystems.com/cn/products/human-ugrp1-scgb3a2-antibody_af3545
Rabbit, anti-tdTomato (Rockland, 600-401-379, 1:1000), http://www.rockland-inc.com/Product.aspx?id=34801
Goat, anti-tdTomato (Rockland, 200-101-379, 1:500), https://www.rockland.com/categories/primary-antibodies/rfp-antibody-pre-adsorbed-600-401-379/
Goat, anti-GFP (Abcam, ab6662, 1:500), http://www.abcam.cn/gfp-antibody-fitc-ab6662.html
Goat, anti-Collagen IV (Millipore, Ab769, 1:500), https://www.merckmillipore.com/CN/zh/product/Anti-Collagen-Type-IV-Antibody,MM_NF-AB769?ReferrerURL=https%3A%2F%2Fwww.bing.com%2F&bd=1
Rat, anti-GFP (nacalai tesque, 04404-84, 1:500), http://www.nacalai.co.jp/global/reagent/Antibodies/GFP_Rat.html
Rabbit, anti-GFP (Invitrogen, A11122, 1:500), https://www.thermofisher.com/antibody/product/GFP-Tag-Antibody-Polyclonal/A-11122
Rat, anti-AGER (R&D system, MAB1179-100, 1:200), https://www.rndsystems.com/cn/products/mouse-rat-rage-antibody-175410_mab1179#product-citations
Rabbit, anti-Sftpc (Millipore, AB3786, 1:200), http://www.millipore.com/publications.nsf/a73664f9f981af8c852569b9005b4eee/e158402e5325b78c852573060076d773/$FILE/AB3786.pdf
Rabbit, anti-ZsGreen (Clontech, 632474, 1:1000), http://www.clontech.com/CN/Products/Fluorescent_Proteins_and_Reporters/Fluorescent_Protein_Antibodies/ibcGetAttachment.jsp?cItemId=27624&fileId=6464523&sitex=10022:22372:US
Rabbit, anti-Ki67 (Abcam, ab15580-100ug, 1:400), https://www.abcam.com/en-us/products/primary-antibodies/ki67-antibody-ab15580
Goat, anti-DTR (R&D system, AF-259-NA, 1:500), https://www.rndsystems.com/cn/products/human-hb-egf-antibody_af-259-na
Mouse, anti-Ace-Tub (Sigma, T7451, 1:300), https://www.sigmaaldrich.cn/CN/zh/product/sigma/t7451
Rabbit, anti-Krt5 (Biolegend, 905504, 1:500), https://www.biolegend.com/en-gb/products/purified-anti-keratin-5-antibody-13378?GroupID=GROUP26
Rabbit, anti-CGRP (Sigma, C8198, 1:500), https://www.sigmaaldrich.com/TW/en/product/sigma/c8198
Goat, anti-PDGFRa (R&D system, AF1062, 1:500), https://www.rndsystems.com/cn/products/mouse-pdgf-ralpha-antibody_af1062
Rat, anti-Lamp3 (NovusBio, 1006F7.05, 1:300), https://www.labome.com/product/Novus-Biologicals/DDX0192P-100.html
Rabbit, anti-FOXJ1 (Abcam, ab235445, 1:300), https://www.abcam.cn/products/primary-antibodies/foxj1-antibody-epr21874-ab235445
Hamster, ant-T1a/Pdpn (DSHB, 8.1.1, 1:100),http://dshb.biology.uiowa.edu/8-1-1_2
Rabbit, anti-Hopx (Abcam, ab307670, 1:100), https://www.scbt.com/p/hop-antibody-e-1
Rabbit, anti-Jagged1 (Abcam, ab7771, 1:100), https://www.abcam.cn/products/primary-antibodies/jagged1-antibody-ab7771
Mouse, anti-MMP9 (Thermo, MA5-15886, 1:200), https://www.thermofisher.cn/cn/zh/antibody/product/MMP9-Antibody-clone-5G3-Monoclonal/MA5-15886
Goat, anti-SPP1/OPN (R&D system, AF808, 1:400), https://www.rndsystems.com/cn/products/mouse-osteopontin-opn-antibody_af808
Rat, anti-CD45(eBioscience, 17-0451-82, 1:200),https://www.thermofisher.com/antibody/product/CD45-Antibody-clone-30-F11-Monoclonal/17-0451-82
Rat, anti-Integrin beta 1 (CD29) (Sigma/Millipore, MAB1997, 1:100), https://www.sigmaaldrich.cn/CN/zh/product/mm/mab1997

Secondary antibodies:
Alexa donkey anti-rabbit 555 (Invitrogen, A31572, 1:1000), https://www.thermofisher.cn/cn/zh/antibody/product/Donkey-anti-Rabbit-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-31572
Alexa donkey anti-rabbit 488 (Invitrogen, A21206, 1:1000), https://www.thermofisher.cn/cn/zh/antibody/product/Donkey-anti-Rabbit-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21206
Alexa donkey anti-rabbit 647 (Invitrogen, A31573, 1:1000), https://www.thermofisher.cn/cn/zh/antibody/product/Donkey-anti-Rabbit-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-31573
Alexa donkey anti-goat 488 (Invitrogen, A11055, 1:1000), https://www.thermofisher.cn/cn/zh/antibody/product/Donkey-anti-Goat-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11055
Alexa donkey anti-goat 555 (Invitrogen, A21432, 1:1000), https://www.thermofisher.cn/cn/zh/antibody/product/Donkey-anti-Goat-

IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21432
Alexa donkey anti-goat 647 (Invitrogen, A21447, 1:1000), https://www.thermofisher.cn/cn/zh/antibody/product/Donkey-anti-Goat-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21447
Alexa donkey anti-mouse 647 (Invitrogen, A31571, 1:1000), https://www.thermofisher.cn/cn/zh/antibody/product/Donkey-anti-Mouse-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-31571
Alexa donkey a-mouse 488 (Invitrogen, A21202, 1:1000), https://www.thermofisher.cn/cn/zh/antibody/product/Donkey-anti-Mouse-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21202
Alexa donkey anti-rat 647 (Invitrogen, A21247, 1:1000), https://www.thermofisher.cn/cn/zh/antibody/product/Goat-anti-Rat-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21247
Alexa donkey anti-rat 488 (Invitrogen, A21208, 1:1000). https://www.thermofisher.cn/cn/zh/antibody/product/Donkey-anti-Rat-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21208

For FACS, antibodies:
CD45 APC-eFluor 780 (eBioscience, 47-0451-82, 1:200), https://www.thermofisher.com/cn/zh/antibody/product/CD45-Antibody-clone-30-F11-Monoclonal/47-0451-82
Ter-119 APC-eFluor 780 (eBioscience, 47-5921-82, 1:200), https://www.thermofisher.com/cn/zh/antibody/product/CD45-Antibody-clone-30-F11-Monoclonal/47-0451-82

Validation

All antibodies used in this study were validation by the manufacturer to confirm their specificity and suitability for the intended applications. The detailed validation methods and data can be accessed through the provided links to the manufacturer's websites.

## Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

| | |
|---|---|
| Cell line source(s) | MLE-12 cell line (sunncell, catalog number: SNL-414), Web-link:https://www.sunncell.com.cn/product/SNL-414 |
| Authentication | The MLE-12 cell line (SNL-414, ATCC CVCL-3751) was authenticated by the supplier via mouse species-specific STR profiling. No further authentication was performed in our laboratory. |
| Mycoplasma contamination | The MLE-12 cell line has been tested negative for mycoplasma contamination |
| Commonly misidentified lines (See ICLAC register) | The MLE-12 cell line used in this study is not listed in the ICLAC Register of Misidentified Cell Lines (version 14) |

## 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 | All animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC, protocol #SIBCB-S374-1702-001-C1) at the Center for Excellence in Molecular Cell Science, Chinese Academy of Sciences, and were performed in accordance with its guidelines. All mice used in this paper were performed on 7–20 weeks old and were kept in a C57BL6/ICR mixed background. Both male and female mice were randomly used for experiments. Sftpc-CreER, Sftpc-DreER, Scgb1a1-CreER, Ager-CreER, Hopx-2A-DreER, p63-DreER, Foxj1-CreER, R26-NR, R26-TLR, R26-tdT, R26-RSR-tdT, R26-RSR-LSL-tdT, R26-Confetti, R26-iDTR, R26-NICD, Rbpj flox/+, Jag1 flox/+ mice lines were previously described, references found in Materials and Methods. The Scgb1a1-DTR-GFP, Ascl1-CreER, Itgb1 flox/+, Rbpj rox/+, Lamp3-CreER, Cldn4-LSL-Dre, Cldn4 flox/+, and Notch2 flox/+ mice lines were generated by Shanghai Model Organisms Center, Inc. (SMOC). For the Scgb1a1-DTR-GFP mouse line, the P2A-Diphtheria Toxin Receptor (DTR)-GFP sequence was inserted into the Scgb1a1 gene locus via homologous recombination. For the Ascl1-CreER mouse line, the CreERT2 sequence was inserted into the Ascl1 gene locus via homologous recombination. For the Itgb1flox/+ mouse line, two loxP sites were added on either side of Exon3 of Itgb1 gene via homologous recombination. For the Rbpjrox/+ mouse line, two rox sites were added on either side of Exon6 and Exon7 of Rbpj gene via homologous recombination.For the Lamp3-CreER mouse line, the 2A-CreER sequence was inserted into the Lamp3 gene locus via homologous recombination. For the Cldn4-LSL-Dre mouse line, the loxP-stop-loxp-Dre sequence was inserted into the Cldn4 gene locus via homologous recombination. For the Cldn4flox/+ mouse line, two loxP sites were added on either side of Exon1 of Cldn4 gene via homologous recombination. For the Notch2flox/+ mouse line, two loxP sites were added on either side of Exon3 of Notch2 gene via homologous recombination. All mice were housed and bred in the Specific Pathogen Free (SPF) animal facility of the Center for Excellence in Molecular Cell Science, under controlled conditions at 22 ± 2 °C, 50 ± 10 % relative humidity, and a 12-h light/dark cycle, with ad libitum access to food and water. Depending on the experimental cohort, mice were used at either 7–20 weeks of age or 6 months of age, and all animals were maintained on a C57BL6/ICR mixed background. Both male and female mice were randomly used for experiments. |
| Wild animals | No wild animals were used in this study. |
| Reporting on sex | No findings applied only to one sex. Both male and female mice were used in this study. |
| Field-collected samples | No field-collected samples were used in this study. |
| Ethics oversight | All animal experiments were conducted in accordance with the procedures approved by the Institutional Animal Care and Use Committee (IACUC) (protocol #SIBCB-S374-1702-001-C1) at the Center for Excellence in Molecular Cell Science, Chinese Academy of Sciences. |

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

## Plants

Seed stocks: NA

Novel plant genotypes: NA

Authentication: NA

## 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 mice were euthanized, and the lungs were perfused with cold PBS through the right ventricle to eliminate blood cells, followed by the injection of protease solution consisting of collagenase type I (500 U/mL; Gibco 17100–017), elastase (4 U/mL; Worthington Biochemical Corporation LS002279), dispase (5 U/mL; BD Biosciences 354235), and DNase I (0.33 U/mL; Worthington Biochemical Corporation LS002139) in DMEM (Gibco, 11965092) through the trachea. Subsequently, the lungs were minced and incubated in 4 mL of protease solution for 30 minutes at 37°C with continuous agitation. The tissue fragments were then dissociated by pipetting during the digestion process, followed by adding equal volume of the DMEM/F12 with 10% FBS to terminate the digestion. After being centrifuged at 1000 × g for 5 minutes at 4°C and washing with DMEM, the precipitate was resuspended in 3 mL enzyme mixture of 0.1% Trypsin-EDTA (Gibco, 25200072) containing DNase I and incubated for 20 minutes at 37°C with agitation.
Next, the digestion was terminated by addition of DMEM/F12 with 10% FBS, after which the cells were sequentially filtered through 100 μm strainers and centrifuged at 1000 × g for 5 minutes at 4°C. The red blood cells were lysed by incubation of red blood cell (RBC) lysis buffer (2–5 mL, eBioscience, 00-4333-57) at room temperature for 5 minutes. The process was ended by adding 8-10mL PBS and then centrifuge at 1000 × g for 5 minutes at 4°C. The precipitate was resuspended in block solution (eBioscience, 14–0161, 1:100) for 5 minutes to block nonspecific antigens prior to further staining. Then the cells were incubated with primary antibodies at 4°C for 30 minutes. The following antibodies were utilized: CD45 APC-eFluor 780 (eBioscience, 47-0451-82, 1:200), Ter-119 APC-eFluor 780 (eBioscience, 47-5921-82, 1:200). Afterwards, the cells were washed with PBS, followed by centrifugation. Subsequently, the cells were resuspended in PBS supplemented with DAPI and DNase I.
Bulk AT2 cells were excluded using Lyso-Tracker by staining the lysosomes in AT2 cells. For Lyso-Tracker staining, the cells were resuspended in DMEM containing LysoTracker (Invitrogen, Lyso-Tracker Deep Red, L12492) and incubated at 37°C for 10 to 15 minutes. Then the cells were centrifuged at 1000 × g for 5 minutes at 4°C, followed by washing with PBS. Subsequently, the cell pellets were resuspended in PBS supplemented with DAPI and DNase I. For scRNA-seq, the AT2-tracer3, BASC-tracer, and Club-tracer cells were sorted from Scgb1a1-CreER;Sftpc-DreER;R26-TLR lungs at 4 weeks and 6 months post-naphthalene injury. For scATAC-seq, the AT2-tracer2 cells were sorted from Scgb1a1-CreER;Sftpc-DreER;R26-NR lungs at 4 weeks and 6 months post-naphthalene injury. All cells in the single-cell suspension were negatively selected for CD45 and Ter119, and then gated the tdT+ZsG– cells as Club-tracer, tdT+ZsG+ cells as BASC-tracer, and tdT–ZsG+Lyso-Trackerlow cells as AT2-tracer3. Sony MA900 and BD FACS Aria were utilized for flow cell sorting. FACS data were analyzed using FlowJo 10.4 (FlowJo LLC). Representative gating strategies used in this study are shown in Supplementary Fig. 1.

Instrument: Sony MA900; BD FACS Aria

Software: FlowJo 10.4

Cell population abundance

The proportion of Club-tracer cells sorted in live CD45– Ter119– cells was about 0.4%.
The proportion of BASC-tracer cells sorted in live CD45– Ter119– cells was about 0.3%.
The proportion of AT2-tracer3 cells sorted in live CD45– Ter119– Lyso-Tracker-low cells was about 5.1%.

Gating strategy

Preliminary FSC/SSC gating were performed for all samples. The AT2-tracer3 cells were defined by DAPI– CD45– Ter119– ZsG + Lyso-Tracker low cells. The BASC-tracer cells were defined by DAPI– CD45– Ter119– ZsG+ tdT+ cells. The Club-tracer cells were defined by DAPI– CD45– Ter119– ZsG– tdT+ cells. Positive and negative staining cells populations were defined by the fluorescence-minus-one method. Representative gating strategies used in this study are shown in Supplementary Fig. 1.

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