

## 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 type="checkbox"/>            | <input checked="" type="checkbox"/> A description of all covariates tested                                                                                                                                                                                                                     |
| <input type="checkbox"/>            | <input checked="" 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 type="checkbox"/>            | <input checked="" 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 | No software was used for collection of data.                                                                                                                                                                                                                                                                      |
| Data analysis   | Graphpad prism 10, FlowJo_v10.6.1 program, Fiji (ImageJ) package:Seurat (R package, version 4.0.0), CellChatCellPhoneDB (actually a Python package, not R), fgseatximport (R package, version 1.16), DESeq2 (version 1.28), GSVA (R package), penalized (R package), GEOquery (R package), tidycmprsk (R package) |

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 RNA-seq data generated in this study were deposited and publicly available in European Genome-Phenome Archive (EGA) at accession number EGAS00001008181 and EGAS00001008182. The data analyzed in this study were obtained from the database of Gene-Expression Omnibus at GSE58831

and from EGA at EGAS00001001926. Customized code used for analysis of the bioinformatic data are available at <https://github.com/LanpengChen/LRMDS-scRNAseqanalysis>.

All other data supporting the findings of this study are cited in Methods, in supplementary documents.

## 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

For our human BM and peripheral blood, we don't report on sex or gender in our study. Sex was not used as covariate in our analysis.

However, the human cell line HS-5, is a cell with fibroblast morphology that was isolated from the stroma of a White, 30-year-old, male patient.

### Reporting on race, ethnicity, or other socially relevant groupings

For our human BM and peripheral blood, the race, ethnicity, or socially relevant groupings are not included in our study. However, the human cell line HS-5, is a cell with fibroblast morphology that was isolated from the stroma of a White, 30-year-old, male patient.

### Population characteristics

All patients included in the study were diagnosed with low risk MDS. Normal bone marrow samples were collected from healthy donors with no clinical complains.

### Recruitment

Patients were recruited based on guideline of HOVON 89 clinical trail (([www.hovon.nl](http://www.hovon.nl); [www.trialregister.nl](http://www.trialregister.nl) as NTR1825; EudraCT No. 2008-002195-10).

### Ethics oversight

The study was approved by the ethics committees of the participating institutions(Central ethics committee (MREC, Medical Research Ethics Committee): METC Amsterdam UMC (central review in the Netherlands) )and was conducted in accordance with the Declaration of Helsinki. All patients gave their written informed consent.

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

No sample size calculations or statistical method were performed to determine the experimental sample size. For cellular studies, the sample size was chosen according to preliminary experiments and our prior experience(Ping Z, et al. Leukemia,2019.) Sample size for animal studies was determined based on our own and published experiences that meaningful differences in key HSC and hematopoietic parameters can be assessed in such a cohort. (Nature,2009; Kenswill et al., Cell Stem Cell, 2021)

### Data exclusions

No data were excluded.

### Replication

All findings have been reproduced in multiple experiments and animals as indicated in the manuscript.

### Randomization

Samples were allocated into experimental groups randomly.

### Blinding

Seeing as all experiments were performed by researchers and care takers, it was not possible to blind these studies.

## 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 &amp; 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 type="checkbox"/>            | <input checked="" 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

Antibody, Clone, Dilution, Manufacture #Catalogue  
 CD45-APC, 2D1, 1:20, eBioscience #15568246  
 CD34-Alexa Fluor 700, 581, 1:50, BioLegend #343526  
 CD33-PE, P67.6, 1:50, BD Biosciences #345799  
 CD117 (C-kit)-PE-Dazzle, 104D2, 1:50, BioLegend #313226  
 CD271-PE, ME20.4, 1:50, BioLegend #345106  
 CD71-Alexa Fluor 700, MEM-75, 1:20, Exbio #A7-235-T100  
 CD235a-PE-Cy7, HI264, 1:50, BioLegend #349112  
 CD31-APC-Cy7, WM59, 1:20, BioLegend #303120  
 CD144-BV421B, 55-7H1, 1:50, BD Biosciences #565671  
 Annexin A1-APC, 74/3, 1:50, BioLegend #831604  
 zombie-BV510, 1:500, BioLegend #423101  
 CD3-PE-Cy5, 1:50, UCHT1, BioLegend #300310  
 CD8-PE-Cy7, SK1, 1:50, BioLegend #344714  
 CD69-BYG584, FN50, 1:50, BioLegend #310906  
 CD69 FITC, FN50, 1:50, BioLegend #310904  
 CCR7-APC, G043H7, 1:50, BioLegend #353214  
 CD45RA-APC-H7, HI100, 1:50, BD Bioscience #560674  
 CD4-BUV395, SK3, 1:50, BD Bioscience #563552  
 Granzyme B-APC-R700, QA16A02, 1:50, BioLegend #372221  
 Granzyme K-BB515, GM26E7, 1:25, BioLegend #370507  
 Perforin-BV421, B-D48, 1:50, BioLegend #353307  
 CD3-PE-Cy7, SK7, BioLegend #344815  
 CD4-Perp-Cy5.5, OKT4, 1:50, eBioscience # 45-0048-42  
 CD8-PE/BYG584, RPA-T8, 1:50, eBioscience, # 12-0088-80  
 CD45-PE-Cy7, HI31, 1:100, BD Biosciences #557748  
 CD38-BB700, HIT2, 1:100, BioLegend #3303504  
 CD90-BYG584, eBio5E10, 1:100, eBioscience #12-0909-42  
 Streptavidin DyLight 594, 1:200, VectorLabs# SA-5594  
 CD11b-Biotin, M1/70, 1:25, BD Biosciences #553309  
 Ter119-Biotin, 1:25, TER-119, BD Biosciences #553672  
 CD3e-Biotin, 1:25, 145-2C11, BD Biosciences #553060  
 CD4 Biotin, 1:25, GK1.5, BD Biosciences #553045  
 CD8-Biotin, 1:25, 53-6.7, BD Biosciences #553029  
 B220-Biotin, 1:25, RA3-6B2, BD Biosciences #553085  
 c-Kit-PE-CF594, 2B8, 1:50, BD Biosciences #562417  
 Sca-1-BV421, D7, 1:100, BioLegend #108120  
 CD48-AF700, HM48-1, 1:100, BioLegend #103426  
 CD150-PE-Cy7, TC15-12F12.2, 1:100, BioLegend #115914  
 CD45.1-APC, A20, 1:100, Sony #1153570  
 CD45.2-PE-Cy7, 104, 1:50, BioLegend #109829  
 Streptavidin Pacific Orange, 1:200, ThermoFisher Scientific #S32365  
 7-AAD-BB700 (PerCP-Cy5.5), 1:100, BioLegend #420404

## Validation

CD45-APC (Bohrer et al., 2022, Cell Reports)  
 CD34-Alexa Fluor 700 (Kenswil et al. 2021. Cell Stem Cell)  
 CD33-PE (Melsen, Themeli et al. Bio-protocol, 2020)  
 CD117 (C-kit)-PE-Dazzle (Ogishi et al. 2023. J Exp Med.)  
 CD271-PE (Horkova et al. 2023. Nat Immunol.)  
 CD71-Alexa Fluor 700 (Weijlet et al., 2022, Cancers (Basel))  
 CD235a-PE-Cy7 (Lütge et al. 2023. Nat Immunol.)  
 CD31-APC-Cy7 (Wu et al. 2022. Nat Commun.)  
 CD144-BV421 (Massidda et al., 2023, STAR Protocols)  
 Annexin A1-APC (Lawrence et al. 2022. Sci Adv.)  
 zombie-BV510 (Lutz et al. 2022. Proc Natl Acad Sci U S A.)  
 CD3-PE-Cy5 (Lafôrets et al. 2023. iScience)  
 CD8-PE-Cy7 (Ritacco et al. 2023. iScience)

CD69-BYG584 (Wang et al. 2023. Sci Adv.)  
CD69 FITC (Taylor et al. 2023. Front Immunol.)  
CCR7-APC (Prockop et al. 2023. J Clin Invest.)  
CD45RA-APC-H7 (Chen et al., 2025 Nat. Commun.)  
CD4-BUV395 (Henrich et al., 2024, Cell Rep. Med.)  
Granzyme B-APC-R700 (Yang et al. 2023, Front Immunol.)  
Granzyme K-BB515 (Vrazo et al. 2015, Blood)  
Perforin-BV421 B(Babl et al. 2023, Front Oncol.)  
CD3-PE-Cy7 (Preglej et al. 2023, Front Immunol.)  
CD4-Perp-Cy5.5 (Agarwal, et al., Cell Rep, 2020.)  
CD8a-PE/BYG584 (Kaiser, et al., Nat Commun, 2024.)  
CD45-PE-Cy7 (Schuster et al., 2025, Nat. Commun.)  
CD38-BB700 (Duhalde Vega et al. 2022, Sci Adv.)  
CD90-BYG584 (Zhu et al., 2024, Cancer Immunol.Immunother.)  
Streptavidin DyLight 594 (Sanchez-Huerta et al., 2025, Cells)  
CD11b-Biotin (Pioli et al., 2025, iScience)  
Ter119-Biotin (Pioli et al., 2025, iScience)  
CD3e-Biotin (Pioli et al., 2025, iScience)  
CD4 Biotin (Borroto et al., 2022, Front. Immunol.)  
CD8-Biotin (Cui et al., 2025, J. Clin. Invest.)  
B220-Biotin (Tai et al., 2024, EMBO Mol. Med.)  
c-Kit-PE-CF594 (Schwarz et al., 2023, FASEB)  
Sca-1-BV421 (Cheong et al. 2023. Nat Commun.)  
CD48-AF700 (Rundberg et al. 2023. iScience.)  
CD150-PE-Cy7 (Le et al. 2023. iScience)  
CD45.1-APC (Li et al. 2010. J. Immunol.)  
CD45.2-PE-Cy7 (Cheng et al. 2023. Nat Immunol.)  
Streptavidin Pacific Orange (Wang et al. 2009. J Immunol Methods)  
7-AAD-BB700 (PerCP-Cy5.5) (Siatra et al. 2023. NPJ Regen Med.)  
All the antibodies used in this study were optimized and validated by suppliers as above and used according to supplied instructions. Most antibodies were re-evaluated and appropriate dilutions were determined by titration.

Eukaryotic cell lines

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

|                                                                   |                                                                                                                                                                                                                                                         |
|-------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Cell line source(s)                                               | OP-9 cells(ATCC Number:CRL-2749,Lot:63544739); HS-5 cells(CRL-3611) used in our ex-vivo experiments were purchased from ATCC                                                                                                                            |
| Authentication                                                    | Not applicable                                                                                                                                                                                                                                          |
| Mycoplasma contamination                                          | Mycoplasma contamination testing is performed regularly on all cell lines used in this study. All tests have consistently negative results, confirming that the cell cultures were free of Mycoplasma contamination throughout the experimental period. |
| Commonly misidentified lines (See <a href="#">ICLAC</a> register) | No commonly misidentified cell lines were used.                                                                                                                                                                                                         |

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      | Donors: C57BL/6J (CD45.2),recipients: trains B6.SJL (CD45.1) were used for transplantations, both were purchased from Charles River Laboratories.<br>Mice were male or female and 3 months old at the time of experiment.<br>Animals were maintained in specific pathogen-free conditions in the Erasmus Animal Facility (EDC). Mice were housed in groups of maximum five animals (100cm2 per animal) under a standard 12 h light/dark cycle, with access to food and water ad libitum and an ambient temperature of 21-23 degrees Celsius with a humidity maintained between 40-70%. All mice were euthanized by cervical dislocation. |
| Wild animals            | No wild animals were used in the study.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
| Reporting on sex        | Sex as a biological variable was not a focus of this study. Both male and female mice were included without intentional bias or selection. Sex was not considered in the experimental design, and data were not disaggregated by sex. No sex-specific effects were hypothesized or observed during the study. Therefore, sex-based analyses were not performed.                                                                                                                                                                                                                                                                          |
| Field-collected samples | No Field-collected samples were used in the study.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
| Ethics oversight        | All work was performed in accordance with Dutch legislation and following institutional ethical approval by Animal Welfare/Ethics Committee of the EDC (IvD and DEC Rotterdam).                                                                                                                                                                                                                                                                                                                                                                                                                                                          |

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

## Clinical data

Policy information about [clinical studies](#)

All manuscripts should comply with the ICMJE [guidelines for publication of clinical research](#) and a completed [CONSORT checklist](#) must be included with all submissions.

|                             |                                                                                                                                                                                                                                                       |
|-----------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Clinical trial registration | www.trialregister.nl; NTR1825 (former ID); NL1715 (recent ID); EudraCT 2008-002195-10                                                                                                                                                                 |
| Study protocol              | van de Loosdrecht, A. A. ; Cremers, E. M.P. ; Alhan, C. et al. / Determinants of lenalidomide response with or without erythropoiesis stimulating agents in myelodysplastic syndromes : the HOVON89 trial. In: Leukemia. 2024 ; Vol. 38. pp. 840–850. |
| Data collection             | van de Loosdrecht, A. A. ; Cremers, E. M.P. ; Alhan, C. et al. / Determinants of lenalidomide response with or without erythropoiesis stimulating agents in myelodysplastic syndromes : the HOVON89 trial. In: Leukemia. 2024 ; Vol. 38. pp. 840–850. |
| Outcomes                    | van de Loosdrecht, A. A. ; Cremers, E. M.P. ; Alhan, C. et al. / Determinants of lenalidomide response with or without erythropoiesis stimulating agents in myelodysplastic syndromes : the HOVON89 trial. In: Leukemia. 2024 ; Vol. 38. pp. 840–850. |

## 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        | <p>mouse sample:</p> <p>Bone marrow: Bones were crushed in 3x2x1mL of FACS buffer (PBS+0.5%FCS) with sterilized mortar and pestle. BM cells were pelleted by centrifuging, and BM supernatant was transferred to a clean Eppendorf tube and snap frozen in liquid nitrogen. BM cell suspension was passed through a 40µM filter. RBCs in BM were lysed in 2mL ACK lysing buffer (Lonza) for 4 min on ice, and washed in 20mL FACS buffer</p> <p>Peripheral blood: PB was collected by cheek puncture monthly and at the end of follow-up periods. Complete blood count was measured on Scil Vet ABC Plus hematology analyzer (Scil Animal Care). Samples were stained with stated antibodies and lysed with IO test buffer conform manufacturer's protocol.</p> <p>human sample:</p> <p>Bone marrow: Viable cryopreserved BM aspirates (mononuclear cell fractions) were thawed in a water bath at 37°C and washed with warm Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% FCS. CD34+ fractions were enriched by the CD34 MicroBead Kit (Miltenyi) and LS MACS separation columns (Miltenyi Biotec) to achieve optimal purity. The CD34-negative fraction after enrichment was collected for T-cell staining.</p> <p>Peripheral blood: PB was collected, mononuclear cells (PBMCs) were isolated using Ficoll density gradient centrifugation. PBMCs were enriched for CD3+ cells with CD3 MicroBeads-based positive selection and column purification, followed by staining with the indicated antibodies.</p> |
| Instrument                | performed on BD FACSAria III flow cytometer (BD Biosciences).                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
| Software                  | FlowJo_v10.6.1 program                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
| Cell population abundance | Reanalyzing the sorted population, 100% purity.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
| Gating strategy           | FSC-A vs SSC-A were used to exclude debris, followed by single cells (SSC-H/W vs SSC-A, FSC-H/W vs FSC-A), viability dye                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |

#### Gating strategy

negative cells, further gating varied depending on tissue and is show in manuscript and supplementary information. All gating was controlled with single staining controls.

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