

Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our [Editorial Policies](#) and the [Editorial Policy Checklist](#).

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

- n/a

Confirmed
- ☐

☒

The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
- ☐

☒

A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- ☐

☒

The statistical test(s) used AND whether they are one- or two-sided
Only common tests should be described solely by name; describe more complex techniques in the Methods section.
- ☐

☒

A description of all covariates tested
- ☐

☒

A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- ☐

☒

A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- ☐

☒

For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted
Give P values as exact values whenever suitable.
- ☒

☐

For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- ☒

☐

For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- ☒

☐

Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

Data collection

NA

Data analysis

All plastic tubes used for nuclei isolation were precoated with 1 % BSA in PBS overnight. Nuclei were isolated from approximately 100 mg of fresh-frozen adult liver tissue. Liver tissue was minced, and then immediately transferred into a homogenizer containing 1 ml of ice-cold homogenization buffer (320 mM Sucrose, 5 mM CaCl2, 3 mM Magnesium Acetate, 10 mM Tris, 0.1 mM EDTA, 0.1% IGEPAL CA-630 (Cat# I8896, Merck), 0.1 mM PMSF (Cat# 10837091001, Roche), 1 μM flavopiridol, 1 mM β-mercaptoethanol and 0.2 U/μl RNase inhibitor in Ultrapure H2O). Tissues were homogenized using a glass douncer with five strokes of pestle A and ten strokes of pestle B. The homogenate was passed through a 70 μm cell strainer into a 50 ml falcon tube, and the strainer was washed with 1.65 ml homogenization buffer to achieve a final volume of 2.65 ml. Subsequently, 2.65 ml of gradient medium (50% Optiprep (Cat# D1556, Merck) with 5 mM CaCl2, 3 mM Magnesium Acetate, 10 mM Tris, 0.1 mM PMSF and 1 mM β-mercaptoethanol in UltraPure H2O) was added to the sample to reach a total volume of 5.3 ml. A 4 ml layer of 29% cushion (29% Optiprep with 150 mM KCl, 30 mM MgCl2, 60 mM Tris and 250 mM Sucrose in UltraPure H2O) was prepared in ultracentrifuge tubes. The homogenized sample was carefully layered on top of the cushion using a low-retention P1000 pipette to avoid disturbing the interface. Centrifugation was performed using an JS13.1 rotor in an Avanti high-speed centrifuge (Beckman Coulter) at 10,000 xg, with low acceleration and maximum deceleration at 4°C for 30 minutes. Following centrifugation, supernatants were gently removed, the nuclei were resuspended in 400 μL of resuspension buffer and passed through a 0.35 μm filter. Nuclei were stained with 1 μg/ml DAPI, and mononuclei were sorted using Aria III cell sorter (BD Biosciences) into 200 μl of 10X resuspension buffer (1% BSA with 0.2 U/μl RNase inhibitor and 1 μM flavopiridol in PBS). After sorting, the volume of each sample was diluted accordingly to obtain 1x resuspension buffer. Next, the nuclei were centrifuged at 400 xg for 3 minutes, followed by 600 xg for 5 minutes at 4 °C. The pellet was resuspended in 100 μl of resuspension buffer for counting, and samples were subsequently diluted to 1100 nuclei/μl. 16,000 nuclei per sample were loaded and used for analysis with the Chromium Single Cell 3' Gene Expression v3.1 Dual Index Kit (Cat# PN-1000269, 10x Genomics) and Gel Beads in Emulsion (GEMs) using the Chromium Next GEM Chip G Single Cell Kit (Cat# PN-1000127, 10x Genomics). After

GEM-RT cleanup, pre-amplification of the sample was performed, producing material for cDNA amplification for gene expression library construction. During the reverse transcription (RT) reaction, cDNA tagging was achieved with 16-nucleotide barcodes and 10-nucleotide molecular identifiers followed by using 35 µl for further cDNA amplification. For snRNA-seq library preparation, 25 % of the total cDNA was used to generate gene expression (GEX) libraries. Libraries were prepared and sequenced according to the manufacturer's protocol using the Dual Index TT Set A (Cat# PN-1000215, 10x Genomics). Final library quality was assessed using a High Sensitivity D5000 assay on a Tape station 4200 (Agilent Technologies) and quantified using the Qubit high-sensitivity dsDNA assay (Invitrogen). Pooled and barcoded libraries were sequenced on an Illumina NextSeq 2000 system with P4 flow cells, achieving an average depth of 30,000 reads per nucleus.

Single-nuclei RNA sequencing data were processed using Cell Ranger (v7.1.0) for read alignment and gene quantification. Downstream analysis was performed using Seurat (v5.0.1) with default pre-processing parameters. Nuclei expressing at least 200 genes in a minimum of three nuclei were retained. Further filtering excluded nuclei with fewer than 600 detected features or more than 30% mitochondrial RNA content. Normalization and scaling followed Seurat's standard pipeline. UMAPs were generated using the first 20 PCs, with clustering performed at a resolution of 0.5. Cell clusters were annotated using a known marker gene panel. Doublet detection and removal were conducted using DoubletFinder (v2.0.4) with an assumed doublet rate of 25%. The optimal pK parameter (0.001) was determined using the paramSweep function. After doublet exclusion, single nuclei were re-embedded with the same UMAP parameters, and clustering was repeated at a resolution of 0.2. Mixed annotation clusters were removed before further analysis. For KC sub-clustering, the first 20 PCs were used with a clustering resolution of 0.3. Differential expression analyses and ORA were performed comparing MP and NP treatment to the Ctrl using a Wilcoxon test. For each cell type, genes with expression levels outside the 0.5th–99.5th percentile were excluded to remove outliers with extremely low or high expression values. Subsequently, for each sample, we retained only genes expressed in at least 1% of cells. The union of these filtered genes across samples was then used for differential expression analysis, which was tested using a two-sided Wilcoxon rank-sum test with Benjamini-Hochberg correction. The ORA utilized the clusterProfiler (v4.10.0) package, querying the Hallmark, Reactome, and Gene Ontology (GO)-Biological Process (BP) databases obtained using the msigdb package (v.7.5.1). To maintain biological relevance, GO BP terms were restricted to the first four hierarchical levels, excluding highly specific child terms (e.g., positive or negative regulation). Gene sets with fewer than 10 or more than 300 genes were omitted. DEGs were defined using adjusted p-value and log2FC thresholds: Hepatocytes: Adjusted $p \leq 0.05$, $\log_2FC \geq 0.75$ or ≤ -0.75 , all other cell types: Adjusted $p \leq 0.1$, $\log_2FC \geq 0.25$ or ≤ -0.25 . As hepatocytes exhibited greater transcriptional differences, they were analysed using more restrictive thresholds. Pathways were considered significant if they had a false discovery rate (FDR) $\leq 10\%$ and included at least three genes (one-sided Hypergeometric over-representation test with Benjamini-Hochberg correction).

To assess treatment-associated changes in lipid metabolism, hepatocytes were analyzed separately using curated mouse gene sets from MSigDB. Gene sets were retrieved using the msigdb R package (7.5.1) and selected based on their annotation to lipid-related processes. Specifically, gene sets were grouped into modules reflecting LPC/PC-related metabolism, LPE/PE-related metabolism, SM/sphingolipid/ceramide metabolism, and hepatic lipid input/output pathways, including lipid transport, lipoprotein metabolism, fatty acid metabolism, cholesterol and bile acid metabolism, and PPAR-associated lipid regulation. Curated gene sets were intersected with the genes detected in hepatocytes. Module scores were calculated using Seurat's AddModuleScore function using default parameters. To summarize treatment effects at the biological replicate level, module scores were averaged per mouse and condition. Treatment-associated changes were quantified as delta module scores, calculated as the mean sample-level module score in each treatment group minus the mean sample-level module score in the PBS control group. In addition, selected gene-level expression changes were visualized as bar plots showing expression fold changes relative to the PBS control condition.

To infer upstream transcriptional regulators associated with treatment-induced transcriptional changes, transcription factor activity was estimated using DoRothEA regulons (1.14.1) together with the VIPER algorithm (1.36.0). Hepatocytes and macrophages were analyzed separately. For each cell type, normalized expression values were pseudobulked by sample and treatment condition to obtain sample-level expression profiles. VIPER was then applied to the pseudobulk expression matrices using DoRothEA transcription factor regulons using default parameters, including all available regulators in each regulon. Treatment-associated regulatory changes were assessed by comparing inferred transcription factor activity scores between each treatment condition and PBS. Statistical differences between treatment and PBS were assessed using two-sided Wilcoxon rank-sum tests on sample-level VIPER activity scores, followed by Benjamini-Hochberg correction for multiple testing. Transcription factors were ranked by adjusted p-value.

Cell-cell communication was assessed using CellChat (v1.6.0) with default parameters. To assess ligand–receptor interactions under each MNP treatment relative to PBS, we first ran CellChat separately on hepatocytes and KCs and subsequently on all cell types combined. In CellChat analysis, genes were considered overexpressed if detected in at least 10% of cells, and a minimum of 10 cells per group was required for interaction inference. In addition, we investigated niche factors as described by Bonnardel et al., 2019, using the gene sets reported in that study. The previously published code used for the analysis reported in this study can be found in gitlab with the project code 85206146.

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 data included in the manuscript can be accessed as follows:

- Lipidomics: At Metabolomics Workbench, study ID ST003776.
- Liver single-nuclei (sn) RNA-sequencing: GSE291616 with token: yjkdeiiudnarfgp
- Histology: <https://doi.org/10.60507/FK2/M42B80>
- Proteomics: PRIDE PXD072158 with token: w28AUhXHO3il

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

In all experiments using mice, sample size calculations were carried out to obtain 0.05 significance level and 80% power according to ethical guidelines for the use of experimental animals by the local institutional animal care committee (Landesamt für Natur, Umwelt und Verbraucherschutz (LANUV), North Rhine-Westphalia).

Data exclusions

Single data points were excluded from analysis if they were identified as outliers according to the Grubbs Outlier test.

Replication

In each figure legend it is specified how many times each experiment was repeated.

Randomization

Mice were randomly assigned to treatment groups.

Blinding

Throughout experiments, mice received a unique ID that identified treatment conditions. Investigators were blinded to condition throughout data collection and analysis.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

Methods

- 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

- n/a Involved in the study
- ☒ ☐ ChIP-seq
 - ☐ ☒ Flow cytometry
 - ☒ ☐ MRI-based neuroimaging

Antibodies

Antibodies used

Antigen Conjugate Clone Cat # Vendor Working concentration (µg/mL)
CD11b PE-Cy7 M1/70 101216 Biolegend 0.25

CD11b BUV661 M1/70 612977 BD Biosciences 1
 CD11b BUV737 M1/70 612800 BD Biosciences 1
 CD11c BV605 N418 117334 Biolegend 1
 CD163 BV421 S15049I 155309 Biolegend 0.25
 CD169 PE-Dazzle594 3D6.112 142423 Biolegend 2
 CD19 APC-Cy7 6D5 115530 Biolegend 1
 CD19 Biotin 6D5 115503 Biolegend 1.25
 CD206 BV711 C068C2 141727 Biolegend 1
 CD31 FITC 390 102406 Biolegend 5
 CD31 BUV661 390 741505 BD Biosciences 1
 CD43 BUV496 S7 741067 BD Biosciences 0.3333
 CD45 BUV805 30-F11 568336 BD Biosciences 0.25
 CD64 FITC X54-5/7.0 139316 Biolegend 0.625
 CD64 PerCP-Cy5.5 X54-5/7.0 139308 Biolegend 4
 CD64 PE-Cy7 X54-5/7.0 139314 Biolegend 2
 CD64 PE-Dazzle594 X54-5/7.0 139320 Biolegend 0.5
 Clec2 APC 17D9 146106 Biolegend 0.25
 Clec2 FITC 17D9 MA5-28218 ThermoFisher Scientific 1
 CX3CR1 APC SA011F11 149008 Biolegend 1
 CX3CR1 BV711 SA011F11 149031 Biolegend 1
 F4/80 BV510 T45-2342 743280 BD Biosciences 2
 F4/80 BV421 BM8 123132 Biolegend 1
 F4/80 APC-Cy7 BM8 123118 Biolegend 0.5
 F4/80 BV711 T45-2342 565612 BD Biosciences 1
 Ki-67 APC SolA15 17-5698-82 Invitrogen 4
 Ly-6C APC-Cy7 HK1.4 128026 Biolegend 1
 Ly-6C BV510 HK1.4 128033 Biolegend 0.5
 Ly-6G PerCP-Cy5.5 1A8 127616 Biolegend 1
 Ly-6G Biotin 1A8 127604 Biolegend 1.25
 MARCO APC 579511 FAB2956A R&D Systems 2
 MerTK PE-Cy7 Mer 151522 Biolegend 0.25
 MHCII AF 700 M5/113.15.2 107622 Biolegend 2.5
 NKp46 Biotin 29A1.4 137616 Biolegend 2.5
 SiglecF BUV395 E50-2440 740280 BD Biosciences 2
 Streptavidin BV785 - 405249 Biolegend 0.5
 Tcr-β APC-Cy7 H57-597 109220 Biolegend 0.5
 Tcr-β Biotin H57-597 109204 Biolegend 1.25
 TER-119 APC/Fire™ 750 TER-119 116250 Biolegend 1
 Tim-4 AF647 RMT4-54 130008 Biolegend 1.25
 Tim-4 BUV-563 RMT4-54 749135 BD Biosciences 1
 Vsig4 PE-Cy7 NLA14 25-5752-82 ThermoFisher Scientific 0.5
 XCR1 APC-Cy7 ZET 148224 Biolegend 1
 XCR1 BV650 ZET 148220 Biolegend 1
 Iba1 - EPR16589 ab178847 Abcam 1.224
 CD31 - 2H8 MA3105 ThermoFisher Scientific 3.3333
 CD68 - FA-11 137001 Biolegend 1.6667
 GFAP - - PA5-16291 Invitrogen 2.1675
 Insulin - - IR00261-2 Agilent Concentration not supplied by manufacturer, used 1/100
 Phalloidin AF488 - A12379 Invitrogen 0.3U/ml
 Donkey α-rabbit AF488 - A21206 Invitrogen 2
 Donkey α-rabbit AF555 - A31572 Invitrogen 2
 Goat α-rabbit AF488 - A-11008 Invitrogen 2
 Goat α-rabbit AF647 - A21244 Invitrogen 2
 Goat α-rat AF647 - 405416 Biolegend 0.5
 Goat α-hamster DyLight™ 649 - 405505 Biolegend 0.5
 Goat α-guinea pig HRP - A18769 Invitrogen 4

Validation

All antibodies used in this study were validated by the manufacturer for the purpose listed in the manuscript. All antibodies used in this study were validated by the manufacturer for use in mouse tissue. Antibodies were titrated in house for optimal performance.

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

As wildtype strain, C57BL/6Jrcc was used. To generate the double fate-mapper Tnfrsf11aCre; Rosa26LSL-YFP (JAX stock #006148; Ms4a3FlpO; Rosa26FSF-tdTomato (JAX stock #032864), we bred Tnfrsf11aCre/+; Rosa26FSF-tdTomato/FSF-tdTomato animals with Ms4a3FlpO; Rosa26LSL-YFP/LSL-YFP animals. Clec4fDTR/+ mice were described by Scott et al., 2016. Ms4a3Cre/+ were described previously and were used to generate Clec4fDTR/+; Ms4a3Cre/+; Rosa26LSL-tdTomato/+ (Jax stock #007914) mice. Finally, Cxcr4CreERT/+ mice and Rosa26LSL-tdTomato LSL-tdTomato (JAX stock #007914) mice were used to generate Cxcr4CreERT2/+; Rosa26LSL-tdTomato/+ mice. All mice were originally of C57BL/6J background and crossed to C57BL/6Jrcc for 2-5 generations. All lines were back-crossed to their respective wildtype line (C57BL/6Jrcc) once per year. Mice were genotyped according to protocols provided by JAX or donating researchers. The animals are housed in individually ventilated cages (IVC) with a floor area of 501 cm². A maximum of 5 animals ≥ 30g are kept per cage. The cages are equipped with bedding and nesting material. A pelleted complete feed

suitable for breeding and husbandry, as well as autoclaved water, are available to the mice ad libitum. Within the cages, a temperature of 21 °C and a relative humidity of 55 ± 15% are maintained via central ventilation, with a 70-fold air exchange rate.

Wild animals

No wild animals were used in this study.

Reporting on sex

Only male mice were used throughout this study, with exception for experiments with Cxcr4-tdTomato mice for fate-mapping. The rationale for choosing young male mice was because they develop diet-induced hepatic steatosis and metabolic dysfunction more rapidly than females. Whether females show comparable size-dependent susceptibility to MNP-induced Kupffer cell and metabolic dysfunction remains to be determined.

Field-collected samples

NA

Ethics oversight

All investigations concerning mouse work conform to the guidelines from Directive 2010/63/EU of the European Parliament on protecting animals used for scientific purposes. In detail, all animal experiments were conducted according to the German law of animal protection and in agreement with the approval of the local institutional animal care committee (Landesamt für Natur, Umwelt und Verbraucherschutz (LANUV), North Rhine-Westphalia, Az 81-02.04.2019.A167 and Az 81-02.04.2023.A255).

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

Mice were anaesthetized and perfused with ice-cold PBS. For flow cytometry analysis, 200 mg of liver and 20mg of spleen were collected. To obtain single-cell suspensions from the cortex and the hippocampus, the brain was cut sagittally into two halves. The cerebellum, brainstem and midbrain were removed leaving only the cortices and the hippocampi. Carefully, hippocampi were dissected using a spatula or an angled tweezer. Brains were dissected in a cold plate and maintained at 4°C. For the liver, brain regions and spleen, the tissue was finely minced and incubated in a digestion mix (PBS containing 1mg/mL Collagenase D (Cat# 1108858001, Roche), 100U/mL DNase I (Cat# DN25, Sigma-Aldrich), 2.4mg/mL of Dispase (Cat# 17105041, Gibco) and 3% fetal calf serum (FCS) (PAN Biotech)) for 30 min at 37 °C, followed by mechanical disruption using a 100 µm cell strainer and washing with FACS buffer (0.5 % BSA, 2 mM EDTA in PBS). For the liver, the cell suspension was first centrifuged at 50 xg for 3 minutes to remove hepatocytes, then supernatants were centrifuged at 400xg for 7 min, 4 °C. Liver and spleen pellets were next resuspended in red blood cell (RBC) lysis buffer (0.1 mM EDTA, 12 mM NaHCO₃, 155 mM NH₄Cl in Aqua Bidest) for 5 minutes on ice, then washed abundantly with FACS buffer before centrifugation. Blocking and surface marker staining were carried out as detailed below.

Bone marrow was isolated from femur and tibia. Following dislocation of the bones and removal of the epiphyses, bone marrow was flushed directly into a 70 µm cell strainer using RPMI. The resulting cell suspension was collected, and the volume was adjusted to 10 mL with RPMI. Cells were centrifuged and the cell pellet was resuspended in RBC lysis buffer and incubated for 5 min on ice. Lysis was stopped through addition of FACS buffer followed by centrifugation. Blocking and surface marker staining were carried out as detailed below.

For analysis of blood, 200 µl were incubated with RBC lysis buffer for 5 minutes on ice, washed with FACS buffer, centrifuged at 400g for 4 °C for 5 min. The RBC lysis was repeated once more before blocking. For the adipose tissue (gWAT, BAT), 200–300 mg of tissue were minced and incubated in a digestion mix containing PBS, 2 mg/ml type-II collagenase (Sigma-Aldrich, C6885-1g), 2.5 mM CaCl₂, and 0.5% BSA for 25 minutes at 200 rpm and 37°C, followed by mechanical disruption using a 100 µm cell strainer and washing with PBS containing 0.5% BSA. Cells were centrifuged and cell pellets were further processed for blocking and surface marker staining as detailed below.

For flow cytometry analysis of intestinal tissue, 1cm of jejunum without Peyer's patches was dissected, opened longitudinally

and washed to remove feces. Mesenteric lymph nodes were harvested and cleaned from surrounding mesentery, and Peyer's patches were dissected from the remaining jejunal tissue. Prior to digestion, epithelial cells were first removed from the intestinal tissue by incubating in HBSS without MgCl₂ (PAN Biotech) containing 1% FCS (PAN Biotech), 100 µg/mL penicillin/ streptomycin (PAN Biotech), 1 mM EDTA (Sigma), and 1 mM DTT (Sigma) for 20 minutes at 37°C with gentle shaking (220rpm). Next, tissue was washed and incubated in HBSS without MgCl₂ (PAN Biotech) containing 1% FCS (PAN Biotech), 100 µg/mL penicillin/streptomycin (PAN Biotech), 1 mM EDTA (Sigma) for 10 minutes at 37°C with gentle shaking. Next, the intestinal tissue removed of epithelial cells, dissected Peyer's patches and mesenteric lymph nodes were finely minced and incubated in a digestion mix (PBS containing 1 mg/mL Collagenase D (Cat# 11088858001, Roche), 100U/mL DNase I (Cat# DN25, Sigma-Aldrich), 2.4 mg/mL of Dispase (Cat# 17105041, Gibco) and 3% fetal calf serum (FCS) (Invitrogen)) for 30 min at 37 °C, followed by mechanical disruption using a 100 µm cell strainer and washing with FACS buffer (0.5 % BSA, 2 mM EDTA in PBS).

All cell pellets were blocked with 1% anti-CD16/32 and 2% rat serum for 15 minutes at 4°C. Cell surface marker staining was performed for 30 minutes at 4°C. For biotinylated antibodies, surface marker staining was carried out in two sequential steps. The complete list of antibodies used can be found in Table 5. All data were analysed using FlowJo (v10.8.1).

Instrument

FACSymphony A5 (BD Biosciences)
LSRII (BD Biosciences)
ID7000 7-laser Spectral Cell Analyzer (Sony Biotechnology)
ID7000 6-laser Spectral Cell Analyzer (Sony Biotechnology)

Software

FlowJo (v10.8.1)

Cell population abundance

Cell population abundances are described for each experiment throughout the manuscript.

Gating strategy

All gating strategies are described in the relevant figure, figure legend and supplementary data. Throughout all experiments, FMOs (fluorescence minus one) were used to determine the delimitation between negative and positive populations.

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