nature portfolio

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Reporting Summary

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

Statistics

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

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒	A description of all covariates tested
☐	☒	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	For FLow cytometry, cells were analyzed using a Fortessa X-20 analyzer (BD Biosciences) and FlowJo software v10 (FlowJo/BD, Ashland, OR, USA). For Immunofluorescence, slides were examined and images were recorded under a confocal microscope Zeiss 710 equipped with an EC Plan-Neofluar 40x/1.30 Oil DIC M27. For gene expression analysis, total RNA from mECs was isolated, reversly transcribed to cDNA using the Thermocycler 2027 (Applied Biosystems) and rt-qPCR carried out using specific primers in a QuantStudioTM 7 Flex Real-Time PCR System (Applied Biosystems). For scRNA-seq: After obtaining a clean single-cell suspension, samples were processed on the Chromium platform (10×) using the Chromium Single Cell 3' Library & Gel Bead Kit v3 kit (10×). After quality controls and quantification on TapeStation instrument (Agilent), libraries were sequenced on NextSeq500 platforms (Illumina) generating around 100,000 reads/cell. Raw sequencing data was demultiplexed with the mkfastq application (Cell Ranger v.3.0.2). Reads were aligned to the reference genome and assigned to genes with cellranger count (Cell Ranger v.3.0.2, genome: refdata-gex-mm10-2020-A), with the expect-cells option set to 10,000.
Data analysis	Immunofluorescence image processing and analysis was carried out using ImageJ/Fiji (versions 2.3.5-2.9.0). A minimum of 3 animals per group was used and at least 3 different fields per animal acquired. Counts from all fields corresponding to one animal were averaged. For quantification of cell numbers per field, individual cells were manually counted using the appropriate channels in combination with DAPI to identify nuclei on ImageJ/Fiji. Flow cytometric data was analyzed using FlowJo software version 10 (BD). Gene expression results were normalized to GAPDH expression using the 2ΔCT (ΔCT = CTreferenceRNA − CTtarget), and values expressed as fold change over the treated group (TGFb). Statistical analyses were performed using GraphPad Prism v8. scRNA-seq data analysis: Seurat 4.3.0.1 was used to analyse scRNA-seq data. All samples were filtered for quality control parameters (cells

with more than 8% reads mapping to mitochondrial genes, feature counts greater than 7000 and smaller 350 and cells with less than 1000 transcripts were filtered out) and DoubletFinder function performed. Applying these filters eliminated dying cells and doublets. Data was then normalized, scaled and dimensionality reduction performed through Principal Component Analysis (PCA) following Seurat's tutorial as evaluated by elbow plots. Cells from all samples were combined and batch correction executed using Harmony (version 1.1), and unsupervised clustering performed. UMAP embedding parameters were based on the top 75 PCs and 0.9 dimensions for visualization. Each cluster was labelled based on canonical skeletal muscle gene expression. Differential expression analysis between conditions was conducted using DESeq2 (version 1.44.0). For each comparison, log2 (fold change) results were shrunken using the apeglm package (version 1.26.1) to remove noise (differentially expressed genes with low counts and/or high dispersion values) while preserving significant differences.
Gene Set Enrichment analysis (GSEA) was conducted using differentially expressed genes (DEG) evaluated by the DEseq2 package. All genes were ranked according to the log2FoldChange and GSEA analysis carried out using R package ClusterProfiler (version 4.12.6) querying the Gene Ontology (GO) and KEGG database. The analysis was performed by setting the minimum gene set to 10 and maximum to 800. GO and KEGG terms were considered significant with a selected cutoff p-value of 0.05.

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 single-cell RNA sequencing data generated in this study has been deposited in the GEO database under accession code GSE291782. Data will be made publicly available upon publication. Any additional information required to reanalyze the data reported in this study is available from the lead contact upon reasonable request. Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Silvia Brunelli (silvia.brunelli@unimib.it)

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	N/A
Reporting on race, ethnicity, or other socially relevant groupings	N/A
Population characteristics	N/A
Recruitment	N/A
Ethics oversight	N/A

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 pre-determine sample size, but sample sizes are consistent with our previous studies and other studies in the field. Single cell RNA sequencing was performed as three biological replicates, more than what is commonly done within the field.
Data exclusions	No data was excluded
Replication	Numbers of biological replicates and independent experiments are indicated in figure legends.
Randomization	No specific randomization method was used.

Blinding	No specific methods were used for blinding.

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	RFP Rabbit 600-401-379 Rockland RFP Chicken 600-901-379 Rockland CD31 Goat α-mouse AF3628 R&D Systems F4/80 Rat α-mouse MCA497 Bio-Rad PE-conjugated rat anti-CD45 BD Biosciences PE-Cy7-conjugated rat anti-CD11b BD Biosciences Col1a1 Rabbit α-mouse PA529569 Invitrogen SPP1 Rabbit α-mouse PA5-141129 Invitrogen Alexa Fluor 568 Donkey α-rat A78946 Invitrogen Alexa Fluor 488 Donkey α-chicken A78948 Invitrogen Alexa Fluor 488 Donkey α-rabbit A32790 Invitrogen Alexa Fluor 555 Donkey α-rabbit A32794 Invitrogen Alexa Fluor 647 Donkey α-goat A32849 Invitrogen Alexa Fluor 555 Donkey α-goat A32816 Invitrogen
Validation	All antibodies are commercially validated and each lot has been tested for performance. Representative flow cytometry dot plots or images are included in commercial data sheets to demonstrate specificity and/or sensitivity. Example of validation statements : BD Biosciences: The specificity is confirmed by using multiple applications that may include a combination of flow cytometry, immunofluorescence, immunohistochemistry or western blot to test a combination of primary cells, cell lines or transfectant models

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	Mouse endothelial cells (mECs) previously isolated from skeletal muscle Ieronimakis N, Balasundaram G, Reyes M. Direct isolation, culture and transplant of mouse skeletal muscle derived endothelial cells with angiogenic potential. PLoS One. 2008;3(3):e0001753
Authentication	No new cell lines were generated in this study. No lines were authenticated.
Mycoplasma contamination	mECs tested negative for mycoplasma contamination during routine checks.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified lines in the ICLAC registry were used for the present 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	The transgenic mouse lines used in the present study were the following (indicated are name of the mouse line, source and RRID and/or reference). Cdh5-CreERT2 - Gift from R.Adams (Wang et al., 2010)

R26tdTomato - The Jackson Laboratory - RRID:IMSR_JAX:007909

All transgenic mouse lines were maintained on a CD45.2 C57BL/6 genetic background. Homozygous transgenic mice expressing a tamoxifen (TAM)-inducible form of Cre recombinase (CRE-ERT2) under the control of the endothelial-specific gene VE-Cadherin (Cdh5) were crossed with R26R-tdTomato homozygous reporter mice. Cre recombination was then induced in double transgenic mice at postnatal days 6-7-8 with three subcutaneous (SC) injections of 25 µl TAM at 10mg/ml (Sigma-Aldrich, St. Louis).
Mice were kept under controlled conditions (12h light/dark cycle and room temperature at 22 °C) and had free access to tap water and standard mice chow.

Wild animals	This study does not involve wild animals.
Reporting on sex	Sex information was not considered in the study design, and was not collected in mice analyzed in this study. No sex-based difference was observed in analysis of adult mice. Hence, findings of the present study apply to both sexes.
Field-collected samples	This study does not involve field collected samples.
Ethics oversight	All animal procedures were performed at San Raffaele Scientific Institute animal facilities in accordance with European Union guidelines and with the approval of the Institutional Ethical Committee and Ministry of Health (Authorization 244/2020-PR).

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

Plants

Seed stocks	N/A
Novel plant genotypes	N/A
Authentication	N/A

Flow Cytometry

Plots

Confirm that:

☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☒ All plots are contour plots with outliers or pseudocolor plots.

☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	Sample preparation is detailed in the Methods section. Briefly, samples were stained and analysed in CMF (Calcium/Magnesium-Free PBS, 10% FBS, 5% Pen/Strep, 2 mM EDTA), incubated for 15 min with 0.5 mg/mL Fc block (1:500) (BD Biosciences, Franklin Lakes, NJ, USA) and labelled with a combination of PE-conjugated rat anti-CD45 (1:400) (BD Biosciences), PE-Cy7-conjugated rat anti-CD11b (1:200) (BD Biosciences) and APC-conjugated rat anti-F4/80 (1:200) (BD Biosciences).
Instrument	Flow cytometry data acquisition was performed on a BD LSR Fortessa X-20
Software	Flow cytometry data was collected with BD FACSDiva software (version 8.0.2). Data was analyzed using FlowJo software version 10 (BD).
Cell population abundance	*Describe the abundance of the relevant cell populations within post-sort fractions, providing details on the purity of the samples and how it was determined.*
Gating strategy	Appropriate fluorescence gating parameters were established with compensation beads (BD Biosciences), unstained, and fluorescence-minus-one (FMO) staining. In all of the samples, doublets were gated out using pulse geometry gates (FSC-H versus FSC-A and SSC-H versus SSC-A), whereas dead cells were excluded based on Hoechst 33258 incorporation. A representative gating strategy is included in the supplementary Fig. S1B: Cells were gated on CD45+.

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