nature portfolio

Corresponding author(s): Joseph M. Miano
Qing Lyu

Last updated by author(s): Sep 26, 2022

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	The images of Figures 1a, 2a, and Extended Data Figures 4a, 5a, 5b (save liver, mammary gland, and ovary), 8d, and 9a were generated on an Olympus IX81 confocal microscope at the University of Rochester. The images of Figures 1b and 6d and Extended Data Figures 3b, 4b, 4c, 5b (liver, mammary gland and ovary), 6 and 10a were generated on a Zeiss LSM 900 at Augusta University. The images of Figure 3e and Extended Data Figure 7 were generated on a Yokogawa CSU-X Spinning Disc Confocal Microscope on an inverted Olympus IX81 platform at University of Missouri. Low magnification images of organs in Extended Data Figure 8c,d were captured with an Olympus dissecting microscope equipped with a CCD camera. Western blots were captured with a Bio-Rad ChemiDoc MP Imaging System qRT-PCR was done with a Bio-Rad CFX96 Real-Time System Genotyping of mice was done on Bio-Rad C1000 Touch Thermal Cyclers Flow cytometry (Extended Data Figure 5a) was performed on a Becton Dickinson Accuri C6 flow cytometer Aortic contractile studies (Figure 6a-c) were done with a DMT Wire Myography System Bulk RNA-seq (Figure 2e and Supplementary Table 2 and Figure 7) was conducted on an Illumina NovaSeq 6000 platform at the Genomics Core of the University of Rochester Long read sequencing was done using Oxford Nanopore's minION Mk1B or a GridION Mk1 systems The immunogold electron microscopy was done using JEOL 1400 Flash transmission electron microscope and image was captured by Gatan One View CCD camera The radiotelemetry recording of blood pressure was measured by DSI Model PA-C10
Data analysis	Images on the IX81 confocal microscope were generated as Z-stacks with Micro-manager 2.0 software. Similar Z-stacked images on the Zeiss

LSM 900 were obtained through ZEN 3.5 Blue software. The images in Figure 3e and Extended Data 7 were obtained from confocal microscopy image stacks using Fiji for Z-Project Function and the Fiji plugin was used for pairwise stitching to stitch three fields of view together to generate 2-D maximum projections for final presentation. For Figure 1biii, the image was processed similarly using Fiji software tools.

All confocal images were processed in Adobe Photoshop (21.1.2) and final figures assembled in Adobe Illustrator (23.1.47).

All graphical data and statistical analyses were done in GraphPad Prism (9.2.0).

Flow cytometry analysis was done with Flowjo software (10.4).

For Nanopore sequencing, fast5 to fastq read conversion was done using guppy (v4.2.2) on MinKNOW (v20.10.3) MinKNOW Core (v4.1.2) and fast base-calling option for the base-call model and minimum Q-score of 7 option for read filtering. See methods for more detailed information.

Following standard bulk RNA-seq analysis at University of Rochester post-sequence analyses include GO annotation with DAVID, transcription factor binding site over-representation with oPPOSUM 3.0, and dot plot analyses was done by QL using free on-line software at http://www.bioinformatics.com.cn/

The bioinformatic data of Fig. 2e was generated at the degPatterns website (https://rdrr.io/github/lpantano/DEGreport/man/degPatterns.html).

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

All raw data are available in the Source files and Bulk RNA-seq data are available through the NCBI Gene Expression Omnibus under Accession numbers GSE138824 (Figure 7) and GSE199244 (Figure 2e and Supplementary Table 2).

Long read informative reads for the mapping of Myh11-Cre (Figure 2d) are found under NCBI Sequence Read Archive BioProject number PRJNA82551.

An up-to-date listing of floxed alleles inactivated with SMC Cre drivers (Supplementary Table 1) is available upon request.

The Itga8-Cre mouse is available, for a modest maintenance fee, upon request to either JMM or LG

Human research participants

Policy information about studies involving human research participants and Sex and Gender in Research.

Reporting on sex and gender	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 sample size calculations were made in this publication. Confocal imaging of the Itga8-Cre and Myh11-Cre occurred over the span of 4.5 years and at two different institutions. The total number of mice analyzed for each strain was in excess of 12 mice per strain with at least 2 samples of every tissue shown in the manuscript for purposes of replication. For vascular tissues (eg, aorta, popliteal lymphatic etc), the sample size is indicated in the legends and during the course of these studies at least 4 independent mice were analyzed in the indicated sexes. Importantly, sex studies could only be conducted in the Itga8-Cre strain because the Myh11-Cre strain has Cre integrated on the Y chromosome. Sample sizes for physiology studies were 5-6 and are indicated in each legend of Figures 4-6. We deemed a minimum number of 5 mice needed for each of these physiology experiments based on historical standard deviations around the means.

Data exclusions	A biological heterozygous control was excluded from analysis in the RNA-seq of Figure 7 because it represented an outlier. The full dataset however is available at the Gene Expression Omnibus of NCBI under GSE138824 (see https://www.ncbi.nlm.nih.gov/).
Replication	All figures (and/or legends) include the number of biological replicates or independent experiments (see also Sample size above).
Randomization	Randomly-selected, age-matched mice were used throughout and where possible, sex studies were conducted. No covariates are indicated in these studies given the carefully controlled nature of the mouse experiments (equal ages, same environmental conditions, etc).
Blinding	OJS was blinded to all mouse genotypes for confocal imaging studies. There was no bias in selecting images for final publication; however, as indicated in the manuscript, two images were uniformly enhanced in order to better capture GFP fluorescence that otherwise would not be indicated in final reproduction. The immunogold EM data were also analyzed in a blind fashion as were the bulk RNA-seq studies. Less subjective assays such as qRT-PCR and Western blotting were necessarily done with knowledge as to each sample.

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

Methods

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

Antibodies

Antibodies used	GFP (1:200), Thermo Fisher, #A-11122; ACTA2 (1:500), Sigma-Aldrich, #A5228; Donkey anti-rabbit AF488 (1:200), Thermo Fisher, #A-21206; Donkey anti-mouse AF647 (1:200), Thermo Fisher, #A-31571; Cre recombinase (1:200), Cell Signaling, #15036S; VCL ; GAPDH (1:5000), Millipore, #MAB374; TUBA1A (1:1000), Sigma-Aldrich, #T5168; HA-tag, Cell Signaling, #1222S; SRF (1:200 IHC, 1:1000 WB), Proteintech, #16821-1-Ap; ITGA8 (1:1000), Santa Cruz, #sc-365798; MYH11 (1:200), Alfa Aesar, #J64817(BT-562); SM-Cy3 Actin (1:200), Sigma-Aldrich, #C6198; Vinculin (1:1000) antibody [N3C1]; GeneTex, #GTX109749.
Validation	Antibodies were validated by the respective providers for the applications described in the manuscript. SRF and ITGA8 antibodies were further validated in the lab by loss-of-function and inclusion of positive/negative control tissues, respectively.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	MOVAS, ATCC #CRL-2797; HCASM, Thermo Fisher #C0175C; ATCC #CRL-1658; mESC 129S6, Sigma-Aldrich #SCR012
Authentication	All cell lines were purchased from commercial providers and authenticated by the expression of cell-restricted markers and general morphology.
Mycoplasma contamination	The cell providers guaranteed cell lines are free from Mycoplasma contamination. No further testing was performed here.
Commonly misidentified lines (See ICLAC register)	None

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	C57BL/6J mice, Jackson Labs (Cat #000664); mTomato/mGFP reporter mice, Jackson Labs (Cat #007676); Sm22-Cre mice, Miano Lab; Myh11-CreERT2, Jackson Labs (Cat #019079); Srf-floxed mice, Miano Lab; Itga8-CreERT2, Miano Lab, available with MTA and fee through Dr. Lin Gan; inducible Myocd mouse of strain C57BL/6J available with MTA from Miano Lab. All mice were maintained in micro-isolator cages containing water and pelleted food (Teklad Global Irradiated 18% Protein Rodent Diet 2918) ad libitum. Rooms

	were temperature and humidity controlled under a 12-hour light (600-1800 hours), 12-hour dark (1800-600 hours) cycle.
Wild animals	No wild animals were used in this study
Reporting on sex	Male and female C57BL/6J mice, male and female Itga8-Cre mice, male and female mT/mG mice, male and female Srf-floxed mice were used in this study; Male Myh11-Cre, and male Sm22-Cre mice were used in this study.
Field-collected samples	No field-collected samples were used in this study
Ethics oversight	All mouse studies were approved by local Institutional Animal Care and Use Committees at the University of Rochester during the period of 2016-2019 (approval #101587); Medical College of Georgia from 2019-present (approval number #2019-1000); and the University of Missouri (approval #27320). An Institutional Biosafety Committee of Augusta University approved the use of Tamoxifen for inducible activation of relevant Cre driver mice (approval #1942). The ethical treatment of mice was in accordance with ARRIVE guidelines.

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

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	Whole blood samples were collected from wild-type, mTmG, Myh11-CreERT2/mTmG, Sm22-Cre/mTmG, and Itga8-CreERT2/mTmG mice and centrifuged for 5 min at 2000x g at room temperature. Buffy coats were carefully transferred to a fresh 1.5ml microfuge tube. 500ul of 1x ACK Lysing Buffer (Thermo, A1049201) was added to lyse red blood cells. Samples were then centrifuged for 5 min at 3000x g at room temperature. Supernatants were carefully aspirated and the pellets were suspended in 1x PBS.
Instrument	Becton Dickinson (BD) Accuri C6
Software	BD Accuri C6, FlowJo (10.4)
Cell population abundance	Flow cytometry was used only for analysis, no FACS sorting.
Gating strategy	We inputted all ungated data for analysis and plot generation, since the goal of this experiment is to identify any potential GFP-positive signals (leakiness) in cells from the Buffy coat (mainly WBC and platelets) of Itga8-Cre mice. We used already-known myeloid leakiness Cre mice (Sm22-Cre) as positive control to show the GFP-positive cells, and used BL6 and mT/mG mice as negative control. See Extended Data Figure 16 and legend to Extended Data Figure 5a for more details.

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