Reporting Summary

Nature Research 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 Research policies, see Authors & Referees 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

☑ zUMIs (version 2.2.1); STAR (version 2.6.1a), samtools (version 1.9); featureCounts from Rsubread (version 1.32.4); R (version 3.5.1); edgeR, bowtie2, chromstaR

Data analysis

Prism GraphPad 8 Software (version: 8.4.2) was used to generate graphs and perform statistical analysis. FlowJo Software (version: 10.7.2) was used for flow cytometry data analysis. ImageJ Software (version: 2.0.0-rc-43/1.50e) was used for imaging data analysis. Wave controller Software from Agilent Technologies (version 2.2.0) was used for SeaHorse data analysis. Integrative Genomic Viewer Software (igV version 2.7.2) was used for gene track views. BD FACSDiva Software (version 8.0.1) was used for cell sorting analysis. Standard and published pipelines [see Data collection] and software were used for NGS data analysis. Packages were cited accordingly.

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/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research 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 list of figures that have associated raw data

- A description of any restrictions on data availability

NGS (RNA- and ATAC-seq as well as CUT&RUN-seq) data that support the findings of this study are available at gene expression omnibus, GEO143580; link: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE143580
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.

- [x] 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**: Sample size was determined based on previously published reports [Le et al., Nat. Cell Biol., 2016; Wu et al., Nature 2016; Mylonas and Tessarz, Life Sci. Alliance, 2018; Dawson et al., Cell Rep., 2012]. No statistical method was used to predetermine sample size.

- **Data exclusions**: The only pre-established exclusion criterion was for replicates that were found to be technically flawed or determined by statistical tests to contain a legitimate outlier data point. When any of the above occurred, the entire replicate was omitted and the whole experiment was repeated, when feasible. The number of final replicates used for each analysis is extensively described in the next section.

- **Replication**: Taking into consideration the above exclusion criterion, all experiments were successfully reproduced at least twice. Final number of biological replicates is clearly indicated in each figure legend.

- **Randomization**: Experimental groups were based on age and/or treatment

- **Blinding**: None of our experiments were blinded, since results were inherently objective and unbiased. Results were subjected to binary interpretation, based on statistical tests. Furthermore, analyses could not be blinded because experiments were performed and analyzed by the same researchers.

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         |
| [ ] | Animals and other organisms |
| [ ] | Human research participants |
| [x] | Clinical data         |

### Methods

| n/a | Involved in the study |
|-----|-----------------------|
| [x] | ChIP-seq              |
| [ ] | Flow cytometry        |
| [x] | MRI-based neuroimaging |

**Antibodies**

| Antibodies used |
|-----------------|
| CD140a-APC (eBioscience, 17140181; LOT 1996382; 1:1000) |
| Sca-1-FITC (eBioscience; 11598182; LOT 1923823; 1:100) |
| Terr-119-PE (eBioscience; 12592182; LOT 4313528; 1:100) |
| CD45-PE (Life Technologies; A16325; LOT 2051673; 1:100) |
| CD34-FITC(eBioscience; 11034181; LOT E00263-1631; 1:100) |
| CD31-APC(eBioscience; 17031180; LOT E071241632; 1:100) |
| CD140a-APC invitrogen; 17140181; LOT 2213095; 1:100) |
| CD29-PE(eBioscience; 120291; LOT E011816363; 1:100) |
| CD44-PE(eBioscience; 12044181; LOT F012381637; 1:100) |
| pan-acetylated-Lysine (Cell Signaling Technology; 94415; LOT 14; 1:400) |
| Fatty Acid Synthase (Cell Signaling Technology; 31895; LOT 2; 1:1000) |
| CBP (Cell Signaling Technology; 73895; LOT 3; 1:1000) |
| AceCS1 (Cell Signaling Technology; 36581; LOT 2; 1:1000) |
| Histone H3 (Cell Signaling Technology; 14269; LOT 6; 1:400) |
| Histone H3 K27ac (Active Motif, 39133; LOT 31416013; 1:300) |
| Histone H3 K2/me3 (Active Motif, 39155; LOT 31814017; 1:300) |
| ACC1 (ProteinTech; 21923-1-AP; LOT 00048856; 1:1000) |
| ALCY (ProteinTech; 15421-1-AP; LOT 00096573; 1:1000) |
| TOMM20 (Santa Cruz Biotechnology; sc-17764; LOT k0117; 1:100) |
| TOMM20 (SigmaAldrich; WH0009804M1; LOT L4221-4F3; 1:200) |
Valiation

CD140a-APC species reactivity: mouse; tested applications: flow cytometry; product citations: 56 https://www.thermofisher.com/antibody/product/CD140a-PDGFRA-Antibody-APC-Monoclonal/17-1401-81

Sca-1- FITC species reactivity: mouse; tested applications: flow cytometry; IF, IHC, WB; product citations: 34; https://www.thermofisher.com/antibody/product/ly-6a-E-Sca-1-Antibody-c1n0-1-Monoclonal/71-5981-82

Treg-119-PE species reactivity: mouse; tested applications: flow cytometry; product citations: 17; https://www.thermofisher.com/antibody/product/TER-119-Antibody-clone-TER-119-Monoclonal/12-5921-82

pan-acetylated Lysine species reactivity: all (including mouse); tested applications: WB, IP, IHC, IF, ChIP, EISA; product citations: 16; https://en.cellsignal.de/products/primary-antibodies/acylated-lysine-antibody/9441

Fatty Acid Synthase species reactivity: human, mouse; tested applications: WB; product citations: 29; https://www.cellsignal.com/products/primary-antibodies/fatty-acid-synthase-antibody3189

CBP species reactivity: human, mouse, rat, monkey; tested applications: WB, IP, IF, ChIP, ChIP-seq; product citations: 28; https://www.thermofisher.com/antibody/product/cbp-065-rabbit-mab/7389

AceCS1 species reactivity: human, mouse, rat, monkey; tested applications: WB, IP; product citations: 18; https://en.cellsignal.de/products/primary-antibodies/acecs1-d19c6-rabbit-mab/3658

Histone H3 species reactivity: human, mouse, rat, monkey; tested applications: WB, IHC, IF, FC, ChIP; product citations: 17; https://en.cellsignal.de/products/primary-antibodies/histone-h3-1b1b2-mouse-mab/14269

ACC1 species reactivity: human, rat, pig; tested applications: WB, IP, IHC, IF, ELISA; product citations: 31; https://www.ptglab.com/products/ACACA-Antibody-21923-1-AP.html#datasheet

ACLY species reactivity: human, mouse, rat; tested applications: WB, IP, IHC, IF, FC, ELISA; product citations: 19; https://www.thermofisher.com/antibody/product/ACLY-Antibody-15471-1-AP.html#datasheet

TOMM20 species reactivity: mouse, rat, human; tested applications: WB, IP, IF, IHC(PI), EISA; product citations: 212; https://www.scbt.com/p/tom20-antibody-10-10 and species reactivity for TOMM20 antibody from Sigma-Aldrich: mouse, rat, human; tested applications: IHC, ELISA, IF, WB; https://www.sigmaaldrich.com/catalog/product/sigma/wh0009840m1?

b-actin species reactivity: mouse, rat, human, avian, bovine, canine, rabbit; tested applications: WB, IHC (P), ELISA; product citations: 7415; https://www.scbt.com/p/beta-actin-antibody-c4

pan-acetylated Histone H3 species reactivity: tested in human and predicted in a wide range of organisms including mouse; tested applications: ChIP, ChIP-seq, DB, WB; product citations: 43; https://www.activemotif.com/catalog/details/39139/histone-h3ac-pan-acetyl-antibody-pab-1

pan-acetylated Histone H4 species reactivity: human and eukaryotes (including mouse); tested applications: WB, ICC ChIP; https://www.merckmillepore.com/DE/de/product/Anti-acetyl-Histone-H4-Antibody,MM_NF-06-866?referrerURL=https%3A%2F%2Fwww.google.com%2F#loveview

Histone H4 species reactivity: Cow, Human; predicted to work with: mouse and rat; tested applications: Flow Cytometry, IHC-P, IF, WB, ChIP, ICC(PI), product citations: 26; https://www.abcam.com/histone-h4-antibody-mabcam-31830-chip-grade-ab31830.html

Citrate Carrier species reactivity: human; predicted to work with: Mouse, Rat, Rabbit, Horse, Chicken, Guinea Pig, Cow, Cat, Gog, S. cerevisiae, D. melanogaster, Zebrafish; tested applications: WB; product citations: 1; https://www.abcam.com/5c25a1-antibody-ab99168.html

Histone H3K27me3 published applications: ChIP, ChIP-Seq, Native ChIP (PMID: 28973448), WB, IF, ELISA, RNA Fish; Reactivity: Human, Mouse, Wide Range Predicted; citations: 203; https://www.activemotif.com/catalog/details/39155/histone-h3-trimethyl-lys27-antibody-pab

Histone H3K27ac published applications: ChIP, ChIP-Seq, Native ChIP (PMIDs: 24013188 & 28245924); WB, IF, IHC(FFPE); Proximity Ligation Assay (PLA); Reactivity: Budding Yeast, Human, Wide Range Predicted; citations: 23; https://www.activemotif.com/catalog/details/39133

LAMP2 species reactivity: Mouse; Recommended Applications: FACS, Immunofluorescence, Immunohistochemistry, Immuneoprecipitation, Western Blot; citations: 22; https://dshb.biology.uiowa.edu/A8L93

PDH1 species reactivity: Mouse, Rat, Human; tested applications: Flow Cytometry (Flow), Immunocytochemistry (ICC/F); citations: 173; https://www.thermofisher.com/antibody/product/CD44-Antibody-clone-IM7-Monoclonal/12-0441-82

CD29-PE species reactivity: mouse, rat; tested applications: Flow Cytometry, ICC/F; citations: 55; https://www.thermofisher.com/antibody/product/CD29-integrin-beta-1-Antibody-clone-eBioHMb1-1-HMb1-1-Monoclonal/12-0291-82

CD44-PE species reactivity: mouse, human; tested applications: Flow Cytometry (Flow), Immunocytochemistry (ICC/F); citations: 173; https://www.thermofisher.com/antibody/product/CD44-Antibody-clone-IM7-Monoclonal/12-0441-82

CD29-PE species reactivity: mouse, rat; tested applications: Flow Cytometry, ICC/F; citations: 55; https://www.thermofisher.com/antibody/product/CD29-integrin-beta-1-Antibody-clone-eBioHMb1-1-HMb1-1-Monoclonal/12-0291-82
Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)  HEK293T were used to generate lentivirus.

Authentication  HEK293T were purchased freshly from ATCC with appropriate authentication.

Mycoplasma contamination  Cell lines are routinely checked for mycoplasma and cells used in this study tested negative.

Commonly misidentified lines  HEK293T have been found to be contaminated with HeLa (Nelson-Rees et al., 1981) - however, cells were freshly purchased from ATCC.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals  C57Bl/6J wild type male mice were used in this study. Mice were 3-4 months old (young cohort) and 18-22 months old (old cohort). Information about the housing conditions of the mice is provided in the respective section of the manuscript.

Wild animals  The study did not involve wild animals.

Field-collected samples  The study did not involve field-collected samples.

Ethics oversight  Landesamt für Natur, Umwelt und Verbraucherschutz Nordrhein-Westfalen (LANUV NRW) approved the protocol used in this paper.

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

Human research participants

Policy information about studies involving human research participants

Population characteristics  all relevant information can be found in Corrigan et al., Cells Tissues Organs 207, 83-96 (2019)

Recruitment  all relevant information can be found in Corrigan et al., Cells Tissues Organs 207, 83-96 (2019)

Ethics oversight  all relevant information can be found in Corrigan et al., Cells Tissues Organs 207, 83-96 (2019)

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

Data deposition

☒ Confirm that both raw and final processed data have been deposited in a public database such as GEO.

☒ Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links

May remain private before publication.

NGS [RNA- and ATAC-seq as well as CUT&RUN] data that support the findings of this study are available at gene expression omnibus, GEO143580; link: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE143580; token: ydyju5qkjhorder

Files in database submission

GSM4711140 H3K27AC Young Sample 1
GSM4711141 H3K27AC Young Sample 2
GSM4711142 H3K27AC Young Sample 3
GSM4711143 H3K27AC Old Sample 1
GSM4711144 H3K27AC Old Sample 2
GSM4711145 H3K27AC Old Sample 3
GSM4711146 H3K27ME3 Young Sample 1
GSM4711147 H3K27ME3 Young Sample 2
GSM4711148 H3K27ME3 Young Sample 3
GSM4711149 H3K27ME3 Old Sample 1
GSM4711150 H3K27ME3 Old Sample 2
GSM4711151 H3K27ME3 Old Sample 3
GSM5266506 H3AC Young Sample 1
GSM5266507 H3AC Young Sample 2
GSM5266508 H3AC Old Sample 1
GSM5266509 H3AC Old Sample 2
GSM5266510 H3AC Old Acetate Sample 1
GSM5266511 H3AC Old Acetate Sample 2

Genome browser session

(bigwig files to upload in IGV are available at: https://figshare.com/s/aeafa250723a74204f96)

Methodology

Replicates

Three replicates per antibody per condition for H3K27ac and H3k27me3 and two replicates for H3ac per condition.

Sequencing depth

Seq depth was between 10-15 million reads per sample [CUT&RUN]

Antibodies

Histone H3 K27ac (Active Motif, 39133; LOT: 31416013)
Histone H3 K27me3 (Active Motif, 39155; LOT: 31814017)
Histone H3acetylation (Active Motif, 39139; LOT: 28S18008)

Peak calling parameters

Peaks were called using chromStat in differential mode between Young and Old for each histone mark (H3K27ac, H3K27me3 and H3ac) with the bin size of 1000, step size of 500 and 15 as minimum mapping quality threshold.

Data quality

We used strict mapping criteria and spike-in controls for normalization as background is very low in CUT&RUN

Software

bowtie2, MarkDuplicates, chromStat, edgeR

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

For cell sorting, cells were harvested, resuspended in HBSS+ buffer and incubated for 45 minutes with the following antibodies from eBioscience: CD140a-APC (#17140181), Sca-1-FITC (#11598180), Ter1-199-PE (#12592182), the Fixable Viability Dye eFlour 450 (#65068314; 1:10000) and the Life Technologies CD45-PE antibody (#A16325). Antibodies Cells were then washed twice with HBSS+ and were filtered through 35μm nylon mesh into 5ml sample tubes. The CD45-TERR119-CD140a+Sca-1+ population was sorted into an appendorf tube containing 500μl a-MEM media, at 4°C. Compensation was done using UltraComp compensation beads (#01222242, Invitrogen,) and Arc beads (#010346, Life technologies).

For FACs-based marker characterization of the young and aged MSCs, cells were stained as described above with the respective
antibodies and analyzed by flow cytometry, using the BD FACSCANTO II instrument (BD Biosciences). Data were collected using FACS-Diva software and analyzed using FlowJo software.

To measure mitochondrial mass, cells were stained with the MitoTracker Deep Red FM probe (Thermo Fisher # M22426) for 30 minutes at 37°C, under hypoxic conditions. They were then washed with PBS, resuspended in a-MEM medium without FBS and phenol red and stained with DAPI (Invitrogen) for dead-cell exclusion. Right afterwards, cells were analyzed by flow cytometry (BD FACSCANTO II, BD Biosciences). Data were collected using FACS-Diva software and analyzed using FlowJo software.

**Instrument**

| FlowJo |

**Software**

| FlowJo |

**Cell population abundance**

At least 10000 cells were collected for each analysis experiment.

**Gating strategy**

Both for cell sorting as well as for cell analysis FSC and SSC were used to gate out cell debris and cell clumps.

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