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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 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	public datasets were download from GEO using fastq-dump : 2.8.0
Data analysis	codes for data analysis is available on GitHub https://github.com/NyxSLY/ASCT Software used for data analysis: FlowJo v. 10 QuantStudio v. 1.3 Trim Galore v. 0.0.4 HISAT v. 2.1.0 STAR v. 2.7 bowtie2 v. 2.2.4 Bismark v. 0.22.3 salmon v. 1.0.0 featureCounts v. 1.5.3 ROSE csaw v. 1.20 TMM v. 3.12 DAVID v. 6.8 HOMER v. 4.8 ChIPseeker v. 1.8.6 clusterProfiler v. 3.0.4 deeptools v. 3.2.0 methylR kit v. 1.16.1 macs2 v. 2.1.0

regioneR v. 1.22
MUSIC
chromHMM v. 1.17
Promoter - 2.0

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 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

All data is deposited under GEO accession number GSE156409

The following secure token has been created to allow review of record GSE156409 while it remains in private status:
whcfeuycfxgdnov

To review GEO accession GSE156409:
Go to https://urldefense.proofpoint.com/v2/url?u=https-3A__www.ncbi.nlm.nih.gov_geo_query_acc.cgi-3Facc-3DGSE156409&d=DwIBAg&c=ZQs-KZ8oxEw0p81sqgiaRA&r=VR7K4KXJBy7qPk56QZAVEg&m=iekXaDM1z34ACfieyTKuGLADNW_3zGF81qjVZp-3rHk&s=oKTt6_be__yRIWrd9mlMkFiGK6F_BTPgfIFo1WlkyDk&e=

We additionally analyzed the following datasets from the GEO database:
E-MTAB-5176
GSE61915
GSE63577
E-GEOD-59966
E-GEOD-464886
EMTAB-4652
GSE113957
SRX393061
PRJNA417856
E-MTAB-4879
SRP053350
GSE104408
GSE99791
GSE63577
GSE83474
GSE52285
GSE47819

We analyzed the following ENCODE datasets (called peaks from ChIP-seq):
ENCFF207AVV
ENCFF753WNT
ENCFF983STO
ENCFF719PKP
ENCFF777ZEH
ENCFF341NJI
ENCFF687AQV
ENCFF088XQT
ENCFF764OZD
ENCFF905PYM
ENCFF333FZO
ENCFF650QJC
ENCFF889AKD
ENCFF815HWK
ENCFF031ZWH
ENCFF004QBE
ENCFF449PID
ENCFF169TCW
ENCFF429BQL
ENCFF440PZY
ENCFF400JCO
ENCFF116OUV

The sequencing data generated in this paper under GEO accession number GSE156409 are shown in the following figures:
Figure 1
Figure 2
Figure 3
Figure 4
Figure 5

Extended Data Figure 1
Extended Data Figure 2
Extended Data Figure 3
Extended Data Figure 4
Extended Data Figure 5

Analysis of the previously published dataset GSE47819 is presented in:
Figure 1
Figure 5
Extended Data Figure 1
Extended Data Figure 5

Analysis of all the other GEO datasets is shown in Extended Data Figure 2.

Analysis of the ENCODE datasets is in Extended Data Figure 3.

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	For MSC cultures, the cell numbers used in experiments were empirically determined by the amount of material needed for sequencing. Mouse sample size for NSC experiments were determined by sufficient statistical power for PCA analysis.
Data exclusions	No data were excluded
Replication	Pilot experiments for RNA-seq, ChIP-seq, and DeCAP-seq were performed and the results were consistent with the primary results reported in the manuscript. Results from pilot experiments were not included in the manuscript due to poor data coverage. Overall, ChIP-seq experiments were replicated 2 or 3 times. RNA-seq was performed in triplicate. WGBS and CMS-IP-seq (for 5hmC) were performed in duplicate. Replication attempts were all successful. hMSC outgrowth was performed multiple times with similar growth rates and senescence each time. Differentiation assays were performed once on this lot of hMSCs; trends in differentiation potential changed consistently over time, and are consistent with changes in differentiation potential during outgrowth of two other lots of hMSCs.
Randomization	Mice were randomly assigned to the young and old groups. For aging studies, hMSCs were randomly allocated during passaging to either be processed to continue culture outgrowth. For the shRNA knockdown, hMSCs were randomly allocated to "NT" and "SETD2" knockdown plates during passaging.
Blinding	Blinding was not possible for some experiments and not applicable for others. Blinding is not applicable for computational analysis of sequencing data. For hMSC aging, early and late passage cells have different morphologies and growth rates, and as experiments are done as cell outgrowth occurs, it is impossible to blind the experimenter. For mice, young and old mice are physically distinct and it is impossible to hide this from experimenters.

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
☒	☐ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

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

Antibodies

Antibodies used

histone H3: Active Motif #61475
histone Millipore #05-928, lot #2884434
H3K27ac: Active Motif #39133, lot #01613007
H3K27me3: Active Motif #39155, lot #23813016
H3K36me3: Active Motif #61101, lot #32412003
H3K4me1: AbCam #ab8895, lot #GR1278894
H3K4me3: Diagenode #C15410030, lot #002
H3K9me3: Active Motif #39765, lot #16513004
TBP: Cell Signaling Technologies #440595, lot #1
Pol2: Active Motif #39097, lot #29613012
EGF: Thermo Fisher #E-35351
Prominin 1: Thermo Fisher #13-1331-80, clone 13A4

Validation

The histone H3, H3K4me1, H3K4me3, H3K9me3, H3K27ac, H3K27me3, H3K36me3, TBP, and Pol2 antibodies are validated for ChIP (and/or ChIP-seq) by their manufacturers; examples of validation are provided on their respective websites.

The EGF antibody is described as suitable for flow cytometry on the manufacturer's website. It has been published for FACS (PubMed ID: 19332781).

The Prominin 1 antibody is validated for flow cytometry on the manufacturer's website.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

HEK293T cells

Authentication

No authentication was used.

Mycoplasma contamination

Cells were not tested for Mycoplasma contamination.

Commonly misidentified lines
(See ICLAC register)

HEK293T cells were used. These cells were only used to produce lentiviruses; no experimental data were derived from the 293T cells.

Animals and other organisms

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

Laboratory animals

Mouse, FVB/n, 7 month and 21 month, males and females

Wild animals

N/A

Field-collected samples

N/A

Ethics oversight

Mice were housed and used for experiments in accordance with a protocol approved by the Stanford University Institutional Animal Care and Use Committee

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.

To review GEO accession GSE156406:
Go to https://urldefense.proofpoint.com/v2/url?u=https-3A__www.ncbi.nlm.nih.gov_geo_query_acc.cgi-3Facc-3DGSE156406&d=DwIBAg&c=ZQs-KZ8oxEw0p81sqgiaRA&r=VR7K4KXJBy7qPk56QZAVEg&m=h4cF4UGqqG0hAMSSQucHTOklORZQtgBx_k2komBNfi4&s=gRBZFKpvuS7BIkhccz2SvA6kv119AfTkiPq1gd86n5M&e=

Enter token evcfskwubnchvqz into the box

Files in database submission

H3_total_O_Batch1
H3_total_O_Batch2
H3K27ac_O_Batch1
H3K27me3_O_Batch2

H3K36me3_O_Batch1
H3K4me1_O_Batch1
H3K4me3_O_Batch1
H3K9me3_O_Batch2
INPUT_O_Batch1
INPUT_O_Batch2
TBP_O_Batch1
H3_total_Y_Batch1
H3_total_Y_Batch2
H3K27ac_Y_Batch1
H3K27me3_Y_Batch2
H3K36me3_Y_Batch1
H3K4me1_Y_Batch1
H3K4me3_Y_Batch1
H3K9me3_Y_Batch2
INPUT_Y_Batch1
INPUT_Y_Batch2
TBP_Y_Batch1
Pol2_Y_Batch1
Pol2_O_Batch1

Genome browser session
(e.g. UCSC)

https://genome.ucsc.edu/s/kuaias/MSC%20ChIP%2Dseq%20for%20review

Methodology

Replicates

no replicate

Sequencing depth

sample total_reads uniquely_mapped_reads length type
H3_total_Y_Batch1 169,478,266 143,838,661 100bp paired-end
H3K4me1_Y_Batch1 152,347,374 134,466,842 100bp paired-end
H3K4me3_Y_Batch1 55,948,246 42,380,527 100bp paired-end
H3K27ac_Y_Batch1 65,435,154 46,203,213 100bp paired-end
H3K36me3_Y_Batch1 144,423,472 123,339,555 100bp paired-end
H3_O_Batch1 169,928,318 145,072,046 100bp paired-end
H3K4me1_O_Batch1 163,513,880 144,900,630 100bp paired-end
H3K4me3_O_Batch1 56,670,376 38,724,338 100bp paired-end
H3K27ac_O_Batch1 44,944,372 37,215,226 100bp paired-end
H3K36me3_O_Batch1 178,873,484 147,589,219 100bp paired-end
INPUT_O_Batch1 64,471,596 55,927,385 100bp paired-end
INPUT_Y_Batch1 198,936,476 173,518,075 100bp paired-end
TBP_Y_Batch1 47257858 34,558,891 100bp paired-end
TBP_O_Batch1 50860266 41,861,896 100bp paired-end
H3K9me3_Y_Batch2 38728058 28910559 100bp paired-end
H3K9me3_O_Batch2 21587242 16479251 100bp paired-end
INPUT_Y_Batch2 72954268 60148607 100bp paired-end
INPUT_O_Batch2 72377436 58568219 100bp paired-end
H3K27me3_Y_Batch2 100132388 81094784 100bp paired-end
H3K27me3_O_Batch2 59187606 49360658 100bp paired-end
H3_total_Y_Batch2 73140580 125231409 100bp paired-end
H3_total_O_Batch2 4651892 7427018 100bp paired-end
Pol2_Y_Batch1 57798554 33696457 100bp paired-end
Pol2_O_Batch1 25409610 20180063 100bp paired-end

Antibodies

histone H3: Active Motif #61475
histone Millipore #05-928, lot #2884434
H3K27ac: Active Motif #39133, lot #01613007
H3K27me3: Active Motif #39155, lot #23813016
H3K36me3: Active Motif #61101, lot #32412003
H3K4me1: AbCam #ab8895, lot #GR1278894
H3K4me3: Diagenode #C15410030, lot #002
H3K9me3: Active Motif #39765, lot #16513004
TBP: Cell Signaling Technologies #440595, lot #1
Pol2: Active Motif #39097, lot #29613012

Peak calling parameters

```
for i in *R1_001.fastq.gz;do

f2=${i/R1/R2}
echo $i
trim_galore --paired $i $f2
done

bowtie2 -p 6 -t -x /Volumes/LACIE/Human_database/hg19/bowtie2_index/hg19 -1 {} -2 {} -S {}.sam

for i in *R1_001_val_1.fq.gz;do
```

```
 f2=${i/R1_001_val_1/R2_001_val_2}
 echo $i
 bowtie2 -p 6 -t -x /Volumes/LACIE/Human_database/hg19/bowtie2_index/hg19 -1 $i -2 $f2 | samtools view -S -b -q 30 - | samtools
sort - -@ 6 -o ${i/_R1_001_val_1.fq/.sorted.bam}
done


mkdir d1_Y.H3K9me3;cd d1_Y.H3K9me3
mkdir chip;mkdir control
samtools view /Volumes/luyang/Histone_Modification_ChIP_seq/hg19/clean_bam/
Y1_H3K9me3.sorted.nomulti.nodup.blacklistTrimmed.bam | MUSIC -pdrocess SAM stdin chip/
samtools view /Volumes/luyang/Histone_Modification_ChIP_seq/hg19/clean_bam/
Y1_input.sorted.nomulti.nodup.blacklistTrimmed.bam | MUSIC -pdrocess SAM stdin control/
mkdir chip/sorted;mkdir chip/dedup;mkdir control/sorted;mkdir control/dedup
MUSIC -sort_reads chip chip/sorted
MUSIC -sort_reads control control/sorted
MUSIC -remove_duplicates chip/sorted 2 chip/dedup
MUSIC -remove_duplicates control/sorted 2 control/dedup
cd chip/dedup;rm KI*;rm GL*
head -25 chr_ids.txt > chr_ids.txt1;mv chr_ids.txt1 chr_ids.txt
cd ../../control/dedup;rm KI*;rm GL*
cd ../..
run_MUSIC.csh -get_optimal_broad_ERs ./chip/dedup ./control/dedup /Volumes/LACIE/Human_database/hg19/
multi_mappability_100
cd ..
mkdir d3_Y.H3K27ac;cd d3_Y.H3K27ac
mkdir chip;mkdir control
samtools view /Volumes/Data1/ChIP_seq_11_2018/bam/remove_duplication/
BM15_S38_L003.sorted.redup.blacklistTrimmed.bam.gz | MUSIC -pdrocess SAM stdin chip/
samtools view /Volumes/Data1/ChIP_seq_11_2018/bam/remove_duplication/
BM12_S35_L003.sorted.redup.blacklistTrimmed.bam.gz | MUSIC -pdrocess SAM stdin control/
mkdir chip/sorted;mkdir chip/dedup;mkdir control/sorted;mkdir control/dedup
MUSIC -sort_reads chip chip/sorted
MUSIC -sort_reads control control/sorted
MUSIC -remove_duplicates chip/sorted 2 chip/dedup
MUSIC -remove_duplicates control/sorted 2 control/dedup
cd chip/dedup;rm KI*;rm GL*
head -25 chr_ids.txt > chr_ids.txt1;mv chr_ids.txt1 chr_ids.txt
cd ../../control/dedup;rm KI*;rm GL*
cd ../..
run_MUSIC.csh -get_optimal_punctate_ERs ./chip/dedup ./control/dedup /Volumes/LACIE/Human_database/hg19/
multi_mappability_100
cd ..
mkdir d3_Y.H3K27me3;cd d3_Y.H3K27me3
mkdir chip;mkdir control
samtools view /Volumes/luyang/Histone_Modification_ChIP_seq/hg19/clean_bam/
Y3_H3K27me3.sorted.nomulti.nodup.blacklistTrimmed.bam | MUSIC -pdrocess SAM stdin chip/
samtools view /Volumes/luyang/Histone_Modification_ChIP_seq/hg19/clean_bam/
Y3_input.sorted.nomulti.nodup.blacklistTrimmed.bam | MUSIC -pdrocess SAM stdin control/
mkdir chip/sorted;mkdir chip/dedup;mkdir control/sorted;mkdir control/dedup
MUSIC -sort_reads chip chip/sorted
MUSIC -sort_reads control control/sorted
MUSIC -remove_duplicates chip/sorted 2 chip/dedup
MUSIC -remove_duplicates control/sorted 2 control/dedup
cd chip/dedup;rm KI*;rm GL*
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cd ../../control/dedup;rm KI*;rm GL*
cd ../..
run_MUSIC.csh -get_optimal_broad_ERs ./chip/dedup ./control/dedup /Volumes/LACIE/Human_database/hg19/
multi_mappability_100
cd ..
mkdir d3_Y.H3K36me3;cd d3_Y.H3K36me3
mkdir chip;mkdir control
samtools view /Volumes/Data1/ChIP_seq_11_2018/bam/remove_duplication/
BM16_S39_L003.sorted.redup.blacklistTrimmed.bam.gz | MUSIC -pdrocess SAM stdin chip/
samtools view /Volumes/Data1/ChIP_seq_11_2018/bam/remove_duplication/
BM12_S35_L003.sorted.redup.blacklistTrimmed.bam.gz | MUSIC -pdrocess SAM stdin control/
mkdir chip/sorted;mkdir chip/dedup;mkdir control/sorted;mkdir control/dedup
MUSIC -sort_reads chip chip/sorted
MUSIC -sort_reads control control/sorted
MUSIC -remove_duplicates chip/sorted 2 chip/dedup
MUSIC -remove_duplicates control/sorted 2 control/dedup
cd chip/dedup;rm KI*;rm GL*
head -25 chr_ids.txt > chr_ids.txt1;mv chr_ids.txt1 chr_ids.txt
cd ../../control/dedup;rm KI*;rm GL*
cd ../..
```

```
run_MUSIC.csh -get_optimal_broad_ERs ./chip/dedup ./control/dedup /Volumes/LACIE/Human_database/hg19/
multi_mappability_100
cd ..
mkdir d3_Y.H3K4me1;cd d3_Y.H3K4me1
mkdir chip;mkdir control
samtools view /Volumes/Data1/ChIP_seq_11_2018/bam/remove_duplication/
BM13_S36_L003.sorted.redup.blacklistTrimmed.bam.gz | MUSIC -pdrocess SAM stdin chip/
samtools view /Volumes/Data1/ChIP_seq_11_2018/bam/remove_duplication/
BM12_S35_L003.sorted.redup.blacklistTrimmed.bam.gz | MUSIC -pdrocess SAM stdin control/
mkdir chip/sorted;mkdir chip/dedup;mkdir control/sorted;mkdir control/dedup
MUSIC -sort_reads chip chip/sorted
MUSIC -sort_reads control control/sorted
MUSIC -remove_duplicates chip/sorted 2 chip/dedup
MUSIC -remove_duplicates control/sorted 2 control/dedup
cd chip/dedup;rm KI*;rm GL*
head -25 chr_ids.txt > chr_ids.txt1;mv chr_ids.txt1 chr_ids.txt
cd ../../control/dedup;rm KI*;rm GL*
cd ../..
run_MUSIC.csh -get_optimal_punctate_ERs ./chip/dedup ./control/dedup /Volumes/LACIE/Human_database/hg19/
multi_mappability_100
cd ..
mkdir d3_Y.H3K4me3;cd d3_Y.H3K4me3
mkdir chip;mkdir control
samtools view /Volumes/Data1/ChIP_seq_11_2018/bam/remove_duplication/
BM14_S37_L003.sorted.redup.blacklistTrimmed.bam.gz | MUSIC -pdrocess SAM stdin chip/
samtools view /Volumes/Data1/ChIP_seq_11_2018/bam/remove_duplication/
BM12_S35_L003.sorted.redup.blacklistTrimmed.bam.gz | MUSIC -pdrocess SAM stdin control/
mkdir chip/sorted;mkdir chip/dedup;mkdir control/sorted;mkdir control/dedup
MUSIC -sort_reads chip chip/sorted
MUSIC -sort_reads control control/sorted
MUSIC -remove_duplicates chip/sorted 2 chip/dedup
MUSIC -remove_duplicates control/sorted 2 control/dedup
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cd ../..
run_MUSIC.csh -get_optimal_punctate_ERs ./chip/dedup ./control/dedup /Volumes/LACIE/Human_database/hg19/
multi_mappability_100
cd ..
mkdir d1_O.H3K9me3;cd d1_O.H3K9me3
mkdir chip;mkdir control
samtools view /Volumes/luyang/Histone_Modification_ChIP_seq/hg19/clean_bam/
O1_H3K9me3.sorted.nomulti.nodup.blacklistTrimmed.bam | MUSIC -pdrocess SAM stdin chip/
samtools view /Volumes/luyang/Histone_Modification_ChIP_seq/hg19/clean_bam/
O1_input.sorted.nomulti.nodup.blacklistTrimmed.bam | MUSIC -pdrocess SAM stdin control/
mkdir chip/sorted;mkdir chip/dedup;mkdir control/sorted;mkdir control/dedup
MUSIC -sort_reads chip chip/sorted
MUSIC -sort_reads control control/sorted
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cd ../..
run_MUSIC.csh -get_optimal_broad_ERs ./chip/dedup ./control/dedup /Volumes/LACIE/Human_database/hg19/
multi_mappability_100
cd ..
mkdir d3_O.H3K27ac;cd d3_O.H3K27ac
mkdir chip;mkdir control
samtools view /Volumes/Data1/ChIP_seq_11_2018/bam/remove_duplication/
BM22_S45_L003.sorted.redup.blacklistTrimmed.bam.gz | MUSIC -pdrocess SAM stdin chip/
samtools view /Volumes/Data1/ChIP_seq_11_2018/bam/remove_duplication/
BM19_S42_L003.sorted.redup.blacklistTrimmed.bam.gz | MUSIC -pdrocess SAM stdin control/
mkdir chip/sorted;mkdir chip/dedup;mkdir control/sorted;mkdir control/dedup
MUSIC -sort_reads chip chip/sorted
MUSIC -sort_reads control control/sorted
MUSIC -remove_duplicates chip/sorted 2 chip/dedup
MUSIC -remove_duplicates control/sorted 2 control/dedup
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multi_mappability_100
cd ..
mkdir d3_O.H3K27me3;cd d3_O.H3K27me3
```

```
mkdir chip;mkdir control
samtools view /Volumes/luyang/Histone_Modification_ChIP_seq/hg19/clean_bam/
O3_H3K27me3.sorted.nomulti.nodup.blacklistTrimmed.bam | MUSIC -pdrocess SAM stdin chip/
samtools view /Volumes/luyang/Histone_Modification_ChIP_seq/hg19/clean_bam/
O3_input.sorted.nomulti.nodup.blacklistTrimmed.bam | MUSIC -pdrocess SAM stdin control/
mkdir chip/sorted;mkdir chip/dedup;mkdir control/sorted;mkdir control/dedup
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MUSIC -sort_reads control control/sorted
MUSIC -remove_duplicates chip/sorted 2 chip/dedup
MUSIC -remove_duplicates control/sorted 2 control/dedup
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cd ../..
run_MUSIC.csh -get_optimal_broad_ERs ./chip/dedup ./control/dedup /Volumes/LACIE/Human_database/hg19/
multi_mappability_100
cd ..
mkdir d3_O.H3K36me3;cd d3_O.H3K36me3
mkdir chip;mkdir control
samtools view /Volumes/Data1/ChIP_seq_11_2018/bam/remove_duplication/
BM23_S46_L003.sorted.redup.blacklistTrimmed.bam.gz | MUSIC -pdrocess SAM stdin chip/
samtools view /Volumes/Data1/ChIP_seq_11_2018/bam/remove_duplication/
BM19_S42_L003.sorted.redup.blacklistTrimmed.bam.gz | MUSIC -pdrocess SAM stdin control/
mkdir chip/sorted;mkdir chip/dedup;mkdir control/sorted;mkdir control/dedup
MUSIC -sort_reads chip chip/sorted
MUSIC -sort_reads control control/sorted
MUSIC -remove_duplicates chip/sorted 2 chip/dedup
MUSIC -remove_duplicates control/sorted 2 control/dedup
cd chip/dedup;rm KI*;rm GL*
head -25 chr_ids.txt > chr_ids.txt1;mv chr_ids.txt1 chr_ids.txt
cd ../../control/dedup;rm KI*;rm GL*
cd ../..
run_MUSIC.csh -get_optimal_broad_ERs ./chip/dedup ./control/dedup /Volumes/LACIE/Human_database/hg19/
multi_mappability_100
cd ..
mkdir d3_O.H3K4me1;cd d3_O.H3K4me1
mkdir chip;mkdir control
samtools view /Volumes/Data1/ChIP_seq_11_2018/bam/remove_duplication/
BM20_S43_L003.sorted.redup.blacklistTrimmed.bam.gz | MUSIC -pdrocess SAM stdin chip/
samtools view /Volumes/Data1/ChIP_seq_11_2018/bam/remove_duplication/
BM19_S42_L003.sorted.redup.blacklistTrimmed.bam.gz | MUSIC -pdrocess SAM stdin control/
mkdir chip/sorted;mkdir chip/dedup;mkdir control/sorted;mkdir control/dedup
MUSIC -sort_reads chip chip/sorted
MUSIC -sort_reads control control/sorted
MUSIC -remove_duplicates chip/sorted 2 chip/dedup
MUSIC -remove_duplicates control/sorted 2 control/dedup
cd chip/dedup;rm KI*;rm GL*
head -25 chr_ids.txt > chr_ids.txt1;mv chr_ids.txt1 chr_ids.txt
cd ../../control/dedup;rm KI*;rm GL*
cd ../..
run_MUSIC.csh -get_optimal_punctate_ERs ./chip/dedup ./control/dedup /Volumes/LACIE/Human_database/hg19/
multi_mappability_100
cd ..
mkdir d3_O.H3K4me3;cd d3_O.H3K4me3
mkdir chip;mkdir control
samtools view /Volumes/Data1/ChIP_seq_11_2018/bam/remove_duplication/
BM21_S44_L003.sorted.redup.blacklistTrimmed.bam.gz | MUSIC -pdrocess SAM stdin chip/
samtools view /Volumes/Data1/ChIP_seq_11_2018/bam/remove_duplication/
BM19_S42_L003.sorted.redup.blacklistTrimmed.bam.gz | MUSIC -pdrocess SAM stdin control/
mkdir chip/sorted;mkdir chip/dedup;mkdir control/sorted;mkdir control/dedup
MUSIC -sort_reads chip chip/sorted
MUSIC -sort_reads control control/sorted
MUSIC -remove_duplicates chip/sorted 2 chip/dedup
MUSIC -remove_duplicates control/sorted 2 control/dedup
cd chip/dedup;rm KI*;rm GL*
head -25 chr_ids.txt > chr_ids.txt1;mv chr_ids.txt1 chr_ids.txt
cd ../../control/dedup;rm KI*;rm GL*
cd ../..
run_MUSIC.csh -get_optimal_punctate_ERs ./chip/dedup ./control/dedup /Volumes/LACIE/Human_database/hg19/
multi_mappability_100
cd ..
```

Data quality

Raw reads were trimmed to remove sequencing adaptors and low quality reads using Trim Galore version 0.4.4 with default parameters (https://www.bioinformatics.babraham.ac.uk/projects/trim_galore/). After mapping, duplicated reads were removed using Picard (http://broadinstitute.github.io/picard). Peak calling was performed using MUSIC with default parameters except that H3K9me3, H3K36me3 and H3K27me3 were performed using the get_motimal_broad_ERs model and peaks were called for the

remaining datasets using get_optimal_punctate_ERs model.

Number of peaks (FDR <0.01)
54403 H3K27ac_O_Batch1
50769 H3K27ac_Y_Batch1
30980 H3K27me3_O_Batch2
37237 H3K27me3_Y_Batch2
12687 H3K36me3_O_Batch1
13070 H3K36me3_Y_Batch1
84867 H3K4me1_O_Batch1
77024 H3K4me1_Y_Batch1
24579 H3K4me3_O_Batch1
21145 H3K4me3_Y_Batch1
25350 H3K9me3_O_Batch2
29154 H3K9me3_Y_Batch2

Software: TrimGalore v. 0.0.4 was used to process reads; bowtie2 v. 2.2.4 was used to map reads; and MUSIC was used for peak calling as described above.

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: Activated and quiescent NSCs (aNSC and qNSC, respectively) were freshly isolated from the subventricular zone of adult (~7 month) and aged (19-21 month) hGFAP-GFP transgenic mice (FVB/N background). SVZs were dissected into PIPES (Ph 7.4) and digested for 10 minutes at 37 degrees with 14U/ml papain. Cells were spun through a 22% Percoll gradient and stained with 1:300 EGF-Alexa 647 (Molecular probes, E-35351), and 1:400 Prominin-1-biotin (eBioscience, 13-1331-80). Dead cells were excluded using propidium iodide. All washes ere performed in HBSS without phenol red and with 1% BSA and 0.1% glucose.

Instrument: BD FACS Aria

Software: FlowJo

Cell population abundance: Approximately 400 cells per animal were collected for library preparation.

Gating strategy: Gating was performed as previously reported in Leeman et al., Science 2018 and Codega et al., Neuron 2014. FACS plots are available upon request.

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