nature portfolio

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Last updated by author(s): Mar 20, 2023

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	BD FACSDiva software (LSRII and Fortessa) or SpectroFlo (Cytek Aurora)was used to collect flow cytometry data. Slidebook 6(3i) was used to collect confocal microscopy data.
Data analysis	Flowjo 10.8.0 for FACS; GraphPad 9.2.0 for statistics; slidebook 6 was used for microscope images; Affymetrix Expression Console v1.1; limma v.3.34.9 ; Trim Galore (version 0.5.0) ; Bowtie 2 (version 2.3.5.1) ; Picard MarkDuplicates function (version 2.19.0); SAMtools (version 1.9); BamTools (version 2.5.1); MACS (version 2.1.2); BEDTools (version 2.27.1); bedGraphToBigWig (version 377); deepTools plotHeatmap (version 3.2.1); DiffBind (version 2.16.0); ChIPseeker (version 1.26.2); clusterProfiler (version 3.18.1); STAR (version 2.7.5a) for sequencing data.

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

RNA-seq, nascent RNA-seq, Cut&Run, ATAC-seq and ChIP-seq data that support the findings of this study have been deposited in the Gene Expression Omnibus (GEO; https://www.ncbi.nlm.nih.gov/geo/) under accession number GSE189563.

Human research participants

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

Reporting on sex and gender	*Use the terms sex (biological attribute) and gender (shaped by social and cultural circumstances) carefully in order to avoid confusing both terms. Indicate if findings apply to only one sex or gender; describe whether sex and gender were considered in study design whether sex and/or gender was determined based on self-reporting or assigned and methods used. Provide in the source data disaggregated sex and gender data where this information has been collected, and consent has been obtained for sharing of individual-level data; provide overall numbers in this Reporting Summary. Please state if this information has not been collected. Report sex- and gender-based analyses where performed, justify reasons for lack of sex- and gender-based analysis.*
Population characteristics	*Describe the covariate-relevant population characteristics of the human research participants (e.g. age, genotypic information, past and current diagnosis and treatment categories). If you filled out the behavioural & social sciences study design questions and have nothing to add here, write "See above."*
Recruitment	*Describe how participants were recruited. Outline any potential self-selection bias or other biases that may be present and how these are likely to impact results.*
Ethics oversight	*Identify the organization(s) that approved the study protocol.*

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 was pre-determined. Sample sizes were chosen according to commonly used standards in the field. The number of independent experiments are indicated in the legend of each figure. Proper negative and whenever possible positive controls were used for each experiment.
Data exclusions	No data were excluded.
Replication	All the experimental finding were reproduced as validated by at least two independent experiments. For ChIP-seq and CUT&RUN experiment, at least two replicates were collected for each group.
Randomization	This is not relevant since we did not use different experimental groups or conditions in our study.
Blinding	The investigators were not blinded to group allocation during data collection or analysis, as there was no subjective measurement in our experiments. This approach is considered standard for experiments of the type performed in this study.

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

For WB: H3 (CST, 9715, 1: 10,000); S10P H3 (Millipore, 06-570, 1:1,000); RNA POLII (Bethyl, A300-653A, 1: 500); SOX2 (R&D Systems, AF2018, 1: 200); EZH2 (CST, 5246, 1: 1,000); SUZ12 (CST, 3737, 1: 1,000); EED (Millipore, 17-10034, 1: 1,000); ARID2 (SIGMA, SAB2702340-100UL, 1: 1,000); PBRM1 (Bethyl, A700-019, 1: 1,000); SMARCE1 (Bethyl, A300-810A, 1: 1,000); SMARCB1 (Bethyl, A301-087A, 1: 1,000); ARID1A (CST, 12354, 1: 1,000), DPF2 (Invitrogen, PA5-21079, 1: 1,000); BRD9 (Active Motif, 61537, 1: 1,000); SMARCA4 (CST, 49360, 1: 1,000); SNRP70 (Abcam, ab83306, 1: 1,000); BRD7 (CST, 14910, 1: 1,000); SMARCC1 (CST, 11956, 1: 1,000).
For ChIP-seq: Five micrograms of antibodies to the following were used: H3K4me3 (CST, 9751S); H3K27ac (Abcam, ab4729); H3K4me1 (Abcam, ab8895); H3K27me3 (CST, 9733); H3K36me3 (CST, 4909); H3K9me3 (Active Motif, 39161); H4K20me3 (Active Motif, 39671); SOX2 (R&D systems, AF2018), CTCF (Abcam, ab70303).
For CUT&RUN: 0.5 ug primary antibodies against SOX2 (R&D systems, AF2018), ESRRB (R&D systems, PP-H6705-00), EZH2 (CST, 5246), ARID1A (SIGMA, HPA005456-100UL), SMARCA4 (Abcam, ab110641), BRD9 (Active Motif, 61537), SMARCB1 (Bethyl, A301-087A), SMARCE1 (Bethyl, A300-810A).
For IHC: OCT4 (Santa Cruz, sc-5279, 1: 50); SOX2 (R&D Systems, AF2018, 1: 20); NANOG (Active Motif, 61419, 1: 200), ESRRB (R&D Systems, PP-H6705-00, 1: 100); SOX1 (R&D Systems, AF3369, 1: 20), NES (MILLIPORE, MAB353, 1: 50), GABA (Invitrogen, PA5-32241, 1: 100), GFAP (Abcam, ab7260, 1: 1,000)
For flow cytometry: 5 ul of PE anti-Histone H3 Phospho (Ser10) Antibody (BioLegend #650808) or PE Mouse IgG2b, κ Isotype Ctrl Antibody (BioLegend #400314) for 1 million cells.

Validation

The specificities of listed FACS antibodies have been validated by the manufacturer by flow cytometry.
The specificities of listed WB antibodies have been validated by the manufacturer by western blot.
The specificities of listed imaging antibodies have been validated by the manufacturer by imaging.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

All cell lines (Sox2-Egfp, Smarca4-Egfp, Smarcb1-Egfp, Smarce1-Egfp, Smarce1-MD, Smarce1-MD (R42A), Smarce1-AID) were derived from AB2.2 mouse embryonic stem cells (https://www.atcc.org/products/scrc-1023).

Authentication

Sox2-Egfp, Smarca4-Egfp, Smarcb1-Egfp, Smarce1-Egfp, Smarce1-MD, Smarce1-MD (R42A), Smarce1-AID were authenticated by PRC-genotyping and western blot. Smarce1-MD, Smarce1-MD (R42A) were also authenticated by Sanger-sequencing. Mitosis-specific degron (MD) and its mutant degron cells and Smarce1-AID mouse ES cells were authenticated by western blot after releasing from mitosis.

Mycoplasma contamination

All cell lines tested negative for mycoplasma contamination.

Commonly misidentified lines
(See ICLAC register)

Cells used are not in the ICLAC database.

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.

All sequencing data that support the findings of this study have been deposited in NCBI's Gene Expression Omnibus (GEO) under accession number GSE189563 (including CUT&RUN, RNA-seq, ATAC-seq and nascent RNA-seq data).

Files in database submission

raw data (.fastq) were provided for the following samples:
asynchronous mESCs_input_1940854
asynchronous mESCs_H3K4me1_1940855
asynchronous mESCs_H3K9me3_1940856
mitotic mESCs_input_1940863
mitotic mESCs_H3K4me1_1940864

mitotic mESCs_H3K9me3_1940865
asynchronous mESCs_input_1884805
asynchronous mESCs_SOX2_1884812
mitotic mESCs_input_1884813
mitotic mESCs_SOX2_1884820
asynchronous mESCs_input_1951682
asynchronous mESCs_H3K4me1_1951683
asynchronous mESCs_H3K9me3_1951684
mitotic mESCs_input_1951688
mitotic mESCs_H3K4me1_1951689
mitotic mESCs_H3K9me3_1951690
asynchronous mESCs_input_1951694
asynchronous mESCs_H3K4me3_1951695
asynchronous mESCs_H3K27me3_1951696
asynchronous mESCs_H3K27ac_1951697
mitotic mESCs_input_1951699
mitotic mESCs_H3K4me3_1951700
mitotic mESCs_H3K27me3_1951701
mitotic mESCs_H3K27ac_1951702
asynchronous mESCs_input_1951704
asynchronous mESCs_H3K4me3_1951705
asynchronous mESCs_H3K27me3_1951706
asynchronous mESCs_H3K27ac_1951707
mitotic mESCs_input_1951709
mitotic mESCs_H3K4me3_1951710
mitotic mESCs_H3K27me3_1951711
mitotic mESCs_H3K27ac_1951712
asynchronous mESCs_input_1983265
asynchronous mESCs_H3K4me1_1983266
mitotic mESCs_input_1983270
mitotic mESCs_H3K4me1_1983271
asynchronous mESCs_input_1983275
asynchronous mESCs_H3K4me1_1983276
mitotic mESCs_input_1983280
mitotic mESCs_H3K4me1_1983281
asynchronous mESCs_input_1996870
asynchronous mESCs_H3K4me3_1996871
asynchronous mESCs_H3K27me3_1996872
asynchronous mESCs_H3K27ac_1996873
mitotic mESCs_input_1996875
mitotic mESCs_H3K4me3_1996876
mitotic mESCs_H3K27me3_1996877
mitotic mESCs_H3K27ac_1996878
asynchronous mESCs_input_1996880
asynchronous mESCs_H3K4me3_1996881
asynchronous mESCs_H3K27me3_1996882
asynchronous mESCs_H3K27ac_1996883
mitotic mESCs_input_1996885
mitotic mESCs_H3K4me3_1996886
mitotic mESCs_H3K27me3_1996887
mitotic mESCs_H3K27ac_1996888
asynchronous mESCs_input_2163925
asynchronous mESCs_H3K9me3_2163926
asynchronous mESCs_H4K20me3_2163927
asynchronous mESCs_H4K20me3_2163928
asynchronous mESCs_H3K36me3_2163930
mitotic mESCs_input_2163931
mitotic mESCs_H3K9me3_2163932
mitotic mESCs_H4K20me3_2163933
mitotic mESCs_H4K20me3_2163934
mitotic mESCs_H3K36me3_2163936
asynchronous mESCs_input_2163937
asynchronous mESCs_H3K9me3_2163938
asynchronous mESCs_H4K20me3_2163939
asynchronous mESCs_H4K20me3_2163940
asynchronous mESCs_H3K36me3_2163942
mitotic mESCs_input_2163943
mitotic mESCs_H3K9me3_2163944
mitotic mESCs_H4K20me3_2163945
mitotic mESCs_H4K20me3_2163946
mitotic mESCs_H3K36me3_2163948
asynchronous mESCs_input_1910803
asynchronous mESCs_SOX2_1910806
mitotic mESCs_input_1910815
mitotic mESCs_SOX2_1910818
asynchronous mESCs_input_1938701

asynchronous mESCs_CTCF_1938706
mitotic mESCs_input_1938710
mitotic mESCs_CTCF_1938715
asynchronous mESCs_input_1940854
asynchronous mESCs_CTCF_1940859
mitotic mESCs_input_1940863
mitotic mESCs_CTCF_1940868

Genome browser session (e.g. UCSC)

https://proteinpaint.stjude.org/chipreview.html

Methodology

Replicates

ChIP-seq, RNA-seq, nascent RNA-seq and CUT&RUN were performed with at least two independent biological replicates as indicated in figure legends.

Sequencing depth

51bp single-end and paired-end reads of ChIP-seq, > 50 million reads for each sample
Raw reads of all samples above have been deposited in NCBI's Gene Expression Omnibus (GEO) under accession number GSE189563 where the sequencing depth information is available.

Antibodies

Five micrograms of antibodies to the following were used: H3K4me3 (CST, 9751S); H3K27ac (Abcam, ab4729); H3K4me1 (Abcam, ab8895); H3K27me3 (CST, 9733); H3K36me3 (CST, 4909); H3K9me3 (Active Motif, 39161); H4K20me3 (Active Motif, 39671); SOX2 (R&D systems, AF2018), CTCF (Abcam, ab70303).

Peak calling parameters

stringent peaks for each replicate were called by MACS2 (V 2.1.1.20160309, with default parameters using Input as control) and filtered with fold enrichment cutoff: 5 and p-value threshold: 1e-9. Peaks that overlapped within replicates were merged (bedtools, V2.17.0, overlap cutoff: 1bp) and the merged peaks were kept as high-confidence peaks for further analysis.

Data quality

Library complexity, cross-correlation, MultiQC and FastQC was tested.

Software

Reads were trimmed with Trim Galore (version 0.6.0) with default parameters.
Reads were aligned to mouse mm10 using BWA (version 0.7.17, default parameters)
Duplicate reads were marked by bamsormadup (biobambam2, V2.0.87).
Uniquely mapped reads for ChIP-seq were obtained using samtools (V0.1.18), with parameter "-q 10 -F1024".
MACS (version 2.1.1) was used to call peaks with default parameters using Input as control.
RPM was applied to the normalized read counts to generate bigwig tracks

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 cycle analysis, 3x106 cells/sample were collected and washed twice in cell suspension buffer (PBS+ 2% FBS) and fixed with 5 ml cold 70% ethanol for at least 1 hour at 4oC. Cells were then washed twice in cold PBS and resuspended in 1 ml of 50 ?g/ml propidium iodide (PI)/PBS buffer supplemented with 0.5 ?g/ml RNase A and incubated overnight at 4oC. Samples were analyzed by 17-color LSR Fortessa (4 Lasers) in the St. Jude flow core. Data were analyzed by FlowJo (Version 10. 7. 1).
for Phospho S10 Histone 3 Staining: 1x106 cells were collected and fixed in the dark for 20 minutes at room temperature with 0.5 ml fixation buffer (BioLegend #420801). Then cells were treated with 1 ml of Intracellular Staining Perm Wash Buffer (BioLegend #421002) twice. Cells were resuspended in 100 ?l of Intracellular Staining Perm Wash Buffer supplemented with 5 ?l of PE anti-Histone H3 Phospho (Ser10) Antibody (BioLegend #650808) or PE Mouse IgG2b, κ Isotype Ctrl Antibody (BioLegend #400314) and incubated for 20 min in the dark at room temperature. Next, cells were washed twice with 2 ml of Intracellular Staining Perm Wash Buffer, resuspended in 0.5 ml of cell suspension buffer, and analyzed by 17-color LSR Fortessa (4 Lasers). Data were analyzed by FlowJo (Version 10. 7. 1).
For cell proliferation: Cells were analyzed following the protocol for the BD Pharmingen BrdU flow kit (APC-BrdU) (552598). Briefly, 1x 106 cells were cultured in 1 ml mESC culture medium and supplemented with 10 ?l of 1 mM BrdU solution. The treated cells were incubated for 30 min at 37oC, 5% CO2 in the incubator. Cells were fixed by BD Cytofix/Cytoperm Buffer and Permeabilized by BD Cytoperm Permeabilization Buffer, and then labelled with APC-conjugated anti-BrdU antibody. After washing, cells were resuspended in 7-AAD solution and analyzed by 17-color LSR Fortessa (4 Lasers). Data were analyzed by FlowJo (Version 10. 7. 1).

Instrument

LSRII, Fortessa (BD Bioscience) or Aurora (Cytek)

Software

Flowjo 10.8.0

Cell population abundance	Purity is computer determined by. A value of % total between 80-90% is obtained. The % total is the % of cells sorted on the stream out of all sorted cells.
Gating strategy	Based on the pattern of FSC-A/SSC-A. Singlets were gated according to the pattern of FSC-H vs. FSC-A. Positive populations were determined by the specific antibodies, which were distinct from negative populations.

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