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	BD FACSDiva software (LSRII and Fortessa) was used to collect flow cytometry data. Slidebook 6(3i) was used to collect confocal microscopy data.
Data analysis	Flowjo 10.7.1 for FACS; GraphPad 9.2.0 for statistics; slidebook 6 was used for microscope images; Trim Galore(version 0.4.4 & 0.6.0); BWA(version 0.7.17); biobambam2(version 2.0.87); BEDTools(version 2.17.0); MACS2(version 2.1.1); STAR(version 2.7.1a); DESeq2(version 1.30.1); RSEM(version 1.3.0); DeepTool(version 3.2.1);ChIPseeker(version 1.26.2);ClusterProfiler(version 3.18.1); GSEA(version 4.1.0); GREAT(version 4.0.4); LOLA(version 1.20.0);chromHMM(version 1.20); Homer(version 4.9.1); FIMO(version 4.11.2) Code used in this study: https://github.com/xiaolongchen0627/SWISNF.mitotic.bookmarking.code

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 (mouse genome assembly mm10).

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 calculation was performed to predetermine sample size. The sample size was selected to maximize the chance of uncovering the mean difference which is also statistically significant.
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). Secondary antibodies for WB: Peroxidase-conjugated AffiniPure Donkey Anti-goat IgG (Jackson Lab, 705-035-003, 1: 10,000); Peroxidase-conjugated AffiniPure Goat Anti-Rabbit IgG (H+L) (Jackson Lab, 111-035-003, 1: 10,000); Peroxidase-conjugated AffiniPure Goat Anti-Mouse IgG (H+L) (Jackson Lab, 115-035-003, 1: 10,000).
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), rabbit mAb IgG XP isotype contro (CST, 3900).
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, clone rat-401, 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.
PE anti-Histone H3 Phospho (Ser10) Antibody (BioLegend #650808): validation for human (by manufacturer) and mouse (https://doi.org/10.7554/eLife.22280), all other information can be found @ https://www.biolegend.com/nl-nl/products/pe-anti-histone-h3-phospho-ser10-antibody-10634
PE Mouse IgG2b, κ Isotype Ctrl Antibody (BioLegend #400314): The MPC-11 immunoglobulin has unknown specificity. This antibody was chosen as an isotype control after screening on a variety of resting, activated, live, and fixed mouse, rat and human tissues. All other information can be found @https://www.biolegend.com/nl-nl/products/pe-mouse-igg2b-kappa-isotype-ctrl-1414
The specificities of listed WB antibodies have been validated by the manufacturer by western blot.
H3 (CST, 9715): validation for H M R Mk Z B Pg. All other information can be found @ https://www.cellsignal.com/products/primary-antibodies/histone-h3-antibody/9715?_=1679261454522&Ntt=9715&tahead=true
S10P H3 (Millipore, 06-570): validation for human. All other information can be found @ https://www.thermofisher.com/antibody/product/49-1041.html?gclid=CjwKCAjw5dqgBhBNEiwA7PryaOFPzLhBTuiUmiIRHKupI6PIqH87Gx61Jg5fxWTDdZVdOhjQVE9ncBoCD-IQAvD_BwE&ef_id=CjwKCAjw5dqgBhBNEiwA7PryaOFPzLhBTuiUmiIRHKupI6PIqH87Gx61Jg5fxWTDdZVdOhjQVE9ncBoCD-IQAvD_BwE:G:s&s_kwcid=AL!3652!3!459737518508!!!g!!!10950825775!106531320406&cid=bid_pca_aup_r01_co_cp1359_pjt0000_bid00000_0se_gaw_dy_pur_con
RNA POLII (Bethyl, A300-653A): validation for Human, Mouse. All other information can be found @ https://www.fortislife.com/products/primary-antibodies/rabbit-anti-rna-polymerase-ii-antibody/BETHYL-A300-653
SOX2 (R&D Systems, AF2018): validation for Human, Mouse, Rat. All other information can be found @ https://www.rndsystems.com/products/human-mouse-rat-sox2-antibody_af2018
EZH2 (CST, 5246): validation for H M R Mk. All other information can be found @https://www.cellsignal.com/products/primary-antibodies/ezh2-d2c9-xp-rabbit-mab/5246
SUZ12 (CST, 3737): validation for H M R Mk. All other information can be found @https://www.cellsignal.com/products/primary-antibodies/suz12-d39f6-xp-rabbit-mab/3737?_=1679261873758&Ntt=3737&tahead=true
EED (Millipore, 17-10034): validation for H, M, R, Op, Ch, Ca, B. All other information can be found @https://www.emdmillipore.com/US/en/product/ChIPAb-EED-ChIP-Validated-Antibody-and-Primer-Set,MM_NF-17-10034?ReferrerURL=https%3A%2F%2Fwww.google.com%2F
ARID2 (SIGMA, SAB2702340-100UL): validation for mouse, human. All other information can be found @ https://www.sigmaaldrich.com/US/en/product/sigma/sab2702340
PBRM1 (Bethyl, A700-019): validation for Human, Mouse. All other information can be found @https://www.fortislife.com/products/primary-antibodies/rabbit-anti-pbrm1-recombinant-monoclonal-antibody-bl-39-2c3/BETHYL-A700-019
SMARCE1 (Bethyl, A300-810A): validation for Human, Mouse. All other information can be found @https://www.fortislife.com/products/primary-antibodies/rabbit-anti-baf57-smarce1-antibody/BETHYL-A300-810
SMARCB1 (Bethyl, A301-087A): validation for Human, Mouse. All other information can be found @ https://www.fortislife.com/products/primary-antibodies/rabbit-anti-smarcb1-snf5-antibody/BETHYL-A301-087
ARID1A (CST, 12354): validation for H M R Mk. All other information can be found @ https://www.cellsignal.com/products/primary-antibodies/arid1a-baf250a-d2a8u-rabbit-mab/12354?_=1679262066257&Ntt=12354&tahead=true
DPF2 (Invitrogen, PA5-21079): validation for Human, Mouse. All other information can be found @https://www.thermofisher.com/antibody/product/DPF2-Antibody-Polyclonal/PA5-21079
BRD9 (Active Motif, 61537): validation for Human, Mouse. All other information can be found @ https://www.activemotif.com/

catalog/details/61537
SMARCA4 (CST, 49360): validation for H M R Mk. All other information can be found @ https://www.cellsignal.com/products/primary-antibodies/brg1-d1q7f-rabbit-mab/49360?_=1679262526041&Ntt=49360&tahead=true
SNRP70 (Abcam, ab83306): validation for Mouse, Rat, Human. All other information can be found @ https://www.abcam.com/products/primary-antibodies/snrp70u1-70k-antibody-ab83306.html
BRD7 (CST, 14910): validation for H. All other information can be found @ https://www.cellsignal.com/product/productDetail.jsp?productId=14910
SMARCC1 (CST, 11956): validation for H M R Mk. All other information can be found @ https://www.cellsignal.com/products/primary-antibodies/smarcc1-baf155-d7f8s-rabbit-mab/11956?_=1679263047173&Ntt=11956&tahead=true
The specificities of listed imaging antibodies have been validated by the manufacturer by imaging.
OCT4 (Santa Cruz, sc-5279): validation for mouse, rat and human. All other information can be found @https://www.scbt.com/p/oct-3-4-antibody-a-9?gclid=CjwKCAjw5dqgBhBNEiwA7PryaFraeq5V88Txe60KQ8RQWrRKGu9kkjjm15J2riDrGNxL5jDMypdwIxoCIPwQAvD_BwE
NANOG (Active Motif, 61419): validation for Human, Mouse. All other information can be found @ https://www.activemotif.com/catalog/details/61419/nanog-antibody-pab
ESRRB (R&D Systems, PP-H6705-00): validation for Human. All other information can be found @ https://www.rndsystems.com/products/human-err-beta-nr3b2-antibody-h6705_pp-h6705-00
SOX1 (R&D Systems, AF3369): validation for Human, Mouse, Rat. All other information can be found @ https://www.rndsystems.com/products/human-mouse-rat-sox1-antibody_af3369
NES (MILLIPORE, clone rat-401, MAB353): validation for M, R. All other information can be found @ https://www.emdmillipore.com/US/en/product/Anti-Nestin-Antibody-clone-rat-401,MM_NF-MAB353
GABA (Invitrogen, PA5-32241): validation for Chemical, Mouse. All other information can be found @ https://www.thermofisher.com/antibody/product/GABA-Antibody-Polyclonal/PA5-32241
GFAP (Abcam, ab7260): validation for Mouse, Rat. All other information can be found @ https://www.abcam.com/products/primary-antibodies/gfap-antibody-ab7260.html

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). E14TG2a mouse ES cells were purchased from ATCC (https://www.atcc.org/products/crl-1821).
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
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asynchronous mESCs_H3K36me3_2163930
mitotic mESCs_input_2163931
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mitotic mESCs_H4K20me3_2163933
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asynchronous mESCs_input_2163937
asynchronous mESCs_H3K9me3_2163938
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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	H3K4me1 ChIP-seq: 4 replicates; H3K4me3 ChIP-seq: 4 replicates; H4K20me3 ChIP-seq: 4 replicates; H3K36me3 ChIP-seq: 2 replicates; H3K9me3 ChIP-seq: 4 replicates; H3K27ac ChIP-seq: 4 replicates; SOX2 ChIP-seq in AB2.2: 2 replicates; SOX2 ChIP-seq in E14TG2a: 3 replicates; CTCF ChIP-seq: 2replicates; ESRRB ChIP-seq: 3 replicates; SOX2 Cut&Run in AB2.2: 4 replicates; SOX2 Cut&Run in E14TG2a: 3 replicates; SOX2 Cut&Run in Smarce1-MD/MR(R42A)@90min: 2replicates for each clone; ESRRB Cut&Run in Smarce1-MD/MR(R42A)@90min: 2replicates for each clone; EZH2 Cut&Run in Smarce1-MD/MR(R42A)@90min: 2replicates for each clone; SOX2 Cut&Run in Smarce1-MD/MR(R42A) treated with DMSO/BRM014: 1 replicates for each clone; SMARCE1 Cut&Run in Smarce1-MD/MR(R42A) treated with DMSO/BRM014: 1 replicates for each clone; SOX2, ESRRB, and EZH2 Cut&Run in Smarce1-AID cells releasing for 30 min: 2 replicates; SMARCE1 Cut&Run in DMSO and 5-Ph-IAA treated Smarce1-AID cells: 2 replicates; ARID1A Cut&Run in asyn and mit: 2 replicates; SMARCA4 Cut&Run in asyn and mit: 2 replicates; BRD9 Cut&Run in asyn and mit: 2 replicates; SMARCB1 Cut&Run in asyn and mit: 4 replicates; SMARCE1 Cut&Run in asyn and mit: 4 replicates; RNA-seq for timecourse SMARCE1 degradation in Smarce1-AID cells: 2 replicates for each time point; ATAC-seq: 2 replicates; RNA-seq for BMP4 rescue experiments: 2 replicates; RNA-seq for Smarce1-MD/MD(R42A) EB formation assay: 2 replicates; for all nascent RNA-seq in the MD and AID system: 2 biological replicates for each clone at each time point.
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.