nature research

Corresponding author(s): C. Thompson and D. Allis

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.

Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. 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
☐	☒	An indication of 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 statistics 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
☐	☒	Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection	No software was used for data collection.
Data analysis	Statistical tests were performed using GraphPad Prism v7.0a (for all bar graphs). For GSEA studies, statistical results are included as part of the default paramaters of the GenePattern GSEAPreranked v6.0.10 module. ImageJ v 1.43u was used for colony formation assays.

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 upon request. 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

RNA-seq, ChIP-seq and ATAC-seq data that support the findings of this study have

been deposited in the Gene Expression Omnibus (GEO) under the accession codes GSE88760 and GSE88769. Previously published sequencing data that were re-analysed here are available under accession codes GSE49847, GSE26833, GSE90895 and GSE56312. Source data for Fig. 4h, Fig. 6a and Supplementary Fig. 4a,h,i have been provided as Supplementary Table 5. All other data supporting the findings of this study are available from the corresponding author on reasonable request.

Field-specific reporting

Please select the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences

For a reference copy of the document with all sections, see nature.com/authors/policies/ReportingSummary-flat.pdf

Life sciences

Study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	Cell biology experiments were performed in triplicate, which extensive experience has shown to be sufficient to determine reproducible results from cultured cells.
Data exclusions	No data were excluded.
Replication	All experiments were reliably reproduced. Each experiment (with the exception of large sequencing experiments) was performed independently at least two times, but usually many more times.
Randomization	Samples were randomly distributed into groups.
Blinding	Blind analysis was used for manual quantification of colony formation assays. In all other cases, analysis was objective and did not require blinding.

Materials & experimental systems

Policy information about availability of materials

n/a	Involved in the study
☐	☒ Unique materials
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☐	☒ Research animals
☒	☐ Human research participants

Unique materials

Obtaining unique materials	Unique materials are available from corresponding authors upon reasonable request.

Antibodies

Antibodies used	Antibodies used for Western blots (at 1:1,000 unless otherwise noted) were pSTAT3 (Cell Signaling Technologies (CST), 9138), STAT3 (CST, 9139) pERK (CST, 4377), ERK (CST, 9102), Tubulin (Sigma, T9026 at 1:10,000), Brd4 (Bethyl Laboratories, A301-985A100 at 1:2,000), Nucleolin (Abcam, ab22758, 1:5000), Nanog (Ebioscience, 14-5761-80) and Actin (Sigma, A3854 at 1:20,000). Antibodies used for ChIP were as follows: H3K9ac (Cell Signaling Technologies, 9649) or H3K27 antibody (Active Motif, 39133) at 0.5–1 µg antibody per ChIP; Brd4 (Bethyl A301-985A50), Med1 (CRSP1/TRAP220, Bethyl A300-793A) and Nanog (Cosmo Bio REC-RCAB002P-F) at 10 ug/mL (5.5 ug in 550 uL for 10 million cells).
Validation	Antibodies were validated as noted by suppliers.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	Mouse ESC lines (ESC1) were generated by B.C. from C57BL/6 x 129S4/SvJae F1 male embryos as previously described (Carey et al., Nature 2015). Nanog-GFP reporter ESCs and iPSCs generated from hematopoietic CD19+ pro-B cells were a gift from R. Jaenisch (MIT). D34 ESCs were generated in the Lowe laboratory as described by Dow et al., PLoS One 2014.
Authentication	Cell lines were not externally authenticated.

Mycoplasma contamination	Cells were routinely tested for mycoplasma.
Commonly misidentified lines (See ICLAC register)	No cell lines used in this study were found in the database of commonly misidentified cell lines that is maintained by ICLAC and NCBI Biosample.

Research animals

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

Animals/animal-derived materials	8 week old outbred ICR female mice from Taconic Farms were used for chimera assays. All animal experiments were carried out according to the protocol approved by the IACUC of Weill Cornell Medical College.

Method-specific reporting

n/a	Involved in the study
☐	☒ ChIP-seq
☐	☒ Flow cytometry
☒	☐ Magnetic resonance imaging

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.*	https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?&acc=GSE95642
Files in database submission	Raw ChIP: ChIP_SL2i-Brd4_1.fastq.gz ChIP_SL2i-Brd4_2.fastq.gz ChIP_SL2i-Brd4-Input_1.fastq.gz ChIP_SL2i-Brd4-Input_2.fastq.gz ChIP_SL2iJQ-Med1_1.fastq.gz ChIP_SL2iJQ-Med1_2.fastq.gz ChIP_SL2iJQ-Med1-Input_1.fastq.gz ChIP_SL2iJQ-Med1-Input_2.fastq.gz ChIP_SL2i-Med1_1.fastq.gz ChIP_SL2i-Med1_2.fastq.gz ChIP_SL2i-Med1-Input_1.fastq.gz ChIP_SL2i-Med1-Input_2.fastq.gz ChIP_SL-Brd4_1.fastq.gz ChIP_SL-Brd4_2.fastq.gz ChIP_SL-Brd4-Input_1.fastq.gz ChIP_SL-Brd4-Input_2.fastq.gz ChIP_SLJQ-Brd4_1.fastq.gz ChIP_SLJQ-Brd4_2.fastq.gz ChIP_SLJQ-Brd4-Input_1.fastq.gz ChIP_SLJQ-Brd4-Input_2.fastq.gz ChIP_SLJQ-Med1_1.fastq.gz ChIP_SLJQ-Med1_2.fastq.gz ChIP_SLJQ-Med1-Input_1.fastq.gz ChIP_SLJQ-Med1-Input_2.fastq.gz ChIP_SL-Med1_1.fastq.gz ChIP_SL-Med1_2.fastq.gz ChIP_SL-Med1-Input_1.fastq.gz ChIP_SL-Med1-Input_2.fastq.gz Processed ChIP: ChIP_SL2i-Brd4_1_val.mm9.sorted.RmDup.10mNorm.bw ChIP_SL2i-Brd4-Input_1_val.mm9.sorted.RmDup.10mNorm.bw ChIP_SL2iJQ-Med1_1_val.mm9.sorted.RmDup.10mNorm.bw ChIP_SL2iJQ-Med1-Input_1_val.mm9.sorted.RmDup.10mNorm.bw ChIP_SL2i-Med1_1_val.mm9.sorted.RmDup.10mNorm.bw ChIP_SL2i-Med1-Input_1_val.mm9.sorted.RmDup.10mNorm.bw ChIP_SL-Brd4_1_val.mm9.sorted.RmDup.10mNorm.bw ChIP_SL-Brd4-Input_1_val.mm9.sorted.RmDup.10mNorm.bw ChIP_SLJQ-Brd4_1_val.mm9.sorted.RmDup.10mNorm.bw ChIP_SLJQ-Brd4-Input_1_val.mm9.sorted.RmDup.10mNorm.bw ChIP_SLJQ-Med1_1_val.mm9.sorted.RmDup.10mNorm.bw

ChIP_SLJQ-Med1-Input_1_val.mm9.sorted.RmDup.10mNorm.bw
ChIP_SL-Med1_1_val.mm9.sorted.RmDup.10mNorm.bw
ChIP_SL-Med1-Input_1_val.mm9.sorted.RmDup.10mNorm.bw

Raw ATAC:
1A-5-DMSO-1_S1_R1_001.fastq.gz
1A-5-DMSO-1_S1_R2_001.fastq.gz
1A-5-DMSO-2_S2_R1_001.fastq.gz
1A-5-DMSO-2_S2_R2_001.fastq.gz
1A-5-JQ1-1_S3_R1_001.fastq.gz
1A-5-JQ1-1_S3_R2_001.fastq.gz
1A-5-JQ1-2_S4_R1_001.fastq.gz
1A-5-JQ1-2_S4_R2_001.fastq.gz
1A_SL_2i_DMSO-1_S7_R1_001_copy2.fastq.gz
1A_SL_2i_DMSO-1_S7_R2_001_copy2.fastq.gz
1A_SL_2i_DMSO-3_S9_R1_001_copy2.fastq.gz
1A_SL_2i_DMSO-3_S9_R2_001_copy2.fastq.gz
1A_SL_2i_JQ1-1_S10_R1_001_copy2.fastq.gz
1A_SL_2i_JQ1-1_S10_R2_001_copy2.fastq.gz
1A_SL_2i_JQ1-2_S11_R1_001_copy2.fastq.gz
1A_SL_2i_JQ1-2_S11_R2_001_copy2.fastq.gz
1A_SL_DMSO-2_S2_R1_001_copy2.fastq.gz
1A_SL_DMSO-2_S2_R2_001_copy2.fastq.gz
1A_SL_DMSO-3_S3_R1_001_copy2.fastq.gz
1A_SL_DMSO-3_S3_R2_001_copy2.fastq.gz
1A_SL_JQ1-1_S4_R1_001_copy2.fastq.gz
1A_SL_JQ1-1_S4_R2_001_copy2.fastq.gz
1A_SL_JQ1-3_S6_R1_001_copy2.fastq.gz
1A_SL_JQ1-3_S6_R2_001_copy2.fastq.gz
5-14-DMSO-1_S9_R1_001.fastq.gz
5-14-DMSO-1_S9_R2_001.fastq.gz
5-14-DMSO-2_S10_R1_001.fastq.gz
5-14-DMSO-2_S10_R2_001.fastq.gz
5-14-JQ1-1_S11_R1_001.fastq.gz
5-14-JQ1-1_S11_R2_001.fastq.gz
5-14-JQ1-2_S12_R1_001.fastq.gz
5-14-JQ1-2_S12_R2_001.fastq.gz
5-14-Nanog-Tg-DMSO-2_S5_R1_001.fastq.gz
5-14-Nanog-Tg-DMSO-2_S5_R2_001.fastq.gz
5-14-Nanog-Tg-DMSO-3_S6_R1_001.fastq.gz
5-14-Nanog-Tg-DMSO-3_S6_R2_001.fastq.gz
5-14-Nanog-Tg-JQ1-1_S7_R1_001.fastq.gz
5-14-Nanog-Tg-JQ1-1_S7_R2_001.fastq.gz
5-14-Nanog-Tg-JQ1-3_S8_R1_001.fastq.gz
5-14-Nanog-Tg-JQ1-3_S8_R2_001.fastq.gz
5-9-DMSO-1_S5_R1_001.fastq.gz
5-9-DMSO-1_S5_R2_001.fastq.gz
5-9-DMSO-2_S6_R1_001.fastq.gz
5-9-DMSO-2_S6_R2_001.fastq.gz
5-9-JQ1-1_S7_R1_001.fastq.gz
5-9-JQ1-1_S7_R2_001.fastq.gz
5-9-JQ1-2_S8_R1_001.fastq.gz
5-9-JQ1-2_S8_R2_001.fastq.gz
5-9-Nanog-Tg-DMSO-1_S5_R1_001.fastq.gz
5-9-Nanog-Tg-DMSO-1_S5_R2_001.fastq.gz
5-9-Nanog-Tg-DMSO-2_S6_R1_001.fastq.gz
5-9-Nanog-Tg-DMSO-2_S6_R2_001.fastq.gz
5-9-Nanog-Tg-JQ1-1_S7_R1_001.fastq.gz
5-9-Nanog-Tg-JQ1-1_S7_R2_001.fastq.gz
5-9-Nanog-Tg-JQ1-2_S8_R1_001.fastq.gz
5-9-Nanog-Tg-JQ1-2_S8_R2_001.fastq.gz
Nanog_Tg_dmso-1_S1_R1_001.fastq.gz
Nanog_Tg_dmso-1_S1_R2_001.fastq.gz
Nanog_Tg_dmso-2_S2_R1_001.fastq.gz
Nanog_Tg_dmso-2_S2_R2_001.fastq.gz
Nanog_Tg_JQ1-1_S3_R1_001.fastq.gz
Nanog_Tg_JQ1-1_S3_R2_001.fastq.gz
Nanog_Tg_JQ1-2_S4_R1_001.fastq.gz
Nanog_Tg_JQ1-2_S4_R2_001.fastq.gz

Processed ATAC:
1A-5-DMSO-1_S1_R1_001_val.mm9.sorted.RmDup.10mNorm.bw
1A-5-DMSO-2_S2_R1_001_val.mm9.sorted.RmDup.10mNorm.bw
1A-5-JQ1-1_S3_R1_001_val.mm9.sorted.RmDup.10mNorm.bw
1A-5-JQ1-2_S4_R1_001_val.mm9.sorted.RmDup.10mNorm.bw

1A_SL_2i_DMSO-1_S7_R1_001_copy2_val.mm9.sorted.RmDup.10mNorm.bw
1A_SL_2i_DMSO-3_S9_R1_001_copy2_val.mm9.sorted.RmDup.10mNorm.bw
1A_SL_2i_JQ1-1_S10_R1_001_copy2_val.mm9.sorted.RmDup.10mNorm.bw
1A_SL_2i_JQ1-2_S11_R1_001_copy2_val.mm9.sorted.RmDup.10mNorm.bw
1A_SL_DMSO-2_S2_R1_001_copy2_val.mm9.sorted.RmDup.10mNorm.bw
1A_SL_DMSO-3_S3_R1_001_copy2_val.mm9.sorted.RmDup.10mNorm.bw
1A_SL_JQ1-1_S4_R1_001_copy2_val.mm9.sorted.RmDup.10mNorm.bw
1A_SL_JQ1-3_S6_R1_001_copy2_val.mm9.sorted.RmDup.10mNorm.bw
5-14-DMSO-1_S9_R1_001_val.mm9.sorted.RmDup.10mNorm.bw
5-14-DMSO-2_S10_R1_001_val.mm9.sorted.RmDup.10mNorm.bw
5-14-JQ1-1_S11_R1_001_val.mm9.sorted.RmDup.10mNorm.bw
5-14-JQ1-2_S12_R1_001_val.mm9.sorted.RmDup.10mNorm.bw
5-14-Nanog-Tg-DMSO-2_S5_R1_001_val.mm9.sorted.RmDup.10mNorm.bw
5-14-Nanog-Tg-DMSO-3_S6_R1_001_val.mm9.sorted.RmDup.10mNorm.bw
5-14-Nanog-Tg-JQ1-1_S7_R1_001_val.mm9.sorted.RmDup.10mNorm.bw
5-14-Nanog-Tg-JQ1-3_S8_R1_001_val.mm9.sorted.RmDup.10mNorm.bw
5-9-DMSO-1_S5_R1_001_val.mm9.sorted.RmDup.10mNorm.bw
5-9-DMSO-2_S6_R1_001_val.mm9.sorted.RmDup.10mNorm.bw
5-9-JQ1-1_S7_R1_001_val.mm9.sorted.RmDup.10mNorm.bw
5-9-JQ1-2_S8_R1_001_val.mm9.sorted.RmDup.10mNorm.bw
5-9-Nanog-Tg-DMSO-1_S5_R1_001_val.mm9.sorted.RmDup.10mNorm.bw
5-9-Nanog-Tg-DMSO-2_S6_R1_001_val.mm9.sorted.RmDup.10mNorm.bw
5-9-Nanog-Tg-JQ1-1_S7_R1_001_val.mm9.sorted.RmDup.10mNorm.bw
5-9-Nanog-Tg-JQ1-2_S8_R1_001_val.mm9.sorted.RmDup.10mNorm.bw
Nanog_Tg_dmso-1_S1_R1_001_val.mm9.sorted.RmDup.10mNorm.bw
Nanog_Tg_dmso-2_S2_R1_001_val.mm9.sorted.RmDup.10mNorm.bw
Nanog_Tg_JQ1-1_S3_R1_001_val.mm9.sorted.RmDup.10mNorm.bw
Nanog_Tg_JQ1-2_S4_R1_001_val.mm9.sorted.RmDup.10mNorm.bw

Genome browser session (e.g. UCSC)

No longer applicable.

Methodology

Replicates

ChIP-seq was performed with a singlicate for each condition, but validation of signal was done by ChIP-qPCR in triplicate, with high concordance. ATAC-seq was performed in duplicate, with two different Tet1/2 double knockouts, hence four ATAC-seq profiles per condition for the knockout experiements.

Sequencing depth

For ChIP-seq, the sequencing depth varied between 18.96 million reads (9.48 mil fragments; ChIP_SL2i-Med1) at the minimum and 73.6 million reads (36.8 mil fragments; ChIP_SL2i-Brd4) at the maximum.
For ATAC-seq, the sequencing depth varied between 33 million reads (16.5 mil fragments; ATACseq_JQ1_2) at the minimum and 215 million reads (107.5 mil fragments; ATAC_5-14-Nanog-Tg-JQ1-1) at the maximum. Paired-end sequencing was used for both ChIP and ATAC.

Antibodies

ChIP; Brd4 (Bethyl A301-985A50, Lot A301-985A50-4 and A301-985A50-5)
Med1 (CRSP1/TRAP220, Bethyl A300-793A, Lot A300-793A-3)
Nanog (Cosmo Bio REC-RCAB002P-F, lot A01OA06)
at 10 ug/mL (5.5 ug in 550 uL for 10 million cells).

Peak calling parameters

Enriched regions were discovered using MACS and scored against matched input libraries (fold change > 2 and p-value < 0.005), filtered for ENCODE black-listed regions, and peaks within 500 bp were merged.

Data quality

Signal-to-noise ratio was measured in 3 ways: 1) cross-correlation coefficient plot, 2) ratio of peak height to background levels in a composite plot over all significant peaks, 3) number of peaks called relative to matched input sample. Counts for peaks meeting fold change and FDR threshold ranged from 897 (inhibitor treatment) to 30,569 (control).

Software

ChIP-seq data was analyzed using samtools, bowtie, Bedtools, bwtools, ngsplot, DESeq2, and R.

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

ESC cell lines were removed from gelatin coated plates using Accutase cell detachment solution (Thermo Fisher A1110501) for 5

Sample preparation	minutes at 37C. They were centrifuged, washed 3x in FACS buffer and resuspended in FACS buffer containing DAPI. They were analyzed on a flow cytometer within 30 minutes.
Instrument	BD LSR Fortessa (for analysis), BD FACSAria III (for sorting)
Software	FACSDiva was used for data acquisition. FlowJo 9.0 was used for data analysis.
Cell population abundance	Clonal cell lines were used for all experiments.
Gating strategy	The gating strategy was initially FSC-A by SSC-A, followed by doublet exclusion using FSC-H and SSC-H, and finally DAPI exclusion using FSC-A by DAPI-A in which DAPI-negative cells were in the lowest quartile of DAPI-A expression. In all cases, DAPI positive cells retained 100x more DAPI than negative cells.

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