nature research

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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 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	none
Data analysis	Example analysis pipeline provided with code within the protocol. Publicly available software bowtie2 2.3.2 (http://bowtie-bio.sourceforge.net/bowtie2/index.shtml), SAMtools 1.3.1 (http://www.htslib.org/download), Picard (https://broadinstitute.github.io/picard/), FastQC v0.11.9 (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/), HOMER v4.11 (http://homer.ucsd.edu/homer/), deepTools (https://deeptools.readthedocs.io/en/develop/index.html)

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

Sequencing data have been deposited at Gene Expression Omnibus with accession code GSE111121, originally generated for Hainer et al. Cell 2019. These data are already available.

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.
Data exclusions	There are no data exclusions
Replication	The exact number of replicates is stated in the relevant legend.
Randomization	No randomization. This is not relevant because the samples form defined groups.
Blinding	No blinding. Blinding is not relevant to the study. The groups were defined and studied by the investigator using standard protocols.

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
☒	☐ Clinical data

Methods

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

Antibodies

Antibodies used	CTCF Millipore, cat# 07-729 From original publication: Antibodies used were H3K4me3 (Millipore 05-745R), H3K27ac (Abcam ab4729), H3K27me3 (Millipore 07-449), CTCF (Millipore 07-729), OCT4 (Thermofisher 701756), SOX2 (Active Motif 39843), NANOG (Active Motif 61419), BRG1 (Bethyl Labs A300-813), SUZ12 (Bethyl Labs A302-407A), IgG (Millipore 06-371)
Validation	Millipore 07-729: Manufacturer has validated in ChIP, WB, ChIP-seq to detect CTCF. Additionally, our analysis is compared to prior ChIP-seq datasets that used alternative antibodies. Finally CTCF has a prominent DNA sequence motif which can be seen from peaks called from data utilizing this antibody. Also confirmed for ChIP-seq in mouse. Used in many publications Millipore 05-745R: confirmed for ChIP-seq in mouse. Used in many publications Abcam ab4729: ChIP-seq grade in mouse. Used in many publications Millipore 07-449: confirmed for ChIP-seq in mouse. Used in many publications, including the original CUT&RUN paper (Skene and Henikoff eLIFE 2017) Millipore 07-729: confirmed for ChIP-seq in mouse. Used in many publications, including the original CUT&RUN paper (Skene and Henikoff eLIFE 2017) Thermofisher 701756: confirmed for ChIP-seq in mouse. Active Motif 39843: confirmed for ChIP-seq in mouse. Active Motif 61419: confirmed for ChIP-seq in mouse. Bethyl Labs A300-813: confirmed for IP in mouse. Used in Ho et al 2009 PNAS. Bethyl Labs A302-407A: confirmed for ChIP-seq in mouse. Used in many publications.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	E14 mouse ES cells
Authentication	These cell lines were not authenticated
Mycoplasma contamination	Cells were previously tested to ensure they were free of mycoplasma
Commonly misidentified lines (See ICLAC register)	n/a

Animals and other organisms

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

Laboratory animals	Mouse (Mus musculus) strain FVB was used for embryo studies. Superovulated females approximately 8 weeks old were mated and zygotes were collected ~17-19 hours post-addition of hCG. All animal studies were performed in accordance with with UMMS guidelines on animal care.
Wild animals	n/a
Field-collected samples	n/a
Ethics oversight	These studies were approved by the Institutional Animal Care and Use Committee of University of Massachusetts Medical School (approval number A-1833).

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.*	uliCUT&RUN data is deposited on GEO (GSE111121), as from original publication (Hainer et al Cell 2019)
Files in database submission	NoAb_500000cells_R1.fq.gz NoAb_50000cells_R1.fq.gz NoAb_5000cells_R1.fq.gz NoAb_500cells_R1.fq.gz NoAb_50cells_R1.fq.gz NoAb_10cells_R1.fq.gz CTCF_500000cells_R1.fq.gz CTCF_50000cells_R1.fq.gz CTCF_5000cells_R1.fq.gz CTCF_500cells_R1.fq.gz CTCF_50cells_R1.fq.gz CTCF_10cells_R1.fq.gz H3K4me3_500000cells_R1.fq.gz H3K4me3_50000cells_R1.fq.gz H3K4me3_5000cells_R1.fq.gz H3K4me3_500cells_R1.fq.gz H3K4me3_50cells_R1.fq.gz H3K4me3_10cells_R1.fq.gz NoAb_500,000cells_rep2_R1.fq.gz NoAb_50,000cells_rep2_R1.fq.gz NoAb_5,000cells_rep2_R1.fq.gz NoAb_500cells_rep2_R1.fq.gz NoAb_50cells_rep2_R1.fq.gz NoAb_10cells_rep2_R1.fq.gz CTCF_500,000cells_rep2_R1.fq.gz CTCF_50,000cells_rep2_R1.fq.gz CTCF_5,000cells_rep2_R1.fq.gz CTCF_500cells_rep2_R1.fq.gz CTCF_50cells_rep2_R1.fq.gz CTCF_10cells_rep2_R1.fq.gz

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NoAb_forIgG_50cells_rep2_1-120.ucsc.bedGraph.gz
Suz12_50000cells_1-120.ucsc.bedGraph.gz
Suz12_50000cells_rep2_1-120.ucsc.bedGraph.gz
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Suz12_50cells_rep2_1-120.ucsc.bedGraph.gz
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IgG_50000cells_rep2_1-120.ucsc.bedGraph.gz

IgG_50cells_1-120.ucsc.bedGraph.gz
IgG_50cells_rep2_1-120.ucsc.bedGraph.gz

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CTCF_1cell_rep4_1-120.ucsc.bedGraph.gz
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CTCF_1cell_rep20_1-120.ucsc.bedGraph.gz
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NoAb_1cell_forN&S_rep2_1-120.ucsc.bedGraph.gz
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NoAb_1cell_forN&S_rep4_1-120.ucsc.bedGraph.gz
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NoAb_1cell_forN&S_rep9_1-120.ucsc.bedGraph.gz
NoAb_1cell_forN&S_rep10_1-120.ucsc.bedGraph.gz
NoAb_1cell_forN&S_rep11_1-120.ucsc.bedGraph.gz
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Nanog_1cell_rep21_1-120.ucsc.bedGraph.gz
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Nanog_1cell_rep26_1-120.ucsc.bedGraph.gz
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Sox2_1cell_rep3_1-120.ucsc.bedGraph.gz
Sox2_1cell_rep4_1-120.ucsc.bedGraph.gz
Sox2_1cell_rep5_1-120.ucsc.bedGraph.gz
Sox2_1cell_rep6_1-120.ucsc.bedGraph.gz
Sox2_1cell_rep7_1-120.ucsc.bedGraph.gz
Sox2_1cell_rep8_1-120.ucsc.bedGraph.gz
Sox2_1cell_rep9_1-120.ucsc.bedGraph.gz
Sox2_1cell_rep10_1-120.ucsc.bedGraph.gz
Sox2_1cell_rep11_1-120.ucsc.bedGraph.gz
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Sox2_1cell_rep13_1-120.ucsc.bedGraph.gz
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Sox2_1cell_rep26_1-120.ucsc.bedGraph.gz

blast_NoAb_rep1_1-120.ucsc.bedGraph.gz
blast_NoAb_rep2_1-120.ucsc.bedGraph.gz
blast_CTCF_rep1_1-120.ucsc.bedGraph.gz
blast_CTCF_rep2_1-120.ucsc.bedGraph.gz
blast_EGFPKD_NoAb_rep1_1-120.ucsc.bedGraph.gz
blast_EGFPKD_NoAb_rep2_1-120.ucsc.bedGraph.gz
blast_EGFPKD_NoAb_rep3_1-120.ucsc.bedGraph.gz
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blast_EGFPKD_Nanog_rep3_1-120.ucsc.bedGraph.gz
blast_EGFPKD_Nanog_rep4_1-120.ucsc.bedGraph.gz
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blast_NanogKD_NoAb_rep2_1-120.ucsc.bedGraph.gz
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blast_NanogKD_NoAb_rep4_1-120.ucsc.bedGraph.gz
blast_NanogKD_Nanog_rep1_1-120.ucsc.bedGraph.gz
blast_NanogKD_Nanog_rep2_1-120.ucsc.bedGraph.gz
blast_NanogKD_Nanog_rep3_1-120.ucsc.bedGraph.gz
blast_NanogKD_Nanog_rep4_1-120.ucsc.bedGraph.gz

Genome browser session
(e.g. UCSC)

n/a

Methodology

Replicates

In the original manuscript:
For Single cell experiments, we performed 120 replicates of CTCF and 24 replicates each of SOX2 and NANOG. For blastocyst experiments, pilot studies for CTCF were performed in duplicate, and four replicates were performed for studies using various knockdowns combined with NANOG uliCUT&RUN.

Sequencing depth

Number of reads followed by uniquely mapped reads to mm10 genome for each sample are as follows (information also included in Supplemental Table 1 in Hainer et al 2019 Cell):

FIG 1:
NoAb_500,000cells 12519390 7724115
NoAb_50,000cells 21124738 13619070
NoAb_5,000cells 22840561 13639479
NoAb_500cells 6721902 2738302
NoAb_50cells 31441140 9944171
NoAb_10cells 22386707 1528715
CTCF_500,000cells 24622812 16910022
CTCF_50,000cells 28244629 19181790
CTCF_5,000cells 86425016 55116921
CTCF_500cells 16011197 7590239
CTCF_50cells 33064993 18592267
CTCF_10cells 52838179 12155437
H3K4me3_500,000cells 18839167 12920028
H3K4me3_50,000cells 24872314 16633943
H3K4me3_5,000cells 9044688 4884909
H3K4me3_500cells 24310031 9846875
H3K4me3_50cells 14117721 1111431
H3K4me3_10cells 39686252 9387951

NoAb_500,000cells_rep2 20593762 14113161
NoAb_50,000cells_rep2 11497226 6962975
NoAb_5,000cells_rep2 16912830 8253228

NoAb_500cells_rep2 9929420 5568228
NoAb_50cells_rep2 15215049 4453136
NoAb_10cells_rep2 12391392 3022213
CTCF_500,000cells_rep2 22683276 15804167
CTCF_50,000cells_rep2 18614886 12554552
CTCF_5,000cells_rep2 14718045 9230719
CTCF_500cells_rep2 14717536 6419888
CTCF_50cells_rep2 13597611 4411910
CTCF_10cells_rep2 4296546 495292
H3K4me3_500,000cells_rep2 13045986 9232784
H3K4me3_50,000cells_rep2 12417936 8656475
H3K4me3_5,000cells_rep2 16750683 10678006
H3K4me3_500cells_rep2 7110613 3467797
H3K4me3_50cells_rep2 21568103 3645797
H3K4me3_10cells_rep2 9512139 1566495

FIG 2:
Fig2_NoAb_50000cells 9345006 5773858
Fig2_NoAb_50cells 10649017 2724091
Sox2_50000cells 11083895 6895275
Sox2_50cells 11853738 5178294
Nanog_50000cells 17263515 11334303
Nanog_50cells 19016839 8492761
Brg1_50000cells 6835700 4202706
Brg1_50cells 3206583 1771877
K27ac_50000cells 7771158 5511044
K27ac_50cells 10034451 3102591
K27me3_50000cells 13393265 8896074
K27me3_50cells 15527909 7857024
Fig2_2NoAb_(for Oct4)_50000cells 13630695 7902256
Fig2_2NoAb_(for Oct4)_50cells 13523229 2474932
Oct4_50000cells 20192783 11134710
Oct4_50cells 12542977 3682460

Fig2_NoAb_50000cells_rep2 25602586 14776121
Fig2_NoAb_50cells_rep2 26177953 3759274
Sox2_50000cells_rep2 25670648 12942925
Sox2_50cells_rep2 37306970 3788437
Nanog_50000cells_rep2 32483165 11903946
Nanog_50cells_rep2 11878529 4292347
Oct4_50000cells_rep2 12058400 7994638
Oct4_50cells_rep2 54221820 5211918
Brg1_50000cells_rep2 35673076 23875169
Brg1_50cells_rep2 16785904 5487185
K27ac_50000cells_rep2 23847927 16297598
K27ac_50cells_rep2 21770144 4344808
K27me3_50000cells_rep2 6612249 4298777
K27me3_50cells_rep2 32824299 13067644
NoAb_forIgG_50000cells 13448716 9008811
NoAb_forIgG_50000cells_rep2 11798458 7014619
NoAb_forIgG_50cells 10024548 4066899
NoAb_forIgG_50cells_rep2 7708842 1211679
Suz12_50000cells 13905377 9229256
Suz12_50000cells_rep2 21037025 13973323
Suz12_50cells 12605631 3665614
Suz12_50cells_rep2 13455514 5941880
IgG_50000cells 14316736 8796196
IgG_50000cells_rep2 16243207 9371814
IgG_50cells 11516348 4964564
IgG_50cells_rep2 5651736 1507348

FIG 3:
NoAb_1cell_rep1 23265110 74478
NoAb_1cell_rep2 27236861 97674
NoAb_1cell_rep3 21219173 50829
NoAb_1cell_rep4 21366345 226075
NoAb_1cell_rep5 64767963 196799
NoAb_1cell_rep6 22255979 143707
NoAb_1cell_rep7 47872691 159939
NoAb_1cell_rep8 18361464 55812
NoAb_1cell_rep9 17025862 54651
NoAb_1cell_rep10 25783042 95596
NoAb_1cell_rep11 51097030 951974
NoAb_1cell_rep12 7306990 144302
NoAb_1cell_rep13 10765019 137413

NoAb_1cell_rep14 15414075 211302
NoAb_1cell_rep15 4560616 15674
NoAb_1cell_rep16 13356346 259673
NoAb_1cell_rep17 7888316 34096
NoAb_1cell_rep18 3622064 29806
NoAb_1cell_rep19 9849232 30348
NoAb_1cell_rep20 7466954 73436
NoAb_1cell_rep21 5306826 18290
NoAb_1cell_rep22 2137806 13625
NoAb_1cell_rep23 3784161 84544
NoAb_1cell_rep24 13008536 71747
NoAb_1cell_rep25 6039547 30242
NoAb_1cell_rep26 5047020 5058
NoAb_1cell_rep27 6531208 7599
NoAb_1cell_rep28 14211594 13236
NoAb_1cell_rep29 6535060 21366
NoAb_1cell_rep30 9381716 4679
NoAb_1cell_rep31 4413640 18879
NoAb_1cell_rep32 7336915 9345
NoAb_1cell_rep33 21868740 44291
NoAb_1cell_rep34 4780084 23385
NoAb_1cell_rep35 1307808 8683
NoAb_1cell_rep36 7309121 15815
NoAb_1cell_rep37 2006659 8410
NoAb_1cell_rep38 1856569 8333
NoAb_1cell_rep39 9015700 42598
NoAb_1cell_rep40 17461032 54256
NoAb_1cell_rep41 5239808 14494
NoAb_1cell_rep42 3567351 5236
NoAb_1cell_rep43 6091229 20725
NoAb_1cell_rep44 43333541 1129419
NoAb_1cell_rep45 5447631 26416
NoAb_1cell_rep46 17642275 450947
NoAb_1cell_rep47 545288 7280
CTCF_1cell_rep1 8058422 33946
CTCF_1cell_rep2 29785051 117613
CTCF_1cell_rep3 39235948 157304
CTCF_1cell_rep4 25623725 167324
CTCF_1cell_rep5 27905711 97412
CTCF_1cell_rep6 34558783 586829
CTCF_1cell_rep7 31415006 114513
CTCF_1cell_rep8 39751033 286283
CTCF_1cell_rep9 31930597 127751
CTCF_1cell_rep10 35583144 252097
CTCF_1cell_rep11 47464970 493706
CTCF_1cell_rep12 49260090 247257
CTCF_1cell_rep13 8114649 207722
CTCF_1cell_rep14 12455981 190767
CTCF_1cell_rep15 20590831 653284
CTCF_1cell_rep16 11065940 376148
CTCF_1cell_rep17 13057364 33945
CTCF_1cell_rep18 15230400 264137
CTCF_1cell_rep19 10923002 195014
CTCF_1cell_rep20 5624046 12828
CTCF_1cell_rep21 7888563 1001971
CTCF_1cell_rep22 6646912 10945
CTCF_1cell_rep23 5616498 17848
CTCF_1cell_rep24 6665353 108780
CTCF_1cell_rep25 3416068 9977
CTCF_1cell_rep26 13061772 53157
CTCF_1cell_rep27 10063453 34834
CTCF_1cell_rep28 20222034 175670
CTCF_1cell_rep29 3094096 18303
CTCF_1cell_rep30 9889585 62981
CTCF_1cell_rep31 7577446 30305
CTCF_1cell_rep32 4857445 15791
CTCF_1cell_rep33 28434201 445419
CTCF_1cell_rep34 16620922 204641
CTCF_1cell_rep35 22746815 99579
CTCF_1cell_rep36 6668233 19208
CTCF_1cell_rep37 6955774 73905
CTCF_1cell_rep38 8409569 21070
CTCF_1cell_rep39 6893828 14734
CTCF_1cell_rep40 10879884 33382
CTCF_1cell_rep41 6990358 41126

CTCF_1cell_rep42 2151399 27091
CTCF_1cell_rep43 4719110 19000
CTCF_1cell_rep44 2739562 44846
CTCF_1cell_rep45 16421665 159443
CTCF_1cell_rep46 2514904 13440
CTCF_1cell_rep47 9281550 10763
CTCF_1cell_rep48 3147939 3462
CTCF_1cell_rep49 16823322 21578
CTCF_1cell_rep50 5203677 176284
CTCF_1cell_rep51 12602737 2393874
CTCF_1cell_rep52 4285786 9742
CTCF_1cell_rep53 26110941 5237
CTCF_1cell_rep54 14015788 20490
CTCF_1cell_rep55 9262580 10743
CTCF_1cell_rep56 23247285 20190
CTCF_1cell_rep57 10189952 12885
CTCF_1cell_rep58 21717639 8101
CTCF_1cell_rep59 10935367 409959
CTCF_1cell_rep60 7657251 14359
CTCF_1cell_rep61 3842865 1934
CTCF_1cell_rep62 38698352 27523
CTCF_1cell_rep63 12287371 11138
CTCF_1cell_rep64 31268573 10424
CTCF_1cell_rep65 3758641 4882
CTCF_1cell_rep66 15850734 15177
CTCF_1cell_rep67 13647142 12074
CTCF_1cell_rep68 7707421 8557
CTCF_1cell_rep69 25195702 12418
CTCF_1cell_rep70 6511340 10373
CTCF_1cell_rep71 19409722 4144
CTCF_1cell_rep72 9506917 23840
CTCF_1cell_rep73 11757049 9091
CTCF_1cell_rep74 11024329 4913
CTCF_1cell_rep75 5698628 8461
CTCF_1cell_rep76 10819532 13896
CTCF_1cell_rep77 8652906 22913
CTCF_1cell_rep78 2178057 6008
CTCF_1cell_rep79 20753776 85460
CTCF_1cell_rep80 8720515 33530
CTCF_1cell_rep81 7734254 26956
CTCF_1cell_rep82 17762621 129075
CTCF_1cell_rep83 44830993 28607
CTCF_1cell_rep84 9748327 6433
CTCF_1cell_rep85 3795644 11738
CTCF_1cell_rep86 1740613 9915
CTCF_1cell_rep87 4703388 21769
CTCF_1cell_rep88 10863160 10176
CTCF_1cell_rep89 8251239 4497
CTCF_1cell_rep90 8110496 8805
CTCF_1cell_rep91 5500707 63340
CTCF_1cell_rep92 2395382 5602
CTCF_1cell_rep93 3781032 14126
CTCF_1cell_rep94 37748446 654482
CTCF_1cell_rep95 16385785 11235
CTCF_1cell_rep96 4829531 14551
CTCF_1cell_rep97 3877652 8730
CTCF_1cell_rep98 29509526 5233
CTCF_1cell_rep99 11050635 9649
CTCF_1cell_rep100 22733078 5766
CTCF_1cell_rep101 4046874 28113
CTCF_1cell_rep102 11056744 7330
CTCF_1cell_rep103 15128411 37079
CTCF_1cell_rep104 7125043 17702
CTCF_1cell_rep105 35176802 38717
CTCF_1cell_rep106 5665082 9264
CTCF_1cell_rep107 22287272 9174
CTCF_1cell_rep108 13333462 7955
CTCF_1cell_rep109 17717525 7440
CTCF_1cell_rep110 15591679 46916
CTCF_1cell_rep111 5126661 11343
CTCF_1cell_rep112 10157718 8044
CTCF_1cell_rep113 9840354 9901
CTCF_1cell_rep114 2054976 12701
CTCF_1cell_rep115 5162319 12165
CTCF_1cell_rep116 5637212 30982

CTCF_1cell_rep117 8417283 22157
CTCF_1cell_rep118 7337851 13838
CTCF_1cell_rep119 44335857 10368
CTCF_1cell_rep120 10290979 5823
NoAb_1cell_forN&S_rep1 19220791 4545
NoAb_1cell_forN&S_rep2 11138973 1256
NoAb_1cell_forN&S_rep3 30159465 3506
NoAb_1cell_forN&S_rep4 10123497 1002
NoAb_1cell_forN&S_rep5 21577700 949
NoAb_1cell_forN&S_rep6 13976915 843
NoAb_1cell_forN&S_rep7 20051488 4716
NoAb_1cell_forN&S_rep8 17917883 11187
NoAb_1cell_forN&S_rep9 9245951 9315
NoAb_1cell_forN&S_rep10 12541873 4820
NoAb_1cell_forN&S_rep11 11293016 5630
NoAb_1cell_forN&S_rep12 3142984 92358
Nanog_1cell_rep1 68117715 574295
Nanog_1cell_rep2 72371907 861323
Nanog_1cell_rep3 11152562 21321
Nanog_1cell_rep4 8669223 30834
Nanog_1cell_rep5 44573865 234500
Nanog_1cell_rep6 10934823 13747
Nanog_1cell_rep7 18949187 16517
Nanog_1cell_rep8 8639013 2284
Nanog_1cell_rep9 17382264 1914
Nanog_1cell_rep10 16930096 4832
Nanog_1cell_rep11 7366442 2775
Nanog_1cell_rep12 14396797 6504
Nanog_1cell_rep13 12473737 2393
Nanog_1cell_rep14 2427303 4030
Nanog_1cell_rep15 10303883 33231
Nanog_1cell_rep16 4048912 2131
Nanog_1cell_rep17 13334284 57854
Nanog_1cell_rep18 2913148 4993
Nanog_1cell_rep19 20806419 3193
Nanog_1cell_rep20 8238323 1591
Nanog_1cell_rep21 8443401 8583
Nanog_1cell_rep22 12209505 14352
Nanog_1cell_rep23 665983 35185
Nanog_1cell_rep24 4162105 6452
Nanog_1cell_rep25 1832338 5207
Nanog_1cell_rep26 879183 3053
Sox2_1cell_rep1 52283680 472339
Sox2_1cell_rep2 15839179 138569
Sox2_1cell_rep3 3233851 5302
Sox2_1cell_rep4 14648677 4985
Sox2_1cell_rep5 9044133 5931
Sox2_1cell_rep6 14375296 1503
Sox2_1cell_rep7 2536082 3867
Sox2_1cell_rep8 7506021 11533
Sox2_1cell_rep9 13417179 9148
Sox2_1cell_rep10 10037743 27332
Sox2_1cell_rep11 32804407 39873
Sox2_1cell_rep12 15034226 14899
Sox2_1cell_rep13 17537185 12571
Sox2_1cell_rep14 8800458 7868
Sox2_1cell_rep15 14909905 6620
Sox2_1cell_rep16 13875212 10189
Sox2_1cell_rep17 4537955 4473
Sox2_1cell_rep18 10830162 5210
Sox2_1cell_rep19 13119087 6977
Sox2_1cell_rep20 2777768 11740
Sox2_1cell_rep21 6402824 6634
Sox2_1cell_rep22 5960732 5592
Sox2_1cell_rep23 10125200 6953
Sox2_1cell_rep24 2917622 3324
Sox2_1cell_rep25 24192050 4361
Sox2_1cell_rep26 7685182 1788

FIG 4:
blast_NoAb_rep1 48402426 134232
blast_NoAb_rep2 10924378 261010
blast_CTCF_rep1 74269402 556739
blast_CTCF_rep2 81540028 468019
blast_EGFP KD_NoAb rep1 31459660 288824

blast_EGFP KD_NoAb rep2 34345741 1489001
blast_EGFP KD_NoAb rep3 39988015 1287868
blast_EGFP KD_NoAb rep4 6197448 1108986
blast_EGFP KD_Nanog rep1 60127971 2001462
blast_EGFP KD_Nanog rep2 22519173 2819130
blast_EGFP KD_Nanog rep3 13662166 1665987
blast_EGFP KD_Nanog rep4 35537566 237104
blast_Brg1 KD_NoAb rep1 7680963 524581
blast_Brg1 KD_NoAb rep2 65438846 3658475
blast_Brg1 KD_NoAb rep3 45326808 467744
blast_Brg1 KD_NoAb rep4 15634958 667208
blast_Brg1 KD_Nanog rep1 28438200 854190
blast_Brg1 KD_Nanog rep2 15083658 1125809
blast_Brg1 KD_Nanog rep3 100185187 14579478
blast_Brg1 KD_Nanog rep4 24195375 4407992
blast_Nanog KD_NoAb rep1 24188051 8207258
blast_Nanog KD_NoAb rep2 36407453 3586859
blast_Nanog KD_NoAb rep3 60034257 7218307
blast_Nanog KD_NoAb rep4 31153933 2323140
blast_Nanog KD_Nanog rep1 25382374 1133411
blast_Nanog KD_Nanog rep2 52788888 1459562
blast_Nanog KD_Nanog rep3 22679561 1530643
blast_Nanog KD_Nanog rep4 8460423 216498

Antibodies

anti-CTCF, millipore, Cat# 07-729, lot 2475698, used at 1:100 final; anti-H3K4me3, millipore, Cat# 05-745R, lot 2848536, used at 1:100 final; anti-Oct4, Invitrogen, Cat# A13998, lot RK2423646, used at 1:100 final; anti-Sox2, Active Motif, Cat# 39843, Lot 19710001, used at 1:100 final; anti-Nanog, Active Motif, Cat# 61419, Lot 04613001, used at 1:100 final; anti-Brg1, Bethyl, Cat# A300-813A, Lot 3, used at 1:100 final; anti-H3K27me3, Millipore, Cat# 07449, Lot JBC1873477, used at 1:100 final; anti-H3K27ac, abcam, Cat# ab4729, Lot GR104852-1, used at 1:100 final; anti-Suz12, Bethyl Labs Cat# A302-407A, Lot 1, used at 1:100 final; and IgG, Millipore, Cat# 06-371, Lot 2967773, used at 1:100 final.

Peak calling parameters

Reads were aligned to mm10 using Bowtie2 with the parameter -X 1000
Peaks were called using the "findPeaks" command in HOMER (http://homer.ucsd.edu/homer/index.html).
Controls were no primary antibody (referred to throughout as "No Antibody") paired with each experiment

Data quality

Mapping of CUT&RUN data is compared to previously published ChIP-seq data throughout.
Peak calling for CUT&RUN data is performed using HOMER "findPeak" commands. The control is the "No Antibody" experiment, paired with each experimental condition.
HOMER uses two parameters to filter peaks against a control experiment. First, it uses a fold change (which is sequencing depth-independent), requiring each putative peak to have 5-fold more normalized tags in the target experiment than the control. In the case where there are no input tags near the putative peak, HOMER automatically sets these regions to be set to the average input tag coverage to avoid dividing by zero. HOMER also uses the poisson distribution to determine the chance that the differences in tag counts are statistically significant (sequencing-depth dependent), requiring a cumulative poisson p-value of 0.0001. This effectively removes peaks with low tag counts for which there is a chance the differential enrichment is found simply due to sampling error.

HOMER assumes the local density of tags follows a Poisson distribution, and uses this to estimate the expected peak numbers given the input parameters much more quickly. Using the expected distribution of peaks, HOMER calculates the expected number of false positives in the data set for each tag threshold, setting the threshold that beats the desired False Discovery Rate, automatically set to 5%.

Software

eads were aligned to mm10 using Bowtie2 with the parameter -X 1000
Unique reads were identified using Picard.
Reads were separated into the following size classes: <120bp for TF occupancy and 150-500bp for nucleosome occupancy using the "awk" command and samtools.
Reads were processed using deepTools to generate heatmaps and metaplots.
Reads were processed using HOMER (http://homer.ucsd.edu/homer/index.html) commands including:
"findPeaks"; "mergePeaks"; "findMotifs"