doi:10.1038/nature19360

Supplementary Tables

Supplementary Table 1. Number of monoclonal reads in each sample

Sample	Number of cells	Total reads	Aligned reads	Monoclonal reads
H3K4me3_P12_rep1	1500	42406818	35819301	7951482
H3K4me3_P12_rep2	1500	37451114	26531389	8494370
H3K4me3_P15_rep1	1500	45562042	40449262	13629478
H3K4me3_P15_rep2	1500	34095651	28941929	11216661
H3K4me3_Oocyte_rep1	1000	64687515	25152463	18678880
H3K4me3_Oocyte_rep2	1000	56962570	45504181	23149474
H3K4me3_2-cell_rep1	1000	24533989	17552064	8237581
H3K4me3_2-cell_rep2	1000	58565093	39766427	6967788
H3K4me3_8-cell_rep1	1500	22409099	13868776	4913167
H3K4me3_8-cell_rep2	1500	57241614	38082347	9971820
H3K27ac_Oocyte_rep1	1000	47940097	30579189	1080322
H3K27ac_Oocyte_rep2	1000	64831957	43908671	2712705
H3K27ac_2-cell_rep1	1000	20018649	14678773	4470385
H3K27ac_2-cell_rep2	1000	58017747	45751884	3163436
H3K27ac_8-cell_rep1	1500	14728967	10072429	2377115
H3K27ac_8-cell_rep2	1500	51818076	36478666	3350257
Input_P12_rep1	1000	38148943	33707795	19403310
Input_P12_rep2	1000	36507990	32250202	18562435
Input_P15_rep1	1000	34694347	30555061	19088000
Input_P15_rep2	1000	36609643	32243154	19265465
Input_oocyte_rep1	1000	77683977	57809011	21063142
Input_oocyte_rep2	1000	60585586	45572881	18133223
Input_2cell_rep1	1000	25693778	18939115	14293389
Input_2cell_rep2	1000	75331284	57525320	22266255
Input_8cell_rep1	1000	23137193	17012428	13090336
Input_8cell_rep2	1000	32939541	24612274	15384924

Supplementary Table 2. KAPA qPCR based quantification of ChIP-seq libraries to assess relative amounts of H3K4me3 ChIP precipitated DNA

Sample	Ct	dCt	Log	Relative amount	Fraction
Oocyte	13.1		0.06	1.15	1.00
2-cell	15.9	2.7	-0.74	0.18	0.16
8-cell	16.6	3.5	-0.95	0.11	0.10
P12	15.9	2.8	-0.76	0.18	0.15
P15	15.4	2.3	-0.61	0.25	0.21

Supplementary Table 3. Summary of WGBS results from P12 and P15

Sample	Bisulfite conversion rate	Coverage of CpG sites	Average CpG methylation	Average read depth of CpG sites
P12	99%	93%	19%	9.06
P15	99%	93%	25%	10.5

Supplementary Table 4. TF motif enrichment at distal *c*REs nearby ZGA genes

TF motif	Consensus sequence	P value
RARg(NR)/ES-RARg-ChIP-Seq(GSE30538)/Homer	AGGTCAAGGTCA	10^{-67}
RLR1?/SacCer-Promoters/Homer	WTTTTCYYTTTT	10^{-45}
Nr5a2(NR)/mES-Nr5a2-ChIP-Seq/Homer	BTCAAGGTCA	10^{-41}
CRX(Homeobox)/Retina-Crx-ChIP-Seq/Homer	GCTAATCC	10^{-38}
Nr5a2(NR)/Pancreas-LRH1-ChIP-Seq(GSE34295)/Homer	BTCAAGGTCA	10^{-36}
Pho2(bHLH)/Yeast-Pho2-ChIP-Seq(GSE29506)/Homer	CCCACGTGCT	10^{-30}
c-Myc/mES-cMyc-ChIP-Seq/Homer	VVCCACGTGG	10^{-27}
n-Myc(HLH)/mES-nMyc-ChIP-Seq/Homer	VRCCACGTGG	10^{-25}
PIF5ox(bHLH)/Arabidopsis-PIF5ox-ChIP-Seq(GSE35062)/Homer	BCACGTGVDN	10^{-21}
ZNF143|STAF(Zf)/CUTLL-ZNF143-ChIP-Seq(GSE29600)/Homer	ATTTCCCAGVAKSCY	10^{-21}
ARE(NR)/LNCAP-AR-ChIP-Seq(GSE27824)/Homer	RGRACASNSTGTYCYB	10^{-20}
Pitx1(Homeobox)/Chicken-Pitx1-ChIP-Seq(GSE38910)/Homer	TAATCCCN	10^{-19}
Max(HLH)/K562-Max-ChIP-Seq/Homer	RCCACGTGGYYN	10^{-18}
Rfx5(HTH)/GM12878-Rfx5-ChIP-Seq(GSE31477)/Homer	SCCTAGCAACAG	10^{-17}
Erra(NR)/HepG2-Erra-ChIP-Seq/Homer	CAAAGGTCAG	10^{-17}
BORIS(Zf)/K562-CTCFL-ChIP-Seq/Homer	CNNBRGCGCCCCCTGSTGGC	10^{-15}
Bcl6(Zf)/Liver-Bcl6-ChIP-Seq(GSE31578)/Homer	NNNCTTTCCAGGAAA	10^{-15}
PR(NR)/T47D-PR-ChIP-Seq(GSE31130)/Homer	VAGRACAKNCTGTBC	10^{-12}
GFY-Staf/Promoters/Homer	RACTACAATTCCCAGAAKGC	10^{-12}
GATA-DR8(Zf)/iTreg-Gata3-ChIP-Seq(GSE20898)/Homer	AGATSTNDNNDSAGATAASN	10^{-11}
Esrrb(NR)/mES-Esrrb-ChIP-Seq/Homer	KTGACCTTGA	10^{-09}
Gfi1b(Zf)/HPC7-Gfi1b-ChIP-Seq/Homer	MAATCACTGC	10^{-08}
FOXA1:AR/LNCAP-AR-ChIP-Seq(GSE27824)/Homer	AGTAAACAAAAAAGAACAND	10^{-08}
AR-halfsite(NR)/LNCaP-AR-ChIP-Seq(GSE27824)/Homer	CCAGGAACAG	10^{-08}
FOXA1(Forkhead)/MCF7-FOXA1-ChIP-Seq/Homer	WAAGTAAACA	10^{-07}
TR4(NR/DR1)/Hela-TR4-ChIP-Seq/Homer	GAGGTCAAAGGTCA	10^{-06}
TATA-box/SacCer-Promoters/Homer	BBHWTATATA	10^{-05}
MafK(bZIP)/C2C12-MafK-ChIP-Seq(GSE36030)/Homer	GCTGASTCAGCA	10^{-05}
FOXA1(Forkhead)/LNCAP-FOXA1-ChIP-Seq(GSE27824)/Homer	WAAGTAAACA	10^{-05}
p53(p53)/Saos-p53-ChIP-Seq/Homer	RRCATGYCYRGRCATGYYYN	10^{-05}

Supplementary Table 5. Genome Ontology analysis of stage restricted putative distal *c*REs

GO biological process	-log10 (P value)
mESC-restricted distal *c*REs	
Posttranscriptional regulation of gene expression	19.52
Stem cell development	16.32
Stem cell differentiation	16.19
Cell junction organization	16.08
Negative regulation of protein kinase activity	14.62
Oocyte restricted distal *c*REs	
Positive regulation of hormone biosyntheic process	25.63
Regulation of hormone biosyntehic process	21.01
Positive regulation of hormone metabolic process	20.66
Regulation of lipid metabolic process	18.53
Regulation of hormone metabolic process	18.25
2-cell restricted distal *c*REs	
Cell cycle	124.44
Protein modification by small protein conjugation or removal	90.70
M phase	88.60
Cell cycle phase	80.80
Cell division	80.38
8-cell restricted distal *c*REs	
Embryonic placenta development	19.08
Plancenta development	15.58
Regulation of circadian rhythm	14.87
Negative regulation of glial cell differentiation	14.40
Regulation of gliogenesis	12.70

Supplementary Table 6. Enriched mouse phenotypes of stage restricted putative distal *c*REs

Mouse phenotypes	-log10(P value)
mESC-restricted distal *c*REs	
Decreased embryo size	50.57
Abnormal embryo size	49.48
Decreased erythrocyte cell number	29.81
Small spleen	29.44
Abnormal erythrocyte cell number	28.22
Oocyte restricted distal *c*REs	
Abnormal cell adhesion	22.97
Abnormal bone volume	19.49
Decreased percent body fat	18.02
Abnpormal cell migration	17.49
Abnormal endometrial gland morphology	17.22
2-cell restricted distal *c*REs	
Embryonic lethality between implantation and placentation	57.21
Complete embryonic lethality before somite formation	47.91
Embryonic lethality before somite formation	47.11
Decreased cell proliferation	42.54
Abnormal cell content or morphology	41.69
8-cell restricted distal *c*REs	
Long tail	18.13
Abnormal allantois morphology	16.07
Abnormal type II pneumocyte morphology	15.05
Enlarged allantois	14.18
Abnormal pulmonary alveoulu epithelial cell morphology	12.31

Supplementary Table 7. Enriched GO biological processes at nearby genes for each TF motif containing stage restricted putative distal *c*REs

Stage	TF motif	Enriched top 3 GO biological processes	P value
Oocyte	Jun-Ap1	negative regulation of signal transduction	7.4e-04
	Jun-Ap1	negative regulation of cell communication	1.4e-03
	Jun-Ap1	response to hormone stimulus	2.2e-03
	BATCH2	negative regulation of signal transduction	1.2e-03
	BATCH2	intracellular signaling cascade	1.4e-03
	BATCH2	actin filament-based process	1.5e-03
	Nr5a2	enzyme linked receptor protein signaling pathway	1.5e-04
	Nr5a2	fatty acid metabolic process	3.8e-04
	Nr5a2	negative regulation of signal transduction	7.4e-04
2-cell	cMyc	protein transport	5.6e-09
	cMyc	establishment of protein localization	8.4e-09
	cMyc	protein localization	3.7e-08
	RARg	protein transport	2.0e-06
	RARg	macromolecule catabolic process	2.4e-06
	RARg	establishment of protein localization	2.7e-06
	Rfx5	cell cycle	2.4e-11
	Rfx5	RNA processing	1.3e-08
	Rfx5	protein localization	2.7E-07
ZGA	RARg	translation	1.2E-10
	RARg	cell cycle	4.6E-10
	RARg	RNA processing	5.2E-10
	RLR1	RNA processing	3.6E-15
	RLR1	cell cycle	3.5E-14
	RLR1	macromolecule catabolic process	8.4E-14
	Nr5a2	macromolecule catabolic process	3.7E-12
	Nr5a2	cellular macromolecule catabolic process	1.7E-11
	Nr5a2	protein catabolic process	9.7E-11
	cMyc	RNA processing	5.0E-16
	cMyc	mRNA metabolic process	1.1E-13
	cMyc	macromolecule catabolic process	1.2E-12
	CRX	RNA processing	6.4E-17
	CRX	cellular macromolecule catabolic process	8.6E-15
	CRX	macromolecule catabolic process	2.4E-14
2/8-cell	RARg	macromolecule catabolic process	3.2E-17
	RARg	cellular macromolecule catabolic process	4.6E-17
	RARg	cell cycle	2.6E-16
	Pho2	macromolecule catabolic process	2.1E-13
	Pho2	cellular macromolecule catabolic process	3.9E-13
	Pho2	protein catabolic process	4.7E-12
	Nr5a2	cellular macromolecule catabolic process	2.3E-17
	Nr5a2	macromolecule catabolic process	4.4E-17
	Nr5a2	cell cycle	1.4E-15
8-cell	RARg	phosphorus metabolic process	2.0E-03
	RARg	phosphate metabolic process	2.0E-03
	RARg	protein transport	2.9E-03
	p53	heart morphogenesis	2.8E-02
	p53	heart development	4.4E-02
	p53	muscle tissue morphogenesis	6.2E-02
	TEAD4	protein transport	2.2E-05
	TEAD4	establishment of protein localization	2.7E-05
	TEAD4	protein localization	4.4E-05
ES-cell	Oct4-Sox2-TCF-Nanog	tube development	7.9E-04
	Oct4-Sox2-TCF-	tube morphogenesis	3.0E-03

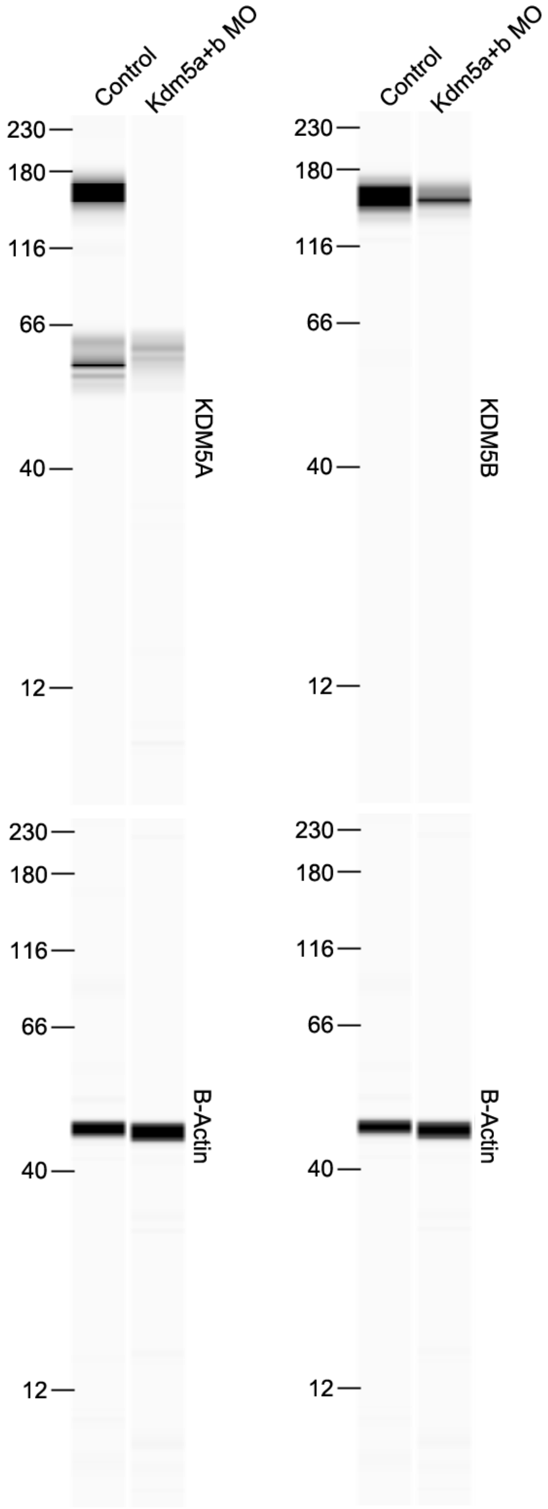
Nanog		
Oct4-Sox2-TCF-Nanog	positive regulation of macromolecule metabolic process	3.2E-03
p53	tolerance induction	1.3E-02
p53	cell division	5.9E-02
Sox2	tube development	2.5E-03
Sox2	establishment of cell polarity	2.6E-03
Sox2	vesicle-mediated transport	2.6E-03

Supplementary Table 8. RNA-seq of Kdm5a and 5b morpholino targeted 2-cell embryos and various controls

Sample	# of embryos	Replicate	Library Type	Uniquely mapped reads
Uninjected	25	rep1	Paired-end	12221992
Uninjected	25	rep2	Paired-end	10578894
α-amanitin	25	rep1	Paired-end	14844407
α-amanitin	25	rep2	Paired-end	11037116
Kdm5a+b MO	25	rep1	Paired-end	24831211
Kdm5a+b MO	25	rep2	Paired-end	19931118
Control MO	25	rep1	Paired-end	28897404
Control MO	25	rep2	Paired-end	19636896

Supplementary Table 9. PANTHER Overrepresentation Test for down-regulated genes after Kdm5a+b MO injection

PANTHER GO-slim biological process	P value
Cell-cell signaling	1.5E-3
Transport	2.6E-2
Metabolic process	2.0E-2



Supplementary Figure 1. Original Simple Western immunoblot images for Kdm5a and Kdm5b morpholino knock-down of KDM5A and KDM5B proteins in 2-cell embryos. Beta-actin is shown as loading control.