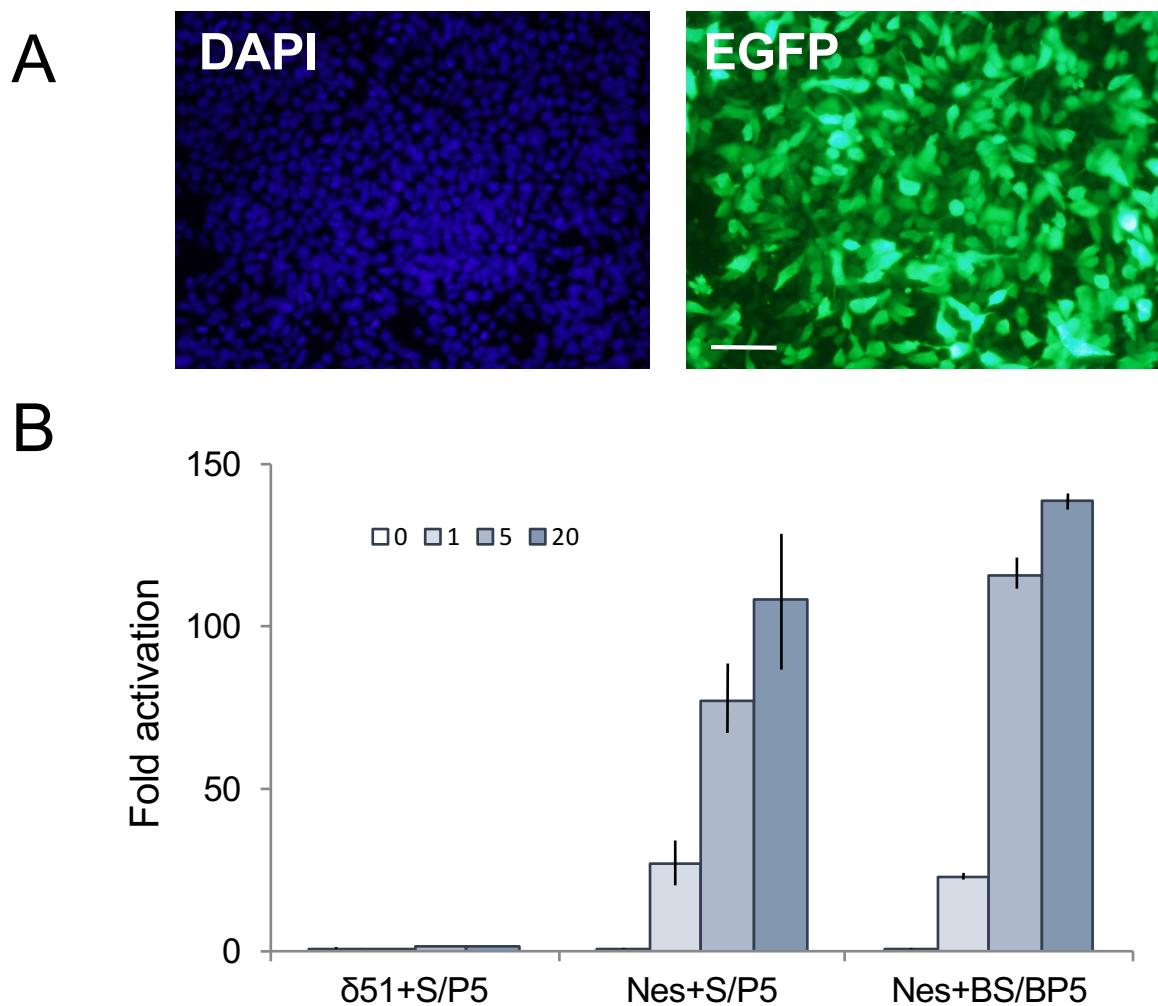
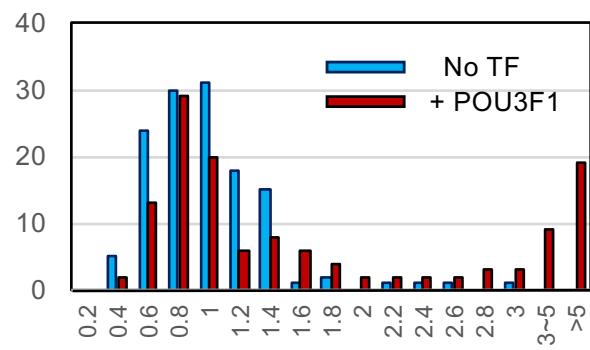
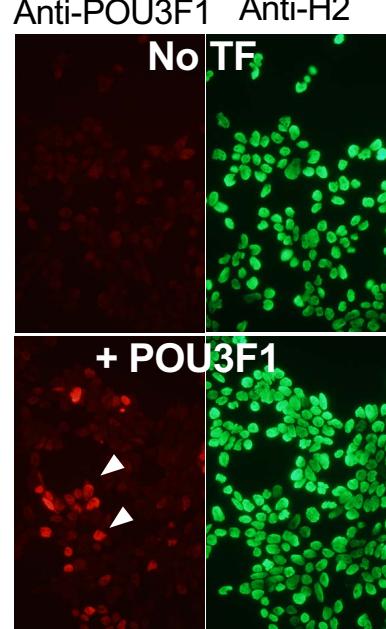
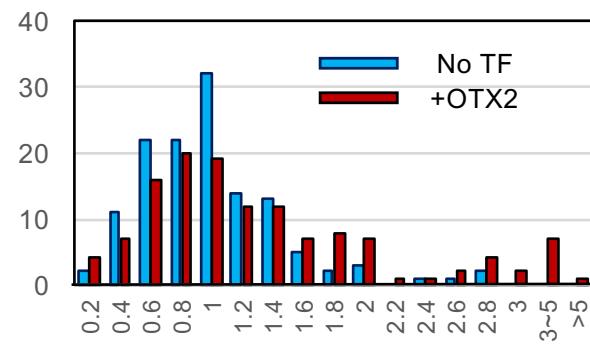
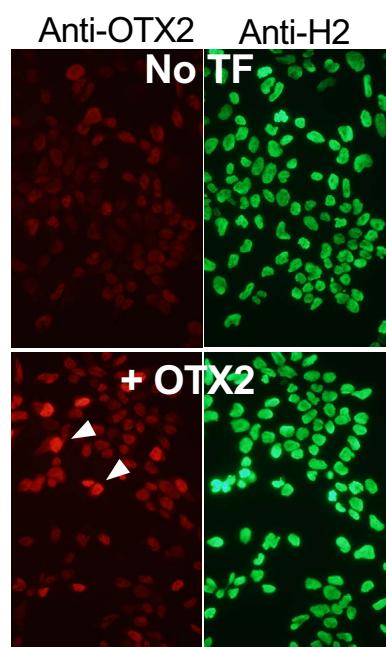
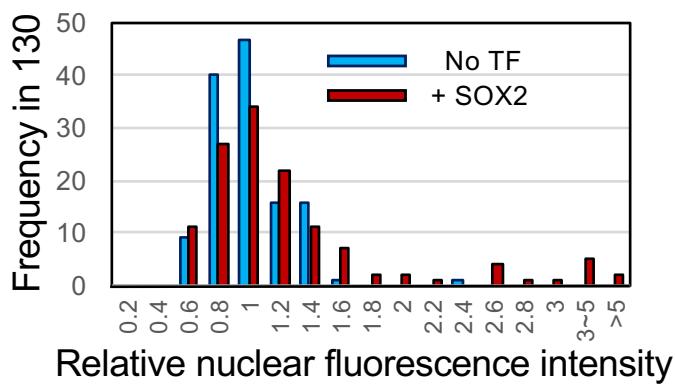
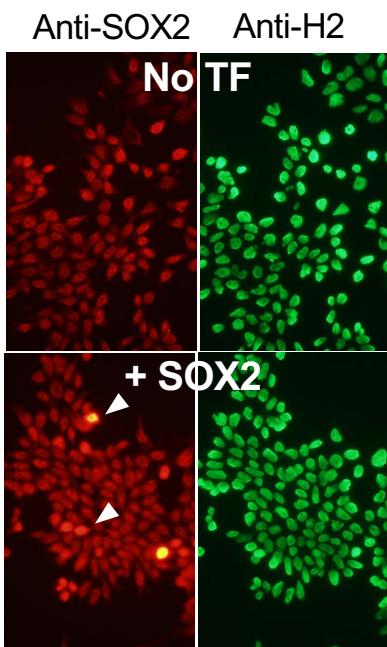
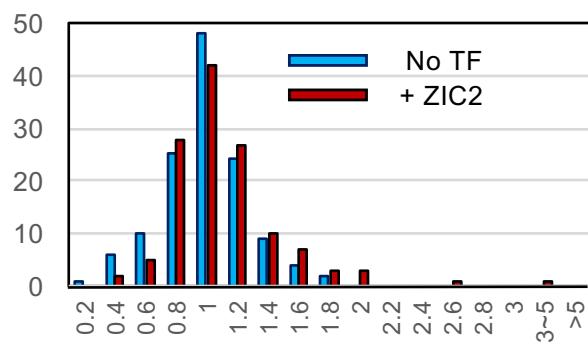
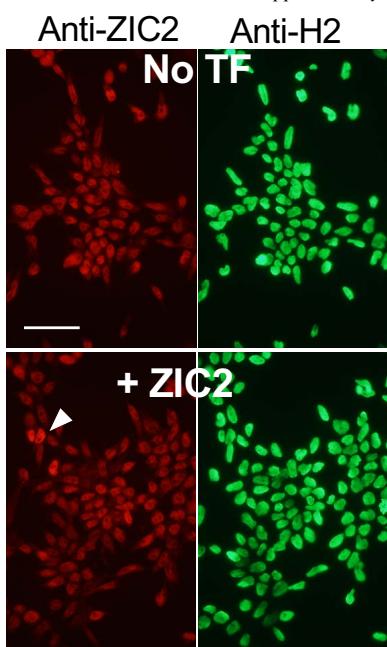


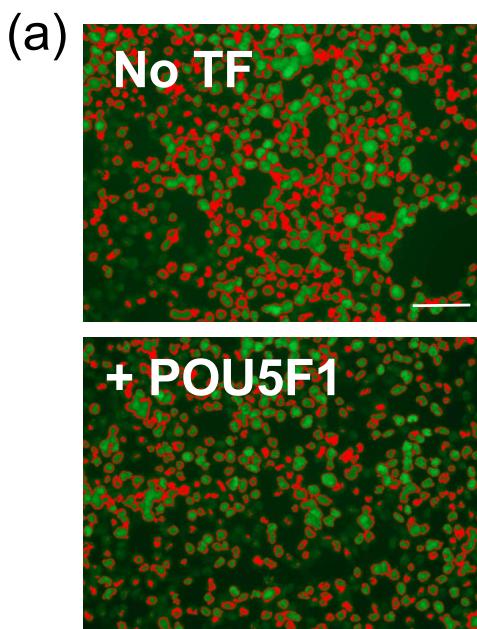
Figure S1



C



D



(b)

Exogenous TF	NANOG immunofluorescence	
	Bright	Dim
None	86% (524/609)	14% (85/609)
ZIC3	87% (503/575)	13% (72/575)
OTX2	83% (513/614)	17% (101/614)
SOX2	85% (625/738)	15% (113/738)
POU5F1	82% (514/628)	18% (114/628)
POU3F1	84% (412/490)	16% (78/490)

Figure S1. Transfection of EpiSCs. (A) Assessing transfection efficiency using CAGGS-EGFP under conditions identical to transfection for ChIP-seq. Comparison of EGFP fluorescence with 4',6-diamidino-2-phenylindole (DAPI) nuclear fluorescence indicated efficiency of 78% in this particular culture. The bar indicates 50 μ m. (B) A luciferase reporter assay demonstrated the full activity of biotinylated SOX2 and POU5F1 in the activation of the *Nestin* enhancer. Luciferase reporters with an octamer of the *Nestin* minimal enhancer Nes30 (Tanaka *et al.* 2004) or without the enhancer (851) were activated with a combination of 0, 1, 5 or 20 ng each of expression vectors for SOX2 (S) or biotinylated SOX2 (BS) and for POU5F1 (P) or biotinylated POU5F1 (BP) in 10T1/2 cells plated in a well of 24-well plate. The mean activation levels for technical duplicate samples were normalized using cotransfected *Renilla* luciferase activity, and the fold activation relative to the basal activity (without exogenous TFs) is shown. The error bars indicate the range of data points. The basal luciferase expression with Nes30 enhancer was 9.3% of that for 851. Details on the transfection conditions and assays have been described previously (Tanaka *et al.* 2004). (C) Analysis of nuclear TF expression levels after transfection with BLRP-tagged exogenous TFs, ZIC2, OTX2, SOX2 or POU3F1, as indicated in Fig. 1C. Nuclei with condensed chromosomes indicating immediately pre/post mitotic stages were excluded from the analysis. When the fluorescence intensity was found to saturate the photorecord, shorter exposure images were used for the analysis. (D) Effect of exogenous TF with BLRP tags on Nanog expression in EpiSCs. (a) Nanog expression was detected in all EpiSC nuclei by immunofluorescence. The expression level that was exceeded in ~85% of control nuclei was set as a threshold and marked red. The bar indicates 50 μ m. (b) The nuclei expressing higher level (Bright) or lower level (Dim) was counted, and the fractions of ‘‘Bright’’ and ‘‘Dim’’ populations after expression of exogenous TFs were compared with the untransfected control.

Figure S2

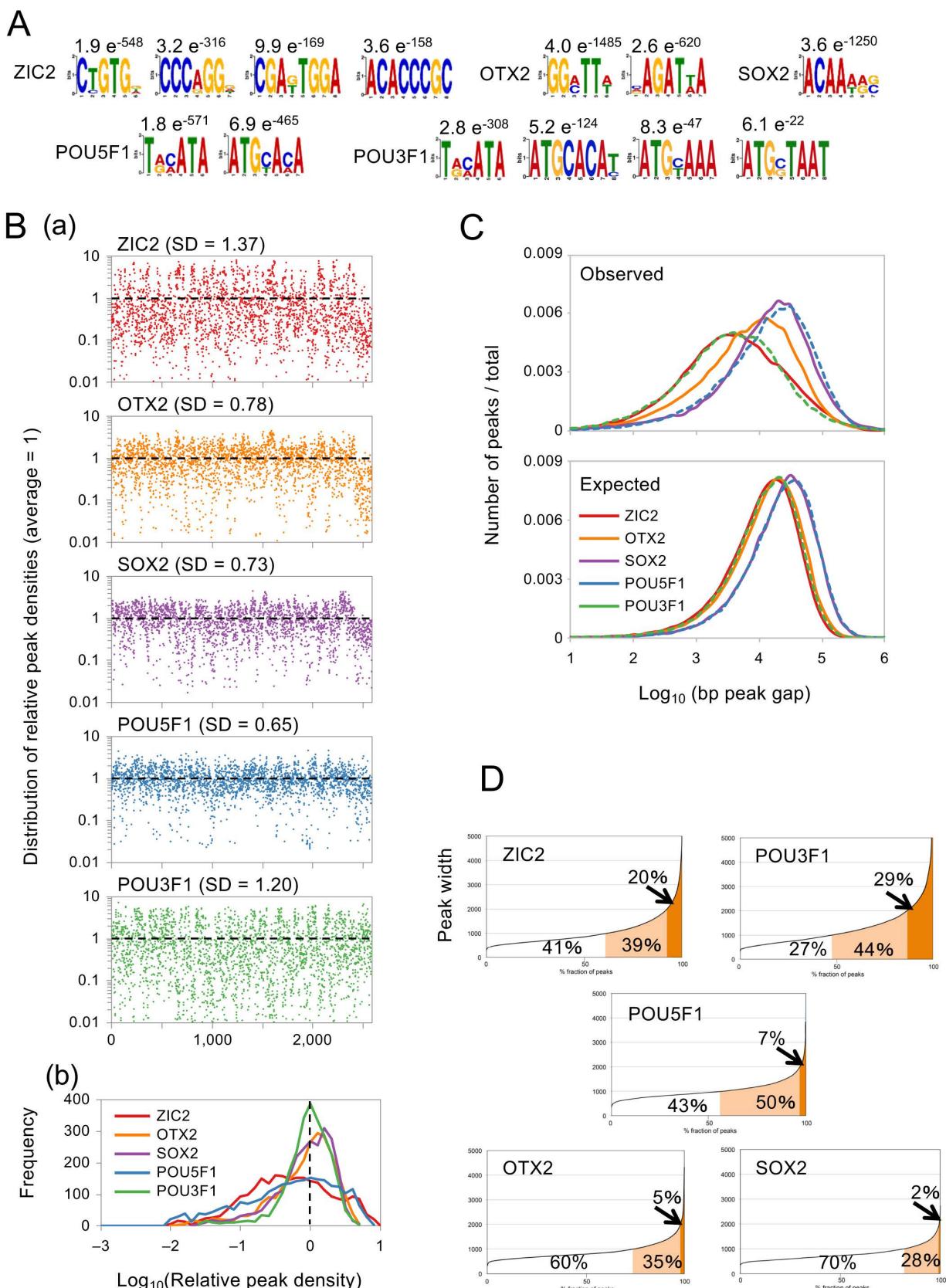


Figure S2. (A) Power-weight matrix (PMW) representation of MEME-ChIP outputs showing sequence motifs highly enriched in ChIP-seq peaks of <1000 bases wide for each TF. Unerased E-values are shown on top of the motifs. (B) Normalized variations of peak densities over the 2585 genomic segments. (a) After normalizing the average peak density to 1, the peak density in each Mb segment was plotted over the entire genome for the five TFs. SD, standard deviation. (b) The summary of the distribution and extents of normalized peak densities represented by histograms. (C) Size distribution of peak gaps, which are distances between the midpoint of nearest peaks. Observed distributions (top) are compared with the expected distributions using randomized peak positions (bottom). (D) Statistics on the peak sizes for all MACS-annotated peaks. The areas representing peaks over 1000 bp (expected to contain several TF binding sites) and over 2000 bp (expected to contain numerous TF binding sites) are colored.

Figure S3

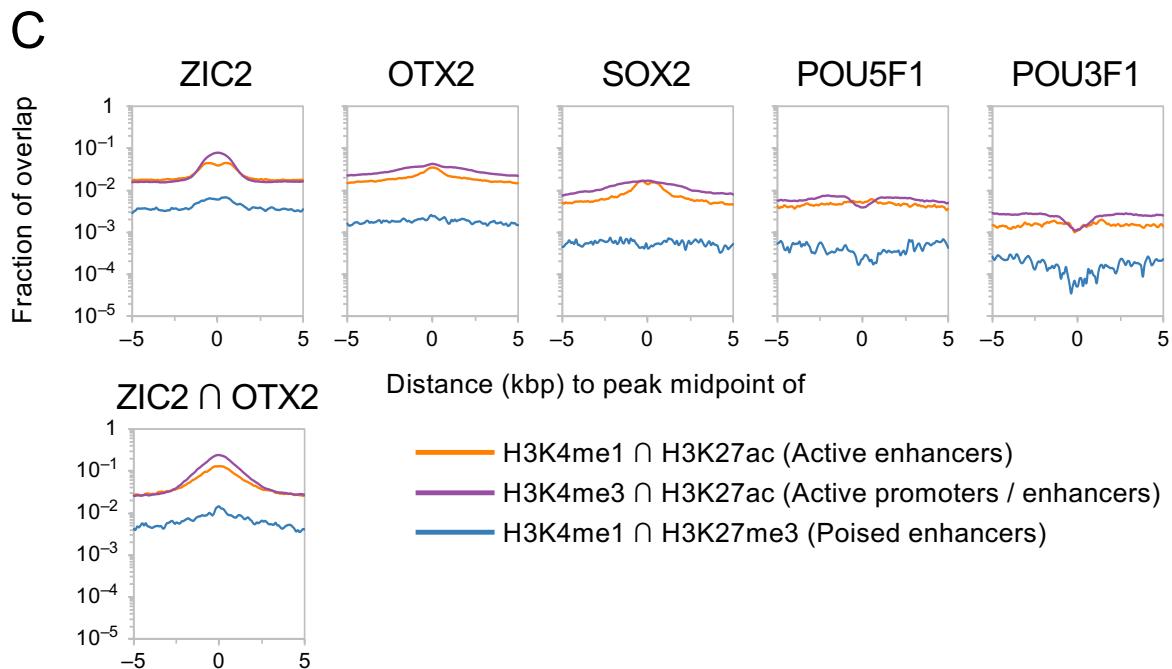
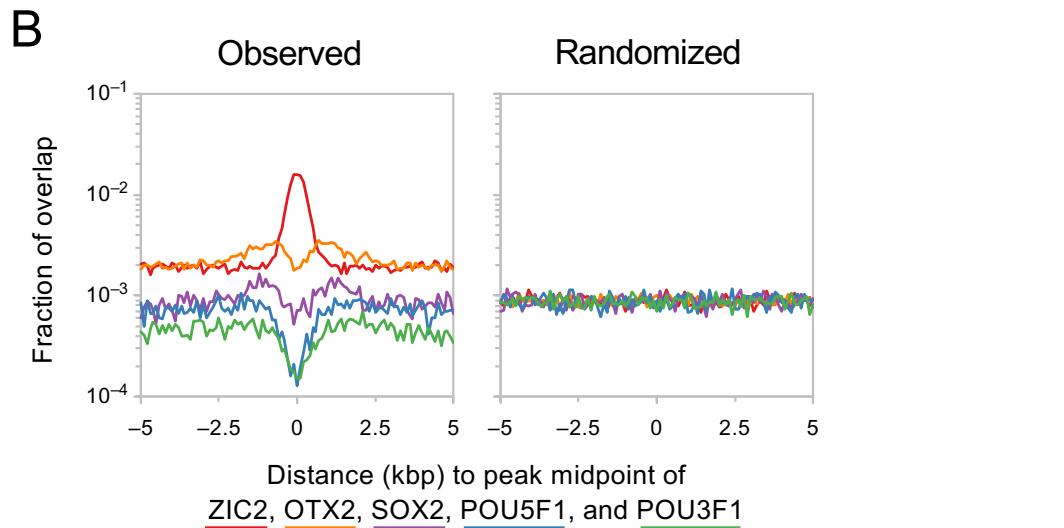
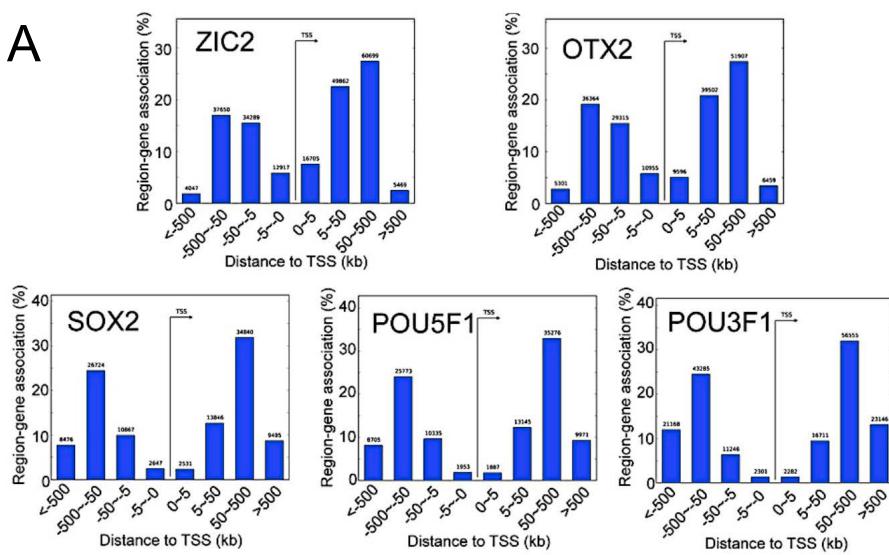


Figure S3. (A) Statistics for the positioning of ChIP-seq summits relative to TSSs of the nearest genes, generated using GREAT software. (B) Aggregation plots illustrating the fraction of relative midpoint positions of the ChIP-seq peaks of TFs indicated by color codes that overlap with TSSs of RefSeq genes. Ordinate, the fraction of overlap in logarithmic scale measured in 100 bp bins. (C) Aggregation plots illustrating the fraction of relative midpoint positions of modified histone H3 ChIP-seq peaks (data taken from Factor *et al.* (2014)) overlapping with the TF ChIP-seq peaks (shown on the top). Ordinate, the fraction of overlap in logarithmic scale measured in 100 bp bins. The decrease of the frequency of the co-occurrence of H3K4me3 and H3K27ac histone modification signatures at the ChIP-seq peak positions of POU3F1 and POU5F1 is accounted for by the low frequencies of positioning of these peaks at TSSs shown in (B).

Figure S4

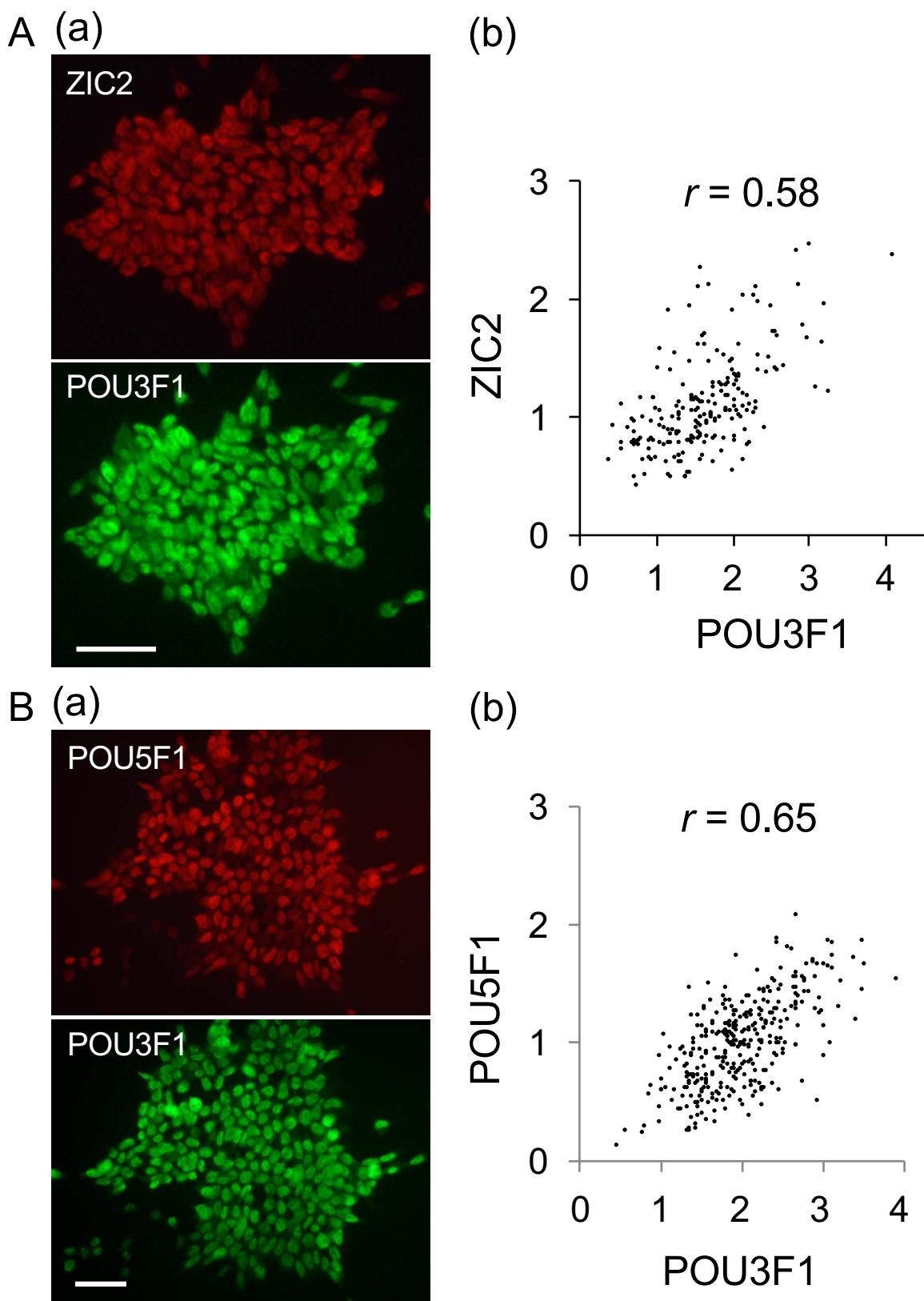


Figure S4. Comparison of expression level variations for ZIC2 vs. POU3F1 (A) and POU5F1 vs. POU3F1 (B) in individual nuclei of EpiSCs. The cultures were stained with respective antibodies, as exemplified by the panels in (a). Anti-ZIC2 also stains ZIC1 and ZIC3, but the major ZIC in EpiSCs is ZIC2 (Iwafuchi-Doi et al., 2014). The bars indicate 50 μ m. (b) Measured relative fluorescence intensities over 211 nuclei in (A) or 330 nuclei in (B) were compared using scatter plots, which gave correlation coefficients of 0.58 and 0.65, respectively.

Figure S5. The nucleotide sequence of pCAGGS-BLRP-BirA with annotation of sequences indicated in Figure 1A.

SmaI

BLRP

His6

NotI

STOP

IRES

BirA

gggatcaatctcgatcgtaatacgttggcgccatgtaatacgtgaattacgtgcgttggactttcaacaagaaggattggcaccttatctgt
cgcgtggaaaagctgataatttattaatcgccagtgaaacttatcattggatgataaaagaaatattggcatttcacgcgaaatagacaacaggg
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GCGCTCCCTGTTCCGACCCCTGCCGTTACCGATACTGTCCGCTTCTCCCTCGGAAGCGTGGCCTTCTCATAGCTCACGCTGTAGGTATCTC
AGTCGGTGTAGGTCGTCGCTCCAAGCTGGCTGTGACGAACCCCCCGTTCAGCCGACCGCTGCCCTATCCGTAACTATCGTCTTGAGTCCA
ACCCGGTAAGACACGACTATGCCACTGGCAGCAGCACTGGTAACAGGATTAGCAGAGCGAGGTATGAGGCGGTGCTACAGAGTTCTGAAGTGGT
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ACTGATCTTCAATCAGCATCTTACTTCACCAGCCTCTGGGTGAGCAAAACAGGAAGGCAAAATGCCGAAAAAGGAAATAAGGGC
GACACGGAAATGTTGAATAACTCATACTCTTCAAT

Table S1. Detailed protocol for ChIP-seq performed using biotinylated TFs expressed in EpiSCs.

A	Handling cells
1	Plate 10^6 Accutase-dissociated EpiSCs in each of five 10 cm dishes using the standard feeder-free EpiSC culture medium.
2	Add DNA-Lipofectamine 2000 complex immediately, and start incubation at 37 °C with 5% CO ₂ .
3	After 8 hours, replace culture medium with the one containing 50 µg/ml biotin to remove DNA-Lipofectamine 2000.
4	After overnight culture, wash the cultures and add 10 ml PBS.
5	Add 270 µl of 37% formaldehyde to give final concentration of 1%.
6	Incubate at room temperature for 10 min, while gently mixing the liquid on a rocker platform.
7	Add 1 ml of 1.25 M glycine to every fixed dish, and incubate further 5 min.
8	After rinse with PBS, add 2 ml of PBS to dishes, remove cells from dishes using cell scraper, and collect cells in 5 dishes into a centrifuge tube.
9	Suspend cells by pipettin, and wash cells twice with PBS by centrifugation at 2,400x g at 4 °C.
10	Remove the supernatant, add 1 ml of ChIP buffer [1 % SDS, 2 mM EDTA, 20 mM Tris-HCl pH 8.1, 150 mM NaCl, 1x protease inhibitor (cOmplete EDTA-free, Roche)] and keep the specimen frozen at -80 °C.
B	ChIP procedure
1	After thawing a sample, transfer it in a 1 ml Covaris vial, and set in a Covaris S2 sonicator.
2	Operate Covaris S2 using following settings: Intensity, 5; Duty cycle, 20%; Cycle burst, 200; Cycle number, 30 sec x40; Bath temperature 7 °C.
3	Centrifuge the sonicated sample at 12,000x g for 10 min at 8 °C.
4	Collect the supernatant, leaving the last ~30 µl.
5	Add 100 µl of ChIP buffer-equilibrated streptavidine-conjugated magnet bead suspension (Dynabeads MyOne Streptavidine) to the supernatant, and mix the suspension gently overnight at 8 °C.
6	Collect beads placing the tube on a magnet for 2 min, and remove supernatant.
7	Suspend beads in 1 ml of wash buffer 1 (2% SDS), swirl the beads gently for 10 min, collect beads and remove supernatant.
8	Repeat step 7.
9	Suspend beads in 1 ml of wash buffer 2 (1% Triton X-100, 1 mM EDTA, 50mM HEPES pH 7.5, 500 mM NaCl), swirl the beads gently for 10 min, collect beads, and remove supernatant.
10	Repeat step 9.
11	Suspend beads in 1 ml of wash buffer 3 (250 mM LiCl, 0.5% NP40, 1 mM EDTA, 10 mM Tris-HCl pH8.1), swirl the beads gently for 10 min, collect beads and remove supernatant.
12	Repeat step 11.
13	Suspend beads in elution buffer (1% SDS, 10 mM EDTA 50 mM Tris-HCl pH 8.1), and swirl the beads gently at 65 °C overnight.
13	Collect beads on a magnet and transfer the supernatant to another tube.
15	Add RNase A at 5 µg/ml, incubate at 37 °C for 30 min, then add proteinase K at 100 µg/ml and incubate at 37 °C for further 30 min. Extract the solution twice with phenol/chloroform and precipitate DNA with ethanol using glycogen as carrier.
16	Resuspend the pellet in 55 µl of 10 mM Tris-HCl pH 8.1, 1 mM EDTA, and incubate at 37 °C for 10 min, among which use 5 µl to determine DNA concentration using Qubit.
17	Proceed to library preparation for sequencing using ~1 ng precipitated DNA and TruSeq ChIP Sample Prep Kit (Illumina).

Table S2. Statistics for Bowtie mapping and MACS peak calling.

DNA	Initial reads	Fractions mapped on the genome	Peaks called	False detection rate
UnChIP control	31,808,283	97%		
ZIC2 ChIP	22,082,410	86%	122,053	0.64%
OTX2 ChIP	34,047,743	91%	103,281	0.80%
SOX2 ChIP	26,163,758	97%	63,751	0.35%
POU5F1 ChIP	25,444,044	89%	62,457	0.10%
POU3F1 ChIP	28,146,846	97%	112,753	0.82%

Table S3. Antibodies used for immunofluorescent analyses.

Primary antibodies

Antibodies	IgG source	Clonarity	Supplier	Product code /reference	Dilutions used
Anti-ZIC2	Rabbit	Polyclonal	Jun Aruga	Inoue <i>et al.</i> 2007	1/1000
Anti-OTX2	Goat	Polyclonal	R&D	AF1979	1/200
Anti-SOX2	Rabbit	Polyclonal	MBL	PM056	1/800
Anti-POU5F1	Goat	Polyclonal	Santa Cruz	sc-1000	1/1000
Anti-POU3F1	Goat	Polyclonal	Santa Cruz	sc-11661	1/200
Anti-POU3F1	Mouse	Monoclonal	Millipore	MABN738	1/400
Anti-NANOG	Rabbit	Polyclonal	Santa Cruz	sc-8628	1/1000
Anti-Histone H2A	Mouse	Monoclonal	MBL	D210-3	1/500

Secondary antibodies

Antibodies	Fluorochromes	IgG source	Supplier	Product code	Dilutions used
Anti-Rabbit IgG	Alexa Fluor 568	Donkey	Abcam	ab175692	1/1000
Anti-Goat IgG	Alexa Fluor 568	Donkey	Abcam	ab175704	1/1000
Anti-Mouse IgG	Alexa Fluor 488	Donkey	Abcam	ab150109	1/1000

Table S4. ChIP-seq and microarray data deposited in public databases and used in this article for analysis.

ChIP-seq

Cells	Specimen	Accession #	References
Mouse ESC1	UnChIP control	GSM1050292	Lodato <i>et al.</i> (2013)
Mouse ESC1	SOX2 ChIP	GSM1050291	Lodato <i>et al.</i> (2013)
Mouse ESC1	UnChIP control	GSM307154	Lodato <i>et al.</i> (2013)
Mouse ESC1	POU5F1 ChIP	GSM307137	Lodato <i>et al.</i> (2013)
Mouse ESC2	UnChIP control	GSM1355163	Buecker <i>et al.</i> (2014)
Mouse ESC2	OTX2 ChIP	GSM1355157	Buecker <i>et al.</i> (2014)
Mouse ESC3	UnChIP control	GSM1499115	Luo <i>et al.</i> (2015)
Mouse ESC3	ZIC2 ChIP	GSM1499116	Luo <i>et al.</i> (2015)
Mouse EpiLC	UnChIP control	GSM1355176	Buecker <i>et al.</i> (2014)
Mouse EpiLC	POU5F1 ChIP	GSM1355167	Buecker <i>et al.</i> (2014)
Mouse EpiLC	OTX2 ChIP	GSM1355169	Buecker <i>et al.</i> (2014)
Human ESC1	SOX2 ChIP	GSM456570	Lister <i>et al.</i> (2009)
Human ESC2	POU5F1 ChIP	GSM518373	Kunarso <i>et al.</i> (2010)
Human ESC3	UnChIP control	GSM1505802	Tsankov <i>et al.</i> (2015)
Human ESC3	SOX2 ChIP	GSM1505766	Tsankov <i>et al.</i> (2015)
Human ESC3	POU5F1 ChIP	GSM1505724	Tsankov <i>et al.</i> (2015)
Mouse ESC1	UnChiP control	GSM594580	Creyghton <i>et al.</i> (2010)
Mouse ESC1	H3K4me1 ChIP	GSM594577	Creyghton <i>et al.</i> (2010)
Mouse ESC1	H3K27ac ChIP	GSM594578	Creyghton <i>et al.</i> (2010)
Mouse EpiSC	UnChIP control	GSM1382221	Factor <i>et al.</i> (2014)
Mouse EpiSC	H3K4me1 ChIP	GSM1382217	Factor <i>et al.</i> (2014)
Mouse EpiSC	H3K4me3 ChIP	GSM1382218	Factor <i>et al.</i> (2014)
Mouse EpiSC	H3K27ac ChIP	GSM1382219	Factor <i>et al.</i> (2014)
Mouse EpiSC	H3K27me3 ChIP	GSM1382220	Factor <i>et al.</i> (2014)
Mouse EpiSC	UnChIP control	GSM1924743	This study
Mouse EpiSC	ZIC2 ChIP	GSM1924744	This study
Mouse EpiSC	OTX2 ChIP	GSM1924745	This study
Mouse EpiSC	SOX2 ChIP	GSM1924746	This study
Mouse EpiSC	POU5F1 ChIP	GSM1924747	This study
Mouse EpiSC	POU3F1 ChIP	GSM1924748	This study

Microarray

Mouse EpiSC	GSM934422 GSM934427	Iwafuchi-Doi <i>et al.</i> (2012)
Mouse ATDC5	GSM1486495	Sugita <i>et al.</i> (2015)
Mouse embryo fibroblasts	GSM1079106	Richter <i>et al.</i> (2013)
Mouse embryo myoblasts	GSM1541934	Unpublished

All microarray data were on the platform of Agilent-028005 SurePrint G3

Mouse GE 8x60K Microarray.