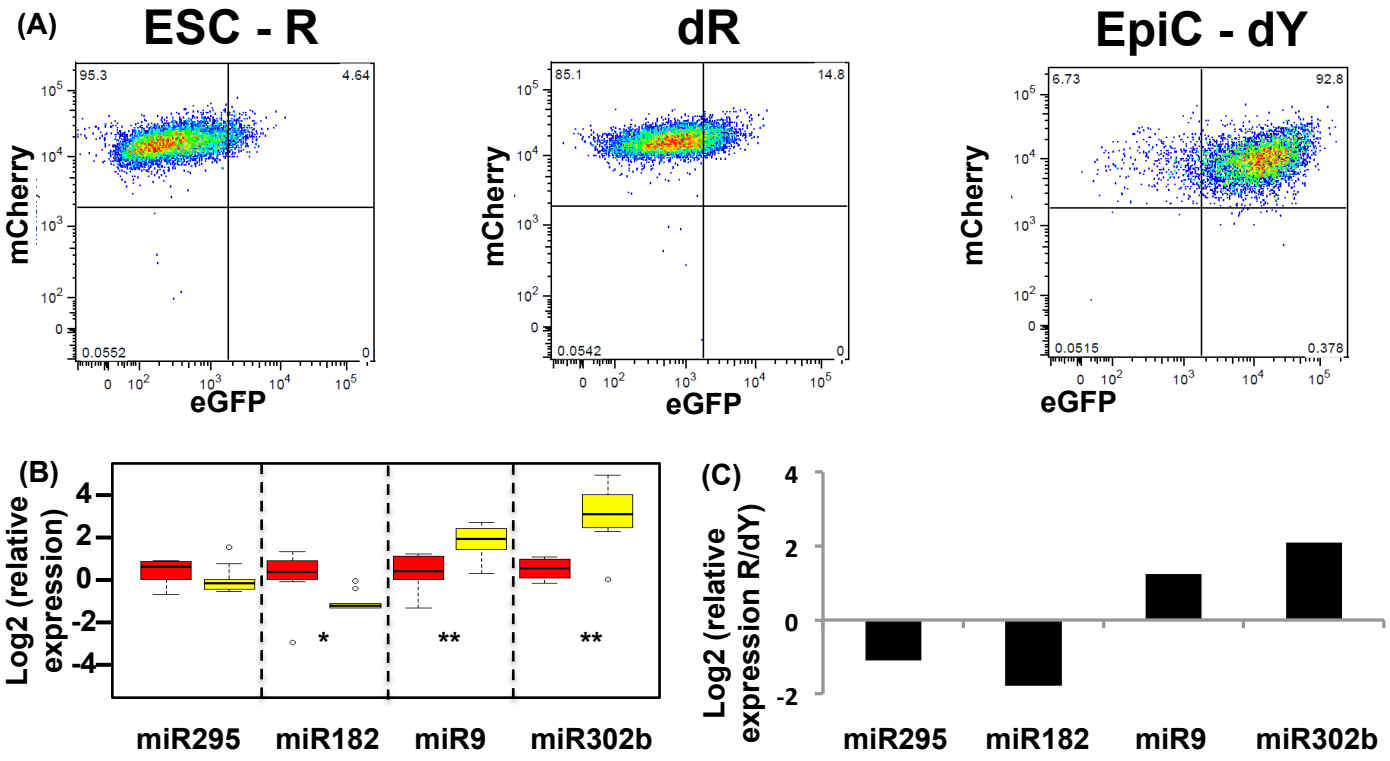
(A)
ESC - R
dR
EpiC - dY
95.3
4.64
0.0552
0
85.1
14.8
0.0542
0
6.73
92.8
0.0515
0.378
mCherry
eGFP
(B)
Log2 (relative expression)
*
**
**
miR295
miR182
miR9
miR302b
(C)
Log2 (relative expression R/dY)
miR295
miR182
miR9
miR302b

Figure S1

Fig S1 (related to Fig 1): *In vitro* ESC differentiation protocol allows for isolation of homogeneous populations

(a) Flow cytometry analysis of mCherry and eGFP levels in cells during the differentiation protocol outlined in Fig. 1a. (b) Single-cell microRNA qPCR for 10 individually sorted ESCs (red boxes), and 11 individually sorted EpiCs (yellow boxes). Primers and Taqman probes for four mature miRNAs were used – miR295, miR182, miR9 and miR302b. Shown are box plots of Ct values normalized to one arbitrarily chosen cell in each case. Line inside each bar represents median and error bars represent standard deviation. Single asterisk (*) represents $p<0.05$, double asterisk (**) represents $p<0.005$ using an unpaired student's T-test. (c) The same miRNAs as b) for a mix of 100 cells. 100 cells were sorted into a single well and processed simultaneously to represent the population average. Data shown is for EpiCs relative to ESCs.

Figure S1

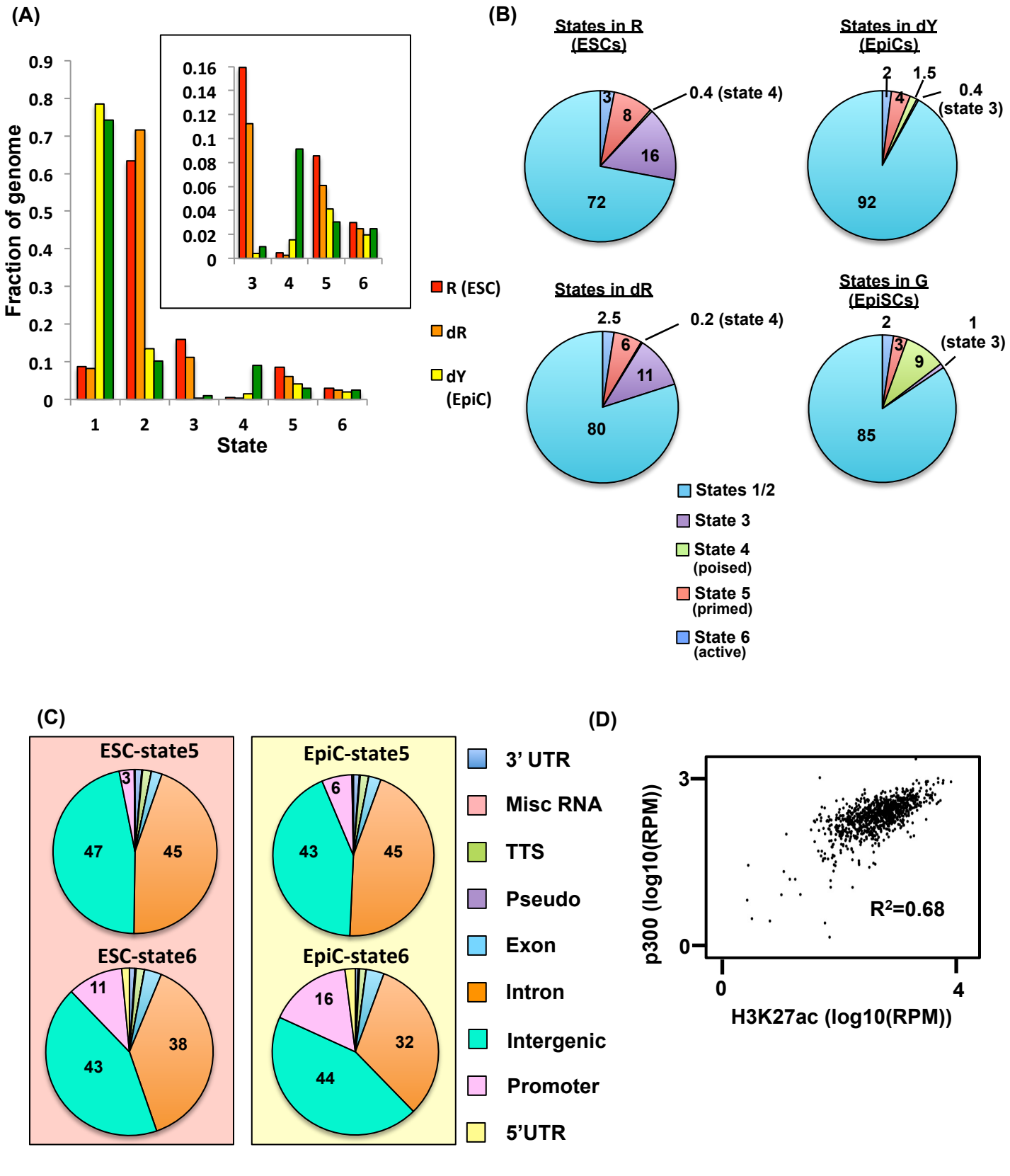
(A)
Fraction of genome
State
R (ESC)
dR
dY (EpiC)
(B)
States in R (ESCs)
0.4 (state 4)
States in dY (EpiCs)
0.4 (state 3)
States in dR
0.2 (state 4)
States in G (EpiSCs)
1 (state 3)
States 1/2
State 3
State 4 (poised)
State 5 (primed)
State 6 (active)
(C)
ESC-state5
EpiC-state5
ESC-state6
EpiC-state6
3' UTR
Misc RNA
TTS
Pseudo
Exon
Intron
Intergenic
Promoter
5'UTR
(D)
p300 (log10(RPM))
H3K27ac (log10(RPM))
R²=0.68

Figure S2

Fig S2 (related to Fig 1): Enhancers transitions in ESCs, dR, EpiCs and EpiSCs
(a) Fraction of genome in states 1-6 in ESCs, dR, EpiCs, and EpiSCs. Inset - states 3-6 shown on a different scale for better visualization. (b) Same as (a) except with data represented as pie charts, with states 1 and 2 combined (percentages also represented numerically inside pie slices). (c) Pie charts of proportion of chromatin in States 5 and 6 of ESC (left) and EpiC (right) that are found in various annotated regions of the genome. Abbreviations: 3'UTR – 3' untranslated region, miscRNA – RNA of unknown function, TTS – transcription termination site, pseudo – annotated pseudogenes, 5'UTR – 5'untranslated region. Numbers inside pie slices are the percentage of the state in the particular feature, included for easier interpretation of the graphs. Data generated using HOMER (Hanna et al., 2002). (d) Scatter plot of RPM of H3K27ac versus p300 at enhancers changing between state 5 and 6 from ESCs to EpiCs. Pearson's correlation R^2 value inset ($p<2.2e-16$).

Figure S2

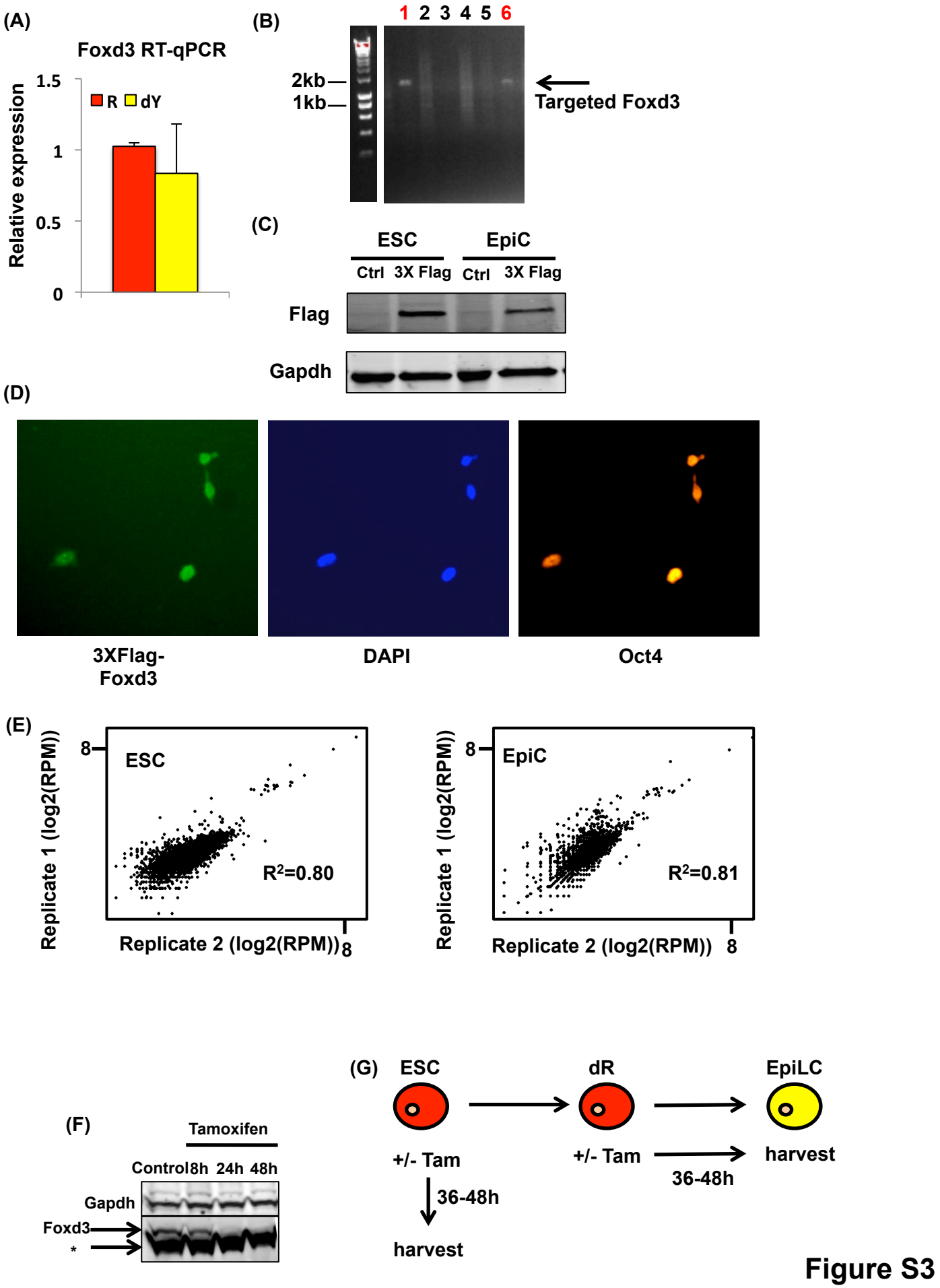
(A)
Foxd3 RT-qPCR
Relative expression
1.5
1
0.5
0
R
dY
(B)
1 2 3 4 5 6
2kb
1kb
Targeted Foxd3
(C)
ESC
EpiC
Ctrl
3X Flag
Ctrl
3X Flag
Flag
Gapdh
(D)
3XFlag-
Foxd3
DAPI
Oct4
(E)
ESC
R2=0.80
Replicate 1 (log2(RPM))
Replicate 2 (log2(RPM))
8
EpiC
R2=0.81
(F)
Tamoxifen
Control
8h
24h
48h
Gapdh
Foxd3
*
(G)
ESC
dR
EpiLC
+/- Tam
+/- Tam
harvest
36-48h
36-48h
harvest

Figure S3

Fig S3 (related to Fig 2): Targeting of Foxd3 with 3XFlag

(a) RT-qPCR for Foxd3 in R (ESCs) and dY (EpiCs) showing no significant change in levels (n=3, student's t-test p=0.46). (b) PCR genotyping of Foxd3 conditional knockout cells with a C-terminal 3X Flag tag targeted on one allele. Foxd3 conditional knockout cells had one allele of Foxd3 knocked in with a C-terminal 3XFlag tag. Primers designed to amplify only targeted allele (see materials and methods). Shown are six independent cell lines, two of which show a positive signal for the targeted allele. (c) Western blot showing expression of 3X Flag-tagged Foxd3 in ESCs and EpiCs. Control cells are non-targeted Foxd3 conditional knockout cells. Gapdh is used as a loading control. (d) Immunocytochemistry analysis showing localization of the 3XFlag-Foxd3 in the nucleus (left), co-localizing with DAPI (middle) and a known nuclear factor Oct4 (right). (e) Scatter plot of RPM of biologic replicates of Foxd3 ChIP-seq in ESCs (top) and EpiCs (bottom). Pearson's correlation R^2 value inset ($p<2.2e-16$). (f) Western blot showing Foxd3 protein depletion during a time-course of Tamoxifen treatment. A number of Foxd3 antibodies were tested in Western blot, and all of them showed large non-specific bands. The antibody that showed the best specific band was used. Both the specific and non-specific bands are shown on the Western. Protein is depleted within 24 hours. (g) Schematic showing timing of Foxd3 knockout in ESCs and EpiCs cells. Importantly, in ESCs, the knockout is performed in the steady state, while in EpiCs, Foxd3 is knocked out prior to reaching the EpiC state.

Figure S3

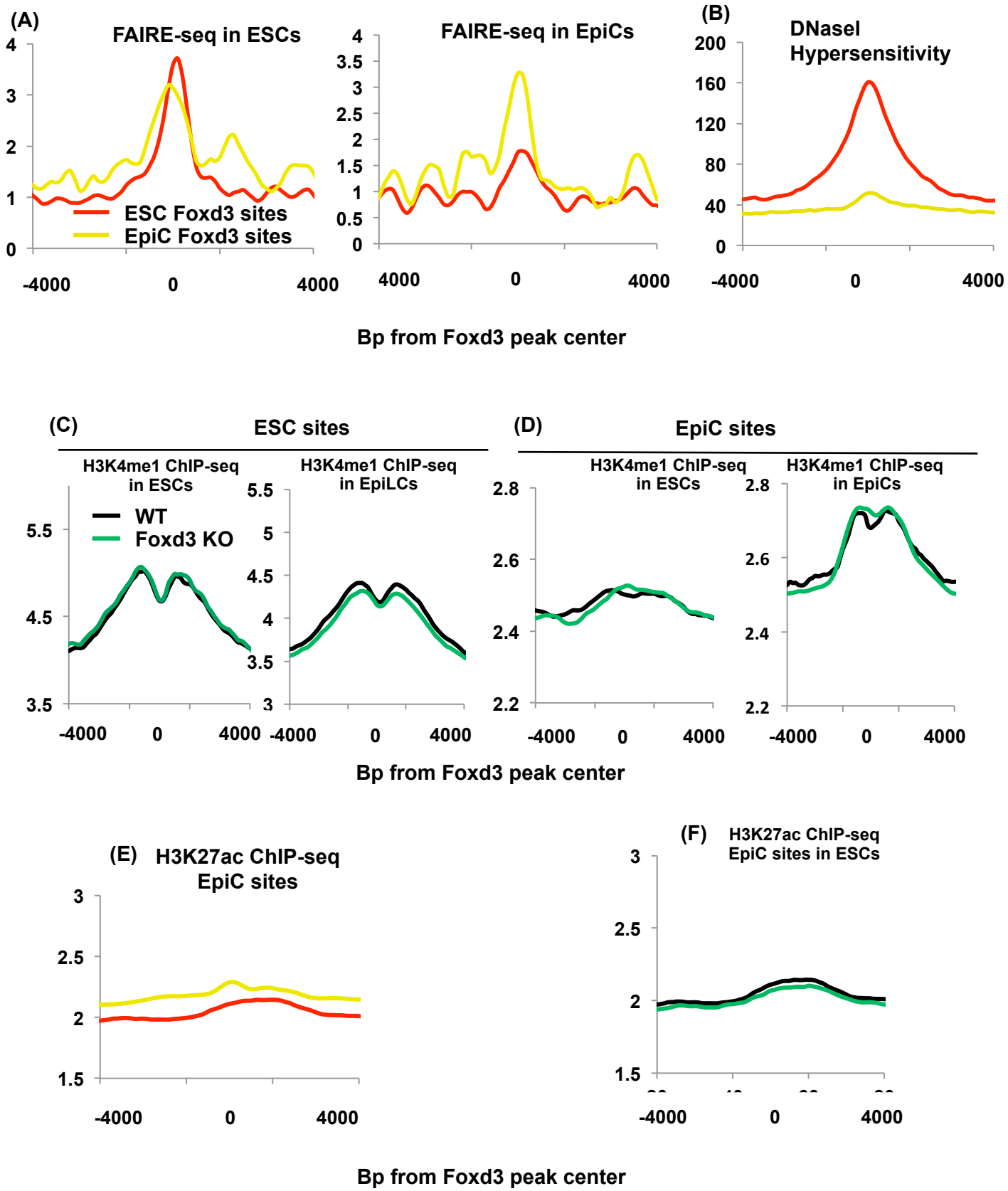
(A)
FAIRE-seq in ESCs
FAIRE-seq in EpiCs
ESC Foxd3 sites
EpiC Foxd3 sites
(B)
DNaseI Hypersensitivity
Bp from Foxd3 peak center
(C)
ESC sites
H3K4me1 ChIP-seq in ESCs
H3K4me1 ChIP-seq in EpiLCs
WT
Foxd3 KO
(D)
EpiC sites
H3K4me1 ChIP-seq in ESCs
H3K4me1 ChIP-seq in EpiCs
Bp from Foxd3 peak center
(E)
H3K27ac ChIP-seq EpiC sites
(F)
H3K27ac ChIP-seq EpiC sites in ESCs
Bp from Foxd3 peak center

Figure S4

Fig S4 (related to Figs 4 and 5): Nucleosome occupancy, H3K4me1 and H3K27ac at Foxd3-bound regions.

(a) Metagene analysis of published FAIRE-seq data in a 1000 bp window surrounding Foxd3-bound sites in ESCs (red line) and EpiCs (yellow line). Data for ESC peaks shown on the left and data for EpiC peaks shown on the right. A moving window average of 500 bp windows and 100bp steps was used. Enirchment was calculated based on coverage data deposited in GEO (GSM1355175) (Liu and Labosky, 2008; Plank et al., 2014). Y-axis – RPM. (b) DNaseI hypersensitivity data for mouse ESCs (average of three data sets) from the ENCODE consortium (Chang and Kessler, 2010; Hochgreb-Hägele and Bronner, 2013; Nitzan et al., 2013; Teng et al., 2008; Thomas and Erickson, 2009; Wang et al., 2011). Metagene analysis for a 4000bp region surrounding the peak is shown, with a moving window average of 500 bp windows and 100bp steps. The y-axis represents an average measure of DNAse hyspersensitivity, as determined by the ENCODE consortium (Abel et al., 2013; Basile et al., 2012). Y-axis – relative counts. (c) Metagene analysis of H3K4me1 ChIP-seq in ESCs (left) and EpiCs (right) data for wild-type and Foxd3 knockout cells using a 4000bp window surrounding Foxd3-bound sites in ESCs. WT (black line). Foxd3 KO (green line). Asterisk (*) denotes paired student's T-test p-values <0.05. Y-axis – RPM. (d) Metagene analysis of H3K4me1 ChIP-seq in ESCs (left) and EpiCs (right) for wild-type and Foxd3 knockout cells using a 4000bp window surrounding Foxd3-bound sites in EpiCs. WT (black line) and Foxd3 KO (green line). Y-axis – RPM. (e) Metagene analysis of H3K27ac ChIP-seq data in ESCs (red line) and EpiCs (yellow line) in a 4000bp window surrounding Foxd3-bound sites in EpiCs. Y-axis – RPM. (f) Metagene analysis of H3K27ac ChIP-seq data in wt and Foxd3 knockout ESCs using a 4000bp window surrounding Foxd3-bound sites in EpiCs. WT (black line) and Foxd3 KO (green line). Y-axis – RPM.

Figure S4

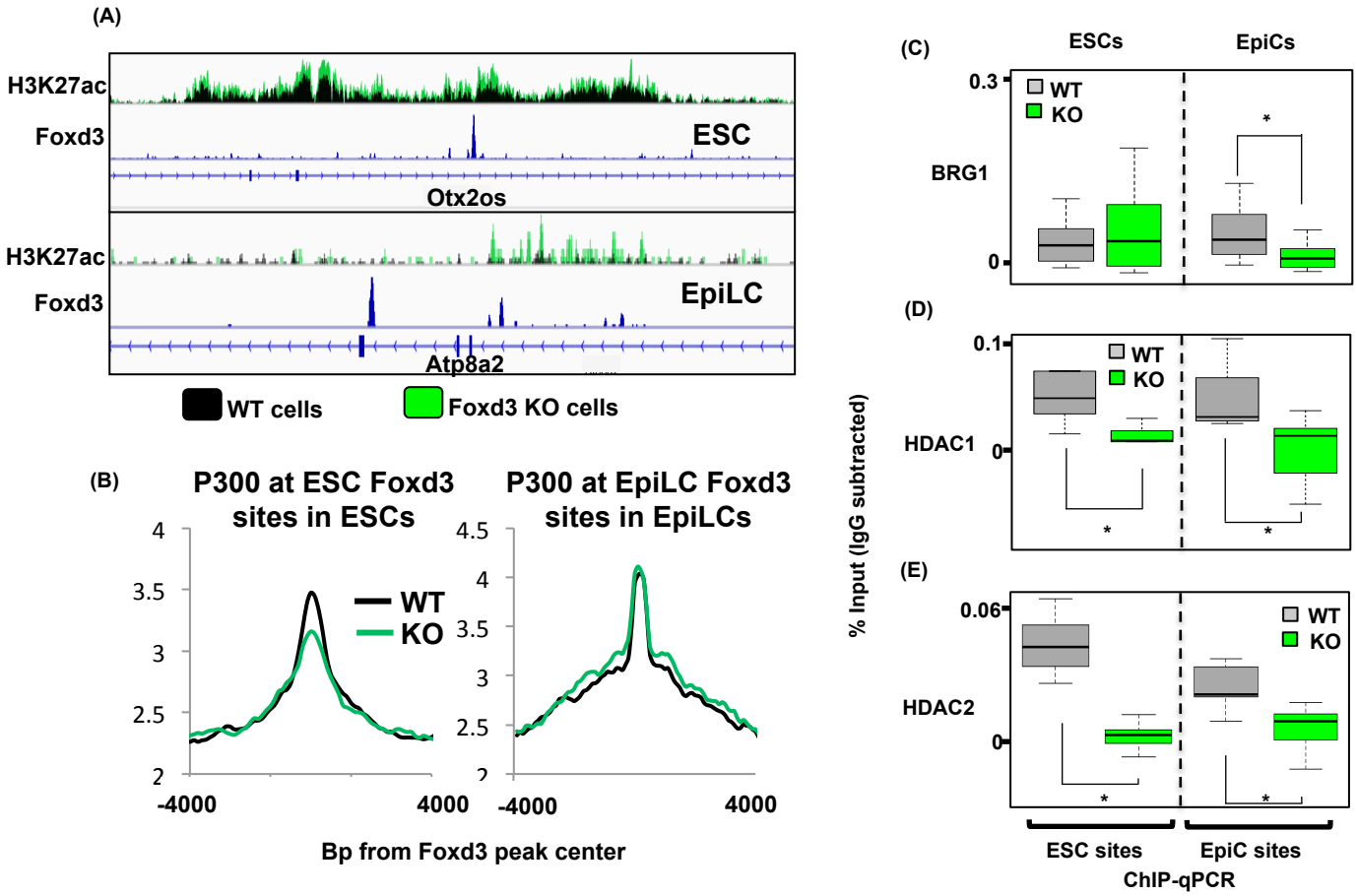
(A)
H3K27ac
Foxd3
ESC
Otx2os
H3K27ac
Foxd3
EpiLC
Atp8a2
WT cells
Foxd3 KO cells
(B)
P300 at ESC Foxd3 sites in ESCs
P300 at EpiLC Foxd3 sites in EpiLCs
WT
KO
4
3.5
3
2.5
2
4.5
-4000
4000
Bp from Foxd3 peak center
(C)
ESCs
EpiCs
0.3
BRG1
0
(D)
0.1
HDAC1
0
(E)
0.06
HDAC2
0
% Input (IgG subtracted)
ESC sites
EpiC sites
ChIP-qPCR

Figure S5

Fig S5 (related to Fig 6): Nucleosome occupancy, H3K4me1, H3K27ac and p300 at Foxd3-bound regions.

(a) Genome browser tracks of Foxd3 and H3K27ac, the latter in WT and Foxd3 KO cells. The non-coding RNA Otx2os is bound by Foxd3 in ESCs, and H3K27ac in WT and KO ESCs is shown (top). The gene Atp8a2 is bound by Foxd3 in EpiCs and H3K27ac in WT and KO EpiCs is shown (bottom). (b) Metagene analysis for p300 at locations of Foxd3 peaks in ESCs (left) and EpiCs (right) cells. Data from wild-type cells is in black, and data from knockout cells is in green. A 4000bp region surrounding the peaks is shown, with a moving window average of 500 bp windows and 100bp steps. Y-axis – RPM. (c) Confirmation of sequencing results with ChIP-qPCR. Box plots of ChIP-qPCR data showing enrichment of Brg1 at six Foxd3 binding sites in ESCs and six Foxd3 binding sites in EpiCs. Data shown for wild-type (WT) and Foxd3 knockout (KO) cells. n=3, Asterisk = $p<0.05$ by paired student's t-test. (d) Same as (c) for HDAC1 (e) Same as (c) for HDAC2

Figure S5

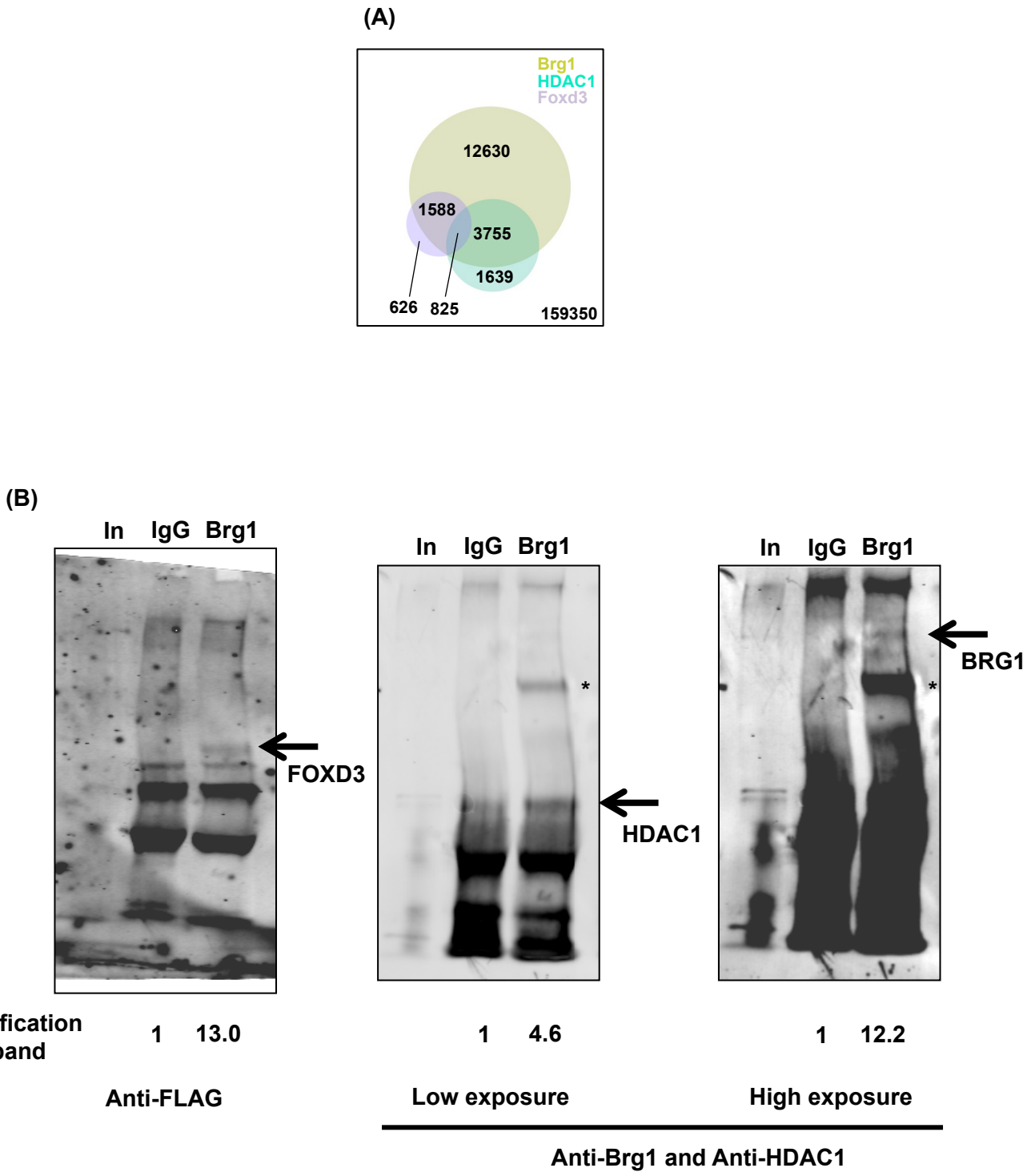
(A)
Brg1
HDAC1
Foxd3
12630
1588
3755
1639
626
825
159350
(B)
In IgG Brg1
FOXD3
In IgG Brg1
HDAC1
In IgG Brg1
BRG1
Quantification of band
1 13.0
1 4.6
1 12.2
Anti-FLAG
Low exposure
High exposure
Anti-Brg1 and Anti-HDAC1

Figure S6

Fig S6 (related to Fig 6): Nucleosome occupancy, H3K4me1, H3K27ac and p300 at Foxd3-bound regions.

(a) Venn diagrams showing overlap of Foxd3, HDAC1 and Brg1 peaks that are found at H3K4me1+ enhancers in ESCs. The total number of enhancers is shown outside the circles. Significance of overlap using Chi-squared test: X-squared = 953387.1, p-value<2.2e-16. (b) Western blots for Flag, Brg1 and HDAC1 following sequential IP for Flag-Foxd3 and then either IgG or Brg1 in ESCs. The HDAC1 and Brg1 western is stained with the same secondary antibody, and is therefore visualized on the same channel. Two exposures of this channel are shown to visualize the two proteins. In – Input (total lysate), IgG – IgG IP, Brg1 – Brg1 IP. Band of correct size is highlighted for each protein with arrow. Band intensity relative to IgG is given below each blot for highlighted protein. *, background band of unknown origin.

Figure S6

EXPERIMENTAL PROCEDURES

Cell lines and cell culture

All ESCs were maintained in Knockout DMEM (Invitrogen) supplemented with 15% Fetal Bovine Serum, Lif and 2i (PD0325901 and CHIR99021). To isolate populations, cells were plated at a density of 400,000 cells/15cm plate on day -1 (ESC state, expressing miR-290 alone, see text). On day 0, Lif and 2i were removed from the media, and on day 1 (16-20 hrs after Lif 2i removal) cells were collected for dR timepoint. On day 3 (approx 50 hours after Lif 2i removal) cells were collected for dY timepoint (EpiCs). EpiSC (G) lines expressing only miR-302, were previously described{Najm:2011wu}. They were grown in 50/50 DMEM F-12/Neurobasal supplemented with N2, B27, bFGF and Activin A. Foxd3 conditional KO cells were treated with 1uM Tamoxifen for 36 hours to induce CreER-dependent excision of the Foxd3 locus. The 3X C-terminal Flag tag was targeted into the Foxd3 locus of the Foxd3 conditional knockout cells using a flip-in construct construct (Addgene 37274) and selected using hygromycin for two weeks. For the initial enhancer profiling, previously described dual-reporter cells were used{Parchem:2014jz}. For all experiments involving Foxd3 (both Foxd3 ChIP-seq and Foxd3 knockout experiments), Foxd3 conditional knockout cells, either with or without one allele targeted with a 3X Flag, were used. Knockout of Foxd3 in ESCs was performed in steady state by treatment with 1uM Tamoxifen for 36-48h. Knockout of Foxd3 in EpiCs was performed similarly, starting 26-48h before the EpiC state is reached during ESC differentiation. Early knockout was performed for a subset of experiemnts, starting 16 hour prior to removal of Lif and 2i. These experiments are tagged KO-l (for knockout-long). Genotyping primers are as follows for the Flag tagged allele: Fwd - TCTTACATCGCGCTCATCAC; Rev - ACCATGATTACGCCAAGCTC (expected band size 1.8kb). These cells were treated identically to the dual-reporter cells, and hence were denoted 'R' (ESC) and 'dY' (EpiC), even though they did not contain the reporters.

Single-cell microRNA qPCR

Single-cell microRNA qPCR was performed as previously described. Briefly, single R or dY cells were sorted into 96-well PCR plate containing RT buffer, using a BD FACSAria II cell sorter. Cells were lysed at 95C, after which RT enzyme was added and the RT protocol was run for 60 cycles. A preamplification reaction using Amplitaq Gold Polymerase was run for 20 cycles, following by Qiagen minElute column purification. Finally, qPCR was performed using specific Taqman probes and primers (see https://urology2008-2012.ucsf.edu/blellochlab/index.htm for sequences).

Chromatin Immunoprecipitation (ChIP)

Nuclei were isolated using nuclear extraction buffer (5 mM PIPES pH 8.0 / 85 mM KCl / 0.5% NP-40) and lysed using 1% SDS/10 mM EDTA/50 mM Tris pH7.4. Cells were sonicated using a BioruptorTM (UCD200), 30 seconds ON, 60 seconds OFF on high for 5 rounds of 10 minutes. Chromatin was verified to be

>80% under 500bp. For MNase ChIP-seq, nuclei were isolated using extract buffer (10 mM HEPES pH 7.9/1.5 mM $MgCl_2$/10 mM KCl/0.5 mM DTT) and chromatin was digested using 500U of MNase per 10 million nuclei for 10 min at room temperature in digestion buffer (10 mM Tris-HCl pH 7.5/15 mM NaCl/60 mM KCl/1mM $CaCl_2$/0.15 mM Spermine/0.5 mM Spermidine). Lysate in dilution buffer (0.5% TritonX-100/2 mM EDTA/20 mM Tris-HCl pH 7.9/150 mM NaCl) equating 5-10 million nuclei per ChIP. Antibodies were used at 5ug/ChIP (except anti H3K4me3 serum where 10ul was used), and pre-incubated with 40ul of a 50/50 slurry of Protein A and Protein G Dynabeads. Beads were washed and incubated with lysate overnight. They were then washed sequentially with the following buffers: Wash 1: 20mM Tris pH=7.9/2 mM EDTA/125 mM NaCl/0.05% SDS/1% Triton X-100; Wash2: 20mM Tris pH=7.9/2 mM EDTA/500 mM NaCl/0.05% SDS/1% Triton X-100; Wash 3: 10mM Tris pH=7.9/1 mM EDTA/250 mM LiCl/1% NP-40/1% NaDeoxycholate, and eluted twice with 100 mM NaBicarbonate/1% SDS. Crosslinks were reversed overnight at 65C, and DNA was purified. Quantitative PCR was performed for gene-specific analysis, or samples were used in library preparation for deep-sequencing.

Sequential ChIP

Protocol adapted from Furlan-Magaril et al{FurlanMagaril:2009fo}. Briefly, cells were crosslinked for 30 min with 3'-dithiobispropionimidate (DTBP), followed by PFA crosslinking, nuclear isolation, sonication and incubation with the first antibody as in the ChIP protocol above. 100 million cells were used per ChIP (scaled up 10-fold from regular ChIP). Beads were washed once for 5 min with Wash 1 (see above), once with 1XTE, and eluted with TE/10 mM DTT. The eluates were diluted 1:20 in dilution buffer (see above) and incubated overnight in the second antibody. Beads were washed once with Wash 1 (see above), once with 1XTE, then eluted as in the ChIP protocol. The remainder of the protocol is identical to the ChIP protocol.

ChIP-seq

ChIP-seq libraries were made as per the Tru-Seq Illumina protocol with minor modifications. Libraries for the initial enhancer screen were sequenced once, and all subsequent libraries in Foxd3 Flag-tagged and Foxd3 conditional KO cells were performed in duplicate or triplicate. And all conclusions made based on the singlicate experiments were verified using the duplicates generated subsequently. Therefore, all ESC and EpiC samples are n=2-4. Briefly, the DNA fragments were converted to phosphorylated blunt ends using End-It DNA repair kit (Epicenter ER0720) and a 3'A overhang was added with Klenow DNA polymerase exo- (NEB M0212S). Illumina adapters were ligated to the fragments using T4 DNA Ligase (Enzymatics L6030-HC-L). Fragments of 200-250bp were then purified using Invitrogen's E-Gel with 2% gel cassettes. PCR was performed with KAPA HotStart DNA polymerase (KR0370), and DNA was purified with a 0.8:1 ratio of AMPPure beads (Agencourt A63880) to DNA. The libraries were quantified by Bioanalyzer and Qubit. The sequencing of the library DNA was performed on the Illumina HiSeq platform according to the manufacturer's

instructions. Each lane of a flowcell contained 2-5 libraries. Sequencing depth for each protein/histone modification was of at least 20X fold coverage.

MNase-qPCR

This assay was adapted from a previously published protocol{Krishnakumar:2010jt}. Briefly, chromatin was crosslinked with PFA and subjected to digestion using 500 units of Micrococcal Nuclease (Worthington) for 10 minutes at room temperature. The DNA was purified using phenol-chloroform extraction and isopropanol precipitation, and mononucleosomes were visualized using agarose gel electrophoresis. Overlapping qPCR primers were designed every 50bp with approximately 100bp amplicons per set for two EpiC enhancers and one ESC enhancer (see Sup Table 4). qPCR was performed and a moving window average of five points was taken across these regions.

Antibodies

The following antibodies were used for ChIP-seq and/or western blotting: Normal Rabbit IgG (Invitrogen 10500C), H3K4me1 (Abcam ab8895), H3K27ac (Abcam ab4729), H3K4me3 (Active Motif 39159), H3K27me3 (Millipore 07-449), p300 (sc-585X), Oct4 (sc-9081X), Pol2 (Santa Cruz sc-900X and sc-899X used at 4:1), Flag (Sigma F1804), HDAC1 (Abcam ab7028), HDAC2 (Abcam ab1832), HDAC3 (Abcam ab7030), Foxd3 (Abcam ab107248), Brg1 (Abcam ab110641), Gapdh (Santa Cruz sc25778).

ChIP-seq data analysis

1) Mapping: Fastq files were mapped to mm10 using the following options in Bowtie 2: -p 4 -q -D 15 -R 10 -L 22 -i S,1,1.15. Mapped files were converted into bam/bed format using samtools and BEDtools{Quinlan:2010km}. All samples were deduplicated to eliminate PCR bias.

2) Visualization: Tracks were created by converting bam files to bigwig using samtools and the UCSC begraph to bigwig script, and visualized using the Integrative Genome Viewer from the Broad Institute.

3) State-finding: ChromHMM was used to identify chromatin states. A total number of 6 states were requested}.

4) Dip-finding: To find nucleosome-depleted regions, the H3K27ac data was used as described previously {Haberland:2009bo}. The algorithm was adapted with a few minor modifications: slope length – 3, dip size – 0.4, height at both ends of dip – 0.2, region of 200bp.

5) Motif-finding: Two programs were used for motif finding RSAT-tool and HOMER, both with default settings{Whyte:2012dd}. In both cases, the background sequence used was the mm10 genome. The search was also repeated with an alternate background set (all unchanging state 5 and 6 regions) and although the significance was lower, the Foxd3 motif was still identified (data not shown), therefore confirming that the result was not due to background bias.

6) Peak finding: MACSv1.4 was used for Foxd3 and Oct4 peak finding{Factor:2014km}. The control data used was ChIP-seq using anti-Flag M2 antibody in an untargeted Flag-negative cell line for Foxd3, and IgG ChIP-seq for

Oct4. The following options were used -s 50 --bw 250 -f BAM -g mm - -m 10 30 -B -q 0.01.
7) Metagene analyses: For each peak, the number of reads per million in a 4000 bp radius for the given data set was extracted. This was done in windows of 100bp. The moving average of each peak was taken, using a window size of 500bp and a step size of 100bp. These individual regions were averaged to create the metagene pattern. In the case of replicates, data from replicates was consolidated and deduplicated together. Example tracks for individual genes can be found in Fig. S5A and supplemental files.
8) Heat maps: For metagene analyses, heat maps were generated using JavaTreeview {Buecker:2014dh}.
9) Venn diagrams: The R package venneuler was used to generate overlapping Venn diagrams of binding sites. The hypergeometric Chi-squared test was used to determine the significance of this overlap.

Quantitative reverse transcription PCR (RT-qPCR)
Total RNA was prepared from WT and Foxd3 knockout (1uM Tamoxifen for 36 hours) using the standard Trizol protocol. RNA was reverse transcribed using the Superscript III First-strand Synthesis kit and standard protocol. Transcript levels were determined with the Sybr Green PCR master mix from Applied Biosystems using the ABI 7900HT 384-well PCR machine.

Microarray analysis
Total RNA was prepared from WT and Foxd3 knockout (1uM Tamoxifen for 36 hours) using the standard Trizol protocol. RNA was sent to the UCLA Neuroscience Genomics Core for reverse-transcription, hybridization to the Illumina Mouse Ref 8 v 2.0 Beadchip, and scanning. Hybridization was performed twice, and some samples were used in both rounds of hybridization to account for technical differences. Data was subsetted and quantile normalized using Sample Network and differential gene expression was performed using the limma package (all the above was performed in RGui) {Benayoun:2011cd, Lam:2013ec, Lalmansingh:2012et}.

Immunoprecipitation (IP)
Cells were collected in the ESCs or EpiCs as per the above protocol. Ten million cells were used per IP. Cells were lysed in 500ul low NP40 lysis buffer (12.5 mM Tris pH7.5 or 7.9/150 mM NaCl/100 uM EDTA/10% glycerol/0.1% NP-40) and with 3X freeze-thaw cycles in liquid nitrogen. Lysate was collected by centrifugation at 13000 RPM for 10 min at 4C. Protein A/G dynabeads were incubated with 10 ug of anti Flag M2, anti HDAC1 or anti Brg1 for two hours. Lysate was incubated with antibody-coated dynabeads overnight. Beads were washed 3 times with low NP40 lysis buffer, and boiled in 5X SDS loading dye with beta-mercaptoethanol. Western blotting against Flag (Sigma M2), HDAC1, HDAC2, HDAC3 and Brg1 (Abcam) was performed (HDAC antibodies used at 1:1000 and Brg1 antibody used at 1:10,000 for Western).

Sequential Immunoprecipitation (sequential IP)

2x10^9 ESCs were crosslinked with EGS (ethylene glycol bis(succinimidyl succinate)) as indicated by the manufacturerand PFA (paraformaldehyde) as above. Cells were lysed followed by MNase digestion of DNA. Sequential IP was performed as per the sequential ChIP protocol above, first with anti-Flag antibody, then with either IgG or anti-Brg1, followed by western for all three proteins. In addition to denaturing of the Flag antibody after the first IP, the lysate was blocked with Flag peptide after the first round of IP, to prevent any excess non-denatured Flag antibody from re-IPing Foxd3.

Primer sequences

Primer sequences for Foxd3 qPCR, six enhancers from ESCs (R) and six from EpiCs (dY), and MNase tiling qPCR.

Gene/location	Forward sequence	Reverse sequence
Foxd3	GTCCGCTGGGAATAACTTTCCGTA	ATGTACAAAGAATGTCCCTCCCACCC
R-Foxd3 site #1	AGCTGCCCACAATAATGTCC	AGAAAGTCTTGCCTGGTGGA
R-Foxd3 site #2	GCAAGGTGAACCTGTCAGTG	GGAGTCCTGCTGAGAAATGC
R-Foxd3 site #3	CCTGCAATTACGCAAAGTCA	CCTAATTGGGGCAGAGACAA
R-Foxd3 site #4	TCACCCTGTGCAGTCTGAAG	ATCTGGACTCTGGTGGCATC
R-Foxd3 site #5	GCTCTCCCTGTGATGGTAGC	CCCAAGGAAAACAGCCATTA
R-Foxd3 site #6	TCCAAGAGCACTGGTCACAG	GACATGGTGGCATGTGTGTT
dY-Foxd3 site #1	ATAGGTGGCCAGCAGCTATG	CTGGCTTGAACAACACAGGA
dY-Foxd3 site #2	TGTGAGCAGTGCTTTTCTCC	GACTTGCACAGTGGGGTTTT
dY-Foxd3 site #3	GGGACTTGTACGGGTCACAG	GCTTTGTCACTGGCTTCCTT
dY-Foxd3 site #4	TGCCTTGACCATCCTTCTCT	GTCTTCTTGGGGTGACCTGA
dY-Foxd3 site #5	TTGAAACACCAACACCCAAA	AGCCTCCTGAAGAGGAAATG
dY-Foxd3 site #6	AAACCACACCAGCCTCAAAG	GTCCCGCAACTCTCTTGTTC
MNasedY2a	TCACAAACGTTCAGGAGCAA	TGTGGCATTCGTAACGTCAT
MNasedY2b	GAGAAAATTGGCTGGCAAAA	GTTCTGGGCTCCCTTGTGTA
MNasedY2c	GATGCCAGCATTTCCTCAAC	TTGCTTGCCTGTCACACTTC
MNasedY2d	TACACAAGGGAGCCCAGAAC	TTGCTTGCCTGTCACACTTC
MNasedY2e	AGGGAGCCCAGAACTTCACT	TTGCTTGCCTGTCACACTTC
MNasedY2f	AGGGAGCCCAGAACTTCACT	TGTGATGGCGCTTGAAATAA
MNasedY2g	GCTTGCCTTAAAATGAAAGGTC	CACAATGCAGTATGTTTTACTACACC
MNasedY2h	GAAGTGTGACAGGCAAGCAA	CAGAAAGCTCATCCTACTGATGT
MNasedY2i	TCAAGCGCCATCACAGATAC	tgacaaccaggaatagccact
MNasedY2j	TCAAGCGCCATCACAGATAC	tgacaaccaggaatagccact
MNasedY2k	TCAAGCGCCATCACAGATAC	tgagccttccaattctggat
MNasedY2l	GCGTAGTtgtagtggctattcc	ggctcaagatctccataccaa
MNasedY2m	gtggctattcctggttgtca	tgaaaggtgtagcccaggtt
MNasedY2n	acccttatctggaggcttgg	cccagatgaaaggtgtagcc
MNasedY2o	tggaggcttggagatcctta	agcagaaggtgtagcccaga
MNasedY2p	aacctgggctacacctttca	actcagtctcacgcagcaag
MNasedY2q	tctgggctacaccttctgct	cagcccaattcccaacaat

MNasedY2r	ggacattggaagaagggagtc	TCAAACAGAATCAAGAAAAACCAA
MNasedY2s	agatccttggacttccattca	TTCAAGCCACATGCTCTCAC
MNasedY2t	ggctgccgactgtaagtcat	TTCAAGCCACATGCTCTCAC
MNasedY2u	ggctgccgactgtaagtcat	TTCAAGCCACATGCTCTCAC
MNasedY2v	TGGTTTTTCTTGATTCTGTTTGAA	CCTGCAGTAACCGACAGAGA
MNasedY2w	TGCAAAGATAGAAGTTGGTGAGA	AGCTTAGGGCAGGAGGAATC
MNasedY2x	GTGAGAGCATGTGGCTTGAA	GGCAATTTACCCATATGCAA
MNasedY2y	CCTGATTCCTCCTGCCCTA	CTTCCACCGGGACAAAGTTA
MNasedY2z	GCTTCCTGATAGCATCACTCG	CTTCCACCGGGACAAAGTTA
MNasedY2aa	TGGGTAAATTGCCTTCTGAGAT	CAGCATTTCCACTCGGGTAT
MNasedY2ab	TTGTAGCTAGGTGAGCCAAAAG	TCCCTGCTTCAAGGAATGTT
MNasedY2ac	TAACTTTGTCCCGGTGGAAG	TTAATGACAGCTGCGTCCTC
MNasedY2ad	ATACCCGAGTGGAAATGCTG	CCGGTTAGAATGGGTCCTTTA
MNasedY2ae	ATTCCTTGAAGCAGGGAGGA	ATAGGCTGGGGAAAGCAGAT
MNasedY2af	AGATAAGGACCAGCGGCTCT	CAGCTGCACTGCTCAAGGT
MNasedY2ag	GGCTTAAATGGGATTGCTTG	CCAGCTTGAAGGTCCATTTT
MNasedY2ah	ATCTGCTTTCCCCAGCCTAT	ATCAGTGGGAGGACACTTGC
MNaseR1a	CAAGCTTTGGAATGTAGGCAAT	GGTGCTTCTCTACGCCAAGT
MNaseR1b	CCACcacacacacacacaca	TCTCCTTTGCTCTCCAGGAA
MNaseR1c	TGACATTTGACAAAGACCAGGA	ATTGACTTTGCTGGCTCACG
MNaseR1d	GCGTAGAGAAGCACCCAGAA	TGGCGGTAATCAAAGTCACA
MNaseR1e	AACACGTGAGCCAGCAAAGT	TGGCGGTAATCAAAGTCACA
MNaseR1f	AACACGTGAGCCAGCAAAGT	TGGCGGTAATCAAAGTCACA
MNaseR1g	AACACGTGAGCCAGCAAAGT	TGGCGGTAATCAAAGTCACA
MNaseR1h	TTGTCCTTTAGTGTAATCGTGGA	TTCAGACGTGGTTAAACCTTGA
MNaseR1i	TTGTCCTTTAGTGTAATCGTGGA	CATTGCTGCTGCTTATGGAA
MNaseR1j	TGCTTGTCTTCACAGCGTTT	CATTGCTGCTGCTTATGGAA
MNaseR1k	CCACGTCTGAACCTTTCTTCC	GATTTTAAAACGGCGAATGC
MNaseR1l	CCACGTCTGAACCTTTCTTCC	TGCATAGCTTTATCCCACGTT
MNaseR1m	TGTTCCATAAGCAGCAGCAA	GCTCCCGGCCTGTTATTAAA
MNaseR1n	TCGCCGTTTTAAAATCCAAC	ACAGACACAGGCGAAACCTT
MNaseR1o	AAGAGATGAACATTTTGCCAGA	GTAATTGCAGGGGAGGAGTG
MNaseR1p	CGGGAGCTCTGTCAGTCAGT	GTGGGAGTGATTGCTGTTGA
MNaseR1q	AAGGTTTCGCCTGTGTCTGT	TGGAATCCGCACTGTTGATA
MNaseR1r	CCTGCAATTACGCAAAGTCA	GGCAGGGGAAAGAAGAAGAA
MNaseR1s	TATCAACAGTGCGGATTCCA	ATGACTTGAACGCGCTTTTT
MNaseR1t	TATCAACAGTGCGGATTCCA	GATGGGCCGAAGAATGACT
MNaseR1u	CTGGTAATTTGGCACCAACG	AGTGAAAAGAGGACGGAGCA
MNaseR1v	AGCGCGTTCAAGTCATTCTT	CTTGCCTTCTGCCAGATTTC
MNaseR1w	TGCTCCGTCCTCTTTTCACT	TCCAGTCGGCATGGTAACTA
MNaseR1x	TGCTCCGTCCTCTTTTCACT	CTCAGCACAGGCTCCAGTC
MNaseR1y	GAAATCTGGCAGAAGGCAAG	AGCATCACGACCAAAGGAGT
MNaseR1z	AGTTACCATGCCGACTGGAG	GCATGCAGTGAGATTTTTCC

MNaseR1aa	CTGCCACACCGAACTCAAT	CAGCGCCTATCACATGCTATT
MNaseR1ab	AGACAGGGCTGTTGGTATGG	CATGGGACAGAACACAGCAT
MNaseR1ac	TGCAAACATGCCAACTCAAT	GGTGCACAGATTCTCATGTCA
MNaseR1ad	GCGCTGCATGGAACTTAGAC	GAAGCAGACCGATGAAAAGG
MNaseR1ae	AAGGGCTGAACAGGGATAAGA	GTACACCAGGCCTGACGACT
MNaseR1af	TGTTCTGTCCCATGATCAACA	GGTTTTGGTTTTGAAATGACC
MNaseR1ag	CCTTTTCATCGGTCTGCTTC	CCAAAGTCCCGTGAGTTCTT
MNaseR1ah	TGGACTGGTCATTTCAAAACC	ATGCTCAGAGGCATTTCCTG

Supplemental Tables

Table S1 (related to Fig. 1):
1) Sequencing statistics. Total number of reads, number of uniquely mapping reads and percentage of uniquely mapping reads for all ChIP-seq data sets. R – ESCs, dR – differentiation day 1, dY – EpiCs, G – EpiSCs.,E – EtOH-treated, T – tamoxifen-treated (Foxd3 KO), T-early – early tamoxifen treatment (Foxd3 early KO), rep – replicate.
2) Results of ChromHMM. Results of ChromHMM that are displayed in a heat map in Fig 1A. The number represent normalized enrichment, with 1 being the maximum average signal for any mark.
3) Dip sites identified in H3K27ac data. Genomic locations (mm10) of all dips identified in transitions from State 5 to State 6 (as determined by ChromHMM). The dips are separated into ESC (R) to dR, dR to EpiCs (dY) and EpiCs (dY) to EpiSCs.
4) Motifs enriched at enhancers transitioning from active to primed and vice versa. Percent enrichment and -log10(p-value) of top 5 known motifs enriched at enhnacers transitioning from State 5 to State 6 and vice versa in ESCs to dR, dR to EpiCs and dY to EpiSCs.

Table S2 (related to Fig. 2):
1) Foxd3 peaks. Genomic locations on mm10 of Foxd3 peaks in ESCs and EpiCs as determined by MACS1.4
2) Motifs at Foxd3 ESC peaks. Identified as true positives by Homer motif analysis. Significance values and percent target sequences containing motifs are shown.
3) Motifs at Foxd3 EpiC peaks. As described for tab 3.
4) Genes near Foxd3 peaks. List of genes near ESCs and EpiCs Foxd3 peaks.

Table S3 (related to Fig. 3):
Oct4 peaks. Genomic locations on mm10 of Oct4 peaks in ESCs and EpiCs as determined by MACS1.4.

Supplemental data files

Supplemental data file 1 (related to Fig. 1)
1) Genome browser tracks for H3K4me1, H3K27ac, H3K27me3, p300, H3K4me3 and Pol2 in R (ESC), dR, dY (EpiC) and G (EpiSC) at Fgf5. The promoter-proximal enhancer is primed (H3K4me1+) and acquires H3K27ac and Pol2 during differentiation (box 1), and three distal enhancers appear de novo in dY (boxes 2-4). Also, p300 levels at the promoter-proximal enhancer do not change drastically despite a large increase in H3K27ac from ESCs to EpiCs.
2) Genome browser tracks for H3K4me1, H3K27ac, H3K27me3, p300, H3K4me3 and Pol2 in R (ESC), dR, dY (EpiC) and G (EpiSC) at Klf4. A general

dismantling of the enhancer chromatin in the region is observed, with H3K27ac dropping rapidly while H3K4me1 persists longer. Even though promoter-proximal H3K27me3 levels increase mildly from ESCs to EpiCs, they increase drastically upon transition to EpiSCs. Again as with Fgf5 enhancers, p300 levels do not correlate with H3K27ac.

Supplemental data file 2 (related to Fig. 2): FIMO analysis for ESC Foxd3 peaks using the Jaspar Foxd3 PWM

Supplemental data file 3 (related to Fig. 2): FIMO analysis for EpiC Foxd3 peaks using the Jaspar Foxd3 PWM

Supplemental data file 4 (related to Fig. 2): Genome browser tracks of Foxd3 in ESCs and EpiCs, and Oct4 in ESCs, dR and EpiCs. The data represents two patterns seen at genes that acquire Oct4 at sites of Foxd3 binding in ESCs. Genes such as Cdh6, Atl7ip2 and Dll1 show increased Oct4 binding in dR but loss in EpiCs, whereas genes such as Hspa9, Wdr33 and Cdca4 only acquire Oct4 in EpiCs. Foxd3 binding precedes maximum Oct4 binding in both cases and is lost upon Oct4 binding.

Supplemental data file 5 (related to Fig. 6): MNase-H3K4me1, H3K27ac, Brg1 and HDAC1 genome browser tracks at representative Foxd3-bound EpiC enhancers plus or minus Foxd3 deletion during the ESC to EpiC transition. For MNase-H3K4me1 tracks, tamoxifen was added 24 hours before initiating differentiation with Foxd3 protein becoming undetectable by western 24 hours into differentiation (eKO or early KO). In contrast, for other tracks tamoxifen was added 24 hours into differentiation causing Foxd3 protein levels to drop during the transition, becoming undetectable just as cells reach the Epi state. This time difference allows for the identification of the three different types of tracks shown, which represent three timepoints of Foxd3 action at enhancers. Type 1, flanking regions remain nucleosome-occluded and hypoacetylated in KO cells. These findings are consistent with Foxd3 binding during late stages of differentiation after depletion of Foxd3 protein in both conditions described above. Type 2, nucleosomes are depleted in the enhancer region in wt and Foxd3 KO. In contrast, H3K27ac increases at enhancer site. This combination of events is consistent with the binding of Foxd3 during the early stages of differentiation. That is, once Foxd3 has bound and nucleosomes have been depleted, loss results in increased acetylation, but little change in nucleosome occupancy. Type 3 has a combination of partial nucleosome depletion and increased acetylation with Foxd3 KO. This intermediate state is consistent with Foxd3 binding at an intermediate stage of differentiation within the time window between Foxd3 loss

in the two conditions described. Metagene analysis shown in main figures represents an average of these different types.