FIGURE S1. Related to Figure 1

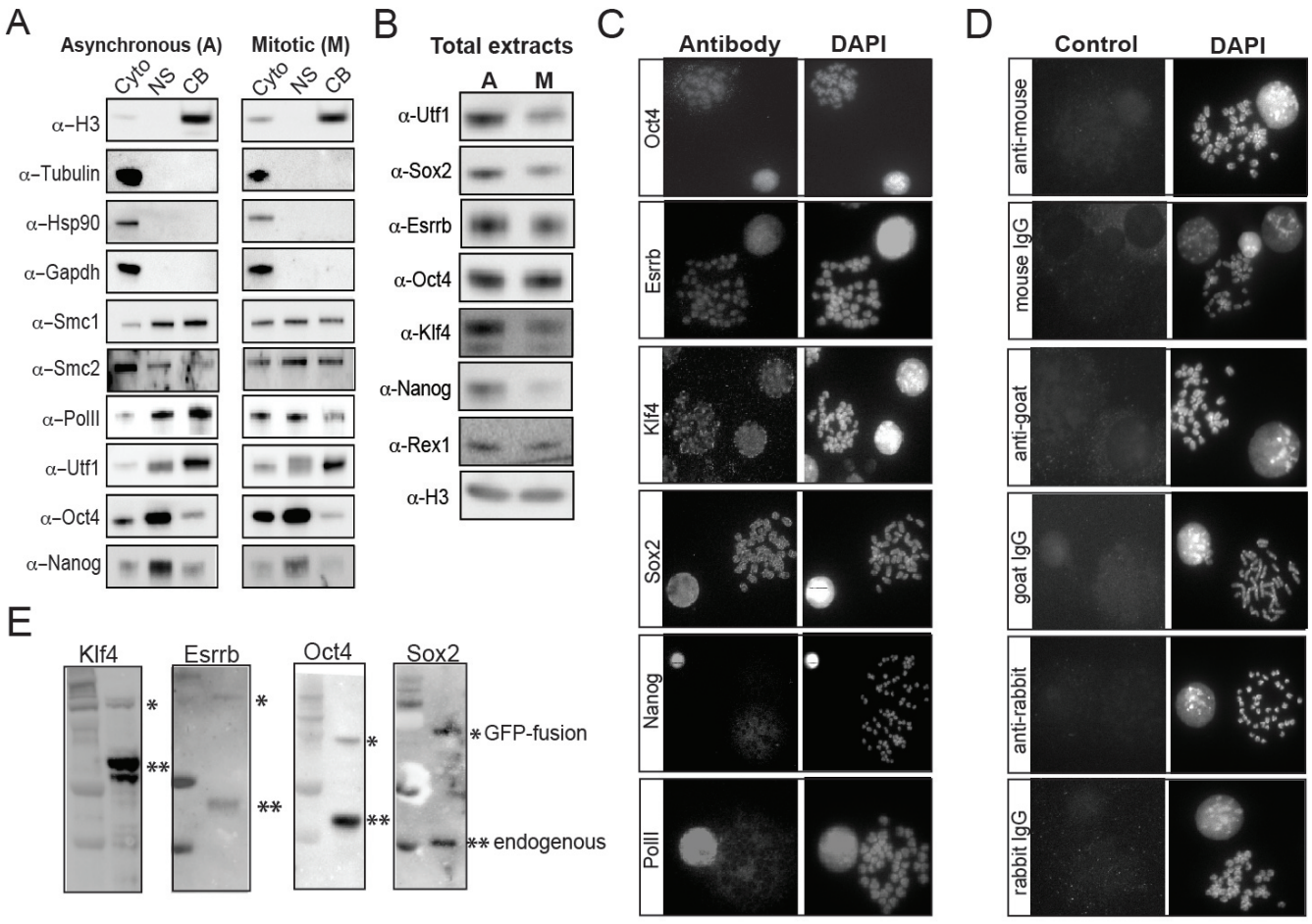

Figure S1 (related to Figure 1) A. Western blot analyses testing the purity of subcellular fractions isolated from asynchronous (A) and mitotic (M) ESCs. Note that the *bona fide* cytoplasmic markers tubulin, Hsp90 and Gapdh were detected only in cytoplasmic fractions (Cyto) and were efficiently depleted from the nuclear soluble (NS) and chromatin-bound (CB) fractions, while Histone 3 (H3) was predominantly enriched in the CB extracts. Nuclear proteins, such as Smc1 and RNA PolII were detected more strongly in the cytoplasmic fraction of mitotic than asynchronous cells due to the lack of nuclear envelope. Condensin subunit Smc2 was mostly detected in the mitotic CB in accordance to its role in mitotic chromatin condensation. Stringent fractionation conditions ensured that the CB extracts contain only the fraction of proteins that are strongly associated to chromatin, whereas loosely associated proteins are released in the NS fraction. **B.** Western blot analyses showing the relative levels of the indicated TFs in the total cellular extracts from asynchronous (A) and mitotic (M) ESCs. **C-D.** Representative immunofluorescence photos on metaphase spreads of ESCs using antibodies against the indicated proteins (C) and the respective IgG isotypes or secondary antibodies (D) as negative controls. In agreement with the biochemical data shown in Figure 1D-E, OCT4, SOX2, KLF4 and ESRRB were associated the mitotic chromosomes, whereas NANOG and RNA Polymerase II (PolII) were excluded from the condensed chromosomes. **E.** Western blot analyses from the transgenic ESCs used for imaging in Figure 1F, showing the relative expression of the chimeric GFP versions (one asterisk) for each of the tested TFs compared to the endogenous ones (two asterisks).

FIGURE S2. Related to Figure 2

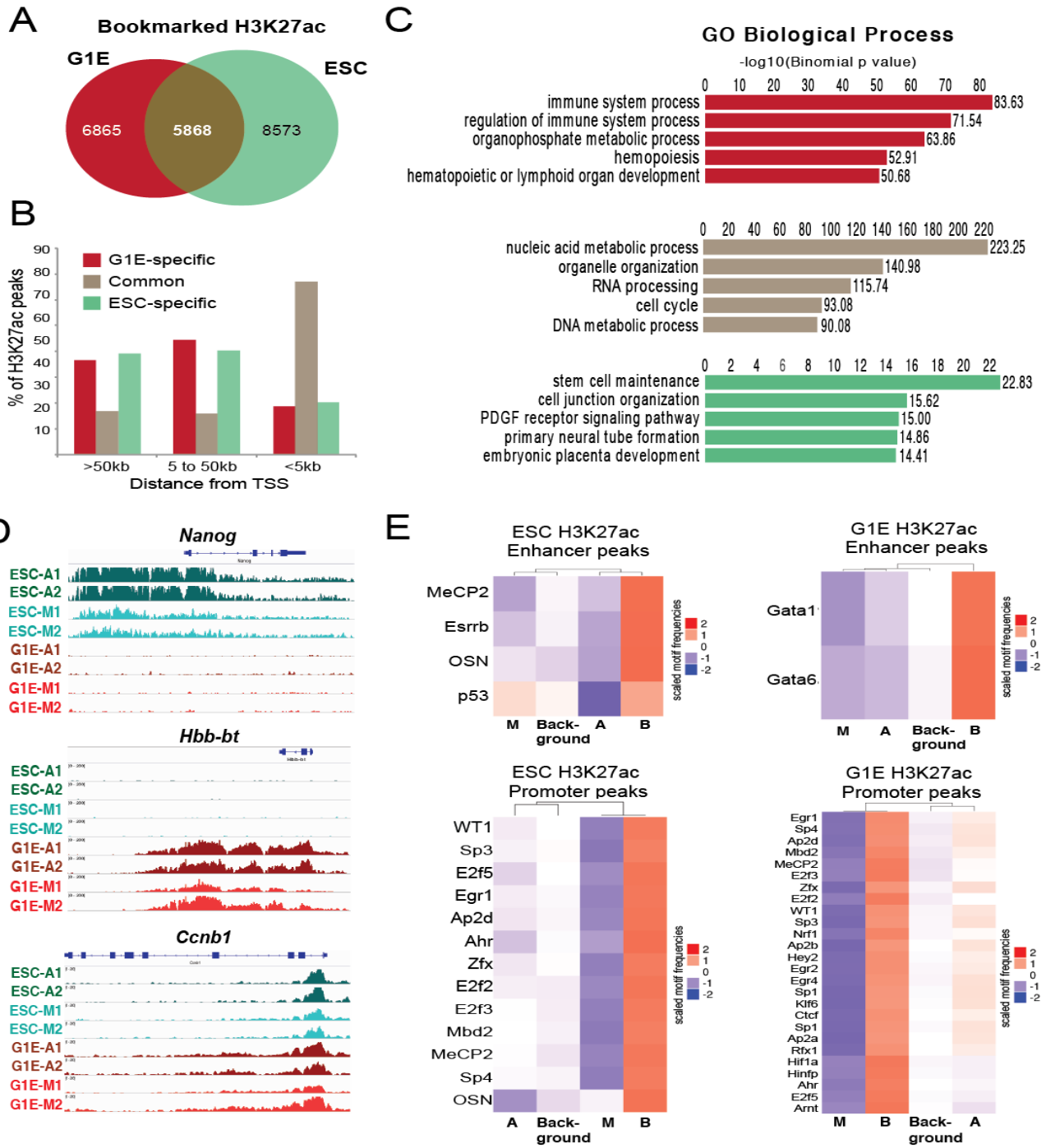

Figure S2 (related to Figure 2) A. Venn diagram depicting the overlap of H3K27ac peaks that were found bookmarked in mouse ESCs and G1E erythroblast cells. For the latter, recently published H3K27ac ChIP-seq datasets were used (Hsiung *et al*. 2016). Only ChIP-seq peaks consistently detected in both biological replicates were considered. **B.** Distribution of G1E-specific (Red), Common (Brown) and ESC-specific (Green) H3K27ac bookmarked peaks relative to Transcription Start Site (TSS). The common peaks were primarily located proximal to TSS, whereas the majority of cell-type specific peaks were >5kb away from TSS. **C.** Top 5 gene ontologies (GO) enriched for each category shown in (A). The common peaks highly enrich for cell cycle and housekeeping genes, whereas the cell-type specific ones enrich for functions related to the respective

cell identity. **D.** Examples of H3K27ac signals in mitotic and asynchronous ES cells and G1E erythroblasts around *Nanog*, *Hbb-bt* and *Ccnb1*. **E.** Heatmaps showing the relative frequencies of known DNA binding motifs that were found enriched among the ESC or G1E bookmarked H3K27ac sites. Motifs for both general and lineage-specifying transcription factors were overrepresented among promoter-proximal H3K27ac peaks (<2.5kb from TSS) (bottom graphs), whereas bookmarked enhancers (H3K27ac peaks >2.5kb for TSS) predominantly enriched for cell-type specific regulators (for raw frequencies see Supplemental table 2).

FIGURE S3. Related to Figure 3

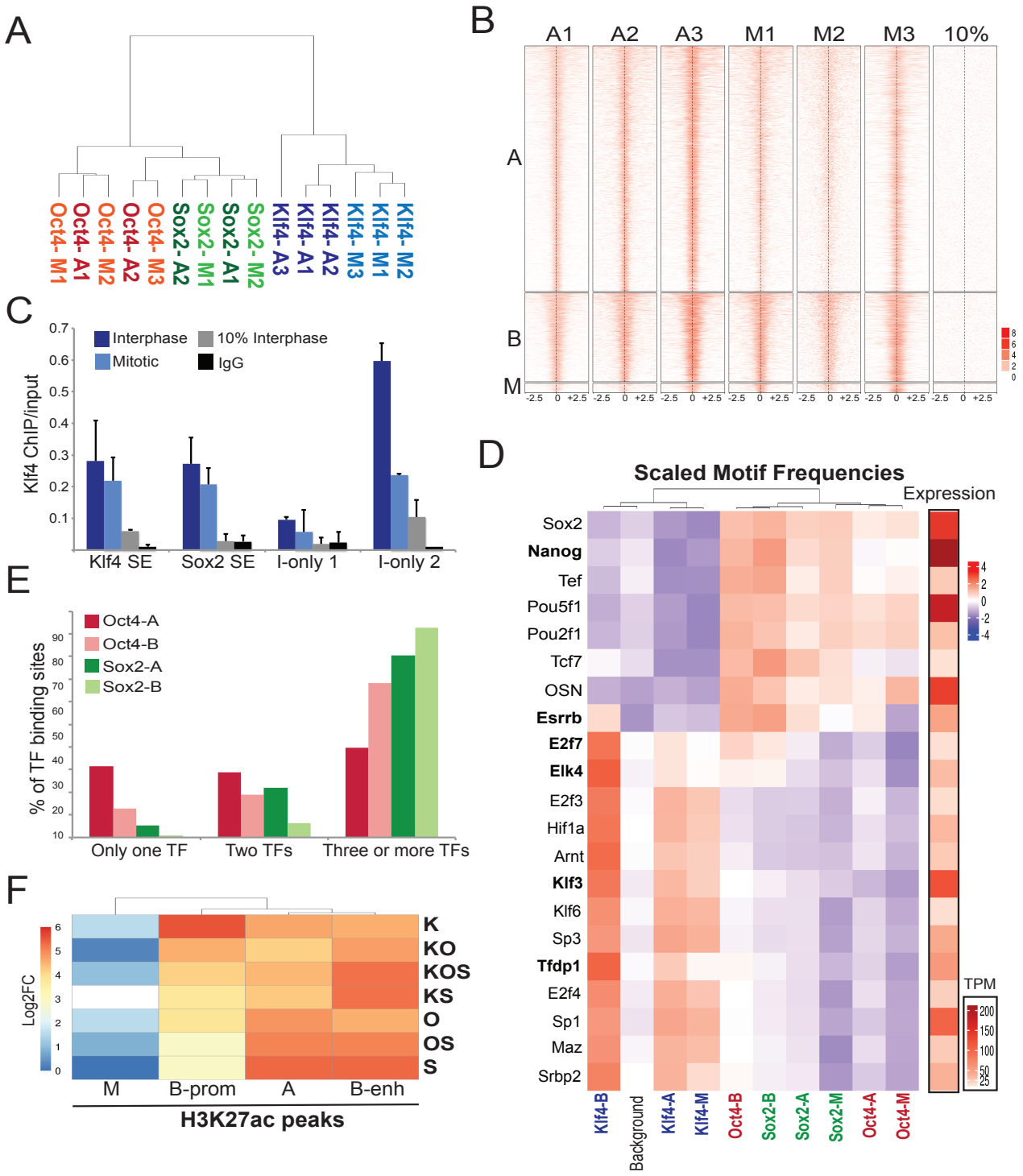

Figure S3 (related to Figure 3) A. Hierarchical clustering of KLF4, OCT4 and SOX2 (KOS) ChIP-seq samples based on Spearman's rank correlation coefficients of binding signals at consistent KOS peaks across replicates. **B.** Enrichment plots (log2 (IP/input)) of KLF4 ChIP-seq signals in three biological replicates of asynchronous (A1, A2, A3) and mitotic (M1, M2, M3) ESCs. ChIP-seq from Interphase ESCs mixed with neuronal progenitor cells (NPCs) in a 1:9 ratio (10% Interphase) was used to control for the potential effect of G2 contamination in mitotic samples. ChIP-seq signals were centered 2.5 kb

upstream/downstream of peak centers. **C.** ChIP qPCR analyses for selected bookmarked or not KLF4 targets, showing the relative levels of KLF4 binding in interphase and mitotic ESCs. The 10% Interphase sample (as described in B) was used as control. **D.** Heatmap showing the relative frequencies of known DNA binding motifs discovered at the unique (A or M) or common (B) binding sites for each of the tested TFs. Motif frequencies are shown as *Z* scores, and raw motif frequencies are reported in Supplemental Table 3. Motifs were filtered based on their p-value ($<10^{-5}$) and the transcriptional activity of the respective transcription factor (>10 Transcripts Per Kilobase Million or TPM > 10; Shen *et al*. 2012). **E.** Barplot showing the percentage of OCT4 and SOX2 binding sites (asynchronous (A) or bookmarked (B)) that overlap with binding of at least one additional TF within 100bp distance. Published ChIP-seq datasets in asynchronous mESCs for ESRRB, OCT4, SOX2 and NANOG were used to determine overlap (Chen et al, 2008; Marson et al, 2008; Whyte et al, 2013). **F.** Enrichment heatmap expressed as log2 ratio of observed over expected frequencies of overlap between bookmarked genomic sites by KOS (individual TF or combinations) and asynchronous-only (A), mitotic only (M) or bookmarked (B) H3K27ac peaks. The expected fraction was estimated from random shuffling (N=50) of the length- and width-matched genomic regions across non-blacklisted genomic regions. B-prom: H3K27ac-bookmarked promoter peaks (<2.5kb from Transcriptional Start Site, TSS); B-enh: H3K27ac-bookmarked enhancer peaks (>2.5kb from TSS).

FIGURE S4. Related to Figure 4

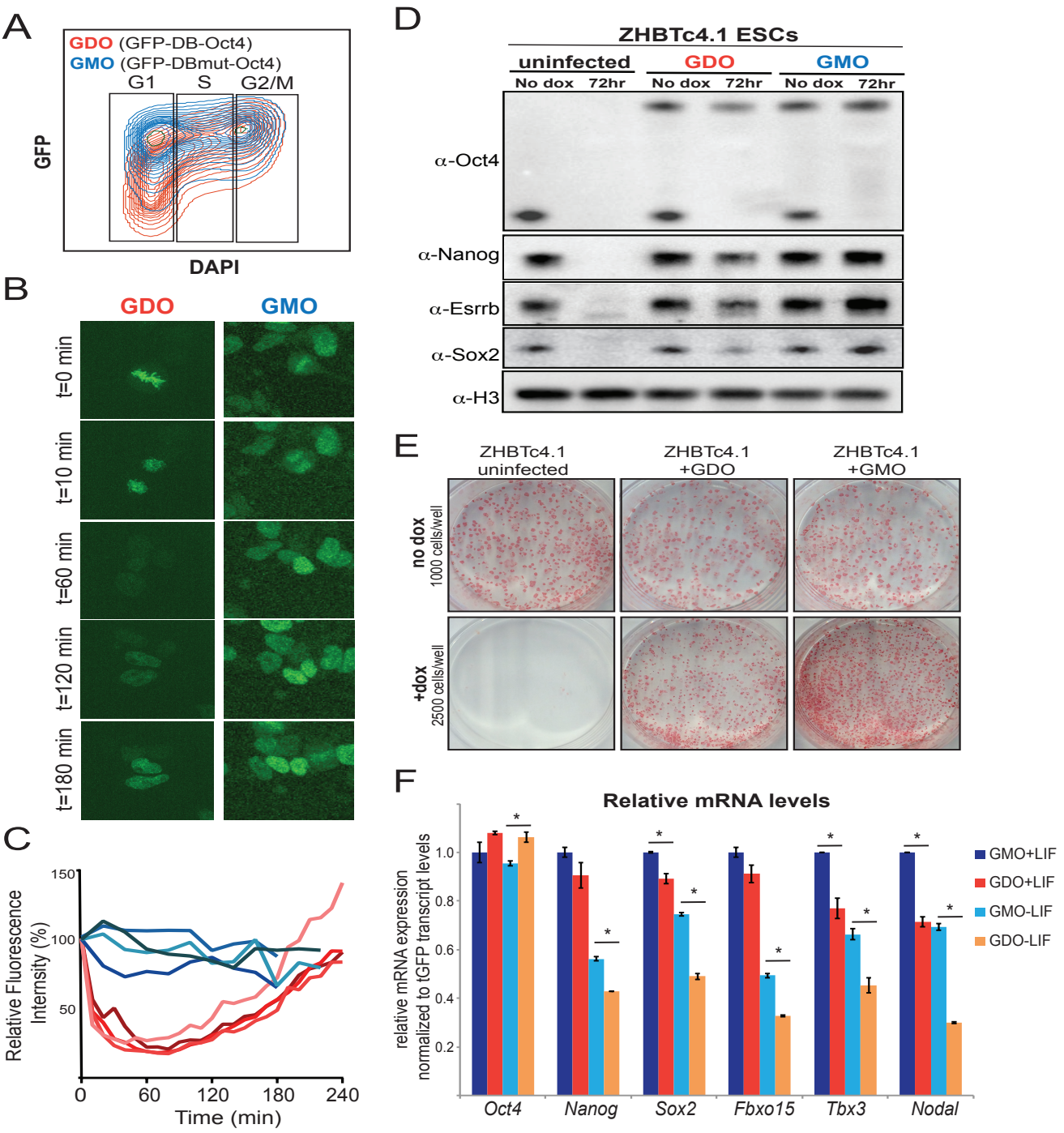

Figure S4 (related to Figure 4) A. FACS analysis of ZHBTc4.1 cells infected with either tGFP-DB-OCT4 (GDO) or tGFP-DBmut-OCT4 (GMO) showing GFP expression levels in relation to DNA content as detected by DAPI. Note that GMO was stably expressed throughout the cell cycle, while GDO was degraded during M-to-G1 transition. **B.** Representative time-lapse images of GDO or GMO infected H2B-mCherry ESCs undergoing cell division. **C.** Quantitation of the live imaging experiments described in (B). The GFP signal intensity expressed as percentage of the intensity during anaphase (time=0) is plotted in a course of 4 hours. Four GDO-expressing cells (red shades) and four GMO-expressing cells (blue shades) are shown. **D.** Representative Western blot experiment, used to quantify

the relative protein levels of selected stem cell regulators in uninfected, GDO or GMO infected ZHBTc4.1 cells with or without treatment with doxycycline (dox). **E.** Alkaline phosphatase staining depicting the self-renewal ability of uninfected, GDO or GMO infected ZHBTc4.1 in the presence or absence of dox. GDO and GMO cells were sorted for GFP expression prior to plating on feeders. 1000 and 2500 cells were plated for the –dox and +dox conditions, respectively. **F**. qRT-PCR results showing the relative expression of the indicated genes in ZHBTc4.1 ESCs infected with either GMO or GDO and cultured for two passages with or without LIF, always in the presence of dox. mRNA levels are plotted as a fraction of the respective ones in the GMO samples with LIF, after normalization to Gapdh and tGFP transcript levels (see primer sequences in Supplemental table 4). Error bars indicate standard deviation from results of 2 technical and 2 biological replicates. Asterisk indicate p-value < 0.05, calculated by two-sample two-sided t-test.

FIGURE S5. Related to Figure 5

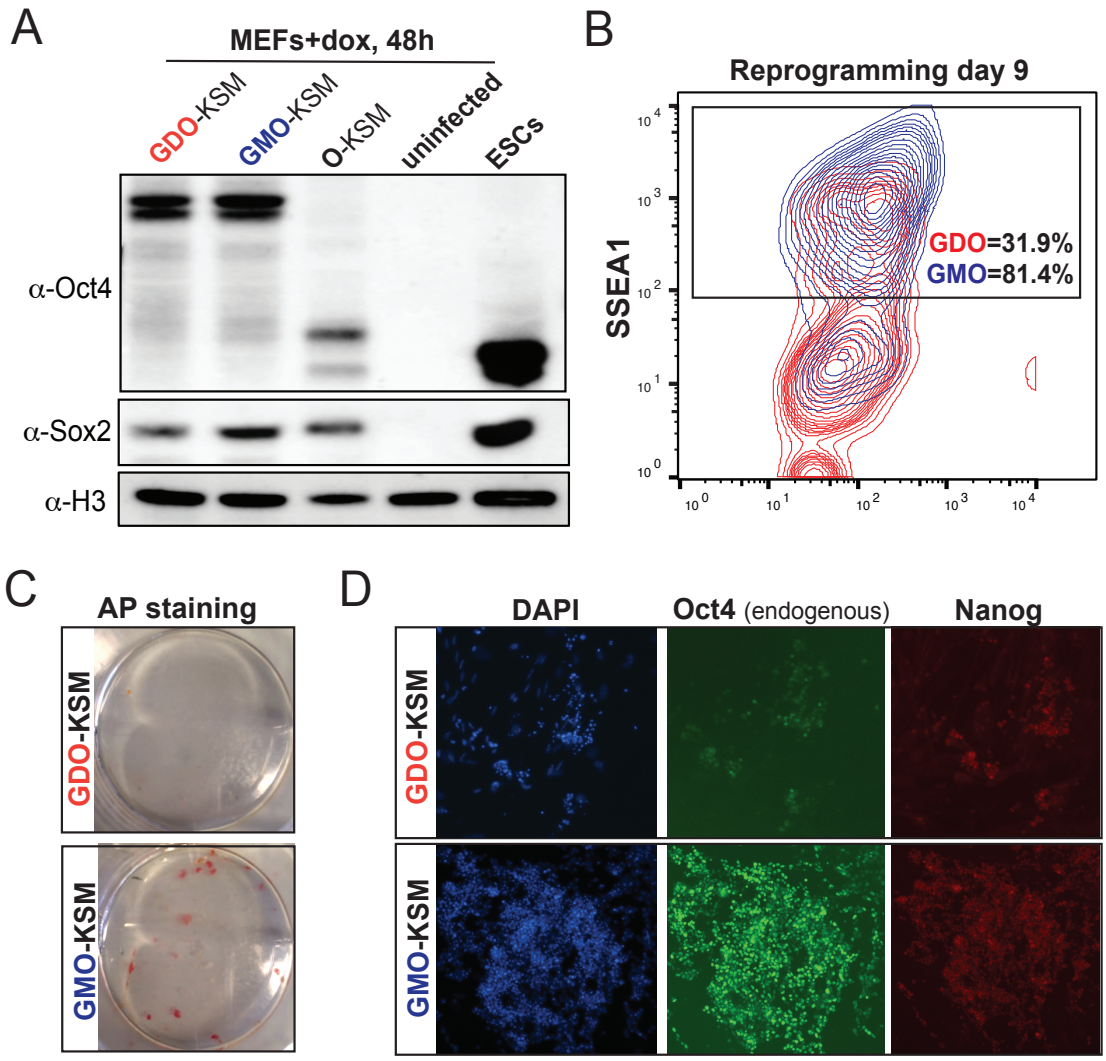

Figure S5 (related to Figure 5) A. Western blot analyses of Rosa26-M2rtTA MEFs infected with pHAGE-tetO-tGFP-DB-OCT4-KSM (GDO-KSM), pHAGE-tetO-tGFP-DBmut-OCT4-KSM (GMO-KSM), or wild type OCT4-KSM and induced with doxycycline (dox) for 72hrs. The chimeric or wild type OCT4 and SOX2 expressed from the polycistronic cassette were detected. Uninfected MEFs and ESCs were used as controls. **B**. FACS analysis showing the percentage of GDO-KSM and GMO-KSM infected MEFs that upregulated the pluripotency marker SSEA-1 after 9 days of dox treatment. **C.** Alkaline phosphatase staining after 12 days of reprogramming and then 4 days of dox withdrawal, which caused a dramatic regression of most of the GDO-KSM iPSC colonies, suggesting that GDO expressing cells failed to reach a stable pluripotent state. **D.** Immunofluorescence images validating the expression of endogenous OCT4 and NANOG in the final GDO-KSM and GMO-KSM iPSCs colonies, which were consistently much fewer in the GDO-KSM conditions (see Figure 5B).

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Cell Culture

V6.5, ZHBTc4.1 (Niwa et al., 2000) and H2B-mCherry expressing ESCs were cultured on irradiated feeder cells in KO-DMEM (Invitrogen) supplemented with L-Glutamine, penicillin-streptomycin, nonessential amino acids, β-mercaptoethanol, 1000 U/ml LIF and 15% heat-inactivated fetal bovine serum (FBS). Prior to synchronization, cells were expanded on gelatinized plates in the presence of 2i, MEK1 inhibitor (PD98059) and Gsk3 inhibitor (Chir99021) for at least 2 passages. Mouse Embryonic Fibroblast (MEF) cultures were established by trypsin-digestion of midgestation (E13.5-15.5) embryos isolated from either the "reprogrammable" mouse strain (Stadtfeld et al., 2010) or the Rosa-M2rtTA strain, followed by culture in DMEM supplemented with 10% FBS, L-Glutamine, penicillin-streptomycin, non-essential amino acids and β-mercaptoethanol.

DNA constructs

The GFP fusions were generated by Gibson assembly strategy using the lentiviral pHAGE-Ef1a vector, turbo GFP (tGFP, Evrogen) and the coding sequence (CDS) of either Pou5f1 (Oct4), Sox2, Klf4 or Esrrb. The respective CDS were PCR amplified from the pHAGE-Ef1a-Stemcca construct (Sommer et al, 2009) or from the FUW-tetO-Esrrb (Addgene). For stable expression, Cells were infected with lentivirus that was produced in 293Ts using the VSV-G and Delta8.9 packaging vectors. For the M-to-G1 degradable (tGFP-DB-OCT4 or GDO) or resistant (tGFP-DBmut-OCT4 or GMO) chimeras of OCT4, we first generated G-blocks (IDT) containing the tGFP in frame with the wild type Cyclin B destruction box (DB) (13-90aa) or a mutated R24A version (DBmut). The G-blocks were then cloned upstream and in frame with the Oct4 CDS into the pHAGE-Ef1a construct for the ZHBTc4.1 ESC experiments or the pHAGE-tetO-Stemcca (Sommer et al., 2009) construct for the reprogramming experiments. For *in vitro* transcription, the tGFP-DBmut-KLF4 fragment was cloned into a pCS2 vector containing a modified multiple cloning site (gift from Dr. Nancy Papalopulu, Manchester University, UK) using the BamHI and BstBI restrictions sites.

Live imaging

For all live imaging, cells were cultured on 35-mm glass bottom dishes (ibidi).
For Figure 1F, V6.5 ESCs infected with lentivirus expressing either tGFP fused to the respective TFs or tGFP alone were partly arrested in mitosis with short nocodazole treatment (2-3 hours) to enrich for mitotic cells. Cell Permeable DNA dye (Vybrant Violet DyeCycle Violet stain-ThermoFisher, 1:2000) was added to the medium 10 minutes prior to live imaging using a Zeiss LSM880 laser scanning confocal microscope.
For the time-lapse movies (Figure S4B and Supplemental Movies 2 and 3), H2B-mCherry ESCs (REF) expressing either GDO or GMO were monitored using either CSU-X Yokogawa spinning disk or LSM880 (Zeiss) confocal microscopes for up to 12 hours (10~15min time intervals). To quantify fluorescence intensity of GDO and GMO, maximum intensity projection files were prepared from z-stacks time series, and fluorescence intensity of GFP after cell division (anaphase as 0-min) was quantified using Metamorph (Molecular Devices) software.
For Figure 1G and related Supplemental movie 1, 2-cell stage embryos were cultured in KSOM-AA microdrops at 37°C in a 5% CO_2 atmosphere on a Zeiss LSM880 microscope equipped with an incubation chamber. Whole-embryo Z-stacks were acquired using an EC Plan-Neofluar 40x/NA1.3 objective. Embryos were imaged for 12-16h at 15-min intervals starting 2h after microinjection.

Mouse husbandry and embryo collection

All animal work was approved by Memorial Sloan Kettering Cancer Center's Institutional Animal Care and Use Committee (IACUC). Mice were housed in a pathogen-free facility under a 12h light/dark cycle. Embryos were obtained from natural matings of outbred CD1 females (Charles River) to CD1 stud males. Embryos were harvested 1.5 days after detection of copulation plug (E1.5, 2-cell stage) by flushing oviducts with 37°C flushing holding media (FHM, Millipore).

In vitro transcription and mRNA microinjection

mRNA for embryo microinjection was transcribed using mMessage mMachine SP6 (Invitrogen) following manufacturer's instructions. Capped mRNA for KLF4-GFP was injected at a concentration of 250ng/µl into one of two blastomeres of 2-cell stage mouse embryos as described (Nagy et al., 2003). Embryos were transferred to microdrops of KSOM-AA medium (Millipore) for overnight culture and live imaging after microinjection. 12 out of 27 injected blastomeres did not divide. Half of the ones that divided showed strong enrichment of KLF4-GFP on the mitotic chromosomes, whereas the rest had no detectable GFP during mitosis.

Virus production and transduction
293T cells were transfected with overexpression constructs along with packaging vectors VSV-G and Delta8.9 using PEI reagent (PEI MAX, Polyscience #24765-2). Supernatant was collected after 48hrs and 72hrs and virus was concentrated using Polyethylglycol (PEG, Sigma # P4338). ESCs (V6.5, ZHBTc4.1 or H2B-cherry) or MEFs (Rosa26-M2-rtTA) were infected in medium containing 5ug/ml polybrene (Millipore, TR-1003-G) followed by centrifugation at 2100rpm for 90 mins at 32^{o}C. GFP positive cells were collected by FACS 3-5 days after infection.

Mitotic arrest and cell cycle analysis
Initial experiments to determine optimal synchronization for each cell line were performed at different nocodazole concentrations (50-200ug/ml), treatment durations (5-24hr) and combination with thymidine treatment prior to nocodazole. The efficiency of synchronization was estimated by FACS (BD FACSCANTOII) after ethanol fixation and staining with H3ser10p antibody (ab47297) and DAPI. For all the experiments included in the manuscript, mitotic arrest was induced as follows: 80-90% confluent mouse ESCs were passaged 1:3 on gelatinized plates the day before synchronization. Nocodazole (100ug/ml) was added to the medium for 7hr prior to collection by mitotic shake-off. In some cases (2 ChIP-seq and 1 fractionation experiment) O/N treatment (~16hrs) with nocodazole was performed without obvious effects on the results. Aliquots from the mitotic and asynchronous populations were retained for estimation of synchronization efficiency by FACS, as described above.

Preparation of metaphase spreads and immunofluorescence experiments
ESCs or reprogrammable MEFs induced with dox for 3 days were treated with nocodazole for 4 or 20 hours, respectively. Cells were collected by trypsinization and resuspended in hypotonic solution (0.075M KCl) for 20 minutes at RT and fixed with methanol: acetic acid (3:1). Fixed cells were spun down at 1200rpm for 5min and pellets were thoroughly resuspended in methanol:acetic acid for 2 additional rounds. Fixed cells were dropped on glass slides, which were previously dipped in warm water and washed in methanol:acetic acid. Slides were air dried for 5-10 minutes and then immediately used for immunofluorescence experiments. Briefly, cells were permeabilized in 0.1% (w/v) Triton X-100 in PBS for 10min at room temperature (RT). After permeabilization, the cells were blocked with 5% (w/v) FBS for 30min. Subsequently, they were incubated overnight at 4^{o}C with primary antibodies or the respective IgG isotypes (see Supplementary table 1). Secondary antibodies conjugated with either Alexa Fluor 488 or 546 (Invitrogen) were used. Images were obtained using Nikon 90i Eclipse with 100X objective.

Cell fractionation
Asynchronous or mitotic ESCs were collected and fractionated using a subcellular protein fractionation kit (ThermoScientific, #78840) following manufacturer's instructions. This protocol combined detergent and salt-based buffers to enable -among others- collection of cytoplasmic, nuclear soluble and chromatin bound fractions. The efficiency and purity of the fractions was evaluated by western blot analysis as shown in Figure S1A using a number of cytoplasmic and chromatin protein markers (see Supplementary Table 1).

ChIP-seq experiments
Asynchronous or mitotic ESC were crosslinked in 1% formaldehyde at RT for 10 minutes and quenched with 125mM glycine for 5 mins at RT. 25-50*10^{6} ESCs were used for the TF ChIPs and 10*10^{6} for H3K27acetylation ChIP. Cell pellets were washed twice in PBS and resuspended in 400ul lysis buffer (10mM Tris pH8, 1mM EDTA, 0.5% SDS) per 20 million cells. Cells were sonicated in a bioruptor device (30 cycles 30sec on/off, high setting) and spun down 10 minutes at 4^{o}C at maximum speed. Supernatants were diluted 5 times with dilution buffer (0.01%SDS, 1.1% triton,1.2mM EDTA,16.7mM Tris pH8, 167mM NaCl) and incubated with the respective antibody (2-3ug/10M cells) (see Supplementary Table 1) O/N with rotation at 4^{o}C. Next day, protein G Dynabeads (ThermoScientific) preblocked with BSA protein (100ng per 10ul Dynabeads) were added (10ul blocked Dynabeads per 10 million cells) and incubated for 2-3 hours at 4^{o}C. Beads were immobilized on a magnet and washed twice in low salt buffer (0.1% SDS,1% triton, 2mM EDTA, 150mM NaCl, 20mM Tris pH8), twice in high salt buffer (0.1% SDS,1% triton, 2mM EDTA, 500mM NaCl, 20mM Tris pH8), twice in LiCl buffer (0.25M LiCl, 1% NP40, 1% deoxycholic acid (sodium salt), 1mM EDTA, 10mM Tris pH8) and once in TE. DNA was then eluted from the beads by incubating with 150ul elution buffer (1% SDS, 100mM NaHCO3) for 20 minutes at 65^{o}C (vortexing every 10min). Supernatants were collected and reverse-crosslinked by incubation at 65^{o}C O/N in presence of proteinase K. After Rnase A treatment for 1hr at 37^{o}C, DNA was purified using the minElute kit (Qiagen). 6-10ng of immunoprecipitated material was used for ChIP-seq library preparation using the KAPA Hyper prep kit (KAPA Biosystems). Libraries were sequenced on an Illumina HiSeq 5000 platform on SE50 mode.

Data Analysis

Mapping, peak calling and peak processing. Generated and published ChIP-seq sequence reads were trimmed for adaptors (cutadapt version 1.8.1) and low-quality ends (sickle version 1.33), respectively. Alignment to the mouse reference genome version mm10 was performed using standard parameters, permitting maximum one mismatch in seed alignment (Bowtie version 2.2.5). Reads marked as positional duplicates or falling into mouseENCODE blacklisted genomic regions (liftOver to mm10; ENCODE Project Consortium 2012) were filtered out. ChIP-seq peaks (enrichment of signals over background) were called at the P value threshold of $p < 10^{-5}$ (MACS version 2.1.1), and peaks detected in more than half of biological replicates were retained for further analysis. For KLF4, all ChIP-seq datasets analyzed (3 for asynchronous or 3 for mitotic ESCs) were generated in house. For OCT4 and SOX2, additional published datasets from Shin et al, 2016 and Whyte et al, 2013, respectively, were integrated to increase stringency. Asynchronous-only or Mitotic-only peaks were unique for the respective sample. Peaks with at least 1bp overlap between asynchronous and mitotic samples were merged and assigned as bookmarked peaks.

ChIP–seq peak annotation and enrichment analysis. Highly confident annotations of transcription factor binding sites were attained from ChIP-seq peaks detected in more than half of published datasets for ESRRB (Chen et al, 2008), OCT4 (Chen et al, 2008; Marson et al; 2008 and Whyte et al, 2013), SOX2 (Chen et al, 2008; Whyet et al, 2013) and NANOG (Chen et al, 2008, Marson et al, 2008 and Whyte et al, 2013). Genome background was generated by random shuffling of length- and width-matched intervals across non-blacklisted regions (bedtools shuffle version 2.25.0) fifty independent times. Enrichments of annotated features were estimated as log2 of ratios of the annotated ChIP–seq percentage relative to the averaged percentage of the randomly shuffled genome background.

ChIP-seq motif enrichment analysis. ChIP-seq peaks were scanned for enrichment of known transcription factor motifs in the mouse HOCOMOCO database (version 10) and additional selected HOMER motifs (version 5.4). Reported motif frequencies at ChIP-seq peaks and background were rescaled as *Z*-values.

ChIP-seq gene ontology (GO) enrichment analysis. Spatial proximity of ChIP-seq peaks to transcript start sites (TSSs) and enriched GOs were uncovered utilizing GREAT (version 3.0.0) web application. We selected the basal plus extension rule for the association of gene ontology annotations with regulatory domains (customized settings: 5 kb upstream and 1 kb downstream of TSSs, further extended both directions by 250 kb). Enrichment of ontology annotations was assessed by binomial test of ChIP-seq peak overlaps with annotated regulatory regions.

Analysis of ChIP-seq overlap with mESC enhancers. Coordinates of enhancers and super-enhancers in mESCs were ascertained from Whyte *et al.* 2013, lifted over from mm9 to mm10 and counted for overlaps minimum of 1 bp with ChIP-seq peaks.

Integrative analysis of ChIP-seq peaks with mouse ESC gene expression. Gene expression in asynchronous mouse ESC (Shen *et al.* 2012) was quantified as transcripts per kilobase million (TPM) in Salmon (version 0.6.0) using GENCODE (version M6, mm10) reference gene annotation. Peaks were assigned with the nearest gene(s) within 100-kb proximity, and if multiple genes were equally close, the expression values were averaged.

Pluripotency Assays

ZHBTc4.1 ES cells either uninfected or infected with pHAGE-tGFP-DB-OCT4 (GDO) or pHAGE-DBmut-OCT4 (GMO) were plated on gelatin without feeders in the presence or absence of doxycycline (2ug/ml) in regular ESC medium (+LIF) or in the absence of LIF (-LIF).

Western blot analysis. After one or two passages in the +LIF conditions described above, cells were trypsinized and resuspended into 1X Laemmli Buffer. Samples were then sonicated for 5 cycles of 30s on, 30s off, boiled for 5min and used for Western Blot analysis using antibodies shown in the Supplementary Table 1.

RNA isolation and qRT-PCR. After two passages in the conditions described above, cells were sorted for GFP expression and replated for 24h prior to collection. RNA was isolated using Qiagen RNAeasy kit and cDNA was prepared using iScript reverse transcription supermix (Biorad). Real Time PCR was performed using the primers listed in Supplementary Table 4.

*Colony formation assays***.** After two passages in the +LIF conditions described above, cells were sorted for GFP expression and then either 1000 (-dox) or 2500 (+dox) GFP+ cells were immediately plated on feeders for 5-10 days in the same conditions (-/+dox). Full scanning of each well was performed using Nikon Biostation CT. Colonies were scored by two independent ways. First, based on the following morphological criteria: *Pluripotent*: 3D-colonies with sharp edges, *Partially*

differentiated: Colonies with flattened edges and presence of large differentiated cells, *Differentiated*: Flat, colonies with distinct, large differentiated cells. Second, based on the partial or full presence of NANOG expressing cells or complete absence of NANOG expression as assessed by immunofluorescence experiments. Finally, Alkaline phosphatase staining was performed using the VectorRed kit (SK5100, VECTOR).

Reprogramming experiments

MEFs carrying the M2-rtTA transgene at the *Rosa26* locus were infected with either pHAGE-tetO-GDO-KSM or pHAGE-tetO-GMO-KSM or pHAGE-tetO-stemcca viruses as described above. After 2 days, $20*10^3$ of the transduced MEFs were plated on gelatinized 6-well plates containing irradiated feeder cells as a monolayer. Doxycycline (2ug/ml) and Ascorbic acid (AA, 50ug/ml, Sigma # A4544) were added for 12-14 days and removed for 4 days prior to colony scoring, as previously described (Stadtfeld et al., 2012). To increase efficiency, one of the experiments was performed in the presence of additional chemicals such as GSK3β antagonist (CHIR99021, Stemgent # 04-0004) and TGF-β antagonist ALK5 inhibitor II (Bar-Nur et al., 2014; Vidal et al., 2014). Infection efficiency was determined by immunofluorescence experiments using OCT4 (Santa Cruz #sc8628), SOX2 (R&D Systems #AF2018), and KLF4 (R&D Systems #AF3158) antibodies. In addition, RT-PCR was used to estimate relative levels of the transgenes for each infection. Colonies were scored under the microscope based on morphology and normalized based on the infection efficiency.

For figures S5B-D, GDO-KSM and GMO-KSM infected cells were sorted based on GFP expression and then plated on feeders in the presence of dox and AA. On day 9, cells were stained for SSEA-1 (BioLegend) and analyzed by FACS. Three days later dox was removed for 4 days and then alkaline phosphatase staining using the VectorRed kit (SK5100, VECTOR) as well as immunofluorescence for OCT4 and NANOG were performed.

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