Supporting Information

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SI Methods

Generation of Histone Deacetylase 1 Mutant cDNAs and Double-Knockout Rescue Experiments. Histone deacetylase 1 (HDAC1) mutant and chimeric cDNAs were generated by PCR and subcloned into a pCAG-IRES-eGFP plasmid using In-Fusion HD EcoDry Cloning Plus kits (Clontech). For rescue experiments, 2.5×10^5 ES cells were transiently transfected with 5 μg of purified DNA using Lipofectamine 2000 (Invitrogen) transfection reagent. ES cells were cultured for 48 h before sorting for GFP-positive (transfected) cells using a BD FACSAria II, which were then treated with 1 μM 4-hydroxytamoxifen (OHT) (for 24 h) and cultured for a further 4 d before cell counting.

Cell Cycle Analysis. For cell cycle distribution, cells were fixed with 70% (vol/vol) ethanol at 4 °C for 1 h and incubated with 50 μg of propidium iodide and RNase A (10 μg/mL) for 30 min. Samples were analyzed using a BD FACSCanto II flow cytometer (BD Biosciences) and BD FACSDiva software. Alternatively, DNA replication analysis was conducted using the Click-iT cell proliferation assay kit (Invitrogen). Briefly, ES cells were incubated with 10 μM 5-ethynyl-2′-deoxyuridine (EdU) for 4 h, fixed, and permeabilized according to the manufacturer's instructions before analysis by flow cytometry as described above.

Immunofluorescence Microscopy. Cells were fixed in paraformaldehyde before being processed for immunofluorescence microscopy as previously described (1). The primary antibody was against α-tubulin (0.3 μg/mL; Sigma), γ-tubulin (0.15 μg/mL; Sigma), and an Alexa Fluor 594 goat anti-rabbit IgG secondary antibody was used (1 μg/mL; Invitrogen). DNA was stained with Hoechst 33258. Cells were imaged using a Leica SP5 laser-scanning confocal microscope. Deconvolution of image stacks was carried out using Huygens Essential software and the *z* stacks were assembled in ImageJ. Cells were "blind" scored as having monopolar spindles when the DNA was arranged in a ring shape with the spindle microtubules radiating from two unseparated spindle poles in the center. "Segregation defects" were defined as either lagging chromosomes in anaphase cells or chromatin bridges in telophase or interphase cells. "DNA abnormalities" describes either micronucleation, the presence of multinucleate cells, or cells with lobed or highly condensed chromatin.

Protein and Enzymatic Analysis. Whole-cell extracts were prepared in IP buffer [20 mM Hepes, pH 7.4, 250 mM NaCl, 0.5% IGEPAL CA-630, and 1× protease inhibitor mix (Sigma)] at 4 °C for 30 min. After centrifugation at 20,000 × *g* for 15 min, histone extracts were prepared from pellets using 0.2 M sulfuric acid overnight at 4 °C as previously described (2). Thirty micrograms of whole-cell extract was separated using SDS/PAGE and membranes were probed with the appropriate antibodies (Table S2). Ten microliters of acid-extracted histone were loaded in each lane and membranes were probed using a panel of anti-acetyl lysine antibodies. The Odyssey Infrared Imaging System was used to quantify protein signal using the appropriate IRDye-conjugated secondary antibodies (LI-COR Biosciences). For immunoprecipitation, 600 μg of whole-cell extract was incubated overnight at 4 °C with antibody-coated (anti-Flag) protein G-Sepharose beads. After four washes in IP buffer, beads were split into two aliquots. One aliquot was used to assess the enzymatic activity of the immunoprecipitates using a commercially available deacetylase assay (Active Motif); the remaining aliquot was resolved by SDS/PAGE and probed with antibodies raised against known components of the immunoprecipitated complexes.

RNA Isolation and Microarray Analysis. Total RNA was isolated from ES cells using TRIzol (Invitrogen) and RNeasy MinElute Cleanup Kit. Comparative gene expression profiles were generated using the Illumina mouseWG-6, version 2, expression BeadChip platform. Labeling of mRNA and hybridization were performed using a standard Illumina protocol. Quality control of total mRNA was performed using a 2100 Bioanalyser (Agilent). Only samples that had an RNA integrity number of 8.6 or higher were selected for processing and array hybridization. Detection *P* values of <0.01 were used to filter all data. Significant differential expression between sample sets was defined as probes that exhibited a robust fold change of ≥1.4 (Fc ≥ 1.4) with an adjusted *P* value of <0.05. Quality analysis and differential expression analyses were performed in Partek Genomics Suit (version 6.5) and ArrayTrack (3). Further analysis of functionally related gene groups among deregulated genes was carried out using the Database for Annotation, Visualization, and Integrated Discovery (DAVID), version 6.7 (4). Gene set enrichment analysis (GSEA) (5) was performed using GSEA software, version 2, along with gene sets from MSigDB. Genes were ranks by the Signal2Noise metric, and 1,000 gene-set permutations were used to calculate the nominal *P* value. Data files from the analysis have been deposited at the Gene Expression Omnibus (GEO) database, accession number GSE52134.

Reverse Transcription and Quantitative Real-Time PCR. RNA (0.5 μg) was reverse transcribed (RT) using Q-Script cDNA SuperMix (Quanta Biosciences). For quantitative real-time PCR (qRT-PCR), cDNA was diluted with an equal amount of diethyl pyrocarbonate-treated H_2O. Multiplex assays were designed using the Universal Probe Library Assay Design Centre (www.roche-applied-science.com). For each reaction, 2 μL of diluted cDNA was used in all subsequent multiplex qRT-PCRs using the Light Cycler probes master mix (Roche) as per the manufacturer's instructions. Reactions were carried out on a Roche Light Cycler 480 under the following conditions: initial denaturation at 94 °C for 10 min, followed by 40 cycles of 94 °C for 10 s, 55 °C for 20 s, and 72 °C for 5 s. A list of primers and probes used for qRT-PCR are listed in Table S3.

1. Faragher AJ, Fry AM (2003) Nek2A kinase stimulates centrosome disjunction and is required for formation of bipolar mitotic spindles. *Mol Biol Cell* 14(7):2876–2889.
2. Dovey OM, Foster CT, Cowley SM (2010) Histone deacetylase 1 (HDAC1), but not HDAC2, controls embryonic stem cell differentiation. *Proc Natl Acad Sci USA* 107(18): 8242–8247.
3. Fang H, et al. (2009) ArrayTrack: An FDA and public genomic tool. *Methods Mol Biol* 563:379–398.
4. Huang W, Sherman BT, Lempicki RA (2009) Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat Protoc* 4(1):44–57.
5. Subramanian A, et al. (2005) Gene set enrichment analysis: A knowledge-based approach for interpreting genome-wide expression profiles. *Proc Natl Acad Sci USA* 102(43):15545–15550.
6. Müller FJ, et al. (2008) Regulatory networks define phenotypic classes of human stem cell lines. *Nature* 455(7211):401–405.

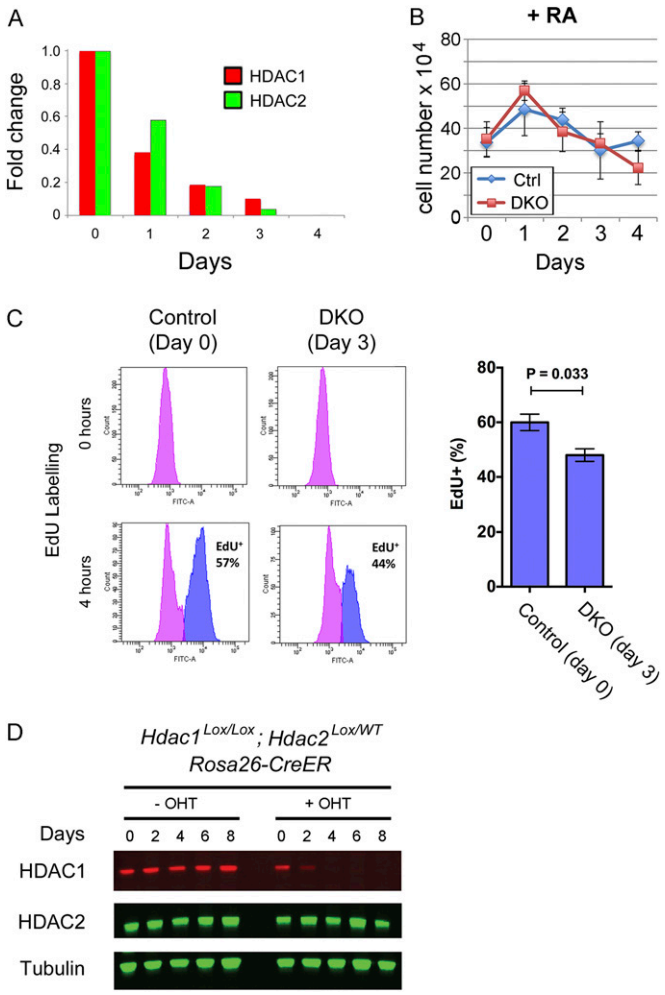


Fig. S1. Quantification of HDAC1 and HDAC2 levels following gene inactivation. (*A*) Quantitative Western blotting was used to quantify the change in HDAC1 and HDAC2 protein level following gene inactivation (0–4 d). Fold change in protein levels relative to α-Tubulin are shown. (*B*) ES cells were treated with 1 μM retinoic acid (RA) for 2 d to induce differentiation and cell cycle withdrawal, before the addition of OHT. Comparative viable cell counts of untreated (Ctrl), and OHT-treated DKO cells are shown for a 4-d period. (*C*) ES cells were treated with the nucleotide analog, EdU, for 4 h to measure entry into S phase. The percentage of EdU-positive cells (EdU^+, colored blue) was quantified by FACS analysis. Representative FACS data are shown in the left panel. Right panel shows mean ($n = 3$) percentage of EdU-positive cells ± SEM. Significance (*P* value) was calculated using a two-tailed *t* test. (*D*) Quantitative Western blot showing loss of HDAC1 and HDAC2 proteins following gene inactivation (0–8 d) in $Hdac1^{Lox/Lox}$; $Hdac2^{Lox/WT}$; *CreER* ES cells. Cells were cultured with, or without, 4-hydroxytamoxifen (OHT) for 24 h to induce the deletion of *Hdac1/2*. α-Tubulin was used to normalize protein loading, and blots were visualized and quantified using an Odyssey scanner.

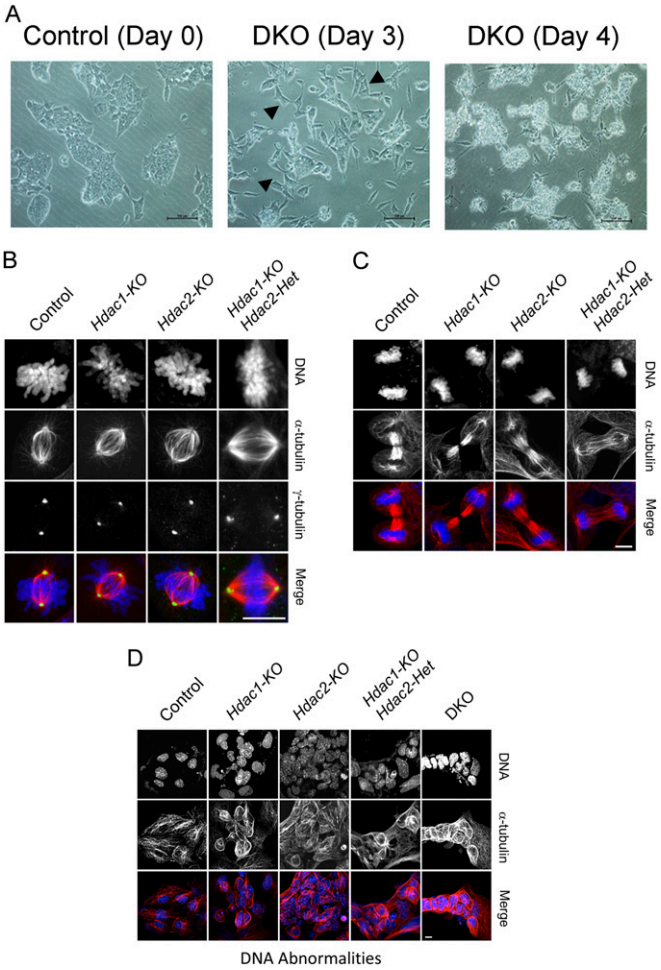


Fig. S2. Loss of HDAC1/ 2 causes a loss of ES cell viability in a cell cycle-dependent manner. (*A*) Phase contrast microscopy was used to take images of *Hdac1/2*-deleted cells at the indicated time points following 4-hydroxtamoxifen (OHT) treatment. The black arrows indicate examples of cells that have undergone a change in morphology. (*B–D*) ES cells were stained with anti-α-Tubulin (red), anti-γ-Tubulin (green), and Hoechst 33258 (blue) to visualize chromosomes during various stages of cell cycle. Experiments were performed on untreated DKO (day 0, Control), single *Hdac1* and *Hdac2* knockout cells, a compound *Hdac1-KO; HDAC2-Het* knockout cell line, and DKO cells following deletion (day 3 after OHT treatment). Images show examples of individual mitotic (*B* and *C*) and interphase (*D*) cells following deletion.

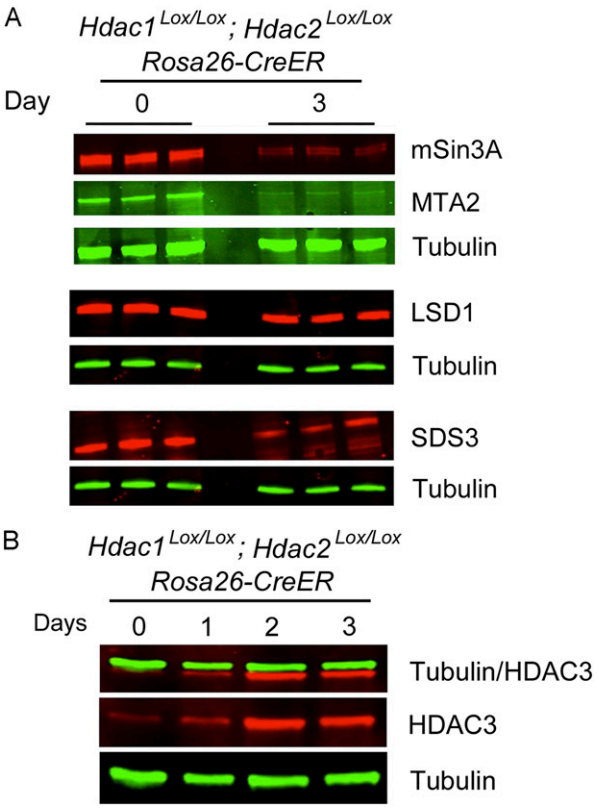
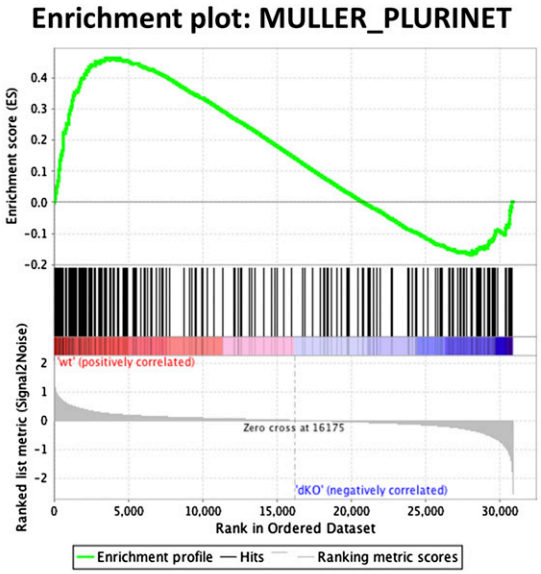


Fig. S3. Loss of HDAC1/2 disrupts corepressor complex integrity. (*A*) Quantitative Western blot showing the relative levels of indicated proteins at day 0, or day 3 following 4-hydroxytamoxifen (OHT) treatment. (*B*) Levels of HDAC3 were measured in *Hdac1/2*-deleted cells over a 3-d time period following OHT treatment. α-Tubulin was used to normalize protein loading, and blots were visualized and quantified using an Odyssey scanner.



Gene Set Enrichment Analysis	Gene set size	Core enrichment size	Enrichment Score	Nominal Enrichment Score	NOM p-value	FDR	Enrichment Cell Type
Muller_PluriNet	296	90	0.47	1.75	<0.001	<0.001	Wt

Fig. S4. GSEA. The 1,708 genes deregulated ≥1.4-fold (adjusted $P < 0.05$) in *Hdac1/2*-deleted cells at day 3 (day 0 vs. day 3) were compared with the "PluriNet" genes, a common set of characteristics shared by all pluripotent stem cell lines (6). Of the 296 PluriNet genes, 90 were significantly enriched in wild-type control ES cells ($P < 0.001$), demonstrating a loss of the pluripotent stem cell phenotype.

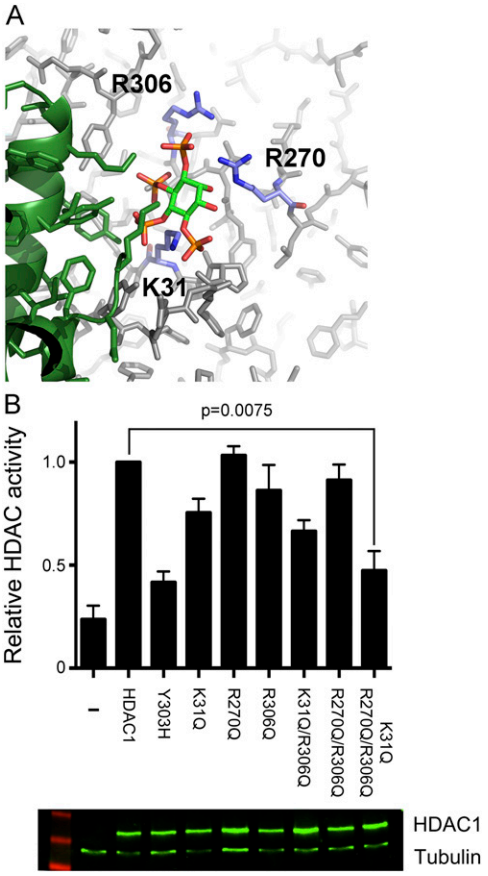


Fig. S5. Cell viability is dependent upon the integrity of the inositol tetraphosphate (IP_4) binding pocket for full activity of HDAC1 in vivo. (*A*) Structure of HDAC1 deacetylase domain with positively charged residues critical for the interaction with IP_4 (K31, R270, and R306) marked in blue. (*B*) Relative deacetylase activity was measured using individual Flag-tagged HDAC1 constructs immunoprecipitated using anti-Flag antisera. All values are means ($n > 3$) ± SEM and are normalized relative to the level of protein expression (shown in the lower panel). Significance (*P* value) was calculated using a two-tailed *t* test. (*Lower*) Western blot performed with anti-FLAG and anti–α-tubulin antisera to determine the relative expression level of individual HDAC1 constructs.

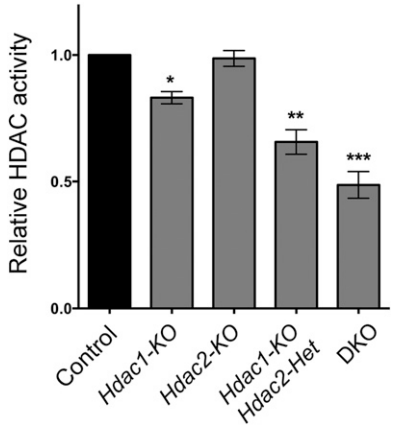


Fig. S6. Total cellular deacetylase activity was measured from cells of the indicated genotype. All values are means ($n > 3$) ± SEM and are normalized relative to the level of α-tubulin in each extract. Significance (*P* value) was calculated using a two-tailed *t* test (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$).

Table S1. List of genes used to assess the pluripotent, or differentiation state of *Hdac1/2*-deleted cells in Fig. 4*E*

Pluripotent genes	Differentiation genes		
Bmp4	*Afp*	*Hoxb3*	*Nkx6-1*
Ccna2	*Alb*	*Hoxb4*	*Nodal*
Cdc42	*Amn*	*Hoxb5*	*Nog*
Cfc1	*Ascl2*	*Hoxb6*	*Otx2*
Chd1	*Cdh5*	*Hoxb7*	*Pax2*
Dppa1	*Cdx2*	*Hoxb8*	*Pax6*
Dppa2	*Chrd*	*Hoxb9*	*Plac1*
Dppa3	*Cldn4*	*Hoxc10*	*Plac1l*
Dppa4	*Dkk1*	*Hoxc11*	*Plac8*
Dppa5	*Dnmt3l*	*Hoxc12*	*Plac8l1*
E2f1	*Eomes*	*Hoxc13*	*Smad2*
Eed	*Esx1*	*Hoxc4*	*Smad3*
Ep300	*Fabp1*	*Hoxc5*	*Snai1*
Esrrb	*Fgf5*	*Hoxc6*	*Snai2*
Esrrbl1	*Fgf8*	*Hoxc8*	*Snai3*
Fgf4	*Foxa2*	*Hoxc9*	*Sox1*
Klf4	*Gata4*	*Hoxd1*	*Sox17*
Klf5	*Gata6*	*Hoxd10*	*Sox18*
Lin28	*Gdf1*	*Hoxd11*	*Sox3*
Mycbp	*Gdf3*	*Hoxd12*	*Sox7*
Nacc1	*Gfap*	*Hoxd13*	*Syp*
Nanog	*Hand1*	*Hoxd3*	*Sypl*
Nanogpd	*Hand2*	*Hoxd4*	*T*
Nr0b1	*Hnf4a*	*Hoxd8*	*Tbx5*
Nr5a2	*Hoxa1*	*Hoxd9*	*Tbx6*
Pou5f1	*Hoxa10*	*Irx3*	*Tead1*
Prdm14	*Hoxa11*	*Lmna*	*Tead2*
Sall4	*Hoxa11s*	*Mesp1*	*Tead3*
Slc2a3	*Hoxa13*	*Mesp2*	*Tead4*
Smad1	*Hoxa2*	*Mixl1*	*Tubb3*
Sox2	*Hoxa3*	*Msi1*	*Vim*
Stat3	*Hoxa4*	*Msi1h*	*Wnt1*
Suz12	*Hoxa5*	*Myh7*	*Wnt3a*
Tcfcp2l1	*Hoxa6*	*Myod1*	
Tert	*Hoxa7*	*Ncam*	
Utf1	*Hoxa9*	*Ncam1*	
Zfp296	*Hoxb1*	*Nes*	
Zfp42	*Hoxb13*	*Neurod2*	
Zfx	*Hoxb2*	*Nkx2-4*	

Table S2. List of antibodies used in this study

Antibody	Clonality	Source	Dilution	Company	Product code
Hdac1	Polyclonal	Rabbit	1:2,000	Santa Cruz	sc-7972
Hdac2	Monoclonal	Mouse	1:2,000	Millipore	05-814
Hdac3	Monoclonal	Rabbit	1:2,000	Abcam	Ab32369
mSin3a	Monoclonal	Rabbit	1:2,000	Abcam	Ab129087
MTA-2	Monoclonal	Mouse	1:2,000	Sigma	M-276
SDS3	Polyclonal	Goat	1:2,000	Bethyle	A300-235A
LSD-1	Polyclonal	Rabbit	1:2,000	Abcam	Ab37165
Oct4	Polyclonal	Rabbit	1:500	Abcam	Ab19857
Nanog	Polyclonal	Rabbit	1:5,000	Bethyle	A300-397A
α-Tubulin	Monoclonal	Mouse	1:5,000	Sigma	TC168
H3	Monoclonal	Mouse	1:2,000	Millipore	05-499
H3K9ac	Monoclonal	Rabbit	1:2,000	Millipore	04-1003
H3K14ac	Monoclonal	Rabbit	1:2,000	Millipore	04-1044
H3K18ac	Monoclonal	Rabbit	1:2,000	Millipore	04-1107
H3K23ac	Polyclonal	Rabbit	1:2,000	Active Motif	39132
H3K27ac	Polyclonal	Rabbit	1:2,000	Active Motif	39135
H3K36ac	Polyclonal	Rabbit	1:2,000	Millipore	07-540
H3K56ac	Polyclonal	Rabbit	1:2,000	Active Motif	39281
H4K5ac	Polyclonal	Rabbit	1:2,000	Active Motif	39699
H4K12ac	Polyclonal	Rabbit	1:2,000	Active Motif	29927
H4K16ac	Polyclonal	Rabbit	1:2,000	Active Motif	39167
H3K9me3	Polyclonal	Rabbit	1:2,000	Abcam	Ab8898
H3K4me2	Polyclonal	Rabbit	1:1,000	Sigma	D5692-200UL

Table S3. List of primers and Universal Probe Library (UPL) hydrolysis probe used for qRT-PCR

Gene	UPL primer sequence	UPL hydrolysis probe	Size, bp
Nanog	Left: gcctccagcagatgcaag	91	75
	Right: ggttttgaaaccaggtcttaacc		
Rex1	Left: ttctctcaatagagtgagtgtgcag	33	68
	Right: aggcgatcctgctttcttc		
Oct4	Left: aatgccgtgaagttggagaa	95	70
	Right: ccttctgcagggctttcat		
Ccdn2	Left: caccgacaactctgtgaagc	17	71
	Right: tccacttcagcttacccaaca		
Gdf3	Left: gggtgttcgtgggaacct	7	78
	Right: ccatcttggaaaggtttctgtg		
Eif5	Left: cgcgttgggtttatgtcttt	46	77
	Right: gctatgtttccccaatacaggt		
Amnionless	Left: tacgagacagtcacgccatc	34	64
	Right: gaggccaggaccaactcc		
Camk2n2	Left: ccagtctgcccaattctga	79	61
	Right: gataccttgggagggaggagt		
Adssi1	Left: aaggccgtgtcattcattg	13	88
	Right: tcagccctttcttctcgttc		
Thy1	Left: aactcttggcaccatgaacc	15	89
	Right: tcaggctggtcaccttctg		
Ly6a	Left: aaggtcaacgtgaagacttcct	72	56
	Right: cctccattgggaactgctac		
Blvrb	Left: cgatgtggacaagactgtgg	104	90
	Right: tcggacattactgtagtgggact		
HMGN3	Left: gcaaatggtgacactaaagttga	98	78
	Right: ttccacgacaattcactctcc		
Med7	Left: tgggataagaaatcggcaaa	7	72
	Right: tgaagatgacaaggaaccaaaa		
Pfn1	Left: ctgtcaccatgactgccaag	18	68
	Right: gatcaaaccaccgtggaca		
Tcf25	Left: ctcaccatgttccctggagt	67	61
	Right: catcaggtcgcacactgc		
MTA2	Left: ccgaagaccctatgcaccta	13	70
	Right: agccttaggaagtcggatcg		
GPS1	Left: gcaggaagatccgcagaa	22	90
	Right: ccactgtagctggctgcata		

Probes were supplied by Roche Diagnostics. Intron-spanning primer sequences [forward (F) and reverse (R)], UPL hydrolysis probe, and length of amplified product are displayed above. UPL reference gene, GAPDH control probe, and primers were used as reference gene in all multiplex reactions (product of Roche Applied Science; catalog #05046211001).

Dataset S1. List of genes deregulated ≥1.4-fold (adjusted $P < 0.05$) in *Hdac1/2*-deleted cells at day 2 (day 0 vs. day 2) and day 3 (day 0 vs. day 3)

Dataset S1

Data files from the analysis have been deposited in the Gene Expression Omnibus (GEO) database (accession number GSE52134).