# Supporting Information

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### SI Materials and Methods

**Construction of Targeting Vectors for the Generation of HDAC1 and HDAC2 Conditional Knockout Alleles.** Both gene-targeting strategies for creation of conditional knockout HDAC1 and HDAC2 alleles followed the same principle. Namely, LoxP sites were added to flank exon 2, a critical exon in the ORF of both genes. All constructs were made using standard "recombineering" techniques and plasmids (1).

***HDAC1 targeting vectors.*** A retrieval construct was made to "capture" a 9,936-bp fragment of the *Hdac1* gene from a 129S7 BAC, using three-way ligation of a pBluescript backbone with a 453-bp Xba/Pac fragment (HD1-5-5′-Xba, TCTAGATCACTTTGTAGACCAGGCTGG; HD1-5-3′-Pac, TTAATTAAACTTCTGCCTTCAAACTTGCC) flanking the 5′ and a 553-bp Pac/HindIII fragment (HD1-3-5′-Pac, TTAATTAAGGGACTTGAACTGTACAGTCC; HD1-3-3′-Hind, AAGCTTGCAGAAACTTGCTCTGTAGCC) flanking the 3′ region of the captured region. The retrieval vector was linearized using a Pac1 restriction digest and then electroporated into DY380 bacteria containing a 129S7 BAC (bMQ-272G3), followed by appropriate selection, to generate a "captured BAC" plasmid containing the 9,936-bp *Hdac1* fragment containing Exon2 on a pBluescript backbone. A cassette containing "FRT.EM7.pgk.Neo.FRT.LoxP" was excised from PL451 (1) using EcoRV/Ale1 restriction sites and then subcloned into the captured BAC using a unique Afe1 restriction site downstream of Exon 2. Finally, a double-stranded oligo containing the 5′ LoxP site was cloned into a unique Csp45I site upstream of Exon 2, to generate the final HDAC1-cKO-Neo construct. HDAC1-cKO-HygΔtk was generated from HDAC1-cKO-Neo using recombineering: The "pgk.em7.HygΔtk" cassette was amplified from pSC5 and electroporated into DY380 bacteria containing HDAC1-ckO-Neo to initiate recombination, followed by hygromycin selection. The size of homology arms and position of LoxP and FRT sites in the final *Hdac1* targeting vectors are outlined in Fig. S1.

***HDAC2 targeting vectors.*** A 3-kb PCR fragment containing the pBluescript backbone flanked by 66 bp of homology, 5′ and 3′ to the region of the HDAC2 gene to be captured was generated using the following primers with pBluecript as a template: pBluCapD2-5′ GCCAAGCAGTACACCAAGAGTGACTTCATCCTATGACTTCTCAGGAACAATTTCCGGAGTCTGTGTTAGGGTGATGGTTCACGTAGTGG and pBluCapD2-3′ GGGATTTAACCTGGATTCTCTAGAAGGCAGCCAGTGCTCCTAACTACTAGTCCAACTTTCCAACACGCTCACTCAAAGGCGGTAATACG. This PCR fragment was then electroporated into DY380 bacteria containing a 129S7 BAC (bMQ-94F13) to retrieve an 11.1-kb fragment of the gene, including exons 1–5, to generate an HDAC2 captured BAC plasmid. A double-stranded oligo containing the 3′ LoxP site was then cloned into a unique BstB1 site downstream of exon 2. Finally, the FRT.EM7.pgk.Neo.FRT.LoxP cassette from pL451 was added upstream of exon 2 by recombineering, using PCR to generate the cassette flanked by 66 bp of homology, 5′ and 3′ to the targeting site, using the following primers and pL451 as a template, D2-Neo-KS-2 GCACAGGCTACTACTGTGTAGTCCTGAGAAATGAAGACAAGAGTGTCTTTATTACCCTGAAAAATGCCCCTCGAGGTCGACGGTATCG and D2-Neo-SK-2 TACATAAGAAGTATTCTTGAAGTACGGAGTAGGATCTAATTTGTCAGCTAGTAGTGCTTCTTGGATGCCGCTCTAGAACTAGTGGATC, to generate the final HDAC2-cKO-Neo construct. HDAC2-cKO-HygΔtk was generated from HDAC2-cKO-Neo using recombineering: The pgk.em7.HygΔtk cassette was amplified from pSC5 and electroporated into DY380 bacteria containing HDAC1-ckO-Neo to initiate recombination, followed by hygromycin selection. The size of homology arms and position of LoxP and FRT sites in the final *Hdac2* targeting vectors are outlined in Fig. S2.

**Generation of *Hdac1* and *Hdac2* Knockout ES Cell Lines.** The outline of the strategy described below is depicted in Figs. S1 and S2. HDAC1-cKO-Neo or HDAC2-cKO-Neo targeting vectors were used to target the first allele of *Hdac1* or *Hdac2*, respectively. The appropriate targeting vector was electroporated into an E14 mouse ES cell line (2) that expresses Cre recombinase and fused to a mutant estrogen receptor ligand-binding domain, from the endogenous ROSA26 locus (CreER-T) (3), and positive clones were identified by G418 selection (180 μg/mL) for 10 days. Following selection, correctly targeted clones were detected by Southern blot screening. Following successful targeting of the first allele, the second allele was targeted using HDAC1-cKO-HygΔtk or HDAC2-cKO-HygΔtk, respectively, following the same principle as above with hygromycin B (100 μg/mL) selection. Double-targeted clones were identified by Southern blot screening. To remove the selection cassettes from double-targeted (single-cell) clones, cells were transiently transfected with a pCAGGS-Flpe plasmid using Lipofectamine 2000, and negative selection was applied using 1-(-2-deoxy-2-fluoro-1-b-D-arabinofuranosyl)-5-iodouracil (FIAU) to identify loss of the thymidine-kinase portion of the "HygΔtk" cassette. Multiple ES cell clones in which the selection cassettes were identified were assessed by Southern blot screening. Addition of 1 μM 4-OHT to the cell culture media was used to induce LoxP recombination and deletion of exon 2. For a detailed outline of Southern blot screening, see Figs. S1 and S2.


1. Liu P, Jenkins NA, Copeland NG (2003) A highly efficient recombineering-based method for generating conditional knockout mutations. *Genome Res* 13:476–484.
2. Hooper M, Hardy K, Handyside A, Hunter S, Monk M (1987) HPRT-deficient (Lesch-Nyhan) mouse embryos derived from germline colonization by cultured cells. *Nature* 326:292–295.
3. Vooijs M, Jonkers J, Berns A (2001) A highly efficient ligand-regulated Cre recombinase mouse line shows that LoxP recombination is position dependent. *EMBO Rep* 2:292–297.

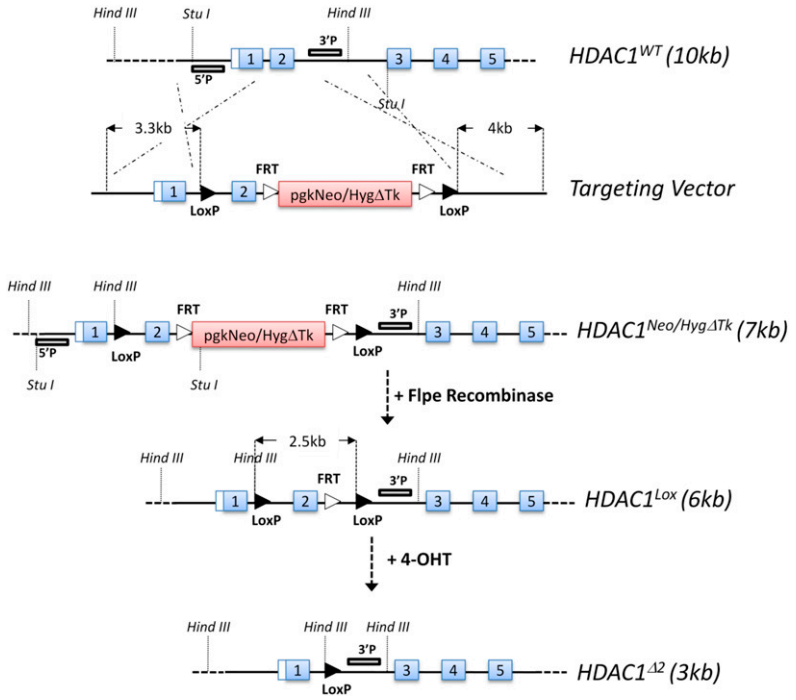


**Fig. S1.** Generation of a conditional Hdac1 allele: outline of strategy to generate a conditional Hdac1 allele. Confirmation of successful integration of the targeting vectors into mouse ES cells was assessed by Southern blot. Exon 2 was flanked by LoxP sites (black triangles) and selection cassettes were flanked by FRT sites (white triangles). Confirmation of successful integration of the targeting vectors into mouse ES cells was assessed by Southern blot. Using the 5′ probe (5′P) on StuI-digested genomic DNA yielded either a 10-kb fragment (*HDAC1*$^{WT}$) or a 7-kb targeted fragment (*HDAC1*$^{Neo/HygΔTk}$). Successfully double-targeted clones (*HDAC1*$^{Neo/HygΔTk}$) were selected and selection cassettes removed by transient transfection of FLPe. Further addition of 4-OHT results in LoxP recombination and excision of exon 2. Southern blot was used to confirm removal of selection cassettes and deletion of exon 2 using the 3′ probe (3′P) on HindIII-digested genomic DNA, yielding either a 6-kb (*HDAC1*$^{Lox}$) or a 3-kb (*HDAC1*$^{Δ2}$) fragment.

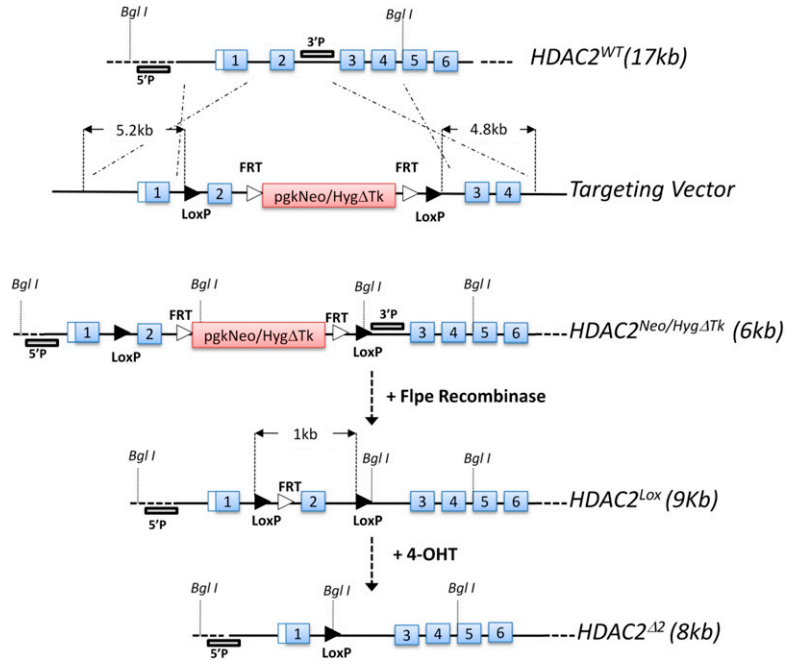


**Fig. S2.** Generation of a conditional Hdac2 allele: outline of strategy to generate a conditional Hdac2 allele. Confirmation of successful integration of the targeting vectors into mouse ES cells was assessed by Southern blot. Exon 2 was flanked by LoxP sites (black triangles) and selection cassettes were flanked by FRT sites (white triangles). Confirmation of successful integration of the targeting vectors into mouse ES cells was assessed by Southern blot. Using the 3′ probe (3′P) on BglI-digested genomic DNA yielded either a 17.1-kb fragment (*HDAC2*$^{WT}$) or a 6-kb targeted fragment (*HDAC2*$^{Neo/HygΔTk}$). Successfully double-targeted clones (*HDAC2*$^{Neo/HygΔTk}$) were selected and selection cassettes removed by transient transfection of FLPe. Further addition of 4-OHT results in LoxP recombination and excision of exon 2. Southern blot was used to confirm removal of selection cassettes and deletion of exon 2 using the 5′ probe (5′P) on BglI-digested genomic DNA yielding either a 9-kb (*HDAC2*$^{Lox}$) or an 8-kb (*HDAC2*$^{Δ2}$) fragment.

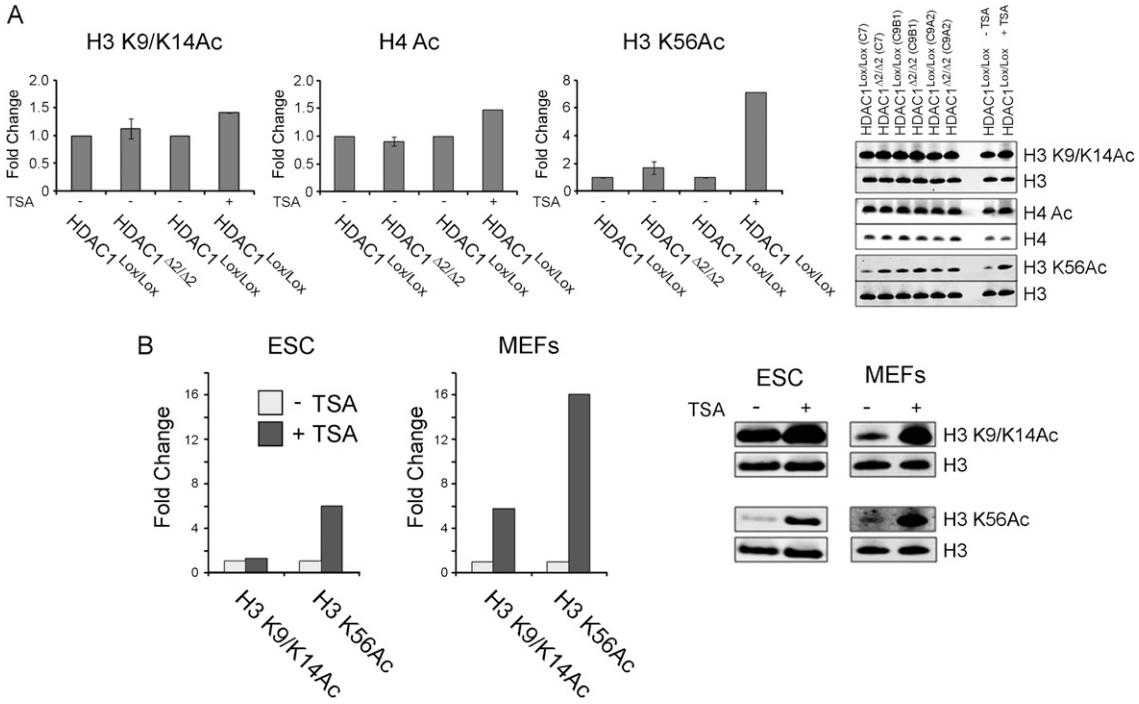


**Fig. S3.** H3K56Ac is regulated by HDAC1 in addition to other zinc-dependent HDAC enzymes. The acetylation status of core histones was detected using quantitative Western blotting. (*A*) Histones were acid extracted from three independent, treatment-matched HDAC1$^{Lox/Lox}$ or HDAC1$^{\Delta 2/\Delta 2}$ clones. Trichostatin A (TSA, 150 nM) was added, as indicated, for 12 h before histone extraction. The signal of specific acetylated lysines was normalized to the total amount of H3 or H4 as appropriate using a LiCOR scanner. (*B*) The acetylation status of histones extracted from wild-type ES cells and mouse embryo fibroblasts (MEFs) was compared with and without TSA treatment as in *A*.

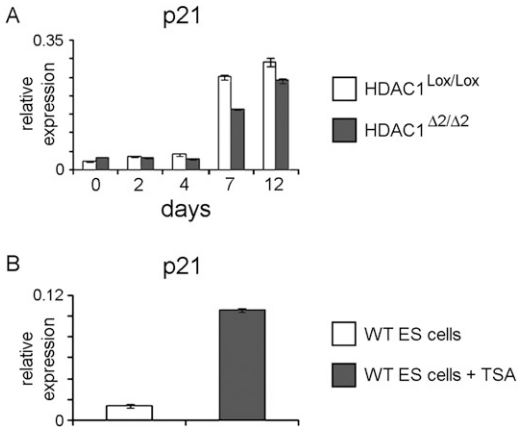


**Fig. S4.** Quantitative RT-PCR analysis of p21 mRNA (*A*) in embryoid bodies at the indicated time points and (*B*) in cycling ES cells with and without trichostatin A treatment (TSA, 30 mM for 24 h). The level of p21 mRNA was measured in a multiplex PCR assay relative to GAPDH, using Universal ProbeLibrary hydrolysis probes.

**Table S1. List of antibodies used, dilutions, manufacturer details, and application**

| Antibody | Clonality | Source | Dilution | Company | Odering code | WB/IP |
|---|---|---|---|---|---|---|
| hdac1 | Polyclonal | Rabbit | 1:2,000 | Santa Cruz | sc-7872 | WB |
| hdac1 | Monoclonal | Mouse | 1:2,000 | Abcam | ab46985 | IP |
| hdac2 | Polyclonal | Rabbit | 1:2,000 | Santa Cruz | sc-7899 | IP |
| hdac2 | Monoclonal | Mouse | 1:2,000 | Millipore | 05–814 | WB |
| hdac3 | Polyclonal | Rabbit | 1:1,000 | Santa Cruz | sc-11417 | WB |
| LSD-1 | Polyclonal | Rabbit | 1:2,000 | Abcam | ab37165 | WB/IP |
| mSin3a | Polyclonal | Rabbit | 1:2,000 | Santa Cruz | sc-767 | WB/IP |
| MTA-2 | Monoclonal | Mouse | 1:2,000 | Sigma | M-7569 | WB/IP |
| α-tubulin | Monoclonal | Mouse | 1:3,000 | Sigma | T5168 | WB |
| H3 | Monoclonal | Mouse | 1:2,000 | Millipore | 05–499 | WB |
| H4 | Monoclonal | Mouse | 1:2,000 | Abcam | ab31827 | WB |
| H4ac | Polyclonal | Rabbit | 1:1,000 | Millipore | 06–866 | WB |
| H4K5ac | Polyclonal | Rabbit | 1:1,000 | Millipore | 06–759MN | WB |
| H4K8ac | Polyclonal | Rabbit | 1:1,000 | Millipore | 06–760MN | WB |
| H4K12ac | Polyclonal | Rabbit | 1:1,000 | Millipore | 06–761 | WB |
| H4K16ac | Polyclonal | Rabbit | 1:1,000 | Millipore | 06–762 | WB |
| H3K9/14ac | Polyclonal | Rabbit | 1:1,000 | Millipore | 06–599 | WB |
| H3K18ac | Polyclonal | Rabbit | 1:1,000 | Millipore | 07–354 | WB |
| H3K27ac | Polyclonal | Rabbit | 1:1,000 | Active Motif | 39132 | WB |
| H3K36ac | Polyclonal | Rabbit | 1:1,000 | Millipore | 07–540 | WB |
| H3K56ac | Polyclonal | Rabbit | 1:1,000 | Active Motif | 39281 | WB |
| H3K4me2 | Polyclonal | Rabbit | 1:1,000 | Abcam | ab32356 | WB |
| H3K9me3 | Polyclonal | Rabbit | 1:1,000 | Abcam | Ab8898 | WB |

IP, immunoprecipitation; WB, Western blot.

**Table S2. Amplicon size, list of primers, and Universal ProbeLibrary hydrolysis probe used in multiplex PCR with as GAPDH control**

| Primer | Universal ProbeLibrary primer sequences | UPL hydolysis probe | Size, bp |
|---|---|---|---|
| Oct3/4 | F: cacgagtggaaagcaactca | 82 | 125 |
| | R: cttctgcagggctttcatgt | | |
| Nanog | F: agcctccagcagatgcaa | 25 | 76 |
| | R: ggttttgaaaccaggtcttaacc | | |
| FGF5 | F: gagccctgaaggaaactcg | 89 | 76 |
| | R: gcgaaacaaaatgacctgact | | |
| Brachyury | F: cgagatgattgtgaccaagaac | 88 | 65 |
| | R: ggcctgacacatttaccttca | | |
| Nkx2.5 | F: gacgtagcctggtgtctcg | 53 | 70 |
| | R: gtgtggaatccgtcgaaagt | | |
| MEF2c | F: tctgccctcagtcagttgg | 77 | 63 |
| | R: cgtggtgtgttgtgggtatc | | |
| MyoD | F: ccaggacacgactgctttct | 52 | 76 |
| | R: cacaccggctgtcctctac | | |
| βIII-tubulin | F: ggcaactatgtaggggactcag | 78 | 87 |
| | R: cctgggcacatacttgtgag | | |
| Nestin | F: tgcaggccactgaaaagtt | 2 | 89 |
| | R: ttccaggatctgagcgatct | | |
| GATA6 | F: ggtctctacagcaagatgaatgg | 40 | 94 |
| | R: tggcacaggacagtccaag | | |
| GATA4 | F: ggaagacaccccaatctcg | 13 | 75 |
| | R: catggccccacaattgac | | |
| HDAC1 | F: gagtacctggagaagatcaagca | 89 | 121 |
| | R: cttcatccccactctcttcg | | |
| HDAC2 | F: ctccacgggtggttcagt | 45 | 71 |
| | R: cccaattgacagccatatca | | |
| p21 | F: tccacagcgatatccagaca | 21 | 90 |
| | R: ggcacactttgctcctgtg | | |

Intron-spanning primer sequences (F, forward; R, reverse), Universal ProbeLibrary hydrolysis probe, and length of amplified product are displayed. Universal ProbeLibrary reference gene, GAPDH control probe, and primers were used as reference genes in all multiplex reactions (product of Roche Applied Science; no. 05046211001). Probes supplied by Roche Diagnostics. UPL, Universal ProbeLibrary.