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Supplemental Data

Deletion of *Histone Deacetylase 3*

Reveals Critical Roles in S Phase Progression

and DNA Damage Control

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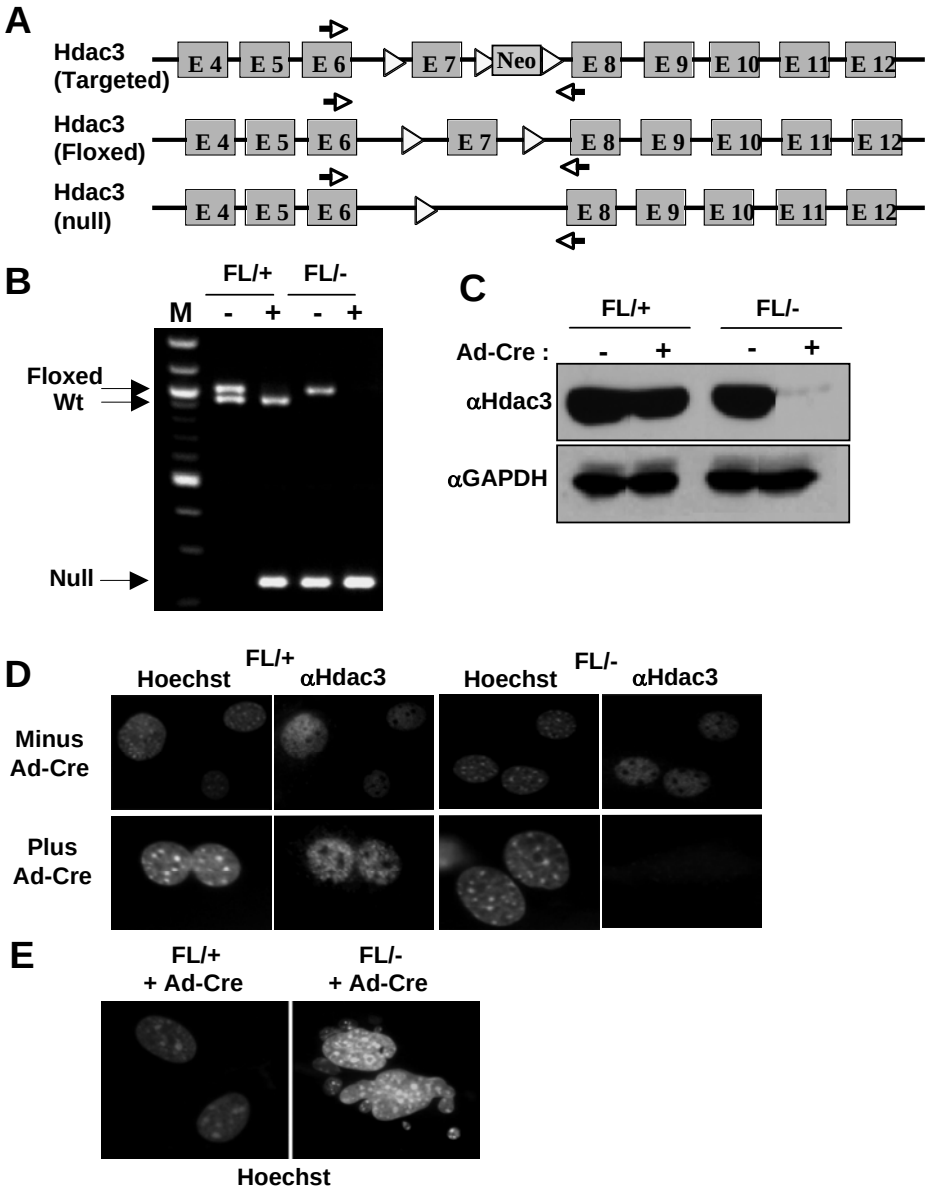


Figure S1. Characterization of *Hdac3*$^{-/-}$ MEFs. (A) Schematic representation of the targeted, floxed (*Hdac3*^{FL}), and null (*Hdac3*$^{-}$) alleles of *Hdac3*. Arrows indicate the primers used for PCR analysis and the arrowheads indicate Lox P sites. (B) PCR analysis of the genomic DNA isolated from *Hdac3*$^{FL/+}$ and *Hdac3*$^{FL/-}$ MEFs either mock infected (-) or 24 hr after Ad-Cre infection (+). PCR was performed with the primers (arrows, panel A) that flank exon 7 to detect the floxed (935 bp), wt (895 bp), and the null (211 bp) bands. M: 100bp ladder (C) Western blot analysis for Hdac3 with protein extracts prepared from MEFs 90 hr after Ad-Cre infection. The levels of GAPDH served as the loading control. (D) Immunofluorescence detection of Hdac3 in *Hdac3*$^{FL/+}$ and *Hdac3*$^{FL/-}$ MEFs. Nuclei are stained with hoechst dye and anti-Hdac3 following 90 hr Ad-Cre infection. (E) Hoechst staining of *Hdac3*$^{+/-}$ and *Hdac3*$^{-/-}$ MEFs was performed to visualize the apoptotic nuclei in *Hdac3*$^{FL/-}$ MEFs following Ad-Cre infection (right hand panel).

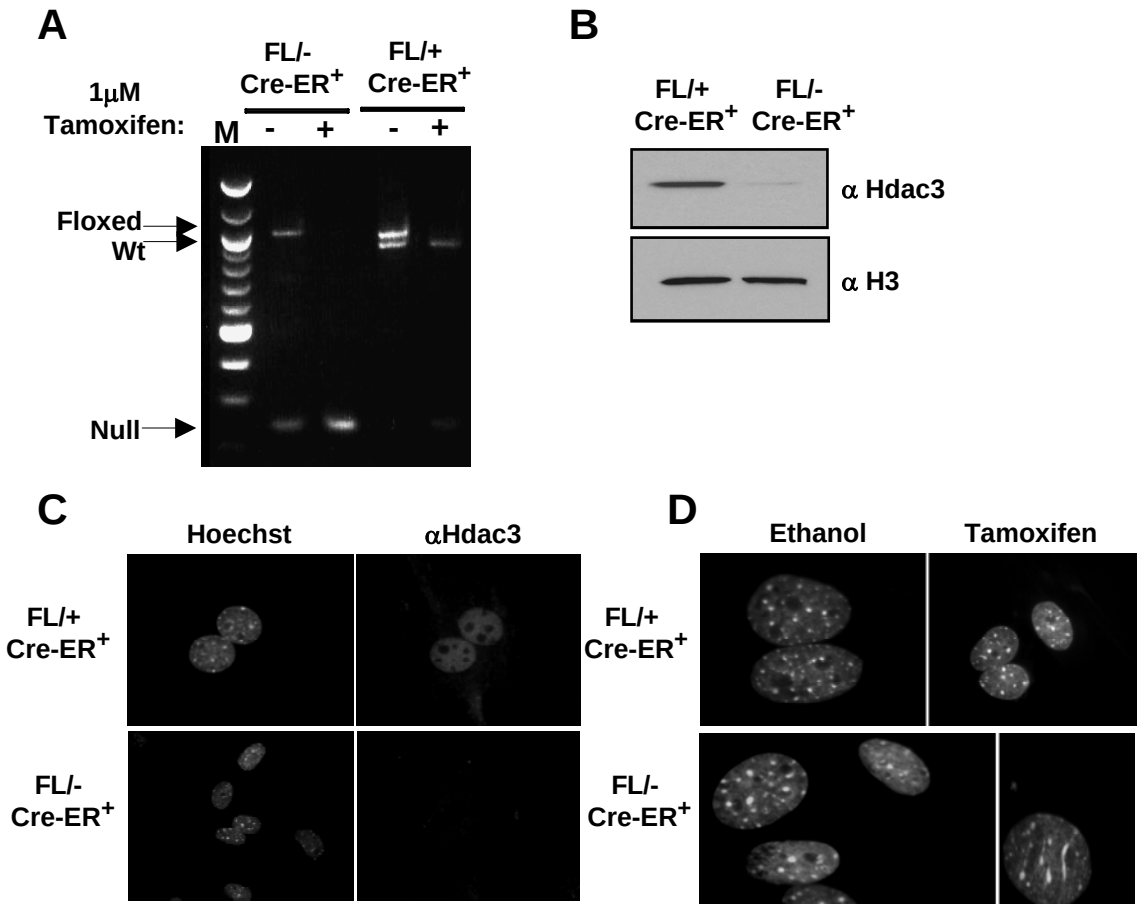


Figure S2. Characterization of Tamoxifen-inducible Cre-ER MEFs. (A) PCR analysis to detect the excision of exon 7 following recombination. PCR analysis of the genomic DNA isolated from *Hdac3*$^{FL/+}$/Cre-ER^{+} and *Hdac3*$^{FL/-}$/Cre-ER^{+} following 24hr of treatment with 1μM tamoxifen. PCR was performed using the primers that flank exon 7 to detect the floxed (935 bp), wt (895 bp), and the null (211 bp) bands. M, 100 bp ladder. (B) Western blot analysis for Hdac3 with nuclear extracts prepared from *Hdac3*$^{FL/+}$/Cre-ER^{+} and *Hdac3*$^{FL/-}$ /Cre-ER^{+} MEFs 90 hr after tamoxifen treatment, where histone H3 served as a loading control. (C) Immunofluorescence staining of Hdac3 in *Hdac3*$^{FL/+}$/Cre-ER^{+} and *Hdac3*$^{FL/-}$/Cre-ER^{+} MEFs 90 hr after tamoxifen treatment. (D) Hoechst staining 90 hr after treatment of *Hdac3*$^{FL/+}$/Cre-ER^{+} and *Hdac3*$^{FL/-}$ /Cre-ER^{+} with tamoxifen.

A. 1.5-fold up in Ad-Cre and tamoxifen treated MEFs (72 hr)

AB gene symbol	Tamoxifen 72hr	Ad-Cre 72hr
Adss	1.8	2.7
Arhgap12	1.6	2.3
Stard4	2.4	3.8
1110030K22Rik	1.8	1.7
Cox7a1	2.1	3.2
Tro	2.3	1.7
Ccl4	1.9	1.8
Ccl6	2.6	8.0
Lss	3.4	1.9
2810003C17Rik	1.5	2.7
Dp1l1	2.0	1.9
Bcl2a1d;Bcl2a1b;Bcl2a1a	2.2	4.4
2010305C02Rik	2.0	2.4
D930010O20Rik	1.9	5.2
Dm15	1.5	1.7
Olfr558	4.7	1.6
Rasgef1b	2.6	2.3
Olfr850	1.7	2.8
Msh3	1.7	2.4
Olfr39	1.7	1.9
Slc16a3	1.6	4.4
6430527G18Rik	1.7	1.7
C1qb	1.5	2.2
Hmgcs1	2.5	1.6
Plf2;Plf;Mrpplf3;Mrpplf4	1.7	1.9
Fdps	1.7	1.7
Eno3	1.8	9.6
Plf2;Plf;Mrpplf3;Mrpplf4	1.7	2.3
Cyp51	2.1	2.0
Scd3;Scd2	1.7	2.2
Nsdhl	2.2	1.5
Acat3	2.3	1.5
C1qg	1.9	2.9
Dhcr24	1.8	2.1
Clecsf8	1.9	1.6
Idi1	2.9	1.9
C1qa	1.9	3.1
Mvd	2.3	1.5
A430103C15Rik	2.0	2.2
Stard4	1.8	3.2
Sc5d	2.2	2.0
Ldlr	1.7	2.1
Cntf	1.8	2.2
Idi1	2.5	2.2
Hsd17b7	2.2	3.0
4632428N05Rik	1.6	2.8

Sc4mol	**1.9**	**1.9**
Gp49b	**1.7**	**3.3**
Lhfp	**2.2**	**1.6**

B. 1.5-fold up in tamoxifen-treated MEFs (72 hr)

AB Gene symbol	Tamoxifen 72hr
Lss	**3.4**
Usp18	**2.2**
Sc4mol	**1.9**
Sqle	**1.9**
Insig1	**2.7**
Fads2	**1.7**
Vav1	**2.6**
Idi1	**2.9**
Plf2;Plf;Mrpplf3;Mrpplf4	**1.7**
Acbd6	**1.7**
2400003L07Rik	**3.6**
2310016A09Rik	**4.7**
Atp5g1	**3.1**
Asc	**1.8**
Olfr1313	**2.3**
Wrn	**1.6**
Mvd	**2.3**
Plscr2	**2.4**
Oit3	**1.9**
AI325941	**2.3**
Nsdhl	**2.2**
Fdps	**1.7**
Fdft1	**2.2**
Scd3;Scd2	**1.7**
Eno3	**1.8**
Hmgcs1	**2.5**
Acat3	**2.3**
Elovl6	**1.8**
Acat2	**2.3**
Stard4	**2.4**
Acvr2b	**1.6**
Mvk	**1.6**
Cyp51	**2.1**
Hsd17b7	**2.2**
Ldlr	**1.7**
Idi1	**2.5**
Saa3	**2.9**
Dhcr24	**1.8**
4933417E01Rik	**1.6**
Myo1g	**1.7**
Olfr51	**2.1**
Prkwnk1	**2.2**
Gbp3	**2.0**

Snf1lk	**2.2**
Psmd8	**15.0**
Hist1h1b	**2.2**
Rasgef1b	**2.6**
BC037432	**1.8**
Tsx	**2.2**
Myo1f	**1.5**
Lhfp	**2.2**
Npm2	**1.6**
Rbbp6;C030034J04Rik	**1.6**
Sc5d	**2.2**
Tm4sf4	**1.6**
2010305C02Rik	**2.0**
Stard4	**1.8**
1110007F12Rik	**1.3**
Pmvk	**2.4**
Clecsf6	**1.5**
Cox7a1	**2.1**
2410018C20Rik	**1.7**
Tpmt	**1.7**
Clecsf8	**1.9**
Adss	**1.8**
Wwox	**4.2**
Mpp2	**2.9**
Gp49b	**1.7**
8030499H02Rik	**2.0**
Cntf	**1.8**
Olfr434	**1.7**
Sat1	**1.5**
Slc25a29	**1.6**
Mgst2	**1.5**
Bcas3	**1.5**
Ms4a6c	**2.0**
Trp53rk	**1.5**
Ptch1	**1.7**
2310030G06Rik	**1.8**
AI854703	**1.7**
4930483J18Rik	**1.7**
6530404N21Rik	**1.6**
Dp1l1	**2.0**

C. 1.5-fold down in tamoxifen-treated MEFs (72 hr)

AB Gene symbol	Tamoxifen 72hr
Syt1	**2.1**
BC021395	**23.6**
Disc1	**1.5**
Ocilrp1	**1.5**
Prss19	**1.6**
Mmp17	**2.4**
Morf4l2	**2.1**

BC050092	**2.1**
Nov	**1.7**
Slc7a1	**2.5**
Tnfrsf18	**2.0**
Strm	**5.6**
Syngr1	**1.8**
2310061G07Rik	**6.2**
D3Ertd300e	**6.1**
Il8rb	**8.8**
9430041C03Rik	**1.9**
2210009G21Rik	**2.8**
1810017F10Rik	**17.6**
Plpi	**2.3**
D030034H08	**2.2**
Rad51l1	**1.6**
Atf7ip	**2.8**
1110031B11Rik	**1.6**
Tifp39	**3.9**
6720466O15Rik	**1.8**
Clstn2	**1.7**
Nos1	**5.5**
3110045G13Rik	**4.8**
Hdac3	**3.8**
Tnfrsf21	**35.7**
Dncic1	**8.5**
2810047L02Rik	**1.8**
Gsg1	**2.9**
Tnn	**2.6**
Fgd6	**2.8**
4931420D14Rik	**3.5**
Kcnd2	**1.2**
Kcnk5	**2.9**
Olfr1388	**1.6**
Gad1	**2.2**
D630003K02Rik	**2.0**
Ppp1r16b	**1.7**
2810457l06Rik	**3.4**
Fgf5	**2.5**
Ing5	**1.8**
Krt2-1	**4.1**
Itgal	**3.1**
Tnni2	**1.6**
E130115E03Rik	**4.0**
Olfr483	**1.6**
1700029l15Rik	**3.5**
A930017N06Rik	**4.1**
Prdm16	**3.1**
A630024J02Rik	**1.3**
Mbnl3	**4.4**
4932417D18Rik	**1.2**
2410127L17Rik	**4.2**
Igsf4c	**2.0**

Grik1	**1.1**
Cnot10	**2.7**
Sox21	**2.2**
8430419L09Rik	**1.6**
Hmgn2	**1.5**
Ly6g5c	**1.5**
2610207I16Rik	**1.6**
AI851790	**1.6**
Olfr1387	**1.8**
A630065K24Rik	**1.7**
4933408F15	**1.6**
Eomes	**1.7**
Timm9	**1.7**
Odf3	**1.4**
Zan	**1.6**
BC020188	**1.7**
Sprr2k	**1.4**
Tnmd	**1.3**
Prkrip1	**1.5**
Asb16	**1.5**
1110058A15Rik	**1.6**
Rgpr	**2.0**
Hapln2	**1.2**
Col2a1	**2.6**
Nppb	**1.7**
Olfr411	**2.1**
Serpinb2	**3.1**
Ccl21a;Ccl21c;Ccl21b	**1.7**
Avpr1a	**1.9**
Tcstv1	**1.6**
Avpr2	**1.4**
2610036L13Rik	**1.3**
Pknox1	**2.0**
2300003P22Rik	**1.7**
Agpt2	**2.2**
Ubtf	**1.4**
D430021I08Rik	**2.5**
C330005M16Rik	**1.6**
Hrh3	**5.1**
Itga9	**1.1**
9930117H01Rik	**2.5**
Gkn1	**4.2**
Il15ra	**2.5**
9830147J24Rik	**1.6**
Zdhhc14	**2.8**
4921530G04Rik	**3.0**
Trib1	**4.2**
Nr2e3	**2.3**
Elk1	**3.7**
Ngef	**2.8**
Pigw	**1.7**
Rag2	**1.7**

Figure S3. List of genes that are differentially expressed in Ad-Cre and tamoxifen-treated *Hdac3*$^{FL/-}$ MEFs. (A) List of genes that are at least 1.5 fold up-regulated in both Ad-Cre and tamoxifen-treated MEFs 72 hr after treatment. (B) List of genes that are at least 1.5 fold up-regulated in tamoxifen-treated MEFs 72 hr after treatment. (C) List of genes that are at least 1.5 fold down-regulated in tamoxifen-treated MEFs 72 hr after treatment. Numbers in the table refer to the fold-increase or decrease in the null MEFs compared to the untreated controls.

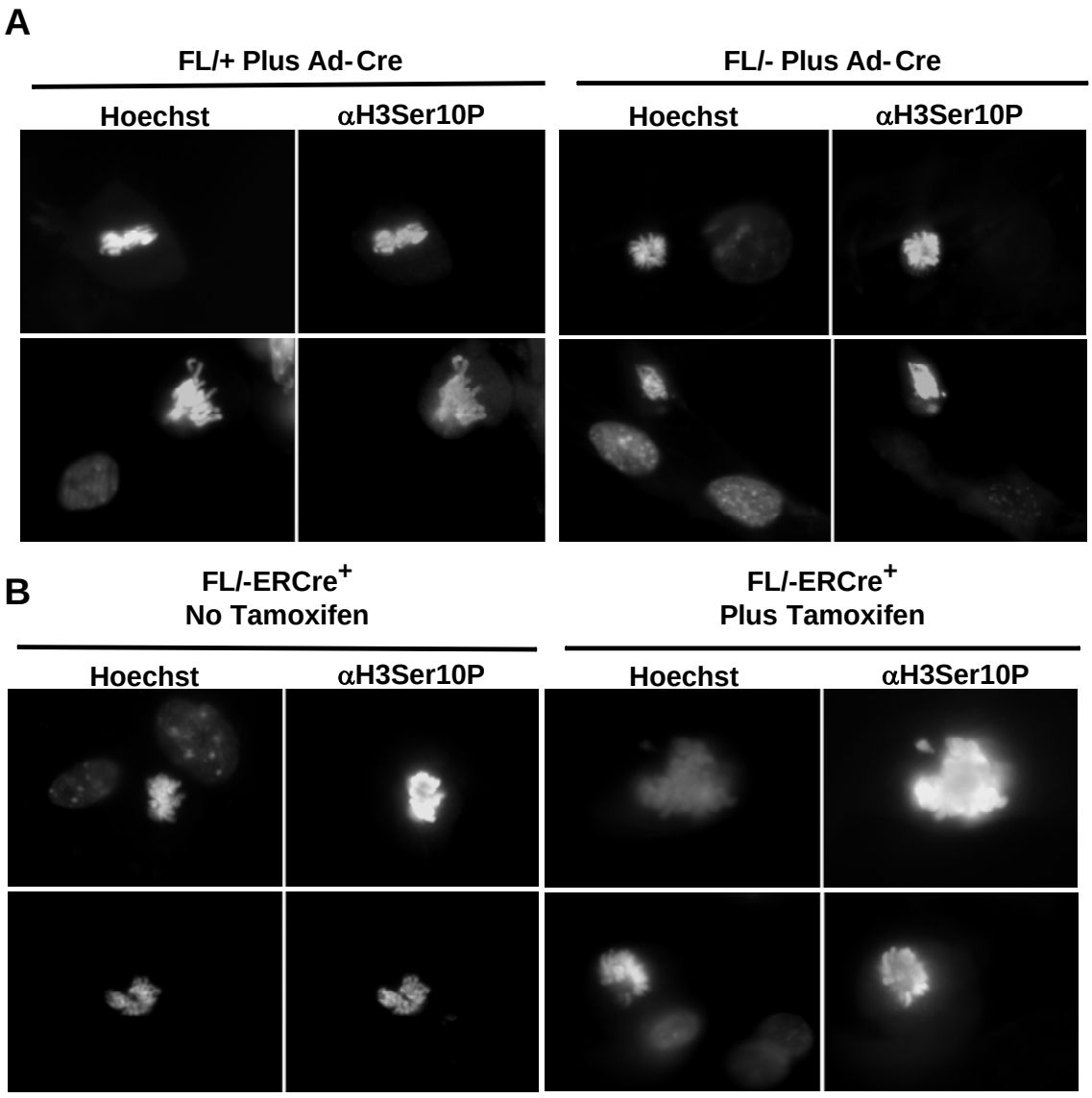


Figure S4. Immunofluoresence of H3ser10 phosphorylation in Ad-Cre infected and tamoxifen-treated MEFs. (A) Immunofluorescence analysis of *Hdac3*$^{FL/+}$ and *Hdac3*$^{FL/-}$ MEFs using anti-phospho H3Ser10 90 hr after Ad-Cre infection. Two different MEF preparations were used for the analysis and representative images are shown. Cells were stained with Hoechst 33258 to visualize the nucleus. (B) Immunofluorescence analysis of H3 Ser10 phosphorylation in *Hdac3*$^{FL/-}$/Cre-ER^{+} and *Hdac3*$^{FL/-}$/Cre-ER^{+} MEFs following tamoxifen or ethanol treatment for 90 hr.

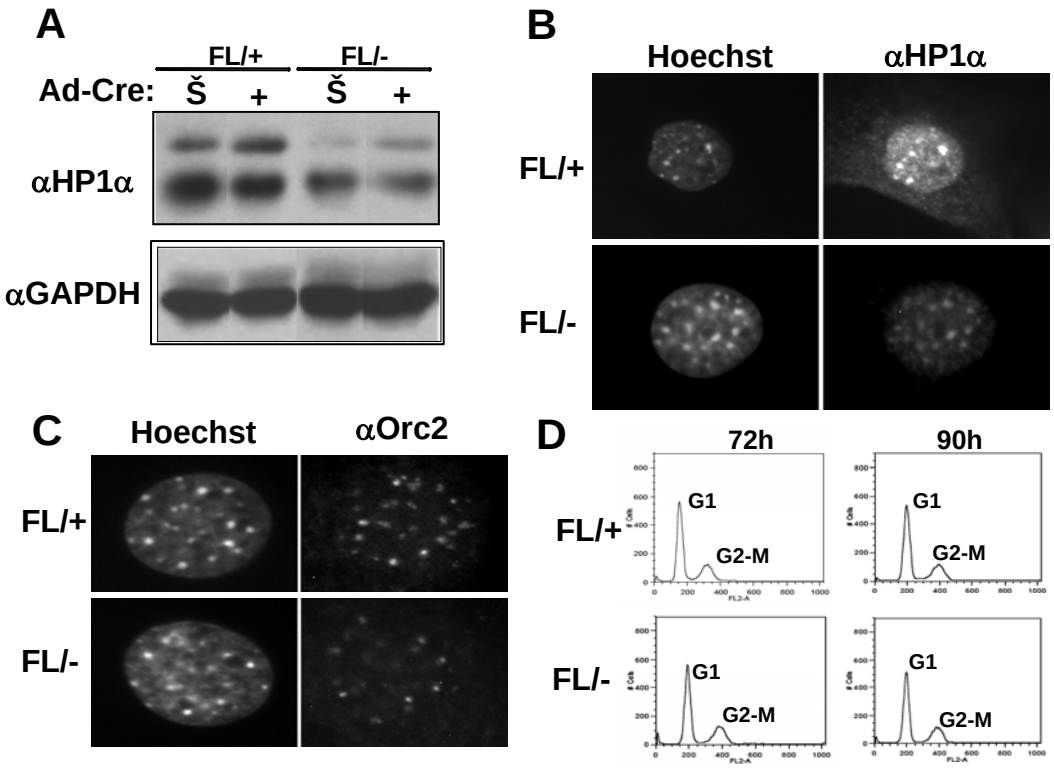


Figure S5. Characterization of mitosis in *Hdac3*$^{-/-}$ MEFs. (A) HP1α levels are unaffected by loss of *Hdac3*. Western analysis of *Hdac3*$^{FL/+}$ and *Hdac3*$^{FL/-}$ MEFs infected with Ad-Cre to detect HP1α levels. The levels of GAPDH were used as a loading control. (B and C) HP1α and Orc2 localize to heterochromatic regions of the nuclei. Immunofluorescence analysis of *Hdac3*$^{FL/+}$ and *Hdac3*$^{FL/-}$ MEFs for the localization of HP1α and Orc2 with the heterochromatic regions present in the nucleus 90 hr after Ad-Cre infection. Hoechst 33258 staining of the nuclei was used to visualize heterochromatic regions. (D) Cell cycle analysis of *Hdac3*$^{-/-}$ MEFs. FACS analysis of Propidium Iodide (PI)-stained *Hdac3*$^{FL/+}$ and *Hdac3*$^{FL/-}$ MEFs was performed 72 hr and 90 hr following Ad-Cre infection. The data was analyzed using Flow-Jo software. The cell cycle analyses were performed three times and similar results were obtained.

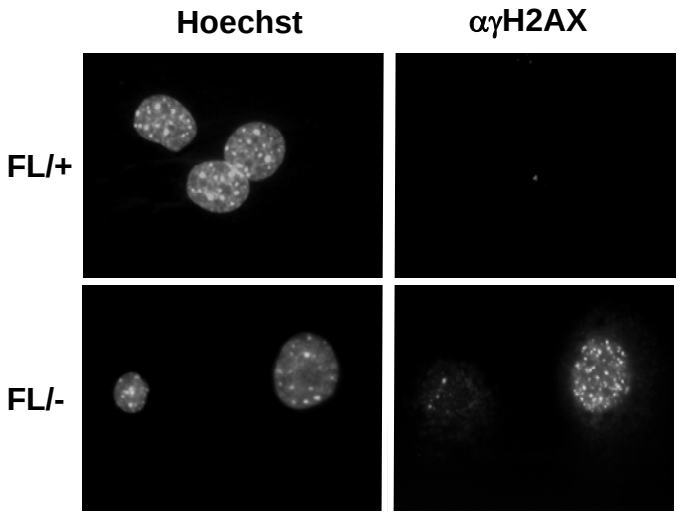


Figure S6. DNA damage in Hdac3$^{-/-}$ MEFs. Immunofluoresence analysis of γH2AX in Ad-Cre infected *Hdac3*$^{FL/+}$ and *Hdac3*$^{FL/-}$ MEFs 72hr post-infection. When 100 cells were counted, an average of 6% contained 20 foci or more in the null cells.

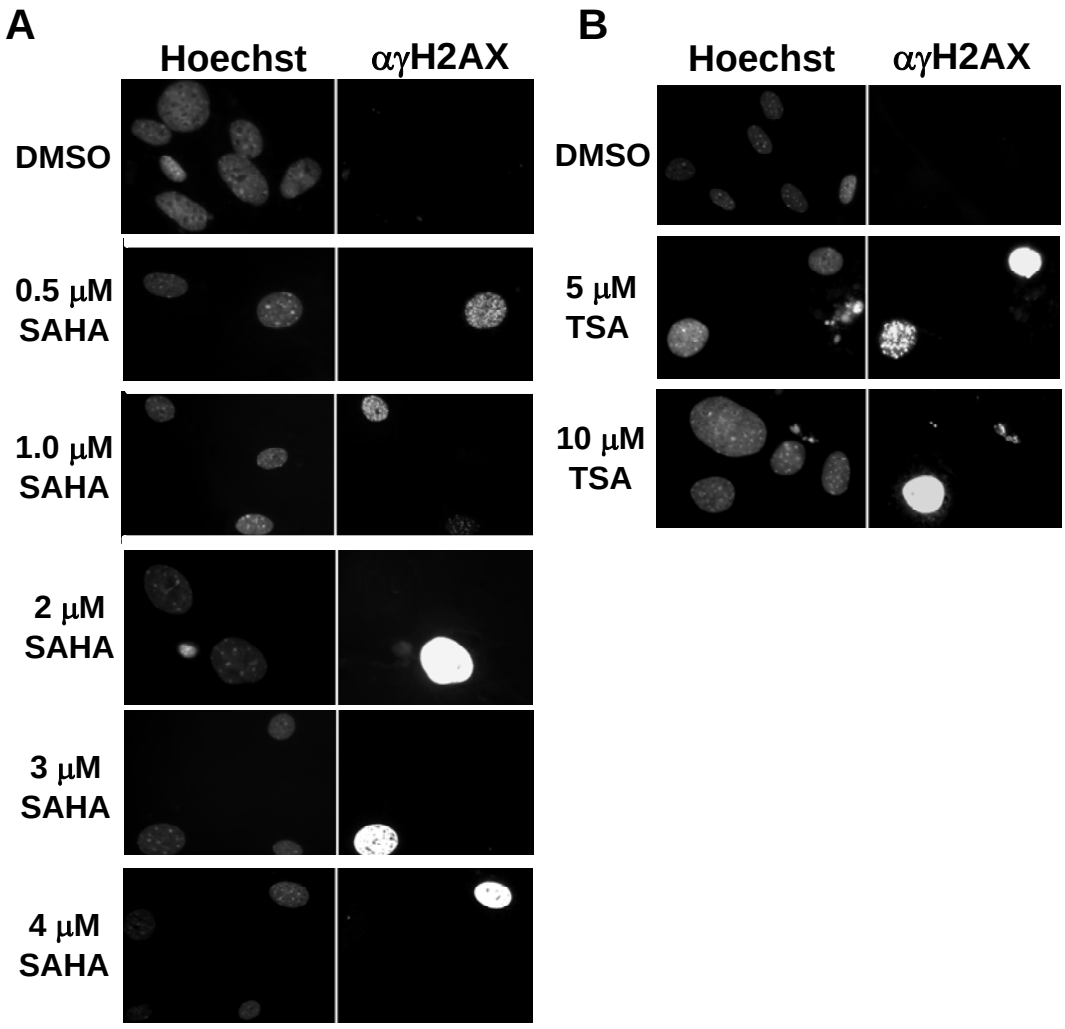


Figure S7. SAHA and TSA treatment cause DNA damage in MEFs. (A) A representative image of the quantitative analysis of γH2AX foci observed in SAHA-treated MEFs shown in Fig. 5F. (B) MEFs were treated with TSA (5 and 10μM) or with DMSO (vehicle) for 24hr and immunofluoresence analysis of γH2AX was performed.

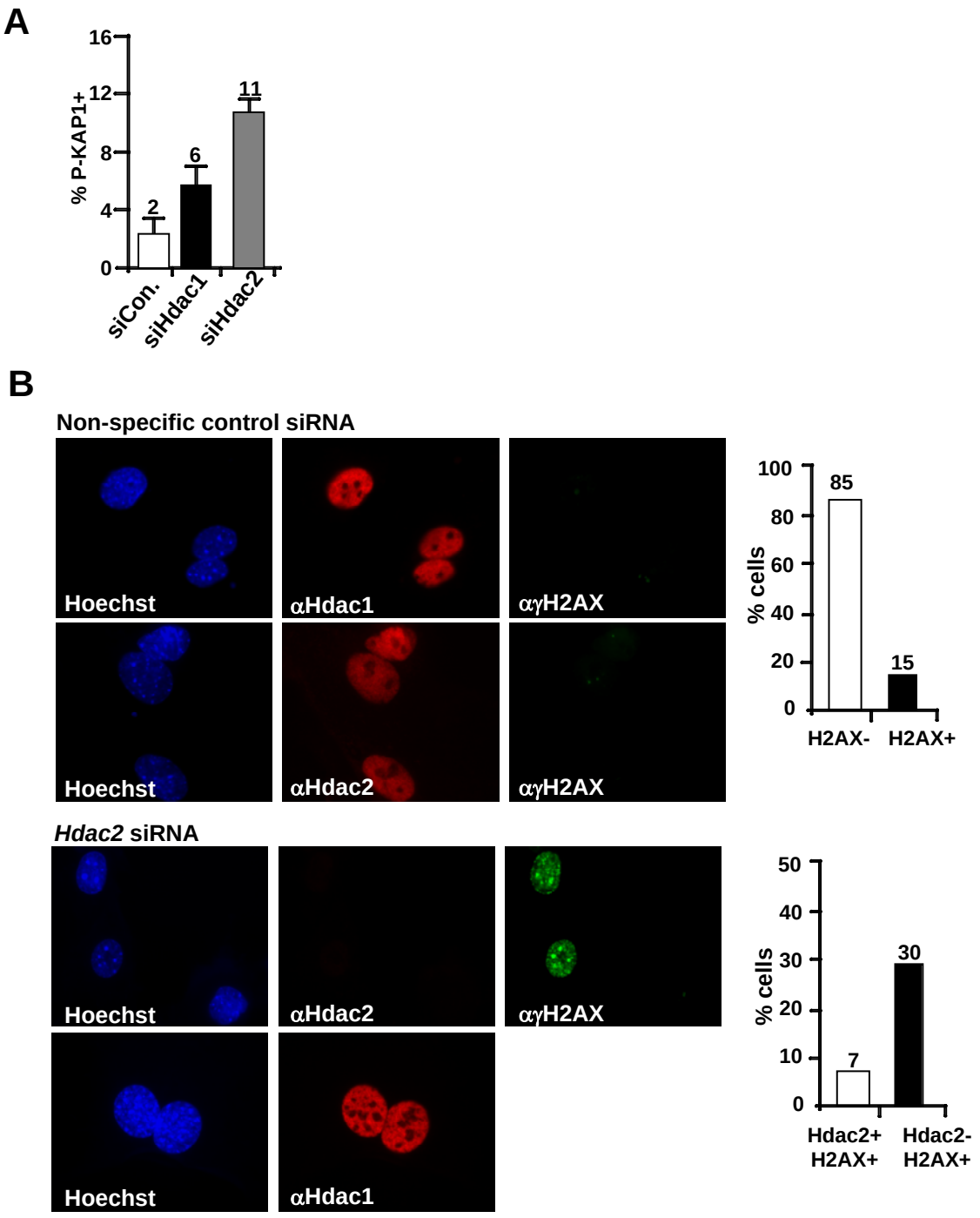
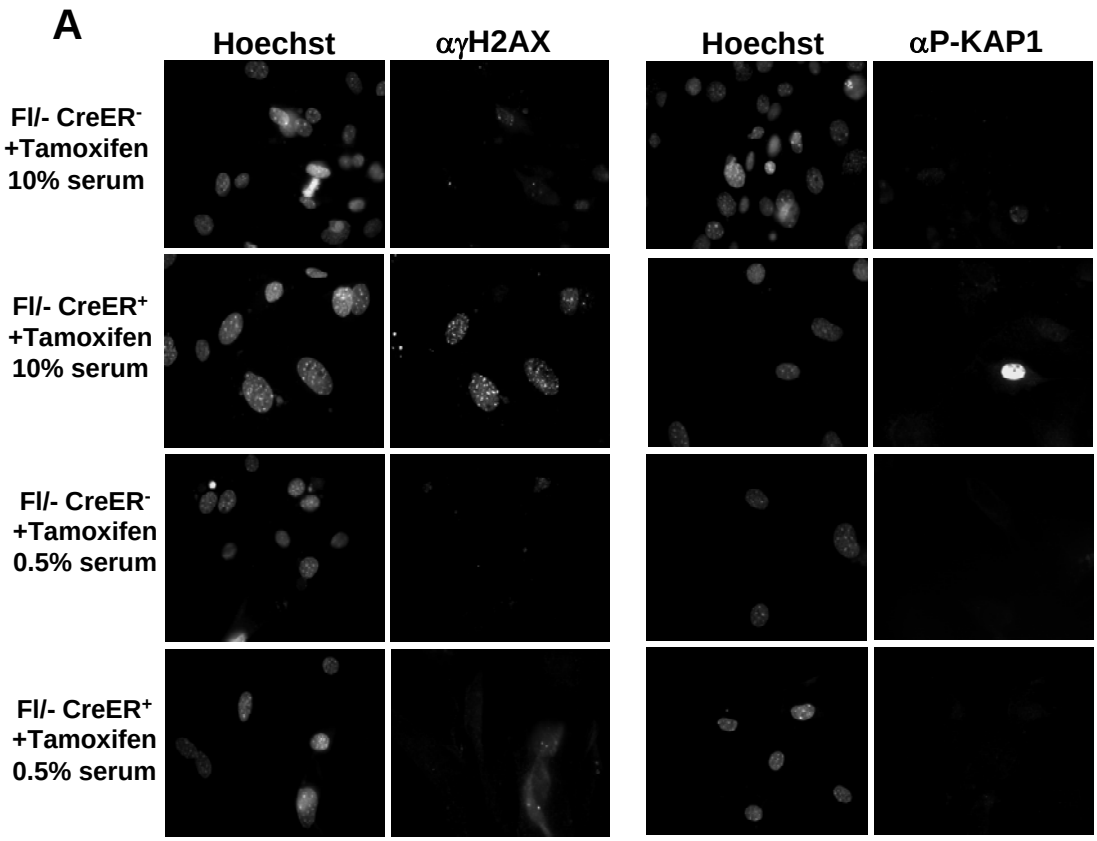


Figure S8. DNA damage in MEFs following *Hdac1* and *Hdac2* siRNA treatment. (A) MEFs were transfected with either siGenome SMART pool for mouse *Hdac2*, mouse *Hdac1*, or with a non-specific control pool (siRNA negative control) and P-Kap1 immunofluoresence analysis was performed at 72hr following transfection. Values

represent the average percent P-Kap1 positive cells from 2 independent experiments ± S.E., in which around 150 cells were counted in total. (B) MEFs were transfected with the non-specific control pool or with *Hdac2* siRNA. MEFs transfected with *Hdac2* siRNAs were scored for the percentage of cells with (Hdac2+) or without (Hdac2-) Hdac2 expression. The percentage of cells with at least 5 γH2AX foci was then determined in Hdac2+, Hdac2-, and control cells.


A
Hoechst
αγH2AX
Hoechst
αP-KAP1
Fl/- CreER- +Tamoxifen 10% serum
Fl/- CreER+ +Tamoxifen 10% serum
Fl/- CreER- +Tamoxifen 0.5% serum
Fl/- CreER+ +Tamoxifen 0.5% serum

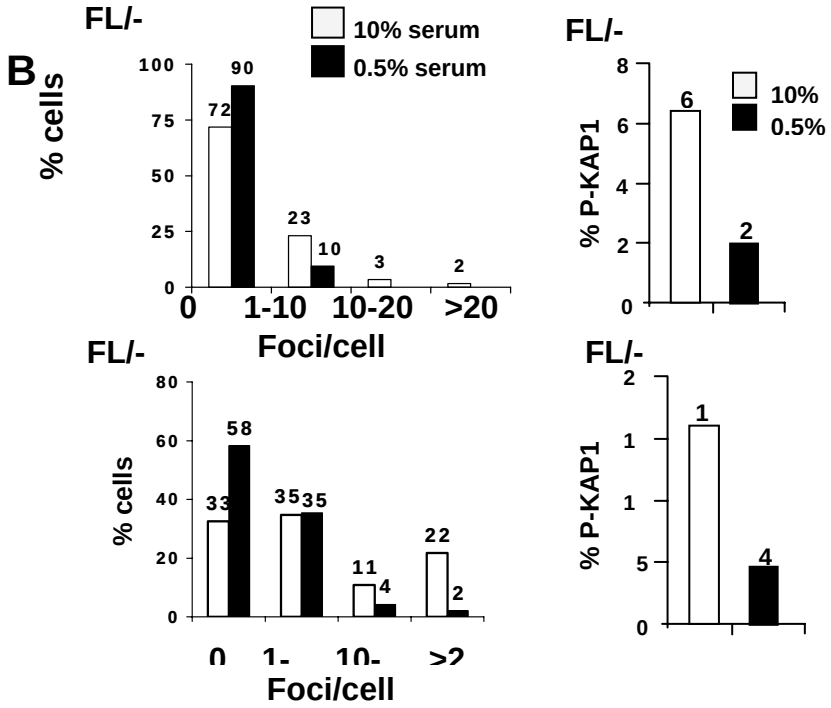

B
FL/-
10% serum
0.5% serum
% cells
100
75
50
25
0
90
72
23
10
3
2
0
1-10
10-20
>20
Foci/cell
FL/-
% P-KAP1
8
6
4
2
0
6
2
10%
0.5%
FL/-
% cells
80
60
40
20
0
58
33
35
35
11
4
22
2
0
1-
10-
>2
Foci/cell
FL/-
% P-KAP1
2
1
1
5
0
1
4

Figure S9. DNA damage is cell cycle dependent in tamoxifen-treated *Hdac3*$^{FL/-}$/Cre-ER^{+} MEFs. (A) A decrease in γH2AX and Kap1 phosphorylation in tamoxifen-treated *Hdac3*$^{FL/-}$/*Cre-ER*$^{+}$ MEFs following serum starvation. *Hdac3*$^{FL/-}$ Cre-ER^{-} and *Hdac3*$^{FL/-}$/Cre-ER^{+} MEFs were treated with 1μM tamoxifen for 48 hr and then the cells were cultured in media containing 0.5% serum for 60 hr prior to immunofluorescence using anti-γH2AX (left panels) or anti-p-KAP1 (right panels). (B) Quantification of the γH2AX foci distribution and p-Kap1 positive cells in serum starved *Hdac3*$^{FL/-}$/Cre-ER^{-} and *Hdac3*$^{FL/-}$/Cre-ER^{+} MEFs. 100 cells were counted for each condition.

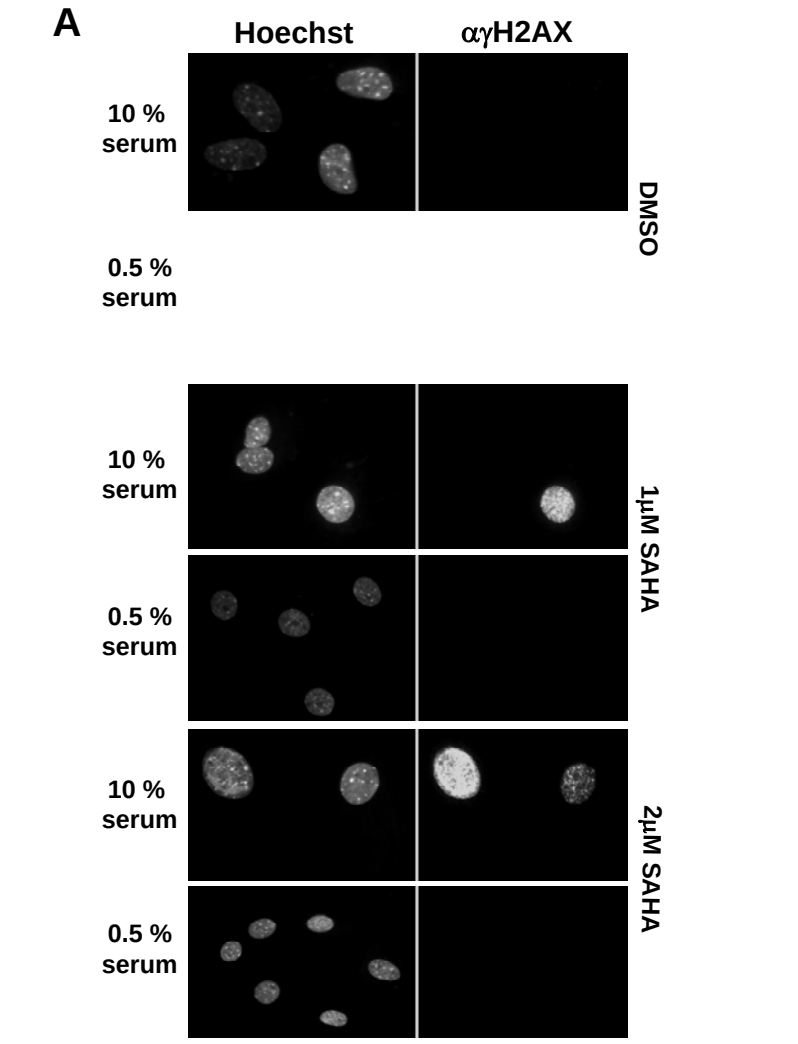

A
Hoechst
αγH2AX
10 % serum
0.5 % serum
DMSO
10 % serum
0.5 % serum
1μM SAHA
10 % serum
0.5 % serum
2μM SAHA
B
0 foci
1-10 foci
10-20 foci
>20 foci
% cells
100
80
60
40
20
0
84
15
0
88
11
1
82
3
2
13
84
11
3
2
72
4
3
20
87
12
2
Serum: 10 0.5 10 0.5 10 0.5
DMSO
1μM
2μM

Figure S10. SAHA-induced DNA damage requires cell cycle progression. (A) MEFs were serum starved for 60hr and then treated with either 1μM SAHA or with 2μM SAHA for 28hr and γH2AX immunofluoresence analysis was performed. (B) Quantification of the γH2AX foci was done by calculating the average percentage of cells with different numbers of γH2AX foci and the data shown is an average percentage ± S.E. obtained with two different MEF isolates.

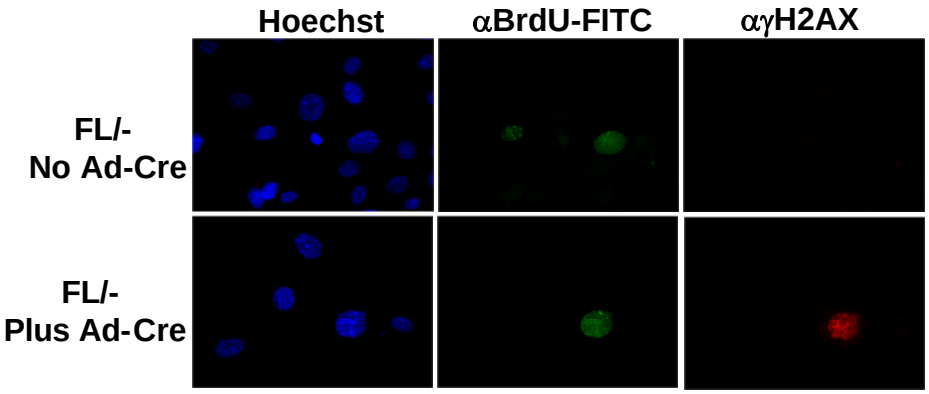


Figure S11. DNA damage in *Hdac3*$^{-/-}$, BrdU positive cells. *Hdac3*$^{FL/+}$ and *Hdac3*$^{FL/-}$ MEFs were infected with Ad-Cre were labeled with a pulse of BrdU. Double immunofluoresence analysis was then performed on these cells with anti-γH2AX and anti-BrdU-FITC antibodies. The experiment was performed with two different MEF preparations and a representative picture from one experiment is shown.

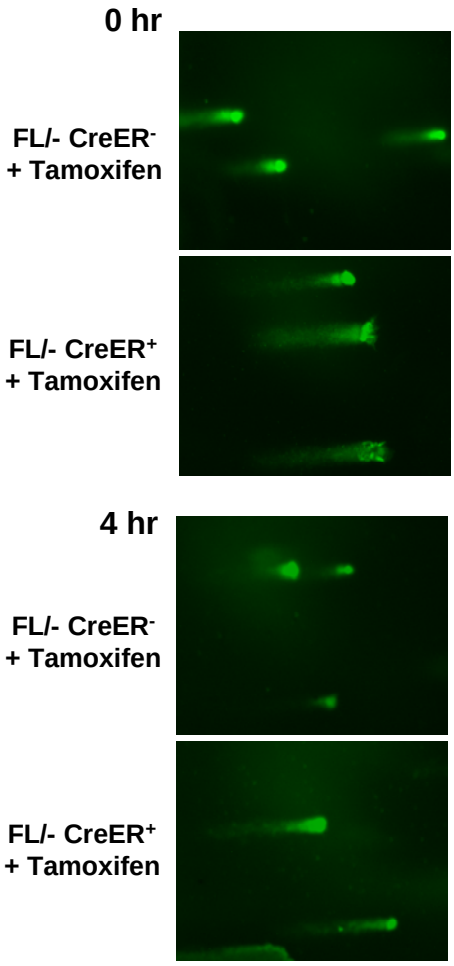


Figure S12. *Hdac3*-null cells are defective in DNA double strand break repair. MEFs (*Hdac3*$^{FL/-}$/Cre-ER^{+} and *Hdac3*$^{FL/-}$/Cre-ER^{-}) were treated with 1μM tamoxifen for 48 hr and then were cultured in media containing 0.5% serum for 60 hr prior to irradiation with 1 Gy of IR. Comet analysis was performed using the Comet assay kit (Trevigen) as per the manufacturer's protocol and representative images of the comets are shown.

Supplemental Experimental Procedures

Generation of the *Hdac3* knockout mice

The targeting construct was designed to flank *Hdac3* exon 7 with LoxP sites and place a floxed *Neo* cassette in the intron between exons 7 and 8. The 5' arm of the targeting construct was generated containing a LoxP site by PCR using splicing by overlap extension (SOE). Initially, two PCR products making up the 5' arm were generated using the primers 5'arm fwd, 5'-CGACTCGAGGTGCAACTGTAAGCCAGGCAGTGG-3' and 5'arm mid rev, 5'-GAATTCACGCGTGGCTGAAATGTGAGCAGGCAGCA-3' and 5'arm rev, 5'-CGAATCGATAAGCCAGCCTGGTCTGTAGCAATA-3' and 5'arm mid fwd, 5'-CGACTCGAGCCGCTGCATGTTCCCTTTCTCCCC-3'. TL1 ES cell DNA was used as the template and the PCR conditions were 94^{o}C, 8 min followed by 30 cycles of 94^{o}C, 1 min, 58^{o}C, 1 min, 72^{o}C, 3 min with a 72^{o}C, 10 min final extension. These PCR products were used as templates for the SOE. The reaction conditions and the primers, containing a LoxP site (italics) and an internal *Mlu*1 site (underlined; added for diagnostic purposes), were as follows: first set of reactions used primers 5' arm fwd, and LoxP Rev, 5'-*ATAACTTCGTATAGCATACATTATACGAAGTTAT*<u>ACGCGT</u>GGCTGAAATGTGAGC AGGCAGCAG-3'; 5'arm rev, and LoxP Fwd, 5'-<u>ACGCGT</u>*ATAACTTCGTATAATGTATGCTATACGAAGTTAT*CCGCTGCATGTTCCCT TTCTCCCC-3'. The PCR conditions for the first set of reactions were 94^{o}C, 8 min followed by 30 cycles of 94^{o}C, 1 min, 58^{o}C, 1 min, 72^{o}C, 3 min with a 72^{o}C, 10 min final extension.

The full-length 5' arm was created by adding the two products of the above reactions together diluted 1:20 with the primers 5'arm fwd and 5'arm rev. PCR reaction conditions were 94^{o}C, 3 min followed by 30 cycles of 94^{o}C, 1 min, 60^{o}C, 45 sec, 72^{o}C, 3 min with a 72^{o}C, 10 min final extension. The product of this reaction was sequenced to verify the presence of the LoxP site and *Mlu*I sites. The approx. 1.8 kb product was subcloned into the *Xho*I and *Cla*I sites of pNTK(A)LP2 creating the 5'arm *pNTK* construct. The *pNTK(A)LP2* vector used for making the targeting construct contains a floxed Neo cassette bounded by multiple cloning sites with *HSV TK* and was provided by the Vanderbilt-Ingram Cancer Center Transgenic Mouse/Embryonic Stem Cell Shared Resource.

The 3' arm was also generated using SOE. The first set of products were generated by the following sets of primers: 3'arm fwd 5'-CGACTCGAGCTGCTTCTTGAGTGCTGGGATTAA-3' and 3'arm mid rev 5'-GTGCTGACATCTGGATGGAGTGTG-3'. The second product was generated by 3'arm rev 5'-ACAGGATCCTGGTTCACTGCTCAGGACAGAGTA-3' and 3'arm mid fwd 5'-GATTCACTCTGGTGTCTCAGCCAT-3'. Reaction conditions for the first round of PCR were 94^{o}C, 3 min, 30 cycles of 94^{o}C, 1 min, 63^{o}C, 45 sec, 72^{o}C, 6 min, with a final extension of 72^{o}C, 10 min. The full-length 3'arm was generated by adding the above two products together after diluting each 3-fold and 8-fold, respectively, and performing PCR with 3'arm fwd and 3'arm rev with the following reaction conditions: 94^{o}C, 3 min, 30 cycles of 94^{o}C, 1 min, 65^{o}C, 45 sec, 72^{o}C, 7 min, with a final extension of 72^{o}C, 10 min. The approx. 3.7 kb product was subcloned into PCR-Script for sequencing. The product was released *by XhoI* and *BamH*I digest and subcloned into the *Sal*I and *BamH*I digested 5'arm *pNTK*.

The targeting construct was restricted with *Not*I for electroporation into TL1 ES cells. Primers for the 3' probe included: 3'probe fwd 5'-CTGAGCAGTGAACCAGTAGACCAC-3' and 3' probe rev 5'-TCACAGCAACTCGAGTGGTCTCAG-3' with reaction conditions 94^{o} C, 3 min, 30 cycles of 94^{o} C, 1 min, 65^{o} C, 45 sec, 72^{o} C, 7 min, with a final extension of 72^{o} C, 10 min. TL1 DNA was used as a template for the reactions. The 3' probe was used to hybridize a Southern blot of *Nhe*I digested ES cell DNA to identify clones that had undergone homologous recombination. Potential positives were identified and a PCR screen examining the 5' end of the targeting construct was performed to ensure proper homologous recombination. This screen used the following primers: 5' outside, 5'-TTAGCCCCAAGCAGCTGTTGATCTG-3' fwd (outside of the arm of the targeting construct) and *Hdac3* 1597 rev, 5'-GGACACAGTCATGACCCGGTC-3' (inside the arm of the targeting construct). This PCR product spans the LoxP site and the *Mlu*I site that was incorporated with the SOE reaction for diagnostic purposes. The PCR products were cut with *Mlu*I to identify the positive clones. Two positive clones were expanded and injected into C57BL/6 blastocysts. The resulting chimeric mice were bred to heterozygosity and crossed with transgenic mice expressing *Cre* ubiquitously from the *EIIA* promoter. Mice that had lost the *Neo* sequences but that retained LoxP sites flanking

exon 7 were identified and used for these studies. Mice that had lost both the *Neo* and exon 7 on one allele (heterozygous) were also identified and bred. PCR screening was used to identify mice carrying a floxed exon 7, wild-type exon 7 and a deleted exon 7. The primers and conditions were as follows: *Hdac3* 1263T 5'-CCACTGGCTTCTCCTAAGTTC-3' and *Hdac3* 2158B 5'-CCCAGGTTAGCTTTGAACTCT-3'. The conditions were 94^{o}C, 5 min, 30 cycles of 94^{o}C, 45 sec, 55^{o}C, 25 sec, 72^{o}C, 1 min 15 sec, followed by 72^{o}C, 10 min. The resulting products floxed exon 7 (935 bp), wild-type exon 7 (895 bp), and deleted exon 7 (211 bp) were resolved on a 2% agarose gel.

siRNA knockdown in MEFs

MEFs were transfected with either siGenome SMART pool for mouse *Hdac2*, siGenome SMART pool for mouse *Hdac1*, or with non-specific control pool (siRNA negative control) using Lipofectamine 2000. 24hr after transfection, the cells were transfected again, and immunofluoresence analysis was done 72hr after the first transfection.

Comet assay

Single cell gel electrophoresis assay was done using the comet assay kit (Trevigen). In brief, MEFs were cultured in the presence of 0.5% serum for 60hr and then exposed to a 1gy of IR. Cells were collected either at 15min (0 hr) or 4 hr following irradiation and resuspended in PBS at a density of 10^5 cells/ml. The assay was done according to the manufacturer's protocol and the cells were stained with Sybr green (Trevigen) following alkaline gel electrophoresis. Images of the comets were captured using the Axiophot microscope and tail moment of the comets was calculated using the Comet Score software.