

Figure S1

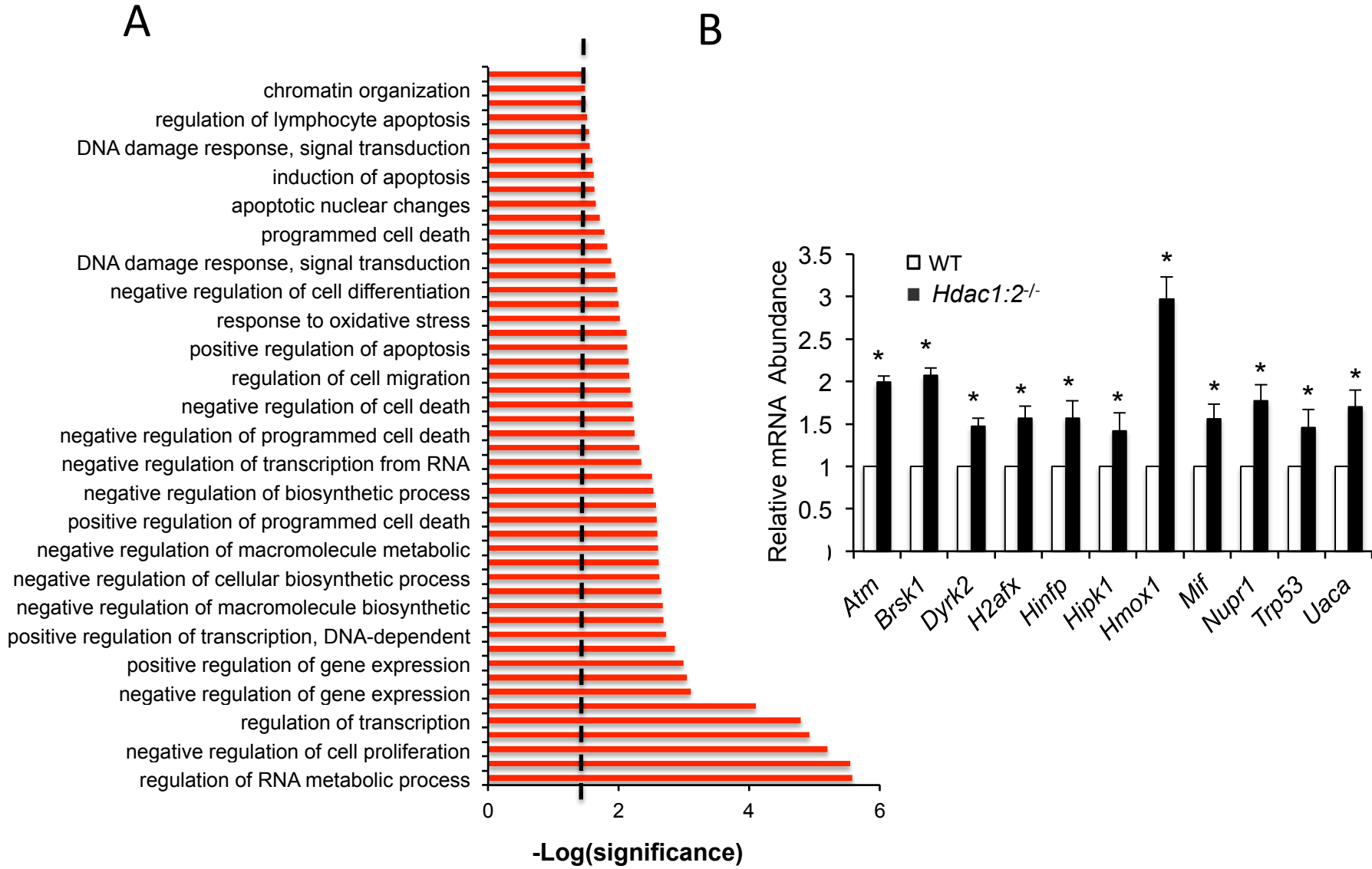


Figure S3

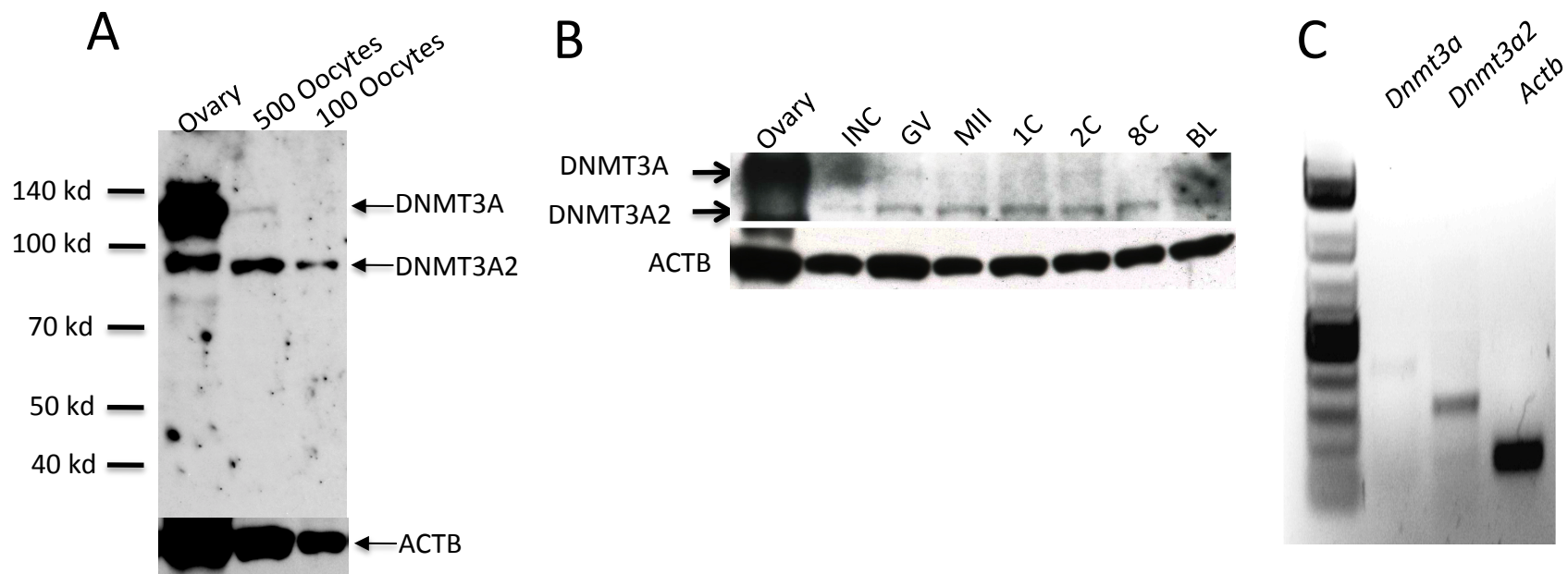


Figure S4

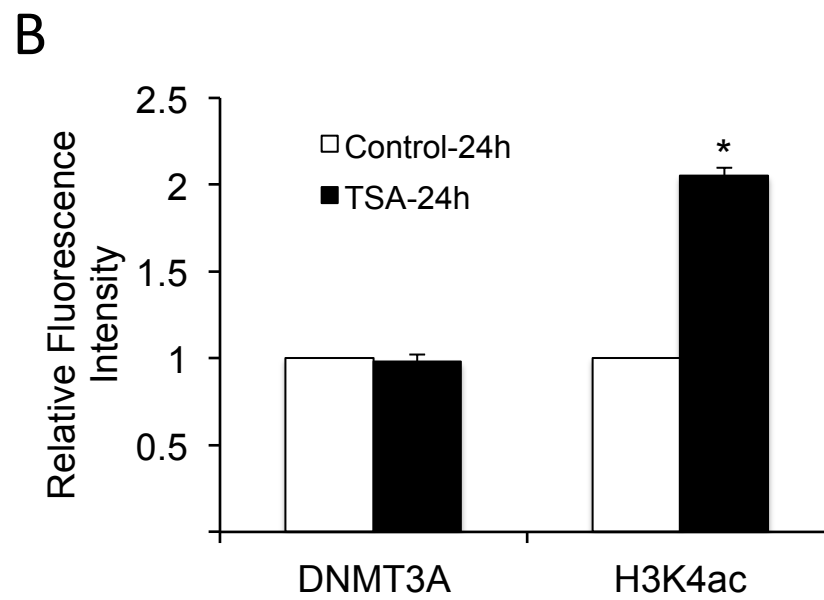
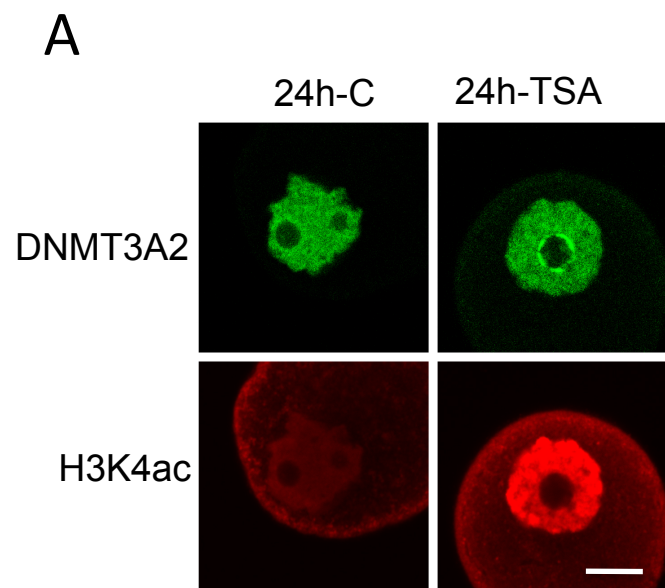
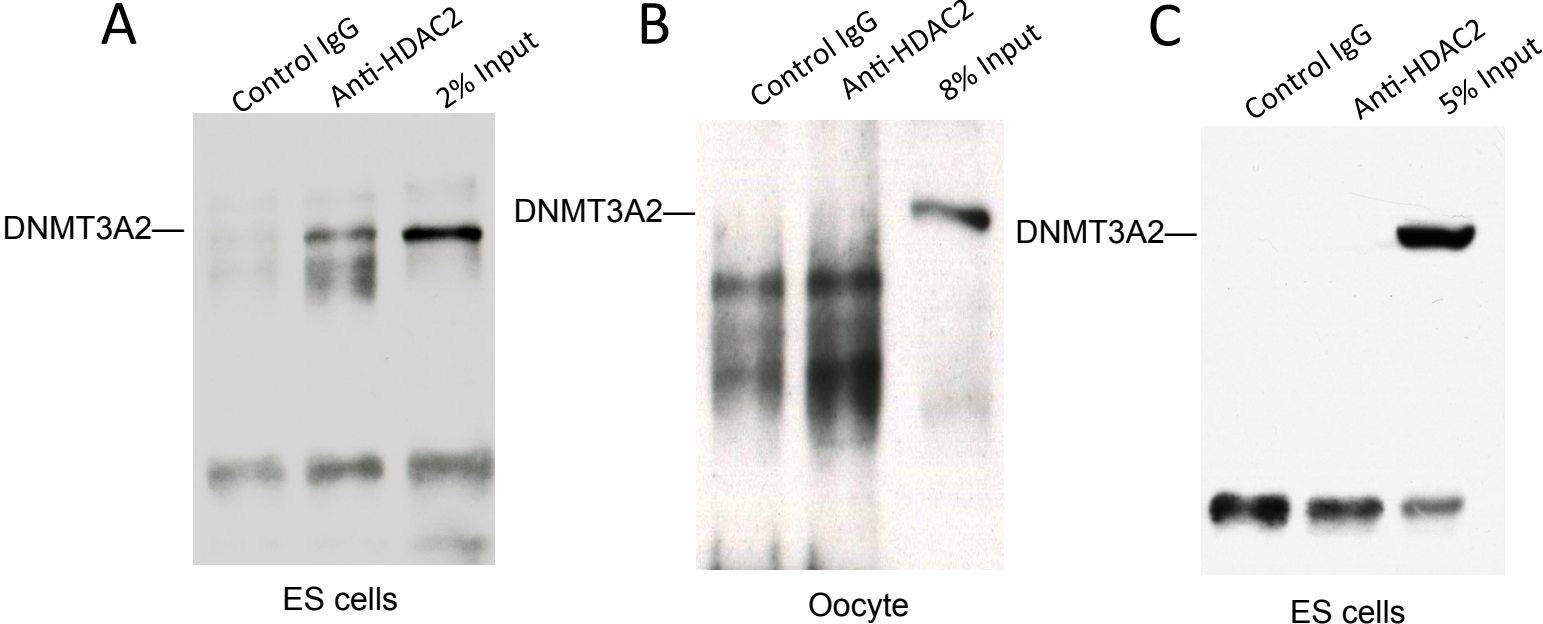


Figure S5



Supplemental Figure Legends

Figure S1

DNA damage pathway genes are misregulated in *Hdac1/2* double mutant oocytes,

Related to Figure 3. (A) Gene ontology analysis was performed with PANTHER.

Significantly ($p < 0.05$) enriched biological processes for up-regulated genes are shown.

Plotted is the $-\log$ (P value) with the threshold (black dashed line) set to 1.3 [$\log(0.05)$].

(B) Up-regulation of DNA damage pathway genes in *Hdac1:2^{-/-}* oocytes. mRNA levels of

DNA damage pathway genes are expressed relative to the WT control, for which

mRNA levels were set at 1, and the data are expressed as mean \pm SEM. (* $p < 0.02$).

Figure S2

***Snrpn* is hypomethylated in growing *Hdac2^{-/-}* oocytes, Related to Figures 2 and 4.**

Bisulfite sequencing analysis of DNA methylation at the ICRs of *Snrpn* in WT and

Hdac2^{-/-} growing oocytes obtained from mice 12 days-of-age. Open circles and filled

circles represent unmethylated and methylated CpG sites, respectively. Each row

represents data from a single DNA molecule. The numbers below each set of DNA

strands indicate the percent of methylated CpG sites.

Figure S3

DNMT3A2 is the predominant DNMT3A isoform expressed in mouse oocytes,

Related to Figure 4.

(A) Western blot analysis of DNMT3A in mouse ovary and oocytes. Samples prepared

from ovary (obtained from mice 18 days-of-age), 500 and 100 full-grown oocytes were

separated by SDS-polyacrylamide gel electrophoresis and protein detected with an anti-

DNMT3A antibody recognizing both DNMT3A and DNMT3A2. Molecular weight

markers are also indicated. **(B)** DNMT3A protein expression profile in oocyte and preimplantation embryos. One hundred forty oocytes/embryos were loaded per lane, and beta-actin (ACTB) was used as a loading control. The experiment was conducted 2 times, and similar results were obtained in each case; a representative experiment is shown. INC, incompetent oocyte; GV, full-grown oocyte; MII, metaphase II; 1C, one-cell embryo; 2C, two-cell embryo; 4C, four-cell embryo; 8C, eight-cell embryo; BL, Blastocyst. **(C)** RT-PCR of *Dnmt3a* and *Dnmt3a2* isoforms in mouse oocytes. A cDNA library was prepared from full-grown oocytes obtained from mice 3 weeks-of-age. PCR was performed with the sense primers specific for *Dnmt3a* and *Dnmt3a2*, and a common antisense primer. *Actnb* was also amplified as an internal control.

Figure S4

Trichostatin A (TSA) treatment has little effect on DNMT3A2 localization in oocytes, Related to Figure 4. **(A)** Immunocytochemical detection of DNMT3A2 (green) and histone H3K4 acetylation (red) after TSA treatment of WT oocytes for 24 h. At least 20 oocytes were analyzed, and the experiment was performed two times using at least three mice per experiment. Shown are representative images. The bar corresponds to 10 μ m. **(B)** Quantification of the data shown in panel A. Nuclear staining intensity of detected proteins in WT oocytes was set to 1, and the data are expressed as mean \pm SEM. (*p < 0.05).

Figure S5

DNMT3A2 interacts with HDAC2, Related to Figure 4. **(A)** Mouse ES cell extract was used for immunoprecipitation with anti-HDAC2 antibody and following electrophoresis the membrane was probed for DNMT3A2. Rabbit IgG was used as a

control. Whole cell extract was used for input control. **(B)** Mouse oocytes (~1800) were lysed and the extracts were used for immunoprecipitation with anti-HDAC2 antibody and following electrophoresis the membrane was probed for DNMT3A2. Rabbit IgG was used as a control. Whole oocyte extract was used for input control. **(C)** Mouse ES cell extract containing an amount of protein equivalent to that from ~1800 oocytes was used and the experiment conducted as in A.

Supplemental Experimental Procedures

mRNA semi-quantitative RT-PCR

Total RNA was extracted from 80 oocytes isolated from mice of 12 d of age by using the PicoPure RNA kit (Arcturus). The isolated RNA was treated with RNase-free DNase (Promega, Madison, WI, USA) at 37 °C for 20 min to avoid contamination of genomic DNA, and was then reverse transcribed into cDNA in 50 µl of reverse transcription reaction mixture containing 2 µg RNA, 2 mM random hexamers, 2 mM dNTPs, 0.01 M dithiothreitol, 5 U RNase inhibitor, and 200 U M-MLV reverse transcriptase (Promega, Madison, WI, USA). The sample was incubated at 37 °C for 1 h and the enzyme was heat-inactivated at 95 °C for 5 min. Two microlitres of cDNA synthesized were then amplified for *Dnmt3a* (accession number, AF068625) and *Dnmt3a2* (accession number, AF480164), the following primers were used as described before (Sakai et al., 2004): *Dnmt3a* sense primer, 5'-CGACTGCGAGGTGGCTTGGGCTG-3', *Dnmt3a2* sense primer, 5'-CTCACACCTGAGCTGTACTGCAGAG-3', and common antisense primer, 5'-CTCCACCTTCTGAGACTCTCCAGAG-3'. The amplification reaction comprised 25

cycles of incubation of the reaction mixture, each cycle comprising denaturation at 94 °C for 1 min, annealing at 60 °C for 1 min, and extension at 72 °C for 1 min. *Actb* was also amplified as an internal control (forward primer, 5'-TTCTACAATGAGCTGCGTGTG-3'; reverse primer, 5'-GGGGTGTGTAAGGTCTCAAA-3'). PCR products were subjected to electrophoresis in 1.5% (w/v) agarose gels, stained with ethidium bromide, and photographed; see Figure S3B.

Quantitative Real-Time RT-PCR

RNA isolation and reverse transcription were performed as above, Real-Time PCR was performed using Sybr Green mix (Applied Biosystems) to analyze the expression of retrotransposons as described previously (Murchison et al., 2007). Two incompetent oocyte equivalents of cDNA was used for each real-time PCR with a minimum of three replicates as well as minus RT and minus template controls for each gene. Quantification was normalized to *Ubf* mRNA, whose transcript abundance is not altered in double mutant *Hdac* oocytes (Ma et al., 2012). The following primers were used as described before (Stein et al., 2015). Primer sequences were: *MT*.fwd: 5'-TGTTAAGAGCTCTGTCGGATGTTG-3'; *MT*.rev: 5'-ACTGATTCTTCAGTCCCAGCTAAC-3'; *SineB1*.fwd: 5'-GTGGCGCACGCCTTTAATC-3'; *SineB1*.rev: 5'-GACAGGGTTTCTCTGTGTAG-3'; *SineB2*.fwd: 5'-GAGATGGCTCAGTGGTTAAG-3'; *SineB2*.rev: 5'-CTGTCTTCAGACACTCCAG-3'; *Line L1* ORF2.fwd: 5'-TTTGGGACACAATGAAAGCA-3'; *Line L1* ORF2.rev: 5'-CTGCCGTCTACTCCTCTTGG-3'; *Iap* LTR.fwd: 5'-TTGATAGTTGTGTTTTAAGTGGTAAATAAA-3'; *IAP* LTR.rev: 5'-AAAACACCACAAACCAAATCTTCTAC-3'; *Ubf*.fwd: 5'-

TCACTAGCACCCACTTGCTC-3'; *Ubf*.rev: 5'-GTGGAGGACGGACATTGGC-3'.

Real-Time PCR analysis was performed with the ABI Taqman Assay-on-demand probe/primer sets as previously described (Stein et al., 2015) to analyze the expression of DNA damage pathway genes. Two replicates were run for each real-time PCR reaction; a minus template served as control. Quantification was normalized to *Ubf* mRNA.

TaqMan probes used for gene expression analysis determined by real-time PCR using TaqMan Gene Expression Assays (Applied Biosystems) were as follows (Assay ID and gene symbol): Mm01177457_m1, *Atm*; Mm01328475_m1, *Brsk1*; Mm00515990_s1, *H2afx*; Mm01168527_g1, *Hinfp*; Mm01166529_m1, *Dyrk2*; Mm03938638_s1, *Mif*; Mm00516005_m1, *Hmox1*; Mm00498104_m1, *Nupr1*; Mm00551207_m1, *Uaca*; Mm01731287_m1, *Trp53/P53*; Mm00456972_m1, *Ubf*.

Antibodies

The following antibodies were used for immunofluorescence and/or immunoblotting blotting: anti-DNMT1 rabbit polyclonal antibody (5032; Cell Signaling; IF, 1:200, WB, 1:1000), anti-DNMT3A rabbit polyclonal antibody (2160; Cell Signaling; IF, 1:200, WB, 1:1000), anti-DNMT3A mouse monoclonal antibody (gift from Guoliang Xu in Shanghai school of biological sciences), anti-DNMT3B mouse monoclonal antibody (IMG-184A, Imgenex; IF, 1:100, WB, 1:1000), anti-DNMT3L rabbit polyclonal antibody (12309, Cell Signaling; IF, 1:100, WB, 1:1000), anti-5-Methylcytosine (5-mC) mouse monoclonal antibody (39649, Active Motif; IF, 1:200), anti-H2A X mouse monoclonal antibody (2372-1; Epitomics; IF, 1:200), anti-5-hydroxymethylcytosine (5-hmC) rabbit polyclonal antibody (39770; Active Motif; IF, 1:100), anti-SIN3A rabbit

monoclonal antibody (7691, Cell Signaling; IF, 1:100, WB, 1:1000). All the other antibodies used in this paper have been described previously (Ma et al., 2012).

Supplemental References

Stein, P., Rozhkov, N.V., Li, F., Cárdenas, F.L., Davydenko, O., Vandivier, L.E., Gregory, B.D., Hannon, G.J., and Schultz, R.M. (2015). Essential Role for endogenous siRNAs during meiosis in mouse oocytes. *PLoS Genet.* 19;11(2):e1005013.