Figure S1

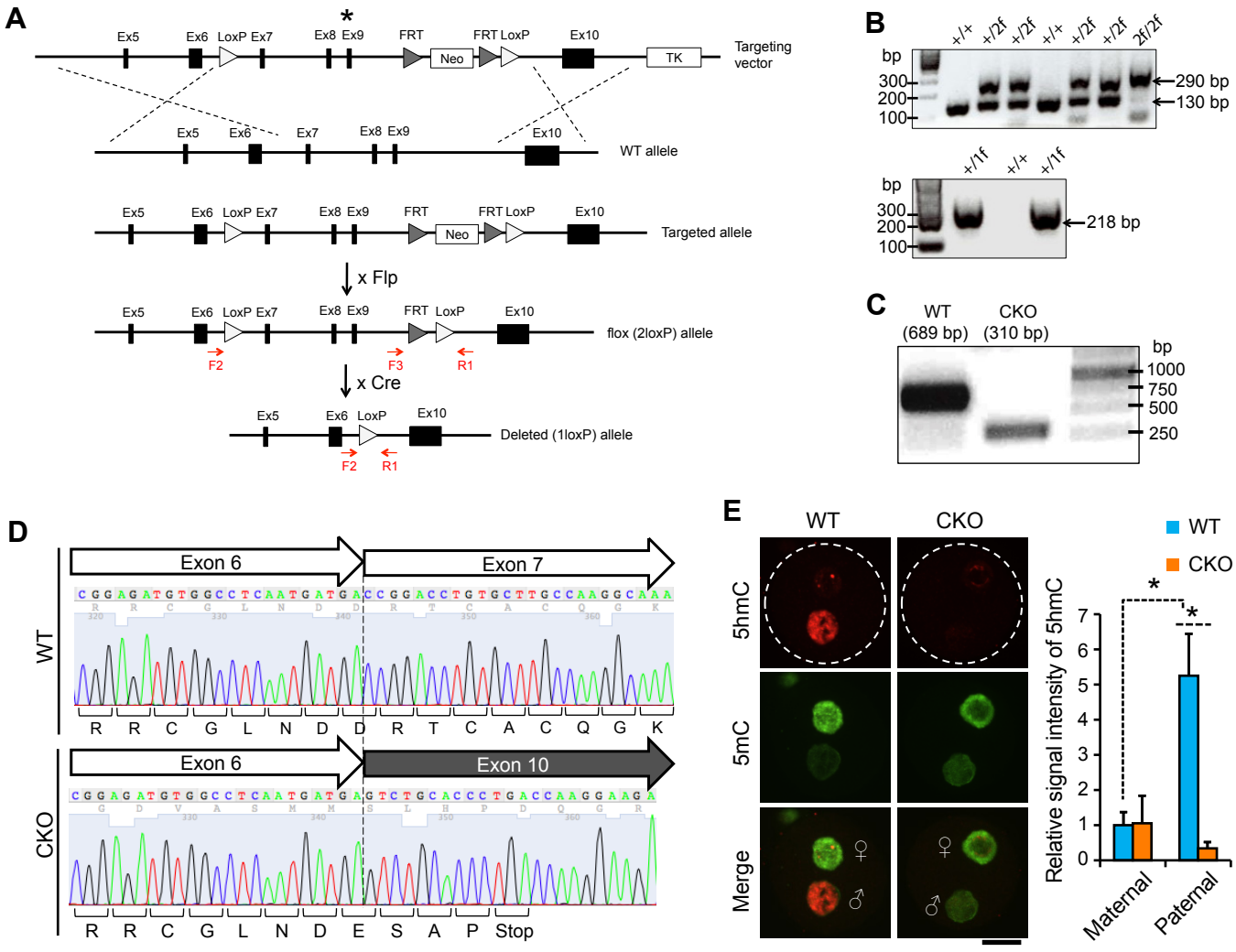

Figure S1. Targeted disruption of Tet3 gene

(A) Strategy for targeted disruption of the Tet3 gene. Black boxes indicate the coding exons of Tet3. The asterisk above exon 9 indicates the catalytic domain of Tet3 that has the iron-binding motif essential for its enzymatic activity. Primers used for mouse genotyping are indicated by red arrows.

(B) Representative images of genotyping of mouse tail DNA. The primer set of F3 and R1 was used to detect WT (130 bp) and 2flox (290 bp) alleles. The primer set of F2 and R1 was used to detect 1flox allele (218 bp).

(C and D) Confirmation of exon 7-9 deletion in CKO oocytes. After cDNA preparation by reverse transcription of total RNA from WT or CKO oocytes, PCR amplification using

primers amplifying exons 6 and 10 were performed (C). The PCR products were then read by Sanger sequencing, which confirmed that exons 7-9 are indeed deleted in CKO oocytes (D). The amino acids translated from the mRNAs are shown. Note that Tet3 protein is truncated in CKO oocytes.

(E) Representative images of zygotes stained with anti-5hmC (red) and anti-5mC (green) antibodies at 8 hours after fertilization. Scale bar, 20 μm. The graph at right represents relative signal intensity of 5hmC. The value of the maternal pronucleus in WT zygotes was set as 1.0. A total of 23 and 18 of WT and CKO zygotes, respectively, were examined. *P<0.01 by Student's t-test. Error bars, s.d.

Related to Figure 1

Figure S2

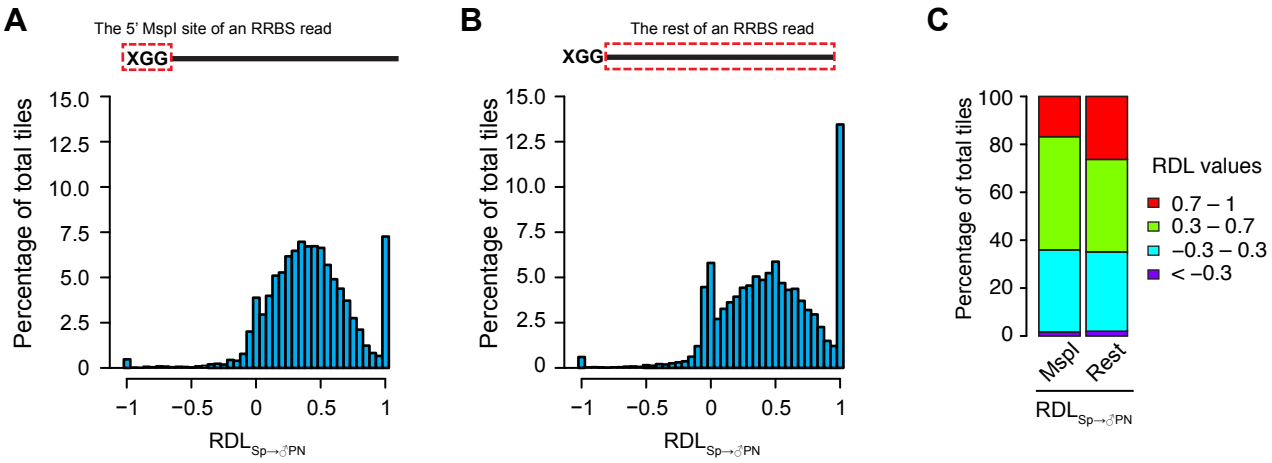

Figure S2. 5fC and 5caC partially contribute to the observed active paternal DNA demethylation.

(A and B) Histograms of $RDL_{Sp\rightarrow♂PN}$ values in WT zygotes using DNA methylation information separately extracted from the MspI site at the 5'-end of RRBS reads (A) or from the rest of the reads (B). Only 100-bp tiles that are methylated in sperm (ML_{Sperm}≥20%) were analyzed. Since the 5' MspI site of an RRBS read is depleted of 5fC/5caC, the decreased percentage of fully demethylated tiles in (A) compared with (B) suggests the contribution of 5fC/5caC in the observed active DNA demethylation. $RDL_{Sp\rightarrow♂PN}$ is defined as $[(ML_{Sp} - ML_{♂PN})/ ML_{Sp}]$. RDL values less than -1 were set to -1.

(C) Distribution of $RDL_{Sp\rightarrow♂PN}$ values in (A) and (B). The RDL values are divided into four groups marked by red (0.7–1), green (0.3–0.7), blue (-0.3–0.3), and purple (<-0.3).

See also Figure 2

Figure S3

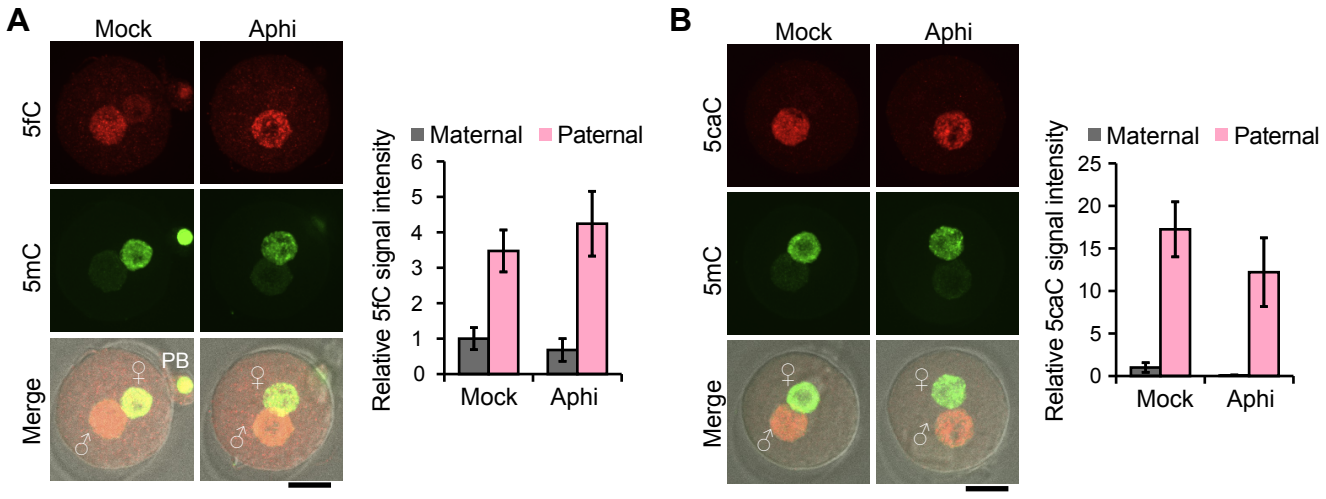

Figure S3. Inhibition of DNA replication does not affect Tet3-mediated 5mC oxidation.

(A and B) Representative images of zygotes stained with anti-5fC (A) or -5caC (B) and anti-5mC antibodies are shown. Zygotes were incubated in the media with or without Aphidicolin. Scale bar, 20 μm. The graphs on the right indicate relative signal intensity of 5fC or 5caC. The value of the maternal pronucleus in Mock zygotes was set as 1.0. For 5fC staining, both Mock and Aphi groups used a total of 19 zygotes. For 5caC staining, Mock and Aphi groups used a total of 19 and 16 zygotes, respectively. Error bars, s.d.

Related to Figure 3

Figure S4

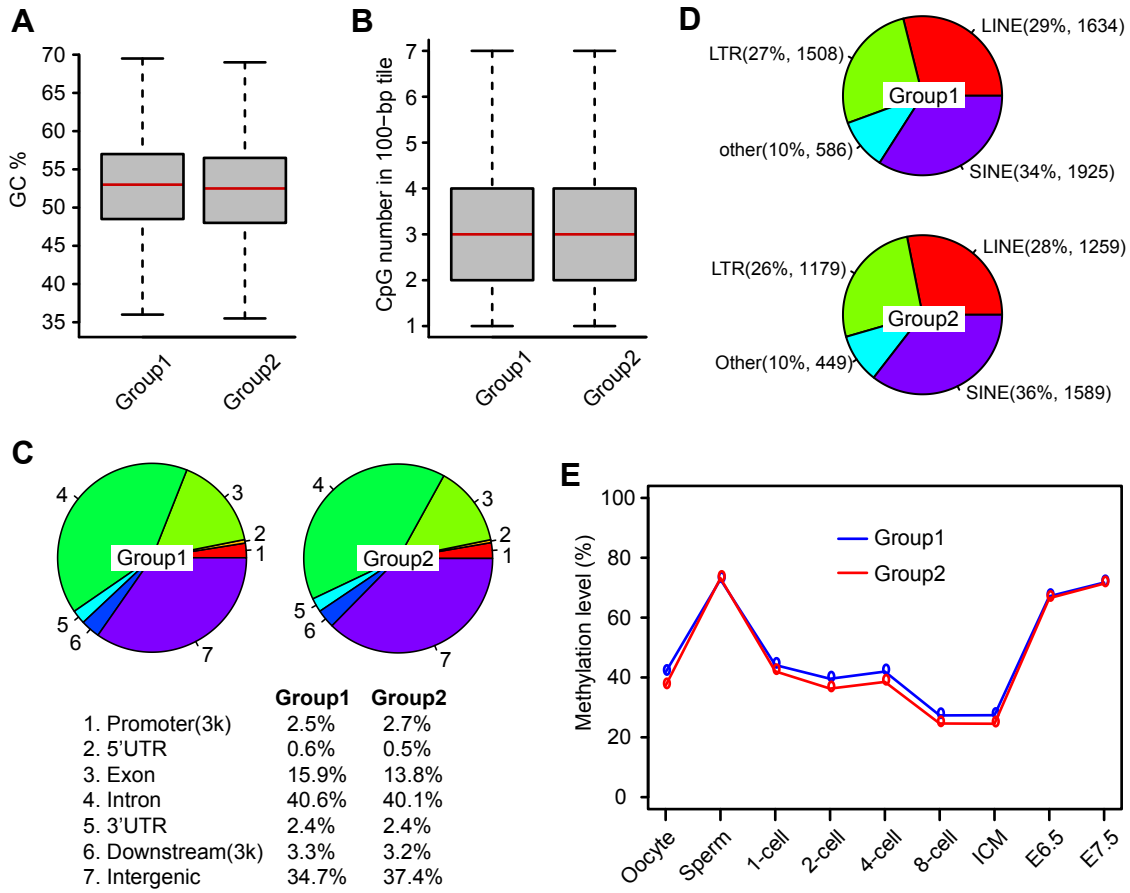

Figure S4. Group1 and Group2 tiles share similar genomic features and dynamic DNA methylation changes during preimplantation development.

(A and B) Boxplot of GC% (A) and the number of CpG dinucleotides (B) in Group1 and Group2 tiles. No significant difference was found. Red line represents the median, boxes and whiskers represent for the 25th and 75th, and 2.5th and 97.5th percentiles, respectively.

(C) Pie charts showing similar distribution of Group1 and Group2 tiles across the whole genome.

(D) Percentage of Group1 and Group 2 tiles overlapping with various types of repeats. In parentheses are the number of tiles overlapping with the indicated repeat and its percentage in all repeat-overlapping tiles. "Other" stands for all other repeats in the RepeatMasker annotation.

(E) Dynamic methylation levels of Group 1 and Group 2 tiles during embryonic development. Related to Figure 4

Table S1. Summary of RRBS libraries, Related to Figure 1,3 and 6

Samples		Total sequencing reads	Mapped reads	Percentage of mapped reads (%)	1x covered CpGs	10x covered CpGs	Bisulfite conversion rate (%)*
Sperm	rep1	29,747,134	18,765,110	63.1	2,254,893	1,328,659	99.8
	rep2	29,180,210	18,485,981	63.4	2,258,808	1,334,837	99.8
WT-Pat	rep1	51,105,126	13,236,983	25.9	1,007,517	815,257	99.4
	rep2	41,712,954	22,688,646	54.4	1,590,377	1,266,998	99.5
CKO-Pat	rep1	44,701,774	21,008,689	47.0	1,659,377	1,259,350	99.5
	rep2	40,313,345	24,819,865	61.6	1,606,509	1,278,377	99.6
WT-Pat-Aphi	rep1	42,227,313	7,534,456	17.8	1,111,922	735,957	99.3
	rep2	29,291,984	17,017,265	58.1	1,047,183	870,950	99.6
CKO-Pat-Aphi	rep1	29,963,268	17,573,136	58.6	1,134,193	922,740	99.4
	rep2	29,963,837	17,204,673	57.4	1,209,495	980,802	99.6
Oocyte	rep1	29,943,617	15,450,790	51.6	1,724,374	1,019,638	97.3
	rep2	28,928,937	17,025,969	58.9	1,679,282	1,053,136	97.3
WT-Mat	rep1	40,854,440	24,055,390	58.9	1,523,143	1,219,347	98.4
	rep2	34,935,651	21,398,069	61.2	1,697,489	1,285,275	98.4
CKO-Mat	rep1	37,996,767	23,255,783	61.2	1,675,651	1,296,135	98.4
	rep2	41,753,044	25,761,755	61.7	1,624,804	1,296,411	98.6
WT-Mat-Aphi	rep1	30,244,242	17,671,244	58.4	1,087,055	871,425	97.2
	rep2	28,133,383	11,389,706	40.5	1,130,535	834,662	97.1
CKO-Mat-Aphi	rep1	28,299,439	16,262,537	57.5	1,204,027	949,076	97.4
	rep2	24,598,965	14,822,370	60.3	1,135,355	886,134	96.9

* Bisulfite conversion rates were estimated by non-converted cytosines in non-CpG context. The lower bisulfite conversion rates of maternal DNA compared with paternal DNA are likely due to the high non-CpG methylation in oocytes.

Table S2. Summary of RNA-Seq libraries, Related to Figure 5

Samples		Total sequencing reads	Mapped reads	Uniquely mapped reads	Percentage of mapped reads (%)	Percentage of uniquely mapped reads (%)
2-cell (WT)	rep1	35,456,463	29,722,881	24,018,174	83.8%	67.7%
	rep2	39,869,462	33,571,526	26,986,698	84.2%	67.7%
2-cell (CKO)	rep1	42,909,250	36,562,645	28,994,981	85.2%	67.6%
	rep2	39,387,774	31,958,686	24,814,813	81.1%	63.0%
Blastocyst (WT)	rep1	47,194,520	42,007,710	38,430,485	89.0%	81.4%
	rep2	52,532,715	46,854,549	42,588,042	89.2%	81.1%
Blastocyst (CKO)	rep1	58,912,595	52,718,203	48,106,791	89.5%	81.7%
	rep2	59,554,859	52,711,629	47,838,721	88.5%	80.3%

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Generation of Tet3 conditional knockout mice

Tet3 conditional knockout targeting vector (pWS-TK6-Tet3CKO) was constructed based on a RED recombinase-mediated recombineering system (Wu et al., 2008). Briefly, BAC clone (RP23-331I23) was modified by introducing two LoxP sites into the regions flanking exons 7-9. The Tet3 gene targeting in the embryonic stem cells (129 background) and the establishment of founder mouse lines were performed by the transgenic core facility at the University of North Carolina at Chapel Hill. The procedure was approved by the IACUC at UNC-CH. A selected marker was removed from the genome by crossing with Flp mice. The mice were then crossed with C57BL/6J mice with a Zp3-Cre transgene. The tail tips were used for genotyping flox (2loxP) and deleted (1loxP) alleles. The primers used for genotyping were the following: Tet3-genotype-F2, 5'-GATTAAAGTCATGCTCCACCACC-3'; Tet3-genotype-F3, 5'-TTCTACGGCTGTGTGTATGAC-3'; Tet3-genotype-R1, 5'-CATACTCACTGACACAGAAAGC-3'. The primers F2 and R1 were used for detection of 1 flox allele. The primers F3 and R1 were used for detection of 2 flox allele.

Reverse transcription and Sanger sequencing

cDNAs from GV oocytes were prepared by using SuperScript III CellsDirect cDNA Synthesis Kit (Life technologies). The deleted region of the Tet3 cDNA was amplified with the following primers: Exon 6 (5'-CCGGATTGAGAAGGTCATCTAC-3') and Exon 10 (5'-AGCGATTGTCTTCCTTGGTCAG-3'). The PCR products were gel extracted and subcloned into TOPO-TA Cloning Kit (Life technologies). After extracting the plasmids, the inserted PCR products were read by Sanger sequencing.

RNA-Seq and data analysis

MII oocytes were collected from superovulated CKO (Zp3-Cre, f/-) or WT (f/f) females. The oocytes were transferred into the HTF medium and fertilized in vitro with sperms from adult CAST/EiJ males. Zygotes that had 2 pronuclei were cultured in KSOM (Millipore). Ten of 2-cell embryos and seven of blastocysts at 30 or 96 hours after

fertilization, respectively, were washed with PBS containing 0.03% BSA. They were then transferred into 0.2 ml tubes and stored at -80°C until use.

To prepare RNA-Seq libraries, frozen 2-cell embryos and blastocysts (2 replicates per sample) were directly lysed and used for cDNA synthesis using SMARTer Ultra Low Input RNA cDNA preparation kit (Clontech) following manufacturer's instructions. After amplification, the cDNA samples were fragmented to an average size of 150 bp using a Covaris M220 sonicator (Covaris). Fragmented DNA was subjected to standard Illumina sequencing library preparation using NEBNext Ultra DNA Library Prep Kit for Illumina (New England Biolabs) following manufacturer's instructions. Single-end 50 bp sequencing was performed on a HiSeq 2500 sequencer (Illumina). Sequencing reads were mapped to the mouse genome (mm9) with TopHat v2.0.10 coupled with Bowtie v2.1.0. For coding genes, the mapped reads were subjected to Cufflinks v2.2.0 with the reference annotation (UCSC gene models) for calculation of expression levels and identification of differentially expressed genes. Expression levels of repeats were estimated by calculating the uniquely mapped reads located in each type of repetitive elements followed by normalization to total uniquely mapped reads. Statistical analyses were implemented with R (http://www.r-project.org/).

REFERENCES

Wu, S., Ying, G., Wu, Q., and Capecchi, M.R. (2008). A protocol for constructing gene targeting vectors: generating knockout mice for the cadherin family and beyond. Nature protocols *3*, 1056-1076.