Supplementary Information

Supplementary Tables

Table. S1 Genomic positions of B1 loci and PCR primers used for analysis of their methylation

Table S2 PCR primers used for methylation analysis of autonomous retrotransposons

Table S3 Real-time PCR primers

Table S4 PCR primers used for methylation analysis of regions around TSS

Supplementary Figure Legends

Fig. S1 Kinetics of the bulk of B1 methylation during germ cell development. A. PCR primers (gray arrows) were designed for the internal B1 sequence (Kato et al. 2007). CpG sites are indicated as lollipops. B. Methylation levels at CpG sites 1 and 2 in PGCs, prospermatogonia, spermatogonia, spermatozoa, and liver of male mice and in fully grown oocytes of female mice were determined by the bisulfite method. Because a substantial number of genomic B1 copies contained CG-to-TG mutations, it is impossible to distinguish unmethylated CG and TG sequences in bisulfite PCR products when analyzing the bulk of B1 copies. Our *in silico* analysis revealed that about 45 and 25% of genomic B1 copies that could be amplified with primers harbor mutations at the CpG sites 1 and 2, respectively (2

bars on the far right). Thus, this type of analysis underestimates the methylation level.

Fig. S2 Northern blot analysis of B1 transcripts. A. Total RNAs (2 μg per lane) isolated from the indicated tissues were separated on 5% denaturing PAGE, transferred onto a membrane, and hybridized against a B1 probe, which was generated from a 108-bp PCR fragment (corresponding to positions 9–116 of the B1 consensus sequence) using primer 1 (5′-GTGGTGGCGCACGCCT-3′), primer 2 (5′-TAGCCCTGGCTGTCCTGGAA-3′), and mouse genomic DNA as a template. The mobility of RNA markers (0.1- to 1-kb RNA ladder from Novagen) is indicated on the left, and the approximately 150-bp B1 band is indicated. The RNA bands denoted by open and closed triangles are 7SL RNA (approximately 299 nucleotides) and 4.5S RNA (approximately 95 nucleotides), respectively, both of which show extensive homology to the 5′ region of the B1 sequence. Thus, they served as internal controls. B. Northern blot of total testis RNAs (2 μg) from *Piwil2/Mili* and *Pld6/MitoPLD* KO mice at P7. At this stage, the number of germ cells are largely unaffected by these mutations. Heterozygous litter mates were used as controls.

Fig. S3 B1 methylation levels in wild-type and mutant prospermatogonia. Methylation levels (y axis) at individual B1 loci in prospermatogonia of *Pld6*$^{-/-}$ (A) and *Piwil2*$^{-/-}$ (B) are plotted against their methylation levels in wild-type prospermatogonia (x axis). Dashed lines

show the y=x slope. Prospermatogonia were collected from seminiferous tubules of P0 or P1 mice by cell sorting (EpCAM-positive for wild-type and *Pld6* KO mice; EGFP-positive for *Piwil2* KO mice containing an *Oct4-EGFP* transgene).

Fig. S4 DNA methylation of autonomous retrotransposons. A. Methylation levels of L1 Gf and A monomers, IAP, and MMERVK10C in wild-type spermatogonia, pachytene spermatocytes, round spermatids, spermatozoa, and liver are shown on a gray scale as in Fig. 1C. B. Effects of *Pld6/MitoPLD* KO mutation on retrotransposon methylation. Methylation of retrotransposons in wild-type and MitoPLD KO spermatogonia are shown for each sequenced clone. Open circles, unmethylated CpG sites; closed circles, methylated CpG sites.

Fig.S5 Effects of genomic features on *Dnmt3a* dependency of B1 methylation. Fold reductions in methylation due to *Dnmt3a* KO mutation at individual B1 loci (dark gray circles) are plotted against LINE density (A), G+C contents (B), and gene density (C) of their flanking genomic regions. B1 loci selected from SINE-poor domains (B1_101 to B1_105) are shown as light gray circles. Methylation levels at 3 paternally methylated regions for genomic imprinting (*H19*, *Dlk1/Meg3*, and *Rasgrf1*) in wild-type and *Dnmt3a* KO spermatogonia were examined as described previously (Kato et al. 2007); results are shown

as open squares. Spearman's correlation coefficients (ρ) are shown on the right of each plot.

Fig.S6 SINE and LINE distributions in the mouse genome and nuclei. A. Local SINE (red) and LINE (green) densities in 2.5-Mb sliding windows (overlap of 2 Mb) in mouse chromosome 1 are shown. The chromosome-banding pattern shown at the bottom was obtained from the UCSC genome browser (http://www.genome.ucsc.edu/). SINE and LINE densities were inversely correlated with each other and roughly correlated with chromosomal bands. Although only chromosome 1 is shown, the other chromosomes showed similar tendencies. B. An immunofluorescence image (600× magnification) of E15.5 testis using anti-OCT4 antibody. Green (i.e., OCT4-positive) indicates prospermatogonial nuclei. This section was adjacent to the one shown in Fig. 4. C and D. FISH images (600× magnification) of the frozen sections using SINE (red) and LINE (green) probes. Both somatic and germ (non-adhering) cells are shown. These images and the one shown in Fig. 4 were obtained from different sections, indicating reproducibility.

Fig. S7 Sequences of analyzed B1 loci. B1 loci are categorized according to their methylation status in spermatozoa. Nucleotides different from those in the consensus sequence are highlighted in green. CpG sites are highlighted in gray.

Fig. S8 Methylation levels at B1 loci and features of their neighboring regions.

Methylation levels at individual B1 loci (B1_001 to B1_062) in spermatozoa are plotted against LINE (A), SINE (B), gene (C), and CpG (F) densities as well as G+C contents (D). Methylation levels at genic and intergenic B1 loci are shown in (E).

Fig. S9 Promoter methylation during germ cell development. Methylation levels at regions around TSS in the indicated cells are shown on a gray scale as in Fig. 1C. Methylation levels were determined by the bisulfite PCR method with primers listed in supplementary Table S4. The names of the neighboring B1 loci are shown on the left. Number of CpG sites in PCR amplicons and gene names are shown on the right.