MORC1 represses transposable elements in the mouse male germline

William A. Pastor1,*, Hume Stroud1,*, Kevin Nee1, Wanlu Liu1, Dubravka Pezic2, Sergei Manakov2, Serena A. Lee1, Guillaume Moissiard1, Natasha Zamudio3, Déborah Bourc’his3, Alexei A. Aravin2, Amander T. Clark1,4 & Steven E. Jacobsen1,4,5

The Microrchidia (Morc) family of GHKL ATPases are present in a wide variety of prokaryotic and eukaryotic organisms but are of largely unknown function. Genetic screens in Arabidopsis thaliana have identified Morc genes as important repressors of transposons and other DNA-methylated and silent genes. MORC1-deficient mice were previously found to display male-specific germ cell loss and infertility. Here we show that MORC1 is responsible for transposon repression in the male germline in a pattern that is similar to that observed for germ cells deficient for the DNA methyltransferase homologue DNMT3L. Morc1 mutants show highly localized defects in the establishment of DNA methylation at specific classes of transposons, and this is associated with failed transposon silencing at these sites. Our results identify MORC1 as an important new regulator of the epigenetic landscape of male germ cells during the period of global de novo methylation.
Two Morc genes in *A. thaliana*, AtMorc1 and AtMorc6, were identified in forward genetic screens for novel transcriptional repressors.\(^1,2\) AtMORC1 and AtMORC6 are required for silencing of a variety of transposons and are essential for higher-order chromatin compaction. The single Morc gene in *Caenorhabditis elegans* was also shown to be required for silencing of a repetitive transgene locus.\(^1\) The founding member of the Morc gene family is mammalian Morc1. MORC1 is highly expressed in the blastocyst and male germline but is not expressed in most differentiated cells.\(^2\) Mice deficient for MORC1 are normal, except that homozygous males are infertile with small testes (hence the name microrchidia).\(^4,5\) Male germ cells in the Morc1 mutant do not undergo successful chromosomal pairing during the zygotene stage of meiosis and instead undergo apoptosis, with no germ cells surviving to complete prophase I.

During germ cell development, most DNA methylation is lost between E8.5 and E13.5. Then, between E13.5 and birth (E19), the genome undergoes global de novo methylation.\(^6-8\) Failure to establish DNA methylation at this time causes transposon upregulation and meiotic failure. Indeed, the meiotic block in the Morc1 mutant is similar to that observed for mice that have defects in DNA methylation and transposon repression, including mice deficient for DNA methyltransferases.\(^9-11\) or the pre-meiotic Piwi-interacting RNA (piRNA) pathway.\(^12,13\) Therefore, we hypothesized that MORC1 might be a critical factor for transposon silencing and DNA methylation in the mouse germline. Here we demonstrate that MORC1-deficient male germ cells undergo transposon derepression starting in late embryogenesis and continuing through the onset of meiosis. We also demonstrate that this phenotype is associated with failed locus-specific de novo methylation targeted specifically towards late-methylating transposon sequences.

**Results**

**MORC1 represses transposons in the male germline.** To further characterize MORC1 we used a previously described FVB/N Morc1 mutant (Morc1\(^{tg/tg}\)) mouse strain in which a tyrosinase gene was integrated into the *Morc1* locus.\(^12\) Transgene insertion resulted in loss of exons 2–4, eliminating a large region of the GHKL ATPase domain including residues predicted to be critical for catalysis and ATP binding.\(^14\) Consistent with previous reports, we found that Morc1\(^{tg/tg}\) mice have a spermatogenesis defect with a complete absence of post-meiotic MIWI2, is also active during this period in promoting transposon silencing.

**piRNA biogenesis occurs normally in the Morc1 mutant.** During this period in germline development (E14.5 to birth), germ cells undergo mitotic arrest and global nuclear reprogramming that arise well before the apparent meiotic onset of meiosis. We hypothesized that MORC1 might be a critical factor for transposon repression in the mammalian germline, acting on many different elements and the IAP-Ey class of retrotransposons, which was not affected in *Morc1\(^{tg/tg}\)* or *Dmnt3l\(^{−/−}\)* mutant testes (Fig. 2f–h).

RNA-Seq data showed a broad transposon derepression defect in *Morc1\(^{tg/tg}\)* starting at E16.5 (Fig. 2c–e and Supplementary Data 2). Quantitative RT–PCR analysis of various transposable element classes gave similar results as RNA-Seq data (Supplementary Fig. 4a). In addition, RNA-Seq on sorted *Morc1\(^{tg/tg}\)* and control germ cells at P10.5 resembled the pattern of transposon derepression observed in whole testes (Supplementary Fig. 4a). Heterozygous *Morc1\(^{tg/+}\)* mice showed no marked increase in transposon expression relative to WT *Morc1\(^{+/+}\)* mice, confirming their validity as littermate controls (Supplementary Fig. 4b).

Different transposon classes showed different patterns of derepression in *Morc1\(^{tg/tg}\)*. Some classes (RLTR4, RLTR6, MuRRs and Em) were upregulated during embryogenesis but silenced even in the knockout at later time points (Fig. 2b). Other transposons (MMERVK10C, GLN and some IAP species) were most highly upregulated at postnatal time points (Fig. 2d). ALLs were upregulated both in late embryogenesis and again at P14.5 after the onset of meiosis (Fig. 2e), which was confirmed by immunofluorescence (Fig. 1d and Supplementary Fig. 2c). These fluctuations in transposon upregulation may reflect differences in the inherent transcriptional programmes of certain transposon classes, as well as varied effectiveness of other, partially redundant transposon repression pathways at different times. In the aggregate, however, these results indicate that MORC1 constitutes a new participant in transposon repression in the mammalian germline, acting on many different elements. Notably, MORC1 silences many transposon classes well after it is downregulated, consistent with it acting through an epigenetic mark such as DNA methylation.

**piRNA biogenesis occurs normally in the Morc1 mutant.** During this period in germline development (E14.5 to birth), germ cells undergo mitotic arrest and global nuclear reprogramming that most notably involves genome-wide de novo DNA methylation mediated by the Dnmt3a/Dnmt3L complex.\(^11,18\) The pre-meiotic piRNA pathway, involving the nuclear PIWI protein Miwi2, is also active during this period in promoting transposon silencing. To evaluate whether MORC1 acts on the same transposon classes as Dnmt3L or Miwi2, we performed RNA-Seq on whole testes from *Dnmt3l\(^{−/−}\)* (ref. 10) and *Miwi2\(^{−/−}\)* (ref. 12) mice and their respective controls at P10.5, and compared this with the *Morc1\(^{tg/tg}\)* P10.5 whole testis data set. *Morc1\(^{tg/tg}\)* and *Dnmt3l\(^{−/−}\)* exhibited derepression of an overlapping set of transposons, primarily long terminal repeat (LTR) retrotransposons, while the *Miwi2\(^{−/−}\)* mutant testes had a milder phenotype and showed derepression of specific LINE elements and the IAP-Ey class of retrotransposons, which was not affected in *Morc1\(^{tg/tg}\)* or *Dmnt3l\(^{−/−}\)* mutant testes (Fig. 2f–h).
The lack of overlap between Morc1 and Miwi2 predicts that piRNAs would be unperturbed in Morc1tg/tg mice. To test this, we performed small RNA sequencing of the testis at E16.5 to examine piRNA production. Our data revealed that the ratio of piRNA/microRNA and the generation of antisense piRNAs were unaltered in Morc1tg/tg (Table 1), indicating that the piRNA pathway remains largely intact in Morc1tg/tg testis at E16.5, and that transposon derepression in Morc1tg/tg is most likely to be independent of the piRNA pathway. In fact, at P10.5 we observed an increase in the fraction of piRNAs derived from LTR retrotransposons, especially of the IAP family, in the MORC1-deficient testis (Supplementary Fig. 5a–c), similar to that observed in Dnmt3l−/−/− mice. These LTR transposon-derived piRNAs corresponded to primary sense piRNAs (Supplementary Fig. 5d,e), suggesting that they are probably more abundant simply because the underlying mRNA species are derepressed in Morc1tg/tg, and some fraction are converted to piRNAs (Supplementary Fig. 5f). Hence, our results indicate that MORC1 acts in a transposon-silencing pathway independent of piRNA production.

Figure 1 | MORC1 is a nuclear protein essential for transposon repression. (a) Domain structure of Morc1 gene and disruption in Morc1f/f allele. Deleted residues predicted to be critical for catalytic activity or ATP binding are denoted. (b) Detection of MORC1 by immunofluorescence (IF) in E16.5 testes. MORC1 is present as a germ cell-specific nuclear protein in the Morc1f/f (het) control but is absent from the Morc1f/f (knockout (KO)). Aberrant expression of IAP GAG (c) and LINE ORF1p (d) in Morc1f/f as detected by IF at P14.5. Note that IAP is overexpressed in most germ cells, whereas LINE is primarily present in the more differentiated cells deeper into the tubule. Scale bars, 20 μm (b–d).
Hypomethylation of transposable elements in \( \text{Morc}^{1\text{tg/tg}} \).

Because of the resemblance between transposons derepressed in \( \text{Morc}^{1\text{tg/tg}} \) and \( \text{Dnmt3l}^{-/-} \) testes (Fig. 2f,g), we sought to examine whether \( \text{Morc1} \) might affect global DNA methylation levels. To address this, we performed whole genome bisulfite sequencing at E16.5, P2.5 and P10.5 on sorted \( \text{Morc1}^{1\text{tg/tg}} \) and control germ cells isolated as above. At E16.5, the germline is undergoing de novo DNA methylation and by P2.5 de novo methylation is largely complete. Between roughly P2.5 and P10.5, germline cells re-enter the cell cycle and either initiate the first wave of spermatogenesis to generate meiotic cells or localize to the basement membrane and generate the long-term

**Table 1** \( \text{piRNA} \) abundance and characteristics in E16.5 \( \text{Morc}^{1\text{het}} \) and \( \text{Morc}^{1\text{KO}} \) testes.

| \( \text{Morc1 het} \) | \( \text{Morc1 KO} \) |
|---------------------|---------------------|
| \( \text{Putative piRNA/miRNA} \) | 0.55 | 0.59 |
| \( \text{Sense/antisense} \) | 1.34 | 1.34 |
| \( \text{Primary/secondary} \) | 4.43 | 3.91 |

\( \text{miRNA, micro RNA; piRNA, Piwi-interacting RNA; smRNA, small RNA.} \) Ratios of putative piRNA/miRNA, sense piRNA/antisense piRNA and primary piRNA/secondary piRNA populations are indicated for smRNA obtained from pooled E16.5 \( \text{Morc}^{1\text{het}} \) and \( \text{Morc}^{1\text{KO}} \) testes. No substantial defect in \( \text{piRNA} \) biogenesis is observed in \( \text{Morc}^{1\text{KO}} \)-deficient testis.

**Figure 2** \( \text{Morc}^{1\text{tg/}} \) shows transposon upregulation resembling \( \text{Dnmt3l}^{-/-} \). (a) Expression of \( \text{Morc1} \) mRNA over development in \( \text{Morc}^{1\text{het}} \), as measured by RNA-seq. (b–e) Overexpression of transposon species over the course of mammalian development, represented as a ratio of expression in the \( \text{Morc}^{1\text{het}} \) and \( \text{Morc}^{1\text{KO}} \) control. Some LTR transposons show upregulation selectively in late embryogenesis (b), while others are overexpressed postnatally (c,d), and LINE elements are overexpressed both during late embryogenesis and again at the onset of meiosis (e). Overexpression of transposons in \( \text{MORC1} \)- (f), \( \text{DNMT3L} \)- (g) and \( \text{MIWI2} \)- (h) deficient whole testis. For a–e, the dotted line indicates a fold change of one. For a–h, two to four replicates per genotype were analysed; all data are RNA-seq from sorted germ cells or whole testis as indicated. Mean + s.e. plotted.
self-renewing spermatogonial stem cell population. In contrast to Dnmt3l−/− mutant germ cells that show a dramatic global reduction in DNA methylation, we found no change in global levels of methylation at any time point in Morc1tg/tg-sorted germ cells (Fig. 3a). Thus, despite the similar morphological phenotypes and transposon expression defects of Morc1tg/tg and Dnmt3l−/− mice, MORC1 does not act by controlling de novo or maintenance methylation at a genome-wide level.

In mammals, DNA methylation is very dynamic and promoter DNA methylation frequently correlates with gene repression. To determine whether there may be localized defects in DNA methylation in Morc1tg/tg, and whether these are associated with derepressed transposons identified by RNA-Seq, we calculated statistically significant differentially methylated regions (DMRs) in the Morc1tg/tg germ cells relative to the Morc1−/− control. At E16.5, we found very few DMRs (Fig. 3b). However, at P2.5 we identified 6,309 hypomethylated regions (Supplementary Data 3) but only 145 hypermethylated regions (Supplementary Data 4), indicating that Morc1tg/tg germ cells have locus and stage-specific DNA methylation defects (Fig. 3b). In addition, the overwhelming majority of regions identified as hypomethylated at P2.5 remain hypomethylated at P10.5 (Fig. 3c) and only a few regions lost methylation between P2.5 and P10.5 (Fig. 3d).

The hypomethylated DMRs in Morc1tg/tg germ cells were highly enriched for LINE and LTR transposons rather than protein-coding genes compared with control regions (see Supplementary Methods), consistent with the transposon expression defects observed in Morc1tg/tg germ cells (Fig. 4a). Indeed, 93.9% of hypomethylated DMRs contained an LTR or LINE, compared with 40.6% of control DMRs. The hypomethylated DMRs were strongly concentrated in the categories of transposons that showed evidence of derepression (Fig. 4b–d) during some stage of development before meiosis.

A partial exception to this trend were IAP elements. Hypomethylated DMRs were strongly enriched for IAP elements and corresponding LTRs (Supplementary Fig. 6a), but there was a poor correspondence between the extent to which a subcategory of IAPs was upregulated and the frequency of overlap with DMRs (Fig. 2d and Supplementary Fig. 6b). This is probably because certain highly similar repetitive elements such as LTR1 give very few uniquely mapping reads and are therefore missing from the data set. To overcome this, we also mapped the BS-seq data to RepBase consensus sequences for relevant transposons. We confirmed hypomethylation of the upregulated IAPLTR1 class (Supplementary Fig. 6c). Mapping to repeat consensus sequences also confirmed hypomethylation of LINE and LTR classes, which frequently overlap with DMRs (Fig. 4c–e).

Only 20 protein-coding genes contained an annotated transcription start site (TSS) within 1 kb of a hypomethylated DMR (Supplementary Data 5) and only 3 contained a TSS within a DMR. Interestingly, all three of these genes (Nebulin, Tmc2 and Cdkl4) contain an RLTR10A transposable element immediately upstream of the TSS and all three genes showed a statistically significant increase in expression (Supplementary Fig. 7 and Supplementary Data 5). Thus, at a very few loci, MORC1 regulates genic expression, probably as a byproduct of its transposon repression activity in the local neighbourhood.
Considering MORC1’s role as local modulator of DNA methylation, we examined changes in methylation in the three well-characterized paternally methylated imprinted loci. Methylation occurred normally at two of the three loci (H19 and Dlk1-Gtl2), but the imprinting control region of Rasgrf1 showed increased transcription and hypomethylation in the Morc1 mutant (Supplementary Fig. 8). Interestingly, this is a transposon-rich area, which has previously been demonstrated as a target of the piRNA pathway (see Discussion below).

Figure 4 | Hypomethylated regions in Morc1tg/tg correspond to upregulated transposon classes. (a) The overlap of hypomethylated DMRs and control regions (randomly selected regions whose methylation is unaffected by loss of MORC1) with genes and transposon classes is indicated. A DMR or control is counted as overlapping with a feature if there was at least one basepair overlap. (b) Overlap of hypomethylated DMRs with transposon classes upregulated in Morc1tg/tg. Metaplot of methylation over LINE (c) and LTR (d) retrotransposons. BS-seq data were mapped to annotated RepBase consensus sequences for each transposon class and methylation is plotted at each CG in the annotated RepBase consensus sequence relative to the consensus sequence. The first 50 bases from each element, which often have low read coverage, are omitted. As it was usually not possible to determine the orientation of LTR-derived reads relative to LTR transposons, the average methylation level for the relevant LTR species is shown. (e) Global hypomethylation of LTR species corresponding to upregulated transposon classes. Again, BS-seq data were mapped to RepBase consensus sequence for these LTRs.
Of the very few hypermethylated loci observed in the Morc1 mutant, most were not conserved across time points and are probably a consequence of biological or statistical noise. However, 15 hypermethylated DMRs were reproducible between P2.5 and P10.5. Nine of these 15 were embedded in 2 transcripts upregulated in Morc1tg/C0; 6 DMRs contained within the body of the Ciklh gene described above and 3 DMRs in an unannotated transcript probably originating from a hypomethylated IAPLTR1 element (Supplementary Fig. 9). These are probably examples of transcriptional run-through from a nearby promoter causing methylation of a locus, a phenomenon that has been described for some imprinted loci25.

DMRs are sites of transposon transcriptional initiation. The highly localized effect of MORC1 on the germline epigenome suggests that MORC1 may function at the transcriptional start sites of transposons to facilitate their silencing and methylation. In support of this, we discovered that hypomethylation in Morc1tg/C0 mutant germ cells was concentrated at the 5′ ends of LINE elements coincident with the location of transcriptional initiation (Fig. 4c)26. Furthermore, LTR transposons, which are typically flanked by LTRs that serve promoter and enhancer functions27, showed hypomethylation on both ends in Morc1tg/C0 (Fig. 4d), and the LTRs themselves are heavily hypomethylated (Fig. 4d,e).

We also noted that hypomethylated DMRs in Morc1tg/C0 germ cells were late targets for de novo methylation during the course of epigenetic reprogramming, since in control Morc1tg/+ cells these genomic regions were also hypomethylated relative to the genome average at E16.5 (Fig. 3c,d and Supplementary Fig. 10). This suggests that these loci are somewhat resistant to de novo methylation. Consistent with this possibility, we also discovered that these Morc1 affected genomic regions have increased H3K4me3 relative to control regions of the genome in early embryogenesis and has an incomplete infertility defect42,43. Thus, the phenotype of transposon derepression and a block in meiosis prophase I superficially resembles the phenotype observed in mice deficient for proteins involved in the pre-pachytene piRNA pathway, including Mili31,32, Miwi2 (ref. 12), MitoPld33, Mov10l1 (refs 34,35), Mael33,36, Tdrkh38, Tdrd9 (ref. 13) and Mvh39,40. What distinguishes Morc1tg/C0 from these characterized pre-pachytene piRNA mutants, however, is the apparent normal piRNA biogenesis in Morc1tg/C0 (Table 1). We do note similarities in the pattern of hypomethylation in Morc1tg/C0 and Mili−/− mutant germ cells, including the Ragsfl imprinting control region24, as well as many of the same transposon families41. The dissimilarity in transposon repression observed in Miwi2−/− and Morc1tg/C0 germ cells (Fig. 2h) suggests that MORC1’s role in the nucleus is independent from the nuclear piRNA pathway mediated by Miwi2. It is possible that MORC1 participates downstream of the nuclear piRNA pathway during embryogenesis and has a separate, piRNA-independent silencing role during the postnatal stages. This could cause Morc1tg/C0 to have a broader transposon derepression phenotype than Miwi2−/−. Alternatively, there may exist a MILL-dependent, MIWI2-independent mechanism for promoting methylation of target loci.

TEx19.1 has also been implicated in transposon repression in the male germline and has no known link to the piRNA pathway42. However, TEx19.1 is cytoplasmic42,43, shows dysregulation only of MMERVK10C elements42,44, and TEx19.1−/− has an incomplete infertility defect42,43. Thus, the Morc1tg/C0 and TEx19.1−/− defects are fairly dissimilar and there is no evidence that they participate in the same pathway.
Figure 5 | Hypomethylated regions in Morc1tg/tg correspond to TSS of transposons active in late embryogenesis. (a) H3K4me3 abundance at E16.5 is calculated within regions identified as hypomethylated DMRs (Hypo DMR) in P2.5 Morc1tg/tg germ cells. (b) Average distributions of uniquely mapping E16.5 RNA-Seq reads (left) and P10.5 RNA-seq reads (right) from individual replicates are plotted over regions identified as hypomethylated at P2.5. (c) Pooled E16.5 ATAC-seq reads are plotted relative to methylation distribution at two loci with hypomethylated DMRs. Each CG is represented as by a bar, with the height of the bar indicating the frequency with which the CG is methylated. A dot at a position indicates no methylation. At least one read must map to the CG for a bar to appear. (d) ATAC-seq reads from individual replicates at E16.5 (left) and P10.5 (right) are plotted relative to DMRs. (e) ATAC-seq read abundance at DMRs and adjacent regions is represented as a boxplot, with each DMR constituting one point in the boxplot. For a–e, DMRs refer to regions hypomethylated in Morc1tg/tg germ cells at P2.5.
Although we have revealed a critical role for MORC1 in transposon silencing, the actual mechanism by which MORC1 promotes DNA methylation in the male germline is unknown. Our study suggests at least three potential routes by which MORC1 represses transposons and facilitates DNA methylation. One possibility is that MORC1 directly silences transcription, perhaps by using its ATPase activity to compact chromatin, thereby repressing H3K4 methylation levels at target sites. This silencing would allow for normal de novo methylation by DNMT3L. A second possibility is that MORC1 could recruit an H3K4 demethylase, which would similarly promote DNA methylation. Either mechanism agrees with our observation that MORC1-hypomethylated DMRs originate from loci with increased H3K4me3 at E13.5. A third non-mutually exclusive possibility is that MORC1 directly recruits the DNA methylation machinery to target loci, mediating methylation and silencing.

In conclusion, a robust genome defense system in the male germline is critical to safeguard genome integrity. We have identified a new participant that acts by facilitating DNA methylation of specific repetitive elements classes.

**Methods**

**Mice.** FVBN-Morc0/0 (Tyr1AV) mice (Morc0/) were recovered from cryopreservation at the Jackson Laboratory and maintained by intercrossing brothers and sisters in the FVB/N background. Male Morc0/0 mice were viable but infertile, whereas female Morc1+/0 mice were viable and fertile. For PCR genotyping, the WT allele was detected as a 347-bp band with the following primers: forward: 5'-ATGCAACATTGAGGGAACAC-3' and reverse: 5'-GCAGGAGTTATCTGATGTCA-3'. The mutant allele was detected as a 244-bp band with the following primers: forward: 5'-AGTTAGCGGTATTTAGTGAGGAGG-3' and reverse: 5'-AGAAAGCTGGCTCTAAACA-3'. PCR conditions involved ten cycles of 94°C, 60°C and 72°C, followed by 28 cycles of 94°C, 50°C and 72°C. For sorting germ cells from E16.5–P2.3, Morc1+/0 females were crossed into the Oct4-IRES-Gfp mixed background. For embryonic staging, timed pregnancies were established and the day a vaginal plug was identified was called embryonic day 0.5 (E0.5). For postnatal time points, the day a litter was first observed was referred to a postnatal day 0.5 (P0.5).

All animal experiments were approved by The UCLA Institutional Animal Care and Use Committee, also known as the Chancellor's Animal Research Committee.

**Antibodies.** Murine Morc1 coiled-coil domain (amino acids 788-950), expressed in and purified from bacteria, was provided by Jianmu Du and Dinhaw Patel (Sloan Kettering). Anti-Morc1 antibody was raised in rabbit in collaboration with Rockland Immunocchemicals. Anti-Line Orf1p antibody was provided by Alex Bortvin (Carnegie Institution for Science) and anti-IAP Gaga antibody was provided by Bryan Cullen (Duke).

**Immunofluorescence.** Whole testes were fixed with 4% paraformaldehyde, immobilized in paraffin and sectioned. After removal of paraffin, sections were stained at the following antibody concentrations: anti-LINE Orf1p (1:300), anti-DNA-PKcs (1:200), TRF1 (1:200), and Use Committee, also known as the Chancellor's Animal Research Committee. All extraneous tissue and the tunica were removed and the seminiferous tubules excised and nucleic acids extracted as above. Extracted samples were reverse transcribed with random hexamers as primer (Life Technologies). The samples were digested with RNAse H in accordance with manufacturer’s protocol. RT–PCR was then performed using iQ SYBR Green (Bio-rad) with 750 nM concentration of each primer. The samples was amplified (PCR programme: 95°C 10 min, 50°C 30 s, 72°C 30 s) with detection of PCR product after each elongation step and determination of melting temperature after the completion of PCR. The reaction was performed using an Agilent Technologies Mx3005p qPCR System (Stratagene). Upregulation of transposon transcript in the mutant is estimated using difference of squares with glyceraldehyde-3-phosphate dehydrogenase as a control.

**Embryonic germ cell purification.** Collection of embryonic tests were performed following institutional approval for appropriate care and use of laboratory animals. Pregnant females were euthanized using CO2 and the embryos removed from the womb and stored on a 10-cm dish filled with chilled 1× PBS. Testicles were removed from the embryos, placed in an individual 15-ml Falcon tube with 3 ml of 0.25% Trypsin, with 3 µl of DNase I 1 Unit per 1 µl (Life Technologies). Testes were incubated for 15 min at 37°C. After incubation, the cells were agitated into suspension gently by pipetting. The trypsin was then quenched using 5 ml MEME/ 10% fetal bovine serum (Life Technologies). The cells were centrifuged at 278g for 5 min and resuspended in 500 µl FACS buffer (1 × PBS 1% BSA). 7-Aminoactinomycin D was added at 1:5 dilution (BD Biosciences) and the cells strained through BD FACS tubes (Corning) before analysis. Green fluorescent protein-positive cells were sorted into Buffer RLT (Qiagen) or ATL (Qiagen) for RNA or DNA extraction, respectively.

**Postnatal germ cell purification.** Pups were euthanized using isoFlurane. The testes were removed using tweezers, placed in a 1.5-ml centrifuge tube and chilled on ice. When all testes had been removed, each pair was placed in 1 ml of type IV collagenase (Invitrogen) in a ultra-low-attachment six-well plate (Corning).

All extraneous tissue and the tunica were removed and the seminiferous tubules were teased apart. The samples were then incubated at 37°C for 15 min and centrifuged for 5 min at 287g. Testes were then resuspended in 500 µl of 0.25% Trypsin (Life Technologies) and incubated for 5 min at 37°C. After the incubation period, the testes were agitated gently into suspension by pipetting. Five hundred microlitres of DMEM/10% fetal bovine serum was added and the samples were centrifuged for 5 min at 200g.

For the P2.3 timepoints, green fluorescent protein-positive cells were sorted as with embryonic time points. To sort germ cells at P10.5, the cells were washed with 1 ml FACS buffer and then resuspended in 500 µl FACS buffer. Cells were then incubated with 1:160 EPCAM PE (Biolegend 118205) and 1:250 µl H2-Kq 647 (Biolegend 115106) on ice for 20 min in the dark, then centrifuged 5 min at 200g and resuspended in 500 µl FACS buffer. DAPI was added (1:1,000, Life Technologies) and the cells were strained through BD FACS tubes (Corning) before analysis. SSC60 EpCAM60 H2-Kq647 cells were sorted into Buffer RLT or ATL for RNA or DNA extraction, respectively.

qRT-PCR of Morc1. For embryonic samples, gonads were pooled from approximately five to seven mice per time point. RNA was extracted by the TRIzol method and DNase-treated (Qiagen) before complementary DNA conversion (Superscript III, Life Technologies). Quantitative amplification of cdna was performed in triplicate using SYBR Green quantitation (PCR primers listed below) on a 7900 HT Fast Real Time PCR System (Applied Biosystems).

**RNA preparation.** RNA for was extracted from whole testes or cells using the RNasey Micro Kit (Qiagen 74004). The material was quantified using a Nanodrop instrument and diluted in RNase-free water. For RNA from whole testis or the Qubit RNA High Sensitivity Assay (Life Technologies) for RNA from sorted germ cells. RNA quality for material from whole testis was assessed by gel electrophoresis and visualization of the 28S and 18S rRNA bands.

DNA preparation. DNA for bisulfite sequencing was extracted using the QIAamp DNA Micro Kit (Qiagen) and quantified using the Qubit dsDNA High Sensitivity Kit (Life Technologies).

**qRT-PCR of retrotransposons.** qRT–PCR for retrotransposons was conducted using published primer sets45. One microgram total RNA was treated with DNase I Amplification Grade (Life Technologies) and converted to cDNA using SuperScript II Reverse transcriptase and random hexamers as primer (Life Technologies). The tissues were digested with RNAse H in accordance with manufacturer’s protocol. RT–PCR was then performed using iQ SYBR Green Mastermix (BioRad) with 750 nM concentration of each primer. The samples was amplified (PCR programme: 95°C 10 min, 50°C 30 s, 72°C 30 s) with detection of PCR product after each elongation step and determination of melting temperature after the completion of PCR. The reaction was performed using an Agilent Technologies Mx3005p qPCR System (Stratagene). Upregulation of transposon transcript in the mutant is estimated using difference of squares with glyceraldehyde-3-phosphate dehydrogenase as a control.

RNA-seq library preparation. RNA from whole testes was processed for sequencing by using a TruSeq RNA Sample Preparation Kit v2 (Illumina) with 250 ng–2 µg total RNA as starting material. Mutant and controls were always matched for starting RNA content. RNA from sorted germ cell was processed using the Ovation Human FFPE RNA-seq Multiple kit (Nugen) using custom primers for depletion of murine RNA provided by the manufacturer, using 10 ng of total RNA. Each library was prepared using RNA from one individual mouse.

Small-RNA isolation and library preparation. Total RNA was isolated from embryonic tests using Ribozol. Thirty micrograms of total RNA was loaded on 12% urea-polyacrylamide (PAGE) gel. The 19-30 nt fraction was excised and snapped to liquid nitrogen in 400 µl 0.4 M NaCl. RNA was eluted from the gel overnight at 16 °C while shaking at 1,000 r.p.m, and precipitated with 3 vol absolute ethanol. Pre-adenylated 3’-linker (5’S/RG/TGAATTTCTCGGGT GCCAAGAACTC/3’dC); 5’-DNA adenylation kit, NEB) was ligated to RNA over-night at 4°C using truncated RNA Ligase 2 (NEB). Ligation reactions were loaded onto 10% urea-PAGE, the 45-56 nt fraction was excised and nucleic acids extracted as above. 5’-Linker (5’S/rG/RuUrCrGrArGrArGrUrCrArCrGrCrGrArCrUrC/3’) was ligated to the samples using RNA Ligase 1 (NEB) overnight at 4°C. Ligation reactions were loaded on 10% Urea-PAA gel, 72–83 nt fraction was excised and nucleic acids extracted as above. Extracted samples were reverse transcribed (primer sequence: 5’-GGAGTTCCTTTGGCCACCGACCAG-3’) and library amplified by PCR using standard Illumina primers. Final libraries were excised from the agarose gel and sequenced.
**Bisulfite library preparation.** Libraries were prepared using the Ovation Ultralow Methylation-Seq Library System (NuGEN). Five to 25 ng DNA was used as starting material. Matched mutant and control samples always contained identical quantities of DNA. Unmethylated Lambda phage DNA (NEB) was spiked in at 0.5% input DNA quantity to determine conversion efficiency, which was consistently >98%. Each library was prepared using DNA from one individual mouse.

**ChiP sequencing.** The ChiP sequencing (ChiP-seq) protocol was adapted from published sources. FACs-sorted cells from an individual mouse were diluted to 292 µl resuspended in 1 x 10^6 at room temperature. Formaldehyde (Sigma) was added to a final concentration of 1% and the sample was incubated for 10 min at room temperature with rocking. One molar glycine was then added to yield a final concentration of 0.1 M and the samples were quenched 30 min with rocking. Cells were then spun at 425 g for 10 min at room temperature. The cell pellet was flash frozen.

After thawing, the cells were resuspended in 200 µl lysis buffer (50 mM Tris-Cl pH 8.0, 20 mM EDTA pH 8.0, 1% SDS, 1 x Complete Protease Inhibitor (Roche)) and incubated on ice for 10 min. Samples were then subjected to a 9-min disruption using a Bioruptor on 'High' setting, with 30:30 s off disruption (hence, 4.5 min of disruption in total). Samples were spun at 14,000 g for 10 min, to remove insoluble material. The soluble sample was diluted to 500 µl with lysis buffer (16.7 mM Tris pH 8, 0.01% SDS, 1.1% TritonX-100, 1.2 mM EDTA, 167 mM NaCl) and 10% of material was saved as input. Sample was precleared with 30 µl Protein A Dynabeads (Life Technologies) and preincubated for 1 h. The cleared material was incubated with 1 µl of HIKAme2 antibody (Millipore 04-745) overnight. The samples were incubated with 30 µl Protein A Dynabeads and the precipitated material was recovered with a magnet. The beads were washed 2 x 4 min with Buffer A (50 mM HEPES pH 7.9, 1% Triton X-100, 0.1% Deoxycholate, 1 mM EDTA, 140 mM NaCl), 2 x 4 min with Buffer B (50 mM HEPES pH 7.9, 0.1% SDS, 1% Triton X-100, 0.1% Deoxycholate, 1 mM EDTA, 500 mM NaCl) and 2 x 4 min with 10 mM Tris/1 mM EDTA. Round material was eluted with 100 µl elution buffer (50 mM Tris pH 8.0, 1 mM EDTA, 1% SDS) at 65°C for 10 min and then eluted a second time with 150 µl elution buffer.

The input samples were thawed and diluted with 200 µl buffer. Cross-linking of ChiP and input samples was reversed by incubating 16 h at 65°C. Samples were then treated with 1.5 µl of 10 mg/ml RNaseA (PureLink RNAase A, Invitrogen 12091-021) for 30 min at 37°C. One hundred micrograms of Proteinase K was then added and the samples treated for 2 h at 56°C. The samples were then purified using a Qiagen MinElute kit.

**ATAC-seq library construction.** Libraries were generated using a method adapted from published protocol. Briefly, FACs-collected cells from individual mice were spun at 500 x g for 5 min at 4°C. Cells were resuspended in 50 µl lysis buffer (10 mM Tris pH 7.4, 10 mM NaCl, 3 mM MgCl2, 0.1% NP40, 1 x Complete Protease Inhibitor (Roche)) and spun at 500 x g for 10 min at 4°C to collect nuclei. The nuclei were resuspended in 2 x Transposase reaction (25 x 10 µg Transposase enzyme) and reacted for 30 min at 37°C on a PCR machine. The material was purified using a Qiagen MinElute protocol, eluting with 14 µl EB (Qiagen).

To amplify ATAC-seq libraries from the treated material, we amplified using the Ad1 primer below and a different Ad2 primer for each sample, which functions within DMRs; (2) WT CG methylation levels are similar as the number of regions per chromosome as DMRs. We defined genes as associated with DMRs when the TSS of an Ensembl transcript was within 1 kb of a DMR.

To align to Repeat consensus sequences, the RepBase consensus sequences for each CG site was calculated. Repeats were obtained from RepeatMasker. Expression values for each repeat family was calculated by adding the scores contained within the repeat body, divided by the total million reads mapped and average length (kb) of repeats within the family.

**RNA-seq analysis.** For all analyses, reads were trimmed to 50 bp and those mapping to ribosomal RNA (GenBank identifiers: 185 NR_003278.2, 28S NR_003279.1, 5S D14832.1, 5.8S K01367.1) by up to three mismatches were discarded.

**Whole-genome bisulfite sequencing.** Reads were split into 500 bp reads before mapping. Reads were mapped to the mm9 genome allowing no mismatches and keeping reads mapped to as many locations in the genome using Bowtie. Each mapping read was assigned a score of 1/n, where n is the number of sites in the genome the read mapped to. Repeats were removed from RepeatMasker. Expression values for each repeat family was calculated by adding the scores contained within the repeat body, divided by the total million reads mapped and average length (kb) of repeats within the family.

**Small RNA sequencing.** Sequence adapters were removed using a custom-designed dynamic programming algorithm that recognizes both exact and inexact matches, and the trimmed reads were aligned to the mm9 genome following a custom suffix array-based procedure. Reads with lengths > 24 nt were considered for piRNA analysis. Based on alignment coordinates, the reads were annotated as derived from exons, introns, transposons and other repeats according to the genome annotation obtained from the UCSC Genome Browser. Reads that had multiple valid alignments were annotated based on ten alignments selected at random, and the majority annotation was assigned as the final annotation. In case of ties, annotation was picked based on a fixed hierarchy principle.

**RESULTS**

**Methylation analysis.** Methylation levels were determined by DESeq47, by using default parameters. For identifying DMRs, the genome was tiled into 500 bp bins and CG methylation levels in knockout and control were compared within each bin. Bins that had a methylation level difference of 50% as well as a false discovery rate < 0.05 calculated by Fisher’s exact test corrected by the Benjamini–Hochberg procedure were selected. DMRs containing at least four cytosines in CG contexts, each covered by at least four reads were retained. Control regions were defined completely randomly, except that control regions have exactly same coverage in CG contexts as the Morc1+/− data within DMRs; (2) WT CG methylation levels are similar as the Morc1+/− data within DMRs (<5%). (3) same number of regions per chromosome as DMRs. We defined genes as associated with DMRs when the TSS of an Ensembl transcript was within 1 kb of a DMR.

**Definition of DMRs**

DMR was defined as a region of the genome in which the CG methylation level is significantly different between two genotypes.

**Identification of DMRs**

DMRs were identified by comparing the CG methylation levels between two genotypes.

**Statistical analysis**

Statistical analysis was performed using statistical software R.

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HiSeq. In keeping with established methodologies\(^30\), reads were aligned to mm9/C0 strands were offset by 24 bp. Duplicated reads were removed with samtools (rmdup function)\(^53\). Previous results show that for Trn transposase, the transposon binds as a dimer and insert two adaptors separated by 9 bp\(^54\). Thus, all reads aligned to the positive strands were offset by +4 bp and all reads aligned to the negative strands were offset by −5 bp.

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Author contributions
W.A.P., K.N., D.P., N.Z. and S.A.L. managed mice, performed dissections and purified DNA and RNA from materials. W.A.P., D.P. and G.M. generated sequencing libraries. H.S., W.L. and S.M. performed bioinformatics analysis. W.A.P. and S.A.L. performed immunofluorescent staining of target tissue. W.A.P, A.T.C. and S.E.J. wrote the manuscript. D.B., A.A.A., A.T.C. and S.E.J. coordinated research.

Additional information
Accession codes: Raw sequencing data, including RNA-seq, whole-genome bisulfite-seq, small RNA-seq, ATAC-seq and ChIP-seq, generated for this study have been deposited in the GEO database under accession number GSE63048.

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Erratum: MORC1 represses transposable elements in the mouse male germline

William A. Pastor, Hume Stroud, Kevin Nee, Wanlu Liu, Dubravka Pezic, Sergei Manakov, Serena A. Lee, Guillaume Moissiard, Natasha Zamudio, Déborah Bourc'his, Alexei A. Aravin, Amander T. Clark & Steven E. Jacobsen

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This Article contains errors in Fig. 2 that were introduced during the production process. The bars of the graph in panel f were inadvertently switched with those of the graph in panel g. The correct version of the figure appears below.
