Maternal DOT1L is dispensable for mouse development

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A battery of chromatin modifying enzymes play essential roles in remodeling the epigenome in the zygote and cleavage stage embryos, when the maternal genome is the sole contributor. Here we identify an exemption. DOT1L methylates lysine 79 in the globular domain of histone H3 (H3K79). Dot1l is an essential gene, as homozygous null mutant mouse embryos exhibit multiple developmental abnormalities and die before 11.5 days of gestation. To test if maternally deposited DOT1L is required for embryo development, we carried out a conditional Dot1l knockout in growing oocytes using the Zona pellucida 3-Cre (Zp3-Cre) transgenic mice. We found that the resulting maternal mutant Dot1lmat−/− offspring displayed normal development and fertility, suggesting that the expression of the paternally inherited copy of Dot1l in the embryo is sufficient to support development. In addition, Dot1l maternal deletion did not affect the parental allele-specific expression of imprinted genes, indicating that DOT1L is not needed for imprint establishment in the oocyte or imprint protection in the zygote. In summary, uniquely and as opposed to other histone methyltransferases and histone marks, maternal DOT1L deposition and H3K79 methylation in the zygote and in the preimplantation stage embryo is dispensable for mouse development.

The earliest stages of embryo development entail the reprogramming of the epigenome in the zygote from the gamete-specific programs of the oocyte and the sperm to totipotency, then zygotic genome activation, and cleavage1–3. Early developmental processes predominantly depend on the supplies provided into the oocyte by the maternal genetic component. Such maternal effects are detected genetically when inactivation of specific genes in the oocyte results in a developmental phenotype4. Because enzymes that catalyze the formation, or removal of covalent modifications of DNA and histone molecules carry out epigenome reprogramming in the early embryo, maternal effect of phenotype is observed for genes that encode chromatin-modifying enzymes5–15.

Disruptor of telomeric silencing 1 (Dot1l) was identified in a yeast genetic screen for suppressors of telomeric silencing16. Overexpression, as well as inactivation of Dot1 suppressed silencing in position effect variegation (PEV) assays using reporter transgenes integrated at specific telomeric and pericentromeric loci15,17–19. Later it was shown using H3K79 methylation-defective mutants and genome-wide expression analysis, that telomeric and pericentromeric silencing is not generally affected by Dot1, as most telomeric and centromeric genes are not subject to H3K79 methylation-dependent natural silencing19. Dot1 catalyzes mono-, di-, and tri-methylation of lysine 79 (H3K79), a residue that is exposed on the surface of the nucleosome in the globular domain of histone 316–19. The H3K79 methyltransferase function is conserved in the orthologous proteins, GRAPPA and Dot1-like (DOT1L) in the fruit fly and mouse, respectively20–22. Loss of function of Dot1l and gpp lead to complete loss of H3K79 methylation, revealing that DOT1L is the sole enzyme in the mouse that methylates H3K7920,22. Dot1l/DOT1L plays roles in transcriptional regulation, cell cycle control, and DNA damage response23–25. The enzymatic steps of Dot1 in generating the H3K79me1, H3K79me2, and H3K79me3 states, are genetically controlled, at least in the yeast24. H3K79me2/me3 is considered an active chromatin mark based on ChiP-chip analysis26. Mixed lineage leukemia gene (MLL) rearrangements (MLL-r) are a major cause of incurable acute lymphoblastic leukemias (ALL). Using a DOT1L inhibitor in MLL-AF4 leukemia cells, H3K79me2/3 was found to be required for maintaining chromatin accessibility, histone acetylation and transcription factor binding, and also for maintaining enhancer-promoter interactions at a subset of H3K79me2/me3 enriched enhancers27. Several lines of evidence suggest, however, that the two marks, H3K79me2 and H3K79me3, may have the opposite readout with regards to gene regulation. Interestingly, gpp mutant flies not only exhibit phenotypes that are consistent with defects in polycomb-type gene repression, but also those with trithorax-type gene activation. These contradictory phenotypes may result from loss of gpp activity in mutants at sites of both active and inactive chromatin domains21. Indeed, whereas H3K79me2 is found in puffs and interbands of Drosophila melanogaster salivary gland polytene chromosomes, where gene activity is observed, H3K79me3 is mainly located in the bands that are inactive. In mouse somatic cells and oocytes, H3K79me2 staining was detected throughout the genome by immunocytochemistry. Whereas H3K79me3 was localized in the pericentromeric heterochromatin regions, H3K79me2 staining was detected throughout the genome by immunocytochemistry. Whereas H3K79me3 was localized in the pericentromeric heterochromatin regions, H3K79me2 staining was detected throughout the genome by immunocytochemistry.
which are devoid of active genes. We showed by immunostaining that the global levels of globular histone marks H3K79me3 and H3K79me2 strongly increased in male but not female mouse fetal germ cells at 15.5 dpc, at the time when epigenome remodeling takes place in the male germ line. We also found that H3K79me3 is localized to DAPI-stained heterochromatin whereas H3K79me2 is localized to DAPI-poor regions in mouse germ cells and somatic cells of the fetal gonad. In addition, we found that H3K79me3 and H3K79me2 generally cluster with repressive and active histone marks, respectively in chromosome-wide ChIP-chip mapping in mouse fibroblasts.

Genomic imprinting allows one parental copy of a gene to be expressed while the second allele is silenced, providing a useful paradigm for understanding epigenetic regulation. We found that H3K79me3 and H3K79me2 occupy reciprocal methylated versus unmethylated parental alleles of differentially methylated regions (DMRs) in the mouse. Maternal genomic imprints at DMRs are established in the growing oocytes. Based on earlier genetic studies, we expect that maternal mutation of Dot1l in the growing oocytes would eliminate the imprinted expression at those genes where the establishment of maternal imprints or the maintenance of maternal or paternal imprints depended on DOT1L.

Whereas Dot1l is not an essential gene in the asexual vegetative life cycle of Saccharomyces cerevisiae, genetic mutations revealed that the orthologous genes are essential in the soma of multicellular organisms such as Drosophila melanogaster, and Caenorhabditis elegans. DOT1L is ubiquitously present in the mouse embryo, suggesting its important function in mouse embryonic development. Deletion of the methyltransferase catalytic domain of DOT1L (exons 5 and 6) by gene targeting resulted in embryonic lethality between 9.5 and 10.5 days post coitum (dpc) due to development abnormalities including stunted growth, defective yolk sac angiogenesis, and dilation of the heart. Another Dot1l knockout model, disrupted the nucleosome binding domain (exon 13) and was embryonic lethal between 10.5 to 13.5 dpc, due to defects in early hematopoiesis. Vessel-remodeling defects were also observed in the extraglandular tissues, which appeared as the consequence of altered hematopoiesis and reduced blood flow in these embryos. In addition to the embryonic phenotypes, tissue specific inactivation of Dot1l revealed that DOT1L is important in several fetal and adult organ systems including erythropoiesis, brain development, and cartilage development. DOT1L plays a role in the development of the cortical plate, by maintaining the progenitor pool, and by affecting the cortical distribution of neurons. Mechanistically, DOT1L activity promotes transcription of genes implicated in asymmetric cell division.

It is not known whether DOT1L has a maternal effect of phenotype. Several lines of evidence predict that DOT1L may be important in the very early stages of embryo development, which depend on maternally supplied DOT1L protein stored in the oocytes. Dot1l transcription and DOT1L protein are present in mouse oocytes and preimplantation embryos, and Dot1l RNA and DOT1L protein levels decrease from the oocyte by the 2-cell stage, remain low at 4-cell stage, and then increase by the blastocyst stage. This pattern suggests that the DOT1L deposition from the oocyte persists during early preimplantation development, and embryonic Dot1l gene activity turns on after the four-cell stage. The Drosophila ortholog, GRAPPA is present in gpp zygotic mutant fly embryos. Interestingly, the severity of the gpp developmental phenotype was enhanced in the fly when the mothers of homozygous mutant males were homozygous for the gpp mutation compared to those from heterozygous mothers. Ooga et al. showed that in the mouse, both H3K79me2 and H3K79me3 decrease soon after fertilization, and the hypomethylated state is maintained in the blastomeres at interphase, except for a transient increase in H3K79me2 at mitosis (M phase). However, H3K79me3 is not detectable throughout preimplantation, even at M phase. H3K79me2 is lost during somatic nuclear transfer into activated oocytes. These results suggested that rapidly eliminating H3K79 methylation after fertilization is important for epigenetic reprogramming of the newly formed oocyte into the totipotent state, which then gives rise to the embryo soma.

Microinjection experiments revealed that (FLAG-tagged) DOT1L is localized in the nucleus at the one-cell and four-cell stages but not at the two-cell stage. This dynamic pattern involves an active export mechanism of DOT1L from the nucleus in 2-cell embryo that requires the C-terminus of DOT1L. DOT1L relocation may be required to regulate the formation of chromocenters (or pericentromeric heterochromatin clusters). Major satellites are found associated with the nucleolar precursor bodies in the one-to two-cell embryos, but they are dissociated from NPB-s and then reassembled in the nucleoplasm after the four-cell stage. Forced expression of the DOT1L caused H3K79 hypermethylation and the formation of chromocenter-like structures at the two-cell stage, and developmental arrest of the embryos at the two-cell stage. These results suggest that the absence of DOT1L, and perhaps the avoidance of chromocenters at the two-cell stage, is essential for early preimplantation development. Similar to the natural events during preimplantation development, inhibition of DOT1L accelerated reprogramming of somatic cells into induced pluripotent stem cells. Reduced H3K79 methylation correlated with the silencing of lineage-specific genes, as well as the upregulation of certain pluripotency genes, such as NANOG and LIN28. This finding is consistent with a role of DOT1L as a barrier to reprogramming.

The question arises, whether any of these processes is essential for embryo development: (1) broad presence of H3K79me2 in the oocyte and in the maternal pronucleus, (2) the elimination of H3K79me2 shortly after fertilization, (3) localization of H3K79me3 at the pericentriolar heterochromatin of germinal vesicle oocytes and metaphase I oocytes (4) reassembly of H3K79me2 to the chromosomes at the M-phase during preimplantation, (5) removal of DOT1L from the nucleus in the process of eliminating chromocenters at the 2-cell stage; (6) a role of DOT1L/H3K79me2 in the newly forming chromocenters at the four-cell stage. We expected to answer some of these questions by a maternal Dot1l knockout experiment. We inactivated Dot1l in the growing oocytes and assessed the developmental potential of the resulting embryos. Surprisingly, we found that maternal contribution of DOT1L in the egg is dispensable from the growing oocyte stage through fertilization and up to the 4-cell stage of mouse embryo development.
Results

Mouse model. We obtained a targeted mouse line from the KOMP depository that allowed testing the maternal effect of Dot1l, as illustrated in Fig. 1A. Flanking exon 2 by loxP sites in the floxed allele allows the conditional inactivation of the Dot1l gene. It was expected that deleting exon 2 using Cre-mediated recombination results in an out-of-frame translated DOT1L protein, a null allele. We identified the floxed (Fig. 1B) and recombined (Fig. 1C) alleles using PCR assays (full gel image displayed in Fig. S1). First we confirmed that the Dot1l+/− mouse line results in embryonic lethality as reported earlier using independently derived Dot1l knockout lines.20,22 We intercrossed Dot1l+/− mice, and dissected the resulting embryos at 9.5, 10.5, and 11.5 days post coitum (dpc). Representative images are shown in Fig. 2A. We found that Dot1l+/− embryos developed normally, but the Dot1l+/− embryos were variably retarded at 9.5 and 10.5 dpc, and were dead and severely retarded at 11.5 dpc (Fig. 2B,C). We did not find any surviving Dot1l−/− pups at birth. This result confirmed the embryonic lethality of the zygotic mutation in the Dot1l mouse line, and verified the suitability of the current mouse line for testing the maternal effect.
Dot1l maternal mutant mice display normal development and fertility. We generated female Dot1l<sup>fl/fl</sup>; TgZp<sup>3- Cre</sup> mice by crossing with the Zp3-Cre transgenic mice<sup>46</sup>. The mouse Zona pellucida 3 (Zp3) gene promoter drives the expression of Cre recombinase in these females, and Cre-excision is specifically targeted to growing oocytes. Zp3-Cre-mediated deletion of Dnmt3a and Dnmt3b occurred in growing oocytes between 3 and 10 days post partum (dpp)<sup>35</sup>. We previously used this strategy to generate Ehmt2mat<sup>−/−</sup> and Setdb1mat<sup>−/−</sup> maternal mutant zygotes<sup>47</sup>. To demonstrate that Cre-recombination inactivated the Dot1l gene in the oocytes we carried out an immunostaining experiment of Dot1l<sup>fl/fl</sup>; TgZp<sup>3- Cre</sup> and control Dot1l<sup>fl/fl</sup> ovaries at 21 dpp (Fig. 3A,B). We counted the oocytes with discernable DAPI DNA staining in the ovary sections of two females in each genotype. Among these, 15/15 Dot1l<sup>−/−</sup> oocytes were positive for H3K79me1/2 staining, and 22/22 Dot1l<sup>−/−</sup> oocytes.

**Figure 2.** Homozygous Dot1l<sup>−/−</sup> mutant embryos die between 9.5 and 10.5 dpc. (A) Representative pictures of Dot1l<sup>+/+</sup>, Dot1l<sup>+/−</sup> and Dot1l<sup>−/−</sup> embryos from a Dot1l<sup>−/−</sup> intercross are shown at different developmental stages, as indicated to the left. (B) The chart displays the distribution of the normal embryos according to the three genotypes, and the retarded embryos among all genotypes at each time point. (C) The chart displays the percentages of each genotype among the retarded embryos at each time point.
Figure 3. Maternal knockout of Dot1l eliminates H3K79me1/2 from Dot1l−/− oocytes. (A) Representative picture of a normal 21-dpp ovary from a Dot1lfl/fl mouse immunostained with an H3K79me1/me2 antibody (red). (B) Representative ovary of a 21-dpp Dot1lfl/fl; TgZp3-Cre female mouse, which carries Dot1l−/− oocytes. (C) Two selected oocytes from the Dot1lfl/fl ovary above (marked by rectangles) are shown stained with an H3K79me1/me2 antibody (red) and counterstained with DNA marker DAPI (blue). (D) Two selected Dot1l−/− oocytes from the Dot1lfl/fl; TgZp3-Cre ovary above are shown stained with an H3K79me1/me2 antibody (red) and counterstained with DNA marker DAPI (blue). The diameter of these oocytes is 66–67 μm. Note the complete absence of H3K79me2 from the Dot1l−/− oocyte nuclei. Granulosa cells are not affected by the oocyte-specific Cre-deletion.
were negative (Fig. 3C,D). This confirms that the Cre-recombination in growing oocytes has eliminated the DOT1L-dependent H3K79me1/2 staining completely by 21 dpp.

To test for maternal effect of phenotype, we crossed Dot1lfl/fl, TgZp3-Cre female mice with J1/1Ms wild type males, and collected embryos at 13.5 dpc. The Dot1lmat–/+ embryos showed normal growth as compared with Dot1lmat–/+ embryos from the control cross using Dot1lfl/fl, TgZp3-Cre mothers (Table 1) and with the control Dot1lfl/fl embryos from Dot1lfl/fl mothers. The heads and the major organs from representative 13.5 dpc Dot1lmat–/+ embryos and control Dot1lfl/fl embryos are shown in Fig. 4. Three Dot1lfl/fl, TgZp3-Cre mothers gave birth to a total of 21 Dot1lmat–/- live pups. All of the Dot1lmat–/- pups were grossly normal in growth and behavior (Table S1).

To detect potential subtle abnormalities caused by maternal DOT1L deficiency, we tested the fertility of the Dot1lmat–/– animals. We intercrossed Dot1lmat–/– females and Dot1lmat–/+ males. Four pairs of these breeders generated a total of 37 live pups from 7 litters, suggesting that the fertility and fecundity of Dot1lmat–/+ mice was not affected. 38% of these pups were Dot1lmat–/–, and 62% were Dot1l+/+, closely matching the Mendelian distribution of 1:2 (Table 2), as would also be expected from a Dot1lmat–/+ intercross (Fig. 2B). The Dot1lmat–/+ and Dot1l+/+ pups appeared grossly normal.

**Dot1l maternal deletion does not enhance the severity of the zygotic deletion.** To test the possibility that maternally deposited DOT1L is only required in the absence of embryo-produced DOT1L, we combined the maternal mutation with the zygotic mutation. We crossed Dot1l+/+, TgZp3-Cre females with Dot1l+/+ males to generate Dot1lmat–/+ and Dot1lmat–/– embryos, which we collected at 9.5 dpc. Five pregnant mothers resulted in a total of 40 embryos. We found that the frequency of retarded Dot1lmat–/– embryos was similar to the frequency of Dot1lmat–/+ embryos generated from the Dot1lmat–/+ intercross (Fig. 5). The frequency of retarded Dot1lmat–/+ and Dot1l+/+ embryos was also similar in the two crosses. These results revealed that there is no extra harm from maternal DOT1L deletion in addition to the zygotic effect.

**Dot1l maternal deletion does not affect the parental allele-specific expression of imprinted genes at 9.5 dpc.** We found earlier a reciprocal marking of the active, and inactive alleles of imprinted genes by the histone marks H3K79me2, and H3K9me3. To test whether maternally deposited DOT1L was important for establishing, or maintaining these marks, we measured the allele-specific transcript levels of a large set of imprinted genes at 9.5 dpc.

Discussion

We report that DOT1L and H3K79 methylation in the egg are dispensable for the first steps of mouse development. This conclusion is based on the findings that the maternal mutant (heterozygous) Dot1lmat–/+ offspring derived from mutant oocytes showed normal growth and fertility, even though no Dot1lmat–/+ homozygous null
Figure 4. *Dot1l*mat−/+, maternal mutant embryos develop normally. (A) Representative pictures depict the head and major organs of a *Dot1l*fl/+ embryo at 13.5 dpc. (B) Representative pictures of a *Dot1l*mat−/+ embryo are shown at 13.5 dpc.
mutant mouse embryo survived beyond 10.5 dpc (Table 1)\textsuperscript{22}. In addition, the lethality phenotype of the maternal-zygotic (homozygous) Dot1l mat–/– mutant embryos was not more severe than that of the zygotic homozygous Dot1l–/– mutant embryos.

The maternal mutation was carried out using the Zp3-Cre transgene\textsuperscript{46}, which is known to effectively inactivate the floxed target in growing oocytes\textsuperscript{14,35}. Cre-excision of the floxed Dot1l alleles in the growing oocytes is expected to have eliminated DOT1L protein and H3K79 methylation from oocytes at subsequent stages, including the germinal vesicle phase and metaphase I oocytes, in the zygote, and the 1-to 4 cell embryo. In the light of our findings, we conclude that the broad presence of H3K79me2 in the oocyte and in the zygote’s maternal pronucleus is dispensable for fertility. The localization of H3K79me3 at the pericentric heterochromatin of germinal vesicle oocytes and metaphase I oocytes is similarly dispensable. Reassembly of H3K79me2 to the chromosomes at the M-phase during preimplantation is not needed for embryo development. DOT1L and H3K79me2 are not required for the newly forming chromocenters at the four-cell stage. Elimination of H3K79me2 shortly after fertilization, and removal of DOT1L from the nucleus in the process of eliminating chromocenters at the 2-cell stage may be essential processes due to the presence of DOT1L during these stages. We cannot exclude the need for these processes in the light of the maternal knockout experiment, which renders them redundant by eliminating DOT1L from the oocyte. Removal of DOT1L from the 2-cell nucleus may, indeed, be necessary to reduce the magnitude of DSB repair and/or enhance totipotency of the 2-cell stage embryo.

Genomic imprinting is mediated by epigenetic modifications that differ on the two parental chromosomes and allow the gene to be expressed from only one parental allele. The methylation imprinting marks at the imprinted DMRs are deposited in the growing oocytes and are maintained in the zygote and later in the maternally inherited chromosome during somatic cell divisions. We deleted Dot1l specifically in growing oocytes to test if DOT1L is needed for maternal imprinting establishment and imprinting maintenance after fertilization. We found that the
YOLK SAC

Dot1l

mat+/(JF1)

Dot1l

mat−/(JF1)

Figure 6. Parental allele-specific expression of the imprinted gene network is undisturbed in the Dot1l maternal mutant embryos. Parental allele-specific transcription was determined for 38 transcripts (marked at the top) in the 9.5 dpc embryo, placenta, and yolk sac using Sequenom allelotyping assays. Dot1lmat+/(JF1) embryos were obtained by crossing Dot1l+/-; TgZp3-Cre females with JF1/Ms (JF1) wild type males. Control Dot1l+/+(JF1) embryos were collected from Dot1l+/+; TgZp3-Cre females crossed with JF1/Ms wild type males. Allelotyping results from duplicate female (F) and male (M) samples are shown from the embryo, placenta, and yolk sac. The color scale to the right shows the percent of each transcript from the maternal allele in the total (maternal + paternal) expression. There was no change in the allelic expression between experimental and control groups.

Material and methods

Mouse breeding and genotyping. All animal experiments were performed according to the National Institutes of Health Guide for the Care and Use of Laboratory animals, with Institutional Care and Use Committee-approved protocols at Van Andel Institute (VAI).

Knockout First mouse line Dot1lfl/fl (KOMP strain 29070) was obtained from KOMP Repository (https://www.komp.org/geneinfo.php?geneid=54455). This mouse was crossed with ROSA26-FLPc mouse B6N.129S4-Sfmbt2f0(+/+) (The Jackson Laboratory JAX# 016226) to remove the LacZ and neo cassettes, resulting in the Dot1l conditional knockout allele (Fig. 1). This line was maintained in a Dot1lfl/fl homozygous breeding scheme.
Female Dot1lfl/fl homozygous mice were crossed with a Zp3-Cre transgenic homozygous male purchased from The Jackson Laboratory (JAX#003651) to generate Dot1lfl/fl; TgZp3-Cre male, then this male further crossed with Dot1lfl/fl females to generate Dot1lfl/fl; TgZp3-Cre mice. Male mice were used as breeders to keep the line. Female Dot1lfl/fl; TgZp3-Cre mice were used as experimental mothers for generating maternal mutant offspring. The second exon of the Dot1l gene was excised in their growing oocytes prior to the completion of the first meiotic division by the Cre recombinase driven by the mouse Zona pellucida 3 (Zp3) gene promoter. Dot1lfl/+; TgZp3Cre or Dot1lfl/fl mice were used as control mothers. All Dot1l mutant mice were maintained in a C57BL/6J genetic background.

To generate heterozygous Dot1lfl/+ mice we crossed Dot1lfl/fl; TgZp3Cre female mice with C57BL/6J wild type males. Dot1lfl/+ mice were gossly normal and fertile as expected.

The three Dot1l genotyping primers (Fig. 1) were: 5′-GTTTGTGCTGTGCTGGACACA-3′; 5′-AGGGA ACAGAAGAGCAGCAGACCTC-3′; 5′-ATGTTTCAGAAAAAGGGTCGAGAT-3′, are shown in Fig. 1. The Cre genotyping primers: 5′-TGCTGTGTTTCAGTGTTGCGGCG-3′ and 5′-TGGCTCTCTCTACACTGCCTGC-3′ resulted a 303 bp PCR product. The embryo sex was determined by Sry genotyping using 5′-ATGGAG GCCCATGTCAAGCG-3′; 5′-TGCCACTCCTGTCAGATTAG-3′ oligos, resulting a 275 bp PCR product.

**Embryo tissue collection.** Female Dot1lfl/fl, TgZp3Cre mice were crossed with males of different genotypes, as specified in the text. The embryo, placenta and yolk sac samples were collected at different developmental stages. Embryos were examined under a Zeiss Discovery 8 dissecting microscope and pictures were taken with ZEISS specified in the text. The embryo, placenta and yolk sac samples were collected at different developmental stages.

**Immunostaining of ovaries.** Ovaries with adjacent tissue were taken out from three weeks old female mice and fixed in 4% paraformaldehyde at 4 °C for overnight, then dehydrated in a graded series of ethanol, and embedded in paraffin. Samples were sectioned at 5-μm thickness and stained with hematoxylin and eosin for histology check. For immunohistochemical analysis, 5-μm sections were deparaffinized and rehydrated in a graded series of ethanol to water, then pretreated by incubating with 1 mg/mL hyaluronidase (Sigma H4272) in PBS for 20 min at room temperature. Antigen retrieval was performed by incubating sections in citrate buffer (10 mM citric acid with 0.05% Tween20 at pH 6.0) heated to 95–100 °C for 40 min. Citrate buffer and samples were then transferred to room temperature and allowed to cool for an additional 60 min. After antigen retrieval, samples were permeabilized with 0.2% TritonX-100 in PBS for 20 min, then blocked with 10% normal goat serum and 1% BSA in PBS for 1 h. Primary antibody (custom preparation of rabbit polyclonal H3K79me1/me2 IgG, Abcam, Cambridge, MA) was diluted 1:500 in PBS and incubated overnight at 4 °C. After washing with PBS, samples were incubated with Alexa 568-conjugated goat anti-rabbit secondary antibody (Invitrogen, Carlsbad, CA) diluted 1:200 in PBS and mounted with Prolong Gold antifade reagent (Invitrogen, P36935) and sealed with coverslip. Fluorescence images were captured for ovaries at 100X magnification and for oocytes at 1000X magnification.

**Allele-specific RNA expression analysis.** Total RNA from each tissue was extracted with RNA Bee (Tel Test), and further cleaned by DNA-freeTM DNA Removal Kit (Ambion) to remove any trace of DNA. The RNA concentration was measured using a Nanodrop spectrophotometer, and 400 ng of total RNA was reverse-transcribed in a 10 μL reaction volume using the SuperScript III random primer synthesis kit (Invitrogen) with random hexamers according to manufacture instruction.

To measure the contribution of each parental allele to total transcript levels we used a multiplex Sequenom (now Agena Bioscience) allelotyping assay as we described earlier31,48. The unextended extend primer (UEP) random hexamers according to manufacture instruction.

Received: 21 May 2020; Accepted: 6 November 2020
Published online: 26 November 2020

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Acknowledgements
We thank Van Andel Institute (VAI) Vivarium for mouse maintenance and husbandry the VAI Pathology Core for tissue embedding and sectioning, and the VAI Microscopy Core for facilitating the microscopy analysis of the ovary sections. We thank Timothy Geddes and Samreen Ahmed (Beaumont Hospital, Detroit) for performing the Sequenom allelotyping assays. We also thank Tie-Bo Zeng for his help with mouse dissection and Nicholas Pierce for his help with mouse genotyping. Funding: This work was supported by VAI.

Author contributions
Conceptualization (P.E.S.); Funding acquisition (P.E.S.); Investigation (J.L., P.E.S.); Methodology (P.E.S.); Supervision (P.E.S.); Validation (J.L., P.E.S.); Visualization (J.L., P.E.S.); Writing-original draft (J.L.); Writing-review and editing (J.L., P.E.S.).

Competing interests
The authors declare no competing interests.

Additional information
Supplementary information is available for this paper at https://doi.org/10.1038/s41598-020-77545-6.

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