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Marcus Curlee December 8, 2020

Hypomorphic maternal LSD1 in mice leads to long-term defects in survival and imprinting

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Abstract

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Oocytes contain proteins which make epigenetic modifications during the maternal-to-zygotic transition, which is the shift from expression of the maternal genome to expression of the zygotic genome. At fertilization, these maternally provided proteins are relied on for the reprogramming of each parental genome, a necessary step for transitioning from the high levels of differentiation in each gamete to a totipotent zygote. LSD1 is an H3K4 demethylase which is present in the female germline and is necessary for successful epigenetic reprogramming. The complete loss of maternal LSD1 is lethal in mice at the 1-2 cell stage, with transcription resembling that of the maternal genome. A partial loss of maternal LSD1 allows mice embryos to survive early development, but their improper reprogramming causes several negative health effects, such as perinatal lethality and defective genomic imprinting. This means that incomplete epigenetic reprogramming can lead to defects which manifest postnatally. In order to further study these long-term effects, our lab developed a new hypomorphic maternal *Lsd1* allele with a mutation in its tower domain. This mutation primarily inhibits LSD1's binding with CoREST, with only minimal impact on LSD1's *in vitro* demethylase activity. Hypomorphic maternal loss of LSD1 increases perinatal lethality and leads to imprinting defects which are maintained throughout development. These results partially phenocopy those of our lab's previous maternal LSD1 partial loss studies, suggesting that LSD1's maternal activity may be CoREST-dependent and that this mouse mutant can serve as a more efficient tool for the in-depth study of phenotypes observed in mice with deficient maternal LSD1. Our results also provide another lens through which to view human mutations in epigenetic enzymes, due to the fact that some phenotypes may be a result of defects which act maternally.

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INTRODUCTION

Introduction to Epigenetics

Epigenetics originated with C. H. Waddington, who coined the term in 1942 as a way to refer to the complex causal mechanisms underlying the relationship between genotype and phenotype (Waddington, 2012). He chose the name partly because of its similarity to the word "epigenesis," the theory that complex tissues develop from undifferentiated origins. During this time, however, the nature of the mechanisms which make up the field of epigenetics were still unknown. Over time, additional information allowed for a more precise definition of "epigenetic trait" to emerge, such as "a stably heritable phenotype resulting from changes in a chromosome without alterations in the DNA sequence" (Berger et al., 2009). In support of this new definition, much continues to be uncovered about the molecular changes that underlie these heritable phenotypes that occur without changes to the DNA sequence.

Chromatin, a complex of DNA and proteins, makes up the foundation on which epigenetic changes occur (Van Steensel, 2011). Nucleosomes are the foundational subunits of chromatin, and their properties can play a major role in controlling gene expression (Cutter and Hayes, 2015). Individual nucleosomes are made up of DNA wrapped around a histone octamer. Epigenetic changes can occur via DNA methylation or through a variety of covalent modifications on the N-terminal tails of histones. For example, these tails can be reversibly modified by methylation, acetylation, phosphorylation, and citrullination (Tessarz and Kouzarides, 2014). Together, these modifications and the proteins that bind to these modifications can affect the way in which DNA is packaged to promote or suppress the expression of nearby genes.

Epigenetic modifications function together with proteins which generally fall into one or more of the following three groups: writers, erasers, and readers. Writers are proteins that are capable of adding epigenetic modifications, and erasers are proteins that are capable of removing epigenetic modifications. Proteins that function as readers can recognize certain epigenetic modifications and mediate their function. They do this by serving as writers or erasers themselves, or by recruiting other proteins to that physically alter the spacing of nucleosomes (Gillette and Hill, 2015). Thus, epigenetic modifications effect transcription by changing the way in which DNA is packaged, either packaging it more tightly, making the genes coded within less accessible for protein activity, or by packaging it more loosely, allowing RNA polymerase and other proteins to more easily transcribe the coded genes (Fomby and Cherlin, 2011).

One epigenetic modification, the methylation of DNA, has a variety of roles. DNA methylation is often found in gene bodies, where it is thought to regulate co-transcriptional splicing and repress intragenic cryptic promoters. A large portion of DNA methylation is also dedicated to repetitive elements, where it is thought to be repressive. In addition, DNA methylation can be found at promoters and other regulatory elements, where it can block transcription by preventing a wide range of transcription factors from properly binding (Greenberg and Bourc'his, 2019).

Histone methylation is another epigenetic mark that can encompass a wide range of possible modifications, producing a variety of effects on cellular activity. While some modifications like H3K9 methylation are generally linked with repression, others such as H3K4 methylation are associated with active transcription (Barski et al., 2007). Histone methylation manipulates transcription levels indirectly, altering chromatin structure or recruiting proteins for further downstream effects, like in the case of H3K36me1 and H3K36me2, which recruit

proteins to remove acetyl groups from nearby histones, eliminating the acetyl groups' transcription-promoting activity (Hyun et al., 2017).

H3K4me as an Active Mark

H3K4me can be found in promoters and throughout the entire open reading frame of active genes, and is generally associated with transcription. Monomethylation, dimethylation, and trimethylation are all present in the open reading frame, with enrichment at different peaks. Monomethylation is highest at the 3' end, dimethylation is highest in the gene body, and trimethylation is highest around the 5' end near the transcription start site (Li et al., 2007). The presence of H3K4me at promoter and coding regions has been verified in the human genome, showing that the basic findings of previous yeast studies of H3K4me carry over to mammalian systems (Liang et al., 2004). Examples of H3K4me outside of these regions also exist, like H3K4me1, which is frequently present at the enhancers of actively transcribed genes (Heintzman et al., 2007).

At first, the relevance of H3K4me to actively transcribed genes seemed to suggest that H3K4me is laid down in order to activate genes. However, further study into the origins of H3K4me on active genes led to a model wherein H3K4me is instead laid down during transcription. This is because of the fact that proteins such as Set-1, which is the H3K4 methyltransferase found in yeast, functions in a complex with RNA polymerase II, which recruits it to active genes where it can then methylate H3K4 (Ng et al., 2003). In this case, H3K4me may serve as a marker of active genes and maintain activation as opposed to instigating *de novo* transcriptional activation.

If the pattern of H3K4me can be maintained through cell division, it could function as a type of epigenetic transcriptional memory of where active gene expression has occurred. However, it would require that epigenetic memory be reprogrammed during processes such as cell differentiation and fertilization in order to allow for cell fate transitions to occur.

LSD1 Removes H3K4me1/2 and Can Erase Transcriptional Memory

In order for the reprogramming of epigenetic memory to occur, H3K4me needs to have the capacity to be removed. LSD1/KDM1A (hereafter referred to as LSD1) was the first protein that was shown to reverse histone methylation. First characterized in 2004, LSD1 was shown to demethylate H3K4me1/2, and this activity represses transcription of the affected genes. This demethylase activity is limited to monomethylated and dimethylated H3K4, with no effect on trimethylated H3K4 (Shi et al., 2004).

Among the strongest pieces of evidence towards LSD1's capacity to reprogram epigenetic memory is the removal of H3K4me1 from enhancer regions during differentiation. For example, in embryonic stem cells (ESCs), open enhancers are marked by H3K4me1 which helps maintain the transcription of genes required to maintain the totipotent state. LSD1 is not required to maintain that ESC state, but rather it is required to remove H3K4me1 during differentiation (Whyte et al., 2012). This suggests that LSD1 is important for cell fate transitions and must remove the memory of the ESC state to enable differentiation.

One of the most major cell fate transitions that occurs every generation is the transition from highly differentiated gametes to a totipotent zygote. Experiments with *C. elegans* showed that mutations in *spr-5*, which is a *C. elegans* ortholog of LSD1, result in progressive sterility

over the course of many generations. This sterility is accompanied by widespread misregulation of spermatogenesis genes and the transgenerational accumulation of H3K4me2 (Katz et al., 2009). These results suggest that H3K4me can function as epigenetic memory, and that H3K4 demethylases are instrumental in resetting epigenetic memory between generations.

LSD1 Function Depends on Complex Composition

Although LSD1 is capable of demethylase activity on its own (Shi et al., 2004), its preference for hypoacetylated substrates means that it often works in a complex with histone deacetylases (HDACs) (Forneris et al., 2006; Shi et al., 2005). These complexes remove expression-promoting acetyl groups from histones while also demethylating active markers on H3K4, creating a coordinated switch in the state of the chromatin from active to repressed.

One example of LSD1 working with these HDAC-containing complexes is through the CoREST complex, in which LSD1 is coupled with HDAC1 and HDAC2. The CoREST-HDAC complex was first characterized in 2001, as a distinct HDAC containing complex which represses transcription by removing histone acetylation (You et al., 2001). CoREST was later found to assist LSD1 in its demethylase activity, while also protecting it from proteasomal degradation (Shi et al., 2005). LSD1 and CoREST were also identified as a requirement for proper cell-lineage differentiation (Wang et al., 2007).

LSD1 also functions similarly as a repressor in the nucleosome remodeling and deacetylase (NuRD) complex. Targets of the NuRD complex include pathways involved with cell signaling, proliferation, and with the epithelial-to-mesenchymal transition (Patel et al., 2018; Wang et al., 2009).

Although LSD1 typically functions as a repressor by way of removing active marks, this is not always the case. In the androgen receptor (AR) complex, LSD1's specificity is altered to remove the repressive mark H3K9me1/2 (Metzger et al., 2005), although it remains unclear if LSD1 truly has this activity *in vivo*. Beyond the limits of even histone methylation as a whole, LSD1 was also shown to demethylate non-histone targets, such as p53, DNMT1, STAT3, and others, demonstrating a wide range of potential LSD1 functions (Amente et al., 2013).

Epigenetic Enzymes Can be Mutated in Humans

Mutations in epigenetic enzymes in mammals are known to cause misregulation of transcription, sometimes with serious deleterious effects. The *Mll* gene is an H3K4 histone methyltransferase which was named after the link between its mutations and mixed linkage leukemia, a severe blood cancer (Slany, 2009). Many of its related H3K4 methyltransferases are also linked to serious disorders in mice. For example, mutations in the *Mll4* gene are linked to reduced H3K4me1 at enhancer regions as well as impaired differentiation in mice (Cao et al., 2018). The *Mll2* gene has not only been linked to transcriptional misregulation and 1-2 cell arrest in mice (Andreu-Vieyra et al., 2010), but also in humans to Kabuki syndrome, a condition which combines intellectual disability, facial malformations, short stature, and other characteristics (Arnaud et al., 2015). Mutations in the *Kdm5c* gene, which codes for an H3K4 demethylase, have a variety of effects on the epigenome, such as increased trimethylation of H3K4, reduced monomethylation of H3K4, and the misregulation of affected genes (Outchkourov et al., 2013). KDM5A, a similar protein which has H3K4me3/me2 histone demethylase activity, is able to inhibit p53 signaling, and its overexpression is associated with high-risk neuroblastomas (Hu et al., 2018).

LSD1 mutations cause intellectual disability and Kabuki-like syndromes in humans, characterized by developmental delays and craniofacial abnormalities. These phenotypes are possibly due to errors in its ability to catalytically function and to bind with transcription factors that are necessary for differentiation (Pilotto et al., 2016). Taken together, the severity of mutations in many different epigenetic modifiers indicate their importance in human development.

Epigenetic Reprogramming at Fertilization and the Maternal-to-Zygotic Transition

Along with their role in other tissues, many epigenetic enzymes are shown to have a particularly important role during the transition from a highly differentiated gamete to a totipotent zygote. Early cloning experiments demonstrate that the egg contains the necessary factors to reprogram a differentiated cell nucleus into a totipotent nucleus. In an experiment done on frogs, researchers produced viable offspring from egg cells with a transplanted somatic nucleus (Gurdon et al., 1958). Later, researchers successfully used transplanted somatic nuclei in cloning experiments on mammals as well (Campbell et al., 1996). Often in these experiments, however, the cloning was not completely efficient. For example, in the experiments by Gurdon et al., embryos derived from their somatic cell nuclear transfer experiments would still inappropriately express genes from the differentiated cell type nucleus that was transplanted. They showed that this inappropriate epigenetic memory was dependent on lysine 4 of histone variant H3.3. This indicates the possible importance of resetting H3K4me in particular at the point of fertilization (Ng. and Gurdon, 2008).

The maternal-to-zygotic transition refers to the shift from expression of the maternal genome to expression of the zygotic genome (Li et al., 2013). In mice, zygotic genome activation takes place largely at the two-cell stage, relying heavily on changes in chromatin structure (Ma et al., 2001). Epigenetic factors, such as histone modifications and DNA methylation undergo necessary changes during the reprogramming necessary in mammals for the maternal-to-zygotic transition. As such, maternal mutations in proteins which enact epigenetic changes can subsequently cause defects during the maternal-to-zygotic transition. Mutations in *Lsd1* (an H3K4 demethylase) and *Brg1* (a chromatin remodeling protein) in mice are both linked to failed zygotic genome activation and arrest within the one-to-two or two-to-four cell stage, and mutations in other chromatin affecting genes such *Mll2* (an H3K4 methyltransferase), *Setdb1* (and H3K9 methyltransferase), and *Ring1* (an H3K27 methyltransferase) are linked to transcriptional misregulation and early developmental arrest (Lee and Katz, 2020). These severe lethal phenotypes indicate the important role of maternally deposited epigenetic regulators in developmental competency post-fertilization.

LSD1 and Epigenetic Reprogramming

Maternally provided LSD1 is necessary for the maternal-to-zygotic transition in mice, playing an essential role in the epigenetic reprogramming necessary for the transition to occur. Without any maternal LSD1 present, embryos arrest at the 1-2 cell stage, proving that embryonic development cannot proceed in its absence (Ancelin et al., 2016; Wasson et al., 2016). In mice, the two-cell stage is the point at which zygotic gene activation (ZGA) becomes the most robust (Schultz, 1993), so lethality at this stage provides evidence for the failure of these embryos to correctly alter transcription. The question remained, however, of whether these effects were

simply due to errors occurring at the oocyte stage being carried over past the point of fertilization. In order to rule out this possibility, further investigation included an experiment wherein wild-type zygotes were treated with a chemical inhibitor of LSD1 activity directly after fertilization. These embryos phenocopied the 1-2 cell arrest phenotype of the previous trials, reinforcing the belief that LSD1 is enabling the maternal-to-zygotic transition due to its functionality post-fertilization, as opposed to its activity at the oocyte stage (Ancelin et al., 2016).

There is also molecular evidence that maternally provided LSD1 is required for the maternal-to-zygotic transition. LSD1-depleted two-cell mouse embryos were more transcriptionally similar to wild-type oocytes than to wild-type embryos, indicating a failure to undergo the maternal-to-zygotic transition (Ancelin et al., 2016; Wasson et al., 2016). Specifically, RNA sequencing data on maternally mutant *Lsd1* embryos revealed an upregulation of genes associated with maternal expression and a downregulation of genes which normally begin getting upregulated as a result of major zygotic gene activation, which occurs at the two-cell stage (Ancelin et al., 2016; Wasson et al., 2016). The same group identified more specific transcriptional problems, such as the *Lsd1* mutant embryos' insufficient silencing of the LINE-1 retrotransposon, which is normally repressed as a part of the maternal-to-zygotic transition (Ancelin et al., 2016). The sum of these transcriptional defects points to the idea that mutant *Lsd1* mouse embryos fail to erase the epigenetic memory associated with oocyte cell transcription.

Introduction to Genomic Imprinting

Genomic imprinting refers to mono-allelic expression depending upon parent of origin (Ferguson-Smith, 2011). DNA methylation and other epigenetic factors consistently repress one specific allele so that for a paternally or maternally expressed gene, all cells experience expression only from the allele inherited from that parent. Imprinted genes are unique as they are activated or inactivated during gamete formation, and do not normally change from this state during development (Reik and Walter, 2001), making them reliable reporters for detecting epigenetic disturbances. The need for each parent to de-methylate or re-methylate the allele inherited from its own opposite-sex parent also creates an opportunity for observing epigenetic malfunctions. Intriguingly, the epigenetic factors that allow for single-allele expression rely on proper reprogramming during the maternal-to-zygotic transition (Li et al., 2013).

Links between histone methylation and DNA methylation are well characterized, granting credence to the notion that perturbations in histone methylation may lead to flaws in genomic imprinting. An example of this connection is DNMT3L, which is one of the proteins relied upon for *de novo* DNA methylation on imprinted genes. This protein interacts with histone H3 while performing its DNA methyltransferase activity, and H3K4 methylation strongly inhibits its function at that site (Ooi et al., 2007). It was also shown that the activity of LSD2, which is an H3K4 histone demethylase, must be present during oogenesis in order for DNA methyltransferases such as DNMT3L and DNMT3A to correctly methylate imprinted alleles (Ciccone et al., 2009). Less direct interactions between histone methylation and DNA methylation also exist. *Lsd1* maternal mutant arrested embryos were shown to overexpress DNMT1, a DNA methyltransferase that normally functions to maintain existing DNA methylation, but is also capable of *de novo* DNA methylation when overexpressed (Vertino et al., 1996; Wasson et al., 2016). These examples provide mechanisms by which defects in histone

methylation may either block the methylation of DNA which is normally methylated or instigate the *de novo* methylation of DNA which is normally left unmethylated.

In mouse embryos with maternally deleted LSD1, RNA sequencing analysis revealed the misregulation of several genes associated with DNA methylation on imprinted genes (Wasson et al., 2016). These include *Tet1*, which converts 5-methylcytosine to 5-hydroxymethylcytosine and plays a role in DNA methylation (Yamaguchi et al., 2013), *Trim28*, which recruits components of the NuRD complex (Czerwińska et al., 2017), and *Dppa3/Stella*, which protects DNA from demethylation (Shin et al., 2017). These findings suggest that improper reprogramming at fertilization may lead to defects in genomic imprinting.

Outstanding Questions

It is known that when LSD1 function is completely lost, mouse embryos arrest at the two-cell stage (Ancelin et al., 2016; Wasson et al., 2016). While this indicates the importance of LSD1's function maternally, a full loss of the maternal protein may not always be what occurs in nature. For example, it was shown that LSD1 protein levels in the oocyte decrease with age in wild-type mice (Shao et al., 2015). If a partial loss of maternal LSD1 protein also occurs in humans during aging, this may result in only a partial loss of LSD1's reprogramming capacity. Our lab seeks to ask: what phenotypes are possible if maternal LSD1's reprogramming function is partially compromised? Would embryos be able to bypass the 1-2 cell arrest caused by complete depletion of maternal LSD1, and would there be any induced defects that persist later in development?

Based on our existing understanding of H3K4 methylation and its ability to interfere with DNA methylation (Ciccone et al., 2009; Ooi et al., 2007; Wasson et al., 2016), it is possible that one of the defects that are detectable in animals that may bypass the 1-2 cell arrest is in genomic imprinting. If there are perturbations, and they are maintained past early development into adulthood, then that would suggest the intriguing possibility that epigenetic defects acquired during early development are heritable across many cell divisions.

MATERIALS AND METHODS

Mouse Husbandry and Genotyping

We used the following four mouse strains*: Zp3-Cre MGI:2176187* (De Vries et al., 2000), *Lsd1*$^{fl/fl}$ *MGI: 3711205* (Wang et al., 2007), *C57BL/6 MGI: 3715241, and Lsd1*^{M448V}. The *Lsd1* forward (F) primer is: GCACCAACACTAAAGAGTATCC. The *Lsd1* reverse (R) primer is: CCACAGAACTTCAAATTACTAAT. The product of a wild-type *Lsd1* allele is 480 base pairs (bp) long. The product of the floxed *Lsd1* allele is 720bp. The product of the deleted Lsd1 allele is 280bp. The Cre forward primer is: GAACCTGATGGACATGTTCAGG. The Cre reverse primer is: AGTGCGTTCGAACGCTAGAGCCTGT. A Cre positive genotype results in a 302bp product and a Cre negative genotype results in 250bp. The M448V forward primer is: CCCAAATGGCATGACATAAA. The M448V reverse primer is: TAAGGCACCAAACCCCTTCT. The M448V allele results in a 386bp product. This allele's point mutation removes a restriction site, and as a result mutants and wild-type mice can be distinguished by incubating their PCR products for 1 hour at 37°C with the HpyAV restriction enzyme. The wild-type allele band sizes are: 72bp, 81bp, 209bp, 24bp. The M448V allele band sizes are: 72bp, 290bp, 24bp. All mouse protocols were approved by the Emory University Institutional Animal Care and Use Committee.

Bisulfite Analysis and Bisulfite-PCR Optimization

DNA was isolated from the heart, brain, and liver of several adult mice from the *Lsd1*$^{+}$, *Lsd1*^{het}, and *Lsd1*^{M448V} crosses. Bisulfite conversion was done on 400ng of DNA through the Zymo EZ DNA Methylation Kit© protocol. The converted DNA samples were then subjected to

PCR amplification in a 15ul reaction. The Zac1 forward primer is: G GGTAGGTAAGTAGTGATAA. The Zac1 reverse primer is: C CTAAAACACCAAAATAACA. Then, TA cloning reactions using a Thermo Fischer TOPO TA cloning kit were set up using 3ul of PCR product from each sample, along with 1ul of salt solution, 1ul of sterile water, and 1ul of TOPO cloning vector. These were allowed 5 minutes to incubate at room temperature. For each TA cloning reaction, 4ul of the incubated mixture was added to a tube of One Shot© *E. coli* cells and then incubated for another 5 minutes on ice. These *E. coli* cells were heat shocked at 42°C for 30 seconds, after which the reactions spent another 5 minutes on ice. 80ul of Xgal was added to pre-warmed LB-Ampicillin plates, in order to serve as a β-galactosidase substrate. 60ul of TA cloning reaction was added to each plate, and these plates incubated overnight at a temperature of 37°C. The next day, each plated had 8-10 white colonies picked and subsequently cultured in 3ml of liquid LB + 150ug Ampicillin, which was left on a shaker overnight at 37°C. Each cell culture that successfully grew *E. coli* was miniprepped through the Qiagen QIAprep kit© protocol. Then, 10ul of each miniprep was digested with EcoRI-HF in a 50ul reaction and each reaction was run on a 1% agarose gel to ensure the success of the protocol. Each undigested miniprep sample was sequencing using the Psomagen sequencing service. Afterwards, each sequence was analyzed using the BiQ Analyzer program (Bock et al., 2005), and a single nucleotide polymorphism between the B6 (A base) and CAST (G base) genetic backgrounds was used in order to distinguish between maternal and paternal alleles.

Immunofluorescence

The heart, lungs, liver, and brain of mice are harvested at postnatal day 0 (p0) and fixed in 4% PFA on ice for varying durations of time. After 2 hours of PBS washes, tissues sit in 30% sucrose solution at 4°C overnight. They are then embedded in OCT compound and cryosectioned at 10µm. Slides are washed with a solution of 10x PBS, 20% Triton-X, and goat serum before adding the primary antibodies: rabbit polyclonal anti-KDM1A (ab17721 1:200), CoREST antibody sc-376567 at 1:200 concentration, and mouse monoclonal anti-GFP (MAC3580). Slides are left in a humidity chamber overnight. The following day, slides are washed 3 times for 20 minutes each in the wash solution at room temperature. Alexa fluor conjugated antibodies are used at 1:500 dilution, then left in a humidity chamber for 1 hour at room temperature. The slides are washed again three times, then mounted and left overnight in a dark chamber before imaging under fluorescence.

Perinatal Lethality

Mouse breeding cages underwent daily observation for the presence of new litters, and when births were discovered, the number of mice born alive were scored. At p1, the litter size in each respective cage was scored again, and the percent lethality was determined by dividing the number of dead mice by the original count of the litter. Mice that died failed to thrive shortly after birth and were often missing visible milk spots. Only litters born from mothers <10 months age were counted in this procedure.

RESULTS

Partial Maternal Loss of LSD1 Causes Long-Term Defects

In order to examine LSD1's maternal role, it was first necessary to verify that LSD1 is present in oocytes. In order to claim that LSD1 plays a role in maternal reprogramming, it must further be verified that it is present in the nucleus, where reprogramming takes place. To test this, former lab members performed immunofluorescence staining for LSD1 on wild-type mouse oocytes (Figure 1A) (Wasson et al., 2016). These experiments showed that LSD1 is present in oocyte nuclei, and that LSD1 is also expressed in the oocyte's surrounding follicle cells.

In previous experiments, two copies of floxed *Lsd1* were combined with a germline-specific *Vasa*-Cre to interrogate phenotypes from a complete maternal loss of LSD1. A complete maternal loss of LSD1 led to embryonic arrest at the 1-2 cell stage (Wasson et al., 2016). However, for reasons that are unclear, a subset of mice instead experienced only a partial loss of LSD1 in the oocyte. When this occurred, embryos were able to bypass the 1-2 cell arrest and make it out to birth (Wasson et al., 2016). These mice born from maternal LSD1 deletion mothers will be referred to as *Lsd1*^{Vasa} M-Z+ (maternal- zygotic+) progeny, and control mice born from Cre negative mothers with floxed *Lsd1* alleles will be referred to as *Lsd1*^{Vasa} M+Z+ progeny.

The first thing that became clear from these *Lsd1*^{Vasa}M-Z+ offspring was that some animals were able to bypass the 1-2 cell arrest seen with a complete maternal loss of LSD1. However, once these animals were born, many of them died shortly after birth. To quantify this lethality phenotype, our lab closely examined *Lsd1*^{Vasa} M-Z+ progeny during the 48 hours following their birth (Wasson et al., 2016). While *Lsd1*^{Vasa} M+Z+ control mice experienced only

a 5% rate of perinatal lethality, *Lsd1*^{Vasa} M-Z+ progeny experienced a 26% rate of perinatal lethality (Figure 1B).

While it was unclear why *Lsd1*^{Vasa} M-Z+ animals were dying, the RNA sequencing data from maternally deficient 2-cell embryos gave some insight in to which gene sets may be misregulated in the partial loss mutants. In particular, several genes associated with imprinting were all misexpressed in arrested embryos (*Tet1, Trim28, Zfp57,* and *Stella*) (Wasson et al., 2016). Additionally, the maintenance DNA methyltransferase *Dnmt1* was overexpressed. When that occurs, *Dnmt1* is shown to have *de novo* methyltransferase activity (Vertino et al., 1996). Collectively, these data indicate that DNA methylation may be perturbed in *Lsd1*^{Vasa} M-Z+ animals that make it out to birth, particularly at imprinted genes. To test this, we used allele-specific bisulfite sequencing on several imprinted candidate genes. Maternal and paternal alleles were identified based on single nucleotide polymorphisms between the B6 and CAST genetic backgrounds, with B6/CAST hybrids used as controls. At the *Zac1* gene, we observed an increase of DNA methylation on the normally unmethylated paternal allele (Figure 1C). While these data were only able to be collected from two animals, these results are consistent with the hypothesis that *Lsd1*^{Vasa} M-Z+ animals have defects in imprinting.

The M448V Mutation Reduces Binding with CoREST

Oocytes from mothers with floxed *Lsd1* alleles meant to be deleted via a *Vasa*-Cre system only rarely experienced the partial loss of maternal protein, and the amount of maternal LSD1 present in these oocytes were likely subject to variation. In addition, due to limited survivors, only two experimental and control mice were able to be used for the purposes of

testing DNA methylation at imprinted genes. Because of the limits of *Lsd1*^{Vasa} M-Z+ progeny for the purposes of studying the effects of LSD1 deficiency beyond the two-cell stage, we decided to create a more tractable model to examine the phenotypes when maternal LSD1 is only partially defective. To address this, our lab used CRISPR-Cas9 to generate a hypomorphic *Lsd1* mouse allele, specifically by creating a point mutation, M448V (Figure 2A). This allele will be referred to as *Lsd1*^{M448V}. This mutation is in LSD1's tower domain, which is an important site for LSD1's binding with other proteins (Forneris et al., 2007; Stavropoulos et al., 2006; Yang et al., 2006). Since the mutation is not in the catalytic site, it only modestly reduces LSD1's independent demethylase activity to 85% *in vitro* (Nicholson et al., 2013). The major effect on function is its ability to bind its partner CoREST, which is reduced to 35% of wild type levels (Nicholson et al., 2013). In other cell types, LSD1 is commonly found in the CoREST complex, working to repress gene expression (Shi et al., 2005). Since the M448V mutation primarily affects CoREST binding, and not demethylase activity, this allele is also useful for investigating whether LSD1 is functioning through CoREST maternally. If LSD1 is indeed functioning through CoREST maternally, we expect that the perinatal lethality and imprinting defect phenotypes from the LSD1 partial loss data should be phenocopied in our hypomorphic LSD1 mice.

To test this, we bred female mice with the *Lsd1*^{M448V} allele over a floxed allele of *Lsd1*. In the presence of an oocyte-specific *Zp3-Cre* allele, the oocyte's floxed allele recombines and leaves the cell's *Lsd1*^{M448V} allele as the only means by which to provide maternal LSD1, which produces protein with a reduced ability to bind CoREST (Figure 2B). F1 offspring from this cross will be referred to as *Lsd1*^{M448V} progeny. All mothers are crossed to a wild-type male,

meaning the progeny will have a wild-type zygotic copy of *Lsd1*. This allows LSD1's maternal activity to be evaluated independently of its zygotic function.

For the first control group, each mother has a *Lsd1*^{M448V} allele over a floxed allele of *Lsd1* and is *Zp3Cre* negative (Figure 2C). In this cross, the maternal LSD1 contribution is derived from one functional copy of *Lsd1* and one hypomorphic *Lsd1*^{M448V} copy. The F1 offspring from this cross will be referred to as *Lsd1*$^{+}$. For the second control group, each mother has a wild type copy of *Lsd1* over a floxed allele of *Lsd1* (Figure 2D). In this cross, the maternal LSD1 contribution is derived from its one functional copy of *Lsd1*. The F1 offspring from this cross will be referred to as *Lsd1*^{het}.

Hypomorphic Maternal LSD1 Causes Perinatal Lethality

In our experiments with the *Lsd1*^{M448V} allele, we asked the question of whether mice with hypomorphic LSD1 phenocopy the perinatal lethality observed with a partial loss of maternal LSD1. To test this, we monitored the rate of perinatal lethality for mice from our *Lsd1*$^{+}$ cross, *Lsd1*^{het} cross, and *Lsd1*^{M448V} cross and compared their rates of lethality between postnatal day 0 (p0) and postnatal day 1 (p1). Progeny from the *Lsd1*$^{+}$ and *Lsd1*^{het} crosses had <10% perinatal lethality, while ~40% of progeny from the *Lsd1*^{M448V} cross died perinatally (Figure 3). This sharp increase in perinatal lethality phenocopies the increase observed in *Lsd1*$^{\text{Vasa}}$ M-Z+ progeny.

Hypomorphic Maternal LSD1 Leads to Imprinting Defects

Because several genes associated with imprinting were found to be misexpressed in our lab's two-cell arrest RNA sequencing dataset (Wasson et al., 2016), and because of the promising data on DNA methylation at imprinted genes in our lab's *Lsd1*^{Vasa} M-Z+ progeny indicated possible imprinting defects (Figure 1C), we chose to investigate the effects of hypomorphic maternal LSD1 on genomic imprinting. In order to determine this, we first harvested the heart, brain, and liver of adult mice from the *Lsd1*$^{+}$, *Lsd1*^{het}, and *Lsd1*^{M448V} crosses. By using allele-specific bisulfite sequencing of the paternally expressed *Zac1* gene on samples from each tissue and utilizing a single nucleotide polymorphism (SNP) to distinguish between the maternal (B6 background) allele and the paternal (CAST background) allele, we gathered data on the DNA methylation patterns of maternal and paternal alleles from each background in heart tissue (Figure 4) brain tissue (Figure 5) and liver tissue (Figure 6). We currently lack the control samples necessary to draw conclusions, but if these data follow the expected DNA methylation patterns, we will observe large discrepancies between *Lsd1*^{M448V} progeny and controls. For example, in heart and liver samples, we would observe that *Lsd1*^{M448V} progeny experience an increase in DNA methylation on the paternal *Zac1* allele when compared to *Lsd1*$^{+}$ and *Lsd1*^{het} progeny, as well as a decrease in DNA methylation on the maternal allele in *Lsd1*^{M448V} liver samples. In brain samples, we would observe that *Lsd1*^{M448V} progeny experience a decrease in DNA methylation on the maternal *Zac1* allele when compared to *Lsd1*$^{+}$ and *Lsd1*^{het} progeny.

DISCUSSION

Partial Loss of Maternal LSD1 Causes Defects Postnatally

The sperm and egg are two highly differentiated cell types that need to come together to form a totipotent zygote at fertilization. Prior studies demonstrated a need to reprogram the epigenetic information from the parental genomes in order for development to succeed (Li et al., 2013). This reprogramming at fertilization largely depends on the maternal RNAs and proteins deposited in the oocyte, since the major wave of zygotic genome activation does not start until the 2-cell stage (Schultz, 1993). Our lab provided significant evidence showing LSD1's relevance to this process. Immunofluorescence staining in mouse oocytes shows that LSD1 is present in the nucleus (Figure 1A) (Wasson et al., 2016). The maternal deletion of *Lsd1* results in a 1-2 cell arrest, with RNA sequencing data showing that the arrested embryos are more transcriptionally similar to oocytes than to wild-type embryos (Wasson et al., 2016). These findings demonstrate that LSD1 is a necessary element for the maternal-to-zygotic transition in mammals, and parallels the findings of other labs which observed 1-2 cell arrest and transcriptome changes is LSD1 negative embryos (Ancelin et al., 2016). Partial maternal loss of maternal LSD1 allows mice to bypass 1-2 cell arrest, but they experience an increase in perinatal lethality and may also experience defective genomic imprinting (Wasson et al., 2016). This suggests that when epigenetic reprogramming at fertilization is partially defective, there are long-term phenotypes which can carry on to later developmental stages.

Maternal Epigenetic Reprogramming via LSD1 May Be CoREST-Dependent

To determine what long-term phenotypes may be possible when maternally LSD1-deficient embryos bypass the 1-2 cell arrest, our lab generated a hypomorphic allele of *Lsd1* that primarily affects binding to CoREST (Figure 2A). These $Lsd1^{M448V}$ progeny were indeed able to bypass the embryonic arrest and exhibit an increased rate of perinatal lethality when compared to control mice (Figure 3). Since these animals have a completely functional zygotic copy of *Lsd1* inherited from the father, this phenotype is due to having hypomorphic LSD1 during the epigenetic reprogramming event at fertilization. These results suggest that incomplete reprogramming at fertilization can create defects that persist throughout development, resulting in long-term health consequences.

The increase in perinatal lethality observed in $Lsd1^{M448V}$ progeny (Figure 3), which rely on hypomorphic maternal LSD1, resembles the increase previously observed in $Lsd1^{\mathrm{Vasa}}$ M-Z+ progeny (Figure 1B), which experienced a partial loss of maternal LSD1. The $Lsd1^{M448V}$ allele does not limit the amount of maternal LSD1 protein, as the partial loss condition does, but rather inhibits LSD1's functionality through a mutation in its tower domain in a residue which binds CoREST. This has some effect on LSD1's ability to demethylate H3K4me1/2 *in vitro*, but the allele's primary effect is on LSD1's ability to bind with CoREST, which is reduced to 35% of its normal level (Nicholson et al., 2013). Given that a partial loss of maternal LSD1 limits LSD1 while the hypomorphic allele primarily perturbs the CoREST binding site of LSD1, the similarity in their resulting phenotypes raises the possibility that LSD1's maternal reprogramming capability is CoREST-dependent. As of now, it is not known what complex LSD1 operates in maternally, but the ability of the $Lsd1^{M448V}$ allele to phenocopy a partial loss of maternal LSD1 provides some evidence in support of the idea that it is functioning in a complex with CoREST.

In order for LSD1's maternal reprogramming capability to be CoREST-dependent, it would first require CoREST to be present the oocyte nucleus. Immunofluorescence imaging of wild-type mouse oocytes in our lab revealed that CoREST is broadly expressed in the nucleus at three different stages of oogenesis (Figure 7). This result establishes the possibility of CoREST-dependent maternal reprogramming in mammalian systems.

Other experiments from our lab have also supported the notion that maternal reprogramming in *C. elegans* is at least partially dependent on CoREST (unpublished data, Brandon Carpenter). The *C. elegans* orthologs for LSD1 and CoREST are SPR-5 and SPR-1, respectively. In worms, SPR-5 functions synergistically with MET-2, an H3K9 methyltransferase that assists in epigenetic reprogramming by adding repressive marks. *met-2* single mutants have no effect on sterility over generations. In contrast, *spr-5; met-2* double mutants are maternal effect sterile in a single generation, demonstrating the importance of those proteins working together to epigenetically reprogram embryos. *met-2; spr-1* double mutants become increasingly sterile across several generations (Figure 8A). While not as severe, the *met-2; spr-1* sterility partially phenocopies the *spr-5; met-2* sterility.

This similarity between both double mutants is also observed at a gene expression level using RNA seq. Comparisons of differentially expressed genes between *met-2*; *spr-1* and *spr-5; met-2* mutants show a high degree of overlap, indicating that they are affecting the same genes (Figure 8B). Of overlapping genes that are upregulated in *met-2; spr-1* animals, *spr-5; met-2* genes are also upregulated, but to a higher degree (Figure 8C). Similarly, overlapping genes that are downregulated in *met-2; spr-1* animals are more downregulated in *spr-5; met-2* animals (Figure 8D). These results demonstrate that while the same genes are affected, *spr-5; met-2* mutant phenotypes are more severe than *met-2; spr-1* mutants. Taken together, these data show

that *met-2; spr-1* animals partially phenocopy the *spr-5; met-2* animals at a transgenerational and molecular level. This reinforces the idea that in worms, in addition to mammals, LSD1 may be at least partially dependent on CoREST for the purpose of maternal reprogramming. As a strategy for examining this possibility further, our lab is currently working towards performing immunoprecipitation of LSD1 in *C. elegans* oocytes followed by mass spectrometry in order to reveal all of the members of the complex LSD1 functions in maternally.

Hypomorphic Maternal LSD1 Leads to Imprinting Defects

Previous studies on the partial loss of maternal LSD1 in mice showed that incomplete epigenetic reprogramming at fertilization may result in lasting defects in DNA methylation at imprinted genes (Wasson et al., 2016). When using allele-specific bisulfite sequencing to examine DNA methylation of the *Zac1* gene in perinatally lethal mouse pups, an increase of DNA methylation on the paternal allele was observed (Figure 1C). Limits of this examination, however, were the fact that only two experimental mice in total were used in the bisulfite analysis, and that the nature of the partial loss phenotype meant that it would be difficult to use it in further studies about the effects of maternal LSD1 deficiency on DNA methylation at imprinted genes.

The observation that our lab's hypomorphic *Lsd1*^{M448V} allele phenocopies the perinatal lethality observed in the partial loss dataset (Figure 3), as well as the fact that this allele can be used on a more consistent basis than the previously used *Lsd1*$^{\text{Vasa}}$ M-Z+ mice, created an opportunity to validate the previous findings on deficient maternal LSD1 and imprinting while also learning more about the nature of these defects. Our allele-specific bisulfite analysis sought

to include tissue-specific analysis which could uncover potential differences between the degree to which imprinting defects are conserved in various tissues throughout development. Specifically, we examined DNA methylation at the *Zac1* gene in heart, brain, and liver tissue for several p0-p3 pups from the $Lsd1^{M448V}$, $Lsd1^{+}$, and $Lsd1^{het}$ crosses. If our controls display the expected DNA methylation patterns, we will observe an increase in DNA methylation on the paternal *Zac1* allele in heart (Figure 4) and liver (Figure 6) tissue for $Lsd1^{M448V}$ progeny when compared to controls. We also would observe a decrease in DNA methylation on the maternal allele of $Lsd1^{M448V}$ progeny in brain tissue (Figure 5), with a minor decrease in DNA methylation on the maternal allele of $Lsd1^{M448V}$ progeny in liver tissue (Figure 6). All tissues and genetic crosses require more replicates before a definitive conclusion can be reached, but if these trends are maintained throughout further study, they would provide strong evidence that hypomorphic maternal LSD1 can cause lasting DNA methylation defects in mammals.

These results hold broad implications regarding the relationship between H3K4 methylation and DNA methylation. It is already known that H3K4 methylation can effect DNA methylation, either by physically inhibiting the function of DNA methyltransferases (Ooi et al., 2007) or by altering gene expression to cause the upregulation of DNMT1, which can lead to *de novo* DNA methylation (Wasson et al., 2016). Our results, however, also suggest that these defects can be very long lasting. Failure to completely remove the necessary H3K4me at fertilization can lead to long-term defects in DNA methylation that persist into adulthood, conserved faithfully throughout millions of cell divisions.

These observed imprinting defects are promising and led us to examine the tissue-specificity of this phenomenon more closely. In order to achieve this, we designed an experiment which utilizes a previously established imprinting reporter mouse line (Stelzer et al., 2016). This

experiment utilizes the *Snrpn* gene, which unlike *Zac1*'s pattern of expression, is expressed through the maternally inherited allele. With GFP inserted after the *Snrpn* imprinted promoter on the maternal allele, we validated that the presence of GFP expression can be used with immunofluorescence techniques to visualize defects in genomic imprinting (Figure 9). We verified the mouse model and completed troubleshooting on our experimental technique by breeding reporter mice which inherit the GFP tagged allele maternally. Because wild-type imprinting of the *Snrpn* gene leaves the maternal allele unmethylated (Figure 9A), we can observe full expression of the GFP tagged *Snrpn* allele (Figure 9B-D) and compare it to GFP negative immunofluorescence staining (Figure 9E-G).

For the experiment itself, we set up two different genetic crosses. Both crosses are between a father with wild-type *Lsd1* carrying the GFP tagged *Snrpn* allele and a mother without the GFP tagged *Snrpn* allele, but with one copy of the hypomorphic $Lsd1^{M448V}$ allele and one floxed *Lsd1* allele. The control group mothers are *Cre* negative, leading to a lack of maternal recombination of the floxed allele and thus a fully functional copy of LSD1 (Figure 9H). Because of this, the control cross is named $Lsd1^{+;\mathrm{GFP}}$. Experimental mothers are *Cre* positive, leading to maternal recombination of the floxed *Lsd1* allele, forcing their oocytes to rely on a single copy of the $Lsd1^{M448V}$ allele as their source of maternal LSD1. Because of this, the experimental cross is named $Lsd1^{M448V;\mathrm{GFP}}$. It is expected that the $Lsd1^{+;\mathrm{GFP}}$ progeny will show no GFP expression due to proper genomic imprinting, but the $Lsd1^{M448V;\mathrm{GFP}}$ progeny may experience some amount of GFP expression due to imprinting defects. Through immunofluorescence imaging, we should be able to observe the effects of this on a tissue-specific and even a cell-specific level.

A Model for LSD1 and Epigenetic Reprogramming at Fertilization

In *C. elegans*, experiments with a mutation in the *spr-5* gene, an *Lsd1* ortholog, revealed that H3K4me can be inherited across multiple generations (Katz et al., 2009). The inability to fully remove the H3K4me epigenetic memory during each reprogramming event at fertilization lead to a buildup of H3K4me2 that grew in intensity with each passing generation. This caused widespread misregulation of genes associated with spermatogenesis and a sterility phenotype which grew in severity as time progressed. The question remained, however, if this generational phenomenon is conserved in mammals.

In mice, the deletion of *Lsd1* causes arrest at the two-cell stage (Ancelin et al., 2016; Wasson et al., 2016), a phenotype which prevents the long-term effects of maternal LSD1 deficiencies from being studied. The occasional partial loss of maternal LSD1 that was observed in some *Lsd1* deletion mice, however, revealed lasting consequences of maternal LSD1 deficiencies in the form of perinatal lethality and defects in genomic imprinting (Wasson et al., 2016). Our experiments with the hypomorphic $Lsd1^{M448V}$ allele reinforce the idea that epigenetic defects caused by improper maternal reprogramming are conserved throughout development and hold long-term health consequences. As a result, we propose that the *C. elegans* model of H3K4me transgenerational epigenetic inheritance holds true in mammalian systems. If this is true, then when a mouse oocyte is not capable of fully removing its H3K4me transcriptional memory of being a differentiated cell during the maternal-to-zygotic transition, that error will be maintained throughout the subsequent offspring's life, leading to indirect defects such as misregulation of imprinted genes (Figure 10).

In the bigger picture, our lab was very interested in studying animals that may have inherited ectopic H3K4me as a method to determine the possible outcomes from incomplete

reprogramming. This is of particular interest because it is unlikely that any humans with completely deficient maternal LSD1 would survive, but partial losses may be a contributor to inherited disease. One mechanism this partial loss of protein could be working through is aging: in mice, LSD1 protein decreases with maternal age (Shao et al., 2015). In humans, maternal age is linked to increased risk of a variety of neurodevelopmental disorders, but the connection between age and the development of those disorders is not fully understood (Heffner, 2004). *De novo* LSD1 mutations in humans are linked to neurological physical disorders similar to Kabuki syndrome (Pilotto et al., 2016). It is unclear whether they are inherited maternally, paternally, or occur post-fertilization. Our data suggest that at minimum, the maternal inheritance of hypomorphic LSD1 in mice can lead to long-term phenotypes. These data raise the intriguing possibility that some of the phenotypes observed in humans may be due to maternal defects in epigenetic enzymes.

Future Directions

Our model of H3K4 methylation being inappropriately inherited in mammals leaves many additional avenues for testing. In *C. elegans* studies, the inappropriate transfer of H3K4me from one generation to another was measured using ChIP assays targeting H3K4me2 (Katz et al., 2009). A similar method could be employed here, utilizing ChIP sequencing at the blastocyst stage to verify the presence of H3K4me buildup in our hypomorphic mouse model. If we observe H3K4me at germline genes in somatic cells, where it typically would be erased during the maternal-to-zygotic transition, this will provide strong evidence that mice which experience defects in maternal reprogramming retain lasting remnants of their mother’s transcriptional memory.

To provide context for any ChIP sequencing results, RNA sequencing at the blastocyst stage may help reveal misexpression patterns in embryos that survive the two-cell stage. Misregulated gene expression is a key consequence of epigenetic defects, and misexpression was observed and characterized in maternal LSD1 deficient two-cell arrest embryos (Ancelin et al., 2016; Wasson et al., 2016). It is possible that in blastocysts, which survived the changes which take place during the two-cell stage's zygotic gene activation, a smaller subset of genes will be misregulated, or genes will be misregulated to a lesser extent. Gathering data on misexpression occurring in embryos that survive the two-cell stage may uncover yet-unknown details as to what is underlying the increased perinatal lethality we observed. It is also possible that patterns of misexpression will overlap with patterns of abnormal H3K4 methylation in *Lsd1^{M448V}* progeny revealed through ChIP sequencing. For example, an increase in H3K4 methylation at germline genes may lead to misexpression of germline genes, a model which would be supported through both sequencing methods. By then performing RNA and ChIP sequencing on *Lsd1^{M448V}* progeny after birth, we may also observe that these defects are maintained to a significant degree.

Because the mutation in our lab's *Lsd1^{M448V}* allele alters LSD1's tower domain, primarily inhibiting its ability to bind with CoREST, it would also be valuable to create an allelic series with mutations in other domains, specifically LSD1's catalytic site. It is possible that these would cause a more severe phenotype, but if results match our data from the *Lsd1^{M448V}* allele, that would suggest that CoREST binding is as crucial for maternal reprogramming as LSD1's catalytic activity.

More broadly, an advantage of our existing *Lsd1^{M448V}* allele is that it can potentially be used to conduct easily repeatable studies of the effects of hypomorphic LSD1 in other tissues. By

using a Cre-Lox system that functions in different cell types, we can examine the effects of hypomorphic LSD1 during differentiation in a variety of circumstances.

Due to the phenotypic consistency and repeatability that our lab's $Lsd1^{M448V}$ allele offers in comparison to the study of partial loss $Lsd1^{\mathrm{Vasa}}$ M-Z+ progeny, we will have the opportunity to study subtler epigenetic defects as research continues. These observations may be more relevant to human disease, as minor defects are more likely to be survived. These data may provide a better understanding of LSD1 related disorders, creating a foundation for the improved treatment of affected people.

FIGURES

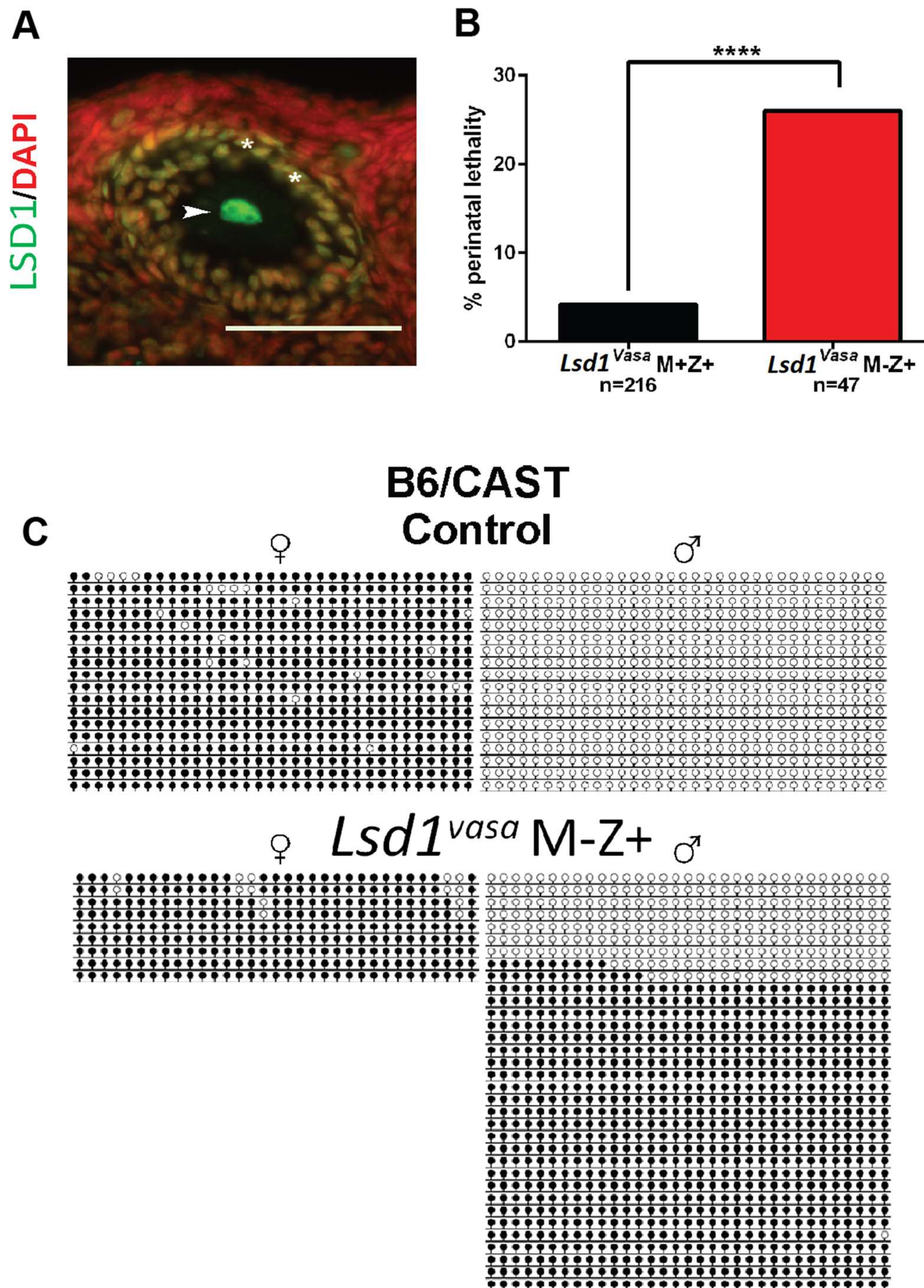

Figure 1. Partial loss of maternal LSD1 leads to long-term defects. (A) A wild-type mouse oocyte nucleus (white arrowhead) and surrounding follicle cells (white asterisks) stained with

anti-LSD1 (green) antibody and DAPI (red). (B) Percentage of newborn pups from *Lsd1*$^{\mathrm{Vasa}}$ M+Z+ heterozygous control and *Lsd1*$^{\mathrm{Vasa}}$ M-Z+ experimental group that died perinatally. n = number of litters analyzed. p-values were calculated with an unpaired t-test where **** = p<0.0001 indicating statistical significance. (C) Allele-specific bisulfite analysis of *Zac1* gene. Each line represents a clone of one allele. Each circle represents a CpG dinucleotide. Closed circles signify methylation and open circles signify a lack of methylation. Maternal and paternal alleles are indicated. Figure 1 is adapted from (Wasson et al., 2016).

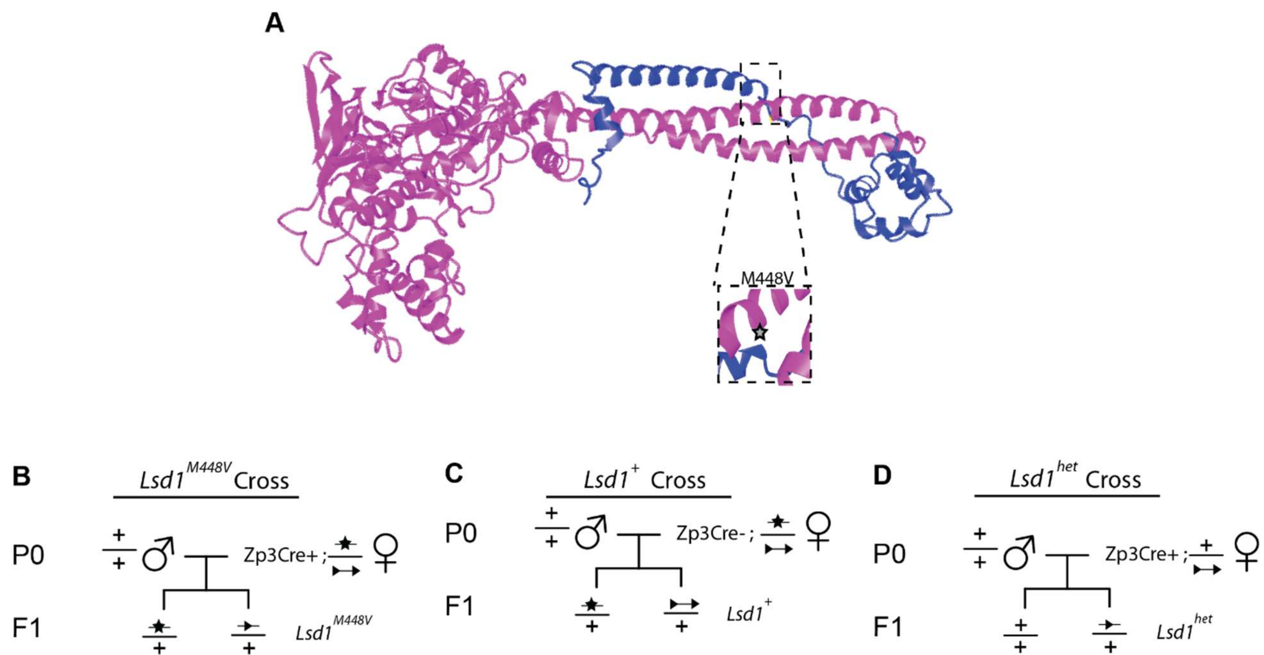

Figure 2. Hypomorphic LSD1 mutation and genetic crosses. (A) Crystal structure of LSD1 (pink) in complex with CoREST (blue). The hypomorphic allele's M448V mutation displayed on a CoREST binding site (star). (B) Genetic cross showing wild-type (+), loxP sites (triangles), and M448V alleles (star). In the P0 *Lsd1*^{M448V} cross, wild-type males are crossed with *Zp3Cre*+ females whose oocytes contain hypomorphic LSD1. (C) In the P0 LSD1^{+} cross, mothers are

Zp3Cre-, contributing one wild-type and one hypomorphic allele maternally. (D) In the P0 *Lsd1*^{het} crosses, mothers are *Zp3Cre*+, contributing one wild-type copy of *Lsd1* maternally.

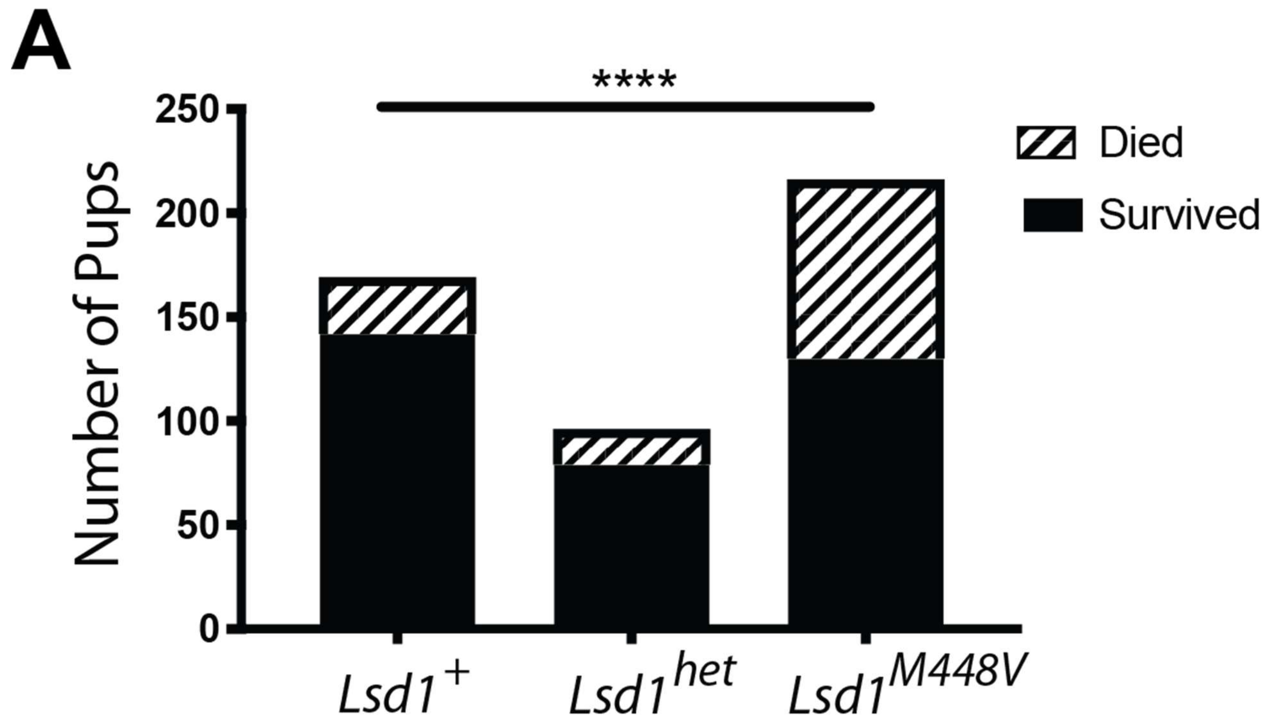

Figure 3. Increased perinatal lethality in *Lsd1*^{M448V} progeny. The proportion of perinatal lethality for progeny from each genetic cross. n= 27, 36, and 15 litters respectively. p values calculated using a chi-square test, **** = p< .00015

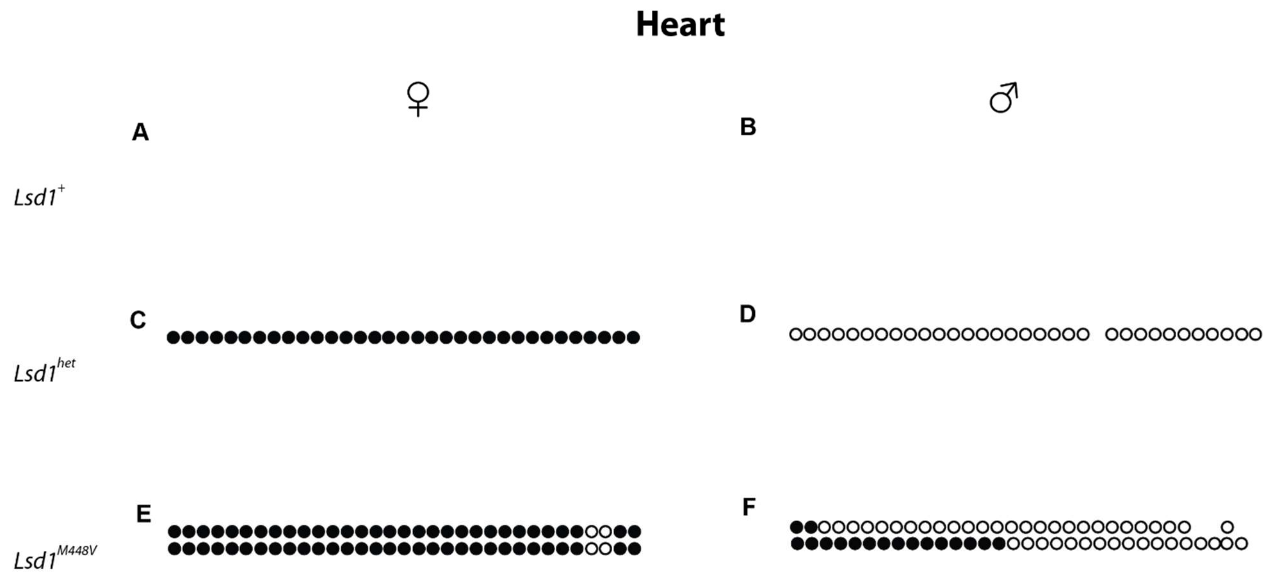

Figure 4. *Zac1* imprinting defects in *Lsd1*^{M448V} progeny heart tissue. (A,B) Allele-specific bisulfite analysis of the *Zac1* gene in *Lsd1*$^{+}$ progeny heart tissue, separated by maternal and paternal alleles, respectively. (C,D) Allele-specific bisulfite analysis of the *Zac1* gene in *Lsd1*^{het} progeny heart tissue. (E,F) Allele-specific bisulfite analysis of the *Zac1* gene in *Lsd1*^{M448V} progeny heart tissue. Each line represents a clone of one allele. Each circle represents a CpG dinucleotide. Closed circles signify methylation and open circles signify a lack of methylation. No circle indicates a lack of sequencing data.

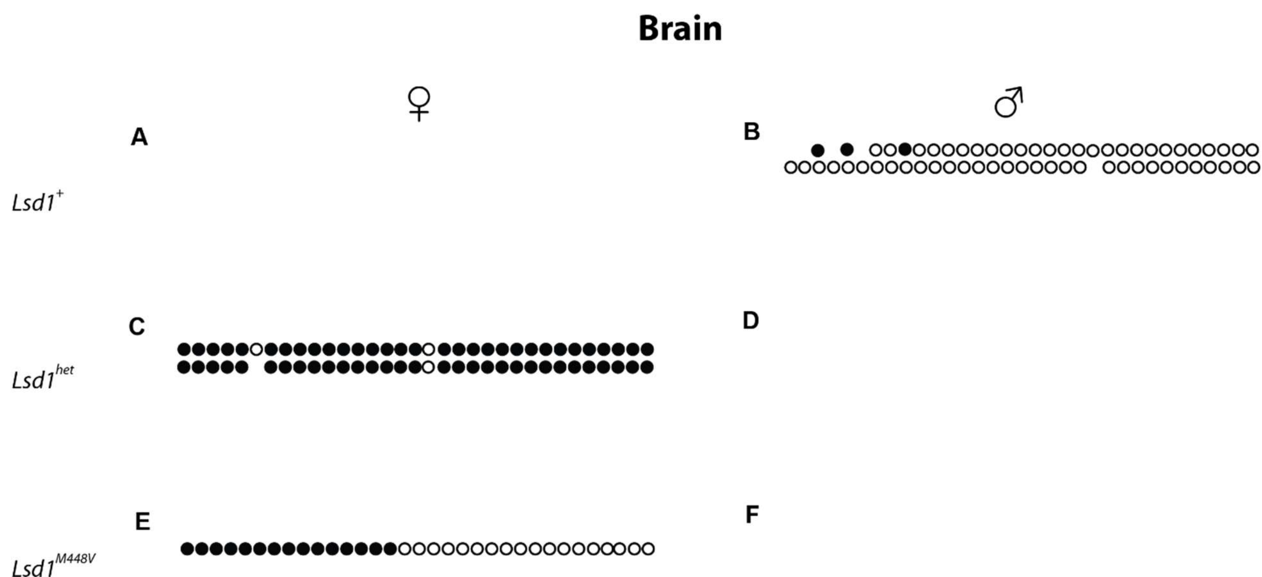

Figure 5. *Zac1* imprinting defects in *Lsd1^{M448V}* progeny brain tissue. (A,B) Allele-specific bisulfite analysis of the *Zac1* gene in *Lsd1^{+}* progeny brain tissue, separated by maternal and paternal alleles, respectively. (C,D) Allele-specific bisulfite analysis of the *Zac1* gene in *Lsd1^{het}* progeny brain tissue. (E,F) Allele-specific bisulfite analysis of the *Zac1* gene in *Lsd1^{M448V}* progeny brain tissue. Each line represents a clone of one allele. Each circle represents a CpG dinucleotide. Closed circles signify methylation and open circles signify a lack of methylation. No circle indicates a lack of sequencing data.

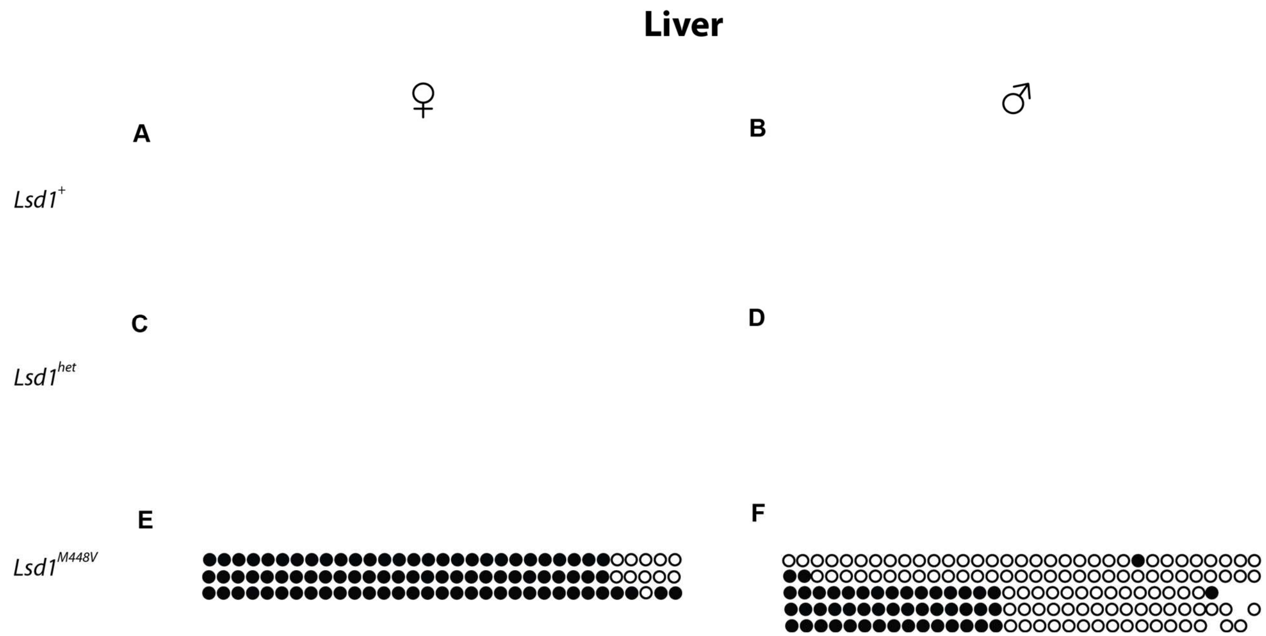

Figure 6. *Zac1* imprinting defects in *Lsd1*^{M448V} progeny liver tissue. (A,B) Allele-specific bisulfite analysis of the *Zac1* gene in *Lsd1*$^{+}$ progeny liver tissue, separated by maternal and paternal alleles, respectively. (C,D) Allele-specific bisulfite analysis of the *Zac1* gene in *Lsd1*^{het} progeny liver tissue. (E,F) Allele-specific bisulfite analysis of the *Zac1* gene in *Lsd1*^{M448V} progeny liver tissue. Each line represents a clone of one allele. Each circle represents a CpG dinucleotide. Closed circles signify methylation and open circles signify a lack of methylation. No circle indicates a lack of sequencing data.

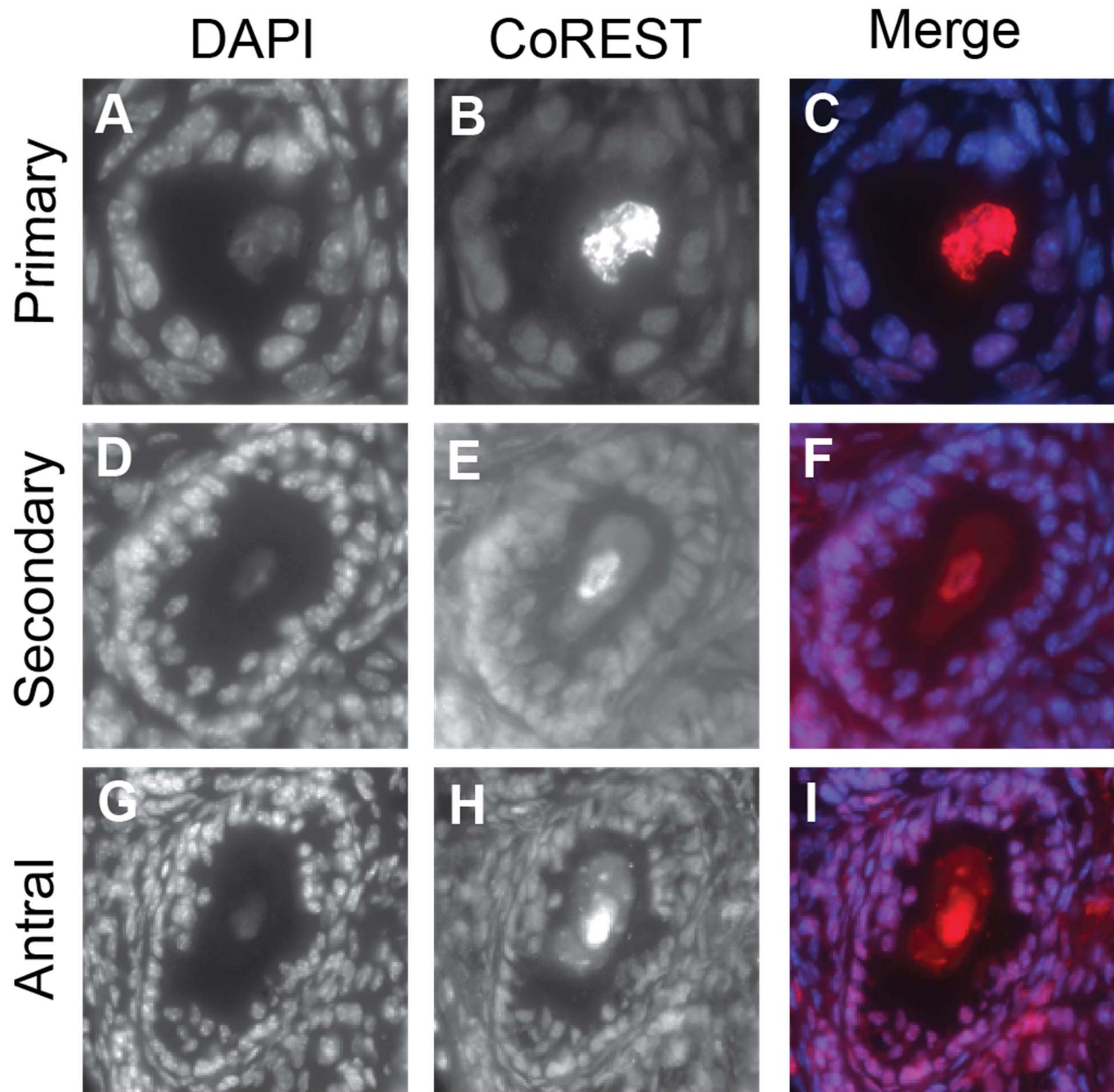

Figure 7. CoREST is broadly expressed in the nucleus at three different stages of oogenesis. Immunofluorescence imaging on three stages of mouse oocyte development: primary (A-C), secondary (D-F), and antral (G-I). DAPI (A,D,G), CoREST (B,E,H), and Merge (C,F.I).

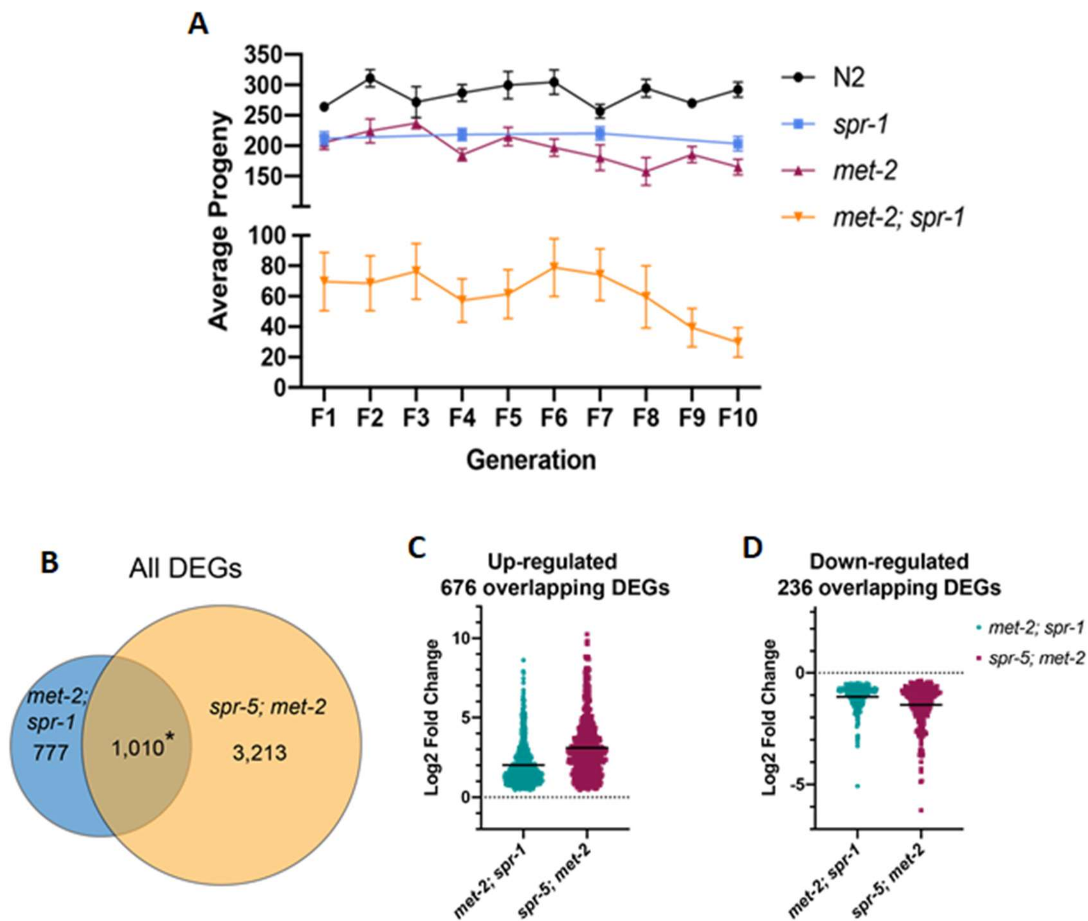

Figure 8. Differentially expressed genes in *spr-5; met-2* progeny are similarly misregulated in *met-2*; *spr-1* progeny, leading to progressive sterility. (A) The average number of total progeny from N2, *met-2, spr-1,* and *met-2; spr-1* mutants over several generations. Error bars represent the standard error of the mean. (B) Depiction of the association between all differentially expressed genes (DEGs) in *met-2; spr-1* and *spr-5; met-2* L1 progeny. Significant over-enrichment in A-C was determined by the hypergeometric test (*P-value < 1.28E-270, *P-value < 2.61E-392, *P-value < 2.16E-72, respectively)**.** Scatter plots display the mean log2 fold change of the 676 up-regulated (C) and 236 down-regulated (D) overlapping differentially expressed genes between *met-2; spr-1* and *spr-5; met-2* L1 progeny.

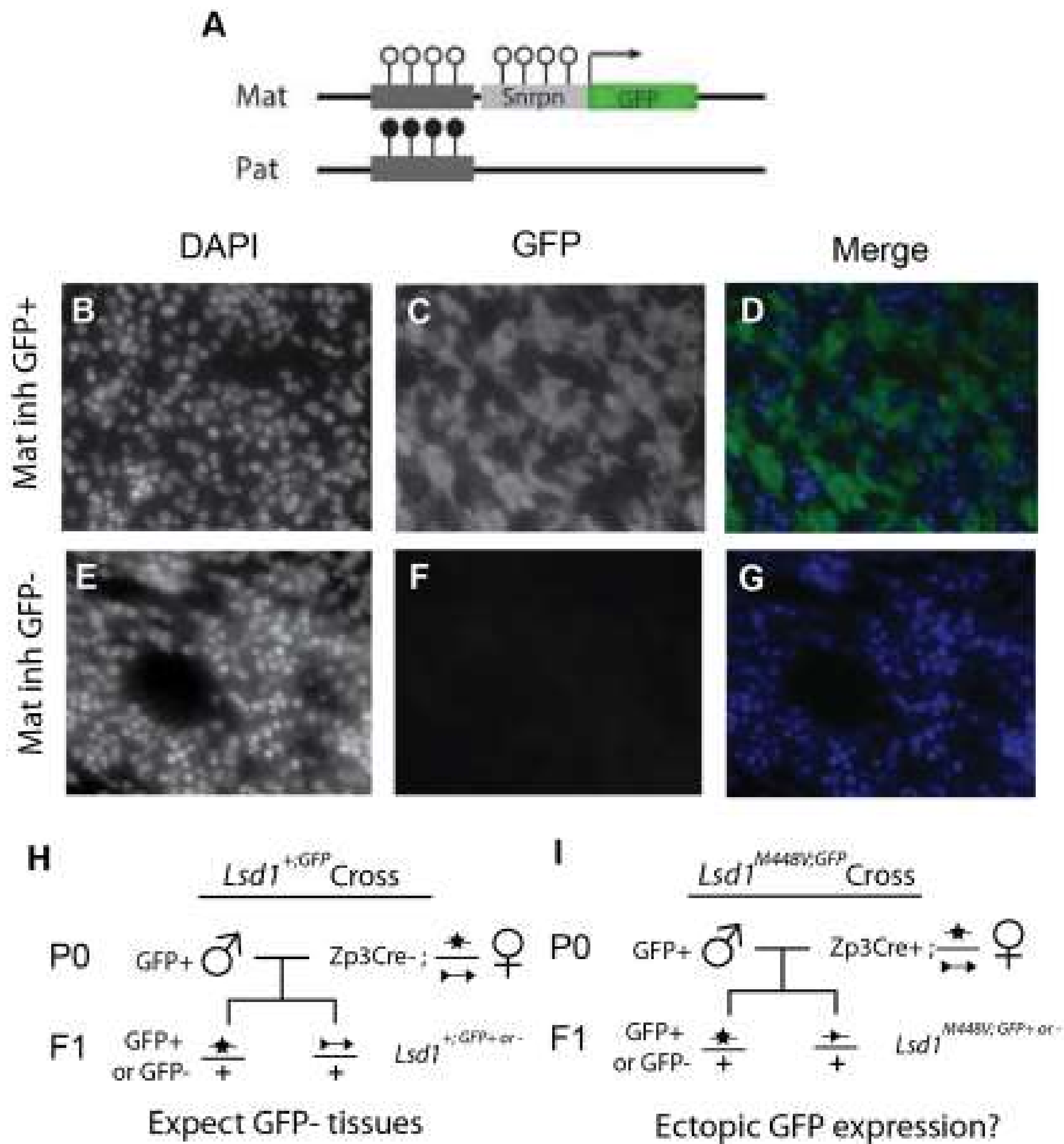

Figure 9. Schematic of Negative and Positive *Snrpn* GPF Imprinting Reporter Mice. (A) Expression of the GFP tagged *Snrpn* gene with maternal inheritance and wild type genomic imprinting. Closed circles signify methylation and open circles signify a lack of methylation. (B-D) DAPI (B), GFP (C) and Merge (D) immunofluorescence imaging for mouse tissue with maternally inherited GFP tagged *Snrpn*. (E-G) DAPI (E), GFP (F) and Merge (G) immunofluorescence imaging for mouse tissue with GFP negative maternally inherited *Snrpn*. (H) Genetic cross showing wild-type *Lsd1* (+), loxP sites (triangles), and M448V alleles (star).

In the P0 *Lsd1*$^{+;GFP}$ cross, mothers are *Zp3Cre*-, contributing one wild-type and one hypomorphic *Lsd1* allele maternally, while fathers provide GFP tagged *Snrpn* (I) In the P0 *Lsd1* $^{M448V;GFP}$ cross, mothers are *Zp3Cre*+, contributing only the hypomorphic *Lsd1* allele maternally. Fathers provide GFP tagged *Snrpn*.

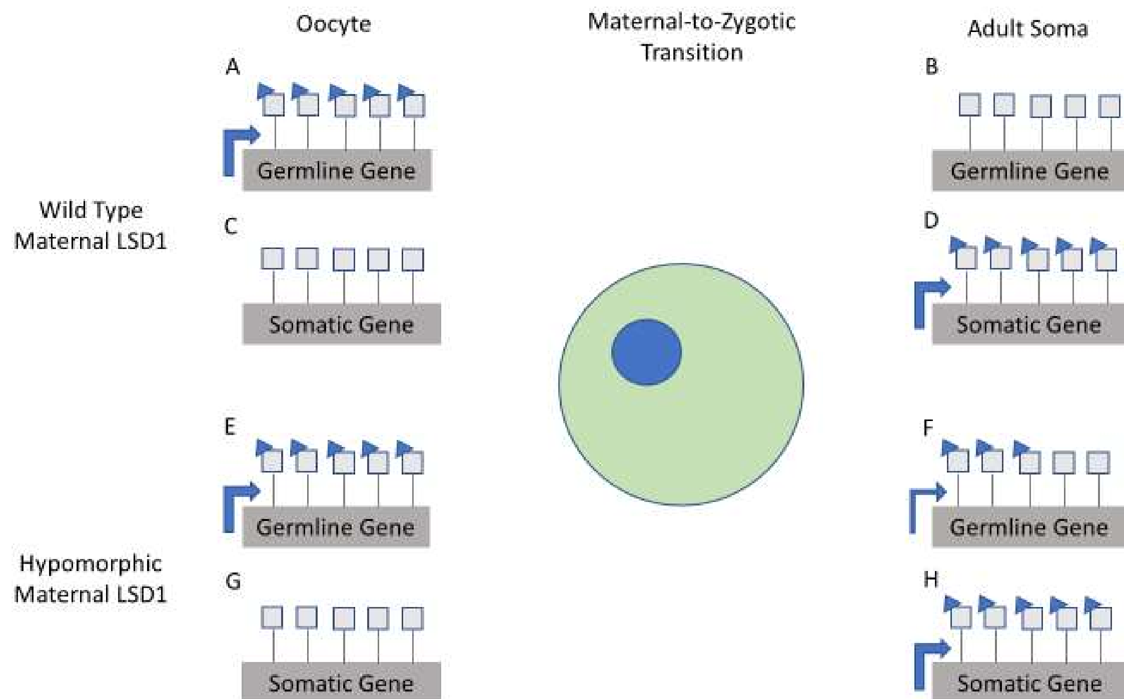

Figure 10. Hypomorphic maternal LSD1 in mammals leads to the incomplete removal of H3K4me transcriptional memory. Symbolic representation of a somatic gene and a germline gene before and after the maternal-to-zygotic transition. Squares represent H3K4, and squares with attached triangles represent mono or di-methylated H3K4. Arrows represent active transcription, with thicker arrows representing higher levels of transcription. For mammals with wild type LSD1 activity (A-D), H3K4me is present on the germline gene before (A) but not after (B) the maternal-to-zygotic transition, due to LSD1's demethylase activity. H3K4me is not

present on the somatic gene before (C) but is present after (D) the maternal-to-zygotic transition. For mammals with hypomorphic maternal LSD1 (E-H), H3K4me is present on the germline gene before the maternal-to-zygotic transition (E) and is partially inherited (F). This results in misregulation of the germline gene. H3K4me is not present on the somatic gene before (G) but is present after (H) the maternal-to-zygotic transition.

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