

Reviewed Preprint

v1 • September 2, 2024

Not revised

✉ For correspondence:

chenh7@sustech.edu.cn

jiawxu@foxmail.com

† These authors contribute equally to this work

Competing interests: No competing interests declared

Funding: See [page 29](#)

Reviewing editor: Falong Lu, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, China

© 2024, Wang et al. This article is distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use and redistribution provided that the original author and source are credited.

Resetting of H3K4me2 during mammalian parental-to-zygote transition

Chong Wang^{1,2,†}, Yang Li^{1,2,†}, Yaqian Wang^{1,2,†}, Yong Shi^{1,2,†}, Xiangrui Meng^{1,2}, Wenbo Li^{1,2}, Jia Guo^{1,2}, Kaiyue Hu^{1,2}, Hao Chen^{2,3} ✉, Jiawei Xu^{1,2} ✉

¹The First Affiliated Hospital of Zhengzhou University & Institute of Reproductive Health, Henan Academy of Innovations In Medical Science, Zhengzhou, China • ²NHC Key Laboratory of Birth Defects Prevention, Zhengzhou, China •

³Department of Human Cell Biology and Genetics, School of Medicine; Shenzhen Key Laboratory of Gene Regulation and Systems Biology, Southern University of Science and Technology, Shenzhen, China

eLife Assessment

This study presents an **important** finding of dynamic reprogramming of global H3K4me2 during mouse oocyte-to-embryo transition. While the H3K4me2 epigenome data is **convincing**, the interpretation and the potential mechanistic claims of the authors are **incomplete** in the current shape with the primary concerns regarding the contribution of Kdm1b or Kdm1a, as well as the specificity of the inhibitor and the antibody. The work will be of interest to researchers interested in epigenetic reprogramming.

<https://doi.org/10.7554/eLife.99417.1.sa4>

Abstract

Upon sperm and oocyte fertilization, the embryo undergoes drastic histone modification reprogramming during pre-implantation development. In this study, we investigated the erasure and re-establishment of H3K4me2 in mouse GV, MII, and embryos using an improved approach called Cleavage Under Targets and Release Using Nuclease (CUT&RUN) with high-throughput sequencing. We found that H3K4me2 extensively occurs as a non-canonical pattern in mouse GV oocytes and early embryos, and parental H3K4me2 was erased by maternal LSD2 during forming the pronucleus in the zygote. The H3K4me2 was erased from GV to MII oocyte and was re-established in the late two-cell stage, removing this epigenetic burden is crucial for ZGA. We then revealed the H3K4me2 locates widespread in the CpG-rich and hypomethylated regulatory regions in the four-cell stage, and the eight-cell stage, but dramatic changes occurred in the inner cell mass (ICM) in mouse embryos. Then, these CpG-rich H3K4me2 regulatory regions became either activated or repressed. To summarize, in this study, we elucidated the pattern of H3K4me2 transition from parent to zygote and H3K4me2 profile during early embryo development. Our findings might provide deeper insights into epigenetic reprogramming during early development and in vitro fertilization in mammalian.

Highlights

1. H3K4me2 showed differential patterns in sperms and oocytes; non-canonical pattern occurred in GV oocytes, but canonical patterns were found in the sperm cells of mice.
2. The H3K4me2 peaks of GV were almost erased in MII oocytes and reconstructed after the major ZGA occurred. Maternal LSD2 was found to regulate H3K4me2 resetting during the parental-to-zygote transition. H3K4me2 may impose an "Epigenetic Burden" on ZGA.

3. Non-canonical H3K4me2 pattern was found in mammalian embryos, however, pervasive H3K4me2 in CpG-rich regulatory regions were found in four-cell and eight-cell stage embryos; The CpG- rich H3K4me2 regulatory regions were either activated or repressed in the ICM.

Introduction

Drastic epigenetic reprogramming occurs during early mammalian embryo development. Epigenetic marks, such as DNA methylation and histone modification, play important roles in the epigenome reprogramming process.^{1, 2} Epigenome reprogramming during the pre-implantation embryo development stage erases the epigenetic memory of oocytes and sperm cells and allows the zygote to establish a new epigenetic landscape, which is relevant for early development and is essential for maternal zygotic transition (MZT) and differentiation.^{1, 3} Deficiencies in the reconstruction of the epigenome can result in developmental defects, such as embryonic arrest.³ Histone modifications are fundamental epigenetic regulators that control gene expression and many key cellular processes.^{4–6} Different types of histone modifications can occur in specific genomic regions and perform distinct functions.³ Most histone modifications occur around transcription start sites (TSSs) in the embryos; this feature is shared by all somatic cells.^{7–9} A similar pattern of histone modification commonly occurs in many species and cell lines.^{10–12} and thus, this distribution pattern is known as the ‘canonical’ pattern.¹³ Highly pervasive H3K27me3 is found in regions depleted of transcription and DNA methylation in oocytes, whereas extensive loss of promoter H3K27me3 occurs at *Hox* and other developmental genes after fertilization, accompanied by global erasure of H3K27me3 from sperm but inheritance of distal H3K27me3 from oocytes; both H3K4me3/H3K27me3 bivalent promoter marks are restored at developmental genes.¹⁴

The K-residues at the N-terminus of histone H3 undergo mono-(H3K4me1), di-(H3K4me2), or tri-methylation (H3K4me3), catalyzed by a family of proteins that contain a SET domain; the methylation of a different number of methyl groups added represents different chromatin status.^{15–17} Different numbers and kinds of histone modification marks are associated with different gene expression states.¹⁸ H3K4me3 is usually enriched near transcription start sites (TSS) that are associated with the activation of nearby genes.¹⁹ H3K4me1 is a global marker of enhancers; regions enriched with H3K4me1 and H3K27ac are identified as super enhancers.^{20–22} H3K4me1/H3K4me2/H3K4me3 is enriched on both sides of the TSS and plays diverse and important roles in regulating gene expression in mammals.^{23, 24} Some studies found that the H3K4me2 distribution pattern is like the H3K4me3 distribution pattern in plants.^{25, 26} Like LSD1, LSD2 (KDM1B) belongs to the FAD-dependent amine oxidase family and shares similar substrate specificity with LSD1, which can function as a H3K4me2 demethylase independently of LSD1.^{27–29} The mechanism underlying H3K4me2 reprogramming in the early development of mammals is still unknown, and the importance of the inheritance of the H3K4me2 programming from parents to offspring needs to be determined.

H3K4me2 can coordinate the recovery of protein biosynthesis and homeostasis following DNA damage, and it is required for the growth and survival of organisms.³⁰ H3K4me2 enrichment in plants is negatively correlated with the gene transcript level. H3K4me2 in rice and arabidopsis specifically distributes over the gene body region and functions as a repressive epigenetic mark.³¹ H3K4me2 was found to be a key epigenetic factor in the chicken PGC cell lineage determination, and a narrow domain activated PGC-specific expression genes and signaling pathways.³² H3K4me2 can occur as early as the embryonic stem cell stage, and its distribution is dynamic and progressive throughout the mouse brain differentiation and tissue development stages.³³ About 90% of the transcription factor binding regions (TFBRs) overlap with the H3K4me2-enriched regions, and along with the H3K27ac-enriched regions, they can greatly reduce false positive predictions of the TFBRs. Additionally, the stronger signals of H3K4me2 enrichment are more likely to overlap with the TFBRs than with weaker signals.³⁴ A subgroup of genes enriched with high levels of H3K4me2 was found within their gene body linked to T cell functions, which was

different from the TSS- centered enrichment of housekeeping genes³⁵. Many regions of the sperm epigenome bearing H3K4me2 are present in spermatogonia, suggesting an early establishment of H3K4me2 in spermatogenesis, which is a highly conserved process³⁶.

In this study, we elucidated the mechanism underlying H3K4me2 reprogramming during the early development of mammals. We optimized the Cleavage Under Target and Release Using Nuclease (CUT&RUN)^{37, 38} methods using H3K4me2 antibody to as few as 50 cells and revealed unique H3K4me2 modification patterns and epigenetic reprogramming that occur during the parental-to-zygote transition in mice. We also showed that H3K4me2 resetting depends on maternal LSD2 and parental H3K4me2 acts as an “Epigenetic Burden” on mammalian ZGA.

Results

Genome-wide profiling of H3K4me2 in mouse oocytes and early embryos

The epigenome underwent significant reprogramming during early development. To determine how H3K4me2 is inherited from the sperm and oocyte by the zygote during early development, we investigated H3K4me2 in mouse gametes and pre-implantation embryos using CUT&RUN methods. We optimized the CUT&RUN protocol (CUT&RUN; Methods) using H3K4me2 antibody. We also acquired high-quality data using only 50 cells. Most peaks could be retrieved using only 50 cells (Figures S1A [↗](#) and S1B [↗](#)). Then, we collected GV oocytes, MII, PN5, early two-cell embryos, late two-cell embryos, four-cell embryos, eight-cell embryos, and the inner cell mass (ICM) of mice. Mouse embryonic stem cells (mESC) were used as control. We profiled H3K4me2 in oocytes and early embryos using CUT&RUN and immunofluorescence (IF) techniques. The results of all replicates were highly consistent (Figure 1A [↗](#); Figures S1A, B, and C [↗](#)). We identified 57,455, 25,073, 110,517, 126,318, 62,575, and 69,154 H3K4me2 peaks in the GV oocytes, late two-cell embryos, four-cell embryos, eight-cell embryos, ICM, and mESC, respectively. Most of the H3K4me2 was erased from GV to MII during oocyte development. The paternal H3K4me2 was also erased after fertilization, and it was re-established in the late two-cell stage (Figures 1A-B [↗](#)). We also demonstrated the H3K4me2, ATAC-seq, and mRNA expression of several developmental key genes (Figure 1C [↗](#)). We found that H3K4me2 positively correlated with H3K4me3 and chromatin accessibility in early embryos (Figures S1D-E [↗](#)). The H3K4me2 genome-wide pattern of four-cell embryos showed a strong association with eight-cell embryos: the H3K4me2 of four-cell and eight-cell embryos was in one cluster, and the correlation was high ($R = 0.93$) (Figures S1F-G [↗](#)).

Distinct H3K4me2 pattern between sperm and oocyte

We systematically characterized the epigenome of mouse gametes. In the analysis, we included published data on DNA methylation³⁹, H3K4me3⁴⁰, and ATAC-seq of early embryos⁴¹, as well as the H3K4me2 data of sperm⁴². Non-canonical patterns of H3K4me3, enriched broad domains in promoters and distal regions, are present in mouse oocytes as broad domains in partially methylated domains (PMDs)^{43, 44}. H3K4me2 shows a non-canonical pattern in the GV oocytes and sperm of mice (Figures 2A-B and E-F [↗](#)). Mature sperm and oocytes are in a state of inactive transcription^{45, 46}; however, strong ChIP-seq and CUT&RUN signal enrichment of H3K4me2 was found to be related to transcription⁴⁷. These findings suggested that H3K4me2 might be deposited during gametogenesis and retained in mature gametes. To confirm this speculation, we performed a K-means cluster analysis for H3K4me2 in gametes. By comparing promoter H3K4me2 at the sperm and GV stages, we found that the promoters could be divided into four groups (Figure 2A [↗](#)). The promoters in the first group (sperm-specific H3K4me2) were involved in fertilization and germ cell development, and these markers were erased in the later stages (Figures 2A [↗](#) and 2C [↗](#)). In contrast, the promoters in the second group (GV-specific H3K4me2) were associated with mitosis and gamete generation. They played a role in GV generation during oocyte development and were re-established in the late stages (Figures 2A [↗](#) and 2C [↗](#)). The promoters in the third group (sperm-GV share H3K4me2) were related to mRNA processing and translation. The signals

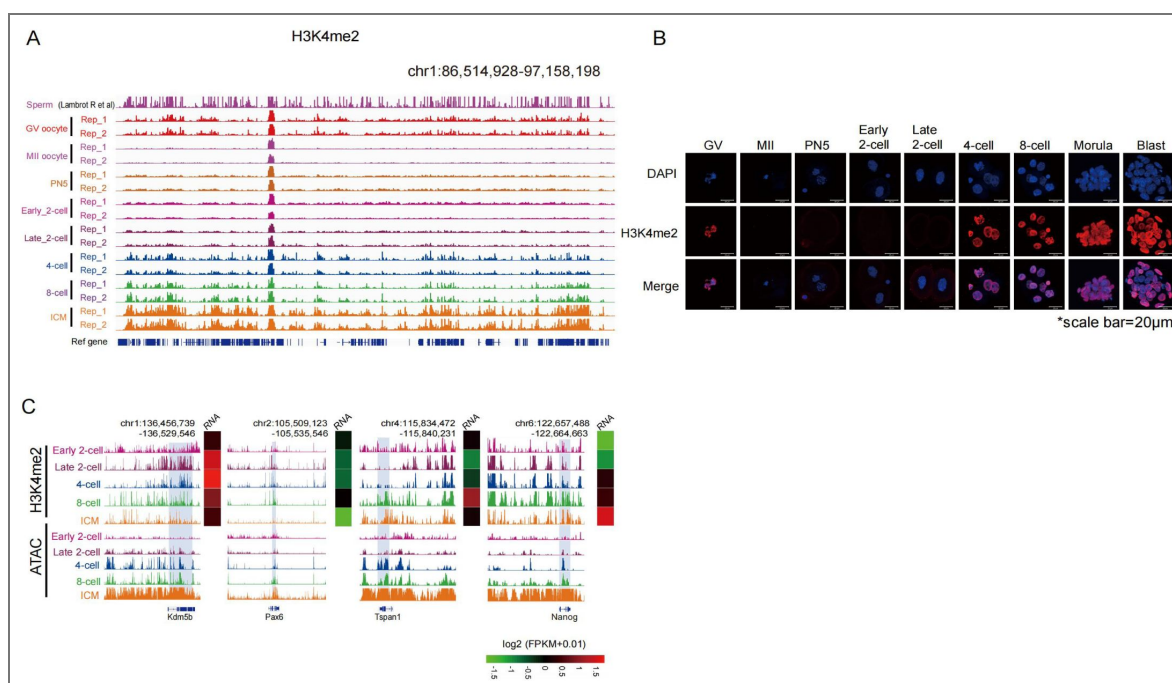


Figure 1. Mapping H3K4me2 in mouse (C57BL6/N) gametes and pre-implantation embryos.

(A) The IGV view shows the global landscape of H3K4me2 signals in mouse sperm, GV oocytes, MII oocytes, and pre-implantation embryos. The H3K4me2 data in sperm was obtained from ENCODE (Lambrot et al.). ICM was isolated from the blastocyst. (B) Immunofluorescence of H3K4me2 in mouse (C57BL6/N) GV oocytes, MII oocytes, PN5 zygote, early two-cell embryo, late two-cell embryo, four-cell embryo, eight-cell embryo, morula, and blastocyst; the scale bar is also shown. (C) H3K4me2 enrichment near typical genes and their expression levels are shown. H3K4me2 signal, ATAC-seq, and gene expression are shown.

of these regions were erased (MII, zygote, and early two-cell stage) and reconstructed (at the late two-cell stage) during development (Figures 2A and 2C). The promoters in the fourth group had a low H3K4me2 level at all stages (Figure 2A).

The H3K4me2 enrichment in these promoters was consistent with the changing dynamics of H3K4me3 (Figure 2A). Specifically, promoters with high CpG density were preferentially marked by H3K4me2 in the sperm-GV share group (Figure 2A). Next, we investigated H3K4me2-marked distal regions in gametes. Sperm-specific groups are involved in gamete generation (Figure 2D). GV-specific distal H3K4me2 showed functions in cell differentiation and mitotic cell cycle (Figure 2D). Both were erased after fertilization and not reconstructed during embryo development (Figure 2B). H3K4me3 signals did not occupy the sperm-specific/GV-specific H3K4me2 distal regions (Figure 2B). The GV-sperm-shared distal H3K4me2 regions were also marked by H3K4me3. Those genes participated in system development, RNA processing, and regulation of gene expression (Figures 2B and 2D). Compared to GV, sperm H3K4me2 was enriched in a fully methylated promoter, but the distal H3K4me2 of both sperm and GV were enriched in the hypomethylated region (Figures S2A-D). These results indicated that differential H3K4me2 modification patterns occurred between sperms and oocytes.

Resetting H3K4me2 in pre-implanted embryos

Significant epigenetic reprogramming and chromosome remodeling associated with H3K4me3, H3K27me3, H3K36me3, and H3K27ac occurs during early mammalian development^{44, 48, 49}. In another study, we found that epigenome reboot occurred during the early developmental stage in humans⁵⁰. We investigated how parental histone mark H3K4me2 at regulatory elements are reprogrammed after fertilization. We found that GV and sperm H3K4me2 peaks were almost erased in PN5 and re-established along with zygotic genome activation (ZGA). This result was further confirmed by IF analysis, which showed that the reconstruction of H3K4me2 might start from the late two-cell stage (Figures 1B and 3B). The H3K4me2 demethylases KDM1B (LSD2) were also highly expressed at the MII stage (Figure S3A). The CUT&RUN data, which showed small H3K4me2 peaks (related to other stages), reappeared at the late two-cell stage (Figure 3A). We concluded that the IF analysis could not detect the newly established weak H3K4me2 signal in the late two-cell stage. H3K4me2 was correlated with the activation of *Prdm9* and *Kmt2c* at the four-cell stage (Figure S3A), and the signal was strong enough to be detected at the four-cell and eight-cell stages (Figures 3A-B) using both the CUT&RUN and IF techniques. The four/eight-cell H3K4me2 targets only partially overlapped with those in sperm or oocytes, and these overlapped targets were mainly enriched in development-associated pathways or processes (Figure 3C). Additionally, gamete-specific H3K4me2 targets were involved in the development and generation of gametes, whereas four/eight cell-specific targets were associated with cell communication and metabolic processes (Figure 3C). These findings indicated that the resetting of H3K4me2 involves distinct sets of genes that are closely related to the development of embryos from mouse gametes to early embryos. About 1% of the four/eight cell H3K4me2 occurred in the hypomethylated regions as early as the late two-cell stage (Figures S3B-D). These results indicated that the resetting of H3K4me2 starts from the late two-cell stage but is mostly re-established at the four/eight-cell stage.

The inhibition of LSD2 increased the expression of H3K4me2, which impaired the development of embryos in vitro

Histone post-translational modifications are coordinated by histone methyltransferases and demethylases⁵¹. KDM1B, also known as LSD2, is the only demethylase homologous to LSD1 in mammals²⁹. Although they share the H3K4me1/2 demethylase function, our analysis showed that *Kdm1a* was rarely expressed during mouse embryonic development (Figure 4A). While, LSD2, which is required to establish the maternal imprinted gene⁵², was highly expressed before the early two-cell stage and decreased after the late two-cell stage (Figures 4A and S3A).

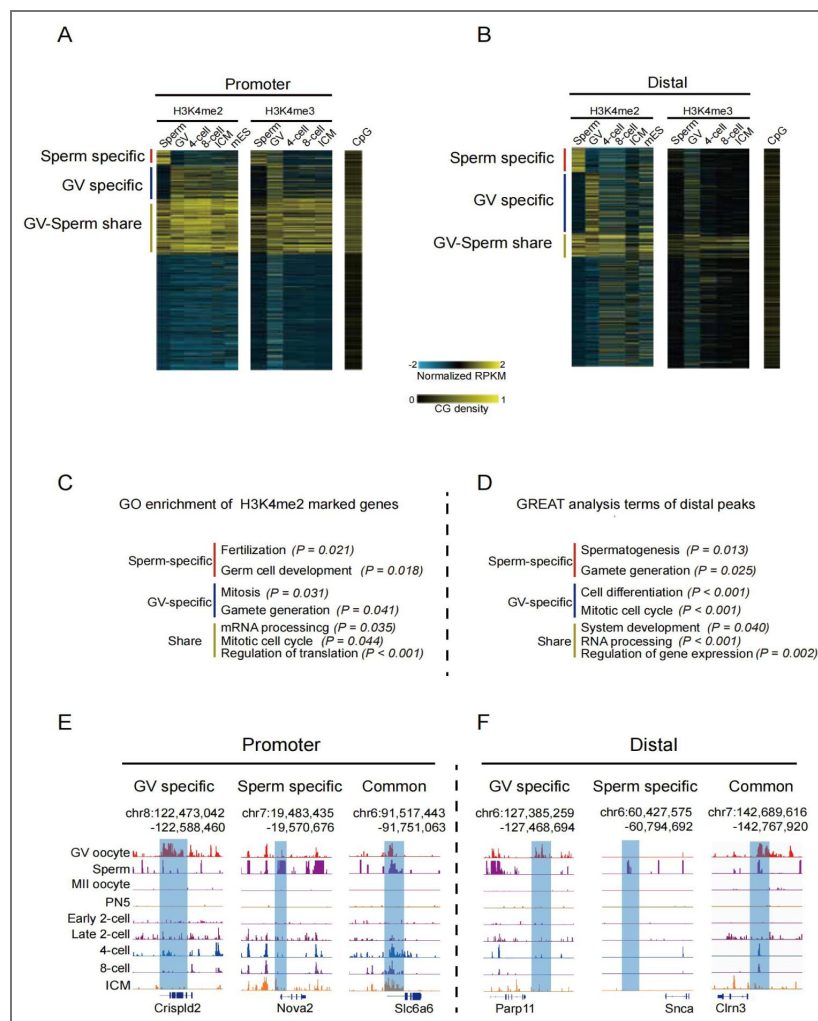


Figure 2. Comparison of H3K4me2 between sperms and oocytes.

(A-B) Heatmaps showing H3K4me2 and H3K4me3 expressed genes between sperm and GV oocyte in the promoter and distal region at different stages. CpG densities are also mapped and shown. (C) The enriched GO terms of H3K4me2 marked genes in GV and sperm-specific or shared genes are shown along with the associated p-values. (D) The results of the GREAT analysis for distal peaks in GV and sperm-specific or shared genes are shown along with the associated p-values. (E-F) The IGV views show H3K4me2 expressions in GV and sperm-specific or shared genes at the representative promoters (left, shaded) or distal accessible regions (right, shaded).

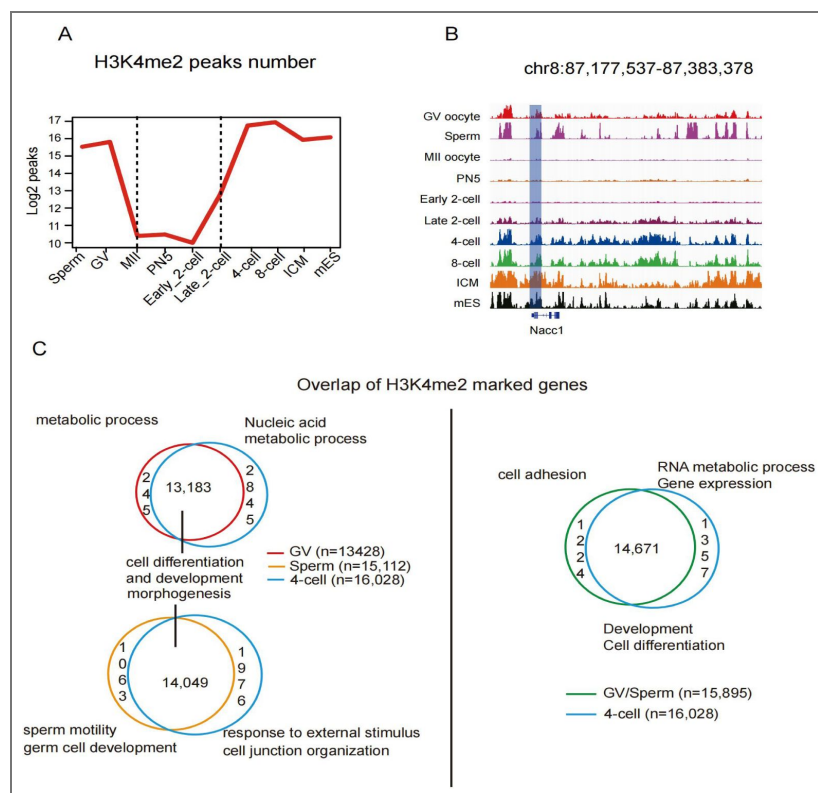


Figure 3. Resetting of H3K4me2 during mouse pre-implantation development.

(A) The number of H3K4me2 peaks in mouse embryo stem cells (mESCs), mouse gametes, and pre-implantation embryos was calculated. (B) The IGV view shows H3K4me2 signals in mESCs, mouse gametes, and pre-implantation embryos; *Ncc1* gene sites are also shown. (C) Venn diagrams illustrate the overlap of H3K4me2 marked genes between sperm/GV oocytes, separately (left) or jointly (right), and four-cell embryos. The enriched GO terms for the shared and non-overlapping genes are also shown.

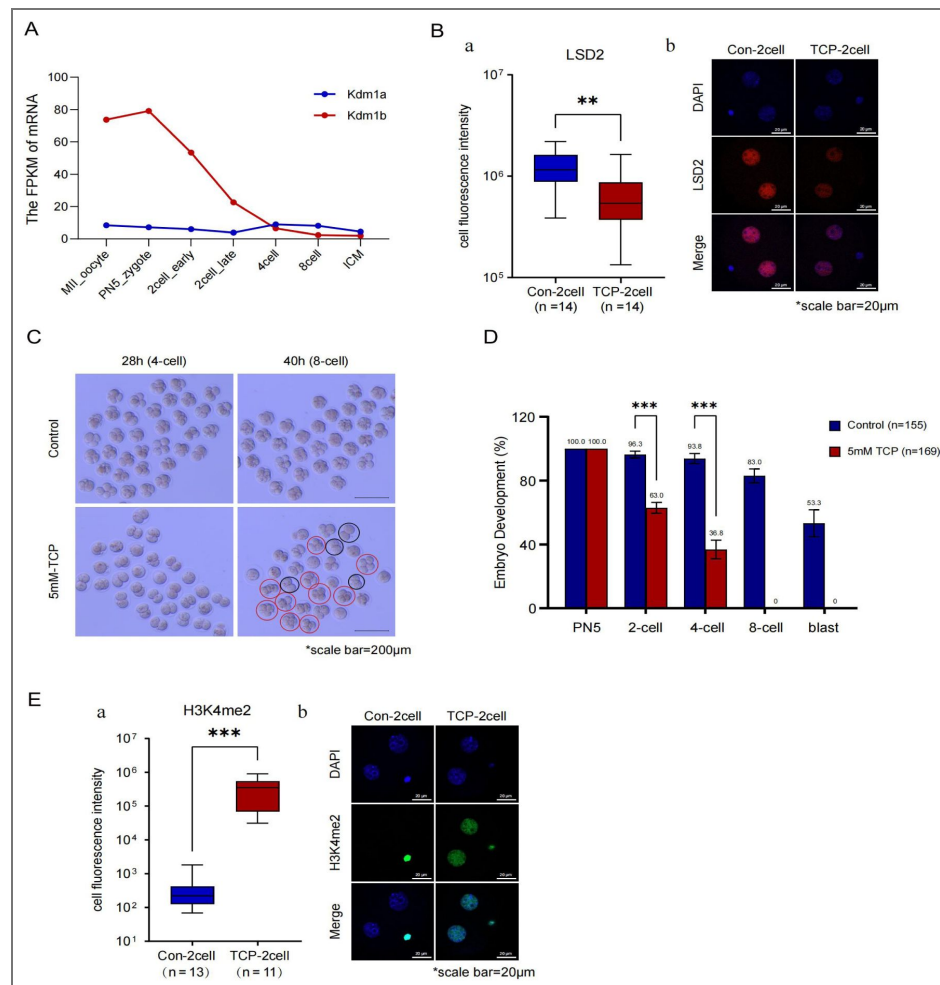


Figure 4. The inhibition of LSD2 resulted in abnormal embryonic development

(A) The dynamic changes in the Kdm1a (blue line) and Kdm1b (red line) levels in oocytes and early embryos (based on published RNA-seq data43). (B) a. The immunofluorescence intensity of LSD2 in the experimental group (5 mM TCP) and the control group are shown using a box diagram. b. Immunofluorescence images showed the immunofluorescence intensity of LSD2 of two-cell embryos in the experimental group (5 mM TCP) (right) and the control group (left); the scale bar is also shown. (C) The developmental morphology of mouse embryos in the experimental group (5 mM TCP) and the control group after 28 h and 40 h of culture are shown along with the scale bar. (D) The figure illustrates the embryonic development of mice at different stages after culture in vitro in the experimental group (5 mM TCP) and the control group. (E) a. The immunofluorescence intensity of H3K4me2 in the experimental group (5 mM TCP) and the control group are shown using a box diagram. b. Immunofluorescence images showed the immunofluorescence intensity of H3K4me2 of two-cell embryos in the experimental group (5 mM TCP) (right) and the control group (left); the scale bar is also shown.

Tranylcypromine (TCP) can inhibit the expression of LSD2⁵³. To investigate the effect of the expression of H3K4me2 on embryonic development, mouse PN5 embryos were cultured in vitro with three different TCP treatment groups (3.6 mM, 5 mM, and 10 mM) and a control group. The inhibition in the presence of 3.6 mM TCP was not prominent, and some embryos could develop to the eight-cell stage at 65 h. When 10 mM TCP was present, embryonic development was strongly inhibited, and the embryo could not develop to the two-cell stage (Figure S4A [4](#)). When 5 mM TCP was present, the embryos could pass through the two-cell stage with a significant developmental delay. Some embryos developed till the four-cell stage, and then, were completely arrested. These results showed that TCP affected embryonic development in a dose-dependent manner and caused developmental arrest. We concluded that the TCP concentration of 5 mM in the experimental group (5 mM TCP group) could be used to determine whether the abnormal expression of H3K4me2 affects the development of embryonic cells, especially the occurrence of ZGA.

We found that adding TCP to culture embryos significantly reduced the expression of LSD2 in embryos (Figure 4B [4](#)). After 28 h of culture, some of the embryos in the Control group developed to the four-cell stage, while those in the 5 mM TCP group remained at the two-cell stage (Figures 4C [4](#) and S4B [4](#)). The development of the embryos was significantly delayed after TCP was added compared to the time taken for the embryos in the Control group to develop. The number of embryos at each stage in the 5 mM TCP group ($n = 169$) and the Control group ($n = 155$) was counted and statistically analyzed (Figure 4D [4](#)). The results showed that 62.96% of embryos in the 5 mM TCP group developed to the two-cell stage (Control: 96.29%), and 36.82% of embryos developed to the four-cell stage (Control: 93.83%). All embryos in the TCP group were arrested at the four-cell stage ($P < 0.001$).

To test whether LSD2 specifically functions as H3K4me2 demethylase in mouse embryos, immunofluorescence staining of cells in the 5 mM TCP group and the Control group was performed using H3K4me1, H3K4me2, and H3K4me3 antibodies, respectively. We calculated the immunofluorescence intensity of individual embryos and found no significant difference in the changes in the expression of H3K4me1 between the 5 mM TCP group and the Control group (Figure S4C [4](#)); also, the changes in the expression of H3K4me3 was not statistically significant (Figures S4C [4](#)). In contrast, when LSD2 expression was inhibited by TCP, the expression of H3K4me2 at the two-cell stage was significantly higher than the expression of H3K4me2 in the cells of the Control group ($P < 0.001$) (Figure 4E [4](#)). Elimination of the demethylase action of LSD2 increased the expression of H3K4me2, which delayed and arrested embryonic development. We also found that LSD2 specifically demethylated H3K4me2 in mammalian embryos.

Parental H3K4me2 was erased by maternal LSD2 during forming the pronucleus in the zygote

During spermatogenesis, most of the histone modifications are replaced by protamine, but a few histones, including H3K4me2, remain conserved⁵⁴. During the early stages of zygote formation, female and male pronuclei are transcriptionally silent, and the development process is regulated by maternal factors. In our study, during the early stage of zygote formation, the expression of H3K4me2 in PN5 was lower than that in PN3 ($P < 0.001$) (Figures 5A-B [4](#)), which indicated that H3K4me2 signaling was gradually reduced during the fusion of sperm and egg. When PN3 embryos were cultured to the PN5 stage under 5 mM TCP conditions, the fluorescence signal of H3K4me2 increased significantly ($P < 0.001$) (Figures 5A-B [4](#)), suggesting that the LSD2 might response the removal of parental H3K4me2 during normal embryonic development. The immunofluorescence values of H3K4me2 in female and male pronuclei were assessed to verify whether the effect of LSD2 inhibition on H3K4me2 was different between male and female pronuclei. When LSD2 was inhibited, the expression of H3K4me2 increased significantly in female and male pronuclei, especially in the male pronucleus (334% increase in the male pronucleus and 155% increase in the female pronucleus) (Figure 5C [4](#)). Sperm carrying H3K4me2 might be more affected by LSD2 demethylase, creating a low H3K4me2 expression environment for zygotic

genome activation. To summarize, parental H3K4me2 was erased by maternal LSD2, the H3K4me2 in the male pronucleus was erased faster than that in the female pronucleus, and maternal LSD2 specifically demethylated H3K4me2.

H3K4me2 participated in the mammalian ZGA and chromatin accessibility regulation

We used the CUT&RUN technology to detect H3K4me2 throughout the genome and performed gene annotation of the differential expression peaks of H3K4me2 in the TCP-treated group and the control group. We found that the H3K4me2 signal increased in 251 genes and decreased in 194 genes (Fold changes > 2, $P < 0.01$) (Figure 6A [↗](#)). The enrichment analysis of the genes from which H3K4me2 differential peaks originated showed that the molecular function was mainly enriched in the transcription regulation of the promoter of RNA polymerase II, and 35 differential genes were involved in this regulation (Figure 6B [↗](#)).

Unlike LSD1, which binds to the promoter region, LSD2 is absent in the promoter region and enriched in the intronic, exonic, and intergenic regions²⁹. The distribution of H3K4me2 changed when LSD2 expression was inhibited (Figures 6C-D [↗](#)). The increase in the H3K4me2 signal mainly occurred in the intergenic and genebody regions (intergenic region: 55.05%, genebody region: 32.66%). We speculated that H3K4me2 located in these regions might be erased by LSD2. However, the deletion of LSD2 did not increase the H3K4me2 signal in the promoter region.

Histone modifications can regulate early embryonic development in mammals by affecting the expression of developmental genes and reprogramming the chromatin structure. RNA-seq data of mouse germ cells and embryos (refer to GEO database: GSE71434) were used to screen minor/major ZGA genes. We examined the gene expression levels of two-cell embryos in the 5 mM TCP group and the Control group (fold changes > 2, $P < 0.05$) and found that 398 genes were up-regulated and 724 genes were down-regulated in the two-cell stage embryos of mice after LSD2 was inhibited (Figures 6E-F [↗](#)). Among these differentially expressed genes, 94 down-regulated genes were major ZGA genes, and 18 up-regulated genes were major ZGA genes. The Zfp825 gene played a positive regulatory role in RNA polymerase II promoter transcription. Inhibition of LSD2 can lead to a reduction in the distribution of H3K4me2 on the Zfp825 gene and a weakening of the expression of Zfp825. These changes may affect the frequency, rate, or extent of RNA polymerase II promoter transcription.

An increase in the expression of H3K4me2 changed the accessibility of chromatin, of which 1,413 peaks were up-regulation and 914 peaks were down-regulated (fold change > 2; $P < 0.05$) (Figure 6G [↗](#)). Peak-related gene screening of the genes near the promoter region (TSS ± 2.5 kb) showed that 240 peak-related genes were up-regulated and 197 peak-related genes were down-regulated. The transcription of genes requires the opening of the tight structure of chromatin to allow the binding of regulatory factors. The up-regulation of H3K4me2 alters the original accessibility state of chromatin, leading to disorders associated with chromatin opening in some developmental genes, which in turn can affect developmental progression. The inhibition of LSD2 expression leads to changes in the distribution of H3K4me2 on genes, abnormal expression of genes associated with ZGA, and disorders related to chromatin opening; these changes together inhibit normal embryonic development.

Non-canonical H3K4me2 in mammalian embryos and pervasive H3K4me2 in CpG-rich regulatory regions in the four-cell and eight-cell embryos of mice

In GV oocytes and post-ZGA stages, H3K4me2 was enriched in both canonical gene promoters and partially methylated domains (PMDs) (Figures 7A-D [↗](#)). Our results showed that H3K4me2 might be re-established in the late two-cell stage, and strong promoter H3K4me2 enrichment might be completed in the four-cell stage and maintained until the eight-cell stage. We also found that the H3K4me2 signals occupied broader regions than those at other stages (Figure 1A [↗](#)). About 65% of these gene promoters were occupied by H3K4me2 in the four-cell and eight-cell stages and the

Figure 5. Parental H3K4me2 was erased by maternal LSD2 during sperm-egg fusion

(A) Immunofluorescence images showed the immunofluorescence intensity of H3K4me2 in single-cell embryos in the experimental group (5 mM TCP) and the control group (C); the scale bar is also shown. (B) The immunofluorescence intensity of H3K4me2 in single-cell embryos in the experimental group (5 mM TCP) and the control group are represented as box and whisker plots. (C) The immunofluorescence intensity of H3K4me2 in male and female embryonic prokaryotes of the experimental group (5 mM TCP) and the control group are represented as box and whisker plots.

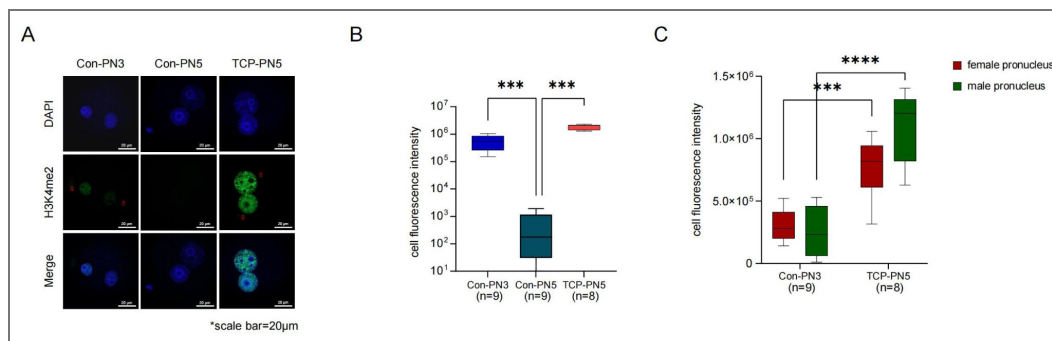
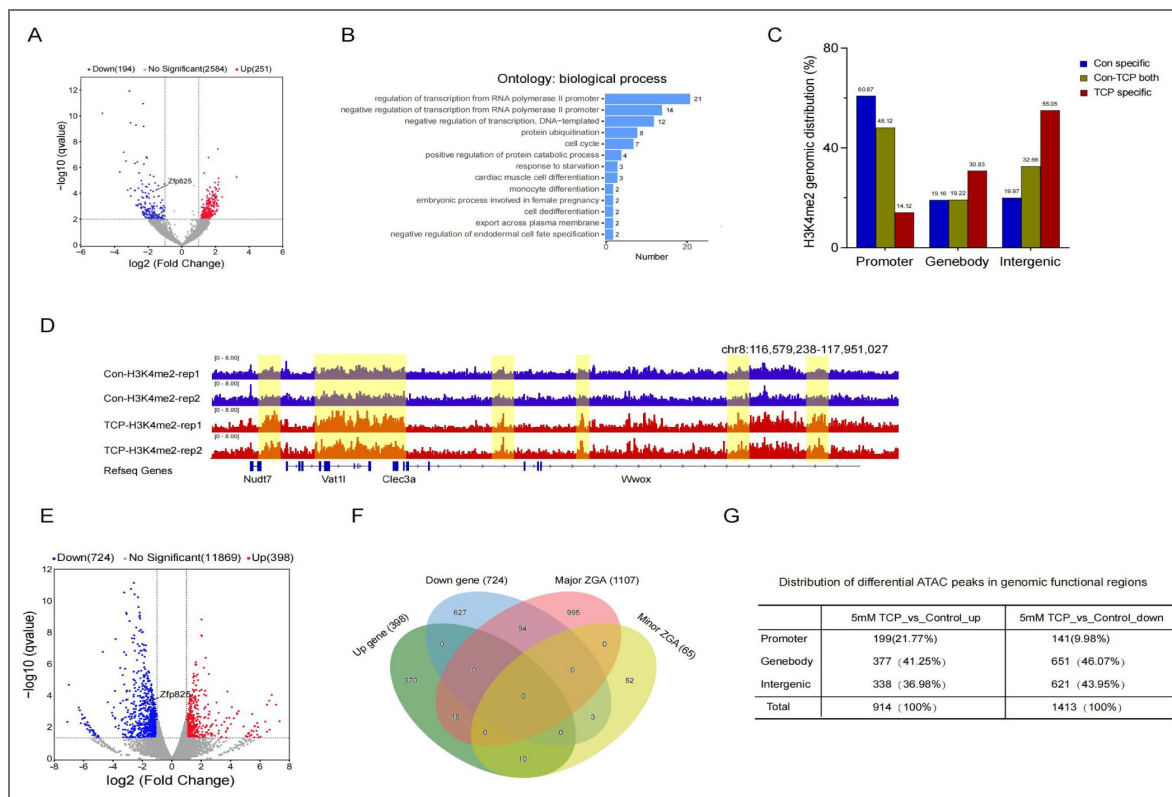


Figure 6. Inhibition of LSD2 affects gene expression

(A) A volcano map of genes with differential H3K4me2 expression after TCP inhibition of LSD2 expression. (B) The GO analysis showed enrichment of biological processes after an increase in the expression of H3K4me2. (C) The peak distribution area of H3K4me2 in the experimental group (5 mM TCP) and the control group (D) The IGV view shows the H3K4me2 signals in typical genes in the experimental group (5 mM TCP) and the control group. (E) A volcano map of the differential expression of H3K4me2 genes in the experimental group (5 mM TCP) and the control group. (F) The Venn diagram illustrates the intersection of differentially expressed genes and minor ZGA and major ZGA genes between the experimental group (5 mM TCP) and the control group (G) The distribution of differential ATAC peaks in the functional regions of the genome.



ICM; these genes played key roles in RNA metabolism and RNA splicing (Figure 7E [↗](#)). We then combined previous H3K4me3 data and chromosome accessibility data and found that these regions overlapped with high CG density regions, H3K4me3 enrichment, and chromatin accessibility regions (Figure 7E [↗](#)). Our results showed that H3K4me2 preferentially occupied hypomethylated and CpG-dense regions (Figures S5A-C [↗](#)).

We further examined how the stage-specific distal peaks were reprogrammed and found that about 35% of the promoters of the genes that were associated with cell differentiation and development did not undergo the large-scale re-established of H3K4me2 in the four-cell, eight-cell, and blastocyst stages (Figure 7E [↗](#)). We identified genes expressed in specific stages and examined their promoter H3K4me2 (Figure S5E [↗](#)). We found promoters with constantly high H3K4me2 that are preferentially CpG-rich, whereas promoters with constantly low H3K4me2 were generally CpG-poor (Figures 7F [↗](#) and S5E [↗](#)). A group of genes showed dynamic promoter H3K4me2 that correlated with gene expression (Figure S5E [↗](#)). These genes played key roles in development, differentiation, and morphogenesis. Therefore, our results indicated that H3K4me2 of the promoter during early embryonic development correlated with gene activities and CpG density.

Next, we investigated distal H3K4me2 signals and found that four/eight-cell embryos also showed widespread distal H3K4me2 signals (Figure 7C [↗](#)). We first identified the distal H3K4me2 regions in mouse gametes and pre-implantation embryos. These regions were relatively CpG-rich (although lower than promoters) and hypomethylated (Figures S5B [↗](#) and S5C [↗](#)). However, compared to promoters, distal H3K4me2 was correlated with CpG density in a continuous and monotonic manner (Figure S5A [↗](#)). To summarize, these results indicated that widespread distal H3K4me2 occurred in CpG-rich and hypomethylated regions in mouse four/eight-cell embryos.

Transcription regulation, CCREs priming, and resolving during maternal-to-zygotic transition

A study showed that most of the transcription factor binding regions overlap with H3K4me2-enriched regions⁵⁵. To investigate the association between H3K4me2 and transcription regulation, we identified stage-specific distal regions and the accessibility in these distal peaks of H3K4me2 (Figure 7F [↗](#)). Then, we identified the binding motifs for transcription factors (TFs) enriched in these distal regions (Figure S5D [↗](#)). TFs in mESC and eight-cell embryos include pluripotency factors involved in the development of the system^{56, 57}. We found that CTF was specifically enriched in eight-cell embryos. The GATA and KLF families are enriched in ICMs.

In a study on humans, we found widespread deposition of H3K4me3 at the promoters, including those for developmental genes, before ZGA. These genes were either activated or repressed after ZGA⁵⁰. Such promoter priming is related to embryonic development. To determine whether this also occurs in mice, we identified specific H3K4me2 target genes in the four/eight-cell stage and compared them to genes that are expressed or inactive at all stages. The H3K4me2 of promoters in the four/eight-cell-specific group decreased in ICM (Figures 8A [↗](#) and S6A [↗](#)). The “active” genes showed strong H3K4me2, H3K4me3, and ATAC-seq signals before and after ZGA (Figures 8A [↗](#) and S6A [↗](#)). Next, we investigated if such priming-resolving epigenetic transition also occurs in the distal regions. Distal H3K4me2 sites were enriched on regulatory elements, as 75.4% sites (compared to 16.2% of random) of them overlapped with candidate cis-regulatory elements (CCREs), identified by ENCODE (Figure 8B [↗](#)).

Enhancers are DNA sequences that increase promoter activity and, in turn, the frequency of gene transcription. The enhancer region is usually modified by H3K27ac (catalyzed by P300/CBP) and H3K4me1 histone 2 (catalyzed by MLL3/4)⁵⁸, whereas the H3K4me3 modification level is low. This modification pattern is the opposite of the pattern found in the promoter region. We compared histone modification to H3K4me2 and H3K27ac and found no significant signal overlap between the two histone modifications at specific developmental stages in the distal region. Distal four/eight-cell H3K4me2 marked CCREs showed high enrichment of chromatin accessibility (Figures 8C [↗](#) and S6B [↗](#)). After the four/eight-cell stage, one class (“active”) remained accessible and was associated with basic biological processes (GREAT analysis). In contrast, the other class

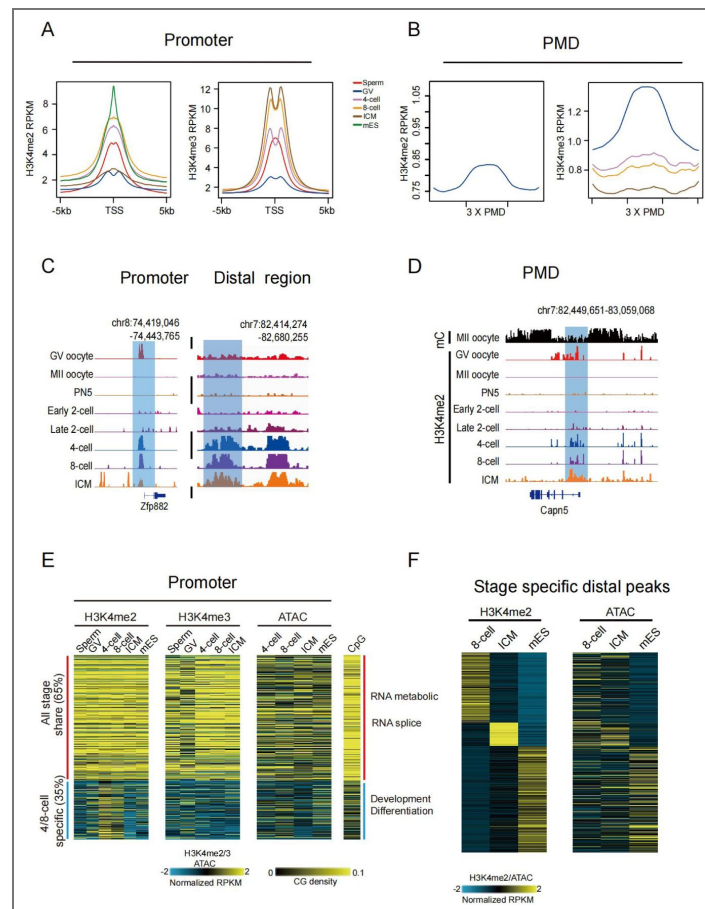


Figure 7. Pervasive H3K4me2 in CpG-rich regulatory regions in mouse embryos.

(A-B) H3K4me2 and H3K4me3 enrichment near TSS (TSS $\pm$ 5 kb) and PMD (3 $\times$ PMD) in mouse gametes, pre-implantation embryos, and mESC. (C) The IGV view shows promoter H3K4me2 signals and widespread distal H3K4me2 signals in mouse oocytes and early embryos. (D) The IGV view shows H3K4me2 signals and the distribution of DNA methylation in mouse oocytes and early embryos at the PMD (partially methylation district) region. (E) Heat maps show the enrichment of H3K4me2 and H3K4me3 at the promoters, including that in all stages shared and four-cell/eight-cell specific. ATAC-seq, promoter CpG density, and GO analysis results are presented. (F) Heat maps of the distal peaks of H3K4me2 expressed in specific stages among eight-cell, ICM, and mESC. ATAC-seq are shown.

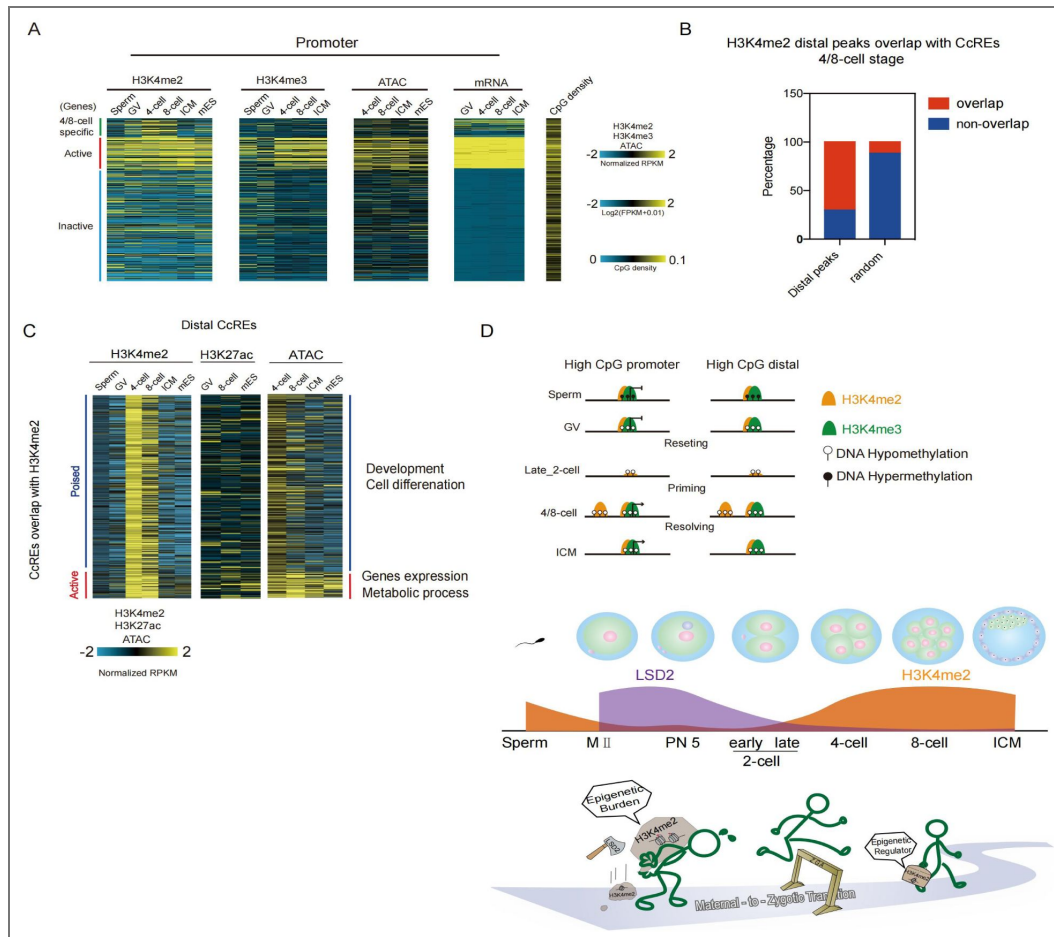


Figure 8. Chromatin states at specific stages and the H3K4me2 mode chart during mouse maternal-to-zygotic transition.

(A) Heat maps of H3K4me2, H3K4me3, and ATAC-seq are shown, “All active” and “All inactive” promoters and the corresponding gene expression are presented. The CpG densities are also shown. (B) The bar graph shows that the H3K4me2 distal peaks overlapped with the CCREs four/eight-cell stage. Random peaks are shown as a control. (C) Heat maps of the chromatin states of the overlap between CCREs and the distal peaks of H3K4me2 and H3K27ac are presented. Clustering was conducted using ATAC-seq, H3K4me2 in the four-cell, eight-cell, and ICM stages. The results of the GREAT analysis for each cluster are shown (right). Data on histone modification of H3K27ac in mice was obtained from the GEO database, GSE72784. (D) Dynamic reprogramming of H3K4me2 during mouse parental-to-zygotic transition. A schematic model of “H3K4me2 resetting” during parental-to-zygotic transition is shown. After fertilization, the parental H3K4me2 is almost globally erased. *De novo* H3K4me2 promoters and distal regions in CpG-rich and DNA hypomethylated regions are re-established at the late two-cell stage, probably as a priming chromatin state. This leads to the resolution of primed promoters to “active” promoters with H3K4me3, or “poised”. A similar transition occurs for putative enhancers.

(Figures 8C [↗](#) and S6B [↗](#); “poised”) was associated with H3K27me3 in the ICM and somatic cells; it was located preferentially near developmental genes. These results indicated that the widespread *de novo* H3K4me2 was re-established following ZGA, and H3K4me2 might be globally reset to a permissive state before it is resolved after the four/eight-cell stage (Figure 8D [↗](#)).

Discussion

Histone modifications are fundamental epigenetic marks. The reprogramming of histone marks plays an important role in early development.⁹ In this study, we profiled the H3K4me2 landscape in mESC, oocytes, and early embryos using a modified low-input, high-sensitivity CUT&RUN method. We found the H3K4me2 showed differential patterns in sperms and oocytes and exhibited non-canonical patterns in GV oocytes but canonical patterns in the sperm in mice.³⁶ Additionally, the GV H3K4me2 peaks were almost erased in MII oocytes, and reconstructed accompanying ZGA occurred. Our results indicated that maternal H3K4me2 was lost during oocyte maturation. The non-canonical H3K4me2 in mammalian embryos and pervasive H3K4me2 in CpG-rich regulatory regions were found in four-cell and eight-cell embryos and in ICM. Hence, we described the first landscape and elucidated the potential function of H3K4me2 during early mammalian development. We also proposed a novel model, known as “Epigenetic Burden”, according to which parental H3K4me2 exhibits an “Epigenetic Burden” of maternal-zygote-transition. This burden is erased by maternal factor LSD2 before ZGA. H3K4me2 plays a regulatory role after ZGA (Figure 8 [↗](#) X). H3K4me2 undergoes a transition that starts from an “Epigenetic Burden”, and then becomes an “Epigenetic regulator” during early histone modification reprogramming.

The paternal epigenetic inheritance is important for development and disease in offspring. Significant epigenome reprogramming occurs and is associated with key processes during early mammalian development.^{1, 3, 9, 14, 39–41, 59–63} Genome-wide H3K4me3 exhibited a non-canonical pattern (ncH3K4me3) in full-grown and mature oocytes, i.e., it occurred as broad peaks at promoters and many distal loci.^{40, 44, 48} We found that H3K4me2 was a non-canonical pattern in the GV oocyte, but it was weakened in the MII oocyte, indicating that H3K4me2 plays an important role during oocyte maturation. H3K4me3 is inherited in pre-implantation embryos and is erased in the late two-cell embryos when the process of canonical H3K4me3 is initiated.^{40, 44} The re-establishment of H3K4me2 mainly starts in the late two-cell stage, and it exists as a non-canonical pattern in the following stages. Several studies have shown that in many species, including mice, zebrafish, and humans, the parental epigenome undergoes dramatic reprogramming after terminally differentiated gamete fertilization, followed by subsequent re-establishment of the embryo epigenome, which leads to epigenetic “rebooting”.⁹ We found that H3K4me2 was erased by LSD2 after fertilization and re-established at the late two-cell stage. Inhibition of the deletion of H3K4me2 resulted in the arrest of embryonic development and abnormal expression of genes related to ZGA. This indicated that H3K4me2 is involved in the reactivation of the embryonic epigenome, and parental H3K4me2 is an “Epigenetic Burden”. H3K4me3 is an active gene expression marker, which mainly marks the gene promoter region. H3K4me1 and H3K4me2 mark enhancer and promoter regions. They have multiple functions for gene activation, according to different enzymes and complexes.^{22, 30, 35, 64–73} Some studies have reported a distinct pattern of H3K4me2 enrichment, spanning from the transcriptional start site (TSS) to the gene body, which marks a subset of tissue-specific genes. This pattern is associated with unique H3K4me2 profile mark tissue-specific gene regulation.^{33, 35} Our results showed non-canonical H3K4me2 in mammalian embryos and pervasive H3K4me2 in CpG-rich regulatory regions of four-cell and eight-cell embryos of mice. We next investigated whether H3K4me2 is associated with active or repressed genes in embryo development and found that promoter H3K4me2 in early mouse embryo development is correlated with gene activities and high CpG density. We also found that promoter H3K4me2 was consistent with H3K4me3.

Histone modification and chromatin accessibility profiles are usually used to identify transcription factor binding regions and motifs. In another study, we found large-scale chromatin accessibility in pre-ZGA human embryos, and these regions contained many transcription factors.³ Around 90% of the transcription factor binding regions overlap with the H3K4me2-enriched regions.

Stronger signals of H3K4me2 enrichment indicate a greater chance of overlap with the transcription factor binding regions³⁴. In this study, we identified the binding motifs for transcription factors enriched in these distal regions, and the CCAAT transcription factor (CTF) was found to be specifically enriched in eight-cell embryos. The GATA and KLF families were enriched in ICMs. GATA is a key regulator of primitive endoderm (PE), which is a part of the ICM⁷⁴, and KLF factors support naive pluripotency⁷⁵. These results showed that stage-specific distal H3K4me2 involves transcription circuitry during mammalian embryonic development.

The state of chromatin influences DNA replication, DNA repair, and gene expression. Accessible chromatin occupies regulatory sequences, such as promoters and enhancers⁷⁶. These elements interact with cell type-specific transcription factors to start transcription processes that determine cell fate⁷⁷. Animal embryos in the early stages undergo extensive histone modification reprogramming, and chromatin remodeling before terminally differentiated gametes develop into pluripotent cells. Some studies have found extensive overlap of accessible promoters and enhancers at the four-cell stage in humans⁵⁰. This finding indicated that crosstalk occurs between histone modification and the chromatin state. In our study, most of the H3K4me2 was re-established in chromatin-accessible regions, including promoters and distal regions at the four-cell stage. Thus, we inferred that H3K4me2 might play an important role in shaping the chromatin state. Our results indicated that crosstalk occurs between accessible chromatin and histone modification.

Dynamic histone modification plays an important role during mammalian maternal-to-zygotic transition (MZT). Approximately 22% of the oocyte genome associated with broad H3K4me3 is negatively correlated with DNA methylation and becomes confined to transcriptional-start-site (TSS) regions in two-cell embryos, accompanied by the onset of major ZGA⁴⁸. Also, H3K4me2 is erased in MII oocytes, and sperm H3K4me2 is erased after fertilization. Similar to the pattern found for H3K4me3, H3K4me2 of the embryo was also re-established accompanied by the onset of major ZGA. The ncH3K4me3 in oocytes overlaps almost exclusively with partially methylated DNA domains⁴⁰. H3K4me2 is also enriched in canonical gene promoters and partially methylated domains (PMDs).

Overall, our findings provided a genome-wide landscape of H3K4me2 modification in mammalian oocytes and pre-implantation embryos. We showed that maternal LSD2 specifically demethylates H3K4me2 after fertilization in mammals. Also, H3K4me2 is erased to a greater extent in the male pronucleus than in the female pronucleus. Based on our results, we proposed a novel model, the “Epigenetic Burden” of histone modification reprogramming during mammalian development. This model can be used to further investigate the epigenetic regulation network in the early developmental stages of mammals.

Materials and methods

Culture of K562 cells

The human K562 cell line was cultured at 37 °C, 5% CO₂, and saturated humidity. The cells were cultured in 1640 culture medium (GIBCO) containing 10% fetal bovine serum and 90% 1640 culture liquid.

Cell culture of mouse ESC

Mouse embryo stem cells (ESC) were cultured on 0.1% gelatin-coated plates without irradiated mouse embryonic fibroblasts (MEFs), using standard ESC medium consisting of DMEM (GIBCO) supplemented with 15–20% fetal bovine serum (FBS, Sigma), 0.1 mM β-mercaptoethanol (Sigma), 0.1 mM MEM non-essential amino acids (Cellgro), 2 mM Glutamax (GIBCO), 1% nucleoside (Millipore), 1% EmbryoMax® Penicillin-Streptomycin Solution (Millipore), and 10 µg/L recombinant leukemia inhibitory factor (LIF) (Millipore). The mouse embryo stem cells were cultured at 37 °C, 5% CO₂, and saturated humidity.

Collection of mouse gametes and early embryos

The mouse oocytes were collected from 4–5-week-old C57BL/6N female mice (purchased from Charles River). The pre-implantation embryos were harvested from 4–5-week-old C57BL/6N females mated with DBA/2 males (eight-week-old when purchased from Charles River). The Germinal vesicle (GV) oocytes were obtained from 4–5-week-old C57BL/6N females, 46–48 h after they were injected with 10 IU of pregnant mare serum gonadotropin (PMSG, Solarbio). The 4–5-week-old C57BL/6N female mice were also administered 10 IU of human chorionic gonadotrophin (hCG) intraperitoneally, 46–48 h after PMSG was administered. Then, they were mated with DBA/2 males. The PN5 zygotes were collected at 28–32 h post-hCG injection. Early two-cell, late two-cell, four-cell, and eight-cell embryos, morulas, and blastocysts were harvested at 32–36 h, 45–48 h, 54–58 h, 60–64 h, 68–75 h, and 84–96 h post-hCG injection. The oocytes in the metaphase of second meiosis (MII) were collected 12–14 h after 10 IU of hCG was injected intraperitoneally. The zona pellucida of all oocytes and embryos was removed using glutathione (GSH Solarbio).

Tranlycypromine treatment of mouse embryos

One-cell embryos were obtained at 24–26 h (PN3) and 28–32 h (PN5) post hCG injection and cultured in the G1 medium in the presence or absence of Tranlycypromine (5 mM). After 48 h, the embryos were transferred to the G2 medium with or without 5 mM TCP. All embryos were maintained at 37 °C in a humidified atmosphere containing 5% CO₂. Then, morphologically normal PN5, late two-cell, and four-cell embryos were collected for further experiments.

Immunofluorescence

The samples (mouse oocytes or pre-implantation embryos) were washed twice in MII medium (Sigma), and 4% PFA was used as a fixation buffer (BOSTER, AR1068) to fix samples at room temperature for about 60 min. Next, the samples were transferred using a mouth pipette and washed once with 1% BSA (Sigma, A1933) in 1×PBS (Corning, 21–040-CVR). The samples were then permeabilized using 0.5% Triton X-100 (Sigma, T8787) in 1×PBS at room temperature for 20 min. Then, they were washed with 1% BSA in 1×PBS and blocked using 1% BSA in 1×PBS at room temperature for 1 h. The blocked samples were incubated with H3K4me2 antibody (Sigma Millipore, 07030) in 1% BSA in 1×PBS diluted at a ratio of 1:500, at room temperature for 2 h or 4 °C overnight. After incubation, they were washed at least five times with 1% BSA in 1×PBS for 3 min. The samples were incubated with Alexa Fluor 568-conjugated donkey anti-rabbit IgG (Invitrogen, A10042) in 1% BSA in 1×PBS diluted at a ratio of 1:200, at room temperature for 50 min in the dark. After incubation, the samples were washed five times with 1% BSA in 1×PBS for 3 min. Finally, the samples were stained using the ProLong[®] Gold Antifade Reagent with DAPI (Cell Signaling, 8961S), using a 4-Chamber Glass Bottom Dish (100 per case), 35 mm Dish with 20 mm Bottom Well (Cellvis, D35C4–20–1-N). The samples were incubated at room temperature for about 24 h in the dark. All images were captured using a Zeiss 700 confocal microscope. Raw images were projected along the Z-axis and processed using the ZEN software (Zeiss) with brightness and contrast adjusted (the same parameters were used for each channel of all samples). In the functional test section, we used the LSD2 antibody (Abcam, ab193080), H3K4me1 antibody (Abcam, ab176877), H3K4me2 antibody (mAb) (Active motif, 39079), and H3K4me3 antibody (Active motif, 39016) as primary antibodies (diluted at a ratio of 1:500). Secondary antibodies (diluted at a ratio of 1:300) used included Goat Anti-Rabbit IgG H&L (Alexa Fluor[®]488) (Abcam, ab150077) and Goat Anti-Mouse IgG H&L (Alexa Fluor[®] 488) (Abcam, ab150113).

Zona pellucida removal and ICM/TE isolation

Before the CUT&RUN experiment was conducted, to guarantee the effect of permeation, the zona pellucida of all oocytes and embryos was chemically removed by glutathione (GSH Solarbio). The zona pellucida was removed after about 10–30 s. Then, the samples were washed twice in PBS (Corning 21–040-CVR) and washed once with wash buffer. The inner cell mass (ICM)s or TEs of day 4–5 blastocysts were isolated mechanically. Before performing CUT&RUN, the separated cells were washed twice with PBS and once with wash buffer, and then, resuspended in wash buffer.

RNA-seq

For RNA-seq, 30–40 two-cell embryos were used for one sample. They were transferred to 1× PBS drops at room temperature and washed thrice to avoid any contamination. The cells were lysed in 1 µL of ice-cold lysis buffer for 10 min. Transcriptome amplification was performed using a Single Cell Full Length mRNA- Amplification Kit (Vazyme, N712). The RNA-seq library was constructed using True Prep DNA Library Prep Kit V2 for Illumina® (Vazyme, TD502). Finally, the library was sequenced using an Illumina Nova 6000 sequencer (Illumina). The data were analyzed following standard RNA-seq procedures.

Generation of miniATAC-seq library

The miniATAC-seq libraries were prepared as previously described³. Cells for miniATAC-seq were prepared in the same way as those prepared for RNA-seq.

CUT&RUN library construction and next-generation sequencing

The CUT&RUN method was used following published protocols^{37, 38} with modifications in antibody binding, adaptor ligation, and DNA purification steps. All samples (including mouse zygotes, embryos with zona pellucida removed, or ESCs) were transferred into 0.2 mL conventional, non-low-binding PCR tubes (Axygen). The samples were resuspended in 60 µL of wash buffer (20 mM HEPES-KOH, pH = 7.5; 150 mM NaCl; 0.5 mM Spermidine; Roche complete protease inhibitor). Concanavalin-coated magnetic beads (Polyscience, 86057) were gently washed thrice or four times and resuspended in binding buffer (20 mM HEPES-KOH, pH = 7.9; 10 mM KCl; 1 mM CaCl₂; 1 mM MnCl₂), and then, 10–15 µL of Concanavalin- coated magnetic beads were added to each sample. The samples were incubated at room temperature (about 25 °C) at 450 rpm for 20 min in a Thermomixer (Eppendorf). The samples were placed on a magnetic stand for removing the supernatant, and then, resuspended in 50 µL antibody buffer (wash buffer plus digitonin 1000:1, 2 mM EDTA, pH = 8.0) with H3K4me2 antibody (Sigma Millipore, 07030) diluted at ratio of 1:100. The samples were incubated at room temperature (23–26 °C) for 2 h at 450 rpm on Thermomixer, or incubated overnight (≥ 16 h) at 4 °C and 450 rpm in a Thermomixer.

Next, the samples were held at the magnetic stand and washed with 190 µL of dig wash buffer (wash buffer plus digitonin, freshly pre-heated, 0.005%–0.01%; tested for each batch) once, and then, washed with 100 µL of the buffer again. The samples were then resuspended in 50 µL of dig wash buffer with PA/G-MNase (to a final concentration of 700 ng/mL), and incubated at room temperature (23–26 °C) on Thermomixer for 1 h, or incubated at 4 °C on Thermomixer for 3 h at 450 rpm. The samples were washed with 190 µL of dig wash buffer twice and then washed again with 100 µL of the buffer at the magnetic stand. Next, 100 µL of dig wash buffer was added and incubated on ice for 3 min. Targeted cleavage was performed on ice by adding 2 µL of 100 mM CaCl₂ for 30 min, and the reaction was stopped by adding 100 µL of 2×stop buffer (340 mM NaCl; 20 mM EDTA, pH = 8.0; 4 mM EGTA, pH = 8.0; 50 µg/mL RNase A; 100 µg/mL glycogen; 0.005%–0.01% preheated digitonin; tested for each batch), and then, the mixture was fully vortexed. The samples were incubated at 37 °C for 30 min for fragment release. The total samples or supernatants were digested by adding 10 ng of carrier RNA (Qiagen, 1068337), 1 µL of 20% SDS, and 2 µL of Proteinase K (Roche). The reaction was performed at 56 °C for 45 min and 72 °C for 20 min in a thermal cycler. DNA was purified using phenol-chloroform, followed by ethanol purification. Then, 200 µL of phenol-chloroform was added, mixed, and centrifuged at 20 °C and 16,000 *g* for 5 min. Next, 200 µL of chloroform was added to each sample. After mixing, the samples were centrifuged at 20 °C and 16,000 *g* for 5 min and transferred to Lo- binding 1.5 mL tubes. Each sample was mixed with 2 µL of glycogen, 20 µL of NaOAC, and 500 µL of 100% ethanol (precooled at –20 °C). The mixture was incubated at –20 °C overnight.

The samples were centrifuged at 16,000 *g* and 4 °C for 20 min, and then, the supernatant was removed. Next, 1 mL of anhydrous ethanol was added to the samples, which were then centrifuged at 4 °C and 16,000 *g* for 10 min. The supernatant was removed by opening the lid of the tubes and air-drying. After the liquid disappeared, purified DNA was used for Tru-Seq library

construction using NEB Next Ultra II DNA Library Prep Kit for Illumina (NEB, E7645S). For the NEB kit, the DNA was resuspended in 25 μ L of ddH₂O, followed by end-repair/A-tailing with 3.5 μ L of End Prep buffer and 1.5 μ L of an enzyme mix. The reaction was conducted at 20 °C for 30 min and 65 °C for 30 min in a PCR machine. Then, 1.25 μ L of diluted adaptors (NEB Multiplex Oligos for Illumina, E7335S) were added and thoroughly vortexed. Next, 15 μ L of Ligation master mix and 0.5 μ L of Ligation enhancer were added, and the mixture was incubated at 16–20 °C for 16–20 min, or at 4 °C overnight. After the ligation reaction was over, the samples were treated with 1.5 μ L of USERM enzyme. The reaction was performed at 37 °C for 15 min in a PCR machine. The samples were purified using 1 $\times$ AMPure beads. The PCR was performed by adding 25 μ L of 2 $\times$ KAPA HiFi Hot Start Ready Mix (KAPA biosystems, KM2602) with 2.5 μ L of primers of the NEB Oligos kit and 2.5 μ L of Illumina universal primers. The conditions used were as follows: 98 °C for 45s, (98 °C for 15s and 60 °C for 10s) with 16–17 cycles, and 72 °C for 1 min. The final libraries were purified using 0.9 $\times$ AMPure beads and used for next-generation sequencing.

Data analysis

CUT&RUN data processing

The single-end CUT&RUN data were mapped to the mm9 genome using Bowtie2 (Version 2.2.9)⁷⁸ with the parameters `-t -q -N 1 -L 25`. The paired-end CUT&RUN data were mapped based on the parameters: `-t -q -N 1 -L 25 -X 1000 --nomixed --no-discordant`. All unmapped reads, non-uniquely mapped reads, and PCR duplicates were removed. For downstream analysis, the read counts were normalized by calculating the number of reads per kilobase of bin per million reads sequenced (RPKM) for 100-bp bins of the genome. The RPKM values were further Z-score normalized for minimizing the batch effect.

ATAC-seq data processing

The ATAC-seq reads were mapped to the mm9 genome using Bowtie2 (version 2.2.2)⁷⁸ with previously described parameters. All unmapped reads, non-uniquely mapped reads, and PCR duplicates were removed. For downstream analysis, the read counts were normalized by calculating the RPKM for 100 bp-bins of the genome. The RPKM values were further normalized by Z-score normalization to minimize the batch effect.

DNA methylation data processing

We used the Bismark software for mapping DNA methylation reads to the mm9 genome. PCR duplicates were removed. For each CpG site, the methylation level was computed as the total methylated counts divided by the total counts across all reads covering the CpG site.

Analysis of the promoter H3K4me2 and H3K4me3 enrichment

Annotated promoters (TSS $\pm$ 2.5 kb) of the mm9 refFlat annotation database from the UCSC genome browser were used. Whole-genome Z-score normalized RPKM scores were used for assessing the data.

Identification of stage-specific distal ATAC-seq peaks

Stage-specific distal ATAC-seq peaks were identified as described previously. Briefly, distal peaks from human embryos and ES cells were merged, and then, the average Z-score was normalized. The RPKM scores were calculated based on them. A Shannon entropy-based method³ was used to identify stage-specific peaks. Peaks with entropy less than 2 were selected as candidates and were further screened using the following criteria: the distal peak had high enrichment at this stage (normalized RPKM > 1) and positive enrichment (normalized RPKM > 0) at no more than two additional stages.

Minor/major ZGA genes and stage-specific genes were screened using the RNA-seq data from mouse germ cells and embryos. ZGA genes were defined as those unexpressed or underexpressed in oocytes (FPKM < 5) but highly upregulated (FPKM > 5, at least three-fold upregulation) in either the one-cell or early two-cell embryo stage (minor ZGA genes) or the late two-cell embryo stage

(major ZGA genes). The genes that were highly expressed in oocytes and considerably upregulated (over five-fold upregulation) during the late two-cell embryo stage were also defined as major ZGA genes.

For stage-specific genes in eight-cell embryos and ICMs, a Shannon entropy-based method³ was used to identify stage specific-genes, as previously described. Maternally expressed genes, defined as those expressed in GV or MII oocytes (FPKM ≥ 1), were excluded from this analysis to avoid the confounding effects of lingering maternal RNAs.

Clustering analysis

We conducted k-means clustering of gene expression levels at various stages using Cluster 3.0 with the parameters `-g 7` (Euclidean distance). Heat maps were generated using Java Treeview 3.0. We conducted hierarchical clustering analysis for H3K4me3 at various stages using the R package “ape” based on Pearson’s correlation between each pair of stages. The distance between the stages was calculated as $1 - \text{Pearson's correlation}$.

Identification of oocyte PMDs

We calculated the average DNA methylation levels for 10-kb bins of the genome and counted the number of CpG dyads in each 10-kb bin. Bins with an average DNA methylation level below 0.6 and above 20 CpG dyads were selected and merged into PMDs using BEDtools (Version v2.25.0)⁷⁹. The promoter regions (± 2.5 kb) were removed from PMDs.

Identification of H3K4me2 peaks

The H3K4me2 peaks were called using MACS2 (Version 2.0.9)⁸⁰ with the parameters `--broad --nomodel --nolambda -g mm 1.87e9/-g hs 2.7e9`. Noisy peaks with weak signals (summed RPKM < 0) were removed before further analyses were conducted.

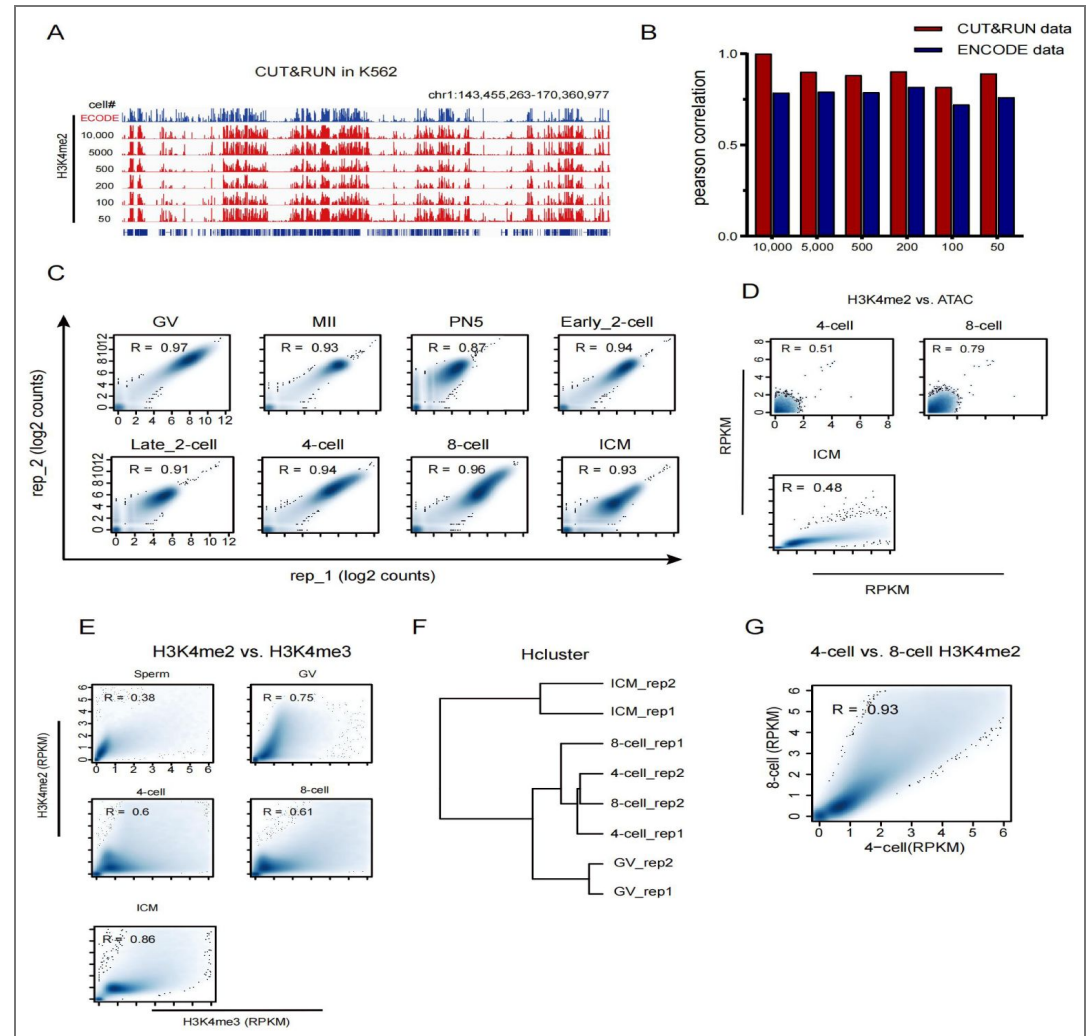
Statistical analysis

All statistical analyses in this study were briefly described in the text and performed using R (v3.6.1) (<http://CRAN.R-project.org/>⁸¹).

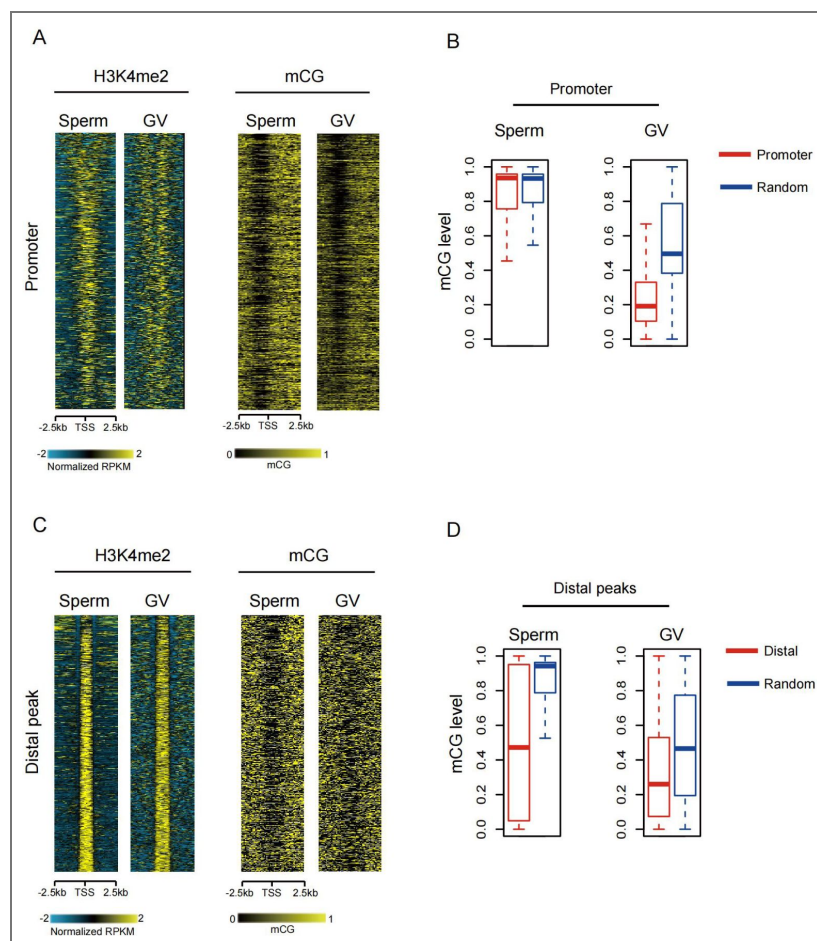
Data availability

All raw sequencing data generated during this work have been submitted to the Genome Sequence Archive in National Genomics Data Center (China National Center for Bioinformatics/Beijing Institute of Genomics, Chinese Academy of Sciences). The GSA accession ID of this paper is CRA004978.

Extended data figures and table



Extended Data Figure 1. Validation of H3K4me2 CUT&RUN data in Human K562 cell line, oocytes and early embryos. (A) The IGV views showing H3K4me2 distributions by CUT&RUN using various numbers of Human K562 cell line. The ENCODE references are added for comparison. GEO accession: GSM733651 . (B) The Pearson correlation coefficients showing the comparison between CUT&RUN of H3K4me2 using various numbers of Human K562 cells and conventional ChIP-seq data from ENCODE. (C) Scatter plots showing the correlations of biological replicates ($n=2$) of H3K4me2 signals (2kb-bin, whole genome) by CUT&RUN for each stage of mouse oocytes and early embryos. The Pearson correlation coefficients are also shown. (D) Scatter plots comparing the H3K4me2 signals with ATAC-seq (Assay for Transposase-Accessible Chromatin with high throughput sequencing) at promoters or distal accessible regions in mouse oocytes and early embryos. Spearman correlation coefficients are also shown. (E) Scatter plots comparing the H3K4me2 signals with H3K4me3 signals in mouse sperm, oocytes and early embryos. Spearman correlation coefficients are also shown. (F) Hierarchical clustering of H3K4me2 mouse oocytes and early embryos. Pearson correlation was used to measure distances. (G) Scatter plots comparing the H3K4me2 signals between 4-cell and 8-cell in mouse embryos. Spearman correlation coefficients are also shown.

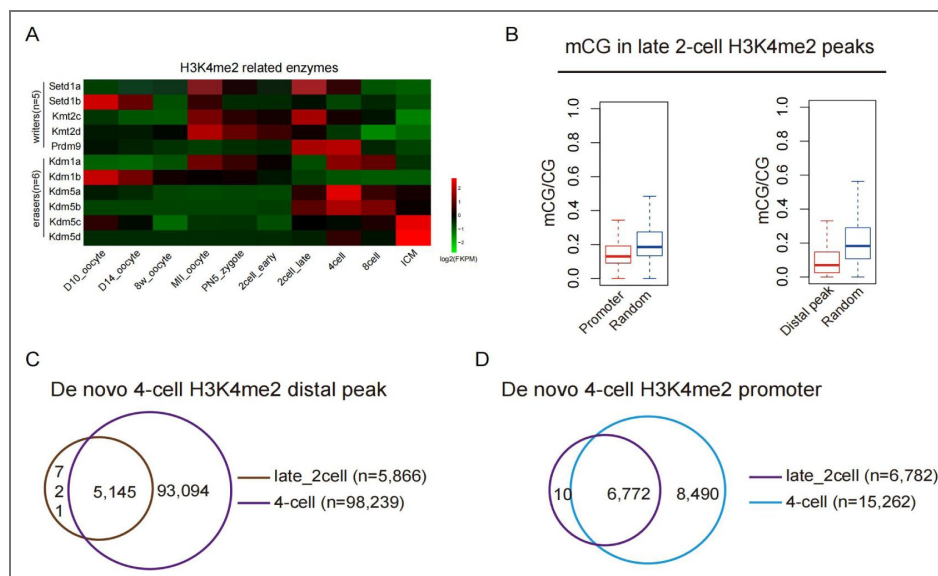


Extended Data Figure 2. H3K4me2 in mouse gametes.

(A) Heatmaps showing H3K4me2 signals at all promoter regions (TSS $\pm$ 2.5kb) in mouse gametes. DNA methylation (TSS $\pm$ 2.5kb) is also mapped. DNA methylation data cited in GEO dataset: GSE56697. (B) The box plots showing DNA methylation level at all promoter regions in mouse gametes. Random peaks are shown as a control. DNA methylation data cited in GEO dataset: GSE56697. (C) Heatmaps showing H3K4me2 signals at all distal regions (peak $\pm$ 2kb) in mouse gametes. DNA methylation (peak $\pm$ 2kb) is also mapped. DNA methylation data cited in GEO dataset: GSE56697. (D) The box plots showing DNA methylation level at all distal regions in mouse gametes. Random peaks are shown as a control. DNA methylation data cited in GEO dataset: GSE56697.

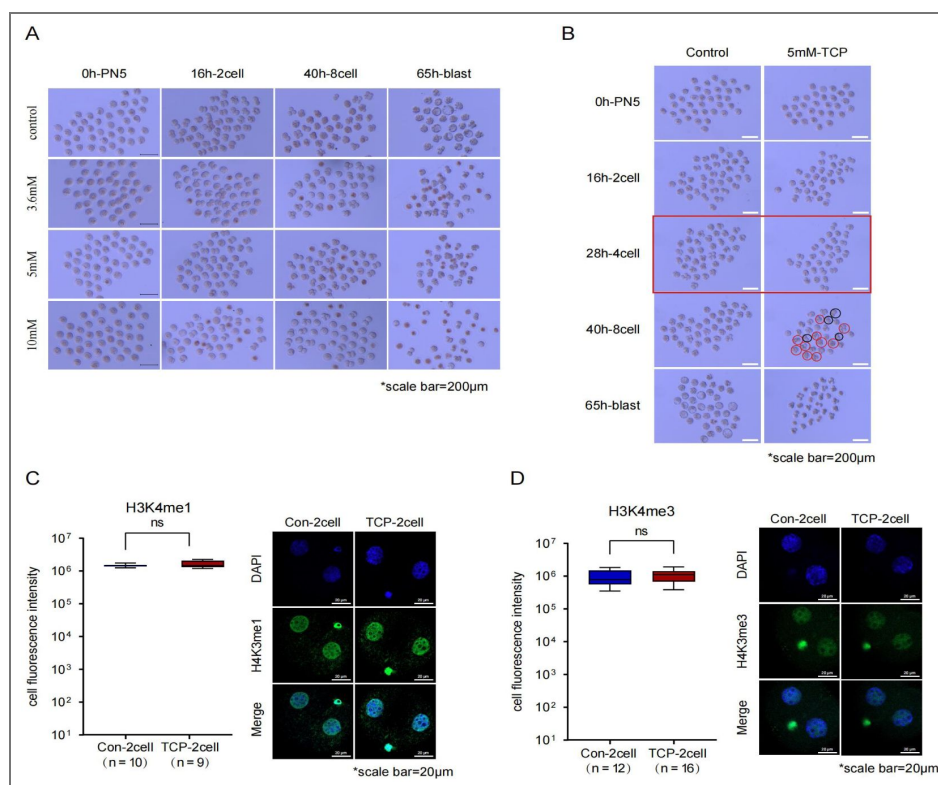
Extended Data Figure 3. H3K4me2 in late 2-cell embryos.

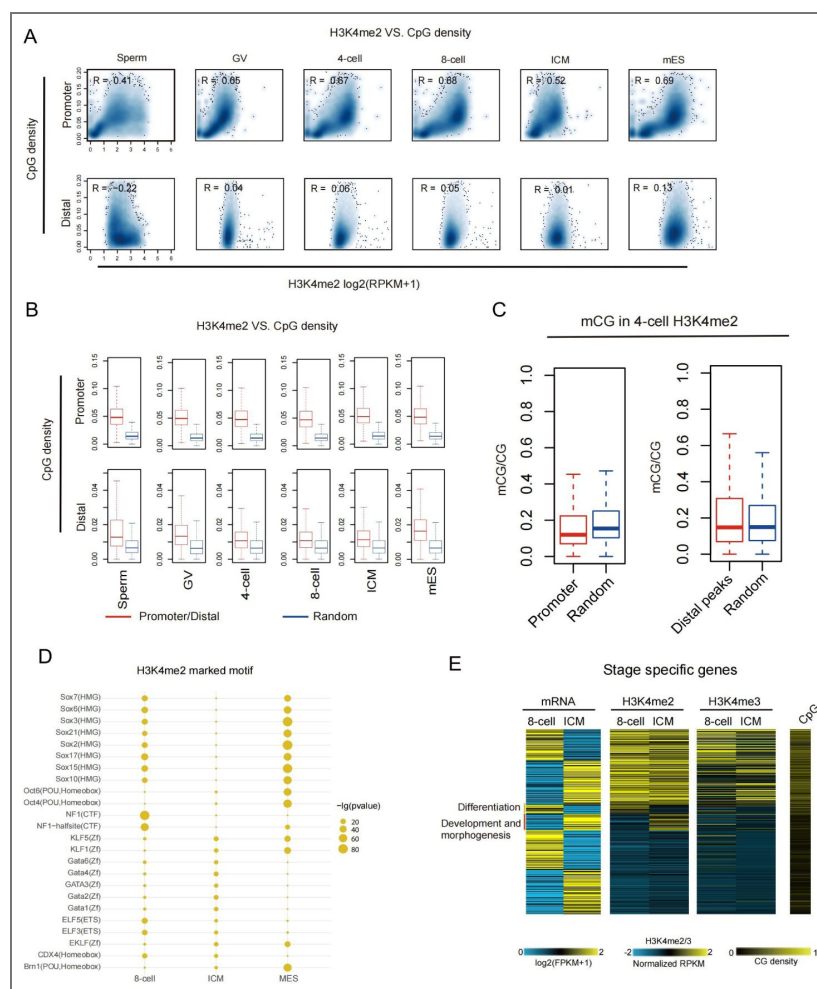
(A) Heatmaps showing the expression of H3K4me2 related regulators in mouse (based on published RNA-seq data43). (B) The box plots showing DNA methylation level at all promoter regions and distal region in mouse 2-cell stage. Random peaks are shown as a control. (C-D) Venn diagrams showing De novo 4-cell H3K4me2 promoter and distal peaks number.



Extended Data Figure 4. Cell culture experiment under TCP treatment

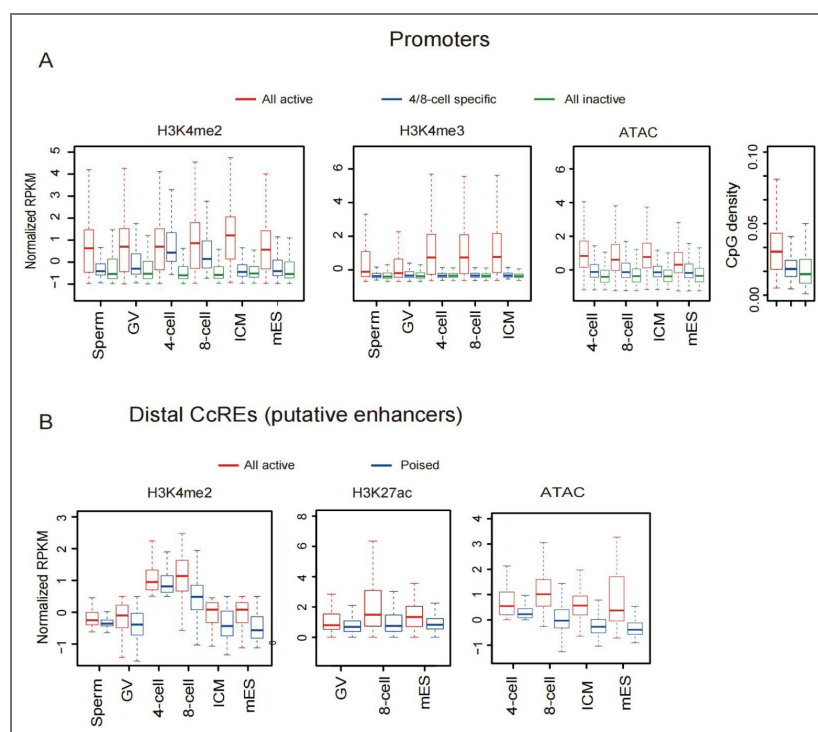
(A) Morphological observation of cells under different concentration of TCP culture, scale bar is also shown. (B) Morphological observation of embryo development after 5mM TCP inhibition of LSD2 expression. (C-D) Immunofluorescence images showed the immunofluorescence intensity of H3K4me1 (C) and H3K4me3 (D) of 2-cell embryos in the experimental group (5mM TCP) and control group, scale bar is also shown.





Extended Data Figure 5. H3K4me2 in mouse 4/8-cell embryos.

(A) Scatter plots comparing the promoter and distal H3K4me2 signals with CpG densities in mouse gametes, 4-cell, 8-cell, ICM and mESC. The Spearman correlation coefficients are also shown. (B) The box plots showing the CpG density and promoter/distal H3K4me2 peaks. Random peaks are shown as a control. (C) The box plot showing DNA methylation in promoter and distal 4-cell H3K4me2 peaks. Random peaks are shown as a control. (D) TF motifs identified from active enhancers in mouse early embryos (8-cell, ICM) and mESC. showing motif enrichment p value < 1e-20 at least at one stage were included. Circle size showing TF enrichment and the expression of the TF is color coded. (E) Heatmaps showing stage-specifically expressed genes further classified by their H3K4me2 states (high, dynamic, and low). RNA-seq, H3K4me3 state, CpG densities and GO analysis results are also mapped and shown.



Extended Data Figure 6. Chromatin states at specific stages and distal CcREs.

(A) Box plots showing H3K4me2, H3K4me3 and ATAC-seq signals, “All active” and “All inactive” promoters in mouse gametes, embryos and mESC. CpG density for each group is also shown (right). (B) The box plot showing the ATAC-seq, H3K4me2 and H3K27ac signals at putative active, poised or random enhancers in mouse gametes, early embryos or mESC, Mouse histone modified H3K27ac data refer to GEO database : GSE72784.

Sample name	Experiment	Replicate	Sample number	Cell number
K562 cell line	H3K4me2CUT&RUN	Rep1	50	50
K562 cell line	H3K4me2CUT&RUN	Rep2	50	50
K562 cell line	H3K4me2CUT&RUN	Rep1	100	100
K562 cell line	H3K4me2CUT&RUN	Rep2	100	100
K562 cell line	H3K4me2CUT&RUN	Rep1	200	200
K562 cell line	H3K4me2CUT&RUN	Rep2	200	200
K562 cell line	H3K4me2CUT&RUN	Rep1	500	500
K562 cell line	H3K4me2CUT&RUN	Rep2	500	500
K562 cell line	H3K4me2CUT&RUN	Rep1	5000	5000
K562 cell line	H3K4me2CUT&RUN	Rep2	5000	5000
K562 cell line	H3K4me2CUT&RUN	Rep1	10000	10000
K562 cell line	H3K4me2CUT&RUN	Rep2	10000	10000
MES cell line	H3K4me2CUT&RUN	Rep1	50	50
MES cell line	H3K4me2CUT&RUN	Rep2	50	50
MES cell line	H3K4me2CUT&RUN	Rep1	100	100
MES cell line	H3K4me2CUT&RUN	Rep2	100	100

Table 1. CUT&RUN experimental sample information

Sample name	Experiment	Replicate	Sample number	Cell number
MES cell line	H3K4me2CUT&RUN	Rep1	200	200
MES cell line	H3K4me2CUT&RUN	Rep2	200	200
MES cell line	H3K4me2CUT&RUN	Rep1	500	500
MES cell line	H3K4me2CUT&RUN	Rep2	500	500
MES cell line	H3K4me2CUT&RUN	Rep1	5000	5000
MES cell line	H3K4me2CUT&RUN	Rep2	5000	5000
MES cell line	H3K4me2CUT&RUN	Rep1	10000	10000
MES cell line	H3K4me2CUT&RUN	Rep2	10000	10000
GV oocyte	H3K4me2CUT&RUN	Rep1	190	190
GV oocyte	H3K4me2CUT&RUN	Rep2	190	190
MII oocyte	H3K4me2CUT&RUN	Rep1	165	165
MII oocyte	H3K4me2CUT&RUN	Rep2	165	165
PN5 zygote	H3K4me2CUT&RUN	Rep1	250	250
PN5 zygote	H3K4me2CUT&RUN	Rep2	250	250
Early_2cell	H3K4me2CUT&RUN	Rep1	86	172
Early_2cell	H3K4me2CUT&RUN	Rep2	86	172
Late_2cell	H3K4me2CUT&RUN	Rep1	98	196
Late_2cell	H3K4me2CUT&RUN	Rep2	98	196
4cell	H3K4me2CUT&RUN	Rep1	65	260
4cell	H3K4me2CUT&RUN	Rep2	65	260

Table 1. (continued)

Sample name	Experiment	Replicate	Sample number	Cell number
8cell	H3K4me2CUT&RUN	Rep1	25	200
8cell	H3K4me2CUT&RUN	Rep2	25	200
ICM	H3K4me2CUT&RUN	Rep1	14	≈ 420
ICM	H3K4me2CUT&RUN	Rep2	14	≈ 420

Table 1. (continued)

Acknowledgements

This work was supported by the National Key R&D Program of China (2019YFA0802200 and 2019YFA0110900 to Jiawei Xu), the National Natural Science Foundation of China 32170819 to Jiawei Xu, Henan University science and technology innovation team to Jiawei Xu), Scientific Research Innovation team of the First Affiliated Hospital of Zhengzhou University Excellent innovation team to Jiawei Xu, Henan Zhongyuan Medical Science and Technology Innovation Development Foundation to Jiawei Xu.

Additional information

Author contributions

Conceptualization: JWX,HC

Methodology: CW, YQW, YL

Experiments performing: CW, YL, YQW, KYH, XRM, WBL

Data NGS (next generation sequencing): CW,YL,YQW

Bioinformatics analysis: YS, JWX, CW, YL, JG

Supervision: JWX

Writing—original draft: JWX, CW, YS, YQW, YL

Writing—review & editing: JWX

Funding

Funder	Grant reference number
National Key R&D Program of China	2019YFA0802200
National Key R&D Program of China	2019YFA0110900
National Natural Science Foundation of China	32170819
Henan University science and technology innovation team	
Scientific Research Innovation team of the First Affiliated Hospital of Zhengzhou University Excellent innovation team	
Henan Zhongyuan Medical Science and Technology Innovation Development Foundation	

Author ORCID iDs

Jiawei Xu: <https://orcid.org/0000-0003-4336-7972>

Note

This reviewed preprint has been updated to use the correct text; previously, a version prior to the one reviewed was presented here.

References

- 1 Reik W., Dean W., Walter J (2001) Epigenetic reprogramming in mammalian development. *Science* **293**:1089-1093 <https://doi.org/10.1126/science.1063443> | PubMed
- 2 Burton A., Torres-Padilla M. E (2014) Chromatin dynamics in the regulation of cell fate allocation during early embryogenesis. *Nat Rev Mol Cell Biol* **15**:723-734 <https://doi.org/10.1038/nrm3885> | PubMed
- 3 Wu J., et al. (2018) Chromatin analysis in human early development reveals epigenetic transition during ZGA. *Nature* **557**:256-260 <https://doi.org/10.1038/s41586-018-0080-8> | PubMed

- 4 Stillman B (2018) Histone Modifications: Insights into Their Influence on Gene Expression. *Cell* **175**:6-9 <https://doi.org/10.1016/j.cell.2018.08.032> | PubMed
- 5 Huang H., Sabari B. R., Garcia B. A., Allis C. D., Zhao Y. (2014) SnapShot: histone modifications. *Cell* **159**:458-458.e451, <https://doi.org/10.1016/j.cell.2014.09.037> | PubMed
- 6 Peterson C. L., Laniel M. A. (2004) Histones and histone modifications. *Curr Biol* **14**:R546-551 <https://doi.org/10.1016/j.cub.2004.07.007> | PubMed
- 7 Hammoud S., et al. (2009) Distinctive chromatin in human sperm packages genes for embryo development. *Nature* **460**:473-478 <https://doi.org/10.1038/nature08162> | PubMed
- 8 Hammoud S., et al. (2014) Chromatin and transcription transitions of mammalian adult germline stem cells and spermatogenesis. *Cell stem cell* **15**:239-253 <https://doi.org/10.1016/j.stem.2014.04.006> | PubMed
- 9 Xia W., Xie W (2020) Rebooting the Epigenomes during Mammalian Early Embryogenesis. *Stem Cell Reports* **15**:1158-1175 <https://doi.org/10.1016/j.stemcr.2020.09.005> | PubMed
- 10 Goldberg A., et al. (2010) Distinct factors control histone variant H3.3 localization at specific genomic regions. *Cell* **140**:678-691 <https://doi.org/10.1016/j.cell.2010.01.003> | PubMed
- 11 Jin C., et al. (2009) H3.3/H2A.Z double variant-containing nucleosomes mark 'nucleosome-free regions' of active promoters and other regulatory regions. *Nature genetics* **41**:941-945 <https://doi.org/10.1038/ng.409> | PubMed
- 12 Mito Y., Henikoff J., Henikoff S (2005) Genome-scale profiling of histone H3.3 replacement patterns. *Nature genetics* **37**:1090-1097 <https://doi.org/10.1038/ng1637> | PubMed
- 13 Ishiuchi T., et al. (2021) Reprogramming of the histone H3.3 landscape in the early mouse embryo. *Nature structural & molecular biology* **28**:38-49 <https://doi.org/10.1038/s41594-020-00521-1> | PubMed
- 14 Zheng H., et al. (2016) Resetting Epigenetic Memory by Reprogramming of Histone Modifications in Mammals. *Mol Cell* **63**:1066-1079 <https://doi.org/10.1016/j.molcel.2016.08.032> | PubMed
- 15 Zentner G. E., Henikoff S (2013) Regulation of nucleosome dynamics by histone modifications. *Nat Struct Mol Biol* **20**:259-266 <https://doi.org/10.1038/nsmb.2470> | PubMed
- 16 Soares L. M., et al. (2017) Determinants of Histone H3K4 Methylation Patterns. *Mol Cell* **68**:773-785.e776, <https://doi.org/10.1016/j.molcel.2017.10.013> | PubMed
- 17 Klose R. J., Zhang Y (2007) Regulation of histone methylation by demethylination and demethylation. *Nat Rev Mol Cell Biol* **8**:307-318 <https://doi.org/10.1038/nrm2143> | PubMed
- 18 Eissenberg J. C., Shilatifard A (2010) Histone H3 lysine 4 (H3K4) methylation in development and differentiation. *Dev Biol* **339**:240-249 <https://doi.org/10.1016/j.ydbio.2009.08.017> | PubMed
- 19 Ruthenburg A. J., Allis C. D., Wysocka J (2007) Methylation of lysine 4 on histone H3: intricacy of writing and reading a single epigenetic mark. *Mol Cell* **25**:15-30 <https://doi.org/10.1016/j.molcel.2006.12.014> | PubMed
- 20 Rickels R., et al. (2017) Histone H3K4 monomethylation catalyzed by Trr and mammalian COMPASS-like proteins at enhancers is dispensable for development and viability. *Nat Genet* **49**:1647-1653 <https://doi.org/10.1038/ng.3965> | PubMed
- 21 Smith E., Shilatifard A. (2014) Enhancer biology and enhanceropathies. *Nat Struct Mol Biol* **21**:210-219 <https://doi.org/10.1038/nsmb.2784> | PubMed
- 22 Creighton M. P., et al. (2010) Histone H3K27ac separates active from poised enhancers and predicts developmental state. *Proc Natl Acad Sci U S A* **107**:21931-21936 <https://doi.org/10.1073/pnas.1016071107> | PubMed
- 23 Hu D., et al. (2013) The MLL3/mLL4 branches of the COMPASS family function as major histone H3K4 monomethylases at enhancers. *Mol Cell Biol* **33**:4745-4754 <https://doi.org/10.1128/mcb.01181-13> | PubMed

- 24 Liu T., et al. (2011) Broad chromosomal domains of histone modification patterns in *C. elegans*. *Genome Res* **21**:227-236 <https://doi.org/10.1101/gr.115519.110> | PubMed
- 25 Du Z., et al. (2013) Genome-wide analysis of histone modifications: H3K4me2, H3K4me3, H3K9ac, and H3K27ac in *Oryza sativa* L. Japonica. *Mol Plant* **6**:1463-1472 <https://doi.org/10.1093/mp/sst018> | PubMed
- 26 van Dijk K., et al. (2010) Dynamic changes in genome-wide histone H3 lysine 4 methylation patterns in response to dehydration stress in *Arabidopsis thaliana*. *BMC Plant Biol* **10**:238 <https://doi.org/10.1186/1471-2229-10-238> | PubMed
- 27 Zheng Y. C., et al. (2015) A Systematic Review of Histone Lysine-Specific Demethylase 1 and Its Inhibitors. *Med Res Rev* **35**:1032-1071 <https://doi.org/10.1002/med.21350> | PubMed
- 28 Yang Z., et al. (2010) AOF1 is a histone H3K4 demethylase possessing demethylase activity-independent repression function. *Cell Res* **20**:276-287 <https://doi.org/10.1038/cr.2010.12> | PubMed
- 29 Fang R., et al. (2010) Human LSD2/KDM1b/AOF1 regulates gene transcription by modulating intragenic H3K4me2 methylation. *Mol Cell* **39**:222-233 <https://doi.org/10.1016/j.molcel.2010.07.008> | PubMed
- 30 Wang S., Meyer D. H., Schumacher B (2020) H3K4me2 regulates the recovery of protein biosynthesis and homeostasis following DNA damage. *Nat Struct Mol Biol* **27**:1165-1177 <https://doi.org/10.1038/s41594-020-00513-1> | PubMed
- 31 Liu Y., et al. (2019) H3K4me2 functions as a repressive epigenetic mark in plants. *Epigenetics & chromatin* **12**:40 <https://doi.org/10.1186/s13072-019-0285-6> | PubMed
- 32 Zhang C., et al. (2021) Narrow H3K4me2 is required for chicken PGC formation. *J Cell Physiol* **236**:1391-1400 <https://doi.org/10.1002/jcp.29945> | PubMed
- 33 Zhang J., Parvin J., Huang K (2012) Redistribution of H3K4me2 on neural tissue specific genes during mouse brain development. *BMC Genomics* **13** <https://doi.org/10.1186/1471-2164-13-S8-S5> | PubMed
- 34 Wang Y., Li X., Hu H (2014) H3K4me2 reliably defines transcription factor binding regions in different cells. *Genomics* **103**:222-228 <https://doi.org/10.1016/j.ygeno.2014.02.002> | PubMed
- 35 Pekowska A., Benoukraf T., Ferrier P., Spicuglia S (2010) A unique H3K4me2 profile marks tissue-specific gene regulation. *Genome Res* **20**:1493-1502 <https://doi.org/10.1101/gr.109389.110> | PubMed
- 36 Lambrot R., Siklenka K., Lafleur C., Kimmins S (2019) The genomic distribution of histone H3K4me2 in spermatogonia is highly conserved in spermdagger. *Biol Reprod* **100**:1661-1672 <https://doi.org/10.1093/biolre/ioz055> | PubMed
- 37 Skene P. J., Henikoff J. G., Henikoff S (2018) Targeted in situ genome-wide profiling with high efficiency for low cell numbers. *Nat Protoc* **13**:1006-1019 <https://doi.org/10.1038/nprot.2018.015> | PubMed
- 38 Skene P. J., Henikoff S (2017) An efficient targeted nuclease strategy for high-resolution mapping of DNA binding sites. *eLife* **6** <https://doi.org/10.7554/eLife.21856> | PubMed
- 39 Wang L., et al. (2014) Programming and inheritance of parental DNA methylomes in mammals. *Cell* **157**:979-991 <https://doi.org/10.1016/j.cell.2014.04.017> | PubMed
- 40 Zhang B., et al. (2016) Allelic reprogramming of the histone modification H3K4me3 in early mammalian development. *Nature* **537**:553-557 <https://doi.org/10.1038/nature19361> | PubMed
- 41 Wu J., et al. (2016) The landscape of accessible chromatin in mammalian pre-implantation embryos. *Nature* **534**:652-657 <https://doi.org/10.1038/nature18606> | PubMed
- 42 Siklenka K., et al. (2015) Disruption of histone methylation in developing sperm impairs offspring health transgenerationally. *Science* **350**:aab2006 <https://doi.org/10.1126/science.aab2006> | PubMed
- 43 Hanna C. W., et al. (2018) MLL2 conveys transcription-independent H3K4 trimethylation in oocytes. *Nature structural & molecular biology* **25**:73-82 <https://doi.org/10.1038/s41594-017-0013-5> | PubMed
- 44 Liu X., et al. (2016) Distinct features of H3K4me3 and H3K27me3 chromatin domains in pre-implantation embryos. *Nature* **537**:558-562 <https://doi.org/10.1038/nature19362> | PubMed

- 45 Li L., Lu X., Dean J (2013) The maternal to zygotic transition in mammals. *Molecular aspects of medicine* **34**:919-938 <https://doi.org/10.1016/j.mam.2013.01.003> | PubMed
- 46 Lee M. T., Bonneau A. R., Giraldez A. J (2014) Zygotic genome activation during the maternal-to-zygotic transition. *Annual review of cell and developmental biology* **30**:581-613 <https://doi.org/10.1146/annurev-cellbio-100913-013027> | PubMed
- 47 Liu Y.-W., et al. (2017) LincRNAFEZF1-AS1 represses p21 expression to promote gastric cancer proliferation through LSD1-Mediated H3K4me2 demethylation. *Molecular cancer* **16**:39 <https://doi.org/10.1186/s12943-017-0588-9> | PubMed
- 48 Dahl J. A., et al. (2016) Broad histone H3K4me3 domains in mouse oocytes modulate maternal-to-zygotic transition. *Nature* **537**:548-552 <https://doi.org/10.1038/nature19360> | PubMed
- 49 Xu Q., et al. (2019) SETD2 regulates the maternal epigenome, genomic imprinting and embryonic development. *Nat Genet* **51**:844-856 <https://doi.org/10.1038/s41588-019-0398-7> | PubMed
- 50 Xia W., et al. (2019) Resetting histone modifications during human parental-to-zygotic transition. *Science* **365**:353-360 <https://doi.org/10.1126/science.aaw5118> | PubMed
- 51 Kaniskan H. U., Martini M. L., Jin J (2018) Inhibitors of Protein Methyltransferases and Demethylases. *Chem Rev* **118**:989-1068 <https://doi.org/10.1021/acs.chemrev.6b00801> | PubMed
- 52 Ciccone D. N., et al. (2009) KDM1B is a histone H3K4 demethylase required to establish maternal genomic imprints. *Nature* **461**:415-418 <https://doi.org/10.1038/nature08315> | PubMed
- 53 Dai X. J., et al. (2020) Tranylcypromine Based Lysine-Specific Demethylase 1 Inhibitor: Summary and Perspective. *J Med Chem* **63**:14197-14215 <https://doi.org/10.1021/acs.jmedchem.0c00919> | PubMed
- 54 Jukam D., Shariati S. A. M., Skotheim J. M (2017) Zygotic Genome Activation in Vertebrates. *Dev Cell* **42**:316-332 <https://doi.org/10.1016/j.devcel.2017.07.026> | PubMed
- 55 Wang Y., Li X., Hu H (2014) H3K4me2 reliably defines transcription factor binding regions in different cells. *Genomics* **103**:222-228 <https://doi.org/10.1016/j.ygeno.2014.02.002> | PubMed
- 56 Dominguez M. H., Ayoub A. E., Rakic P (2013) POU-III transcription factors (Brn1, Brn2, and Oct6) influence neurogenesis, molecular identity, and migratory destination of upper-layer cells of the cerebral cortex. *Cerebral cortex* **23**:2632-2643 <https://doi.org/10.1093/cercor/bhs252> | PubMed
- 57 Sarkar A., Hochedlinger K (2013) The sox family of transcription factors: versatile regulators of stem and progenitor cell fate. *Cell stem cell* **12**:15-30 <https://doi.org/10.1016/j.stem.2012.12.007> | PubMed
- 58 Field A., Adelman K. (2020) Evaluating Enhancer Function and Transcription. *Annual review of biochemistry* **89**:213-234 <https://doi.org/10.1146/annurev-biochem-011420-095916> | PubMed
- 59 Xavier M. J., Roman S. D., Aitken R. J., Nixon B (2019) Transgenerational inheritance: how impacts to the epigenetic and genetic information of parents affect offspring health. *Hum Reprod Update* **25**:518-540 <https://doi.org/10.1093/humupd/dmz017> | PubMed
- 60 Chen Q., Yan W., Duan E (2016) Epigenetic inheritance of acquired traits through sperm RNAs and sperm RNA modifications. *Nat Rev Genet* **17**:733-743 <https://doi.org/10.1038/nrg.2016.106> | PubMed
- 61 Zhang B., et al. (2018) Widespread Enhancer Dememorization and Promoter Priming during Parental-to-Zygotic Transition. *Molecular cell* **72** <https://doi.org/10.1016/j.molcel.2018.10.017> | PubMed
- 62 Chen Z., Djekidel M., Zhang Y. J. N (2021) Distinct dynamics and functions of H2AK119ub1 and H3K27me3 in mouse pre-implantation embryos. *Nat Genet* **53**:551-563 <https://doi.org/10.1038/s41588-021-00821-2> | PubMed
- 63 Xia W., et al. (2019) Resetting histone modifications during human parental-to-zygotic transition. *Science* **365**:353-360 <https://doi.org/10.1126/science.aaw5118> | PubMed
- 64 Agarwal S., et al. (2021) KDM1A maintains genome-wide homeostasis of transcriptional enhancers. *Genome Res* **31**:186-197 <https://doi.org/10.1101/gr.234559.118> | PubMed
- 65 Ye R., Cao C., Xue Y. J. E. (2020) Enhancer RNA: biogenesis, function, and regulation. *Essays Biochem* **64**:883-894 <https://doi.org/10.1042/ebc20200014> | PubMed

- 66 Kang Y., Kim Y., Kang J., Kim A. J. F. (2021) Histone H3K4me1 and H3K27ac play roles in nucleosome eviction and eRNA transcription, respectively, at enhancers. *FASEB J* **35**:e21781 <https://doi.org/10.1096/fj.202100488R> | PubMed
- 67 Kang H., et al. (2020) Dynamic regulation of histone modifications and long-range chromosomal interactions during postmitotic transcriptional reactivation. *Genes Dev* **34**:913-930 <https://doi.org/10.1101/gad.335794.119> | PubMed
- 68 Ishihara H., et al. (2019) Primed histone demethylation regulates shoot regenerative competency. *Nat Commun* **10**:1786 <https://doi.org/10.1038/s41467-019-09386-5> | PubMed
- 69 Murphy P., Wu S., James C., Wike C., Cairns B. J. C (2018) Placeholder Nucleosomes Underlie Germline-to-Embryo DNA Methylation Reprogramming. *Cell* **172**:993-1006.e1013 <https://doi.org/10.1016/j.cell.2018.01.022> | PubMed
- 70 Yan J., et al. (2018) Histone H3 lysine 4 monomethylation modulates long-range chromatin interactions at enhancers. *Cell Res* **28**:204-220 <https://doi.org/10.1038/cr.2018.1> | PubMed
- 71 Soldi M., et al. (2017) Chromatin proteomics reveals novel combinatorial histone modification signatures that mark distinct subpopulations of macrophage enhancers. *Nucleic Acids Res* **45**:12195-12213 <https://doi.org/10.1093/nar/gkx821> | PubMed
- 72 Dorigi K., et al. (2017) Mll3 and Mll4 Facilitate Enhancer RNA Synthesis and Transcription from Promoters Independently of H3K4 Monomethylation. *Mol Cell* **66**:568-576.e564, <https://doi.org/10.1016/j.molcel.2017.04.018> | PubMed
- 73 Kaikkonen M., et al. (2013) Remodeling of the enhancer landscape during macrophage activation is coupled to enhancer transcription. *Mol Cell* **51**:310-325 <https://doi.org/10.1016/j.molcel.2013.07.010> | PubMed
- 74 Morrissey E. E., et al. (1998) GATA6 regulates HNF4 and is required for differentiation of visceral endoderm in the mouse embryo. *Genes & development* **12**:3579-3590 <https://doi.org/10.1101/gad.12.22.3579> | PubMed
- 75 Weinberger L., Ayyash M., Novershtern N., Hanna J. H (2016) Dynamic stem cell states: naive to primed pluripotency in rodents and humans. *Nature reviews. Molecular cell biology* **17**:155-169 <https://doi.org/10.1038/nrm.2015.28> | PubMed
- 76 Gross D. S., Garrard W. T (1988) Nuclease hypersensitive sites in chromatin. *Annual review of biochemistry* **57**:159-197 <https://doi.org/10.1146/annurev.bi.57.070188.001111> | PubMed
- 77 Rivera C. M., Ren B. (2013) Mapping human epigenomes. *Cell* **155**:39-55 <https://doi.org/10.1016/j.cell.2013.09.011> | PubMed
- 78 Langmead B., Salzberg S. L (2012) Fast gapped-read alignment with Bowtie 2. *Nat Methods* **9**:357-359 <https://doi.org/10.1038/nmeth.1923> | PubMed
- 79 Quinlan A. R., Hall I. M (2010) BEDTools: a flexible suite of utilities for comparing genomic features. *Bioinformatics* **26**:841-842 <https://doi.org/10.1093/bioinformatics/btq033> | PubMed
- 80 Zhang Y., et al. (2008) Model-based analysis of ChIP-Seq (MACS). *Genome Biol* **9**:R137 <https://doi.org/10.1186/gb-2008-9-9-r137> | PubMed

Peer reviews

Reviewer #1 (Public Review):

By mapping H3K4me2 in mouse oocytes and pre-implantation embryos, the authors aim to elucidate how this histone modification is erased and re-established during the parental-to-zygotic transition, as well as how the reprogramming of H3K4me2 regulates gene expression and facilitates zygotic genome activation.

Employing an improved CUT&RUN approach, the authors successfully generated H3K4me2 profiling data from a limited number of embryos. While the profiling experiments are very

well executed, several weaknesses, particularly in data analysis, are apparent:

(1) The study emphasizes H3K4me2, which often serves as a precursor to H3K4me3, a well-studied modification during early development. Analyzing the new H3K4me2 dataset alongside published H3K4me3 data is crucial for comprehensively understanding epigenetic reprogramming post-fertilization and the interplay between histone modifications. However, the current analysis is preliminary and lacks depth.

(2) Tranylcypromine (TCP) is known as an irreversible inhibitor of monoamine oxidase and LSD1. While the authors suggest TCP inhibits the expression of LSD2, this assertion is questionable. Given TCP's potential non-specific effects in cells, conclusions related to the experiments using TCP should be made with caution.

(3) Some batches of H3K4me2 antibody are known to cross-react with H3K4me3. Has the H3K4me2 antibody used in CUT&RUN been tested for such cross-reactivity? Heatmaps in the figures indeed show similar distribution for H3K4me2 and H3K4me3, further raising concerns about antibody specificity.

(4) Certain statements lack supporting references or figures (examples on page 9 can be found on line 245, line 254, and line 258).

(5) Extensive language editing is recommended to clarify ambiguous sentences. Additionally, caution should be taken to avoid overstatement - most analyses in this study only suggest correlation rather than causality.

<https://doi.org/10.7554/eLife.99417.1.sa3>

Reviewer #2 (Public Review):

Chong Wang et al. investigated the role of H3K4me2 during the reprogramming processes in mouse preimplantation embryos. The authors show that H3K4me2 is erased from GV to MII oocytes and re-established in the late 2-cell stage by performing Cut & Run H3K4me2 and immunofluorescence staining. Erasure and re-establishment of H3K4me2 have not been studied well, and profiling of H3K4me2 in germ cells and preimplantation embryos is valuable to understanding the reprogramming process and epigenetic inheritance.

(1) The authors claim that the Cut & Run worked for MII oocytes, zygotes, and the 2-cell embryos. However, it is unclear if H3K4me2 is erased during the stage or if the Cut & Run did not work for these samples. To support the hypothesis of the erasure of H3K4me2, the authors conducted immunofluorescence staining, and H3k4me2 was undetected in the MII oocyte, PN5, and 2-cell stage. However, the published papers showed strong staining of H3K4me2 at the zygote stage and 2-cell stage ((Ancelin et al., 2016; Shao et al., 2014)). The authors need to cite these papers and discuss the contradictory findings.

The authors used 165 MII oocytes and 190 GV oocytes for the Cut & Run. The amount of DNA in MII oocytes is halved because of the emission of the first polar body. Would it be a reason that H3K4me2 has fewer H3K4me2 peaks in MII oocytes than GV oocytes?

In Figure 3C, 98% (13,183/13,428) of H3K4me2 marked genes in GV oocytes overlap with those in the 4-cell stage. Furthermore, 92% (14,049/15,112) of H3K4me2 marked genes in sperm overlap with those in the 4-cell stage. Therefore, most regions maintain germ line-derived H3K4me2 in the 4-cell stage. The authors need to clarify which regions of germ line-derived H3K4me2 are maintained or erased in preimplantation embryos. Additionally, it would be interesting to investigate which regions show the parental allele-specific H3K4me2 in preimplantation embryos since the authors used hybrid preimplantation embryos (B6 x DBA).

(2) The authors claim that Kdm1a is rarely expressed during mouse embryonic development (Figure 4A). However, the published paper showed that KDM1a is present in the zygote and 2-cell stage using immunostaining and western blotting ((Ancelin et al., 2016)). Additionally, this paper showed that depletion of maternal KDM1A protein results in developmental arrest at the two-cell stage, and therefore, KDM1a is functionally important in early development. The authors should have cited the paper and described the role of KDM1a in early embryos.

(3) The authors used the published RNA data set and interpreted that KDM1B (LSD2) was highly expressed at the MII stage (Figure S3A). However, the heat map shows that KDM1B expression is high in growing oocytes but not at 8w_oocytes and MII oocytes. The authors need to interpret the data accurately.

(4) All embryos in the TCP group were arrested at the four-cell stage. Embryos generated from KDM1b KO females can survive until E10.5 (Ciccone et al., 2009); therefore, TCP-treated embryos show a more severe phenotype than oocyte-derived KDM1b deleted embryos. Depletion of maternal KDM1A protein results in developmental arrest at the two-cell stage ((Ancelin et al., 2016)). The authors need to examine whether TCP treatment affects KDM1a expression. Western blotting would be recommended to quantify the expression of KDM1A and KDM1B in the TCP-treated embryos.

(5) H3K4me2 is increased dramatically in the TCP-treated embryos in Figure 4 (the intensity is 1,000 times more than the control). However, the Cut & Run H3K4me2 shows that the H3K4me2 signal is increased in 251 genes and decreased in 194 genes in the TCP-treated embryos (Fold changes > 2, $P < 0.01$). The authors need to explain why the gain of H3K4me2 is less evident in the Cut & Run data set than in the immunofluorescence result.

References

- Ancelin, K., ne Syx, L., Borensztein, M., mie Ranisavljevic, N., Vassilev, I., Briseñ o-Roa, L., Liu, T., Metzger, E., Servant, N., Barillot, E., Chen, C.-J., Schü le, R., & Heard, E. (2016). Maternal LSD1/KDM1A is an essential regulator of chromatin and transcription landscapes during zygotic genome activation. <https://doi.org/10.7554/eLife.08851.001> 
- Ciccone, D. N., Su, H., Hevi, S., Gay, F., Lei, H., Bajko, J., Xu, G., Li, E., & Chen, T. (2009). KDM1B is a histone H3K4 demethylase required to establish maternal genomic imprints. *Nature*, 461(7262), 415-418. <https://doi.org/10.1038/nature08315> 
- Shao, G. B., Chen, J. C., Zhang, L. P., Huang, P., Lu, H. Y., Jin, J., Gong, A. H., & Sang, J. R. (2014). Dynamic patterns of histone H3 lysine 4 methyltransferases and demethylases during mouse preimplantation development. *In Vitro Cellular and Developmental Biology - Animal*, 50(7), 603-613. <https://doi.org/10.1007/s11626-014-9741-6> 
<https://doi.org/10.7554/eLife.99417.1.sa2>

Reviewer #3 (Public Review):

Summary:

This study explores the dynamic reprogramming of histone modification H3K4me2 during the early stages of mammalian embryogenesis. Utilizing the advanced CUT&RUN technique coupled with high-throughput sequencing, the authors investigate the erasure and re-establishment of H3K4me2 in mouse germinal vesicle (GV) oocytes, metaphase II (MII) oocytes, and early embryos.

Strengths:

The findings provide valuable insights into the temporal and spatial dynamics of H3K4me2 and its potential role in zygotic genome activation (ZGA).

Weaknesses:

The study primarily remains descriptive at this point. It would be advantageous to conduct further comprehensive functional validation and mechanistic exploration.

Key areas for improvement include enhancing the innovation and novelty of the study, providing robust functional validation, establishing a clear model for H3K4me2's role, and addressing technical and presentation issues. The text would benefit from the introduction of a novel conceptual framework or model that provides a clear explanation of the functional consequences and molecular mechanisms underlying H3K4me2 reprogramming in the transition from parental to early embryonic development.

While the findings are significant, the current manuscript falls short in several critical areas. Addressing major and minor issues will significantly strengthen the study's contribution to the field of epigenetic reprogramming and embryonic development.

<https://doi.org/10.7554/eLife.99417.1.sa1>

Author response:

Public Reviews:

Reviewer #1 (Public Review):

By mapping H3K4me2 in mouse oocytes and pre-implantation embryos, the authors aim to elucidate how this histone modification is erased and re-established during the parental-to-zygotic transition, as well as how the reprogramming of H3K4me2 regulates gene expression and facilitates zygotic genome activation.

Employing an improved CUT&RUN approach, the authors successfully generated H3K4me2 profiling data from a limited number of embryos. While the profiling experiments are very well executed, several weaknesses, particularly in data analysis, are apparent:

(1) The study emphasizes H3K4me2, which often serves as a precursor to H3K4me3, a well-studied modification during early development. Analyzing the new H3K4me2 dataset alongside published H3K4me3 data is crucial for comprehensively understanding epigenetic reprogramming post-fertilization and the interplay between histone modifications. However, the current analysis is preliminary and lacks depth.

Thank you very much for your valuable suggestions. The data of histone H3K4me3 in humans and mice has been published, and our previous data revealed the unique pattern of H3K4me3 during early human embryos and oocytes (Science. 2019 Jul 26;365(6451):353-360.). So, this study mainly focuses on the localization of H3K4me2 in mouse oocytes and preimplantation embryos, how it is erased and re-established during mammalian parental-to-zygote transition, and its function. The combined analysis of H3K4me2 and H3K4me3 is not our main work, but it is not ruled out that there may be new discoveries between these two histones. Previously, our data tended to show that the H3K4me2 not only acts as a precursor of H3K4me3, but also plays its role independently.

(2) Tranylcypromine (TCP) is known as an irreversible inhibitor of monoamine oxidase and LSD1. While the authors suggest TCP inhibits the expression of LSD2, this assertion is questionable. Given TCP's potential non-specific effects in cells, conclusions related to the experiments using TCP should be made with caution.

Thank you for pointing this out, and we thank the reviewer again for the important suggestion. We found that the previous study (.Binda C, Valente S, Romanenghi M, Pilotto S, Cirilli R, Karytinis A, Ciossani G, Botrugno OA, Forneris F, Tardugno M, Edmondson DE, Minucci S, Mattevi A, Mai A. Biochemical, structural, and biological evaluation of tranylcypromine derivatives as inhibitors of histone demethylases LSD1 and LSD2. J Am Chem Soc. 2010 May 19;132(19):6827-33.) indicated that TCP was a non-reversible inhibitor of LSD1 and LSD2 (Human LSD2/KDM1b/AOF1 Regulates Gene Transcription by Modulating Intragenic H3K4me2 Methylation, Mol Cell. 2010 Jul 30; 39(2): 222–233.), but according to our data, the content of LSD1 was very low in the early stages of mouse embryos, which mainly inhibited the function of LSD2.

(3) Some batches of H3K4me2 antibody are known to cross-react with H3K4me3. Has the H3K4me2 antibody used in CUT&RUN been tested for such cross-reactivity? Heatmaps in the figures indeed show similar distribution for H3K4me2 and H3K4me3, further raising concerns about antibody specificity.

We thank the reviewer for the insightful comments. The H3K4me2 antibody was purchased from Millipore (cat. 07030). Figure 2A shows the specific enrichment area of H3K4me2 in promoter and distal region. Some batches of H3K4me2 antibody are known to cross-react with H3K4me3, but the H3K4me2 antibody we used in our CUT&RUN seems to have Low cross-reactivity.

(4) Certain statements lack supporting references or figures (examples on page 9 can be found on line 245, line 254, and line 258).

Thank you for pointing this out, and we will add references to support the statement in the paper as suggested.

(5) Extensive language editing is recommended to clarify ambiguous sentences. Additionally, caution should be taken to avoid overstatement - most analyses in this study only suggest correlation rather than causality.

Thank you for your kind comments. We will revise the expression in the manuscript later.

Reviewer #2 (Public Review):

Chong Wang et al. investigated the role of H3K4me2 during the reprogramming processes in mouse preimplantation embryos. The authors show that H3K4me2 is erased from GV to MII oocytes and re-established in the late 2-cell stage by performing Cut & Run H3K4me2 and immunofluorescence staining. Erasure and re-establishment of H3K4me2 have not been studied well, and profiling of H3K4me2 in germ cells and preimplantation embryos is valuable to understanding the reprogramming process and epigenetic inheritance.

(1) The authors claim that the Cut & Run worked for MII oocytes, zygotes, and the 2-cell embryos. However, it is unclear if H3K4me2 is erased during the stage or if the Cut & Run did not work for these samples. To support the hypothesis of the erasure of H3K4me2, the authors conducted immunofluorescence staining, and H3K4me2 was undetected in the MII oocyte, PN5, and 2-cell stage. However, the published papers showed strong staining of H3K4me2 at the zygote stage and 2-cell stage ((Ancelin et al., 2016; Shao et al., 2014)). The authors need to cite these papers and discuss the contradictory findings.

The authors used 165 MII oocytes and 190 GV oocytes for the Cut & Run. The amount of DNA in MII oocytes is halved because of the emission of the first polar body. Would it be a reason that H3K4me2 has fewer H3K4me2 peaks in MII oocytes than GV oocytes?

First of all, thank you for your valuable advice. The published papers showed strong staining of H3K4me2 at the zygote stage and 2-cell stage (Ancelin et al., 2016), which is interesting. I think we may have used different parameters in the confocal laser shooting process. We used the same parameter to continuously shoot the blastocyst stage from the GV stage. If we only shot the fertilized egg and the 2-cell stage, I think we may also see weak fluorescence at the 2-cell stage under different parameters. We will refer to this reference and discuss it in the resubmitted version.

Moreover, you mentioned the H3K4me2 has fewer H3K4me2 peaks in MII oocytes than GV oocytes, because the MII expelled the polar body. There is no problem with this logic. However, the first polar body expelled from the MII stage is still in the zona pellucida, and we also collected the polar body in the CUT&RUN experiment; Therefore, compared to GV, the DNA content of MII samples is not halved. After further discussion, we believe that the reduction of H3K4me2 peaks in MII stage compared with GV stage may be closely related to oocyte maturation. It is the specific modification of histones in different forms at different times that affects the chromatin structure change appropriately with the different stages of meiosis. At present, it has been confirmed that H3K4me3 gradually decreases from GV to MII stage during the maturation of human oocytes. H3K27me3 did not change from GV to MII stage.

In Figure 3C, 98% (13,183/13,428) of H3K4me2 marked genes in GV oocytes overlap with those in the 4-cell stage. Furthermore, 92% (14,049/15,112) of H3K4me2 marked genes in sperm overlap with those in the 4-cell stage. Therefore, most regions maintain germ line-derived H3K4me2 in the 4-cell stage. The authors need to clarify which regions of germ line-derived H3K4me2 are maintained or erased in preimplantation embryos. Additionally, it would be interesting to investigate which regions show the parental allele-specific H3K4me2 in preimplantation embryos since the authors used hybrid preimplantation embryos (B6 x DBA).

Thank you very much for your suggestion. Further analysis of which regions show the parental allele-specific H3K4me2 in preimplantation embryos will make the study more interesting. We will discuss this in depth in resubmitted vision.

(2) The authors claim that Kdm1a is rarely expressed during mouse embryonic development (Figure 4A). However, the published paper showed that KDM1a is present in the zygote and 2-cell stage using immunostaining and western blotting (Ancelin et al., 2016). Additionally, this paper showed that depletion of maternal KDM1A protein results in developmental arrest at the two-cell stage, and therefore, KDM1a is functionally important in early development.

The authors should have cited the paper and described the role of KDM1a in early embryos.

In the analysis of this experiment, we believe that in the early embryonic development of mice, the expression of KDM1A is lower than that of KDM1B, which is relative. Similarly, the transcriptome data we cite also show that KDM1A is expressed at elevated levels during oocyte maturation and fertilization compared to immature oocytes. In addition, the effects of loss of maternal KDM1a on embryonic development were not discussed. We believe that the absence of maternal KDM1b blocks embryonic development, and we will cite and discuss the references later.

(3) The authors used the published RNA data set and interpreted that KDM1B (LSD2) was highly expressed at the MII stage (Figure S3A). However, the heat map shows that KDM1B expression is high in growing oocytes but not at 8w oocytes and MII oocytes. The authors need to interpret the data accurately.

After re-checking the data, we found that there was a problem with the normalization method of our heat map, and we will re-make the heatmap and submit it in the modified version. With reference to Figure 4A, the content of Kdm1b is indeed higher than that of Kdm1a.

(4) All embryos in the TCP group were arrested at the four-cell stage. Embryos generated from KDM1b KO females can survive until E10.5 (Ciccone et al., 2009); therefore, TCP-treated embryos show a more severe phenotype than oocyte-derived KDM1b deleted embryos. Depletion of maternal KDM1A protein results in developmental arrest at the two-cell stage (Ancelin et al., 2016)). The authors need to examine whether TCP treatment affects KDM1a expression. Western blotting would be recommended to quantify the expression of KDM1A and KDM1B in the TCP-treated embryos.

We will further dig the transcriptome data to confirm the specificity of TCP to KDM1b. In addition, the intervention of TCP on the whole fertilized egg in this study increased the H3K4me2 content, and the embryo development retarding effect was more significant than that obtained by crossing with normal paternal lines after knocking down KDM1B from the mother.

(5) H3K4me2 is increased dramatically in the TCP-treated embryos in Figure 4 (the intensity is 1,000 times more than the control). However, the Cut & Run H3K4me2 shows that the H3K4me2 signal is increased in 251 genes and decreased in 194 genes in the TCP-treated embryos (Fold changes > 2, $P < 0.01$). The authors need to explain why the gain of H3K4me2 is less evident in the Cut & Run data set than in the immunofluorescence result.

Thanks a lot for your question. In the experimental group, the fluorescence value of H3K4me2 in IF was increased by 1000 times (Figure 4E), and the expression of H3K4Me2-related genes in CR was up-regulated and down-regulated for a total of 445 changes (Figure 6A). In our opinion, as a semi-quantitative analysis, immunofluorescence cannot be compared with the quantitative analysis method of CR because of the different analysis models and threshold Settings.

References

- Ancelin, K., ne Syx, L., Borensztein, M., mie Ranisavljevic, N., Vassilev, I., Briseñ o-Roa, L., Liu, T., Metzger, E., Servant, N., Barillot, E., Chen, C.-J., Schü le, R., & Heard, E. (2016). Maternal LSD1/KDM1A is an essential regulator of chromatin and transcription landscapes during zygotic genome activation. <https://doi.org/10.7554/eLife.08851.001> 
- Ciccone, D. N., Su, H., Hevi, S., Gay, F., Lei, H., Bajko, J., Xu, G., Li, E., & Chen, T. (2009). KDM1B is a histone H3K4 demethylase required to establish maternal genomic imprints. *Nature*, 461(7262), 415-418. <https://doi.org/10.1038/nature08315> 
- Shao, G. B., Chen, J. C., Zhang, L. P., Huang, P., Lu, H. Y., Jin, J., Gong, A. H., & Sang, J. R. (2014). Dynamic patterns of histone H3 lysine 4 methyltransferases and demethylases during mouse preimplantation development. *In Vitro Cellular and Developmental Biology - Animal*, 50(7), 603-613. <https://doi.org/10.1007/s11626-014-9741-6> 

Reviewer #3 (Public Review):

Summary:

This study explores the dynamic reprogramming of histone modification H3K4me2 during the early stages of mammalian embryogenesis. Utilizing the advanced CUT&RUN technique coupled with high-throughput sequencing, the authors investigate the erasure

and re-establishment of H3K4me2 in mouse germinal vesicle (GV) oocytes, metaphase II (MII) oocytes, and early embryos.

Strengths:

The findings provide valuable insights into the temporal and spatial dynamics of H3K4me2 and its potential role in zygotic genome activation (ZGA).

Weaknesses:

The study primarily remains descriptive at this point. It would be advantageous to conduct further comprehensive functional validation and mechanistic exploration.

Key areas for improvement include enhancing the innovation and novelty of the study, providing robust functional validation, establishing a clear model for H3K4me2's role, and addressing technical and presentation issues. The text would benefit from the introduction of a novel conceptual framework or model that provides a clear explanation of the functional consequences and molecular mechanisms underlying H3K4me2 reprogramming in the transition from parental to early embryonic development.

While the findings are significant, the current manuscript falls short in several critical areas. Addressing major and minor issues will significantly strengthen the study's contribution to the field of epigenetic reprogramming and embryonic development.

<https://doi.org/10.7554/eLife.99417.1.sa0>