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Supplementary Materials for

Depletion of oocyte dynamin-related protein 1 shows maternal-effect abnormalities in embryonic development

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Figs. S1 to S4
Tables S1, S4 and S5
Legends for tables S2 and S3

Other Supplementary Material for this manuscript includes the following:

Tables S2 and S3

Fig. S1

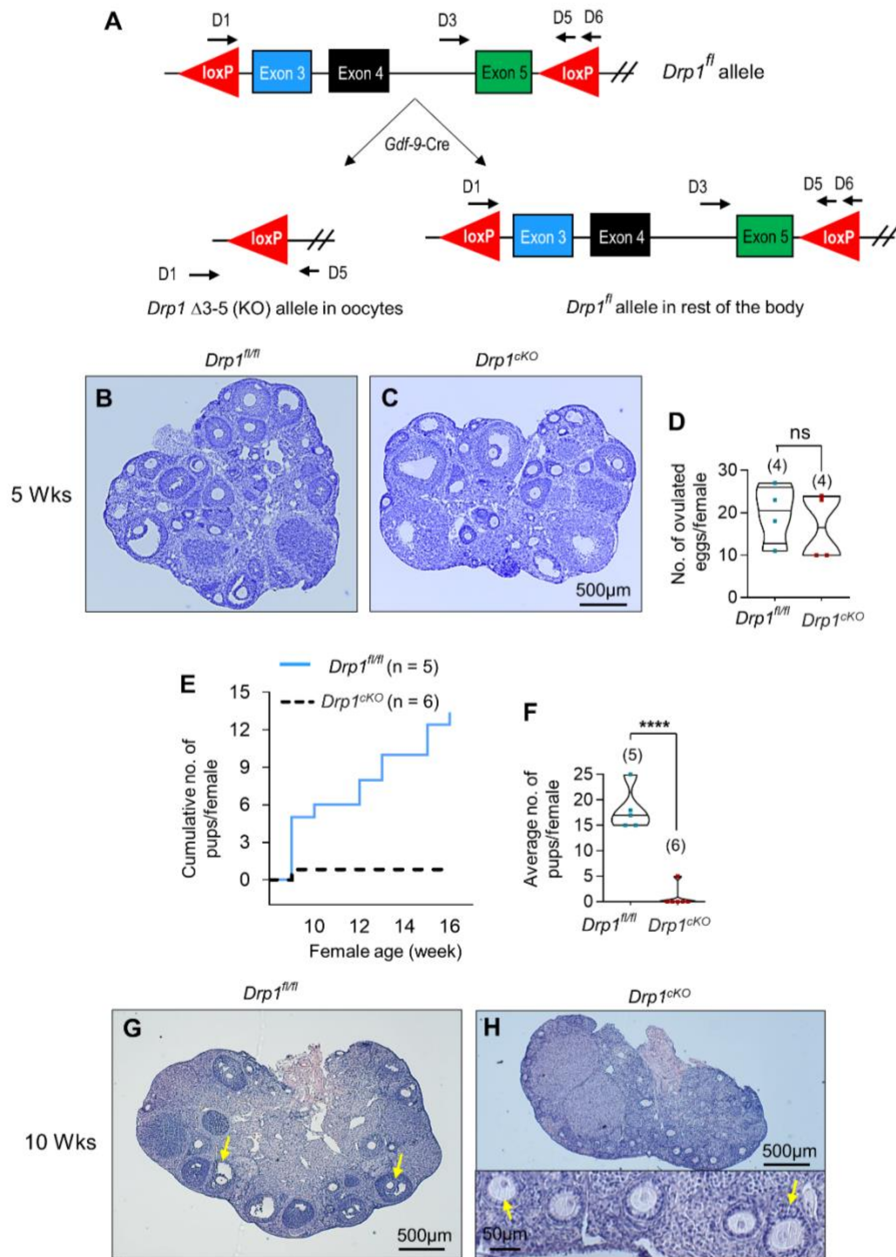

Fig. S1. *Drp1* deletion strategy in oocytes and assessment of fertility and ovary development. (A) Schematic representation of *Drp1* alleles and deletion of exons 3, 4 and 5 and creation of a *Drp1* Δ3-5 allele by *Gdf-9*-Cre-mediated recombination in oocytes. D1, D3, D5 and D6 indicate primers for genotyping. Comparable ovary morphology with follicles at different developmental stages in 5-week-old *Drp1*$^{fl/fl}$ (B) and *Drp1*^{cKO} (C) mice. Paraffin sections of 5 µm thickness were prepared and stained with hematoxylin. (D) Average number of ovulated eggs/4-5-week-old females. Four females per genotype were superovulated. (E) Comparison of cumulative number of pups per *Drp1*$^{fl/fl}$ (n = 5 females, blue line) and *Drp1*^{cKO} (n = 6 females, black dashed line). (F)

Average total number of pups per female during testing period. Numbers of females used are shown. Results show mean ± SD and *P* values determined by two-tailed Student's *t*-test (D and F). Control *Drp1*$^{fl/fl}$ mice at 10 week of age contained antral follicles (G, arrows) but ovaries of *Drp1*^{cKO} mice lacked bigger follicles and only contained primary or early secondary follicles (H, arrows, inset).

Fig. S2

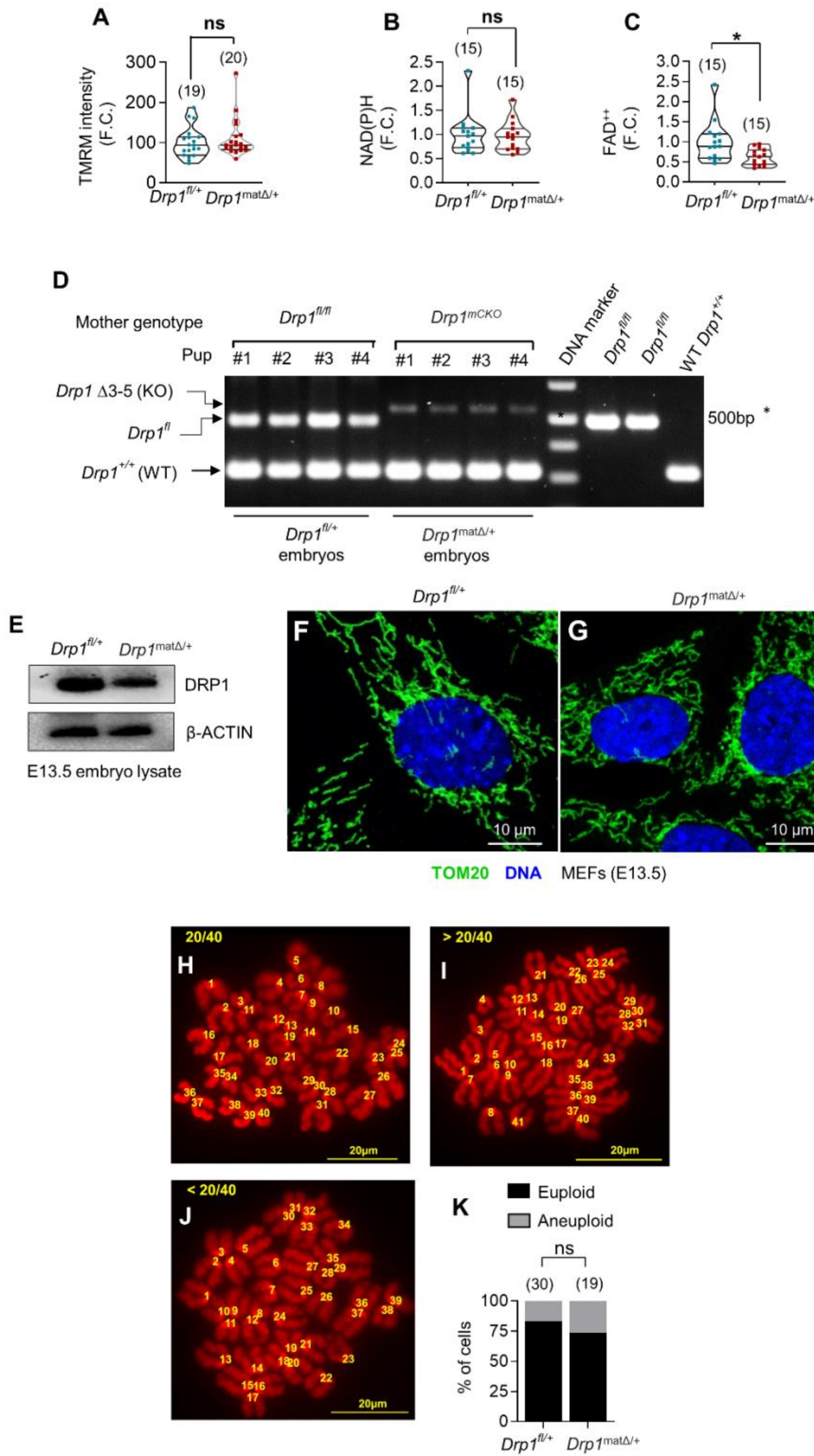

Fig. S2 continued

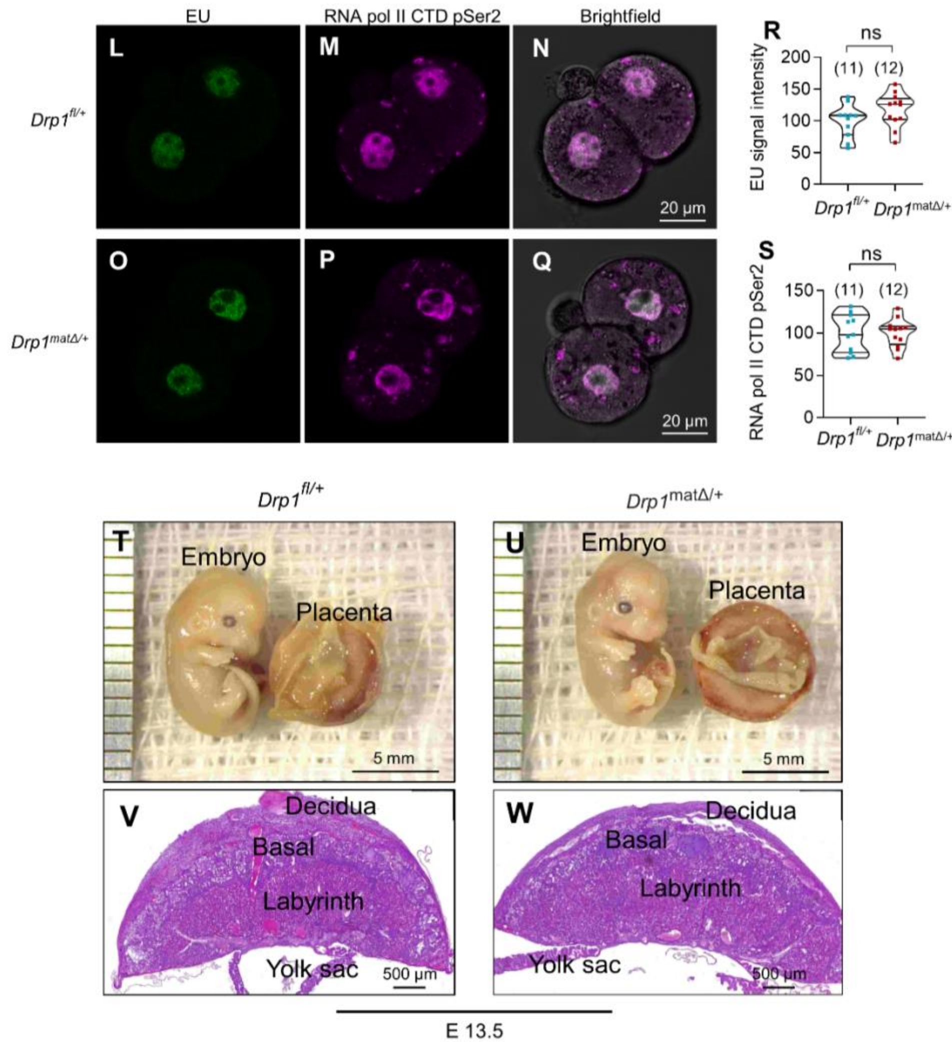

Fig. S2. Analysis of embryos and placentae. (A) TMRM intensity measurement in blastocysts. (B) NAD(P)H and (C) FAD^{++} levels in blastocysts. Total number of blastocysts analyzed are shown. (D) PCR-genotyping of offspring from *Drp1*^{cKO} and *Drp1*$^{fl/fl}$ females that were mated with wild-type C57BL/6J male mice, using primers indicated in A, where deletion of *Drp1* exons 3-5 in one allele of the genomic DNA was seen in all pups, as indicated by the PCR bands of approximately 539 bp. (E) Western blots of E13.5 *Drp1*$^{fl/+}$ and *Drp1*$^{mat\Delta/+}$ embryo lysates showing recovery of Drp1 in the embryos after fertilization of *Drp1*$^{\Delta/\Delta}$ oocytes by wild-type males. Level of β-actin was used as loading control. Fetal fibroblasts were derived from E13.5 control *Drp1*$^{fl/+}$ (F) and *Drp1*$^{mat\Delta/+}$ (G) embryos and mitochondria and DNA were labelled with TOM20 (green) and Hoechst (blue) respectively. (H-K) Chromosome spread in 4-cell stage zygotes. Photographs of chromosome spreads showing the examples of blastomeres with (H) normal chromosome number, (I) increased chromosome number and (J) reduced chromosome number. (K) Comparison of percentages of euploid or aneuploid *Drp1*$^{fl/+}$ and *Drp1*$^{mat\Delta/+}$ blastomeres. (L-S) Comparison of transcriptional activity between *Drp1*$^{fl/+}$ and *Drp1*$^{mat\Delta/+}$ 2-cell zygotes. Immunofluorescence detection of EU incorporation in (L) *Drp1*$^{fl/+}$ and (O) *Drp1*$^{mat\Delta/+}$ zygotes. RNA pol II CTD pSer2

labeling in (M) *Drp1*$^{fl/+}$ and (P) *Drp1*$^{mat\Delta/+}$ zygotes. Merged images of (N) *Drp1*$^{fl/+}$ and (Q) *Drp1*$^{mat\Delta/+}$ zygotes. (R) Quantification of EU and (S) RNA pol II CTD pSer2 levels. Representative images are shown. Numbers indicate the total number of zygotes analyzed. Results show mean ± SD and *P* values determined by two-tailed Student's *t*-test (R, S). ns, not significant. Photographs of *Drp1*$^{fl/+}$ (T) and *Drp1*$^{mat\Delta/+}$ (U) E13.5 embryo and placenta. Representative Haematoxylin and Eosin stained *Drp1*$^{fl/+}$ (V) and *Drp1*$^{mat\Delta/+}$ (W) E13.5 placenta sections.

Fig. S3

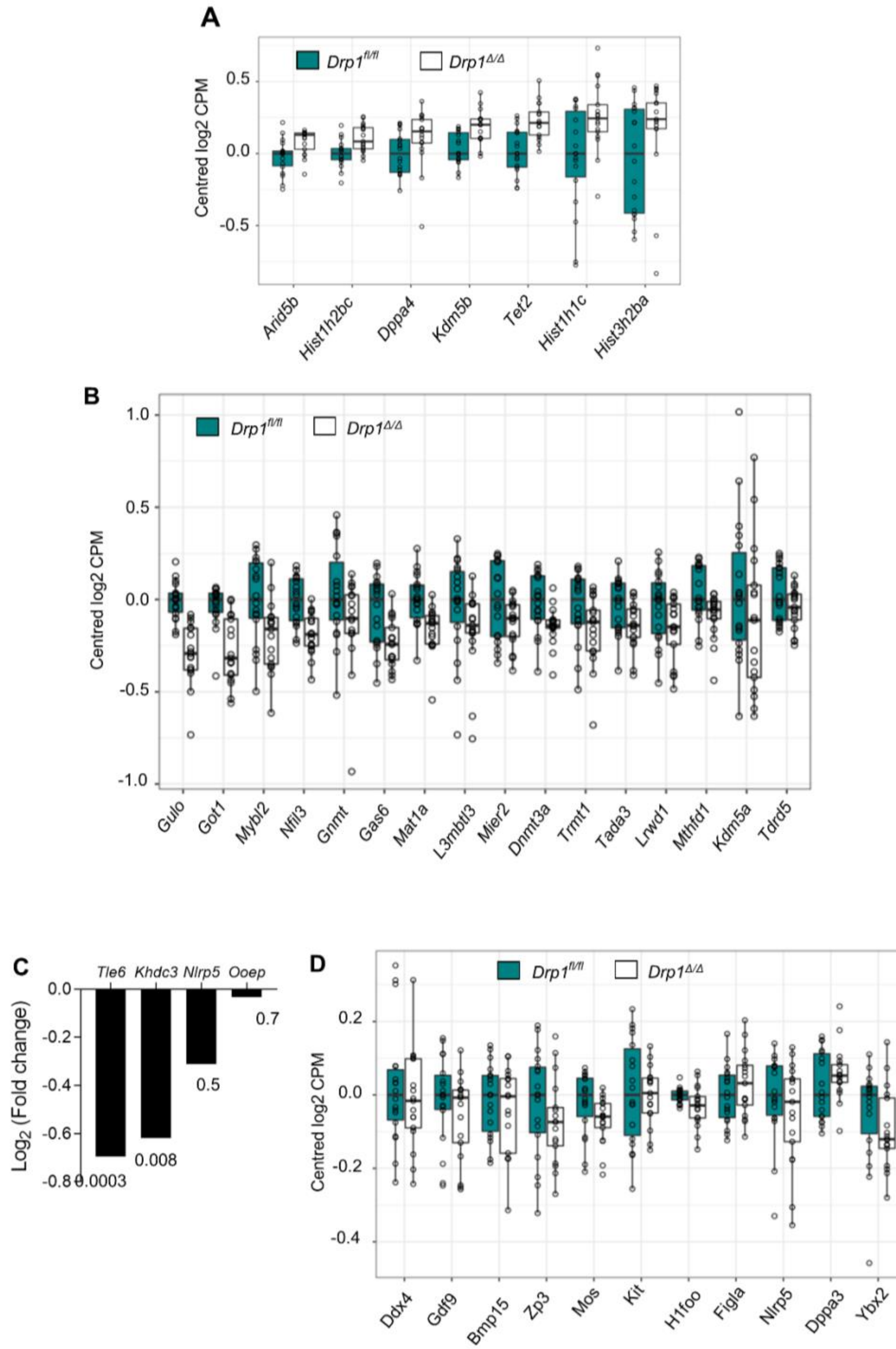

Fig. S3 continued

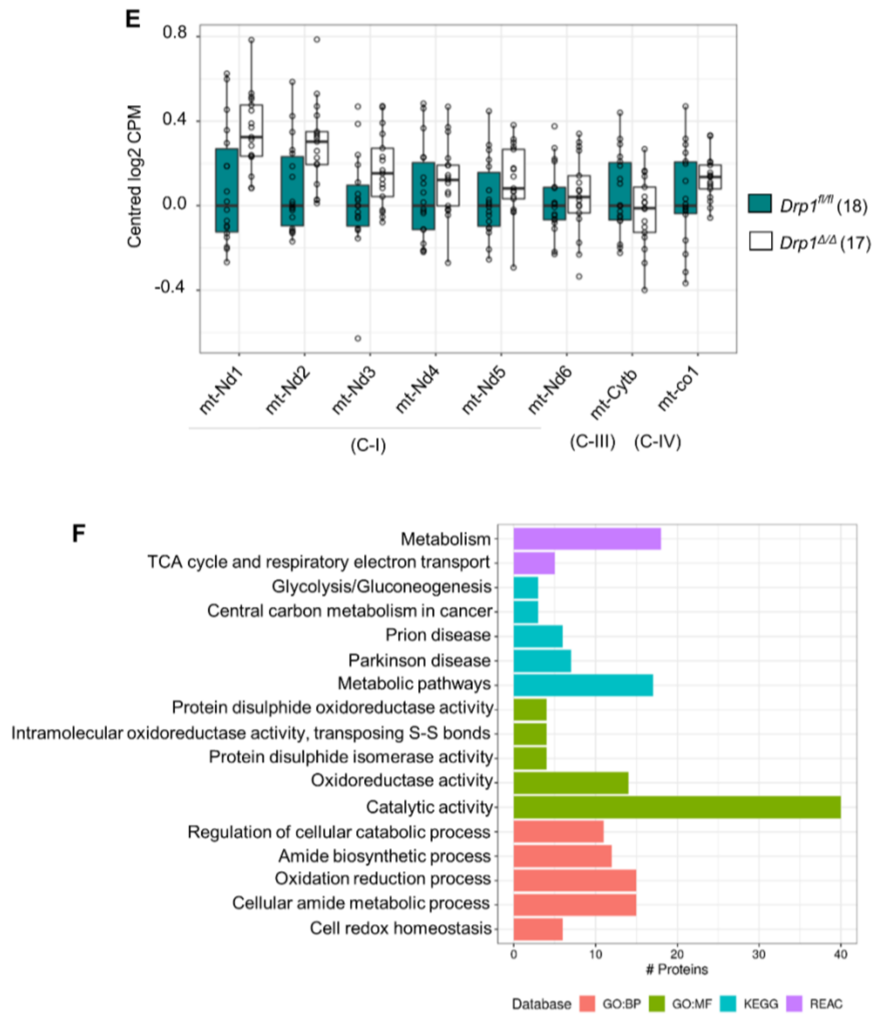

Fig. S3. Gene expression analysis between *$Drp1^{fl/fl}$* and *$Drp1^{\Delta/\Delta}$* oocytes. (A-E) Single cell transcriptomic analysis in individual germinal vesicle stage oocytes. Significantly upregulated (A) and downregulated (B) epigenetic regulators in *$Drp1^{\Delta/\Delta}$* oocytes compared to *$Drp1^{fl/fl}$* oocytes. (C) Log2 fold change in Subcortical Maternal Complex members between *$Drp1^{fl/fl}$* and *$Drp1^{\Delta/\Delta}$* oocytes. (D) No significant changes in the expression of oocyte-specific genes between *$Drp1^{fl/fl}$* and *$Drp1^{\Delta/\Delta}$* oocytes. (E) No significant changes in the expression of mtDNA-encoded genes between *$Drp1^{fl/fl}$* and *$Drp1^{\Delta/\Delta}$* oocytes. Number of single oocytes analyzed per genotype is shown. (F) Proteomic analysis of MII stage oocytes. Enrichment analysis of differentially abundant proteins between *$Drp1^{fl/fl}$* and *$Drp1^{\Delta/\Delta}$* oocytes. GO, gene ontology; BP, biological process; MF, molecular function; KEGG, Kyoto Encyclopedia of Genes and Genomes; REAC, reactome.

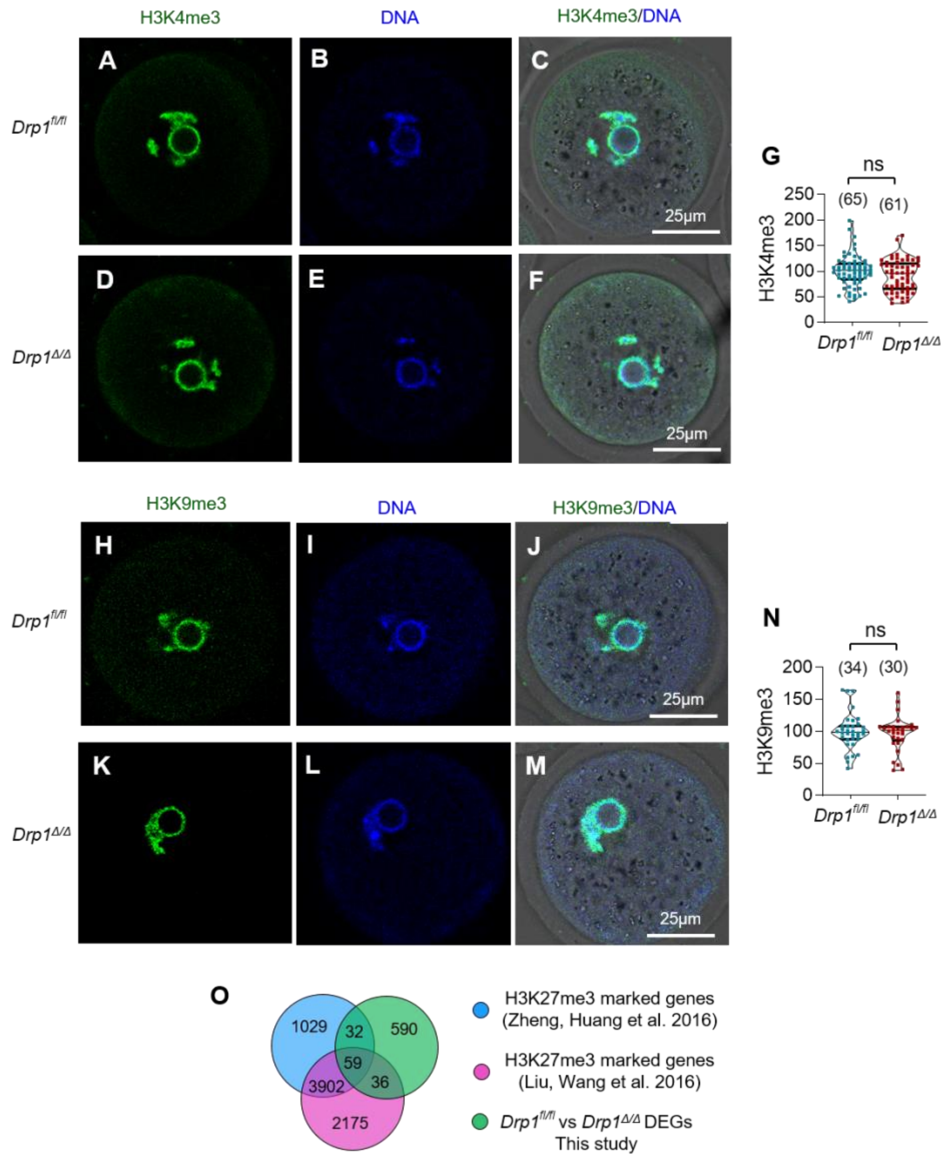

Fig. S4. Comparison of nuclear epigenetic marks between *Drp1$^{fl/fl}$* and *Drp1$^{\Delta/\Delta}$* oocytes. Immunofluorescence (IF) showing H3K4me3 (A, green), DNA (B, blue) and merged (C) in *Drp1$^{fl/fl}$* oocytes. IF showing H3K4me3 (D, green), DNA (E, blue) and merged (F) in *Drp1$^{\Delta/\Delta}$* oocytes. (G) Quantification of H3K4me3 fluorescence. (H) Quantification of H3K9me3 fluorescence. IF showing H3K9me3 (H, green), DNA (I, blue) and merged (J) in *Drp1$^{fl/fl}$* oocytes. IF showing H3K9me3 (K, green), DNA (L, blue) and merged (M) in *Drp1$^{\Delta/\Delta}$* oocytes. (N) Quantification of H3K9me3 fluorescence. Results show mean ± SD and *P* values determined by two-tailed Student's *t*-test (G, N). (O) Venn diagram showing the differentially expressed genes between *Drp1$^{fl/fl}$* and *Drp1$^{\Delta/\Delta}$* oocytes in this study matching with previously published genes known to be marked with H3K27me3 in mouse oocytes.

Table S1. Developmental potential of *Drp1*$^{mat\Delta/+}$ embryos after natural mating.

Mating (Embryo genotype)	Number of			
	Plugged	Pregnancies	Average implantation per pregnancy (Mean ± SD)	% resorption of implants (resorbed of total)
Drp1$^{fl/fl}$ ♀ x WT ♂ (***Drp1***$^{fl/+}$)	17	16 (94%)	8 ± 1.1	3% (4/129)
Drp1$^{fl/fl}$, *Gdf9-Cre* ♀ x WT ♂ (***Drp1***$^{mat\Delta/+}$)	25	15 (60%)*	4.2 ± 2.3	76% (48/63)

Plugged, found with vaginal plug. Pregnancy, uteri containing live or dead embryos at dissection. * Chi square test, $P < 0.0001$.

Table S2. Proteomic Excel file (separate file)

Table S3. H3K27me3 marked genes (separate file)

Table S4. Developmental potential of *Drp1matΔ/+* embryos after embryo transfer.

Mating (Embryo genotype)	Number of				
	2-cell	Transfers	Pregnancies	Total Implantations (% of 2-cells)	Overall viable embryos (% of 2-cells)
Drp1$^{fl/fl}$ ♀ x WT ♂ (***Drp1***$^{fl/+}$)	74	9	8 (89%)	40 (54%)	39 (53%)
Drp1$^{fl/fl}$, *Gdf9-Cre* ♀ x WT ♂ (***Drp1***$^{mat\Delta/+}$)	65	8	4 (50%)	14 (22%)	8 (12%)*

Transfers, to pseudopregnant recipients; Pregnancies, recipients with implantation sites (% of 2-cells transferred); Viable embryos, live embryos at the expected developmental stage (% of 2-cell zygotes). * Chi square test, $P < 0.0001$.

Table S5. Developmental potential of reconstituted zygotes.

Group	Number of				
	2-cell	Transfers	Pregnancies	Implantations	Viable embryos
KO Pn. in WT Cyt (**KO Pn**)	178	17	9 (53%)	61 (34%)	24 (13%)
WT Pn. in KO Cyt (**KO Cyt**)	182	17	15 (88%)	75 (41%)	49 (27%)