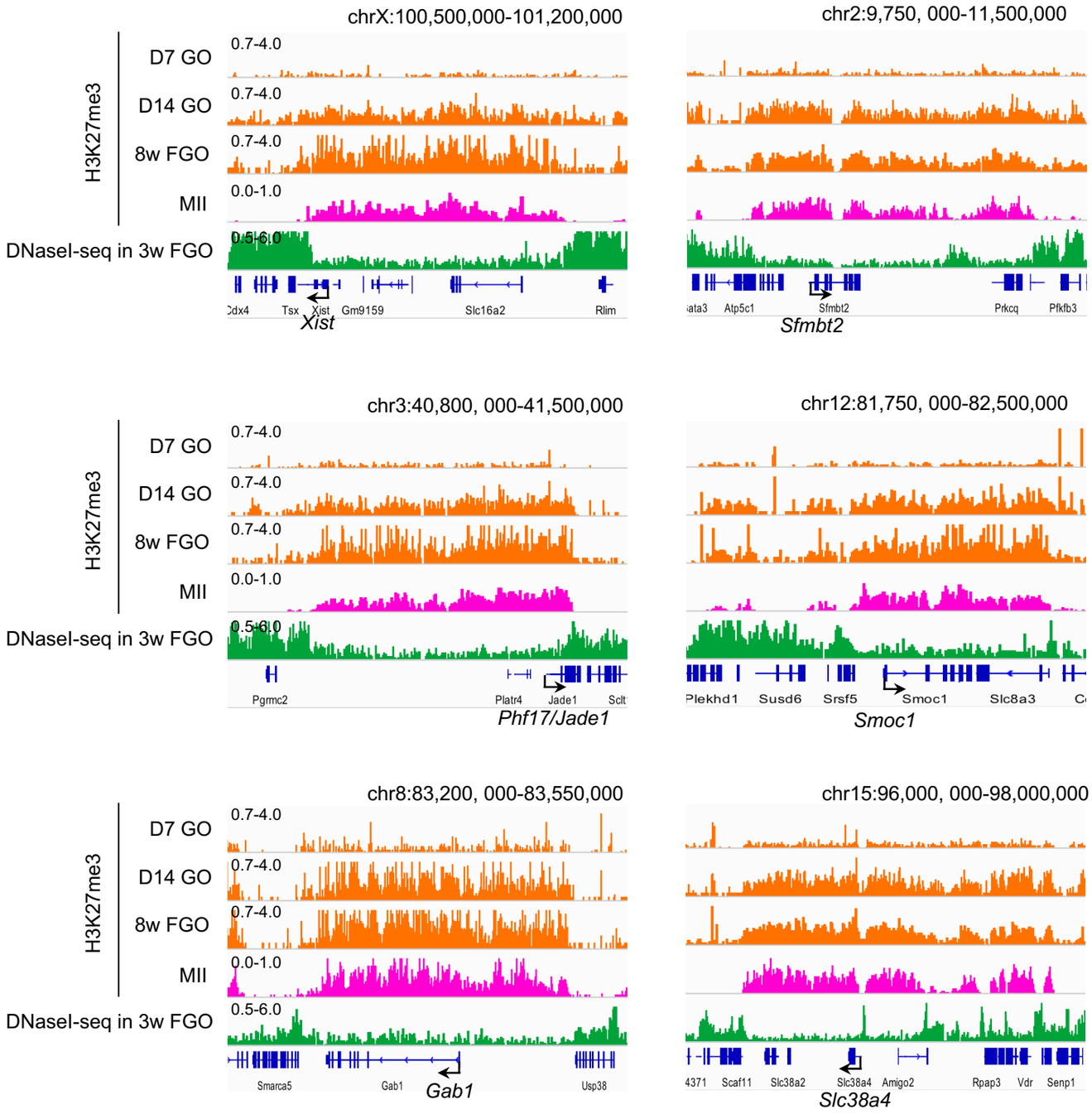


Figure S1. Maternal H3K27me3 domains are established during oocyte growth.

Genome browser view of H3K27me3 ChIP-seq and DNaseI-seq at H3K27me3 imprinted genes. D7 GO, growing oocytes from 7-day old females. D14 GO, growing oocytes from 14-day old females. 8w FGO, fully-grown oocytes from 8-week old females. MII, MII-stage oocytes. The H3K27me3 ChIP-seq datasets of D7 GO, D14 GO, and 8w FGO were obtained from GSE76687 (Zheng et al. 2016) and that of the MII oocytes was obtained from GSE73952 (Liu et al., 2016). The DNaseI-seq dataset was obtained from GSE92605 (Inoue et al. 2017a).

Supplemental_Fig_S2.

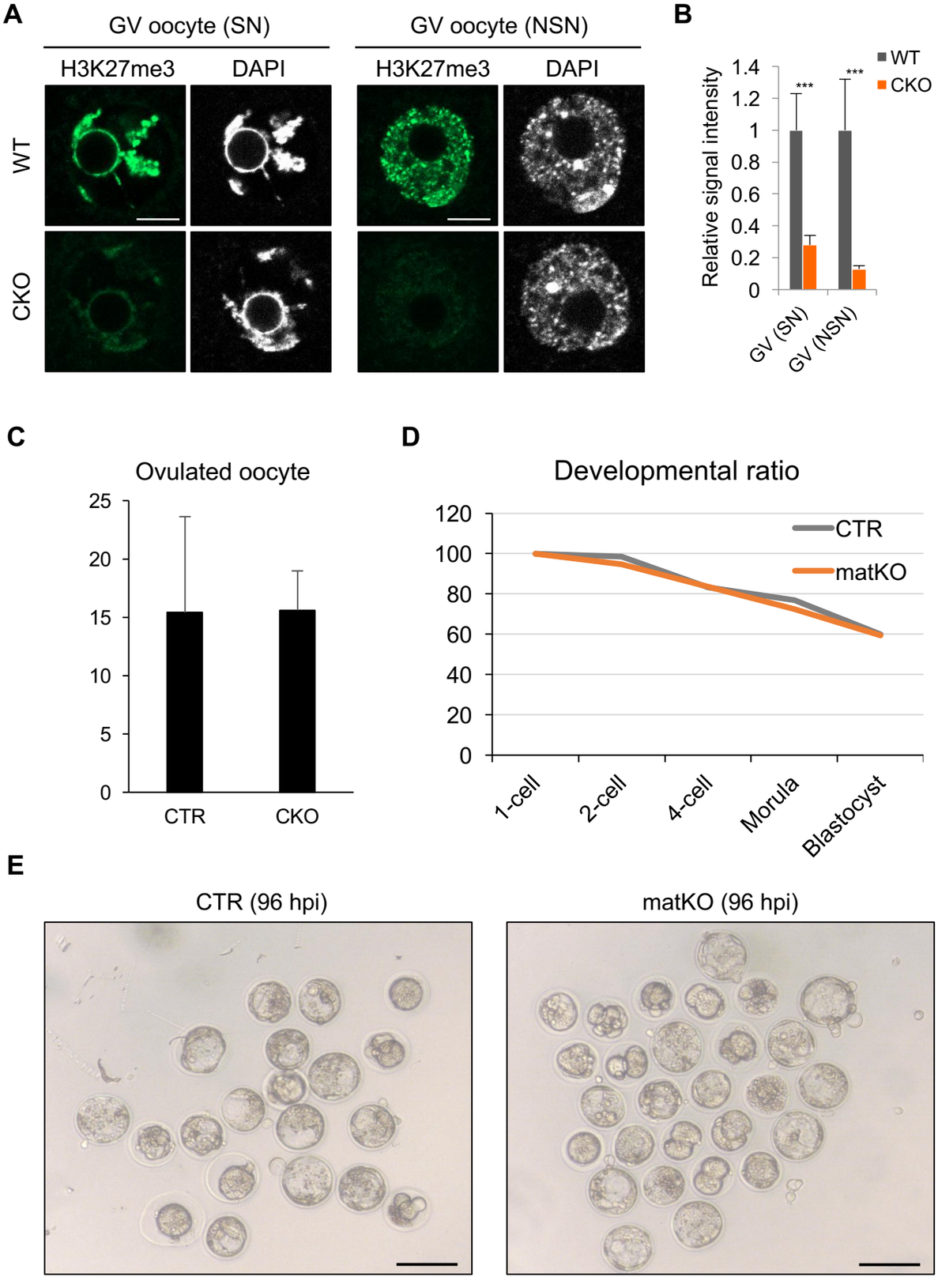


Figure S2. The number of ovulated oocytes and the preimplantation developmental ratio

(A) The number of superovulated MII oocytes in CTR and CKO. Error bars indicate SD. The experiments were repeated 5 times with 3-10 females used in each experiment.

(B) Percentages of *in vitro* fertilized derived embryos that reach the indicated stages at 28 (2-cell), 48 (4-cell), 78 (morula), and 96 (blastocyst) hours post-insemination (hpi). The number of 1-cell zygotes was set as 100%. The experiments were repeated 4 times with 11-68 embryos used in each experiment.

(C) Representative images of control and *Eed* matKO embryos at 96 hpi. Scale bar, 100μm

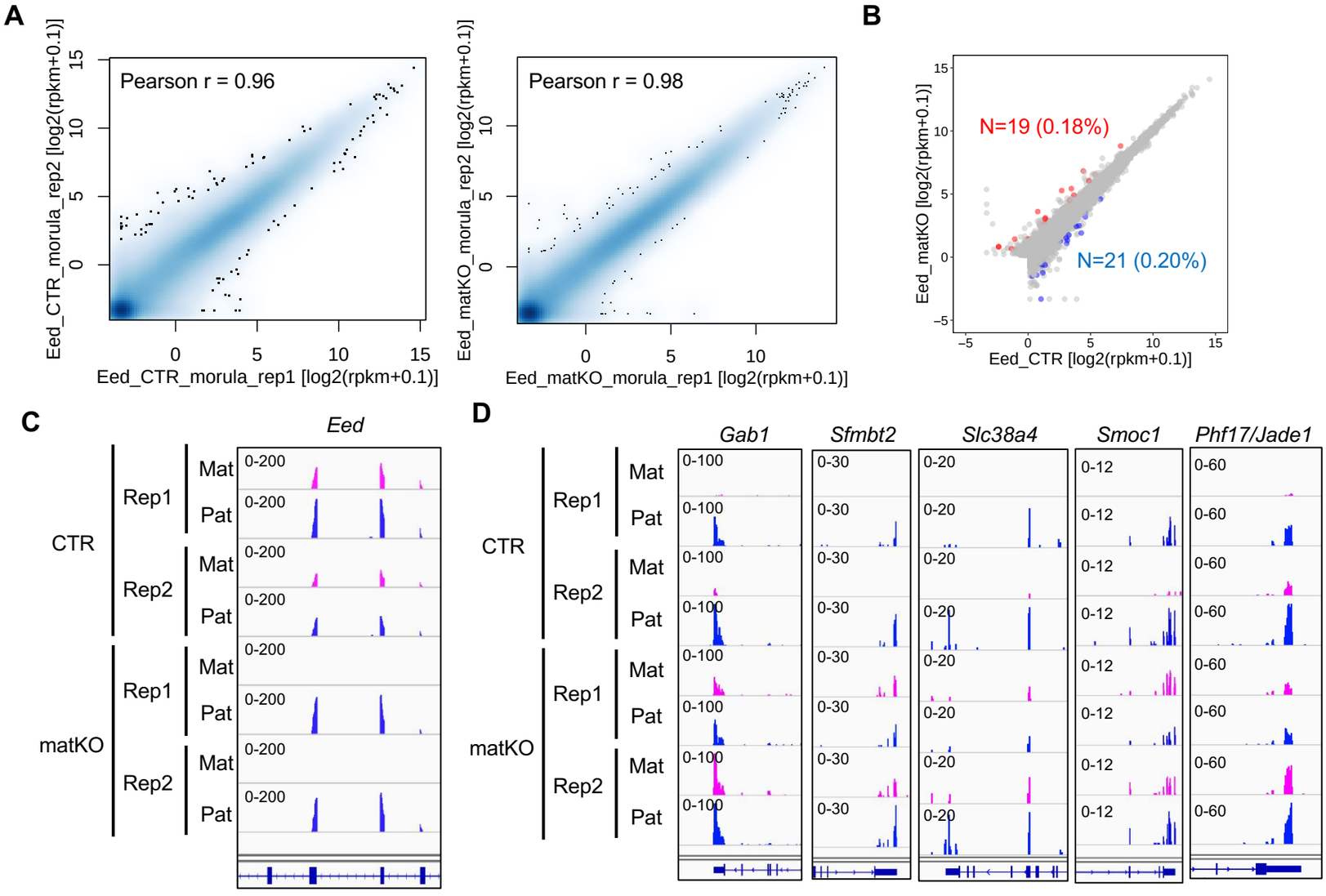


Figure S3. Loss of H3K27me3 imprinting in *Eed* matKO morula embryos

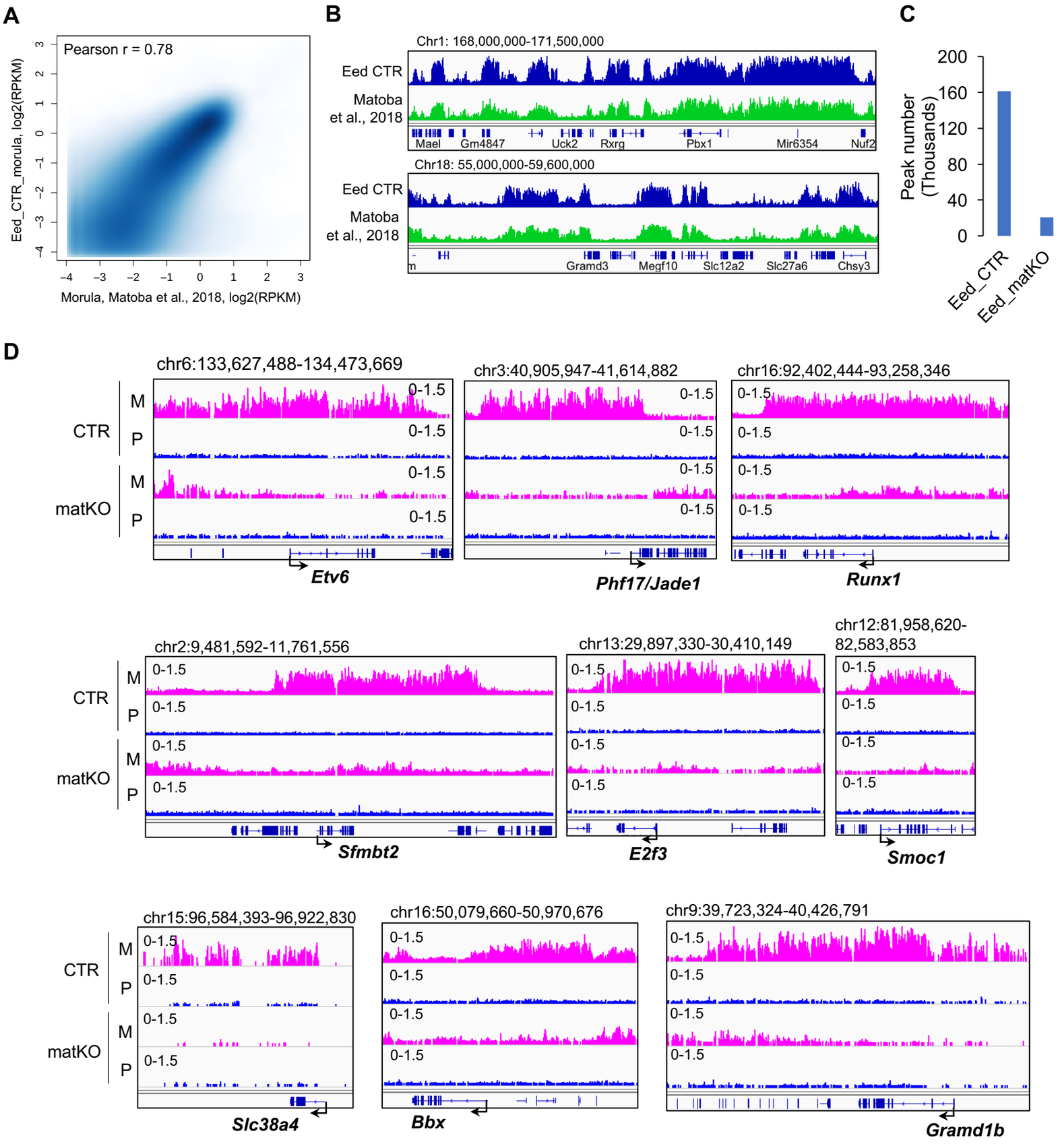
(A) Scatter plot depicting the correlation between biological duplicate of morula RNA-seq samples.

(B) Scatter plot comparing RNA-seq data of CTR and *Eed* matKO embryos. Red and blue dots indicate up- and down-regulated genes, respectively, with (p-value < 0.05 & fold change > 2) as the cutoffs.

(C) Genome browser view of RNA-seq data of *Eed* in morula embryos.

(D) Genome browser view of RNA-seq data at H3K27me3-dependent imprinted genes.

Supplemental_Fig_S4.



D (continued)

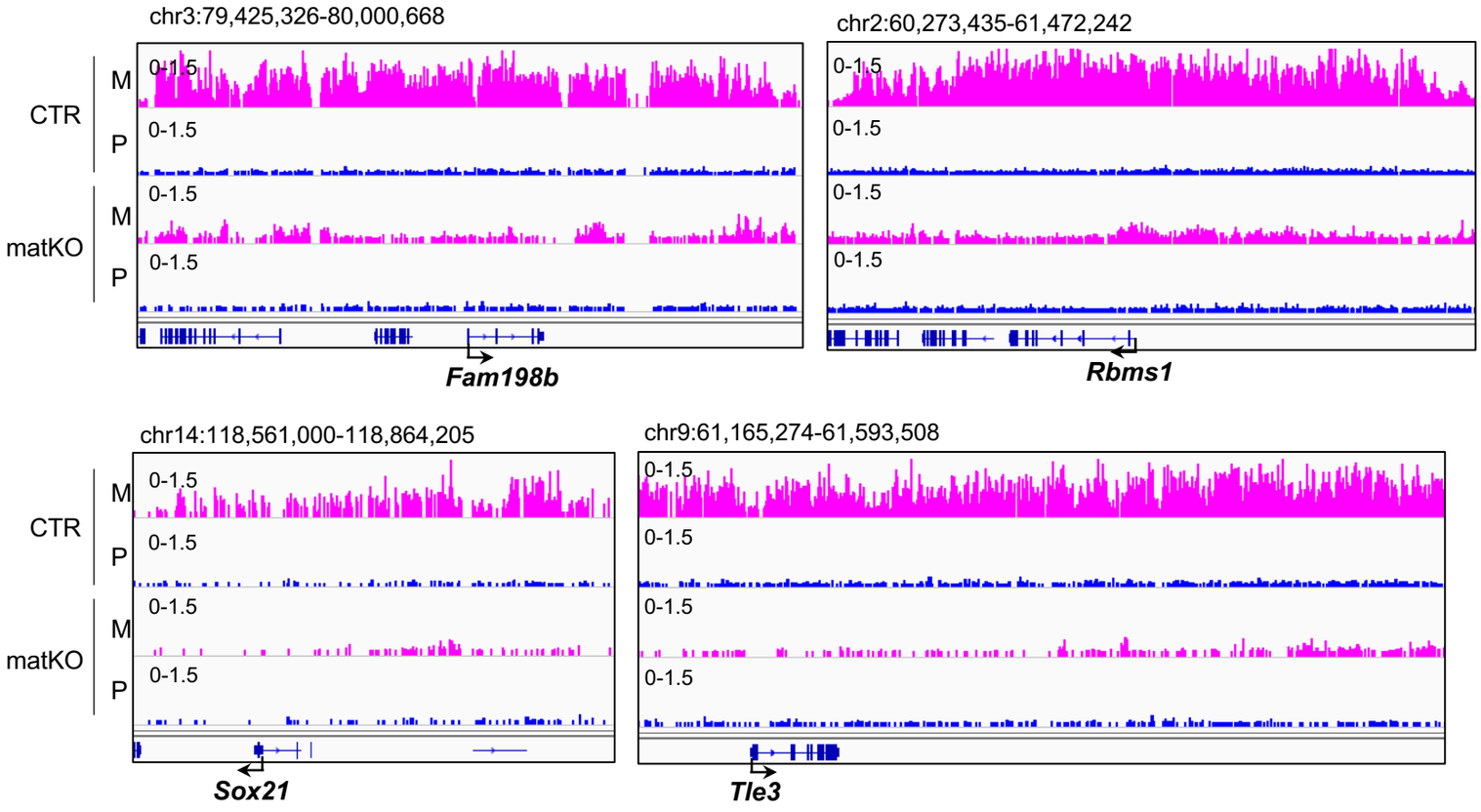


Figure S4. Loss of maternal H3K27me3 domains at the H3K27me3-dependent imprinted loci in *Eed* matKO morula embryos.

(A) Scatter plot showing a correlation between H3K27me3 peaks of a published dataset (Matoba et al. 2018) and *Eed* CTR morula embryos.

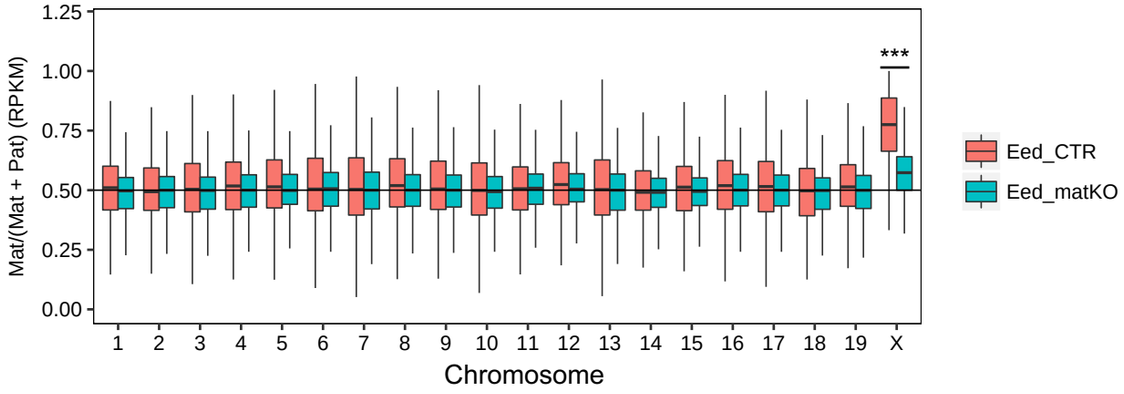
(B) Genome browser views of two representative loci showing high similarity of H3K27me3 enrichment between *Eed* CTR morula embryos and the public dataset.

(C) The number of H3K27me3 peaks identified in *Eed* CTR and matKO morula embryos using MACS2.

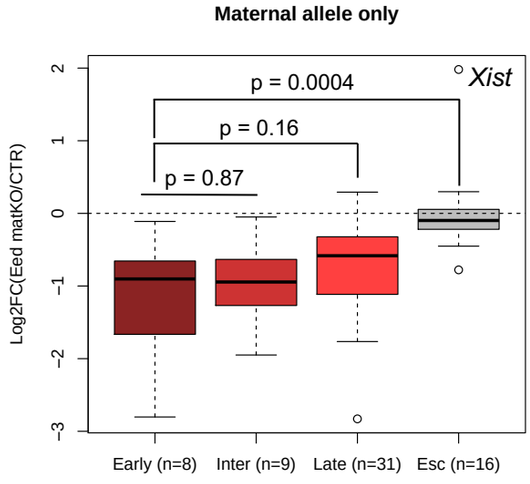
(D) Genome browser view of the 14 H3K27me3-dependent imprinted loci (Fig. 1E) showing loss of maternal H3K27me3 domain in *Eed* matKO morula embryos.

Supplemental_Fig_S5.

A



B



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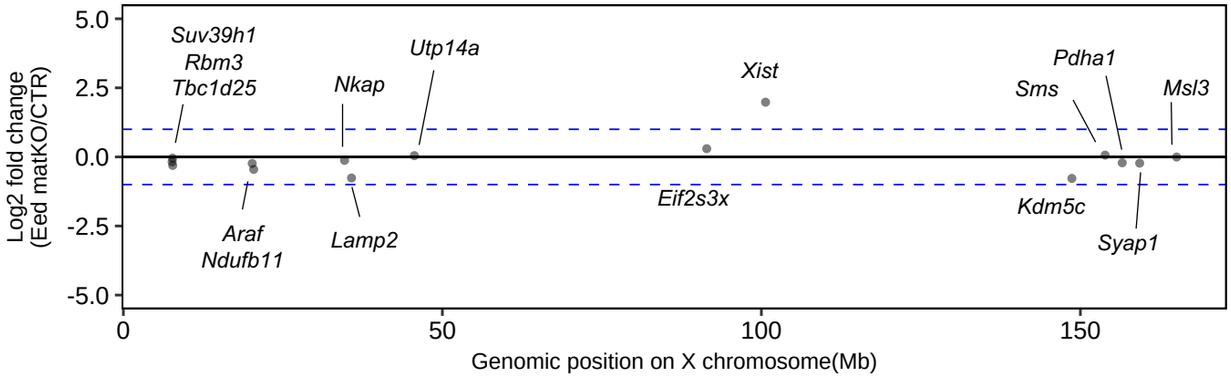


D

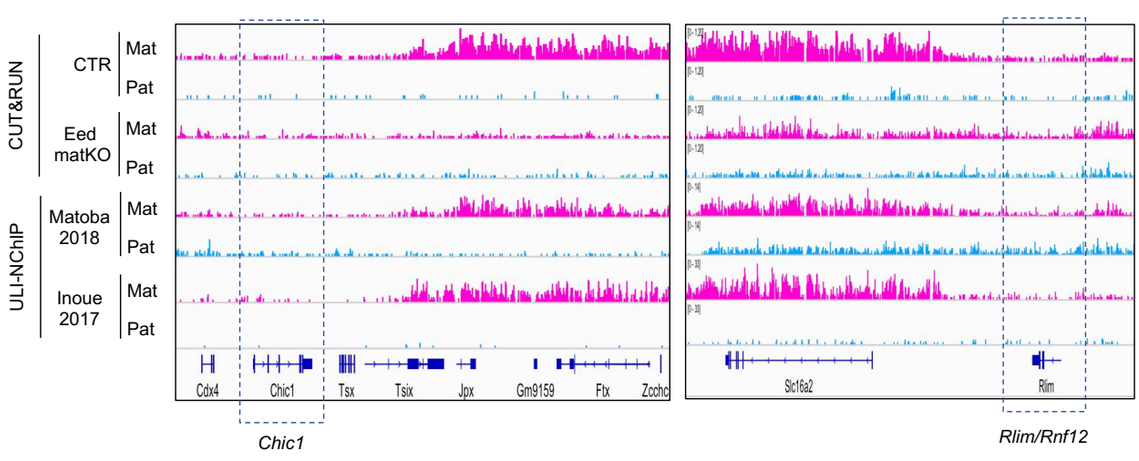


Figure S5. Maternal XCI in *Eed* matKO morula embryos

(A) Box plot showing the maternal allelic expression ratio [Mat/(Mat+Pat)] of individual chromosomes in CTR and *Eed* matKO embryos. Box edges and whiskers indicate the 25^{th}/75^{th} and 2.5^{th}/97.5^{th} percentiles, respectively. ***, $p<4.7 \times 10^{-91}$; (Mann-Whitney-Wilcoxon Test).

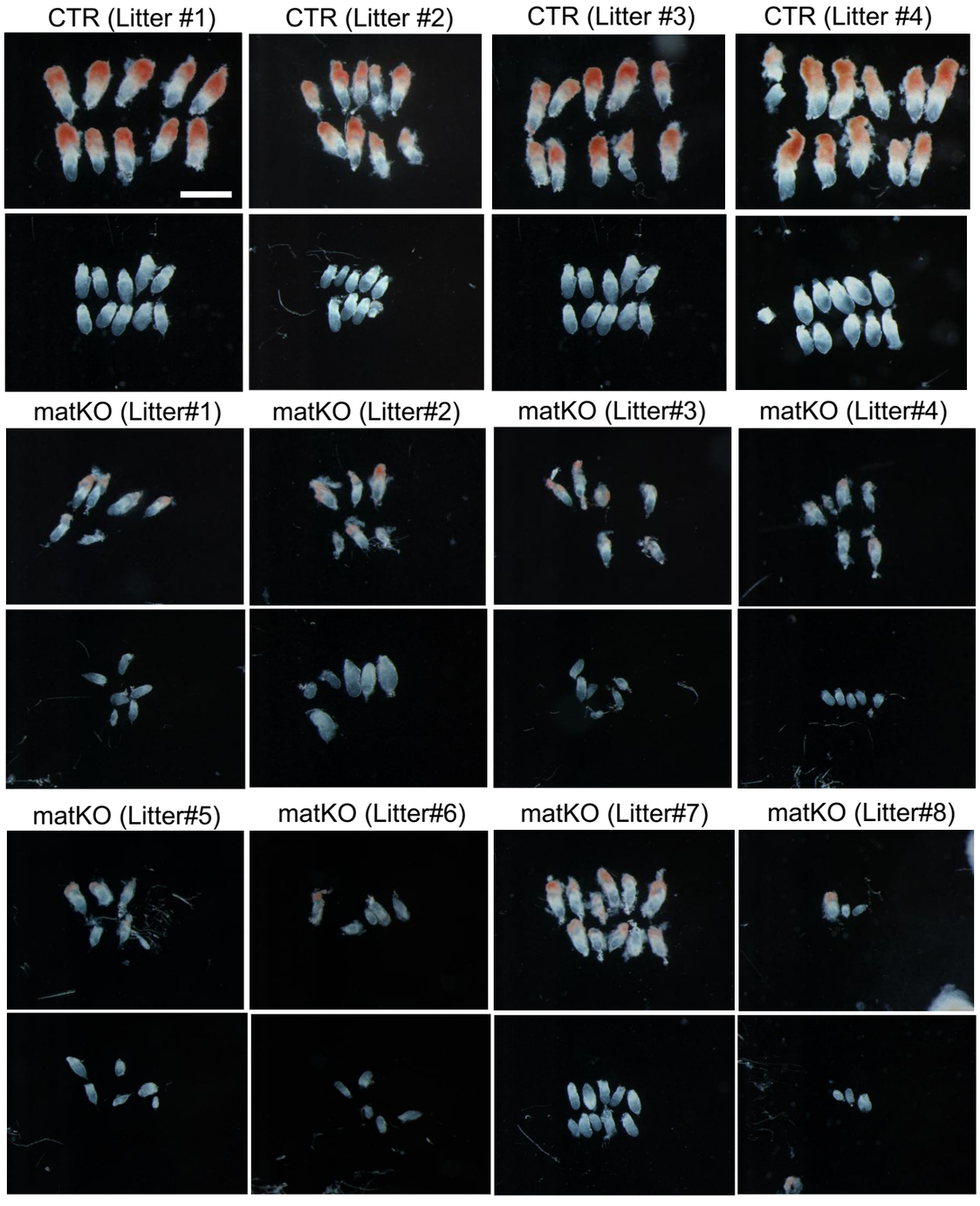
(B) Box plot showing the expression level changes of different category of X-linked genes in *Eed* matKO morula embryos. Early, intermediate, and late silenced genes and imprinted XCI escapees were obtained from Borensztein et al., 2017. Genes with enough SNP reads (SNP reads RPKM > 0.5) were analyzed. Middle lines in the boxes represent the medians. Box edges and whiskers indicate the 25th/75th and 2.5th/ 97.5th percentiles, respectively. Mann-Whitney-Wilcoxon Test p-values are as indicated. Early = early silenced genes; Inter: intermediately silenced genes; Late: late silenced genes; Esc: escapees.

(C) The relative expression levels of known escapees between CTR and *Eed* matKO morula embryos. The expression levels of the maternal allele were analyzed. Dotted lines indicate 2-fold of differential expression.

(D) Genome browser view of H3K27me3 CUT&RUN or low-input ChIP-seq (Matoba et al. 2018, Inoue et al., 2017b) at early silenced X-linked genes.

Supplemental_Fig_S6.

A



B

Mother	Father	No. of litters	No. of decidua (Ave±SD)	No. of embryos (Ave±SD)	No. of females	No. of males
Eed$^{fl/fl}$	B6	7	67 (9.6±1.0)	59 (8.4±1.5)	29/53 (55%)	24/53 (45%)
Gdf9^{Cre}, *Eed*$^{fl/fl}$	B6	11	94 (8.5±1.6)	67 (6.1±2.0)	31/48 (65%)	17/48 (35%)
Eed$^{fl/fl}$	PWK	3	35 (11.7±1.1)	35 (11.7±1.1)	13/27 (48%)	14/27 (52%)
Gdf9^{Cre}, *Eed*$^{fl/fl}$	PWK	5	46 (9.2±0.8)	42 (8.4±1.5)	20/33 (61%)	13/33 (39%)

Figure S6. *Eed* matKO embryos show growth retardation and male-biased sublethality at E6.5

(A) Representative images of E6.5 embryos. For each litter, top and bottom panels showing the embryos before or after removing ectoplacental cones and Reichert membranes. Scale bar, 1 mm.

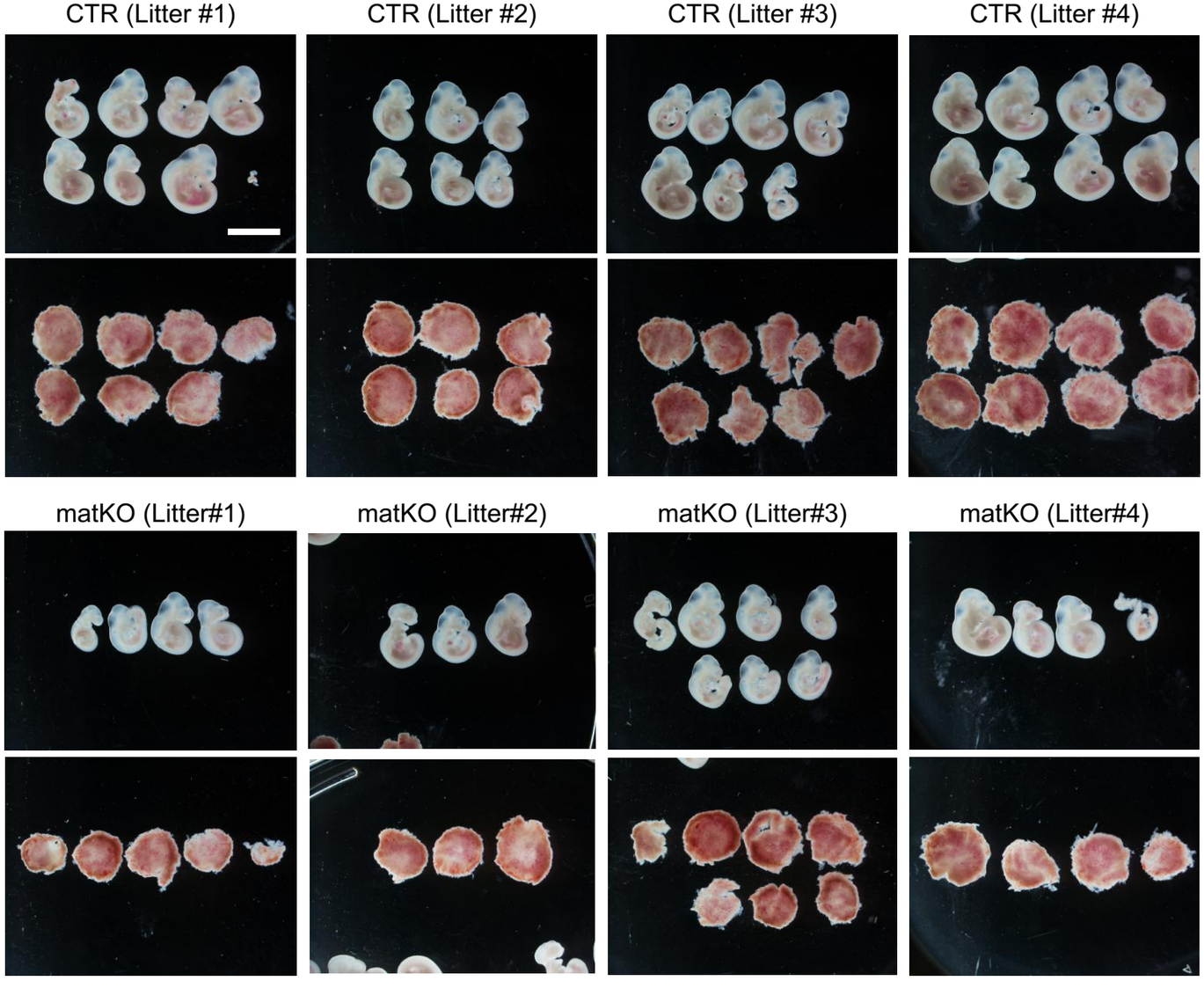
(B) Summary of E6.5 dissection.

Supplemental_Fig_S7.

A

Mother	Father	No. of litters	No. of Decidua (Ave±SD)	No. of absorbed (Ave±SD)	No. of embryos (Ave±SD)	No. of females (%)	No. of males (%)
Eed$^{fl/fl}$	B6	6	55 (9.2±1.6)	8 (1.3±1.5)	47(7.8±1.8)	20/47 (43%)	27/47 (57%)
Gdf9^{Cre}, *Eed*$^{fl/fl}$	B6	5	35 (7.0±2.6)	9 (1.8±1.5)	26 (5.2±2.2)	19/26 (73%)	7/26 (27%)

B



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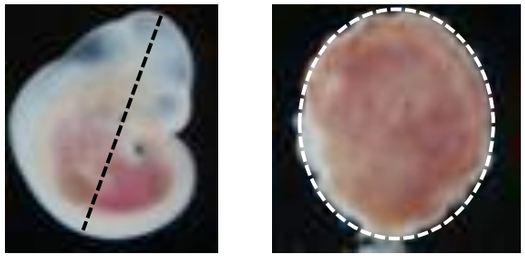


D

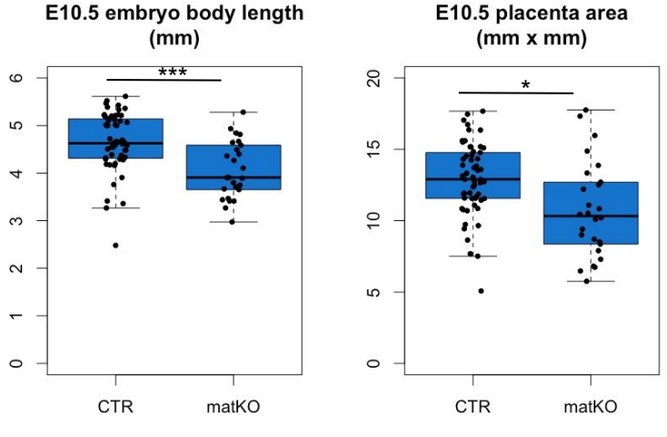


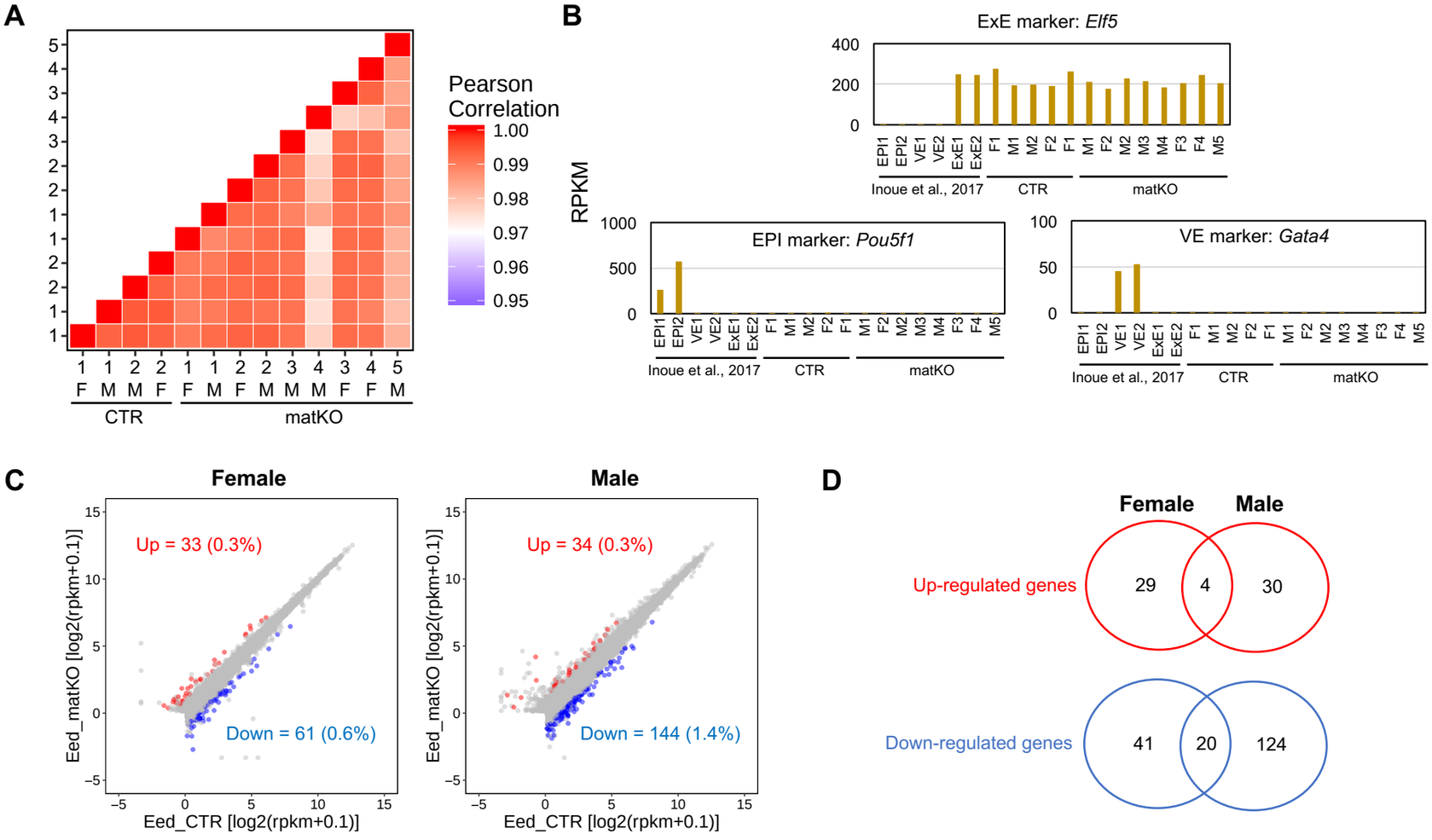
Figure S7. Dissection of *Eed* matKO embryos at E10.5.

(A) Summary of E10.5 dissection.

(B) Representative images of E10.5 embryos. For each litter, top and bottom panels show the fetus and the placenta, respectively. Scale bar, 5mm.

(C) Illustration to show how to quantify the embryo body length and placental size.

(D) Box plot showing the embryo body length and placenta size. Each black point represents each individual embryo or placenta. Student t-test p-values, *** = 8.1×10^{-5}, * = 0.001.



E

Commonly down-DEGs in both sexes

Gene name	Chr		Gene name	Chr
Acyp2	chr11		Prdm5	chr6
Dhrs7	chr12		Calca	chr7
Gpx2	chr12		Cited1	chrX
Rnase1	chr14		Klhl13	chrX
Khdrbs3	chr15		Nr0b1	chrX
Mal2	chr15		Nudt11	chrX
Pla2g16	chr19		Pir	chrX
Ttpa	chr4		Tmsb15b1	chrX
Prom1	chr5		Tmsb15b2	chrX
Bcl2l14	chr6		Xlr4c	chrX

Commonly up-DEGs in both sexes

Gene name	Chr
Thg1l	chr11
Lcp1	chr14
C430002E04Rik/Platr4	chr3
Naa11	chr5

Figure S8. Characterization of transcriptomes in *Eed* matKO extra-embryonic ectoderm (ExE) at E6.5.

(A) Correlation between biological replicates of RNA-seq samples. M, male. F, female.

(B) Expression levels of cell lineage marker genes. A public dataset of epiblast (EPI), visceral endoderm (VE), and ExE were used as a control.

(C) Scatter plot comparing RNA-seq in CTR and *Eed* matKO ExEs. Red and blue dots indicate up- and down-regulated genes, respectively, with (p value < 0.05 & fold change > 2) as a cutoff.

(D) Vienn diagram showing DEG numbers in male and female *Eed* matKO ExEs.

(E) The list of commonly down-and up-regulated DEGs in both sexes of *Eed* matKO ExEs.

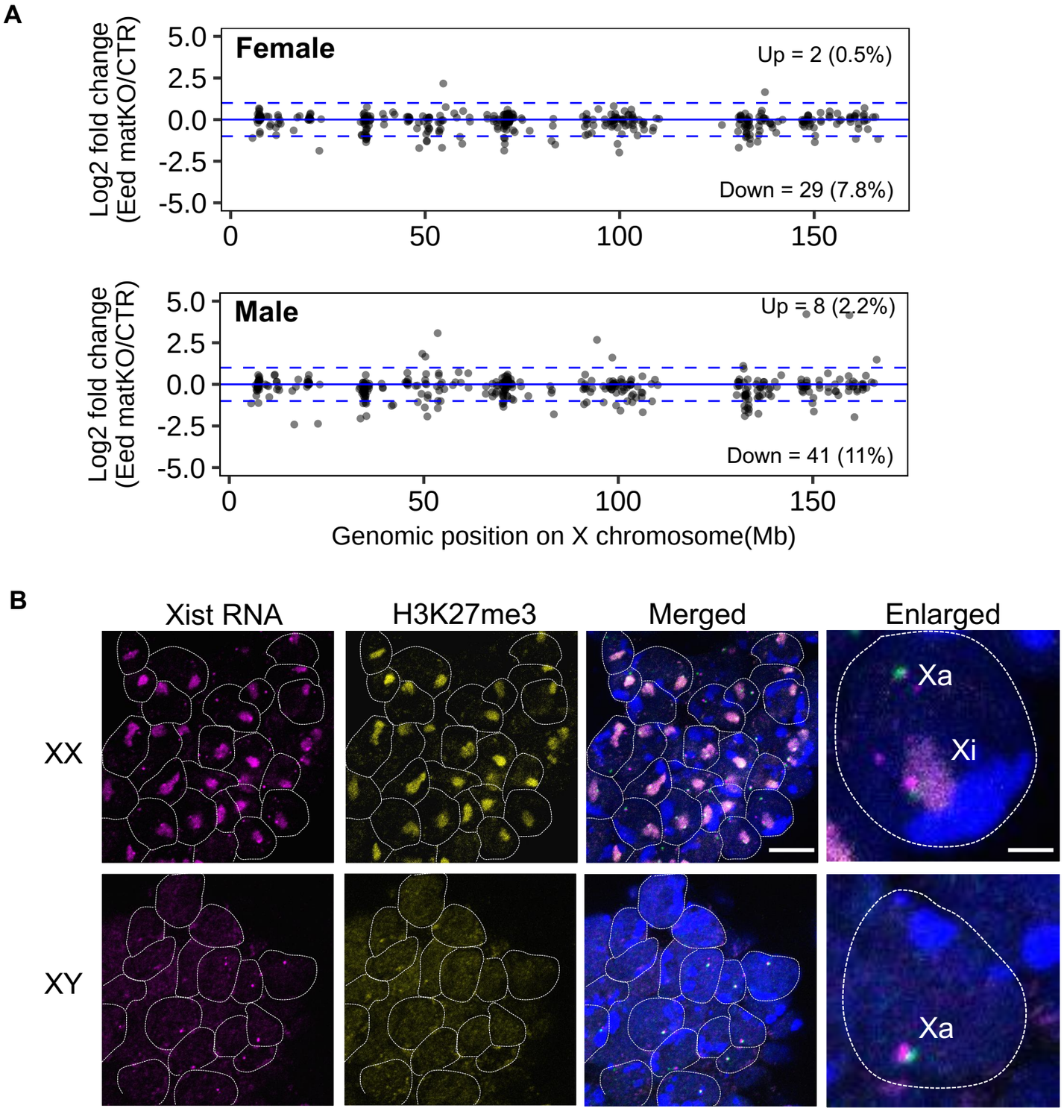


Figure S9. Characterization of XCI in *Eed* matKO extra-embryonic ectoderm (ExE) at E6.5.

(A) Relative expression levels of X-linked genes between CTR and *Eed* matKO ExEs. Each dot represents an individual gene (RPKM>1). Dashed lines indicate 2-fold differential expression.

(B) Representative images of immuno (H3K27me3)-RNA (*Xist*)/DNA(*Rnf12*) FISH in ExEs of female (XX) and male (XY) E6.5 embryos. Blue color indicates DAPI. Scale bars in the merged and enlarged images are 20 and 5 μm, respectively. Xi, inactive X chromosome. Xa, active X chromosome.

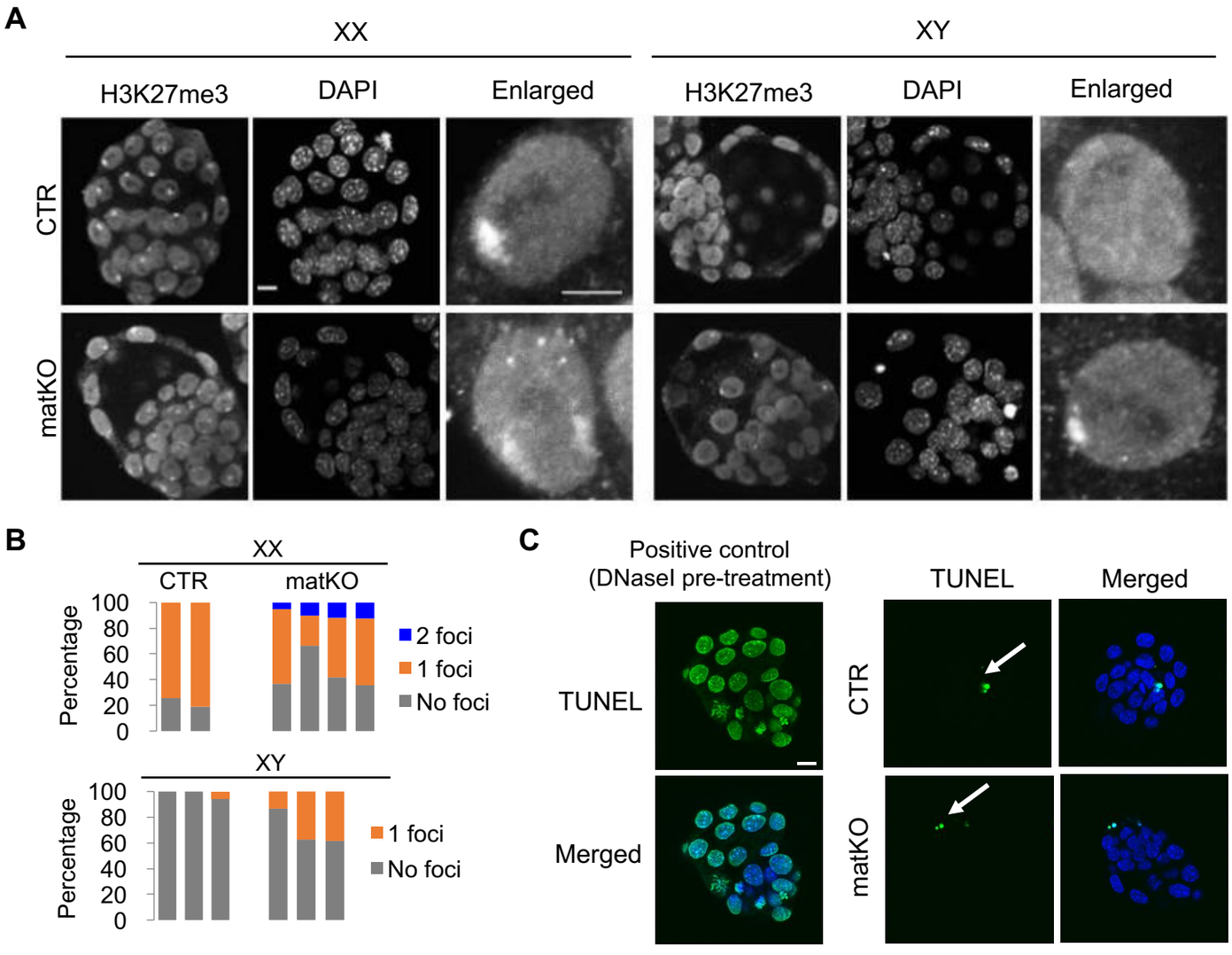


Figure S10. H3K27me3 immunostaining and TUNEL assay in E3.5 embryos.

(A) Representative images of E3.5 blastocysts immunostained with an anti-H3K27me3 antibody. Scale bars in the H3K27me3 images and enlarged images are 10 and 5 μm, respectively.

(B) Quantification of H3K27me3 foci. Each bar represents an individual embryo. The numbers of embryos examined were 5 (CTR) and 7 (matKO).

(C) Representative TUNEL assay images in E3.5 embryos (right panel). The number of control and matKO embryos examined were 3 and 3, respectively. The positive control embryo (left panel) was prepared by pre-treatment with DNaseI to create double DNA strand breaks. The white arrows indicate polar bodies. Scale bar, 10 μm.