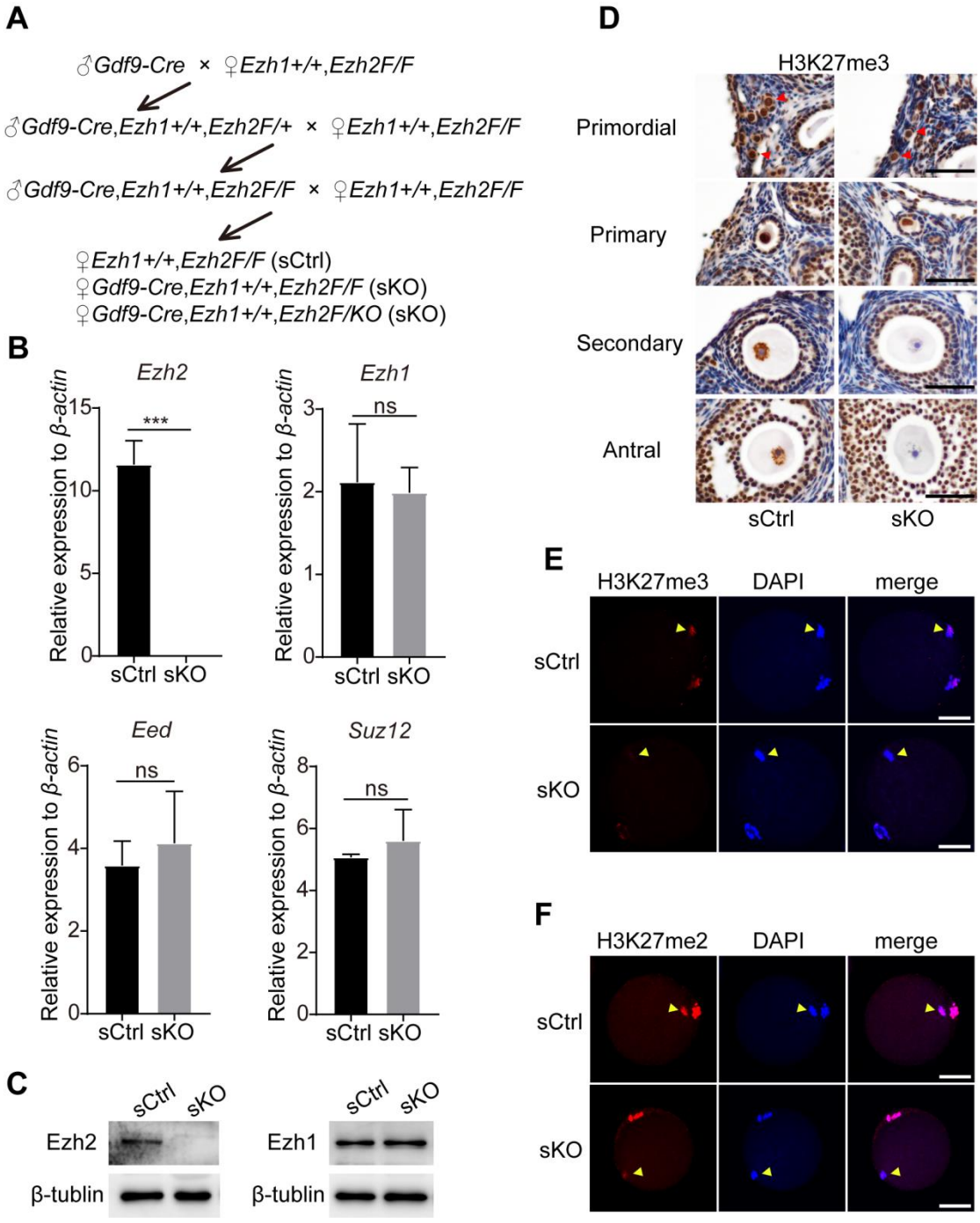

Fig. S1. Generation of maternal *Ezh2* deficient mice and knockout efficiency in oocytes. (A) Breeding scheme used to generate sCtrl and sKO mice. **(B)** Real-time PCR analysis of mRNA level for *Ezh2*, *Ezh1*, *Eed* and *Suz12* in sCtrl and sKO oocytes. Data are presented as the mean ± SD. Unpaired *t* test: ns, not significant; ***$P < 0.001$. **(C)** Levels of Ezh2 and Ezh1 protein in oocytes as assessed by western blot analysis; β-tublin was used as loading control. **(D)** H3K27me3 in

oocytes from primordial, primary, secondary and antral follicles checked by immunohistochemical (IHC) staining. Ovary sections were stained with anti-H3K27me3 antibody and counterstained with hematoxylin. The primordial follicles were indicated by red arrows. Scale bars, 50 μm. **(E and F)** H3K27me3 and H3K27me2 in super-ovulated oocytes measured by immunofluorescence (IF) staining. The chromosomes of oocytes were indicated by yellow arrows. Number of oocytes examined for H3K27me3: sCtrl, n=27; sKO, n=21. For H3K27me2: sCtrl, n=16; sKO, n=20. Scale bars, 50 μm.

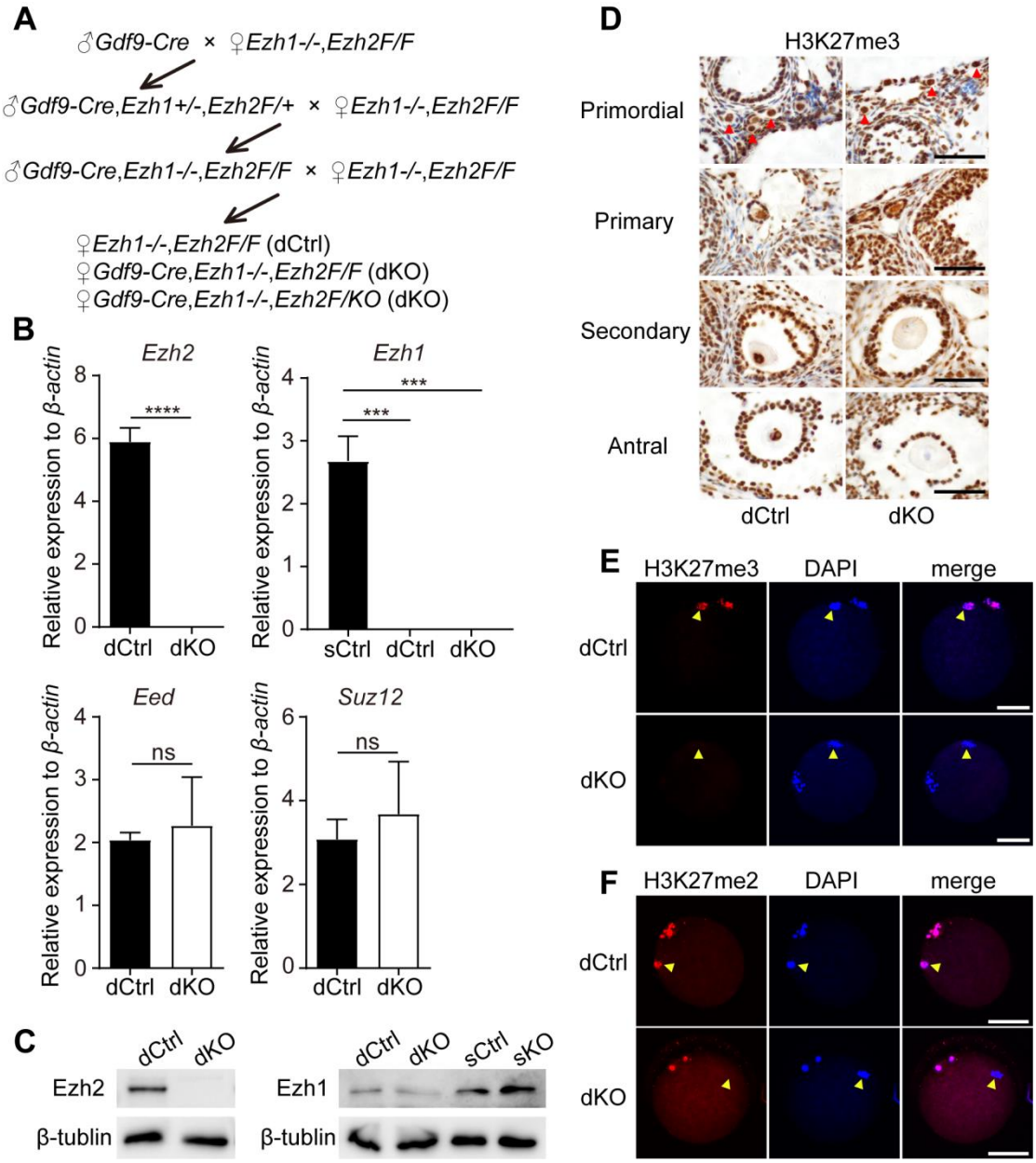

Fig. S2. Generation of maternal *Ezh1/2* deficient mice and knock out efficiency in oocytes. (A) Breeding scheme used to generate dCtrl and dKO mice. **(B)** Real-time PCR analysis of *Ezh2*, *Ezh1*, *Eed* and *Suz12* transcripts in dCtrl and dKO oocytes. Data are presented as the mean ± SD. Unpaired *t* test: ns, not significant; ***$P < 0.001$; ****$P < 0.0001$. **(C)** Levels of Ezh2 and Ezh1 protein in oocytes as assessed by western blot analysis; β-tublin was used as loading control. **(D)** IHC staining showed H3K27me3 modification in oocytes from primordial, primary, secondary and antral follicles. Ovary sections were stained with anti-H3K27me3 antibody and then counterstained with hematoxylin. The primordial follicles were indicated by red arrows. Scale bars, 50 μm. **(E and F)** IF analysis of H3K27me3 and H3K27me2 in super-ovulated oocytes. The chromosomes of oocytes were indicated by yellow arrows. Number of oocytes examined for H3K27me3: dCtrl, n=16; dKO, n=28. For H3K27me2: dCtrl, n=24; dKO, n=32. Scale bars, 50μm.

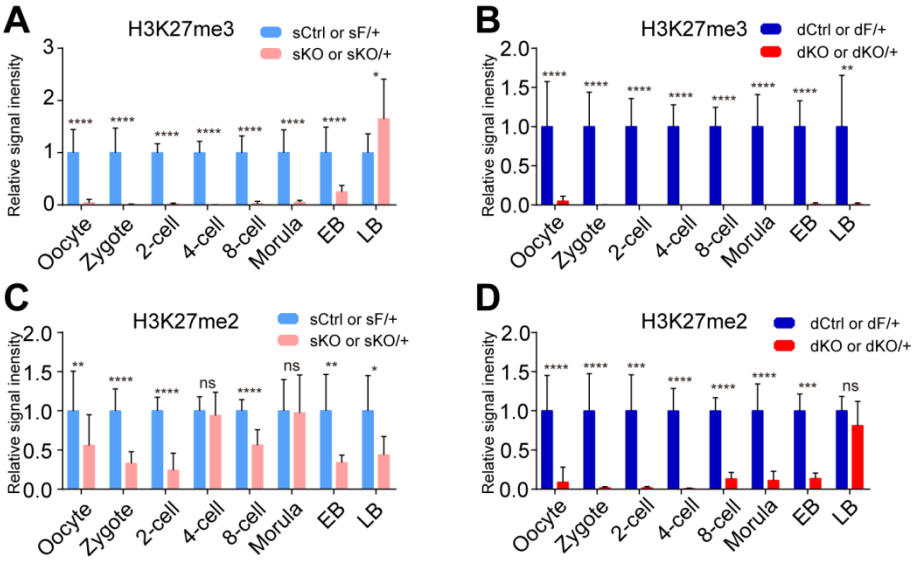

Fig. S3. The relative level of H3K27me3 and H3K27me2 signals in oocytes and embryos. (A) Relative H3K27me3 signal intensity in single knockout groups. (B) Relative H3K27me3 signal intensity in double knockout groups. (C) Relative H3K27me2 signal intensity in single knockout groups. (D) Relative H3K27me2 signal intensity in double knockout groups. EB, early blastocyst; LB, late blastocyst. The average siganls of control oocytes or embryos were set as 1.0. Results are presented as mean ± SD. Mann Whitney test or unpaired *t* test: ns, not significant; **P* < 0.05; ***P* < 0.01; ****P* < 0.001; *****P* < 0.0001.

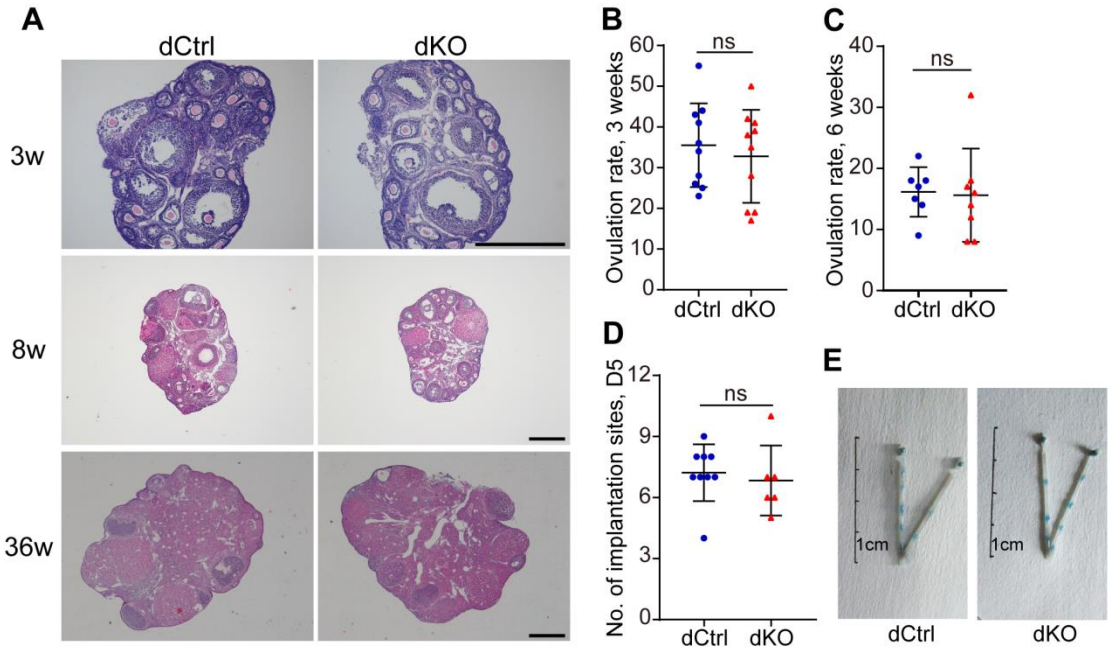

Fig. S4. Ovary morphology, ovulation rate and implantation site in dCtrl and dKO female mice. (A) Ovary sections stained with hematoxylin-rosin (HE) from 3-, 8-, and 36-wk-old mice. 3-wk-old mice were treated with PMSG for 46 hours. Scale bars, 500 μm. **(B and C)** The number of ovulated oocytes per female mouse with superovulation treatment. **(D)** The number of implantation sites on Day 5 of pregnancy in dCtrl and dKO mice. **(E)** Representative images of uteri with implantation sites on Day 5. Scale bars, 1cm. Results are presented as mean ± SD. Statistical comparisons of values were made using unpaired *t* test (B and C) or Mann Whitney test (D). ns, not significant.

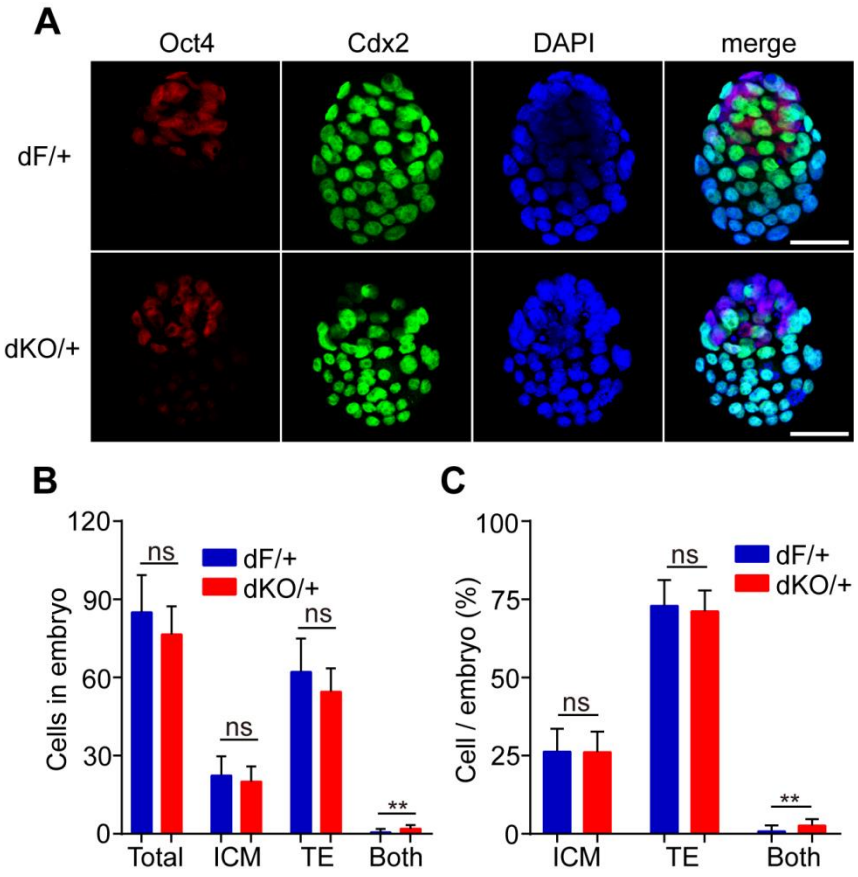

Fig. S5. The normal differentiation of trophectoderm (TE) and the inner cell mass (ICM) in dKO/+ embryos at E3.75. (A) Representative images of Oct4 and Cdx2 immunostaining in embryos at E3.75. Inner cell mass (ICM) were stained with Oct4 (red) and trophectoderm (TE) cells were stained with Cdx2 (green). Number of total embryos: dF/+, n=13; dKO/+, n=11. Scale bars, 50 μm. **(B)** The number of different cell parts in embryos at E3.75. Results are presented as mean ± SD. Statistical comparisons of values were made using Mann Whitney test (Total and Both) or unpaired *t* test (ICM and TE). ns, not significant; $^{**}P < 0.01$. **(C)** The proportion of different cell parts at E3.75. Both: mix-expression of Oct4 and Cdx2. Results are presented as mean ± SD. Statistical comparisons of values were made using Mann Whitney test (Both) or unpaired *t* test (ICM and TE). ns, not significant; $^{**}P < 0.01$.

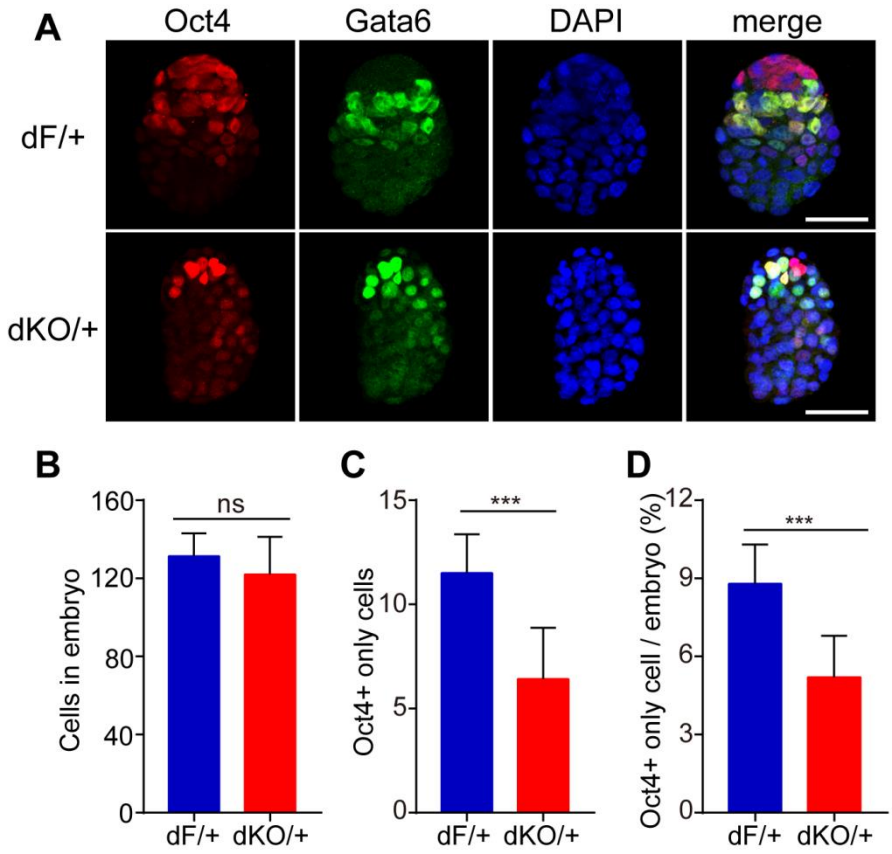

Fig. S6. Oct4+ only cells were decreased at E4.5. (A) Representative images of Oct4 and Gata6 in embryos at E4.5. ICM cells were labled by Oct4 (red) and PrE cells were marked by Gata6 (green). Number of total embryos: dF/+, n=6; dKO/+, n=19. Scale bars, 50 μm. **(B)** The average number of total cells in embryos. Results are presented as mean ± SD. Mann Whitney test: ns, not significant. **(C)** Decreased Oct4+ only cells in dKO/+ embryos. Results are presented as mean ± SD. Mann Whitney test: ***$P < 0.001$. (D) Ratio of EPI cells classified by Oct4+ only cells. Results are presented as mean ± SD. Mann Whitney test: ***$P < 0.001$.

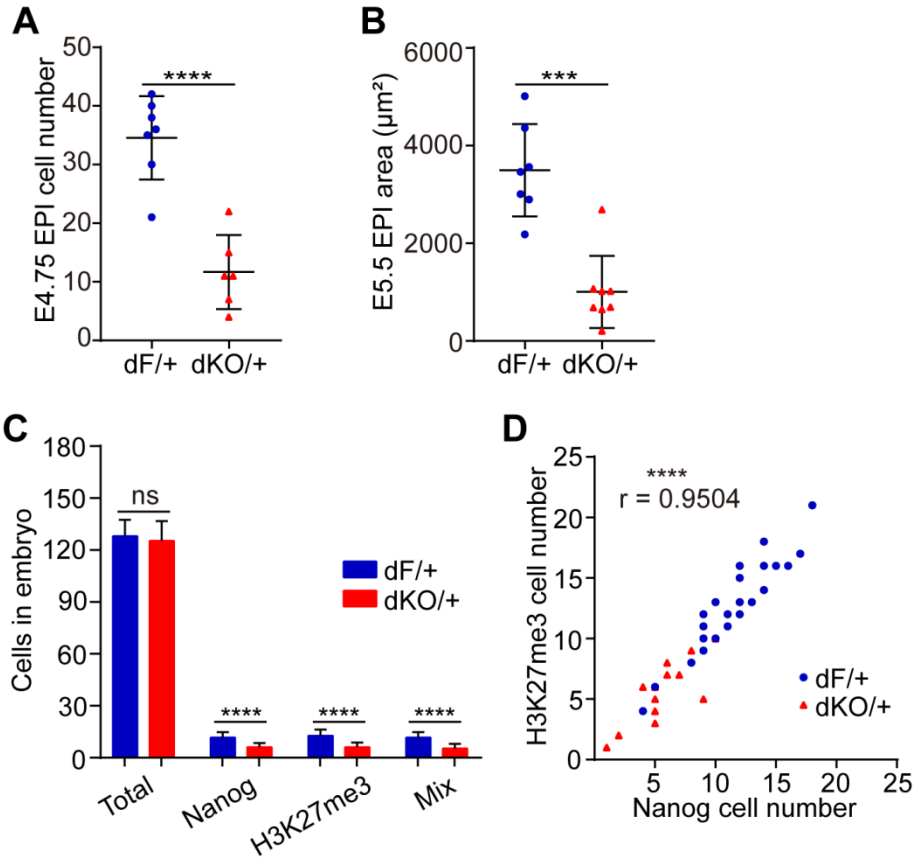

Fig. S7. Decreased EPI cells from E4.5 to E5.5. (A) EPI cell number at E4.75. Number of total embryos: dF/+, n=7; dKO/+, n=6. Results are presented as mean ± SD. Unpaired *t* test: ****$P < 0.0001$. **(B)** EPI area at E5.5. Results are presented as mean ± SD. Mann Whitney test: ***$P < 0.001$. **(C)** Decreased Nanog and H3K27me3 cells in dKO/+ embryos. Mix: cells showed both Nanog and H3K27me3 staining. Results are presented as mean ± SD. Unpaired *t* test: ns, not significant; ****$P <$ 0.0001. **(D)** Correlation analysis of Nanog and H3K27me3 cell numbers. Number of total embryos: dF/+, n=31; dKO/+, n=17. The cell numbers is highly correlated; Pearson r = 0.9504. ****$P < 0.0001$.

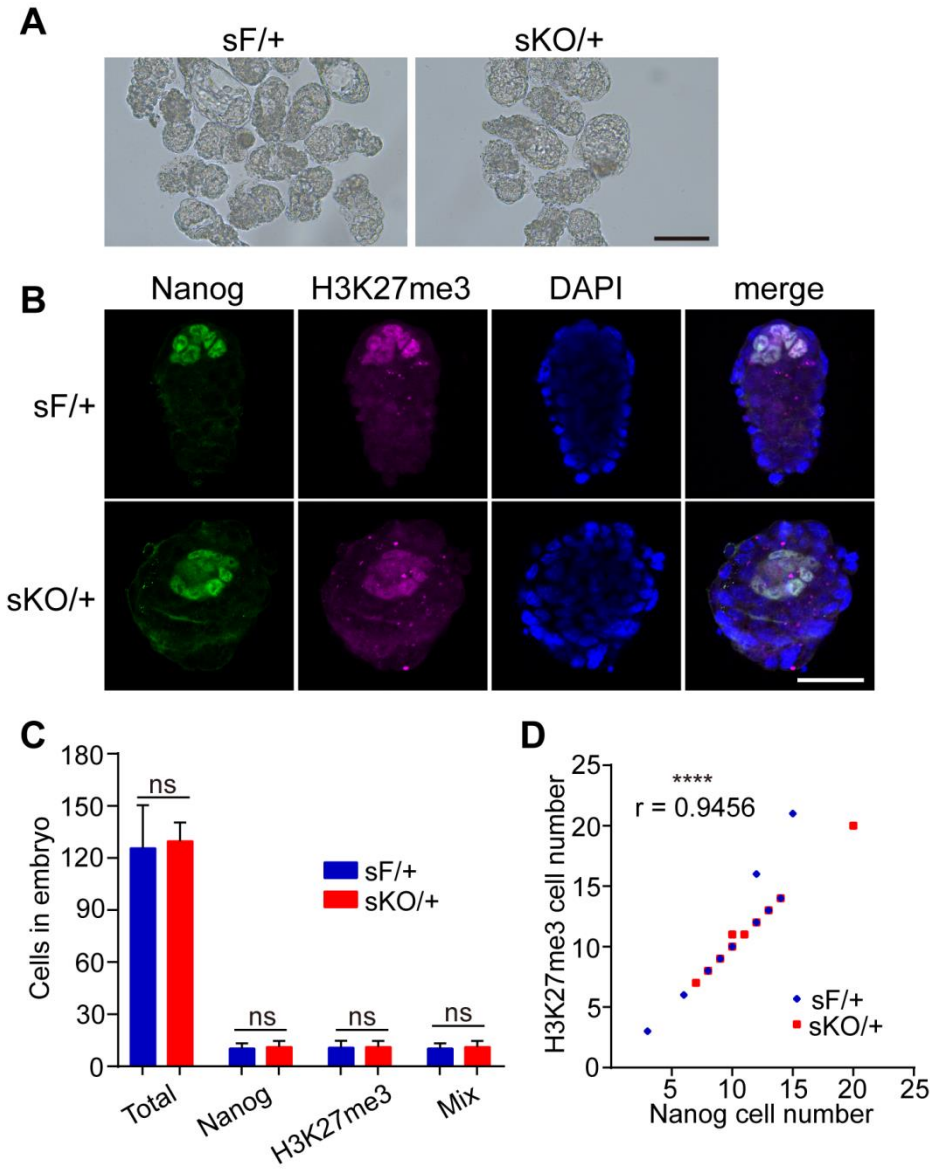

Fig. S8. Normal epiblast cells in sKO/+ Embryos at E4.5. (A) Representative images of flushed embryos at E4.5. Scale bars, 100 μm. **(B)** Representative images of late blastocysts immunostained with Nanog and H3K27me3. Number of total embryos: sF/+, n=31; sKO/+, n=35. Scale bars, 50 μm. **(C)** Quantitative analysis of Nanog and H3K27me3 cells. Mix: cells showed both Nanog and H3K27me3 staining. Results are presented as mean ± SD. Mann Whitney test: ns, not significant. **(D)** Correlation analysis of Nanog and H3K27me3 cell numbers. Number of total embryos: sF/+, n=31; sKO/+, n=35. The cell numbers is highly correlated; Pearson r = 0.9456. ****$P < 0.0001$.

Table S1. Embryo transfer

Group	No. of recipients	No. of blastocysts transferred	Pups per littter	Term pups (%)
dF/+	13	170	6.31±2.32	82 (48.24%)
dKO/+	9	111	2.22±1.86***	20 (18.02%)****

Pups per recipient are recorded as mean ± SD. Statistical comparisons were made using Mann Whitney test, ***$P < 0.001$. Comparisons between dF/+ and dKO/+ about Pups (%) were by χ^2 test, ****$P < 0.0001$.

Table S2. Primers

Gene	Sequence (5′ to 3′)	Reference
Primers for genotype		
Ezh2-F	GCTAGAAGCATTCCCCACAC	
Ezh2-R	CTGGCTCTGTGGAACCAAAC	
Ezh2-R (within *lacZ*)	ATGGGCCTCATAGTGACAGG	
Ezh1-F	GATGCCCTCAACCAGTACTC	
Ezh1-R	TTTATATCACGCACCCACAC	
Ezh1-R (within *lacZ*)	TAAAGCGAGTGGCAACATGG	
Gdf9-Cre-F	TCTGATGAAGTCAGGAAGAACC	
Gdf9-Cre-R	GAGATGTCCTTCACTCTGATTC	
Internal control-F	CAAATGTTGCTTGTCTGGTG	
Internal control-R	GTCAGTCGAGTGCACAGTTT	
Primers for real-time PCR		
Ezh2-F	TGACCCTGACCTCTGTCTCACG	
Ezh2-R	TCAGACGGTGCCAGCAGTAAGT	
Ezh1-F	AGCGATGCTGTGTTTCTGGA	
Ezh1-R	GGCGCTTCCGTTTTCTTGTT	
Eed-F	ATGCCATTGTATGCTGGAAACC	
Eed-R	CACTGGCTGTAATCAAATCGCC	
Suz12-F	TCTCATCGAAATTCCAGAACAAGC	
Suz12-R	CAAGCTATGAGATTCTTGCTCTCC	
β-actin-F	GTGACGAGGCCCAGAGCAAGAG	(1)
β-actin-R	CGTACATGGCTGGGGTGTTGAAGG	

Table S3. Antibodies

Antibody	Source	Concentration
Rabbit polyclonal Anti-Ezh2	Abcam, ab186006	WB: 1:500
Mouse monoclonal Anti-beta tublin	Proteintech, 66240	WB: 1:2000
Rabbit monoclonal Anti-H3K27me3	CST, 9733	IHC: 1:2500
		IF: 1:400
Rabbit monoclonal Anti-Vcam1	Abcam, ab134047	IHC: 1:1000
Rabbit polyclonal Anti-integrin alpha 4	Santa Cruz, sc14008	IHC: 1:1000
Rabbit monoclonal Anti-H3K27me2	CST, 9728	IF: 1:400
Rabbit monoclonal Anti-Oct4	Abcam, ab200834	IF: 1:200
Mouse monoclonal Anti-Cdx2	Biogenex, CDX-88	IF: 1:200
Rabbit polyclonal Anti-Nanog	Abcam, ab80892	IF: 1:200
Goat polyclonal Anti-Gata6	R&D systems, AF1700	IF: 1:200
Alexa Fluor 594 Goat Anti-Rabbit IgG	ZSBIO, ZF-0516	IF: 1:200
Alexa Fluor 488 Goat Anti-Mouse IgG	ZSBIO, ZF-0516	IF: 1:200
Alexa Fluor 568 Donkey Anti-Rabbit IgG (H+L)	Abcam, ab175693	IF: 1:500
Alexa Fluor 594 Donkey Anti-Rabbit IgG (H+L)	YEASEN, 34212ES60	IF: 1:200
Alexa Fluor 488 Donkey Anti-Goat IgG (H+L)	YEASEN, 34306ES60	IF: 1:200
Alexa Fluor 647 Donkey Anti-Mouse IgG (H+L)	YEASEN, 34113ES60	IF: 1:200

Table S4. Putative H3K27me3-dependent imprinted genes

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Reference

1. Ciccone DN, Su H, Hevi S, Gay F, Lei H, Bajko J, et al. KDM1B is a histone H3K4 demethylase required to establish maternal genomic imprints. Nature. 2009;461(7262):415-8.