

Supplementary Information for

Maternal EHMT2 is essential for homologous chromosome segregation by regulating *Ccnb3* expression in oocyte meiosis

(Meng et al.)

Includes:

Supplementary Figure. 1-7

Video S1-4

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CCNB3 antibody information

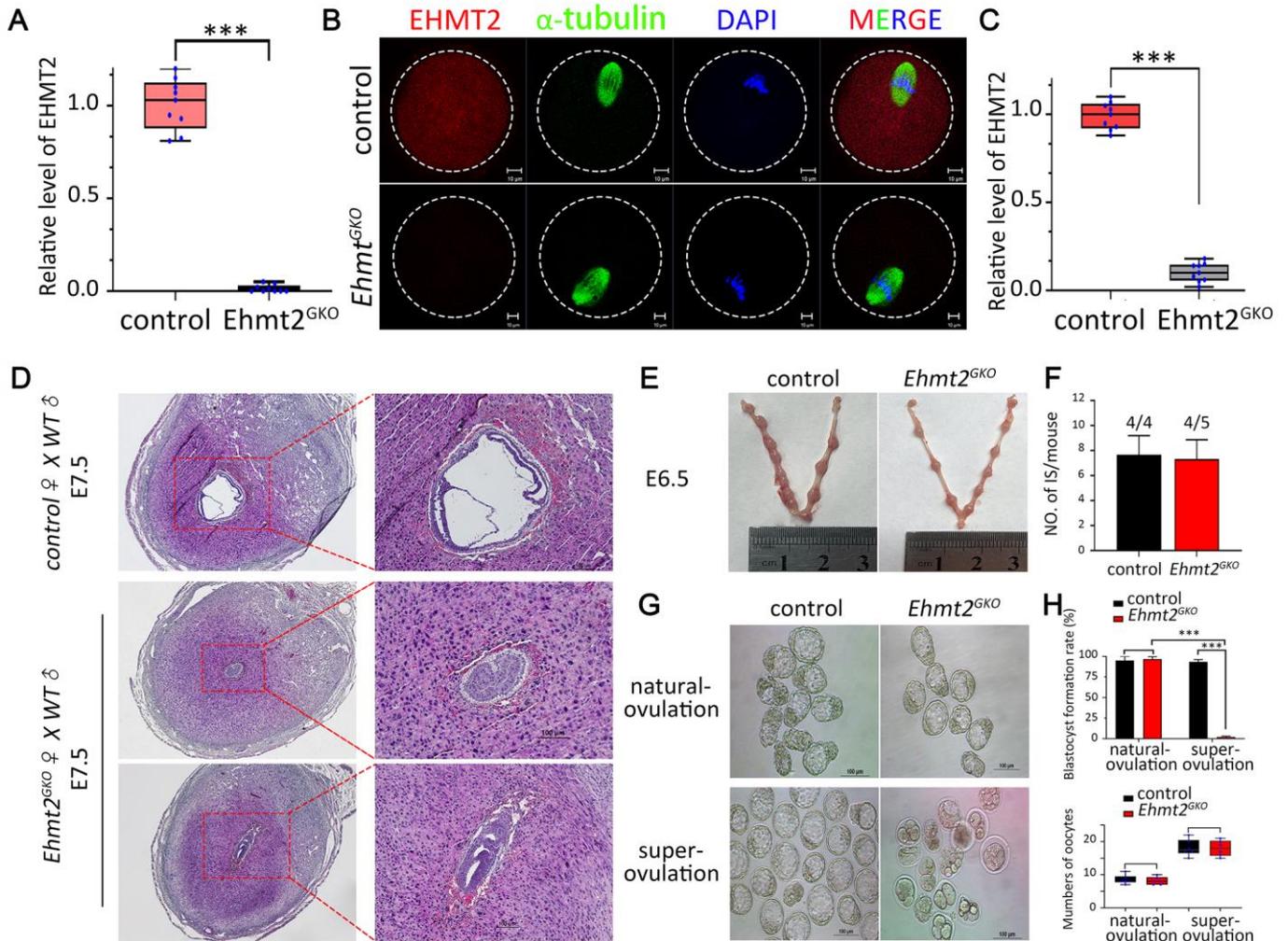


Figure S1. Embryos lacking maternal EHMT2 have developmental defects.

(A) Relative fluorescence intensity of EHMT2 at Germinal vesicle (GV) stage control oocytes and *Ehmt2^{GKO}* oocytes. Error bars, S.D. ***P < 0.001 by two-tailed Student's t tests. (B) The signal of EHMT2 in control oocytes and *Ehmt2^{GKO}* oocytes. GV oocytes were cultured for about 9h and subjected to immunofluorescence staining for EHMT2 (red), α -tubulin (green) and DAPI (blue). Scale bars: 10 μ m. All of the experiments were repeated at least three times, and representative results are shown. (C) Relative fluorescence intensity of EHMT2 at MI-AI stage control oocytes and *Ehmt2^{GKO}* oocytes. Error bars, S.D. ***P < 0.001 by two-tailed Student's t tests. (D) Histologic analysis of control and *Ehmt2^{GKO}* embryos at E7.5. Note growth malformation and retardation at E7.5. (E-F) Representative uterus, number of implantation sites and implantation rate at E6.5 in control and *Ehmt2^{GKO}* mice. (G) Both control mice and *Ehmt2^{GKO}* female mice undergo natural ovulation and superovulation, mate with WT male mice, and then recover embryos from the uterus at day E3.5. (H) Upper is the blastocyst formation rate of control and *Ehmt2^{GKO}* embryos derived from natural ovulation and superovulation, which recovered from the uterus at day E3.5. Lower is numbers of oocytes derived from natural ovulation and superovulation.

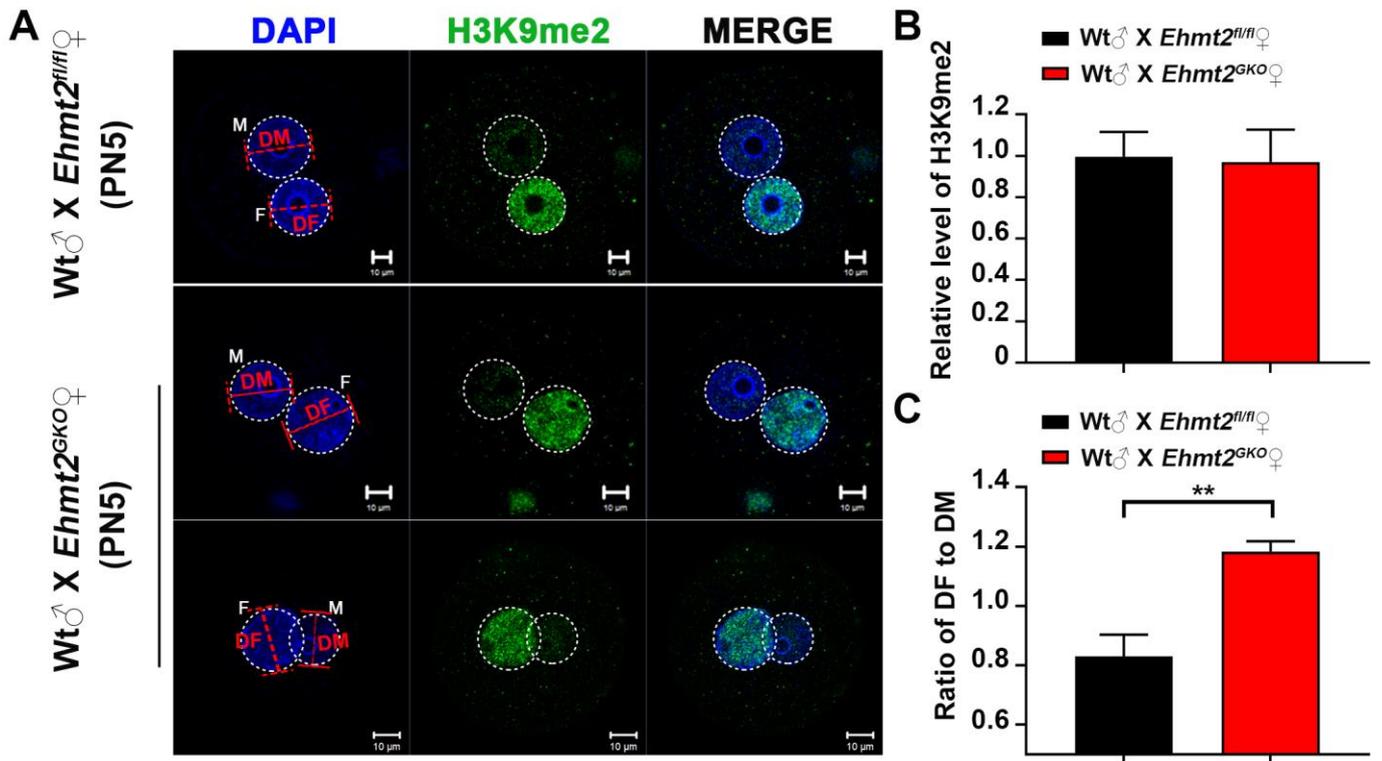


Figure S2. Compensatory establishment of H3K9me2 in *Ehmt2^{GKO}* zygotes.

(A) The H3K9me2 state of zygotes at PN5 after loss of maternal EHMT2. (B) Relative fluorescence intensity of H3K9me2 at PN5 stage control and *Ehmt2^{GKO}* zygote maternal pronucleus. n=25. (C) The ratio of female pronucleus to male pronucleus in control zygotes and *Ehmt2^{GKO}* zygotes.

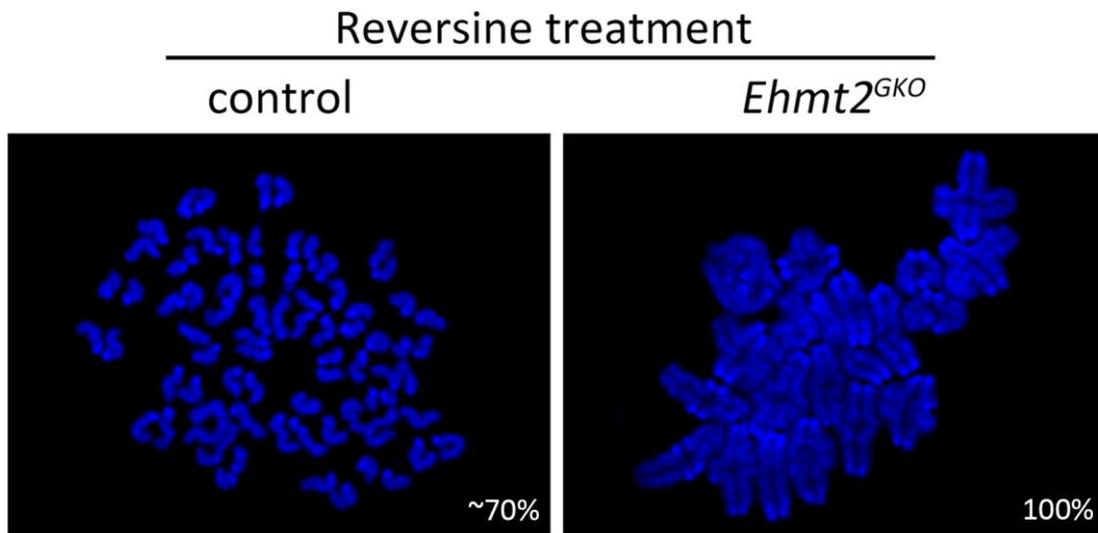


Figure S3. The metaphase I arrest was not due to the failure of SAC inactivation in *Ehmt2^{GKO}* oocytes.

Chromosome spreads were prepared at GVBD+12h of reversine-treated control oocytes and reversine-treated *Ehmt2^{GKO}* oocytes. Reversine was added at GVBD, respectively.

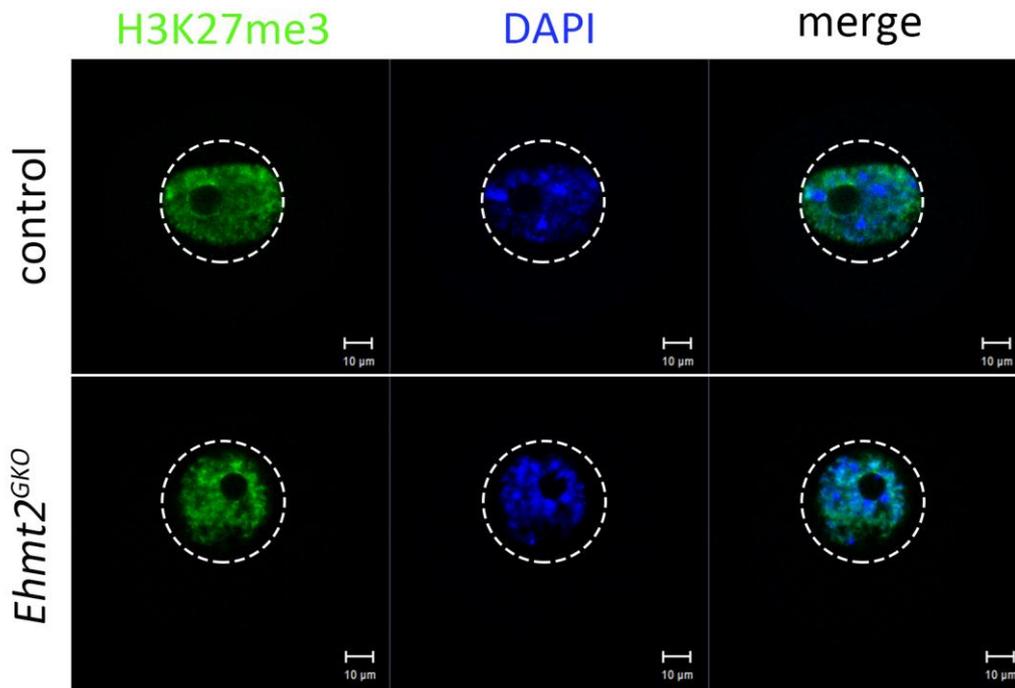


Figure S4. There is no obvious change in the H3K27me3 relative fluorescence intensity in *Ehmt2^{GKO}* oocytes

Representative images of the signal of H3K27me3 in control and *Ehmt2^{GKO}* GV oocytes.

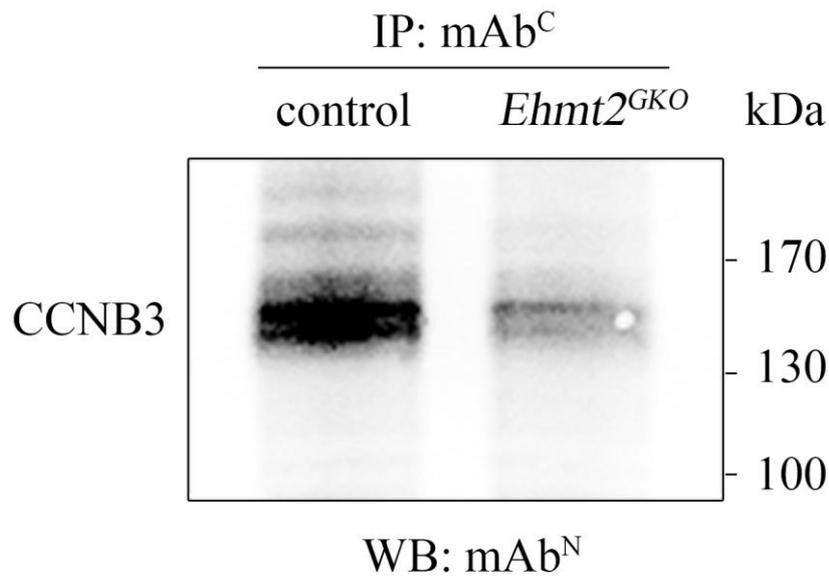


Figure S5. CCNB3 protein level decreased in *Ehmt2^{GKO}* female.

The CCNB3 level of adult control ovary and *Ehmt2^{GKO}* ovary extracts by immunoprecipitation and western blotting (IP-WB), respectively. mAb^C represents the antibody with CCNB3-C-terminal (aa1067-1080) as the antigen, mAb^N represents the antibody with CCNB3-N-terminal (aa110-123) as the antigen.

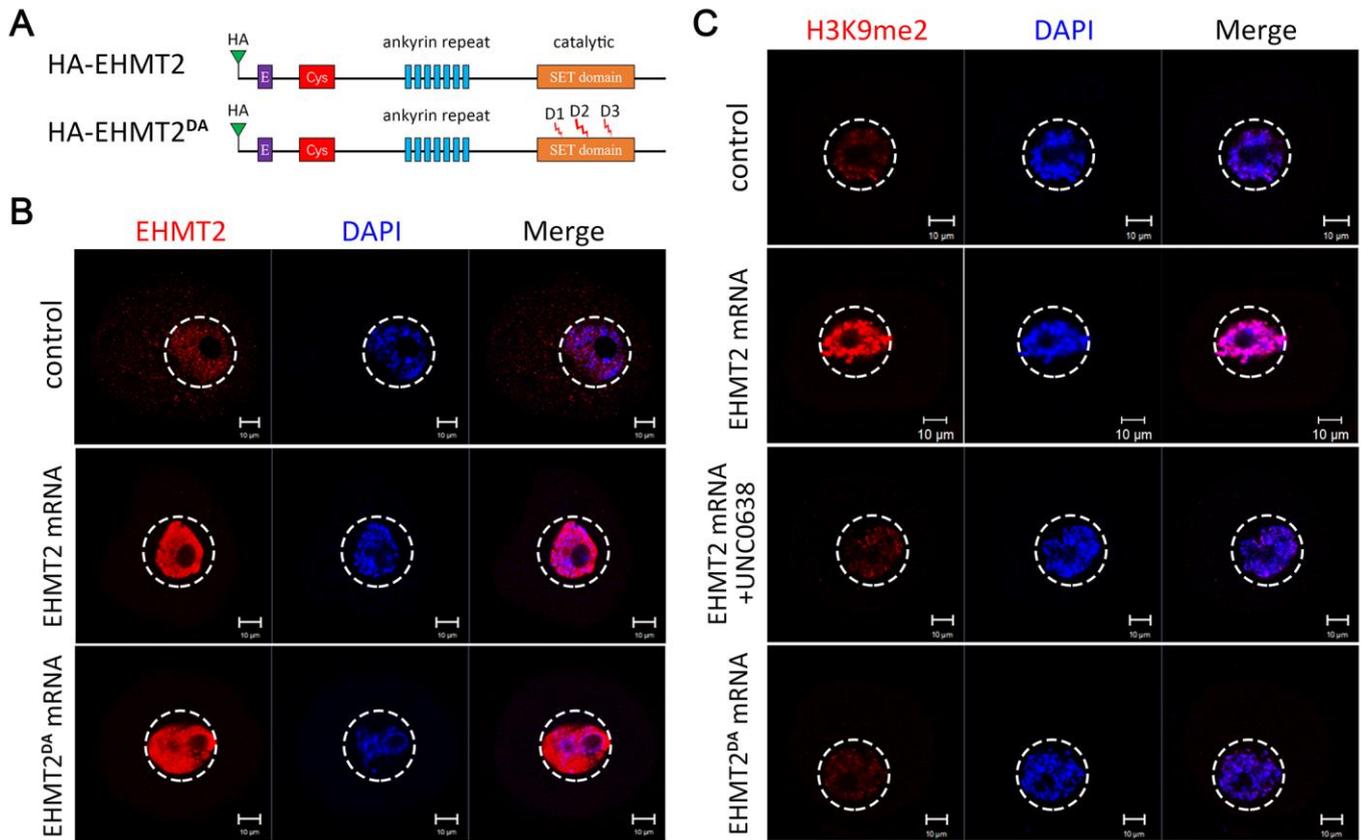


Figure S6. UNC0638 could inhibits histone H3K9me2 methyltransferase activity of EHMT2.

(A) Schematic representation of HA-tagged EHMT2, HA-tagged EHMT2 containing three points mutant. (D1 represents D1078A, D2 represents D1083A, D3 represents D1088A) in SET domain without catalytic activity. (B) Representative images of the control oocytes microinjected with EHMT2 mRNA and EHMT2^{DA} mRNA, respectively. Oocytes were double stained for EHMT2 antibody (red) and DAPI (blue). Scale bar, 10 μm. (C) Representative images of the H3K9me2 state in control oocytes, control oocytes microinjected with EHMT2 mRNA, EHMT2 mRNA injected oocytes treated with UNC0638 and control oocytes microinjected with EHMT2^{DA} mRNA, respectively. Scale bar, 10 μm.

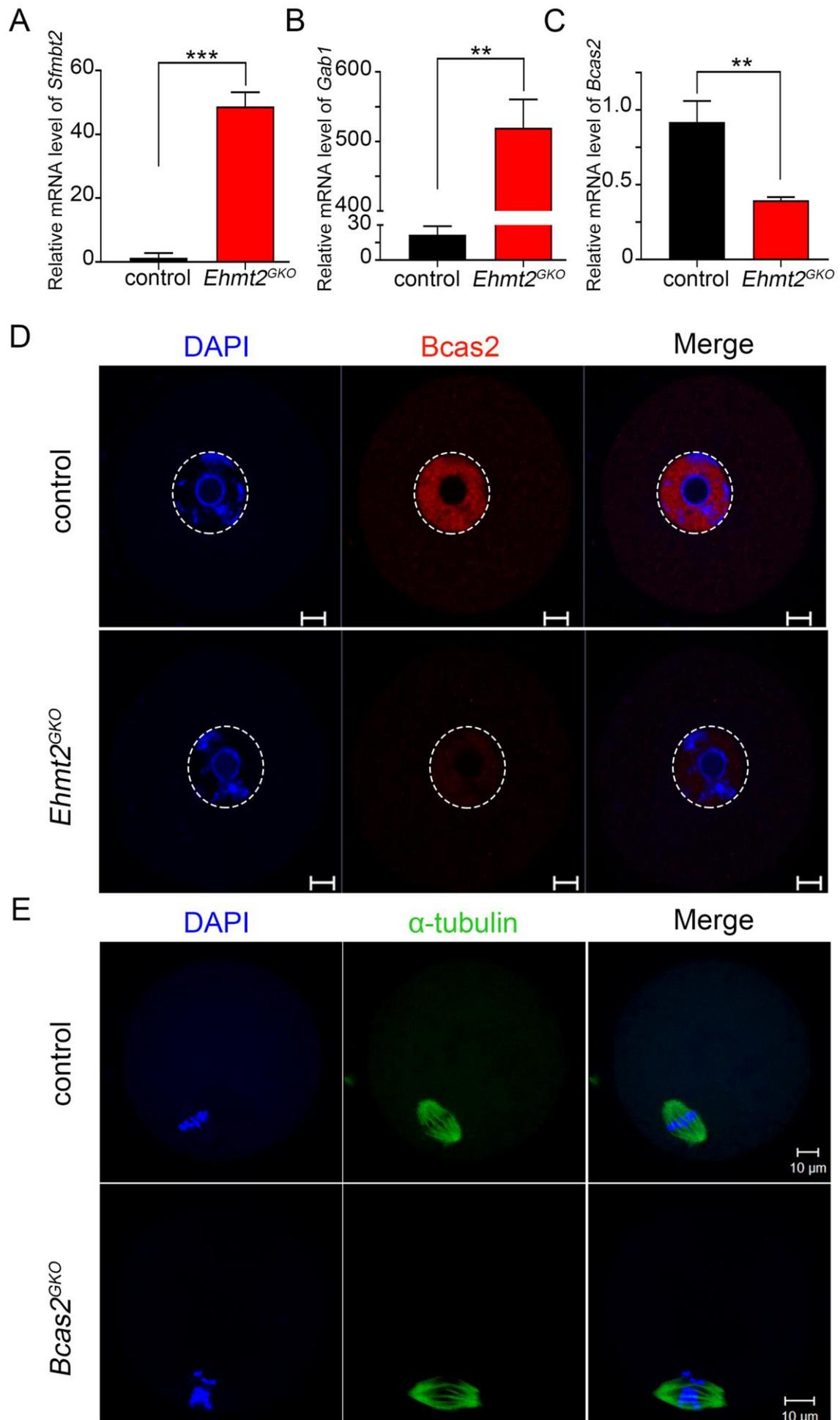


Figure S7. Other factors might be involved in the chromosome alignment process

(A-C) Comparison of the *Sfmbt2*, *Gab1* and *Bcas2* expression levels (by FPKM values) in control and *Ehmt2^{GKO}* oocytes, respectively. (D) Representative images of the BCAS2 state in control oocytes and *Ehmt2^{GKO}* oocytes, respectively. Scale bar, 10 μ m. (E) Superovulated oocytes derived from control and *Bcas2^{GKO}* female mice were used for DAPI (blue) and α -tubulin (green) immunofluorescent staining, respectively. Scale bar, 10 μ m.

Video S1. Time-lapse of Separase sensor in control oocyte.

Video S2. Time-lapse of Separase sensor in *Ehmt2^{GKO}* oocyte.

Video S3. Time-lapse of Securin-mCherry in control oocyte

Video S4. Time-lapse of Securin-mCherry in *Ehmt2^{GKO}* oocyte

Supplementary Table1

Differentially expressed genes in control and *Ehmt2^{GKO}* superovulated oocytes

Supplementary Table2

Ctcf siRNA and related primer sequence information

CCNB3 antibody information

NCBI link: https://www.ncbi.nlm.nih.gov/nucore/NM_183015.3

Antibody application: **WB** and **IP**

Antigen-#1-N: aa110-123: **SNMEKEFILDIPNK**

Antigen-#2-C: aa1067-1080: **ESRTDNSSAIMPSS**

CCNB3 protein sequence:

MPPPLLPKRSKLETEKAQSNKITPREEQQSEKIGKSNHAASSSSSTQGAVKRRSVFEDVTNASHSQC
VQSKEDNIELKSHVSKRTKKGVEVTQKKIKSSKMGHVTSLS**SNMEKEFILDIPNK**PKTLTTEEPSVF
QKTLVLNEEPATKETCLMRKTLKSCAFHQETLLMEKPLTLLVETEDYNEFDTELMTSKKKDKPEDPT
IIEEMTDLKKSVIRKVTLTSSPLWLKNKHVVQEEKPVIQEKSSFKKISLVSNNVTTKEKPPVKKPHFR
KKKPTTEMKSLQEPSLEEKYNTQEDASILKKPQVLQENTNNKDATLTPVTFKGGHSANEATHK
KPSSSKNNPDPQGKGTNLRPLRVHPVTYENEPMSKKSTTKKKDSHFHGSPVLPDKHSPQMEVSTV
KKSLALPNPTTEEKMLHFPVATVLEKQHNMGAPCLKKPSPLRKQQQLPKRRRFFSNSAVQETVIRK
PLFFKMSTTEKDPSPQWPSALPKKHISPGELSKQKKQHVS PKHNMEEDSQCWLDSAFKKQLSREEP
ASTHTPLKLEMQQAITKETGFHLRNPLVLPVTSEAKSLTKEPPSFREQNTSLLKRKSTHTITLQQA
QSEWQEMTDEDNRNLF SIKPGSHRKEPIPEFLQNPLPPNENCLISQKLSHSMPFASQKTTSQERHRKE
SVASNDKKNFFSQDLFSPFSSADEDTLKFHKS LDFQE QVDRKND SHKKMFDSQDSVSEEE SFLRKL
CKDRCSSTEELS QERTVALEQEFLLIKILNENTSSDVDEPLSHQSPHIQNHSDTTKEALEASEALEAPE
ALETLEALVASEDLEEPLNILEELSTENMVALMKMLVTEDESTKDSFSGNYTAAREAHAEKSLLEE
TSINEAATLKESLSSQEKHRAELVTVLKELLVLMKNPSLKRVALAFQENPSNNVETLLREVLALVENS
TADESTLQEKPKTKTDVTPKELLALEENSSNKKANPMDLSLFDHKPDTEMGEIARMVLTDEEYNIDT
LYERVLALSQGLIAADQLSFTDLQNFEETKIVDEEEFFKSFLVFENKNSPNMSSNAF**ESRTDNSSAIM**
PSSKAFNPVENSNPYVSSSKSFKSTLGAKETEITIQDSDSLERIEKEGQDPLLNTIYAKDVFNYLKER
EEKFLVQKYMDGQMELTSDMRAILVDWLVEIQGSFQMTHETLYLAVKIMDLYLMKAQCKKNHLQL
LGSTTYMIAAKFEESYPPSLSEFLFICEDMYEKS DMVSLESSILQTLNFDINIPTAYNFLRRYASCIHAS

MKTLTLSRFICEMTLQEYIEERPSKLAAASFILALYMRNLSNCVPTLEYFTGYKMAELHILVRKLN
HLLNFRSHSILKNVFEKYSEETYFEVAKIPPLSKQDLENLLNCALFH

Ctcf-siRNA sequence used i

	sense (5'→3')
Ctcf (M)-476-siRNA	GCAGUGUACAGAUGGUAUTT
Ctcf (M)-1671-siRNA	CCACUUGCGAAAGCAGCAUTT

qPCR primer sequence

Ctcf-qPCR forward primer
GATCCTACCCTTCTCCAGATGAA

Ehmt2 point mutant (D1078A&D1083A&D1088A)

Ehmt2 point mutant forward primer
TCTTACCTCTTCGCTTTAGATAACAAGGATGGCGAGC
Note: KOD-Plus- Mutagenesis Kit (Toyobo, Cat:SMK-101)

n this study

antisense (5'→3')

AUUACCAUCUGUACACUGCTT

AUGCUGCUUUCGCAAGUGGTT

(5'→3')

reverse primer

GTACCGTCACAGGAACAGGT

38A) primer sequence (5'→3')

reverse primer

AGCATCCTCTCTCACAGCAGCCTCGGCATCAGAGATCAGCT