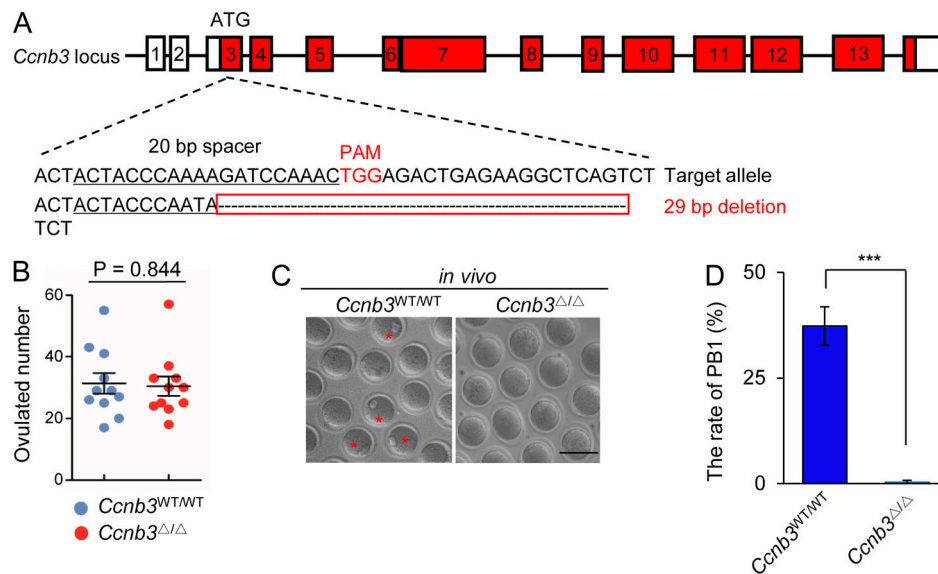# Supplemental material

Figure S1.   **Characters of *Ccnb3* mutant mice. (A)** Schematic diagram for constructing *Ccnb3* mutant mice using CRISPR/Cas9. **(B)** Superovulation of WT and *Ccnb3* mutant mice. The number of superovulated oocytes of *Ccnb3*[△/△] mice was similar to the WT; P = 0.844. **(C and D)** Oocytes with *Ccnb3* mutation failed to extrude PB1 by in vivo superovulation. The asterisk indicates the PB1. At least 300 oocytes were collected and measured in each group. Bars, 20 µm. Unpaired two-tailed Student's *t* test. Error bars represent mean ± SD; ***, P < 0.001.
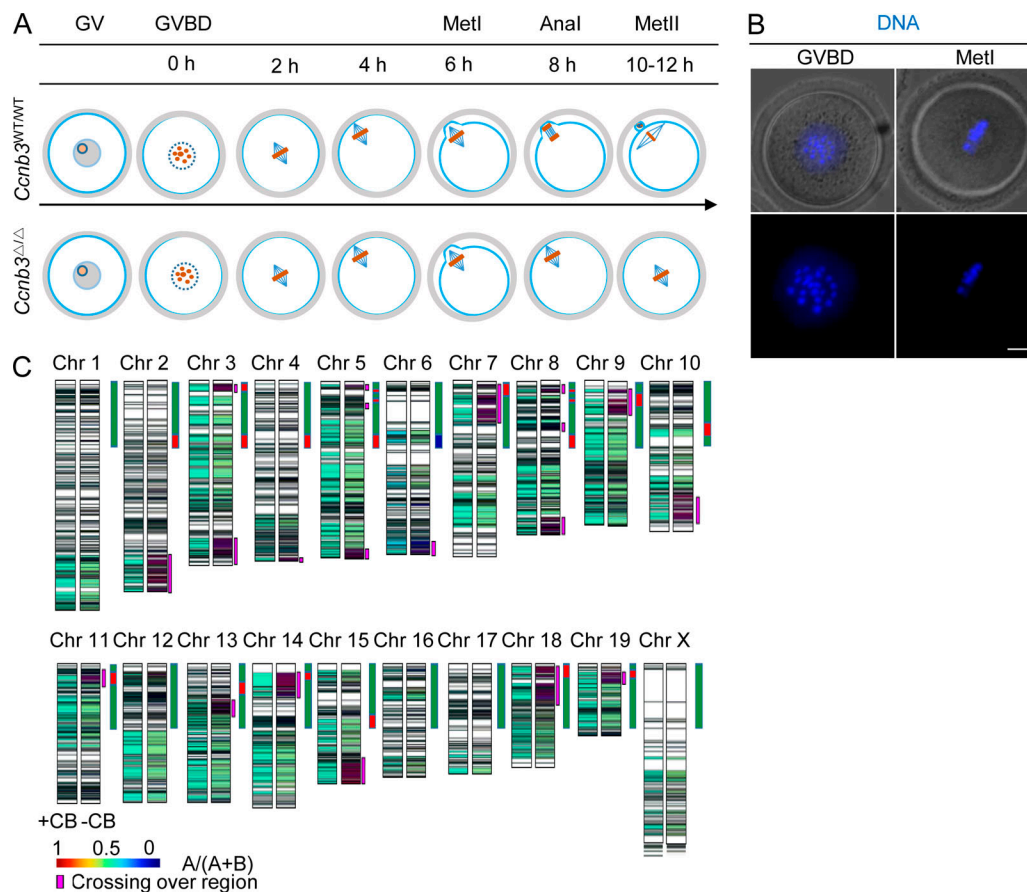
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Figure S2. **Development of *Ccnb3* mutant oocytes in meiosis. (A)** Schematic outline of IVM process of mouse oocytes. **(B)** Oocyte developmental capacity was measured by Hoechst 33342 staining. Oocytes were incubated in M2 medium to resume meiosis; the developmental capacity was measured by Hoechst 33342 staining after 2 and 8 h of GVBD, which represent GVBD and MetI stages of oocytes, respectively. At least 100 oocytes were measured in each group. Bar, 20 μm. **(C)** Whole genome sequencing analysis of *Ccnb3*△/△ ESCs. The 4N ESCs (with CB treatment, referred to as +CB) and 2N ESCs (without CB treatment, referred to as −CB) are sequenced separately. Only the heterozygous sites in 4N ESCs and the corresponding sites in 2N ESCs are shown. As calculated by the base frequency ratio in one genome locus, the heterozygous sites are marked in range from green to blue, and the homozygous sites are marked in red or blue as well as the missing site marked in blank. The homozygous regions are inferred as the crossing-over region through the homozygous sites in 2N ESCs.
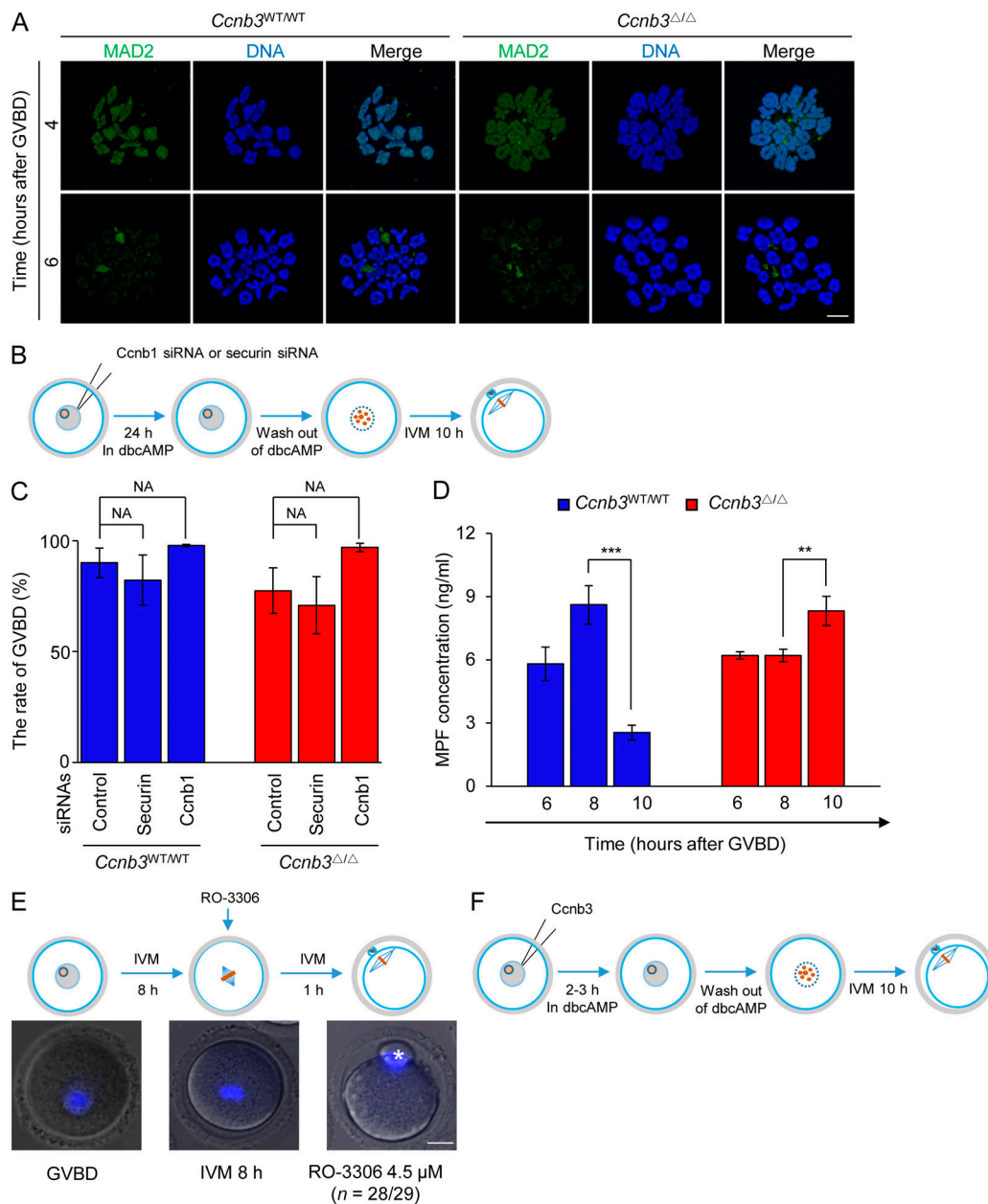
Figure S3. **Rescue of MetI arrest caused by *Ccnb3* mutation. (A)** Immunofluorescence staining of MAD2 during IVM. At least 30 oocytes used for immunofluorescence staining. Both WT and *Ccnb3* mutant oocytes showed similar expression pattern during maturation. **(B)** Schematic diagram for Ccnb1 siRNA or securin siRNA injection. GV-stage oocytes were isolated in M2 medium containing dbcAMP that inhibits GVBD and released into inhibitor-free medium after 24 h of injection. The concentration of Ccnb1 siRNA and securin siRNA is 50 μM. **(C)** The efficiency of the GVBD after Ccnb1 siRNA and securin siRNA injection. At least 150 oocytes for each group. Error bars represent mean ± SD; NA ($P > 0.05$; Student's t test). **(D)** Quantification the MPF concentration of oocytes during IVM (6, 8, and 10 h after GVBD; $n = 40$ oocytes per group, three repeats). Unpaired two-tailed Student's t test. Error bars represent mean ± SD; **, $P < 0.01$; ***, $P < 0.001$. **(E)** Schematic diagram for RO-3306 treatment (top). Reduction of CDK1 activity by addition of RO-3306 (a specific inhibitor of CDK1 activity) in *Ccnb3*$^{\triangle/\triangle}$ oocyte recovered the MetI arrest (bottom). The asterisk indicates the extruded PB. **(F)** Schematic diagram for Ccnb3 mRNA injection. GV-stage oocytes were isolated in M2 medium containing dbcAMP that inhibits GVBD and released into inhibitor-free medium after 2 h of injection. The concentration of Ccnb3 mRNA is 100 ng/μl. Bars: 5 μm (A); 20 μm (E).

Table S1. **Developmental capacity of *Ccnb3*$^{\triangle/\triangle}$ oocytes after ICSI**

Group	No. of oocytes cultured	2-cell (%)	4-cell (%)	8-cell (%)	Morula (%)	Blastocyst (%)
Ccnb3$^{WT/Y}$ × *Ccnb3*$^{WT/WT}$	73	73	70	66	65	59 (80.8)
Ccnb3$^{WT/Y}$ × *Ccnb3*$^{\triangle/\triangle}$	46	41	39	35	33	31 (75.6)
Ccnb3$^{\triangle/Y}$ × *Ccnb3*$^{\triangle/\triangle}$	85	78	73	67	64	59 (75.6)

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Table S2. **sgRNAs and primers designed in the study**

Assay	Name	Sequence 5′–3′
sgRNA	sgRNA-Ccnb3-1	ACTACCCAAAAGATCCAAAC
		GTTTGGATCTTTTGGGTAGT
	sgRNA-Ccnb3-2	ATCCAAACTGGAGACTGAGA
		TCTCAGTCTCCAGTTTGGAT
	sgRNA-Ccnb3-3	TAACAAGATCACACCTAGAG
		CTCTAGGTGTGATCTTGTTA
	sgRNA-Ccnb3-4	TCATCTTCATTTTCAACTCA
		TGAGTTGAAAATGAAGATGA
	sgRNA-Ccnb3-5	TAGTCAATGTGTCCAGTCAA
		TTGACTGGACACATTGACTA
	sgRNA-Ccnb3-6	GCCTAAAACACTAACTACTG
		CAGTAGTTAGTGTTTTAGGC
RT-PCR	Ccnb1	AAATAAGGCCAAGGTCAGTATG
		AACAGTACCTTTTCCAGTACCT
	Ccnb3	ACCACTACTACCCAAAAGATCCA
		AGTTACATCTTCAAACACTGATC

Table S3. **Reagent or resource used in the study**

Reagent or Resource	Source	Identifier
Antibodies		
Mouse anti-Cyclin B3	Presented from Scott laboratory	N/A
Rabbit anti-CDK1	Abcam	No. ab32094
Rabbit anti-Cyclin B1	Abcam	No. ab181593
Mouse anti-alpha Tubulin	Sigma	No. T6199
Goat anti-rabbit IRDye 800CW	Licor	No. 926-3221
Goat anti-mouse IRDye 800CW	Licor	No.926-32210
Anti-rabbit IgG HRP	Rockland	No. 18-8816-33
Rabbit anti-MAD2	Abcam	No. ab180579
Rabbit anti-BUB3	Abcam	No. ab133699
Mouse anti-SECURIN	Abcam	No. ab3305
Rabbit anti-REC8	Abcam	No. ab192241
siRNAs		
Ccnb1 siRNA Silencer Select	Thermo Fisher	s114071; No. 4390771
Ccnb1 siRNA Silencer Select	Thermo Fisher	s114072; No. 4390771
Ccnb1 siRNA Silencer Select	Thermo Fisher	s114073; No. 4390771
Ccnb1 siRNA	Thermo Fisher	153133; No. AM16708
Securin siRNA Silencer Select	Thermo Fisher	132000
Securin siRNA Silencer Select	Thermo Fisher	1320003
Securin siRNA Silencer Select	Thermo Fisher	1320003
Ccnb3-mus-1532 (F: 5′-GCAAGCCAUUGUUCUUUAATT-3′; R: 5′-UUAAAGAACAAUGGCUUGCTT-3′)	Sangon Biotech	N/A
Ccnb3-mus-2229 (F: 5′-CCAUAAGUCUUUGGACUUUTT-3′; R: 5′-AAAGUCCAAAGACUUAUGGTT-3′)	Sangon Biotech	N/A
Ccnb3-mus-3971 (F: 5′-GCAUGAAGACACUAACCUUTT-3′; R: 5′-AAGGUUAGUGUCUUCAUGCTT-3′)	Sangon Biotech	N/A
Reagent or Resource	Source	Identifier
siRNAs (Continued)		
NC (F: 5′-UUCUCCGAACGUGUC ACGUTT-3′; R: 5′-ACGUGACACGUUCGGAGAATT-3′)	Sangon Biotech	N/A
Software and Algorithms		
SnapGene		
Quantity One		
ImageJ		
DNASTAR		
PrimerBank	https://pga.mgh.harvard.edu/primerbank/	https://pga.mgh.harvard.edu/primerbank/
Plasmids		
Securin-EGFP	Youbio Biotech	No. G148185
Ccnb1-EGFP	Youbio Biotech	No. G144604
Ccnb3-EGFP	Youbio Biotech	No. 35391

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