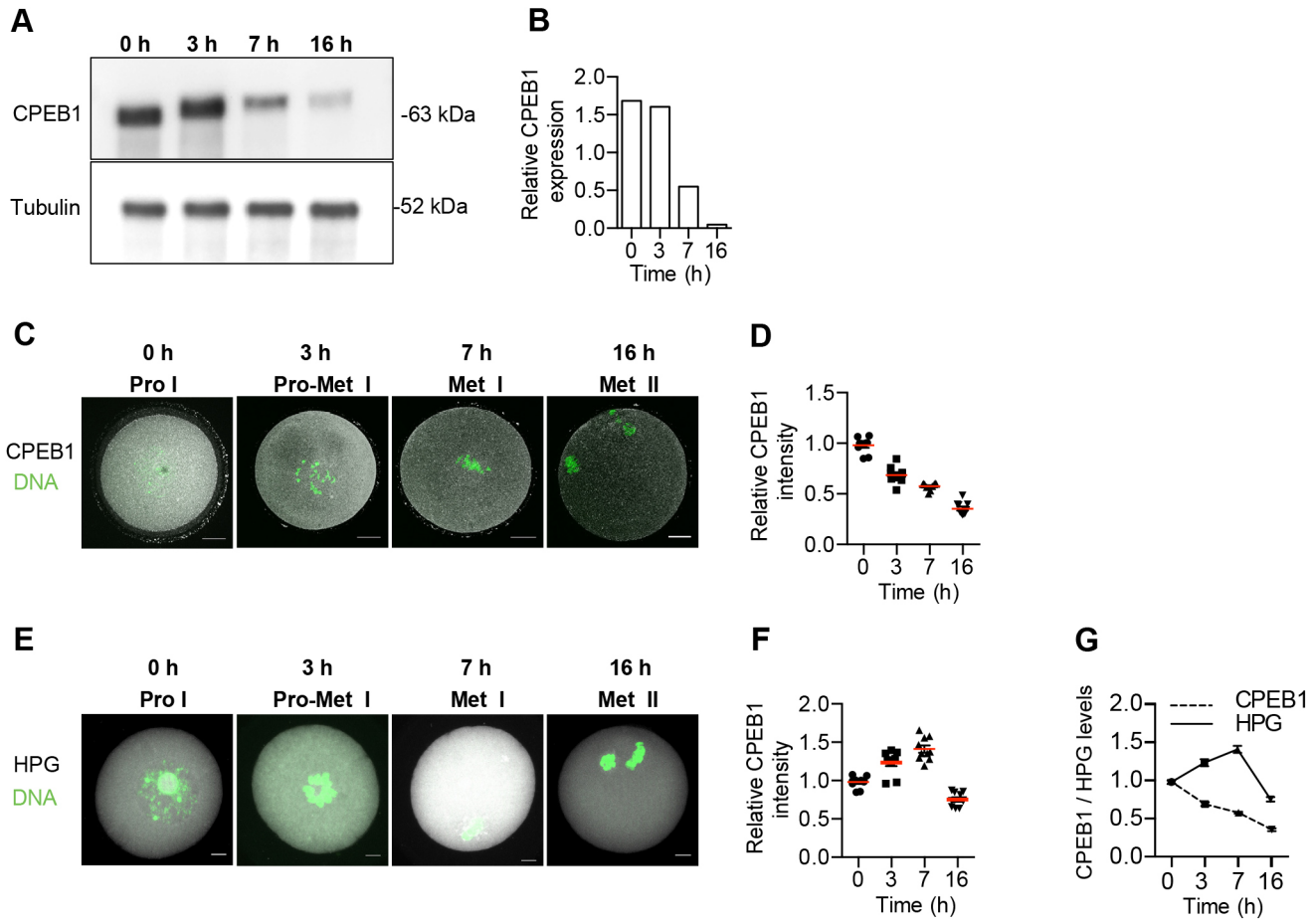
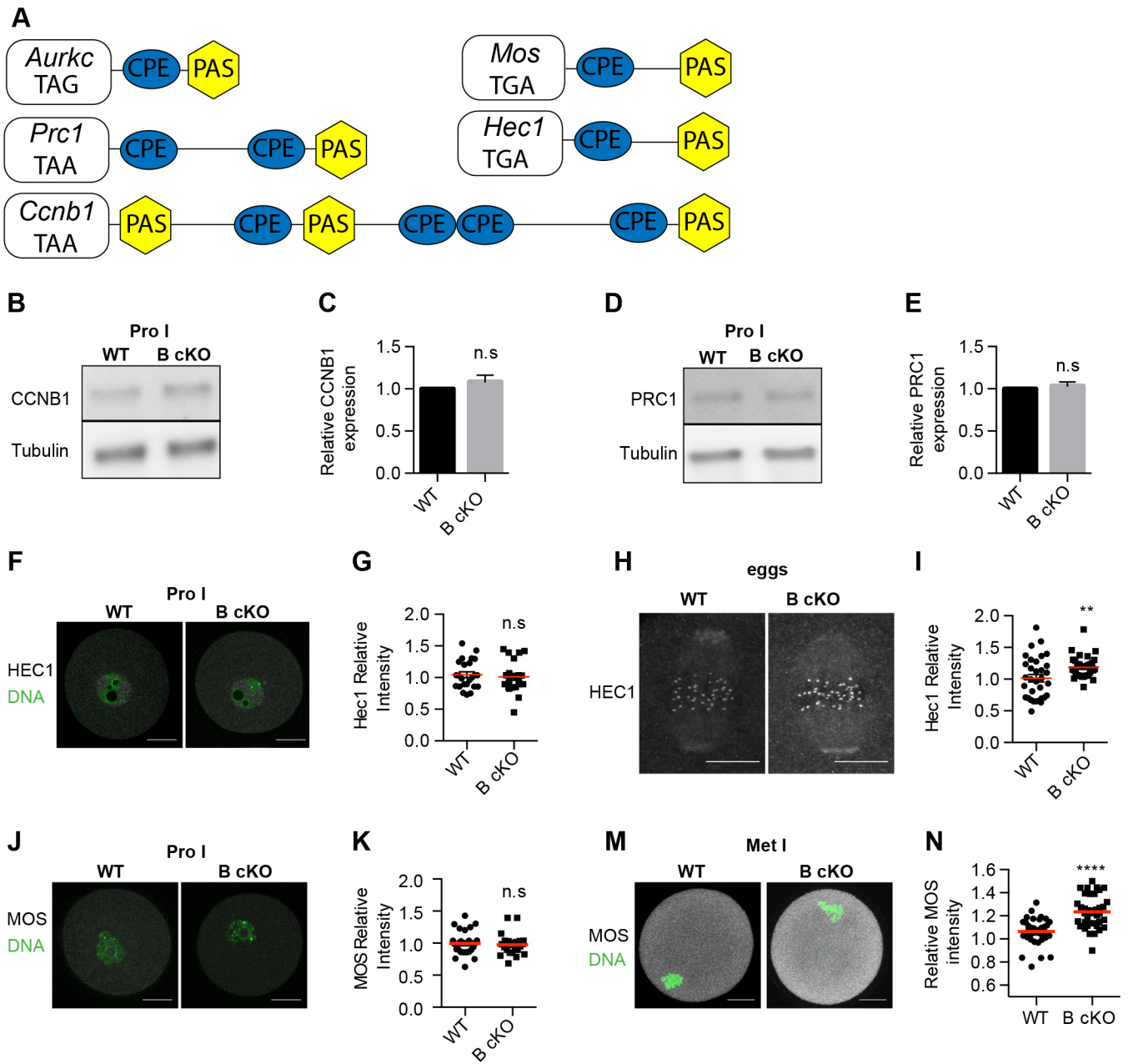


**Fig. S1.** Experimental controls. A) Prophase I oocytes from WT mice were collected and *in vitro* matured with DMSO or cycloheximide (CHX, 20 $\mu$ M) for 7 h. B) Relative pixel intensity of HPG from A. (Number of oocytes, DMSO: 22 and CHX is 22, Unpaired student's t-test, two tailed, \*\*\*\* $p < 0.001$ ). C) Prophase I oocytes from WT, B cKO and B cKO/hetA were collected and stained with anti-HPG (Gray) and with DAPI (DNA, green). D) Relative pixel intensity of HPG from C. (Number of oocytes, WT: 25; B cKO: 22, B cKO het A: 25, One-Way ANOVA). E) Time required for completion of meiotic maturation via polar body extrusion (PBE) in WT, B cKO and B cKO hetA oocytes (Number of oocytes, WT: 25; B cKO: 32 and B cKO hetA: 20 One-way ANOVA, non-significant  $p = 0.1628$ ). These experiments were repeated 3 times with total of 3-4 mice/genotype.

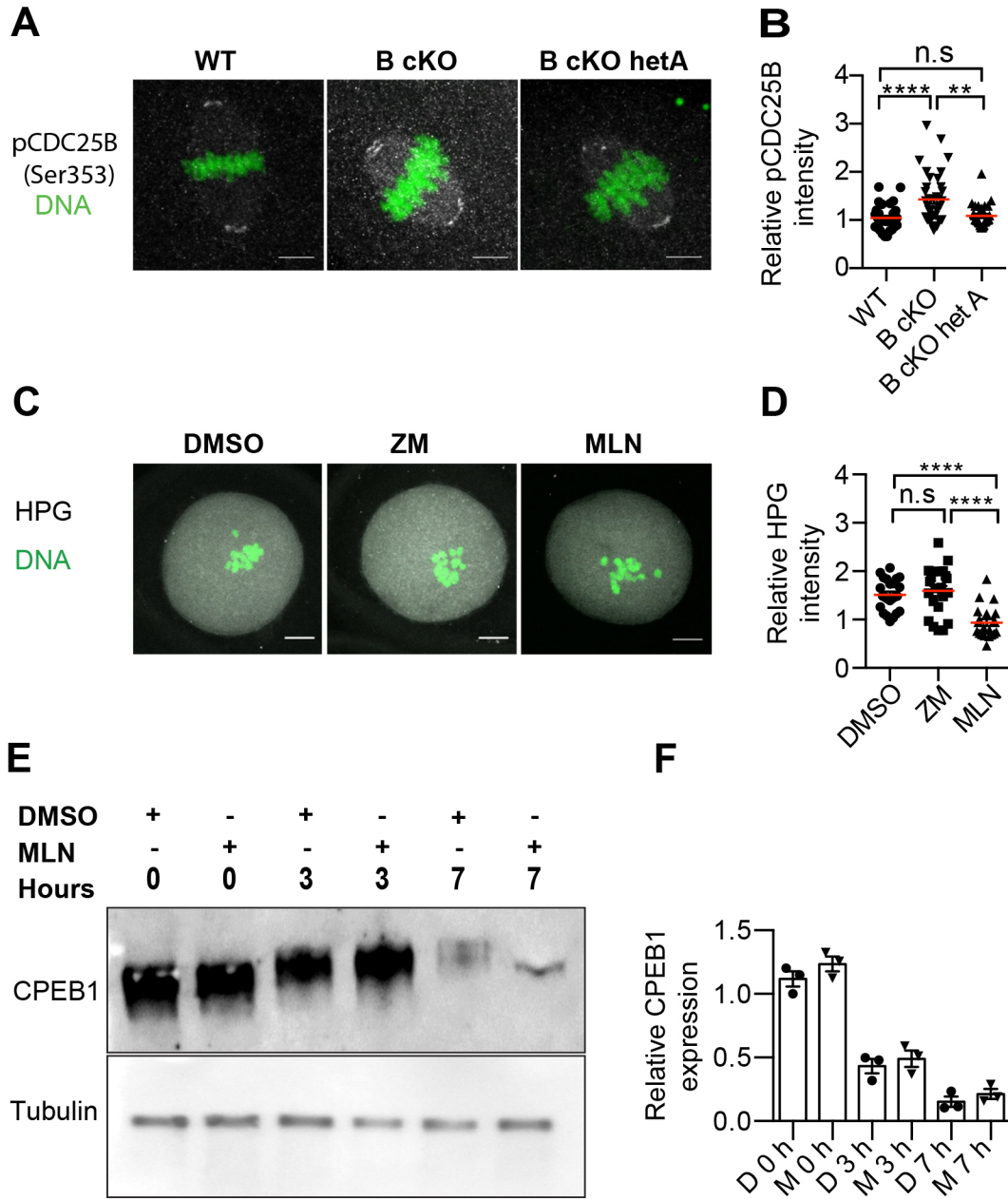


**Fig. S2:** CPEB1 stability and phosphorylation dynamics and translation levels during mouse oocyte meiotic maturation. A-G) Prophase I oocytes from WT mice were collected and *in vitro* matured. Oocytes were collected at the indicated time points to obtain oocytes at major cell cycle phases where translation dynamics differ: prophase I (0h), pro-Metaphase I (3h), Metaphase I (7h) and Metaphase II (16h). A) Prophase I oocytes from WT mice were collected and matured for the indicated time points prior to lysis and resolution by SDS-PAGE and membranes were probed with anti-CPEB1 (30 oocytes/lane).  $\alpha$ -Tubulin served as a loading control. B) Relative CPEB1 expression from A. Values normalized to 0h. This experiment was conducted 3 times with a total of 3-5 WT mice. C) Oocytes matured *in vitro* for the indicated amount of time were stained with anti-CPEB1 (gray) and DAPI (DNA, green) to confirm meiotic stage. Shown are representative confocal z-projections. D) Relative pixel intensity of CPEB1 from C. Values normalized to 0h. E) Oocytes matured *in vitro* for the indicated amount of time were labeled with HPG to detect translation and stained with anti-HPG (gray) and DAPI (DNA, green) to confirm meiotic stage. Shown are representative confocal z-projections. F) Relative pixel intensity of HPG from E. G) Overlay of CPEB1 and HPG levels, values from E and F. Scale bar is 10 $\mu$ m.

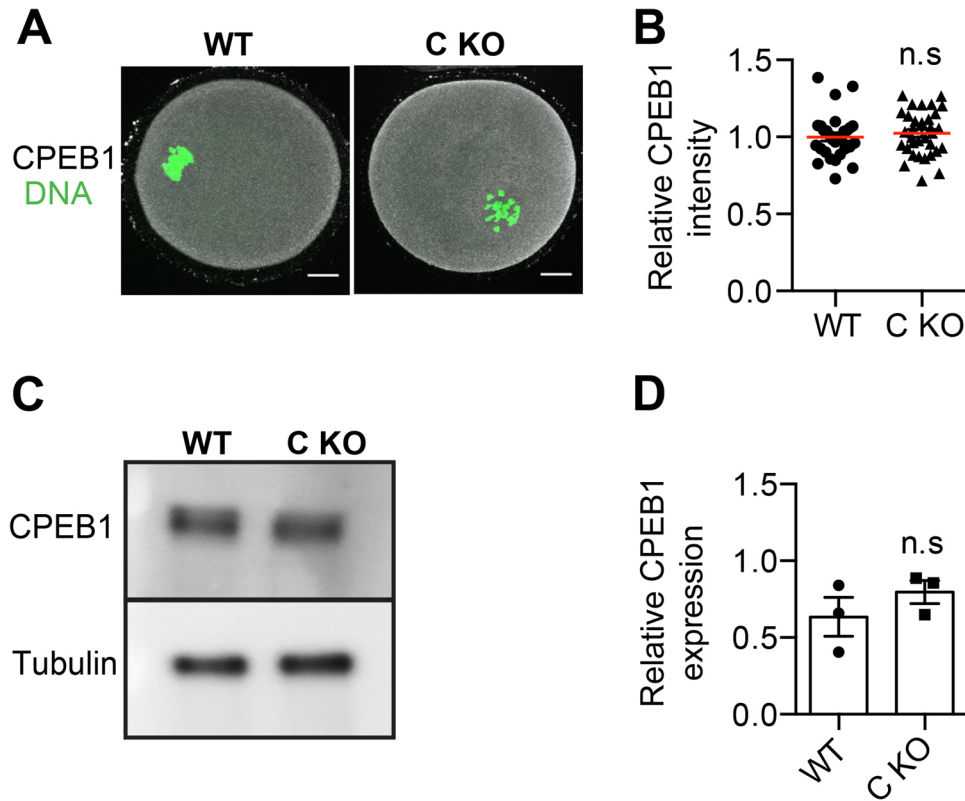


### Fig. S3. CPE-containing candidate genes in WT and B cKO oocytes

A) Schematic representation of the 3' UTR present in the mouse *Aurkc*, *Ccnb1*, *Hec1*, *Mos* and *Prc1*. Polyadenylation sequences (PAS) are in yellow. Consensus CPEs are in blue. (B) Prophase I oocytes from WT and B cKO were lysed and resolved by SDS-PAGE prior to western blotting to detect CCNB1 (25 oocytes/lane). (C) Relative CCNB1 expression from B (Unpaired student's t-test, two tailed, n.s: not-significant). (D) Prophase I oocytes from WT and B cKO were lysed and resolved by SDS-PAGE prior to western blotting to detect PRC1 (25 oocytes/lane). (E) Relative PRC1 expression from D. Values normalized to  $\alpha$ -tubulin (Unpaired student's t-test, two tailed, n.s: not-significant). (F) Prophase I oocytes from WT and B cKO were stained with anti-HEC1 (gray) and DAPI (DNA, green) to confirm meiotic stage. Shown are representative confocal z-projections. (G) Relative pixel intensity of HEC1 from F (Number of oocytes; WT-24 and B cKO-22, (Unpaired student's t-test, two tailed, n.s: not-significant). (H) Met II eggs from WT and B cKO were stained with anti-HEC1 (gray). Shown are representative confocal z-projections. (I) Relative pixel intensity of HEC1 from F (Number of oocytes; WT-33 and B cKO-34, Unpaired student's t-test, two tailed \*\*p <0.01). (J) Prophase I oocytes from WT and B cKO were stained with anti-MOS (gray) and DAPI (DNA, green) to confirm meiotic stage. Shown are representative confocal z-projections. (K) Relative pixel intensity of MOS from J (Number of oocytes; WT-24 and B cKO-22, Unpaired student's t-test, two tailed, n.s: not-significant). (M) Confocal images of Met I oocytes from WT and B cKO stained with anti-MOS (gray) and DAPI (green). (N) Relative intensity of MOS from M. Values normalized to WT (Number of oocytes: WT-32; B cKO-35).



**Fig. S4. AURKB/C inhibition does not affect translation.** A) IVM Met II eggs from WT, B cKO and B cKO hetA were stained with anti-pCDC25B (Ser353) (gray) and DAPI (DNA, green) to confirm meiotic stage. Shown are representative confocal z-projections. B) Relative pixel intensity of pCDC25B from A (Number of oocytes; WT-32, B cKO-36 and B cKO hetA -29, One-way ANOVA, n.s: not-significant, \*\* $p < 0.01$ , \*\*\*\* $p < 0.0001$ ). This experiment was repeated 3 times with total of 3 mice/genotype). C) Prophase I oocytes from WT mice were collected and matured to metaphase of meiosis I. Oocytes were matured in presence of DMSO, 5 $\mu$ M ZM447439 (ZM) or 1 $\mu$ M MLN8237 (MLN) and stained to detect HPG (gray) to assess translation DAPI (DNA, green) to confirm meiotic stage. Shown are representative confocal z-projections. D) Relative pixel intensity of HPG from C Number of oocytes: DMSO- 24; ZM-22; MLN- 21. One-way ANOVA, n.s. = not-significant, \*\*\*\*  $p < 0.0001$ . E) WT oocytes were in vitro matured with or without MLN and 25 oocytes were collected per group after 0, 3 and 7 hours of maturation. Oocytes were lysed and resolved by SDS-PAGE, then membranes were probed with anti-CPEB1. F) Relative CPEB1 expression from E. Values normalized to  $\alpha$ -tubulin. These experiments (C-E) were repeated 3 times with 2-3 mice per experimental replicate. Scale bar is 10  $\mu$ m.



**Fig. S5. Loss of AURKC does not affect CPEB1 function.** A) Prophase I oocytes from WT and AURKC KO oocytes mice were collected and matured to metaphase of meiosis I and stained with anti-CPEB1 (gray) and DAPI (DNA, green). Shown are representative confocal z-projections. B) Relative pixel intensity of CPEB1 from A. Values normalized to WT (Number of oocytes: WT- 29; C KO- 33, (Unpaired student's t-test, two tailed, n.s. = not significant). Scale bar is 10  $\mu$ m. C) Prophase I oocytes from WT and AURKC KO mice were matured to Metaphase I and prior to western blotting to detect CPEB1 (30 oocytes/lane).  $\alpha$ -Tubulin served as a loading control. D) Quantification of CPEB1 expression after normalizing values from C to  $\alpha$ -tubulin (Unpaired student's t-test, two tailed, n.s. = not significant). These experiments were repeated 3 times with 1 mouse per genotype for each experimental replicate.





**Sequence of Firefly-Luciferase-AurkC-3'UTR (Mutated):**

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### **Sequence of Firefly-Luciferase-Ccnb1-3'UTR**

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**Sequence of Firefly-Luciferase-Ccnb1-3'UTR (Mutated)**

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