

## EXOSC10 mediated RNA degradation sculpts the transcriptome during oocyte growth-to-maturation transition

Di Wu\*, Cameron Palmer and Jurrien Dean  
Laboratory of Cellular and Developmental Biology, NIDDK,  
National Institutes of Health, Bethesda, Maryland 20892  
\*corresponding author: di.wu2@nih.gov

### Supplementary Figures

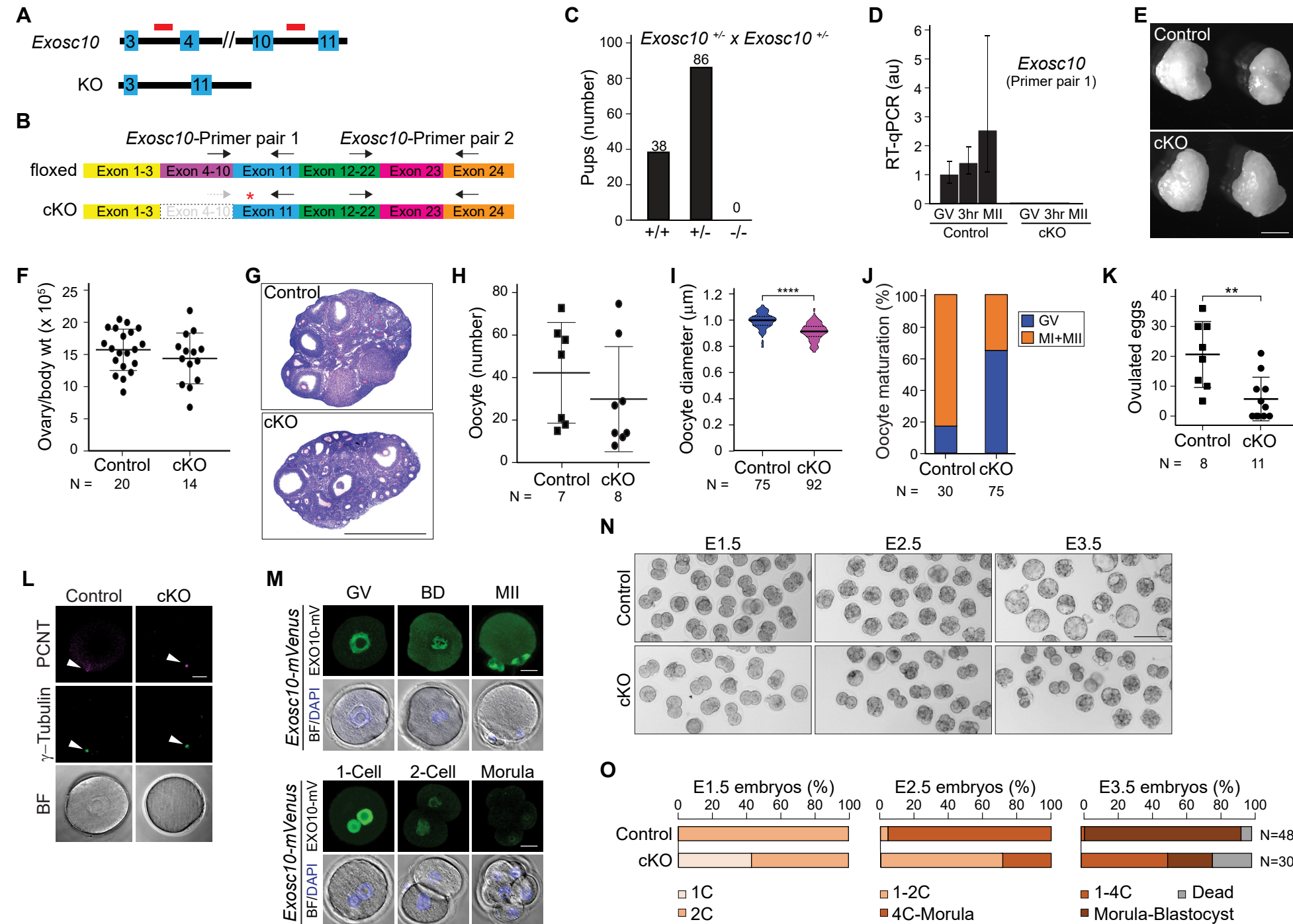
**Figure S1. Characterization of *Exosc10* oocyte-conditional knockout mice.** (A) Schematic of the strategy to generate *Exosc10* knockout allele using CRISPR/Cas9 in which two gRNAs were used to delete exons 4-10. (B) Schematic of mRNAs generated from the floxed and cKO alleles. In the latter, exons 4-10 are absent, and an early termination occurs in exon 11. (C) Number of pups with indicated genotypes. No *Exosc10* homozygous null pups survived to term. (D) qPCR of *Exosc10* in single oocytes obtained from controls and cKO females. Two primer pairs were used (see B). Primer pair 1, shown here, detects the region deleted after loxP recombination; primer pair 2 detects region shared by the mRNAs transcribed from the *Exosc10* floxed and cKO allele. (E) Representative ovaries (12 weeks) dissected from control and cKO mice. (F) Ovary/body weight ratio of control and cKO females (12 weeks). Numbers of ovaries indicated below each group. (G) Histology of periodic acid-Schiff stained ovaries in E. (H) Quantification of fully-grown oocytes in single (2  $\mu$ m) sections from G. Number of ovaries indicated below each group. (I) Diameter of control and cKO oocytes. The horizontal lines represent the median and quartiles. (J) *Ex vivo* maturation of control and cKO GV oocytes through meiosis (MI, MII). Number of oocytes indicated below each group. (K) Number of ovulated eggs collected from control and cKO female oviducts after hormonal stimulation with eCG and hCG. Number of females indicated below each group. (L) Immunostaining of microtubule-organizing center (MTOC) proteins pericentrin (PCNT) and  $\gamma$ -tubulin, indicated by arrowheads. (M) Confocal fluorescence and bright-field images of EXOSC10-mVenus localization during control oocyte maturation and pre-implantation development. (N) *Ex vivo* culture of pre-implantation development of the embryos derived from control and cKO female mice. Bright-field images were obtained at E1.5, E2.5 and E3.5. (O) Quantification of the embryonic progression at each time point in N. In F, H, K, the horizontal lines represent the mean and standard deviation. Scale bars: 1 mm in E, G; 20  $\mu$ m in L, M; 100  $\mu$ m in N. \*\*\*\*  $p < 0.0001$ , \*\*  $p < 0.05$ , two-tailed Student's t-test.

**Figure S2. EXOSC10 degrades poly(A) RNA in oocytes.** (A, C, E) Confocal fluorescence images showing poly(A) RNA FISH, bright-field and DAPI of wildtype oocytes at GV, GV+3hr and MII stages in A, of oocytes overexpressing *mVenus*, *Exosc10-mVenus* or *dExosc1-mVenus* cRNA at GV, GV+3hr stages in C, and of oocytes derived from controls and cKO females at GV, GV+3hr in E. The oocytes at GV+3hr stage were obtained by *ex vivo* culture of GV oocytes in maturation medium for 3 hr. (B, D, F). Quantification of overall poly(A) RNA fluorescence of A, C, E, respectively. The horizontal lines in represent the mean and standard deviation. In B, Nc1, negative control 1, without probe incubation; Nc2, negative control 2, treated by RNase A before probe incubation. Number of oocytes indicated below each group. \*\*\*\*  $p < 0.0001$ , \*  $p < 0.05$  in D by one-way ANOVA. Scale bars: 20  $\mu$ m in A, C, E.

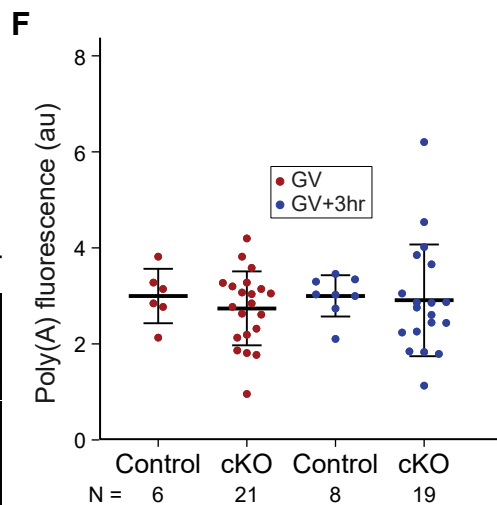
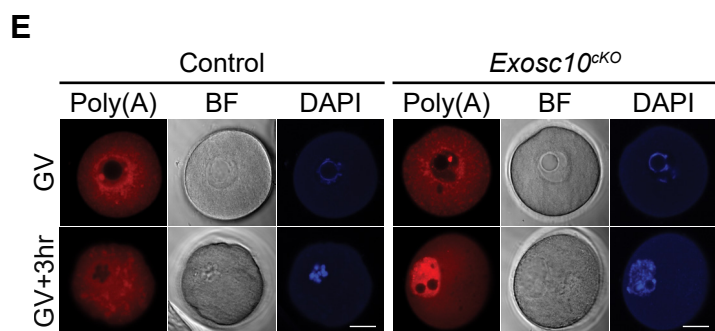
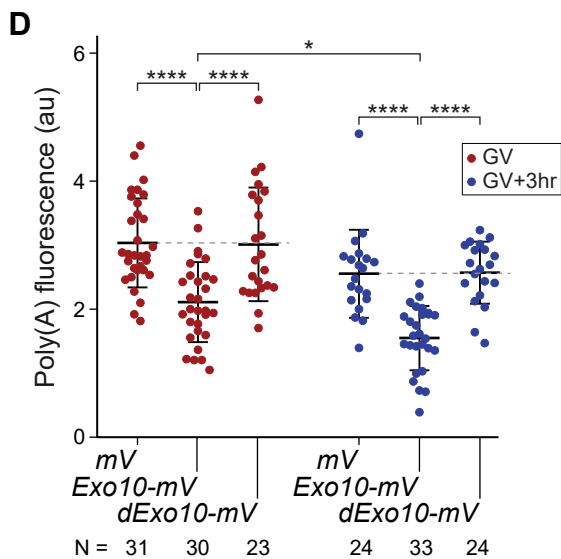
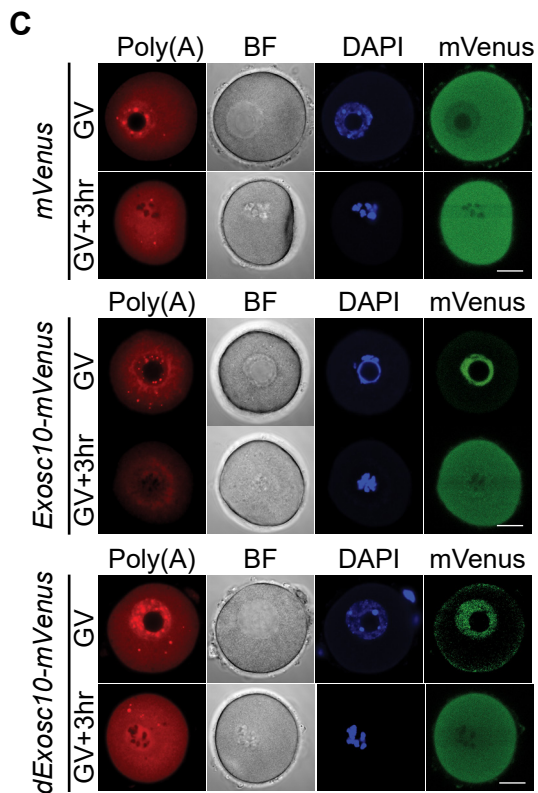
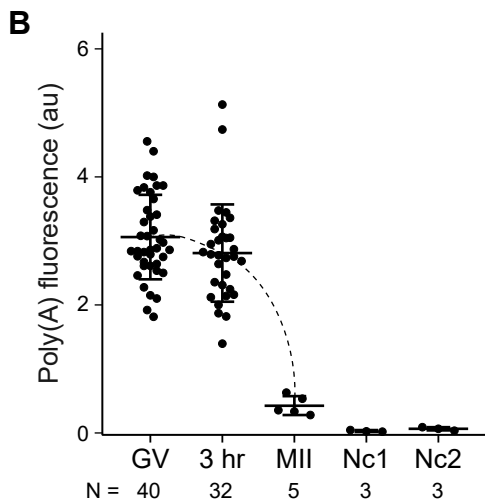
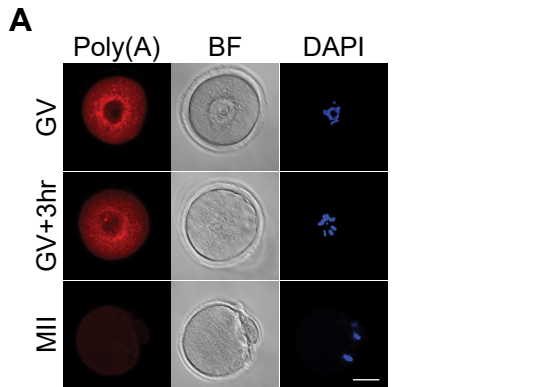
**Figure S3. Validation of linear amplification of 64 RNA-seq libraries by ERCC RNA spike-in mix.** Each scatter plot represents the linear regression of ERCC reads with the original concentration. The red number is the covariant of coefficient and the blue number is the ERCC components (92 in total) detected. Libraries are from GV\_ctr, control GV oocytes; GV.3\_ctr, control GV oocytes incubated for 3 hr; MII\_ctr, control MII oocytes; GV\_cKO, cKO GV oocytes; GV.3\_cKO, cKO GV oocyte incubated for 3 hr; MII\_cKO, cKO MII oocytes.

**Figure S4. Overexpression of *Rab5a* and *Rab5c* cRNA failed to phenocopy *Exosc10*<sup>cKO</sup> oocytes.** (A) Brightfield images after *ex vivo* culture (0, 3 hr, 20 hr) of wildtype oocytes injected with cRNA of *Rab5a-mVenus*, *Rab5c-mVenus*, *Rab5a-mVenus/Rab5c-mVenus* and *mVenus*. (B) Bar graphs of oocyte maturation from GVBD (BD) to MII in A in two independent experiments. The total number of oocytes is indicated above each bar graph. (C-D) Fluorescent images of oocytes after co-injection of cRNA of *Rab5a-mVenus* and *Rab5c-mVenus* which failed to decrease Rab7. Scale bars: 100  $\mu$ m in A, C, D.

**Figure S5. cAMP level does not increase in *Exosc10*<sup>cKO</sup> oocytes.** (A) A bar graph of the mean and standard error of log<sub>2</sub> fold change of *Gpr12* (G protein-coupled receptor 12), *Gpr3* (G protein-coupled receptor 3), *Pde3a* (phosphodiesterase 3A), *Adcy3* (adenylate cyclase 3), *Adcy9* (adenylate cyclase 9) from single oocyte RNA-seq data sets. \*\*\*\*  $\rho < 0.0001$ , \*\*\*  $\rho < 0.001$ , \*\*  $\rho < 0.01$ , \*  $\rho < 0.05$ , n.s. no significance, which are the  $\rho$ -adjust values in the DESeq2 analysis. (B) Confocal fluorescence images of cAMP in control and *Exosc10* cKO GV oocytes. Scale bars: 20  $\mu$ m.

**Figure S1**

# Figure S2

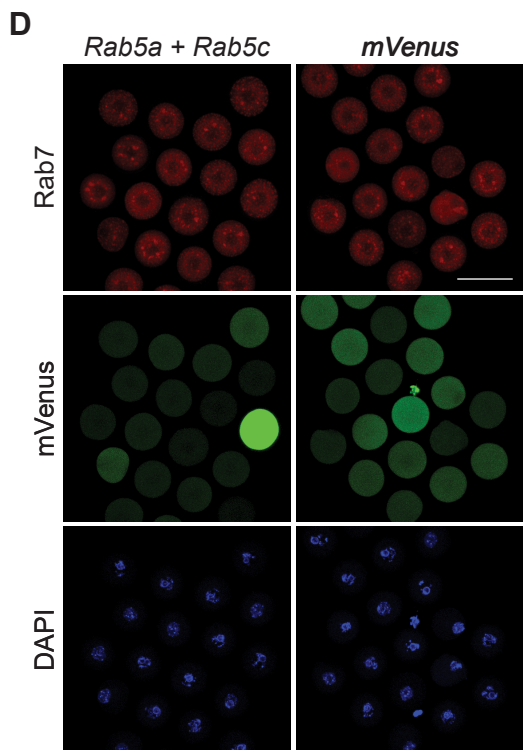
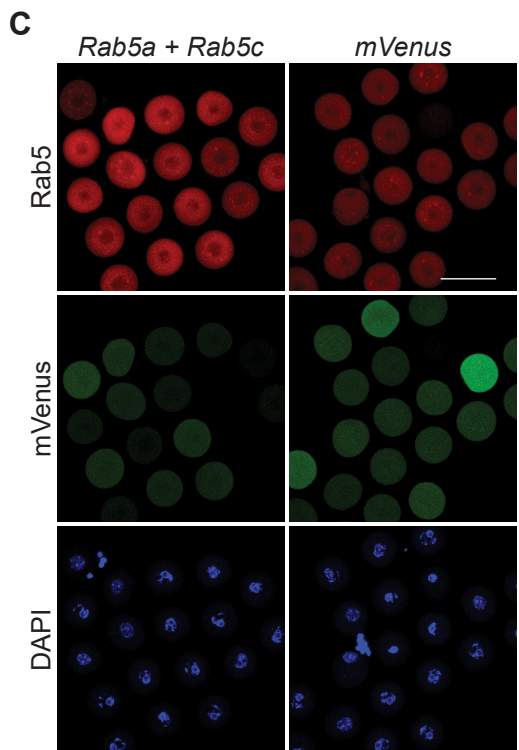
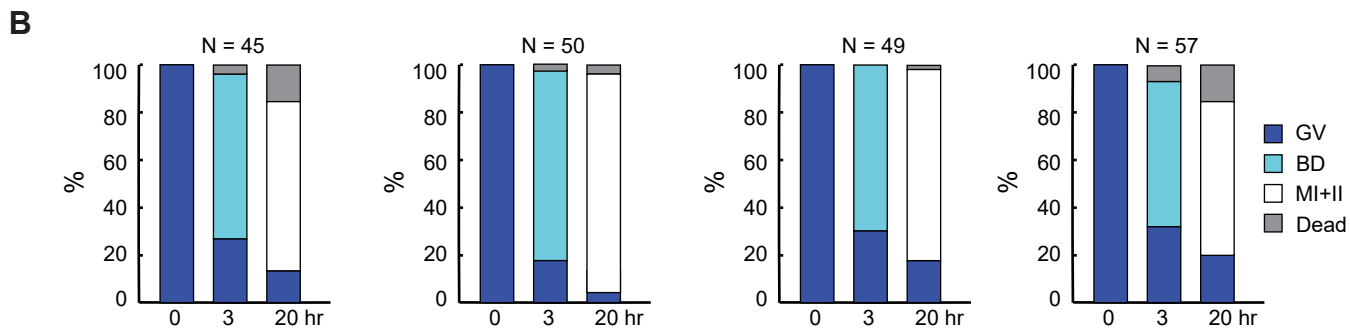
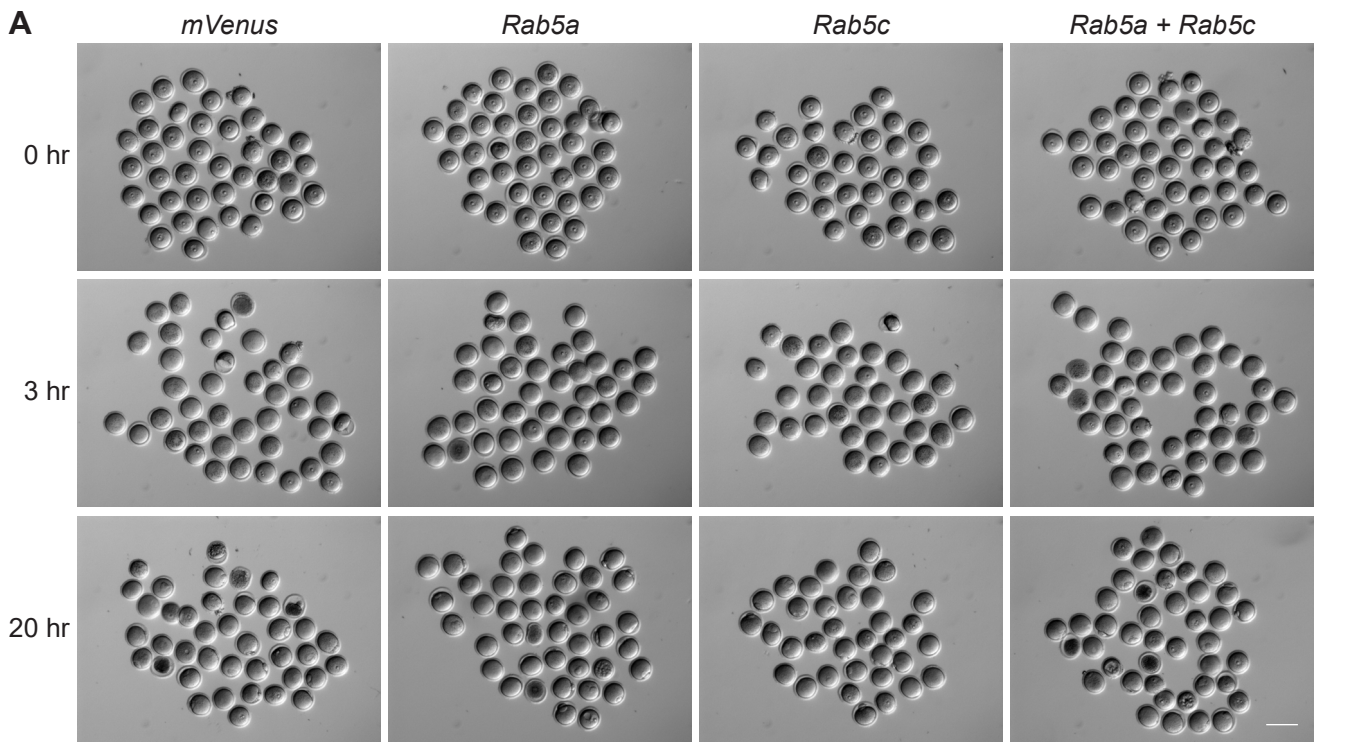




# Figure S3



# Figure S4



# Figure S5

