

Expanded View Figures

Figure EV1.

Figure EV1. The effect of MOS variants on promoting oocyte meiosis maturation.

- A The diagram showing the experimental procedure. Geminal vesical (GV) oocytes were microinjected with FLAG-tagged MOS, MOS^{Asn95Lys}, MOS^{Met139Thr} and MOS^{Arg246His}, MOS^{Cys320Ter}, or FLAG mRNAs combined with mCherry mRNAs, and maintained in milrinone-treated M2 medium for 24 h. The number of GV oocytes, geminal vesical breakdown (GVBD), or metaphase II (MII) oocytes was counted.
- B The representative bright-field images and fluorescent images were captured at 24 h after microinjection using a Nikon microscope. Over 100 oocytes in each group. Scale bar = 100 μ m.
- C The percentage of meiosis resumption (GVBD and MII) oocytes at 24 h after microinjection with different MOS mRNAs in (B). N number was labeled in each graph.
- D Western blot analysis of FLAG, pERK1/2, and mCherry using oocytes after microinjection with different *MOS* mRNAs combined with mCherry mRNAs for 24 h. Histone H3 and DDB1 were used as loading controls. *N* = 100 oocytes per sample.

Data information: For C, data are expressed as mean \pm SD. One-way ANOVA followed by *post hoc* Tukey's test for multiple comparisons, ***P < 0.0001. Source data are available online for this figure.



D			simos + FLAG-MOS mRNA			
	siNC	siMos	Wild-type	Asn95Lys	Met139Thr+Arg246His	s Cys32Ter
FLAG		\mathbf{O}		O		8
pERK1/2/DAPI	3					5



Figure EV2. The RNAi effect of Mos and the rescuing effects of different MOS variants in oocytes.

- A Schematic diagram of oocyte cultures and experimental procedure. Mouse GV oocytes were microinjected with siRNAs and cultured for 24 h in the presence of 2.5 µM milrinone, followed by microinjection of *MOS* variants mRNAs, and cultured for 4 h before release to fresh M2 medium for another 14 h culture. Then, the RNAi efficiency of *siRNA* targeting mouse *Mos* and rescuing effects of different human *MOS* variants were determined.
- B The representative images showing the decreased pERK1/2 (red) and abnormal spindle (FITC-α-tubulin, green) after *Mos* siRNA injection. DAPI (blue) was stained for visualization of DNA. Scale bar = 10 μm.
- C RT-qPCR results showing the mouse Mos mRNA expression levels in oocytes with negative control siRNAs or Mos siRNAs (n = 3).
- D Representative immunofluorescence images showing the FLAG (red) and pERK1/2 (green) levels in mature oocytes after microinjection of *siMos* or combined with indicated human *MOS* variants in GV oocytes. At least 30 oocytes each group were used for immunofluorescence. Scale bar = $10 \ \mu$ m.

Data information: In C, data are expressed as mean \pm SD of three biological replicates. ***P = 0.0002 (unpaired two-tailed Student's *t*-test). Source data are available online for this figure.