

## APPENDIX

### **Biallelic mutations in *MOS* cause female infertility characterized by human early embryonic arrest and fragmentation**

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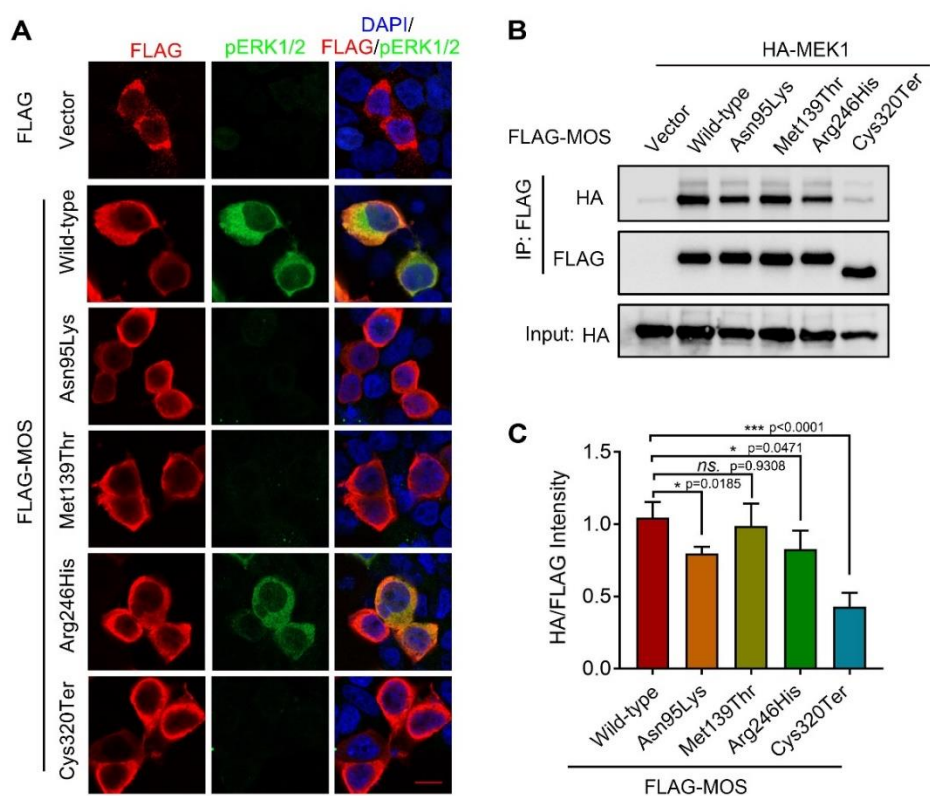
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## Appendix Figure S1



### Appendix Figure S1. The effects of *MOS* variants on subcellular localization, ERK1/2 activation and the binding capacity with MEK1.

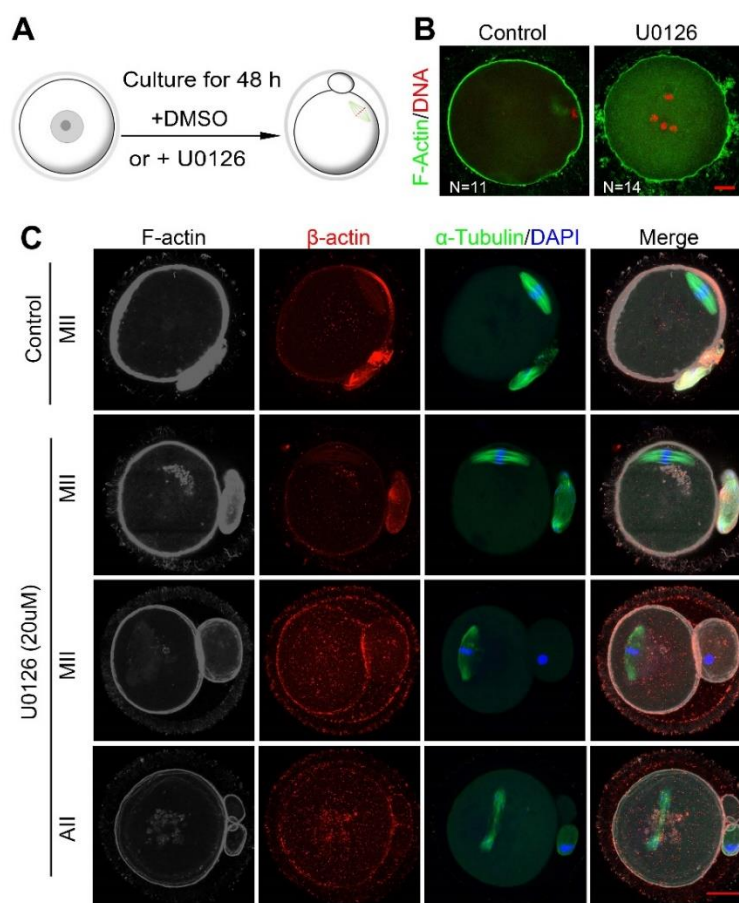
**A.** Representative confocal images labeled with FLAG (red), pERK1/2 (green) and DAPI (blue) in HEK293 cells after transfection of indicated *MOS* variants. Scale bar=10  $\mu$ m.

**B.** Anti-FLAG immunoprecipitation of extracts from HEK293 cells transiently expressing HA-MEK1 in combination with FLAG-tagged *MOS* or indicated *MOS* variants. Input and immunoprecipitates (IP) were analyzed using specified antibodies. Two independent experiments were performed.

**C.** Bar graph quantifying the mean intensity for HA relative to FLAG signal after IP described in (B).

Data information: For (C), data are expressed as mean  $\pm$  SD. One-way ANOVA, followed by *post hoc* Tukey's test for multiple comparisons, ns., no significance; \*,  $P < 0.05$ ; \*\*\*,  $P < 0.001$ .

## Appendix Figure S2



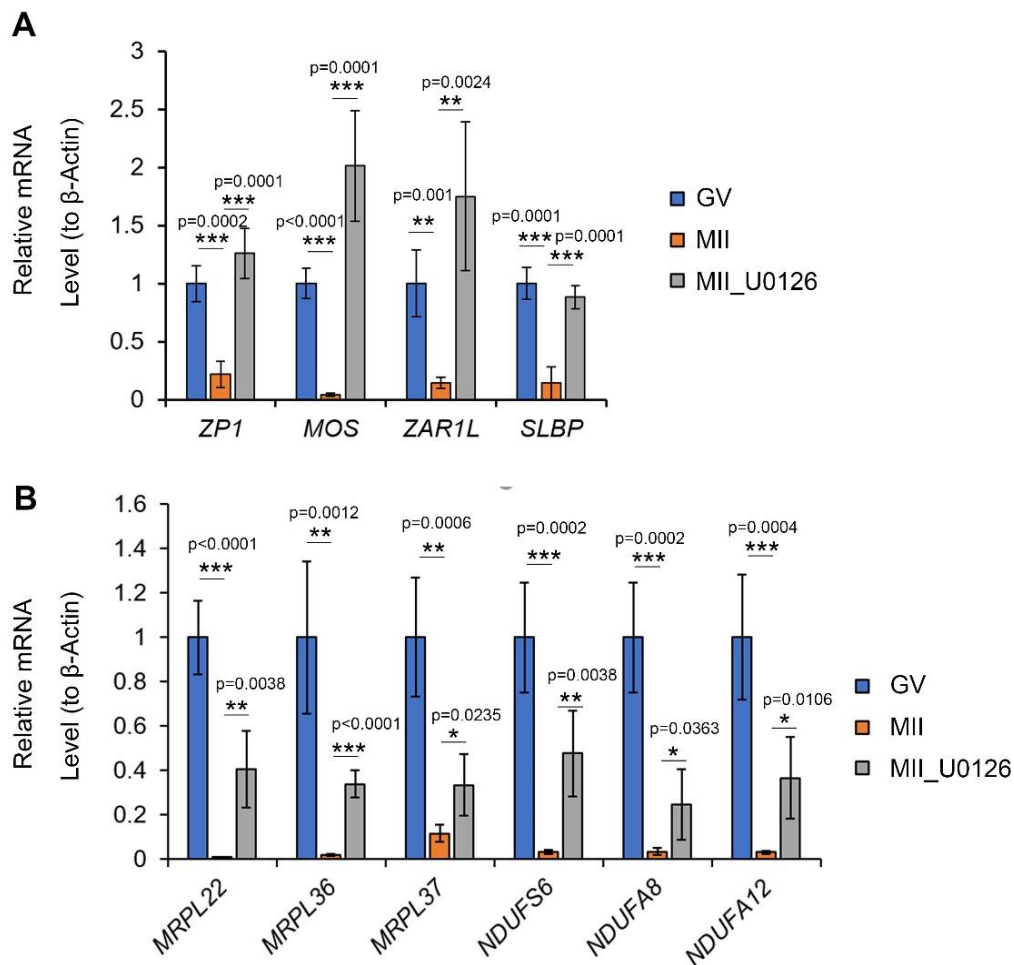
### Appendix Figure S2. ERK1/2 inactivation causes oocyte cytoskeleton assembly disorder during meiotic maturation.

**A.** The diagram of experimental procedure. The immature (geminal vesical breakdown, GVBD) human oocytes were cultured in a medium containing DMSO or 20  $\mu$ M U0126 for 48 h.

**B.** The immunofluorescence of F-actin (green) and DNA (red) in human oocytes. Scale bar=20  $\mu$ m.

**C.** Mouse GV oocytes were cultured in M2 medium with or without 20  $\mu$ M U0126 for 14 h *in vitro*. Mature oocytes were fixed and immuno-stained with antibodies against  $\beta$ -actin (red) and FITC- $\alpha$ -tubulin (green), as well as co-stained with phalloidin (gray) and DAPI (blue). Scale bar=20  $\mu$ m. MII, metaphase II; AII, anaphase II.

## Appendix Figure S3



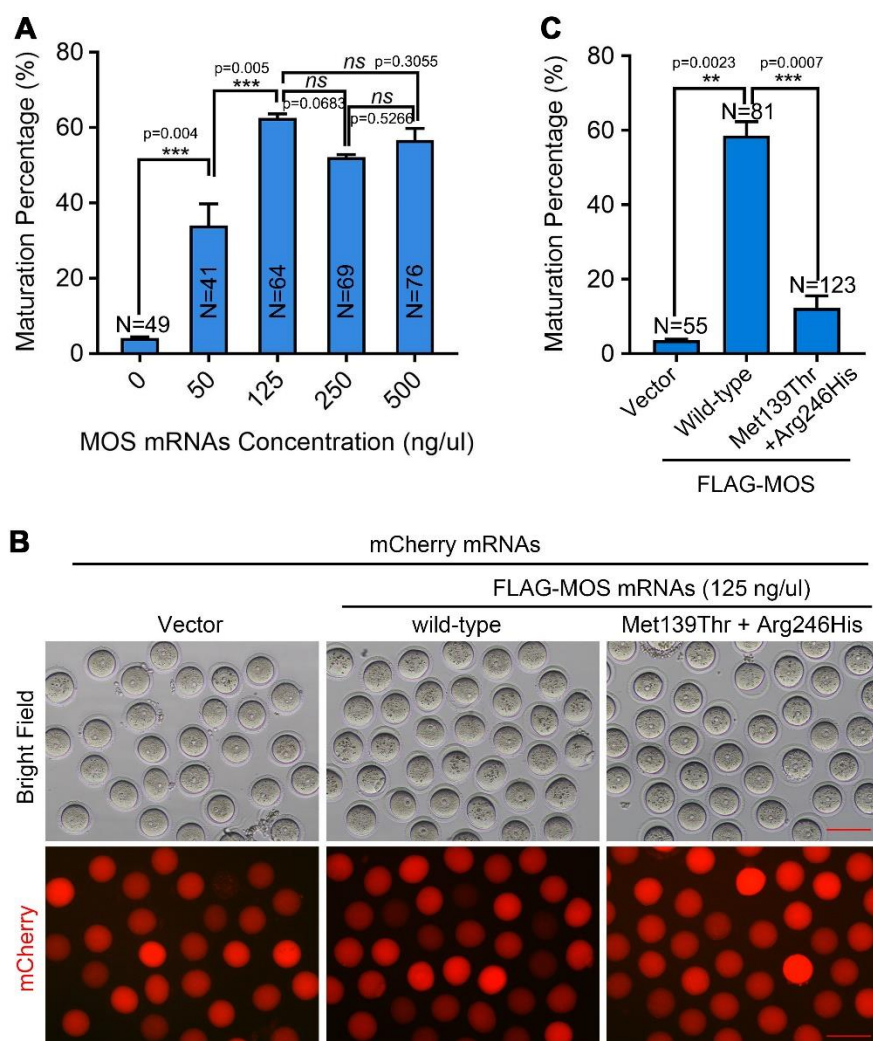
### Appendix Figure S3. ERK1/2 inactivation retarded some maternal genes degradation during human oocyte maturation.

**A-B.** RT-qPCR results showing the relative mRNA level of selected oocyte-specific genes (A) and genes relative to mitochondrial function (B).  $\beta$ -Actin was used as an internal control.

Data information: Data are expressed as mean  $\pm$  SD of two biological replicates.

Unpaired two-tailed Student's *t* test was used for statistical analysis. \*,  $P<0.05$ ; \*\*,  $P<0.01$ ; \*\*\*,  $P<0.001$ . Detailed *P* value as indicated.

## Appendix Figure S4.



### Appendix Figure S4. The dose effect of MOS mRNAs for promotion of meiosis maturation.

**A.** The statistical result of oocyte meiotic maturation percentage after microinjection with mCherry (500 ng/ul) and different concentrations (0, 50, 125, 250 and 500 ng/ul) of wild-type *MOS* mRNAs. The oocyte number per group was labeled.

**B.** The representative images showing the oocyte morphology and mCherry signal after microinjection with mCherry in combined with 125 ng/ul wild-type *MOS* mRNAs or the two variants in patient 2. Scale bar=100  $\mu$ m.

**C.** The statistical result of oocyte meiotic maturation percentage after microinjection with mCherry (500 ng/ul) and 125 ng/ul wild-type *MOS* mRNAs or the combined two variants found in patient 2. The oocyte number per group was labeled.

Data information: Data are expressed as mean $\pm$ SD. One-way ANOVA with *post hoc* Tukey's test for multiple comparisons in (A) and unpaired two-tailed Student's *t* test (C) were used for statistical analysis. ns., no significance; \*\*,  $P<0.01$ ; \*\*\*,  $P<0.001$ . Detailed *P* value as indicated.

**Appendix Table S1. Menstrual Cycles and Basic Sex Hormone Levels of Patients with EEAF.**

<b>Patients</b>	<b>Menstrual cycles (days)</b>	<b>hFSH (mIU/mL)</b>	<b>hLH (mIU/mL)</b>	<b>E2 (pg/mL)</b>	<b>Prog (ng/mL)</b>	<b>Testo (ng/mL)</b>	<b>PRL (ng/mL)</b>
Patient 1	29-30	6.02	6.30	41.03	0.45	4.67	12.61
Patient 2	29-30	8.70	3.95	14.00	0.5	0.51	12.79
Patient 3	26-28	6.77	4.41	53.84	0.76	0.4	21.86

Abbreviations are as follows: hFSH, human follicle-stimulating hormone; hLH, human luteinizing hormone; E2, estradiol; Testo, testosterone; Prog, progestin; PRL, prolactin.



**Appendix Table S2. Primers and siRNA Oligos List**

Target Gene	Species	Oligo Sequences (5'-3')	Application
<i>MOS</i>	Human	Forward: ATTGACTGGGAGCAGGTGTG	PCR primers for identification of c.285C>A (p.Asn95Lys) variant
		Reverse: AACTCCATGATGATGGTCCCTA	
<i>MOS</i>	Human	Forward: CTTACCGCGGTGTTCTCTGTG	PCR primers for identification of c.416T>C (p.Met139Thr) and c.737G>A, (p.Arg246His) variant
		Reverse: CCACCACCGCGTACAGTAT	
<i>MOS</i>	Human	Forward: GTACTCACTAGATGTTGTGAACGG	PCR primers for identification of c.960C>A, (p.Cys320Ter) variant
		Reverse: TTCTTCGACATCTCCACTTCC	
<i>MOS</i>	Human	Forward: CAAGTGCACCAAGAAACGACTAGCATCTCG	Mutagenesis primers for c.285C>A (p.Asn95Lys) variant
		Reverse: CGAGATGCTAGTCGTTTCTTGGTGCACTTG	
<i>MOS</i>	Human	Forward: GGGACCATCATCACGGAGTTCGGTGG	Mutagenesis primers for c.416T>C (p.Met139Thr) variant
		Reverse: CCACCGAACTCCGTGATGATGGTCCC	
<i>MOS</i>	Human	Forward: CATACACCCACCACGCCCCGGAG	Mutagenesis primers for c.737G>A (p.Arg246His) variant
		Reverse: CTCCGGGGCGTGGTGGGTGTATG	
<i>MOS</i>	Human	Forward: CGTCATCCAGCGCTGATGGAGACCCAGCGC	Mutagenesis primers for c.960C>A, (p.Cys320Ter) variant
		Reverse: GCGCTGGGTCTCCATCAGCGCTGGATGACG	
<i>Mos</i>	Mouse	Forward: TGGCTGGTTTTGAGAATCAAGG	RT-qPCR primers
		Reverse: GTCACATGAGACACTAGGGAGA	
<i>Gapdh</i>	Mouse	Forward: AGGTCGGTGTGAACGGATTTG	RT-qPCR primers
		Reverse: TGTAGACCATGTAGTTGAGGTCA	

N/A	N/A	Sense: TTCTCCGAACGTGTCACGTTT	siRNA against negative control
<i>Mos</i>	Mouse	Sense: GGAAAGACACTGCAGAACA	siRNAs against mouse <i>Mos</i>
		Sense: GCCTAGGTACCATAATCAT	
		Sense: CCATCAAGCAAGTAAACAA	
<i>MOS</i>	Human	Forward: TCACTAGATGTTGTGAACGGC	RT-qPCR primers
		Reverse: TCAGAGCAACCGAAGTCACTAA	
<i>ZP1</i>	Human	Forward: ACGACCTGGGGTTACCCTG	RT-qPCR primers
		Reverse: AGCTGCATTCCCTTGATCCC	
<i>ZARIL</i>	Human	Forward: CTATGGCTTGTACCAGGGTTATG	RT-qPCR primers
		Reverse: CTTGTAAGGGTCAATGCAGTAGT	
<i>SLBP</i>	Human	Forward: GACCCGAGAGCTTTACCACTC	RT-qPCR primers
		Reverse: GGCACAGTAGACATAGACTCCT	
<i>MRPL22</i>	Human	Forward: CGAGGAATGTCTATTGACCAGGC	RT-qPCR primers
		Reverse: CGGATGCGTTTCAGGCACT	
<i>MRPL36</i>	Human	Forward: TGCGCTCACTTCTCTCACC	RT-qPCR primers
		Reverse: ACAGTAGACGTACCACCGACC	
<i>MRPL37</i>	Human	Forward: TCCCCTGGATAGGGTGTACG	RT-qPCR primers
		Reverse: GAGCGGTAGAACCTTGGGT	
<i>NDUFS6</i>	Human	Forward: TTCGGTTTGTAGGTCGTCAGA	RT-qPCR primers
		Reverse: CCATCGCACGCTATCACCC	
<i>NDUFA8</i>	Human	Forward: CCCAACAAGGAGTTTATGCTCT	RT-qPCR primers
		Reverse: CACAGTGACGTTTTATCTGCCT	
<i>NDUFA12</i>	Human	Forward: GGTCTCCGAGGCTATCTACGG	RT-qPCR primers
		Reverse: GGAGGCACCATGCTTCCATC	
<i>ACTB</i>	Human	Forward: CATGTACGTTGCTATCCAGGC	RT-qPCR primers
		Reverse: CTCCTTAATGTCACGCACGAT	