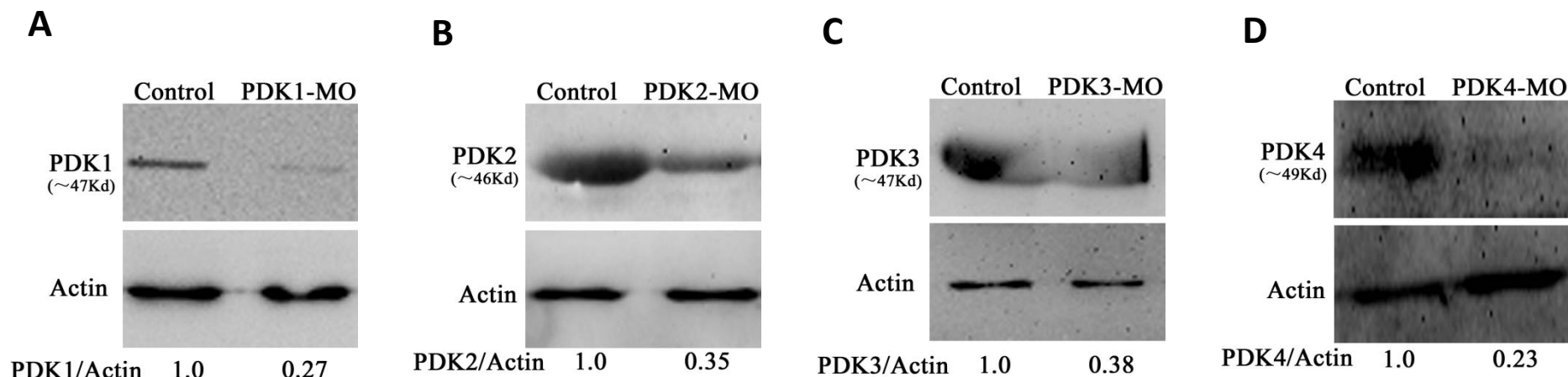


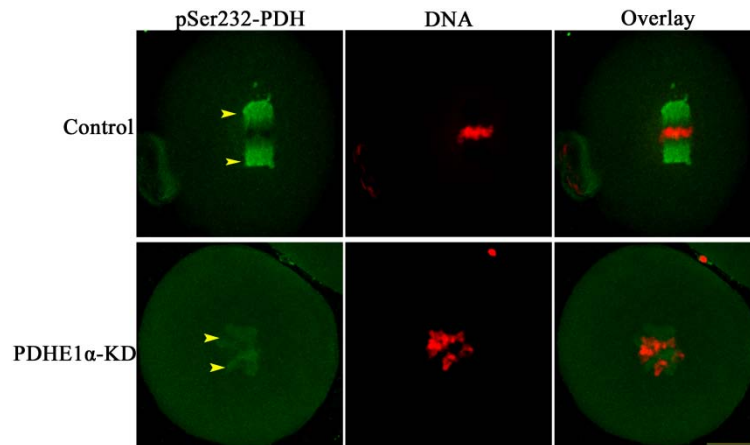
Figure S1



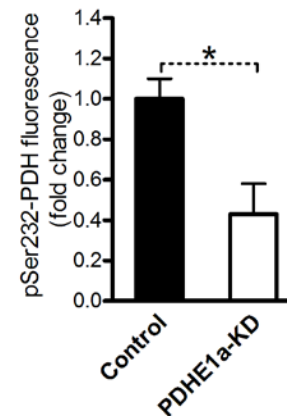
Suppl Fig. 1 Knockdown of endogenous PDK protein expression with morpholino injection. Fully-grown oocytes injected with PDK morpholinos (PDK-MO) were arrested at GV stage with milrinone for 20 hours to facilitate blocking of mRNA translation, and then matured in milrinone-free medium. A sham MO standard was injected as control. Western blot showing partial knockdown of PDK1 (A), PDK2 (B), PDK3 (C) and PDK4 (D) after morpholino injection, with actin as a loading control. Band intensity was calculated using Image J software, and the ratio of PDK/Actin expression was normalized and values are indicated. All experiments were repeated at least three times and only the representative gel images are shown.

Figure S2

A

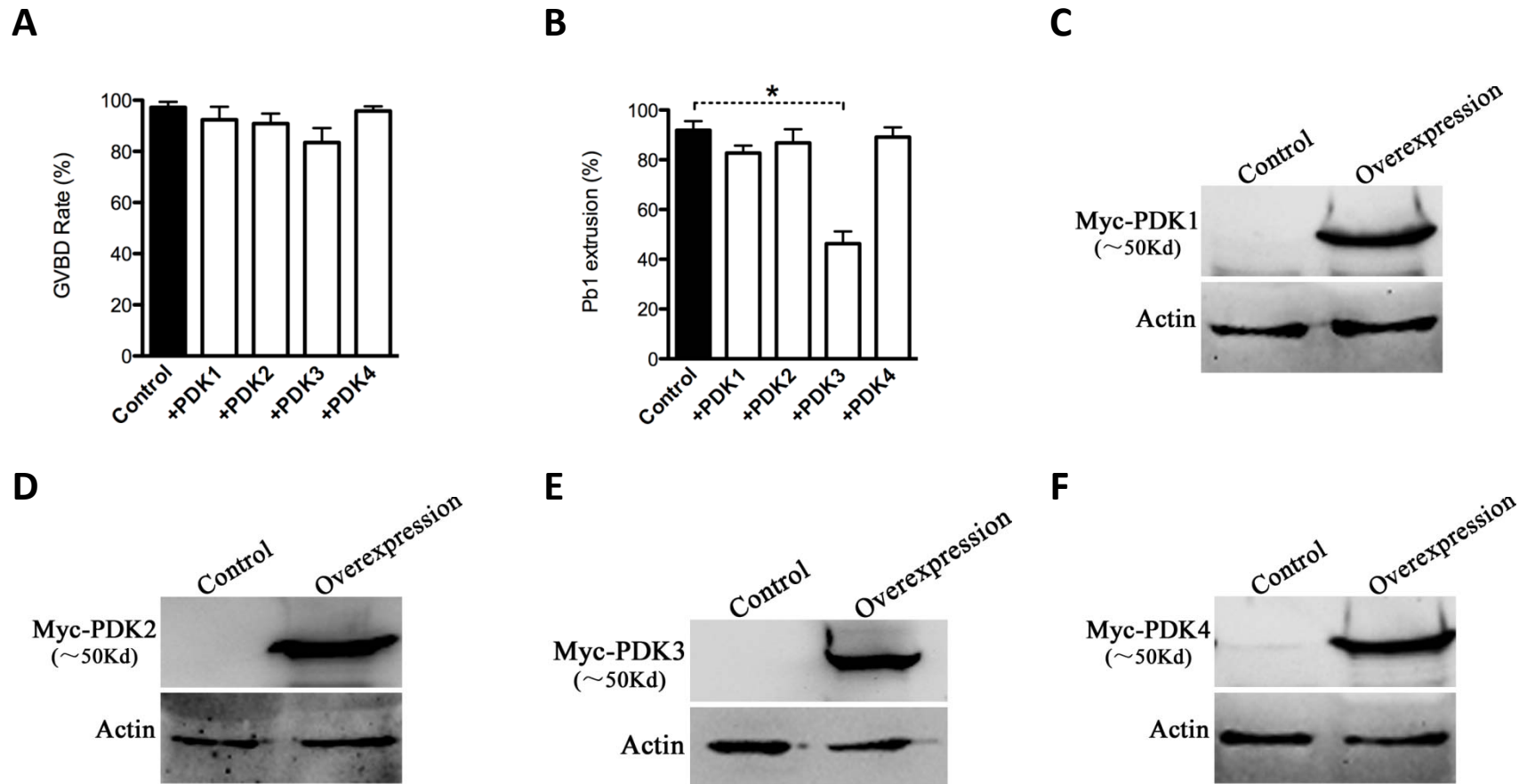


B



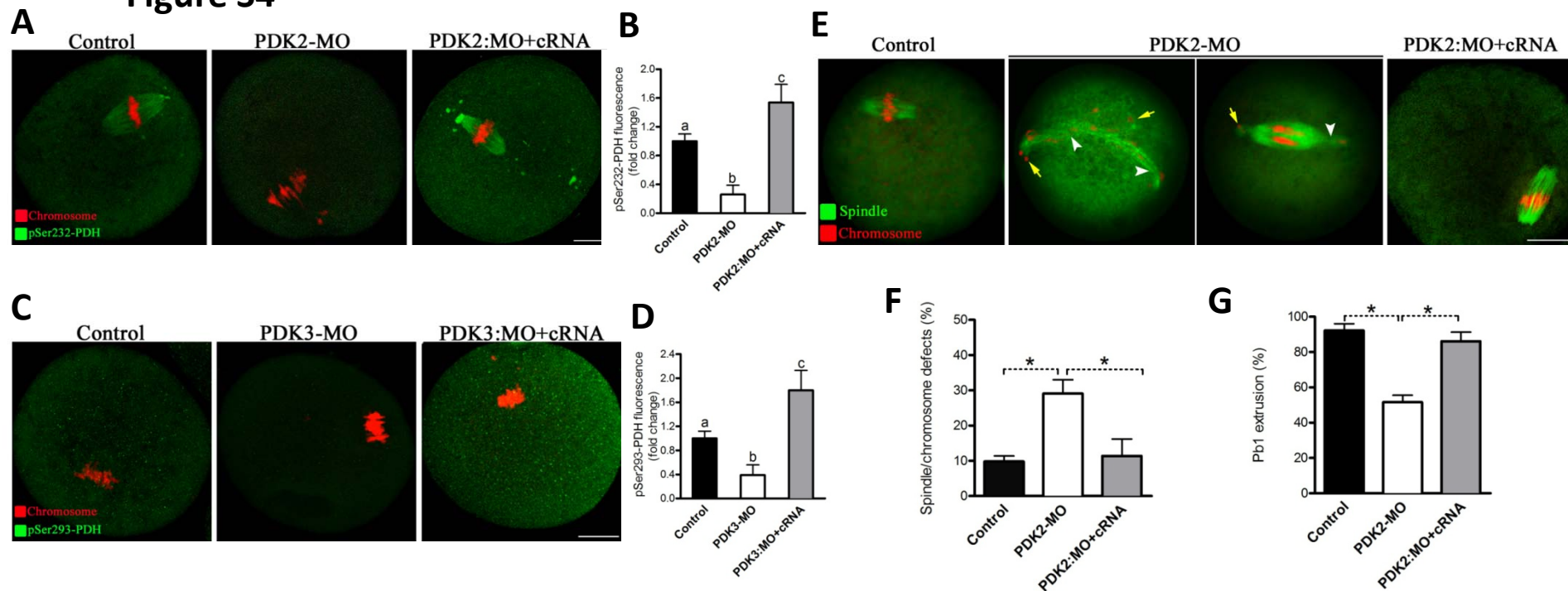
Suppl Fig. 2 Effects of PDHE1 α knockdown on pSer232-PDH staining in mouse oocytes. Fully-grown oocytes injected with PDHE1 α morpholino were arrested at GV stage with milrinone for 20 hours to facilitate blocking of mRNA translation, and then matured in milrinone-free medium. A sham MO standard was injected as control. (A) Control and PDHE1 α -KD MII oocytes were stained with pSer232-PDH antibody (green) and counterstained with PI for chromosome (red). Representative confocal sections are shown. (B) Quantification of pSer232-PDH fluorescence in A. Data are expressed as the mean \pm SD from three independent experiments in which at least 50 oocytes were analyzed. * $p < 0.05$ vs controls. Scale bar: 20 μ m.

Figure S3



Suppl Fig. 3 Effects of PDK overexpression on maturational progression. PBS (control group) or exogenous Myc-PDK mRNA (overexpression group; +PDK) was injected into fully-grown oocytes, which were arrested for 20 hours with milrinone to allow synthesis of new Myc-PDK protein, and then cultured in milrinone-free medium to evaluate the meiotic progression. (A-B) Quantitative analysis of GVBD and Pb1 extrusion in control (n=90), PDK1 (n=120), PDK2 (n=105), PDK3 (n=116) and PDK4 (n=120)-overexpressing oocytes. The graph shows the mean \pm SD of the results obtained in three independent experiments. * $p < 0.05$ vs control. (C-F) Western blot analysis showed that exogenous Myc-PDK1-4 protein was efficiently overexpressed, probing with anti-Myc Tag antibody.

Figure S4



Suppl Fig. 4 Effects of exogenous PDK mRNA expression on the phosphorylation of Ser232/293-PDH and spindle/chromosome organization in MO-injected oocytes. cRNA was microinjected into fully-grown oocytes 2h after MO injection, and the immunofluorescence staining with pSer232/293-PDHE1 α antibody, maturational progression, and spindle/chromosome organization were examined after milrinone treatment and in vitro maturation. A sham MO standard was injected as control. (A) Control, PDK2-MO and PDK2:MO+cRNA injected oocytes were stained with pSer232-PDH antibody (green) and counterstained with PI for chromosome (red). (B) Quantification of pSer232-PDH fluorescence in A. (C) Control, PDK3-MO and PDK3:MO+cRNA injected oocytes were stained with pSer293-PDH antibody (green) and counterstained with PI for chromosome (red). (D) Quantification of pSer232-PDH fluorescence in C. For (B) and (D), data are expressed as the mean \pm SD from three independent experiments in which at least 60 oocytes were analyzed. (E) Control, PDK2-MO and PDK2:MO+cRNA injected oocytes were stained with α -tubulin antibody to visualize the spindle (green) and counterstained with PI to visualize chromosomes (red). (F) Quantitative analysis of control, PDK2-MO and PDK2:MO+cRNA oocytes with abnormal spindle and chromosomes. (G) Quantitative analysis of Pb1 extrusion in control, PDK2-MO and PDK2:MO+cRNA oocytes. For (F) and (G), data are expressed as mean percentage \pm SD from three independent experiments in which at least 100 oocytes were analyzed. * or different superscript letters indicate significant differences. Scale bar: 20 μ m.

Table S1 Primer sequences of genes for qRT-PCR

<i>Gene</i>	<i>Primer sequence</i>
GAPDH	Forward Primer: 5' –CTTTGTCAAGCTCATTTCTTGG – 3' Reverse Primer: 5' –TCTTGCTCAGTGTCTTGC – 3'
PDK1	Forward Primer: 5' –GACTGTGAAGATGAGTGACCG – 3' Reverse Primer: 5' –CAATCCGTAACCAAACCCAG – 3'
PDK2	Forward Primer: 5' –AAGAGATCAACCTGCTTCCTG – 3' Reverse Primer: 5' –GCATCTGTGAACTGGCTTAGAG – 3'
PDK3	Forward Primer: 5' –CGCCATTACAAGACCACTCC – 3' Reverse Primer: 5' –CAGAGACTTCAGAGACAGCAC – 3'
PDK4	Forward Primer: 5' –AGTGACTCAAAGACGGGAAAC – 3' Reverse Primer: 5' –GTGTGAGGTTAATTCTGGCG – 3'

Table S2 Primer sequences of genes for cDNA amplification

<i>Gene</i>	<i>Primer sequence</i>
PDK1	Forward Primer: 5' –GGGGGCCGGCCG ATGAGGCTGGCAAGGCT – 3' Reverse Primer: 5' –GGGGGCGCGCC TTAAGAGCTTCGGAATGTGG – 3'
PDK2	Forward Primer: 5' –GGGGGCCGGCCGATGCGCTGGGTCCGG – 3' Reverse Primer: 5' –GGGGGCGCGCCCTAGCTGACCCGATACGTCG – 3'
PDK3	Forward Primer: 5' –GGGGGCCGGCCGATGCGGCTCTTCTACCGGCT – 3' Reverse Primer: 5' –GGGGGCGCGCCCTAGAAAGTTCTATTACTCT – 3'
PDK4	Forward Primer: 5' –GGGGGCCGGCCGATGAAGGCAGCCCGCTTC – 3' Reverse Primer: 5' –GGGGGCGCGCCTCACACTGCCAGCTTCTCCT – 3'

Table S3 Primer sequences of genes for site-directed mutagenesis of PDHE1a
The sections responsible for the mutation are highlighted yellow.

<i>Gene</i>	<i>Primer sequence</i>
PDHE1a	Forward Primer: 5' –GGGGGCCGGCCGATGAGGAAGATGCTTG – 3' Reverse Primer: 5' –GGGGGCGCGCCTTAAGTACTGACTTAAAC – 3'
PDH-Ser232A	Forward Primer: 5' –CTATGGCATGGGGACG GCT GTTGAGAGAGCAGC – 3' Reverse Primer: 5' –GCTGCTCTCTCAACAGCCGTCCCCATGCCATAG – 3'
PDH-Ser232D	Forward Primer: 5' –CTATGGCATGGGGACG GAT GTTGAGAGAGCAGCAG – 3' Reverse Primer: 5' –CTGCTGCTCTCTCAACATCCGTCCCCATGCCATAG – 3'
PDH-Ser293A	Forward Primer: 5' –CGCTACCATGGACAC ACC ATGAGTGACCCTGGA – 3' Reverse Primer: 5' –TCCAGGGTCACTCATGGTGTGTCCATGGTAGCG – 3' Forward Primer: 5' –CCGCTACCATGGACAC GCC ATGAGTGACCCTGG – 3' Reverse Primer: 5' –CAGAGCCTCGTGGTACCTCTCCTCGGTGGCGTT – 3'
PDH-Ser293D	Forward Primer: 5' –CGCTACCATGGACAC AAC ATGAGTGACCCTGGA – 3' Reverse Primer: 5' –TCCAGGGTCACTCATGGTGTGTCCATGGTAGCG – 3' Forward Primer: 5' –CGCTACCATGGACAC GAC ATGAGTGACCCTGGA – 3' Reverse Primer: 5' –TCCAGGGTCACTCATGTCGTGTCCATGGTAGCG – 3'