

## **Supporting information**

### **PIP5K1A–PIP2–RhoA Signaling Orchestrates Membrane Remodeling during Early Porcine Embryo Development**

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#### **The file includes:**

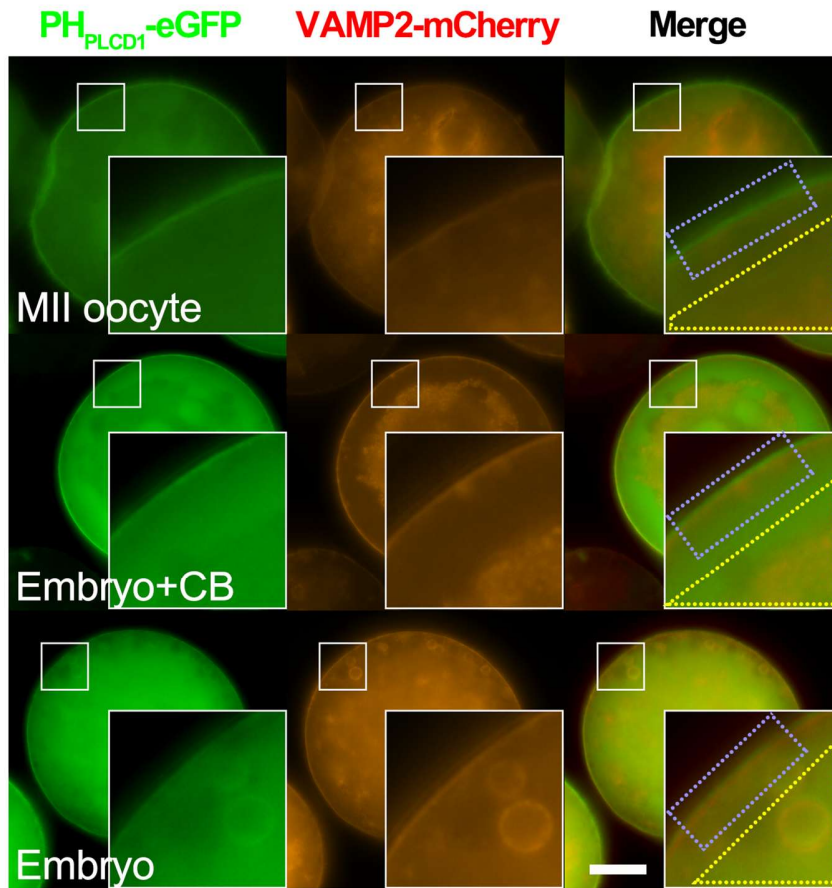
Figures. S1 to S8

Tables S1 and S2

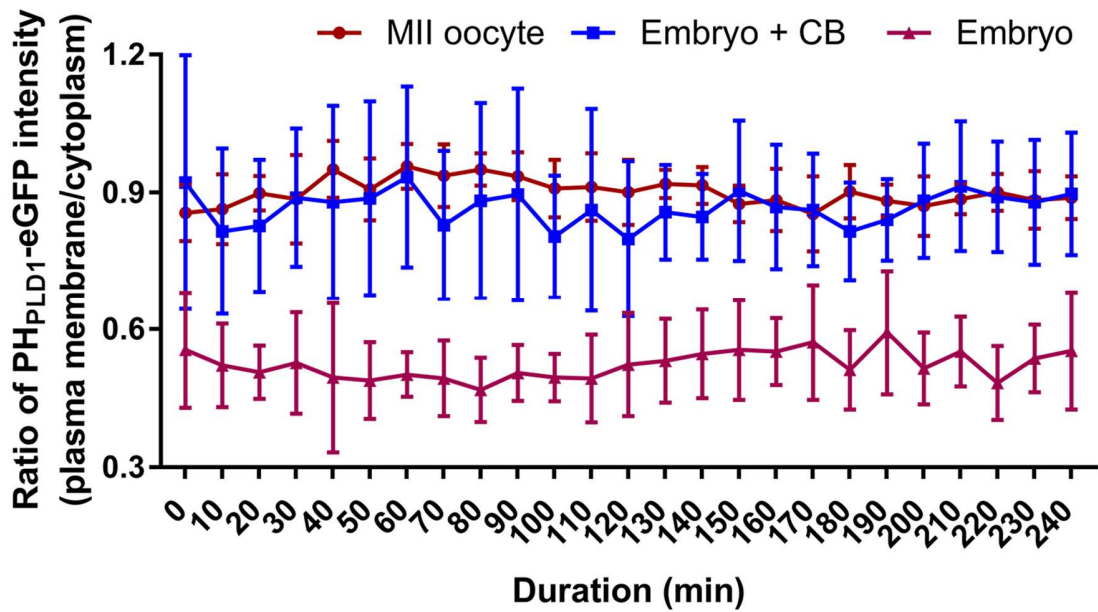
#### **Other Supplementary Materials for this manuscript include the following:**

Movie S1 to S5

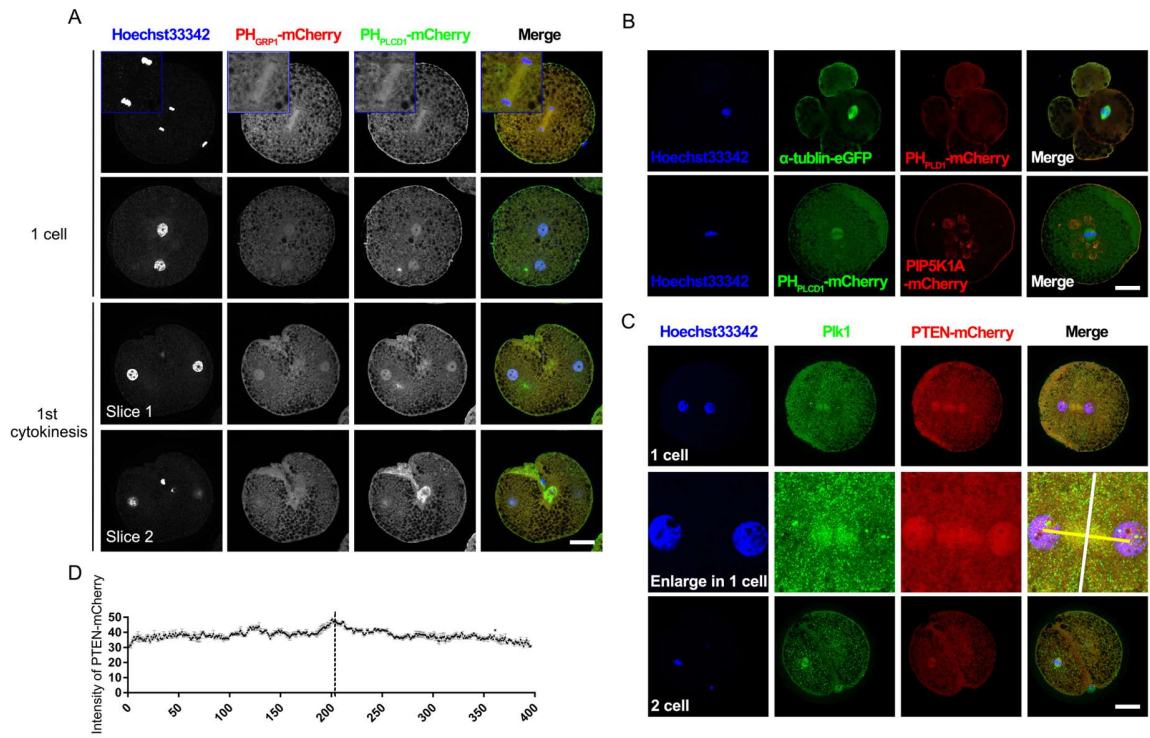
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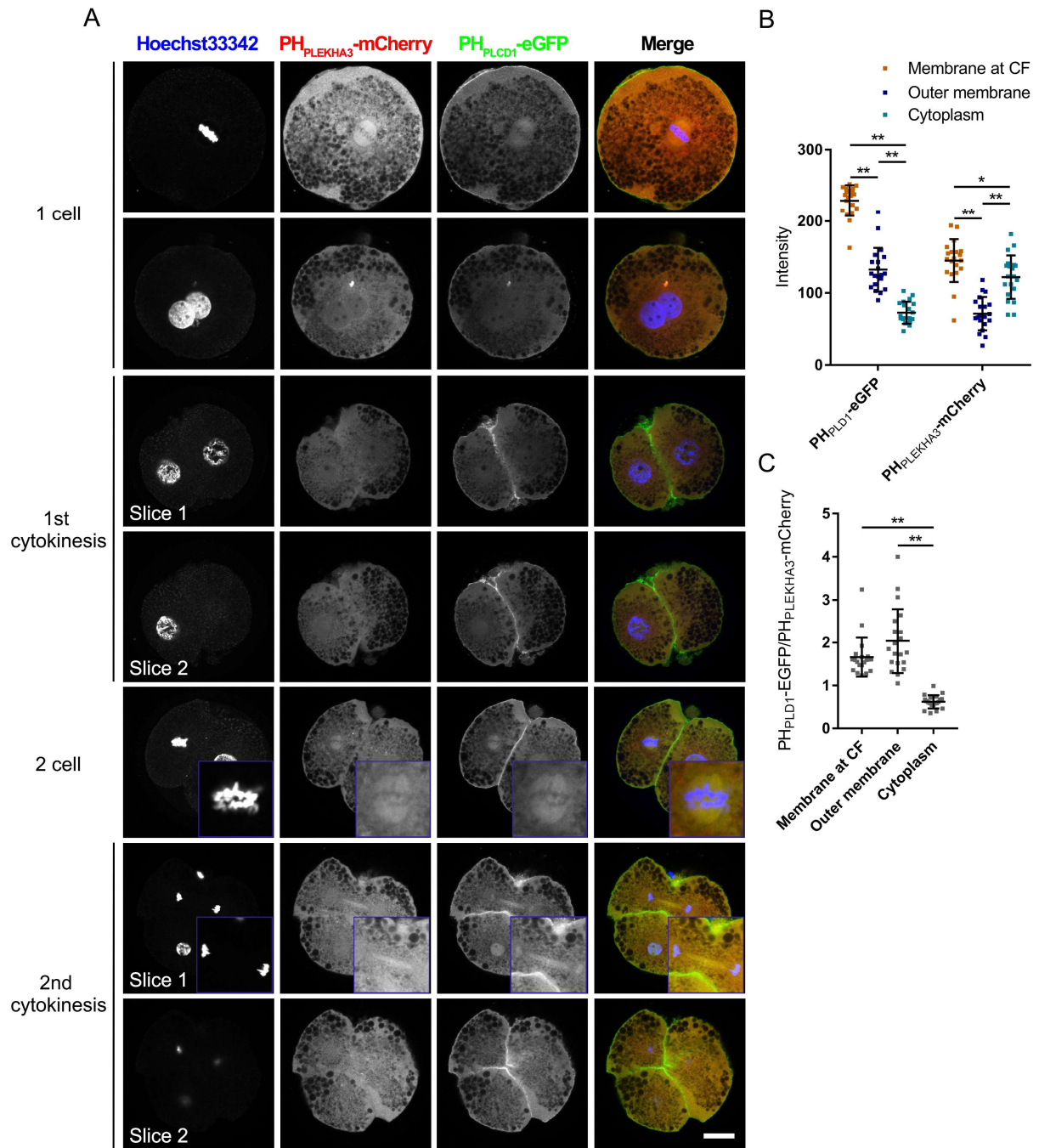
B



**Figure S1. Live imaging of PH<sub>PLCD1</sub>-eGFP and VAMP2-mCherry in MII oocytes and embryos. (A)** The Distribution of PH<sub>PLCD1</sub>-eGFP and VAMP2-mCherry in MII oocytes and embryos. CB, cytochalasin B. Scale bar = 25  $\mu$ m. **(B)** Ratio of PH<sub>PLCD1</sub>-eGFP in the cytoplasm to the plasma membrane.

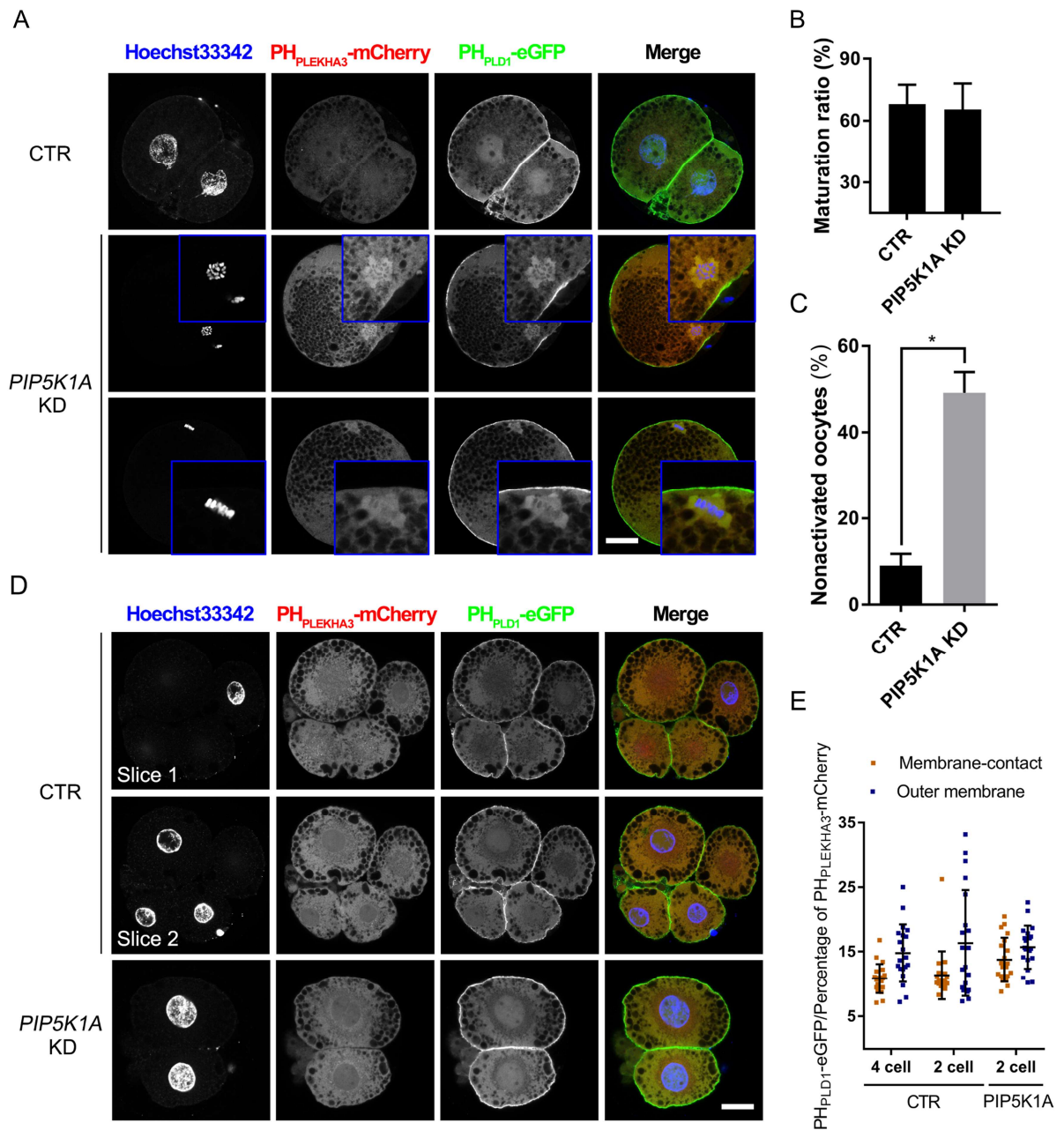


**Figure S2. Localization of PIP3, PIP2, and their related proteins in the nucleus or at the spindle.** (A) Localization of PH<sub>GRP1</sub>-mCherry (a PIP3 marker) and PH<sub>PLCD1</sub>-eGFP in the nucleus and spindle. (B) Subcellular localization of PIP2 and PIP5K1A in the spindles. (C) Subcellular localizations of Plk1 (spindle) and PTEN. (D) The intensity of PTEN-mCherry along the spindle axis (yellow line) in Figure S2C. The dotted line represents the white line in Figure S2C. Scale bar = 25  $\mu$ m.



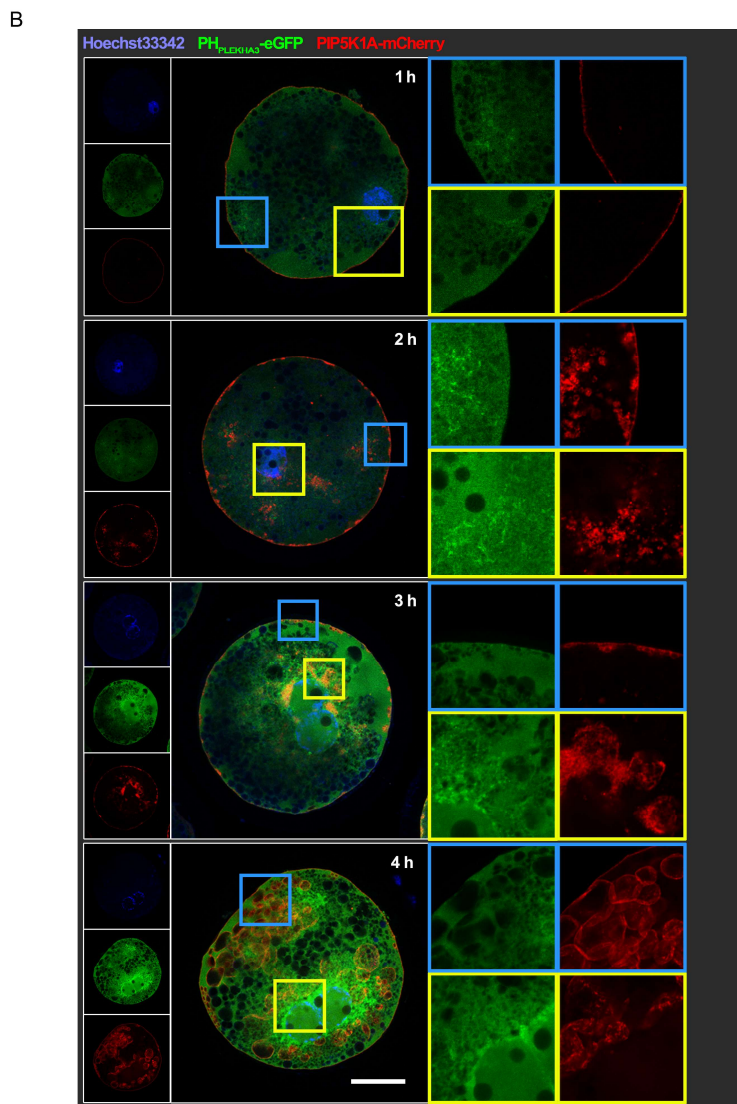
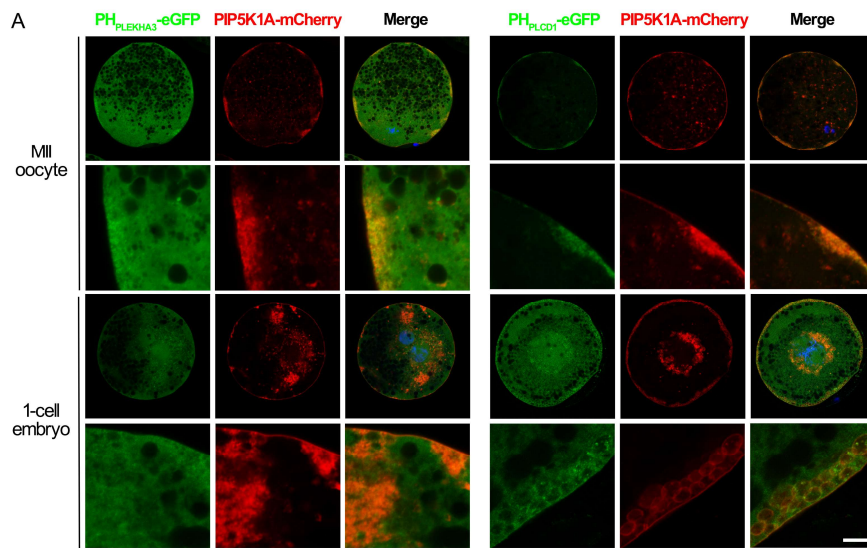
**Figure S3. Localization of PI4P and PIP2 at the membrane and spindle. (A)** Localization of  $PH_{PLEKHA3}$ -mCherry and  $PH_{PLCD1}$ -eGFP at the membrane and spindle during 1-cell, 2-cell, and cytokinesis stages. **(B)** The intensities of  $PH_{PLEKHA3}$ -mCherry and  $PH_{PLCD1}$ -eGFP in different

embryonic regions during cytokinesis. (C) Intensity ratio of PH<sub>PLCD1</sub>-eGFP to PH<sub>PLEKHA3</sub>-mCherry in different regions of the embryo during cytokinesis. Scale bar = 25  $\mu$ m.

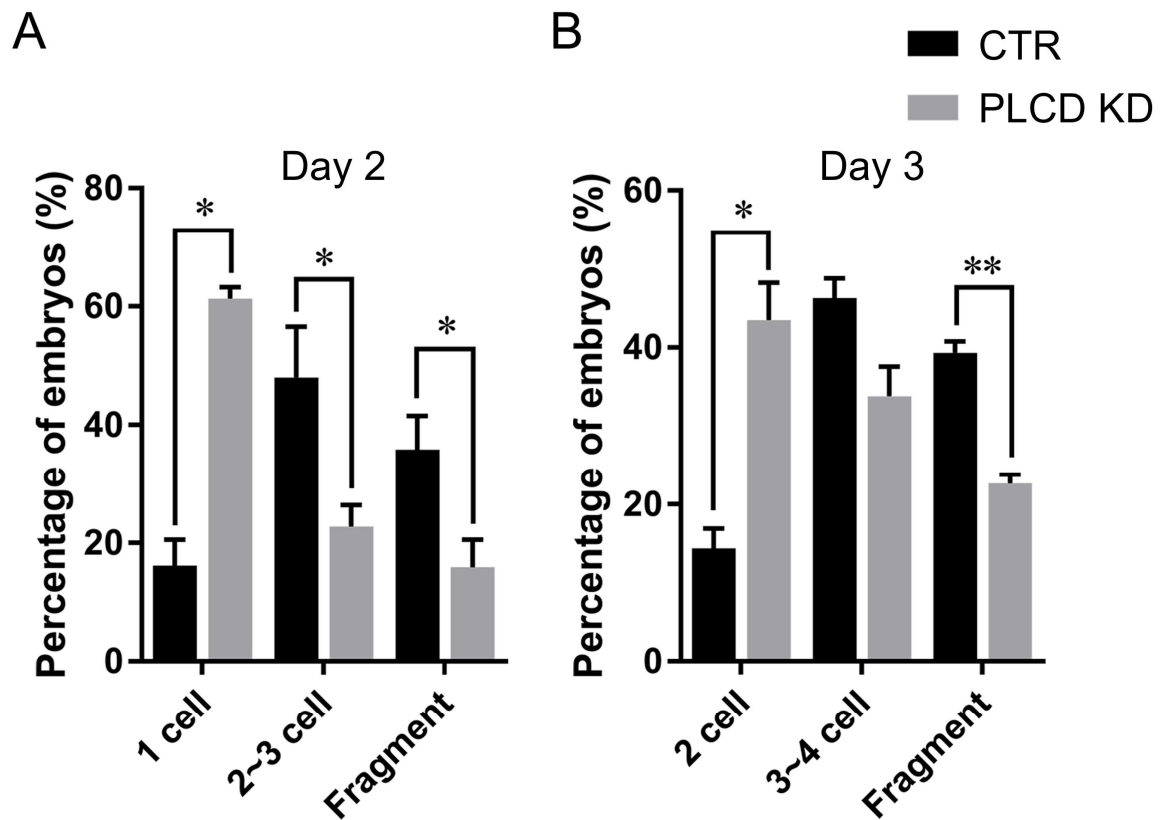


**Figure S4. Effects of *PIP5K1A* knockdown in oocytes and embryos on PI4P and PIP2. (A)** PH<sub>PLEKHA3</sub>-mCherry and PH<sub>PLD1</sub>-eGFP expression 44 h after PIP5K1A knockdown in GV oocytes and 1 d after parthenogenetic electrical stimulation for oocyte activation. Scale bar = 25  $\mu$ m. **(B)** Maturation ratio of GV stage oocytes at 44 h after PIP5K1A knockdown. **(C)** Percentage of non-activated GV oocytes 44 h after PIP5K1A knockdown and 1 d after parthenogenetic

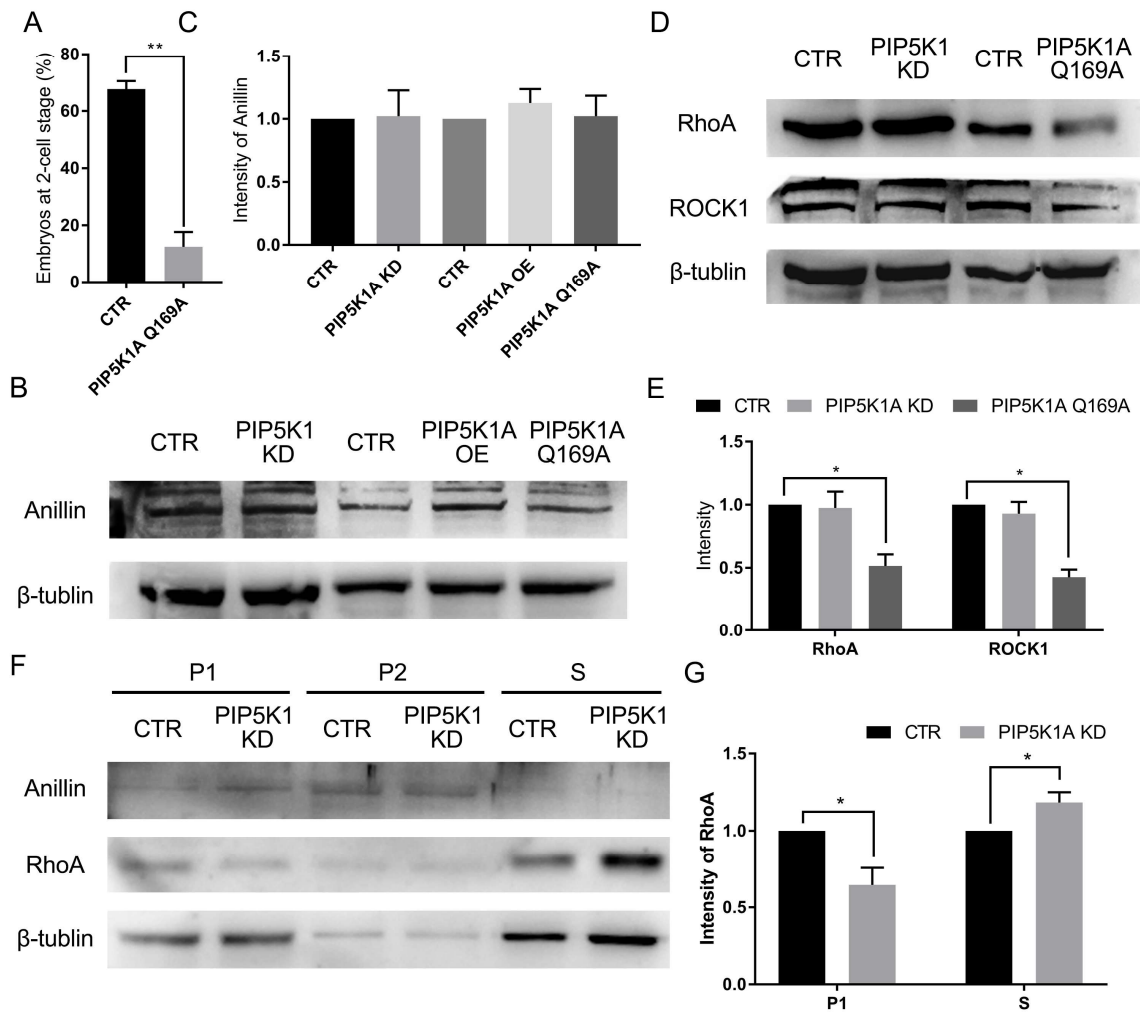
electrical stimulation. **(D)** PH<sub>PLEKHA3</sub>-mCherry and PH<sub>PLCD1</sub>-eGFP 1 d after PIP5K1A knockdown of 1-cell embryos. Scale bar = 25  $\mu$ m. **(E)** Intensity ratio of PH<sub>PLCD1</sub>-eGFP to PH<sub>PLEKHA3</sub>-mCherry in different regions of the embryo (Figure S4D).



**Figure S5. Effects of *PIP5K1A* overexpression on PIP2 and PI4P.** (A) Localization of PH<sub>PLEKHA3</sub>-mCherry, PH<sub>PLCD1</sub>-eGFP, and PIP5K1A-mCherry 4 h after exogenous expression in MII oocytes. Scale bar = 25  $\mu$ m. (B) The localization of PH<sub>PLEKHA3</sub>-eGFP and PIP5K1A-mCherry after their exogenous expression in embryos. Scale bar = 25  $\mu$ m.

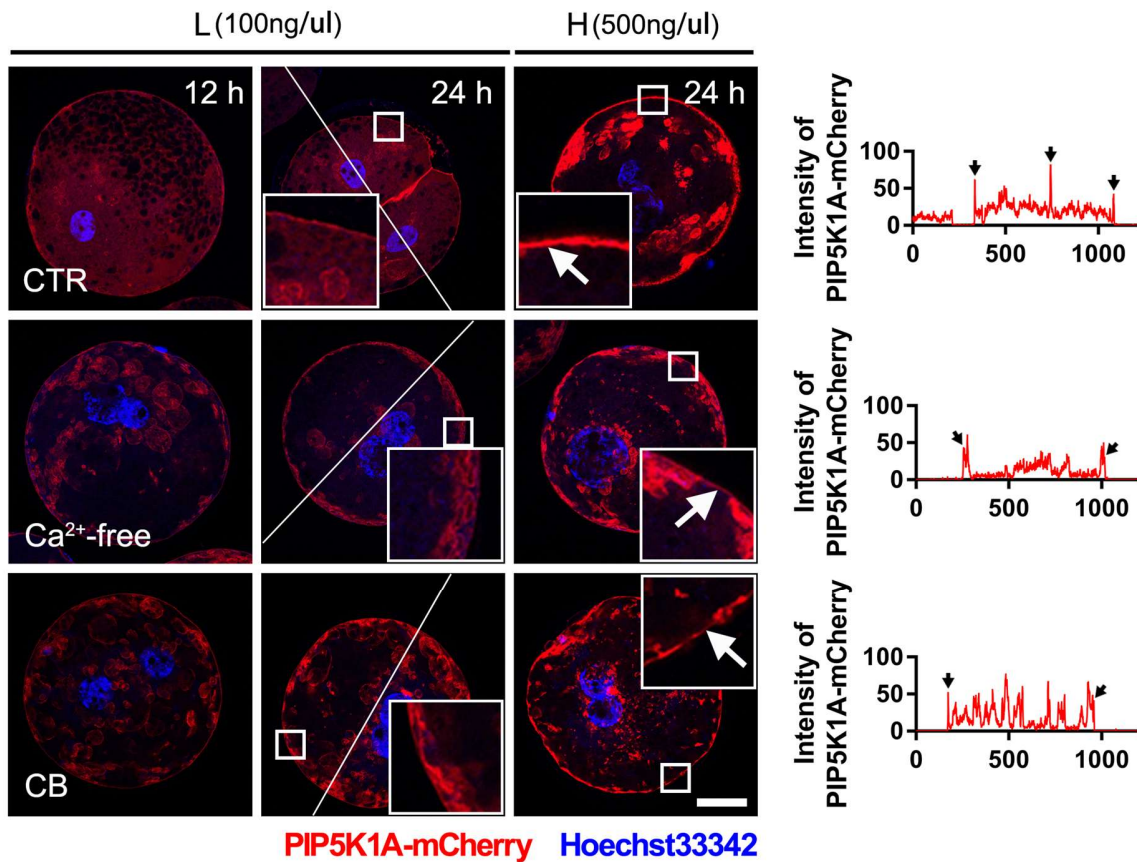


**Figure S6. Effects of *PLC* knockdown on embryo development. (A)** Porcine embryo development on day 2. **(B)** Porcine embryonic development on day 3.



**Figure S7. Regulation of PIP5K1A on RhoA signaling.** (A) The ratio of embryos on day 2 after expression of the PIP5K1A<sup>Q169A</sup> mutant. (B) Western blot for anillin in PIP5K1A KD, PIP5K1A OE, and PIP5K1A mutant groups. (C) Anillin protein levels after PIP5K1A knockdown, overexpression, or inactivation. (D) Western blot for RhoA and ROCK1 in the PIP5K1A KD and PIP5K1A mutant groups. (E) RhoA and ROCK1 protein levels in PIP5K1A KD and PIP5K1A mutant groups. (F) Western blot for anillin and RhoA in different cell fractions following PIP5K1A knockdown. (G) Anillin and RhoA protein levels in different cell

fractions after PIP5K1A knockdown. P1: Membranous precipitate 1. P2: Membranous precipitate 2. S: Cytoplasmic supernatant.



**Figure S8. Localization of PIP5K1A in different treatment groups.** PIP5K1A-mCherry was microinjected into 1-cell embryos, and then checked at 12 h and 24 h. L, low concentration of microinjected PIP5K1A-mCherry (100 ng/uL); H, high concentration of microinjected PIP5K1A-mCherry (500 ng/uL). The white arrow indicates the plasma membrane. In the right graphs, the intensity of PIP5K1A-mCherry was tested at the line in the L group (24 h); the dotted lines indicate the position of the plasma membrane, and the black arrow indicates the plasma membrane. Scale bar = 25  $\mu$ m.

**Table S1.** Primers used in the RT-PCR and vector construction.

Gene name	Sequence (5'→3')	Application
<i>PIP5K1A</i>	GACAACAAAGGCGGAAGTG	RT-PCR
	GGTGAGAAATAAGGGTCAGGAAT	
	GAATTAATACGACTCACTATAGGGAGACACTA	Production of dsRNA
	CAATGACTTTCGGTTCA	
	GAATTAATACGACTCACTATAGGGAGACGGCT	
	GGATACTGTGCTT	
	CAAGTCCGGAATGGCATCTGAGGTGCTTTATG	Production of cRNA
	TGAGCTCGAGTCAGTGGATAAACTCTGACTCT	
	GTAAAAT	
	TGGACGAGCTGTACAAGTCCGGAATGCTGTGC	
	TGTATGAGAAGAACCAAACAGGTTGAAAAAA	Production of Q169A-mutated cRNA
	ATGATGAGGACCAAAGATTATGGCATCTGAG	
	GTGCTTTATG	
	CCGAGGGTTCGCGTTGAGGTTTCATGTAGTATC	
	CTGG	cRNA
	AACCTCAACGCGAACCCCTCGGACTTTGCTG	
	AGAATTCGAAGCTTGAGCTCGAGTCAGTGGAT	
	AAACTCTGACTCTG	
	GGCTTTGTAGGTTGAGCCGGCGAGGTCATATT	cRNA
	TGATATGCATCTTGAC	

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	GCCGGCTCAACCTACAAAGCCCGGGCTTCTCA	Production of
	GAAAGAGC	PIPB-mutated
		cRNA
	CAAGTTGTTAACAAACCTGTAAGACTGTAGAA	Production of
	TGT	LOOP-mutated
	TTACAGGTTTGTTAACAACCTGGAGCACTCTT	cRNA
	GGAAAGC	
	GACCACAATTCATCTTCTTCCTT	RT-PCR
	CGTCAAGCACAGAGGCATT	
	GAATTAATACGACTCACTATAGGGAGATCACA	
<i>PIP5K1B</i>	GAGAACCCAGACACAA	Production of
	GAATTAATACGACTCACTATAGGGAGACGTCA	dsRNA
	AGCACAGAGGCATT	
	CATGCAATGTGGAGATAGTGGT	RT-PCR
<i>PIP5K1C</i>	GCGTCCTCCTCGTCTGAG	
	AGGAAGAAGTGGAGTGTGAAGAG	RT-PCR
<i>PIP4K2A</i>	GGAGCTGTTGAGCGTGTTT	
	CCCAAGAAGGAGGTTTATTTCA	RT-PCR
<i>PIP4K2B</i>	CAGGGTTCACAGTCGAGATTT	
	CCCTGGAGAGTTTGAATCCTT	RT-PCR
<i>PIP4K2C</i>	GCGTGAGCTGCCTTCTTC	
	AAAGACAAGGCCAACCGATA	RT-PCR
<i>PTEN</i>	GGATCAGAGTCAGTGGTGTCAG	

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	GAATTAATACGACTCACTATAGGGAGACCCTCT	
	TACTGCCTCCAAC	
	GAATTAATACGACTCACTATAGGGAGAGCTGTG	
	GTGGATTATGGTCT	Production of
	GAATTAATACGACTCACTATAGGGAGAAAAGG	dsRNAs
	GACGAACTGGTGTAATG	
	GAATTAATACGACTCACTATAGGGAGACTGGAT	
	TTGATGGCTCCTCTAC	
	CAAGTCCGGACACCTCCCGCTCCTGGAG	Production of
	TGAGCTCGAGTCAGACTTTTGTAATTTGTGTAT	cRNA
	GCTGATCTTC	
	GACGAGCTGTACAAGTCCGGAATGGACTCAG	Production of
<i>PLCD1</i>	GCCGGGACTTC	cRNA for PH
	GAATTCGAAGCTTGAGCTCGAGTTATCACATT	domain
	CCCTGAAGATCTTCCG	
	GACGAGCTGTACAAGTCCGGAATGGAGGGGGT	Production of
<i>PLEKHA3</i>	TCTGTAC	cRNA for PH
	GAATTCGAAGCTTGAGCTCGAGTTACTAAGTC	domain
	CTAGTATCAGTCAAACAGG	
	GACGAGCTGTACAAGTCCGGATTCAACCCCGA	Production of
<i>GRP1</i>	CCGGGAG	cRNA for PH
	GAATTCGAAGCTTGAGCTCGAGTCAACTGATA	domain
	CTGGCTCTGATGGACTTCATC	

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	TGGACGAGCTGTACAAGTCCGGAGTCGGCAAC	
<i>VAMP2</i>	CGCTGCGAC	Production of
	AGAATTCGAAGCTTGAGCTCGAGTCAGGGGG	cRNAs
	GAAACCTCTCCAAC	

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**Table S2. Concentrations of microinjected RNAs and their purposes.**

Name	Concentration (ng/ $\mu$ L)	Purpose
	120	Localization
<i>PIP5K1A-mCherry</i> cRNA	200	Overexpression
	500	
<i>PIP5K1A(Q169A)-mCherry</i> cRNA	500	Overexpression
<i>PIP5K1A(PIPBA<math>\Delta</math>)-mCherry</i> cRNA	500	Overexpression
<i>PIP5K1A(LOOP<math>\Delta</math>)-mCherry</i> cRNA	500	Overexpression
<i>PIP5K1A</i> dsRNA	1350	Knockdown
<i>PIP5K1B</i> dsRNA	1250	Knockdown
<i>PTEN-mCherry</i> cRNA	450	Localization
<i>PTEN</i> dsRNA	1280	Knockdown
<i>PH<sub>PLCD1</sub>-mCherry</i> or <i>-eGFP</i> cRNA	550	Localization
<i>PH<sub>PLEKHA3</sub>-mCherry</i> or <i>-eGFP</i> cRNA	450	Localization
<i>PH<sub>GRP1</sub>-mCherry</i> cRNA	500	Localization
<i>VAMP2-mCherry</i> or <i>-eGFP</i> cRNA	370	Localization
<i>Utrophin-mCherry</i> cRNA	200	Overexpression
	600	
<i>UtrCH-mCherry</i> cRNA	560	Localization
<i>mCherry</i> cRNA	50	Localization
	450	Overexpression
<i>eGFP</i> cRNA	50	Localization
	500	Overexpression

**Movie S1. Dynamics of PH<sub>PLCD1</sub>-eGFP and UtrCH-mCherry in oocytes and embryos. (A)**

Dynamic changes of PH<sub>PLCD1</sub>-eGFP and UtrCH-mCherry in oocytes and CB-treated or CB-free embryos when live imaging started at 3 h after microinjection. **(B)** Increased levels of exogenous UtrCH-mCherry induce the formation of a dynamic structure of PH<sub>PLCD1</sub>-eGFP. Embryos in video Ba were obtained 3 h after they were microinjected with 100 ng/μL UtrCH-mCherry, but embryos in videos Bb and Bc were obtained 3 h and 12 h after they were microinjected with 500 ng/μL UtrCH-mCherry. Dots and bubbles in Bb exhibited polar movement toward one site on the membrane. A long time in the Bc resulted in the nucleation of large fibers with weak dynamics in PH<sub>PLCD1</sub>-eGFP and UtrCH-mCherry. **(C)** Nuclei with PH<sub>PLCD1</sub>-eGFP and UtrCH-mCherry formed and their polar movement toward one site at the membrane when live imaging started 6 h after microinjection. **(D)** PH<sub>PLCD1</sub>-eGFP and UtrCH-mCherry aggregate at the cleavage furrow during cytokinesis. However, the cytokinesis-flattened embryos were incomplete. **(E)** Fragmented embryos exhibited phenotypes similar to cytokinesis in S1D. **(F)** Overgrowth of nuclei with PH<sub>PLCD1</sub>-eGFP and UtrCH-mCherry did not trigger deepening compared with that in Movie S1D. Scale bar = 25 μm. Time showed as “minutes: seconds”.

**Movie S2. Effects of *PIP5K1A* knockdown on dynamics of PH<sub>PLCD1</sub>-eGFP and UtrCH-**

**mCherry. (A)** Bubble formation of PH<sub>PLCD1</sub>-eGFP and UtrCH-mCherry in embryos when live imaging started 3 h after microinjection. Internalized bubbles were reduced in the *PIP5K1A* knockdown group. **(B)** Formation of nuclei with PH<sub>PLCD1</sub>-eGFP and UtrCH-mCherry when live imaging was initiated 12 h after microinjection. These nuclei were reduced in the *PIP5K1A* knockdown group. Scale bar = 25 μm. Time showed as “minutes: seconds”.

**Movie S3. Effects of exogenous *PIP5K1A* overexpression on PI4P and PIP2.** (A) The dynamics of PH<sup>PLEKHA3</sup>-eGFP in oocytes and embryos of the exogenous PIP5K1A-mCherry overexpression group. (B) The dynamics of PH<sup>PLCD1</sup>-eGFP in oocytes and embryos of the exogenous PIP5K1A-mCherry overexpression group. (C) Control group for the exogenous PIP5K1A-mCherry overexpression group in videos S3A and S3B. The dynamics of PH<sup>PLEKHA3</sup>-mCherry and PH<sup>PLCD1</sup>-Egfp are shown at the interphase (Movie S3Ca) and during cytokinesis (Movie S3Cb). (D) Internalization of PH<sup>PLCD1</sup>-eGFP from the plasma membrane into the cytoplasm, triggered by excess exogenous PIP5K1A-mCherry, resulted in the loss of contractile force, expansion of the plasma membrane, and subsequent death of the embryo. Scale bar = 25  $\mu$ m. Time showed as “minutes: seconds”.

**Movie S4. Mutation of exogenous *PIP5K1A* on PIP2.** (A) Dynamics of PH<sup>PLCD1</sup>-eGFP in embryos of the PIP5K1A<sup>K365N/K366N</sup>-mCherry expression group at interphase. (B) Dynamics of PH<sup>PLCD1</sup>-eGFP in embryos of the PIP5K1A<sup>K365N/K366N</sup>-mCherry expression group during cytokinesis. (C) The effect of excess PIP5K1A<sup>K365N/K366N</sup>-mCherry on the dynamics of PH<sup>PLCD1</sup>-eGFP. PH<sup>PLCD1</sup>-eGFP changed from dots to bubbles, aggregated, and moved into the cytoplasm. (D) PIP5K1A<sup>K365N/K366N</sup>-mCherry and PH<sup>PLCD1</sup>-eGFP aggregated at one position on the plasma membrane. Scale bar = 25  $\mu$ m. Time showed as “minutes: seconds”.

**Movie S5. Regulation of PIP5K1A<sup>K365N/K366N</sup>-mCherry on cytokinesis.** (A) PIP5K1A<sup>K365N/K366N</sup>-mCherry aggregated in the plasma membrane during anaphase. (B) Aggregated PIP5K1A<sup>K365N/K366N</sup>-mCherry initiated cleavage furrow and recruitment of the

residual spindle. (C) Aggregated PIP5K1A<sup>K365N/K366N</sup>-mCherry did not capture spindles during abnormal cytokinesis. Scale bar = 25  $\mu\text{m}$ . Time showed as “minutes: seconds”.