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Protein deubiquitination during oocyte maturation influences sperm function during fertilization, anti-polyspermy defense and embryo development

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Supplementary Material

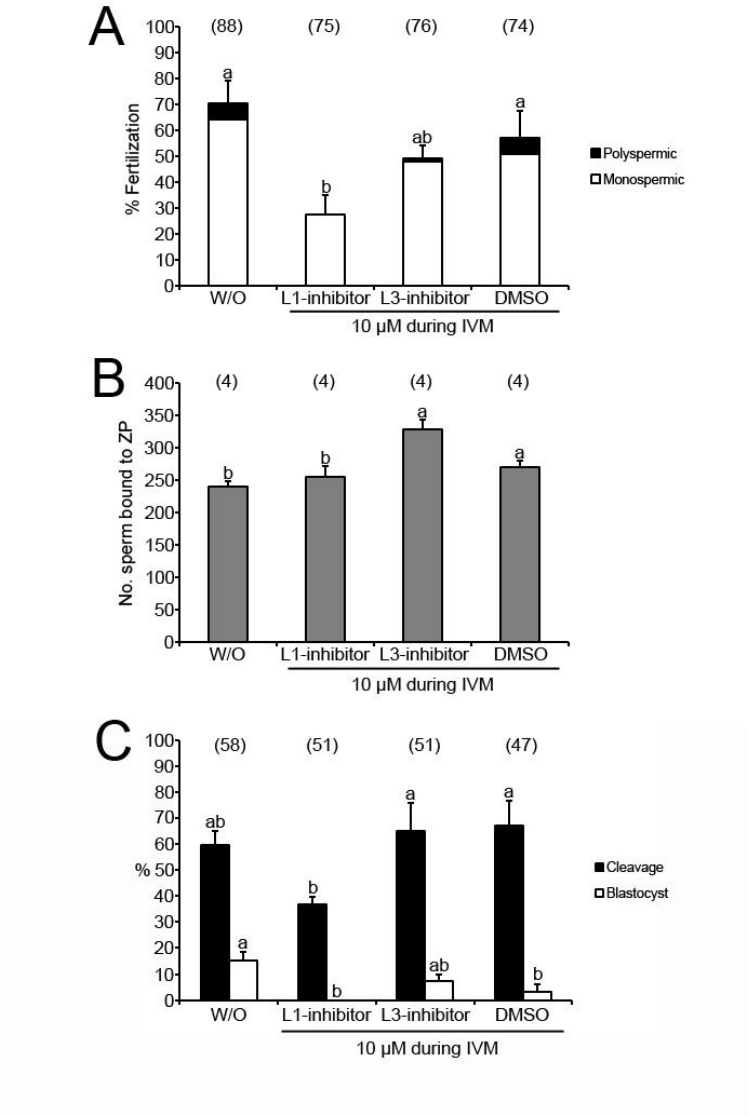

Fig. S1. Fertilization and embryo development of porcine oocytes matured in the presence/absence of 10 µM L1-inhibitor or L3-inhibitor. (**A**) Oocytes matured in the presence of L1-inhibitor or L3-inhibitor, were fertilized for 6 hrs, and examined to assess fertilization rates. Diagram indicates % monospermic (□) and % polyspermic (■) fertilization. (**B**) Oocytes were incubated with $1\times x10^5$ spermatozoa/ml in the presence of L1-inhibitor or L3-inhibitor for 30 min. The number of spermatozoa bound to ZP was counted. (**C**) Embryo development of porcine oocytes matured with 10 µM L1-inhibitor or L3-inhibitor. Diagram indicates % cleaved embryos (■) and % blastocysts (□). Each experiment was repeated four times. All values are expressed as the mean ± SEM. Different superscripts a & b in each diagram denote a significant difference at $p<0.05$. Numbers of cultured/inseminated ova are indicated in parentheses.

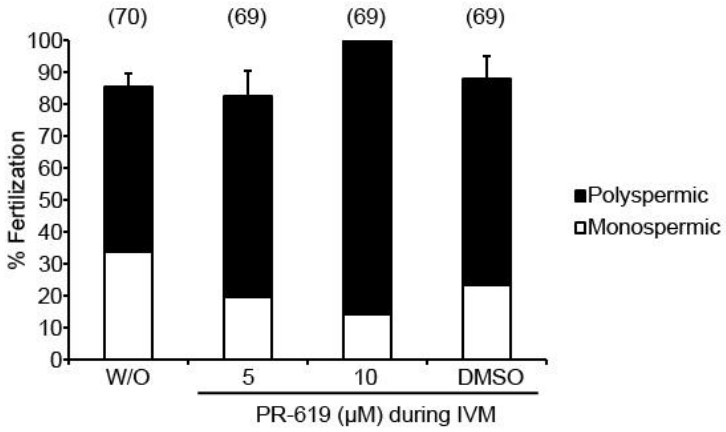

Fig. S2. Effect of general deubiquitinase inhibitor PR-619 on porcine IVM/IVF. Oocytes were cultured in the presence/absence of PR-619 for the entire IVM period. After IVM, only the oocytes that reached MII were selected and fertilized for 6 hrs. Diagram indicates % monospermic (□) and % polyspermic (■) fertilization. Values are expressed as the mean percentages ± SEM. Each experiment was repeated three times. Numbers of oocytes are indicated in parentheses.

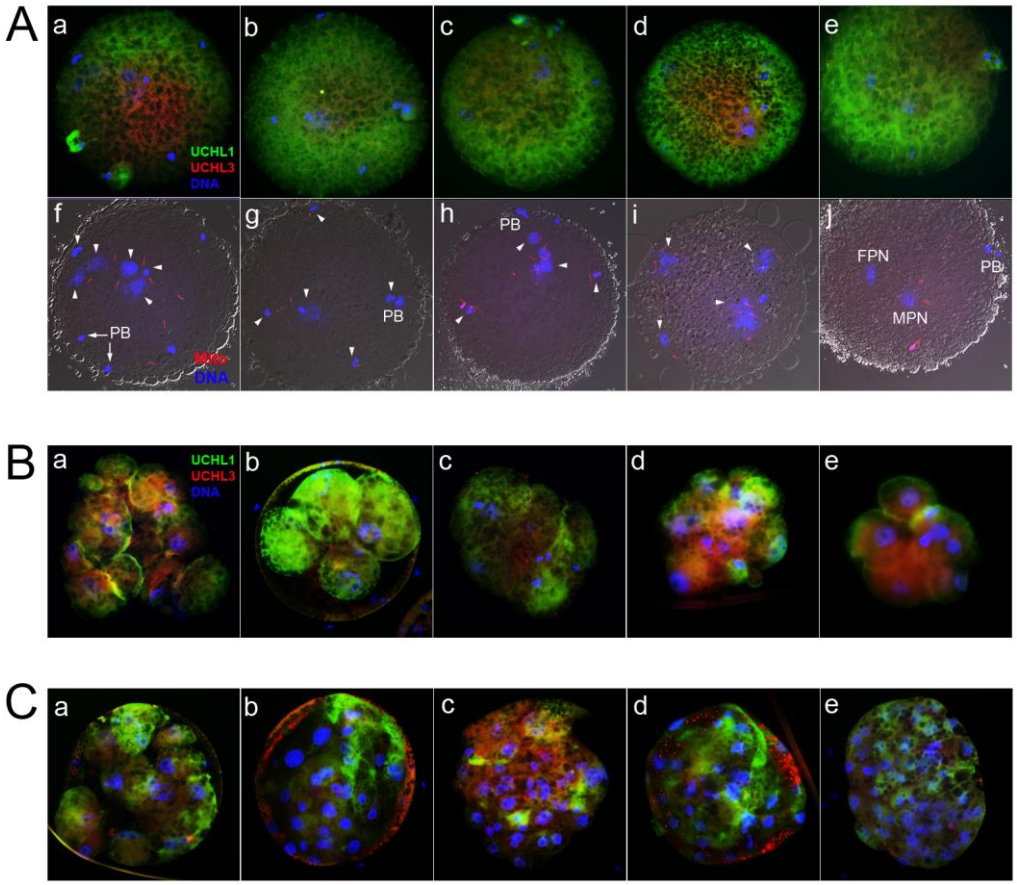

Fig. S3. Localizations of UCHL1 and UCHL3 in the fertilized oocytes, embryos and blastocysts. (**A**) Sperm incorporation and pronucleus formation in the oocytes matured with DUB inhibitors after IVF. Oocytes were matured in the absence (a)/presence of 20 μM L1-inhibitor (b), 100 μM L3-inhibitor (c), 10 μM PR-619 (d) or DMSO (e; vehicle control) and then fertilized. UCHL1, UCHL3 and DNA were labeled with GAM-IgG-FITC (green), GAR-IgG-Cy5 (red) and DAPI (DNA), respectively (**a-e**). In some experiments, spermatozoa were pre-labeled with MitoTracker® Red CMXRos to detect sperm mitochondria (red; **f-j**). (**B**) Oocytes were matured in the absence (a)/presence of 20 μM L1-inhibitor (b), 100 μM L3-inhibitor (c), 10 μM PR-619 (d) or DMSO (e), fertilized, and cultured. Embryos were fixed and labeled with antibodies after IVC for 48 hrs (a-e). (**C**) Embryos were cultured in the absence (a)/presence of 5 μM L1-inhibitor (b) or 5 μM L3-inhibitor (c). However, no blastocyst developed from oocytes cultured in the presence of PR-619. A blastocyst derived from oocytes matured with 5 μM PR-619 (d), and a blastocyst cultured in the presence of vehicle, DMSO (e) is shown. Original magnification ×400.