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Supplementary Material

Induction of autophagy promotes porcine parthenogenetic embryo development under low oxygen conditions

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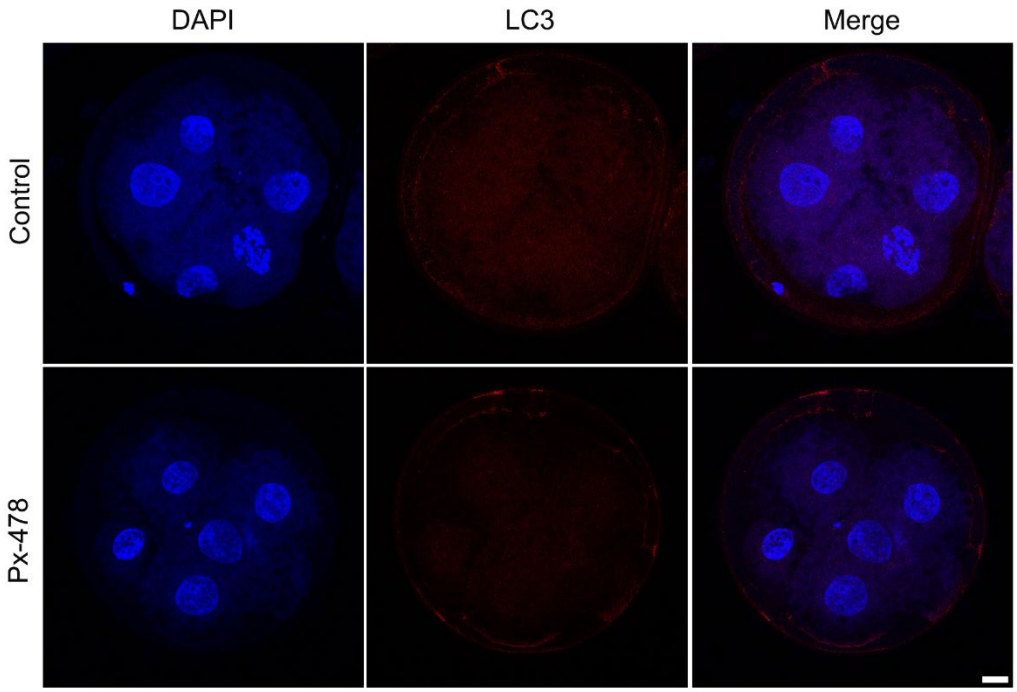

Figure S1. Low oxygen induced HIF-1α expression promotes autophagy during embryonic development. The effect of Px-478 on autophagy in pig PA embryos (48h after activation). Immunofluorescence of embryos was imaged using anti-LC3 (red) and DAPI (blue). Bar = 100 μm.

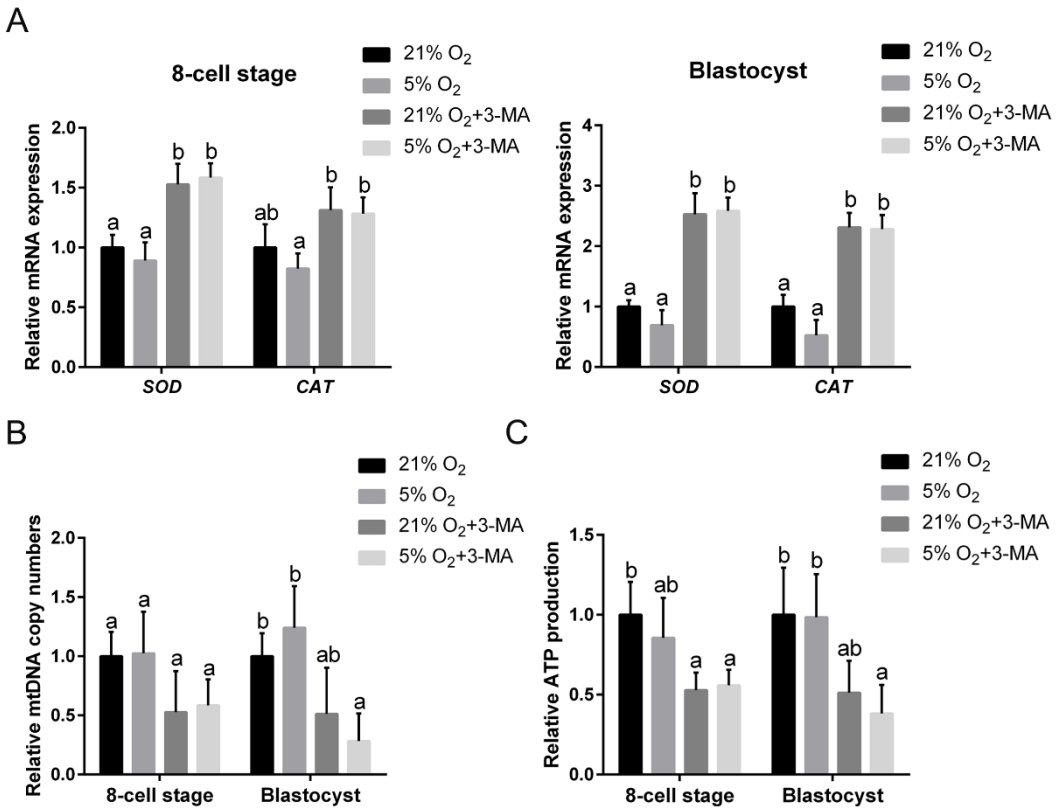

Figure S2. Low oxygen induced autophagy regulates ROS level during embryonic development. (A) The effect of low oxygen induced autophagy on redox state. The anti-oxidative related genes were determined by real time qPCR. The data are means ± S.E.M. (B) The effect of low oxygen induced autophagy on mtDNA copy numbers. The relative mtDNA copy numbers were detected by real time qPCR and normalized to the amount of β-globin. (C) The effect of low oxygen induced autophagy on ATP production. ATP level was detected as described in material and method section. The ATP experiments were conducted in triplicate, and the relative expression data were normalized to embryo number per sample. Data are presented as means ± S.E.M of three independent experiments. Different lowercase letters represent the difference of expression levels that are significant ($p < 0.05$).

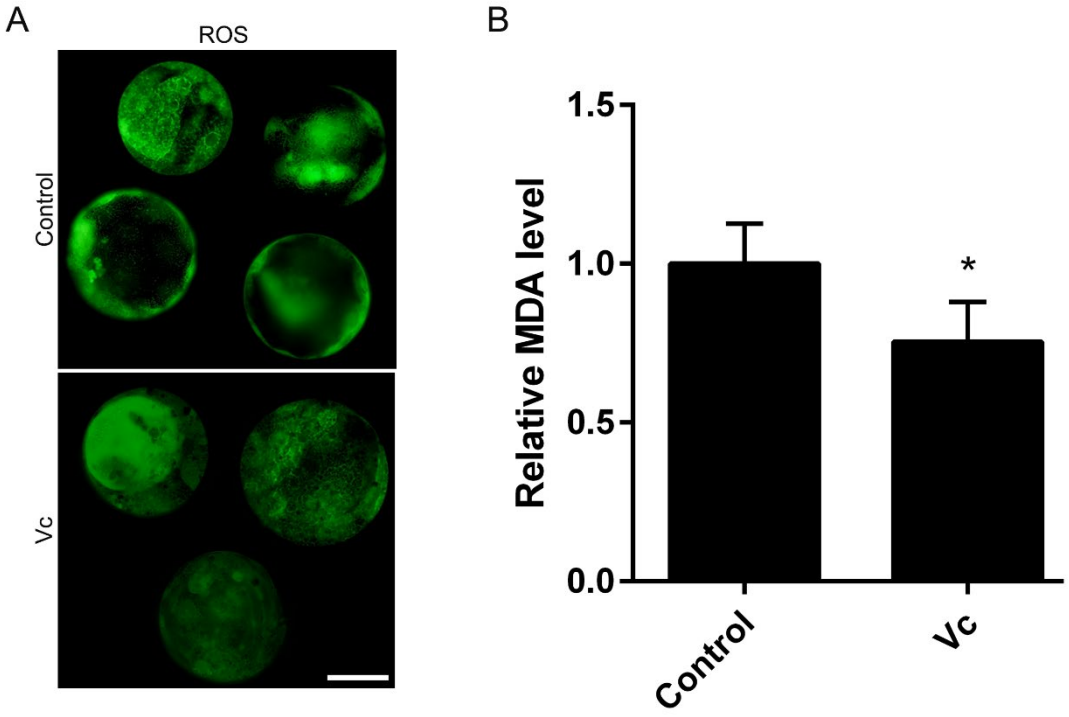

Figure S3. Vc downregulates oxidative stress during embryonic development. (A) Vc decreased the ROS level. Immunofluorescence of embryos was imaged using DCHF-DA (green). Bar = 100 μm. (B) Embryos were treated with Vc, the MDA level was detected as described in material and methods. MDA level was determined and calculated relative to that of the control group. Data are presented as means ± S.E.M of three independent experiments. * $p < 0.05$.

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Table S1. Primer sequences used in real-time PCR

Primer ID	Forward primer sequence (5'-3')	Reverse primer sequence (5'-3')
ATG5	CAC TGC CTC CTG TGT CTT CA	CAC TGC CTC CTG TGT CTT CA
ATG12	TCT CCC CAG AAA CTG CCA CT	GGT TCG CTC TAC AGC CCA TT
BECN1	TTC GCG TCT CCT GAA TCA CCT GTA	AGT GCC TAA CTC AAG GCT GCA GAT
ATG7	CGG ATG GTG AAC CTC AGC GA	CAT ACA GCG GCT GCC TCA CAG
BNIP3	TCC TGG GTA GAA CTG CAC TTC	GCT GGG CAT CCA ACA GTA TTT
OCT4	GAA GGT GTT CAG CCA AAC GAC	CGA TAC TTG TCC GCT TTC
SOX2	AAC CAG AAG AAC AGC CCA GAC	TCC GAC AAA AGT TTC CAC TCG
NANOG	CCT CCA TGG ATC TGC TTA TTC	CAT CTG CTG GAG GCT GAG GT
KLF4	CAG TTC GGC TAT AAC ACT GGT G	GCC CCC GAC AGA GAA GAT G
eIF1A	GGT GTT CAA AGA AGA TGG GCA AGA G	TTT CCC TCT GAT GTG ACA TAA CCT C
CAT	CTT CTC CGT CGT GTT CTC TG	GTC CAG AAG AGC CTG AAT GC
SOD	GAG ACC TGG GCA ATG TGA CT	CTG CCC AAG TCA TCT GGT TT
EGFP	TGA ACC GCT CGA GCT GAA GGG	TCC AGC AGG ACC ATG TGA TCG C