Supplementary Information

Supplementary Figures:

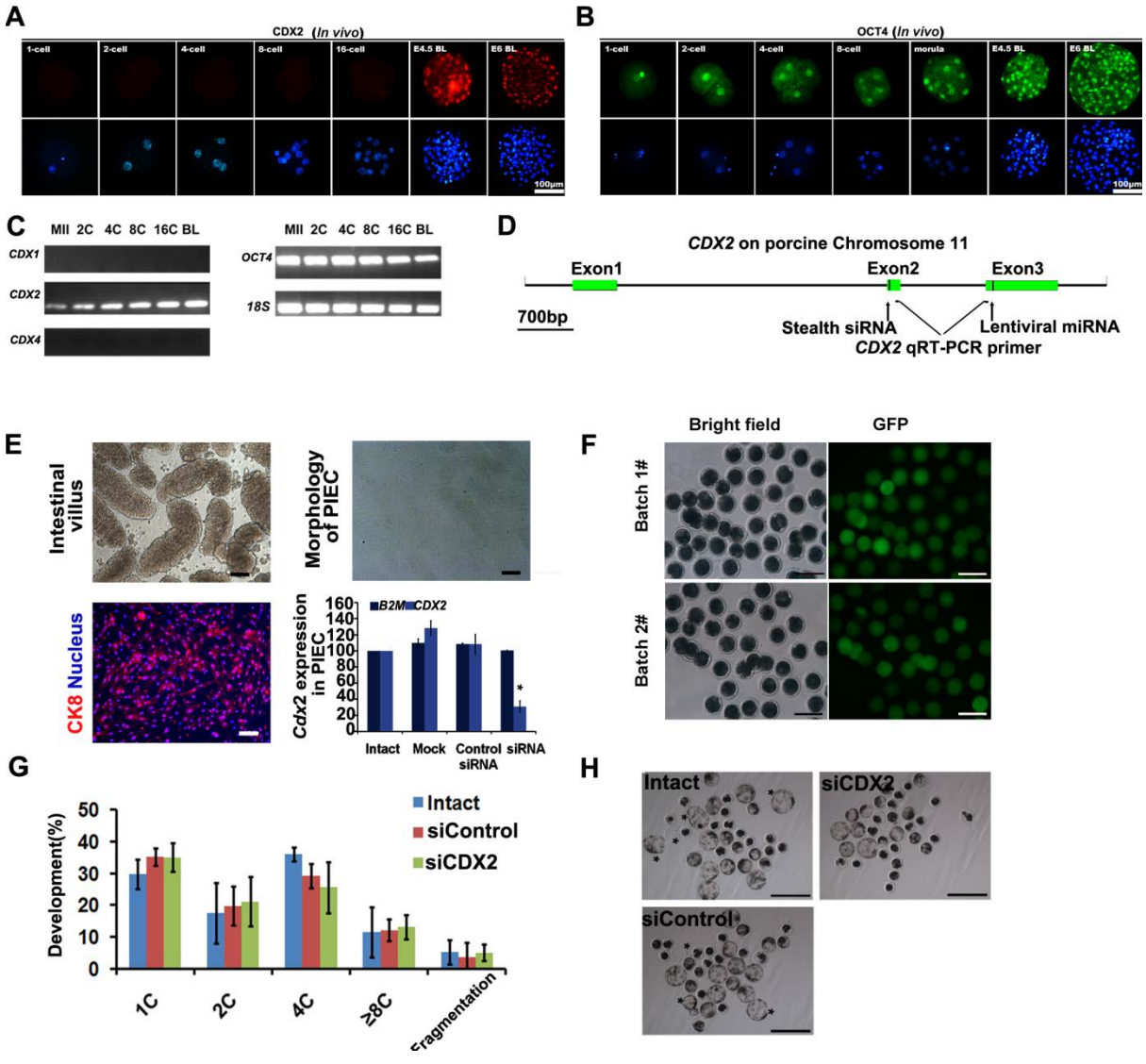

Fig. S1 The effects of *CDX2* knockdown on porcine embryonic development. (A-B) IF assays shows the *in vivo* expression pattern of (A) OCT4 (green) and (B) CDX2 (red). (C) RT-PCR proved the *CDX2* and *OCT4* mRNA expression throughout the development and the absence of *CDX1* and *CDX4* expression in porcine early stage embryos. (D) Illustration of locus targeted by CDX2 interfering tools and primers for qPCR. This study used two methods to knockdown *CDX2*: Stealth siRNA injection in zygotes and miRNA- expressing lentivirus mediated CDX2 knockdown. “Stealth siRNA” and “Lentiviral miRNA”

labeled their target locus. (E) Stealth siRNA also could effectively repress CDX2 expression in porcine intestinal epithelial cells (PIEC). Bar, 100μm. (F) Injection of GFP mRNA into porcine zygotes has shown that our injection efficiency is close to 100%. Bar, 100μm. (G) The morphology of embryos at D6.5. Bar, 500μm. (H) Embryonic development at D3.

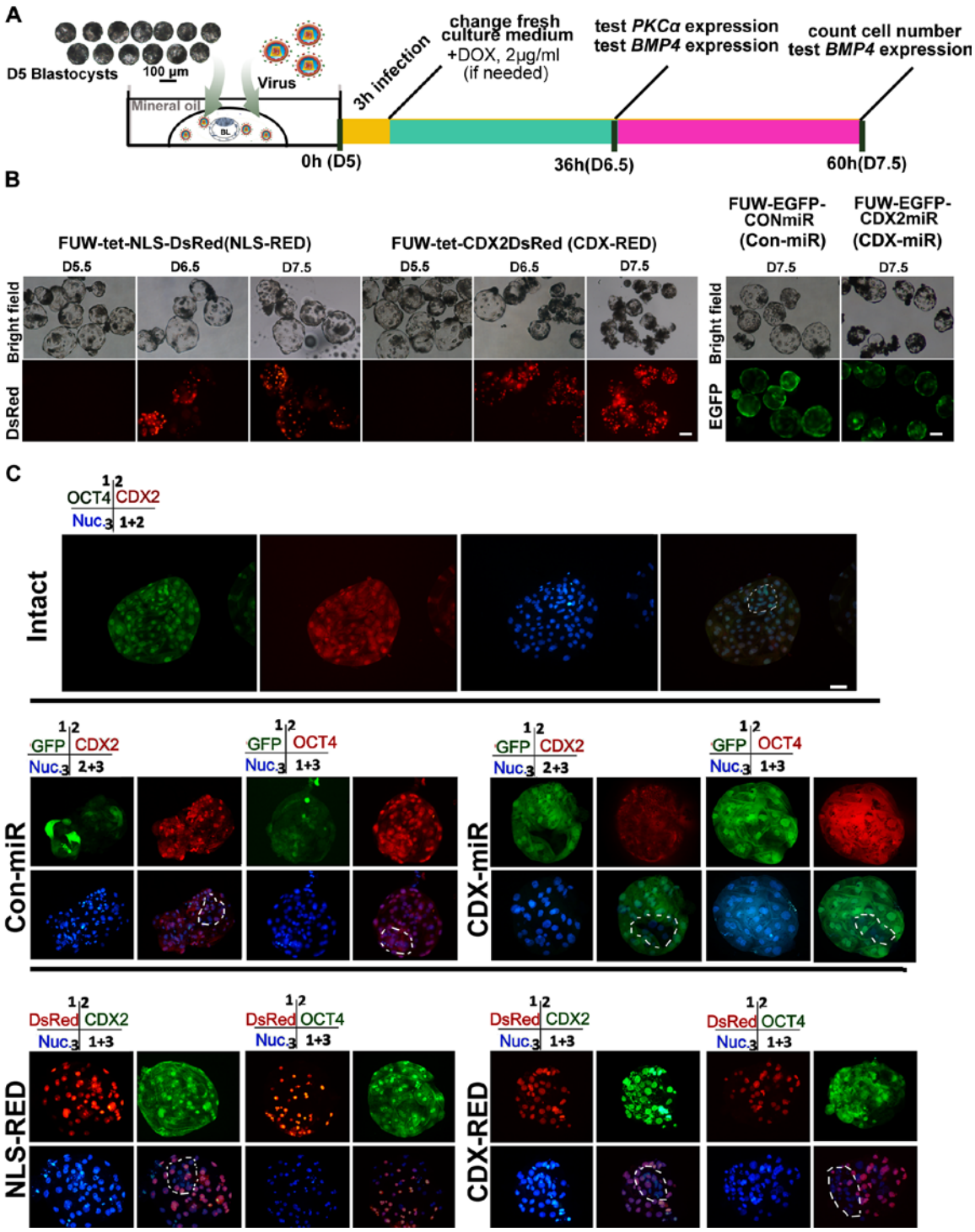

Fig. S2 Lentivirus mediated TE specific *CDX2* regulation. (A) The procedure of lentivirus infection and following experiments. (B) The status of blastocysts after lentivirus transfection. (C) IF assay shows the CDX2 and OCT4 expression after TE specific lentivirus infection. Bar, 50μm.

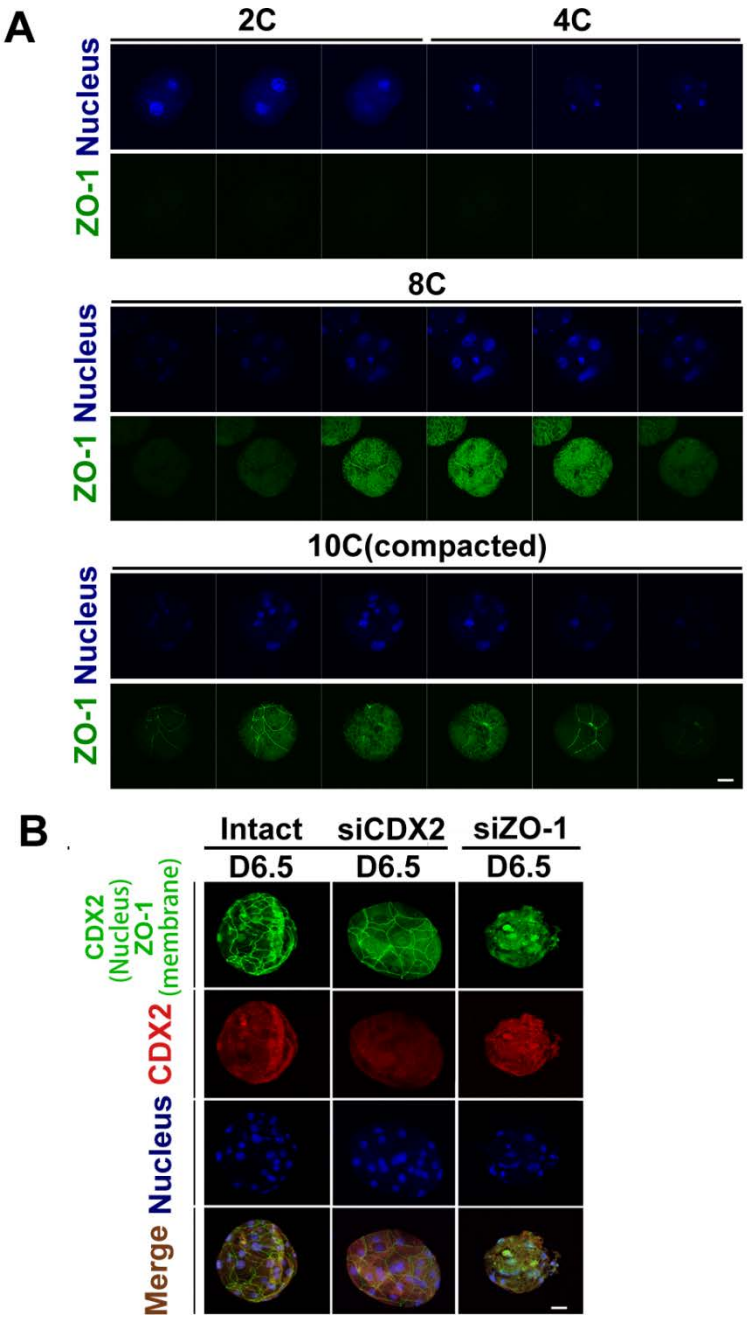

Fig. S3 CDX2 expression and the tight junction formation are independent events. (A) IF assay against ZO-1 in cleavage stage porcine embryos indicates that the formation of tight junction in porcine embryos is earlier than the CDX2 accumulation. (B) IF results show that the formation of tight junction (marked by ZO-1) and CDX2 expression are independent, because RNA interference against each of them does not affect the another one. Bar, 50μm.

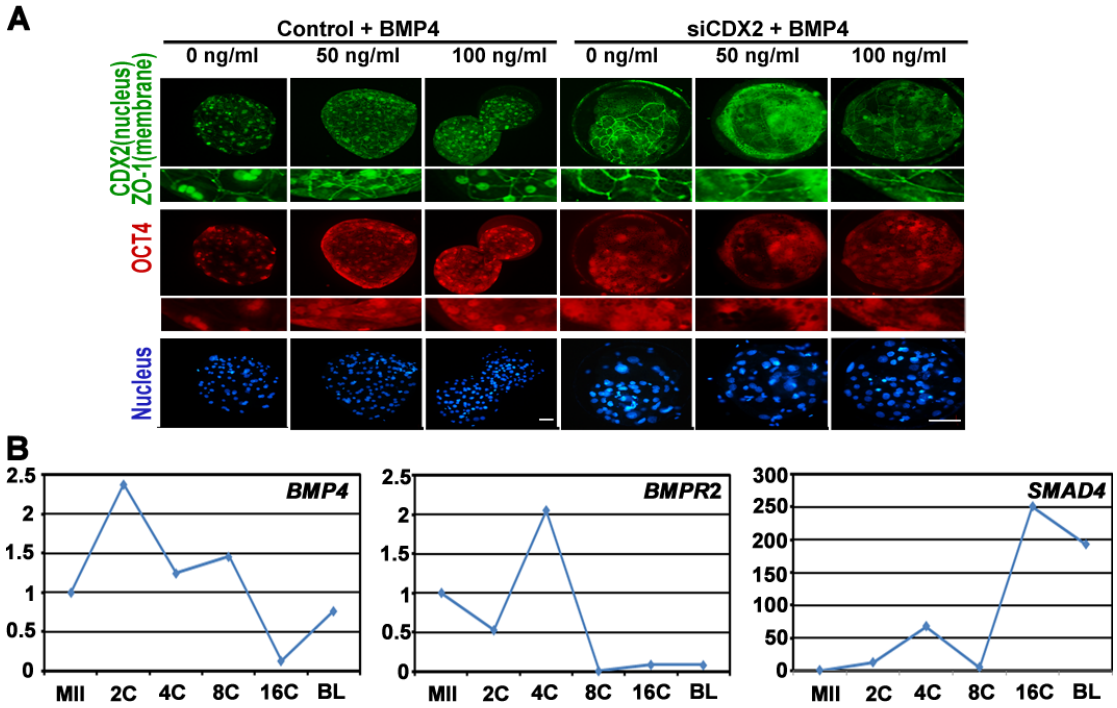

Fig. S4 BMP4 signaling is active in porcine blastocysts. (A) IF assay was used to calculate the blastocyst cell numbers after BMP4 supplement and at the same time prove CDX2 absence in siCDX2 blastocysts. Bar, 50μm. (B) The mRNA expression patterns of *BMP4*, *Smad4* and *BMPR2* throughout early porcine embryo.

Supplementary Tables:

Table S1. Primers used for qPCR

Gene	GeneBank No.(Ensemble ID)	Primer pairs
CDX2	AM778830	F: 5'AGTCGCTACATCACCATTCGGAG3' R: 5'GCTGCTGTTGCTGCAACTTCTTC3 '
POU5F1	NM_001113060	F: 5'GAAGCTGGACAAGGAGAAGCTGGAG3 ' R: 5'ATGGTCGTTTGGCTGAACACCTTC3 '
NANOG	AY596464	F: 5'CCTCCATGGATCTGCTTATTC3 ' R: 5'CATCTGCTGGAGGCTGAGGT 3 '
SOX2	NM_001123197	F: 5'AACCAGAAGAACAGCCCAGAC3 ' R: 5'TCCGACAAAAGTTTCCACTCG3 '
GATA4	NM_214293	F: 5'ATGAAGCTCCATGGTGTCCC 3 ' R: 5'ACTGCTGGAGTTGCTGGAAG3 '
GATA6	NM_214328	F: 5'TTGGTTATTCCCGAATTTCTCCG3 ' R: 5'CATTCCTGCAAACTGGGTGATAC3 '
CDH1	NM_001163060.1	F: 5'TGCTGCTCCTGCTCCTTATTCG3 ' R: 5'CTGGTCCTCTTCTCCACCTCCT3 '
ZO-1	AJ318101.1	F: 5'AGTGGCGTTGACACGTTCTCTG3 ' R: 5'ACCACGGTGTGACCATCCTCAT3 '
PRKCA	XM_005668672.1	F: 5'GGAGACAGCCTTCCAACAACCT3 ' R: 5'TGTCGGCGAGCATCACCTTC3 '
ATP1B1	AJ401029.1	F: 5'TGTGCCCAGCGAACTCAAAGAA3 ' R: 5'CCAACCATTCGAGCCTGAACCT3 '
EOMES	XM_003132081.2	F: 5'TGGACTCAATCCTACTGCCCACTAC3 ' R: 5'TTTGCCGCAGGTCACCCACTT3 '
ELF5	NM_001243711.1	F: 5'TCCTCCAGAACATTCGCTCACAAG3 ' R: 5'TGATGAGAACTTTGGAGGCTTGTTC3 '
CDH3	（ENSSSCT00000026686.1）	F: 5'GTCACAGACCAGAACGACCACAAG3 ' R: 5'CATCGTCCTCATCGGTGGCTGT3 '
HAND1	NM_001014428.1	F: 5'CCGAGCTGCGCGAGTGCAT3 ' R: 5'TTGGCCAGCACGTCCATCAGGT3 '
GCM1	XM_001927486.5	F: 5'CCTTTCTCCTCACCTATACCTCTC3 ' R:

Table S2. Antibodies Used for IF and WB assays

Primary antibody			
Immunogen	Source	Dilution	Description
a peptide mapping near the N-terminus of Oct-3/4 of human origin	sc-8628 Santa Cruz	IF:1:50	OCT4, Goat polyclonal IgG
human CDX2 recombinant protein	ab-88129 Abcam	IF:1:50 WB:1:500	CDX2, Rabbit monoclonal IgG
Human E-Caherin	ab-1416 Abcam	IF:1:50	E-CADHERIN, Mouse monoclonal IgG1
Human ZO-1	339188 Invitrogen	IF:1:500	Mouse monoclonal antibody conjugated to Alexa Fluor 488
A synthetic peptide corresponding to internal region of human NANOG	PAB6837 Abnova	IF:1:50	NANOG, Goat polyclonal
a peptide mapping near the C-terminus of Sox-2 of human origin	sc-17320 Santa Cruz	IF:1:100	SOX2, Goat polyclonal IgG
peptide region of the Human ZFP42 protein sequence according to NP_777560	SAB2102769 Sigma	IF:1:100	ZFP42(REX1), Rabbit polyclonal
Human Cytokeratin-18	BM0032 Boster	IF:1:100	CK8, Mouse monoclonal IgG
a peptide from the p17 fragment corresponding to the cleaved region of caspase-3 human caspase-3	G7481 Promega	IF:1:500	Active- CASPASE3, Rabbit polyclonal
synthesized peptide derived from human Catenin-β	SAB4500543 Sigma	IF:1:100	endogenous levels of total CATNB (Catenin-β), Rabbit polyclonal IgG
the C-terminus of PKC ζ of rat origin	sc-216 Santa Cruz	IF:1:50	PKCζ, Rabbit polyclonal IgG
residues surrounding Thr567 of human ezrin,	3149P CST	IF:1:500 WB:1:1000	Phospho-Ezrin (Thr567), Rabbit monoclonal IgG
amino acids 311-586 mapping at the C-terminus of human Ezrin	sc-20773 Santa Cruz	IF:1:500 WB:1:1000	Total Ezrin, Rabbit polyclonal IgG
the C-terminus of PKCα of human origin	sc-208 Santa Cruz	IF:1:500 WB:1:100	PKCα, Rabbit polyclonal IgG

Secondary antibody	
Name	Source
Alexa Fluor 546 Donkey Anti-Rabbit IgG	Molecular Probe A10040
Alexa Fluor 546 Donkey Anti-Mouse IgG	Molecular Probe A10036
Alexa Fluor 546 Donkey Anti-Goat IgG (H+L)	Molecular Probe A11056
Alexa Fluor 488 Donkey Anti-Goat IgG (H+L)	Molecular Probe A11055
Alexa Fluor 488 Doncky Anti-Rabbit IgG (H+L)	Molecular Probe A21206
Alexa Fluor 488 Donkey Anti-Mouse IgG (H+L)	Molecular Probe A21202
Anti-Rabbit IgG (whole molecule) –Peroxidase antibody produced in goat	Sigma A9169
Anti-Mouse IgG (whole molecule) –Peroxidase antibody produced in rabbit	Sigma A9044

Note Commercial antibodies have been tested and found to not work in pig including :

OCT4: sc-8629 Santa Cruz; O8389 Sigma
CDX2: AB4123 Millipore; 3977S Cell Signaling
NANOG: sc-33760 Santa Cruz; ab21624 Abcam
SOX2: ab97959 Abcam
ZFP42: ab50828 Abcam