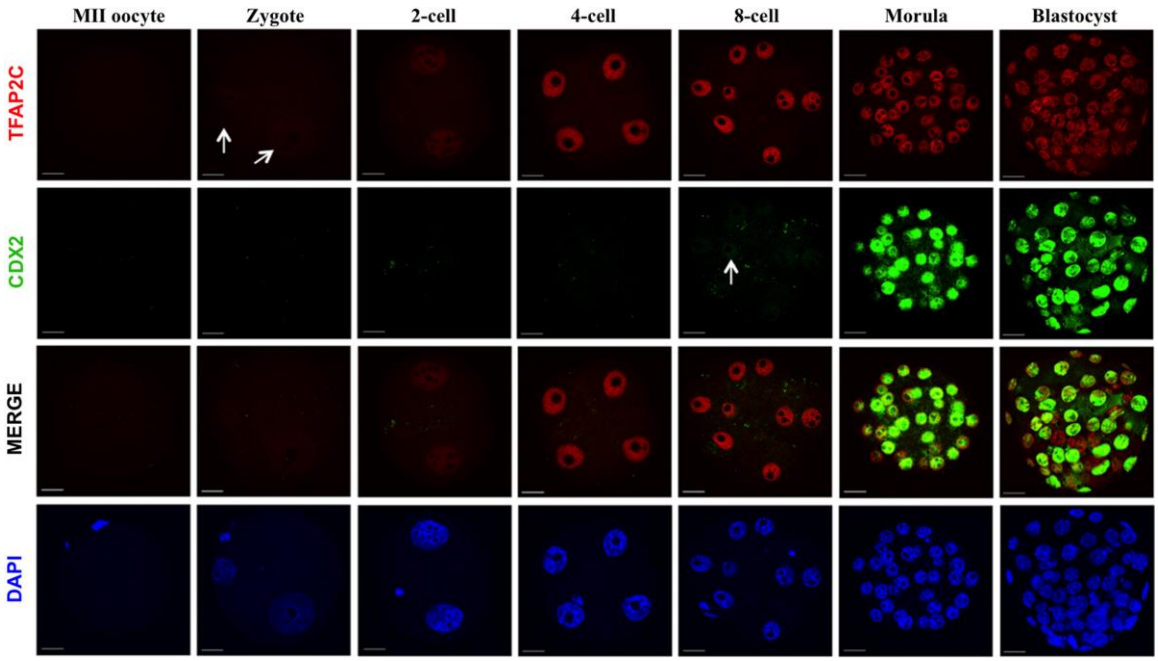

Fig. S1. TFAP2C expression precedes and overlaps CDX2 in preimplantation embryos.

Immunofluorescence analysis of TFAP2C and CDX2 during mouse preimplantation development. TFAP2C protein first appeared at the 1-cell stage, while CDX2 protein first appeared at the 8-cell stage. Nuclei were counterstained with DAPI. Scale bars = 20 μm. A total of two biological replicates were performed.

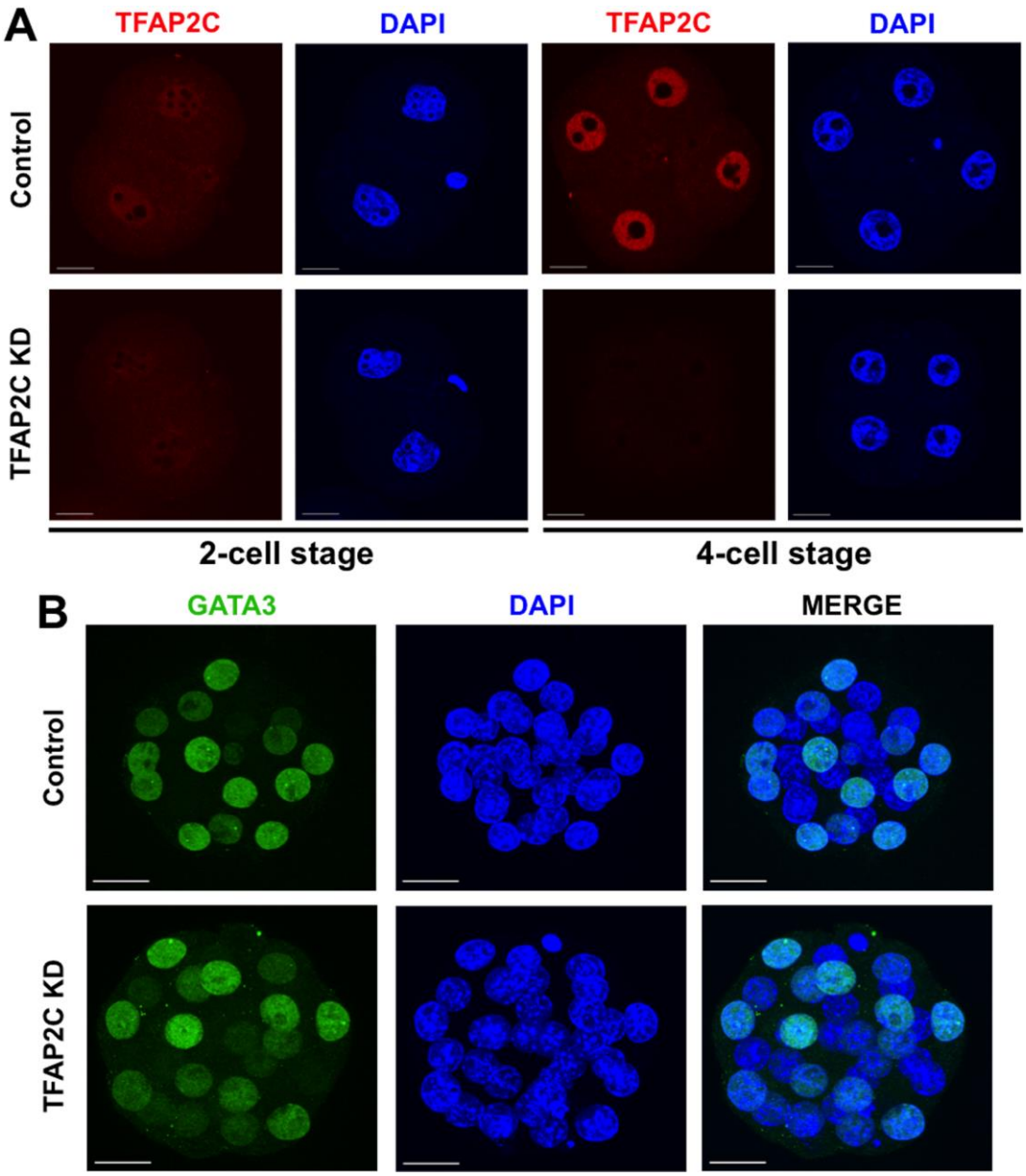

Fig. S2. Temporal reduction in TFAP2C protein following microinjection of *Tfap2c* siRNA and GATA3 expression in TFAP2C KD morulae.

(A) Immunofluorescence analysis of TFAP2C protein in 2-cell and 4-cell embryos from zygotes microinjected with *Tfap2c* siRNA. TFAP2C protein was strongly downregulated by the 4-cell stage. (B) Evaluation of GATA3 protein in TFAP2C KD and control morulae. GATA3 was unchanged in TFAP2C KD embryos. Nuclei were counterstained with DAPI. Scale bars = 20 μm. A total of two biological replicates were performed.

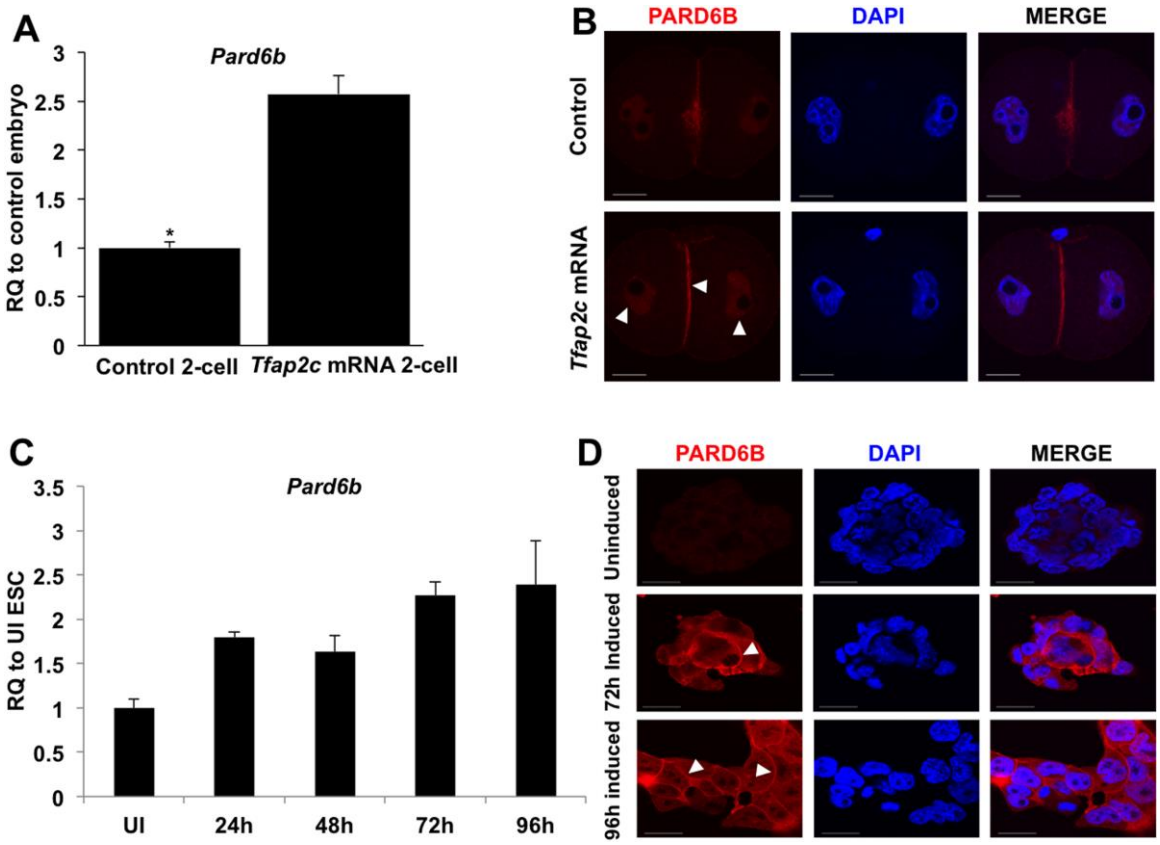

Fig. S3. Overexpression of TFAP2C induces *Pard6b* mRNA and protein during early development.

(A) Real-time qPCR analysis of *Pard6b* expression in 2-cell embryos from zygotes microinjected with 25ng/µl of *Tfap2c* mRNA. (B) Immunofluorescence analysis of PARD6B in 2-cell embryos from zygotes microinjected with 25ng/µl of *Tfap2c* mRNA. Nuclei were counterstained with DAPI. Scale bars = 20 µm. (C) Real-time qPCR analysis of *Pard6b* expression in *Tfap2c*-inducible ESCs. (D) Immunofluorescence analysis of PARD6B in *Tfap2c*-inducible ESCs. Nuclei were counterstained with DAPI. Scale bars = 20 µm.

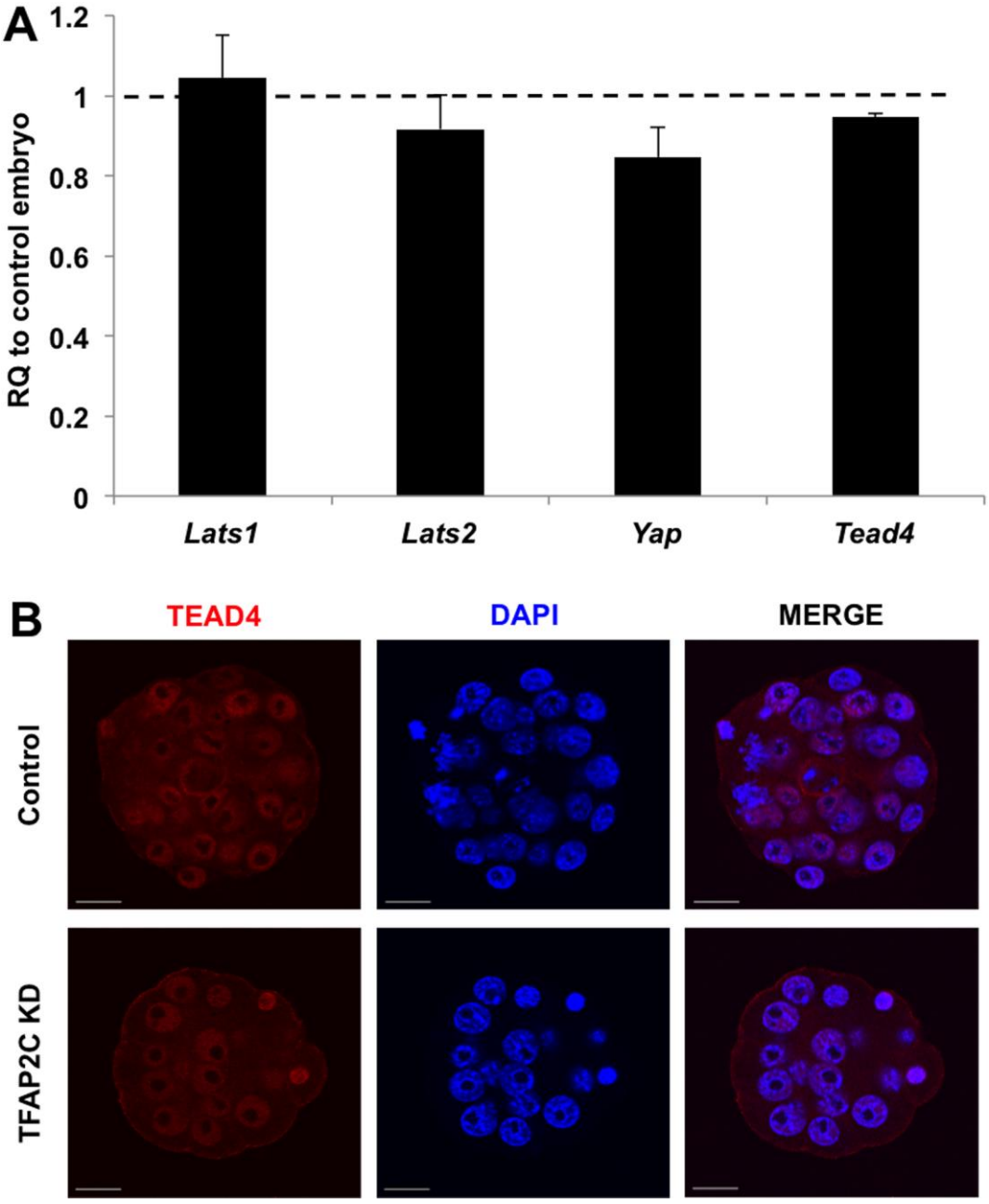

Fig. S4. TFAP2C does not directly regulate the expression of key HIPPO signaling pathway members.

(A) Real-time qPCR analysis of *Lats1, Lats2, Yap, Tead4* transcripts in TFAP2C KD morula. Expression is relative to control embryos. RQ, relative quantification. (B) Immunofluorescence analysis of TEAD4 in TFAP2C KD and control morulae. Nuclei were counterstained with DAPI. Scale bar = 20 μm. A total of two biological replicates were performed using 24-28 embryos per group.

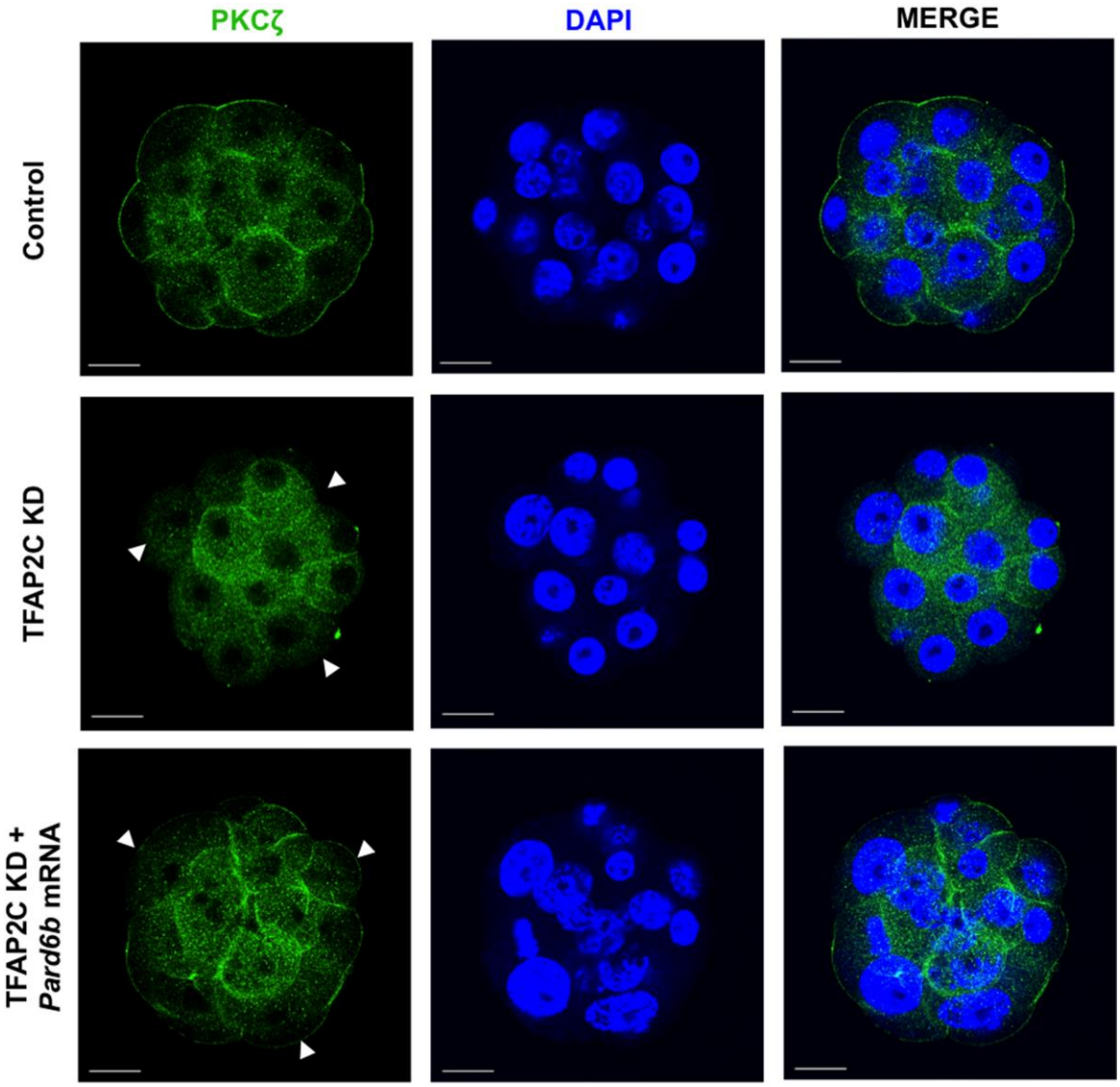

Fig. S5. PARD6B rescue can restore PKCζ localization to the apical region in TFAP2C KD embryos.

Immunofluorescence analysis of PKCζ localization in TFAP2C KD, PARD6B rescued TFAP2C KD, and control morulae. Nuclei were counterstained with DAPI. Scale bar = 20 μm. A total of two biological replicates were performed using 20-24 embryos per group.

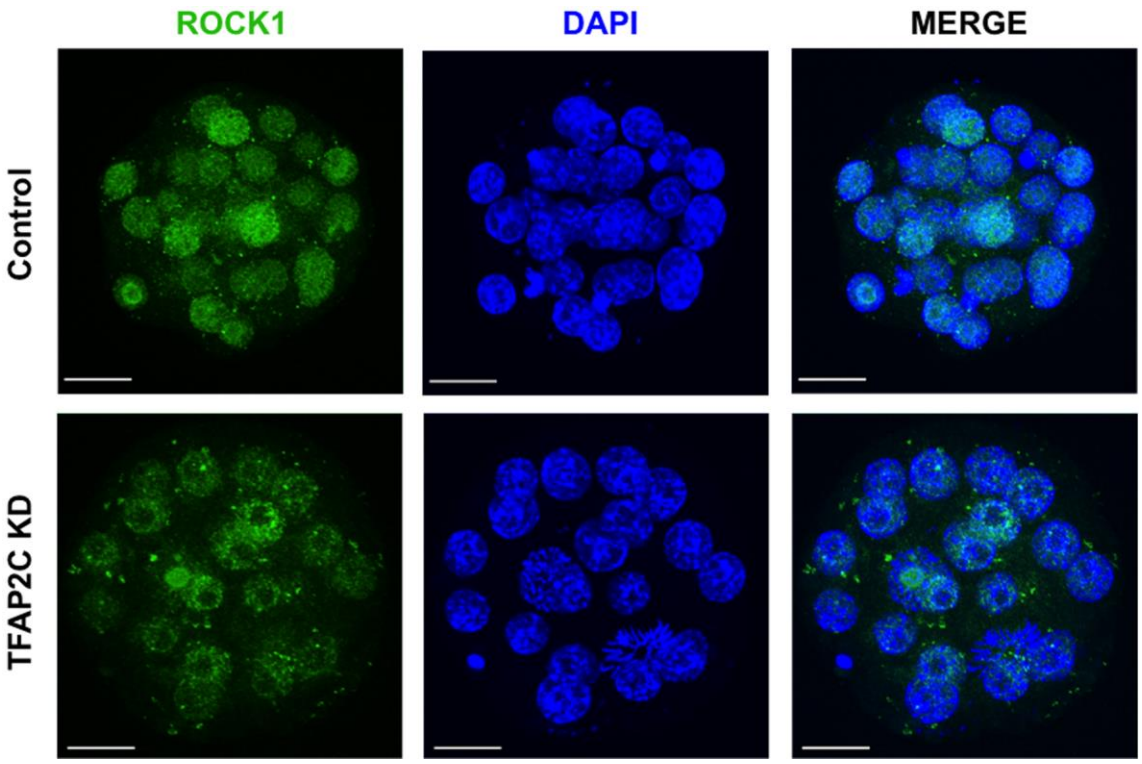

Fig. S6. ROCK1 protein is reduced in TFAP2C KD morulae.

Immunofluorescence analysis of ROCK1 in TFAP2C KD and control morulae. Nuclei were counterstained with DAPI. Scale bar = 20 μm. A total of two biological replicates were performed.

Table S1. Primers and probes

SYBR-Green primers for quantitative RT-PCR analysis:

Species	Gene	Forward (5'-3')	Reverse (5'-3')
Mouse	*Lats1*	TTTGCAGGCTGCTGGCTTTG	AGACATCTGCTCTCGACGAG
Mouse	*Lats2*	TGCGAGTCATCAAGCAGACC	ACTTGGCTCTACTGCTGTGC
Mouse	*Yap*	GTCCTCCTTTGAGATCCCTGA	TGTTGTTGTCTGATCGTTGTGAT

TaqMan probes for quantitative RT-PCR analysis:

Species	Gene	Catalog number
Mouse	*Tfap2c*	Mm00493473-m1
Mouse	*Cdx2*	Mm01212280-m1
Mouse	*Rock1*	Mm00485745-m1
Mouse	*Rock2*	Mm01270843-m1
Mouse	*Limk1*	Mm00440191-m1
Mouse	*Limk2*	Mm01187665-m1
Mouse	*Ubtf*	Mm00456972-m2

Primers for quantitative ChIP analysis:

Species	Genomic location	Primer sequences
Mouse	*Cdx2* intron 1 TFAP2C proximal motif	Forward: 5'-TCACAGCGACCTCTCATCTG-3' Reverse: 5'-AGGGGGAGGAGAACCTCAG-3'
Mouse	*Cdx2* intron 1 TFAP2C distal motif	Forward: 5'-ATCTAAGGGGTGGGAGTTGC-3' Reverse: 5'-TGGTTTGCAAAGGTTTTTACC-3'

Table S2. Antibodies

Primary Antibody	**Species**	**Vendor**	**Cat.no. and Dilution**
TFAP2C	Rabbit	Santa Cruz Biotechnology	sc-8977 (1:100)
CDX2	Mouse	Biogenex	AM-392 (1:25)
PARD6B	Rabbit	Santa Cruz Biotechnology	sc-67393 (1:100)
PKCζ	Mouse	Santa Cruz Biotechnology	sc-17781 (1:100)
pERM	Rabbit	Cell Signaling Technology	3149 (1:100)
YAP	Mouse	Abnova	H00010413-M01 (1:100)
pYAP	Rabbit	Cell Signaling Technology	4911 (1:100)
TEAD4	Mouse	Abcam	ab58310 (1:100)
GATA3	Mouse	BD Pharmingen	558686 (1:100)
F-actin	Amanita phalloides	Molecular Probes	A12380 (1:40)
Normal control IgG	Rabbit	Millipore	12-370 (1:100)

Secondary Antibody	**Species**	**Vendor**	**Cat.no.**
Alexa Fluor 488 anti-mouse IgG	Goat	Molecular Probes	A11001 (1:2000)
Alexa Fluor 488 anti-mouse IgG	Chicken	Molecular Probes	A21200 (1:2000)
Alexa Fluor 488 anti-rabbit IgG	Goat	Molecular Probes	A11008 (1:2000)
Alexa Fluor 594 anti-mouse IgG	Donkey	Molecular Probes	A21207 (1:2000)
Anti-rabbit IgG-HRP	Goat	Thermo Scientific	31460 (1:5000)