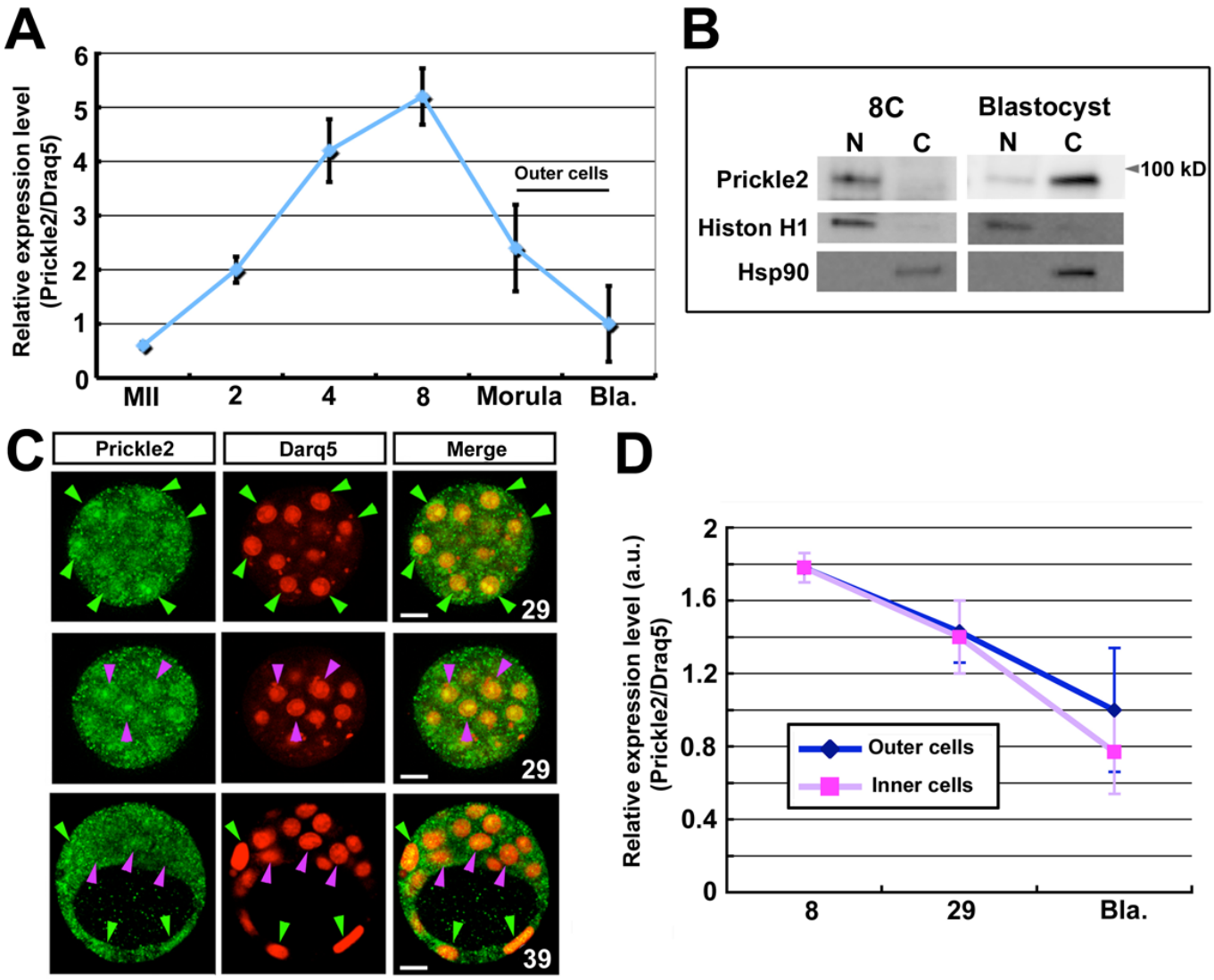


Fig. S1. Expression of Pk2 during preimplantation development. (A) Relative expression level of nuclear Pk2 at serial stages. Theses values were normalized to ratio of fluorescence intensity of Pk2 per that of Draq5 at the 2-cell stage. Data are represented as means and the error bars represent the s.d.. (B) Western blotting with nuclear (N) and cytoplasmic (C) extracts at the compacted 8-cell stage (8C) and blastocyst stage. HistonH1 was a marker for the nucleus. Hsp90 was a marker for cytoplasm. Western blotting was carried out two independent experiments. (C) IHC for Prickle2 at 29-cell stage and blastocyst. Nuclei (red) were stained with Draq5 (29-cell stage, n = 12; blastocyst, n = 8). Green arrowheads were indicated outer cells. Merge arrowheads were indicated inner cells. (D) Relative expression level of nuclear Pk2 in outer and inner cells after compaction. Error bars indicate s.d. (n>20 blastomeres). Scale bars, 10 μm in C.

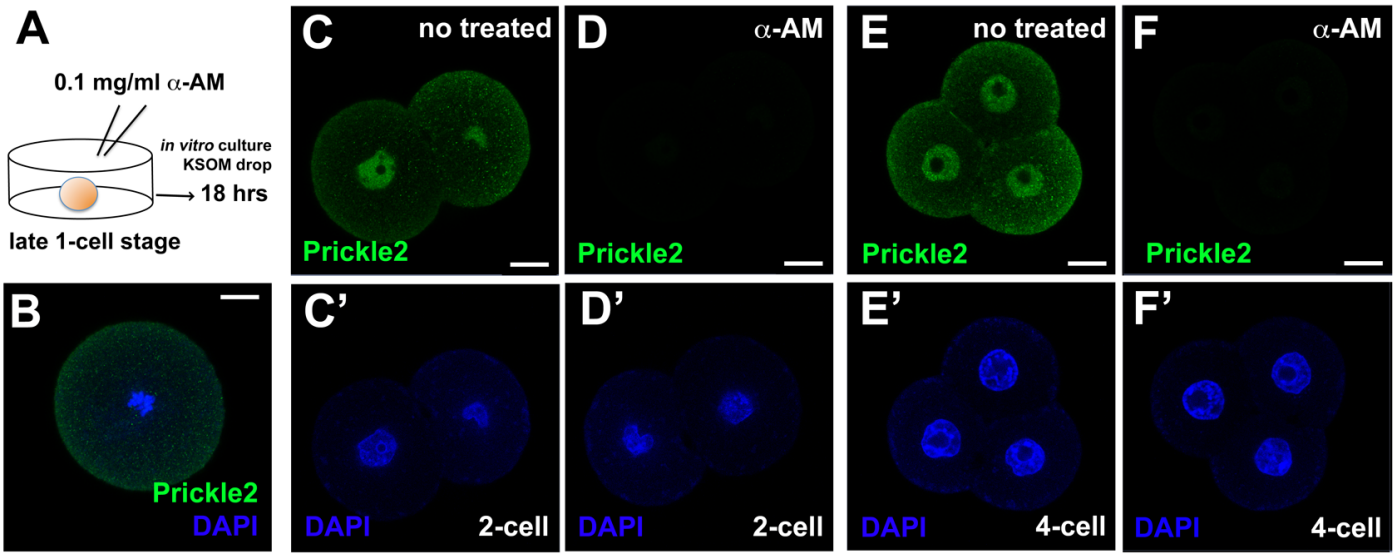


Fig. S2. Analysis of expression pattern of Pk2 during preimplantation development. (A) Scheme for α-amanitine (α-AM) treatment *in vitro* culture. (B-F) Immunohistochemistry (IHC) for Pk2 in representative embryos, with or without exposure to α-AM. Note, nuclear Pk2 signal were absent (D; n = 21/21, F; n = 19/22). Nuclei were stained with DAPI (B, C'-F'). Images were taken of a central plane through the embryo. Scale bars, 10 μm.

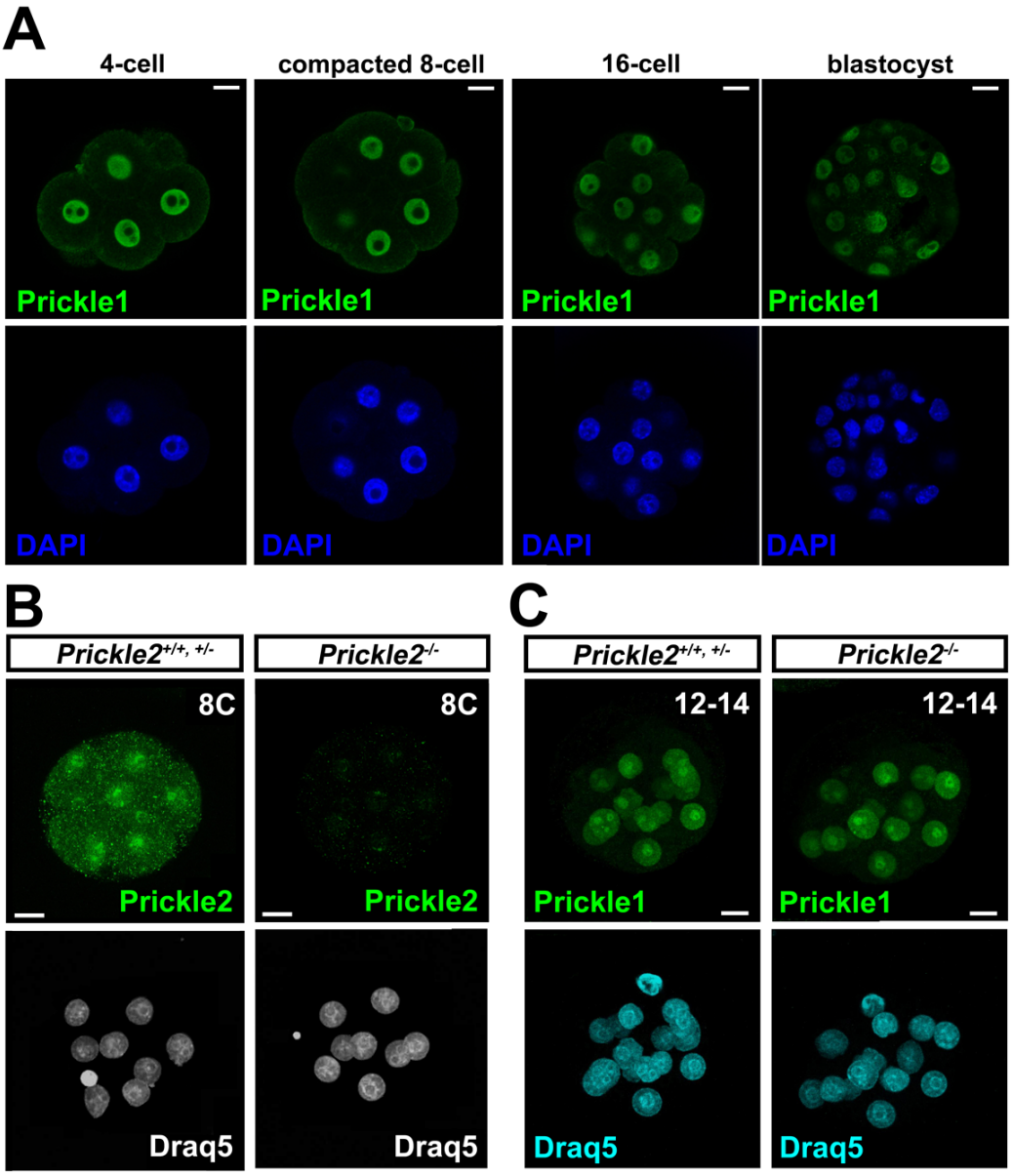


Fig. S3. Expression of Pk1 and Pk2 during pre-implantation development. (A) IHC for Pk1 in representative embryos at serial stages. (B) IHC for Pk2 at the compacted 8-cell stage in the control (n = 39/43) and *Pk2*$^{-/-}$ (n = 15/15) embryos. (C) IHC for Pk1 at the 12-14-cell stage in the control (n = 23/23) and *Pk2*$^{-/-}$ (n = 5/5) embryos. Nuclei were stained with DAPI (A) or Draq5 (B and C). Images were taken of a central plane through the embryo. Scale bars, 10 μm.

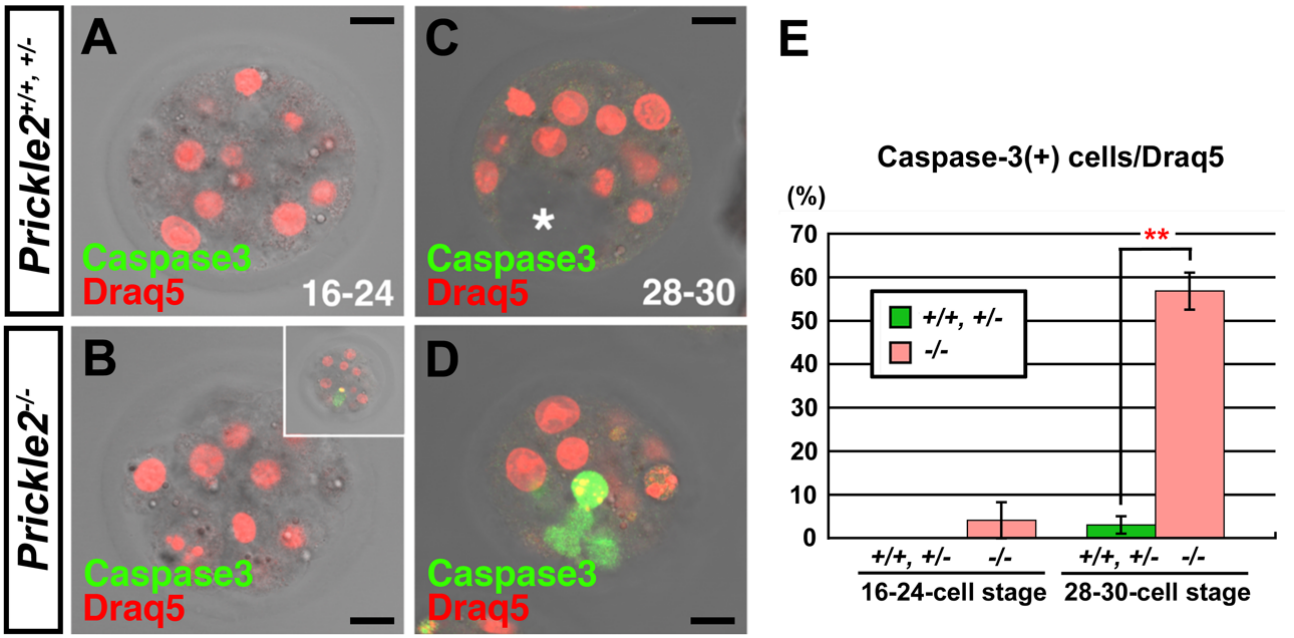


Fig. S4. The phenotypes of *Pk2*$^{-/-}$ embryos at late morula stages. (A-D) IHC for cleaved caspase-3 in the control and *Pk2*$^{-/-}$ embryos. Note, the number of cleaved caspase-3 positive increased throughout the embryo (n = 8/8). *Inset* in B indicates *Pk2*$^{-/-}$ embryos with cleaved caspase-3 positive blastomeres (n = 2/6). Asterisk indicates the blastocyst cavity. (E) Measurement of cleaved caspase-3-positive cells at the indicated stages. Error bars indicate s.d. (n>40 blastomeres). Scale bars, 10 μm.

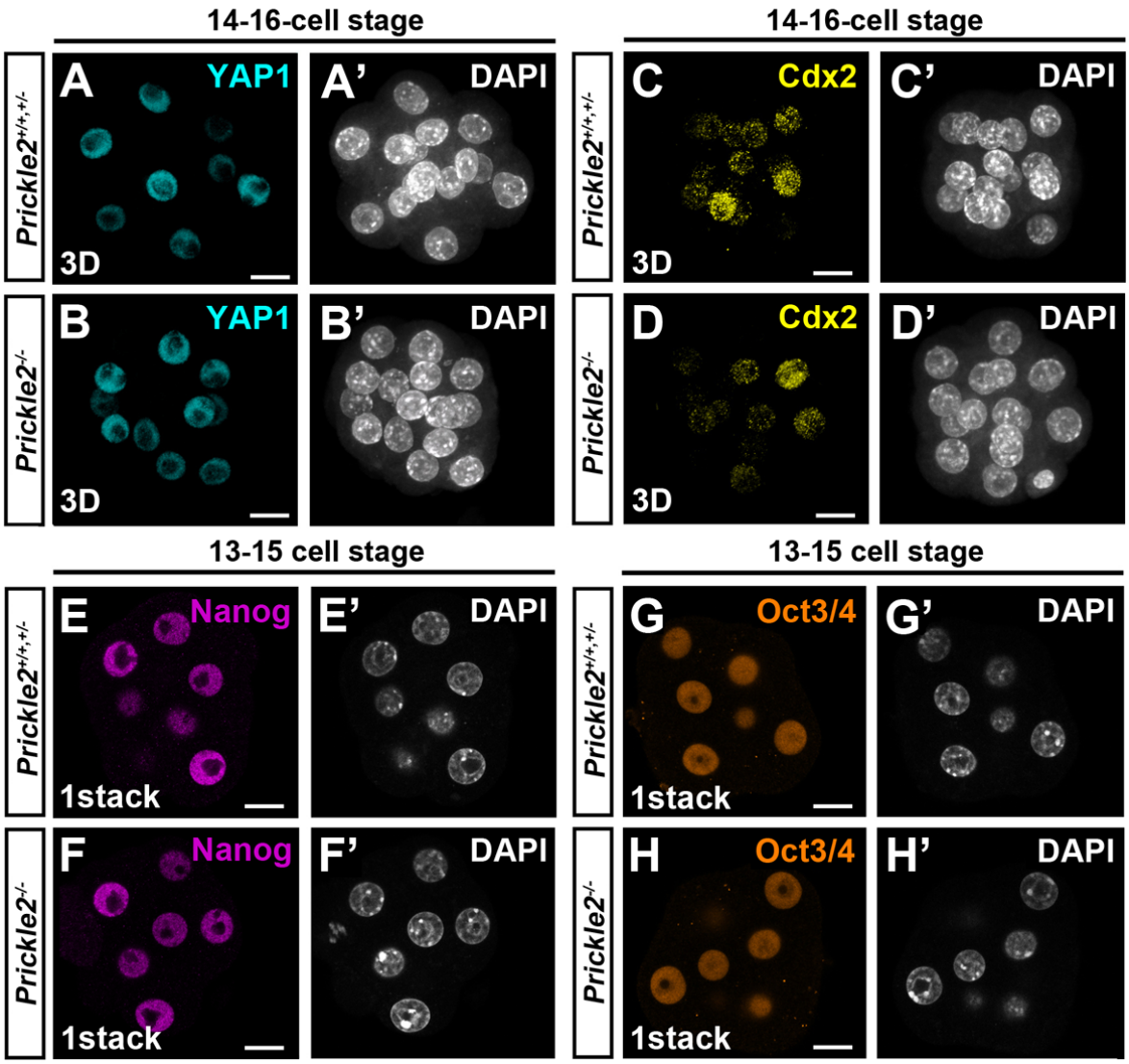


Fig. S5. Expression of Yap1, Cdx2, Nanog and Oct3/4 after compaction in *Pk2*$^{-/-}$ embryos. (A and B) IHC for YAP1 in control and *Pk2*$^{-/-}$ (n = 6/6) embryos at the stages indicated. (C and D) IHC for Cdx2 in control and *Pk2*$^{-/-}$ (n= 6/6) embryo at the indicated stage. (E and F) IHC for Nanog in control and *Pk2*$^{-/-}$ (n =9/10) embryos at the indicated stages. (G and H) IHC for Oct4 in control and *Pk2*$^{-/-}$ (n =8/8) embryos at the early morula stage. (A'-H') The number of blastomeres in control and *Pk2*$^{-/-}$ embryos was counted by DAPI staining. Each image was a Z-series projection of the confocal sections of embryos (3D) (A-D') or taken in a central plane through the embryo (E-H'). Scale bars, 10 μm.

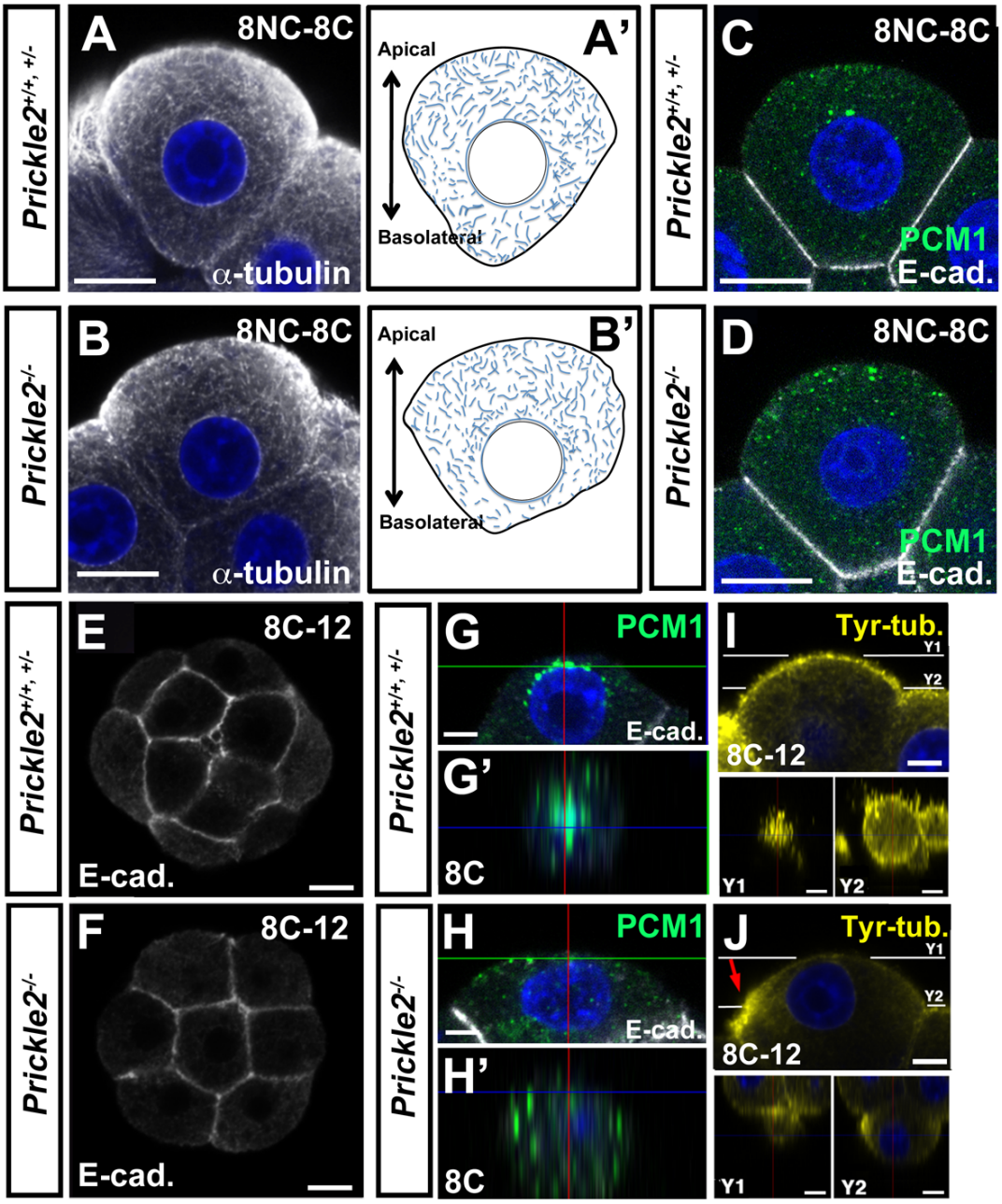


Fig. S6. *Pk2* mediates redistribution of the microtubular architecture within the cell cortex during compaction. (A and B) IHC for α-tubulin in control and *Pk2*$^{-/-}$ (n= 22/24 blastomeres, n = 8) embryos at the indicated stages. (C and D) Double IHC for PCM1 and E-adherin (C and D), in control and *Pk2*$^{-/-}$ (32/32 blastomeres, n = 4) embryos at the indicated stages. (A' and B') Traced drawings of cells stained for α-tubulin antibodies. (E and F) IHC for E-cadherin in control and *Pk2*$^{-/-}$ (n = 12/12) embryos at the indicated stages. (G -H') Double IHC for PCM1 and E-cadherin in control and *Pk2*$^{-/-}$ (27/32 blastomeres, n = 4) blastomeres. The lower panels are vertical sections of the parts indicated by red and green lines in the upper panels. (I and J) IHC for tyrosinated-tubulin (Tyr-tubulin) in control and *Pk2*$^{-/-}$ (19/24 blastomeres, n = 3) blastomeres. Red arrow indicates a local accumulation of Tyr-tubulin staining. The lower panels are vertical sections of the parts indicated by white lines in the upper panels (Y1 and Y2). Scale bars, 10 μm in A-F; 5 μm in G-J.

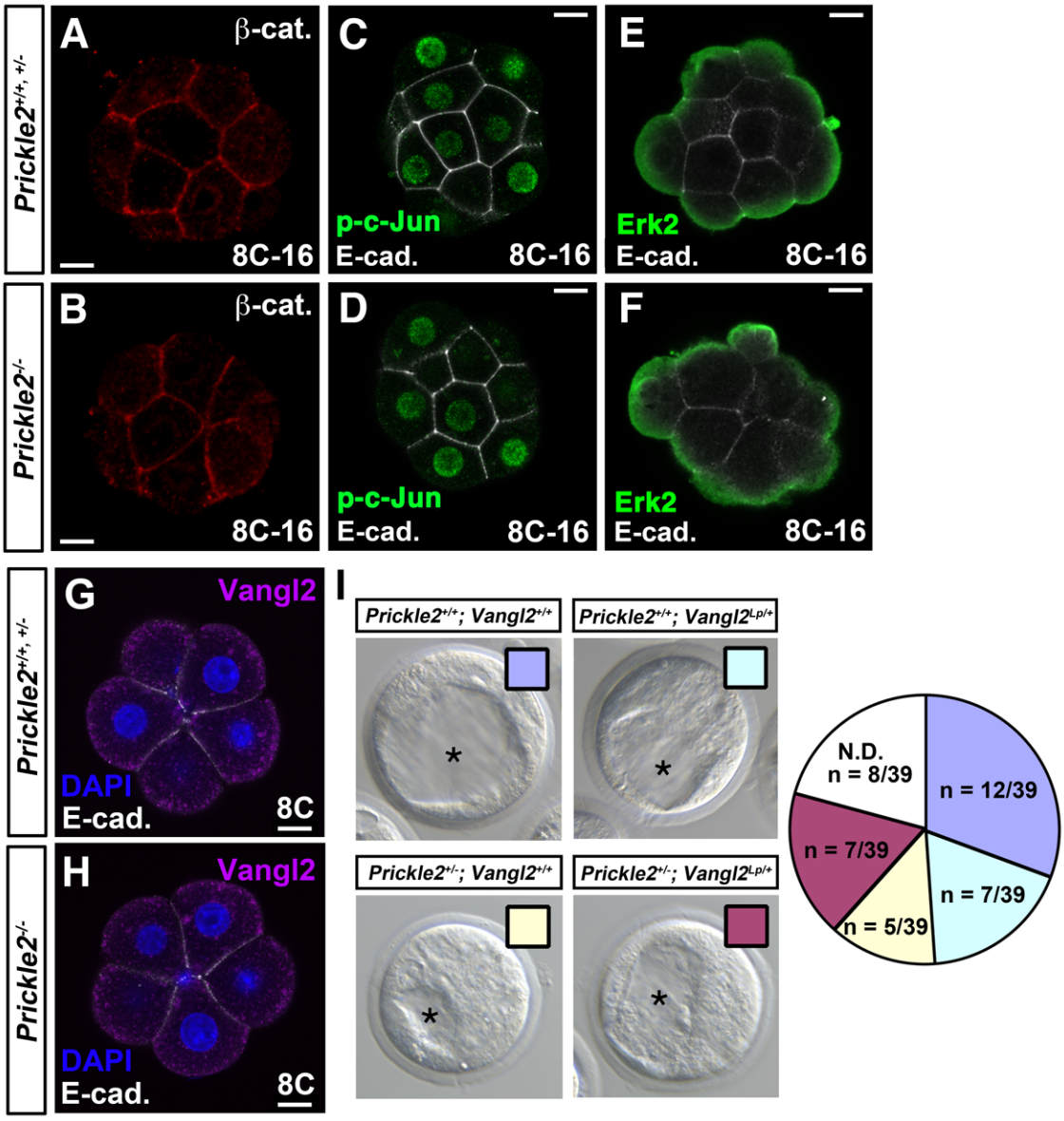


Fig. S7. Several signaling pathways underlying blastocyst cavity formation in *Pk2*$^{-/-}$ embryos. (A and B) IHC for β-catenin at the early morula stage in control and *Pk2*$^{-/-}$ (n = 3/3) embryos. (C and D) Double IHC for phosphorylated c-Jun (p-c-Jun) at the early morula stage in control and *Pk2*$^{-/-}$ (n = 3/3) embryos. (E and F) Double IHC for Erk2 and E-cadherin at the early morula stage in control and *Pk2*$^{-/-}$ (n = 3/3) embryos. (G and H) IHC for Vangl2 at the compacted 8-cell stage in control and *Pk2*$^{-/-}$ (n = 4/5) embryos. (I) Gross observation of *Pk2*$^{+/-}$; *Vangl2*$^{Lp/+}$ embryos *in vitro*.

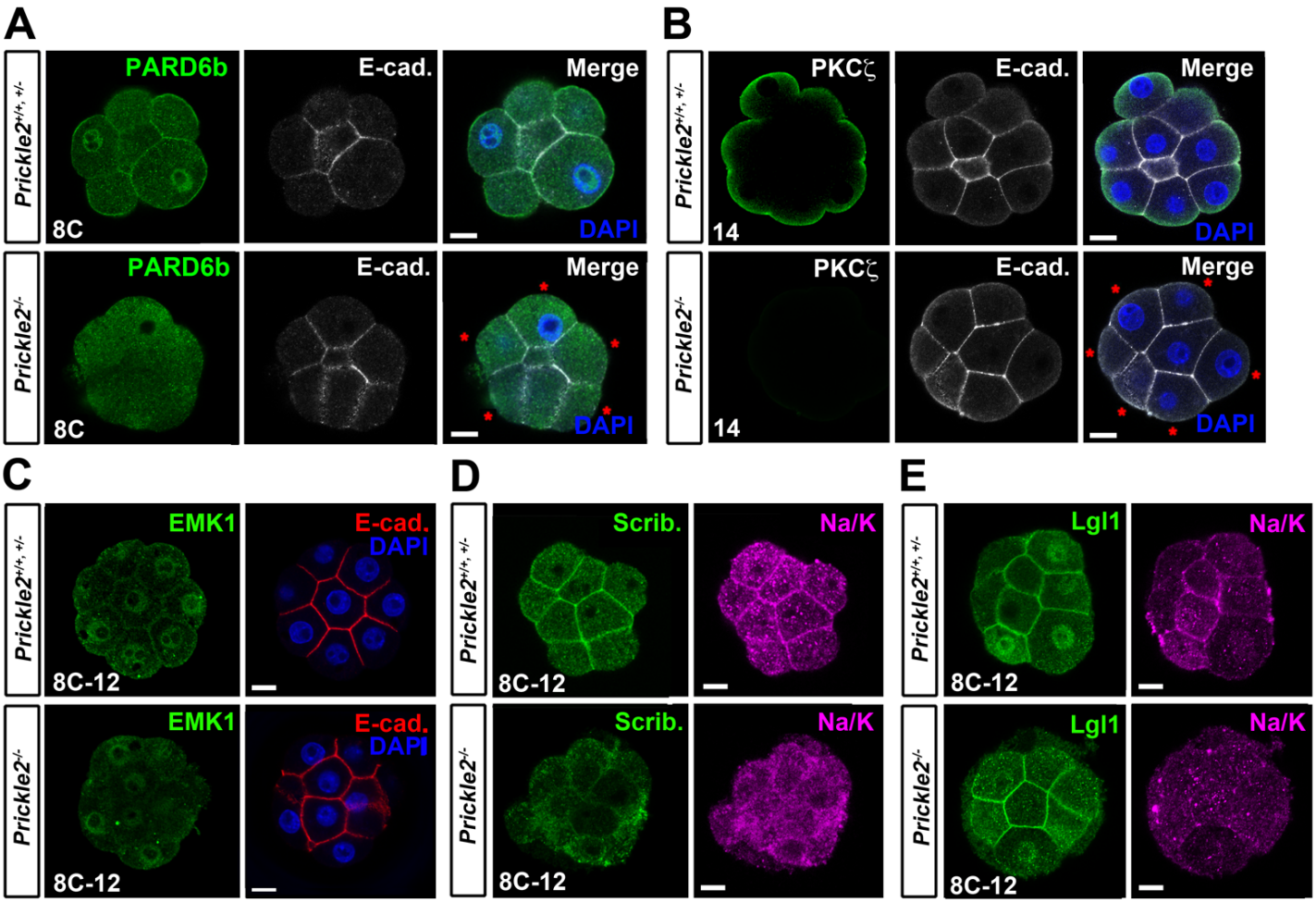


Fig. S8. Whole views of IHC for the cell polarity regulator (PARD6b, PKCζ, EMK1, Scribble, Lgl1) and E-cadherin in control and *Pk2*$^{-/-}$ embryos. Red asterisks show blastomeres with disrupted PARD6b or PKCζ distributions on the apical membrane of *Pk2*$^{-/-}$ embryos. Scale bars, 10 μm.

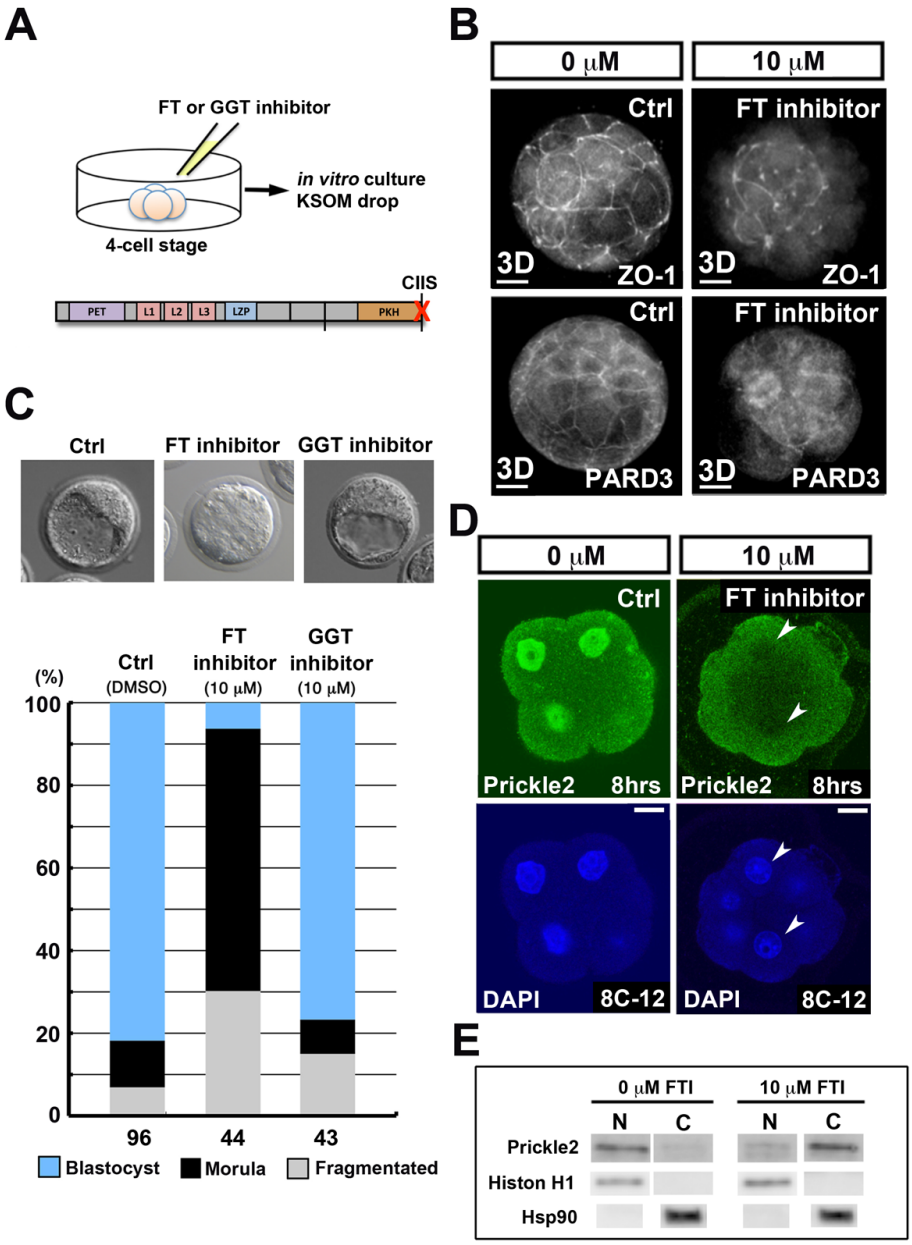


Fig. S9. Cell polarity is disrupted in FT inhibitor-treated embryos. (A) Mouse embryos were treated from the 4-cell stage and observed for nuclear Pk2 protein after 8 hrs or for blastocyst cavity formation after 17 hrs. (B) IHC for ZO-1 and PARD3 in control and FT inhibitor-treated embryos (ZO-1; n = 4/6, PARD3; n = 12/14). (C) Effect of FT or GGT inhibition on blastocyst formation is shown as the percentage and actual number of embryos after about 24 hrs of *in vitro* culture. (D) IHC for Pk2 in control and FT inhibitor-treated embryos at the indicated stage. White asterisks indicate significant loss of the nuclear Pk2 signal (n = 12/12). (E) Western blotting with nuclear (N) and cytoplasmic (C) extracts in control and FT inhibitor-treated embryos at the late 8-cell stage. Western blotting were carried out two independent experiments. Scale bars, 10 μm in B and D.

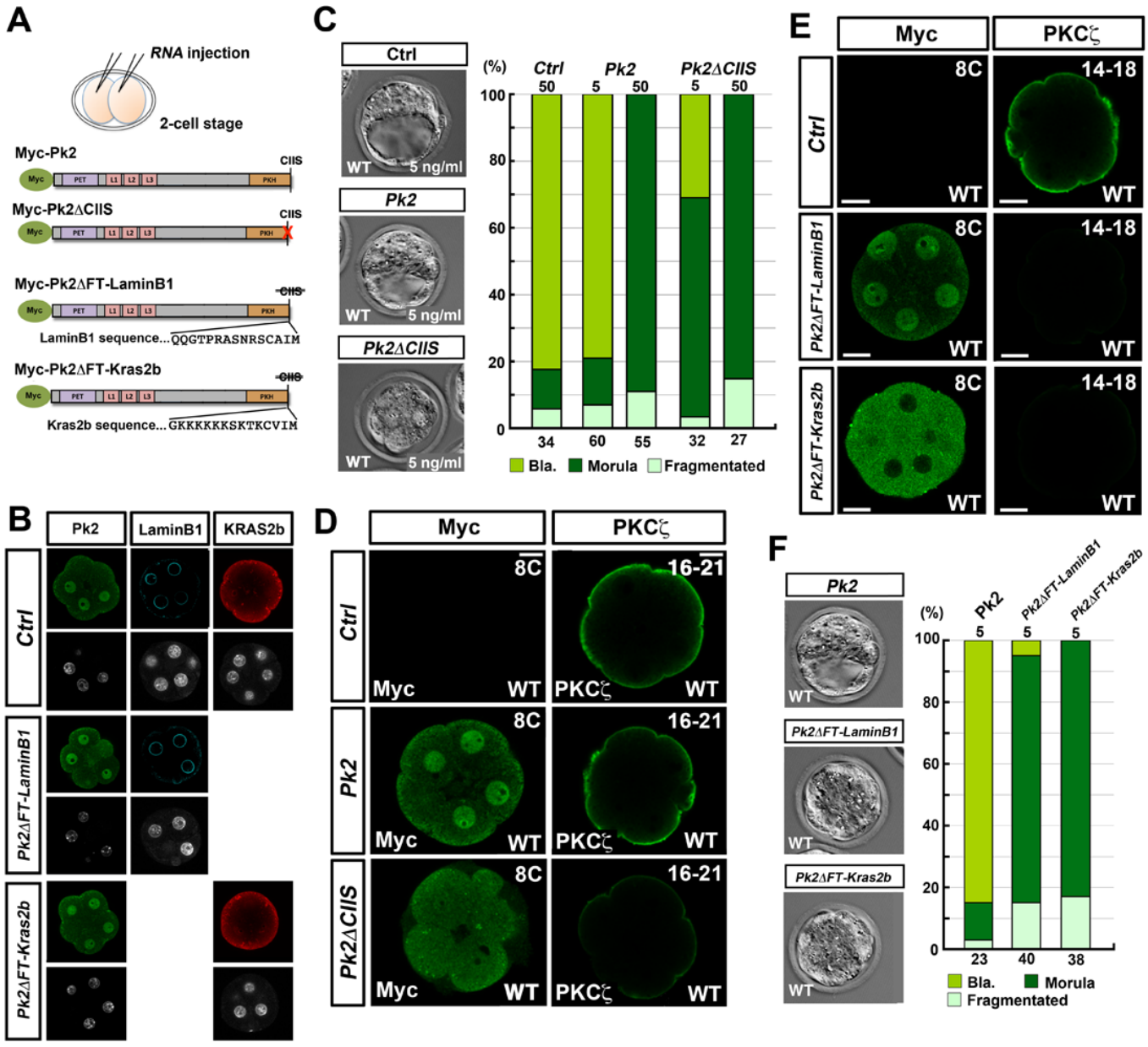
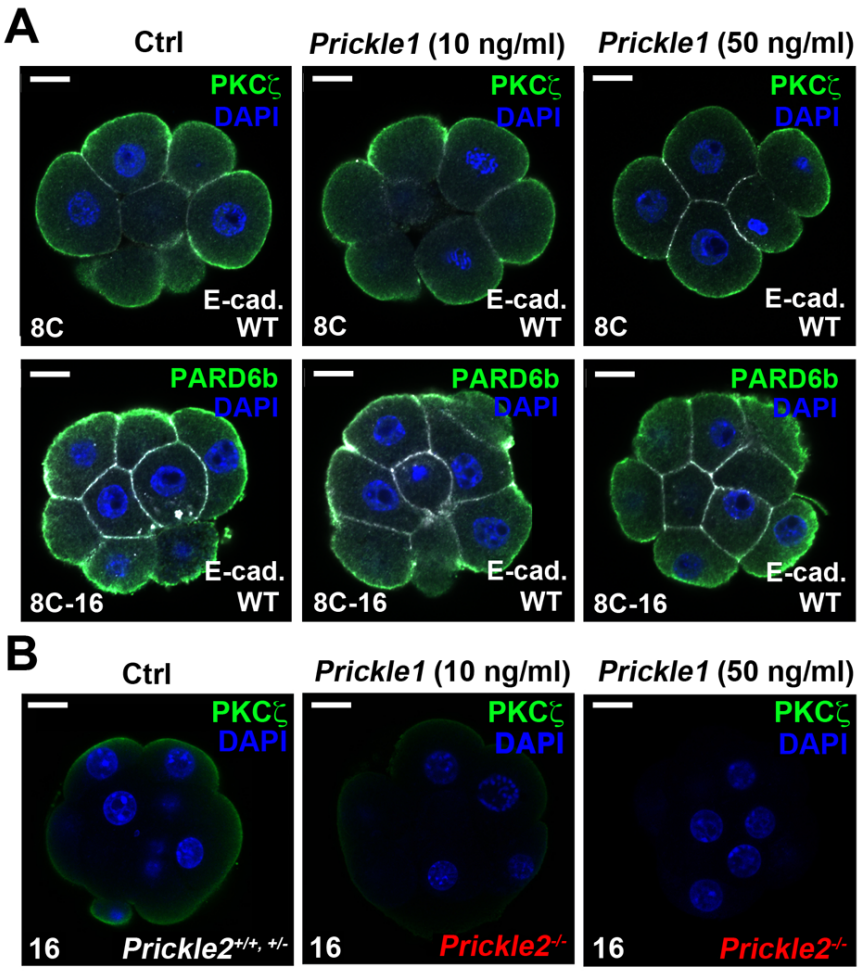


Fig. S10. Nuclear translocation of Pk2 is prerequisite for preimplantation development. (A) 6xMyc-tagged full-length and various mutant *Pk2 RNAs* were synthesized and injected into each blastomere at the 2-cell stage in wild-type embryo. (B) The localization of Pk2 (left panels), LaminB1 (middle panels) and KRAS2b (right panels) in embryos receiving injections of indicated *RNA*. Nuclei were stained with DAPI. (C) DIC images of *EGFP* (*Ctrl*), full-length, and CIIS-deleted forms of *Pk2 (Pk2ΔCIIS) RNA*-injected embryos. Graph summarizing the effects of *Pk2 RNA* injection on blastocyst cavity formation. Numbers represent the total number of embryos examined in each category. (D) IHC for Myc and PKCζ in the embryos receiving 5 ng/ml injections of *Ctrl* and *Pk2ΔCIIS 2 RNA*-injected embryos at 8-12-cell stage. (E) DIC images and graph summarizing the effect of the embryos receiving 5 ng/ml injections of *Ctrl RNA* or *RNA* for *Pk2* in which the C-terminus was replaced with that of *LaminB1 (Pk2ΔFT-LaminB1)* or *kras2b (Pk2ΔFT-Kras2b)* at the indicated stages. Numbers represent the total number of embryos examined in each category. (F) IHC for Myc and PKCζ in embryos receiving 5 ng/ml injections of EGFP *RNA* (Ctrl), or *RNA* for *Pk2ΔFT-LaminB1 or Pk2ΔFT-Kras2b* at the indicated stages. Scale bars, 10 μm in C and E.



C

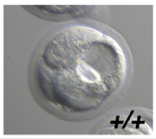
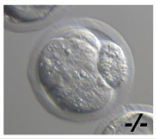
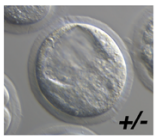
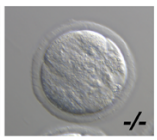
	Prickle2$^{+/+,\ +/-}$	*Prickle2*$^{-/-}$	*Prickle2*$^{+/+.\ +/-}$	*Prickle2*$^{-/-}$
	10 ng/ml *Prickle1*	10 ng/ml *Prickle1*	50 ng/ml *Prickle1*	50 ng/ml *Prickle1*
Blastocyst	30（71.4）	0（0）	14 (35.9)	0(0)
Morula	9（21.4）	4 (40)	21 (53.8)	4 (66.7)
Flagmentation	3（7.2）	6 (60)	4 (10.3)	2 (33.3)
total	42（100）	10（100）	39 (100)	6 (100)

Fig. S11. Effect of wild-type and *Pk2*$^{-/-}$ embryos receiving injections of *Pk1 RNA* and *Pk1 c*DNA. (A) IHC for PKCζ or PARD6b and E-cadherin in wild-type embryos at the indicated stage. Full-length *Pk1 RNA* was injected into the each blastomere of wild-type two-cell stage embryos. (B) IHC for PKCζ in representative embryos at the 16-cell stage. Full-length *Pk1* cDNA was injected into the pronuclei of one-cell-stage of control or *Pk2*$^{-/-}$ embryos. (C) Table showing the effect of full-length *Pk1* DNA-injected control or *Pk2*$^{-/-}$ embryos. Scale bars, 10 μm in A and B.

Table S1. Genotyping of *Pk2*$^{-/-}$ mutant mice

A

Prickle2+/- crosses (1st-3rd backcross on B6)

	line 1 (#64)			line 2 (#134)		
	+/+	+/-	-/-	+/+	+/-	-/-
P0	21	40	0	7	18	0
E7.5	11	24	0	10	29	0
E4.5	n.d.			4	11	0
E3.5 (*in vitro*)	 			 		

B

Prickle2+/- crosses (1st backcross on CBA)

	P9		
Genotype	Number	Frequency(%)	Expected(%)
+/+	42	32.8	25
+/-	84	65.6	50
-/-	2	0	25
Total	128	100	100

Prickle2+/- crosses (2nd backcross on CBA)

	P0		
Genotype	Number	Frequency(%)	Expected(%)
+/+	18	37.5	25
+/-	30	62.5	50
-/-	0	0	25
Total	48	100	100

Prickle2+/- crosses (3rd backcross on CBA)

	P0		
Genotype	Number	Frequency(%)	Expected(%)
+/+	24	34.8	25
+/-	45	65.2	50
-/-	0	0	25
Total	69	100	100

C

Prickle2+/- crosses (N6, B6) x 1st backcross on CBA

	P5		
Genotype	Number	Frequency(%)	Expected(%)
+/+	21	26.9	25
+/-	48	61.5	50
-/-	9	11.6	25
Total	78	100	100

Prickle2+/- crosses (N6, B6) x 2nd backcross on CBA

	P0		
Genotype	Number	Frequency(%)	Expected(%)
+/+	10	28.6	25
+/-	23	65.7	50
-/-	2	5.7	25
Total	35	100	100

D

Prickle2+/- crosses (3rd backcross on B6)

	P0		
Genotype	Number	Frequency(%)	Expected(%)
+/+	28	32.6	25
+/-	58	67.4	50
-/-	0	0	25
Total	86	100	100

Prickle2+/- crosses (6th backcross on B6)

	P0		
Genotype	Number	Frequency(%)	Expected(%)
+/+	21	30.0	25
+/-	46	65.7	50
-/-	3	4.3	25
Total	70	100	100

Prickle2+/- crosses (8th backcross on B6)

	P0		
Genotype	Number	Frequency(%)	Expected(%)
+/+	20	25	25
+/-	38	47.5	50
-/-	22	27.5	25
Total	80	100	100

Table S2. Antibodies used in this study

Antibody	Source	Dilution	Company (Cat.#)
Antibodies for IHC			
Prickle2	Rabbit	1:300	Gifted from Dr. J. Axelrod
Prickle1	Rabbit	1:200	Gifted from Dr. A.G. Bassuk
α-tubulin (DM1A)	Mouse	1:200	Sigma (T9026)
Tyrosylated tubulin (YL1/2)	Rat	1:200	Abcam (ab 6160)
E-cadherin (ECCD2)	Rat	1:400	MERCK (205604)
PCM1	Mouse	1:200	Cell Signaling (#5213)
PARD6B (M-64)	Rabbit	1:50	Santa Cruz (sc-67393)
PARD3	Rabbit	1:200	Upstate (#07-330)
PKCζ (C-20)	Rabbit	1:50	Santa Cruz (sc-216)
Na^+/K^+ ATPase α-1	Mouse	1:200	Millipore (#05-369)
phospho-PKCζ (Thr410/403)	Rabbit	1:200	Cell Signaling (#9378)
EMK1 (MARK2)	Mouse	1:200	abcam (ab77698)
Scribble (H-300)	Rabbit	1:50	Santa Cruz (sc-28737)
Vangl2	Sheep	1:200	R&D systems (AF4815)
RhoA (119)	Rabbit	1:50	Santa Cruz (sc-179)
Cdx2	Mouse	1:500	BioGenex (MU392A-UC)
Yap1	Mouse	1:500	Abnova (H00010413-M01)
Nanog	Rabbit	1:200	COSMO BIO Co., LTD. (RCAB0002P-F)
Oct3/4 (C-10)	Mouse	1:50	Santa Cruz (sc5279)
Myc (9E10)	Mouse	1:200	BIOMOL (SA-294)
p42 MAP Kinase (Erk2)	Mouse	1:200	Cell Signaling (#9108)
Lgl1(Hugl-1) (M-102)	Rabbit	1:50	Santa Cruz (sc-67244)
β-catenin	Rabbit	1:200	Sigma (C2206)
Active Caspase-3	Rabbit	1:200	BD Pharmingen (559565)
Phospho-c-Jun (Ser73)	Rabbit	1:200	Cell Signaling (#9164)
ZO-1	Rabbit	1:200	ZYMED (LTD.61-7300)
Lamin B1	Rabbit	1:200	abcam (ab16048)
KRAS2b	Rabbit	1:200	ProteinTech Group, Inc. (16155-1-AP)
Draq5	---------	--------	biostatus (BOS-889-001)
DAPI	---------	--------	Invitrogen (D3571)
Alexa Fluor 555 phallodin	---------	1:50	Invitrogen (A34055)
Immunobloing			
Prickle2	Rabbit	1:2000	abcam (ab65964)
HistonH1	Rabbit	1:5000	abcam (ab61243)
Hsp90	Rabbit	1:5000	Cell Signaling (#4874)
Inhibitors			
α-Amanitin			Wako (010-22961)
FT inhibitor (B581)			Chemicon (#344510)
GGT inhibitor (GGTI-298)			Chemicon (#345883)

Table S3A. Primers used for constructing deletion or mutation of C terminal site of *Prickle2* gene

Name	Orientation	Sequence
Pk2ΔFT-LaminB1	F	5′- CCG CTC GAG ACA GCA GGG AAC CCC AAG AGC ATC CAA TAG AAG CTG TGC AAT TAT GTA ATC TAG AGC -3′
	R	5′- GCT CTA GAT TAC ATA ATT GCA CAG CTT CTA TTG GAT GCT CTT GGG GTT CCC TGC TGT CTC GAG CGG -3′
Pk2ΔFT-Kras2	F	5′- CCG CTC GAG TGG TAA AAA GAA GAA AAA GAA GTC AAA GAC AAA GTG TGT AAT TAT GTA ATC TAG AGC -3′
	R	5′- GCT CTA GAT TAC ATA ATT ACA CAC TTT GTC TTT GAC TTC TTT TTC TTC TTT TTA CCA CTC GAG CGG -3′
Pk2ΔCIIS	F	5′- TGG GGG TAG AGG AGT GAG GGG AAG TAG CGT -3′
	R	5′- TAA AAT CAC TAG TGA GGC CGC CTG CAG GTC -3′

F, forward; R, reverse

Table S3B. Primer Sequences for RT-PCR

Gene	Orientation	Sequence
Prickle2	F	5′- GAC CTC ATC TAC TTT TAC CAA -3′
	R	5′- TAC TAC CAC CCA CTT TAT TCT -3′
Cdx2	F	5′- GCA GTC CCT AGG AAG CCA AGT GA -3′
	R	5′- CTC TCG GAG AGC CCA AGT GTG -3′
Yap1	F	5′- CCT TCT TCA AGC CGC CCG GAG-3′
	R	5′- CAG TGT CCC AGG AGA AAC AGC-3′
Nanog	F	5′- CAC CCA CCC ATG CTA GTC TT -3′
	R	5′- ACC CTC AAA CTC CTG GTC CT -3′
Oct3/4	F	5′- CAC GAG TGG AAA GCA ACT CA -3′
	R	5′- AGA TGG TGG TCT GGC TGA AC -3′
gapdh	F	5′- CCC ACT AAC ATC AAA TGG GG -3′
	R	5′- CCT TCC ACA ATG CCA AAG TT -3′

F, forward; R, reverse