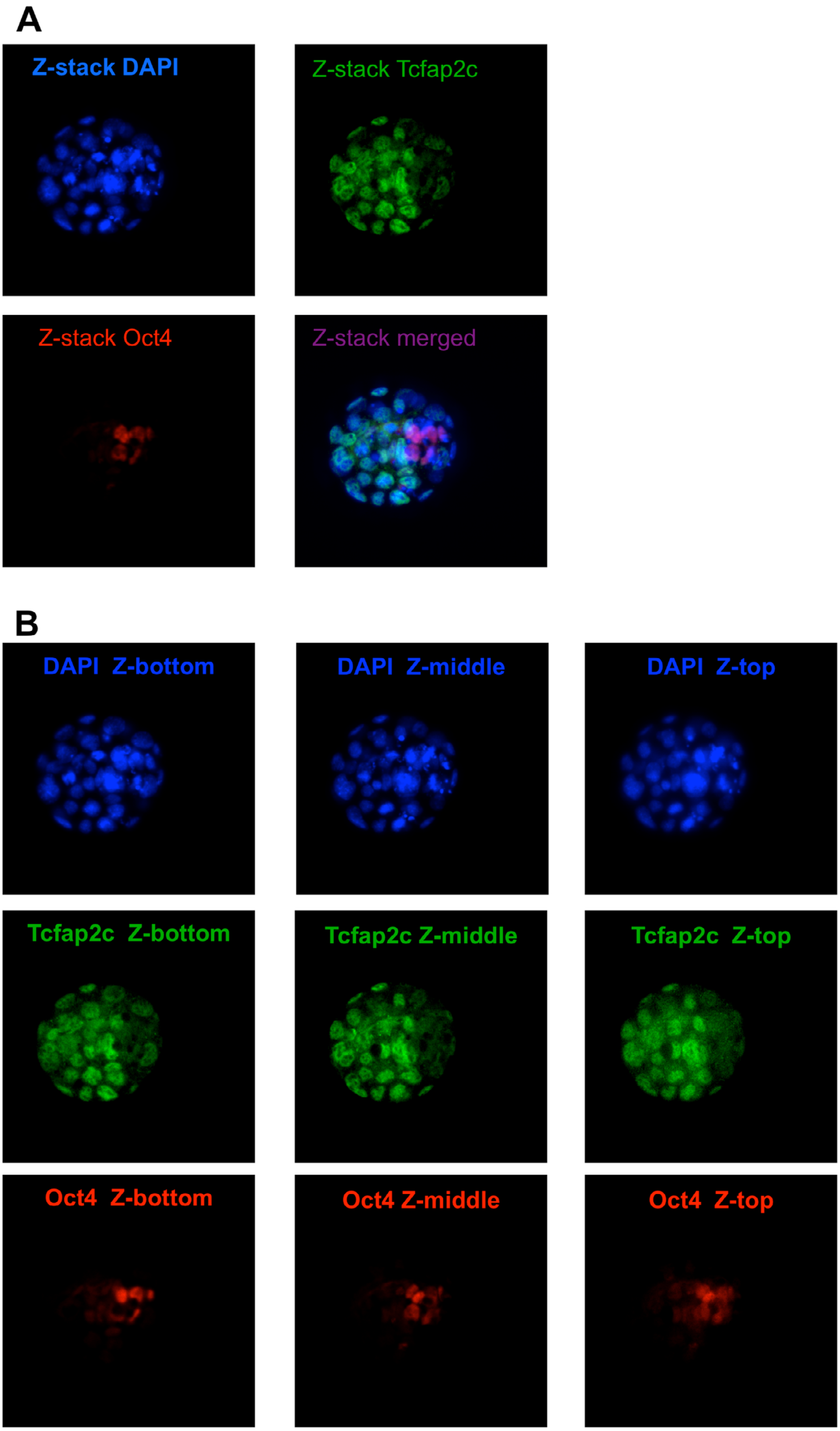

Fig. S1. Heterogeneous localization of Tcfap2c protein in mouse blastocysts. (**A**) Combined *z*-stack showing the localization of Tcfap2c in the mural TE. Tcfap2c is absent in the Oct4-positive ICM and surrounding polar TE. (**B**) Individual optical sections of a blastocyst stained for Tcfap2c, Oct4 and with DAPI.

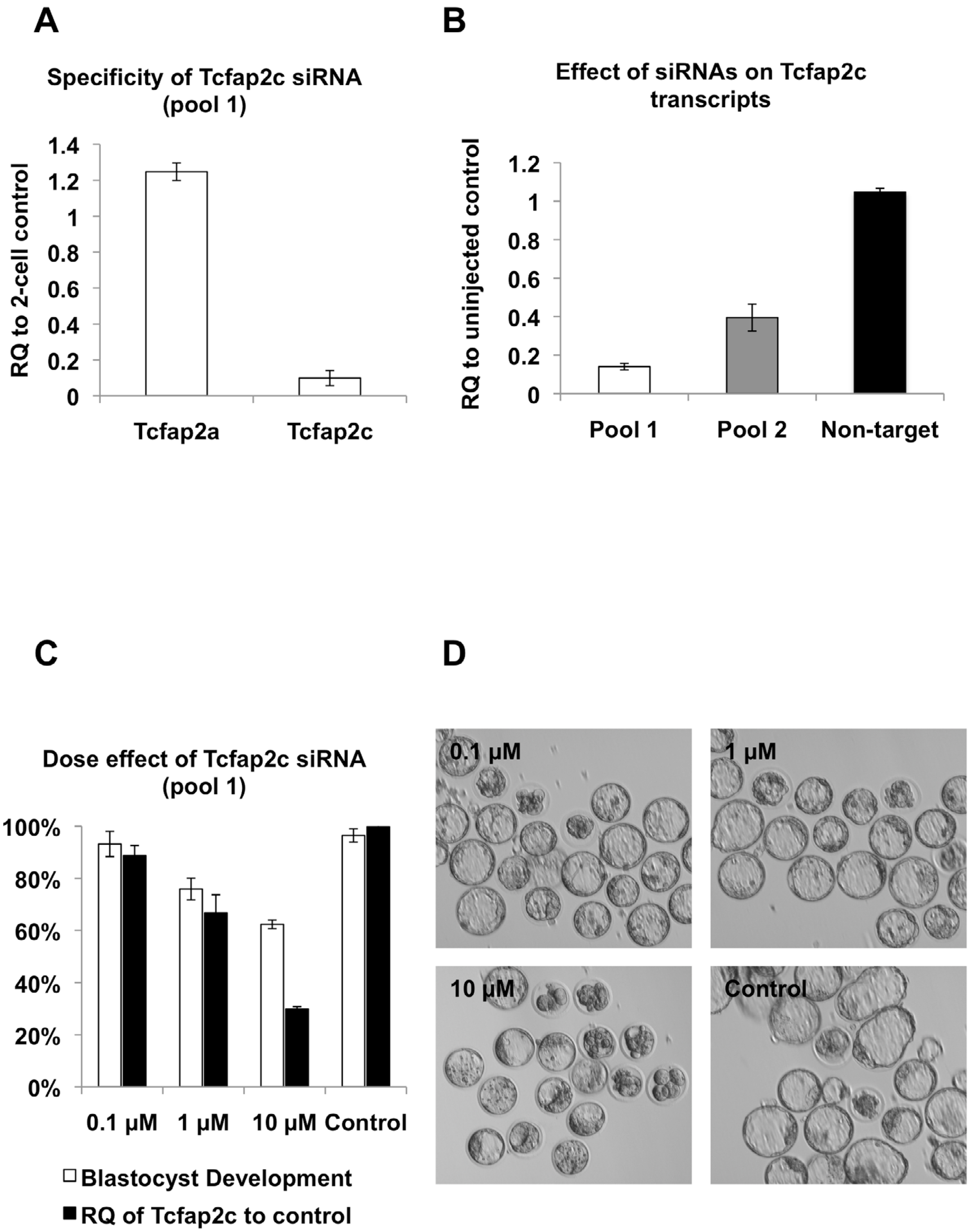

Fig. S2. Control experiments demonstrating specificity of siRNA. (**A**) qRT-PCR analysis of *Tcfap2a* and *Tcfap2c* transcripts in two-cell embryos microinjected with 100 µM *Tcfap2c* siRNA. (**B**) qRT-PCR analysis of *Tcfap2c* transcripts in embryos injected with non-targeting siRNA, *Tcfap2c* siRNA (pool 1), or *Tcfap2c* siRNA (pool 2). Expression is relative to control uninjected embryos. (**C**) Effect of different concentrations of *Tcfap2c* siRNA on *Tcfap2c* transcripts and blastocyst formation. (**D**) Brightfield images of embryos injected with different concentrations of *Tcfap2c* siRNA at 120 hph. Error bars represent mean ± s.e.m.

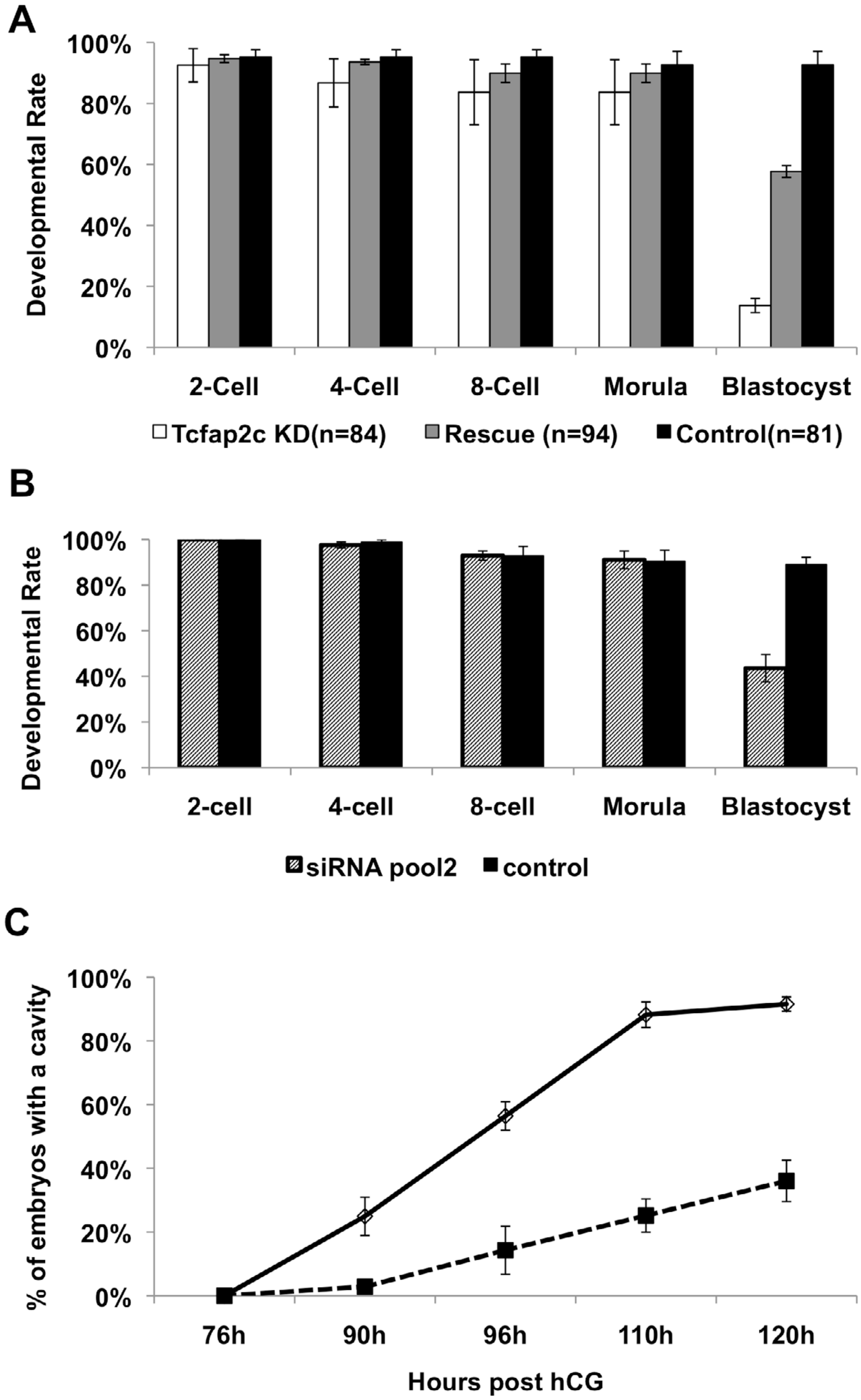

Fig. S3. siRNA-mediated knockdown of Tcfap2c in mouse preimplantation embryos. (**A**) Effect of *Tcfap2c* siRNA on mouse preimplantation development (*Tcfap2c* siRNA pool 1). A second set of embryos was co-injected with both *Tcfap2c* siRNA (pool 1) and *Tcfap2c* mRNA (rescue). (**B**) Developmental rates of preimplantation embryos injected with a second pool of *Tcfap2c* siRNA. (**C**) Timing of cavity formation in Tcfap2c KD embryos (*Tcfap2c* siRNA pool 1). Error bars represent mean ± s.e.m.

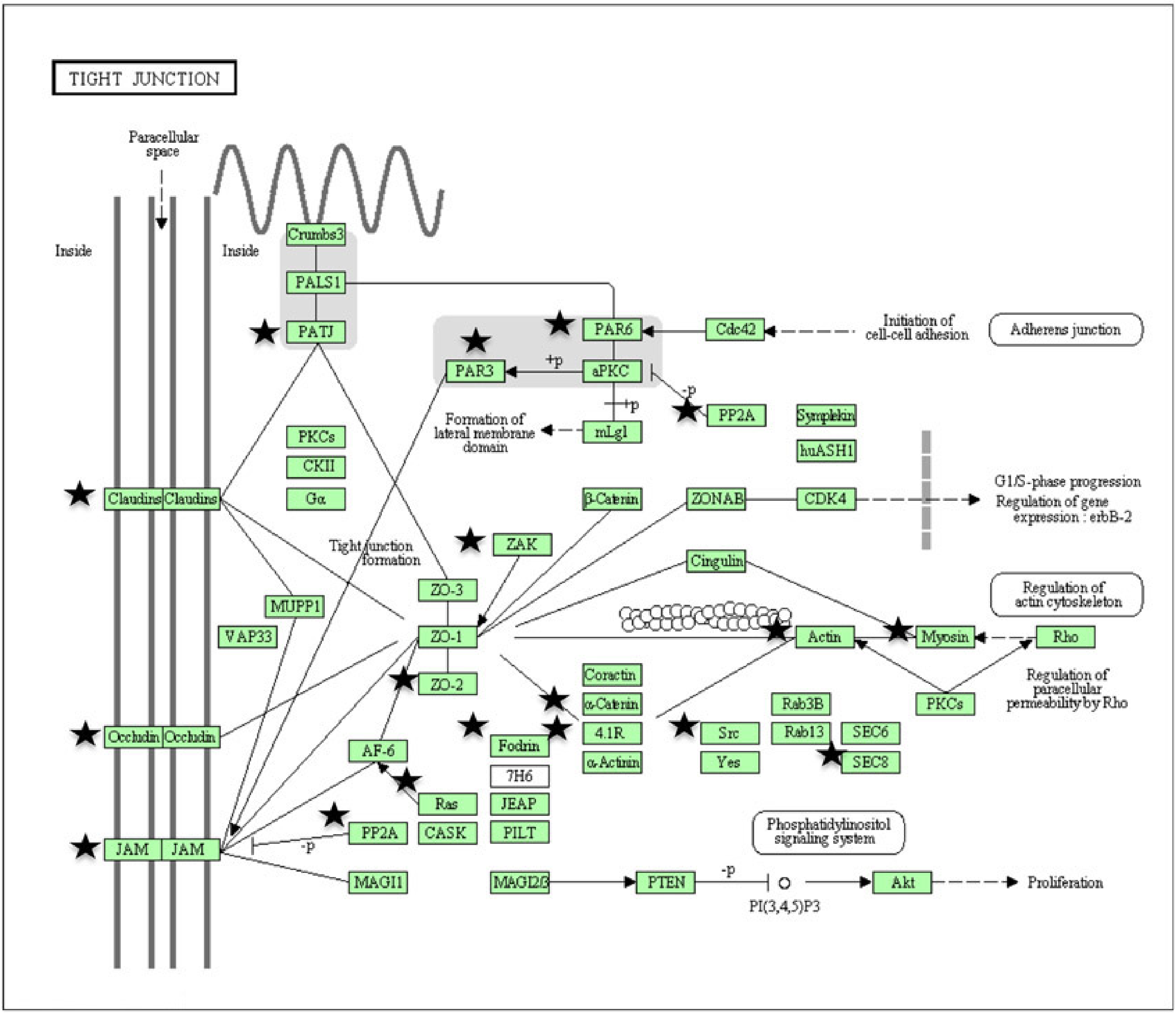

Fig. S4. Tight junction pathway map. Tcfap2c target genes are found in TJ and paracellular sealing pathways. Genes selected using the DAVID pathway analysis tool are marked with a star. Pathway map 04530 is adapted with permission from the KEGG database (http://www.genome.jp/kegg-bin/show_pathway?map04530).

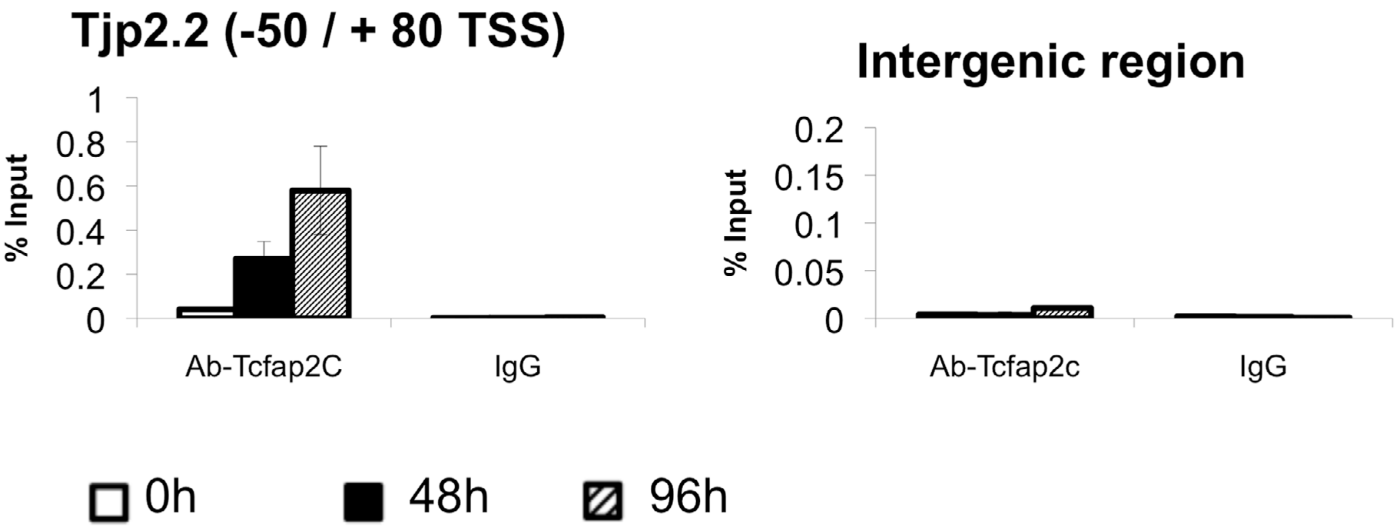

Fig. S5. ChIP analysis of Tcfap2c-bound genes. The promoter regions of *Tjp2* variant 2 and *Fn1* were occupied by Tcfap2c in Cdx2-inducible ES cells, but enrichment was not observed at an intergenic region (negative control). Error bars represent mean ± s.e.m.

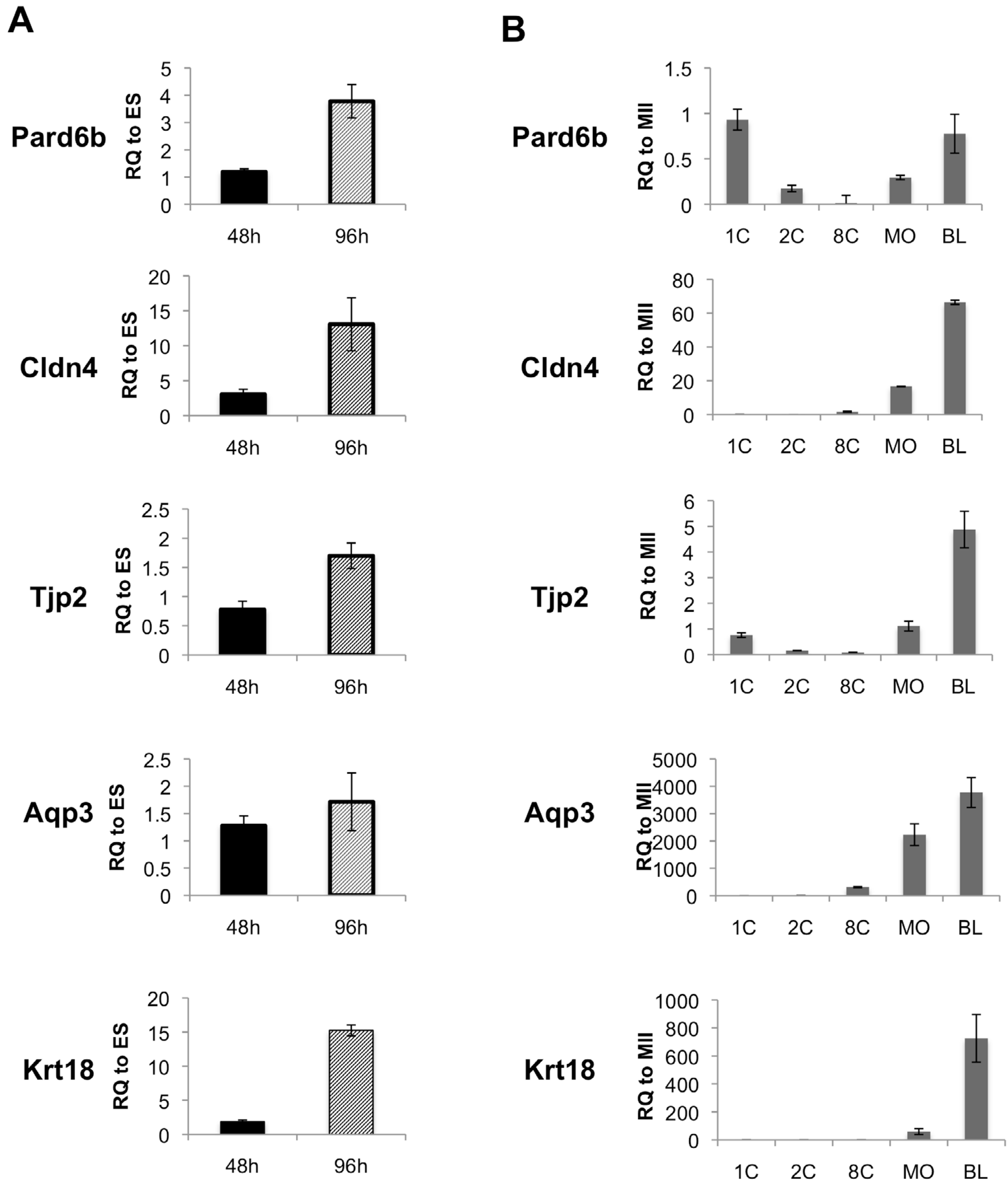

Fig. S6. Tcfap2c target genes are upregulated during ES cell differentiation into TE-like cells and during blastocyst formation. (**A**) Real-time RT-PCR analysis of Tcfap2c target genes in Cdx2-inducible ES cells at 0, 48 and 96 hours post-induction. Key blastocyst formation genes are upregulated during ES cell differentiation into TE-like cells. (**B**) Real-time RT-PCR analysis of *Pard6b*, *Tjp2*, *Cldn4*, *Aqp3* and *Krt18* transcripts in one-cell, two-cell, four-cell, eight-cell, morula, and blastocyst stage embryos. Error bars represent mean ± s.e.m.

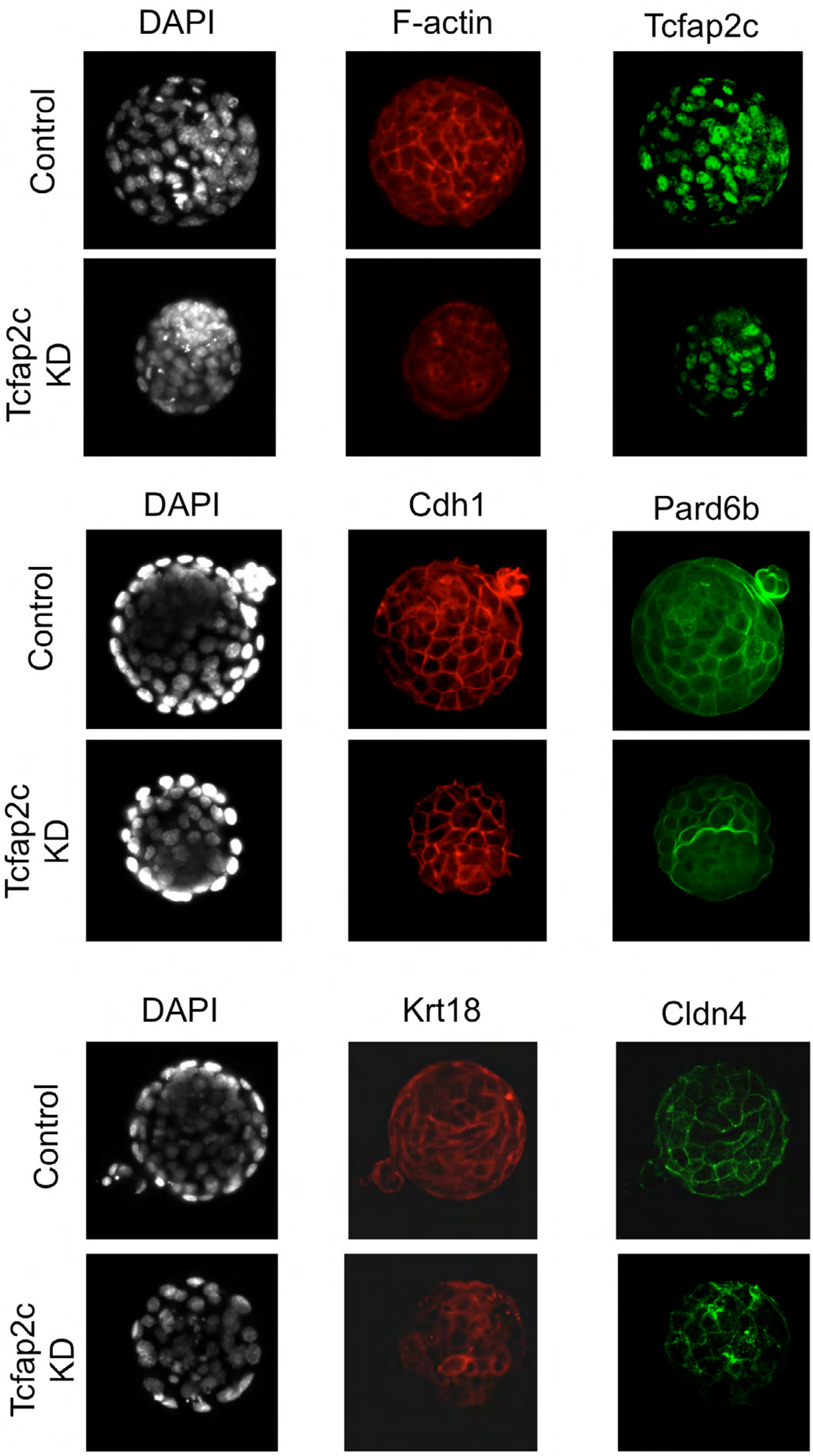

Fig. S7. Expression and subcellular localization of Pard6b, Cldn4 and Krt18 are disrupted in Tcfap2c KD blastocysts. Tcfap2c KD embryos that developed into blastocysts were stained for Tcfap2c, Pard6b, Cdh1, Cldn4, Krt18 and F-actin. Embryos were counterstained with DAPI.

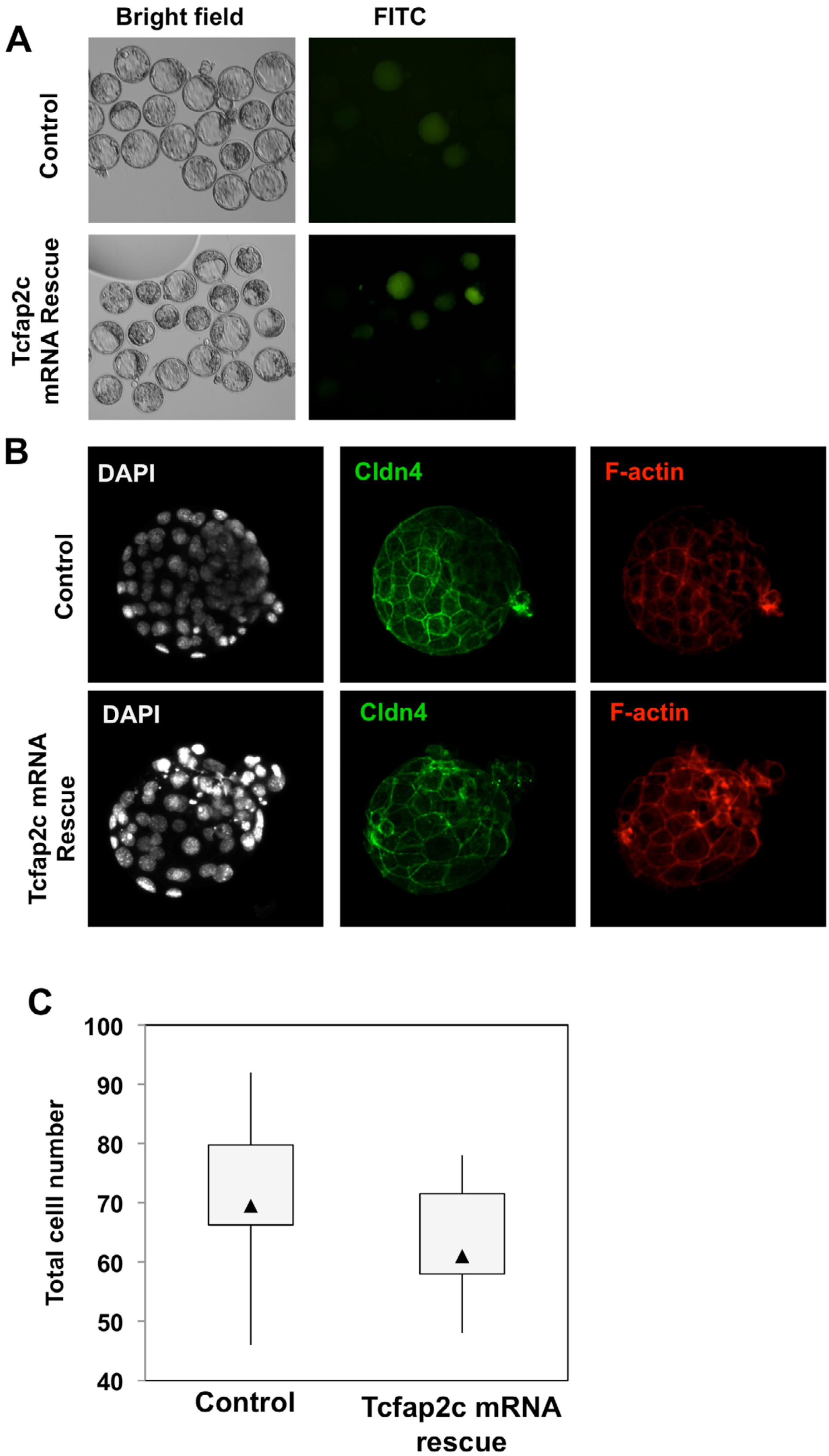

Fig. S8. Injection of *Tcfap2c* mRNA into Tcfap2c KD embryos restored paracellular sealing and TJ assembly. *Tcfap2c* mRNA-rescued blastocysts were subjected to an FITC-dextran assay and stained for TJ and cytoskeleton proteins. (**A**) Brightfield and FITC images of control and rescued blastocysts. (**B**) ICC analysis of Cldn4 and F-actin in control and rescued blastocysts. Embryos were counterstained with DAPI. (**C**) Box plot analysis of total cell numbers in *Tcfap2c* mRNA-rescued and control blastocysts. Small triangle denotes median; bars represent maximum and minimum.

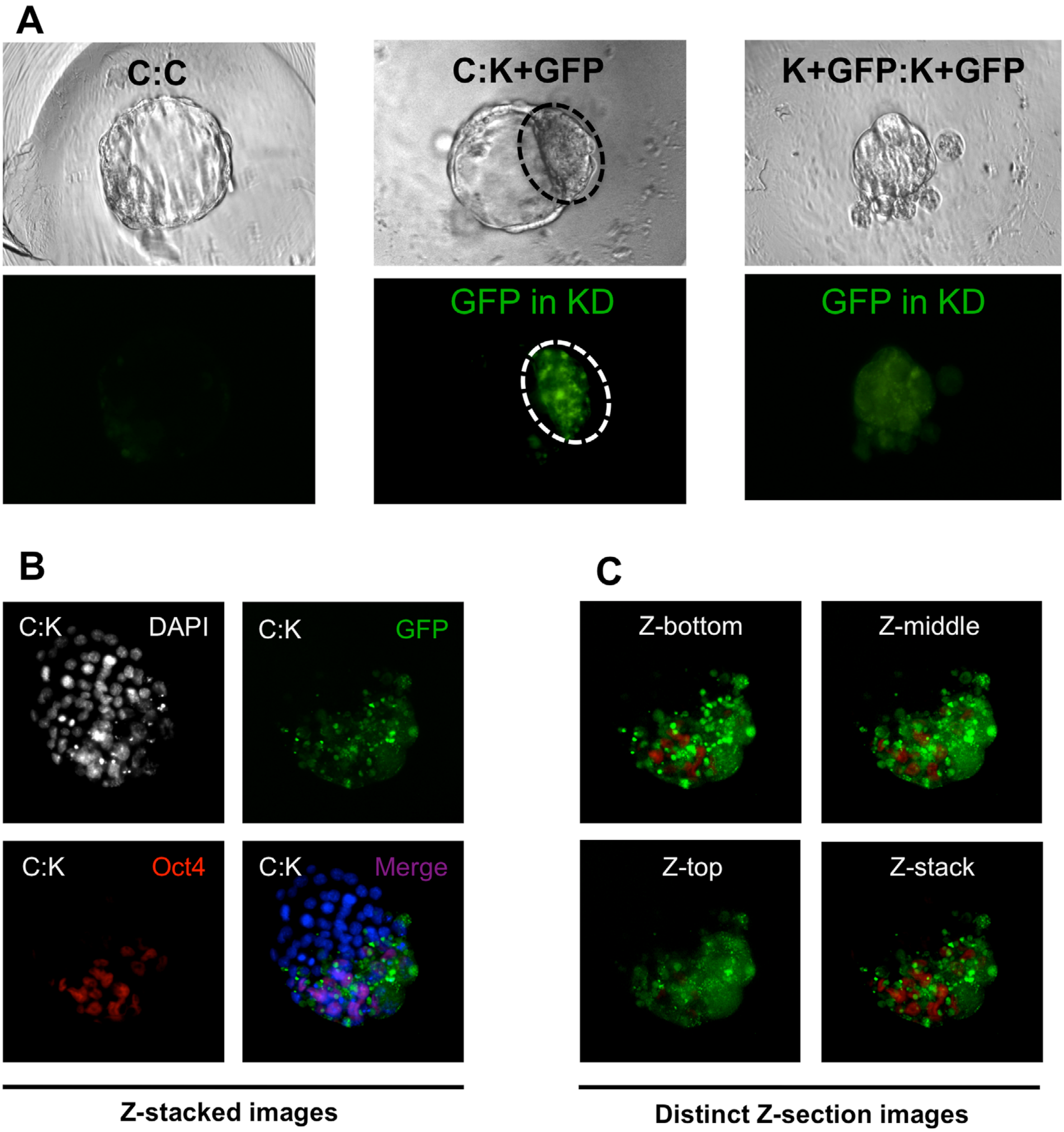

Fig. S9. Tcfap2c KD blastomeres allocate to the ICM in chimeras. GFP^+/Tcfap2c KD eight-cell embryos were aggregated with control eight-cell embryos (C:K) and cultured to the blastocyst stage. (**A**) GFP^+/Tcfap2c KD blastomeres contribute to the ICM. Dashed oval denotes GFP^+ ICM. (**B**) Combined *z*-stack showing the localization of GFP (Tcfap2c KD cells) and Oct4 in the ICM. Embryos were counterstained with DAPI. (**C**) Individual optical sections showing the colocalization of GFP (Tcfap2c KD cells) and Oct4 in the ICM of C:K blastocysts.

Table S1. Primer sequences

Gene	Forward	Reverse
Cdh1	TCCTTGTTCGGCTATGTGTC	GGCATGCACCTAAGAATCAG
beta-catenin	GTTCGCCTTCATTATGGACTGCC	ATAGCACCCTGTTCCCGCAAAG
Pard6b	ATGATGACAACTACCACAAGGCGG	GGACGAGCCGAGATATGAAGATGC
Pard3	GAGACTCTACGGAGGTCCATGT	TCGGTCATCCAGTTCTGTCTCG
Inadl	CAGCAAGTATGCCTTGGTCT	GTGGTCTAGGGTTTCCACCT
Cldn4	CGCTACTCTTGCCATTACG	ACTCAGCACACCATGACTTG
Cldn6	CATTACATGGCCTGCTATTC	CACATAATTCTTGGTGGGATATT
Cldn7	AGGGTCTGCTCTGGTCCTT	GTACGCAGCTTTGCTTTCA
Ocln	ACGTCGTGGACCGGTATC	AAAAACAGTGGTGGGGAAC
F11r	ACTGGGATTCTCCTGGATTC	CCACCAAAGAGCCAAGACTA
Jam2	TGAGGCAGCTCTAGCAGTTT	CTGTGTTCAGCTCCTCCAGT
Tjp1	TTTGGGCTGTGCATCTGA	TGCTTTATTGCTGCAGAGG
Tjp2	CCCCGAGGAGGAGGAGTA	GGGTCCCCACCGTATCTC
Atp1b3	CTGCACTCTTCACATTCACA	CACAGAATATGGCTTTAGGA
Aqp3	GTGGCTCAGGTGGTGCTCAG	CACATTGCGAAGGTCACAGCG
Aqp9	GAAGGACCGAGCCAAGAAGAAC	AGCAATAGAGCCACATCCAAGG
Krt18	GGGTCCTTCTGCATCTGGAG	GGCATCGTTGAGACTGAAATC
Fn1	TGTAGGAGAACAGTGGCAGAAA	CAGGTCTACGGCAGTTGTCA
Rock2	GGTACCTGTACATGGTGATGGAGT	TGCAAGCACTACTTCAGCAGTG
P21	CCGTTGTCTCTTCGGTCCC	CATGAGCGCATCGCAATC
P53	GCGTAAACGCTTCGAGATGTT	TTTTTATGGCGGGAAGTAGACTG
Hdac1	GTGCCTGCTTAGGAGCTCTG	CCTCCACCCTACAGAATTGG

ChIP primers	Forward	Reverse
Pard6b	GGGCACTCAGCATGAACC	GCCACTCTGCGTCCTCAC
Cldn4	GGCTGGGGGAGATGATAGTT	CCACAGGTGCTGCAGTTAAA
Tjp2	ATAGGCACTTGTCCCCCTTT	CAGGTACTCGCCTGGAGAAG
Aqp3	GTGCCTTGCGCTAGCTACTT	GTGAAGCATCTCCCCACAAC
Krt18	GTGAGGATGGCAGGTATGCT	GCGAGAGACCAGAACAGGAG
p21	TGTCTGGATATCGCTGTGGA	AAGGAGTGGGTTGGTCCTG
Intergenic negative control	TTTTCAGTTCACACATATAAAGCAGA	TGTTGTTGTTGTTGCTTCACTG

Table S2. Putative Tcfap2c target genes

Table S3. Enriched GO terms and biological processes of putative Tcfap2c target genes in blastocysts

GO term	Description	*P*-value	Enrichment
GO:0060644	Mammary gland epithelial cell differentiation	1.65E–04	101.86
GO:0071345	Cellular response to cytokine stimulus	2.37E–04	10.8
GO:0019221	Cytokine-mediated signaling pathway	8.53E–04	11.88

Table S4. Pathway analysis of genes bound by Tcfap2c and upregulated at the blastocyst stage

Pathway	Gene counts	*P*-value
Tight junction	24	4.83E–07
Regulation of actin cytoskeleton	26	1.68E–04
Focal adhesion	24	4.04E–04
Amino sugar and nucleotide sugar metabolism	9	0.002641929
Adherens junction	12	0.002738967
Pathways in cancer	30	0.006226075

Table S5. Tcfap2c target genes with known functions/phenotypes

Gene	Type of protein	Function/phenotype	Manipulations/methods	References
Pard6b	Regulation of junction assembly and polarization/cytoplasmic adaptor protein	Embryos failed to form blastocyst cavity; abnormal Tjp1 localization; Prkcz absent	shRNA plasmid injection	(Alarcon, 2010)
Cldn4	Tight junction-associated transmembrane protein	Absent or immature blastocoel cavity without expansion	Specific inhibitor treatment	(Moriwaki et al., 2007)
Cldn6	Tight junction-associated transmembrane protein	Absent or immature blastocoel cavity without expansion	Specific inhibitor treatment	(Moriwaki et al., 2007)
Cldn7	Tight junction-associated transmembrane protein	Expressed during preimplantation development; function in preimplantation development?	RT-PCR	(Moriwaki et al., 2007)
Tjp1	Cytoplasmic adaptor protein/regulation of junction assembly	KO embryos failed to develop beyond E11.5; KD embryos exhibited a decrease in blastocyst formation	Knockout, siRNA microinjection	(Katsuno et al., 2008; Sheth et al., 2008)
Tjp2	Cytoplasmic adaptor protein/regulation of junction assembly	KO embryos die around gastrulation; KD embryos showed delayed blastocoel formation and upregulation of Tjp1	Knockout, siRNA microinjection	(Katsuno et al., 2008; Sheth et al., 2008; Xu et al., 2008)
Atp1b1	Ion gradient	KD embryos fail to develop to the blastocyst stage; abnormal distribution of Ocln and Tjp1	siRNA injection	(Madan et al., 2007)
Atp1b3	Ion gradient	A gene preferentially expressed in human TE and associated with blastocyst formation in bovine	Human microarray data; a subtractive cDNA library in bovine	(Adjaye et al., 2005; Goossens et al., 2007)
Atp1a1	Ion gradient	KO embryos underwent compaction and cavitation, but showed aberrant blastocyst formation such as cell dissociation in vitro and peri-implantation lethality in vivo	Knockout	(Barcroft et al., 2004)
Apq3	Water channel protein	Aqp3 is localized on the basolateral membrane domain of the TE; mediates trans-trophectodermal water movement during cavitation	Hyperosmotic treatment	(Barcroft et al., 2003)
Apq9	Water channel protein	Aqp9 is localized at the apical membrane of the TE; mediates trans-trophectodermal water movement during cavitation	Hyperosmotic treatment	(Barcroft et al., 2003)
Cdh1	Adhesion	KO of zygotic Cdh1 results in failure to cavitate after compaction; maternal KO showed delayed compaction but normal development by zygotic expression	Knockout	(Larue et al., 1994; Riethmacher et al., 1995; de Vries et al., 2004)
Ocln	Tight junction-associated transmembrane protein	Inhibition of blastocoel formation and impaired paracellular sealing of TE; Occludin–/– mice born with no gross phenotype in the expected Mendelian ratios	Neutralizing antibodies	(Saitou et al., 2000; Kim et al., 2004)
Inadl/Patj	Regulation of junction assembly and polarization/cytoplasmic adaptor protein	Disruption of Patj expression in mice leads to delayed tight junction formation as well as defects in cell polarization	siRNA transfection	(Shin et al., 2005)
Jam2	Tight junction-associated transmembrane protein	Jam2 interacts with Tjp1 and Par3 in endothelia cells; role in blastocyst formation?	In vitro GST-pulldown assay	(Ebnet et al., 2003)
F11r/Jam 1	Tight junction-associated transmembrane protein	Compacted embryo treated with neutralizing antibodies showed delayed blastocoel cavity formation	Neutralizing antibodies	(Thomas et al., 2004)
Fn1	Tight junction-associated protein	Expression is upregulated during blastocyst formation in bovine; role in preimplantation development?	A subtractive cDNA library in bovine	(Goossens et al., 2007)
Rock2	Downstream effector of small GTPase	Mouse embryos treated with an inhibitor developed to the morula stage, but fail to form blastocysts	Specific inhibitor of Rho-kinase treatment	(Kawagishi et al., 2004)
Krt18	Cytoskeleton, intermediate filament	KD bovine embryos exhibit a decrease in blastocyst formation	dsRNA injection	(Goossens et al., 2007)