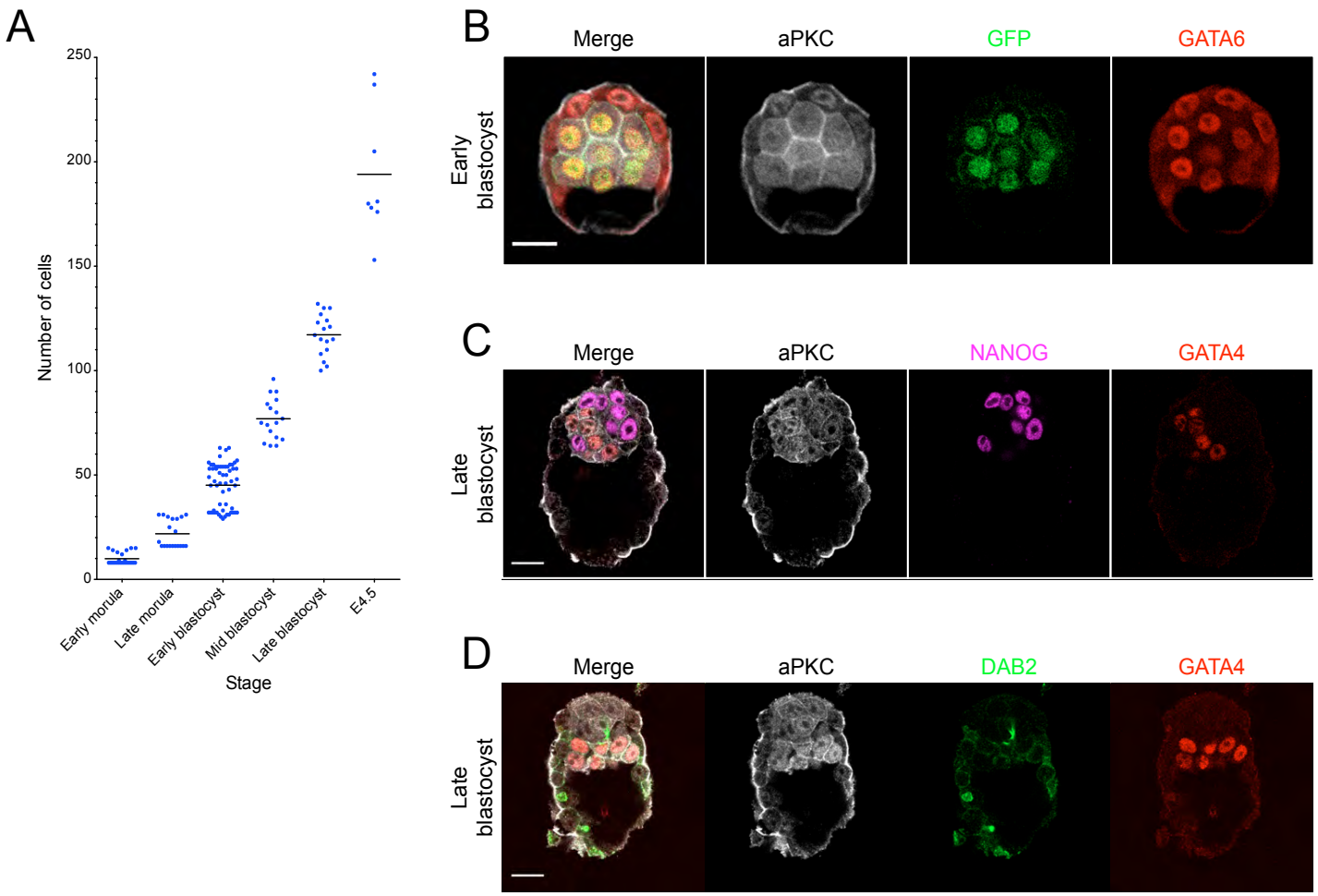


Figure S1. (A) Total cell counts for embryo staging. Embryos were collected and fixed every 6h and grouped in categories according to their number of cells, as described (Plusa et al., 2008). Each dot represents one embryo. Mean cell number±s.d., for late morula: 21.85±6.71 cells; for early blastocysts: 45.14±10.60 cells; for mid blastocysts: 76.94±9.93 cells; for late blastocysts: 117.2±10.01 cells; for E4.5 blastocysts: 194±31.40 cells. (B) Early blastocyst stained for aPKC and GATA6. Most cells at this stage display GATA6 staining. All ICM cells express similar levels of GATA6, *Pdgfra* (visualised as nuclear GFP) and aPKC. Membrane GFP: GPI-tagged GFP, nuclear GFP: H2B-GFP expressed from the *Pdgfra* locus (*Pdgfra*$^{H2B\text{-}GFP}$). (C) Late blastocyst stained for aPKC, NANOG and GATA4. Note the mutually exclusive NANOG and GATA4 distribution and the higher levels of aPKC in the GATA4+ population. (D) Late blastocyst stained for aPKC, DAB2 and GATA4. Note that DAB2 does not yet appear polarised whereas some cells begin to display apical aPKC. Scale=20μm.

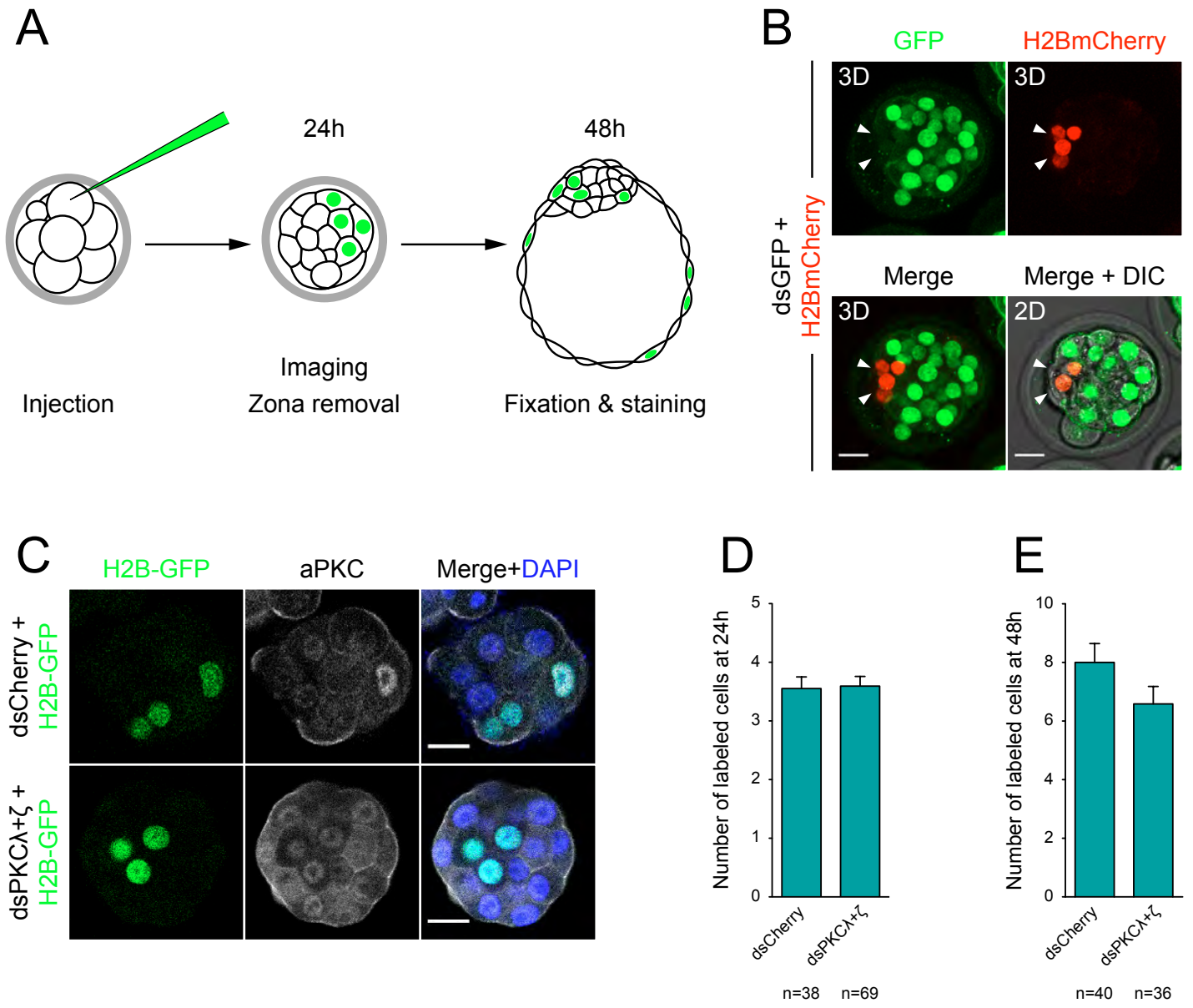


Figure S2. (A) Schematic of dsRNA injection. (B) Live images of a control *CAG::H2B-GFP/CAG::H2B-GFP; CAG::GFP-GPI/+* embryo injected with dsGFP and H2B-mCherry as a tracer. Arrowheads point at the injected clone, showing reduced levels of both nuclear and membrane GFP. Fluorescence panels are maximum intensity projections (3D), Merge + DIC displays a single medial plane. (C) Immunofluorescence for aPKC in representative embryos fixed 24h post-injection (hpi). Note the reduced levels of cytoplasmic aPKC in the experimental embryo. (D) Total number of injected cells per embryo 24hpi. (E) Total number of injected cells per embryo 48hpi. Bar charts display mean+SEM. n: number of embryos. Scale=20μm.

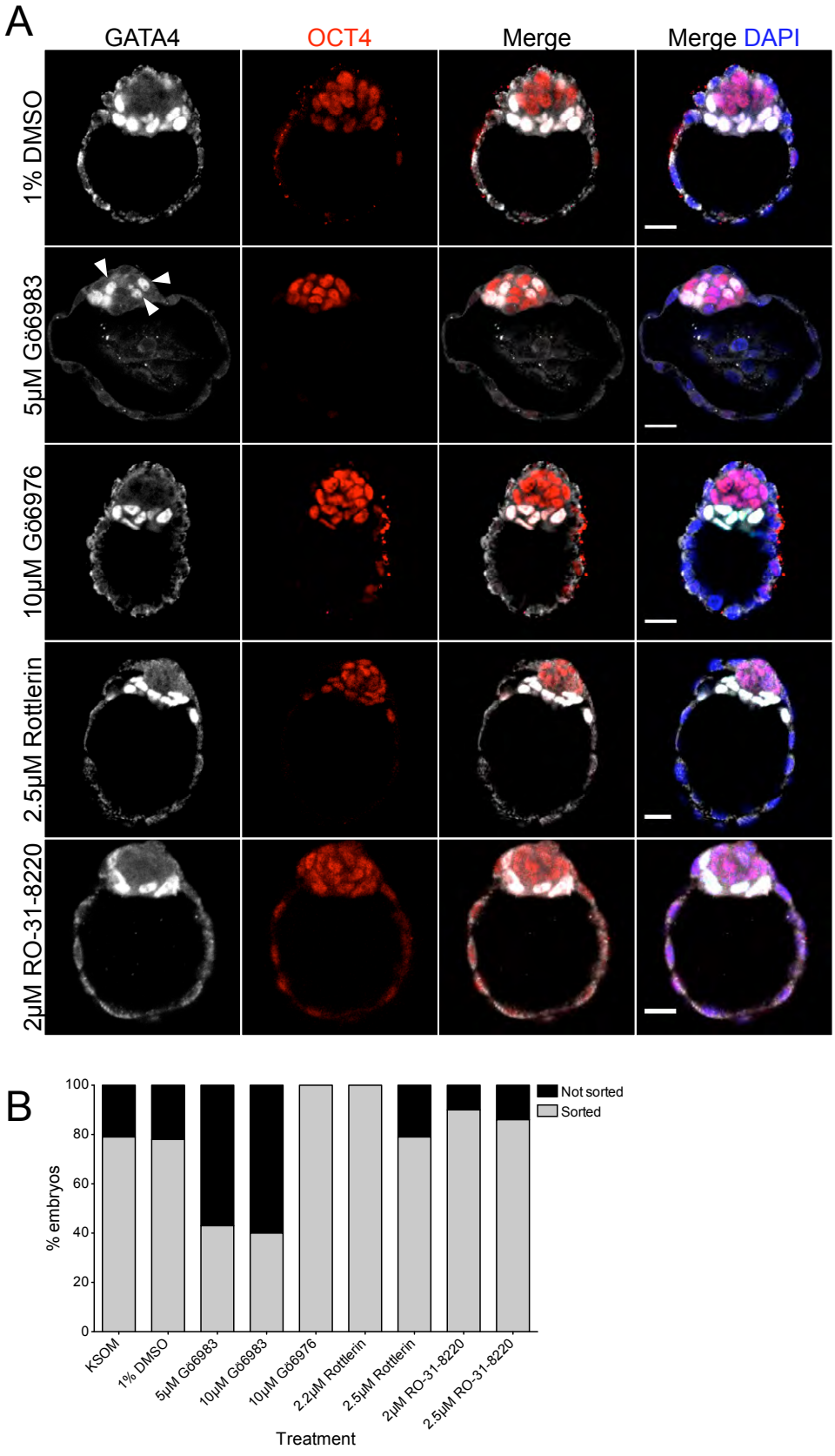


Figure S3. (A) Immunofluorescence for GATA4 and OCT4 in representative embryos treated with different PKC inhibitors as described in Figure 4A (see also Tables 2 and S2). Arrowheads point at PrE cells scattered throughout the ICM in embryos treated with 5µM Gö6983. (B) Percentage of embryos showing a sorted PrE layer for each concentration of the inhibitor or control conditions (see also Table 2 for number of embryos and Table S2 for details on each inhibitor's targets and specificity). Embryos treated with Gö6983 displayed a sorted PrE in a significantly lower number of cases than either the controls or other PKC inhibitors (X^2 test, $p<0.0001$ when comparing Gö6983 to all the other groups). See Table 2 and Materials and Methods for details on the statistical analysis.

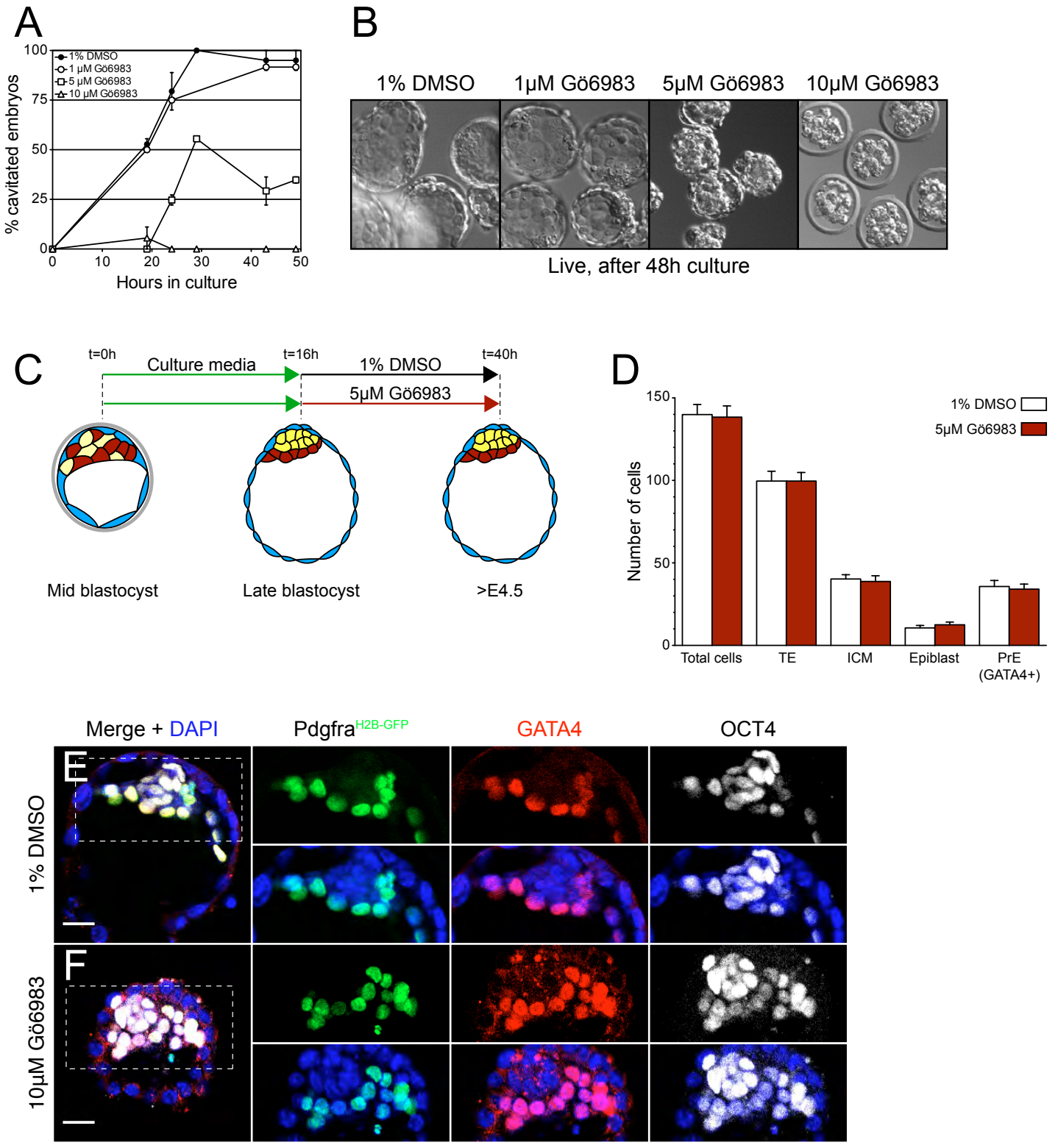


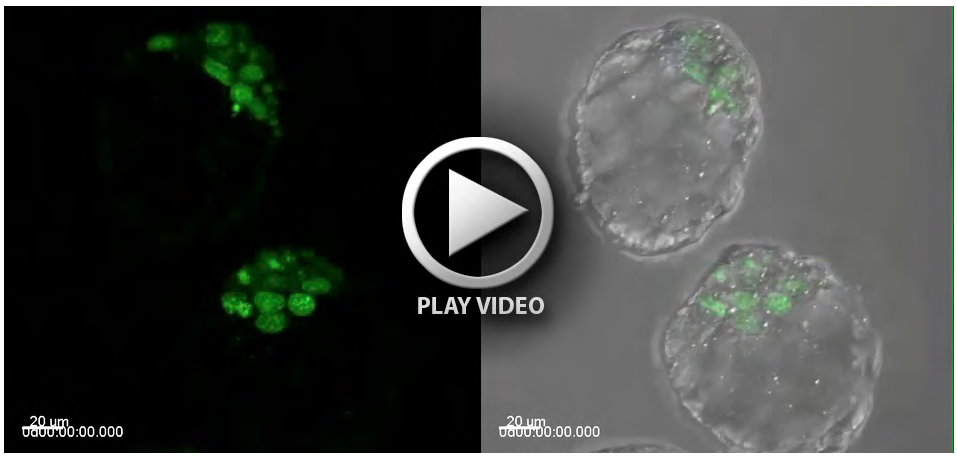
Figure S4. (A) Cavitation of 8-cell stage embryos cultured in control conditions or with increasing concentrations of Gö6983 for 48h. (B) Images after culture for 48h. Embryos treated with 5µM of Gö6983 fail to expand the cavity and show defects in the TE, although the ICM is not damaged. Lower concentrations (1µM) of the inhibitor are comparable to control conditions, whereas higher concentrations (10µM) are lethal at these stages. (C) Schematic of late treatment with Gö6983, from the late blastocyst (sorted PrE) for 24h. (D) Cell counts for each blastocyst lineage after treatment with 10µM Gö6983. No significant difference in the number of cells was found for any of the lineages between control and experimental embryos (unpaired Student's t-test). n=11 embryos for 1% DMSO, n=8 embryos for 10µM Gö6983. (E, F) Late blastocysts stained for GATA4 and OCT4 after culture from the late blastocyst in 1% DMSO or 10µM Gö6983, as shown in (C). Insets: detail of ICMs. Each channel also overlaid with nuclear staining in all panels. Scale=20µm.

Movie 1. Z-stack through the mid blastocyst in Figure 1B showing aPKC and GATA4 staining.

Movie 2. Z-stack through the late blastocyst in Figure 1C showing aPKC and GATA4 staining.

Movie 3. Z-stack through the E4.5 blastocyst in Figure 1D showing aPKC and GATA4 staining.

Movie 4. Control embryos cultured in the presence of 1% DMSO from the mid to late blastocyst as shown in Figure 4B. Whole Z-stacks were acquired every 15 minutes over a period of 16h. Maximum intensity projections are shown for GFP only and GFP+DIC. Movie shown at 4 frames per second (fps).



Movies 5 and 6. Experimental embryos cultured in the presence of 5μM Gö6983 from the mid to late blastocyst as shown in Figures 4C, D. Whole Z-stacks were acquired every 15 minutes over a period of 16h. In Movie 5 a cell that loses its position at the ICM surface and dies deeper in the ICM is highlighted with a pink spot. A cell highlighted in blue comes into contact with the cavity, but fails to maintain its position and migrates back into the ICM. A cell marked in yellow remains anchored at the ICM surface but eventually dies, as GFP+ cells that fail to reach the ICM surface by the late blastocyst. In Movie 6, note how cells remain scattered throughout the ICM. Although they display visibly lower cell death, they fail to form a monolayer at the ICM surface. Maximum intensity projections are shown for GFP only and GFP+DIC. Movie shown at 4fps.

Movie 7. Z-stack through a representative control embryo treated with 1% DMSO and stained for GATA4 and OCT4 (see also Figure 5A').

Movies 8 and 9. Z-stacks through representative embryos treated with 5μM Gö6983. In Movie 8, note the GFP+ cells scattered throughout the ICM and displaying pan-cellular distribution of GATA4 (see also Figure 5B'). In Movie 9, GFP+, GATA4+ PrE cells cluster on a side of the ICM without forming a monolayer.

Table S1. Antibodies used for immunofluorescence

Epitope	Host	Dilution	Company	Clone	Catalog #
PKCζ	rabbit	1:200	Santa Cruz	C-20	sc-216
GATA4	goat	1:100	Santa Cruz	C-20	sc-1237
GATA4	rabbit	1:100	Santa Cruz	H112	sc-9053
GATA6	goat	1:100	R&D		AF1700
Nanog	rat	1:100	eBioscience	eBioMLC-51	14-5761
NANOG	rabbit	1:300	Cosmo Bio		RCAB0001P
OCT4	mouse	1:100	Santa Cruz	C-10	sc-5279
DAB2	mouse	1:200	BD		BD-610464
E-cadherin	rat	1:300	Sigma	DECMA-1	U3254
LRP2	rabbit	1:75	Sigma		HPA005980

Table S2. Comparison of the specificities for the PKC inhibitors used

		cPKCs			nPKCs					aPKCs	
Inhibitor	**Cat #**	**PKCα**	**PKCβ**	**PKCγ**	**PKCδ**	**PKCε**	**PKCη**	**PKCθ**	**PKCμ**	**PKCλ**	**PKCζ**
Gö6983	365251	7 nM	7 nM	6 nM	10 nM				20 μM		60 nM
Gö6976	365250	2.5 nM	6 nM						20 nM		
Rottlerin	557370	30-45 μM	30-45 μM	30-45 μM	3-6 μM	100 μM		3-6μM			100 μM
RO-31-8220	557520	5 nM	10-25 nM	10-25 nM		10-25 nM					

Blastocysts were treated with broad-range PKC inhibitors to assess the specificity of Gö683 and its effect on aPKC. IC_{50} values or ranges are given in nM or μM for each inhibitor-enzyme combination. IC_{50} indicates the concentration necessary to reduce the enzyme's activity to 50% in an *in vitro* assay. Lower IC_{50} values indicate a higher affinity for the enzyme. IC_{50} values as provided by the manufacturer. All inhibitors were purchased from Calbiochem.