SUPPLEMENTARY INFORMATION

SUPPLEMENTARY FIGURES

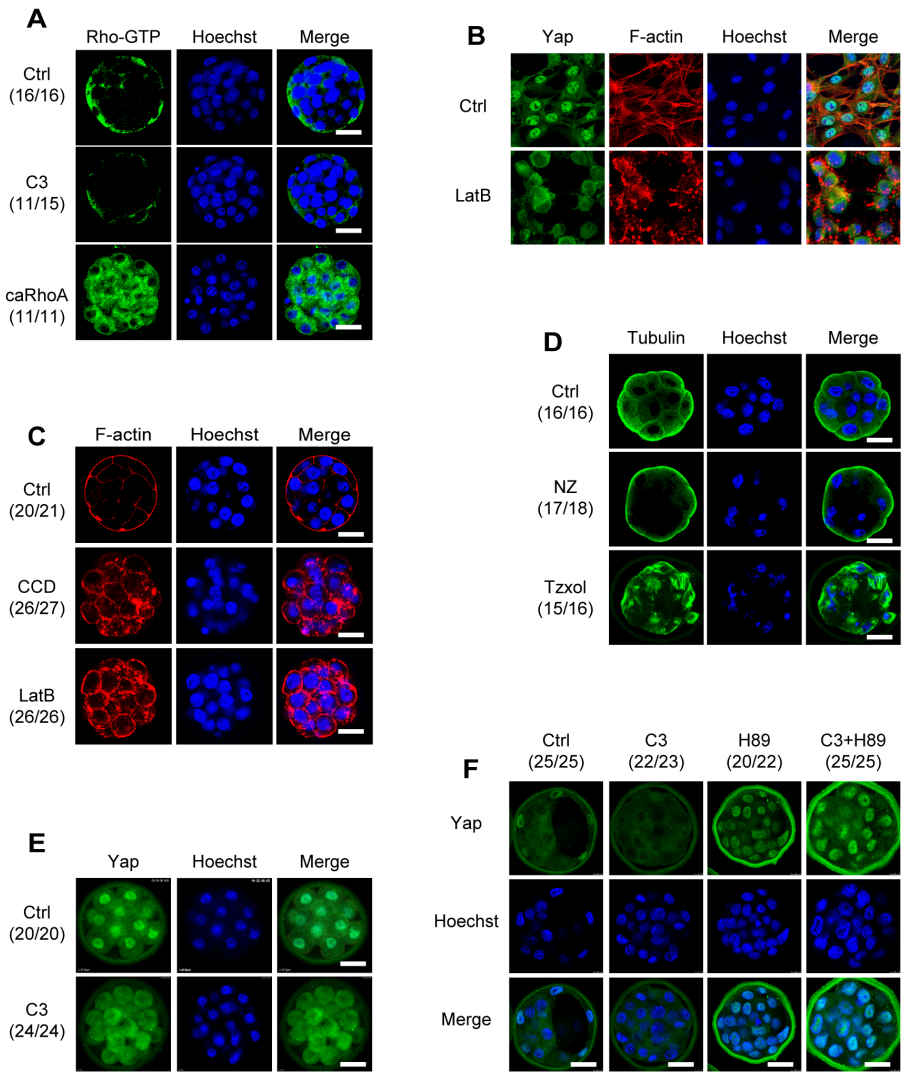

Fig. S1. Related to Fig. 2. Cell contacts, but not $G\alpha_s$-coupled GPCRs, suppress Rho signaling in ICM cells. (A) Verification of the specificity of the Rho-GTP affinity assay. Late morula / early blastocysts treated with C3 or injected with 10 ng/µl caRhoA mRNA, were subjected to Rho-GTP affinity assay. Bars: 25 µm. (B) Immunofluorescence staining of 3T3 cells treated with or without LatB for 2 hours. (C) Immunofluorescence staining of F-actin in embryos treated with CCD and LatB. (D) Immunofluorescence staining of tubulin in embryos treated with nocodazole (NZ) and taxol. Embryos were treated as described in Fig. 2C. (E) Late morula and early blastocysts were treated with calcium free medium to dissociate blastomeres, and then treated with or without C3 for 2 hours, followed by immunofluorescence analysis. (F) Late morula and early blastocysts were treated with PKA inhibitor H89 alone, Rho inhibitor C3 alone, or the combination of H89 and C3 for 2 hours, followed by immunofluorescence analysis. Bars: 25 µm.

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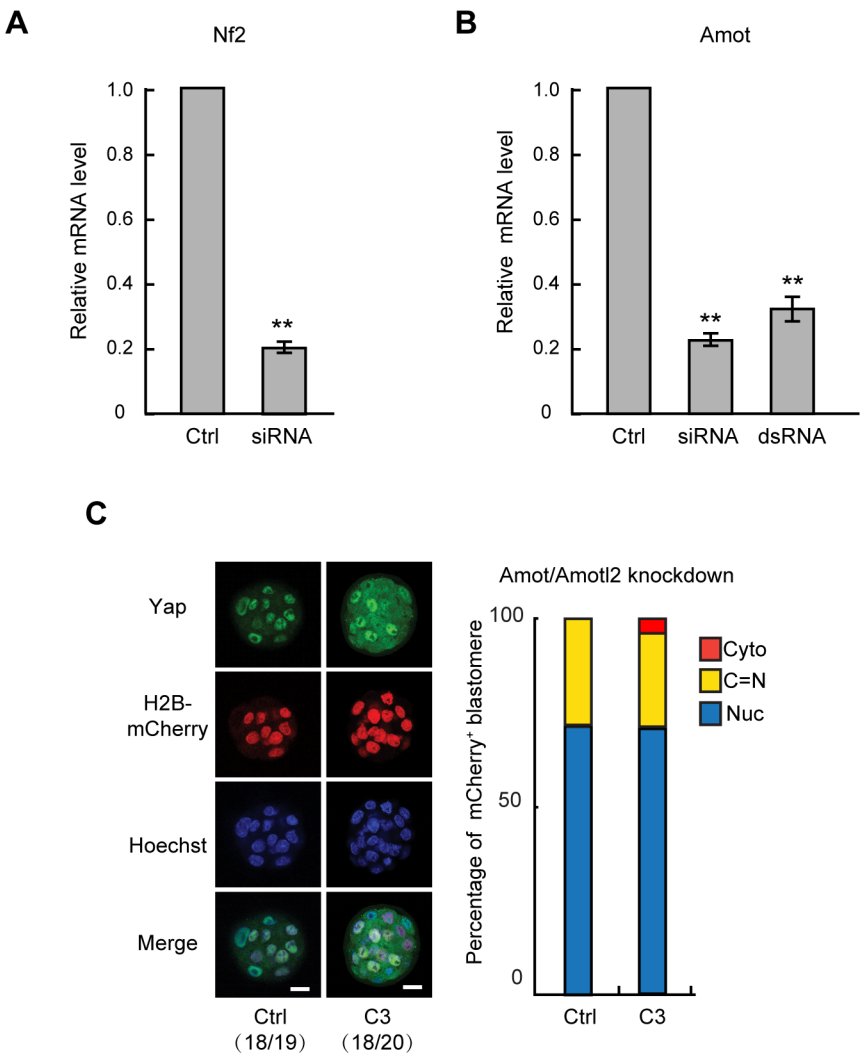

Fig. S2. Related to Fig. 3. Validation of the knockdown efficiency for *Nf2* and *Amot*, and the redundancy of Amot and Amotl2. (A) *Nf2* siRNA or negative control siRNA were transfected into embryonic stem cells. Twenty-four hours after transfection, cells were harvested. RNA was purified from these cells and subjected to quantitative RT-PCR analysis. (B) Similar to (A), except that *Amot* siRNA, *Amot* dsRNA, or negative control siRNA were transfected into embryonic stem cells. Data are shown as mean ± SD (n = 3). (C) One blastomere of the 2-cell embryo was injected with both Amot and Amotl2 siRNA, together with H2B-mCherry mRNA. At the late morula stage, embryos were treated with or without C3 for 2 hours, and fixed for immunofluorescence assay. mCherry fluorescent signals mark the progeny cells from the injected 2-cell blastomere. Left panels show the representative images. Right panel summarizes the data from about 20 embryos and more than 100 mCherry positive blastomeres.

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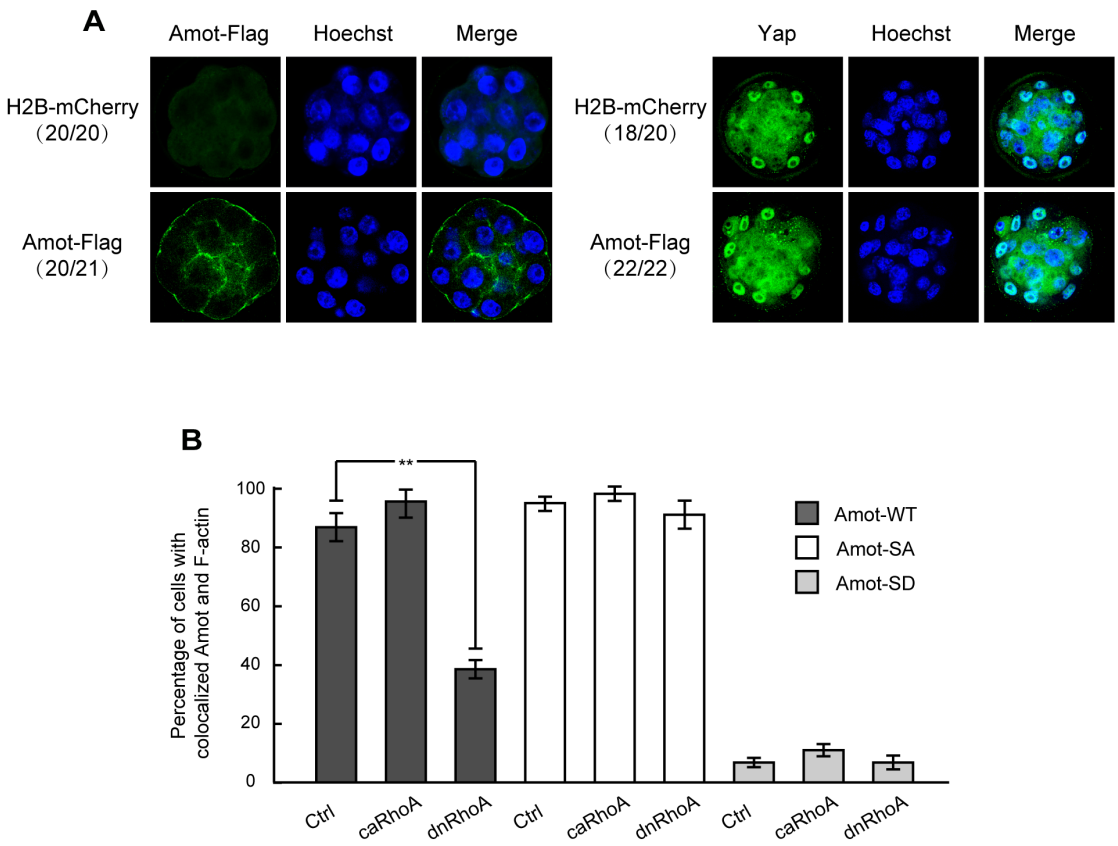

Fig. S3. Related to Fig. 4. Rho regulates the colocalization of Amot and Nf2. (A) Zygotic Amot-Flag mRNA Injection does not Yap distribution in the blastocyst. Zygotes were injected with 200 ng/µl Amot-Flag mRNA or H2B-mcherry mRNA. Blastocyst stage embryos were subject to immunofluorescence detection of Amot and Yap. Bars: 25 µm. (B) Cells with colocalized Amot and F-actin are counted from the images shown in Fig. 4C. The percentages of cells with colocalized Amot and F-actin are plotted.

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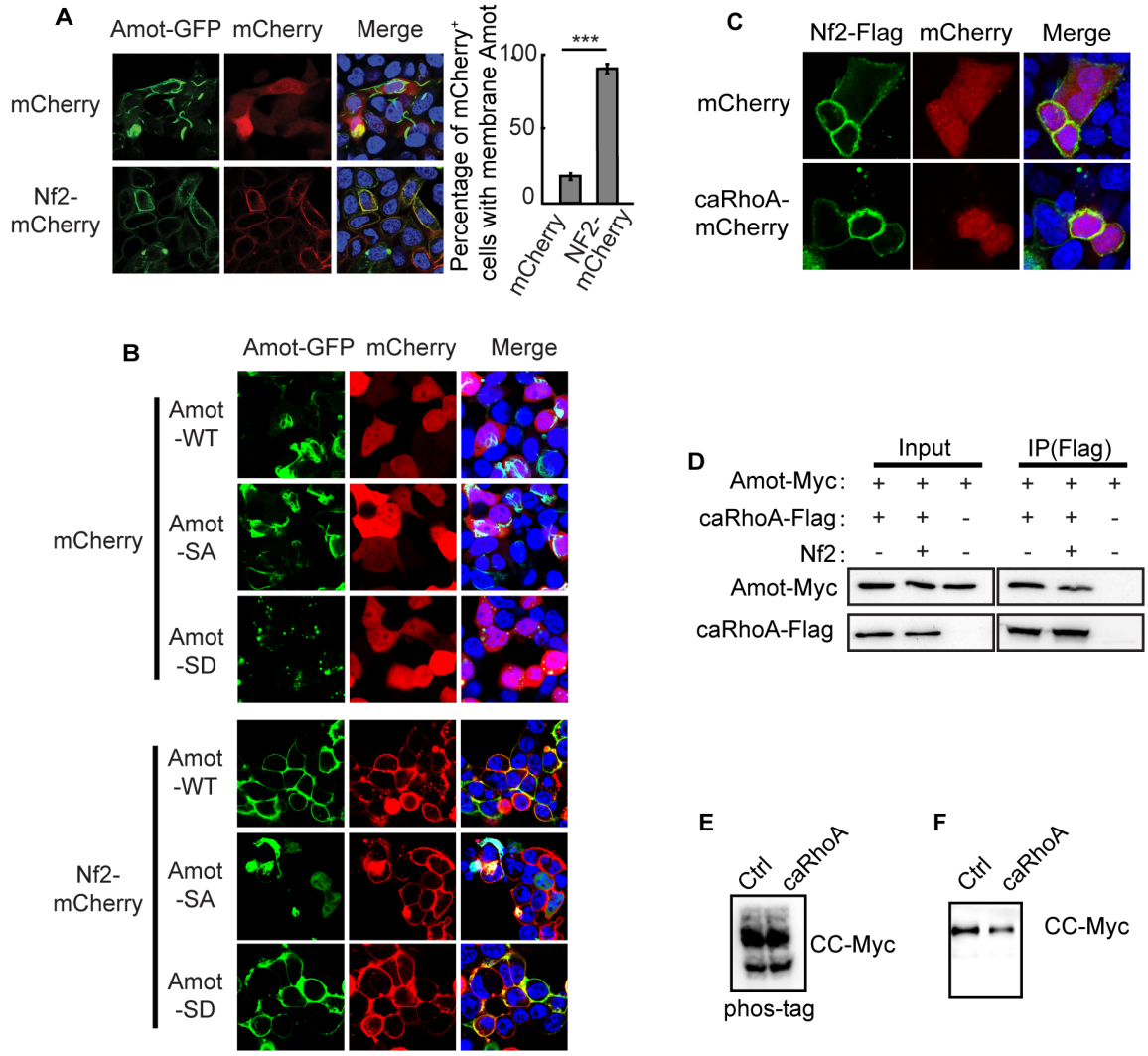

Fig. S4. Related to Fig. 5. Nf2 recruits Amot to the plasma membrane. (A) Similar to Fig. 5A, except that the experiments were carried out with Hela cells, but not with HEK293T cells. (B) GFP tagged Amot-WT, SA, and SD, were expressed, with mCherry or Nf2-mCherry, in HEK293T cells. Confocal images were taken to visualize the localization of Amot and Nf2. (C) Nf2-Flag expression vector, together with plasmids expressing mCherry or caRhoA-mCherry, was transfected into HEK293T cells. Twenty-four hours later, immunofluorescence staining with Flag antibody was performed to visualize the distribution of Nf2. mCherry signals indicate the transfected cells. (D) Plasmids expressing caRhoA-Flag and Amot-Myc, with empty or Nf2 expression vector, were co-transfected into HEK293T cells. Twenty-four hours after transfection, cells were harvested and subjected to co-IP experiment with anti-Flag M2 beads. (E-F) caRhoA does not change the electrophoretic mobility of Amot CC domain in phos-tag (E) or regular SDS-PAGE (F) gel. Plasmid expressing CC-Myc, with empty or caRhoA expression vector, was transfected into HEK293T cells. Cells were harvested at 24 hours after transfection, and cell lysates were prepared for phos-tag (E) or regular SDS-PAGE (F) electrophoresis.

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SUPPLEMENTARY TABLE

Table S1. Working concentrations of inhibitors and activators

Signal Pathway	Inhibitor / activator	Working Concentration
Ras-MAPK	PD98059	1 μM
	PD0325901	1 μM
PI3K	MK2206	3 μM
RhoA-ROCK	C3 transferase	1 μg/ml
	CCG1423	10 μM
	Y27632	20 μM
PKC	D-sphingosine	2.5 μM
	GÖ 6976	1.32 μM
	Ro-31-8220	5 μM
PKA	H89	10 μM
	KT5720	5 μM
GSK3	CHIR	3 μM
GPCRs	PMA	10 ng/ml
	LPA	10 μM
	Ki16425	10 μM
PPase	Okadaic acid	0.25 μM

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