Supplementary Figure Legends

Figure S1: IQGAP1 depletion induces spindle misorientation.
MDCK cells were transfected with three different siRNA heteroduplexes targeting canine IQGAP1. The mean angle formed between the mitotic spindle and the apicobasal axis was: control = 75.53° ± 2.69, siRNA#1 = 51.22° ± 4.14; siRNA#2 = 45.47° ± 4.57, siRNA#3 = 48.02° ± 4.73 (n=3, >40 cysts per experiment; values represent the mean ± s.d.)

Figure S2: The absence of IQGAP1 does not affect apical vesicle transport.
(A) MDCK cells were transfected with a Slp2a siRNA or IQGAP1 siRNA and cysts were then grown for 72 h, fixed and stained for β-catenin (green), Podxl (red) and ToPro3 (blue). White arrows indicate internal vesicle accumulation. Cysts forming a single lumen were quantified: control, 74.6 ± 4.3%; siSlp2a, 53.28 ± 0.6%; siIQGAP1, 55.1 ± 3.5% (n=3, >50 cysts per experiment; values represent the mean ± s.d.). Vesicle accumulation was quantified: control, 37.4 ± 10%; siSlp2a, 61.21 ± 6.1%; siIQGAP1, 34.7 ± 8.9% (n=3, >50 cysts per experiment; values represent the mean ± s.d.). (B) Apical biotinylation assay. The apical recovery of Podxl was analyzed in control and IQGAP1-silenced cells at 0, 30, 60 and 90 min after trypsin treatment. Pixel intensity of biotinylated Podxl at each time point was represented relative to no trypsinized cells (NT). (C) Calcium switching experiments to quantify trans-epithelial resistance (TER) or impedance, using an electrical cell-substrate impedance system (ECIS) in monolayers (Lo et al, 1995). Control or IQGAP1-depleted cells were polarized in monolayers for 72h, and they were left overnight
in low-calcium medium to disrupt their cell-cell interactions and the internalization of apical proteins (Vega-Salas et al, 1987, 1988). After the addition of calcium, normal TER levels were recovered similarly in control and IQGAP1-silenced cells, whereas cells in which Cdc42 was silenced, a protein implicated in tight junction formation at cell-cell contacts (Joberty et al, 2000; Wells et al, 2006), showed a marked delay in TER recovery (Suppl. Fig. 2A) and in the exocytosis of apical proteins (Rodríguez-Fraticelli et al, 2010). Control (grey), siIQGAP1 (red) and siCdc42 (pink) are represented, the values representing the mean ± s.d. from 3 different experiments. Error bars represent the s.d.: *p < 0.05; **p<0.01. Scale bars, 7 µm.

Figure S3: IQGAP1 depletion does not affect interphase centrosome positioning.
A) MDCK cells were transfected with control or IQGAP1 siRNA and grown in 3D cultures. After 72 h the cysts were stained for actin (red), γ-tubulin (green) and β-catenin (blue). (B) The mean distance of the centrosomes with respect to the apical membrane: control = 0.542 ± 0.11, siIQGAP1 = 0.528 ± 0.10 (n=3; >50 cells per experiment. Values represent the mean ± s.e.m.). (C) Mean distance between the centrosomes of the cells: control = 1.025 ± 0.22, siIQGAP1 = 0.856 ± 0.19 (n=3; >50 cells per experiment. Values represent the mean ± s.e.m.). Scale bars, 5 µm.

Figure S4: Ser1441 phosphorylation does not mediate the apical translocation of IQGAP1 induced by PMA treatment.
(A) Scheme showing the region of human IQGAP1 thought to be phosphorylated by aPKC after exposure to PMA, and a comparison with dog IQGAP1. (B) Cysts stably
expressing IQGAP1-S1441A GFP were treated for 15 min with DMSO (control) or 4 µM PMA, and then stained for actin (red) and IQGAP1 (blue). The basolateral (arrows) and apical (arrowheads) localization of the mutant construct is indicated. Scale bars, 5 µm.

**Figure S5: IQ motifs are necessary for the efficient binding of IQGAP1 to the EGFR.**

(A) The interaction of IQGAP1 and IQGAP1 ΔIQm with EGFR. (B) IQGAP1 interacts with EGFR in the presence and absence of EGF stimulation. (C) MDCK cells forming cyst for 72 h were treated for 24h with EGF, fixed and stained for actin (red) and ToPro3 (blue). White arrows indicate the new lumens formed and the clusters of cells invading the lumen. (D) Percentage of cysts forming normal lumens was represented.

**Figure S6: EGF treatment induces tubulogenes**sis.

MDCK cells were grown in 3D collagen cultures as described previously (Leroy and Mostov, 2007), and after 10 days they were treated for 24 h with EGF. The cells were then fixed and stained for IQGAP1 (green), actin (red) and β-catenin (blue). Scale bars, 5 µm.