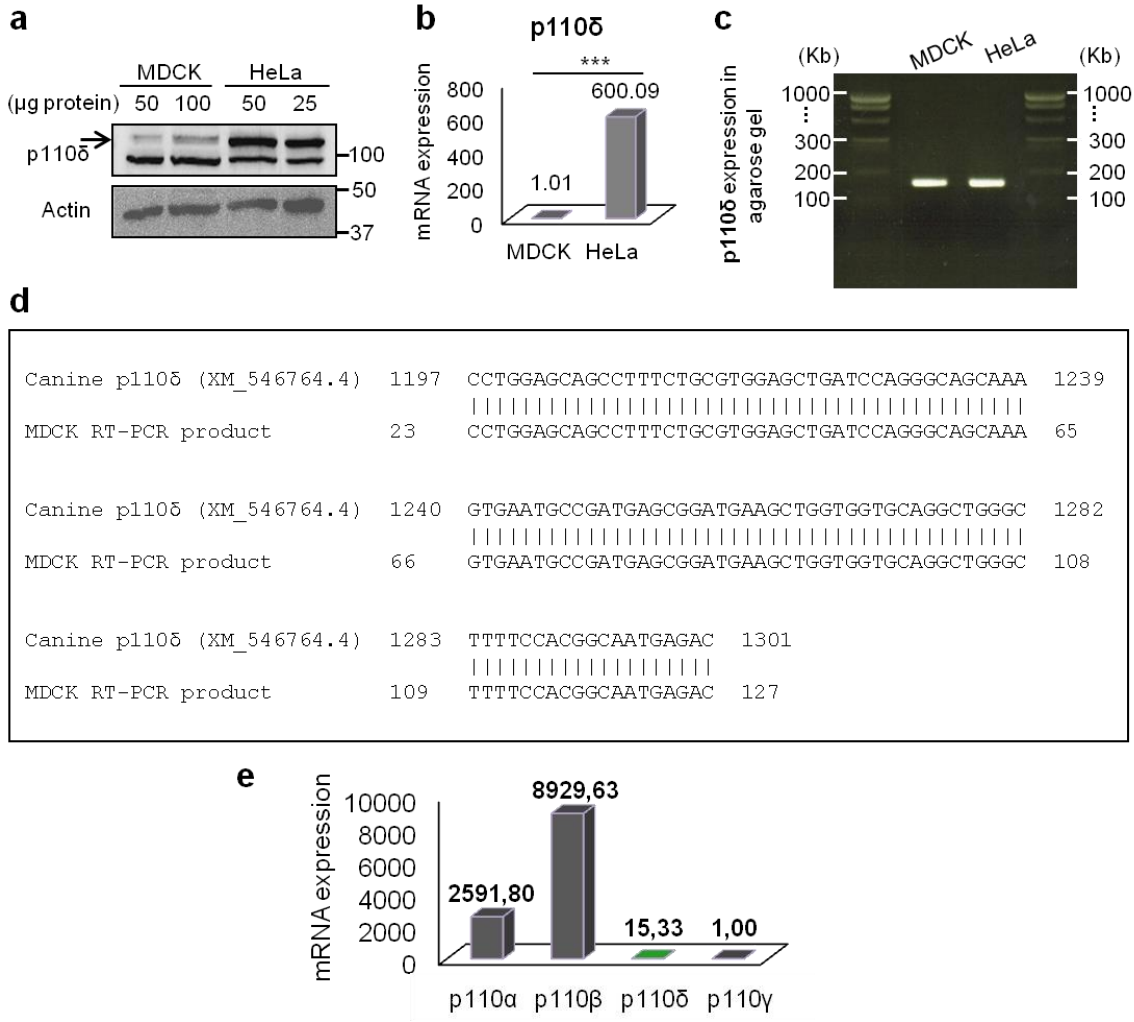
Supplementary Figure 1

Inhibitor (Target)	Concentration (µM)	Open lumen (%)	Multi lumens (%)	No lumen (%)	Small (%)	Inverted (%)
No treatment		81.92±3.71	NS	18.08±3.71	NS	NS
PI-103 (p110α/β/γ/δ)	0.1	76.01±2.7	6.73±2.82	15.8±4.3	1.45±1.29	NS
	0.5	74.72±2.68	4.76±2.03	18.47±4.04	2.05±0.96	NS
	1	53.8±4.93	5.04±2.88	24.12±8.94	17.04±3.85	NS
	5	25.75±4.16	4.93±3.39	22.66±5.31	46.66±4.22	NS
	10	6.97±3.43	3.45±2.01	27.26±5.08	62.33±5.75	NS
AS-605240 (p110γ)	0.1	77.28±7.75	22.72±7.75	NS	NS	NS
	0.5	67.86±6.3	22.21±7.76	NS	9.93±4.69	NS
	1	59.58±6.54	19.98±4.92	NS	20.44±6.71	NS
	5	20.23±3.52	18.94±3.75	12.82±4.28	48.01±8.06	NS
	10	8.56±5.43	15.05±5.45	39.72±5.57	36.68±5.6	NS
TGX115 (p110β/δ)	0.1	70.94±6.24	6.03±2.05	21.55±5.1	NS	1.49±0.14
	0.5	59.77±5.58	10.03±2.10	27.13±5.14	NS	3.07±1.95
	1	45.61±3.62	19.54±4.77	31.19±5.59	NS	3.66±1.49
	5	37.34±6.95	18.33±4.89	39.46±9.73	NS	4.87±2.03
	10	32.87±8.74	12.62±3.53	48±9.75	NS	6.52±2.43
IC87114 (p110δ)	0.1	71.5±5.58	NS	25.36±4.42	NS	3.14±2.07
	0.5	63.6±2.76	NS	32.73±3.84	NS	3.67±2.26
	1	69.07±9.05	NS	11.86±3.27	NS	19.07±6.92
	5	56.17±4.95	NS	23.33±5.97	NS	20.5±3.8
	10	11.67±5.69	NS	54.02±8.3	NS	34.31±6.34
CAL-101 (p110δ)	0.1	81.58±3.57	NS	14.8±4.38	NS	3.63±2.37
	0.5	79.2±5.81	NS	16.91±6.09	NS	3.9±2.55
	1	63.86±6.48	NS	22.56±7.35	NS	13.58±3.07
	5	60.33±7.16	NS	19.33±5.91	NS	20.33±4.03
	10	35.38±5.42	NS	27.49±6.56	NS	37.13±5.11

Supplementary Figure 1 | Effect of isoform-selective inhibitors of PI3K on apico-basal polarity of MDCK cells.

MDCK cells were plated on Matrigel to form cysts, 24 h after plating cells were treated for 72 h with different concentrations (from 0.1 to 10 µM) of isoform-selective inhibitors of PI3K: PI-103, AS- 605240, TGX115, IC87114 and CAL-101. The images of cysts presented different phenotypes (Normal lumen, multi lumen, no lumen, small cysts with no lumen, and inverted polarity) were presented in Fig.1. The percentage ± s.e.m of each of phenotype as a function of the concentration of inhibitor is presented in the figure. The data were calculated from 3 independent experiments. NS = not seen.

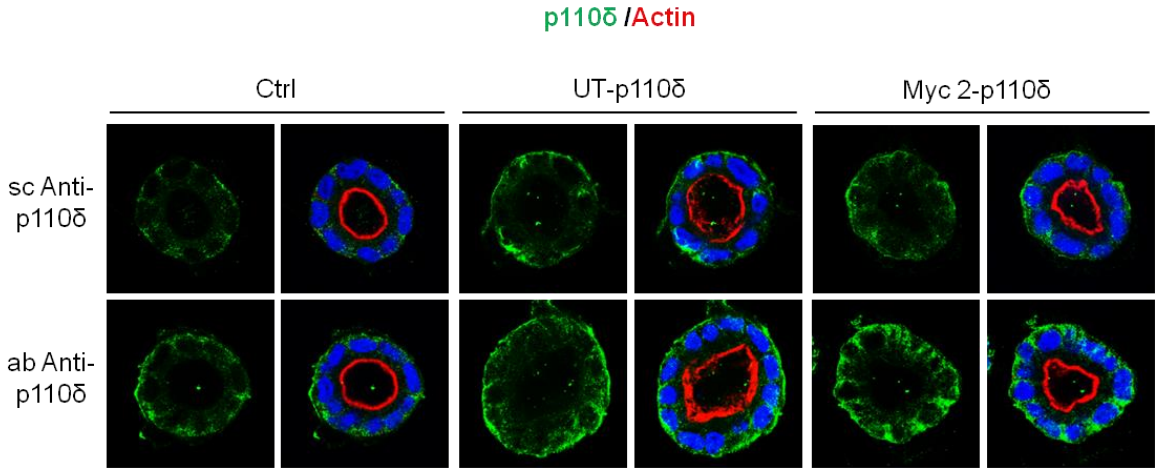
Supplementary Figure 2



Supplementary Figure 2 | p110δ expression in MDCK cells.

(**a**) Immunoblot analysis of p110δ in MDCK and HeLa cells lysates using p110δ specific antibody (sc7176). 50 and 100 µg or 50 and 25 µg of lysate from MDCK and HeLa cells respectively were loaded on the gel and actin was used as a loading control. (**b**) qRT-PCR analysis of p110δ mRNA expression in MDCK and HeLa cells. (**c**) Products of RT-PCR in (**b**) were collected and migrated in agarose gel electrophoresis. The gels were visualized on a U.V. trans-illuminator by staining the DNA with a fluorescent dye (ethidium bromide). The DNA molecular weight markers were indicated at both side of the photograph. (**d**) The product of qRT-PCR from MDCK cells (in **b**) was purified and sequenced. The alignment of the obtained sequence with the canine p110δ sequence in database (XM_546764.4) was shown. (**e**) Total RNA from MDCK cells was analyzed by qRT-PCR for p110δ, p110α, p110β and p110γ expression and normalized to GAPDH (see Methods).

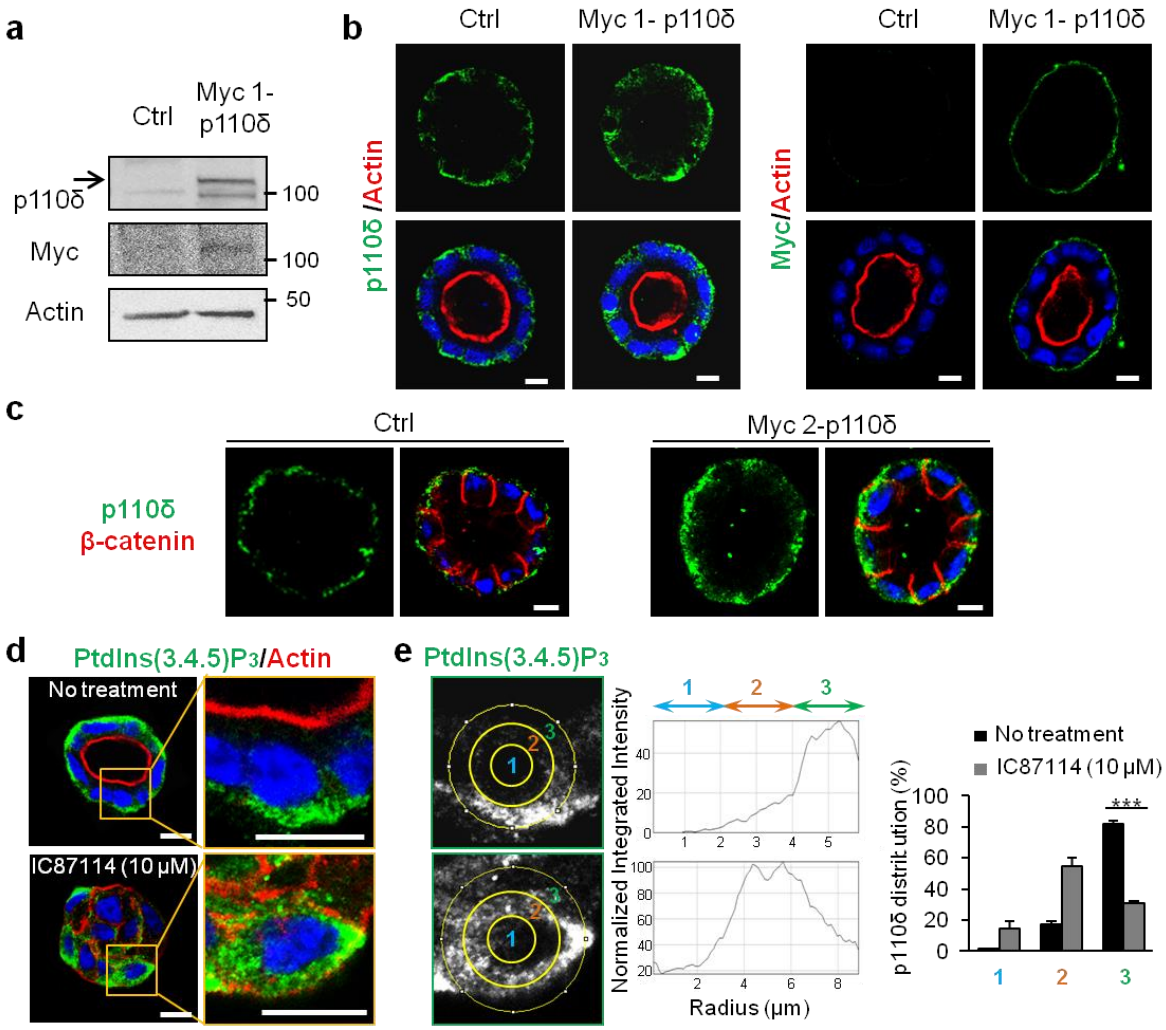
Supplementary Figure 3



Supplementary Figure 3 | Different staining of p110δ in MDCK cysts.

MDCK cells were transfected or not (Ctrl) with UT-p110δ or Myc 2-p110δ cDNAs (see methods) and plated on Matrigel to form cysts and stained for p110δ (green), actin (red) and Hoechst (blue). Two different p110δ specific antibodies (sc7176 and ab32401) were used for p110δ staining.

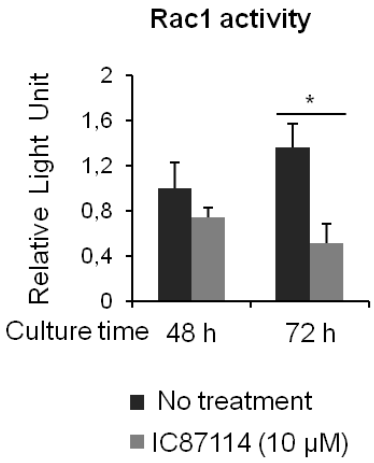
Supplementary Figure 4



Supplementary Figure 4 | p110δ regulates PtdIns(3,4,5)P_3 membrane localization

MDCK cells were transfected or not (Ctrl) with 1 µg of Myc 1-p110δ cDNA and p110δ was analyzed (**a**) either by immunoblot, using anti p110δ (sc7176) and anti Myc. Actin is used as loading control, (**b**) or by immunofluorescence on cysts using anti p110 δ (ab32401) or Myc (green), actin (red) and Hoechst (blue). (**c**) Cysts from MDCK cells transfected or not (Ctrl) with 1 µg of Myc 2-p110δ cDNA were stained for p110δ (green) using anti p110δ (ab32401), β -catenin (red) and Hoechst (blue). (**b**, **c**) Single confocal section through the middle of cyst is shown and the scale bar represents 10 µm. (**d**) Analysis of PtdIns(3,4,5)P_3 (green), actin (red) and Hoechst (blue) in MDCK cysts treated or not for 72 h with IC87114 at 10 µM. A higher magnification is presented in the right column. (**e**) Signal of PtdIns(3,4,5)P_3 in an individual cell and the plot of distribution were analyzed as described in Fig; 4b. Values from analyzed of 20 cells were represented in histogram as mean ± s.e.m. Triple asterisks indicate student t test P<0.0001.

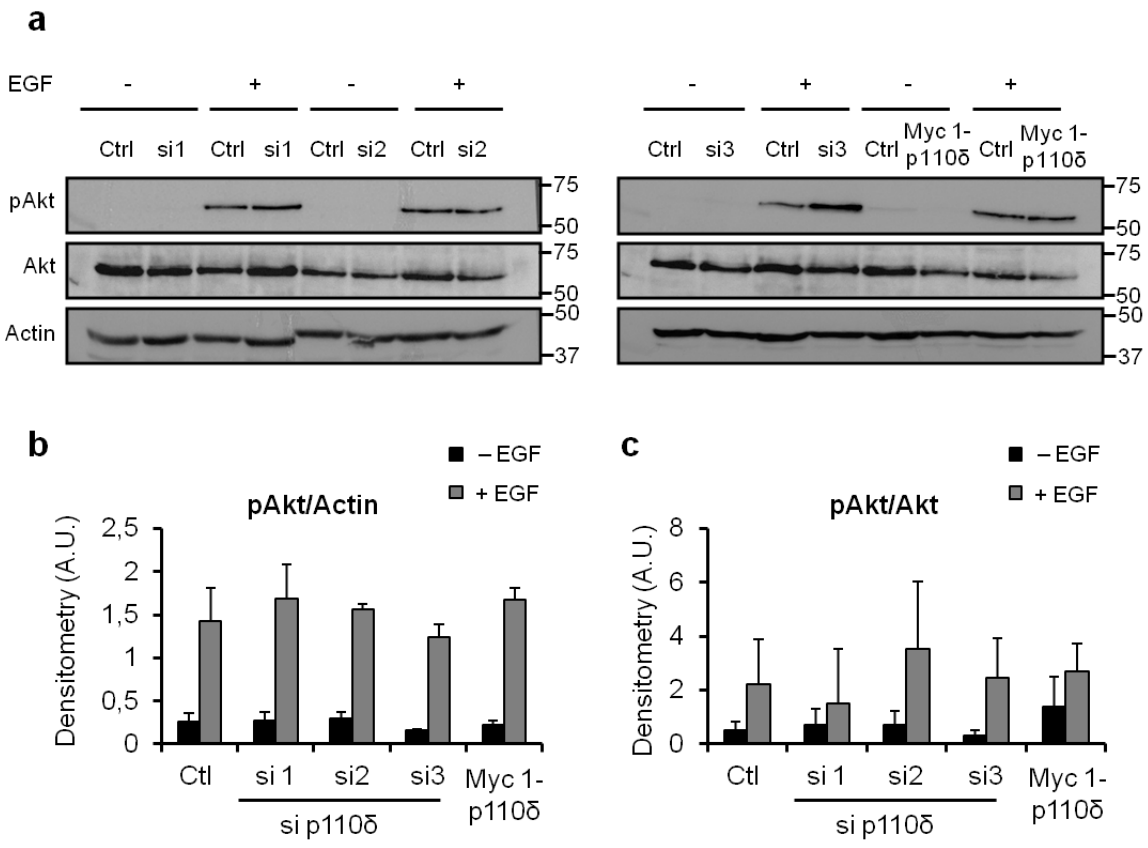
Supplementary Figure 5



Supplementary Figure 5 | p110δ regulates Rac1 activation.

MDCK cells were plated on Matrigel to form cyst and treated or not 24 h after plating with IC87114 (10 µM) and grown for another 48 and 72 h with a daily change of the medium and the lysates of cells were used to quantify Rac1 activation using G-LISA Biochem Kit (luminescence based). Intensity was measured using luminometer and normalized to data from 48 h of culture with no treatment. Values from three independent experiments are expressed as mean ± s.e.m. Asterisk indicates student t test $P<0.05$.

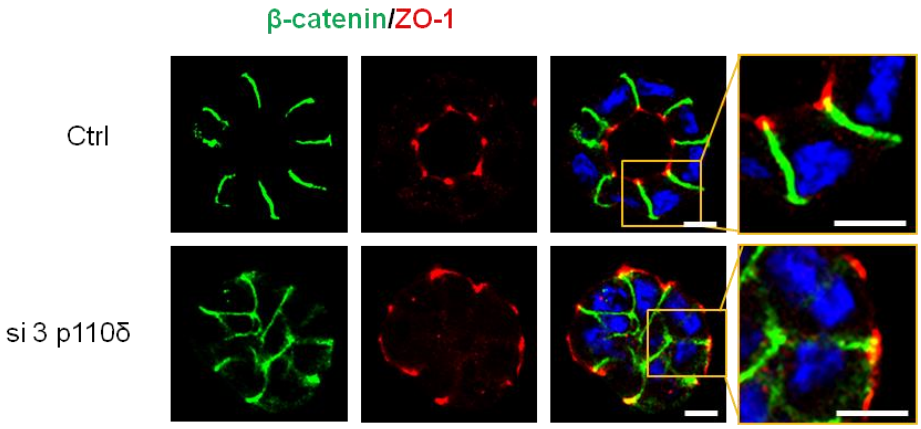
Supplementary Figure 6



Supplementary Figure 6 | p110δ expression doesn't affect the phosphorylation status of Akt.

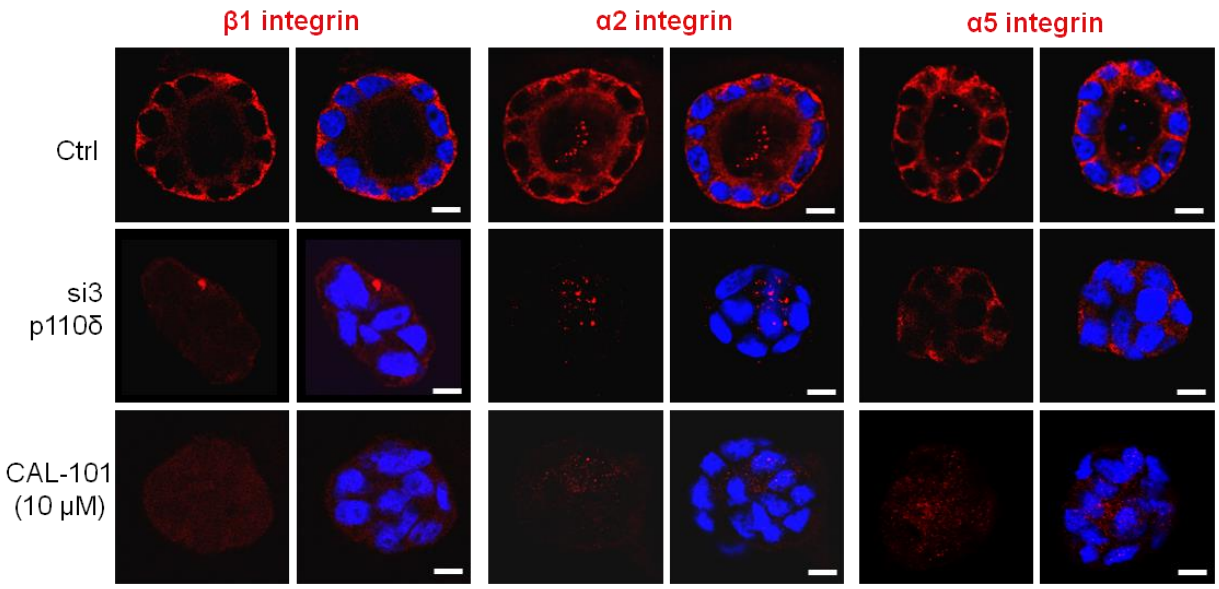
(**a**) MDCK cells grown on plastic dishes were transfected or not (Ctrl) with 100 pmol of three different si p110δ (si1, si2, si3), and with Myc 1-p110δ cDNA. 48h after transfection, cells were treated or not for 15 min with EGF (100 ng/ml) and analyzed by immunoblot for pAkt and Akt . Actin is used as loading control. The densitometry analysis was normalized to actin (**b**) and to total Akt (**c**), and data from 2 independent experiments were represented as arbitrary unit (A.U.) in the histograms. Values from three independent experiments are expressed as mean ± s.e.m.

Supplementary Figure 7



Supplementary Figure 7 | ZO-1 staining in p110δ depleted and invert polarized cysts. MDCK cells transfected or not with 100 pmol of p110δ si3 RNA were grown in Matrigel for 4 days to form cysts and then stained for β -catenin (green), ZO-1 (red) and Hoechst (blue) as indicated on the pictures. A confocal section through the middle of cyst is shown. A higher magnification of the data in the orange square is presented in the right panels. The scale bar represents 10 μm.

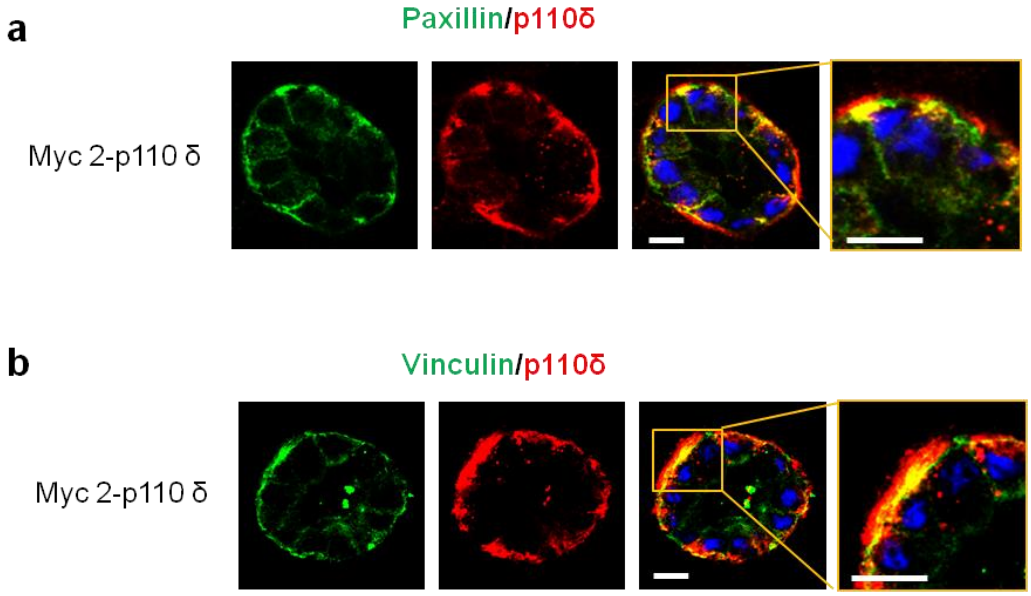
Supplementary Figure 8



Supplementary Figure 8 | Effect of p110δ on β1, α2 and α5 integrins.

MDCK cells transfected or not with 100 pmol of p110δ si3 RNA or treated with CAL-101 were grown in Matrigel for 4 days to form cysts, then fixed and stained for β1, α2 and α5 integrins (red) and Hoechst (blue) as indicated on the pictures. A confocal section through the middle of cyst is shown. The scale bar represents 10 μm.

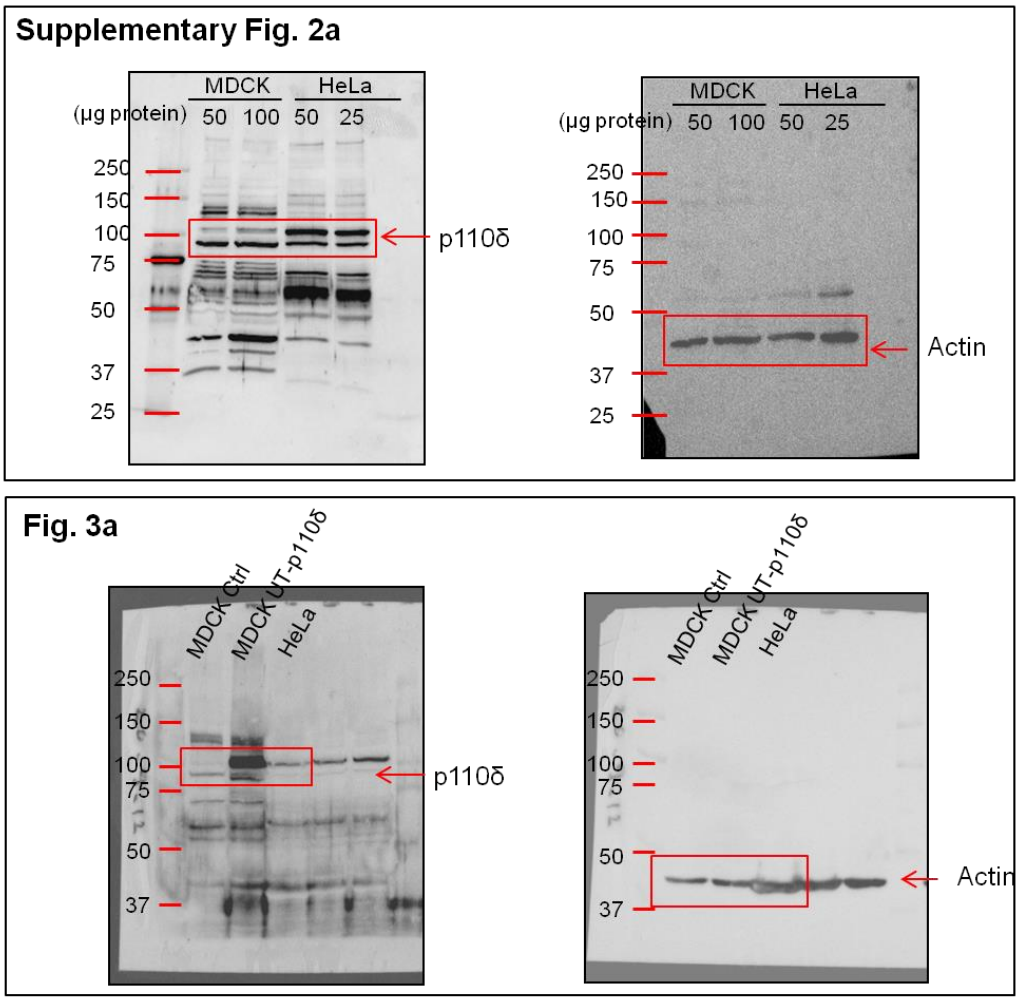
Supplementary Figure 9



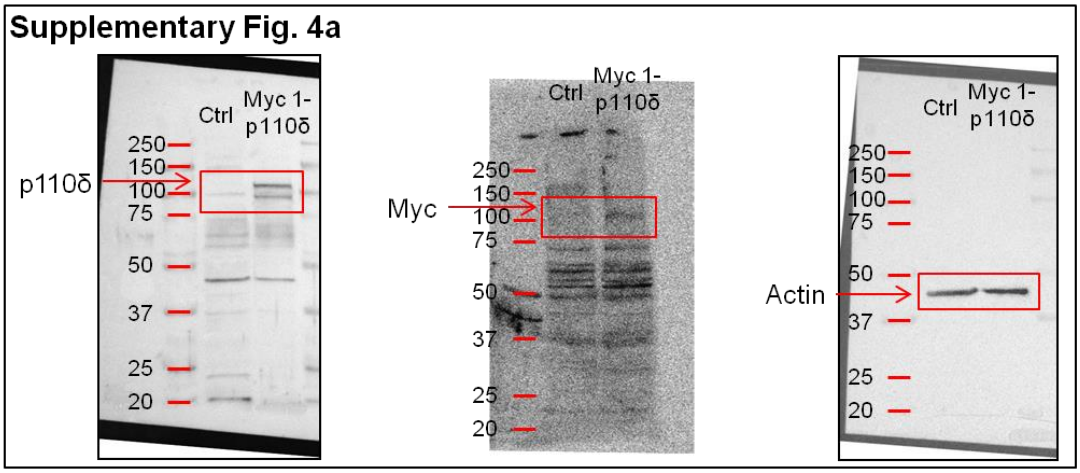
Supplementary Figure 9 | Paxillin and vinculin are partly colocalized with p110δ

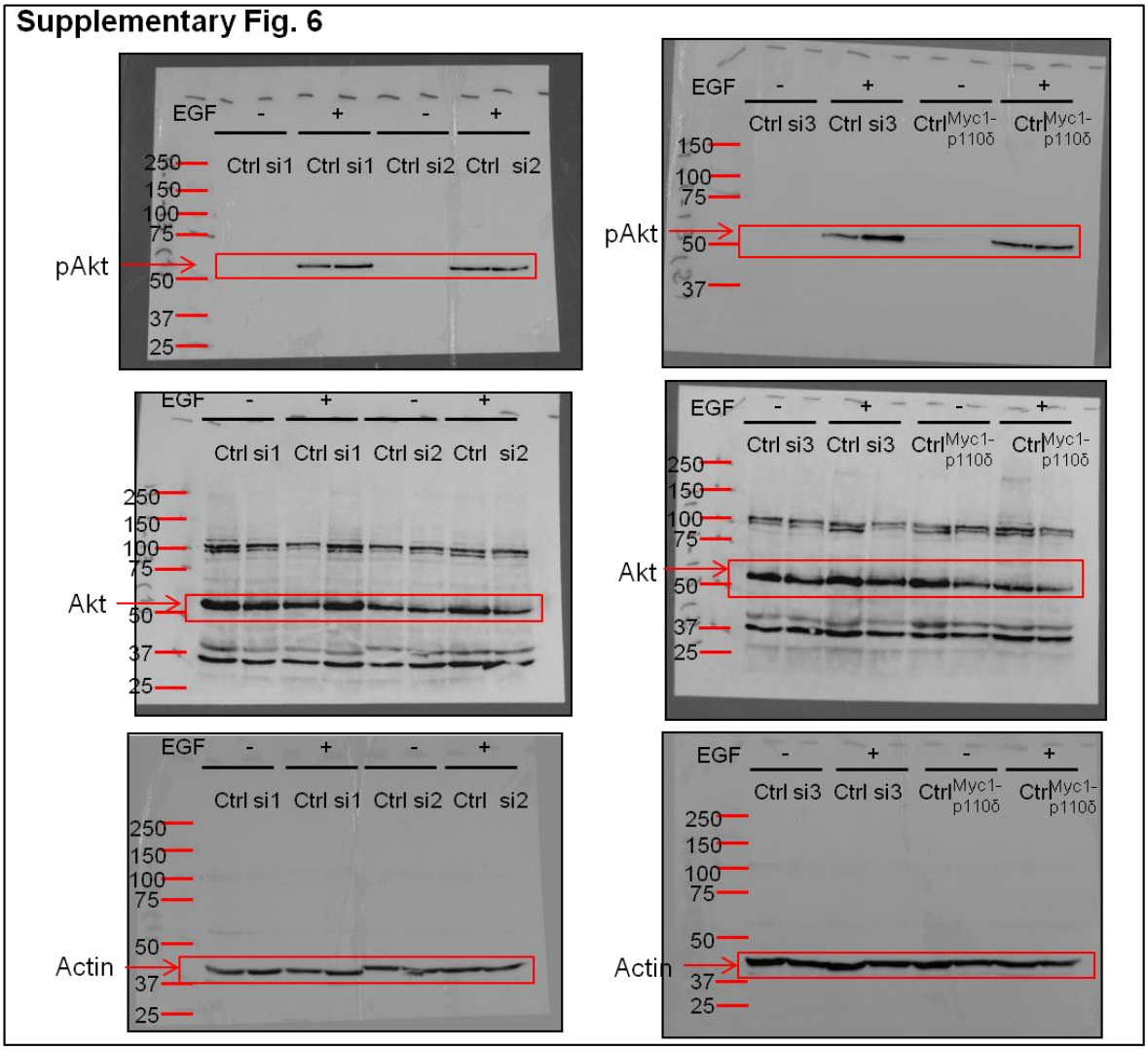
(**a**) MDCK cells transfected with 1 µg of myc 2-p110δ cDNA were grown on Matrigel for 4 days to form cysts, then fixed and stained for Paxillin (green), p110δ (red) using a p110δ-specific antibody (ab32401) and Hoechst (blue) as indicated on the pictures. A confocal section through the middle of cyst is shown. A higher magnification of the data in the orange square is presented in the right column. The scale bar represents 10 µm. (**b**) same as (**a**) for vinculin staining (green).

Supplementary Figure 10

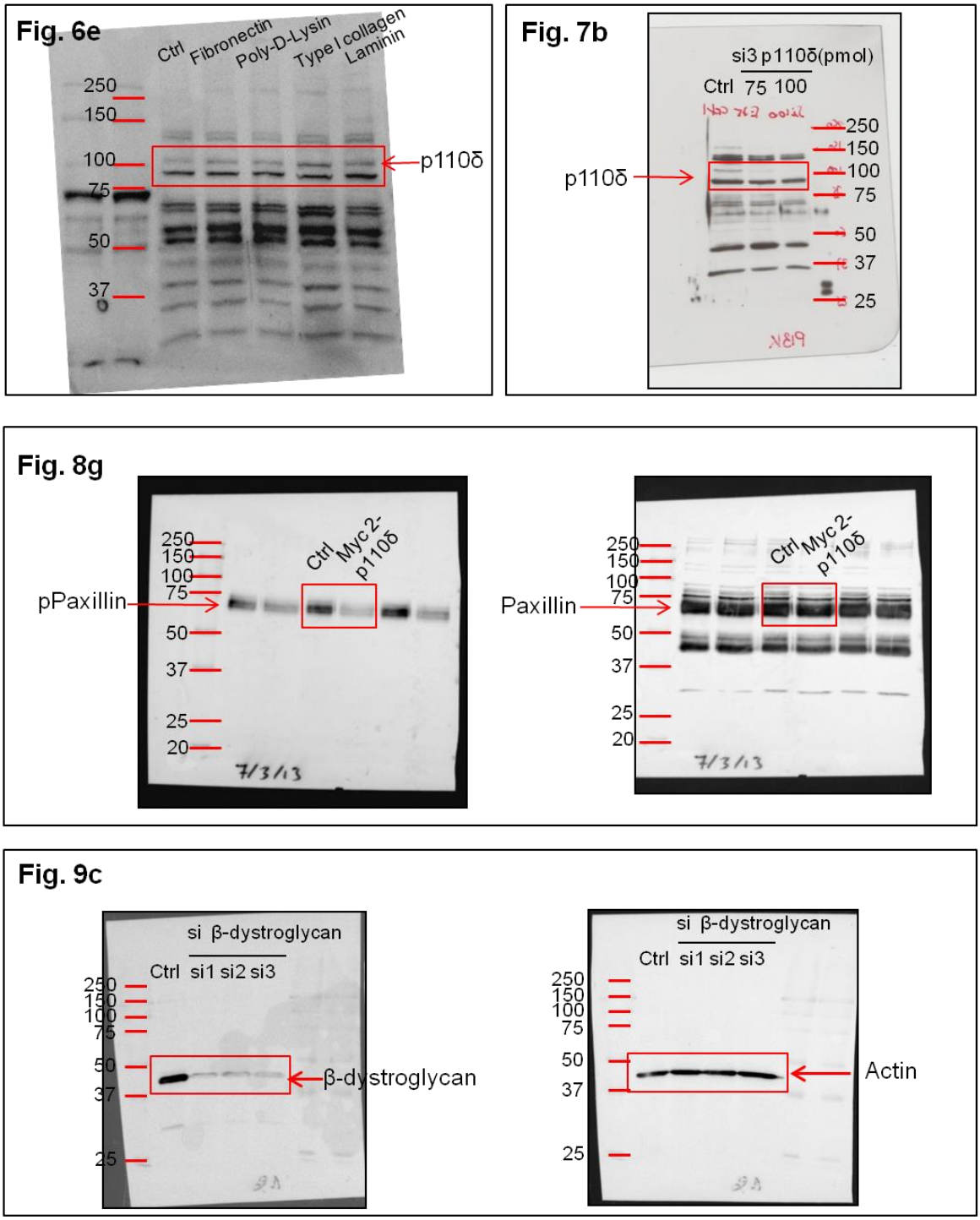


Supplementary Figure 10 continued





Supplementary Figure 10 continued



Supplementary Figure 10: Uncropped images of immunoblot

Red box indicated the cropped part included in the corresponding figure.

Supplementary Table 1

For RT-PCR the following primers are used:

	Sequence (5' to 3')
Human p110δ forward:	TGCCAAACCACCTCCCATTCCT
Human p110δ reverse:	CATCTCGTTGCCGTGGAAAAGC
Canine p110δ forward:	CACCAAACCACCCCCCATTCC
Canine p110δ reverse:	CGTCTCATTGCCGTGGAAAAGC
Canine p110α forward:	GAAGCACCTAAATAGGCAAGTTG
Canine p110α reverse:	GAGCATCCATGAAATCTGGTCGC
Canine p110β forward:	GGCATGGGTAAATACGATGG
Canine p110β reverse:	GCAAAGCTGTTGCATTTTCA
Canine p110γ forward:	TTCCTGTGCTGGCTACTGTG
Canine p110γ reverse:	GGGTTAGCACAAATGGCACT
Canine GAPDH forward:	GCCAAGAGGGTCATCATCTC
Canine GAPDH reverse:	GCTAGAGGAGCCAAGCAGTT
Human GAPDH forward:	AGCCACATCGCTCAGACAC
Human GAPDH reverse:	GCCCAATACGACCAAATCC