FIGURE LEGENDS FOR SUPPLEMENTARY MATERIAL

Figure 1. Polycystin-1 does not confer resistance to anoikis in MDCK cells

a) Three negative controls (MDCKtTA, F6, F2) and three *PKD1*+ cell lines (C8/68, G7/36, G3) were plated at equal densities in the absence of serum on plates coated with a PolyHema solution (6% in ethanol). All cell lines initially grew as clusters in suspension under these conditions, as previously described, but all had dissociated into single dead or dying cells after 96h.We compared the relative fraction of cells undergoing apoptosis at four time points post plating (24, 48, 72, 96h) using the Cell Death detection ELISA kit. The results are shown as optical density (OD) measured at the suggested wavelength (490nm) and normalized for the total protein content of each sample tested. No differences were observed at any time-point between cells expressing and not expressing *PKD1*.

b) Same experiment as in **(a)** except we estimated the viability of cells using the trypan blue exclusion method (Jan Y et al , *Cell*, 116, 751-762, 2004.). We have presented representative data from the 96h timepoint. On top, only individual cells are seen. On the bottom, results are expressed as percentage of alive versus dead cells.

Figure 2. Cells expressing Polycystin-1 do not grow or survive suspended in agar.

a,b) In order to test whether expression of *PKD1* would enable epithelial cells to survive when suspended in agar, we plated 5000 cells of each positive (C8/68, G7/36, G3) and negative clone (MDCKtTA, F6, F2) in a mixture of 0.5% agar +/- serum in DMEM. Photomicrographs were taken at the end of 1 week in culture. **(a)** A few foci (arrowheads) were observed in both the *PKD1*-negative MDCK cell lines and the positive control A431 but not in the *PKD1*+ MDCK lines. In contrast, no structures were observed for any of the cell lines cultured without serum **(b).**

Figure 3. Cells expressing polycystin-1 are resistant to apoptosis induced by a variety of stimuli.

a,b) In order to test whether expression of Polycystin-1 prevents death from apoptosis induced by different stimuli, we treated the cells in the presence or absence of serum (a and b) as negative and positive controls respectively, or in the presence of 20 J/cm2 of UV light (a) or 2ng /ml of TNFalpha (b). After 8 and 12 hrs respectively, cells were collected and apoptosis assessed as described in the legend for Supplementary Figure 1a. Polycystin-1 expressing cells were protected from apoptosis induced by either UV-light induced or TNFalpha under the stated conditions.

Figure 4. HepG2 stable transfectants expressing polycystin-1 are resistant to apoptosis in an Akt dependent manner.

a) In order to test whether DN-Akt was able to prevent apoptosis in HepG2 cells we generated stable transfectants expressing *PKD1* ($HepG2^{PKD1Zeo}$, clone A15) as well as negative controls expressing Zeocin only ($HepG2^{Zeo}$, clone E19). Stable transfectants were generated as previously described (19). Total RNA was extracted from single isolates and treated in the presence (RT+) or absence (RT-) of reverse transcriptase. Primers specific for beta-actin were used to check the quality of the RNA preparation (top) while primers specific for the recombinant, flag-tagged form of *hPKD1* (see Methods) were employed to check for *PKD1* expression (bottom). Water served as negative control in both PCRs (-Ctrl), while pCI-ß-PKD1-Flag (6) plasmid served as positive control (Ctrl+, bottom panel).

b) Expression of Polycystin-1 in HepG2 cells prevents death from apoptosis induced by different stimuli, We treated the cells in the presence of 60 J/cm2 of UV light (left) or 2ng /ml of TNFalpha (right). After 8 hrs cells were collected and apoptosis assessed either by TUNEL or anti-active caspase 3 antibody. Polycystin-1 expressing cells were protected from apoptosis induced by either UV-light induced or TNFalpha under the stated conditions.

c) Phosphorylation of Akt is enhanced in HepG2^{PKD1Zeo} cells as compared to the controls. The A15 *PKD1+* clone and E19, *PKD1-* cells were transiently transfected with HA-Akt. Western blots using anti phosphoser273-Akt antibodies revealed higher phosphorylation levels in the A15 as compared to E19 clone. Membranes were then stripped and re-probed using an anti-HA antibody as a loading control.

d) HepG2 control (E19) and HepG2^{PKD1Zeo} (A15) were transiently transfected using a mock-, a wild type (WT-Akt) or a Dominant Negative (DN_Akt) Akt contruct. Apoptosis was induced using UV light like in (c) and cells were stained with an anti-HA antibody (in red) to identify transfected cells, with a TUNEL assay (in green) to visualize apoptotic cells and counterstained with DAPI (blue nuclei) to visualize all the cells present in the field. Very few apoptotic cells are visibile in mock-transfected A15 cells, while a considerable number is visible in E19 cells treated under the same conditions. Similar results were observed when the WT-Akt construct was transfected in these two cell lines, while higher rates of HA-positive/TUNEL positive cells are visible in A15 when DN-Akt is transfected in these cells. Quantification of the experiment is shown in Fig 2d of the text.