Supplemental Methods

cAMP assay

Levels of cAMP in kidney tissue of WT mice fed *ad libitum* (AL) and *Pkd1*$^{RC/RC}$ mice fed AL or 40% food restriction (FR) for 6 months were determined using the Direct cAMP ELISA kit (Enzo Life Sciences) according to the manufacturer instructions. Briefly, 50 mg of snap-frozen tissue was homogenized in 0.5 mL of 0.1 M HCl, debris removed by centrifugation, and supernatant diluted 1:3 in HCl for use in the assay. ELISA results were normalized for protein content and expressed as pmol cAMP per mg protein.

MTT assay

Pkd1$^{del2/del2}$ and WT MEFs were plated in 96 well plates (3,000 per well) in 150 µl of media and allowed to attach for 24 h. For glucose dependence studies, medium was changed to DMEM containing 4.5 g/L (equivalent to 25 mM), 1.0 g/L, or 0.2 g/L glucose with or without glutamine (4 mM), with 10% FBS, penicillin and streptomycin, and cells were incubated for 72 h at 37°C. MTT 20 µl per well of 5 mg/mL was then added for 3 h and the resulting formazan crystals were dissolved in dimethyl sulfoxide (DMSO, 200 µl per well). Absorbance was measured at 560 nm. Cell number was assessed in parallel studies.

TUNEL assay

Pkd1$^{del2/del2}$ and WT MEFs were plated in 8-well chamber slides at 2 x 10^4 cells per well and incubated at 37 °C. After 24 hours of seeding, cells were given the medium (DMEM/F12, 10% FBS) containing various concentrations of glucose and glutamine. Cells were then

cultured for 12 hours and washed twice with PBS. TUNEL staining was performed by using the ApopTag Peroxidase *In Situ* Apoptosis Detection Kit (S7100, Millipore, MA) according to manufacturer's instructions.

Serum insulin and IGF-1 levels

Mouse serum was collected from WT AL, *Pkd1*$^{RC/RC}$ AL and *Pkd1*$^{RC/RC}$ FR mice and stored at -80C. The amounts of insulin and IGF-1in the serum were measured using the Ultra-Sensitive Mouse Insulin ELISA and IGF-1 ELISA kits, both from Crystal Chem, Inc., according to the manufacturer instructions.

Supplemental Table 1A. Body and organ weights of *Pkd1*$^{RC/RC}$ mice fed *ad libitum* (AL) or 40% food restriction (FR) for 2 months.

	Body (g)	Heart (g)	2 Kidneys (g)
AL	26.2 ± 0.6	0.12 ± 0.005	0.51 ± 0.03
40% FR	17.8 ± 0.7***	0.11 ± 0.003	0.28 ± 0.01***
% Difference	-32.2	-7.8	-45.1

40% FR was instituted in 5 month old mice for 2 months. Values are means ± SEM, n=5 mice per group. ****P*< 0.001 compared to AL.

Supplemental Table 1B. Comparison of ratios for kidneys/heart weight and kidneys/body weight in *Pkd1*$^{RC/RC}$ mice fed *ad libitum* (AL) or 40% food restriction (FR) for 2, 3, and 6 months.

	2 months FR		3 months FR		6 months FR	
	Kidneys/ Heart	Kidneys/ Body‡	Kidneys/ Heart	Kidneys/ Body‡	Kidneys/ Heart	Kidneys/ Body‡
AL	4.2 ± 0.2	1.9 ± 0.1	3.8 ± 0.3	2.4 ± 0.2	5.1 ± 0.3	2.8 ± 0.2
40% FR	2.5 ± 0.1***	1.6 ± 0.02**	2.5 ± 0.05**	1.6 ± 0.05**	2.4 ± 0.2**	1.8 ± 0.1***
% Difference	-40.6	-18.9	-35.9	-32.8	-52.8	-36.2

40% FR was instituted in 5 month old mice for 2 months, in 5.5 month old mice for 3 months, and in 1.5 month old mice for 6 months. Values are means ± SEM, n=5-12 mice per group. ‡ Kidneys/body weight ratios are multiplied by 100. ***P*< 0.01, ****P*< 0.001 compared to AL.

Supplemental Table 2. Blood chemistry in *Pkd1*$^{RC/RC}$ mice fed AL or 40% FR.

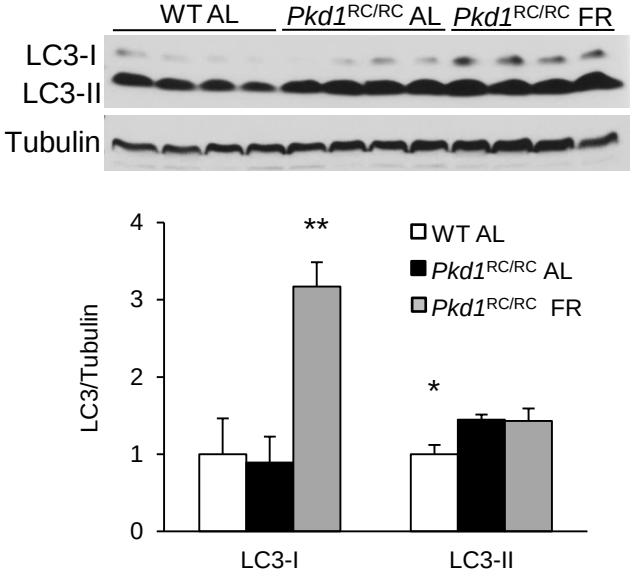
	AL	FR
Glucose (mg/dL)	207 ± 17	173 ± 10
Na^+ (mM)	152 ± 2	148 ± 1.0
K^+ (mM)	6.7 ± 0.3	6.4 ± 0.3
Cl^- (mg/dL)	117 ± 2.0	117 ± 2.0
Ca^{++} (mg/dL)	9.1 ± 0.3	7.6 ± 0.2 ***
BUN (mg/dL)	25.5 ± 1.0	17.4 ± 1.9 **
Creatinine (mg/dL)	0.3 ± 0.1	0.2 ± 0.03
Albumin (g/dL)	1.6 ± 0.1	1.8 ± 0.1 **
Total protein (g/dL)	4.7 ± 0.2	4.1 ± 0.1 *

Data are means ± SEM. *P<0.05, **P<0.01, ***P<0.001 vs *Pkd1*$^{RC/RC}$ mice on AL.

Supplemental Table 3. TaqMan Gene Expression Assays.

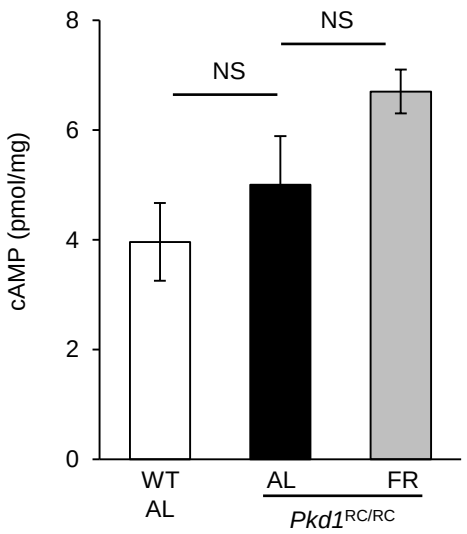
Gene Symbol	Probe ID
Mcp-1	Mm00441243_g1
Il-6	Mm00446190_m1
Tnfa	Mm00443258_m1
Mmp2	Mm00439498_m1
Col1a1	Mm00801666_g1
Tgfb1	Mm01178820_m1
Ngal	Mm01324470_m1
p16	Mm00494449_m1
Glut1	Mm00441480_m1
Glut4	Mm01245502_m1
Hk1	Mm00439344_m1
Hk2	Mm00443385_m1
Pkm	Mm00834102_gH
Ldha	Mm01612132_g1
Pdha	Mm00468675_m1
Pck1	Mm01247058_m1
Igf1	Mm00439560_m1
Igf1r	Mm00802831_m1
Gapdh	4352932E

Supplemental Figure 1



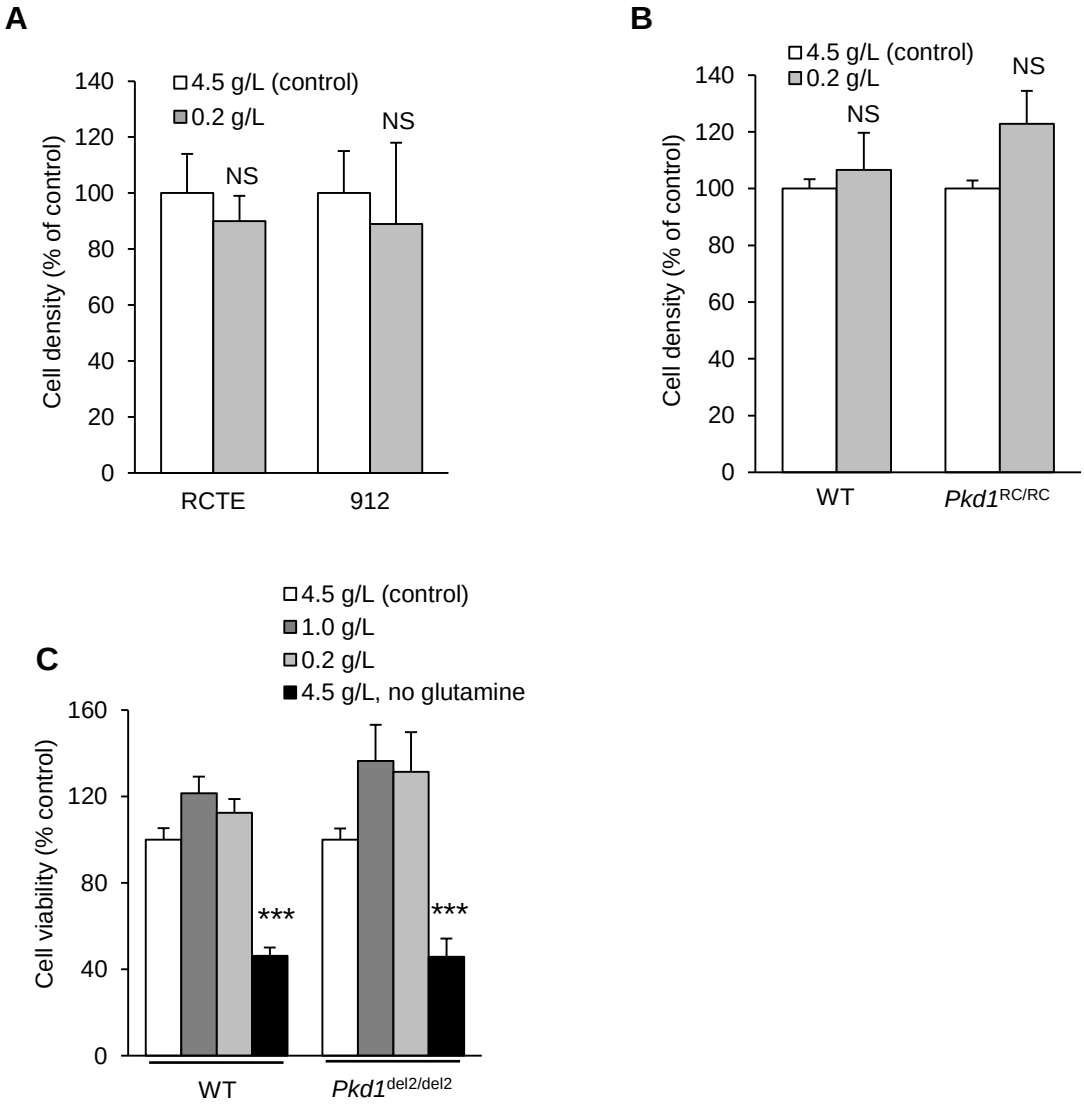
Supplemental Figure 1. Modulation of LC3 expression in *Pkd1*$^{RC/RC}$ kidneys. 3 month old *Pkd1*$^{RC/RC}$ mice were placed on 40 % FR diet for 1 week. Kidneys lysates were prepared from FR mice along with age-matched AL-fed WT and *Pkd1*$^{RC/RC}$ controls, followed by immunoblotting for LC3 and tubulin. Quantitative analysis of bands was performed by densitometry using ImageJ software. Each gel lane was obtained from an independent mouse (n=4). Data are means ± SEM. **P*< 0.05, ***P*< 0.01 compared to *Pkd1*$^{RC/RC}$ AL group.

Supplemental Figure 2



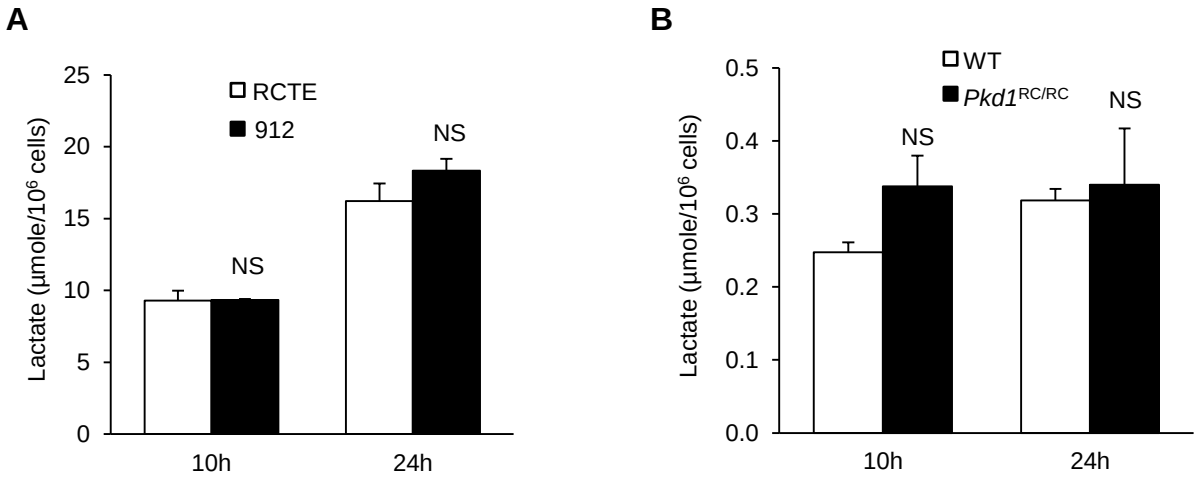
Supplemental Figure 2. FR does not decrease cAMP levels in *Pkd1*$^{RC/RC}$ mice. cAMP levels in kidneys of WT mice fed AL (n=4) and *Pkd1*$^{RC/RC}$ mice fed AL or 40% FR diet (n=8 per group) for 6 months were measured by ELISA and normalized to total protein. Data are means ± SEM, n = 4-5. NS, not significant compared to *Pkd1*$^{RC/RC}$ AL.

Supplemental Figure 3



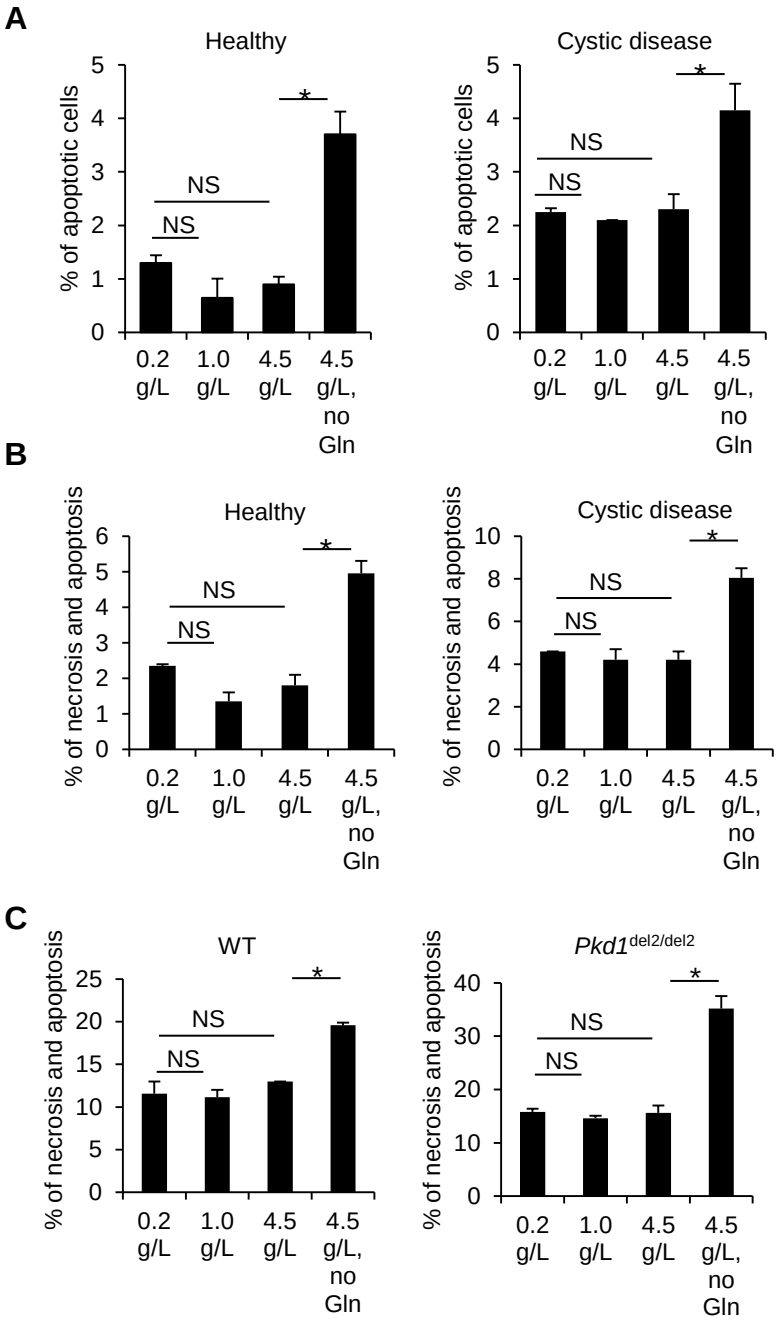
Supplemental Figure 3. Glucose needs of human cells and MEFs are not different for cell proliferation. (**A-B**) Human renal cortical tubular epithelial cells (RCTE) and PKD 912 cystic epithelial cells (**A**) or WT and *Pkd1*$^{RC/RC}$ MEFs (**B**) were cultured in the presence of high (4.5 g/L) and low (0.2 g/L) glucose, and cell density was determined 48 or 72 hours later by counting. (**C**) *Pkd1*$^{del2/del2}$ and WT MEFs were cultured in the presence of various doses of glucose and glutamine, and cell viability was determined 72 hours later by MTT assay. Data are means ± SEM for n=4-6 per group, and expressed relative to high glucose control for each cell type. All comparisons of high and low glucose were NS, no significant difference, ***$P < 0.001$ for comparison of high glucose with and without glutamine. Experiments were performed at least 4 times.

Supplemental Figure 4



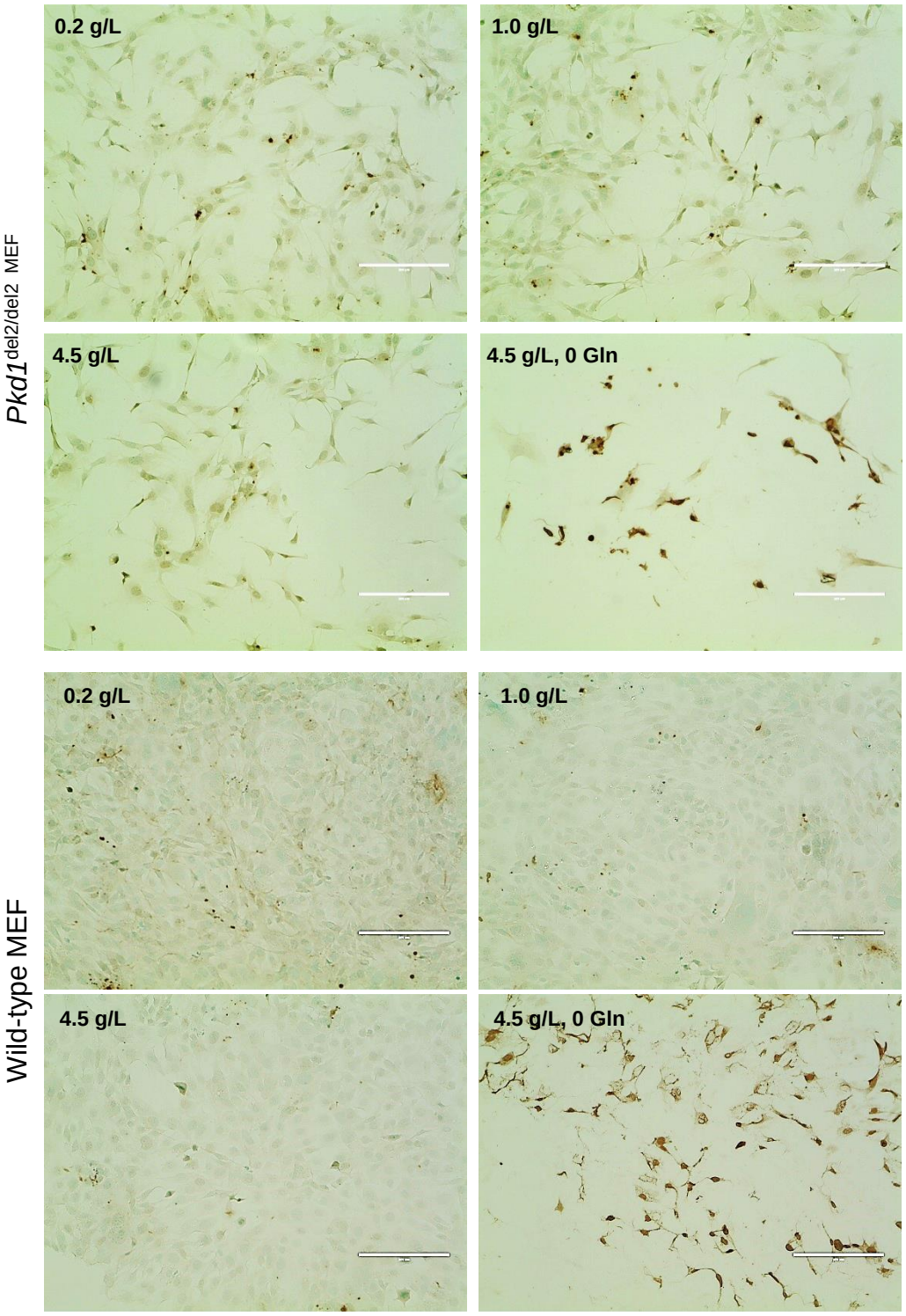
Supplemental Figure 4. Lactate production in WT and PKD cells is not different. (**A-B)** Conditioned medium was collected from (**A**) human renal cortical tubular epithelial cells (RCTE) and PKD null cyst lining cells (912) or (B)WT and *Pkd1*$^{RC/RC}$ MEFs after 10 and 24 hours of exposure to cells. Lactate measurements were normalized to cell counts. Data are means ± SEM. Experiments were performed 4 times in triplicate. NS= not significant compared to WT control.

Supplemental Figure 5



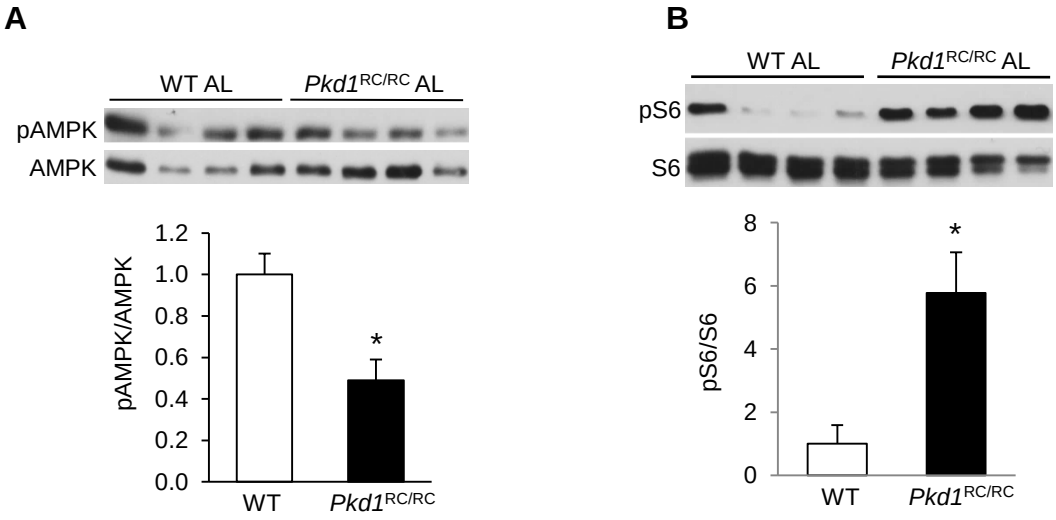
Supplemental Figure 5. Glucose needs of (**A,B**) human cystic/ healthy cells and (**C**) *Pkd1*$^{del2/del2}$ / WT cells are not different. (**A,B**) Human renal cortical tubular epithelial cells (RCTE)/ cystic epithelial cells from ADPKD patients were cultured in the presence of various doses of glucose and glutamine (Gln). Apoptosis alone (**A**) and apoptosis + necrosis (**B**) was determined 48 hours later by Annexin V assay and propidium iodide (PI). (n=6 per group, *P*> 0.05 for all comparisons, ns= not significant). (**C**) Graph showing the percentage of apoptosis + necrosis in *Pkd1*$^{del2/del2}$ and WT MEFs after treating with various concentrations of glucose and glutamine (Gln). Apoptosis and necrosis were determined 48 hours after treatment by Annexin V and PI assay. (n=6 per group, *P*> 0.05 for all comparisons, NS= not significant)

Supplemental Figure 6



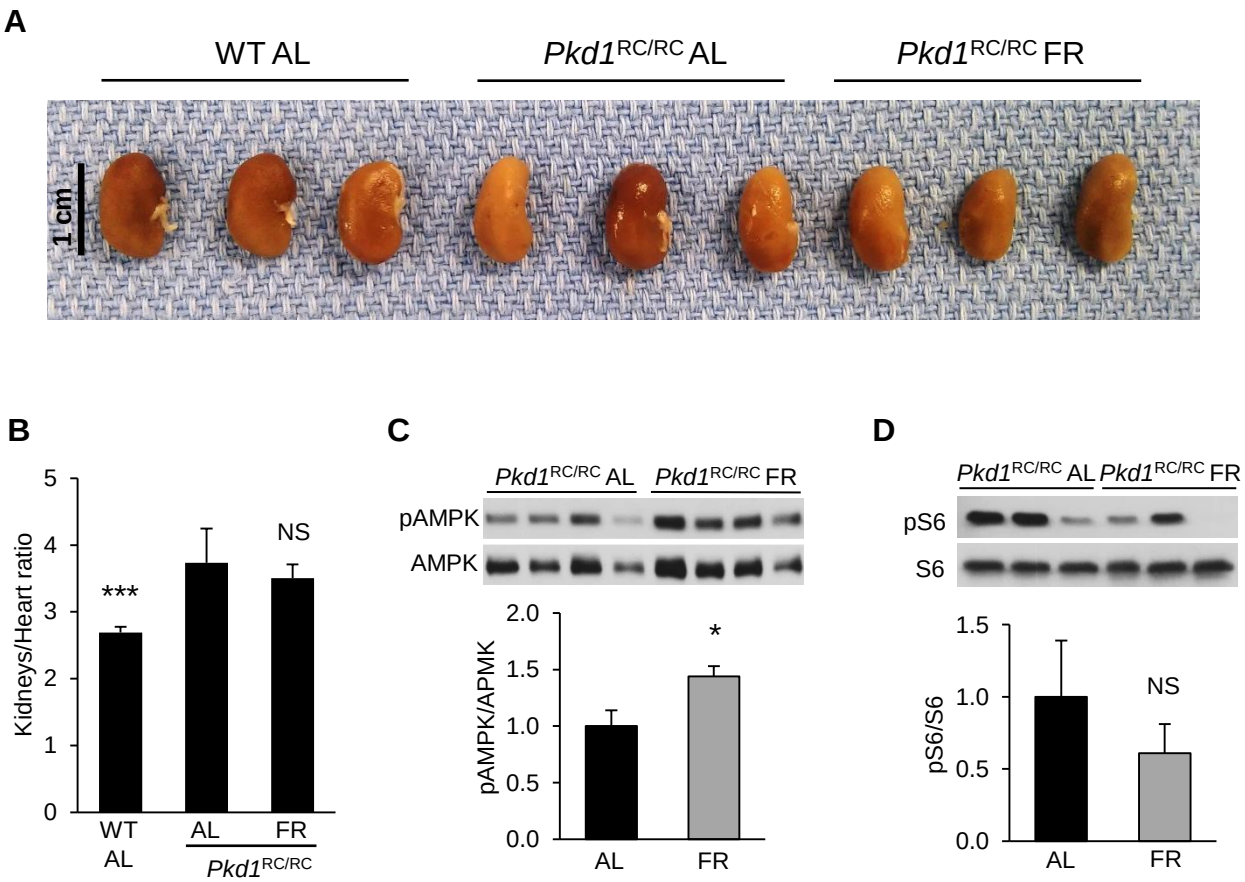
Supplemental Figure 6. Glucose needs of WT and *Pkd1*$^{del2/del2}$ MEF cells are not different. WT/ *Pkd1*$^{del2/del2}$ MEFs were cultured in the presence of various doses of glucose and glutamine. Apoptosis was determined 48 hours after treatment by TUNEL assay and observed under microscopy. n=6 per group. Gln= Glutamine, Scale bars, 200 µm.

Supplemental Figure 7



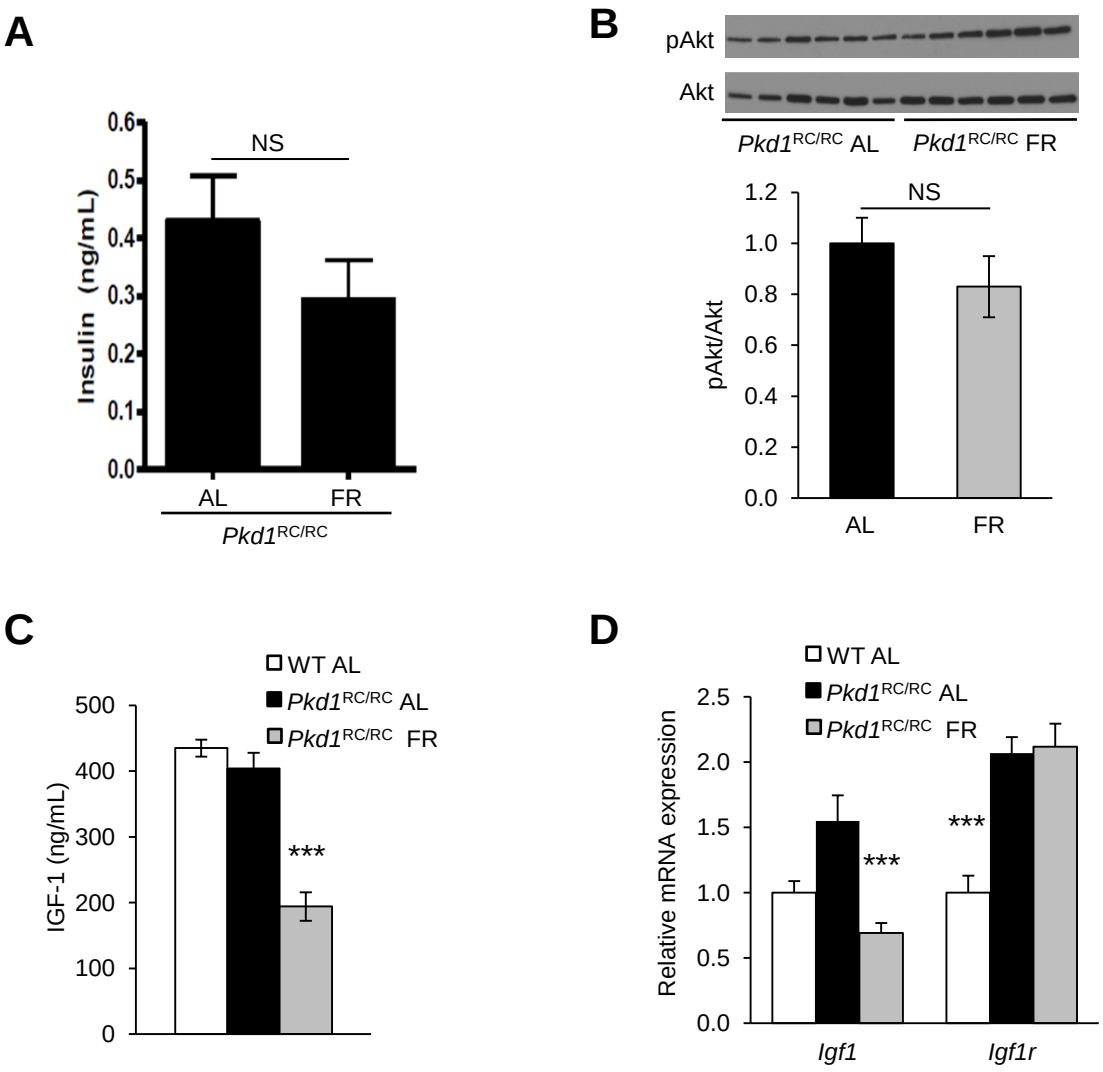
Supplemental Figure 7. The AMPK pathway is inhibited and the mTOR pathway is activated in the kidneys of *Pkd1*$^{RC/RC}$ mice. Comparison of phosphorylated (p) and total AMPK (A) and S6 (B) by immunoblotting in kidney lysates from 7.5 month old WT and *Pkd1*$^{RC/RC}$ mice fed AL. Quantitative analysis of pAMPK/AMPK (A) and pS6/S6 (B) by densitometry; graph shows means ± SEM for n=4 mice per group. *P< 0.05 compared to WT mice.

Supplemental Figure 8



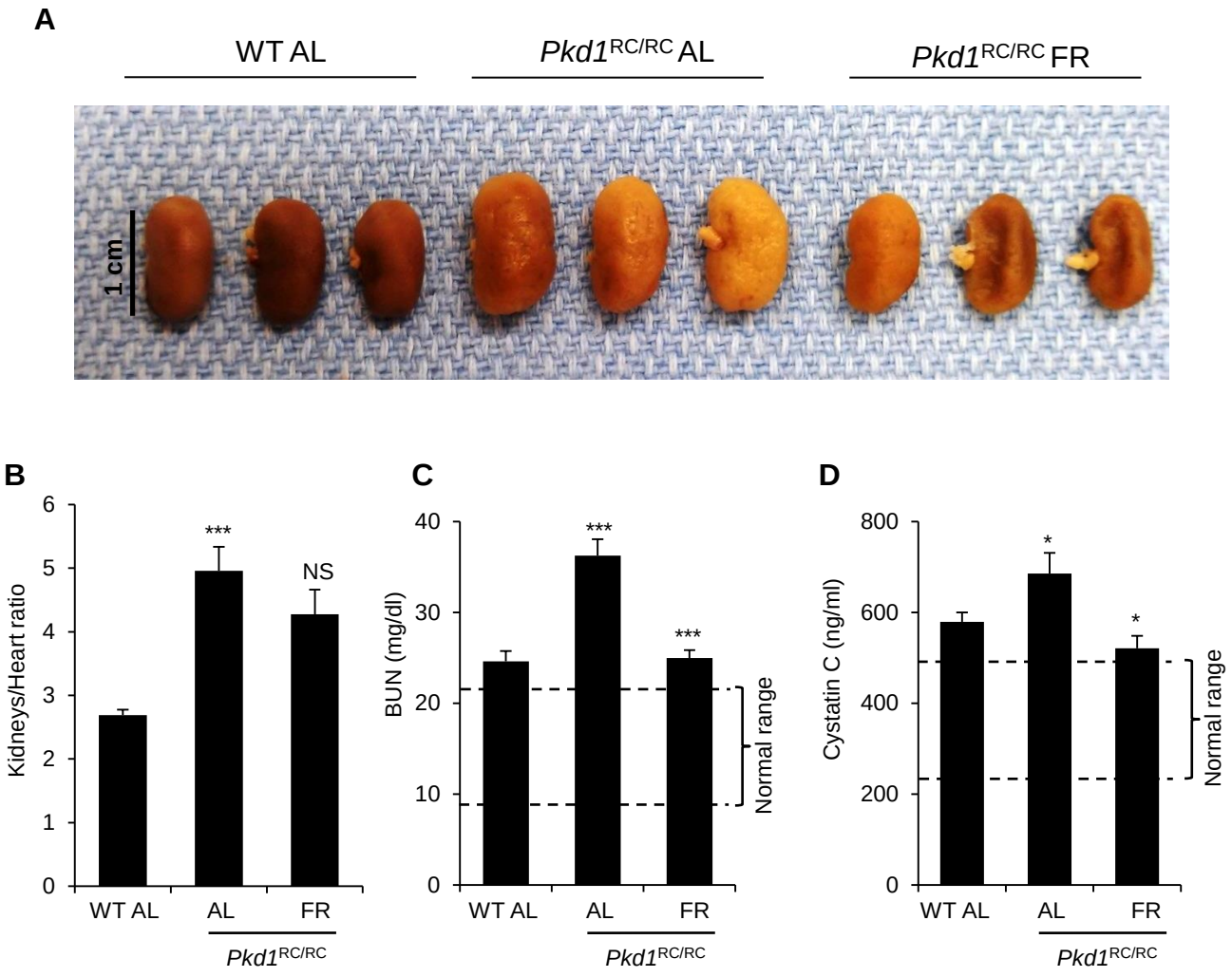
Supplemental Figure 8. The AMPK pathway is activated and the mTOR pathway shows an inhibitory trend in *Pkd1*$^{RC/RC}$ kidneys after 1 week of FR. 3 month old *Pkd1*$^{RC/RC}$ mice were fed AL or 40% FR diet for 1 week; age-matched WT mice received AL diet. (**A**) Kidney images and (**B**) kidneys/heart weight ratios show not appreciable difference between *Pkd1*$^{RC/RC}$ on AL and FR after 1 week. (**C-D**) Comparison of phosphorylated (p) and total AMPK (**C**) and S6 (**D**) in kidney lysates by immunoblotting. Densitometric analysis of pAMPK/AMPK (**C**) and pS6/S6 (**D**); graphs show means ± SEM for n=3-4 mice. NS (not significant), **P*< 0.05, ****P*< 0.001 vs *Pkd1*$^{RC/RC}$ AL.

Supplemental Figure 9



Supplemental Figure 9. Six week old *Pkd1*$^{RC/RC}$ mice were fed AL or 40% FR for six months. (**A**) Serum insulin levels in *Pkd1*$^{RC/RC}$ mice on AL or FR diet were measured by ELISA (n=5 mice per group). (**B**) Comparison of phosphorylated (p) and total Akt by immunoblotting in kidney extracts of *Pkd1*$^{RC/RC}$ mice on AL or FR. Each gel lane is obtained from an independent mouse. Graph shows quantitative analysis of bands by densitometry. (**C**) ELISA analysis of serum IGF-1 levels in WT mice on AL and *Pkd1*$^{RC/RC}$ mice on AL or FR (n=5-7 mice per group). (**D**) mRNA expression of *Igf1* and *Igf1r (*IGF-1 receptor) determined by real-time PCR and expressed relative to *Gapdh* in kidneys of WT mice on AL (n=4) and *Pkd1*$^{RC/RC}$ mice on AL or FR (n=8 per group). Data are means ± SEM. NS, not significant, ***P<0.001 compared to *Pkd1*$^{RC/RC}$ AL.

Supplemental Figure 10



Supplemental Figure 10. FR treatment at the late stage of ADPKD. 9 month old *Pkd1*$^{RC/RC}$ mice were fed AL or 40% FR for 6 weeks (n=6 per group). Mice were scarified at the end of the study. (**A**) Pictures showing the kidney of age-matched WT mice on AL diet and *Pkd1*$^{RC/RC}$ mice on AL or FR diet. Scale bar, 1 cm. (**B**) Comparison of kidneys/heart weight ratio in WT AL with *Pkd1*$^{RC/RC}$ AL and FR. ***P< 0.001 for WT AL vs *Pkd1*$^{RC/RC}$ AL , NS= not significant for *Pkd1*$^{RC/RC}$ AL vs FR. (**C,D**) FR treatment improves kidney function when introduced in 9 month old *Pkd1*$^{RC/RC}$ mice (i.e., at the well-developed stage of the cystic disease). Kidney function was determined by plasma BUN and Cystatin C. (n=6 per group). Data are means ± SEM. *P< 0.05, ***P< 0.001 for WT AL vs *Pkd1*$^{RC/RC}$ AL and *Pkd1*$^{RC/RC}$ AL vs FR.