SUPPLEMENTAL MATERIAL

Functional polycystin-1 dosage governs autosomal dominant polycystic kidney disease severity

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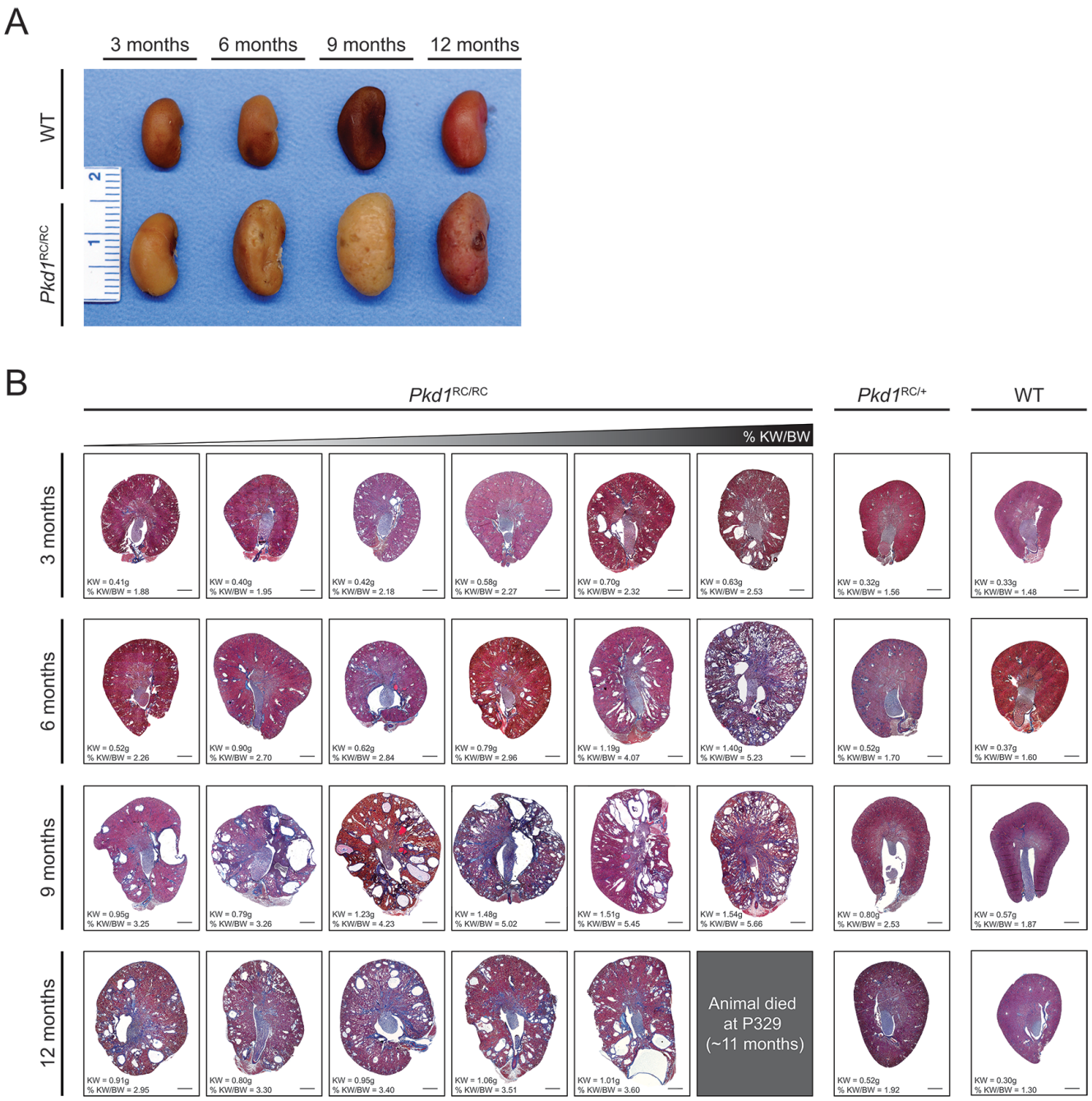
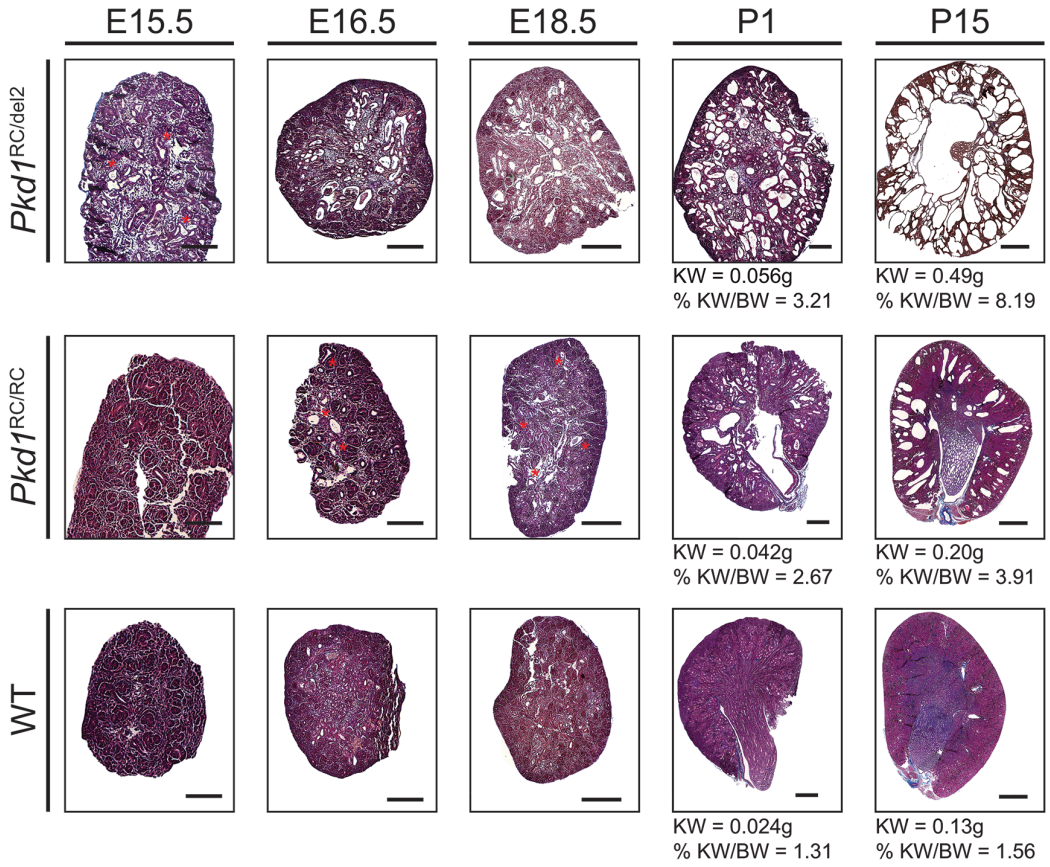
CONFLICT OF INTEREST

The authors have declared that no conflict of interest exists.

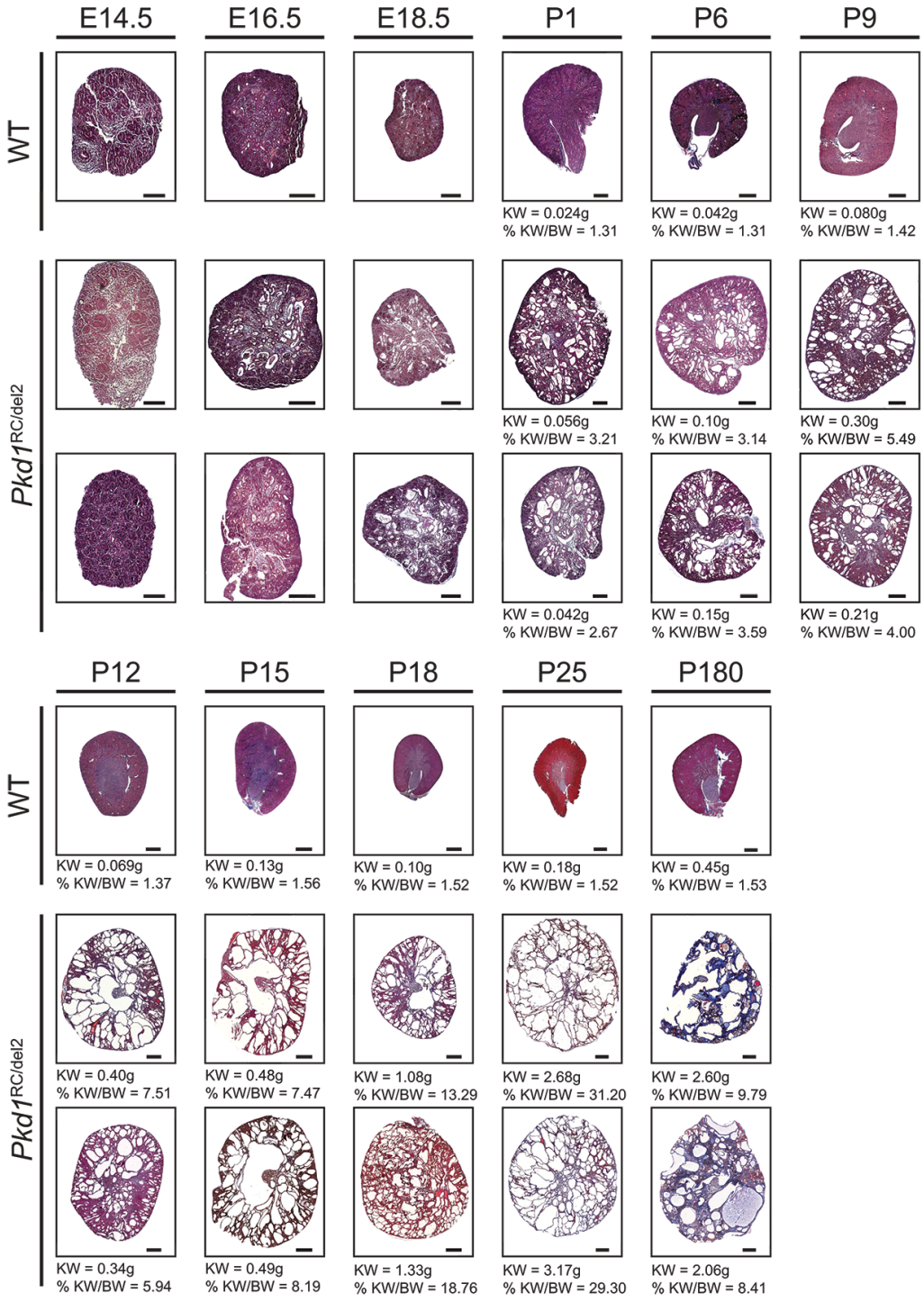


Supplemental Figure 1
Illustration of disease progression and variation in *Pkd1*$^{RC/RC}$ mice. (A) Gross images of WT and *Pkd1*$^{RC/RC}$ kidneys at 3, 6, 9, and 12 months. Macroscopic cysts become visible by 6 months. Ruler shows the SI unit cm. (B) Masson Trichrome stained kidney histology sections of all *Pkd1*$^{RC/RC}$ mice used for physiological and histological analysis in Figures 2 and 3. The illustration highlights the observed disease variability among animals at the same age, and the progressive disease severity throughout aging in these outbred animals. Scale bars = 1mm.

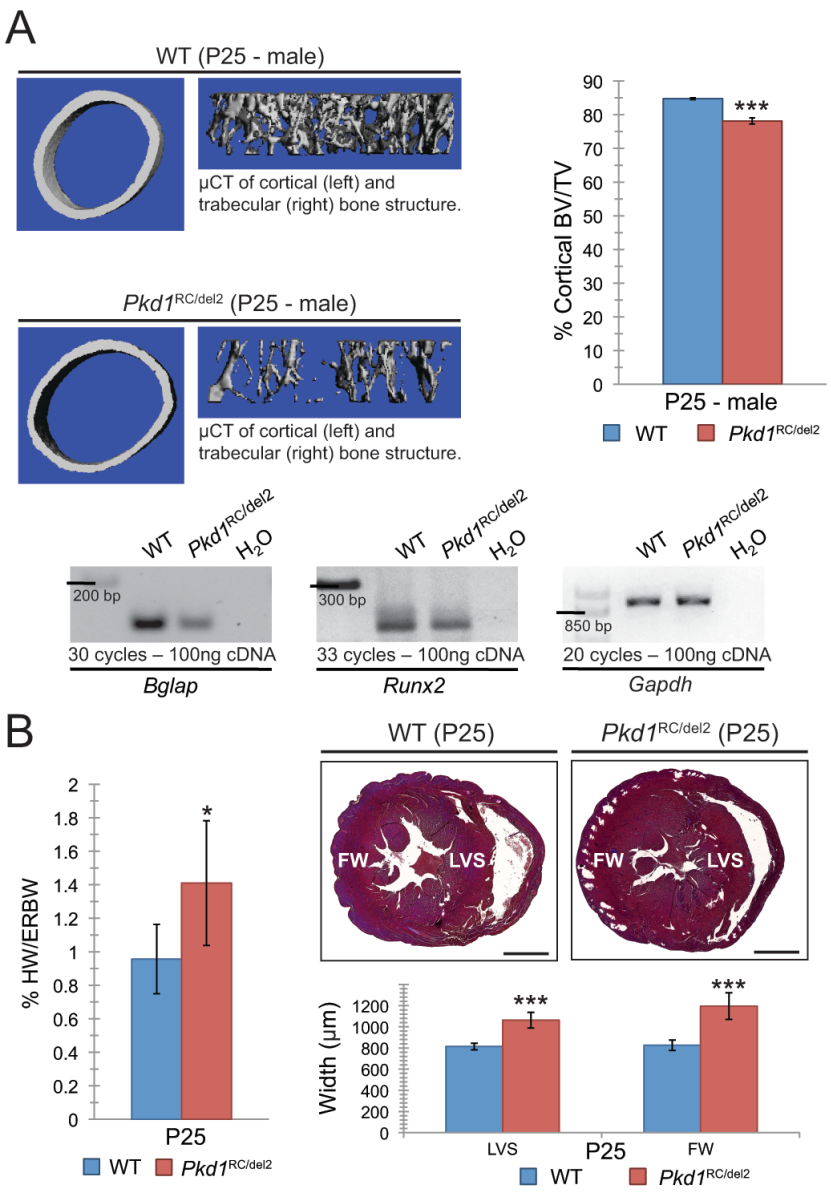


Supplemental Figure 2

Cyst development during embryogenesis and the first postnatal month in *Pkd1*$^{RC/RC}$ and *Pkd1*$^{RC/del2}$ animals. During embryonic development kidney cysts (*) are detectable in *Pkd1*$^{RC/del2}$ mice by E15.5 and *Pkd1*$^{RC/RC}$ animals by E16.5. Subsequently, cystogenesis proceeded much more rapidly in *Pkd1*$^{RC/del2}$ versus *Pkd1*$^{RC/RC}$ animals. *Pkd1*$^{RC/RC}$, nonetheless, have a significant cyst burden during renal development and, indeed, have a higher %KW/BW at P15 than at 1 month (Figure 2C). This is probably because PT cysts dominate the cyst burden at the early stages, but then decline as CD cysts become the major adult disease manifestation (Figure 6A, C). Scale bars: E15.5 = 100μm, E16.6 = 150μm, E18.5 and P1 = 250μm, P15 = 750μm.

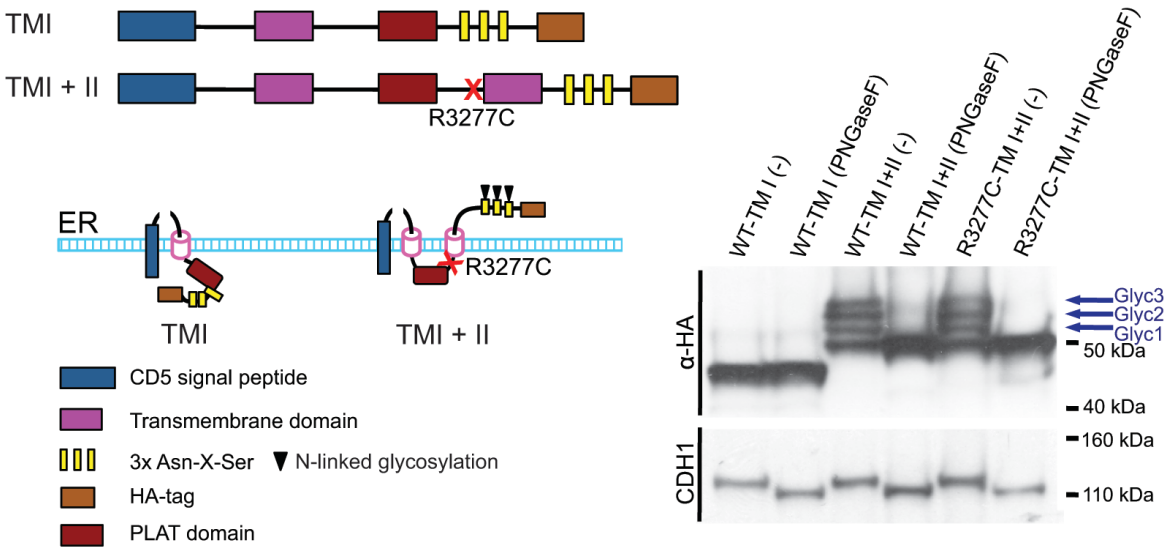


Supplemental Figure 3
Detailed illustration of disease variation among non-littermate *Pkd1*$^{RC/del2}$ animals at various time points. Masson Trichrome stained histology sections of one WT and two non-littermate *Pkd1*$^{RC/del2}$ animals at various ages. Cyst burden progressively increased with age until P25. Subsequently, the kidneys become highly fibrotic (P180). The model shows only moderate disease variability among animals. Scale bars: E14.5 and E16.5 = 150µm, E18.5 and P1 = 250µm, P6 and P9 = 500µm, P12 and 15 = 750µm, P18 to P180 = 1mm.

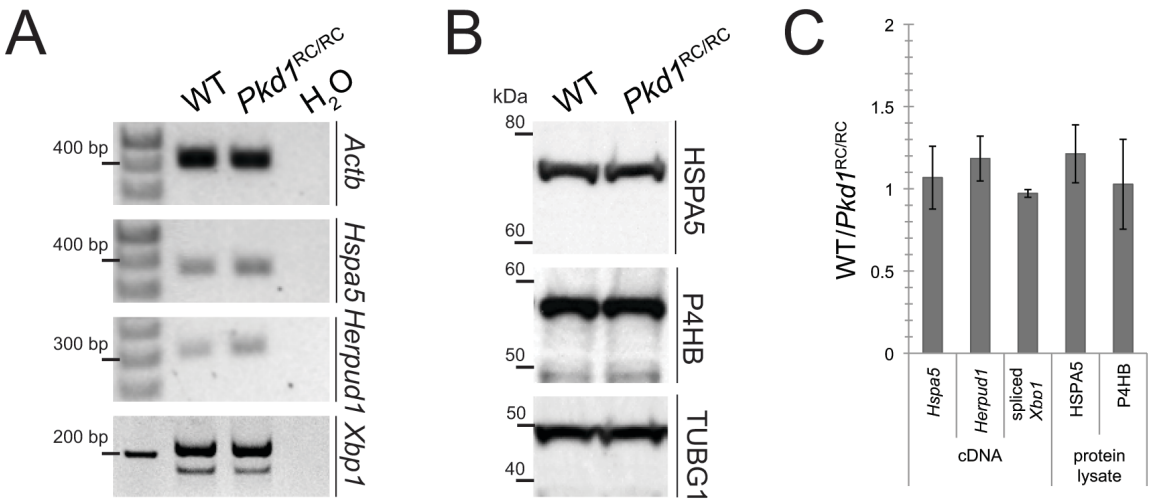


Supplemental Figure 4

The *Pkd1* p.R3277C allele causes two extrarenal phenotypes. (A) *Pkd1*$^{RC/del2}$ animals develop a osteopenia like phenotype characterized by a significant decrease in cortical (left µCT image + graph) and trabecular (right µCT image) bone mass – a phenotype known to be associated with a reduction of *Pkd1* in mice(1, 2). BV: Bone volume, TV: Tissue volume. The lower panel shows a reduction of expression by semi-quantitative RT-PCR analysis of *Bglap* (an osteoblast specific protein regulating bone mineralization and Ca^{2+} homeostasis – 42% reduction, $p<0.01$) and *Runx2* (a transcription factor regulating osteoblast differentiation – 22% reduction, $p<0.05$) (n=3, *Gapdh* serves as a control). This reduced expression of these major mRNAs involved in osteoblast maintenance highlights the importance of PC-1 in bone(2, 3). (B) *Pkd1*$^{RC/del2}$ animals show early signs of LVH. LVH is a known cardiovascular complication in ADPKD patients and is often characterized by an increase in heart weight (right) due to the thickening of the myocardium (left)(4). Heart weight (HW) was measured as percent extra renal body weight (ERBW = BW - KW) and myocardium thickness was measured along the free wall (FW) and the left ventricular septum (LVS), n=7/group, Scale bars = 1mm. No significant accumulation of fibrosis was noted in the LV myocardium of *Pkd1*$^{RC/del2}$ animals, which would indicate cardiac failure. Taken together, this data suggests that *Pkd1*$^{RC/del2}$ animals likely develop hypertension. Statistical values were obtained by Student t-test (*$p<0.05$, ***$p<0.001$), error bars indicate ± SD.



Supplemental Figure 5
The *Pkd1* p.R3277C allele does not affect membrane integration of PC1. Left panel: Diagram of the in-vitro expression construct and the assay layout. The three C-terminal glycosylation tags can only be properly glycosylated if the second TM of PC-1 integrates successfully into the ER membrane. Right panel: WB of a 24h HEK293T cell lysate showing that the mutant p.R3277C construct is glycosylated similarly to the WT, highlighting that the close proximity of the *Pkd1* p.R3277C allele to the second TM does not modify the integration efficiency of this domain. CDH1 (E-cadherin, loading control)



Supplemental Figure 6
The reduced folding ability of *Pkd1* p.R3277C does not trigger ER stress in primary CD cells. (A) Semi-quantitative RT-PCR of ER-stress response genes *Hspa5*, *Herpud1* and cleaved *Xbp1*. (B) WB of ER-stress response proteins Hspa5 and P4hb. (C) Quantification of (A) and (B) depicted as normalized relative ratio between WT and *Pkd1*$^{RC/RC}$. RT-PCR was normalized to *Actb*. Cleaved *Xbp1* was normalized to total *Xbp1*, and protein levels were normalized to Tubg1, n=3/group. A slight increase in expression of ER-stress response genes was noted in *Pkd1*$^{RC/RC}$ cells compared to WT, however none reached significance. A duplicate of this experiment was performed on whole kidneys with consistently similar results (data not shown). Statistical values were obtained by Student t-test, error bars indicate ± SD.

Supplemental Table 1
Summary of measurements obtained from WT, *Pkd1*$^{RC/+}$, and *Pkd1*$^{RC/RC}$ mice to document PKD severity over a one year time period

	KV (mm^3)	WT		*Pkd1*$^{RC/+}$		*Pkd1*$^{RC/RC}$		
		Mean	*SD*	*Mean*	*SD*	*Mean*	*SD*	*P-value*[A]
Age in months	3	214.10	37.37	-	-	507.79	173.39	0.0113
	6	249.40	68.51	-	-	713.24	278.93	0.0126
	9	263.18	86.34	-	-	741.78	310.84	0.0184
	12	256.62	53.16	-	-	638.16	88.96	1.36E-04
	%KW/BW	**WT**		***Pkd1*$^{RC/+}$**		***Pkd1*$^{RC/RC}$**		
		Mean	*SD*	*Mean*	*SD*	*Mean*	*SD*	*P-value*[A]
Age in months	3	1.49	0.20	1.52	0.13	2.19	0.24	0.00142
	6	1.66	0.24	1.62	0.13	3.35	1.10	0.0185
	9	1.66	0.24	1.94	0.45	4.48	1.07	9.36E-04
	12	1.62	0.26	1.58	0.33	3.35	0.25	1.94E-05
	cAMP (pmol/mg protein)	**WT**		***Pkd1*$^{RC/+}$**		***Pkd1*$^{RC/RC}$**		
		Mean	*SD*	*Mean*	*SD*	*Mean*	*SD*	*P-value*[A]
Age in months	3	2.89	0.46	3.06	0.29	3.80	1.04	0.142
	6	2.99	0.18	3.51	0.59	5.02	1.29	0.0152
	9	3.35	0.87	4.19	0.49	6.35	1.41	0.00550
	12	3.54	0.81	4.14	0.40	6.11	0.15	2.00E-04
	BUN (mg/dl)	**WT**		***Pkd1*$^{RC/+}$**		***Pkd1*$^{RC/RC}$**		
		Mean	*SD*	*Mean*	*SD*	*Mean*	*SD*	*P-value*[A]
Age in months	3	32.03	4.16	31.61	6.08	31.41	6.30	0.868
	6	30.49	3.57	32.10	7.72	37.75	15.99	0.406
	9	25.98	3.23	25.46	2.52	57.08	8.80	1.60E-04
	12	27.58	2.51	30.97	7.03	56.98	11.74	2.68E-04

[A]Significance compared to WT.

Supplemental Table 2
Summary of renal function measurements obtained from WT and *Pkd1*$^{RC/del2}$ mice, highlighting early onset PKD

		WT			*Pkd1*$^{RC/del2}$			
%KW/BW		*Mean*	*SD*	*n*	*Mean*	*SD*	*P-value*[A]	*n*
Age in days	P1	1.29	0.14	9	3.05	0.60	1.53E-06	5
	P6	1.25	0.05	8	3.27	0.68	7.99E-07	8
	P12	1.23	0.18	5	7.07	1.00	1.68E-07	7
	P18	1.57	0.14	12	19.00	6.24	2.81E-08	6
	P25	1.50	0.11	8	24.63	7.07	1.38E-08	14
	P180	1.66	0.24	5	9.10	0.98	8.68E-05	2
		WT			***Pkd1*$^{RC/del2}$**			
cAMP (pmol/mg protein)		*Mean*	*SD*	*n*	*Mean*	*SD*	*P-value*[A]	*n*
Age in days	P1	4.87	1.54	6	8.42	1.34	0.00166	6
	P6	7.62	2.01	8	12.45	4.39	0.0122	9
	P12	5.38	1.61	5	48.91	15.25	9.23E-05	7
	P18	5.02	0.92	10	74.52	21.36	4.94E-08	6
	P25	4.55	1.19	9	127.07	49.61	4.20E-07	13
	P180	2.99	0.18	5	9.31[B]	3.79	0.0185	2
		WT			***Pkd1*$^{RC/del2}$**			
BUN (mg/dl)		*Mean*	*SD*	*n*	*Mean*	*SD*	*P-value*[A]	*n*
Age in days	P1	14.69	10.10	5	17.86	11.11	0.663	5
	P6	14.61	2.28	5	18.81	6.08	0.192	5
	P12	18.40	5.72	5	30.37	8.56	0.0261	6
	P18	21.37	3.54	5	86.40	20.09	1.74E-04	5
	P25	23.04	7.00	6	177.90	49.98	4.85E-06	9
	P180	30.49	3.57	5	313.60	37.15	4.97E-05	2

[A]Significance compared to WT.
[B]The significantly decreased cAMP levels in surviving *Pkd1*$^{RC/del2}$ animals (P25 vs. P180) are likely associated with increasing kidney fibrosis and the presence of only few remaining functional renal tubules.

Supplemental Table 3
Summary of PCNA measurements in P18 WT and *Pkd1*$^{RC/del2}$ animals[A]

Individual animals	Stain	WT	*Pkd1*$^{RC/del2}$			
		Non-dil CD	Non-dil CD	SC	MC	LC
1	*PCNA*	8	18	25	40	77
	DAPI	768	1156	748	928	1411
2	*PCNA*	5	15	20	36	87
	DAPI	759	864	612	876	1452
3	*PCNA*	8	19	18	28	87
	DAPI	713	1028	674	760	1721
Mean (%PCNA/DAPI)		**0.94**	**1.71**	**3.09**	**4.03**	**5.50**
SD		**0.25**	**0.15**	**0.37**	**0.32**	**0.47**
P-value relative to WT		**-**	**0.00965**	**0.00110**	**1.87E-04**	**1.19E-04**

[A]PCNA measurements of 100 non-dilated CD (Non-dil) in WT and 100 non-dil, 20 small (<50nuclei, SC), medium (50-200 nuclei, MC), and large (>200 nuclei, SC) CD cysts in *Pkd1*$^{RC/del2}$ animals.

Supplemental Table 4
Summary of cilia length measurements in CD using SEM[A]

Measuments are in µm	Individual animals 1	2	3	Mean	SD
WT P25 + 12 months (Mean)[B]	2.05	1.96	1.93	**1.98**	**0.064**
SD	1.03	0.35	0.30	***P-value***	
n	52	56	54	***WT vs. Pkd1$^{RC/del2}$***	***0.00122***
Age	P25	P25	12mo	-	-
***Pkd1$^{RC/del2}$* P25 (Mean)[C]**	5.52	4.28	5.12	**4.98**	**0.63**
SD	1.13	0.78	1.10	***P-value***	
n	115	116	122	***Pkd1$^{RC/del2}$* vs. *Pkd1$^{RC/RC}$***	***0.00659***
***Pkd1$^{RC/RC}$* 12 months (Mean)[D]**	3.21	2.90	2.94	**3.02**	**0.17**
SD	0.67	0.59	0.52	***P-value***	
n	101	100	109	***WT vs. Pkd1$^{RC/RC}$***	***5.65E-04***

[A]Cilia measurements of >100 cilia in non-dilated (~40), dilated (~30), and cystic (~30) CD in the case of *Pkd1*$^{RC/del2}$/*Pkd1*$^{RC/RC}$ and >50 cilia of non-dilated CD in WT animals (P25 and 12mo of age).
[B]WT (P25) vs. WT (12mo) p=0.643
[C]*Pkd1*$^{RC/del2}$ (P25 #3): cystic CD – 5.03µm, dilated CD – 5.27µm, non-dilated CD: 5.16µm (cystic/dilated vs. normal p=0.559)
[D]*Pkd1*$^{RC/RC}$ (12mo #3): cystic CD – 2.96µm, dilated CD – 3.01µm, non-dilated CD: 2.89µm (cystic/dilated vs. normal p=0.319)

Supplemental Table 5
Summary of cilia length measurements in bile duct using SEM[A]

Measuments are in µm	Individual animals 1		2	3	Mean	SD
	BD	M[C]	BD	BD		
WT P25 + 12 months (Mean)[B]	3.47	-	3.49	3.67	**3.54**	**0.11**
SD	0.15	-	0.25	0.50	***P-value***	
n	51	-	53	53	***WT vs. Pkd1$^{RC/del2}$***	***0.0929***
Age	P25	-	P25	12mo	-	-
***Pkd1$^{RC/del2}$* P25 (Mean)**	3.47	-	3.18	3.28	**3.31**	**0.15**
SD	0.17	-	0.18	0.26	***P-value***	
n	62	-	64	9	***Pkd1$^{RC/del2}$* vs. *Pkd1$^{RC/RC}$***	***0.406***
***Pkd1$^{RC/RC}$* 12 months (Mean)**	3.50	6.24	3.36	3.24	**4.09**	**1.44**
SD	0.61	1.57	0.28	0.54	***P-value***	
n	77	17	71	63	***WT vs. Pkd1$^{RC/RC}$***	***0.554***

[A]Cilia measurements of >50 cilia in >5 bile ducts (BD)/animal
[B]WT(P25) vs. WT (12mo) p=0.220
[C]Cilia length measurements of one microhamartoma (M) (WT vs. M p=1.49E-06)

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