Supplemental material

Methods

Mass spectrometry

One part of whole urine was mixed with four parts of acetone and precipitated overnight at -20°C. Samples were centrifuged at 1500 rpm (~500 x g) for 30 min. The supernatant was discarded and the pellet was dried at room temperature for 30 min. Pellets from acetone-precipitated urine or uEVs were lysed by sonication in 12 mM Sodiumdeoxycholate (SDC), 12 mM sodium laurylsarcosinate (SLS) in 100 mM Tris/HCl (pH 9.0).[1] Disulfide bonds were reduced in 5 mM dithiothreitol at 50°C for 30 minutes and free sulfhydryl groups were then alkylated in 10 mM iodoacetamide for 30 min in the dark at room temperature. The protein mixture was diluted 5 times with 50 mM ammonium bicarbonate and digested with trypsin overnight at 37°C. Digestions were acidified with trifluoroacetic acid to a concentration of 0.5% to precipitate the SDC and SLS, which was subsequently removed by centrifugation. Tryptic peptides were fractionated by HILIC and Sixteen 6 ml fractions were collected and ultimately divided into eight fractions, desalted and lyophilized. Each fraction was then analyzed by LC-MS. For quantitative dimethyl labeling of uEVs (identification cohort), desalting and reductive dimethylation was performed on the SPE cartridge (as described previously [2]) before peptide fractionation by HILIC. All LC–MS/MS analyses were performed on a Q Exactive mass spectrometer (Thermo Scientific, San Jose, CA). A total of eight dried HILIC fractions were resuspended in 0.5% formic acid in 3% acetonitrile and loaded onto an easy spray column (Pepmap RSLC, C18, 2 um, 75 um x 25 cm, Thermo Fisher Scientific). Peptides were separated using the Thermo Fisher Scientific Easy Nano LC1000 (buffer A=0.1 % formic acid and buffer B=0.1 % formic acid in acetonitrile) using a gradient of 0-28% buffer B for 90 min, with a flow

rate of 300 nl/min. Each Data collection cycle in the Q Exactive consisted of 1 full MS scan (300-1750 m/z) followed by 15 data dependent MS/MS scans.

Mouse models of ADPKD

iKsp-*Pkd1*^{del} mice have a homozygously bred kidney specific tamoxifen inducible Cre and two *Lox*P sites that flank exons 2-11 of the *Pkd1* gene.[3, 4] Tamoxifen (Sigma-Aldrich, T5648) was dissolved in 10% ethanol in sunflower oil by sonication and administered by oral gavage at either *Post-Natal* day (P)10-12 (6 mg/kg), P18-20 (150 mg/kg) or P40-42 (200 mg/kg). To follow the onset of renal failure, 30µl blood from the tail vein was used to measure blood urea using Reflotron technology (Kerkhof Medical Service, Apeldoorn, The Netherlands). Mice having a blood urea > 20 mmol/L were considered to be at the onset of renal failure and were euthanized by cervical dislocation. P10 mice generally have severe PKD at an age of 33 days. P18 mice develop renal failure approximately 12 weeks after Tamoxifen, and P40 mice develop renal failure approximately 16 weeks after Tamoxifen. In addition, P40 mice were also euthanized at 11 weeks after Tamoxifen administration at which the mice have mild PKD.[5] Local animal experimental committee of the Leiden University Medical Center and the Commission Biotechnology in Animals of the Dutch Ministry of Agriculture approved the experiments performed. Protein extraction was performed as described previously. [5] In short: kidneys were homogenized in RIPA without detergents using Magnalyser technology (Roche, Woerden, The Netherlands) and sonication. Next, an equal amount of RIPA with detergents (final concentration 1% DOC, 1% NP-40, 1% Triton X100) was added and the samples were incubated for 45 minutes at 4°C. The lysates were centrifuged at 14.000 g for 10 minutes and the supernatant was used for immunoblot analyses.

Genetic analysis of ADPKD patients

Genomic DNA was isolated from peripheral blood samples using PUREGENETM nucleic acid purification chemistry on the AUTOPURE LS 98 Instrument (Qiagen, Venlo, The Netherlands). Mutation analysis of PKD1 and PKD2 involved direct sequencing of all coding exons, except exon 1 and 12 of PKD1, including flanking intron sequences and Multiplex Ligation-dependent Probe Amplification (MLPA). The ADPKD mutation data base (http://pkdb.mayo.edu/) was used as a source of information about known PKD1 and PKD2 variants. Sequence analysis of exons 2-32 of PKD1 was performed using Long Range (LR) PCR's followed by nested PCR's. For the analysis of exons 33-46 of PKD1 and PKD2 LR PCR was not necessary. Details on primer sequences and PCR conditions are available upon request. Amplicons were sequenced using the BigDye terminator (Life Technologies, Bleiswijk, The Netherlands) method followed by electrophoresis on the ABI3730 DNA Analyzer (Applied Biosystems, Bleiswijk, The Netherlands). Analysis of the sequence data was performed using SeqScape software (Applied Biosystems). For the detection of larger deletions and duplications two commercially available MLPA kits (P351-B2 and P352-C1; MRC-Holland, Amsterdam, The Netherlands) were used according to the manufacturer's instructions.

References

1. Masuda, T, Saito, N, Tomita, M, Ishihama, Y: Unbiased quantitation of Escherichia coli membrane proteome using phase transfer surfactants. *Mol Cell Proteomics,* 8**:** 2770-2777, 2009.
2. Wilson-Grady, JT, Haas, W, Gygi, SP: Quantitative comparison of the fasted and re-fed mouse liver phosphoproteomes using lower pH reductive dimethylation. *Methods,* 61**:** 277-286, 2013.
3. Lantinga-van Leeuwen, IS, Leonhard, WN, van de Wal, A, Breuning, MH, Verbeek, S, de Heer, E, Peters, DJ: Transgenic mice expressing tamoxifen-inducible Cre for somatic gene modification in renal epithelial cells. *Genesis,* 44**:** 225-232, 2006.
4. Lantinga-van Leeuwen, IS, Leonhard, WN, van der Wal, A, Breuning, MH, de Heer, E, Peters, DJ: Kidney-specific inactivation of the Pkd1 gene induces rapid cyst formation in developing kidneys and a slow onset of disease in adult mice. *Hum Mol Genet,* 16**:** 3188-3196, 2007.
5. Leonhard, WN, van der Wal, A, Novalic, Z, Kunnen, SJ, Gansevoort, RT, Breuning, MH, de Heer, E, Peters, DJ: Curcumin inhibits cystogenesis by simultaneous interference of multiple signaling pathways: in vivo evidence from a Pkd1-deletion model. *Am J Physiol Renal Physiol,* 300**:** F1193-1202, 2011.

Supplementary Table 1: patient characteristics

Identification group		Age	Gender	PKD 1 mutation	eGFR (ml/min/1.73m2)	Ht TKV (ml/m)	UAlbumin (mg/mol creat)
	Healthy subject 1	59	F				
	Healthy subject 2	55	M				6,739
	Healthy subject 3	44	F				0,886
	Healthy subject 4	55	F				0,394
	Healthy subject 5	56	M				2,653
	Healthy subject 6	26	M				0,191
	ADPKD 1	58	F	Hetrozygous c.6736C>T, p.Gln2246*	51	552,8	0,500
	ADPKD 2	51	M	Hetrozygous c.1418_1419del, p.Val473fs	30	1050,8	1,556
	ADPKD 3	44	F	Hetrozygous c.1198C>T, p.Arg400*	46	N.A.	6,272
	ADPKD 4	55	F	Hetrozygous c.5101A>G p.Asn1701Asp	49	285,0	1,359
	ADPKD 5	60	M	No mutation identified, but no mutations in *PKD2* and phenotype consistent with ADPDK †	62	1347,7	0,059
	ADPKD 6	24	M	Hetrozygous c.8791+1G>T	47	2271,0	8,218
Confirmation group 1							
	Healthy subject 7	58	F				0,267
	Healthy subject 8	61	M				0,671
	Healthy subject 9	52	F				0,698
	Healthy subject 10	41	F				0,377
	Healthy subject 11	43	M				0,353
	Healthy subject 12	43	M				0,476
	ADPKD 7	59	F	Hetrozygous c.9569-1G>C	41	2184,7	1,607
	ADPKD 8	50	F	Hetrozygous c.1141G>T p.Glyc381Cys	38	1318,7	1,444
	ADPKD 9	60	F	No mutation identified, but no mutations in *PKD2* and phenotype consistent with ADPDK †	46	N.A.	4,091
	ADPKD 10	44	M	Hetrozygous c.7973_7974 del p.Val2658fs	49	1749,5	3,152
	ADPKD 11	47	M	Hetrozygous c.11606T>A p.Leu3869Gln	57	880,4	2,167
	ADPKD 12	42	M	Hetrozygous c.4968_4969delinsC p.Arg1657fs	68	1272,3	1,529
	CKD 1	60	F		36		22,167
	CKD 2	50	F		35		0,220
	CKD 3	56	F		44		0,667
	CKD 4	43	M		51		1,214
	CKD 5	44	M		54		0,168
	CKD 6	40	M		54		24,522
Confirmation group 2							
	ADPKD 13	58	M	Hetrozygous c.12167G>A, p.Trp4056*	35	758,3	0,897
	ADPKD 14	40	F	Hetrozygous c.6913C>T, p,Gln2305*	47	1287,0	15,769
	ADPKD 15	51	M	Hetrozygous c.9409C>T, p.His3137Tyr	33	778,3	0,510
	ADPKD 16	48	F	Hetrozygous c.4797C>G p.Tyr1599*	60	844,1	7,500
	ADPKD 17	41	M	Hetrozygous c,5154_5163dup p.Met1722fs	46	2624,6	5,493
Confirmation group 3							
	Healthy 13	29	F				9,76
	Healthy 14	32	M				1,20
	Healthy 15	29	F				7,40
	Healthy 16	30	M				7,12
Stage 1	ADPKD 18	23	F	Hetrozygous c.2542C>T p.Gln848*	125,2	709,3	5,63
	ADPKD 19	24	F	Hetrozygous c.1286G>T, p.Trp429Leu + Hetrozygous c. 8293C>T p.Arg2765Cy	108,69	626,6	2,88
	ADPKD 20	25	M	Hetrozygous c.4957C>T, p.Glu1653*	114,2	782,2	10,88
	ADPKD 21	27	M	Hetrozygous c.7915C>T p.Arg2639*	106,3	528,0	9,67
	ADPKD 22	34	M	Hetrozygous c.4957C>T, p.Glu1653*	93,28	631,5	1,61
	ADPKD 23	34	F	Hetrozygous c. 5266G>T, p.Glu1756*	111,12	456,3	14,15
Stage 3-4	ADPKD 24	37	F	Hetrozygous c.6795C>G p.Tyr2265*	63	861,8	14,51
	ADPKD 25	48	F	Hetrozygous c.4797C>G p.Tyr1599*	60	844,1	0,83
	ADPKD 26	42	F	Hetrozygous c.3296-2A>C	54	646,8	0,50
	ADPKD 27	50	F	Hetrozygous c.2269del p.Gln757fs	52	763,9	1,26
	ADPKD 28	38	F	Hetrozygous c.11343C>A p.Tyr3781*	49	1244,0	0,35
	ADPKD 29	39	M	Hetrozygous c.11537+2T>G	46	943,1	4,81
	ADPKD 30	56	M	Hetrozygous c.12167G>A, p.Trp4056*	46	758,3	1,28
	ADPKD 31	46	F	Hetrozygous c.6913C>T p.Gln2305*	42	1287,0	3,60
	ADPKD 32	43	M	Hetrozygous c.4968_4969delinsC p.Arg1657fs	32	3384,1	0,68
	ADPKD 33	42	M	Hetrozygous c.5011dup p.Asp1671fs	28	2693,2	0,56
	ADPKD 34	39	M	Hetrozygous c.2180T>C p.Leu727Pro	24	2796,9	1,52

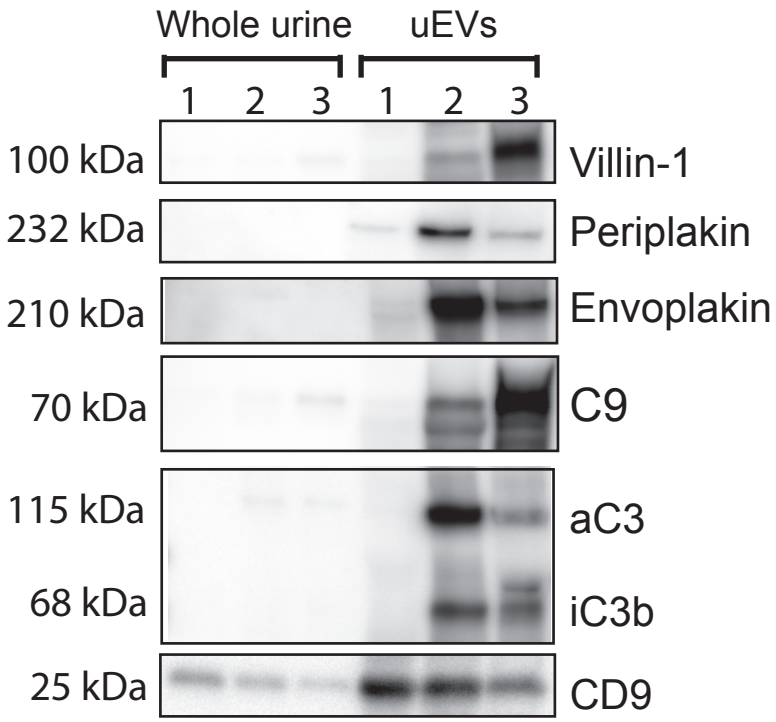
Footnote

† Previous literature confirms that more elaborate genetic analysis in ADPDK patients in whom genotyping did not identify a mutation, usually yields a *PKD1* mutation (Rossetti S, J Am Soc Nephrol, 2012; Paul BM, Kidney Int, 2014)

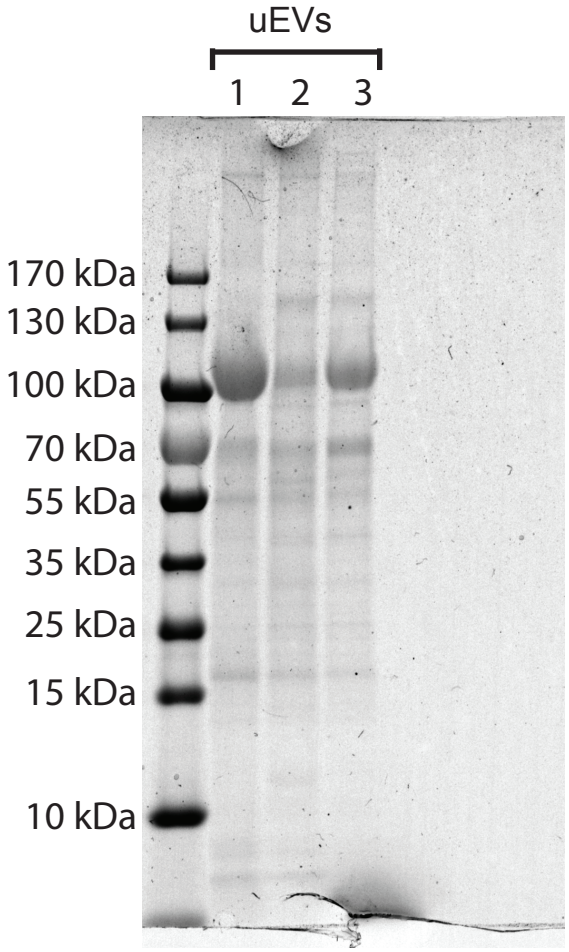
Supplementary Table 2: Characteristics of wild-type and kidney-specific-tamoxifen-inducible *Pkd1* -deletion mice

	#	Day tamoxifen	Day of sacrifice	Localization of cysts	2KW/BW	Blood urea (mmol/l)
Wild type	1		138		1.32	9.0
	2		135		1.17	9.1
	3		83		1.24	10.6
	4		33		1.16	N.D.
	5		33		1.10	N.D.
iKsp-Pkd1del P10	1	10	33	Mostly distal	2.26	23.2
	2	10	33	Mostly distal	2.31	9.4
	3	10	33	Mostly distal	2.91	9.5
iKsp-Pkd1del P18	1	18	94	All segments	11.79	> 50
	2	18	113	All segments	11.26	> 50
	3	18	110	All segments	10.50	33.2
iKsp-Pkd1del P40 mild	1	40	117	Mostly proximal	2.90	9.9
	2	40	117	Mostly proximal	2.25	11.2
	3	40	117	Mostly proximal	2.30	10.0
iKsp-Pkd1del P40 severe	1	40	149	Mostly proximal	7.10	24.3
	2	40	134	Mostly proximal	6.82	22.3
	3	40	153	Mostly proximal	6.57	24.3

Supplementary figure 1



Supplementary figure 2



Protocol for urinary extracellular vesicle isolation

Isolation protocol

- The following isolation solution was used (all compounds were purchased from SigmaAldrich):
    - o 10 mM triethanolamine (0.5ml of 1M stock)
    - o 250 mM sucrose(25ml of 0.5M Stock)
    - o ddH20 was added to 45ml
    - o pH was adjusted to 7.6 with 1M NaOH
    - o ddH20 was added to 50 ml
- To remove whole cell membranes and other high density particles, urine samples were centrifuged at 17,000 x g for 15 minutes at 4°C in 70 ml polycarbonate centrifuge bottles (Beckman Coulter, Woerden, The Netherlands) in an ultracentrifuge (Beckman L8-70M ultracentrifuge, Rotor 45 Ti).
- The supernatant was stored at 4°C (Supernatant 1).
- The pellet was suspended in 360 µl dithiothreitol (500 mg/ml) and 450 µl isolation solution and incubated for 5 minutes at 37°C and vortexed until the pellet was dissolved.
- The dissolved pellet was centrifuged again at 17,000 x g for 15 minutes at 4°C in 10 ml polycarbonate tubes (Rotor 70.1 Ti) .
- This supernatant was pooled with Supernatant 1 and centrifuged in 70 ml polycarbonate tubes at 200,000 x g for 2 hours at 4°C.
- The supernatant was discarded and the pellet was suspended in MilliQ or laemmli buffer and stored at -80°C until further analysis.