The genetic background significantly impacts the severity of kidney cystic disease in the *Pkd1*$^{RC/RC}$ mouse model of autosomal dominant polycystic kidney disease

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Running Title: Genetic background *Pkd1*$^{RC/RC}$ model

Supplementary Methods

Source of animal lines and diet

Wildtype C57BL/6J and BalbC/cJ animals were obtained from Jackson Laboratory (Bar Harbor, ME) and 129S6/SvEvTac animals from Taconic Biosciences (Rensselaer, NY). Animals were maintained on the 5053 – PicoLab Rodent Diet 20 (LabDiet, St. Louis, MO). Breeding Animals were fed the high-protein 5058 – PicoLab Mouse Diet 20 (LabDiet, St. Louis, MO), and experimental animals had access to this feed until weaned at approximately 4 weeks of age. Mouse colonies were kept at a constant 22.8° with 34% humidity and a strict 12-hour light/dark cycle.

Histological analysis

Animals were sacrificed by carbon dioxide exposure as per Mayo Clinic IACUC guidelines. Body and organ weights were recorded before fixation in 4% paraformaldehyde. Fixed tissue was embedded in paraffin, cut into 4μm sections, and stained with Masson trichrome stain for histological analysis. CA and CN were calculated from three distinct Masson trichrome stained left kidney cross-sections from each mouse. The CA was calculated as a percentage of the total cross-sectional area of the kidney using the NIS elements software. A cyst was defined as >2000 μm^2 in adult mice and a dilated tubule as >500 μm^2 in P1-P18 mice. Cyst number was determined as previously described.[1] FA was determined from eight Picrosirius Red stained cortical images and recorded as a percentage of the total captured region using the MetaMorph software. Biliary lesions, defined as multiple bile ducts grouped together (biliary hamartomas) and/or as greatly enlarged bile ducts, were identified by visualization of at least three Masson trichrome stained liver sections from each background and evaluated by an experienced liver pathologist (Dr. Roger Moreira). The incidences of biliary dysgenesis were recorded with the mean hepatic dysgenesis loci per section reported.

Immunofluorescence labeling

Immunofluorescence labeling of paraffin embedded sections was prepared as previously described.[2] The primary reagents were, biotinylated-LTA (1:250, Vector Laboratories), AQP2 antibody (1:500; ProSci), and PCNA antibody (1:250; Cell Signaling), with DAPI employed as a nuclear counterstain. Secondary antibodies (Alexa Fluor, Invitrogen) were used at a 1:500 dilution. For proliferation analysis, cyst linings were determined to be the layer of cells surrounding a dilated tubule of >500 μm^2 in P1-P18 mice and a PCNA-positive cell was determined to be any cell in a cyst lining that had an intense PCNA signal when compared to the surrounding DAPI

signal. Proliferation was also measured in P18 WT CD tubule linings for all backgrounds apart from F1(129/B6). The percentage of proliferating cystic epithelial cells was calculated as the number of PCNA positive cells/the total number of cells in the cyst linings and averaged for each mouse. For each kidney, 20-50 cysts were quantified for analysis and were selected from throughout the kidney using an overlay-grid.

BUN and cAMP measurements

Blood was collected by cardiac puncture after mice were euthanized by CO^2, heparin added to prevent clotting, and was centrifuged for 20min at 1500g to collect plasma. Diluted plasma was used to determine BUN using the colorimetric BUN assay (BUN-Urea, BioAssay Systems). cAMP levels were performed using 30-70mg of pulverized, flash-frozen kidney tissue (Direct cAMP EIA kit, ENZO). Both assays were performed according to the manufacturers' protocols.

Magnetic Resonance Imaging and Analysis

Magnetic resonance imaging scans in all animals were performed in a Bruker Advance 700 Mhz (16.4T) vertical bore nuclear magnetic resonance spectrometer following procedures approved by the IACUC.[3] TKV was calculated from axial slices of the kidney using Analyze 12.0 software and using an automated method.[4] Cyst volume was determined by multiplying the fraction of CA by the final TKV. TKV was corrected for mouse length (Ln), measured from the nose to the base of the tail, for the preclinical study.

Supplementary References

1. Olson RJ, Hopp K, Wells H, et al. Synergistic Genetic Interactions between Pkhd1 and Pkd1 Result in an ARPKD-Like Phenotype in Murine Models. *J Am Soc Nephrol.* 2019;**30**(11):2113-27. 10.1681/ASN.2019020150.
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Supplementary Tables

Table S1: Details of cystic kidney disease in the different genetic backgrounds in *Pkd1*$^{RC/RC}$ mice by sex

Endpoint	B6		BC		F1(BC/B6)		129		F1(129/B6)	
KW/BW%	**Males**	**Females**	**Males**	**Females**	**Males**	**Females**	**Males**	**Females**	**Males**	**Females**
1m	1.65 ± 0.17	1.58 ± 0.05	2.12 ± 1.05	3.04 ± 0.55†	1.84 ± 0.42	2.07 ± 0.19	2.34 ± 0.25	2.56 ± 0.70	2.00 ± 0.28	1.60 ± 0.23
3m	1.96 ± 0.26	1.73 ± 0.21	3.22 ± 0.61††	3.25 ± 1.13††	2.10 ± 0.13	1.75 ± 0.11	3.77 ± .063†††	2.39 ± 0.38	2.26 ± 0.19	3.20 ± 1.61†
6m	1.93 ± 0.25	2.02 ± 0.33	3.51 ± 0.34†††	3.95 ± 0.72†††	2.53 ± 0.45	2.27 ± 0.27	4.15 ± 0.76†††	5.71 ± 2.44†††	2.47 ± 0.07	4.15 ± 0.71††† **
9m	2.08 ± 0.12	2.34 ± 0.26	3.15 ± 0.32††	4.81 ± 0.49††† **	3.20 ± 0.42††	4.00 ± 0.57†† **	3.66 ± 0.98†††	4.54 ± 0.39†††	1.73 ± 0.21	3.77 ± 0.82†***
12m	2.43 ± 0.23	4.31 ± 0.53***	3.28 ± 0.67†	4.92 ± 0.89**	3.02 ± 0.23	3.82 ± 0.29**	3.72 ± 1.32†††	4.82 ± 0.29	2.12 ± 0.27	3.59 ± 0.48**
CA%										
1m	3.21 ± 1.30	3.04 ± 1.01	15.56 ± 5.32†	19.58 ± 5.75†††	6.54 ± 4.41	3.95 ± 2.61†	15.01 ± 5.44†	16.10 ± 8.45†††	9.20 ± 4.36	4.85 ± 2.27
3m	5.30 ± 2.41	3.10 ± 1.25	18.91 ± 8.27††	17.39 ± 3.57†††	7.13 ± 4.04	12.60 ± 5.73	32.12 ± 16.63†††	19.43 ± 7.89†††	7.87 ± 3.14	9.63 ± 5.27
6m	5.71 ± 4.77	3.96 ± 2.14	23.87 ± 6.32†††	22.60 ± 2.99†††	10.70 ± 4.09	8.23 ± 2.79	28.44 ± 5.31†††	32.03 ± 14.07†††	*14.66 ± 4.59*	18.20 ± 4.70^{X}†††
9m	3.91 ± 3.03	7.16 ± 3.63	22.03 ± 2.25†††	20.22 ± 2.56††	18.41 ± 5.66††	16.74 ± 1.45†	24.65 ± 5.35†††	25.53 ± 3.65†††	5.53 ± 2.38	17.00 ± 5.25†† **
12m	8.09 ± 4.46	20.77 ± 4.93***	27.65 ± 13.29†††	26.98 ± 8.51	13.20 ± 1.47	18.84 ± 4.40	16.90 ± 8.29	18.90 ± 3.15	13.44 ± 4.92	29.20 ± 5.08***
CN										
1m	14 ± 8	20 ± 7	79 ± 19	104 ± 37†	47 ± 27	24 ± 11	79 ± 24	52 ± 15	29 ± 10	19 ± 6
3m	19 ± 3	18 ± 8	162 ± 60†††	134 ± 52††	46 ± 28	76 ± 27	110 ± 24††	73 ± 14	39 ± 11	66 ± 44
6m	17 ± 9	71 ± 37*	189 ± 26†††	220 ± 37†††	151 ± 58†††	71 ± 21**	146 ± 37†††	186 ± 54††	*86 ± 42*	88 ± 24^{X}
9m	*47 ± 7*	89 ± 36^{X}	220 ± 21^{X}	333 ± 73††† **	182 ± 30^{X}	244 ± 29†††	*156 ± 8*$_{X}$	243 ± 27^{X}†††	78 ± 44$_{X}$	143 ± 21††
12m	66 ± 39	94 ± 8	212 ± 58†††	181 ± 43†	185 ± 31†††	241 ± 38†††	159 ± 17††	235 ± 48††† *	141 ± 42††	269 ± 0.4††† ***
BUN										
1m	16.6 ± 2.5	18.8 ± 3.5	19.3 ± 1.2	22.4 ± 4.8	16.0 ± 2.9	16.8 ± 4.1	18.6 ± 3.8	19.4 ± 5.1	17.2 ± 2.0	13.9 ± 0.7
3m	23.3 ± 1.0	24.8 ± 1.8	22.6 ± 5.4	18.4 ± 2.3	19.8 ± 3.6	16.2 ± 1.8	25.2 ± 4.4	20.8 ± 3.0	24.0 ± 2.2	*20.5 ± 2.6*
6m	24.8 ± 3.3	23.2 ± 2.3	20.0 ± 5.7	24.8 ± 0.5	21.2 ± 3.8	16.0 ± 2.5	28.2 ± 4.3	26.4 ± 5.7	29.3 ± 2.8	*33.7 ± 3.1*
9m	24.5 ± 5.1	24.7 ± 6.4	33.8 ± 9.0†	30.4 ± 6.5	22.2 ± 4.4	19.8 ± 4.8	*27.7 ± 4.7*	32.2 ± 4.8^{X}	27.7 ± 2.1	21.6 ± 1.1
12m	20.4 ± 2.0	40.6 ± 12.8***	40.8 ± 16.0†††	32.2 ± 17.9	20. ± 6.1	25.4 ± 4.5†††	24.8 ± 4.9	35.0 ± 7.4*	31.2 ± 4.2††	*48.2 ± 10.5*
FA%										
1m	1.44 ± 1.19	0.99 ± 0.40	1.30 ± 1.02	0.90 ± 0.47	0.56 ± 0.07	0.65 ± 0.68	0.54 ± 0.59	0.72 ± 0.55	0.27 ± 0.18	0.11 ± 0.07
3m	0.55 ± 0.35	1.76 ± 0.23	0.59 ± 0.35	1.76 ± 2.23	0.26 ± 0.17	0.83 ± 0.41	1.27 ± 0.72	1.59 ± 0.41	1.54 ± 0.53	*1.04 ± 0.58*^{X}
6m	1.67 ± 0.54	1.50 ± 0.82	3.00 ± 0.90	1.73 ± 0.54	1.41 ± 0.93	0.72 ± 0.22	2.36 ± 1.00	4.50 ± 1.75	0.67 ± 0.83	1.28 ± 1.35
9m	2.77 ± 0.98	5.68 ± 1.66	6.22 ± 5.36	2.61 ± 1.56	2.35 ± 1.74	7.68 ± 2.73**	5.88 ± 3.28	6.30 ± 1.81	1.64 ± 0.44	4.78 ± 2.73*
12m	11.11 ± 3.95	9.81 ± 2.08	6.05 ± 2.61††	10.58 ± 3.94	3.85 ± 0.12†††	8.74 ± 4.61*	11.16 ± 3.24	17.18 ± 4.10††† ***	3.84 ± 1.61†††	*12.74 ± 0.34*^{X}
cAMP										
1m	7.09 ± 4.28	6.83 ± 4.14	3.08 ± 1.29	4.28 ± 1.89	10.05 ± 2.24	8.97 ± 1.31	10.18 ± 2.91	17.15 ± 5.24	6.97 ± 2.95	7.72 ± 0.91
3m	4.61 ± 1.60	6.83 ± 2.06	2.60 ± 1.15	5.02 ± 2.64	7.45 ± 1.51	6.96 ± 1.14	17.93 ± 4.98†††	10.60 ± 3.91	3.84 ± 0.41	4.74 ± 1.15
6m	8.47 ± 2.97	7.18 ± 2.49	9.21 ± 4.86	8.43 ± 5.79	6.56 ± 1.68	12.24 ± 6.05	33.90 ± 13.85†††	*27.84 ± 5.56*$_{X}$	13.16 ± 5.69	8.10 ± 1.13
9m	13.72 ± 7.13	19.27 ± 4.85	11.19 ± 2.27	11.93 ± 2.33	11.90 ± 4.73	18.30 ± 5.29	50.45 ± 6.86†††	38.44 ± 17.13†††	8.95 ± 2.12	12.98 ± 5.80
12m	*14.31 ± 4.51*	20.03 ± 6.82^{X}	24.21 ± 9.56$_{X}$	21.94 ± 11.67	14.34 ± 6.32$_{X}$	23.70 ± 11.93*	*30.37* $_{X}$	40.57 ± 14.31†††	6.95 ± 1.43$_{X}$	*10.59 ± 0.06*^{X}$_{X}$
TKV										
1m	466 ± 91	432 ± 97	*1109 ± 235*	*848*	NA	*403*	676 ± 256	518 ± 97	519 ± 71	377 ± 63
3m	*697 ± 238*	579 ± 125^{X}	*1581* $_{X}$	*1272 ± 241*	827 ± 155$_{X}$	666 ± 179	1422 ± 303 *$_{X}$	928 ± 416†	1044 ± 335$_{X}$	954 ± 200††
6m	944 ± 221	660 ± 114**	*1344*	*1105*	NA	*1021*	*1639 ± 121*	1704 ± 196^{X}†††	826 ± 128	1295 ± 474††† *
9m	717 ± 31	791 ± 146	NA	*2122*	NA	NA	*1649 ± 5*	1966 ± 319†††	1339 ± 207†††	1637 ± 177†††
12m	*729 ± 70*	956 ± 115^{X}	NA	NA	NA	NA	*1510 ± 114*$_{X}$	*1961*	1166 ± 241$_{X}$	1531 ± 1209††

One-way ANOVA followed by Bonferroni multiple comparison test between males and females. *p<0.05, ** p<0.01, ***p<0.001.
Two-way ANOVA followed by Dunnett's multiple comparison test to age-matched and sex-matched B6 mice. † p<0.05, †† p<0.01, ††† p<0.001
BUN, mg/dL; cAMP, pmol/mg protein, TKV, mm^3, NA, data not available.
Significance was not determined for groups with N<3, that are italicized. ^{X} Could not be statistically analyzed between males and females, $_{X}$ Could not be statistically analyzed between age-matched and sex-matched B6 mice. Number of animals per group are shown in Table 1.

Table S2: Progression of disease by sex compared to 1m within each genetic background

Endpoint	B6		BC		F1(BC/B6)		129		F1(129/B6)	
KW/BW%	**Males**	**Females**	**Males**	**Females**	**Males**	**Females**	**Males**	**Females**	**Males**	**Females**
1m	1.65 ± 0.17	1.58 ± 0.05	2.12 ± 1.05	3.04 ± 0.55	1.84 ± 0.42	2.07 ± 0.19	2.34 ± 0.25	2.56 ± 0.70	2.00 ± 0.28	1.60 ± 0.23
3m	1.96 ± 0.26	1.73 ± 0.21	3.22 ± 0.61*	3.25 ± 1.13	2.10 ± 0.13	1.75 ± 0.11	3.77 ± .063	2.39 ± 0.38	2.26 ± 0.19	3.20 ± 1.61*
6m	1.93 ± 0.25	2.02 ± 0.33	3.51 ± 0.34*	3.95 ± 0.72	2.53 ± 0.45*	2.27 ± 0.27	4.15 ± 0.76*	5.71 ± 2.44**	2.47 ± 0.07	4.15 ± 0.71***
9m	2.08 ± 0.12*	2.34 ± 0.26**	3.15 ± 0.32	4.81 ± 0.49**	3.20 ± 0.42***	4.00 ± 0.57***	3.66 ± 0.98	4.54 ± 0.39*	1.73 ± 0.21	3.77 ± 0.82**
12m	2.43 ± 0.23***	4.31 ± 0.53***	3.28 ± 0.67*	4.92 ± 0.89*	3.02 ± 0.23***	3.82 ± 0.29***	3.72 ± 1.32	4.82 ± 0.29*	2.12 ± 0.27	3.59 ± 0.48**
CA%										
1m	3.21 ± 1.30	3.04 ± 1.01	15.56 ± 5.32	19.58 ± 5.75	6.54 ± 4.41	3.95 ± 2.61	15.01 ± 5.44	16.10 ± 8.45	9.20 ± 4.36	4.85 ± 2.27
3m	5.30 ± 2.41	3.10 ± 1.25	18.91 ± 8.27	17.39 ± 3.57	7.13 ± 4.04	12.60 ± 5.73**	32.12 ± 16.63*	19.43 ± 7.89	7.87 ± 3.14	9.63 ± 5.27
6m	5.71 ± 4.77	3.96 ± 2.14	23.87 ± 6.32	22.60 ± 2.99	10.70 ± 4.09	8.23 ± 2.79	28.44 ± 5.31	32.03 ± 14.07*	*14.66 ± 4.59*^{X}	18.20 ± 4.70***
9m	3.91 ± 3.03	7.16 ± 3.63	22.03 ± 2.25	20.22 ± 2.56	18.41 ± 5.66**	16.74 ± 1.45***	24.65 ± 5.35	25.53 ± 3.65	5.53 ± 2.38	17.00 ± 5.25***
12m	8.09 ± 4.46	20.77 ± 4.93***	27.65 ± 13.29	26.98 ± 8.51	13.20 ± 1.47	18.84 ± 4.40***	16.90 ± 8.29	18.90 ± 3.15	13.44 ± 4.92	29.20 ± 5.08***
CN										
1m	14 ± 8	20 ± 7	79 ± 19	104 ± 37	47 ± 27	24 ± 11	79 ± 24	52 ± 15	29 ± 10	19 ± 6
3m	19 ± 3	18 ± 8	162 ± 60	134 ± 52	46 ± 28	76 ± 27*	110 ± 24	73 ± 14	39 ± 11	66 ± 44
6m	17 ± 9	71 ± 37*	189 ± 26**	220 ± 37**	151 ± 58**	71 ± 21*	146 ± 37*	186 ± 54***	*86 ± 42*^{X}	88 ± 24
9m	*47 ± 7*^{X}	89 ± 36**	220 ± 21	333 ± 73***	182 ± 30	244 ± 29***	*156 ± 8* ^{X}	243 ± 27***	78 ± 44	143 ± 21**
12m	66 ± 39**	94 ± 8***	212 ± 58**	181 ± 43	185 ± 31***	241 ± 38***	159 ± 17**	235 ± 48***	141 ± 42**	269 ± 0.4***
BUN										
1m	16.6 ± 2.5	18.8 ± 3.5	19.3 ± 1.2	22.4 ± 4.8	16.0 ± 2.9	16.8 ± 4.1	18.6 ± 3.8	19.4 ± 5.1	17.2 ± 2.0	13.9 ± 0.7
3m	23.3 ± 1.0*	24.8 ± 1.8	22.6 ± 5.4	18.4 ± 2.3	19.8 ± 3.6	16.2 ± 1.8	25.2 ± 4.4	20.8 ± 3.0	24.0 ± 2.2*	*20.5 ± 2.6*^{X}
6m	24.8 ± 3.3**	23.2 ± 2.3	*20.0 ± 5.7*^{X}	24.8 ± 0.5	21.2 ± 3.8	16.0 ± 2.5	28.2 ± 4.3*	26.4 ± 5.7	29.3 ± 2.8***	*33.7 ± 3.1*^{X}
9m	24.5 ± 5.1**	24.7 ± 6.4	33.8 ± 9.0	30.4 ± 6.5	22.2 ± 4.4	19.8 ± 4.8	27.7 ± 4.7*	32.2 ± 4.8**	27.7 ± 2.1**	21.6 ± 1.1
12m	20.4 ± 2.0	40.6 ± 12.8***	40.8 ± 16.0*	32.2 ± 17.9	20.0 ± 6.1	25.4 ± 4.5**	24.8 ± 4.9	35.0 ± 7.4***	31.2 ± 4.2***	*48.2 ± 10.5*
FA%										
1m	1.44 ± 1.19	0.99 ± 0.40	1.30 ± 1.02	0.90 ± 0.47	0.56 ± 0.07	0.65 ± 0.68	0.54 ± 0.59	0.72 ± 0.55	0.27 ± 0.18	0.11 ± 0.07
3m	0.55 ± 0.35	1.76 ± 0.23	0.59 ± 0.35	1.76 ± 2.23	0.26 ± 0.17	0.83 ± 0.41	1.27 ± 0.72	1.59 ± 0.41	1.54 ± 0.53	*1.04 ± 0.58*^{X}
6m	1.67 ± 0.54	1.50 ± 0.82	3.00 ± 0.90	1.73 ± 0.54	1.41 ± 0.93	0.72 ± 0.22	2.36 ± 1.00	4.50 ± 1.75*	0.67 ± 0.83	1.28 ± 1.35
9m	2.77 ± 0.98	5.68 ± 1.66**	6.22 ± 5.36	2.61 ± 1.56	2.35 ± 1.74	7.68 ± 2.73*	5.88 ± 3.28*	6.30 ± 1.81**	1.64 ± 0.44	4.78 ± 2.73*
12m	11.11 ± 3.95***	9.81 ± 2.08***	6.05 ± 2.61	10.58 ± 3.94***	3.85 ± 0.12**	8.74 ± 4.61**	11.16 ± 3.24***	17.18 ± 4.10***	3.84 ± 1.61**	*12.74 ± 0.34*^{X}
cAMP										
1m	7.09 ± 4.28	6.83 ± 4.14	3.08 ± 1.29	4.28 ± 1.89	10.05 ± 2.24	8.97 ± 1.31	10.18 ± 2.91	17.15 ± 5.24	6.97 ± 2.95	7.72 ± 0.91
3m	4.61 ± 1.60	6.83 ± 2.06	2.60 ± 1.15	5.02 ± 2.64	7.45 ± 1.51	6.96 ± 1.14	17.93 ± 4.98	10.60 ± 3.91	3.84 ± 0.41	4.74 ± 1.15
6m	8.47 ± 2.97	7.18 ± 2.49	9.21 ± 4.86	8.43 ± 5.79	6.56 ± 1.68	12.24 ± 6.05	33.90 ± 13.85**	*27.84 ± 5.56*	13.16 ± 5.69*	8.10 ± 1.13
9m	13.72 ± 7.13	19.27 ± 4.85**	11.19 ± 2.27	11.93 ± 2.33	11.90 ± 4.73	18.30 ± 5.29	50.45 ± 6.86***	38.44 ± 17.13	8.95 ± 2.12	12.98 ± 5.80*
12m	*14.31 ± 4.51*^{X}	20.03 ± 6.82***	24.21 ± 9.56	21.94 ± 11.67***	14.34 ± 6.32	23.70 ± 11.93*	*30.37*	40.57 ± 14.31	6.95 ± 1.43	*10.59 ± 0.06*^{X}
TKV										
1m	466 ± 91	432 ± 97	*1109 ± 235*	848	NA	*403*	676 ± 256	518 ± 97	519 ± 71	377 ± 63
3m	*697 ± 238*^{X}	579 ± 125	*1581*^{X}	*1272 ± 241*^{X}	827 ± 155	666 ± 179	1422 ± 303	928 ± 416	1044 ± 335	954 ± 200**
6m	944 ± 221**	660 ± 114**	*1344*^{X}	*1105*^{X}	NA	1021.22	*1639 ± 121*^{X}	1704 ± 196***	826 ± 128	1295 ± 474***
9m	717 ± 31	791 ± 146***	NA	*2122*^{X}	NA	NA	*1649 ± 5* ^{X}	1966 ± 319***	1339 ± 207***	1637 ± 177***
12m	*729 ± 70*^{X}	956 ± 115***	NA	NA	NA	NA	*1510 ± 114* ^{X}	1961	1166 ± 241	1531 ± 1209***

One-way ANOVA followed by Dunnett's multiple comparison test comparing sex-matched animals to 1-month mice within each background. *p<0.05, **p<0.01, ***p<0.001.
BUN, mg/dL; cAMP, pmol/mg protein, TKV, mm^3, NA, data not available.
Significance was not determined for groups with N<3, that are italicized. ^{X} Could not be statistically analyzed between males and females, $_{x}$ Could not be statistically analyzed between age-matched and sex-matched B6 mice. Number of animals per group are shown in Table 1.

Table S3: Comparison of sham vs. tolvaptan-treated mice

Endpoint	Sham	Tolvaptan	Significance[1]
Initial TKV (mm^3)	331 ± 89	343 ± 89	0.6378
Initial LnTKV (mm^3/cm body length)	44.7 ± 10.6	46.9 ± 10.8	0.4927
KW/BW%	2.76 ± 0.72	2.20 ± 0.44	**0.0020**
Final LnTKV (mm^3/cm body length)	99.5 ± 25.8	75.5 ± 21.2	**0.0010**
Change LnTKV% (%ΔLnTKV)	126.3 ± 45.5	64 ± 40.0	**<0.0001**
Cyst Number	147 ± 55	111 ± 39	**0.0113**
Cyst Volume (mm^3)	197 ± 128	120 ± 1105	**0.0125**
Cystic Area%	19.73 ± 7.80	16.09 ± 5.83	0.0733
cAMP (pmol/mg total protein)	9.94 ± 4.23	7.74 ± 3.17	**0.0481**
BUN (mg/dL)	23.7 ± 7.1	19.6 ± 4.6	**0.0225**

Unpaired t-test compared to sham treated animals. Significant values are bolded.

Table S4: Sex-specific comparison of sham vs. tolvaptan-treated mice

Endpoint	Sham		Tolvaptan	
	Males	**Females**	**Males**	**Females**
Initial BW (g)	12.0 ± 3.0	12.1 ± 2.0	11.9 ± 2.1	12.3 ± 2.7
Initial LnTKV (mm^3/cm body length)	42.8 ± 11.6	46.7 ± 9.6	45.4 ± 8.9	48.3 ± 12.7
Final BW (g)	27.4 ± 3.2***	22.7 ± 1.7	25.9 ± 2.2***	20.8 ± 1.9 †
Final LnTKV (mm^3/cm body length)	109.7 ± 27.5	89.3 ± 20.3	89.0 ± 19.7 † ***	62.0 ± 12.6 †††
Change LnTKV% (%ΔLnTKV)	160.8 ± 36.3***	91.7 ± 20.1	96.1 ± 26.4 ††† ***	31.2 ± 16.6 †††
KW (g)	0.81 ± 0.31*	0.59 ± 0.13	0.62 ± 0.15**	0.42 ± 0.11 ††
KW/BW%	2.87 ± 0.84	2.66 ± 0.58	2.35 ± 0.40	2.05 ± 0.45 ††
Cyst Volume (mm^3)	223 ± 157	171 ± 90	153 ± 70*	87 ± 47 ††
Cyst Number	149 ± 64	145 ± 46	122 ± 34	100 ± 41 †

Unpaired t-test comparing males vs. females within treatment groups. ***p<0.001.
Unpaired t-test comparing sex-matched mice across treatment groups. † p<0.05, †† p<0.01, ††† p<0.001

Supplementary Figures

Figure S1

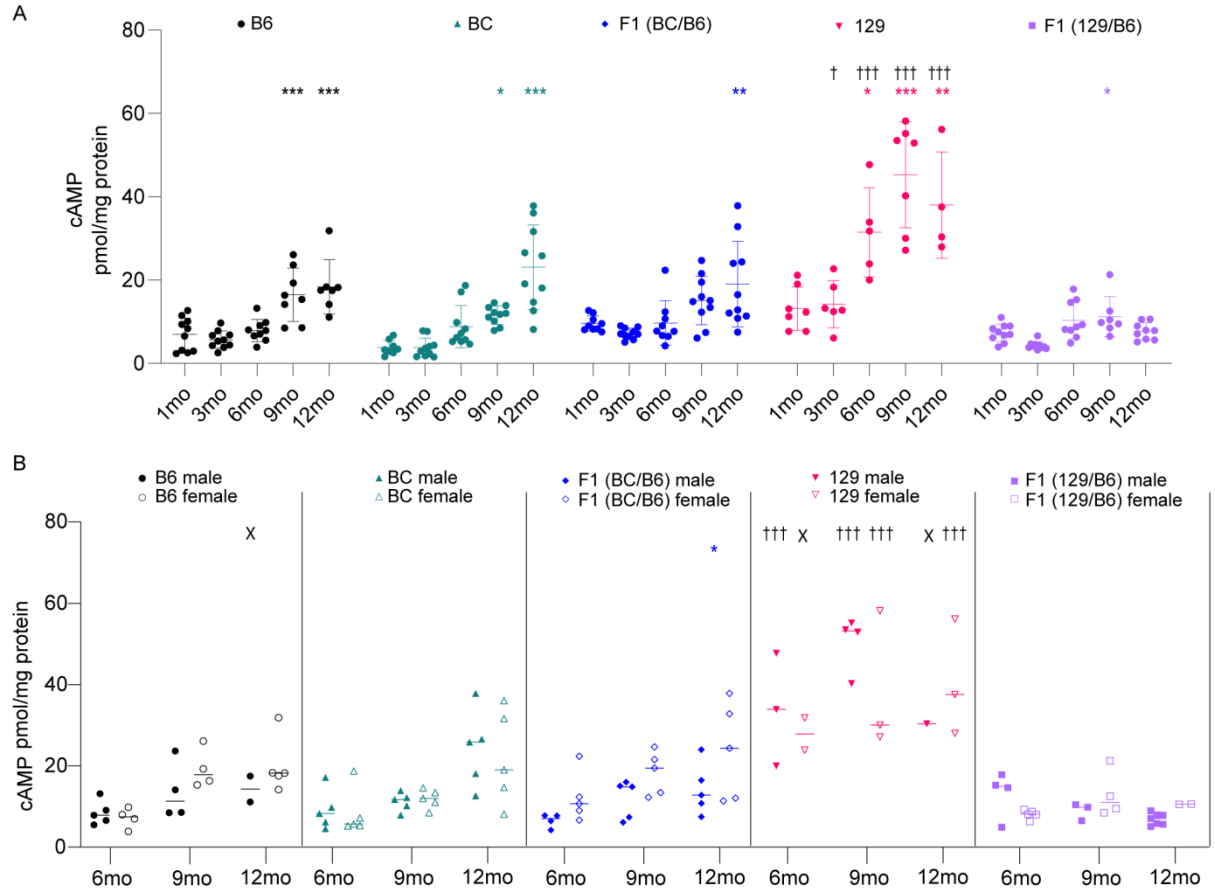

Figure S1. Analysis of kidney cAMP across different genetic backgrounds in *Pkd1*$^{RC/RC}$ mice.

(**A**) Kidney cAMP was determined in mice from each background at 1, 3, 6, 9 and 12m. Mean values +/- SD are shown on the scatter plot. The significance of the difference in the cAMP level in each background compared to the 1m baseline value was performed using a one-way ANOVA followed by Dunnett's multiple comparison test: * $p<0.05$, ** $p<0.01$, *** $p<0.001$. The significance of cAMP levels in each background were also determined compared to age matched B6 data using a two-way ANOVA followed by Dunnett's multiple comparison test: $^{\dagger}p<0.05$, $^{\dagger\dagger\dagger}p<0.001$. (**B**) Mice at each age and background were analyzed for sex specific differences in cAMP levels. Two-way ANOVA analysis followed by Bonferroni's multiple comparison test between males and females for age-matched mice of each genetic background: *$p<0.05$. Each timepoint in each background was also compared to corresponding B6 mice to determine differences per sex in cystic disease using two-way ANOVA analysis followed by Dunnett's multiple comparison test:

$^{\dagger}p<0.05$, $^{\dagger\dagger\dagger}p<0.001$ Statistical analysis only performed on groups that contained $N \geq 3$, and groups not analyzed are marked with X. Number of animals per group are shown in Table 1.

Figure S2

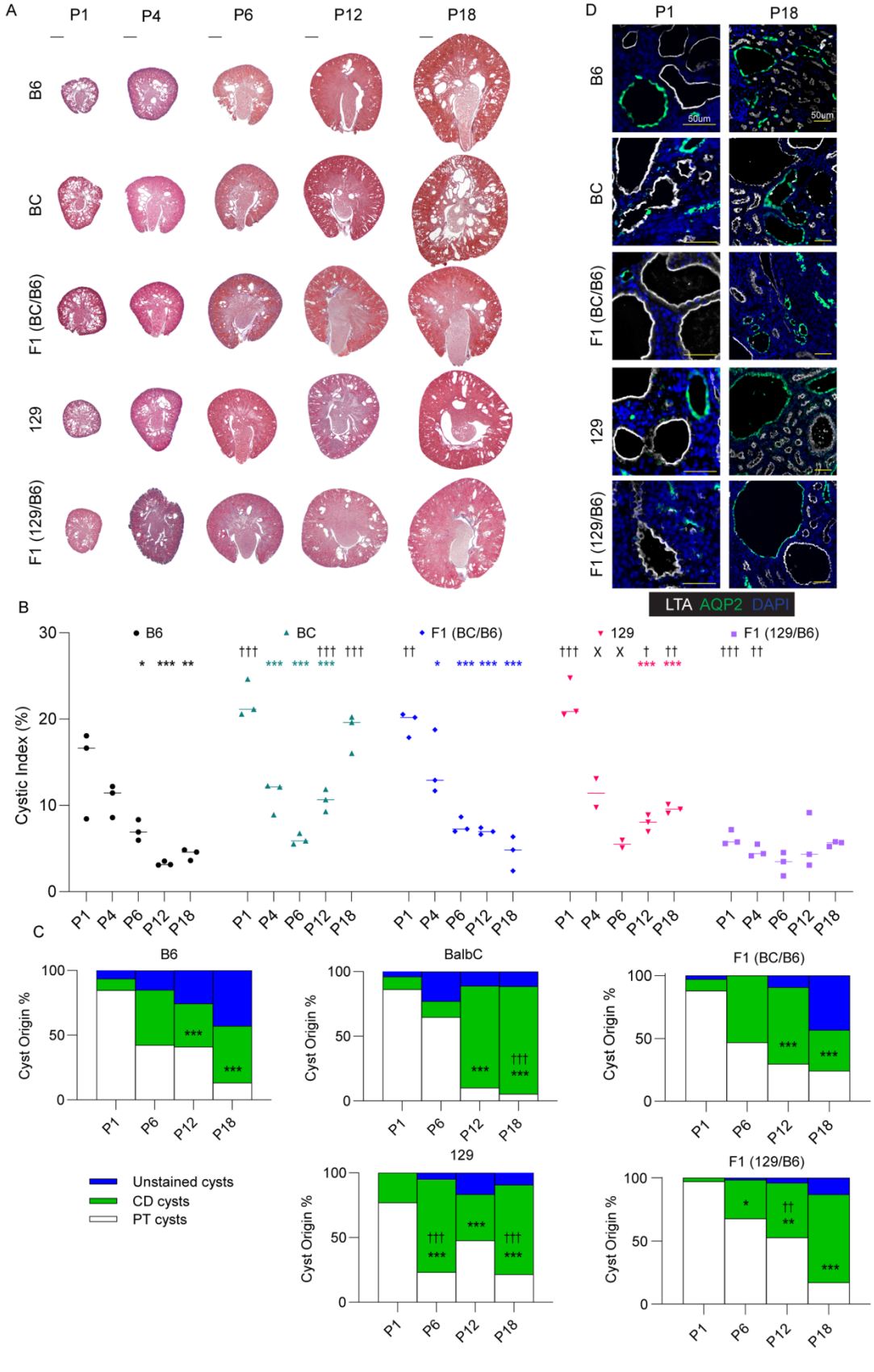
A
P1
P4
P6
P12
P18
B6
BC
F1 (BC/B6)
129
F1 (129/B6)
D
P1
P18
50um
LTA AQP2 DAPI
B
B6
BC
F1 (BC/B6)
129
F1 (129/B6)
Cystic Index (%)
C
B6
BalbC
F1 (BC/B6)
129
F1 (129/B6)
Cyst Origin %
Unstained cysts
CD cysts
PT cysts

Figure S2. Characterization of cystic disease in early perinatal *Pkd1*$^{RC/RC}$ mice across the genetic backgrounds.

(**A**) Masson Trichrome stained cross-sectional images of mice at P1, P4, P6, P12 and P18 in the different backgrounds. Scale bar 250μm. (**B**) CA measured from kidney cross sections for each background and timepoint shown as a scatter plot with the mean indicated. Change in cystic disease compared to the P1 baseline within each genetic background was analyzed with one-way ANOVA analysis followed by Dunnett's multiple comparison test: *p<0.05, **p<0.01, ***p<0.001. Each background was also compared to age matched B6 mice to determine differences in cystic disease by two-way ANOVA analysis followed by Dunnett's multiple comparison test compared to age matched B6 mice: †p<0.05, ††p<0.01, †††p<0.001. (**C**) Tubular segment origin of cysts for each of the backgrounds determined by LTA (proximal tubule; PT; white) or AQP2 (collecting duct; CD; green) staining or unstained/another segment (blue). Difference in %CD cysts compared to P1 within each genetic background was analyzed with one-way ANOVA followed by Dunnett's multiple comparison test: *p<0.05, **p<0.01, ***p<0.001. Difference in %CD cysts from age matched B6 mice was determined by two-way ANOVA analysis followed by Dunnett's multiple comparison test: ††p<0.01, †††p<0.001. (**D**) Examples of immunofluorescence imaging of cyst linings to determine origin of cysts (LTA, PT [white]; AQP2, CD [green]; DAPI, nuclei [blue]). Scale bar 50μm. Statistical analysis only performed on groups that contained N≥3, and groups not analyzed are marked with X.

Figure S3

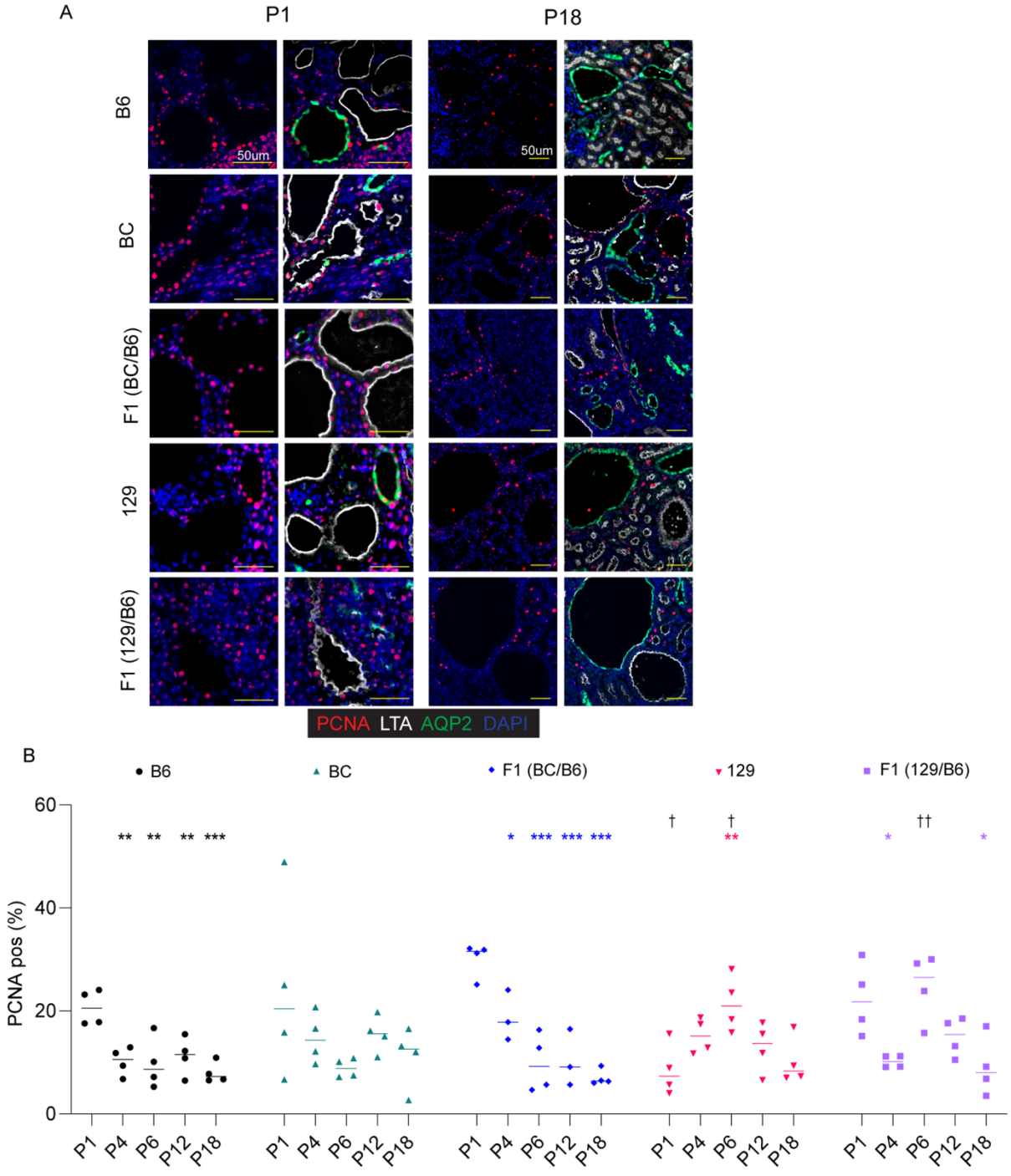

Figure S3. Proliferation in cyst linings in early postnatal mice.

(**A**) Immunofluorescence imaging of cyst linings detected for PCNA (red), LTA (PT; white), AQP2 (CD; green) and DAPI (blue) to determine proliferating cystic epithelia (%PCE). PCNA positive nuclei were counted as representative of cell undergoing proliferation and only determined in cyst

linings. Cysts were identified as tubules with a diameter >200um^2. Scale bar 50μm. (**B**) Quantitative analysis of PCNA positive cells in cyst linings showing mean values. Three wildtype animals were collected and analyzed at P18 for each background, except F1(129/B6). Mean P18 wildtype %PCNA positive tubule epithelial cells: B6, 2.6; BC, 5.6; F1(BC/B6), 3.0; and 129, 1.3. The significance of differences in PCE compared to P1 baseline within each background was tested with a one-way ANOVA followed by Dunnett's multiple comparison test: *p<0.05, **p<0.01, ***p<0.001. The significance of differences between each background compared to age matched B6 data was determined with a two-way ANOVA followed by Dunnett's multiple comparison test: †p<0.05, ††p<0.01.

Figure S4

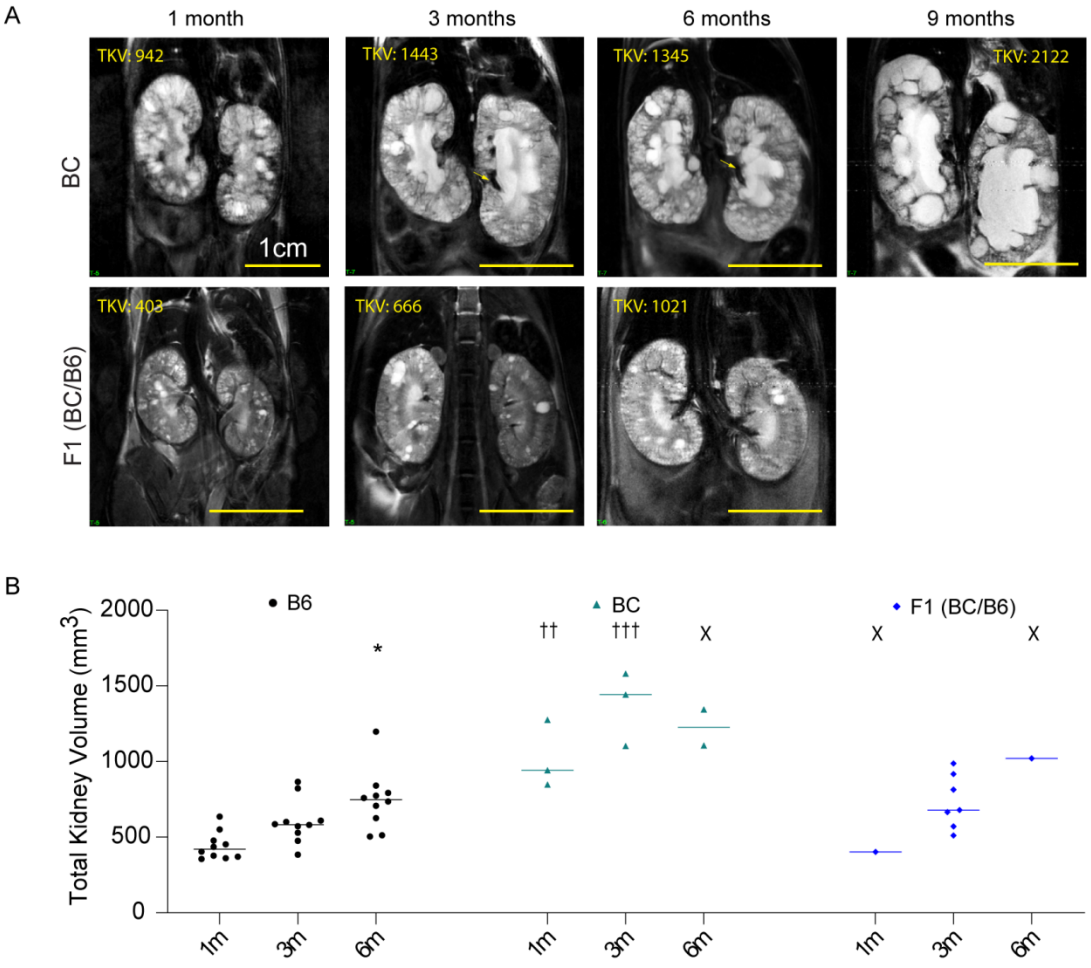

Figure S4. Comparison of live *Pkd1*$^{RC/RC}$ mice in the BC and F1(BC/B6) backgrounds over time by MR imaging.

(**A**) Representative kidney coronal MR sections from BC and F1(BC/B6) mice. Scale bar 1cm. Note that different animals were imaged at each timepoint. (**B**) Quantification of TKV determined from MR images. Arrow indicates urine retention. The significance of differences in TKV within each background compared to 1m baseline was determined with a one-way ANOVA followed by Dunnett's multiple comparison test: *$p<0.05$. The significance of differences in TKV in each background compared to age matched B6 mice was determined with a two-way ANOVA followed by Dunnett's multiple comparison test: $^{††}p<0.01$, $^{†††}p<0.001$. Statistical analysis only performed on groups that contained $N\geq3$, and groups not analyzed are marked with X.

Figure S5

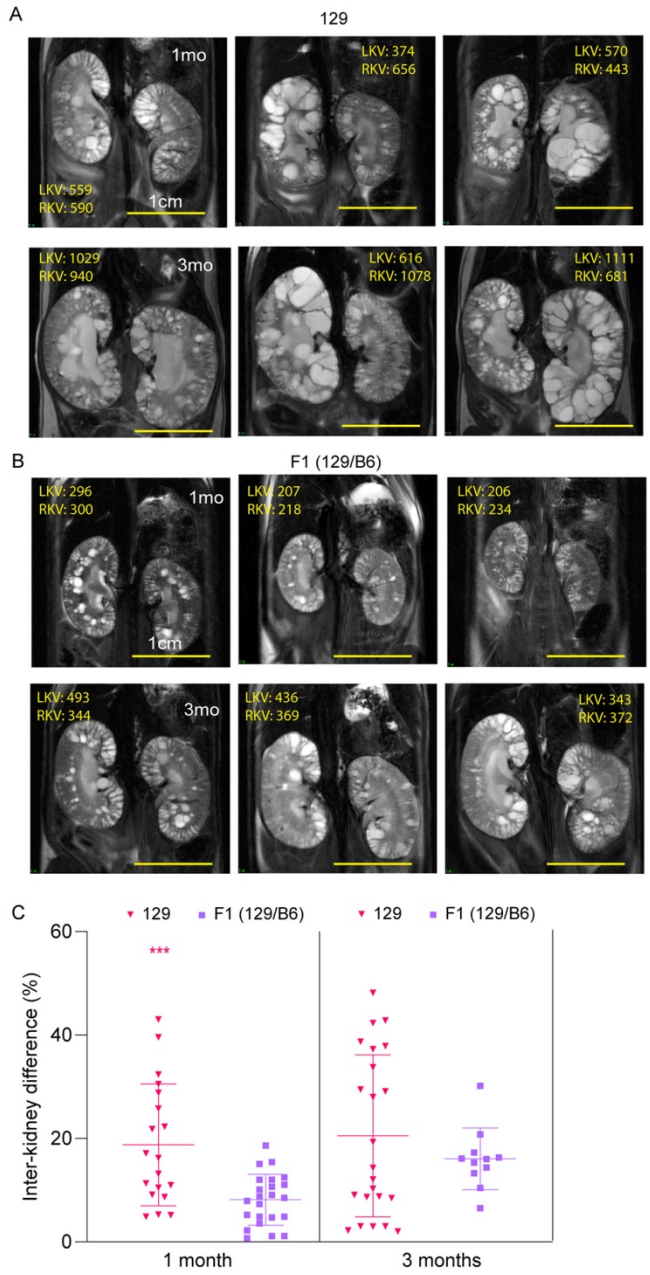

Figure S5. Visualization of inter-kidney volume variability in individual 129 and F1(129/B6) *Pkd1*$^{RC/RC}$ mice.

Coronal MR sections of kidneys from 129 (**A**) and F1(129/B6) (**B**) *Pkd1*$^{RC/RC}$ mice at 1m and 3m exhibiting inter-kidney cystic variability especially in the 129-line. The left kidney volumes (LKV) and right kidney volumes (RKV) are shown. Scale bar 1cm. (**C**) Chart showing the inter-kidney difference in TKV by percentage for each mouse. Significant difference in 129 vs. F1(129/B6) at 1m and 3m was determined using an unpaired t-test ***p<0.001.

Figure S6

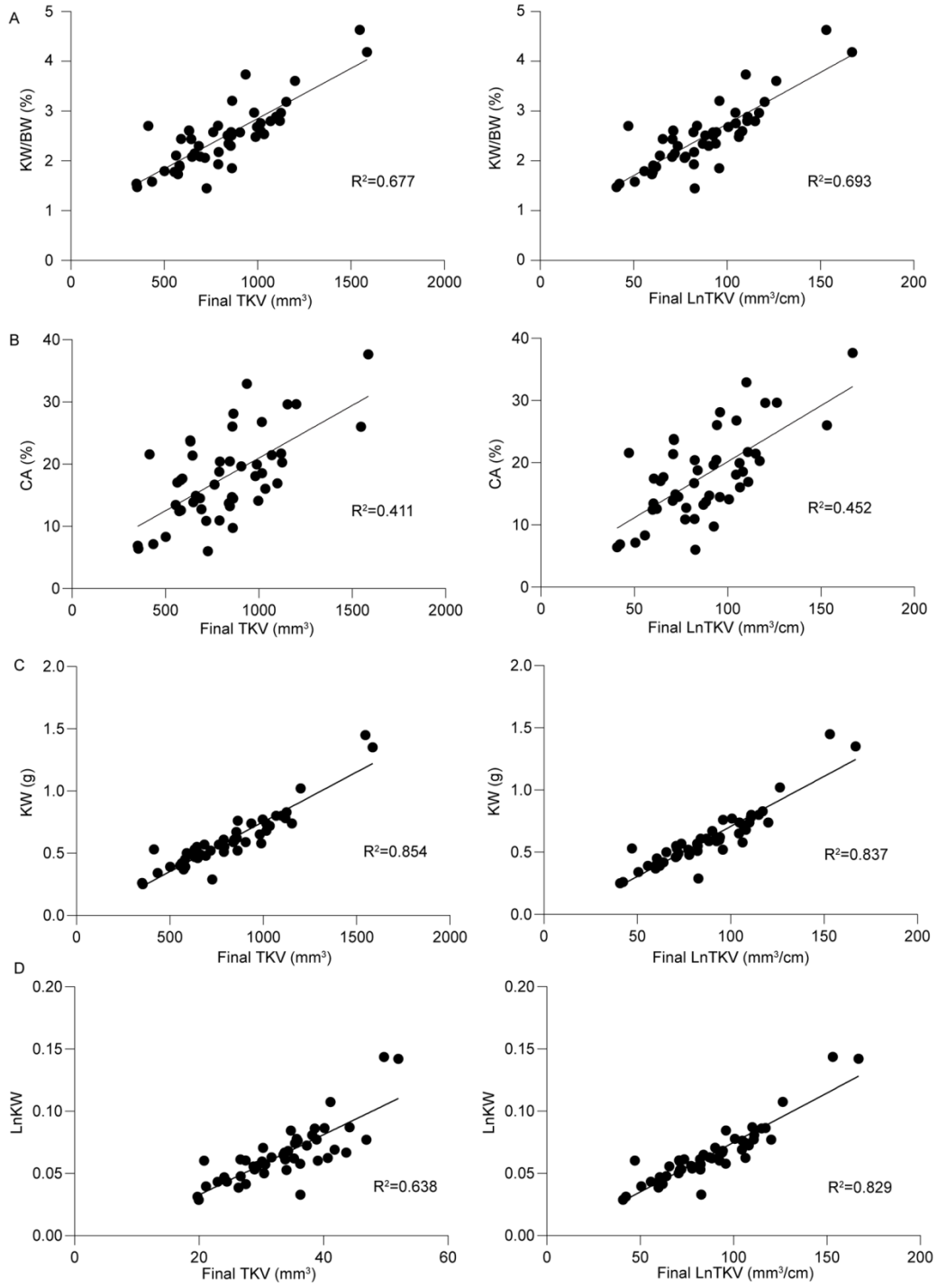

Figure S6. Comparison of final TKV and LnTKV to KW/BW (A), Cystic area (CA; B), KW (C) and LnKW (D).

Combined data from the preclinical trial comparing these endpoints at 13w. R^2 determined by linear regression analysis.

Figure S7

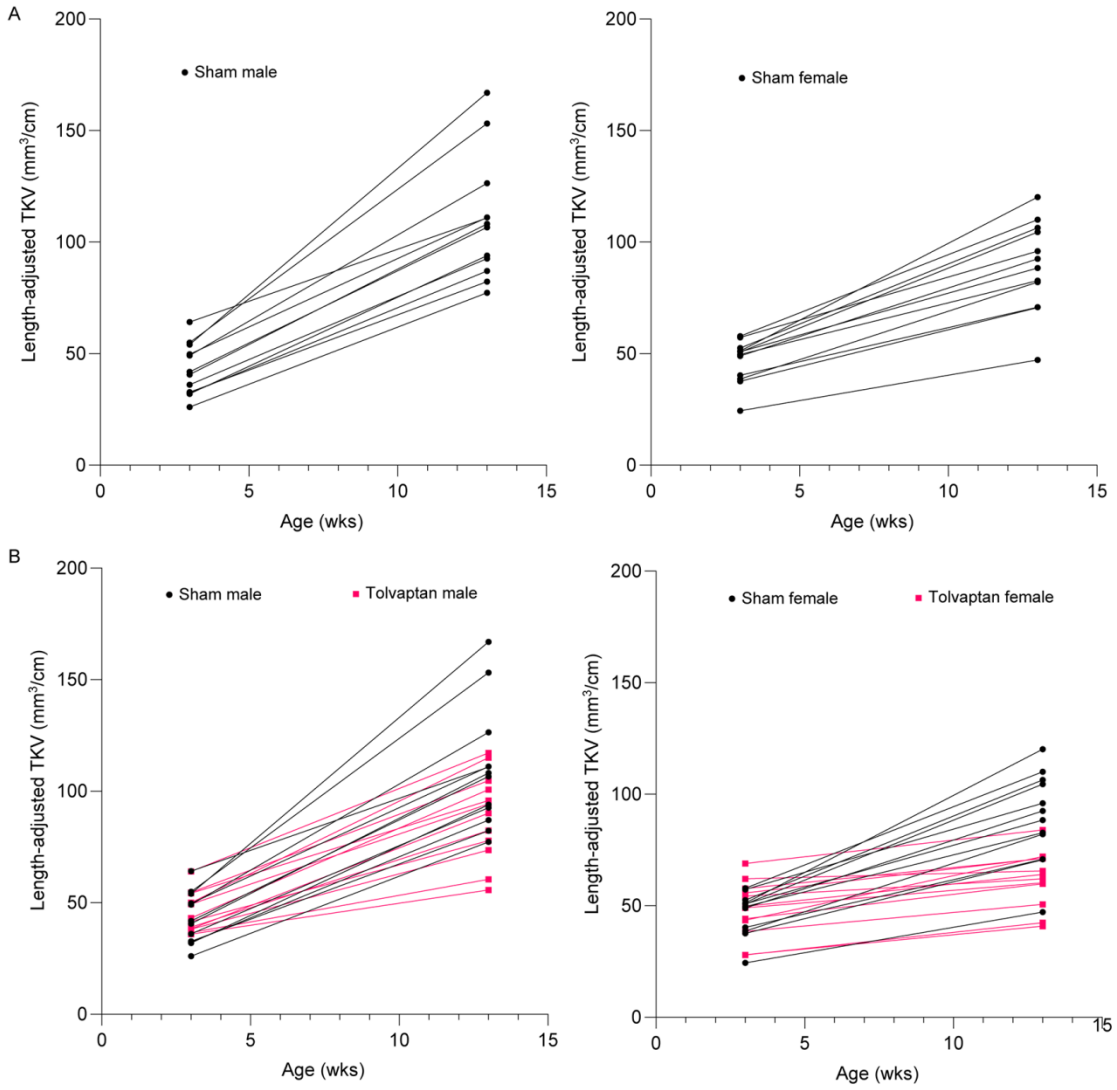

Figure S7. Change in LnTKV from 3 to 13 weeks.

(**A**) Change in sham treated *Pkd1*$^{RC/RC}$ F1(129/B6) males and females from start (3w) to end (13wks) of preclinical study. (**B**) Change in LnTKV in both sham and tolvaptan-treated males and females during the 10-week study. A paired t-test comparing animals at 3w and 13w resulted in $p<0.001$ for all four groups.

Figure S8

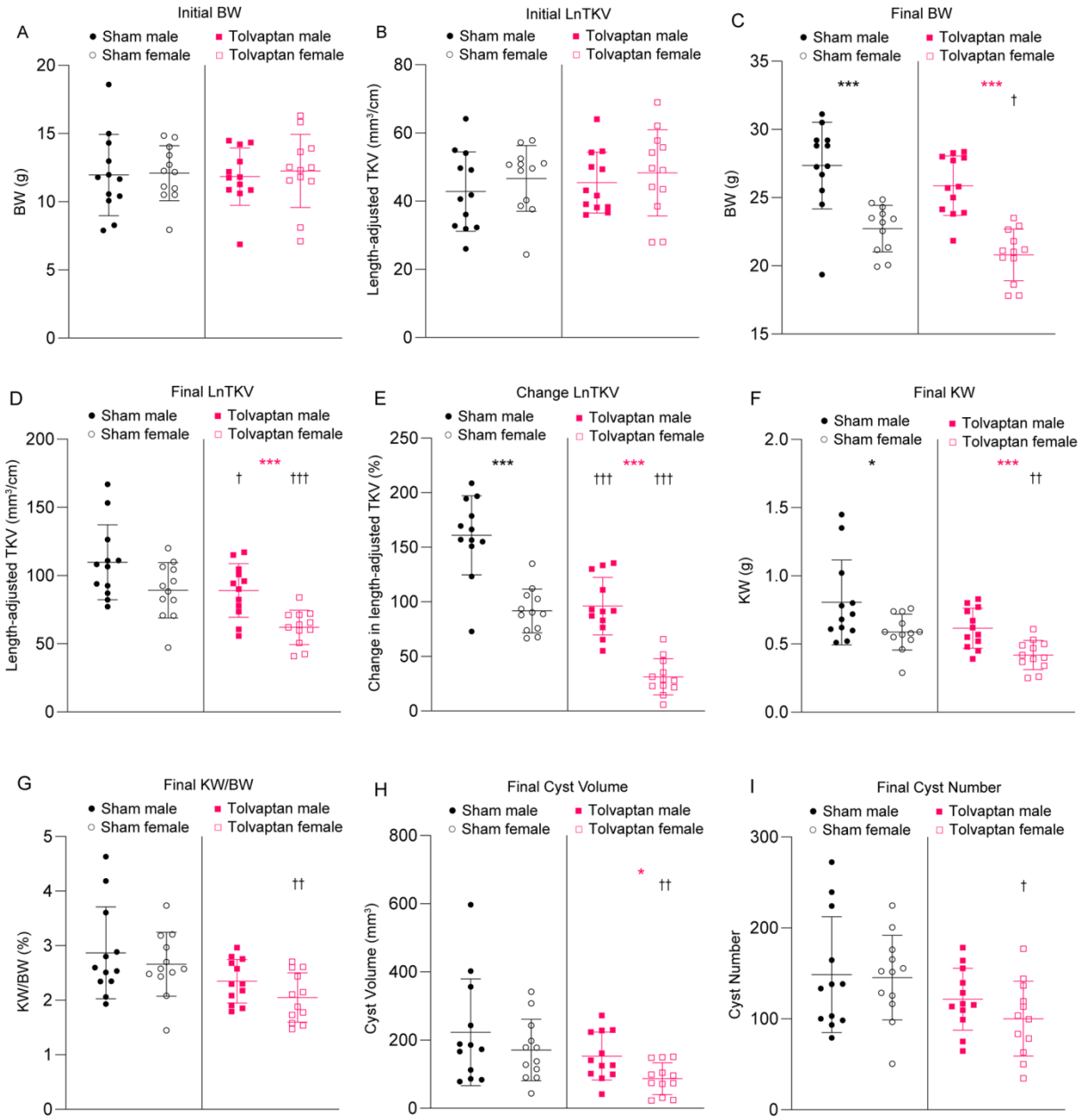

Figure S8. **Preclinical testing of tolvaptan in F1(129/B6) *Pkd1*$^{RC/RC}$ mice, sex specific data.** Initial BW (3w) (**A**) and LnTKV (**B**) in the sham and tolvaptan treated male and female groups. Corresponding final data (13w) for the endpoints: BW (**C**), LnTKV (**D**), %ΔLnTKV (**E**), KW (**F**), KW/BW (**G**), CV (**H**), and CN (**I**). Significant differences between males vs. females within treatment groups was determined using an unpaired t-test *p<0.05, ***p<0.001. Differences between sex-matched animals between treatment groups were also determined using an unpaired t-test †p<0.05, ††p<0.01, †††p<0.001. (**Table S4**)