

Supplementary Tables:*Supplementary Table 1: List of Antibodies, Lectins and Stains*

Antibodies, Lectins and Stains	Source	ID
Biotinylated Dolichos Biflorus Agglutinin (DBA) 1:250 (IF)	Vector Laboratories	B-1035, RRID:AB_2 314288
Biotinylated Lotus Tetragonolobus Lectin (LTL) 1:250 (IF)	Vector Laboratories	B-1325, RRID:AB_2 336558
Mouse anti-Acetylated tubulin 1:300 (IF)	Sigma-Aldrich	T6793, RRID:AB_4 77585
Rabbit anti-AKT 1:100 (IF) 1:1,000 (WB)	CST	#9272, RRID:AB_3 29827
Rabbit anti-pAKT (T308) 1:100 (IF)	Abcam	ab38449, RRID:AB_7 22678
Rabbit anti-pAKT (T308) 1:1000 (WB)	CST	#2965, RRID:AB_2 255933
Rabbit anti-pAKT (S473) 1:100 (IF) 1:1,000 (WB)	CST	#4060, RRID:AB_2 315049
Mouse anti-AURKA (IAK1) 1:100 (IF), 1: 2,000 (WB)	BD Bioscience	610939, RRID:AB_3 98252
Rabbit anti-pAURKA/B/C (T288) 1:1000 (WB)	CST	#2914, RRID:AB_2 061631
Rabbit anti-actin 1: 5,000 (WB)	Sigma-Aldrich	A2066, RRID:AB_4 76693

Mouse anti-g-H2AX 1:200 (IF) 1:1000 (WB)	Abcam	ab22551, RRID:AB_4 47150
Rabbit anti-Ki67 1:500 (IF)	Abcam	ab16667, RRID:AB_3 02459
Rabbit anti-p53 1:250 (IF)	Lecia Biosystems	p53-CM5p, RRID:AB_2 744683
Rabbit anti-Pericentrin 1:500 (IF)	Biolegend	923701, RRID:AB_2 565440
Rabbit anti-PDK1 (PDK1) 1:100 (IF)	Thermofisher Invitrogen	PA5-79801, RRIF:AB_2 746916
Rabbit anti-THP (UMOD) 1:50-100 (IF)	SCBT	H-135 (discontinue d), RRID:AB_2 212378
Rabbit anti-UMOD (THP) 1:500 (IF)	Abcam	ab207170
Mouse anti-Uroplakin-III 1:50 (IF)	Progen	651108, RRID:AB_1 543140
Mouse anti-V5 clone V5-10 1:1000 (WB)	Sigma-Aldrich	V8012, RRID:AB_2 61888
Rabbit anti-V5 1:100 (IF)	CST	#13202, RRID:AB_2 687461
Goat anti-V5 conjugated agarose bead (IP)	Abcam	Ab1229, RRID:AB_3 08681

Alexa Fluor® donkey anti-mouse IgG (H+L) 488 1:600 (IF)	Molecular Probes	A21202, RRID:AB_1 41607
Alexa Fluor® donkey anti-mouse IgG (H+L) 555 1:600 (IF)	Molecular Probes	A31570, RRID:AB_2 536180
Alexa Fluor® donkey anti-mouse IgG (H+L) 647 1:600 (IF)	Molecular Probes	A31571, RRID:AB_1 62542
Alexa Fluor® goat anti-mouse IgG1 (H+L) 488 1:600 (IF)	Molecular Probes	A21121, RRID:AB_ 2535764
Alexa Fluor® goat anti-mouse IgG2b (H+L) 555 1:600 (IF)	Molecular Probes	A21147, RRID:AB_ 2535783
Alexa Fluor® donkey anti-rabbit IgG (H+L) 488 1:600 (IF)	Molecular Probes	A21206, RRID:AB_2 535792
Alexa Fluor® donkey anti-rabbit IgG (H+L) 555 1:600 (IF)	Molecular Probes	A31572, RRID:AB_1 62543
Alexa Fluor® donkey anti-rabbit IgG (H+L) 647 1:600 (IF)	Molecular Probes	A31573, RRID:AB_2 536183
Dolichos Biflorus Agglutinin (DBA)- FITC 1:50-100 (IF)	Vector Laboratories	FL-1031, RRID:AB_2 336394
Donkey anti-rabbit IgG (H+L) HRP 1:10,000 (WB)	Millipore	AP182P, RRID:AB_9 2591
Goat anti-mouse IgG (H+L) HRP 1:10,000 (WB)	Millipore	P0447, RRID:AB_2 617137
Streptavidin, Alexa Fluor® 488 1:600 (IF)	Molecular Probes	S11223
Streptavidin, Alexa Fluor® 555 1:600 (IF)	Molecular Probes	S21381

Streptavidin, Alexa Fluor® 647 1:600 (IF)	Molecular Probes	S21374
DAPI (4',6-diamidino-2-phenylindole) 1:5,000 (IF)	DAKO	D1306, RRID:AB_2 629482

Supplementary Figures

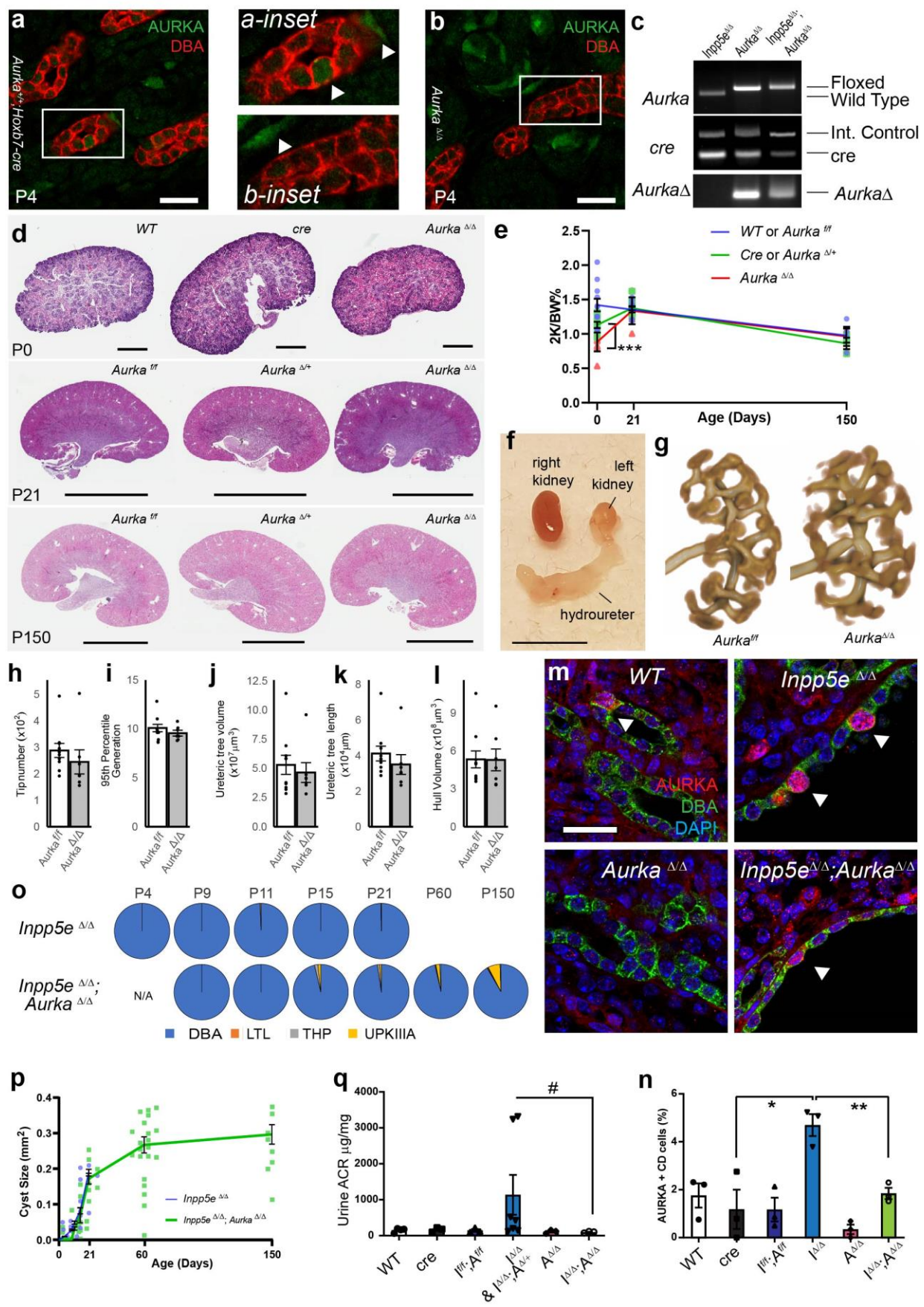


Fig S1: Deletion of *Aurka* has no kidney phenotype & extended characterisation of neonatal JS PKD mice

(a,b) Kidney sections stained for DBA, AURKA and DAPI in animals of ages and genotypes indicated (scalebar = 20µm). Inset arrowheads indicate AURKA+ve cells. (c) Genomic PCR analysis of *Aurka* wildtype, floxed and Δ/Δ loci with *cre* and internal controls. (d) Kidney H&E sections from wild type, *cre*, *Aurka* $\Delta/+$ and/or *Aurka* Δ/Δ mice at indicated ages (scalebar = 0.5 mm at P0, 4 mm P21 and P150). (e) Combined kidney weight over total body weight percentage (2K/BW%) ($p = 0.0005$). (f) Image of hydroureter and hydronephrosis occasionally observed in *Aurka* Δ/Δ mice. (G-L) OPT analysis of E14.5 embryonic kidneys assessing parameters including 3D renders of kidneys (g), tips number (h), branch generations (i), tree volume (j), tree length (k) and kidney hull volume (l). (m) Kidney sections stained for DBA, DAPI and AURKA at P21, Arrowheads indicate AURKA+ve CD cells in cysts (scalebar = 50 µm). (n) Quantification of % AURKA +ve collecting duct cells at P21. (left to right p-values 0.0153, 0.0059). (o) Identity of cyst cell identity following staining with DAPI, DBA and UP3AIII. (p) Quantification of the cyst cross sectional area over time. (r) Quantification of the urine Albumin Creatinine Ratios (ACR) in µg/mg. (p -value = 0.0551). All graph data indicate mean $\pm$ S.E.M. (a-c) $n=3$, (d-e) $n=3-12$, (g-l) $n=7-10$, (m,n) $n=3$, (o-p) $n=3-23$, (q) $n=3-7$. Exact n values and data point values provided in Source Data File.

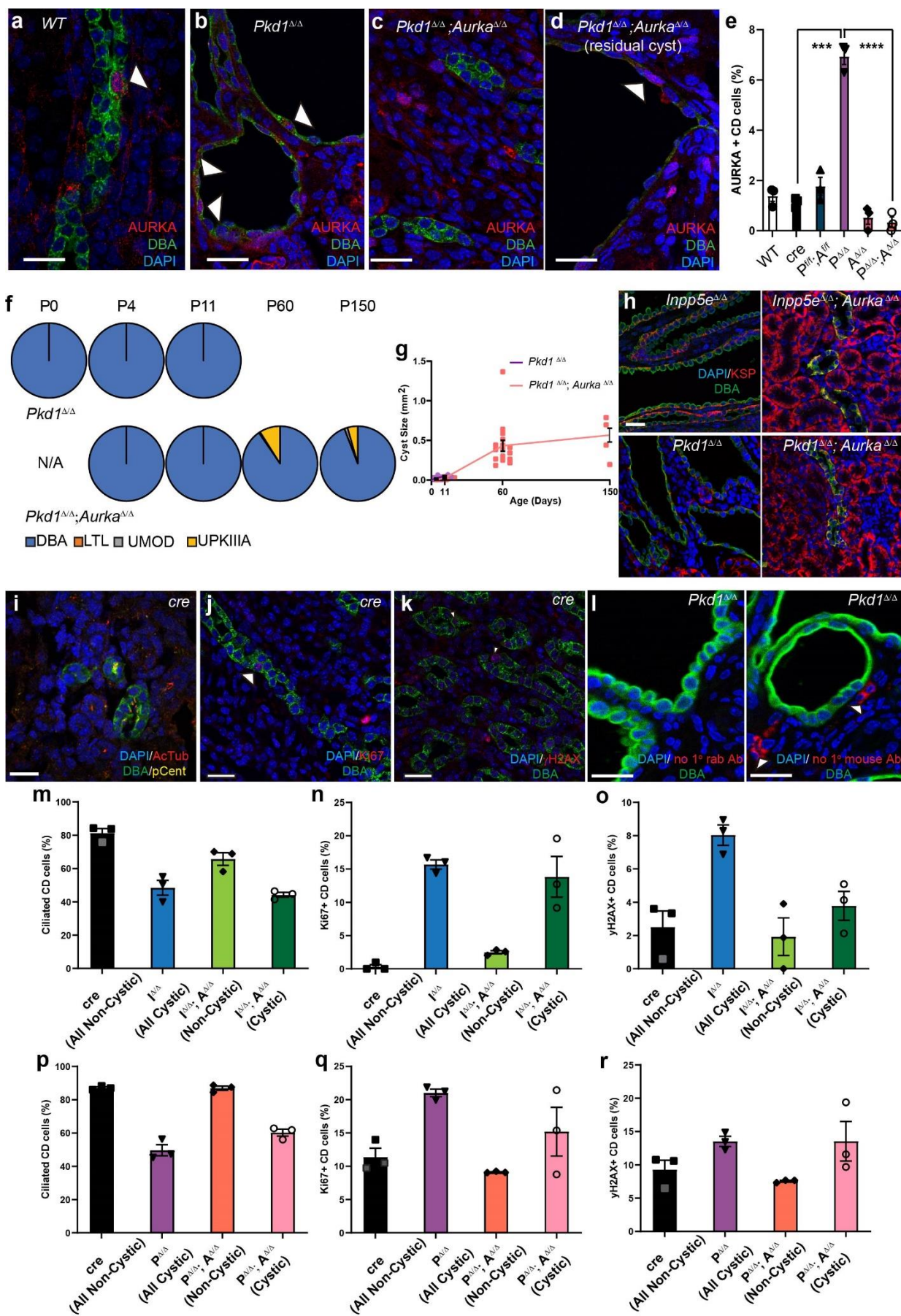


Fig S2: Extended characterisation of neonatal ADPKD mice

a-d) P4 Immunofluorescent imaging of AURKA expression in WT collecting ducts, *Pkd1*^{Δ/Δ} cysts and residual cysts in *Pkd1*^{Δ/Δ}; *Aurka*^{Δ/Δ} mutants but not normal collecting ducts. Arrowheads indicate AURKA+ve cells. e) Quantitation of AURKA expressing collecting duct cells across indicated genotypes at P4. P values from left to right; p=0.0006 and 0.0001. f) Kidney sections stained with DAPI, DBA, THP and LTL to determine cyst identity. g) Quantification of the cyst cross sectional area over time. h) Immunostaining of P21 *Inpp5e*^{Δ/Δ}, P11 *Pkd1*^{Δ/Δ} and rescued counterpart kidney sections for DAPI, DBA and KSP. i-k) P4 immunofluorescent imaging of control *cre* collecting ducts, labelled as indicated. l) P11 secondary only control imaging of *Pkd1*^{Δ/Δ} cysts without rabbit or without mouse primary antibody, as indicated. Non-specific staining was low for anti-rabbit IgG secondary antibodies. Anti-mouse IgG secondary antibody did however label occasional interstitial mouse cells (arrowheads) but rarely tubular cells. m-o) P21 *Inpp5e* model data for % Ciliated CD cells, % Ki67 CD cells and % γH2AX CD cells highlighting behaviour of cystic and non-cystic regions in *Inpp5e*^{Δ/Δ}; *Aurka*^{Δ/Δ} mutants. p-r) P11 *Pkd1* model data for % Ciliated CD cells, % Ki67 CD cells and % γH2AX CD cells highlighting behaviour of cystic and non-cystic regions in *Pkd1*^{Δ/Δ}; *Aurka*^{Δ/Δ} mutants. Bars = 25 μm. All graph data indicate mean ± S.E.M. (a-e) n =3, (f-g) n=3-16, (h-r) n=3. Exact n values and data point values provided in Source Data File.

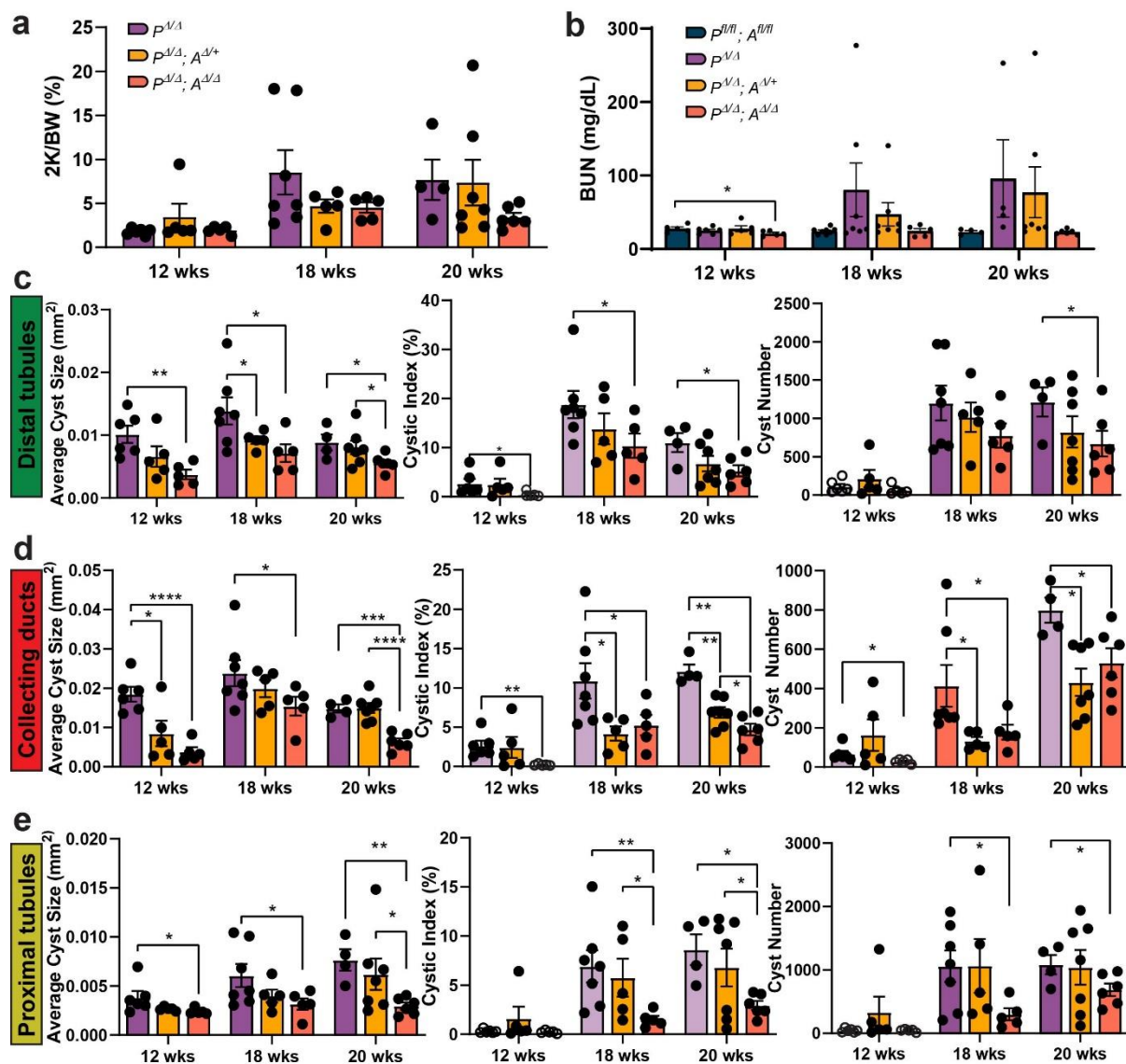


Fig S3: Extended characterisation of adult onset ADPKD mice

(a) 2KW/BW ratios. (b) Assessment of Blood Urea Nitrogen (BUN) as a measure of kidney function mice of indicated genotypes at fixed experimental time point. $p=0.0241$ (c) The average cyst size, cystic index and cyst number per cross section for tubule cyst identity in distal tubules at 12, 18 and 20-weeks. P values from left to right: average cyst size – 0.0019, 0.0384, 0.0134, 0.0376, 0.0355; cystic index – 0.0384, 0.0238, 0.0248; cyst number 0.0354. (d) The average cyst size, cystic index and cyst number per cross section for tubule cyst identity in collecting ducts at 12, 18 and 20-weeks. P values from left to right: average cyst size – 0.0001, 0.0170, 0.0330, 0.0002, 0.0001; cystic index – 0.0057, 0.0125, 0.0290, 0.0023, 0.0002, 0.0261; cyst number – 0.0283, 0.0194, 0.0365, 0.0021, 0.0129. (e) The average cyst size, cystic index and cyst number per cross section for tubule cyst identity in proximal tubules at 12, 18 and 20-weeks. P values from left to right: average cyst size – 0.0425, 0.0283, 0.0091, 0.0451; cystic index – 0.0079, 0.0462, 0.0159, 0.0456; cyst number – 0.0113, 0.0385. All graphs indicate mean + S.E.M. (a-e) $n=4-8$. Exact n values and data point values provided in Source Data File.

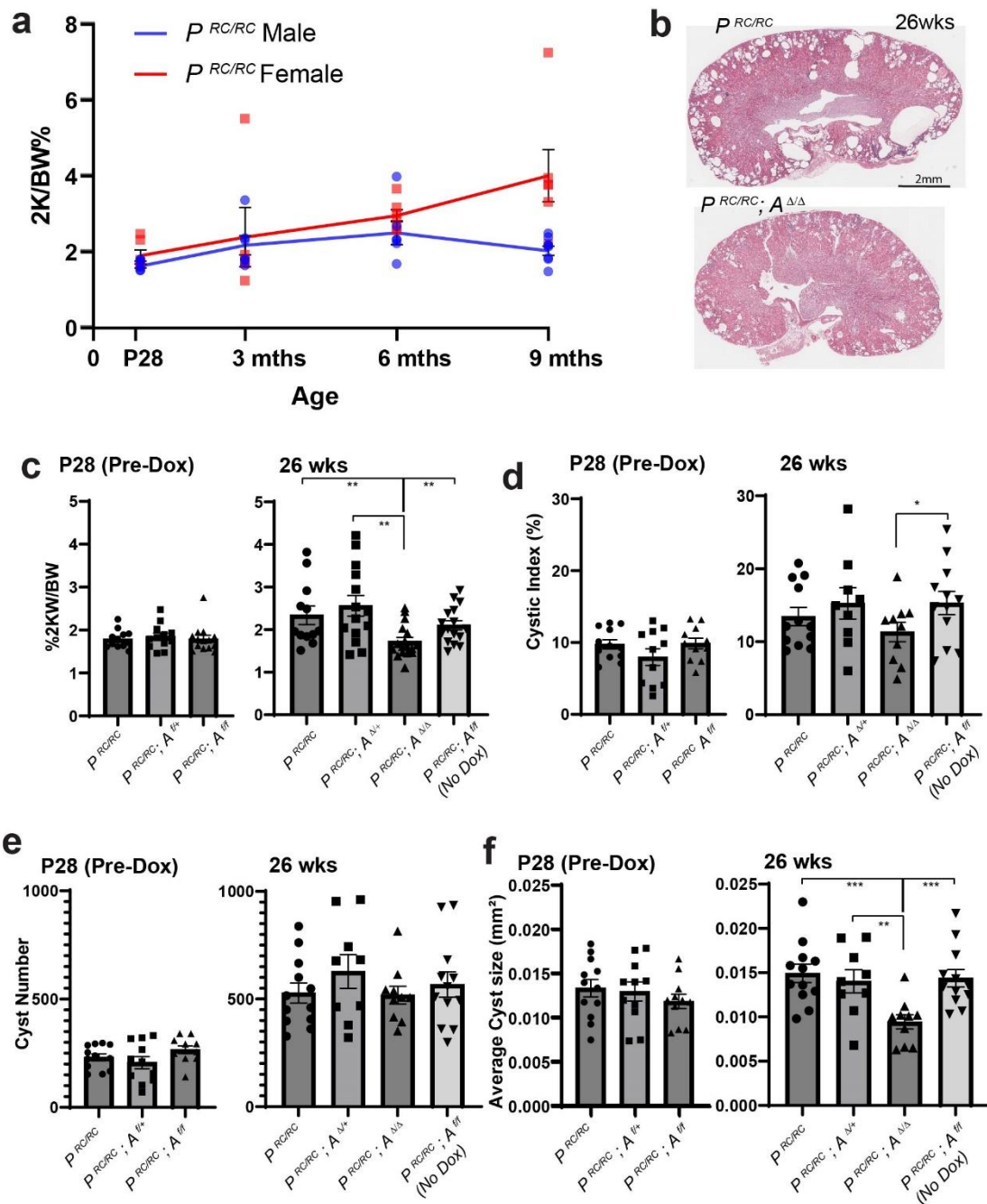


Fig S4: Characterisation of adult onset ADPKD RC mice

(a) 2K/BW ratios of male and female $Pkd1^{RC/RC}$ mice over time, showing sex divergence from 6 to 9 mths of age. (b) H&E sections of 26 week old $Pkd1^{RC/RC}$ ($P^{RC/RC}$) with and without *Aurka* ($A^{\Delta/\Delta}$) mice. c-f) Quantification of 2K/BW, cystic index, cyst number and size at postnatal day 28 (P28) and 26 weeks of age (22 weeks after doxycycline treatment to delete *Aurka*). (c-d) P values from left to right = 0.0099, 0.0026, 0.0063, 0.0352. (e-f) P values from left to right less than 0.0002, 0.0050, 0.0005. All graphs indicate mean + S.E.M. (a) n=5-8, (b-f) n = 9-14. Exact n values and data point values provided in Source Data File.

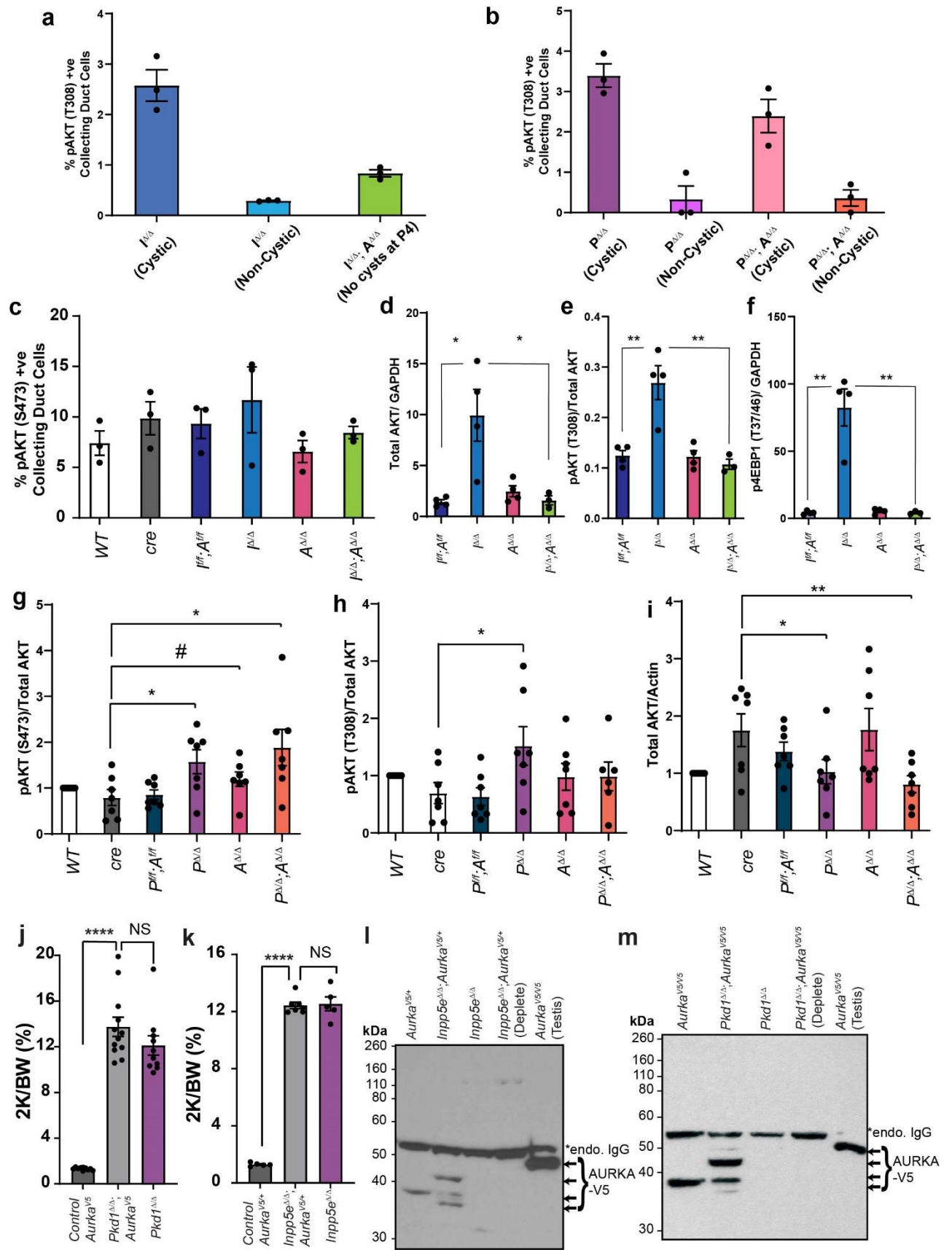


Fig S5: AKT activity and extended COIP data

(a,b) Quantification of % pAKT (T308) +ve collecting duct cells at P4, split by genotype and cystic and non-cystic tubules. (c) Quantification of pAKT (S473) +ve collecting duct cells in *Inpp5e* model at P4. (d) AlphaLISA analysis of Total AKT over total GAPDH from P21 whole kidney lysates from *Inpp5e* model ($p=0.0219$ for *Inpp5e^{Δ/Δ}* kidneys relative to floxed control and $p=0.0222$ relative to *Inpp5e^{Δ/Δ};Aurka^{Δ/Δ}*). (e) AlphaLISA analysis of pAKT (T308) ratio from whole kidney lysates ($p=0.0094$ for *Inpp5e^{Δ/Δ}* kidneys relative to floxed control and $p=0.0066$ relative to *Inpp5e^{Δ/Δ};Aurka^{Δ/Δ}* kidneys) at P21. (f) AlphaLISA analysis of p4EBP1 (T37/46) over total GAPDH from whole kidney lysates ($p=0.0054$ for *Inpp5e^{Δ/Δ}* kidneys relative to both floxed controls and *Inpp5e^{Δ/Δ};Aurka^{Δ/Δ}* kidneys) at P21. g-i) Densitometry of Western blots of P4 kidney lysates from *Pkd1^{Δ/Δ};Hoxb7* mice. Western blots represent biological replicates/individual animals with each independent experiment. Each WT control sample within a replicate set was defined as 1. Blots were repaired by probing, stripping and reprobing of the same membrane to generate datasets. g) pAKT (S473)/Total AKT ratio (left to right $p=0.0159$, $p=0.0598$, $p=0.0175$). (h) pAKT (T308)/Total AKT ratio (*Pkd1^{Δ/Δ}* vs *cre* $p=0.0295$). Single outlier ($>Q3+1.5 \times IQR$) removed from *Pkd1^{Δ/Δ};Aurka^{Δ/Δ}* dataset. (i) Total AKT expression level (left to right $p=0.0334$, $p=0.0085$). (j,k) Comparison of combined kidney to body weight percentage (2K/BW%) in mice of indicated genotypes (*Aurka^{V5/V5}* relative to *Pkd1^{Δ/Δ};Aurka^{V5/V5}* mice, $p \text{ value}=6.6 \times 10^{-9}$. *Aurka^{V5/+}* relative to *Inpp5e^{Δ/Δ};Aurka^{V5/+}* mice, $p \text{ value}=5.0 \times 10^{-10}$). (l,m) Input lysates from P11 (*Pkd1*) and P15 (*Inpp5e*) mouse kidneys of indicated genotypes. Mouse testis from adult *Aurka^{V5/V5}* mice was included as a positive control. Asterisked upper band is mouse endogenous IgG in tissue extracts detected by the anti-mouse IgG secondary antibody used to identify mouse monoclonal anti-V5. All graph data indicate mean $\pm$ S.E.M. (a-c) $n=3$, (d-f) $n=3-4$, g-i) $n=6-7$, (j) $n=10-12$, (k) $n=5-7$, (l,m) $n=3$, NS= not significant. Individual western blot scans, n and data point values provided in supplementary material- Source Data File. All graph data indicate mean $\pm$ S.E.M.

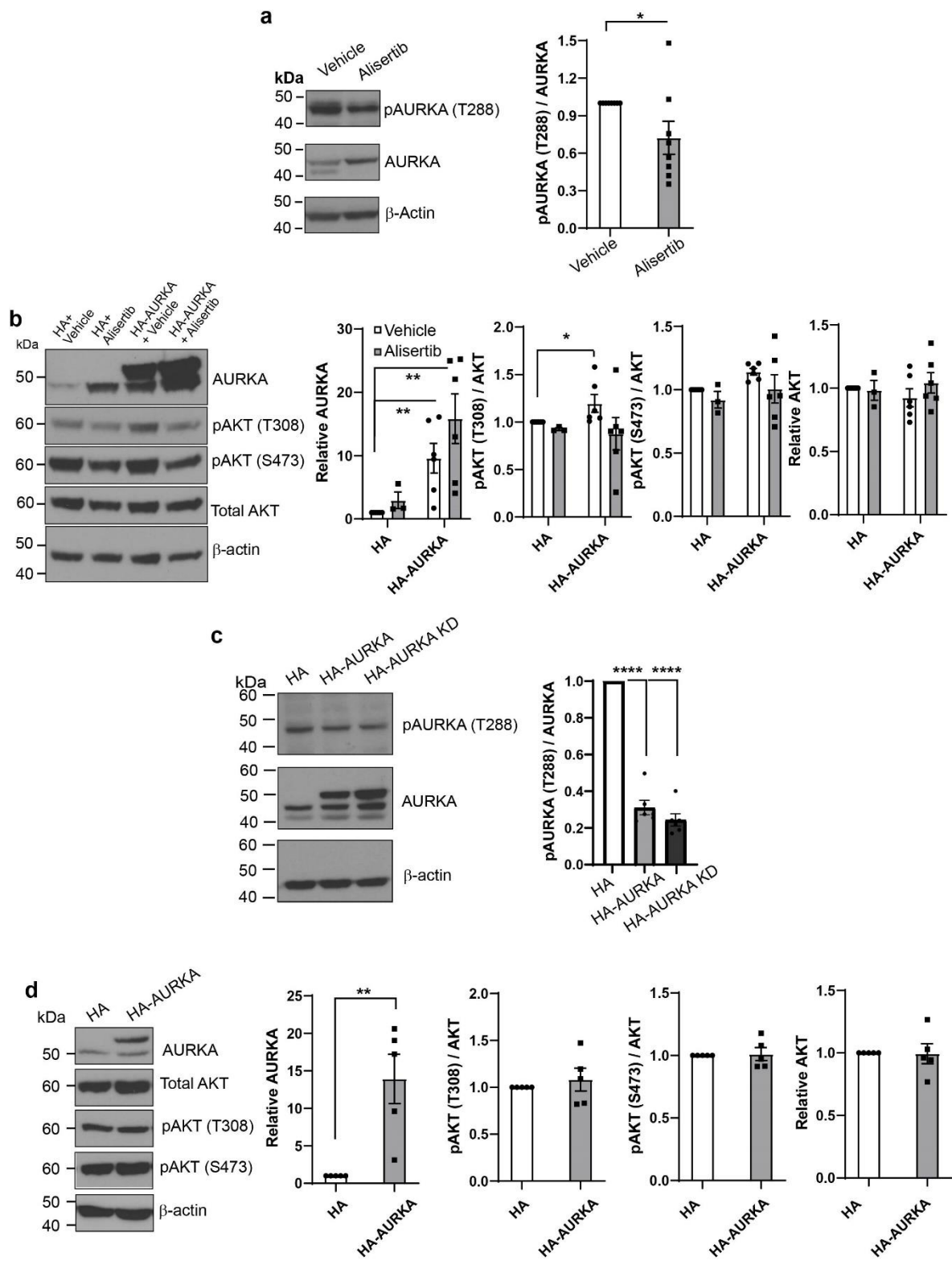


Fig S6: AURKA regulates pAKT (T308) extended

(a) Western blots and densitometry of mIMCD3 cell lysates treated with Alisertib (1 μ M) or vehicle for 48 hrs under growth conditions. Alisertib reduces the pAURKA (T288) ratio ($p = 0.0372$, $n=8$). (b) Western blots and densitometry of mIMCD3 cell lysates transfected with HA or HA-AURKA expression plasmids, with 24 hrs exposure to Alisertib (1 μ M) or DMSO vehicle. AURKA is over-expressed ($p = 0.0073$, 0.0064). HA-AURKA expression increases pAKT (T308)/total AKT ratio ($p = 0.0455$), this does not occur with 24 hrs of Alisertib exposure. pAKT (S473)/total AKT ratio and relative AKT expression remain similar with Alisertib ($n=3-6$). (c) Western blots and densitometry of mIMCD3 cell lysates treated with HA, HA-AURKA or HA-AURKA KD expression plasmids, then cultured under growth conditions. Neither HA-AURKA or HA-AURKA KD show significant phosphorylation at T288, whereas only a band consistent with endogenous AURKA does, suggesting over-expressed AURKA is predominantly maintained in a kinase inactive state. HA-AURKA and HA-AURKA KD both reduce relative phosphorylation at T288 over total AURKA levels, p -value = 5.5×10^{-6} and 1.61×10^{-6} , $n=6$. (d) Western blots and densitometry of serum starved (24hr) mIMCD3 cell lysates transfected with HA or HA-AURKA expression plasmids probed for AURKA, total AKT, pAKT (T308), pAKT (S473) and actin. AURKA is overexpressed (p -value = 0.0086) but no changes in pAKT (T308)/total AKT, pAKT (S473)/total AKT ratios or relative AKT expression are observed, $n=5$. Western blots represent biological replicates with each independent experiment set run on a separate gel or two replicates on a gel. Each control sample within a replicate set was then defined as 1. Western membranes were cut to 40-60kDa interval before antibody probing, stripping and reprobing. Due to limits of stripping and reprobing, some replicates for S6b and S6d were probed across repeat gel sets performed in parallel. S6a and S6c were reprobes of the same membrane. Individual western blot scans, n and data point values provided in supplementary material- Source Data File. All graph data indicate mean $\pm$ S.E.M.

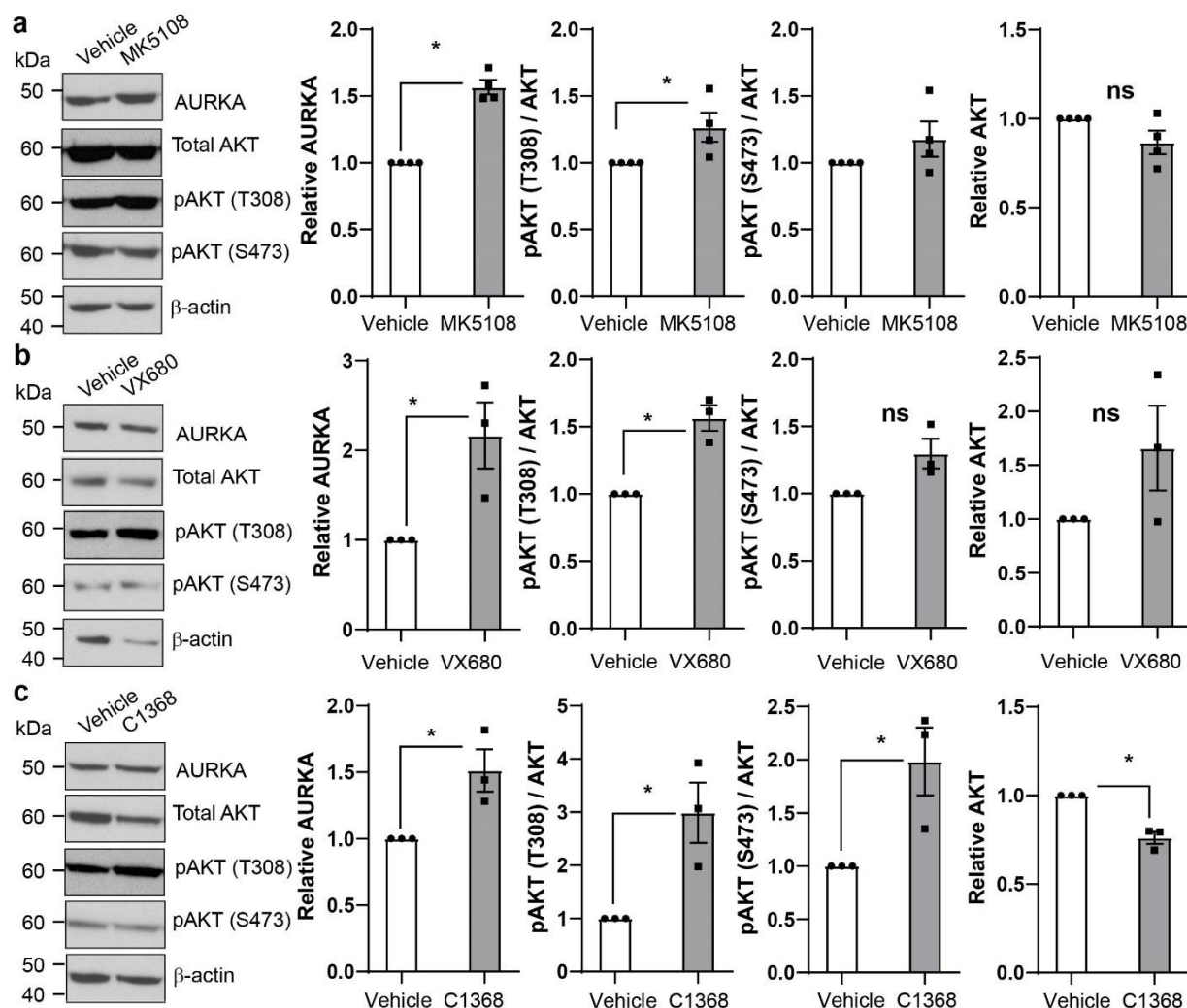


Fig S7: AURKA regulates pAKT (T308) extended Part2

(a-c) Western blots and densitometry of mIMCD3 cell lysates treated with AURKA kinase inhibitors MK5108 (1 μ M), VX680 (500nM), C1368 (2 μ M) or vehicle for 48 hrs under growth conditions. All inhibitors promoted AURKA upregulation ($p = 0.0009$, 0.0464 , 0.0420 respectively) and increases in the pAKT (T308)/AKT ratio ($p = 0.0464$, 0.0135 , 0.0361 respectively). In contrast, the pAKT (S473)/AKT ratio was not statistically different with MK5108 and VX680, but altered with C1368 ($p=0.0455$). Total AKT levels were not statistically different with MK5108 and VX680, but were significantly reduced by C1368 ($p<0.0103$), MK5108 $n=4$, VX680 & C1368 $n=3$. All graph data indicate mean $\pm$ S.E.M. Western blots represent biological replicates with each independent experiment set stored and run on the same gels. Each control sample within a replicate was defined as 1. Blots were repaired by probing, stripping and reprobing of the same membrane to generate datasets. Exact n and data point values provided in Source Data File.

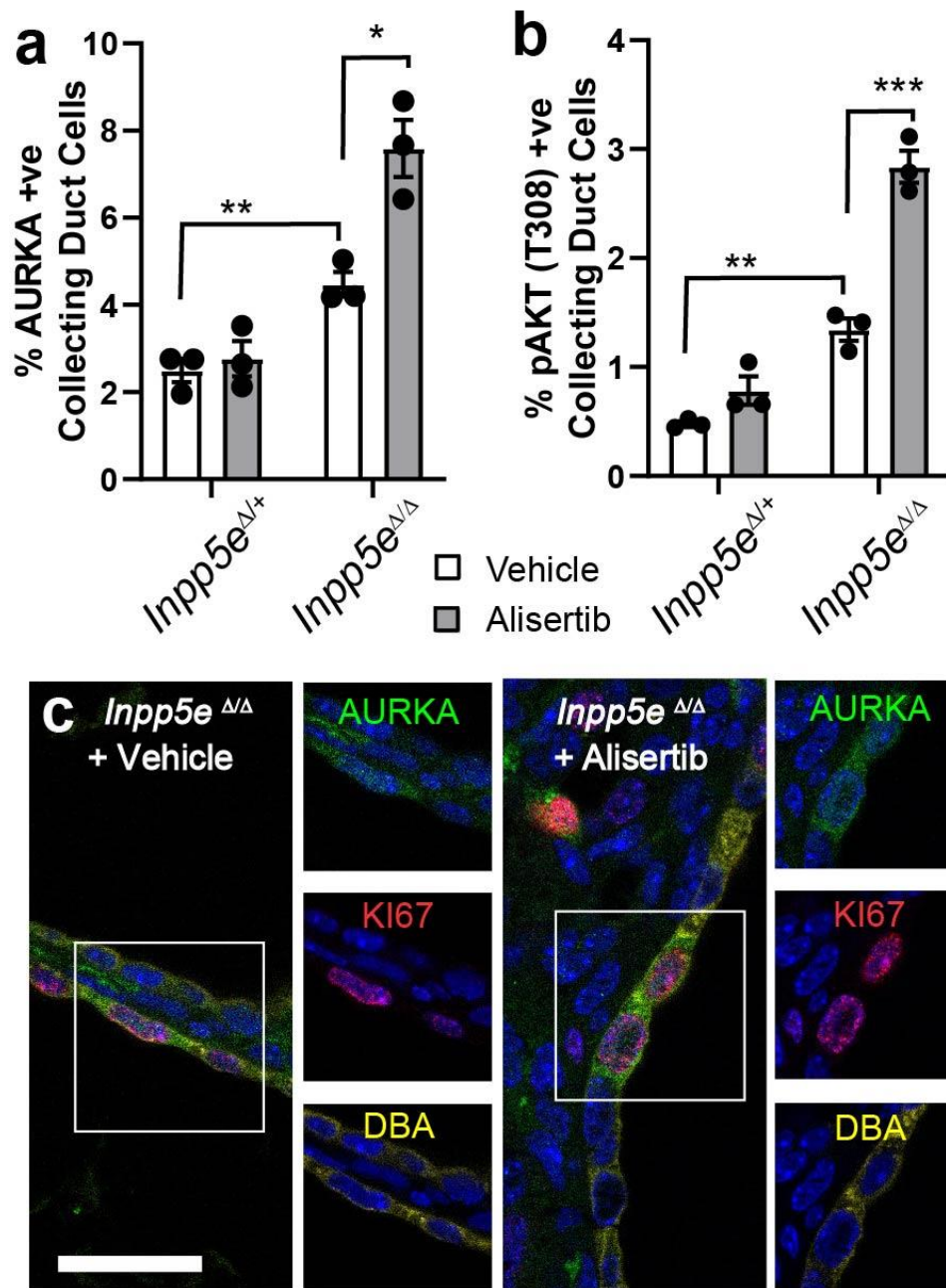


Fig S8: Extended characterisation of Alisertib treatments

(a,b) Quantification of AURKA (a) and pAKT (T308) (b) +ve collecting duct cells. P values from left to right; $p=0.0035$, $p=0.0134$, $p=0.0051$, $p=0.0009$. (c) Immunostaining of Ki67/AURKA in collecting duct cells marked with DBA during vehicle or Alisertib treatment of P15 *Inpp5e^{Δ/Δ}* mice (bar = 25 μm). All graph data indicate mean $\pm$ S.E.M. Abbreviation pT308 = pAKT (T308). White bars = vehicle; Grey bars = Alisertib treatment. a-c) $n=3$. All data from P15. Exact n and data point values provided in Source Data File.

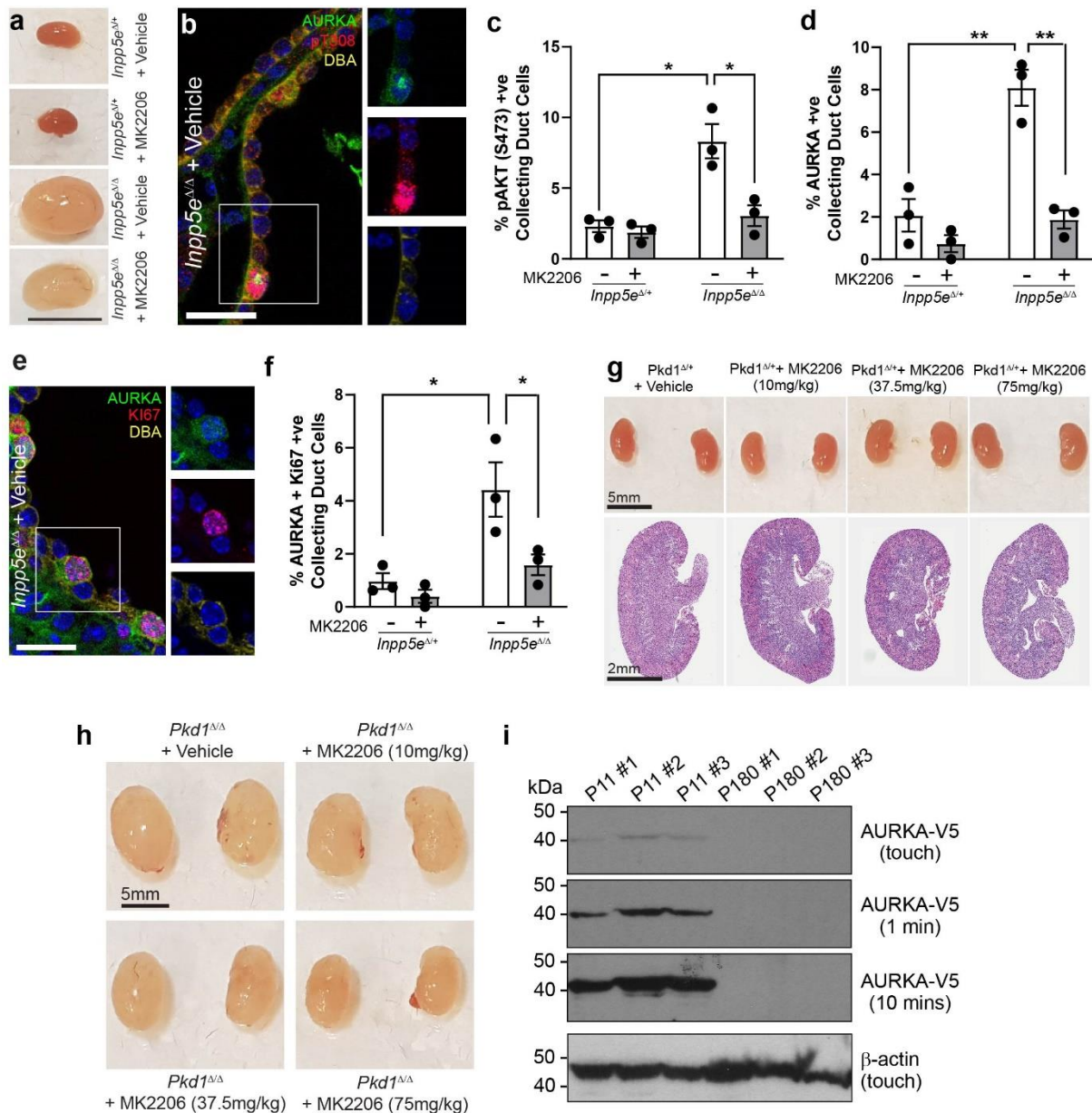


Fig S9: Extended characterisation of MK2206 treatments

(a,g,h) Representative images of mouse whole kidneys or sections from P15 *Inpp5e* or P11 *Pkd1* mice orally treated with vehicle or MK2206. Whole kidney photo bar = 0.5 cm, H&E bar = 2 mm. (b) Staining for AURKA, DBA and pAKT (T308) in mice exposed to vehicle (bar = 25 μm). (c,d) Quantification of the proportion of pAKT (S473) +ve or AURKA+ve collecting duct cells in *Inpp5e*^{Δ/Δ} and control mice at P15, following treatment with Vehicle or MK2206 (n=3) p values left to right, 0.0140, 0.0146, 0.0032, 0.0038. (e) Staining for AURKA, DBA and Ki67 in mice exposed to vehicle (bar = 25 μm). (f) Quantification of the proportion of Ki67 or AURKA+ve collecting duct cells in *Inpp5e*^{Δ/Δ} and control mice at P15, following treatment with Vehicle or MK2206 (n=3) p values left to right 0.0340, 0.0476. (i) Detection of AURKA in tissue extracts from P11 and 6 month old mouse kidneys of *Aurka*^{V5/V5} mice noting exposure time. (g-h) n =4-12. All graph data indicate mean ± S.E.M. Abbreviation pT308 = pAKT (T308). Exact n and data point values provided in Source Data File.