Supplementary table 1: List of the 11 compounds selected amongst the 51 hits of the *in cellulo* drug screen. The molecules were chosen based on their mode of action, toxicity and physicochemical properties.

	Name	Class	Pertinent Effect(s)	Toxicity theshold			Safe concentrations
				Inhibition of proliferation	Apopoptic bodies	Permeabilized cells	
A	Alprostadil	Synthetic prostaglandin	TGF-β inhibitor, decreased cilia beat, actin & RhoA inhibitor, canonical Wnt agonist	≥ 40µM	None up to 80µM	≥ 80µM	< 40µM
B	Cyproheptadine hydrocloride	First-generation H1 antihistamine	CyclinD2 expression inhibitor, decreased cilia beat, apoptosis inhibitor	≥ 5µM	≥ 20µM	≥ 40µM	< 5µM
C	Ethopropazine hydrocloride	Muscarinic receptor antagonist	Autophagy	≥ 10µM	≥ 80µM	≥ 80µM	< 10µM
D	Fluticasone propionate	Corticosteroid	Decreased cilia beat, Smo agonist	NA*	≥ 40µM	NA*	< 40µM
E	Methotrexate	Antimetabolite	Actin stress fibers disassembly	None up to 80µM	None up to 80µM	None up to 80µM	Up to 80µM
F	Mycophenolic Acid	Inosine monophosphate dehydrogenase inhibitor	JNK activator, MAPK/NF-κB inhibitor	None up to 80µM	≥ 40µM	None?	< 40µM
G	Paclitaxel	Spindle poison	Microtubule depolymerization inhibitor	≥ 10nM	≥ 100nM	≥ 100nM	< 10nM
H	Pyrimethamine	Antiprotozoal	STAT3 inhibitor	≥ 10µM	None up to 80µM	≥ 20µM	< 10µM
I	Simvastatin	HMG CoA Reductase inhibitor	RhoGTPases inhibition, actin modulation	≥ 1µM	≥ 1µM	≥ 1µM	< 1µM
J	Tropisetron hydrochloride	Serotonin 5-HT3 receptor antagonist	JNK inhibitor, increased tight-junction formation, antifibrotic (skin)	≥ 40µM	≥ 80µM	None up to 80µM	< 40µM
K	Verapamil	Non-Dihydropyridine Calcium channel blocker	RhoGTPases inhibition & cAMP elevation, mTor, autophagy inducer	None up to 80µM	≥ 5µM?	None up to 80µM	Up to 80µM

* False positive beyond 5µM

Supplementary table 2: Genotype and clinical details of individuals from whom URECs were derived.

	Genotype status	Clinical Status	Renal Failure (Stage)	Sex	Age (y)	% Ciliated cells
Pt1	del Hom *NPHP1*	*Nephronophthisis*	4	M	26	19
Pt2	del Hom *NPHP1*	*Nephronophthisis*	4	M	15	32
Pt3	del Hom *NPHP1*	*Nephronophthisis*	3	F	15	21
Pt4	Het c.1884+1G>T + Het c.1252-2A>G *NPHP1*	*Nephronophthisis*	4	F	13	50
Pt5	del Hom *NPHP1*	*Nephronophthisis*	4	M	18	38
Pt6	del Hom *NPHP1*	*Nephronophthisis*	4	F	16	40
Pt7	del Het + Het c.1078C>T - p.Q360* *NPHP1*	*Nephronophthisis*	1	M	9	43
Pt8	del Hom *NPHP1*	*Nephronophthisis*	1	M	23	49
Pt9	del Het + Het c.70-1G>A *NPHP1*	*Nephronophthisis*	4	F	8	32
Pt10	del Hom *NPHP1*	*Nephronophthisis*	4	F	17	45
Pt11	del Hom *NPHP1*	*Nephronophthisis*	4	M	30	47
CTL1		Minimal change disease	/	F	9	65
CTL2		No renal anomalies	/	F	14	39
CTL3		No renal anomalies	/	F	21	66
CTL4		No renal anomalies	/	M	10	74
CTL5		No renal anomalies	/	M	9	75
CTL6		No renal anomalies	/	F	11	62
CKD1		Hemolytic uremic syndrome	5	M	12	65
CKD2		Detrusor urethral sphincter dyssynergia	4	M	17	87
CKD3		Twin-to-twin transfusion syndrome	2	F	14	57
CKD4		Right renal hypoplasia/left renal dysplasia/bilateral vesicoureteral reflux	4	M	18	89
CKD5		Renal hypodisplasia with repeated urinary infections	3	F	20	64

Pt: *NPHP1* patient, CTL: control individual, CKD: non-ciliopathy chronic kidney disease patients, del: deletion, Hom: Homozygous, Het: heterozygous, M: male, F: female

Supplementary table 3: Primers used for RT-qPCR.

Gene name	Species	Forward primer (5' to 3')	Reverse primer (5' to 3')
Col1a1	Mouse	CTGACGCATGGCCAAGAAGA	GGGACCCTTAGGCCATTGTG
Col3a1	Mouse	GGACCAGCAGGAACTAATGGTAT	GTTCTCCAGGTGATCCATCTTT
Lcn2	Mouse	TCCTCAGGTACAGAGCTACAA	GCTCCTTGGTTCTTCCATACA
Kim1	Mouse	GAGAGTGACAGTGGTCTGTATTG	TCATAGCAGCCACCTTCATTC
Acta2	Mouse	CATGCGTCTGGACTTGGCTG	GACAATCTCACGCTCGGCAGTAG
Tgfβ1	Mouse	GGGAAGCAGTGCCCGAACCC	TGGGGGTCAGCAGCCGGTTA
Nphp1	Mouse	GTGAGCATCAGCAGGAAAGA	CTTCTTCCTCTCCACCAGTTTC
Nphp1 (genotyping)	Mouse	ACCGTTTAGTGCAACCTGTG	CATCACGTCTTCCATGCACT
THBS1	Human	CCCAGATCAGGCAGACACAG	TACTGGCAGTTGTCCCGTTC
LAMC2	Human	CGCAGCTCTGCAGAATACAG	AGACCCATTTCGTTGGACAG
ITGA2	Human	GCAACATCCCAGACATCCCA	CTTTCGTAGCACTTCGTCGC
HAS3	Human	TACATCCAGGTGTGCGACTC	CCTACTTGGGGATCCTCCTC
FLNA	Human	GTGCCAGCCGAATTCAGTAT	CACATAAGCCACACCACAGG
ABLIM3	Human	CAGTCCATGGCCAGCAGTAA	CACTTGAAGCAGCTGACGTG
PKIA	Human	TGATATCCTGGTTTCCTCTGCA	GGCTTCCCCACTTTGTTCTG
CPT1A	Human	CCATGCCATCCTGCTTTACA	ATCGTGGATCCCAAAAGACG
PGC1A	Human	CCAAAGGATGCGCTCTCGTTCA	CGGTGTCTGTAGTGGCTTGACT
DEPTOR	Human	CAGCATGTGTCCAACAAGCA	GGGGGACTTTTCATTGAGCA
ATOH8	Human	TGACTACAGTGCCGACCACA	TCACTCCTTGCGCTTCTTGG

Supplementary material

NPHP1_KD MDCK and NPHP1_KD IMCD3 cell lines

NPHP1_KD MDCK cell line was previously described, and NPHP1_KD IMCD3 was generated following the same procedures (1). Briefly, shRNA construct targeting mouse *Nphp1* mRNA sequence (5‘-TGAAAGACGCTCTCAAAGTTTTTCAAGAGAAACTTTGA-GAGCGTCTTTCTTTTTTC-3’) was cloned into the lentiviral pSICOR vector, kindly provided by Tyler Jacks, following published instructions (2). Lentiviruses were produced in HEK239T cells, and mIMCD3 cells were infected at an MOI of 20 in the presence of 8mg/mL polybrene.

Antibodies

The following antibodies were used in the study with the following dilution: acetylated α-Tubulin (1/1000e ; Sigma, T6793), AQP2 (1/200e ; Santa Cruz, sc9882), CP110 (1/400e ; Proteintech, 12780-1-AP), polyglutamination modification GT335 (1/5000e ; AdipoGen, AG-20B-0020-C100), Ki67 (1/200e ; Santa Cruz, sc7846), NCC/SLC12A3 (1/200e ; Sigma, HPA028748), NPHP1 (1/100e for immunofluorescence and 1/1000e for Western Blot ; Bicell scientific, 90001), SV40 (1/200e ; Calbiochem, DP02-200UG), p27Kip1 (1/100e ; BD Transduction Lab, 610242).

Primary Screen of the Prestwick Library molecules

- *Cell culture, compound treatment and immunofluorescence*

The Prestwick Chemical Library, containing 1200 mostly FDA-approved drugs, was purchased from Prestwick Chemical Inc. (Illkirch, France) at a concentration of 10 mM in dimethyl sulfoxide (DMSO). To study the effect of the drugs on wound closure and ciliogenesis, 50µL of compounds were added to culture medium for a final drug concentration

of 10µM and 0.5% DMSO. Our preliminary experiments showed that the final 0.5% DMSO had no effect on these two cellular processes. All treatments were performed twice independently.

NPHP1_KD IMCD3 were seeded into 96-well plastic microplates (Packard Viewplate, Perkin Elmer) at a density of 30,000 cells/well in 100µL of DMEM/F12 supplemented with 10% FBS. Cell plates were then incubated at 37°C, 5% CO2 in a 95% humidified Cytomat 6000K (Thermo Fisher Scientific) for 24h. On the following day, cells were washed and incubated overnight at 37°C in serum-free medium. Next day, the cell monolayer was automatically scratched in a straight line to create a "wound" using a 96-pin tool (V&P Scientific, Inc.) mounted on the TeMO (Tecan). Living cells were immediately labeled with Calcein AM (Molecular Probes) and imaged with an automated fluorescent microscope (InCell 2000, GEH) at 2x magnification (T0 of the migration assay). Cells were then incubated with drugs from the Prestwick Library for 9h at 37°C to allow wound closure.

NPHP1_KD MDCK were seeded into 96-well plastic microplates (Packard Viewplate, Perkin Elmer) at a density of 7,500 cells/well in 100µl of MEM alpha supplemented with 10% FBS, 1% Glutamax and 1% Penicilline/Streptomycine. Cells were then incubated at 37°C, 5% CO2 in a 95% humidified Cytomat 6000K (Thermo Fisher Scientific) for 120h, followed by the incubation with drugs from the Prestwick Library for 48h at 37°C.

For immunofluorescence, cells were fixed 15 minutes with 4% PFA, quenched in 50mM NH_4Cl, and permeabilized 15 minutes with PBS – 0.2% BSA – 0.05% saponin, followed by incubation with DAPI and anti-acetylated αtubulin antibody for primary cilium detection.

- *Data acquisition, quantification and analysis*

For the wound-healing assay, images (T0-T9h) were acquired with a 2x objective (InCell 2000, GEH), which provided sufficient resolution to determine the extent of migration. If

disruption of the cell monolayer was observed, images were discarded from the analysis. Image analysis was automated using Cell Developer Toolbox (GEH). For each image, the area of the wound was quantified, and difference of area between T0 and end point was calculated to obtain the migration rate.

For the primary cilium analysis in mIMCD3, images were acquired using a 20x objective. Primary cilium detection was performed using CellProfiler software. Primary cilium analysis focused on the number of cilium per well, and its length variation according to drug treatment. For the primary cilium analysis in mIMCD3 and MDCK cells, images were acquired using a 20x objective. Primary cilium detection was performed using CellProfiler software for mIMCD3 cells and IN Cell analyser 1000 Workstation (GEH) software for MDCK cells, in order to quantify the percentage of ciliated cells and length of cilia. Drugs that cause cell toxicity were discarded from the analysis.

Positive hits for each compound were identified as follows. Data were first corrected through median polishing using log or logit functions. B-score normalization was then applied to each replicate, separately, and including corrections for plates, rows and columns (3, 4). Median and median absolute deviation (MAD) were computed and used to compute Robust Z-scores (RZ-scores). For each sample, according to the formula score = (pertubator value-median)/(1.4826 * Median MAD). RZ-scores were calculated for each compound when compared with the tested population. A compound is identified as a ‘hit’ if the RZ-score is above 2 or below -2 for both replicate experiments.

Drug toxicity on URECs

Control cells were incubated with a large range of drug concentrations 1µM to 40µM to perform time-lapse imaging for 48h using IncuCyte® Cytotox Reagents to label both apopoptic cells and dying cells (membrane integrity loss). Images were analyzed with

Incucyte Zoom Software (Essen Bioscience). Drug toxicity was assumed when a decreased final confluence (corresponding to decreased proliferation) and/or increased apoptosis or cell permeability were observed compared to 0.01% and 0.5% DMSO conditions.

Zebrafish experiments

Zebrafish were maintained at 28.5°C under standard protocols. The following transgenic lines were used: Tg(wt1b:eGFP)^{li1} (5) and Tg(bactin:arl13b-GFP)^{hsc5Tg} to label proximal pronephros and cilia, respectively (5, 6). Translation-blocking *nphp4* morpholinos (Gene Tools LLC, Philomath, OR, USA) were injected into one-cell stage *Tg(wt1b:eGFP)* embryos at 0.4pmol per embryo (7). At 10 hours post fertilization (hpf), embryos were transferred into 12-well plates (20 embryos/well) and incubated with drugs diluted in 1% DMSO Egg-Water in presence of phenylthiourea (to block pigmentation). At 24hpf, drugs were renewed, and dead embryos were removed. Pronase was used at 36hpf to help hatching. Between 48hpf and 54hpf. GFP-positive embryos were individually positioned on the dorsal side in 96-well plates using a previously published method to perform automated imaging of the proximal pronephros with Opera Phenix HSC system (Perkin Elmer) (8). Pronephros morphology was evaluated by two independent observers using a double-blind approach. For whole-mount immunofluorescence, DMSO and Alprostadil-treated *Tg(bactin:arl13b-GFP)* embryos were fixed at 24hpf in 4% PFA, blocked in PBS – 0.3% Triton – 4% BSA for 1h at room temperature and incubated with phalloidin (1/200^{e}) at 4°C overnight. After several washes in PBS – 0.3% Triton, embryos were mounted, and confocal images were acquired using a Spinning Disk microscope (40x or 63x, Zeiss). Z-stacks were performed to acquire the whole cloaca region, and projections were assembled and analyzed using Image J software to determine cilium length.

Mice urine and plasma analyses

24-hour urine samples were collected using metabolic cages. At the time of sacrifice, kidneys were removed for morphological, protein, and mRNA studies. Plasma creatinin and Blood Urea Nitrogen (BUN) was determined on an automatic analyzer (Konelab 20i, Thermo Fisher Scientific).

Immunostaining of human kidney sections

Renal kidney tissue from 3 patients suffering from nephronophthisis were collected from 1973 to 1998. Immunostaining was performed as described in kidney mice section on human kidney biopsies from 3 affected individuals carrying *NPHP1* mutations and one control individual. Confocal images were acquired using a Spinning Disk microscope (40x or 63x, Zeiss).

cAMP dosage in URECs

Cells were incubated during 15 minutes with 0.04% DMSO, 25µM Forskolin or 2µM Alprostadil. cAMP production was measured using cAMP-Glo™ Assay (V1501, Promega) following the recommendations of the manufacturer.

Western blot analysis

Kidneys were lysed in 50mM Tris HCl pH8 – 200mM NaCl – 1mM EDTA pH8 – 1mM EGTA – 1mM DTT – 1% SDS. Cells were lysed in 50mM Tris HCl pH7.4 – 150mM NaCl – 0.1% SDS – 1% Triton – 0.5% sodium deoxycholate. Lysis buffer was supplemented with protease and protein phosphatase inhibitor tablets (04-693-159-001 and 04-906-845-001 respectively, Roche). Protein content was determined with the BCA Protein Assay kit (23225, Thermo Fisher Scientific). Equal amounts of protein were resolved on gels under reducing

conditions, transferred and incubated with primary and secondary antibodies, and visualized using the Fusion Fx7 (Vilber) imaging system or the Odyssey ® CLx imaging system (LI-COR Biosciences). Quantifications were done using Image Studio Lite software (LI-COR Biosciences).

Microarray data analysis

Affymetrix Human ClariomD data were normalized using quantile normalization with adjustment based on the median intensity of probes with similar GC content (using Affymetrix Power Tools). Background correction was made using the antigenomic probes. Only probes targeting exons annotated from FAST DB v2016_1 transcripts were selected. Probes were considered as expressed if the DABG P value ≤ 0.05 (Detection Above Background P values were calculated using Affymetrix Power Tools) in more or equal than 60% of samples in at least one of the two compared experimental condition (e.g. RLT 10µM). Genes were considered as expressed if more or equal than 50% of their probes are expressed. Minimum 4 selected probes were required to assess gene expression. When possible, only high-specific probes were selected (i.e. not overlapping with repeat regions; not cross-hybridizing; and $40 \leq GC\% \leq 60$). In addition, if possible, only probes targeting constitutive gene regions were selected (i.e. targeting at least 75% of transcripts of a given gene). We performed a paired Student's t-test to compare gene intensities in the different biological replicates. Genes were considered significantly regulated when fold change was ≥ 1.5 and uncorrected P value ≤ 0.05.

- *Unsupervised analysis*

The PCA has been performed using "prcomp" function in R and the 2 first dimensions were plotted. The clustering has been performed using "dist" and "hclust" functions in R, using

Euclidean distance and Ward agglomeration method. Bootstraps have been realized using "pvclust" package in R, with the same distance and agglomeration method, using 1000 bootstraps.

- *Sample reproducibility study*

Pearson correlation tests have been performed for each pair of samples using "cor.test" function in R. Heatmaps and clusterings were performed with "dist" and "hclust" functions in R using Euclidean distance and Ward agglomeration method. Bootstraps were realized as described in "Unsupervised analysis" method. Analysis for enriched GO terms, KEGG pathways and REACTOME pathways were performed using the automated meta-analysis tool Metascape (http://metascape.org/).

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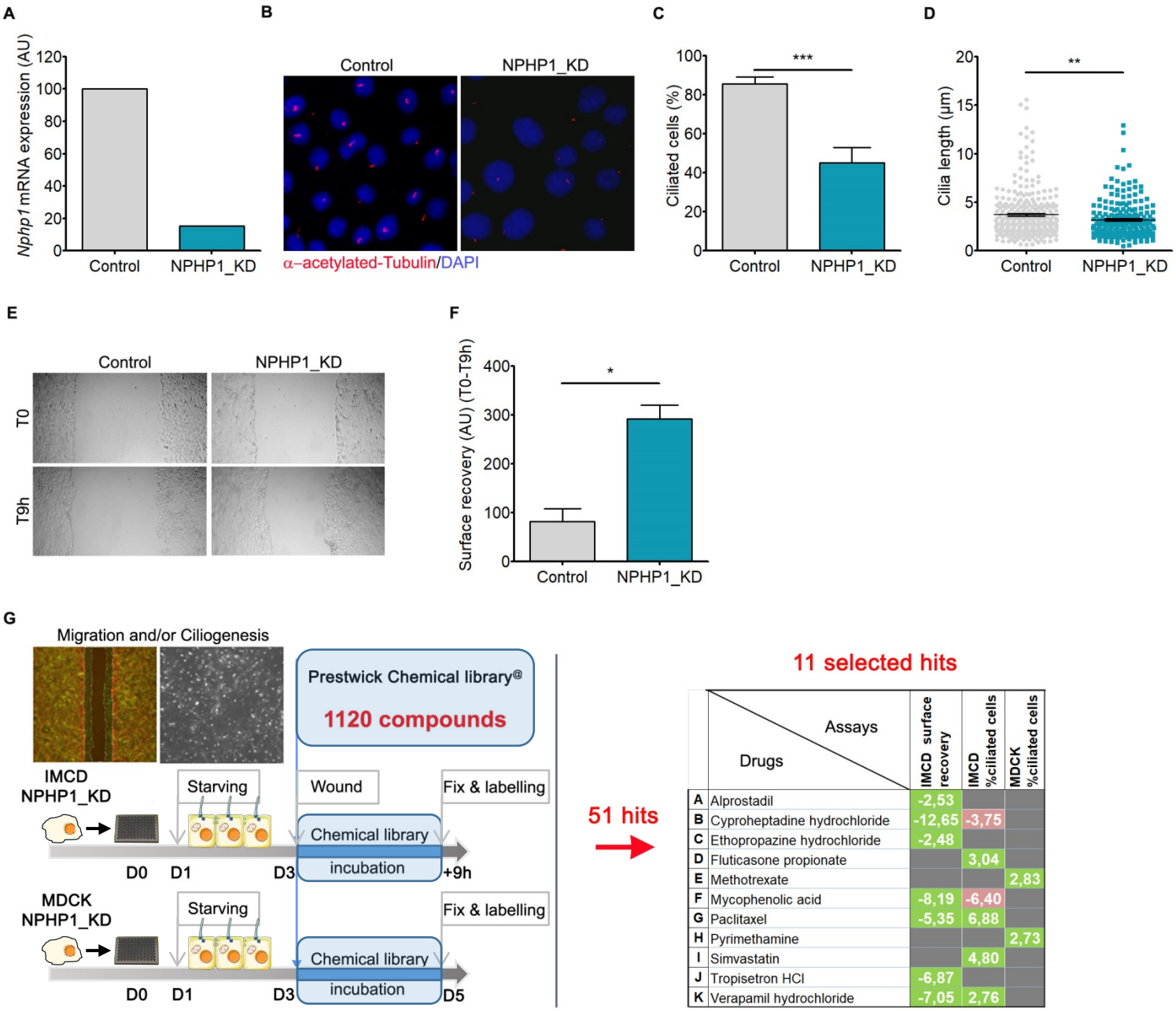
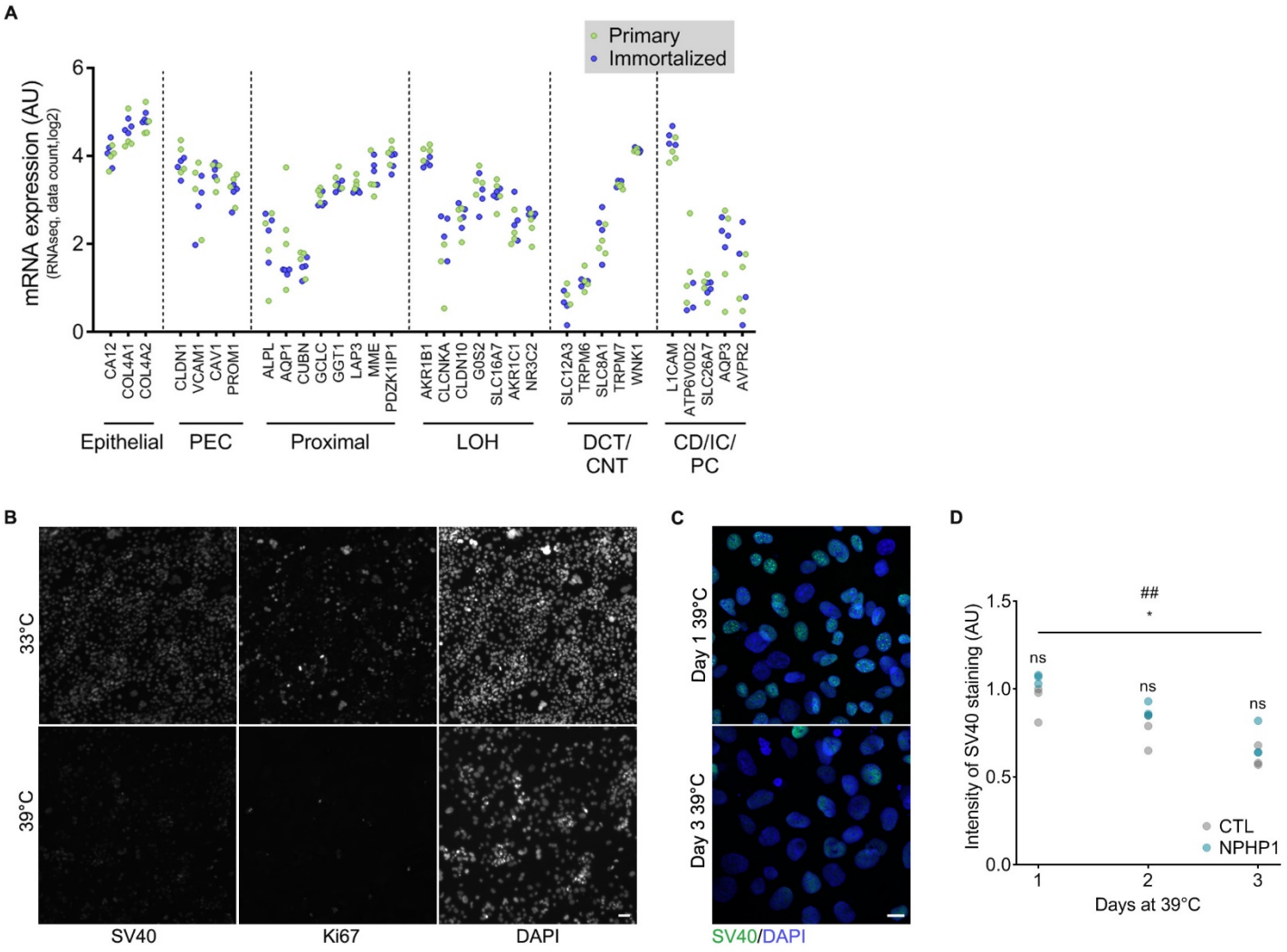


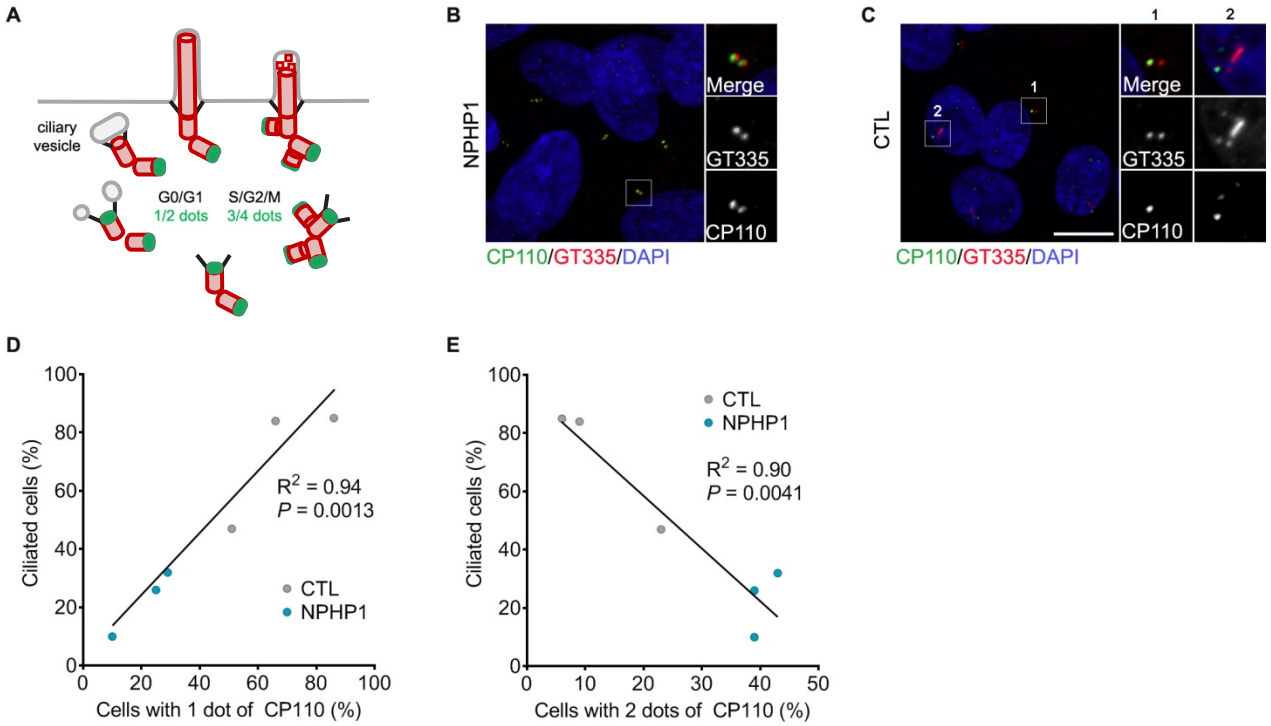
Supplementary Figure S1: Ciliogenesis and migration defects in NPHP1_KD mIMCD-3 cells

(A) qRT-PCR analysis of *Nphp1* expression in polyclonal control and *Nphp1* knock-down (NPHP1_KD) mIMCD3 cells. **(B)** Representative images of immunofluorescence of primary cilia (acetylated α-Tubulin, red) and nuclei (DAPI, blue) in control and NPHP1_KD mIMCD3 cells cultured for 48 hours after serum starvation. **(C)** Quantification of the percentage of ciliated cells. $n = 2$ experiments. Fisher's exact test: $***P < 0.001$. **(D)** Quantification of cilium length. $n = 2$ experiments. Student t-test: $*P < 0.01$. **(E)** Representative images of wound healing assay at T = 0h and T = 9h after scratch on control and NPHP1_KD mIMCD3 cells cultured for 18 hours after serum starvation. **(F)** Quantification of scratch surface recovery as seen in (E). $n = 2$ experiments. Mann-Whitney test: $*P < 0.05$. **(G)** Left panel: Schematic representations of the primary screens. Molecules from the Prestwick library were tested for their effects on migration and/or ciliogenesis in mIMCD3 and MDCK cells, in which the expression of *Nphp1* was stably knockdown (NPHP1_KD). In mIMCD-3 cells, compounds were added for 9h after wounding and both migration and ciliogenesis were measured. In MDCK cells, compounds were added for 2 days and ciliogenesis was analyzed. Right panel: Quantification of the effect of the 11 selected hits on cell migration (surface recovery) or ciliogenesis (% of ciliated cells). RZ score were calculated for each compound when compared to DMSO treatment. A compound is identified as a 'positive hit' (green) if its RZ-score is above 2 (for ciliogenesis) or below -2 (surface recovery) for both replicate experiments. A non-significant effect of a compound on ciliogenesis or cell migration will be indicated by a RZ-score between -2 and 2, whereas a negative ciliogenesis effect (red) will be indicated by a RZ score < -2. **(C-D, F)** Bars indicate mean ± SEM. AU: arbitrary unit.



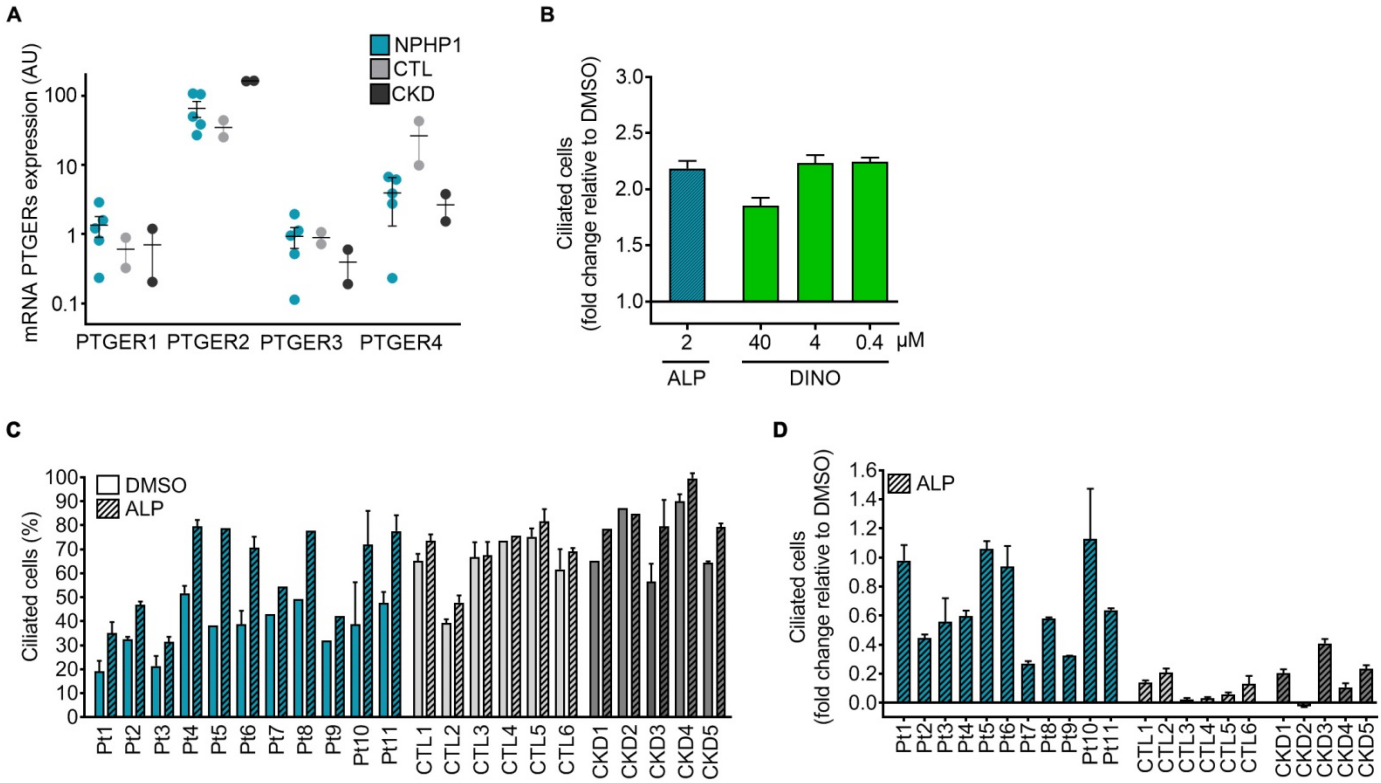
Supplementary Figure S2: Characterization of URECs

(A) Expression data of epithelial and renal markers issued from comparative RNAseq analysis of primary (green) and immortalized (blue) URECs from 4 individuals (2 NPHP1 patients and 2 control individuals). Mann-Whitney test: not significant. AU: arbitrary unit. **(B)** Representative images of immunofluorescence of one immortalized UREC line derived from NPHP1 patient (Pt1) cultured for five days at 33°C (permissive temperature) or 39°C (restrictive temperature) and stained with anti-SV40 large T antigen and anti-Ki67 antibodies. **(C)** Representative images of immunofluorescence of immortalized URECs cultured for one to three days at 39°C (restrictive temperature) and stained with anti-SV40 large T antigen (green) antibody and nuclei (DAPI, blue). **(D)** Quantification of SV40 large T antigen nuclear expression in immortalized URECs derived from NPHP1 patients (NPHP1, n = 3) and control individuals (CTL, n = 3). n = 2 experiments. Unpaired Student t-test: $^{*}P < 0.05$ (NPHP1 day 1 vs NPHP1 day 3), $^{\#\#}P < 0.01$ (CTL day 1 vs CTL day 3), ns: not significant (NPHP1 vs CTL). AU: arbitrary unit. Dots indicate mean. **(A, D)** Each dot represents one individual cell line. **(B, C)** Scale bar: 10µm. PEC: Parietal Epithelial Cells, LOH: Loop Of Henle, DCT: Distal Convoluted Tubule, CNT: Connecting Tubule, CD: Collecting Duct, IC: Intercalated cells, PC: Principal cells.



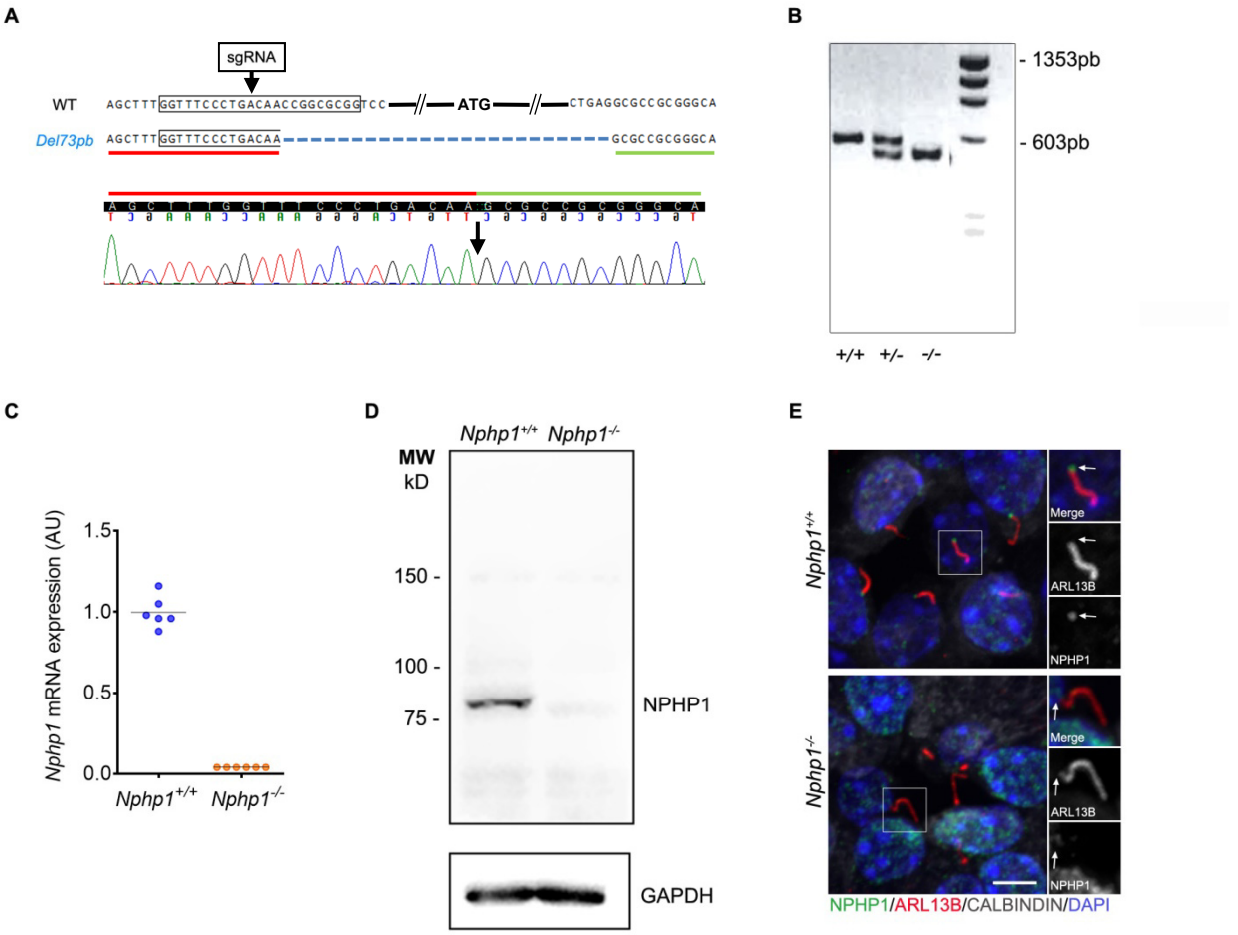
Supplementary Figure S3: Characterization of the URECs ciliary defect

(A) Scheme of CP110 cell cycle localization during ciliogenesis and cilia disassembly. **(B, C)** Representative images of immunofluorescence of immortalized URECs grown for five days at 39°C and stained for CP110 (green), cilia and basal bodies (GT335, red) and nuclei (DAPI, blue). Example of cells with centrosomes presenting with two (B) and one CP110 dot (C). Scale bar: 10µM. **(D, E)** Correlation between the percentage of ciliated cells and the percentage of cells with one (D) or two dots (E) of CP110. Dots indicate mean. *n* = 3 experiments. Pearson's test (*r* = 0.9700 for one dot, *r* = -0,9471 for two dots). Each dot represents one individual cell line.



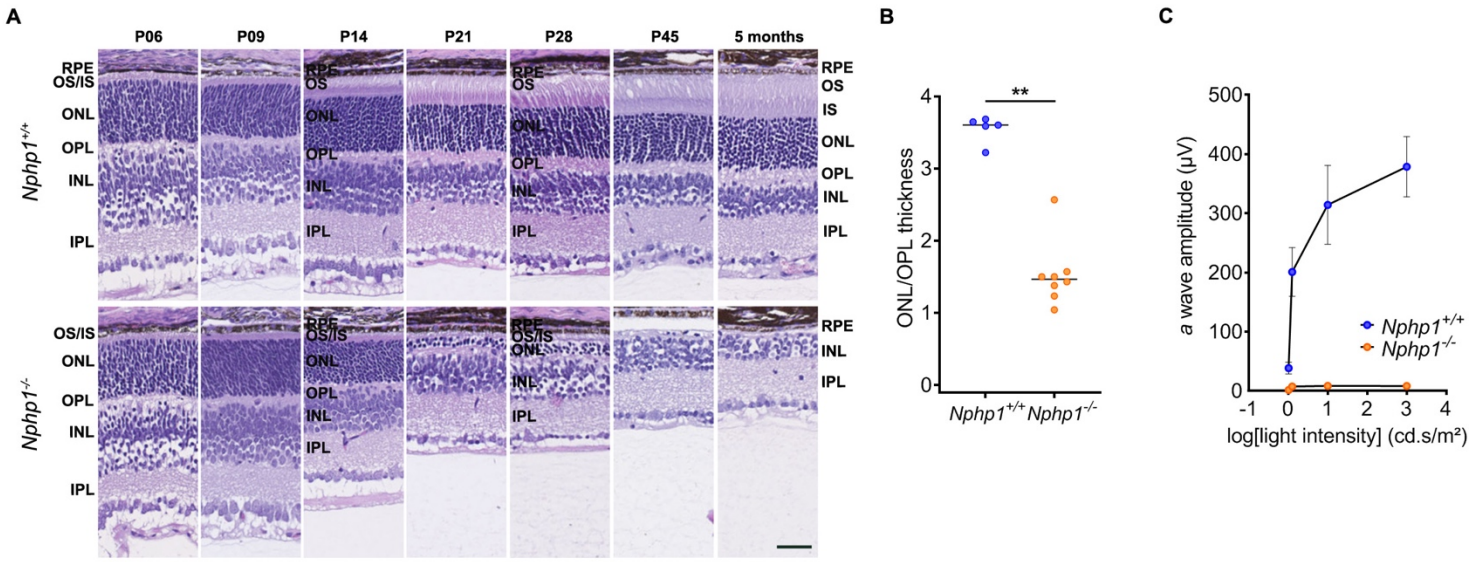
Supplementary Figure S4: Validation of prostaglandin signaling as a target in *NPHP1* URECs

(A) qRT-PCR analysis of EP receptors (*PTGER1-4*) expression in immortalized URECs devired from NPHP1 patients (NPHP1, *n* = 5), control individuals (CTL, *n* = 2) and CKD patients (CKD, *n* = 2). AU: arbitrary unit. **(B)** Quantification of ciliogenesis 5 days after seeding in one immortalized NPHP1 UREC line (Pt1) exposed for 2 days to 2µM Alprostadil or increasing concentrations of Dinoprostone. Bars indicate mean ± SEM. *n* = 2 experiments. **(C-D)** Effect of Alprostadil on ciliogenesis, evaluated by the percentage of ciliated cells (C) or the fold change of ciliated cell relative to DMSO (D) in immortalized NPHP1 (Pt1-11), CTL (CTL1-6) and CKD (CKD1-5) URECs. *n* = 1–15 experiments, with a mean of 3.5 experiments per cell line. ALP: Alprostadil, DINO: Dinoprostone.



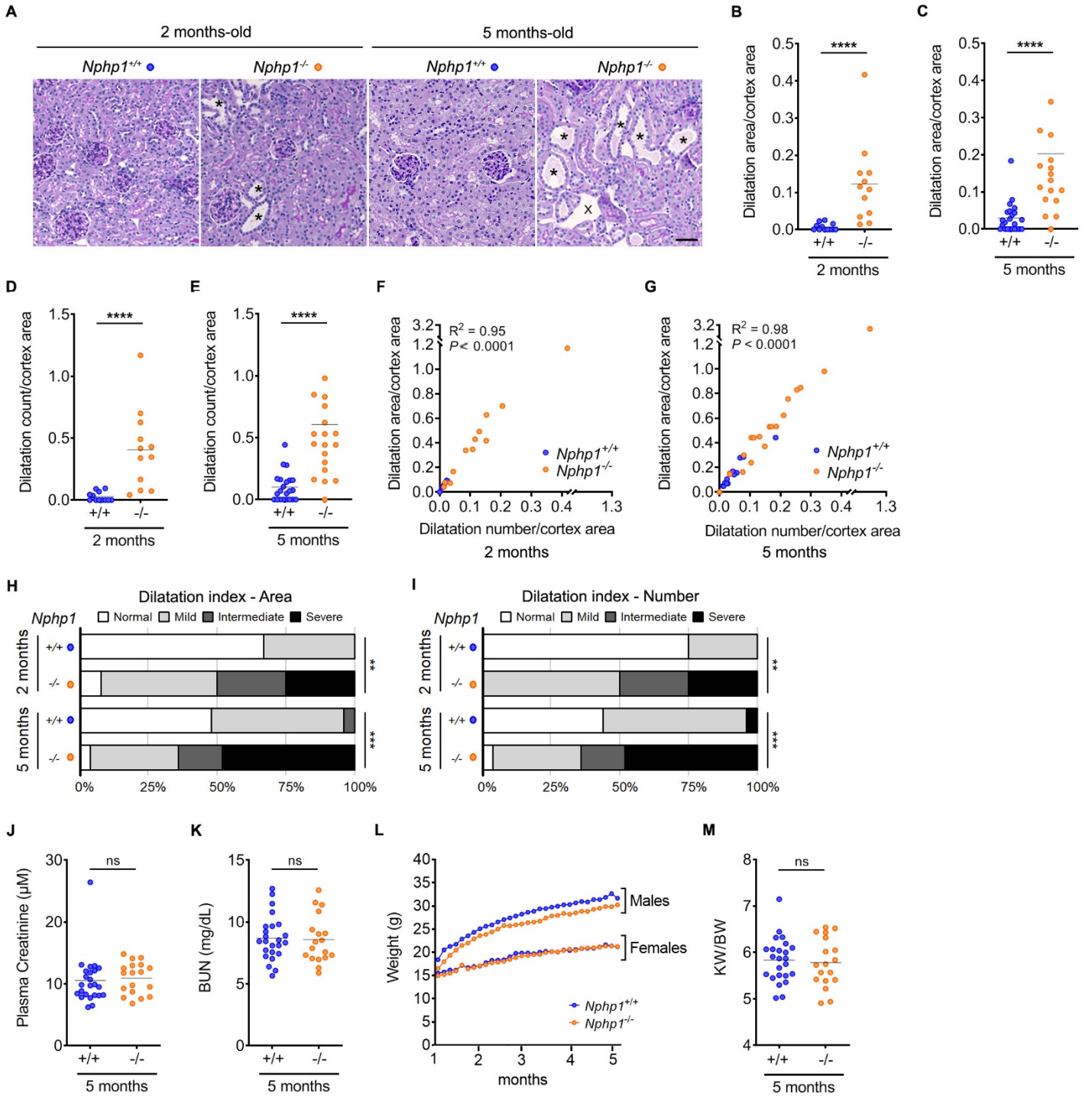
Supplementary Figure S5: *Nphp1*$^{-/-}$ mice generation using CRISPR/Cas9 technology

(A) Schematic representation of the deletion strategy to obtain *Nphp1*$^{-/-}$ mice using CRISPR/Cas9. Guide RNAs targeted sequence upstream exon 1 leads to a 73bp deletion encompassing the start codon ATG in exon 1 (Del 73pb) (Sanger Sequencing). **(B)** Genotypes were based on PCR amplification of total genomic DNA with primers framing the deletion (PCR fragment of 603pb for *Nphp1*$^{+/+}$). **(C)** qRT- PCR analysis of *Nphp1* expression in kidneys from *Nphp1*$^{+/+}$ and *Nphp1*$^{-/-}$ 5 months-old mice. n = 5 mice per genotype. Each dot represents one individual mouse. Bars indicate means. AU: arbitrary unit. **(D)** Western blot analysis of NPHP1 expression in *Nphp1*$^{+/+}$ or *Nphp1*$^{-/-}$ mice testis. **(E)** Representative images of immunostaining of NPHP1 (green), primary cilia (ARL13B, red), DCT/CNT (Calbindin, green) and nuclei (DAPI, blue) in *Nphp1*$^{+/+}$ or *Nphp1*$^{-/-}$ mice. Scale bar: 10µM. DCT: Distal Convoluted Tubule, CNT: Connecting Tubule.



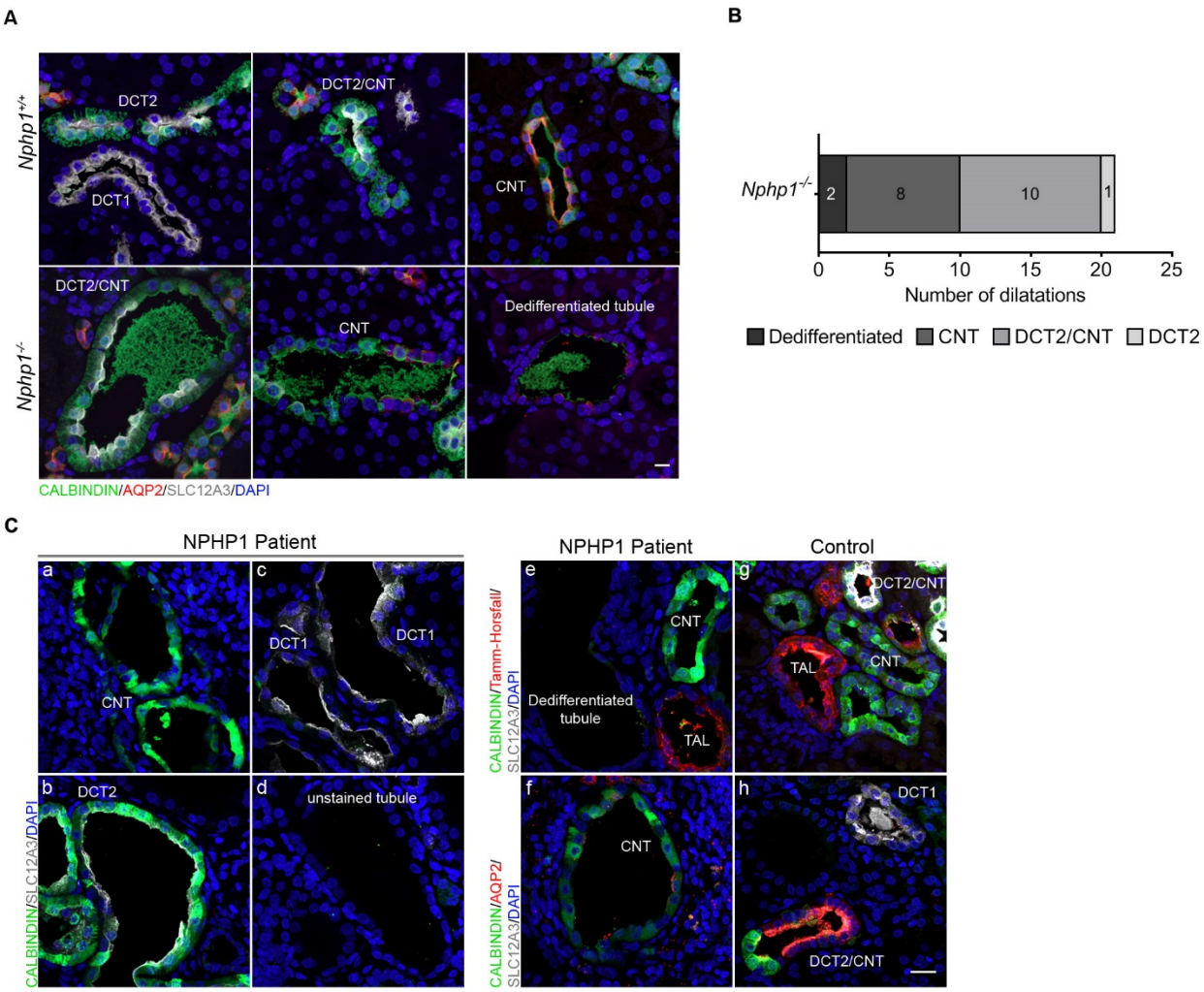
Supplementary Figure S6: Analysis of retina phenotype of *Nphp1*$^{+/+}$ and *Nphp1*$^{-/-}$ mice

(A) H&E staining of *Nphp1*$^{+/+}$ and *Nphp1*$^{-/-}$ retinas from P6 to P45 and 5 months-old mice. Scale bar: 50µm. **(B)** Quantification of ONL/OPL thickness ratio in retinas from P21-old *Nphp1*$^{+/+}$ (n = 3) and *Nphp1*$^{-/-}$ (n = 8) mice treated with vehicule (5% Solutol). Each dot represents one individual mouse. Mann-Whitney test: *P < 0.05. **(C)** Scotopic *a*-wave amplitude *versus* stimulus intensity (log) function in P21-old *Nphp1*$^{+/+}$ (n = 4) and *Nphp1*$^{-/-}$ (n = 4) mice retinas. Dots indicate mean ± SEM. RPE: Retinal Pigmented Epithelium, OS: Outer Segment, IS: Inner Segment, ONL: Outer Nuclear Segment, OPL: Outer Plexiform Layer, INL: Inner Nuclear Segment, IPL: Inner Plexiform Layer.

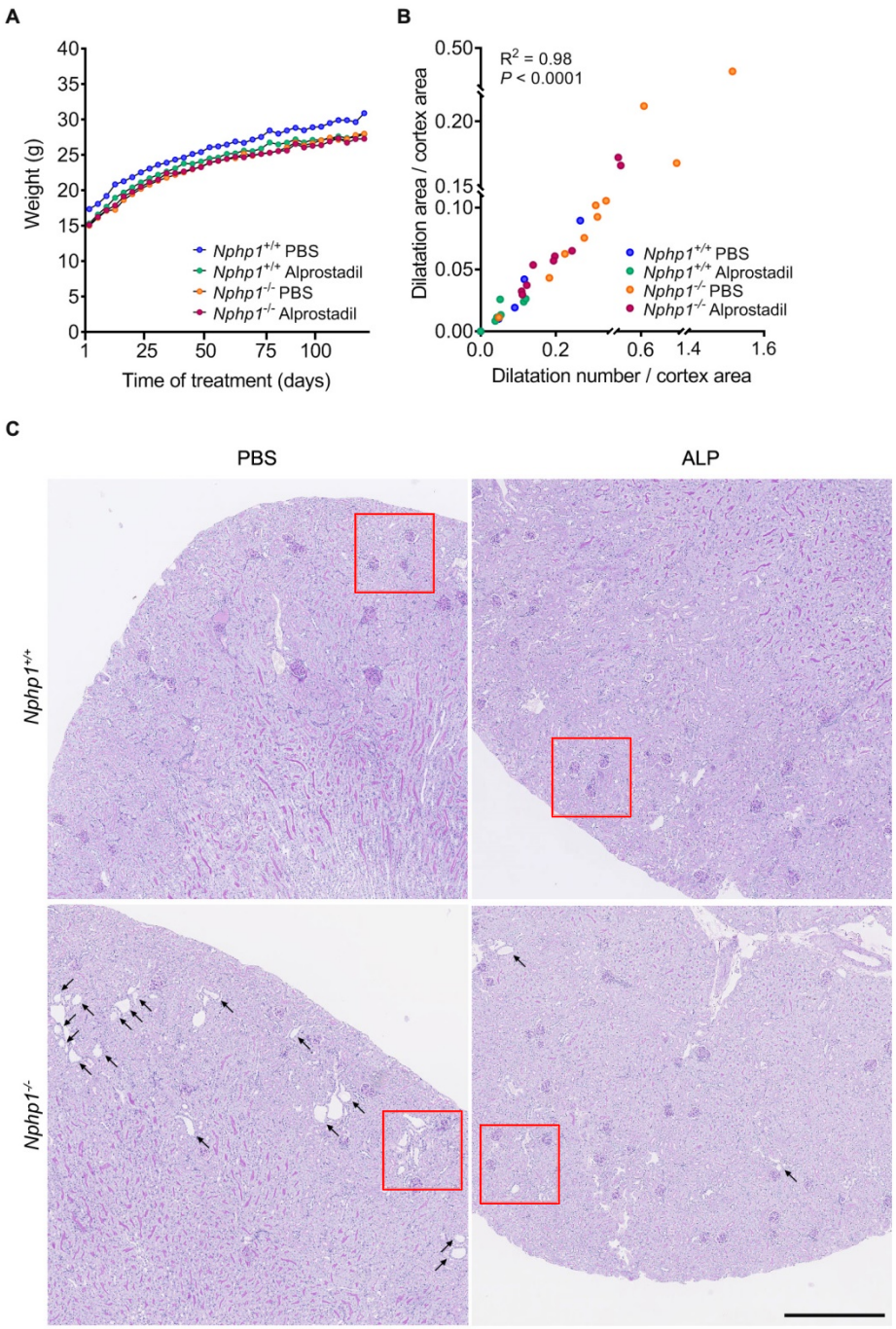


Supplementary Figure S7: Analysis of kidney phenotype of *Nphp1*$^{+/+}$ and *Nphp1*$^{-/-}$ mice

(A) Representative images of PAS staining of kidney sections from 2 and 5 months-old *Nphp1*$^{+/+}$ and *Nphp1*$^{-/-}$ mice. Scale bar: 50µm. * = tubular dilatations; X = blood vessels. **(B-E)** Quantification of the total area covered by dilatation/cortex area (B, C) and the number of dilatation/10µm^2 in cortex (D, E) in *Nphp1*$^{+/+}$ and *Nphp1*$^{-/-}$ mice at 2 and 5 months-old. **(F, G)** Scatter dot plots correlating the number of dilatation/10µm^2 in cortex to the total area covered by dilatations/cortex area in 2 months (F) and 5 months (G) -old *Nphp1*$^{+/+}$ mice. Pearson's test (r = 0,9770 for 2 months-old, r = 0.9914 for 5 months-old). **(H, I)** Dilatation index for the total area covered by dilatations/cortex area (H) and the number of dilatation/10µm^2 in cortex (I). Chi-squared test: $^{**}P < 0.01$, $^{***}P < 0.001$. **(J, K)** Plasma creatinine (J) and blood urea nitrogen (BUN) (K) of 5 months-old *Nphp1*$^{+/+}$ and *Nphp1*$^{-/-}$ mice. **(L)** Body weight increase in *Nphp1*$^{+/+}$ and *Nphp1*$^{-/-}$ mice from 1 month to 5 months-old. **(M)** Kidney weight to body weight ratio (KW/BW) of 5 months-old *Nphp1*$^{+/+}$ and *Nphp1*$^{-/-}$ mice. **(B-M)** 2 months-old: males (n = 6), females (n = 6), 5 months-old: males (n = 12)), females (n = 12)) and *Nphp1*$^{-/-}$ (2 months-old: males (n = 7), females (n = 5), 5 months-old: males (n = 8), females (n = 10). **(B-E, J, K, M)** Mann-Whitney test: $^{****}P < 0.0001$, ns: not significant. **(B-G, J, K, M)** Each dot represents one individual mouse.

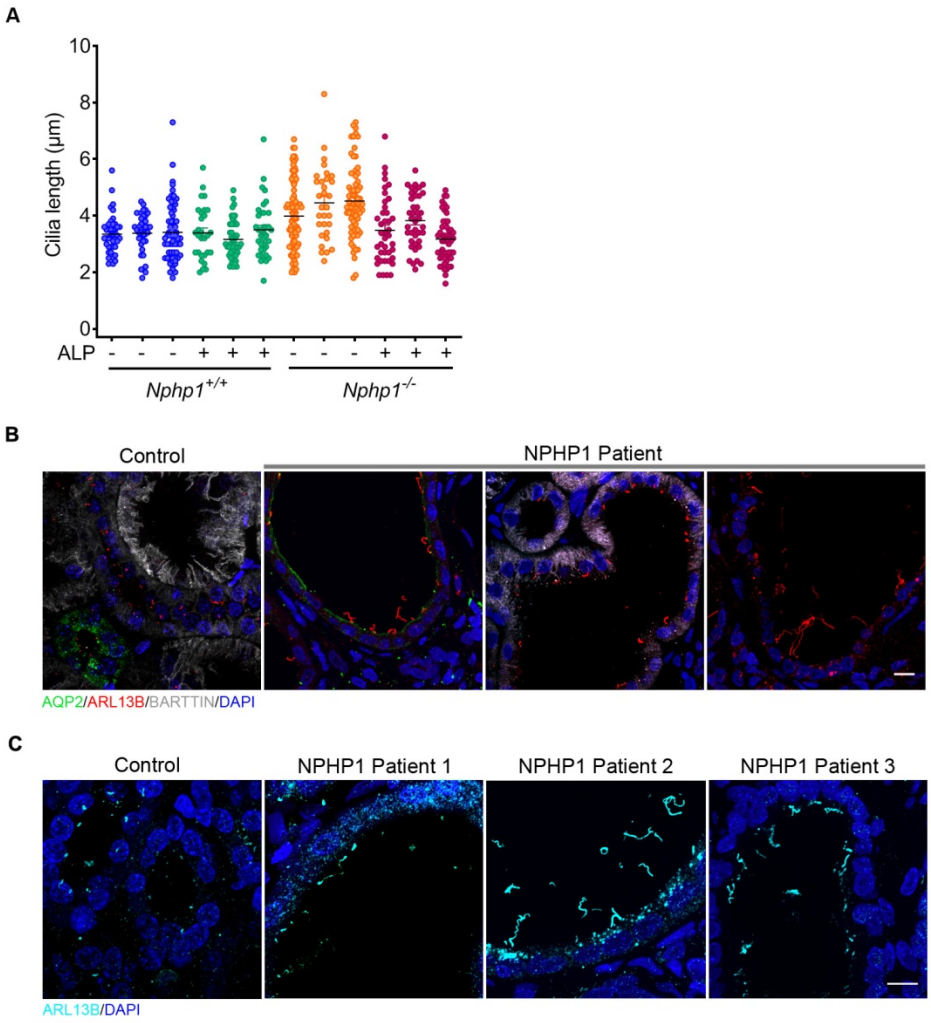


Supplementary Figure S8: Localization of tubular dilatations in *Nphp1*$^{-/-}$ mouse and *NPHP1* patient kidneys
(A) Representative images of immunostaining of mouse kidney tubules stained with anti-SLC12A3 (DCT, grey), anti-Calbindin (DCT and CNT, green), anti-AQP2 (CNT and CCD, red) antibodies and DAPI (nuclei, blue). Scale bar: 50µm. **(B)** Quantification of the number of dilatations in each segment of the nephron in *Nphp1*$^{-/-}$ animals (n = 4). **(C)** Representative images of (a-d) immunostaining of human kidney tubules from *NPHP1* patient stained with anti-SLC12A3 (grey), anti-Calbindin (green) antibodies and DAPI (blue); (e-g) immunostaining of human kidney tubules from *NPHP1* patient (e) and control individual (g) with anti-SLC12A3 (gray), anti-Calbindin (green), anti-Tamm-Horsfall (TAL, red) antibodies and DAPI (blue); (f-h) immunostaining of human kidney tubules from *NPHP1* patient (f) and control individual (h) with anti-SLC12A3 (grey), anti-Calbindin (green), anti-AQP2 (red) antibodies and DAPI (blue). Scale bar: 20µm. TAL: Thick Ascending Limb of the Loop of Henle, DCT: Distal Convoluted Tubule, CNT: Connecting Tubule, CCD: Cortical Collecting Duct.



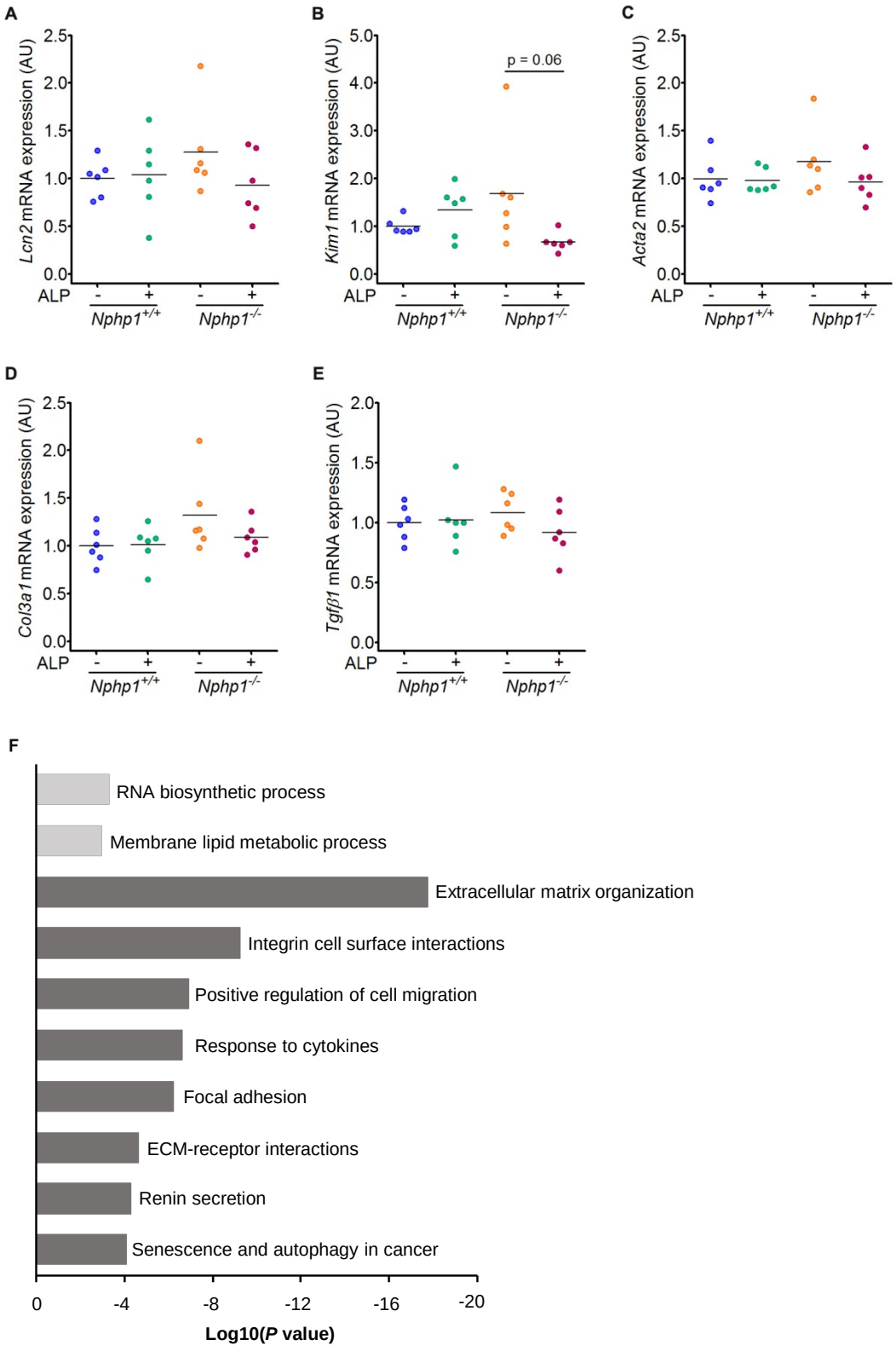
Supplementary Figure S9: Impact of Alprostadil treatment on body weight and tubular dilatations in *Nphp1*$^{+/+}$ and *Nphp1*$^{-/-}$ mice

(A) Body weight increase in *Nphp1*$^{+/+}$ and *Nphp1*$^{-/-}$ mice injected daily with vehicle (PBS) or Alprostadil (80µg/kg) from 1 month to 5 months-old. **(B)** Scatter dot plot correlating the number of dilatation/10µm^2 in cortex to the total area covered by dilatations/cortex area in *Nphp1*$^{+/+}$ and *Nphp1*$^{-/-}$ mice treated with PBS or Alprostadil (ALP). Pearson's test (r = 0.9932). **(C)** PAS staining of kidney sections used in Figure 3 at lower magnificence. Scale bar: 50µm. Arrows indicate dilatations. **(A, B)** n = 9–10 male mice for each genotype/treatment.



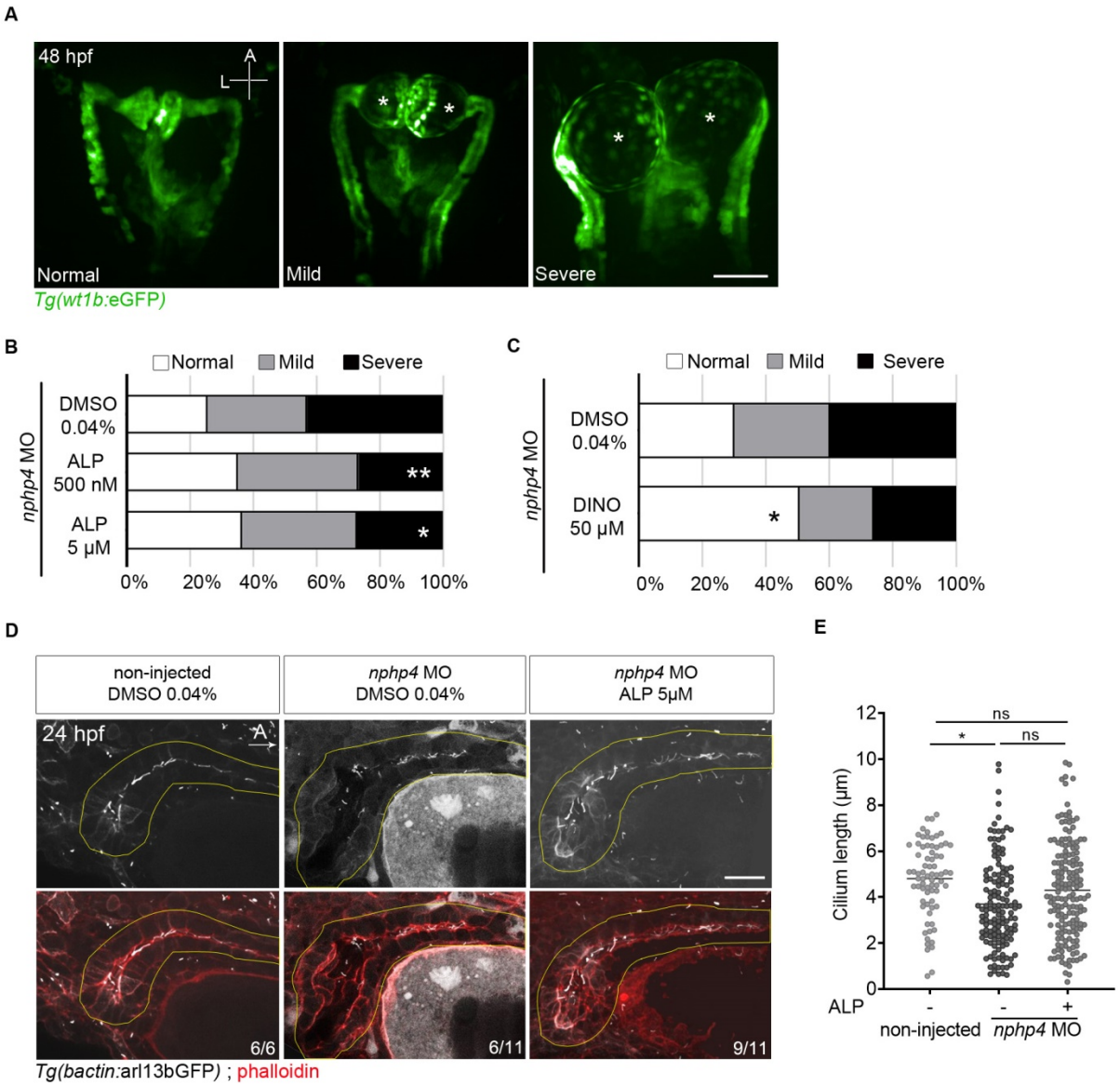
Supplementary Figure S10: Impact of Alprostadil treatment on ciliogenesis in *Nphp1*$^{-/-}$ mice and cilia length in *NPHP1* patient kidneys

(A) Effect of Alprostadil (ALP) on primary cilium length in connecting and distal convoluted tubules of *Nphp1*$^{+/+}$ and *Nphp1*$^{-/-}$ mice at 5 months-old. *n* = 3 male mice for each genotype/treatment. Each dot represents one cilium. **(B)** Representative images of immunostaining of human kidney sections from NPHP1 patient and control individual stained with anti-AQP2 (CNT and CCD, green), anti-ARL13B (cilia, red), anti-BARTTIN (TAL, DCT and CNT, grey) antibodies and DAPI (nuclei, blue). Scale bar: 10µm. **(C)** Immunostaining of a control and three additional NPHP1 patient's kidney sections stained with anti-ARL13B (cyan) antibody and DAPI (blue). Scale bar: 20µm. ALP: Alprostadil, TAL: Thick Ascending Limb of the Loop of Henle, DCT: Distal Convoluted Tubule, CNT: Connecting Tubule, CCD: Cortical Collecting Duct.



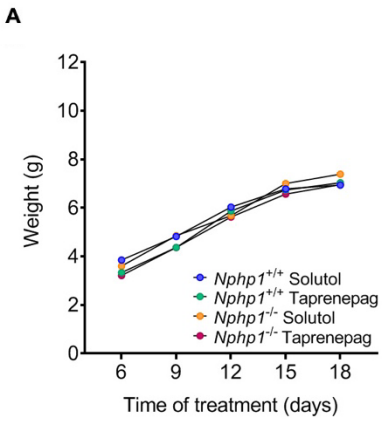
Supplementary Figure S11: Impact of Alprostadil treatment on pro-fibrotic markers and global RNA-seq from kidney of *Nphp1*$^{+/+}$ and *Nphp1*$^{-/-}$ mice

(A-E) qRT-PCR analysis of *Lcn2*, *Kim1*, *Acta2*, *Col3a1* and *Tgfβ1* expression from 5 months-old *Nphp1*$^{+/+}$ and *Nphp1*$^{-/-}$ mice kidneys treated or not with Alprostadil (ALP). One-way ANOVA followed by Holm-Sidak's post-test: not significant. Each dot represents one individual mouse. Bars indicate mean. AU: arbitrary unit. **(F)** Pertinent down- (light grey) or up-regulated (dark grey) selected pathways or relevant processes involving the 480 significantly differentially expressed genes detected by RNAseq in 5 months-old *Nphp1*$^{-/-}$ mice (n = 5) in comparison to *Nphp1*$^{+/+}$ mice (n = 4). Pathways were highlighted using Metascape. ALP: Alprostadil.

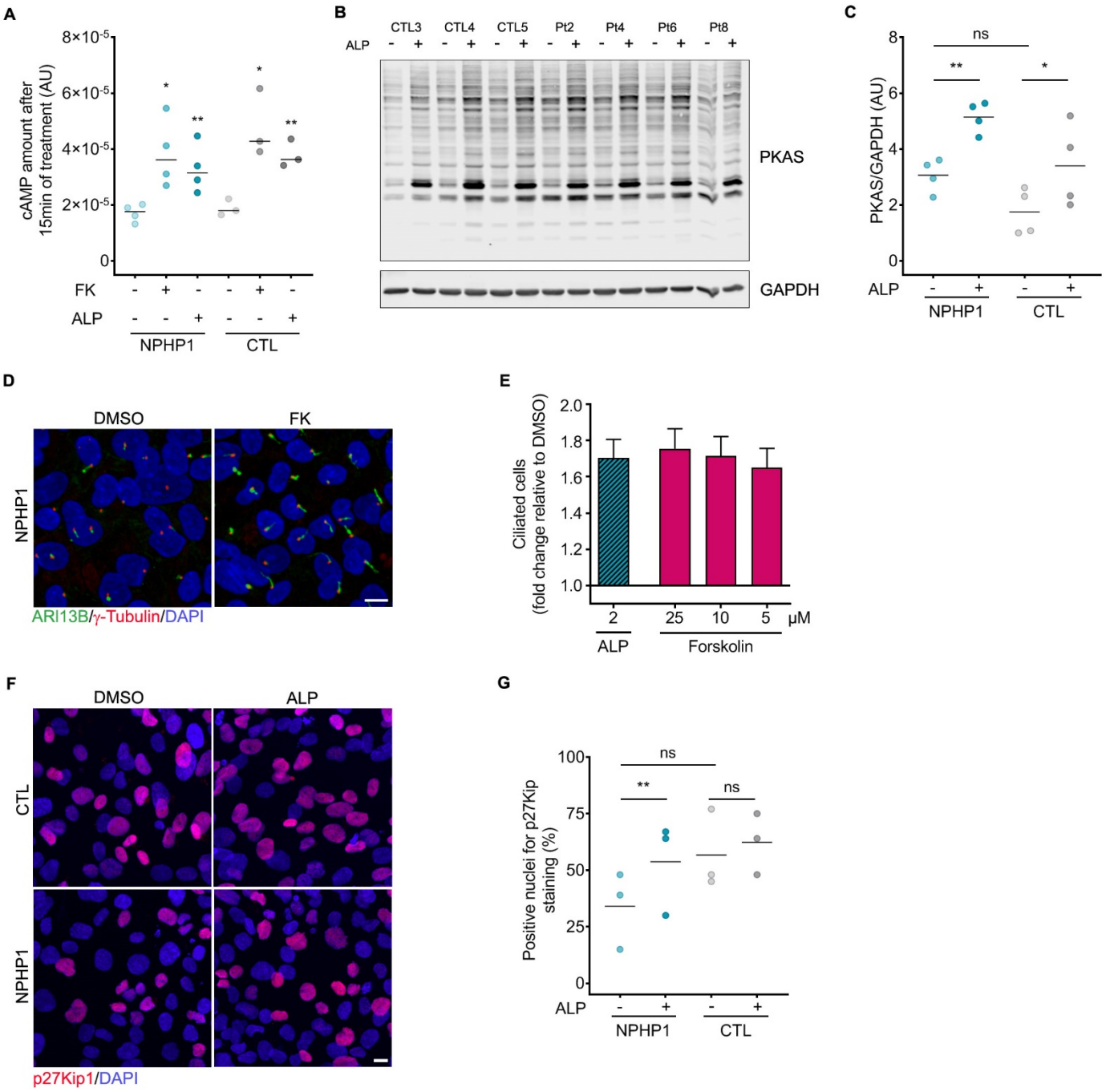


Supplementary Figure S12: *In vivo* validation of prostaglandin signaling as a target in zebrafish

(A) *nphp4* morpholino-injected embryos were treated from 10 to 48 hours post fertilization (hpf), and morphology of glomeruli and proximal pronephros was assessed using *Tg(wt1b:*GFP*)* transgenic embryos at 48-54 hpf. Three different categories were established depending on the absence (normal) or presence of mild or severe glomerular dilations (asterisks). Representative images of dorsal views, anterior to the top. Scale bar: 50µm. **(B)** Treatment with Alprostadil significantly reduced the percentage of severe dilations at 500nM (*n* = 71 embryos) and 5µM (*n* = 99 embryos) compared to control DMSO (0.04%) (*n* = 86 embryos). **(C)** Treatment with Dinoprostone (50µM) (*n* = 77 embryos) significantly increased the percentage of normal proximal pronephros compared to DMSO (0.04%) (*n* = 93 embryos). **(D)** *nphp4* morpholino-injected embryos were treated with Alprostadil (5µM) from 10 to 24 hpf and fixed prior to immunostaining. Tubule lumens were labelled with phalloidin, and cilia with *Tg(bactin:arl13bGFP)* transgene. *nphp4* knockdown leads to dilation of the cloaca region, that is partially restored with Alprostadil treatment. Representative images of side views, anterior to the right. Scale bar: 20µm. **(E)** Quantification of cilium length in the distal part of the pronephros of *nphp4* morpholino-injected embryos upon Alprostadil (*n* = 14 embryos) or DMSO (*n* = 11 embryos) treatment, compared to DMSO-treated non-injected animals (*n* = 6 embryos). Bars indicate mean. Mann-Whitney test: **P* < 0.05, ns = not significant. Each dot represents cilia length. ALP: Alprostadil, DINO: Dinoprostone. **(B-C)** *n* = 3–5 experiments. Fisher's exact test (Normal/Mild compared to Severe (B), Normal compared Mild/Severe (C)): **P* < 0.05, ***P* < 0.01.



Supplementary Figure S13: Impact of Taprenepag treatment on *Nphp1*$^{+/+}$ and *Nphp1*$^{-/-}$ mice body weight
(A) Body weight increase in *Nphp1*$^{+/+}$ and *Nphp1*$^{-/-}$ mice injected every 3 days with vehicle (5% Solutol) or Taprenepag (18mg/kg) from P6 to P21.



Supplementary Figure S14: cAMP pathway and p27Kip1 upon Alprostadil treatment in NPHP1 URECs

(A) cAMP amount after 15 minutes of treatment with DMSO (0.04%), Forskolin (FK, 25µM) or Alprostadil (2µM) in immortalized URECs derived from NPHP1 patients (NPHP1, *n* = 4) and control individuals (CTL, *n* = 3). Paired Student t-test (NPHP1/CTL DMSO vs NPHP1/CTL FK and ALP): **P* < 0.05, ***P* < 0.01. **(B)** Representative image of Western blot analysis showing all phospho-PKA substrates using PKAS antibody in immortalized NPHP1 (Pt) and CTL URECs after 15 minutes of treatment with DMSO (0.04%) or Alprostadil (2µM). **(C)** Quantification of the relative abundance of PKAS in immortalized NPHP1 (*n* = 4) and CTL (*n* = 3) URECs. **(D)** Representative images of immunofluorescence of primary cilia (ARL13B, green), basal bodies (γ-Tubulin, red) and nuclei (DAPI, blue) in immortalized NPHP1 URECs 5 days after seeding treated for 2 days with DMSO (0.04%) or Forskolin (25µM). **(E)** Quantification of ciliogenesis in one immortalized NPHP1 UREC line (Pt6) exposed for 2 days to Alprostadil (2µM) or increasing concentrations of Forskolin. Bars indicate mean ± SEM. *n* = 2 experiments. **(F)** Representative images of immunofluorescence of p27Kip1 (red) and DAPI (blue) in immortalized NPHP1 URECs 4 days after seeding treated for 1 day with DMSO (0.04%) or Alprostadil (2µM). **(G)** Quantification of the percentage of p27Kip1 positive nuclei in immortalized NPHP1 (*n* = 3) and CTL (*n* = 3) URECs. **(A, C, G)** Each dot represents one individual cell line. Bars indicate mean. *n* = 3 experiments. **(D, F)** Scale bar: 10µM. **(C, G)** Mann-Whitney test (NPHP1 vs CTL) and paired Student t-test (DMSO vs ALP): **P* < 0.05, ***P* < 0.01, ns: not significant. ALP: Alprostadil. AU: arbitrary unit.