Supplementary Information

Materials and Methods

Cell culture

Conditionally immortalized podocytes (SVI; CLS Cell Line Service, Eppelheim, Germany) were handled as described previously.[1] Podocytes were cultivated at 33°C in RPMI 1640 Medium (Thermo Fisher Scientific, Waltham; MA, USA) supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin, and 0.1 mg/ml streptomycin. To induce differentiation, podocytes were maintained at 38 °C for at least 2 weeks. All experiments were performed using differentiated podocytes.

Transfection of cultured podocytes

Knockdown of palladin (PalldKD) was achieved using Silencer® Select siRNAs (Ambion®, Thermo Fisher Scientific). Cells were seeded on collagen IV (0.1 mg/ml; BD Bioscience, San Jose, CA, USA) coated glass coverslips and transfected with Palld1 siRNA (s90889), Palld2 siRNA (s90890) and control siRNA (4390846), respectively (final concentration 20 nM). For transfection, the K2® Transfection System (Biontex Laboratories GmbH, Munich, Germany) was used according to the manufacturer's instructions. After 72 hours, cells were used for experiments, except regulation studies of cytoskeletal genes were performed at double transfected PalldKD cells. Transfection efficiency was examined by RT-PCR, qRT-PCR, Western blot analysis and/or immunofluorescence staining.

Immunofluorescence staining of cultured podocytes

Cultured podocytes were fixed with 2% paraformaldehyde (PFA) for 10 min at room temperature, permeabilized by 0.3% Triton X-100 and blocked with blocking solution (2% FBS, 2% bovine serum fraction V, 0.2% fish gelatine in PBS). Cells were incubated with the following primary antibodies for 1 hour: rabbit anti-palladin (10853-1-AP, Proteintech Group, Manchester, UK; 1:150), mouse anti-synaptopodin (61094, Progen Biotechnik GmbH, Heidelberg, Germany; 1:100), rabbit anti-vinculin (V9131, Sigma-Aldrich, St. Louis, MO, USA; 1:100) and rabbit anti-α-actinin-4 (0042-05, immunoGlobe Antikörpertechnik GmbH, Himmelstadt, Germany; 1:100). Bound

antibodies were visualized with Alexa Fluor® 647-conjugated (Thermo Fisher Scientific; 1:300) or Cy3-conjugated secondary antibodies (Dianova/ Jackson Immuno Research, Hamburg, Germany; 1:300).
To visualize F-actin, cells were stained with Alexa Fluor® 488-phalloidin (Thermo Fisher Scientific; 1:100) and for nuclei staining DAPI (Sigma-Aldrich; 1:100) was used. Finally, cells were embedded in Mowiol (40 ml PBS, 10 g Mowiol (Carl Roth, Karlsruhe, Germany) and 20 ml glycerol). All experiments were performed at room temperature.
Images were acquired using a Leica TCS SP5 confocal laser scanning microscope (Leica Microsystems, Wetzlar, Germany).

Actin dynamics and focal adhesion analysis

For actin dynamic studies, podocytes were seeded on collagen IV coated glass coverslips and transfected with control or Palld1 siRNA. After 3 days confluent cells were incubated with latrunculin A, cytochalasin D and jasplakinolide, respectively (final concentrations 0.5 µM, Sigma-Aldrich) for 5-25 min. Podocytes were stained for F-actin, nuclei and palladin to confirm knockdown efficiency.
Images were taken with a Leica TCS SP5 confocal laser scanning microscope (1024 x 1024 pixel) at 400x magnification (1 pixel (pxl) edge length = 0.38 µm). To investigate effects on the actin cytoskeleton, imaged cells were analyzed with the software F_Seg.[2]

Briefly, images were pre-processed to enhance the signal to noise ratio and to identify fiber-like structures (width ≥ 2 pxl, length-width ratio 3:1). A fiber-like structure with length ≥ 6 pxl and width ≥ 2 pxl was defined as an actin filament. Length and width were measured for each identified filament. Additionally, the F-actin distribution was determined by fluorescence intensity and clustered in thin actin filaments (length ≥ 6 pxl, width 2-5 pxl) and thick actin filaments (length ≥ 6 pxl, width 6-20 pxl), respectively. The leftover F-actin structures were classified as “nonfiber-like” F-actin. Over 150 cells were analyzed for each point in time.

For quantification of focal adhesions area, cultured podocytes were stained for vinculin. Over 60 cells per group were studied in an automated fashion by the developed custom software “Focal Contact Segmentation and Analysis Tool” as described previously.[3]

Wound assay

Podocytes were seeded in a collagen IV coated culture insert attached on a coated µ-Dish (Ibidi GmbH, Planegg, Germany). After 3 days, cells were transfected with control and Palld1 siRNA, respectively. The cell migration was investigated immediately after removing the insert at 38°C maintained by a temperature control system (Life Imaging Services, Basel, Switzerland). The system was mounted on a Leica DMI 6000B fluorescence microscope (Leica Microsystems). Images were taken every 10 min for 22 hours with an integrated digital camera and processed with Volocity software (PerkinElmer, Waltham, MA, USA). Afterwards, cells were fixed and stained for F-actin, nuclei and palladin.

Isolation of mouse glomeruli

Glomeruli were isolated with magnetic Dynabeads as described previously.[4]

RNA extraction, RT-PCR/qRT-PCR

Samples from transfected cells/ glomeruli/ kidneys/ zebrafish larvae were processed in Tri-Reagent (Sigma-Aldrich) according to the manufacturer's instructions. For cDNA synthesis, 1 µg of isolated total RNA was reverse transcribed using the QuantiTect Reverse Transcription Kit (Qiagen, Hilgen, Germany). Transcription of zebrafish RNA was performed using SuperScript Reverse Transcriptase (Thermo Fisher Scientific).

For reverse transcriptase-PCR (RT-PCR) Taq Polymerase (Peqlab/ VWR International, Radnor, PA, USA) was used for cells/ mice samples and Platinum® Taq DNA Polymerase (Thermo Fisher Scientific) for zebrafish samples, respectively. RT-PCR was performed on a Mastercycler gradient (Eppendorf AG, Hamburg, Germany).

Quantitative real-time PCR (qRT-PCR) was performed on a LightCycler Nano (Roche Diagnostics GmbH, Mannheim, Germany) using iTaq Universal SYBR Green Supermix (Bio-Rad Laboratories GmbH, Hercules, California USA). For PCR, the respective primers were used (see Supplementary Table 1). Relative mRNA expression was calculated by normalizing values to the housekeeping genes *Gapdh* or *eef1a1l1.*

Protein isolation and Western blot analysis

For protein isolation, transfected cells/ glomeruli/ kidney were taken up in RIPA buffer (Sigma-Aldrich) supplemented with Halt Protease Inhibitor (Thermo Fisher Scientific; 1:100). Lysis was done for 20 min by shaking at 1,400 rpm at 4°C in a Thermomixer Comfort (Eppendorf AG). Lysed samples were centrifuged and supernatants quantified using the Qubit®2.0 Fluorometer (Thermo Fisher Scientific). Adjusted protein amounts were blotted as described previously.[3] Blots were washed in 1xTBST (50 mM Tris, 150 mM NaCl, 10 mM $CaCl_2$, 1 mM $MgCl_2$ supplemented with Tween-20 0.5%) and blocked with 5% milk powder (in 1xTBST) for 1 hour at room temperature. Moreover, primary antibodies anti-palladin (10853-1-AP, Proteintech Group, 1:3000) and anti-Gapdh (sc-25778, Santa-Cruz; 1:4000) were diluted in 5% milk powder (in 1xTBST) and incubated with the blots overnight at 4°C. Afterwards, blots were incubated with secondary antibody goat anti-rabbit IgG-HRP (sc-2030, Santa Cruz, 1:17500) for 45 min, incubated with the Clarity™ Western ECL Blotting Substrate (Bio-Rad) and the reaction visualized on X-ray films (GE Healthcare, Little Chalfont, UK). Protein expression was normalized to Gapdh as a housekeeping protein.

Human kidney biopsies

Kidney biopsies from the Department of Nephropathology (Institute of Pathology, University Hospital Erlangen, Germany) were used. The use of remnant kidney biopsy material was approved by the Ethics Committee of the Friedrich-Alexander-University of Erlangen-Nürnberg, waiving the need for retrospective consent for the use of archived material. Colocalization studies of palladin and synaptopodin were performed on biopsies of controls (n=3), FSGS patients (n=4) and DN patients (n=5). 3-7 glomeruli per biopsy were analyzed depending on biopsies.

Generation of PodoPalld-/- and PodoPalld-R26R mice

To generate a podocyte-specific palladin knockout (PodoPalld-/-) mouse, 2.5P-Cre mice (kindly provided by Marcus Moeller[5]) and mice containing a *loxP* site flanked exon in the palladin gene were mated. The following exon in the palladin gene is flanked by *loxP* sites: GGGGTTCCCA AAGAAGTCCA GTAGAACTGC TAGAATTGCC TCTGATGAGG AGATTCAAGG CACAAAGGAT GCTGTCATCC

AAGACCTGGA ACGGAAGCTT CGCTTCAAGG AGGACCTTCT GAACAATGGC CAACCG (Ensemble Gene ID: ENSMUSG00000058056 or NCBI Gene ID: 72333). For experiments, PodoPalld-/- mice with heterozygous Cre-recombinase expression were used. Mice without Cre-recombinase expression were used as controls (PodoPalld+/+). Experiments were done with six month old male mice with C57BL/6 genetic background (at least n=3 of each group).

Additionally, PodoPalld mice were mated with R26R1M mice (kindly provided by Max-Planck Institute of Immunobiology and Epigenetics, Freiburg, Germany) containing a *lacZ* gene flanked by *loxP* sites, to verify podocyte specific Cre-recombinase expression.[51] Experiments were performed on two month old male/ female PodoPalld-R26R1M $^{(cre+/-,\ lacZ/lacZ)}$ and PodoPalld-R26R1M $^{(cre-/-,\ lacZ/lacZ)}$ mice (at least n=3 of each group). Mice were housed as described previously.[6]

Genotyping of mice was performed with Phire® Animal Tissue Direct PCR Kit (Finnzymes/Thermo Fisher Scientific) in accordance to the manufacturer's instructions using primers shown in Supplementary Table 1.

Mouse model of nephrotoxic serum-induced glomerulonephritis

Two to three month old male PodoPalld+/+ and PodoPalld-/- mice were retro-orbitally injected with 17 µl of nephrotoxic serum (NTS) per gram of body weight over two consecutive days (n=8 per group). Controls were injected with PBS (n=4 per group). Urine samples were obtained every 4 days. On day 12, mice were anesthetized with ketamine (100 mg/kg) – xylazine (10 mg/kg) and blood samples were retro-orbitally obtained.

All mice were handled in strict accordance with good animal practice as defined by the relevant national animal welfare bodies of France, and all animal work was approved by the appropriate committee of the National Institute for Health and Medical Research (INSERM) and the Pierre and Marie Curie University (Paris, France) (Agreement number: B752001).

Renal function

Urine samples were collected at days 0, 4, 8 and 12 and blood samples before sacrifice. Proteinuria was measured with a Konelab analyzer (Thermo Fisher

Scientific) and normalized to urine creatinine. Blood urine nitrogen (BUN) and plasma creatinine levels were measured with an enzymatic method (Konelab analyzer) and expressed in mM and µM, respectively.

Histology and X-gal staining

For kidney removal mice were sacrificed. The samples were dehydrated and embedded into paraffin by standard procedures. Paraffin sections (4 µm) were performed on a Leica SM 2000R (Leica Microsystems). After deparaffinization, sections were rehydrated and H&E staining was performed. Sections were mounted in Eukitt (Fluka/Sigma-Aldrich) and imaged with an Olympus BX50 microscope (Olympus Europe, Hamburg, Germany).

Samples were snap-frozen in liquid-nitrogen using Tissue-Tek (Sakura, Staufen, Germany) and cut on a Leica CM-3050-S Cryostat. Cryosections (7 µm) were used for immunofluorescence staining. For detection of β-galactosidase activity, sections of PodoPalld-R26R mice (9 µm) were fixed with 2% glutaraldehyde for 10 min at room temperature, washed in 1xPBS and and incubated in X-gal solution (1xPBS with 2 mM $MgCl_2$, 5 mM potassium ferrocyanide, 5 mM potassium ferricyanide, 1 mg/ml X-gal in DMF)[7] in a humidified chamber for 1 h at 37°C. The slides were washed in 1xPBS, counterstained with Nuclear Fast Red for 1 min.

Immunofluorescence staining and immunohistochemistry of kidney sections

After deparaffinization, sections of mouse kidneys and human biopsies were rehydrated and unmasked in citrate buffer (0.1 M, pH 6.0) by heating for 5 min in a pressure cooker.

For immunofluorescence staining sections were blocked with blocking solution (2% FBS, 2% bovine serum fraction V, 0.2% fish gelatine in PBS) and incubated with the following primary antibodies overnight at 4°C: rabbit anti-palladin (10853-1-AP, Proteintech Group; 1:150), rabbit anti-palladin622 (Palld622, 1:150), mouse anti-synaptopodin (61094, Progen Biotechnik GmbH, 1:10), rabbit anti-α-actinin-4 (0042-05, immunoGlobe Antikörpertechnik GmbH, 1:150) and guinea pig anti-nephrin (GP-N2, Progen Biotechnik GmbH, 1:100). Bound antibodies were visualized with Alexa Fluor® 488- or Cy3-conjugated secondary antibodies (Jackson Immuno

Research/ Dianova; 1:600). Additionally, nuclei were stained with 1 mg/100 ml Hoechst 33342 (Sigma-Aldrich) for 5 min.
Kidney cryosections were blocked with blocking solution and stained for F-actin using Alexa Fluor® 488-phalloidin (Thermo Fisher Scientific; 1:100). Sections were embedded in Mowiol (Carl Roth).
Images were acquired using a Leica TCS SP5 confocal laser scanning microscope (Leica Microsystems) and superresolution microscope (Elyra PS.1, Carl Zeiss Microscopy, Jena, Germany).

For immunohistochemistry (IHC), the Vectastain kit (SP-2001, Vector Laboratories, Burlingame, CA, USA) was used following manufacturer's instructions. Palladin was detected using rabbit anti-palladin antibody (10853-1-AP, Proteintech Group; 1:850). Visualization was performed with DAB substrate kit (SK-4100; Vector Laboratories) followed by nuclear staining with hematoxylin and mounting in Eukitt (Sigma-Aldrich). In controls, PBS was used instead of primary antibody.
Images were acquired using an Olympus BX50 microscope (Olympus Europe).

Glomerular morphology analysis of mouse kidney

For electron microscopy, kidneys were embedded in EPON 812 (SERVA, Heidelberg, Germany). Ultrathin sections were cut and contrasted with 5% uranyl acetate and lead citrate. All grids were examined with a LIBRA® 120 transmission electron microscope (Carl Zeiss Microscopy).

Furthermore, the frequency of glomerular abnormalities was investigated more precisely using Richardson's (Azur II/ Methylene blue) stained semithin sections of mouse kidneys. Over 50 glomeruli of PodoPalld+/+ mice and PodoPalld-/- mice were categorized into (i) glomeruli with normal morphology, (ii) dilated capillaries and (iii) affected podocytes (podocytes with cyst and enlarged sub-podocyte space).

For further evaluation of the glomerular morphology, the slit diaphragm density was used as a direct and sensitive marker for foot process effacement, as described previously.[8] Structured illumination microscopy (SIM) image stacks of 20 glomeruli in three individual mice per group were acquired using a Zeiss Elyra PS.1 system (Carl Zeiss Microscopy) equipped with a 63x (NA 1.4) oil immersion objective. Z-Stacks were recorded with a size of 2,430 x 2,430 pixel^2 (78.35x78.35 µm^2) with a slice-to-slice distance of 0.2 µm of approximately 3.5 µm using the 561 nm laser, with 2.4%

laser power and an exposure time of 100 ms. The 34 µm period grating was shifted and rotated 5 times on every frame. 3D SIM reconstruction was performed with the Zeiss ZEN Software using following parameters: Baseline Cut, SR Frequency Weighting: 1.3; Noise Filter: -5.6; Sectioning: 96, 84, 83. For automatic assessment of the slit diaphragm density, multiple areas per image stack with a plan view on the slit diaphragm were segmented and measured using a custom-made FIJI-based macro which establishment was described earlier.[8] The slit diaphragm density was stated as length of the slit diaphragm per glomerular capillary area in μm^{-1}. Means of both groups were compared using unpaired Student's *t*-test using Prism 5 (GraphPad, La Jolla, CA, USA).

Zebrafish stocks

Zebrafish of the AB background were bred in a pH- and temperature-controlled facility as previously described.[9] Zebrafish lines were grown and mated at 28.5°C and eggs were directly collected in E3 media (5 mM NaCl, 0.17 mM KCl, 0.33 mM $CaCl_2 \cdot 2H_2O$, 0.32 mM $MgSO_4 \cdot 7H_2O$). For further experiments, eggs were incubated in a TB15-incubator (Thermo Fisher Scientific) on 28.5°C/100% humidity and E3 media were replaced daily.

Injection of morpholinos

For injection of specific morpholinos (MO), the concentration of morpholinos was set to 0.5 mM with injection solution (100 mM KCl, 10 mM HEPES) and phenol red solution (Sigma-Aldrich). This mix was incubated for 5 min at 65°C and centrifuged. The supernatant was loaded to a Femtotip injection needle (Eppendorf AG) and connected to a Femtojet microinjector (Eppendorf AG) with pressure control. The morpholinos were injected in the yolk of fertilized eggs.[10]

Morpholinos were synthesized by Gene Tools LLC (Philomath, OR, USA) and the following sequences were used: control morpholinos (CtrlMO) 5'-CCTCTTACCTCAGTTACAATTTATA-3' and palladin morpholinos (PalldMO) 5'-TGTCATTCCAGCTCCCGTCCTGCAT-3'.

Staining and live cell imaging of zebrafish larvae

For fluorescence microscopy, whole mount staining of 1 day past fertilization old zebrafish larvae was performed. Larvae were fixed in methanol/ aceton (1:1)

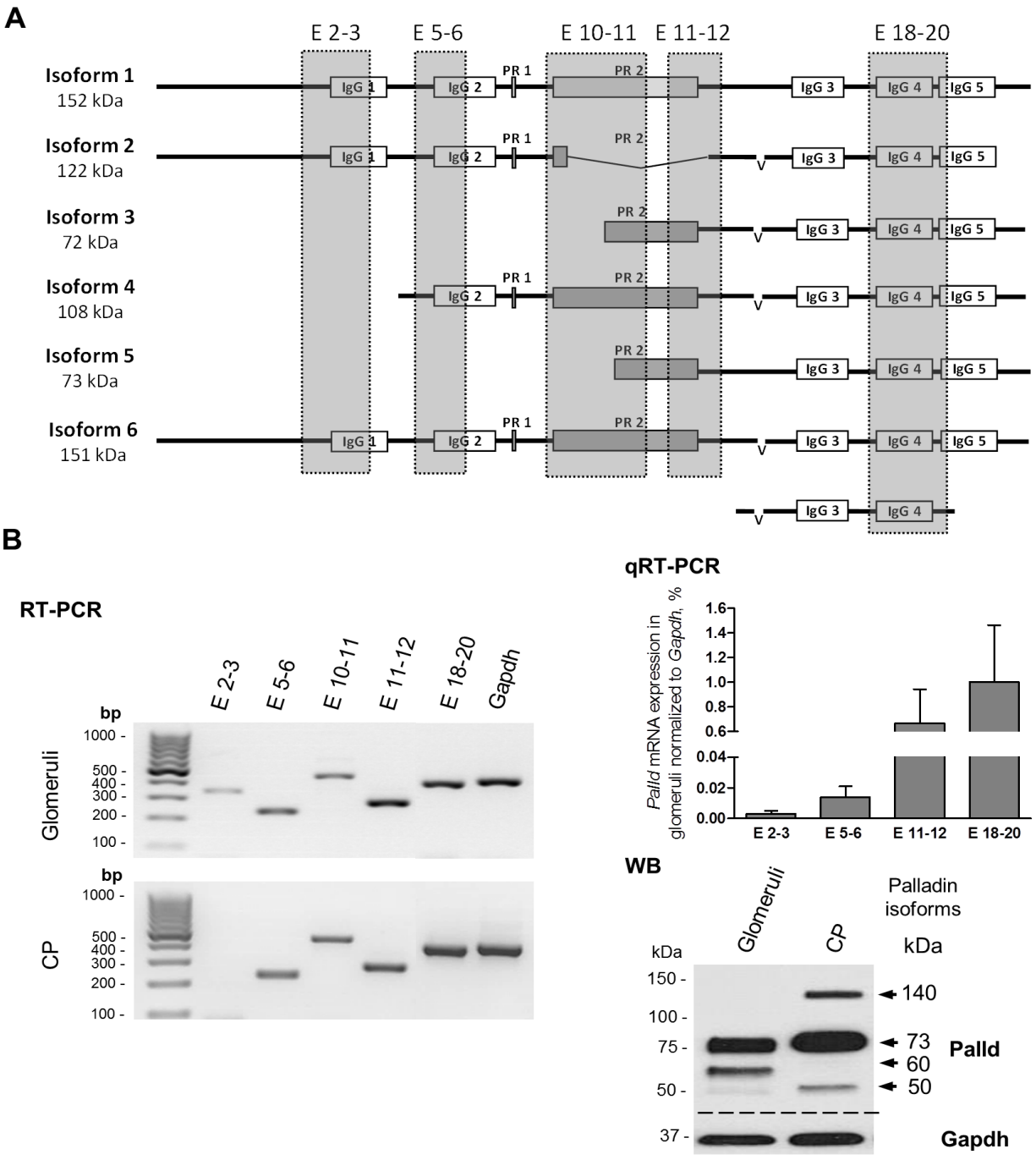
overnight on ice and permeabilized by 0.3% Triton X-100 for 30 min at 36°C. Afterwards larvae were incubated with rabbit anti-palladin622 antibody (1:300) and Alexa Fluor® 488-phalloidin (Thermo Fisher Scientific; 1:250) for 2 hours at 36°C. For palladin detection larvae were incubated with Alexa Fluor® Cy3-conjugated secondary antibody (Jackson Immuno Research; 1:500). Samples were mounted in Mowiol (Carl Roth).

The cellular migration of flat epithelial layers was investigated using 30 hpf zebrafish larvae. Images were taken every 12 min for 1 hour with Leica Application Suite Software using a Leica TCS SP5 confocal laser scanning microscope (Leica Microsystems).

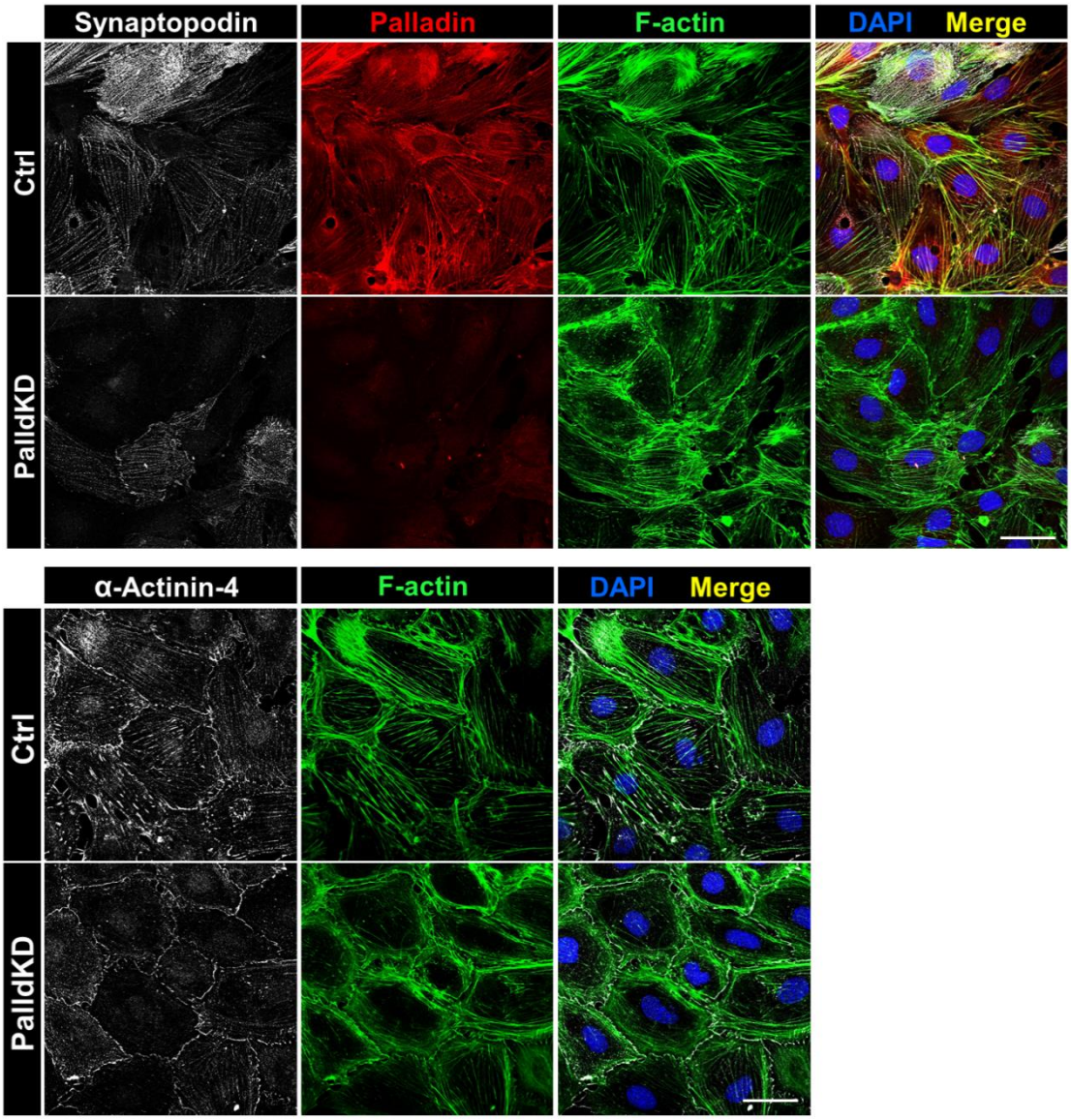
Statistical analysis

All data are given as means±SD or ±SEM, analyzed by unpaired Student's *t*-test with repeated measurements. Differences were regarded as significant at a *p*-value <0.05.

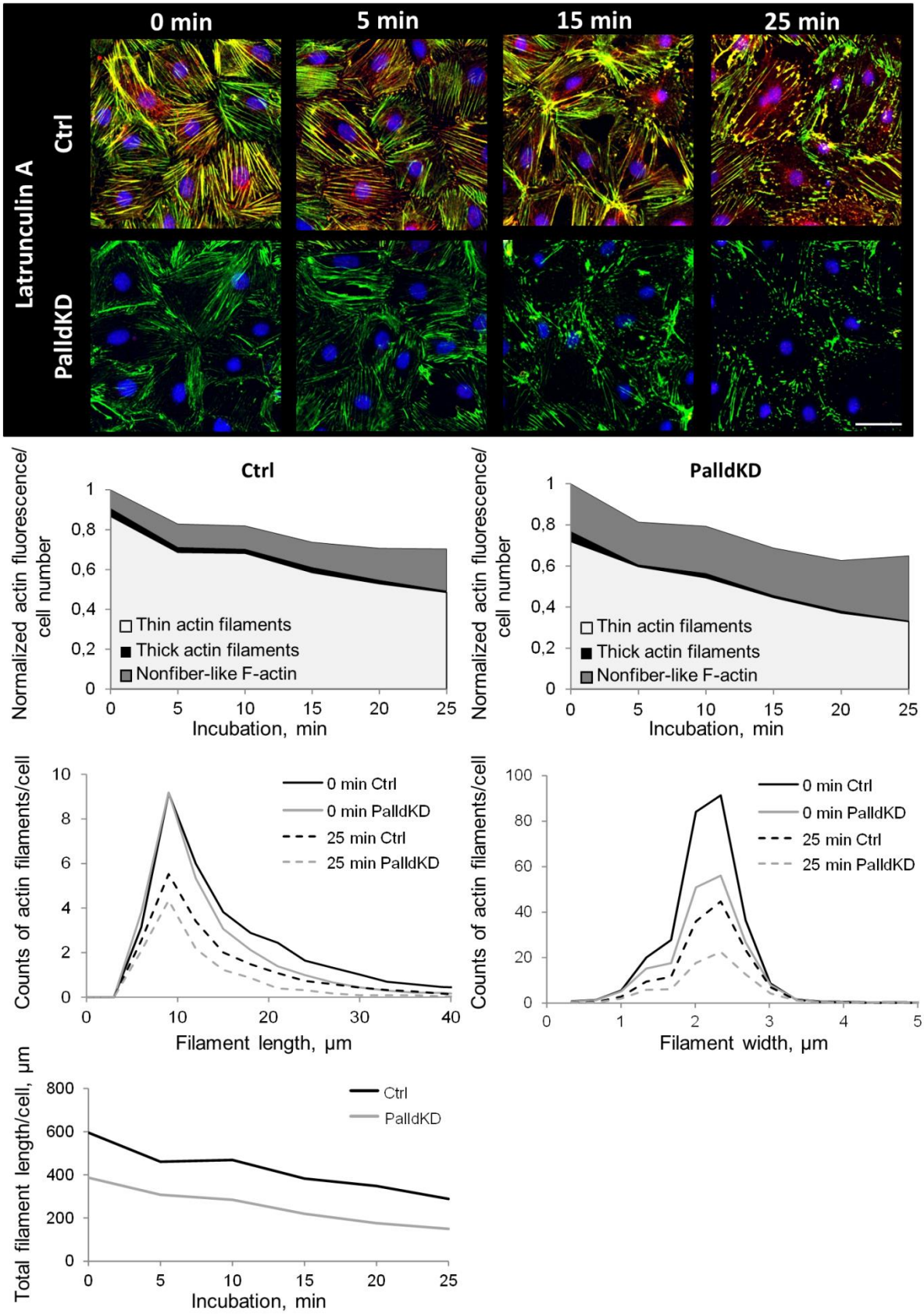
Supplementary Figures



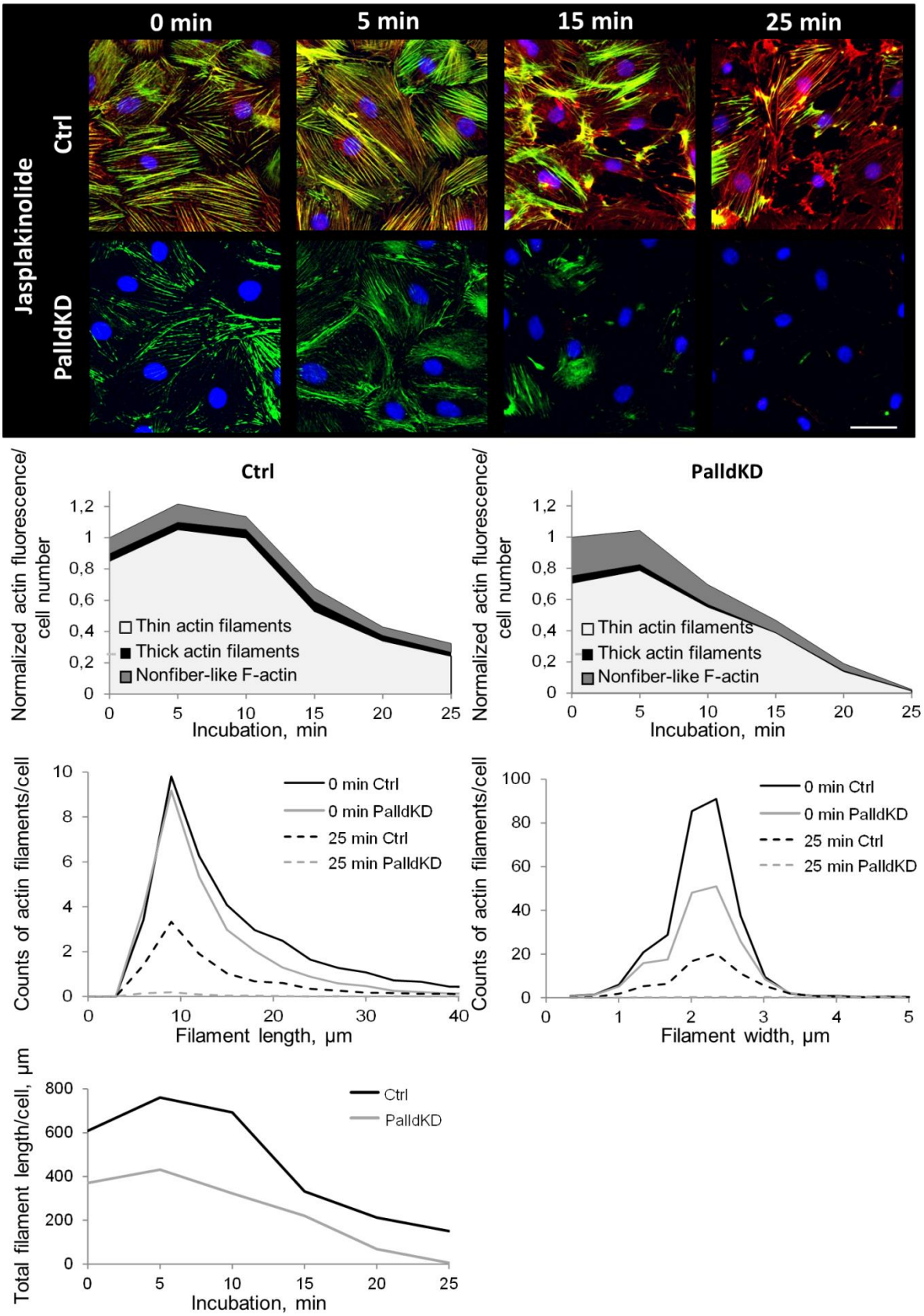
Supplementary Figure 1. Different palladin isoforms are expressed in differentiated murine podocytes. (A) The graphic is a scaled overview of existing palladin isoforms in mice (data were obtained from Uniprot ID Q9ET54 and D3Z1J5). Most of the isoforms contain proline-rich regions (PR) and all of them immunoglobulin-like domains (Ig). The Grey bars show the localization of the exon (E) spanning primer pairs detecting different regions in the palladin isoforms that were used for RT-PCR and qRT-PCR analyses in Figure 2B. (B) A signal for E 2-3 was detected in glomeruli but not in CP by RT-PCR analysis. All other primer pairs (E 5-6, E 10-11, E 11-12, E 18-20) showed specific signals in glomeruli as well as in CP. In podocytes of isolated glomeruli, we found the expression of a 73 kDa, 60 kDa and a 50 kDa isoform by Western blot (15 µg/lane). In contrast, CP expressed a 50 kDa, 73 kDa as well as a 140 kDa isoform (10 µg/lane).



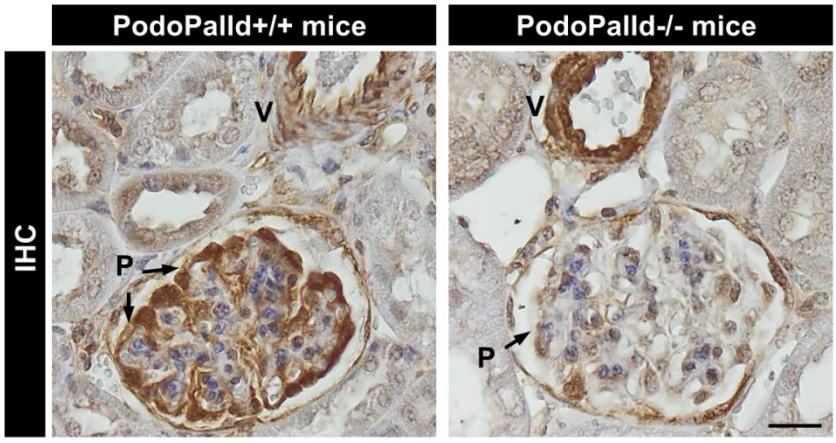
Supplementary Figure 2. Knockdown of palladin in cultured podocytes resulted in a down-regulation of synaptopodin and α-actinin-4. Cultured podocytes with PalldKD showed a weaker fluorescence signal for synaptopodin and α-actinin-4 compared to control-transfected (Ctrl) podocytes in the immunofluorescence staining. Scale bars represent 50 µm.



Supplementary Figure 3. Incubation of cultured podocytes with latrunculin A. Podocytes were incubated with latrunculin A (0.5 µM) for 25 min. Panels on top show fixed cells at different points in time, stained for F-actin (green), palladin (red) and nuclei (blue). After 25 min, PalldKD had less thin actin filaments and more nonfiber-like F-actin. Furthermore, we observed a reduction of the long and thick actin filaments as well as total filament length in PalldKD (graphics in the middle). Treatment with latrunculin A reduced the total filament length in PalldKD and Ctrl podocytes similarly (graphic on the bottom). Scale bar represents 50 µm.



Supplementary Figure 4. Incubation of cultured podocytes with jasplakinolide. Cultured mouse podocytes were incubated with jasplakinolide (0.5 µM) for 25 min and stained for F-actin (green), palladin (red) and nuclei (blue). After 25 min, PalldKD podocytes lost all actin filaments in contrast to Ctrl podocytes. Scale bar represents 50 µm.



Supplementary Figure 5. Confirmation of the palladin KO in PodoPalld-/- mice. The panels show an immunohistochemistry staining of kidney paraffinsections. PodoPalld+/+ mice exhibit strong palladin-expressing podocytes (P) and vascular smooth muscle cells (V). In contrast, the palladin signal is absent in podocytes of PodoPalld-/- mice but vessels are still positive for palladin. Scale bar represents 20 µm.

Supplementary Movies

Supplementary Movie 1. Migration of control podocytes. The migration of control podocytes into the gap of a migration chamber is followed by time lapse microscopy. Pictures were taken every 10 min over 22 hours.

Supplementary Movie 2. Migration of palladin knockdown podocytes. The migration of palladin knockdown podocytes into the gap of a migration chamber is followed by time lapse microscopy. Pictures were taken every 10 min over 22 hours.

Supplementary Movie 3. Migration of epithelial cells in CtrlMO zebrafish embryo. Epithelial cells of a CtrlMO zebrafish embryo showed a flat morphology and a directed migration was observed by time lapse microscopy. Pictures were taken every 12 min over 1 hour.

Supplementary Movie 4. Migration of epithelial cells in PalldMO zebrafish embryo. Epithelial cells of a PalldMO zebrafish embryo showed a rounded morphology and an undirected migration was observed by time lapse microscopy. Pictures were taken every 12 min over 1 hour.

Supplementary Table

Supplementary Table 1. Primer for RT-PCR and qRT-PCR

Gene	SE/ AS	Primersequence	PCR-product
cultured podocytes			
Palld	SE	5'-CTCTCGATCACGGGACAGTGGAGAT-3'	403 bp
	AS	5'-GTATCCATCAGCAACCCCCGTGTTC-3'	
Vinc	SE	5'-TTACCTCATTGACGGCTCTAGG -3'	296 bp
	AS	5'- GAACAGGAAGCAGCTCTTTGAC -3'	
Synpo	SE	5'-TCCCAGGCCTTCCTTCTC-3'	210 bp
	AS	5'-AGGGGGACATTGGTGGAG-3'	
Actn4	SE	5'-CAGCAGAAGCTGGAGGACTT-3'	244 bp
	AS	5'-GTTCCAGCCTACGGATTTCA-3'	
Itgb1	SE	5'- CAACTGGTTTCCTGGATTGG-3'	253 bp
	AS	5'-TTTGAGAGCCTCTGGGATTC-3'	
Tln1	SE	5'-AAGGCTCTCTGTGGCTTCAC-3'	147 bp
	AS	5'-CTGACAGGCCATCTGAATTG-3'	
Actb	SE	5'-TGGCTCCTAGCACCATGAAG-3'	193 bp
	AS	5'-AACGCAGCTCAGTAACAGTCC-3'	
Rac1	SE	5'-CTGCCAATGTTATGGTAGATGG-3'	153 bp
	AS	5'-TTTCAAATGATGCAGGACTCAC-3'	
Nphs1	SE	5'-GCCACCACCTTCACACTGAC-3'	233 bp
	AS	5'-AGACCACCAACCGCAAAGAG-3'	
Gapdh	SE	5'-GAAGCCCATCACCATCTTCCAGGAG-3'	420 bp
	AS	5'-GTGGATGCAGGGATGATGTTCTGGG-3'	
palladin isoforms			
Palld exon 2	SE	5'-CTGGAGATGGACGCAGAAGTCAAGC-3'	334 bp
Palld exon 3	AS	5'-CCTCAAAGGCCTCAGCAATGACCAA-3'	
Palld exon 5	SE	5'-CTGTGATTCAGCCCCTATCTGT-3'	230 bp
Palld exon 7	AS	5'-CGGAGAGTCTTGGATCTCACTT-3'	
Palld exon 10	SE	5'-GCATGGCAGCTCTTCAAATGCA-3'	439 bp
Palld exon 11	AS	5'-GCGTAGTTGAAGGAGCCGGA-3'	
Palld exon 11	SE	5'-ACCTAGCTCCACCTCACACTGC-3'	276 bp
Palld exon 12	AS	5'-TGGCCATTGTTCAGAAGGTCCT-3'	
Palld exon 18	SE	5'-CTCTCGATCACGGGACAGTGGAGAT-3'	403 bp
Palld exon 20	AS	5'-GTATCCATCAGCAACCCCCGTGTTC-3'	
(based on palladin Transcript ID ENSMUST00000121785.8 or RefSeq ID NM_001293772.1)			
zebrafish larvae			
palld	*SE*	5'-CCGCATCCTACAGAAGAAGC-3'	128 bp
	AS	5'-CCGAGCCATATTCGTTTGTT-3'	
eef1a1l1	SE	5'-CTGGAGGCCAGCTCAAACAT-3'	87 bp
	AS	5'-ATCAAGAAGAGTAGTACCGCTAGCATTAC-3'	

genotyping mice

Cre[11]	SE	5'-GCATTACCGGTCGATGCAACGAGTGAT-GAG-3'	408 bp
	AS	5'-GAGTGAACGAACCTGGTCGAAATCAGTGCG-3'	
Pod-Cre	SE	5'-GGTTGGCACCCCTCTAGCATGACATTAGGA-3'	~500 bp
	AS	5'-TCATCACTCGTTGCATCGACCGGTAATGCA-3'	
(kindly provided by Dr. Marcus J. Moeller, Germany)			
Palld_PLGP3	SE	5'-GCTTCGCTTCAAGGAGGACCTTCTG-3'	~500 bp
Palld_PLGP4	AS	5'-TGTATATCATGTTGTGGTGTCAGCC-3'	
R26R[12]	SE	5'-AAAGTCGCTCTGAGTTGTTAT-3'	~300 bp
	AS	5'-GCGAAGAGTTTGTCCTCAACC-3'	

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