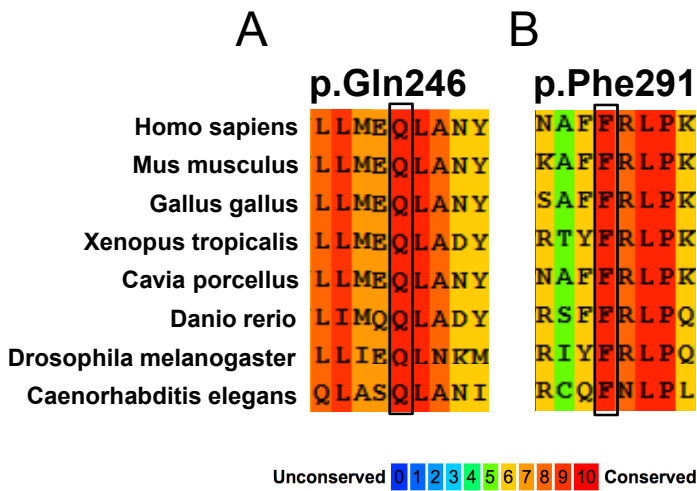


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## Supplemental Data

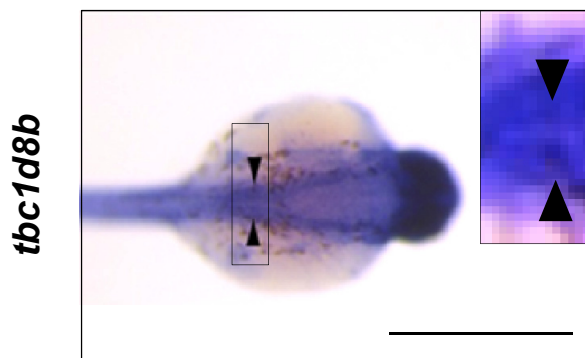
### ***TBC1D8B* Loss-of-Function Mutations Lead to X-Linked Nephrotic Syndrome via Defective Trafficking Pathways**

**Guillaume Dorval, Valeryia Kuzmuk, Olivier Gribouval, Gavin I. Welsh, Agnieszka Bierzynska, Alain Schmitt, Stéphanie Miserey-Lenkei, Ania Koziell, Shuman Haq, Alexandre Benmerah, Géraldine Mollet, Olivia Boyer, Moin A. Saleem, and Corinne Antignac**



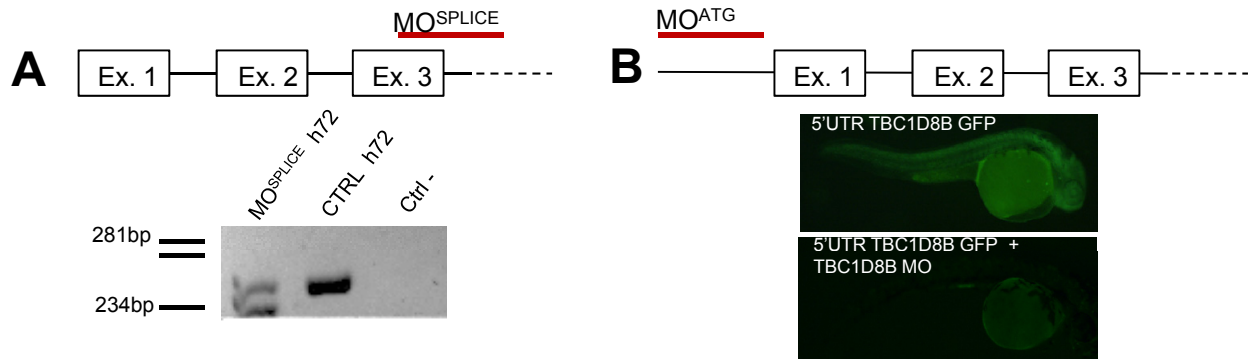
### Figure S1- TBC1D8B amino-acids conservation

Amino-acids alignments of p.Gln246 and p.Phe291 revealed conservation of both amino-acids from *C. elegans*.



### Figure S2 – *tbc1d8b* in situ hybridization in zebrafish larvae

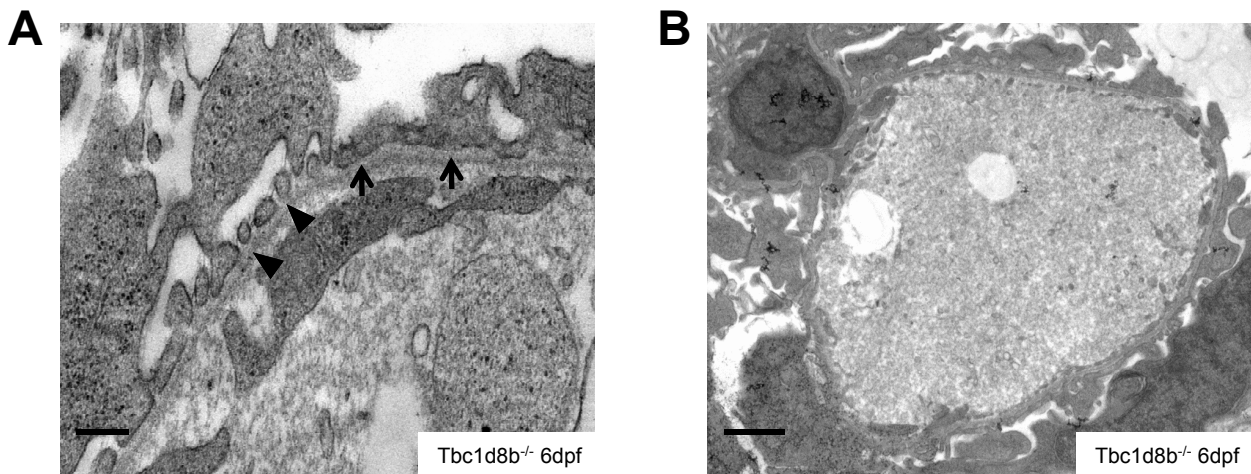
*In situ* hybridization experiments revealed expression in the brain, neural tubes, pectoral fins and the pronephric glomeruli at 24hpf. Square shows pronephric glomeruli at higher magnification. Black arrowheads indicate unfused glomeruli on each side of median line. Scale bar 500µm.



**Figure S3 - Verification of both initiation blocking and splice MO specificity**

(A) PCR from  $MO^{SPLICE}$  cDNA specimen showed an extra-band at 224bp compared to WT cDNA.

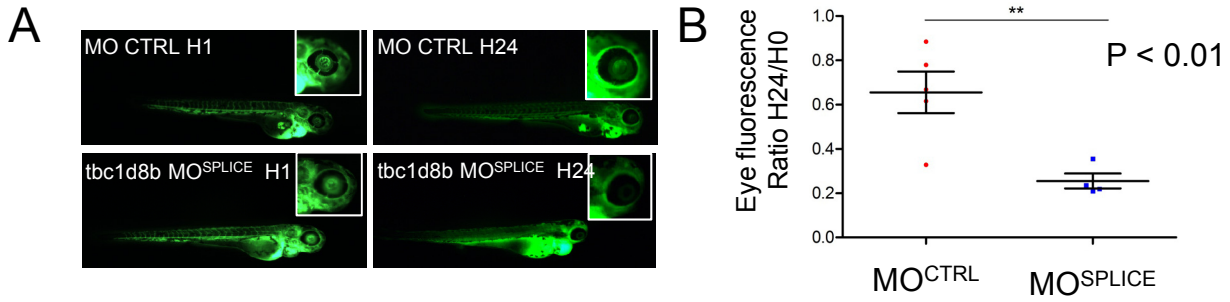
(B) Embryos injected with  $MO^{ATG}$  and its corresponding sequence mRNA fused to GFP showed a significant decreased in fluorescence compared to embryos injected with mRNA only.



**Figure S4 - Electron microscopy of zebrafish glomeruli**

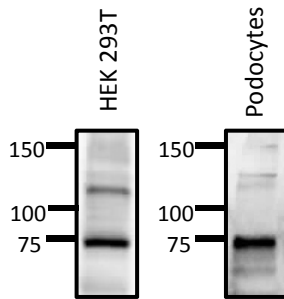
Electron microscopy revealed at high magnification (A-5000X) an alternance of regular foot processes (arrowheads) and foot process effacement (arrows). This architecture is found all around glomerular basement membrane at lower magnification (B- 1500X).

Scale bar = 0.2 $\mu$ m



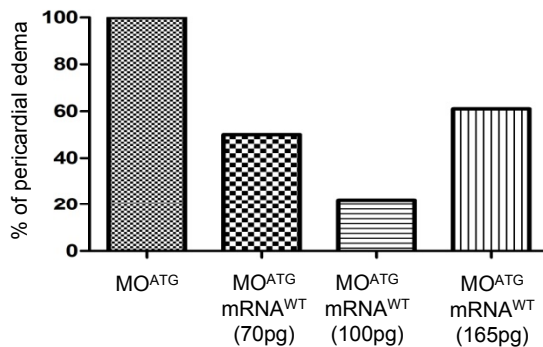
### Figure S5 – Dye filtration eye assay in zebrafish

Measurements of fluorescence in retinal vein of WT and morphants zebrafish revealed a significant decreased in morphants, suggesting a pathological protein loss by fish (\*\*,  $p < 0.01$ ).



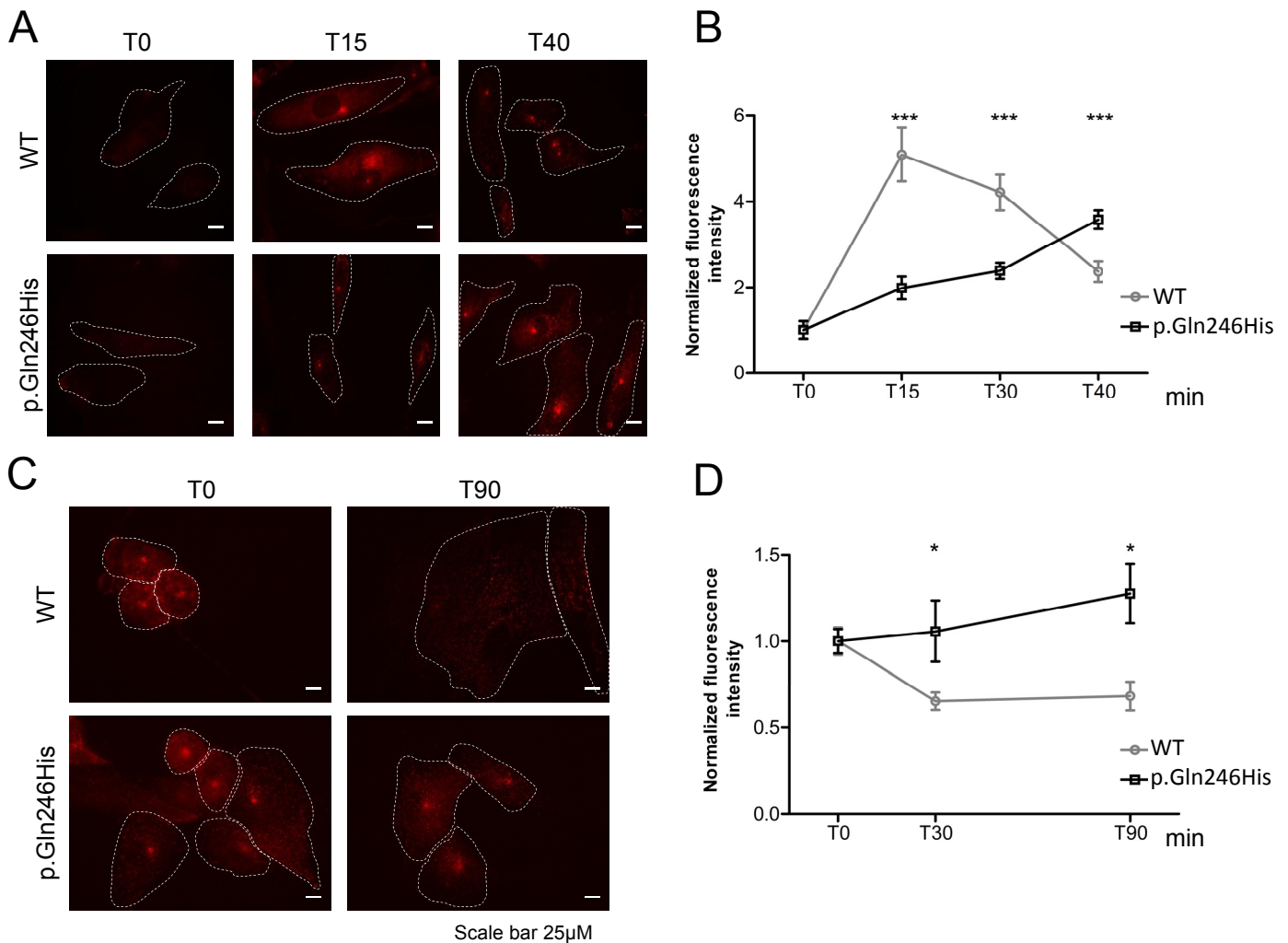
### Figure S6 -TBC1D8B expression in cultured podocytes and KEK293T cells

Western-blot of TBC1D8B revealed expression of a band around 75kDa in cultured differentiated podocytes, demonstrating the expression of the isoform 2 expected to migrate at 73kDa. In HEK293T cells, both isoforms were present even if the shorter one seems to be predominant (not quantified).



### Figure S7 – Dose-response effect of TBC1D8B human mRNA injection in morphants zebrafish

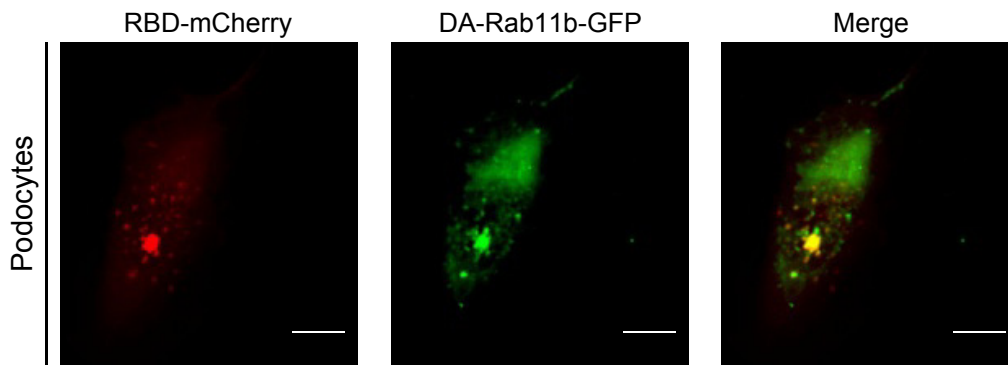
Injection of TBC1D8B WT mRNA in zebrafish morphants induced a dose-dependent response. Dose > 100pg induced embryo toxicity with aberrant phenotype such as cyclops.



**Figure S8- Transferrin assay in a i HUX fibroblasts harboring the p.Gln246His mutation and control**

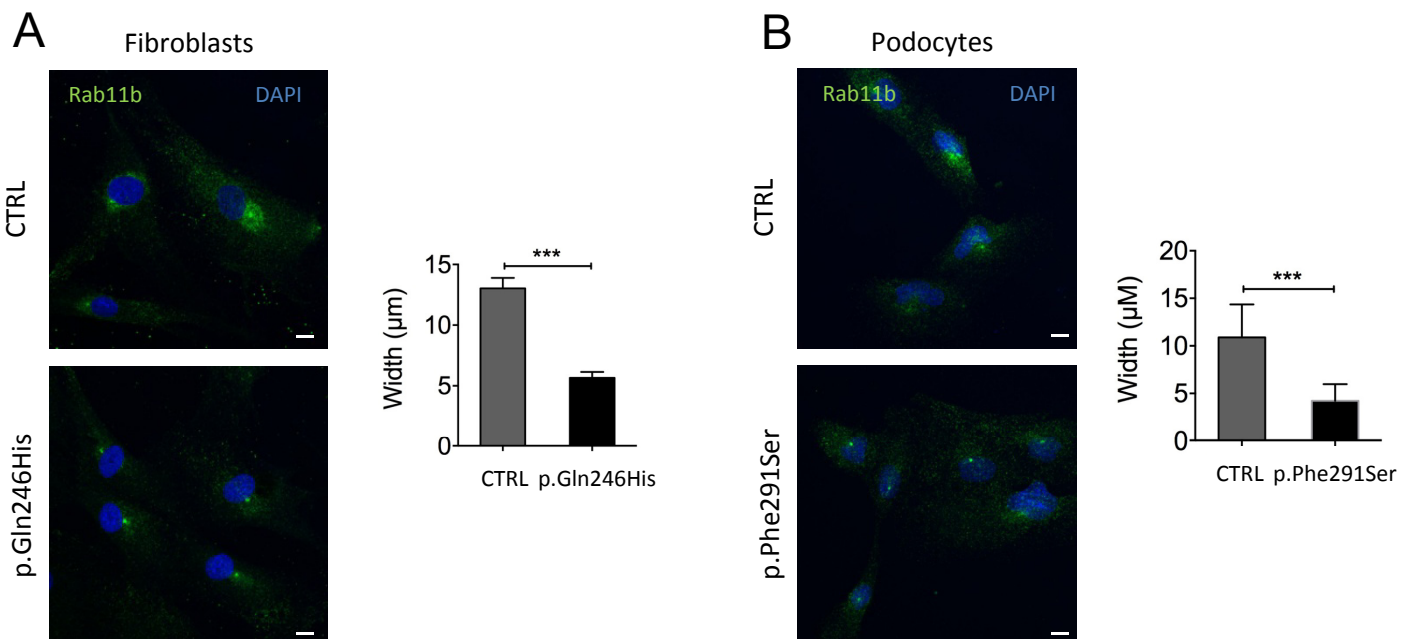
A-B After incubation with fluorescent labeled transferrin, intensity rapidly increased in control fibroblasts whereas fluorescence increasing was significantly slower in mutated cells harboring the p.Gln246His mutation. Graph B represents the quantification of normalized fluorescence in both mutated and control cells (\*\*\*, $p < 0.001$ , mean $\pm$ SEM). (Scale bar 25 $\mu$ m)

C-D Measuring of labeled-transferrin recycling in WT and p.Gln246His fibroblasts revealed a maintenance of fluorescence 90min after internalization in mutant cells while it was almost negative in control podocytes (\*\*\*, $p < 0.01$  at 90min, mean $\pm$ SEM). (Scale bar 25 $\mu$ m)



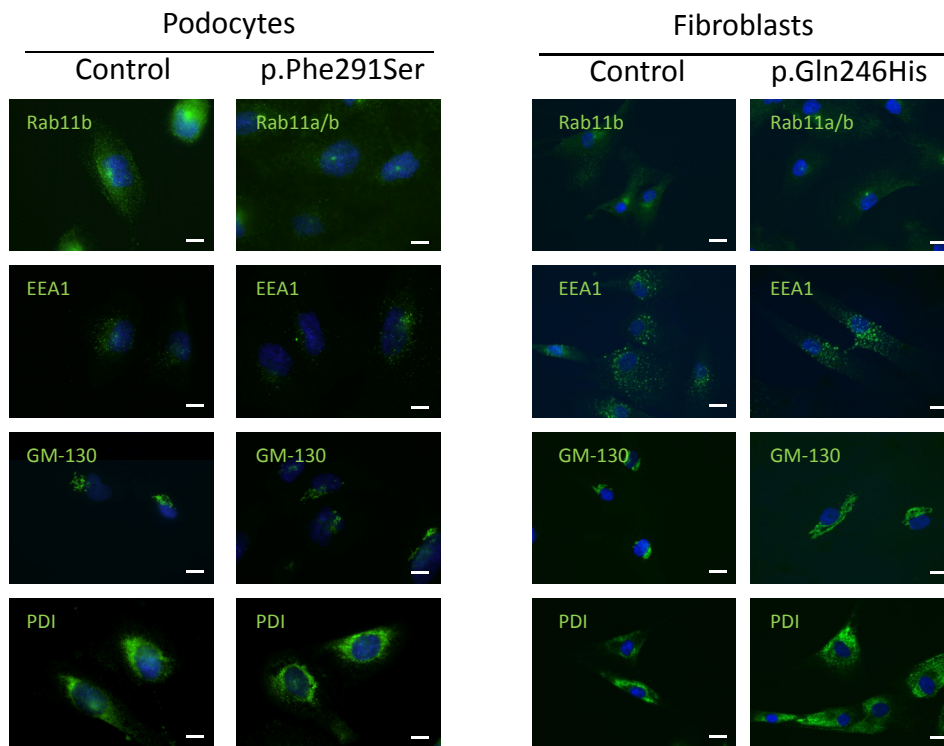
**Figure S9- Cellular localization of DA-Rab11b and the Rab-Binding-Domain (RBD) of TBC1D8B in podocytes**

In co-transfected podocytes, RBD-mCherry showed a partial colocalization with DA-Rab11b-GFP at the perinuclear recycling compartment. Scale bar 10 $\mu$ M



**Figure S10- Cellular localization of endogenous Rab11b**

In mutant fibroblasts and podocytes, Rab11b exhibits a restricted localization to the perinuclear compartment compared to WT cells. Scale bar 10 $\mu$ M



**Figure S11 – Immunofluorescence staining of cellular compartment in *UZZWYX* [bX]j [Xi Ug and controls**

In *UZZWYX* and control fibroblasts and podocytes, only Rab11b (on the top line) exhibited a particular phenotype described in the figure S10. Staining of early endosomes (EEA1), golgi (GM-130) or endoplasmic reticulum (PDI) are the same in *UZZWYX* and controls.

<b>Primers</b>	<b>Sequence</b>
TBC1D8B Rab Binding Domain F for pCS2-GFP	TAGGGATCCGCCACCATGGGACGTGGTGTAGTAGT TGTTTCG
TBC1D8B Rab Binding Domain R for pCS2-GFP	TAGGAATTCCGGTCAAAGAACCTGTTTAAGGCTGT C
TBC1D8B Rab Binding Domain F for pmCherry-NI	TAGGGTACCGCCACCATGGGACGTGGTGTAGTAGT TGTTTCG
TBC1D8B Rab Binding Domain R for pmCherry-NI	ATGGGATCCAGGTCAAAGAACCTGTTTAAGGCTGT C
Rab11b F for pCS2-GFP	TAGGGATCCGCCACCATGATGGGGACCCGGGACG ACG
Rab11b R for pCS2-GFP	TAGGAATTCCGCAGGTTCTGGCAGCACTGCA
MO <sup>SPLICE</sup> F	GCTTGCTGGTCCGGAACCTTGG
MO <sup>SPLICE</sup> R	AACAGGCGTTCAAAGCGAAGC
<b>Site-directed mutagenesis primers</b>	<b>Sequence</b>
pCS2-Rab11b-GFP F - c.209A>T - Gln70Leu	CACCGCTGGCCTGGAGCGCTACC
pCS2-Rab11b-GFP R - c.209A>T - Gln70Leu	GGTAGCGCTCCAGGCCAGCGGTG
pCR-TBC1D8B full F - c.738G>C - Gln246His	TAATGGCATAGTTTGCCAGGTGTTCCATAAGAAGG TATG
pCR-TBC1D8B full R - c.738G>C - Gln246His	CATACCTTCTTATGGAACACCTGGCAAACCTATGCC ATTA
pCR-TBC1D8B full F - c.872T>C - Phe291Ser	CTTTGGGCAGCCTAGAAAAGGCATTAATGCTCA CTGTGG
pCR-TBC1D8B full R - c.872T>C - Phe291Ser	CCACAGTGAGCAATTTAATGCCTTTTCTAGGCTGC CCAAAG
<b>Primers for WISH probes</b>	<b>Sequence</b>
Tbc1d8b F WISH1	GTTTGGTTTGCCGCAGAGAG
Tbc1d8b R WISH1	AATTAACCCTCACTAAAGGTCTCTGGAACCTCCTCG CAC
Tbc1d8b F WISH2	ACGGGAATATCAGCTCTGCG
Tbc1d8b R WISH2	TAATACGACTCACTATAGGGAAGATGGAAGGCTGA CCGTG
Tbc1d8b genotyping F	GCATCTTGCCATTGCTGATATTG
Tbc1d8b genotyping R	CAAGCCAACAGACATCTGTGAACC

**Table S1 – List of primers used for experiments**



## Methods

### ES and Mutation Calling

ES of DNA from families was performed using the Agilent SureSelect All Exon 51Mb V5 capture-kit and HiSeq2500 (Illumina) sequencer (paired-end reads: 2x75 bases). Obtained sequences were aligned to the human genome (National Center for Biotechnology build 37/hg19) using the Lifescope suite from Life Technologies. Substitution and variation calls were made with the Genome Analysis Toolkit pipeline. Variants were then annotated using the Paris Descartes University Bioinformatics platform software system. We assumed the causal variant (1) segregates with disease status; (2) is novel or has a minor allele frequency <1/1000 in dbSNP, 1000 Genome Project, gnomAD; (3) is not found in >10/2049 projects from our in-house database and (4) alters a protein's amino-acid sequence and is likely to disrupt the function of that protein according to PolyPhen2, SIFT, and MutationTaster.

### Plasmids and cell culture

Human TBC1D8B ORF was obtained from Source BioScience (IRCMp5012H0832D) in a pCR-blunt II TOPO backbone (pCR-TBC1D8B-full). The Rab-Binding-Domain (aa1392 to 1412) of TBC1D8B was amplified using specific primers (Table S1) and then subcloned inframe into *Bam*HI and *Eco*RI sites of plasmid pCS2-GFP (pCS2-RBD-GFP), into *Kpn*I and *Bam*HI sites of plasmids pmCherry-N1 (Clontech) (pN1-RBD-mcherry) and pCDNA3.1(+)/Myc-His C from Thermofischer (pCDNA-RBD-Myc). Zebrafish *tbc1d8b* 5'UTR and the 73 first bases of the cDNA were subcloned inframe into *Bam*HI and *Xho*I sites of pCS2-GFP (PCS2-5'UTR-GFP) using primers listed in the table S1. Rab11b cDNA was amplified from a human podocyte cell line<sup>1</sup> using specific primers (Table S1) and then subcloned into *Bam*HI and *Eco*RI sites of the pCS2-GFP plasmid (pCS2-Rab11b-GFP). All plasmids pDest-CA-rab5-GFP and pDest-CA-rab11a-GFP corresponding to the constitutively active form of each Rab were a kind gift from Dr. Stéphanie Miserey-Lenkei. Site-directed mutagenesis (QuikChange kit, Stratagene) was used to generate the missense mutations used in this study: p.Gln246His and p.Phe291Ser for TBC1D8B, and p.Gln70Leu for Rab11b to obtain the constitutively active form of Rab11b. All constructs were verified by Sanger sequencing.Human.

Embryonic Kidney 293T (HEK293T) cells were maintained in Dulbecco's Modified Eagle Medium (DMEM, Gibco®, Life Technologies) supplemented with 10% fetal bovine serum (FBS), glutamine and penicillin/streptomycin. Fibroblasts obtained from skin biopsies from affected and control individuals were cultured in OptiMEM (Gibco®, Life Technologies) supplemented with 10% FBS, glutamine, penicillin/streptomycin. Conditionally immortalized human podocytes (Saleem MA *et al.* 2002)<sup>1</sup> were

routinely cultured at 33°C in RPMI-1640 (Gibco<sup>®</sup>, Life Technologies), supplemented with 10% FBS, 1% insulin/transferrin/selenium, glutamine and penicillin/streptomycin (all from Life Technologies), and then, for experiments, cells were thermoswitched to 37°C for 10-14 days differentiation. Cells used in this study were tested mycoplasma-free.

### **Antibodies, reagents and chemical compounds**

The following antibodies were used in this study: mouse monoclonal antibodies (mAb) against GFP [Roche, Cat. No. 118144600001, used at 1/2000 for western blot (WB)], c-myc epitope (Thermo Fisher, MS-139-P1, used at 1/1000 for WB), and synaptopodin [Progen, Cat. No. 65194, used at 1/40 for immunofluorescence (IF)], and rabbit polyclonal antibodies against TBC1D8B (Abcam, ab121780, used at 1/50 for IF and ab179995, used at 1/1000 for WB) and Rab11b (Atlas Antibodies HPA054396, used at 1/200 for IF). Alexa-fluor 647 Phalloidin from ThermoFischer was used for IF (catA22287). For IF, Alexa Fluor-conjugated secondary antibodies (donkey anti-rabbit Alexa Fluor 488 and anti-mouse Alexa Fluor 546) were obtained from Life Technologies (ThermoFischer). Alexa-Fluor 555- or 546-conjugated transferrin (Life Technologies, Cat.No T35352 and T23364, respectively, used at 25µg/mL) was used for trafficking experiments.

### **Immunoprecipitation and immunoblotting**

HEK293T cells were transiently co-transfected with pcDNA3.1-RBD-myc and either pDest-CA-Rab5/11a-GFP or pCS2-CA-Rab11b-GFP using Lipofectamine Reagent 2000 (ThermoFischer Scientific) according to the manufacturer instructions. Forty-eight hours post transfection, cells were lysed in 50 mM Tris-HCl pH8, 150 mM NaCl, 1% NP-40 supplemented with 10 mM protease inhibitor cocktail (Sigma) and incubated for 20 min at 4 °C. After centrifugation (10min, 10 000 rpm, 4°C), protein dosage was performed using the BCA protein assay kit (Thermo Scientific). The c-myc tag was immunoprecipitated using the µMACS<sup>™</sup> Epitope Tag Protein Isolation Kit (Miltenyi Biotec). Briefly, fresh lysates (1mg of protein) were incubated with magnetic beads coupled to a myc antibody. For immunoprecipitation of endogenous TBC1D8B, HEK293T lysates were incubated with rabbit anti-TBC1D8B antibodies (1µg), followed by a 30-min incubation with magnetic beads-coupled to protein A. Immunoprecipitated proteins were isolated using µMACS<sup>™</sup> Separation Columns in a magnetic µMACS<sup>™</sup> separator and subsequently eluted with 1X Laemmli buffer. Lysates (50µg) and immunoprecipitated samples were loaded on a 8-16 % Mini-PROTEAN<sup>®</sup> TGX<sup>™</sup> precast gel (BioRAD) and transferred to a PVDF membrane (GE Healthcare). Immunoblotting was performed using the indicated primary antibodies overnight at 4°C and the secondary antibodies for 1 hour at room temperature. Signals were detected using ECL reagents (Amersham Biosciences) and acquired with a Fusion Fx7 imaging system (Vilber Lourmat).

### **Immunofluorescence and vesicular trafficking studies**

Podocytes were grown on type I collagen-coated coverslips and either used to study the subcellular localization of endogenous TBC1D8B, or transiently transfected the next day using FuGENE® HD Transfection Reagent (Promega) or used for endocytosis and recycling experiments. Cells were fixed in 4% paraformaldehyde (PFA) for 20 min at room temperature and alternatively permeabilized with 0.3% Triton TX-100 (Sigma) in 1X PBS for 4 min (for endogenous TBC1D8B) or directly incubated in blocking buffer containing 5% bovine serum albumin (BSA, Sigma) for 1h at room temperature. Cells were then incubated in the appropriate primary and secondary antibodies for 1h each at room temperature. Nuclei were stained with Hoechst (#33342, Sigma) at room temperature for 8 min.

For transferrin endocytosis (uptake) / recycling (chase), we performed two different analyses:

For endocytosis study (transferrin uptake), fibroblasts were starved at 37°C for 60 min in transferrin-free OptiMEM medium supplemented with 1% Bovine Serum Albumin (BSA) and podocytes were starved in Serum-Free-Medium devoid of transferrin (SFM, Gibco®, Life Technologies) with 1% Bovine Serum Albumin (BSA) for 60 min. Cells were then incubated on ice at 4°C in the same ice-cold medium enriched with Alexa-Fluor (AF-555)-conjugated transferrin at a final concentration of 25µg/mL. This step allows transferrin to bind its receptor but inhibit internalization. After 30 min incubation, cells were washed in cold 1X PBS. Cells were then incubated at 37°C in their respective medium (with transferrin) to allow transferrin internalization, and blocked in 4% PFA at different time points. Mutated cells were compared to control cells for each time point.

For recycling study (chase study), cells were incubated in Alexa-conjugated transferrin-enriched appropriate medium (25µg/mL) supplemented with 1% BSA for 60min at room temperature for fibroblasts and 120min for podocytes. Cells were then washed with 1X PBS, incubated in transferrin-free medium and then fixed in 4% PFA at different time points. For endocytosis and recycling, all time points' results were normalized to T0.

As transferrin endocytosis / recycling kinetics are dependent on cell types, the use of different time points for transferrin endocytosis and chase in podocytes vs fibroblasts were based on the manufacturer instructions (30 to 60 min for endocytosis) and literature when available<sup>1,2,3</sup>, and empirically on our personal lab experience. Experiments were repeated 3 times and at least 20 cells were analyzed in each time point experiment. Microscope settings were the same within each independent experiment. The integrated fluorescence intensity in each independent cell was measured and the background fluorescence intensity was subtracted to calculate corrected total cell

fluorescence (CTCF) using the following equation  $CTCF = \text{integrated density} - (\text{area of selected cell} \times \text{mean background fluorescence per unit area})$ . All time point values were normalized to T0 (maximum intensity). Whereas microscope settings allow to easily detecting a 50% decrease of fluorescence in cells, they were imprecise for lower values. Thus recycling assay was stopped when the fluorescence intensity in WT cells reached 50% of the intensity measured at T0.

Immunofluorescence studies of endogenous TBC1D8B were performed as described above on 10  $\mu\text{m}$ -frozen sections of human embryonic kidney biopsies obtained from The Department of Pathology of Necker-Enfants Malades Hospital (Paris, France).

### **Scratch assay and adhesion assay**

This was performed as previously described by Vollenbroeker *et al.*, 2009<sup>2</sup>. In brief, confluent differentiated human podocytes were cultured in six-well plates and scratched with two lines at 90° angle with a sterile 0.4-mm 200- $\mu\text{l}$  Gilson style extension length tip. Images of the same field were captured at 0 and 12 hours.

Medium was removed, and cells were trypsinized until all cells were suspended, which was optically controlled. Cells were plated into 96-well plates and three experimental wells were assigned as the 100% attachment control, to which 20%, 50% and 100% of the total volume of cells were added. Cells were left to adhere for 45 min at 37°C. Control wells for 100% attachment were fixed with 100  $\mu\text{l}$  4% PFA for 20 min at room temperature. Thereafter, the plate was tapped to remove loose and non-adherent podocytes, and washed twice with 100  $\mu\text{l}$  PBS, and the experimental wells were then fixed with 100  $\mu\text{l}$  4% PFA for 20 min. Cells were stained with 100  $\mu\text{l}$  of 0.1% crystal violet in 2% ethanol for 60 minutes at room temperature. Absorbance on the plate was measured at 570nm in a plate reader. Results were depicted as a percentage of 100% adherent cells and normalized against the adhesion of the human wild type podocytes.

### **Zebrafish**

#### **Breeding and embryo collection**

Adult zebrafish were maintained in system water at 28 °C, pH 7 and conductivity of 500 $\mu\text{S}$  on a 14 h light/10 h dark cycle. They were bred by natural crosses and embryos were maintained at 28 °C in embryo medium (0.1 g/L Instant Ocean Sea Salts, 0.1 g/L sodium bicarbonate, 0.19 g/L calcium sulphate, 0.2 mg/L methylene blue, H<sub>2</sub>O) until the desired developmental stage was reached.

## Morpholinos

To knockdown the expression of *tbc1d8b*, *tbc1d8b* splice-blocking morpholino (MO<sup>SPLICE</sup>-GAGCAAATAACCATCTCACCTTA) targeting the exon3-intron3 boundary, and translation-blocking morpholino (MO<sup>ATG</sup>-TTGAGCCACATGCTGGAATTTGTCT) targeting the ATG codon were designed and ordered from GeneTools, LCC. The following mismatch control morpholino (MO<sup>CONTROL</sup>-CTAGGACGGACACGTGTTACCCAGG) was used as a negative control. Dose-response experiments were performed to determine the optimum concentration of morpholino which produced no toxic effects. A 8 ng/embryo dose of the *tbc1d8b* splice morpholino yielded a reproducible phenotype, whereas injection of the control did not result in any detectable phenotype at the same dose. A dose of 1.6 ng/embryo was determined for the *tbc1d8b* translation-blocking morpholino. The morpholinos were injected into embryos at the 1–2 cell stage with phenol red as a vehicle to visualise injections. After microinjection, embryos were maintained as described above. Embryo development was evaluated at 24 hpf, 48 hpf, 72 hpf and 96hpf.

Splice morpholino specificity was checked by RT-PCR. Briefly, mRNA was isolated using Qiagen Extraction Kit and then treated with DNase I. Five micrograms of total RNA was reverse-transcribed using Superscript II (Life Technologies) with oligo(dT) primers. PCR was the performed using the primers listed in Table S1.

For translation-blocking morpholino specificity check, mRNA was synthesized from pCS2-5'UTR-GFP using the SP6 mMessage mMachine Kit from Thermo Fisher. Briefly, 5 µg of plasmid were linearized (*NotI*) and purified in 15µL H<sub>2</sub>O. 6 µL were used to synthesize mRNA. After synthesis, mRNA yield was measured using a nanodrop technology. MO<sup>ATG</sup> specificity was checked by co-injection of the newly synthesized mRNA with the MO<sup>ATG</sup>. The absence of GFP confirmed the efficacy of MO<sup>ATG</sup>.

## Whole-mount *in situ* hybridization (WISH)

WISH probes were synthesized using specific primers listed in the table S1. T3 enzyme was used for *tbc1d8b* transcription and digoxigenin labeling. For whole-mount *in situ* hybridization, embryos were fixed in PFA 4% overnight at 4 °C and processed as described in Thisse and Thisse, 2008{Thisse, 2008 #506}.

## Proteinuria analyses

For proteinuria screening, embryos were injected at 96hpf directly in the cardinal vein with both a FITC-labeled 500kDa and Texas-Red-labeled 10kDa dextrans (20mg/mL each - 4nL).

For the eye dye filtration assay, fluorescence was measured in retinal vein at 1hpi and 24hpi. Normalized CTCF were calculated as described previously and compared for each condition.

For tubular assay, fish were fixed 6hours after dextran injection in 4% PFA and embedded in technovit® 7100 from EMS (#14653) according to the manufacturer protocol.

### **Rescue experiments**

mRNA was synthesized from pCR-TBC1D8B-full WT and mutated after *BamHI* restriction using the T7 promoter. Briefly, 1µg of linearized plasmid was used for mRNA synthesis using the mMessage mMachine Kit from ThermoFischer. Each newly synthesized mRNA was then titrated and co-injected with MO<sup>ATG</sup> and MO<sup>SPLICE</sup> in the cytoplasm of 1 cell-stage embryos and phenotype was screened at day 4.

### **Transmission electron microscopy and Imaging**

For transmission electron microscopy, human samples were washed three times in 0.1 M cacodylate buffer at 4°C and postfixed in 1% osmium tetroxide in 0.1 M cacodylate buffer before dehydration with ethanol. This was followed by embedding them in Araldite resin. Zebrafish embryos were fixed in 4% PFA and 4% glutaraldehyde in PBS overnight at 4°C, and then dehydrated through a graded ethanol series. Samples were embedded in epoxy resin (Electron Microscopy Sciences). For all samples ultra-thin sections (50-90 nm) were obtained using a Reichert Ultracut S (Leica, Germany), and these were placed on 75-mesh grids (Electron Microscopy Sciences) and conventionally stained with uranyl acetate and lead citrate (Electron Microscopy Sciences). Observations and images were collected using a JEOL 1011 transmission electron microscope operating at 80 kV and equipped with a GATAN Orius camera 1000 for zebrafish and digital micrographs were taken on a Philips 100CS microscope for human samples.

Depending on the experiments performed on human or zebrafish tissue, samples were examined with different microscopes: a Leica M165FC stereoscope, an epi-illumination microscope (DMI 6000, Leica) with a cooled charge-coupled device (CCD) camera (MicroMax, Princeton Instruments), a spinning disc confocal composed by a Yokogawa CSU-X1 spinning disk scanner coupled to a Zeiss Observer Z1 inverted microscope through a Hamamatsu Orca Flash 4.0 sCMOS Camera, and a Leica SP5-II confocal laser scanning microscope attached to a Leica DMI 6000 inverted epifluorescence microscope.

## Statistical analysis

Statistical analysis of at least three independent experiments was done using the one-sample two tailed *t* test and proportions were compared using the Chi2 test. *p* values: \*, *p*<0.05; \*\*, *p*<0.01; \*\*\*, *p*<0.001. GraphPad Prism 5 software was used to perform all statistical analyses (mean± SEM).

## Additional references

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2. Vollenbroeker, B., George, B., Wolfgart, M., Saleem, M.A., Pavenstadt, H., and Weide, T. (2009). mTOR regulates expression of slit diaphragm proteins and cytoskeleton structure in podocytes. *Am J Physiol Renal Physiol* 296, F418-426.
3. Rapaport D, Lugassy Y, Sprecher E, Horowitz M. Loss of SNAP29 impairs endocytic recycling and cell motility. *PLoS One*. 2010;5(3):e9759.
4. Romano S, Maffei P, Bettini V, et al. Alstrom syndrome is associated with short stature and reduced GH reserve. *Clin Endocrinol (Oxf)*. 2013;79(4):529-536.
5. Hermle T, Schneider R, Schapiro D, et al. GAPVD1 and ANKFY1 Mutations Implicate RAB5 Regulation in Nephrotic Syndrome. *J Am Soc Nephrol*. 2018;29(8):2123-2138.