Supplemental Material

Supplemental Methods 1.

Histology

Kidneys were fixed in 4% paraformaldehyde/0.1 M PBS, paraffin-embedded and 3 µm sections were cut using a Leica RM2265 microtome (Leica Biosystems, Wetzlar, Germany). Hematoxylin and eosin (H&E), Periodic acid-Schiff (PAS) and Azan stainings were performed following standard procedures. 7 µm thick sections from kidney samples snap frozen in liquid nitrogen without prior fixation were prepared using a Leica CM3050 cryostat and fixed with methanol for 10 min at -20 °C. Target Retrieval (Dako, Hamburg, Germany) was used for Antigen-retrieval at 100°C for 10 minutes, blocking and antibody-incubations were performed with either 5% skimmed milk or 1% BSA and carried out as described[1]. For visualization with DAB, endogenous peroxidase was blocked with Peroxidase Solution (Dako) and hemalaun was used for nuclear counterstaining. Lectin staining was performed according to the DIG Glycan Differentiation Kit (Roche, Basel, Switzerland). Sialidase treatment was carried out with 0.25 U/ml *Arthrobacter ureafaciens (A.u.)* sialidase (E.Y. Laboratories, San Mateo, CA, USA) for 2 h at 37°C prior to lectin staining. Digital micrographs were taken with Zeiss Axiovert 200 M microscope equipped with an AxioCamMRm digital camera and AxioVison software (Zeiss, Oberkochen, Germany) or a Leitz Orthoplan microscope (Leica, Wetzlar, Germany) equipped with a digital imaging camera Leica EC3.

[1]Weinhold B, Sellmeier M, Schaper W, Blume L, Philippens B, Kats E, *et al*.: Deficits in sialylation impair podocyte maturation. *J Am Soc Nephrol* 23: 1319-1328, 2012

Supplemental Methods 2.

Cell surface biotinylation and EndoH treatment in HEK 293 cells

Generation and cultivation of HEK *CMAS*$^{-/-}$ and HEK 293 wildtype cells (kindly provided by the Institute of Cell Biology, Hannover Medical School) was described before.[2]

The expression plasmid for the stable expression of nephrin-flag in HEK 293 cells was generated as follows. The vector p-mNPHS1-Flag-CMV5a served as template for amplification of the coding sequence of murine nephrin sequence with C-terminal Flag-tag by PCR with the primers KMB7 and KMB8 (Supplemental Table 2). The fragment was subcloned into a pcDNA3 vector to give rise to pcDNA3-mNPHS1-Flag, harboring a neomycin resistance. HEK cells were transfected with pcDNA3-mNPHS1-Flag and cell clones with stable expression were selected with G418 (Merck, Darmstadt, Germany). HEK cells were tested for mycoplasma contamination with a negative result.

For western blot analysis HEK cells were lysed in RIPA buffer. For sialidase treatment an aliquot of the samples was incubated with 0.05 U *A.u*. sialidase per mg protein at 37°C for 30 min prior to western blot analysis.

Biotinylation of cell surface proteins of wildtype and *CMAS*$^{-/-}$ HEK 293 cell surface proteins stably expressing nephrin-Flag was performed using the *Pierce Cell Surface Protein Isolation Kit* (Thermo Scientific) according to the manufacturer's protocol. Cells were incubated with 0.25 mg ml^{-1} EZ-Link Sulfo-NHS-SS-Biotin solution for 30 min at 4°C, biotinylation was stopped, cells were lysed in RIPA buffer and biotinylated proteins were affinity purified with NeutrAvidin Agarose. Adjusted protein amounts were analyzed by SDS-PAGE and western blotting. For time course experiments cells were cultivated for defined time periods after biotinylation, counted, harvested and lysed in HBSS containing 1 % Triton-X 100, 0.02 mM PMSF, aprotinin and leupeptin. The amount of biotinylated nephrin-Flag was determined according to Turvy and Blum[3] in a capture ELISA using Flag-M2 antibody and 50 mU ml^{-1} Streptavidin-HRP with TMB substrate (Roche) for detection at 450 nm. Endo H (New England Biolabs) treatment was performed following the manufacturer's protocol.

[2]Abeln M, Borst KM, Cajic S, Thiesler H, Kats E, Albers I, *et al.*: Sialylation Is Dispensable for Early Murine Embryonic Development in Vitro. *Chembiochem* 18: 1305-1316, 2017

[3]Turvy DN, Blum JS: Biotin labeling and quantitation of cell-surface proteins. *Curr Protoc Immunol* Chapter 18: Unit 18 17, 2001

Supplemental Table 1. Antibodies and lectins

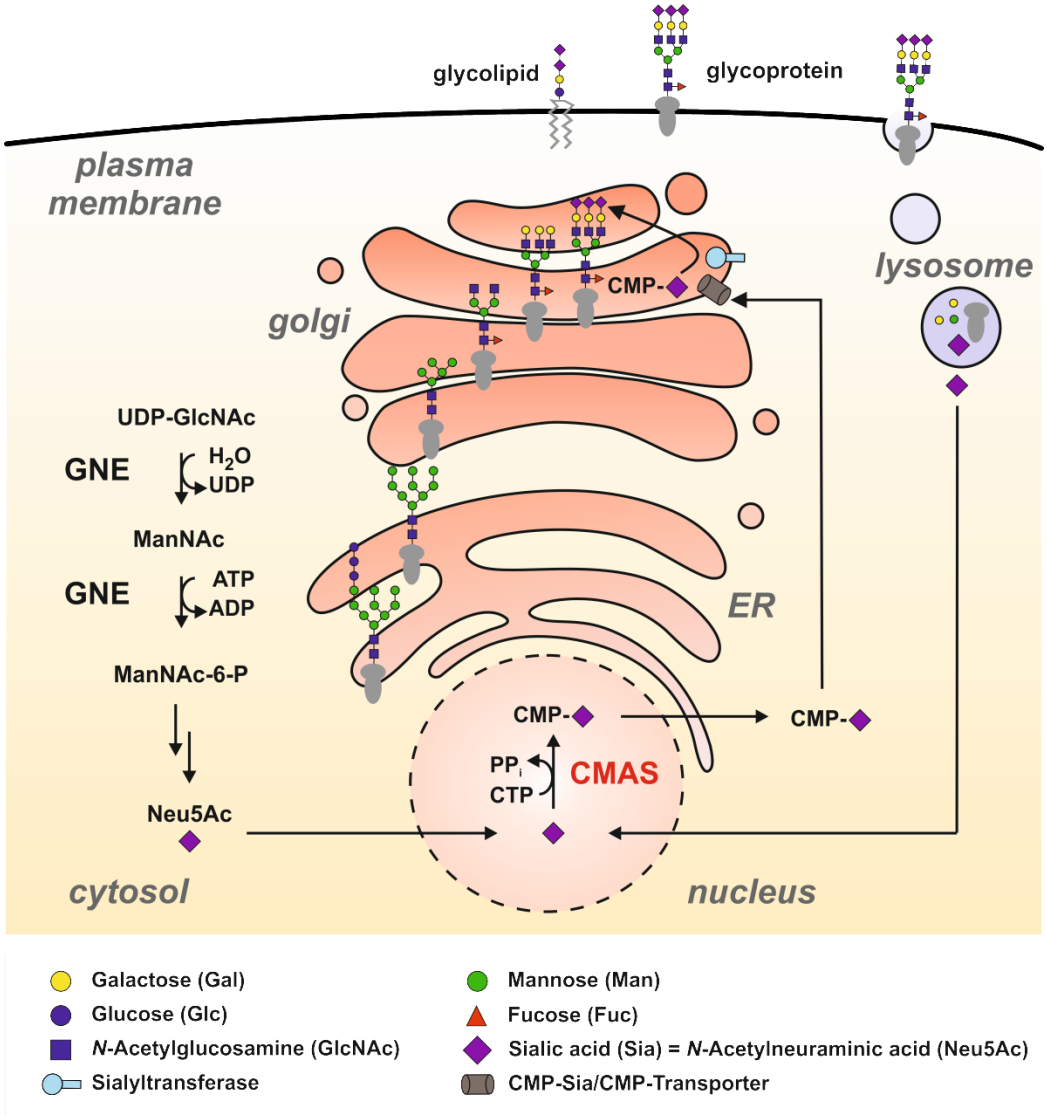
Primary antibody	Source	Manufacturer/Reference	Catalog no.	Dilution
β-actin	mouse	Millipore	MAB1501	1:100000 (WB)
albumin	goat	abcam	ab19194	1:1000 (WB)
α-smooth muscle actin	mouse	Sigma	A2457	1:500 (IHC)
β1-integrin	goat	Santa Cruz Biotechnology	sc-6622	1:1000 (WB)
caveolin 1	mouse	BD Biosciences	610407	1:100 (IHC)
Clathrin (light chain)	rabbit	Ahle et al. 1988[4]	-	1:200 (IF)
CMAS	rabbit	Schaper et al. 2012[5]	-	1:15.000 (WB)
digoxigenin -HRP	-	Roche	11633716001	1:8000 (WB)
EEA1	rabbit	Santa Cruz Biotechnology	sc-33585	1:500 (IF)
flag-M2	rabbit	Cell Signaling	2368	1:5000 (WB) 1:50 (ELISA)
GAPDH	mouse	Santa Cruz Biotechnology	sc-25778	1:2000
giantin	rabbit	BioLegend	PRB-114C	1:200 (IF)
LAMP-1	rat	Santa Cruz Biotechnology	sc-19992	1:500 (IF)
neph1	rabbit	Millipore	ABS1511	1:200 (IF)
nephrin	guinea pig	PROGEN Biotechnik	GP-N2	1:2000 (WB) 1:5000 (IF)
podocalyxin	goat	R&D Systems	AF1556	1:1000 (WB) 1:200 (IF)
podocin	rabbit	abcam	ab50339	1:300 IF
synaptopodin	goat	Santa Cruz Biotechnology	sc-21537	1:350 (WB) 1:800 (ICH) 1:50 (IF)
CD60b(UM4D4)-FITC	mouse	Ancell/Hölzel	212-040	1:25 (IF)
VEGFA	rabbit	abcam	ab52917	1:50 (IF)
WT1	rabbit	abcam	ab89901	1:500 (ICH) 1:50 (IF)
ZO-1	rat	DSHB (Developmental Studies Hybridoma Bank)	rR26.4C	1:20 (IF)
Lectin	**Sugar specificity**	**Manufacturer**	**Catalog no.**	**Dilution**
MAA-digoxigenin	Neu5Ac-α2,3-R	Roche	11210238001	1:500 (WB)
PNA- digoxigenin	β1,3-Gal-R	Roche	11210238001	1:1000 (WB)
SNA- digoxigenin	Neu5Ac-α2,6-R	Roche	11210238001	1:8000 (WB)
SNA-biotinylated	Neu5Ac-α2,6-R	Vector	B-1015	1:50000 (IHC)
SNA-TRITC	Neu5Ac-α2,6-R	EY Laboratories	R-6802-1	1:200 (IF)
Secondary antibody	**Source**	**Manufacturer**	**Catalog no.**	**Dilution**
goat-AP	rabbit	Jackson	305-055-003	1:5000 (WB)
goat-HRP	donkey	Santa Cruz	sc-2020	1:15000 (WB)
goat-Alexa488	donkey	Invitrogen	A11055	1:400 (IF)
guinea pig-HRP	goat	Jackson	106-035-003	1:15000 (WB)
guinea pig-Cy3	donkey	Jackson	706-165-148	1:500 (IF)
guinea pig-Alexa488	donkey	Jackson	706-545-148	1:100 (IF)
mouse-AP	goat	Dianova	115-055-003	1:5000 (WB)
mouse-Alexa488	goat	Molecular Probes	A 11029	1:400 (IF)
rabbit-AP	goat	Jackson	111-055-003	1:5000 (WB)
rabbit-HRP	goat	Jackson	111-035-003	1:15000 (WB)
rabbit-Cy3	sheep	SIGMA	C2306	1:500 (IF)
rabbit-Alexa568	goat	Molecular Probes	A11036	1:500 (IF)
rabbit-Alexa488	donkey	Molecular Probes	A21206	1:200 (IF)
rat-Alexa488	goat	Molecular Probes	A11006	1:200 (IF)

[4]Ahle S, Mann A, Eichelsbacher U, Ungewickell E: Structural relationships between clathrin assembly proteins from the Golgi and the plasma membrane. *EMBO J* 7: 919-929, 1988

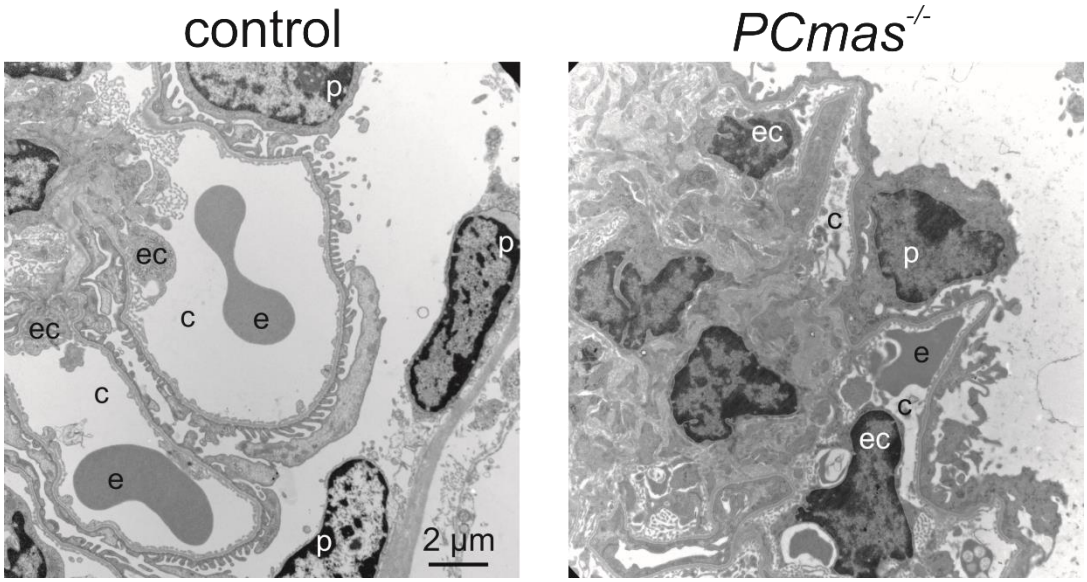
[5]Schaper W, Bentrop J, Ustinova J, Blume L, Kats E, Tiralongo J, *et al*.: Identification and biochemical characterization of two functional CMP-sialic acid synthetases in Danio rerio. *J Biol Chem* 287: 13239-13248, 2012

Supplemental Table 2. Primers

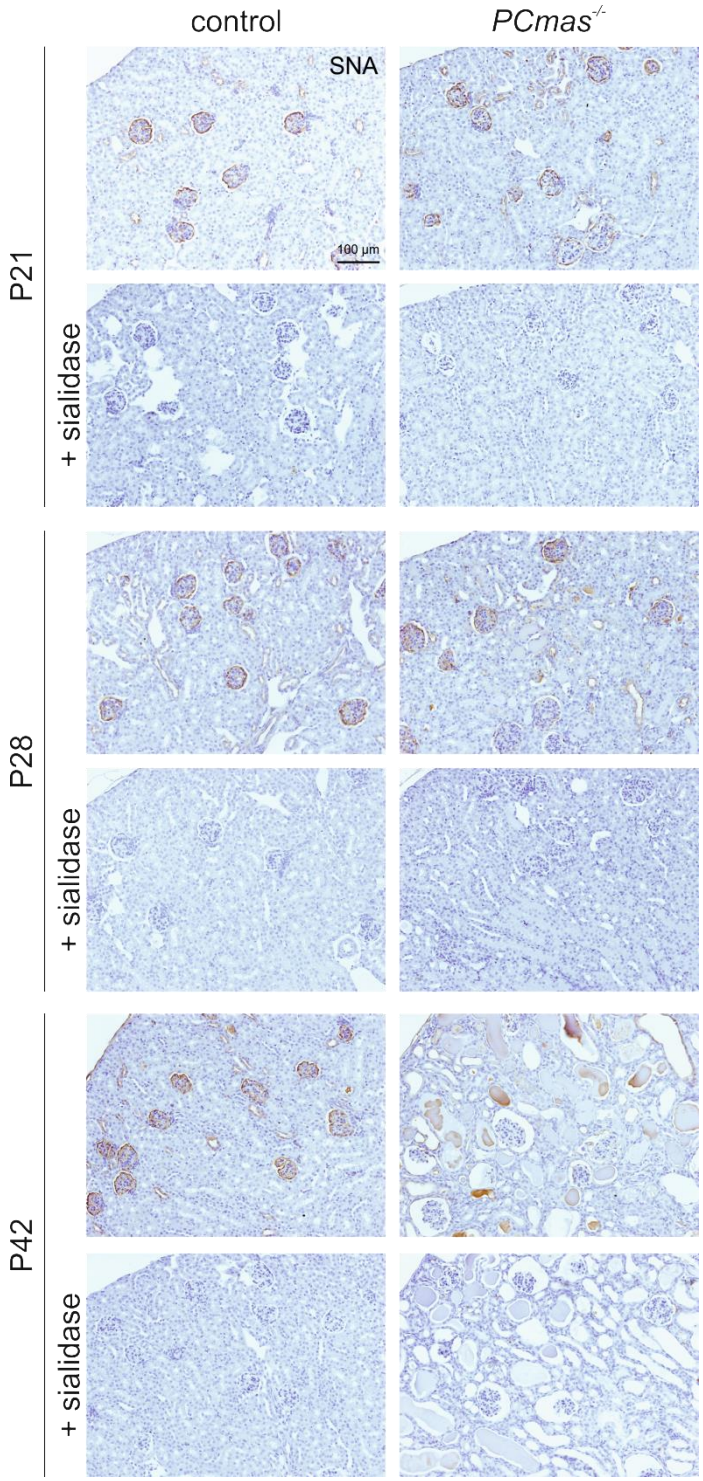
Gene	Primer	Sequence
Cre Fw	BW F51	5´- ggc gga tcc gaa aag aag -3´
Cre Rev	BW B54	5´- ccc ggc aaa aca ggt agt -3´
PST Fw (internal control for *Cre* PCR)	LW 13	5´- ctc agt tct ggc tat ttc ttt tgt -3´
PST Rev (internal control for *Cre* PCR)	LW 14	5´- gag ctc aca acg act ctc cga gc -3´
Cmas Fw	BW F59	5´- agc gcc tgt gta ccc ctc tta -3´
Cmas Rev	BW B58	5´- gcg agc agc aag tga gca -3´
Immorto Fw	BW F86	5´- cct ctg agc tat tcc aga agt agt g -3´
Immorto Rev	BW B87	5´- tta gag ctt taa atc tct gta ggt ag -3´
STX Fw (internal control for *Immorto* PCR)	STX cond1b	5´- cct aga tgg gtt ggt gtt gc -3´
STX Rev (internal control for *Immorto* PCR)	STX cond2	5´- gag aca gca act aga gga ata aca -3´
Nphs1-Flag Fw	KMB 7	5´- caa gcg aag ctt atg gga gct aag gaa gcc aca g -3´
Nphs1-Flag Rev	KMB 8	5´- acc gcc tct aga cta ctt gtc atc gtc gtc ctt g -3´
Cmas Fw	KMB 77	5´- gtc tgc att ctg agg gga gt -3´
Cmas Rev	KMB 78	5´- aga gca caa cac aga agg ct -3´



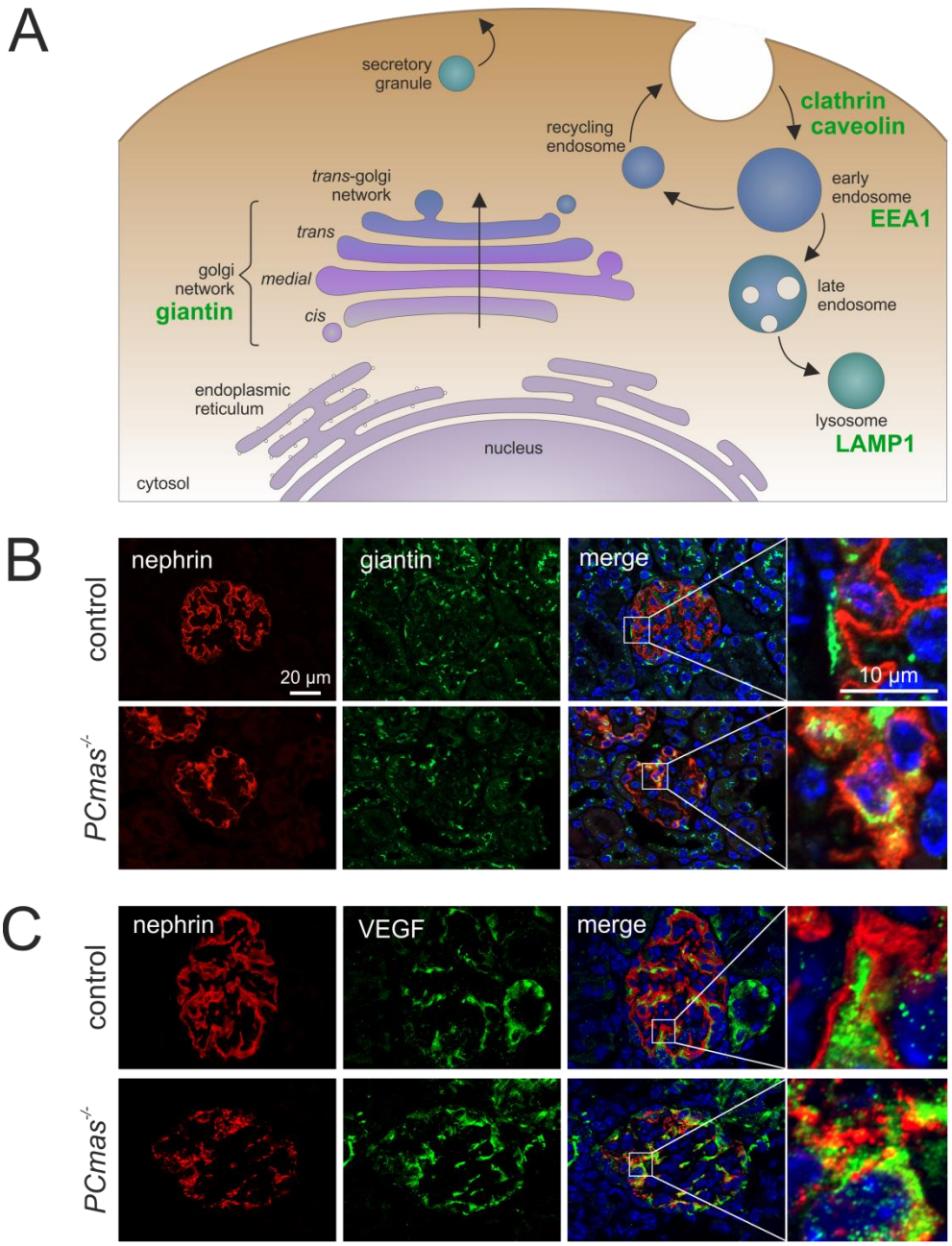
Supplemental Figure 1. Biosynthesis of sialylated glycoconjugates. *De novo* biosynthesis of Sia is started by the conversion of UDP-*N*-Acetylglucosamine (UDP-GlcNAc) to *N*-Acetylmannosamine (ManNAc), which is further phosphorylated to ManNAc6P. Both reactions are catalyzed by the bifunctional enzyme UDP-GlcNAc epimerase – ManNAc kinase (GNE). After two additional steps, free *N*-Acetylneuraminic acid (Neu5Ac, Sia) is activated by CMP-Sia synthase (CMAS) to its cytidine monophosphodiester CMP-Sia. Sia activation by CMAS is non redundant and crucial for the biosynthesis of both, sialylated glycoproteins and glycolipids, since only the high energy nucleotide sugar is accepted by sialyltransferases as donor substrate. CMP-Sia is transported into the golgi apparatus by the CMP-Sia transporter und transferred in the golgi compartment to nascent glycoconjugates, which are subsequently transported to the cell surface. Endocytic recycling of sialoglycoconjugates includes lysosomal degradation and transport of Sia into the cytoplasm for further activation by CMAS. Dietary ManNAc supplementation permits a bypass of the GNE reaction and therefore a rescue of mouse models with mutations in the GNE but does not allow rescue of CMAS deficient mice.



Supplemental Figure 2. Obliteration of glomerular capillary lumen in *PCmas*$^{-/-}$ kidney sections. Transmission electron micrographs of ultrathin sections of the renal cortex of control and *PCmas*$^{-/-}$ kidney specimen sampled at P42. Podocyte (p), capillary lumen (c), Endothelial cell (ec), erythrocyte (e).

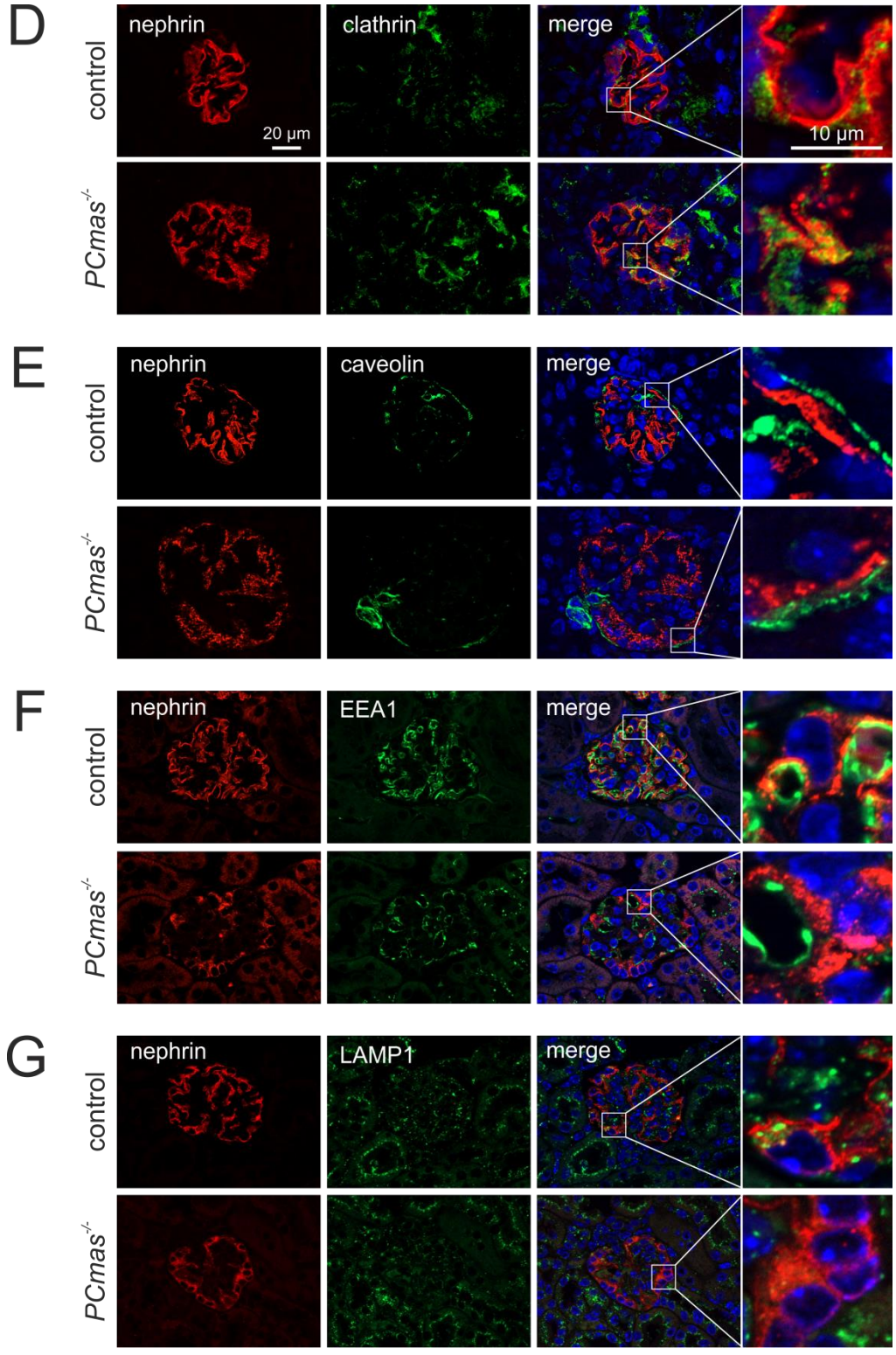


Supplemental Figure 3. Loss of sialylation in *PCmas*$^{-/-}$ kidney sections is apparent to different degrees in different glomeruli at P28. Immunohistochemical staining of paraffin-embedded kidney sections of control and *PCmas*$^{-/-}$ mice at P21, P28 and P42 with biotinylated lectin SNA, specific for α2,6-linked Sia. Lectin specificity was verified by sialidase treatment prior to staining. Labeling with biotinylated Lectin was visualized by streptavidin-HRP binding and development with DAB/H_2O_2. In control mice, glomeruli and especially the podocytes were highly α2,6-sialylated. While no differences between control and *PCmas*$^{-/-}$ mice were visible at P21, glomeruli in the inner rim of the cortex of *PCmas*$^{-/-}$ mice lost Sia at P28 and the glomeruli at the outer rim were still sialylated. At P42, all glomeruli lost SNA staining in mutant mice.

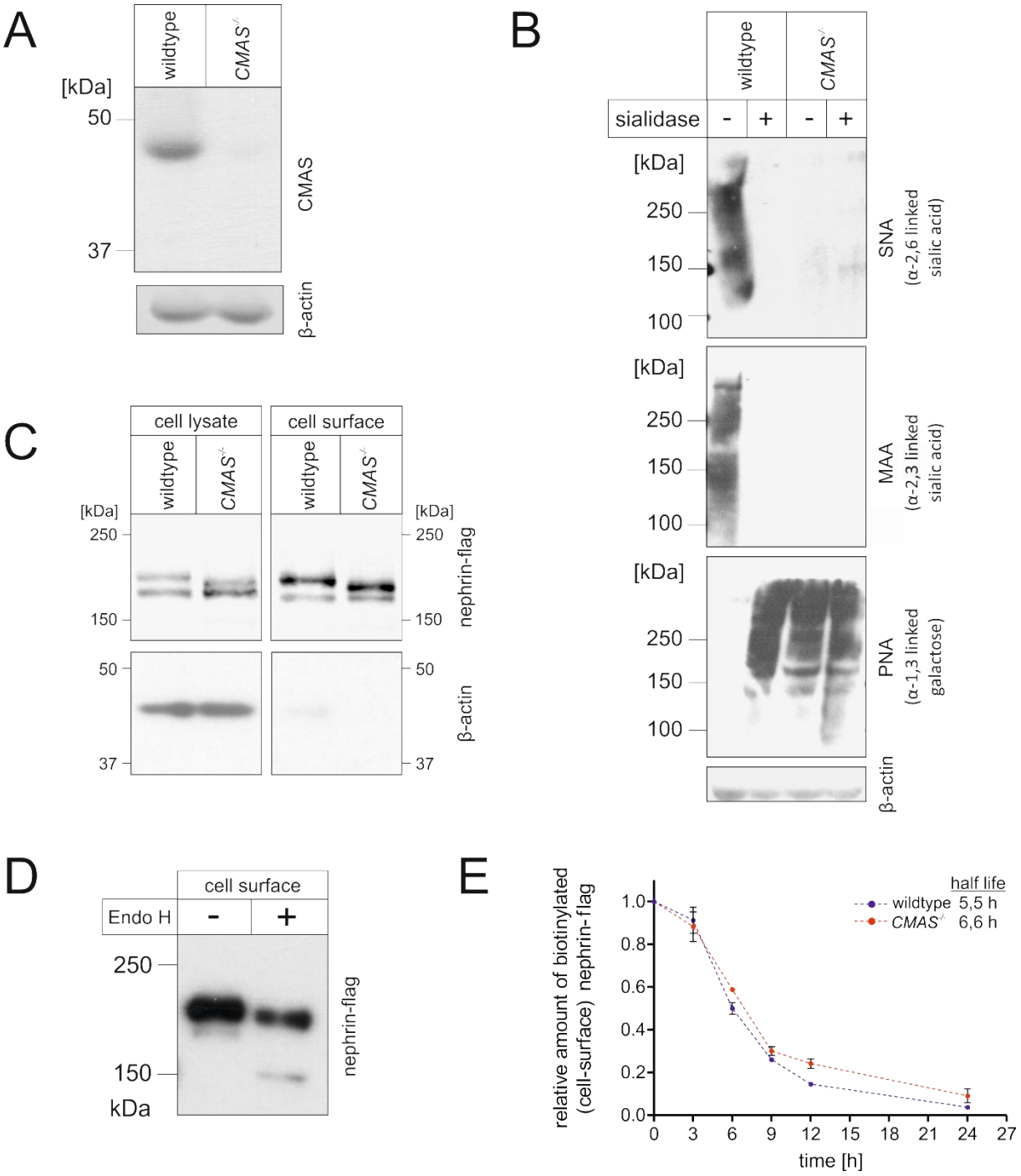


Supplemental Figure 4. Mislocalized nephrin is partially associated to compartments of the secretory and endocytic pathway. **(A)** Schematic representation of the endocytic and secretory pathway and vesicle affiliation of the applied marker proteins (green) (modified from Casey *et al.*[6]). Indirect immunofluorescent costaining of nephrin (red) with introduced compartment marker proteins (green) on paraffin-embedded renal tissue sections from control and *PCmas*$^{-/-}$ mice sampled at P28: **(B)** giantin as marker for the golgi apparatus and **(C)** VEGF as marker for the secretory pathway, **(D)** clathrin as marker for clathrin-mediated endocytosis, **(E)** caveolin as marker for lipid-raft mediated endocytosis, **(F)** EEA1 as marker for early endosomes and **(G)** LAMP1 as marker for lysosomes. Nuclei were stained with DAPI. Compared to wildtype mice, enhanced colocalization of nephrin was only observed with markers of the secretory pathway as well as with clathrin for clathrin-mediated endocytosis in *PCmas*$^{-/-}$ mice, indicating altered turnover of asialo-nephrin.

[6]Casey JR, Grinstein S, Orlowski J: Sensors and regulators of intracellular pH. *Nat Rev Mol Cell Biol* 11: 50-61, 2010

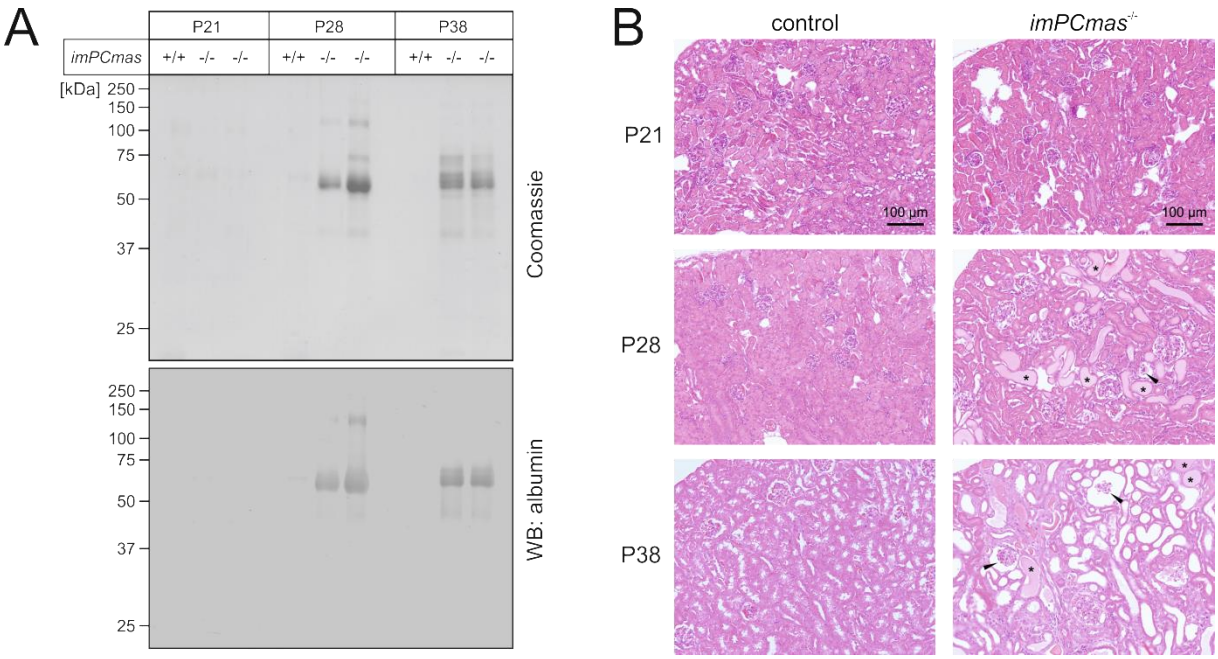


Supplemental Figure 4.

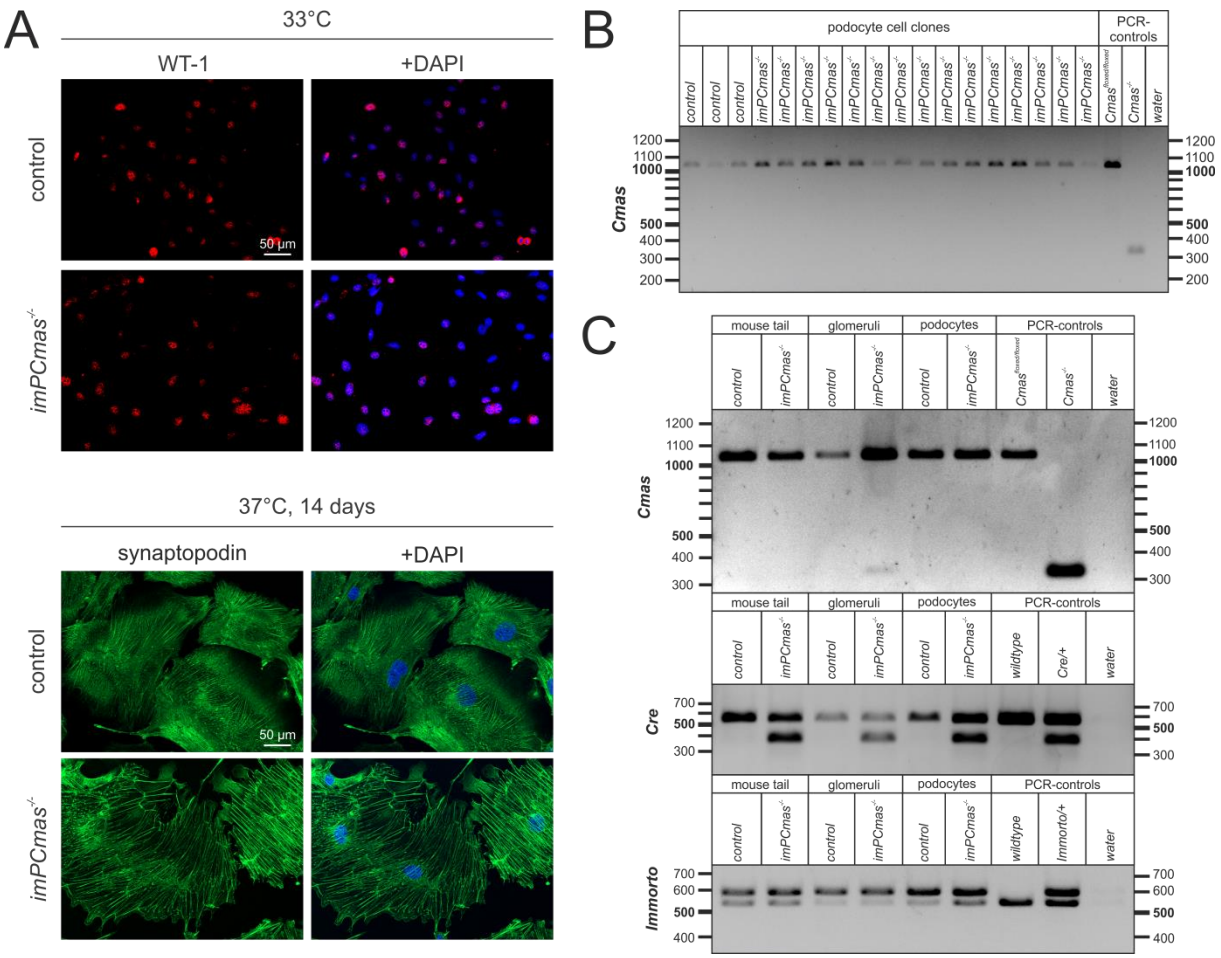


Supplemental Figure 5.

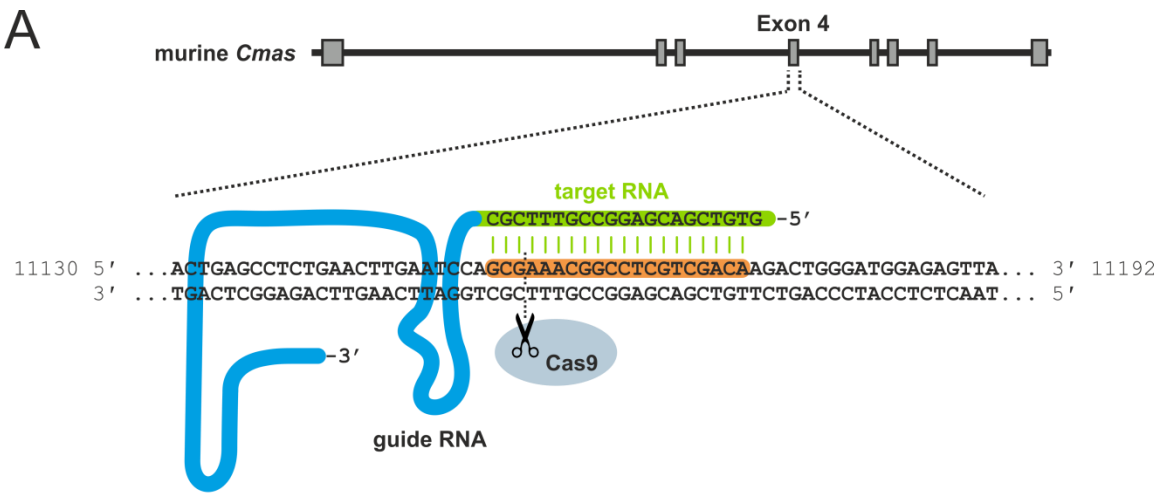
Supplemental Figure 5. Biochemical characterization of wildtype and *CMAS*$^{-/-}$ HEK 293 cells stably expressing nephrin-Flag and analysis of nephrin trafficking and half-life in these cell lines. Whole cell lysates were analyzed by Western blotting regarding **(A)** CMAS expression and **(B)** cell surface sialylation. Actin staining was used as loading control. Loss of CMAS expression in *CMAS*$^{-/-}$ HEK 293 cells resulted in lack of cell surface sialylation, as depicted by lack of signals with the Sia-specific lectins *Sambucus nigra* agglutinin (SNA) and *Maackia amurensis* agglutinin (MAA) recognizing, α2,6- and α2,3-linked Sia, respectively. In line with loss of sialylation, *CMAS*$^{-/-}$ HEK 293 cells showed increased signals of galactose-capped glycans as depicted with peanut agglutinin (PNA). Specificity of lectin staining was confirmed by sialidase treatment. **(C)** Nephrin cell surface presentation as a function of sialylation was investigated by a cell surface biotinylation assay in stably transfected wildtype and *CMAS*$^{-/-}$ HEK 293 cells. Cell surface proteins were biotinylated, cells were lysed and biotinylated proteins were separated with NeutrAvidin agarose. Lysate and cell surface fractions were separated by SDS-PAGE and analyzed by immunoblot stained for Flag-tagged nephrin. Actin staining served as control for purity of the cell surface fraction. In both cell lines, Flag-tagged nephrin was detected as a doublet in Western blot analysis of surface fraction and cell lysate, representing different glycoforms (further analyzed in (D)). Of note, asialo-nephrin in *CMAS*$^{-/-}$ HEK293 cells was detected at the cell surface in an amount similar to sialylated nephrin in wildtype cells. **(D)** Western blot analysis of EndoH treated samples after cell surface biotinylation. Biotinylated cell surface proteins from HEK 293 wildtype cells were treated with Endo H to remove immature mannose-rich N-glycans. Proteins were separated by SDS-PAGE and analyzed in an immunoblot stained for nephrin-Flag. The upper bands represent mature, glycosylated nephrin, the lower band represents an EndoH-sensitive immature glycoform of nephrin and is shifted to a mass of 150 kDa after EndoH treatment. **(E)** Half-life of biotinylated nephrin in HEK wildtype and *CMAS*$^{-/-}$ cells. After biotinylation of cell-surface proteins, cells were cultivated and harvested at different time points. In three individual experiments the quantification of biotinylated nephrin in the lysate was performed by a capture ELISA. The calculated half-lives of sialo- ($t_{1/2}$ = 5.5 h) and asialo-nephrin ($t_{1/2}$ = 6.6 h) differed only slightly, indicating that neither nephrin cell surface presentation nor nephrin endocytosis was dependent on sialylation in HEK 293 cells.



Supplemental Figure 6. *imPCmas*$^{-/-}$ mice develop a phenotype identical to *PCmas*$^{-/-}$ mice. **(A)** Analysis of urine samples of control and *imPCmas*$^{-/-}$ mice at P21, P28 and P38 by SDS-Page and Coomassie staining (top) and albumin immunoblot (bottom), respectively. *PCmas*$^{-/-}$ mice develop albuminuria around P28. **(B)** H&E staining of paraffin-embedded kidney sections of control and *imPCmas*$^{-/-}$ mice at P21, P28 and P38. In line with the onset of proteinuria around P28, glomeruli with enlarged Bowman's space, and tubuli filled with protein casts were visible at P28 and even more pronounced at P38.



Supplemental Figure 7. Outgrowth of *imPCmas*$^{-/-}$ glomeruli proves to be wildtype podocytes. Analysis of podocyte cell clones obtained from isolated control and *imPCmas*$^{-/-}$ glomeruli. **(A)** Indirect immunofluorescence staining for podocyte markers on podocyte cell clones grown under permissive (WT1) and non-permissive (synaptopodin) conditions. **(B)** PCR analysis of the *Cmas* gene. **(C)** Genotyping of mouse tail, glomeruli immediately after isolation and glomerular outgrowth (podocytes) from control and *imPCmas*$^{-/-}$ mice. The *Immorto* allele was detectable in all samples from control and *imPCmas*$^{-/-}$ mice while the *Cre* allele was only present in glomerular samples from *imPCmas*$^{-/-}$ mice. Genomic DNA from tail biopsies of wildtype, *Cmas*$^{floxed/floxed}$ (1.06 kb), *Cre/+* (372 bp, with internal control band 530 bp) and *Immorto/+* (600 bp, with internal control band 364 bp) mice and genomic DNA from *Cmas*$^{-/-}$ ES cells (315 bp) served as controls for the amplification of the specific PCR fragments. A water control without DNA served as negative control.



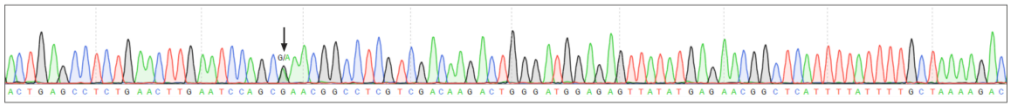
B

wildtype

target site

...ACTGAGCCTCTGAACTTGAATCCAGCGAAACGGCCTCGTCGACAAGACTGGGATGGAGAGTTATATGAGAACGGCTCATTTTATTTTGCTAAAAGACATTTGA...
T E P L N L N P A K R P R R Q D W D G E L Y E N G S F Y F A K R H L

***Cmas*$^{-/-}$ clone 1**



allele 1 ...ACTGAGCCTCTGAACTTGAATCCAGCG-AACGGCCTCGTCGACAAGACTGGGATGGAGAGTTATATGAGAACGGCTCATTTTATTTTGCTAAAAGACATTTGA...
T E P L N L N P A N G L V D K T G M E S Y M R T A H F I L L K D I *

allele 2 ...ACTGAGCCTCTGAACTTGAATCCAGC-AAACGGCCTCGTCGACAAGACTGGGATGGAGAGTTATATGAGAACGGCTCATTTTATTTTGCTAAAAGACATTTGA...
T E P L N L N P A N G L V D K T G M E S Y M R T A H F I L L K D I *

***Cmas*$^{-/-}$ clone 2**

allele 1 ...ACTGAGCCTCTGAACTTGAATCCAGCG-AACGGCCTCGTCGACAAGACTGGGATGGAGAGTTATATGAGAACGGCTCATTTTATTTTGCTAAAAGACATTTGA...
T E P L N L N P A N G L V D K T G M E S Y M R T A H F I L L K D I *

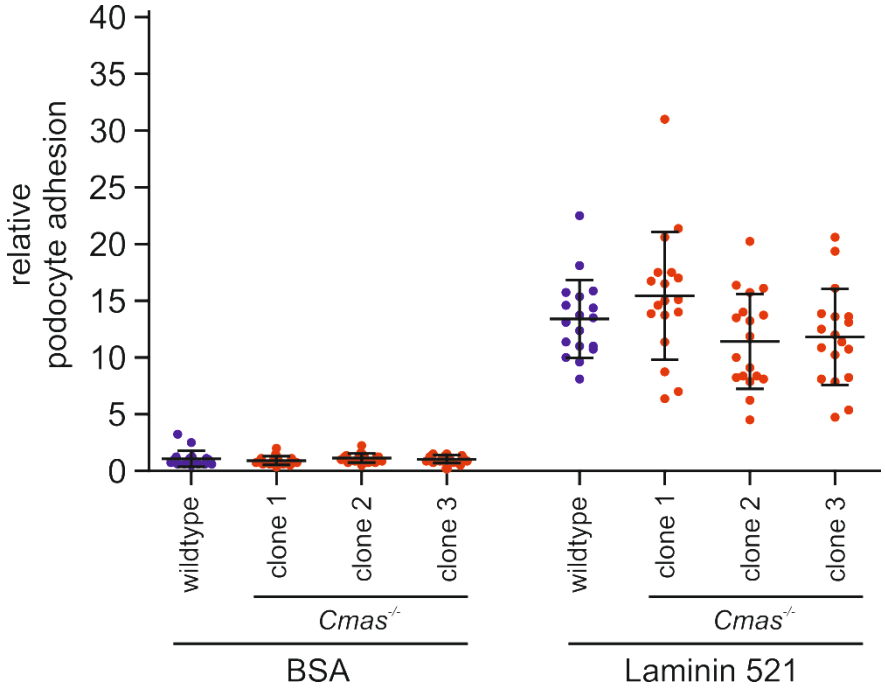
allele 2 ...ACTGAGCCTCTGAACTTGAATCCAGCGAAAACGGCCTCGTCGACAAGACTGGGATGGAGAGTTATATGAGAACGGCTCATTTTATTTTGCTAAAAGACATTTGA...
T E P L N L N P A K T A S S T R L G W R V I *

***Cmas*$^{-/-}$ clone 3**

allele 1 ...ACTGAGCCTCTGAACTTGAATCCAG--AAACGGCCTCGTCGACAAGACTGGGATGGAGAGTTATATGAGAACGGCTCATTTTATTTTGCTAAAAGACATTTGA...
T E P L N L N P E T A S S T R L G W R V I *

allele 2 ...ACTGAGCCTCTGAACTTGAATCCAGCG-AACGGCCTCGTCGACAAGACTGGGATGGAGAGTTATATGAGAACGGCTCATTTTATTTTGCTAAAAGACATTTGA...
T E P L N L N P A N G L V D K T G M E S Y M R T A H F I L L K D I *

Supplemental Figure 8. Generation of immortalized murine *Cmas*$^{-/-}$ podocyte cell lines by use of CRISPR/Cas9 technology. A sequence in exon 4 of the murine *Cmas* gene was chosen as target sequence (highlighted in orange) for the Cas9 enzyme. (B) Sequencing analysis of exon 4 of three independent *Cmas*$^{-/-}$ clones revealed frameshifts and premature stop codons (*) in all cases due to deletion or insertion of one or two nucleotides in all alleles.



Supplemental Figure 9. *In vitro* adhesion assay (laminin). Cell adhesion assay of wildtype and *Cmas*$^{-/-}$ podocytes to laminin 521 and BSA as control. Three individual experiments were conducted with each 6 technical replicates per cell line.