Supplemental data

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Supplemental Table 1: Primers

Name	Use	Species	Sequence (5'-3')
GuideRNARAB7humanfw	Guide RNA forward for CRISPR/Cas9 to target Rab7	human	CACCGTTGCTGAAGGTTATCATCT
GuideRNARAB7humanrev	Guide RNA reverse for CRISPR/Cas9 to target Rab7	human	AAACGGATGATAACCTTCAGCAAC
RAB7a humanseqfwd	Sequencing Primers forward	human	CACCGTTATCATCCTGGGAGATT

RAB7a humanseqrev	Sequencing Primer reverse	human	AAACGAATCTCCCAGGATGATAA
Serpine1 (Pai1) fwd	Forward primer for RT-qPCR	mouse	CACAGGCACTGCAAAAGGTC
Serpine1 (Pai1) rev	Reverse primer for RT-qPCR	mouse	GGATTGTCTCTGTCGGGTTGT
Lcn2 fwd	Forward primer for RT-qPCR	mouse	ACGGACTACAACCAGTTCGC
Lcn2 rev	Reverse primer for RT-qPCR	mouse	AATGCATTGGTCGGTGGGG
Gapdh fwd	Forward primer for RT-qPCR	mouse	CCTGGAGAAACCTGCCAAGTA
Gapdh rev	Reverse primer for RT-qPCR	mouse	AAGTCGCAGGAGACAACCTG
Ccl2 fwd	Forward primer for RT-qPCR	mouse	AGCTGTAGTTTTTGTCACCAAGC
Ccl2 rev	Reverse primer for RT-qPCR	mouse	TGCTTGAGGTGGTTGTGGAA

Supplemental Table 2: List of antibodies and imaging dyes used in this study.

WB= used in Western blot analysis, IF= used in immunofluorescence staining

Antigen	Species	Provider	Working Dilution
Lamp1	Rabbit	OriGene Technologies Inc.	1:1000 (WB)
LAMP1	Rabbit	46843 Cell Signaling	1:300
Lamp2a H4B4	Mouse	Santa Cruz Biotechnology, Heidelberg	1:1000 (WB), 1:100 (IF)
LC3B	Mouse	Medical & Biological laboratories Co., LTD.	1:1000 (WB)
Sequestosome-1 p62	Mouse	BD Biosciences, Franklin Lakes, NJ, USA	1:1000 (WB)
Sequestosome-1 P62	Guinea pig	Progene	1:1000(WB)
SQSTM1/p62	Rabbit	23214, Cell Signaling	1:100 (IF)
SYNPO (Synaptopodin)	Mouse	65194, Progene	1:200 (IF)
Rab7	Rabbit	Cell Signaling Technology, Inc., Danvers, USA	1:1000 (WB)
Rab7	Rabbit	Santa Cruz Biotechnology, Heidelberg	1:1000 (WB)
Rab7	Rabbit	ab137029, Abcam	1:150 (IF)
Nephrin	guinea pig	Progen	1:100 (IHC), 1:500 (WB)
RILP	Rabbit	*Abcam*	1:1000 (WB)
WT1	Rabbit	ab15249, Abcam	1:200 (IF)
V1G1	Mouse	Santa Cruz Biotechnology, Heidelberg	1:250 (WB)
A-Actinin4	Rabbit	Enzo Life Sciences GmbH, Lörrach	1:1000 (WB)
B-Tubulin	Mouse	Sigma-Aldrich Chemie GmbH, Munich	1:1000 (WB)
Alexa Fluor 488 conjugated alpha-mouse IgG	Goat	Invitrogen Life Technologies GmbH, Darmstadt	1:1000

Alexa Fluor 488 conjugated alpha-Rabbit IgG	Goat	Invitrogen Life Technologies GmbH, Darmstadt	1:1000 (IF)
Alexa Fluor 594 conjugated alpha-mouse IgG	Goat	Invitrogen Life Technologies GmbH, Darmstadt	1:1000 (IF)
Alexa Fluor 594 conjugated alpha-Rabbit IgG	Goat	Invitrogen Life Technologies GmbH, Darmstadt	1:1000 (IF)
Alexa Fluor 594 conjugated phalloidin	-	Invitrogen Life Technologies GmbH, Darmstadt	1:300 (IF)
DAPI (4',6-diamidine-2-phenylindole)	-	Invitrogen Life Technologies GmbH, Darmstadt	1:5000 (IF)
Hoechst 33342 trihydrochloride, trihydrate	-	Thermo Scientific Inc., Waltham, USA	10mg/ml
HRP-conjugated alpha-mouse IgG	Goat	Jackson Immunoresearch Laboratories, Inc., Suffolk, Great Britain	1:1000 (WB)
HRP-conjugated alpha-Rabbit IgG	Goat	Jackson Immunoresearch Laboratories, Inc., Suffolk, UK	1:1000 (WB)
LysoTracker green DND-26	-	Thermo Scientific Inc., Waltham, USA	50 nM
LysoTracker Red	-	Thermo Scientific Inc., Waltham, USA	50 nM
Wheat germ agglutinin texas red tm x-conjugate	-	Thermo Scientific Inc., Waltham, USA	1 mg/ml
PageRuler Plus Prestained Protein Ladder	-	Thermo Scientific Inc., Waltham, USA	

Supplemental Table 3: Components for ligation of guide RNAs.

Reagent	Amount
Guide RNA fw & rev	8µl each
10x PNK buffer	2µl
5mM ATP	1µl
Polynucleotide kinase	1µl

Supplemental Table 4: Components for Digestion with BbSI for generating a CRISPR construct for Rab7 KO.

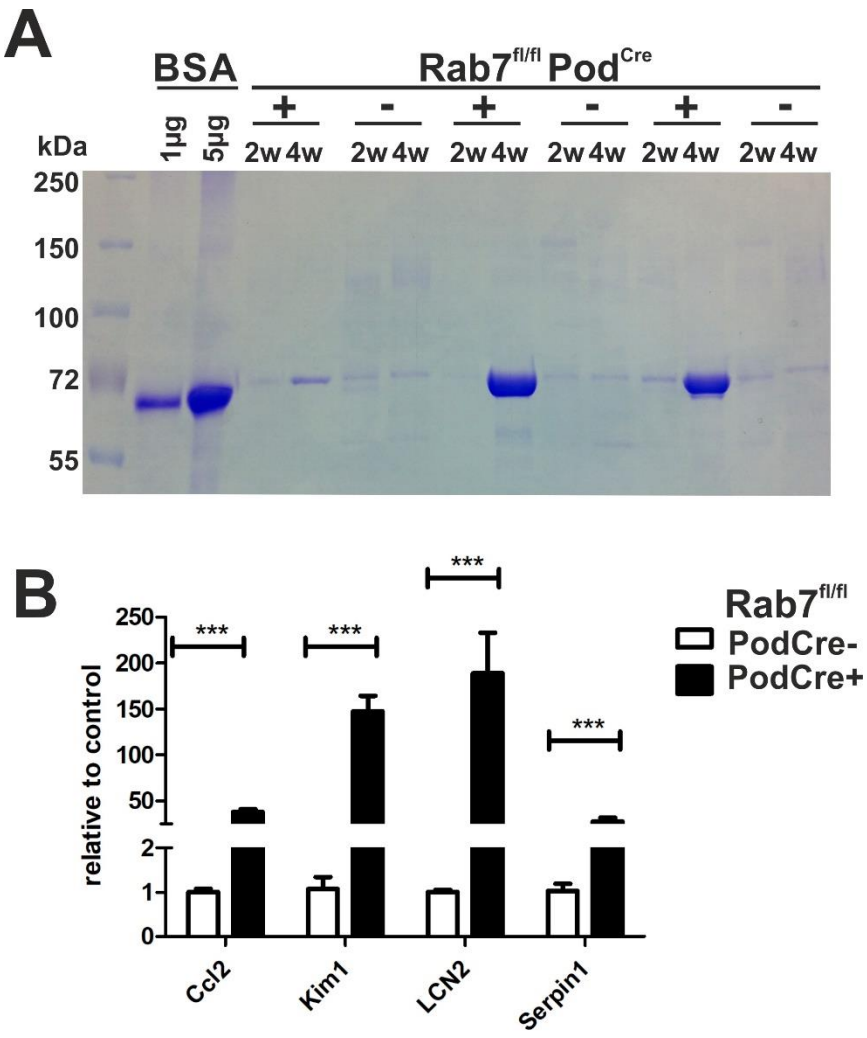
Reagent	Amount
Px330-459 plasmid	50 ng
Annealed PCR product (Suppl. Table. 2)	0,5 µl
10x T4 Ligation Buffer	1 µl
*Bb*SI	0,5 µl
T4 Ligase	0,5 µl
H_2O	6,5 µl
5 min 37 °C, 5min 23 °C for 30x cycles	

Supplemental Table 5: Average injury scores in 8-week-old Rab7 depleted mice.

Analysis was done from paraffin sections. 0 = < 5 %; 1 = 5-25 %; 2 = 25-50 %; 3 = >50 %; N= 5, Statistical analysis was performed by Mann Whitney test with **p =0.0079

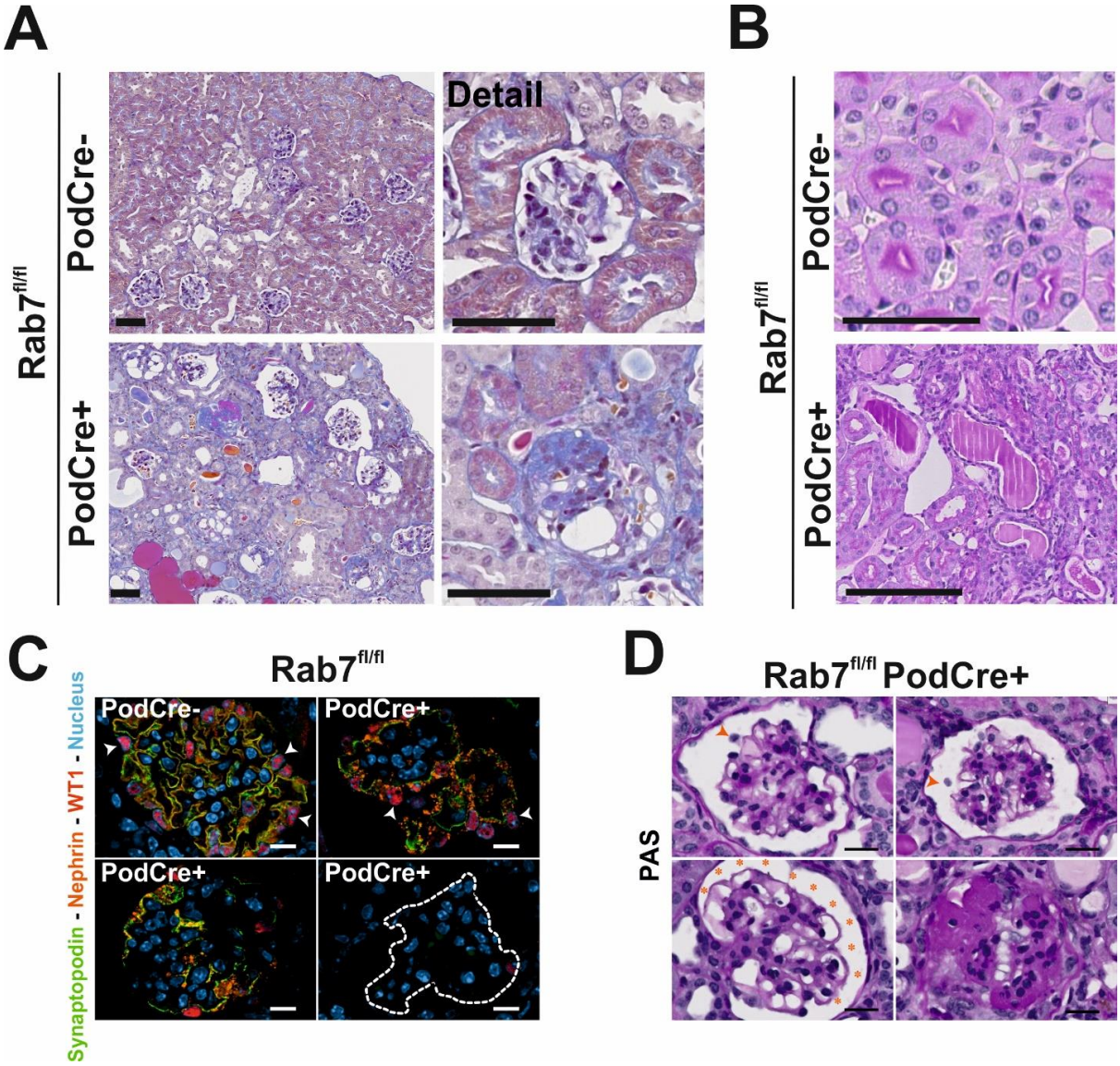
	Cntr [average score]	KO [average score]
Interstitial fibrosis	0	1.2**
Tubular atrophy	0	1.4 **
Interstitial inflammation	0	1**
Proteinaceous casts	0	2.8**

Supplemental Figures and Legends



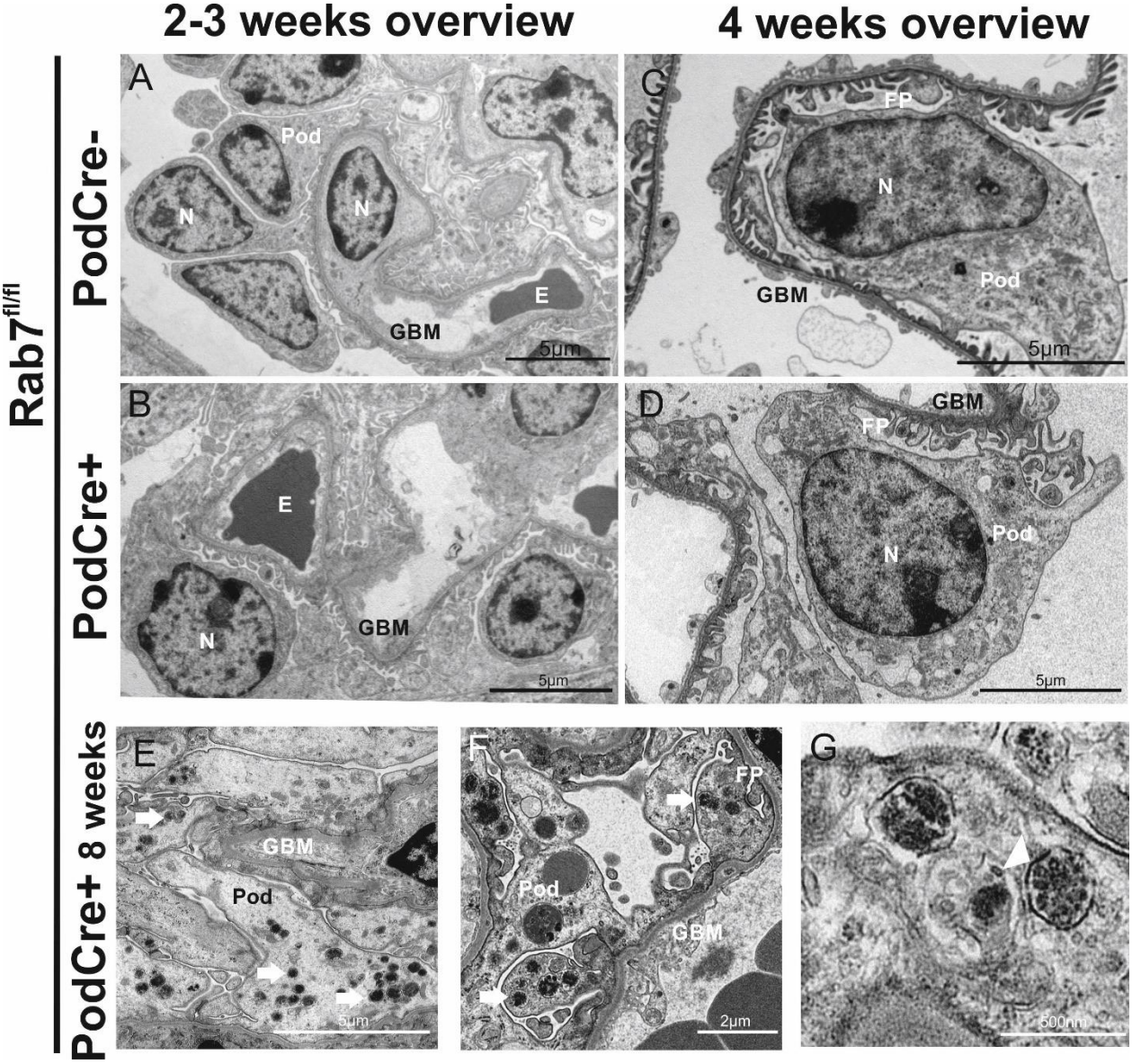
Supplemental Figure 1: Rab7 depletion in podocytes in mice results in proteinuria and upregulation of renal injury markers.

(A) SDS-PAGE with Coomassie staining of urines of Rab7$^{fl/fl}$;*PodCre+* and control mice to detect proteinuria. The samples were respectively collected from the same animal at 2 and 4 weeks of age from a total of 3 mice per group. As control, a standard BSA concentration of 1 and 5 µg was used. N=3. **(B)** Total RNA was isolated and analyzed by qPCR. Rab7$^{fl/fl}$;*Pod-Cre+* mice show increasing injury markers at 8 weeks after birth. Shown is Mean with SEM, *** $p<0.0001$ in a two-way ANOVA, N=4.



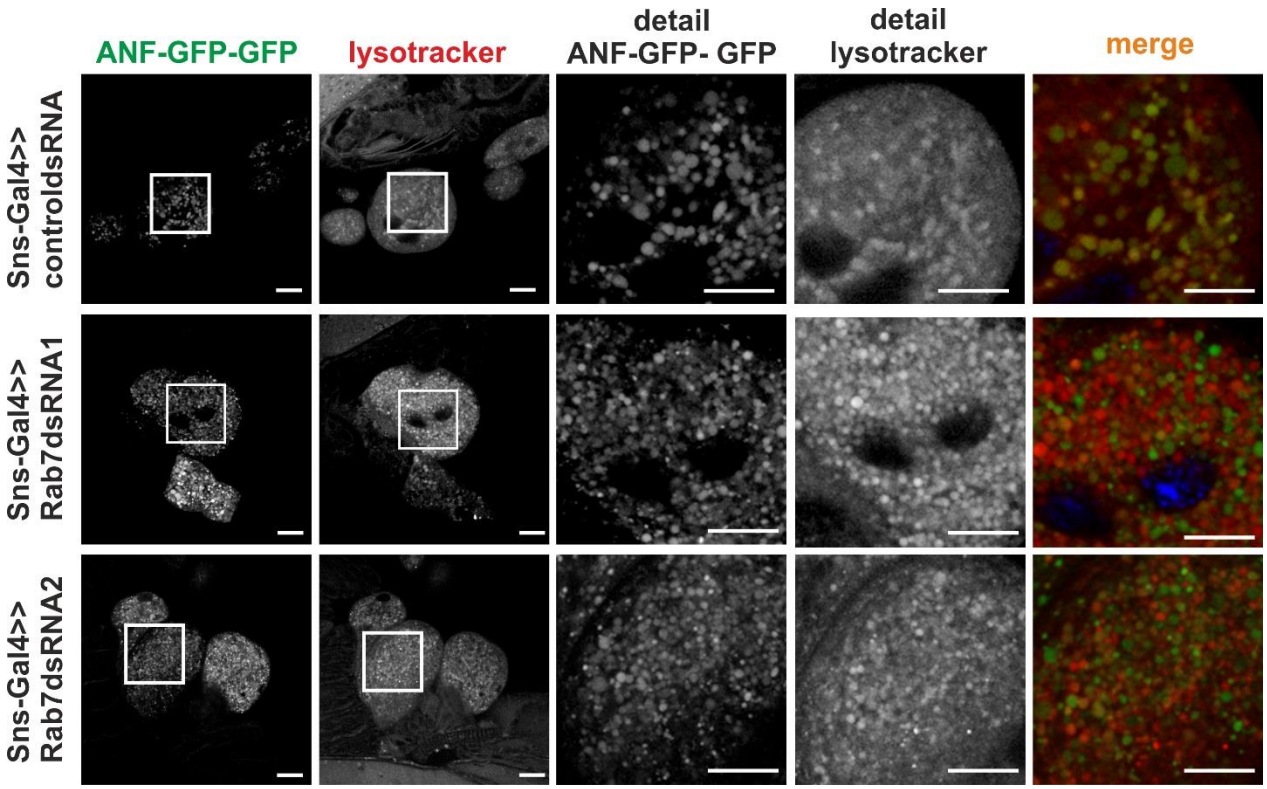
Supplemental Figure 2: Rab7 depletion in podocytes leads to podocyte loss.

(A) SFGO staining of 8-week-old kidney paraffin sections. Rab7$^{fl/fl}$;*PodCre+* mice show dilated Bowman's space and global interstitial fibrosis. Quantification is added in Suppl. Tab. ST5. Scale bar 50 µm (B) PAS staining of 8-week-old kidney cryosections of Rab7$^{fl/fl}$;*PodCre+* and control mice with the focus on proteinaceous casts, Scale bar 50 µm. The quantification is shown in Suppl. Table ST5. (C/D) Podocyte depletion in Rab7$^{fl/fl;}$*PodCre+* and control mice by immunofluorescence and PAS staining, (C) spectrum of podocyte depletion from representative glomeruli in Rab7$^{fl/fl}$;*PodCre+* animals, white arrowheads indicate representative podocyte nuclei. Dashed line indicates glomerular tuft area. (D) Spectrum of podocyte depletion from representative glomeruli in Rab7 KO animals. (upper left) Orange arrow indicates podocyte cell body bulging/detachment into the urinary space; (upper right) orange arrowhead indicates free floating/detached cell in the urinary space; (lower left) orange asterisks indicate glomerular capillaries without visible podocyte nuclei; (lower right) fully sclerosed glomerulus and concomitant podocyte depletion.



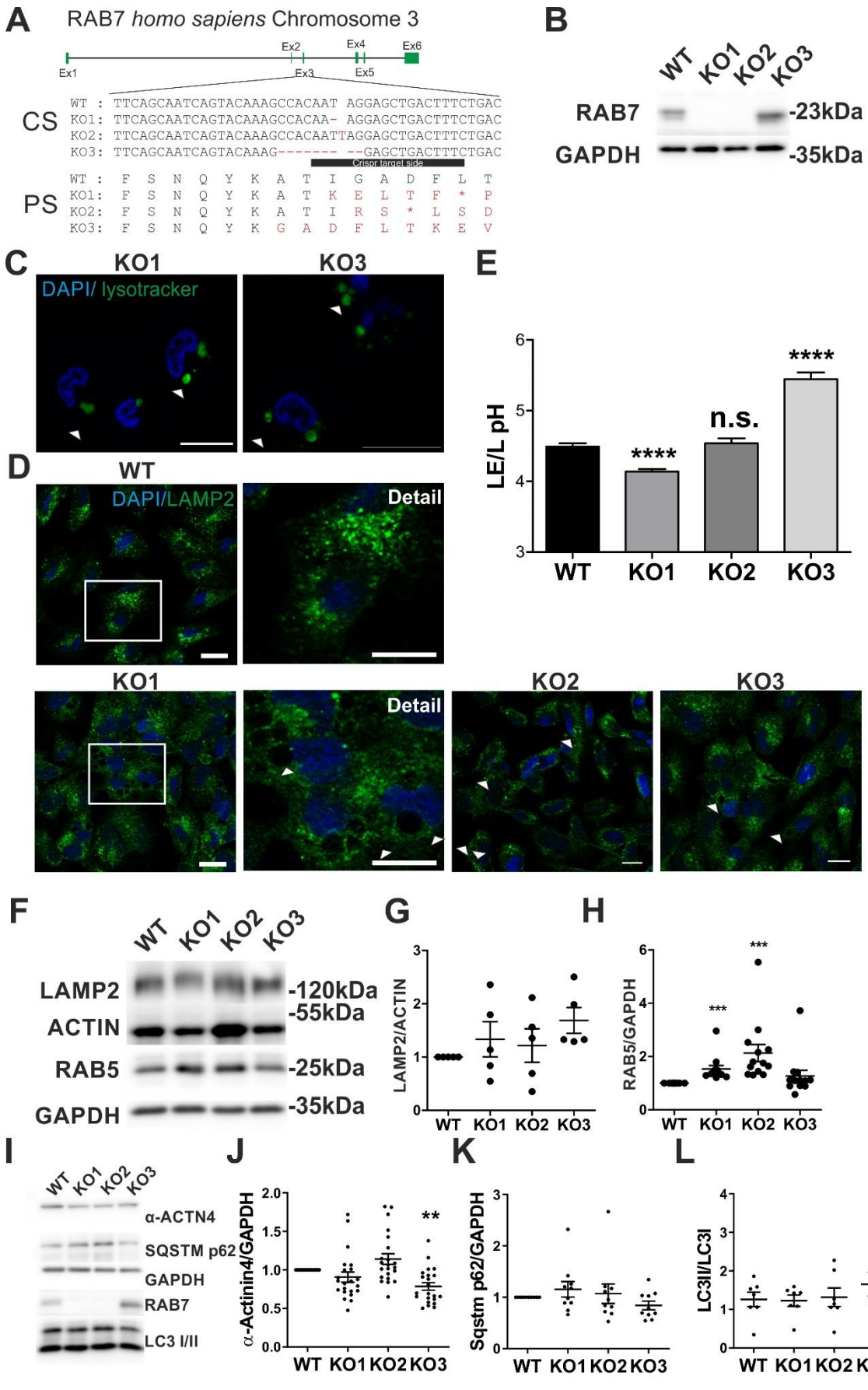
Supplemental Figure 3: Additional information: Rab7 KO mice podocytes accumulate unprocessed late-endosome-like vesicles (ULLVS).

(A/B) Ultrastructural analyses: TEM images of 2-3-week-old mice. The SD is still intact in Rab7$^{fl/fl}$;*PodCre+* mice. (C/D) TEM images of 4-week-old mice. The GBM looks slightly thickened and FPE with disruption of the SD is partly detectable. (E/F) TEM images: Detailed view of the accumulating vesicles in 8-week-old Rab7$^{fl/fl}$;*PodCre+* mice. Accumulating ULLVs are spread throughout the podocyte (white arrow). The appearance of accumulating vesicles did not change during disease progression; we still observed large single membrane vesicles composed of intraluminal vesicles (white arrows). (G) TEM images: Higher magnification of the ULLV structures found in podocytes. Pod = podocyte; GBM = glomerular basement membrane; N = nucleus; B = blood vessel; FP = foot process.



Supplemental Figure 4: Rab7 knockdown in *Drosophila* nephrocytes results in vesicle accumulation.

Uptake of secreted ANF-GFP-GFP into nephrocytes. Shown are prepared control (*dsRNA* for Or83b) and Rab7 knockdown nephrocytes (*dsRNA1* and *2*) showing an accumulation of ANF-GFP-GFP in Rab7 knockdown nephrocytes. Acidic compartments were stained with LysoTracker Red. Knockdown in nephrocytes was accomplished by employing *Sns-Gal4*. Zoom-in images are shown to the right. Scale bar: 10 µm. N=3.

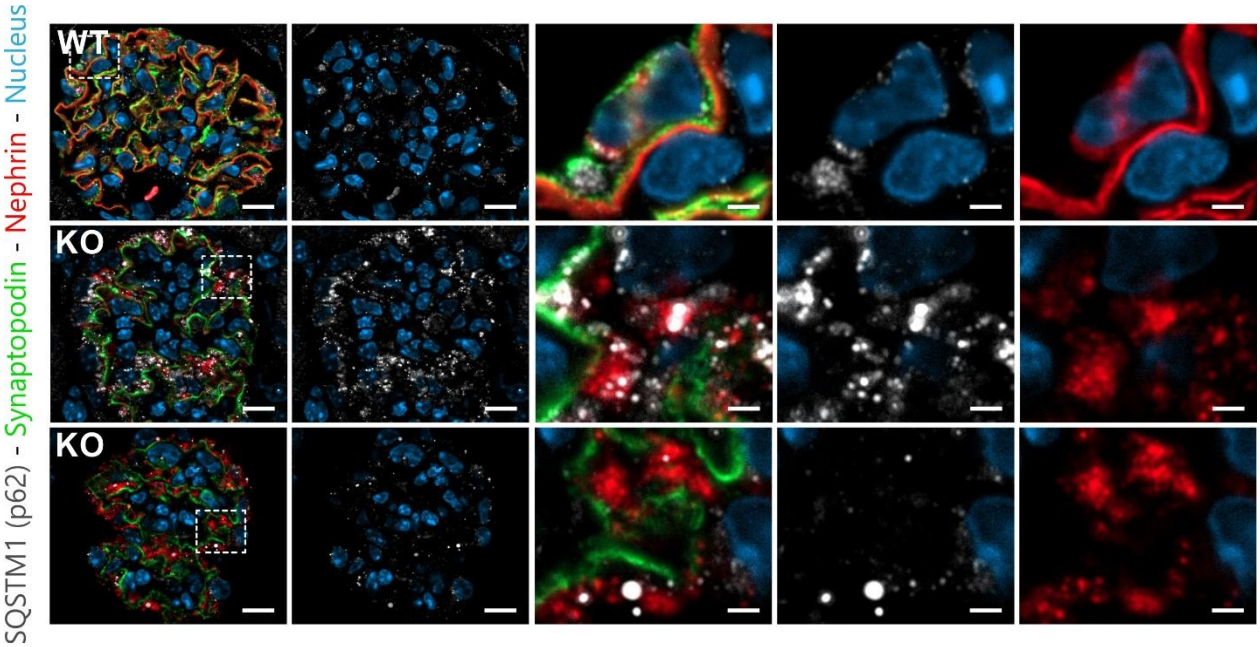

A
RAB7 homo sapiens Chromosome 3
Ex1
Ex2
Ex3
Ex4
Ex5
Ex6
CS
WT : TTCAGCAATCAGTACAAAGCCACAAT AGGAGCTGACTTTCTGAC
KO1: TTCAGCAATCAGTACAAAGCCACAA- AGGAGCTGACTTTCTGAC
KO2: TTCAGCAATCAGTACAAAGCCACAATTAGGAGCTGACTTTCTGAC
KO3: TTCAGCAATCAGTACAAAG------- --GAGCTGACTTTCTGAC
Crispr target side
PS
WT : F S N Q Y K A T I G A D F L T
KO1: F S N Q Y K A T K E L T F * P
KO2: F S N Q Y K A T I R S * L S D
KO3: F S N Q Y K G A D F L T K E V
B
WT
KO1
KO2
KO3
RAB7
-23kDa
GAPDH
-35kDa
C
KO1
KO3
DAPI/ lysotracker
D
WT
DAPI/LAMP2
Detail
KO1
Detail
KO2
KO3
E
LE/L pH
****
n.s.
****
WT
KO1
KO2
KO3
F
WT
KO1
KO2
KO3
LAMP2
-120kDa
ACTIN
-55kDa
RAB5
-25kDa
GAPDH
-35kDa
G
LAMP2/ACTIN
H
RAB5/GAPDH
***
***
I
WT
KO1
KO2
KO3
α-ACTN4
SQSTM p62
GAPDH
RAB7
LC3 I/II
J
α-Actinin4/GAPDH
**
K
Sqstm p62/GAPDH
L
LC3II/LC3I

Supplemental Figure 5: RAB7 depletion in podocytes disrupts the fine-tuning of intraluminal acidification.

(A) RAB7 editing confirmation by sequencing in three *CRISPR/Cas9*-edited clones with different protein sequences. Clone 1 and 2 exhibited frameshift mutations, while clone 3 contained a deletion of 3 amino acids. AS: coding DNA sequence, PS: amino acids sequence **(B)** RAB7 expression was detected by Western blotting. As clone KO3 showed an in-frame deletion, its expression is recognized by anti Rab7 antibodies in WB analyses. GAPDH served as loading control. **(C/D)** Images of WT and RAB7 KO cell lines. Cells were stained by the live cell tracker LysoTracker green (C). The RAB7 depleted human podocyte cell lines 1 and 3 show enlarged LysoTracker positive vesicles. (D) Additional immunofluorescence staining with anti-Lamp2 for cell line 2 and 3. N=3 Immunofluorescence analyses using with antibodies against LAMP2 reveals huge LAMP2-positive vesicles in RAB7 depleted cell lines (white arrowheads); Scale bar 20 µm.**(E)** Determination of the lysosomal pH by dextranes attached to pH sensitive dyes as described before by Canton and Grinstein [25]. LE = late endosomes, L = lysosomes, shown is mean with SEM; ***p< 0.0001 in a Kruskal- Wallis test, N=2.

(F-H) Western blot analysis shows that LAMP2 expression is not significantly altered in Rab7 KO lines. The expression of the early endosome marker RAB5 is slightly increased in Rab7 KO cell lines 1 and 2. Blots were quantified from at least 3 independent experiments and normalized to GAPDH and ACTIN and the control cell line. N>3.

(I-L) Western blots: Autophagy markers were detected by WB using antibodies against Rab7, p62/Sqstm1, LC3I/II. α-Actinin-4 and GAPDH served as loading controls. P62 was slightly upregulated in RAB7 KO cell lines 1 and 2 and downregulated in cell line 3. The LC3II/LC3I ratio was determined and does not show changes in cell line 1 and 2 but an increase in cell line 3. Quantification was done from at least three independent experiments.



Supplemental Figure 6: Altered nephrin distribution in podocyte-specific Rab7 depleted mice only minorly co-localizes with p62/ Sqstm1 positive structures.

Images show p62/Sqstm1 distribution in glomeruli of 8- week old WT control and Rab7$^{fl/fl}$;*PodCre+* mice. Nephrin (red), Synaptopodin (Synpo, green), and Nuclei (Dapi, blue) were stained as co-marker. Nephrin staining shows a vesicular like pattern and translocation towards the podocyte cell body apical of Synpo in KO animals. Synpo shows intact localization to the basolateral podocyte compartment. Analysis of Sqstm1 shows enriched signals in some glomeruli of Rab7$^{fl/fl}$,*PodCre+* mice, but only minor co-localization with Nephrin positive structures. P61/Sqstm1 signals had a wide spectrum in expression levels when comparing several glomeruli.