Supplemental material

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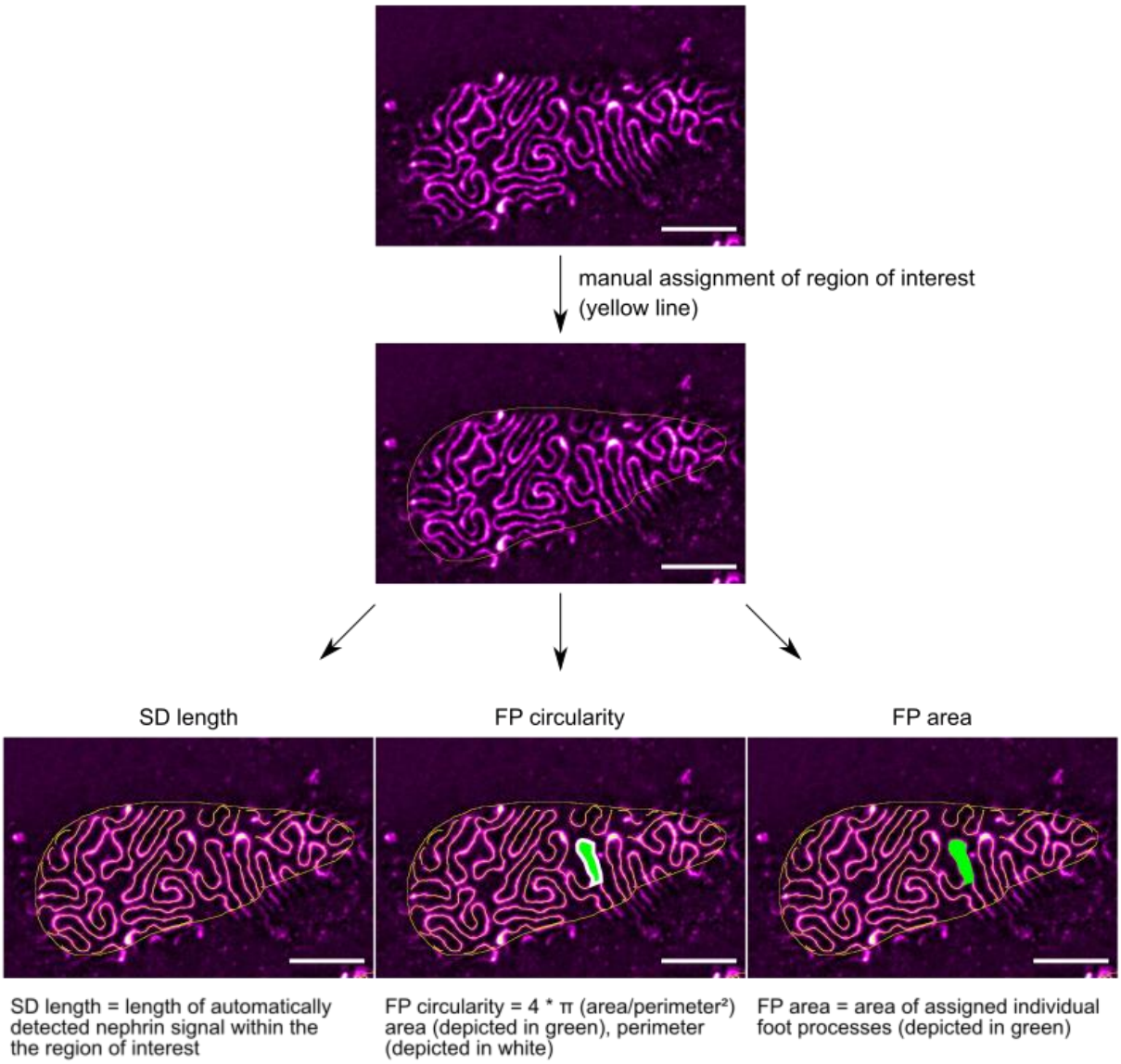
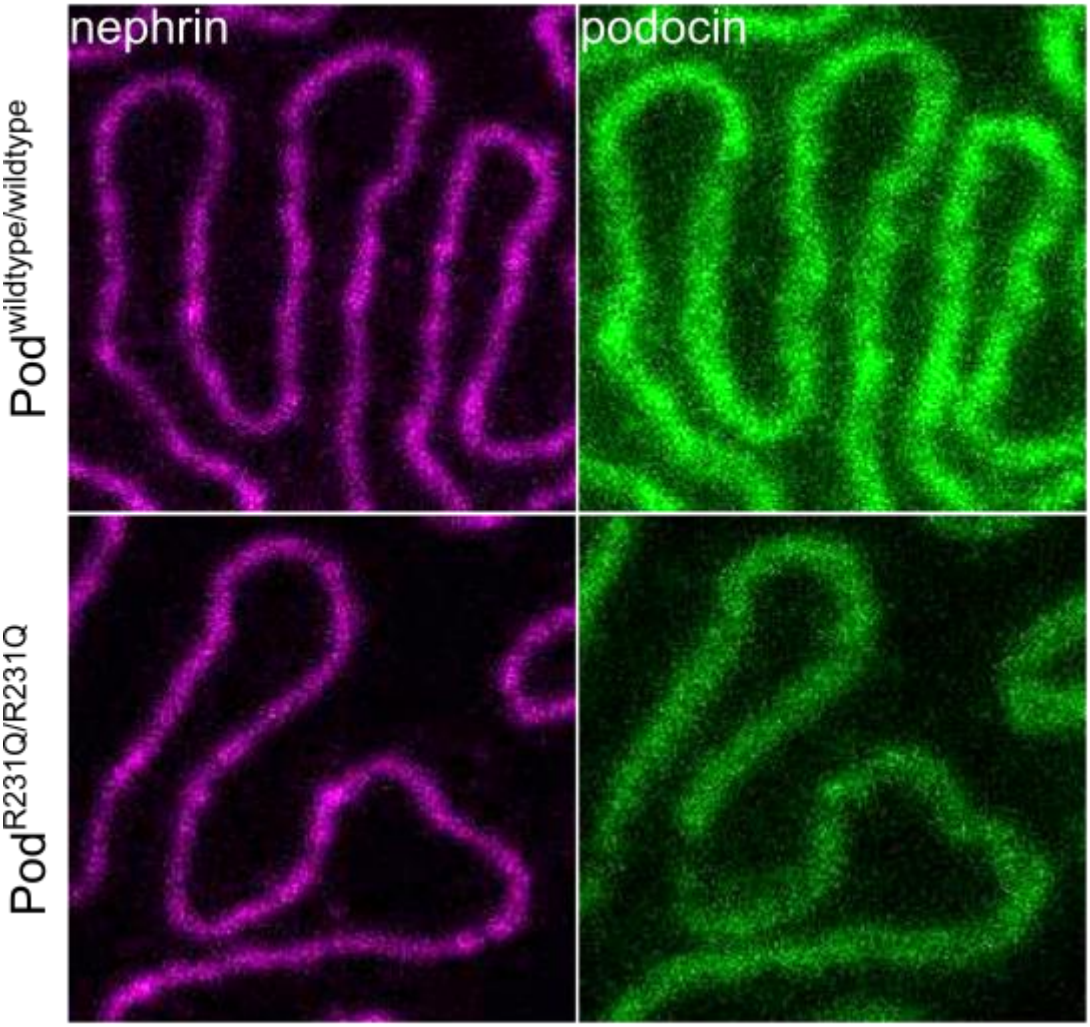
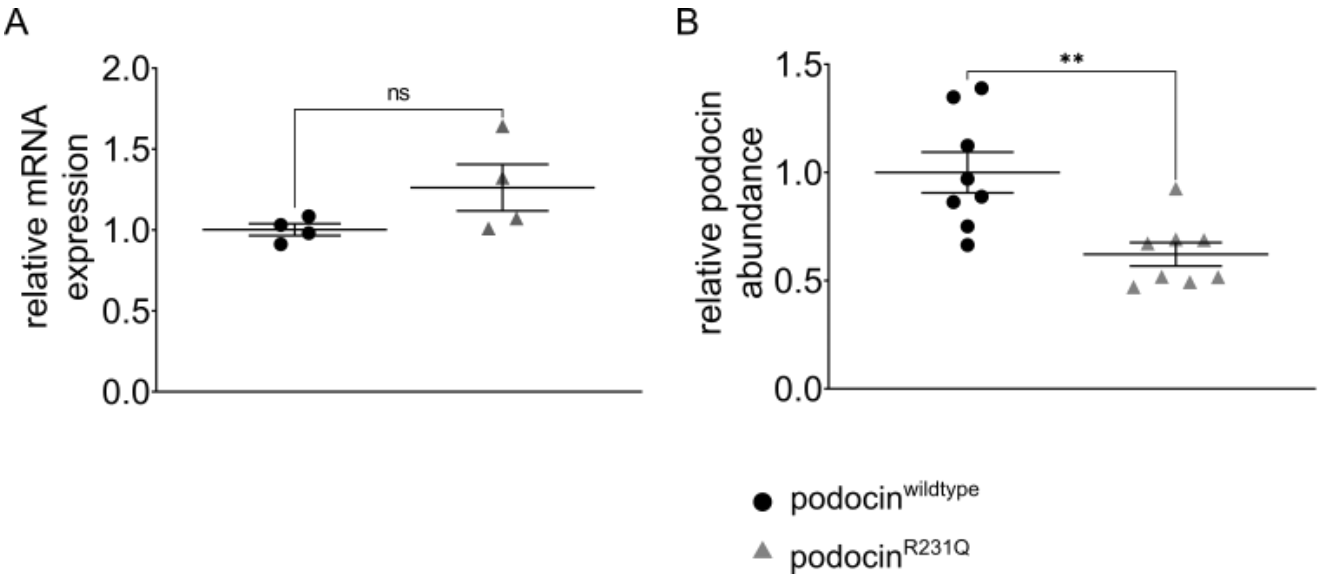
Suppl Fig. 1-3



Suppl. Fig 1: Quantification of morphological parameters. Workflow of the quantification of SD length, FP circularity and FP area. The region of interest is manually assigned. The nephrin signal within the region of interest is automatically detected and measured to obtain values for the SD length per area. For FP circularity and area, individual foot processes are assigned by closing the the foot process at its base using the line tool. The area of each foot process is automatically quantified, the circularity is automatically calculated using the perimeter (depicted in white) and the area (depicted in green). One individual foot process is highlighted for illustration purposes. Scale bars correspond to 2 µm.



Suppl. Figure 2: The fluorescence intensity ratio of podocin to nephrin is decreased in *Pod*$^{R231Q/R231Q}$ mice as compared to wildtype litter mates. STED-microscopy using equal laser power after immunolabeling nephrin and podocin reveals lower a lower fluorescence intensity of podocin relative to nephrin in *Pod*$^{R231Q/R231Q}$ mice (lower panels). Panels are 2 x 2 µm in size.



Suppl. Fig. 3: PodocinR231Q is less abundant in immortalized human cultured podocytes. (A) Quantitative PCR of podocin cDNA in human cultured podocytes expressing FLAG-tagged podocin^{wildtype} (black circles) or podocin^{R231Q} (grey triangles). HPRT was used as house-keeping gene. mRNA levels were not significantly different (n = 4 biological replicates). (B) Abundance of FLAG-tagged podocin^{wildtype} (black circles) or podocin^{R231Q} (grey triangles) in human cultured podocytes as determined by densitometry in western blots normalized to human actin. FLAG-tagged podocin^{R231Q} was significantly less abundant than podocin^{wildtype} (n = 8 biological replicates). Mann-Whitney-U-Test were used to determine statistical significance in (A) and (B). ** $p < 0.01$, Data are presented as mean ± SEM.