Supplemental Table 1. Filtering process for variants from normal reference sequence (VRS) following WES in families A1389, A982, A1436 and B324 affected with nephrotic syndrome.

FAMILY	**A1389**	**A982**	**A982**	**A1436**	**B324**
Individual	**A1389-21**	**A982-21**	**A982-22**	**A1436-24**	**B324-21**
Consanguinity	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
[a]# of homozygosity peaks	**22**	**16**	**21**	**24**	**15**
Cumulative Homozygosity by descent[b] [Mb]	**256**	**128**	**425**	**228**	**370**
[b]Hypothesis from mapping: homozygous (H), heterozygous (h)	**H**	**H**	**H**	**H**	**H**
Total reads (millilon)	**68.3**	**80.1**	**177.4**	**93.5**	**82.5**
Matched reads in pairs	**96.5%**	**91.1%**	**86.0%**	**86.5%**	**94.3%**
Matched reads in broken pairs	**3.5%**	**5.0%**	**8.4%**	**9.1%**	**4.6%**
Total number variants detected	**346,026**	**452,352**	**609,501**	**629,747**	**426,047**
Reject common dbSNP137; MAF>1%	**141,683**	**208,082**	**357,108**	**448,975**	**215,740**
Keep VF>=55% AND Cov >=2	**62,950**	**122,768**	**239,401**	**244,034**	**129,135**
Keep non-synonymous and splice (+6-3/CLC)[c]	**1,295**	**2,455**	**2,738**	**2,090**	**1,299**
Keep located in homozygosity peaks	**156**	**179**	**901**	**137**	**99**
(Located within Splice Site)	**4**	**13**	**161**	**2**	**20**
(Deletion/Insertion)	**43**	**89**	**65**	**14**	**10**
(Stop gained/Stop lost)	**0**	**6**	**4**	**2**	**0**
(Missenses)	**109**	**71**	**671**	**119**	**69**
Remaining variants after inspection	**11**	**14**	**9**	**13**	**12**
Sanger confirmation / Segregation	**4**	**1**	**1**	**2**	**2**
Remaining genes	***KANK1, SOC5, TBC1D2B, GRIP1***	***KANK2***	***KANK2***	***KANK4, THBS3***	***KANK4, THBS3***

[a]See **Figure 1**.

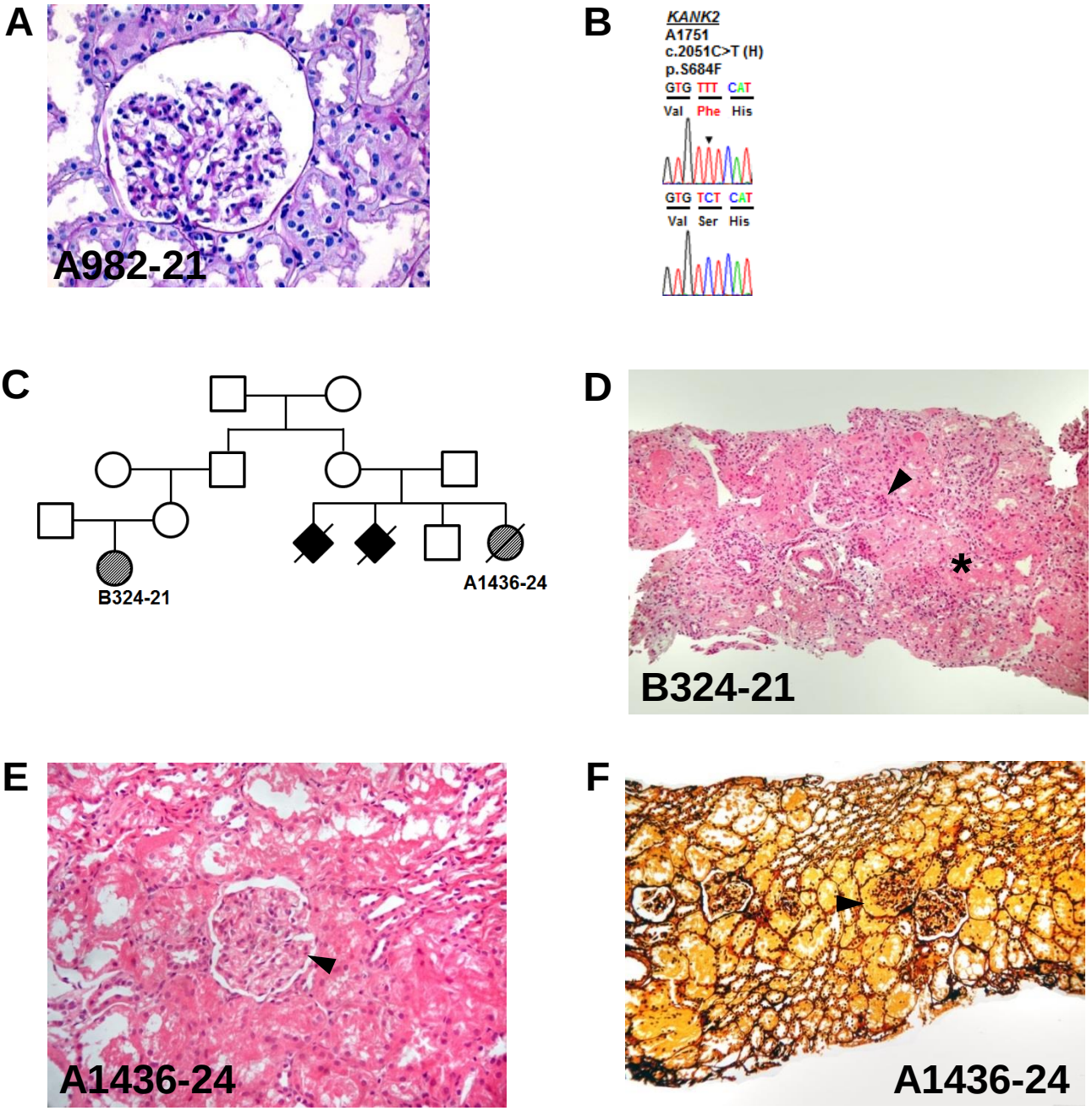
[b]Evaluation for homozygous variants was done in regions of homozygosity by descent.

[c]For variants which potentially affect spicing process, +6 to -3 bases of exon-intron junction were examined using CLC Genomics Workbench (CLC bio).

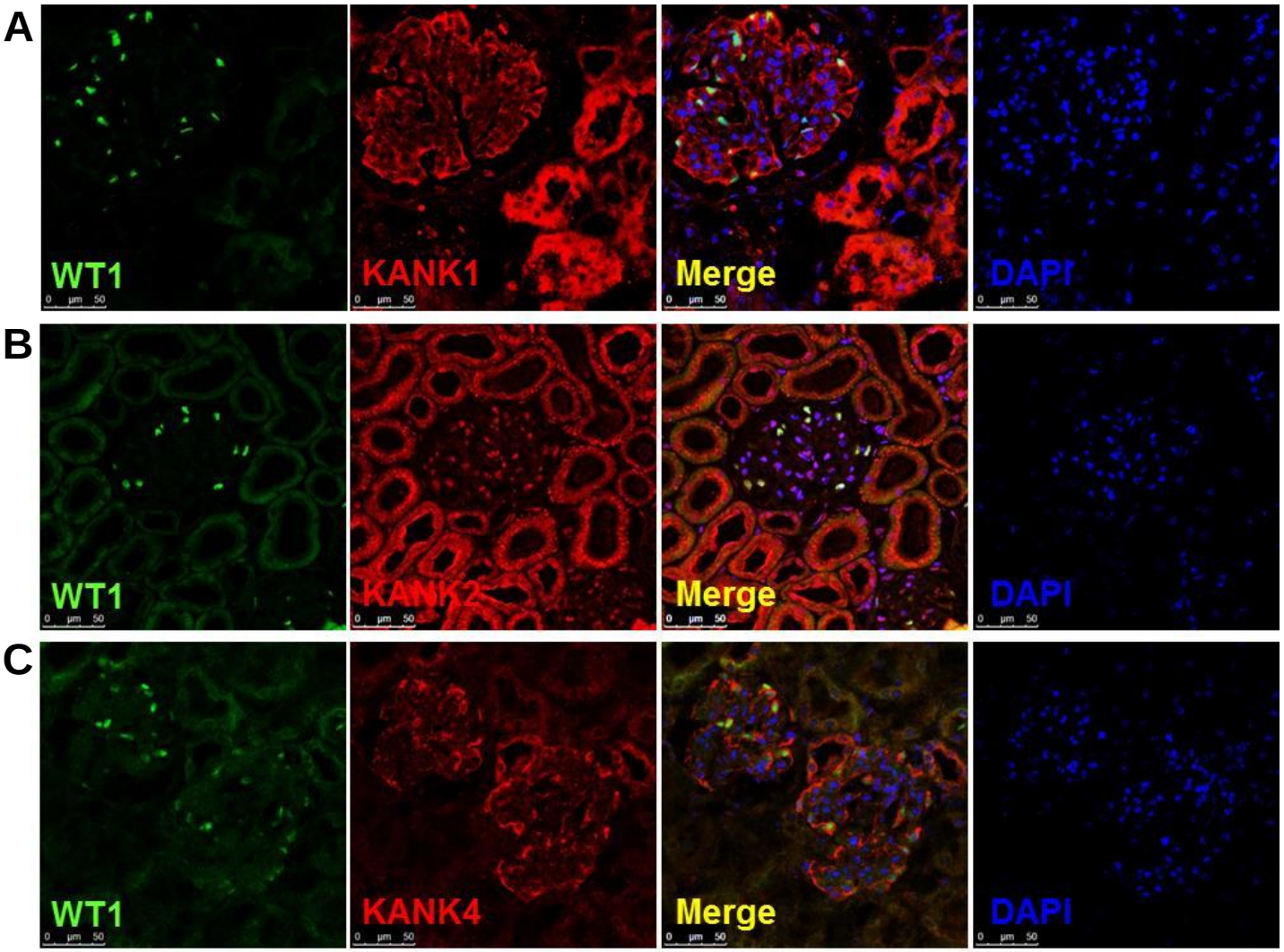
Cov, coverage; SNPs, single nucleotide polymorphism; VF, variant frequency;

Supplemental Table 2. Oligonucleotide sequences Used in this study.

Morpholino oligonucleotide (MO)		
kank2 ATG MO	ATGAAGCACCTGAGCCATGATTGTC	
kank2 e2i2 MO	CTCCATGCCTTGAAAAACAGTGAA	
Reverse transcription PCR		
kank2	TTGTCCACACCTGTCTCCC	GAGCACTCTGTAGCTCTTGC
Target sequences of siRNAs		
KANK1 siRNA	GGTCAGTCACGGACGGATA	
	GGATAAAGGAGTTCCGGCA	
	CAGAGAAGGACATGCGGAT	
	GAAGTCAGCGTCTGCGAAA	
KANK2 siRNA	GAACGGGACTTGGGCATGC	
	GACGAGAGCCCTACATCAT	
	CAGCTCACAGTACAACTTA	
	CGTGCGATCTATCATGAAA	
Kank2 siRNA	AAGAAGATCAGCATTACAGAA	
	AAGAAGATCAGCATTACAGAA	

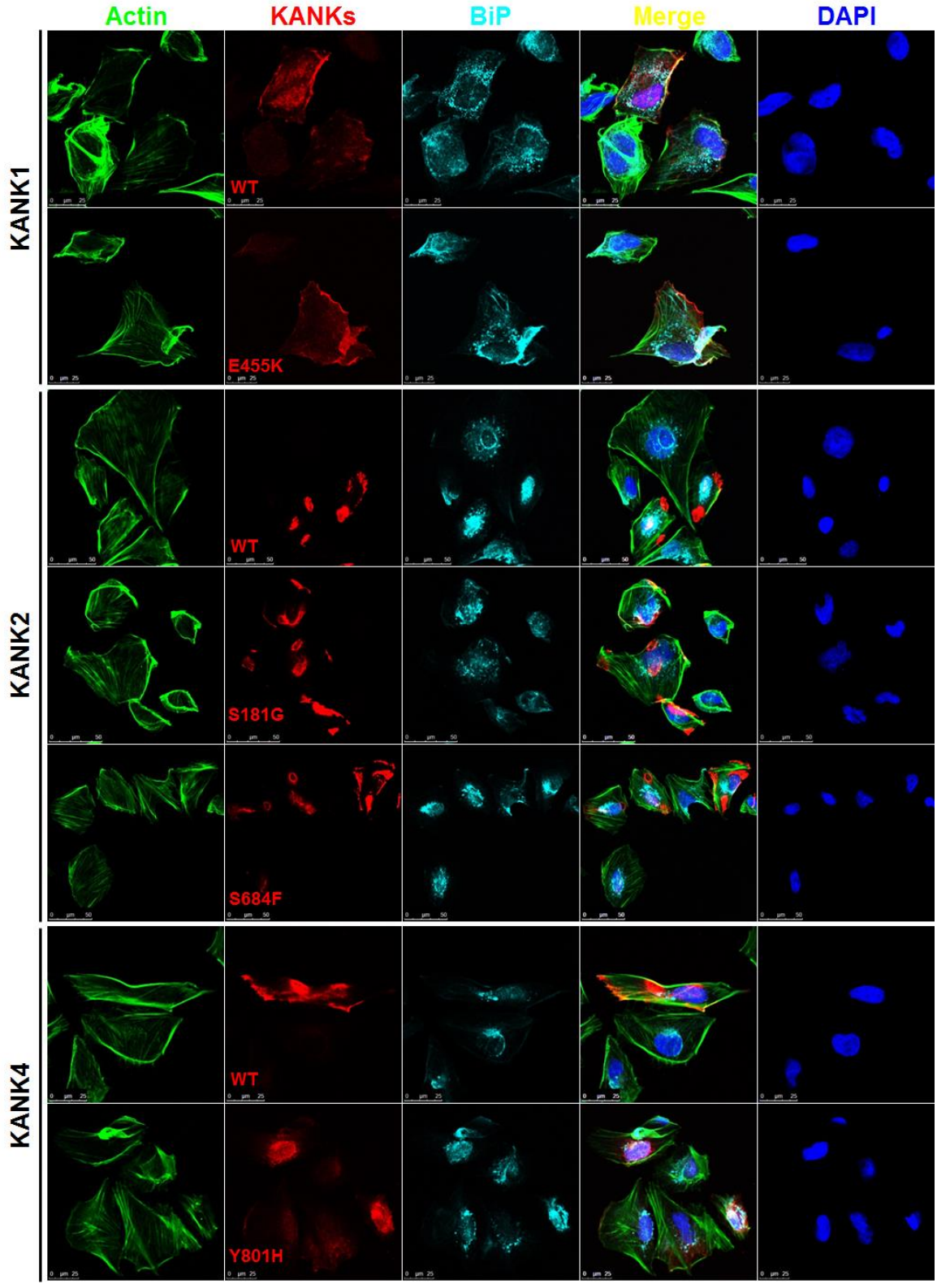


Supplemental Figure 1. Clinical information of families with nephrotic syndrome.
(A) Renal histology of A982-21 shows minimal change disease.
(B) Chromatogram of a *KANK2* mutation identified in individual A1752-21 with SSNS. Gene symbol (underlined), family number, mutation, and predicted translational changes are given (see also **Table 1**). Sequence traces are shown for the variant above normal control. Mutated nucleotide is indicated by arrowheads.
(C) Pedigree of B324 and A1436, who are maternal cousins.
(D) Renal histology of B324-21 shows focal segmental glomerulosclerosis (FSGS) (arrowhead) and tubulointerstitial fibrosis (asterisk).
(**E-F**) Renal histology of A1436-24 shows FSGS (arrowhead).



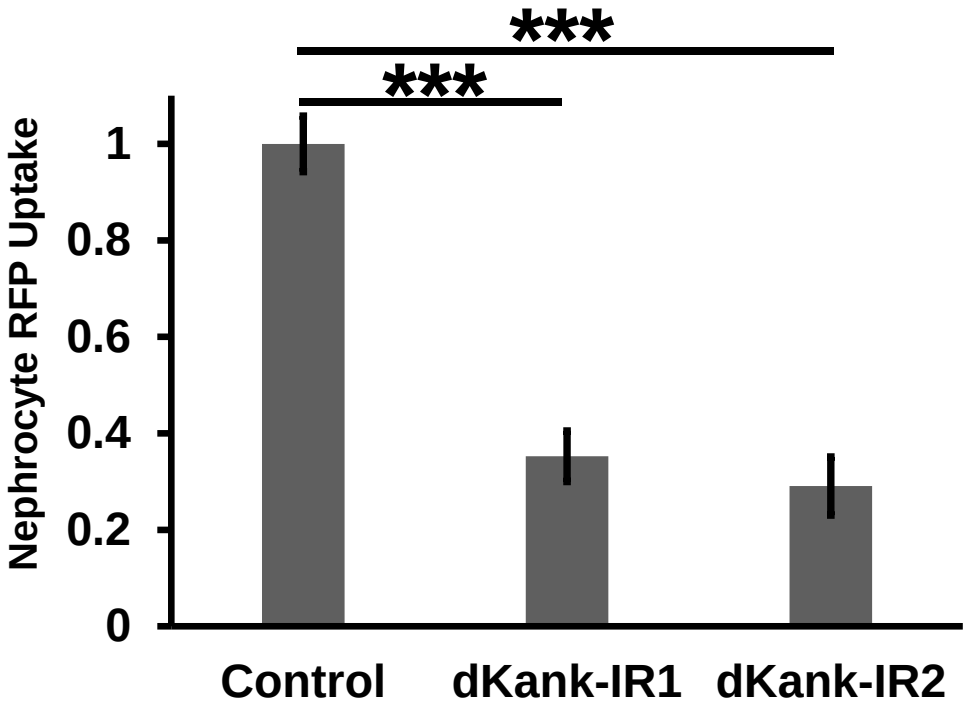
Supplemental Figure 2. Localization of KANK2, KANK2 and KANK4 in adult rat kidney.

(A) Coimmunofluorescence of KANK1 with WT1, which stains the nuclei of podocytes.
(B) Coimmunofluorescence of KANK2 with WT1.
(C) Coimmunofluorescence of KANK4 with WT1.

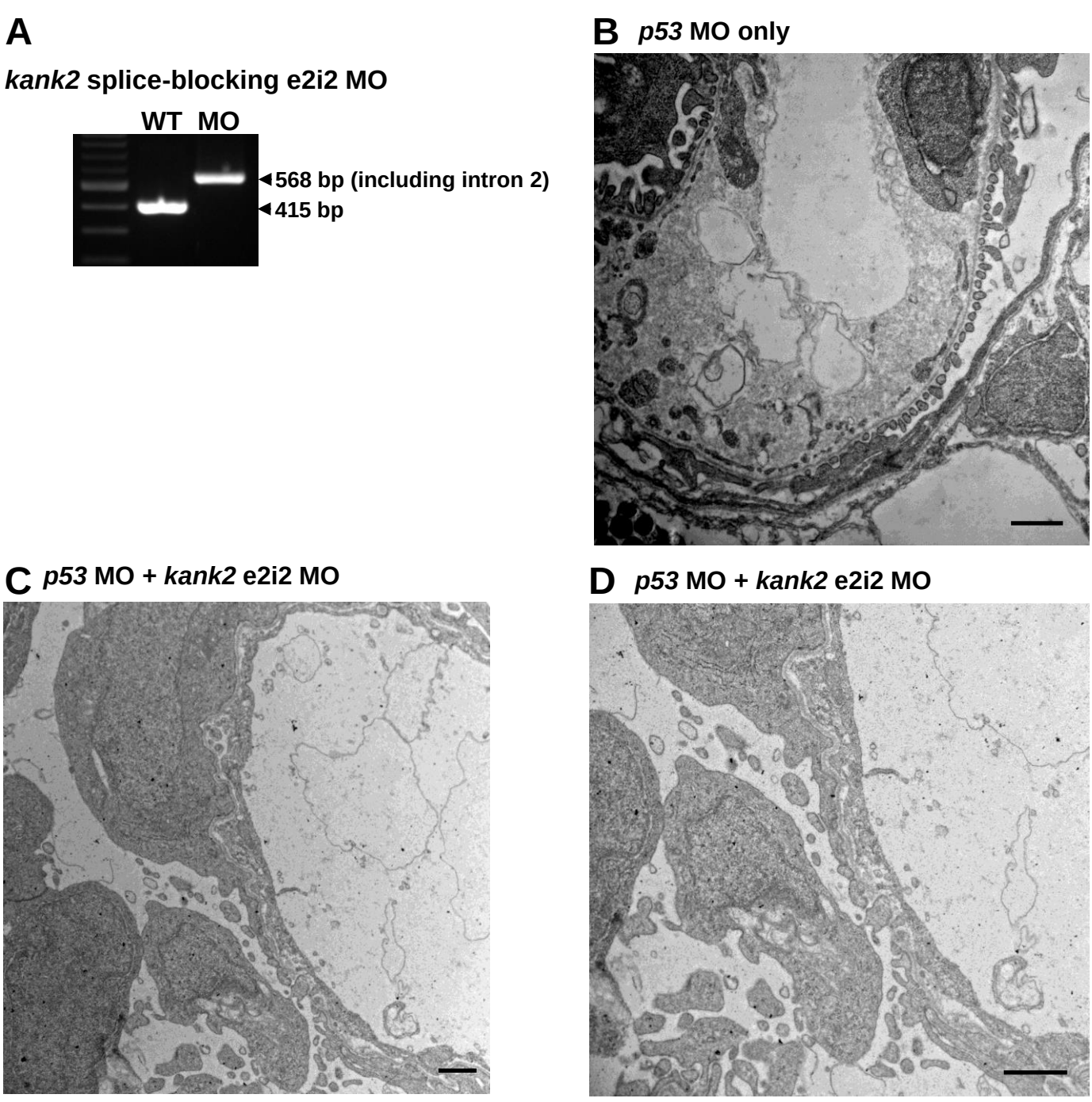


Supplemental Figure 3. Immunofluorescence of KANK1, KANK2 and KANK4 in culture human podocytes.

Podocytes were transfected with Myc-tagged wild type and variant clones of KANK1, KANK2 and KANK4. Cells were fixed with 4% paraformaldehyde, permeabilized with 0.1% SDS and immunostained with Alexa Fluor 488 phalloidin for actin, an ER maker BiP and an anti-Myc antibody. Overexpressed KANK proteins predominantly localize to cytoplasm of podocytes, but none of variant KANK protein localization shows any distinction from wild type (WT).



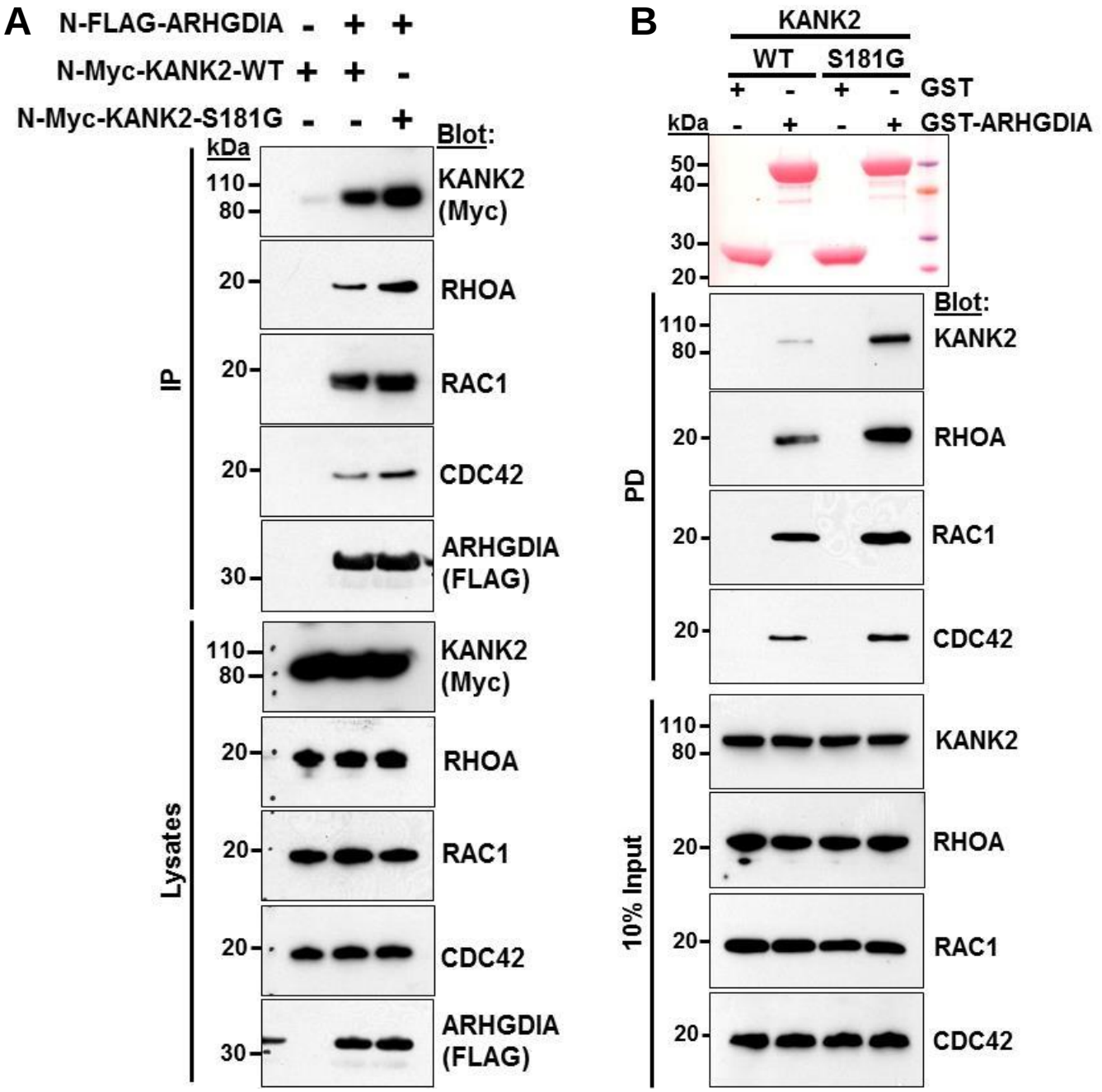
Supplemental Figure 4. RNAi knockdown of *dKank* using Dot-Gal4 significantly reduced the fluorescent protein uptake in nephrocytes. The ANF-RFP intensities of dKank-IR1 and dKank-IR2 were normalized to the control. Data were expressed as means ± standard error (***p<0.001).



Supplemental Figure 5. *kank2* knockdown in zebrafish.

(A) RT-PCR was performed to detect the transcript of *kank2* in 48 hpf embryos. In embryos (WT) injected with 0.2 mM of *p53* MO only the normal splicing product (415 bp) appeared. In contrast, in embryos (MO) injected with 0.2 mM e2i2 MO targeting the donor site of intron 2, a splice product of 568 bp was detected that had retained intron intron 2 as confirmed by direct sequencing of the RT-PCR product.

(B-D) TEM images of control zebrafish injected with p53 MO (B) and of zebrafish injected with p53 and *kank2* e2i2 MOs (C-D). D is the inset of E. These TEM images are representative of two control and three *kank2* morphants. Scale bars are 2 μm.

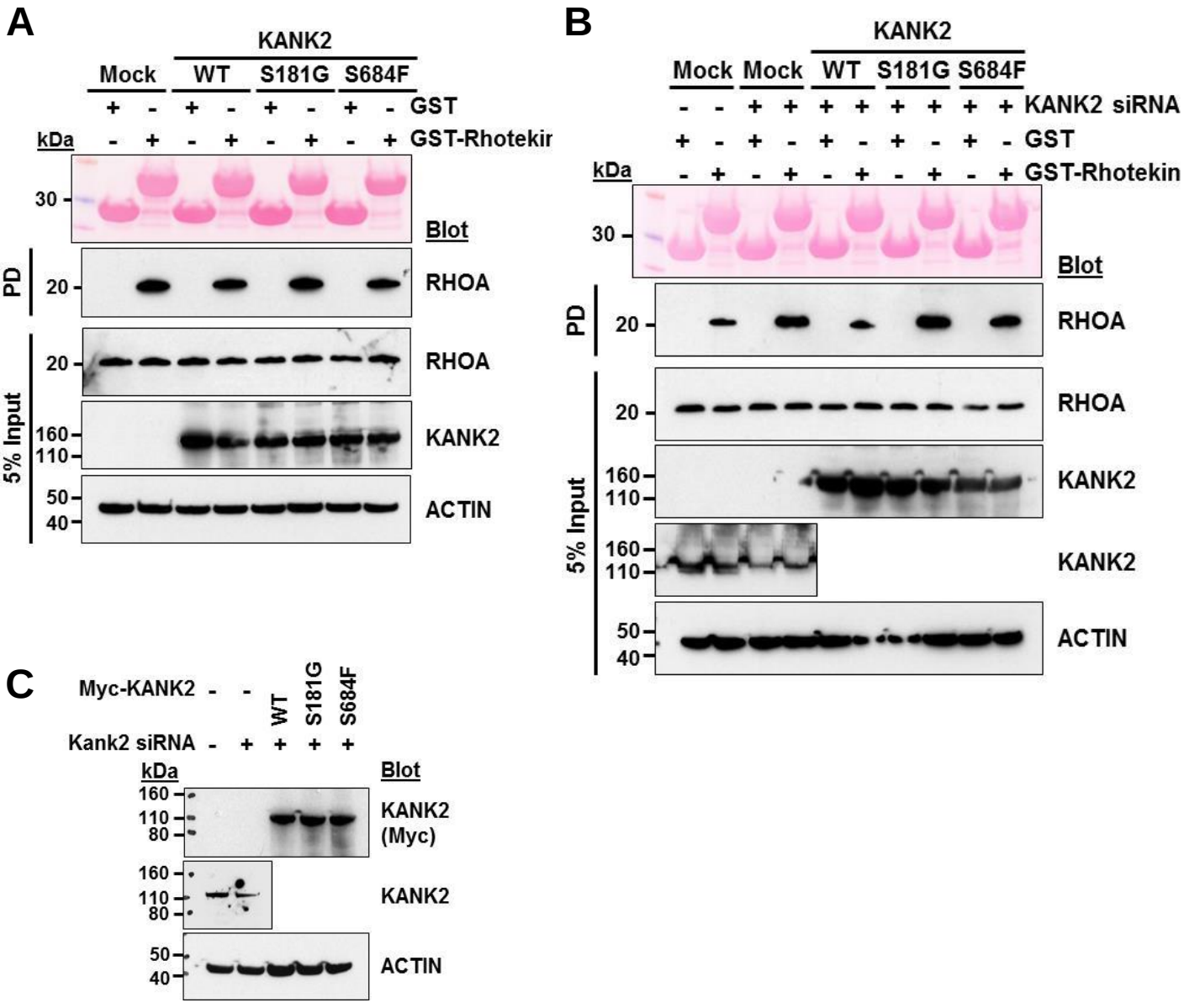


Supplemental Figure 6. **Effects of KANK2 p.S181G variant protein on protein-protein interaction.**

(A) Interaction of wild type (WT) and p.S181G of KANK2 with ARHGDIA and RHO GTPases in cultured human podocytes. FLAG-tagged WT ARHGDIA, Myc-tagged KANK2 (WT and p.S181G) constructs were transfected into podocytes and, using an anti-FLAG antibody, were coimmunoprecipitated with endogenous RHO proteins. Note that, compared to KANK2 WT, the KANK2 p.S181G showed stronger affinity to ARHGDIA.

(B) GST pulldown of purified ARHGDIA with KANK2 (WT and p.S181G). Podocytes were transfected with Myc-tagged KANK2 constructs. KANK2 p.S181G showed enhanced interaction with ARHGDIA. Note that when KANK2 p.S181G was present, the amounts of endogenous RHOA, RAC1 and CDC42, pulled down by ARHGDIA, increased.

IP and PD denote immunoprecipitation and pulldown, respectively. All IP and PD experiments are representative of more than 3 experiments.



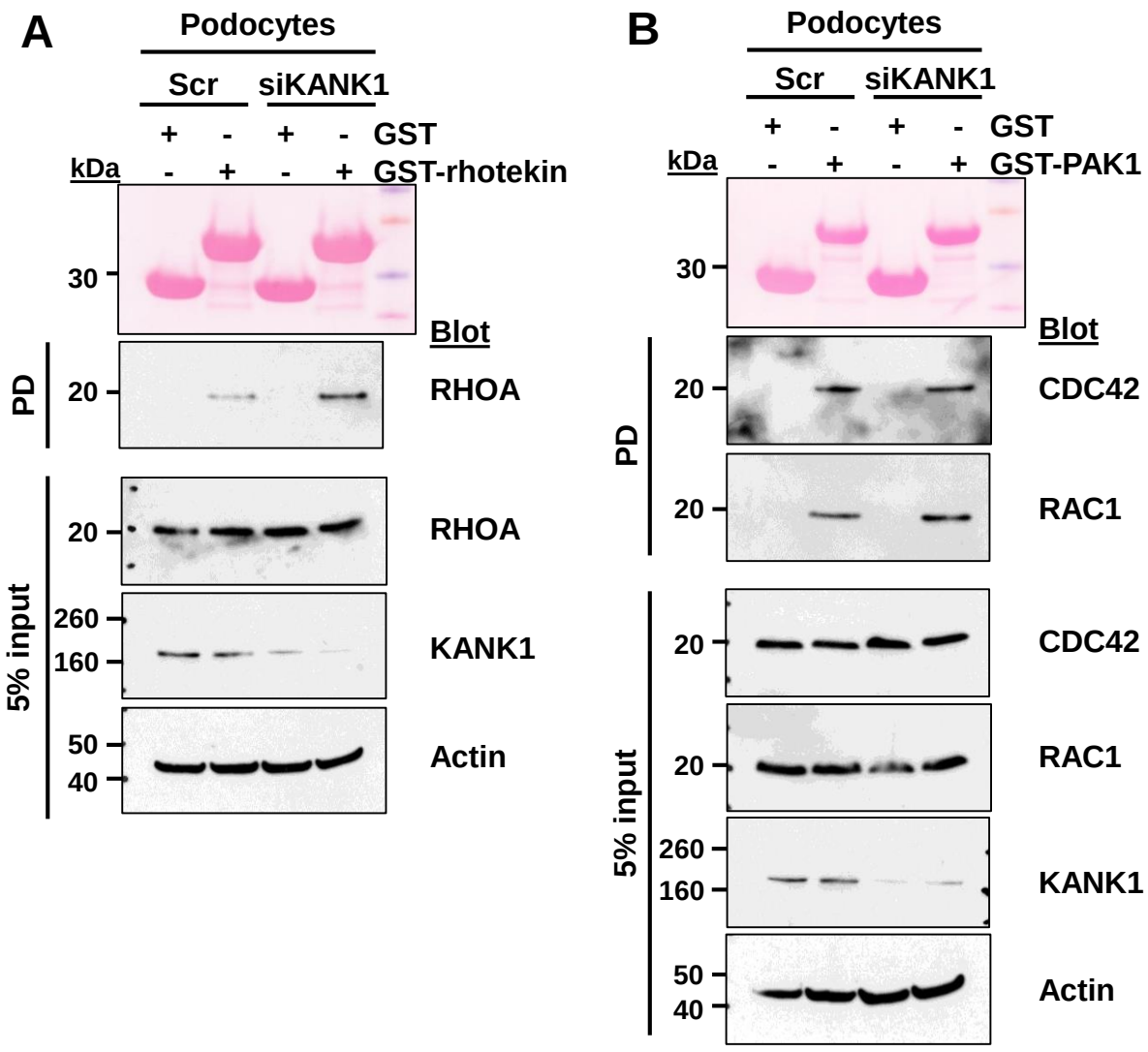
Supplemental Figure 7. **Effects of *KANK2* mutations on RHOA activity.**

(A) Effect of KANK2 overexpression on RHOA activity. Overexpression of KANK2 wild type (WT), p.S181G or p.S684F protein does not affect active GTP-bound RHOA in murine cultured podocytes.

(B) Effect of *Kank2* knockdown on RHOA activity. Kank2 knockdown in murine podocytes resulted in increased GTP-bound RHOA. This increased RHOA was reversed by overexpression of wild type KANK2, but not by overexpression of p.S181G or p. S684F KANK2. IP and PD denote immunoprecipitation and pulldown, respectively.

(C) Efficiency of *Kank2* knockdown in cultured murine podocytes. Upon transfection of Kank2 siRNA, KANK2 is significantly reduced. The upper panel blot also shows the expression level of rescue constructs.

All PD and Western blot are representative of more than 3 experiments.

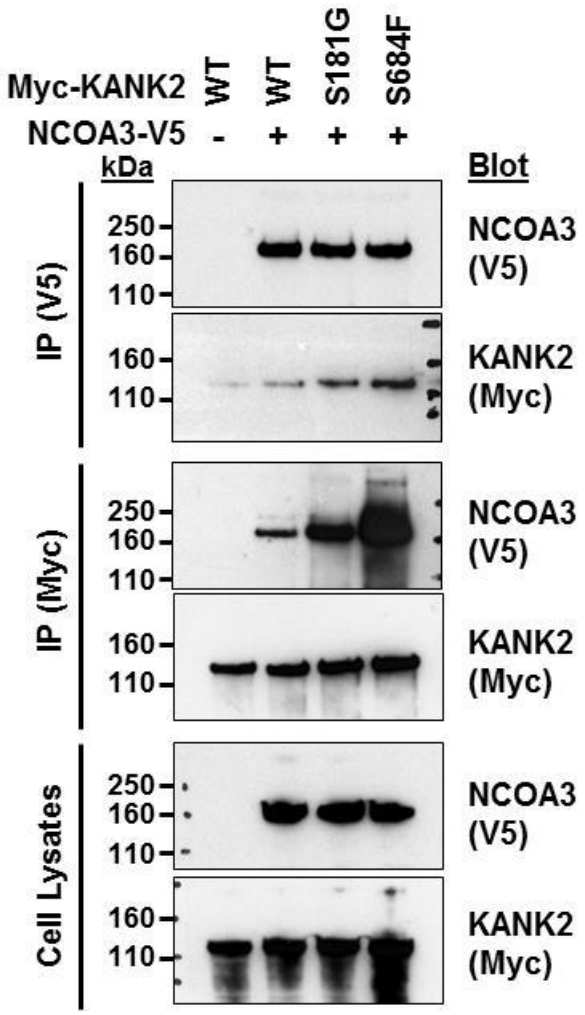


Supplemental Figure 8. Effects of KANK1 knockdown on RHO GTPases in cultured human podocytes.

(A) Active GTP-bound RHOA precipitated from cultured human podocytes transfected with scrambled (Scr) or KANK1 siRNA using a GST-Rhotekin (RBD) pulldown assay. Ponceau red staining at the top shows the GST proteins used. Compared to control podocytes, podocytes transfected with KANK1 siRNA exhibited an increase in GTP-bound form of RHOA. The efficiency of knockdown by siRNA was confirmed by immunoblotting with an anti-KANK1 antibody (second to lowest panel).

(B) Active GTP-bound forms of RAC1 and CDC42 precipitated from podocytes podocytes transfected with scrambled (Scr) or KANK1 siRNA using a GST-PAK1 (CRIB) pulldown assay. Cells transfected KANK1 siRNA exhibited increased active RAC1, but not CDC42.

PD denotes pulldown and PD experiments are representative of more than three experiments.



Supplemental Figure 9. **Effects of *KANK2* mutations on interaction with NCOA3**
Coimmunoprecipitation with Myc-tagged KANK2 and V5-tagged NCOA3 shows that KANK2 and interacts with NCOA3 when transiently overexpressed in human cultured podocytes. Coimmunoprecipitation was performed using both V5 (upper two panels) and Myc (middle two panels) antibodies. None of the two human KANK2 mutant proteins (p.S181G and p.S684F) abrogate this interaction. IP denotes immunoprecipitation and IP experiments are representative of three experiments.

Supplemental Methods

The sources for the clinical data and the blood samples were:

A1389-21 and **A1751-21**: Dr. Henry Fehrenbach (Memmingen)

A982 (-21; -22): Dr. Julia Hoefele (Martinsried) and Dr. Lutz T. Weber (Cologne)

A1436-24 and **B324-21**: Dr. Ludmila Podracka (Kosice) and Dr. Andrej Boor (Kosice)