SUPPLEMENTARY MATERIALS

A rare autosomal dominant variant in regulator of calcineurin type1 (*RCAN1*) gene confers enhanced calcineurin activity and may cause FSGS

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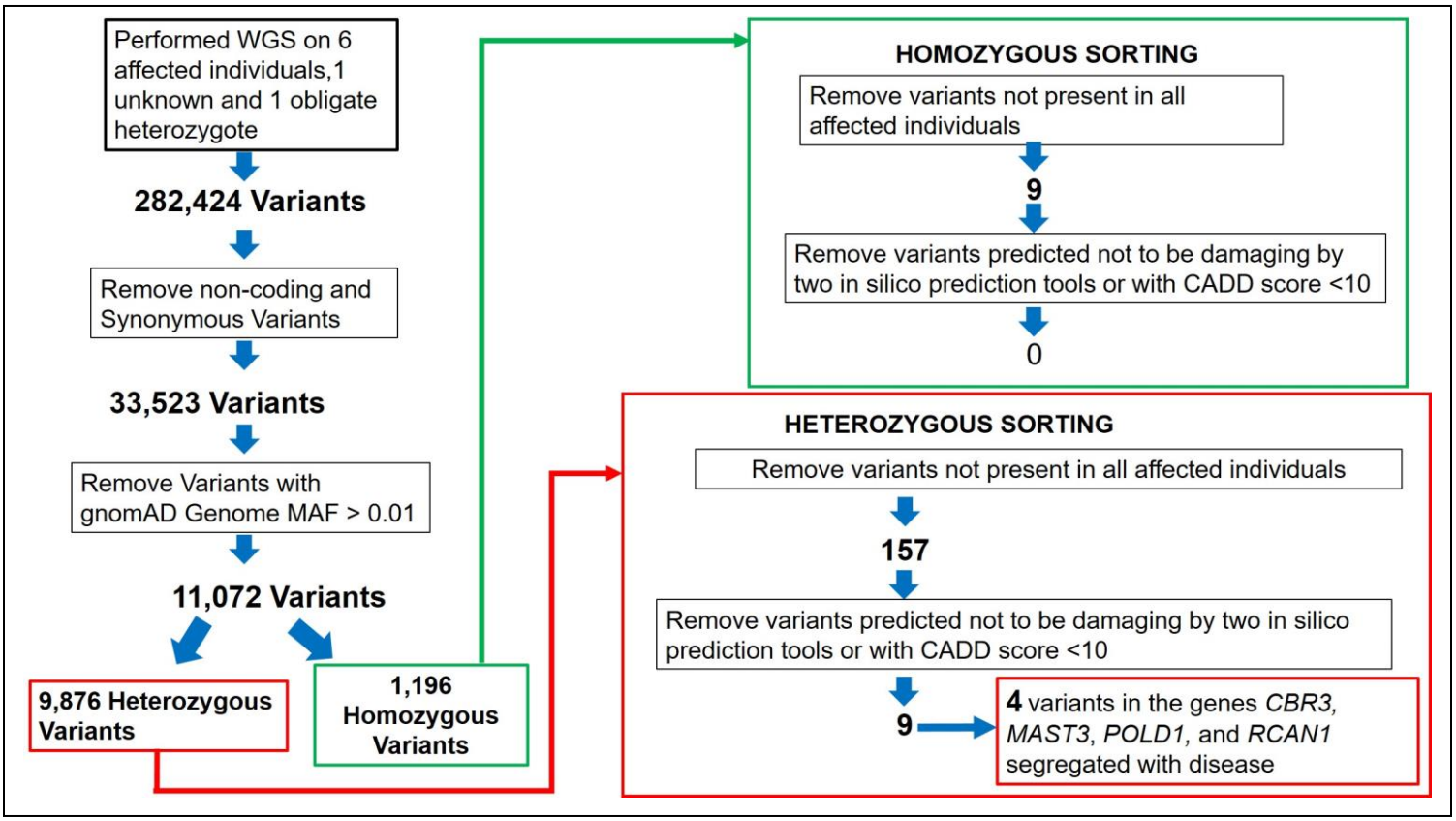
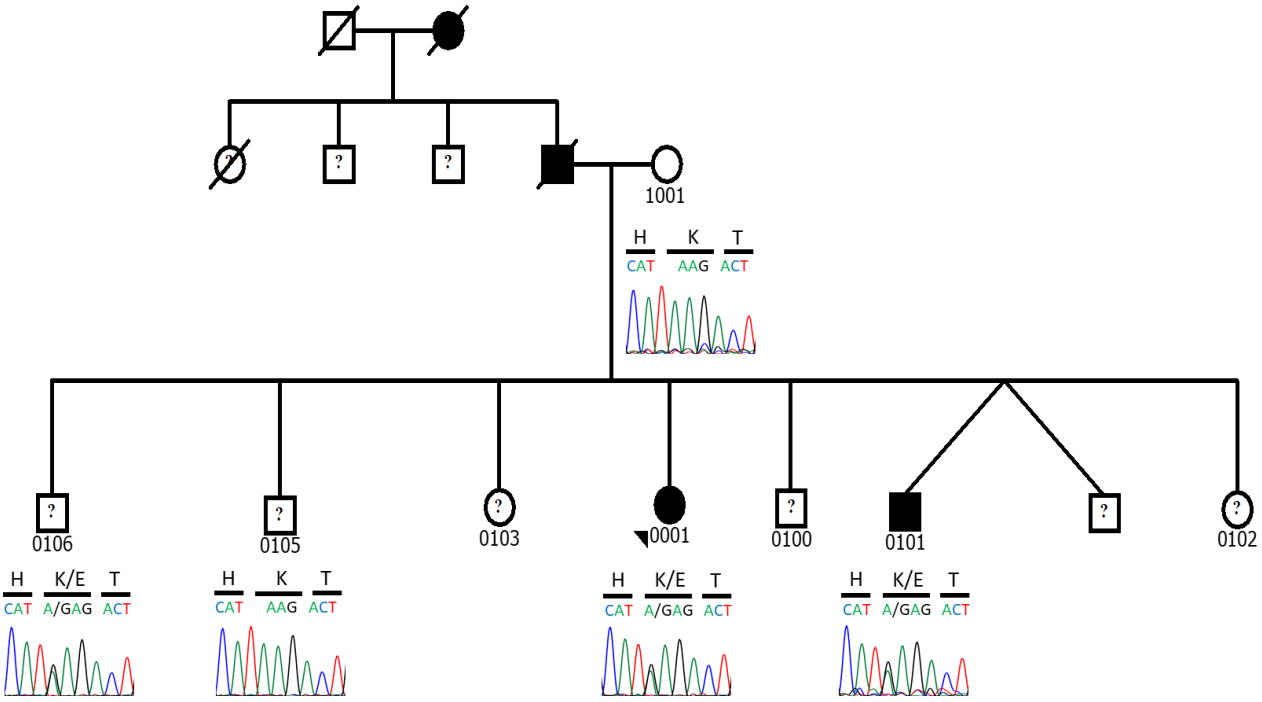
Supplementary Figure 1: Filtering of rare variants in WGS data from Family 40030

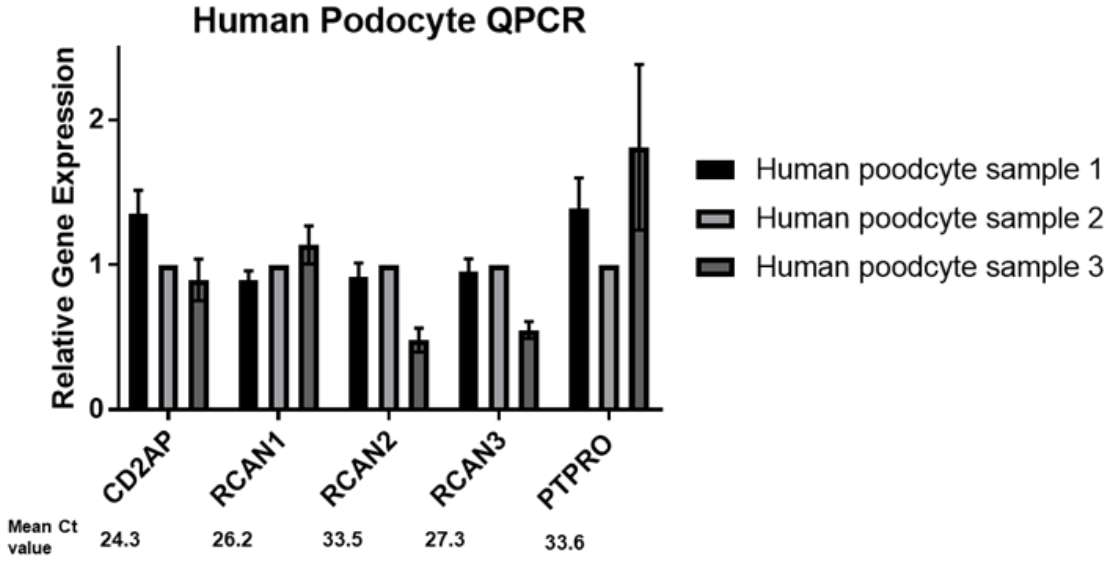


Supplementary Figure 2: Pedigree of second FSGS family with segregating RCAN1 variant



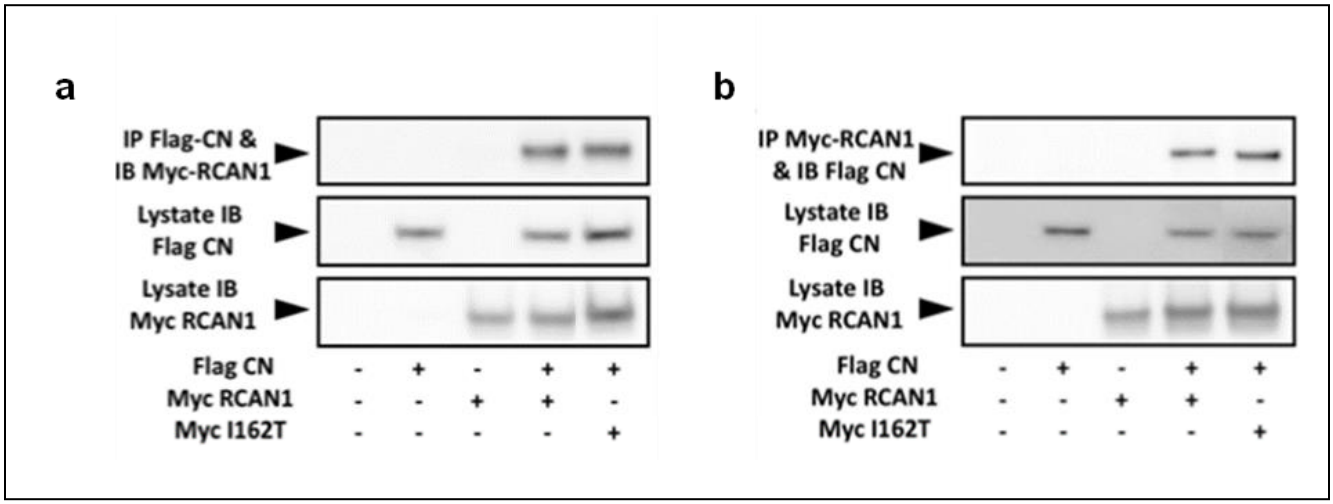
Supplementary Figure 3: *RCAN* gene expression in cultured Human podocytes

Quantitative Real-Time PCR analysis of three independent samples of differentiated conditionally immortalized human podocytes revealed that *RCAN1, RCAN2,* and *RCAN3* are expressed in the podocyte. The mean Ct values for each gene are listed to provide a relative comparison of *RCAN* genes expression to known podocyte genes *CD2AP* and *PTPRO (GLEPP1)*. These values suggest that *RCAN1-3* are expressed at a similar level to CD2AP and *PTPRO*. This analysis was repeated in triplicate with multiple wells per sample for each replicate.



Supplementary Figure 4: RCAN1 and CN binding

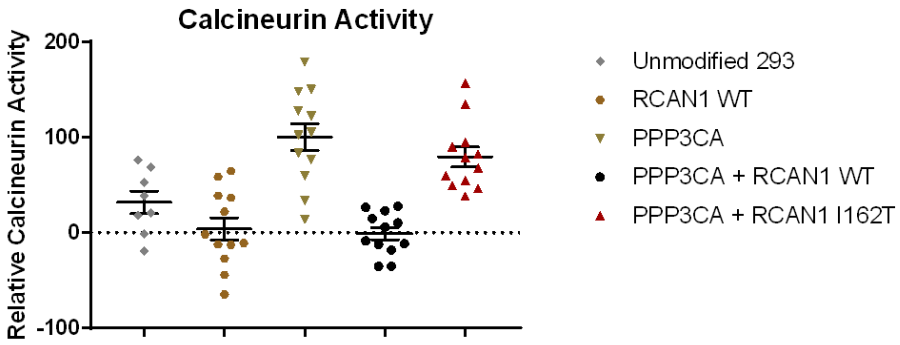
To determine if the *RCAN1* mutations affected docking of RCAN1 and calcineurin, we transfected human embryonic kidney cells (HEK293) cells with a flag-tagged calcineurin construct and myc-tagged *RCAN1* constructs as indicated. Immunoprecipitation studies were then performed as described in the Methods Section. (a) Immunoprecipitation of the calcineurin construct co-immunoprecipitated equal amounts of wild type and mutant RCAN1 proteins. (b) Conversely, immunoprecipitation of either wild type or mutant *RCAN1* constructs co-immunoprecipitated equal amounts of the calcineurin protein.



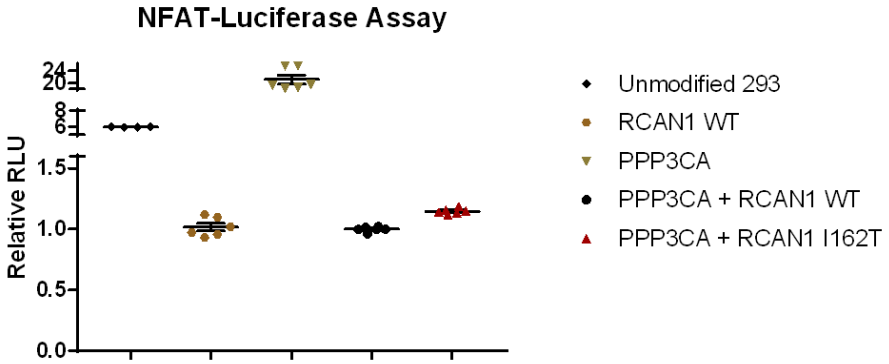
Supplementary Figure 5: *RCAN1* single transfection calcineurin activity.

To ensure that the calcineurin activity and NFAT luciferase activity assays were working appropriately, we examined the activity of unmodified HEK293 cells as well as the single transfections of *RCAN1* WT or *PPP3CA*. (a&b) Both the calcineurin activity (a) and NFAT expression (b) decreased in *RCAN1* WT expressing cells and increased in *PPP3CA* expressing cells compared to unmodified cells, demonstrating the efficacy of the assays.

a

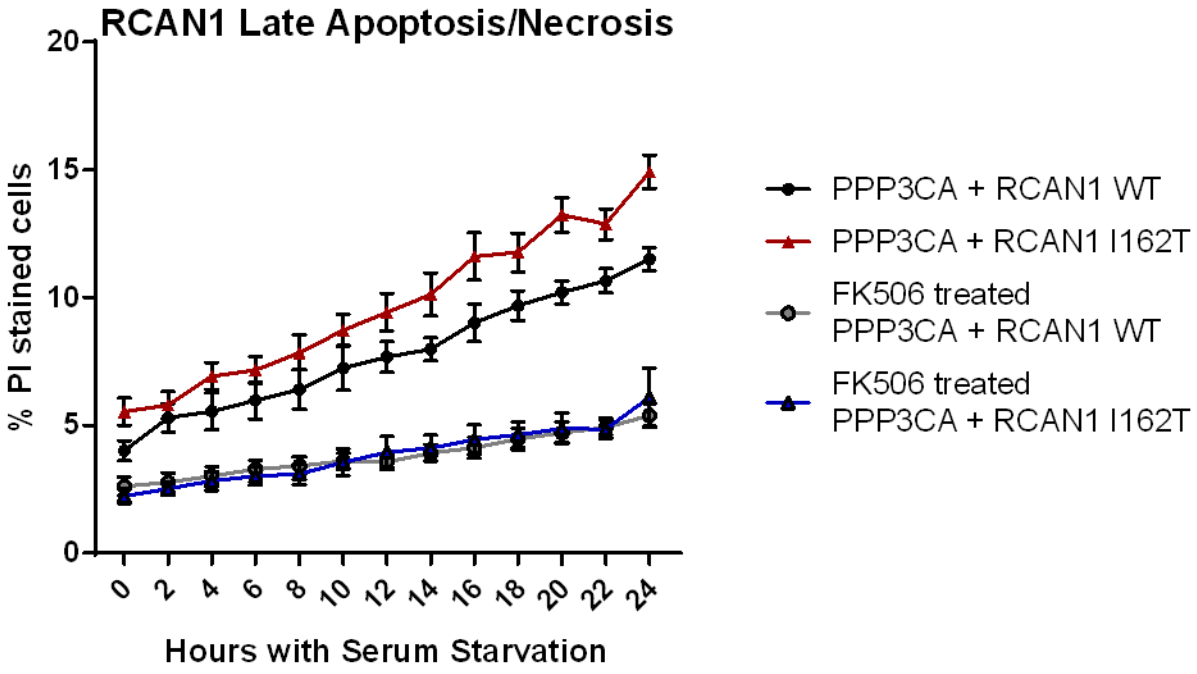


b

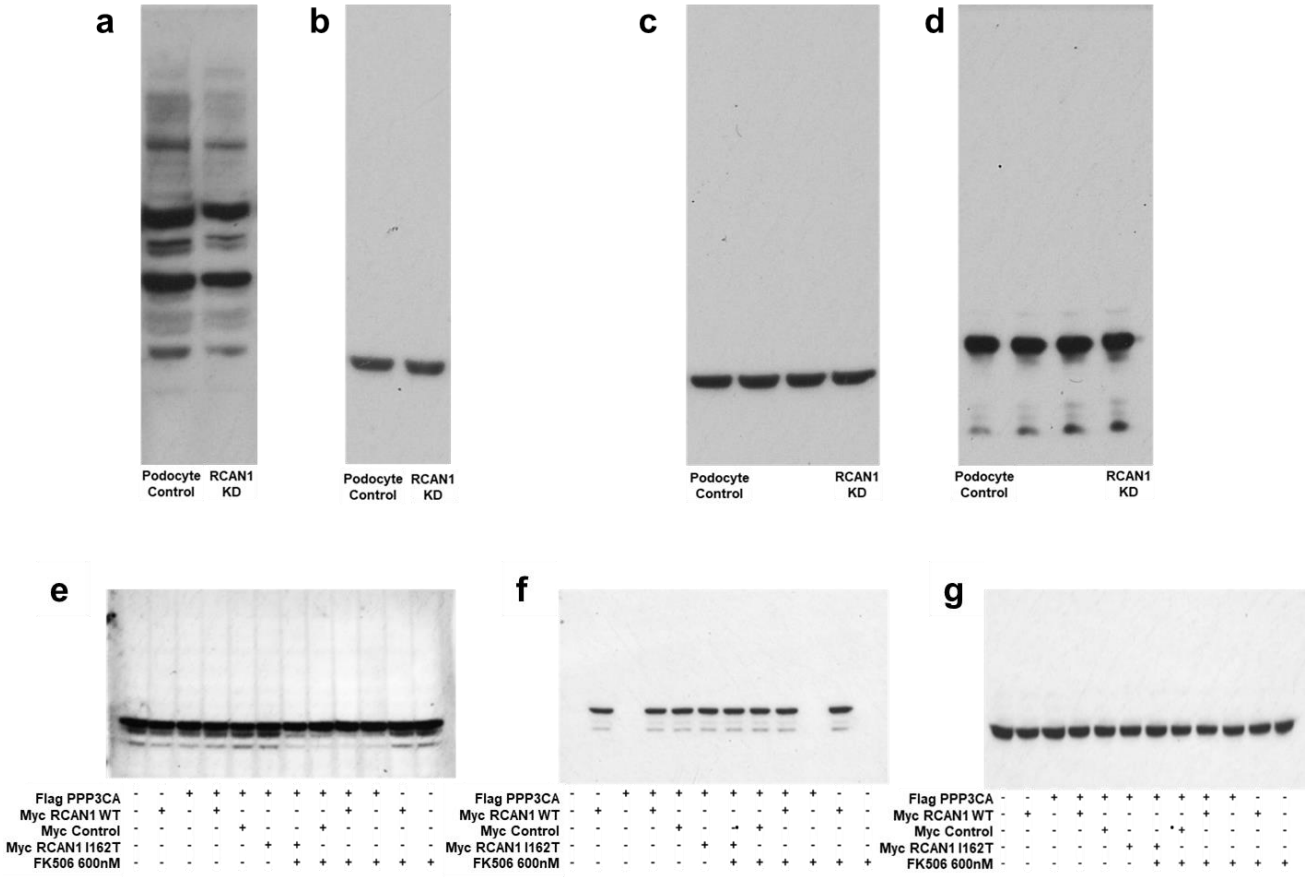


Supplementary Figure 6: Late Apoptosis/Necrosis

In addition to apoptosis readings using cleaved caspase 3 reporters, live cell automated imaging of propidium iodide staining was simultaneously used to measure late apoptosis/necrosis levels in HEK293 cells transfected with *PPP3CA* and either WT *RCAN1* (black circle) or I162T (red triangle) mutants. The I162T expressing cells displayed increased late apoptotic/necrotic cells compared to WT *RCAN1* expressing cells (p=0.0152 at 24 hours respectively, two-way ANOVA). This increased cell death was eliminated when the cells were treated with 1uM of FK506 (blue triangle) (p>0.4 for all time points in both mutants).



Supplementary Figure 7: Unmodified western blots for RCAN1 (a), β-actin (b), Caspase 3 (c), and β-actin (d) in conditionally immortalized podocytes. Unmodified western blots for Caspase-3 (e), Myc-tag (f), and β-actin (g) in 293 cells.



Supplementary Table 1: Segregating variants found in Family 40030

Gene	HGVSc	HGVSp	gnomAD (Eur)	MAF	CADD	Polyphen	SIFT	Mut Taster
RCAN1	**c.485T>C**	**p.I162T**	**2/128738**	**0.00001**	**26.7**	**Probably Damaging**	**Damaging**	**Disease causing**
MAST3	c.1160G>A	p.R387H	49/122646	0.0003	26.0	Possibly Damaging	Damaging	Disease causing
POLD1	c.653G>A	p.R218H	41/124252	0.0003	32.0	Possibly Damaging	Damaging	Disease causing
CBR3	c.605 C>A	p.T202K	28/129144	0.0002	34.0	Probably Damaging	Damaging	Disease causing

GRCH37: RCAN1-001: ENST0000031380

Supplementary Table 2: Evolutionary conservation of *RCAN1* variant residues

	I162				K128		
Human	S	I	L		H	K	T
Rhesus	S	I	L		H	K	T
Mouse	S	I	L		H	K	T
Dog	S	I	L		H	K	T
Elephant	S	I	L		H	K	T
Chicken	S	I	L		H	K	T

Supplementary Table 3: Description of cohorts

Cohort	Individuals/ Family	Race* White/Black/ Asian/Others	Histology* FSGS/MCD/ Others /Unknown	Therapy* response SRNS/SSNS/ Unknown
Duke	547/392	182/66/119/25	118/55/29/190	110/195/87
Boston Children's Hospital	138/114	114/0/0/0	7/0/107/0	7/0/107
NEPTUNE cohort	627/627	332/149/67/79	183/168/129/147	NA++
Beth Israel Hospital, Boston	524/337	216/31/21/69	337/0/0	337/0/0
Toronto General Hospital , Canada	193/193	NA	NA	42/45/106
UK NephroS cohort	NA	NA	NA	NA

* Findings in proband

NA: Not available

++: Partial or complete remission ever (Yes/No/Unknown) 451/98/78

Supplementary Table 4: Heterozygous missense variants in *RCAN1-3* genes in patients with nephrotic syndrome

Gene	**rsID**	**Exon**	**Nucleotide**	**Protein**
RCAN1		1	c.A107C	p.L36R
RCAN1		1	c.A109C	p.S37A
RCAN1	rs749675544	1	c.C115T	p. A39T
RCAN1	rs377673728	2	c.C368T	p.R123K
RCAN1	rs140515920	2	c.A382G	p.K128E
RCAN1	rs146806035	3	c.C448T	p.H150Y
RCAN1	rs145120179	3	c.C458T	p.P153L
RCAN1	rs1178734954	3	c.T485C	p.I162T
RCAN2		4	c.C445A	p.P149T
RCAN2		5	c.G596C	p.G199A
RCAN2	rs201948840	5	c.C700T	p.R234H
RCAN2	rs138769310	5	c.G721T	p.V241L
RCAN2		5	c.A728C	p.N243H
RCAN3	rs114568126	4	c.C391T	p.R131W
RCAN3		4	c.A482T	p.E161V
RCAN3		4	c.T517G	p.C173G
RCAN3	rs779074675	5	c.G566C	p.G189A
RCAN3		5	c.A605C	p.E202A
RCAN3	rs201998230	5	c.G668T	p.R223L
RCAN3	rs201388228	5	c.G673A	p.D225N

GRCH37:

RCAN1-001: ENST00000313806.4,

RCAN2-002: ENST00000371374.1,

RCAN3-001: ENST00000374395.4

Supplementary Table 5: Members of the Nephrotic Syndrome Study Network (NEPTUNE)

NEPTUNE Enrolling Centers

Cleveland Clinic, Cleveland, OH: K Dell[*], J Sedor[**], M Schachere[#], J Negrey[#]
Children's Hospital, Los Angeles, CA: K Lemley[*], E Lim[#]
Children's Mercy Hospital, Kansas City, MO: T Srivastava[*], A Garrett[#]
Cohen Children's Hospital, New Hyde Park, NY: C Sethna[*], K Laurent[#]
Columbia University, New York, NY: G Appel[*], A Pradhan[#]
Emory University, Atlanta, GA: L Greenbaum[*], C Wang**, C Kang[#]
Harbor-University of California Los Angeles Medical Center: S Adler[*], J LaPage[#]
John H. Stroger Jr. Hospital of Cook County, Chicago, IL: A Athavale[*], M Itteera
Johns Hopkins Medicine, Baltimore, MD: M Atkinson[*], S Boynton[#]
Mayo Clinic, Rochester, MN: F Fervenza[*], M Hogan**, J Lieske[*], V Chernitskiy[#]
Montefiore Medical Center, Bronx, NY: F Kaskel[*], M Ross[*], P Flynn[#]
NIDDK Intramural, Bethesda MD: J Kopp[*], J Blake[#]
New York University Medical Center, New York, NY: H Trachtman[*], O Zhdanova**, F Modersitzki[#], S Vento[#]
Stanford University, Stanford, CA: R Lafayette[*], K Mehta[#]
Temple University, Philadelphia, PA: C Gadegbeku[*], S Quinn-Boyle[#]
University Health Network Toronto: M Hladunewich**, H Reich**, P Ling[#], M Romano[#]
University of Miami, Miami, FL: A Fornoni[*], C Bidot[#]
University of Michigan, Ann Arbor, MI: M Kretzler[*], D Gipson*, A Williams[#], J LaVigne[#]
University of North Carolina, Chapel Hill, NC: V Derebail[*], K Gibson[*], E Cole[#], J Ormond-Foster[#]
University of Pennsylvania, Philadelphia, PA: L Holzman[*], K Meyers**, K Kallem[#], A Swenson[#]
University of Texas Southwestern, Dallas, TX: K Sambandam[*], Z Wang[#], M Rogers[#]
University of Washington, Seattle, WA: A Jefferson[*], S Hingorani**, K Tuttle**[§], M Bray[#], M Kelton[#], A Cooper[#§]
Wake Forest University Baptist Health, Winston-Salem, NC: JJ Lin*, Stefanie Baker[#]

Data Analysis and Coordinating Center: M Kretzler, L Barisoni, J Bixler, H Desmond, S Eddy, C Gadegbeku, B Gillespie, D Gipson, L Holzman, V Kurtz, M Larkina, J Lavigne, S Li, CC Lienczewski, J Liu, T Mainieri, L Mariani, M Sampson, M Wladkowski, A Williams, J Zee

Digital Pathology Committee: Carmen Avila-Casado (UHN-Toronto), Serena Bagnasco (Johns Hopkins), Joseph Gaut (Washington U), Stephen Hewitt (National Cancer Institute), Jeff Hodgin (University of Michigan), Kevin Lemley (Children's Hospital LA), Laura Mariani (University of Michigan), Matthew Palmer (U Pennsylvania), Avi Rosenberg (NIDDK), Virginie Royal (Montreal), David Thomas (University of Miami), Jarcy Zee (Arbor Research) Co-Chairs: Laura Barisoni (Duke University) and Cynthia Nast (Cedar Sinai)

*Principal Investigator; **Co-investigator; [#]Study Coordinator
[§]Providence Medical Research Center, Spokane, WA

Supplementary Protein Modeling (Movies 1-2): Movie files showing the predicted 3D structures of wildtype *RCAN1* (Supplementary Movie 1), and *RCAN1* I162T (Supplementary Movie 2).

Supplementary Apoptosis (Movies 2-5): Movie files of HEK 293 cells transfected with *PPP3CA* and either *RCAN1* WT (Supplementary Movie 3), and *RCAN1* I162T (Supplementary Movie 4) undergoing serum starvation (4hr-24hr of starvation). Cell death can be observed and quantified with green fluorescence indicating cleaved caspase 3 activity (apoptosis) which is then followed by red propidium idiode staining (late apoptosis/necrosis). Reduced apoptosis can be observed in *RCAN1* I162T (Supplementary Movie 5) cells treated with 1µM FK506.