

Supplemental Data

***DAAM2* Variants Cause Nephrotic Syndrome**

via Actin Dysregulation

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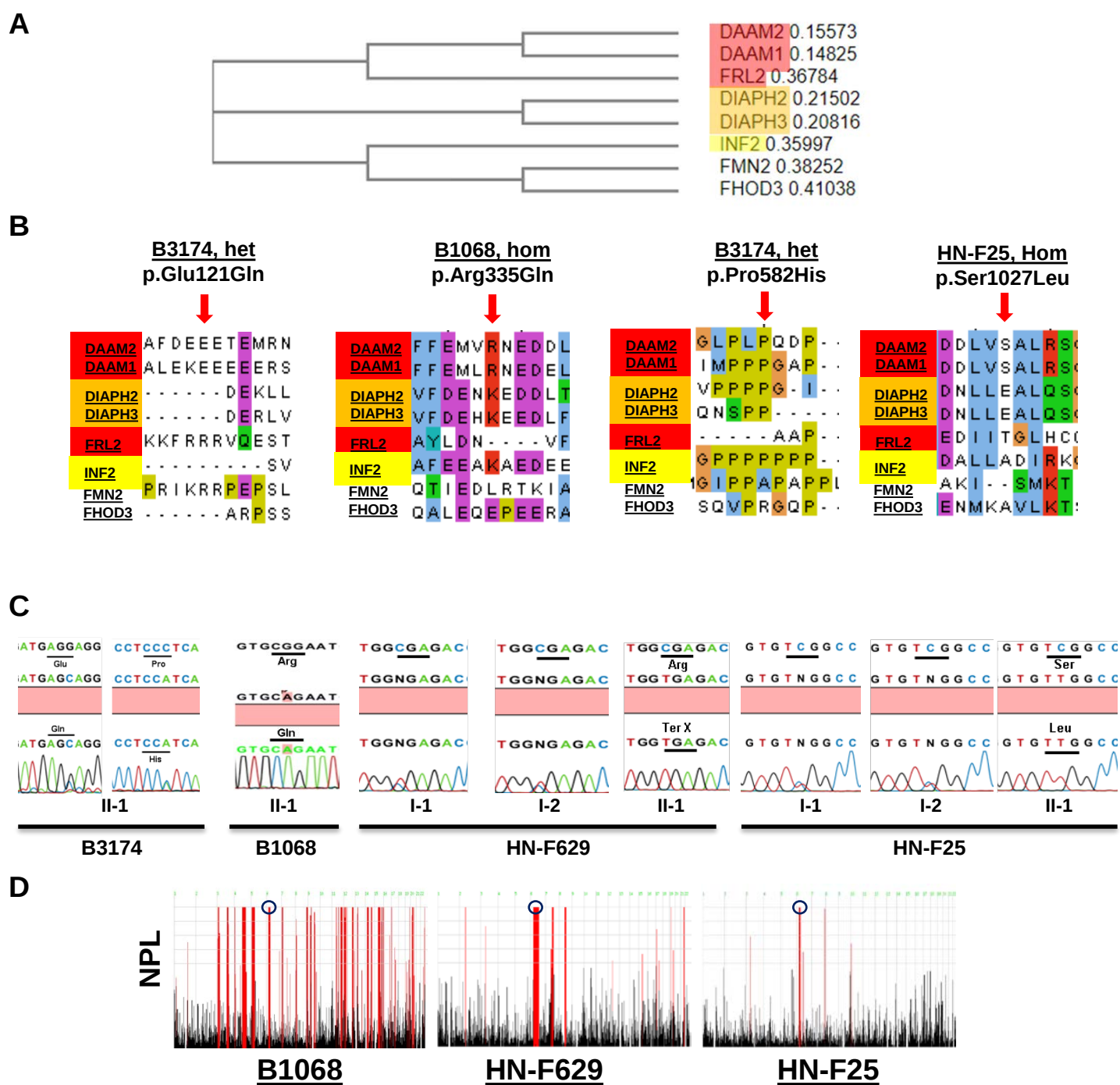


Figure S1. Domain conservation, pedigree structure and homozygosity mapping of variants in *DAAM2* found in individuals with SRNS.

(A) Phylogenetic tree of close *DAAM2* paralogues. Relatedness by sequence conservation taken from the dendrogram is reflected from top to bottom and in colors. Closest paralogues are *DAAM2*, *DAAM1* and *FRL2* (red), *DIAPH2*, *DIAPH3* (orange) and *INF2* (yellow). *FMN2* (*FRL1*), *DIAPH2*, *DIAPH3* and *INF2*. (B) Amino acid conservation among *DAAM2* paralogues for mutated amino acid positions found in individuals with SRNS (see Table 1). in positions of individuals with SRNS variants. (C) Sanger confirmation and segregation of *DAAM2* variants. (D) Homozygosity mapping of four families with *DAAM2* variants showing high degree of homozygosity for three families reported to be consanguineous. *DAAM2* is located within a peak of homozygosity by descent on chromosome 6 (blue circle) in these families. Family A3174 is not shown because it was not reported as consanguineous and has low degree of homozygosity, with no peak at chromosome 6.

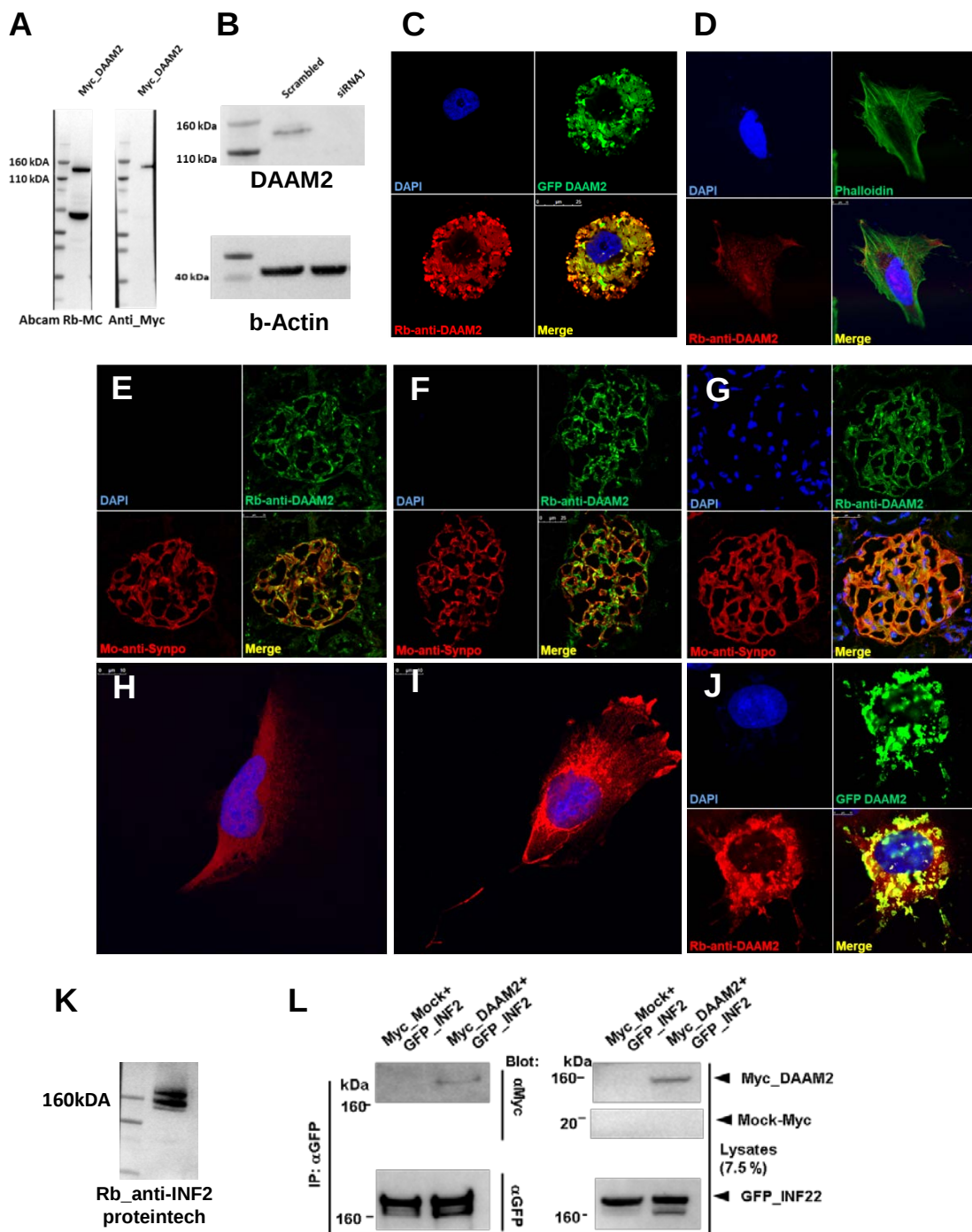


Figure S2: DAAM2 Antibody, podocyte expression and INF2 co-precipitation confirmation.

Western blot analysis confirms detection of overexpressed Myc_DAAM2 by anti_DAAM2 rabbit monoclonal antibody (abcam) (**A**). Knock down of DAAM2 by siRNA confirms endogenous DAAM2 expression in podocytes and specificity of abcam antibody to DAAM2 (**B**). Abcam antibody detects over-expressed GFP_DAAM2 in podocytes stained by immunofluorescence (IF) imaged by confocal microscope (**C**). Abcam Antibody confirms endogenous expression of DAAM2 in cultures human podocytes using IF staining. Phalloidin fibers shown in relation to cytoplasmic expression of DAAM2 (**D**). Confirmation of DAAM2 expression and co-localization with synaptopodin in adult rat glomerular podocytes, when detected by custom made antibody (Boston molecules) against a C-terminal epitope (**E**), N-terminal epitope (**F**) and by abclonal anti-DAAM2 antibody (**G**). Endogenous DAAM2 (**H**) and over-expressed GFP_DAAM2 (**I**, **J**) is detected by SantaCruz antibody in cultured human podocytes. INF2 is expressed in HEK cells (**K**). GFP_INF2 precipitates Myc_DAAM2 in HEK cells ('reverse' experiment of fig. 2f).

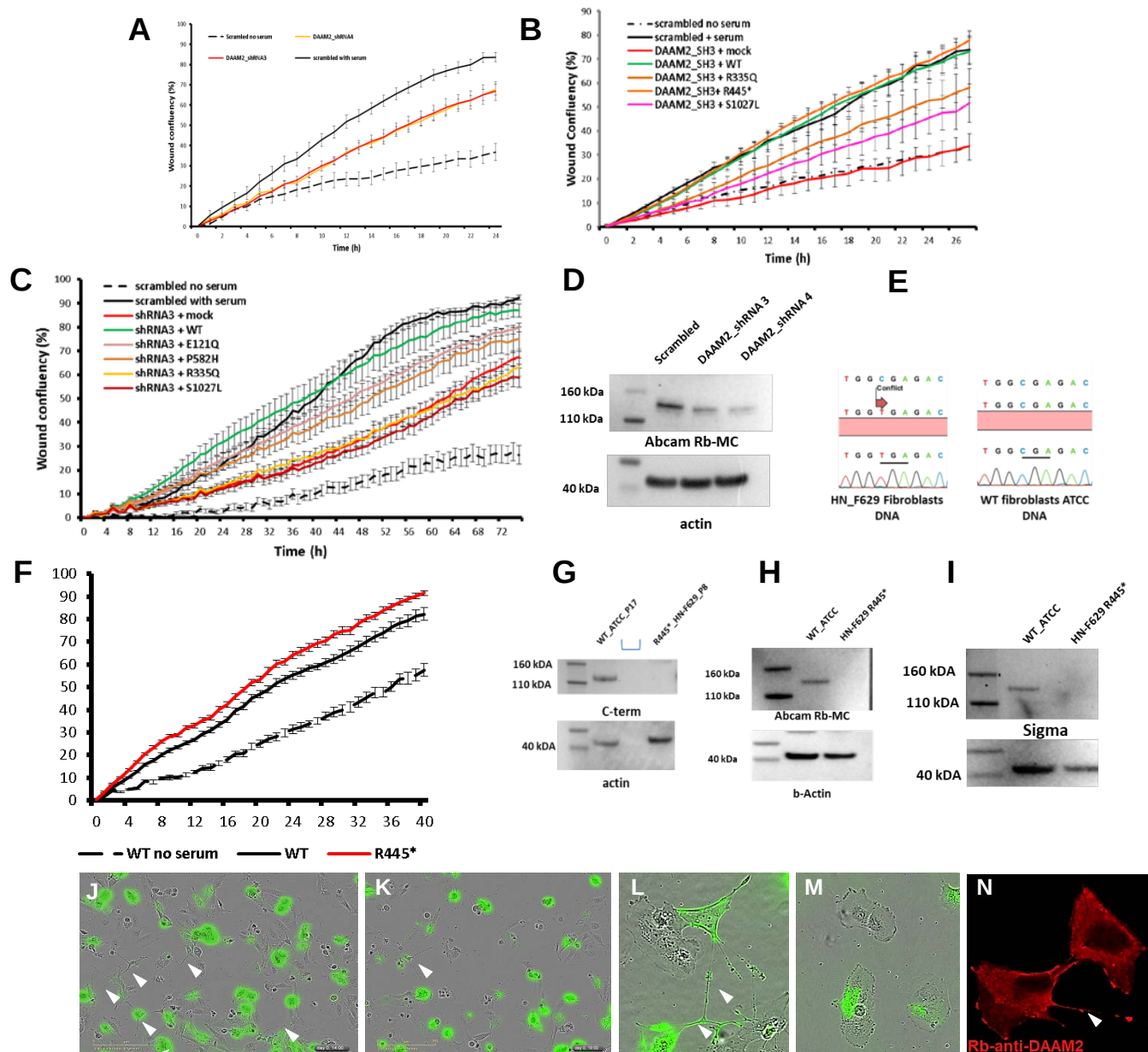


Figure S3. DAAM2 knock-down confirmation in shRNA cell line, PMR and FMR repeats and filopodia formation images.

Knockdown of *DAAM2* by 2 independent shRNAs reduces podocyte migration rate (**A**). Overexpression of murine cDNA representing wt-*DAAM2* and variant p.Arg445* from individual with SRNS- fully rescue migration defect caused by shRNA induced *DAAM2*-knock-down, while cDNA representing variants p.Arg335Gln and p.Ser1027Leu- only partially so (**B,C**). shRNA treatment result in stable reduction in band size corresponding to *DAAM2* protein in 2 independent shRNA lines (**D**). Sanger sequencing of skin fibroblasts confirms homozygous nonsense variant of individual HN-F629 (**E**). Fibroblasts cultured from individual's HN-F629 skin, migrate faster than control adult skin cultured fibroblasts (**F**). Individual HN-F629 derived fibroblasts show no band on WB analysis corresponding to *DAAM2*, but healthy fibroblasts do, as detected by 3 independent antibodies in 3 independent experiments: custom made- Boston molecules, C-terminal epitope (**G**), Sigma (**H**), abcam (**I**). (**J-N**): Example images of filopodia formation as imaged by the Incucyte Videomicroscopy system. Over-expression of WT-GFP_*DAAM2* cDNA induces filopodia (arrowheads **J,L**) to a higher extent than p.Arg335Gln-GFP_*DAAM2* cDNA (**K**) or GFP-mock (**M**). Image of an GFP_*DAAM2* over expressing podocytes, taken by a confocal microscope showing filopodia formation in podocytes (arrowheads, **N**). Error bars= SEM.

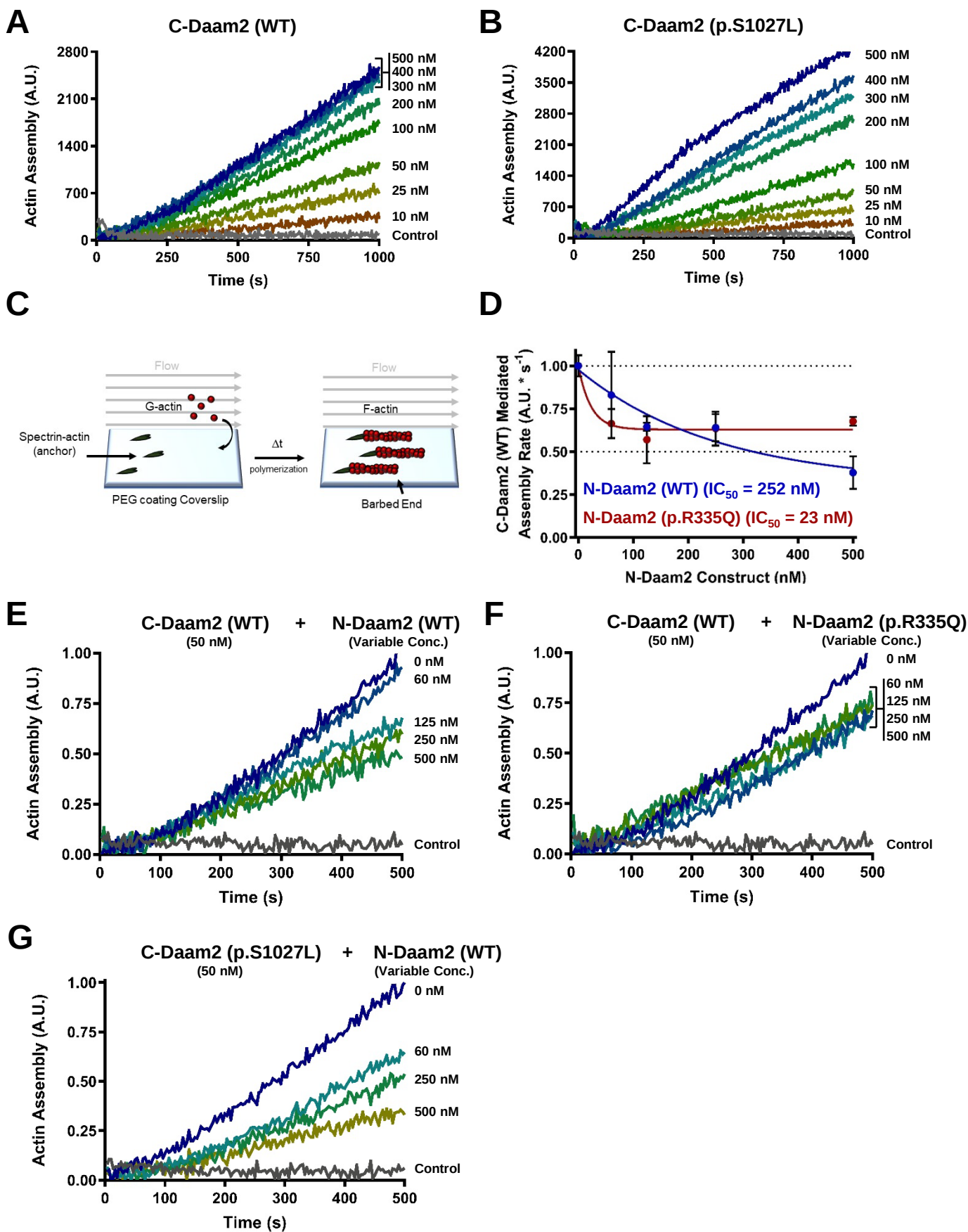


Figure S4.

Figure S4. *Daam2* variants have altered effects on actin filament nucleation and elongation

(A,B) Bulk actin assembly assays comparing the effects of different concentrations of C-Daam2 (WT) **(A)** and C-Daam2 (p.S1027L) **(B)** on the polymerization rate of 2 μ M G-actin (10% pyrene labeled) in the presence of 5 μ M profilin. Increase in fluorescence corresponds to increase in actin assembly. Three of the curves for C-Daam2 (WT) and three of the curves for C-Daam2 (p.S1027L) also appear in Figure 5B. **(C)** Schematic of the microfluidic-assisted TIRF microscopy setup used for the analysis shown in Figure 5D and 5E. Spectrin-actin seeds were passively adsorbed to the surface and used to nucleate actin filaments by flowing in actin monomers and profilin. Thus, filaments were anchored at their pointed ends and grew at their distal free barbed ends. Once filaments reached $\sim 5 \mu$ m in length, C-Daam2 (WT or p.S1027L) was flowed in (without actin) and allowed to cap filament barbed ends for 30 s. Then, the same G-actin-profilin mix as above was flowed in, and the barbed end elongation rate was monitored for 10-15 min. **(D)** Quantification of bulk actin assembly assays, performed as above in A and B, comparing the inhibitory effects of different concentrations of N-Daam2 (WT) and N-Daam2 (p.R335Q) on the actin assembly activity of 50 nM C-Daam2 (WT). N-Daam2 (p.R335Q) is more effective than N-Daam2 (WT) in inhibiting C-Daam2 (WT) activity, suggesting that the variant enhances formin autoinhibition. One of the curves also appears in Figure 5F. **(E, F)** Bulk assays, performed as above, comparing the inhibitory effects of different concentrations of N-Daam2 (WT) **(E)** and N-Daam2 (p.R335Q) **(F)** on the actin assembly activity of 50 nM C-Daam2 (WT). **(G)** Bulk assays, performed as above, comparing the inhibitory effects of different concentrations of N-Daam2 (WT) on the actin assembly activity of 50 nM C-Daam2 (p.S1027L). All experiments were performed two times. For D, the data were averaged, and error bars represent SD. For all remaining panels, the data shown are from one experiment, and are representative of the results observed in both independent experiments

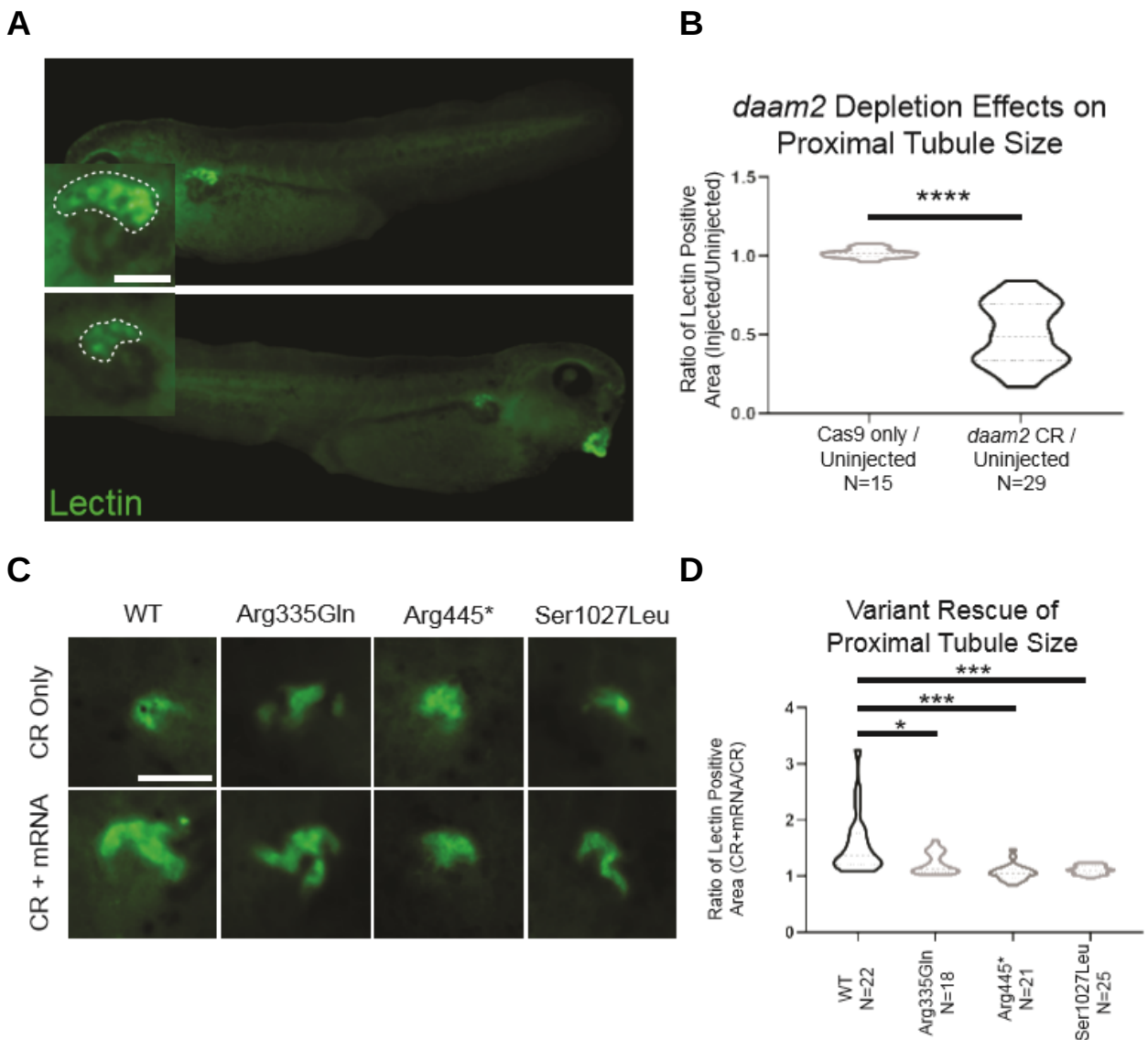


Figure S5. Mosaic knockout of *daam2* results in pronephric abnormalities.

Xenopus embryos were injected with Cas9 together with CRISPR guide RNA (gRNA) oligonucleotides targeting *daam2* in one cell at the 4-cell stage. Abnormalities in pronephric development, specifically formation of small proximal pronephric segments were observed and the ratio of this area between morphant and control sides at stages 38-39 was calculated. The proximal pronephros was detected using staining with fluorescein conjugated lectin. **(A)** Mosaic knockout of *daam2* results in abnormalities of proximal pronephric development. Upper panel displays the uninjected control side. Lower panel displays the *daam2* CRISPR injected side. **(B)** Ratio of proximal pronephric area of injected vs uninjected sides. **(C)** Injection of wildtype mRNA of DAAM2 restores lectin signal as a marker of restored pronephric development. Injection of mRNA reflecting human variants p.R335Q and p.Ser1027Leu also restores lectin signal but injection of mRNA reflecting p.R445* mutant, fails to do so. **(D)** Ratio of proximal pronephric area of mRNA injected vs CRISPR only sides. * $p < 0.05$, *** $p < 0.0005$, **** $p < 0.0001$ by Student's t test. Scale bars depict 100 μm .

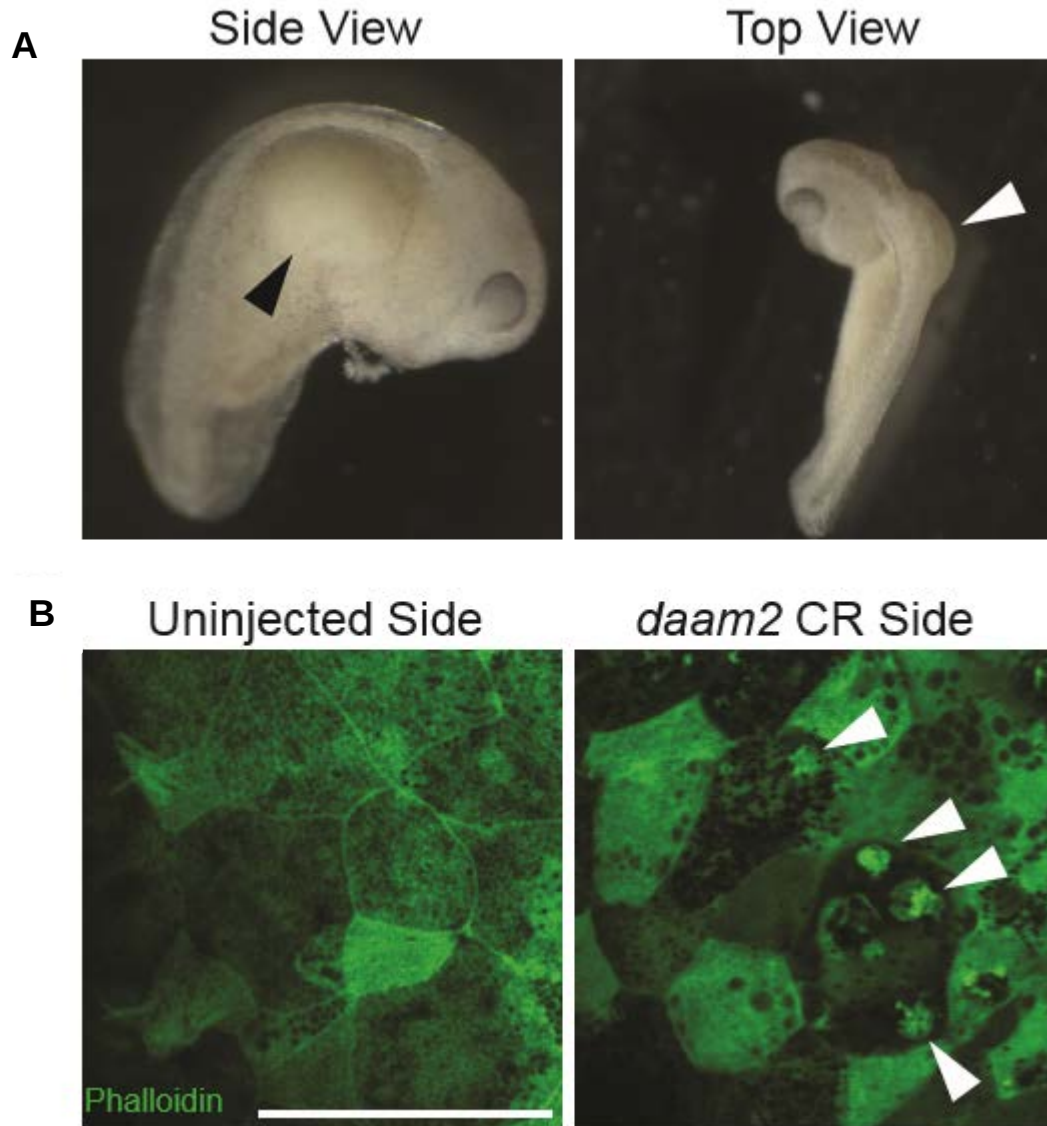


Figure S6. Mosaic knockout of *daam2* results in actin distribution defects.

Xenopus embryos were injected with Cas9 together with CRISPR guide RNA (gRNA) oligonucleotides targeting *daam2* in once cell at the 4-cell stage. Abnormalities in actin distribution were observed at stages 31-32. Actin was detected using staining with Alexa488 conjugated phalloidin. **(A)** Mosaic knockout of *daam2* results in abnormalities within the epidermal tissue architecture apparent as bulging tissue. Left panel displays the side view of this bulging tissue. Right panel displays a top view of the bulging tissue. **(B)** Phalloidin staining reveals abnormal distribution of actin in the mosaic *daam2* knockout epidermis including actin conglomerates (white arrow heads), which are absent from regions of the embryo not targeted with *daam2* CRISPR. Scale bar depicts 100 μ m.

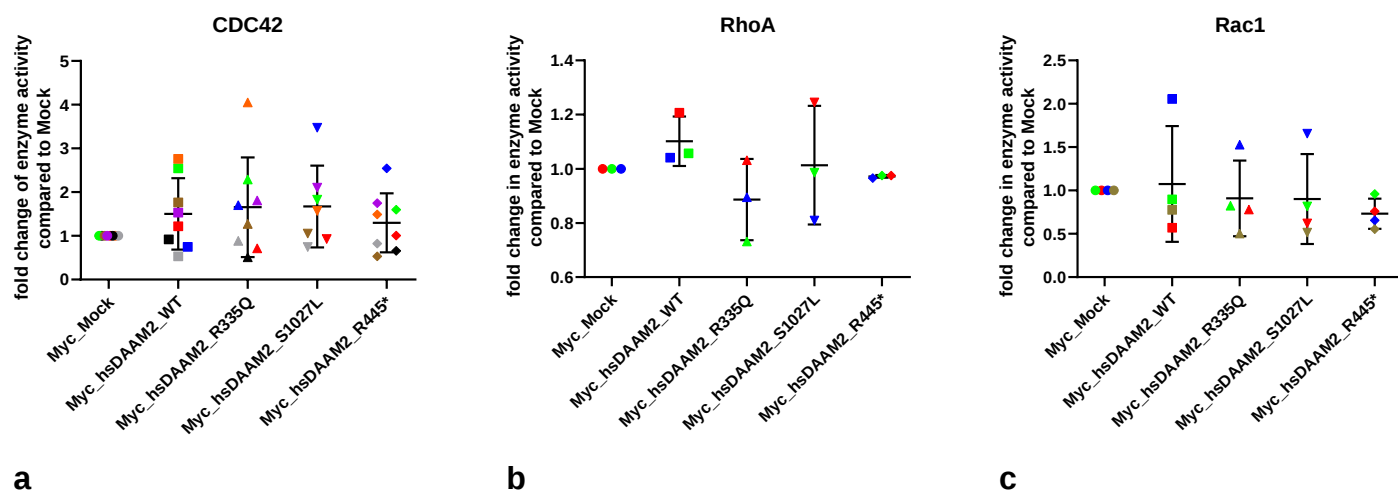


Figure S7: Overexpression of DAAM2 constructs does not significantly alter enzyme activity of the small GTPases CDC42, RhoA and Rac1 in human podocytes.

(a) Overexpression of neither Myc-tagged DAAM2 wild type (WT) nor the three DAAM2 mutants identified in individuals resulted in a significant change in active CDC42. Active CDC42 was measured by G-LISA assay in conditionally immortalized human podocytes.

(b) Overexpression of neither Myc-tagged DAAM2 wild type (WT) nor the three DAAM2 mutants identified in individuals with SRNS resulted in a significant change in active RhoA. Active RhoA was measured by G-LISA assay in conditionally immortalized human podocytes.

(c) Overexpression of neither Myc-tagged DAAM2 wild type (WT) nor the three DAAM2 mutants identified in individuals with SRNS resulted in a significant change in active Rac1. Active Rac1 was measured by G-LISA assay in conditionally immortalized human podocytes.

Error bars are defined as the standard error of at least three independent experiments, indicated by individual colors. Data are presented as the mean $\pm$ SD

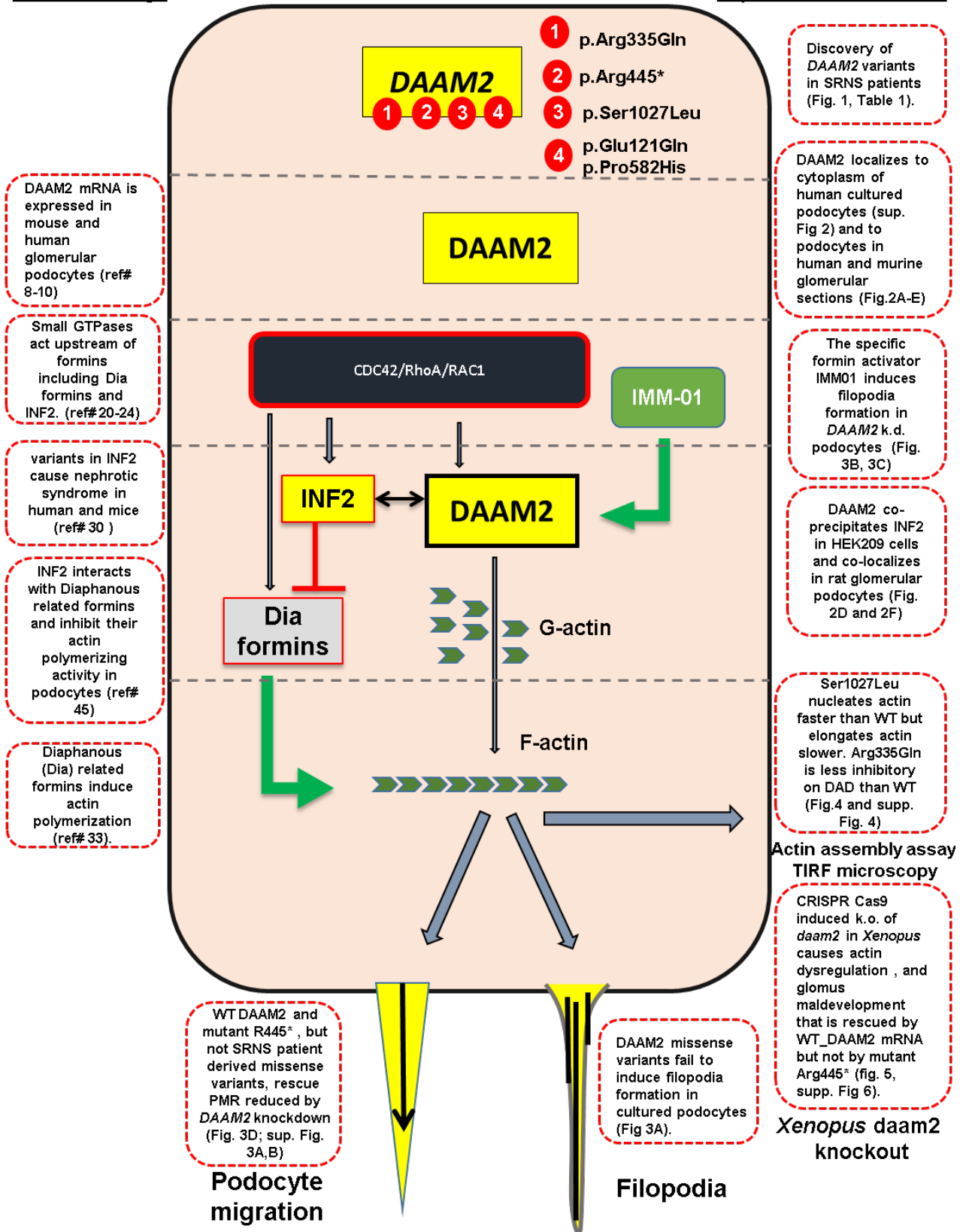


Figure S8. Schematic representation of the roles of DAAM2 in regulation of actin cytoskeleton structures in podocytes.

Table S1: primers used for cloning and PCR

Primer	type	sequence
DAAM2_G1004A_F	mutagenesis	CCAGGTCATCCTCATTCTGCACCATCTCGAAGAAG
DAAM2_G1004A_R	mutagenesis	CTTCTTCGAGATGGTGAGAAATGAGGATGACCTGG
mDaam2_mut_F	mutagenesis	CCAGGTCATCCTCATTCTGGACCATCTCAAAGAAG
mDaam2_mut_R	mutagenesis	CTTCTTTGAGATGGTCCAGAAATGAGGATGACCTGG
hDAAM2_S1027L_fw	mutagenesis	CGCAGGGCCAACACCAGGTCATCGAAC
hDAAM2_S1027L_rev	mutagenesis	GTTCCGATGACCTGGTGTGGCCCTGCG
hDAAM2_Arg445*_fw	mutagenesis	CTTCTCTGCTGGTCTCACCAGTCTTCACTTCAT
hDAAM2_Arg445*_rev	mutagenesis	ATGAAGTGAACAGTGGTGAGACCAGGAGAGAAG
mDaam2_S1027L_fw	mutagenesis	AGCGCAGGGCTAACACTAGGTCATCAAACCTCGC
mDaam2_S1027L_rev	mutagenesis	GCGAGTTTGATGACCTAGTGTAGCCCTGCGCT
mDaam2_Arg445*_fw	mutagenesis	CTTCTCTGCTGGTCTCACCAGTCTTCACTTC
mDaam2_Arg445*_rev	mutagenesis	GAGGTGAAGCAGTGGTGAGACCAGGAGAGAAG
DAAM2_Glu121Gln_F	mutagenesis	CTCATCTCCGTCTCTGCTCATCAAACGCGTAC
DAAM2_Glu121Gln_R	mutagenesis	GTACGCGTTTGATGAGCAGGAGACGGAGATGAG
DAAM2_Pro582His_F	mutagenesis	TAGGGGTCTCTGATGGAGGGGACGGC
DAAM2_Pro582His_R	mutagenesis	GCCTGCCCTCCATCAGGCCCTA
DAAM2_fix-frame_GST_fw	mutagenesis	CCCGGAATTCCTGGTCGACATGGCCC
DAAM2_fix-frame_GST_rev	mutagenesis	GGGCCATGTCGACCGGGAATTCGCGG
DAAM2_fix-frame_SNAP_fw	mutagenesis	CCCGGAATTCCTGGTCGACCTGTATC
DAAM2_fix-frame_SNAP_rev	mutagenesis	GATACAGGGTCGACCGGGAATTCGCGG
hDAAM2_shRNA3R_Fw	mutagenesis	TCCTCGTTAGAAAGCTTCAATTTTGAGAGAAGTATGATTCAGTTTGGGCCCTCCGGCC
hDAAM2_shRNA3R_Rev	mutagenesis	GGCCGGAGGGCCCAAACTGAATCATACTTCTCTCAAAATGAAGCTTTCTAACGAGGA
h_DAAM2_sh3_F	shRNA	GATCCGCATCATCTCTTCTTCAAGTGAAGCTTGGAAAGAAGGATGATGCTTTTACGCGTG
h_DAAM2_sh3_R	shRNA	AATTCACGCGTAAAAAGCATCATCTCTTCTTCAAGTTTCTGACTTGGAAAGAAGGATGATGCG
h_DAAM2_sh4_F	shRNA	GATCCGCATCTTTGATACCTTCTTCCGAAGCAAGAAGGTATCAAGATGCTTTTACGCGTG
h_DAAM2_sh4_R	shRNA	AATTCACGCGTAAAAAGCATCTTTGATACCTTCTTGTCTCGGCAAGAAGGTATCAAGATGCG
DAAM2_FH1_DAD_Fw	PCR	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCAACCCTGTATCTTCCCCACCAC
DAAM2_DID_rev	PCR	GGGGACCATTGTGACAAGAAAGCTGGGCTCTGAGCTGTGAGAGTTCACTGAG
Hs_DAAM2_DAD-shrtPCR_Fw	PCR	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCAACCCTGAAGGAGCAGAGGGAAC
DAAM2_N-terminal_to_FH1_for_BamH1	PCR - pGEX	GGATCCATGGCCCCCG
DAAM2_N-terminal_to_FH1_rev_EcoR1	PCR - pGEX	GAATTCCTCAGCTGTGAGAGTTCACTGAG
DAAM2_FH1_to_C-terminal_for_BamH1	PCR - pGEX	GGATCCCTGTATCTTCCCCACCAC
DAAM2_FH1_to_C-terminal_rev_closed_EcoR1	PCR - pGEX	GAATTCCTCAATAATTAGCCGTTTATTGC
DAAM2_FH1_to_C-terminal_rev_open_EcoR1	PCR - pGEX	GAATTCATAATTAGCCGTTTATTGC
DAAM2_N-terminal_to_FH1_for_Sall_unspoverh	PCR - pGEX	TAAGCAGTCGACATGCCCCCG
DAAM2_N-terminal_to_FH1_rev_NotI_unspoverh	PCR - pGEX	TGCTTAGCGCGCGCTCAGCCTGTGAGAGTTCACTGAG
DAAM2_FH1_to_C-terminal_for_Sall_unspoverh	PCR - pGEX	TAAGCAGTCGACATGCTTCTTCCCCACCAC
DAAM2_FH1_to_C-terminal_rev_closed_NotI_unspoverh	PCR - pGEX	TGCTTAGCGCGCGCTCAATAATTAGCCGTTTATTGC
DAAM2_FH1_to_C-terminal_rev_open_NotI_unspoverh	PCR - pGEX	TGCTTAGCGCGCGCATAATTAGCCGTTTATTGC
DAAM2_N-terminal_to_FH1_for_Sall	PCR - pGEX	DAAM2_N-terminal_to_FH1_for_SallPCR - pGEX
DAAM2_N-terminal_to_FH1_rev_NotI	PCR - pGEX	GCGGCCGCTCAGCCTGTGAGAGTTCACTGAG
DAAM2_FH1_to_C-terminal_for_Sall	PCR - pGEX	DAAM2_FH1_to_C-terminal_for_SallPCR - pGEX
DAAM2_FH1_to_C-terminal_rev_closed_NotI	PCR - pGEX	GCGGCCGCTCAATAATTAGCCGTTTATTGC
DAAM2_FH1_to_C-terminal_rev_open_NotI	PCR - pGEX	GCGGCCGCTCAATAATTAGCCGTTTATTGC
DAAM2_delta-C-term_rev_CI	for GST-precipitation	TCAGCTGTGAGAGTTCACTGAG
hDAAM2_DeI_N-term_F	for HexaHis	CCTGTATCTTCCCCACCAC
hDAAM2_PCR_Fw	PCR- pDONR	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCAACATGGCCCCCG
hDAAM2_PCR_R_CI	PCR- pDONR	GGGGACCATTGTGACAAGAAAGCTGGGTCTCAATAATTAGCCGTTTATTGCC
hDAAM2_PCR_R_Op	PCR- pDONR	GGGGACCATTGTGACAAGAAAGCTGGGTCTCAATAATTAGCCGTTTATTGCC
mDaam2_PCR_Fw	PCR- pDONR	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCAACATGGCCCTCCGCAAG
mDaam2_PCR_R_CI	PCR- pDONR	GGGGACCATTGTGACAAGAAAGCTGGGTCTCAATAATTAGCCTGTTTATGGC
mDaam2_PCR_R_Op	PCR- pDONR	GGGGACCATTGTGACAAGAAAGCTGGGTCTCAATAATTAGCCTGTTTATGGC
Hs_DAAM2_seq_R1	sequencing	CAATGAGTGAGGTGTGGATG
Hs_DAAM2_seq_F1	sequencing	GTCGTGGAAGACCTGAAGAC
Hs_DAAM2_seq_F2	sequencing	CTTCGAGATGGTGCGGAATG
Hs_DAAM2_seq_F3	sequencing	CTCAATGACAACCAATGACCTG
Hs_DAAM2_seq_F4	sequencing	CAAGTTCATCCAGAGAAGAG
Hs_DAAM2_seq_F5	sequencing	GCCCAAGTGAAGTTTGTCC
mo_Daam2_R1	sequencing	GAGAGTTGTTTCATCAGAGCCTTG
mo_Daam2_F1	sequencing	ATGAGGTTGTGACCCGCTTC
mo_Daam2_F2	sequencing	GAGCTAGCCAGGAGGTTTGAC
mo_Daam2_F3	sequencing	CCATCTCATGACCCCTCTCC
mo_Daam2_F4	sequencing	CTAGAAGAGCACAAACATGAGATTG
mo_Daam2_F5	sequencing	CCAAAGTCAACCTGGCAGAGC
DAAM2_C-term_seq_rev	sequencing	TGAGTGGGACGTCACTGCTG
Daam2_mice_WT_Fw	sequencing	GGGACCTTTGTGCAAGTGTCTCTCAAA
Daam2_mice_WT_Rev	sequencing	GAGCGATGATGCTGATGGCCTCAG
Daam2_mice_KO_Fw	sequencing	AGGCGCATAACGATACCACGAT
Daam2_mice_KO_Rev	sequencing	CCACAACGGGTTCTCTGTT
DAAM2_DAD-seq_Fw	sequencing	CTGAAGGAGCAGAGGGAAC