

Supplemental Data for Akilesh *et al.*

Supplemental Movie S1. Live cell imaging of YFP-actin transduced undifferentiated podocyte cultured at 33°C. The cell exhibits active membrane ruffling. An image was captured every 15 seconds for 20 minutes to generate the movie. Magnification 400x.

Supplemental Movie S2. Live cell imaging of YFP-actin transduced differentiated podocyte cultured at 37°C. The cell exhibits minimal membrane ruffling. An image was captured every 15 seconds for 20 minutes to generate the movie. Magnification 400x.

Supplemental Movie S3. Live cell imaging of control knockdown podocyte (fLuc) transduced with YFP-actin and differentiated at 37°C. The cell exhibits minimal membrane ruffling. An image was captured every 15 seconds for 20 minutes to generate the movie. Magnification 400x.

Supplemental Movie S4. Live cell imaging of Arhgap24 knockdown podocyte (line 451) transduced with YFP-actin and differentiated at 37°C. Arhgap24 knockdown results in increased membrane ruffling even after differentiation. An image was captured every 15 seconds for 20 minutes to generate the movie. Magnification 400x.

Supplemental Movie S5. Live cell imaging of Arhgap24 knockdown podocyte (line 756) transduced with YFP-actin and differentiated at 37°C. Arhgap24 knockdown results in increased membrane ruffling even after differentiation. An image was captured every 15 seconds for 20 minutes to generate the movie. Magnification 400x.

Supplemental Figure Legends

Supplemental Figure 1 Hamster and rabbit antisera are specific for Arhgap24. **(A)** HEK293 cells were transfected with FLAG-tagged Arhgap24 (+) or left untransfected (–). Cell lysates were then immunoblotted with Armenian hamster antiserum generated against Arhgap24. This antiserum detects the predicted 95 kD band. Preabsorption of the antiserum with GST-Arhgap24 quenches the signal. **(B)** Cultured podocytes (600x magnification) and **(C)** mouse glomeruli (200x magnification) were stained with rabbit antiserum generated against Arhgap24. The specific staining is abolished by preincubating the antiserum with GST-Arhgap24 antigen.

Supplemental Figure 2 Arhgap24 is expressed in the E13 embryonic kidney. Arhgap24 is expressed in the comma (C), S-shaped body (S) and capillary loop (Cap) stages of glomerular development and co-localizes with synaptopodin, a marker of podocytes. Original magnification 200x.

Supplemental Figure 3 Pedigree information for T97I and P417A variations. DNAs from one family for the T97I variation and three families for the P417A variation were available for sequence analysis. The presence of kidney disease (clinical and/or biopsy proven) is denoted by the filled-in symbols. If DNA was analyzed for sequence variation, the result of a non-synonymous variation is denoted within the individuals symbol (Wt = wildtype; Ht = heterozygous). The reference protein sequence (NP_001020787) was

considered wildtype. In no instance did the variation consistently correlate with kidney disease.

Supplemental Figure 4 Sequencing chromatogram of *ARHGAP24* from the proband of the family depicted in Figure 6 of the main text. The first four rows represent forward and reverse sequencing across codon 158 in the two siblings with FSGS. Both individuals are heterozygous at this position. Sequencing of the same region in the forward direction is shown in rows five and six for two control individuals. The CAG → CGG change results in a substitution of arginine for glutamine (Q158R).

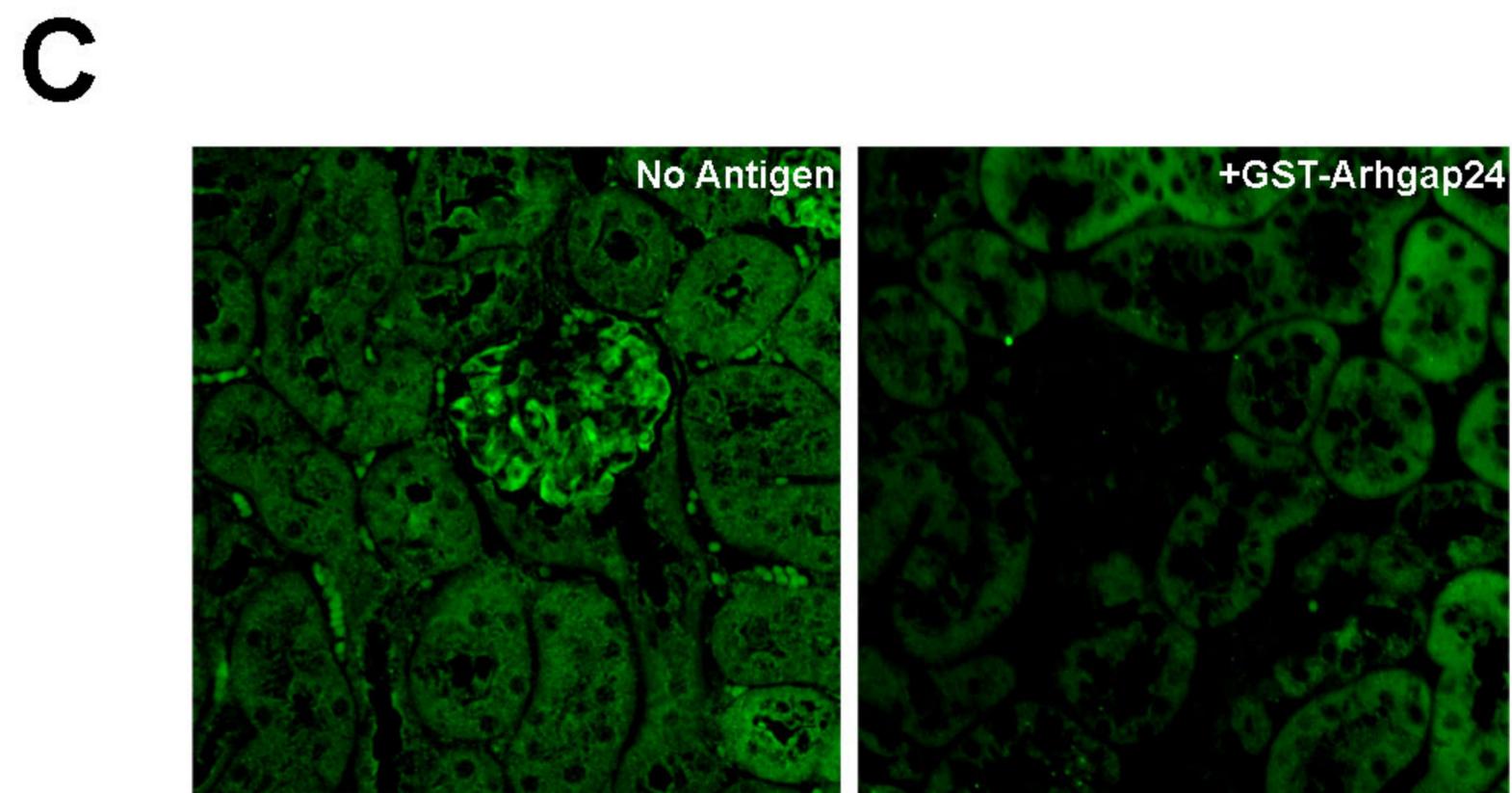
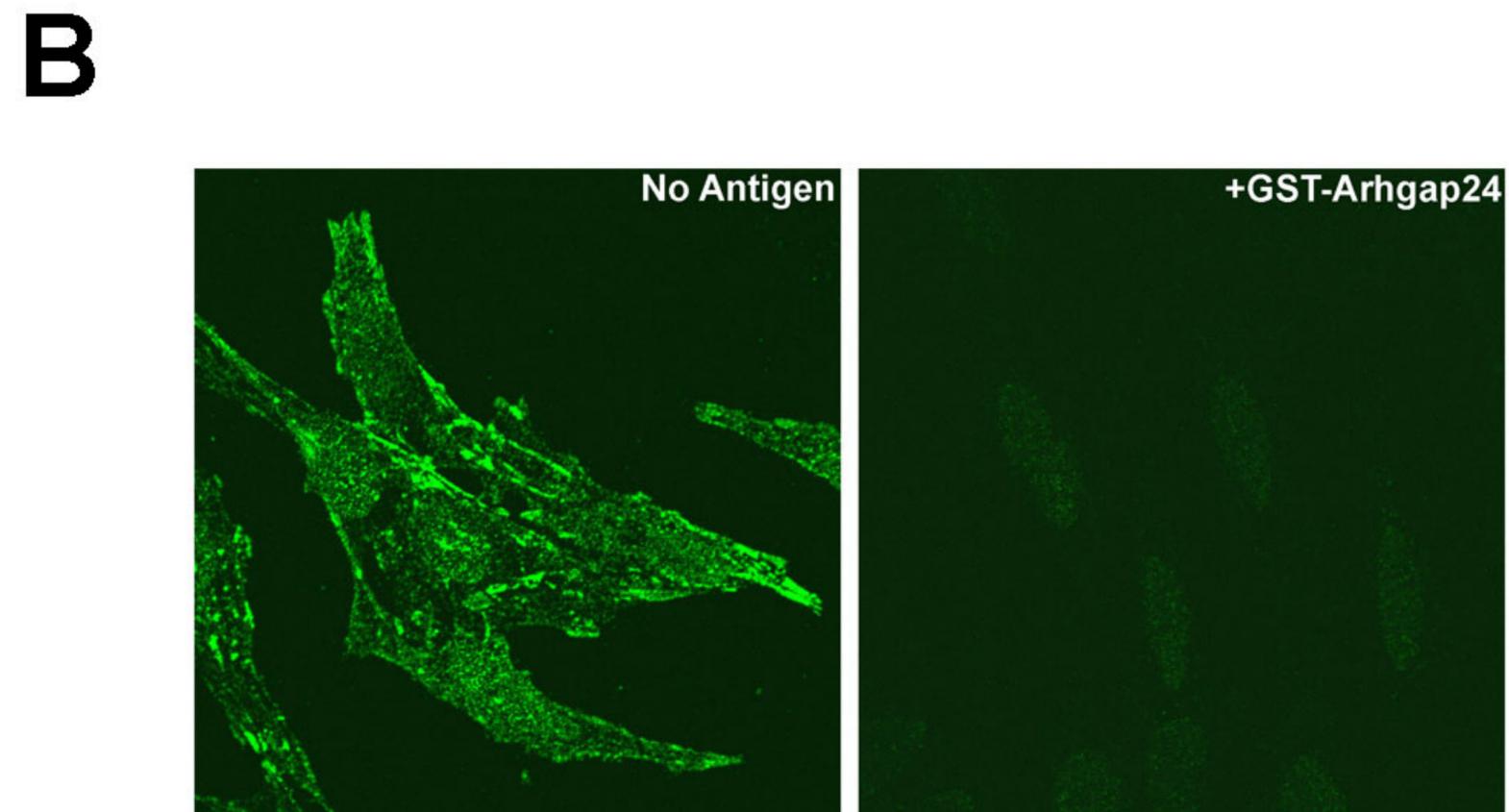
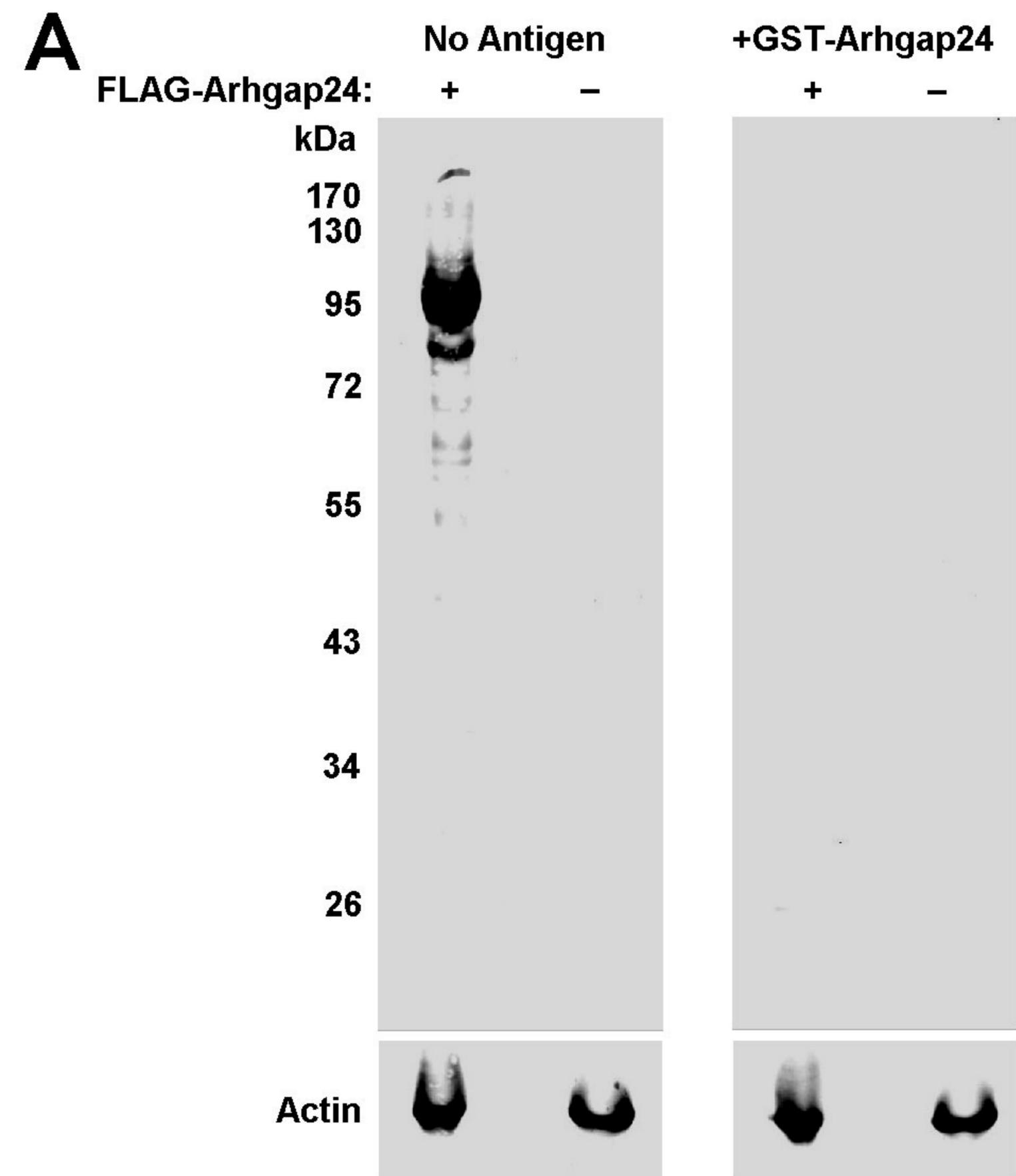
Supplemental Table 1. Non-synonymous SNPs identified in the transcript of isoform 1 of *ARHGAP24* from over a 1000 human genomes sequenced (www.1000genomes.org). SNPs in bold are known polymorphisms in the gene.

<u>Residue</u>	<u>SNP ID</u>	<u>Alleles</u>
Variation		
R142H	rs36067390	G/A
Q359R	LWK_P3:4:87134907:G	A/G
D368G	YRI_P3:4:87134934:G	A/G
P417A	rs35521695	C/G
V420I	LWK_P3:4:87135089:A	G/A
K421Q	YRI_P3:4:87135092:C	A/C
G473V	YRI_P3:4:87135249:T	G/T
R483H	LWK_P3:4:87135279:A	G/A
R499Q	TSL_P3:4:87135327:A	G/A
F539L	YRI_P1:4:87135446:C	T/C
G618S	CEU_P3:4:87135683:A	G/A
R622H	JPT_P3:4:87135696:A	G/A
M696K	JPT_P3:4:87140739:A	T/A
E721*	rs13292	G/T

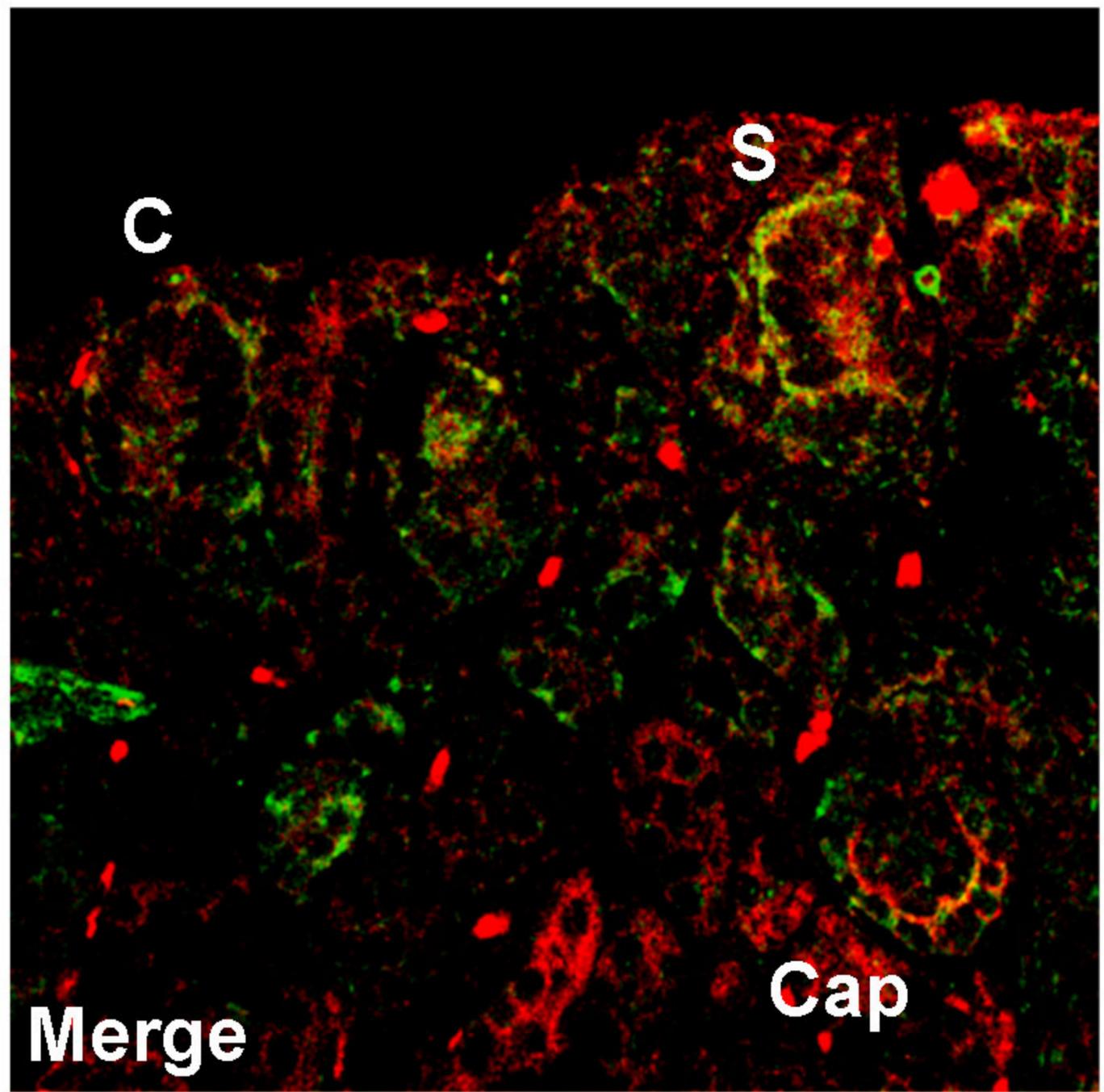
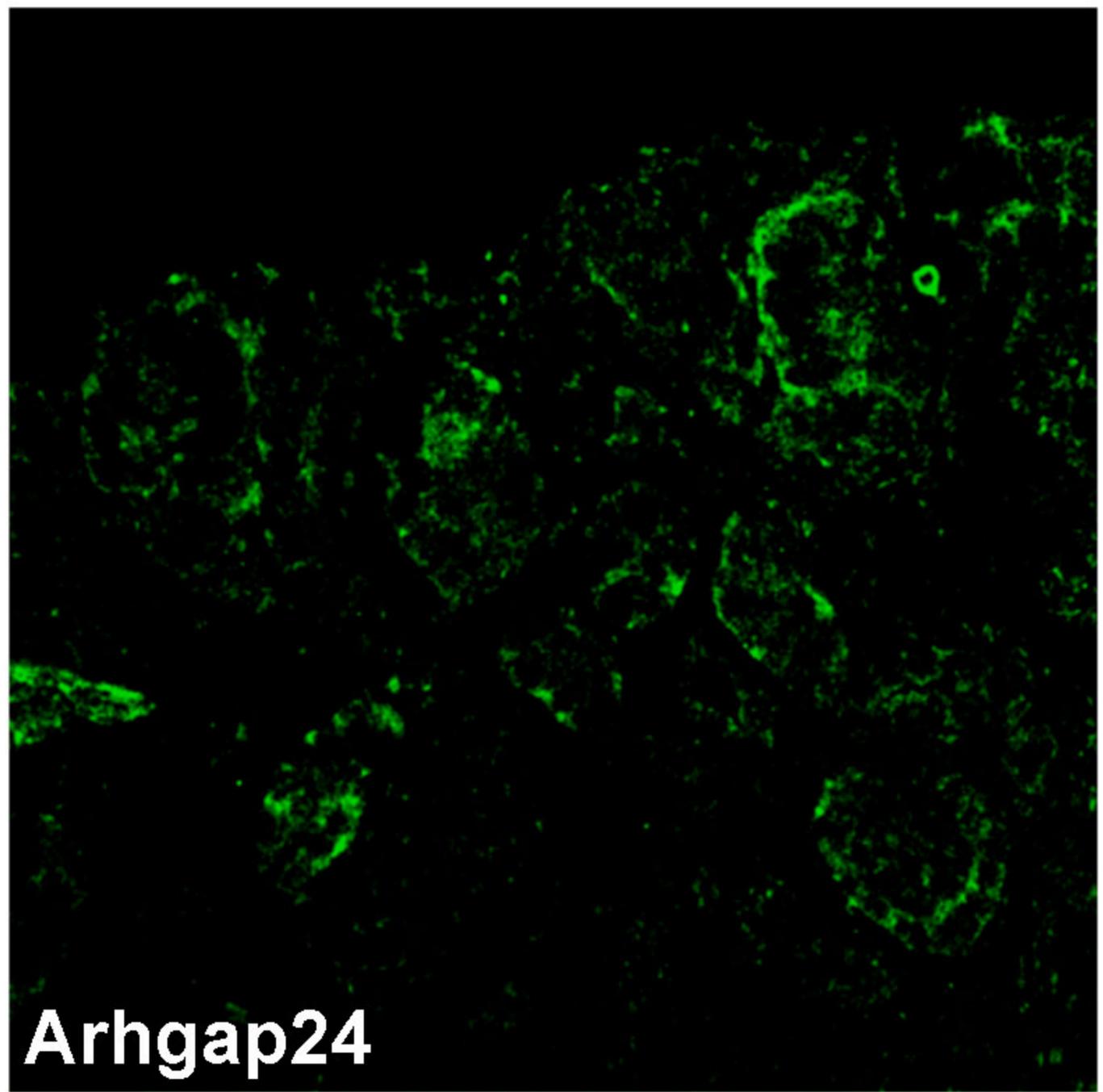
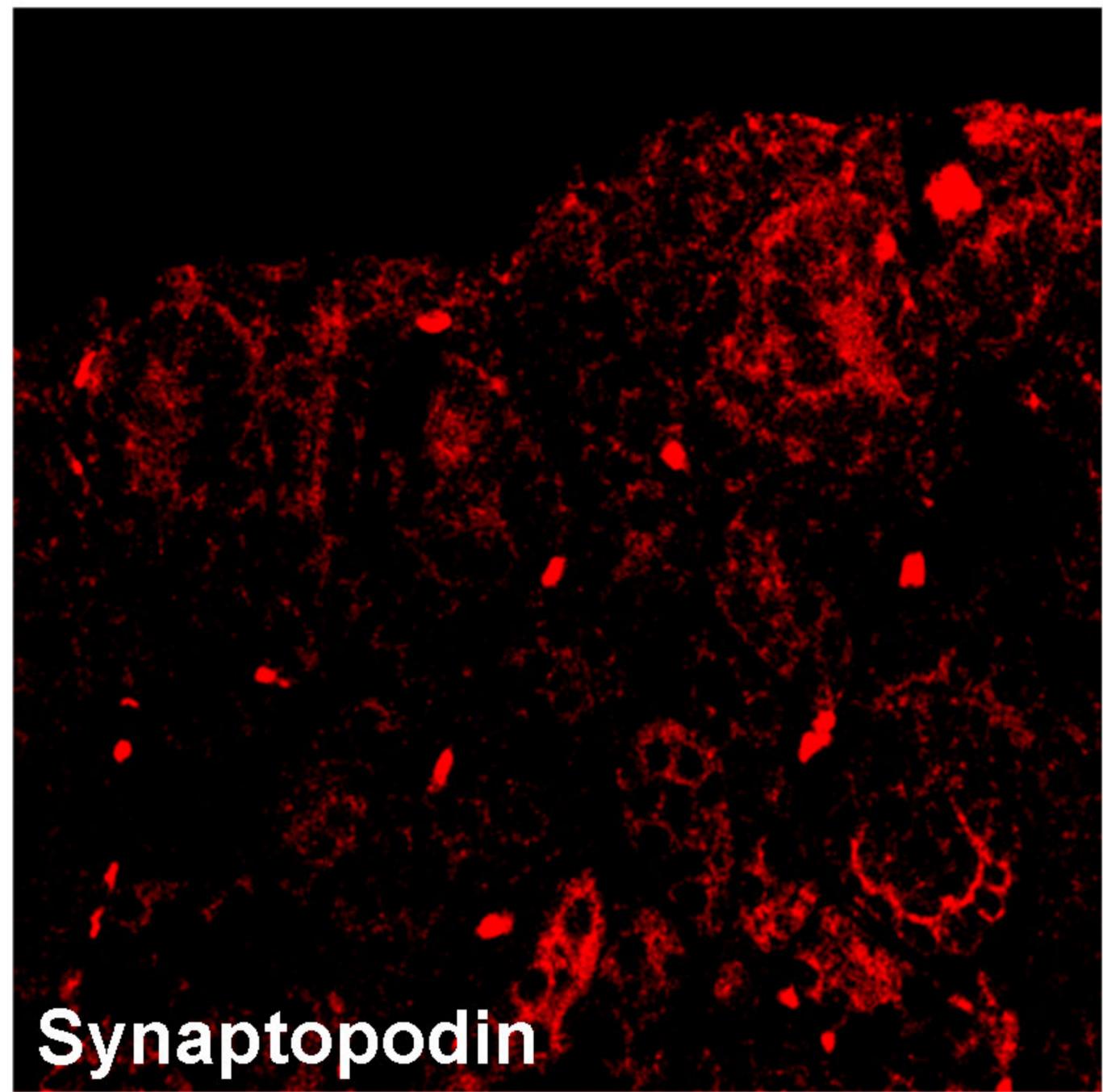
Supplemental Table 2. List of genes that are differentially expressed by at least five-fold between undifferentiated and differentiated cultured podocytes. Arhgap24 was chosen for further analysis given the magnitude of upregulation upon differentiation and by examination of the probeset hybridization pattern. See main text for additional details.

<u>Probe Set</u>	<u>Gene</u>	<u>Accession</u>	<u>Undifferentiate d (33°C) MBEI</u>	<u>Differentiate d (37°C) MBEI</u>	<u>Fold chang e</u>
1423571_at	SIpr1: sphingosine-1-phosphate receptor 1	BB133079	783.38	36.76	-21.31
1424588_at	Srgap3: SLIT-ROBO Rho GTPase activating protein 3	AF481964	71.52	755.51	10.56
1424842_a_at	Arhgap24: Rho GTPase activating protein 24	BC025502	139.93	2541.26	18.16
1433553_at	Garnl3: GTPase activating RANGAP domain-like 3	BB131106	15.72	249.36	15.86
1434911_s_at	Arhgap19: Rho GTPase activating protein 19	BG072763	521.6	89.05	-5.86
1435033_at	Arhgef4: Rho guanine nucleotide exchange factor (GEF) 4	AV024939	71.91	359.56	5
1456337_at	Arap2: ArfGAP with RhoGAP domain, ankyrin repeat and PH domain 2	AV291818	68.35	345.45	5.05

Supplemental Figure 1

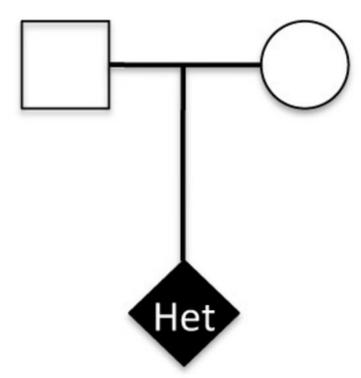


Supplemental Figure 2

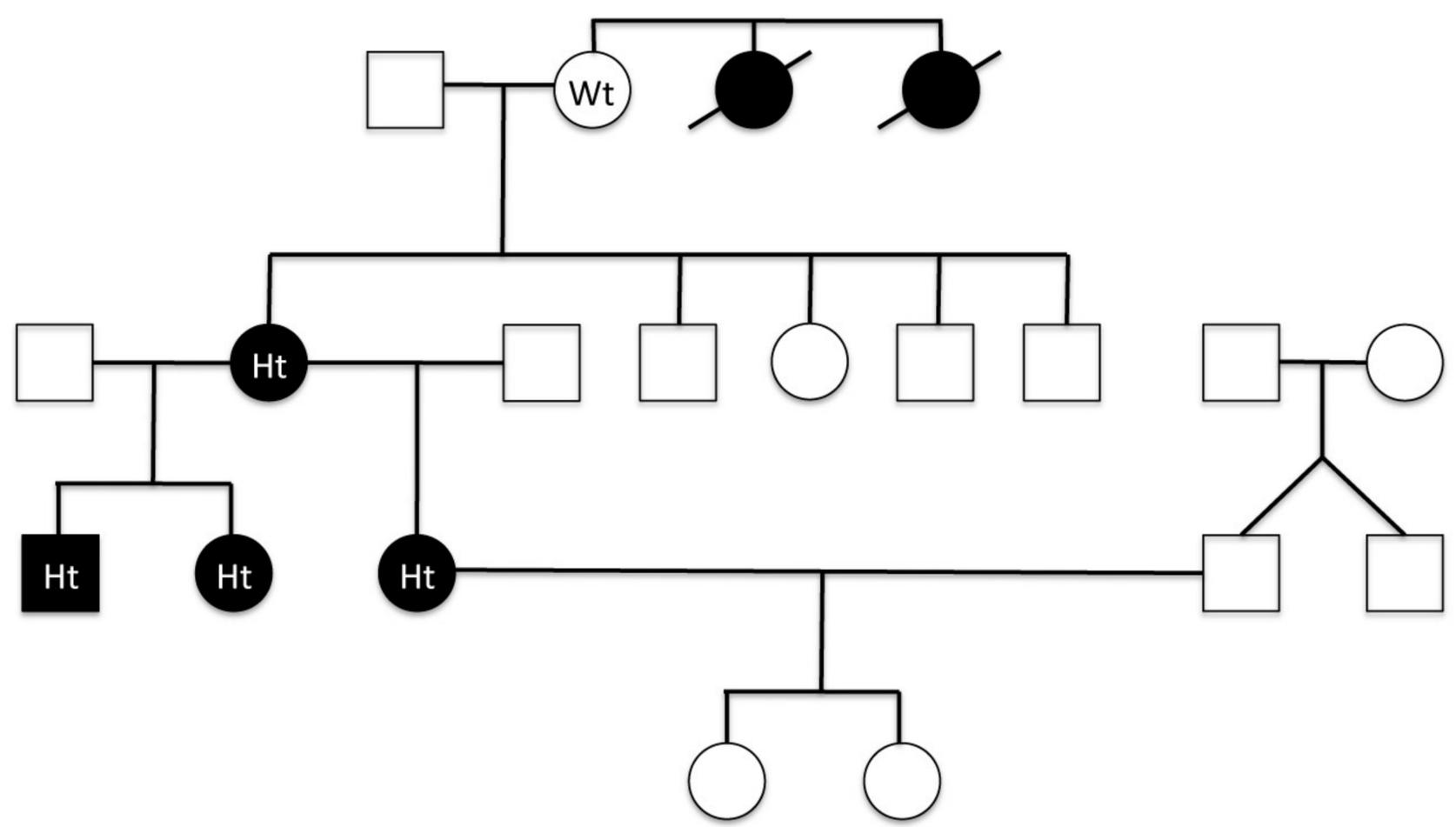


Supplementary Figure 3

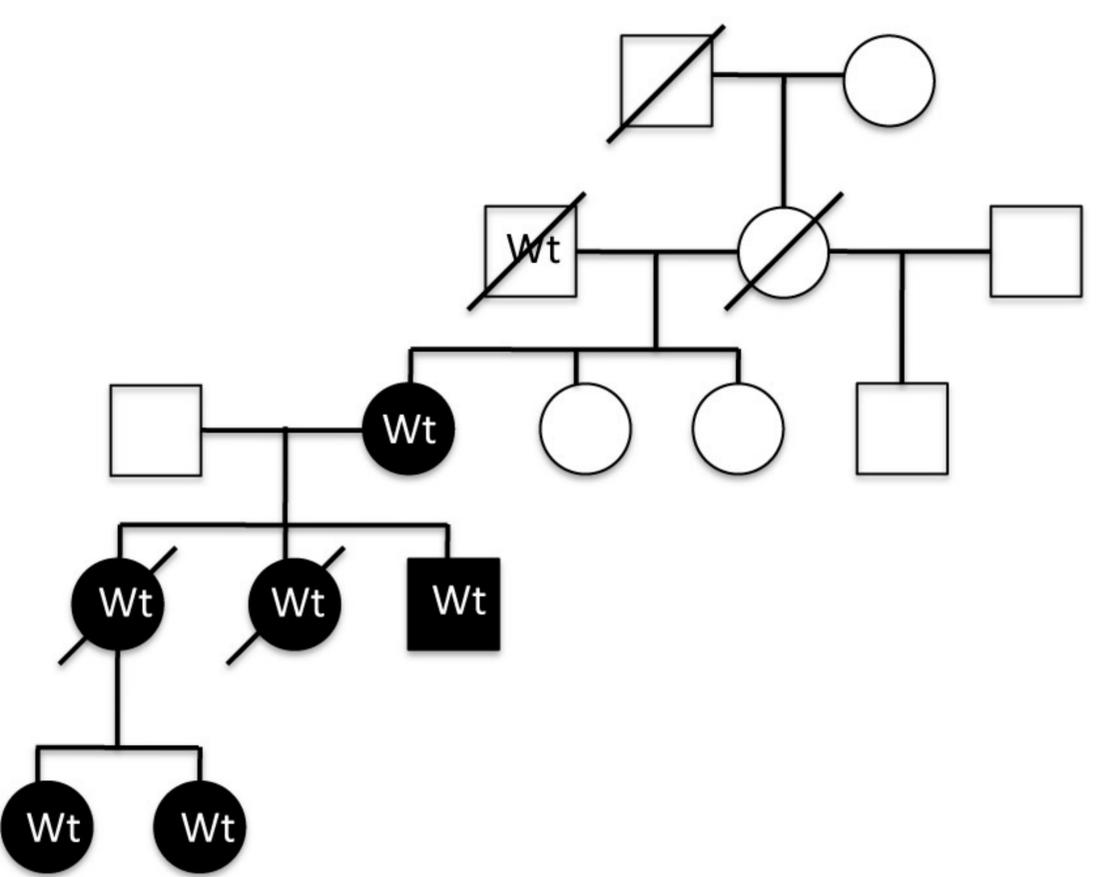
T97I



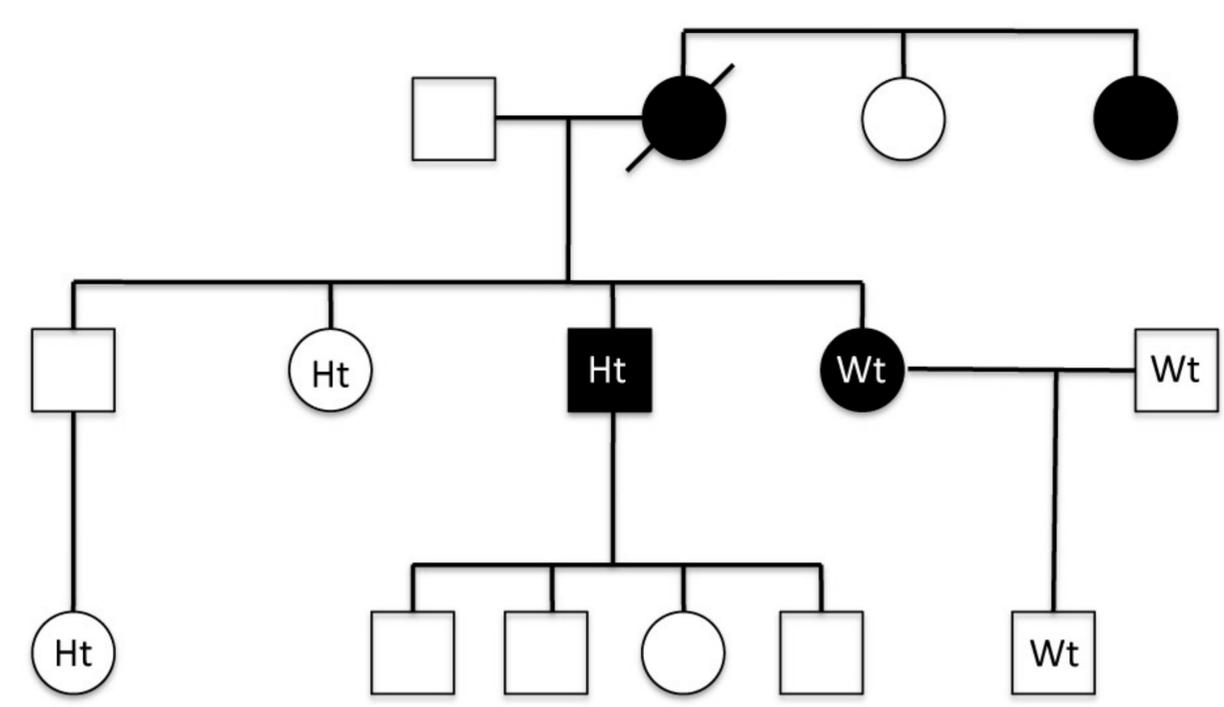
P417A Pedigree #1



P417A Pedigree #2



P417A Pedigree #3



Supplemental Figure 4

