Supplementary Material

Steroid-sensitive nephrotic syndrome candidate gene CLVS1 regulates podocyte oxidative stress and endocytosis

Brandon M Lane[1], Megan Chryst-Stangl[1], Guanghong Wu[1], Mohamed Shalaby[2], Sherif El Desoky[2], Claire C Middleton[1], Kinsie Huggins[1], Amika Sood[3], Alejandro Ochoa[3], Andrew F Malone[4], Ricardo Vancini[5], Sara E Miller[5], Gentzon Hall[1,6], So Young Kim[7], David N Howell[5], Jameela A Kari[2], Rasheed Gbadegesin[1,6]

Supplementary Table 1: Homozygous Variants found in a family with hereditary SSNS and *in-silico* data

Gene (variant)	**gnomAD Allele Frequency**	**Homozygous in gnomAD**	**Homozygous variant in unaffected family members?**	**CADD Score**	**SIFT**	**Polyphen**	**Mut. Taster**	**Conservation**
CLVS1 (H310Y)	15 / 281974	No	No (0)	22.6	Damaging	Possibly Damaging	Disease Causing	Frog
COL6A1 (P827L)	6 / 267418	No	Yes (1)	27.0	Damaging	Probably Damaging	Polymorphism	Frog
MX2 (A516T)	14 / 282404	No	Yes (2)	27.0	Damaging	Probably Damaging	Disease Causing	Lamprey
EML4 (D243Y)	NA	No	Yes (1)	25.9	Damaging	Probably Damaging	Disease Causing	Lamprey

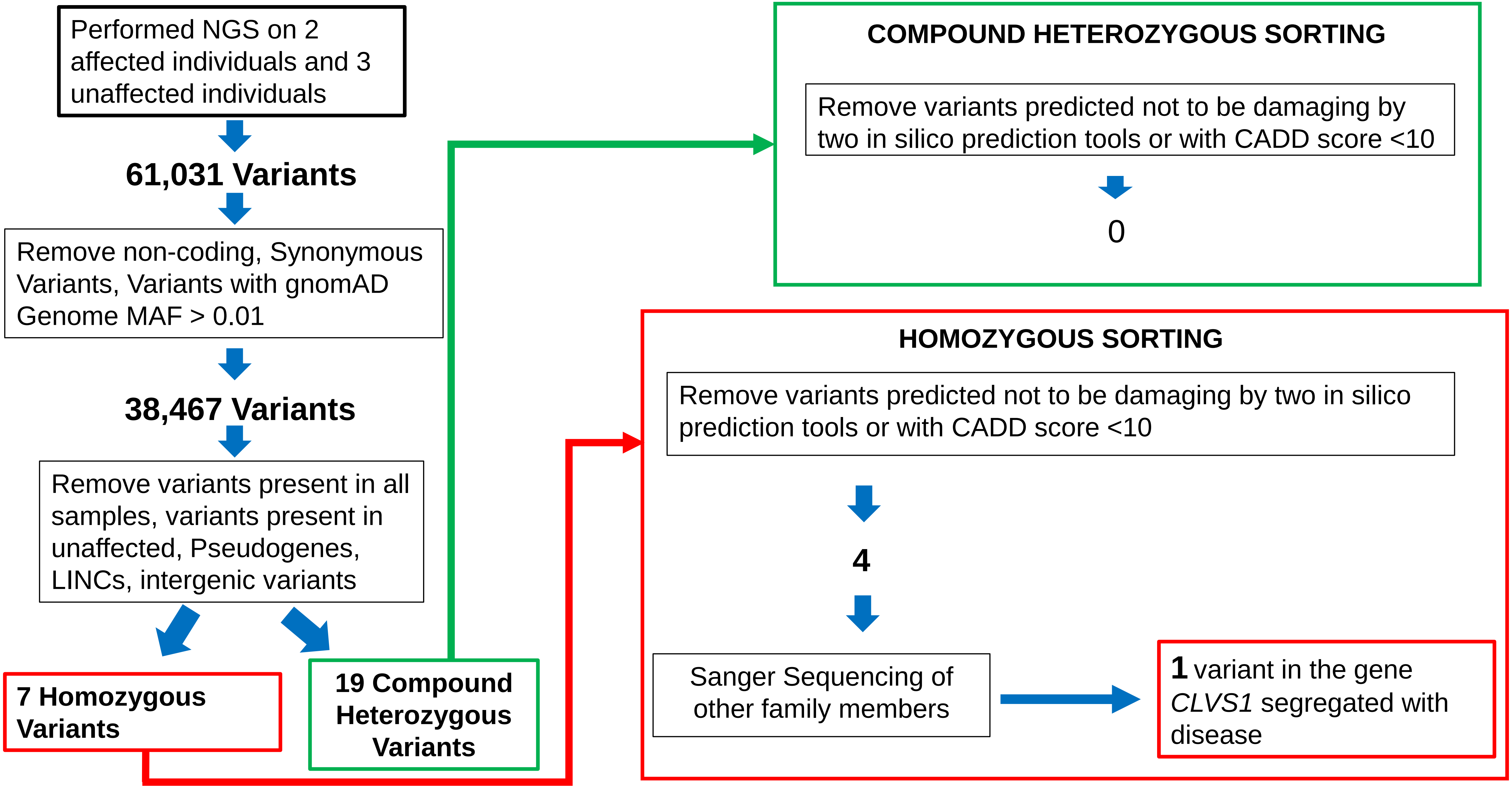
Supplementary Table 2: Summary of Homozygosity Mapping in a Consanguineous Family with Hereditary SSNS

Chromosome	Homozygosity Region*	Size (MB)	Candidate Genes in Homozygosity Regions	Known Genes in Homozygosity Regions
1	20,639,207 - 27,023,656	6.38	0	0
4	98,761,900 - 100,334,066	1.57	0	0
8	54,138,291 - 77,616,519	23.48	1 *(CLVS1)*	0
10	97,987,378 - 108,338,917	10.35	0	1 *(PAX2)* +
21	42,317,771 - 46,950,863	4.63	1 *(MX2)*	0

* Based on Human GRCH37/hg19

\+ *PAX2* is an autosomal dominant gene

Supplementary Figure 1: Variant Filtering



Performed NGS on 2 affected individuals and 3 unaffected individuals

61,031 Variants

Remove non-coding, Synonymous Variants, Variants with gnomAD Genome MAF > 0.01

38,467 Variants

Remove variants present in all samples, variants present in unaffected, Pseudogenes, LINCs, intergenic variants

7 Homozygous Variants

19 Compound Heterozygous Variants

COMPOUND HETEROZYGOUS SORTING

Remove variants predicted not to be damaging by two in silico prediction tools or with CADD score <10

0

HOMOZYGOUS SORTING

Remove variants predicted not to be damaging by two in silico prediction tools or with CADD score <10

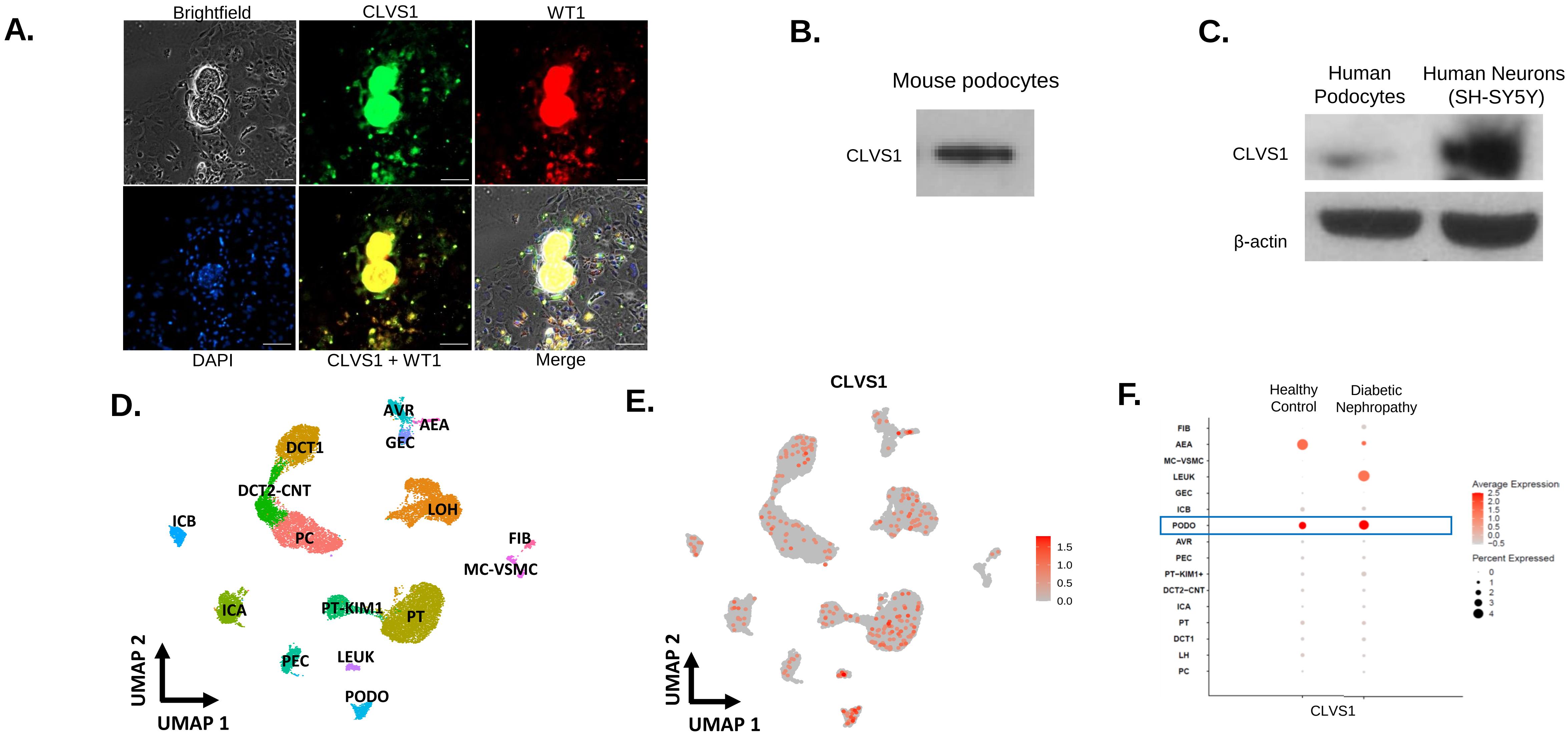
4

Sanger Sequencing of other family members

1 variant in the gene *CLVS1* segregated with disease

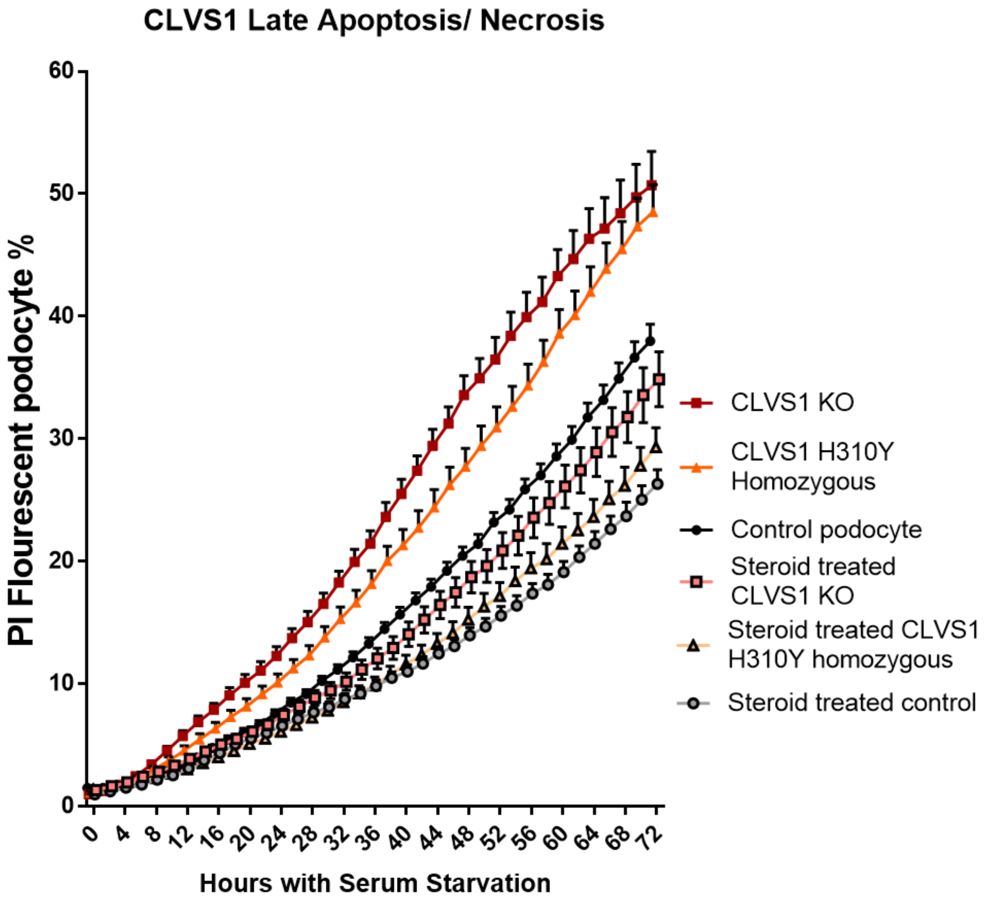
Supplementary Figure 2: *CLVS1* Expression in podocytes

***CLVS1* is expressed in podocytes**. **A)** Immunofluorescence imaging of extracted mouse glomeruli revealed co-localization of CLVS1 and WT1 proteins in podocytes (bars =100µM). **B)** *CLVS1* expression was confirmed in the extracted mouse podocytes with immunoblotting. **C)** *CLVS1* is highly expressed in human neuron cell lines (SH-SY5Y) compared to the conditionally immortalized human podocyte cell lines. **D-F**) Analysis of publicly available human kidney single cell sequencing data confirmed expression of *CLVS1* in podocytes. (26)



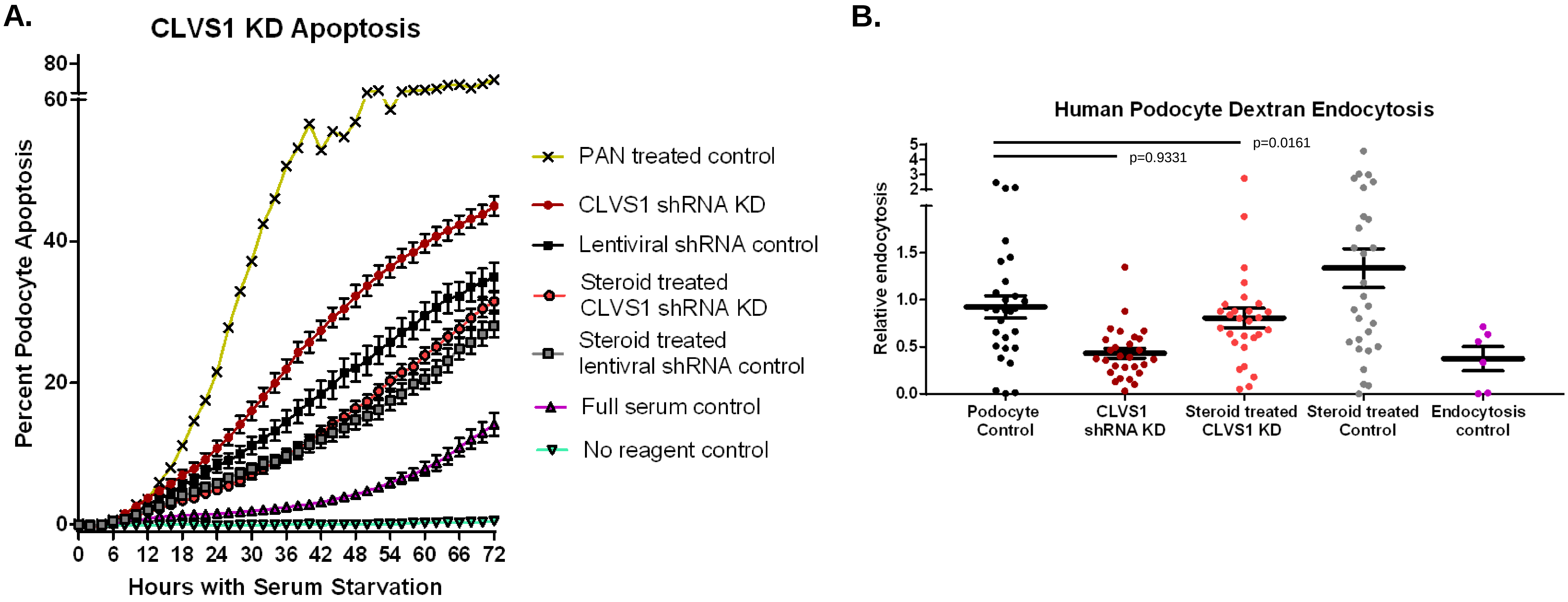
Supplementary Figure 3: *CLVS1* Late Apoptosis/Necrosis

***CLVS1* KO and H310Y KI increases total cell death in podocytes.** Quantification of Propidium revealed an increase in podocyte late apoptosis/necrosis in podocytes with *CLVS1* KO and homozygous H310Y KI podocytes compared to controls (*p<0.05 for all time points after 20 hours, N>25 for each group, two-way ANOVA) which was rescued by treatment with 1µM Dexamethasone.



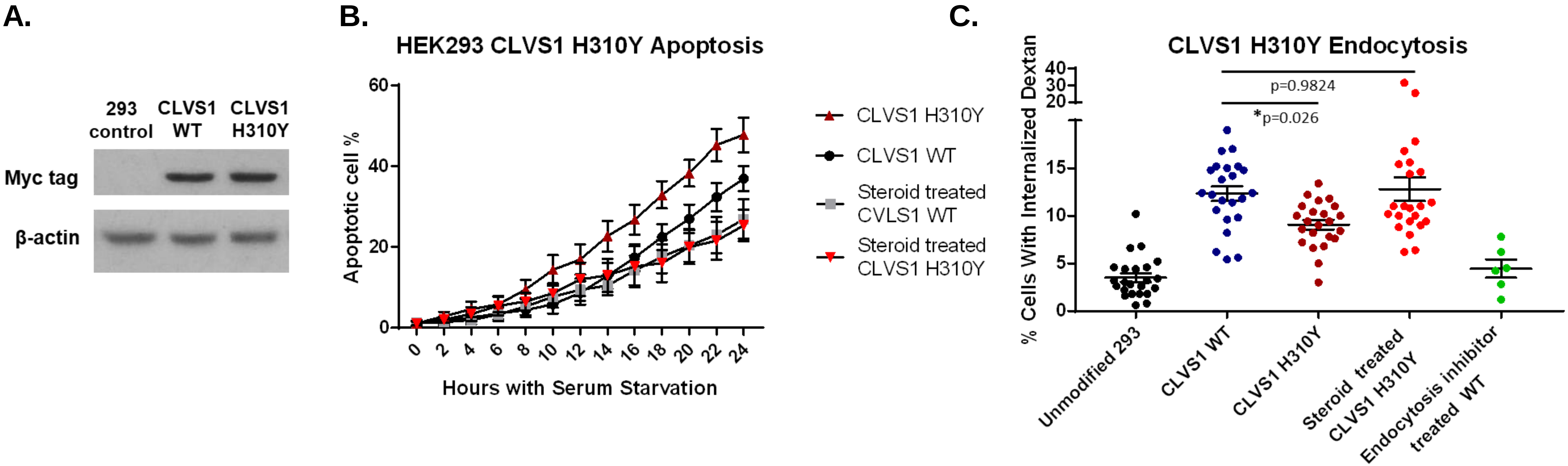
Supplementary Figure 4: *CLVS1* KD Podocyte Apoptosis and Endocytosis

***CLVS1* knockdown increases podocyte susceptibility to apoptosis and endocytosis**. **A)** Quantification of cleaved caspase 3 revealed an increase podocyte susceptibility to serum starvation induced apoptosis in podocytes with *CLVS1* knockdown (KD) compared to controls (*$p<0.05$ for all time points after 30 hours, N>15 for each group, two-way ANOVA) which was rescued by treatment with 1μM Dexamethasone. **B)** Similar to *CLVS1* KO podocytes, *CLVS*1 KD also decreased endocytosis of fluorescently labeled phRODO molecules ($p=0.0161$, $n=28$ each, one-way ANOVA) that could be rescued with steroid treatment ($p=0.9331$) .



Supplementary Figure 5: HEK293 Cell CLVS1 Analysis

The *CLVS1* p.H310Y variant decreases cellular endocytosis and viability. A) Western blot showing similar levels of Myc-tagged *CLVS1* expression in WT and p.H310Y transfected HEK293 cells. **B)** Overexpression of the *CLVS1* p.H310Y variant increased susceptibility to apoptosis in HEK293 cells (n>12, p<0.05 for all time points > 14hours, two-way ANOVA) that could be rescued with 1µM Dexamethasone. (p>0.05 for all time points, two-way ANOVA) **C)** *CLVS1* p.H310Y expressing HEK293 cells displayed decreased internalization of internalized fluorescently labeled dextran molecules (pHrodo) compared to their respective controls (n=23, p=0.026, one-way ANOVA). These endocytosis deficiencies can be restored with pre-treatment with 1µM Dexamethasone (n=23, p=0.9824, one-way ANOVA).



Supplementary Figure 6: Protein Binding Predictions

The *CLVS1* p.H310Y variant alters ligand binding. A-B) Protein ligand predictions and biological annotations of the target protein by COFACTOR and COACH based on the I-TASSER structure prediction revealed that one of the top clavesin-1 ligands (A), VIV, was disrupted by the H310Y mutation (B). **C)** Two views of a model of the alpha tocopherol transport protein bound to a wild type clavesin-1 molecule in the region predicted to be disrupted by the structural effects of H310Y.

A.

Predicted CLVS1 WT Ligands

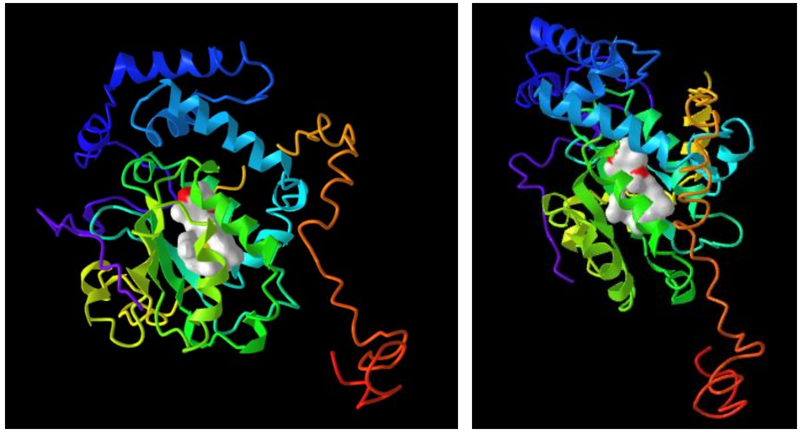
Rank	C-score	Cluster size	PDB Hit	Ligand Name
1	0.32	17	3hy5A	RET
2	0.2	14	1r5lA	VIV
3	0.13	7	3w67B	3PT
4	0.05	5	3b7nA	B7N
5	0.04	3	1oizA	TRT

B.

Predicted CLVS1 H310Y Ligands

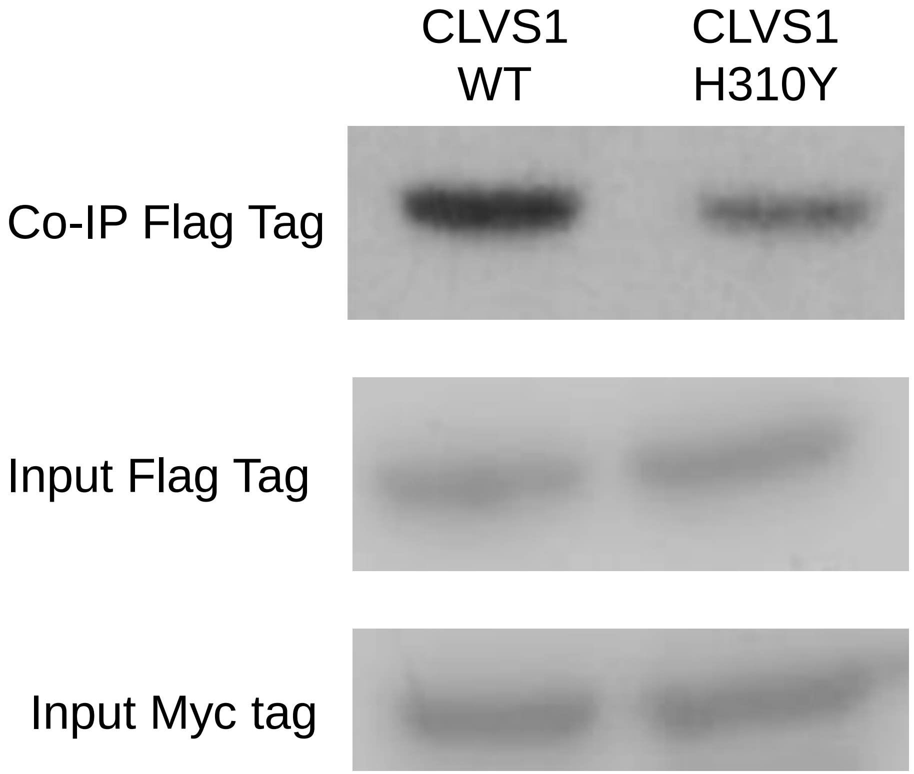
Rank	C-score	Cluster size	PDB Hit	Ligand Name
1	0.26	16	3hy5A	RET
2	0.21	15	1oizB	TRT
3	0.07	6	3w67B	3PT
4	0.05	5	3b7nA	B7N
5	0.04	3	4j7qA	B7N

C.



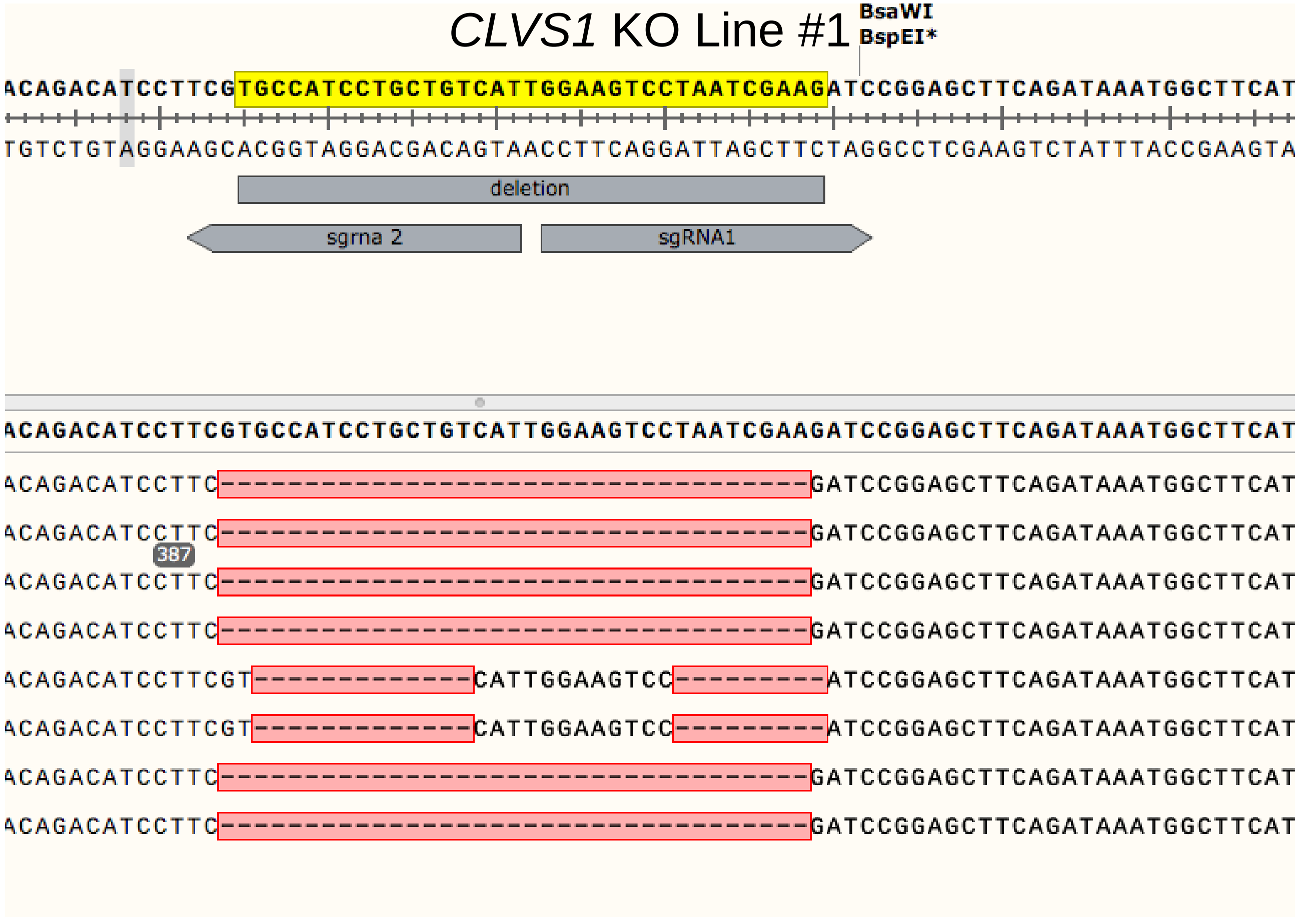
Supplementary Figure 7: Co-IP Myc Tag Pulldown

The *CLVS1* p.H310Y variant decreases binding to αTTP. Co-immunoprecipitation studies revealed a decrease in Flag-tagged αTTP bound to the immunoprecipitated Myc-tagged clavesin-1 when both are expressed at equivalent levels in HEK293 cells.

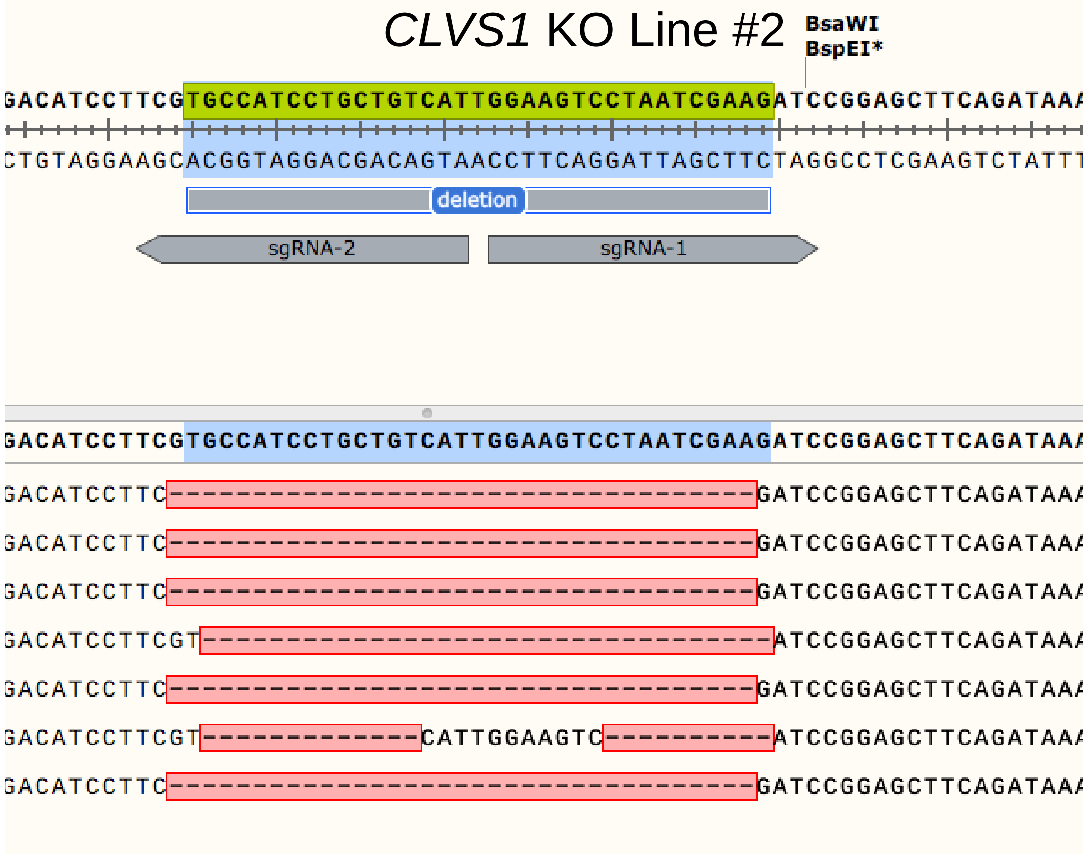


Supplementary Figure 8: *CLVS1* KO lines

***CLVS1* KO Podocyte Lines**. **A**) Schematic depicting the targeting strategy for KO line creation with primers and sgRNA as well as the deletions present in each of the two *CLVS1* KO podocyte lines that were examined in this study.



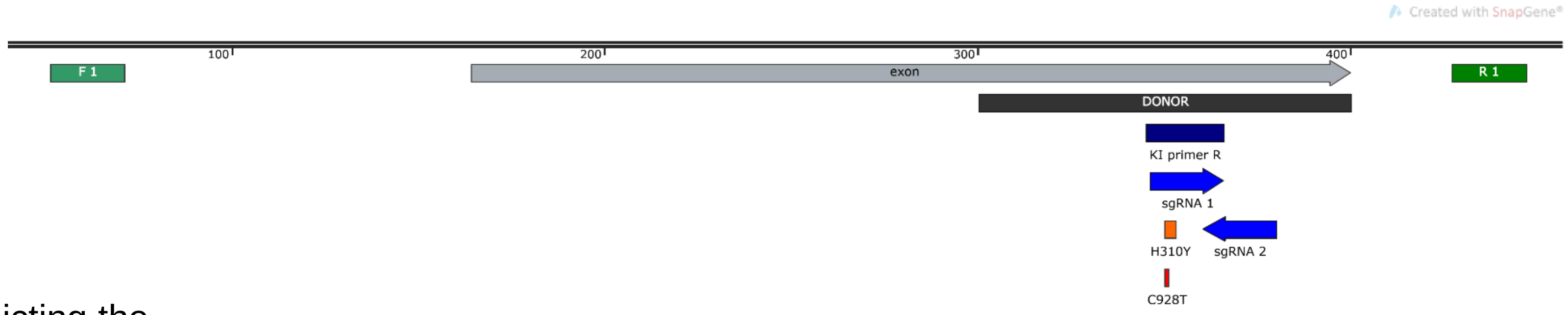
6/8 clones have an out of frame 35bp deletion
2/8 clones have a 22bp deletion



5/7 clones have an out of frame 35bp deletion
1/7 clones have a 23bp deletion
1/7 clones have a 34bp deletion

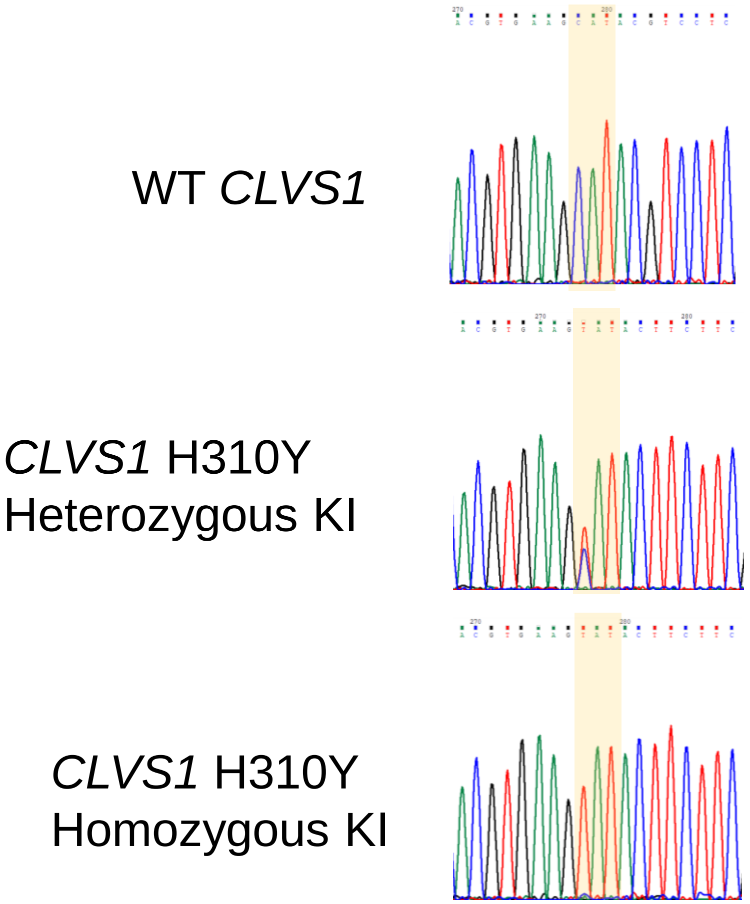
Supplementary Figure 9: *CLVS* H310Y construct

A.



***CLVS1* H310Y KI Podocyte Lines**. **A**) Schematic depicting the targeting strategy for KI line creation with donor oligo, primers, sgRNAs to create the C928T mutation resulting in the p.H310Y variant. **B**) Sequencing analysis identified a clone with a heterozygous C928T mutation and another clone with a homozygous mutation resulting in H310Y (codon highlighted in orange). Additional sequencing identified a heterozygous *CLVS1* variant upstream of the H310Y variant in the homozygous KI line that is predicted to be benign by Polyphen *in silico* analysis (0.014).

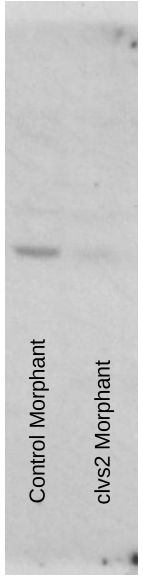
B.



Supplementary Figure 10: Uncropped Western Blots

Fig 2

clvs2



β-actin

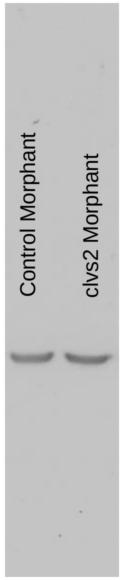
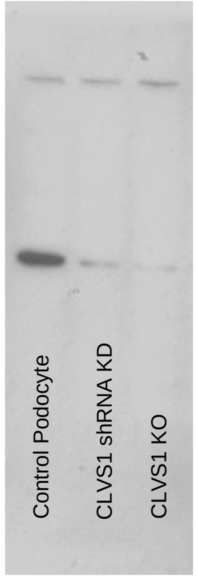


Fig 3

CLVS1



β-actin

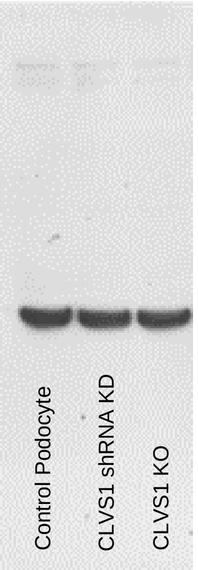
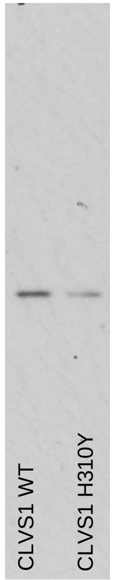


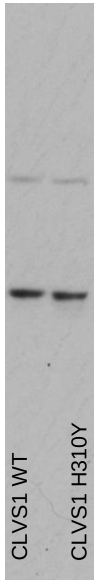
Fig 6

Myc Tag
Co-IP

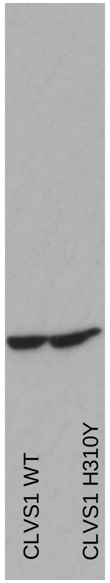


Flag Tag
Input
Lysate

Myc Tag
Input
Lysate



β-actin
Input
Lysate



Supplementary Videos Legend

Videos depict human *CLVS1* podocyte cell lines during serum starvation with images taken every 2 hours for 72 hours. Apoptosis can be visualized when a caspase 3 substrate is cleaved and fluoresces green. Late apoptosis and necrosis can be viewed through red Propidium iodide fluorescence.

Supplementary Video 1: Control podocytes

Supplementary Video 2: *CLVS1* KO podocytes

Supplementary Video 3: Steroid treated *CLVS1* KO podocytes