Supplementary

Table S1 Biochemical analyses of infant blood and urine are conducted at TTUHSC hospital laboratories

Variables	Description
Creatinine	Always WNL (0.2–0.3 mg/dL)
Albumin	0.5 g/dL (DOL 2); was always between 1.5–2.6 g/dL despite regular albumin infusion (normal range 0–14 days old: 3.3–4.5 g/dL)
Urine protein/creatine ratio	164 mg/mg (DOL 5) (nephrotic range Upr/Cr ratio >2 mg/mg)
Urinary protein	2,144 mg/dL (DOL 5)
Urinary creatinine	<13 mg/dL (DOL 5)
Triglyceride	173–792 mg/dL [reference range 0–9 yrs: <75 mg/dL]
TSH (thyroid stimulating hormone) and free T4 (thyroxine 4)	9.11 mIU/mL and 40.81 ng/dL respectively at DOL 54 when synthroid was started (normal levels TSH 1 week–3 months: 720–11,000 mIU/mL; free T4 31 days–12 months: 0.48–2.34 ng/dL)

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Table S2 Gene Panel for Focal Segmental Glomerulosclerosis and Nephrotic Syndrome (Mayo Clinic Website)

Gene	Reference transcript	Gene	Reference transcript
ACTN4	NM_004924.6	*LMX1B*	NM_002316.4
ALG1	NM_019109.5	*MAGI2*	NM_012301.4
ANLN	NM_018685.5	*MYH9*	NM_002473.5
APOL1	NM_003661.4	*MYO1E*	NM_004998.4
ARHGAP24	NM_001025616.3	*NPHS1*	NM_004646.3
ARHGDIA	NM_001185077.3	*NPHS2*	NM_014625.4
CD2AP	NM_012120.3	*NUP107*	NM_020401.4
CLCN5	NM_000084.5	*NUP133*	NM_018230.3
COL4A3	NM_000091.5	*NUP160*	NM_015231.2
COL4A4	NM_000092.5	*NUP205*	NM_015135.3
COL4A5	NM_000495.5	*NUP85*	NM_024844.5
COQ2	NM_015697.8	*NUP93*	NM_014669.5
COQ6	NM_182476.3	*OCRL*	NM_000276.4
COQ8B	NM_024876.4	*PAX2*	NM_003987.4
CRB2	NM_173689.7	*PDSS2*	NM_020381.4
CUBN	NM_001081.4	*PLCE1*	NM_016341.4
DGKE	NM_003647.3	*PLCG2*	NM_002661.5
EMP2	NM_001424.6	*PMM2*	NM_000303.3
FAN1	NM_014967.5	*PODXL*	NM_001018111.3
FAT1	NM_005245.4	*PTPRO*	NM_030667.3
FN1	NM_212482.3	*SCARB2*	NM_005506.4
INF2	NM_022489.4	*SGPL1*	NM_003901.4
ITGA3	NM_002204.4	*SMARCAL1*	NM_014140.4
ITGB4	NM_000213.5	*TBC1D8B*	NM_017752.3
KANK2	NM_001136191.3	*TRPC6*	NM_004621.6
LAMA5	NM_005560.6	*TTC21B*	NM_024753.5
LAMB2	NM_002292.4	*WDR73*	NM_032856.4

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Table S3 The examination results of a panel of 54 genes (Table S2) reveal four genes and their nucleotide IDs (hg19) tested for variants through DNA sequencing in the congenital nephrotic syndrome case study

Gene name/description	NCBI gene ID	NCBI -nucleotide ID	Variant	Zygosity	Clinical significance
NPHS1: NPHS1 adhesion molecule, nephrin	4868	NM_004646.3	c.2150A>G; p.Tyr717Cys(p.Y717C); Chr19(GRCh37):g36335067T>C	Heterozygous	Variant of uncertain significance
NPHS1: NPHS1 adhesion molecule, nephrin	4868	NM_004646.3	c.3024A>G; p.Arg1008= (pR1008=); Chr19(GRCh37):g36330224T>C	Heterozygous	Variant of uncertain significance
ALG1: ALG1 chitobiosyldiphosphodolichol beta-mannosyltransferase	56052	NM_019109.5	c.739C>T; p.Arg247Cys (p.F247C; Chr16(GRCh37):g5128017C>T	Heterozygous	Variant of uncertain significance
CRB2: Crumbs cell polarity complex component 2	286204	NM_173689.7	c.1654G>T; p.Ala552Ser (P.A552S); CHr9(GRCh37):g.126132986G>T	Heterozygous	Variant of uncertain significance
NUP160: Nucleoporin 160	23279	NM_015231.2	c.3271C>T; p.Arg1091Cys (p.1091C); CHr9(GRCh37):g.47819349G>A	Heterozygous	Variant of uncertain significance

The Mayo Clinic performed the analysis.

Table S4 Regulatory elements, CTCF, TFBS (Transcription Factor Binding Site) and CpG Islands (CGI) are identified in the enhancers and silencers located in the forward strand of the NPHS1 genomic region. The JASPAR tool identifies regulatory elements such as TFBS and CTCF motifs. CpG Islands (CGI) are identified by EMBOSS Cpgplot and EMBOSS Newcpgreport

DNA Sequence	Type	Size (nt)	TFBS #	CTCF motif	CGI
LOC127891330	Enhancer	641	1926	8	2
LOC127891331	Enhancer	642	1957	10	0
LOC129664432	Silencer	253	694	1	1
LOC129664433	Silencer	176	525	4	0

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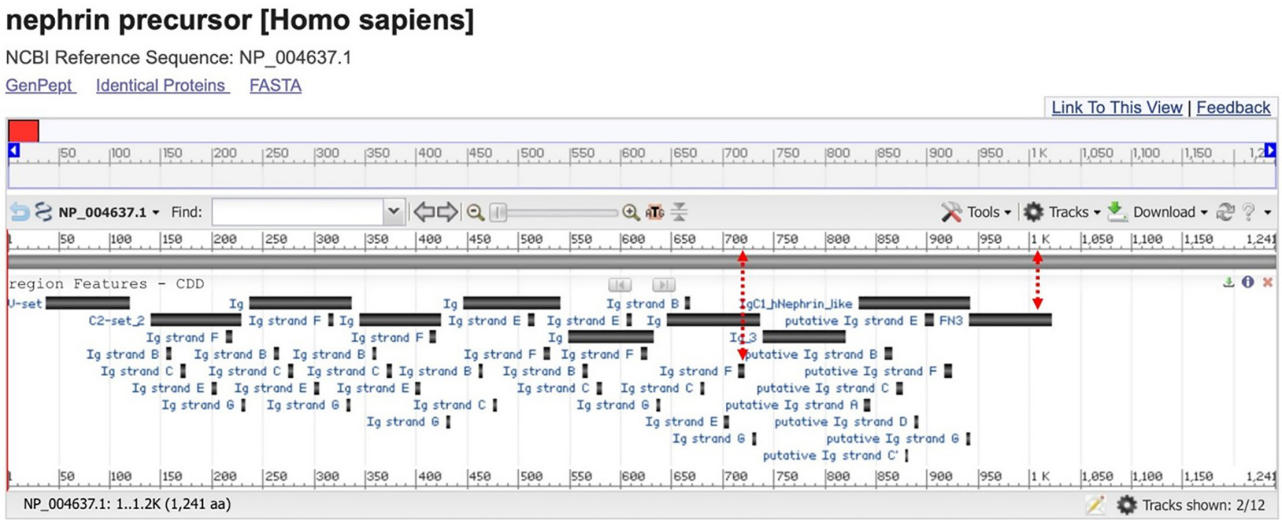

Figure S1 The locations of the two NPHS1 variants along the nephrin precursor are indicated by red arrows. The exon 16 variant (c.2150A>G) may alter the function of the nephrin Ig strand F due to the substitution of tyrosine with cysteine at position 717 (p.Tyr717Cys). The exon 22 variant (c.3024A>G; p.Arg1008=) does not alter nephrin because it is of a synonymous type. Data from the nephrin precursor [Homo sapiens] NCBI reference sequence NP_004637.1.

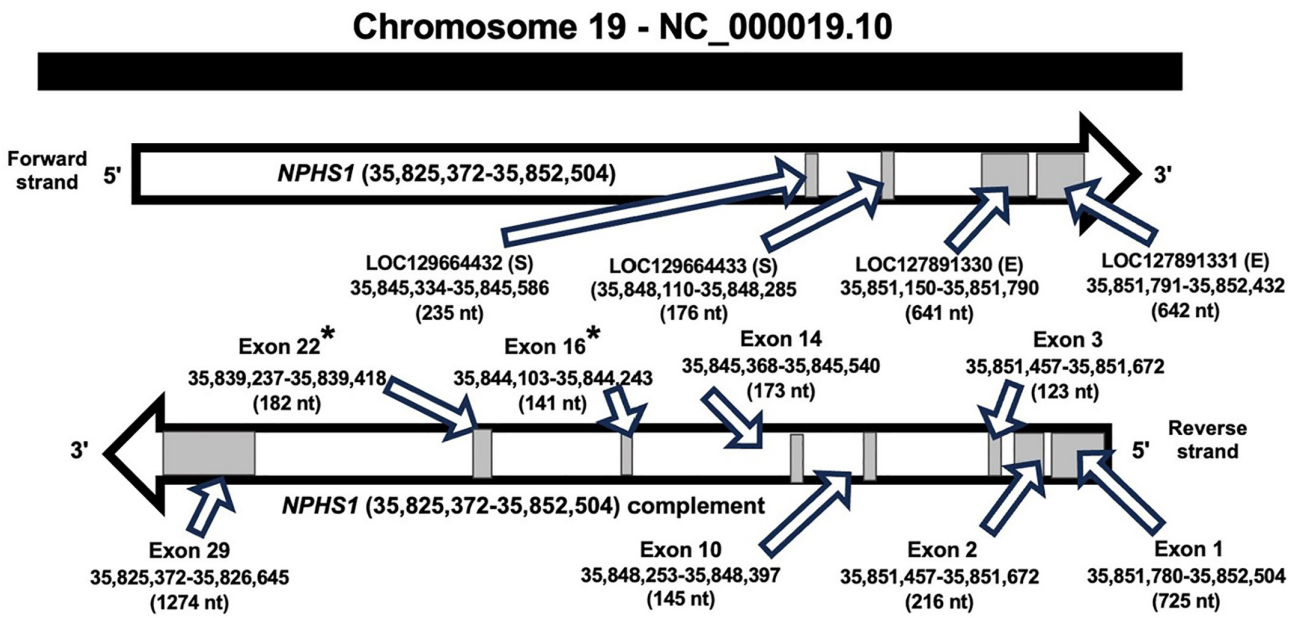

Figure S2 The genomic context of the *NPHS1* locus. Positions of *NPHS1* exons on the reverse strand, as well as enhancers and silencers on the forward strand. Variant (c.2150A>G; p.Tyr717Cys) is mapped to exon 16; variant (c.3024A>G; p.Arg1008=) is mapped to exon 22. Data are adapted from the Gene-NCBI database for human assembly GRCh38.p14 (GCF_000001405.40) updated on 23-Nov-2023. * Indicate VUS in the exon; nt, nucleotide.

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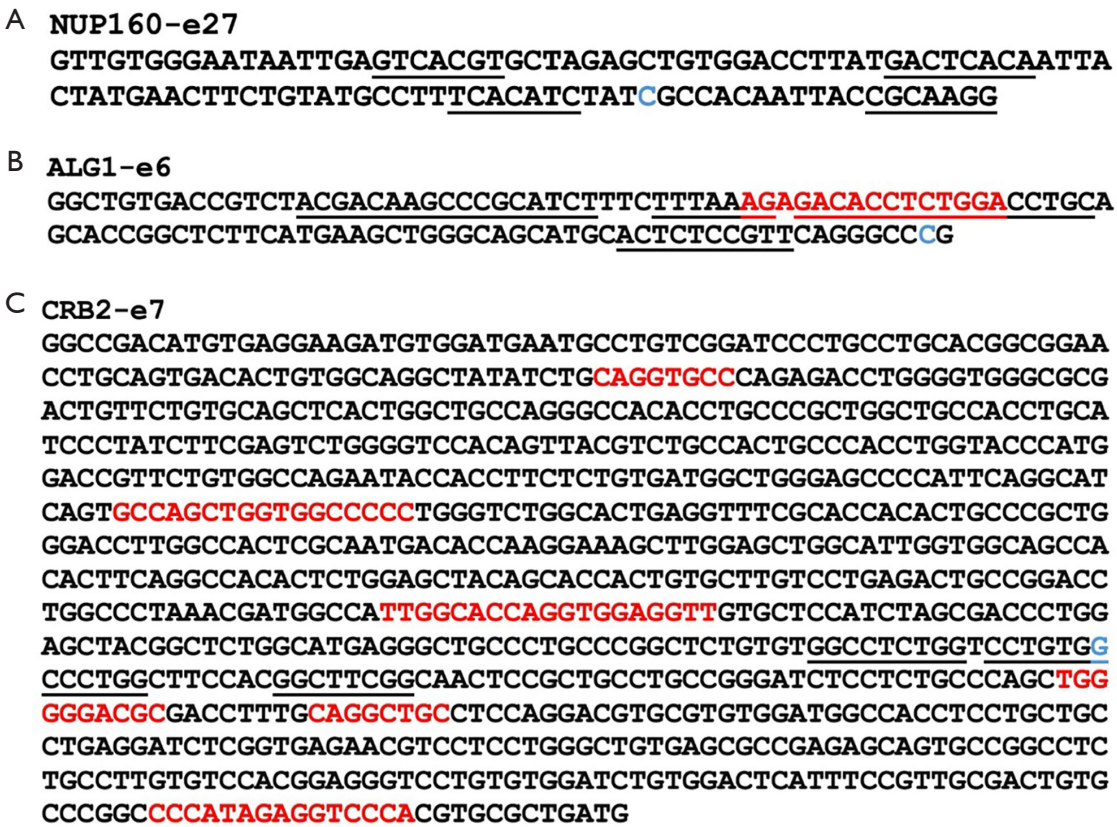

Figure S3 The identified ESE and CTCF motifs in NUP160 exon-27 (A), ALG1 exon-6 (B), and CRB2 exon-7 (C). The CTCF motifs are highlighted in red, ESE motifs are underlined, and the normal base of the mutated nucleotide is highlighted in blue. The CTCF motifs were identified by the JASPAR database (/jaspar.elixir.no). The exonic splicing enhancer (ESE) motifs were identified by ESEfinder 3 (esefinder.ahc.umn.edu).

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