## Supplemental data

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A


ССTGCAGGAATTTACACTCСACTGGTCTCTCTCATTACAGCCTGGGCCGAGCTGGTTAGGTGA GCTCCATAAAACCCAAAGGGACTTGGTGTCAGGAGAGGACATGGAGGGGGCTGAGTGA-

B
SRNS-associated NPHS1 mutation: p.L1240fs1286X
$\frac{- \text {-LTG }}{\text {-Leu }} \frac{\text { CCC }}{\text { Pro }} \frac{\text { TTC }}{\text { Phe }} \frac{\text { GAG }}{\text { Glu }} \frac{\text { CTG }}{\text { Leu }} \frac{\text { AGG }}{\text { Arg }} \frac{\text { GGA }}{\text { Gly }} \frac{\text { CAT }}{\text { His }} \frac{C T C}{\text { Leu }} \frac{A A C}{\text { Asn }} \frac{\text { CCC }}{\text { Pro }} \frac{\text { ATT }}{\frac{\text { Ile }}{} \frac{\text { GTC }}{\text { Val }} \frac{\text { CTG }}{\text { Leu }} \frac{\text { CAC }}{\text { His }} \frac{\text { CTG }}{\text { Leu }} \frac{C A G}{\text { Gln }} \frac{\text { GAA }}{\text { Glu }} \frac{\text { TTT }}{\text { Phe }}}$ (aa1240)
ACA CTC CAC TGG TCT CTC TCA TTA CAG CCT GGG CCG AGC TGG TTA GGT GAG CTC CAT Thr Leu His Trp Ser Leu Ser Leu Gin Pro Gly Pro Ser Trp Leu Gly Glu Leu His

$$
\frac{\text { AAA }}{\text { Lys }} \frac{\operatorname{ACC}}{\text { Thr }} \frac{\text { CAA }}{\text { Gln }} \frac{A G G}{\text { Arg }} \frac{\text { GAC TTG }}{\text { Asp }} \frac{\text { GTG TCA }}{\text { Val }} \frac{\text { GGA }}{\text { Ser }} \frac{\text { GAG }}{\text { Gly }} \frac{\text { GAC }}{\text { Glu }} \frac{\text { ATG }}{\text { Asp }} \frac{\text { GAG }}{\text { Glu }} \frac{\text { GGG }}{\text { Gly }} \frac{\text { GCT }}{\text { Ala }} \frac{\text { GAG TGA- }}{\text { Glu }} \frac{\text { (aa1285) }}{\text { Stop }}
$$



Figure S1. The SRNS-associated NPHS1 mutation p.L1240fs1286X impairs PDZbinding motif (PBM) that is required for MAGI1 binding. (A-B) Amino acid sequence analysis of the SRNS-associated NPHS1 mutation p.L1240fs1286X. The 16 deleted nucleotides (c.3720_3735C) in the disease mutation are shown in red (A). The additional coding sequence and corresponding amino acids are shown in cyan (B). (C) GST-pull down assay showing that the PBM of the p.L1240fs1286X mutation could not bind to MAGI1-PDZ3.
MAGI1_PDZ3_mouse CQIPDYQEQDIFLWRKETGFGFRILGGNEPGEPIYIGHIVPLGAADTDGRLRSGDELICV
MAGI1 PDZ3 human CPIPDYOEODIFLWRKETGFGFRILGGNEPGEPIYIGHIVPLGAADTDGRLRSGDELICV
MAGI1 PDZ3 rat COIPDYOEODIFLWRKETGFGFRILGGNEPGEPIYIGHIVPLGAADTDGRLRSGDELICV
MAGI1_PDZ3_COW NRLPDYQEQDIFLWRKETGFGFRILGGNEPGEPIYIGHIVPLGAADTDGRLRSGDELICV
MAGI1_PDZ3_fish SRLPDYQEQDIFLWRKDTGFGFRILGGNEPGEPIYIGHIVKYGAADEDGRLRSGDELICV
MAGI1 PDZ3 worm YNQKPSDLITVSLIRKPVGFGFRLLGGVESKTPLSVGQIVIGGAAEEDGRLQEGDEIVEI
MAGI1_PDZ3_fly


Figure S2. Amino acid sequence alignment of PDZ3 domain of MAGI1 from
different species. The totally conserved and conserved residues are color in red and green, respectively.


Figure S3. Gly at $\mathbf{- 3}$ position of PBM is the key determinant for other targets recognition by MAGI1-PDZ3. (A) Sequence alignment of PBMs of other targets of MAGI1 PDZ3. (B) ITC-based affinity measurements of bindings of MAGI1-PDZ3 to wild type and G-3A mutant of ESAM-PBM.

Table S1. Data collection and refinement statistics

| Data collection and processing |  |
| :---: | :---: |
| Complex | Nephrin-PBM/MAGI1-PDZ3 |
| Source | SSRF-BL18U1 |
| Wavelength(A) | 0.97791 |
| Space group | I23 |
| Unit cell (a,b,c, A ) | 93.731, 93.731,93.731 |
| Unit cell ( $\alpha, \beta, \gamma, \circ$ ) | 90.000,90.000,90.000 |
| Resolution range ( A ) | 50.00-1.78 (1.81-1.78) |
| No. of unique reflections | 13086 (683) |
| Redundancy | 3.9 (3.6) |
| $\mathrm{I} / \sigma(\mathrm{I})$ | 16.16(5.44) |
| Completeness (\%) | 98.1 (99.9) |
| $\mathrm{R}_{\text {merge }}(\%)^{\mathrm{a}}$ | 6.9 (17.2) |
| Wilson_B | 18.68 |
| Structure refinement |  |
| Resolution ( A ) | 46.865-1.780 (1.917-1.780) |
| $\mathrm{R}_{\text {work }}{ }^{\mathrm{b}} / \mathrm{R}_{\text {free }}{ }^{\mathrm{c}}$ (\%) | 17.41 (17.44)/19.85 (21.24) |
| rmsd bonds ( A )/angles $\left(^{\circ}\right.$ ) | 0.009/1.369 |
| Number of reflections |  |
| Working set | 13083(2490) |
| Test set | 639 (129) |
| Number of protein atoms | 856 |
| Number of solvent atoms | 88 |
| Average B factor ( $\AA^{2}$ ) | 22.9 |
| Ramachandran plot(\%) |  |
| Most favored regions | 98.96 |
| Additionally allowed | 1.04 |
| Generously allowed | 0 |

Numbers in parentheses represent the value for the highest resolution shell.
${ }^{\text {a }} \mathrm{R}_{\text {merge }}=\sum\left|\mathrm{I}_{\mathrm{i}}-\mathrm{I}_{\mathrm{m}}\right| / \sum \mathrm{I}_{\mathrm{i}}$, where $\mathrm{I}_{\mathrm{i}}$ is the intensity of the measured reflection and $\mathrm{I}_{\mathrm{m}}$ is the mean intensity of all symmetry related reflections.
${ }^{\mathrm{b}} \mathrm{R}_{\text {cryst }}=\Sigma| | \mathrm{F}_{\text {obs }}\left|-\left|\mathrm{F}_{\text {calc }}\right| / \Sigma\right| \mathrm{F}_{\text {obs }} \mid$, where $\mathrm{F}_{\text {obs }}$ and $\mathrm{F}_{\text {calc }}$ are observed and calculated structure factors.
${ }^{\mathrm{c}} \mathrm{R}_{\text {friee }}=\Sigma \mathrm{T}| | \mathrm{F}_{\text {obs }}\left|-\left|\mathrm{F}_{\text {calc| }}\right| / \Sigma \mathrm{T}\right| \mathrm{F}_{\text {obs }}$, where T is a test data set of about $5 \%$ of the total reflections randomly chosen and set aside prior to refinement.

