

## Supplementary Data

Tyrosine phosphorylation-dependent activation of TRPC6 regulated by PLC- $\gamma$ 1 and Nephrin:  
Effect of mutations associated with focal segmental glomerulosclerosis

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### SUPPLEMENTAL FIGURE LEGENDS

**Figure S1.** The peptide coverage of PLC- $\gamma$ 1 by mass spectrometry analysis.

Tryptic digest of a protein band in Fig. 2A were analyzed by liquid chromatograph-mass/mass spectrometry (LC-MS/MS; Q-STAR Elite, Applied Biosystems). Peptide ions were analyzed with ProteinPilot<sup>TM</sup> (ver. 3.0) software. In the analysis, the score for PLC- $\gamma$ 1 was 60.65, and matched peptides with over 95% confidence (underlined in the figure) covered 30.9% of total human PLC- $\gamma$ 1 sequence.

**Figure S2.** Nephrin regulation of TRPC6 trafficking is not dependent on PLC- $\gamma$ 1 catalytic activity.

A, B. Indicated plasmids were transfected into cultured podocytes, and surface biotinylation assay was performed. For the indicated samples, cells were pretreated with 10  $\mu$ M U73122 for 15 min.

**Figure S3.** Nephrin does not affect tyrosine phosphorylation of TRPC6 by Fyn.

Indicated plasmids were expressed in cultured podocytes, and HA- or FLAG-immunoprecipitates were subjected to western blot with indicated antibodies.

### SUPPLEMENTAL MATERIALS AND METHODS

Following oligonucleotides were used to make mutant plasmids.

HA-TRPC6 Y to F substitution mutants.

Y31F : gagccaggacttttgctgatgg      ccatcagcaaaaagtctctggctc

Y50F : gccaccgttgctactacc      ggtagtagccaacggtggc

Y85F : cgaggaccagcattcatgtttaatg      cattaaacatgaatgctggctctcg  
Y107F : gcagctgaattggcaacatcccag      ctgggatgttgccaaattcagctgc  
Y206F : cagcaagatgactttttgcctatgatg      catcataggcaaaaaagtcattcttgctg  
Y208F : gactttatgcctttgatgaagatggg      cccatctcatcaaaggcataaaagtc  
Y284F : caagtcagcattcctgtcattgtcc      ggacaatgacaggaatgctggacttg  
Y895F : ctcaagtctccgtttgaactcc      ggagttcaaacggagacttgag

HA-TRPC6 FSGS mutants.

P112Q : tggcaacatccaagtggctggaag      cttccagaccacttgatgttgcca  
N143S : ggctgtggccagtgagcacttg      ccaagtgtcactggccacagcc  
S270T : ccttcagccacactagatccaggatc      gatcctggatctagtgtggctgaagg  
K874X : ggcccagattgattaggagcagatgagg      cctcatcgctctcctaatcaatctgggcc  
R895C : catctcaagtctctgttatgaactcc      ggagttcataacagagacttgagatg  
E897K : gtctccgttataaactcctgaagag      ctctcaaggagttataacggagac

HA-TRPC6 deletion (98-104) mutant.

gcctgtctattgaggtgaatattggcaac      gttgccatattcagcctcaatagacaggc

Flag-Nephrin deletion (1216-1227) mutant.

ccctatgaccttgcctacgaccaggtggcagc      gctgccacctggctgtagcgaaggtcataggg

Flag-PLC- $\gamma$ 1 mutants.

N\*(R586K) : ccttcctagtgaagaaagtgagac      gtctcactttcttactaggaagg  
C\*(R694K) : cttcctgtgaagaagcgaacg      cgttgcgcttctcaccaggaag  
Del(N-SH2) : ctcgagcgagaagccacagacgaatg      cattcgtctgtgcttctcgtcagag  
Del(C-SH2) : gcccatgagagcaaaaacgaggagc      gcctcctcgttttgcctcatgggc

GST-Nephrin-CD deletion mutants.

1102-1211 : gaatggaccctaaagaccttgcctg      cagcgaaggtcttaggggtccattc  
1102-1215 : ctatgaccttgcctgacctgaggctccag      ctggacctcaggtcagcgaaggtcatag  
1102-1227 : gaggggaatctaggaccaggtgg      ccacctggctcctagattcccctc

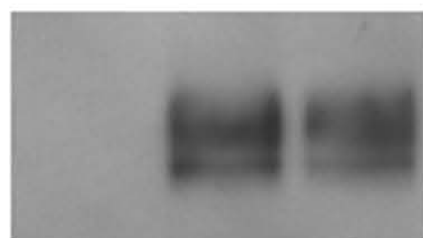
1 MAGAASPCANGCGPGAPSDAEVLHLCRSLEVGTVMTLFYSKKSQRPERKT  
FQVKLETROITWSRGADKIEGAIDIREIKEIRPGKTSRDFDRYQEDPAFR  
101 PDQSHCFVILYGMEFRLKTLSLOATSEDEVNMWIKGLTWLMEDTLOAPT  
LOIERWLRKQFYSVDRNREDRISAKDLKNMLSQVNYRVPNMRFLRERLTD  
201 LEQRSGDITYGQFAQLYRSLMYSAQKTMDLPFLEASTLRAGERPELCRVS  
LPEFQQFLLDYQOGELWAVDRLQVQEFMLSFLRDPLREIEEPYFFLDEFVT  
301 FLFSKENS VWNSQLDAVCPDTMNNPLSHYWISSHNTYLTGDQFSSESSL  
EAYARCLRMGCRCIELDCWDGPDGMPVIYHGHTLTTKIKFSDVLHTIKEH  
401 AFVASEYPVILSIEDHCSIAQQRNMAQYFKKVLGDTLLTKPVEISADGLP  
SPNOLKRKILIKHKKLAEGSAYEEVPTSMMYSENDISNSIKNGILYLEDP  
501 VNHEWYPHYFVLTSSKIYYSEETSSDOGNEDEEEPKEVSSSTELHSNEKW  
FHGKLGAGRDGRHIAERLLTEYCIETGAPDGSFLVRESETFVGDYTLSFW  
601 RNGKVQHCRHSRQDAGTPKFFLTDNLVFDSDLYDLITHYQQVPLRCNEFE  
MRLSEPVPTNAHESKEWYHASLTRAQAEHMLMRVPRDGAFLVRKRNEPN  
701 SYAISFRAEGKIKHCRVQOEGQTVMLGNSEFDSLVDLISYYEKHPLYRKM  
KLRYPINEEALEKIGTAEPDYGALYEGRNPGFYVEANPMPTFKCAVKALF  
801 DYKAOREDELTFIKSAIONVEKQEGGWWRGDYGGKKQLWFPSNYVEEMV  
NPVALEPEREHLDENSP LGDLLRGVLDVPACQIAIRPEGKNNRLFVFSIS  
901 MASVAHWSLDVAADSQEELQDWVKKIREVAOTADARLTEGKIMERRKKIA  
LELSELVVYCRPVPFDEEKIGTERACYRDMSSFPETKAEKYVNKAKGKKF  
1001 LOYNRLOLSRIYPKGQRLDSSNYDPLPMWICGSQLVALNFQTPDKPMQMN  
QALFMTGRHCGYVLQPSTMRDEAFDPFDKSSLRGLEPCAISIEVLGARHL  
1101 PKNGRGIVCPFVEIEVAGAEYDSTKOKTEFVVDNGLNPVWPAKPFHFQIS  
NPEFAFLRFVVYEEDMFSDQNFLAQATFPVKGLKTGYRAVPLKNNYSEDL  
1201 ELASLLIKIDIFPAKENGDLSPFSGTSLRERGS DASGQLFHGRAREGSFE  
SRYQQPFEDFRISQEHLDHFDSRERRAPRRTRVNGDNRL

Figure S1.

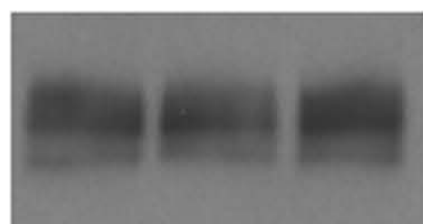
**A**

HA-TRPC6	+	+	+
Fyn	-	+	+
U73122	-	-	+

Biotin-TRPC6

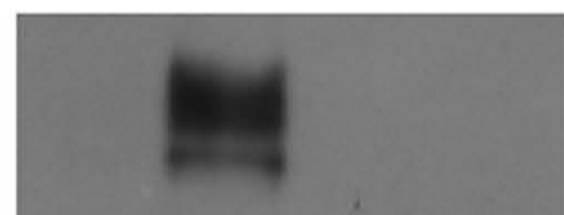


Total-TRPC6

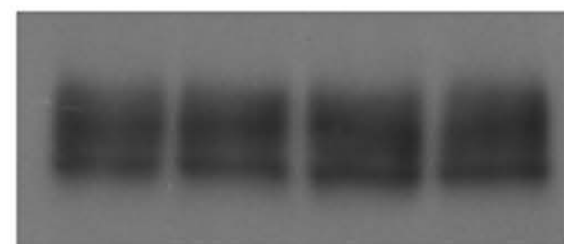
**B**

HA-TRPC6	+	+	+	+
Flag-Nephrin	-	-	+	+
Fyn	-	+	+	+
U73122	-	-	-	+

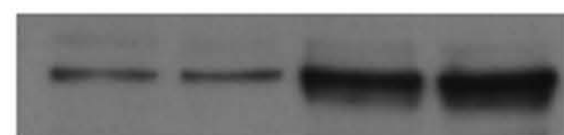
Biotin-TRPC6

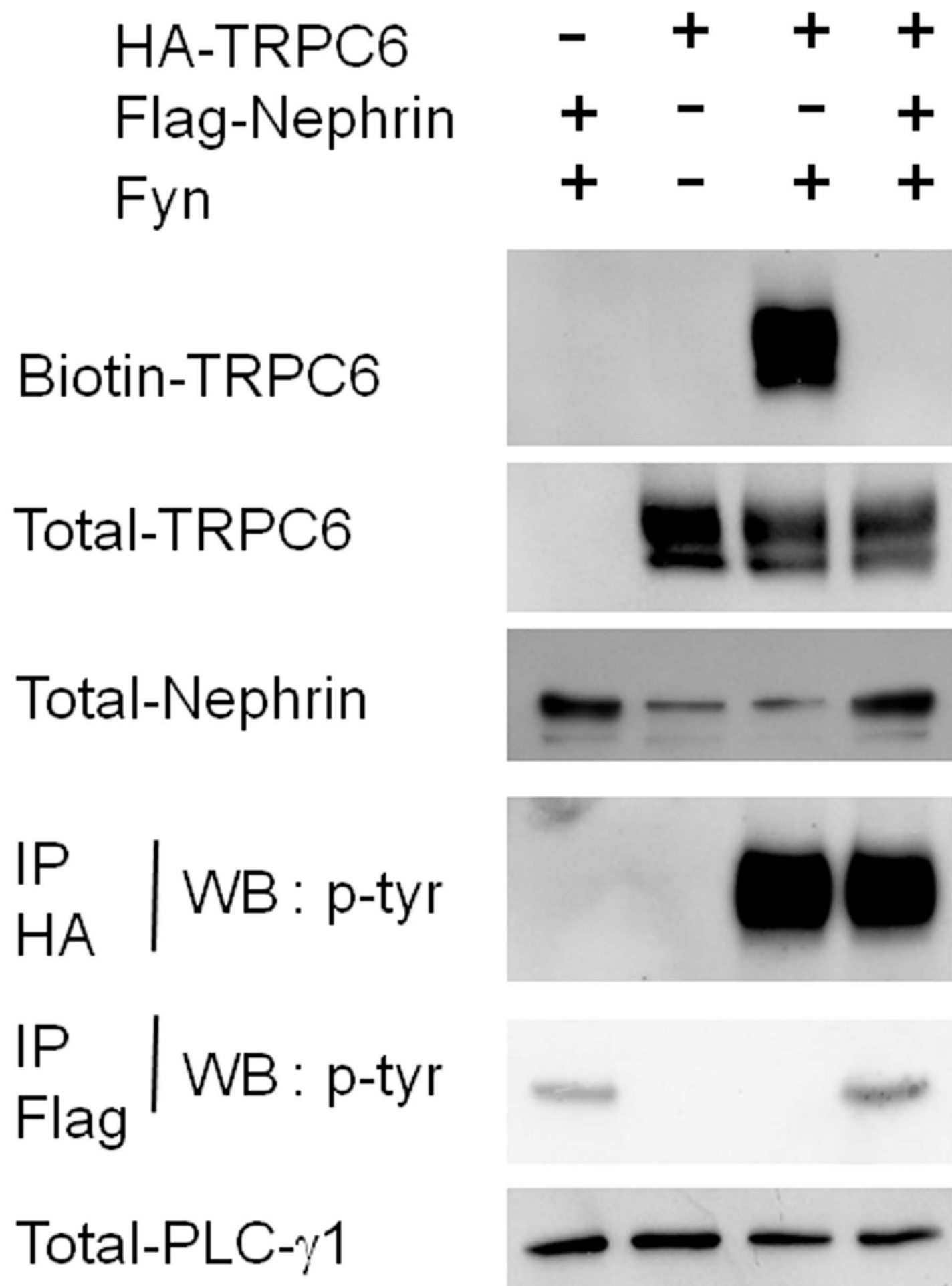


Total-TRPC6



Total-Nephrin

**Figure S2**



**Figure S3**