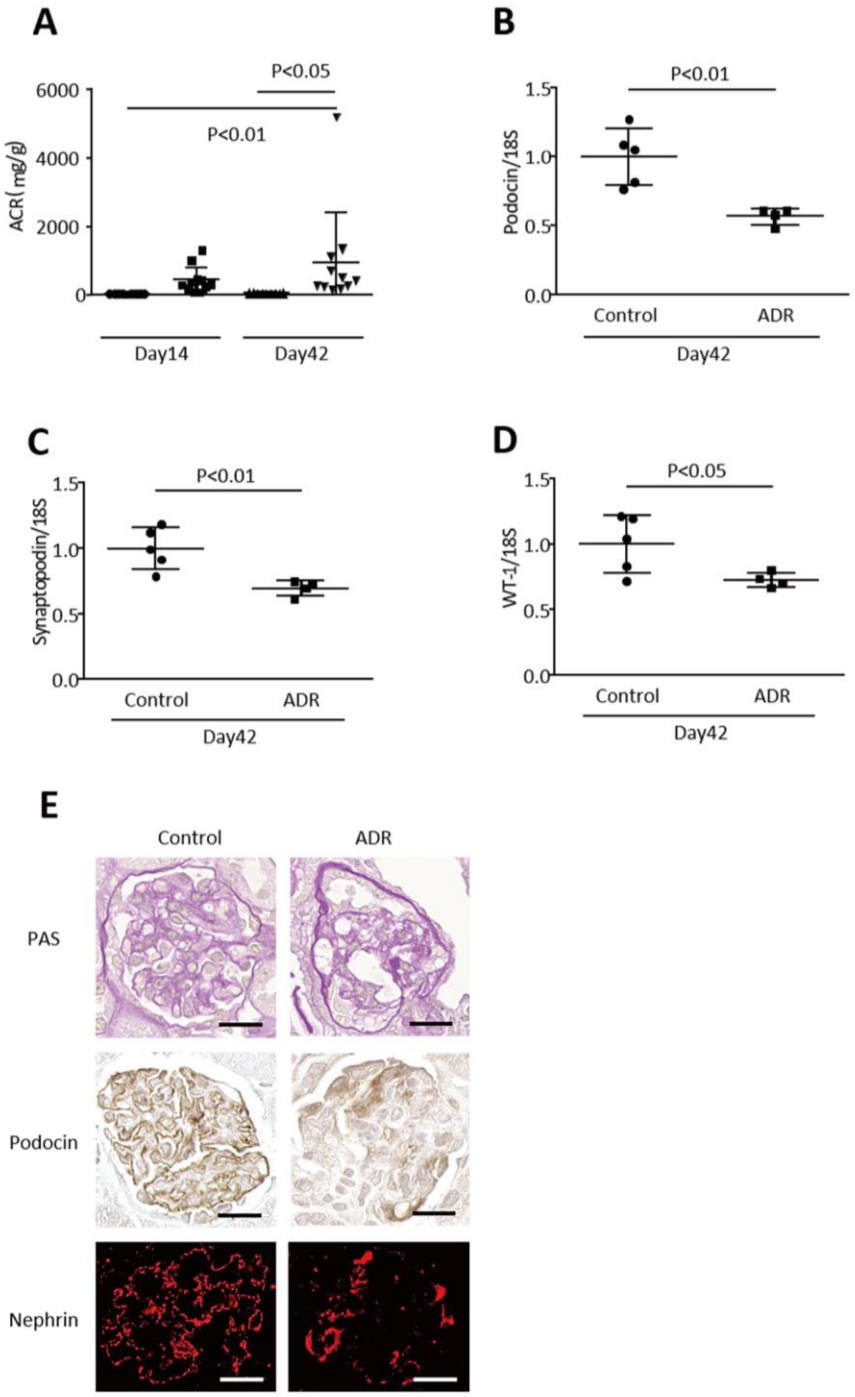
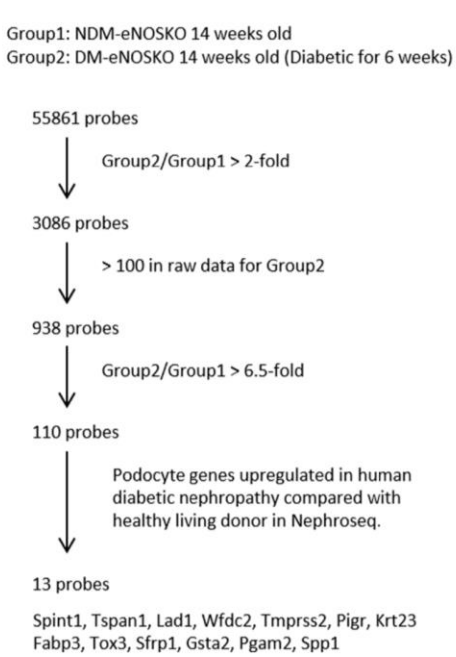
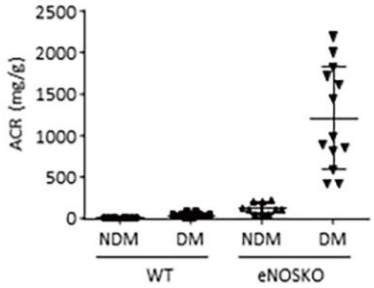


Figure S1. In order to further examine the specific mechanism, we first investigated serine protease activities in the kidney using the Adriamycin (ADR) murine nephropathy model. ADR at a dose of 10.5 mg/kg body weight was injected into the tail veins of male BALB/c mice at 8 weeks of age. At 10 or 14 weeks of age, all mice were sacrificed. Glomerular RNA was collected and evaluated as described in Materials and Methods. (A) ADR-induced nephropathy mice showed a significant increase in urinary albumin excretion on day 42. Podocyte injury was also confirmed by significant reductions in mRNA expressions of Podocin, Synaptopodin, and Wilms tumor protein (WT-1: a transcription factor for the specific activation of a glomerular differentiation program in renal precursors (31)) in mice with ADR-induced nephropathy compared with those in control mice (B-D). ADR was also induced glomerulosclerosis in mice, in which Podocin and Nephrin were down-regulated and pathologically dislocated in the glomeruli (E).

A



B



C

Microarray

	Fold change Group2 / Group1
Spint1	6.5
St14 (Matriptase)	5.3
Prss8 (Prostasin)	3.7 (13.9)
Hpn (Hepsin)	4.3
Hgfac	12.1

Serine protease activity

D

GeneSymbol	normalized G1	normalized G2	Flags G1	Flags G2	raw G1	raw G2
Spint1	-3.173	-0.570	Not Detected	Detected	32.136	219.756
Spint1	-2.000	0.705	Detected	Detected	72.483	531.887
St14 (Matriptase)	-1.966	0.444	Detected	Detected	74.204	443.724
Prss8	-1.381	0.535	Detected	Detected	111.262	472.583
Prss8	-3.425	0.443	Not Detected	Detected	26.989	443.332
Hpn	1.320	3.386	Detected	Detected	723.701	3408.850
Hgfac	-4.980	-1.401	Not Detected	Detected	9.181	123.557

Figure S2. (A) Samples were glomerular RNA extracted from mice shown in Table S2. After microarray analysis, several steps were applied to extract key genes. Final 13 genes are shown. (B) Urinary albumin/creatinine ratio in wild type and eNOS KO mice with/without diabetes in Figure 3A-C is shown. (C) (D) Fold changes between two groups and gene expression data about HAI-1 and TTSPs included in Figure 1B are shown here. NDM, non-diabetes; DM, diabetes

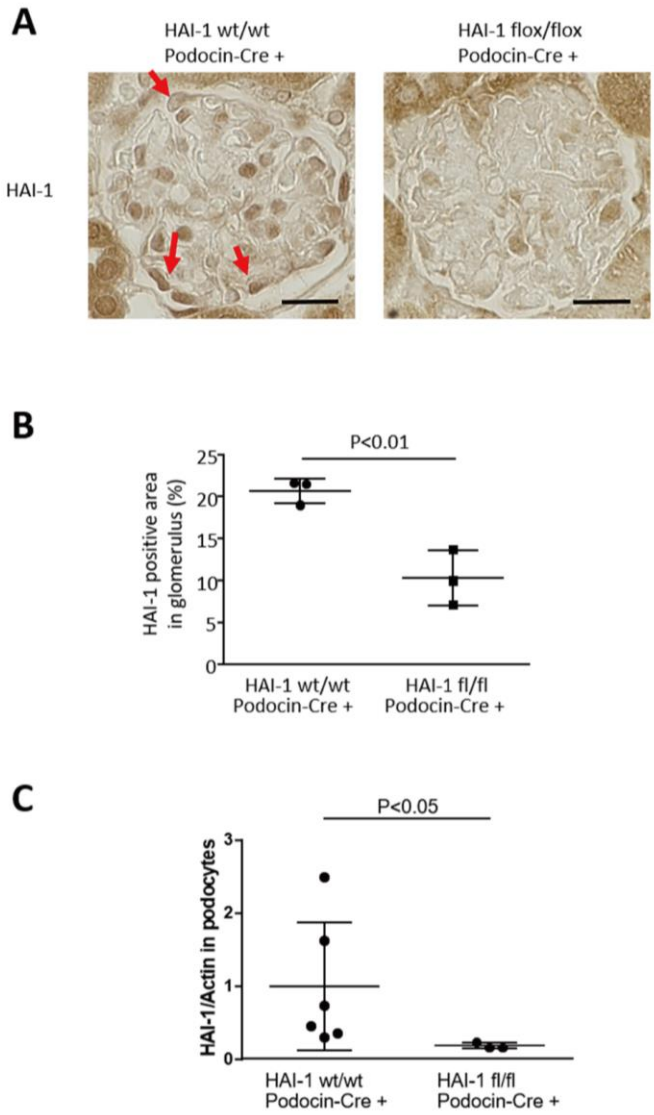


Figure S3. The mouse with podocyte specific HAI-1 knockout mice. (A) In immunohistochemistry, HAI-1 flox/flox: Podocin-Cre (+) mice showed a reduction in HAI-1 expression after injection of Tamoxifen. Red arrows show HAI-1 expression in podocytes. (B) HAI-1 positive area per glomerulus significantly decreases in HAI-1 flox/flox: Podocin-Cre (+) after tamoxifen treatment. (C) HAI-1 mRNA expression was significantly reduced in the podocytes of podocyte-specific HAI-1 KO mice compared with in podocytes of WT mice. Bar: 20μm

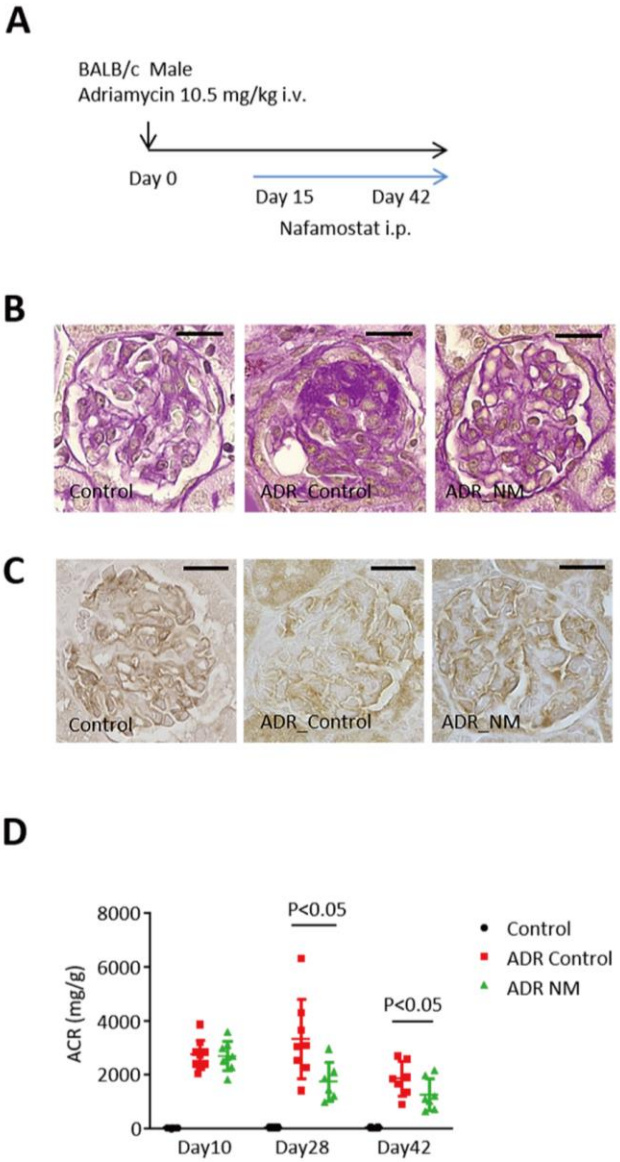


Figure S4. (A) We treated ADR-induced nephropathy mice with NM from day 15 to day 42. (B) PAS staining showed severe glomerular sclerosis in mice with ADR nephropathy. Treatment with Nafamostat mesilate (NM) ameliorated glomerular injury in ADR mice. (C) Immunohistochemistry shows that Podocin expression was reduced in ADR mice, but it was ameliorated by NM treatment. (D) ADR mice develop albuminuria, which was suppressed by NM. Data of ACR from Control and ADR Control is the same as those used in Figure 4C.

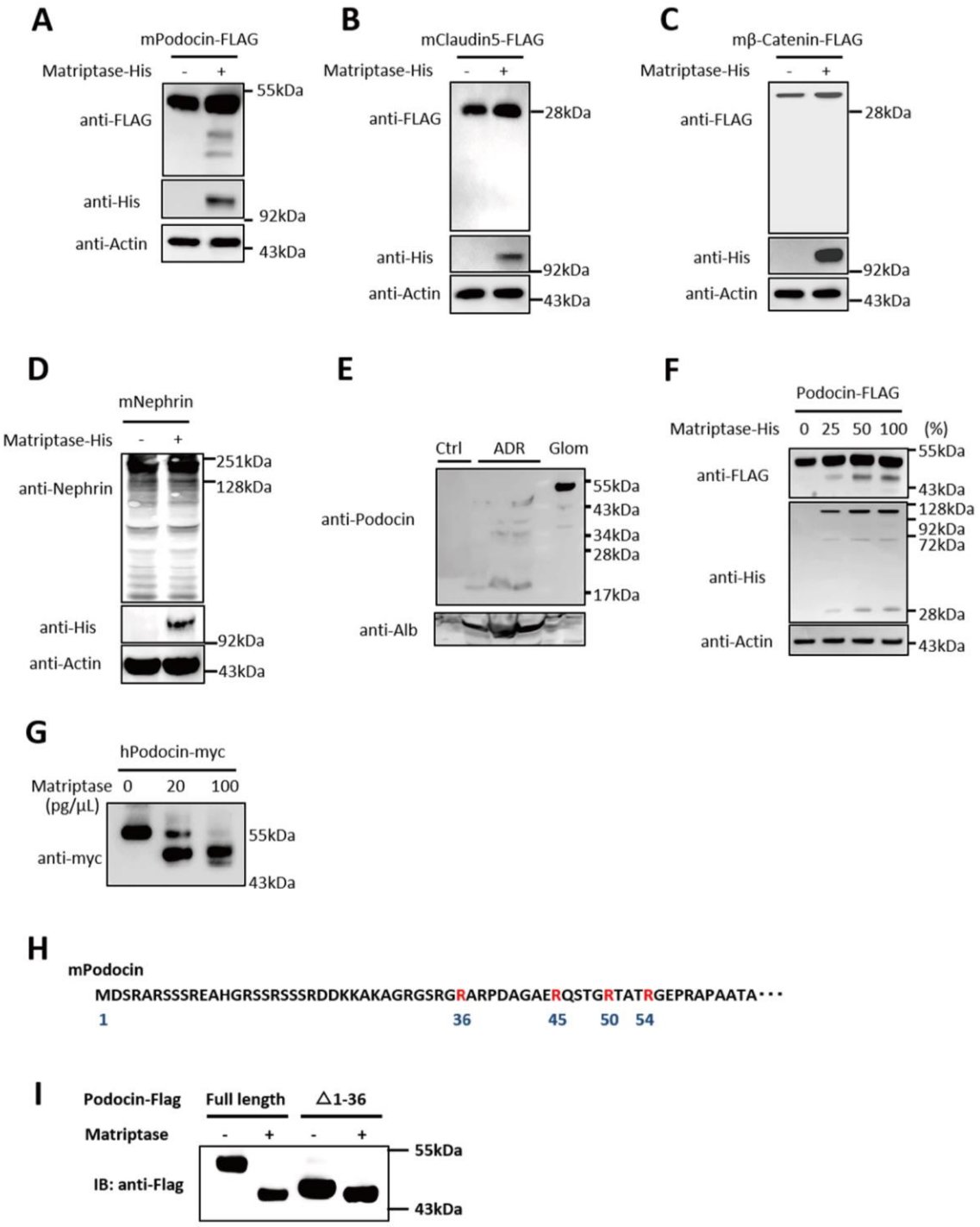


Figure S5. (A) Co-expression of FLAG-Podocin and Matriptase in HEK293 gave rise to the cleaved form of Podocin. (B, C) A full-length cDNA of Claudin 5 or β-Catenin was cloned into pFLAG-CMV-6a. Claudin5 or β-Catenin was overexpressed with Matriptase in HEK293 cells. No effect of Matriptase on Claudin 5 and β-Catenin were shown. (D) HeLa cells were infected by adenovirus for expressing Nephrin-DsRed and transfected with Matriptase. No effect of Matriptase on Nephrin protein was shown. (E) Urinary protein concentrated with ITSIPrep Total Protein Isolation Kit (ITSI BIOSCIENCE, Johnstown, PA) contained the cleaved Podocin in ADR nephropathy mice. Glomerular (Glom) protein and anti-albumin antibody (Exocell) were used. (F) Matriptase cleaved Podocin in a dose-dependent manner in HEK293. (G) Human Podocin-tagged with myc extracted from HEK293 cells was cleaved by Matriptase in a dose-dependent manner. (H) N-terminal 64 amino acids of mouse Podocin are shown highlighting Arginine, which could be a cleavage site for Matriptase, in red. (I) Podocin (full-length) and deleted type of Podocin (△1-36) were cleaved by Matriptase, producing △N-mPodocin.

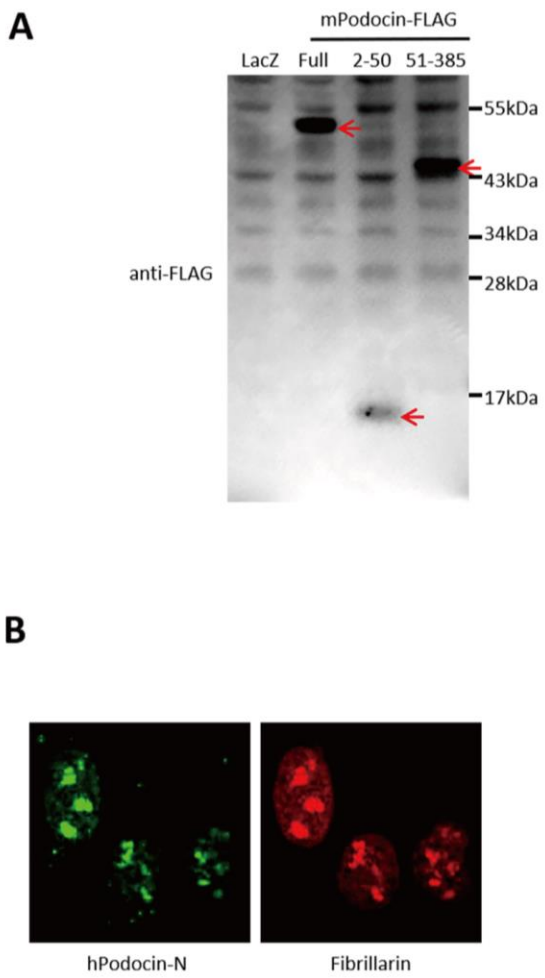


Figure S6. (A) Transfection of FLAG-mPodocin or FLAG-mPodocin(2-50) or FLAG-Podocin(51-385) in MDCK are shown. Anti-FLAG antibody detected each protein as a result of western blotting. (B) N-terminal fragment of human Podocin tagged with 3xFLAG and Fibrillarin showed the same intracellular pattern in U2OS cells.

Table S1. General characteristics of mice with ADR nephropathy.

	Control			ADR		
Age	8 week	10 week	14 week	8 week	10 week	14 week
Body weight (g)	23.9±1.2	24.6±1.3	27.7±1.6	23.0±0.7	23.8±1.0	24.6±1.3
Albumin creatine ratio (mg/g)	ND	16.7±5.6	31.4±7.8	ND	599.4±246.5	1067.7±325.0

ADR, adriamycin; ND, not done

Table S2. General characteristics of mice with diabetic eNOSKO mice.

	eNOSKO mice					
	NDM			DM		
Age	8 week	10 week	14 week	8 week	10 week	14 week
Body weight (g)	21.0±1.0	26.1±0.7	26.3±1.2	19.3±1.0	22.7±4.0	24.0±3.2
Blood glucose (mg/dl)	ND	121.3±19.1	105.3±1.5	ND	402.0±179.9	506.7±10.0
Systolic blood pressure (mmHg)	ND	116.5±12.3	128.2±8.8	ND	126.9±18.7	125.5±13.6
Diastolic blood pressure (mmHg)	ND	92.1±10.9	105.0±10.1	ND	103.2±15.1	99.5±10.8
Urine albumin (μg/day)	60.5±5.3	96.5±30.8	73.3±43.0	83.8±45.2	474.6±592.3	223.4±104.6
Albumin creatine ratio (mg/g)	263.2±55.5	415.2±69.6	231.5±162.4	314.3±163.4	1505.5±1792.0	573.2±287.9

NDM, non-diabetic mellitus; DM, diabetic mellitus; eNOSKO, eNOS knock-out; ND, not done