Deletion of protein tyrosine phosphatase SHP-1 restores SUMOylation of podocin and reverses the progression of diabetic kidney disease

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Both clinical and experimental data suggest that podocyte injury is involved in the onset and progression of diabetic kidney disease (DKD). Although the mechanisms underlying the development of podocyte loss are not completely understood, critical structural proteins such as podocin play a major role in podocyte survival and function. We have reported that the protein tyrosine phosphatase SHP-1 expression increased in podocytes of diabetic mice and glomeruli of patients with diabetes. However, the *in vivo* contribution of SHP-1 in podocytes is unknown. Conditional podocyte-specific SHP-1–deficient mice (Podo-*SHP-1*$^{-/-}$) were generated to evaluate the impact of SHP-1 deletion at four weeks of age (early) prior to the onset of diabetes and after 20 weeks (late) of diabetes (DM; *Ins2*$^{+/C96Y}$) on kidney function (albuminuria and glomerular filtration rate) and kidney pathology. Ablation of the *SHP-1* gene specifically in podocytes prevented and even reversed the elevated albumin/creatinine ratio, glomerular filtration rate progression, mesangial cell expansion, glomerular hypertrophy, glomerular basement membrane thickening and podocyte foot process effacement induced by diabetes. Moreover, podocyte-specific deletion of SHP-1 at an early and late stage prevented diabetes-induced expression of collagen IV, fibronectin, transforming growth factor-β, transforming protein RhoA, and serine/threonine kinase ROCK1, whereas it restored nephrin, podocin and cation channel TRPC6 expression. Mass spectrometry analysis revealed that SHP-1 reduced SUMO2 post-translational modification of podocin while podocyte-specific deletion of SHP-1 preserved slit diaphragm protein complexes in the diabetic context. Thus, our data uncovered a new role of SHP-1 in the regulation of cytoskeleton dynamics and slit diaphragm protein expression/stability, and its inhibition preserved podocyte function preventing DKD progression.

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KEYWORDS: diabetic nephropathy; podocin; podocytes; SHP-1; SUMO2

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Translational Statement

Diabetic kidney disease remains a major health concern affecting the quality of life of patients with diabetes. Current treatments that specifically target podocyte dysfunction and loss, which are hallmarks of glomerular disease, are still lacking. Our study provides evidence that preventing diabetes-induced protein tyrosine phosphatase Src homology region 2 domain-containing phosphatase 1 (SHP-1) expression and activity restores the expression and stability of podocin, a crucial structural protein for podocyte function. Our data also uncovered a totally new mechanism by which SHP-1 can disrupt protein stability by modulating the SUMOylation state of specific protein.

Diabetic kidney disease (DKD) remains the leading cause of kidney failure worldwide and an independent risk factor for all-cause and cardiovascular mortalities in patients with diabetes.[1] Kidney complication was traditionally thought to result from interactions between hemodynamic and metabolic factors.[2] However, current knowledge indicates that the extent of kidney damage in patients with diabetes is not completely explained by increased systemic

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and intraglomerular pressure induced by metabolic factors. Clear evidence indicates that the pathogenesis of DKD is multifactorial; both genetic (or epigenetic) and local factors are responsible for triggering a complex series of pathophysiological events.[3,4] However, it remains obscure why some subjects with diabetes develop nephropathy whereas others never progress to kidney failure.

The glomerular epithelial cell, also known as the podocyte, is a terminally differentiated specialized cell with interdigitating foot processes that wrap around the glomerular capillary tuft to form an integral component of the glomerular filtration barrier.[5] Podocytes also maintain the basement membrane filtration process.[6] Thus, it is with no surprise that podocyte damage causes leakage of proteins into the urine, representing the first clinical manifestation of glomerular diseases.[7,8] Mechanisms leading to podocyte dysfunction have been widely studied and vary from inflammation to hemodynamic and metabolic changes as well as genetic mutations.[6] Careful morphometric examination of kidney biopsies of subjects with diabetes has revealed that the density of podocytes was significantly reduced in patients with a short duration of diabetes before the onset of microalbuminuria, which can serve as a strong predictor of kidney disease progression.[9,10] Based on both clinical and animal data, it is well accepted that podocyte dysfunction and loss are hallmarks of glomerular disease in diabetic mice and humans and play an important role in the development of DKD. Therefore, additional experimental studies are needed to understand the mechanisms of diabetes-induced podocyte dysfunction, which will identify new targets to prevent kidney disease in patients with diabetes.

In almost all cases, podocytes manifest dysfunction by undergoing morphologic changes. Extensive research has shed light on the molecular signaling that is involved in the complex architectural and functional properties of podocytes.[11] The nephrin-neph1-podocin complex has been widely recognized as a signal transducing receptor complex, which is deregulated in many glomerular diseases including diabetes.[11] The activation of phosphoresidues within the cytoplasmic domain of nephrin and neph1 has been previously studied.[12] Although multiple post-translational modifications could influence nephrin-neph1-podocin–receptor activation, the dephosphorylation of this complex is less studied. Previous groups including us have reported that protein tyrosine phosphatases (PTP) such as PTP1B, Src homology region 2 domain-containing phosphatase 1 (SHP-1), and SHP-2 can regulate nephrin phosphorylation.[13-15] SHP-1 is a cytosolic PTP expressed primarily in hematopoietic and epithelial cells.[16] SHP-1 contains 2 N-terminal SH2 domains, a single phosphatase domain, and a C-terminal tail encompassing 2 sites for tyrosine phosphorylation.[17] SHP-1 has been shown to dephosphorylate a wide spectrum of phosphoproteins involved in hematopoietic and epithelial cell signaling of the tyrosine kinase receptor family. Diabetes has been shown to increase the expression of SHP-1 in the retina[18] and ischemic muscle.[19] In the kidney, SHP-1 expression and activity are elevated in podocytes after high glucose (HG) exposure and diabetes, affecting growth factor signaling.[20,21] However, because of lack of specific inhibitors of SHP-1, it is currently unknown if reducing SHP-1 activity may prevent podocyte dysfunction. In addition, it remains to be proven *in vivo* whether inhibition of SHP-1 can prevent the development and halt the progression of DKD.

METHODS

Additional methods such as reagents, antibodies, mice genotyping, blood glucose, urinary albumin, glomerular filtration rate (GFR), tissue preparation, renal pathology, transmission and scanning electron microscopy, immunohistochemistry and immunofluorescence of paraffin and OCT sections, immunoprecipitation, immunoblot and quantitative polymerase chain reaction analyses, phosphatase assay, podocytes culture, and immunofluorescence can be found in the Supplementary Methods.

Human tissue study

After written consent, kidney tissues were obtained from 24 (12 nondiabetic and 12 diabetic) patients who underwent a nephrectomy for clear cell carcinoma at the Centre Hospitalier de l'Université de Sherbrooke. Blood and urine samples were obtained within the month before the surgery. To assess kidney function, the estimated GFR (eGFR) was calculated with the CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) formula based on serum creatinine levels. Blood and urine samples were obtained within the month before the surgery. The urine samples were sent to the biochemistry laboratory where they were analyzed for total proteins (by a turbidimetric method), urinary albumin (by an immunoturbidimetric method), and creatinine (by an enzymatic method; Roche Diagnostics). Data were used to study SHP-1 levels and renal function in the human kidney. In addition, PTPN6 gene expression was evaluated in another cohort. Detailed information about the recruitment, data collection, and RNA-seq processing is available as previously published.[22]

Animals and experimental design

To determine the specific role of SHP-1 in podocytes, a mouse carrying the podocin-rtTA transgene (specific to podocytes) and a TetOn-Cre transgene (provided by Susan E. Quaggin, Northwestern University) was bred to SHP-1 flox mice (JAX stock number: 008336; B6.129P2-Ptpn6tm1Rsky/J). Transgenic mice (podocin-rtTA-Cre^{+}-Ptpn6$^{flox/flox}$, backcrossed 10 generations in C57BL/6 background) was also bred with diabetic heterozygous male Ins2$^{+/C96Y}$ (Akita) mice (JAX stock number: 003548; C57BL/6-Ins2^{Akita}/J). Akita mice develop diabetes at 4 weeks of age. Mice were treated with or without doxycycline (625 mg/kg; chow diet) for 2 weeks at 4 weeks of age, a point where nephrogenesis is completed, or at 20 weeks of age (after 16 weeks of uncontrolled diabetes). Control mice (podocin-rtTA-Cre^{+}-Ptpn6$^{+/+}$; Podo-SHP-1$^{+/+}$), which do not possess the flox sequence within the SHP-1 gene, and podocyte SHP-1–deficient mice (Podo-SHP-1$^{-/-}$) were killed at 20 and 28 weeks of age. Treatment with doxycycline at different time points was used to determine whether the tissue-specific knockout of SHP-1 in podocytes will prevent (treatment at 4 weeks of age) or delay (treatment at 20 weeks of age) podocyte dysfunction and glomerular pathology. Throughout the study period, animals were provided with free access to water and standard rodent chow (Envigo Teklad).

Isolation of human glomeruli

Renal cortex was minced and successively passed through a 250 μm, 200 μm, and 150 μm sieves. The cells on the top of 150 μm sieve were collected with phosphate-buffered saline, washed, and centrifuged for 10 minutes at 500 *g* as previously reported.[23] The pellet was resuspended in radioimmunoprecipitation a buffer and ground for total protein extraction.

Mass spectrometry

Kidney cortex proteins of 3 nondiabetic Podo-SHP-$1^{+/+}$, 3 diabetic Podo-SHP-$1^{+/+}$, and 3 diabetic mice with specific SHP-1 deletion (Podo-SHP-$1^{-/-}$) were solubilized in 8 M urea/10 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid pH 8.0 lysis buffer. After bicinchoninic acid protein quantification, 50 μg of proteins were reduced by the addition of dithiothreitol (5 mM final concentration) and samples were boiled for 2 minutes at 95 °C followed by a 30-minute incubation at room temperature. Chloroacetamide was added to a final concentration of 7.5 mM, incubated in the dark for 20 minutes, and then 150 μl of NH_4HCO_3 was added. Proteins were digested with 1 μg of trypsin overnight at 30 °C. The samples were next acidified with trifluoroacetic acid to reach a final concentration of 0.2%, and samples were desalted using C18 tip columns (Pierce Cat number: 87784). Peptides were quantified by nanoscale liquid chromatography coupled to tandem mass spectrometry (nanoElute; Bruker Daltonics) followed by analysis with the MaxQuant software by the proteomic platform of the Université de Sherbrooke. Additional details of the gradient and the acquisition parameters are available in the Supplementary Methods. Data were processed with the Perseus software of MaxQuant. The mass spectrometry proteomics data have been deposited to the ProteomeXchange consortium via the PRIDE[24] partner repository with the dataset identifier "PXD035343." For subsequent analyses, we have selected proteins that are modulated (either increased or decreased) by diabetes and reversed by the absence of SHP-1 in diabetic animals (DM-Podo-SHP-$1^{-/-}$) with changes higher than 0.5-fold. Analyses were also performed using the STRING and TISSUES databases.

Study approval

The human study was approved by the Centre Intégré Universitaire de Santé et de Services Sociaux de l'Estrie–CHU de Sherbrooke Research Ethics Board and was conducted in accordance with the Declaration of Helsinki (protocol number: 2016-1294). Gene expression data in microdissected kidney tubules were approved by the institutional review board of the University of Pennsylvania.[22] All animal experiments were conducted in accordance with the Canadian Council of Animal Care and Institutional Guidelines and were approved by the Animal Care and Use Committees of the Université de Sherbrooke according to National Institutes of Health guidelines.

Statistical analyses

In vivo and *in vitro* data are presented as the mean ± SD for each group. Statistical analysis was performed by nonpaired 1-way analysis of variance, followed by Tukey's test corrections for multiple comparisons. Data in each group were checked for normal distribution using the D'Agostino and Pearson normality test based on $P = 0.05$. For analysis with human data, statistical analyses were carried out by the CRCHUS biostatistics service and conducted using the SPSS V26 software (IBM Corp.) for descriptive, comparative, and correlation analysis. Continuous variables are presented as mean ± SD or median [interquartile range], respectively. Categorical variables are expressed as a percentage and absolute numerical values. Normality of continuous variables was determined with the Shapiro-Wilk W-test. A Wilcoxon test was performed when comparing continuous variables with repeated measures, followed by a Bonferroni correction in the case of multiple comparisons, whereas a Mann-Whitney *U*-test was performed to compare independent groups. Correlation analyses were performed using a Spearman test. A *P* value of <0.05 was considered statistically significant.

RESULT

SHP-1 in human DKD

Because SHP-1 expression is elevated in vascular tissues of diabetic mice as compared with nondiabetic counterparts,[21] we wanted to validate if this was also the case in human kidneys. Patients without and with diabetes were recruited. Baseline characteristics of the patients are listed in Table 1. Gender and age were similarly distributed. As expected, patients with diabetes were characterized by higher body mass index and blood pressure levels as well as elevated albumin-to-creatinine and protein-to-creatinine ratios (Table 1). Levels of expression of SHP-1 were measured in the human kidney cortex samples of both groups. Our data showed that SHP-1 mRNA ($P = 0.0028$; Figure 1a) and protein ($P = 0.0012$; Figure 1b) levels were significantly increased in the kidney cortex of patients with diabetes by 1.4-fold and 3.5-fold, respectively, as compared with those without diabetes. More precisely, elevated SHP-1 protein expression in patients with diabetes was also confirmed in isolated glomerular extracts ($P = 0.0070$; Figure 1c). Although there was no difference in eGFR between groups, SHP-1 mRNA levels strongly correlated with eGFR ($\rho = -0.5044$) in patients with diabetes (Figure 1d), an observation that was weaker in patients without diabetes

Table 1 | Characteristics of NDM and DM participants

Characteristic	NDM (n = 12)	DM (n = 12)	*P* value
Gender: female/male	5/7	5/7	0.544
Age, yr	61 ± 13	62 ± 10	0.989
Active smoking, n (%)	1 (8)	0	0.498
HTN, n (%)	2 (16)	10 (83)	<0.0001
ACEi-ARB, n (%)	2 (16)	10 (83)	<0.0001
BP, mm Hg	130 ± 16/72 ± 9	139 ± 13/79 ± 9	0.002
BMI, kg/m^2	26 ± 6	33 ± 7	0.019
eGFR, ml/min per 1.73 m^2	71 [52–94]	65 [44–93]	0.484
ACR, mg/mmol	1 [0.5–3.5]	4.3 [1.4–19.6]	0.001
PCR, g/g	0.1 [0.1–0.1]	0.13 [0.1–0.33]	0.002
HbA1C, %	5.9 ± 0.3	7.9 ± 1.2	<0.0001
Years of diabetes	0	8 [6–10]	<0.0001
SHP-1 mRNA levels	1.0 [0.9–1.6]	1.7 [1.4–2.1]	0.0028

ACEi, angiotensin-converting enzyme inhibitor; ACR, albumin-to-creatinine ratio; ARB, angiotensin receptor blocker; BP, blood pressure; BMI, body mass index; DM, diabetes; eGFR, estimated glomerular filtration rate; HbA1c, glycated hemoglobin A1c; HTN, hypertension; NDM, without diabetes; PCR, protein-to-creatinine ratio; SHP-1, Src homology 2 domain-containing protein tyrosine phosphatase 1.
Data are presented as mean ± SD for normally distributed data, and median [interquartile range] for non-normally distributed data.
The χ^2 test was used for categorical variable such as gender and active smoking, and *P* values were estimated by the Mann-Whitney *U*-test or the Wilcoxon test wherever applicable with the Bonferroni correction.

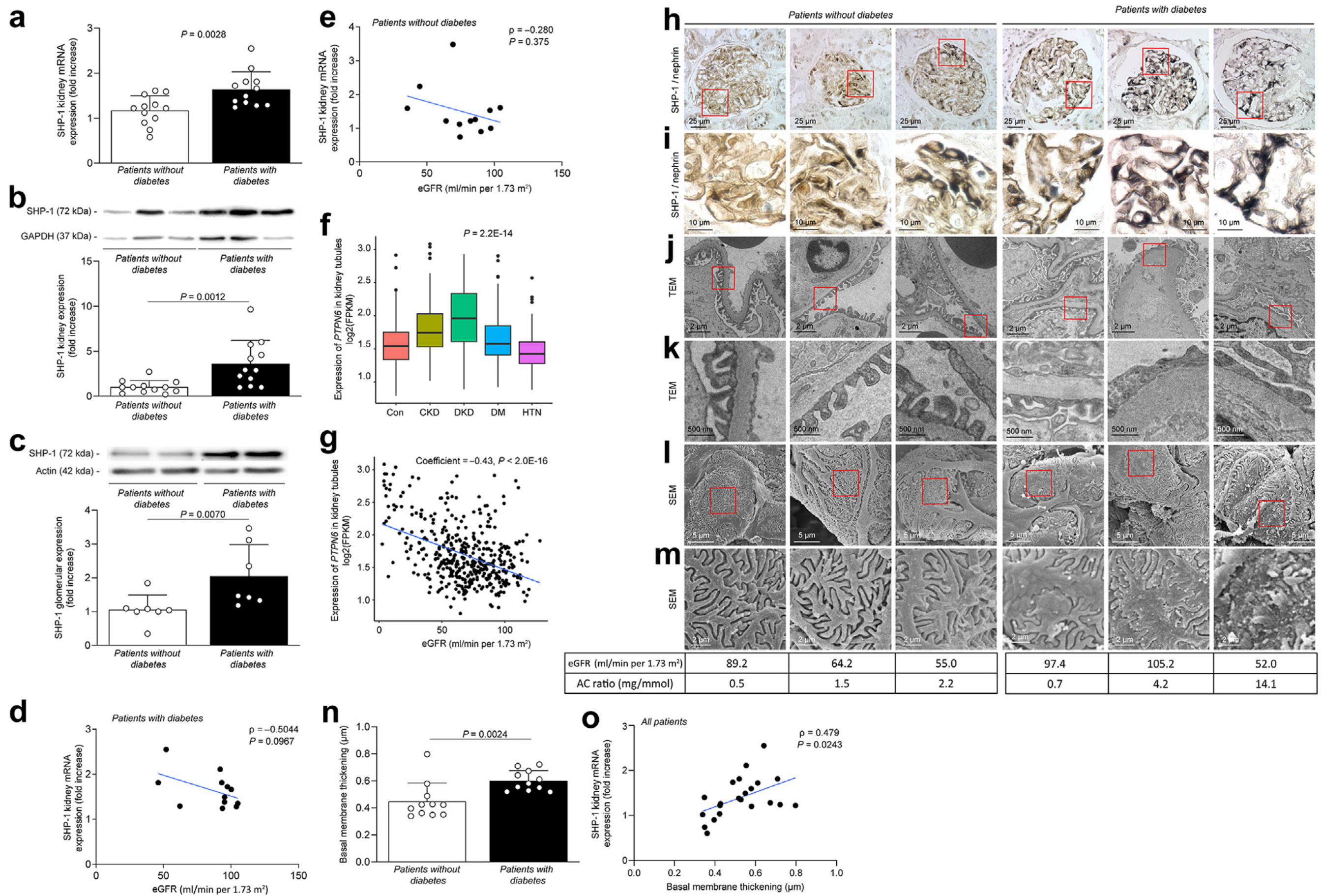

Figure 1 | Src homology 2 domain-containing protein tyrosine phosphatase 1 (SHP-1) (a) mRNA and protein levels of human (b) kidney cortex and (c) glomeruli. SHP-1 mRNA levels are correlated with estimated glomerular filtration rate (eGFR) of patients (**d**) with diabetes, (**e**) without diabetes, and (**g**) patients enrolled in RNA sequencing study. (**f**) PTPN6 (SHP-1) RNA expression after RNA sequencing of control (orange), chronic kidney disease (CKD; yellow), diabetic kidney disease (DKD; green), diabetes mellitus (DM; blue), or hypertension (HTN; purple) patients. (**h,i**) Coimmunostaining of SHP-1 (blue) and nephrin (light brown) of glomeruli of patients with and without diabetes. (**j,k**) Transmission and (**l,m**) scanning electron microscopy images with quantification of the (**n**) glomerular basal membrane thickening of patients with and without diabetes that correlated (**o**) with SHP-1 expression. The red squares indicate higher magnification area. Results are shown as mean ± SD of 12 (**a,b,d,e**), 7 (**c**), and 11 (**h–o**) samples per group. AC, albumin to creatinine; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; SEM, scanning electron microscopy; TEM, transmission electron microscopy. To optimize viewing of this image, please see the online version of this article at www.kidney-international.org.

(ρ = −0.280; Figure 1e). These data suggest that SHP-1 may contribute to the development of DKD. These findings were corroborated in a larger study cohort in which gene expression was profiled in microdissected human kidney tubules (n = 432) using RNA sequencing.[22] Demographic and clinical data of human kidney samples used for this analysis are listed in Table 2. Data showed that patients with DKD exhibited the highest expression levels of PTPN6 gene as compared with control participants and even patients with diabetes and without kidney disease (P = 2.2E–14; Figure 1f). When correlated with eGFR, SHP-1 gene expression remained inversely correlated with eGFR (ρ = −0.43; P < 2E–16; Figure 1g). SHP-1 expression was also evaluated in kidney cross-sections by immunohistochemistry. Figure 1h and i reveals that SHP-1 staining (blue) was predominantly in the podocytes (brown) of patients with diabetes. Interestingly, the increase of SHP-1 expression was positively associated with the elevated albumin-to-creatinine ratio and negatively associated with the eGFR decline in patients with diabetes, and to a lesser extent in patients without diabetes. Furthermore, enhanced SHP-1 staining was accompanied with podocyte damage indicated by foot process effacement and glomerular basement thickening (Figure 1j–m). Basal membrane thickening was significantly increased by 1.5-fold (P = 0.0024; Figure 1n) in patients with diabetes compared with patients without diabetes, which strongly correlated with increased SHP-1 mRNA levels (ρ = 0.479; Figure 1o).

Glomerular dysfunction and structural alterations caused by diabetes can be prevented and restored by removing SHP-1 specifically in podocytes

We have generated a podocyte-specific SHP-1–deficient mouse without (*Ins2*$^{+/+}$) or with diabetes (*Ins2*$^{+/C96Y}$; Supplementary Figure S1A–F) to evaluate the *in vivo*

Table 2 | Demographic and clinical data of human kidney samples used for analysis[22]

Demographic characteristics	Primary cohort (n = 432)	Control (n = 78)	CKD (n = 88)	DKD (n = 70)	DM (n = 94)	HTN (n = 102)
Gender, female, n (%)	160 (36.6)	27 (34.6)	31 (35.2)	34 (48.6)	33 (35.1)	34 (33.3)
Age, yr, mean ± SD	61.4 ± 12.5	52.9 ± 13.6	63.2 ± 12.6	68.5 ± 9.8	62.7 ± 9.7	60.1 ± 11.7
Race, n (%)						
Asian	7 (1.6)	2 (2.6)	0 (0)	2 (2.9)	2 (2.1)	1 (1.0)
White	290 (67.0)	45 (57.7)	66 (71.6)	45 (64.3)	66 (70.2)	70 (68.6)
African American	77 (17.8)	13 (16.6)	17 (19.1)	14 (20.0)	15 (16.0)	18 (17.7)
Hispanic	7 (1.6)	2 (2.6)	0 (0)	2 (2.9)	1 (1.1)	2 (2.0)
Multiracial	20 (4.6)	10 (12.8)	2 (2.3)	2 (2.9)	3 (3.2)	3 (2.9)
Unknown	32 (7.4)	6 (7.7)	6 (6.8)	5 (7.1)	7 (7.5)	8 (7.8)
Clinical characteristics						
Diabetes type 1, n (%)	15 (3.5)	0	0	9 (12.9)	6 (6.4)	0
Diabetes type 2, n (%)	149 (34.4)	0	0	61 (87.1)	88 (93.6)	0
Hypertension, n (%)	317 (73.2)	0	69 (78.4%)	65 (92.9)	80 (85.1)	102 (100)
Baseline GFR, ml/min per 1.73 m^2, mean ± SD	67.3 ± 26.7	85.5 ± 16.1	40.9 ± 18.5	38.1 ± 16.4	82.2 ± 15.0	82.8 ± 15.5
Interstitial fibrosis, % of area ± SD	14.3 ± 21.7	6.6 ± 11.0	24.5 ± 29.9	27.0 ± 30.3	9.8 ± 10.6	6.7 ± 8.9

CKD, chronic kidney disease; DKD, diabetic kidney disease; DM, diabetes mellitus; GFR, glomerular filtration rate; HTN, hypertension.

contribution of SHP-1 to podocyte function and diabetic kidney disease. We also confirmed that doxycycline treatment had no influence in GFR response in both nondiabetic and diabetic *Ins2*$^{+/C96Y}$ mice (Supplementary Figure S1G). In our first set of experiments, the SHP-1 gene was removed before the development of diabetes (at 4 weeks of age). Body weight and systemic glucose were not different between diabetic (*Ins2*$^{+/C96Y}$) and diabetic PodoSHP-1$^{-/-}$ mice at 28 weeks of age (Supplementary Table S1). After 24 weeks of uncontrolled diabetes, 24-hour urinary albumin excretion (Figure 2a) and GFR (Figure 2b) were elevated by 12-fold and 2-fold, respectively, in diabetic *Ins2*$^{+/C96Y}$ mice compared with their littermate controls (*Ins2*$^{+/+}$). Deletion of SHP-1 in podocytes at 4 weeks of age reduced urinary albumin excretion and GFR by 73% ($P = 0.0016$) and 43% ($P < 0.0001$), respectively, in diabetic mice. Our conditional podocyte-specific SHP-1 knockout mice also allowed us to investigate if SHP-1 is involved in the progression of DKD by evaluating kidney function in nondiabetic (*Ins2*$^{+/+}$) and diabetic (*Ins2*$^{+/C96Y}$) mice at 20 weeks of age. As expected, *Ins2*$^{+/C96Y}$ mice exhibited elevated urinary albumin levels and GFR as compared with control littermates (Figure 2c and d). Furthermore, a subgroup of diabetic mice was then treated with doxycycline at 20 weeks of age and killed at 28 weeks of age to determine if removing the SHP-1 gene when there is already the presence of glomerular dysfunction can cease or reverse the progression of DKD. Untreated 28-week-old *Ins2*$^{+/C96Y}$ mice displayed a 2.8-fold and 125% increase in urinary albumin excretion ($P = 0.0011$) and GFR ($P = 0.0384$), respectively, as compared with 20-week-old *Ins2*$^{+/C96Y}$ mice. Removing SHP-1 uniquely in podocytes of diabetic mice at 20 weeks of age stopped the progression of albuminuria and completely restored GFR as compared with untreated *Ins2*$^{+/C96Y}$ podo-SHP-1$^{+/+}$ (Figure 2c and d). Interestingly, 28-week-old *Ins2*$^{+/C96Y}$ podo-SHP-1$^{-/-}$ exhibited similar levels of urinary albumin as diabetic mice at 20 weeks of age, suggesting that the deletion of SHP-1 was able to halt albuminuria progression as well as the rise of GFR. Histologically, diabetes caused a 1.4-fold increase ($P = 0.0161$) in glomerular hypertrophy (Figure 3a and g) and a 2.7-fold increase ($P < 0.0001$) in mesangium expansion (Figure 3b and e) as well as induced collagen type IV (Figure 3c) and transforming growth factor beta expression (Figure 3d) as compared with nondiabetic mice. Interestingly, these structural changes were prevented by the deletion of SHP-1 specifically in podocytes at the early (4 weeks) and late stages (20 weeks) of diabetes (Figure 3a–h).

Deletion of SHP-1 prevented diabetes-induced podocyte injury and loss

To evaluate podocyte damage, electron microscopy was used to measure foot process width and glomerular basement membrane (GBM) thickening. Foot process effacement and GBM thickening (Figure 4a and b) were significantly increased by 1.6-fold ($P < 0.001$; Figure 4c) and 1.5-fold ($P < 0.0001$; Figure 4d) in diabetic mice compared with their littermate controls. Excision of the SHP-1 gene only in podocytes completely prevented foot process effacement (Figure 4c) and significantly reduced GBM thickening (Figure 4d) caused by diabetes. Glomerular structures were also evaluated in our reversibility model (20-week-old doxycycline treatment). Both podocyte effacement (Figure 4e and g) and GBM thickening (Figure 4f and h) were worsened with diabetes duration. Remarkably, 28-week-old diabetic mice treated with doxycycline at 20 weeks of age displayed a similar foot process structure as the nondiabetic 20-week-old mice (Figure 4g), whereas GBM thickening was comparable with 20-week-old diabetic mice. These data suggest that inhibition of SHP-1 may have restored podocyte structure and ceased GBM thickening. In addition, we assessed diabetes-related

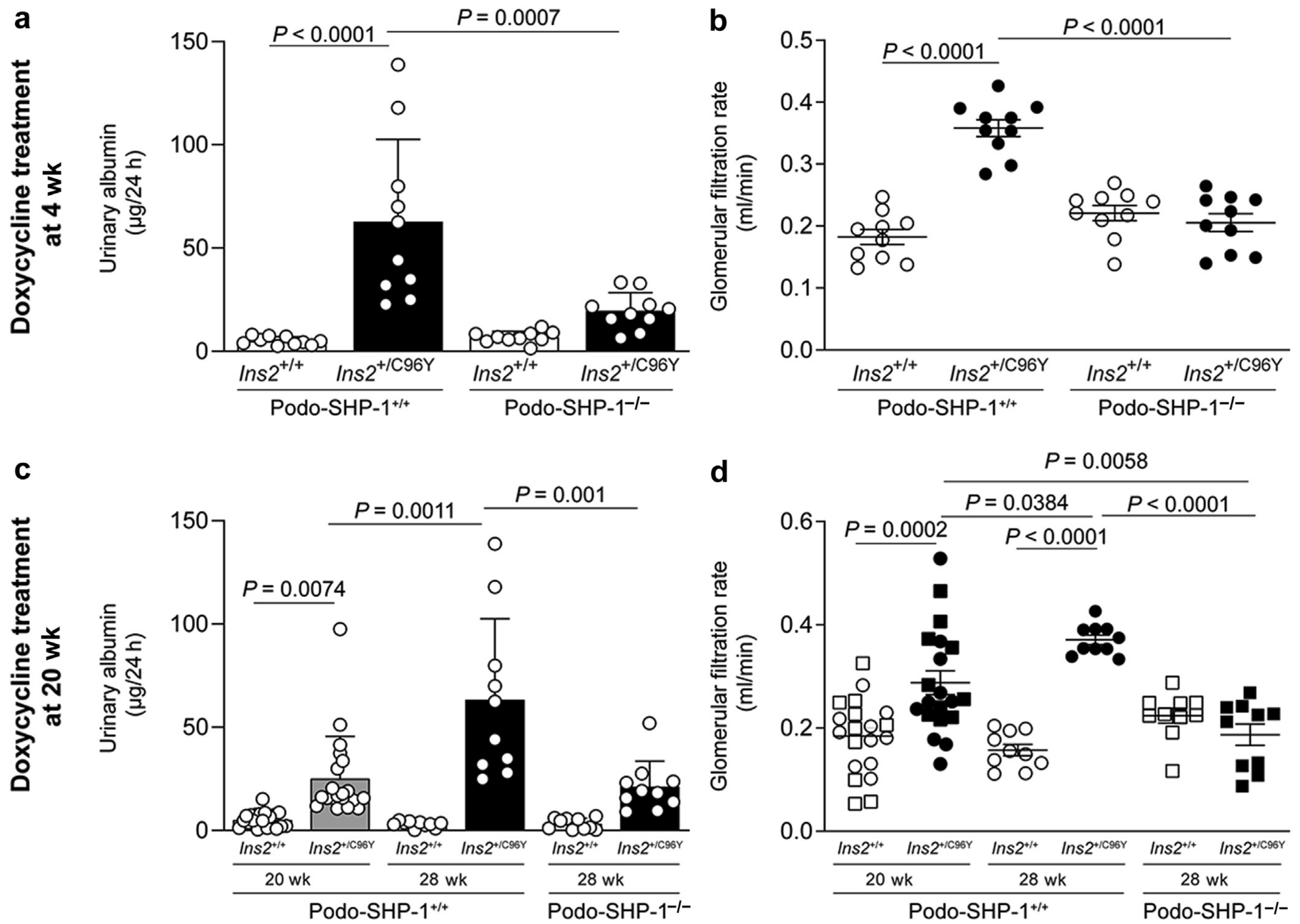

Figure 2 | (a) Urinary albumin levels and (b) glomerular filtration rate were measured at 20 and 28 weeks of age in nondiabetic (*Ins2*$^{+/+}$; white bars) and diabetic (*Ins2*$^{+/C96Y}$; black bars) mice, without (Podo-SHP-1$^{+/+}$) or with podocyte-specific deletion of SHP-1 (Podo-SHP-1$^{-/-}$) induced at 4 (a,b) and 20 weeks (c,d) of age. For the reversibility group (**c,d**), urinary albumin levels and glomerular filtration rate were measured in all mice at 20 weeks of age without (*Ins2*$^{+/+}$; white bars) or with diabetes (*Ins2*$^{+/C96Y}$; gray bars). Then, half of diabetic mice were treated with doxycycline at 20 weeks of age, and urinary albumin levels and glomerular filtration rate were evaluated at 28 weeks of age. Results are shown as mean ± SD of 10 (**a,b**), 10 to 20 (**c,d**) mice per group. SHP-1, Src homology 2 domain-containing protein tyrosine phosphatase 1.

podocyte loss with immunostaining of WT-1, a podocyte marker. Diabetic mice exhibited a 14% reduction in podocyte count per glomeruli (Figure 5a and b; $P = 0.0262$), which was not observed in diabetic Podo-SHP-1$^{-/-}$ treated with doxycycline at 4 weeks of age. Even in diabetic mice treated with doxycycline at 20 weeks of age, late deletion of SHP-1 was able to prevent podocyte shedding (Figure 5c and d; $P = 0.0421$).

Inhibition of SHP-1 prevented diabetes-generated extracellular matrix remodeling

Diabetes induced a 1.6-fold increase in SHP-1 mRNA expression ($P = 0.0157$; Figure 6a) and a 2.2-fold increase in SHP-1 phosphatase activity ($P = 0.0331$; Supplementary Figure S2) in mice renal cortex. This elevation was mainly in podocytes because removing SHP-1 gene specifically in podocytes completely prevented the surge of SHP-1 expression and phosphatase activity in diabetes. Diabetes also enhanced the mRNA expression of Coll IV (1.5-fold; $P = 0.0261$; Figure 6b), fibronectin (2.3-fold; $P = 0.0005$; Figure 6c), and transforming growth factor beta (2.2-fold; $P = 0.0068$; Figure 6d), which was blunted in diabetic podocyte-specific SHP-1–deficient mice treated with doxycycline at 4 weeks of age. Diabetes has been shown to affect the actin cytoskeleton pathway and the expression of podocyte-specific membrane markers. Our data showed that the mRNA expression of RhoA (Figure 6e), Rho-associated protein kinase 1 (ROCK1; Figure 6f), nephrin (Figure 6g), podocin (Figure 6h), and transient receptor potential channel 6 (Figure 6i) were altered by diabetes, and the absence of the SHP-1 gene in podocytes was able to protect these markers from diabetes-induced modulations. Interestingly, using our mouse model of reversibility, we demonstrated that despite an increase in collagen type IV (Figure 7a), fibronectin (Figure 7b), and transforming growth factor beta (Figure 7c) expression in diabetic mice at 20 weeks of age, late deletion of SHP-1 specifically in podocytes (Figure 7d) halted diabetes-induced upregulation of these genes. Similar to the

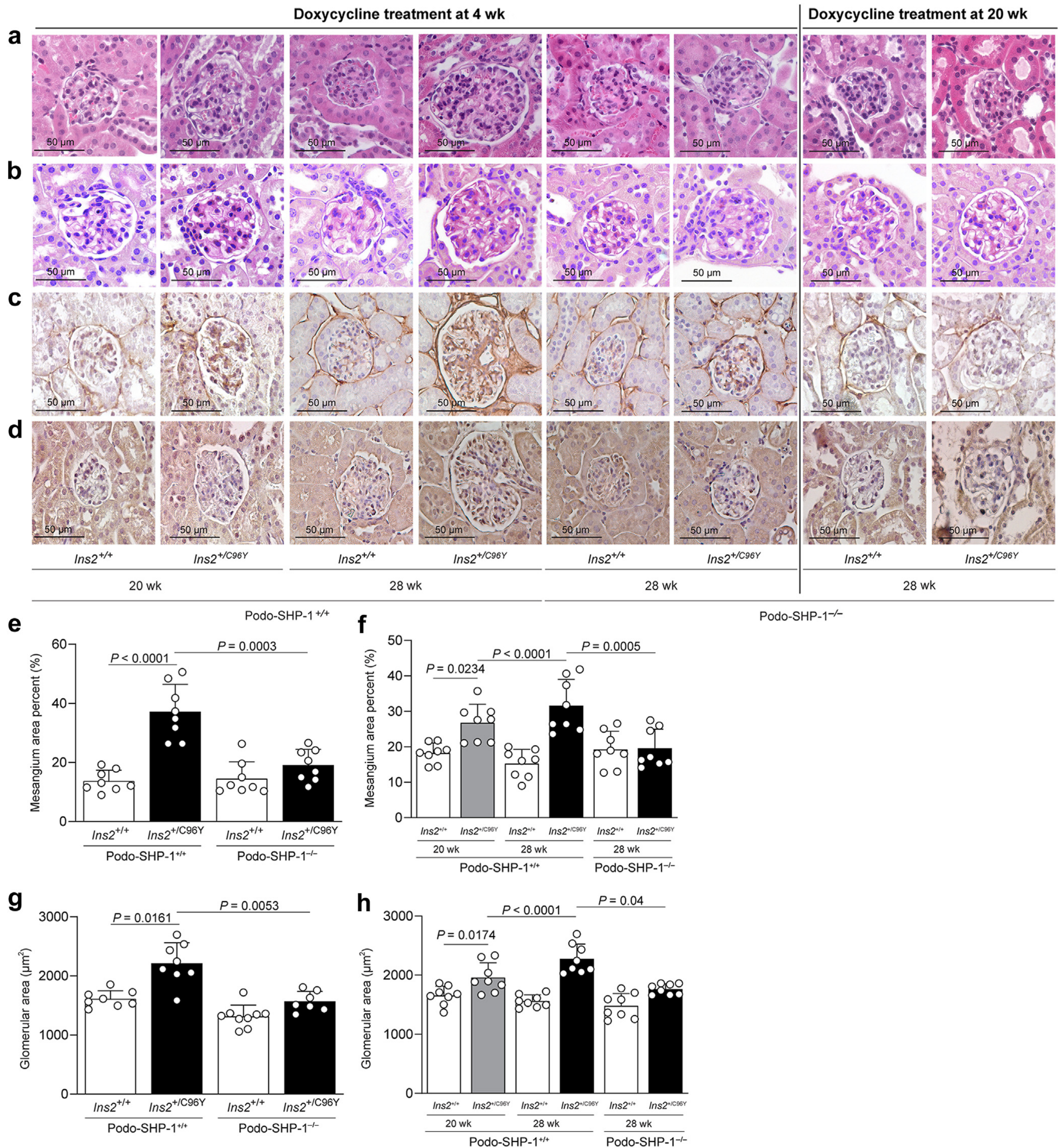

Figure 3 | Kidney cross-sections of 20- and 28-week-old nondiabetic (*Ins2*$^{+/+}$; white bars) and diabetic (*Ins2*$^{+/C96Y}$; gray [20 weeks of age] and black [28 weeks of age] bars) mice, without (Podo-SHP-1$^{+/+}$) or with podocyte-specific deletion of SHP-1 (Podo-SHP-1$^{-/-}$) induced at 4 or 20 weeks of age were stained with (a) hematoxylin and eosin, (b) periodic acid–Schiff to quantify (e,f) mesangial expansion and (g,h) glomerular size. Antibody against (**c**) collagen type IV and (**d**) transforming growth factor beta were used for immunochemistry staining of the glomerular area. Results are shown as mean ± SD of 8 mice per group. SHP-1, Src homology 2 domain-containing protein tyrosine phosphatase 1. To optimize viewing of this image, please see the online version of this article at www.kidney-international.org.

early deletion of SHP-1, late ablation of SHP-1 in diabetic mice stopped mRNA expression alterations of markers associated with actin cytoskeleton signaling and remodeling such as RhoA (Figure 7e), ROCK1 (Figure 7f), and markers of the slit diaphragm such as podocin (Figure 7g) and transient receptor potential channel 6 (Figure 7h). Importantly, neither diabetes

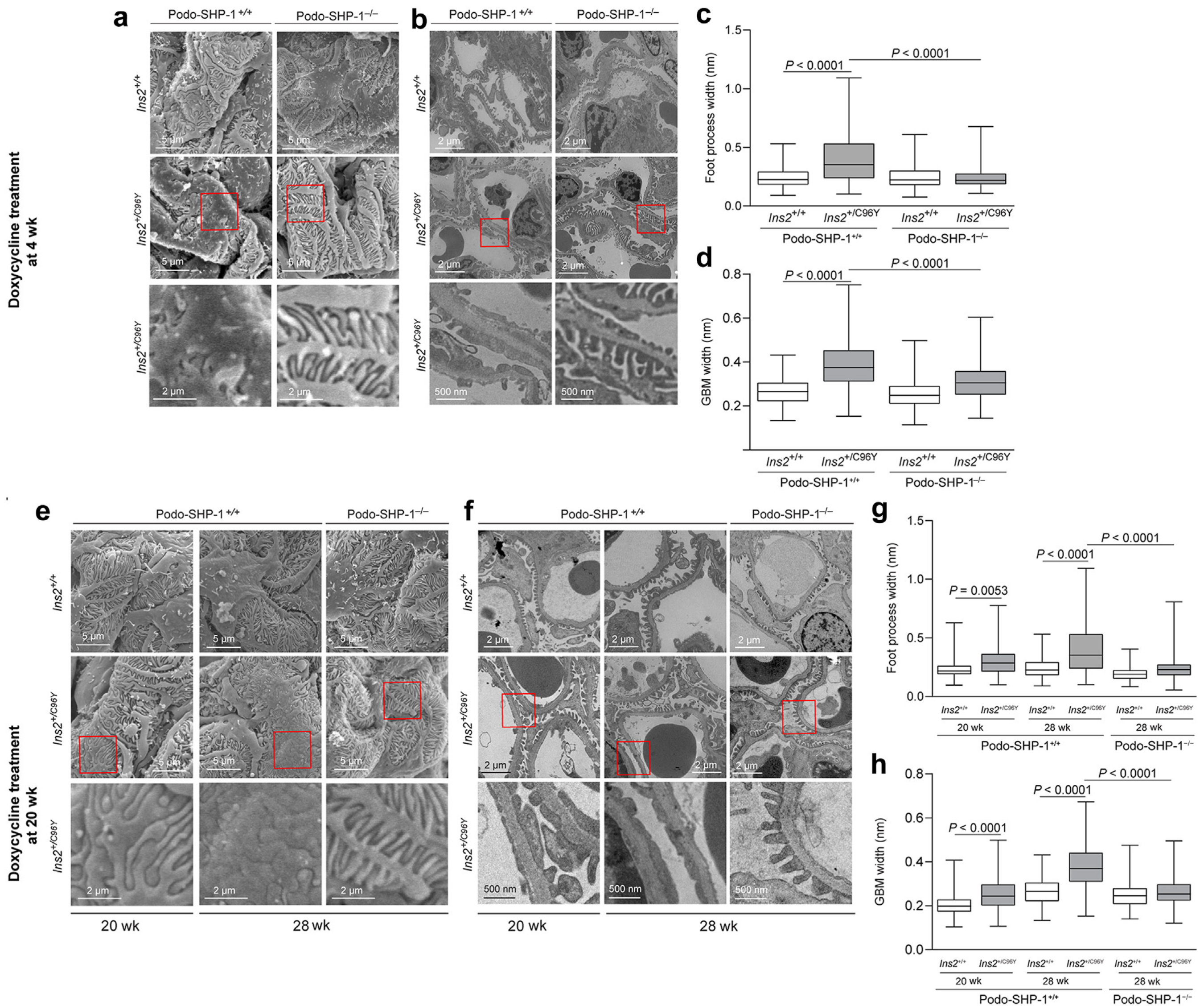

Figure 4 | (a,e) Scanning and (b,f) transmission electron microscopy of 20- and 28-week-old nondiabetic (*Ins2*$^{+/+}$; white bars) and diabetic (*Ins2*$^{+/C96Y}$; gray [20 weeks of age] and black [28 weeks of age] bars) mice, without (Podo-SHP-1$^{+/+}$) or with podocyte-specific deletion of SHP-1 (Podo-SHP-1$^{-/-}$) induced at 4 and 20 weeks of age. (**c,g**) Foot process effacement and (**d,h**) glomerular basement thickening (GBM) were quantified using the ImageJ software. The red squares show higher magnification area. Results are shown as mean ± SD of 10 to 15 consecutive measurement of distance between foot process (**c,g**) or GMB width (**d,h**) of 9 mice per group. SHP-1, Src homology 2 domain-containing protein tyrosine phosphatase 1. To optimize viewing of this image, please see the online version of this article at www.kidney-international.org.

nor the absence of SHP-1 expression in podocyte had influenced the expression of SHP-2, the closest tyrosine phosphatase protein of SHP-1 (Figure 7i).

Identification of a novel mechanism: SHP-1 is responsible for diabetes-induced reduction of essential slit diaphragm structural protein expression

To further investigate the molecular mechanisms of SHP-1 in podocytes, we performed mass spectrometry analyses of the kidney cortex of our treated and untreated diabetic mice. As shown in Figure 8a, several proteins were positively or negatively modulated by diabetes and some of these changes were prevented by the absence of SHP-1. From this list, proteins that are known to have been involved in the kidney function and structure were identified to be increased by diabetes and reserved by the deletion of SHP-1 (blue dots) or decreased by diabetes and restored by the absence of SHP-1 in podocytes (green dots). A complete list of proteins is available in Supplementary Tables S2 and S3. Interestingly, protein expressions such as podocin and focal adhesion kinase have been restored by 130% and 70%, respectively, by the deletion of SHP-1 in a diabetic condition (Figure 8b and c). In addition, our results revealed that small ubiquitin-related modifier 2 (SUMO2) protein was reduced in diabetic mice (−1.7-fold; $P = 0.005$) and restored by the deletion of SHP-1 (Figure 8b and c).

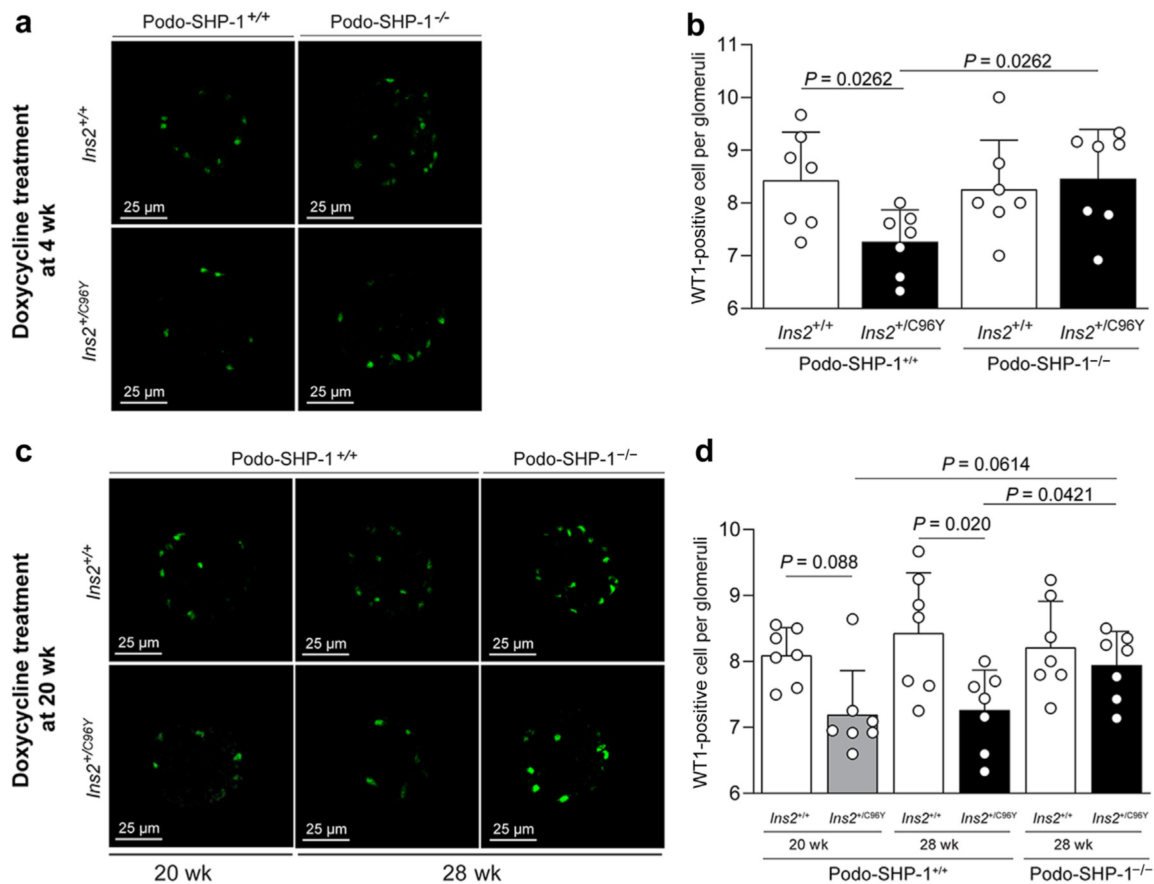

Figure 5 | (a,c) Immunofluorescence of podocyte nucleus (WT1; green) and (b,d) cell count in the glomerulus (same average size) of 20- and 28-week-old nondiabetic (*Ins2*$^{+/+}$; white bars) and diabetic (*Ins2*$^{+/C96Y}$; gray [20 weeks of age] and black [28 weeks of age] bars) mice, without (Podo-SHP-1$^{+/+}$) or with podocyte-specific deletion of SHP-1 (Podo-SHP-1$^{-/-}$) induced at 4 and 20 weeks of age. Results are shown as mean ± SD of 6 to 12 glomeruli of 7 mice per group. SHP-1, Src homology 2 domain-containing protein tyrosine phosphatase 1. To optimize viewing of this image, please see the online version of this article at www.kidney-international.org.

SUMOylation is a post-translational modification involved in a variety of cellular processes such as protein stability, regulation of gene transcription, and cell death.[25] Our immunoblot analyses confirmed the significant reduction of SUMO2 and podocin in renal glomeruli of diabetic *Ins2*$^{+/C96Y}$ mice (Figure 8d), an observation that was also corroborated in the kidney cortex of diabetic patients (Figure 8e). Moreover, our data suggested that decreased SUMO2 expression was associated with reduced mRNA (Figures 6h and 7g) and protein levels of podocin (Figure 8d), an effect that was prevented by the absence of the SHP-1 gene in podocytes. Furthermore, our immunofluorescence and immunoprecipitation assays demonstrated that diabetes prevented the association of SUMO2 with podocin, reducing podocin and SUMO2 abundance, an effect that was abolished with the deletion of SHP-1 in podocytes (Figure 8f and g). A previous study reported that SUMOylation by SUMO1 leads to p53 activation and apoptosis during cellular stress.[26] In contrast to SUMO2, our data showed that SUMO1 was enhanced in the kidney cortex of patients with diabetes and diabetic mice compared with their nondiabetic counterparts, and associated with podocin, which was blunted with the absence of SHP-1 specifically in podocytes (Supplementary Figure S3). Although nephrin was undetermined in the mass spectrometry analysis, its regulation has been shown to be critical for the preservation of the integrity of the slit diaphragm. Thus, we evaluated nephrin expression and phosphorylation in our groups. Our data demonstrated that nephrin expression and phosphorylation are decreased by 55% in diabetic mice compared with nondiabetic counterparts ($P = 0.0079$; Supplementary Figure S4). Both early and late deletion of the SHP-1 gene in podocytes were able to prevent the diabetes-induced reduction of nephrin phosphorylation and expression (Supplementary Figure S4). The importance of SUMOylation of podocin and implication of SHP-1 in podocin expression was evaluated in mouse podocytes. Cultured mouse podocytes were exposed to HG levels (25 mmol/l) or infected with adenoviral vector of the native form of SHP-1. We confirmed that protein expression of podocin and SUMO2 were decreased in podocytes exposed to HG levels and overexpression of SHP-1, whereas overexpression of the dominant negative form of SHP-1 prevented HG-induced podocin and

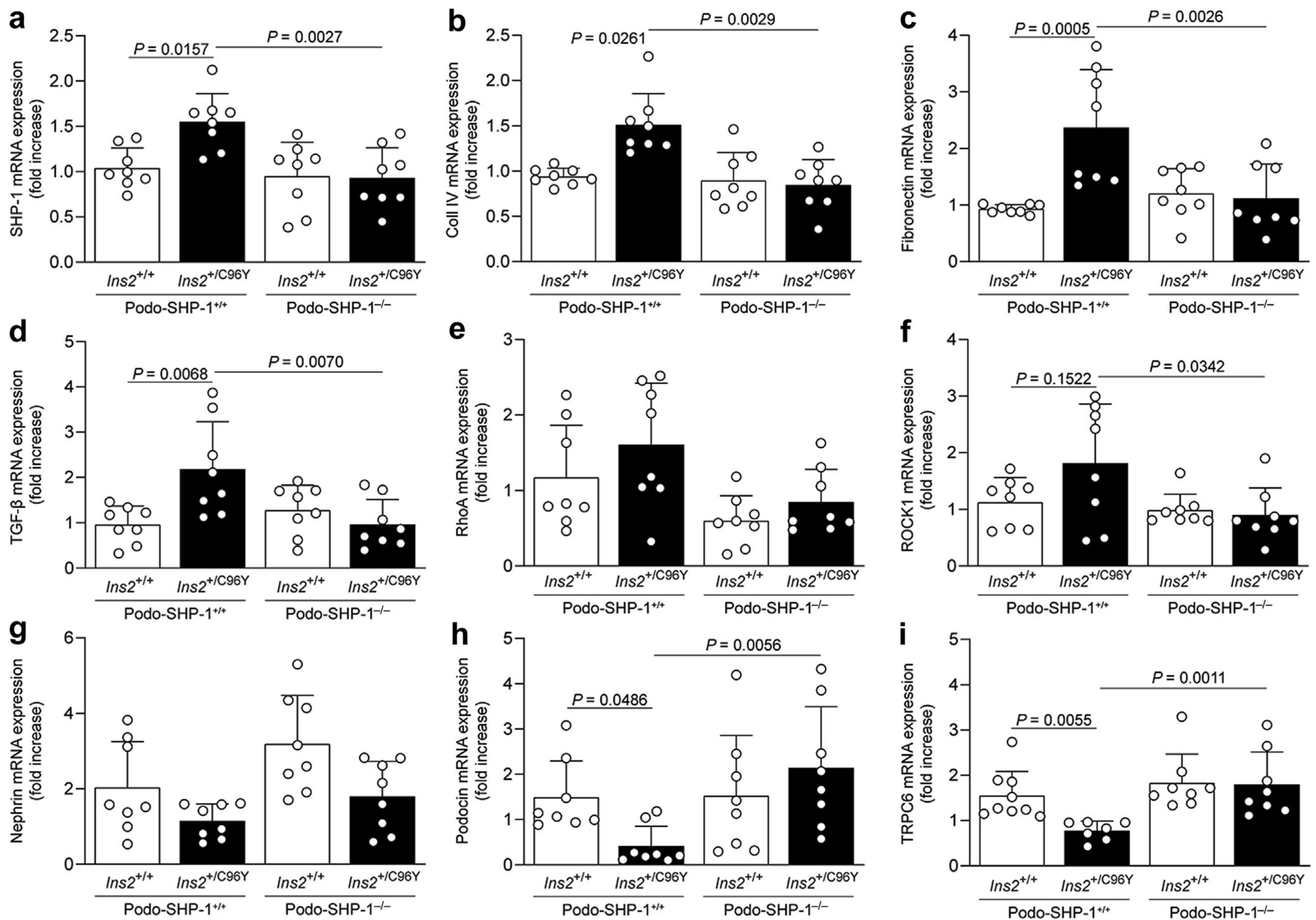

Figure 6 | Quantitative mRNA expression of (a) Src homology 2 domain-containing protein tyrosine phosphatase 1 (SHP-1), (b) collagen type IV (Coll IV), (c) fibronectin, (d) transforming growth factor beta (TGF-β), (e) RhoA, (f) Rho-associated protein kinase 1 (ROCK1), (g) nephrin, (h) podocin, and (i) transient receptor potential channel 6 (TRPC6) of 28-week-old nondiabetic (*Ins2*$^{+/+}$; white bars) and diabetic (*Ins2*$^{+/C96Y}$; black bars) mice, without (Podo-SHP-1$^{+/+}$) or with podocyte-specific deletion of SHP-1 (Podo-SHP-1$^{-/-}$) induced at 4 weeks of age. Results are shown as mean ± SD of 8 mice per group.

SUMO2 reduction. Interestingly, inhibition of SUMOylation (purple bar) exhibited a similar decrease in podocin expression (Supplementary Figure S5A and B). In addition, we confirmed that inhibition of SUMOylation, HG exposure, and overexpression of SHP-1–induced podocyte injury measured F-actin remodeling, an effect that can be prevented by the overexpression of the dominant negative form of SHP-1 under HG conditions (Supplementary Figure S5C).

DISCUSSION

Podocytes play an essential role in building and maintaining the filtration process of the glomerulus. Thus, it is not surprising that damage to podocytes ultimately leads to the development of glomerular disease such as focal segmental glomerulosclerosis and DKD.[27,28] These diseases usually progress to chronic kidney disease and kidney failure. Podocyte injury results in the effacement and shedding of their foot processes due to the breakdown of proteins that compose the slit diaphragms. Any changes in these protein expressions and signaling properties will prompt podocyte detachment, cell death, and glomerular disease progression.[11] Over the past few years, many laboratories, including us, have investigated the molecular mechanisms of podocyte injury in DKD. These include growth factor signaling pathways (vascular endothelial growth factor and insulin) and expression of structural cytoskeleton proteins involved in the actin remodeling process such as nephrin-Neph1-podocin.[20,29-31] Gene mutations within these growth factor ligands or receptors and within proteins that regulate podocyte actin cytoskeletal dynamics have resulted in glomerulosclerosis and pathology similar to patients with DKD. Thus, improving our knowledge of the molecular mechanisms that occur during podocyte injury may offer new opportunities to identify novel therapeutic targets, which could potentially be used to restore podocyte function. The timing of therapeutic intervention in patients with DKD remains a challenge and proves to be a significant issue because podocyte injury appears before the detection of proteinuria in humans.

Our group has studied the role of SHP-1, a PTP that is upregulated in podocytes exposed to elevated glucose levels

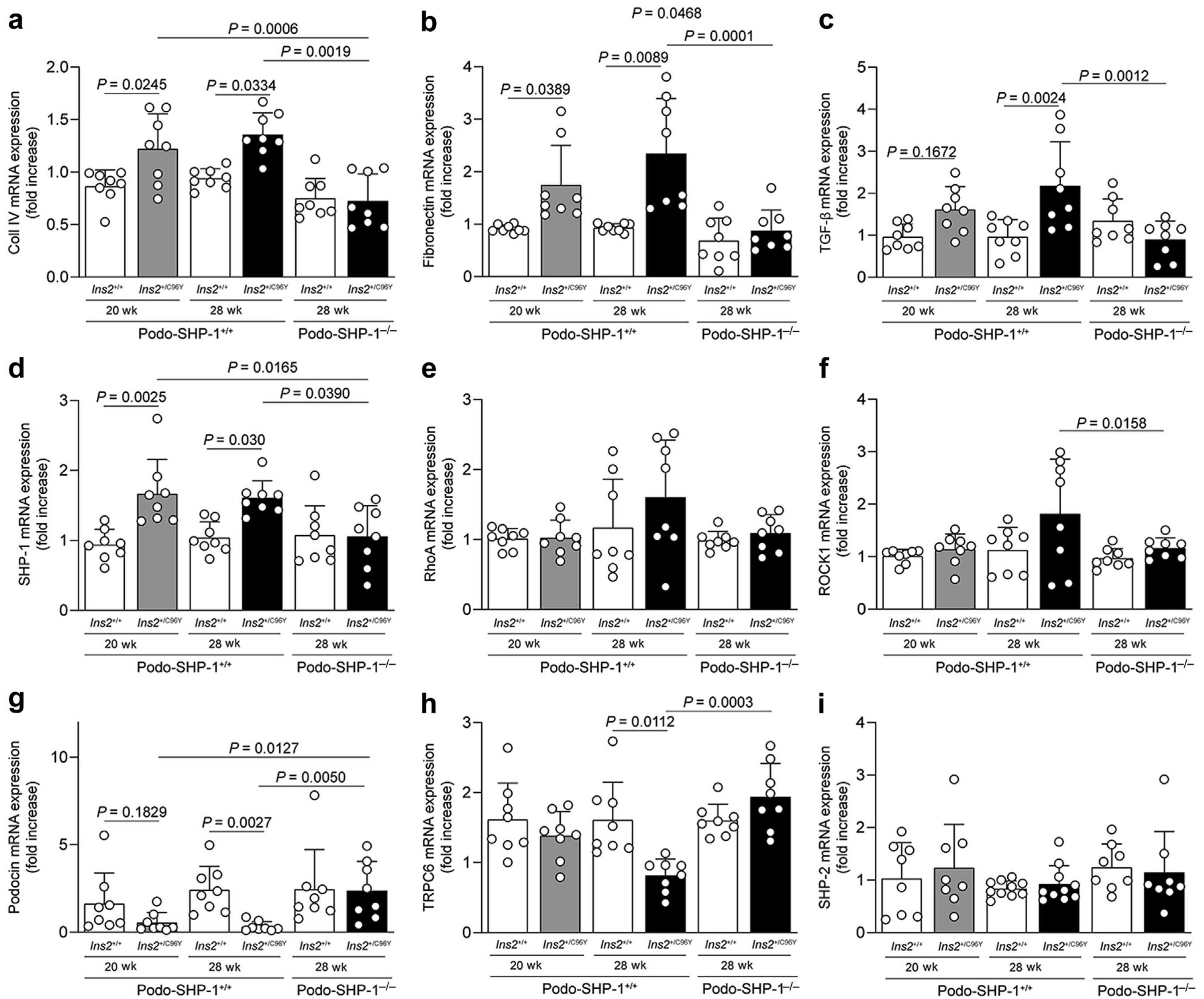

Figure 7 | Quantitative mRNA expression of (a) collagen type IV (Coll IV), (b) fibronectin, (c) transforming growth factor beta (TGF-β), (d) Src homology 2 domain-containing protein tyrosine phosphatase 1 (SHP-1), (e) RhoA, (f) Rho-associated protein kinase 1 (ROCK1), (g) nephrin, (h) podocin and (i) transient receptor potential channel 6 (TRPC6) of 20- and 28-week-old nondiabetic (*Ins2*$^{+/+}$; white bars) and diabetic (*Ins2*$^{+/C96Y}$; gray [20 weeks of age] and black [28 weeks of age] bars) mice, without (Podo-SHP-1$^{+/+}$) or with podocyte-specific deletion of SHP-1 (Podo-SHP-1$^{-/-}$) induced at 20 weeks of age. Results are shown as mean ± SD of 8 mice per group.

and the glomeruli of diabetic mice.[20,21] For the first time, our current study provided evidence that SHP-1 expression is increased in the glomeruli of diabetic patients. Astonishingly, the podocyte-specific deletion of SHP-1 can prevent or even reverse the progression of DKD in mice. SHP-1 is known to interact with the insulin[32] and vascular endothelial growth factor[33] receptors. However, because the diabetic mice were deficient in insulin (due to the insulin 2 gene mutation), preservation of the podocyte structure and function cannot be attributed to the maintenance of the insulin signaling pathway. In addition, because controversies exist regarding the presence of the vascular endothelial growth factor receptor in podocytes, preserved vascular endothelial growth factor signaling in the absence of SHP-1 may have little influence on podocyte function. Previous reports have shown that tyrosine phosphatases such as PTP1B and SHP-1 are capable of modulating protein expression and activity involved in the slit diaphragm and actin cytoskeleton remodeling.[13,14] Thus, our current study further investigated the influence of SHP-1 in the regulation of podocyte structural proteins.

Podocyte function is dependent on the plasticity of its cytoskeletal architecture. Signaling pathways that trigger actin cytoskeleton remodeling involved in maintaining foot process structure in human podocyte disease are still largely underexplored. The Rho family of small GTPases are known regulators of cytoskeletal dynamics and have been shown to participate in maintaining podocyte adhesion junctions.[34] Previous studies have reported that the deregulation of RhoA associated with Rac1 activity is detrimental to podocyte

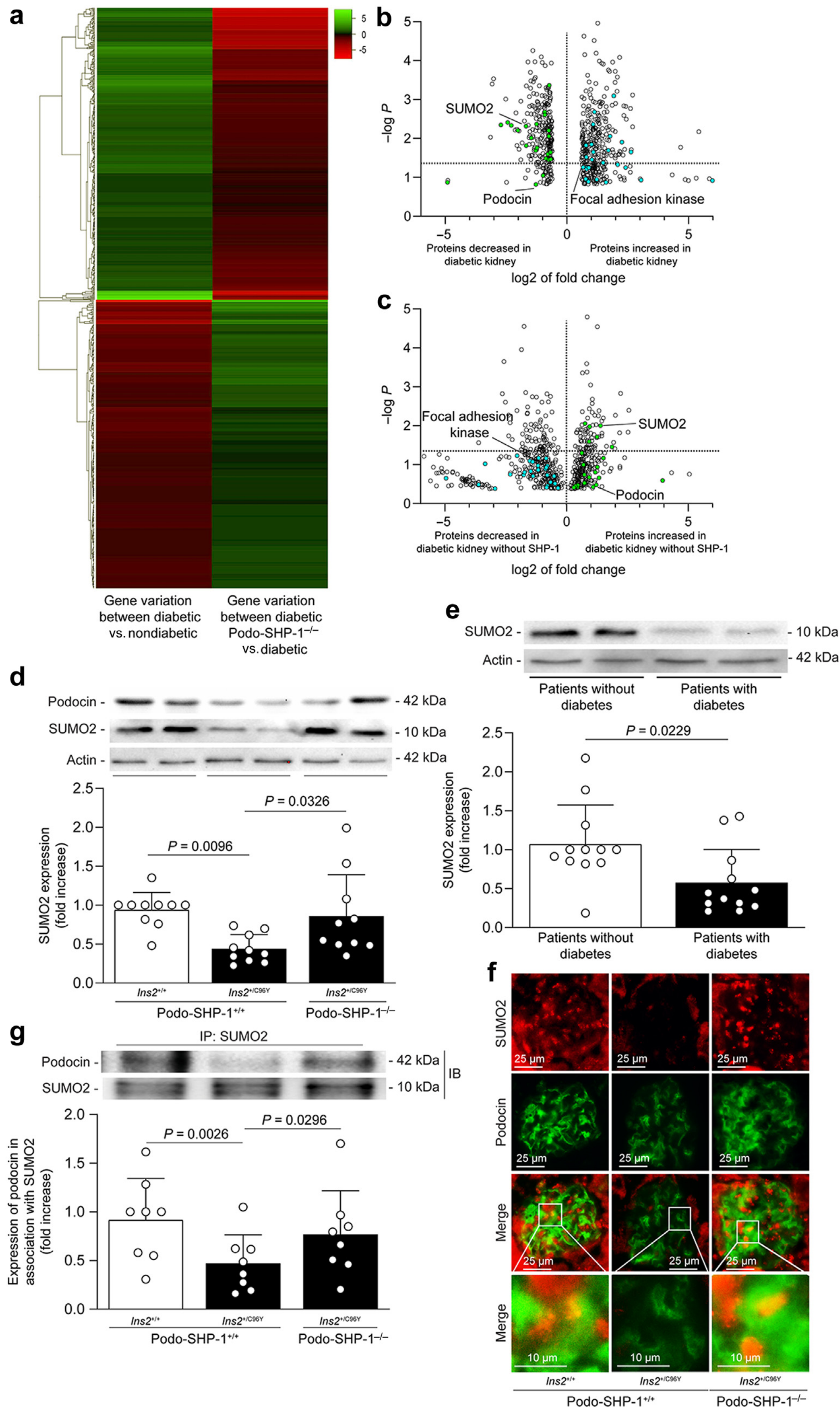

Figure 8 | Mass spectroscopy data analysis presented in the (a) heat map and (b,c) volcano plot in log of fold change between (left column of a and b) 28-week-old diabetic (*Ins2*$^{+/C96Y}$) and nondiabetic (*Ins2*$^{+/+}$) mice and (right column of a and c) diabetic Podo-SHP-1$^{-/-}$ compared with diabetic Podo-SHP-1$^{+/+}$. Proteins listed that are associated with kidney function and structure are identified to be increased by diabetes and reversed by the deletion of SHP-1 (blue dots) or decreased by diabetes and restored (continued)

structure and is associated with foot process effacement.[35,36] Constitutive activation of RhoA in podocytes resulted in reduced nephrin expression, cell death, and proteinuria.[37] One of the major downstream mediators of RhoA, ROCK1, participates in the regulation of the actin cytoskeleton, and its activation has been linked to multiple forms of glomerular diseases.[38,39] Interestingly, our study demonstrated that RhoA and ROCK1 expression were elevated in the kidney cortex of diabetic mice, and the absence of SHP-1 was able to reduce their expression to basal levels. Accordingly, it is not surprising that the reduction of RhoA signaling blunted podocyte injury in a mouse model of chronic kidney disease.[40,41] A previous study reported that preservation of RhoA signaling in lipopolysaccharide-induced proteinuria can be achieved via synaptopodin.[42,43] Synaptopodin is a proline-rich actin binding protein that, in interaction with the actin-associated protein α-actinin-4, plays an important role in cross-linking actin filaments in podocytes.[44,45] Synaptopodin also participates in the contractile properties of the podocyte actin cytoskeleton by simultaneously regulating RhoA and small GTPase cell division control protein 42 homologue.[46] In association with elevated RhoA and ROCK1 expression, our results indicate that synaptopodin expression is reduced in diabetic mice. This agrees with previous observations that downregulation of synaptopodin occurs in glomeruli of patients with idiopathic nephrotic syndrome and other glomerular diseases.[47,48] Therefore, preservation of its expression with the deletion of SHP-1 could represent an antiproteinuric pathway by halting the progression of podocyte dysfunction and DKD.

Although podocin gene mutation is associated with the most common genetic form of hereditary nephrotic syndrome,[49] patients with DKD also have reduced podocin expression.[50,51] Podocin, a member of the stomatin protein family, is exclusively expressed in podocytes of developing and mature glomeruli and is located in the cytosolic side of the slit diaphragm.[52] It acts together with other transmembrane adhesion proteins such as nephrin and Neph1 to form a protein complex. Importantly, podocin interaction is required for effective signaling through nephrin and its associated proteins.[53] Indeed, a previous investigation reported that podocin promotes efficient nephrin signaling by facilitating the recruitment of nephrin to detergent-resistant lipid microdomains of the plasma membrane.[54] Our study demonstrated that the expression of podocin and the phosphorylation of nephrin are reduced in diabetes and prevented by the deletion of SHP-1. Moreover, podocin binds and regulates the transient receptor potential channel 6, which is also critically involved in proteinuric kidney disease.[55] Altogether, podocin serves as a scaffold connecting junction protein within the slit diaphragm and actin cytoskeleton. Interestingly, our data revealed that SUMO2 is downregulated in a diabetic state and rescued by the inhibition of SHP-1. SUMOs are a family of small proteins that covalently attach to and detach from other proteins to affect their function.[56] Therefore, protein SUMOylation, a post-translational modification, is implicated in a variety of biological processes such as transcriptional regulation, cell cycle progression, cell death, stress response nuclear-cytosolic trafficking, and protein stability.[57] Over the past few years, a large number of clinical studies have demonstrated the relationship between SUMO modifications and pathologic diseases such as neurodegenerative diseases, cancer, cardiomyopathy, and diabetes.[58-61] Evidence suggests that SUMO1 and SUMO2/3 play distinct physiological roles.[62] SUMO2 has the particularity capacity of forming chains on target substrates, conferring distinct mechanisms of action as compared with SUMO1.[63] Although previous studies have shown that SUMOylation by SUMO1 can occur in podocytes,[64] we showed for the first time that SUMO2 is decreased, whereas SUMO1 is increased, in the kidney of diabetic mice and patients with diabetes. This decrease is directly associated with the reduction of podocin. Under resting conditions, cells maintained limited amount of unconjugated SUMO1 proteins, whereas SUMO2 is found to be rapidly mobilized and is essential for cell survival after heat-induced injury (72-75 of A). SUMO1 has been shown to either enhance or suppress protein transcriptional activity.[26,65] In contrast, SUMO2 can counteract detrimental actions of stress-induced protein misfolding, which is viewed as a protective mechanisms.[62] Furthermore, SUMOylation has been demonstrated to be essential for normal nephrin localization, stability, and proper function,[66] suggesting that deSUMOylation of nephrin reduced its membrane expression at the slit diaphragm. Indeed, cultured podocytes treated with SUMO inhibitor induced the reduction of podocin and caused podocyte injury. Because SUMOylation is a reversible process, restoring SUMOylation of podocin in our podocyte-specific SHP-1–deficient mice may lead to podocin expression recycling to stabilize the slit diaphragm and actin cytoskeleton signaling pathway in addition to the preservation of nephrin phosphorylation. Using another model of podocyte injury, other investigators have reported that the nephrin-neph1-podocin complex can regulate the actin-cleaving protein Cofilin-1 to reconstitute regular foot process architecture.[67] Therefore, inhibition of SHP-1 may have preserved podocin complex interaction with actin-associated proteins, which is essential for regenerative actin remodeling that presumably occurs after early podocyte injury. Furthermore, our study

Figure 8 | (continued) by the absence of SHP-1 in podocytes (green dots). (**d**) Immunoblot analysis of small ubiquitin-related modifier 2 (SUMO2) and podocin protein expression in the mouse kidney cortex and (**e**) human kidney cortex. (**f**) Colocalization by immunofluorescence of podocin (green) and SUMO2 (red) in mouse glomeruli. (**g**) Immunoprecipitation assay of SUMO2 associated with podocin in the mouse kidney cortex of 28-week-old nondiabetic (*Ins2*$^{+/+}$), diabetic (*Ins2*$^{+/C96Y}$), and diabetic mice with podocyte-specific deletion of SHP-1 (Podo-SHP-1$^{-/-}$). Results are shown as mean ± SD of 3 (**a–c**) and 8 (**d, f, and g**) mice per group and 12 (**e**) human samples. SHP-1, Src homology 2 domain-containing protein tyrosine phosphatase 1. To optimize viewing of this image, please see the online version of this article at www.kidney-international.org.

uncovered a new role of SHP-1 in the modulation of podocin expression via SUMO2 in the kidney. Besides SUMOylation, other post-translational modifications may regulate podocin stability. A previous study demonstrated that ubiquitin ligase Ubr4 controls stability of podocin/MEC-2 supercomplexes.[68] Our mass spectrometry analysis also revealed that neural precursor cell expressed developmentally downregulated protein 8, involved in NEDDylation, a process similar to ubiquitination, was slightly elevated in diabetic mice. However, neural precursor cell expressed developmentally down-regulated protein 8–bind association with podocin was unaffected (Supplementary Figure S6).

In conclusion, this study provides evidence that the deletion of SHP-1 in podocytes exposed to diabetes for several months restores SUMOylation of podocyte structural proteins to preserve their stability and reverse the progression of DKD. Therefore, inhibition of SHP-1 may prevent diabetes-induced deregulation of podocyte function and may offer additional tools to protect patients who may not benefit from standard kidney care.

DISCLOSURE

All the authors declared no competing interests.

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AUTHOR CONTRIBUTIONS

FL, MR, and BD performed experiments and collected samples and experimental data. AG performed animal care. FL, AMC, JM, SH, and RS recruited patients, performed the process, and analyzed human tissue samples. HL and KS provided the RNA-seq data of a previously published human kidney cohort. DL and F-MB performed mass spectrometry analysis. FL, MR, AMC, and PG analyzed the data and wrote the manuscript.

SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Supplementary Methods.

Supplementary Table S1. Body weight and blood glucose levels of nondiabetic (Ins2$^{+/+}$) and diabetic (Ins2$^{+/C96Y}$) mice, without or with deletion of Src homology 2 domain-containing protein tyrosine phosphatase 1 (SHP-1). Data are shown as N = 12 mice per group ± SD.

Supplementary Table S2. List of proteins decreased in diabetic mice compared with nondiabetic mice and reversed with the deletion of Src homology 2 domain-containing protein tyrosine phosphatase 1 (SHP-1) specifically in podocytes that were identified in the kidney tissue by the STRING and TISSUES databases.

Supplementary Table S3. List of proteins increased in diabetic mice compared with nondiabetic mice, and reversed with the deletion of Src homology 2 domain-containing protein tyrosine phosphatase 1 (SHP-1) specifically in podocytes that were identified in the kidney tissue by the STRING and TISSUES databases.

Supplementary Table S4. Primer sequence used for genotyping and Src homology 2 domain-containing protein tyrosine phosphatase 1 (SHP-1)-specific deletion validation.

Supplementary Table S5. Mouse (m) and human (h) primer sequence used for quantitative polymerase chain reaction.

Supplementary Figure S1. Src homology 2 domain-containing protein tyrosine phosphatase 1 (SHP-1)-specific deletion was established by genotyping of (**A**) Tet-ON Cre and (**B**) podocin-rtTa transgenes as well as (**C**) loxP sequences within the SHP-1 (Ptpn6) gene. (**D**) Schematic of the excision PCR strategy to confirm the ablation of the SHP-1 gene. (**E**) After doxycycline treatment, excision of exon 1(II) to 9 of the SHP-1 gene was determined with the PCR amplification product of 400 bp generated with the F1/R2 primers (Supplementary Table S4). (**F**) Immunofluorescence of SHP-1 (green) and WT1 (red) of diabetic Podo-SHP-1$^{+/+}$ and diabetic Podo-SHP-1$^{-/-}$ mouse glomeruli. The white arrows show colocalization of SHP-1 and WT1 in podocytes. (**G**) Glomerular filtration rate of nondiabetic (white) and diabetic (black) Podo-SHP-1$^{+/+}$, without (circles) or with (squares) doxycycline treatment. Results are shown as mean ± SD of 10 (g) mice per group.

Supplementary Figure S2. Src homology 2 domain-containing protein tyrosine phosphatase 1 (SHP-1) phosphatase activity in 28-week-old nondiabetic (Ins2$^{+/+}$; white box) and diabetic (Ins2$^{+/C96Y}$; black box) mice, without (Podo-SHP-1$^{+/+}$) or with podocyte-specific deletion of SHP-1 (Podo-SHP-1$^{-/-}$) induced at 4 (blue box) and 20 (purple box) weeks of age. Results are shown as mean ± SD of 7 mice per group.

Supplementary Figure S3. Immunoblot analysis of SUMO1 protein expression in the (**A**) human kidney cortex and (**B**) mouse glomeruli. (**C**) Immunoprecipitation assay of podocin and SUMO1 of the kidney cortex of 28-week-old nondiabetic (Ins2$^{+/+}$), diabetic (Ins2$^{+/C96Y}$), and diabetic mice with podocyte-specific deletion of Src homology 2 domain-containing protein tyrosine phosphatase 1 (SHP-1) (Podo-SHP-1$^{-/-}$). Results are shown as mean ± SD of 12 human samples (**A**) and 8 mice per group (**B,C**).

Supplementary Figure S4. (**A**) Immunoblot and (**B**) quantification of total and phosphotyrosine (Y1176-1193) nephrin expression in the kidney glomeruli of 28-week-old nondiabetic (Ins2$^{+/+}$; white box), diabetic (Ins2$^{+/C96Y}$; black box) without (Podo-SHP-1$^{+/+}$) or with podocyte-specific deletion of SHP-1 (Src homology 2 domain-containing protein tyrosine phosphatase 1; Podo-SHP-1$^{-/-}$) induced at 4 (blue box) or 20 weeks of age (purple box). Results are shown as mean ± SD of 8 mice per group.

Supplementary Figure S5. (**A**) Immunoblot and (**B**) quantification, and immunofluorescence of (**C**) podocin and (**D**) F-actin (phalloidin) protein expression in mouse podocytes exposed to normal (white) or high glucose (black), with or without infection of dominant negative form (gray) or wild-type (blue) form of Src homology 2 domain-containing protein tyrosine phosphatase 1 (SHP-1) adenoviral vectors or treated with SUMOylation inhibitor 2-D08 100 μM (purple). Results are shown as mean ± SD of 6 independent experiments.

Supplementary Figure S6. (**A**) Immunoblot and (**B**) quantification of co-immunoprecipitation assays of neural precursor cell expressed developmentally down-regulated protein 8 (NEDD8) with podocin of

the kidney cortex of 28-week-old nondiabetic (Ins2$^{+/+}$), diabetic (Ins2$^{+/C96Y}$), and diabetic mice with podocyte-specific deletion of Src homology 2 domain-containing protein tyrosine phosphatase 1 (SHP-1) (Podo-SHP-1$^{-/-}$). Results are shown as mean ± SD of 4 mice per group.

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