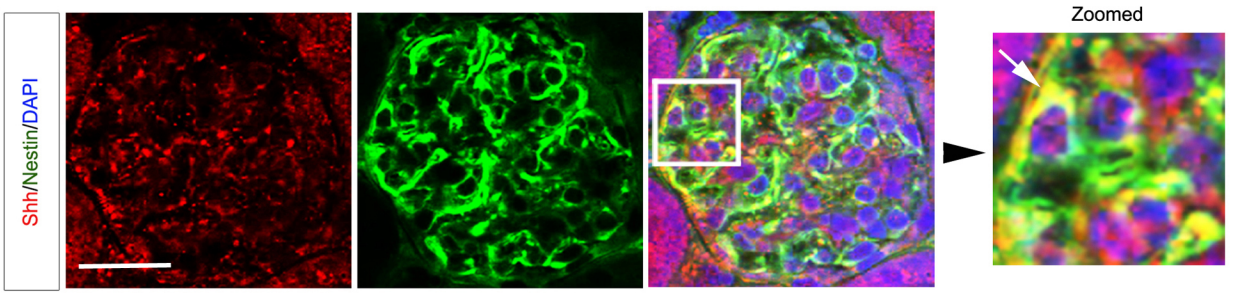
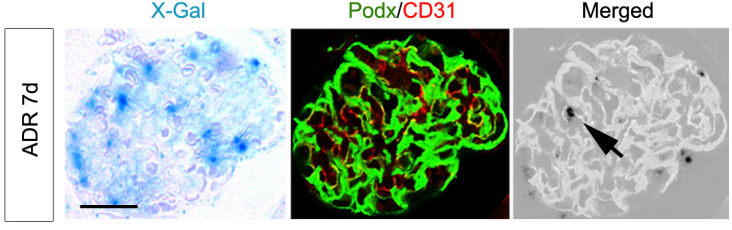
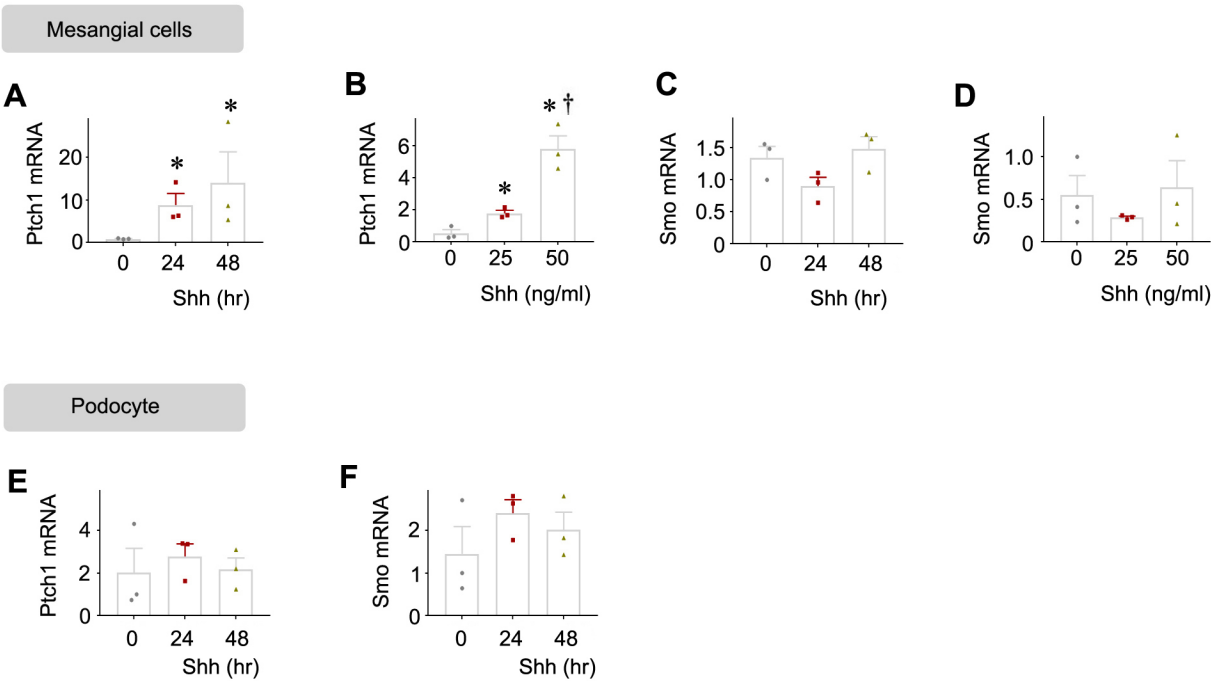


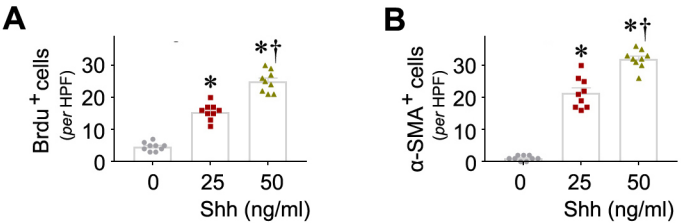
Supplemental Figure 1. Col-localization of Shh and nestin in glomerulardocytes in proteinuric CKD. Double immunofluorescence staining for Shh (red) and nestin (green), a podocyte -specific marker, in the injured glomeruli after ARDR injection. Boxed areas are enlarged and presented. Arrow indicate souble staining. Scale bar, 25 μm.



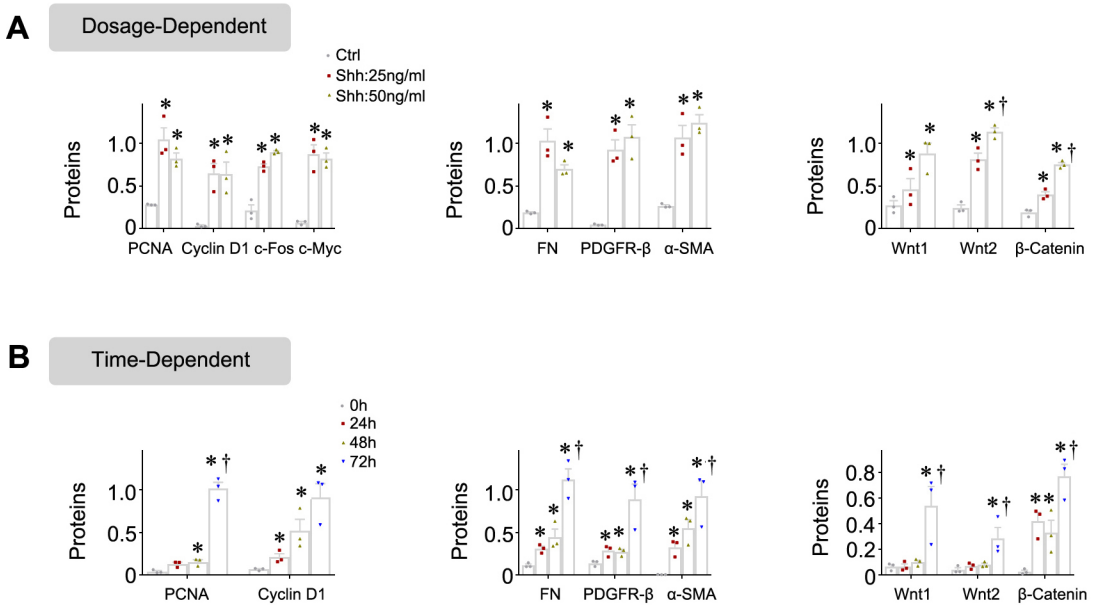
Supplemental Figure 2. Hedgehog-responding cells in the glomeruli are non-endothelial and non-podocytes.
Co-staining of X-Gal with endothelial marker (CD31, red) and podocyte marker (podocalyxin, green) at 7 days after ADR. Black arrow indicates hedgehog-responding cells in the glomeruli. Scale bar, 25 μm.



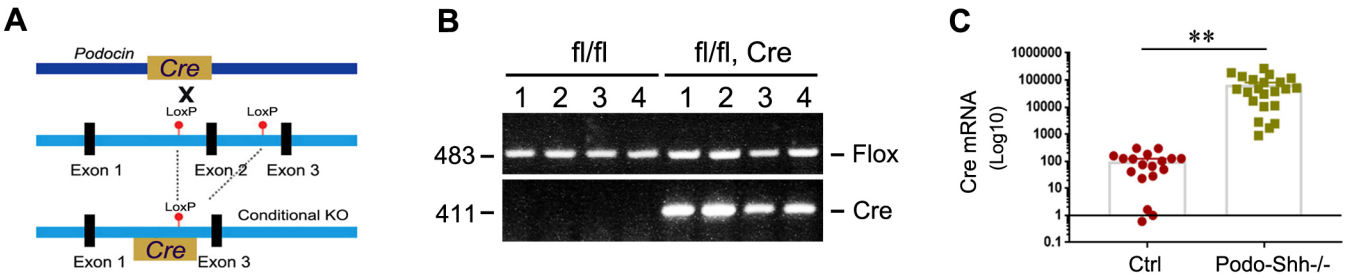
Supplemental Figure 3. Expression of Shh receptors in mesangial cells and podocytes in vitro. (A, B) qPCR shows that Shh induced Ptch1 mRNA expression in cultured mesangial cells in a dose- and time-dependent manner. **P* < 0.05 versus controls (n=3), †*P* < 0.05 versus Shh at 25 ng/ml (n=3, *t* test). (**C**, **D**) Shh did not significantly affect Smo mRNA expression in mesangial cells. (**E**, **F**) Shh did not affect Ptch1 and Smo mRNA expression in podocytes.



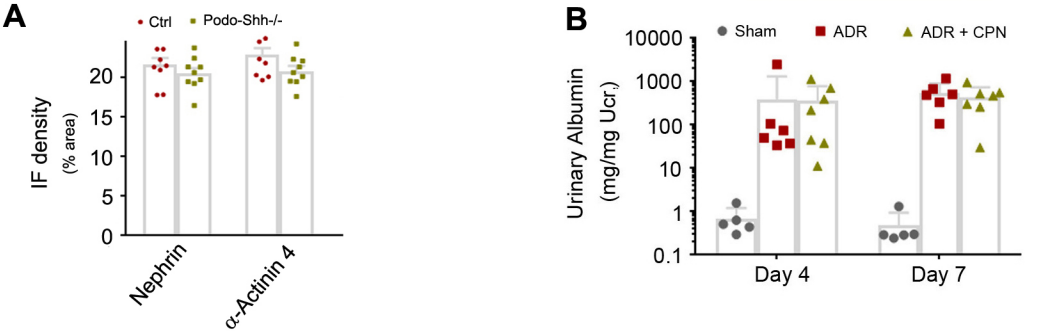
Supplemental Figure 4. Quantification of BrdU and αSMA-positive mesangial cells after Shh treatment. Mesangial cells were incubated with different doses of Shh for 2 days. BrdU+ (**A**) and αSMA+ (**B**) cells per high power field (HPF) were counted. Three fields per treatment were counted, and each treatments were repeated 3 times. **P* < 0.05 versus controls, †*P* < 0.05 versus 25 ng/ml of Shh. Student-Newman-Keuls test.



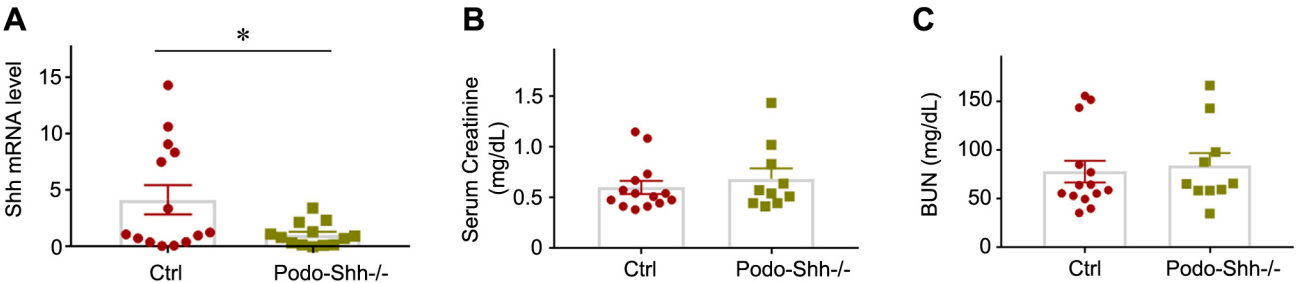
Supplemental Figure 5. Quantification of protein expression in mesangial cells after Shh treatment. (**A**) Mesangial cells were treated with different doses of Shh (0, 25 and 50 ng/ml) for 2 days. Expression of various proteins were assessed by Wstern blotting. **P* < 0.05 versus controls (n=3), †*P* < 0.05 versus 25 ng/ml of Shh (n=3). (**B**) Mesangial cells were treated with 50 ng/ml of Shh for various perids of time as indicated. Expression of various proteins were assessed by Wstern blotting.**P* < 0.05 versus controls (n=3), †*P* < 0.05 versus 48 hr (n=3). Student-Newman-Keuls test.



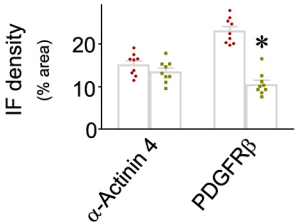
Supplementary Figure 6. Generation and characterization of conditional knockout mice with podocyte-specific ablation of Shh. (**A**) Experimental design. (**B**) Mouse genotyping. (**C**) qPCR analyses revealed a marked expression of Cre recombinase mRNA in Podo-Shh-/- mice. Student's *t* test.



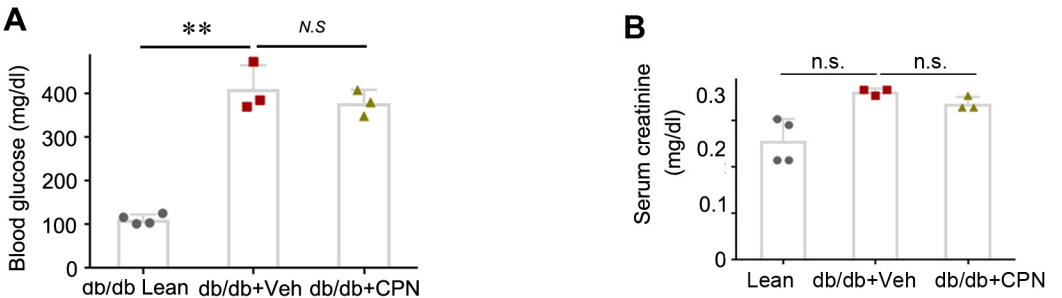
Supplemental Figure 7. (A) Loss of Shh in podocytes does not affect nephrin and and α-actinin 4 expression in the glomeruli at 7 days after ADR injection. Quantification of nephrin and α-actinin 4 staining in control and podo-Shh-/- mice is shown as the relative IF intensity (percentage of area). n=5-7. **(B) Pharmacologic inhibition of Shh by CPN had little impact on uirnary protein levels at day 4 and day 7 after ADR (n=5-7).**



Supplementary Figure 8. Podocyte-specific deletion of Shh does not affect kidney function at 5 weeks after ADR injection. (**A**) qPCR showed the reduction of renal Shh mRNA in Podo-Shh-/- mice, compared to controls. *$P < 0.05$. (**B**, **C**) There was no difference in serum creatinine (**B**) and blood urea nitrogen (BUN) (**C**) betwen control and Podo-Shh-/- mice at 5 weeks after ADR injection (n = 10-15). Student's *t* test.



Supplemental Figure 9. Quantification of α-actinin 4 and PDGFRβ staining in control and podo-Shh-/- mice at 5 weeks after ADR.
Relative IF intensity (% area) is shown. **P* < 0.05 versus controls (n=9). Student's *t* test.



Supplementary Figure 10. Inhibition of Shh signaling by CPN does not affect blood glucose and serum creatinine in db/db mice. (**A**) In 6 months old *db/db* mouse, adminitration with CPN for 2 weeks did not significantly affect blood glucose levels. (**B**) CPN did not significantly affect serum creatinine levels in db/db mice. Student-Newman-Keuls test.

Supplemental Table 1. Demographic Characteristics of the Participants Included in Shh IHC staining

No.	Gender	Age	Diagnosis	Scr (μmol/L)	e-GFR (ml/min/1.73m²)	Plasma Albumin (g/L)
1	Female	63	MN	59	94.9	24.7
2	Male	52	MN	85	87.2	18.6
3	Female	30	MN	51	130.5	18
4	Female	63	MN	92	56.8	23.6
5	Female	38	IgAN	52	121.7	37.5
6	Female	61	IgAN	115	44.2	26.9
7	Female	22	IgAN	41	178.8	26.9
8	Male	49	FSGS	278	22.5	17.2
9	Female	37	FSGS	112	50.5	33.3
10	Female	48	LN,SLE	56	106.1	34.8

The representative micrographs of Shh staining from those patients are selected and presented in Figure 8.
Abbreviation: Scr, serum creatinine; e-GFR, estimated glomerular filtration rate; MN, membranous nephritis; IgAN, IgA nephropathy; FSGS, focal and segmental glomerulosclerosis; LN, lupus nephritis; SLE, systemic lupus erythematosus.

Supplemental Table 2. Demographic Characteristics of the Participants Included in the Shh ELISA

Characteristic		Efficacy Cohort	
		Healthy person(N=35)	CKD patients (N=27)
Gender--No.(%)			
	Male	20(57.1)	13(48.1)
	Female	15(42.9)	14(51.9)
Age at entry--years*			
	Mean±SEM	42.38±3.05	40.13±3.48
	Range	21-72	17-77
Clinical Diagnosis--No.(%)†			
			Primary: IgAN, FSGS, MN, Secondary: LN, DKD, HTN.

*****There were no significant between-group differences at baseline. The serum shh ELISA included data from all participants.

Supplemental Table 3. Nucleotide sequences of the primers used for RT-PCR

Mouse gene	Primer Sequence 5' to 3'	
	Forward	Reverse
Shh	CGGCAGATATGAAGGGAAGA	TCATCACAGAGATGGCCAAG
Gli-1	CTCGACCTGCAAACCGTAAT	GTGGTACACAGGGCTGGACT
Gli-2	CTCATGGCAAGCCACCCCACC	CCGCTTGCGGCTCAGTCGTG
Gli-3	ACATCTGGCGGAGCCCTCTCT	CGGCCCTCATGATGTCTGGCA
Smoothened	CGGACTCGCAGGAGGAAGCG	GGGTACGGCTGGGCAACTCC
Ptch1	CAGCCGAGACAAGCCCATCGAC	ATGTTGGCCTGGGAGGCAGC
Ptch2	ACTGGTGGCCTTTGGGGCTCT	GAGGTGTACGCAGCCTCTTCCC
Hhip	CAACCTGCCCAGCCACTGACC	GACACCTCCATGACGGCACGC
Nephrin	ACCTGGACGACATGGAGAAG	ATCGGACAACAAGACGAACC
Podocin	GGAAGCTGAGGCACAAAGAC	AGCGACTGAAGAGTGTGCAA
CD2AP	AGGTAGAAGAAGGCTGGTGGA	GTGAGGTAGGGCCAGTCAAA
Podocalyxin	GCCTTCACTTGCTCTTACGG	AAAACCCGGTCATAACCACA
WT1	CATCCAGGCAGGAAAGTGT	TGCAGTCAATCAGGTGTGCT
Fibronectin	CGA GGT GAC AGA GAC CAC AA	CTG GAG TCA AGC CAG ACA CA
α-SMA	GAGGCACCACTGAACCCTAA	CATCTCCAGAGTCCAGCACA
FSP-1	AGCTACTGACCAGGGAGCTG	TCATTGTCCCTGTTGCTGTC
Collagen I	ATCTCCTGGTGCTGATGGAC	ACCTTGTTTGCCAGGTTCAC
Collagen III	AGGCAACAGTGGTTCTCCTG	GACCTCGTGCTCCAGTTAGC
β-actin	CAGCTGAGAGGGAAATCGTG	CGTTGCCAATAGTGATGACC