Online Supplemental Materials

REDD1 ablation attenuates development of renal complication in diabetic mice

Sunilkumar et al, 2022

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Table S1. Antibody information

Assay	Antibody	Source	Dilution	Catalog #	Lot#
Western blotting	REDD1	ProteinTech	1:500	10638-1-AP	95508
	Podocin	Sigma	1:2000	P0372	115575
	GAPDH	Santa Cruz	1:2000	sc-47724	H1021
	phospho GSK3β (Ser 9)	Cell Signaling	1:1000	5558	10
	GSK3β		1:1000	12456	10
	phospho glycogen synthase (Ser 641)		1:1000	3891	2
	Cleaved caspase 3		1:1000	9664	47
	Phospho-S6 Ribosomal Protein (Ser240/244)		1:1000	5364	8
	S6 Ribosomal Protein		1:1000	2317	4
	HA-tag		1:1000	3724	10
	Actin		1:1000	4970	19
	Goat anti-rabbit IgG-HRP	Bethyl Laboratories	1:10000	A120-101	44
	Goat anti-mouse IgG-HRP		1:10000	A90-116	43
IHC	Fibronectin	Cell Signaling	1:1000	26836	3
	Nitro-Tyrosine		1:1000	9691	3
	phospho GSK3β (Ser 9)		1:500	5558	10
	GSK3β		1:1000	12456	10
	WT1		1:100	83535	1
	Donkey anti-Rabbit IgG-Alexa Fluor 647	Jackson	1:1000	711-605-152	159933

Table S2. PCR primer sequences

Species	Target	Forward sequence (5' - 3')	Reverse sequence (5' - 3')
Mouse	REDD1	GGGATCGTTTCTCGTCCTCC	ATGAGGAGTCTTCCTCCGGC
	TGFβ1	CCCGAAGCGGACTACTATGC	CATAGATGGCGTTGTTGCGG
	Fibronectin	CGACGTGACAGAGACCACAA	CTGGAGTCAAGCCAGACACA
	αSMA	GAGGCACCACTGAACCCTAA	CATCTCCAGAGTCCAGCACA
	Collagen 1A1	GAGAACCAGCAGAGCCA	GAACAAGTGACAGAGGCATA
	E-cadherin	GGTTTTCTACAGCATCACCG	GCTTCCCCATTTGATGACAC
	GAPDH	GGTGGTCTCCTCTGACTTCAACA	GTTGCTGTAGCCAAATTCGTTGT
Human	REDD1	CTCTTCGCCCTCGTCCTTG	TCCAGGTAAGCCGTGTCTTC
	GCLC	GTTCTCAAGTGGGGCGATGA	TTCTCCCCAGACAGGACCAA
	GCLM	ATGGCCTGTTCAGTCCTTGG	CTCGTGCGCTTGAATGTCAG
	HO-1	TGACCCATGACACCAAGGAC	GGGGGCAGAATCTTGCACTTT
	NQO-1	TGATATTCCAGTTCCCCCTGC	TGGCAGCGTAAGTGTAAGCA
	GAPDH	GTTGTCTCCTGCGACTTCA	TGCTGTAGCCGTATTCATTG

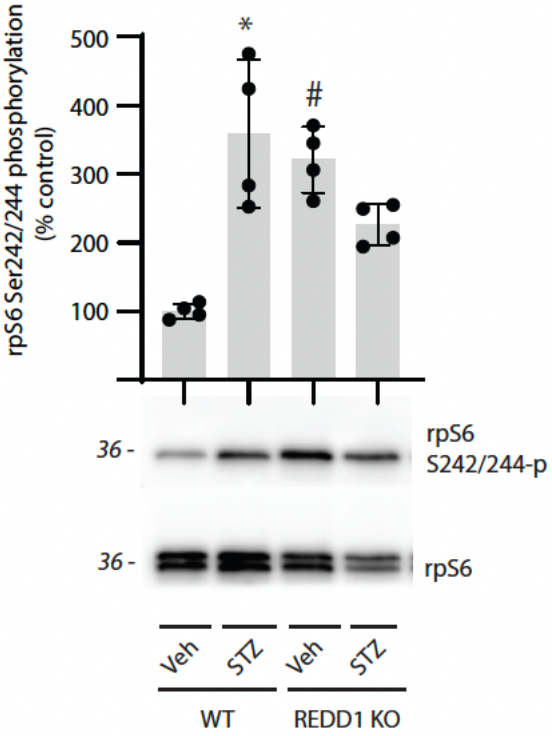

Figure S1: Analysis of ribosomal protein S6 phosphorylation in renal tissue lysates. Diabetes was induced in wildtype (WT) and REDD1 knockout (KO) mice by administration of streptozotocin (STZ). All analyses were performed 16 weeks after mice were administered STZ or a vehicle (Veh). Phosphorylation of ribosomal protein S6 at ser 240/244 was assayed in renal tissue homogenates by western blotting. Data are presented as means ± SD. Statistical significance was denoted as * $p < 0.05$ *vs.* Veh, # $p < 0.05$ *vs.*WT.

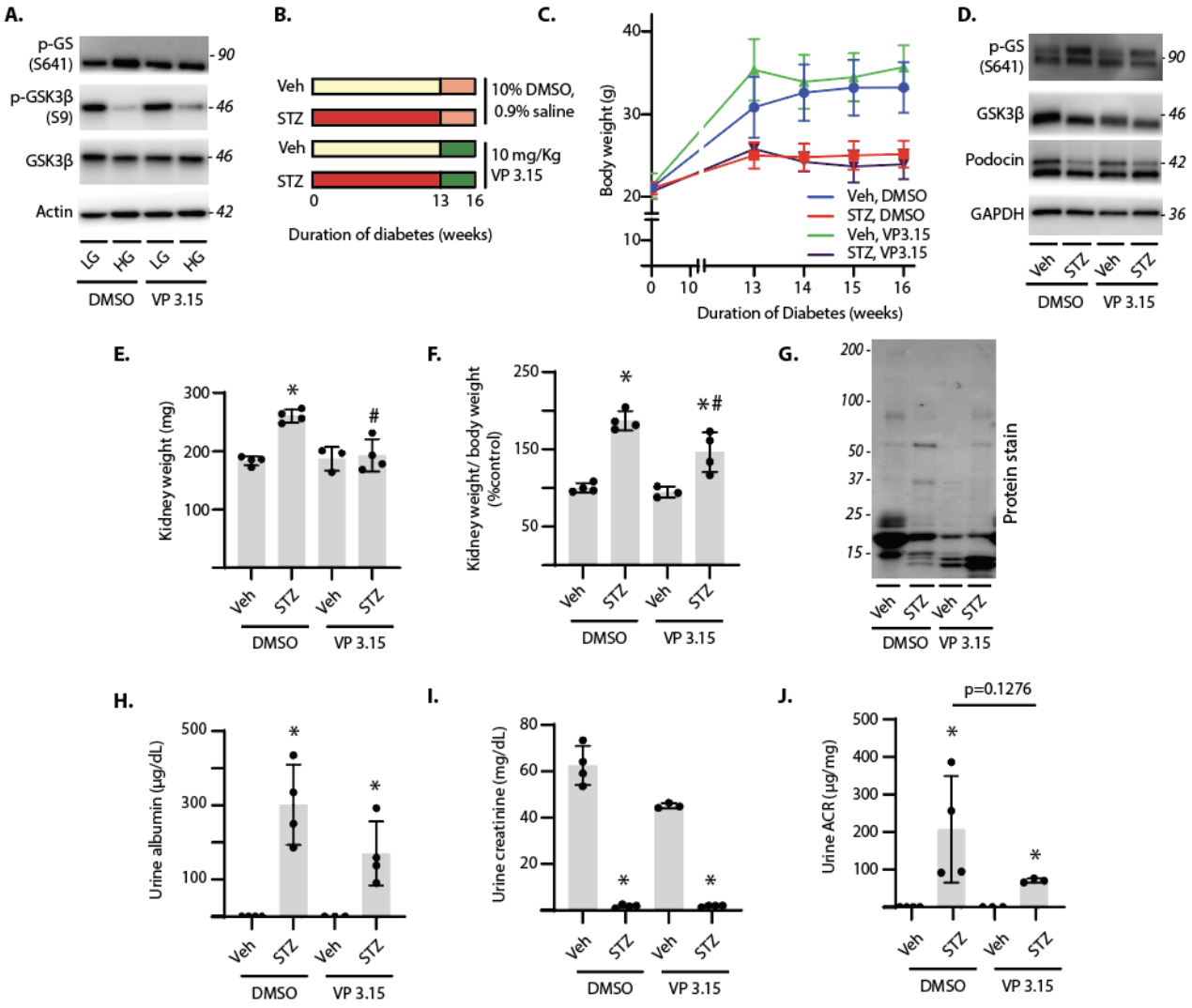

Figure S2: GSK3β inhibition attenuates diabetes-induced renal dysfunction. *A,* Conditionally immortalized human podocytes (CHIP) were exposed to hyperglycemic conditions in the presence or absence of 1 μM VP3.15. Glycogen Synthase (GS) phosphorylation at Ser641, Glycogen Synthase Kinase 3β (GSK3β) phosphorylation at Ser9, GSK3β, REDD1 and actin expression were evaluated by western blotting. Representative blots are shown with molecular mass indicated on the *right* of each blot. *B-J*, Mice were administered either streptozotocin (STZ) to induce diabetes or a vehicle (Veh) control. After 13 weeks of diabetes, mice were administered 10 mg/kg VP3.15 or DMSO vehicle alone daily for 3 weeks (B). Body weights of mice were compared during VP3.15 treatment (C). After 16 weeks of diabetes, GS phosphorylation, GSK3β, REDD1, podocin and GAPDH were determined in kidney tissue homogenates by western blotting (D). Kidney weights were compared (E*)*. Urinary protein was separated by SDS-PAGE and visualized by protein stain (*G*). Urine was assayed for albumin (H) and creatinine levels (I). Urine albumin to creatinine ratio (ACR) was determined (J). Data are presented as means ± SD. Statistical significance was denoted as * $p < 0.05$ *vs.* Veh; # $p < 0.05$ *vs*. DMSO.

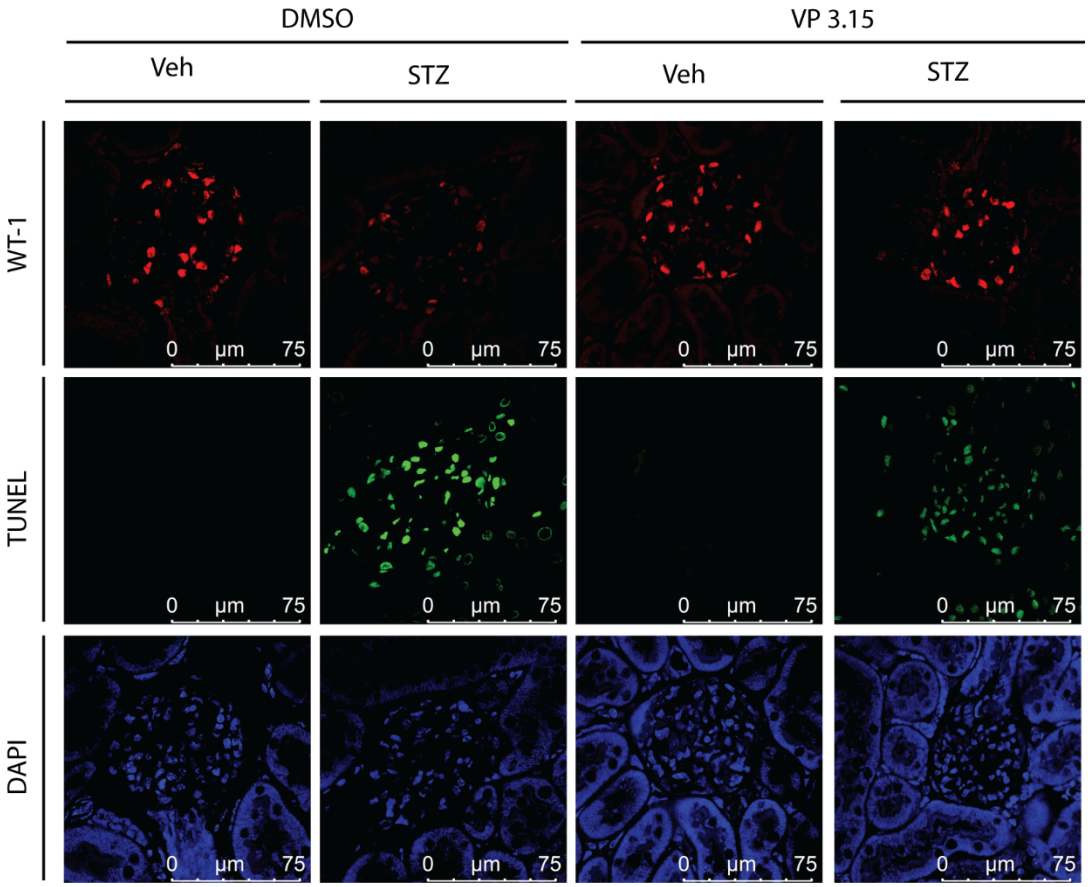

Figure S3: GSK3β inhibition prevents diabetes-induced podocyte loss. Diabetes was induced by administration of streptozotocin (STZ). Non-diabetic mice received a vehicle control (Veh). After 13 weeks of diabetes, mice received either 10 mg/Kg VP3.15 or DMSO vehicle alone daily for 3 weeks. After 16 weeks of diabetes formalin-fixed paraffin-embedded sections (6 µm) were prepared from kidneys. Immunohistochemistry was used to localize the podocyte marker WT1 (*red*) and TUNEL staining (*green*) was used to detect apoptotic nuclei. Sections were counterstained with DAPI (*blue*) and imaged using the Leica SP8 confocal laser microscope. Representative micrographs are shown (600 X magnification; scale bar 75 µm).