SUPPLEMENTARY DATA FILE

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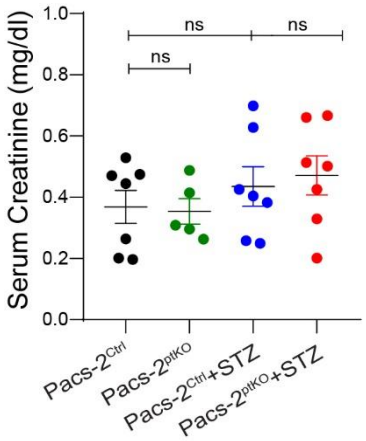
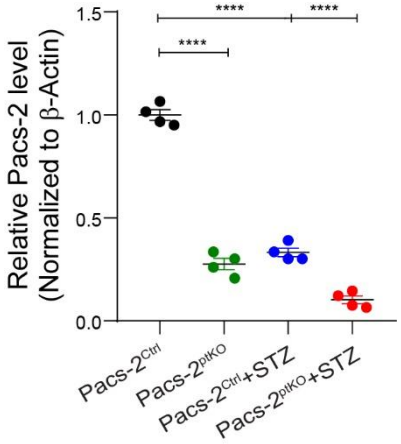
Supplementary Figure 1



Supplementary Figure 1 – PT-specific Pacs-2 deficiency did not affect serum creatinine. Serum creatinine of indicated groups of mice (n=5 or 7). Data are presented as means ± SEMs. ns, not significant.

Supplementary Figure 2



Supplementary Figure 2 – Pacs-2 mRNA levels in non-diabetic and diabetic Pacs-2^{Ctrl} mice and Pacs-2^{ptKO} mice. qPCR analysis of Pacs-2 in kidney cortices of indicated mice (n=4). Data are presented as means ± SEMs. ****$P < 0.0001$.

Supplementary table 1: Primer sequences used for used for experiment.

Primer			Sequence (5'-3')
P1	Mus musculus	F	CAGGGTCGGTGGAAACTCGTTT
P2	Mus musculus	R	GCCTCTATCTGTAGGCTTGTGGG
P3	Mus musculus	F	CGCTCTGCTCTTGCCACTTCCT
P4	Mus musculus	R	AGTCTACCCCGCATTTCCTCCC
Cre	Mus musculus	F	ACCTGAAGATGTTCGCGATTATCT
Cre	Mus musculus	R	ACCGTCAGTACGTGAGATATCTT
Pacs-2	Mus musculus	F	GAAAACCCAAGAAGCAGCGG
Pacs-2	Mus musculus	R	GCTCCGAATCCAAGACCTCC
β-actin	Mus musculus	F	CAAGCAGGAGTACGATGAGTC
β-actin	Mus musculus	R	AACGCAGCTCAGTAACAGTC

Supplementary Table 2. Antibodies used for experiment.

Antibodies		
Anti-megalin	Abcam	ab76969
Anti-PACS-2	Proteintech	19508-1-AP
Anti-Drp1	Proteintech	12957-1-AP
Anti-Fis1	Proteintech	10956-1-AP
Anti-Mfn1	Proteintech	13798-1-AP
Anti-Mfn2	Proteintech	12186-1-AP
Anti-LC3B	Cell Signaling Technology	3868s
Anti-TOM20	Proteintech	11802-1-AP
Anti-TIM23	Proteintech	11123-1-AP
Anti-BECN1/Beclin1	Proteintech	11306-1-AP
Anti-PINK1	Novus Biologicals	BC100-494PEP
Anti-Parkin	Cell Signaling Technology	2132s
Anti-p62	Cell Signaling Technology	5114s
Anti-COX4	Proteintech	11242-1-AP
Anti-Flag	Cell Signaling Technology	8146T
Anti-BCL2	Proteintech	12789-1-AP
Anti-BAX	Proteintech	60267-1-Ig
Anti-Cleaved-Caspase 3	Cell Signaling Technology	9661S
Anti-GAPDH	Proteintech	60004-1-Ig
Anti-β-ACTIN	Proteintech	60008-1-Ig
Anti-VDAC1	Proteintech	66345-1-Ig
Anti-IP3R1	Proteintech	19962-1-AP
Anti-PACS-2	Invitrogen	PA5-72866
Anti-LC3β	Santa Cruz Biotechnology	sc-271625
Anti-Calnexin	Proteintech	66903-1-Ig
Anti-Drp1	Abcam	ab184247
Anti-LC3B	Proteintech	18725-1-AP
Anti-BECN1/Beclin1	Proteintech	66665-1-Ig
Anti-Fibronectin	Abcam	ab2413

Supplementary Table 3. Clinical characteristics, PACS-2 expression levels and IFTA in control subjects and patients with DN. SCr, serum creatinine; yr, year; F, female; M, male; BUN, blood urea nitrogen; eGFR, estimated glomerular filtration Rate; FBG, fasting blood glucose; HbA1c, hemoglobin A1c; CHOL, total cholesterol; TG, triglycerides; IOD, integrated option density; IFTA: interstitial fibrosis and tubular atrophy; NA, not applicable/not available; Data are expressed as mean ± SD.

Control group (n=9)														
No	Age (yr)	Sex	SCr (mmol/L)	BUN (mmol/L)	Uric acid (mmol/L)	Proteinuria (g/24h)	eGFR (mL/min/ 1.73m^2)	FBG (mmol/L)	HbA1c (%)	HbA1c (mmol/mol)	CHOL (mmol/L)	TG (mmol/L)	PACS-2 expression (IOD)	IFTA (%)
1	29	M	88.0	5.8	325.0	0.16	117.47	NA	NA	NA	3.72	2.42	57.83	5
2	13	F	49.0	4.3	337.0	0.08	152.40	6.64	NA	NA	4.22	0.94	52.23	0
3	18	M	77.5	7.1	471.8	NA	105.86	5.50	NA	NA	9.52	4.00	54.63	0
4	16	F	48.3	4.3	140.0	NA	129.44	4.85	NA	NA	9.30	2.21	55.45	0
5	18	M	102.0	5.6	486.0	NA	78.73	5.25	5.1	32	15.39	1.75	47.46	5
6	23	F	53.0	4.8	310.4	0.20	121.51	5.10	NA	NA	2.89	0.70	30.95	5
7	48	F	55.5	3.9	186.9	0.02	112.83	5.38	NA	NA	4.20	2.01	54.02	5
8	47	F	61.5	5.8	318.9	0.06	114.57	4.97	4.8	29	4.55	2.11	39.98	5
9	58	M	80.9	6.3	522.9	1.92	88.47	NA	NA	NA	6.43	5.45	29.79	20
mean ± SD	30 ± 17		68.4 ± 19.3	5.3 ± 1.1	344.3 ± 130.8	0.41 ± 0.74	113.5 ± 21.6	5.38 ± 0.60	4.95 ± 0.21	30.50 ± 2.12	6.69 ± 4.04	2.40 ± 1.48	46.93 ± 10.76	5.0 ± 6.1
DN group (n=25)														
No	Age (yr)	Sex	SCr (mmol/L)	BUN (mmol/L)	Uric acid (mmol/L)	Proteinuria (g/24h)	eGFR (mL/min/ 1.73m^2)	FBG (mmol/L)	HbA1c (%)	HbA1c (mmol/mol)	CHOL (mmol/L)	TG (mmol/L)	PACS-2 expression (IOD)	IFTA (%)
1	63	F	72.1	6.7	418.6	18.70	81.63	NA	10.9	96	7.57	2.76	40.04	10
2	54	M	368.8	22.3	330.3	3.40	14.38	NA	5.7	39	NA	NA	10.08	70
3	53	M	72.5	5.2	332.8	0.30	112.81	NA	10.6	92	3.89	1.82	35.70	30
4	39	M	74.0	4.4	358.0	1.20	114.27	27.21	15.4	145	4.33	1.61	42.64	20
5	61	M	85.0	6.1	352.0	4.10	72.90	9.15	7.1	54	11.51	3.31	36.23	20
6	62	M	195.5	9.2	481.6	8.00	30.27	NA	NA	NA	3.04	0.57	15.86	60
7	68	M	176.0	7.5	590.8	2.40	35.78	11.59	9.0	75	5.68	1.95	34.50	40
8	58	M	193.0	9.5	348.0	NA	32.29	5.27	7.6	60	6.61	2.61	28.17	60
9	31	M	98.7	4.8	499.2	NA	87.25	4.47	NA	NA	3.80	7.37	15.07	30

10	57	M	181.0	10.7	453.0	NA	31.72	10.04	5.0	31	3.73	1.74	46.07	30
11	56	M	143.1	12.4	352.0	6.20	39.60	5.65	6.4	46	4.41	1.10	13.48	60
12	38	M	97.5	7.4	625.6	NA	87.44	NA	NA	NA	5.39	4.96	35.85	40
13	52	M	120.9	5.6	174.6	NA	57.00	5.87	8.0	64	5.04	1.20	15.82	70
14	52	M	247.8	11.8	372.2	NA	28.22	NA	6.8	51	2.79	3.00	9.82	80
15	53	M	100.6	10.8	341.8	0.93	72.65	7.71	6.1	43	6.01	0.99	13.10	10
16	43	F	96.4	7.4	383.2	5.88	54.80	8.63	6.9	52	4.85	2.18	24.91	60
17	52	M	177.4	11.6	427.6	2.47	34.64	NA	7.7	61	4.97	3.58	14.96	40
18	46	M	191.0	7.4	493.0	4.91	33.86	6.21	7.4	57	5.62	2.90	22.90	60
19	28	M	77.2	5.8	544.7	NA	136.80	4.27	5.8	40	4.39	1.42	38.06	5
20	58	M	132.0	9.7	395.0	5.12	48.30	NA	6.5	48	4.49	2.61	27.70	30
21	57	F	105.0	8.7	324.0	6.60	50.04	NA	6.4	46	5.85	1.82	26.69	40
22	49	F	179.0	4.9	383.0	NA	29.70	15.92	6.0	42	6.56	1.82	32.35	70
23	62	M	70.0	3.8	467.0	0.11	89.93	4.56	6.5	48	3.87	0.99	18.41	20
24	52	M	145.0	7.8	356.0	2.38	46.63	5.81	8.7	72	4.49	1.76	25.26	30
25	47	F	102.0	7.2	426.0	NA	44.88	7.73	8.6	70	3.34	2.2	20.24	50
mean ± SD	51 ± 10		140.1 ± 68.9	8.3 ± 3.8	409.2 ± 96.2	4.54 ± 4.46	58.71 ± 31.78	8.76 ± 5.80	7.69 ± 2.29	60.55 ± 25.06	5.10 ± 1.81	2.35 ± 1.45	25.76 ± 10.85	41.4 ± 21.5