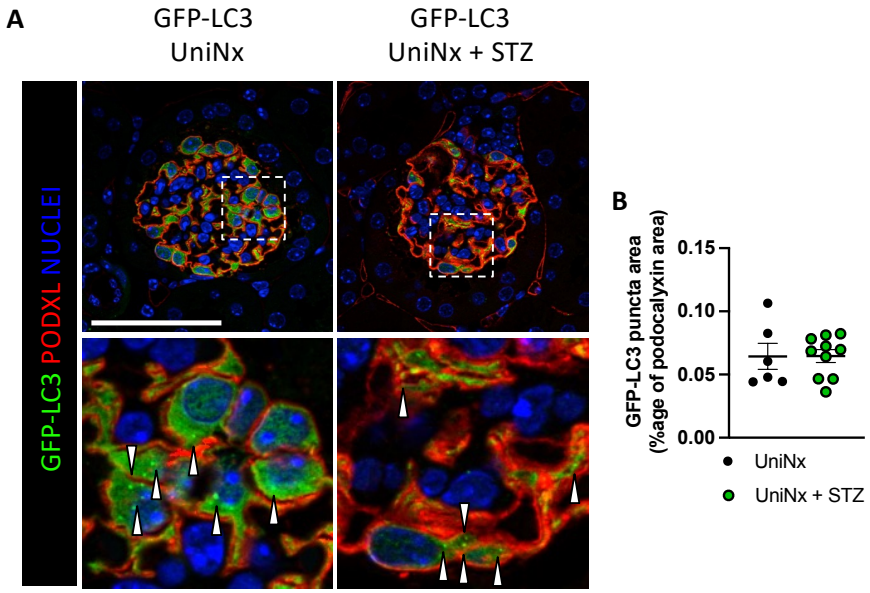
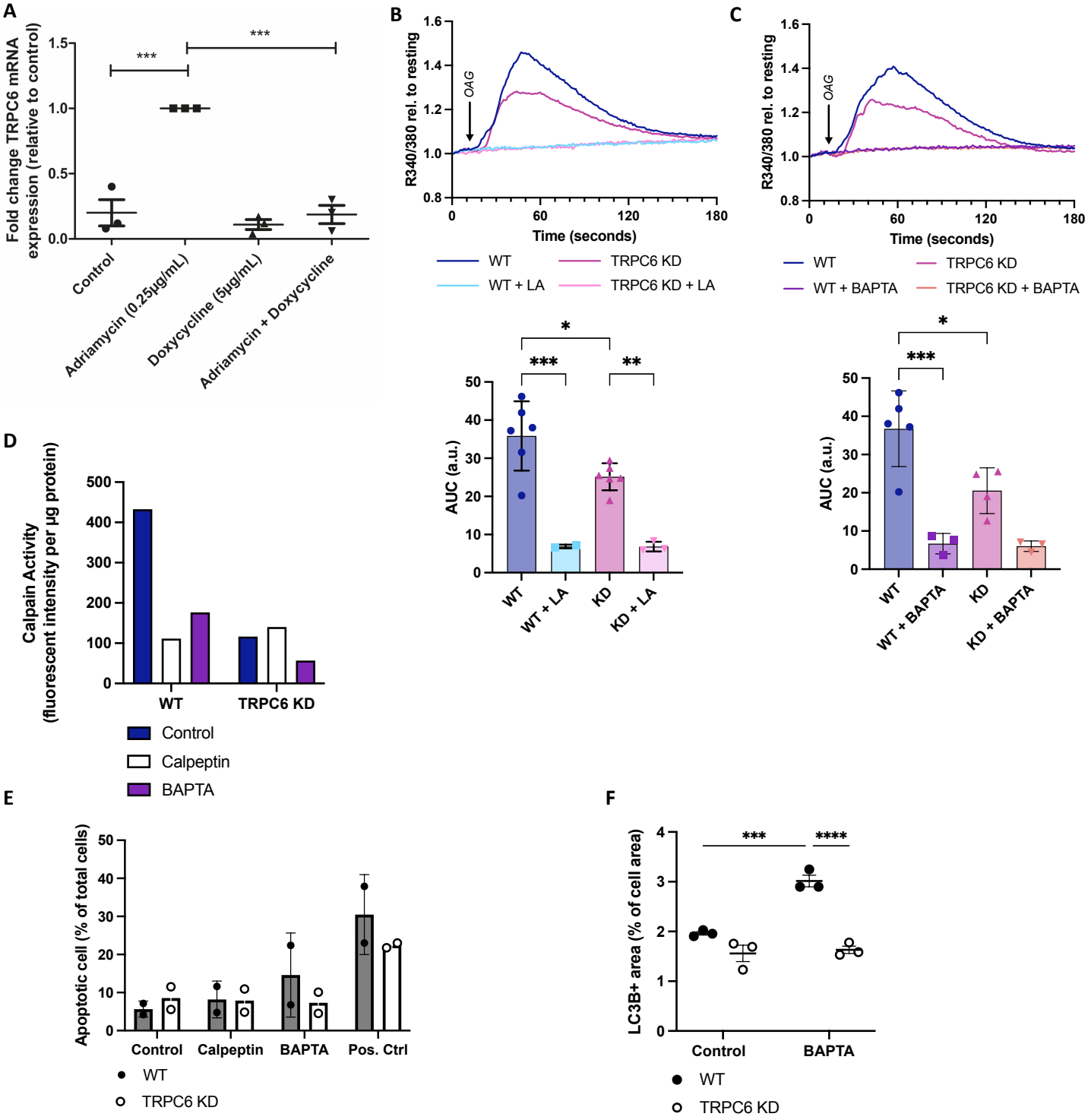
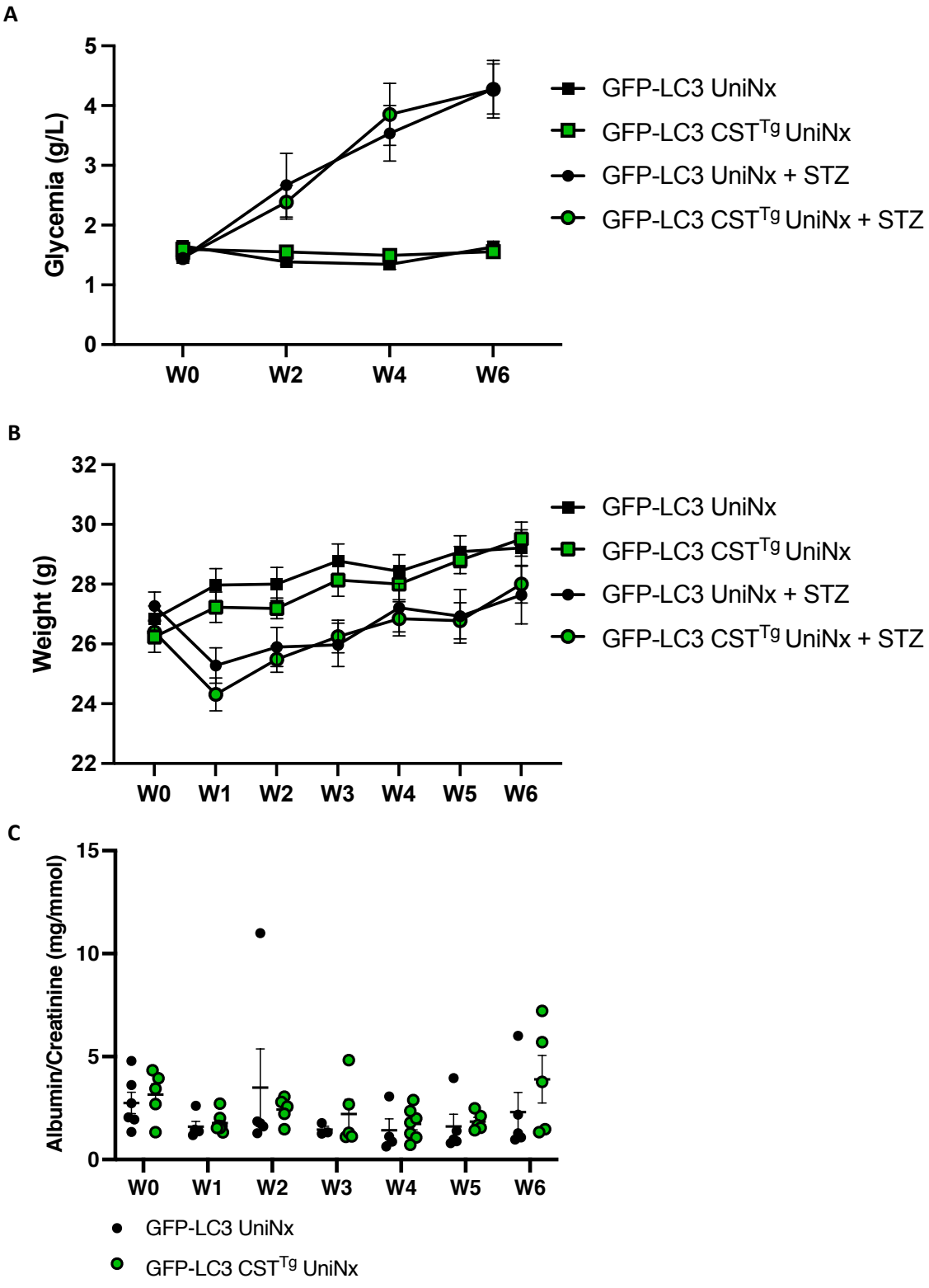
List of Supplemental Materials



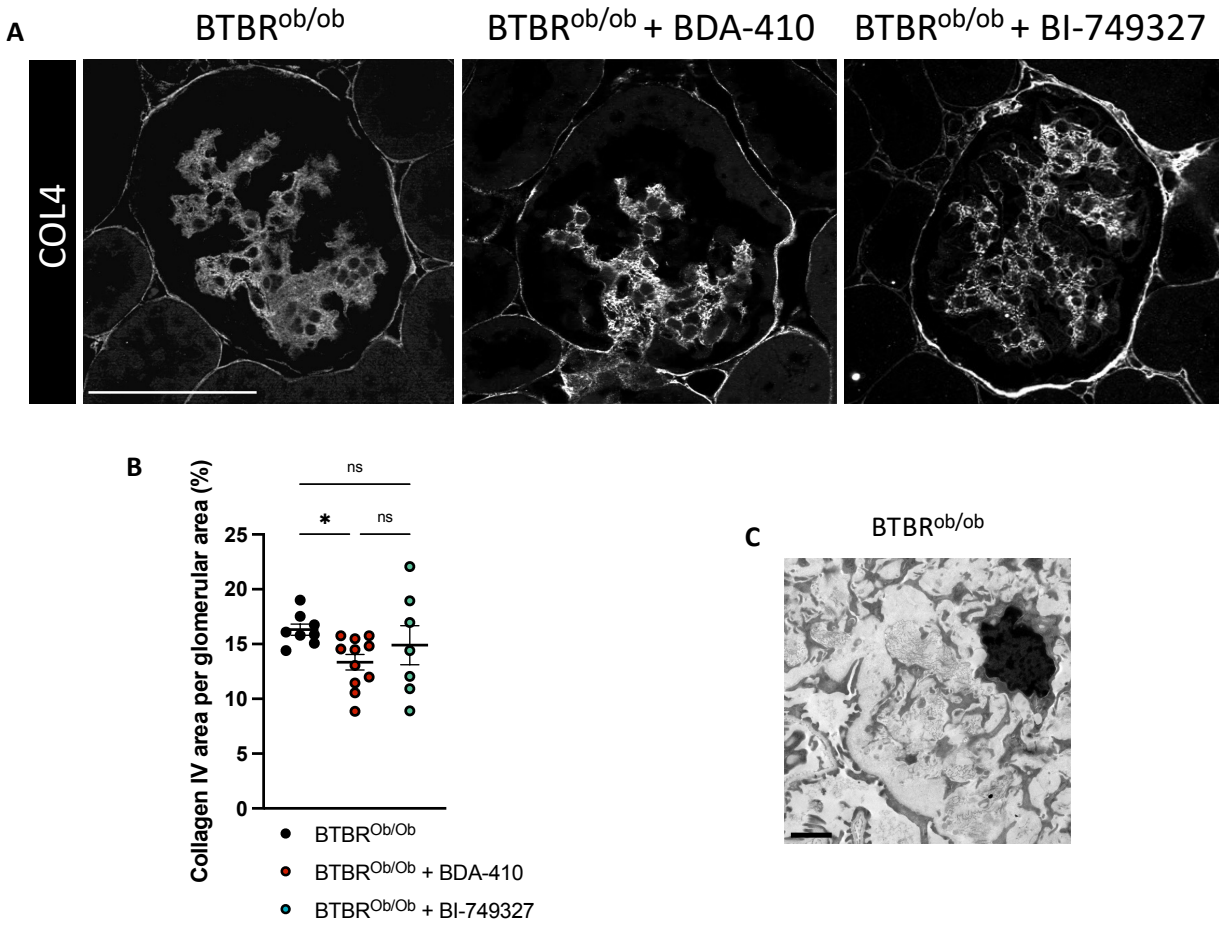
Supplementary Figure 1: Autophagic flux assessment in type diabetes mellitus in mice. A. Representative immunofluorescence of GFP-LC3 (green) and PODXL (red) expression in glomerulus from GFP-LC3 UniNx mice and GFP-LC3 UniNx +STZ mice. Nuclei are stained in blue with Hoechst. Scale bar: 50µm **B.** Quantification of GFP-LC3 punctiform area within PODXL area in type I diabetes model. n = 6 GFP-LC3 UniNx mice, n = 10 GFP-LC3 UniNx + STZ. Values are presented as individual plots and mean ± SEM. Unpaired *t* test: NS, p>0.05.



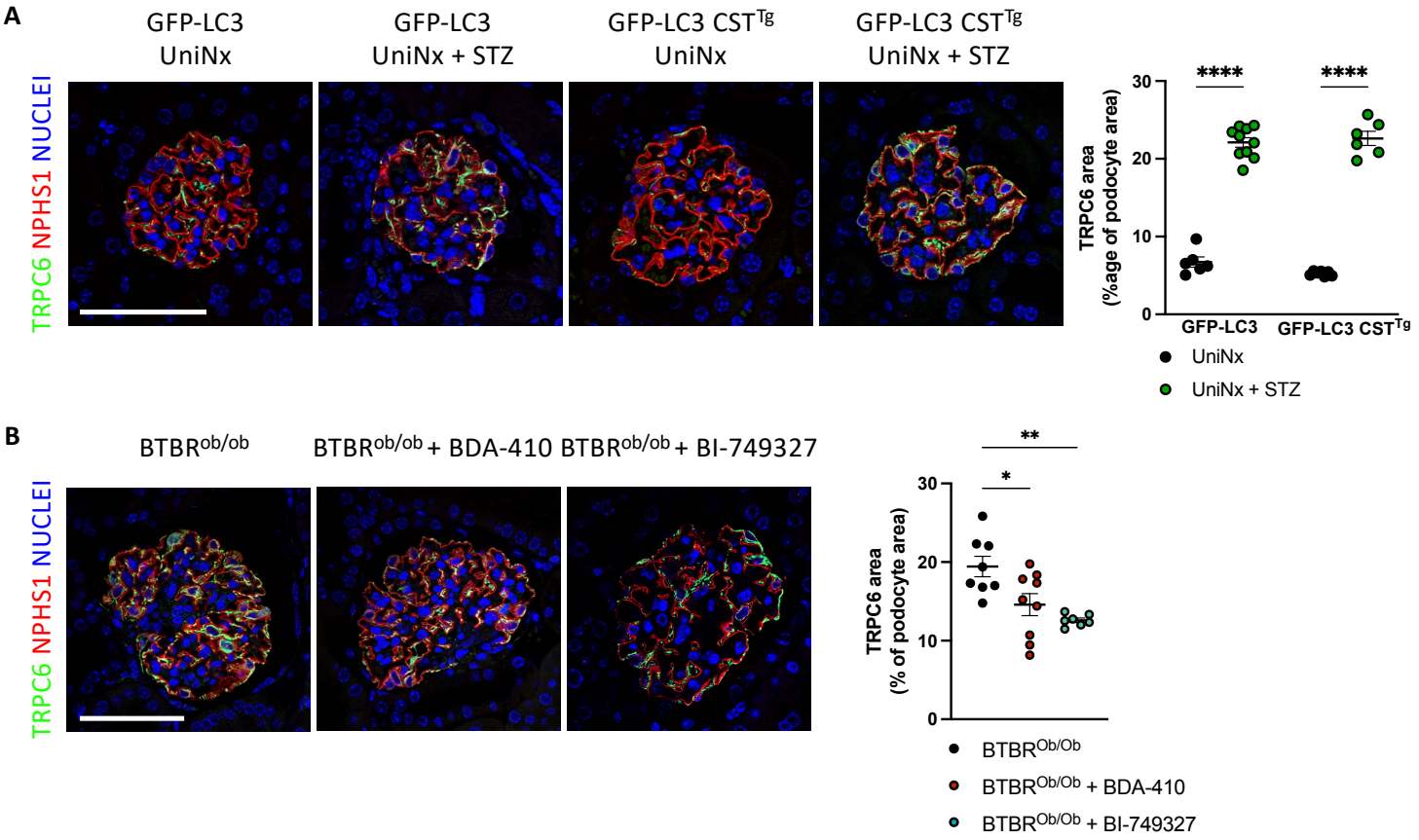
Supplementary Figure 2: Validation of TRPC6 KD construction in MPC-5 podocytes. (A) Fold change in *Trpc6* mRNA expression in MPC-5 podocytes expressing a tetracyclin-inducible TRPC6 shRNA construct. Adriamycin treatment induced *Trpc6* mRNA expression in podocytes not treated with doxycycline, whereas doxycycline-treated podocytes exhibit lower *Trpc6* mRNA expression with or without Adriamycin treatment. (B) OAG-mediated Ca2+ response in WT and TRPC6 KD podocytes. Cells were treated or not with larixyl acetate (LA) as a positive control of TRPC6 blockade. One-way ANOVA: P<0.0001. Tukey Post hoc test: WT vs. WT + LA, p=0.0003; WT vs. TRPC6 KD, p= 0.02; TRPC6 KD vs. TRPC6 KD + LA, p=0.0045. (C) OAG-mediated Ca2+ response in WT podocytes treated or not with the Ca2+ chelator BAPTA. One-way ANOVA: P=0.0002. Tukey Post hoc test: WT vs. WT + BAPTA, p=0.0004; WT vs. TRPC6 KD, p= 0.02. (D) Calpain activity in WT podocytes treated or not with BAPTA or calpeptin. (E) Flow cytometry measurement of apoptosis in WT and TRPC6 KD podocytes after treatment with calpeptin or BAPTA. Staurosporin was used as a positive control of apoptosis. (F) Quantification of the LC3B+ puncta area in WT and TRPC6 KD podocytes treated or not with BAPTA. 2-way ANOVA: BAPTA effect, p=0.0009; TRPC6 KD effect, p<0.0001. Post hoc Tukey test: WT vs. WT BAPTA, p=0.0006; WT BAPTA vs. TRPC6 BAPTA, p<0.0001



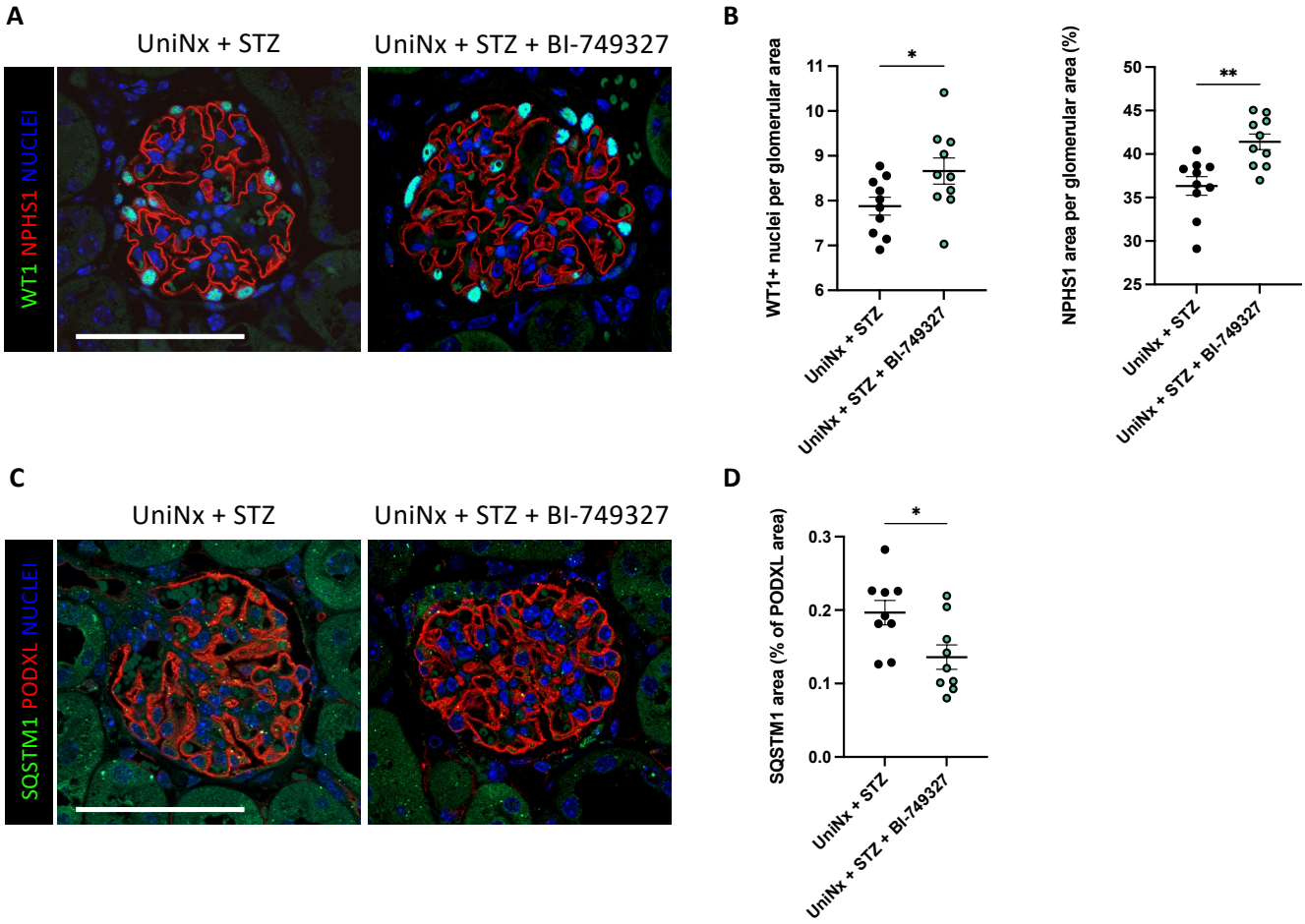
Supplementary Figure 3: Biological parameters in mice with calpastatin overexpression. A. Fed glycemia in GFP-LC3 UniNx and GFP-LC3 CST^{Tg} UniNx after being injected or not with STZ. Values are presented as mean ± SEM. **B.** Body mass of GFP-LC3 UniNx and GFP-LC3 CST^{Tg} UniNx after being injected or not with STZ. Values are presented as mean ± SEM. **C.** Evolution of the albumin-to-creatinine ratio in GFP-LC3 UniNx and GFP-LC3 CST^{Tg} UniNx mice. Values are presented as individual plots and mean ± SEM.



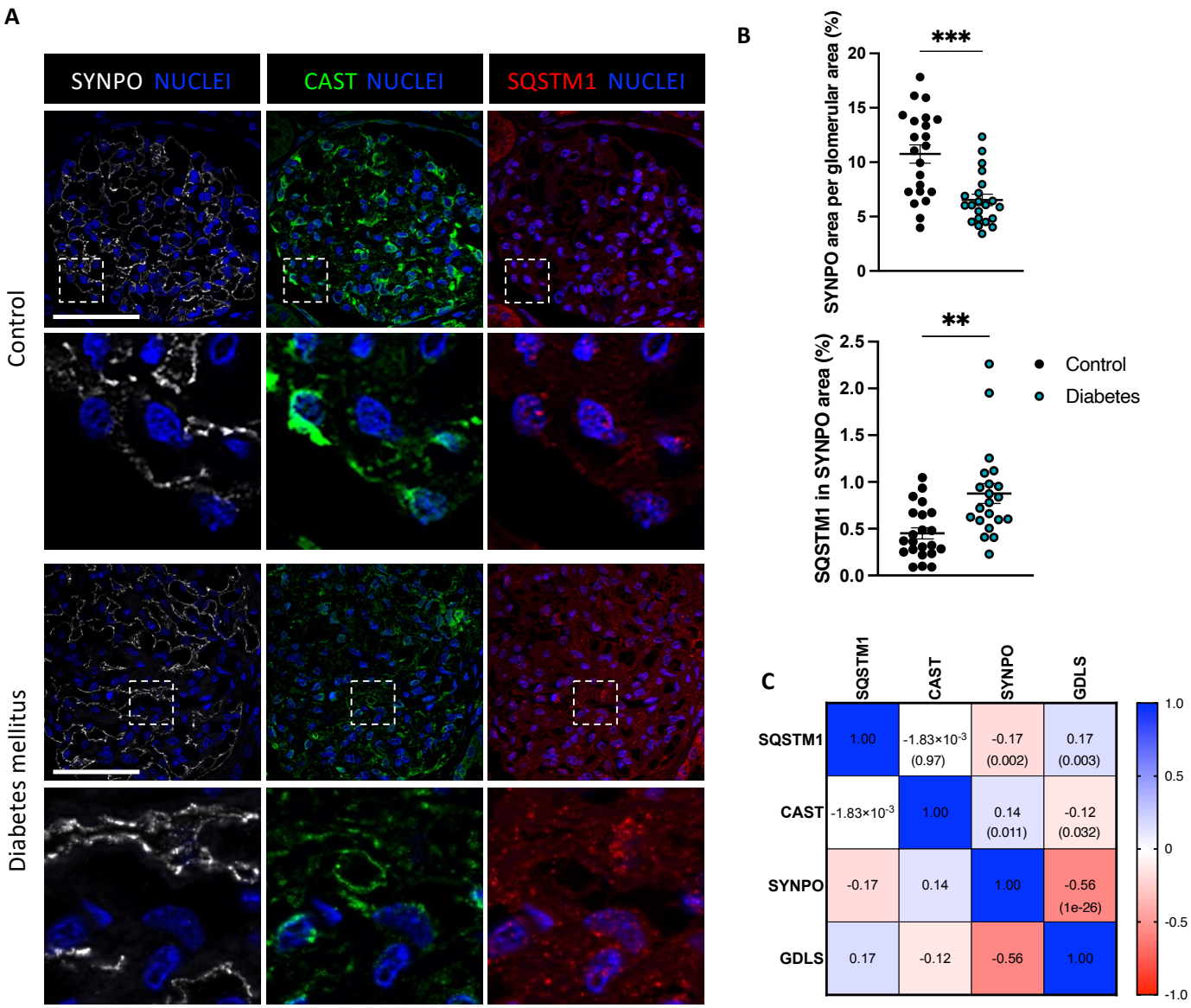
Supplementary Figure 4: Pharmacological calpain-1 inhibition with BDA-410 or TRPC6 inhibition with BI-749327, prevent glomerulosclerosis in type II diabetes model. A. Representative immunofluorescence of Collagen 4 expression in glomeruli from $BTBR^{ob/ob}$ mice treated or not with BDA-410 or BI-749327 for 6 weeks. **B**. Associated quantification. n =8 $BTBR^{ob/ob}$, n =11 $BTBR^{ob/ob}$ + BDA-410, n=7 BI-749327. Values are presented as individual plots and mean ± SEM. One-way ANOVA: p=0.1712. Fisher LSD post hoc test: $BTBR^{ob/ob}$ vs. $BTBR^{ob/ob}$ BDA-410, p= 0.04 **C**. Transmission electron microscopy images of mesangial cells showing fibrosis in $BTBR^{ob/ob}$ mice. Scale bar: 2µm.



Supplementary Figure 5: TRPC6 expression in diabetes models. A. Representative immunofluorescence and associated quantification of TRPC6 (Green) and NPHS1 (red) expression in glomeruli from uni-nephrectomized (UniNx) GFP-LC3 and GFP-LC3 CST^{Tg} mice treated or not with streptozotocin (+STZ). Nuclei are stained in blue with Hoechst. Scale bar: 50µm. Values are presented as individual plots and mean ± SEM. Two-way analysis of variance: Genotype, p=0.5; Treatment, p<0.0001. Tukey's test: p<0.0001 for GFP-LC3 UniNx vs. GFP-LC3 UniNx + STZ and GFP-LC3 CSTTg UniNx vs. GFP-LC3 CSTTg UniNx + STZ. **B.** Representative immunofluorescence and associated quantification of TRPC6 (Green) and NPHS1 (red) expression in glomeruli from BTBR$^{ob/ob}$ mice treated or not with BDA-410 or BI-749327 for 6 weeks. Nuclei are stained in blue with Hoechst. Scale bar: 50µm. Values are presented as individual plots and mean ± SEM. One-way analysis of variance: **, p=0.002. Fisher LSD test: *, p=0.019 for BTBR$^{ob/ob}$ vs. BTBR$^{ob/ob}$ + BDA-410 and **, p=0.002 for BTBR$^{ob/ob}$ vs. BTBR$^{ob/ob}$ + BI-749327.

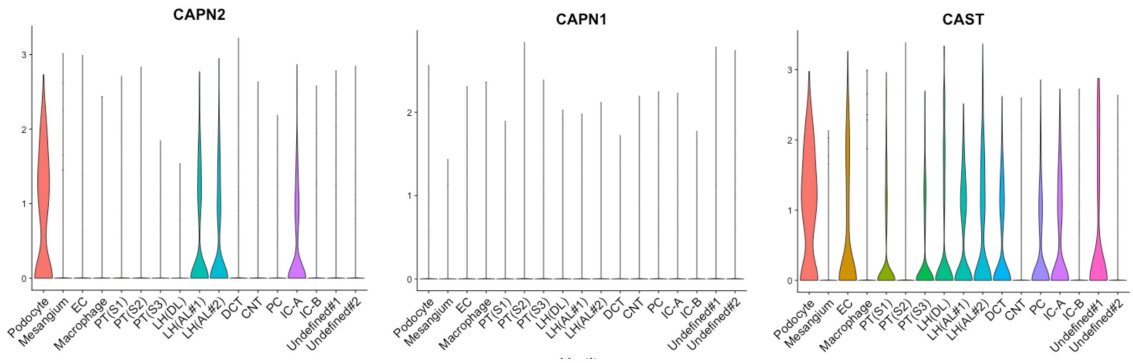


Supplementary figure 6 : Pharmacological TRPC6 inhibition with BI-749327 prevents podocyte injury and restores glomerular autophagic flux in type I diabetes model. A. Representative immunofluorescence of WT1 (Green) and NPHS1 (Red) expression in glomeruli from C57B6J mice UniNx 6 weeks after being injected with STZ and treated or not with BI-749327. Nuclei are stained in blue with Hoechst. **B.** Quantification of the number of WT1+ nuclei and NPHS1 area per glomerular area. n = 10 UniNx + STZ, n = 10 UniNx + STZ + BI-749327. Values are represented as individual plots and mean ± SEM. Unpaired t test: For WT1 *p=0.0401, for NPHS1 **p=0.0019. **C.** Representative immunofluorescence of SQSTM1 (Green) and PODXL (Red) expression in glomeruli from C57B6J mice UniNx after being injected with STZ and treated or not with BI-749327. Nuclei are stained in blue with Hoechst. **D.** Quantification of SQSTM1 area in PODXL area n = 10 UniNx + STZ, n = 10 UniNx + STZ + BI-749327. Values are represented as individual plots and mean ± SEM. Unpaired t test: For SQSTM1 *p=0.0202.



Supplementary Figure 7: CAST expression, autophagic flux assessment and podocyte injury in patients with diabetic kidney disease. A. Representative immunofluorescence of SYNPO (white), CAST (green), and SQSTM1 (red) expression in glomerulus from control and diabetic patients. Nuclei are stained in blue with Hoechst. Scale bar: 50µm **B.** Quantifications of SYNPO and SQSTM1 expressions. Values are presented as individual plots of each patient and mean ± SEM. ** p<0.01, *** p<0.001. **C.** Correlation matrix of the glomerular areas of SQSTM1, CAST, SYNPO and the glomerular diabetic lesion score (GDLS) in patients by individual glomerular staining. Numbers in squares represent: Pearson's r (p value).

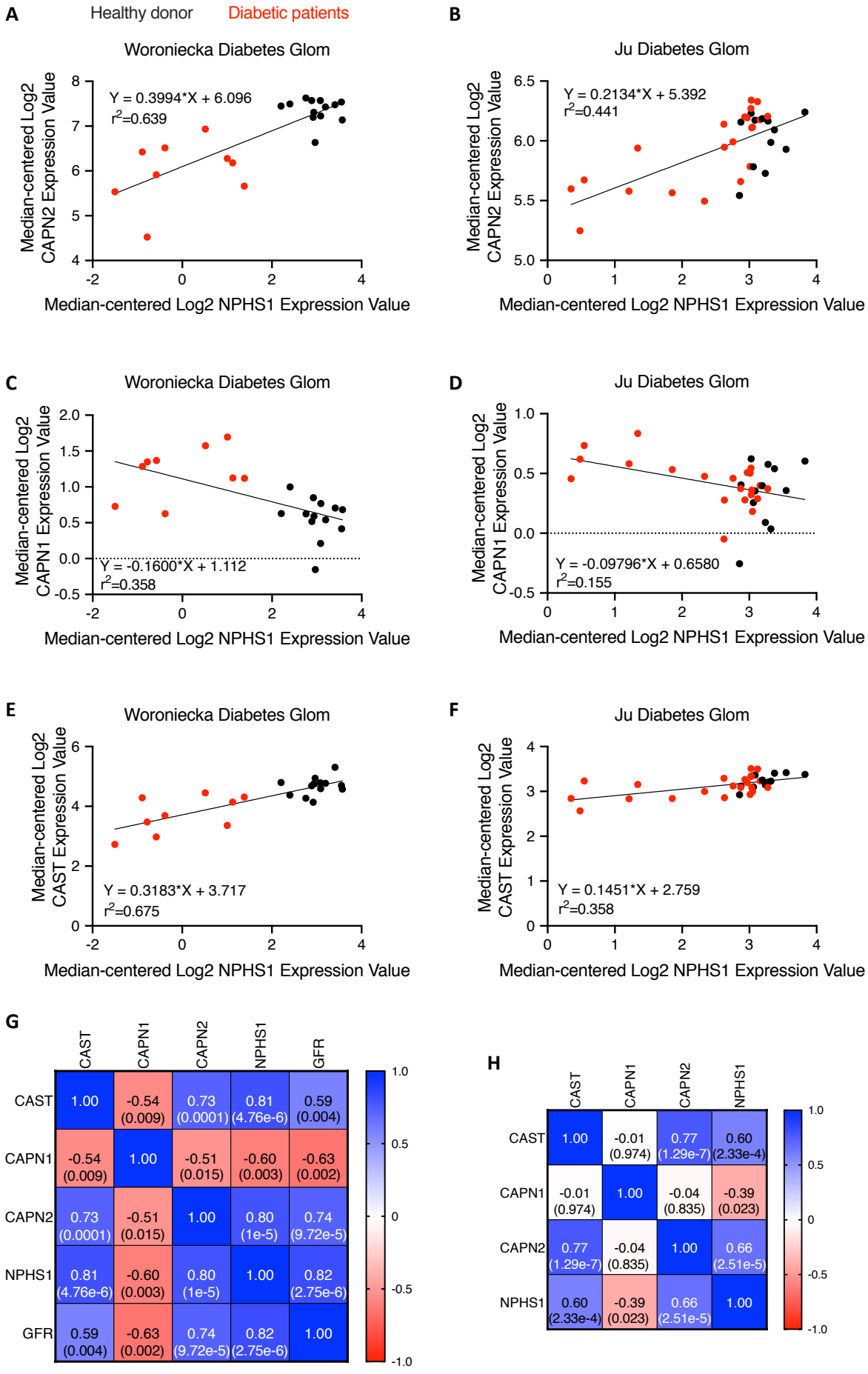
A



B

RNAseq	cluster name	cell count in cluster	median expression	cells expressing (%)	fold change	p value	adjust p value	gene
scRNAseq	Podocyte	118	5.13	72.9	2.61	0	0	NPHS1
			0.186	19%	0.53	5.90e-52	NS	TRPC6
			0.831	83%	1.74	8.33e-43	NS	CAPN2
			0.203	NS	NS	NS	NS	CAPN1
			0.661	66,1%	1.02	8.58e-18	NS	CAST
snRNAseq	Podocyte	1028	19.3	94.2%	4.19	0	0	NPHS1
			2.09	47.8%	1.46	9.79e-192	2.91e-187	TRPC6
			3.39	73.0%	1.18	4.80e-141	1.43e-136	CAPN2
			0.232	NS	NS	NS	NS	CAPN1
			2.87	66.9%	0.474	2.20e-36	6.55e-32	CAST

Supplementary Figure 8: Single cell RNAseq database. A. Expression of CAPN2, CAPN1 and CAST in single cell RNAseq from the Kidney Interactive Transcriptomics public database. **B.** Expression of NPHS1, TRPC6, CAPN2, CAPN1 and CAST in single cell and single nuclei RNAseq from the KPMP public database.



Supplementary Figure 9: Nephroseq transcriptomic database. Expression of CAPN2 (**A-B**), CAPN1 (**C-D**) and CAST (**E-F**) in glomeruli from the Woroniecka Diabetes Glom (**A, C, E**) and the Ju diabetes Glom (**B, D, F**) database. **G-H.** Correlation matrix of the expression of CAST, CAPN1, CAPN2, NPHS1 (and the GFR for **G**) in the Woroniecka Diabetes Glom (**G**) and the Ju diabetes Glom (**H**) database.

Gender	Age (years)	Creat (umol/L)	UPCR (g/mmol)	UACR (mg/mmol)	eGFR (mL/min)	Patients	Patient group	Diabetes	ID
F	48	90	0.012	2.4	59	Renal Transplant Routine Biopsy (RTRB)	Control		C1
M	63	148	1.737	1116	31	Diabetic Kidney Disease (DKD)	DKD	T2 DM	D1
M	48	166	0.06	12	39	RTRB	Control		C2
F	32	91	0.13	6.1	62	RTRB	Control		C3
M	61	326	0.039		17	DKD	DKD	T2 DM	D2
M	49	70	0.058	46.5	103	DKD	DKD	T2 DM	D3
M	65	141	0.017	3	44	RTRB	Control		C4
F	56	446	1.81	928	9	DKD	DKD	T2 DM	D4
M	64	115	0.21	38.5	56	RTRB	Control	Diabetes	C5
M	77	147	0.021	8	40	RTRB	Control		C6
F	46	80	0.004	1	76	RTRB	Control		C7
M	70	357	0.688	444	15	DKD	DKD	T2 DM	D5
M	65	119	0.67	584	53	DKD	DKD	T2 DM	D6
F	42	90	0.013	1.5	85	RTRB	Control		C8
M	77	339	0.451	364.2	15	DKD	DKD	T2 DM	D7
F	46	104	0.011	0.9	49	RTRB	Control		C9
M	67	77	0.39	44	88	DKD	DKD	T2 DM	D8
M	61	846	0.772	545	6	DKD	DKD	T2 DM	D9
M	71	870	0.4	243	5	DKD	DKD	T2 DM	D10
M	60	150	0.036	6.8	40	RTRB	Control		C10
M	47	146	1.25	750	45	DKD	DKD	T2 DM	D11
F	39	96	0.043	0	56	RTRB	Control		C11
M	64	113	0.015	0	57	RTRB	Control		C12
F	34	200	0.03	11.2	27	DKD	DKD	T1 DM	D12
F	30	76	0.012	0.5	78	RTRB	Control		C13
F	60	93	0.019	3.9	53	RTRB	Control		C14
F	61	60	0.011	0	95	RTRB	Control		C15
F	69	147	0.141	69	31	DKD	DKD	T2 DM	D13
M	56	100	0.043	0	67	RTRB	Control		C16
M	38	453	0.931	578.7	17	DKD	DKD	T1 DM	D14
F	20	82	0.008	3	89	RTRB	Control		C17
F	55	126	0.034	4.7	38	RTRB	Control	Diabetes	C18
F	40	161	0.012	1	43	RTRB	Control		C19
M	69	300	0.027	18.3	18	DKD	DKD	T2 DM	D15
M	55	190	0.974	717	33	DKD	DKD	T2 DM	D16
M	31	97	0.013	0	89	RTRB	Control		C20
M	72	304	0.733	543.8	18	DKD	DKD	T2 DM	D17
F	50	395	0.503	315.9	14	DKD	DKD	T2 DM	D18
M	64	312	0.023	13.3	18	DKD	DKD	T2 DM	D19
F	68	164	0.248	169.5	27	DKD	DKD	T2 DM	D20
F	70	519	0.672	379	7	DKD	DKD	T2 DM	D21
F	23	92	0.011	2.9	66	RTRB	Control		C21
F	43	61	0.101	54.2	93	Uncharacterized proteinuria	PROT		P2
M	57	216	0.036	22	27	Uncharacterized proteinuria	PROT		P3
F	73	115	0.008	2	41	Uncharacterized proteinuria	PROT		P4
	53	104	0.41	291	65	DKD	DKD	T2 DM	D22
M	64	132	0.119	101.3	47	Membranous Nephropathy (MN)	PROT		P5
F		55	0.368	280	97	MN	PROT		P6
F		95	0.7	595	55	MN	PROT		P7
M	51	178	0.107	82.6	35	MN	PROT		P8
M	46	488	0.772	509.2	11	MN	PROT		P9
	69	215	0.709	590	26	MN	PROT		P10
M	30	74	0.331	323	108	MN	PROT		P11
M	52	73	0.45	420	98	MN	PROT		P12
F	31	66	0.116	94.4	91	MN	PROT		P13
F	49	88	0.153	0.11	59	MN	PROT		P14
F		78	0.412	335	75	Minimal Change Disease (MCD)	PROT		P15
M	50	138	1.018	727.6	47	MCD	PROT		P16
M	43	94	0.44	295	81	MCD	PROT		P17
M	54	83	0.804	226	66	MCD	PROT		P18
F	22	52	0.7	700	120	MCD	PROT		P19
M	26	130	1.01	802	58	MCD	PROT		P20
M	29	74	0.989	728	119	MCD	PROT		P21
M	38	172	0.613	1400	38	MCD	PROT		P22

Supp Table 1: Patients characteristics

Descriptives

	disease	Age (years)	BMI (kg/m2)	UACR (mg/mmol)	eGFR (mL/min)
N	Control	21	19	21	21
	DKD	22	20	21	22
	PROT	21	20	21	21
Mean	Control	48.0	24.2	4.58	61.0
	DKD	60.4	28.1	389	30.1
	PROT	47.3	26.5	400	66.3
Std. error mean	Control	3.34	0.934	1.83	3.98
	DKD	2.43	0.930	69.5	5.57
	PROT	3.12	0.888	75.7	7.00

Supp Table 2: Patients descriptive statistics

Descriptives

	disease	SQSTM1	TRPC6	SYNPO	CAST
N	Control	19	19	19	21
	DKD	21	21	21	21
	PROT	21	21	21	21
Mean	Control	0.522	3.51	14.9	4.87
	DKD	1.17	6.82	7.12	4.02
	PROT	0.713	5.57	10.3	5.68
Std. error mean	Control	0.0853	0.399	1.01	0.404
	DKD	0.0914	0.662	0.845	0.363
	PROT	0.0330	0.275	0.618	0.399

Supp Table 3: Stainings descriptive statistics. DKD: diabetic kidney disease, PROT: proteinuric disease

Kruskal-Wallis

	χ^2	df	p	ε^2
Age (years)	8.5937	1	0.003	0.2261
BMI (kg/m2)	0.8359	1	0.361	0.0232
UACR (mg/mmol)	0.0235	1	0.878	6.53e-4
eGFR (mL/min)	10.4373	1	0.001	0.2747
TRPC6	1.4885	1	0.222	0.0363
SYNPO	8.1520	1	0.004	0.1988
SQSTM1	13.3059	1	<.001	0.3245
CAST	6.6485	1	0.010	0.1622

Supp Table 4: Analyses of variances for DKD and proteinuric patients. Non parametric Kruskal-Wallis analyses of variances. BMI: body mass index, UACR: urinary-to-creatinine ratio, eGFR: estimated glomerular filtration rate.

Model Fit Measures

Model	Deviance	BIC	R^2_N
1	48.5	55.9	0.246
2	42.5	53.6	0.393
3	32.1	46.9	0.605
4	30.0	48.5	0.641

Supp Table 5: Binomial logistic regression. Comparison of models. Model 1: dependent variable= SYNPO, Model 2: dependent variables= SYNPO + CAST, Model 3: dependent variables= SYNPO + CAST + SQSTM1, Model 4: dependent variables= SYNPO + CAST + SQSTM1 + TRPC6 . BIC: Bayesian Information Criterion, R^2_N Nagelkerke's R^2.

Comparison					
Model		Model	χ^2	df	p
1	-	2	5.96	1	0.015
2	-	3	10.45	1	0.001
3	-	4	2.08	1	0.149

Supp Table 6: Model comparison.

Predictor	Estimate	SE	Z	p
Intercept	1.976	3.001	0.658	0.510
SYNPO	0.211	0.146	1.443	0.149
CAST	0.580	0.330	1.755	0.079
SQSTM1	-4.751	2.161	-2.199	0.028
TRPC6	-0.418	0.324	-1.288	0.198

Supp Table 7: Model coefficients. Estimate represents the log odds of disease = PROT vs. Disease = DKD

	Predicted		
Observed	DKD	PROT	% Correct
DKD	17	3	85.0
PROT	4	17	81.0

Supp Table 8: Prediction with Model 4. Cut-off value =0.6