

Supplementary Information

GRP78 contributes to the beneficial effects of SGLT2 inhibitor on proximal tubular cells in DKD

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Supplementary Table 1

Primary antibody for western blotting	catalog No. RRID	company	dilution	KO/KD validated
Albumin Rabbit Polyclonal antibody	16475-1-AP AB_2168001	Proteintech	1:5000	no
GRP78/BIP Polyclonal antibody	11587-1-AP AB_2119855	Proteintech	1:1000	Yes
Anti-GRP78 BiP antibody [EPR4040(2)]	ab108613 AB_10859806	abcam	1:1000	no
rat monoclonal anti-GRP78 (76-E6)	sc-13539 AB_627698	Santa Cruz Biotechnology	1:200	no
anti-Calnexin antibody	C4731 AB_476845	SIGMA	1:2000	no
SGLT1 antibody [N3C3]	GTX105367 AB_10624670	Gene Tex	1:1000	no
SGLT2 Rabbit Polyclonal antibody	24654-1-AP AB_2750601	Proteintech	1:1000	Yes
Monoclonal Anti-Golgi 58K Protein	G2404 AB_477002	SIGMA	1:1000	no
anti-LAMP1 antibody (rabbit polyclonal)	ab24170 AB_775978	abcam	1:1000	no
anti-LAMP2 antibody (H4B4) (mouse monoclonal)	ab25631 AB_470709	abcam	1:500	no
Tom20 (D8T4N) Rabbit mAb	#42406 AB_2687663	CST	1:2000	no
VDAC1 (D73D12) Rabbit mAb	#4661 AB_10557420	CST	1:1000	no
anti-GAPDH (14C10)	#2118 AB_561053	CST	1:5000	no
anti-αTubulin (DM1A)	#3873 AB_1904178	CST	1:5000	no
E-Cadherin (24E10) Rabbit mAb	#3195 AB_2291471	CST	1:1000	no
E-cadherin Rabbit Polyclonal antibody	20874-1-AP	Proteintech	1:5000	Yes

	AB_10697811			
N-cadherin Rabbit Polyclonal antibody	22018-1-AP AB_2813891	Proteintech	1:2000	Yes
Phospho-AMPKα (Thr172)(40H9) Rabbit mAb	#2535 AB_331250	CST	1:1000	no
AMPKα Antibody	#2532 AB_330331	CST	1:1000	Yes (KD)
Phospho-eIF2α (Ser51) (D9G8) XP Rabbit mAb	#3398 AB_2096481	CST	1:1000	no
rabbit polyclonal anti-eIF2α	#9722 AB_2230924	CST	1:1000	no
ATF4 (D4B8) Rabbit mAb	#11815 AB_2616025	CST	1:1000	no
CHOP (L63F7) Mouse mAb	#2895 AB_2089254	CST	1:1000	no
anti-Bax	#2772 AB_10695870	CST	1:1000	Yes (KO)
smooth muscle actin Polyclonal antibody	14395-1-AP AB_2223009	Proteintech	1:1000	Yes
Phospho-NF-κBp65(Ser536) (93H1) Rabbit mAb	#3033 AB_331284	CST	1:10000	no
NF-κBp65 (D14E12) XP Rabbit mAb	#8242 AB_10859369	CST	1:1000	Yes (KO)
Phospho-Akt (Ser473) (D9E) XP Rabbit mAb	#4060 AB_2315049	CST	1:1000	no
Akt Antibody	#9272 AB_329827	CST	1:1000	Yes (KD)
Integrin beta 1/CD29 antibody	GTX128839 AB_2885823	Gene Tex	1:5000	Yes
mouse monoclonal anti-FLAG M2	F1804 AB_262044	SIGMA	1:1000	

Knockout (KO) and Knockdown (KD) Validation data are obtained from updated antibody specification datasheets and manufacturers' websites or technical support information.

Supplementary Table 2

Primary antibody	catalog No. RRID	company	dilution	KO/KD validated
Immunofluorescence				
BIP (C50B12) Rabbit mAb (Alexa Fluor 647 Conjugated)	#57163	CST	1:75	no
Immunohistochemistry				
BiP (C50B12) Rabbit mAb	#3177 AB_2119845	CST	1:200	no
anti-Fibronectin antibody	ab2413 AB_2262874	abcam	1:500	no
TIM-1/KIM-1/HAVCR Antibody	NBP1-76701 AB_11037459	Novus Biologicals	1:100	Yes
Integrin beta 1/CD29 antibody	GTX128839 AB_2885823	Gene Tex	1:1000	Yes

Knockout (KO) and Knockdown (KD) Validation data are obtained from updated antibody specification datasheets and manufacturers' websites.

Supplemental Figure 1. Metabolic and urinary data of mice.

a. body weight (g); **b.** blood glucose (mg/dL); **c.** kidney weight (g) / body weight (g) ratio; **d.** urine volume (mL/day); **e.** food intake (g/day); **f.** serum creatinine (Cr) (mg/dL); **g.** creatinine clearance (Ccr) (ml/min/kg). N=6-13. CTRL; Citrate buffer administrated control mouse, STZ; streptozotocin (STZ) -induced diabetic mouse, CTRL+CANA; Canagliflozin administrated control mouse, STZ+CANA; Canagliflozin administrated STZ-induced diabetic mouse. One-way ANOVA followed by Tukey-Kramer test is used. All Data are presented as mean \pm S.D., **p<0.01, ****p<0.0001. **h and i.** Western blot for albumin using urine samples (**f**). Analysis was performed with loading doses of each sample proportional to the 24-hour urine volume to simulate daily albumin excretion. Densitometry quantification of Western blot (**g**). N=5-8. One-way ANOVA followed by Tukey-Kramer test is used. All Data are presented as mean \pm S.D., *p<0.05,

Supplementary Figure 2. GRP78 as a SGLT2-inteactive molecule.

a. Kidney lysate of STZ-induced diabetic mouse were immunoprecipitated with IgG or SGLT2 antibody. Blot with GRP78. **b.** Kidney lysate of STZ-induced diabetic mouse were immunoprecipitated with IgG or GRP78 antibody. Blot with SGLT2 antibody. The exposure time to detect SGLT2 band was different between immunoprecipitated and input protein. Two membranes with different exposure times are presented side by side for the band of SGLT2 with input samples.

Supplementary Figure 3. Immunofluorescence staining of mouse kidney and electron microscopy of mouse proximal tubular cells.

a. Double immunofluorescence staining of mouse kidney for GRP78; green signal and ER

marker Calnexin; red signal. CTRL; Citrate buffer administrated control mouse, STZ; streptozotocin (STZ) -induced diabetic mouse, CTRL+CANA; Canagliflozin administrated control mouse, STZ+CANA; Canagliflozin administrated STZ-induced diabetic mouse. Bar=50 μ m. Statistical analysis of the merged area in tubular cell of 4-5 different mice per group is shown in the right column. * p <0.05, *** p <0.001. **b.** Electron microscopy of mouse proximal tubular cells. Asterisk; ER. Bar=1 μ m.

Supplementary Figure 4. Canagliflozin suppresses ER stress enhancement induced by SERCA inhibitor, thapsigargin, in HK2 cells.

a. Western blot analysis of HK2 cells. The expression of both SGLT1 and SGLT2 were confirmed. HK2 cells were cultured with DMSO or 1 μ M thapsigargin (TG) or 1 μ g/ml tunicamycin (TM) for 24 hr. Canagliflozin was co-administrated at 0 μ M or 3 μ M for 24 hr. **b.** Western blot analyses. **c.** Densitometry quantification of Western blot. N=3, One-way ANOVA followed by Tukey-Kramer test is used. All Data are presented as mean \pm S.D., * p <0.05, ** p <0.01, *** p <0.001, **** p <0.0001.

Supplementary Figure 5. Canagliflozin (CANA) inhibits cell damages of tubular cells in STZ-induced diabetic mice.

a. TUNEL assay. The number of TUNEL positive cells per slice were counted across multiple fields per mouse and counts from different 4-5 mice were used for statistics. Statistical analysis was shown in the right column. N=4-5. Bar=100 μ m. **b.** Immunohistochemical staining for KIM-1. KIM-1 positive regions were averaged across multiple fields per mouse and values from different 6-9 mice were used for statistics. Statistical analysis was performed on different mice as shown in the right column. N=6-9. Bar=100 μ m. **c.** Western blotting using

kidney tissues. Densitometry quantification of Western blot is shown in the right column. N=3. CTRL; Citrate buffer administrated control mouse, STZ; streptozotocin (STZ) -induced diabetic mouse, CTRL+CANA; Canagliflozin administrated control mouse, STZ+CANA; Canagliflozin administrated STZ-induced diabetic mouse. All data are presented as mean \pm S.D. One-way ANOVA followed by Tukey-Kramer test is used. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

Supplementary Figure 6. Canagliflozin (CANA) inhibits fibrosis of STZ induced diabetic kidney.

Immunohistochemical staining of fibronectin. Bar=100 μm . Fibronectin positive area was measured and statistically analyzed as shown in the right column.

CTRL; Citrate buffer administrated control mouse, STZ; streptozotocin (STZ) -induced diabetic mouse, CTRL+CANA; Canagliflozin administrated control mouse, STZ+CANA; Canagliflozin administrated STZ-induced diabetic mouse. N=5-10. One-way ANOVA followed by Tukey-Kramer test is used. All Data are presented as mean \pm S.D. * $p < 0.05$, ** $p < 0.01$.

Supplementary Figure 7. Urinary secretion of GRP78 in mice.

- a. Western blot for GRP78 using urine samples. Analysis was performed with loading doses of each sample proportional to the 24-hour urine volume to simulate daily GRP78 excretion.
- b. Densitometry quantification of Western blot (**g**). N=4. P values were determined by unpaired *t* test, * $p < 0.05$.

Supplemental Figure 8. Canagliflozin (CANA) promotes endoplasmic reticulum (ER) robustness.

In diabetic kidney disease, ER stress is enhanced and regulated by unfolded protein responses (UPR). Excessive and prolonged ER stress leads to UPR failure and cell death. Knockdown of GRP78, which is major player of UPR, also promotes UPR failure. CANA plays a cytoprotective role in reducing excessive ER stress and promoting ER robustness.

Supplemental Figure 9. Graphical abstract.

Canagliflozin protects proximal tubular cells and inhibits EMT and interstitial fibrosis via following mechanisms. (1) Canagliflozin protects proximal tubular cells by enhancing AMPK phosphorylation, improving ER robustness, and suppressing EMT coordinating with GRP78.

(2) Canagliflozin suppress the secretion of soluble secreted GRP78 (sGRP78) from tubular cells and inhibit sGRP78-induced phosphorylation of p65NFkB and Akt in surrounding tubules. (3)

Canagliflozin contributed to the maintenance of the appropriate amount and localization of integrins $\beta 1$ in tubular cells.

Supplemental Figure 10.

Reproducibility experiment of Figure 2b and Figure 8f using another mouse kidney.

Supplemental Figure 11. Uncropped images