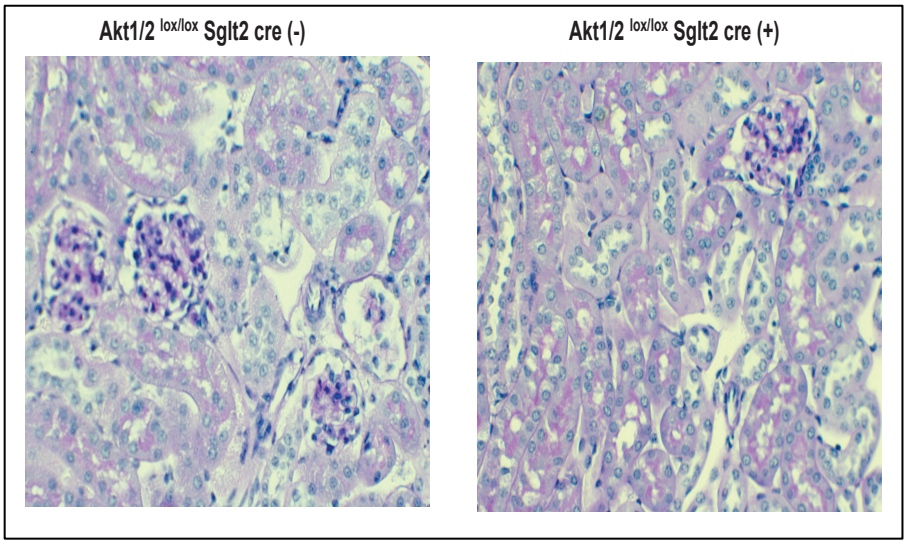
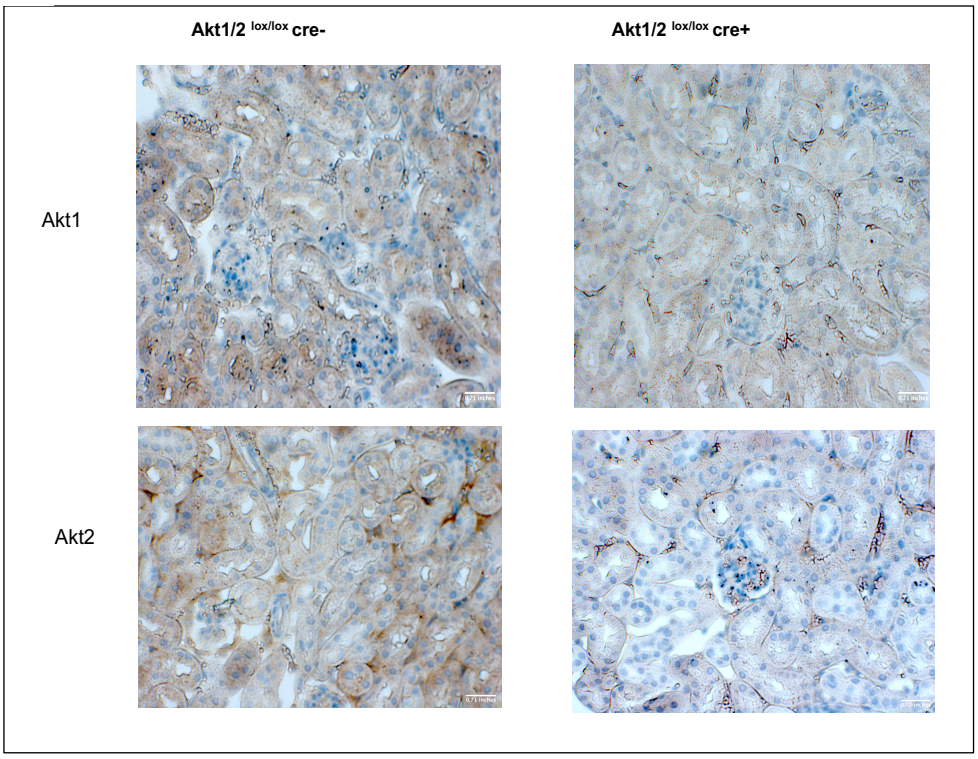
Supplemental Fig-S2

A



B

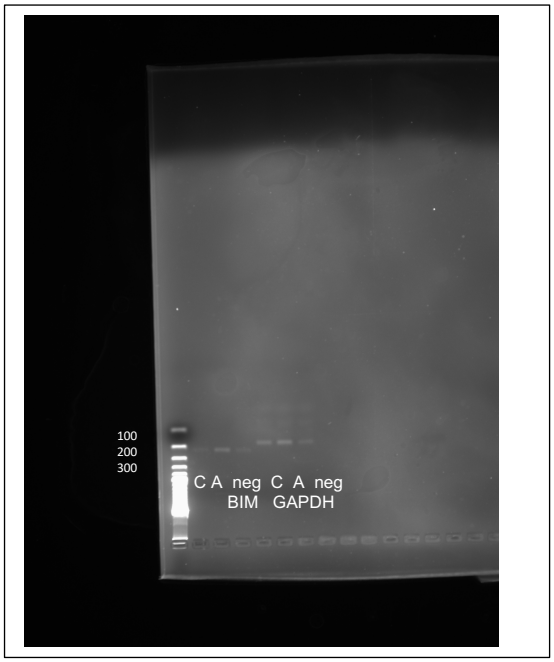


C

	Akt1/2$^{lox/lox}$SGLT cre	Akt1/2$^{lox/lox}$SGLT cre + ip albumin
BUN (mg/dl)	34.35±3.01	34.47± 2.37
Creatinine (mg/dl)	0.28±0.03	0.38± 0.03

Fig-2a: Akt ½ $^{lox/lox}$ cre- and Akt ½ $^{lox/lox}$ cre+ mouse displayed normal histology
Fig-2b: Akt ½ $^{lox/lox}$ cre+ exhibited decreased expression of both Akt1 and Akt2 by immunohistochemistry
Fig-2c: Albumin overload Akt ½ $^{lox/lox}$ cre+ maintained normal kidney function. Mean ± SEM n=3

Supplemental Fig-5



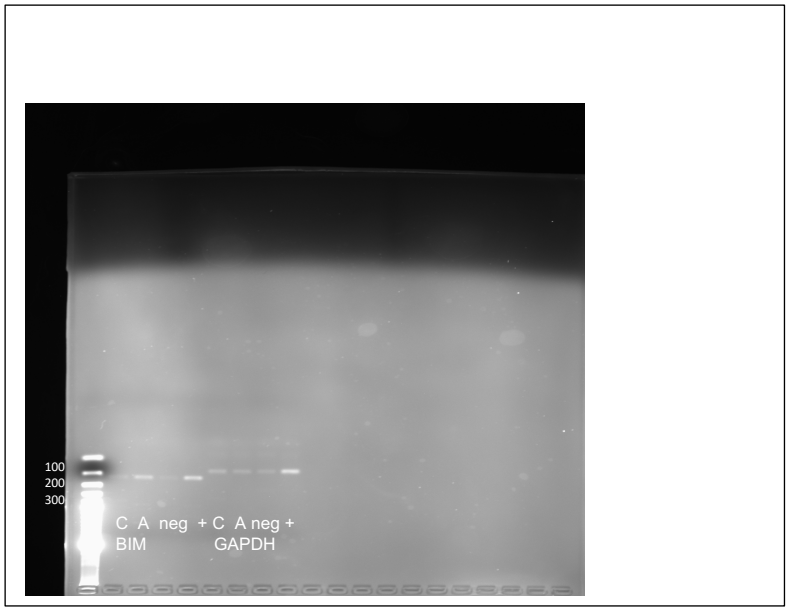


Fig-5: CHIP experiments demonstrated Foxo-1 induced BIM transcription in response to albumin overload in PTEC.

In-vitro albumin overload model: Media was changed to serum free for 16 hours and followed by incubation with 10mg/ml of endotoxin free human albumin (Sigma) for 6, 16 and 24 hours to determine the time course of phosphorylated Akt expression and cleavage of caspase-3. For the rest of *in vitro* albumin overload experiments, HKC-8 cells were incubated with in media albumin for 24 hours and control cells were maintained in serum free media.
Preparation of cell lysates: Cells were washed with PBS and incubated for 30 min on ice-cold lysis buffer [20 nM HEPES, pH 7.4, 2 mM EGTA, 1% Triton X-100, 400 M PMSF, 50 mM NaF, 2 M microcystin LR, complete protease inhibitor (Roche), 10 ng/μl leupeptin, 10 ng/μl aprotinin, 4 ng/μl elastatinal, 2.5mM phenanthroline, 100μM L-1ltosylamido-2phenylethyl chloromethyl ketone], and sonicated for 10 s 3 times and cleared by centrifugation at 4°C for 10 min at 15,000 g. The supernatant was retained and used for Western blotting experiments as published previously
Western blotting: HKC-8 cell lysates were processed and probed with p-Foxo1-Thr24, p-Foxo1-Ser 256, p-Foxo3-Ser253 and p-Foxo3-Ser318, caspase 9, caspase-3 and BIM antibodies (Cell Signaling).
Transfection experiments: Plasmids were mixed with Lipofectamine-2000 (Invitrogen) in Opti-MEM reduced serum media (Invitrogen) and incubated overnight. Media was changed to F12: DMEM with 5% FBS the following day. Experiments were performed 48 hours after transfection. Cells were maintained in serum free media (SFM) for 16 hours before the experiments.

Chromatin immunoprecipitation (CHIP) experiments: HKC-8 cells were subjected to albumin overload as outlined above. The harvested chromatin was immunoprecipitated by use of Foxo1 antibody (Abcam). After immunoprecipitation, the protein-DNA cross-links were reversed, and the DNA was purified. PCR was performed using the BIM primers below.

forward 5′-AGGCAGAACAGGAGGAGA-3′

reverse 5′-AACCCGTTTGTAAGAGGC-3′

Input was used as positive control and cell lysates subjected to immunoprecipitation with IgG was used as negative control.

Animal model of albumin overload: Wild type C57BL/6 (female and male) mice at 6-8 week of age and littermate controls underwent intraperitoneal low endotoxin BSA (10 mg/g body weight, dissolved in saline) versus comparable volume of saline for 5 consecutive days/week for 6 weeks. Immunohistochemistry and H&E staining were performed on deparaffinized kidney tissue through xylene and descending grades of ethanol, fixed with 4% formaldehyde/PBS for 30 min at 4°C, permeabilized with 0.2% Triton X-100/PBS for 15 min at 4°C.

Human formalin fixed paraffin embedded kidney biopsy samples from these patients were obtained from the Cincinnati Children's Hospital Medical Center Discover Together Biobank with IRB approval. The slides were blocked and permeabilized in PBS with 0.3% triton with 10% normal donkey serum for one hour, followed by incubation overnight in pser473-Akt (1:100, Cell Signaling). Slides were then incubated in secondary antibody as well as fluorescein labeled lotus tetragonolobus lectin (LTL,1:250) for one hour to identify proximal tubules. Slides underwent three washes in PBST for 5 minutes each, followed by DAPI nuclear staining, and then protected with Prolong Gold Antifade reagent and covered.

Quantification of Akt p-Ser473 expression on patient kidney biopsies: Z-stack confocal images were obtained with the 20X objective using the Nikon A1 inverted confocal microscope. The images were processed using NIS-Elements AR 5.2.00 software. The mean fluorescent intensity of single tubules was determined by creating a proximal tubule Binary using General Analysis tool based on LTL signal followed by converting binaries to a region of interest for each individual

tubule. Signal intensity within this region of interest was measured by the Elements software and exported into Excel. Twenty proximal tubules were measured for each patient sample.

Antibodies:

Cs_Caspase-3 Antibody #9662-17, 19, 35

Cs-Cleaved Caspase-3 (Asp175) (5A1E) Rabbit mAb #9664-17, 19

Cs-Cytochrome-c -4272

Cs-Akt-9272

Cs-phospho thr 308 Akt - 4056

CS-Phospho-Akt (Ser473) (D9E) XP® Rabbit mAb #4060

Cs-Bax Antibody #2774

Cs-Bim Antibody #2819

Cs-FoxO3a (75D8) Rabbit mAb #2497

Cs-Phospho-FoxO1 (4)/FoxO3a (Thr32)/FoxO4 (Thr28) (4G6) Rabbit mAb #2599

Cs-Phospho-FoxO1 (Ser256) Antibody #9461

Cs-Phospho-FoxO3a (Ser318/321) Antibody #9465

Cs- COX IV (3E11) Rabbit mAb #4850

Abcam-(ab52857) **Anti-FOXO1A antibody [EP927Y]**