

## Supporting Information

### A Novel Fluorogenic Assay for the Detection of Nephrotoxin-Induced Oxidative Stress in Live Cells and Renal Tissue

Kamalika Mukherjee<sup>1\*</sup>, Tak Ian Chio<sup>2#</sup>, Han Gu<sup>2‡</sup>, Dan L. Sackett<sup>3</sup>, Susan L. Bane<sup>2\*</sup> and Sanja Sever<sup>1\*</sup>

<sup>1</sup> Division of Nephrology, Massachusetts General Hospital and Harvard Medical School, Boston, MA

<sup>2</sup> Department of Chemistry, Binghamton University, State University of New York, Binghamton, NY

<sup>3</sup> *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD

\*Corresponding authors:

Kamalika Mukherjee

E-mail: kmukherjee1@mgh.harvard.edu; kmukher1@binghamton.edu

Susan L. Bane

Phone: 607-777-2927

E-mail: sbane@binghamton.edu

Sanja Sever

Phone: 617-724-8922

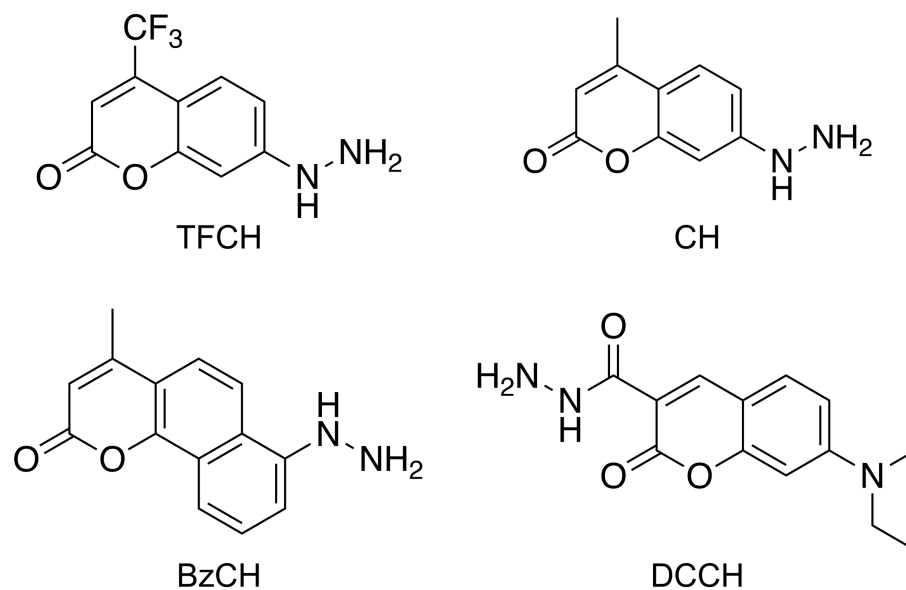
E-mail: ssever@mgh.harvard.edu

#### Present Addresses

<sup>#</sup> Department of Pathology and Laboratory Medicine, Institute on Aging and Center for Neurodegenerative Disease Research, University of Pennsylvania School of Medicine, Philadelphia, PA 19104

<sup>‡</sup> AstaTech, Inc. Keystone Business Park  
2525 Pearl Buck Road. Bristol PA, 19007

## Supplementary Figures



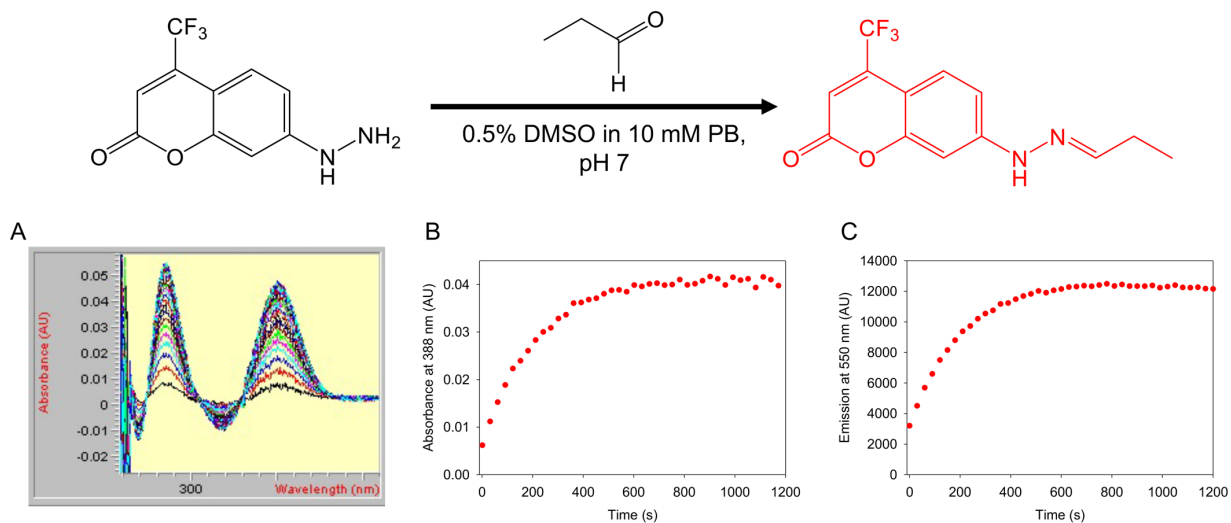
**Figure S1. Structures of alpha-effect amines.**

TFCH: 4-trifluoromethyl-7-hydrazinyl-2H-chromen-2-one

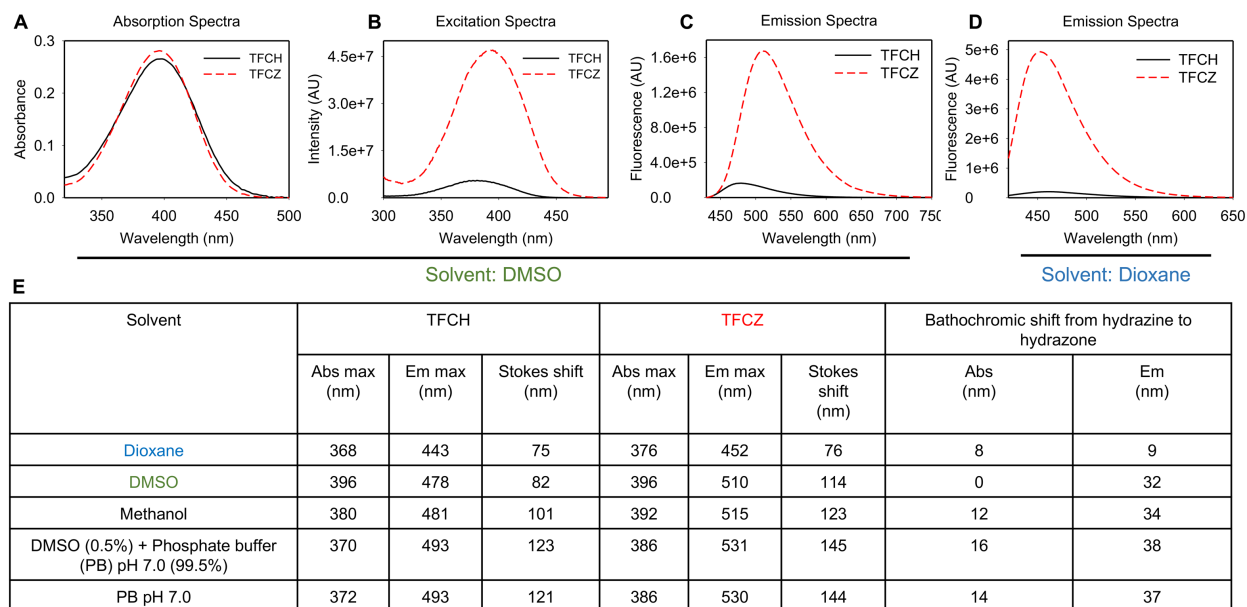
CH: 7-hydrazinyl-4-methyl-2H-chromen-2-one

BzCH: 7-hydrazinyl-4-methyl-2H-benzo[h]chromen-2-one

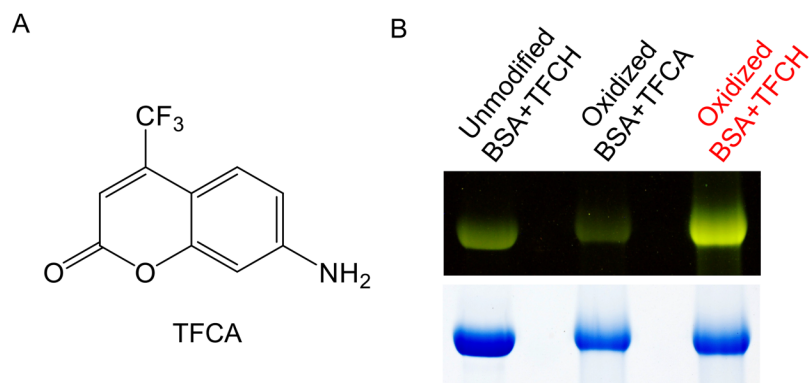
DCCH: Coumarin-3-carbohydrazide



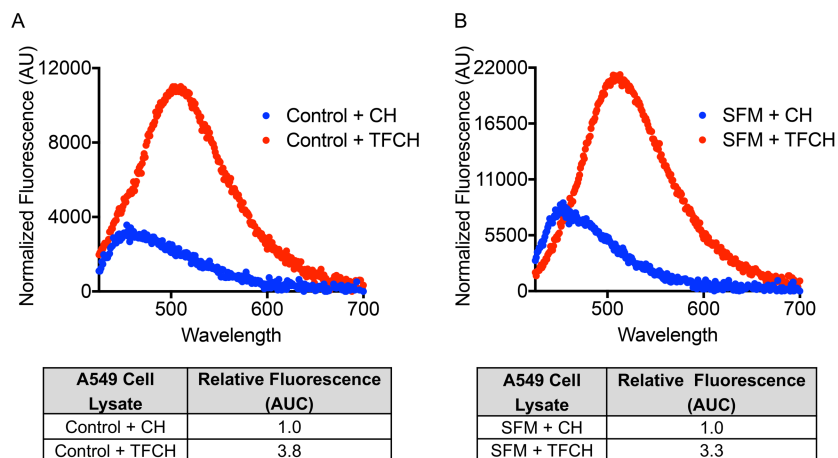
**Figure S2. TFCH undergoes reaction with a model aliphatic aldehyde (propanal) in neutral aqueous solution to form a fluorescent hydrazone.** Hydrazone formation between 5  $\mu$ M TFCH and 500  $\mu$ M propanal was monitored by absorption difference spectroscopy (A). Data from a single wavelength (388 nm) from A plotted as a function of time (B). Hydrazone formation between 5  $\mu$ M TFCH and 500  $\mu$ M propanal was monitored by fluorescence spectroscopy (C). The sample was excited at 388 nm and the increase in emission at 550 nm was recorded over time.



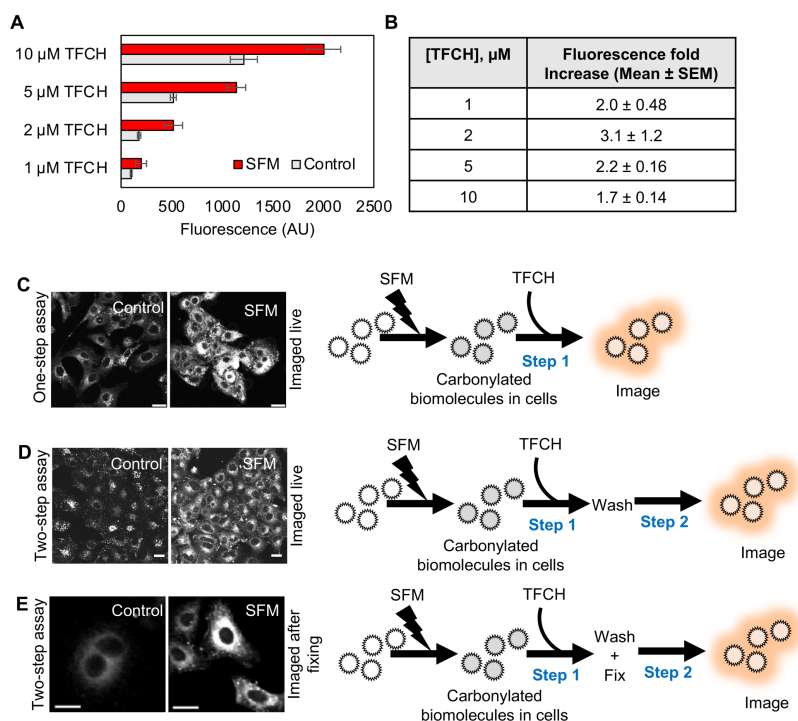
**Figure S3. Photochemical properties of TFCH and TFCZ.** Absorption (A), excitation (B), emission (C) spectra of 10  $\mu$ M TFCH and TFCZ in DMSO. Emission spectra of TFCH and TFCZ in dioxane (D). For the emission spectra, samples were excited at 405 nm (C-D). Spectroscopic (absorption and emission) characterization of TFCH and TFCZ in different solvents (E).



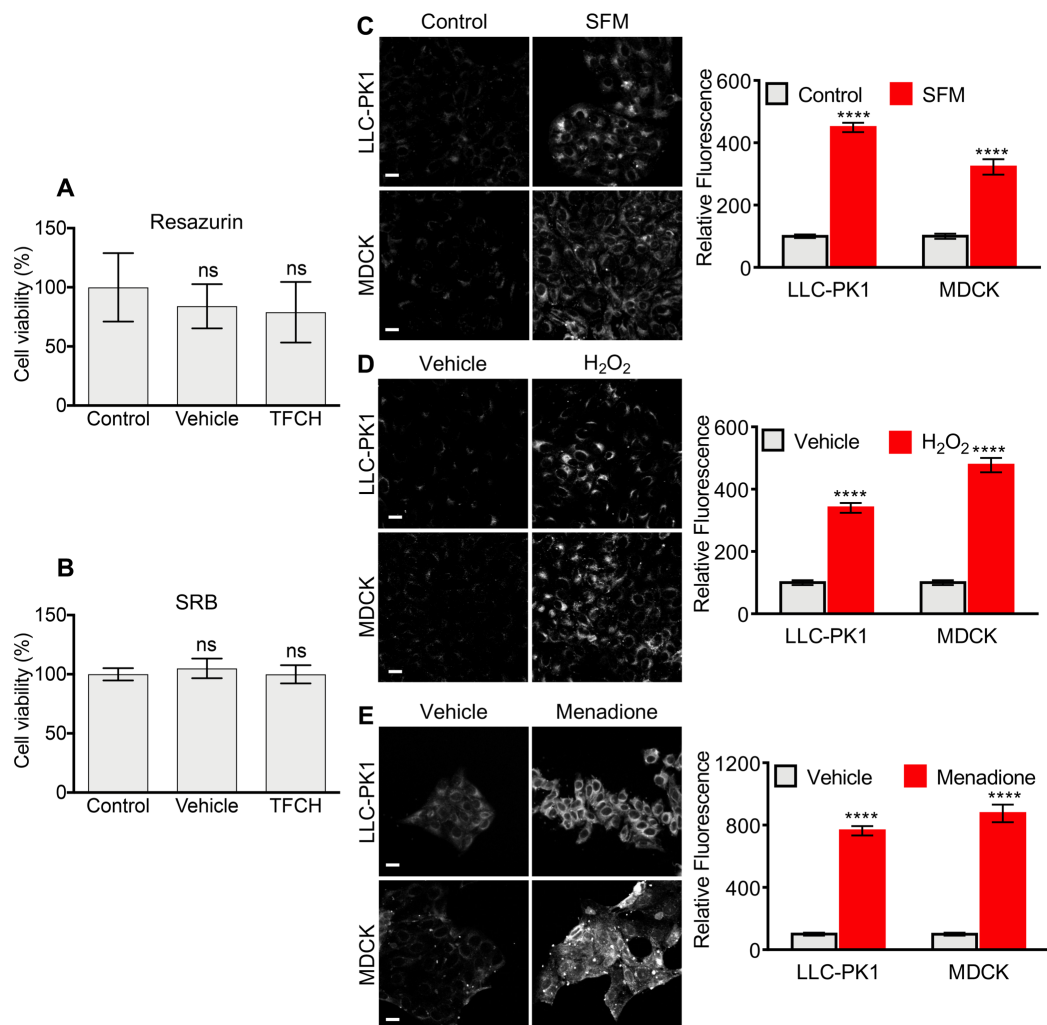
**Figure S4. Carbonylated biomolecules can be detected by TFCH in an isolated system.** TFCH was allowed to react with oxidized bovine serum albumin (BSA) as described in the *Methods* and subjected to SDS-PAGE analysis. Unmodified BSA incubated with TFCH or oxidized BSA incubated with TFCA (A) was used as controls. The upper panel shows the SDS-PAGE gel imaged under long-wavelength UV and the lower panel shows the Coomassie stained gel (B).



**Figure S5. TFCH displays better sensitivity to cellular carbonylated biomolecules than CH.** A549 lung cancer cells grown in standard media (A) or serum free media (SFM) (B) were incubated with 20  $\mu$ M CH or TFCH for 2.5 h. The cells were then washed, lysed, and an emission spectrum of each sample was recorded (excited at 405 nm). The spectra were normalized by total protein representing total cell number. The relative fluorescence of each sample is reported as the total area under the curve (AUC). Note that the fluorescence in the control samples is due to the presence of inherent carbonyls in cancer cells<sup>1</sup>.



**Figure S6. TFCH serves as a tool for detecting oxidative stress-induced carbonylation in live cells by HCS- and HTS-compatible assays.** A plate-reader based assay for detecting SFM-induced carbonylation (A-B). A549 cells were grown in standard media (control) or SFM for 24 h before adding the stated concentration of TFCH for 90 min. The cells were rinsed with PBS and the emission was recorded at 525 nm (excitation: 405 nm). Graph shows relative fluorescence due to serum starvation detected by different concentrations of TFCH (A). Error bars represent SEM. The fold increase in fluorescence was calculated as the ratio of signal generated in the SFM treated samples and the control samples (B). A one-step assay for visualizing biomolecule-carbonyls in live cells (C). Serum-starved or control cells were allowed to react with 2  $\mu\text{M}$  TFCH for 30 min before imaging the live cells without rinsing out excess fluorophore. Scale bar, 20  $\mu\text{m}$ . A two-step assay for visualizing biomolecule-carbonyls (D-E). A549 cells grown in standard media (control) or SFM for 24 h were allowed to react with 20  $\mu\text{M}$  TFCH for 30-60 min. In the second-step, excess fluorophore was removed; the cells were washed and either imaged live (D) or fixed, processed (as described in the *Methods*), and imaged (E). Scale bar, 20  $\mu\text{m}$ .



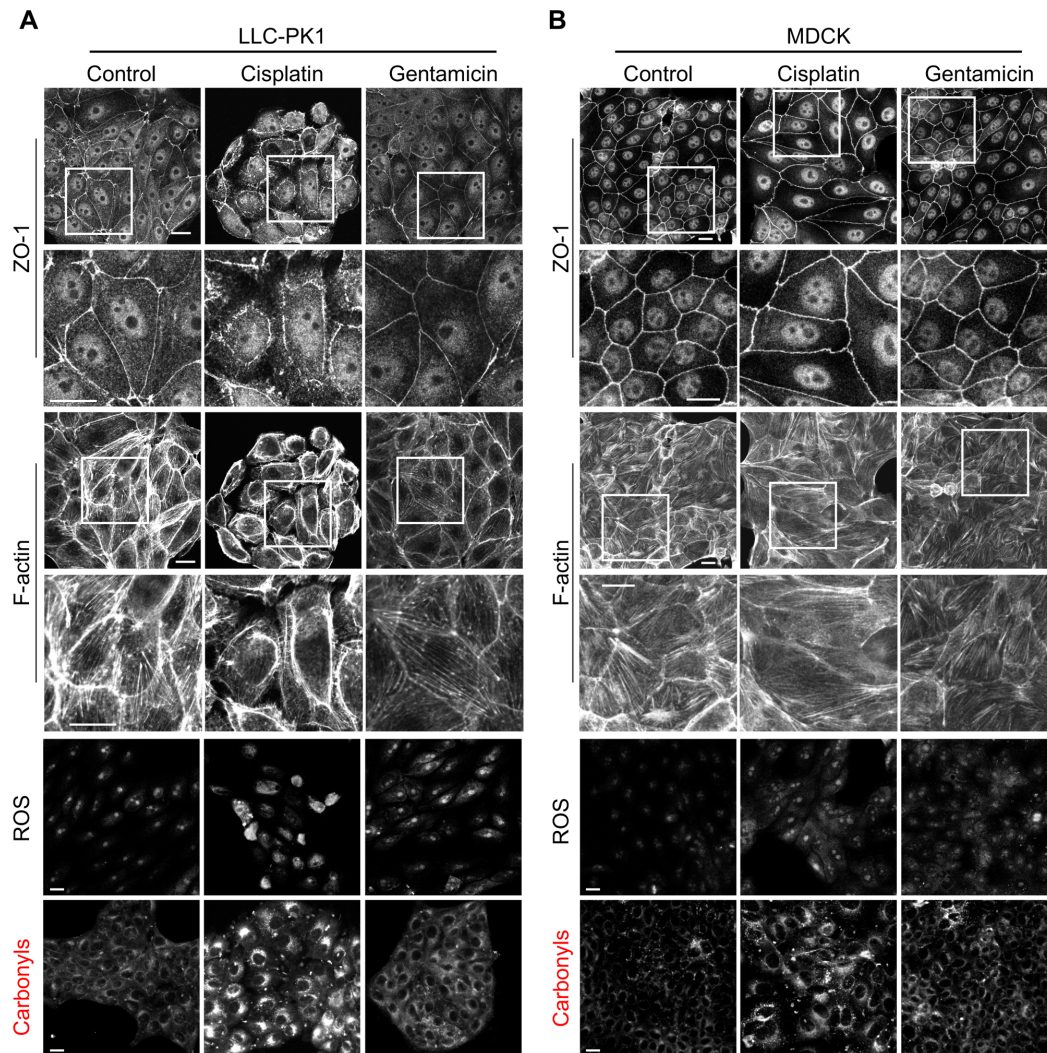
**Figure S7. TFCH is a live cell compatible probe for detecting OS-induced carbonylation in kidney cells.**

*TFCH is not cytotoxic (A-B).* Resazurin (A) or SRB (B) assay was performed after MDCK cells were exposed to media without added treatment (control), with vehicle (0.5%, v/v, DMSO) or with TFCH (20  $\mu$ M, the highest concentration used) for 24 h. Error bars represent SD. One-way ANOVA with Dunnett's multiple comparisons test was performed to compare each treatment with the control;  $P > 0.05$  was considered not significant (ns).

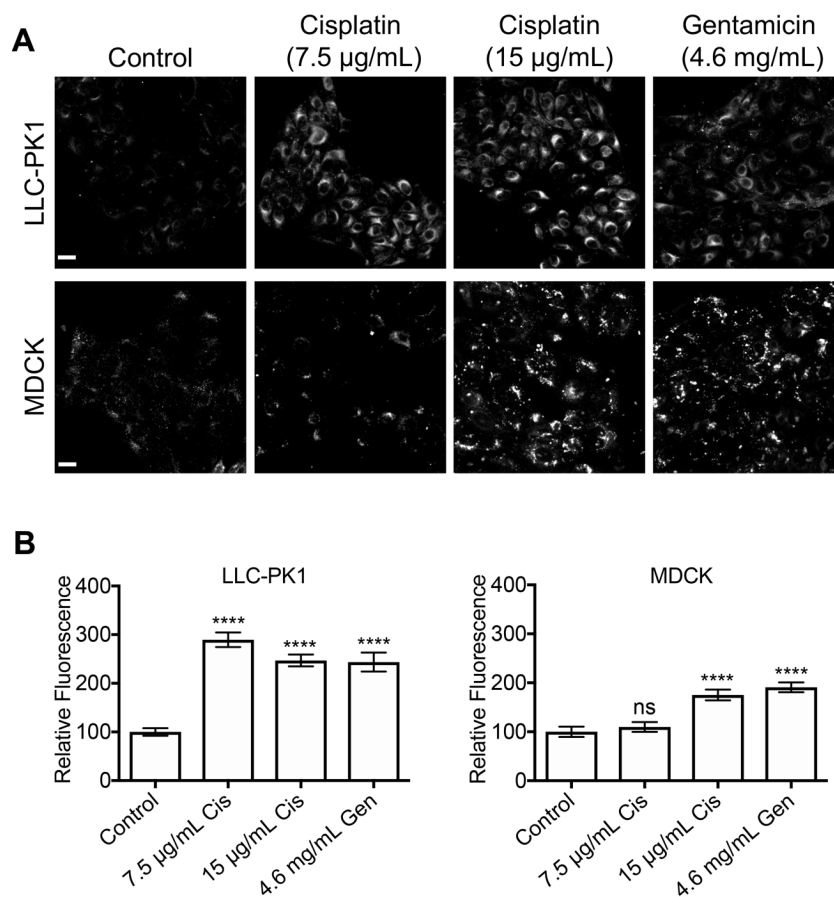
*TFCH detects oxidative stress-induced carbonylation in kidney cells (C-E).* SFM (C)-, H<sub>2</sub>O<sub>2</sub> (D)- or menadione (E)-induced carbonylation was detected by incubating live LLC-PK1 or MDCK cells with 20  $\mu$ M TFCH for 30-60 min. Cells were rinsed with PBS, fixed, and processed before imaging. Scale bar, 20  $\mu$ m.

Bar graphs showing quantification of cellular carbonyls detected by TFCH in LLC-PK1 or MDCK cells. At least three independent experiments were performed, and fluorescence associated with > 100 cells were quantified. An unpaired t-test with Welch's correction was performed. \*\*\*\* $P < 0.0001$ . Error bars represent SEM.

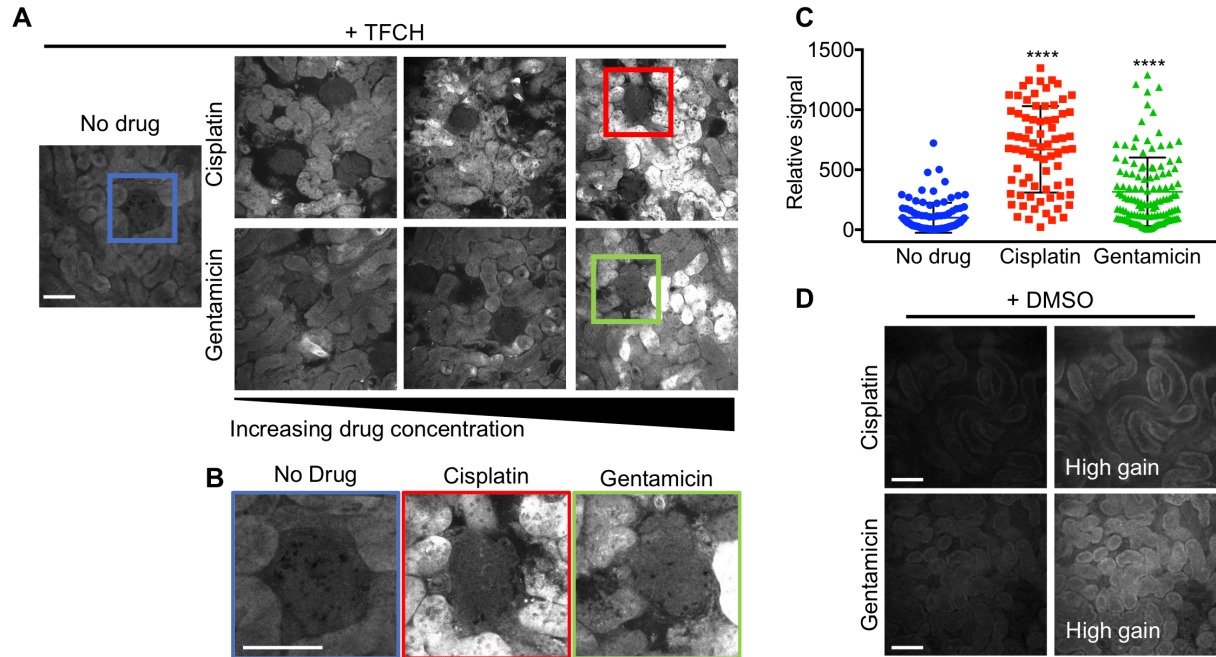




**Figure S8: Photomicrographs showing the effect of cisplatin and gentamicin in renal epithelial cells.** Status of ZO-1, F-actin, ROS, and biomolecule-carbonyls (detected by TFCH) in LLC-PK1 (A) and MDCK (B) cells. Renal cells were treated with vehicle (cell culture media; no drug; control), cisplatin (1.5  $\mu\text{g}/\text{mL}$ ) or gentamicin (0.58  $\text{mg}/\text{mL}$ ) for 24 h before performing the assays as described in the *Methods*. Note that the continuous ZO-1 staining pattern in the control LLC-PK1 cells becomes discontinuous upon cisplatin treatment. Similarly, F-actin is mostly associated with the cell periphery, while the central region of the cells shows diffuse staining pattern in LLC-PK1 cells treated with cisplatin. Gentamicin in LLC-PK1, and both drugs in MDCK cells do not alter ZO-1 and F-actin staining pattern. Scale bar, 20  $\mu\text{m}$ .



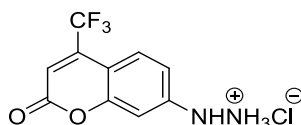
**Figure S9: Drug-induced carbonylation can be detected within hours by TFCH.** LLC-PK1 and MDCK cells were allowed to grow for 3 h in the presence of cisplatin (Cis) or gentamicin (Gen). TFCH (20  $\mu\text{M}$ ) was added to the medium for an additional 60 min. The cells were then rinsed with PBS, fixed, processed, and imaged as described in the *Methods*. One-way ANOVA with Dunnett's multiple comparisons test was performed to compare each treatment with the control. \*\*\*\* $P < 0.0001$ . Error bars represent SEM. Scale bar, 20  $\mu\text{m}$ .



**Figure S10: Drug-induced oxidative injury detected in kidney tissue slices by TFCH.** Dose-dependent increase in carbonylation detected by TFCH (2  $\mu$ M) (A). Cisplatin concentrations used: 37.5, 75, 150  $\mu$ g/mL; gentamicin concentrations used: 1.2, 2.3, 4.6 mg/mL. Insets of (A) showing distribution (glomerular versus renal tubule) of biomolecule-carbonyls (fluorescence) in the tissue section (B). Quantification of renal tubule associated fluorescence in control (no drug), cisplatin (150  $\mu$ g/mL), or gentamicin (4.6 mg/mL) treated tissue slices (C). Error bars represent SD. Control samples (D): tissue sections treated with cisplatin (150  $\mu$ g/mL) or gentamicin (4.6 mg/mL) were exposed to vehicle (0.5% DMSO, v/v), instead of TFCH. An unpaired t-test with Welch's correction was performed to compare the control with each treatment. \*\*\*\* $P < 0.0001$ . Error bars represent SD. Scale bar, 100  $\mu$ m.

## Synthesis and characterization of TFCH and TFCZ

### 4-Trifluoromethyl-7-hydrazinyl-2H-chromen-2-one (TFCH)



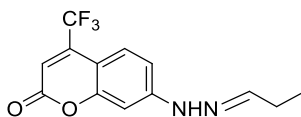
7-Amino-4-(trifluoromethyl) coumarin (500 mg, 2.18 mmol) was dissolved in 1.50 mL concentrated HCl and stirred for 10 min at -10 °C. A chilled solution of sodium nitrite (181 mg, 1.2 eq) in 600  $\mu$ L water was added dropwise to keep the temperature of reaction below 0 °C. The solution was stirred for 1 h at -10 °C. Stannous chloride dihydrate (1.57 mg, 3.8 eq) was dissolved in 1.50 mL concentrated HCl and chilled on ice. This cold stannous chloride HCl solution was then slowly added to the diazonium solution and the temperature of reaction was maintained below 0 °C. The reaction was stirred for 1.5 h at -10 °C. The yellow slurry was then filtered and washed with cold water and cold ethanol. TFCH HCl salt was collected as a light yellow solid (350 mg, 57% yield).

**<sup>1</sup>H NMR** (400 MHz, DMSO-d<sub>6</sub>)  $\delta$ : 10.49 (br, **NH**, 3H), 9.32 (s, **NH**, 1H), 7.59 (d, J = 8.0 Hz, 1H), 7.00 (m, 2H), 6.77 (s, 1H).

**<sup>13</sup>C NMR** (400 MHz, DMSO-d<sub>6</sub>)  $\delta$ : 159.25, 155.98, 151.00, 126.06, 123.59, 120.86, 112.53 (q, CF<sub>3</sub>), 111.87, 105.97, 100.31.

**MS-ESI<sup>+</sup>**: C<sub>10</sub>H<sub>7</sub>F<sub>3</sub>N<sub>2</sub>O<sub>2</sub> [M + H]<sup>+</sup> calcd.: 245.17, found: 245.12.

### 7-(2-Propylidenehydrazinyl)-4-(trifluoromethyl)-2H-chromen-2-one (TFCZ)



The TFCH HCl salt (20 mg, 0.082 mmol) was dissolved in 600  $\mu$ L methanol and 20  $\mu$ L of trifluoroacetic acid. Propionaldehyde (58.7  $\mu$ L, 10 eq) was added to the solution which was stirred further for 30 min. The yellow precipitate was filtered and washed with cold methanol. The TFCZ was dried by air and collected as yellow solid (15 mg 64% yield).

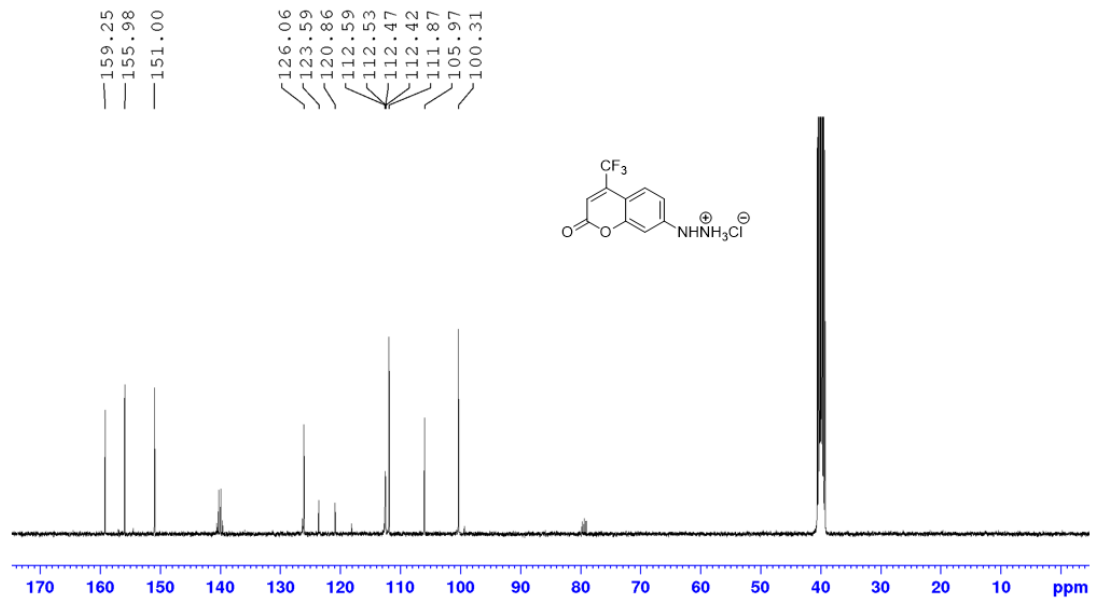
$^1\text{H NMR}$  (400 MHz, DMSO- $d_6$ )  $\delta$ : 10.66 (s, **NH**, 1H), 7.50 (dd,  $J = 9.0, 2.1$  Hz, 1H), 7.37 (t,  $J = 4.9$  Hz, 1H), 6.94 (dd,  $J = 9.0, 1.7$  Hz, 1H), 6.86 (d,  $J = 2.0$  Hz, 1H), 6.57 (s, 1H), 2.30 (dq,  $J = 7.5, 5.0$  Hz, 2H), 1.08 (t,  $J = 7.4$  Hz, 3H).

$^{13}\text{C NMR}$  (400 MHz, DMSO- $d_6$ )  $\delta$ : 159.65, 156.80, 150.37, 147.22, 126.43, 123.75, 121.01, 110.27, 109.71 (q,  $\text{CF}_3$ ), 104.11, 97.76, 25.76, 11.21.

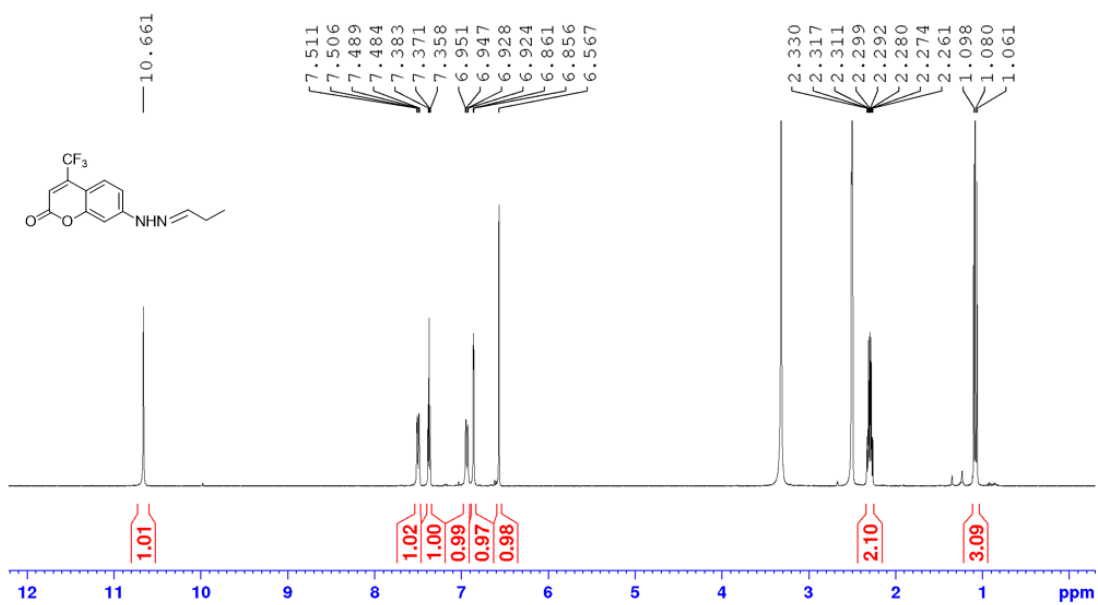
**MS-ESI $^+$** :  $\text{C}_{13}\text{H}_{11}\text{F}_3\text{N}_2\text{O}_2$   $[\text{M} + \text{H}]^+$  calcd.: 285.08, found: 285.11.



$^1\text{H NMR}$  spectrum of TFCH.



<sup>13</sup>C NMR spectrum of TFCH.



<sup>1</sup>H NMR spectrum of TFCZ (hydrazone product of TFCH and propionaldehyde).

## Methods

### TFCH reaction with propanal.

Equal volumes of 10  $\mu\text{M}$  TFCH and 1 mM propanal in 10 mM PB, pH 7 containing 0.5% DMSO placed in separate compartments of a dual chambered quartz cell in an absorption spectrophotometer (Hewlett-Packard 8453). The sample was zeroed and the solution was quickly mixed by inversion. Spectra were obtained over time until a steady state was reached. Note, the solutions are diluted by a factor of two and the path length is doubled; therefore, only the differences in the absorbance of the solution after mixing are recorded.

Hydrazone formation between 5  $\mu\text{M}$  TFCH and 500  $\mu\text{M}$  propanal in 10 mM PB, pH 7 containing 0.5% DMSO was monitored using Synergy Mx microplate reader (BioTek). The sample was excited at 388 nm and the emission at 550 nm was recorded over time until a steady state was reached.

### Spectroscopic characterization of TFCH or TFCZ

$\sim 10$   $\mu\text{M}$  TFCH or TFCZ (hydrazone of TFCH and propanal) was prepared in DMSO or other solvents (1,4-dioxane; methanol; 10 mM sodium phosphate buffer (PB) pH 7; or PB, pH 7 containing 0.5% DMSO, v/v). Absorption spectra of these samples were taken at room temperature (RT) using a Hewlett-Packard 8452A diode array spectrophotometer equipped with the Olis Globalworks software. Emission and excitation spectra were recorded at RT using a Jobin Yvon Horiba FluoroMax-3 spectrofluorometer. Fluorescence spectra were collected using a  $2 \times 10$  mm fluorescence cuvette, positioned for the light to pass through the shorter path. Emission spectra (405 nm excitation) were corrected by subtracting the solvent's fluorescence followed by normalization to account for any differential absorption of TFCH and TFCZ at the excitation wavelength.

### Detection of carbonylated BSA by TFCH

Bovine serum albumin (BSA) was oxidized as described previously<sup>2</sup>. Commercially available unmodified BSA or oxidized BSA was allowed to react with TFCH for 3.5 h. In parallel, oxidized BSA was allowed to react with TFCA for the same period of time. The final concentrations of the protein and the fluorophore in each reaction were 2  $\mu\text{g}/\mu\text{L}$  and 300  $\mu\text{M}$ , respectively. The samples were then subjected to SDS-PAGE analysis. The gel was imaged under long wavelength UV prior to Coomassie blue staining and imaging to account for protein loading. The major band representing BSA is shown in the illustration.

### **Cell culture**

A549 cells were grown in F12K media (ATCC); MDCK cells were grown in DMEM:F-12 (ATCC); and LLC-PK1 cells were grown in DMEM with 4.5 g/L glucose, L-glutamine, and sodium pyruvate (Corning). All standard cell culture media were supplemented with 10% FBS (ThermoFisher Scientific, Sigma or Atlanta Biologicals) and 1% antibiotic-antimycotic (ThermoFisher Scientific). All serum-free media (SFM) were supplemented only with 1% antibiotic-antimycotic. Cells were maintained in a mammalian cell culture incubator. LLC-PK1 cells were grown for 72 h and MDCK cells were grown overnight on collagen-coated coverglasses or 96-well plates prior to treatment.

### **Relative fluorescence of CH and TFCH labeled carbonyls in cellular environment**

Control (cells grown in standard media) or serum-starved (cells grown in SFM for 24 h) A549 cells were incubated with 20  $\mu$ M CH or TFCH for 2.5 h. The cells were then washed and lysed in PB-S buffer (10 mM PB, pH 7, supplemented with 1% SDS, and protease inhibitor). Each sample was excited at 405 nm and the emission spectra were recorded. The emission associated with the lysis buffer alone was subtracted and the spectra were normalized by total protein. GraphPad Prism was used to calculate the total area under the curve (AUC) of each spectrum.

### **Platereader assay by TFCH**

A549 cells were grown in standard media or SFM for  $\sim$ 24 h. Media were then discarded and the respective media containing 1, 2, 5, or 10  $\mu$ M TFCH in 0.5% DMSO (v/v) were added and incubated for 1.5 h. The cells were rinsed once with Dulbecco's phosphate buffered saline (DPBS) and the fluorescence of the samples in DPBS were read in a Synergy Mx microplate reader (BioTek). The samples were excited at 405 nm and the emission was measured at 525 nm.

The cells were then subjected to a sulforhodamine B (SRB) assay, as described before<sup>2</sup>. Optical density (OD) was recorded at 570 nm ( $OD_{570}$ ), which was used to represent cell number. The fluorescence intensity recorded at 525 nm ( $F_{525}$ ), representing TFCH-associated carbonyls, was then normalized to account for variable cell densities by taking the ratio of  $F_{525}$  and  $OD_{570}$ . The background signal generated by the samples treated with vehicle (0.5% DMSO, v/v) was subtracted from the signal of those treated with TFCH. Two independent experiments, each with six intra-experimental replicates, were performed. The average of the control samples treated with 1  $\mu$ M TFCH was set to 100 in each independent experiment. The graph shows the mean  $\pm$  SEM of the fluorescence of the sample (mean of 12 wells) relative to the average of the control treated with 1  $\mu$ M TFCH.

The fold increase in fluorescence was calculated as the ratio of signal generated in the serum starved samples and the unstarved control samples. The data in the corresponding table are reported as mean  $\pm$  SEM of experimental replicates.



## **TFCH-mediated detection and visualization of cellular carbonyls generated by different models of oxidative stress**

### **SFM-induced**

Control (cells grown in standard media) or serum-starved (cells grown in SFM) A549 cells were incubated with 2  $\mu\text{M}$  TFCH in 0.5% DMSO for 30 min and imaged immediately, without washing out excess fluorophore<sup>3</sup>.

In another set, A549 cells were grown in standard media or SFM for 24 h followed by incubation with 20  $\mu\text{M}$  TFCH in 0.5% DMSO for 30 min. The cells were washed and imaged live at room temperature within 20 min after fluorophore incubation using a Zeiss confocal microscope. Ex: 405 nm; Em: LP 420.

In a different set, the control and the serum starved (24 h) cells (A549, MDCK, LLC-PK1) were allowed to react with 20  $\mu\text{M}$  TFCH for 40-60 min. The cells were then rinsed with PBS and fixed with 4% paraformaldehyde in PBS (PFA solution) for 15 min. The cells were washed twice more with PBS, mounted with ProLong<sup>TM</sup> Gold antifade mountant (ThermoFisher Scientific), and cured overnight. The cells were imaged using a confocal microscope.

In an independent experimental set, MDCK cells were incubated with standard media (control) or SFM for 1.5 h and then allowed to react with 20  $\mu\text{M}$  CH, BzCH, DCCH or TFCH for 30 min. The cells were rinsed, fixed, processed, and imaged as described previously. CH used in this work was either purchased from Cayman Chemicals or synthesized in the Bane lab<sup>2</sup>, BzCH was synthesized in the Bane lab<sup>4</sup>, and DCCH was purchased from Sigma.

### **Hydrogen peroxide-induced**

MDCK or LLC-PK1 cells were treated with vehicle (water) or 400  $\mu\text{M}$  hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) for 2 h. The media was replaced with fresh media containing 20  $\mu\text{M}$  TFCH in 0.5% DMSO (v/v). After a 30 min incubation, the cells were washed, fixed, processed, and imaged as described previously.

### **Menadione-induced**

MDCK or LLC-PK1 cells were treated with vehicle (DMSO, 0.05% v/v) or 100  $\mu\text{M}$  menadione for 1 h prior to the addition of 20  $\mu\text{M}$  TFCH in 0.5% DMSO (v/v). After 30 min, the cells were washed, fixed, mounted, and imaged as described previously.

### **Gentamicin and Cisplatin-induced**

LLC-PK1 or MDCK cells grown on collagen-coated coverglasses were treated with 0.15  $\mu\text{g}/\text{mL}$  of cisplatin (*cis*-Diamineplatinum(II) dichloride, Sigma) or 0.58  $\text{mg}/\text{mL}$  of gentamicin (gentamicin sulfate salt, Sigma) for 24 h. A final concentration of 20  $\mu\text{M}$  TFCH in 0.5% (v/v) DMSO was added to the samples and incubated for 40-60 min before washing, fixing, processing, and imaging. Cells that did not receive any drug treatment were used as a control.

In another experimental set, LLC-PK1 or MDCK cells were allowed to grow for 3 h in the presence of stated concentrations of cisplatin (Cis) or gentamicin (Gen). TFCH (20  $\mu$ M) was then added to the medium for an additional 60 min, washed, fixed, and processed before imaging.

### **CellROX Green-mediated detection of reactive oxygen species (ROS) in different models of oxidative stress**

All treatment methods employed for preparing the samples for subsequent staining with CellROX Green (ThermoFisher Scientific) were the same as that of TFCH. The CellROX Green staining was performed according to the manufacturer's instructions. Briefly, the cells were treated with 5  $\mu$ M CellROX Green for 30 min, washed, fixed, processed (as described previously) before imaging.

### **Image acquisition, quantification and analysis for cell-based assays using TFCH and CellROX Green**

Cells stained with TFCH were excited using the 405 nm laser and the emission was monitored using LP420 or between 420-700 nm. Cells stained with CellROX Green were excited using the 488 nm laser and emission was monitored using LP505.

All images were obtained using a Zeiss or a Leica confocal microscope. For visual clarity, the photomicrographs were processed using identical parameters for each cell line within an experimental set. Thresholded photomicrographs were used for quantification of signal/cell (IntDen/cell) using Fiji/ImageJ (NIH). Data are reported as mean  $\pm$  SEM of signal per cell relative to the average of the control. The average of the control in each experimental set was set to 100. Data from a minimum of 2 experimental sets, with a minimum of 30 cells per sample per set, are reported in the graph. Where indicated, a two-tailed unpaired t-test with Welch's correction or a one-way ANOVA with Dunnett's multiple comparisons test was performed using GraphPad Prism to compare each treatment with the control. The percent difference in mean and standard error was also calculated using GraphPad Prism. \* $p$ <0.05, \*\* $p$ <0.01, \*\*\* $p$ <0.001, \*\*\*\* $p$ <0.0001, and not significant (ns) was  $p$ >0.05.

### **Resazurin-based assay**

Cisplatin or gentamicin drug stocks were prepared fresh in appropriate cell culture media prior to each experiment. LLC-PK1 or MDCK cells grown on collagen-coated 96-well plates (100  $\mu$ L media/well) were treated with only media (control), cisplatin or gentamicin by adding an additional 100  $\mu$ L of fresh media supplemented with or without drugs. In parallel, a separate set of wells that did not contain cells were similarly treated for background subtraction. After 20-22 h, the cell viability assay was initiated using the *In vitro* toxicology assay kit, Resazurin based (Sigma-Aldrich) following the manufacturer's instruction. In brief, 22  $\mu$ L of the reagent was added to each well and incubated for 4 h. The samples were excited at 560 nm and emission was recorded at 590 nm using a Cytation 5 imaging reader (BioTek). Emission of the treated wells without cells was subtracted from the emission of those containing cells. Each experimental

set consisted of control cells (no drug treatment), and treatment with cisplatin or gentamicin. The graph represents the effect of drugs on cell viability relative to the control (without drug treatment). The average of the control in each experimental set was set to 100%. Error bars show SEM. Where indicated, an unpaired t-test with Welch's correction was performed and the percent difference in mean and standard error was calculated using GraphPad Prism.

### **SRB-based assay**

The drug treatment was performed as described in the resazurin-based assay section. After 24 h incubation with different drug concentrations, a SRB-based assay was performed as described before with minor modifications<sup>2</sup>. Experimental layout and data presentation are the same as that of the resazurin-based assay. Where indicated, an unpaired t-test with Welch's correction was performed and the percent difference in mean and standard error was calculated using GraphPad Prism.

### **Effect of TFCH on cell viability**

MDCK cells were treated with only media (control), vehicle (0.5%, v/v, DMSO) or TFCH (20  $\mu$ M, the highest concentration used) for 24 h before performing a resazurin- or SRB-based assay. One-way ANOVA with Dunnett's multiple comparisons test was performed to compare each treatment with the control using GraphPad Prism.

### **Actin and ZO-1**

LLC-PK1 and MDCK cells grown on collagen-coated coverglasses were treated with different concentrations of drugs. After 24 h, the samples were processed for immunocytochemistry to assess the status of ZO-1 and actin. A polyclonal ZO-1 antibody (ThermoFisher Scientific), followed by AlexaFlour 488 goat anti-rabbit IgG (H+L), was used to stain ZO-1. Subsequently, the cells were stained with Rhodamine Phalloidin and Hoechst 33342 to probe for F-actin and the nucleus, respectively. Cells were imaged using a Zeiss confocal microscope. The intensity of actin-stress fibers within each cell (IntDen/cell) was quantified by Fiji/ImageJ (NIH) in thresholded images. The intensity (IntDen) of cell membrane-associated ZO-1 was quantified by selecting a fixed region of interest drawn with the "rectangle" tool. Emission channels representing actin and ZO-1 staining are presented in the figure. For visual clarity, the photomicrographs were processed using identical parameters for each cell line within an experimental set. Graphs represent the intensity of actin stress fiber per cell or ZO-1 intensity, both relative to the control. The average of the control was set to 100. An unpaired t-test with Welch's correction was performed and the percent difference in mean and standard error was calculated using GraphPad Prism.

### Detection of carbonyls in rat tissue slices

Male Sprague-Dawley rats were purchased from Charles River Laboratories. Rat kidney slices were prepared and maintained live as described elsewhere with minor modifications<sup>5</sup>. The kidney tissue slices were treated with different concentrations of cisplatin (37.5, 75, 150 µg/ mL) or gentamicin (1.2, 2.3, 4.6 mg/mL) in HBSS. After 1 h, TFCH (final concentration: 2 µM) was added to each sample and incubated for 30 min at 37 °C. Multiple control samples were processed in parallel. To assess the basal level of carbonyls in the kidney slices, untreated (no drug) samples incubated with TFCH (final concentration: 2 µM) were used. To ensure that the fluorescence detected was not contributed by residual drug itself, tissue samples were exposed to the highest concentrations of cisplatin or gentamicin used and treated with DMSO (vehicle of TFCH, 0.5%, v/v) for 30 min. At the end of the fluorophore or vehicle treatment, all samples were washed with PBS and incubated with a periodate-lysine paraformaldehyde (PLP) fixative for 20 min. The samples were washed 4-5 times with PBS before mounting with ProLong<sup>TM</sup> Gold antifade mountant (ThermoFisher Scientific) and cured overnight. The samples were imaged using a Zeiss confocal microscope. Where indicated, representative photomicrographs were assembled (stitched) from multiple sections of the renal cortex. For visual clarity, the photomicrographs presented in a set were processed using identical parameters unless otherwise mentioned.

Thresholded photomicrographs of untreated (control), cisplatin (150 µg/ mL), or gentamicin (4.6 mg/mL) treated kidney slices were used for quantification of renal tubule-associated fluorescence. The fluorescence intensity (IntDen) of the renal tubule was quantified by selecting a fixed region of interest drawn with the “rectangle” tool in Fiji/ImageJ software. Data are reported as mean ± SD of signal relative to the average of the control. The average of the control was set to 100. A two-tailed unpaired t-test with Welch’s correction was performed using GraphPad Prism. Based on this analysis, \*\*\*\* $p < 0.0001$ .

Animal experiments were approved by the institutional committee on Research Animal Care, in accordance with the National Institutes of Health guide for the care and use of the laboratory animals.

### References:

1. Aryal, B.; Rao, V. A., Specific protein carbonylation in human breast cancer tissue compared to adjacent healthy epithelial tissue. *PLoS One* **2018**, *13* (3), e0194164.
2. Mukherjee, K.; Chio, T. I.; Sackett, D. L.; Bane, S. L., Detection of oxidative stress-induced carbonylation in live mammalian cells. *Free Radic Biol Med* **2015**, *84*, 11-21.
3. Mukherjee, K.; Chio, T. I.; Bane, S. L., Visualization of oxidative stress-induced carbonylation in live mammalian cells. *Methods Enzymol* **2020**, *641*, 165-181.
4. Mukherjee, K.; Chio, T. I.; Gu, H.; Banerjee, A.; Sorrentino, A. M.; Sackett, D. L.; Bane, S. L., Benzocoumarin Hydrazine: A Large Stokes Shift Fluorogenic Sensor for Detecting Carbonyls in Isolated Biomolecules and in Live Cells. *ACS Sensors* **2017**, *2* (1), 128-134.
5. Bouley, R.; Breton, S.; Sun, T.; McLaughlin, M.; Nsumu, N. N.; Lin, H. Y.; Ausiello, D. A.; Brown, D., Nitric oxide and atrial natriuretic factor stimulate cGMP-dependent membrane insertion of aquaporin 2 in renal epithelial cells. *J Clin Invest* **2000**, *106* (9), 1115-26.

