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1. Supplementary Methods

Cell culture conditions for permeability studies and high throughput screening

HPTEC medium was supplemented with 100 IU/ml penicillin, 100 µg/ml streptomycin, 36 ng/ml hydrocortisone, 10 ng/m epidermal growth factor, 1% insulin-transferrin-selenium and 4 pg/ml triiodothyronin on collagen coated tissue culture plates (6 µg/cm^2) at 37°C in a humidified 5% CO_2 incubator. HK-2 medium was supplemented with 0.05 mg/ml bovine pituitary extract (BPE) and 5 ng/ml human recombinant epidermal growth factor (EGF). The medium was replaced one day after seeding and then every second day. For permeability studies, the cells were seeded on HTS transwell (Corning Life Science) according to manufacturer's protocol. The cells were cultured on Poly-D-Lysine coated black clear bottom 384-well plate (Corning Life Science) for high throughput screening (HTS) experiments.

Live cell imaging and measurement of oxidative stress (ROS) and number of dead cells

Automated live-cell, multi-color image acquisition was performed on an Operetta® High Content Imaging System (Perkin Elmer, Walthalm, MA) using a 20X objective. Quantitative image analysis was performed using Columbus 2.4.2 Software (Perkin Elmer, Walthalm, MA). Evaluated measurements included the texture of the cells in digital phase contrast images (exposure time 50 ms, normalization by Kernel, SER Edge-filtered, 1px), mean ROS intensity per well (max. excitation/ emission wavelength: 500/ 550 nm, exposure time 60 ms), mean number of TOTO-3 Iodine positive stained cells per well (max. excitation/ emission wavelength: 642/ 660 nm, exposure time 60 ms), and mean TMRM intensity per well (max. excitation/ emission wavelength: 577/ 590 nm, exposure time 60 ms). The average of these numerical measurements from four biological replicates (two wells per condition, two fields per well) was normalized to the average of the 0.5% DMSO treated control wells.

Gene expression profiling using L1000 platform

The method involves ligation-mediated amplification (LMA) using locus specific probes engineered to contain unique molecular barcodes, universal biotinylated primers, and 5.6-micron optically-addressed polystyrene microspheres coupled to capture probes complementary to the barcode sequences. Specific probes were annealed to HPTEC cDNA, ligated by a taq ligase, amplified, and then hybridized to barcoded Luminex beads. Beads are analyzed using Luminex FLEXMAP 3D® technology by identifying specific bead region and measuring the density of the hybridized probes on each bead with laser beams. Data were calibrated and normalized to 80 invariantly expressed transcripts in each plate.

Immunofluorescence staining

All primary antibodies were monoclonal, apart from anti-AQP1 (polyclonal) and were used at following dilutions: mouse anti-ZO-1 (1:100), mouse anti-N-cadherin (1:50), mouse anti-cytokeratin 18 (1:100), rat anti-E-cadherin (1:50), and mouse anti-HO-1 (1:100, 1:50). The cells were exposed with either FITC-conjugated donkey anti-rat IgG, or Alexa Fluor 488-conjugated donkey anti-mouse IgG at a dilution of 1:200 for 1h at room temperature. Cells were mounted with Vectashield-DAPI mounting medium (Vector Laboratories) or DAPI solution (10 µg/ml in PBS) and examined by ECLIPSE 90i microscope (Nikon Instruments Inc.) and ImageXpress Micro (Molecular Device, Sunnyvale, CA).

Semiquantitative and real-time PCR

Total RNA was isolated using QIAshredder™ and RNeasy® Mini Kit (Qiagen) and transcribed into cDNA using QuantiTect Reverse Transcription Kit (Qiagen). Semiquantitative PCR was performed with HotStarTaq Master Mix Kit (Qiagen) using a C1000™ Thermal Cycler (Bio-Rad Laboratories) under standard cycling conditions. The PCR products were separated by electrophoresis on a 1.5% agarose gel containing 0.5 µg ethidium bromide per ml and visualized with a BioRad Imager (Biorad) under UV light. Real-time PCR (qPCR) was carried out on a CFX96 Touch™ PCR Detection System (Bio-Rad Laboratories) using QuantiFast®SYBR® Green PCR Kit (Qiagen). All samples were measured in duplicates and normalized against GAPDH. Changes in the mRNA expression were calculated using the

ΔΔCt method relative to 0.5% dimethyl sulfoxide (DMSO) control. Primers for specific genes were designed using MacVector software (MacVector Inc., Cary, NC) and are listed in Supplementary Table 2.

Transport Activity

The integrity of the cell monolayer was determined by measuring the transepithelial electrical resistance (TEER) using the Millicell® ERS-2 meter (Millipore). TEER of cell-free filter was subtracted from all samples and the values are expressed in ohms per square centimeter. Confluent monolayers were used to evaluate the permeability and transport properties of HPTECs. The amount of [^{14}C]-sucrose was calculated as apical to basolateral (A-B) or basolateral to apical (B-A) transport. Fluxes across the monolayers were calculated as nmol/well. For the flow cytometer based measurement of rhodamine 123m a minimum of 20,000 events were collected per sample, and the samples were gated on forward scatter versus side scatter to exclude clumps and debris. Amount of glucose transport was measured after 5, 15, 30, 45, 60, 90, and 120 min of incubation with 100, 200, and 500 μM 2-NBDG (2-(N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)-2-deoxyglucose). Cells were lysed with 0.1 mL of cell lysis buffer (1 % sodium deoxycholate, 40 mM KCl, and 20 mM Tris [pH 7.4]) for 10min at RT and 90μl supernatant was transferred into black 96-well plates. Fluorescence of 2-NBDG was measured at 485/528 nm (ex/em) using a Synergy H1 microplate reader (BioTek Instruments, Inc.) and the amount of the intracellular concentration of 2-NBDG was calculated based on a standard curve. 2-NBDG concentration was normalized to DNA content after adding 1 μg/ml Hoechst into each well and measurement of the fluorescent intensity at 360/460 nm (ex/em).

Enzymatic Assays

γ-Glutamyl transferase (GGT) activity was determined by the measurement of p-nitroaniline (pNA) released from the L-γ-glutamyl-pNA substrate using a colorimetric assay from Biovision (Milpitas, CA). Cells were treated with 25 or 50 μM $CdCl_2$ for 24 h and the release of pNA was measured spectrophotometrically at 418 nm every 5 min for up to 2 h using the SpectraMax Paradigm microplate reader (Molecular Devices). GGT activity is expressed as nanomoles of pNA per minute and milligram of

protein. Glutathione (GSH) levels were measured with GSH-Glo assay (Promega Corporation) in cells treated with 10 or 50 μM $CdCl_2$ for 6, 12 and 24 h. The luminescent signal was read using the luminometer (Turner Biosystems), and the amount of GSH was calculated based on the standard curve. All experiments was performed in triplicate and repeated three times independently.

Computational data mining of rat Toxicogenomic databases

Association between Hmox1 and Havrc1 and renal pathology: Since multiple doses are often required to observe pathology, only data derived from DrugMatrix or TG-Gates animals dosed for more than one day were selected for analysis. There were 252 kidney samples in the DrugMatrix data set having both gene expression data and pathology assessment, dosed >1 day. Of the 252 samples with pathology determinations, 39 animals displayed "kidney_cortex, tubule, necrosis" (Supplementary File 1, tab 1). In the case of the TG-GATES data set, all >1 day kidney samples with a pathology determination of "kidney:necrosis:renal tubule", were analyzed. A total of 1856 samples met this standard (Supplementary File 1, tab 2). Twenty-two were marked positive for renal tubule necrosis and 1834 were negative (Supplementary File 1, tab 2). Prior to the analysis, Affymetrix microarray kidney gene expression sample files from the DrugMatrix and TG-GATEs were normalized separately using the RMA algorithm. The association between pathology, and *Hmox1 and Havrc1* expression were evaluated using a Welch test (unpaired, unequal variance t–test) in Genespring 12.6.

Association between Hmox1 and established transcriptional biomarkers of kidney toxicity: To determine the association between expression of *Hmox1* and other validated transcriptional, such as *Havrc1*, a linear regression was performed using all available Affymetrix kidney gene expression data from DrugMatrix and TG-GATEs. The regression analysis was performed in Microsoft Excel using the "regression" statistical function.

3D human kidney microphysiological system (MPS)

Cell culture: Epithelial cell culture media consisted of Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 (DMEM/F12; Life Technologies; CA), supplemented with insulin (10 µg/mL), transferrin (5.5 µg/mL), selenium (6.7 ng/mL), penicillin (100 units/mL), streptomycin (100 µg/mL), amphotericin B (0.25 µg/mL), and hydrocortisone (50 nM).

Cell seeding in Nortis Device: The metal blocking rod was removed from the device, and mouse collagen type IV (Corning Inc., Tewksbury, MA) at 5 µg/mL for 1 hour was used for priming the device lumen. Confluent PTECs were digested with 0.05% trypsin-EDTA to obtain single cell suspensions. The cells were washed, counted and resuspended at a concentration of 15-20 x 106 cells/mL.

2. Supplementary Figures

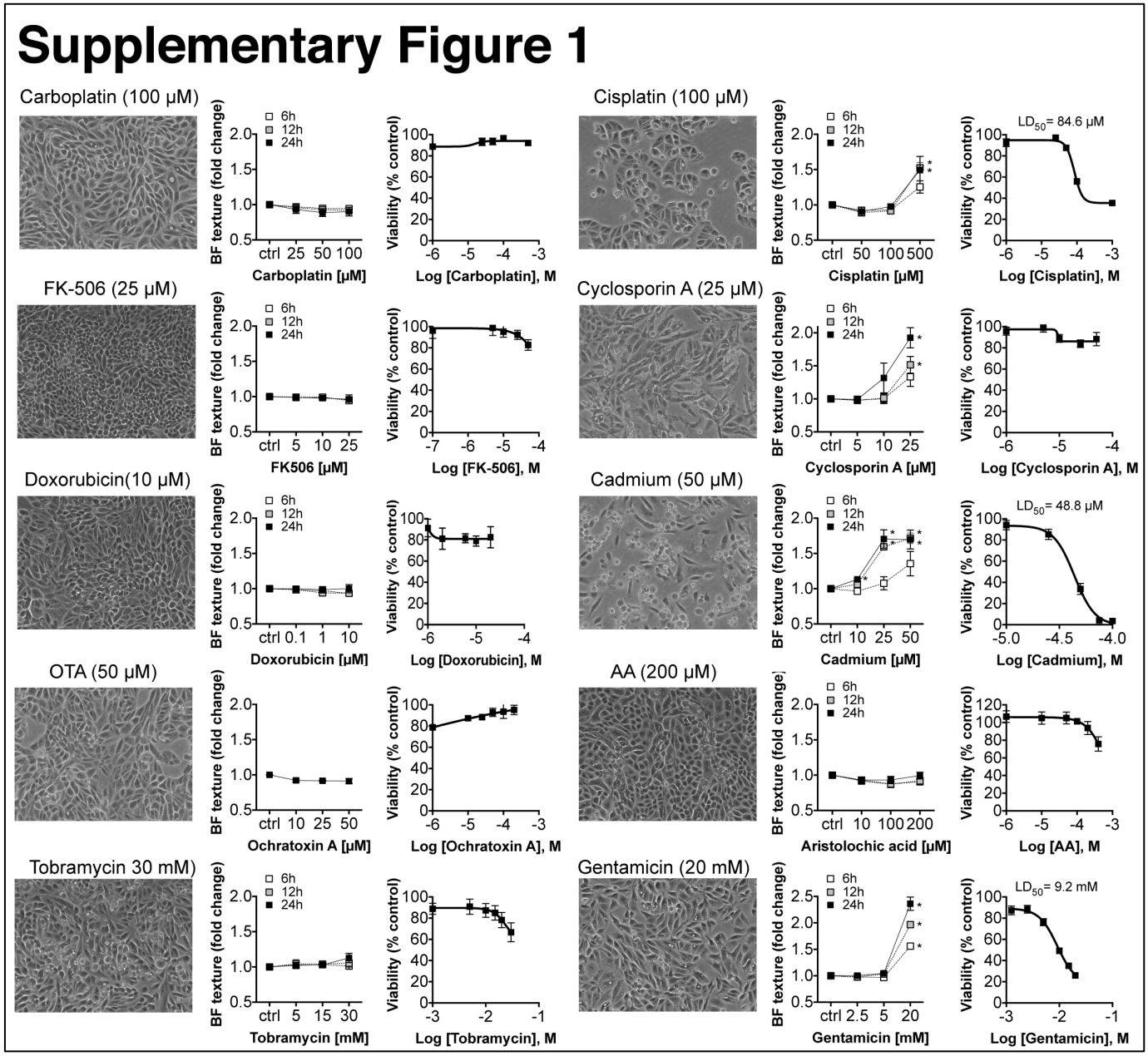

Figure S1. Dose response and time course data for cell viability following kidney toxic and non toxic compounds in HPTECs. Effects of nine nephrotoxic model compounds vs. non-toxic control carboplatin on morphology and cell viability were assessed using cellular imaging of cells and ATP measurement (CellTiterGlo assay). Images were acquired 24 h after treatment on Nikon TE2000 using 20x objective. Structural changes in HPTECs were assessed using texture based image analysis measuring the pattern corresponds to ‘edges’ parameter using Perkin Elmer Operetta platform after 6, 12

and 24 h (SER Edge, 1px; exposure time: 50 ms; magnification: 10x). Calculated SER edge values of triplicates in three independent experiments for each condition (six fields per well) were analyzed using Columbus 2.4.2 Software, and normalized to average of the 0.5% DMSO controls. Data are presented as mean ± SEM fold change (n=3, *p<0.05).

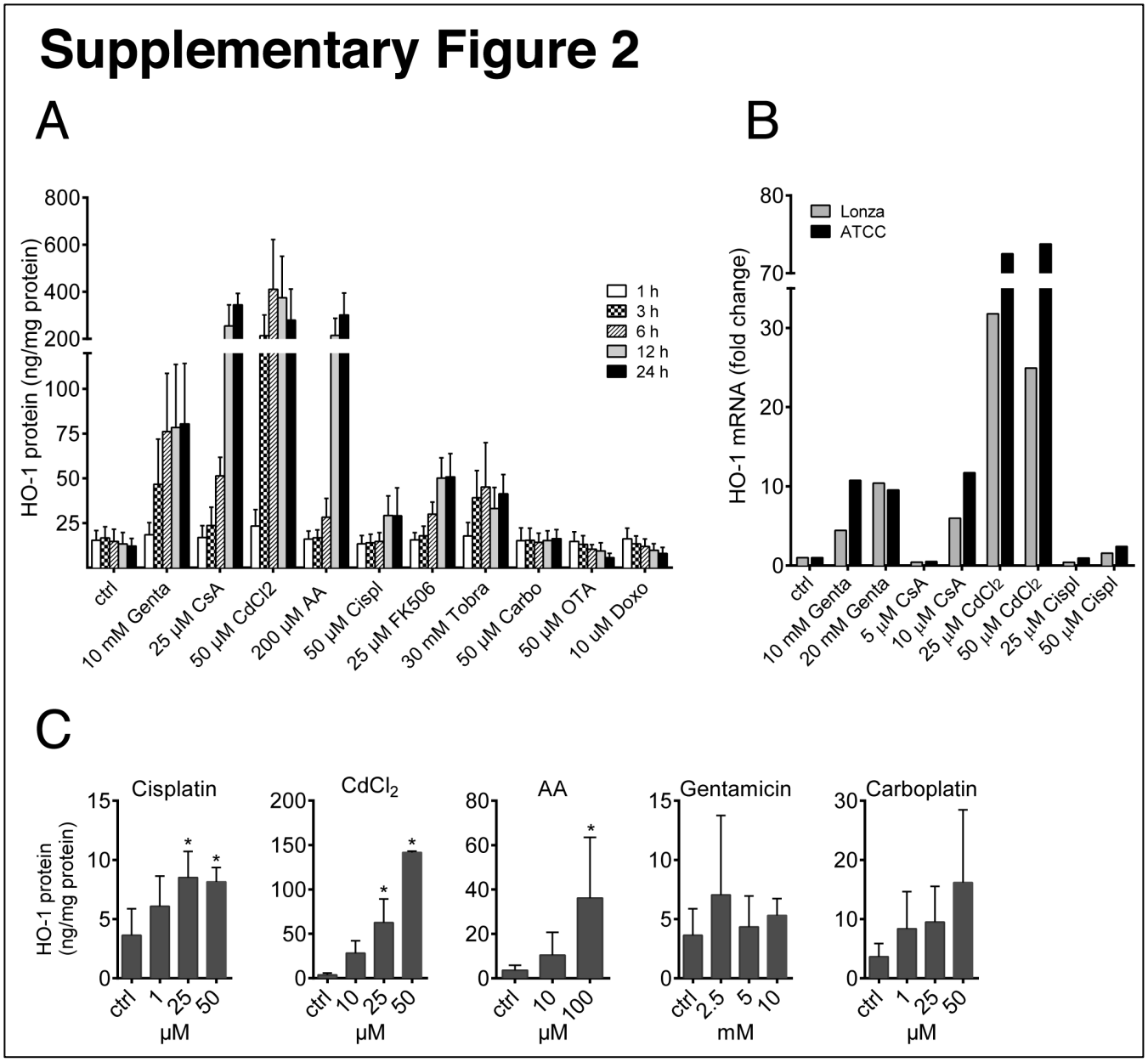

Figure S2. Additional HO-1 profiles. (A) HO-1 protein significantly increased in HPTECs in a time-dependent manner following exposure to diverse kidney toxic compounds. Changes of HO-1 protein were measured by ELISA and presented as mean±SD (n=3, *p<0.05). **(B)** Upregulation of HO-1 gene expression in primary human RPTEC from ATTC (0.5% FBS) and Lonza (0.5% FBS) was detected after incubation with gentamicin, CsA, cadmium chloride and cisplatin for 24 h. The fold change values compared to controls (0.5% DMSO) were calculated by normalizing the values against GAPDH. Results are presented from one single experiment. **(C)** Concentrations of HO-1 protein in HK-2 cells after

incubation with model compounds for 24h. Changes of HO-1 protein were measured by ELISA and presented as mean ±SD of 3 individual replicates measured in duplicate.

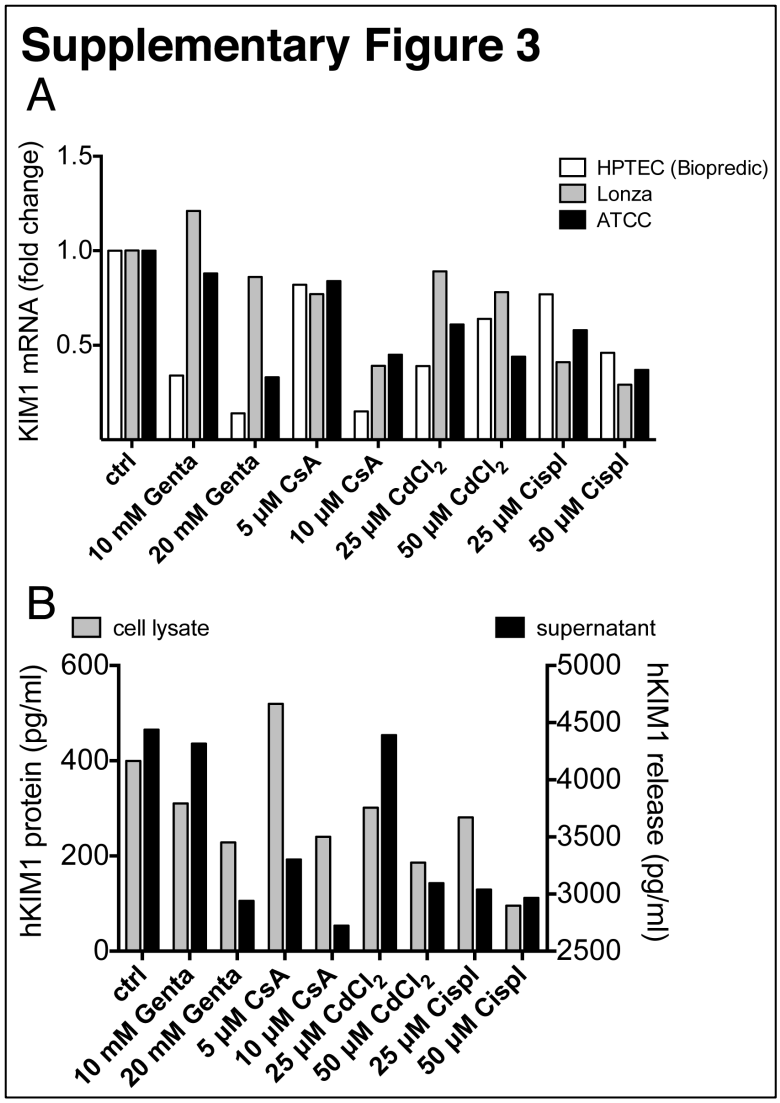

Figure S3. KIM-1 expression and secretion in proximal tubular epithelial cells after incubation with several nephrotoxic compounds. (A) KIM-1 mRNA expression was assessed by qPCR 24 h following the incubation of gentamicin, CsA, cadmium chloride, and cisplatin in HPTECs, primary human RPTEC from ATTC and Lonza and did not show a dose-dependent increase in response to a toxic stimulus. (B) KIM-1 protein level and its secretion into cell media of HPTEC, quantified using Luminex bead assay, also demonstrate that KIM-1, a well known biomarker for kidney injury *in vivo*, is not induced in cells cultured *in vitro* in correlation to cytotoxicity. Data are presented from one representative experiment.

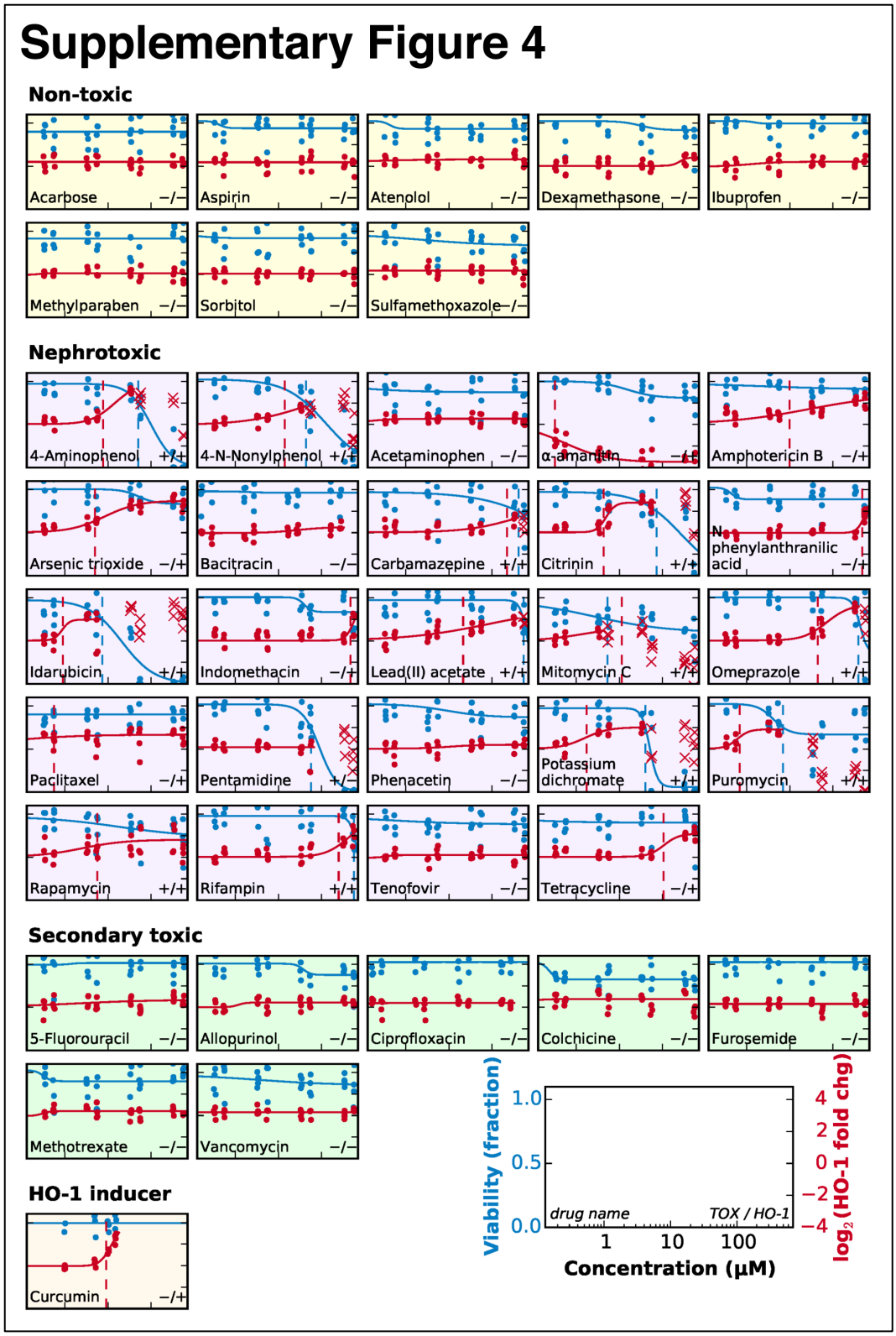

Figure S4. Raw cell viability and HO-1 induction data and fitted logistic curves for 39 drugs and Curcumin (a non-toxic HO-1 inducer). Blue and red vertical dotted lines indicate the IC25 and HO-1 (fold change of significant HO-1 induction), respectively. Crosses in the HO-1 induction data indicate

discarded data (see methods). Scales and labels are indicated on the schematic plot in the lower-right corner. The drugs are grouped by clinical toxicity classes using the colors from Figure 5D/E. Data are normalized to DMSO control.

3. Supplementary Tables

Table S1. List of compounds used in the study.

Discovery panel		
Non-toxic	**PT-toxic**	
Carboplatin	Aristolochic acid Cadmium chloride Cisplatin Cyclosporin A Doxorubicin Gentamicin Ochratoxin A Tacrolimus (FK-506) Tobramycin	
Validation panel		
Non-toxic	**PT-toxic**	**Sec. mech. toxic**
Acarbose Aspirin Atenolol Dexamethasone Ibuprofen Methylparaben Sorbitol Sulfamethoxazole	4-Aminophenol 4-N-Nonylphenol Acetaminophen Amanitin Amphotericin B Arsenic trioxide Bacitracin Carbamazepine Citrinin N-Phenylanthranilic acid Idarubicin Indomethacin Lead(II) acetate trihydrate Mitomycin C Omeprazole Paclitaxel Pentamidine isethionate Phenacetin Potassium dichromate Puromycin dihydrochloride Rapamycin Rifampin Tenofovir Tetracycline	5-Fluorouracil Allopurinol Ciprofloxacin Colchicine Furosemide Methotrexate Vancomycin

Table S2. List of primers used for RT-PCR.

GENE	F/R	SEQUENCE
cDNA specific primers used for semi-quantitiative PCR		
Kidney-specific cadherin (KSP)	F	5'-GGC TGT GGC TGC TTT GTG TC
	R	5'-CAC CTG GTC ATT CTC ATC CTT CAC
Megalin	F	5'-GCA TTT TCG CTG TGG AAG TGG
	R	5'-CGT GGT CGC ACC TGT ATT CAC
Aquaporin-1 *(AQP1)*	F	5'-ATC ACA CAC GAA TTC AGC AAC CAC
	R	5'-ACA CCC CCA TAT GAG GCT TGA C
Multidrug resistance protein 2 (MRP2)	F	5'-CAG ACC TGC CAC TTT GTT TTG AG
	R	5'-CGA GAG AAT CCA GAA TAG GGA CAG
P-glycoprotein (MDR1)	F	5'-ATG GTG GTG GGA ACT TTG GC
	R	5'-GAA ACC TGA ATG TAA GCA GCA ACC
Organic cation transporter 2 (OCT2)	F	5'-ATA CAG TTG GGC TCC TGG TG
	R	5'-GAG GCG GGT AGA GAT TTT CC
GAPDH	F	5'- GGA GCG AGA TCC CTC CAA AAT
	R	5'- GGC TGT TGT CAT ACT TCT CAT GG
cDNA specific primers used for real-time PCR		
Hemeoxygenase-1 (HMOX-1)	F	5'- CTT CAC CTT CCC CAA CAT TGC
	R	5'- TTC TAT CAC CCT CTG CCT GAC TGC
Kidney Injury Molecule-1 (KIM-1)	F	5'- CTG CAG GGA GCA ATA AGG AG
	R	5'- TCC AAA GGC CAT CTG AAG AC
GAPDH	F	5'- ATT GCC CTC AAC GAC CAC TTT G
	R	5'- TCT CTC TTC CTC TTG TGC TCT TGC

4. Data File S1: Computational Mining of the Toxicogenomic databases (UPLOADED SEPARATELY)