Supplementary:

Figure S1: Quntitative cytotoxicity% of LIVE/DEAD® staining

Figure S2: Negative Control for Immunodetection of AL-I adducts.

Figure S3: AA-I toxicity with or without hepatic metabolism in rat MPS.

Figure S4: AA-I induced NQO1 expression in human hepatocytes

Figure S5: AL-I-NOH treatment in human MPS

Figure S6: OAT1/3 uptake of AL-I-$NOSO_3$

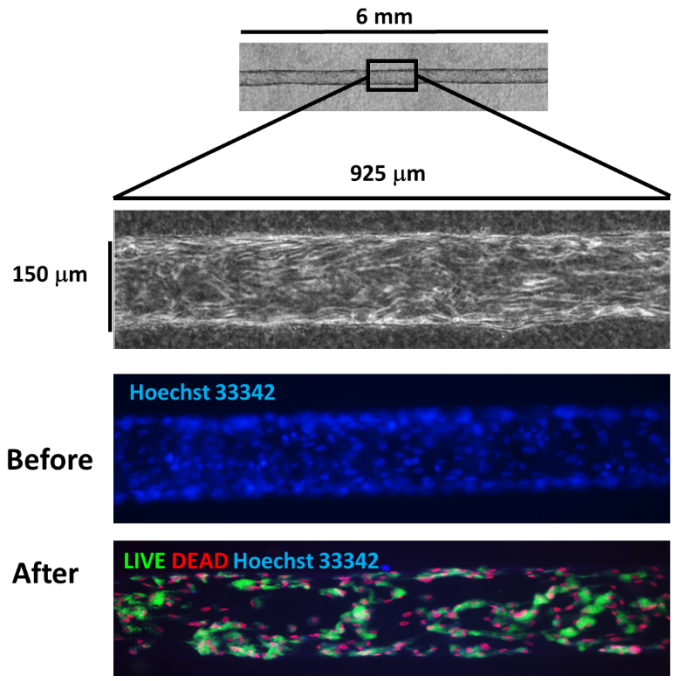
Figure S7: Mass Spec chromatograph of AL-I-$NOSO_3$

Table S1: Demographics information for human hepatocytes and PTEC donors

Table S2: The list of human cells used in different experiments

Figure S1. Quntitative cytotoxicity% of LIVE/DEAD® staining

The whole PTECs-formed kidney tubule with 95-100% confluence is 6 mm in length. A randomly picked image of kidney tubule under microscope with a 100x magnitude (Hoechst 33342 stained, 222 ± 5 rat cells vs. had 209 ± 9 human cells, N=5) in an area of 150 x 925 μm^2 (wide x length). All kidney MPS chips were checked for the confluency before the treatment. Only kidney MPS chips with 95-100% confluence can be allowed for the later treatment. After the treatment, LIVE/DEAD® staining was performed and imaged under microspore with a 100x magnitude from a randomly picked area of kidney tubule for assessing cytotoxicity. Cytotoxicity % was calculated from an equation of [# of dead cells + # of lost cells]/ [# of initial total cells] in a field with size of 150 x 925 μm^2.



$$\text{Cytotoxicity\%} = \frac{\#\text{ of Dead cells} + \#\text{ of lost cells } (\Delta\text{Hoechst 33342})}{\#\text{ of Original cells (Hoechst 33342)}}$$

Fig. S2: Negative control for immunodetection of AL- adducts in human kidney cells.
The protocol for AL-I-adduct detection was identical to Figure 2, with the exclusion of primary antibody.

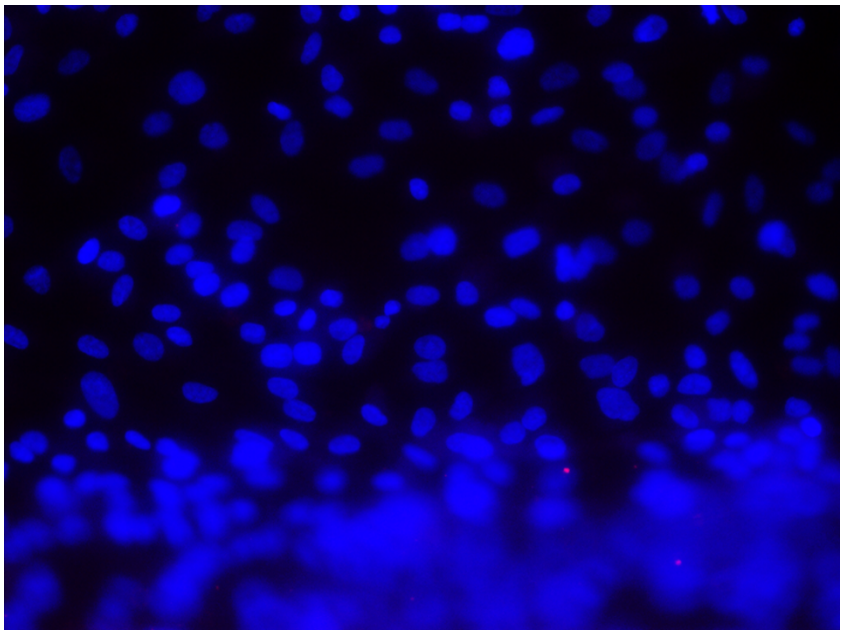
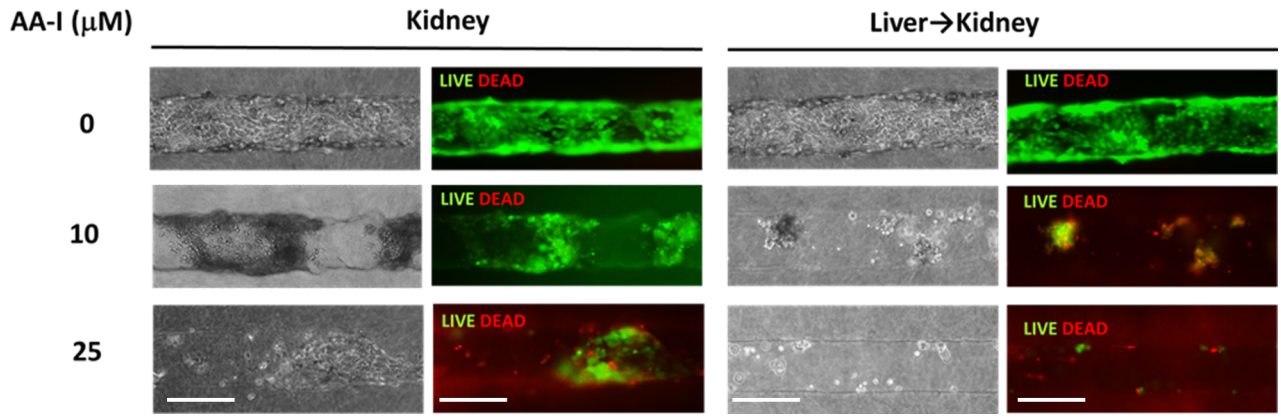
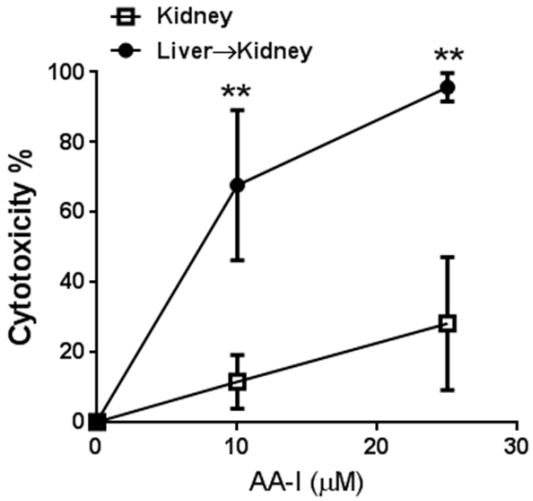


Fig. S3. AA-I rat nephrotoxicity with or without hepatic metabolism. **(A)** Representative phase contrast and LIVE/DEAD® images of rat PTECs cultured in MPS after AA-I treatment for 24 hours, (**B**) Quantitative cytotoxicity of images in A (N=4 per treatment group). **(C)** KIM-1 and ALT levels in rat MPS effluents (N=4 pertreatment group).**(D)** Representative LIVE/DEAD® staining of rat PTECs cultured in MPS after dicumarol (10 μM) and AA-I (25 μM) co-treatment for 24 hours (N= 5 per treatment). Quantitative results presented as average ± SD. All experiments were independently repeated at least three times. Statistical significance in (B) and (C) was calculated using t-test (*: $p<0.05$; **: $p< 0.01$). Bar in (A) and (D) = 150 μm.. Bar = 150 μm.

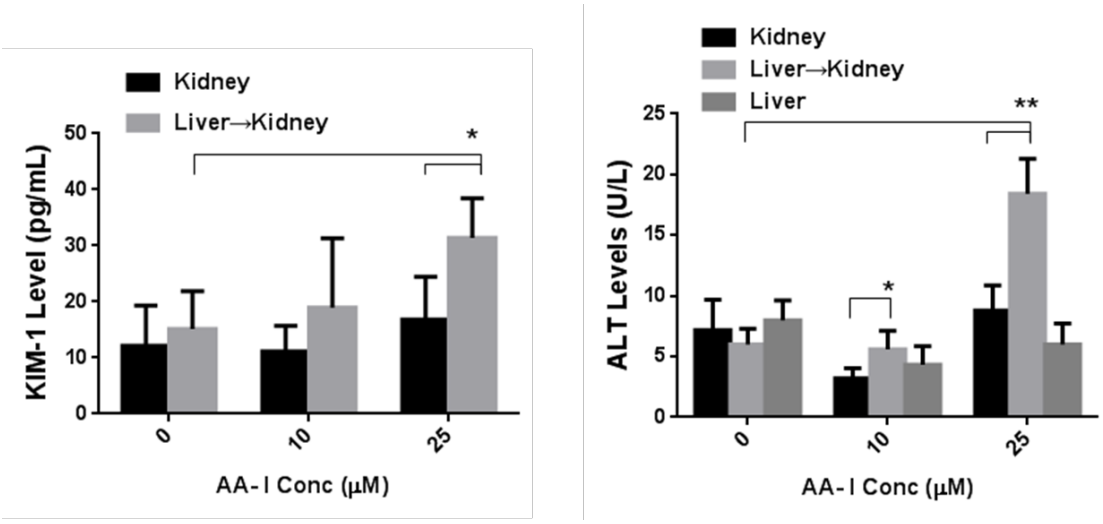
(A)



(B)



(C)


Kidney
Liver→Kidney
KIM-1 Level (pg/mL)
50
40
30
20
10
0
*
0
10
25
AA-I Conc (μM)
Kidney
Liver→Kidney
Liver
ALT Levels (U/L)
25
20
15
10
5
0
**
*
0
10
25
AA-I Conc (μM)

(D)

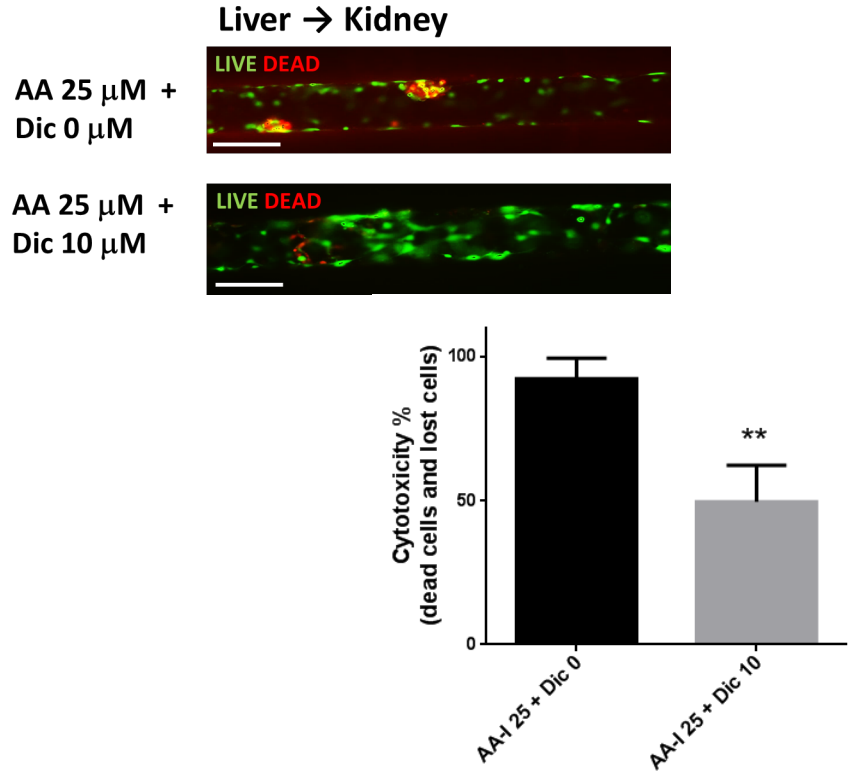

Liver → Kidney
AA 25 μM +
Dic 0 μM
LIVE DEAD
AA 25 μM +
Dic 10 μM
LIVE DEAD
Cytotoxicity %
(dead cells and lost cells)
100
50
0
**
AA-I 25 + Dic 0
AA-I 25 + Dic 10

Fig. S4: AA-I induced NQO1 expression in human hepatocytes. Representative ICC staining of NQO1 in human hepatocytes cultured in MPS with or without 25 μM AA-I treatment for 24 hours. N= 2 indepent experiments with 2MPS per group, per experiment.

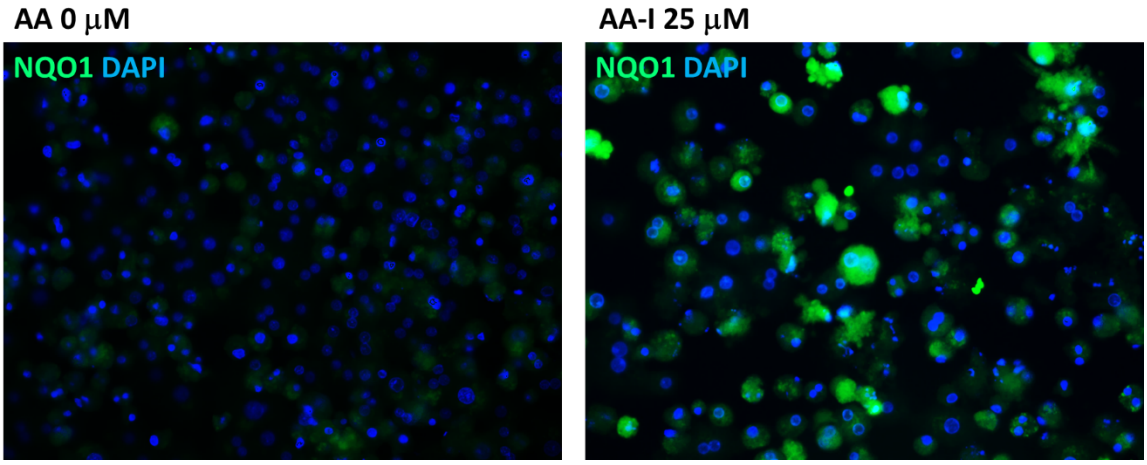
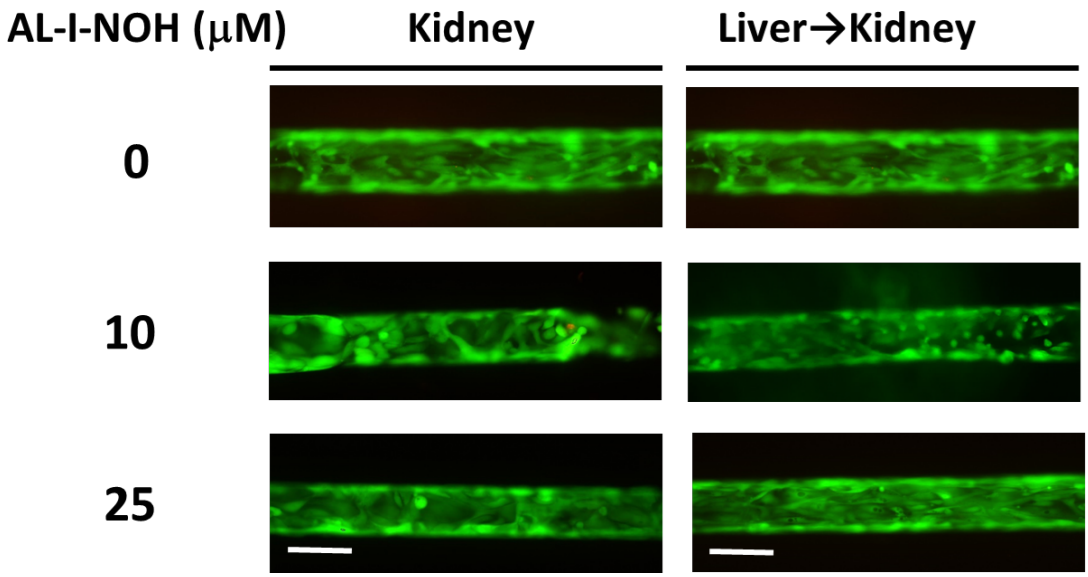


Fig. S5: AL-I-NOH treatment in human MPS. (A) Representative LIVE/DEAD® staining of PTECs cultured in MPS after AL-I-NOH treatment for 24 hours, and cytotoxicity% (N= 3). **(B)** Quantitative results presented of images in A (N=3 per treatment group).

(A)



(B)

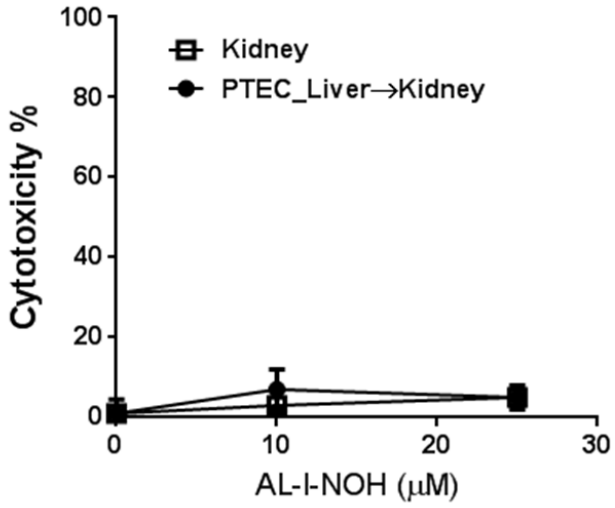


Figure S6: OAT1/3 uptake of AL-I-NOSO$_3$. Uptake of 0.5 μM AL-I-NOSO3 in OAT1 or OAT3Transfected HEK Cells vs. mock transfection in the presence of 2 mM Probenecid. Quantitative results are presented as average ± SD. Statistical significance was calculated using t-test (**: $p < 0.01$).

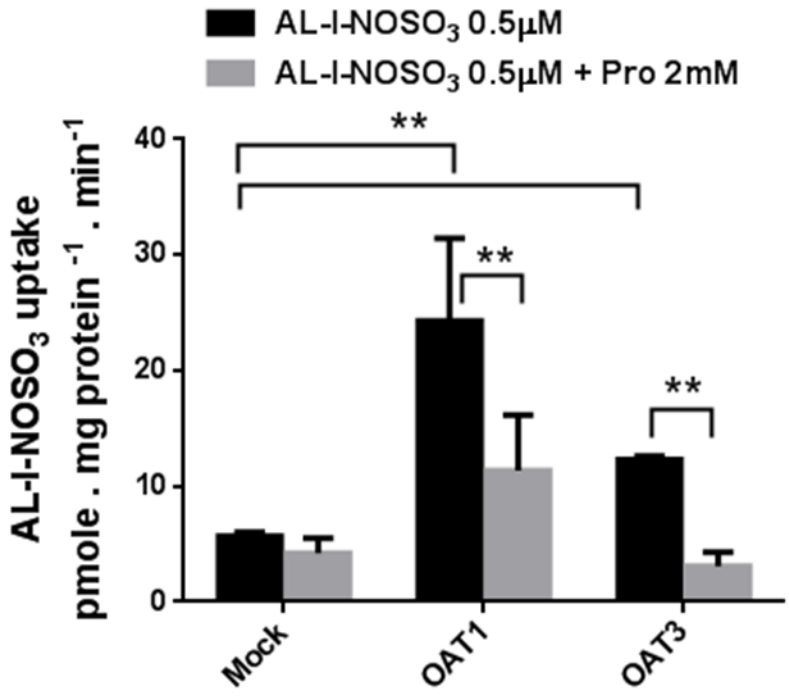


Fig. S7: Mass Spec chromatograph of AL-I-NOSO$_3$

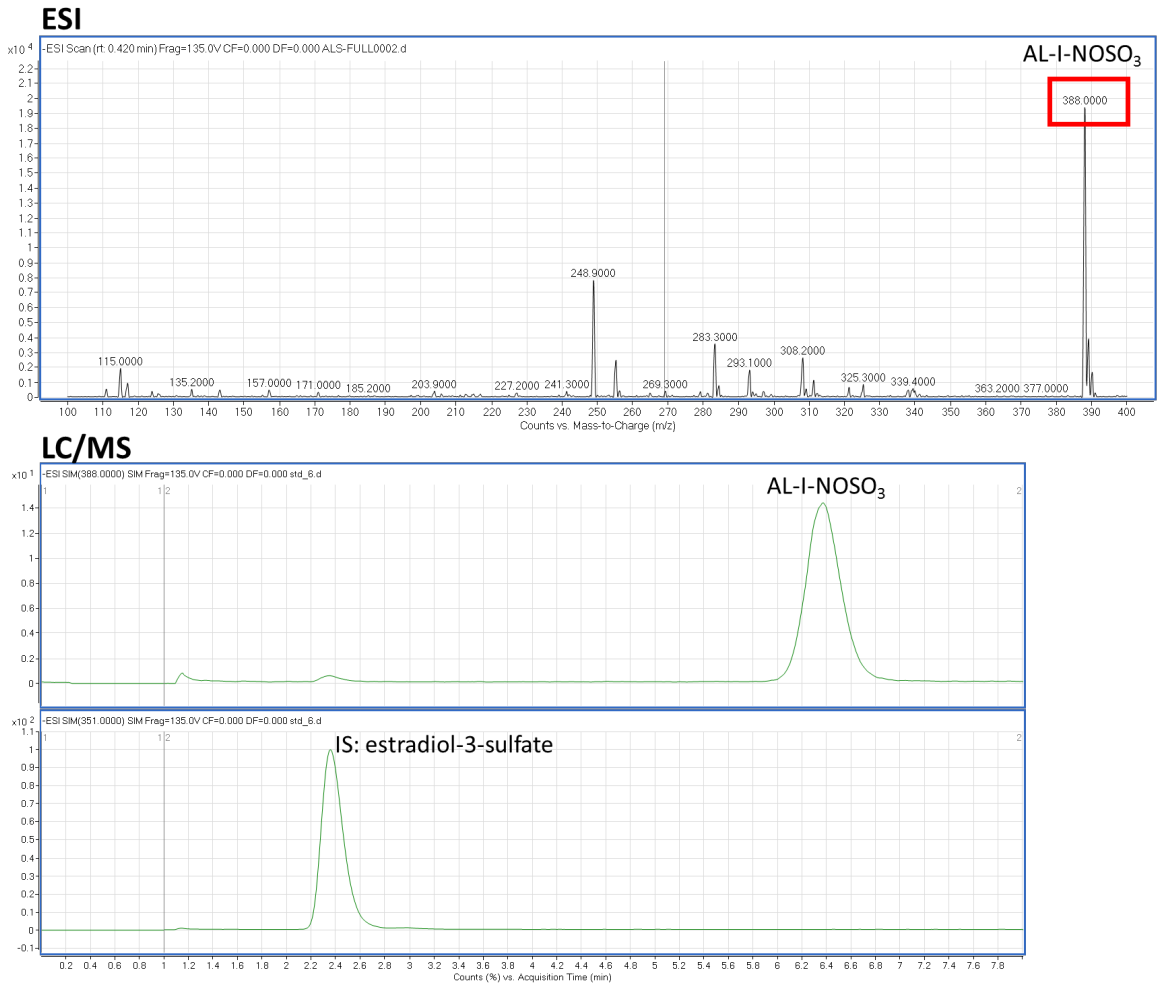


Table S1: Demographics information for human hepatocytes and PTEC donors

Human Cryopreserved Hepatocytes from TRL:

Sample Number	Age	Sex	Race	BMI	Tobacco Use	Alcohol Use	HIV, HBV, HCV Serology	Cause of Death
HUM4096A	34	Female	Caucasian	25	No	Yes	Negative	Anoxia
HUM4097A	53	Female	Caucasian	35	No	Yes	Negative	Anoxia

Human Fresh-isolated Hepatocytes from LTCDS:

Sample Number	Age	Sex	Race	Cause of Surgery
15-002	Femal	37	Caucasian	colon cancer mets to the liver. She has a history of chemo

Human PTEC:

PTEC ID#	Sex	Age	Race	Cause of Surgery
Him-20	Male	62	Caucasian	Transitional cell carcinoma - no kidney tumor.
Him-23	Male	62	Hispanic	Other active medical problems: HTN, DM, hyperlipidemia, but normal renal function
Him-25	Male	49	Caucasian	Presented with renal mass—path shows clear cell renal CA.
Bio 13	Male	57	Caucasian	No other active medical problems
Bio 26	Male	38	Caucasian	

Table S2: The list of human cells used in different experiments

Experiments	Cell Types	N=1	N=2	N=3	N=4	N=5	N=6
LIVE/DEAD® stain of AA-I treatment	Hepatocytes:	15-002	HUM 4096A	HUM 4096A	HUM 4097B		
	PTEC:	Him 20	Him 25	Him 25	Bio 13		
ICC of AL-I DNA Addcuts in AA-I treated	Hepatocytes:	HUM 4097B	HUM 4097B	HUM 4096A			
	PTEC:	Bio 13	Bio 26	Him 23			
KIM-I	Hepatocytes:	15-002	HUM 4096A	HUM 4096A	HUM 4096A	HUM 4096A	HUM 4096A
	PTEC:	Him 20	Him 25	Him 20	Him 25	Him 25	Him 25
LIVE/DEAD® stain of AA-I + Dic treatment	Hepatocytes:	HUM 4097B	HUM 4097B	HUM 4096A	HUM 4097B	HUM 4097B	
	PTEC:	Bio 26	Bio 26	Him 23	Bio 13	Bio 13	
LIVE/DEAD® stain of AL-I-NOSO3 treatment	Hepatocytes:	HUM 4097B	HUM 4097B	HUM 4096A	HUM 4097B	HUM 4097B	
	PTEC:	Bio 26	Bio 26	Him 23	Bio 13	Bio 13	
ICC of AL-I DNA Addcuts in AL-I-NOSO3 treated	Hepatocytes:	HUM 4097B	HUM 4097B	HUM 4097B			
	PTEC:	Bio 26	Bio 13	Bio 13			
LIVE/DEAD® stain of AL-I-NOSO3 + Pro treatment	Hepatocytes:	HUM 4097B	HUM 4097B	HUM 4096A	HUM 4097B	HUM 4097B	HUM 4097B
	PTEC:	Bio 26	Bio 26	Him 23	Bio 13	Bio 13	Bio 26