ASCCT-ESTIV & JSAAE Joint Webinar

Development of *in vitro* evaluation model for drug-induced proximal tubular injury using human proximal tubular epithelial cell spheroid

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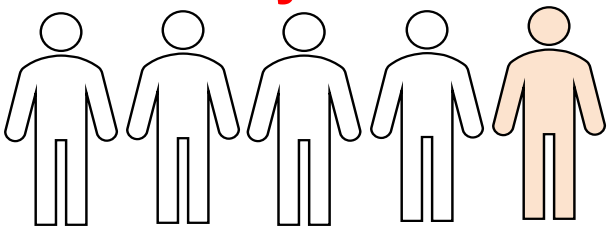
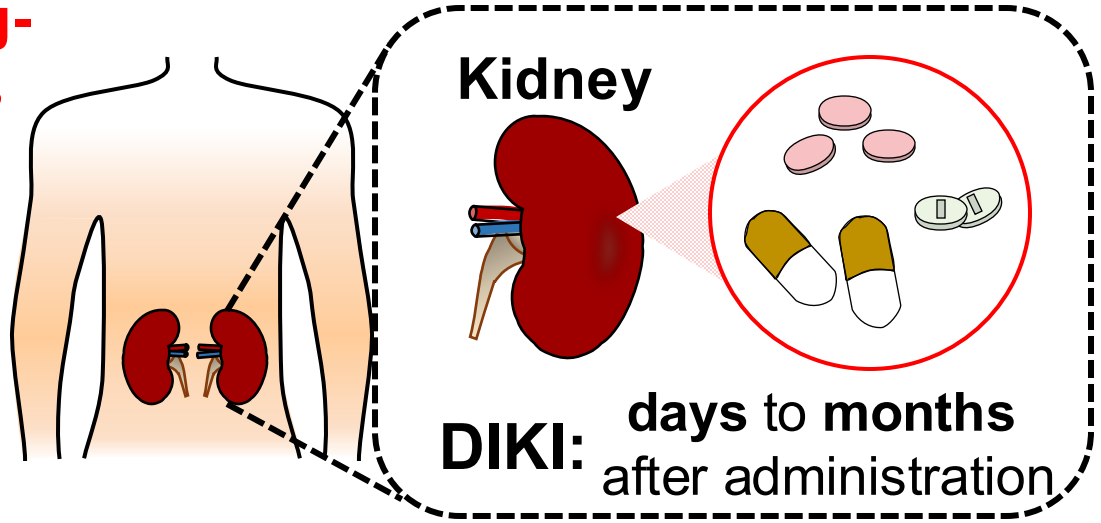
Drug-induced kidney injury (DIKI)

In clinical

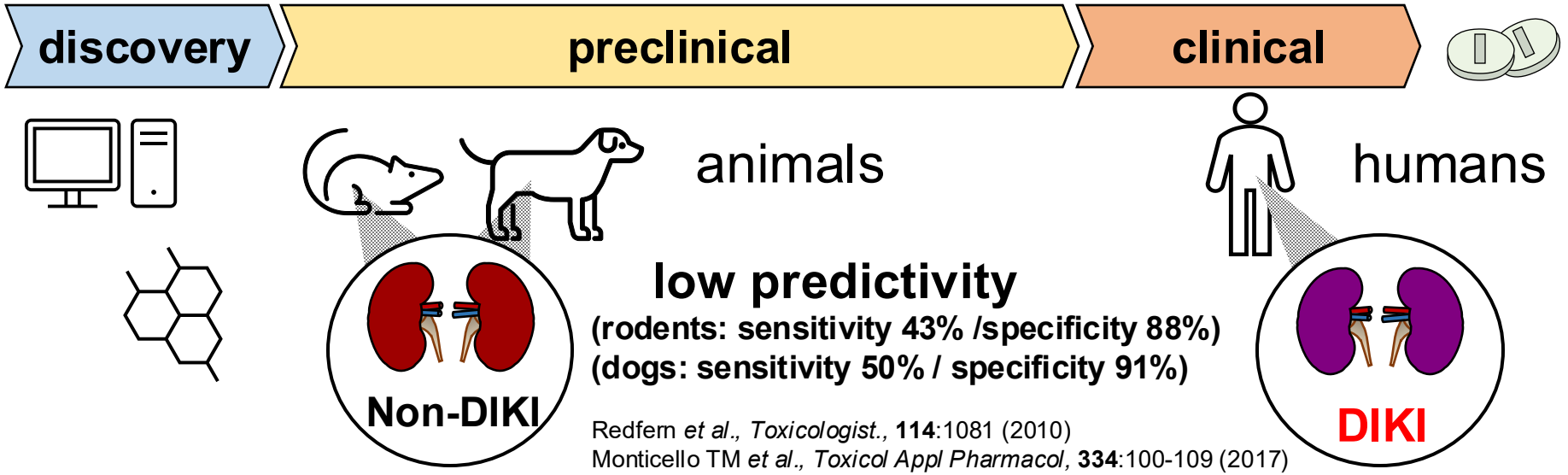
Twenty-five percent of drug-induced kidney damage is caused by medications.



Mehta *et al., Kidney Int.,* **66**(4):1613-21 (2004)

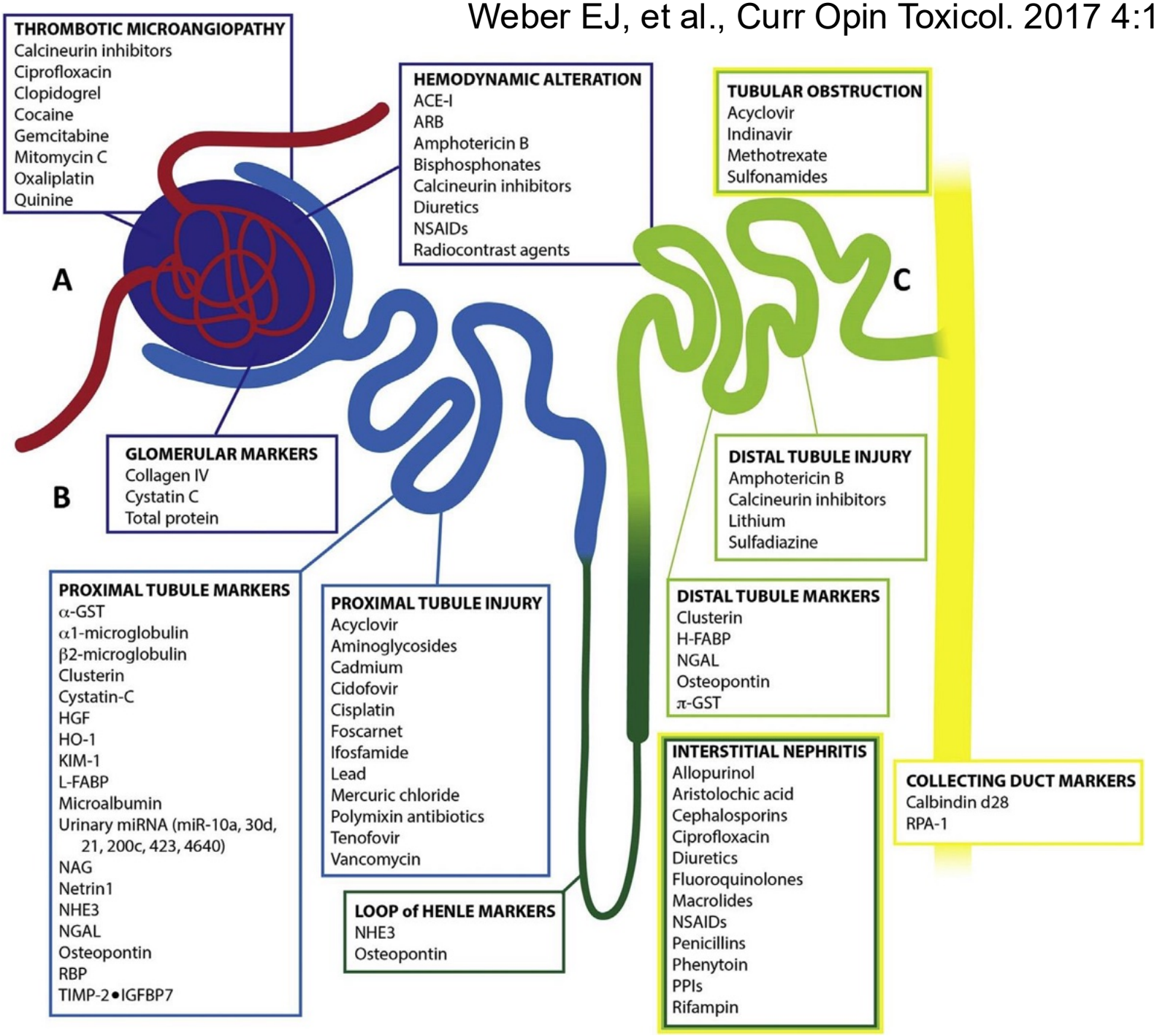


In drug development



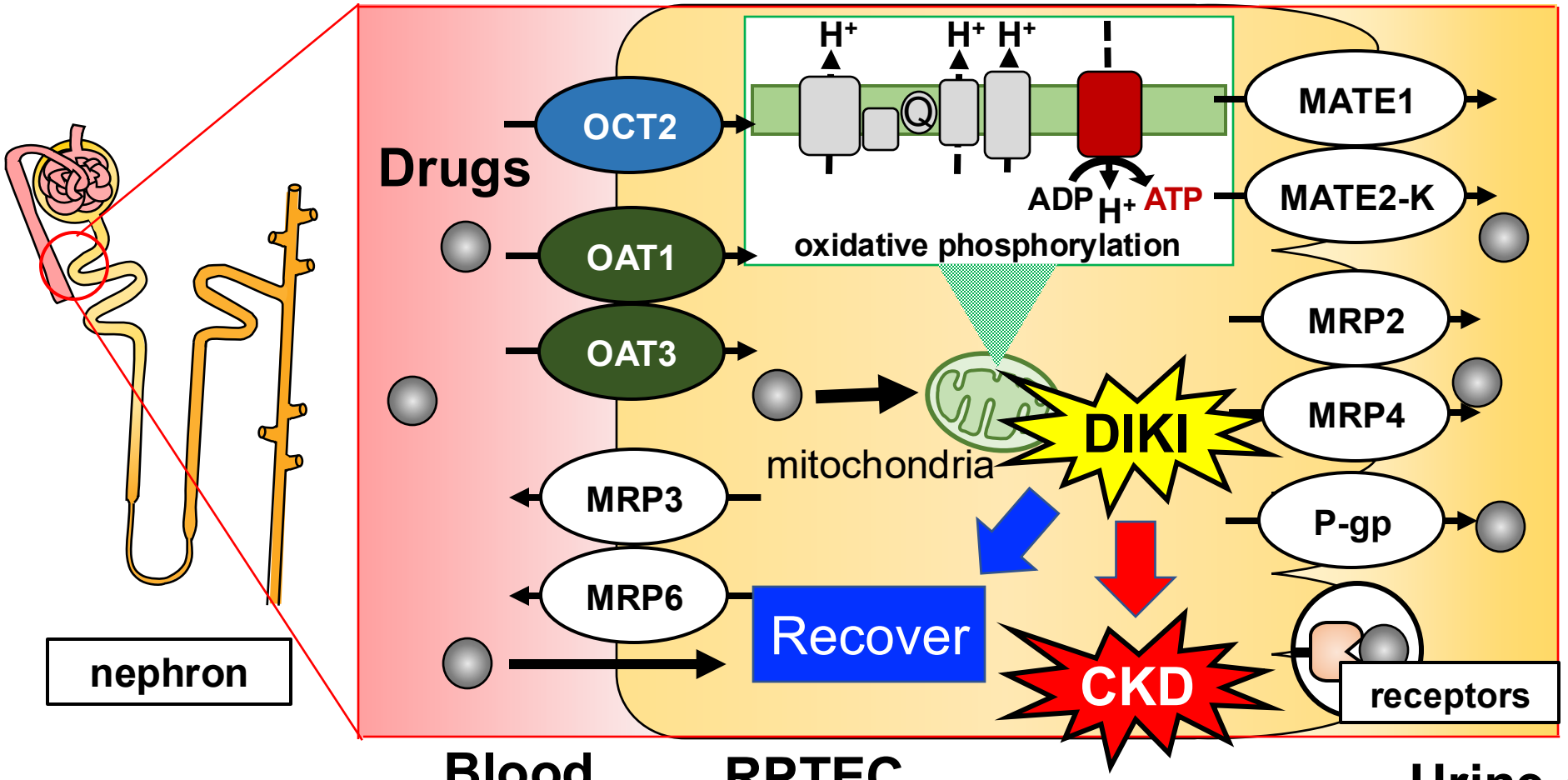
Redfern *et al., Toxicologist.,* **114**:1081 (2010)
Monticello TM *et al., Toxicol Appl Pharmacol,* **334**:100-109 (2017)

Weber EJ, et al., Curr Opin Toxicol. 2017 4:16-21.

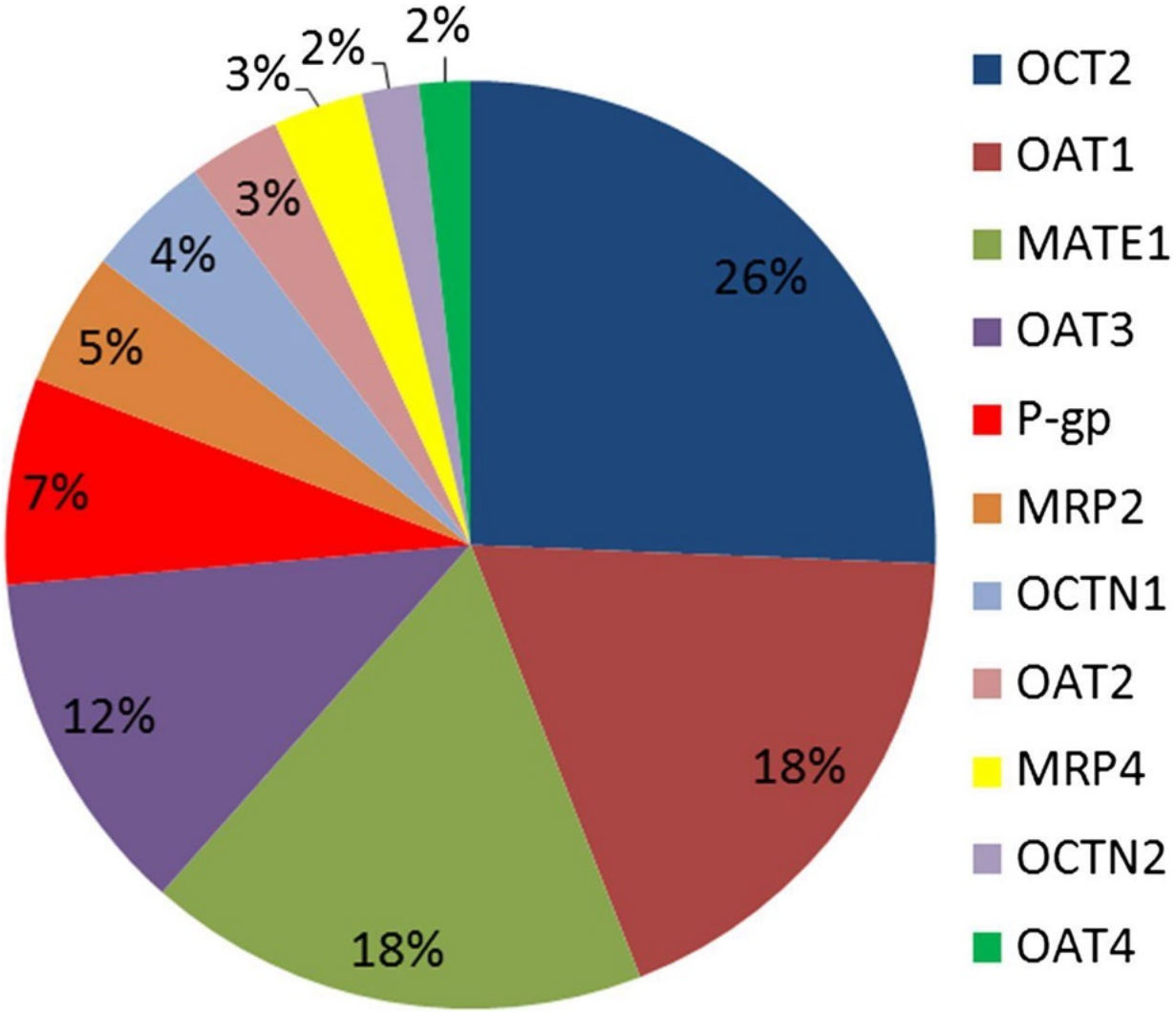


Factors influencing the development of DIKI

Drug distribution by transporters (TPs)
Expression change of TPs
Aerobic respiration
Progression to CKD or recovery



Relative transporter expression in the human kidney cortex



Prasad B. *et al., Drug Metab Dispos*, 2016, 44(12):1920-1924.

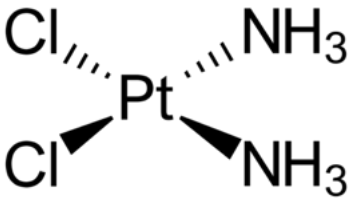
Substrate and inhibitors of renal transporters

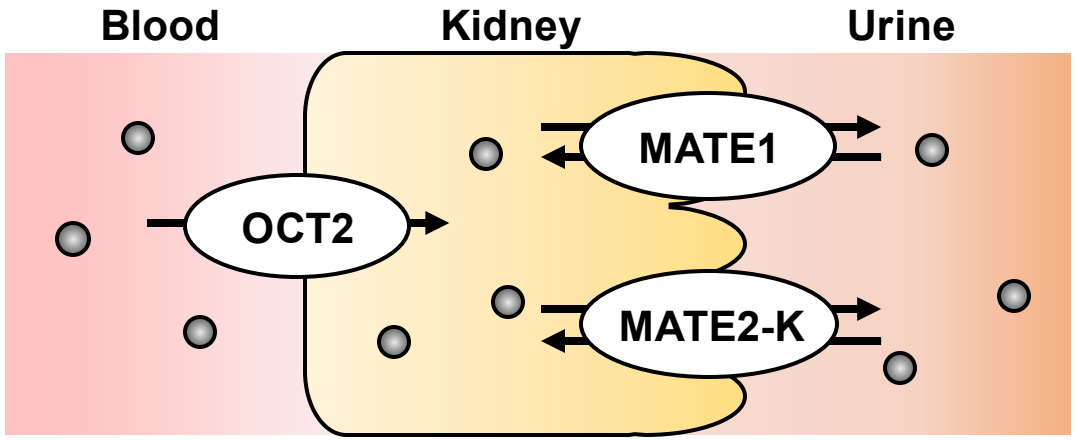
Transporter/alias (Gene)	Selected substrates	Selected inhibitors
OAT1 (*SLC22A6*)	Para-aminohippurate, adefovir, cidofovir, zidovudine*, lamivudine*, zalcitabine*, acyclovir*, tenofovir*, ciprofloxacin*, methotrexate*	Probenecid*, novobiocin
OAT3 (*SLC22A8*)	Oestrone-3-sulphate, non-steroidal anti-inflammatory drugs, cefaclor, ceftizoxime, furosemide*, bumetanide*	Probenecid*, novobiocin
OCT2 (*SLC22A2*)	*N*-Methylpyridinium, tetraethylammonium, metformin*, pindolol, procainamide, ranitidine amantadine, amiloride, oxaliplatin, varenicline*	Cimetidine*, pilsicainide, cetirizine*, testosterone, quinidine
PEPT2 (*SLC15A2*)	Glycylsarcosine, cephalexin, cefadroxil, bestatin, valacyclovir, enalapril, aminolevulinic acid, captopril, dipeptides, tripeptides	Zofenopril, fosinopril
MATE1 *(SLC47A1)*	Metformin, *N*-methylpyridinium, tetraethylammonium	Quinidine, cimetidine, procainamide
MATE2-K *(SLC47A2)*	Metformin, *N*-methylpyridinium, tetraethylammonium	Cimetidine, quinidine, pramipexole

The International Transporter Consortium, *Nat Rev Drug Discov*, 2010, **9**(3):215-36.

Drug transporters for platimun-induced nephrotoxicity

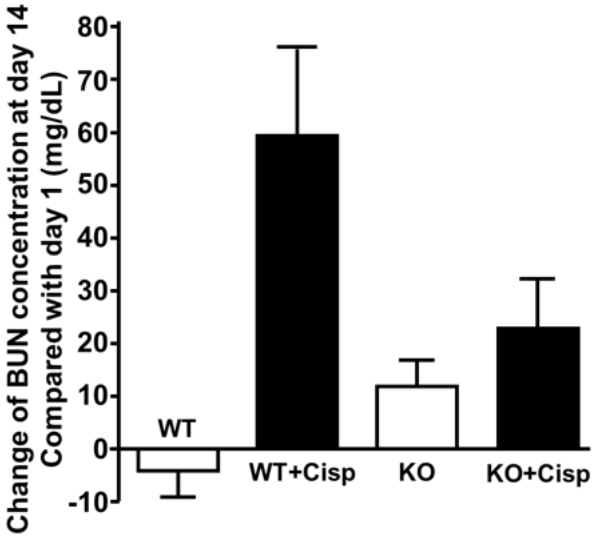
Cisplatin



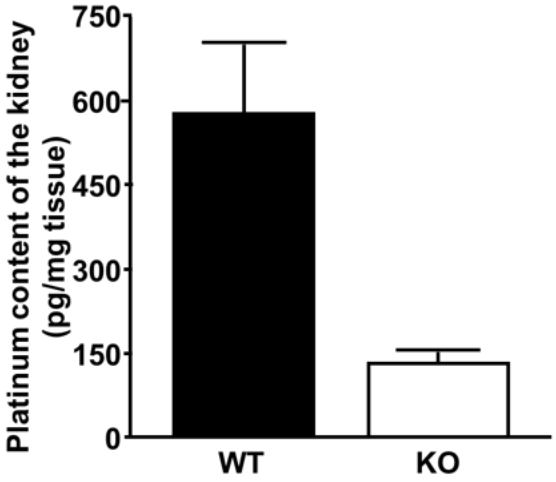


Oct2 (*Slc22a2*) knockout (KO) mice[1)]

BUN

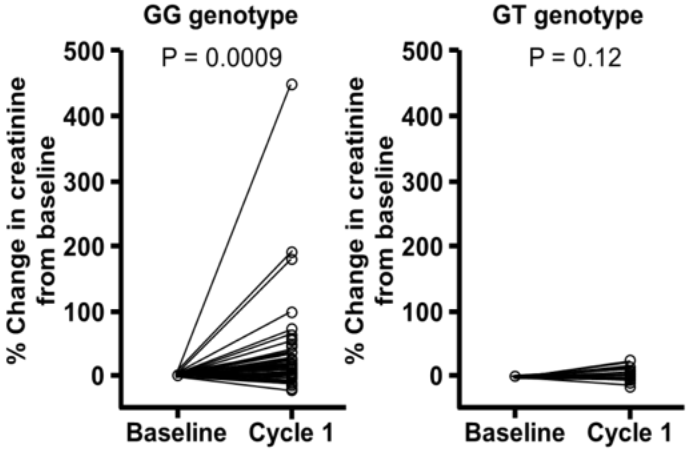


Cisplatin in kidney



SLC22A2 808G>T genotype[2)]

Serum creatinien



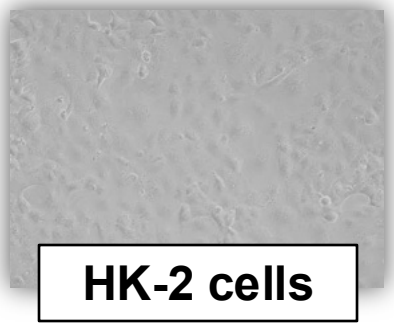
DIKI; drug-induced kidney injury

1) Ciarimboli G. *et al., Am. J. Pathol.*, **176**: 1169-80 (2010)
2) Filipski KK. *et al., Clin. Pharmacol. Ther.*, **86:** 396-402(2009)

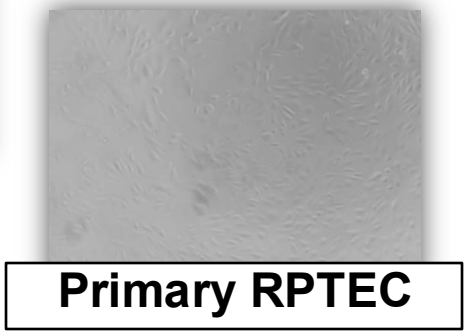
Challenges of *in vitro* DIKI evaluation

in vitro DIKI evaluation

Human kidney-2 cells (HK-2 cells)

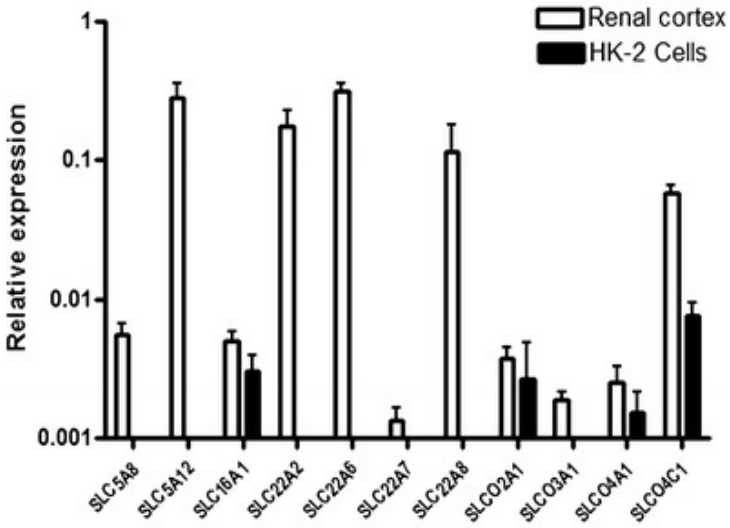


Human primary renal proximal tubule epithelial cells



Transition of drugs into RPTEC

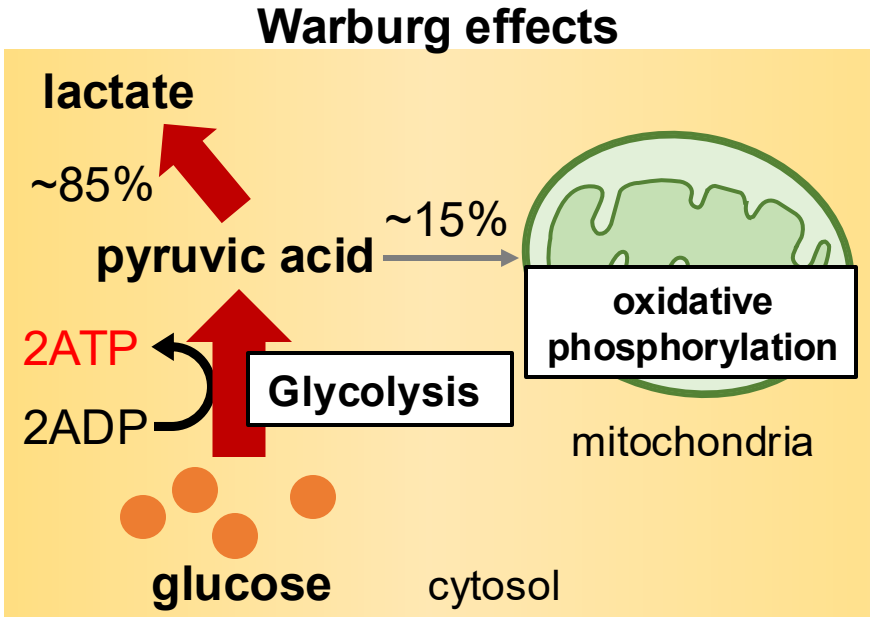
Low level of transporter expression



Jenkinson SE *et al., Pflugers Arch.*, **464**: 601-611 (2012)

Energy synthesis process

Low kidney physiological response



Human RPTEC spheroids: 3D-RPTEC

Cell source

Primary human RPTECs

Created after culture growth of frozen cells

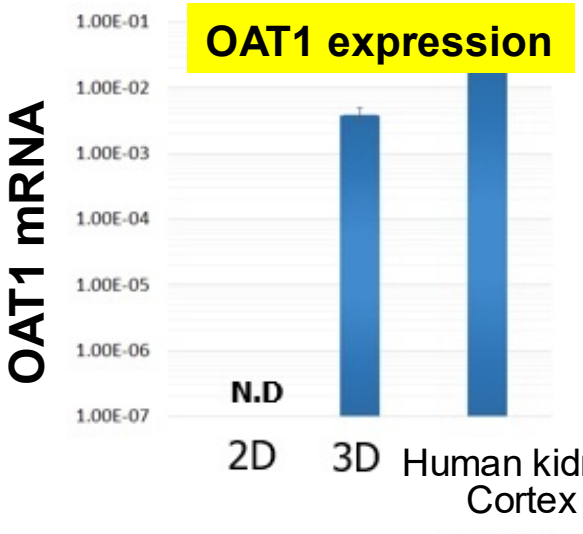
Character

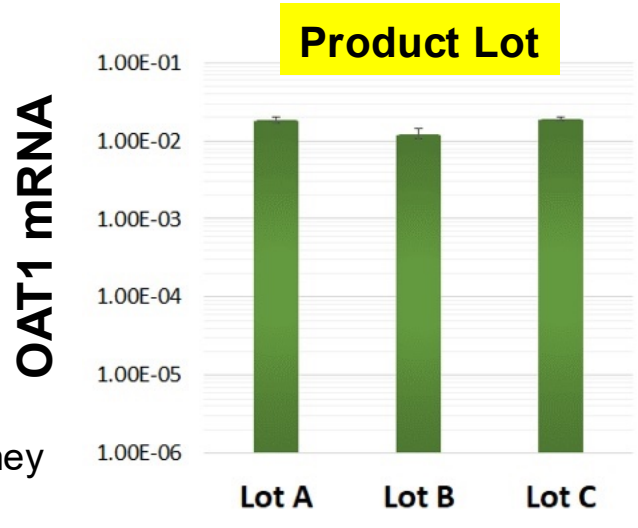
Improved expression of 5 drug transporters including OAT1

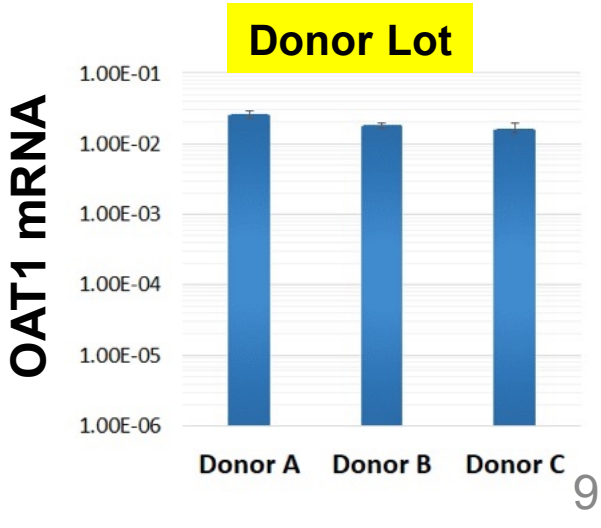
Able to culture maintaining OAT1 expression over 40 days

protein expression of 71% of transporters are within 5-fold of human renal cortex

1) Ishiguro *et al.*, ***Drug Metab Dispos***, 2023 51:1177



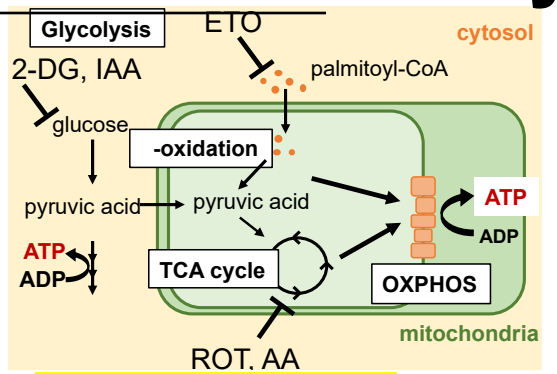


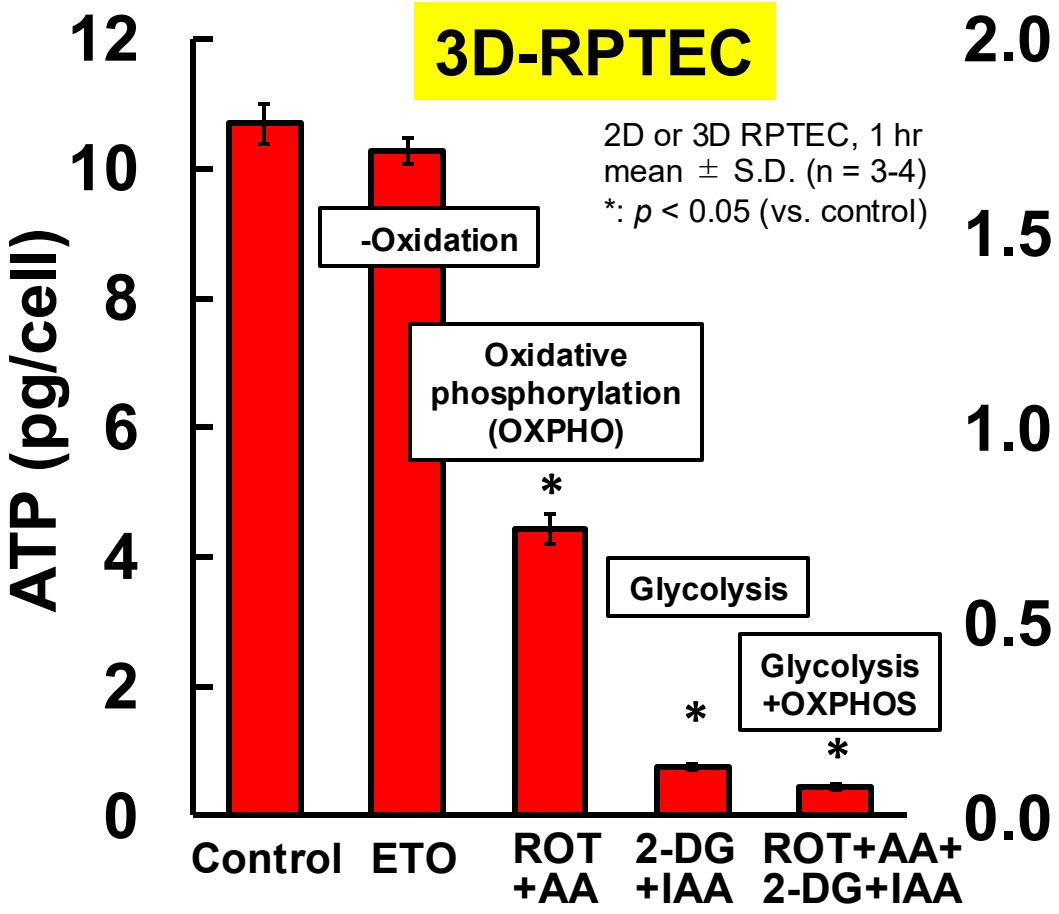


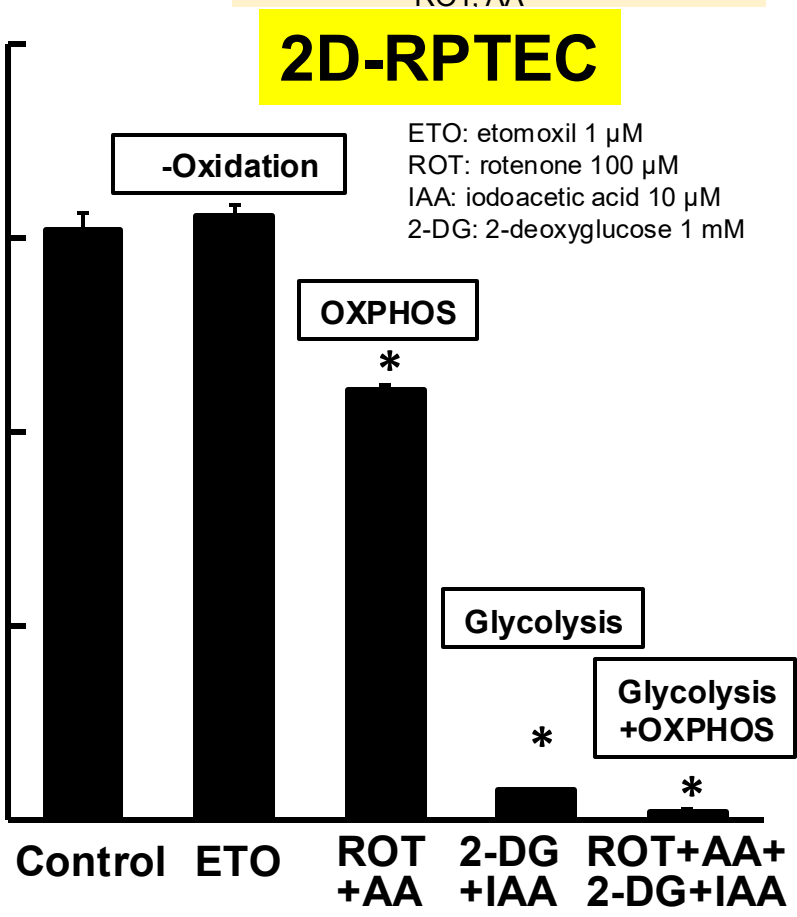
Characterization of the ATP Synthesis Pathway

ATP in 3D-RPTEC was synthesized from the citric acid cycle and glycolysis.

was higher in the 3D culture system compared to the 2D culture system.

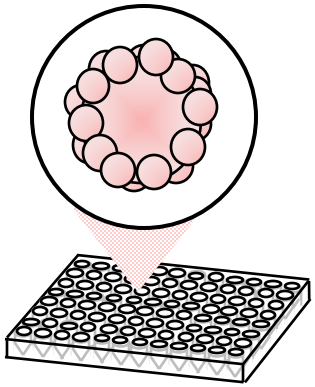


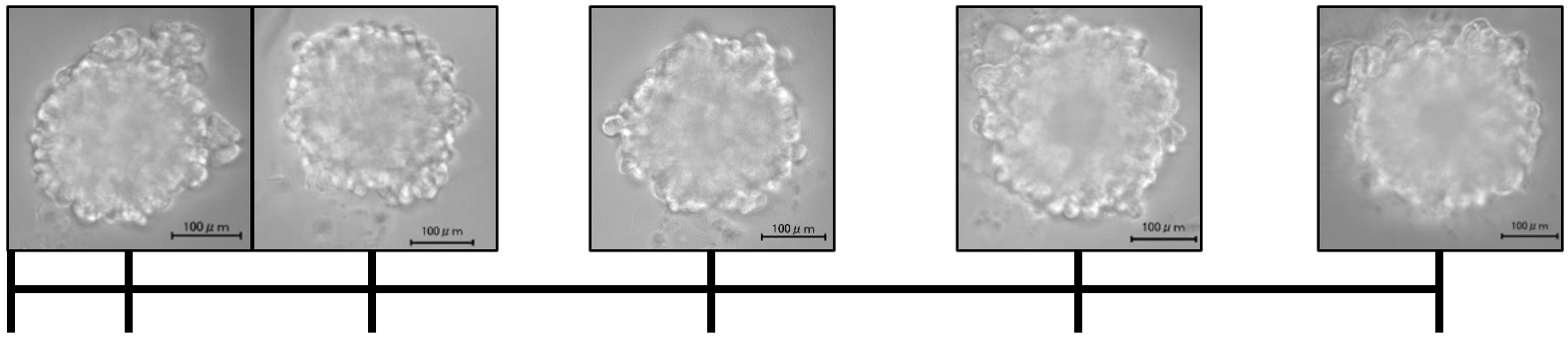




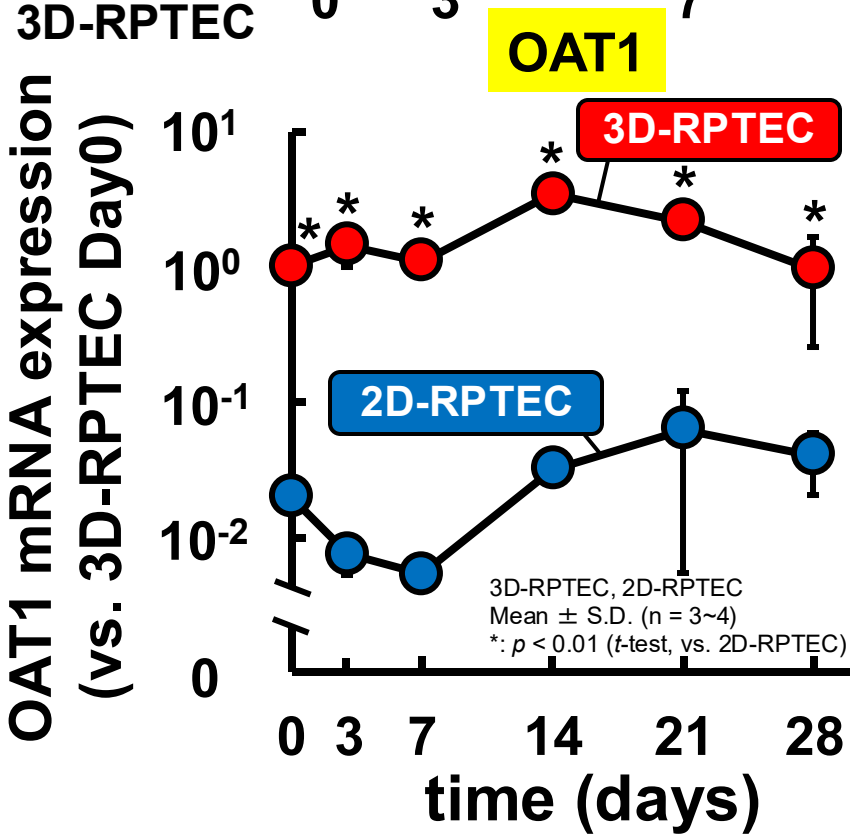
Arakawa *et al.*, ***J Pharm Sci***, 2024, 13:3255-3264.

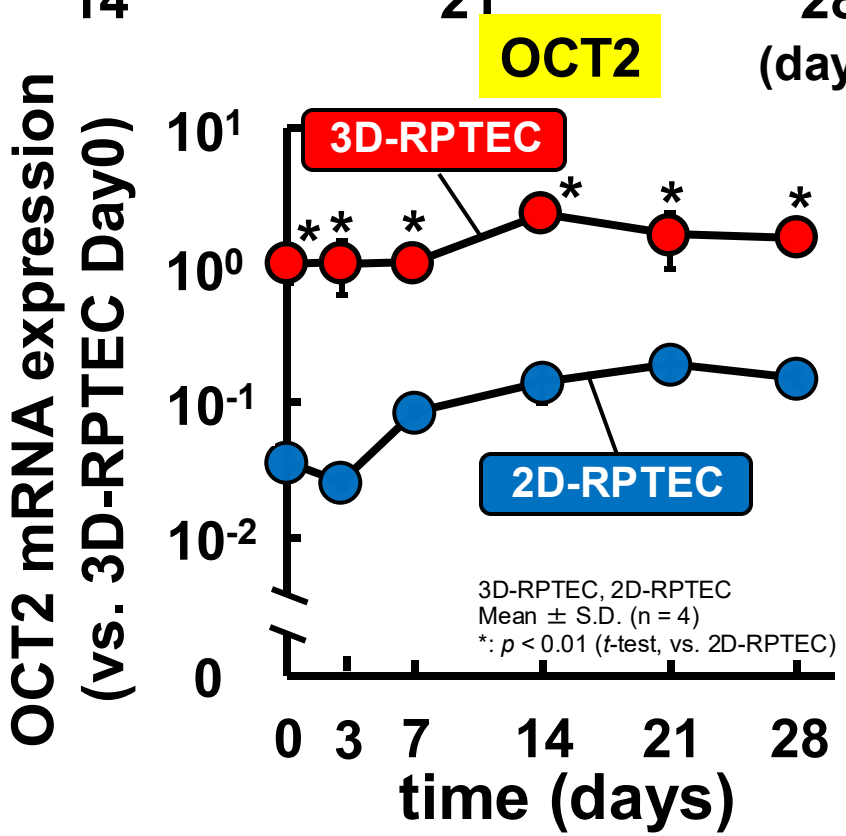
Characteristics of long-term cultured 3D-RPTEC





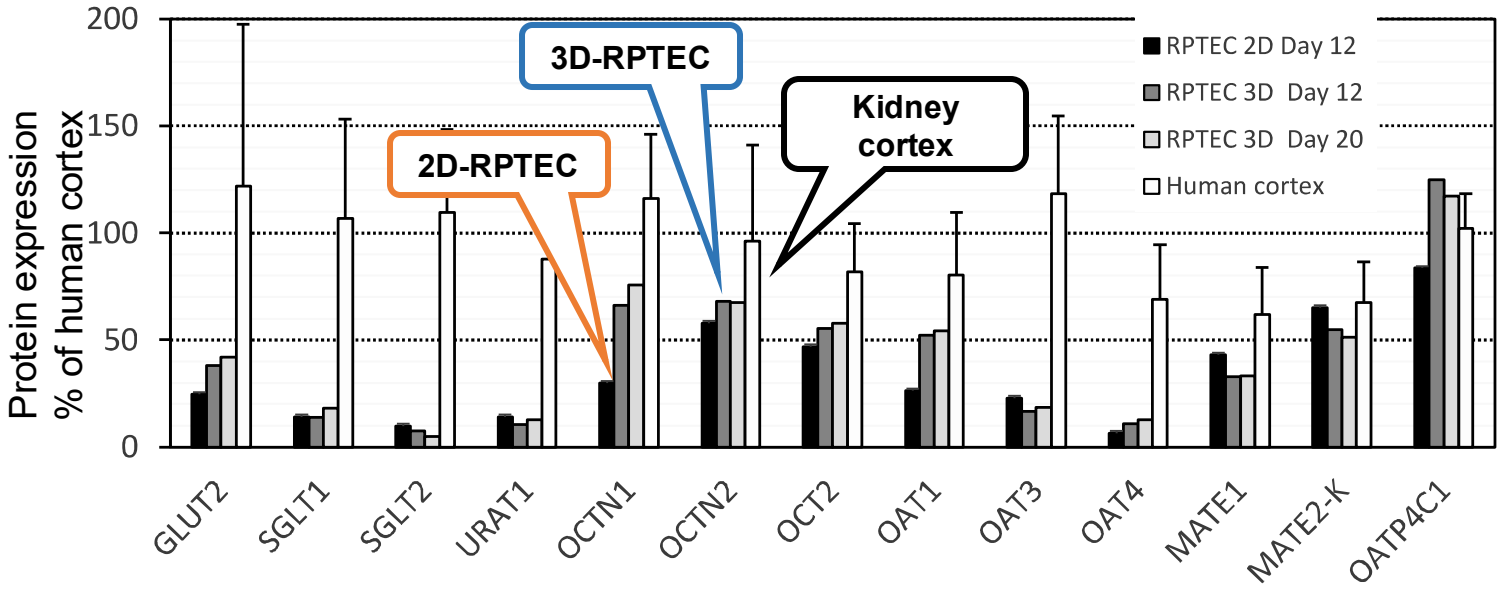
3D-RPTEC

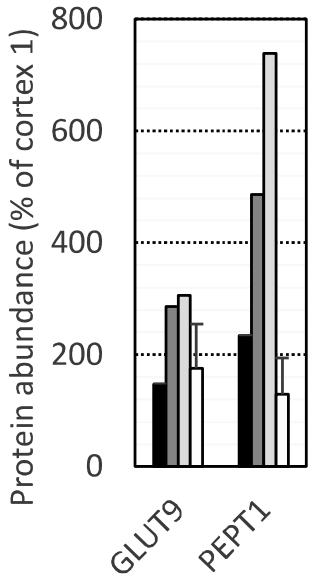




Characterization of Drug Transporter Expression

71% of transporters had protein content within 5 times that of the human renal cortex[1)]
Megalin/cubilin involved in the endocytosis pathway of polymers is expressed.
OAT1 and OCT2 are highly expressed, while OAT3 and SGLT2 are low.

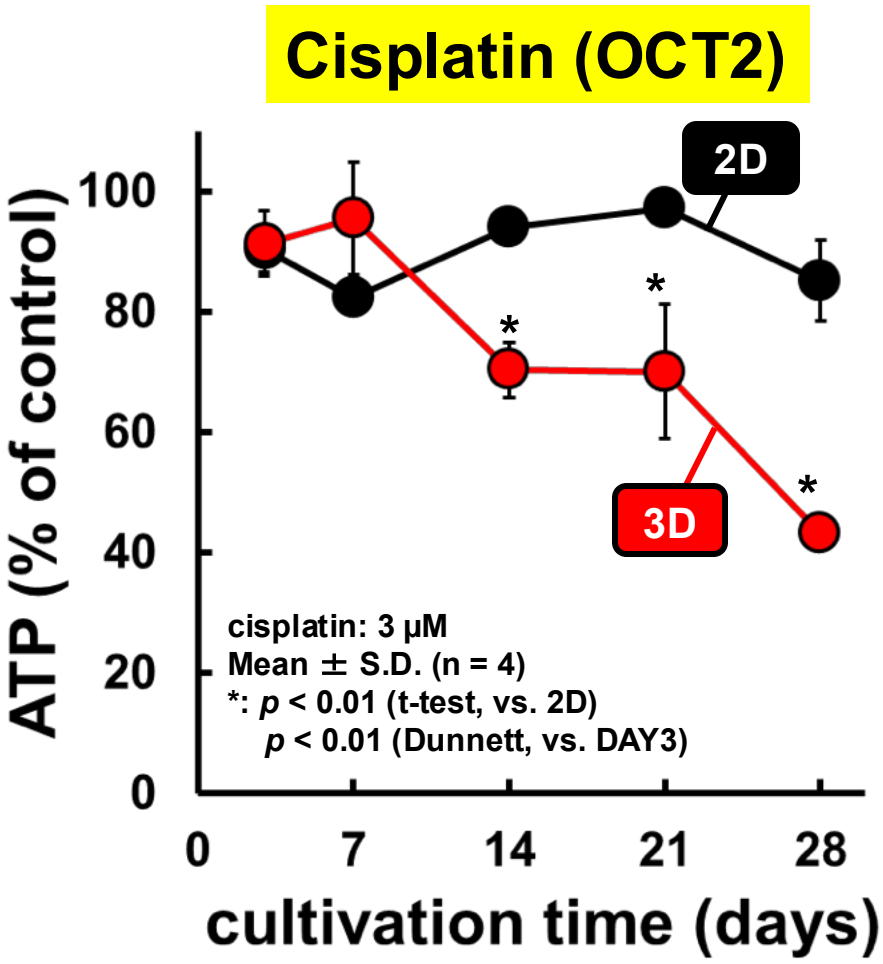


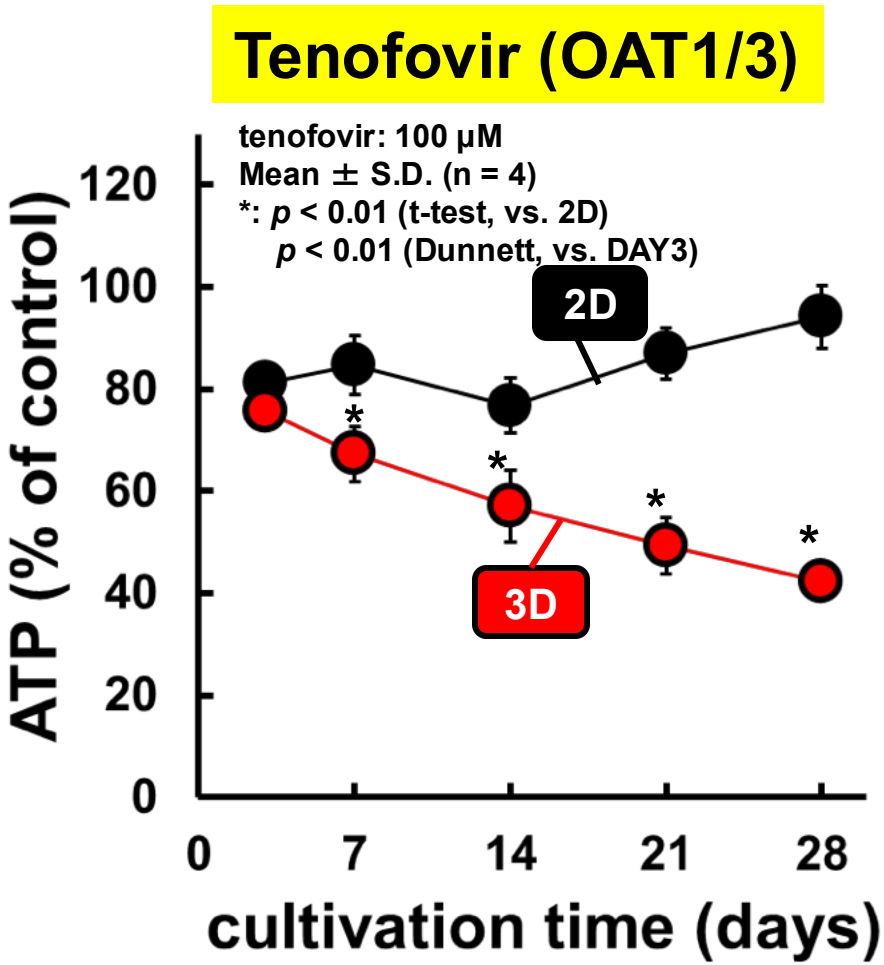


1) Ishiguro *et al., Drug Metab Dispos,* 51, 1177-87 (2023).

Exposure of OAT1 and OCT2 substrate drugs

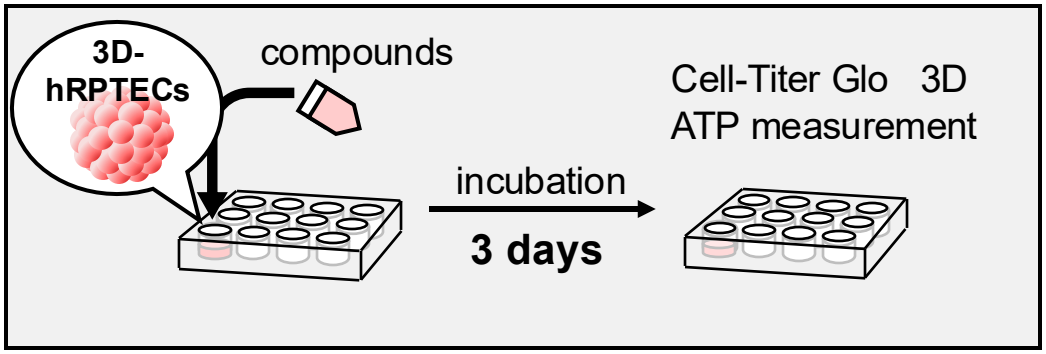
A decrease in intracellular ATP levels of 3D-RPTEC was observed after 28 days of exposure to cisplatin (OCT2) and tenofovir (OAT1).

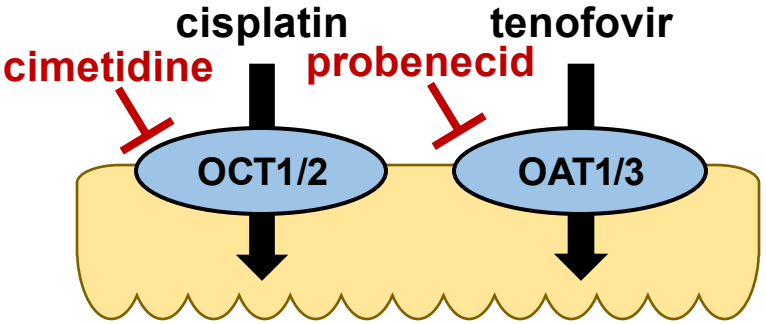




Arakawa *et al., J Pharm Sci*, 2024, 113:3255-3264

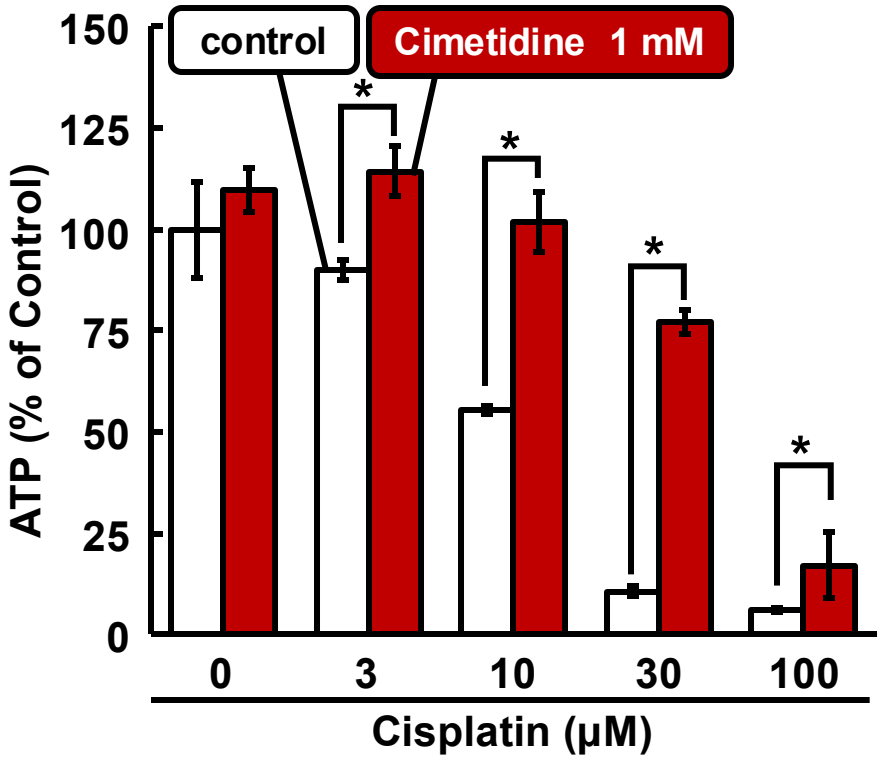
Effects of Cisplatin and Tenofovir on ATP Content in the Presence of Transporter Inhibitors



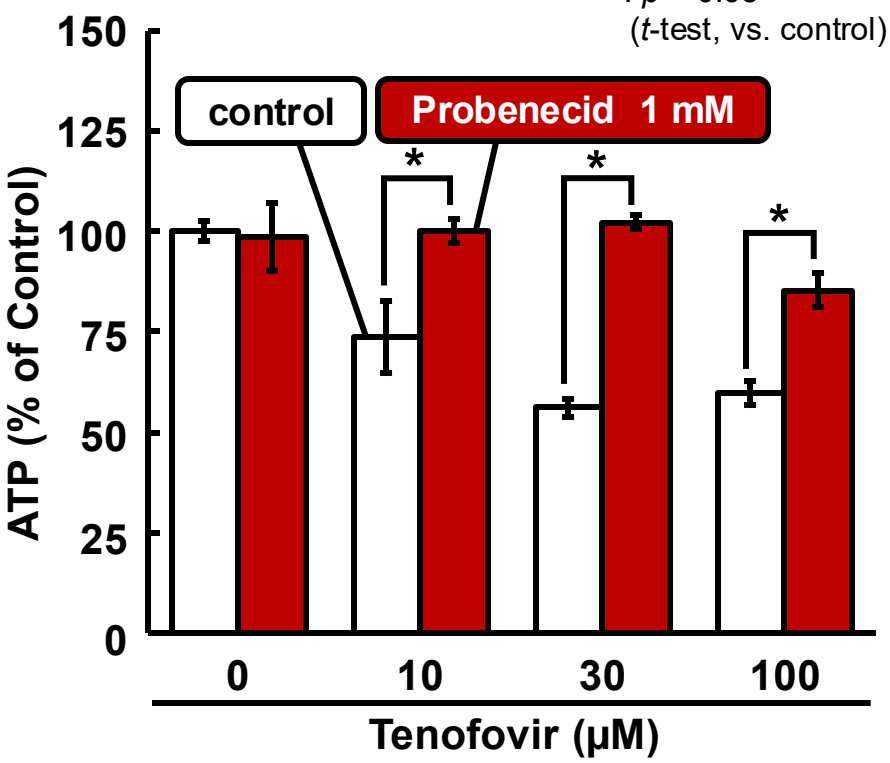


3D-hRPTECs, 3 days
mean ± S.D. (n = 4)
*: $p < 0.05$
(*t*-test, vs. control)

Cisplatin



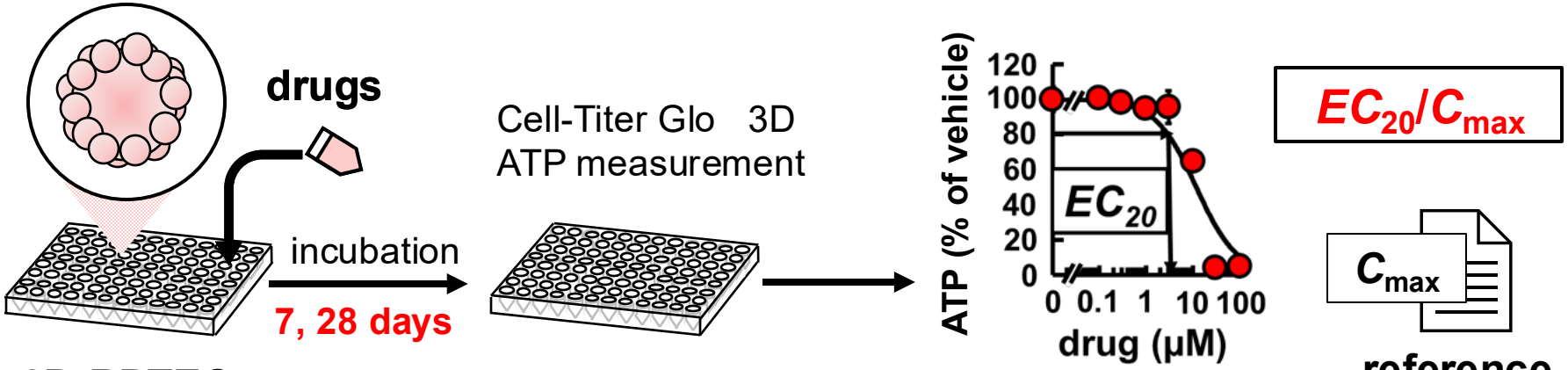
Tenofovir



Conducting cytotoxicity tests using 3D-RPTEC[1)]

Cell: 3D-RPTEC (1 spheroid/well)
Studied drugs: Selected with the cooperation of Dr. Kohei Matsushita (phathologist), referring to the papers in the following slide (Pfizer and Takeda's in vitro test system for drug-induced kidney injury[2,3)])
Biomarker: Intracellular ATP Amount　Cell-Titer Glo 3D ATP measurement, Promega

1) Arakawa *et al., J Pharm Sci*, 2024, 113:3255-3264.
2) Rana *et al., Chem Res Toxicol*, 2019, 32:156-167.
3) Bajaj *et al., Toxicology*, 2020, 442:152535.



EC_{20}: The ratios of ATP 20% decreasing concentration

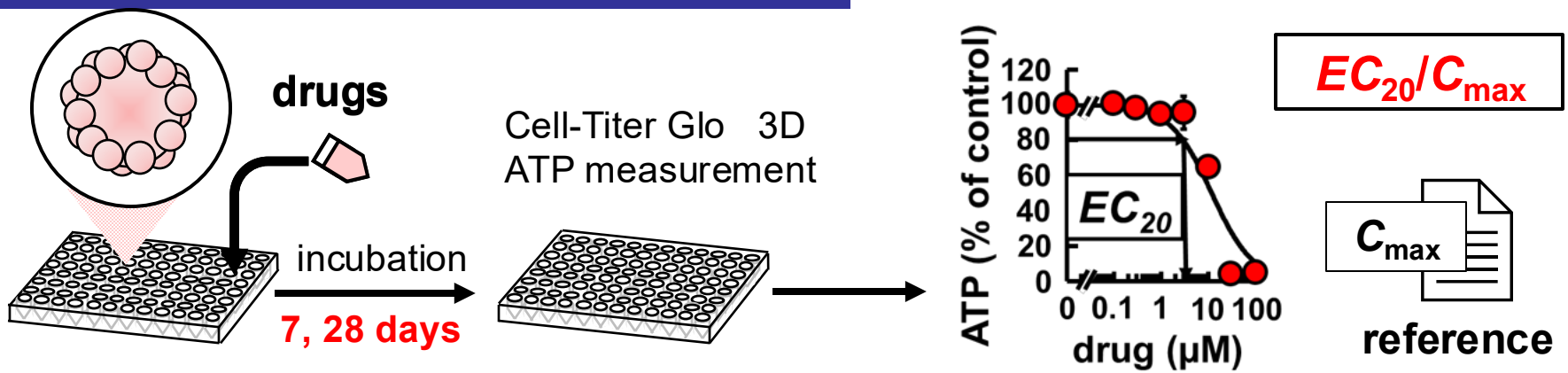
Compounds to be examined (17 positive subjects, 15 negative subjects)

drugs	category	C_{max} (μM)	RPTEC injury	DIKI target	Renal PK factor	Memo
adefovir	antiviral	0.0670	positive	proximal tubule	OAT1	-
carboplatin	antineoplastic	67.3	positive	proximal and distal tubule		-
cephaloridine	antibiotics	57.8	positive	proximal tubule	OAT3	Lipid peroxidation
cephalothin	antibiotics	54.7	positive	proximal tubule	OAT3	-
cisplatin	antineoplastic	8.26	positive	proximal tubule	OCT2/MATE1	DNA damage, mitochondrial injury etc.
cyclosporin A	immunosuppressive	0.210	positive	proximal tubule	P-gp	mitochondrial injury, fibrosis via epithelial mesenchymal transition
gentamycin	antibiotics	18.8	positive	proximal tubule	megalin	Lysosomal injury
mitomycin C	antineoplastic	1.77	positive	proximal tubule (animal), vascular endothelium, glomerulus		-
omeprazole	proton pump inhibitor	3.18	positive	proximal tubule (animal), Interstitial inflammation		-
oxaliplatin	antineoplastic	8.06	positive	proximal tubule	OCT2/MATE1	-
pentamidine	antiprotozoal	0.900	positive	proximal tubule		-
rifampicin	antibiotics	12.2	positive	proximal tubule Interstitial inflammation		-
tacrolimus	immunosuppressive	0.0325	positive	proximal and distal tubule	P-gp	endoplasmic reticulum and lysosomal injury
tenofovir	antiviral	0.926	positive	proximal tubule	OAT1, MRP2/4	mitochondrial injury
tetracycline	antibiotics	3.67	positive	proximal tubule, Interstitial inflammation	OCT2	-

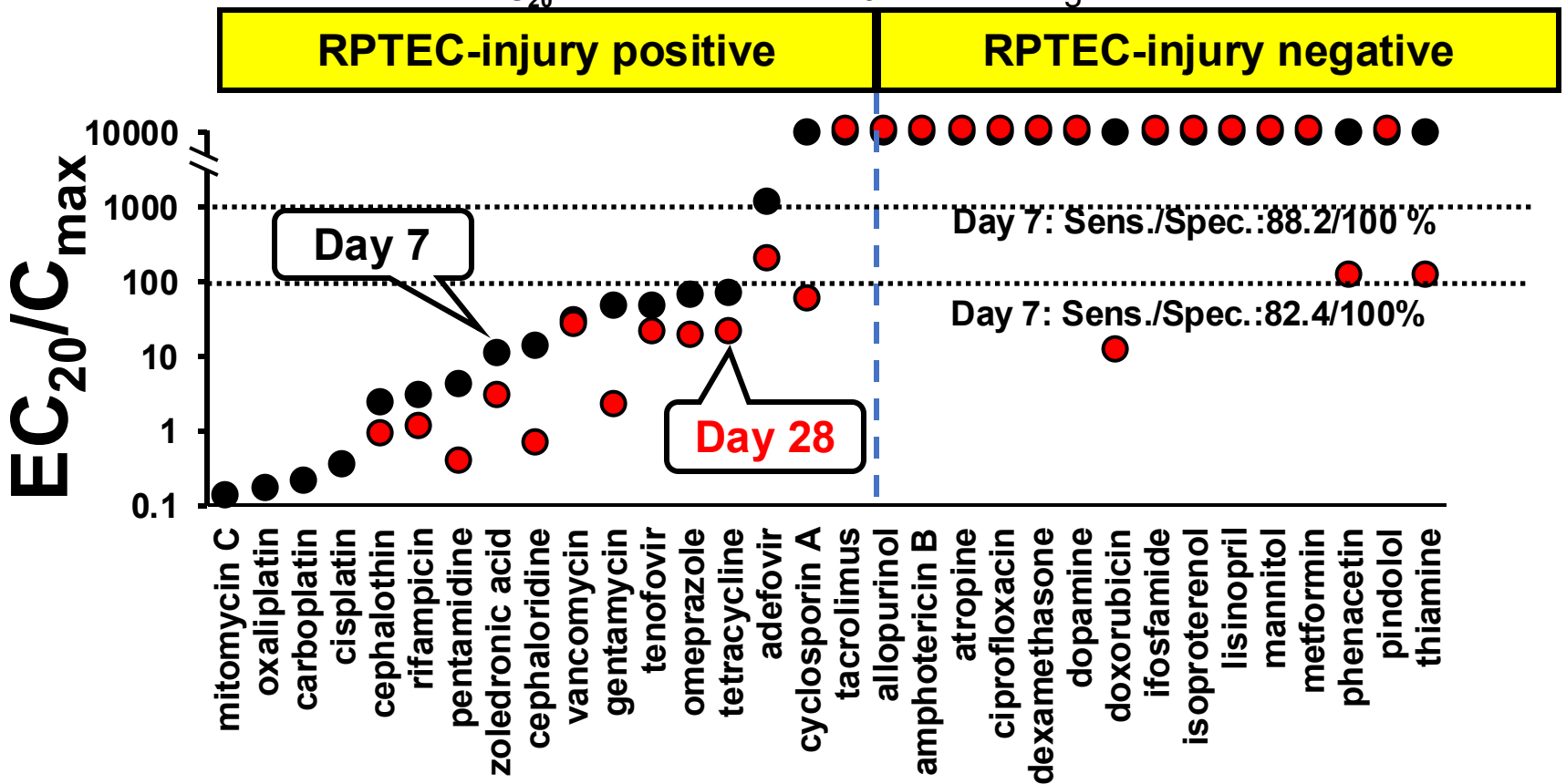
Drugs	category	C_{max} (μM)	RPTEC injury	DIKI target	Renal PK factor	Memo
vancomycin	antibiotics	38.0	positive	proximal tubule	megalin	Lysosomal injury
zoledronic acid	bisphosphonate	0.970	positive	proximal tubule		-
allopurinol	xanthine oxidase inhibitor	73.5	negative	distal tubule	URAT1	-
amphotericin B	antifungal	10.8	negative	distal tubule		Tubular acidosis
atropine	anticholinergic agent	0.0900	negative	non-nephrotoxicant		
ciprofloxacin	antibiotics	12.1	negative	collecting duct?	OAT3, MRP2	crysterization
dexamethasone	steroids	0.680	negative	glomerulus (animal)		-
dopamine	inotropic agents	0.0770	negative	non-nephrotoxicant		
doxorubicin	antineoplastic	0.0370	negative	podocyte	P-gp	
ifosfamide	antineoplastic	199	negative	proximal tubule	OCT2	mitochondrial injury DIKI by its metabolite produced in liver
isoproterenol	1 2 adrenergic agonists	0.946	negative	pre-kidney		decrease in blood presure
lisinopril	ACE inhibitor	0.0860	negative	pre-renal		decrease in blood presure
mannitol	osmotic diuretics	43.1	negative	proximal tubule		osmotic nephrosis
metformin	anti-diabetic drugs	12.4	negative	medullary interstitial cell (animal, CYP metabolite)	OCT2, MATE1/2K	-
phenacetin	analgesic	38.4	negative	proximal tubule and distal tubule (animal)		DIKI by its metabolite produced in liver
pindolol	adrenergic antagonist	0.210	negative	non-nephrotoxicant		
thiamine	vitamin	6.80	negative	non-nephrotoxicant		

Arakawa *et al., Drug Metab Pharmacokinet*, 2025, 101056

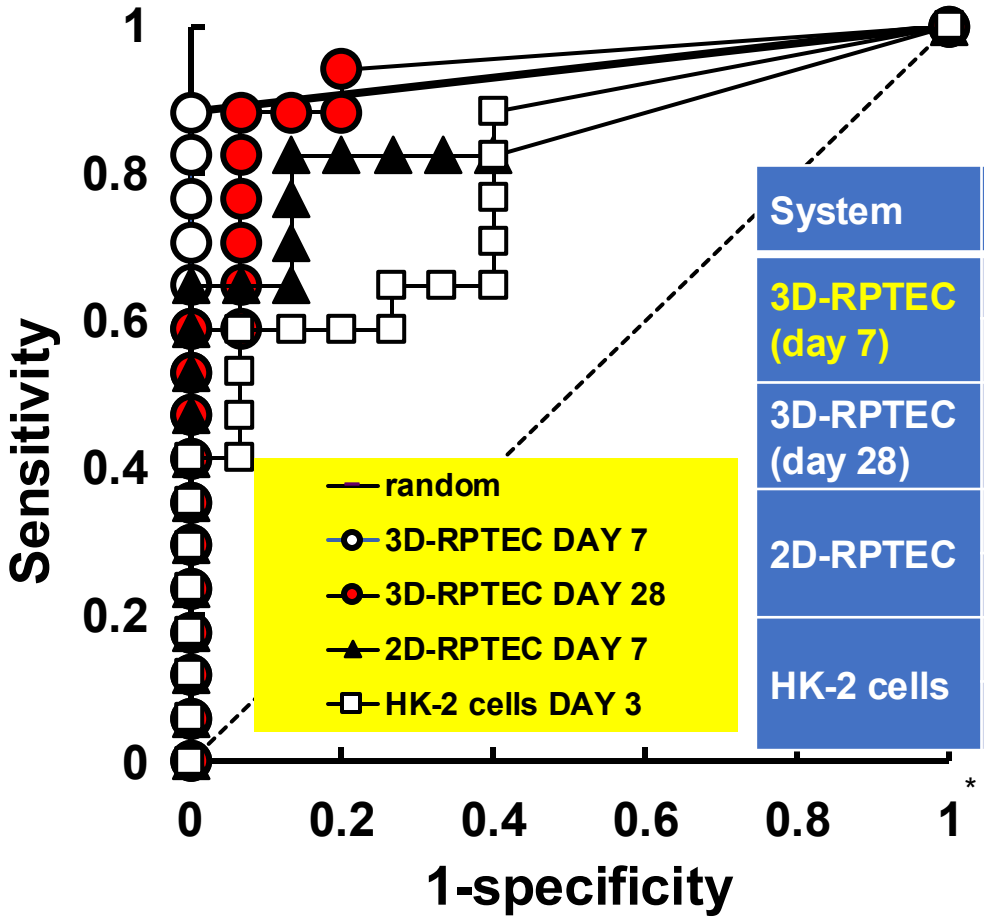
Evaluation of RPTEC-injury by 3D-RPTECs



EC_{20}: The ratios of ATP 20% decreasing concentration



Comparison of prediction rates using ROC-AUC curves



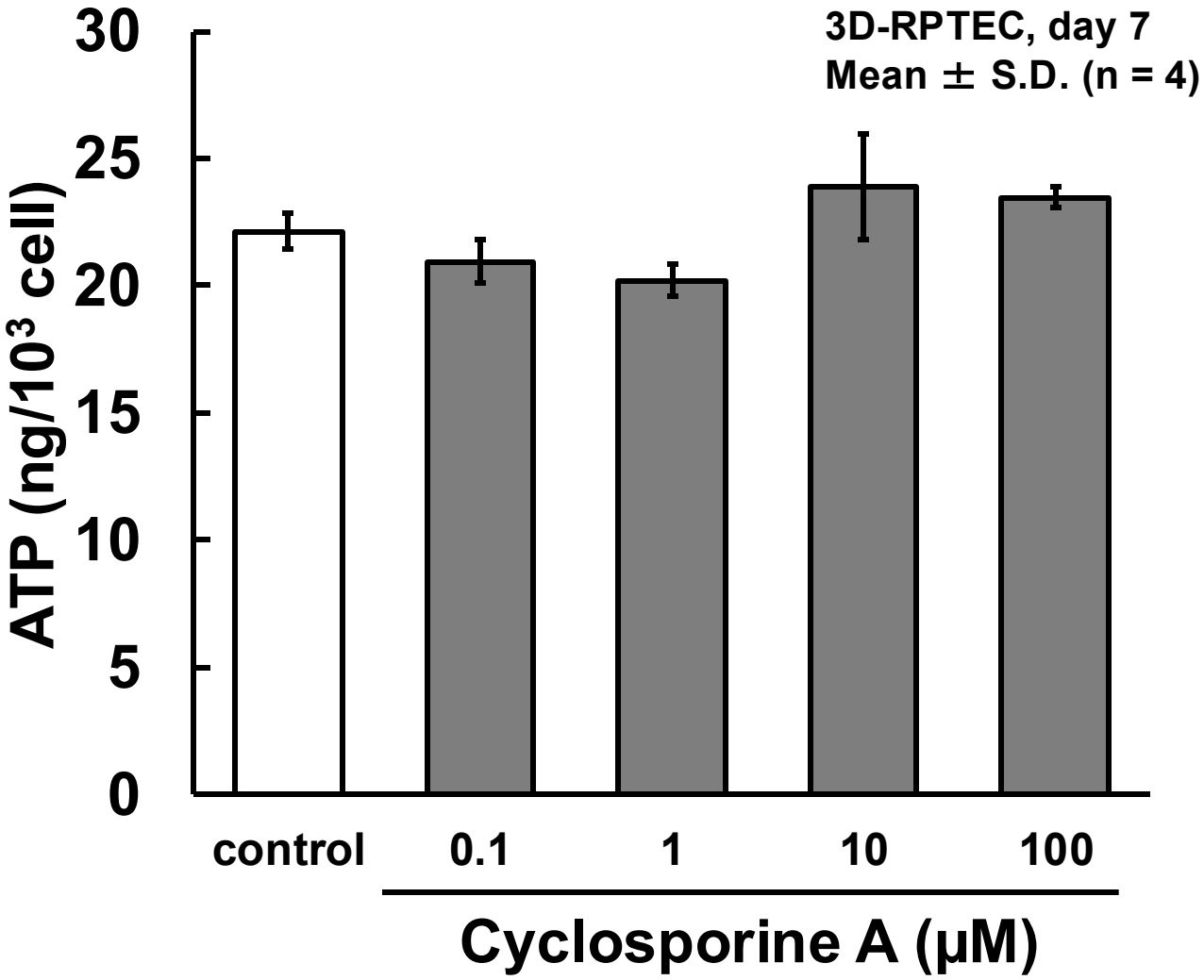
System	Sensitivity	Specificity	Cut-off value	AUC	Youden Index
3D-RPTEC (day 7)	82.4%	100%	100	0.941	0.824
	88.2%	100%	1110*		0.882
3D-RPTEC (day 28)	88.2%	93.3%	100*	0.933	0.816
2D-RPTEC	82.0%	87.0%	49.4*	0.853	0.69
	82.0%	67.0%	100		0.49
HK-2 cells	58.8%	93.3%	14.4*	0.796	0.522
	71.0%	60.0%	100		0.306

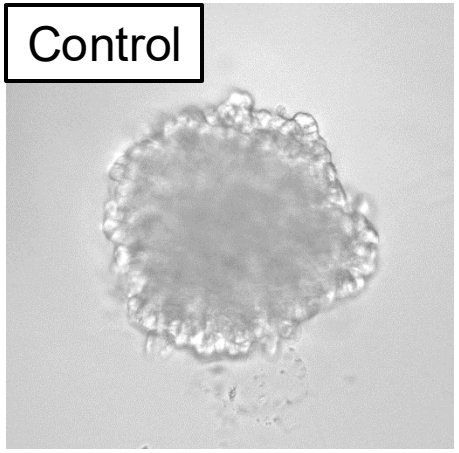
* indicates the cut-off values for giving the highest Yaudan Index

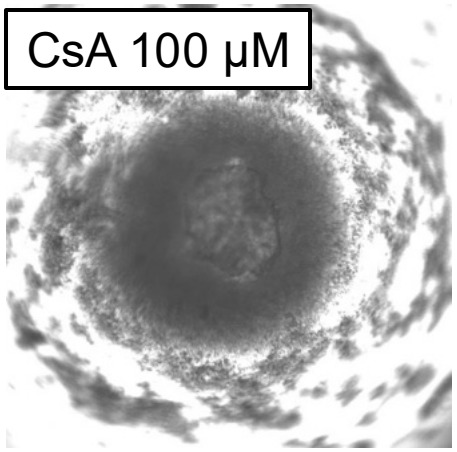
The 7-day exposure assessment using 3D-RPTEC demonstrated higher predictive accuracy compared to HK-2 cells and 2D-RPTEC.

Effect of cyclosporin A exposure in 3D-RPTECs

Seven days of exposure to cyclosporin A did not result in a decrease in intracellular ATP levels.



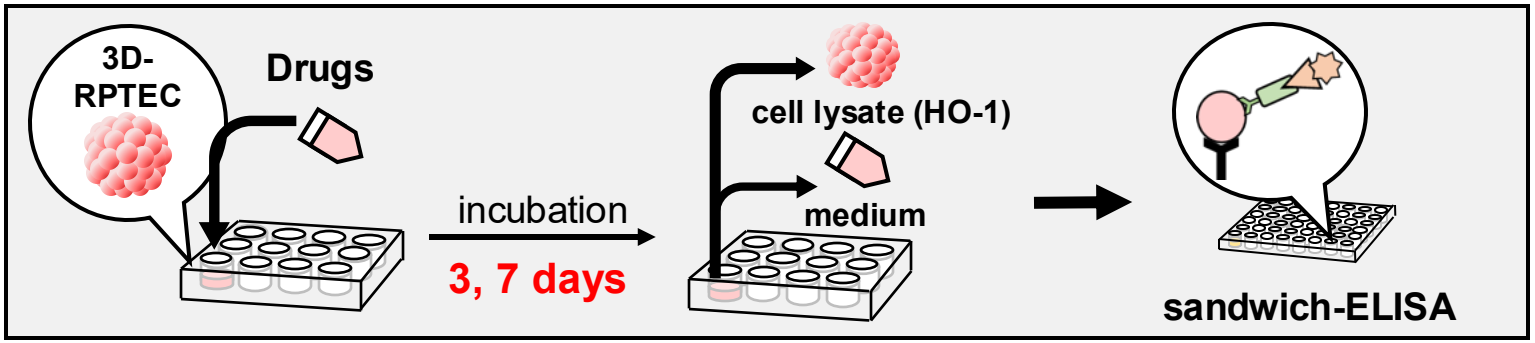


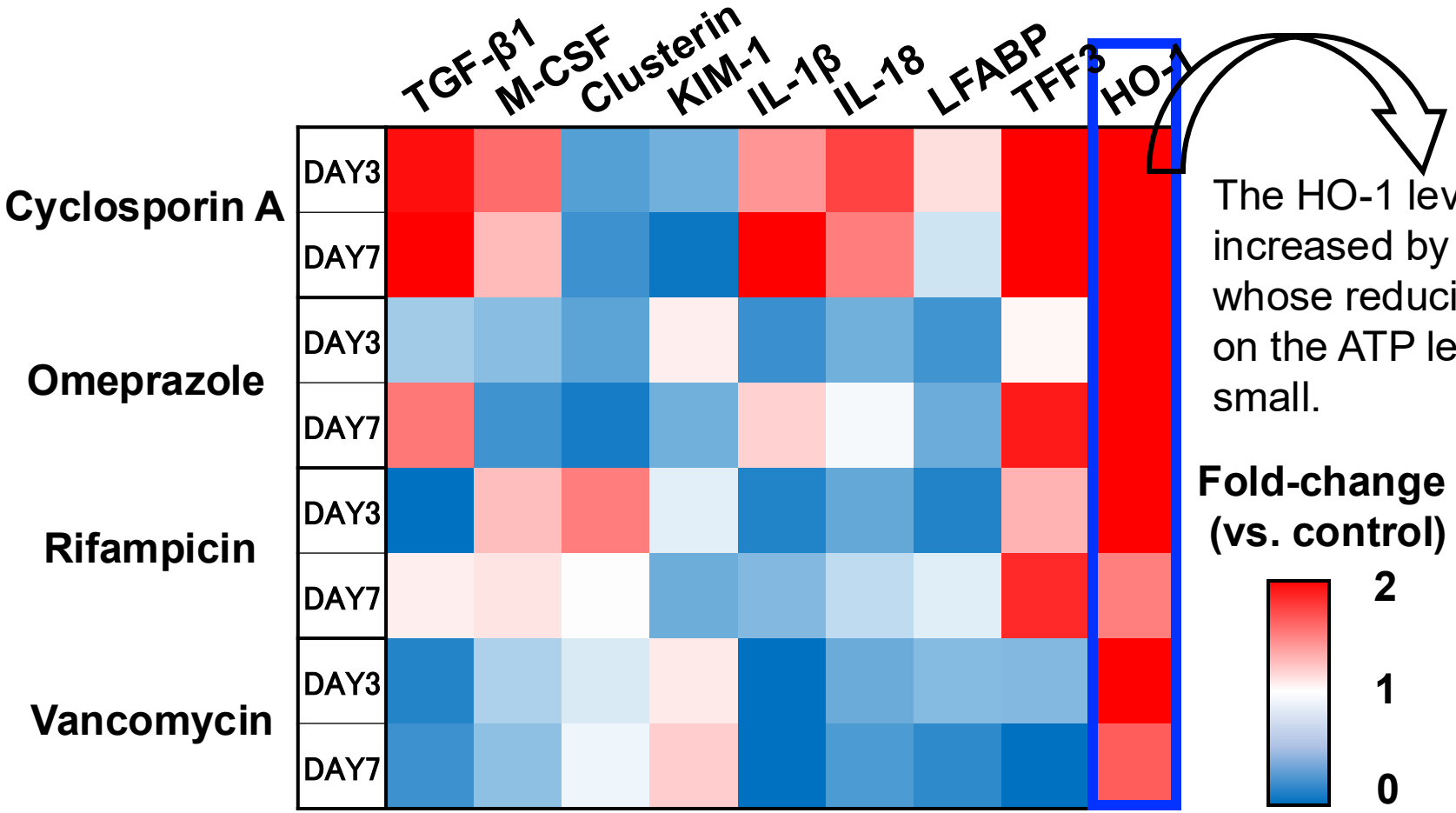


Renal Toxic markers by ELISA

Renal toxicity marker	Indicator
TGF-	fibrosis
M-CSF	inflammation
Clusterin (CLU)	proximal tubular damage
KIM-1	proximal tubular damage
IL-1	inflammation
IL-18	inflammation
L-FABP	oxidative stress
TFF3	entire kidney damage
HO-1	Oxidative stress, inflammation

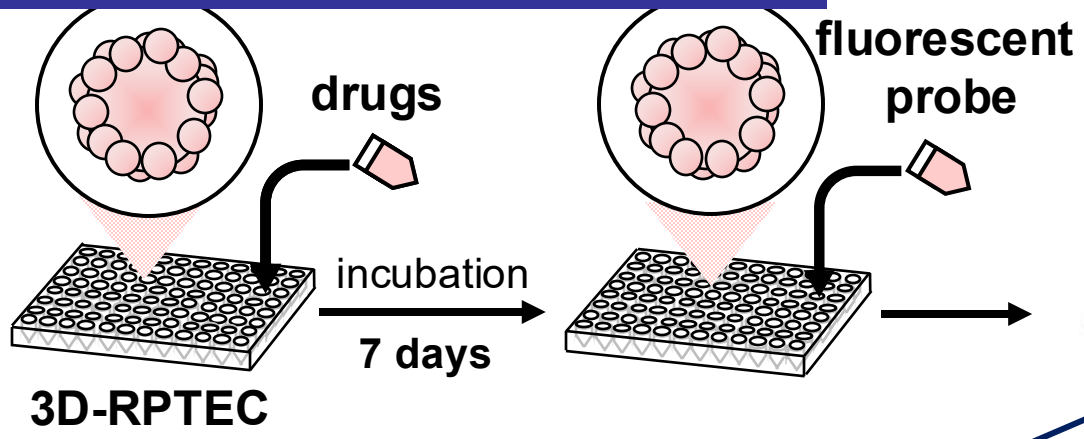
Changes of various toxic markers





The HO-1 level was increased by the drugs whose reducing effect on the ATP level was small.

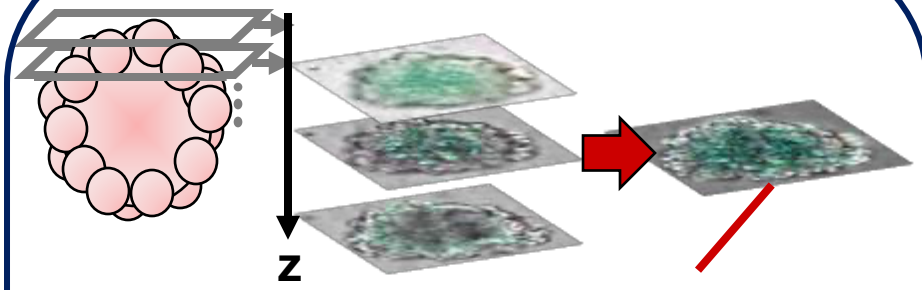
High content analysis (HCA)



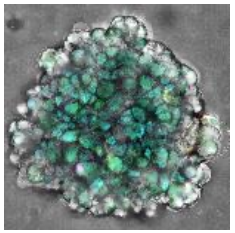
Quantification

confocal microscope

Fluorescent probe	Target
Hoechst 33342	double-stranded DNA
CellROX Green	DNA oxidative stress
CellROX DeepRed	cellular oxidative stress
LysoTracker	acidic organelles
ERTracker	sulphonylurea receptors of ATP-sensitive K+ channels
MitoTracker	mitochondrial membrane potentials
Phalloidin	actin filament

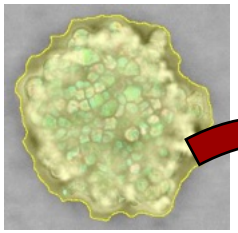


consolidates 21 sliced images into one (MaxIP)



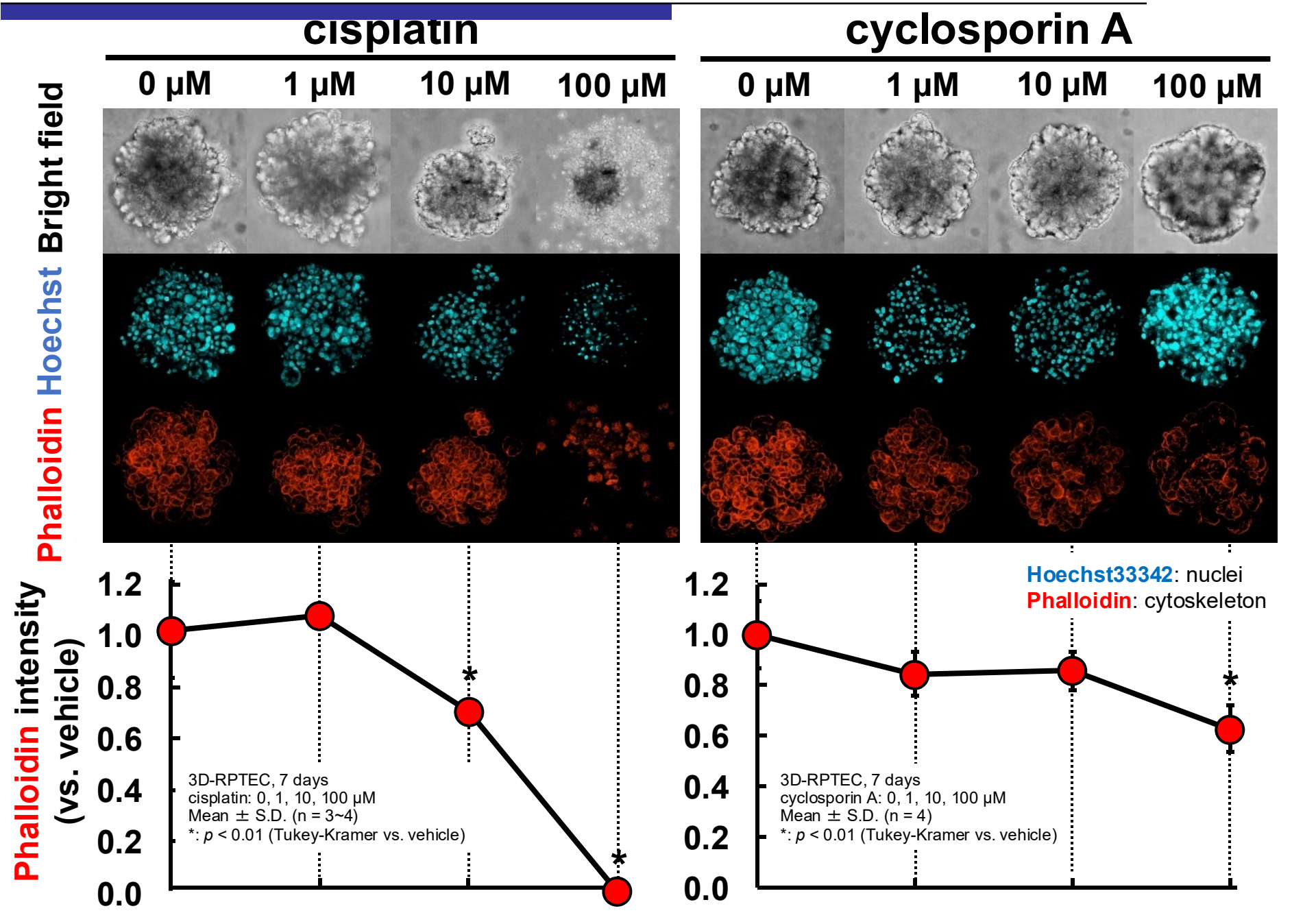
MaxIP

image processing

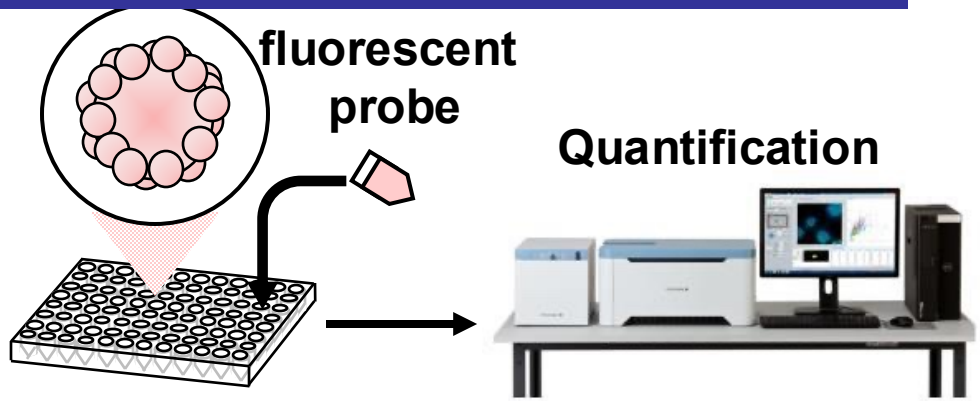


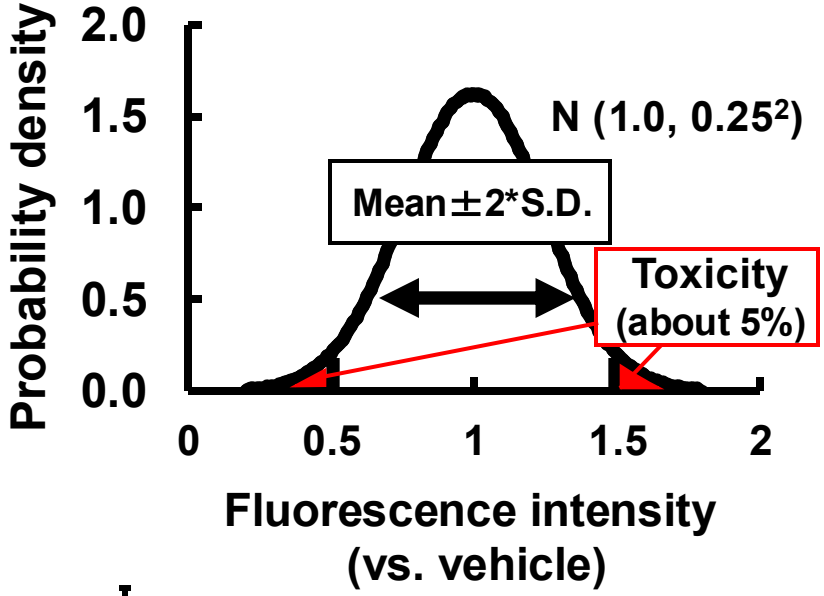
Quantification

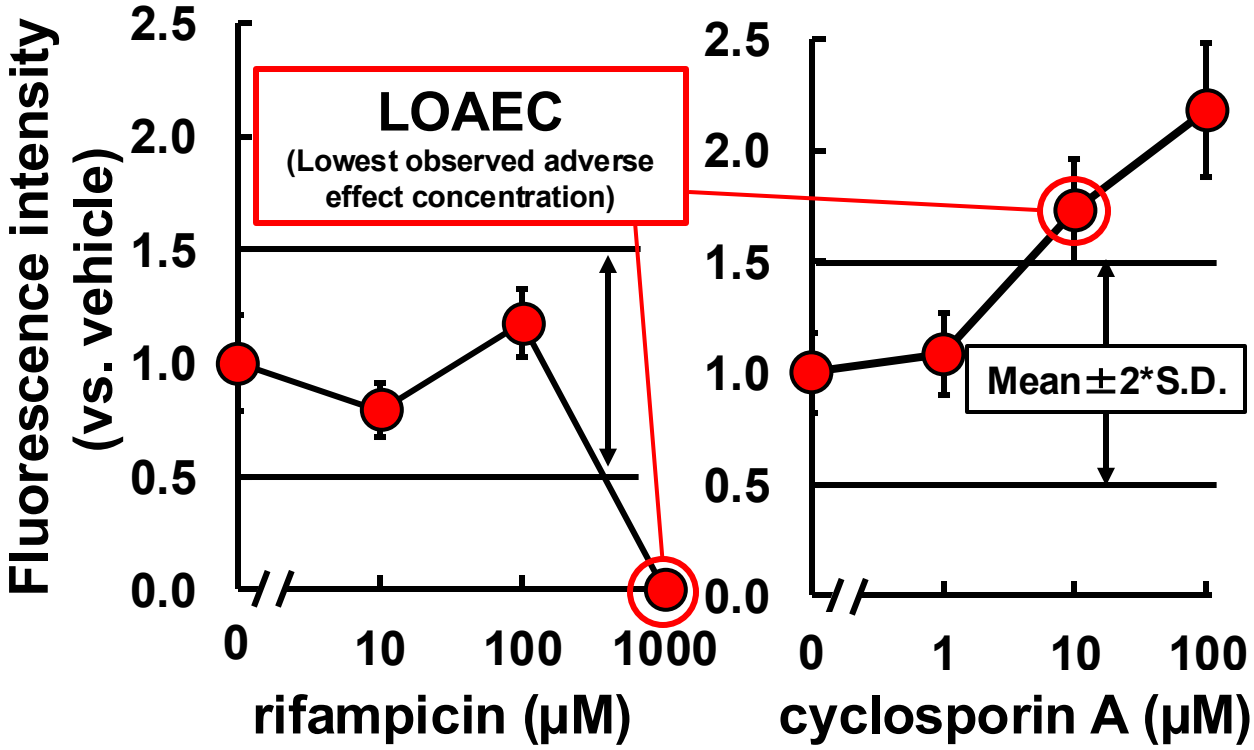
Effects of drugs on organelles in 3D-RPTEC



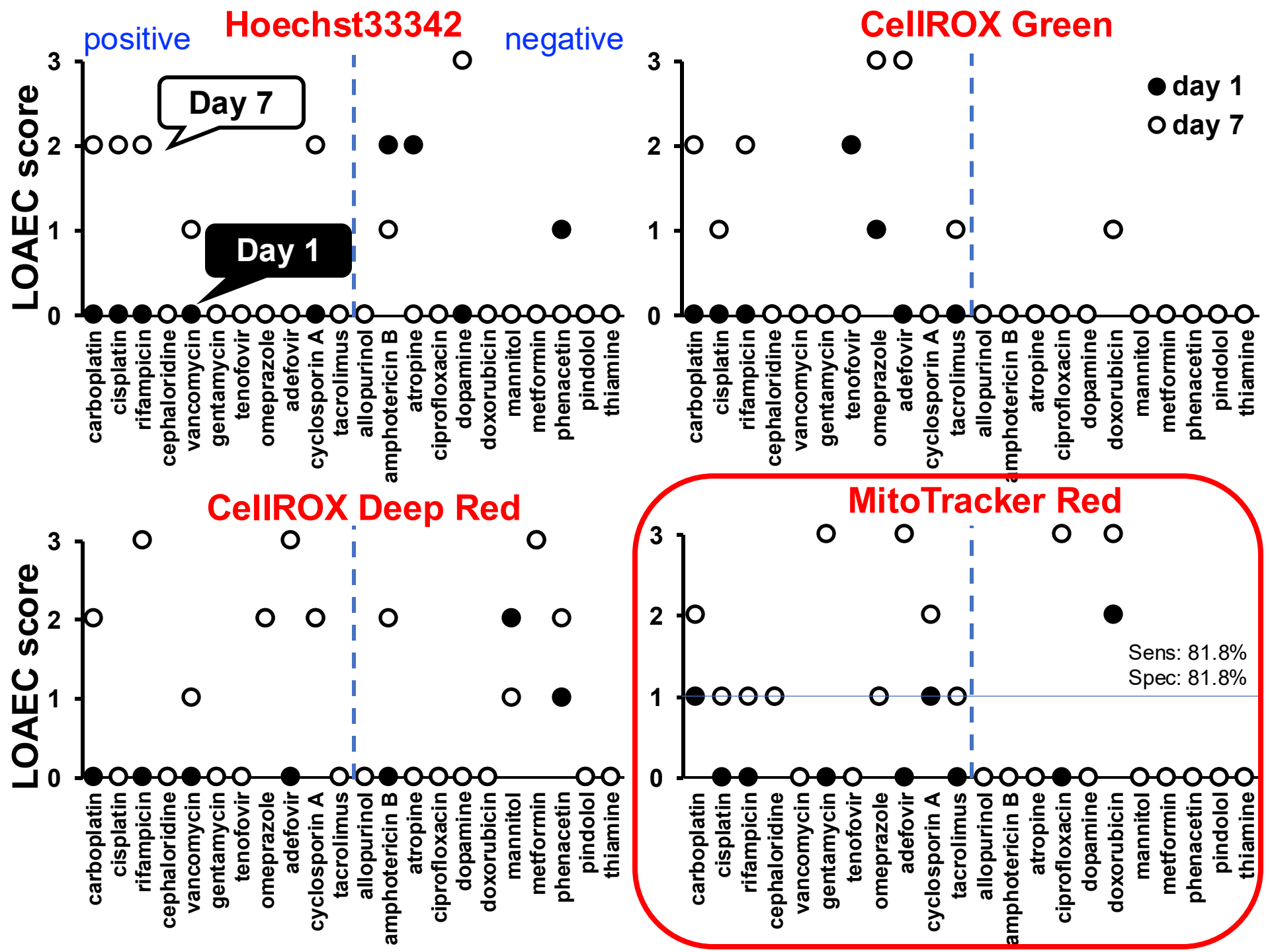
High content analysis (HCA)





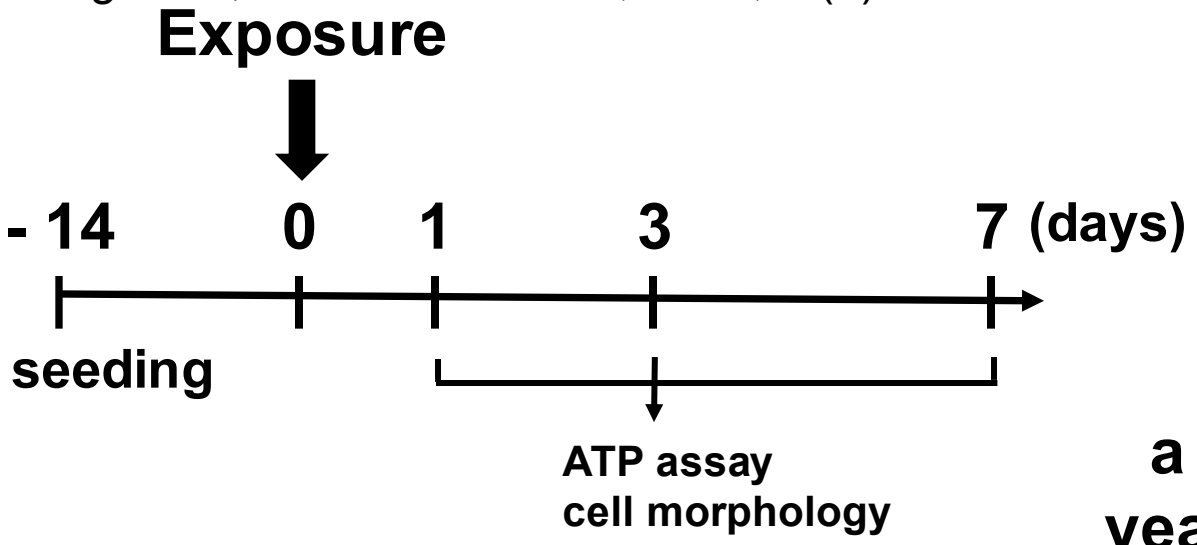


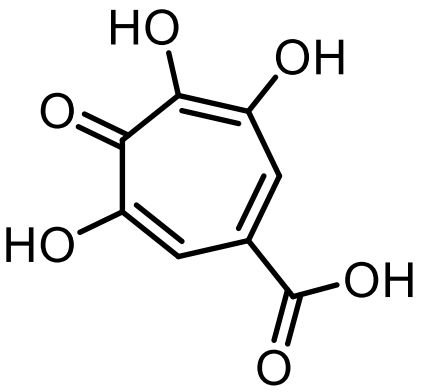
LOAEC based score	
3	within 1* C_{max}
2	within 10* C_{max}
1	within 100* C_{max}


Hoechst33342
positive
negative
Day 7
Day 1
LOAEC score
CellROX Green
day 1
day 7
CellROX Deep Red
MitoTracker Red
Sens: 81.8%
Spec: 81.8%
carboplatin
cisplatin
rifampicin
cephaloridine
vancomycin
gentamycin
tenofovir
omeprazole
adefovir
cyclosporin A
tacrolimus
allopurinol
amphotericin B
atropine
ciprofloxacin
dopamine
doxorubicin
mannitol
metformin
phenacetin
pindolol
thiamine

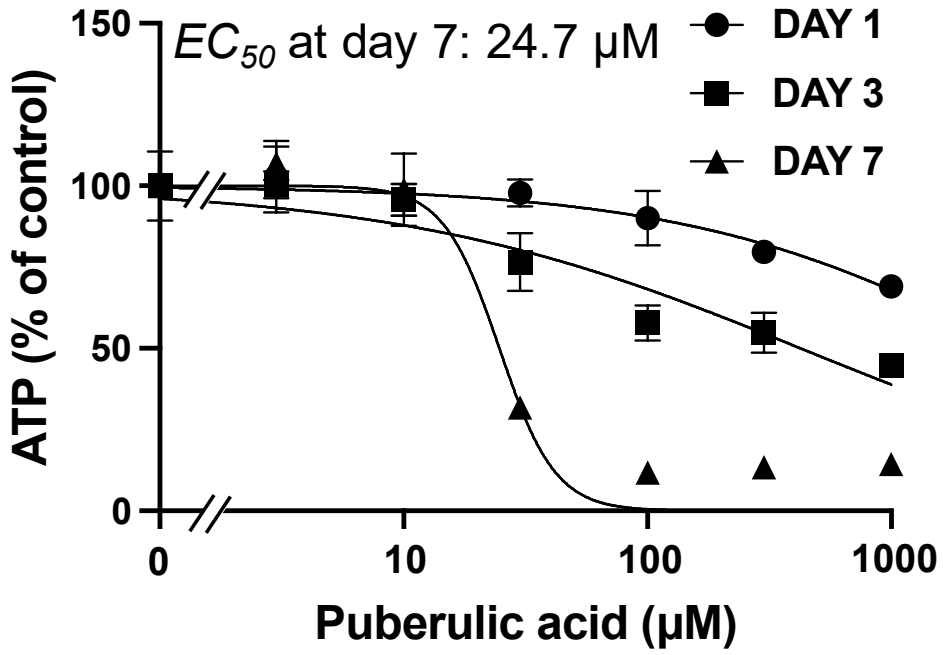
Evaluation of puberulic acid by 3D-RPTECs

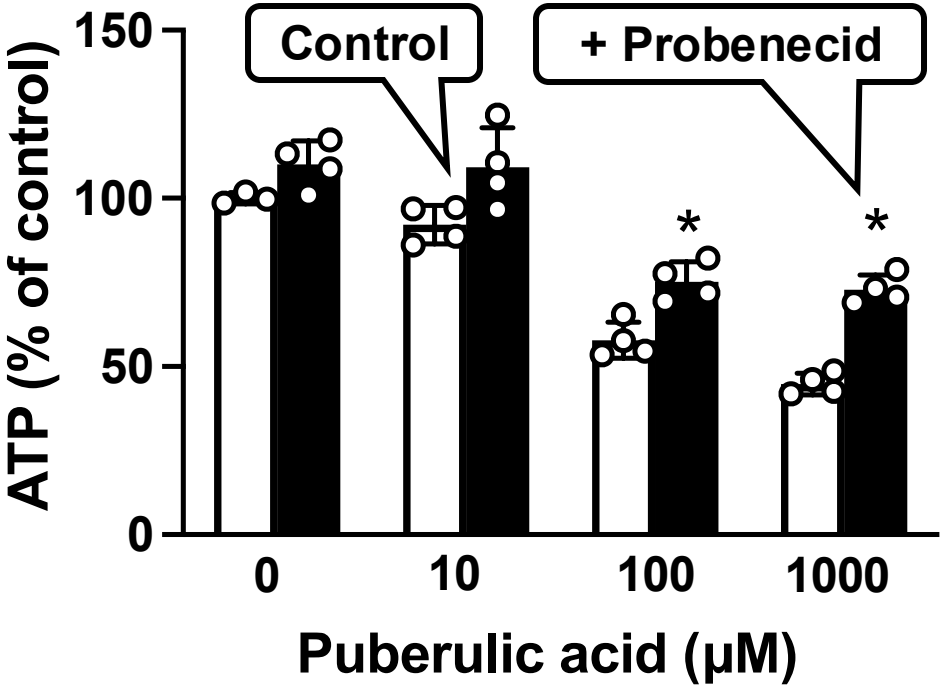
Peng *et al.*, ***Biol Pharm Bull***, 2025;48(6):928-931





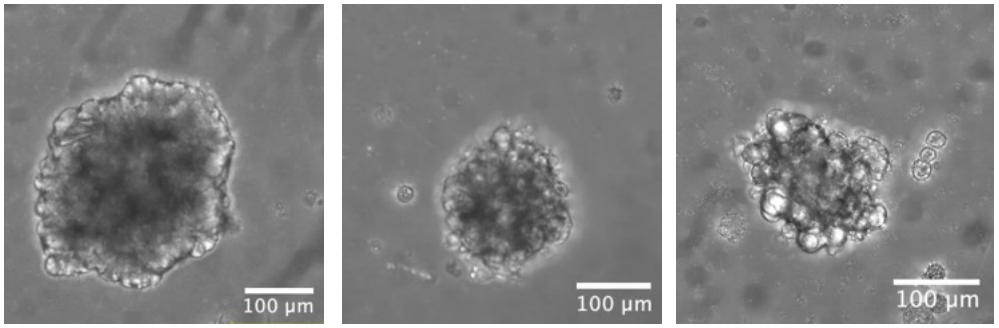
Puberulic acid
a concomitant in red-yeast rice supplement





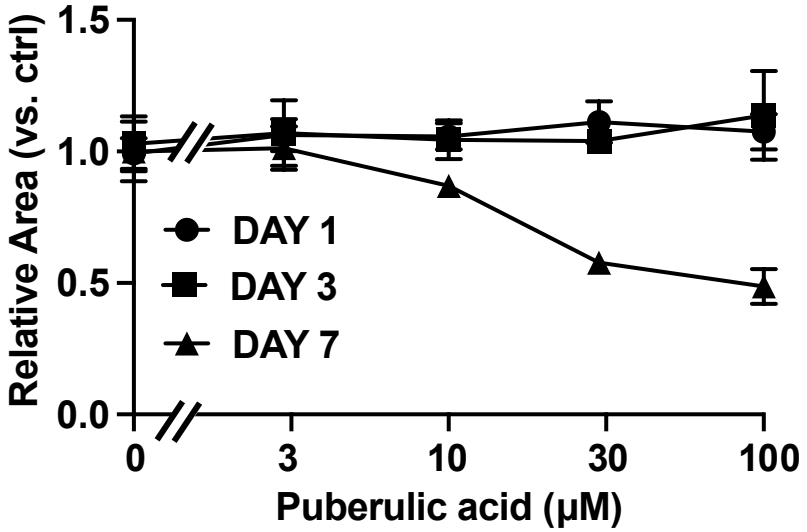
Nephrotoxicity Assessment of Puberulic Acid

Cell Area

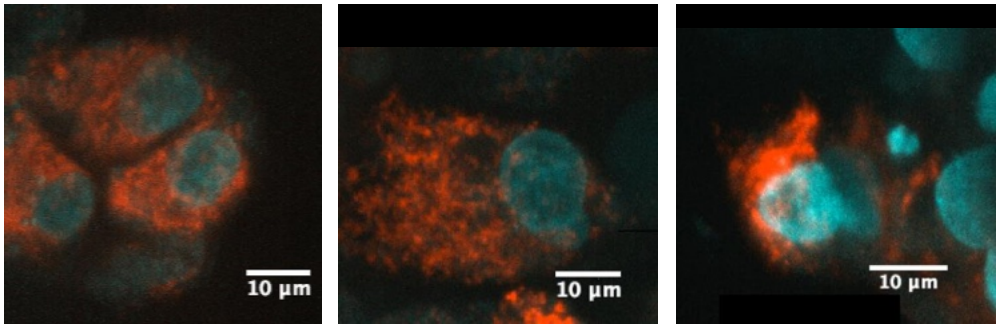


Control 30 μM 100 μM

Puberulic acid (Day 7)

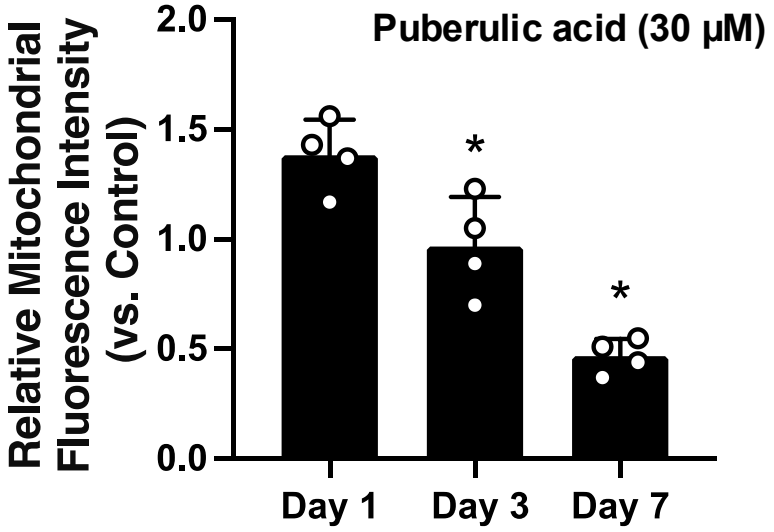


Mito-tracker Intensity



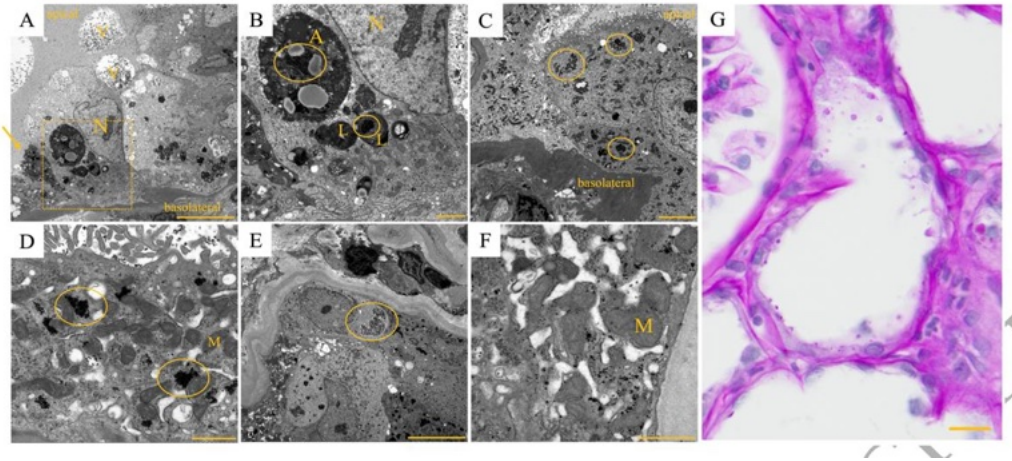
Control Day 3 Day 7

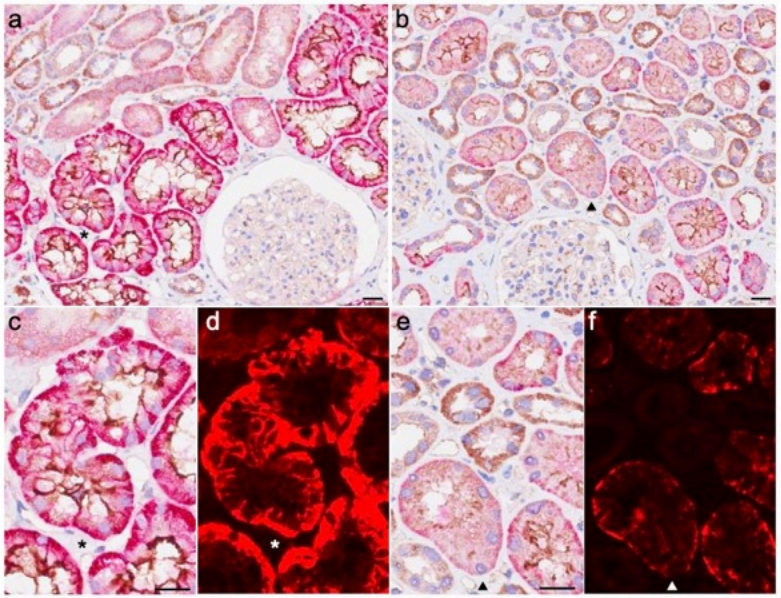
Puberulic acid (30 μM)



Peng *et al.*, ***Biol Pharm Bull***, 2025;48(6):928-931

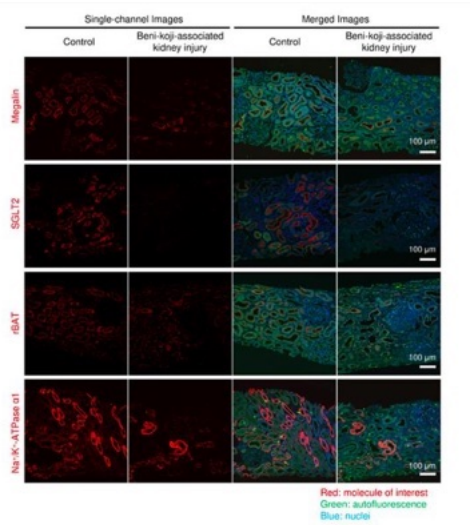
Kobayashi Pharmaceutical: Renal pathological anatomical findings of patients who ingested red yeast rice food.





Supplemental Figure 3. Immunohistochemical analysis of glucose transporter expression.

Immunohistochemistry demonstrating sodium-glucose cotransporter 2 (SGLT2) stained with 3,3'-diaminobenzidine tetrahydrochloride (DAB) and glucose transporter type 2 (GLUT2) stained with Fast Red in a case of minor glomerular abnormality (a) and gluconeogenetic acute tubular injury in a patient with acute kidney injury (AKI) linked to red yeast rice supplement use (b). Fast Red staining (c, e) was visualized using immunofluorescence microscopy (Texas Red fluorescent filter) (d, f). Asterisks and arrowheads indicate the same lesion. Scale bars represent 20 μm.



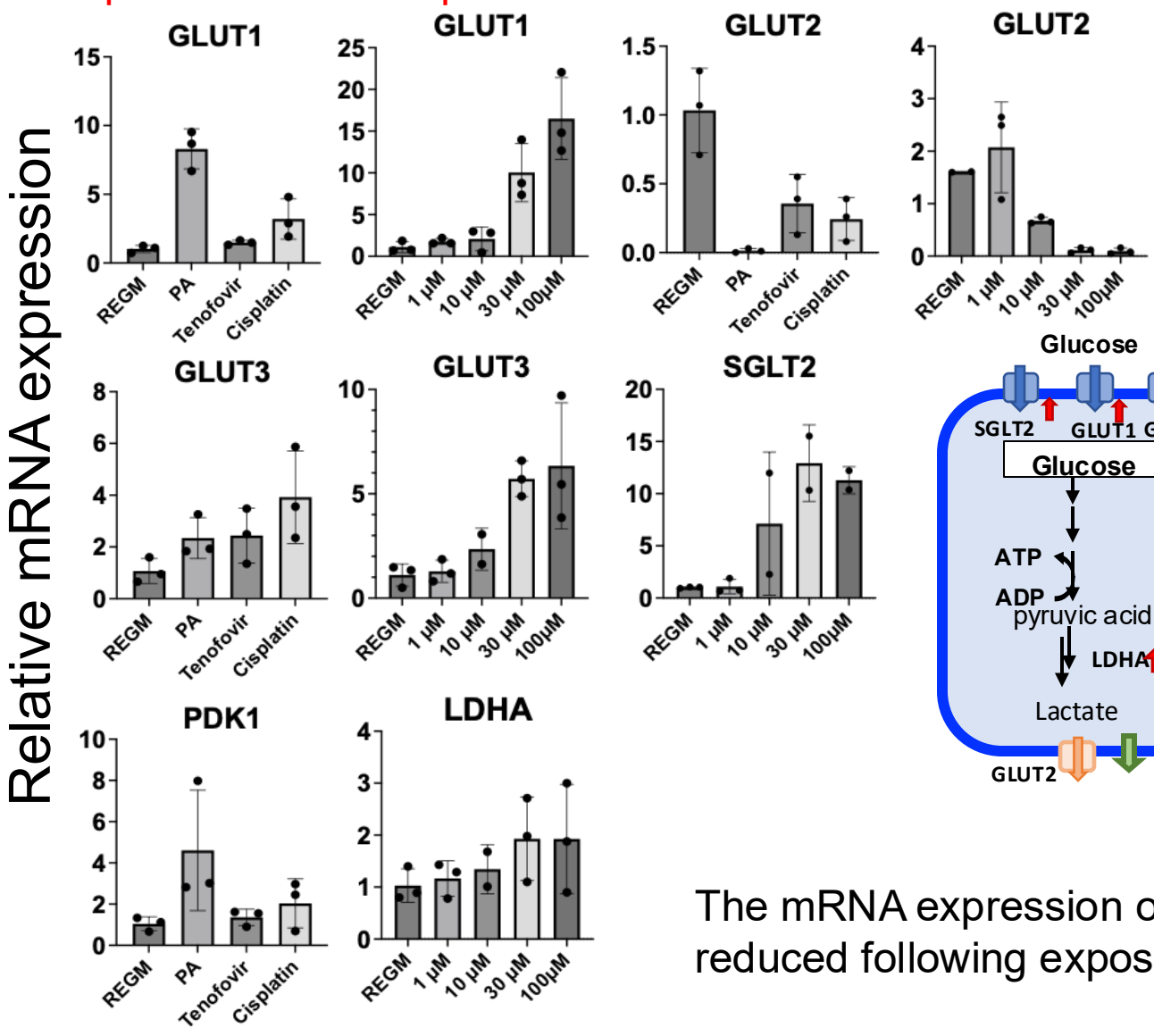
Supplementary Figure S2. Expression of megalin, SGLT2, rBAT, and Na^+/K^+-ATPase α1 in Beni-koji tablet-associated kidney injury

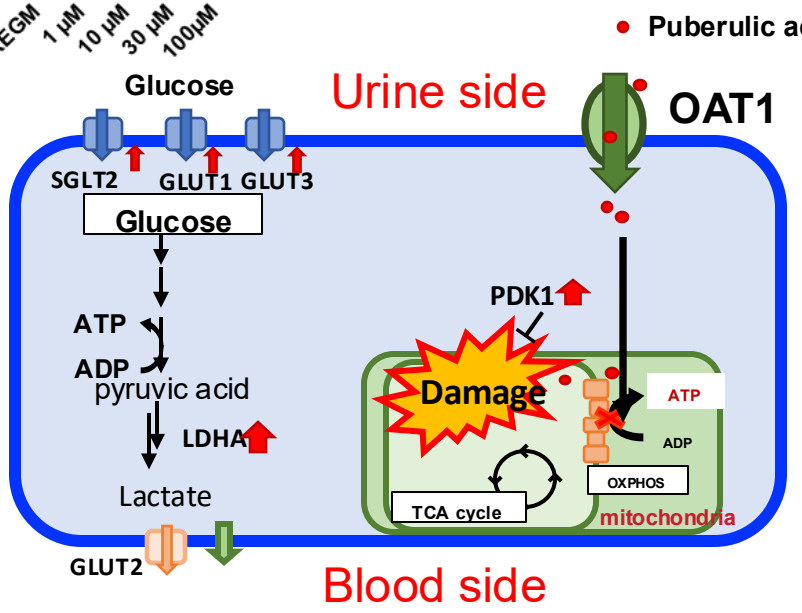
The following were observed in the proximal tubules of patients who took red yeast rice food:
Glycogen accumulation
Decreased expression of GLUT2

Ushimaru *et al.*, *Kidney Int Rep*. 2024 10:269-270.

Changes in mRNA Levels

PA: puberulic acid 30 μM

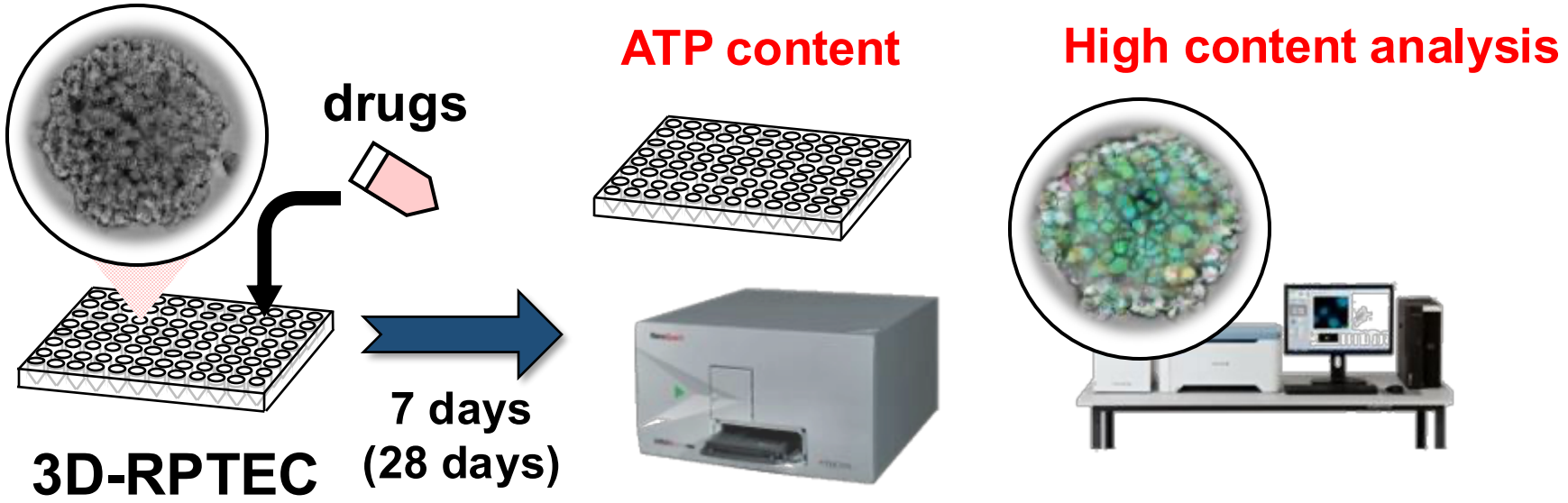




The mRNA expression of GLUT2 was dramatically reduced following exposure to puberulic acid.

Conclusion and Future Prospects

- Changes in intracellular ATP levels in 3D-RPTECs could be used as an indicator to assess drug-induced proximal tubular injury with high predictive performance.
- A domestic ring study of the cytotoxicity test using 3D-RPTECs obtained high inter-laboratory reproducibility.
- We will continue to validate their usefulness in drug and chemical safety.



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