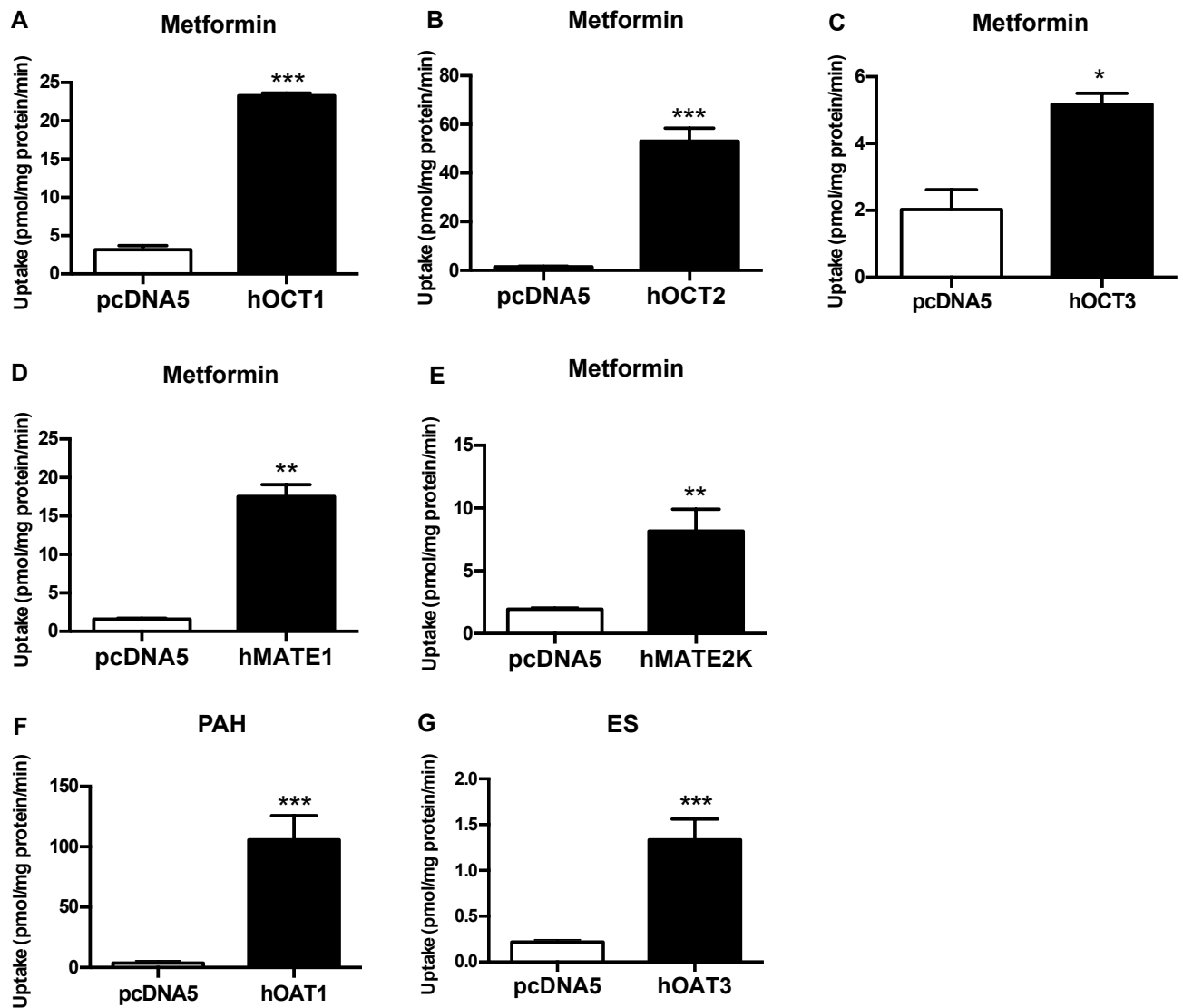Supplemental Data for **Drug Metabolism and Disposition**

Atenolol Renal Secretion Is Mediated by Human Organic Cation Transporter 2 (hOCT2) and Multidrug and Toxin Extrusion Proteins (hMATEs)

Jia Yin, Haichuan Duan, Yoshiyuki Shirasaka[1], Bhagwat Prasad, and Joanne Wang

Supplemental Figure 1. Functional characterization of HEK293 cells stably expressing selected drug transporters. The uptake of probe substrate for each drug transporter was measured in both transporter-transfected and pcDNA5-transfected control HEK293 cells. **(A) - (C)**: uptake of 6.1 μM metformin for 2 min by hOCT1, hOCT2, and hOCT3. **(D)** and **(E)**: uptake of 6.1 μM metformin for 5 min by hMATE1 and hMATE2-K. **(F)**: uptake of 2 μM PAH for 2 min by hOAT1. **(G)**: uptake of 0.06 μM ES for 2 min by hOAT3. Data are presented as

mean ± SD. Uptake in transporter-expressing cells were compared with that in control cells, ***

P < 0.001, ** P < 0.01, * P < 0.05.

Supplemental Table 1: Surrogate peptides of hOCT1, hOCT2, hMATE1 and hMATE2-K and

their MS/MS parameters for protein quantification.

Protein	Compound Name	Parent Ion	Product Ions	Fragmentor	Collision Energy	LLOQ (fmol on column)
hOCT1	LSPSFADLFR	576.7	476.6, 855	25	16	0.20
	LSPSFADLF<u>R</u>	581.8	778.4, 865.4	25	23	
hOCT2	LNPSFLDLVR	587.34	228.13, 946.54	140	15	0.62
	LNPSFLDLV<u>R</u>	592.34	228.13, 956.54	140	15	
hMATE1	GGPEATLEVR	514.9	617.3, 817.6	130	14	0.64
	GGPEATLEV<u>R</u>	519.9	827.6, 627.3	130	15	
hMATE2-K	TPEEAHALSAPTSR	489.7	618.3, 460.2	130	15	2.50
	TPEEAHALSAPTS<u>R</u>	494.7	628.3, 470.2	130	15	

Supplemental Table 2: Comparison of K_m and V_{max} values derived from Michaelis-Menten (MM) equation and MM with a diffusional component.

Transporter	MM fitting [a]			MM with diffusional component fitting [b]		
		V_{max}			V_{max}	
	K_m (μM)	(pmol/min/mg protein)	R^2	K_m (μM)	(pmol/min/mg protein)	R^2
hOCT1	128 ± 16	1355 ± 40	0.9404	128 ± 50	1356 ± 156	0.9404
hOCT2	280 ± 41	1836 ± 71	0.9715	278 ± 120	1838 ± 284	0.9714
hMATE1	32 ± 5	323 ± 12	0.9641	37 ± 9	318 ± 17	0.9630
hMATE2-K	76 ± 14	500 ± 22	0.9730	78 ± 21	500 ± 26	0.9730

[a.] Data are fitted to $V = V_{max}*S / (K_m + S)$ where V is the velocity of uptake, V_{max} is the maximum velocity of uptake, S is the substrate concentration, and K_m is the Michaelis-Menten constant. [b.] Data are fitted to $V = V_{max}*S / (K_m + S) + P_{dif}*S$ where a first order diffusional process is included. The P_{dif} is the nonsaturable passive diffusion rate constant.