Supplemental Data

Journal Title

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Article Title

Impact of direction of transport on the evaluation of inhibition potencies of multidrug and toxin extrusion protein 1 (MATE1) inhibitors

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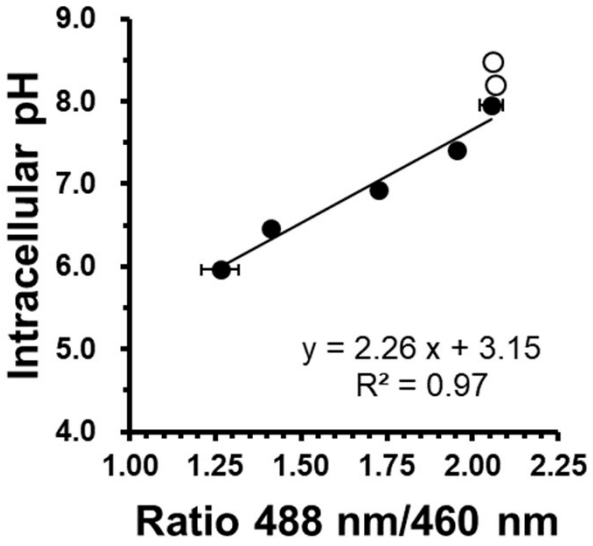
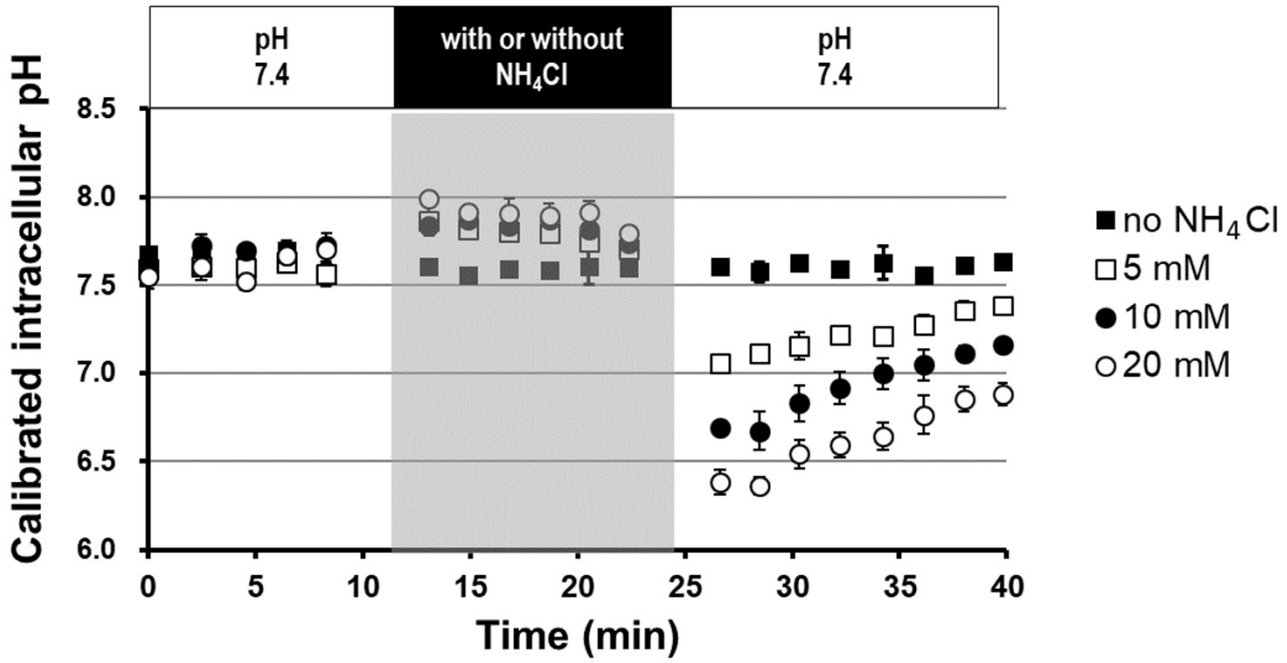


Supplemental Figure 1: Correlation between intracellular pH and ratio of fluorescence intensity of BCECF at 488 and 460 nm.

MATE1-expressing HEK293 cells were pre-loaded with BCECF-AM at 37° C for 30 min. The ratio of fluorescence intensities of excitation at 488 nm and 460 nm was measured. Intracellular pH was calibrated using standardized pH buffers (pH 6.0 – 8.0, closed circle) containing 10 μM nigericin. Data from pH 8.2 and 8.5 buffers (open circle) was not included due to a limitation of the method. Each point represents the mean value ± S.D. (n = 3).

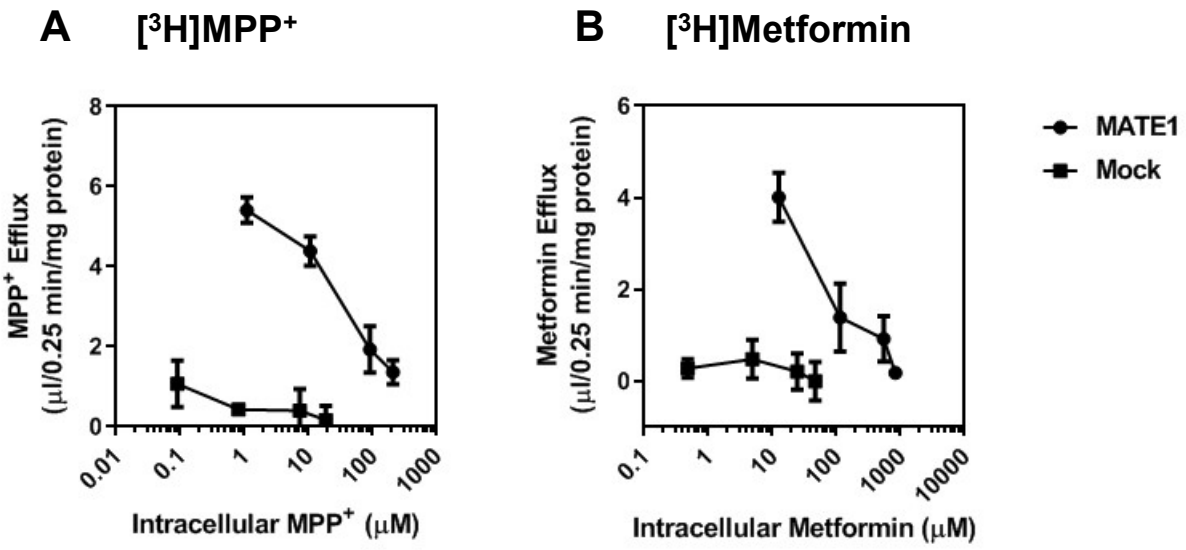


Supplemental Figure 2: The effect of various concentration of NH_4Cl on intracellular pH in MATE1-expressing HEK293 cells.

MATE1-expressing cells were pre-loaded with BCECF-AM at 37° C for 30 min. The ratio of fluorescence intensities of excitation at 488 nm and 460 nm was measured.. Each point represents the mean value ± S.D. (n = 3).

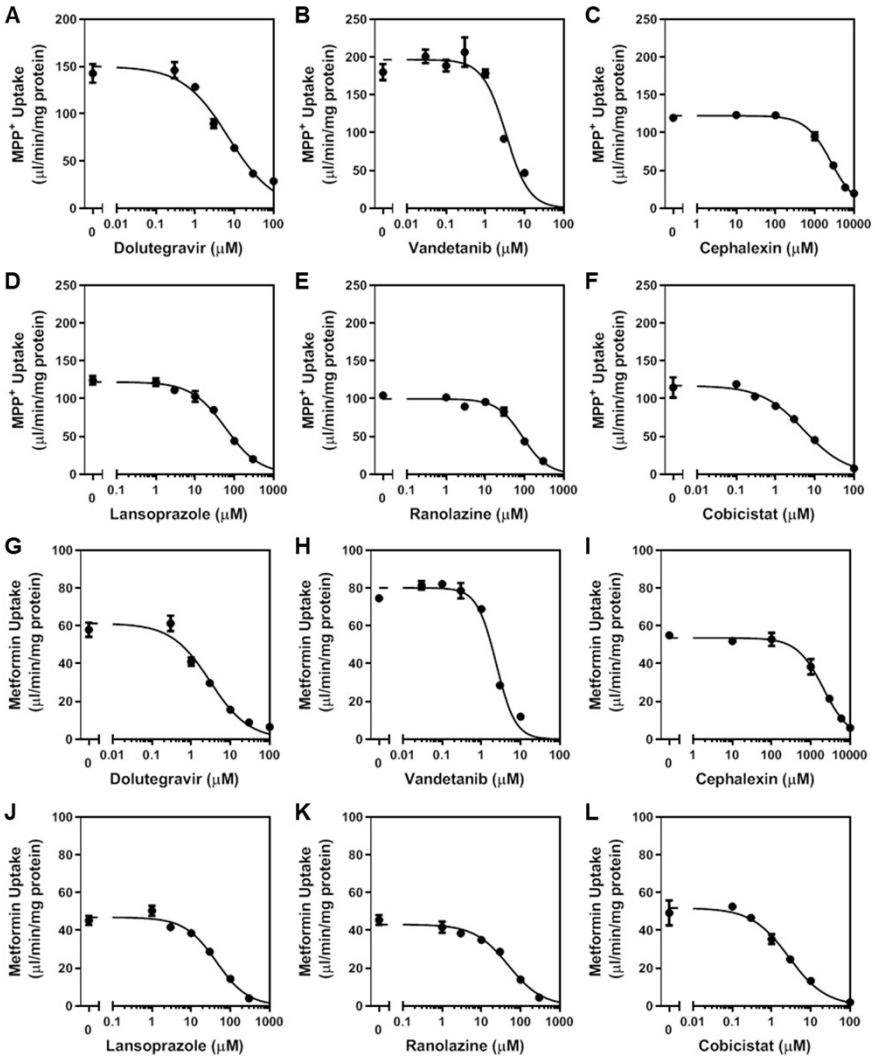
Equilibrium 37˚C	Pre-load 37˚C	Efflux 37˚C	Termination ice-cold
Aspirate culture media ↓ Wash (x 3) with NH_4Cl free buf ↓ Add 500 µL NH_4Cl free buf ↓ **30min**	Aspirate NH_4Cl free buf ↓ Add 250 µL NH_4Cl free buf with substrate ↓ **10min**	Aspirate NH_4Cl free buf with substrate ↓ Add 250 µL 20 mM NH_4Cl buf with or without inhibitor ↓ **0.25min**	Aspirate 20 mM NH_4Cl buf with or without inhibitor ↓ Wash (x3) with ice-cold NH_4Cl free buffer ↓ Dry up ↓ Cell collection

Supplemental Figure 3: Assay procedure of developed efflux assay.



Supplemental Figure 4: Concentration-dependent efflux of [^{3}H]MPP$^+$ (A) and [^{3}H]metformin (B).

Efflux of [^{3}H]MPP$^+$ (nominal concentrations: 0.05, 0.5, 5, 15 µM) and [^{3}H]metformin (nominal concentrations: 0.75, 7.5, 37.5, 75 µM) were determined in MATE1-expressing HEK 293 cells (closed circles) and Mock-HEK cells (closed squares) for 0.25 min. Intracellular concentrations of substrates were determined by assuming that the cellular volume per milligram protein as 6.5 µL [Gillen et al., 1999] . Each point represents the mean value ± S.D. (n = 3).



Supplemental Figure 5: Inhibitory effects of various compounds on the uptake of [^{3}H]MPP$^+$ and [^{14}C]metformin.

Uptake of [^{3}H]MPP$^+$ (1 μM, 1 min, A-F) and and [^{14}C]metformin (10 μM, 1 min, G-L) was determined in the absence and presence of indicated inhibitors at pH 7.4 in 20 mM NH_4Cl pre-pulse condition. Each point represents the mean value ± S.E. (n = 3).