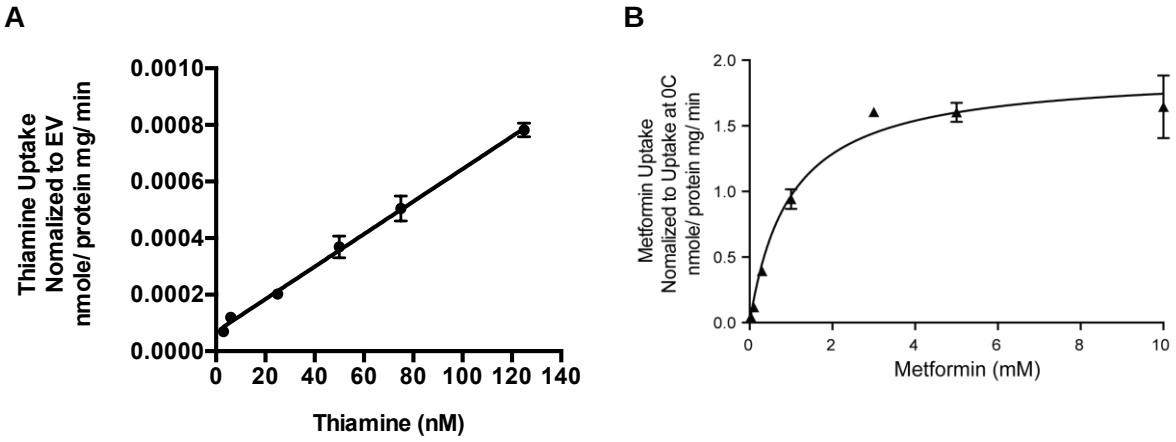
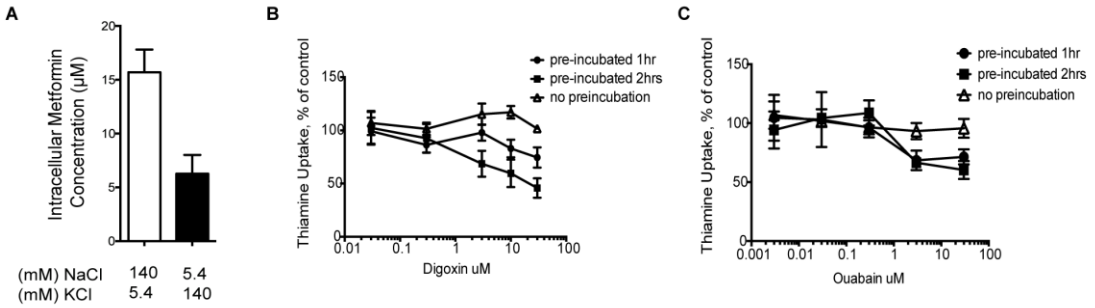
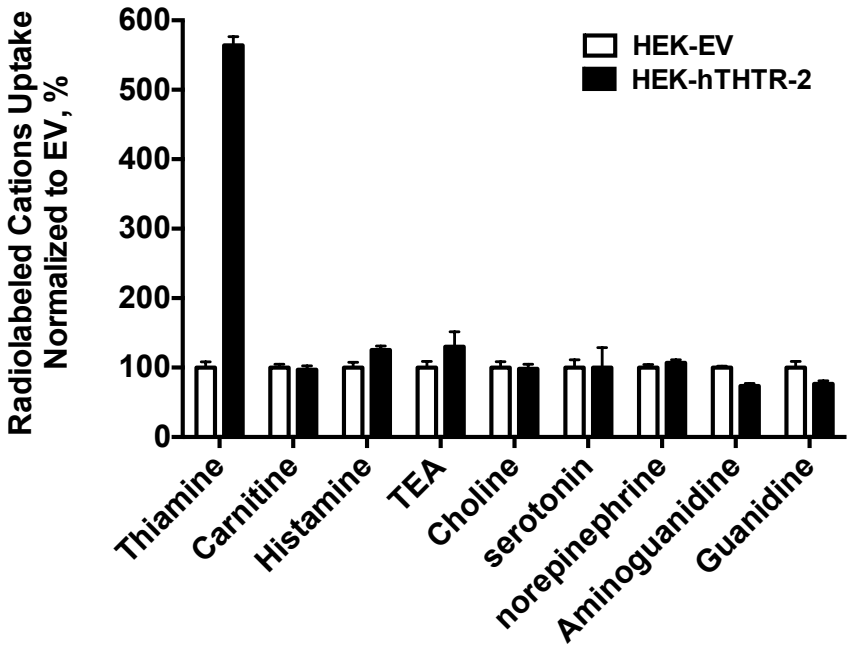
Supplemental Figures.



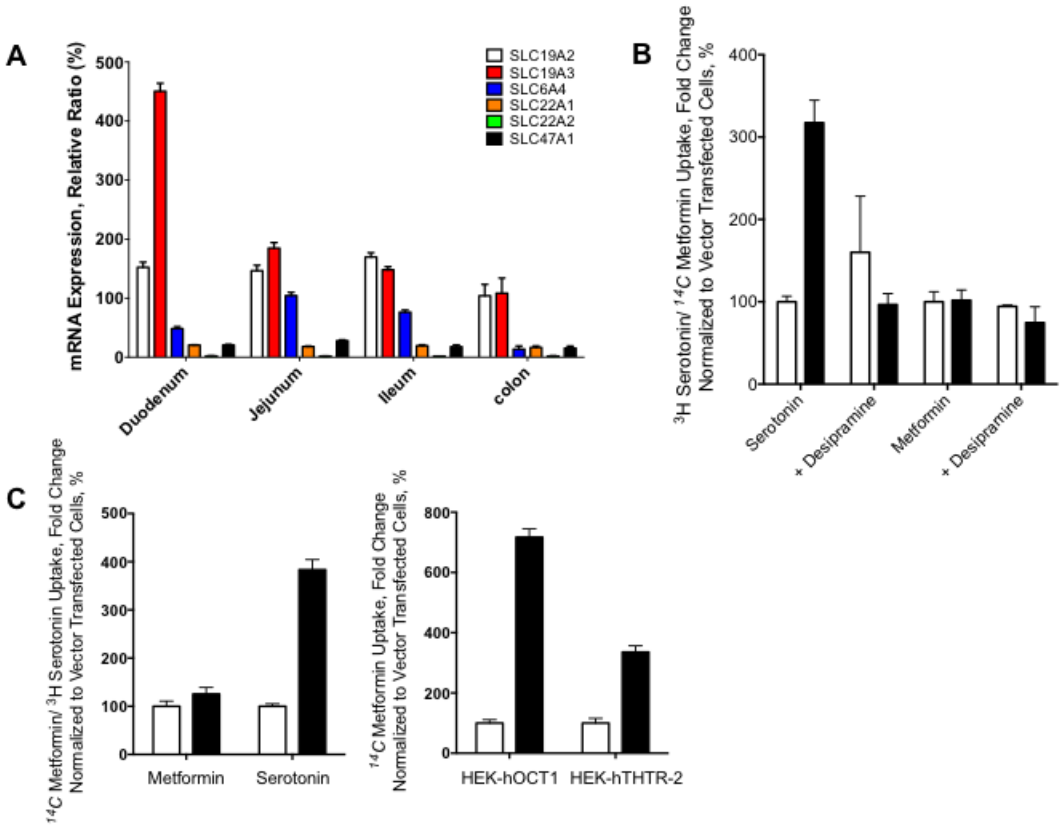
SP-1. A. Kinetic characterization of hTHTR-2 mediated thiamine uptake. The initial rate (i.e. 3 mins) of thiamine uptake by HEK-hTHTR-2 stable cell lines over the range of 3 to 125 nM. **B.** Kinetic characterization of hTHTR-2 mediated metformin uptake. The initial rate of metformin uptake in HEK-hTHTR-2 cells increased with concentration and was saturable. Cells were incubated with concentrations ranging from 0.1 mM to 10 mM for 7 minutes at 37°C (black circle) or 0°C (empty square). The saturable metformin uptake rate was calculated as the difference in accumulation between the uptake at 37°C and 0°C and data were fit to a Michaelis-Menten equation. Data shown are the mean ± SD.



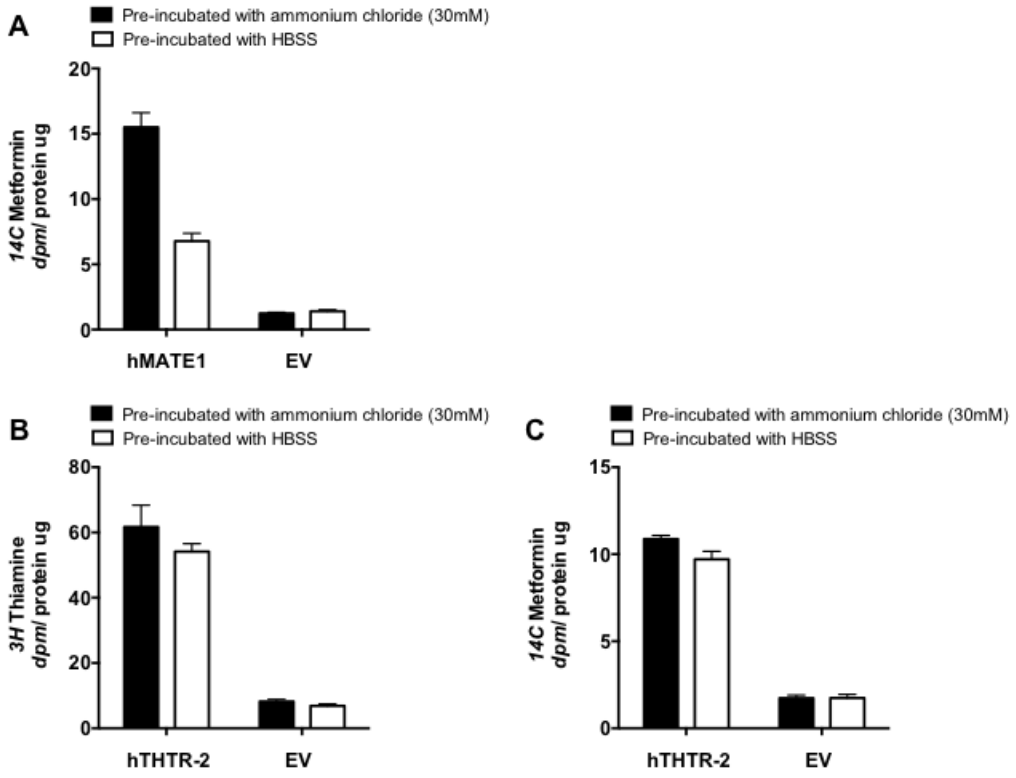
SP-2. The effect of electrochemical gradient on hTHTR-2 mediated uptake. **(A)** Cells were incubated in normal HBSS buffer with 140 mM NaCl and 5.4 mM KCl (empty bar) or KCl replacement buffer with 140 mM KCl and 5.4 mM NaCl (black bar) for 20 minutes. Data are presented as the difference in intracellular concentrations between hTHTR-2 over-expressing and empty vector cells. **(B)** Cells were pre-incubated with digoxin (30 μM, 10 μM, 3 μM, 300 nM, 30 nM, and 0 (control)) or **(C)** ouabain (30 μM, 3 μM, 300 nM, 30 nM, 3 nM and 0 (control)) for no, 1 or 2 hours pre-incubation before exposure to uptake buffer. Data are presented as the difference between hTHTR-2 over-expressing and empty vector cells. Data shown are the mean ± SD for a representative experiment of n= 2.



SP-3. Radiolabeled uptake of various organic cations in hTHTR-2 over-expressing cells. Cells were incubated in the uptake buffer containing radiolabeled compounds for 5 minutes. Data shown are the mean ± SD for three replicates in a representative experiment.



SP-4. Metformin uptake in human SERT overexpressing cells. **(A)** The relative mRNA levels of SLC19A2, SLC19A3, SLC6A4, SLC22A1, SLC22A2, and SLC47A1 were determined by real-time PCR. The mRNA expression level of SLC19A2 in colon was set to 100%. Data are presented as mean ± SD and pooled samples are from 5 to 39 individuals. **(B)** Stably overexpressing human SERT HEK cells were incubated in the uptake buffer containing ^{3}H serotonin (positive control) or ^{14}C metformin with or without 50 µM desipramine for 10 minutes. **(C)** Transient transfected SERT (pCMV6-XL4 vector), hOCT1 (pcDNA5/FRT vector), and hTHTR-2 (pcDNA5/FRT vector) HEK cells were incubated in the uptake buffer containing ^{3}H serotonin (positive control) or ^{14}C metformin for 10 minutes. Data shown are the mean ± SD for a representative experiment of n= 3.



SP-5. The effect of pre-incubation with ammonium chloride on hTHTR-2 mediated thiamine and metformin uptake. Cells were pre-incubated with HBSS buffer in the absence (white bar) or presence (black bar) of ammonium chloride (30 mM) for 30 minutes before exposure to uptake buffer. hMATE1 stable over-expressing cells **(A)** were incubated with ^{14}C metformin (5 µM) for 5 minutes. hTHTR-2 over-expressing cells were incubated with ^{3}H thiamine (25 nM) **(B)** and ^{14}C metformin (5 µM) **(C)** for 5 minutes. Data shown are the mean ± SD for a representative experiment of n= 2.