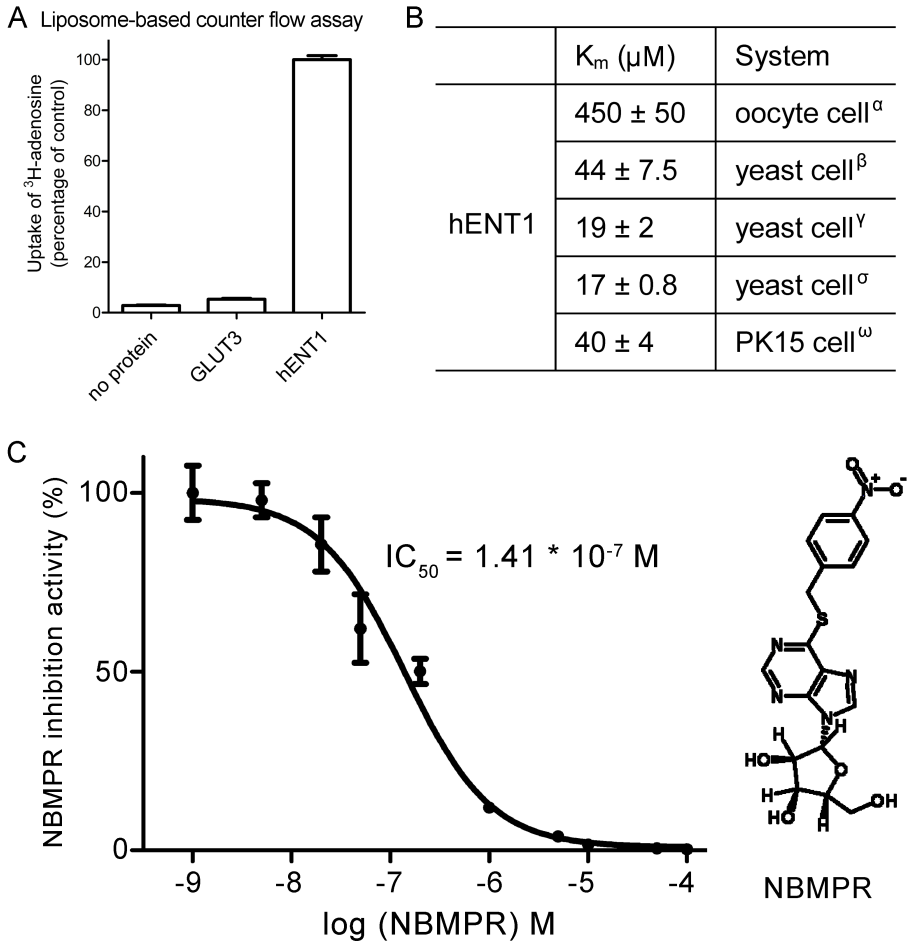

Figure S1. Transport activity of hENT1. (A) hENT1, but not the control membrane transporter GLUT3, efficiently transported adenosine. No protein refers to the condition where protein-free liposomes were used. (B) Reported K_m values for hENT1. The K_m values of hENT1 for adenosine were previously reported in studies using *Xenopus* oocyte, yeast, and PK15 cell. $^{\alpha}$Study published in ref. (Aseervatham et al., 2015), $^{\beta}$Study published in ref. (Endres et al., 2004), $^{\gamma}$Study published in ref. (Visser et al., 2007), $^{\sigma}$Study published in ref. (Visser et al., 2005), and $^{\omega}$Study published in ref. (Ward et al., 2000). (C) NBMPR inhibits the transport activity of hENT1 *in vitro* in a dose-dependent manner. Uptake of ^{3}H-adenosine into the hENT1-incorporated liposomes is subject to competition by the addition of increasing concentrations of the inhibitor NBMPR.

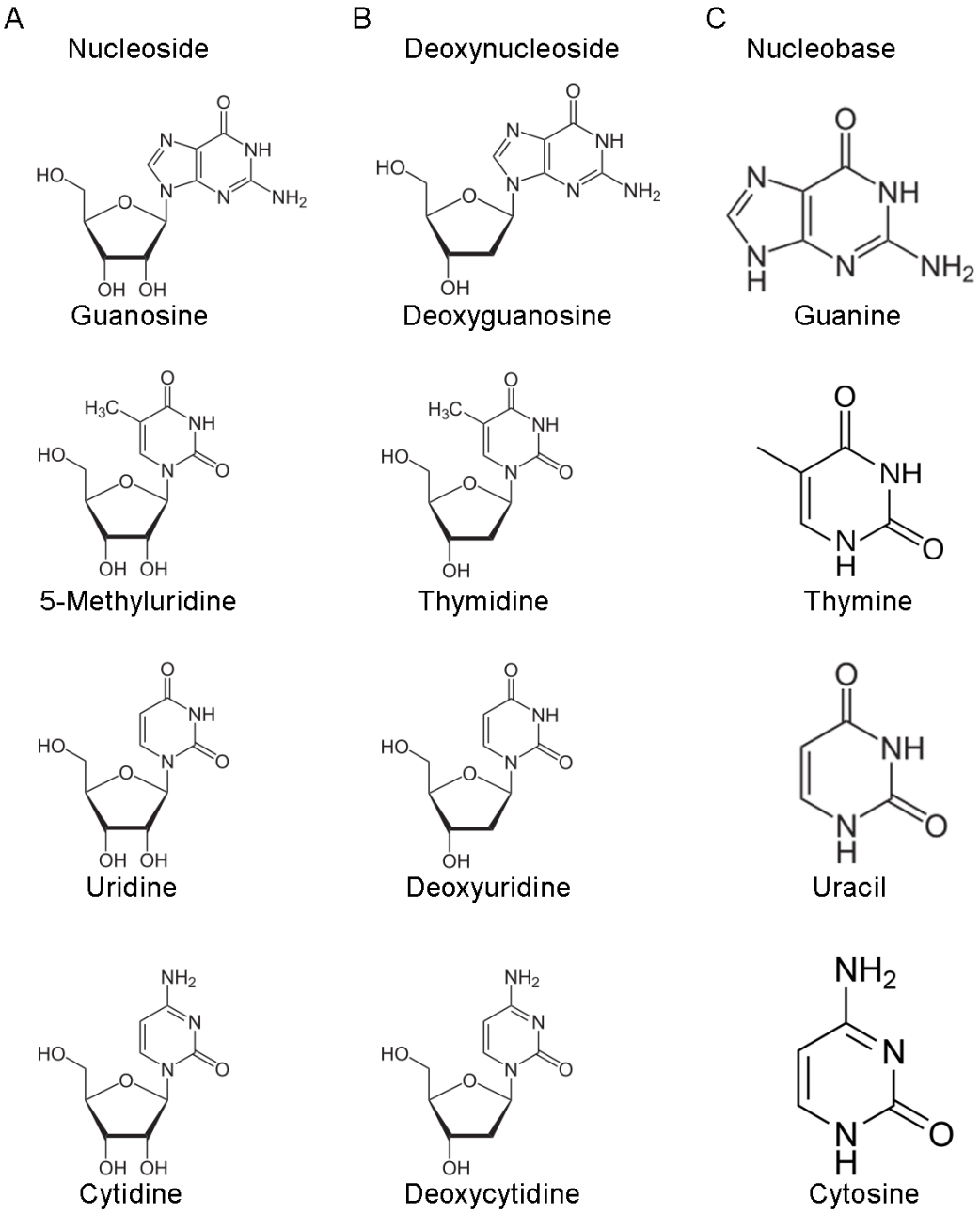

Figure S2. Chemical structures of representative nucleosides and nucleobases.

Nucleobases (C) are nitrogen-containing biological compounds, which link a 5-carbon sugar, either ribose or deoxyribose to form nucleosides (A) or deoxynucleosides (B).

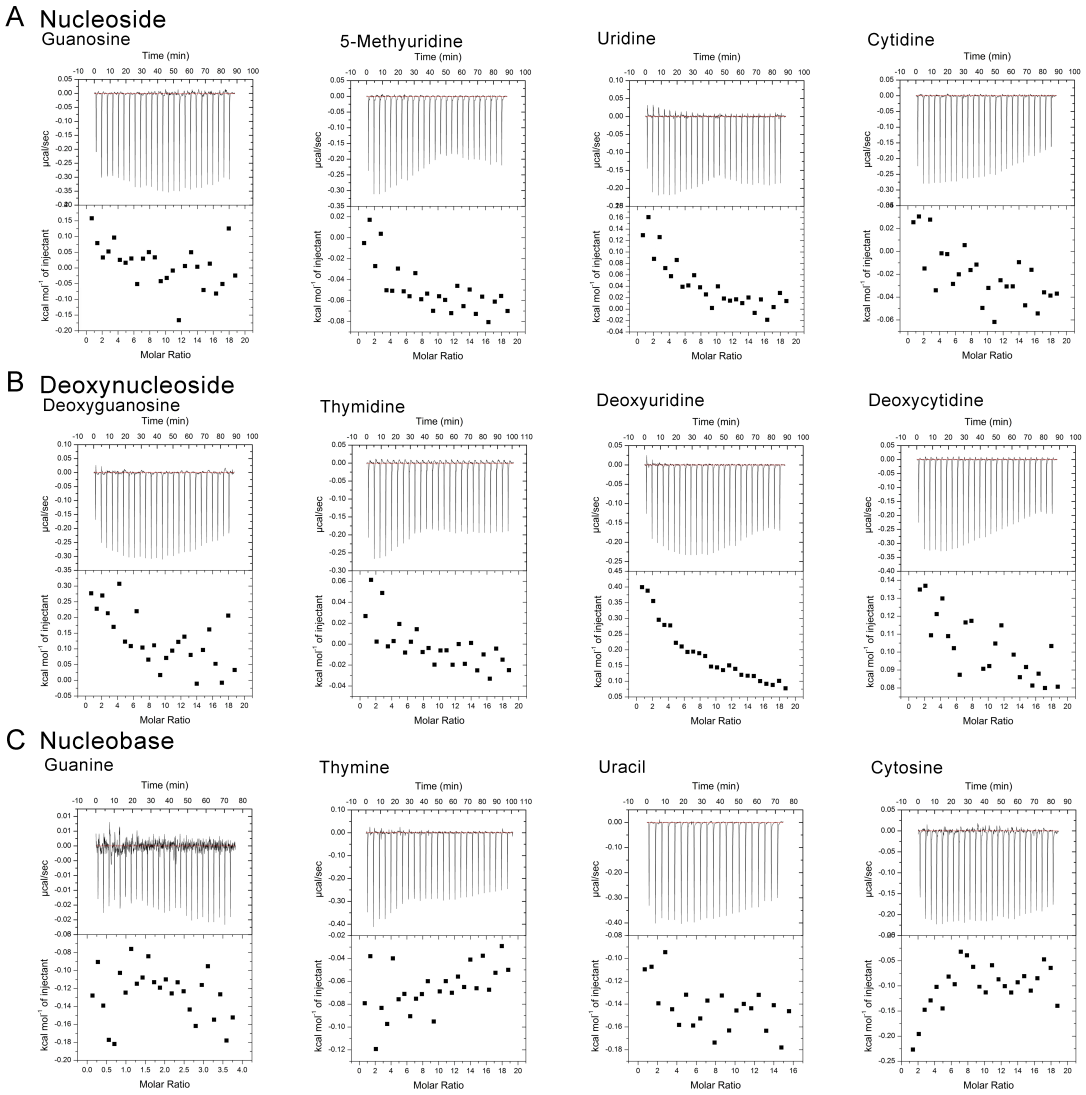

Figure S3. ITC analyses of nucleoside and nucleobase binding by hENT1. (A) None of the nucleosides examined exhibited detectable binding to hENT1 by isothermal titration calorimetry (ITC). Shown here from left to right are the ITC data for guanosine, 5-methyluridine, uridine, and cytidine. The ITC data for each nucleoside includes the original titration isotherm curves (upper panel) and the processed data points (lower panel). (B) None of the deoxynucleosides examined exhibited detectable binding to hENT1 by isothermal titration calorimetry (ITC). (C) None of the nucleobases examined exhibited detectable binding to hENT1 by isothermal titration calorimetry (ITC).

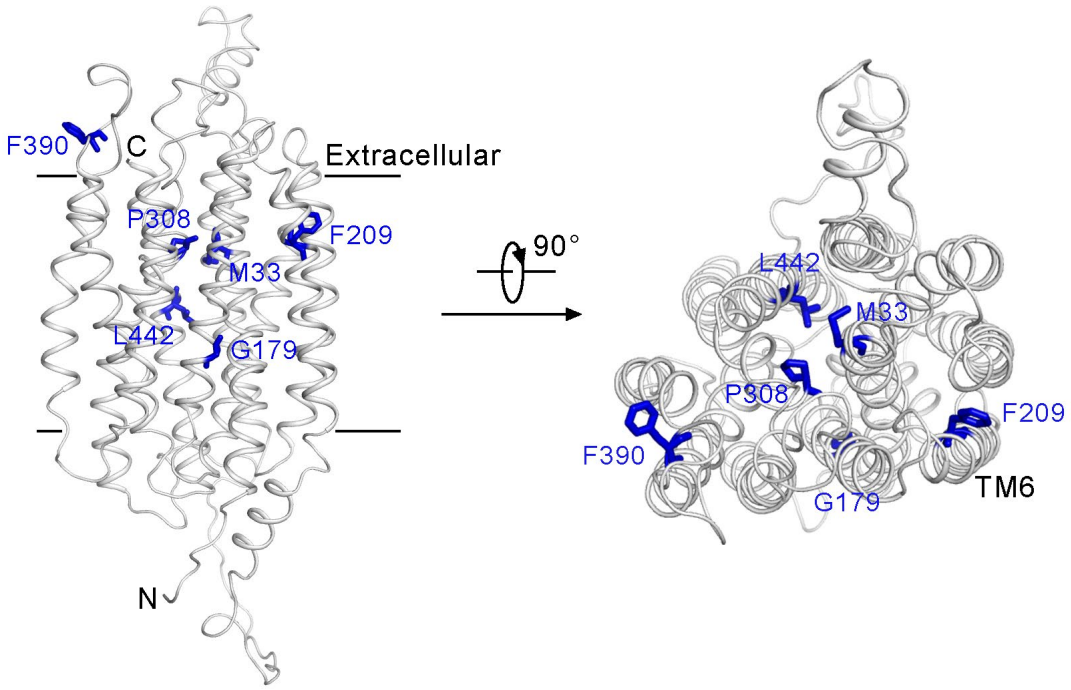

Figure S4. The structural model of hENT1. The structural model of hENT1 was generated as described in Materials and Methods. Two perpendicular views of hENT1 are shown by cartoon. Six representative residues in hENT1 are showed by sticks and labelled blue.

Supplementary References

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