SUPPLEMENTAL FIGURE LEGENDS

Fig. 1. Formation of an amphiphilic helix at the N-terminus of hENT3. Amino acids (indicated by single letter codes; *dotted circles*) 31-48 (*left)* and 32-49 (*right)* of hENT3 were analyzed by Edmundson helical wheel arrangement using GENETYX software. Note the positively charged residues (indicated by '+' sign) and hydrophobic residues (*filled circles*) align at opposite ends of the helix. Leucine residues capable of redirecting intracellular hENT3 to the cell surface are highlighted (*full circles*).

Fig. 2. Trafficking of LLAA-hENT3 and Δ36hENT3 YFP-tagged hENT3 mutant constructs to the cell surface. 3T3 mouse fibroblasts were transfected with pEYFP-LLAAhENT3 and pEYFP-Δ36hENT3 expression constructs and immunocytochemical analysis performed with 1:1000 anti-hENT3 polyclonal antibody. Note extensive colocalization (*yellow in merged images*) of YFP fluorescence of the fusion proteins (*green*) with the hENT3 antibody staining (*red*) in these cells. *Arrowheads* indicate localization of Δ36hENT3 and LLAhENT3 to the plasma membrane. Nuclei stained with DAPI are *blue.* Original magnification X40.

Fig. 3. A. Comparison of anti-hENT3 polyclonal antibody staining with staining obtained from C- and N-terminal directed hENT3 antibodies. HeLa cells grown on glass coverslips were fixed and immunostained with anti-hENT3 rabbit polyclonal (*left panels)* and with either anti- $hENT3_{N\text{-}terminus}$ (*top right*) or anti-$hENT3_{C\text{-}terminus}$ (*bottom right*) goat polyclonal antibodies. *Arrows* (*left panels*) indicate hENT3 staining pattern consistent with mitochondrial staining. Note distinct mitochondrial-like hENT3 staining pattern recognized by anti-hENT3 rabbit polyclonal antibody but not as well by the C- or N-terminus directed hENT3 antibodies. Nuclei stained with DAPI are

blue. Original magnification 60X. B. Comparison of hENT3 recognition patterns in western blots by various hENT3 antibodies. Total (T; 10 μg), mitochondrial (M; 20 μg) and the corresponding cytosolic fractions (C; 20 μg) were separated from HeLa cell lysates and subjected to western blotting analysis. Immunoblots were separately probed with 1:2000 anti-hENT3 rabbit polyclonal (*top*) antibody, 1:2000 anti-hENT3$_{\text{N-terminus}}$ (*middle*) goat polyclonal antibody or 1:2000 anti-hENT3$_{\text{C-terminus}}$ (*bottom)* goat polyclonal antibody. Note identification of hENT3 band (~65 kDa) by all three antibodies under denatured conditions of mitochondrial fractions.

Fig. 4. Transient expression of YFP-hENT3 in various cell types. Various cell lines grown in glass coverslips were transfected with pEYFP-hENT3 plasmid construct. Fluorescence arising from YFP-hENT3 was visualized directly under the microscope (*green)*. Note presence of only vesicular (dotted) staining of YFP-hENT3 in these cell types. Nuclei stained with DAPI are blue. Original magnification X60.

Fig. 5. Inhibition of ^{3}H-adenosine transport by nucleobases using Δ36hENT3 cRNA injected oocytes. Transport of ^{3}H-adenosine (1 μM) for 30 minutes was measured in Xenopus oocytes 24 h after injection of Δ36 hENT3 cRNA. Measurements were conducted after incubation of oocytes in Na+-free buffers at pH 6.5 in the absence (control) or presence of non-labeled nucleobases (1 mM). Note reduction in ^{3}H-adenosine transport into oocytes in the presence of high concentration of all three nucleobases. Values represent mean ±SE from two experiments.