Supplemental methods

Determination of substrate specificity of HUT-1 protein. The *hut-1* gene was subcloned into YEP352GAPII vector with three copies of HA epitope tag at the position corresponding to the carboxyl terminus of the expressed protein. Yeast transformation, subcellular fractionation, and nucleotide-sugar transport assays were performed as described previously (1). A *Saccharomyces cerevisiae* strain W303-1a (MATa, ade2-1, ura3-1, his3-11, 15, trp1-1, leu2-3, 112, and can1-100) was transformed by the lithium acetate procedure (2). Transformants were selected at 30 °C in a synthetic defined uracil-deficient medium. For the subcellular fractionation, the transformants were grown in the uracil-deficient medium at 30 °C. Cells were harvested, treated with zymolysase100T, and homogenized in ice-cold lysis buffer (0.8 M sorbitol in 10 mM triethanolamine, pH 7.2, 5 μg/ml pepstatin A, and 1 mM phenylmethylsulfonyl fluoride). The lysate was centrifuged at 1,000 x g for 10 min to remove unlysed cells and cell-wall debris. The supernatant was then centrifuged at 100,000 x g for 1 h at 4 °C to yield the microsome fraction. Two hundred micrograms of the protein from the microsome fraction was incubated at 30 °C for 5 min in 100 μl of reaction buffer (20 mM Tris-HCl, pH 7.5, 0.25 M sucrose, 5.0 mM $MgCl_2$, 1.0 mM $MnCl_2$, and 10 mM 2-mercaptoethanol) containing 1 μM radiolabeled substrate. After incubation, the radioactive substrate incorporated into the microsomes was trapped using a 0.45-μm nitrocellulose filter, and the radioactivity was measured by liquid scintillation. For each sample, the activity was calculated as the difference between the observed value and the background value obtained from the same assay at 30 °C for 0 min. The expression of HUT-1 protein was confirmed by Western Blot Analysis. Fifty micrograms of the protein from each sample was added to 3xSDS sample buffer (New England Biolabs Inc., Beverly, MA) and then incubated at room temperature for 2 h. The samples were fractionated on a 2-15% gradient SDS-polyacrylamide gel (Daiichi Pure Chemicals Co., Ltd., Tokyo, Japan) and were electrotransferred onto a polyvinylidene difluoride membrane. The HA-tagged proteins were immunostained with mouse anti-HA mAb and horseradish peroxidase-conjugated anti-mouse IgG mAb. The bound horseradish peroxidase was detected using ECLplus (Amersham Biosciences) according to the manufacturer's protocol.

REFERENCES

1. Kamiyama, S., Sasaki, N., Goda, E., Ui-Tei, K., Saigo, K., Narimatsu, H., Jigami, Y., Kannagi, R., Irimura, T., and Nishihara, S. (2006) Molecular cloning and

characterization of a novel 3'-phosphoadenosine 5'-phosphosulfate transporter, PAPST2. *J. Biol. Chem.* **281**, 10945-10953

2. Ito, H., Fukuda, Y., Murata, K., and Kimura, A. (1983) Transformation of intact yeast cells treated with alkali cations. *J. Bacteriol.* **153**, 163-168

Supplemental figure legends

Figure S1. Phylogenetic tree analysis of SLC35 sequences in *H. sapiens*, *D. melanogaster*, *C. elegans*, *A. thaliana*, *S. cerevisiae* and *S. pombe*.

The phylogenetic tree was constructed using ClustalW (http://align.genome.jp/). Subfamilies of SLC35B are depicted in gray circles. Previously identified substrates are indicated as colored asterisks: light green, UDP-Gal; blue, UDP-Glc; purple, UDP-Xyl and UDP-GlcNAc; brown, UDP-GlcA; and red, PAPS. The scale bar represents the evolutionary distance. Branch lengths are proportional to phylogenetic distance.

Figure S2. Effect of *hut-1* RNAi on HUT-1::EGFP expression.

EGFP fluorescence levels of F1 animals treated with control (left) and *hut-1* (right) RNAi for 48 h at 25°C were quantified with the COPAS Biosort. The fluorescence and TOF were expressed in arbitrary units.

Figure S3. RNAi-mediated knockdown of *hut-1* does not induce heat shock stress.

Effect of control (left), *hut-1* (middle) and *daf-21* (a *Caenorhabditis elegans* homologue of Hsp90, right) RNAi after 72 h of culture at 25 °C on GFP driven by the *hsp-16.2* promoter. Strong GFP induction in pharynx was observed in *daf-21* RNAi treated animals but not in control and *hut-1* RNAi animals. Scale bar = 50 μm.

Figure S4. hUGTrel1 protein rescues the ER stress phenotype in *hut-1* RNAi worms.

(*A*) DIC (upper) and fluorescence (bottom) images of an L4 *hut-1* RNAi worm with (left) or without (right) expression of hUGTrel1, which contains the P_{hsp-4}*::gfp* reporter. RNAi was treated for 72 h at 20°C. Scale bar = 50 μm. (*B*) Quantitation of the mean GFP level per animal. GFP images were thresholded and the intensity of GFP fluorescence in individual worms was measured with MetaMorph software. Values are expressed as mean±SEM. NS indicates no statistically significant difference ($P>0.05$; Student's t test). A double asterisk indicates a statistically significant difference ($P<0.005$).

Figure S5. Expression patterns of HUT-1::EGFP.

Representative expression patterns of P_{hut-1}*::*HUT-1::EGFP. Anterior is to the left. (*A* and *A'*) An L4 larva showing EGFP fluorescence in seam cells. (*B* and *B'*) An L4 larva showing EGFP fluorescence in spermatheca. (*C* and *C'*) Head regions of an L3 larva

showing EGFP fluorescence in gland cells. This animal did not show EGFP expression in the intestine possibly due to mosaicism of the extrachromosomal array. An asterisk indicates autofluorescence of the intestinal cells. (*D* and *D'*) An L4 larva showing EGFP fluorescence in DTC. Scale bar = 50 μm.

Figure S6. Characterization of HUT-1 by yeast expression.

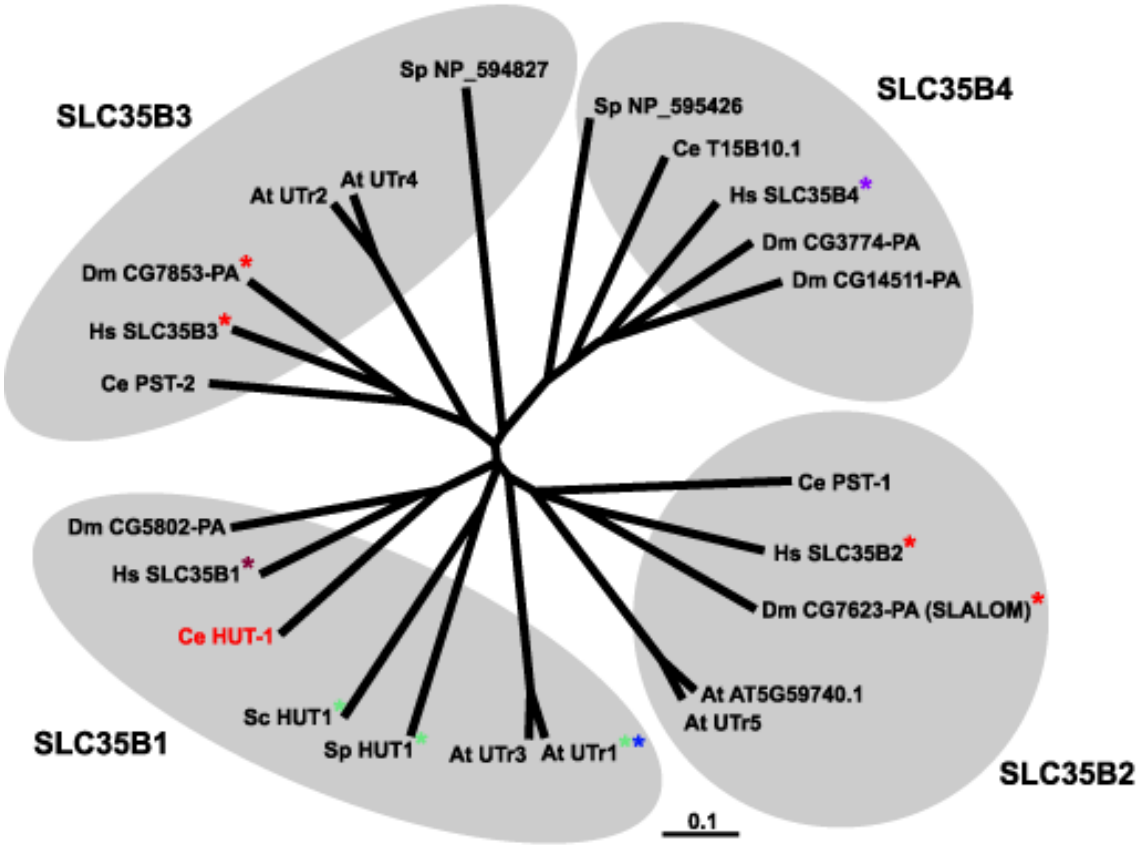
(A) Expression state of HUT-1 protein in the microsomal fraction. Each fraction prepared from yeast cells expressing either the mock vector (lane 1-3) or HA-tagged HUT-1 (lane 4-6) was loaded on SDS-PAGE and subjected to immunoblot analysis using anti-HA antibody. (B) Transport activity of HUT-1 for various nucleotide-sugars and PAPS. Each microsomal fraction was measured as described in Supplemental methods.

Supplemental Table 1: Primer used for reporter analysis

Construction		Primer sequences (5'- -3')
P_{hut-1}::egfp	Forward	5'- TTGTTCTTAATTTCACTTTATTTGACG –3'
	Reverse	5'- CATCTGGAAGGGAAGCATTT –3'
P_{hut-1}::hut-1::egfp	Forward	Same to *P_{hut-1}::egfp* Forward
	Reverse	5'- TTTATTATGCACTTTTGGCTCAG –3'
P_{eft-4}::hut-1::venus	Forward	5'- TGGCGGCCGCCCAAAAAACCATGAGACGCC –3'
	Reverse	5'- TGGCGGCCGCATTTATTATGCACTTTTGGCTC –3'
egfp::hut-1	Forward	5'- TCCCTCGAGGCCAAAAAACCATGAGACGCCACAT –3'
	Reverse	5'- GAAGGATCCTTATTTATTATGCACTTTTGGCTCAGTTG –3'
egfp::SP12	Forward	5'- TCCCTCGAGGGACGGAATGATTGCAATGCTC –3'
	Reverse	5'- GAAGGATCCTTATTTCGTCTTCTTTGTCTC –3'
P_{eft-4}::egfp::hut-1	Forward	5'- TAGTGGCGGCCGCATGGTGAGCAAGGGCGAGGA –3'
	Reverse	Same to *egfp::hut-1* Reverse
P_{eft-4}::egfp::SP12	Forward	Same to *P_{eft-4}::egfp::hut-1* Forward
	Reverse	5'- GAAGGATCCTTATTTCGTCTTCTTTGTCTC –3'
P_{aman-2}::aman-2::egfp	Forward	5'- CTGCTCCGCGTCATTTTTA –3'
	Reverse	5'- TTCTTTTTCTTCATCAAAATCTACCG –3'
P_{eft-4}::hUGTrel1	Forward	5'- TGGCGGCCGCGCCTCTAGCAGCTCCCTGGT –3'
	Reverse	5'- CTAGATCTCTAGTGGGATGTCTTCTTAGCT –3'
P_{eft-4}::schut1	Forward	5'- TGGCGGCCGCGCGGGAAGTACATCCAGTTT –3'
	Reverse	5'- AGAGATCTCTACGCAGATTTTGCCTTCG –3'
P_{eft-4}::sphut1	Forward	5'- TGGCGGCCGCGCTGGCTTTATGCGACAATT –3'
	Reverse	5'- CTAGATCTCTATGATGCCTTTTTTTTCG –3'
P_{eft-4}::ZK896.9	Forward	5'- TGGCGGCCGCGGGCTCACAAAAGCAGAAAC –3'
	Reverse	5'- CTAGATCTTTATGATTTGCTGCTCTCGAC –3'
P_{eft-4}::sqv-7	Forward	5'- TGGCGGCCGCACGTCAACAGTACAGTCACC –3'
	Reverse	5'- CTAGATCTTCAGTTCCTTGGCTTGTGCA –3'

Restriction recognition sites are underlined.

Figure S1



Identified transport substrates
*UDP-Gal *UDP-Glc
*UDP-Xyl and -GlcNAc
*UDP-GlcA *PAPS

Ce; *C. elegans*
Hs; *H. sapiens*
Dm; *D. melanogaster*
At; *A. thaliana*
Sp; *S. pombe*
Sc; *S. cerevisiae*

Figure S2

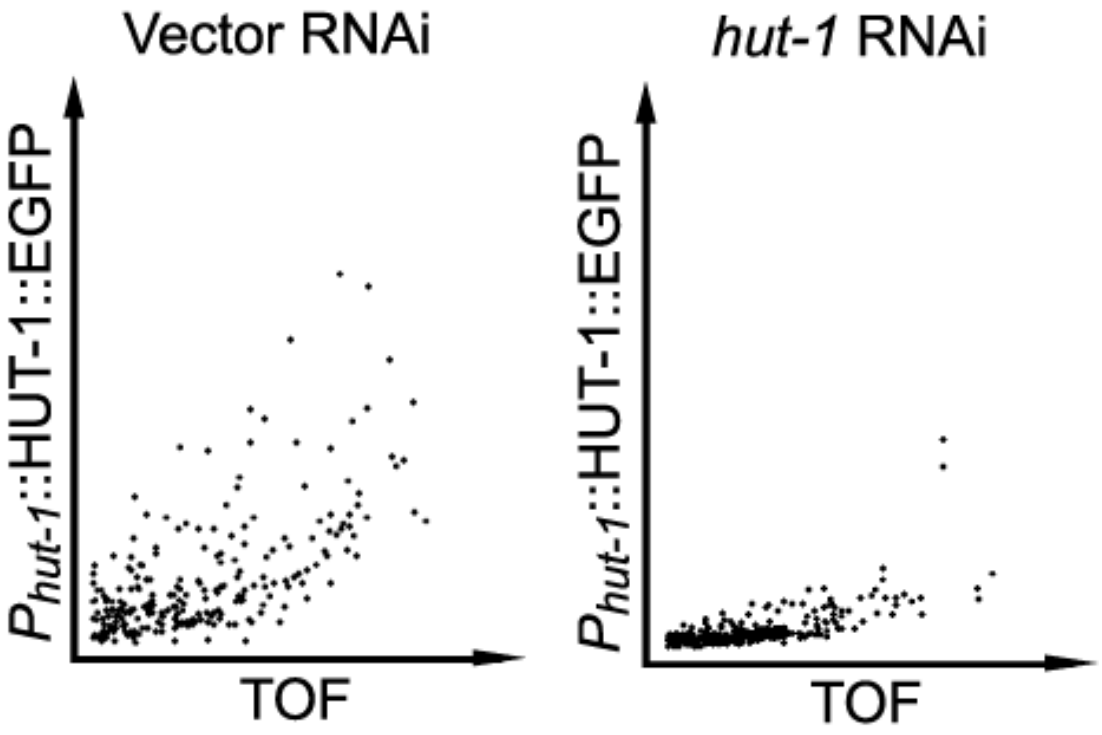

Vector RNAi
hut-1 RNAi
P_{hut-1}::HUT-1::EGFP
TOF
P_{hut-1}::HUT-1::EGFP
TOF

Figure S3

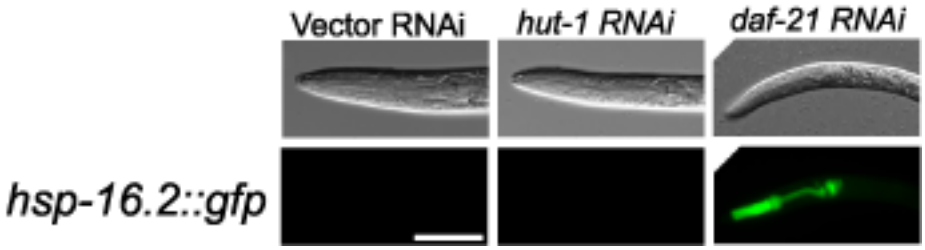
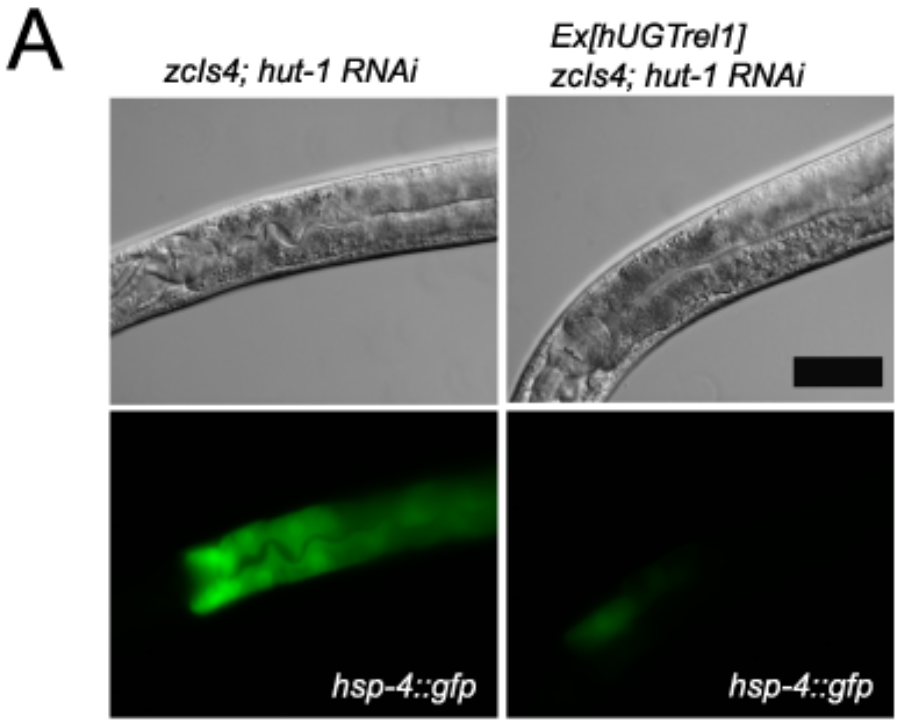


Figure S4


A
zcIs4; hut-1 RNAi
Ex[hUGTrel1]
zcIs4; hut-1 RNAi
hsp-4::gfp
hsp-4::gfp

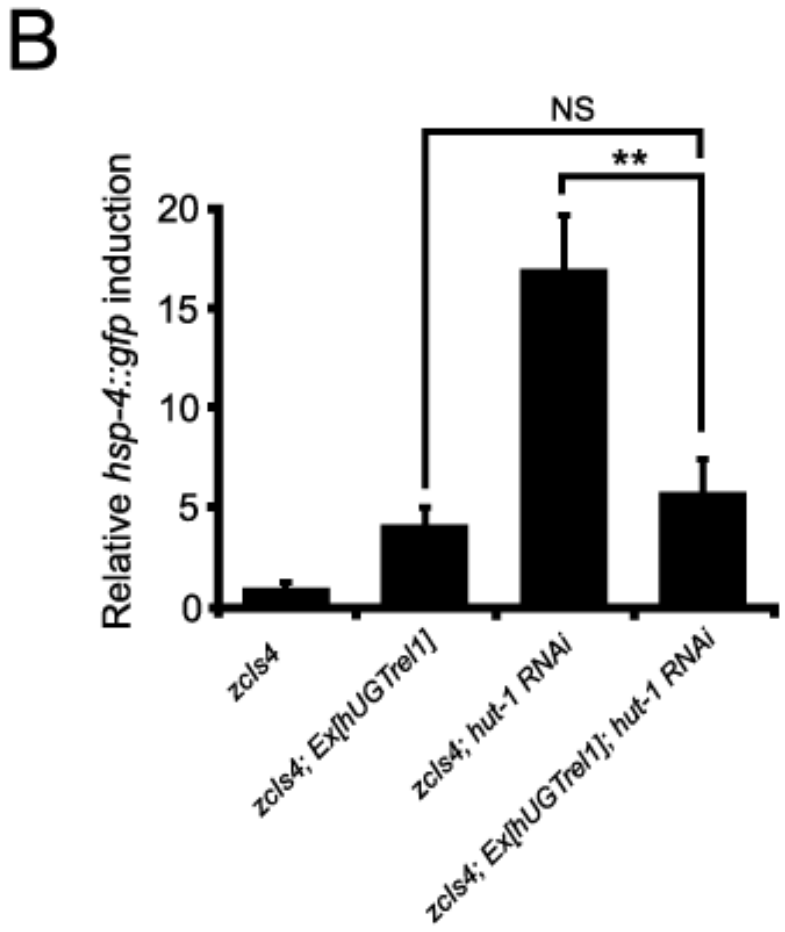

B
NS
**
Relative hsp-4::gfp induction
20
15
10
5
0
zcIs4
zcIs4; Ex[hUGTrel1]
zcIs4; hut-1 RNAi
zcIs4; Ex[hUGTrel1]; hut-1 RNAi

Figure S5

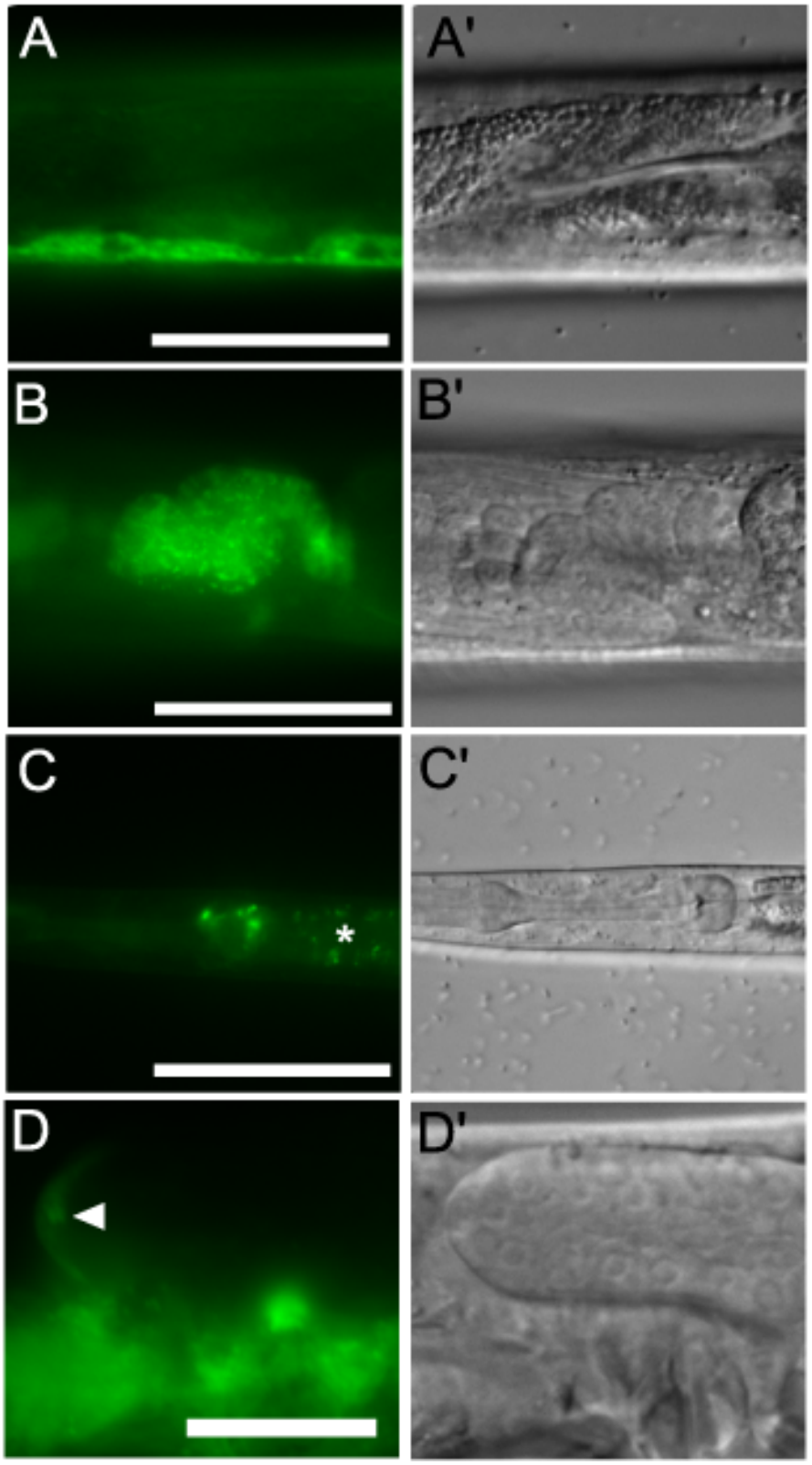
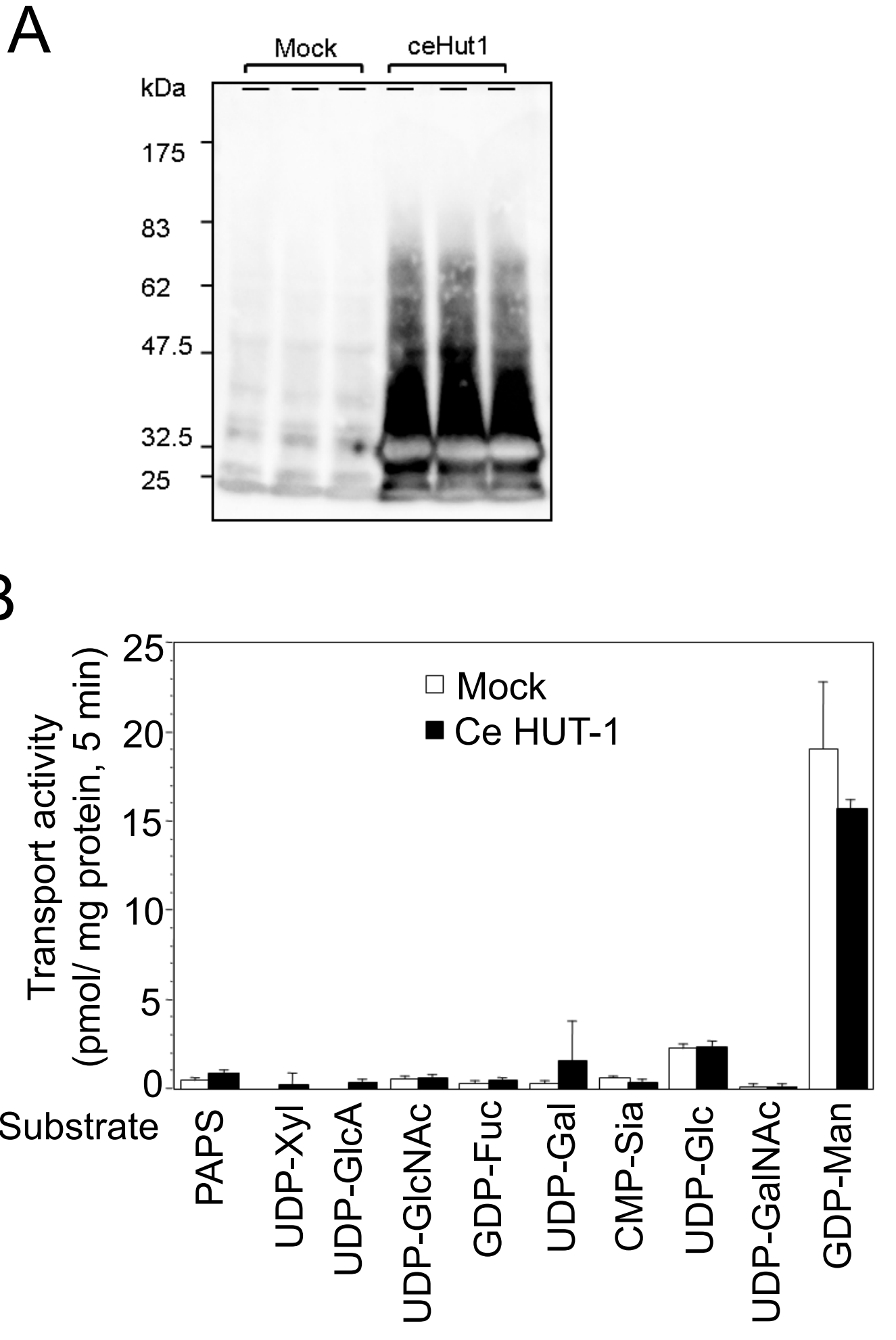


Figure S6


A
Mock
ceHut1
kDa
175
83
62
47.5
32.5
25
B
Transport activity
(pmol/ mg protein, 5 min)
25
20
15
10
5
0
Mock
Ce HUT-1
Substrate
PAPS
UDP-Xyl
UDP-GlcA
UDP-GlcNAc
GDP-Fuc
UDP-Gal
CMP-Sia
UDP-Glc
UDP-GalNAc
GDP-Man