GLUT-4 NH_2-TMD- $_{486}$TPS**LL**EQEVKPST<u>ELE</u>YLGP$_{505}$

Gap1 NH_2-TMD- $_{573}$DLD**LL**KQEIA<u>EE</u>KAIMATKP$_{593}$

Fig. S1. The C-terminal cytosolic domains of GLUT4 and Gap1 contain acidic clusters membrane-distal to the dileucine motifs that are required for their regulated trafficking. Clusters of acidic amino acid residues membrane distal to their respective dileucine signals (spaced by 5-8 residues) have previously been identified in GLUT4, IRAP and PC6B (Shewan et al., 2000, *Biochem J.* 350, 99-107). The above alignment shows that the C-terminal cytosolic tail of Gap1 also contains this feature (underlined). Position of the trans membrane domain (TMD) is indicated.

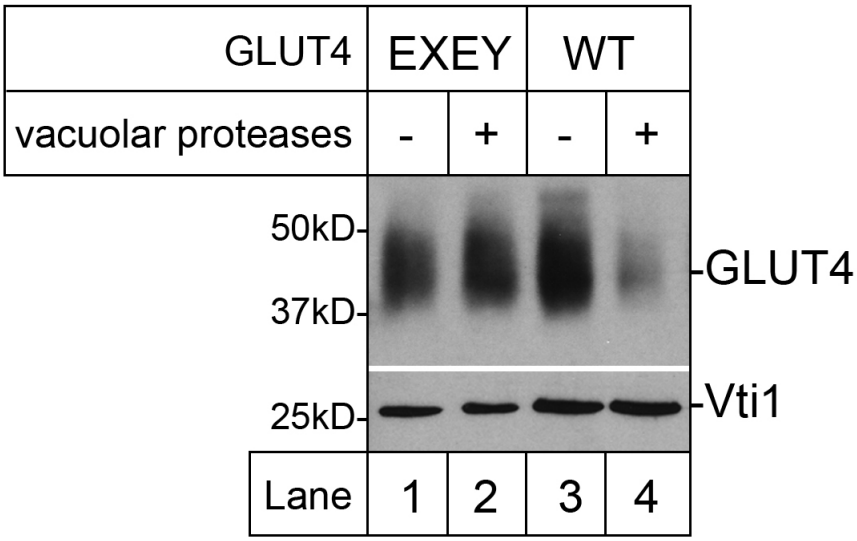

Fig. S2. Endosomal sorting of GLUT4 in yeast requires the C-terminal acidic motif. Mutation of E_{499}, E_{501} and Y_{502} to A (to create GLUT4-EXEY) has previously demonstrated a role for the ELEY motif in the intracellular sequestration of GLUT4 by regulating transport between endosomes and the TGN (Shewan et al., 2003, *Miol Biol Cell*. 14, 973-986). We introduced these same mutations in the context of our yeast expression vector (pCAL4) and used immunoblot analysis to compare the levels of EXEY-GLUT4 to those of wild-type (WT) GLUT4 expressed in yeast cells containing (+) or lacking (-) active vacuolar proteases (RPY10 and SF838-9D) grown in media containing ammonium sulphate as a nitrogen source. Lysates from these cells were immunoblotted for GLUT4 and Vti1 (loading control). In contrast to wild-type GLUT4, which gets degraded by vacuolar proteases when expressed in cells grown on a rich source of nitrogen such as ammonium sulphate (Lamb et al., 2010, *Traffic*. 11, 1445-1454. and Figs 1, 2 and 3 of this study), EXEY-GLUT4 is not degraded under these conditions (compare lanes 2 and 4).

Table S1. Sequences of the C-terminal cytosolic tails of GLUT4 and Gap1.

S. cerevisiae Gap1 (residues 548- 602) KIYKRNWKLFIPAEKMDIDTGRREVDLDLLKQEIAEEKAIMATKPRWYRIWN FWC – COOH
Human GLUT4 (residues 471-509) TRGRTFDQISAAFHRTPSLLEQEVKPSTELEYLGPDEND – COOH

N.B. The sequence of this portion of mouse GLUT4 is identical with the exception of residue 484: H in human, R in mouse).

Genbank accession numbers: Human GLUT4, NP_001033.1; mouse GLUT4, NP_033230.2; Gap1, CAA82113.1.