SUPPLEMENTARY INFORMATION

SUPPLEMENTARY METHODS

Isolation of deletion mutations

rabs-5 (ok1513) was isolated by knockout consortium. Other deletion mutations used in this work were isolated as previously described (Gengyo-Ando & Mitani, 2000). Primers used in nested PCR screening for detecting *vps-45 (tm246*) are as follows; first round: 5'-ATGGGTGTACTGATCGTGAT-3', 5'-ATTTGCGTATGCACTGACCA-3', second round: 5'-ATTTGCGTATGCACTGACCA-3', 5'-TCTCCTGCTCTACTTCTGCT-3'. Primers for screening *rabs-5 (tm2036)* are as follows; first round: 5'-ATGGGTGTCCCCGAGAGGAT-3', 5'-ATGACGGTCCTGCTCTATTG-3', second round: 5'- ACAGTGATGACGTCGTCTAG-3', 5'-CTATTGCGATTGAGATGCTC-3'. Primers for screening *syn-13 (tm2037)* are as follows; first round: 5'-CCTTCAAAGACATCTCTCCC-3', 5'-ATTCCTGCTTGGGAGATCAA-3', second round: 5'- CCGATACTACTTGAATAGGC-3', 5'-

GAAAGGCCACGCACCAGCTA-3'. Primers for screening *syn-16 (tm1560)* are as follows; first round: 5'- AAATAGAGGCCCGAACAGAG-3', 5'-ATCGCAGCCGATATGCAAGA-3', second round: 5'-ACTTTAGGCCAATGCAGCAT-3', 5'-TCGTTCGCTGGATAACGTCA-3'. Sequence analysis of the deleted regions was performed as previously described (Gengyo-Ando & Mitani, 2000). These deletion alleles were backcrossed at least four times.

Transgenes and Germ-Line Transformation.

A genomic fragment containing 1 kb upstream and 2.5 kb downstream of the *vps-45* gene (C44C1.4) in addition to the *vps-45* coding sequence was amplified. The lethal and endocytosis phenotypes at the restrictive temperature (25 °C) in *vps-45 (tm246)* were fully rescued by this genomic clone. The expression vectors pFX_EGFPT, pFX_DsRedmT, and pFX_DsRedxT were used for constructing reporter plasmids (Gengyo-Ando *et al*, 2006). To make a functional *vps-45::EGFP* fusion, a 5-kb fragment of the *vps-45* region was PCR amplified and cloned in 5' to EGFP in pFX_EGFPT. For coelomocyte-specific expression of VPS-45 or RAB-5 (Q78L),

unc-122 promoter sequence was used as previously described (Fares & Greenwald, 2001b). The rescue constructs for *vps-45* orthologs and *rabs-5* were made by subcloning these coding sequences into downstream of the *vps-45* promoter. Standard microinjection techniques were used to generate stable transgenic *C. elegans* lines carrying extrachromosomal DNA arrays. Rescue and expression constructs were injected at 20 - 100 ng/μl. pRF4 *[rol-6d]* or *Pgcy-10::DsRed* or *Punc-122::DsRedx* were used as co-transformation markers. Where indicated, extrachromosomal arrays were integrated into the genome by irradiation with ultraviolet light (254 nm) as previously described (Mitani, 1995). The transgenic arrays constructed for this study are; *tmIs104* and *tmIs105 [vit-2::EGFP,rol-6d]*, *tmIs268 [Punc-122::vps-45(+), rol-6d]*, *tmEx146 [vps-45(+)* genome, *rol-6d*], *tmEx144 [vps-45(+)::EGFP, rol-6d], tmEx1315[vps-45(+)::DsRedm, rol-6d], tmEx1387 [Pvps-45::rabs-5(+),Punc-122::DsRedx*, *Pgcy-10::DsRed], tmEx1384 [Punc-122::rab-5 (Q78L), Punc-122::DsRedx*, *Pgcy-10::DsRed], tmEx568, tmEx569, tmEx759 [Pvps-45::vps-45(+) (C. elegans vps-45), rol-6d], tmEx213, tmEx214, tmEx215, tmEx752 [Pvps-45::mVps-45 (murine ortholog), rol-6d], tmEx757, tmEx758,

tmEx1354 [Pvps-45::vps45 (yeast ortholog), *rol-6d*]. *bIs34 [rme-8::GFP]*(Zhang *et al*, 2001) was kindly provided by Dr. Grant. *arIs37 [Pmyo-3::ssGFP]*(Fares & Greenwald, 2001a) and *pwIs50* [*lmp-1::GFP*] (Treusch *et al*, 2004) were obtained from *Caenorhabditis* Genetics Center.

Microscopy

Fluorescence images were obtained using a BX51 microscope (Olympus Optical Co., Ltd, Tokyo, Japan) equipped with a Sensys CCD camera (Photometrics) and an IP-lab software (Nippon Roper, Tokyo, Japan). Confocal images were obtained using Zeiss LSM310 or Zeiss 510 META confocal microscope systems (Carl Zeiss MicroImaging).

Transmission Electron Microscopy (TEM)

Wild type or mutant larvae were fixed with 2.5% glutaraldehyde, 1% paraformaldehyde, 0.2 M trehalose, and 1 mM $MgCl_2$ in 50 mM cacodylate buffer (pH 7.4). They were then postfixed with 1% osmium tetroxide in 100 mM cacodylate buffer,

mounted in agarose, and embedded in Epon 812 plastic resin. Transverse sections were cut at 70-120 nm thickness, counterstained with uranyl acetate and/or lead citrate, and examined in transmission electron microscopes JEOL JEM-1010 at 80 kV or Hitachi H-7100 at 75 kV.

Analysis of the RAB-5 (Q78L) -induced large endosomes

arIs37;tmEx1384, arIs37;vps-45 (tm246); tmEx1384, arIs37;rabs-5 (ok1513); tmEx1384, *syn-13 (tm2037) arIs37;tmEx1384, arIs37;syn-16 (tm1560); tmEx1384, syn-13 (tm2037) arIs37;syn-16 (tm1560);tmEx1384* and *arIs37;unc-64 (e264);tmEx1384* were used for this assay. Knockdown of the *syn-1, syn-2, syn-3, syn-4, VF39H2L.1* and *T10H9.3* was performed with feeding RNAi basically according to the standard method (Timmons & Fire, 1998). *tmEx1384* has an extrachromosomal array which co-expresses the RAB-5 (Q78L) and DsRed in the coelomocytes. The fluorescent images of the coelomocytes with red fluorescence were taken from L1 or early L2 larva grown at 15 °C, and the largest vesicles that accumulate GFP were analyzed by an IP-lab software. GFP-filled vesicles with a diameter of 1.5 μm or more were counted as

RAB-5 (Q78L)-induced large endosomes. In the coelomocytes of *arIs37*, we rarely detected the GFP-filled vesicles with a diameter of more than 1 μm at the same larval stage. In the coelomocytes with red fluorescence, percentage of the coelomocyte having large endosomes per total coelomocyte was analyzed. Assay was independently performed at three times and 20-50 coelomocytes were used in each strain.

Isolation of the DNA clones

The cDNA clones encoding *vps-45, syn-1, syn-2, syn-3, syn-4, syn-13, syn-16, VF39H2L.1, T10H9.3*, *unc-64* and *rabs-5* were obtained by RT-PCR using a mixed-stage *C. elegans* Bristol N2 cDNA as a template. The cDNA clone encoding mammalian vps45 (mVps45) was amplified from mouse brain cDNA. The genome clone encoding the entire coding sequence of yeast Vps45 was amplified from *S. cerecisiae* genome DNA. All coding sequences used in this work were verified by sequencing.

Yeast two-hybrid assay

Yeast two-hybrid assay was performed using the Matchmaker two-hybrid system 3 (Clontech). Full-length cDNA encoding the *vps-45* (amino acids 1-547), murine mVps45 (amino acids 1-570), and yeast vps45 (amino acids 1-577) were cloned into the pGBKT7. cDNA fragments encoding cytoplasmic domains of nine syntaxins of *C. elegans* were cloned into the pGADT7, respectively; *syn-1* (amino acids 1-277), *syn-2* (amino acids 1-266), *syn-3* (amino acids 1-389), *syn-4* (amino acids 1-257), *syn-13* (amino acids 1-253), *syn-16* (amino acids 1-306), VF39H2L.1 (amino acids 1-200) and *unc-64* (amino acids 1-266). Full-length cDNA encoding the *rabs-5* (amino acids 1-563) was also cloned into the pGADT7. The yeast strain Y187 was transformed by the lithium acetate method with both bait and prey plasmids. Transformants were streaked on a filter paper and subjected to the β-galactosidase assay as described in the manufacturer's protocol. For each experiment, at least four independently derived colonies were tested.

Co-immunoprecipitation

HEK293T cells were transiently transfected with the expression vectors as indicated using Lipophectamine 2000 (Invitrogen). After incubation for 22 h, the transfectants were lysed with lysis buffer containing 1% Triton X100, 50 mM Tris-HCl (pH 7.5), 50 mM NaCl, 0.1 mM phenyl methylsulphonyl fluoride and 1 μg/ml of leupeptin and pelleted. The supernatants were precleared with Sepharose 4B agarose, then allowed to bind anti-Flag antibody conjugated to agarose beads (Sigma) for 2 hr at 4 °C. The immunoprecipitants were washed three times with wash buffer containing 1% Triton X100, 50 mM Tris-HCl (pH 7.5), 50 mM NaCl. The immunoprecipitates (IP) or whole cell lysates (Input) were analyzed by western blotting using anti-Flag antibody (M2, Sigma) and anti-Myc antibody (9E10, Santa Cruz biotechnology).

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