Supplementary Methods

***C. elegans* Strains**

Strains of *C. elegans* were cultured at 20°C using standard protocols. The N2 Bristol strain was used as the wild-type strain. The following strains were used in this work: linkage group I (LG I): *mtm-1(ok742)*, *vps-34(h797)*, *epg-8(bp251)*; LG II: *laat-1(qx42)*, *lgg-1(bp500)*, *epg-5(tm3425)*; LG III: *mtm-3(tm4475)*, *cup-5(bp510)*, *epg-6(bp242), mtm-6(ok330)*; LG V: *atg-9(bp564)*, *mtm-9(ok3523), atg-18(gk378)*; LG X: *mtm-5(ok469)*, *atg-2(bp576)*.

The *vps-34*-deficient strain, *vps-34(h797)*, is maintained as *dpy-5(e61) vps-34(h797)*; *qxEx* [*vps-34(+)*; P_{sur-5}SUR-5-GFP]. Non-green embryos were scored as *vps-34(lf)* (*dpy-5(e61) vps-34(h797)*). The *tm4475* allele of *mtm-3* contains a 402 bp deletion and 12 bp insertion that removes the entire 3rd exon and intron and generates an early stop codon, resulting in a truncated protein containing only the first 66 amino acids. It is probably a null mutation of *mtm-3* and has been out-crossed with the wild type N2 strain at least 6 times before further analysis. The *mtm-3(tm4475)* mutants are viable but display a 36% embryonic lethality and 45% larval arrest. The *ok742* allele of *mtm-1* contains a 1314 bp deletion that removes the region from intron 4 to exon 7 of the *mtm-1* gene, resulting in a truncated MTM-1 protein containing only the N-terminal PH-GRAM domain. It is likely a null mutation of *mtm-1* which causes embryonic lethality and larval arrest. The deletion alleles of *mtm-6 and mtm-9* , *ok330* and *ok3523*, cause protein truncations that remove the majority of the catalytic phosphatase domain of MTM-6 and 9, and are therefore very likely to be strong loss-of-function or null mutations. The *ok469* allele of *mtm-5* contains a 665 bp deletion that removes the region from

exon 7 to 9, resulting in a truncated MTM-5 protein lacking the conserved myotubularin domain.

adIs2122 (P_{lgg-1}GFP::LGG-1) was a gift from C. Kang (University of Texas). Other strains carrying integrated or transgenic arrays used in this study are as follows: *qxIs293*(P_{mtm-3}GFP::MTM-3), *qxIs503*[P_{mtm-3}GFP::MTM-3(C459S)], *qxEx4098*(P_{ced-1}ATG-18::GFP), *qxEx5931*[P_{ced-1}ATG-18(FKKG)::GFP], *qxEx5932*[P_{ced-1}ATG-18(FTTG)::GFP], *bpIs151*(P_{sqst-1}SQST-1:: GFP), *bpIs242*(C33D9.6::GFP) and *bpIs244*(C17E4.2::GFP), *arIs37*(P_{myo-3}ssGFP), *bIs1* (VIT-2::GFP).

Starvation assay

Embryos were synchronized, collected and incubated in deionized distilled water at 20 °C. At each time point, an aliquot of each sample was placed on an OP50-seeded plate. After 3 days at 20°C, the number of animals surviving to L4 or adulthood was counted. At least 300 animals were quantified each day.

RNAi

To inactivate *vps-34, mtm-3, atg-18, rab-5* and *rab-7* by RNAi, dsRNA (corresponding to bases 560 to 1390 of the *vps-34* cDNA, 1151 to 1774 of the *mtm-3* cDNA, 340 to 818 of the *atg-18* cDNA, 32 to 590 of the *rab-5* cDNA, and 147 to 609 of the *rab-7* cDNA) was synthesized in vitro (~1000 ng/µl) and injected into the gonad of young adult hermaphrodites. The phenotype of embryos laid between 12 to 24 h post-injection was examined.

Examination of expression levels of GFP::MTM-3 and ATG-18::GFP

To determine expression levels of GFP::MTM-3 and ATG-18::GFP, mixed-stage worms that express GFP::MTM-3, GFP::MTM-3(C459S) or ATG-18::GFP were collected and worm lysates were prepared for western blot analysis using anti-GFP antibody. Tubulin was used as the internal control.

Microscopy and imaging analysis

DIC and fluorescent images were captured with a Zeiss Axioimager A1 equipped with epifluorescence and an AxioCam monochrome digital camera and were processed and viewed using Axiovision Rel. 4.7 software (Carl Zeiss, Inc.). For confocal images, a Zeiss LSM 510 Pascal inverted confocal microscope with 488 and 543 lasers and a Zeiss LSM 510 Meta inverted confocal microscope with 405, 488 and 543 lasers were used and images were processed and viewed using LSM Image Browser software.

Transmission Electron Microscopy (TEM)

Embryos were treated with chitinase [20 mg/ml chitinase, 130 mM KCl, 2.5 mM $MgCl_2$, 2.5 mM $CaCl_2$, 5 mM HEPES (pH 7.5)] for 5 min and then fixed in 2.5% glutaraldehyde, 1% paraformaldehyde in 0.1 M sucrose on ice followed by fixing for 90 min at 4℃ in M9 containing 0.5% OsO_4 and 0.5% $KFe(CN)_6$. Each worm was embedded in a small block of 3% agarose. After dehydration with graded ethanol solutions, worms were embedded in EMbed812 (Electron Microscopy Sciences). 70-nm ultrathin sections were stained with 2%

uranyl acetate for 30 min and lead citrate for 10 min.

Plasmid construction

To generate the GFP::MTM-3 reporter, a 2.5 kb promoter region of *mtm-3* was inserted into the pPD49.26-gfp vector via the Bam HI site, followed by ligation with the 2.9 kb *mtm-3* cDNA through the KpnI site. To obtain the ATG-18::GFP reporter, 2.1 kb of *atg-18* genomic DNA was cloned into $P_{ced\text{-}1}$gfp through the Kpn I site. The C459S mutation of MTM-3 and the FKKG/FTTG mutations of ATG-18 were introduced into the above constructs using PCR-based site-directed mutagenesis.