Supplementary Materials for

LAAT-1 is the Lysosomal Lysine/Arginine Transporter that Maintains Amino Acid Homeostasis

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Materials and Methods

*C. elegans* strains

Strains of *C. elegans* were cultured using standard protocols (25). The N2 Bristol strain was used as the wild-type strain. Mutations used are described in *C. elegans II* (26) unless otherwise indicated. Linkage group II (LG II): *laat-1* (*qx42, qx111, qx66, gt3, gt6, gt12, ctms-1(ok813)* (this study), *gcn-2(ok871)* (19). LG III: *cup-5(ar465)* (19). LG IV: *ced-3(n717)*. The following reporter strains were used in this study: *qxIs354* (P*ced-1 LAAT-1::GFP*), *qxIs352* (P*ced-1 :LAAT-1::mCHERRY*), *qxIs281* (P*ced-1 CTNS-1::GFP*), *qxIs66* (P*ced-1 :GFP::RAB-7*), *bIs1* (VIT-2::GFP) (11), *pwlIs281* (CAV-1::GFP) (28), *bIs34* (RME-8::GFP) (20), *cdIs40* (GFP::CUP-5) (19), *qxEx2726* (P*hsp::CHERRY*), *qxEx1736* (P*hsp::Lact-C2*).

Isolation, mapping and cloning of *laat-1*

Mutants were generated by using standard ethylmethane sulfonate mutagenesis protocols. The *qx42* mutant was identified by increased cell corpses at embryonic stages; *qx111* and *qx66* were identified by enhanced accumulation of GFP::LGG-1 (which labels autophagosomes) in the intestine of *tat-1(qx30)* mutants. The *gt3, gt6* and *gt12* mutants were isolated from a screen for enhanced germline apoptosis 28-30 h post irradiation using acridine orange staining as described previously (31). All mutants were backcrossed with N2 animals at least four times before further analysis. *qx42* was mapped to the left of *lin-31(-6.04)* on LG II using *lin-31(-6.04) unc-4(1.75)* as the three-factor mapping marker. Single nucleotide polymorphism (SNP) mapping was then performed to place *qx42* to the left of *hw16087(-16.04)*. Transformation rescue experiments were performed using long PCR fragments covering this region and one product containing the open reading frame of *Y43H11AL.2* was found to possess the rescuing activity. *gt3, 6,* and *12* were mapped to the left arm of LG II via SNP mapping. *gt12* was identified as an allele of *Y43H11AL.2* by array comparative genome hybridization (32). Sequencing of *Y43H11AL.2* in *gt12* mutants identified a C to T transition that resulted in a stop codon at amino acid 63. Sequencing of *Y43H11AL.2* in *gt3* and *gt6* identified G to A changes that resulted in the replacement of Gly215 by Glu and Gly84 by Asp, respectively. We identified a C to T change in *qx66* and a G to A transition in *qx111* which resulted in the replacement of Pro59 by Leu and Trp25 by a premature stop codon, respectively.

Quantification of cell corpses, cell death and cell corpse duration

The number of germ cell corpses in one gonad arm at various adult ages was scored as described (33). The occurrence of somatic and germ cell death as well as duration of cell corpses were examined as described previously (8, 34, 35). To distinguish apoptotic cells from enlarged lysosomes in *laat-1(qx42)* embryos, dying cells were identified by both raised-button morphology and labeling of GFP::Lact-C2 which binds surface-exposed phosphatidylinerine on apoptotic cells.

Examination of lysosomes and cargo degradation
Accumulation of VIT-2::GFP, CAV-1::GFP, RME-2::GFP, SEPA-1::GFP and T12G3.1::GFP in embryos was quantified at 4-fold (SEPA-1::GFP and T12G3.1::GFP), 1.5-fold (CAV-1::GFP), or 200-cell stage (RME-2::GFP) by collecting fluorescent images with a Zeiss LSM 5 Pascal inverted confocal microscope and determining the average fluorescence intensity per pixel using Axiovision Rel. 4.7 software (Carl Zeiss, Inc.). Five different regions were quantified in each embryo and 10 embryos were scored for each strain.

To examine lysosomes, *C. elegans* embryos were stained by Lysotracker Red (1:1000, Invitrogen, USA) before examination. Lysotracker Red accumulation was quantified by determining the number of Lysotracker Red-positive puncta within a unit area of 50 µm². 20 animals were examined for each strain. To examine lysosomes in *C. elegans* embryonic cells, embryos were treated with chitinase (0.5 units, Sigma, USA) for 20-40 min to partially release cells from the egg shell before staining by Lysotracker Red. To quantify lysosome volume, 3-D fluorescent images of *C. elegans* embryos expressing NUC-1::mCHERRY were collected by an Andor Revolution® XD spinning disk microscope (Andor Technology plc.) and volumes of NUC-1::mCHERRY-positive puncta were quantified by Imaris software (Bitplane, Andor Technology, plc.). At least 100 lysosomes were quantified for each strain. To examine lysosomes in coelomocytes, *C. elegans* strains carrying P*hsps*CHERRY and endosomal marker RME-8::GFP or P*hsps*CHERRY and lysosomal marker GFP::CUP-5 were used. CHERRY is expressed upon heat-shock treatment and secreted into the body cavity from where it is taken up by coelomocytes through endocytosis and sent to lysosomes via vesicular transport (36). Lysosomes are identified by accumulation of CHERRY and labeling of CUP-5 but not RME-8.

**Antibody generation and immunostaining**

VIT-2(83-620) or CPL-1(161-337) protein tagged with six Histidine residues (VIT-2(83-620)-His₆ or CPL-1(161-337)-His₆) was expressed in and purified from *E. coli* and used to raise rat polyclonal antibodies against VIT-2 or CPL-1. The anti-CPL-1 antibody recognized both processed (27 KD, active form) and unprocessed CPL-1 (38 KD) in a western blot analysis using the lysosomal fraction isolated from *C. elegans* embryos. The VIT-2 antibody was further purified as described previously (36), and recognized a single band of expected size (170 KD) using a lysate prepared from mix-staged wild-type worms. The immunostaining experiment with anti-VIT-2 (1:500) or anti-GFP antibodies (1:200, Roche, USA) was performed as described previously (36).

**Microscope and image 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.). A 100x Plan-Neofluar objective (NA1.30) was used with Immersol 518F oil (Carl Zeiss, Inc.). For confocal images, a Zeiss LSM 5 Pascal inverted confocal microscope with 488, 543, 514, 458, and 405 lasers was used and images were processed and viewed using LSM Image Browser software. Images of the same reporter/antibody staining in different strains were taken with the same exposure time.
Purification of lysosomes and quantification of amino acid accumulation

Lysosomes were isolated and purified from *C. elegans* embryos using a Lysosome Isolation Kit as per the manufacturer’s instructions (LYSISO1, Sigma, USA). The crude lysosomal fraction was purified by a density gradient built up with Optiprep Density Gradient Medium Solution and Sucrose (bottom to top: 27, 22.5, 19, 16, 12 and 8%). The enrichment of lysosomes in fractions separated by the density gradient (bottom to top: B1-6) was determined by examining acid phosphatase activity (BioVision, USA) and processing of cathepsin with anti-CPL-1 antibodies after being normalized to the same total protein concentration. As shown in fig. S8, A and B, fractions B4-6 showed the highest acid phosphatase activity, while B3-6 contained abundant processed CPL-1. Fractions B4-6 were therefore combined, and from them purified lysosomes were precipitated, washed, and re-suspended in the extraction buffer. The purified lysosomal fraction was analyzed by western blots using antibodies that detect mitochondria (anti-HSP-60, Developmental Studies Hybridoma Bank, Univ. of Iowa, USA), endosomes (anti-RME-1, Developmental Studies Hybridoma Bank, Univ. of Iowa, USA) or nuclei (anti-HEL-1) (37). As shown in fig. S8C, no signal was detected by anti-RME-1 or anti-HEL-1 while a weak band was detected by anti-HSP-60, indicating that the majority of the above organelles was eliminated from the lysosomal fraction. The cytosolic and purified lysosome fractions from different strains were normalized to the same total protein concentration and amino acids were quantified by amine reactive isotope coded tags (iTRAQ® Reagents, Applied Biosystem, USA) in combination with LC/MS/MS (Beijing Amino Acid Medical Research CO., LTD, China). The ratio of amino acid concentration in purified lysosomal versus cytosolic fractions was determined to compare lysosomal amino acid accumulation in different strains.

Cysteamine treatment

Synchronized L1 larvae were cultured on NGM plates spotted with OP50 culture, each containing 10 mM cysteamine (Sigma, USA). Lysosome morphology in coelomocytes was examined 48 h post L4/adult molt. At least 15 animals were scored for each strain/treatment. Liquid-cultured worms treated with 10 mM cysteamine were collected and the lysosome fraction was prepared and analyzed for accumulation of cystine, cysteine and mixed disulfide of cysteine-cysteamine by iTRAQ® Reagents coupled with LC/MS/MS as described above. 3 independent experiments were performed with similar results. Data from one experiment is shown in Fig. 3, D and E.

Examination of embryonic development and protein synthesis

To examine embryonic development, 20 young adults (24 h post L4/adult molt) were placed on one NGM plate and incubated at 20ºC for 2 h. After adult animals were removed, the number of hatched embryos was determined every hour between 10 and 19 h. The percentage of viable embryos was determined by dividing the number of larvae (examined 36 h post egg laying) by the total number of laid embryos. At least three independent experiments for each strain/treatment were performed.

Amino acid supplementation was performed by including free amino acids (Ameresco, USA) at various concentrations in both NGM plates and OP50 culture spotted onto NGM plates. L4 larvae were placed on the amino acid-supplemented plates and cultured for at least one generation before embryonic development/lethality was
examined. Lysine and arginine were supplied at various concentrations (10 mM, 50 mM, and 100 mM each) in laat-1(qx42) mutants and accelerated embryonic development was only observed when both of them were added at 100 mM.

Protein synthesis rate was determined by FRAP (Florescence Recovery After Photo-bleaching) as described previously (22) with modifications. Briefly, embryos carrying P_{laat-1}::mCherry were collected at the comma or 1.5-fold stage, photo-bleached (100% 543 nm laser to 30-50% of initial emission intensity) and monitored for fluorescence recovery by time lapse recording (2.5 min interval for 20 min post bleaching) using a LSM 5 Pascal inverted confocal microscope (Carl Zeiss, Inc.). Fluorescence intensity of the bleached region was measured every 5 min post bleaching by Image J and the percentage of fluorescence recovery was determined as Ft-Fp/Fb-Fp.

Ft: fluorescence intensity at different time points after bleaching
Fp: fluorescence intensity immediately after bleaching
Fb: fluorescence intensity before bleaching

At least 20 embryos were examined for each strain/treatment.

**Cell culture and transfection**

COS-7 cells were cultured under 5% CO2 in glucose-rich Dulbecco’s Modified Eagle Medium (DMEM, Hyclone, USA) supplied with 10% fetal calf serum (FCS, Hyclone, USA) and 100 U/ml penicillin/streptomycin (Gibco, USA). For transfection, 2x10^6 COS-7 cells were transfected with 10 µg plasmid in 500 µl jetPrime DNA transfection buffer containing 30 µl jetPrime DNA transfection reagent (PolyPlus transfection, France). To generate stable cell lines, transfected cells (24 h post transfection) were trypsinized, centrifuged, resuspended, and plated with 200 µg/ml hygromycin (Invitrogen, USA) included for selection. The medium was changed every 4 days and clones were selected two weeks later.

**Amino acid uptake assay**

Stable COS-7 cell lines expressing EGFP (control), LAAT-1(Δ299-304)::EGFP, LAAT-1(P59LΔ299-304)::EGFP, PQLC2(ΔLL)::EGFP, or PQLC2(P55LΔLL)::EGFP were used for the amino acid uptake assays performed as described previously with modifications (4). Cells were placed in 24-well culture plates with 6 replicates for each cell line and cultured for 48-96 h without hygromycin before being assayed for amino acid uptake. Meanwhile, cells were cultured for the same period of time in 10 cm petri dishes to determine protein expression level by western blot analysis (fig. S9, I and J).

To examine amino acid uptake, culture medium was removed and cells were then incubated in 200 µl uptake buffer containing fresh DMEM and 10% FCS with pH adjusted to 4.5 by citric acid and supplied with unlabeled and isotope-labeled amino acids. We observed high levels of lysine and arginine uptake in all cell lines including EGFP-expressing control cells when the uptake assay was performed in the buffer reported previously for cystine uptake (5 mM D-glucose, 140 mM NaCl, 1 mM MgSO4, 20 mM potassium phosphate, pH 5.6) (4). We reasoned that the high uptake of lysine and arginine in this buffer may be due to the stimulation of plasma membrane cationic amino acid transporters by serum starvation and/or deprivation of nutrients provided by the culture medium. Indeed, we observed a similarly high uptake of lysine and arginine in all cell lines when lysine- or arginine-free DMEM and serum were used. Consistent with
this, we saw a gradually reduced uptake of lysine or arginine in the control but not the LAAT-1-expressing cells when increased amounts of unlabeled lysine/arginine were added back to the reaction buffer consisting of lysine/arginine-free DMEM and serum. We therefore included both DMEM and 10% FCS in the uptake buffer and supplied both unlabeled and isotope-labeled lysine/arginine to inhibit the activity of endogenous plasma membrane cationic amino acid transporters. Specifically, we incubated cells in 200 µl uptake buffer as described above and supplied unlabeled lysine or arginine to a final concentration of 4 mM and 2 mM, respectively (4 fold more than that in DMEM), and 24.6 pmol [³H]L-lysine (2 µCi, PerkinElmer, USA) or 34 pmol [³H]L-arginine (1.5 µCi, PerkinElmer, USA) per well. The uptake reaction was stopped 20 minutes later by two brief washes with 500 µl ice-cold wash buffer (5 mM D-glucose, 140 mM NaCl, 1 mM MgSO₄, 20 mM potassium phosphate, pH 7.4). The cells were lysed with 200 µl of 0.1 M NaOH and the radioactivity was counted by liquid scintillation in Optiphase-supermix using a WALLAC MicrobetaTrilux 1450 LSC & Luminescence Plate Counter (PerkinElmer, USA). Each sample was measured three times to get the average value. 6 replicates for each cell line were examined in each experiment and at least 3 independent experiments were performed. For histidine and cystine uptake, each well was supplied with unlabeled histidine or cystine to a final concentration of 1 mM (4 fold more than that in DMEM) and 40 pmol [³H]L-histidine (2 µCi, American Radiolabeled Chemicals, Inc.) or 1.67 nmol [³⁵S]L-cystine (1 µCi, American Radiolabeled Chemicals, Inc.). For alanine, glutamic acid and cysteine uptake, 19.8 pmol [³H]L-alanine (1 µCi, PerkinElmer, USA), 26.8 pmol [³H]L-glutamic acid (2 µCi, PerkinElmer, USA) or 1.54 pmol [³⁵S]L-cysteine (1.65 µCi, PerkinElmer, USA) was supplied in each well.

For western blot analysis, cells were collected, centrifuged and washed with ice-cold PBS before incubating with cell lysis buffer for 30 min at 4ºC. The resulting cell lysate was resolved by SDS polyacrylamide gel electrophoresis, transferred to PVDF membrane, and blotted with anti-Tubulin (Sigma, USA) or anti-GFP antibodies (Roche, USA).

**Statistical analysis**

The standard error of the mean (SEM) was used as y error bars for bar charts plotted from the mean value of the data. Data derived from different genetic backgrounds were compared by Student’s two way unpaired t-test. Data were considered statistically different at *P*<0.05. *P*<0.0001 is indicated with double asterisks and *P*<0.05 with single asterisks.

**Plasmid construction**

The 2 kb promoter sequence of *laat-1* was amplified using PBL517/158 and cloned into pPD49.26-mcherry or pPD49.26 through the Pst I and Xma I sites. The resulting P*ₜₐ₇*₁ and P*ₜₐ₇*₁mCHERRY was then ligated with the genomic fragment of *laat-1* amplified with PBL514/516 through the Xma I and Kpn I sites to generate P*ₜₐ₇*₁LAAT-1 and P*ₜₐ₇*₁LAAT-1::mCHERRY, respectively. P*ₜₐ₇*₁LAAT-1::GFP and P*ₜₐ₇*₁LAAT-1::mCHERRY were constructed by ligating the *laat-1* genomic fragment with P*ₖₑ₅₄*₁GFP and P*ₖₑ₅₄*₁mCHERRY through the Xma I and Kpn I sites, respectively. To express *laat-1* in mammalian cells, the full length and truncated cDNA fragments of *laat-1* were amplified using primers PHWD665/PBL540 and PHWD666/665 and cloned into pCMV-
EGFP via the Sal I and Kpn I sites to generate pCMV-LAAT-1::EGFP and pCMV-LAAT(Δ299-304)::EGFP, which lacks the lysosomal target motif. The full length and truncated cDNA of human PQLC2 was amplified from HEK 293 cells using primers PBL554/599 and PBL554/778 and cloned into pCMV-EGFP through the EcoR I and Xho I sites to get pCMV-PQLC2::EGFP and pCMV-PQLC2(ΔL288L289)::EGFP in which the lysosomal target motif is disrupted. The P59L mutation of LAAT-1 and the P55L mutation of PQLC2 were introduced by site-directed mutagenesis using primers PBL804/805 and 806/807, respectively. To generate stable cell lines expressing LAAT-1 and PQLC2, the laat-1::egfp and PQLC2-egfp fragments described above were digested out from the pCMV-EGFP vector and cloned into pCDNA-Hgro through the EcoR I and Not I sites. To construct pET21b-cpl-1(161-337) and pET21b-vit-2(83-620), corresponding cDNA fragments of cpl-1 and vit-2 were amplified using primers PHWD940/941 and PHWD423/424, respectively and cloned into pET21b through the Nhe I and Xho I sites.

**Primers used for plasmid construction**

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| PBL806 | getttgctgcatctacctc
tccagttctcaaaaaagctacaag |
| PBL807 | ctttgaggctttgtgaaactgbaAaggatagtcagcagcaaaag |
| PBL770 | gcCTCGAGagctgggggctcaagc |
| PHWD423 | gcGCTAGCtacgagctcacgagatgccttc |
| PHWD424 | gcCTCGAGaaagtacggatcatctgggttaa |
| PHWD665 | tagcGTGACatgaacgggccggtttaag |
| PHWD666 | gtGTTACatcggagtcgcttggagtatgtcccttgtgtcaaggt |
| PHWD940 | ttgGCTAGCtacgagctcacgagatgccttc |
| PHWD941 | taggCTCGAGagcatggagctattggagccctgg |

Restriction sites are in capital letters; each site has 2-4 protective 5’ nucleotides. Nucleotides altered by site-directed mutagenesis are in boxed capital letters.
Fig. S1. laat-1(qx42) mutants accumulate corpse-like objects representing cell corpses and enlarged lysosomes

(A to D) qx42 (B, D, arrows) was isolated due to accumulation of many refractile corpse-like objects in embryos. This phenotype is only partially suppressed by loss of ced-3, the *C. elegans* caspase that blocks almost all apoptosis in worms (38), suggesting that some of the refractile bodies may not represent apoptotic cells. (E to G) qx42 embryos accumulate lysotracker-positive structures. Similar to CUP-5, a lysosomal membrane protein, loss of which causes accumulation of many refractile bodies representing both

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cell corpses and enlarged lysosomes (39), we found that lysotracker red stained significantly more and larger structures in qx42 embryos (F, arrows) than in wild type (E, arrowheads) and this was unaffected by loss of ced-3 (G), indicating that qx42 contains enlarged lysosomes. Quantification of lysotracker-positive puncta is shown as mean ± SEM in (G). 20 animals were scored for each strain. **P<0.0001. Lysosome volumes are significantly larger in qx42 than in wild type, and the increased volume was unaffected by loss of ced-3 as shown in Fig. 1D, confirming that qx42 mutants accumulate enlarged lysosomes. Therefore, the refractile corpse-like objects in qx42 embryos likely represent both cell corpses and enlarged lysosomes. (H to K) Confocal fluorescent images of L1 larvae (H, I) or adults (J, K) of wild type (H, J) or laat-1(qx42) (I, K) expressing NUC-1::mCHERRY are shown. Enlarged lysosomes labeled by NUC-1::mCHERRY are observed in laat-1(qx42) (arrows) but not wild-type worms which contain small NUC-1 puncta or tubules (arrowheads). (L to R) Multiple laat-1 alleles show the enlarged lysosome phenotype. Confocal fluorescent images of embryos in wild type (L), laat-1(qx66) (M), qx111 (N), gt3 (O), gt6 (P), and gt12 (Q) expressing NUC-1::mCHERRY. Arrowheads indicate lysosomes in wild type and arrows point to enlarged lysosomes in laat-1 mutants. Quantification of lysosome volume is shown as mean ± SEM in (R). At least 100 lysosomes were quantified. **P<0.0001. In panels (E-Q), structures indicated by yellow arrows or arrowheads are shown at x4 magnification in the insets. In panel J, the upper inset shows NUC-1-positive puncta and the bottom one shows aggregated NUC-1. Scale bars represent 20 µm in panels J and K, and 5 µm in other panels.
Fig. S2. Cell corpse degradation but not engulfment or cell death is defective in *laat-1* mutants

(A to D) *laat-1(qx42)* mutants affect clearance of somatic cell corpses. To examine whether embryonic cell death is affected in *qx42* mutants, we labeled apoptotic cells with secreted GFP::Lact-C2 (which binds surface-exposed phosphatidylserine on apoptotic cells, thus distinguishing them from enlarged lysosomes). Cell deaths (A) and duration of C1, C2 and C3 corpses which undergo programmed death at mid-embryogenesis (40) (B) were monitored in wild-type and *laat-1(qx42)* embryos. The y-axis in (A) indicates cell deaths occurring at certain time points as shown on the x-axis and the total numbers of cell deaths are shown in parentheses. The appearance of refractile bodies positive for GFP::Lact-C2 was normal in *qx42* mutants (A), suggesting that embryonic cell death is not affected. The duration of C1, C2 and C3 (B) was quantified in at least 10 embryos and they persisted significantly longer in *qx42* mutants, suggesting that clearance of embryonic cell corpses is defective. CED-1, the phagocytic receptor, clusters around apoptotic cells during engulfment and is released from apoptotic cell-containing phagosomes shortly after engulfment (41, 42). The formation (C) and duration (D) of CED-1::GFP rings around cell corpses were monitored in wild-type, *laat-1(qx42)* and
*ced-6(n2095)* embryos. At least 25 cell corpses were quantified. The y-axis in (D) indicates the number of cell corpses around which the CED-1::GFP ring persisted for a certain period of time as shown on the x-axis. The average duration time of CED-1::GFP (± SEM) is shown in parentheses. Formation and duration of the CED-1::GFP ring around cell corpses were normal in *qx42* mutants, indicating that cell corpse internalization is unaffected. The *ced-6* mutants, which are defective in cell corpse engulfment (43), significantly delayed CED-1 clustering around cell corpses and its release from phagosomes. (E to H) *laat-1(qx42)* (F), but not wild-type (E) or *laat-1(qx42); ced-3(n717)* (G) animals accumulate germ cell corpses (arrows). Quantification is shown in (H). At least 15 animals were scored for each genotype and time point. (I) Cell deaths in the bend region of U-shaped gonads (left panel) were quantified in wild-type and *laat-1(qx42)* animals 24 h post L4/adult molt for 200 min. At least 10 animals were scored for each strain. (J) Duration of 19 and 21 germ cell corpses from wild-type and *laat-1(qx42)* animals were followed, respectively. Average duration (± SEM) is shown in parentheses. Germ cell death induction was unaffected but germ cell corpses persisted much longer in *qx42* mutants, suggesting that cell corpse removal is defective. (K) Germ cell corpses were quantified in wild type and *laat-1* mutants at 24 h, 36 h, and 48 h post L4/adult molt. At least 15 animals were scored for each strain at every time point. (L to O) DIC and fluorescent images of the germline in wild-type (L-L’’) and *laat-1(qx42)* (M-M’’) animals expressing H2B::GFP and NUC-1::mCHERRY. Arrows indicate normal nuclei and arrowheads point to apoptotic cells. Scale bars: 5 µm. The degradation of apoptotic cells in phagolysosomes, indicated by disappearance of H2B::GFP, was quantified and shown in (N) and (O). In panels (B, C, H, I, K, N, O), data are shown as mean ± SEM. Data derived from different mutant backgrounds were compared to wild type. **P<0.0001, *P<0.05; all other points had P>0.05.
**Fig. S3. laat-1 (qx42) mutants are defective in yolk degradation**

(A to B') Yolk uptake is unaffected in *laat-1(qx42)* mutants. Wild-type (A, A') and *laat-1(qx42)* (B, B') oocytes (arrows) contain a similar level of VIT-2::GFP. (C to H') Confocal fluorescent images of embryos in wild type (C-C'', E-F') and *laat-1(qx42)* (D-D'', G-H') with (C-D'') or without ectopically expressed VIT-2::GFP (E-H') and stained by anti-GFP (C-D'') or anti-VIT-2 antibodies (E-H'). DAPI staining shows nuclei in each embryo. Endogenous VIT-2 accumulates in early *laat-1(qx42)* embryos but is mostly degraded at the 1.5-fold stage, indicating that yolk degradation is affected but not totally blocked. Scale bars represent 20 µm in panels A and B, and 5 µm in other panels.
Fig. S4. Endocytic cargo proteins RME-2 and CAV-1 are not properly degraded in *laat-1* mutants

(A to F’) CAV-1::GFP (A-B’) and RME-2::GFP (C-F’), which are present in wild-type oocytes (arrowheads) and one-cell stage embryos, but are quickly degraded during embryogenesis (A, A’, C, C’, E, E’), persist in *laat-1(qx42)* embryos (thick arrows, B, B’, D, D’, F, F’). Thin arrows indicate spermtheca. (G to I’’) Confocal fluorescent images of wild-type (G-G’’) and *laat-1(qx42)* embryos (H-I’’) expressing NUC-1::mCHERRY and CAV-1::GFP (G-H’’) or RME-2::GFP (I-I’’). GFP overlapped with mCHERRY in *laat-1(qx42)* embryos (arrows), indicating lysosomal accumulation of CAV-1 and RME-2. Structures indicated by yellow arrows are shown at ×4 magnification in the insets. (J and K) Quantification of CAV-1::GFP and RME-2::GFP accumulation. At least 10 embryos were scored for each strain. Data are shown as mean±SEM. **P<0.0001. (L to O’’) Confocal fluorescent images of embryos in wild type (L-L’’, N-N’’) and *laat-1(qx42)* (M-M’’, O-O’’) expressing CAV-1::GFP (L-M’’) or RME-2::GFP (N-O’’) and stained by anti-GFP antibodies. DAPI staining shows nuclei in each embryo. GFP fluorescence and anti-GFP staining were observed in *laat-1(qx42)* but not wild-type embryos. Scale bars represent 20 µm in panels A-D and 5 µm in other panels.
Fig. S5. *laat-1* mutants accumulate autophagic cargo

(A to C) SEPA-1::GFP accumulates in a *laat-1(qx42)* embryo (B, B’) but not a wild-type embryo (A, A’). Quantification is shown in (C). At least 10 embryos were scored for each strain. Data are shown as mean ± SEM. **P<0.0001. (D to D’’) Confocal fluorescent images of *laat-1(qx42)* embryos expressing SEPA-1::GFP and NUC-1::mCHERRY. SEPA-1::GFP overlapped with NUC-1::mCHERRY (arrows), indicating lysosomal accumulation of SEPA-1. Structures indicated by yellow arrows are shown at x4 magnification in the insets. (E to H’’) Confocal fluorescent images of wild-type (E-E’’, G-G’’) and *laat-1(qx42)* (F-F’’, H-H’’) embryos expressing SEPA-1::GFP (E-F’’) or T12G3.1::GFP (G-H’’) and stained by anti-GFP antibodies. DAPI staining shows nuclei in each embryo. GFP fluorescence and anti-GFP staining were observed in *laat-1(qx42)* but not wild-type embryos. Scale bars: 5 µm.
**Fig. S6. Molecular cloning of laat-1**

(A) Cloning of laat-1. The top bar indicates the genetic map of the laat-1 genomic region. The laat-1 gene structure is shown with filled boxes representing exons and thin lines indicating introns. The arrow delineates the direction of transcription. The rescue of laat-1(qx42) by different reporters is shown at the bottom. At least 15 animals (transgenic and non-transgenic) from each independent line were scored for the rescue of the persistent corpse-like object phenotype. (B to E) Confocal fluorescent images of wild-type (B) and laat-1(qx42) (C-E) embryos expressing NUC-1::mCHERRY (B, C) or NUC-1::mCHERRY with LAAT-1::GFP (D) or LAAT-1(Δ299-304)::GFP (E). Lysosomes indicated by yellow arrows are shown at x4 magnification in the insets. The enlarged lysosome phenotype was rescued by expression of LAAT-1::GFP (D) but not LAAT-1(Δ299-304)::GFP (E). Scale bars: 5 μm. (F) Sequence alignment of *C. elegans* (c.e) LAAT-1, human (h.s) PQLC2 and yeast (s.c) YOL092W. Identical residues are shaded in black and similar ones in gray. Red boxes indicate the 7 transmembrane domains. The blue box delineates the lysosomal targeting motif. The PQ loop repeats are shaded in green and mutations identified in different laat-1 alleles are marked. (G) The gene structure of ctns-1 is shown with filled boxes representing exons and thin lines indicting introns. The arrow shows the direction of transcription. The red bar below the transcript shows the size and position of the deleted region in the deletion allele *ok813*. 
Fig. S7. LAAT-1 is widely expressed and localizes to lysosomes
(A to C'') Fluorescent images of sheath cells (A-A'', C-C'') and hypodermal cells (B-B'') in wild type expressing LAAT-1::mCHERRY and CTNS-1::GFP (A-B'') or LAAT-
1::GFP and stained by lysotracker red (C-C’’). LAAT-1 colocalizes with CTNS-1 and lysotracker red (arrows). (D to E’’) Fluorescence images of wild-type embryos expressing NUC-1::mCHERRY and LAAT-1::GFP (D-D’’) or LAAT-1(Δ299-304)::GFP (E-E’’). LAAT-1::GFP labels lysosomes as indicated by NUC-1::mCHERRY (arrows), while LAAT-1(Δ299-304)::GFP localizes to plasma membranes (arrowheads). Lysosomes indicated by yellow arrows are shown at x4 magnification in the insets. (F to M’) DIC and fluorescent images of wild-type animals transgenic for P_{laat-1}mCHERRY. mCHERRY fluorescence was observed in various cell types. Scale bars in all panels represent 5 µm.
Fig. S8. Lysosome purification

(A and B) Lysosomes were prepared from *C. elegans* embryos. Acid phosphatase activity (A) and processing of CPL-1 revealed by western blot analysis using anti-CPL-1 antibodies (full length: 38 KD, processed active form: 27 KD) (B) were used to determine the enrichment of lysosomes in different fractions separated by a density gradient as described in Materials and Methods. Fractions B4-6 were combined and from them purified lysosomes were precipitated. (C) The purified lysosomal fraction (PLF) was examined by western blots using anti-CPL-1 or antibodies that detect mitochondria (anti-HSP-60), endosomes (anti-RME-1) or nuclei (anti-HEL). Whole worm lysate (WL) was loaded as positive controls for different antibodies.
**Fig. S9. PQLC2 and LAAT-1 localize to lysosomes in COS-7 cells**

(A) The ratio of amino acid concentration in lysosomal versus cytosolic fractions prepared from embryonic lysates was determined and normalized as 1 fold in wild type (y-axis). (B) DIC and fluorescent images of wild-type and *ctns-1(ok813)* coelomocytes expressing secreted CHERRY (ssCHERRY) and the endosomal marker RME-8::GFP. Lysosomes are labeled by CHERRY (arrows) but not RME-8 which marks endosomes (arrowheads). Insets show endosomes indicated by yellow arrowheads. Scale bars: 5 µm. (C to H’’) Fluorescent images of COS-7 cells expressing LAMP1::mCHERRY and wild-type or mutated PQLC2::EGFP (C-E’’) or LAAT-1::EGFP (F-H’’), which carry the point mutation that disrupts the PQ loop repeat and/or lack the lysosome sorting motif. Wild-type PQLC2 and LAAT-1 mainly localize to lysosomes labeled by LAMP1::mCHERRY (arrowheads), while those that lack the lysosome sorting motif associate with plasma membranes (arrows). Lysosomes indicated by yellow arrowheads are shown at 4x magnification in the insets. Scale bars: 5 µm. (I and J) The expression level of GFP-tagged proteins in COS-7 cell lines used for amino acid uptake assay was examined by western blot. Samples in lane 1 in (I) and (J) were prepared from EGFP-expressing cells. Samples in lanes 2 and 3 in (I) were from cells expressing PQLC2(ΔLL)::EGFP and PQLC2(P55LΔLL)::EGFP, and in (J) were from cells expressing LAAT-1(Δ299-304)::EGFP and LAAT-1(P59LΔ299-304)::EGFP. α-Tubulin was used as the internal control.
Fig. S10. LAAT-1 and PQLC2 transport histidine

The uptake of histidine, glutamic acid, alanine, cystine and cysteine was examined in COS-7 cells transfected with LAAT-1- (A-E) or PQLC2- (F-J) constructs as indicated. Expression of LAAT-1(Δ299-304)::EGFP or PQLC2(ΔLL)::EGFP led to increased uptake of histidine but not glutamic acid, alanine, cystine or cysteine. **$P<0.0001$; all other points had $P>0.05$. 
Fig. S11. External supplements of both lysine and arginine rescue embryonic retardation in laat-1 mutants

(A) Externally supplied proline (P), arginine (R), or lysine (K) fail to reverse the embryonic retardation in laat-1(qx42) mutants. At least 100 embryos were examined for each strain/treatment. (B) Externally supplied lysine (K) plus arginine (R) but not glycine (G) reversed the embryonic retardation in laat-1(qx111) mutants. At least 88 embryos were examined in each experiment. (C-C”) Fluorescent images of a laat-1(qx42) embryo expressing VIT-2::GFP and NUC-1::mCHERRY with externally supplied free lysine (K) and arginine (R). The accumulation of VIT-2::GFP in enlarged lysosomes (arrows) was still observed. Structures indicated by yellow arrows are shown at x4 magnification in the insets. Scale bars represent 5 µm. (D) Externally supplied glycine (G) failed to accelerate the protein synthesis rate in laat-1 mutants. The protein synthesis rate was measured as described in Fig. 4 and Materials and Methods. The y-axis indicates the percentage of fluorescent recovery at certain time points as shown on the x-axis. At least 20 embryos were measured for each strain/treatment. Data are shown as mean ± SEM. **P<0.0001. (E) Cartoon illustrating LAAT-1 function. LAAT-1 maintains cytosolic lysine (K) and arginine (R) availability needed for normal embryonic development in wild type (WT, left). Loss of laat-1 function causes accumulation of lysine and arginine in lysosomes, thus limiting the cytosolic availability of the two amino acids. This leads to retarded development in laat-1(1f) mutant embryos (middle) and embryonic lethality in laat-1(1f) gcn-1(1f) worms in which the amino acid response (AAR) pathway is impaired (right).
References


