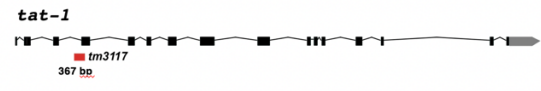


Table of protein sequences for DSR2, bATP8A2, hATP8A1, hATP11C, and their isoforms (TAT-1a, TAT-1b, TAT-1c) across various species. Includes accession numbers and residue counts.



- Conserved residue
TAT-1a specific mutation
TAT-1c specific mutation
E116K
P1132L
V1150M
L1192S

Fig. S1. Alignment of P4-ATPase protein sequences. The protein sequences from three TAT-1 isoforms of *C. elegans* are aligned with those of P4-type ATPases from *Drosophila melanogaster* (DSR2), a bovine P4-ATPase (ATP8A2), and two human P4-ATPase homologues (ATP8A1, ATP11C). The positions and the nature of 16 TAT-1 substitutions analyzed in this study are shown. The size and the position of the *tm3117* deletion mutation in the *tat-1* gene are also depicted.

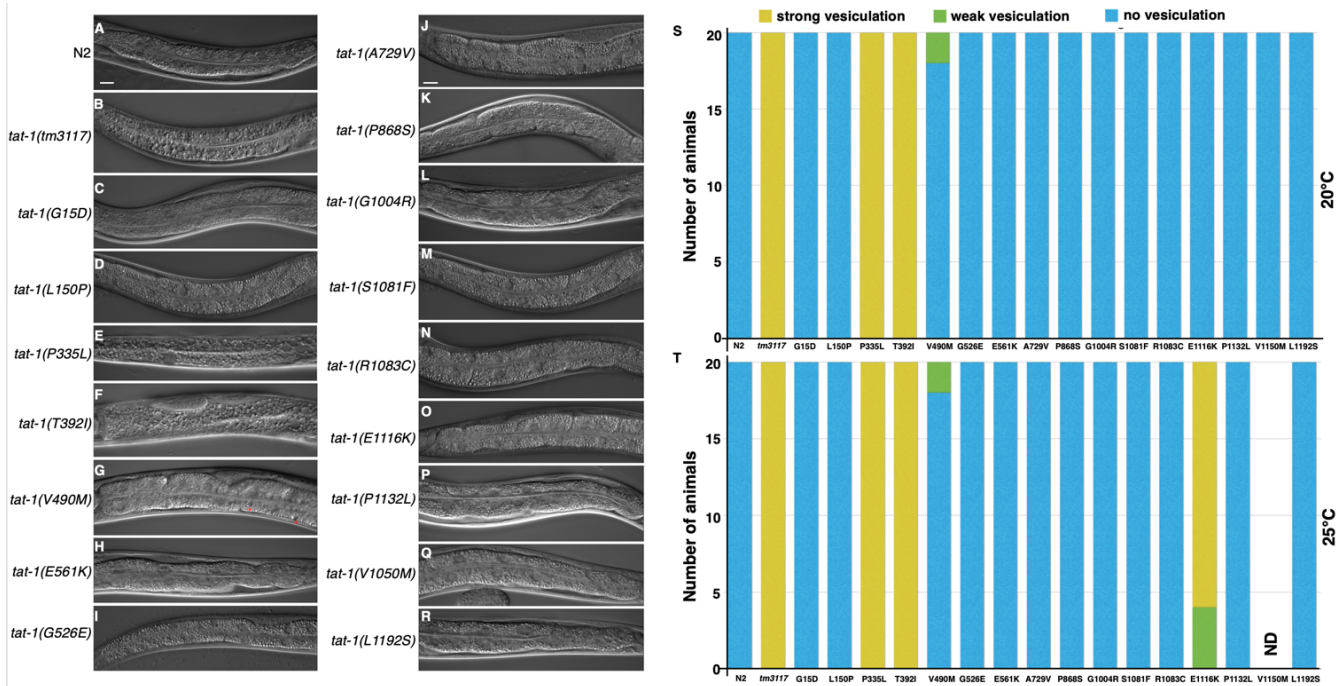


Fig. S2. The vacuolar gut phenotypes of all TAT-1 mutants. (A-R) Representative images of the intestines of two control strains (N2 and *tm3117*) and sixteen *tat-1* mutants grown at 20°C are shown. The red asterisks indicate two vacuoles seen in the TAT-1(V490M) mutant (G). Scale bar, 10 μm for all images. (S, T) Gut vesiculation defects of all *tat-1* mutants scored at 20°C (S) and 25°C (T) are shown. 20 animals were scored for each strain. Yellow, green and blue colors indicate strong, weak, and no vesiculation gut defects, respectively. ND, not determined due to sterility at 25°C.

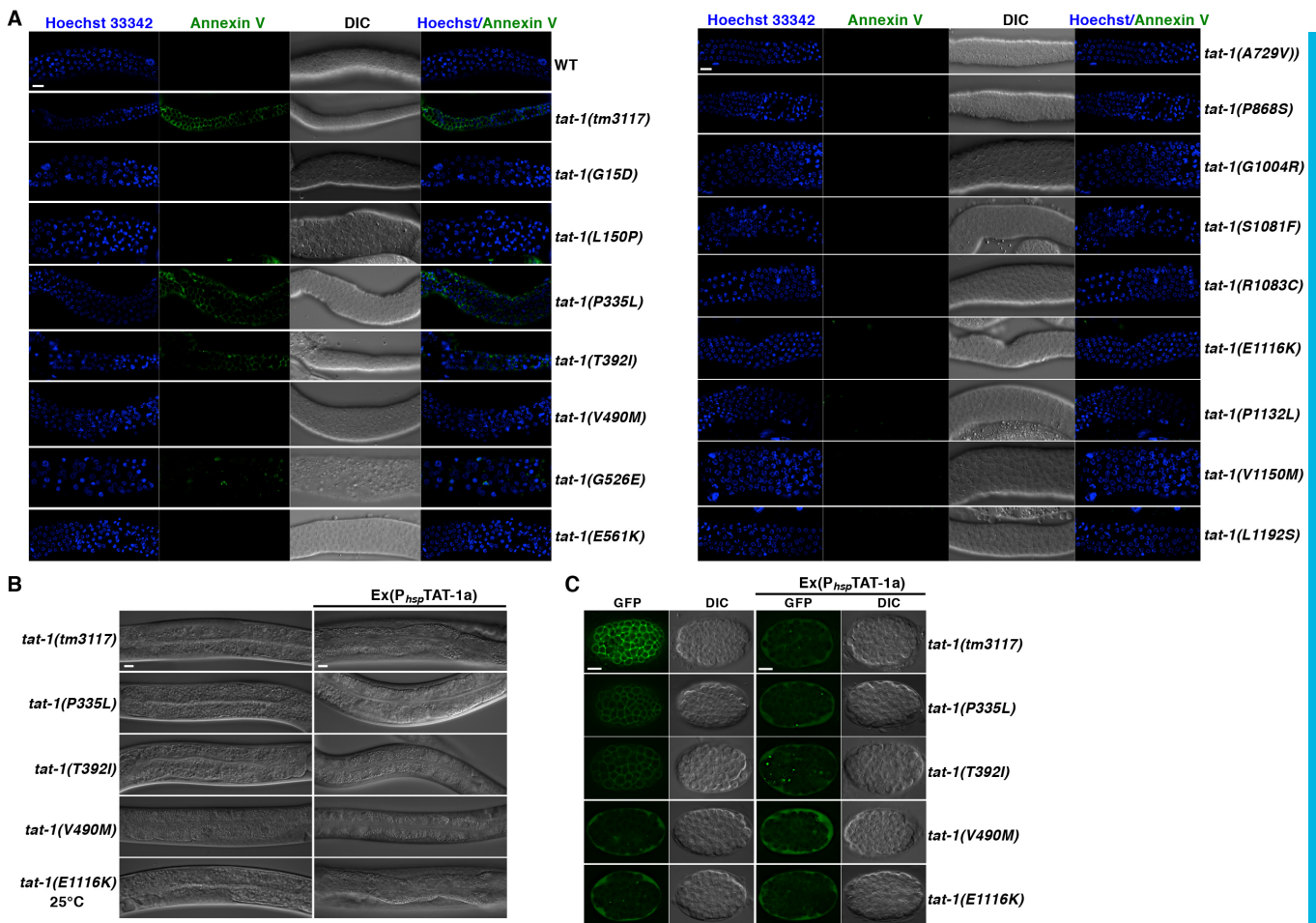


Fig. S3. PS staining in germ cells from all *tat-1* mutants and rescue of selected *tat-1* mutants.

(A) Representative images of dissected gonads from the indicated animals stained with Annexin V (green) to visualize externalized PS, Hoechst 33342 (blue) to show nuclei, and merged images of the Hoechst 33342 and the Annexin V staining. The bright-field DIC images of the gonads are also shown. The N2 (wildtype) and *tat-1(tm3117)* strains were included as controls, respectively. Images acquired from animals grown at 20°C. Scale bar, 10 μm for all the images. (B) Rescue of the vacuolar gut defects in *tat-1* mutants. Representative DIC images of the intestines of five *tat-1* mutants as indicated without (left column) or with (right column) the extrachromosomal transgenic array containing the *P_{hsp}TAT-1a* construct. Images were captured at the L4 stage after two heat shock treatments (see Materials and Methods). The *tat-1(E1116K)* animals shown in the last row were grown at 25°C. Scale bar, 10 μm for all images. (C) Rescue of the ectopic PS exposure defect in *tat-1* mutants. Representative GFP and DIC images of comma stage *smIs434* embryos carrying the indicated *tat-1* mutations without (first and second columns) or with (third and fourth columns) the extrachromosomal transgenic array containing the *P_{hsp}TAT-1a* construct. Images of sGFP::Lact staining (GFP) and corresponding DIC images (DIC) are shown. Heat shock treatment was performed at 33°C for 45 min. Images were captured 2 h after heat shock treatment. Scale bars, 10 μm for all images.

Table S1. Analysis of the potential impact of 16 new *tat-1* mutations on the functions of *tat-1*

Allele name	Amino acid change	Conserved amino acid	Predicted impact (PROVEAN)	Predicted impact (PolyPhen-2)	Predicted impact (SIFT)	Predicted impact (all three combined)
<i>gk625246</i>	G15D	Yes	Neutral	Possibly Damaging	Not Tolerated	2/3 deleterious
<i>gk412448</i>	L150P	Yes	Deleterious	Possibly Damaging	Not Tolerated	3/3 deleterious
<i>gk314197</i>	P335L	Yes	Deleterious	Probably Damaging	Not Tolerated	3/3 deleterious
<i>gk396853</i>	T392I	Yes	Deleterious	Probably Damaging	Not Tolerated	3/3 deleterious
<i>gk188335</i>	V490M	Yes	Neutral	Benign	Not Tolerated	2/3 neutral
<i>gk810250</i>	G526E	Yes	Neutral	Possibly Damaging	Not Tolerated	2/3 deleterious
<i>gk896724</i>	E561K	Yes	Deleterious	Benign	Not Tolerated	2/3 deleterious
<i>gk617264</i>	A729V	Yes	Deleterious	Possibly Damaging	Not Tolerated	3/3 deleterious
<i>gk188324</i>	P868S	Yes	Deleterious	Probably Damaging	Not Tolerated	3/3 deleterious
<i>gk188319</i>	G1004R	Yes	Deleterious	Probably Damaging	Tolerated	2/3 deleterious
<i>gk378291</i>	S1081F	No	Neutral	Probably Damaging	Tolerated	2/3 neutral
<i>gk583692</i>	R1083C	No	Neutral	Probably Damaging	Not Tolerated	2/3 deleterious
<i>gk339292</i>	E1116K	No	Deleterious	Probably Damaging	Not Tolerated	3/3 deleterious
<i>gk188307</i>	P1132L	No	Neutral	No Data	No Data	1/1 neutral
<i>gk520472</i>	V1150M	No	Neutral	No Data	No Data	1/1 neutral
<i>gk441133</i>	L1192S	No	Neutral	No Data	No Data	1/1 neutral

The predicted negative impact of the mutations was evaluated using PROVEAN, SIFT, and PolyPhen-2, three popular mutation analysis programs as described in Materials and Methods.