Supporting Information for:

Tyrosine aminotransferase is involved in the oxidative stress response by metabolizing *meta*-tyrosine in *Caenorhabditis elegans*

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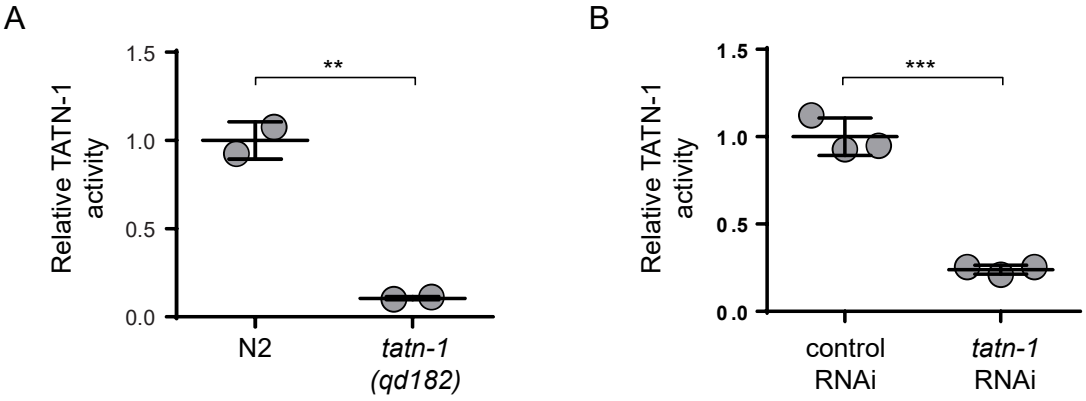

Figure S1. Reduction in TATN-1 enzymatic activity with gene mutation or RNAi knockdown

A. The relative TATN-1 activity measured in protein lysates from adult N2 and *tatn-1(qd182)* mutant worms. Mean enzymatic activity and standard deviation relative to N2 worms are shown. ** represents $p < 0.01$ by *t*-test. B. The relative TATN-1 activity measured in protein lysates from adult N2 worms treated with control or *tatn-1* RNAi. Mean enzymatic activity and standard deviation relative to control RNAi-treated N2 worms are shown. *** represents $p < 0.001$ by *t*-test.

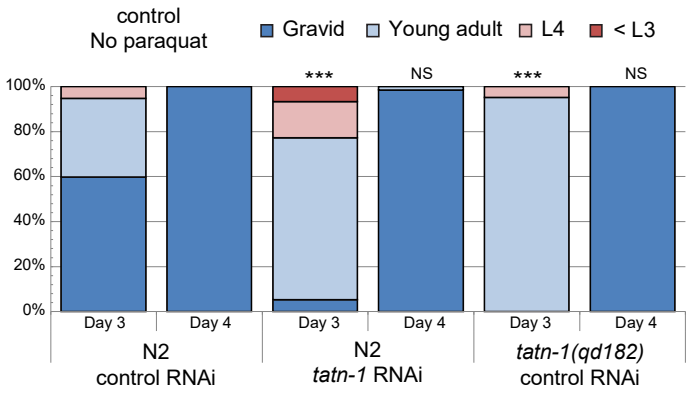

Figure S2. Reduction of TATN-1 activity slightly delays development even in the absence of paraquat

Results of a developmental assay using N2 and *tatn-1(qd182)* mutant worms treated with control or *tatn-1* RNAi in the absence of paraquat. Approximately 150 worms were scored per condition, and the percentage of worms in the indicated developmental stage per day are shown. *** represents $p < 0.001$ for the comparison of worms deficient in TATN-1 activity as result of *tatn-1* RNAi treatment or the *tatn-1(qd182)* genetic mutation to N2 worms treated with control RNAi of the same chronological age using a chi-square test.

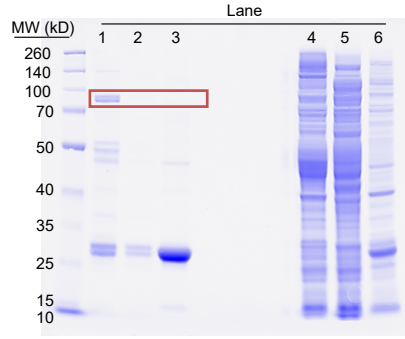

Figure S3. Semi-purified TATN-1 following expression in *E. coli*

Image of SDS-PAGE gel showing partially purified TATN-1::GST (81.6 kD, red box) extracted from bacterial cells induced to express a plasmid harboring *tatn-1* cDNA fused to GST (lane 1). Lane 2 is a negative control with these same cells but without induction. Lane 3 is induction of bacterial cells expressing only GST (27.5 kD). Lanes 4-6 are cell lysates from the same bacteria as lanes 1-3, respectively.

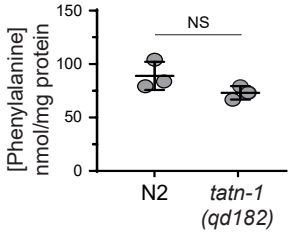

Figure S4. No elevation in phenylalanine concentrations in worms grown on plates supplemented with 4 mM *m*-tyrosine

Results from LC/MS-MRM quantification of the concentration of free free phenylalanine within N2 and *tatn-1(qd182)* mutant worms grown on NGA plates supplemented with 4 mM *m*-tyrosine. Approximately 1500 worms were collected per biological replicate, and the amino acid concentrations were normalized to the concentration of soluble protein measured per sample. Mean phenylalanine concentration and standard deviation are shown. NS represents no statistical significance as deter-

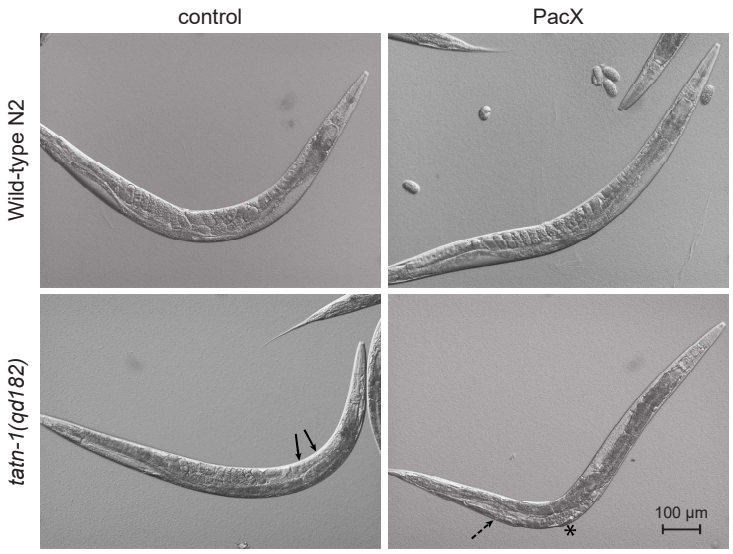

Figure S5. *tatn-1* mutants treated with PacX have a phenotype similar to worms gown on plates supplemented with *m*-tyrosine

Representative images of *tatn-1(qd182)* and N2 worms treated with control and PacX on day one of adulthood. Note the greatly reduced number of embryos within the uterus of PacX-treated *tatn-1(qd182)* worms (indicated by asterisk). Additionally, while control-treated *tatn-1* mutants exhibit normal appearing mature oocytes (solid arrows), the mature oocytes of PacX-treated *tatn-1* mutant animals are more difficult to distinguish (dashed arrow).

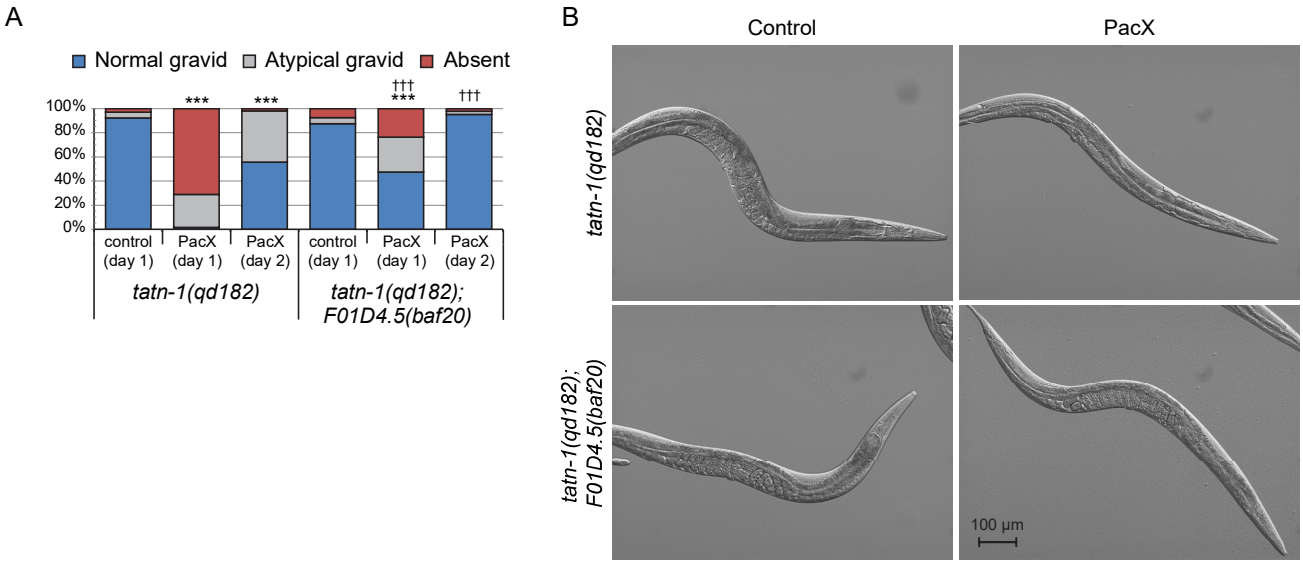

Figure S6. Forward genetic screen reveals the *F01D4.5* mutant to be resistant to PacX treatment

A. Assessment of the number of embryos present in adult *tatn-1(qd182)* and *tatn-1(qd182)*; *F01D4.5(baf20)* worms fed bacteria expressing PacX or control on days 1 and 2 of adulthood. Approximately 150 worms were scored per condition, and the percentage of worms within each condition that were scored as normal gravid (≥ 6 embryos within the uterus), abnormal gravid (< 6 embryos), or absent (0 embryos) are displayed. *** represents $p < 0.001$ for the comparison of the effects of control and PacX treatment within the same strain by chi-square test. ††† represents $p < 0.001$ for the comparison of the effects of PacX between genotypes by chi-square test. B. Representative images of worms quantified in A.

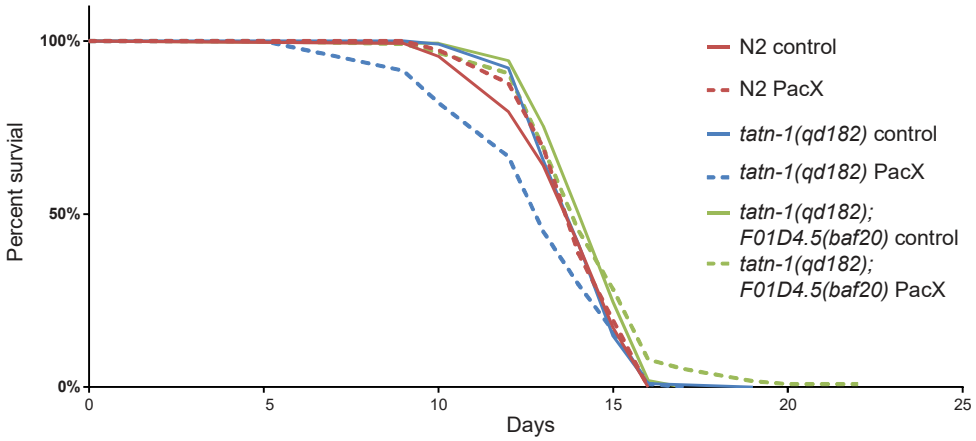

Figure S7. PacX-treated *tatn-1* mutants have a shortened lifespan

Kaplan-Meier survival curve for N2, *tatn-1(qd182)*, and *tatn-1(qd182);F01D4.5(baf20)* worms treated with either control bacteria or PacX-expressing bacteria. Mean lifespan (days) for each genotype and treatment are as follows: control-treated N2 (13.9), PacX-treated N2 (14.1), control-treated *tatn-1(qd182)* (14.2), PacX-treated *tatn-1(qd182)* (13.1), control-treated *tatn-1(qd182); F01D4.5(baf20)* (14.5), PacX-treated *tatn-1(qd182); F01D4.5(baf20)* (14.5). p = 0.007 for *tatn-1(qd182)* treated with control versus PacX; p = 0.60 for N2 treated with control versus PacX; and p = 0.42 for *tatn-1(qd182); F01D4.5(baf20)* treated with control versus PacX by log-rank test.

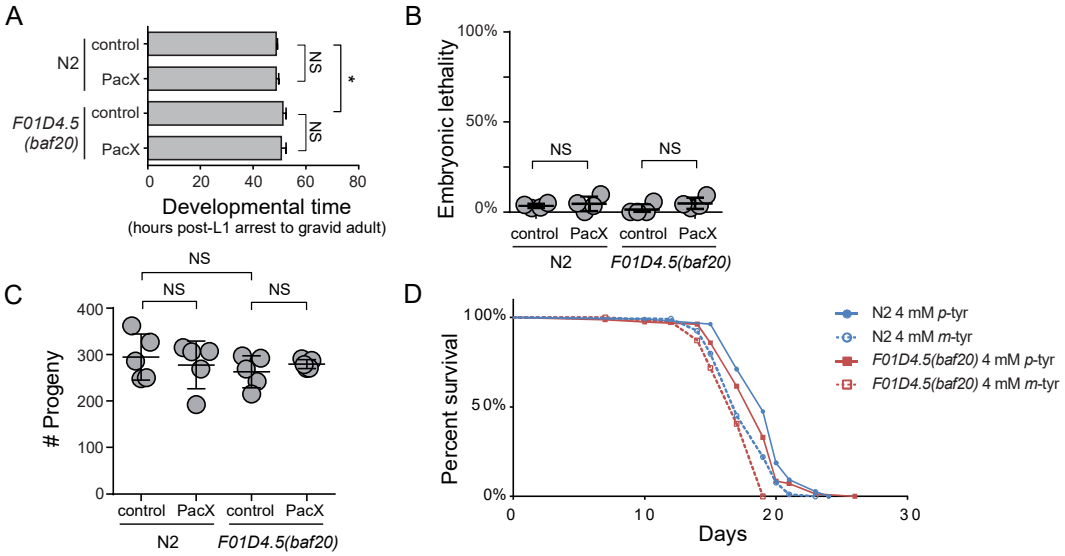

Figure S8. The *F01D4.5(baf20)* mutant shows little difference from N2 with regards development, fertility, or lifespan

A. The developmental time for wild-type N2 and *F01D4.5(baf20)* was measured during treatment with control or PacX expressing bacteria. For N2, 48.8 hours on control and 48.8 on PacX. For *F01D4.5(baf20)*, 51.3 on control and 50.8 on PacX. * represents p = 0.05 by one-way ANOVA with Tukey's post-hoc test. NS represents not signficant. B. The percentage of embryos that died after egg laying from mothers treated with control or PacX expressing bacteria. For N2, 3.4% on control and 4.5% on PacX. For *F01D4.5(baf20)*, 1.4% on control and 4.8% on PacX. NS represents not significant by one-way ANOVA with Tukey's post-hoc test. C. Fertilty of mothers grown on control or PacX expressing bacteria. For N2, 295 on control and 278 on PacX. For *F01D4.5(baf20)*, 263 on control and 279 on PacX. NS represents not significant by one-way ANOVA with Tukey's post-hoc test. D. Kaplan-Meier curves showing the lifespan of N2 and *F01D4.5(baf20)* after treatment with *p*-tyrosine (N2 median survival 19 days and *F01D4.5(baf20)* 18 days, p = 0.05 by log-rank test) or *m*-tyrosine (N2 median survival 17 days and *F01D4.5(baf20)* 17 days, p = 0.03 by log-rank test).

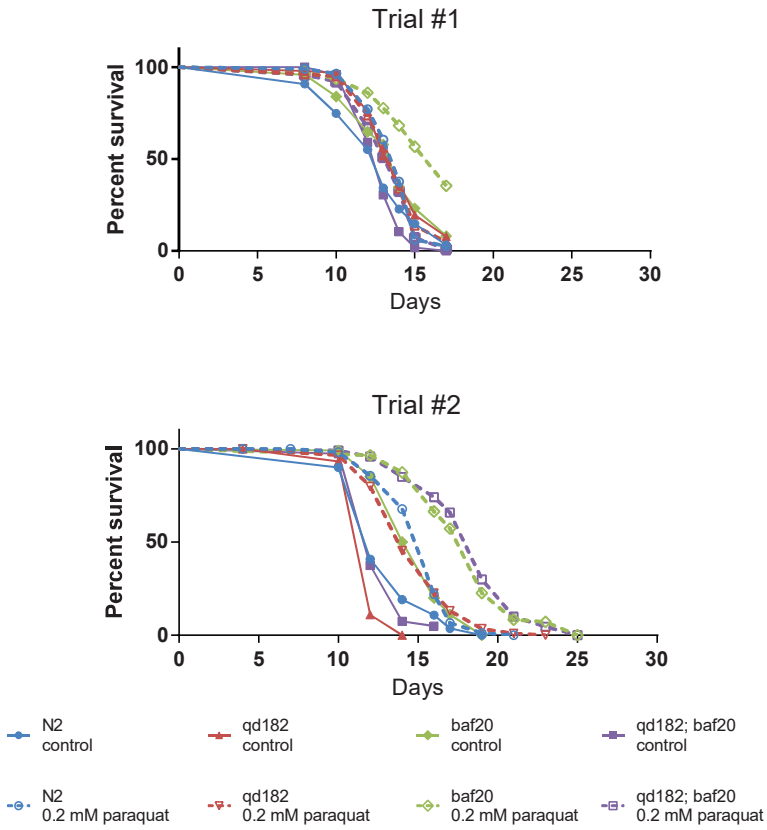

Figure S9. Mild paraquat treatment extends lifespan of *tatn-1(qd82); F01D4.5(baf20)* worms but has uncertain effects on the lifespan of *tatn-1(qd182)* worms

Kaplan-Meier curves showing the lifespans of N2, *tatn-1(qd182)*, *F01D4.5(baf20)*, and *tatn-1(qd181); F01D4.5(baf20)* worms treated with 0.2 mM paraquat compared with an untreated control from two independent experiments. Trial #1 showed paraquat treatment to extend the lifespan of N2 (median survival of control-treated 13 days and paraquat-treated 14 days, $p = 0.01$) , *F01D4.5(baf20)* (median survival of control-treated 14 days and paraquat-treated 17 days, $p < 0.001$), and *tatn-1(qd182); F01D4.5(baf20)* worms (median survival of control-treated 13 days and paraquat-treated 14 days, $p < 0.001$) but not *tatn-1(qd182)* worms (median survival of control-treated 14 days and paraquat-treated 14 days, $p = 0.54$). Trial #2 showed paraquat treatment extended the lifespans of each strain with the largest effect seen for *tatn-1(qd182); F01D4.5(baf20)* worms (median survival of N2 control-treated 12 days and paraquat-treated 16 days, $p < 0.001$; *tatn-1(qd182)* control-treated 12 days and paraquat-treated 14 days, $p < 0.001$; *F01D4.5(baf20)* control-treated 15 days and paraquat-treated 19 days, $p < 0.001$; *tatn-1(qd182);F01D4.5(baf20)* control-treated 12 days and paraquat-treated 19 days, $p < 0.001$). All reported *p*-values were calculated by log-rank test.

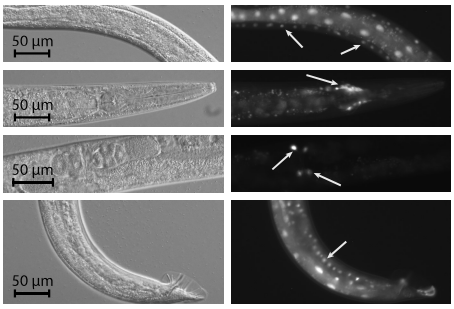

Figure S10. Expression of *F01D4.5* in additional tissues

Images showing the expression of the *F01D4.5p::GFP* transcriptional reporter in additional cells and tissues, including the hypodermis (top), neurons (2nd from top), vulva (2nd from bottom), and male germline (bottom). Arrows highlight GFP within nuclei of the indicated tissue or cells, which is likely due to the inclusion of the first exon of *F01D4.5* in the construct. All images were captured at 20X magnification, and the scale bar indicates 50 microns.

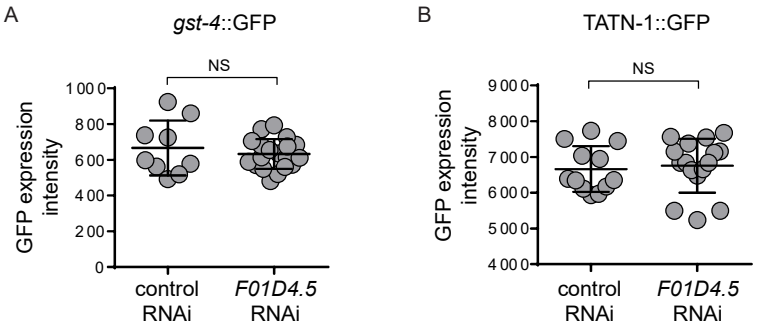

Figure S11. No effect of *F01D4.5* RNAi on SKN-1 activity or TATN-1 expression

A. Average fluorescence intensity of *gst-4::GFP; tatn-1(qd182)* transgenic worms treated with either control or *F01D4.5* RNAi. N = 9-20 worms per RNAi treatment. Mean fluorescent intensity and standard deviation are displayed. p = 0.45 by *t*-test . B. Average fluorescence intensity of TATN-1::GFP in transgenic worms treated with either control or *F01D4.5* RNAi. N = 12-16 worms per RNAi treatment. Mean fluorescent intensity and standard deviation are displayed. p = 0.73 by *t*-test.

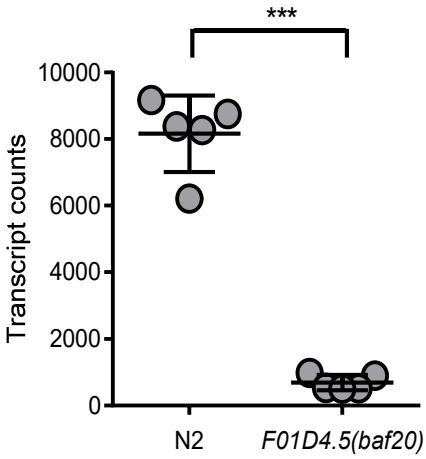

Figure S12. Reduced expression of *T27E7.1* in the *F01D4.5(baf20)* mutant compared to N2

Nanostring was used to measure the expression of *T27E7.1* in RNA extracted from wild-type N2 and *F01D4.5(baf20)* mutants. Mean transcript counts and standard deviation are displayed. N = 5 biologic replicates per genotype. *** represents $p < 0.0001$ by *t*-test. This difference represents a log(2) fold change of -3.57 compared to the log(2) fold change of -3.34 determined by RNA-seq.

Table S1. Differentially expressed genes between N2 and *F01D4.5(baf20)* worms

Results of whole transcriptome profiling by RNA-sequencing. Log2(fold-change) is indicative of the gene expression in *F01D4.5(baf20)* worms compared to N2 worms. Note the reduction in F01D4.5 expression (blue).

Gene ID	Gene name	log2(FC)	adjusted p-value
T27E7.1		-3.343	1.6E-92
F57A10.2		2	4.5E-21
Y71F9AL.4		0.912	2.1E-12
Y82E9BR.22		-0.787	2.2E-09
F33H2.7		0.788	4.3E-08
Y17D7C.3		-0.787	5.7E-08
F01D4.5b		-1.283	1.2E-07
K07F5.12		0.654	5.6E-07
C25D7.3	*sdc-3*	0.625	1.4E-06
B0513.3a	*rpl-29*	-0.769	7.3E-06
F10E7.7.2	*rpl-33*	-0.741	5.9E-05
F37H8.2		-0.855	1.5E-04
M04F3.3		0.583	1.8E-04
Y37E3.8a		-0.701	1.8E-04
F02A9.3.3	*far-2*	-0.545	5E-04
W09C5.6a	*rpl-31*	-0.362	9.6E-04
F58E10.5	*end-3*	1.042	1.5E-03
K11H12.4		-0.855	1.5E-03
F52G3.1		0.575	1.5E-03
E04D5.1a.2	*eif-2a*	-0.832	1.5E-03
Y11D7A.7		0.751	1.5E-03
C56A3.1	*grl-17*	1.036	1.6E-03
F57G4.4	*fbxa-191*	0.796	1.6E-03
B0281.4		0.816	1.7E-03
T04H1.9	*tbb-6*	-0.82	2.1E-03
F15A4.12.2		-0.984	2.6E-03
ZC317.7		-0.748	2.9E-03
M162.12		0.892	3.7E-03
C14C6.5		-0.821	3.9E-03
Y11D7A.10d		-0.871	4.1E-03
Y73B6BL.12		0.858	5.8E-03
Y48G1A.1		-0.404	6E-03
F54H12.1a	*aco-2*	-0.8	9E-03

Gene ID	Gene name	log2(FC)	adjusted p-value
T20D4.3		-0.625	1.1E-02
Y82E9BR.3.2		-0.826	1.3E-02
F14B4.1		0.653	1.5E-02
Y37H2A.14		-0.764	1.5E-02
Y82E9BR.15	*elc-1*	-0.315	1.5E-02
Y82E9BR.16a		-0.438	1.5E-02
K04C1.3		-0.59	1.8E-02
ZK673.1a		-0.723	1.9E-02
F54D11.3		0.632	2.1E-02
C34B7.1		0.659	2.2E-02
F21C10.4		0.812	2.4E-02
C04F12.1		0.539	2.6E-02
F37C12.3.2		-0.588	2.6E-02
Y73B6BL.288		0.778	2.6E-02
F53F4.5b	*fmo-4*	-0.648	3.2E-02
D2096.1b	*timm-17b.2*	0.696	3.2E-02
F25E5.5		-0.605	3.4E-02
Y73F8A.12		0.851	3.4E-02
F28B12.2f	*egl-44*	-0.826	3.6E-02
B0491.6b		-0.746	3.7E-02
F21D9.2		0.42	3.8E-02
ZK652.4.3	*rpl-35*	-0.49	3.8E-02
K02E10.2b	*hid-1*	-0.793	3.8E-02
Y17G9B.1	*npp-26*	0.834	3.8E-02
ZK177.2		-0.787	3.9E-02
ZK370.4b		0.676	4.1E-02
M117.6		-0.531	4.1E-02
K02D10.7		-0.472	4.3E-02
Y67D8C.23		0.73	4.3E-02
F57G4.8	*fbxa-192*	0.594	4.4E-02
F01D5.1		-0.683	4.5E-02

Table S2. Expression of amino acyl-tRNA synthetases in N2 worms compared to *F01D4.5(baf20)* mutants

The expression of amino acyl-tRNA synthetases as indicated by the results of RNA-seq analysis. Red highlights genes whose expression is up in *F01D4.5(baf20)* mutants compared to N2 utilizing an unadjusted p-value. Green highlights genes that are downregulated in these mutants. Note that *fars-1*, along with *yars-1*, is downregulated in *F01D4.5(baf20)* worms

Amino acid	Gene name	Gene ID	log2(FC)	unadjusted p-value
Alanine	*aars-1*	W02B12.6a	0.0185	0.9013
Arginine	*rars-1*	F26F4.10c	-0.0944	0.3762
	rars-2	C29H12.1	-0.0116	0.9399
Asparagine	*nars-1*	F22D6.3a	0.0035	0.9685
Aspartic acid	*dars-2*	F10C2.6	0.1261	0.2194
Glutamic acid	*ears-1*	ZC434.5.1	-0.2802	0.1662
	ears-2	T07A9.2	0.1663	0.0303
Glutamine	*qars-1*	Y41E3.4a	-0.2777	0.1009
Glycine	*gars-1*	T10F2.1a	0.0014	0.9921
Isoleucine	*iars-1*	R11A8.6.1	0.1607	0.4705
	iars-2	C25A1.7a	0.0932	0.2781
Leucine	*lars-1*	R74.1.2	0.0116	0.8511
Lysine	*kars-1*	T02G5.9c.1	-0.1157	0.4715
Methionine	*mars-1*	F58B3.5a	-0.1686	0.3116
		F58B3.5b	0.0993	0.6417
		F58B3.5c	-0.0190	0.9295
Phenylalanine	*fars-1*	T08B2.9a	-0.5690	0.0084
	fars-3	F22B5.9	0.0062	0.9243
Proline	*pars-1*	T20H4.3a	0.3744	0.0251
Serine	*sars-1*	C47E12.1.1	0.1994	0.2448
	sars-2	W03B1.4a	-0.1029	0.3777
		W03B1.4b	0.4200	0.0600
Threonine	*tars-1*	C47D12.6a	-0.0480	0.8295
		C47D12.6b.3	-0.1499	0.4057
Tryptophan	*wars-1*	Y80D3A.1	-0.0020	0.9822
	wars-2	C34E10.4a	-0.3544	0.1116
		C34E10.4b	0.1961	0.0977
Tyrosine	*yars-1*	Y105E8A.19	0.0042	0.9410
	yars-2	K08F11.4a	-0.4730	0.0274
Valine	*vars-1*	ZC513.4	0.0355	0.6445

Table S3. List of primers used in the construction of reporter strains

CRISPR-edited F01D4.5::mKate2	
5’ arm forward:	5’-ACGTTGTAAAACGACGGCCAGTCGCCGGCAGGGAATCACGAAGCAACTGT-3’
5’ arm reverse:	5’-CATCGATGCTCCTGAGGCTCCCGATGCTCCTTTGTTTTTGGCACATGGGT-3’
3’ arm forward:	5’-GAGCAGAAGTTGATCAGCGAGGAAGACTTGGGTACAGACATTCCAGACAAAATAC-3’
3’ arm reverse:	5’-GGAAACAGCTATGACCATGTTATCGATTTCGACGTGAAAACTCGCTCCTT-3’
sgRNA pJW1285 forward:	5’-GTACCCATGTGCCAAAAACAAGTTTAAGAGCTATGCTGGAAAC-3’
sgRNA pJW1285 reverse:	5’-CAAGACATCTCGCAATAGG-3’
***F01D4.5p::*GFP**	
Promoter forward:	5’-TTCTGCAGGTGGGTGTCTTGCCTTGATT-3’
Promoter reverse:	5’-AGCCCGGGGTATTTGAGTGGCCATATTTT-3’

Dataset S1: Full results of the characterization of gonadal morphology of wild-type and *tatn-1(qd182)* transgenic worms treated with PacX expressing bacteria or *m*-tyrosine