allele	*mg412*	*tm2707*	*ok3445*	*let-25(mn25)*
Phenotype	Supernumerary molt	L1 arrest in molt	L1 arrest, identical to tm2707	L1 arrest, identical to tm2707
exon	4	4	4	4
Genotype ATG is 1	A 1090 G	1489- 2047=558 bp in frame deletion and 12bp insertion ACGGATATCACC	636 in frame deletion and 10 bp GCTCCTACAC inserted= out of frame immediately after end of insertion	G 1412 A
Protein	Threonine 364 Alanine T364A	186 AA deletion (497-682), 4 AA insertion	213 AA deletion (448-660) leading to a stop codon 23 AA after deletion insert point	Glycine 471 Argenine G471R
Confirmed genotype through a cross, by pcr and/or sequencing	Yes (recessive), sequenced	Yes (recessive), PCR confirms arrested are homozygous for genotype	ND, ND	ND, Sequencing confirms arrested are homozygous
Rescued	Rescued with genomic fragment Additionally this allele on a transgene rescues the tm2707 L1 arrest	Rescued with genomic fragment and exon 3 GFP translational fusion- arrest vs. rescued genotypes confirmed by PCR	ND	ND

Supplemental Table 1. *pqn-47* alleles

Summary of *pqn-47* alleles identified and characterized in this study. phenotype, exon, genotype, amino acid change, degree of confirmation by sequencing loci after backcrossing, or PCR based assays, and if rescue with F59B10.1 has been confirmed.

Species	Accession RefSeq	Gene	Other names	Blast Rank by species	Blast CE PQN-47 as Query		Reciprocal Top Blast Hit
					coverage	E value	
C. elegans	NP_496262.2	PQN-47	F59B10.1	1	100%	0	PQN-47
C. briggsae	XP_002631278.1	CBR-PQN-47		1	99%	0	PQN-47
C. elegans	NP_509709.2	F21A10.2		2	98%	0	F21A10.2
C. briggsae	XP_002644190.1	CBG17173		2	91%	0	F21A10.2
H. *sapiens*	NP_037411.1	C11orf9	myelin gene regulatory factor	1	75%	2e-85	PQN-47
M. *musculus*	NP_001028653.1	MRF	Gm98	1	72%	8e-85	PQN-47
H. *sapiens*	XP_001718110.2	C12orf28		2	72%	4e-79	F21A10.2
M. *musculus*	NP_001028505.1	Gm239	LOC237558	2	72%	7e-84	PQN-47
X. *laevis*	NP_001087759.1	MRF		1	63%	2e-85	PQN-47
D. *melanogaster*	NP_611893.3	CG3328		1	53%	6e-77	PQN-47
Ciona *intestinalis*	XP_002120171.1			1	52%	6e-75	F21A10.2
D. *rerio*	XP_002667695.2	172183		1	52%	5e-72	PQN-47
Dictyostelium *discoideum*	XP_001134496.1			1	42%	1e-21	F21A10.2
D. *rerio*	NP_001188321.1			2	33%	4e-60	PQN-47
Monosiga *brevicollis* MX1	XP_001742100.1			1	28%	8e-45	PQN-47
Monosiga *brevicollis* MX1	XP_001742246.1			2	20%	5e-27	F21A10.2
Dictyostelium *discoideum*	XP_638706.1	rcdK	ORFveg132	2	13%	1e-17	F21A10.2
S. *cerevisiae*	NP_014933.1	GAM1	SNF2	Not found	16%	0.04	Not found
S. *cerevisiae*	NP_011992.1	Ndt80	DAS1	Not found	9%	0.86	Not found

Supplemental Table 2. Orthologues and paralogues of *pqn-47*

PQN-47 (NP_496262.2) BLASTp scores; first and second highest score in each species retrieved, and scores reported for *pqn-47* vs each hit with BLASTP 2.2.25+. Reciprocal BLAST done on each orthologue against the C. *elegans* genome, and top hit reported. Ntd80 and GAM1 are not related to *pqn-47* nor its bonfide mammalian orthologues C11orf9 or MRF. Monosiga brevicollis is likely to be a fragment due an incomplete genome sequence.

	A. Rescue		B. Precocious alae					C. Adult death
			lL3 to eL4	early L4	e-mid L4	mid-late l4	lL4	
	L1 arrest in molt (48hrs 25C)		Staged based on progress of gonad migration after reflex, back towards vulva					(72 hrs 25C)
	no array	array	0 to < 1/3	1/3 to <1/2	1/2 way	> 1/2	at vulva	
Wildtype			0%(8)	0%(1)	0%(12)	35%(65)	86%(7)	0%(424)
pqn-47(t*m2707)* ex.PQN-47::GFP line A	100%(174)	17%(250)						18%(538)
pqn-47(t*m2707)* ex.PQN-47::GFP line B	100%(36)	7%(40)						
pqn-47(t*m2707)* Is. PQN-47::GFP			0%(3)	43%(14)	42%(31)	97%(37)	100%(2)	25%(460)
lin-41			ND	27%(19)	0%(4)	30% (20)	ND	
pqn-47(t*m2707) /mnCi*								1%(132)
			Staged based on vulval inversion development					
				u	pyramid	christmas tree		
Wildtype				0%(11)	35%(17)	55%(18)		
pqn-47(t*m2707)* Is. PQN-47:GFP				45%(22)	48%(25)	83%(40)		
lin-41				13%(15)	50%(2)			

Supplemental Table 3. Over-expression of PQN-47 rescues *tm2707* L1 molt and causes precocious cuticle alae and adult lethality

(A)tm2707 L1 molt arrest is rescued by extra-chromosomal (ex.) and integrated (Is) arrays of PQN-47::GFP. (B**)** Strains bearing PQN-47 have precocious alae, sometimes faint, when wild type L4 worms of the same stage (based on vulval morphology) and degree of gonad reflux (more than half way back towards vulva) do not have any cuticle alae, but same aged *lin-41* worms with similarly staged vulvas and gonads, do have adult alea, though often with gaps. Gonad arms reflex and start migrating towards the vulva in late L3, at the same time as vulval morphogenesis begins. Although these two developmental programs usually are coordinated with molts and cuticle synthesis, they can be uncoupled, and so neither can be used as un-ambiguous references for developmental stage. Earlier stage classes are to the left and later to the right. Worms staged based on gonad migration, after reflex, back towards vulva, and expressed as proportion of distance to vulva. % of each class of stage of worm showing adult specific cuticle alae (faint and patchy included). Number of worms in each class in parenthesis. (upper panel) or Worms staged based vulval morphogenesis. (lower panel). (C) percentage dead due to bursting as gravid adults at 72 hours post L1 synch at 25^0 C

		NLS	Nuclear export signal (Leptomycin B target)	Localization prediction	localization	Secreted SP?	Non-classic secreted?	Unstructured Domain	Transmembrane Domain # of TM domains, and orientation
Prediction server used for analysis		Predict NLs	NetNES 1.1 prediction	TargetP	Wolf PSORT	*SignalP 3.0 Server*	Secretome 2.0 Server mamalian	Globplot2	*TMpred*
pqn-47 NP_496262	*C. elegans*	none	none	none	cyto	no	no	1st third	1
F21A10.2 NM_171762.2	*C. elegans*	none	none	none	plasma mem	no	no	1st ¼, 3rd 1/4	1
C11orf9 NP_037411.1	human	none	1	none	cyto	no	no	1st third and end	2
Gm98 NP_001028653.1	mouse	none	1	none	cyto	no	no	1st ¼ and 3rd 1/4	1
C12orf28 XP_001718110.2	human	none	2, but interspaced	none	cyto	no	no	Some 1st 1/4	2
Gm239 NP_001028505.1	mouse	none	1	none	cyto	no	no	1st 1/4	2
CG3328 NP_611893.3	*Drosophila*	1	1	none	cyto	no	yes	Some 1st and last third	2
LOC100329855 XP_002667695.2	Zebra fish	none	none	none	cyto	no	yes	1st 1/3rd	none
Mrf NP_001087759.1	xenopus	none	none	none	cyto	no	no	1st and last 3rd	1
GAM1 NP_014933	SC	3-5	none	mito	nuc	no	no	1st and last 3rd	none
ndt80 NP_011992.1	SC	none	none	none	nuc	no	no	3rd quarter	none
daf-16 NM_001026422.3	*C. elegans*	none	5 in a row and 1	mito	nuc	no	yes	lots	1
pqn-47 XP_002631278	*Briggsae*	none	8 in a row	none	cyto	no	no	1st 1/4th	1
Reference		*Murat Cokol, Rajesh Nair and Burkhard Rost.* EMBO Reports 1: 411-415.	**Analysis and prediction of leucine-rich nuclear export signals** *Tanja la Cour, Lars Kiemer, Anne Mølgaard, Ramneek Gupta, Karen Skriver and Søren Brunak* **Protein Eng. Des. Sel.**, 17(6):527-36, **2004.**	**Locating proteins in the cell using TargetP, SignalP, and related tools** Olof Emanuelsson, Søren Brunak, Gunnar von Heijne, Henrik Nielsen	Paul Horton, Keun-Joon Park, Takeshi Obayashi, Naoya Fujita, Hajime Harada, C.J. Adams-Collier, & Kenta Nakai, "WoLF PSORT: Protein Localization Predictor", *Nucleic Acids Research*. doi:10.1093/nar/gkm259. 2007.	**Improved prediction of signal peptides: SignalP 3.0.** *Jannick Dyrløv Bendtsen, Henrik Nielsen, Gunnar von Heijne and Søren Brunak.* **J. Mol. Biol.**. 340:783-795. **2004.**	**Feature based prediction of non-classical and leaderless protein secretion** *J. Dyrløv Bendtsen, L. Juhl Jensen, N. Blom, G. von Heijne and S. Brunak* **Protein Eng. Des. Sel.**. 17(4):349-356. **2004**	GlobPlot: exploring protein sequences for globularity and disorder Nucleic Acid Res 2003 - Vol. 31. No.13 (OpenAccess)	K. Hofmann & W. Stoffel (1993) *TMbase - A database of membrane spanning proteins segments Biol. Chem. Hoppe-Seyler 374,166*

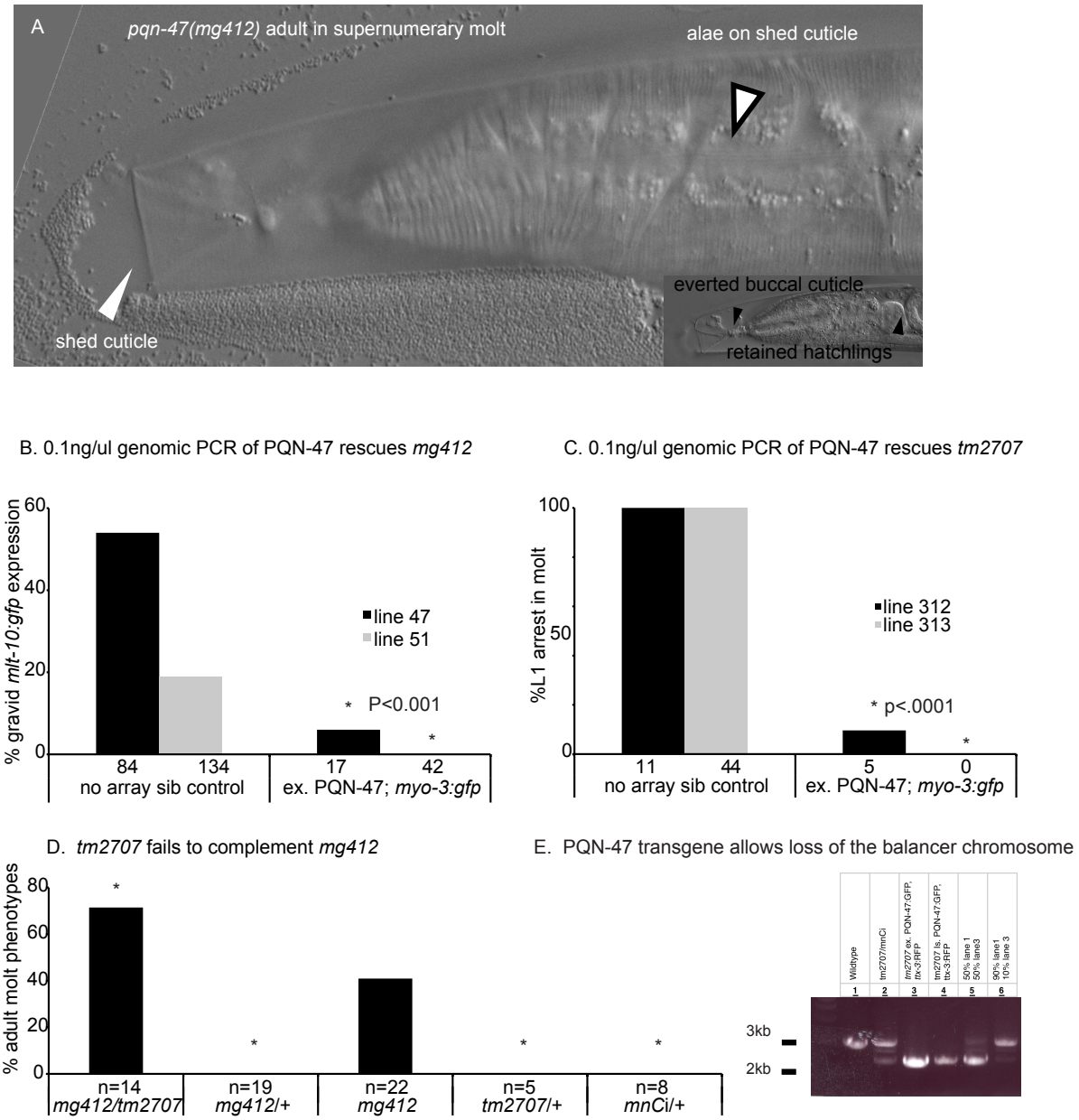
Supplemental Table 4. ***In silico*** **analysis** ***pqn-47*** **has no known domains and is highly unstructured.**

pqn-47 and its orthologues and paralogues were queried for domains and motifs using the servers listed in the top row. DAF-16 is a positive control for the programs to correctly identify features of interest based on sequence alone.

	A. Percentage young adults (with zero embryos) with alae (n)	B. # of seam cells in young adults with no embryos	C. Percentage young adults (0-10 eggs) expressing *col-19:gfp* (n, % dead next day)
Wildtype	100% (9)	17 (n=19)	96% (n=23,0)
pqn-47(mg412)	100% (17)	17 (n=18)	100% (n=20, 35)
lin-29 sqt-2		20 (n=11)	
let-7(mg279)		23 (n=12)	

Supplemental Table 5. *mg412* supernumerary molt out of a fully differentiated adult hypodermis and cuticle: Alae, seam, *col- 19*::gfp.

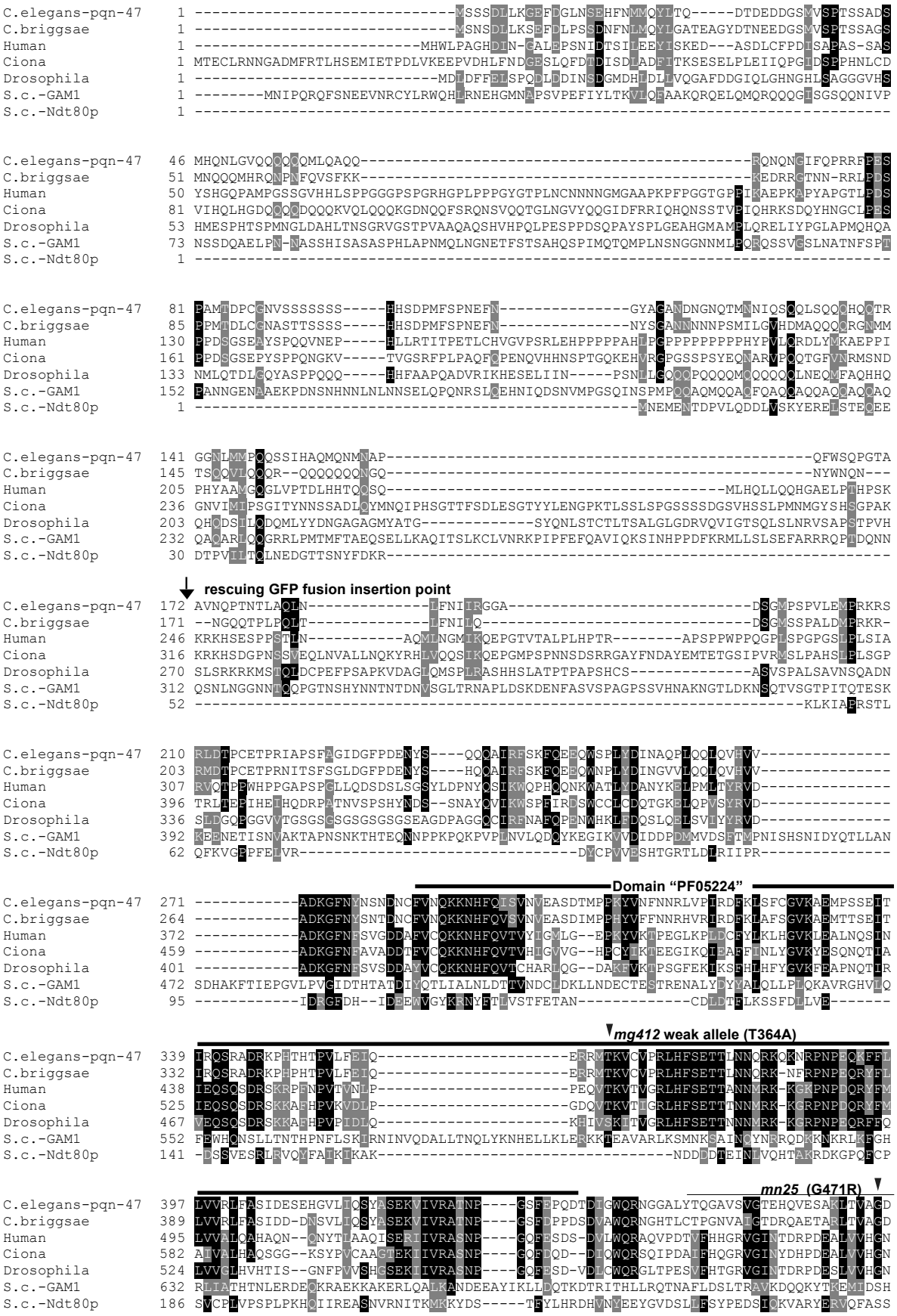
mg412, unlike retarded heterochronic mutants that continue molting as reproductive adults, does so out of an a partially adult cuticle. Animals were grown as synchronized cultures and further age controlled by examining number of eggs and vulval morphology. (A) % of young adults with no embryos with alae (B) number of seam cells in young adults with a mature vulva but no embryos number as identified by seam cell gfp expression (C) % young adults with 0-10 eggs expressing adult cuticle collagen, *col-19p::gfp*. *let-7(mg279)* does not express *col-19::gfp* as an adult. *lin-29* does not have alae as a young adult (Ambros)

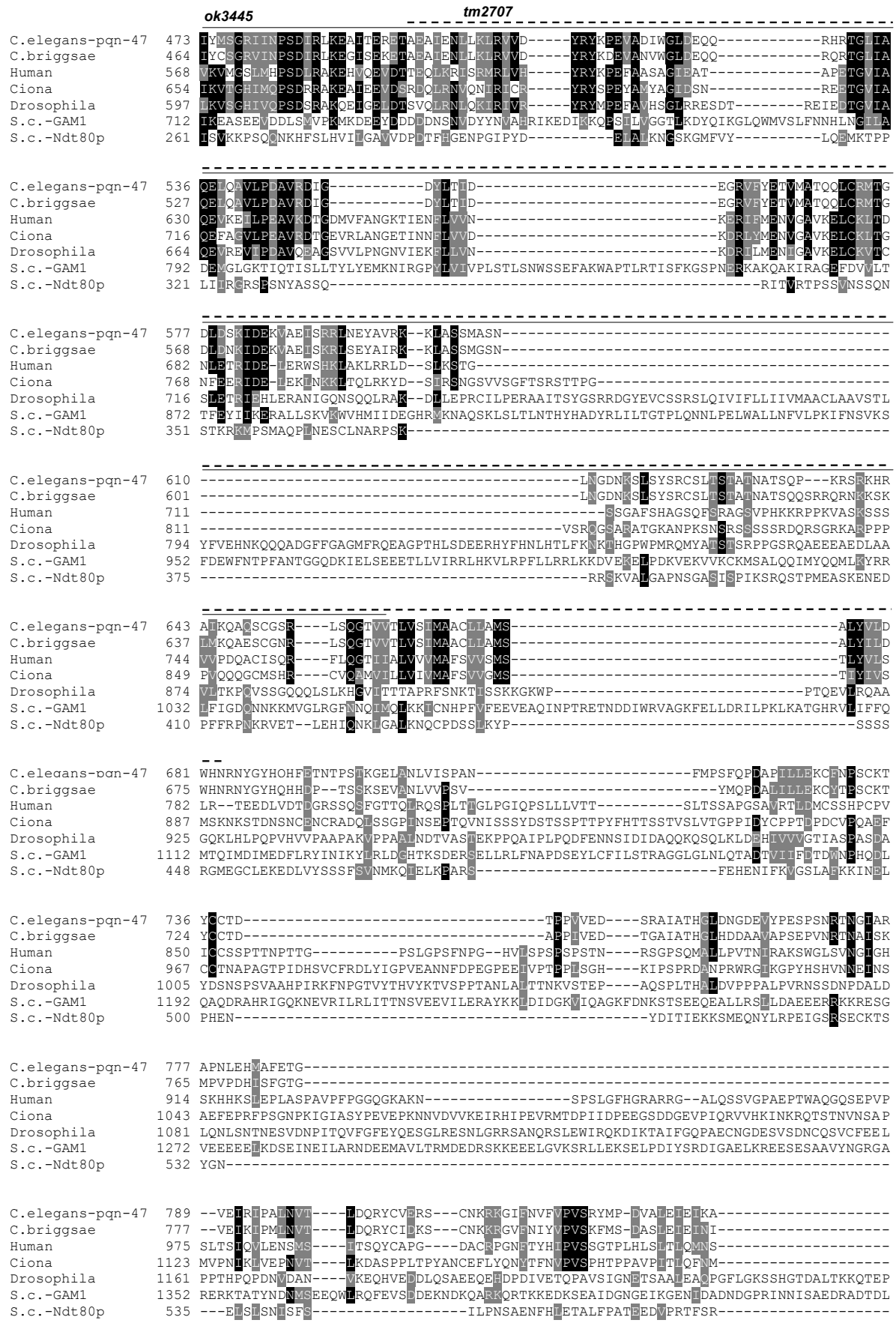


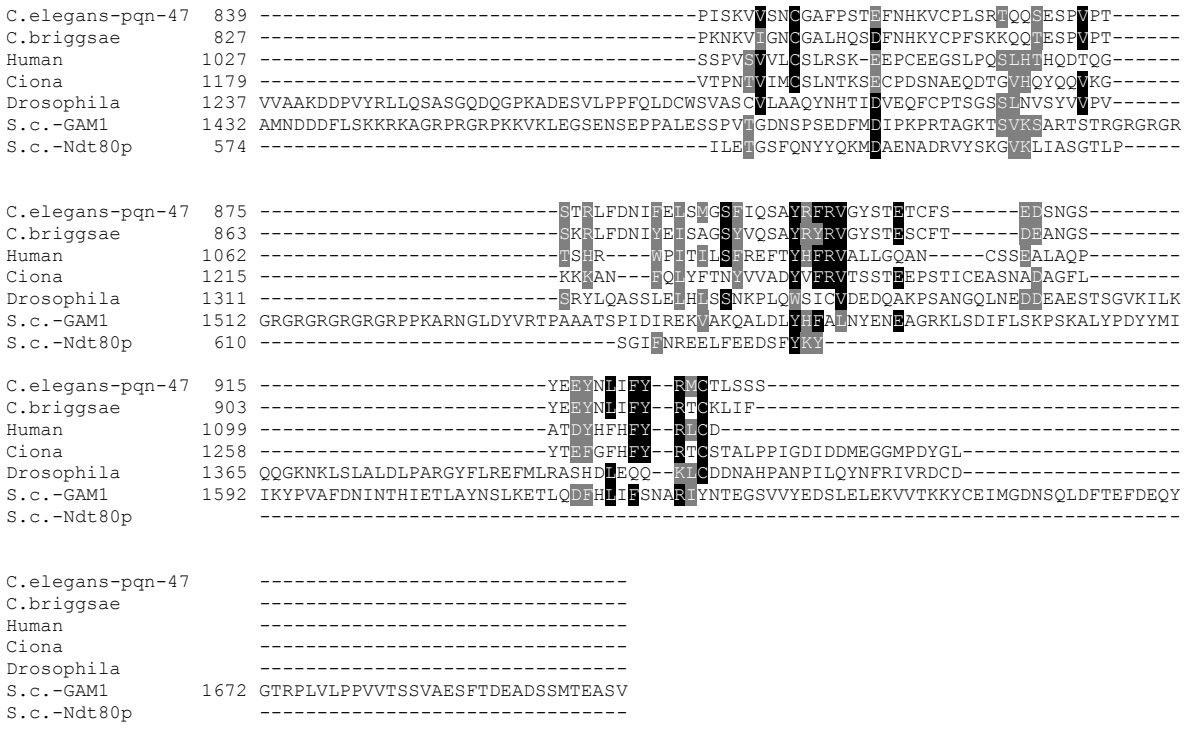
Supplemental Figure 1. Details of *pqn-47*(*mg412)* supernumerary molt, and complementation and rescue of *pqn-47* alleles with F59B10.1

(A) *pqn-47(mg412)* animals attempt to molt as adults, out of an adult cuticle as alae (outlined arrow head) can be seen on the outer and shed cuticle. The inset shows a different focal plane of the same animal. (B) Young adults and gravid adults were sorted based on the presence of co-injection marker *myo-3p::gfp*, then scored for *mlt-10p::gfp-pest* expression over the next 6 hrs. *

denotes P<0.0001 by Fisher's exact test. Multiple independent lines showed similarly significant rescue. (C) L1 molt and non-*molt* animals were picked and then scored for having the array or not based on the co-injection marker *myo-3p::gfp*. Arrested animals had lost the array and animals that grew up had it (D) *mg412mlIs12* males crossed to *tm2707/mnC1*, *Ismlt-10p::gfp-pest*. L1 F1 cross progeny with *mlIS12* were singled and examined for phenotypes throughout their lives. Observation of either *mlt* or *dpy* phenotypes in their F2 offspring allowed the unambiguous determination of the genotype of the singled F1 from the complementation cross. *mg412/tm2707* progeny were not found to have larval molts, rather they had an earlier onset of the retarded phenotypes than the *mg412* allele. Male cross F1 expressed *mlt-10p::gfp-pest* as adults (same age as gravid hermaphrodites) and burst at similar frequency, but because their genotype cannot be determined easily via phenotypic analysis of their progeny, their numbers are excluded from this figure. Category *mg412*/+ has a wild type copy from the balancer chromosome mnC1 (E) PCR confirms the genotype of the *tm2707* rescued worms. *tm2707* lines that are rescued with the PQN-47::GFP, have lost the mnCi chromosome, and therefore become homozygous for the *tm2707* deletion. This PCR only detects endogenous loci because the 3' oligo lies beyond 3'UTR used in the *pqn-47* rescuing construct. Other oligo pairs can also detect the rescuing array.

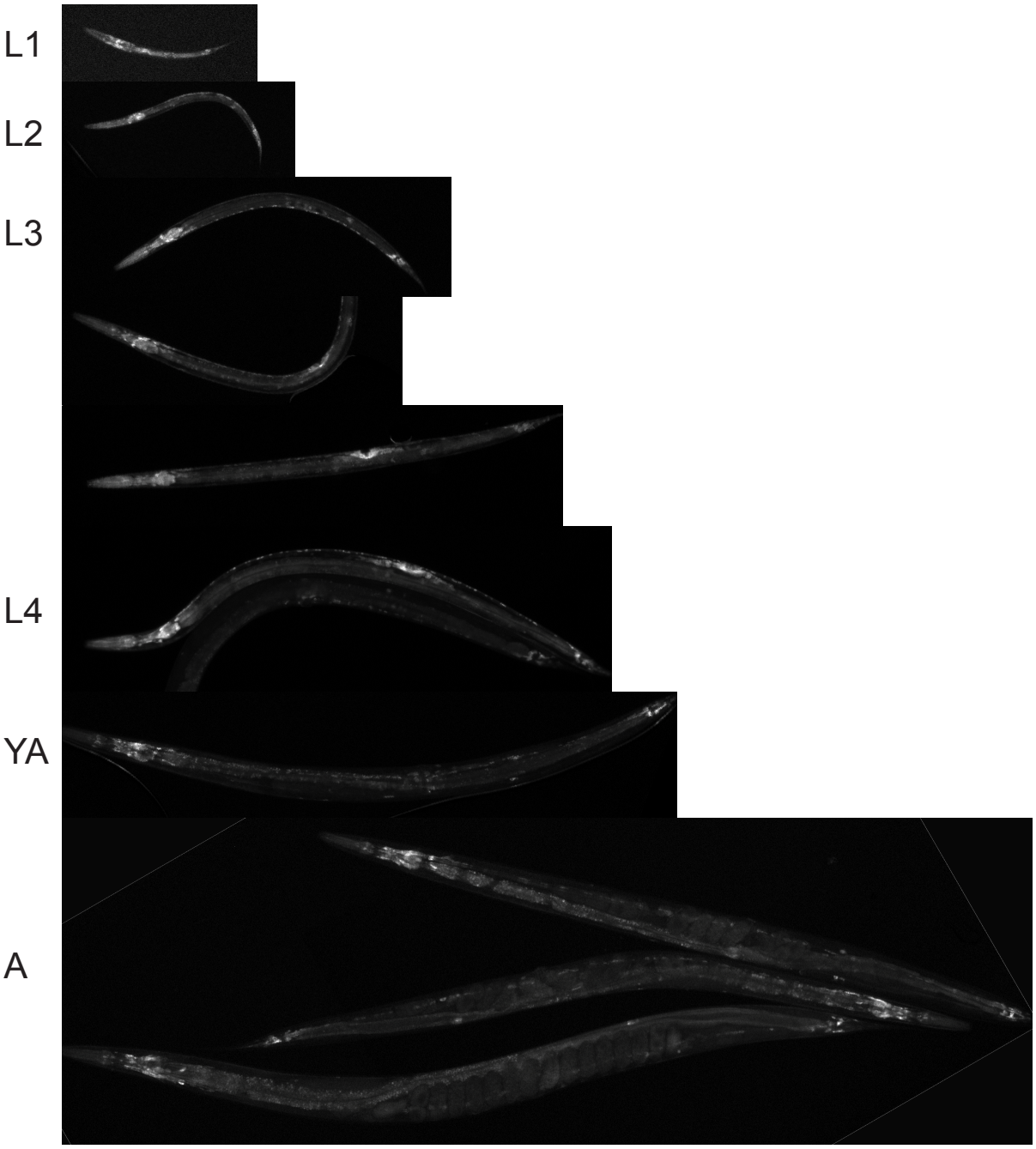

rescuing GFP fusion insertion point
Domain "PF05224"
mg412 weak allele (T364A)
mn25 (G471R)


ok3445
tm2707
C.elegans-pqn-47
C.briggsae
Human
Ciona
Drosophila
S.c.-GAM1
S.c.-Ndt80p



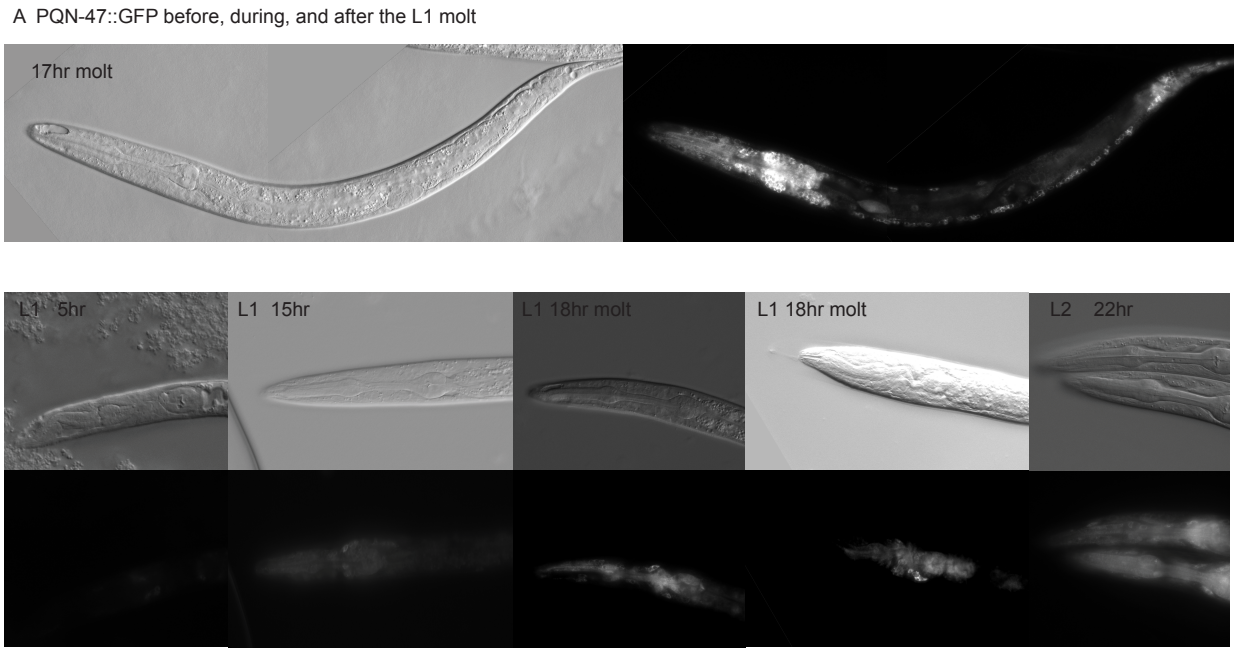
Supplemental Figure 2. Complete alignment of *pqn-47* with animal orthologues and spurious yeast homologues

The PQN-47 protein is highly conserved in animals, though not significantly in Fungi, especially around the mis-annotated central domain PF05224 (thick line) in which all of our mutations are located. Identical residues shaded black and similar with gray. Arrow heads indicate locations of point mutations *mg412* and *mn25*, and deletions are indicated by a thin line (*ok3445)* or dashed line (*tm2707*). GFP was inserted in frame in the coding sequence in an area of low conservation to generate the translational protein fusion used in this study, as C terminal fusions were not able to rescue the mutant phenotypes (data not shown). Protein alignment by CLUSTAL W (2), then BOXSHADE 3.21



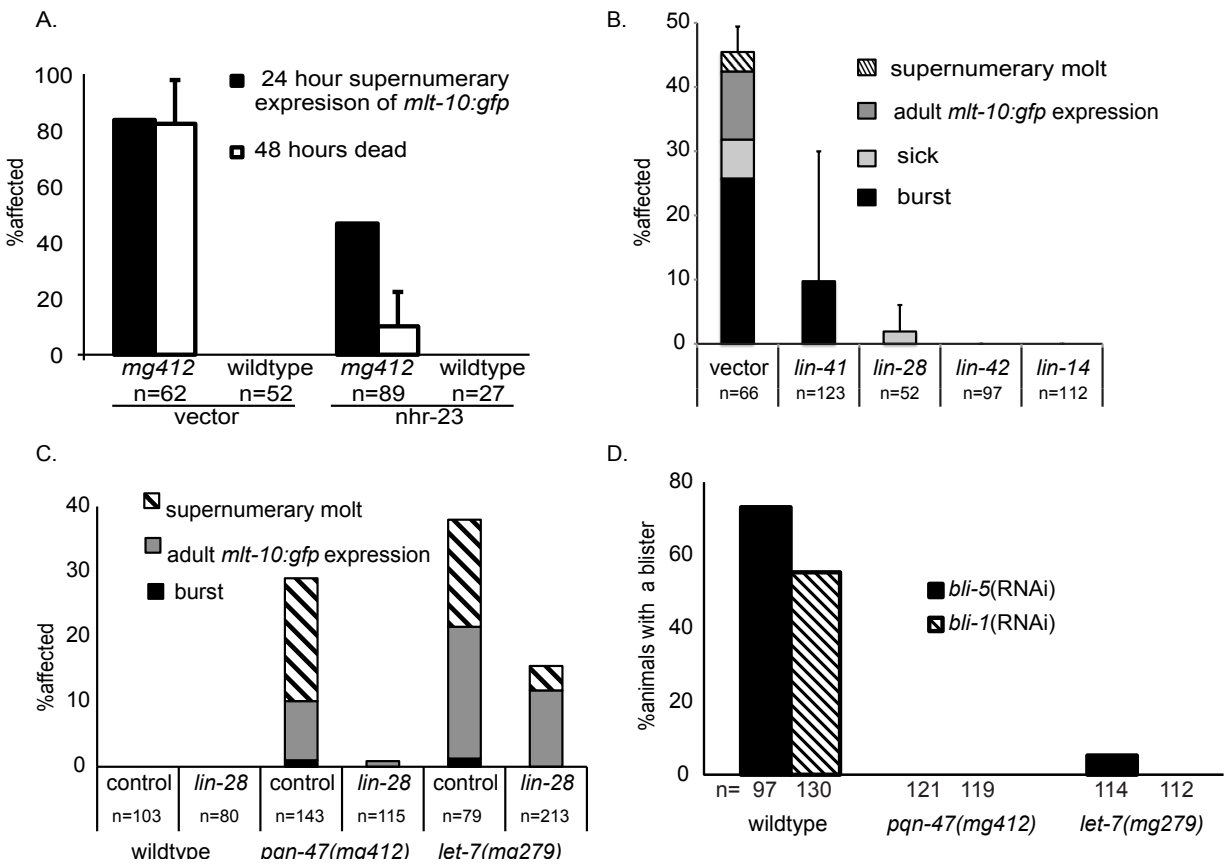
Supplemental Figure 3. PQN-47::GFP expression through development

Mixed stage PQN-47::GFP worms were sorted with a COPAS Biosort according to size and photographed with the same exposure.



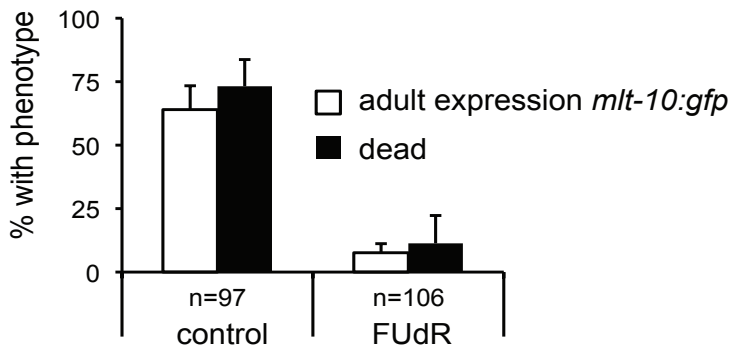
Supplemental Figure 4. PQN-47expression thoughout the L1 molt

(A)PQN-47::GFP (B) promoter reporter *pqn-47p::gfp-pest*



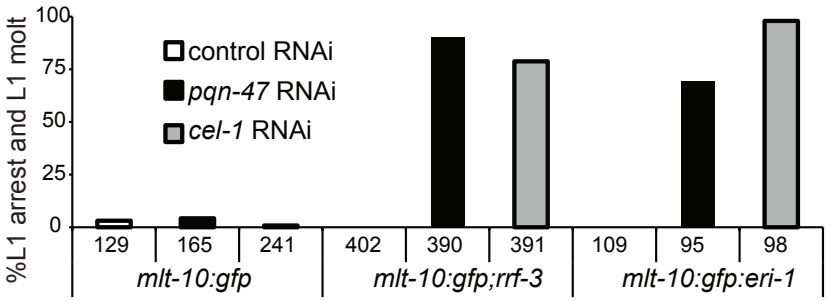
Supplemental Figure 5. *pqn-47* interactions with heterochronic and molt genes

(A) Post developmental (L4) knockdown of *nhr-23* (RNAi) suppresses adult *mlt-10p::gfp-pest* expression and subsequent death of *pqn-47(mg412)* (B) *pqn-47(mg412)* adult phenotypes are suppressed by precocious heterochronic gene RNAi knockdown (C) *lin-28(RNAi)* suppresses the phenotypes of *pqn-47(mg412).* (D) adult molting mutants *pqn-47(mg412)* and *let-7(mg279)* potently suppress *bli-1* and *bli-5* blister phenotypes.



Supplemental Figure 6. DNA replication is required for the supernumerary adult molt of weak allele *mg412*

Late L4 treatment with FUDR suppresses *mg412* adult molts and identifies intestinal endoreduplications as important, because intestinal endoreduplications are the only DNA synthesis event after mid L4.



Supplementary Figure 7. Two generation feeding of pqn-47 RNAI causes L1 arrest only in strains that are more sensitive to RNAi

The L1 arrest and molt phenotype was only achieved after 2 generation RNAi feeding to *eri* strains, suggesting that knockdown in neurons (which are refractory to RNAi in normal strains) may be important for this first larval stage arrest. L4s were put on RNAi of the control vector, *pqn-47* or *cel-1* RNAi, and allowed to lay eggs overnight at 20 degrees, then removed. F1 were allowed to grow for 30 more hours, and then scored for the stage of arrest (molt and not molt). Numbers of animals of each type listed below. *cel-1* RNAi only works in *eri* strains. This was found for additional *eri* strains not shown.