

allele	<i>mg412</i>	<i>tm2707</i>	<i>ok3445</i>	<i>let-25(mn25)</i>
Phenotype	Supernumerary molt	L1 arrest in molt	L1 arrest, identical to <i>tm2707</i>	L1 arrest, identical to <i>tm2707</i>
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 Arginine 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 <i>tm2707</i> 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	
<i>C. elegans</i>	<a href="#">NP_496262.2</a>	PQN-47	F59B10.1	1	100%	0	PQN-47
<i>C. briggsae</i>	<a href="#">XP_002631278.1</a>	CBR-PQN-47		1	99%	0	PQN-47
<i>C. elegans</i>	<a href="#">NP_509709.2</a>	F21A10.2		2	98%	0	F21A10.2
<i>C. briggsae</i>	<a href="#">XP_002644190.1</a>	CBG17173		2	91%	0	F21A10.2
<i>H. sapiens</i>	<a href="#">NP_037411.1</a>	C11orf9	myelin gene regulatory factor	1	75%	2e-85	PQN-47
<i>M. musculus</i>	<a href="#">NP_001028653.1</a>	MRF	Gm98	1	72%	8e-85	PQN-47
<i>H. sapiens</i>	<a href="#">XP_001718110.2</a>	C12orf28		2	72%	4e-79	F21A10.2
<i>M. musculus</i>	<a href="#">NP_001028505.1</a>	Gm239	LOC237558	2	72%	7e-84	PQN-47
<i>X. laevis</i>	<a href="#">NP_001087759.1</a>	MRF		1	63%	2e-85	PQN-47
<i>D. melanogaster</i>	<a href="#">NP_611893.3</a>	CG3328		1	53%	6e-77	PQN-47
<i>Ciona intestinalis</i>	<a href="#">XP_002120171.1</a>			1	52%	6e-75	F21A10.2
<i>D. rerio</i>	<a href="#">XP_002667695.2</a>	172183		1	52%	5e-72	PQN-47
<i>Dictyostelium discoideum</i>	<a href="#">XP_001134496.1</a>			1	42%	1e-21	F21A10.2
<i>D. rerio</i>	<a href="#">NP_001188321.1</a>			2	33%	4e-60	PQN-47
<i>Monosiga brevicollis</i> MX1	<a href="#">XP_001742100.1</a>			1	28%	8e-45	PQN-47
<i>Monosiga brevicollis</i> MX1	<a href="#">XP_001742246.1</a>			2	20%	5e-27	F21A10.2
<i>Dictyostelium discoideum</i>	<a href="#">XP_638706.1</a>	rcdK	ORFveg132	2	13%	1e-17	F21A10.2
<i>S. cerevisiae</i>	<a href="#">NP_014933.1</a>	GAM1	SNF2	Not found	16%	0.04	Not found
<i>S. cerevisiae</i>	<a href="#">NP_011992.1</a>	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. Ndt80 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 (72 hrs 25C)
	L1 arrest in molt (48hrs 25C)		IL3 to eL4	early L4	e-mid L4	mid-late L4	IL4	
	no array	array	Staged based on progress of gonad migration after reflex, back towards vulva					
Wildtype			0 to < 1/3	1/3 to <1/2	1/2 way	> 1/2	at vulva	0%(424)
<i>pqn-47(tm2707)</i>			0%(8)	0%(1)	0%(12)	35%(65)	86%(7)	
ex. PQN-47::GFP line A	100%(174)	17%(250)						18%(538)
<i>pqn-47(tm2707)</i>								
ex. PQN-47::GFP line B	100%(36)	7%(40)						
<i>pqn-47(tm2707)</i>			0%(3)	43%(14)	42%(31)	97%(37)	100%(2)	25%(460)
Is. PQN-47::GFP								
<i>lin-41</i>			ND	27%(19)	0%(4)	30% (20)	ND	1%(132)
<i>pqn-47(tm2707) /mnCi</i>								
			Staged based on vulval inversion development					
			u	pyramid	christmas tree			
Wildtype			0%(11)	35%(17)	55%(18)			
<i>pqn-47(tm2707)</i>			45%(22)	48%(25)	83%(40)			
Is. PQN-47::GFP								
<i>lin-41</i>			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 alae, 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<sup>0</sup> 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
<b>Prediction server used for analysis</b>		Predict NLS	NetNES 1.1 prediction	TargetP	Wolf PSORT	SignalP 3.0 Server	Secretome 2.0 Server mamalian	Globplot2	TMpred
<b>pqn-47</b> NP_496262	<i>C. elegans</i>	none	none	none	cyto	no	no	1 <sup>st</sup> third	1
<b>F21A10.2</b> NM_171762.2	<i>C. elegans</i>	none	none	none	plasma mem	no	no	1 <sup>st</sup> ¼, 3 <sup>rd</sup> 1/4	1
<b>C11orf9</b> NP_037411.1	human	none	1	none	cyto	no	no	1 <sup>st</sup> third and end	2
<b>Gm98</b> NP_001028653.1	mouse	none	1	none	cyto	no	no	1 <sup>st</sup> ¼ and 3 <sup>rd</sup> 1/4	1
<b>C12orf28</b> XP_001718110.2	human	none	2, but interspaced	none	cyto	no	no	Some 1 <sup>st</sup> 1/4	2
<b>Gm239</b> NP_001028505.1	mouse	none	1	none	cyto	no	no	1 <sup>st</sup> 1/4	2
<b>CG3328</b> NP_611893.3	<i>Drosophila</i>	1	1	none	cyto	no	yes	Some 1 <sup>st</sup> and last third	2
LOC100329855 XP_002667695.2	Zebra fish	none	none	none	cyto	no	yes	1 <sup>st</sup> 1/3rd	none
<b>Mrf</b> NP_001087759.1	xenopus	none	none	none	cyto	no	no	1 <sup>st</sup> and last 3rd	1
<b>GAM1</b> NP_014933	SC	3-5	none	mito	nuc	no	no	1 <sup>st</sup> and last 3rd	none
<b>ndt80</b> NP_011992.1	SC	none	none	none	nuc	no	no	3 <sup>rd</sup> quarter	none
<b>daf-16</b> NM_001026422.3	<i>C. elegans</i>	none	5 in a row and 1	mito	nuc	no	yes	lots	1
<b>pqn-47</b> XP_002631278	<i>Briggsae</i>	none	8 in a row	none	cyto	no	no	1 <sup>st</sup> 1/4th	1
<b>Reference</b>		Murat Cokol, Rajesh Nair and Burkhard Rost. <a href="#">EMBO Reports 1: 411-415.</a>	<b>Analysis and prediction of leucine-rich nuclear export signals</b> Tanja la Cour, Lars Kiemer, Anne Mølgaard, Ramneek Gupta, Karen Skriver and Søren Brunak <b>Protein Eng. Des. Sel.</b> , 17(6):527-36, 2004.	<b>Locating proteins in the cell using TargetP, SignalP, and related tools</b> 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", <i>Nucleic Acids Research</i> . doi:10.1093/nar/ukm269. 2007.	<b>Improved prediction of signal peptides: SignalP 3.0.</b> Jannick Dyrlov Bendtsen, Henrik Nielsen, Gunnar von Heijne and Søren Brunak <b>J. Mol. Biol.</b> , 340:783-795, 2004.	<b>Feature based prediction of non-classical and leaderless protein secretion</b> J. Dyrlov Bendtsen, L. Juhl Jensen, N. Blom, G. von Heijne and S. Brunak <b>Protein Eng. Des. Sel.</b> , 17(4):349-356, 2004	GlobPlot: exploring protein sequences for globularity and disorder <a href="#">Nucleic Acid Res 2003 - Vol. 31, No. 13 (OpenAccess)</a>	<b>TMbase - A database of membrane spanning proteins segments</b> K. Hofmann & W. Stoffel (1993) <i>Biol. Chem. Hoppe-Seyler</i> 374,166

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

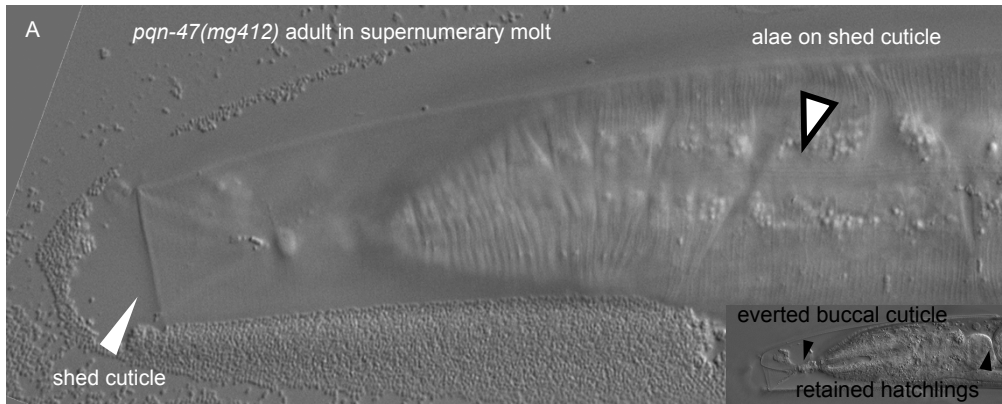


*pqn-47* and its orthologues and paralogues were queried for domains and motifs using the servers listed in the top row. DAF-16 is a positive control for the programs to correctly identify features of interest based on sequence alone.

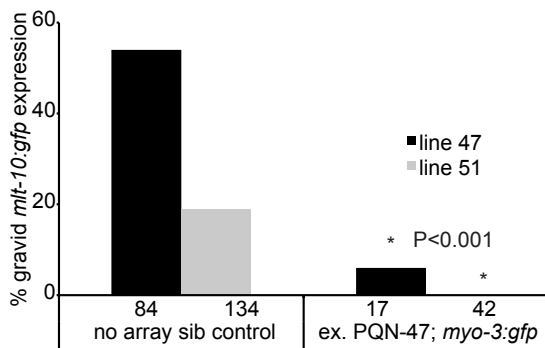
	A. Percentage young adults (with zero embryos) with alae (n)	B. # of seam cells in young adults with no embryos	C. Percentage young adults (0-10 eggs) expressing <i>col-19::gfp</i> (n, % dead next day)
Wildtype	100% (9)	17 (n=19)	96% (n=23,0)
<i>pqn-47(mg412)</i>	100% (17)	17 (n=18)	100% (n=20, 35)
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

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

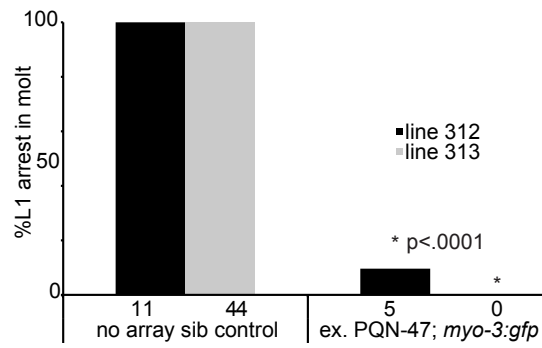
*mg412*, unlike retarded heterochronic mutants that continue molting as reproductive adults, does so out of an a partially adult cuticle. Animals were grown as synchronized cultures and further age controlled by examining number of eggs and vulval morphology. (A) % of young adults with no embryos with 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)



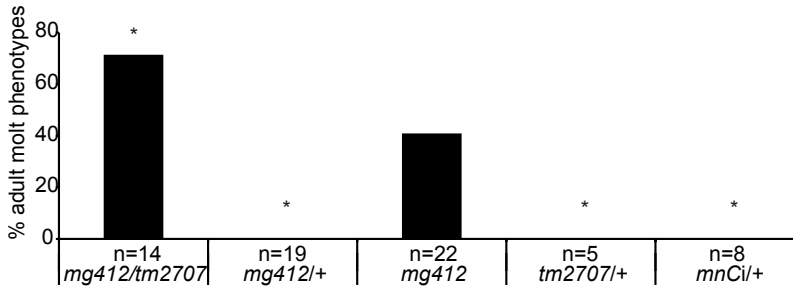
B. 0.1ng/ul genomic PCR of PQN-47 rescues *mg412*



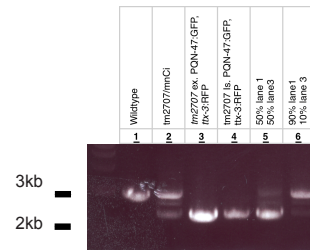
C. 0.1ng/ul genomic PCR of PQN-47 rescues *tm2707*



D. *tm2707* fails to complement *mg412*



E. PQN-47 transgene allows loss of the balancer chromosome



**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) *mg412mlls12* males crossed to *tm2707/mnC1, Ismlt-10p::gfp-pest*. L1 F1 cross progeny with *mlls12* 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.

C.elegans-pqn-47 1 -----VSSSDLLKCEFTGLNSEHFNMMQYLTQ-----DTDEDDGSMVSEPTSSADS  
 C.briggsae 1 -----VNSDILKSEFLPSNDNFNLMQYLGATEAGYDTNEEDGSMVSEPTSSAGS  
 Human 1 -----MHWLPAGHDIN-CALGPSNIDTSLILEEYTSKED---ASDLCFPDISAPAS-SAS  
 Ciona 1 MTECLRNNGADMFRTLHSEMIETPDLVKEEPVDHLFNDGESLQFDIDISDLADFTKSESEPLEI IQPGIDSEPHNLCD  
 Drosophila 1 -----MDIFFELSPQDLIDINSDGMHDLDLVQGAFFDDGIQLGHNGHLSAGGGVHS  
 S.c.-GAM1 1 -----MNIPIRQRFSENEEVNRCYLWRQHNRNEHGMNIPSVPEFIYLTKVILQFAAKQRQELQMRQQQCISGSQQNIVP  
 S.c.-Ndt80p 1 -----

C.elegans-pqn-47 46 MHQNLGVQQQQQMLQAQQ-----RQNQNIQFQPRRFES  
 C.briggsae 51 MNQQQMHRQNPNFQVSFKK-----KEDRRKTNN-RRIPDS  
 Human 50 YSHGQPAMPGSSGVHLSPPGGGPPGRHGLPLPPGYGTPLNCNNNGMGAAKPPFGGTGPEIKAEPKAPYAPGTIPDS  
 Ciona 81 VIHQHLHGDOQQDQQQKVQLQQKQDNGQFSRQNSVQQTGLNGVYQQGIDFRRIQHQNSTVPIQHRKSQDYHNGCLPES  
 Drosophila 53 HMESPTSPMNGLDAHLTNSGRVGTSPVAAQAQSHVHPQLPESPPDSQPAYSPLGEAHGMAMLQRELIYPLGLAPMQHQA  
 S.c.-GAM1 73 NSSDQAELEPNIASSHISASASPHLAPNMQLNGETFTSTSAHQSPIMQTMPLNSNGGNMMLPQRQSSVSLNATNFSPT  
 S.c.-Ndt80p 1 -----

C.elegans-pqn-47 81 PAMTDFPCNVSSSSSSS-----HSDPMFSPNEFN-----GYAGANIDNGNQTMMNIQSQQLSQOCHOCTR  
 C.briggsae 85 PPMTDLCGNASTTSSSS-----HSDPMFSPNEFN-----NYSGANNNNPSMILGVHDMAQQQQRGNMM  
 Human 130 PPDGSGSEAYSPQVNEP-----HLLRTITPETLCHVGVPSRLEHPPPPPAHIEGPPPPPPPPHYVPLCRDLYKAEFPI  
 Ciona 161 PPDGSGSEYSPQVNGKV-----TVGSRFPLPAQFEPENQVHHNSPTGQKEHVRGPGSSPSYEONARVPOQTGFVNRMSND  
 Drosophila 133 NMLQTDLQGYASPPQQQ-----HFAAPQADVRIKHESELIIN-----PSNLLGQQPQQQQMCOQQOCLNEQIFAQHHQ  
 S.c.-GAM1 152 PANNGENAIEKPDNSNHNMLNLNNSLQPNRSLGHNIDQSNVMPGSIINSFMPQCAQMQQAQFQAQQAQQAQQAQQAQ  
 S.c.-Ndt80p 1 -----MNEMLTDFVLQDDLVSKYERESTGCEE

C.elegans-pqn-47 141 GGNLMMPOQSSIIHAQMNMNAP-----QFWSQPGTA  
 C.briggsae 145 TSCQVTLQOOR--QQQQQQNGQ-----NYWYNQN---  
 Human 205 PHYAAMGQGLVPTDLHHTQCSQ-----MLHQLLQQHGAELTPHPSK  
 Ciona 236 GNVIIMPSGITYNSSADLYMNQIPIHSGTTFSDLESPTYLENGPKTLSSLSPGSSSSDGSVHSSLPMMNGYSHSGPAK  
 Drosophila 203 QHIDSLQDQMLYDNGAGAGMYATG-----SYQNLSTCLTSLALGLGDRVQVIGTSQLSLNRVSAFSTPVH  
 S.c.-GAM1 232 QAQARLQQRRLPMTMTAEQSELLKQITSLKLVNRKPIPFQVAVIQKSNHPPDFKRMLLSLSEFARRRQPTDQNN  
 S.c.-Ndt80p 30 DTPVITLQLNEDGTTSNYFDR-----

↓ rescuing GFP fusion insertion point

C.elegans-pqn-47 172 AVNQPTNTLAQLN-----LFNITRGGG-----DSGMPSPVLEMPKRKS  
 C.briggsae 171 --NGQQTPLPOLT-----LFNIIQ-----DSGMSSPALDMPKRK-  
 Human 246 KRKHSEPPSTLN-----AQMINGMTRKEPQGTVTALPLHPTR-----APSPWPQGLSPGGSPLPSIA  
 Ciona 316 KRKHSDGPNSSVEQLNVALLNQYRHLVQOSTKQEPGMPSPNNSDSRRGAYFNDAEMTETGSIPIVRMSLPAHSLPLSGP  
 Drosophila 270 SLSRKRKMSIQLDCPEFSPAPKVDAGIQMSPLRASHHSLATPTPAPSHCS-----ASVSPALSAVNSQADN  
 S.c.-GAM1 312 QSNLNGGNNTQPGTNSHYNNTNTDNVSGLTRNAPLDSKDFENFASVSPAGPSSVHNAKNGTLDKNSQTVSGTPIQTESK  
 S.c.-Ndt80p 52 -----KLIKIAERSTL

C.elegans-pqn-47 210 RLDTPCETPRIAPSFAGIDGFPDENYS-----QQCAIRESKFQEEQWNSPLYDINAQPLQQQOVHVW-----  
 C.briggsae 203 RMDTPCETPRNITSFSGLDGFPDENYS-----HQCAIRESKFQEEQWNSPLYDINGVVLQQQOVHVW-----  
 Human 307 RVQTEFWHPGAPSPQLLQDSDSLSSGYLDPNYQSIKWQPHQONKRWATLYDANYKELPMLTYRVD-----  
 Ciona 396 TRLTEPIHEHQDRPPTNVSPSHYNDSSNAYQVKKWSPETRDSWCCICDQTKGELQPVSYRVD-----  
 Drosophila 336 SLLGQGGVVTGSGSSGSGSGSGSEAGDPAGGQCIRENAQFENWHLKLDQSLQSLVHYRVD-----  
 S.c.-GAM1 392 KENETISNVAKTAPNSNKTHTEQNNPPKQKVPVILNVLDQYKEGKIVVDIDDDPMVDSFETMPNISHSNIIDYQTLNAN  
 S.c.-Ndt80p 62 QPKVGFPEFIVR-----DPCVVEHSHTGRTLDRIIPR-----

Domain "PF05224"

C.elegans-pqn-47 271 -----ADKGFNYSNDCNFVNOCKKNHFQISVNVNASDTPMFKYVFNRRNLVPTRDEKLSFCGVKAEEMPSSEIT  
 C.briggsae 264 -----ADKGFNYSNDCNFVNOCKKNHFQVSVNVNASDIMPPIYVFNRRNRVTRDEKLSFCGVKAEEMTTSEIT  
 Human 372 -----ADKGFNYSVGDADFVQCKKNHFQVTVYIGLGG--EPKYVKTEPEGLKPLDLKHLGKLEALNQSIN  
 Ciona 459 -----ADKGFNEAVADDTFVQCKKNHFQVTVHIGVVG--HPCYIKTEEGIKOTEAEFFINLYGVRYESQNQTIA  
 Drosophila 401 -----ADKGFNYSVSDDAYVQCKKNHFQVTVCHARIQG--DAKEVKTSPGFETKSKSHLHFYGVKFEAPNQITR  
 S.c.-GAM1 472 SDHAKFTIEPGVLPVGLDTHATDHYQTLIALNLDTVNDCKDKLNDCECTETRENALYDHALQLLPLQKAVRGHVLIQ  
 S.c.-Ndt80p 95 -----IDRGEDH--IDEEWVGYKKNYFTLVSTFETAN-----CDLDTLKSSEFDLQVE

↓ mq412 weak allele (T364A)

C.elegans-pqn-47 339 IROSRADRKPHHTHTPVLFETIQ-----ERRMTKVCFVRLHFSETTLNNQRKQKRNPNPQRFFL  
 C.briggsae 332 IROSRADRKPHHTHTPVLFETIQ-----ERRMTKVCFVRLHFSETTLNNQRK-NRPNPQRFFL  
 Human 438 IEQSQSDRSKRPNPVTNPL-----PEQVTKVTVGRLHFSETTANNMRK-KGKPNPDQRVFM  
 Ciona 525 IEQSQSDRSKKAHPVKVDLP-----GDQVTKVTVGRLHFSETTANNMRK-KGRPNPDQRVFM  
 Drosophila 467 IEQSQSDRSKKAHPVPIDLIQ-----KEIVSKITVGRHFSETTANNMRK-KGRPNPQRFFQ  
 S.c.-GAM1 552 FEWHQNSLLTNTHPNLSKRNINVDALLTNQYKHNHELLKLEKKTTEAVARLKSMMNKSAINCYNRQDKKRRLLKFGH  
 S.c.-Ndt80p 141 --SSVESRLRQVFAKIKAK-----NDDDETEINLVQHTAKRDKGPOFCP

mm25 (G471R) ↓

C.elegans-pqn-47 397 LVVRLFASIDSEHGVLQSYASEKVIIVRASNF-----GSFEPQDITDIGNQRNGGALYTOGAVSVGTEHQVESARLTVAGD  
 C.briggsae 389 LVVRLFASIDDDNSVLIQSYASEKVIIVRASNF-----GSFEPDSDVAWQRNGHTLCTPGNVALGTPDRQAETARLTVAGD  
 Human 495 LVVALQAHAQN--QNYTLAAQISERIIVRASNF-----GCFESDS--DVLWQRAQVPDITVHHGRVGINITDRPDEALVHGN  
 Ciona 582 AVVALQAQSGG--KSYFVCAAGTEKIIVRASNF-----GCFEQD--DIQWQRSQIPDAIFHQGRVGINIDHPDEALVHGN  
 Drosophila 524 LVVGLHVHTIS--GNFPVVSCHGSEKIIVRASNF-----GCFESD--VDLQWQRGLTPESVHTGRVGINITDRPDESLVHGN  
 S.c.-GAM1 632 RLATHTNLERDCKRAEKKAKERLQALKANDEEAYIKLLDQTKDTRIHTLLRQTNAFILDSLTRAVKDDQKYTREREMDSH  
 S.c.-Ndt80p 186 SWCPFLVPSPLPKHIIIREASNVNRIITRMKYKYS-----TEYLHRDHVNIIEYGVDSLSLISYPEDSDQKVARYEIVQNFASS

ok3445

tm2707

C.elegans-pqn-47 473 IYMSGRIINPSDRLRKEA... YRYKPEWADIWGLEEQQ... RHRTGLIA
C.briggsae 464 IYCSGRVINPSDRLRKEG... YRYKDEVANVWGLEEQQ... RQRTGLIA
Human 568 IYKVMGSLMHPSDRLRA... YRYKPEFAASAGTEAT... APETGVIA
Ciona 654 IKVTGHIQPSDRRAKBA... YRYSPBYAYAGLDSN... REETGVIA
Drosophila 597 IKVSGHIVQPSDSRAKQ... YRYMPEFAVHSGLRRES... REIETGVIA
S.c.-GAM1 712 IYKASEEVDLDSVVKMK... YRYKPEFAVHSGLRRES... REIETGVIA
S.c.-Ndt80p 261 ISYVKKPSQGNKHFSLV... YRYKPEFAVHSGLRRES... REIETGVIA

C.elegans-pqn-47 536 QELQAVLPDAVRDITG... DMLTID... EGRVVFYETVMATQQLCRM...
C.briggsae 527 QELQAVLPDAVRDITG... DMLTID... EGRVVFYETVMATQQLCRM...
Human 630 QELQAVLPDAVRDITG... DMLTID... EGRVVFYETVMATQQLCRM...
Ciona 716 QEFAGVLPDAVRDITG... DMLTID... EGRVVFYETVMATQQLCRM...
Drosophila 664 QEFAGVLPDAVRDITG... DMLTID... EGRVVFYETVMATQQLCRM...
S.c.-GAM1 792 QEFAGVLPDAVRDITG... DMLTID... EGRVVFYETVMATQQLCRM...
S.c.-Ndt80p 321 QEFAGVLPDAVRDITG... DMLTID... EGRVVFYETVMATQQLCRM...

C.elegans-pqn-47 577 DLNPKIDEKVAEISRR... KLASMASN...
C.briggsae 568 DLNPKIDEKVAEISRR... KLASMASN...
Human 682 DLNPKIDEKVAEISRR... KLASMASN...
Ciona 768 DLNPKIDEKVAEISRR... KLASMASN...
Drosophila 716 DLNPKIDEKVAEISRR... KLASMASN...
S.c.-GAM1 872 DLNPKIDEKVAEISRR... KLASMASN...
S.c.-Ndt80p 351 DLNPKIDEKVAEISRR... KLASMASN...

C.elegans-pqn-47 610 LINGDNKSLSYRCSL... STANATSQP... KRSRKHR
C.briggsae 601 LINGDNKSLSYRCSL... STANATSQP... KRSRKHR
Human 711 LINGDNKSLSYRCSL... STANATSQP... KRSRKHR
Ciona 811 LINGDNKSLSYRCSL... STANATSQP... KRSRKHR
Drosophila 794 LINGDNKSLSYRCSL... STANATSQP... KRSRKHR
S.c.-GAM1 952 LINGDNKSLSYRCSL... STANATSQP... KRSRKHR
S.c.-Ndt80p 375 LINGDNKSLSYRCSL... STANATSQP... KRSRKHR

C.elegans-pqn-47 643 ALKQAESCGRS... LSQGTVVTLVLSMAAC... ALYVLD
C.briggsae 637 ALKQAESCGRS... LSQGTVVTLVLSMAAC... ALYVLD
Human 744 ALKQAESCGRS... LSQGTVVTLVLSMAAC... ALYVLD
Ciona 849 ALKQAESCGRS... LSQGTVVTLVLSMAAC... ALYVLD
Drosophila 874 ALKQAESCGRS... LSQGTVVTLVLSMAAC... ALYVLD
S.c.-GAM1 1032 ALKQAESCGRS... LSQGTVVTLVLSMAAC... ALYVLD
S.c.-Ndt80p 410 ALKQAESCGRS... LSQGTVVTLVLSMAAC... ALYVLD

C.elegans-pqn-47 681 WHNRNYGYHOFHT... TNPSTK... FMPSFQ...
C.briggsae 675 WHNRNYGYHOFHT... TNPSTK... FMPSFQ...
Human 782 WHNRNYGYHOFHT... TNPSTK... FMPSFQ...
Ciona 887 WHNRNYGYHOFHT... TNPSTK... FMPSFQ...
Drosophila 925 WHNRNYGYHOFHT... TNPSTK... FMPSFQ...
S.c.-GAM1 1112 WHNRNYGYHOFHT... TNPSTK... FMPSFQ...
S.c.-Ndt80p 448 WHNRNYGYHOFHT... TNPSTK... FMPSFQ...

C.elegans-pqn-47 736 YCCTD... SRAIATH... YPESPSNR...
C.briggsae 724 YCCTD... SRAIATH... YPESPSNR...
Human 850 YCCTD... SRAIATH... YPESPSNR...
Ciona 967 YCCTD... SRAIATH... YPESPSNR...
Drosophila 1005 YCCTD... SRAIATH... YPESPSNR...
S.c.-GAM1 1192 YCCTD... SRAIATH... YPESPSNR...
S.c.-Ndt80p 500 YCCTD... SRAIATH... YPESPSNR...

C.elegans-pqn-47 777 APNLEHMAFETG...
C.briggsae 765 APNLEHMAFETG...
Human 914 APNLEHMAFETG...
Ciona 1043 APNLEHMAFETG...
Drosophila 1081 APNLEHMAFETG...
S.c.-GAM1 1272 APNLEHMAFETG...
S.c.-Ndt80p 532 APNLEHMAFETG...

C.elegans-pqn-47 789 --VEIKIPALNVT... IDQRYC... CNKRG...
C.briggsae 777 --VEIKIPALNVT... IDQRYC... CNKRG...
Human 975 --VEIKIPALNVT... IDQRYC... CNKRG...
Ciona 1123 --VEIKIPALNVT... IDQRYC... CNKRG...
Drosophila 1161 --VEIKIPALNVT... IDQRYC... CNKRG...
S.c.-GAM1 1352 --VEIKIPALNVT... IDQRYC... CNKRG...
S.c.-Ndt80p 535 --VEIKIPALNVT... IDQRYC... CNKRG...

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C.elegans-pqn-47  839  -----PISKVSNCGAFPSTEFNHKVCPLSRQQEESPVPT-----
C.briggsae      827  -----PKNKVGNCGALHQSDFNHKYCPFSKKQQEESPVPT-----
Human          1027 -----SSPVSVVLCSLRSKDEPCEEGSLPQSLHHQDQTGG-----
Ciona          1179 -----VTPNIVIMCSLNTKSECPDSNAEQDTGVHQYQQVKG-----
Drosophila     1237 VVAAKDDPVYRLLQSASGQDQGPKADESVLPFFQLDCWSVASCVLAAQYNHTIIVEQFCPTSGSINVSYYVFPV-----
S.c.-GAM1      1432 AMNDDDFLSKKRKAGRPRGRPKKVKLEGSENSEPPALESSPVVGDNSPSEDFMDIPKPRTAGKTSVKSARTSTRGRGRGR
S.c.-Ndt80p    574  -----ILEGSGFQNYQKMDAENADRVSYKGVKLIASGTLP-----

C.elegans-pqn-47  875  -----STLFDNIETESMGSFIQSAYNFRVGYSTETCFSEESNGS-----
C.briggsae      863  -----SKLFDNIYETISAGSYVQSAYRFRVGYSTESCFTDEANGS-----
Human          1062 -----TSR---WPTTILSFREFTYHFRVALLGOAN---CSSSEALAQP-----
Ciona          1215 -----KKKAN---FQLYFTNYVVADYFRVTSSTEEPSTICEASNADAGFL-----
Drosophila     1311 -----SRYLQASSLETHISSNKPLQMSICVDEDEQAKPSANGQLNEDDEAESTSGVKILK
S.c.-GAM1      1512 GRGRGRGRGRGRPPKARNGLDYVRTPAAATSPIDIREKVAQALDLYHFAINYENIAGRKLSDIFLSKPSKALYPDYMI
S.c.-Ndt80p    610  -----SGIENREELFEEDSFYKY-----

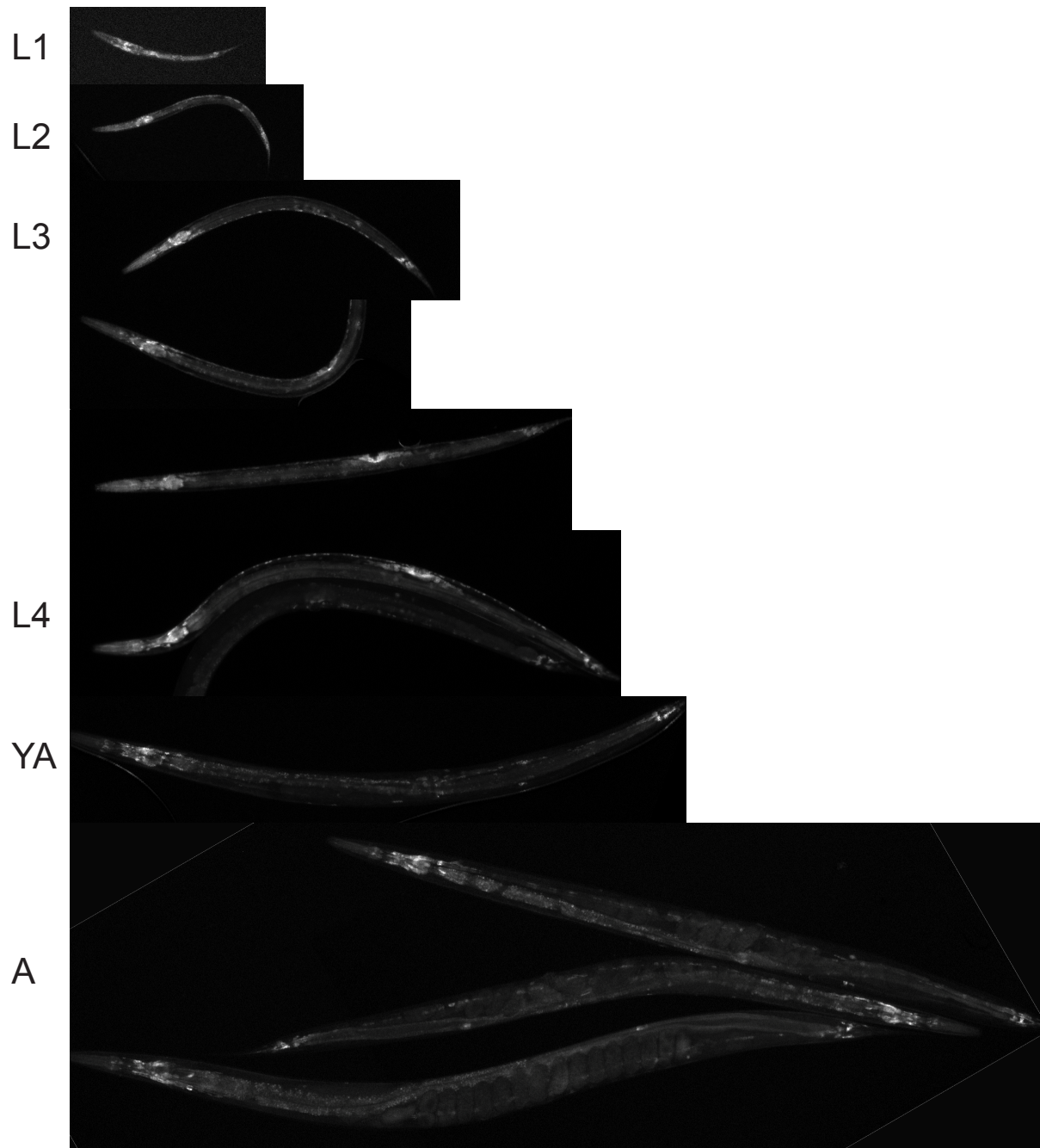
C.elegans-pqn-47  915  -----YEEYNLIIFY--RMCTLSSS-----
C.briggsae      903  -----YEEYNLIIFY--RTCKLIF-----
Human          1099 -----ATDYHFHY--RLCD-----
Ciona          1258 -----YTEEGFHFY--RTOSTALPPIGDIDDMEGGMPDYGL-----
Drosophila     1365 QQGKNKLSLALDLPARGYFLREFMLRASHDLTEQQ--KICDDNAHPANFILQYNFRIVRDCD--
S.c.-GAM1      1592 IKYPVAFDNINTHIETLAYNSLKETLQDEHLEISNARTIYNTEGSVVYEDSLELEKVVTKKYCEIMGDNSQLDFTEFDEQY
S.c.-Ndt80p

C.elegans-pqn-47  -----
C.briggsae -----
Human -----
Ciona -----
Drosophila -----
S.c.-GAM1      1672 GTRPLVLPVVVTSSVAESFTDEADSSMTEASV
S.c.-Ndt80p -----

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**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

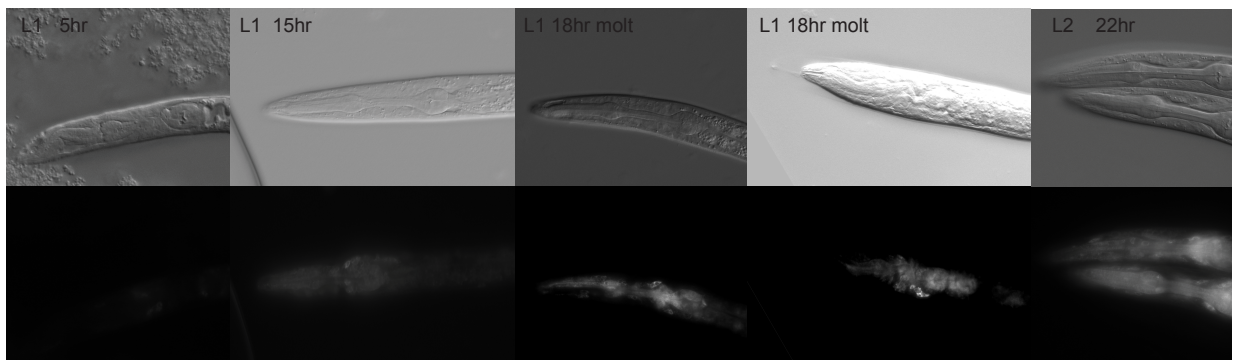
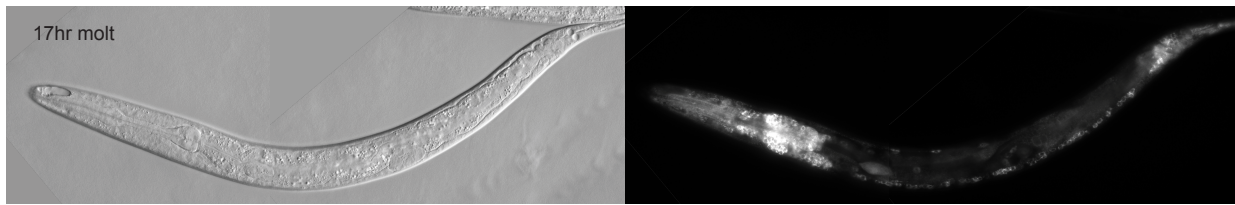


**Supplemental Figure 3. PQN-47::GFP expression through development**

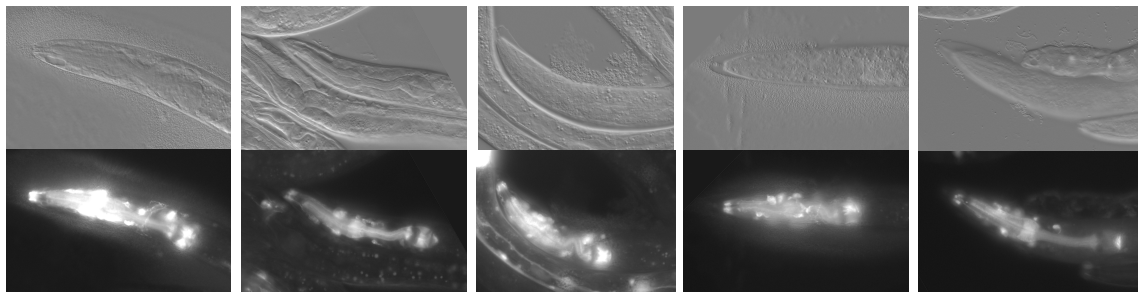
Mixed stage PQN-47::GFP worms were sorted with a COPAS Biosort according to size and photographed with the same exposure.



A PQN-47::GFP before, during, and after the L1 molt

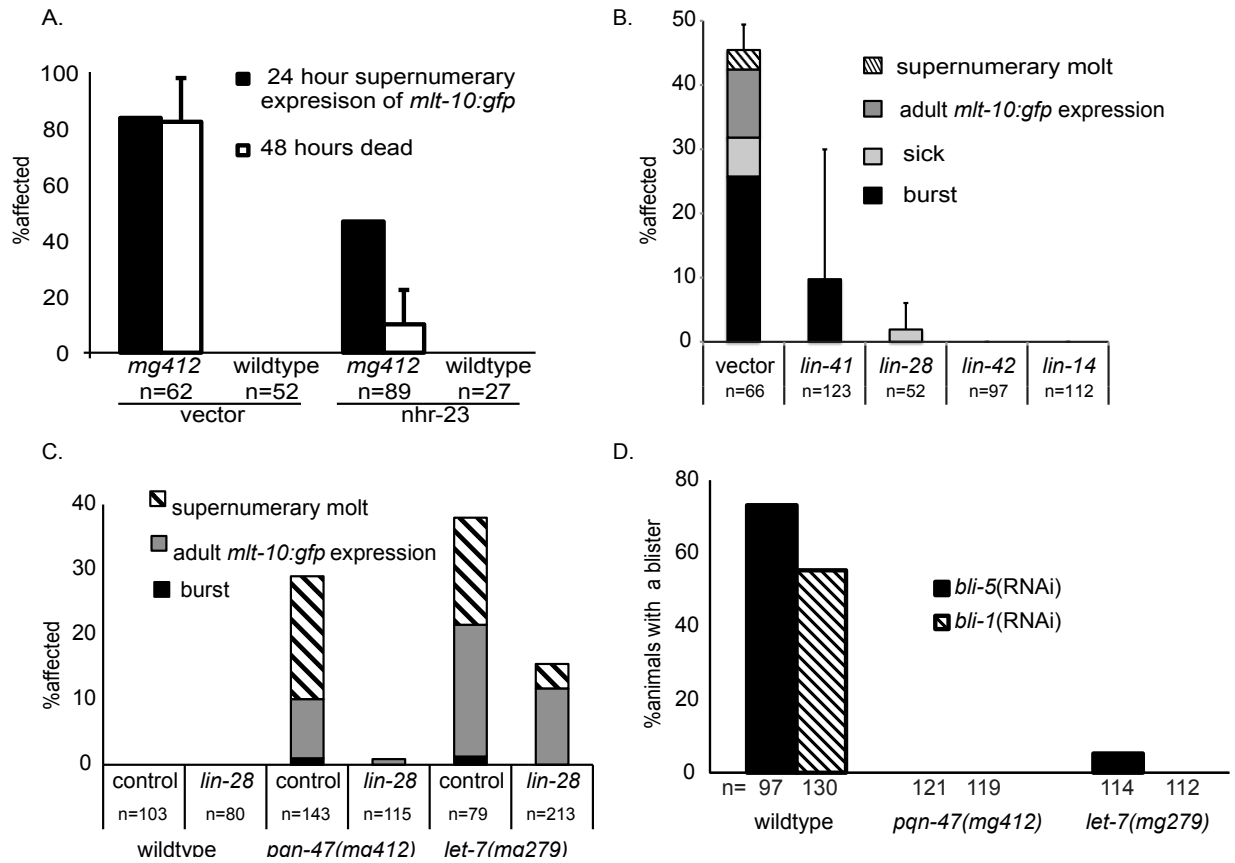


B promoter *pqn-47:gfp*:PEST showing progression through the L1 molt (18hr staged by cuticle sedding)



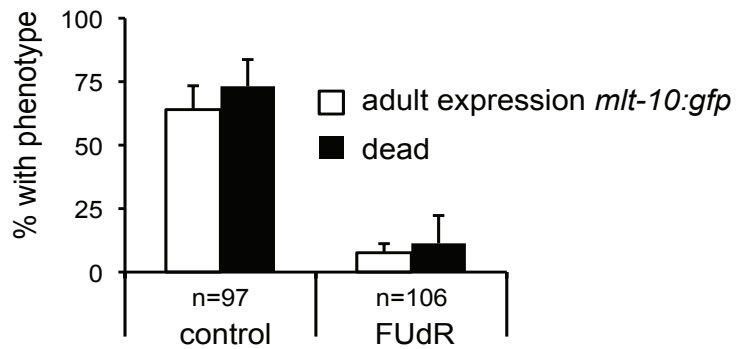
### Supplemental Figure 4. PQN-47 expression throughout the L1 molt

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



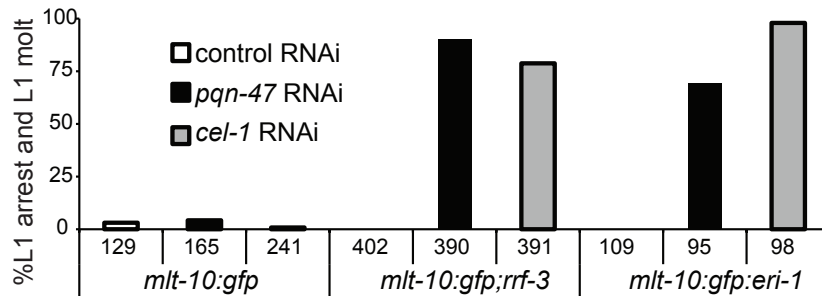
### Supplemental Figure 5. *pqn-47* interactions with heterochronic and molt genes

(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.