Table S1. Viability and fertility of *pup* mutants at 20°C

Genotype	Maternal Gen	Avg. clutch size ± SEM	% Progeny viable	% Progeny sterile
WT	M+Z- F1	260±9	100	0
	M-Z- F2	268±10	100	0
	M-Z- F3	257±4	100	0
pup-1(tm1021)	M+Z- F1	214±19	99	2
	M-Z- F2	188±23^	97	12
	M-Z- F3	177±21^	80	17
pup-2(tm4344)	M+Z- F1	260±12	83	1
	M-Z- F2	212±8^	89	3
	M-Z- F3	211±10^	85	3
pup-1/-2(om129)	M+Z- F1	108±8^*$	80	46
	M-Z- F2	55±7^*$	55	51
	M-Z- F3	43±12^*$	58	55

Experiments were performed at 20°C. N=10-57 complete clutches were counted per genotype. ^ indicates $P<0.05$ compared to WT of the same generation. * indicates $P<0.05$ compared to *pup-1* mutant of the same generation. $ indicates $P<0.05$ compared to *pup-2* mutant of the same generation.

Table S2. Viability and fertility of *pup* M+Z- individuals

Maternal Genotype	Avg. clutch size ± SEM	% viable M+Z-	% Sterile M+Z-
pup(+)/qC1g	95±7	95	0
pup-1(tm1021)/qC1g	99±10	98	0
pup-2(tm4344)/qC1g	121±8	84^	0
pup-1/-2(om129)/qC1g	109±14	87	0

Experiments were performed at 25°C. Clutch size includes the total number of viable and nonviable progeny of three offspring genotypes: *pup/pup*, *pup/qC1g*, and *qC1g/qC1g.* Non-green progeny are *pup/pup* M+Z- offspring of the *pup/qC1* mother. N=12-24 complete clutches were counted per genotype. A one-way ANOVA plus Dunnett's multiple comparison post-ANVOA test indicates that the average clutch size for each mutant is not significantly different from *pup(+)/qC1.* ^ indicates P<0.05 calculated by Z-test compared to *pup(+)/qC1* (*P*<0.05).

Supplementary materials and methods

Comparison of *pup-1(tm1021)* and *pup-1(gg519)*

After we had completed our analysis of *pup-1(tm1021)*, it was reported that another deletion allele, *pup-1(gg519)*, becomes 100% sterile in two generations at 25°C (Spracklin et al. 2017). This report led us to do a side-by-side comparison of the two mutants. We obtained homozygous *pup-1(gg519)* mutant strain from the Kennedy lab and generated a balanced *pup-1(gg519)/qC1gfp* strain. We then analyzed brood sizes and germline phenotypes of M+Z- and M-Z- offspring at 25°C following the same protocol we had used for *pup-1(tm1021)*. Although brood sizes are generally smaller than for *pup-1(tm1021)*, embryonic viability is the same and we were able to maintain the strain for 9-10 generations at 25°C. We also note that these animals did not exhibit the germ cell loss phenotype, but instead appeared to be sterile due to gametogenesis defects (Fig. S2). *pup-3(0)* substantially suppressed the brood size defect and sterility (Fig. S2), as was observed for *tm1021.* We also amplified and sequenced the *pup-2* gene present in the *gg519* strain in order to eliminate the possibility of a *pup-2* mutation that might contribute to the strain phenotype. We did not detect a mutation in *pup-2*. Based on our experience with other *pup* alleles, we suspect that maintaining *gg519* as a homozygote indefinitely at lower culture temperatures led to a gradual reduction in fertility that contributed to the rapid loss of fertility at 25°C as reported (Spracklin et al. 2017).

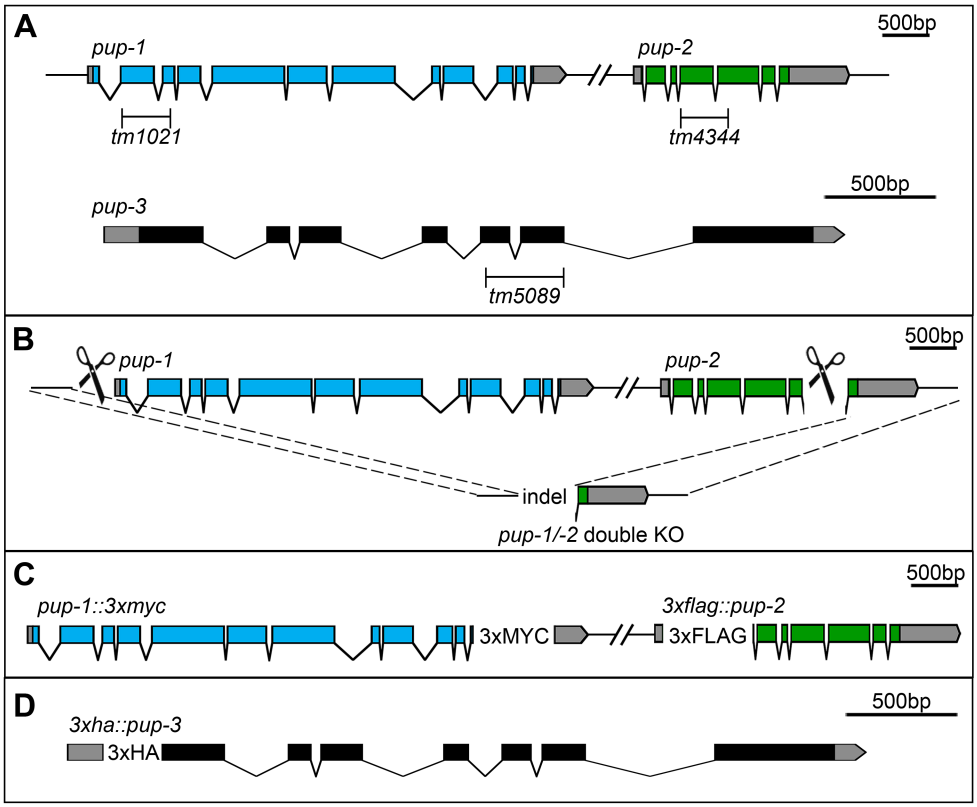
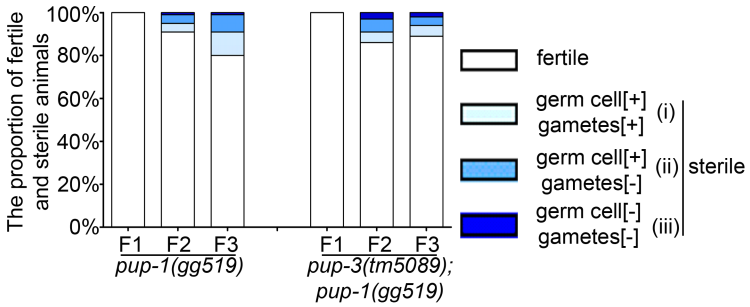


Figure S1. Schematic illustration of the CRISPR-Cas9 strategies used to generate mutations and epitope-tagged strains. Boxes and lines represent exons and introns, respectively. Scissors mark predicted Cas9 cleavage sites. (A) Diagram of *pup-1*, *pup-2*, and *pup-3* intron-exon organization with position of *tm1021*, *tm4344*, and *tm5089* deletion alleles indicated. The 3' end of *pup-1* and 5' end of *pup-2* are located ~5.4 kb apart. (B) Location of CRISPR-Cas9 cut sites relative to *pup-1* and *pup*-2, and structure of the deletion. The *om129* deletion is 14.7 kb. (C) Diagram of the *pup-1 – pup-2* region after addition of epitope tags. We generated strains with only *pup-1* (*omIs8*) or *pup-2* (*omIs7*) containing an epitope tag as well as doubly-tagged strains (EL629). (D) Diagram of *3xha*-tagged *pup-3* gene (*omIs9*). A strain was also generated containing *3xflag* at the N-terminus rather than *3xha* (*omIs10*).



Genotype	Maternal Gen	Avg clutch size±SEM	% Progeny viable
pup-1(gg519)	F1	61 ± 13	90
	F2	32 ± 8*	91
	F3	23 ± 5*	83
pup-3(tm5089); pup-1(gg519)	F1	147 ± 10#%	96
	F2	57 ± 6	90
	F3	37 ± 8	90

Figure S2. *pup-1(tm2021)* and *pup-1(gg519)* mutants have similar phenotypes. Histograms display % fertile and % sterile F1 (M+Z-), F2 (M-Z-), and F3 (M-Z-) adults raised at 25°C. Table provides brood size data and % viable progeny produced by these animals raised at 25°C. Note that *pup-3(0)* partially suppresses the *pup-1(gg519)* phenotype. Statistical significance was calculated by Student t-test. * indicates P<0.05 compared to *pup-1(tm1021)* of the same generation. Number of *pup-1(gg519)* single mutant and *pup-3; pup-1(gg519)* double mutant broods scored, respectively, were: F1 n=15, 16; F2 n=15, 17; F3 n=15, 19. # indicates P<0.05 compared to *pup-3(tm5089)* of the same generation. % indicates P<0.05 compared to *pup-3(tm5089); pup-1(tm1021)* of the of the same generation. See Table 1 and Supplemental text.

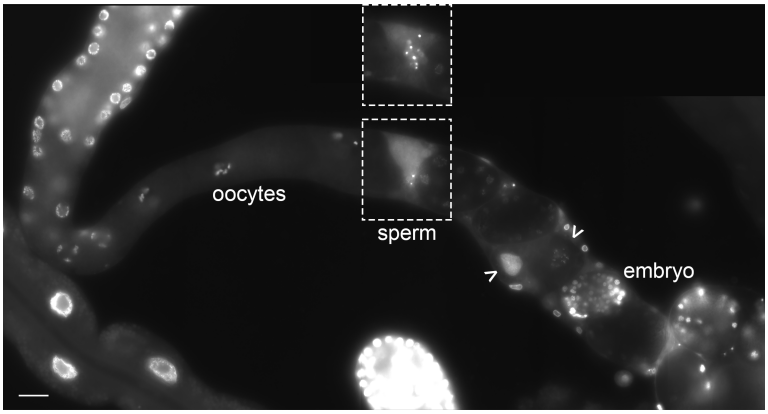


Figure S3. *pup* mutants have polyploid oocytes. Image shows a representative DAPI-stained gonad from a dissected *pup-1/-2(0)* adult with an intact uterus. Polyploid oocytes are visible in the uterus (arrow head). Sperm are located in the spermatheca. Inset, sperm present in a different focal plane. A total 62 adults were examined for the presence of polyploid oocytes in the oviduct versus uterus. Scale bar = 16μm.

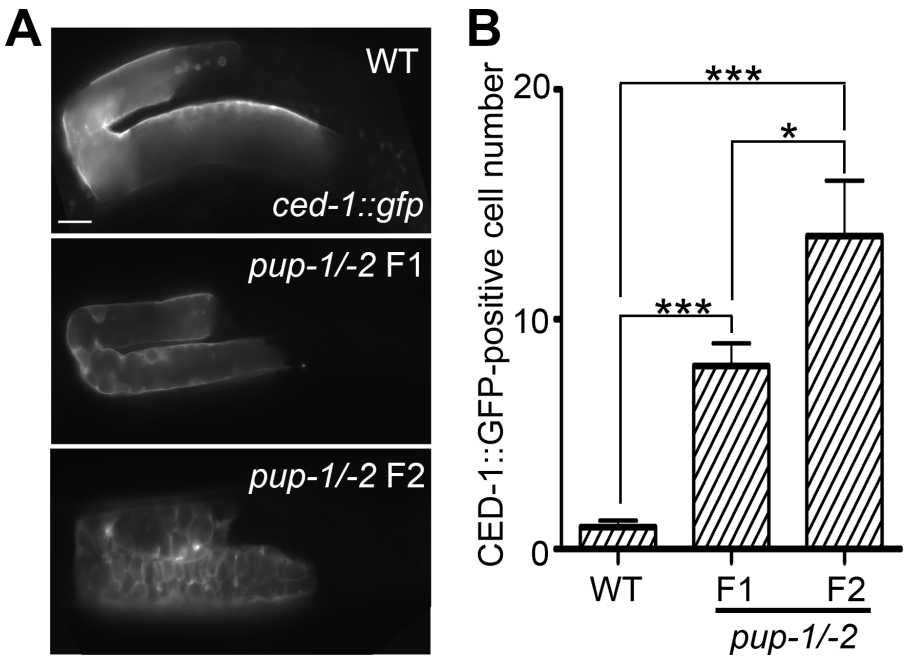


Figure S4. Apoptosis is elevated in *pup-1/-2(0)* gonads. (A) Representative images of *Plim-7ced-1::gfp* expression in the gonad of wild type and *pup-1/-2* F1 and F2 animals raised at 25°C. Scale bar = 16 µm. (B) Quantification of CED-1::GFP-positive cells in wild type (n=25), *pup-1/-2* F1 (n=33) and *pup-1/-2* F2 (n=29) animals. A one-way ANOVA plus Bonferroni's multiple comparison post-hoc test was used. * P<0.05, *** P<0.001.

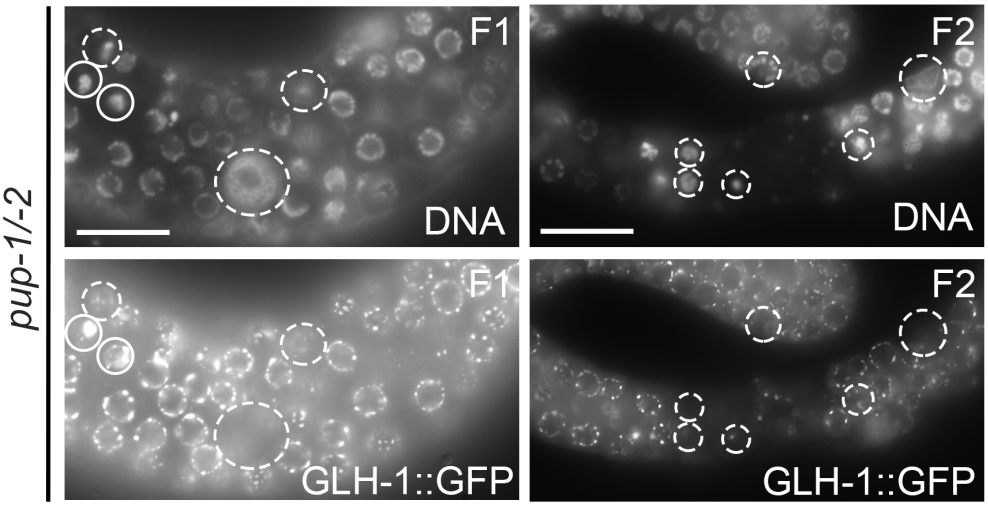


Figure S5. *pup-1/-2(0)* germ cells with absent/reduced P granule expression have abnormal nuclear morphology. Images show germ cells in hermaphrodites raised at 25°C and stained with DAPI to visualize DNA. *pup-1/-2(0)* mutants are the F2 generation. Dashed circles indicate examples of nuclei with little/no GLH-1::GFP expression; solid circles indicate nuclei with abnormal (clumped) GLH-1::GFP distribution. N=32 gonad arms. Scale bar =16 μm.

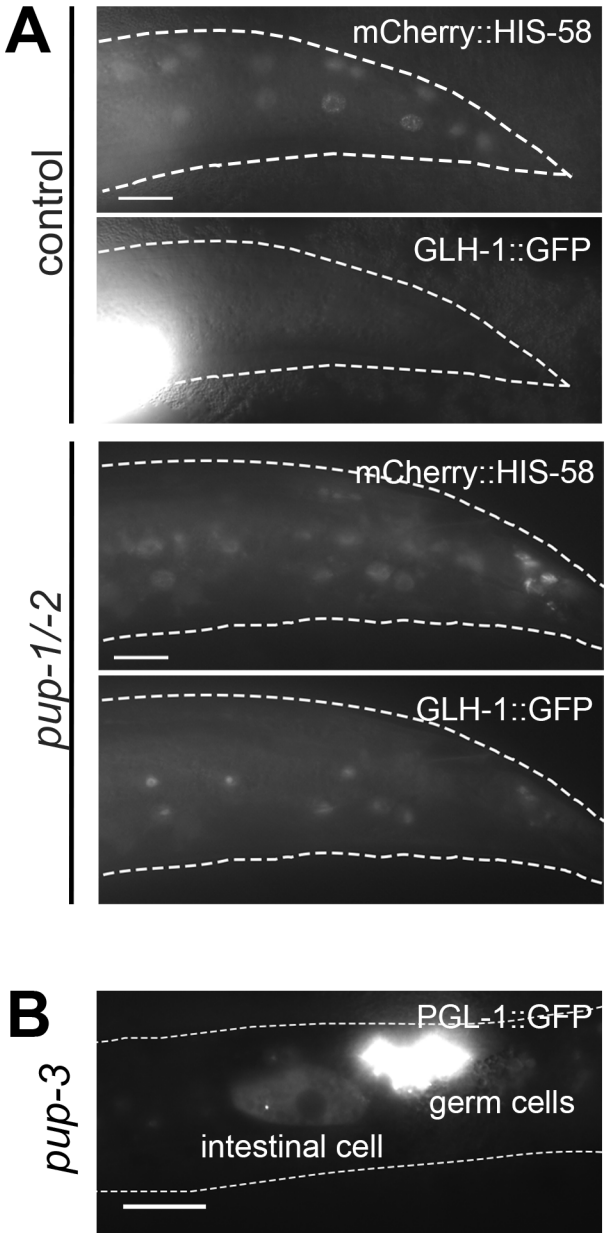


Figure S6. Somatic expression of P granule proteins in *pup-1/-2(0)* and *pup-3(0)* mutants. (A) Expression of GLH-1::GFP in somatic cells in the *pup-1/-2(0)* tail. The strain contains *mCherry::his-58* to visualize chromatin. The control genotype is *pup-1/-2(0)/qC1*. N=26 adults. (B) Expression of PGL-1::GFP in *pup-3(0)* intestinal cells. N=43 adults. Scale bar = 16 μm.

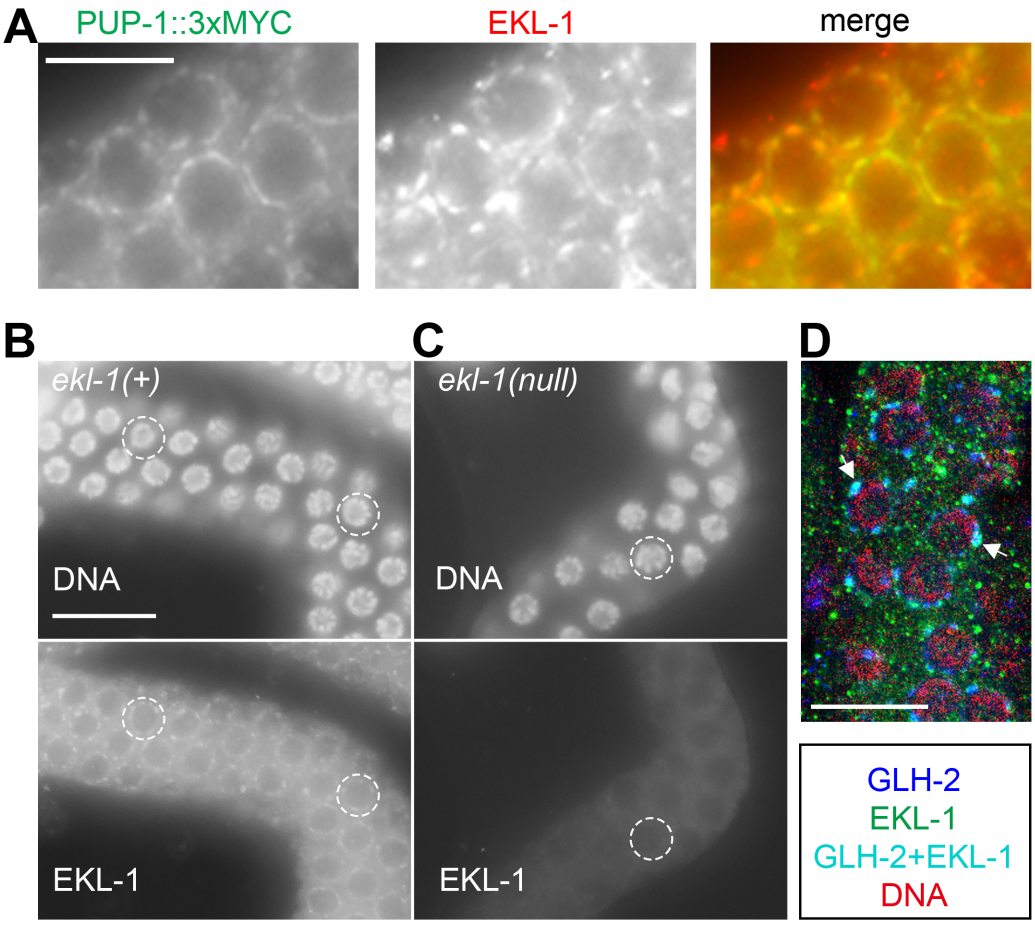


Figure S7. PUP-1 and EKL-1 localize to germline P granules. (A) Co-localization of PUP-1::3xMYC and EKL-1 (n=23 gonad arms). Dissected (B) *ekl-1(+)* and (C) *ekl-1(om83)* gonads immunolabeled with anti-EKL-1. Note perinuclear EKL-1 foci in *ekl-1(+)* and their absence in the *ekl-1(om83)* null mutant (n=31 *ekl-1(+)*, 23 *ekl-1(om83)* adults). (D) Confocal image of *ekl-1(+)* gonad co-labeled with anti-EKL-1 and anti-GLH-2 (n=24). DNA is visualized with DAPI (red). Arrows indicate examples of GLH-2 (blue) co-localizing with EKL-1 (green). Scale bar = 16 μm.

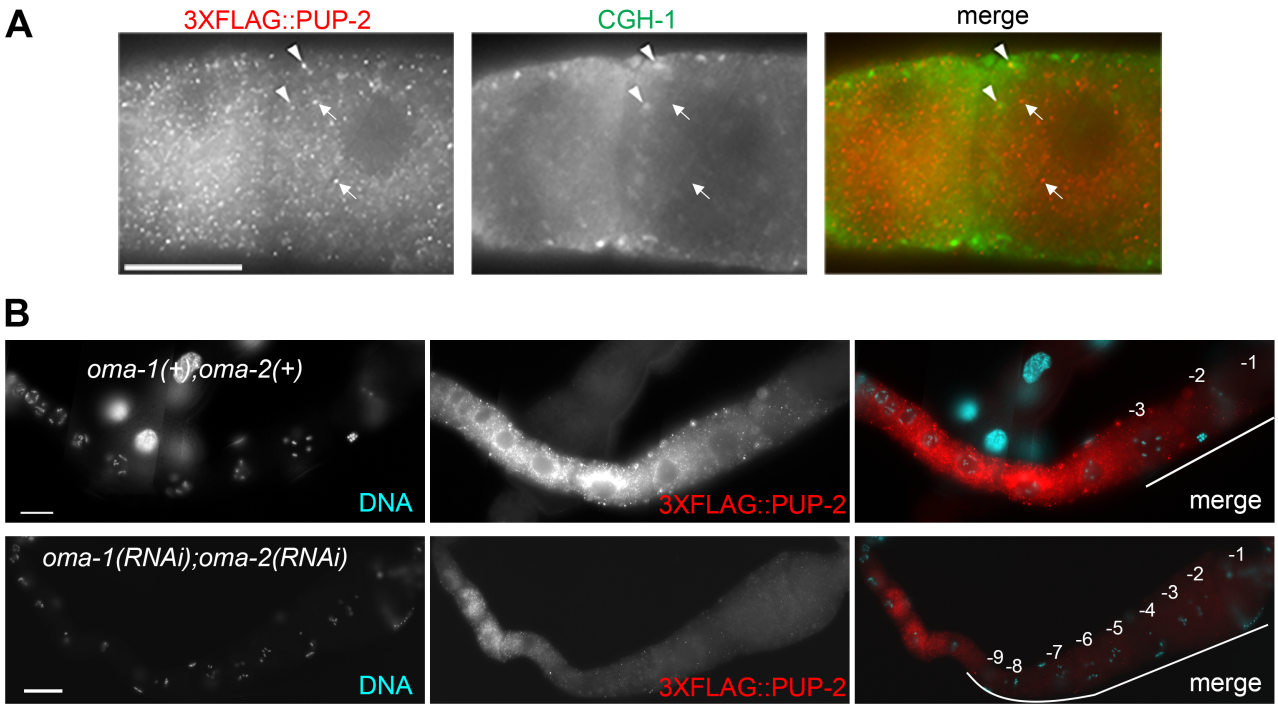


Figure S8. Exploration of PUP-2 expression in developing oocytes. (A) Relative location of 3xFLAG::PUP-2 and CGH-1 foci. Images show dissected germline tissue immunolabeled with anti-FLAG and anti-CGH-1 antibody (n=27 gonad arms). Animals were raised at 25°C. Arrows indicate examples of PUP-2 foci that do not contain CGH-1. Arrowheads indicate rare co-labeling of both proteins. (B) Downregulation of PUP-2 abundance in -1, -2, and -3 oocytes is independent of OMA-1 and OMA-2 activity. *3xflag::pup-2* L1 hermaphrodites were treated with *oma- 1/oma-2* dsRNA at 25°C until adulthood, and their gonads were dissected and immunolabeled with anti-FLAG antibody (n=25 gonad arms). 3xFLAG::PUP-2 abundance drops in late-stage oocytes in control and *oma-1/-2(RNAi)* germ lines. Bars indicate oocytes at equivalent stages in control and *oma-1/-2(RNAi)* gonads. Note that *oma-1/-2(RNAi)* causes accumulation of late-stage oocytes in the oviduct (Detwiler et al., 2001). Scale bar = 16 µm.

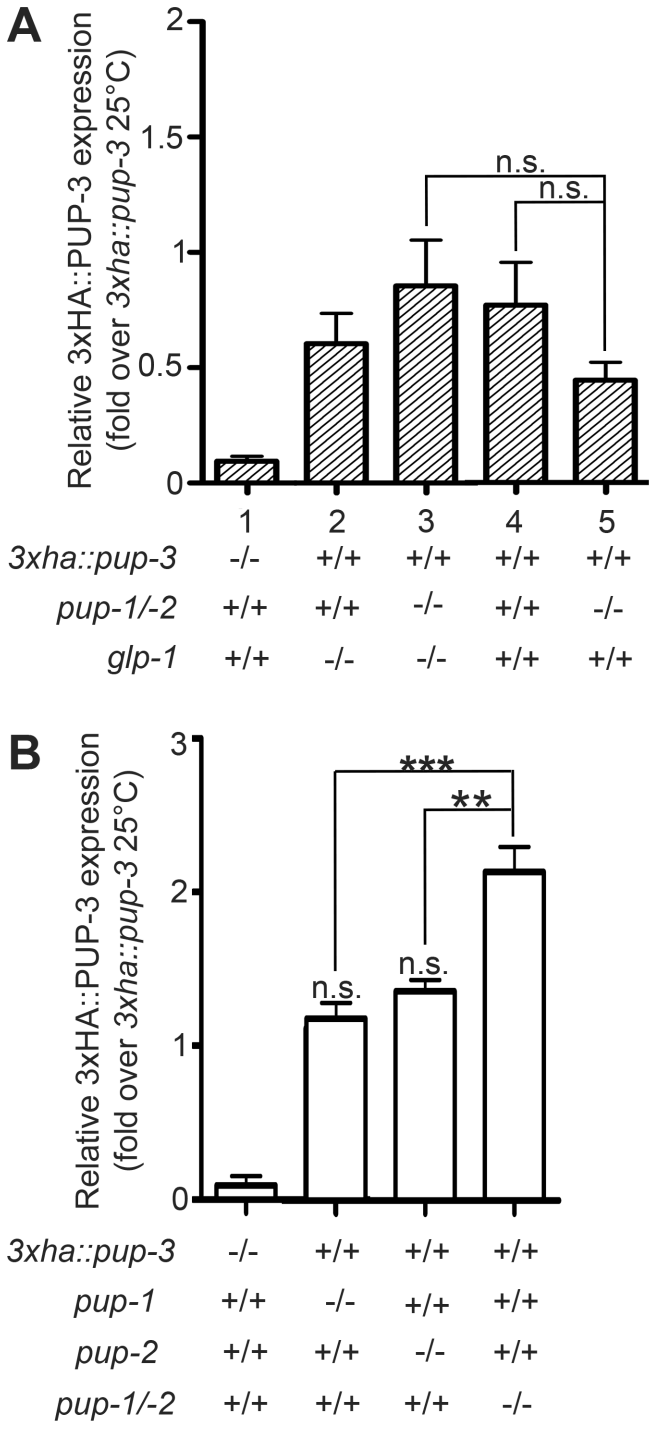


Figure S9. 3xHA::PUP-3 abundance under various conditions. (A) 3xHA::PUP-3 expression is not significantly elevated in *pup-1/-2(0)* adults raised at 20°C. Histogram indicates the abundance of 3xHA::PUP-3 in each strain normalized to control 3xHA::PUP-3 abundance at 25°C (see Fig. 6). *pup-1/-2(om129)* double mutants were assayed in the F2 (M-Z-) generation. N=3 biological replicates. (B) Comparison of 3xHA::PUP-3 expression in *pup-1* and *pup-2* single mutants and *pup-1/-2(0)* double mutant at 25°C. 3xHA::PUP-3 expression in the single mutants is not significantly different from the expression in the *3xha::pup-3* 25°C control (indicated n.s. above each bar). ** P<0.01, *** P<0.001 by one-way Anova and Tukey post-hoc test. N=3 biological replicates.