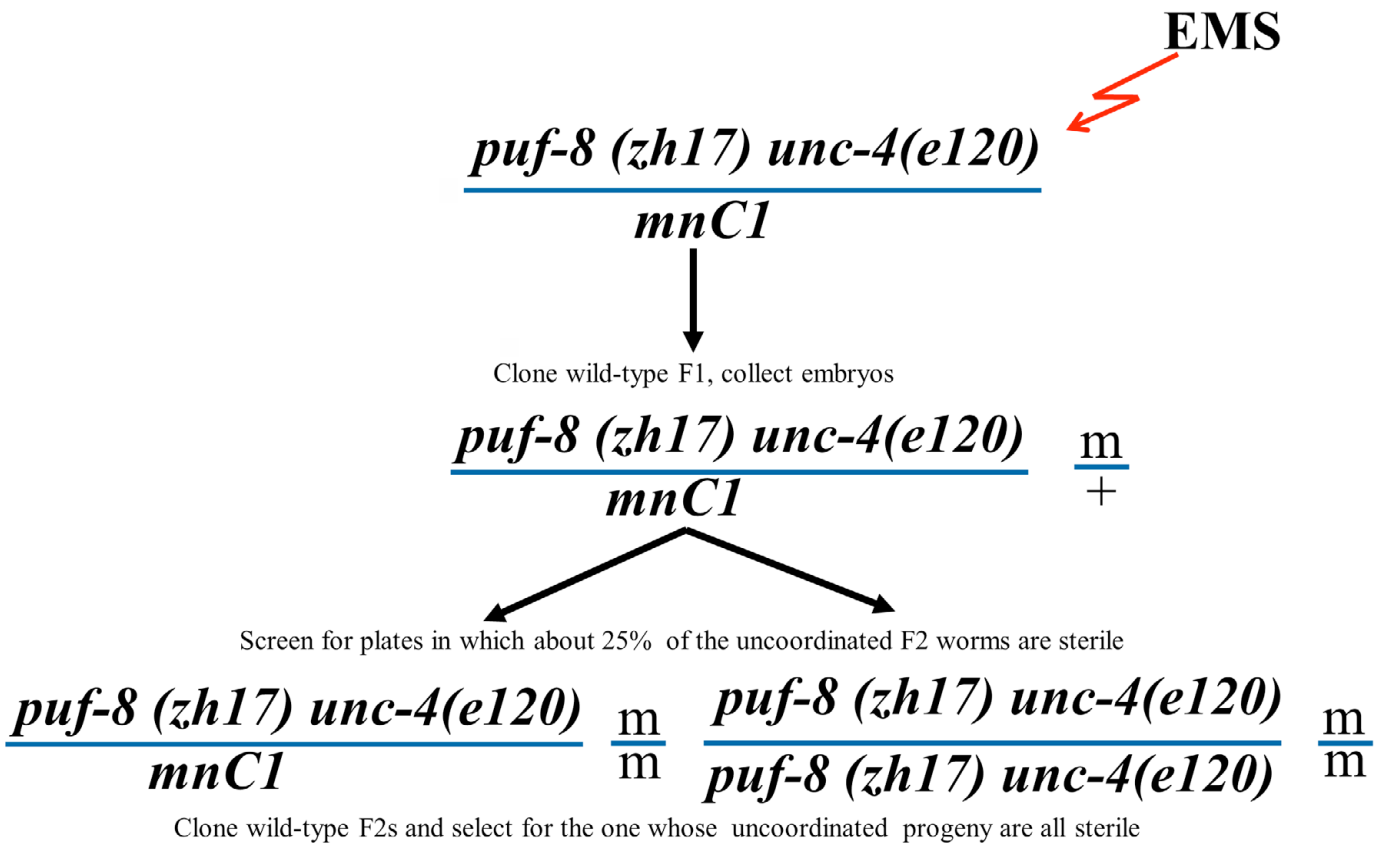
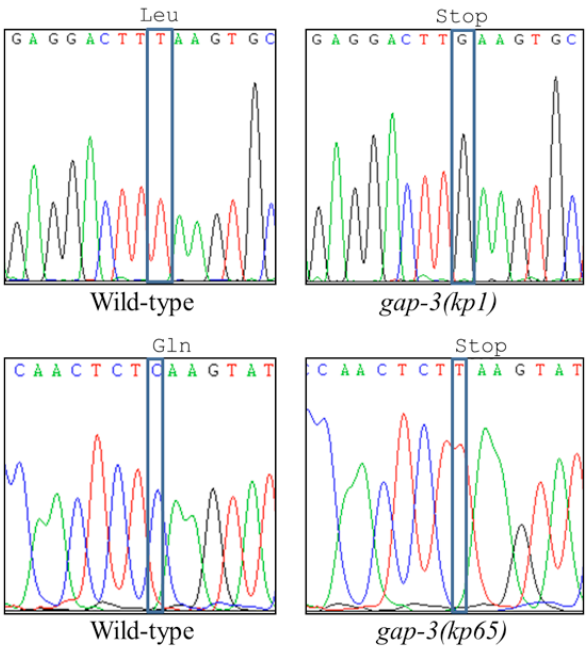


Fig. S1. The genetic screen. In total, 10,639 F1 worms, corresponding to 21,278 haploid genomes, were screened and 71 mutants, designated with allele numbers *kp-1* through *kp-71,* were isolated. Based on the germline phenotype, the mutants were broadly classified into three classes: class I, reduced germline; class II, tumourous germline; and class III, masculinized germline (mog) (see Fig. 1 and Table S1). So far, 37 of these alleles have been tested through complementation analysis; these 37 have been assigned to 29 different complementation groups. This screen also yielded new alleles of already known synthetic mutants of *puf-8.* The Hansen laboratory (University of Calgary, Canada) has isolated an allele of *puf-8* as an enhancer of the tumour phenotype observed in weak gain-of-function alleles of *glp-1* (Racher and Hansen, 2012). Both *glp-1* and three of our class II alleles, *kp16*, *kp31* and *kp43*, are on chromosome III. Therefore, we tested whether these three alleles map to *glp-1* by complementation. All three alleles failed to complement *ar202*, a known allele of *glp-1*, confirming that these three are indeed new alleles of *glp-1*. Mutations in *fbf-1* and *lip-1* have been shown to display synthetic phenotype with *puf-8(–)* (Bachorik and Kimble, 2005; Morgan et al., 2010). Complementation analysis confirmed that *kp8* and *kp12* are alleles of *fbf-1* and *lip-1*, respectively.

A



B

MHSSENTTPNSSTEGPLFLKTHTNPTPPDISPNDVWYHGELNEHDANHLLQGTSPGAFLVRQTGQTRFYLSILDQ
DGYPKHLPIRQLTPPHYFFEGKRFETLRDIVDAYQLAVIPVRKGGIMPINGEIQETKYLCVRRFEQREIDEMNSE
IGDILSVCETDETGWMLGRNETSGSVGIILRSHLEPLITEVADLSDLPYFYDTVSTDMVQYSPIGTFLLRRSSKG
TDTYALLVKTQFDLVEKFLIVGCPSRGFSLAGRPFPTIGHVLTRYCDRAISGGVRLTHAVCVKHQRKSSSRRSTA
EVRWPPFIGNETASMSSSQYDDGLREQKPRRMFRGTSIDTALASSSSFLTSSTAMPSPSTTVSTGIPEHLIDRFH
DEDVNNHPETSSSTTTVAATVALRKSREEKQWKECWLTLSDIPGGSSQLSVFDSMGSKLRQQVDLSTCTLFWLDE
SVFCADGCLFLSPSFPQQQPPTLYLCFRPYTAFLKWIRILRSRTIYQDVPPPMLQGTISVGEPNSQVSILSIEID
*kp65 **
KFRSELLKSDMFYSAQVSLNGVKIGVSNAFAPAGNKGPNEMPVVVIDSKFVIPCIPTCSTNIQLSMNSHSSIGKR
GRPCGVTVTICLNENNESICQSQADSTGFVFRAQRNRCPVLPLDRYKPLLDMIRECPSSILSWPSQVLPSHLKQF
MYTCISHLYALNPQFMSQVIRRIINDILVTSTAEDVFRKDSLATGIITQCLRHLFKTPFDEFLLENAQFSQSLKQ
QNLDSGGGAVELLVNFVDQRLLTIPLATRLLTIAAECAGCRFGDEQHEQHLIKRTLSALLILRVLNPIIFSTLNT
*kp1 **
GIGSQIAKLVQISANSAASQTPTDSLTPAASTIRRMFDRIIFLVNQQQPAESPEDSEIGEVHTEWISCLTYMIGH
SLTIRSATSSEDVHLPLPVLELIRLHHL*

Fig. S2. Mutations in *kp1* and *kp65* alleles disrupt the GTPase-activating domain (GAP) of GAP-3. (**A**) Electropherograms showing the wild-type and mutant sequences around the mutated nucleotide. (**B**) Amino acid sequence of GAP-3. The amino acids changed into STOP (*) codons is highlighted in red. The GAP domain sequence is underlined.

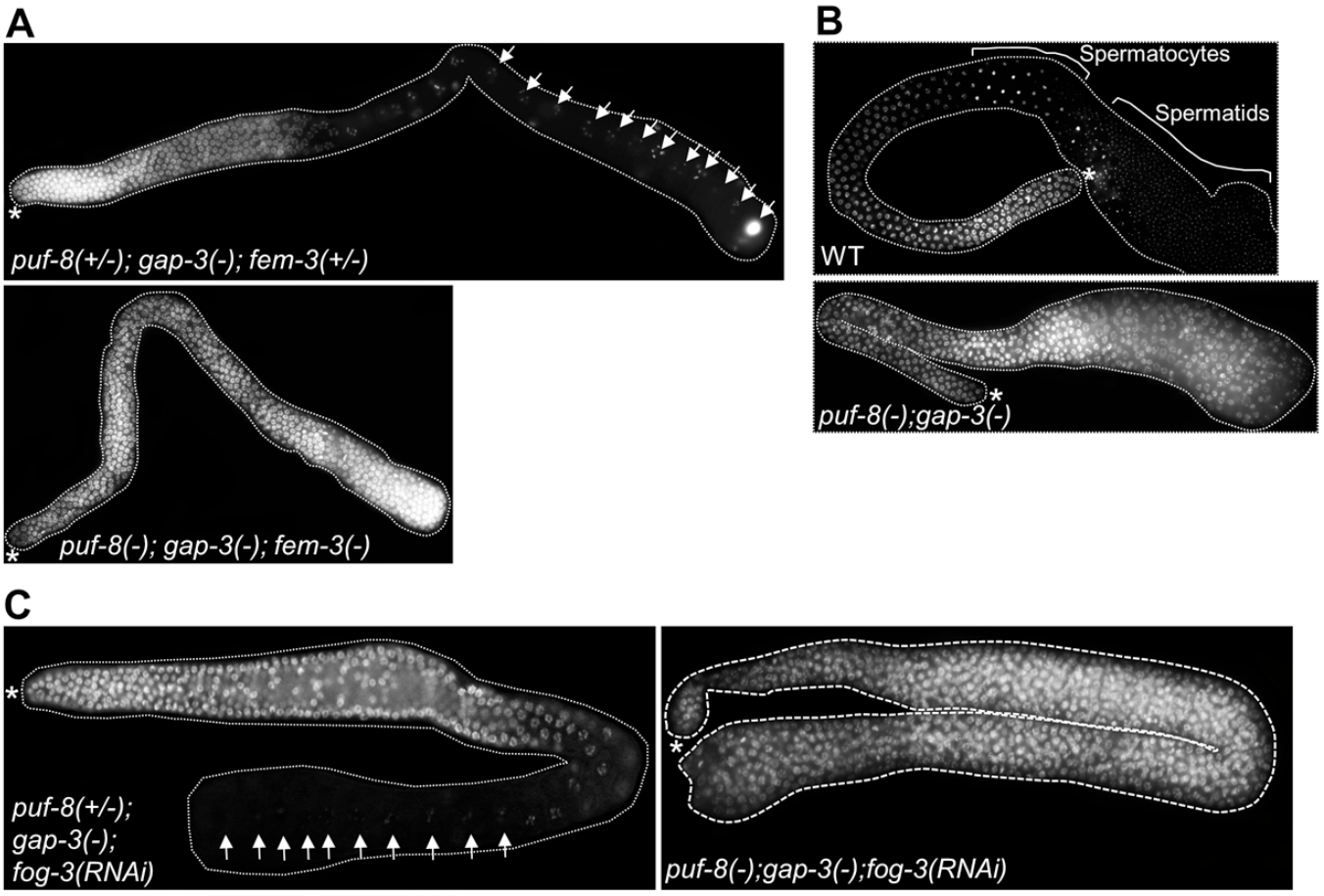


Fig. S3. Overproliferation phenotype of *puf-8(–); gap-3(–)* is not dependent on germ cell sex. (**A-C**) Dissected DAPI-stained gonads of the indicated genotypes are shown. Although the gonads in A and C are from hermaphrodite animals, the ones in B are from males. Arrows in A,C indicate oocyte nuclei. In this, as well as in Fig. S4, the distal part of the gonad is oriented towards the left; the distal end is indicated by an asterisk. Although gametes are present in the feminized [*fem-3(–)* and *fog-3(–)*] hermaphrodites and males heterozygous for *puf-8* [*puf-8(+/–)*], they are completely absent in the *puf-8(–/–)* homozygotes; instead, the proximal region of the *puf-8(–/–)* homozygous gonads are filled with mitotically proliferating cells. Hermaphrodites carrying loss-of-function in *fem-3* or *fog-3* fail to produce sperm, and make only oocytes. Hence these mutants are said to 'feminize' the hermaphrodite germline (Ahringer and Kimble, 1991; Chen et al., 2000).

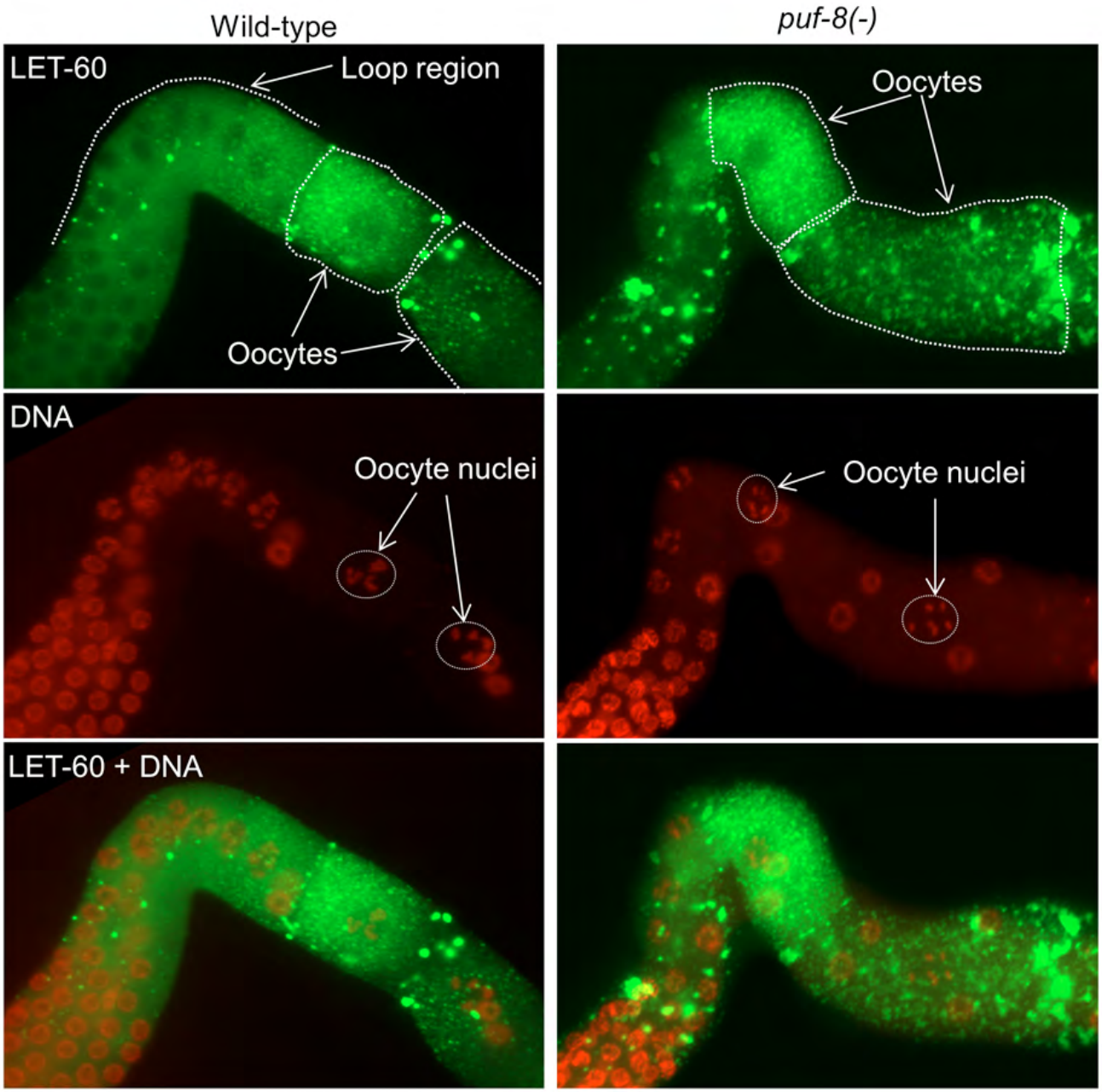


Fig. S4. Expression pattern of LET-60 in the wild-type and *puf-8(–)* gonads. Dissected gonads stained with anti-LET-60 antibodies and DAPI. Only the loop region and the first couple of oocytes are shown. Although the LET-60 level in the oocytes is approximately the same in the wild-type and *puf-8(–)*, the punctate structures stained by the anti-LET-60 antibodies are larger and more numerous in the loop region of *puf-8(–)* gonad when compared with the wild type. See Fig.7 for the LET-60 expression pattern in the distal part of the gonad. Owing to strong LET-60 signals in the region shown above, the duration of exposure for the acquisition of LET-60 images shown here was about one-third of that in the images in Fig. 7.

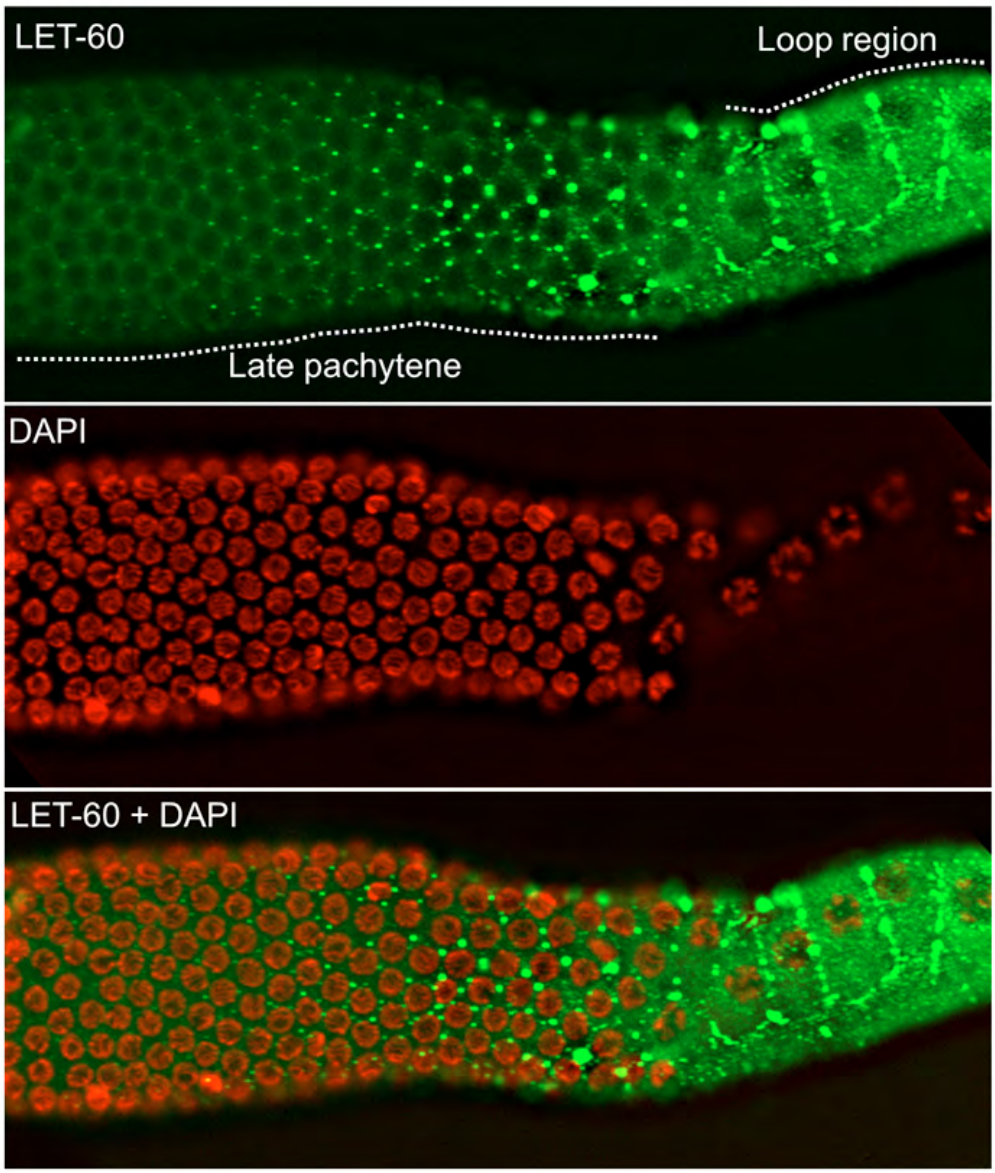


Fig. S5. Expression pattern of LET-60 in the pachytene region of wild-type gonads. Dissected gonad stained with anti-LET-60 antibodies and DAPI. Only the proximal half of the distal gonad is shown. Although the LET-60 signal is fainter in the distal (left) part of the late pachytene region, distinct puncta at the corners of the hexagonal germ cells are noticeable in most cells of the late pachytene region. Membrane localization of LET-60 is more readily observable in the rectangular immature oocytes at the loop region.

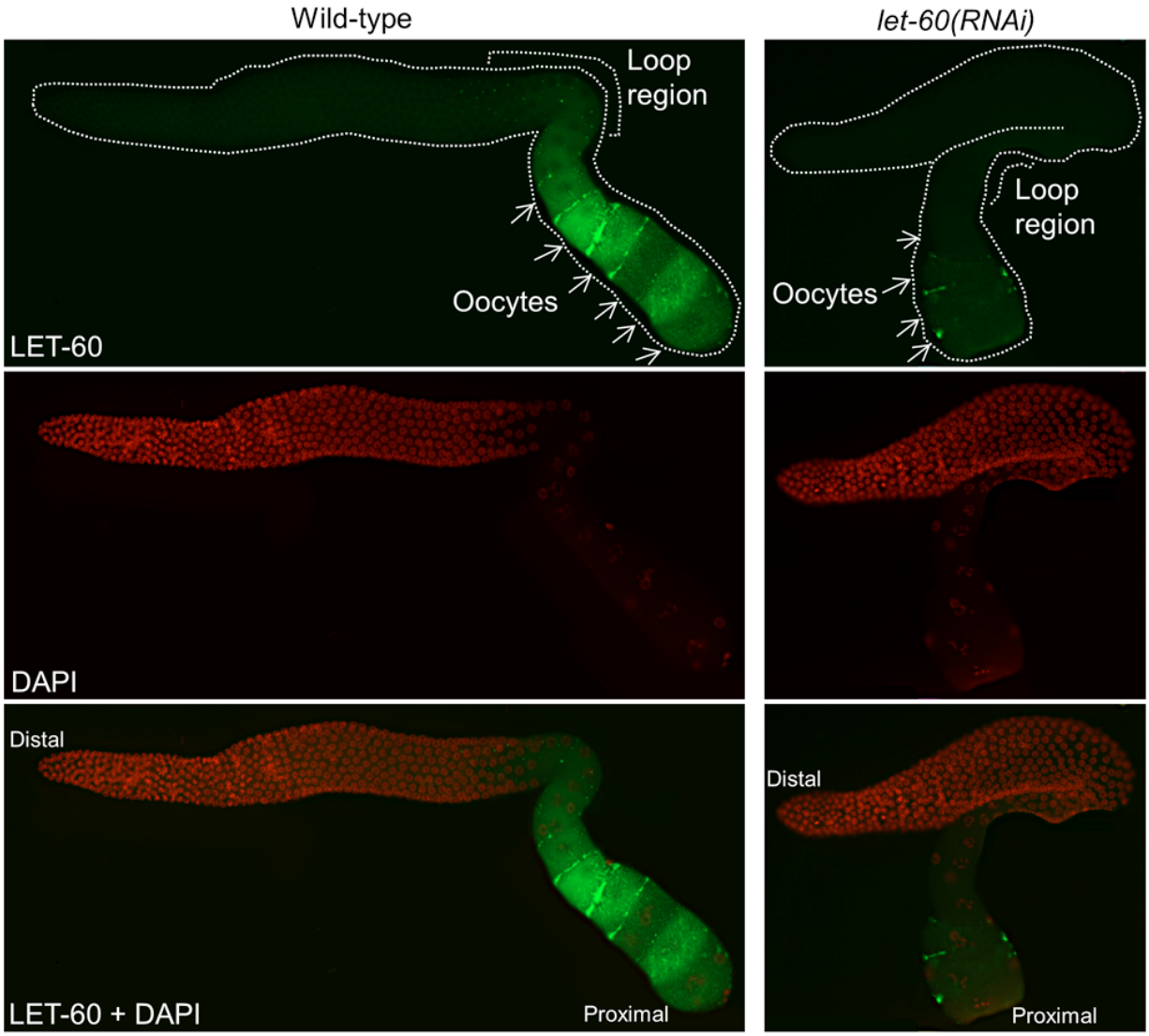


Fig. S6. Anti-LET-60 antibody specifically recognizes LET-60. Dissected gonads stained with anti-LET-60 antibodies and DAPI. Although LET-60 expression in the loop region and oocytes are readily visible in the wild-type gonad, LET-60 is undetectable in the loop region of *let-60(RNAi)* gonad; even in the oocytes, only a weak signal is visible in parts of the cell membrane.

Table S1. Classification of mutants that show synthetic sterility with *puf-8(–)*

Class	Germline defect	Number of mutants*	Penetrance
I	Reduced germline	20 (16)	~100%
II	Tumorous germline	7 (3)	20-100%
III	Mog (masculinization of germline)	10 (10)	15-100%

*Numbers in parentheses are the number of complementation groups for each class.

Table S2. *C. elegans* strains used in this study

3′UTR fusions			
Strain	**Transgene**	**Genotype**	**Reference**
IT285	*pie-1 prom:gfp:H2B:let-60 3'utr*	*unc-119(ed3) III; kpIs(pSV6)*	This study
IT734	*pie-1 prom:gfp:H2B:let-60 3'utr*	*puf-8(ok302) unc-4(e120) / mnC1* II	This study
IT460	*pie-1 prom:gfp:H2B:let-60 (SubC) 3'utr*	*unc-119(ed3) III; kpIs(pSV25)*	This study
ORF fusions			
IT543	*pie-1 prom:gfp:gap-3 ORF:hip-1 3'utr*	*unc-119(ed3) III; kpIs(pSV39)*	This study
IT654	*pie-1 prom:gfp:gap-3 ORF:hip-1 3'utr*	*dpy-5(e61) gap-3(kp-1) I*	This study
IT722	*puf-8 prom:puf-8 ORF:9xHA:gfp:puf-8 3'utr*	*unc-119(ed3) III; kpIs(pAK9)*	This study
Other strains			
Strain	**Genotype**		**Reference**
IT60	*puf-8(zh17) unc-4(e120) / mnC1* II		(Ariz et al., 2009)
JH1500	*puf-8(ok302) unc-4(e120) / mnC1* II		(Subramaniam and Seydoux, 2003)
IT540	*gap-3(kp1) I; puf-8(zh17) unc-4(e120) / mnC1* II		This study
IT770	*gap-3(kp65) I; puf-8(zh17) unc-4(e120) / mnC1* II		This study
IT124	*fbf-1(ok91) fbf-2(q704) puf-8(zh17) unc-4(e120)/mIn1[mIs14 dpy-10(e128)] II.*		This study
IT136	*fbf-1(ok91) fbf-2(q704) unc-4(e120)/mIn1[mIs14 dpy-10(e128)] II.*		This study
IT190	*dpy-5(e61)5 gap-3(kp-1) I; fbf-1(ok91) fbf-2(q704) unc-4(e120)/mIn1[mIs14 dpy-10(e128)] II.*		This study
IT191	*dpy-5(e61) gap-3(kp-1) I; fbf-1(ok91) fbf-2(q704) puf-8(zh17) unc-4(e120)/mIn1[mIs14 dpy-10(e128)] II*		This study
BA606	*spe-6(hc49) unc-25(e156)III; eDp6(III;f)*		(Varkey et al., 1993)
IT208	*dpy-5(e61) gap-3(kp-1)/hT2[dpy-18(h662)] I ; puf-8(zh17)unc-4(e120)/mnc1 II ; +/hT2; +/hT2[bli-4(e937)] III*		This Study
IT769	*gap-3(kp-1)/hT2[dpy-18(h662)] I ; puf-8(zh17)unc-4(e120)/mnc1 II ; spe-6(hc49) unc-25(e156)/hT2[bli-4(e937)] III*		This study

Table S3. Sequences of PCR primers used in this study: primers for generating GFP:GAP-3 fusion

Primer name	Sequence	Description
KS33575	tctggatccactagtatgcactcaagcgagaatac	Forward primer for upstream CDS fragment of *gap-3*
KS3102	cacagccagctgataggcatc	Reverse primer for upstream CDS fragment of *gap-3* from genomic DNA (to include first intron)
KS3576	tctggcgcccaaatgatgaagccgaatgagc	Reverse primer for downstream CDS fragment of *gap-3* from cDNA
KS2092	tttcctcaacaacagccgccg	To check orientation of *gap-3* cDNA fragment in pSV37

Table S4. List of the genes screened by RNAi for rescue of the germline tumour phenotype of *puf-8(–); gap-3(–)*

Sequence	Gene name	Functional description*
ZK1067.1	***let-23***	Encodes an EGF-receptor-family transmembrane tyrosine kinase
ZK792.6	***let-60***	Encodes a member of the GTP-binding RAS protooncogene family
C05D11.4	***let-756***	Encodes fibroblast growth factor (FGF)-like ligand required for progression through early larval development
F36H1.4	***lin-3***	Encodes a member of the EGF family of peptide growth factors
F43C1.2	***mpk-1***	Encodes a mitogen-activated protein (MAP) kinase an ERK ortholog
F26E4.1	*sur-6*	Encodes a regulatory (B) subunit of serine/threonine protein phosphatase 2A (PP2A-B)
F52C12.5	*elt-6*	Erythroid-Like Transcription factor family
F55A8.1	*egl-18*	Encodes a member of the GATA-family of transcription factors
C08C3.1	*egl-5*	Encodes a homeodomain transcription factor, orthologous to Drosophila Abd-B and the vertebrate Hox9-13 proteins
Y54G11A.10	*lin-7*	Encodes a protein that contains a PDZ domain and an L27 domain, two protein interaction domains that likely serve as organizational centers for large macromolecular complexes in polarized cells
F39B2.4	*sur-2*	Encodes a novel protein that is orthologous to the Drosophila and human MED23 mediator subunits
F26F4.3	*rom-1*	Rhomboid (drosophila) related
C48A7.1	*egl-19*	Encodes the pore-forming alpha1 subunit of a voltage-gated calcium channel orthologous to the alpha subunit of mammalian l-type calcium ion channels
M110.5	*dab-1*	Encodes an ortholog of the cytoplasmic adaptor protein DISABLED, required for normal molting and meiotic arrest;
F38H4.9	*let-92*	Encodes a homolog of PP2AC, the catalytic subunit of protein phosphatase 2A (PP2A)
AC7.2	*soc-2*	Encodes a leucine-rich repeat protein; soc-2 functions downstream in the let-60/Ras and egl-15/FGF receptor signaling pathways to positively and negatively regulate signaling through these pathways, respectively
C09H6.2	*lin-10*	Encodes a PDZ and PTB domain-containing protein that is homologous to mammalian Munc interacting proteins (Mint1, OMIM:602414) and is required for polarized protein localization
M01D7.7	*egl-30*	Encodes an ortholog of the heterotrimeric G protein alpha subunit Gq (Gq/G11 class)
R01H10.8	*cnk-1*	Encodes a protein that contains a SAM domain, a PDZ domain, and a PH domain.
C07H6.7	*lin-39*	Encodes a homeodomain protein homologous to the Deformed and Sex combs reduced family of homeodomain proteins

*www.wormbase.org

Core components of RAS/MAPK signaling are shown in bold, and the rest are positive regulators of RAS/MAPK signaling.

Table S5. Sequences of PCR primers used in this study: primers for generating GFP-3' UTR fusions

Primer name	Sequence	Description
KS2833	tctgggccctcagcatcgtgagcgtcacg	Forward primer for *let-60*
KS2834	tctgggccctcatgctccgatggcgaggtc	Reverse primer for*let-60*
KS3437	ggatcctgtgtgtgtgtgtgtgtgtgtgtaacggaacgaccggcagat	Reverse for upstream fragment for substitution C
KS3438	ggatcctgtgtgtgtgtgtgtgtgtgtgtatatagatacaattagacccc	Forward for downstream fragment for substitution C

Table S6. Sequences of PCR primers used in this study: primers used for testing PUF-8 : *let-60* 3' UTR interaction

Primer name	Sequence	Description
Primers used for preparing in vitro transcription templates used in the mobility shift assays		
KS2808	ccaaggggttatgctaggaag	Common forward primer for synthesizing in-vitro transcription templates of the fragments of *let-60* 3'UTR
KS3295	tcgaagagtatgtagaggcgg	Reverse primer for fragment 1
KS3296	ccgcctctacatactcttcga	Forward primer for fragment 2
KS3098	ggccaattttggaacgagg	Reverse primer for fragment 2
KS3297	gcatccgaacccctcctcg	Forward primer for fragment 3
KS3299	aacggaacgaccggcagatgc	Reverse primer for fragment 3
KS3298	tggcctcatctattttcag	Forward primer for fragment 4
KS3100	gaatgcaatacgagggagc	Reverse primer for fragment 4
KS3300	gctccctcgtattgcattc	Forward primer for fragment 5
KS3097	cctcatctttcgcttagccc	Common forward primer for upstream fragment for substitutions in fragment 4
KS3433	ggatcctgtgtgtgtgtgtgtgtgtgtgtgtgtcgaggaaggggaataaagcatc	Reverse primer for upstream fragment of substitution A
KS3434	ggatcctgtgtgtgtgtgtgtgtgtgtgtgttctgccggtcgttccgttt	Forward primer for downstream fragment of substitution A
KS3435	ggatcctgtgtgtgtgtgtgtgtgtgtgtgtcttctgaaaatagatgaggcc	Reverse primer for upstream fragment of substitution B
KS3436	ggatcctgtgtgtgtgtgtgtgtgtgtgtgtcctctcatttttgtcaag	Forward primer for downstream fragment of substitution B
KS3437	ggatcctgtgtgtgtgtgtgtgtgtgtgtgtaacggaacgaccggcagat	Reverse primer for upstream fragment of substitution C
KS3438	ggatcctgtgtgtgtgtgtgtgtgtgtgtgtatatagatacaattagacccc	Forward primer for downstream fragment of substitution C
KS3432	taatacgactcactatagggagaagatgctttattccccttcctcg	Common forward primer, with T7 promoter sequence, for synthesizing template for in-vitro transcription of substituted Fragment 4

KS3441	taaaatgtagaggaatagag	Common reverse primer for synthesizing template for in vitro transcription of substituted Fragment 4
Primers for PUF-8:9×HA:GFP transgene construct pAK9		
KS3788	tctactagttacccatacgacgtcccagactacgcctatccgtatgatgttccggattatgct	Forward primer amplify HA sequence for pAK8
KS3789	tctactagtcccgggggcgtagtctgggacgtcgtatgggtaagcataatccggaacatcatacggata	Reverse primer for HA sequence for pAK8
KS3791	tctcccgggtacccatacgacgtcccagactacgcctatccgtatgatgttccggattatgct	Forward primer for HA sequence for pAK10
KS3792	tctcccggggcgcgcggcgtagtctgggacgtcgtatgggtaagcataatccggaacatcatacggata	Reverse primer for HA sequence for pAK10
KS3794	tctgcgcgctacccatacgacgtcccagactacgcctatccgtatgatgttccggattatgct	Forward primer for HA sequence for pAK11
KS3795	tctgcgcgcagcgtagtctgggacgtcgtatgggtaagcataatccggaacatcatacggata	Reverse primer for HA sequence for pAK11
Primers for reverse transcription and RT-PCR		
KS2096	gcattagcggccgcgaaattaatacgactcactatagggaga(t)21 v	Anchored oligo dT primer
KS3297	gcatccgaacccctcctcg	Forward for *let-60* mRNA
KS3299	aacggaacgaccggcagatgc	Reverse for *let-60* mRNA
KS1102	tctaagcttatggctcaaacaaagccgat	Forward for *pie-1* mRNA
KS2495	aatctgacgacgattcgagc	Reverse for *pie-1* mRNA