

## SUPPLEMENTARY INFORMATION

### SUPPLEMENTARY MATERIALS AND METHODS

#### **RNAi experiments and germ cell counts:**

RNAi by bacterial feeding was performed as described (Timmons et al., 2001).

*lin-12* RNAi [Ahringer clone sjj\_R107.8; (Kamath et al., 2003)] in N2, *rrf-1(pk1417)* and *rrf-3(pk1426)* strains: Animals were fed control (L4440) or *lin-12* RNAi for two generations: after L1 synchronization by hatch-off, early adults (prior to egg-laying) were transferred to new RNAi plates overnight and then removed to limit progeny that were then scored as early adults. RNAi experiments evaluated for vulval morphology were scored in one generation as adults after L1 hatch-off onto RNAi plates.

*hlh-2* RNAi: Two *hlh-2* RNAi clones were created according to (Chesney et al., 2009).

Synchronized early third larval stage worms (L3) were fed control (L4440) or *hlh-2* RNAi clones and scored as fourth larval stage worms (L4) for *lag-2* DTC expression analysis or as early adults to count the number of nuclei in the proliferative zone. Similarly to (Chesney et al., 2009), two *hlh-2* RNAi clones showed similar effects and were analyzed together.

**Germ nuclei counts:** See main text for methods used to determine germ nuclei counts in the proliferative zone.

**TABLE S1****A. Strains used in this study:**

<b>Strain</b>	<b>Genotype</b>	<b>Reference</b>
N2	Wild-type	(Brenner, 1974)
CB1372	<i>daf-7(e1372)</i>	(Swanson and Riddle, 1981)
DR40	<i>daf-1(m40)</i>	(Swanson and Riddle, 1981)
GR1269	<i>daf-7(e1372); daf-3(e1376)</i>	(da Graca et al., 2004)
GC1147	<i>daf-1(m40); daf-3(e1376)</i>	(Dalfo et al., 2012)
GC1149	<i>daf-5(e1386); daf-1(m40)</i>	(Dalfo et al., 2012)
GC832	<i>glp-1(e2141)</i>	(Hutter and Schnabel, 1994)
PD8488	<i>rrf-1(pk1417)</i>	(Sijen et al., 2001)
NL2099	<i>rrf-3(pk1426)</i>	(Simmer et al., 2003)
NF2168	<i>tkIs11 [Pmig-24::Venus ]</i>	(Tamaï and Nishiwaki, 2007)
GR1311	<i>daf-3(mgDf90)</i>	(Patterson et al., 1997)
GC909	<i>rrf-1(pk1417); glp-1(e2141)</i>	(Korta et al., 2012)
GC1172	<i>xnSi1[Pmex-5::GFP-PH:: nos-2(3')] II; unc-119(ed3); nals37[pGC457 Plag-2(3kb)::mCherry-PH::let-858(3'); unc-119(+)]</i>	(Chihara and Nance, 2012); This study
GC1412	<i>naSi8 [pGC680 Plag-2(3kb)::GFP-PH::let-858(3')] II</i>	This study
GC1418	<i>daf-7(e1372); naSi8 [pGC680 Plag-2(3kb)::GFP-PH::let-858(3')] II</i>	This study
GC1419	<i>daf-7(e1372); daf-3(e1376); naSi8 [pGC680 Plag-2(3kb)::GFP-PH::let-858(3')] II</i>	This study
GC1420	<i>daf-1(m40); xnSi1[Pmex-5::GFP-PH:: nos-2(3')] II; unc-119(ed3); nals37[pGC457 Plag-2(3kb)::mCherry-PH::let-858(3'); unc-119(+)]</i>	This study
GC1421	<i>daf-5(e1386); daf-1(m40); xnSi1[Pmex-5::GFP-PH:: nos-2(3')] II; unc-119(ed3); nals37[pGC457 Plag-2(3kb)::mCherry-PH::let-858(3'); unc-119(+)]</i>	This study
GC1347	<i>unc-119(ed3); nals81[pGC630 Plag-2(2kb)::GFP-PH::let-858(3'); unc-119(+)]</i>	This study
GC1349	<i>daf-7(e1372); unc-119(ed3); nals81[pGC630 Plag-2(2kb)::GFP-PH::let-858(3'); unc-119(+)]</i>	This study
GC1359	<i>unc-119(ed3); nals84[pGC642 Plag-2(1kb)::GFP-PH::let-858(3'); unc-119(+)]</i>	This study
GC1362	<i>unc-119(ed3); nals87[pGC643 Plag-2(0.5kb)::GFP-PH::let-858(3'); unc-119(+)]</i>	This study
GC1422	<i>daf-7(e1372); daf-3(e1376); unc-119(ed3); nals81[pGC630 Plag-2(2kb)::GFP-PH::let-858(3'); unc-119(+)]</i>	This study

GC1423	<i>daf-7(e1372); unc-119(ed3); nals84[pGC642 Plag-2(1kb)::GFP-PH::let-858(3'); unc-119(+)]</i>	This study
GC1424	<i>daf-7(e1372); daf-3(e1376); unc-119(ed3); nals84[pGC642 Plag-2(1kb)::GFP-PH::let-858(3'); unc-119(+)]</i>	This study
GC1425	<i>daf-7(e1372); unc-119(ed3); nals87[pGC643 Plag-2(0.5kb)::GFP-PH::let-858(3'); unc-119(+)]</i>	This study
GC1426	<i>daf-7(e1372); daf-3(e1376); unc-119(ed3); nals87[pGC643 Plag-2(0.5kb)::GFP-PH::let-858(3'); unc-119(+)]</i>	This study
GC1427	<i>unc-119(ed3); nals96[pGC644 mutPlag-2(3kb)::GFP-PH::let-858(3'); unc-119(+)]</i>	This study
GC1429	<i>naSi8 [pGC680 Plag-2(3kb)::GFP-PH::let-858(3')] II; unc-119(ed3); nals96[pGC644 mutPlag-2(3kb)::GFP-PH::let-858(3'); unc-119(+)]</i>	This study
GC1430	<i>daf-7(e1372); naSi8 [pGC680 Plag-2(3kb)::GFP-PH::let-858(3')] II; unc-119(ed3); nals96[pGC644 mutPlag-2(3kb)::GFP-PH::let-858(3'); unc-119(+)]</i>	This study
GC1431	<i>unc-119(ed3) ;nals98[pGC681 Plag-2(405bp)::GFP-PH::let-858(3'); unc-119(+)]</i>	This study
GC1433	<i>daf-7(e1372); unc-119(ed3); nals98[pGC681 Plag-2(405bp)::GFP-PH::let-858(3'); unc-119(+)]</i>	This study
GC1434	<i>daf-7(e1372); daf-3(e1376); unc-119(ed3); nals98[pGC681 Plag-2(405bp)::GFP-PH::let-858(3'); unc-119(+)]</i>	This study
GC1435	<i>unc-119(ed3); nals100[pGC682 Plag-2(1kbΔDBS)::GFP-PH::let-858(3'); unc-119(+)]</i>	This study
GC1437	<i>daf-7(e1372); unc-119(ed3); nals100[pGC682 Plag-2(1kbΔDBS)::GFP-PH::let-858(3'); unc-119(+)]</i>	This study
GC1438	<i>daf-7(e1372); daf-3(e1376); unc-119(ed3); nals100[pGC682 Plag-2(1kbΔDBS)::GFP-PH::let-858(3'); unc-119(+)]</i>	This study
GC1439	<i>unc-119(ed3); nals102[pGC683 Plag-2(1kbΔCons)::GFP-PH::let-858(3'); unc-119(+)]</i>	This study
GC1443	<i>daf-7(e1372); unc-119(ed3); nals102[pGC683 Plag-2(1kbΔCons)::GFP-PH::let-858(3'); unc-119(+)]</i>	This study
GC1445	<i>daf-7(e1372); daf-3(e1376); unc-119(ed3);nals102[pGC683 Plag-2(1kbΔCons)::GFP-PH::let-858(3'); unc-119(+)]</i>	This study
GC1447	<i>unc-119(ed3); nals106[pGC684 Plag-2(1kbΔDBS+ΔCons)::GFP-PH::let-858(3'); unc-119(+)]</i>	This study

GC1450	<i>daf-7(e1372); unc-119(ed3); nals106[pGC684 Plag-2(1kbΔDBS+ΔCons)::GFP-PH::let-858(3'); unc-119(+)]</i>	This study
GC1451	<i>daf-7(e1372); daf-3(e1376); unc-119(ed3); nals106[pGC684 Plag-2(1kbΔDBS+ΔCons)::GFP-PH::let-858(3'); unc-119(+)]</i>	This study
GC1452	<i>daf-7(e1372); xnSi1[Pmex-5::GFP-PH:: nos-2(3')] II; unc-119(ed3); nals37[pGC457 Plag-2(3kb)::mCherry-PH::let-858(3'); unc-119(+)]</i>	This study
GC1453	<i>daf-1(m40); daf-3(e1376); xnSi1[Pmex-5::GFP-PH:: nos-2(3')] II; unc-119(ed3); nals37[pGC457 Plag-2(3kb)::mCherry-PH::let-858(3'); unc-119(+)]</i>	This study

## B. Plasmids used in this study:

Plasmid	Description	Reference/Construction
pPD129.36	Control RNAi (L4440)	(Timmons and Fire, 1998)
sjj_R107.8	<i>lin-12(RNAi)</i>	(Kamath et al., 2003)
pGC457	<i>Plag-2(3kb)::mCherry-PH::let-858(3')</i>	GFP-let-858 was cut out from pPD117.01 using AgeI/Apal and inserted into pJK590. The resulting plasmid was then cut with SmaI/SpeI and ligated with mCherry-PH, which was cut with SpeI from pDC05 (Chihara and Nance, 2012).
pGC630	<i>Plag-2(2kb)::GFP-PH::let-858(3')</i>	A 2kb fragment of the <i>lag-2</i> promoter was amplified from pGC457 using primers GCo1928 and GCo1907. A fragment containing GFP-PH was amplified using primers GCo1909 and GCo1910. The 3' UTR of <i>let-858</i> was amplified from pGC399 (McGovern et al., 2009) using GCo1911 and GCo1912. All the pieces were ligated into pJN566

		(Armenti et al., 2014), digested with Pmel, using Gibson assembly.
pGC642	<i>Plag-2(1kb)::GFP-PH::let-858(3')</i>	A 1kb fragment of the <i>lag-2</i> promoter was amplified from pGC457 using primers GCo1930 and GCo1907. GFP-PH was amplified from pGC630 using GCo1909 and GCo1910. The 3' UTR of <i>let-858</i> was amplified from pGC457 using GCo1911 and GCo1912. All the pieces were ligated into pJN566, digested with Pmel, using Gibson assembly.
pGC643	<i>Plag-2(0.5kb)::GFP-PH::let-858(3')</i>	A 0.5kb fragment of the <i>lag-2</i> promoter was amplified from pGC457 using primers GCo1931 and GCo1907. GFP-PH was amplified from pGC630 using GCo1909 and GCo1910. 3' UTR of <i>let-858</i> was amplified from pGC457 using GCo1911 and GCo1912. All the pieces were ligated into pJN566, digested with Pmel, using Gibson assembly.
pGC644	<i>mutPlag-2(3kb)::mCherry-PH::let-858(3')</i>	pGC457 was mutagenized using site-directed mutagenesis with Pfu polymerase. 3 pairs of primers were used: GCo1866 and GCo1867, GCo1868 and GCo1869, GCo1870 and GCo1871.
pGC680	<i>Plag-2(3kb)::GFP-PH::let-858(3')</i>	A 3kb fragment of the <i>lag-2</i> promoter was amplified from pGC457 using primers GCo1906 and

		GCo1907. GFP-PH was amplified from pGC630 using GCo1909 and GCo1910. 3' UTR of <i>let-858</i> was amplified from pGC457 using GCo1911 and GCo1912 primers. All the pieces were ligated into pJN566, digested with PmeI, using Gibson assembly.
pGC681	<i>Plag-2(450bp)::GFP-PH::let-858(3')</i>	The vector was amplified from pGC642 using primers GCo2149 and GCo2147 and self-ligated using T4-DNA ligase.
pGC682	<i>Plag-2(1kbΔDBS)::GFP-PH::let-858(3')</i>	The vector was amplified from pGC642 using primers GCo2145 and GCo2146 and was ligated with gBlock GCo2383 using Gibson assembly.
pGC683	<i>Plag-2(1kbΔCons)::GFP-PH::let-858(3')</i>	The vector was amplified from pGC642 using primers GCo2145 and GCo2146 and was ligated with gBlock GCo2384 using Gibson assembly.
pGC684	<i>Plag-2(1kbΔDBS+ΔCons)::GFP-PH::let-858(3')</i>	The vector was amplified from pGC642 using primers GCo2145 and GCo2146 and was ligated with gBlock GCo2385 using Gibson assembly.
GHUC-1	<i>Plag-2(600bp) prey</i>	600 bp upstream of the <i>lag-2</i> ATG was purchased as a gBlock ( <i>Lag2 wt</i> ), digested and cloned between the NotI and EcoRI restriction sites of the GHUC reporter vector (see below), placing the fragments upstream of the HIS3-GFP cassette. See additional notes regarding

		B1H vectors in D below.
GHUC-2	<i>Plag-2ΔDBS</i> prey	600 bp upstream of the <i>lag-2</i> ATG was amplified from pGC682 using primers <i>Lag2 600 5p</i> and <i>Lag2 600 3p</i> listed below. These were digested and cloned between the NotI and EcoRI restriction sites of the GHUC reporter vector. See additional notes regarding B1H vectors in D below.
GHUC-3	<i>Plag-2ΔCons</i> prey	600 bp upstream of the <i>lag-2</i> ATG was amplified from pGC683 using primers <i>Lag2 600 5p</i> and <i>Lag2 600 3p</i> listed below. These were digested and cloned between the NotI and EcoRI restriction sites of the GHUC reporter vector. See additional notes regarding B1H vectors in D below.
GHUC-4	<i>Plag-2Δboth</i> prey	600 bp upstream of the <i>lag-2</i> ATG was amplified from pGC684 using primers <i>Lag2 600 5p</i> and <i>Lag2 600 3p</i> listed below. These were digested and cloned between the NotI and EcoRI restriction sites of the GHUC reporter vector.
GHUC-5	<i>Plag-2scrDBS</i> prey	600 bp upstream of the <i>lag-2</i> ATG was purchased as a gBlock with the DBS sequence scrambled ( <i>Lag-2-scrDBS</i> ), digested and cloned between the NotI and EcoRI restriction sites of the GHUC reporter vector. See additional notes regarding B1H

		vectors in D below.
GHUC-6	<i>Plag-2-scrCons</i> prey	600 bp upstream of the <i>lag-2</i> ATG was purchased as a gBlock with the Cons sequence scrambled ( <i>Lag-2-scrCons</i> ), digested and cloned between the <i>NotI</i> and <i>EcoRI</i> restriction sites of the GHUC reporter vector. See additional notes regarding B1H vectors in D below.
GHUC-7	<i>Plag-2-scrBoth</i> prey	600 bp upstream of the <i>lag-2</i> ATG was purchased as a gBlock with the DBS and Cons sequences scrambled ( <i>Lag-2-scrBoth</i> ), digested and cloned between the <i>NotI</i> and <i>EcoRI</i> restriction sites of the GHUC reporter vector. See additional notes regarding B1H vectors in D below.
pB1Hw2-Daf3250	DAF-3-N' bait	The coding sequence of amino acids 1-250 of Daf3 were amplified with primers <i>Daf3 5p</i> and <i>Daf3 250 3p</i> from clone # F25E2.5C of the transcription factors collection from Open Biosystems, catalog # OCE4818-202528301. The coding sequence for these amino acids and an N-terminal linker were cloned between the <i>NotI</i> and <i>XbaI</i> sites of the pB1Hw2 vector. See additional notes regarding B1H vectors in D below.
pGC692	<i>hlh-2</i> RNAi 5' clone	A 5' fragment of the <i>hlh-2</i> mRNA was amplified using primers GCo2416 and

		GC02417 (according to (Chesney et al., 2009). pPD129.26 was digested with EcoRV and ligated with <i>hh-2</i> fragment using Gibson assembly.
pGC693	<i>hh-2</i> RNAi 3' clone	A 3' piece of the <i>hh-2</i> mRNA was amplified using primers GCo2418 and GCo2419 (according to (Chesney et al., 2009). pPD129.26 was digested with EcoRV and ligated with <i>hh-2</i> fragment using Gibson assembly.

### C. Primers and gBlocks used in this study:

Name	Sequence
GCo1866	cgctcaaataaatgtccag
GCo1867	GGAATGCATTGAACATGAGAAG
GCo1868	GTTTGTTCCTTCCCCTTCCC
GCo1869	ggaaaatggctgatggccttg
GCo1870	ggtgtgagtgaagatccttgg
GCo1871	gccggaaaactaaaaatg
GCo1906	aagattttcattagagaatgtctagaactagGCCGTTactggcgctactccacc ttt
GCo1907	cggccaaattgaaaagtgttgtggctctagcaaagctcaaggcgactataagtt cg
GCo1909	tttcctctaagtatcccgaaacttatagtcgacccgttagttgtctagaagccaaca
GCo1910	ttaaaattgaaaattcaacgacgtggcgctgcataccttaTTTGTATAGTTCA TCCA
GCo1911	TGCTGGGATTACACATGGCATGGATGAACATACAAAtaaggatgt cgacgccaacgtc
GCo1912	CGCACCGTACGTCTCGAGTgtaaaacgacggccagtGTTccaagcgagg acaattctca
GCo1928	aagattttcattagagaatgtctagaactagGCCGTTttcggAACgtctcatta ca
GCo1930	aagattttcattagagaatgtctagaactagGCCGTTtaaattagttcgaatt cc
GCo1931	aagattttcattagagaatgtctagaactagGCCGTTgcctgcctatctataacc ta
GCo2145	cgacgaacgacttgtcaataaaaattg
GCo2146	ccttgtcagtcgtcaagaacatac
GCo2147	AAACGGCctagtcttagacattctc
GCo2149	5' Phos-ctctcaagtattcttacacgtac

GCo2383	cagataaattgcacaattttattgcacaagtcgtcgccgtgtttttaaaatgttggcaaagattgtgaagtccctgttagttaacactctaagttactccaaagactctacctgcctacgcctatcataaccctagtcgttatcacctactcgctgcatagttgatgtacctatataacagttcataaatgaatttgtcaaaaattccactctcaagtatttttacacgtacttatttgacaaatcctgtcagtcgtcaagaacacatcacatcgaaaggcgcaat
GCo2384	cagataaattgcacaattttattgcacaagtcgtcgccgtgttttgcgtcaaatgtggcaaagattgtgaagtccctgttagttaacactctaagttactccaaagactctacctgcctacgcctatcataaccctagtcgttatcacctactcgctgcatagttgatgtacctatataacagtcactctcaagtatttttacacgtacttatttgacaaatcctgtcagtcgtcaagaacacatcacatcgaaaggcgcaat
GCo2385	cagataaattgcacaattttattgcacaagtcgtcgccgtgtttttaaaatgttggcaaagattgtgaagtccctgttagttaacactctaagttactccaaagactctacctgcctacgcctatcataaccctagtcgttatcacctactcgctgcatagttgatgtacctatataacagtcactctcaagtatttttacacgtacttatttgacaaatcctgtcagtcgtcaagaacacatcacatcgaaaggcgcaat
GCo2416	aattaatacgactcactatagggagaccggcagatctgtatggcgatccaaatagccaacttacg
GCo2417	cggccccccctcgaggtcgacggtatcgataagctgtatcttgcgttggaaaggtaacc
GCo2418	aattaatacgactcactatagggagaccggcagatctgtatggcttggagataccaacttg
GCo2419	cggccccccctcgaggtcgacggtatcgataagctgtataaccgtggatgtccaaactgcgc
Daf3 5p	aataaaGCGGCCGCGGACTACAAGGATGACGACGACAAGTTCCGGACCGTTCCAAGACACCCCCCATGGTACCAAGCTAATAGCAACTTCTCTTC
Daf3 250 3p	ggcggtTCTAGACTTACCTCTGCCAACAAATCATAGT
Lag2 600 5p	aataaaGCGGCCGCAAATAATTGCACAATTTTATTGC
Lag2 600 3p	ccgcccGAATTCTTCTGAAAAAAGGCAAATTG
gBlock: <i>Lag2 wt</i>	aataaaGCGGCCGCAAATAATTGCACAATTTTATTGCACAAGTCGTTCGTCGTTCTGTTTTTCGCTGTCAAATGTTGGCAAAGATTGTGAAGTCCCCTGTAGTTAACACTCTAAGTTACTCCAAAGACTTCTACCTGCCTACCTGCCTGCCTATCTATAACCTAGTGCCTATCACC TACTCGCTGCATAGTTGATGTACCTATAAACAGTTCAAAATGAATTTGTCAAATCCACTCTCAAGTATTCTACACGTACTTATTGTACAAATCTTGTACAATCCTTGTCAGTCGCTGCAAGAACATACACATCGAAGAGGCGCAATCGAAACACAGGTGTTGCCGTTGATCGTCTCCCGCCCCGCCTGTTGGCGGGACGGGTGTCGGTCACCACCACATCATATTGTTGGACACACTTGCACATCCGGTTCACACCCGATTACCGCATCGGGGCTTGATCTGGGGCGGTATTGGATCTTTGTTATGTAGATTTTCTCGCCGTTATGATTGGATTATTTTCTCTTATCTTGAGACTTGTAATCTTGCCTGATTGCTAGCTAGCCA AAACTTCACTGTTCTTTTCTCTCTAAGTATTCCCAGACTTATAGTCGACCTTGAGCTTGCTAGAAGCCAACAACACTTTCAAAATTGCCCTTTTCAAGAAAGAATTCCcgccc
gBlock: <i>Lag-2-scrDBS</i>	aataaaGCGGCCGCAAATAATTGCACAATTTTATTGCACAAGTCGTTCGTCGTTCTGTTTTTC <del>ctatcact</del> AAAATGTTGGCAAAGATTGTGAAGTCCCCTGTAGTTAACACTCTAAGTTACTCCAAAGACTTCTACCTGCCTACCTGCCTGCCTATCTATAACCTAGTGCCTATCACCTACTCGCTGCATAGTTGATGTACCTATAACAGTTCAAAATGAAT

	TTTGTCAAAATTCCACTCTCAAGTATTCTACACGTACTTATTTGT ACAAATCCTGTCAGTCGCTGCAAGAACATACACATCGAAGAGGC GCAATCGAAACAACAGGTGTCGCCCGTTGATCGTCTCCCCGCC CCGCCTGTTGGCGGGACGGGTGTCGGTCACCACCAACATCA TATTGTTGGACACACTTGACATCCGGTTACACCCGATTACCG CATCGGGGTCTTGATCTGGGGCGGCTATTGGATCTTTGTTATG TAGATTTTTCTCGCCGTTATGATTTGGATTATTTCTCTTAT CTTGCAGTCGACTTGTAAATCTTGCCTGATTGCTAGCTAGCCAAA ACTTCACTTGTCTTTCTCTAAGTATTTCCGAACCTTAT AGTCGACCTTGAGCTTGCTAGAACCAACACACTTTCAAATT GCCTTTTCAGAAAGAATTCCggccc
gBlock <i>Lag-2</i> -scrCons	aataaaGCGGCCGCAAATAAATTGACAATTATTGACAAGTC GTTCGTCGTTCTGTTTTTCGCTGTCAAATGTTGGCAAAGATT GTGAAGTCCCCTGTAGTTAACACTCTAAGTTACTCCAAGACTT CTACCTGCCTACCTGCCTGCCTATCTATAACCTAGTGCCTATCACC TACTCGCTGCATAGTTGATGTACCTATATAACAGT <b>ctgcgggca</b> <b>gccccactggggcct</b> CACTCTCAAGTATTCTACACGTACTTATTTG TACAAATCCTGTCAGTCGCTGCAAGAACATACACATCGAAGAGG CGCAATCGAAACAACAGGTGTCGCCCGTTGATCGTCTCCCCGC CCGCCTGTTGGCGGGACGGGTGTCGGTCACCACCAACATC ATATTGTTGGACACACTTGACATCCGGTTACACCCGATTACC GCATCGGGGTCTTGATCTGGGGCGGCTATTGGATCTTTGTTAT GTAGATTTTTCTCGCCGTTATGATTTGGATTATTTCTCTTA TCTTGCAGTCGACTTGTAAATCTTGCCTGATTGCTAGCTAGCCAA AACTTCACTTGTCTTTCTCTAAGTATTTCCGAACCTTAT TAGTCGACCTTGAGCTTGCTAGAACCAACACACTTTCAAATT TGCCTTTTCAGAAAGAATTCCggccc
gBlock: <i>Lag-2</i> -scrBoth	aataaaGCGGCCGCAAATAAATTGACAATTATTGACAAGTC GTTCGTCGTTCTGTTTT <b>ctatact</b> AAAATGTTGGCAAAGATTG TGAAGTCCCCTGTAGTTAACACTCTAAGTTACTCCAAGACTTCT ACCTGCCTACCTGCCTGCCTATCTATAACCTAGTGCCTATCACCTA CTCGCTGCATAGTTGATGTACCTATATAACAGT <b>ctgcgggcaaggc</b> <b>cccaactggggcct</b> CACTCTCAAGTATTCTACACGTACTTATTTGTA CAAATCCTGTCAGTCGCTGCAAGAACATACACATCGAAGAGGCG CAATCGAAACAACAGGTGTCGCCCGTTGATCGTCTCCCCGCC GCCTGTTGGCGGGACGGGTGTCGGTCACCACCAACATCATA TTGTTGGACACACTTGACATCCGGTTACACCCGATTACCGCA TCGGGGTCTGATCTGGGGCGGCTATTGGATCTTTGTTATGTA GATTTTTCTCGCCGTTATGATTTGGATTATTTCTCTTATCT TGCAGTCGACTTGTAAATCTTGCCTGATTGCTAGCTAGCCAAAC TTTCACTTGTCTTTCTCTAAGTATTTCCGAACCTTATAG TCGACCTTGAGCTTGCTAGAACCAACACACTTTCAAATTG CCTTTTCAGAAAGAATTCCggccc
M02389	gcacaagtgcgtcggtcg
M02390	gggacttcacaatcttgccaac
M02391	acgtgtagaatacttgagagt
M02388	atgtacctatataacagttcataaatg
M02339	tgcgtgttatattatc
M02340	taaagagtggtgaaa
M02392	tttccaccaactcttaatttc

MO2393	aacttccaccgccccatgttg
MO2262	cacactgtactcattgttctg
MO2263	aggttaactaaagatagtgaag
MO2398	ctgtgacatcggtatggatggac
MO2399	ccgtcggttcgcattgagcac
MO2402	gatgaggaagtggatattaccag
MO2403	acgcatactcattattcgattc

#### D. Additional Notes on B1H plasmids:

MRB1H vector (Kanamycin) based plasmids: There are multiple reporters in this vector including GFP, HIS3, URA3, and mCherry (G-H-U-C). For this study we utilized the HIS3-GFP cassette and were only concerned with the activation of GFP. We cloned our inserts between NotI and EcoRI placing them upstream of the promoter that drives the HIS3-GFP cassette. When activated by recruitment of polymerase, HIS3 and GFP are transcribed from the same promoter but an internal Shine-Dalgarno sequence allows each protein to be translated separately.

pB1Hw2 vector (Ampicillin) based plasmids: As first described in (Noyes et al., 2008), the pB1Hw2 vector allows for the expression of a protein of interest as a C-terminal fusion to the omega subunit of bacterial polymerase. In this way omega functions as the activation domain by recruiting the rest of the polymerase only when the protein of interest binds a DNA sequence near a promoter. The “w2” version of this plasmid signifies the use of a weak promoter to drive expression of the omega – protein of interest fusion (w5 and wL versions of the plasmid exist for higher levels of expression). The protein of interest and a stop codon are typically cloned between the KpnI and XbaI sites of the plasmid for in-frame expression. However, in the case of Daf3, there is an internal KpnI site so we cloned into the upstream NotI sequence, recreating the linker between NotI and KpnI on the primer utilized.

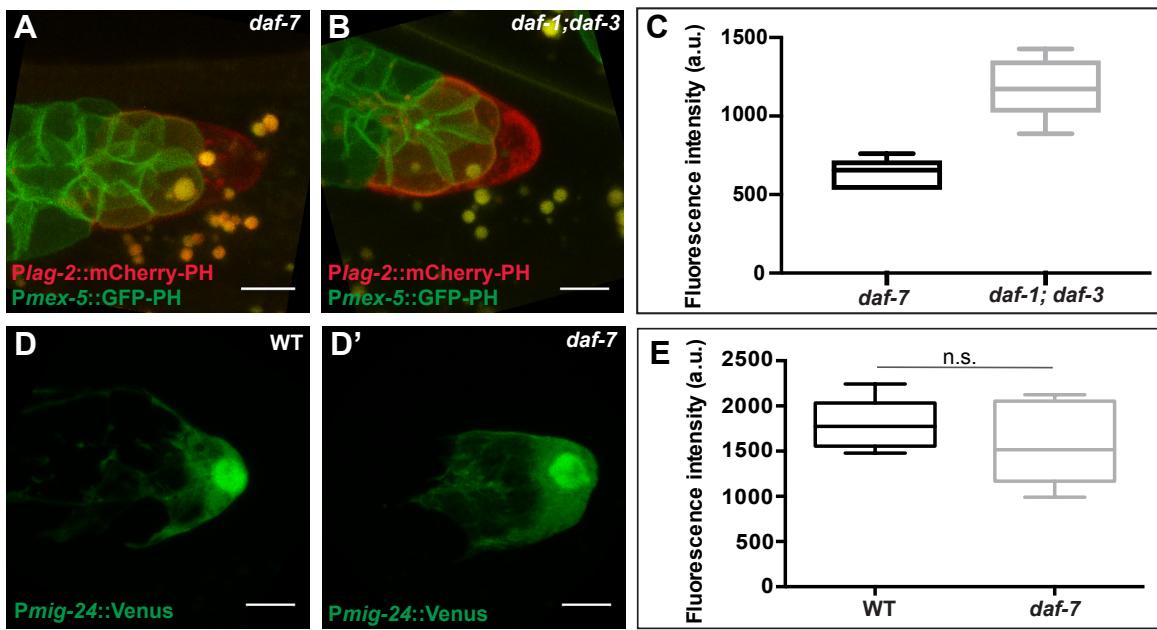
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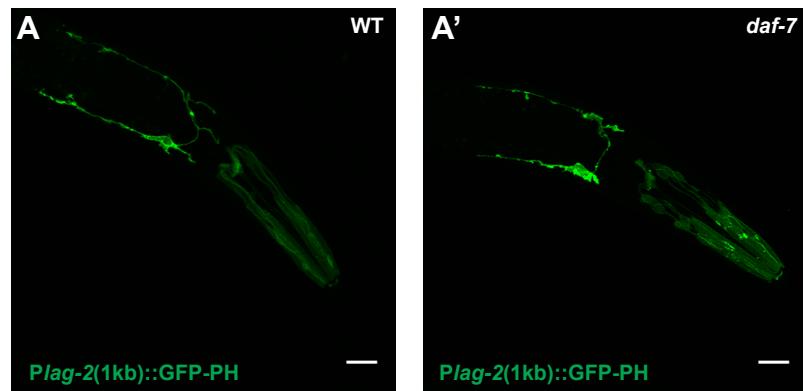
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## SUPPLEMENTARY FIGURES



**Figure S1: DAF-7/TGF $\beta$  signaling promotes *lag-2* DTC expression but not *mig-24*.**

(A-C) Expression of *lag-2* (3 kb) reporter *nals37* in distal gonad arms of (A) *daf-7* and (B) *daf-3; daf-1* mutant animals. *naSi1* [*Pmex-5::GFP-PH*] marks germ cell membranes. (C) Quantification of the mCherry signal. (D-E) Comparative expression of *mig-24* promoter in (D) wild type and (D') *daf-7* animals. All images are Z-projections of 0.46  $\mu$ m confocal stacks. Scale bar is 5  $\mu$ m. Mutant alleles: *daf-1(m40)*, *daf-5(e1386)*, *daf-7(e1372)*, *daf-3(e1376)*. (F) Quantification of Venus signal in the DTC. “n.s.” represents “not significant” p>0.05, two-tailed Student’s t-test. N  $\geq$  15 animals; one DTC scored per animal. Error bars represent S.E.M.



**Figure S2: Neuronal expression of *lag-2* reporter is not changed in *daf-7* animals.**  
Expression of the *nals84* (1 kb) reporter in head neurons of (A) wild type and (B) *daf-7(e1372)* animals. Scale bar is 20  $\mu$ m.

**Plag-2** **TGCGCTGTC**aaaatgtggcaaagattgtgaagtcccctgtagttaacactctaaggta  
ctccaaagacttctacacctgcctacacctgcctatctataaccttagtgccatatcaccta  
ctcgctgcatagttgatgtacctatataacagt**TCATAAAATGAATTGTCAAAATTC**

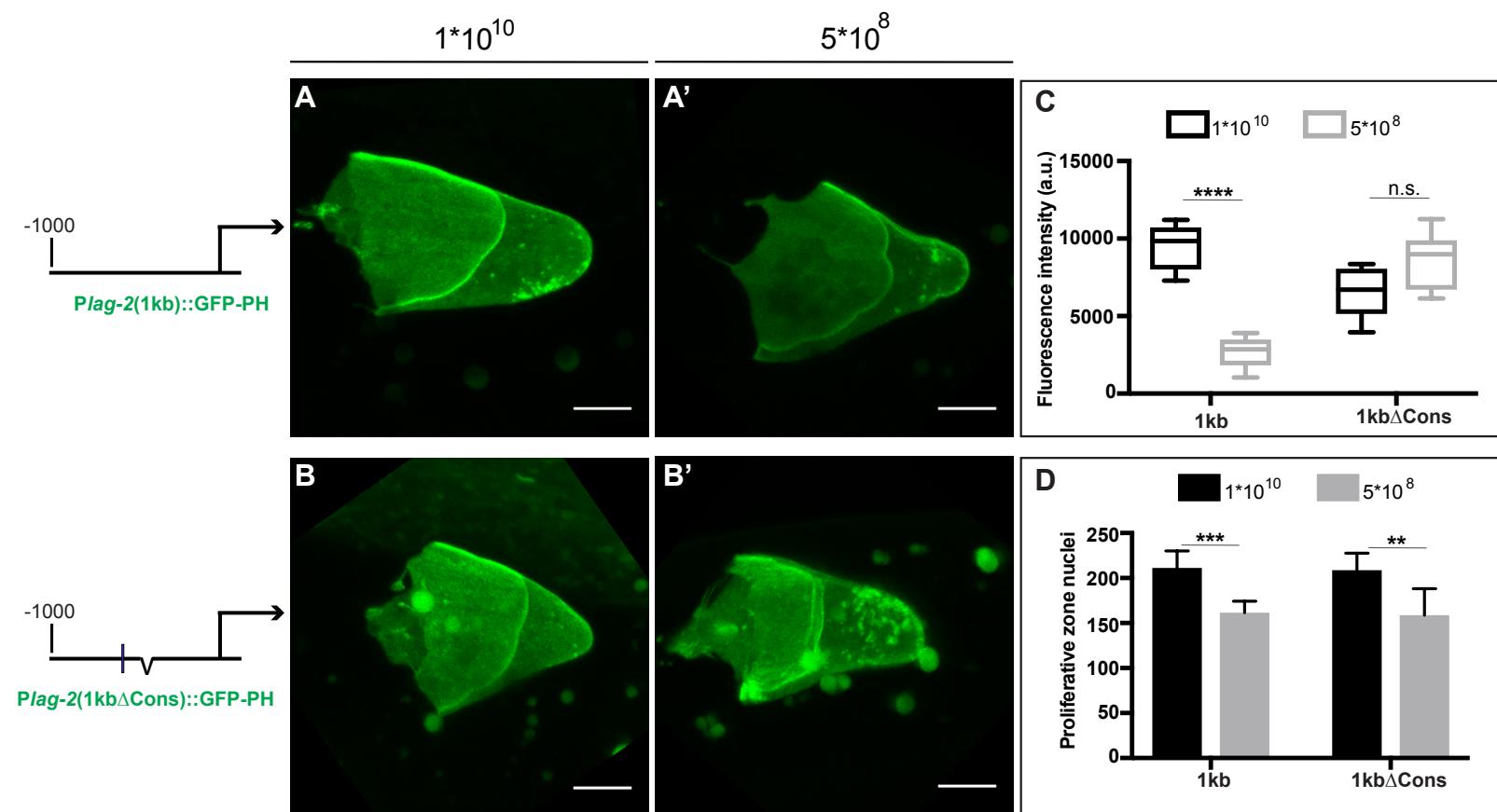
**Posm-9** ctgcgt**GTCTA**tattatcaattttcaccaac**TCTTTAATTTCAGAAACC**

**DBS**    **GTCTG**  
“rule”

**Cons**    **CT [AT] TAA[AT] TTN (0,3) [AC] AN (0,2) TTTTG[CT] CATAA[TA]C[TC]**  
“rule”

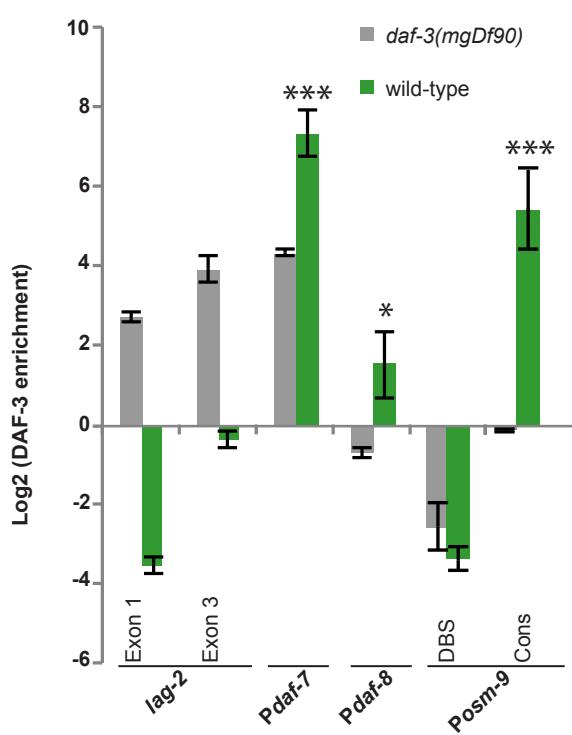
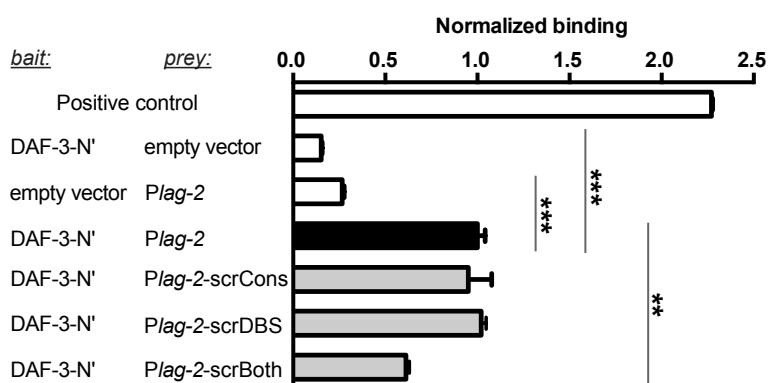
**Figure S3: Related DBS and Cons sequences in the upstream regions of *osm-9* and *lag-2*.**

Sequence in capital letters and in blue and red boxes indicate the DBS and Cons sequences, respectively. Additional related sequence is underlined in *Posm-9*. Bioinformatic analysis identified the related sequences using a flexible version of the depicted “rules” (Sims et al., 2016).



**Figure S4: The Cons sequence is required for *lag-2* reporter response to low food in the DTC.**

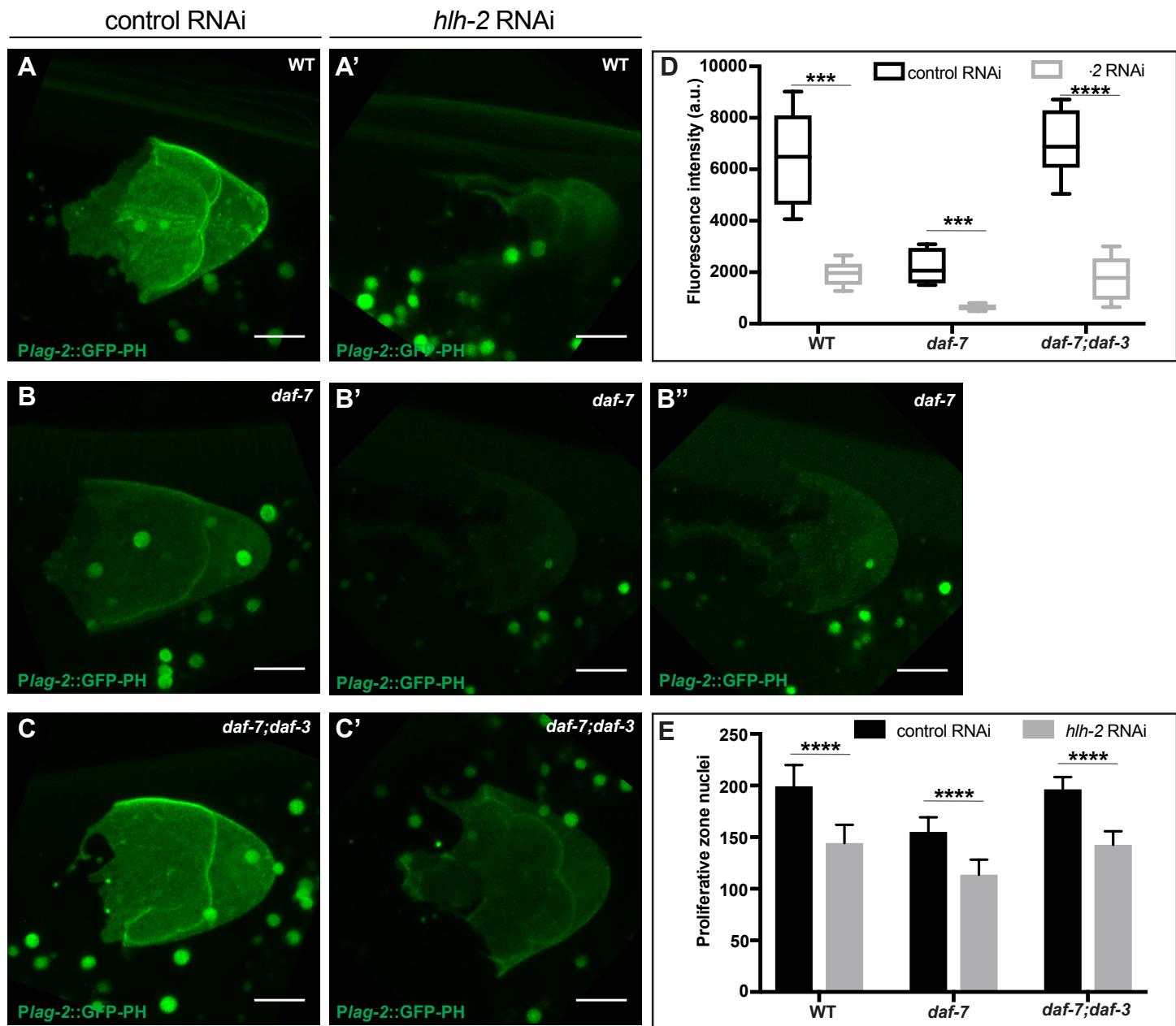
(A, B) Representative images of reporter GFP expression driven by 1 kb of the *lag-2* upstream region (*nals84* in strain GC1359) or 1kbΔCons (*nals102* in strain GC1439) in the DTC of late L4 animals reared from early L3 on high ( $1 \times 10^{10}$ ) or low ( $5 \times 10^8$ ) concentrations of OP50 bacteria. All images are Z-projections of 0.46 μm confocal stacks. Scale bar is 5 μm. (C) Quantification of the GFP signal in the DTC from experiments represented in panels A-B. (D) Number of proliferative zone nuclei per gonad arm in early adults (collected from the same plates as animals in previous panels) reared from early L3 on high or low bacterial concentrations. Statistics: “n.s.” is  $p>0.05$ , \*\*\* $p<0.001$ , \*\*\*\* $p<0.0001$ , two-tailed Student’s t-test.  $N \geq 15$  animals, one DTC scored per animal (GFP quantifications) or one gonadal arm per animal (proliferative zone quantifications). Error bars represent S.E.M.

**A****B**

**Figure S5: DAF-3 binds *lag-2* promoter.**

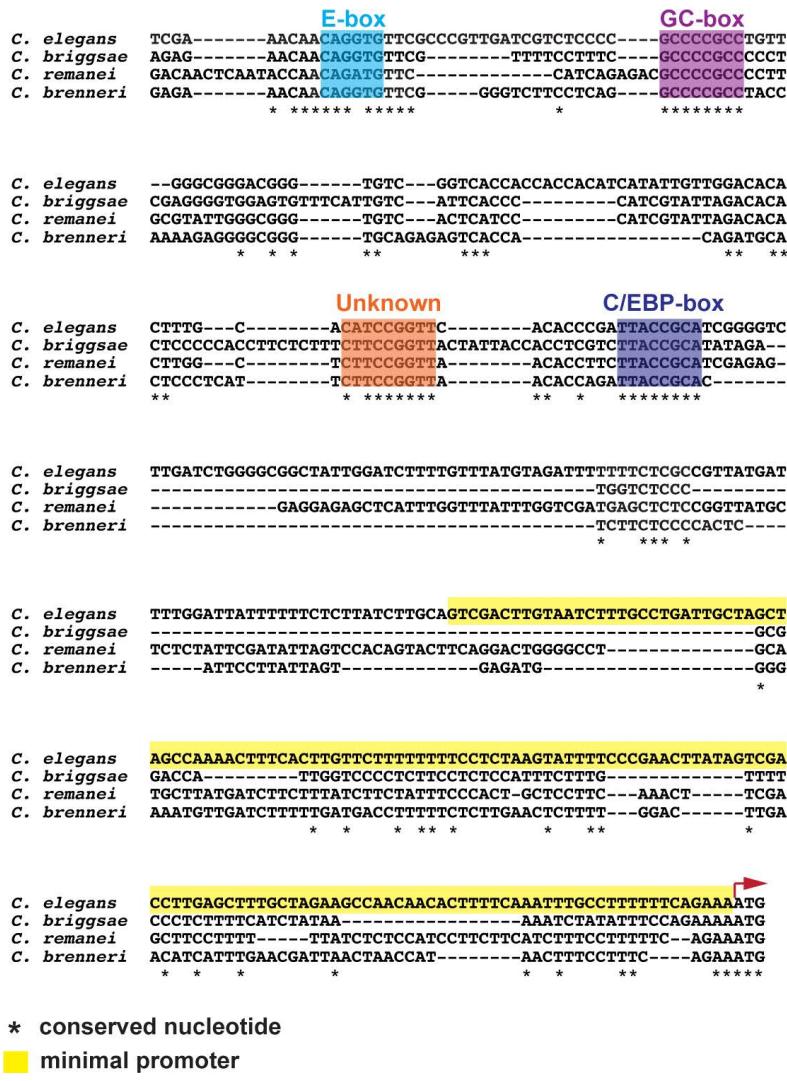
(A) Log2 normalized enrichment of DAF-3 SMAD binding to exons of *lag-2* (as negative controls), to upstream regulatory regions of *daf-7* and *daf-8* (as positive controls), to *osm-9* DBS (as a negative control in animals that have not passed through dauer) and to *osm-9* Cons in wild-type and *daf-3(mgDf90)* strains. Bar graph represents IP-qPCR data, normalized to DAF-3 enrichment at the actin *act-2* promoter (Park et al., 2010). N ≥ 2 biologically independent trials. Significant enrichment in wild-type compared to *daf-3(mgDf90)* is indicated by \*p<0.05, \*\*\*p<0.001, Student's t-test. Error bars represent S.E.M.

(B) Bacterial one-hybrid assay in which N-terminal fragment (up to 250 amino acids, DAF-3-N') of DAF-3 was used as bait and 600 bp upstream of the *lag-2* ATG [Plag-2(600bp)] was used as prey. Bar graphs represent FACS analysis data of different baits and preys binding, normalized to DAF-3-N' and Plag-2(600bp) (Plag-2) binding. Binding of the transcription factor *Zif268* to its consensus target was tested as a strong positive control. Binding of DAF-3-N' to empty prey vector and binding of empty bait vector to Plag-2 serve as negative controls. Binding of DAF-3-N' to Plag-2 with scrambled Conserved motif (Plag-2scrCons), scrambled DBS motif (Plag-2scrDBS) or both (Plag-2scrBoth) was tested as well. For sequences of scrambled motifs see Material and Methods. Mean fluorescence for each sample was normalized to the fluorescence of DAF-3-N' paired with the WT Plag-2 promoter to provide a comparative measure of fluorescence with and without the mutated sequences. \*\*p<0.01, \*\*\*p<0.001, Student's t-test. Error bars represent S.E.M.

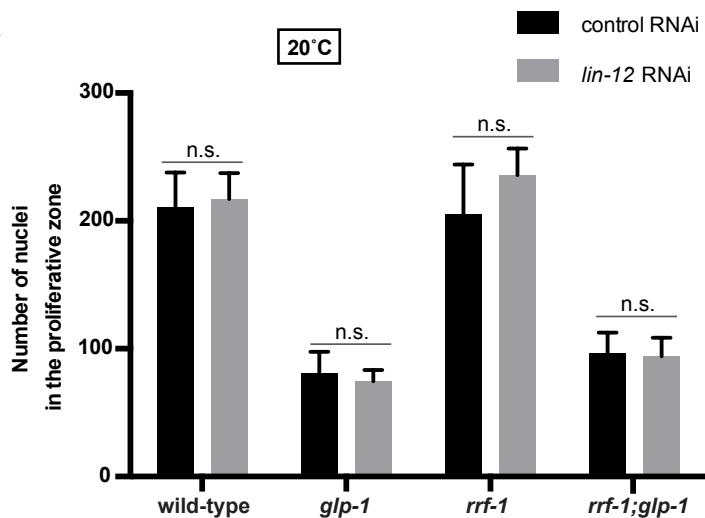
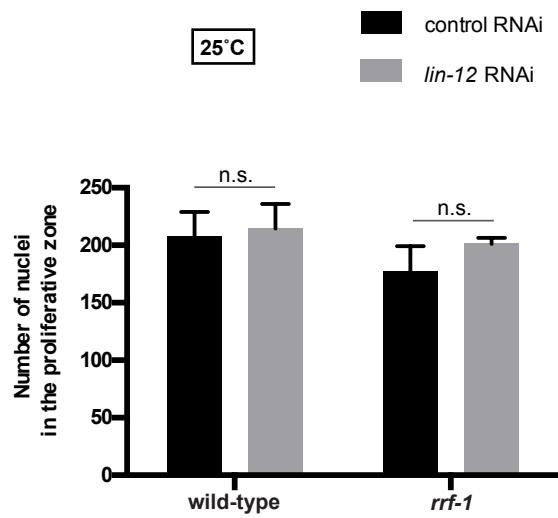
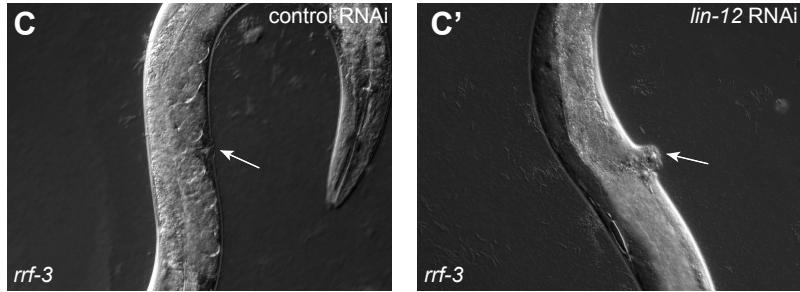


**Figure S6: DAF-7/TGF $\beta$  pathway regulation of *lag-2* DTC expression and germline development occurs in parallel to regulation by HLH-2.**

Expression levels of the *lag-2* single copy reporter (*naSi8*) in (A, A') wild type, (B, B') *daf-7* (B'' is the same image as B' but with enhanced brightness to show residual GFP signal) and (C, C') *daf-7; daf-3* animals treated with control or *hhl-2* RNAi. (D) Quantification of GFP signal in the DTC. (E) Number of nuclei in the proliferative zone of early adults treated with control or *hhl-2* RNAi. All images are Z-projections of 0.46  $\mu\text{m}$  confocal stacks. Scale bar is 5  $\mu\text{m}$ . Mutant alleles: *daf-7(e1372)*, *daf-3(e1376)*. Statistics: \*\*\*p<0.001 \*\*\*\*p<0.0001, two-tailed Student's t-test. N  $\geq$  15 animals, one DTC scored per animal (GFP quantifications) or one gonadal arm per animal (proliferative zone counts). Error bars represent S.E.M.

**Figure S7: Conserved motifs in the upstream region of *lag-2***

Multiple sequence alignment of ~400 bp upstream of the *lag-2* ATG from different nematode species. Alignment was made using MUSCLE (<http://www.ebi.ac.uk/Tools/msa/muscle/>). The conserved motifs: GC-box/Sp1 binding site and C/EBP-box were identified using MatInspector ([https://www.genomatix.de/online\\_help/help\\_matinspector/matinspector\\_help.html](https://www.genomatix.de/online_help/help_matinspector/matinspector_help.html)). The positions of the E-box/HLH-2 binding site and minimal promoter in *lag-2* promoter are shown as previously reported (Chesney et al., 2009; Karp and Greenwald, 2003; Karp and Greenwald, 2004; Zhang and Greenwald, 2011). We note that the Cons sequence (Fig. S3) is not highly conserved between species, as are these motifs.

**A****B****C**

**Figure S8: Germline-directed LIN-12 RNAi does not affect the number of proliferative germ cells in the early adult.**

(A-B) Number of nuclei in the proliferative zone of early adults (just after the L4/Adult molt) treated with control (L4440) or *lin-12* RNAi at (A) 20°C and (B) 25°C. Mutant alleles were: *glp-1*(e2141), *rrf-1*(pk1417). Animals were fed RNAi-inducing bacteria for two generations: mothers were fed from the first larval stage (L1) and, after transfer to fresh RNAi bacteria, their progeny were scored as adults. The *glp-1*(e2141) mutant at 20°C is a sensitized condition for loss of germline stem cells; reduced TGF $\beta$  signaling dramatically enhances this phenotype (Dalfo et al., 2012). (A) N  $\geq$  12 and (B), N  $\geq$  15 gonad arms. Error bars represent S.E.M; n.s. indicates no significant difference ( $p>0.05$ ) between animals treated with control and *lin-12* RNAi. (C, C') To ensure efficacy of our RNAi reagent, we tested the ability of the same culture of RNAi-inducing bacteria to cause *lin-12* mutant phenotypes. Phenotypes were only observed in the *rrf-3* mutant background that augments somatic RNAi (Simmer et al., 2003; Simmer et al., 2002). Representative images of *rrf-3*(pk1426) animals treated with (C) control or (C') *lin-12* RNAi at 20°C. Normal vulva phenotype in (C) was 100% penetrant (N  $\geq$  100), and disrupted vulva in (C') was 90% penetrant (N  $\geq$  50). Arrows point to vulva.