SUPPLEMENTAL MATERIAL

Table S1. Summary of CRISPR/Cas9 alleles

Allele name	crRNA(s)	Mutation
ma388	5' LCE crRNA 3' LCE crRNA	X:14743758<ctgtcaccgcaaatc[Δ55bp]ttggacctatttttt>X:14743674
ma403	SA1 crRNA	X:14743856<atagtggaaatcatg[Δ14bp]aacctcatctgctgg>X:14743827
ma406	IVT 5' previously published crRNA IVT 3' previously published crRNA	X:14743877<tgctagtcttcacca[Δ178bp]acctatttttttaa>X:14743680
ma408*	IVT mutLCE crRNA	X:14743741<caggatctcacactt**T**t**G**cc**A**caacacct**G**ta**TT**t**G**atccgcat**T**ta**TT**t**G**gattggacctatttt>X:14743676
ma422**, ma423***	IVT ma422 crRNA IVT ma423 crRNA	**ma422** X:14743981<cggtaatgtatctgg[Δ6bp]taatctaatcgtatgtactgtt[Δ2bp]gtaatgtatccatg[Δ2bp]gccgtttgacatttct[Δ2bp]tggtaagatgtgcaa>X:14743888 **ma423** X:14743873<agtcttcaccattgt[Δ1bp]gatagtggaaatcatgtttatttc[Δ1bp]ggggaacctcatctg>X:14743817
ma485 ****	5' LCE crRNA 3' LCE crRNA	X:14743758<ctgtcaccgcaaatc[Δ55bp]ttggacctatttttt>X:14743674

*made by replacing 55bp deletion of ma388 with mutated LCE; nucleotide changes are capitalized and in bold.

**ma422 was made using IVT ma422 crRNA in the background of ma409 (not used for experiments in this article) which has a 113bp deletion of SA1-2.

***ma423 was made using IVT ma423 crRNA in the background of ma422 by replacing the 113 bp deletion (ma409) with mutated SA1-2.

****ma485 was made in a *let-7(mg279)* background and is the same deletion as ma388

Table S2. Oligonucleotides used in this study

Oligo ID	Name	Sequence
oCN66	let-7 RT	ATCTAATTATCAAGAGCAAGTTCAAATGT
oCN59	SL1 F	GGTTTAATTACCCAAGTTTGAG
oCN61	LCE R	GAGGTGTTGAGGTAGAAGTGTGAG
oCN342	LCE probe	GGATGAGGTAGAGGTGTTGAGGTAGGGCGGG
oCN343	SL1-LCE probe	TTCCCCTCAAACTTGGGTAATTAAACCGGCGGG
oCN345	let-7 probe	AACTATACAACCTACTACCTCAGGCGGG
oCN347	5.8s rRNA probe	GAACCAGACGTACCAACTGGAGGCCCGGCGGG
	oligo (dT) 20	TTTTTTTTTTTTTTTTTTTT
oCN70	SL1-pri-let-7 F	GGTTTAATTACCCAAGTTTGAGGCAAG
oCN71	SL1-LCE F	GGTTTAATTACCCAAGTTTGAGGGGAA
oCN356	TSO 5'RACE	GTTCAGAGTTCTACAGTCCGACGATCrGrGrG
oCN357	Rd1 SP	GTTCAGAGTTCTACAGTCCGACGATC
oCN216	pri-let-7 F	CAAGCAGGCGATTGGTG
oCN63	pri-let-7 R	CGAAGAGTTCTGTCTCCGGTAAGG
oCN339	T7 SL1-LCE F	TAATACGACTCACTATAGGGGTTTAATTACCCAA GTTTGAGGGGAACCTCATCTGCTGGG
oCN337	T7 pri-let-7 F	TAATACGACTCACTATAGGATTCTAGATGAGTA GCCCACCTAGCAG
	tracrRNA	IDT Alt-R™ CRISPR tracrRNA
	dpy-10 crRNA	IDT Alt-R™ CRISPR crRNA /AlTR1/rGrCrUrArCrCrArUrArGrGrCrArCrCrArCrGrA rGrGrUrUrUrUrArGrArGrCrUrArUrGrCrU/AlTR2/
oCN181	5' LCE crRNA	IDT Alt-R™ CRISPR crRNA /AlTR1/rGrGrCrUrGrUrCrArCrCrGrCrArArArUrCrArU rCrGrUrUrUrUrArGrArGrCrUrArUrGrCrU/AlTR2/
oCN182	3' LCE crRNA	IDT Alt-R™ CRISPR crRNA /AlTR1/rArArArArArArArArUrArGrGrUrCrCrArArUr CrGrGrUrUrUrUrArGrArGrCrUrArUrGrCrU/AlTR2/

oCN87	ΔLCE HR	GCCGTCTGGCACCAAGTGGGCTGTCACCGCAAA TCTTGGACCTATTTTTTTTAAATTCTTCAAATAA AAAC
oCN180	SA1 crRNA	IDT Alt-R™ CRISPR crRNA /AlTR1/rGrArArArUrCrArUrGrUrUrUrArUrUrUrUrCr ArGrGrUrUrUrUrArGrArGrCrUrArUrGrCrU/AlTR2/
oCN77	ΔSA1 HR	GCTAGTCTTCACCATTGTAGATAGTGGAAATCAT GAACCTCATCTGCTGGGCAACTACTCCAACATG CGTG
oCN183	T7 promoter	TAATACGACTCACTATAG
oCN184	IVT dpy-10 crRNA	CAAAACAGCATAGCTCTAAAACCTCGTGGTGCC TATGGTAGCCTATAGTGAGTCGTATTA
oCN198	IVT 5' previously published crRNA	CAAAACAGCATAGCTCTAAAACCTATCTACAAT GGTGAAGACCTATAGTGAGTCGTATTA
oCN199	IVT 3' previously published crRNA	CAAAACAGCATAGCTCTAAAACCGATTGGACCT ATTTTTTTCCTATAGTGAGTCGTATTA
oCN201	Previously published HR	TGCAATAGTTCCAATTGCTAGTCTTCACCAACCT ATTTTTTTTAAATTCTTCAAATAAAA
oCN210	IVT mutLCE crRNA	CAAAACAGCATAGCTCTAAAACAGATTTGCGGT GACAGCCCTATAGTGAGTCGTATTA
oCN209	IVT mutLCE HR	GCCGTCTGGCACCAAGTGGGCTGTCACCGCAAA TCATCAGGATCTCACACTTTTGCCACAACACCTG TATTTGATCCGCATTTATTTGGATTGGACCTATT TTTTTTAAATTCTTCAAATAAAAA
oCN239	IVT ma422 crRNA	CAAAACAGCATAGCTCTAAAACGATACATTACC GATACAACCCTATAGTGAGTCGTATTA
oCN248	mutSA3-6 HR	GAACTGTATTCGGAGAACTGTTGTATCGGTAAT GTATCTGGAATAATCTAATCGTATGTACTGTTGT AATGTATCCATGGCCGTTTGACATTTCTTGGTAA GATGTGCAATAGTTCCAATTGCTAGTCTT

oCN266	IVT ma423 crRNA	CAAAACAGCATAGCTCTAAAACCAAATCATCAG GATCTCACCTATAGTGAGTCGTATTA
oCN265	mutSA1-2 HR	GAGGTGTTGAGGTAGAAGTGTGAGATCCTGATG ATTTGCGGTGACAGCCCACTTGGTGCCAGACGG CATTCCCTAGGCGACACGCATGTTGGAGTAGTT GCCCAGCAGATGAGGTTCCCCGAAAATAAACAT GATTTCCACTATCACAATGGTGAAGACTAGCAA TTGGAACTATTGCACATCT
oCN397	mir-241 synthetic	rUrGrArGrGrUrArGrGrUrGrCrGrArGrArArArUrGrA
	gpd-1 QPCR F	GATGGACCAATGAAGGGAAT
	gpd-1 QPCR R	GTCGTACCAAGAGACGAGCTT
	let-7 synthetic	rUrGrArGrGrUrArGrUrArGrGrUrUrGrUrArUrArGrUr U
	mir-48 synthetic	rUrGrArGrGrUrArGrGrCrUrCrArGrUrArGrArUrGrCrG rA
	mir-84 synthetic	rUrGrArGrGrUrArGrUrArUrGrUrArArUrArUrUrGrUr ArGrA

Table S3. *C. elegans* strains used in this study

Strain Name	**Strain Description**	**Genotype**
MT355	*lin-14(gf)*	*lin-14(n355)* X
VT965	*lin-14(lf)*	*lin-14(n179)* X
VT1295	*lin-28(0)*/*lin-28* null	*lin-28(n719)* I; *maIs105* V
VT1367	wild type/WT	*maIs105* [*col-19::gfp*] V
VT3594	*lin-28(0); lin-46(0)*	*lin-28(n719)* I; *lin-46(ma164), maIs105* V
VT3609	*lin-4(lf)*	*lin-4(e912)* II; *maIs105* V
VT3616	ΔLCE	*let-7(ma388)* X; *maIs105* V
VT3666	ΔSA1	*let-7(ma403)* X; *maIs105* V
VT3669	previously published deletion	*let-7(ma406)* X; *maIs105* V

VT3675	ΔSA1; *lin-28(0)*	*let-7(ma403)* X; *lin-28(n719)* I; *maIs105* V
VT3678	mutLCE	*let-7(ma408)* X; *maIs105* V
VT3718	mutSA1-6	*let-7(ma422ma423)* X; *maIs105* V
VT3719	mutLCE; *mir-48(0)*	*let-7(ma408)* X; *miR-48(n4097), maIs105* V
VT3720	ΔLCE; *mir-48(0)*	*let-7(ma388)* X; *miR-48(n4097)*, *maIs105* V
VT3721	ΔLCE; *mir-48(0) mir-241(0)*	*let-7(ma388)* X; *miR-48 miR-241(nDF51)*, *maIs105* V
VT3837	*mir-48(0)*	*mir-48(n4097)*, *maIs105* V
VT3838	mutSA1-6; *mir-48(0)*	*let-7(ma422ma423)* X; *mir-48(n4097)*, *maIs105* V
VT3839	*let-7* locus' ORF::GFP transgene	*maEx264* [*unc-119+; plet-7::let-7 locus with ORF::GFP*]; *unc-119(ed3)* III
VT3840	*mir-48(0) mir-241(0)*	*mir-48 mir-241 (nDf51)*, *maIs105* V
VT3902	*let-7(mg279ΔLCE)*	*let-7(mg279ma485)* X; *maIs105* V
VT3903	LCE transgene; ΔLCE; *mir-48(0)*	*maEx267 [rol-6(su1006); unc-119+, plet-7::let-7 locus without mature let-7 sequence]; let-7(ma388)* X*; mir-48(n4097), maIs105* V

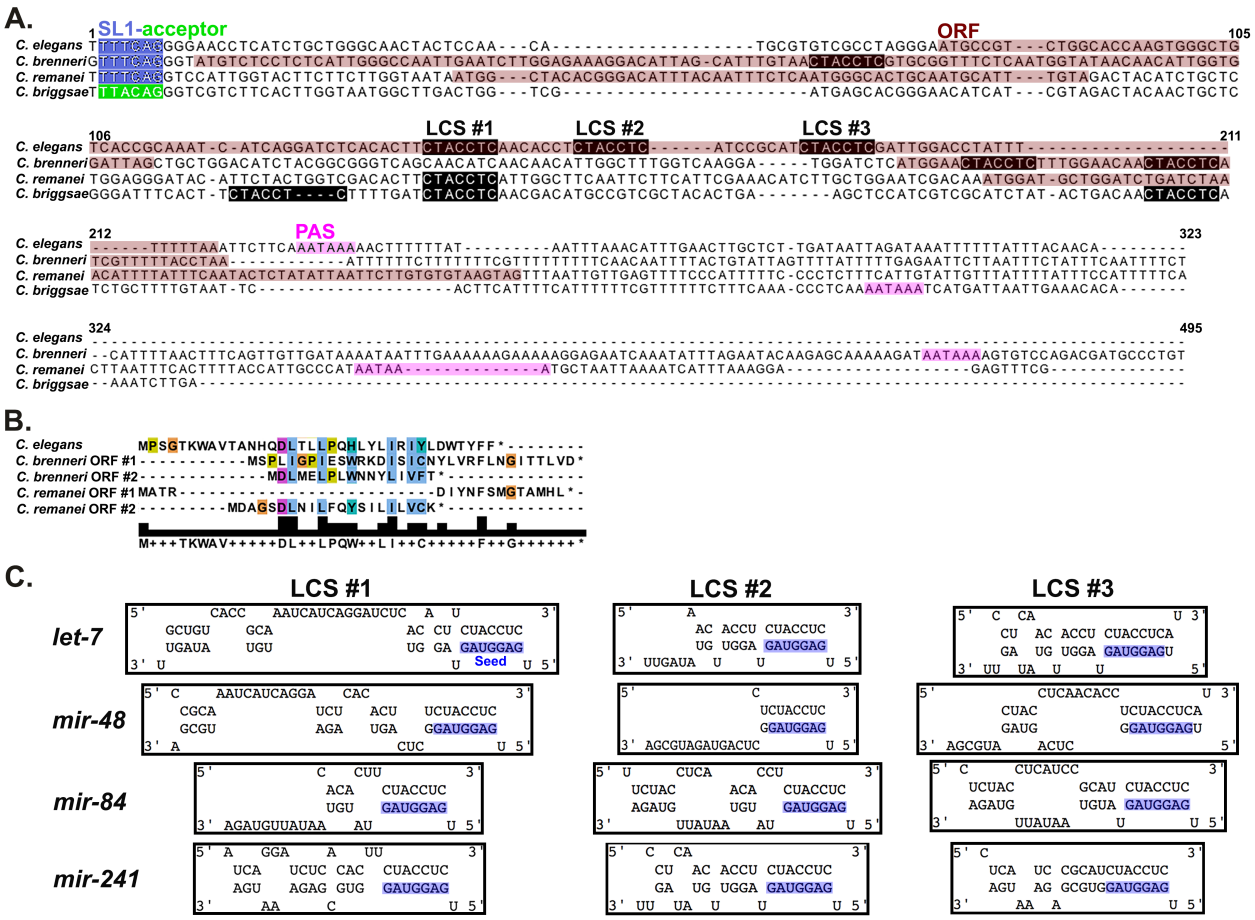

Figure S1. The LCE and SL1 splice acceptor sequences are conserved among *Caenorhabditis* species.

(A) Genomic alignment of the regions downstream (3') from the *pre-let-7* stem-loop of four *Caenorhabditis* species' *let-7* loci. Highlighted in blue is the canonical SL1-acceptor sequence. Highlighted in green is a non-canonical SL1-acceptor sequence. Red shading marks potential open reading frames (ORF). Black shading indicates is *let-7 complementary sequence* (LCS). Predicted polyadenylation signals (PAS) are shaded in pink.

(B) Amino acid alignment of the potential ORFs of the *SL1-LCE*s.

(C) Predicted RNA hybridization of *let-7fam* microRNAs (bottom strands; 5' to the right) with the three LCSs in *C. elegans SL1-LCE*. Highlighted in light blue is the seed sequence of each microRNA.

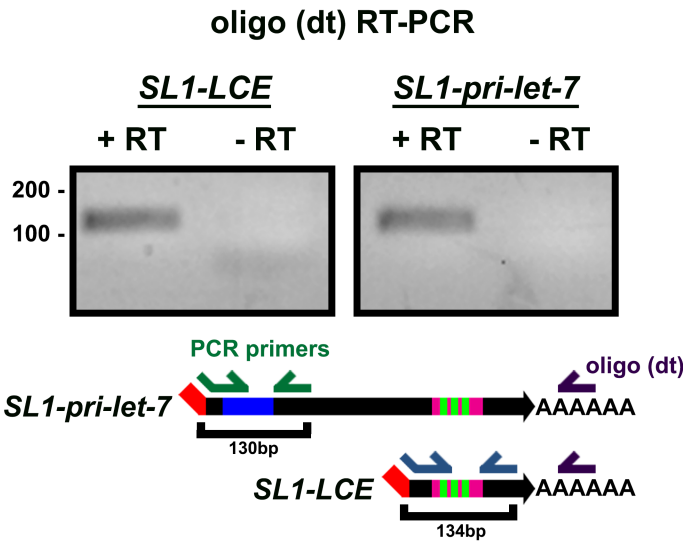

Figure S2. *let-7* locus transcripts are polyadenylated.

Non-quantitative RT-PCR of cDNA made from total RNA from a mixed-population of WT animals with (+) or without (-) RT in the cDNA synthesis step. The left panel shows products of a PCR reaction using primers (green in the diagram) specific for *SL1-pri-let-7*; the right panel shows products of a PCR reaction using primers (turquoise in the diagram) specific for *SL1-LCE*. Numbers mark dsDNA ladder bands in bp.

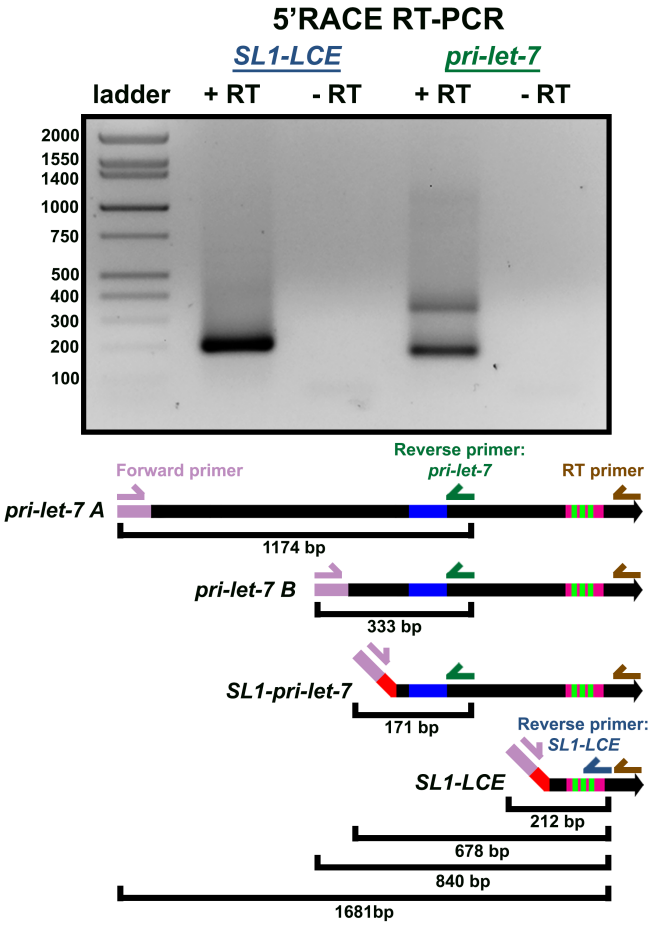

Figure S3. The *SL1-LCE* is the only detectable transcript from the *let-7* locus that does not contain *pre-let-7*.

Non-quantitative 5'RACE RT-PCR of cDNA made from total RNA from molting L2 WT animals (24 hours after plating) with (+) or without (-) RT in the cDNA synthesis step. The left two lanes show products of PCR reactions using a primer pair (turquoise and pink) that is expected to amplify all LCE containing *let-7* transcripts. Note: *SL1-LCE* is preferentially amplified because of the short product produced from *SL1-LCE* compared to the products produced from *pri-let-7* isoforms. The right two lanes show products of PCR reactions using a primer pair (green and pink) that is expected to amplify *pri-let-7* isoforms only. Note: two *pri-let-7* isoforms (*B* and *SL1*) are preferentially amplified due to their shorter product sizes. Numbers mark dsDNA ladder bands in bp.

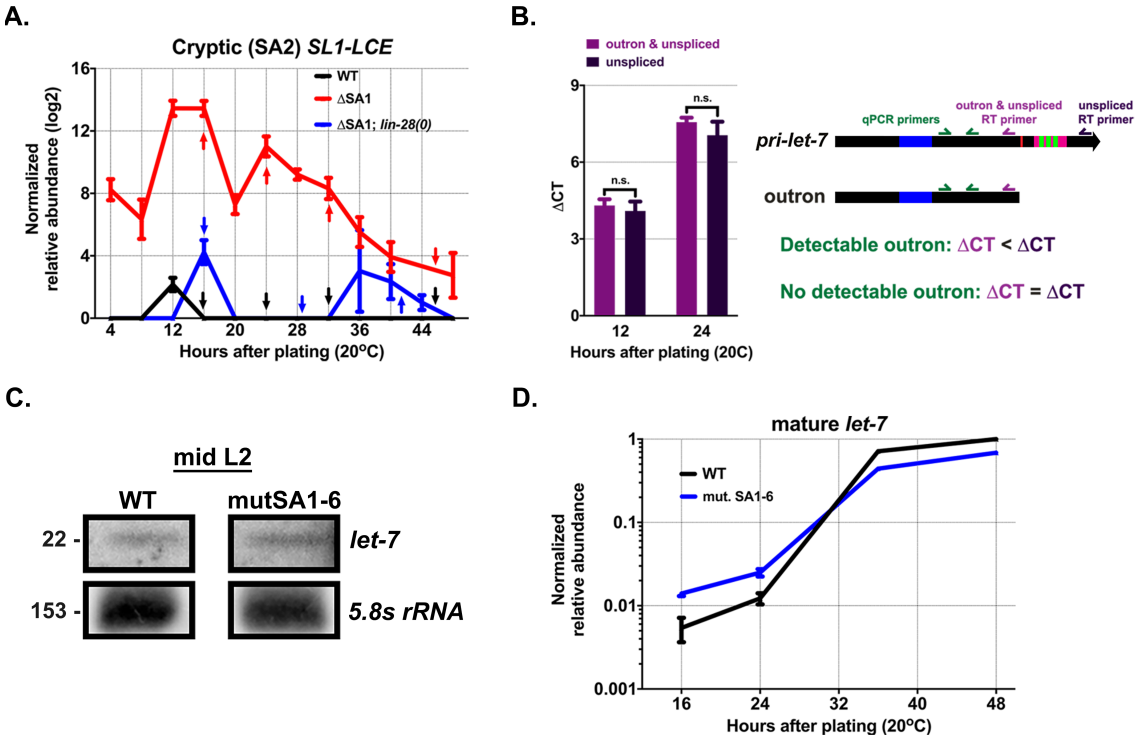

Figure S4. Deletion of the canonical splice acceptor result in the use of non-canonical splice acceptors, the *pri-let-7* outron in not detectable in L1 and L2 larvae, and mutations that reduce LCE trans-splicing display elevated levels of mature *let-7* in the L1 and L2 stages and reduced levels in the L3 and L4 stages, compared to WT.

(A) qRT-PCR developmental profile of the levels of an *SL1-LCE* transcript (*Cryptic(SA2)SL1-LCE*) that contains SL1 spliced to a cryptic SA sequence (TTGTAG). *Cryptic(SA2)SL1-LCE* levels were determined for samples from wild type (black), ΔSA1 (red), and doubly-mutant ΔSA1; *lin-28(0)* (blue) animals throughout development. Data are represented as mean ±SD. n's = 3 biological replicates. Arrows mark the times of larval molts.

(B) qRT-PCR analysis of cDNA synthesized from WT mid-L1 (12 hours after plating) and molting-L2 (24 hours after plating) either upstream (outron & unspliced RT primer) or downstream (unspliced RT primer) of the canonical splice acceptor. Data are represented as mean ±SD. n = 3's biological replicates. Statistical significance was determined using a two-tailed Student's t test.

(C) Total RNA from mid-L2 (20 hours after plating) WT and mutSA1-6 animals analyzed by northern blotting with a probe for *let-7* mature microRNA. Numbers mark RNA sizes in nt.

(D) FirePlex miRNA analysis of *let-7* levels in WT and mutSA1-6 animals throughout development. Data are represented as mean ±SD. n = 3.

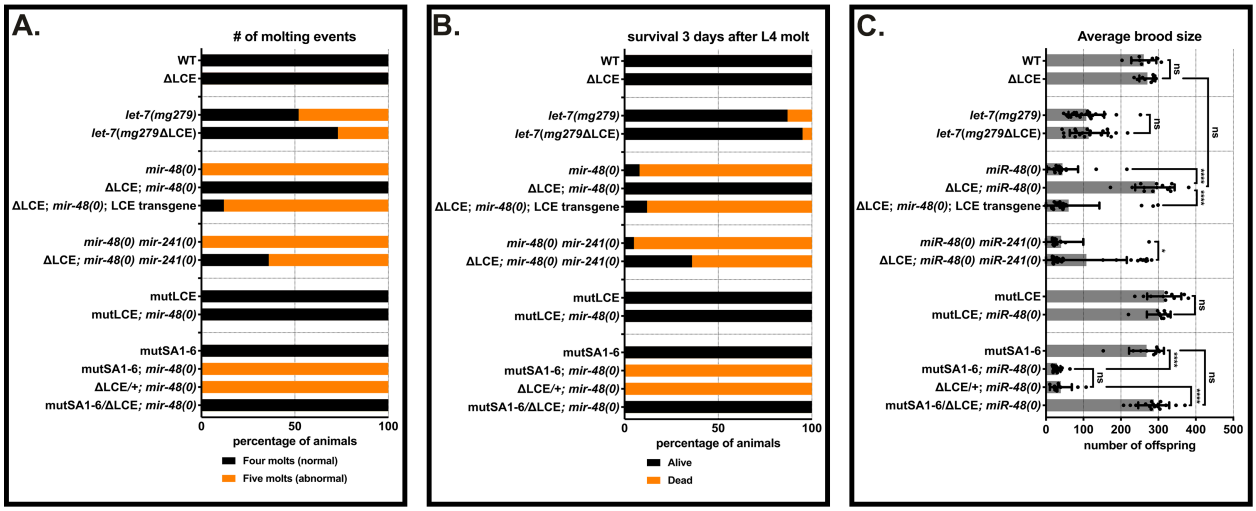

Figure S5. Inhibition of *SL1-LCE* function, either by deletion of the LCE, or by mutations of LCE-proximal trans-splicing acceptor sequences, suppresses multiple phenotypes associated with the retarded development of *mir-48(0)* animals.

Deletion (ΔLCE) or mutation (mutLCE) of the LCE or mutation in the SAs (mutSA1-6) suppresses (A) the extra molt, n's from top (WT) to bottom (mutSA1-6/ΔLCE; *mir-48(0)*): 9, 10, 23, 12, 9, 22, 26, 18, 15, 10, 15, 10, 10, 11, and 16, (B) adult lethality, n's from top (WT) to bottom (mutSA1-6/ΔLCE; *mir-48(0)*): 9, 10, 23, 12, 9, 22, 26, 18, 15, 10, 15, 10, 10, 11, and 16, and (C) reduced brood size of *mir-48(0)* animals, n's from top (WT) to bottom (mutSA1-6/ΔLCE; *mir-48(0)*): 9, 9, 23, 20, 26, 14, 26, 18, 33, 9, 29, 10, 29, 11, and 16 animals. The graphs are quantifications of each respective phenotype observed for each genotype. Statistical significance was determined using a two-tailed Student's t test. P-values: ns > 0.05, * = ≤ 0.05, **** ≤ 0.0001.

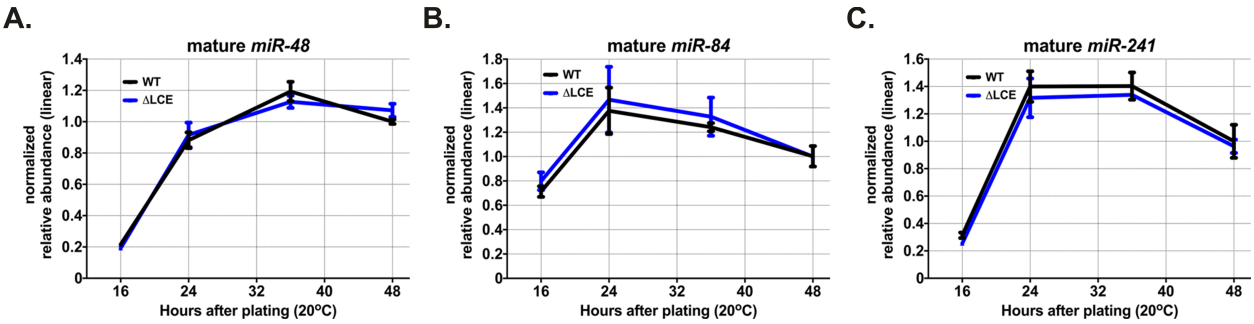

Figure S6. Deletion of LCE sequences from the *let-7* locus does not detectably change the levels of *let-7fam* microRNAs.

FirePlex miRNA analysis of (A) *mir-48*, (B) *mir-84*, and (C) *mir-241* levels in WT and LCE deletion animals throughout development. Data are represented as mean ±SD. n's = 3 biological replicates.

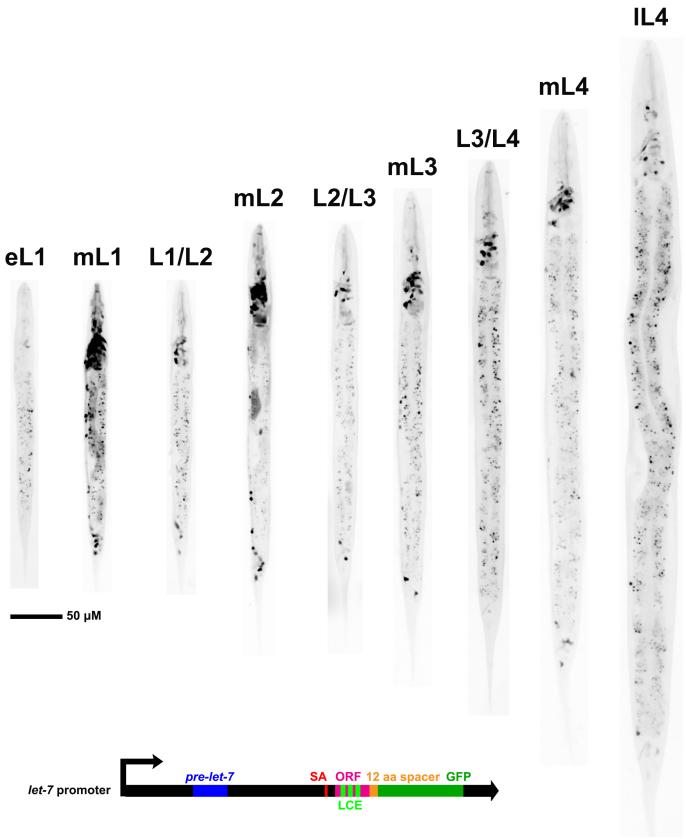

Figure S7. Temporal profile of expression of a GFP-tagged LCE ORF recapitulates that observed for *SL1-LCE*.

Express of GFP in animals carrying an *let-7* locus LCE ORF::GFP transgene throughout development. Images are of representative animals. Note: puncta observed in the intestine is from autoflourescence.