Supplemental materials

# Alternative splicing of the *Caenorhabditis elegans lev-11* tropomyosin gene is regulated in a tissue-specific manner

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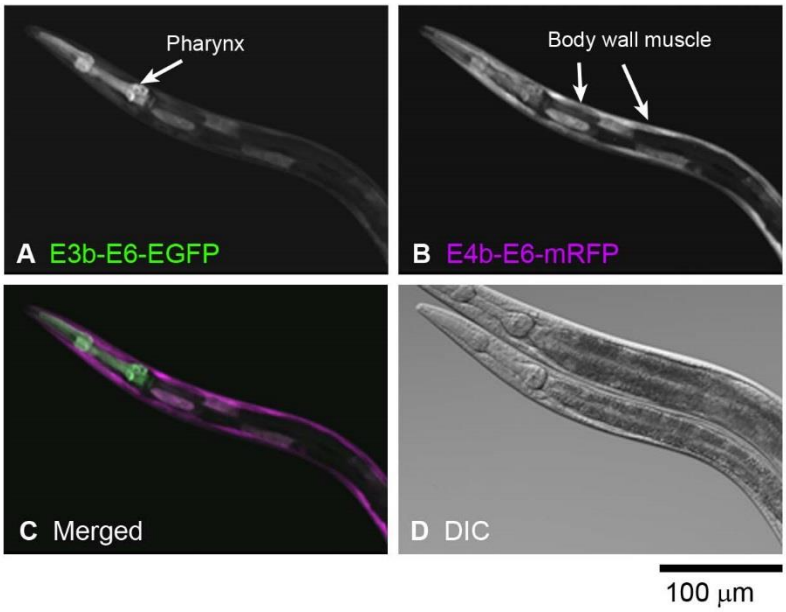
Supplemental Figure S1

Supplemental Tables S1-S3




**Supplemental Fig. S1. Fluorescence splicing reporter analysis for *lev-11* E4s and E5s using a ubiquitous promoter.** The reporter minigenes, LEV-11 E3b-E6-EGFP and LEV-11 E4b-E6-mRFP (see Fig. 3 for the structures), were examined under the control of the ubiquitous *eef-1A.1* promoter. Fluorescence images show expression of E3b-E6-EGFP (A) and E4b-E6-mRFP (B) in L4 larvae. A merged image of EGFP in green and mRFP in magenta (C) and a DIC image (D) are shown. Bars, 100 μm.

**Supplemental Table S1. Worm strains**

| Strain | Transgene | Genotype |
|---|---|---|
| KH1506 | E3b-E6-EGFP<br>E4b-E6-mRFP<br>in all tissues | *lin-15 (n765) X; ybEx1506 [eef-1A.1::LEV-11E3b-E6-EGFP eef-1A.1::LEV-11E4b-E6-mRFP lin-15(+)]* |
| KH1866 | E5a-ECFP<br>E5b-Venus<br>E5c-HcRed<br>in all tissues | *lin-15 (n765) X; ybEx1866 [eef-1A.1::LEV-11E4aE4bxE5aE6-ECFP eef-1A.1::LEV-11E4aE4bxE5bE6-VENUS eef-1A.1::LEV-11E4aE4bxE5cE6-HcRed lin-15(+)]* |
| KH2171 | E9a-mCherry<br>ΔE9a-EGFP<br>in all tissues | *ybEx2171 [eef-1A.1::LEV-11E8E9aE9b-GGS6-EGFP eef-1A.1::LEV-11E8E9aE9b-(+1)GGS6-mCherry lin-15 (+) pRG5271Neo]* |
| KH2445 | E3b-E6-EGFP<br>E4b-E6-mRFP<br>in body wall muscles | *smg-2 (yb979) I; ybEx2445 [myo-3::LEV-11E3b-E6-EGFP myo-3::LEV-11E4b-E6-mRFP pBSKII(-)]* |
| KH2447 | E3b-E6-EGFP<br>E4b-E6-mRFP<br>in pharyngeal muscles | *smg-2 (yb979) I; ybEx2447 [myo-2::LEV-11E3b-E6-EGFP myo-2::LEV-11E4b-E6-mRFP pBSKII(-)]* |
| KH2449 | E3b-E6-EGFP<br>E4b-E6-mRFP<br>in neurons | *smg-2 (yb979) I; ybEx2449 [rgef-1::LEV-11E3b-E6-EGFP rgef-1::LEV-11E4b-E6-mRFP pBSKII(-)]* |
| KH2479 | E3b-E6-EGFP<br>E4b-E6-mRFP<br>in intestine | *smg-2 (yb979) I; ybEx2479 [gst-42::LEV-11E3b-E6-EGFP gst-42::LEV-11E4b-E6-mRFP pBSKII(-)]* |

**Supplemental Table S2. Sequences of the primers used to detect endogenous LEV-11 mRNAs in the RT-PCR assays and molecular cloning.**

| Position | Direction | Sequence |
|---|---|---|
| Exon 1[1] | Forward | 5'-CACCATGGACGCCATTAAGAAGAAGATG-3' |
| Exon 3b[1] | Forward | 5'-CACCATGTCGAAGGTAAACAAGGAGG-3' |
| Exon 9a | Reverse | 5'-TCTCCTGGACGGTCTGTTCG-3' |
| Exon 9b | Reverse | 5'-TCATCATCTCACACAGCAAGTGA-3' |
| Exon 9c | Reverse | 5'-TTAATATCCGGAGAGCTCTTGGAAG-3' |

[1]Underlines indicate CACC sequences for directional TOPO cloning.

**Supplemental Table S3. Sequences of primers used in plasmid construction.**

Primers used to amplify genomic fragments.

| Amplified fragments | Sequence |
|---|---|
| *lev-11* exon 3b – exon 6 | 5′-AAAAGCAGGCTctagacaccATGgGTCAGGCCCGTGAG-3′ |
| | 5′-CCATGGTGGCTCTAGACTCTCCGGCCTCGGCACG-3′ |
| *lev-11* exon 4a – exon 6 | 5′-AAAAAGCAGGCTccaccatgGTGCTTGTGGAGGAGGAC-3′ |
| | 5′-TATACAAAGTTGTCTCTCCGGCCTCGGCACG-3′ |
| *lev-11* exon 4b – exon 6 | 5′-AAAAGCAGGCTctagacaccATGgCCCTCCTCGAGGAAGA-3′ |
| | 5′-CCATGGTGGCTCTAGACTCTCCGGCCTCGGCACG-3′ |
| *lev-11* exon 8 – exon 9b | 5′-AAAAAGCAGGCTccaccatgGAGGTCGACAGACTCGAAG-3′ |
| | 5′-TATACAAAGTTGCACGGAGTTCTGAAATATTGAGTC-3′ |

Underlines indicate parts of *att*B sequences for BP cloning or *Xba* I recognition site (exon 6 Reverse). Lower case indicates nucleotides for introducing Kozak's consensus.

Primers used for mutagenesis.

| Mutation | Sequence |
|---|---|
| *lev-11* exon 4b – 5' splice site | 5′-GACGAaTCTGAGCGaTAtGCaTTGAGATGAG-3′ |
| | 5′-CTCATCTCAAtGCaTAtCGCTCAGAtTCGTC-3′ |
| *lev-11* exon 5a – termination codon | 5′-TGCCAACTTCCTCtAGACTCAAGTCGA-3′ |
| | 5′-TCGACTTGAGTCTaGAGGAAGTTGGCA-3′ |
| *lev-11* exon 5c – termination codon | 5′-GAGCAATCAGATTtAaATGGATGATGACA-3′ |
| | 5′-TGTCATCATCCATtTaAATCTGATTGCTC-3′ |
| *lev-11* exon 5b – termination codon | 5′-GGAGCGCGCCAACtaaGTTGAGGCCCAAC-3′ |
| | 5′-GTTGGGCCTCAACttaGTTGGCGCGCTCC-3′ |
| *lev-11* exon 9a – termination codon | 5′-GATCCAAGGATCCcAAATAGTACGGTA-3′ |
| | 5′-TACCGTACTATTTgGGATCCTTGGATC-3′ |

Lower case indicates nucleotides for disrupting potential splice sites (exon 4b), introducing termination codons (exon 5s), or disrupting a termination codon (exon 9b).