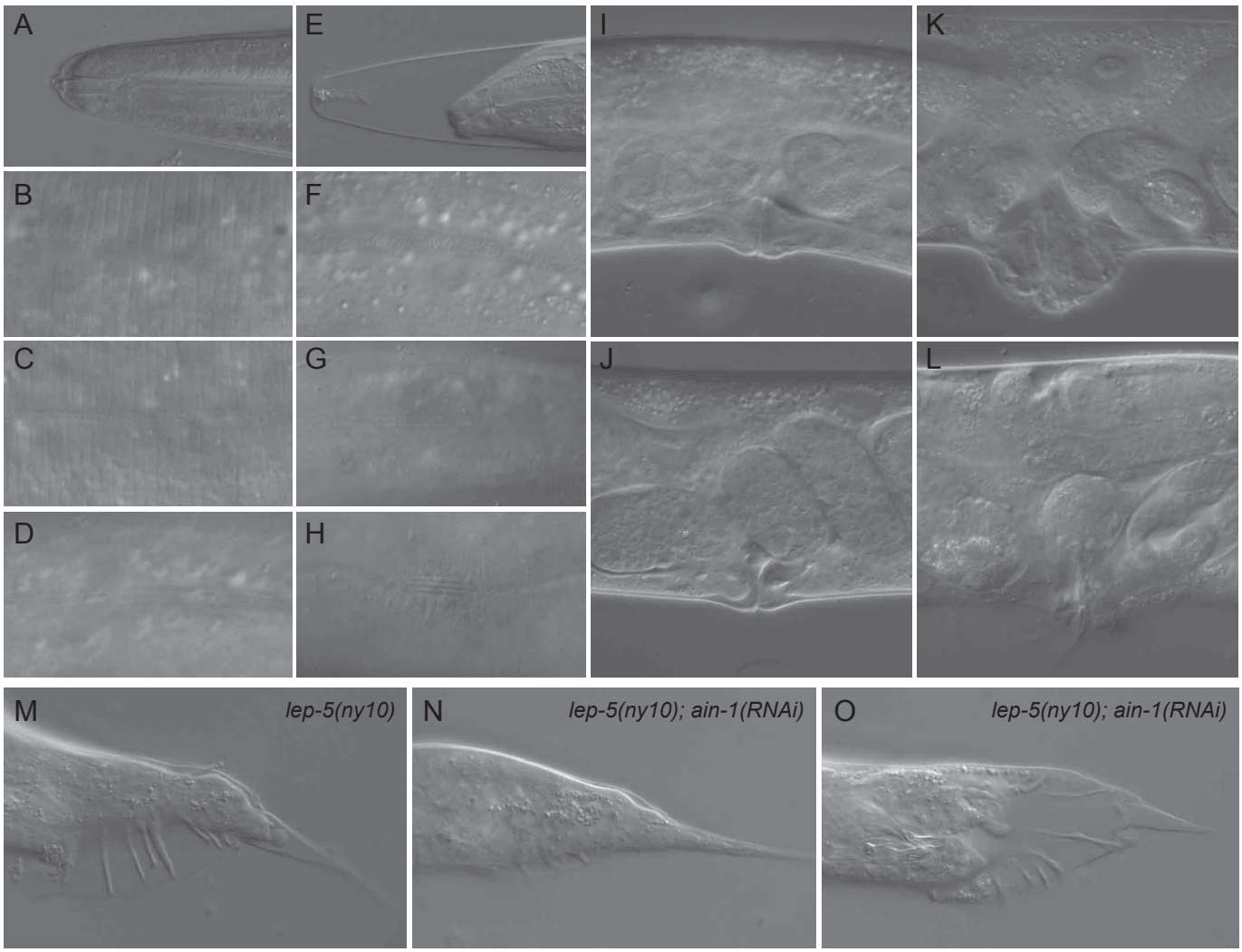

Figure S1. Additional *lep-5(ny10)* phenotypes, related to Figure 1.
(A, E): Head of a hermaphrodite undergoing a supernumerary molt. (B-D) Lateral fields of hermaphrodites before the supernumerary molt. There are no alae (B), incomplete alae (C) or very weak alae (D) in the anterior part of the body; the posterior part of the body usually lacks alae. (F-H) lateral fields of hermaphrodites after the supernumerary molt: Alae are disorganized (F) or incomplete (G and H). (I, J): vulva before (I) and after initiation of the supernumerary molt (J). (K, L) vulva after completion of the supernumerary molt; the animals are Pvul and Egl. (M) Adult male tail with normal fan and Lep tail tip undergoing delayed TTM. (N) Tail of an adult male after *ain-1* RNAi treatment. The fan is narrow and the rays are short, indicating a defect in anterior retraction. (O) Tail of an adult male after *ain-1* RNAi treatment in ventral view showing extreme ectopic retraction of the tail tissue. Rays and phasmids are attached to the cuticle and appear as long processes.

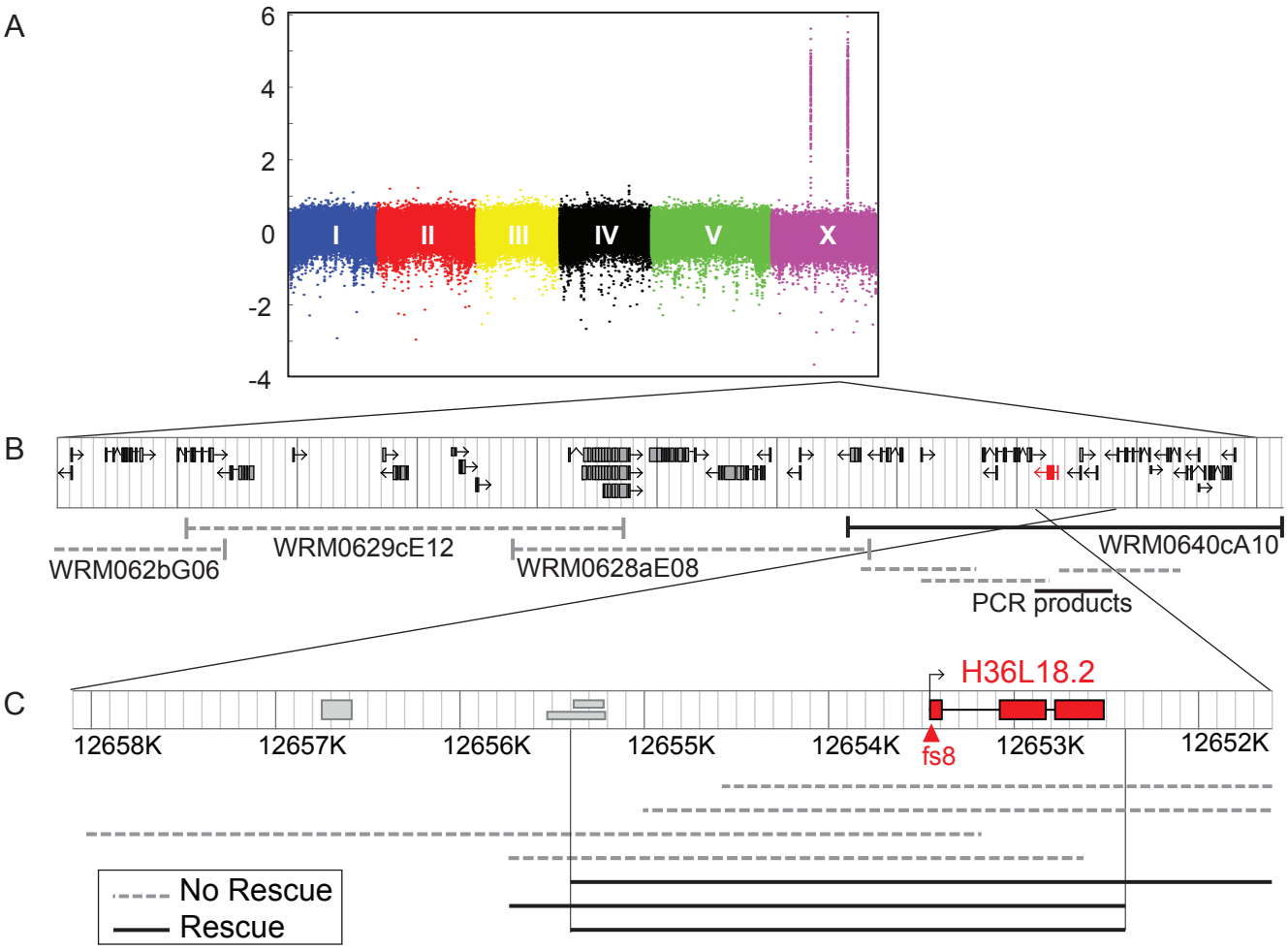

Figure S2. Mapping of *lep-5*, related to Figure 2. (A) Rainbow plot showing the result of array comparative genomic hybridization with DNA from strain DF70 carrying the *lep-5(ny10)* mutation compared to DNA from the control CB4088 strain. DF70 shows two deletions in chromosome X. Subsequent analysis determined that *lep-5(ny10)* is associated with the right deletion. (B) The 80Kb region deleted in *lep-5(ny10)* contains 32 predicted genes and one pseudogene in wild-type DNA. Dashed and solid lines indicate fosmids and PCR products used in experiments to rescue the *lep-5(ny10)* phenotype. (C) Genomic region containing H36L18.2/*lep-5* and graphic display of fragments used in rescue experiments with *lep-5(fs8)*. A minimum length of 1970 nucleotides upstream of the transcription start site of *lep-5* was required for successful rescue, indicating that this region contains important regulatory sites. The point mutation in *lep-5(fs8)* is marked by a red triangle.

B

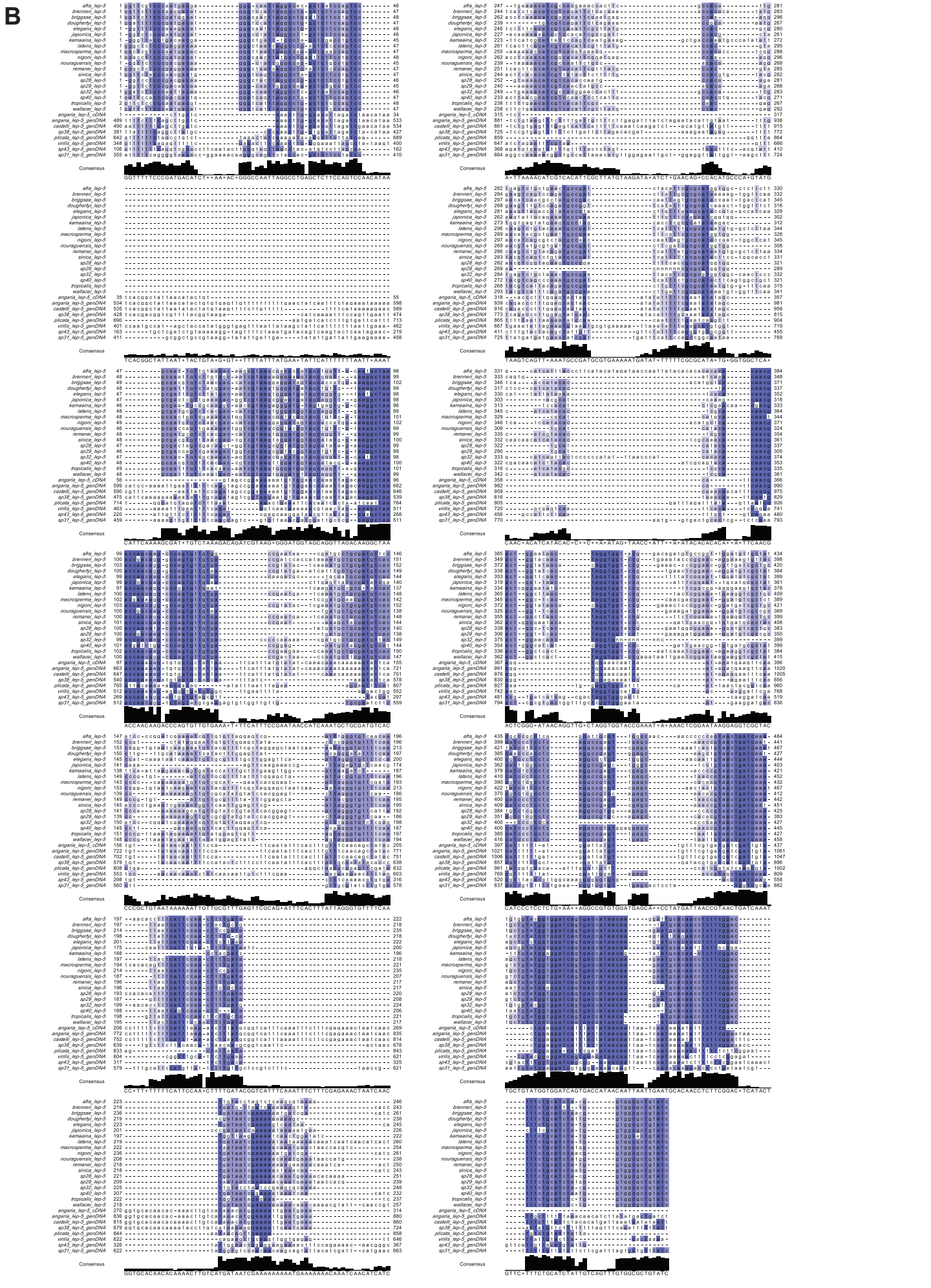
afra_lep-5
brenneri_lep-5
briggsae_lep-5
dougherhtj_lep-5
elegans_lep-5
japonica_lep-5
kamaaina_lep-5
latens_lep-5
macrosperma_lep-5
nigoni_lep-5
nouraguensis_lep-5
remanei_lep-5
sinica_lep-5
sp28_lep-5
sp29_lep-5
sp32_lep-5
sp40_lep-5
tropicalis_lep-5
wallacei_lep-5
angaria_lep-5_cDNA
angaria_lep-5_penDNA
castelli_lep-5_penDNA
sp38_lep-5_penDNA
pilicata_lep-5_penDNA
vinilis_lep-5_penDNA
sp43_lep-5_penDNA
sp31_lep-5_penDNA
Consensus

Figure S3. Alignments for *lep-5* orthologs from *Caenorhabditis* species; related to Figure 2. (A) ClustalOmega alignment of *lep-5* sequences from 19 *Caenorhabditis* species. The manually added SL1 sequence is in lowercase letters. Sequencing gaps of unknown size in *Caenorhabditis* sp. 29 and *C.* sp. 32 are indicated by "N". Invariant positions are marked by asterisks. The most highly conserved regions are at the 5'- and 3'-ends of the sequence. (B) MAFFT alignment of *lep-5* coding sequences, cDNA, and genomic sequences from 26 *Caenorhabditis* species. Sequences were identified and aligned as described in the Supplemental Experimental Procedures. The first 19 *lep-5* coding sequences from the *Elegans* supergroup (Kiontke et al., 2011) are identical to those in Figure S3. Portions of those 19 *lep-5* sequences shown here correspond to the following subsequences: *C. afra*, nt 1-563; *C. brenneri*, nt 1-517; *C. briggsae*, nt 1-547; *C. doughertyi*, nt 1-507; *C. elegans*, nt 1-524; *C. japonica*, nt 1-481; *C. kamaaina*, nt 1-498; *C. latens*, nt 1-531; *C. macrosperma*, nt 1-511; *C. nigoni*, nt 1-547; *C. nouraguensis*, nt 1-491; *C. remanei*, nt 1-521; *C. sinica*, nt 1-527; *C.* sp. 28, nt 1-503; *C.* sp. 29, nt 1-472; *C.* sp. 32, nt 1-506; *C.* sp. 40, nt 1-521; *C. tropicalis*, nt 2-503; and *C. wallacei*, nt 1-534. The portion of the *C. angaria lep-5* cDNA sequence shown corresponds to nt 1-484. Other *lep-5* genomic sequences come from the following genome assemblies (given as source URLs), scaffolds within each assembly, and nucleotide coordinates within each scaffold. *C. angaria*: ftp://ftp.wormbase.org/pub/wormbase/releases/WS250/species/c_angaria/PRJNA51225/c_angaria.PRJNA51225.WS250.genomic.fa.gz; Cang_2012_03_13_00001, nt 687,640-688,300. *C. castelli*: http://bang.bio.ed.ac.uk/caenorhabditis/caenorhabditis_castelli_JU1956/caenorhabditis_castelli_JU1956_clcSE_1.fna; caenorhabditis_castelli_JU1956_contig_8515; nt 5,033-4,388 (reverse complement of genomic sequence). *C.* sp. 38: http://bang.bio.ed.ac.uk/caenorhabditis/caenorhabditis_sp38_JU2809/caenorhabditis_sp38_JU2809_SPADES assembly.fna; caenorhabditis_sp38_JU2809_NODE_347_length_42521_cov_117.126_ID_693; nt 32,100-32,703. *C. plicata*: http://bang.bio.ed.ac.uk/caenorhabditis/caenorhabditis_plicata_SB355/caenorhabditis_plicata_SB355_clcPEcleaned_2.fna; caenorhabditis_plicata_SB355_contig_1792; nt 2,077-2,506. *C. virilis*: http:bang.bio.ed.ac.uk caenorhabditiscaenorhabditis_virilis_JU1968caenorhabditis_virilis_JU1968_clcSE_1.fna; caenorhabditis_virilis_JU1968_contig_2216; nt 27,994-27,457 (reverse complement of genomic sequence). *C.* sp. 43: http://bang.bio.ed.ac.uk/caenorhabditis/caenorhabditis_sp43_NIC1070/caenorhabditis_sp43_NIC1070_clcSE_1.fna; caenorhabditis_sp43_NIC1070_contig_49733; nt 106-641. *C.* sp. 31: http://bang.bio.ed.ac.uk/caenorhabditis/caenorhabditis_sp31_JU2585/caenorhabditis_sp31_JU2585_clcSE_1.fna; caenorhabditis_sp31_JU2585_contig_6880; nt 9,510-8,889 (reverse complement of genomic sequence).

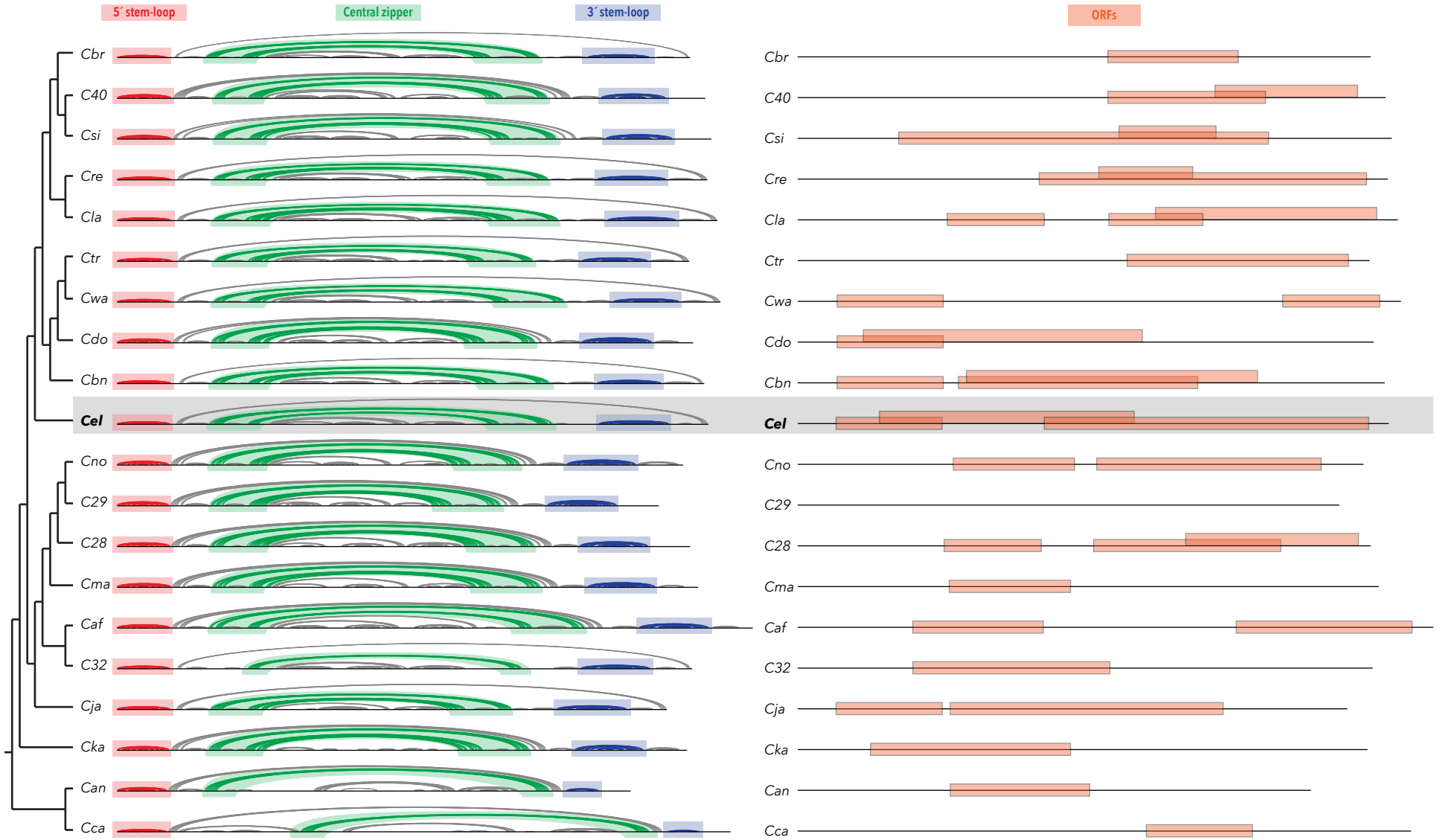

Figure S4. Conservation of predicted *lep-5* secondary structure (left; 5' stem-loop, central zipper, and 3'-stem-loop) and non-conservation of predicted ORFs (right) among 20 species of *Caenorhabditis*; related to Figure 2. Turbofold predictions of secondary structures in linear format drawn using VARNA are shown at left for species of *Caenorhabditis*, showing their phylogenetic position (cladogram at far left). Relative positions of ORFs predicted for each *lep-5* ortholog are depicted as orange bars at right. Abbreviations for species are: *Cbr: C. briggsae, C40: C.* sp. 40, *Csi: C. sinica, Cre: C. remanei, Cla: C. latens, Ctr: C. tropicalis, Cwa: C. wallacei, Cdo: C. doughertyi, Cbn: C. brenneri, Cel: C. elegans, Cno: C. nouraguensis; C29: C.* sp. 29*; C28: C.* sp. 28, *Cma: C. macrosperma, Caf: C. afra, C32: C.* sp. 32, *Cja: C. japonica, Cka: C. kamaaina, Can: C. angaria, Cca: C. castelli*. See Supplemental Experimental Procedures for details about secondary structure and ORF predictions.

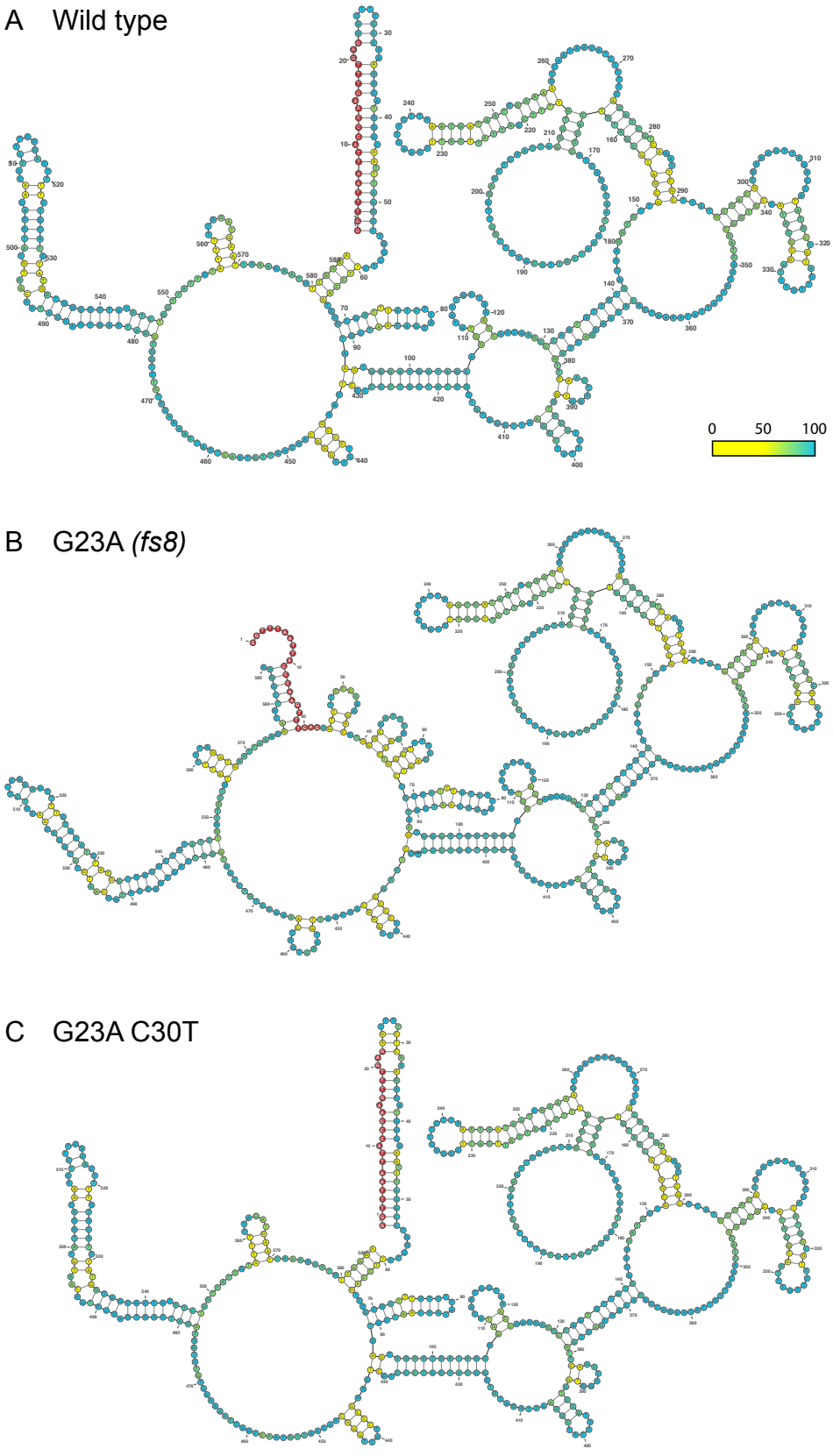

Figure S5. Predicted structures of *lep-5* RNAs indicating base-pairing confidence, related to Figure 2. Shown are predictions for the secondary structure of full-length (A) wild-type *lep-5*, (B) *lep-5*(G23A), and (C) *lep-5*(G23A C30T). SL1 sequence is shown in red. Other nucleotide positions are colored according to base-pairing confidence as predicted by Turbofold, with yellow and blue representing low and high confidence, respectively.

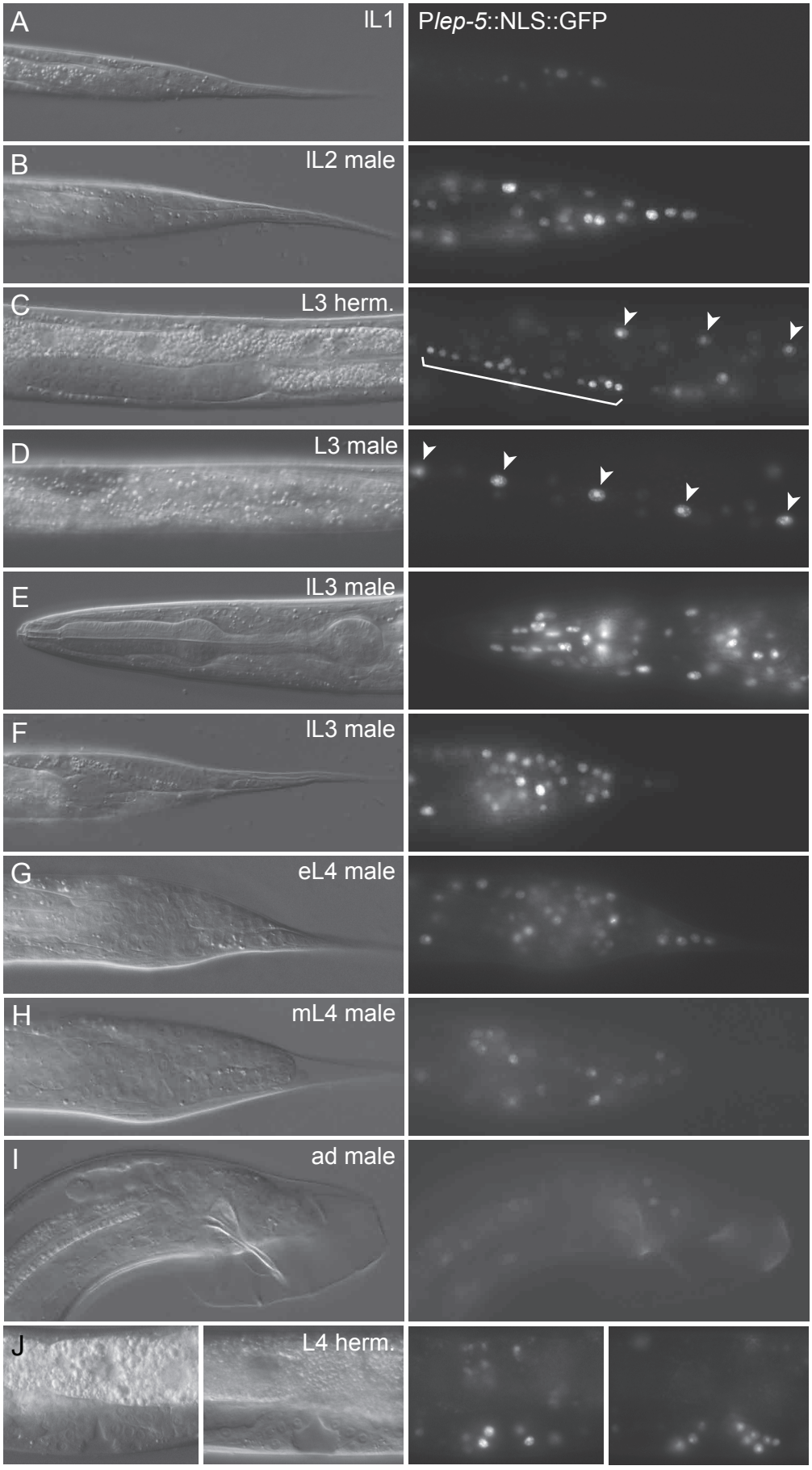

Figure S6. Spatiotemporal expression of P*lep-5*::NLS::GFP, related to Figure 3. (DIC images left, fluorescent images right.) Expression begins to be faintly visible in late L1 animals (A). It becomes bright during L2 and remains bright until late L3 (C-F). Expression diminishes in early-mid-L4 (G, H) and is almost gone in adults (I). Expression is observed in the tail epidermis including the tail tip cells (B, F, G)), in the pharnyx muscles (E), nerve cells in the pharynx (E) and cloacal region (F) and in the ventral nerve cord (C, bracket). The reporter is also expressed in seam cells (arrowheads in C and D) and in the vulva cells during vulva morphogenesis (J).

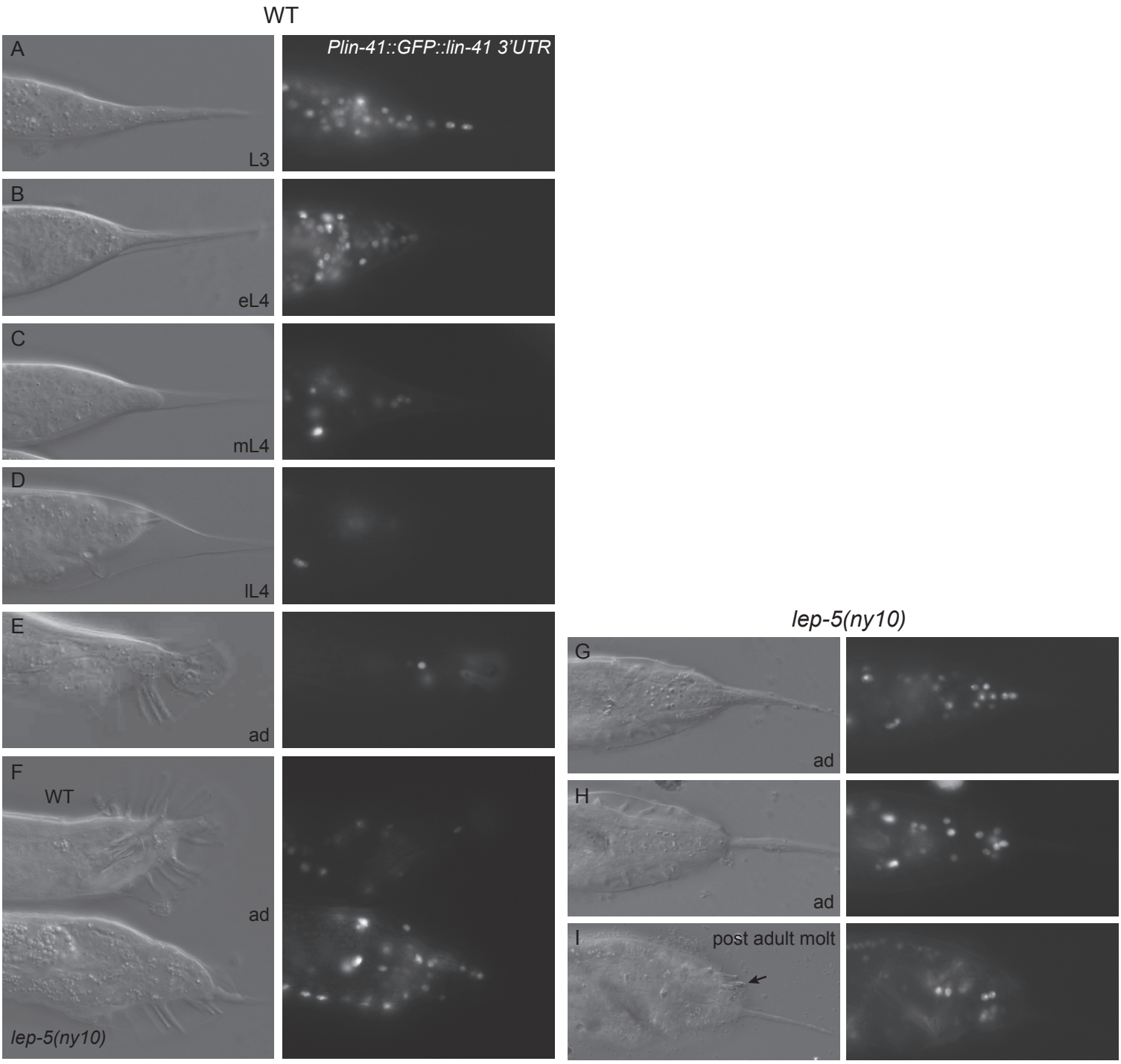

Figure S7. *lin-41* expression persists into adulthood in *lep-5* mutants, related to Figure 4.
Expression of a transcriptional P*lin-41*::NLS::GFP::lin-41 3'UTR reporter in wild type and *lep-5(ny10)* mutant animals (left panels show DIC images for comparison). in wild type (A-E), the reporter is expressed brightly in many nuclei of the tail, including the tail tip cells in larvae as early as L2. It diminishes after the L3 stage and is restricted to few cells in late L4 and adults. (A-E, top animal in F). In *lep-5(ny10)* mutants (bottom animal in F, G-I), expression remains bright in adults and is even visible in older animals that have undergone an ectopic molt (I; arrow points to a ray which remains a finger-like process after molt of the fan).

Table S1. Primers, guide RNAs, and ssODN repair templates, related to STAR Methods

Rescue experiments:
pCC1-forward: ggatgtgctgcaaggcgattaagttgg (to test fosmids)
H36L18.2_F: gggcgaaatgagctttgaatgatttgttcgtgg
H36L18.2_R: gaccagataaaggtacgtgagcgagattatggtattccg
lep-5F1: gtaattcgcgtttctaggtg
lep-5R1: gggcgaaatgagctttga
lep-5F2: gaccagataaaggtacgtgag
lep-5R2: tgctacaagagcaaagtattta
lep-5F3: cttcaaacacaactgctcttc
lep-5R3: tagttgaaccagtcgttgtgt
lep-5F4: aaagtacatgcgaacttgtgt
lep-5R4: gggcgaaatgagctttga
lep-5F5: cttcaaacacaactgctcttc
lep-5R5: accagcatatagagttttgca
lep-5F6: aaagtacatgcgaacttgtgt
lep-5R6: accagcatatagagttttgca
attBlep-5F6: *ggggacaactttgtatagaaagttg*aaagtacatgcgaacttgtgt
attBlep-5R6: *ggggactgctttttttgtacaaacttg*accagcatatagagttttgca

Secondary structure modifications:
KKlp5_expr-9: caaaagtacatgcgaacttgtgtgc
KKlp5_expr-10: cggtcactttggttccattgaatc
KKlp5_expr-1: cacttcaaacacaactgctcttccttatc
KKlp5_23A+30T-R: gcccatgtctttgagaaaactctgaaaattgaaaataatcgataacttaaattcg
KKlp5_23A+30T-F: aatttaagttatcgattattttcaattttcagagttttctcaaagacatg
KKOLp5-8a: ccattatgaaacccagtcgtaagcg
KKlp5_23C_R: ccatgtctttgggaaaacgctgaaaattgaaaataatcgataacttaaattcg
KKlp5_23C_F: aatttaagttatcgattattttcaattttcagcgttttcccaaagacatg
KKlp5_23C+30G-R: gcccatgtctttgcgaaaacgctgaaaattgaaaataatcgataacttaaattcg
KKlp5_23C+30G-F: aatttaagttatcgattattttcaattttcagcgttttcgcaaagacatg
RHOLp5-2: gcttgttggtttagcattacacc
KKlp5_Cbr-AR: ctaagttgcccatgccatcgggaagaaccctgaaaattgaaaataatcgataacttaaattcg
KKlp5_Cbr-BF: tatttatcgaatttaagttatcgattattttcaattttcagggttcttcccgatggcatgggc
KKlp5_Cbr-nR: ctctcgtcatggaaaacaacaaaag
KKOLp5-1 taatttaggtactggctgttggtttatgaag
KKlp5_Can-AR: gttagatctaatgtgccaagtttgagcaagctgaaaattgaaaataatcgataacttaaattcg
KKlp5_Can-BF: cgaatttaagttatcgattattttcaattttc*ag*cttgctcaaacttggcacattag
KKlp5_Can-nR: tcagcaaagataaatattggagaatatcgg

Site-directed mutagenesis
AA4,5,7F: gcagcatcttgtcacaacact**a**ggctt**a**tt**a**gtttagcattacacctgctat
AA4,5,7R: atagcaggtgtaatgctaaac**t**aa**t**aagcc**t**agtgttgtgacaagatgctgc
AA14,15F: gattatttgatgggtgacatc**ct**atc**a**cagcatcttgtcacaacactg
AA14,15R: cagtgttgtgacaagatgctg**t**gat**ag**gatgtcacccatcaaataatc

Deletions
Δ1F: *gtacgggacgtc*tatctggttattgtatttttagatg
Δ1R: *gtacgggacgtc*atccaccagacagcttttgatcag
Δ2F: *gtacgggacgtc*gtcaataacatcttttcacatcg
Δ2R: *gtacgggacgtc*caccctaattgaactccaagc

CRISPR

lep-5_5'_crRNA_1: gatgggtgaaatgaaaaggg
lep-5_3'_crRNA: ggacttttctgcatctatgg
KK_CR_lep-5_1: acgcgatgggcagaaatagtagc
KK_CR_lep-5_2: ttcggtcccaacattctttaattc
lep-5_fs18_crRNA: **mUmCAAAUUGUUGCGUUUUGCU**GUUUUAGAGCUAUGCUGUUUUG
lep-5_fs18_ssODN: cgatgtcacccatcaaataatcaaattgttgcgtta**aagctt**ggagttcaattagggtgttttccattattcattc
lep-5-fs18-detect-F: tgtgcaccacaatttcgcaa
lep-5-fs18-detect-R: tccaccagacagcttttgatca
lep-5_fs19_crRNA_1: **mAmCUGAUCAAAAGCUGUCUGG**GUUUUAGAGCUAUGCUGUUUUG
lep-5_fs19_crRNA_2: **mGmGACUUUUCUGCAUCUAUGG**GUUUUAGAGCUAUGCUGUUUUG
lep-5_fs19_ssODN: tgttgggcaacaattgtaactgatcaaaagctgt**ctgcag**gcgctgtatctggttattgtatttttagatgaat
lep-5-fs19-detect-F: tcgtcaaattcgcttatgccac
lep-5-fs19-detect-R: taagcggtcactttggttccat
lep-5_fs21_crRNA: **mAmUUUUCAGACAAUCGUAAGU**GUUUUAGAGCUAUGCUGUUUUG
lep-5_fs21_ssODN: aattgctcaatttttgtattttcagacaatcgtatctg**gcatgc**ttggaggtgtaatgctaaaccaacaagcccagtgttgtg
lep-5_fs22_crRNA: **mUmGCCCAACAAGGCCUGAGGA**GUUUUAGAGCUAUGCUGUUUUG
lep-5_fs22_ssODN: caactaggtggtccgttttatcggaattggatgtcccaa**gcatgc**ctcgacaggccttgttgggcaacaattgtaactgatcaa
lep-5-fs21-detect-F: tggtaagtttgcaggtggct
lep-5-fs21-detect-R: aactttcgcgtggcataagc
lep-5-fs22-detect-F: acaagcccagtgttgtgaca
lep-5-fs22-detect-R: ccagtcgtaagcggtcactt

Transcriptional reporter

KKkp5_GFPA-R: tcctcctgaaaatgttctatgttatgttagtatcaccctgaaaattgaaaataatcg
KKlp5_GFPB-F: cgaatttaagttatcgattattttcaattttcagggtgatactaacataacatagaacattttc
MN-lin-44_8: aacaaaaataggggtgggagcacagg
RHOLp5-7: cagcattgcagttaattctcttg
MN-lin-44_9: gaagctaaaaaacaaagaaattaagagaag

qPCR

Lp5qPCR_F1: ccaaagacatgggcaatttagg
Lp5qPCR_R1: ggcttgttggtttagcattacacc
Y45F10D.4 F: gtcgcttcaaatcagttcagc
Y45F10D.4 R: gttcttgtcaagtgatccgaca
lin28 FW2: tcgacggtagtatcggaggg
lin28 RV2: gaggtgttggtgacgggag
Luc-qPCR_F: gcaaaacgcttccatcttcc
Luc-qPCR_R: tccacaaccttcgcttcaaa
cdc-42F: ctgctggacaggaagattacg
cdc-42R: ctcggacattctcgaatgaag

C. angaria lep-5

Can_lep-5_F: cgagaaactaatcaacggtgc
Anchored Oligo(dT): atgttgacgcagccagtgac(T)20vn (v=a,c,g)
SL1_primer: ggtttaattacccaagtttgag
Can_lep-5_R2: tacaatcgatcatcacgaaacaacataatcc
Can_lep-5_Rn: gtttccatgtttatgacaagtttgtg (for heminested PCR with SL1_primer).