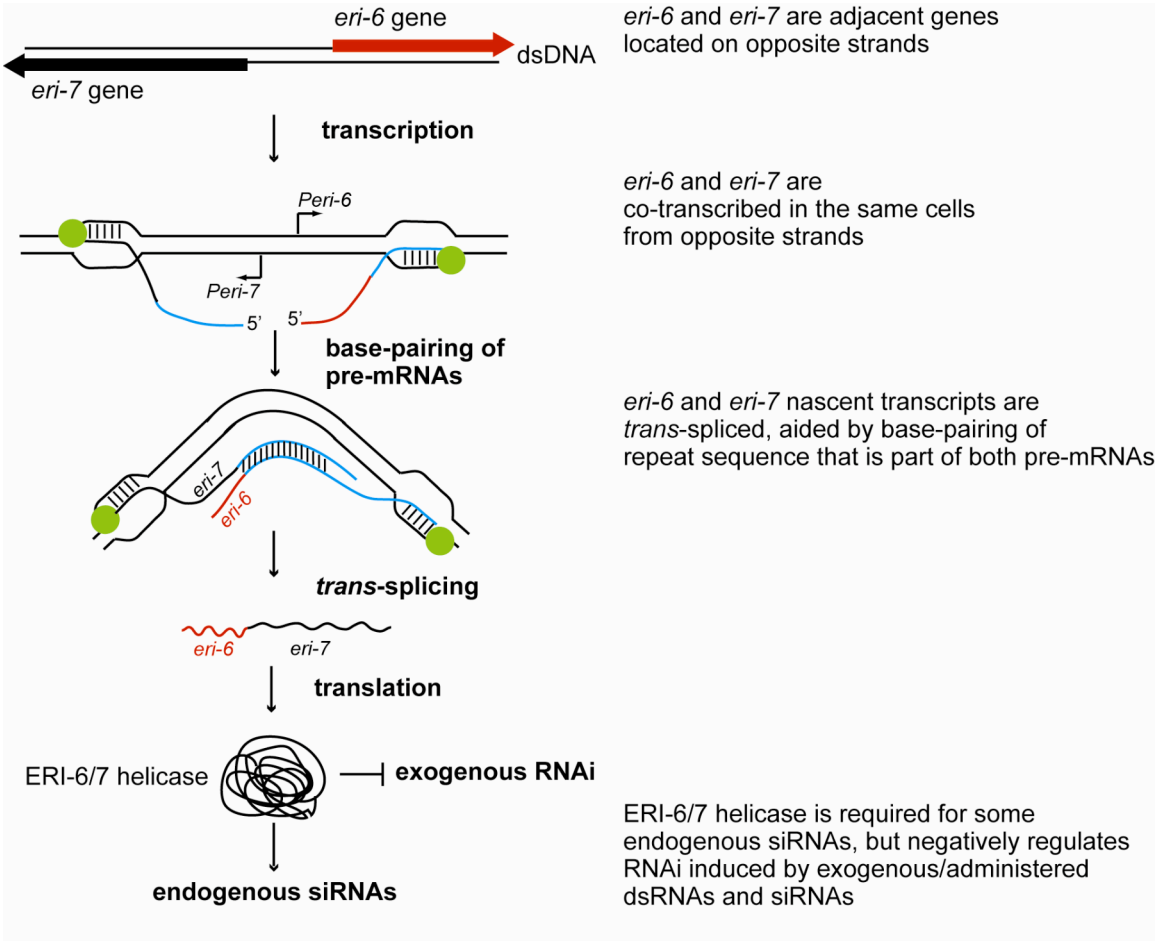
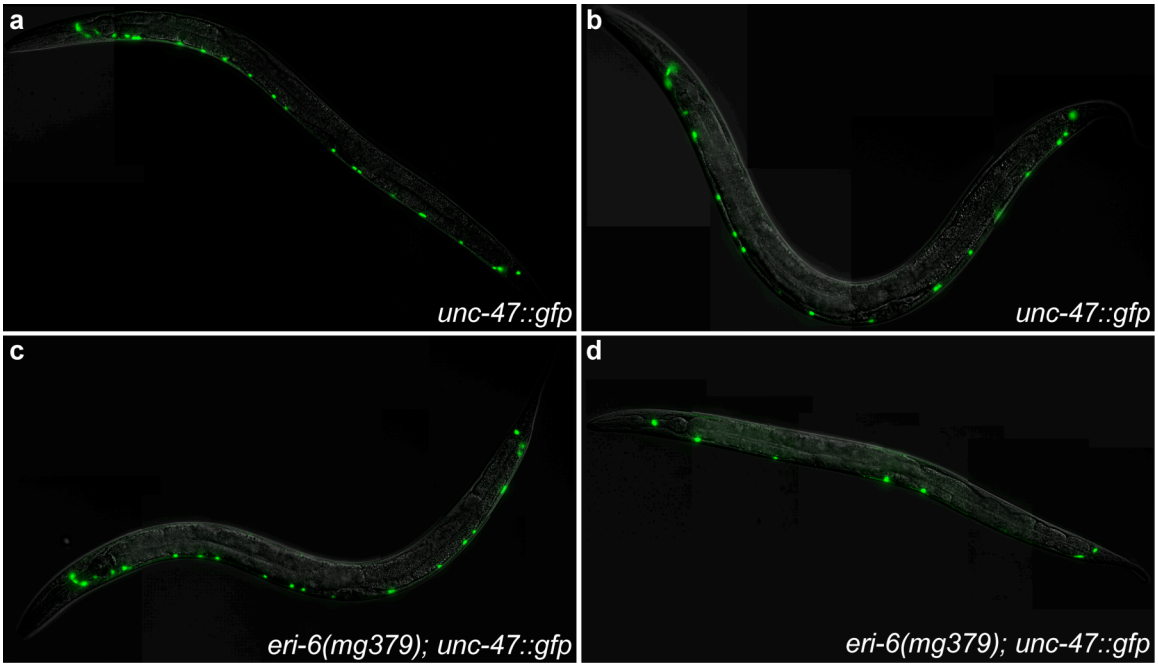
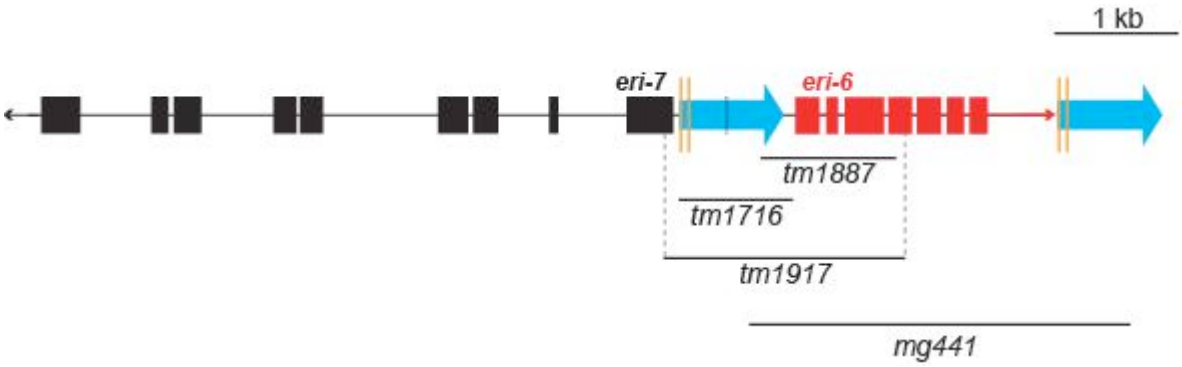
Supplementary Figures and Legends



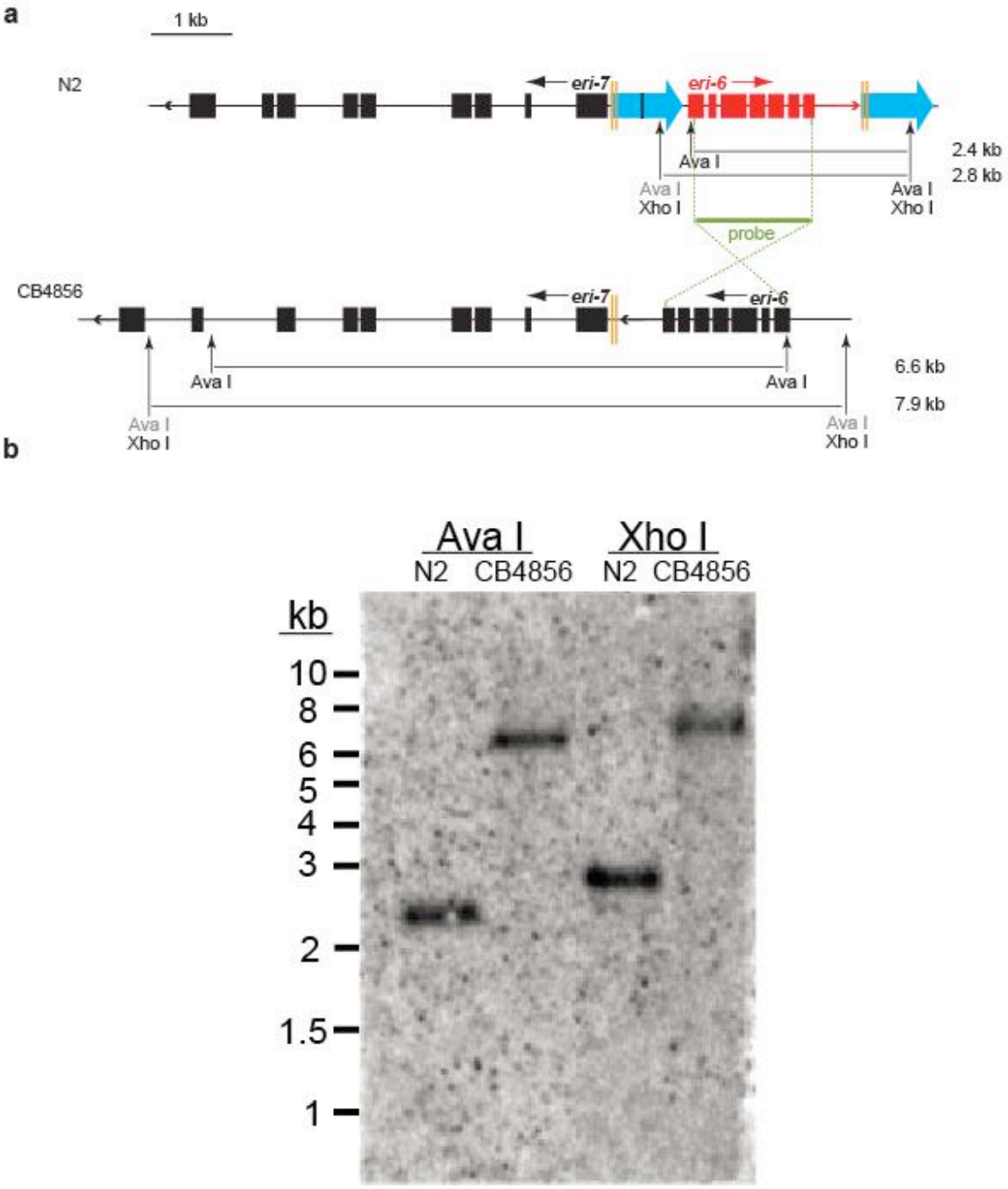
Supplementary Figure 1. Schematic of the main findings of this paper.



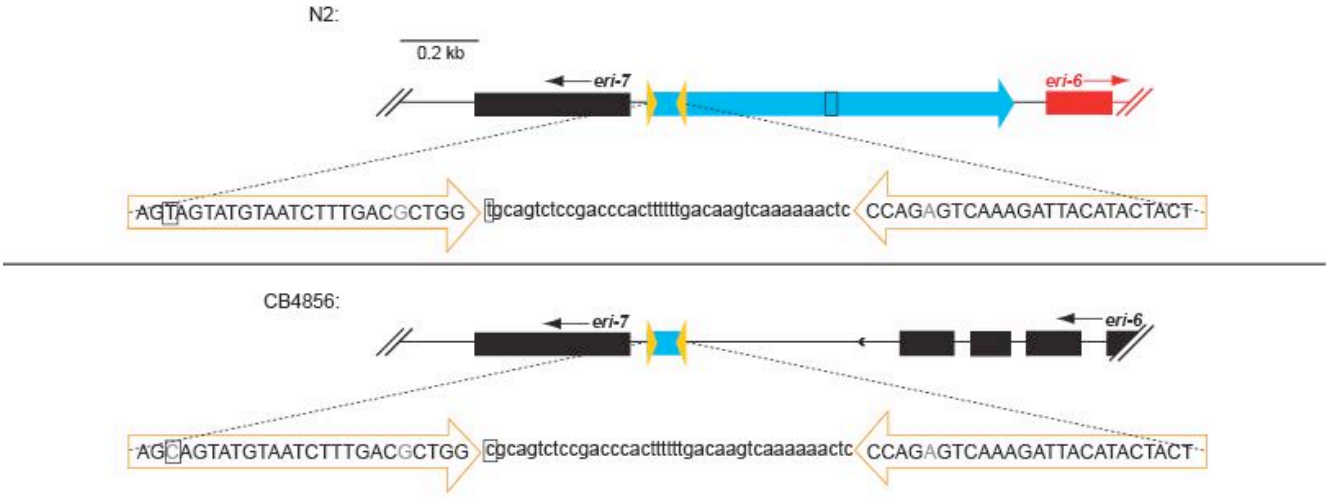
Supplementary Figure 2. *eri-6(mg379); unc-47::gfp* hypersensitivity to *gfp* dsRNA. Wild-type and *eri-6*(*mg379)* worms carrying an integrated *unc-47::gfp* transgene were grown on bacteria expressing either control vector dsRNA (**a, c**) or dsRNA derived from *gfp* (**b, d**). *eri-6(mg379)* animals were backcrossed to wild-type N2 animals six times, and a non-mutagenized *unc-47::gfp* transgene was subsequently crossed back into the *eri-6(mg379)* strain. Representative L4 progeny are shown. Worms were fed from the L1 stage.



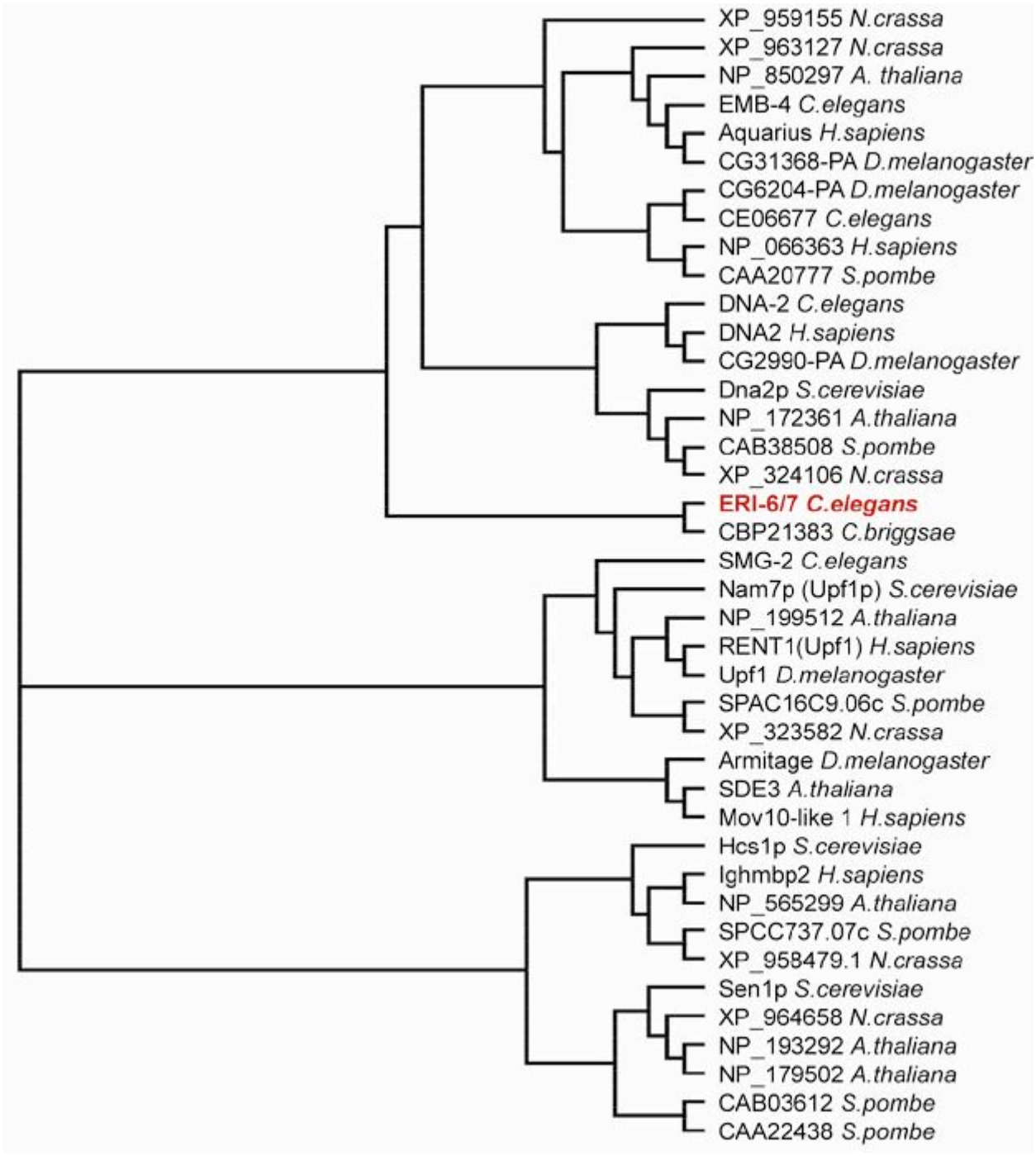
Supplementary Figure 3. ***eri-6/7* deletion alleles.** The blue arrows indicate the 5' 935-bp and 3' 926-bp direct repeats that flank *eri-6*. Open rectangle within blue arrow is an exon predicted[44] that could not be confirmed by RT-PCR. The orange lines designate the 25-bp inverted repeats.



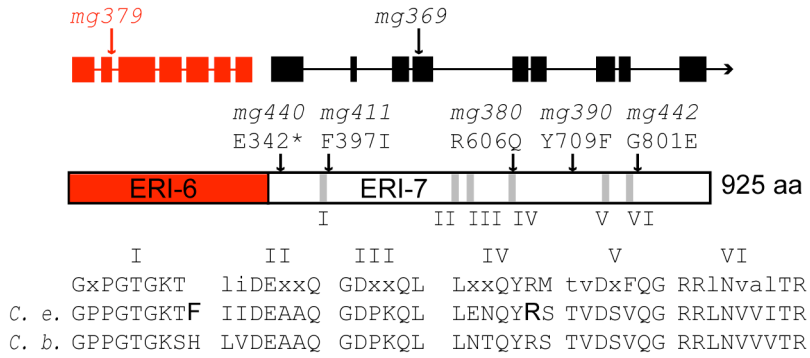
Supplementary Figure 4. ***eri-6/7*** **Southern blot to confirm the *eri-6* gene orientation.** **a**, Schematic of *eri-6/7* locus in N2 and CB4856, showing predicted digest products of restriction enzymes Ava I and Xho I. Sites not relevant for products in grey. Probe used, covering most of the *eri-6* gene, shown in green. Blue arrows are direct repeats. In orange are inverted repeats. **b,** Southern blot confirming antiparallel orientation of *eri-6* and *eri-7* in N2, and parallel orientation in CB4856.



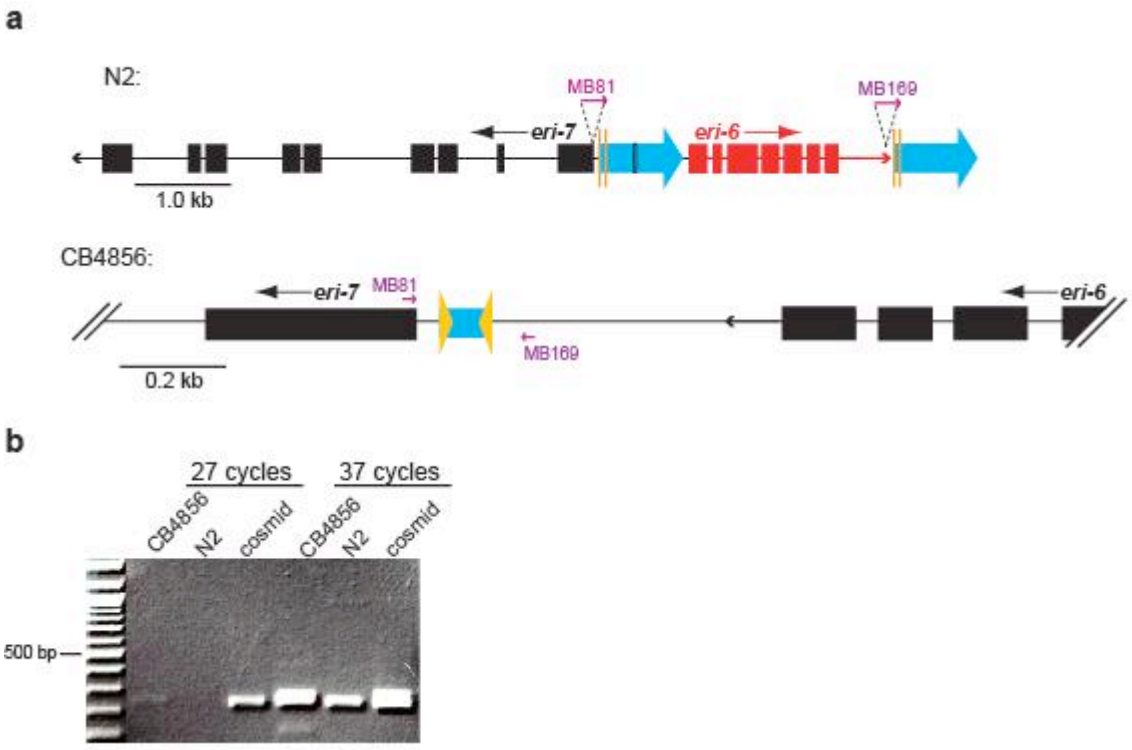
Supplementary Figure 5. ***eri-6/7*** **locus inverted repeat sequence in** ***C. elegans*** **strains N2 and CB4856.** SNPs between N2 and CB4856 are in rectangles. In grey are nucleotide mismatches between the inverted repeat arms. The blue arrow designates the ~930-bp direct repeat that resides upstream (33393 to 34328 of the C41D11 cosmid) and downstream (C41D11 36226 to 37152) of *eri-6* (C41D11 34407 to 35688, from start codon to predicted stop). The orange arrow heads designate the 25-bp inverted repeats.



Supplementary Figure 6. **A phylogenetic tree of superfamily I helicases.** Complete helicase protein sequences were aligned. In bold red is *C. elegans* ERI-6/7.



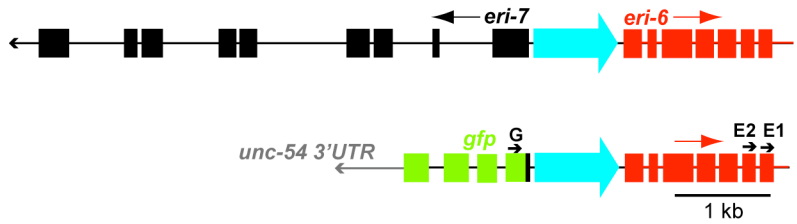
Supplementary Figure 7. *eri-6/7* encodes a superfamily I helicase (COG1112). The *eri-6/7* mutations identified include the following: (i) mutations that specify early stop codons due to a frameshift (*mg369, mg379*), (ii) a nonsense mutation (*mg440* E342*), (iii) and mutations resulting in amino acid substitutions in, or close to, highly conserved motifs in the ATPase and helicase domains (*mg380* R606Q, *mg390* Y709F, *mg411* F397I and *mg442* G801E). The consensus conserved motifs[43] found in superfamily I helicases are compared to the sequences found in *C. elegans* and *C. briggsae*.



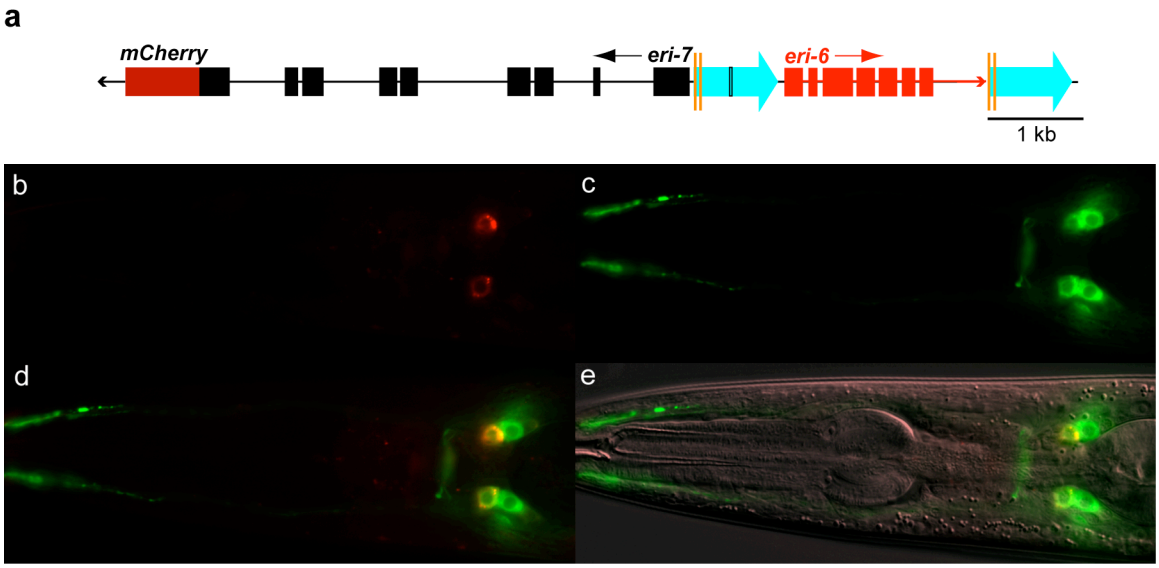
Supplementary Figure 8. **PCR assay to detect genomic rearrangement.** Blue arrow designates the direct repeat. Inverted repeats are in orange. **a**, schematic of *eri-6/7* locus showing where primers (in purple) anneal in N2 and CB4856. MB81 primes at the first nucleotide of the first *eri-7* exon, 41 nt 5' of the inverted repeat present in both N2 and CB4856. MB169 primes in the *eri-6* 3'-UTR, 51 nt 5' of the second inverted repeat in N2, or 51 nt 3' of the inverted repeat in CB4856. **b,** Ultraviolet transilluminator photograph of ethidium bromide-stained agarose gel after electrophoresis of PCR products generated using primers MB81 and MB169 on genomic DNA (CB4856, N2) or cosmid DNA (C41D11).

Consensus 5'-splice site	***a/c a g / G U R a G u u u***
eri-6 5'-splice site	*g c a* / g u a c g g u u
Consensus 3'-splice site	**u U U u C A G / r**
C41D11.1b 3'-splice site	c u c a c a g / g
C41D11.1c 3'-splice site	a u u c c a g / a
C41D11.1d 3'-splice site	a u u c c a g / g

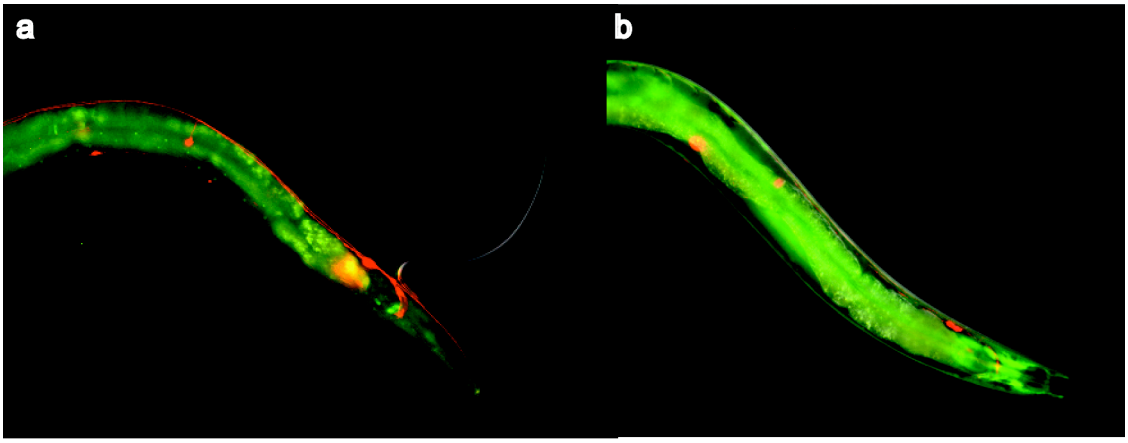
Supplementary Figure 9. **Alignment of splice sites of *eri-6* splice variants and *C. elegans* consensus sequences.** In italics is the ORF sequence of the *eri-6/7* fusion. Consensus sequences are as published[45]. Capital letters are bases that are found in >75% of the splice sites. "/" is the exon/intron boundary.



Supplementary Figure 10. Schematic of the *trans*-splicing reporter in which a *gfp* gene without ATG was cloned 3' of the first two codons of *eri-7*.



Supplementary Figure 11. Cytoplasmic expression of ERI-6/7::mCherry. **a,** Schematic of a construct in which *mCherry* was cloned into the *eri-6/7* locus to produce a full-length translational fusion of ERI-6/7::mCherry. **b**, *mCherry* expression is seen in one neuron in the head. **c**, DiO staining is green. **d**, The merged pictures show the bilateral ASK neurons expressing ERI-6/7::mCherry, predominantly or exclusively in the cytoplasm. **e**, Merged fluorescence and Nomarski pictures showing the position of the neurons in the head of the worm.



Supplementary Figure 12. Induction of the *eri-6/7 trans*-splicing reporter in *rde-1*(RNAi). Transgenic worms carrying the reporter (Supplementary Fig. 9) were fed on **(a)** HT115 *E. coli* expressing dsRNA from vector without an insert or **(b)** with an insert corresponding to the *rde-1* gene. Animals were fed starting at the L1 stage. Representative progeny are shown. GFP expression is induced in multiple tissues, *e.g.* in the head. Body fluorescent signal in **a** is gut autofluorescence.

Supplementary Methods

Southern blotting. 3 μg of genomic DNA was digested used for Southern blotting was done according to Sambrook (Sambrook et al Molecular Cloning). Two probes were used: the *eri-6* probe spans the whole *eri-6* gene, the *eri-7* probe spans the first two exons of *eri-7*.

Genomic rearrangement PCR assay. 100 ng of mixed-stage N2 genomic, RNase A-treated DNA was used for each reaction. Finnzymes Phusion™ High-Fidelity PCR Kit was used with the following cycling conditions: 98ºC for 30 s; between 27 and 38 cycles of [98ºC 10 s, 57ºC 15 s, and 72ºC 30 s]; 72ºC 5 - 7 min. MB81: 5'-ATTTGTATTTTGCTTCTGGTTT-3'. MB169: 5'-TAAGTTTTCCTAAAATGATTTC-3'. Primers were used at a final concentration of 0.5 μM.

Supplementary Tables

Supplementary Table 1. *eri-6/7* alleles confer a generally-enhanced sensitivity to dsRNAs.

Analysis	Genotype	Fed or injected (*) dsRNA	Phenotype scored	Score
Generally Enhanced RNAi	*unc-47::gfp*	control vector	No. GFP^+ neurons	17.8 ± 0.9
	unc-47::gfp	*gfp*	No. GFP^+ neurons	18.9 ± 0.3
	eri-6(mg379); unc-47::gfp	control vector	No. GFP^+ neurons	18.9 ± 0.2
	eri-6(mg379); unc-47::gfp	*gfp*	No. GFP^+ neurons	6.1 ± 0.2
	Wild type	*lin-1*	% Muv	1.1 ± 0.2
	eri-6(mg379)	*lin-1*	% Muv	66 ± 5.3
	eri-7(mg369)	*lin-1*	% Muv	78.1 ± 2.4
	eri-7(mg411)	*lin-1*	% Muv	57.9 ± 6.7
	Wild type	*unc-22* siRNA*	% Twitching	0
	eri-1(mg366)	*unc-22* siRNA*	% Twitching	30
	eri-7(mg369)	*unc-22* siRNA*	% Twitching	18
Non-Complementation	Wild type	control vector	No. progeny	181± 19
	Wild type	*hmr-1*	No. progeny	129 ± 15
	eri-6(mg379)	control vector	No. progeny	209 ± 20
	eri-6(mg379)	*hmr-1*	No. progeny	0 ± 1
	eri-7(mg369)	control vector	No. progeny	192 ± 22
	eri-7(mg369)	*hmr-1*	No. progeny	0 ± 1
	eri-6(mg379) / +	control vector	No. progeny	212 ± 12
	eri-6(mg379) / +	*hmr-1*	No. progeny	90 ± 21
	eri-7(mg369) / +	control vector	No. progeny	172 ± 16
	eri-7(mg369) / +	*hmr-1*	No. progeny	96 ± 9
	eri-6(mg379) / eri-7(mg369)	control vector	No. progeny	240 ± 11
	eri-6(mg379) / eri-7(mg369)	*hmr-1*	No. progeny	0
Epistasis	Wild type	*lin-1*	% Muv	1 ± 0.2
	eri-7(mg411)	*lin-1*	% Muv	53 ± 6.7
	mut-7(pk204)	*lin-1*	% Muv	0
	eri-7(mg411); mut-7(pk204)	*lin-1*	% Muv	0

	rrf-1(pk1417)	*lin-1*	% Muv	0
	eri-7(mg411) rrf-1(pk1417)	*lin-1*	% Muv	0
	rde-4(ne299)	*lin-1*	% Muv	0 ± 0.2
	eri-7(mg411); rde-4(ne299)	*lin-1*	% Muv	2
	rde-1(ne300)	*lin-1*	% Muv	0
	eri-7(mg411); rde-1(ne300)	*lin-1*	% Muv	0

Worms of the indicated genotypes were fed bacteria expressing the indicated dsRNA. In *gfp* RNAi experiments, L1s were fed and their progeny scored at the L4 stage for ventral cord GFP fluorescence. In *hmr-1* RNAi experiments, L1s were fed and scored as adults for brood size. In *lin-1* RNAi experiments, L1s were fed and scored as adults in the following generation for protruding-, bursting-, and/or multi-vulvae (Muv) phenotypes. Scores are the mean ± s.e.m. *unc-22* siRNAs (23 bp with 2 nt, 3' overhangs) were injected and progeny scored. Wild type worms are N2 Bristol.

Supplementary Table 2. Enhanced transgene silencing in *eri-6/7* mutants.

Transgene	**Background**	**Phenotype**	
		Rol	**GFP**
mgIs30[*]	wt	94.5 ± 1.1% Rol	
mgIs30[*]	*eri-7(mg411)*	11.9 ± 4.1% Rol	
mgIs30[*]	*eri-6(mg379)*	1.3 ± 0.9% Rol	
mgIs30[*]	*eri-7(RNAi)*	-	
mgIs30[*]	*eri-6(RNAi)*	-	
pkIs1582[†]	wt	++	
pkIs1582[†]	*eri-7(mg411)*	-	
sur-5::gfp	wt		GFP in most cells
sur-5::gfp	*eri-7(RNAi)*		weaker GFP in mid-body

[*]*mgIs30* is an integrated array of the following genotype: *rol-6(su1006)::lin-14 3'UTR, col-10::lacZ, lim-6::gfp)*. [†]*pkIs1582* is an integrated array of the following genotype: *rol-6(su1006), let-858::gfp*.

Silencing of transgene's (*mgIs30*) rolling phenotype by *eri-6/7* genetic alleles and silencing of transgenes' (*mgIs30* and *pkIs1582*) rolling phenotype by *eri-6/7(RNAi)*. Numerical scores are the mean ± s.e.m. Rol +/- scores are based on the following designations: (-) very few rollers; (+) <25% rollers; (++) 25-75% rollers, and (+++) >75% rollers. For RNAi experiments, L1 worms were fed on bacteria expressing dsRNA and scored as L4 in the following generation.

Supplementary Table 3. Rescue of *eri-6* and *eri-7* mutations by a transgenic *eri-6/7* cDNA. The *eri-6/7* cDNA was cloned in between the *eri-6* promoter (starting 1.1 kb upstream of the *eri-6* start codon) and the *eri-7* 3'-UTR (as annotated[44]). This construct was injected into worms of the genotype *eri-6(mg379)*; *mgIs30*. Three transgenes (*mgEx748*, *mgEx749*, *mgEx750*) were assayed for rolling. Subsequently, one cDNA transgene (*mgEx749*) was crossed into *eri-7(mg411)*; *mgIs30* and assayed for rolling. 15 animals were assayed for rolling transgenic progeny. Fraction rolling worms was averaged.

Genotype	Fraction rolling	Standard error
eri-6(mg379); mgIs30	0.0	
mgEx748-50; eri-6(mg379); mgIs30	0.80	0.08
eri-7(mg411); mgIs30	0.08	0.02
mgEx749; eri-7(mg411); mgIs30	0.93	0.02

Supplementary Discussion

Analysis of *eri-6/7* mutants

The *mg379* mutation affects the 5' splice site at exon/intron 2 of *eri-6*. RT-PCR analysis showed that this usually leads to incorporation of intron 2 into the *eri-6* mRNA, resulting in a frameshift and early stop codon in *eri-6*. Other variants include use of an alternative splice site which removes the last codon of exon 2 resulting in loss of a valine that is conserved in *C. briggsae*, and a variant that incorporates the first 4 nt of intron 2 before splicing to exon 3, resulting in a frameshift and early stop codon. *mg369* contains 2 lesions: a substitution in exon 4 of *eri-7* (at position 31801 of the C41D11 cosmid), 5 nt downstream of a 1-nt insertion that leads to a frameshift and early stop codon. t*rans*-spliced *eri-6/7* mRNAs can be detected in both mutants.

Four deletion alleles were obtained (Supplementary Fig. S3): (i) *tm1716* (33365 to 34357 of cosmid C41D11) removes *eri-6/7* intergenic sequence alone (putative promoter); (ii) *tm1887*, (34155 to 34990 of cosmid C41D11) is a deletion of part of *eri-6* coding and upstream sequence; (iii) *tm1917* (33299 to 35149 of cosmid C41D11) is a deletion of part of *eri-7*, the *eri-6/7* intergenic region, and part of *eri-6*; and (iv) a spontaneous allele, *mg441*, deletes the *eri-6* locus. RT-PCR analysis showed that *mg441*

does not express *eri-6*. All deletion mutants show an enhanced RNAi phenotype after six outcrosses to wild-type N2. Southern blot analysis showed that the *tm1716, tm1887* and *tm1917* alleles have the respective deletions as reported by the National Bioresource Project for the Experimental Animal Nematode *C. elegans* (Japan, Mitani), but also have a complete copy of *eri-6* (data not shown). PCR and sequencing of the *tm1716, tm1887* and *tm1917* alleles within the respective true-breeding homozygous, backcrossed strains using sets of primers that are external, internal, or both, relative to the deletion boundaries, reveal DNA segments with the predicted deletion, as well as DNA species that are wild type. This wild-type copy is presumably linked, as it is still present after extensive backcrossing. RT-PCR analysis showed that *eri-6* is expressed in all three mutants, but the *eri-6/7* fused mRNA is not. These deletion alleles are probably the result of complex rearrangements during the mutagenesis process, as has been reported to occur (National Bioresource Project for the Experimental Animal Nematode *C. elegans*).

Verification of genome structure and exploring DNA rearrangement

To verify the genome structure as found by sequencing of *C. elegans* Bristol N2[44], Southern blotting was done on N2 and CB4856 genomic DNA, using a probe spanning *eri-6* (Supplementary Fig. S4), and a probe spanning the first two exons of *eri*-7 (data not shown). We also performed extensive PCR analyses and sequencing. These experiments confirmed the genome structure of the N2 strain, and revealed an evolutionarily divergent gene structure in CB4856.

The Southern blot confirmed an antiparallel *eri-6* and *eri-7* open reading frame gene structure in N2, consistent with the *C. elegans* genome project annotation, and demonstrated that this is the *eri-6/7* gene structure for the observable majority of somatic plus germline cells.

However, it remained a possibility that the *eri-6/7* fusion RNAs are transcribed from rearranged DNA in a small subpopulation of cells; for example, less than 10% of the cells. This chimeric DNA could be the result of a genomic rearrangement mechanism initiated in certain cells or at particular developmental stages, perhaps mediated at the *eri-6/7* locus by the short inverted repeats that lie within the ~930-bp direct repeats that

flank *eri-6* (Fig. 1b and Supplementary Fig. S5). We have addressed this possibility with extensive PCR-probing of mixed-stage, genomic DNA preparations. Given the repetitive nature of the *eri-6/7* locus, we sought to minimize the probability of generating spurious or artifactual PCR products, which could potentially be produced if the replicating DNA polymerase extending from both primers were to fall off the template, perhaps after encountering secondary structure within the inverted repeats, thereby creating single-stranded DNA with homologous 3' ends that could anneal and be extended in a manner similar to splicing by overlap extension (SOE) PCR. We therefore used a highly processive DNA polymerase and designed primers corresponding to non- repetitive sequence (Supplementary Fig. S8a) that would generate a short amplicon (~230 bp). We also used long extension times (relative to what is normally required for the polymerase) and maximal polymerase concentrations in order to minimize probability of the enzyme falling off the template early. In *C. elegans* CB4856 (Hawaii), there are two single nucleotide polymorphisms (SNPs) within the 25-bp inverted repeats (Supplementary Fig. S5), which allow for confirmation that a given PCR product is specific to the targeted strain and not due to contamination by DNA of another *C. elegans* isolate. We determined the minimum number of cycles required to amplify the 230-bp amplicon in CB4856 under a given set of conditions (see Supplementary Methods), as visualized by eye with ethidium-stained agarose gel electrophoresis. 27 cycles were required to obtain a visible PCR product from CB4856 (Supplementary Fig. S8b), where *eri-6* and *eri-7* are *in cis*. In *C. elegans* N2, no such PCR amplicon was generated by these primers in 27 cycles, consistent with the Southern blotting results. We then asked whether, using the same pair of primers, we could amplify an analogous *cis* fragment in N2, where *eri-6* and *eri*-7 are mainly or always *in trans*, within 37 cycles (10 additional cycles over the CB4856 minimum), which should be 1000 times more sensitive (given 100% efficiency) and thus able to detect a rearranged locus even if it were to occur in a single cell of the ~1000 cells of the worm. We reproducibly obtained a *cis* product as a discrete band of expected size in N2, though significantly less intense than the CB4856 product, and subsequent sequencing revealed that it indeed contained the N2-specific SNPs. However,

these same primers, MB81 and MB169, also amplify the same 230-bp product from our cosmid templates of the region—both C41D11 (Supplementary Fig. S8b) and T01C5 (data not shown). The cosmids, amplified in *Escherichia coli* (*E. coli*), should be clonal representations of a single DNA species, yet both the expected *trans* (N2) orientation and the *cis* (CB4856) orientation are robustly amplified.

One possible mechanism by which the *eri-6* and *eri-7* sequences could become juxtaposed *in cis* in a PCR of DNA cloned in *E. coli* (and N2) would be through the inverted repeat sequence stimulating either the DNA polymerase falling off the template despite our precautions; or through the related phenomenon of template-switching of the polymerase occurring to yield the *cis* orientation artifactually. Template-switching, though usually associated with reverse transcription polymerase, has been demonstrated to occur in *in vitro* DNA synthesis by *Thermus aquaticus* (Taq) DNA polymerase between a replicating strand and a second, complimentary strand, which can be either the existing homologous template strand, or other complementary nascent strands present in the reaction mixture[46]. Template-switching is dependent of the concentration of substrate, which is determined by the starting template concentration and the number of amplification cycles. We believe the *cis* band we see after 37 cycles in N2 (but not 27 cycles) that is even more robustly amplified from the clonal cosmid DNA (that was to be the negative control) is the result of concentration-dependent template-switching.

The other, much less likely possibility would be that the direct repeats flanking *eri-6* mediate DNA rearrangement in both *E. coli* and *C. elegans*, and that this very rare rearrangement in *C. elegans* is sufficient to generate all of the *eri-6/7* fused mRNA we have detected. Given that the *eri-6/7* RNA shows evidence of RNA editing, and that the DNA rearrangement that we detect and believe to be an artifact would remove the dsRNA from the *eri-6/7* primary transcript, the mRNA *trans*-splicing model is much more favored by our data. In addition, a rearrangement in *E.coli* is unlikely because the cosmid C41D11 was isolated from *E. coli* 1046, a *recA*- strain.

Bioinformatic search for *eri-6* splice variants

A database of 370,197 *C. elegans* ESTs was constructed and searched for *eri-6* sequences by blastn. This database contains ESTs that are not present in the *C. elegans* genome database[44], because these ESTs match two unlinked genes. No *eri-6* splice variants other than the four described in Fig. 1a were found.

Non-complementation and the search for interchromosomal *trans*-splicing

Interchromosomal *trans*-splicing of *eri-6* and *eri-7* is unlikely to occur efficiently, given the lack of complementation between the predicted null alleles *eri-6(mg379)* and *eri-7(mg369)*. *trans*-heterozygous animals (harboring a single copy of each allele) are phenotypically similar to strains carrying the homozygous, single alleles (Table 1). *eri-6(tm1887)* (Supplementary Fig. S3 and Supplementary Discussion on analysis of *eri-6/7* mutants) and *eri-7(mg411)* also fail to complement (data not shown). Animals heterozygous for either *eri-6* or *eri-7 in trans* to a wild-type chromosome are only slightly Eri (Table 1), and *eri-6/7* hemizygous animals, harboring a copy of the *tm1917* allele (Supplementary Fig. S3) that deletes part of both *eri-6* and *eri-7*, *in trans* to a wild-type chromosome, are not Eri (data not shown), suggesting that *eri-6/7* is not a haploinsufficient locus, and that noncomplementation is not simply the result of disrupting either gene copy at both the *eri-6* and *eri-7* loci. Therefore, noncomplementation is likely the result of a failure of the wild-type copies of *eri-6* mRNA and *eri-7* mRNA (transcribed from homologous chromosomes in the *trans*-heterozygotes) to efficiently *trans*-splice. Alternatively, as wild-type gene dosage would be predicted to be maximally 25% if interchromosomal *trans*-splicing is equally efficient as intrachromosomal *trans*-splicing, this may not be sufficient for *eri-6/7* gene function.

To detect potential, interchromosomal *trans*-splicing, we cloned PCR-amplified *eri-6/7* cDNA that was isolated from eri-*7(mg369) eri-6*(+) / *eri-7*(+) *eri-6(mg379) trans*-heterozygous animals. Sequencing revealed that one out of twenty-six clones contained an *eri-6*/*eri-7* allele-complement consistent with interchromosomal *trans*-splicing [being either completely wild type or having the *eri-6(mg379)* and *eri-7(mg369)* molecular lesions]. However, in control cDNA made from a 1:1 mixture of RNA isolated from *eri-6(mg379)* homozygous animals and RNA isolated from *eri-7(mg369)* homozygous

animals, we found that three out of fifty clones either contained both the *eri-6(mg379)* and *eri-7(mg369)* molecular lesions, or contained neither of them (completely wild type). Therefore it is likely that the clone that seemed to be the product of interchromosomal *trans*-splicing is actually a result of an RT-PCR artifact, such as template-switching by the reverse transcriptase[47]. We conclude that interchromosomal *trans*-splicing is very inefficient or does not occur.

Additional technical information. Figure 2. b, Shown is average of 3 lines carrying extrachromosomal array of the 9.1-kb or 8.2-kb fragment in the *eri-7(mg411); mgIs30* background, or at least 3 F2 from 2 lines after crossing the arrays into the *eri-6(mg379); mgIs30* background. **c,** The *eri-7* promoter (the sequence upstream of exon 1 up to the *eri-6* start codon) was fused to *gfp*, while the *eri-6* promoter (the same sequence in the reverse orientation) was fused to *mrfp*. These constructs express in the hypodermis and a couple of head neurons. The merged image shows almost complete overlap between the *eri-6* and *eri-7* expression. **d,** editing was observed in 11/11 clones

Supplementary Notes

References

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