Supporting Information

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SI Materials and Methods

Strains Used. N2 wild-type, HC46 *ccIs4251 [myo-3::gfp]*; *mIs11 [myo-2::gfp]*, HC114 *sid-1*(*qt9*); *mIs11*; *ccIs4251*; *qtIs3 [myo-2::gfp-hpRNA]*, HC195 *nrIs20 [sur-5::gfp]*, HC196 *sid-1*(*qt9*), HC565 *ccIs4251*; *mIs11*; *sid-1*(*qt9*), HC566 *nrIs20*; *sid-1*(*qt9*), HC567 *nrIs20*; *eri-1*(*mg366*), HC568 *nrIs20*; *eri-1*(*mg366*); *sid-1*(*qt9*), HC570 *juIs73*; *ccIs4251*; *sid-1*(*qt9*); *qtEx116[pRF4+pHC337]*, HC573 *juIs73*; *ccIs4251*; *sid-1*(*qt9*), and HC575 *mIs11*; *ccIs4251*; *qtIs3*; *qtIs5 sid-1*(*qt9*)

Transgenes. In all cases except one (see below), transgenic animals were healthy and appeared morphologically normal.

Plasmids: pRF4 (23) or pHC183 (14) were used to obtain transgenic animals that roll or that express DsRed in the body-wall muscles (bwm), respectively.

To express *gfp*-hpRNA in the bwm: A 1:1 mix (0.035 mg/ml each) of pHC172 (14), which has the *gfp* coding region as an inverted repeat sequence under the control of the *myo-3* promoter, and pHC183 was injected into HC195 or HC566 animals to generate transgenic lines.

To express *gfp*-hpRNA in all neurons: The inverted-repeat sequence targeting *gfp* was subcloned from pHC172 under the control of the ≈3.4 kb promoter of the pan-neuronally expressed gene F25B3.3 (S1) to generate pHC337. A mix of pHC337 (0.04 mg/ml) and the coinjection marker PF25B3.3::DsRed (0.008 mg/ml) was injected into HC195 and HC566 animals to generate transgenic lines. A 1:1 mix of pHC337 and pRF4 (0.04 mg/ml each) was injected into HC573 animals to generate transgenic lines and a representative rolling transgenic line was designated as HC570.

To express *sid-1*(+) cDNA in the bwm: The SID-1 cDNA with a C-terminal FLAG tag was cloned as an NheI/KpnI fragment under the control of the myo-3 promoter in pPD96.52 (gift of Andrew Fire, Stanford University) to generate pHC355. A 1:1 mix of pHC355 and pHC183 (0.04 mg/ml each) was injected into HC114, HC573, or rolling HC570 animals to generate transgenic lines. Some double transgenic animals produced by injecting rolling HC570 animals showed growth and morphological defects.

PCR fusion products: Promoter sequences were amplified with Expand Long Template (ELT) polymerase (Roche) and either noncoding sequences or coding sequences along with 3′UTR were amplified with PfuUltra II Fusion polymerase (Stratagene) by using primers that generate ≈50 bp overlap between the two PCR fragments. The fusion products were generated with ELT polymerase by using the amplified promoter and the coding sequences as template and nested primers. In some cases, the two PCR fragments were fused in vivo). The specific templates and primers used to generate the various PCR fusion products are detailed below. The sequences of all primers used (P1–P41) are provided in Table S1.

To make the coinjection marker Pmyo-2::DsRed: The *myo-2* promoter was amplified from pHC168 (14) with primers P1 and P3. The DsRed2 cDNA along with unc-54 3′UTR was amplified from pHC183 (14) with primers P4 and P5. The fusion product was generated with primers P2 and P6. Transgenic animals generated using this marker showed bright DsRed2 fluorescence in pharyngeal muscles and sometimes showed faint fluorescence in bwm, likely because of trace expression of pHC183, the template used to amplify DsRed cDNA.

To make the coinjection marker Psid-2::DsRed: The *sid-2* promoter was amplified from wild-type genomic DNA (gDNA) with primers P23 and P25. The DsRed2 cDNA along with unc-54 3′UTR was amplified from pHC183 with primers P26 and P5. The two PCR products were mixed at 1:1 ratio (0.008 mg/ml each) for injection into animals to generate transgenic lines where the PCR products would be fused in vivo.

To make the coinjection marker PF25B3.3::DsRed: The F25B3.3 promoter was amplified from gDNA with primers P39 and P40; and DsRed2 cDNA along with unc-54 3′UTR was amplified from pHC183 with primers P41 and P5. The two PCR products were mixed at 1:1 ratio (0.008 mg/ml each) for injection into animals to generate transgenic lines where the PCR products would be fused in vivo.

To express *gfp*-dsRNA in the pharynx: (a) Pmyo-2::gfp-sense: The *myo-2* promoter was amplified from gDNA with primers P1 and P7. *gfp* coding sequence was amplified from pHC168 (14) with primers P8 and P9. The fusion was generated with primers P2 and P10. (b) Pmyo-2::gfp-antisense: The *myo-2* promoter was similarly amplified with primers P1 and P12 and *gfp* coding sequence was similarly amplified with primers P11 and P13. The fusion product was generated with primers B2 and P14. A 1:1 mix of Pmyo-2::gfp-sense and Pmyo-2::gfp-antisense (0.025 mg/ml each) along with Pmyo-2::DsRed (0.008 mg/ml) was injected into HC46 or HC565 animals to generate transgenic lines.

To express *unc-22*-dsRNA in the pharynx: (a) Pmyo-2::unc22sense: The *myo-2* promoter was amplified from pHC168 with primers P1 and P15. 589 bp of *unc-22* coding sequence was amplified from gDNA with primers P16 and P17. The fusion product was generated with primers P2 and P18. (b) Pmyo-2::unc-22antisense: The *myo-2* promoter region was similarly amplified with primers P1 and P20; and the 589 bp of *unc-22* coding sequence was similarly amplified with primers P19 and P21. The fusion product was generated with primers C2 and P22. A 1:1 mix of Pmyo-2::unc-22sense and Pmyo-2::unc22antisense (0.024 mg/ml each) along with Pmyo-2::DsRed (0.008 mg/ml) was injected into N2 or HC196 animals to generate transgenic lines.

To express *gfp*-dsRNA in the gut: (a) Psid-2::gfp-sense: The *sid-2* promoter was amplified from gDNA with primers P23 and P27. *gfp* coding sequence was amplified from pHC168 with primers P28 and P9. The fusion product was generated with primers P24 and P10. (b) Psid-2::gfp-antisense: The *sid-2* promoter region was similarly amplified with primers P23 and P30 and *gfp* coding sequence was similarly amplified with primers P29 and P13. The fusion product was generated with primers E2 and P14. A 1:1 mix of Psid-2::gfp-sense and Psid-2::gfp-antisense (0.025 mg/ml each) along with Psid-2::DsRed (0.008 mg/ml) was injected into HC195 or HC566 animals to generate transgenic lines.

To express *gfp*-dsRNA in the bwm: (a) Pmyo-2::gfp-sense: The *myo-3* promoter was amplified from gDNA with primers P31 and P33. *gfp* coding sequence was amplified from pHC168 with primers P34 and P9. The fusion product was generated with primers P32 and P10. (b) Pmyo-3::gfp-antisense: The *myo-3* promoter region was similarly amplified with primers P31 and P36 and *gfp* coding sequence was similarly amplified with primers P35 and P13. The fusion product was generated with primers P32 and P14. A 1:1 mix of Pmyo-3::gfp-sense and Pmyo-3::gfp-antisense (0.034 mg/ml each) along with pHC183 (0.034 mg/ml) was injected into HC195 animals to generate transgenic lines.

To express *sid-1*(+) cDNA in the gut: The sid-2 promoter was amplified from gDNA with primers P23 and P37; and *sid-1*(+) cDNA along with unc-54 3′UTR was amplified from pHC355 with primers P38 and P5. A 1:1 mix of the two PCR products

(0.008 mg/ml each) along with Psid-1::DsRed (0.008 mg/ml) was injected into HC575 to generate transgenic lines where the *sid-2* promoter is fused to *sid-1*(+) cDNA in vivo.

Microscopy. For Fig. 1*D*, similar distributions were obtained when the number of brightly fluorescent gut nuclei per worm was counted independently by two researchers (data not shown). For Fig. 3, 30 transgenic animals from each of three transgenic lines (total of 90 animals) were analyzed per genotype. Animals were scored as showing silencing of GFP expression in nonred tissues if GFP expression was detectably dimmer than in control animals when scored at a fixed magnification. For Fig. S1, 37 confocal slices that encompass the entire worm were collected using exposure times that avoid saturation of pixels by the brightest cells on an Axiovert 200 microscope (Zeiss) and the total fluorescence was measured by using Image J (National Institutes of Health). A maximum of three L4 worms were analyzed per slide and 10 animals were analyzed per genotype. The background fluorescence for each slide was calculated as the total fluorescence in 37 slices in a region where there were no worms. Background fluorescence of the slide was subtracted from the total fluorescence for each worm to obtain its average total fluorescence plotted in Fig. S1*D*.

Feeding RNAi. L1-L2 staged animals were fed *E. coli* that express *gfp*-hpRNA with an *unc-22* loop (pPD126.25). The animals were grown at 20 °C and were assayed 4 days later for GFP expression and *unc-22* silencing. *E. coli* that do not express any hpRNA or dsRNA was used as control. Among animals that express GFP and that were >3 days past L2, $\approx 30\%-60\%$ showed reduced GFP fluorescence in the gut even when fed control OP50 *E. coli*.

***Unc-22* Silencing.** For Fig. 2, Starved animals were transferred via an agar chunk to a plate with OP50 *E. coli* and 1.5 days later, young adults were assayed. Transgenic or control animals that twitch in response to 1 μl of 3 mM Levamisole (in water) within 10 seconds were scored as twitching. Sixty animals per genotype or 20 animals for each of 3 independent transgenic lines (total 60) were scored.

1. Altun-Gultekin Z, *et al*. (2001) A regulatory cascade of three homeobox genes, ceh-10, ttx-3 and ceh-23, controls cell fate specification of a defined interneuron class in *C. elegans*. *Development* 128:1951–1969.
2. Hobert O (2002) PCR fusion-based approach to create reporter gene constructs for expression analysis in transgenic *C. elegans. Biotechniques* 32:728–730.
3. Kemp BJ, *et al*. (2007) In vivo construction of recombinant molecules within the *Caenorhabditis elegans* germ line using short regions of terminal homology. *Nucleic Acids Res* 35:e133.

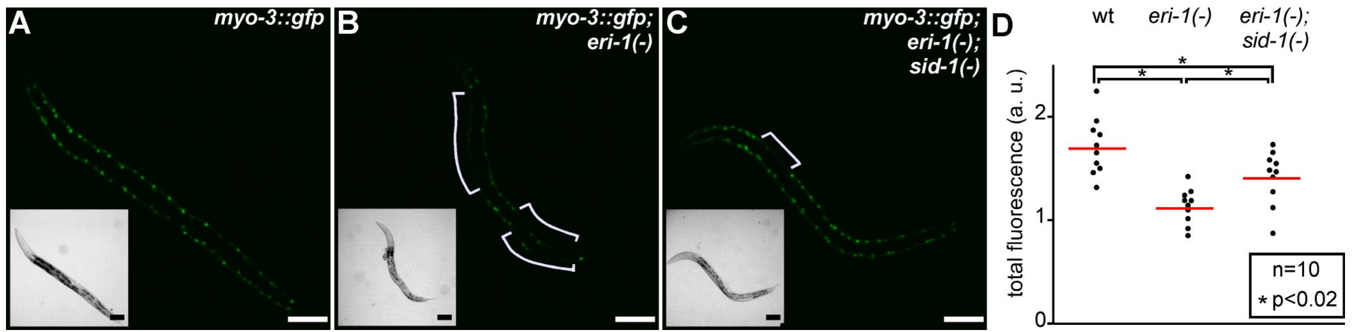

Fig. S1. The extent of silencing of a tissue-specific transgene depends on SID-1. Representative wild-type (*A*), *eri-1*(-) (*B*), and *eri-1*(-);*sid-1*(-) (*C*) animals of the fourth larval (L4) stage that express GFP in body-wall muscles (*bwm*::*gfp*) are shown. *Insets*: bright-field images. Regions showing silencing in the bwm (brackets in *B* and *C*) are indicated. Scale bar: 100 μm. (*D*) Quantification of the extent of transgene silencing for each genotype shown in *A*–*C*. Total fluorescence per animal was measured for 10 L4 animals. The average fluorescence per animal (red line) for each genotype was significantly ($P < 0.02$; *t* test) different from that of the others.

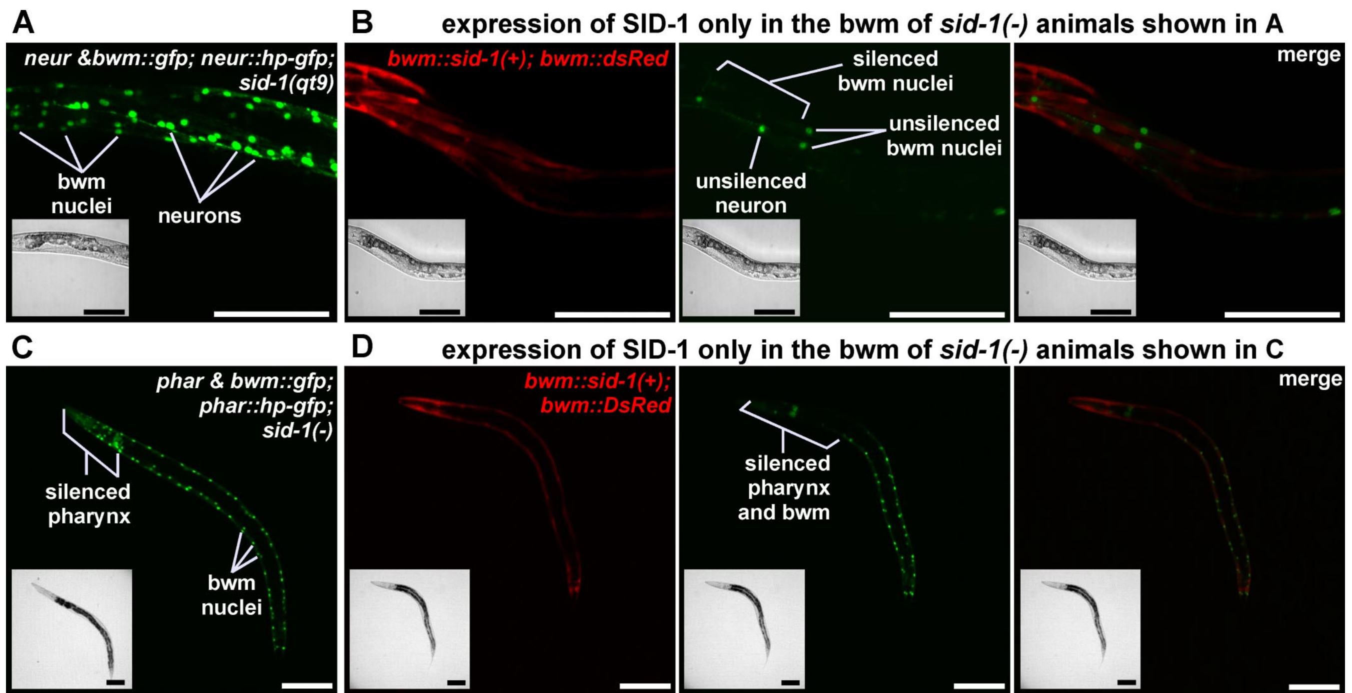

Fig. S2. Expression of *gfp*-hpRNA in either neurons or pharynx of *sid-1*(-) animals can silence *gfp* in *sid-1*(+) bwm. (*A*) Representative *sid-1*(-) L4 animal that expresses GFP in a subset of neurons and in the bwm, but that expresses *gfp*-hpRNA only in all neurons (*neur*::*hp-gfp*). No silencing of GFP expression is detected. *Inset*: bright-field image. (*B*) Representative L4 animal of the same genotype as in (*A*), but in which wild-type *sid-1* cDNA (*sid-1*(+)) and DsRed have been coexpressed in some of the bwm cells. Red channel showing specific but mosaic expression of *sid-1*(+) in the bwm (*Left*); green channel showing specific silencing of GFP expression only in the bwm cells that express *sid-1*(+) (*Middle*); and merge of images from both channels (right) are shown. *Insets*: bright-field images. Scale bars: 100 μm. (*C*) Representative *sid-1*(-) L4 animal that expresses GFP in the pharynx and bwm, but that expresses *gfp*-hpRNA only in the pharynx (*phar*::*hp-gfp*). Only GFP expression in the pharynx is silenced as indicated. Inset: bright-field image. (*D*) Representative L4 animal of the same genotype as in (*C*), but in which wild-type *sid-1* cDNA (*sid-1*(+)) and DsRed have been coexpressed in the bwm. Red channel showing specific expression of *sid-1*(+) in the bwm (*Left*); green channel showing silencing of GFP expression in the pharynx and the anterior half of bwm (*Middle*); and merge of images from both channels are shown. *Insets*: bright-field images. Scale bars: 100 μm.

PNAS PNAS PNAS PNAS

Table S1. Primers used to generate PCR fragments for making transgenes

Name	Sequence
P1	CGAGGCATTTGAATTGGGGG
P2	GGTGGTGGACAGTAACTGTC
P3	CGTTCTCGGAGGAGGCCATCCGAATCGATAGGATCTCGG
P4	CCGAGATCCTATCGATTCGGATGGCCTCCTCCGAGAACG
P5	CGGTCATAAACTGAAACGTAAC
P6	CCTTATCATATGTTACGTTTCAG
P7	GAAAAGTTCTTCTCCTTTACTCATCCGAATCGATAGGATCTCGG
P8	CCGAGATCCTATCGATTCGGATGAGTAAAGGAGAAGAACTTTTC
P9	TTCATCCATGCCATGTGTAATC
P10	GTAATCCCAGCAGCTGTTAC
P11	CCGAGATCCTATCGATTCGGTTCATCCATGCCATGTGTAATC
P12	GATTACACATGGCATGGATGAACCGAATCGATAGGATCTCGG
P13	ATGAGTAAAGGAGAAGAACTTTTC
P14	GAAGAACTTTTCACTGGAGTTG
P15	CAATGTTGCCAAATCACTTTCGCCGAATCGATAGGATCTCGG
P16	CCGAGATCCTATCGATTCGGCGAAAGTGATTTGGCAACATTG
P17	CTTGATTTGGAATGGAACCTTC
P18	GGAACCTTCACAACACATGG
P19	CCGAGATCCTATCGATTCGGCTTGATTTGGAATGGAACCTTC
P20	GAAGGTTCCATTCCAAATCAAGCCGAATCGATAGGATCTCGG
P21	CGAAAGTGATTTGGCAACATTG
P22	GGCAACATTGGAGACTGATG
P23	CTGCCTATTGGGACTCAACG
P24	CAACGGGCGAGGAATCTTC
P25	CGTTCTCGGAGGAGGCCATTTCCTGAAAATATCAGGGTTTTG
P26	CAAAACCCTGATATTTTCAGGAAATGGCCTCCTCCGAGAACG
P27	GAAAAGTTCTTCTCCTTTACTCATTTCCTGAAAATATCAGGGTTTTG
P28	CAAAACCCTGATATTTTCAGGAAATGAGTAAAGGAGAAGAACTTTTC
P29	CAAAACCCTGATATTTTCAGGAATTCATCCATGCCATGTGTAATC
P30	GATTACACATGGCATGGATGAATTCCTGAAAATATCAGGGTTTTG
P31	GGTCGGCTATAATAAGTTCTTG
P32	CCCGACAAAACATGAGTATTTC
P33	GAAAAGTTCTTCTCCTTTACTCATCAAGGGTCCTCCTGAAAATG
P34	CATTTTCAGGAGGACCCTTGATGAGTAAAGGAGAAGAACTTTTC
P35	CATTTTCAGGAGGACCCTTGTTCATCCATGCCATGTGTAATC
P36	GATTACACATGGCATGGATGAACAAGGGTCCTCCTGAAAATG
P37	AAATTATCAAATAAACACGAATCATTTCCTGAAAATATCAGGGTTTTG
P38	CAAAACCCTGATATTTTCAGGAAATGATTCGTGTTTATTTGATAATTT
P39	CGATAATCTCGTGACACTCG
P40	CGTTCTCGGAGGAGGCCATCGTCGTCGTCGTCGATGC
P41	GCATCGACGACGACGACGATGGCCTCCTCCGAGAACG