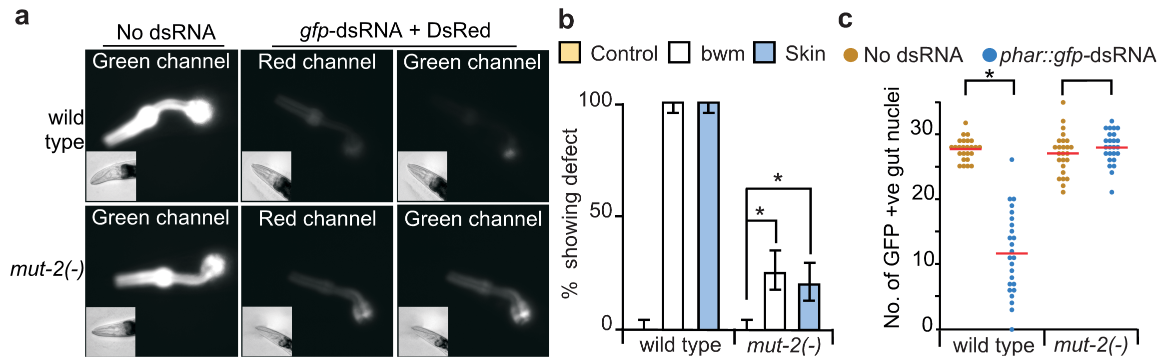
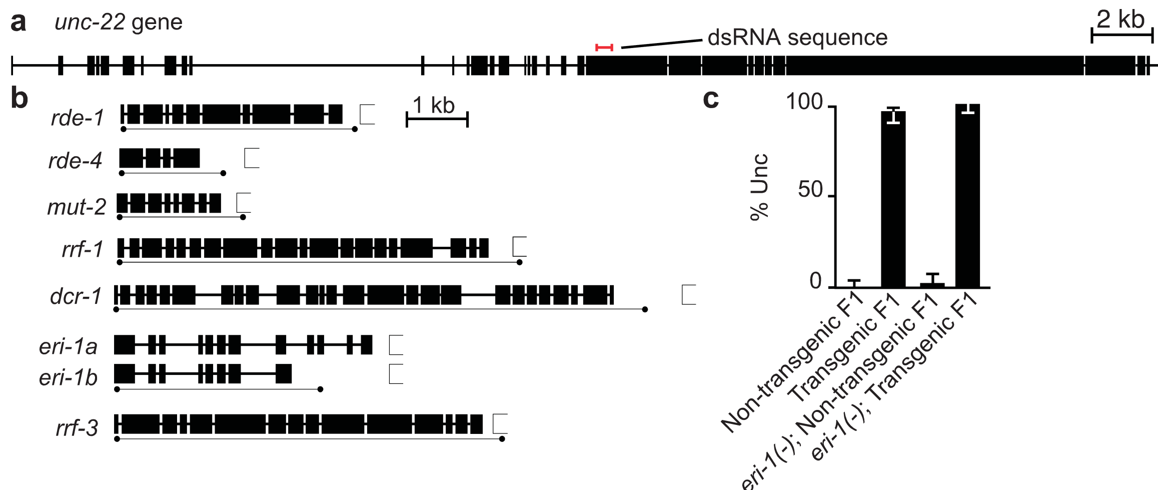
Two classes of silencing RNAs move between *C. elegans* tissues.

Antony M Jose, Giancarlo A Garcia, and Craig P Hunter.

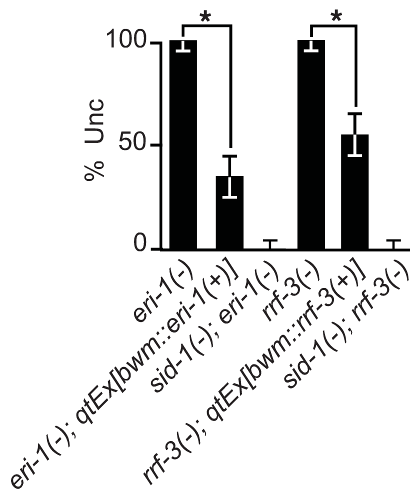
Supplementary Figures, Figure Legends, and Tables.



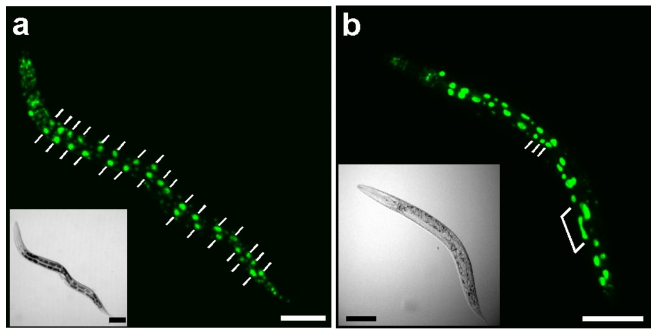
Supplementary Figure 1 MUT-2 is required for efficient RNAi. (**a**) Representative images of the pharynx of wild-type and *mut-2(-)* animals that express GFP in the pharynx (left panels) and that in addition coexpress *gfp*-dsRNA and DsRed in the pharynx (middle and right panels). The representative animals presented in these grey scale images show that wild-type animals silence *gfp* expression (green channel) more potently than *mut-2(-)* animals even in response to lower levels of *gfp*-dsRNA (red channel). (**b**) Feeding RNAi of wild-type and *mut-2(-)* animals. L4 animals were fed either L4440 (control) or dsRNA targeting the body-wall muscle gene *unc-22* (bwm) or the skin gene *dpy-7* (skin) and the percentage of L4 progeny that showed the corresponding defects were determined. Error bars indicate 95% confidence intervals and asterisks indicate significant differences (P>0.05). (**c**) A representative transgene that expresses *gfp*-dsRNA in the pharynx (*phar::gfp-dsRNA*) in *sur-5::gfp* animals was crossed into *mut-2(-); sur-5::gfp* animals and the number of brightly fluorescent gut nuclei were counted. n=25 L4 animals. Averages (red bars), significant differences (brackets and *, P<0.05) and similar values (brackets) are indicated.



Supplementary Figure 2 Schematics of constructs used and inheritance of silencing in the transgenic strain used in **Fig 1**. (**a-b**) Structures of *unc-22* and RNAi pathway genes. Thin line, introns; thick line, exons; and red line, dsRNA sequence. Regions amplified to rescue each RNAi pathway gene are indicated below gene structures as thin lines with terminal circles (primer positions). Open brackets indicate start of the gene 3' to RNAi pathway gene. (**c**) L4 animals of the representative *neur::u22ds* transgenic line used in **Fig. 1** in wild-type and *eri-1(-)* background were allowed to have progeny. *Unc-22* silencing was measured as in **Fig. 1** in L4 staged progeny that either have or do not have the *neur::u22ds* transgene as indicated by presence or lack of DsRed expression, respectively. Error bars indicate 95% confidence intervals.



Supplemental Figure 3 Inhibitors of RNAi reduce silencing due to mobile RNAs. The representative transgene used in **Fig. 1** to express *unc-22*-dsRNA under the control of the neuronal *rgef-1* promoter (*neur::u22ds*) was crossed into the single- and double-mutant backgrounds indicated. Silencing of *unc-22* was measured (% Unc) in these animals and in animals with the corresponding RNAi gene rescued in bwm of single mutants. n=100 L4 animals. 95% confidence intervals (error bars) and significant differences (brackets and *, P<0.05) are indicated. See **Supplementary Fig. 2** for details of constructs used.



Supplemental Figure 4 Variation in number and morphology of gut nuclei as indicated by *sur-5::gfp* expression. (**a**) *sur-5::gfp* expression in a wild-type L4 animal with normal gut nuclei indicated by lines. (**b**) *sur-5::gfp* expression in a *rde-4(-)* L4 animal with regions of supernumerary (lines) and fewer (bracket) nuclei than normal. The reason for the variations in the number of *sur-5::gfp*-marked nuclei is currently unclear. Animals such as those in (**b**) occur in the case of various genotypes, including wild-type, suggesting that the reason for this variation is likely environmental. Interestingly, a role for RNAi-related genes and retinoblastoma genes in the control of nuclear divisions in the *C. elegans* gut has been demonstrated[36]. Scale bar, 50 μm.

Supplementary Table 1 Interdependence of RNAi pathway genes for mobile RNA production.

Genotype	% animals with gut silencing (n>100)[a]

rde-1(-); sur-5::gfp; qtEx[bwm::mut-2(+)]	0
rde-1(-); sur-5::gfp; qtEx[bwm::rde-4(+)]	0
rde-4(-); sur-5::gfp; qtEx[bwm::mut-2(+)]	0
mut-2(-); sur-5::gfp; qtEx[bwm::rde-4(+)]	0

[a]In each case, more than 20 animals from each of five transgenic lines were examined and the percentage of animals showing detectable silencing of GFP expression in the gut was measured.

Supplementary Table 2 Primers used for PCR

P1	CGAGGCATTTGAATTGGGGG
P2	CGTTCTCGGAGGAGGCCATCCGAATCGATAGGATCTCGG
P3	CCGAGATCCTATCGATTCGGATGGCCTCCTCCGAGAACG
P4	CGGTCATAAACTGAAACGTAAC
P5	GGTGGTGGACAGTAACTGTC
P6	CTGAAACGTAACATATGATAAGG
P7	CGATAATCTCGTGACACTCG
P8	CGTTCTCGGAGGAGGCCATCGTCGTCGTCGTCGATGC
P9	GCATCGACGACGACGACGATGGCCTCCTCCGAGAACG
P10	GACACTCGTTTCCGATACCC
P11	GAAAAGTTCTTCTCCTTTACTCATCGTCGTCGTCGTCGATGC
P12	GCATCGACGACGACGACGATGAGTAAAGGAGAAGAACTTTTC
P13	CAATGTTGCCAAATCACTTTCGCGTCGTCGTCGTCGATGC
P14	GCATCGACGACGACGACGCGAAAGTGATTTGGCAACATTG
P15	CTTGATTTGGAATGGAACCTTC
P16	GGAACCTTCACAACACATGG
P17	GAAGGTTCCATTCCAAATCAAGCGTCGTCGTCGTCGATGC
P18	GCATCGACGACGACGACGCTTGATTTGGAATGGAACCTTC

P19	CGAAAGTGATTTGGCAACATTG
P20	GGCAACATTGGAGACTGATG
P21	GGTCGGCTATAATAAGTTCTTG
P22	CGGGAAAATTCGAGGACATCAAGGGTCCTCCTGAAAATG
P23	CATTTTCAGGAGGACCCTTGATGTCCTCGAATTTTCCCG
P24	GTGAAATCACCTGCAGAGAG
P25	CCCGACAAAACATGAGTATTTC
P26	CACCTGCAGAGAGAAAACATTTT
P27	GATCTTTATTTGGTTGAGACATCAAGGGTCCTCCTGAAAATG
P28	CATTTTCAGGAGGACCCTTGATGTCTCAACCAAATAAAGATC
P29	CCTTGCTAGTTATCGTCTCC
P30	AGTTATCGTCTCCGTAATTCG
P31	CGTTAGTTTGGTTAAATCCATCAAGGGTCCTCCTGAAAATG
P32	CATTTTCAGGAGGACCCTTGATGGATTTAACCAAACTAACG
P33	CACTGCAGAGAATGAGTGTG
P34	GTAGAGGTCAGAGGCATAG
P35	ATCATTATCAAACGGGAGCATCAAGGGTCCTCCTGAAAATG
P36	CATTTTCAGGAGGACCCTTGATGCTCCCGTTTGATAATGAT
P37	CTGTGAGCAGTAGTACAAGTG
P38	GCAGTAGTACAAGTGAACCG
P39	CGGCTCATCTGCGCTCATCAAGGGTCCTCCTGAAAATG
P40	CATTTTCAGGAGGACCCTTGATGAGCGCAGATGAGCCG
P41	GCAAGACCGATAATAGAGGAT
P42	ACTGAAAACGCCAGAAACTAG
P43	CGGGAAAATTCGAGGACATCGTCGTCGTCGTCGATGC
P44	GCATCGACGACGACGACGATGTCCTCGAATTTTCCCG

P45 GATCTTTATTTGGTTGAGACATCGTCGTCGTCGTCGATGC

P46 GCATCGACGACGACGACGATGTCTCAACCAAATAAAGATC

P47 CGTTAGTTTGGTTAAATCCATCGTCGTCGTCGTCGATGC

P48 GCATCGACGACGACGACGATGGATTTAACCAAACTAACG

P49 CCATGACTTCGTTCCGACATCAAGGGTCCTCCTGAAAATG

P50 CATTTTCAGGAGGACCCTTGATGTCGGAACGAAGTCATGG

P51 GGCTTACCTGGTATCTTTGATC

P52 ACCTGGTATCTTTGATCTCTG

P53 CAGCTCTTACCCTGACCATCAAGGGTCCTCCTGAAAATG

P54 CATTTTCAGGAGGACCCTTGATGGTCAGGGTAAGAGCTG

P55 GCAGACTTCTTATCGGTGTG

P56 CAGCTCTTACCCTGACCATCGTCGTCGTCGTCGATGC

P57 GCATCGACGACGACGACGATGGTCAGGGTAAGAGCTG

Supplementary Methods

DNA constructs and transgenic animals. Co-injection markers: (a) pHC183[5]: Plasmid with the *myo-3* promoter cloned 5' of DsRed2 cDNA.

(b) pHC488: The *myo-2* promoter region was amplified from genomic DNA with the primers P1 and P2. The DsRed2 coding sequence along with *unc-54* 3´UTR sequence was amplified from pHC183 with primers P3 and P4. The fusion product was generated with P5 and P6 and then cloned into a T/A cloning vector (StrataClone, Stratagene) to make pHC488.

(c) *Prgef-1::DsRed*: The *rgef-1* promoter was amplified with P7 and P8. The DsRed2 coding sequence along with *unc-54* 3´UTR sequence was amplified from pHC183 using P9 and P4. The fusion product was generated with P10 and P6.

(d) *Prgef-1::GFP*: The *rgef-1* promoter was amplified with P7 and P11. The GFP coding sequence along with *unc-54* 3' UTR sequence was amplified from HC46[5] genomic DNA with P12 and P4. The fusion product was generated with P10 and P6.

To express *gfp*-dsRNA in pharyngeal muscles*: Pmyo-2::gfp-sense*, and *Pmyo-2::gfp-antisense* were made as in ref. 8. A 1:1 mix of *Pmyo-2::gfp-sense* and *Pmyo-2::gfp-antisense* (0.01 mg/ml each) along with pHC488 (0.038 mg/ml) was injected into HC195 and PD4792 animals to generate transgenic lines.

To express *unc-22*-dsRNA in neurons (*neur::u22ds*)*:* (a) *Prgef-1::unc-22sense*: The *rgef-1* promoter was amplified with P7 and P13. An ~560 bp *unc-22* sequence was amplified from genomic DNA with P14 and P15. The fusion product was generated with P10 and P16. (b) *Prgef-1::unc-22antisense*: The *rgef-1* promoter was amplified with P7 and P17. The same ~560 bp *unc-22* sequence was amplified from genomic DNA with P18 and P19. The fusion product was generated with P10 and P20. A 1:1:1 mix of *Prgef-1::unc-22sense*, *Prgef-1::unc-22antisense*, and *Prgef-1::DsRed* (0.01 mg/ml each) was injected into N2 animals to generate transgenic lines.

To express *rde-1(+)* in bwm cells [*Pmyo-3::rde-1(+)*]: The *myo-3* promoter was amplified from pHC183 with P21 and P22. The *rde-1* coding and 3´UTR sequences were amplified from genomic DNA with P23 and P24. The fusion product was generated with P25 and P26. A mix of *Pmyo-3::rde-1* (0.01 mg/ml) and pHC183 (0.038 mg/ml) was injected into WM27, HC70, and HC782 animals with the *qtEx136* transgene to generate transgenic lines.

To express *mut-2(+)* in bwm cells [*Pmyo-3::mut-2(+)*]: The *myo-3* promoter was amplified from pHC183 with P21 and P27. The *mut-2* coding and 3´UTR sequences were amplified from genomic DNA with P28 and P29. The fusion product was generated using P25 and P30. A mix of *Pmyo-3::mut-2* (0.01 mg/ml) and pHC183 (0.038 mg/ml)

was injected into WM30 and HC784 animals with the *qtEx136* transgene to generate transgenic lines.

To express *rde-4(+)* in bwm cells [*Pmyo-3::rde-4(+)*]: The *myo-3* promoter was amplified from pHC183 with P21 and P31. The *rde-4* coding and 3´UTR sequences were amplified from genomic DNA with P32 and P33. The fusion products were generated with P25 and P34. A mix of *Pmyo-3::rde-4* (0.01 mg/ml) and pHC183 (0.038 mg/ml) was injected into WM49 and HC783 animals with the *qtEx136* transgene to generate transgenic lines.

To express *rrf-3(+)* in bwm cells [*Pmyo-3::rrf-3(+)*]: The *myo-3* promoter was amplified from pHC183 with P21 and P35. The *rrf-3* coding and 3´UTR sequences were amplified from genomic DNA with P36 and P37. The fusion product was generated with P25 and P38. A mix of *Pmyo-3::rrf-3* (0.01 mg/ml) and pHC183 (0.038 mg/ml) was injected into NL2099 animals with the *qtEx136* transgene to generate transgenic lines.

To express *eri-1(+)* in bwm cells [*Pmyo-3::eri-1(+)*]: The *myo-3* promoter was amplified from pHC183 with P21 and P39. The *eri-1* coding and 3´UTR sequences were amplified from genomic DNA with P40 and P41. The fusion product was generated with P25 and P42. A mix of *Pmyo-3::eri-1* (0.01 mg/ml) and pHC183 (0.038 mg/ml) was injected into GR1373 animals with the *qtEx136* transgene to generate transgenic lines.

To express *rde-1(+)* in neuronal cells [*Prgef-1::rde-1(+)*]: The *rgef-1* promoter was amplified from genomic DNA with P7 and P43. The *rde-1* coding and 3´UTR sequences were amplified from genomic DNA with P44 and P24. The fusion product was generated with P10 and P26. A mix of *Prgef-1::rde-1* (0.01 mg/ml) and pHC183 (0.038 mg/ml) was injected into WM27 animals with *qtEx136* and into HC782 animals with *qtEx136* to generate transgenic lines.

To express *mut-2(+)* in neuronal cells [*Prgef-1::mut-2(+)*]: The *rgef-1* promoter was amplified from genomic DNA with P7 and P45. The *mut-2* coding and 3´UTR

sequences were amplified from genomic DNA with P46 and P29. The fusion product was generated using P10 and P30. A mix of *Prgef-1::mut-2* (0.01 mg/ml) and pHC183 (0.038 mg/ml) was injected into WM30 animals with *qtEx136* and into HC784 animals with *qtEx136* to generate transgenic lines.

To express *rde-4(+)* in neuronal cells [*Prgef-1::rde-4(+)*]: The *rgef-1* promoter was amplified from genomic DNA with P7 and P47. The *rde-4* coding and 3´UTR sequences were amplified from genomic DNA with P48 and P33. The fusion products were generated with P10 and P34. A mix of *Prgef-1::rde-4* (0.01 mg/ml) and pHC183 (0.038 mg/ml) was injected into WM49 animals with *qtEx136* and into HC783 animals with *qtEx136* to generate transgenic lines.

To express *rrf-1(+)* in bwm cells [*Pmyo-3::rrf-1(+)*]: The *myo-3* promoter was amplified from pHC183 with P21 and P49. The *rrf-1* coding and 3´UTR sequences were amplified from genomic DNA with P50 and P51. The fusion product was generated with P25 and P52. A mix of *Pmyo-3::rrf-1* (0.01 mg/ml) and pHC183 (0.038 mg/ml) was injected into HC780 animals with the *qtEx136* transgene and into HC781 animals with the *qtEx136* transgene to generate transgenic lines.

To express *dcr-1(+)* in bwm cells [*Pmyo-3::dcr-1(+)*]: The *myo-3* promoter was amplified from pHC183 with P21 and P53. The *dcr-1* coding and 3´UTR sequences were amplified from genomic DNA with P54 and P55. A 1:1 mix of the PCR products (0.01 mg/ml each) and pHC183 (0.038 mg/ml) was injected into HC779 animals with the *qtEx136* transgene to generate transgenic lines.

To express *dcr-1(+)* in neuronal cells [*Prgef-1::dcr-1(+)*]: The *rgef-1* promoter was amplified from genomic DNA with P7 and P56. The *dcr-1* coding and 3´UTR sequences were amplified from genomic DNA with P57 and P55. A 1:1 mix of the PCR products (0.01 mg/ml each) and pHC183 (0.038 mg/ml) was injected into HC779 animals with the *qtEx136* transgene to generate transgenic lines.

To express DsRed in bwm cells of HC737 animals, 0.038 mg/ml of pHC183 was injected to generate transgenic lines.

In most cases, transgenic lines were easily generated and transgenic animals were healthy, and appeared morphologically normal.

Supplementary Reference

36. Grishok, A. & Sharp, P. A. Negative regulation of nuclear divisions in *Caenorhabditis elegans* by retinoblastoma and RNA interference-related genes. *Proc. Natl. Acad. Sci. USA* **102**, 17360-17365 (2005).