SUPPLEMENTARY MATERIALS AND METHODS

Strains Used

N2  
Wild type

AMJ8  
Juls73[Punc-25::gfp] III

AMJ57  
sid-1(qt9) V; jamEx12[Pmyo-3::sid-1(+);unc-54 3'UTR & pH183] (strain generated in 6 after multiple passages)

AMJ58  
rde-1(ne219) I; jamEx1[Pmyo-3::rde-1(+);rde-1 3'UTR & Pmyo-3::DsRed2:unc-54 3'UTR]

AMJ66  
rde-4(ne301); jamEx4[Pmyo-3::rde-4(+);rde-4 3'UTR & pH183] (strain generated in 23 after multiple passages).

AMJ151  
rde-4(ne301); mls11[Pmyo-2::gfp::unc-54 3'UTR & gut::gfp::unc-54 3'UTR & pes-10::gfp::unc-54 3'UTR] jamls3[Pmyo-2::DsRed::unc-54 3'UTR] jamls4[Pmyo-3::rde-4(+);rde-4 3'UTR & pH183]] II (Outcrossed to AMJ8)

AMJ162  
rde-4(ne301); jamEx24[Pnas-9::rde-4(+);rde-4 3'UTR & Pnas-9::gfp::unc-54 3'UTR]

AMJ188  
rde-4(ne301); jamEx3[Prgef-1::rde-4(+);rde-4 3'UTR & Prgef-1::gfp::unc-54 3'UTR]

AMJ190  
rde-4(ne301) III; oxSi221[Peft-3p::gfp + cb-unc-119(+)] II

AMJ210  
jamEx24[Pnas-9::rde-4(+);rde-4 3'UTR & Pnas-9::gfp::unc-54 3'UTR]

AMJ212  
rde-4(ne301) III; jamEx47[Pwrt-2::rde-4(+);rde-4 3'UTR & Pwrt-2::gfp::unc-54 3'UTR]

AMJ217  
rde-4(ne301) III; jamEx52[Punc-54::rde-4(+);rde-4 3'UTR & Punc-54::gfp::unc-54 3'UTR]

AMJ220  
rde-4(ne301) III; jamEx55[Psid-2::rde-4(+);rde-4 3'UTR & Psid-2::gfp::unc-54 3'UTR]

AMJ229  
rde-4(ne301) III; oxSi221 II; jamEx4

AMJ237  
rde-4(ne301) III; jamEx65[Pmyo-3::rde-4(+);rde-4 3'UTR & Pmyo-3::gfp::unc-54 3'UTR]

AMJ238  
rde-4(ne301) III; jamEx66[Pmyo-3::rde-4(+);rde-4 3'UTR & Pmyo-3::gfp::unc-54 3'UTR]

AMJ239  
rde-4(ne301) III; jamEx67[Pmyo-3::rde-4(+);rde-4 3'UTR & Pmyo-3::gfp::unc-54 3'UTR]

AMJ268  
rde-4(ne301) III; sid-1(qt9) V; jamEx3
AMJ269  rde-4(ne301) III; sid-1(qt9) V
AMJ290  rde-4(ne301) III; jamEx89[Punc-119c::rde-4(+):rde-4 3'UTR & Punc-119c::gfp::unc-54 3'UTR]
AMJ303  jamEx77[Pnas-9::gfp::unc-54 3'UTR]
AMJ311  rde-4(ne301) III; sid-1(qt9) V; jamEx12
AMJ314  oxSi221 II; unc-119(ed3) III (?); rde-1(ne219) V
AMJ331  oxSi221 II; unc-119(ed3) III (?); rde-1(ne219) V; jamEx1
AMJ343  rde-4(ne301) III; sid-1(qt9) V; jamEx3 jamEx12
AMJ383  eri-1(mg366) IV; jamEx77
AMJ385  nrde-3(tm1116) X; jamEx77
AMJ488  ergo-1(tm1860) V; jamEx77
AMJ565  jamSi6 II; rde-4(ne301) III; unc-119(ed3) III (?)
AMJ749  bli-1(jam14) II
AMJ783  jamEx194[Pmyo-3::DsRed::unc-54 3'UTR] – line 1
AMJ784  jamEx195[Pmyo-3::DsRed::unc-54 3'UTR] – line 2
AMJ785  jamEx196[Pmyo-3::DsRed::unc-54 3'UTR] – line 3
AMJ788  rde-1(ne219) V; jamEx199[Psid-2::rde-1(+):rde-1 3'UTR & Psid-2::gfp::unc-54 3'UTR]
AMJ793  jamEx203[Prgef-1::bli-1-dsRNA & Prgef-1::gfp::unc-54 3'UTR]
AMJ804  rde-4(ne301) III; K08F4.2(K08F4.2::gfp) IV
AMJ805  oxSi221 II; unc-119(ed3) III(?); jamEx196
AMJ821  nrde-3(tm1116) X; jamEx203
AMJ822  sid-1(qt9) V; jamEx203
AMJ824  rde-4(ne301) III; K08F4.2(K08F4.2::gfp) IV; jamEx4
AMJ912  jamSi28[Pmyo-3::rde-4(+):rde-4 3'UTR] II; rde-4(ne301) III
EG4322  tti5605 II; unc-119(ed3) III
EG6070  oxSi221 II; unc-119(ed3) III
**Transgenesis**

To express rde-4(+) in the body-wall muscle under the myo-3 promoter:

The wild-type rde-4 gene was expressed under the control of the myo-3 promoter from extrachromosomal arrays (AMJ66 (23), AMJ237, AMJ238, and AMJ239) or from an integrated array (AMJ151)).

Expression from extrachromosomal arrays: To make Pmyo-3::rde-4(+)::rde-4 3’UTR, the myo-3 promoter (Pmyo-3) was amplified with primers P28 and P31, and rde-4(+) was amplified with primers P30 and P4. The two PCR products were used as templates to generate the Pmyo-3::rde-4(+) fusion product with primers P29 and P32. To make Pmyo-3::gfp::unc-54 3’UTR, gDNA from a strain that expresses Pmyo-3::gfp::unc-54 3’UTR (HC150 (ccls4251 [pSAK2 (Pmyo-3::nlsGFP-LacZ) & pSAK4 (Pmyo-3::mitoGFP), & dpy-20 subclone]) I; qtls3 [pBMW14(Pmyo-2::GFP::unc-22--PFG)] III; mls11 [Pmyo-2::gfp, gut::gfp, pes-10::gfp] IV sid(qt25)) was used as template to directly amplify the fusion product with the primers P33 and P34 using Long-Template Expand Polymerase (Roche). WM49 animals were microinjected with a 1:1 mixture (10 ng/µl) of Pmyo-3::rde-4(+)::rde-4 3’UTR and Pmyo-3::gfp::unc-54 3’UTR in 10 mM Tris (pH 8.5) to generate three independent transgenic lines (AMJ237, AMJ238, and AMJ239).

Expression from an integrated array: A strain with two spontaneous integration events that generated jamls3 and jamls4 was designated as AMJ151 (rde-4(ne301) III; mls11 jamls3 jamls4 IV). Microinjection of pH448 at 38 ng/µl in 10 mM Tris (pH 8.5) into rde-4(ne301) III; mls11 generated
jamIs3. Subsequent microinjection of a mix of Pmyo-3::rde-4 and pHC183 (as described earlier in (23)) generated jamIs4. The resultant strain was then outcrossed by mating with AMJ8 (juls73) to generate juls73/rde-4(ne301) heterozygotes and picking their self progeny that lack juls73.

To express rde-4(+) in the body-wall muscle under the unc-54 promoter:

To make Punc-54::rde-4(+)::rde-4 3'UTR, the unc-54 promoter (Punc-54) was amplified with primers P22 and P24, and rde-4(+) and rde-4 3'UTR was amplified with primers P23 and P4. The two PCR products were used as templates to generate the Punc-54::rde-4(+)::rde-4 3'UTR fusion product with primers P25 and P5. To make Punc-54::gfp::unc-54 3'UTR, Punc-54 was amplified using primers P22 and P27 and gfp::unc-54 3'UTR was amplified from pPD95.75 using primers P26 and P8. The two PCR products were used as templates and Punc-54::gfp::unc-54 3'UTR fusion product was generated using the primers P25 and P13. WM49 animals were microinjected with a 1:1 mixture of P7, and P12 a and P11, and P10. The resultant transgenic lines. A representative transgenic line was designated as AMJ217.

To express rde-4(+) in the hypodermis under the nas-9 promoter:

To make Pnas-9::rde-4(+)::rde-4 3'UTR, the nas-9 promoter (Pnas-9) was amplified using the primers P1 and P3, and rde-4(+) and rde-4 3'UTR was amplified using the primers P2 and P4. The two PCR products were used as templates to generate the Pnas-9::rde-4(+)::rde-4 3'UTR fusion product with primers P40 and P5. To make Pnas-9::gfp::unc-54 3'UTR, Pnas-9 was amplified with primers P1 and P7, and gfp::unc-54 3'UTR was amplified from pPD95.75 using the primers P6 and P8. WM49 animals were microinjected with a 2:1:1 mixture of Pnas-9::rde-4(+)::rde-4 3'UTR (10 ng/µl), Pnas-9 (with gfp overlap) (5 ng/µl) and gfp (with Pnas-9 overlap) (5 ng/µl) in 10 mM Tris (pH 8.5) to generate transgenic lines. A representative transgenic line was designated as AMJ162.

To make strain AMJ210, AMJ162 was crossed with AMJ8 males. F2 cross progeny that were homozygous for juls73 (which is linked to rde-4(+) and contained the jamEx24[Pnas-9::rde-4(+)::rde-4 3'UTR & Pnas-9::gfp::unc-54 3'UTR] transgene were passaged for one generation to ensure homozygosity of juls73 and then crossed with N2 males. A representative F2 progeny of this cross that lacked juls73 (i.e. was homozygous for rde-4(+)) but contained the jamEx24[Pnas-9::rde-4(+)::rde-4 3'UTR & Pnas-9::gfp::unc-54 3'UTR] transgene was designated as AMJ210.

To express rde-4(+) in the hypodermis under the wrt-2 promoter:

To make Pwrt-2::rde-4(+)::rde-4 3'UTR, the wrt-2 promoter (Pwrt-2) was amplified using the primers P9 and P11, and rde-4(+)::rde-4 3'UTR was amplified using the primers P10 and P4. The two PCR products were used as templates to generate the Pwrt-2::rde-4(+)::rde-4 3'UTR fusion product with primers P12 and P5. To make Pwrt-2::gfp::unc-54 3'UTR, Pwrt-2 was amplified using primers P9 and P15, and gfp::unc-54 3'UTR was amplified from pPD95.75 using primers P14 and P8. The two PCR products were used as templates to generate Pwrt-2::gfp fusion product with primers P12 and P13. WM49 animals were microinjected with a 1:1 mixture (10 ng/µl) of Pwrt-2::rde-4(+)::rde-4 3'UTR and
Pwrt-2::gfp::unc-54 3'UTR in 10 mM Tris (pH 8.5) to generate transgenic lines. A representative transgenic line was designated as AMJ212. This strain grew slowly for the first ~4 generations, but became comparable to other strains in later generations.

To express rde-4(+) in the intestine under the sid-2 promoter:

To make Psid-2::rde-4(+):rde-4 3' UTR, the sid-2 promoter (Psid-2) was amplified using the primers P16 and P18, and rde-4(+) along with rde-4 3'UTR was amplified using the primers P17 and P4. The two PCR products were used as templates and the Psid-2::rde-4(+):rde-4 3'UTR fusion product was generated using the primers P19 and P5. To make Psid-2::gfp::unc-54 3'UTR, Psid-2 was amplified using the primers P16 and P21, and gfp::unc-54 3'UTR was amplified from pPD95.75 using the primers P20 and P8. The two PCR products were used as templates and the Psid-2::rde-4(+):rde-4 3'UTR fusion product was generated using the primers P19 and P13. WM49 animals were microinjected with a 1:1 mixture (10 ng/µl) of Psid-2::rde-4(+):rde-4 3'UTR and Psid-2::gfp::unc-54 3'UTR in 10 mM Tris (pH 8.5) to generate transgenic lines. A representative transgenic line was designated as AMJ220.

To express rde-4(+) in the neurons under the rgef-1 promoter:

To make Punc119::rde-4(+):rde-4 3' UTR, the unc-119 promoter (Punc119) was amplified using the primers P39 and P35, and rde-4(+)::rde-4 3'UTR was amplified using the primers P38 and P4. The two PCR products were used as templates to generate the Punc119::rde-4(+):rde-4 3'UTR fusion product with primer P41 and P5. To make Punc119::gfp::unc-54 3'UTR, Punc119 was amplified using primers P39 and P36, and gfp::unc-54 3'UTR was amplified from pBH34.21 using the primers P37 and P42. The two PCR products were used as templates and the Punc119::rde-4(+)::rde-4 3'UTR & Prgef-1::gfp::unc-54 3'UTR progeny were isolated and designated as AMJ188.

To express rde-1(+) in the body-wall muscles under the myo-3 promoter:

As described in (23).

To express rde-1(+) in the intestine under the sid-2 promoter:

To make Psid-2::rde-1(+):rde-1 3' UTR, the sid-2 promoter (Psid-2) was amplified using the primers P16 and P65, and rde-1(+)::rde-1 3'UTR was amplified using the primers P68 and P45. The two PCR
products were used as templates to generate the \textit{Psid}:-\textit{rde-1(+)::rde-1 3'UTR} fusion product with primers P19 and P46. Coinjection marker \textit{Psid}:-\textit{gfp::unc-54 3'UTR}, was made as described for AMJ220. WM27 animals were microinjected with a 1:1 mixture (10 ng/\(\mu\)l) of \textit{Psid}:-\textit{rde-1(+)::rde-1 3'UTR} and \textit{Psid}:-\textit{gfp::unc-54 3'UTR} in 10 mM Tris (pH 8.5) to generate transgenic lines. A representative transgenic line was designated as AMJ788.

**To express \textit{rde-1(+) in the hypodermis under the wrt-2 promoter:**

To make \textit{Pwrt-2::rde-1(+)::rde-1 3'UTR}, the \textit{wrt-2} promoter (\textit{Pwrt-2}) was amplified using the primers P9 and P43, and \textit{rde-1(+)::rde-1 3'UTR} was amplified using the primers P44 and P46. The two PCR products were used as templates to generate the \textit{Pwrt-2::rde-1(+)::rde-1 3'UTR} fusion product with primers P12 and P46. \textit{Pwrt-2::gfp::unc-54 3'UTR} was made as described for AMJ212. WM27 animals were microinjected with a 1:1 mixture (10 ng/\(\mu\)l) of \textit{Pwrt-2::rde-1(+)::rde-1 3'UTR} and \textit{Pwrt-2::gfp::unc-54 3'UTR} in 10 mM Tris (pH 8.5) to generate transgenic lines. Three representative transgenic lines were designated as AMJ631, AMJ632, and AMJ633.

**To express \textit{rde-1(+) in neurons under the rgef-1 promoter:**

Made as described in (23).

**To express \textit{gfp in the hypodermis under the nas-9 promoter:**

To make \textit{Pnas-9::gfp::unc-54 3'UTR}, the \textit{nas-9} promoter (\textit{Pnas-9}) was amplified with primers P1 and P6, and \textit{gfp::unc-54 3'UTR} was amplified from pPD95.75 using primers P47 and P8. The two PCR products were used as templates and \textit{Pnas-9::gfp::unc-54 3'UTR} fusion product was generated using the primers P40 and P13. N2 animals were microinjected with a \textit{Pnas-9::gfp::unc-54 3'UTR} in 10 mM Tris (pH 8.5) to generate transgenic lines. A representative transgenic line was designated as AMJ303.

**To express \textit{DsRed in the body-wall muscle under the myo-3 promoter:**

N2 animals were microinjected with pHC183 (\textit{Pmyo-3::DsRed::unc-54 3'UTR}, made as described in (23)) in 10 mM Tris (pH 8.5) to generate 3 transgenic lines designated as AMJ783, AMJ784 and AMJ785.

**To express \textit{sid-1(+) in the body-wall muscles under the myo-3 promoter:**

As described in (6).

**To express \textit{rde-4(+) in the hypodermis under the nas-9 promoter from a single-copy transgene:**

EG4322 animals were microinjected with a mixture of pJM6 (22.5ng/\(\mu\)l) and the coinjection markers pCFJ601 (50ng/\(\mu\)l), pMA122 (10 ng/\(\mu\)l), pGH8 (10 ng/\(\mu\)l), pCFJ90 (2.5 ng/\(\mu\)l), and pCFJ104 (5 ng/\(\mu\)l) (plasmids described in (27)) to generate a transgenic line as described earlier (27). This isolated line was crossed into AMJ8 males and the resulting \textit{rde-4(+)::juls73} male progeny were crossed to WM49,
and homozygozed for the single-copy insertion and \textit{rde-4(-)} to generate AMJ565. The integration of \textit{Pnas-9::rde-4(+):rde-4 3'UTR} in AMJ565 was verified by genotyping for the presence of \textit{Pnas-9::rde-4(+)} using primers P53 and P54.

\textbf{To express \textit{rde-4(+)} in the body-wall muscle under the \textit{myo-3} promoter from a single-copy transgene:}

EG4322 animals were microinjected with a mixture of pPR1 (22.5ng/µl) and the coinjection markers pCFJ601 (50ng/µl), pMA122 (10 ng/µl), pGH8 (10 ng/µl), pCFJ90 (2.5 ng/µl), and pCFJ104 (5 ng/µl) (plasmids described in (27)) to generate a transgenic line as described earlier (27). This isolated line was crossed into AMJ8 males and the resulting \textit{rde-4(+)/juls73} male progeny were crossed to WM49, and homozygozed for the single-copy insertion and \textit{rde-4(-)} to generate AMJ912. The integration of \textit{Pmyo-3::rde-4(+):rde-4 3'UTR} in AMJ912 was verified by genotyping for the presence of an insertion at the Mos site using primers P82-P84.

\textbf{To express \textit{bli-1-dsRNA} in the neurons under the \textit{rgef-1} promoter:}

To make \textit{Prgef-1::bli-1-dsRNA} sense strand, the \textit{rgef-1} promoter (\textit{Prgef-1}) was amplified with primers P67 and P68 and a 1kb region in exon 3 of \textit{bli-1} was amplified using primers P69 and P70. The two PCR products were used as templates and \textit{Prgef-1::bli-1-dsRNA} sense fusion product was generated using the primers P71 and P72. To make \textit{Prgef-1::bli-1-dsRNA} antisense strand, the \textit{rgef-1} promoter (\textit{Prgef-1}) was amplified with primers P67 and P73 and \textit{bli-1} was amplified using primers P74 and P75. The two PCR products were used as templates and \textit{Prgef-1::bli-1-dsRNA} antisense fusion product was generated using the primers P71 and P76. N2 animals were microinjected with a 1:1:1 ratio of sense \textit{Prgef-1::bli-1-dsRNA}, antisense \textit{Prgef-1::bli-1-dsRNA} and \textit{Prgef-1::gfp::unc-54 3'UTR} (as described in (23)) in 10 mM Tris (pH 8.5) to generate transgenic lines. A representative transgenic line was designated as AMJ793.
Figure S1. Silencing by feeding RNAi of some genes is reduced in tissues expressing RDE-4 or RDE-1 from a repetitive transgene. (A) Silencing by feeding RNAi of some endogenous genes is reduced in tissues expressing rde-4(+) or rde-1(+) from a repetitive transgene. Wild-type animals, mutant animals (rde-1(-), top or rde-4(-), bottom) or mutant animals with tissue specific rescue in the hypodermis (Ex[Pwrt-2::rde(+)]) were fed dsRNA against hypodermal genes (dpy-7 or bli-1, green) and the fractions of animals that showed silencing (fraction silenced) were determined. Asterisks indicates p<0.01 (compared to wild-type animals). (B and C) Representative images of animals that express gfp (black) in all somatic cells (Peft-3::gfp) in a rde-4(-) background (B) or with rde-4 rescued in the muscles (Pmyo-3::rde-4(+)) (C) that were fed either control dsRNA (control RNAi) or dsRNA against gfp (gfp RNAi) are shown. Insets are brightfield images, scale bar = 50 µm. Also see Figure 1B and methods for details on generation of the inverted grey-scale images presented in all figures. (D) Silencing of bli-1 and dpy-7 is inhibited by the expression of RDE-4 from a repetitive transgene in the hypodermis. Wild-type animals and rde-4(-) animals that express rde-4(+) along with gfp in the hypodermis (Ex[Pnas-9::rde-4(+)]) from extrachromosomal repetitive DNA (array) were fed dsRNA against hypodermal genes (dpy-7 or bli-1, green). The fractions of animals either with or without the arrays that showed silencing (fraction silenced) were determined. Also see Figure 1C. Asterisks indicates p<0.01 (compared to wild-type animals). Error bars indicate 95% confidence intervals (CI), n>22 animals.
Figure S2. Silencing by feeding RNAi of some genes is reduced in tissues expressing a repetitive transgene of unrelated sequence. (A-C) Representative images of animals with gfp expression in all somatic cells (GFP) that were fed gfp RNAi (A) or animals that in addition express DsRed in the body-wall muscle (Pmyo-3::DsRed) that were fed control RNAi (B) or gfp RNAi (C) are shown. Expression of gfp or DsRed is shown in black except in merged images in (B) and (C) that show overlap of gfp and DsRed expression (DsRed = magenta and GFP = green). Insets are brightfield images and scale bar = 50 µm. (D) Quantification of gfp silencing in animals with or without DsRed expression in body-wall muscles. In animals expressing DsRed in body-wall muscles (Ex[Pmyo-3::DsRed]), gfp fluorescence was brighter in body-wall muscles than in other tissues. Red lines indicate median GFP fluorescence. Asterisks indicates p<0.01 (compared to wild-type animals). (E) Silencing of unc-22 and unc-54 can be inhibited by the expression of a repetitive transgene of unrelated sequence in body-wall muscles. Two additional lines (also see Figure 1D) of wild-type animals expressing DsRed in body-wall muscles (Ex[Pmyo-3::DsRed]) from extrachromosomal repetitive transgenes (array) were fed dsRNA against body-wall muscle genes (unc-22 or unc-54, magenta). The fractions of animals either with or without the arrays that showed silencing (fraction silenced) were determined (n=50). Error bars indicate 95% CI and asterisks indicate p<0.01 (compared to animals without array).
Figure S3. Genes that show robust inhibition of silencing upon expression of an unrelated transgene require the nuclear RNAi pathway for complete silencing. (A) Inhibition of bli-1 silencing by expression of any repetitive DNA can be relieved by the loss of some components of the endogenous RNAi pathway. (Left) Schematic of endogenous RNAi. Aberrant RNA recruits the RNA-dependent RNA polymerase RRF-3, the exonuclease ERI-1, the endonuclease DCR-1, the primary Argonaute ERGO-1, and the secondary Argonautes (e.g. NRDE-3) to cause silencing. (Right) Loss of eri-1 or rrf-3 but not of ergo-1 or nrde-3 relieves inhibition of bli-1 silencing caused by hypodermal expression of repetitive DNA. Extent of silencing (fraction Bli) in response to bli-1 feeding RNAi of animals with or without an extrachromosomal array that expresses gfp in the hypodermis (Ex[Pnas-9::gfp]) in a wild-type, rrf-3(-), eri-1(-), ergo-1(-) or nrde-3(-) background were determined (n>28 animals). Error bars indicate 95% CI and asterisks indicate p<0.01 (compared to wild-type animals with or without the array, respectively). (B) Genes that show robust inhibition of silencing upon expression of a repetitive transgene appear to also require the nuclear Argonaute NRDE-3 for complete silencing. Wild-type animals and nrde-3(-) animals were fed dsRNA against unc-22, unc-54, or bli-1 and the fractions of animals that showed silencing (fraction silenced) were determined. Error bars indicate 95% CI and asterisks indicate p<0.01 (compared to wild-type animals with or without the array, respectively). (C) Silencing of bli-1, by ingested dsRNA requires multiple components of the nuclear RNAi pathway. Wild-type, nrde-3(-), nrde-2(-), nrde-1(-), or nrde-4(-) animals were fed dsRNA against bli-1 and the percentage of gravid adult animals that showed silencing (% Bli) was determined (n = number of gravid adults scored for silencing). (D) Silencing of bli-1 by neuronal dsRNA requires the nuclear Argonaute nrde-3. Silencing of bli-1 by dsRNA against bli-1 expressed under a neuronal promoter (Ex[Prgef-1::bli-1-dsRNA]) in a wild-type, sid-1(-), or nrde-3(-) background were measured as in (C).
Figure S4. Silencing of bli-1 upon feeding RNAi in wild-type animals and in animals with tissue-specific rescue of rde-4 in non-hypodermal cells results in unique patterns of blisters that differ from those in bli-1(-) animals. (A) Design of Cas9-based genome editing to generate a bli-1 null mutant. The bli-1 gene (exons, blue boxes; introns, grey lines) was targeted by a single-guide RNA (sgRNA) that cuts within the gene (orange *) and was repaired with a double-stranded...
DNA template (green flanking the gene). (B) Results from Cas9/sgRNA injection into wild-type worms to generate a bli-1 null mutant. Wild-type animals were simultaneously targeted for edits in dpy-10 and bli-1 (co-conversion strategy (28)), resulting in progeny that displayed Rol, DpyRol and Dpy defects (indicative of dpy-10 editing) with or without blisters as indicated. (C) A representative animal that illustrates scoring of bli-1 silencing in each section of the worm. The animal was divided into 8 sections (a through h, see Figure 3C) and each section was scored for presence of a full blister (black), partial blister (grey), or no blister (white) as indicated in the inset. Scale bar = 50 µm. (D) Wild-type animals display a stereotyped pattern of susceptibility to bli-1 feeding RNAi. Progeny of wild-type animals targeted by Cas9-based genome editing, bli-1 null mutant animals, and wild-type animals exposed to feeding RNAi were scored for blister patterns as described in Figure 3C (n>45 gravid adult animals). Unlike sections in the progeny of animals that were injected with Cas9/sgRNA or in bli-1 null mutants, sections in wild-type animals that were subject to bli-1 feeding RNAi showed a stereotyped frequency of blister formation (a > b > … > h). (E) RDE-4 expression in intestinal cells near the tail correlates with blister formation in rde-4(-) hypodermis near the tail upon bli-1 RNAi. (F and G) Blister scoring method to detect variations in the pattern of blister formation upon bli-1 feeding RNAi. (F) The pattern of blister formation in rde-4(-) animals with rde-4(+) expressed in the intestine (Psid-2) was examined (step I) and animals following the stereotyped order of susceptibility to bli-1 RNAi (a > b > … > h) were removed (step II). The remaining animals, which show variant susceptibility to bli-1 feeding RNAi, were aggregated (step III and IV) and collapsed into a heat map (step V) to assess frequency of variant blisters in each section. Grey bounding box (step II-step IV) indicates the total number of worms that showed blister formation in each strain (n = 50 gravid adult animals) (G) The pattern of bli-1 silencing in rde-4(-) animals with RDE-4 expressed from neuronal promoters (Prgef-1 & Punc-119) or body-wall muscle promoters (Pmyo-3 & Punc-54) were examined and variant blisters were isolated as in (F) (n = 50 adult animals). (H) The pattern of blisters that result from silencing of bli-1 in rde-4(-) hypodermis are characteristic of the tissue that expresses RDE-4. Patterns of blister formation in response to ingested bli-1 RNAi were examined in wild-type animals or in rde-4(-) animals that express RDE-4 under neuronal promoters (Prgef-1 or Punc-119, orange) or under body-wall muscle promoters (Pmyo-3 or Punc-54, magenta). For each strain, sections with full (black) or partial (grey) blister formation in animals that showed a variation in the order of susceptibility (i.e. were not a > b > … > h) were plotted. Grey bounding box and n are as in (F).
Figure S5. Expression of RDE-1 in one somatic tissue can enable silencing of *Peft-3::gfp* in other mutant somatic tissues. Representative images of animals with *gfp* expression (black) in all somatic cells (*Peft-3::gfp*) in a wild-type background (A and C), *rde-1(-)* background (A), or *rde-1(-)* background with *rde-1(+) expressed in body-wall muscles (*Ex[Pmyo-3::rde-1(+)]*) (B and C). Animals were fed control RNAi or *gfp* RNAi for one generation (A & B) or for two generations (C). Insets are brightfield images and scale bar = 50 µm.
Figure S6. Expression of RDE-4 in the somatic tissues of parents does not typically enable feeding RNAi in rde-4(-) progeny. (A-D) Progeny of wild-type animals, rde-4(-) animals, or rde-4(-) animals expressing RDE-4 in the muscle (Pmyo-3 or Punc-54), hypodermis (Pnas-9), or intestine (Psid-2) were fed dsRNA (F1-only feeding RNAi) against unc-22, unc-54, act-5, or bli-1 and the fractions of animals that showed silencing (fraction silenced) were determined. Also see Supplementary Table S1. Error bars indicate 95% CI and asterisks indicate p<0.01 (animals without array compared to rde-4(-) animals). (E) Parental expression of RDE-4 from an integrated Pmyo-
3::rde-4(+) transgene also enables feeding RNAi of unc-22 in rde-4(-) progeny. The rde-4(+) progeny (Is[myo-3::rde-4(+)]/Ø) and rde-4(-) progeny (Ø/Ø) of parent animals expressing RDE-4 from an integrated repetitive array in the muscle (Is[myo-3::rde-4(+)]/Ø) were subjected to feeding RNAi (F1 RNAi) of unc-22 and the fractions of animals that showed silencing were determined (fraction silenced). 100% of wild-type and 0% of rde-4(-) control animals showed silencing of unc-22. Error bars indicate 95% CI and n>15 animals.

Figure S7. Co-expression of an unrelated gene from a repetitive transgene with RDE-4 from a single-copy transgene is insufficient to silence genes in rde-4(-) somatic tissues. Animals that express RDE-4 from a single-copy transgene in the hypodermis (Si[Pnas-9::rde-4(+)]) and additionally gfp from a repetitive transgene (Ex[Pnas-9::gfp]) in the hypodermis were fed dsRNA against unc-22 (magenta), act-5 (blue) or bli-1 (green). Silencing was scored as in Figure 2A. Also see Figure 4C and Supplementary table S1. Error bars indicate 95% CI and n>49 animals.

SUPPLEMENTARY TABLES

Table S1. Feeding RNAi and defects scored.

| Gene | Expression     | Defect scored upon RNAi                                                                 |
|------|----------------|----------------------------------------------------------------------------------------|
| unc-22 | body-wall muscle | L4 or young adults twitch within 2 minutes in response to 3mM levamisole (Sigma Aldrich). |
| unc-54 | body-wall muscle | Inability to move backwards upon touching the head and lack of sinusoidal movement.       |
| act-5  | intestine       | Failure to develop to the 4th larval stage in 3 days.                                    |
| dpy-7  | hypodermis     | Short, fat L4- or adult-staged animals                                                   |
| bli-1  | hypodermis     | Presence of fluid-filled blisters on adults                                               |
| pos-1  | germline       | Animals that laid >90% unhatched eggs                                                    |
| par-1  | germline       | Animals that laid >66% unhatched eggs                                                    |
| par-2  | germline       | Animals that laid >80% unhatched eggs                                                    |
| Name | Sequence |
|------|----------|
| P1   | gttccattcatggaatcttagag |
| P2   | ttcatctttgtctaatctatggaatgtaaccaatctaatggaac |
| P3   | cgagttaatatccttactggaatgtaaccaatctaatggaac |
| P4   | cactgcagagaatgagag |
| P5   | gtagaggtcagagggcatag |
| P6   | ttcatctttgtctaatctatggaatgtaaccaatctaatggaac |
| P7   | gaaaagtattttacttatggaatgtaaccaatctaatggaac |
| P8   | cggtcataaactgaaacgttaac |
| P9   | tcaaggaactgtaac |
| P10  | acctgcattttttttctggaatgtaaccaatctaatggaac |
| P11  | cgagttaatatccttactggaatgtaaccaatctaatggaac |
| P12  | ctgaacttcaaatggtgtg |
| P13  | ctgaacgtaacatattgataagg |
| P14  | acctgcattttttttttctggaatgtaaccaatctaatggaac |
| P15  | gaaaagtattttacttatggaatgtaaccaatctaatggaac |
| P16  | aattggatgagagagccttg |
| P17  | caaaaccctgtatatctttctggaatgtaaccaatctaatggaac |
| P18  | cgagttaatatccttactggaatgtaaccaatctaatggaac |
| P19  | ctgcctatgggaacttcaac |
| P20  | caaaaccctgtatatctttctggaatgtaaccaatctaatggaac |
| P21  | gaaaagtattttacttatggaatgtaaccaatctaatggaac |
| P22  | ttgttccgcaaatattgc |
| P23  | ccaattggaagagaacttggatgtaaccaatctaatggaac |
|   | Sequence                                    |
|---|--------------------------------------------|
|P24| cgtagttgtgtaaatccatgatctctgcctttcaaatgg    |
|P25| ggtatgagagagttgccagag                   |
|P26| ccattggaagagcgagaactgtaaatggagaagaacttttc |
|P27| gaaaaagtctttctcttaacatgattttctgctctttcaaatgg |
|P28| aggctgcaaaaaagatcagg                      |
|P29| tctctcgtacgtgtaactctcactctgcgggc        |
|P30| cgaccactagatccatctgaaatggatcttaacaaactacg |
|P31| cgttagttgtagttatcactttctagatgtagatcggctc |
|P32| agagagctcgaggtagaggtcagaggtcagagagcatag |
|P33| ggtcggctataataagflcttg                   |
|P34| cggtcataaactgaaacgttaac                  |
|P35| cgttagttgtaaatctctgaaaaagaggttcttc      |
|P36| gttctctttgagcagctctgaaaagaggttttc       |
|P37| gaaaaactcttttttttctgatgactctccttaagagaagc |
|P38| gaaaaactctttttttttctgatggaatttaacaaactacg |
|P39| gtcaaaatgaacagacgccg                     |
|P40| tttctcgccctctaaagaaac                   |
|P41| aaatgacacagagcggacg                    |
|P42| ctgaaacgtaacatatgataagg                 |
|P43| cggtcataaactgaaacgttaac                 |
|P44| tttctcgagatgtcctgaattttccgga           |
|P45| gctcaactggagaagtgtga                     |
|P46| gcaggtgatttcacagacttc                  |
|P47| gaaaaagtctctctctttactcatgaagatggagaagaattgatg  |
|P48| tgctatgaggcagaggtaac                   |
| P49 | cgtagtttggttaaatccattgaacagaatgatgaattgcg |
|-----|------------------------------------------|
| P50 | cgcaatttcacttctgttcaatgtagatctttccaaactaacg |
| P51 | tctctccctgaggatcttcaaatgtggaattacaactcagt |
| P52 | gagagaactagtgtagggtacagggctag |
| P53 | ccattgatctttgcccaactc |
| P54 | gctttctgttggtccg |
| P55 | attaggtgacacatagattatactctgctgttgagctgtt tagagccagaaatagcaag |
| P56 | tggcaccgagtcgggtgc |
| P57 | attaggtgacacatatagctaccatatggccacacgaggttt tagagctaagaaatagcaag |
| P58 | cgctcgctctcaaatcag |
| P59 | agactagaatgtagagttagatgaggtagatcactac |
| P60 | gtatgtgtatctaactcacttaactaactaataaaaaactctaggtct |
| P61 | cccatcttcccagatatcc |
| P62 | atatatagactgcgggtcaggtgg |
| P63 | tcccttcatgtcttgcacaac |
| P64 | cacttgaacttcaatcggcaagtgagaatgactggaaaccgcatgcggtgcctatggtagcggagcttcaccagcaacagccta |
| P65 | tcgggaaaatctcaggacacttctgtcgaatataatactcaggggttt |
| P66 | aaaaaacctgtatatcctcaggaatggtttctgcagtttcccga |
| P67 | cgataatctgtgacactcg |
| P68 | gagagggtgcacgggaacccggtctgtcgtcgtcatg |
| P69 | gcacacgacgcagcaggtttccgtgcacccctc |
| P70 | tttctccaggttagtccagg |
| P71 | ccccttatctcagcacac |
| P72 | gttgaccatctttgacgtt ccg |
SUPPLEMENTARY DISCUSSION

Challenges and limitations in interpreting experiments that use repetitive transgenes. Partial characterization of the molecular structure of repetitive transgenes in *C. elegans* by Southern blotting (51) or Illumina sequencing (42) reveal that they can be made of >100 copies with at least some copies including complex rearrangements. These rearrangements can create unique sequences in promoter regions and within coding regions. When promoter or 3’ UTR sequences are changed, it can result in the expression of a gene in unintended tissues even when well-characterized promoters were used to make the repetitive transgene. When coding sequences are changed, it can result in the production of new variants of a protein with different biochemical properties that can result in unpredictable functions.

Repeats are associated with heterochromatin factors and histone modifications, some of which can also occur upon feeding RNAi at the target locus (e.g. H3K9me3 (68)). Recent reports suggest that loss of H3K9me3 can cause RNA:DNA hybrid-associated instability of the repeats (69). This possibility could alter inferences about the repetitive transgene-dependent inhibition observed for feeding RNAi of some genes (Supplementary Figure S3). For example, the bli-1 silencing observed in *eri-1(-)* mutants despite expression from repetitive transgenes could be associated with changes in DNA and/or chromatin. Furthermore, when two repetitive transgenes are used (Figure 4), chromatin factors could be titrated from one transgene by the other, resulting in changed levels of expression.