ONLINE METHODS

Strains Used. N2 wild-type, HC196 *sid-1(qt9)*, WM30 *mut-2 or rde-3(ne298)*, WM27 *rde-1(ne219)*, WM49 *rde-4(ne301)*, NL2099 *rrf-3(pk1426)*, GR1373 *eri-1(mg366)*, HC70 *rde-1(ne219); mIs11[Pmyo-2::gfp]; ccIs4251[Pmyo-3::gfp]; qtIs3[Pmyo-2::hp-gfp]*, PD4792 *mIs11[myo-2::GFP, gut::GFP, pes-10::GFP]*, HC195 *nrIs20[sur-5::gfp]*, HC731 *sid-1(qt9); eri-1(mg366)*, HC732 *sid-1(qt9); rrf-3(pk1426)*, HC733 *mut-2(ne298); sid-*

1(qt9); nrIs20, HC734 *sid-1(qt9); rde-4(ne301); nrIs20*, HC735 *mut-2(ne298); mIs11*, HC736 *qtEx136[Prgef-1(F25B3.3)::unc-22sense; Prgef-1::unc-22antisense; Prgef-1::DsRed line 8],* HC737 *rde-4(ne301); nrIs20,* HC738 *rde-1(ne219); nrIs20,* HC739 *mut-2(ne298); nrIs20*, HZ202 *dcr-1(bp132); wIs51[scm-1::GFP]*[24], RB798 *rrf-1(ok589),* NL2098 *rrf-1(pk1417),* HC779 *dcr-1(bp132)* [outcrosssed with N2 twice], HC780 *rrf-1(ok589)* [outcrossed with N2 twice], HC781 *rrf-1(pk1417)* [outcrossed with N2 twice], HC782 *sid-1(qt9) rde-1(ne219)*, HC783 *sid-1(qt9); rde-4(ne301)*, HC784 *sid-1(qt9); mut-2(ne298)*.

Strain constructions and analyses of transgenics. Double mutants were made using standard genetic approaches and were verified by genotyping using DNA sequencing or PCR analysis. Additional strains were constructed by crossing representative transgenes into various genetic backgrounds. These include strains generated by (1) crossing HC736 into either single mutants (WM27, HC196, WM27, WM49, NL2099, GR1373, HC779, HC780, HC781) or double mutants (HC731, HC732, HC782, HC783, HC784); (2) crossing a representative line that coexpresses *rde-4(+)* and DsRed2 in bwm cells of WM49 into HC734 and HC737; (3) crossing a representative line that coexpresses *rde-1(+)* and DsRed2 in bwm cells of WM27 into HC738; (4) crossing a representative line that coexpresses *mut-2(+)* and DsRed2 in the bwm cells of WM30 into HC733 and HC739; and (5) crossing a representative line that coexpresses *gfp*-dsRNA and DsRed2 in the pharynx of HC195 and PD4792 into HC739 and HC735, respectively. To avoid bias due to observed phenotypic defects, cross progeny or re-homozygosed progeny were either selected using the DsRed2 co-injection markers or selected randomly and the genotype was determined subsequently by PCR.

30-35 animals from three independent transgenic rescue lines were analyzed (n=100) in all cases except for the rescue of *rde-4(+)* in neurons of *rde-4(-)* animals,

where a representative rescue line was crossed into *rde-4(-)* animals with the *neur::u22ds* transgene and 100 double-transgenic animals were analyzed.

Microscopy. Fluorescent images shown are projections of Z-series that were acquired using a Ziess spinning-disc confocal microscope except in **Supplemental Fig. 1a**, where widefield fluorescent images taken using a dissecting fluorescent microscope are shown. Images for strains that are being compared in all figures were acquired under the same non-saturating exposure conditions and, with the exception of the DsRed inset in **Fig. 2b**, then adjusted identically using Image J (NIH) and Photoshop (Adobe) software for display.

RNAi assays. To measure the extent of GFP silencing, we used a dissecting fluorescent microscope to count the number of brightly fluorescent gut nuclei in animals of the fourth larval stage (L4 stage) that are visible at a fixed magnification. The 2 nuclei that are located below 2 other nuclei in the first segment of the intestine (Int 1) are not easily resolved at this level of magnification and were not counted in this assay. Silencing in **Fig. 1c** was measured at 25°C, since some silencing of pharyngeal GFP is observed at lower temperatures, which is consistent with previous reports of RDE-1-independent silencing[20]. For feeding RNAi, L4-staged animals were fed bacteria that express L4440 control dsRNA or dsRNA matching a target gene on agar plates that contain 1mM isopropyl β-D-1-thiogalactopyranoside (IPTG). The percentage of the resultant L4 progeny that showed the corresponding defects was determined. For *act-5* silencing, the number of animals that survive beyond L4 on day 5 for each genotype are expressed as a percentage of L4 and older animals on day 5 of the same genotype on L4440. To measure *unc-22* silencing in response to expressed *unc-22*-dsRNA or *unc-22* feeding RNAi, we determined the percentage of L4-staged animals that twitched within 3 minutes in 3 mM levamisole (Sigma Aldrich) or on RNAi feeding plates without levamisole, respectively.

DNA constructs and transgenic animals. PCR fragments for transgenic expression[33] and transgenic animals[34] were generated using standard methods as in ref. 8. Briefly, PCR fragments corresponding to the coding sequences and 3´UTR were amplified and fused to promoter sequences using overlap extension PCR[32]. These fragments were then purified using a PCR clean-up column (Qiagen) and injected along with appropriate co-injection markers into *C. elegans* to generate transgenic lines. The specific primers used for PCR (**Supplementary Table 2**) and the specific concentrations and co-injection markers used for injections are detailed in **Supplementary Information**.

Statistical Analysis. Statistical significance of differences in average numbers of gut nuclei was calculated using Student's t-test. For all other assays, 95% confidence intervals for single proportions were calculated using Wilson's estimates with continuity correction[35] and significant differences were determined using Wilson's pooled estimates.

Methods-only References

33. Hobert, O. PCR fusion-based approach to create reporter gene constructs for expression analysis in transgenic *C. elegans*. *Biotechniques* **32**, 728-730 (2002).

34. Mello, C. C., Kramer, J. M., Stinchcomb, D., & Ambros, V. Efficient gene transfer in C. elegans: extrachromosomal maintenance and integration of transforming sequences. *EMBO J* **10**, 3959-3970 (1991).

35. Newcombe, R. G. Two-sided confidence intervals for the single proportion: Comparison of seven methods. *Statist. Med.* **17**, 857-872 (1998).