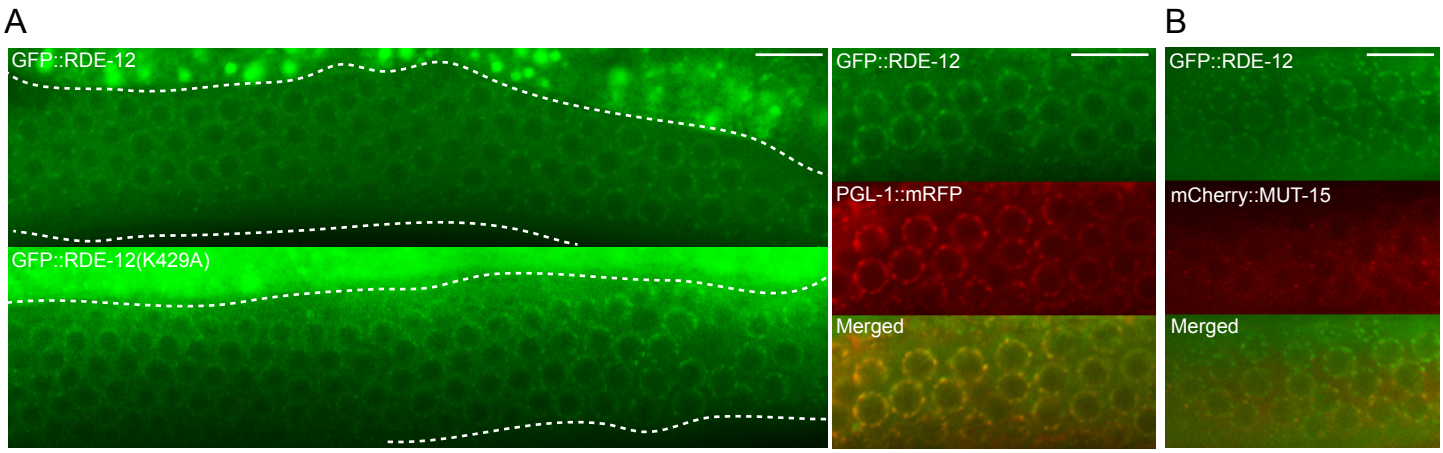

Figure S1. Localization of GFP::RDE-12 in germline

(A) Fluorescence micrographs showing GFP::RDE-12 (left upper), GFP::RDE-12 (K429A) (left lower), and GFP::RDE-12 co-localization with PGL-1::mRFP (right). The dashed lines in the micrographes indicate the position of the germ line.

(B) Fluorescence micrographs showing GFP::RDE-12 co-localization with mCherry::MUT-15. Scale bars: 10 μm (A, B).

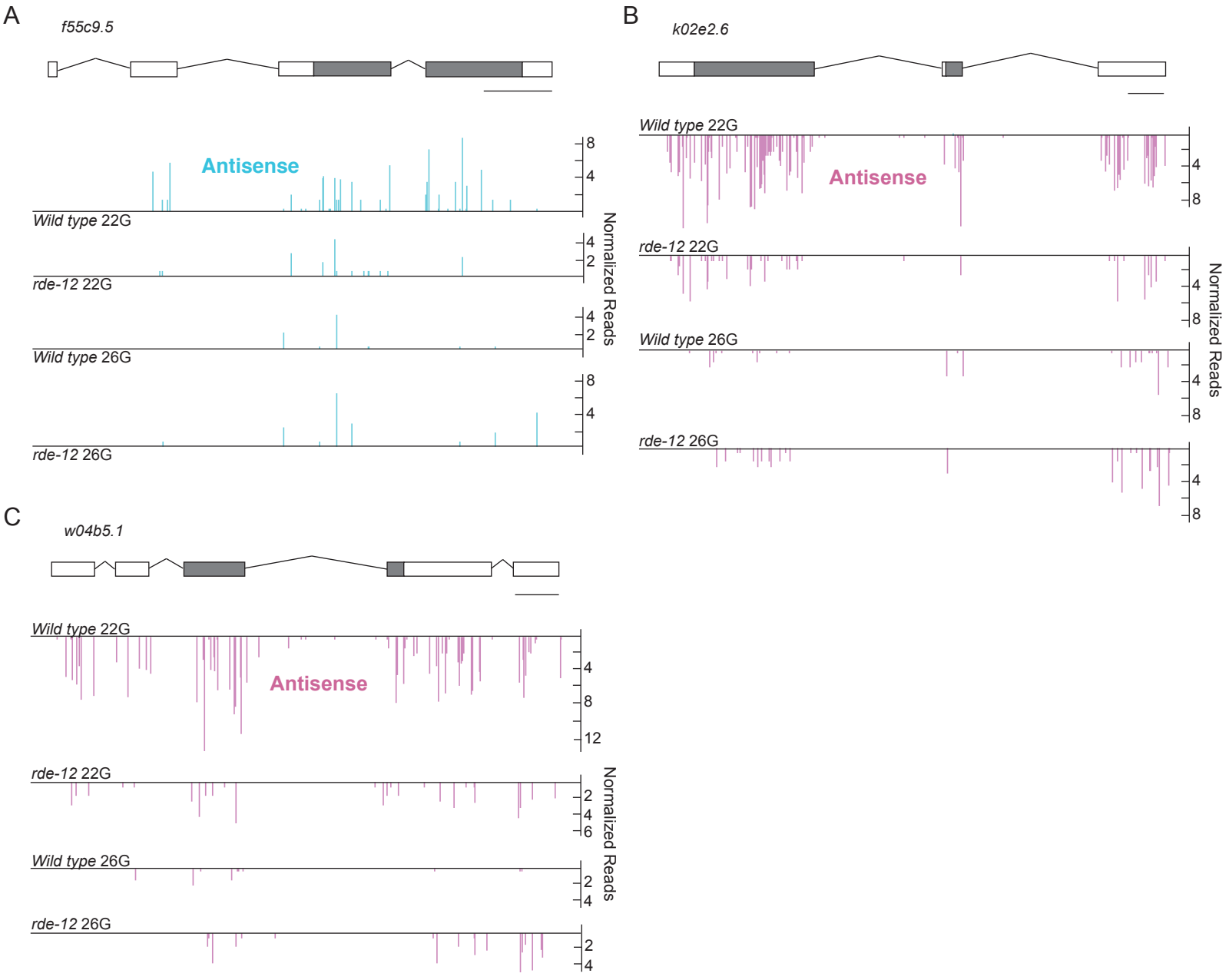

Figure S2. *rde-12* is required for ERGO-1-dependent 22Gs, but not 26Gs

(A-C) Genome browser view showing *f55c9.5* (A), *k02e2.6*(B) and *w04b5.1*(C) aligned above deep-sequencing read-density plots. Small-RNA distributions in *wild-type* and *rde-12(tm3644)* mutant strains are indicated on a $\log_2$ scale. The color is based on genome browser conventions, where the Watson strand is blue and Crick strand is pink in the genome browser. The coding and untranslated regions are indicated by gray and white boxes, respectively. The scale bars below the gene structure indicate 100 bases.

Supplemental Table 1

Gene	*rde-12*	*Wild type*	*rde-12/WT*	Pathway
F55A4.4	20.5787	533.0621	0.0372	ERGO-1
F07G6.6	284.4349	7155.7039	0.0382	ERGO-1
W04B5.2	547.3922	13363.9705	0.0393	ERGO-1
K02E2.6	1315.8506	27082.6184	0.0463	ERGO-1
F55C9.3	304.2971	4500.4413	0.0633	ERGO-1
Y37E11B.2	1977.3755	27524.9681	0.067	ERGO-1
ZK402.3	152.2111	2088.6738	0.0679	ERGO-1
ZK402.2	94.2597	1293.178	0.0679	ERGO-1
ZK402.5	153.6564	2039.9513	0.07	ERGO-1
ZK380.5	122.0527	1172.2173	0.0943	ERGO-1
F52D2.8	16.4629	154.5708	0.0963	ERGO-1
F39E9.7	964.0902	7678.8076	0.1115	ERGO-1
F55C9.5	337.3412	2438.1077	0.1215	ERGO-1
Y17D7B.4	739.6725	5281.9965	0.1228	ERGO-1
K06B9.6	1234.2226	8189.5354	0.131	ERGO-1
F31A9.2	26.9651	171.1147	0.1361	ERGO-1
E01G4.5	83.7338	526.9063	0.1371	ERGO-1
CD4.8	347.0224	2121.2038	0.1406	ERGO-1
H09G03.1	180.2406	1071.1644	0.144	ERGO-1
W05H12.2	216.8361	1191.7431	0.1539	ERGO-1
C36A4.11	928.3812	4524.5647	0.1703	ERGO-1
F52D2.6	32.8657	150.05	0.1797	ERGO-1
F39F10.4	24.1267	106.1892	0.1851	ERGO-1
C01A2.1	155.4473	587.782	0.2092	ERGO-1
ZC132.4	56.7687	188.1396	0.2318	ERGO-1
Y47H10A.5	3004.7782	15134.3261	0.1657	RDE-1

List of the target genes whose small RNA levels are reduced more than 3-fold in the *rde-12* mutants compared *to wild type* in the ERGO-1 and RDE-1 pathways. The minimal read cutoff is 20 in this analysis.

Supplemental Experimental Procedures

Strains

EG4322	*Mos1(ttTi5605) II; unc-119(ed9) III*
FX03644	*rde-12(tm3644) V*
FX03679	*rde-12(tm3679) V*
WM300	*neSi111 [gfp::rde-12, cb-unc-119(+)] II; unc-119(ed3) III*
WM301	*neSi112 [gfp::rde-12, cb-unc-119(+)] II; unc-119(ed3) III; rde-12(tm3644) V*
WM302	*neSi113 [flag::rde-12, cb-unc-119(+)] II; unc-119(ed3) III*
WM303	*neSi114 [flag::rde-12, cb-unc-119(+)] II; unc-119(ed3) III; rde-12(tm3644) V*
WM304	*neSi115 [gfp::rde-12(K429A), cb-unc-119(+)] II; unc-119(ed3) III; rde-12(tm3644) V*
WM305	*neSi116 [flag::rde-12(K429A), cb-unc-119(+)] II; unc-119(ed3) III; rde-12(tm3644) V*
WM306	*neSi117 [gfp::rde-12, cb-unc-119(+)] II; axIs1488 [mCherry::patr-1, unc-119(+)]; rde-12(tm3644) V*
WM307	*neIs99 [ha::rde-1; rde-4::flag, rol-6(su1006)]; pkIs2289[unc-22 22si, unc-119(+)]*
WM308	*neIs99 [ha::rde-1; rde-4::flag, rol-6(su1006)]; pkIs2289[unc-22 22si, unc-119(+)]; rde-12(tm3644) V*

***rde-12* transgenic lines**

Genomic sequence (LGV:13659825..13670374) encompassing the operon CEOP5398 which includes *f58g11.1* and *f58g11.2* was amplified from the cosmid f58g11 and *C.elegans* genomic DNA and inserted into a modified version of pCFJ151 (B1496) for MosSCI on LGII [S1]. The *gfp* gene amplified from pPD95.75 (Addgene) or 3x*flag* sequence (GATTACAAAGACCATGATGGTGACTATAAGGATCATGATATTGACTATAAAGACGATGACGATAAG) was inserted either after the initiation codon or before the stop codon of *f58g11.2*. The *gfp* and *flag*-tagged *f58g11.2* constructs were present

at 10 ng/μl in the injection mixture. The K429A mutation was introduced into these plasmids by site-directed mutagenesis.

Small RNA cloning and Computational Analysis

Size-selected small RNAs were treated with tobacco acid pyrophosphatase and ligated to a 5′ linker containing a 4 nt barcode as well as a 3′ linker (miRNA Cloning Linker 1, IDT) as described [S2]. PCR was used to add Illumina adapters and amplify the libraries, which were then sequenced on an Illumina GAIIx at the UMass Medical School Deep Sequencing Core. A custom Perl script was used to remove the 5′ barcode and the 3′ adapter sequences. If the 3′ adapter was not identified, then incomplete 3′ adapters (CTGTA, CTGT, CTG, or CT) were removed. Reads of at least 17 nt in length were mapped to the *C. elegans* genome (WormBase release WS215) and miRBase 16 using Bowtie 0.12.7 with the parameter '-v 3 -a --best --strata -m 400'. A custom Perl script was used to perform a post-match analysis, only allowing mis-matches with reads ≥19 nts: at most one mismatch for 19–23 nt, two for 24–30 nt and three for ≥31nt. The Bowtie parameter '-a --best –strata' was used to return only the best matches. The read count of each sequence was normalized to the total number of reads that match the genome. To account for differences in sequencing volume between samples, we normalized the total of matched non-structural RNAs to 5 million reads. A custom script and Bioperl was used to draw the scatter plot. The single nucleotide histograms of the start sites of matched reads were obtained using a custom Perl script and the generic genome browser 1.70. All scripts are available upon request.

Virus assay

C. elegans was infected with Orsay virus as previously described [S3]. Briefly, wild-type (N2), *rde-1(ne300), rde-12(tm3644)* and *rde-12(tm3679)* mutant animals were infected with Orsay virus for 3 days at 20°C from L1 stage. Total RNA was extracted from adult worms with TRIzol (Life Technologies) and the level of viral transcripts was measured by RT-qPCR as described [S4]. Abundance of Orsay viral RNAs was first normalized to *act-3*, and fold enrichment was determined relative to N2 infection. At least three biological replicates were performed for each strain. PCR primers used to detect Orsay virus are:

CMo18191 ACCTCACAACTGCCATCTACA
CMo18192 GACGCTTCCAAGATTGGTATTGGT

Immunoprecipitation

Synchronous adult *flag::wago-1* worms were dounced in a stainless steel homogenizer to prepare worm total lysate. FLAG::WAGO-1 protein complexes were immunoprecipitated with M2 anti-FLAG antibodies (Sigma, F1804) from 20 mg of lysate, essentially as described [S2], and eluted from Protein G Dynabeads (Life Technologies) with an excess of FLAG peptide (Sigma, F4799). The eluent was subjected to SDS-PAGE, and the protein bands stained with colloidal Blue (Life Technologies, LC6025) were cut from the gel. Proteins were identified by microcapillary LC/MS/MS at the Taplin Mass Spectrometry Facility (Harvard Medical School, MA).

RNA immunoprecipitation (RIP)

Worms grown on *E.coli* HT115 strain expressing *sel-1* or control (L4440) dsRNA were suspended in RNA IP buffer [20mM HEPES-potassium hydroxide, pH 7.3; 110mM potassium acetate, 1mM EDTA; 0.5% Triton X; 0.1% Tween 20; 90μg/ml PMSF; 0.2μg/ml Pepstatin A; one tablet/5ml Complete Protease Inhibitor Cocktail Tablets (Roche); 0.02U/μl SUPERaseIn (Life Technologies)] and dounced in a stainless steel homogenizer to obtain total lysate. The protein concentration of each cell extract was adjusted to 20 mg/ml. Input samples were removed and 10 μg of RDE-12 antibodies or rabbit IgGs were added to immunoprecipitate RDE-12 protein-RNA complexes. RNAs were extracted with Trizol from immunoprecipitates, and cDNA was generated using a mix of *sel-1* antisense primers (CMo12538, CMo12540, CMo12542, CMo12544, and CMo12546) and Superscript III Reverse Transcriptase (Life Technologies). Quantitative PCR was performed as described [S4] using an ABI 7500 Fast Real-Time PCR instrument and primers CMo12541 and CMo12542. *sel-1* RIP with RDE-12 antibodies was first normalized to *act-3* RIP, and fold enrichment was determined relative to negative control with rabbit IgGs.

Primers used in RIP

CMo12538: ATTGCGGCCTCATCAACTCG

CMo12540: CCTGGTTGCATATGGATTGTTGG
CMo12542: CCTGAATGGACCAACGGGAAT
CMo12544: CCAAATCAGCTTTCGACACATCA
CMo12546: CCTTCGCCAACTTCGTGCAT
CMo12541: AGAGAAGGAAGAGTTGCAGCTCATAGA
CMo12542: CCTGAATGGACCAACGGGAAT

Microscopy

For wide-field live images, transgenic worms were mounted in dH_2O on RITE-ON glass slides (Beckton Dickinson). Epi-fluorescence and differential interference contrast (DIC) microscopy were performed using an Axioplan2 Microscope (Zeiss). Images were captured with an ORCA-ER digital camera (Hamamatsu) and AxioVision (Zeiss) software. Confocal images were obtained using a Nikon TE2000-E2 microscope equipped with a Yokogawa CSU10b spinning disk confocal scan head and custom laser launch, acousto-optic tunable filter (NEOS Technologies) and relay optics (Solamere Technology Group, Salt Lake City, Utah). Multi-wavelength confocal z-series were acquired with a Nikon 60X or 100X VC Plan Apo oil objective (NA = 1.4) or a 100X objective and 1.5x tube lens with a QImaging Rolera MGi EMCCD camera with an EM gain of 3800. The final pixel sizes were 0.1826 microns/pixel, 0.1084 microns/pixel and 0.0721 microns/pixel for the 60X, 100X, and 100X objective with 1.5X tube lens, respectively. The z step was 0.2 microns, and the exposure time varied from 40 to 250 msec, depending on the fluorophore. Metamorph Software version 7.4 (Molecular Devices) controlled the microscope hardware and image acquisition. Images were analyzed with Metamorph by subtracting the average intensity in a 128 x 128 pixel region away from the stained embryos on a plane-by-plane basis. Volume rendering was performed with the Metamorph 4D viewer, and the black level was set to exclude pixels with intensity values 3-fold lower than the average signal intensity for each fluorophore.

Immunostaining

The following antibodies were used for immunostaining: Anti-nuclear pore complex proteins mouse antibodies, mAb414 (Jackson Immunoresearch Laboratories), anti-DsRed rabbit antibodies (Clontech, 632496), anti-GFP rabbit polyclonal antibodies conjugated with Alexa Fluor 488 (life technologies, A-21311), Anti-GFP mouse

antibody 3E6 (Life Technologies, A11120), anti-mouse antibodies conjugated with Alexa Fluor 594 (Life Technologies, A-11005), anti-rabbit antibodies conjugated with Alexa Fluor 594 (Life Technologies, A-11037), anti-mouse antibodies conjugated with Alexa Fluor 488 (Life Technologies, A-21131). Antibodies were diluted 1/500 for use.

Supplemental References

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