SUPPLEMENTAL INFORMATION

piRNAs initiate an epigenetic memory of non-self RNA in the *C. elegans* germline

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SUPPLEMENTAL FIGURE LEGEND

Figure S1. Small RNA profile of *gfp*::*csr-1* transgenic lines, related to Figure 3
Small RNA density along the *gfp* and *cdk-1* coding regions of wild-type and indicated transgenic lines. Vertical bars represent the 5′ nt of a small RNA, and the height of each bar indicates the number of reads that start at that position. The strand is represented by color; sense (pink) and antisense (light blue). Scale bar indicates 20 reads per million. The strain *neSi9 gfp*::*csr-1* (*RNAe*) (GFP-) was generated by crossing *neSi9 gfp*::*csr-1* (GFP+) to *neSi10 gfp*::*csr-1(RNAe*) (GFP-). The strain *neSi8 gfp*::*csr-1* (GFP+) was generated by crossing an *rde-3* mutation into the strain *neSi8 gfp::csr-1(RNAe)* (GFP-) and then removing the *rde-3* mutation by crossing *rde-3; neSi8 gfp*::*csr-1* to a wild-type strain.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Strain List

EG4322	*Mos1(ttTi5605) II; unc-119(ed3) III*
EG5003	*unc-119(ed3) III; Mos1(cxTi10882) IV*
WM189	*avr-14(ad1302) I; Mos1(tTTi5605) II; unc-119 (ed3) III; glc-1(pk54::Tc1) avr-15(ad1051) V*
WM238	*neSi8 [gfp::csr-1(RNAe), cb-unc-119(+)] II; unc-119(ed3) III.*
WM239	*neSi9 [gfp::csr-1, cb-unc-119(+)] II; unc-119(ed3) III.*
WM240	*neSi10 [gfp::csr-1(RNAe), cb-unc-119(+)] IV; unc-119(ed3) III.*
WM241	*neSi11 [gfp::cdk-1(RNAe), cb-unc-119(+)] II; unc-119(ed3) III.*
WM242	*neSi12 [cdk-1::gfp, cb-unc-119(+)] II; unc-119(ed3) III.*
WM269	*neSi13 [gfp::cdk-1, cb-unc-119(+)] II; prg-1(tm872) I; unc-119(ed3) III.*
WM243	*neSi14 [cdk-1::gfp(RNAe), cb-unc-119(+)] II; unc-119(ed3) III.*
WM247	*neSi8 [gfp::csr-1(RNAe), cb-unc-119(+)] II; hcp-6(mr17) I.*
WM248	*neSi11 [gfp::cdk-1(RNAe), cb-unc-119(+)] II; hcp-6(mr17) I.*
WM249	*neSi8 [gfp::csr-1(RNAe), cb-unc-119(+)] II; rde-3(ne3370) I.*
WM250	*neSi11 [gfp::cdk-1(RNAe), cb-unc-119(+)] II; rde-3(ne3370) I.*
WM251	*neSi8 [gfp::csr-1(RNAe), cb-unc-119(+)] II; rde-1(ne300) V.*
WM252	*neSi11 [gfp::cdk-1(RNAe), cb-unc-119(+)] II; rde-1(ne300) V.*
WM253	*neSi8 [gfp::csr-1(RNAe), cb-unc-119(+)] II; mes-3(bn35) dpy-5(e61) I / hT2 [bli-4(e937) let-?(q782) qIs48]* (I; III)
WM254	*neSi11 [gfp::cdk-1(RNAe), cb-unc-119(+)] II; mes-3(bn35) dpy-5(e61) I / hT2 [bli-4(e937) let-?(q782) qIs48]* (I; III) .
WM255	*neSi8 [gfp::csr-1(RNAe), cb-unc-119(+)] II; dpy-11(e224) mes-4(bn23) unc-76(e911)V/nT1[unc-?(n754) let-?](IV;V).*

WM256 *neSi11 [gfp::cdk-1(RNAe), cb-unc-119(+)] II; dpy-11(e224) mes-4(bn23) unc-76(e911)V/nT1[unc-?(n754) let-?](IV;V).*

WM257 *neSi8 [gfp::csr-1(RNAe), cb-unc-119(+)] II; unc-32 mut-7(ne4255)III.*

WM258 *neSi11 [gfp::cdk-1(RNAe), cb-unc-119(+)] II; unc-32 mut-7(ne4255)III.*

WM259 *neSi8 [gfp::csr-1(RNAe), cb-unc-119(+)] II; hpl-1(tm16224) X.*

WM260 *neSi11 [gfp::cdk-1(RNAe), cb-unc-119(+)] II; hpl-1(tm16224) X.*

WM261 *neSi8 [gfp::csr-1(RNAe), cb-unc-119(+)] II; hpl-2 (tm1489) III; hpl-1(tm16224) X.*

WM262 *neSi11 [gfp::cdk-1(RNAe), cb-unc-119(+)] II; hpl-2 (tm1489) III; hpl-1(tm16224) X.*

WM263 *neSi8 [gfp::csr-1(RNAe), cb-unc-119(+)] II; hpl-2 (tm1489) III.*

WM264 *neSi11 [gfp::cdk-1(RNAe), cb-unc-119(+)] II; hpl-2 (tm1489) III.*

WM265 *neSi8 [gfp::csr-1(RNAe), cb-unc-119(+)] II; wago-1(tm1414) I.*

WM266 *neSi11 [gfp::cdk-1(RNAe), cb-unc-119(+)] II; wago-1(tm1414) I.*

WM246 *neSi8 [gfp::csr-1(RNAe), cb-unc-119(+)] II; nrde-3(tm1116) X.*

WM244 *neSi11 [gfp::cdk-1(RNAe), cb-unc-119(+)] II; wago-9(tm1200) dpy-17(e164) III.*

WM245 *neSi8 [gfp::csr-1(RNAe), cb-unc-119(+)] II; wago-9(tm1200) dpy-17(e164) III.*

WM267 *neSi11 [gfp::cdk-1(RNAe), cb-unc-119(+)] II; wago-1(tm1414) I; wago-9(tm1200) dpy-17(e164) III.*

WM268 *neSi11 [gfp::cdk-1(RNAe), cb-unc-119(+)] II; wago-9(tm1200) dpy-17(e164) III; wago-10(tm1186) V.*

WM270 *neSi8 [gfp::csr-1(RNAe), cb-unc-119(+)] II; unc-24(e138) teIs1[oma-1::gfp, unc-119(+)] IV.*

WM271 *neSi11 [gfp::cdk-1(RNAe), cb-unc-119(+)] II; unc-24(e138) teIs1[oma-1::gfp, unc-119(+)] IV.*

WM272	*neSi11[gfp::cdk-1 (RNAe), cb-unc-119(+)] II; neIs2[wrm-1::gfp, rol-6(su1006)]*
WM273	*neSi14[cdk-1::gfp(RNAe), cb-unc-119(+)] II; neIs2[wrm-1::gfp, rol-6(su1006)]*
WM274	*neSi8 [gfp::csr-1(RNAe), cb-unc-119(+)] II; met-1*(*n4337*) I/*hT2*[*bli-4(e937) let-?(q782) qIs48*] (I;III); *met-2(n4256)* III/hT2[*bli-4(e937) let-?(q782*) *qIs48*] (I;III)

MosSCI targeting constructs

csr-1: A 7.0 kb DNA fragment (*Spe*I – *Bst*Z171) from cosmid (F20D12) containing the entire *csr-1* gene was inserted into a modified version of pCFJ151 (B1496) for LGII or pCFJ178 (B1777) for LGIV (Frokjaer-Jensen et al., 2008). The *gfp* coding region amplified from pPD95.75 (addgene) or 3x*flag* sequence (GATTACAAAGACCATGATGGTGACTATAAGGATCATGATATTGACTATAAA GACGATGACGATAAG) was inserted in a *Sma*I site created by site-direct mutagenesis immediately after the initiation codon in the first exon of the *csr-1* gene. The *csr-1* constructs were present at 50 ng/ml in the injection mixture.

rde-3: A 4.4 kb DNA fragment (*Sal*I – *Bam*HI) from cosmid (K04F10) containing the entire *rde-3* coding sequence was cloned into the B1496 vector. A *Bgl*II site was engineered by site-directed mutagenesis immediately after the initiation codon or before the stop codon of *rde-3* to insert *gfp* or 3x*flag* sequence. The *rde-3* constructs were present at 50 ng/ml in the injection mixture.

cdk-1: A 1.8 kb fragment (LGIII: 9747058-9748900) containing the entire *cdk-1* coding region was amplified by PCR and cloned into the B1496 vector. A *Bgl*II site was engineered by site-directed mutagenesis immediately after the initiation codon or before

the stop codon of *cdk-1* to insert the *gfp* sequence. The *cdk-1* constructs were present at 10 or 50 ng/ml in the injection mixture.

pie-1: A 2.6 kb fragment (LGI: 12426792-12429422) containing the *pie-1* coding region and a 3.7 kb fragment (pID3.01B, addgene) between *Sca*I and *Not*I sites containing the *pie-1* promoter and *gfp* coding region were combined and inserted into the B1496 vector. The *pie-1* constructs were present at 10 or 50 ng/ml in the injection mixture.

wago-9: A 5.3 kb fragment (LGIII: 4171941-4177280) containing the entire *wago-9* coding region was cloned into the B1496 vector. A *Bam*HI site was created by site direct mutagenesis immediately after the initiation codon of *wago-9* to insert the *gfp* coding region or 3x*flag* sequence. The *wago-9* constructs were present at 10 ng/ml in the injection mixture.

MosSCI by heat-shock, ivermection selection

Several lines that stably transmit the Rol phenotype were used for each MosSCI construct. Starved L1 worms were plated to fifteen 60 mm plates at a density of ~300 worms per plate and grown until the L4 or young-adult stage. Heat-shock induction of Mos Transposase was performed by placing plates in a Ziploc bag and submerging in a 35°C water bath for 1.5 hours. After heat shock, worms were propagated for 2 generations at 25°C. F3 embryos were harvested with a brief hypochlorite treatment, washed with M9 and seeded onto 60 mm NGM plates containing 2 ng/ml ivermection to select against animals carrying the extrachromosomal array. After 3 days at 25°C, wild-type looking worms (non-Unc, non-Rol) were manually picked and transgene insertions were homozygosed for phenotypic analysis.

Primers for qPCR

Primer sequences used for qPCR are following:

cmo15847m CCGGGAGTAAAGGAGAAGAA (M1 forward)
cmo15848m ATCACCTTCACCCTCTCCAC (M1 reverse)
cmo15851m ATGGTGTTCAATGCTTCTCG (M2 forward)
cmo15852m TGTAGTTCCCGTCATCTTTGA (M2 reverse)
cmo15863m GGTGATGTTAATGGGCACAA (P1 forward)
cmo15864m TTAAACTTACCCATGGAACAGG (P1 reverse)
cmo15865m TTTCAGCCAACACTTGTCACT (P2 forward)
cmo15866m TCATGCCGTTTCATATGATCT (P2 reverse)
cmo15935m TCTTTGGTTGTCTGCGTCTC (H3K9me negative control, forward)
cmo15936m AAGTTGGTGGTGTGTGTTGG (H3K9me negative control, reverse)
cmo15933m CAACCCCGTTTAGTCCAATG (H3K9me positive control, forward)
cmo15934m AATGCTCTGGGGCTAGATTG (H3K9me positive control, reverse)

Small RNA cloning and Computational Analysis

Small RNA libraries were prepared using a ligation-dependent method with a 5′ adapter containing a 4 nt barcode and a 3′ adapter added to the RNA as described (Gu et al., 2009). The normal cloning protocol was modified to be suitable for cloning from minute quantities of RNA, such as obtained from a few dissected germ lines (W Gu and C Mello, unpublished). Libraries were sequenced on an Illumina GAII 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: one mismatch for 19 – 21 nt, two for 22 – 24 nt and three for ≥25 nt. The Bowtie parameter '-a --best –strata' was used to return only the best matches. The read count of each sequence was normalized to the number of matches in 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 nt histogram for the start site of matched RNA was obtained using a custom Perl script and the generic genome browser 1.70. All scripts are available upon request.