Supplemental Information

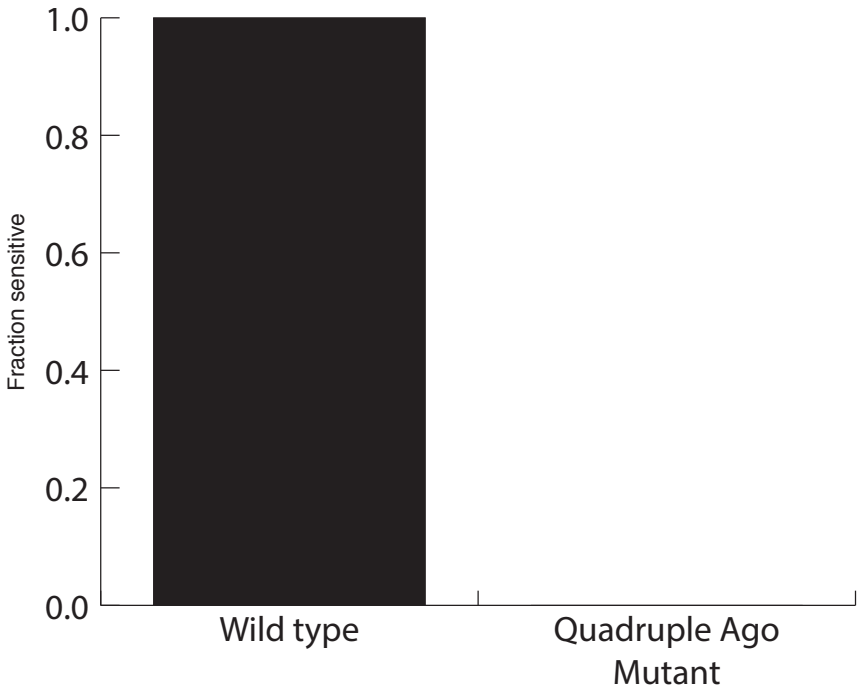
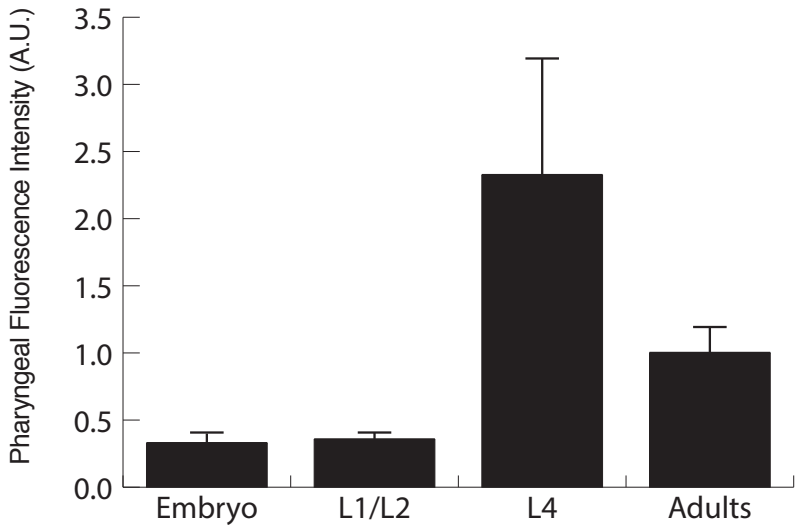
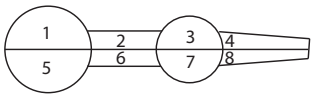


Figure S1. Cytoplasmic argonautes are required for RNAi, related to Figure 1. HC1054 [*sago-1 (tm1195), sago-2 (tm894), ppw-1(tm914), wago-4(tm1019)]* quadruple mutant is resistant to *act-5* feeding RNAi. N=10.

A



B



C

Fraction of Pharynx Silenced 0/8 1/8 3/8 6/8

Figure S2. Heat-shock promoter expression in the pharynx and scoring GFP silencing in the pharynx, Related to Figure 2. A) The hsp-16.2 promoter is not more strongly expressed in the pharynx of young worms. Pharyngeal GFP fluorescence intensity 24 hours post heat-shock in *hsp-16.2::GFP* worms at different stages. Error bars represent standard deviation; n=19-22 animals per developmental stage. B) To score pharyngeal silencing, the pharynx was divided into eight sections. C) The number of strongly silenced sections, out of eight, was determined. Arrowheads point to silenced sections. Scoring was done blind to the identity of the worm. Scale bars represent 5 μm.

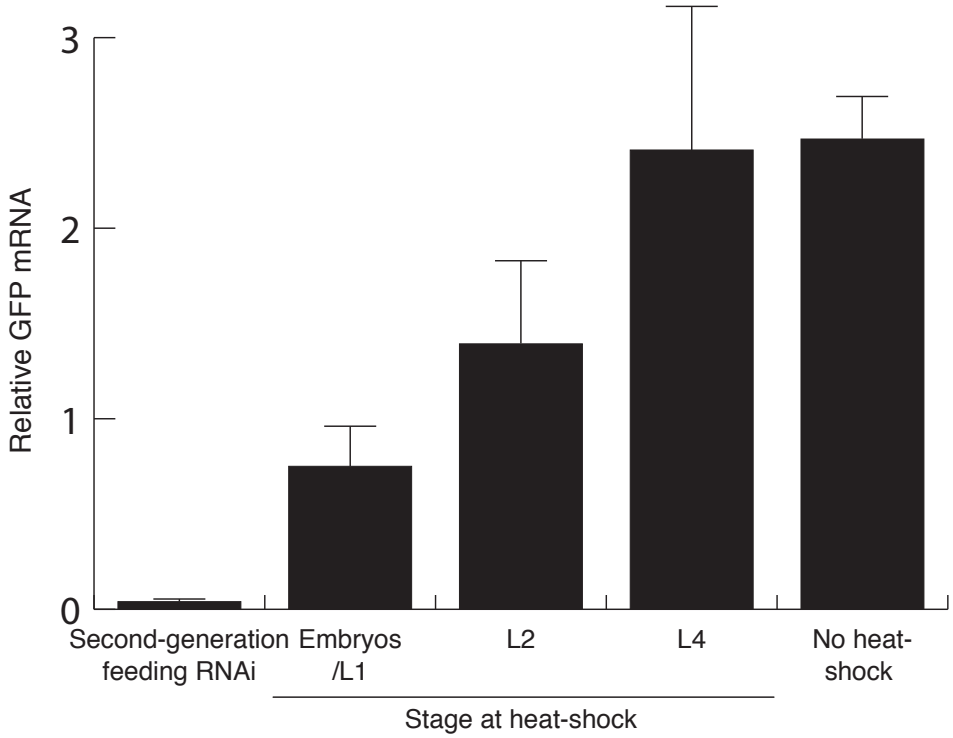


Figure S3. GFP mRNA is reduced when embryos are induced to express GFP dsRNA, but not when older worms are, Related to Figure 3. Worms were either fed GFP dsRNA for two generations, or heat-shocked at the indicated times. For feeding RNAi, worms were normalized to animals treated with mock RNAi, while the heat-shock experiments, heat-shocked animals with the *hsp-16.2::GFP* dsRNA array were normalized to worms lacking the array. RNA levels were measured 48 hours after GFP dsRNA induction. N=5-11. Bars represent SEM.

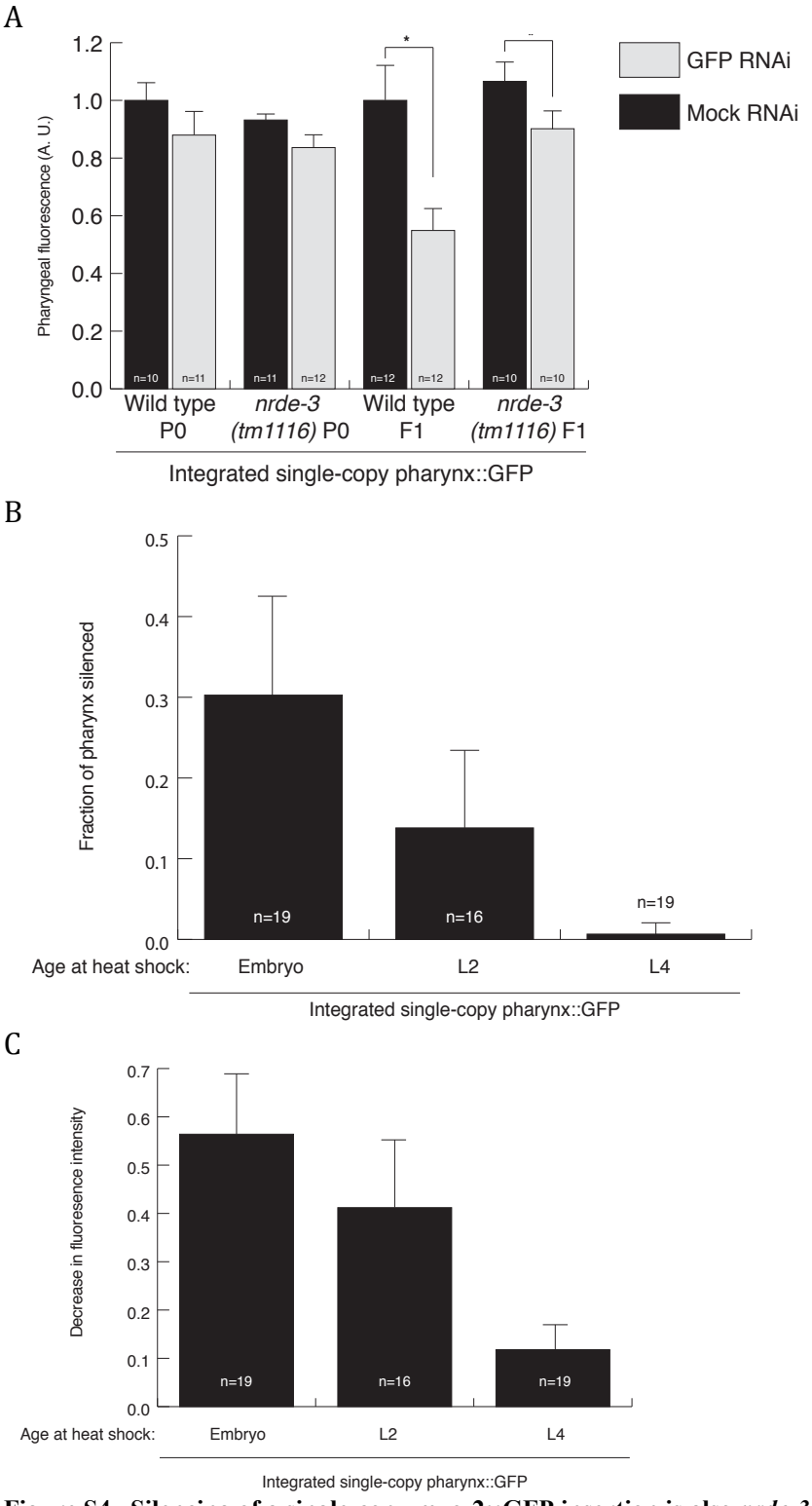


Figure S4. Silencing of a single-copy myo-2::GFP insertion is also *nrde-3* dependent and has a critical period, Related to Figures 1 and 2. A) Silencing of a single-copy *myo-2::GFP* insertion by feeding RNAi either in the first or second generation, normalized to wild type fluorescence intensity on mock RNAi. B) Fraction of pharyngeal muscle silenced, as scored in figure S2C. C) Decrease in fluorescence intensity, compared to an age-matched, heat-shocked worm lacking the GFP-hairpin. Error bars represent standard deviation, n = animals scored.

	First-generation RNAi			Second-generation RNAi	
Genotype	**pha-4 RNAi**	**pharyngeal GFP RNAi**	**BWM GFP RNAi**	**pha-4 RNAi**	**pharyngeal GFP RNAi**
N2	-	-	+	++	+
sid-1	-	-	-	-	-
rde-1	-	-	-	-	-
rrf-1	-	-	NT	++	-
rde-12	-	-	+/-	-	-
MAGO (four cytoplasmic argonautes)	-	-	+	-	+
hrde-1	-	NT	NT	++	NT
nrde-2	-	NT	NT	-	NT
nrde-3	-	-	+ *	-	-
rrf-3	+/-	NT	NT	++	NT
eri-1	-	+/-	NT	++	+
lin-15ab	+	+	NT	++	+
lin-35	+	NT	NT	++	NT
lin-35, nrde-2	-	NT	NT	-	NT
Maternal *sid-1(-)*	NA	NA	NA	NT	-

Table S1. Summary of sensitivity of various RNAi mutants to feeding RNAi, Related to Figure 5. Embryos were placed on *E. coli* expressing either *pha-4* or *GFP* dsRNA. For *pha-4* RNAi, worms were scored as ++ if arrested prior to L2, + if arrested prior to L4, and +/- if only a fraction of worms arrested prior to L4. See Figures 1 and 5. For body-wall muscle RNAi, +/- represents that worms were only partially silenced. +* represents that vulval muscle were not silenced, see Figure 4. For maternal *sid-1* tests, *sid-1(qt78)* young adult hermaphrodites were fed GFP dsRNA expressing bacteria and crossed to male wild-type worms with the myo-2::GFP array. The cross progeny were reared on OP50 and scored for silencing. NT: Not tested. NA: Not applicable.

RNA Abundance (FKPM)			
	Intestine	**Body-wall Muscle**	**Pharyngeal Muscle**
Cytoplasmic Argonautes			
C04F12.1/vrsa-1	0.94	0.23	0.06
wago-4	0.09	1.86	0.16
ppw-1	3.33	3.15	**0**
ppw-2	0.27	0.99	0.11
sago-1	6.83	0.36	**0**
sago-2	1.8	0.26	**0**
Systemic RNAi genes			
sid-1	2.09	0.82	0.96
sid-2	3.96	0	0
Primary siRNA processing			
dcr-1	0.81	0.82	0.55
rde-1	1.9	0.94	0.39
Secondary siRNA amplification			
rde-10	0.57	0.62	0.54
rde-11	1.77	0.94	0.31
rde-12	1.72	1.47	0.94
rrf-1	1.15	0.52	0.14
Nuclear RNAi pathway			
nrde-1	1.08	0.35	0.43
nrde-2	1.77	1.39	0.88
nrde-3	1.52	0.26	0.37
nrde-4	2.79	0.52	0.86

Table S2. RNA abundance levels of various RNAi-related genes, in FKPM, in the intestine, body-wall muscle and pharynx, Related to Figure 1. Data is from Blazie et al., 2015.

Supplemental Experimental Procedures

Strains used in this study

Strain name	Genotype	Reference
N2 Bristol	Wild type	Brenner, 1974
HC57	*mIs3[Pmyo2:: gfp-hp RNA]; mIs11[myo-2p::GFP + pes-10p::GFP + gut-promoter::GFP]; ccIs4251[(pSAK2) myo-3p::GFP::LacZ::NLS + (pSAK4) myo-3p::mitochondrial GFP + dpy-20(+)]*	Winston et al., 2002
HC1050	*mIs11, ccIs4251*	This study
HC1051	*rde-1 (ne219); mIs11; ccIs4251*	This study
HC1052	*rde-12(qt131); mIs11; ccIs4251*	This study
HC1053	*nrde-3 (tm1116); mIs11; ccIs4251*	This study
HC1054	*sago-1 (tm1195); sago-2 (tm894); ppw-1(tm914); wago-4(tm1019); mIs11; ccIs4251,*	This study
HC1077	*rde-1 (ne219); mIs11;*	This study
HC1078	*rde-12 (qt131); mIs11;*	This study
HC1079	*nrde-3 (tm1116); mIs11;*	This study
HC1095	*sago-1 (tm1195); sago-2 (tm894); ppw-1(tm914); wago-4(tm1019); mIs11;*	This study
HC1055	*qtEx197[hsp-16.2::GFP dsRNA, myo-3::mCherry, NeoR]; mIs11*	This study
HC1056	*qtEx198[hsp-16.2::GFP dsRNA, myo-3::mCherry, NeoR]; mIs11, nrde-3(tm1116)*	This study
HC1057	*qtEx198, mIs11*	This study
PD4443	*ccIs4443 [arg-1::GFP + dpy-20(+)]*	Kostas and Fire, 2002
HC1082	*ccIs4443; nrde-3(tm1116)*	This study
HC1065	*eri-1 (mg366); qtEx197; mIs11*	This study
HC1066	*lin-15ab (n765), qtEx197, mIs11*	This study
HC1062	*eri-1 (mg366), mIs11, ccIs4251*	This study
HC1063	*lin-15ab (n765) mIs11, ccIs4251,*	This study
GR1373	*eri-1 (mg366)*	Kennedy et al., 2004
MT8189	*lin-15ab (n765)*	Wang et al., 2005
MT10430	*lin-35 (n745)*	Lu and Horvitz, 1998
HC1068	*lin-35 (n745), nrde-2 (gg91)*	This study
NL2099	*rrf-3(pk1426)*	Simmer et al., 2002
TJ375	*gpIs1[hsp-16.2p::GFP]*	Rea et al., 2005
HC1073	*f32b4.4(-), myo-2p::GFP*	This study
HC1094	*f32b4.4(-), myo-2p::GFP, nrde-3 (tm1116)*	This study
WM27	*rde-1 (ne219)*	Tabara et al., 1999
HC445	*sid-1(qt9)*	Winston et al., 2002
NL2098	*rrf-1(pk1417)*	Sijen et al., 2001
HC820	*rde-12(qt131)*	Yang et al., 2014
FX1200	*hrde-1(tm1200)*	Buckley et al., 2012
YY186	*nrde-2(gg91)*	Guang et al., 2010
WM156	*nrde-3(tm1116)*	Gu et al., 2009

qPCR. RNA, collected as in Ly et al., 2005, was treated with Dnase I (Roche) (30° C 20 minutes), heat inactivated (75° C for 10 minutes) and reverse transcribed using random hexamers and Thermoscript RT (Invitrogen). 2 uL of the resulting cDNA (diluted 1:10 in water) was used in a 25 uL QuantiTect SYBR Green (Qiagen) reaction. qPCR was performed using an Eppendorf Mastercycler Realplex4 and Noiseband quantification with the following PCR cycle: 15 minutes 95° C, 15 seconds 94° C, 30 seconds 56° C, 35 seconds 72° C, read, cycle to step 2 for 40 cycles. Analysis was performed using the ΔΔCT method. Primers specific to GFP mRNA, and not the GFP-hairpin were used. GFP mRNA levels was normalized to *act-1* mRNA levels. To calculate the decrease in GFP, heat-shocked worms were normalized to heat-shocked worms lacking the array.

DNA constructs. We used pCFJ104 to express mCherry in the body-wall muscle and pCFJ910 to express NeoR, which confers G418 resistance to worms (Frokjaer-Jensen et al., 2014).

To create the hsp-16.2p::GFP-hairpin plasmid pHC236, the GFP hairpin was cut out of pPD126.25 using NotI and AgeI. This fragment was inserted into pPD118.26, which had been digested with NotI and BspEI.

Primers:
act-1 qPCR F : ACGCCAACACTGTTCTTTCC
act-1 qPCR R: GATGATCTTGATCTTCATGGTTGA
Myo-2 GFP qPCR F: AGCTCCCGAGATCCTATCG
Myo-2 GFP qPCR R: ATTGGGACAACTCCAGTGAAA

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