

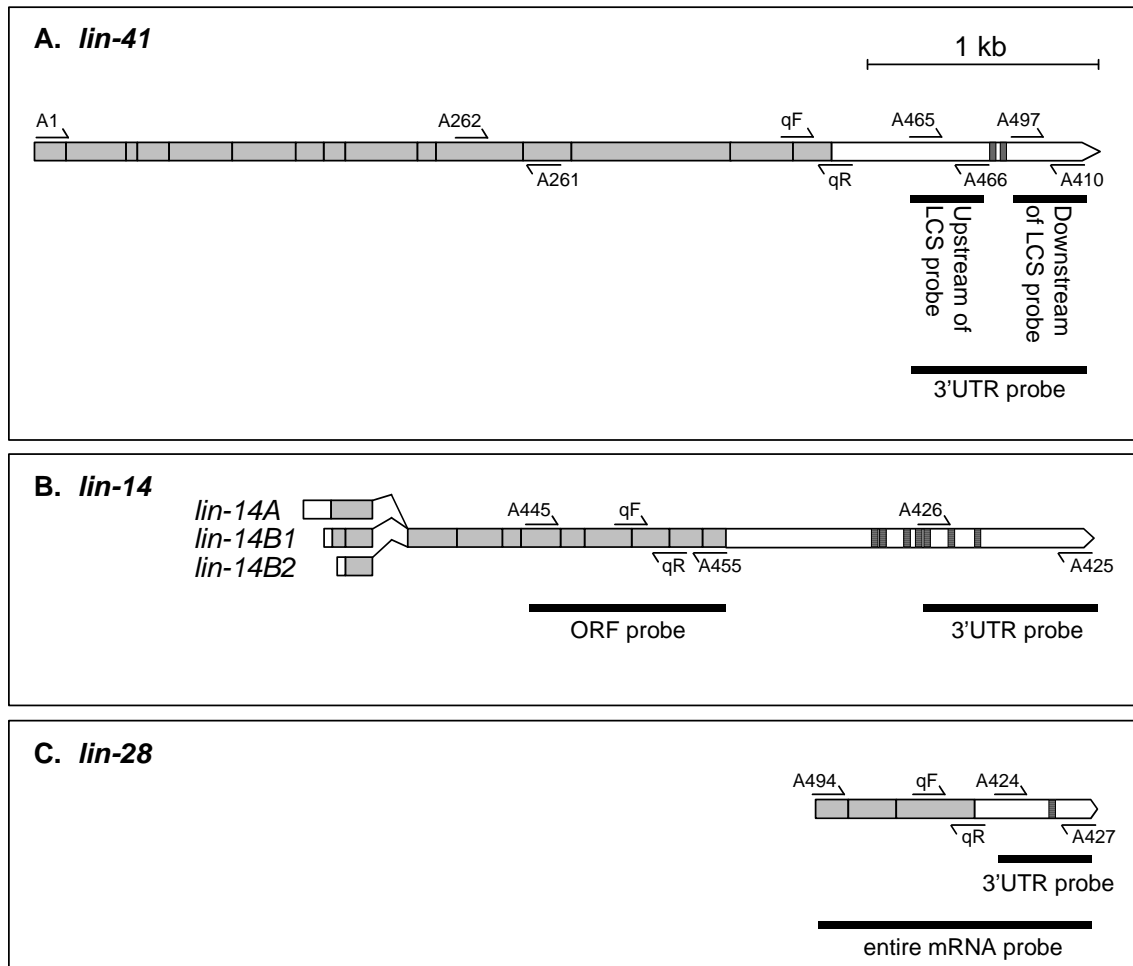
Supplemental Data

Regulation by *let-7* and *lin-4* miRNAs

Results in Target mRNA Degradation

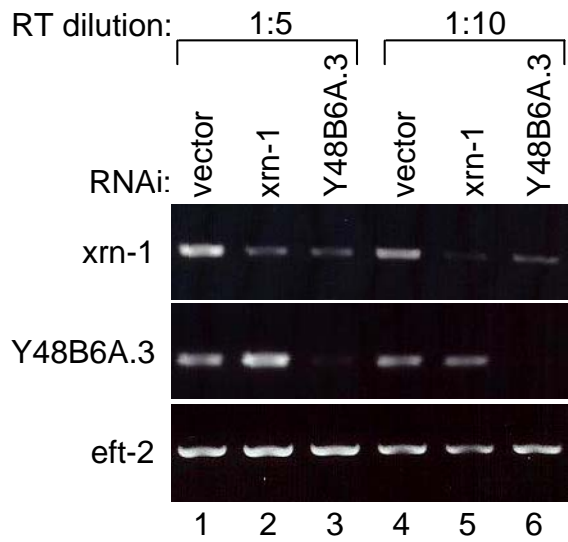
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Figure S1. MicroRNA Target Genes and Relative Primer Positions



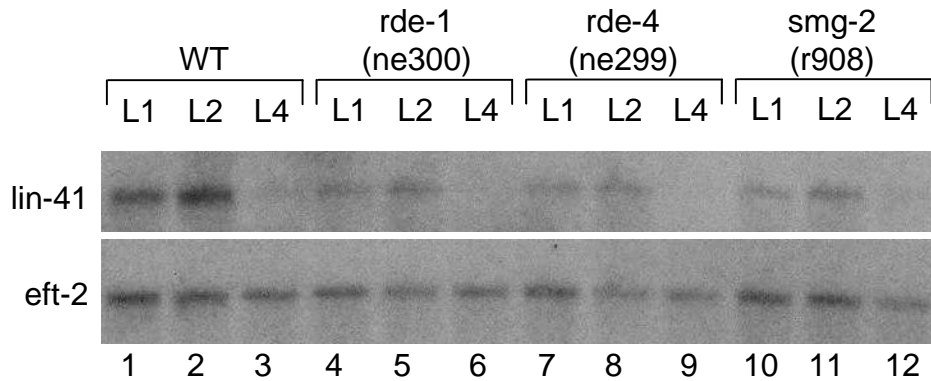
Introns were omitted to conserve space and exons are represented as gray boxes. The relative positions and names of primers used in this study are indicated by horizontal arrows; primers with "q" prefaces were used for qRT-PCR experiments and "A" prefaces for standard PCR. The LCS's in the *lin-41* 3' UTR (A) are indicated by boxes filled with black and white stripes and the *lin-4* complementary elements in *lin-14* (B) and in *lin-28* (C) are indicated with boxes filled with gray and black stripes. The regions used as probes for the Northern analyses are indicated by thick black lines.

Figure S2. Effectiveness of RNAi against 5'-3' Exonuclease Genes



RNA was isolated from *rrf-3* worms fed vector control or dsRNA expressing bacteria against *xrn-1* or Y48B6A.3 and analyzed by RT-PCR for relative levels of *xrn-1*, Y48B6A.3 or control *eft-2* mRNAs. Two template dilutions are shown.

Figure S3. Regulation of *lin-41* mRNA in RNAi and nonsense mediated decay deficient worms.



RNA was isolated from WT, *rde-1(ne300)*, *rde-4(ne299)* and *smg-2(r908)* mutant worm strains and subjected to Northern analyses to detect *lin-41* mRNA. The blot was re-probed for *eft-2* control mRNA.

Table S1. Quantitative Real Time PCR (qRT-PCR) Analyses of miRNA Target mRNA Levels

Trial	Strain	Stage	Gene of Interest	$\Delta\Delta C_T$	$\Delta\Delta C_T$ St. dev	Fold Modulation $2^{(-\Delta\Delta C_T)}$	Range	
1	WT 25°C	L2	<i>lin-41</i>	-	0.06	1.00	0.96 - 1.04	
	WT 25°C	L4	<i>lin-41</i>	2.96	0.05	0.13	0.12 - 0.13	
	<i>let-7(n2853)</i> 25°C	L2	<i>lin-41</i>	-	0.16	1.00	0.90 - 1.11	
	<i>let-7(n2853)</i> 25°C	L4	<i>lin-41</i>	1.40	0.10	0.38	0.35 - 0.41	
2	WT 25°C	L2	<i>lin-41</i>	-	0.09	1.00	0.94 - 1.06	
	WT 25°C	L4	<i>lin-41</i>	2.73	0.02	0.15	0.15 - 0.15	
	<i>let-7(n2853)</i> 25°C	L2	<i>lin-41</i>	-	0.03	1.00	0.98 - 1.02	
	<i>let-7(n2853)</i> 25°C	L4	<i>lin-41</i>	0.77	0.04	0.59	0.57 - 0.60	
1	WT 20°C	L1	<i>lin-14</i>	-	0.03	1.00	0.98 - 1.02	
	WT 20°C	L2	<i>lin-14</i>	1.11	0.09	0.46	0.44 - 0.49	
	<i>lin-4(e912)</i> 20°C	L1	<i>lin-14</i>	-	0.03	1.00	0.98 - 1.02	
	<i>lin-4(e912)</i> 20°C	L2	<i>lin-14</i>	0.5	0.14	0.71	0.64 - 0.78	
	<i>lin-14(n355gf)</i> 20°C	L1	<i>lin-14</i>	-	0.07	1.00	0.95 - 1.05	
	<i>lin-14(n355gf)</i> 20°C	L2	<i>lin-14</i>	0.22	0.01	0.86	0.85 - 0.87	
	WT 20°C	L1	<i>lin-28</i>	-	0.07	1.00	0.95 - 1.05	
	WT 20°C	L2	<i>lin-28</i>	1.78	0.09	0.29	0.27 - 0.31	
	<i>lin-4(e912)</i> 20°C	L1	<i>lin-28</i>	-	0.05	1.00	0.97 - 1.03	
	<i>lin-4(e912)</i> 20°C	L2	<i>lin-28</i>	0.85	0.13	0.56	0.51 - 0.61	
	2	WT 20°C	L1	<i>lin-14</i>	-	0.08	1.00	0.94 - 1.06
		WT 20°C	L2	<i>lin-14</i>	1.88	0.20	0.27	0.24 - 0.31
		<i>lin-4(e912)</i> 20°C	L1	<i>lin-14</i>	-	0.07	1.00	0.96 - 1.05
		<i>lin-4(e912)</i> 20°C	L2	<i>lin-14</i>	-0.84	0.07	1.79	1.71 - 1.87
<i>lin-14(n355gf)</i> 20°C		L1	<i>lin-14</i>	-	0.06	1.00	0.96 - 1.05	
<i>lin-14(n355gf)</i> 20°C		L2	<i>lin-14</i>	-1.95	0.15	3.85	3.47 - 4.27	
WT 20°C		L1	<i>lin-28</i>	-	0.10	1.00	0.93 - 1.07	
WT 20°C		L2	<i>lin-28</i>	1.56	0.09	0.34	0.32 - 0.36	
<i>lin-4(e912)</i> 20°C		L1	<i>lin-28</i>	-	0.08	1.00	0.95 - 1.05	
<i>lin-4(e912)</i> 20°C		L2	<i>lin-28</i>	-0.80	0.08	1.74	1.65 - 1.84	

Results for trials using two independent RNA samples are shown as fold modulation comparing earlier to later stages of development in wildtype or mutant worm strains. Samples were normalized to *eft-2* and the earlier stage was set to a value of one for calculations of fold modulation (see Experimental Procedures).