SUPPLEMENTARY INFORMATION

LIN-28 co-transcriptionally binds primary *let-7* to regulate miRNA maturation in *C. elegans*

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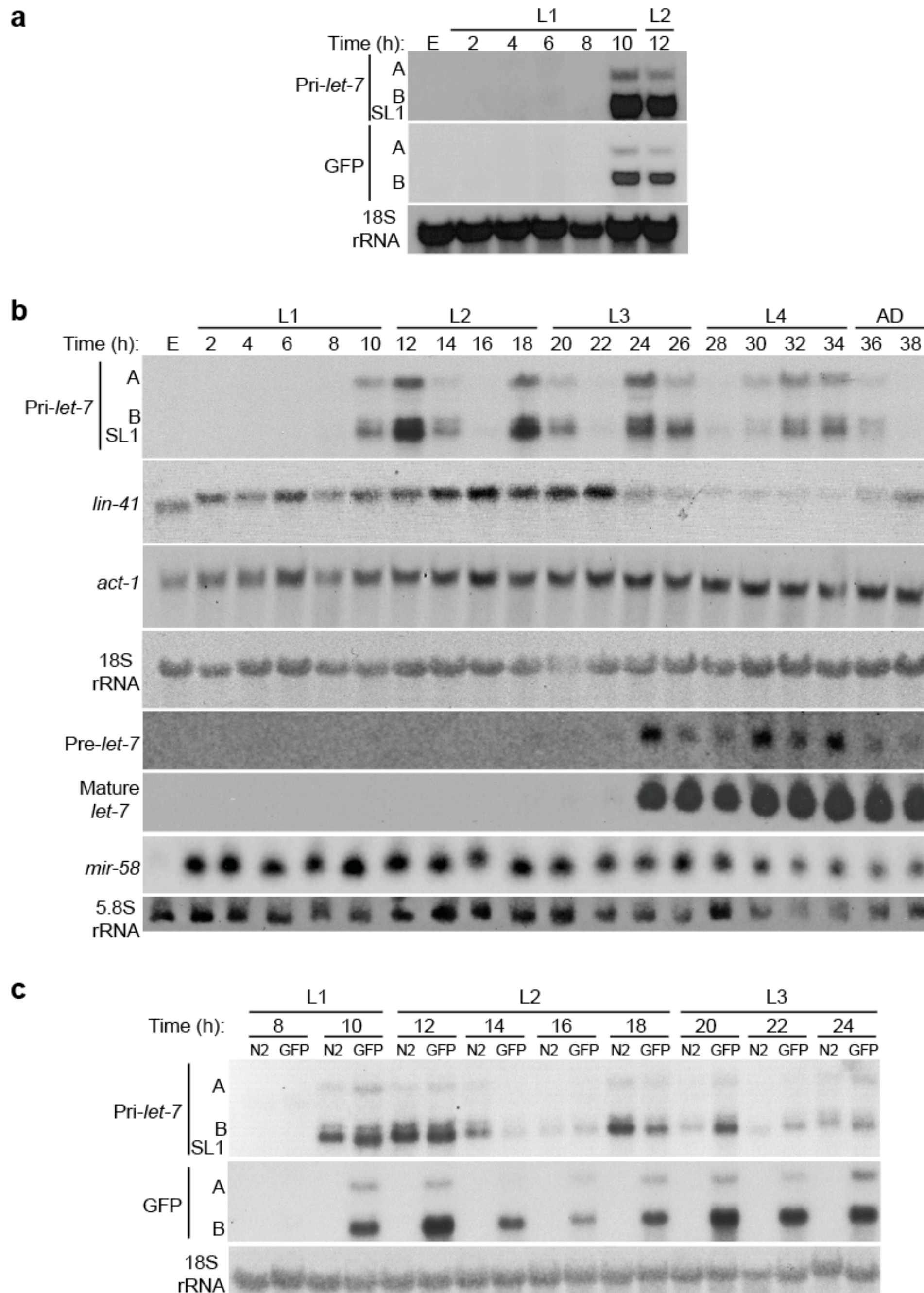
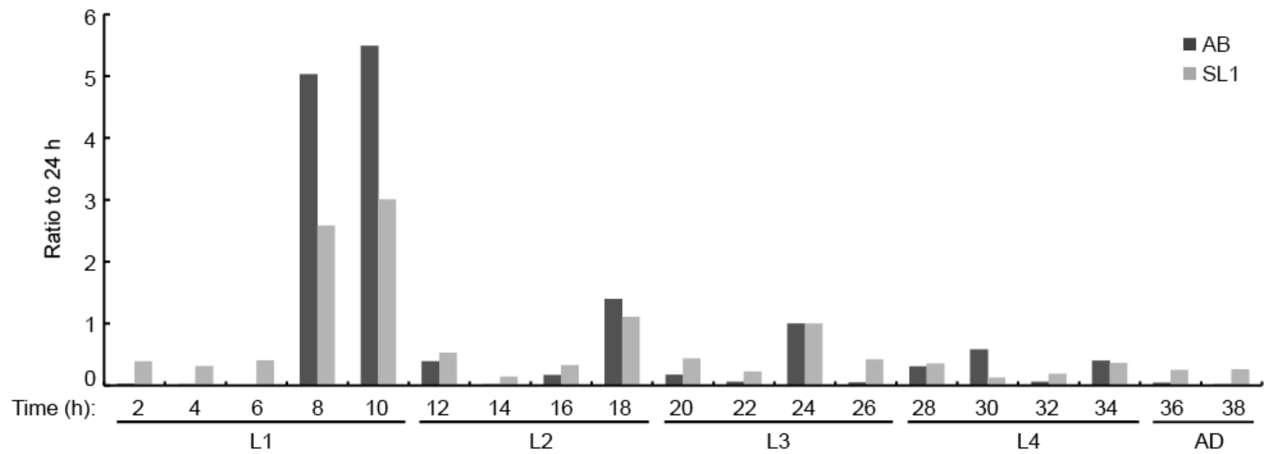
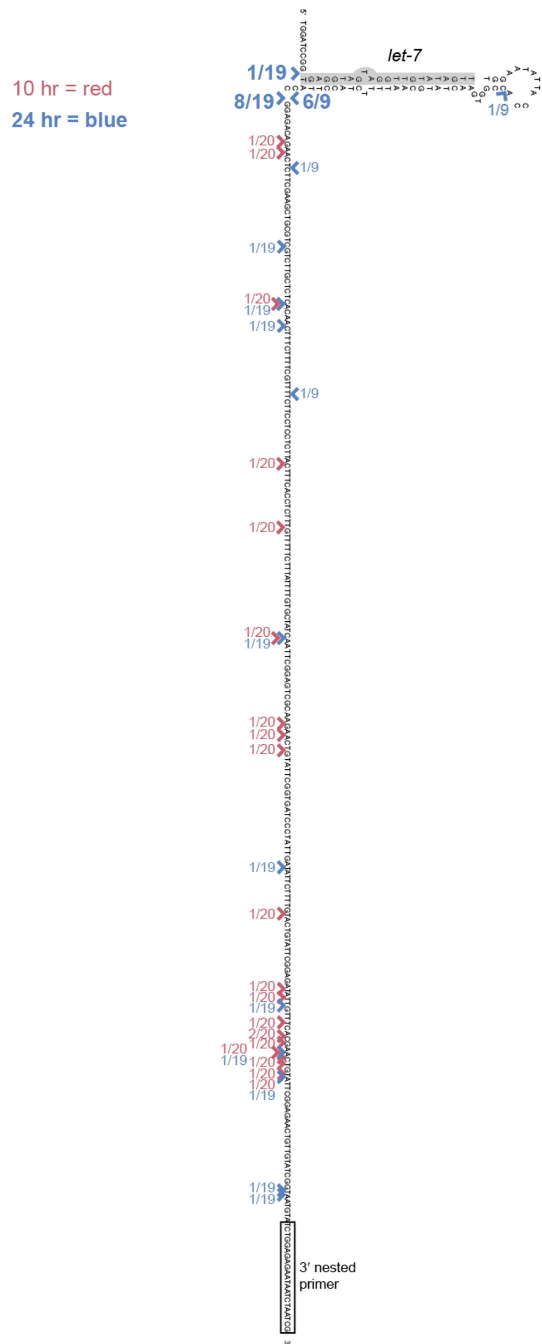


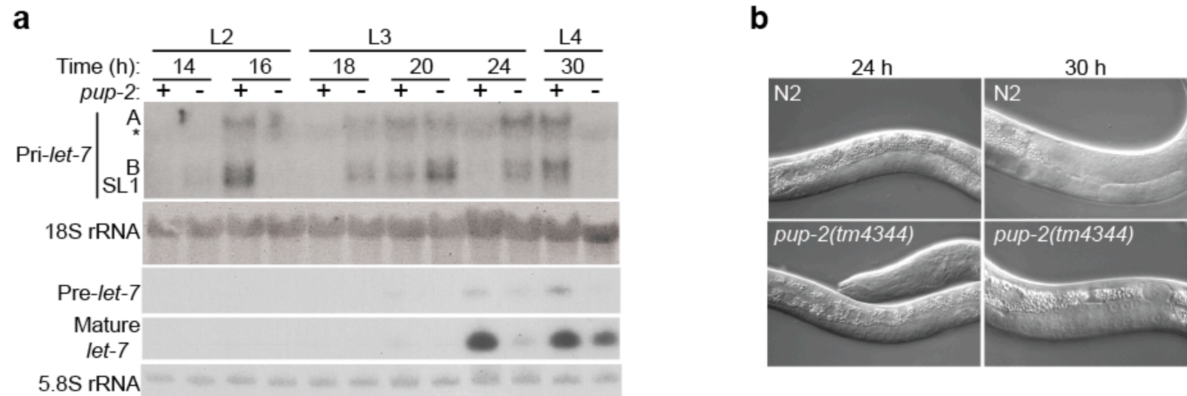
Supplementary Figure 1. Northern blot analysis of *let-7* expression. (**a**) Independent, replicate experiment of **Figure 1b**. Total RNA was isolated from synchronized transgenic worms and analyzed by agarose northern blotting as in **Figure 1b**. (**b**) Independent, replicate experiment of **Figure 1c**. Total RNA was isolated from embryos (E) or synchronized WT N2 worms and analyzed by agarose or PAGE northern blotting. (**c**) The entire northern blot from **Figure 1c** is shown.



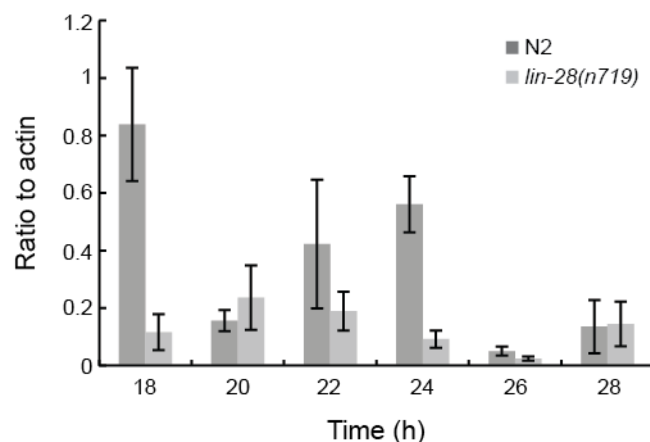
Supplementary Figure 2. qRT-PCR analysis of *let-7* expression. Total RNA was isolated from synchronized WT N2 worms at the indicated time points and analyzed by qRT-PCR with primers specific to the indicated pri-*let-7* isoforms and actin. Samples shown were also analyzed by agarose northern blotting in **Figure 1b**.



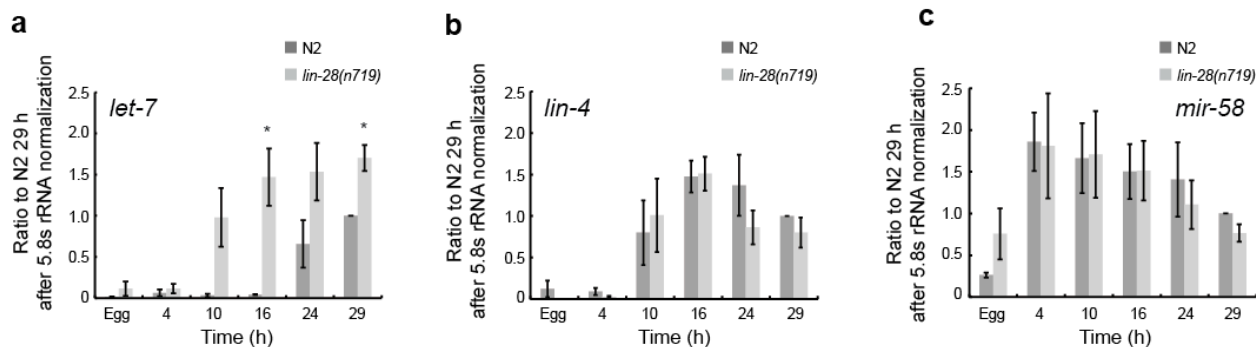
Supplementary Figure 3. 5′ and 3′ RACE clones. Sequencing results of Drosha cleavage products from two independent experiments are mapped onto primary *let-7* sequence. The mature *let-7* sequence is highlighted in grey, and the 3′ nested primer used for 5′ RACE cDNA synthesis is boxed. The number of clones that mapped to a cleavage site out of the total number of clones sequenced is shown next to each cleavage site marked with an arrowhead. Red and blue cleavage sites correspond to 10 and 24 hr time points, respectively. Cleavage sites to the right or left of the pri-*let-7* sequence correspond to 3′ or 5′ RACE analysis, respectively. Expected Drosha cleavage sites are in bold.



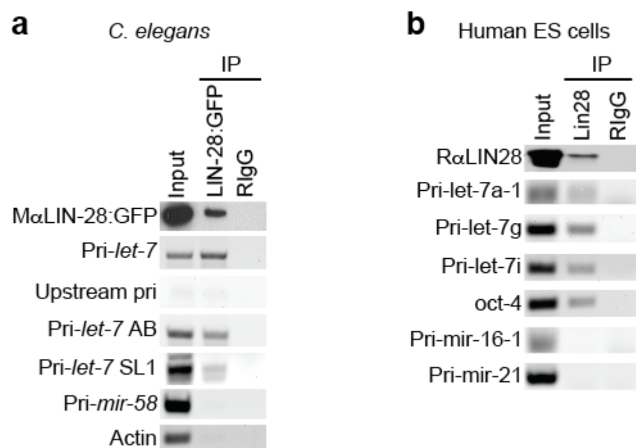
Supplementary Figure 4. Analysis of *let-7* levels in *pup-2(tm4344)* worms. (**a**) Total RNA was isolated from synchronized N2 (+) or *pup-2(tm4344)* mutant (-) worms at the indicated time points and analyzed by northern blotting as described in **Figure 2c**. The asterisk marks a background 18s rRNA band. (**b**) Representative images of gonad development in N2 and *pup-2(tm4344)* worms at the indicated time points. Development of *pup-2(tm4344)* worms is 2-8 hours delayed relative to N2.



Supplementary Figure 5. qRT-PCR analysis of pri-*let-7* levels in N2 and *lin28(n719)* worms. Total RNA was isolated from synchronized N2 or *lin-28(n719)* worms at the indicated time points and analyzed by qRT-PCR. The average ratio of total pri-*let-7* to actin from three, independent experiments is shown. Error bars shown s.e.m.



Supplementary Figure 6. Effect of *lin-28* on mature *let-7*, *lin-4* and *mir-58* miRNAs. Analysis of *let-7* (**a**), *lin-4* (**b**), and *mir-58* (**c**) levels in N2 versus *lin-28(n719)* worms. The average ratios of mature miRNA at each time point compared to the 29 h N2 time point after normalization to 5.8s rRNA were calculated from three independent experiments, and were analyzed by Student's t-tests (*, p<0.05). Error bars show s.e.m.



Supplementary Figure 7. Additional samples showing that LIN-28 binds endogenous *let-7* primary transcripts in *C. elegans* and human ES cells. (**a**) Independent, replicate experiment of **Figure 4c**. RIP was performed on synchronized PQ272 worms collected at 10 h as described in **Figure 4c**. Samples were analyzed by RT-PCR or western blotting as described in **Figure 4c**. (**b**) Independent, replicate experiment of **Figure 4d**. RIP was performed on undifferentiated HUES6 cells as described in **Figure 4d**. Samples were analyzed by RT-PCR or western blotting as described in **Figure 4d**.

SUPPLEMENTARY TABLES

Supplementary Table 1. Primers used for cloning

Purpose	Lab Designation	Sequence
plet-7B::GFP cloning	A632	ACTGAGAAGCTTCTCCCTCTTTTAAGCCTG
plet-7B::GFP cloning	A608	ACTGAGGGTACCAAACCGCTGCTAGGTGGCTACTC

Supplementary Table 2. Primers used for northern probes

Probe	Lab Designation	Sequence
pri-*let-7*	A62 A63	GGCTCCATGGATACATTACTCAACAG GGATCATCAATCAAGTGTGCACTG
GFP	A406 A407	CACTGGAGTTGTCCCAATTCTTG GCGGTTTCTTTGAATTTGGCGGC
18s	A839 A840	GCGTACGGCTCATTAGAGCAGATATCAC GGTCAGAACTAGGGCGGTATCTAATCG
5.8s	A479 A480	CTAGCTTCAGCGATGGATCGGTTGC GAACCAGACGTACCAACTGGAGGCCC
lin-41	A155 A156	GGGCATGCTTCCTGCACGCCCCTCCC GGGCGAGCGCTTCAGCCAAATCCCC
act-1	A810 A811	GTGTTCCCATCCATTGTCGGAAGAC GCACTTGCGGTGAACGATGGATGGG
pup-2	A2273 A2274	CGAAGAACGGTATCCAGGA TAAGAGAGCCGTAGAAAGAAAAATC
let-7	A1114 starfire	AACTATACAACCTACTACCTCA
lin-4	A1916 starfire	TCACACTTGAGGTCTCAGGGA
mir-58	A2071 starfire	TGCCGTACTGAACGATCTCA

Supplementary Table 3. Primers used for RACE

Primer	Lab Designation	Sequence
P1	5′ nest*	GGACACTGACATGGACTGAAGGAGTA
P2	A127 (lanes 1-5) A2013 (lanes 6-8)	GAGTAGCCCACCTAGCAGCGGTCG CAAGCAGGCGATTGGTGGA
P3	A1987	CACGAACTGTATTCGGAGA
P4	A1693	CGATTAGATTATTCTCTCCAGA
3′ RACE cDNA	A468	CTACTCCTTCAGTCCATGTCAGTGTCC
3′ RACE PCR	A706 A468	TGAGGTAGTAGGTTGTATAGTT CTACTCCTTCAGTCCATGTCAGTGTCC
3′ RACE nested PCR	A1977 A469	GGTTGTATAGTTTGGAATATTACCA CATGTCAGTGTCCTCGTGCTCCAGTC
5′ RACE cDNA	A62	GGCTCCATGGATACATTACTCAACAG
5′ RACE PCR	5′ RACE* A1692	CGACTGGAGCACGAGGACACTGA CTCAACAGTACATACGATTAG
5′ RACE nested PCR	5′ nest* A1693	GGACACTGACATGGACTGAAGGAGTA CGATTAGATTATTCTCTCCAGA

*, Included in the GeneRacer Kit (Invitrogen, L1500).

Supplementary Table 4. Primers used for RT-PCR and qPCR

Target	Lab Designation	Sequence
pri-*let-7* (RT-PCR)	A41 A62	CAGGCAAGCAGGCGATTGGTGGACGG GGCTCCATGGATACATTACTCAACAG
pri-*let-7* (qRT-PCR)	A2013 A42	CAAGCAGGCGATTGGTGGA GACGCAGCTTCGAAGAGTTCTGTC
pre-*let-7*	A706 A308	TGAGGTAGTAGGTTGTATAGTT GTAAGGTAGAAAATTGCATAGTTC
upstream pri-*let-7*	A632 A1364	ACTGAGAAGCTTCTCCCTCTTTTAAGCCTG CGATATCAAAACATCTTCGAAAGGACAG
pri-*let-7* AB (RT-PCR)	A127 A62	GAGTAGCCCACCTAGCAGCGGTCG GGCTCCATGGATACATTACTCAACAG
pri-*let-7* AB (qRT-PCR)	A2016 A42	GTCTAATTTAACAACAAGTACTAATCCATT GACGCAGCTTCGAAGAGTTCTGTC
pri-*let-7* SL1 (RT-PCR)	A90 A62	GGTTTAATTACCCAAGTTTGAG GGCTCCATGGATACATTACTCAACAG
pri-*let-7* SL1 (qRT-PCR)	A90 A42	GGTTTAATTACCCAAGTTTGAG GACGCAGCTTCGAAGAGTTCTGTC
pri-*mir-58*	A2111 A2112	GGCTTCAGTGGCTCCTCT CGTTTAGTGCGCACATTCGGCAA
actin	A810 A2144	GTGTTCCCATCCATTGTCGGAAGAC GTGAGGAGGACTGGGTGCTCTT
20kb upstream pri-*let-7*	A2295 A2296	CATCTCACCTTATTCCAGGAGAAAAC CAAAATGATCCGGTGAATGATCCAGT
pri-*mir-47*	A1551 A1552	GCTTCCTGGCCTGCAGTGGCATCTAC ACACGGGAACACTCGTAGTGTTAAAG
hsa-pri-let-7a-1	A2287 A2288	GATTCCTTTTCACCATTCACCCTGGATGTT TTTCTATCAGACCGCCTGGATGCAGACTTT
hsa-pri-let-7g	A2324 A2325	GTTCCTCCAGCGCTCCGTT CCATTACCTGGTTTCCCAGAGA
hsa-pri-let-7i	A2326 A2327	GTGCCTCCCCGACACCAT GTGAAACTAACGGTTTCCGTGGT
hsa-oct-*4*	A2299 A2300	GCCGGTTACAGAACCACACT GTGGAGGAAGCTGACAACAA
hsa-pri-mir-16-1	A2303 A2304	TAATACGACTCACTATAGGTGATAGCAATGTCAGCAGTG GTAGAGTATGGTCAACCTTA
hsa-pri-mir-21	A2301 A2302	GTTCGATCTTAACAGGCCAGAAATGCCTGG ACCAGACAGAAGGACCAGAGTTTCTGATTA
hsa-pre-let-7a-1	A706 A9	TGAGGTAGTAGGTTGTATAGTT TCCCAGTGGTGGGTGTGACCCTAAA
hsa-pre-let-7g	A2286 A2285	GGCAAGGCAGTGGCCTGTACAGTT TGAGGTAGTAGTTTGTACAGTT
hsa-pre-let-7i	A2281 A2282	TGAGGTAGTAGTTTGTGCTGTT AGCAAGGCAGTAGCTTGCGCAG

SUPPLEMENTARY METHODS

***C. elegans* RNA Immunoprecipitation (RIP).** PQ272 worms were crosslinked in a Spectrolinker XL-10000 UV Crosslinker with an energy output of 3 kJ/m^2 at a distance of ~10 cm, frozen on dry ice, mechanically homogenized in lysis buffer [150 mM NaCl, 25 mM HEPES pH 7.5, 0.2 mM DTT, 10% glycerol, 40 U / μl RNAsin, 1% Triton X-100, and protease inhibitor cocktail (Roche)], and spun at 12,000 x g for 15 min. Equal lysate amounts were precleared with Protein G Dynabeads (Invitrogen), before incubation at 4°C overnight with GFP (kind gift from R. Gassmann and A. Desai) or IgG (Caltag Laboratories) polyclonal, crosslinked Protein G Dynabeads (Invitrogen) blocked with sheared salmon sperm DNA. Beads were washed twice with low salt wash buffer (1x PBS pH 7.4, 0.1% SDS, 0.5% sodium deoxycholate, and 0.5% NP-40), high salt wash buffer (5x PBS pH 7.4, 0.1% SDS, 0.5% sodium deoxycholate, and 0.5% NP-40), and proteinase K buffer (100 mM TrisCl pH 7.4, 50 mM NaCl, and 10 mM EDTA), before treatment with proteinase K (Invitrogen) and urea, and RNA extraction with Trizol (Invitrogen). RNA was treated with RQ1 DNase (Promega) and re-extracted before cDNA synthesis with random primers and Superscript II (Invitrogen). PCR was performed with primers listed in **Supplementary Table 4**.

ES cell RNA Immunoprecipitation (RIP). HUES6 cells were lysed in 1X RIPA buffer (Millipore) containing 1X protease inhibitor cocktail (Sigma), and spun at 14,000 x g for 10 min. Equal amounts of pre-cleared lysate were incubated for 3 hours at 4°C with LIN-28 (Abcam) or IgG (Caltag Laboratories) polyclonal antibodies, prebound to Protein G Dynabeads (Invitrogen). Beads were washed, treated with proteinase K and urea, and

RNA extracted as described above. cDNA synthesis and PCR was performed as described above.

ES cell fractionation. HUES6 cells were washed in cold PBS, pH 8 and incubated in Buffer A [10 mM HEPES, pH 7.9, 10 mM KCl, 1 mM DTT, 0.1 mM EDTA, 1X protease inhibitors (Sigma)] containing 0.05% NP40 on ice for 5 min. Centrifugation at 500 x g for 5 min at 4°C yielded a cytoplasmic supernatant fraction. The resulting nuclear pellet fraction was washed with cold Buffer A before being resuspended in an equal volume of Buffer A. NP40 and sodium deoxycholic acid were added to both fractions to yield a final concentration of 1% and 0.5% respectively. Equal amounts of each fraction were removed for preclearing and RIP as described above.

Chromatin Immunoprecipitation (ChIP). PQ272 or pD4792 worms were incubated in 5 ml M9 (42 mM Na_2HPO_4, 22 mM KH_2PO_4, 85.5 mM NaCl, and 1 mM $MgSO_4$) with 1% formaldehyde for 30 min (Pol II and MIgG ChIP) or 0.5% formaldehyde for 20 min (GFP and RIgG ChIP), and 125 mM glycine for 5 min before freezing on dry ice. Worms were incubated on ice for 10 min in ChIP lysis buffer [50 mM TrisCl pH 8, 1% SDS, 10 mM EDTA and protease inhibitor cocktail (Sigma)], sonicated 5 times for 10 sec with a Sonic Dismembrator (Fisher Scientific), and spun at 12,000 x g for 10 min. Equal amounts of lysate were precleared with Protein G Dynabeads (Invitrogen) in ChIP dilution buffer (0.01% SDS, 1.1% Triton X-100, 1.2 mM EDTA, 16.7 mM TrisCl pH 8, and 167 mM NaCl), and incubated at 4°C overnight with GFP (kind gift from R. Gassmann and A. Desai) or RNA pol II (Covance) antibodies. Antibodies were immunoprecipitated by incubation with Protein G Dynabeads (Invitrogen) for 1 hr at 4°C. Beads were washed

once with low salt wash buffer (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM TrisCl pH 8, and 150 mM NaCl), once with high salt wash buffer (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM TrisCl pH 8, and 500 mM NaCl), once with LiCl wash buffer (0.25 M LiCl, 1% NP-40, 1% sodium deoxycholate, 1 mM EDTA, and 10 mM TrisCl pH 8), twice with 1x TE pH 8 and twice in elution buffer (1% SDS and 0.1 M $NaHCO_3$). Eluates were incubated at 65°C for 4 hrs in 125 mM NaCl, treated with proteinase K (Invitrogen), and DNA extracted by phenol:chloroform treatment and isopropanol precipitation. qPCR was performed with primers listed in **Supplementary Table 4**.