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Supplementary Materials for

The *lin-4* MicroRNA Targets the LIN-14 Transcription Factor to Inhibit Netrin-Mediated Axon Attraction

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The PDF file includes:

Fig. S1. Global survey of microRNA presence and abundance during *C. elegans* development.
Fig. S2. Responsiveness of a *lin-14 3′UTR* reporter to ectopic expression of *lin-4* microRNA in AVM neurons.
Fig. S3. Responsiveness of a *lin-14 3′UTR* reporter to ectopic expression of *miR-125a* in AVM neurons.
Fig. S4. *lin-4* and *lin-14* mutations do not affect the timing of AVM axon outgrowth.
Fig. S5. The effect of the LIN-14 transcription factor on activity of the promoters of various *unc-40* pathway genes in AVM neurons.
Table S1. *lin-4* mutation does not affect AVM cell fate.
Table S2. *C. elegans* strains used in this study.
Table S3. Stem-loop RT primers and forward PCR primers.

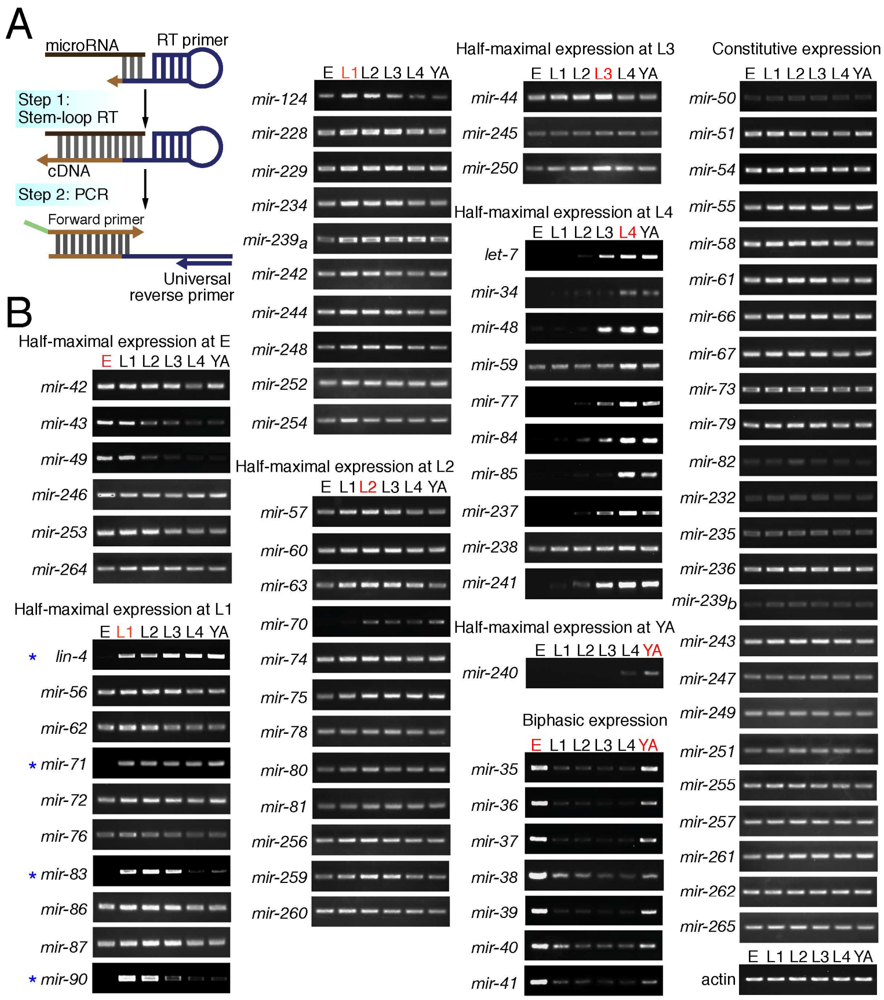

Fig. S1. Global survey of microRNA presence and abundance during *C. elegans* development. (**A**) MicroRNA quantification involved two steps, stem-loop reverse transcription (RT) followed by conventional polymerase chain reaction (PCR). The stem-loop RT primer binds to the 3' portion of the mature microRNA and is reverse transcribed with reverse transcriptase. Subsequently, the RT product is quantified with conventional PCR using a microRNA-specific forward primer and a universal reverse primer. The forward primer is tailed at 5' end to increase the melting temperature (Tm). (**B**) Stem-loop RT-PCR analysis of RNA isolated from populations of staged animals. Equal amounts of RNA from staged N2 animals were used in the RT-PCR amplification of microRNA and actin transcripts. E, L1, L2, L3, L4, and YA indicate the embryonic, the first larval, the second larval, the third larval, the fourth larval, and the young adult stages, respectively. A representative experiment of three experiments is shown for each. MicroRNAs were clustered based on the timing of their half-maximal abundance.

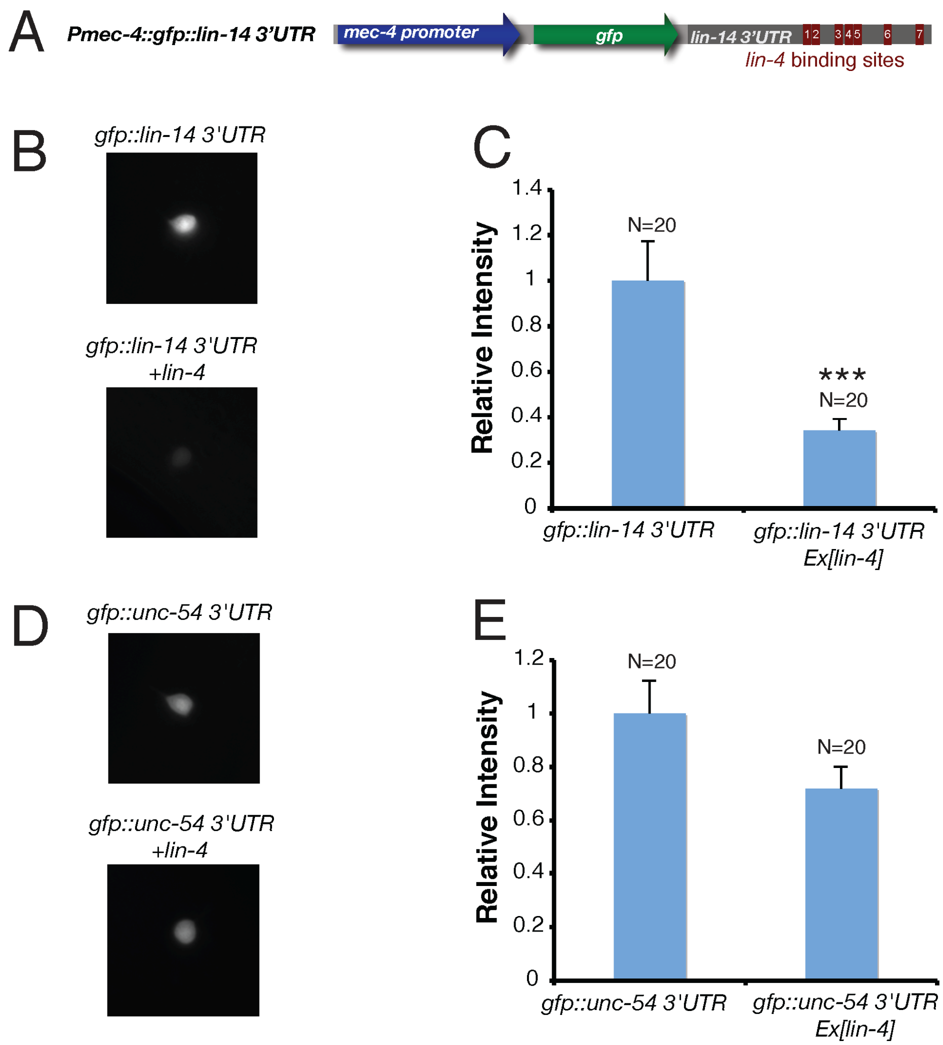

Fig. S2. Responsiveness of a *lin-14 3'UTR* reporter to ectopic expression of *lin-4* microRNA in AVM neurons. (**A**) Organization of the *Pmec-4::gfp::lin-14* 3'UTR reporter construct. On the basis of computational analysis, seven *lin-4* microRNA binding sites were introduced into the *lin-14* 3'UTR. (**B**, **C**) Representative animals (B) and quantification (C) of the reporter with the *lin-14* 3'UTR in the presence or absence of ectopic *lin-4*. (**D**, **E**) Representative animals (D) and quantification (E) of the reporter with the *unc-54* 3'UTR (control) in the presence or absence of ectopic *lin-4*. In (C and E), relative intensity was calculated by normalizing the fluorescence intensity of the reporter to that in the wild-type control. error bars indicate the s.e.m. Asterisks represent $p < 0.001$ by Student's t-Test**.** N represents the number of animals.

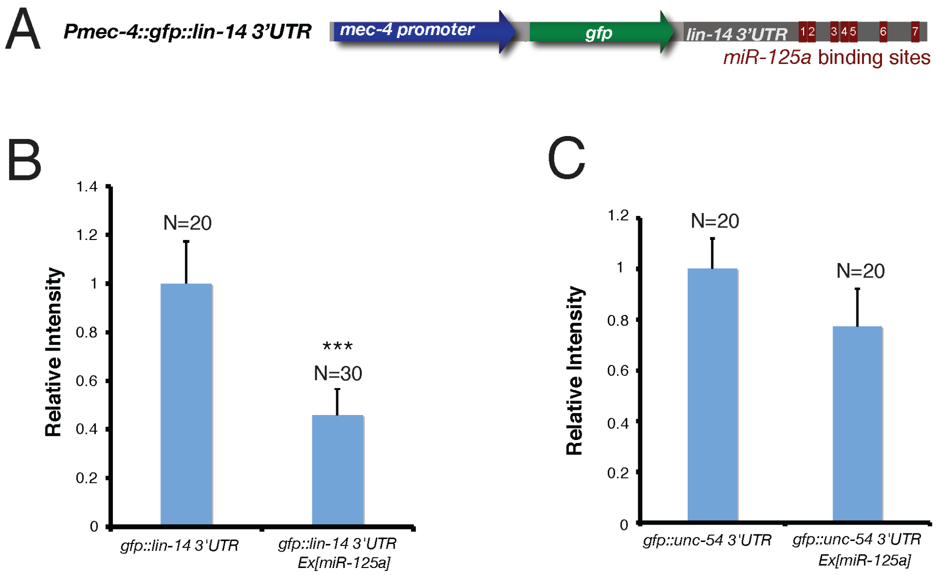

Fig. S3. Responsiveness of a *lin-14 3'UTR* reporter to ectopic expression of *miR-125a* in AVM neurons. (**A**) Organization of *Pmec-4::gfp::lin-14* 3'UTR reporter construct. On the basis of computational analysis, seven *miR-125a* binding sites were introduced into the *lin-14* 3'UTR. (**B**, **C**) Quantification of expression of the *lin-14* 3' UTR reporter with the miR-125a binding sites or the control reporter with the *unc-54* 3'UTR in the presence or absence of ectopic *miR-125a*. Relative intensity was calculated by normalizing the fluorescence intensity of the reporter to that in the wild-type control. Error bars indicate the s.e.m. Asterisks represent $p < 0.001$ by Student's t-Test**.** N represents the number of animals.

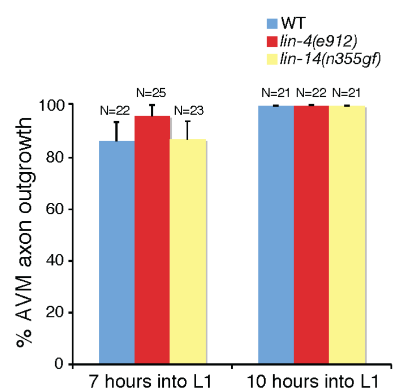

Fig. S4. *lin-4* and *lin-14* mutations do not affect the timing of AVM axon outgrowth. Neither *lin-4* loss-of-function nor *lin-14* gain-of-function mutations affected the timing of AVM axon outgrowth. AVM axon outgrowth was visualized using the *zdIs5(Pmec-4::gfp)* marker. 5hr into the L1 stage, *Pmec-4::gfp* was not yet visible in AVM axons. N represents the number of animals.

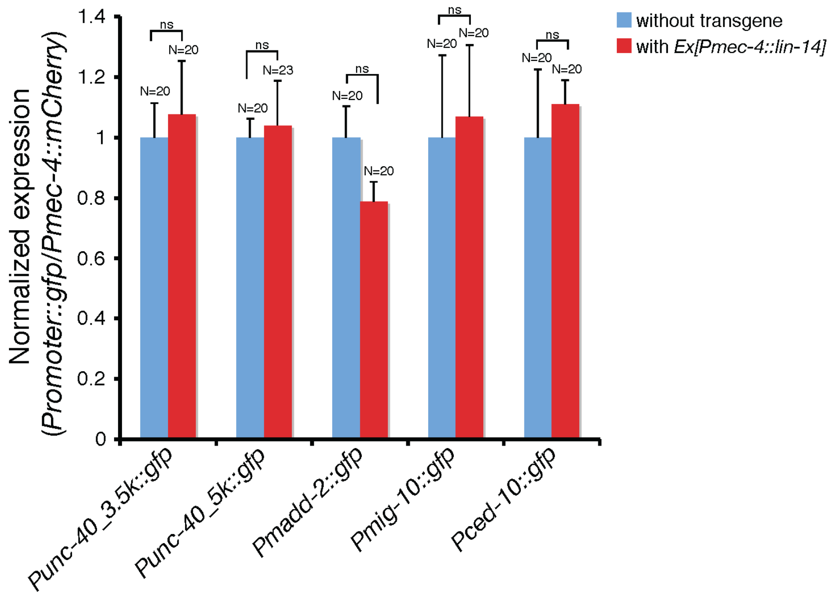

Fig. S5. The effect of the LIN-14 transcription factor on activity of the promoters of various *unc-40* pathway genes in AVM neurons. LIN-14 did not affect the activity of gene reporters driven by the promoters of various components of the *unc-40* pathway. ns indicates not significant by Student's t-Test. The fluorescence intensity of the promoter reporter was normalized by that of the *Pmec-4::mCherry* control reporter. Normalized expression was calculated by normalizing the fluorescence intensity of the promoter reporter to that in the wild-type animals.

Table S1. *lin-4* mutation does not affect AVM cell fate.

AVM cell identity	WT	*lin-4(e912)*
AVM cell position	56/56	81/81
AVM axon ventral guidance	56/56	81/81
AVM axon anterior growth	56/56	81/81
AVM marker expression (*Pmec-4::gfp*)	56/56	81/81
AVM marker expression (*Pmir-90::gfp*)	58/58	58/58

Table S2. *C. elegans* strains used in this study.

Strain	Mutations	Integrated transgenes	Extrachromosomal transgenes
SK4005		*zdIs5 I [mec-4::gfp]*	
XN67	*lin-4(e912) II*	*zdIs5 I*	
XN608	*dcr-1(mg375) III*	*zdIs5 I*	
XN44	*alg-1(gk214) X*	*zdIs5 I*	
XN46	*alg-2(ok304) II*	*zdIs5 I*	
XN244	*lin-14(n355) X*	*zdIs5 I*	
XN232	*lin-14(n360) X*	*zdIs5 I*	
CX5494	*slt-1(ok255) X*	*zdIs5 I*	
CX5300	*unc-6(ev400) X*	*zdIs5 I*	
XN715	*eva-1(tm974) I*	*zdIs4 IV [mec-4::gfp]*	
CX4760	*sax-3(ky123) X*	*zdIs5 I*	
CX5299	*unc-40(e1430) I*	*zdIs5 I*	
XN878	*madd-2(ky592) V*	*zdIs5 I*	
XN648	*dcr-1(mg375) III; slt-1(ok255) X*	*zdIs5 I*	
XN650	*dcr-1(mg375) III; unc-6(ev400) X*	*zdIs5 I*	
XN69	*alg-1(gk214) slt-1(ok255) X*	*zdIs5 I*	
XN447	*unc-6(ev400) alg-1(gk214) X*	*zdIs5 I*	
XN72	*alg-2(ok304) II; slt-1(ok255) X*	*zdIs5 I*	
XN73	*alg-2(ok304) II; unc-6(ev400) X*	*zdIs5 I*	
XN83	*lin-4(e912) II; slt-1(ok255) X*	*zdIs5 I*	
XN137	*lin-4(e912) II; unc-6(ev400) X*	*zdIs5 I*	
XN717	*eva-1(tm974) I; lin-4(e912) II*	*zdIs4 IV*	
XN1351	*lin-4(e912) II; sax-3(ky123) X*	*zdIs5 I*	
XN613	*unc-40(e1430) I; lin-4(e912) II*	*zdIs5 I*	
XN248	*lin-14(n355) slt-1(ok255) X*	*zdIs5 I*	
XN353	*unc-40(e1430) I; lin-14(n355) X*	*zdIs5 I*	
XN241	*lin-14(n360) slt-1(ok255) X*	*zdIs5 I*	
XN322	*lin-4(e912); lin-14(n360) slt-1(ok255)X*	*zdIs5 I*	
CX5169	*unc-6(ev400) slt-1(eh15) X*	*zdIs5 I*	
XN612	*lin-4(e912); unc-6(ev400) slt-1(eh15) X*	*zdIs5 I*	
XN298	*lin-4(e912) II; slt-1(ok255) X*	*zdIs5 I*	*xrEx70[Pmec-4::lin-4; Pofm-1::rfp]*
XN1295	*lin-4(e912) II; slt-1(ok255) X*	*zdIs5 I*	*xrEx434[Punc-54::lin-4; Podr-1::rfp]*
XN1352	*lin-4(e912) II; slt-1(ok255) X*	*zdIs5 I*	*xrEx[Pajm-1::lin-4; Podr-1::rfp]*
XN281	*slt-1(ok255) X*	*zdIs5 I*	*xrEx47[Pmec-4::hbl-1; Podr-1::rfp]*
XN729	*slt-1(ok255) X*	*zdIs5 I*	*xrEx161[Pmec-4::lin-41; Podr-1::rfp]*
XN292	*slt-1(ok255) X*	*zdIs5 I*	*xrEx77[Pmec-4::lin-14; Podr-1::rfp]*
XN315	*unc-6(ev400) X*	*zdIs5 I*	*xrEx77*

XN1048			*xrEx384[Plin-4::gfp; Pmec-4::mCherry]*
XN1233			*xrEx425[Plin-14::gfp; Pmec-4::mCherry]*
VL396	*unc-119(ed3) III*		*wwEx29[Pmir-83::gfp; unc-119(+)]*
VT1598	*unc-119(ed3) III*	*maIs227[Pmir-90::gfp; unc-119(+)]*	
XN490			*xrEx120[Pmec-4::lin-14; Podr-1::gfp]; xrEx138[Punc-40_3.5k::gfp; Pmec-4::mCherry; Podr-1::rfp]*
XN1312			*xrEx77; xrEx441[Punc-40_5k::gfp; Podr-1::gfp; Pmec-4::mCherry]*
XN781			*xrEx120; xrEx201[Pmadd-2::gfp; Pmec-4::mCherry; Podr-1::rfp]*
XN762			*xrEx77; xrEx185[Pced-10::gfp; Pmec-4::mCherry; Podr-1::gfp]*
XN470			*xrEx120; xrEx127[Pmig-10::gfp]; xrEx384[Pmec-4::mCherry; Podr-1::rfp]*
XN818			*xrEx293[Pmec-4::lin-14::mCherry; Podr-1::rfp]; xrEx285[Pmec-4::unc-40::gfp; Podr-1::gfp]*
XN820			*xrEx293; xrEx287[Pmec-4::unc-40::gfp; Podr-1::gfp]*
XN819			*xrEx293; xrEx286[Pmec-4::unc-40::gfp; Podr-1::gfp]*
XN620			*xrEx177[Pmec-4::gfp::lin-14 3'UTR; Pmec-4::mCherry::unc-54 3'UTR; Podr-1::rfp]*
XN640	*lin-4(e912) II*		*xrEx177*
XN894			*xrEx177; xrEx69[mec-4::lin-4; Pofm-1::rfp]*
XN619	*unc-40(e1430) I; slt-1(ok255) X*	*zdIs5 I*	*xrEx77*
XN749	*madd-2(ky592) V; slt-1(ok255) X*	*zdIs5 I*	*xrEx77*
XN580	*unc-34(gm104) V; slt-1(ok255) X*	*zdIs5 I*	*xrEx77*
XN556	*mig-10(ct41) III; slt-1(ok255) X*	*zdIs5 I*	*xrEx77*
XN579	*ced-10(n1993) IV; slt-1(ok255) X*	*zdIs5 I*	*xrEx77*
XN687	*mig-10(ct41) III; slt-1(ok255) X*	*zdIs5 I*	*xrEx77; xrEx1[Pmec-7:FP4-mito; Podr-1:gfp]*
XN657	*ced-10(n1993) IV; slt-1(ok255) X*	*zdIs5 I*	*xrEx77; xrEx1*
XN1348		*xrIs34 II (MosSCI[Pmec-4::unc-40::gfp])*	
XN1349		*xrIs34 II (MosSCI[Pmec-4::unc-40::gfp])*	*xrEx77*
XN1350		*xrIs34 II (MosSCI[Pmec-4::unc-40::gfp])*	*xrEx293*

Table S3. Stem-loop RT primers and forward PCR primers.

microRNA		Primer sequence
let-7	RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGAACTATAC
	F	CGGCGGTGAGGTAGTAGGTTGT
lin-4	RT	TCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGTCACACTT
	F	CGGCGGTCCCTGAGACCTCAA
mir-34	RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGACCAGCTA
	F	CGGCGGAGGCAGTGTGGTTA
mir-35	RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGACTGCTAG
	F	CGGCGGTCACCGGGTGGAAACT
mir-36	RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGCATGCGAA
	F	CGGCGGTCACCGGGTGAAAATT
mir-37	RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGACTGCAAG
	F	CGGCGGTCACCGGGTGAACACT
mir-38	RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGACTCCAGT
	F	CGGCGGTCACCGGGAGAAAAAC
mir-39	RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGCAAGCTGA
	F	CGGCGGTCACCGGGTGTAAATC
mir-40	RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGTCAGCTAA
	F	CGGCGGTCACCGGGTGTACATC
mir-41	RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGTAGGTGAT
	F	CGGCGGTCACCGGGTGAAAAAT
mir-42	RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGTCTGTAGA
	F	CGGCGGTCACCGGGTTAACATC
mir-43	RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGGCGACAGC
	F	CGGCGGTATCACAGTTTACTTGC
mir-44	RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGAGCTGAAT
	F	CGGCGGTGACTAGAGACACAT
mir-48	RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGTCGCATCT
	F	CGGCGGTGAGGTAGGCTCAGTAG
mir-49	RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGTCTGCAGC
	F	CGGCGGAAGCACCACGAGAAGC
mir-50	RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGCCCAAGAA
	F	CGGCGGTGATATGTCTGGTATT
mir-51	RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGAACATGGA
	F	CGGCGGTACCCGTAGCTCCTATC
mir-54	RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGCTCGGATT
	F	CGGCGGTACCCGTAATCTTCATAA
mir-55	RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGCTCAGCAG
	F	CGGCGGTACCCGTATAAGTTTCT
mir-56	RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGCTCAGCGG
	F	CGGCGGTACCCGTAATGTTTCC
mir-57	RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGACACACAG
	F	CGGCGGTACCCTGTAGATCGAGCT
mir-58	RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGATTGCCGT
	F	CGGCGGTGAGATCGTTCAGTAC
mir-59	RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGCATCATCC
	F	CGGCGGTCGAATCGTTTATCAGG
mir-60	RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGTGAACTAG
	F	CGGCGGTATTATGCACATTTTCT
mir-61	RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGGATGAGTA
	F	CGGCGGTGACTAGAACCGTTA
mir-62	RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGCTGTAAGC
	F	CGGCGGTGATATGTAATCTAGC
mir-63	RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGTTTCCAAC
	F	CGGCGGTATGACACTGAAGCGAGT

mir-66	RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGTCACATCC
	F	CGGCGGCATGACACTGATTAGGG
mir-67	RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGTCTACTCT
	F	CGGCGGTCACAACCTCCTAGAAAG
mir-70	RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGATGGAAAC
	F	CGGCGGTAATACGTCGTTGGTGT
mir-71	RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGTCACTACC
	F	CGGCGGTGAAAGACATGGG
mir-72	RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGTCAGCTAT
	F	CGGCGGAGGCAAGATGTTGGCAT
mir-73	RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGACTGAACT
	F	CGGCGGTGGCAAGATGTAGGCAG
mir-74	RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGTGTAGACT
	F	CGGCGGTGGCAAGAAATGGCAG
mir-75	RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGTGAAGCCG
	F	CGGCGGTTAAAGCTACCAACCG
mir-76	RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGTCAAGGCT
	F	CGGCGGTTCGTTGTTGATGAAG
mir-77	RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGTGGACAGC
	F	CGGCGGTTCATCAGGCCATAGC
mir-78	RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGGCACAAAC
	F	CGGCGGTGGAGGCCTGGTTGT
mir-79	RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGAGCTTTGG
	F	CGGCGGATAAAGCTAGGTTACC
mir-80	RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGTCGGCTTT
	F	CGGCGGTGAGATCATTAGTTGAA
mir-81	RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGACTAGCTT
	F	CGGCGGTGAGATCATCGTGAAA
mir-82	RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGACTGGCTT
	F	CGGCGGTGAGATCATCGTGAAA
mir-83	RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGTTACTGAA
	F	CGGCGGTAGCACCATATAAATT
mir-84	RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGTACAATAT
	F	CGGCGGTGAGGTAGTATGTAAT
mir-85	RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGGCACGACT
	F	CGGCGGTACAAAGTATTTGAAAAG
mir-86	RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGGACTGTGG
	F	CGGCGGTAAGTGAATGCTTTGCC
mir-87	RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGGCACACCT
	F	CGGCGGGTGAGCAAAGTTTCAG
mir-90	RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGAGGGGCAT
	F	CGGCGGTGATATGTTGTTTGAAT
mir-124	RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGTGGCATTC
	F	CGGCGGTAAGGCACGCGGTGA
mir-228	RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGCCGTGAAT
	F	CGGCGGAATGGCACTGCATGAAT
mir-229	RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGCGATGGAA
	F	CGGCGGAATGACACTGGTTATCTTTT
mir-232	RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGTCACCGCA
	F	CGGCGGTAAATGCATCTTAACTG
mir-234	RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGAAGGGTAT
	F	CGGCGGTTATTGCTCGAGAAT
mir-235	RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGTCAGGCCG
	F	CGGCGGTATTGCACTCTCCCCG
mir-236	RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGAGCGTCAT
	F	CGGCGGTAATACTGTCAGGTAAT
mir-237	RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGAGCTGTTC
	F	CGGCGGTCCCTGAGAATTCTCGA

mir-238	RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGTCTGAATG
	F	CGGCGGTTTGTACTCCGATGCCA
mir-239a	RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGCCAGTACC
	F	CGGCGGTTTGTACTACACATAGG
mir-239b	RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGCAGTACTT
	F	CGGCGGTTTGTACTACACAAAA
mir-240	RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGAGCGAAGA
	F	CGGCGGTACTGGCCCCCAAATC
mir-241	RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGTCATTTCT
	F	CGGCGGTGAGGTAGGTGCGAG
mir-242	RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGTCGAAGCA
	F	CGGCGGTTGCGTAGGCCTTTG
mir-243	RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGGATATCCC
	F	CGGCGGCGGTACGATCGCGGCGG
mir-244	RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGCATACCAC
	F	CGGCGGTCTTTGGTTGTACAAAGT
mir-245	RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGGAGCTACT
	F	CGGCGGATTGGTCCCCTCCAAG
mir-246	RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGGCTCCTAC
	F	CGGCGGTTACATGTTTCGGGT
mir-247	RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGAGAAGAGA
	F	CGGCGGTGACTAGAGCCTATTC
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	F	CGGCGGATACACGTGCACGGATAA
mir-249	RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGGGCAACGC
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mir250	RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGTGCCAACA
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mir-251	RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGAATAAGAG
	F	CGGCGGTTAAGTAGTGGTGCCGCT
mir-252	RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGTTACCTGC
	F	CGGCGGATAAGTAGTAGTGCCGC
mir-253	RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGCCTTCCCA
	F	CGGCGGTTAGTAGGCGTTGTG
mir-254	RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGCCTACAGT
	F	CGGCGGTGCAAATCTTTCGCGAC
mir-255	RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGCTGTAAAA
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mir-262	RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGATCAGAAA
	F	CGGCGGGTTTCTCGATGTTT
mir-264	RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGCATAACAA
	F	CGGCGGGGCGGGTGGTTGTT
mir-265	RT	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGATACCACC
	F	CGGCGGTGAGGGAGGAAGGG