

Cell-type-specific profiling of loaded miRNAs from *Caenorhabditis elegans* reveals spatial and temporal flexibility in Argonaute loading

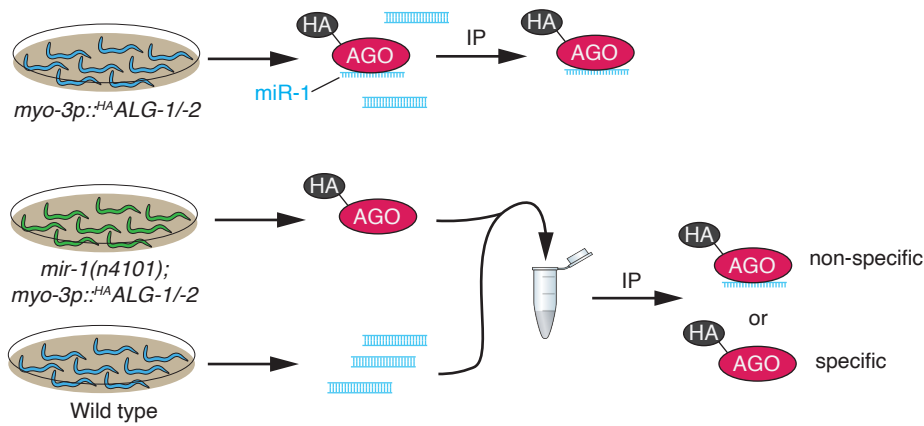
Christopher A Brosnan^{1,2,*}, Alexander Palmer¹ and Steven Zuryn^{1,*}.

Supplementary Information:

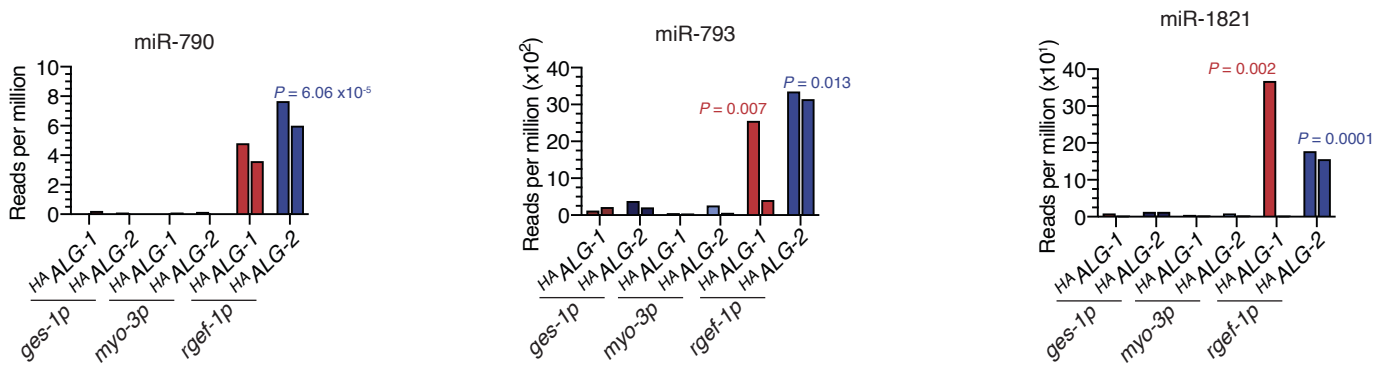
Supplementary Figure 1
Supplementary Figure 2
Supplementary Figure 3
Supplementary Figure 4
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Supplementary Figure 7
Supplementary Figure 8
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Supplementary Table 1
Supplementary Table 2

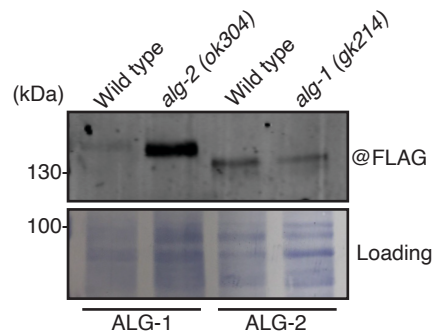
Supplementary Note 1



Supplementary Figure 1. Schematic representation of experimental design from Figure 1e to demonstrate intact and unaltered *in vivo* AGO:miRNA complexes during immunoprecipitation process. Standard AGO:miRNA IP is shown at the top with expected loading of miR-1 when either ^{HA}ALG-1 or ^{HA}ALG-2 is immunoprecipitated specifically from the body wall muscles. *mir-1(n4101);myo-3p::^{HA}ALG-1/2* were homogenized and combined at a ratio of 1:1 with miR-1-expressing wild-type homogenates. After immunoprecipitation, the two possible scenarios are shown on the right as either non-specific or specific complexes. Figure 1e clearly demonstrates that specific complexes are formed.

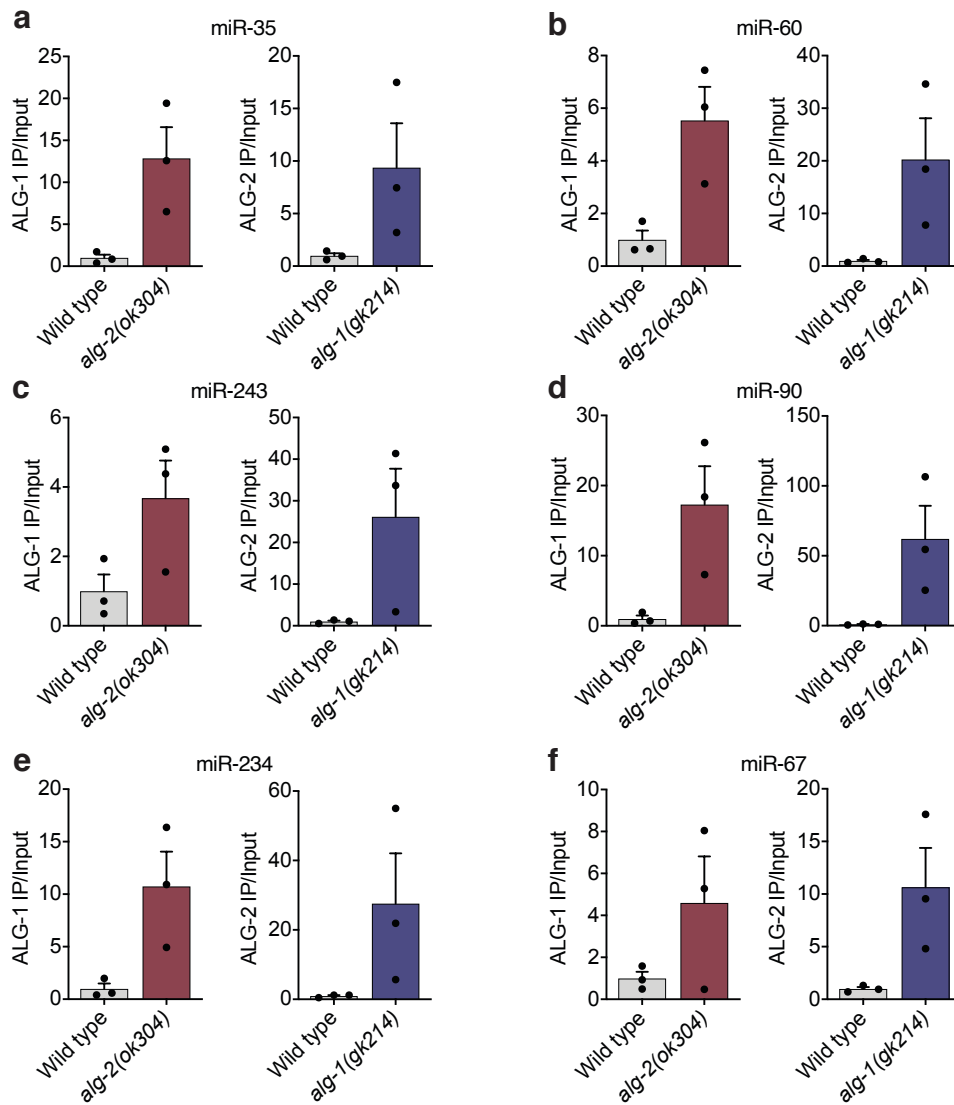


Supplementary Figure 2. Sensitivity of cell-type-specific AGO loading as illustrated in neurons. Plot of reads counts per million in individual cell types of miR-790 (left), miR-793 (middle) and miR-1821 (right) in either ALG-1 or ALG-2. Biologically independent replicate libraries are shown separately, two bars. *P* values are shown for significant loading and were calculated by fitting a two-tailed negative binomial model to processed read counts, according to edgeR pipeline for pairwise comparisons between multiple groups.

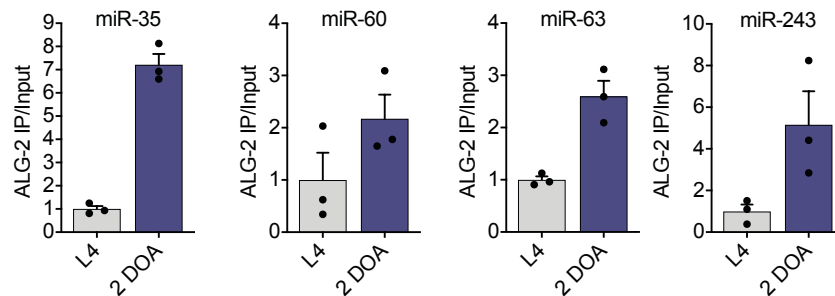


Supplementary Figure 3. Expression of ALG-1 and ALG-2 in corresponding mutant backgrounds.

Western blot analysis of CRISPR generated *CRISPR-Cas9* 3XFLAG::GFP::ALG-1 (ALG-1) in a wild-type or *alg-2(ok304)* mutant background. The same is shown for *CRISPR-Cas9* 3XFLAG::RFP::ALG-2 (ALG-2) in a wild-type or *alg-1(gk214)* background. Loading represents total protein stained with Coomassie blue. ALG levels were confirmed by western blots on two separate occasions with identical results.

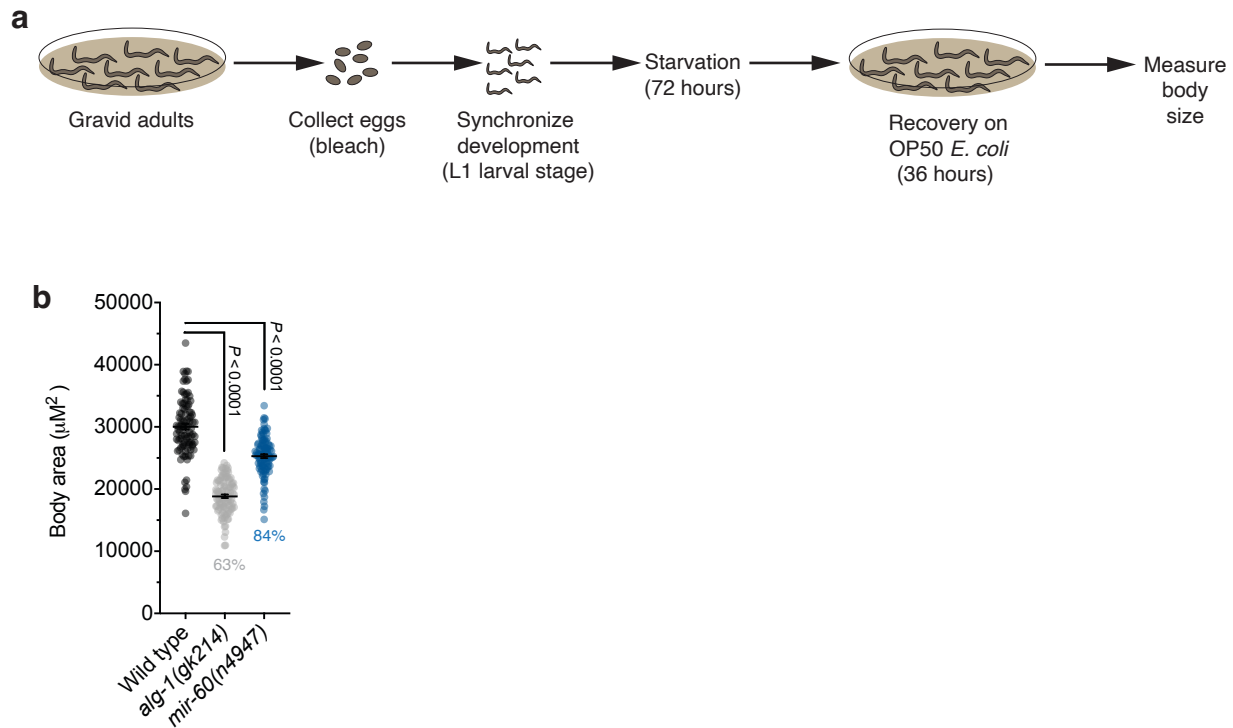


Supplementary Figure 4. Quantification of miRNA re-loading between ALG-1 and ALG-2. (a) Re-loading of the constitutively expressed miR-35 into ALG-1 in an *alg-2(ok304)* mutant (left – grey vs red) or into ALG-2 in an *alg-1(gk214)* mutant (right – grey vs blue). (b) Re-loading of the intestine-specific miRNA miR-60. (c) Re-loading of intestine-specific miR-243. (d) Neuronal-enriched miR-90 re-loading between ALG-1 and ALG-2. (e) Neuronal-specific miR-234 re-loading between ALG-1 and ALG-2. (f) Re-loading of muscle-specific miR-67. Each bar represents the average enrichment of immunoprecipitation over input for the indicated miRNA in the indicated genetic background. Error bar represent $\pm$ s.e.m. for 3 biological immunoprecipitation replicates.

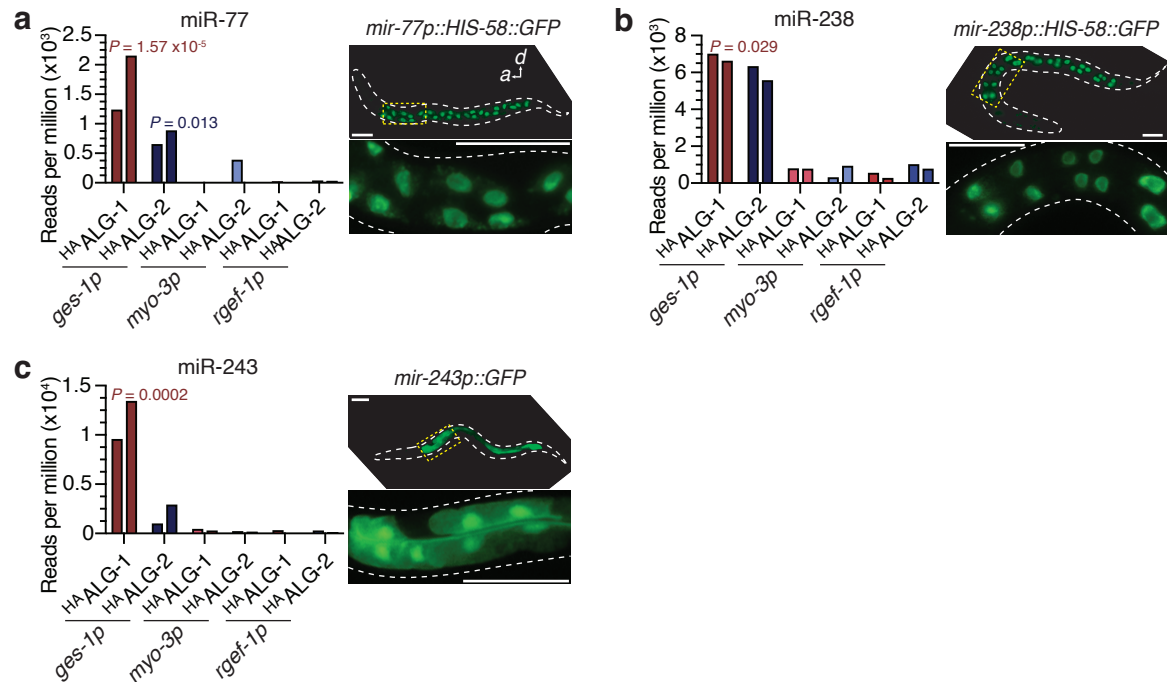


Supplementary Figure 5. Reloading of miRNAs into ALG-2 during ageing.

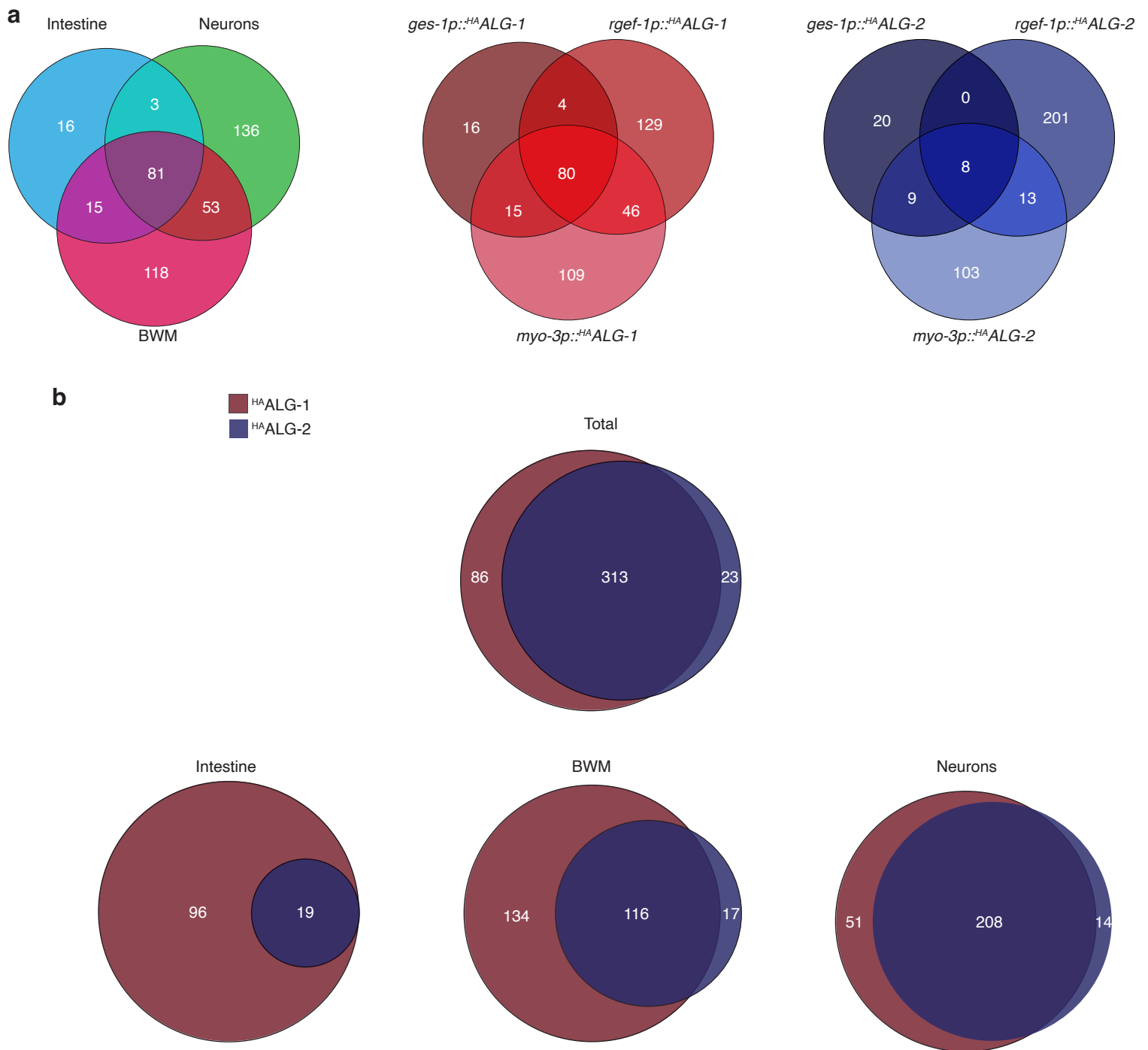
qRT-PCR of the indicated miRNAs in either ALG-2 immunoprecipitated L4 (grey) or 2-day-old adult (2 DOA) animals. Each bar represents the average enrichment of immunoprecipitation over input for the indicated miRNA at either L4 or 2 DOA animals. Error bar represent +/- s.e.m. for 3 biological immunoprecipitation replicates.



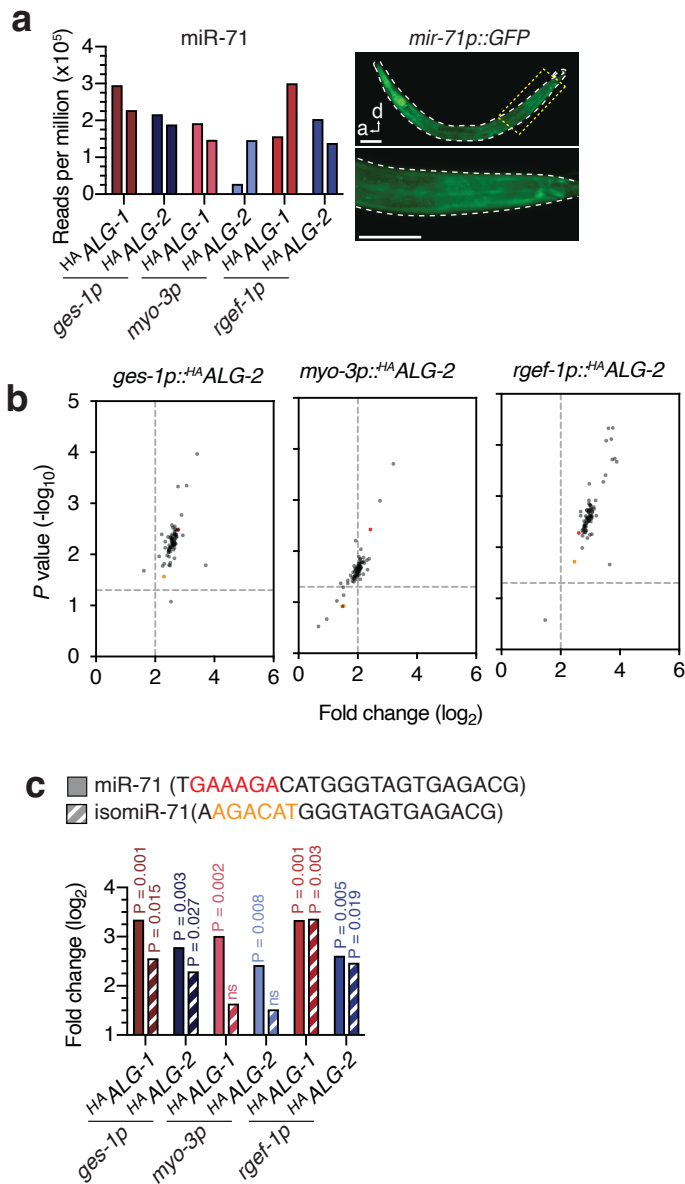
Supplementary Figure 6. Recovery from starvation stress. (a) Scheme of workflow used to assay recovery from starvation stress. Animals were hatched in M9 buffer and deprived of food for 72 hours. L1 larvae were then allowed to recover for ~36 hours prior to measuring body size. (b) Quantification of body area of non-starved animals (no 72 hour starvation period). Percentages shown are the relative average body areas of animals compared to wild type. Error bars represent $\pm$ s.e.m. P values represent one-way ANOVA with Tukey's multiple comparisons test. $n > 30$ biologically independent animals for each strain tested.



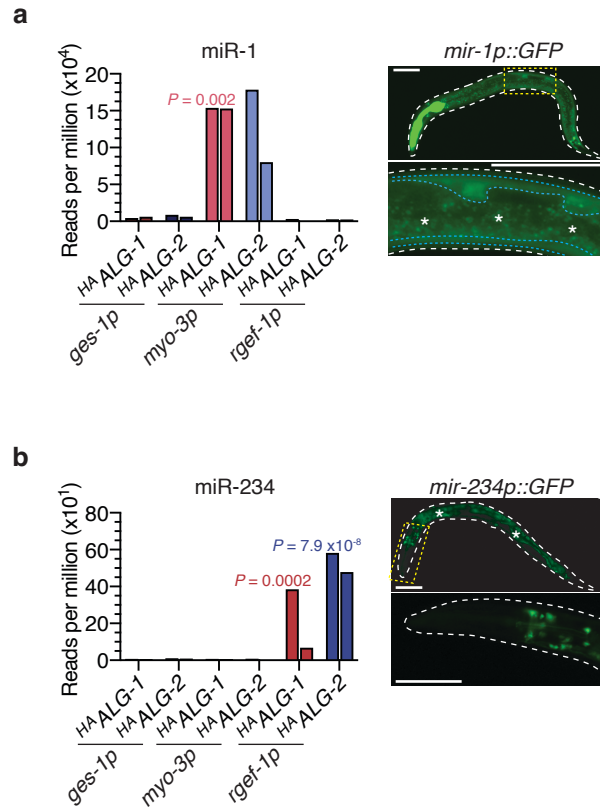
Supplementary Figure 7. Confirmation of intestine-expressed and AGO-loaded miRNAs. (a) Intestine-specific loading of miR-77 shown by plots of read counts per million in the indicated cell-type-specific AGO libraries (left). Promoter GFP fusion image (right) shows intestine-specific expression of miR-77. (b) Intestine-specific loading of miR-238 shown by plots of read counts per million in the indicated cell-type-specific AGO libraries (left). Promoter GFP fusion image (right) shows intestine-specific expression of miR-238. (c) Intestine-specific loading of miR-243 shown by plots of read counts per million in the indicated cell-type-specific AGO libraries (left). Promoter GFP fusion image (right) shows intestine-specific expression of miR-243. Graphs show two biological replicate experiments, two bars. Scale bars, 50 μ m.



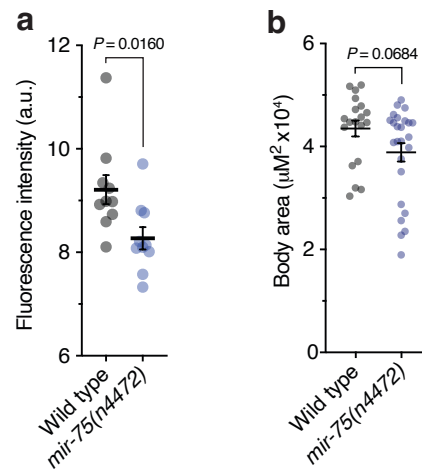
Supplementary Figure 8. Cell- and AGO-specific distribution of loaded isomiRs.
(a) Venn diagram representation of cell-type specific isomiRs (left), ALG-1 loaded cell-type specific (middle) or ALG-2 loaded cell-type specific (right) isomiRs. **(b)** AGO-specific loading of isomiRs independent of cell-type (top) or AGO-specific loading with the indicated cell-types (bottom). BWM, body wall muscle.



Supplementary Figure 9. miR-71 and isomiR variations in cell-type-specific loading. (a) Read counts per million in individual AGO- and cell-type-specific libraries for miR-71 showing ubiquitous enrichment. Live animal image (right) of the promoter of miR-71 fused to GFP showing ubiquitous expression. Scale bars, 50 μ m. (b) Volcano plots of miR-71 (red) and miR-71 isomiRs (black and orange) in individual ALG-1- and cell-type-specific libraries. Orange dot corresponds to specific isomiR-71 shown in (c). P values were calculated by fitting a two-tailed negative binomial model to processed read counts, according to edgeR pipeline for pairwise comparisons between multiple groups. (c) AGO- and cell-type-specific $\log_2$ fold changes of miR-71 and isomiR-71, which unlike miR-71 is not enriched in body wall muscle and has an alternative seed sequence (highlighted in red and orange font). For (a) and (c), graphs show two biological replicate experiments, two bars.

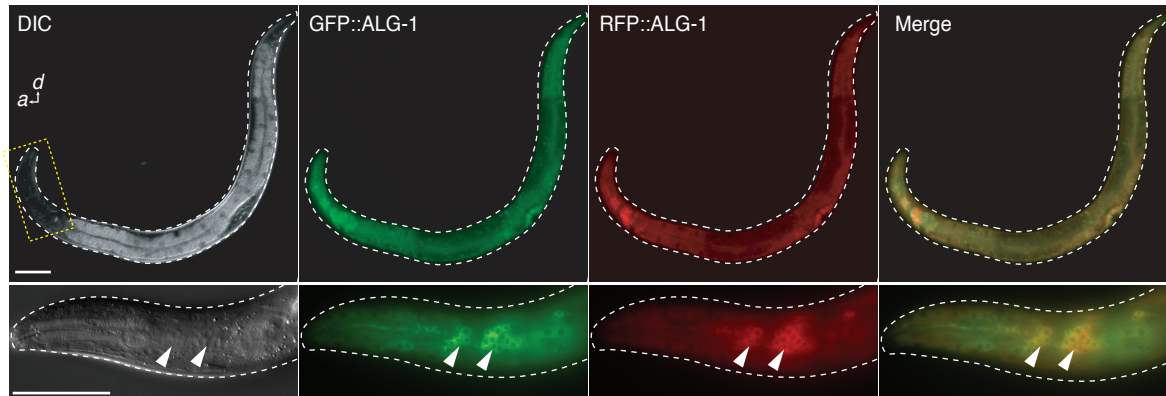


Supplementary Figure 10. Cell-type-specific loading indicates cell-type-specific function. (a) Muscle specific loading of miR-1 shown by plots of read counts per million in the indicated cell-type-specific AGO libraries (left). Promoter GFP fusion image (right) shows body wall muscle (outlined in dashed blue line in close up image), in addition to pharyngeal expression of miR-1. **(b)** Neuron-specific loading of miR-234 shown by plots of read counts per million in the indicated cell-type-specific AGO libraries (left). Promoter GFP fusion image (right) shows neuron-specific expression of miR-1. Graphs show two biological replicate experiments, two bars. Scale bars, 50 μ m. Asterisks indicate intestine autofluorescence.

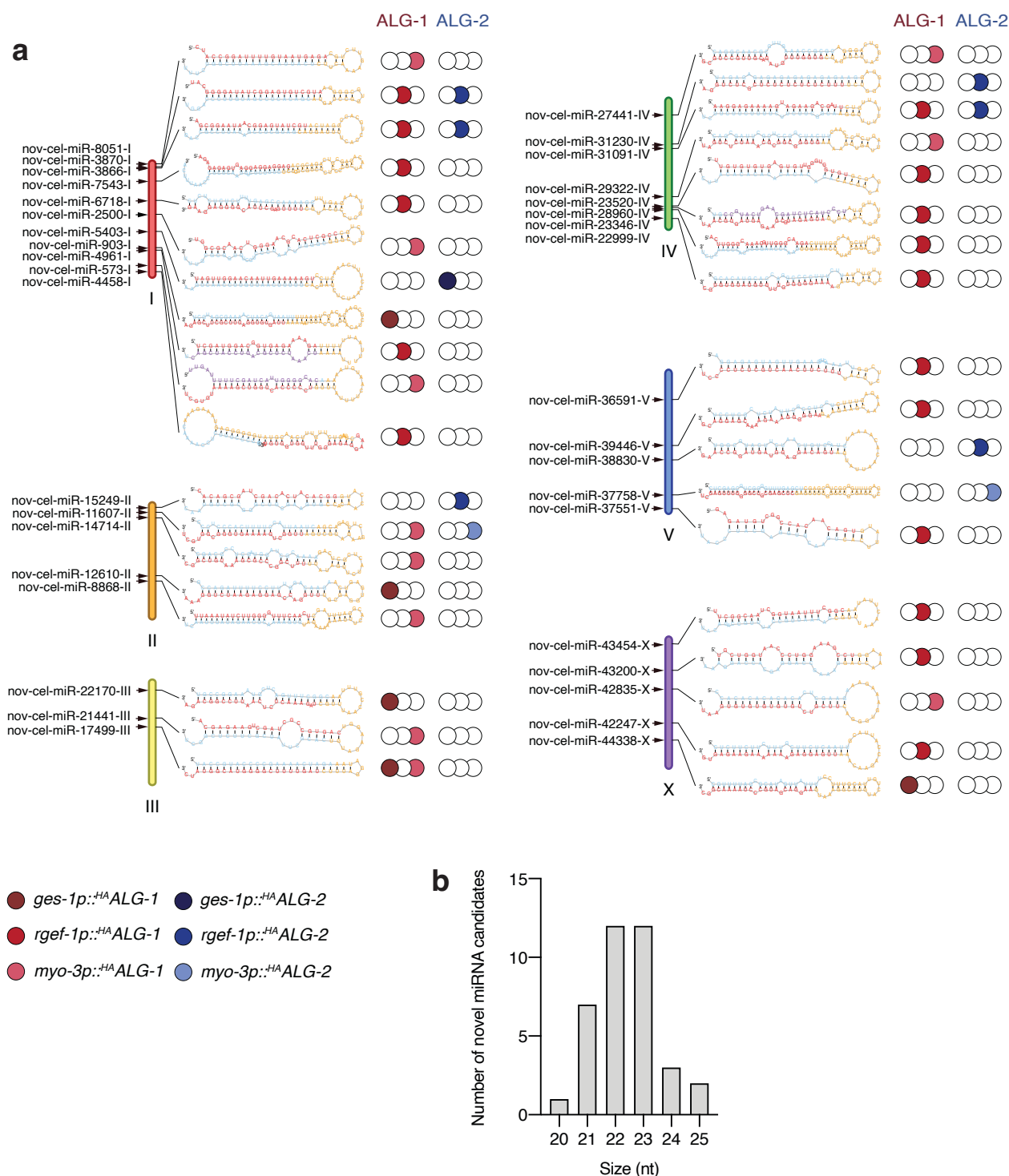


Supplementary Figure 11. Fat content and starvation recovery of *mir-75(n4472)*. (a) Fat content of intestine of wild-type and *mir-75(n4472)* animals, as assessed by Oil Red O staining. a.u., arbitrary units. $n=10$ independent animals. (b) Body area measurement of starvation-recovered wild-type and *mir-75(n4472)* worms. $n \geq 19$ independent animals. Error bars represent $\pm$ s.e.m. P values represent two-way unpaired Student's t -test.

CRISPR-Cas9 FLAG::GFP::ALG-1; *CRISPR-Cas9* FLAG::RFP::ALG-2



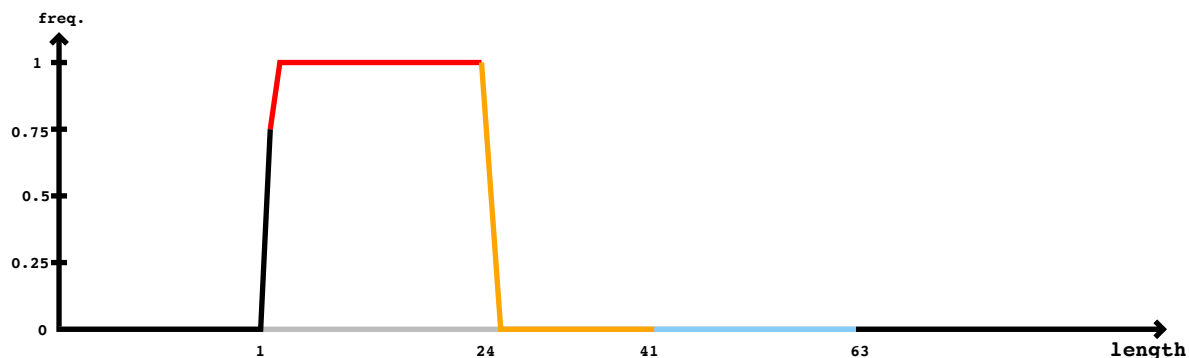
Supplementary Figure 12. Enrichment of ALG-2 expression in the neurons of the head. Representative confocal images of *CRISPR-Cas9* 3XFLAG::GFP::ALG-1; *CRISPR-Cas9* 3XFLAG::RFP::ALG-2 transgenic L4 stage worms. Arrows indicate head ganglia. Scale bars, 50 μ m. Images represent what was seen in at least 4 different imaged animals on multiple imaging sessions.



Supplementary Figure 13. Genomic location, pre-miRNA structure, cell-type-specific loading and AGO-specific loading of novel miRNA candidates. (a) Novel miRNA candidate name and genomic location (left). Predicted secondary structure of novel pre-miRNA transcript (middle). Colors (see key at bottom) indicate ALG-1 cell-type-specific (left) or ALG-2 cell-type-specific mature novel miRNA candidate loading patterns. **(b)** Size distribution of novel miRNA candidates.



Supplementary Figure 14. Stem loop structure, read counts and miRNA annotation as determined by miRDeep2 software of novel miRNA candidates.

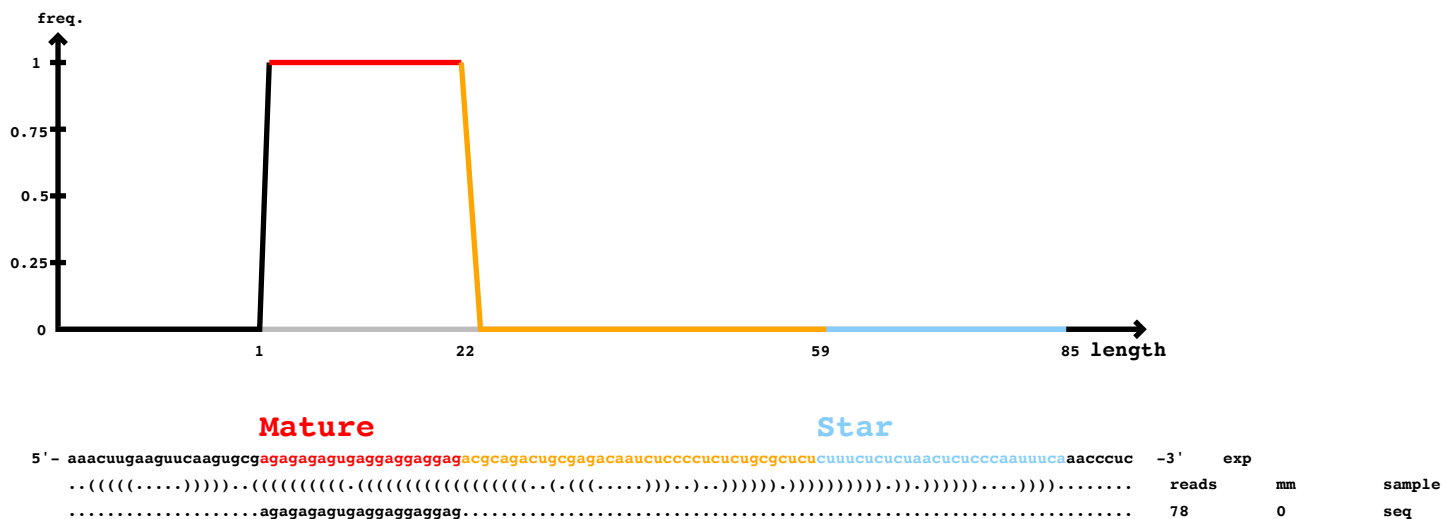
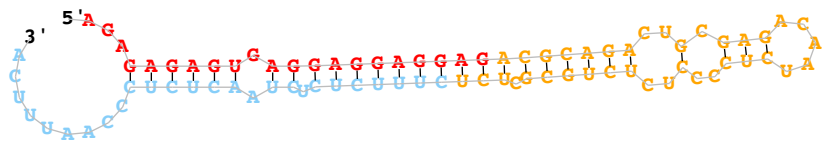


Supplementary Figure 14. Stem loop structure, read counts and miRNA annotation as determined by miRDeep2 software of novel miRNA candidates.

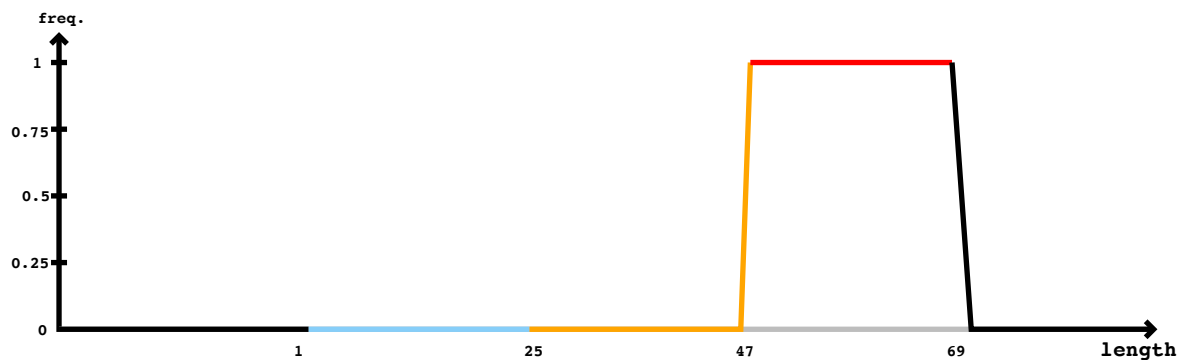
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Supplementary Figure 14. Stem loop structure, read counts and miRNA annotation as determined by miRDeep2 software of novel miRNA candidates.

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 Score for cons. seed :
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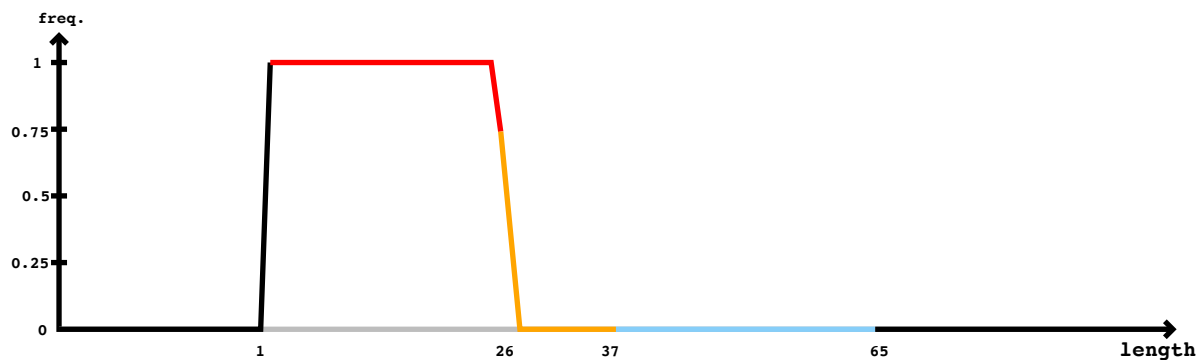
Supplementary Figure 14. Stem loop structure, read counts and miRNA annotation as determined by miRDeep2 software of novel miRNA candidates.



Mature

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Supplementary Figure 14. Stem loop structure, read counts and miRNA annotation as determined by miRDeep2 software of novel miRNA candidates.

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Supplementary Figure 14. Stem loop structure, read counts and miRNA annotation as determined by miRDeep2 software of novel miRNA candidates.



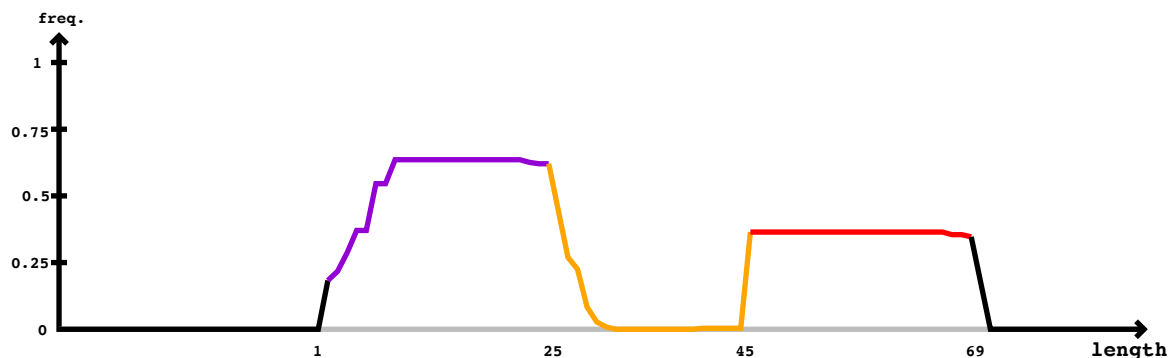
Supplementary Figure 14. Stem loop structure, read counts and miRNA annotation as determined by miRDeep2 software of novel miRNA candidates.

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Supplementary Figure 14. Stem loop structure, read counts and miRNA annotation as determined by miRDeep2 software of novel miRNA candidates.

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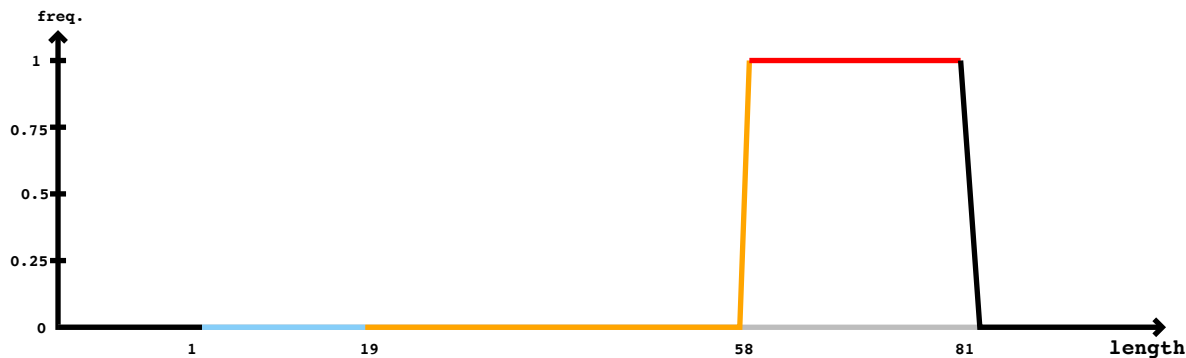
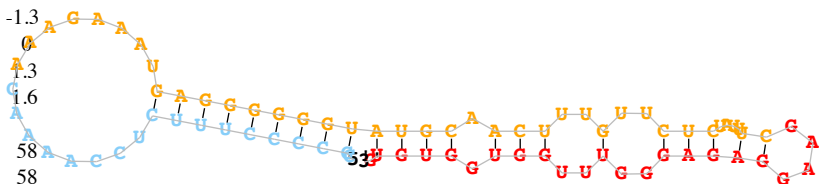


Mature

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	uuggucuuuuuuagacguugauuag <u>u</u> gauuuuucgauc <u>au</u> uuggggc <u>ac</u> aaaaauguaacuuuuuuuug <u>uc</u> ccccacugggucgaaaaugugcuuacauuaacgaauaaaau		exp	
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Supplementary Figure 14. Stem loop structure, read counts and miRNA annotation as determined by miRDeep2 software of novel miRNA candidates.

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 Score for cons. seed :
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 Loop read count : 0
 Star read count : 0



Star

Mature

5'

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-3'

exp

reads

mm

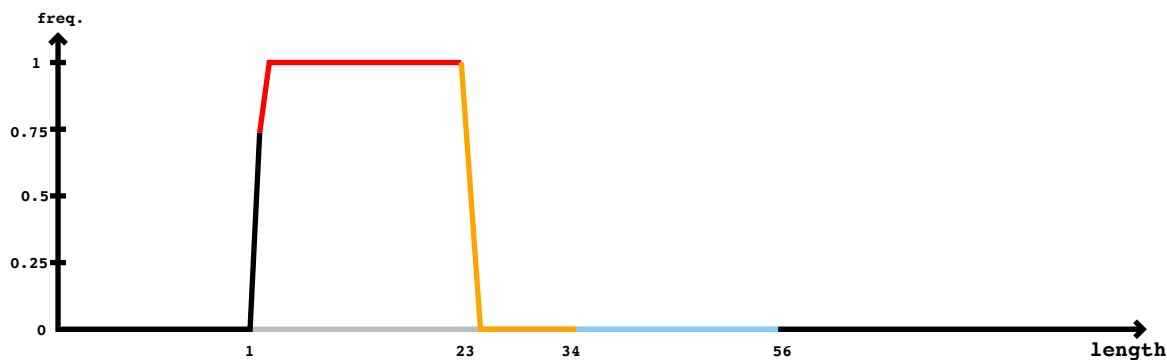
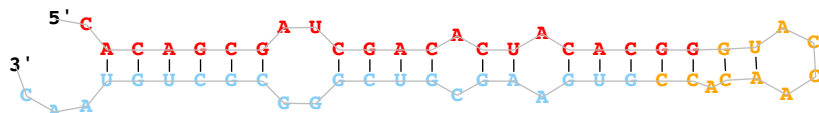
sample

seq

58

0

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Mature read count : 201
Loop read count : 0
Star read count : 0



Mature										Star																	
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		((((((((.....)))))).....(((((((.....((((((.....)))))))))..)))))..))))).....((((((((((((.....))))))))).....																				reads		mm		sample	
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		acagcgaucgacacuuacacgg.....																				53		0		seq	

Supplementary Figure 14. Stem loop structure, read counts and miRNA annotation as determined by miRDeep2 software of novel miRNA candidates.

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5'	uuuuuuuuucucgagcuuccuugcagc	cgagcgcucucgauggaagcga	aaagacucgcucaucuugauuuuuu	uucggcccgcgaaaugcggc	ugccaggcaagcgugcaauu	-3'	exp
.....	(((((((	(((((((	(((	(((	(((	reads	mm
.....	(((	(((	(((	(((	(((	30	0
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.....	uuucggcccgcgaaaugcggc		112	1	seq		

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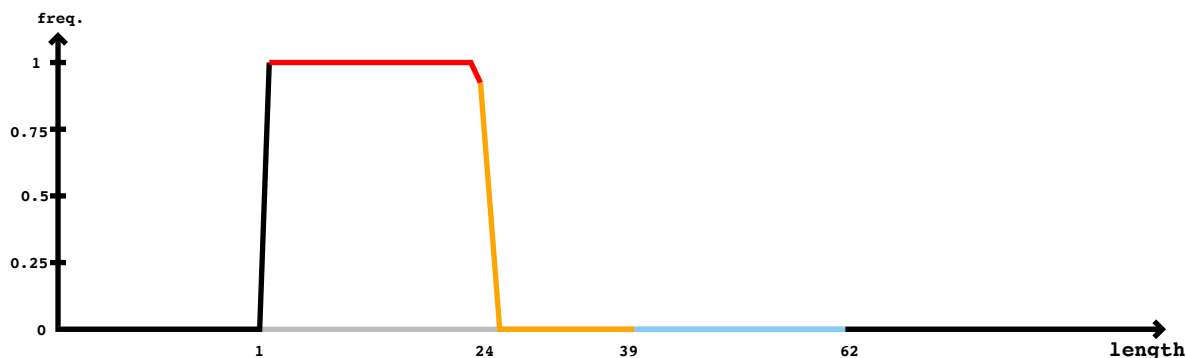
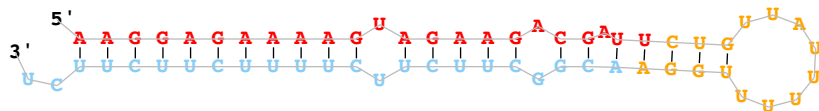


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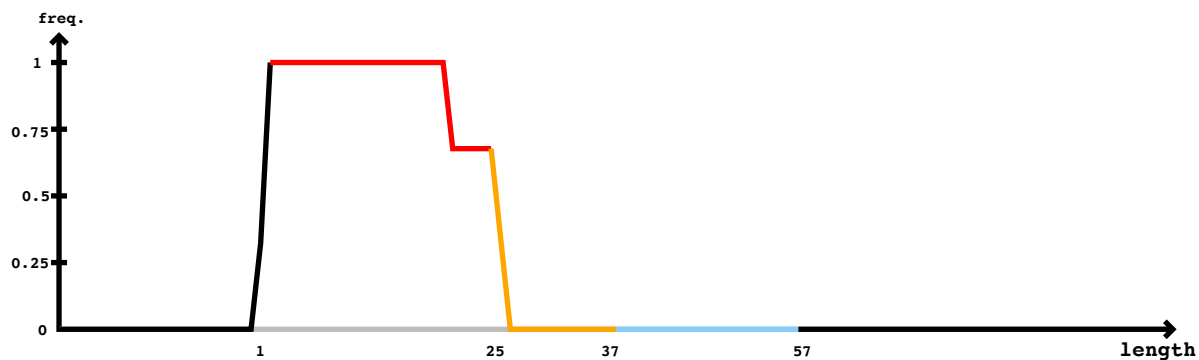
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Score for cons. seed :
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Mature read count : 1707
Loop read count : 0
Star read count : 0



Mature										Star																							
5'	uucugagcauugcaaaaaagaggagaaaaguagaagacgauucuguuauuuuuuggaacgggcuccuccuuuuuccuccuccuuguugagcuuuuuucacacuuuuugcuucugu																														-3'	exp	
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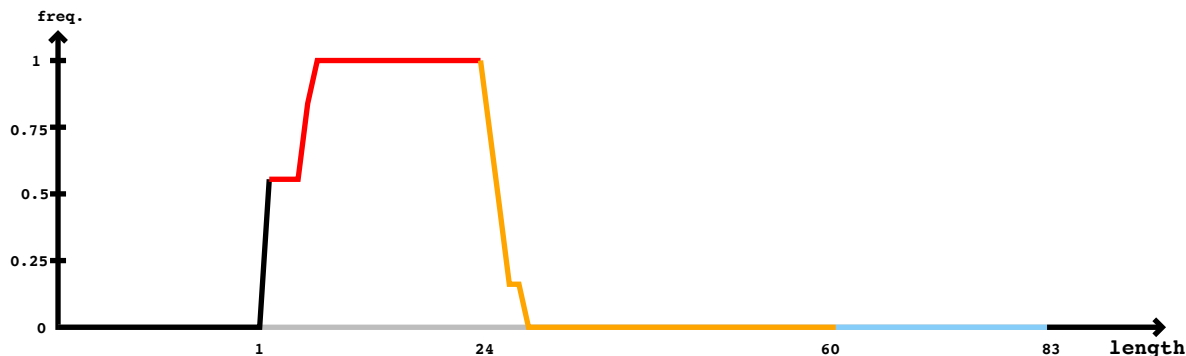
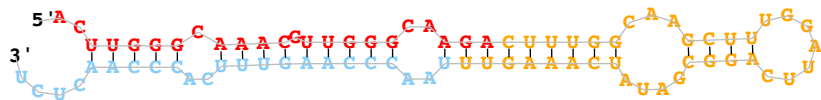
Star

Supplementary Figure 14. Stem loop structure, read counts and miRNA annotation as determined by miRDeep2 software of novel miRNA candidates.

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Supplementary Figure 14. Stem loop structure, read counts and miRNA annotation as determined by miRDeep2 software of novel miRNA candidates.

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 Loop read count : 0
 Star read count : 0



Mature										Star									
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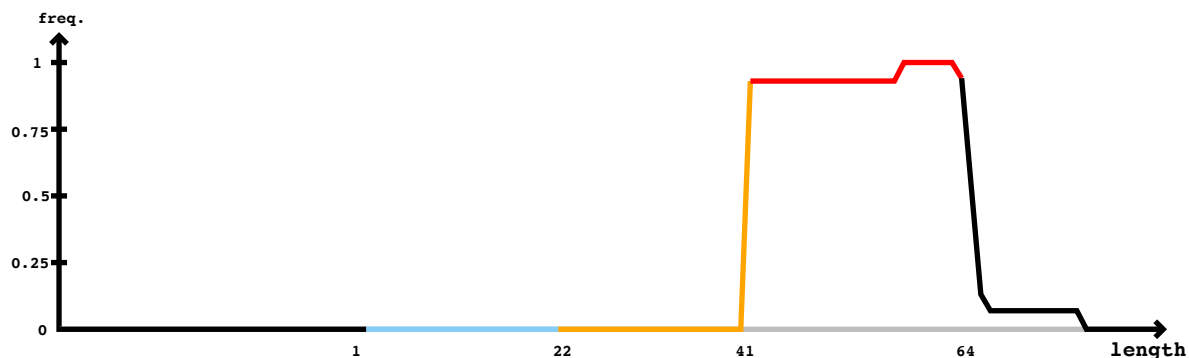
Supplementary Figure 14. Stem loop structure, read counts and miRNA annotation as determined by miRDeep2 software of novel miRNA candidates.



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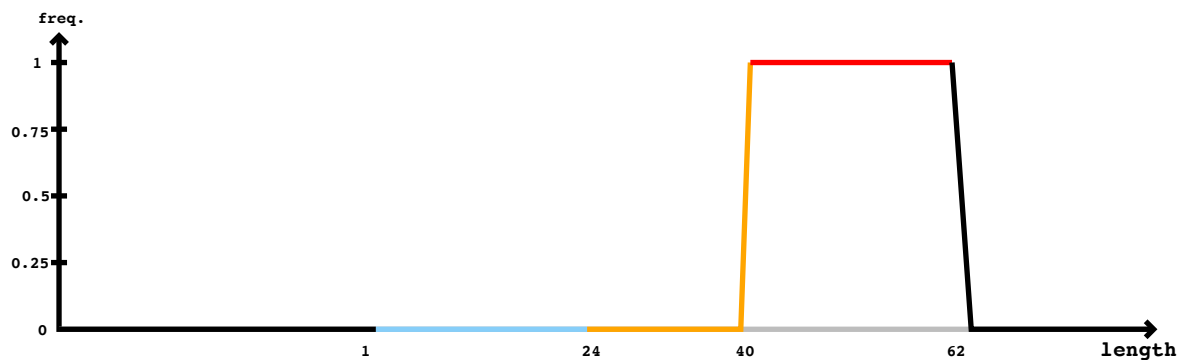


Supplementary Figure 14. Stem loop structure, read counts and miRNA annotation as determined by miRDeep2 software of novel miRNA candidates.



Star		Mature			
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Supplementary Figure 14. Stem loop structure, read counts and miRNA annotation as determined by miRDeep2 software of novel miRNA candidates.

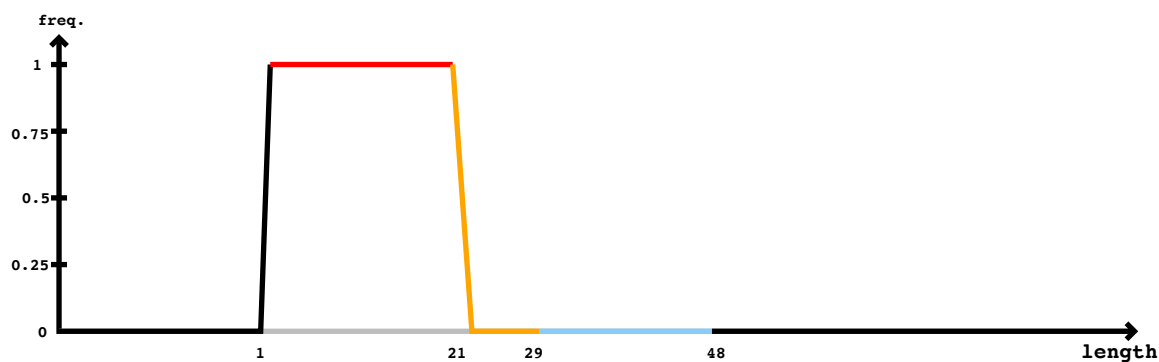


Supplementary Figure 14. Stem loop structure, read counts and miRNA annotation as determined by miRDeep2 software of novel miRNA candidates.

5' C U C G G A G C G U C G G G C U U U G A G C U C G A G C C G U C C G G G U U U G A G U C
3' U C G A G U U U G G C C U G C G A G C U C G A G U U U G G G C U G C G A G C U C



Supplementary Figure 14. Stem loop structure, read counts and miRNA annotation as determined by miRDeep2 software of novel miRNA candidates.



Star

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Supplementary Figure 14. Stem loop structure, read counts and miRNA annotation as determined by miRDeep2 software of novel miRNA candidates.



Supplementary Figure 14. Stem loop structure, read counts and miRNA annotation as determined by miRDeep2 software of novel miRNA candidates.



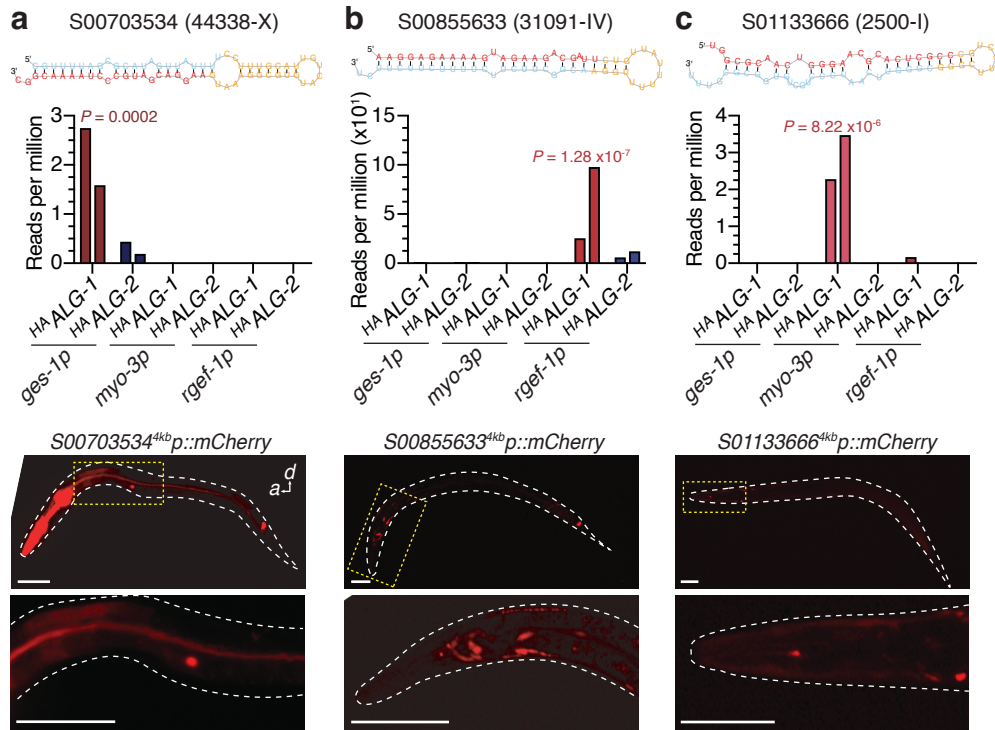
Supplementary Figure 14. Stem loop structure, read counts and miRNA annotation as determined by miRDeep2 software of novel miRNA candidates.



Supplementary Figure 14. Stem loop structure, read counts and miRNA annotation as determined by miRDeep2 software of novel miRNA candidates.

[illegible]

Supplementary Figure 14. Stem loop structure, read counts and miRNA annotation as determined by miRDeep2 software of novel miRNA candidates.



Supplementary Figure 15. Examples of novel miRNA candidate cell-type specific expression and loading. (a-c) Predicted secondary structures of pre-miRNAs, read counts per tissue- and AGO-type loading, and representative images of promoter mCherry fusion strains for (a) intestine-specific (b) neuronal-specific, and (c) body wall muscle-specific novel miRNAs. For (a) to (c), graph shows reads per million in indicated cell types of two biological replicate experiments, two bars. Scale bars, 50 μm .

Supplementary Table 1: List of the generated transgenic strains used in this study

Strain Name	Genotype
SJZ284	<i>foxSi62[ges-1p::HA::ALG-1::SL2::HIS-58::GFP::tbb-2 3'UTR (oxti177)IV]</i>
SJZ285	<i>foxSi63[ges-1p::HA::ALG-2::SL2::HIS-58::GFP::tbb-2 3'UTR (oxti177)IV]</i>
SJZ286	<i>foxSi64[rgef-1p::HA::ALG-1::SL2::HIS-58::GFP::tbb-2 3'UTR (oxti177)IV]</i>
SJZ287	<i>foxSi65[rgef-1p::HA::ALG-2::SL2::HIS-58::GFP::tbb-2 3'UTR (oxti177)IV]</i>
SJZ290	<i>foxSi68[myo-3p::HA::ALG-1::SL2::HIS-58::GFP::tbb-2 3'UTR (oxti177)IV]</i>
SJZ291	<i>foxSi69[myo-3p::HA::ALG-2::SL2::HIS-58::GFP::tbb-2 3'UTR (oxti177)IV]</i>
SJZ1001	<i>mir-60(n4947)II; foxSi166[elt-2p::mir-60::SL2::HIS-58::GFP::tbb-2 3'UTR (oxti177)IV]</i>
SJZ1002	<i>mir-60(n4947)II; foxSi167[rgef-1p::mir-60::SL2::HIS-58::GFP::tbb-2 3'UTR (oxti177)IV]</i>
SJZ1003	<i>mir-60(n4947)II; foxSi168[myo-3p::mir-60::SL2::HIS-58::GFP::tbb-2 3'UTR (oxti177)IV]</i>
SJZ845	<i>fox30[3xFLAG::GFP::alg-1]</i>
SJZ846	<i>alg-2(ok304)II; fox30[3xFLAG::GFP::alg-1]X</i>
SJZ844	<i>ap43[3xFLAG::mKate2::alg-2]II; alg-1(gk214)]X</i>
SJZ1048	<i>foxEx276[mir-77p::mir-77::SL2::HIS-58::GFP::tbb-2 3'UTR]</i>
SJZ1051	<i>foxEx279[mir-238p::mir-238::SL2::HIS-58::GFP::tbb-2 3'UTR]</i>
SJZ1036	<i>alg-1(gk314)X; foxEx253[eft-3p::HA::ALG-2::SL2::HIS-58::GFP::tbb-2 3'UTR]</i>
SJZ1037	<i>alg-1(gk314)X; foxEx255[ges-1p::HA::ALG-1::SL2::HIS-58::GFP::tbb-2 3'UTR]</i>
SJZ1038	<i>foxSi63[ges-1p::HA::ALG-2::SL2::HIS-58::GFP::tbb-2 3'UTR (oxti177)IV]; alg-1(gk314)X</i>
SJZ998	<i>foxSi134[rgef-1p::FLAG::RPL-18::SL2::HIS-58::GFP::tbb-2 3'UTR (oxti177)IV]</i>
SJZ1000	<i>alg-2(ok304)II; foxSi134[rgef-1p::HA::RPL-18::SL2::HIS-58::GFP::tbb-2 3'UTR (oxti177)IV]</i>
SJZ832	<i>foxEx230(pS00703534(4kb)::mCherry)</i>
SJZ783	<i>foxEx212(pS00855633(4kb)::mCherry)</i>
SJZ786	<i>foxEx215(pS01133666(4kb)::mCherry)</i>

Supplementary Table 2: List of primers used for this study

	Name	Sequence (5' to 3')
Genotyping	sz6-oCFJ1529	TATCGTAAATCGGCGCGAGC
	sz13-oCFJ1491	gtcactcaaacgatgcaga
	sz14-oCFJ1492	gcaatttcggcaatttcagt
	miR-60-F	ATGATGCATGCAACTAAGTC
	miR-60-F1	CACAATTCTCTTTTATAGTG
	miR-60-R	AGCCTTTGTCAATCCGTTGC
	RB574-F	GACTTAGCCTAGGCAAGCCC
	RB574-F1	CGATGCCAGTCATCGAATTGC
	RB574-R	GCTTACTAGCTTACAAATCTC
	VC446-F	CCAAAAATGCATGACGATC
	VC446-F1	TGATCTAGAAGTAAGTTTTTCATCG
	VC446-R	GAACATCGCTAAATGTTTGTGG
	miR-60-F	ATGATGCATGCAACTAAGTC
	miR-60-F1	CACAATTCTCTTTTATAGTG
	miR-60-R	AGCCTTTGTCAATCCGTTGC
Probes	miR-2	GCACATCAAAGCTGGCTGTGATA
	Lin-4	TCACACTTGAGGTCTCAGGGA
	U6	ATCTTCTCTGTATTGTTCC
Cloning	EFT3p-F	ATGGATCCGCACCTTTGGTCTTTTATTG
	EFT3p-R	ATCTCGAGTGAGCAAAGTGTTTCCCAACTG
	myo-3p-F	ATGGATCCCGGCTATAATAAGTTCTTGAA
	myo-3p-R	ATCTCGAGTCTAGATGGATCTAGTGGTC
	rgef-1p-F	ATGGATCCCGTTTCCGATACCCCTTATA
	rgef-1p-R	CAGATATCCTTTACTGCTGATCGTCGTCG
	ges-1p-F	ATGGATCCCGGCTATAATAAGTTCTTGAA
	ges-1p-R	ATCTCGAGCTGAATCAAAGATAAGATATG
	unc-25p-F	CAGGATCCGCATGCAAAAAACACCCACTT
	unc-25p-R	CACTCGAGTTTTGGCGGTGAAGTGAAGCTT
	FI-RPL18-F	ATGGATTATAAAGACGATGACGATAAGATGGGTATTGATATCAACCAC
	RPL18-SL2-R	AAAGTAGGATGAGACAGCTTAGTTCTGTAGGCTCTGG
	FI-F	ATGGATTATAAAGACGATGACGATAAG
	HA-ALG1-F	ATGTATCCGTATGATGTTCTGATTATGCTgaggatccgaggaATGTCCGGCGGGCCCAATATTTG
	ALG1-2-R	GGGGACCACTTTGTACAAGAAAGCTGGGcTTAAGCAAAGTACATGACGTTG
	HA-ALG2-F	ATGTATCCGTATGATGTTCTGATTATGCTgaggatccgaggaATGTTCCCTCTGCTGTACAC
	ALG2-2-R	GGGGACCACTTTGTACAAGAAAGCTGGGcTTAGGCAAAATACATGACGTTG
	allgene-HA-1-F	GGGGACAAGTTTGTACAAGAAAGCAGGCTtaAGAAAAAATGTATCCGTATGATGTTCC
	SL2-2-F	GGGGCAGCTTTCTGTACAAGTGGGAATGCCACCAAGCCATCTG
	tbb2-3-R	GGGGACAACCTTTGTATAAAAGTTGctaTGAGACTTTTTCTTGGCGG
	F-RPL18-F	ATGGATTATAAAGACGATGACGATAAGATGGGTATTGATATCAACCAC
	RPL18-SL2-R	AAAGTAGGATGAGACAGCTTAGTTCTGTAGGCTCTGG
	ALG1-1	ACGTTGTAAACGACGGCCAGTCGCGGCTTGTGCTGTTCTATCCGAA
	ALG1-2	TCCAGTGAAACATTCTTCTCTTACTCATGAGAGGATTGAAAGCGCTGGGTTGG
	ALG1-3	CGTGATTACAAGGATGACGATGACAAGAAATGGCTGCCGAAGTGAACAAC
	ALG1-4	TCACACAGGAAACAGCTATGACCATGTTATCAACGTTCTCACAGACAG
	Cas9-sg-ALG1-F	AGCGCTTTCAATCCCTCTCAGTTTTAGAGCTAGAAATAGCAAGT
	Cas9-sg-ALL-R	CAAGACATCTCGCAATAGG
	pri-miR60-F	CTCGAAAAACCGCTTGTCTTG
	pri-miR60-SL2-R	GTGAAAGTAGGATGAGACAGCCGATTCTCAAGTCTTGAAAC
	CD-3	TTTTTCTGCTAGCTTAATTAAGCCGGCGGCCCTCAACTTTCTATACAAAGTTGATAGC
	elt2p-NotI-F	ATGCGGCGCGGATCCCCGGGCTGCAGGA
	elt2p-NaeI-R	ATGCGGCTCTATAATCTATTTTCTAGT
	miR-67p-NotI-F	AAGCGGCGCGGTGAGCTTCGATACTTTTTTC
	miR-67p-PacI-R	ACTTAATTAAAGTTGGAATACCAGAGAATT
	miR-77p-NotI-F	ATCGCGCGCCGCACTTCGAGTTTTTGAAATCCG
	miR-77-NheI-R	CTAGCTAGCCAACTCTATACCAATTGG
	miR-238p-NotI-F	ATCGCGCGCCCAAAAGAAGCTTGAGTTAC
	mCh-F	ATGGTCTCAAAGGGTGAAGAAAG
	unc54-R	AAACAGTTATGTTTGGTATATTGG
	miR-238-NheI-R	CTAGCTAGCCATGGCTCATAACTATCTG
	AP4-S00855633-R	ATCTTCTTACCCTTTGAGACCATCGTGGAATATCACTGTCCAACC
	AP5-S00855633-F	TTGAATGCGAGAAGAGAGATACGGA
	AP6-S00855633-F*	CTTAAAGGGGGGAACCGCTCG
	AP10-S01133666-R	ATCTTCTTACCCTTTGAGACCATGACAGGAGGTTGCTTATGAATGC
	AP11-S01133666-F	TGGAATGTGCTGTAATCTTGAG
	AP12-S01133666-F*	TTCATGTGCGTCGTGTTGTTTGG
	AP15-S00703534-R	ATCTTCTTACCCTTTGAGACCATGCTTCTTGGGGGAAAGTCAATAG
	AP16-S00703534-F	GTGTCGAAGGAAAAGTTGCTTGAG
	AP17-S00703534-F*	CTTTCATGTTCTGTTTCGCGCAG
q-RT-PCR	IK-44	CAGGTCCAGTTTTTTTTTTTTTTVN
	miR-1-3p-F	CGCAGTGGAAATGAAAGAAAT
	miR-1-3p-R	CAGGTCCAGTTTTTTTTTTTTTTTACAT
	miR-67-3p-F	CGCAGTCACAACCTCCTAGAAAG
	miR-67-3p-R	CAGGTCCAGTTTTTTTTTTTTTTTCTACT
	miR-71-5p-F	CGCAGCGGTACGATCGCGGCG
	miR-71-5p-R	CAGGTCCAGTTTTTTTTTTTTTTTGATATCC
	miR-90-3p-F	CGCAGTGATGTTGTTTGAATG
	miR-90-3p-R	CAGGTCCAGTTTTTTTTTTTTTTTAGGGG
	miR-791-3p-F	CGCAGTTTGGCACTCCGAGATAGGCAA
	miR-791-3p-R	CAGGTCCAGTTTTTTTTTTTTTTTGCTT
	Lsy-6-3p-F	CGCAGTTTTGTATGAGACGCAATTCGA
	Lsy-6-3p-R	CAGGTCCAGTTTTTTTTTTTTTTTCGAAA
	miR-60-3p-F	CGCAGTATTATGCACATTTTCT
	miR-60-3p-R	CAGGTCCAGTTTTTTTTTTTTTTTGAAC
	miR-83-3p-F	CGCAGTAGCACCATATAAAT
	miR-83-3p-R	CAGGTCCAGTTTTTTTTTTTTTTTACTG
	miR-35-3p-F	CAGTCACCGGGTGGAACATA
	miR-35-3p-R	CAGGTCCAGTTTTTTTTTTTTTTTACTGC
	miR-234-3p-F	CGCAGTTATTGCTCGAGAATA
	miR-234-3p-R	CAGGTCCAGTTTTTTTTTTTTTTAAGGG

Cell-type-specific profiling of loaded miRNAs from *Caenorhabditis elegans* reveals spatial and temporal flexibility in Argonaute loading

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Supplementary Note 1:

Identification of novel miRNA candidates in *C. elegans*

The sensitivity of detection combined with the cell-type-specific resolution of our approach enabled us not only to identify a large set of isomiRs that were loaded into silencing complexes, but also to discover 37 new candidate miRNA loci in this set (~15% of the total number previously identified in *C. elegans*, Supplementary Fig. 13 and 14) that satisfied stringent criteria (including nucleotide length) and mapped across all chromosomes in the *C. elegans* genome (Supplementary Data 3, Supplementary Data 5 and Supplementary Fig. 13). Importantly, aside from loading into AGO proteins, each of the mature miRNA sequences mapped to loci predicted to form low energy hairpin secondary structures typical of pri-miRNAs (Supplementary Fig. 13 and 14). Interestingly, the novel candidate miRNAs also demonstrated a high degree of cell- and AGO-type specificity, suggesting that they have specialized activities, which in addition to their relatively low read count levels may have hindered their discovery until now (Supplementary Fig. 13 and 14, Supplementary Data 3 and 5). To further validate their authenticity and determine their expression patterns, we generated transgenic strains carrying transcriptional fluorescent reporters (*mir-promoter^{Akb}::mCherry*) of three examples of novel miRNA candidates that were loaded into AGOs in either the intestine, BWM, or nervous system. Each of the miRNA reporters exhibited an expression pattern that was consistent with its spatially unique AGO interactions (Supplementary Fig. 15; intestine, neuron and BWM respectively). This confirmed that the putative *cis*-regulatory regions selected to drive the reporter were transcriptionally functional, indicative of an expressible miRNA locus that matched their spatial loading patterns.