

Specific microRNAs Regulate Heat Stress Responses in *Caenorhabditis elegans*

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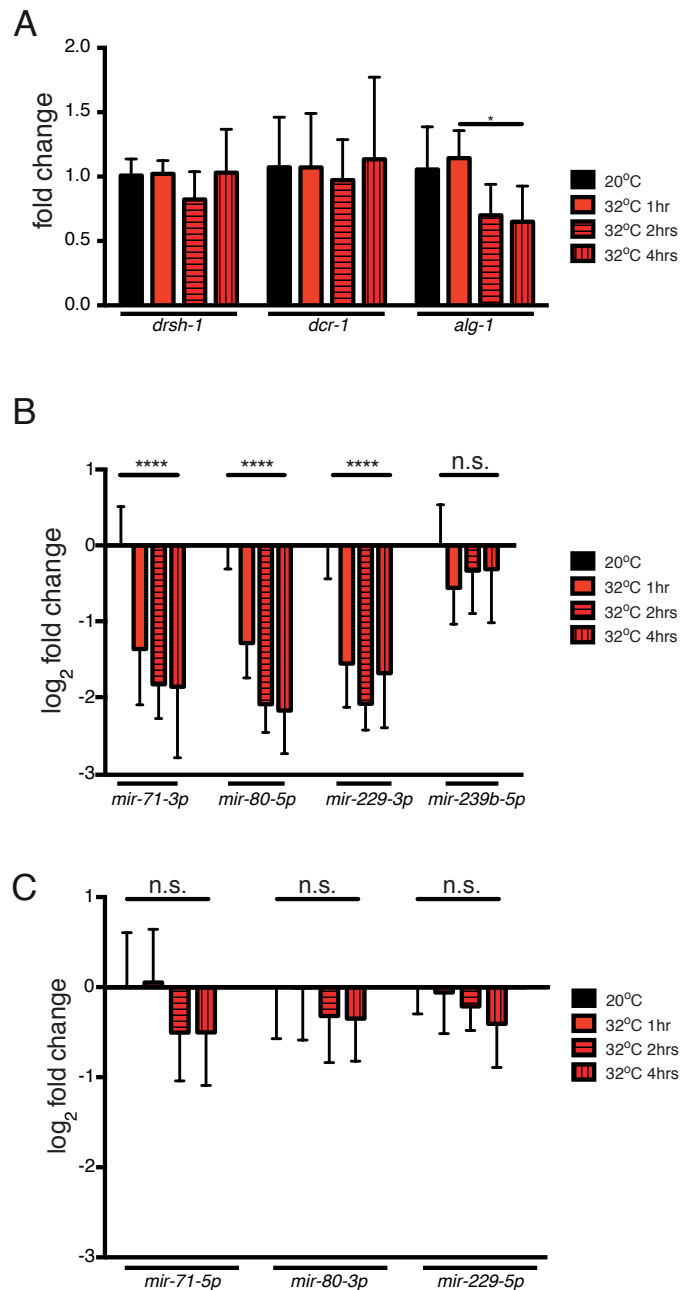


Figure S1. qRT-PCR Analysis

(A) miRNA biogenesis machinery factors *drsh-1*, *dcr-1* and *alg-1* are not markedly thermoregulated. Data was normalized to two reference mRNAs namely *gpd-1* and *gpd-4* and are presented as mean fold changes \pm SEM. Statistical significance was assessed by two-way ANOVA analysis followed by Tukey's multiple comparisons test. * $p < 0.05$.

(B-C) *mir-71-3p*, *mir-80-5p*, *mir-229-3p* are rapidly thermoregulated at 32°C, whereas *mir-239b-5p* is not (B). miRNAs opposite to those shown to be regulated in RNA sequencing

are not thermoregulated (C). *mir-239b3p* is not presented on the figure as it was below the level of detection (C). Data was normalized to two reference miRNAs namely *mir-86-5p* and *mir-794-5p* and are presented as log₂ means ± SEM. Statistical significance was assessed by two-way ANOVA analysis followed by Tukey's multiple comparisons test. ****p<0.0001, n.s - not significant.

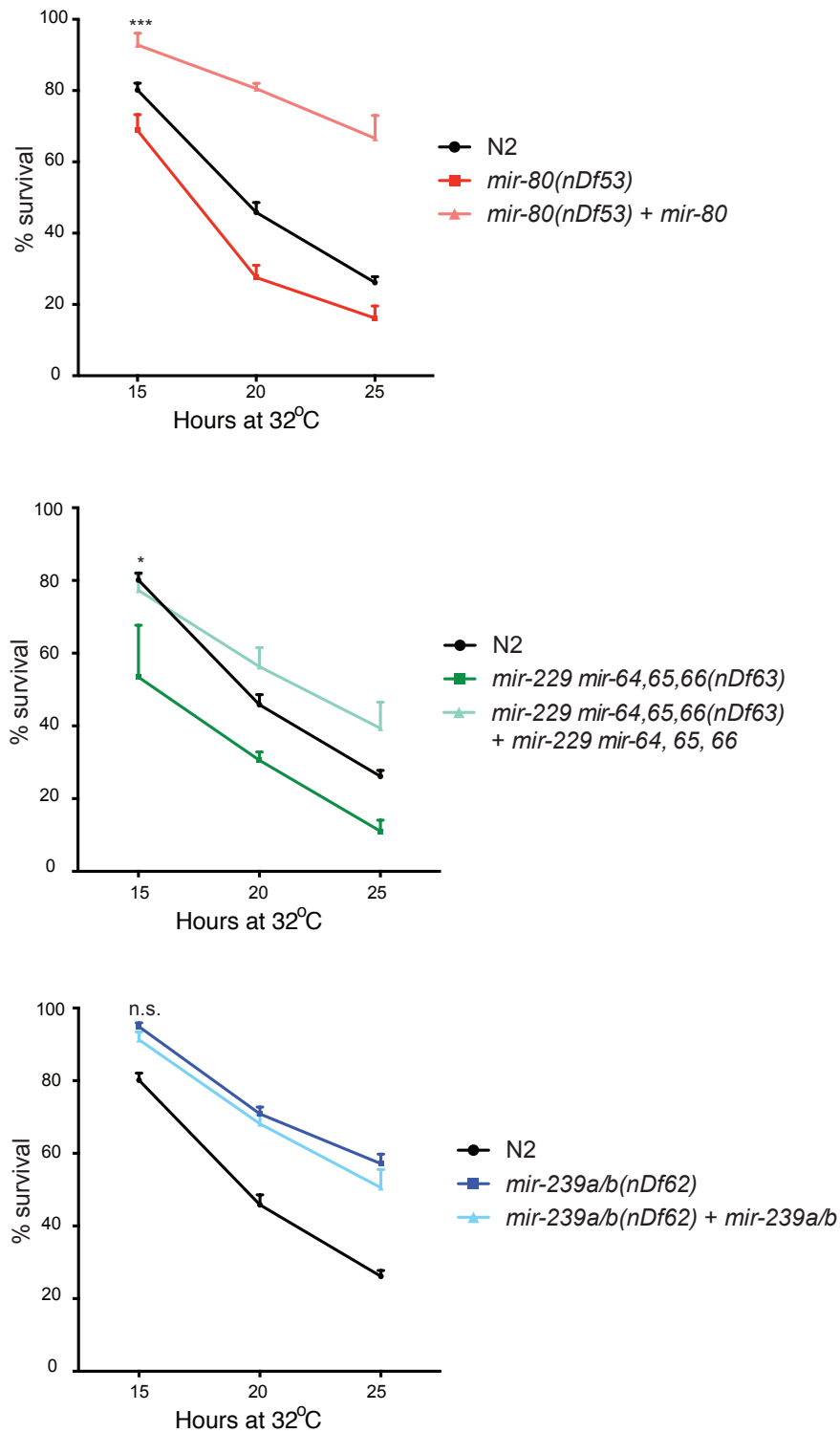


Figure S2. Rescue of miRNA Heat Stress Phenotypes

(a-c) Heat stress phenotypes of *mir-80(nDf53)* and *mir-229, 64-66(nDf63)* are rescued by transgenic expression of the respective miRNAs under the control of their own regulatory sequences. We were unable to rescue the *mir-239a/b(nDf62)* heat resistance phenotype.

One representative rescue line is shown for each, three lines were scored - data not shown. Data are presented as means \pm SEM and statistical significance was assessed by 2way ANOVA followed by Tukey's multiple comparison test. $n > 110$, * $p < 0.05$, *** $p < 0.001$, n.s. - not significant.

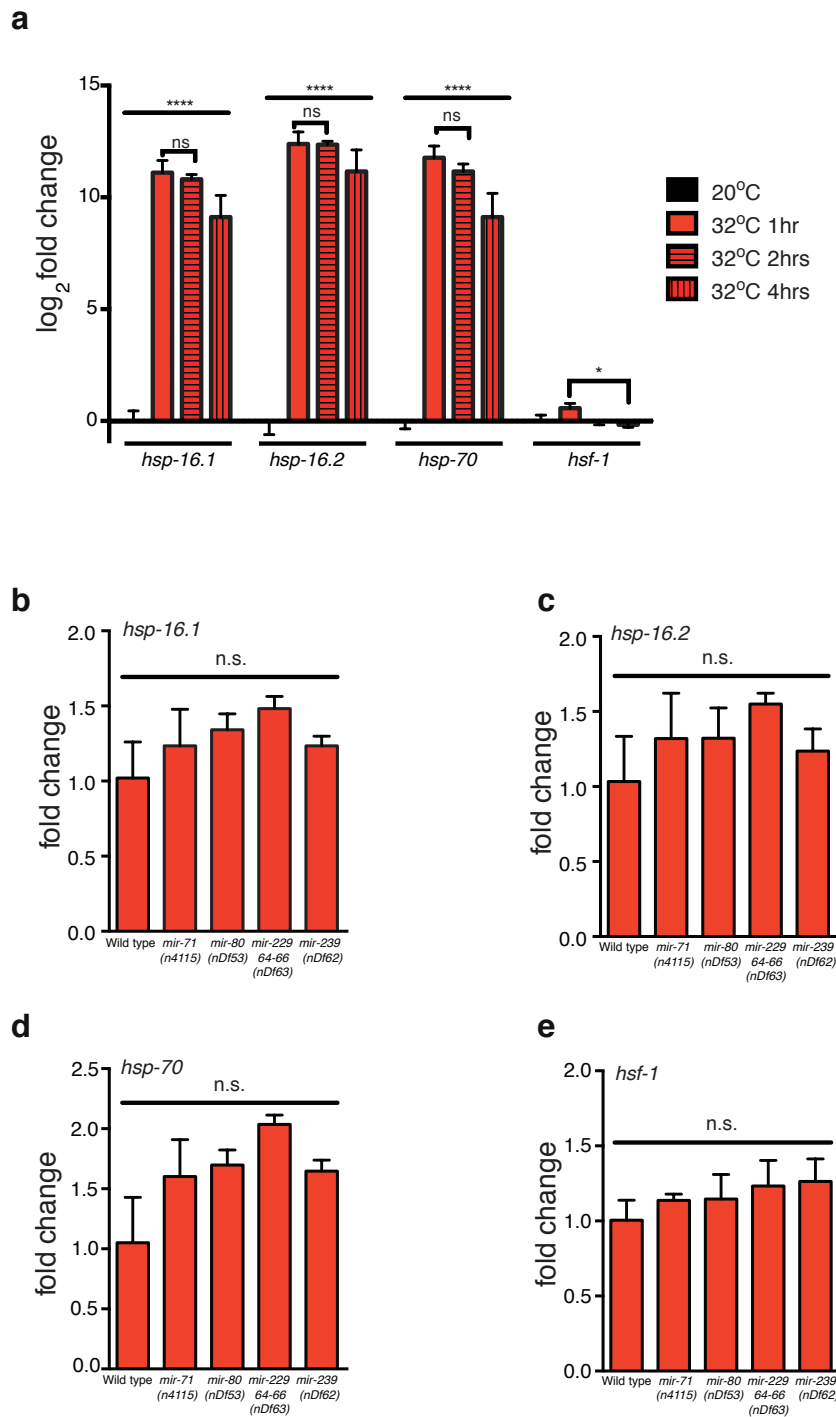


Figure S3. Molecular Analysis of Thermoregulated miRNA Function

(a) qRT-PCR analysis showing relative expression levels of *hsp-16.1*, *hsp-16.2*, *hsp-70* and *hsf-1* in wild type animals at 20°C and after a 32°C heat stress for 1, 2 and 4 hrs.

(b-e) qRT-PCR analysis showing relative expression levels of *hsp-16.1*, *hsp-16.2*, *hsp-70* and *hsf-1* in TRM mutant backgrounds.

Data are normalized to two reference genes, *gpd-1* and *gpd-4* in A; *pmp-3* and *cdc-42* in (a-e); and are presented as log₂ means ± SEM (a) and means ± SEM (b-e). Statistical significance was assessed by two-way ANOVA analysis followed by Tukey's multiple comparisons test. Three independent biological replicates were used for RNA isolation.

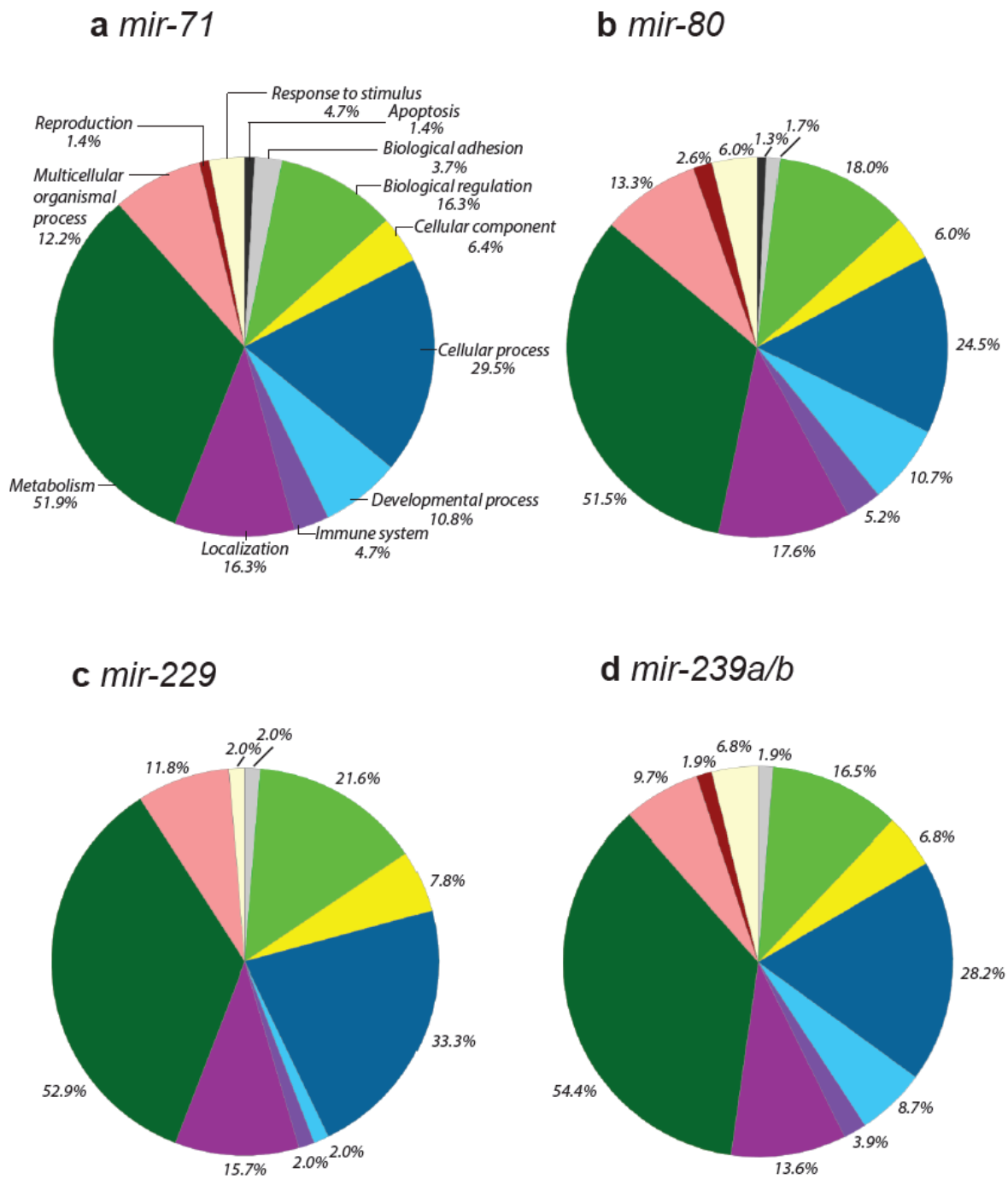


Figure S4. The majority of predicted targets for *mir-71*, *mir-80*, *mir-229* and *mir-239* are genes involved in metabolic processes

(a-d) Pie charts of the broad biological functions of predicted miRNA targets for *mir-71*, *mir-80*, *mir-229* and *mir-239*. Biological functions were categorized using the PANTHER classification system and results are depicted as percent of process hits against total number of hits, a number of the predicted targets fall into multiple categories.

miRNAs regulated by heat stress
Read counts >150 and fold change >1.9

miRNA	Fold change	Normalized read counts in 35°C	Normalized read counts in 20°C	Sequence
<i>mir-83-3p</i>	4.901	1387.692	339.952	UAGCACCAUUAUAAAUUCAGUAA
<i>mir-239b-5p</i>	3.772	13328.593	4300.273	UUUGUACUACACAAAAGUACUG
<i>mir-49-3p</i>	3.398	480.841	167.802	AAGCACACGAGAAAGCUGCAGA
<i>mir-249-3p</i>	2.270	454.128	241.705	UCACAGGACUUUUUGAGCGUUGCC
<i>mir-1820-5p</i>	2.101	538.486	311.261	UUUUGAUUGUUUUUCGAUGAUUGCG
<i>mir-77-5p</i>	-7.089	600.349	5312.306	GAUGGUUGUGCUCUGAGGAAAU
<i>mir-81-5p</i>	-6.644	185.588	1593.691	CGGUUUUCACCGUGAUCUGAGA
<i>mir-86-3p</i>	-5.618	281.193	2006.678	CUGGGCUCAGAUUCGCUUAGGC
<i>mir-52-3p</i>	-4.789	239.014	1460.666	CACGUUACAAGAAAGGUAAGC
<i>mir-237-3p</i>	-3.599	206.677	952.041	CUGUCGAGUUUUGUCAAGGACC
<i>mir-80-5p</i>	-3.574	4501.915	19706.831	AGCUUUCGACAUGAUUCUGAAC
<i>mir-54-5p</i>	-3.269	1259.748	5085.381	AGGAUUGAGACGACGAGAACA
<i>mir-58-5p</i>	-3.251	627.062	2537.039	UGCCCUACUCUUCGCAUCUCA
<i>mir-84-3p</i>	-2.561	392.265	1258.955	CACAAUGUUUCAACUACUCGGC
<i>mir-74-5p</i>	-2.248	160.280	462.544	CGGGCUUCAUCUCUUUCCAGC
<i>mir-799</i>	-2.128	178.558	485.150	UGAACCCUGAAUAAAGCUAGUGG
<i>mir-229-3p</i>	-2.105	3420.724	8830.079	AGAAAGGUUUCGGGUGUCAUAG
<i>mir-71-3p</i>	-1.959	2056.933	4954.095	UAUCACUAUUCUGUUUUUCGC

miRNAs from the opposite strands to those regulated by heat stress

miRNA	Fold change	Normalized read counts in 35°C	Normalized read counts in 20°C	Sequence
<i>mir-80-3p</i>	1.632	104000.991	77189.291	UGAGAUCAUUAGUUGAAAGCCGA
<i>mir-239b-3p</i>	n.a.	2.811	0.000	GCACUUUUGUGGUGUCAAAAA
<i>mir-54-3p</i>	1.396	6730.377	5887.009	UACCCGUAAUCUUAUAAUCCGAG
<i>mir-1820-3p</i>	1.354	5.623	4.347	AACCAUUGUAAACAUAACAAGA
<i>mir-84-5p</i>	1.334	15451.608	14112.824	UGAGGUAGUAUGUAAUUAUGUAGA
<i>mir-81-3p</i>	1.309	501027.178	461700.298	UGAGAUCUUCGUGAAAGCUAGU
<i>mir-74-3p</i>	1.270	376.799	365.166	UGAGAUCGUUCAGUACGGCAAU
<i>mir-52-5p</i>	1.238	20231.905	19899.848	CACCCGUACAUAUGUUUCCGUGCU
<i>mir-49-5p</i>	-1.573	172.934	345.169	CGCAGUUUGUUGUGAUGUCUCC
<i>mir-83-5p</i>	-1.470	14.059	33.908	ACUGAAUUUAUGUGUGUACUUGA
<i>mir-77-3p</i>	-1.218	8160.248	12134.838	UUCAUCAGGCCAUAGCUGUCCA
<i>mir-86-5p</i>	-1.218	6436.529	6457.365	UAAGUGAAUGCUUUGCCACAGUC
<i>mir-237-5p</i>	-1.192	98214.020	99827.020	UCCCUAGAGAAUUCGAAACAGCU
<i>mir-249-5p</i>	n.a.	0.000	0.860	AGCAACGCACAACGUCUUCUGUG
<i>mir-58-3p</i>	-1.125	349938.852	375488.779	UGCCCUACUCUUCGCAUCUCA
<i>mir-71-5p</i>	-1.123	10685.370	11601.868	UGAAAGACAUGGGUAGUGAGACG
<i>mir-229-5p</i>	-1.096	38831.479	43064.460	AAUGACACUGGUUAUCUUUCCAUCCG
<i>mir-799</i>	n.a.	n.a.	n.a.	n.a.

Table S1. miRNA Sequencing

Table listing miRNAs with >150 normalized read counts, regulated >2-fold according to regression analysis and corresponding miRNAs originating from opposite strands, lacking regulation in heat stress. Data analysis was performed by miRDeep2^{31,32}.

2 trials combined							
Strain	Number of animals (n)	Median lifespan (days)	Mean lifespan (days)	Maximal lifespan (days)	p value against N2	% of mean lifespan change against N2	comments
N2	97	10	10.8	19		NA	
<i>mir-71</i>	91	8	7.6	13	< 0.0001	29.63	p value based on survival curve
<i>mir-80</i>	101	9	10.3	17.5	n.s.	4.63	
<i>mir-229</i>	114	7	8.3	16	< 0.0001	23.15	p value based on survival curve
<i>mir-239</i>	98	10	10.1	16	n.s.	6.48	
trial 1							
N2	42	8	9.6	19		NA	
<i>mir-71</i>	41	7	7.1	12		26.04	
<i>mir-80</i>	52	9	9.4	16		2.08	
<i>mir-229</i>	57	7	7.5	17		21.88	
<i>mir-239</i>	42	8	9.3	14		3.12	
trial 2							
N2	55	11	11.7	19		NA	
<i>mir-71</i>	50	8	8	14		31.62	
<i>mir-80</i>	49	12	11.3	19		3.42	
<i>mir-229</i>	57	8	8.8	15		24.79	
<i>mir-239</i>	56	11	11.2	18		4.27	

Table S2. Lifespan Analysis at 25°C

C. elegans miRNA mutant strains backcrossed to N2 three times were assayed at 25°C.

Life span analysis was repeated twice (n>90-118) with similar effects. Mean life span values and statistical analyses of life span assays are shown. Data are presented as means ± SEM and statistical significance was assessed by 2way ANOVA followed by Tukey's multiple comparison test. n>110, *p<0.05, **p<0.01, ****p<0.0001, n.s. - not significant.

Target predictions for miRNAs regulated in RNAseq

miRNA	PicTar score	Description
mir-71-3p		
vha-15	7,0828	required for systemic RNAi, probably during endocytotic RNAi uptake
F55C12.1	5,547	-
mec-2	4,1877	mechanical stimuli
pmk-3	4,0565	MAPK pathways are involved in stress response and innate immunity
che-12	3,8608	Involved in converting environmental stimuli into neuronal activity
otub-1	3,5318	unknown
R07G3.8	3,5134	unknown
Y9C2UA.1	3,4858	unknown
tram-1	3,2744	unknown
saps-1	3,2176	required for proper spindle positioning in embryos
T10B9.9	3,1354	unknown
Y106G6G.4	3,1043	Homology to cell wall mannoprotein
Y57G11C.38	3,0901	unknown
mlc-3	3,0719	activity is required for both embryonic and postembryonic development
nol-5	3,0548	Homology to protein involved in pre-rRNA processing
vha-12	3,0001	suppresses necrotic neurodegeneration
diat-1	2,9843	-
ZK652.8	2,9652	unknown
nhr-10	2,717	predicted as a nuclear hormone receptor
nos-2	2,6601	nos-2 affects incorporation of primordial germ cells into the somatic gonad
flp-32	2,4339	unknown
RIT3P.239	2,3482	RIT3P.239
nmy-1	2,2331	required for embryonic elongation and establishment of normal body morphology
F42G8.10	1,9847	unknown
T14B1.1	1,8882	unknown
F43G9.3	1,6074	homology to mitochondrial carrier protein
C10G11.6	1,5017	unknown
wdfy-2	1,4856	essential for wild-type levels of endocytosis
dnj-1	1,3428	homology of protein which is a co-chaperone for Hsp40
F49H12.5	1,1724	
mir-80-5p		
rpl-10	4,4451	required for embryonic viability
hpo-26	2,8216	unknown
lin-14	2,5154	required for specifying the division timings of cells during postembryonic development
rpl-17	2,3675	activity is required for embryonic and germline development
mdt-28	2,2071	unknown
pab-2	1,529	pab-2 encodes a polyadenylate-binding protein 1 homolog
rsp-7	1,0777	required for constitutive splicing and influence alternative splicing regulation.
mir-229-3p		
cutl-16	7,2138	unknown
peb-1	5,9701	required for proper morphogenesis of the pharynx, vulva
unc-130	3,2383	member of the forkhead domain family of transcription factors
ran-1	3,2085	transport of proteins into nucleus
eif-3.C	3,0867	encodes a putative c subunit of translation initiation factor 3 that is required for fertility
sqv-5	2,8202	encodes a chondroitin synthase that both initiates and elongates chondroitin chains
sas-5	2,6646	required for centrosome duplication in <i>C. elegans</i>
C02D5.2	2,1529	unknown
pat-9	1,8232	nuclear zinc finger protein critical for the assembly of muscle attachments
F17C11.10	1,7794	unknown
Iron-1	1,7783	homology to leucine-rich repeat-containing G-protein coupled receptor 5
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rpa-4	7,3299	unknown
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K11D9.3	5,4969	unknown
mnk-1	5,3609	mnk-1 encodes the <i>C. elegans</i> ortholog of the Mnk MAP kinase-interacting kinase
npri-3	4,7594	unknown
C15C8.7	4,5803	unknown
Y53F4B.14	4,3808	unknown
R151.4	4,1425	homology to Fibroblast growth factor receptor
hecd-1	4,0372	HECD1 ubiquitin ligase homolog
hsp-12.1	4,0325	member of the small heat shock family of proteins
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F49H12.5	1,1724	
mir-80-5p		
rpl-10	4,4451	required for embryonic viability
hpo-26	2,8216	unknown
lin-14	2,5154	required for specifying the division timings of cells during postembryonic development
rpl-17	2,3675	activity is required for embryonic and germline development
mdt-28	2,2071	unknown
pab-2	1,529	pab-2 encodes a polyadenylate-binding protein 1 homolog
rsp-7	1,0777	required for constitutive splicing and influence alternative splicing regulation.
mir-229-3p		
cutl-16	7,2138	unknown
peb-1	5,9701	required for proper morphogenesis of the pharynx, vulva
unc-130	3,2383	member of the forkhead domain family of transcription factors
ran-1	3,2085	transport of proteins into nucleus
eif-3.C	3,0867	encodes a putative c subunit of translation initiation factor 3 that is required for fertility
sqv-5	2,8202	encodes a chondroitin synthase that both initiates and elongates chondroitin chains
sas-5	2,6646	required for centrosome duplication in <i>C. elegans</i>
C02D5.2	2,1529	unknown
pat-9	1,8232	nuclear zinc finger protein critical for the assembly of muscle attachments
F17C11.10	1,7794	unknown
Iron-1	1,7783	homology to leucine-rich repeat-containing G-protein coupled receptor 5
T23B12.11	1,646	unknown
byn-1	1,4897	homolog of mammalian BYSTIN-LIKE
F13D12.9	1,3538	unknown
C34F6.7	1,1971	unknown
ncs-2	1,0461	Neuronal Calcium Sensor family
mir-239b-5p		
F53C11.4	7,5134	unknown
rpa-4	7,3299	unknown
nlp-24	6,5062	Neuropeptide-Like Protein
sta-2	5,6183	unknown
K11D9.3	5,4969	unknown
mnk-1	5,3609	mnk-1 encodes the <i>C. elegans</i> ortholog of the Mnk MAP kinase-interacting kinase
npri-3	4,7594	unknown
C15C8.7	4,5803	unknown
Y53F4B.14	4,3808	unknown
R151.4	4,1425	homology to Fibroblast growth factor receptor
hecd-1	4,0372	HECD1 ubiquitin ligase homolog
hsp-12.1	4,0325	member of the small heat shock family of proteins
fat-1	4,0217	encodes an omega-3 fatty acyl desaturase
pfn-1	3,9521	required for embryonic cytokinesis
mdt-15	3,8211	MDT-15 participates in basal and fasting-induced transcription of numerous other metabolic genes

Target predictions for miRNAs regulated in RNAseq

miRNA	PicTar score	Description
mir-71-3p		
vha-15	7,0828	required for systemic RNAi, probably during endocytotic RNAi uptake
F55C12.1	5,547	-
mec-2	4,1877	mechanical stimuli
pmk-3	4,0565	MAPK pathways are involved in stress response and innate immunity
che-12	3,8608	Involved in converting environmental stimuli into neuronal activity
otub-1	3,5318	unknown
R07G3.8	3,5134	unknown
Y9C2UA.1	3,4858	unknown
tram-1	3,2744	unknown
saps-1	3,2176	required for proper spindle positioning in embryos
T10B9.9	3,1354	unknown
Y106G6G.4	3,1043	Homology to cell wall mannoprotein
Y57G11C.38	3,0901	unknown
mlc-3	3,0719	activity is required for both embryonic and postembryonic development
nol-5	3,0548	Homology to protein involved in pre-rRNA processing
vha-12	3,0001	suppresses necrotic neurodegeneration
dlat-1	2,9843	-
ZK652.8	2,9652	unknown
nhr-10	2,717	predicted as a nuclear hormone receptor
nos-2	2,6601	nos-2 affects incorporation of primordial germ cells into the somatic gonad
flp-32	2,4339	unknown
RIT3P.239	2,3482	RIT3P.239
nmy-1	2,2331	required for embryonic elongation and establishment of normal body morphology
F42G8.10	1,9847	unknown
T14B1.1	1,8882	unknown
F43G9.3	1,6074	homology to mitochondrial carrier protein
C10G11.6	1,5017	unknown
wdfy-2	1,4856	essential for wild-type levels of endocytosis
dnoj-1	1,3428	homology of protein which is a co-chaperone for Hsp40
F49H12.5	1,1724	
mir-80-5p		
rpl-10	4,4451	required for embryonic viability
hpo-26	2,8216	unknown
lin-14	2,5154	required for specifying the division timings of cells during postembryonic development
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R151.4	4,1425	homology to Fibroblast growth factor receptor
hecd-1	4,0372	HECD1 ubiquitin ligase homolog
hsp-12.1	4,0325	member of the small heat shock family of proteins
fat-1	4,0217	encodes an omega-3 fatty acyl desaturase
pfn-1	3,9521	required for embryonic cytokinesis
mdt-15	3,8211	MDT-15 participates in basal and fasting-induced transcription of numerous other metabolic genes

Table S3. miRNA Target Predictions

Target predictions for: 1) Thermoregulated miRNAs and 2) miRNAs on the opposite strand to that regulated by heat stress.

Transcript	qRT-PCR oligonucleotides	
	Forward primer	Reverse primer
<i>mir-71-3p</i>	cgagatcactattctgt	ccagtttttttttgcga
<i>mir-71-5p</i>	agtgaaagacatgggtagtg	tcacgttttttttttcgtc
<i>mir-80-3p</i>	cgagtgagatcattggtg	gttttttttttttcggcttc
<i>mir-80-5p</i>	cagagtttcgacatgattc	tcacgttttttttttcag
<i>mir-229-3p</i>	gagaaaggtatcgggtgtc	tcacgttttttttttcagtc
<i>mir-229-5p</i>	gaatgacacatggtatcttcc	tcacgttttttttttcgatg
<i>mir-239b-3p</i>	gcaggcactttgtgg	tcacgttttttttttcgac
<i>mir-239b-5p</i>	cgagtttgactacaca	gtccagtttttttttttcagt
<i>mir-86-5p</i>	agtaagtgatcttgcaca	gtccagtttttttttttgc
<i>mir-794-5p</i>	cagtgagtgatctcgtgtg	gtccagttttttttttttgagga
<i>drsh-1</i>	ccactcggatglatgac	gtccaacactcatcaggc
<i>dcr-1</i>	tagtgattagtcgctctcg	caccaattccaagcttggc
<i>alg-1</i>	tcctggaggaaactatcaaac	gctgatactcggattaactc
<i>hsp-16.1</i>	ggctcagatggaacgtcaa	tgccaactttgatcattgta
<i>hsp-16.2</i>	tgcaaatctcctcatctgag	tggttaaacctgagacgttga
<i>hsp-70</i>	cggtattatcaaaatggaaggtt	tacgagcgctgactctt
<i>hsf-1</i>	tcagacagttgaatgacgg	ctgactcgtctctgctcg
<i>gpd-1</i>	atcacgtgttttaacgcac	atgagtcctcgtatgacccg
<i>gpd-4</i>	tcacgtatttcaacgcac	atgagtcctcgtatgacccg

Genotyping oligonucleotides					
Mutant allele	Forward primer	Reverse primer	Amplicon size (bp)	Reverse primer - wild type band only	Amplicon size (bp)
MT12993 <i>mir-71</i> (<i>n4115</i>)	ggaaggaacagttacatagtc	cactggatgacgttagttaagc	wild type-520 mutant-460	NA	NA
MT13949 <i>mir-80</i> (<i>nDf53</i>)	gcatccaatcgagcgcag	NA	NA	cacacggacggactgcc	592
MT16494 <i>mir-229, mir-64-66</i> (<i>nDf63</i>)	cgtaaatcgccacattgcc	NA	NA	gggatccacacagagaatc	543
MT15312 <i>mir-239a/b</i> (<i>nDf62</i>)	ggagtagcaccagtg	NA	NA	ggtacagtgccaagaagacaca	876
Rescue PCR oligonucleotides					
Mutant allele	Forward primer	Reverse primer	Amplicon size (bp)		
RJP999 <i>mir-229, mir-64-66</i> (<i>nDf63</i>)	ggagtcgagccaatattctagc	ggagtcgagccaatattctagc			3396
RJP1014 <i>mir-239a/b</i> (<i>nDf62</i>)	catcgctgctctctcc	ctaatgcaacgctctcaa			2208
RJP936 <i>mir-80</i> (<i>nDf53</i>)	gattcccaagaaccattgc	ggatcagggagacatcac			2377

Table S4. List of Oligonucleotides Used in This Study

Strain name	miRNA gene	Chromosome	allele	# outcrossed
MT12993	<i>mir-71</i>	I	<i>n4115</i>	0x
RJP907	<i>mir-71</i>	I	<i>n4115</i>	3x
MT13949	<i>mir-80</i>	III	<i>nDf53</i>	0x
RJP936	<i>mir-80</i>	III	<i>nDf53</i>	3x
MT16494	<i>mir-229, mir-64-66</i>	III	<i>nDf63</i>	2x
RJP999	<i>mir-229, mir-64-66</i>	III	<i>nDf63</i>	5x
MT15312	<i>mir-239a/b</i>	X	<i>nDf62</i>	0x
RJP1014	<i>mir-239a/b</i>	X	<i>nDf62</i>	3x
RJP1742	rpEx741 [mir-80;myo-2::mCherry]; mir-80(<i>nDf53</i>)III	III	<i>nDf53</i>	n/a
RJP1661	rpEx710 (mir-229;myo-2::mCherry);mir-229, 64, 65, 66(<i>nDf63</i>)III	III	<i>nDf63</i>	n/a
RJP1741	rpEx740 [mir-239a mir-239b;myo-2::mCherry]; mir-239a mir-239b (<i>nDf62</i>) X	X	<i>nDf62</i>	n/a

Table S5. List of Strains Used in This Study