Supplementary Data

The microRNA miR-124 controls gene expression in the sensory nervous system of *Caenorhabditis elegans*

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I. Supplementary Text

Analysis of t1 nucleotide conservation in nematodes and vertebrates

We identified conserved 3′UTR matches to the seed sequence (positions 2-7) of highly conserved *C. elegans* miRNA families (TargetScan, Release 5.1) (1) as described in the Methods section of the main text. For each conserved seed match we assessed whether the identity of the *C. elegans* t1 nucleotide was conserved in *C. briggsae*, *C. remanei* and *C. brenneri*. We then used a one-sided binomial test to assess whether, among conserved t1 nucleotides, any given nucleotide occurred more often than expected, based on trinucleotide background frequencies obtained from the 3′UTRs of genes that included at least one conserved seed match against the miRNA family under investigation. The rationale of this analysis was based on the assumption that conserved seed matches were mostly genuine target sites. To further enrich the considered sites for functional target sites we repeated the analysis for the subset of conserved seed matches that were also flanked by a conserved t8 match.

We repeated the analysis for highly conserved miRNA families in vertebrates using multiple sequence alignments of human 3′UTRs with mouse, rat, dog and chicken that were extracted from a 23 species multiple alignment of 3′UTR sequences downloaded from the TargetScan website (2). For site conservation, human sites were required to overlap with sites in the aligned orthologous sequences from all four other species.

Results are shown in Supplementary Figures 4 and 5. As reported previously, in vertebrates there appeared to be a strong preference for an adenosine among

conserved t1 nucleotides for most miRNA families (1) (Supplementary Figure 5). Some individual miRNA families showed a preference for nucleotides other than adenosine when considering conserved seed matches. However, in many cases this apparent preference for other nucleotides disappeared when considering conserved seed matches flanked by a conserved t8 match, suggesting the observation might be due to seed matches that are not genuine target sites. In nematodes a preference for a t1 adenosine appeared to be less prevalent (Supplementary Figure 4). However, the results of the more informative analysis of conserved seed matches flanked by a conserved t8 match were difficult to interpret as the number of such sites for individual miRNA families was small.

Supplementary Figure Legends

Supplementary Figure 1. Expression of host gene *trpa-1* is not abrogated in *mir-124* mutants

trpa-1 expression in wild-type (WT) and *mir-124* mutant animals as assessed by qRT-PCR with oligos that span the intron that includes the miRNA. miR-124 was amplified as a control. *trpa-1* and miR-124 values were normalized to *ama-1* and miR-52 respectively. Color bars correspond to the arithmetic mean from three biological replicates, error bars indicate standard error of the mean.

Supplementary Figure 2. Quality control of GFP sorting

GFP mRNA expression in sorted GFP+ and GFP- cells from wild-type (WT) and *mir-124* mutant animals, as assessed by qRT-PCR. Values were normalized to *ama-1*. Green bars correspond to the arithmetic mean from three biological replicates, error bars indicate standard error of the mean. GFP mRNA was undetectable in GFP- cells

from wild-type and *mir-124* mutant animals.

Supplementary Figure 3. Assessment of miR-124 expression in sorted cells

Expression of mature miR-124 in sorted GFP+ and GFP- cells from wild-type (WT) and *mir-124* mutant animals, as assessed by qRT-PCR. Values were normalized to miR-52, a ubiquitous miRNA. Blue bars correspond to the median from three biological replicates, error bars represent the range of data values. miR-124 is enriched ~5 times in *mir-124* promoter::GFP wild-type cells confirming that *mir-124* promoter::GFP recapitulates endogenous *mir-124* expression.

Supplementary Figure 4. t1 nucleotide conservation in nematodes

Shown are results for conserved seed matches (left) and conserved matches to miRNA positions 2-8 (right). Heatmaps of negative $\log_{10}$ transformed *P*-values were based on a one-sided binomial test, testing for overrepresentation of A, C, G and U among conserved t1 nucleotides. The percentage of t1 nucleotides that are conserved is shown in the left margin. The number of conserved t1 nucleotides and the miRNA sequence at positions 1-8 are given in the right margin. miRNA families were ordered according to percentage of t1 conservation.

Supplementary Figure 5. t1 nucleotide conservation in vertebrates

See explanations for Supplementary Figure 4.

II. Supplementary Tables

Supplementary Table 1. Strains	
Strain	Genotype
N2 (Bristol)	wild type
MT13292	*mir-124 (n4255) IV (3)*
SX621	*mjIs27 V [pmir-124::GFP + lin-15 (+)] ; lin-15AB (n765) X*
SX620	*mir-124 (n4255) IV ; mjIs27 V ; lin-15AB(n765) X*
SX392	*mjEx142 [pmir-124::mcherry]*
SX324	*mir-124 (n4255) IV ; lin-15B (n765) kyIs141 [osm-9::GFP5 + lin-15 (+)] X*
SX322	*kyIs140 [str-2::GFP + lin-15 (+)] I ; mir-124 (n4255) IV*
SX323	*mir-124 (n4255) IV ; lin-15B (n765) kyIs105 [str-1::GFP + lin-15 (+)] X*
SX325	*mir-124 (n4255) IV ; egIs [pdat-1::GFP]*
SX344	*mir-124 (n4255) IV ; lin-15B(n765) X; adEx1262[gcy-5::GFP lin-15(+)].*
SX459	*egIs1 [pdat-1::GFP] ; mjEx142 [pmir-124::mcherry]*
SX460	*mjIs27 [pmir-124::GFP + lin-15 (+)] , oyIs44 [odr-1::RFP] V*
SX458	*mjEx142 [pmir-124::mcherry] , adEx1262 [gcy-5::GFP + lin-15(+)]*
SX1019	*mjEx142 [pmir-124::mcherry] , kyIs141 [osm-9::GFP5 + lin-15 (+)] X*
SX1020	*mjEx142 [pmir-124::mcherry] , kyIs39 [sra-6::GFP + lin-15 (+)]*
CX3716	*lin-15B (n765) kyIs141 [osm-9::GFP5 + lin-15 (+)] X (4)*
CX3695	*kyIs140 [str-2::GFP + lin-15 (+)] I (5)*
PY2417	*oyIs44 [odr-1::RFP] V (6)*
CX3553	*lin-15B (n765) kyIs105 [str-1::GFP + lin-15 (+)] X (7)*
BZ555	*egIs [pdat-1::GFP] (8)*
CX3465	*kyIs39 [sra-6::GFP + lin-15 (+)] (9)*
DA1262	*lin-15B(n765) X; adEx1262[gcy-5::GFP lin-15(+)] (10)*

Supplementary Table 2. Identified miR-124 expressing neurons		
NEURON	**NEURON FUNCTION**	**NEURONAL MARKER**
AWA	Volatile chemotaxis (11)	*osm-9::GFP*
AWC	Volatile chemotaxis, navigation (11)	*str-2::GFP, odr-1::RFP*
AWB	Volatile avoidance (7)	*str-1::GFP, odr-1::RFP*
ADE, PDE	Mechanosensory (12)	*dat-1::GFP*
ASE	Water soluble chemotaxis (13)	*gcy-5::GFP (ASER)*
ASG, ASJ, ADL	Dauer entry (ASG) and exit (ASJ) (14), Avoidance from Cd^{2+} and Cu^{2+} (15)	*osm-9::GFP*
ASH, ASI, PVQ	Osmotic avoidance (ASH) (16), chemotaxis to lysine (ASI) (13)	*sra-6::GFP*
ASK	Chemotaxis to lysine (13)	*osm-9::GFP*
PHA, PHB	Avoidance (antagonistic) (17)	*osm-9::GFP*
IL1	Mechanosensation (18)	-
PVD *	Stretch-sensing mechanosensation (19)	-
I6 *	Unknown	-
HSN * (weak expression)	Egg laying (20)	-
Identified miR-124 expressing neurons with known functions and neuronal cell fate markers used for cell identification and characterisation in *mir-124* mutants. All neurons except those marked with an asterisk are ciliated.		

Supplementary Table 3. Oligos used for qRT-PCR	
Universal miRNA primer	CTCAAGTGTCGTGGAGTCGGCAA
miR-124 Fwd	ACACTCCAGCTGGGTAAGGCACGCGGTGAA
miR-124 Rev	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGTGGCATTC
miR-52 Fwd	ACACTCCAGCTGGGCACCCGTACATATGTT
miR-52 Rev	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGAGCACGGA
ama-1 Fwd	TGATCCGATGAATGATGGAA
ama-1 Rev	TTCCATTCTGCGTTGATGTC
gfp Fwd	GTTCCATGGCCAACACTTG
gfp Rev	TCGAGAAGCATTGAACACCA
trpa-1 Fwd (exon 5)	CAACTACTTGAATGGGATATTCGAC
trpa-1 Rev (exon 6)	TTTCCAGAATCCACGGCTAC

III. Supplementary References

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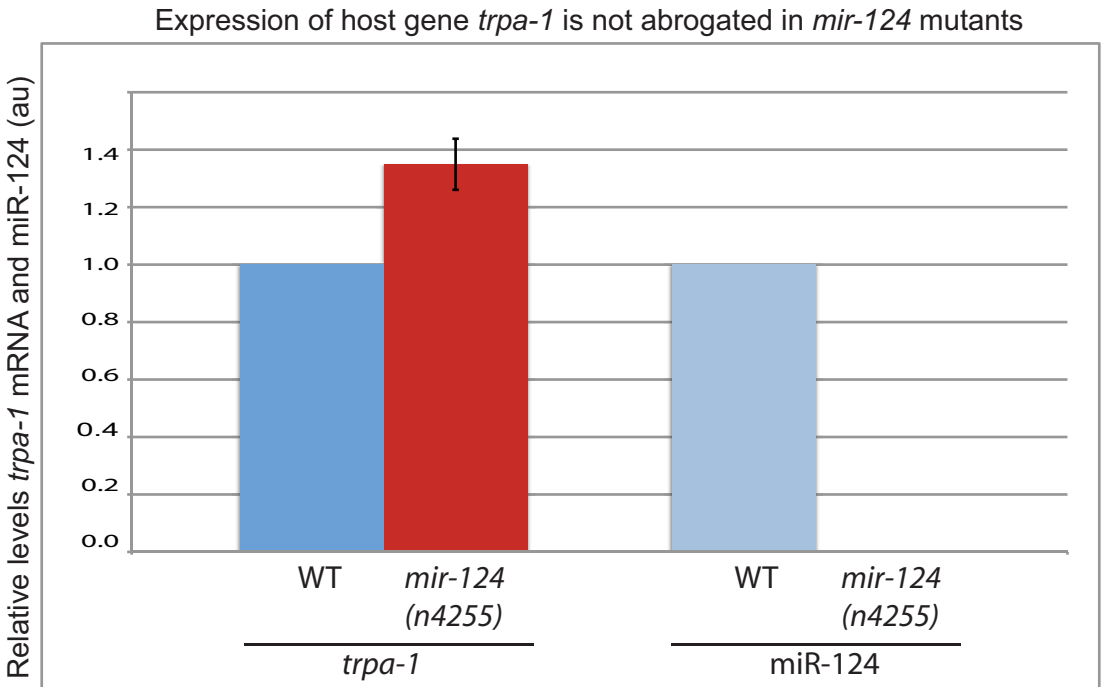
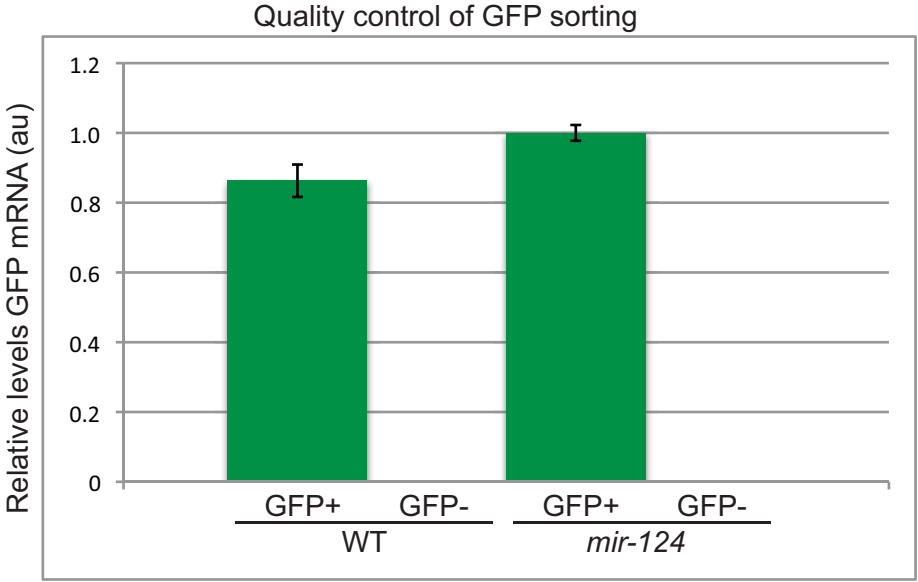
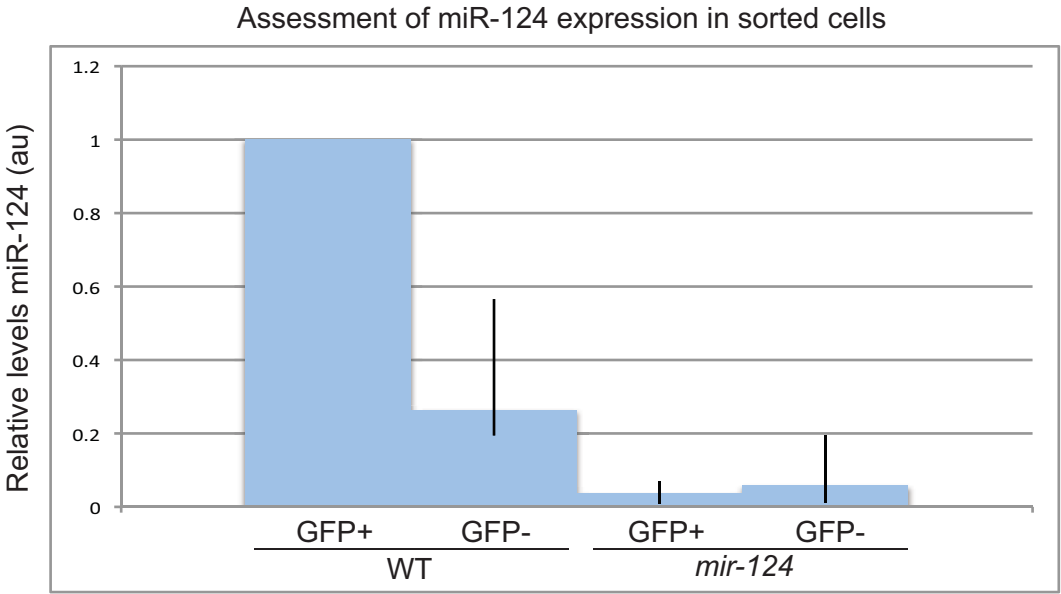
Supplementary Figure 1



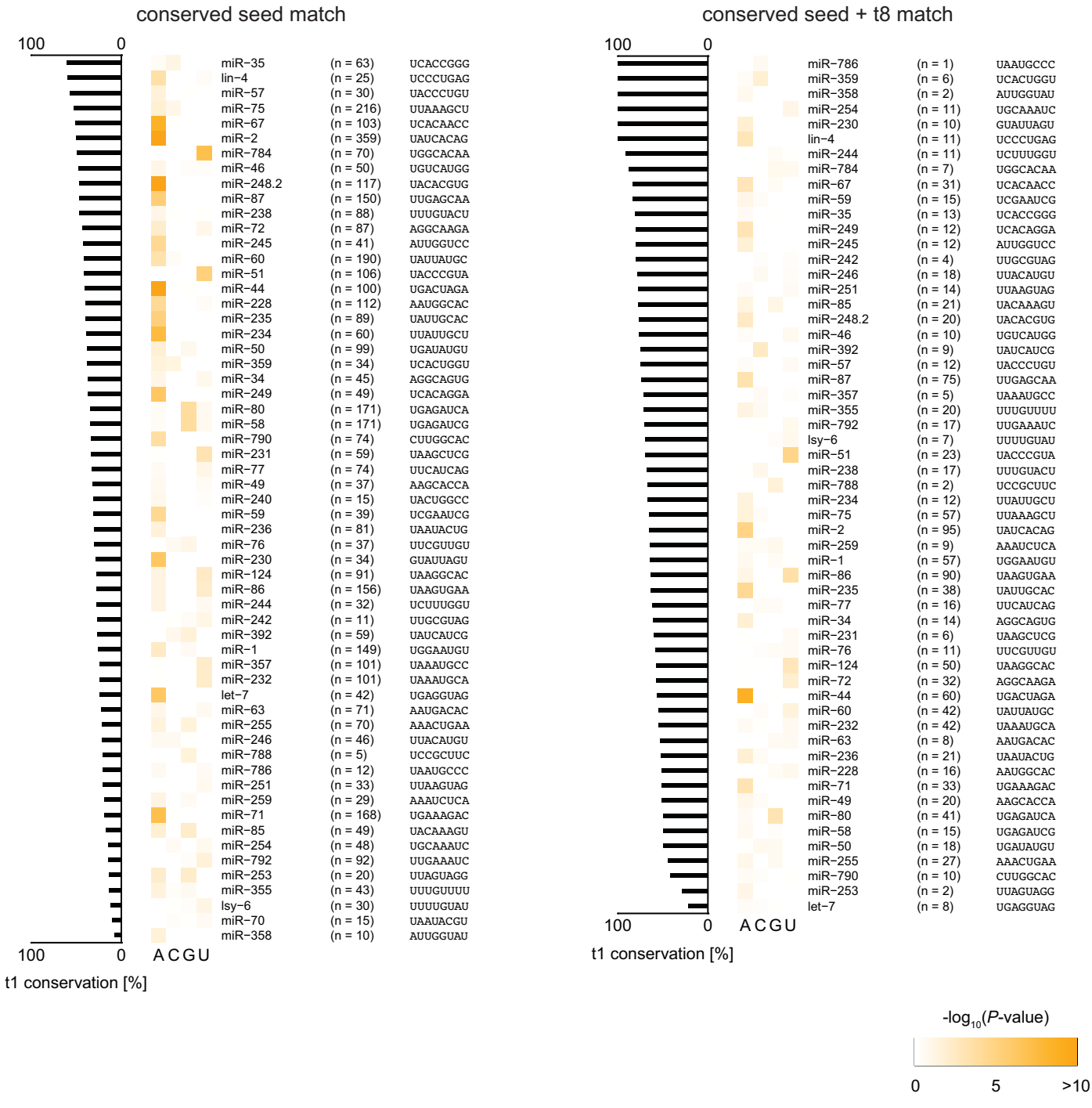
Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 5