Electronic Supplementary Material (ESI) for Nanoscale.

Supporting Information:

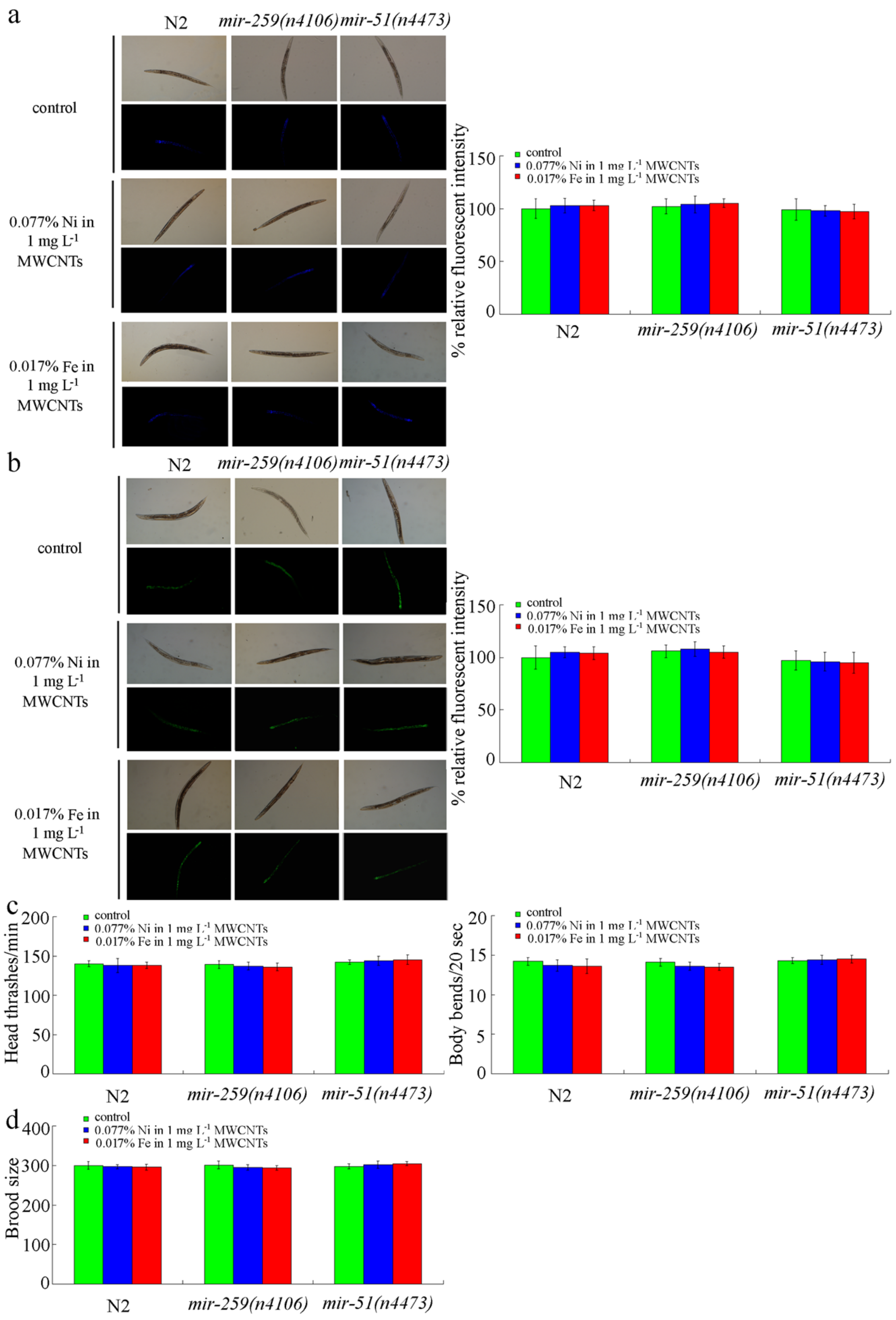

Fig. S1 Effects of Ni and Fe in MWCNTs on wild-type and mutant nematodes. (a) Effects of Ni and Fe in MWCNTs on intestinal autofluorescence in wild-type and mutant nematodes. (b) Effects of Ni and Fe in MWCNTs on intestinal ROS production in wild-type and mutant nematodes. (c) Effects of Ni and Fe in MWCNTs on locomotion behavior in wild-type and mutant nematodes. (d) Effects of Ni and Fe in MWCNTs on brood size in wild-type and mutant nematodes. Bars represent means ± S.E.M.

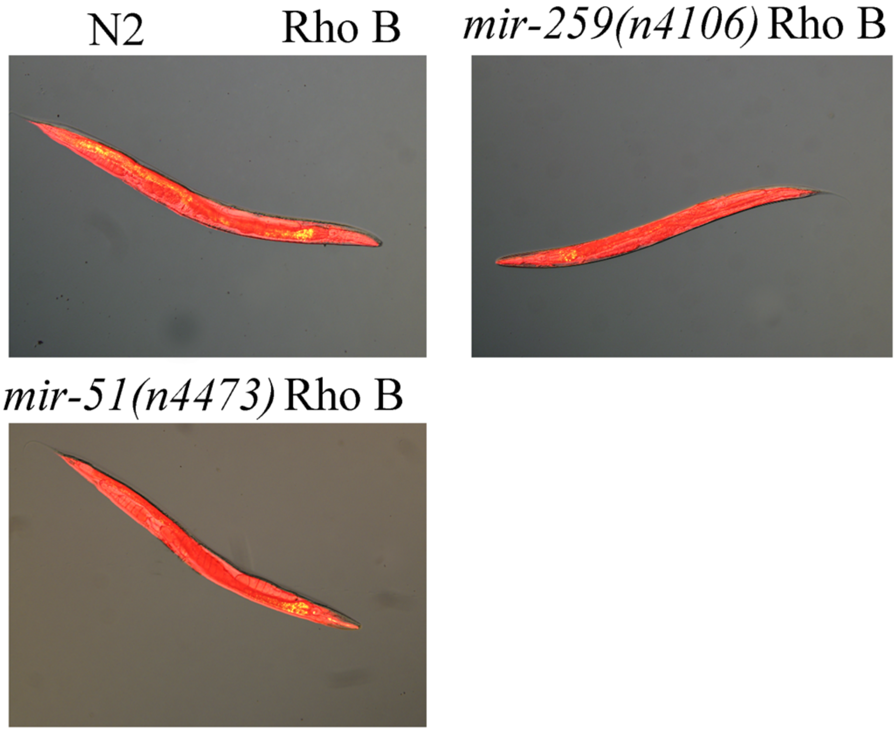

Fig. S2 Distribution of Rho-B in wild-type and mutant nematodes.

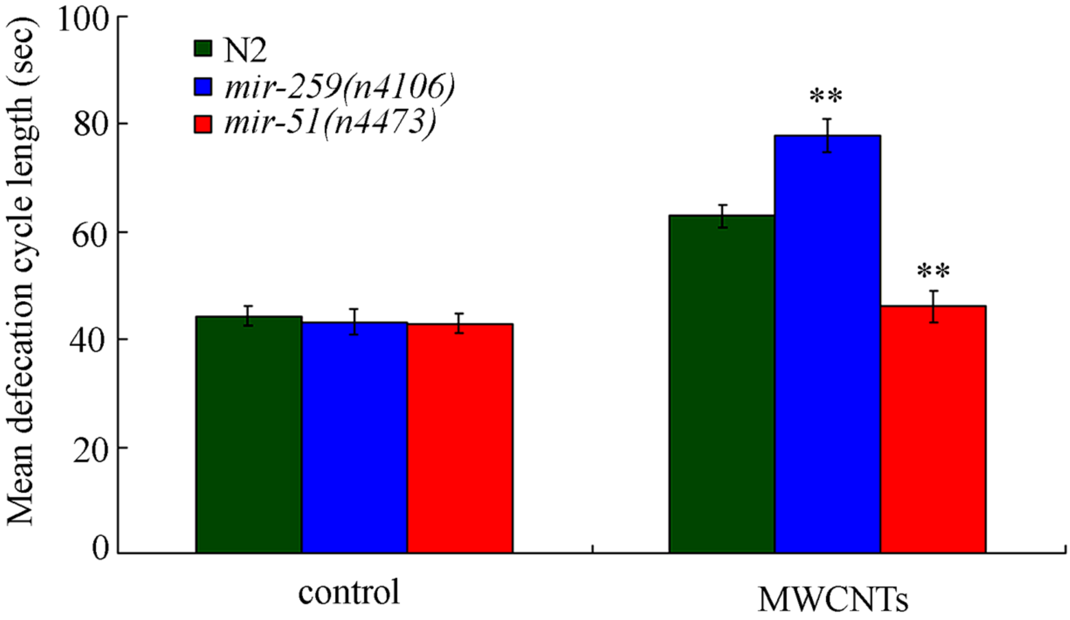

Fig. S3 Mean defecation cycle length in wild-type, *mir-259(n4106)* and *mir-51(n4473)* mutant nematodes exposed to 1 mg L^{-1} of MWCNTs. Bars represent mean ± S.E.M. ** $P < 0.01$.

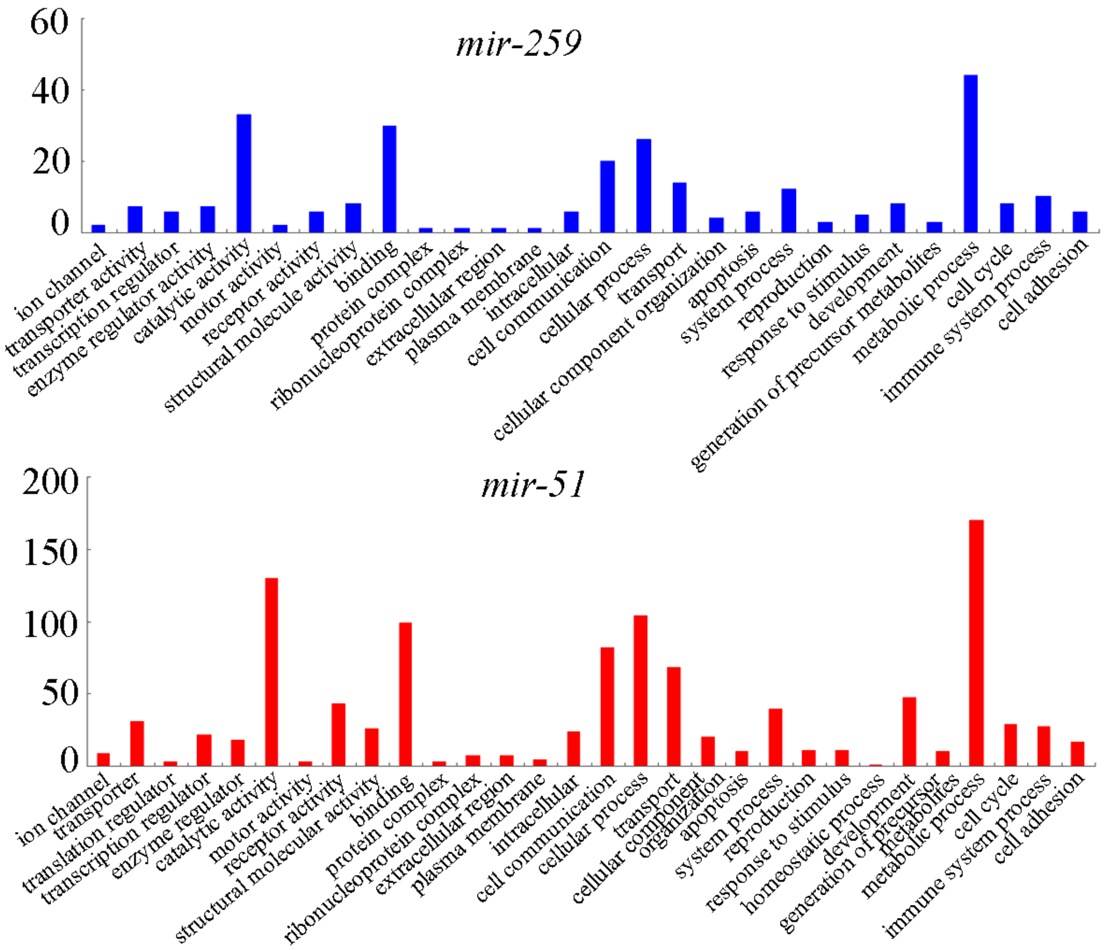

Fig S4. Gene ontology terms with gene counts based on *mir-259* and *mir-51* in nematodes.

Table S1. The dysregulated miRNAs after MWCNTs exposure

Gene ID	Accession	Fold changes (FC)	Log FC	Regulation
MI0005189_1	*mir-789-5p*	10.93611	3.451028	down
MI0008200_1	*mir-1830-3p*	5.871368	2.553697	down
MI0000013_1	*mir-42-5p*	5.419725	2.43822	down
MI0005190_1	*mir-789-3p*	5.419725	2.43822	down
MI0000303_2	*mir-228-3p*	4.877751	2.286216	down
MI0000022_1	*mir-51-3p*	4.74226	2.245575	down
MI0007985_1	*mir-1823*	4.74226	2.245575	down
MI0005201_2	*mir-800-5p*	4.064793	2.023182	down
MI0005197_1	*mir-796*	3.645372	1.866066	down
MI0017536_1	*mir-4806-3p*	3.645372	1.866066	down
MI0000044_1	*mir-73-5p*	3.387328	1.760148	down
MI0000054_1	*mir-83-5p*	3.161507	1.660612	down
MI0010960_2	*mir-2210-3p*	3.048595	1.608145	down
MI0000319_2	*mir-243-3p*	2.889494	1.530817	down
MI0000304_2	*mir-229-3p*	2.874097	1.523109	down
MI0005194_1	*mir-793*	2.840423	1.506106	down
MI0000035_2	*mir-64-3p*	2.734029	1.451029	down
MI0000002_2	*lin-4-3p*	2.709862	1.43822	down
MI0000759_1	*mir-360-5p*	2.709862	1.43822	down
MI0005198_2	*mir-797-3p*	2.709862	1.438219	down
MI0000016_2	*mir-45-5p*	2.617481	1.388179	down
MI0005879_1	*mir-1018*	2.605637	1.381636	down
MI0000028_1	*mir-57-3p*	2.37113	1.245575	down
MI0010973_2	*mir-2220-3p*	2.37113	1.245575	down
MI0000032_1	*mir-61-3p*	2.274349	1.185454	down
MI0000754_1	*mir-355*	2.263702	1.178684	down

MI0000018_1	*mir-47-5p*	2.176538	1.122035	down
MI0000325_1	*mir-249-3p*	2.167889	1.116291	down
MI0000303_1	*mir-228-5p*	2.156886	1.10895	down
MI0007983_1	*mir-1821*	2.112099	1.078677	down
MI0000043_1	*mir-72-5p*	2.067725	1.048044	down
MI0000003_1	*mir-1-3p*	2.056293	1.040046	down
MI0000031_2	*mir-60-5p*	2.032397	1.023182	down
MI0005191_2	*mir-790-3p*	2.032397	1.023182	down
MI0010966_2	*mir-2216-5p*	2.032397	1.023182	down
MI0000027_2	*mir-56-5p*	2.000887	1.00064	down
MI0000008_2	*mir-37-3p*	2.031124	1.022278	up
MI0000011_2	*mir-40-3p*	2.047955	1.034184	up
MI0010964_1	*mir-2214-5p*	2.06021	1.042792	up
MI0000007_2	*mir-36-3p*	2.135786	1.094767	up
MI0000006_2	*mir-35-3p*	2.159425	1.110647	up
MI0000009_2	*mir-38-3p*	2.2153	1.147502	up
MI0000048_1	*mir-77-5p*	2.283326	1.191137	up
MI0000336_2	*mir-259-5p*	2.583157	1.369136	up
MI0010964_2	*mir-2214-3p*	2.583157	1.369136	up
MI0000010_2	*mir-39-3p*	2.611428	1.384839	up
MI0000023_2	*mir-52-3p*	2.690448	1.427846	up
MI0000331_1	*mir-255-5p*	2.690448	1.427846	up
MI0000752_1	*mir-353*	2.690448	1.427846	up
MI0007981_2	*mir-1819-3p*	2.690448	1.427846	up
MI0010969_1	*mir-2209b-3p*	2.690448	1.427846	up
MI0017713_1	*mir-4926*	2.690448	1.427846	up
MI0000010_1	*mir-39-5p*	3.153464	1.656937	up
MI0000007_1	*mir-36-5p*	3.247398	1.699284	up
MI0000337_1	*mir-260*	3.44421	1.784173	up

MI0000014_1	*mir-43-5p*	4.035672	2.012809	up
MI0017542_2	*mir-4812-5p*	4.035672	2.012809	up
MI0017535_2	*mir-4805-3p*	5.380896	2.427846	up
MI0017723_1	*mir-4937*	5.380896	2.427846	up
MI0000009_1	*mir-38-5p*	16.97503	4.085343	up
MI0000006_1	*mir-35-5p*	36.90224	5.205637	up
MI0000012_1	*mir-41-5p*	45.73762	5.515309	up

Table S2. Gene ontology (GO) terms with gene counts more than 50 based on down-regulated miRNAs in MWCNTs exposed nematodes

No.	GO term	GO ID	Count
1	biological process	GO:0008150/0000004/0007582	376
2	molecular function	GO:0003674/0005554	321
3	cellular component	GO:0005575/0008372	290
4	cell part	GO:0044464	281
5	cell	GO:0005623	281
6	cellular process	GO:0009987/0008151/0050875	253
7	binding	GO:0005488	211
8	biological regulation	GO:0065007	196
9	multicellular organismal process	GO:0032501/0050874	196
10	developmental process	GO:0032502	194
11	regulation of biological process	GO:0050789/0050791	186
12	multicellular organismal development	GO:0007275	182
13	metabolic process	GO:0008152	170
14	membrane	GO:0016020	165
15	membrane part	GO:0044425	160
16	intrinsic to membrane	GO:0031224	145
17	integral to membrane	GO:0016021	145
18	intracellular	GO:0005622	138
19	catalytic activity	GO:0003824	137
20	embryonic development	GO:0009790/0009795	134
21	embryonic development	GO:0009792	131
22	localization	GO:0051179	127
23	primary metabolic process	GO:0044238	127
24	cellular metabolic process	GO:0044237	124
25	reproduction	GO:0000003/0019952/0050876	120

26	intracellular part	GO:0044424	118
27	regulation of cellular process	GO:0050794/0051244	105
28	intracellular organelle	GO:0043229	96
29	organelle	GO:0043226	96
30	regulation of biological process	GO:0048518/0043119	94
31	post-embryonic development	GO:0009791	94
32	nematode larval development	GO:0002119	92
33	larval development	GO:0002164	92
34	establishment of localization	GO:0051234	90
35	anatomical structure development	GO:0048856	89
36	regulation of growth	GO:0040008	88
37	positive regulation of growth	GO:0045927	85
38	transport	GO:0006810/0015457/0015460	85
39	macromolecule metabolic process	GO:0043170/0043283	83
40	cellular component organization	GO:0016043	81
41	growth	GO:0040007/0048590	75
42	intracellular membrane-bounded organelle	GO:0043231	75
43	membrane-bounded organelle	GO:0043227	75
44	protein binding	GO:0005515/0045308	74
45	locomotion	GO:0040011	73
46	cellular macromolecule metabolic process	GO:0044260/0034960	72
47	positive regulation of growth rate	GO:0040010	71
48	regulation of growth rate	GO:0040009	71
49	metal ion binding	GO:0046872	67
50	cation binding	GO:0043169	67
51	ion binding	GO:0043167	67
52	anatomical structure	GO:0009653	66

	morphogenesis		
53	reproductive process	GO:0022414	66
54	protein metabolic process	GO:0019538/0006411	65
55	nucleic acid binding	GO:0003676	62
56	cytoplasm	GO:0005737	62
57	hydrolase activity	GO:0016787	62
58	regulation of metabolic process	GO:0019222	61
59	nucleus	GO:0005634	58
60	signaling	GO:0023052	58
61	system development	GO:0048731	57
62	response to stimulus	GO:0050896/0051869	54
63	cellular protein metabolic process	GO:0044267	54
64	transition metal ion binding	GO:0046914	53
65	regulation of cellular metabolic process	GO:0031323	51
66	organ development	GO:0048513	51
67	nitrogen compound metabolic process	GO:0006807	51
68	regulation of macromolecule metabolic process	GO:0060255	50
69	cellular nitrogen compound metabolic process	GO:0034641	50

Table S3. Gene ontology (GO) terms with gene counts more than 50 based on up-regulated miRNAs in MWCNTs exposed nematodes

No.	GO term	GO ID	Count
1	biological process	GO:0008150/0000004/0007582	126
2	cellular component	GO:0005575/0008372	113
3	cell part	GO:0044464	110
4	cell	GO:0005623	110
5	molecular function	GO:0003674/0005554	104
6	cellular process	GO:0009987/0008151/0050875	77
7	membrane	GO:0016020	74
8	membrane part	GO:0044425	70
9	integral to membrane	GO:0016021	67
10	intrinsic to membrane	GO:0031224	67
11	multicellular organismal process	GO:0032501/0050874	66
12	developmental process	GO:0032502	65
13	binding	GO:0005488	64
14	multicellular organismal development	GO:0007275	59
15	biological regulation	GO:0065007	57
16	regulation of biological process	GO:0050789/0050791	55
17	metabolic process	GO:0008152	55

Table S4. Signaling pathways with the gene number (matched with entitylist) more than 2 based on down-regulated miRNAs in MWCNTs exposed nematodes

No.	Pathway	Counts	*p* value
1	Oxidative phosphorylation	12	0.000001
2	Alkaloid biosynthesis II	5	0.000022
3	Drug metabolism - other enzymes	6	0.000002
4	Calcium signaling pathway	5	0.000072
5	Folate biosynthesis	3	0.000469
6	Wnt signaling pathway	5	0.000821
7	Sulfur metabolism	2	0.001446
8	Glycerophospholipid metabolism	3	0.003004
9	Ubiquitin mediated proteolysis	5	0.003857
10	Ether lipid metabolism	2	0.006252
11	Citrate cycle (TCA cycle)	3	0.006852
12	Reductive carboxylate cycle (CO2 fixation)	2	0.00758
13	Hedgehog signaling pathway	2	0.009023

Table S5. Signaling pathways with the gene number (matched with entitylist) more than 2 based on up-regulated miRNAs in MWCNTs exposed nematodes

No.	Pathway	Counts	*P* value
1	Cysteine metabolism	4	0.000001
2	Alanine and aspartate metabolism	4	0.000003
3	Alkaloid biosynthesis I	3	0.000009
4	Phenylalanine, tyrosine and tryptophan biosynthesis	3	0.000012
5	Phenylalanine metabolism	3	0.000021
6	Proteasome	4	0.000022
7	Tyrosine metabolism	3	0.000093
8	Arginine and proline metabolism	3	0.000122
9	Wnt signaling pathway	4	0.000122
10	Pyrimidine metabolism	4	0.000139
11	Glutamate metabolism	3	0.000248
12	Purine metabolism	4	0.000865
13	p53 signaling pathway	2	0.001795
14	Inositol phosphate metabolism	2	0.002594
15	RNA polymerase	2	0.004231
16	Cell cycle	3	0.004439
17	Phosphatidylinositol signaling system	2	0.00499
18	Glycine, serine and threonine metabolism	2	0.006235
19	DNA polymerase	2	0.008591

Table S6. Information for nematode strains used in the present study[57]

Strain	miRNA(s)	Allele	chromosome	Deletion size (bp)
N2	wild-type			
MT13372	*mir-42-44*	*nDf49*	II	1103
MT14446	*mir-228*	*n4382*	IV	1026
MT14450	*mir-51*	*n4473*	IV	1504
MT13078	*mir-73-74*	*nDf47*	X	326
MT15022	*mir-83*	*n4638*	IV	823
MT15454	*mir-243*	*n4759*	IV	1102
MT13016	*mir-64, mir-229*	*nDf52*	III	652
DR721	*lin-4*	*e912*	II	not determined
MT15018	*mir-360*	*n4635*	X	1307
MT13433	*mir-45*	*n4280*	II	1495
VC347	*mir-57*	*gk175*	II	474
MT14875	*mir-61, mir-250*	*nDf59*	V	1142
MT16316	*mir-355*	*n4618*	V	1106
VC328	*mir-47*	*gk167*	X	1110
VC514	*mir-35*	*gk262*	II	1268
MT13015	*mir-72*	*n4130*	II	968
MT12954	*mir-1*	*n4101*	I	380
MT16471	*mir-60*	*n4974*	II	787
MT14119	*mir-35-41*	*nDf50*	II	1261
MT16311	*mir-77*	*n4286*	II	1036
MT12969	*mir-259*	*n4106*	V	529
MT12945	*mir-52*	*n4100*	IV	398
MT14919	*mir-260*	*n4601*	II	911

Table S7. Gene ontology (GO) terms with gene counts based on the *mir-259*

No.	GO term	GO ID	Count
1	ion channel activity	GO:0005216	2
2	transporter activity	GO:0005215	7
3	transcription regulator activity	GO:0030528	6
4	enzyme regulator activity	GO:0030234	7
5	catalytic activity	GO:0003824	33
6	motor activity	GO:0003774	2
7	receptor activity	GO:0004872	6
8	structural molecule activity	GO:0005198	8
9	binding	GO:0005488	30
10	protein complex	GO:0043234	1
11	ribonucleoprotein complex	GO:0030529	1
12	extracellular region	GO:0005576	1
13	plasma membrane	GO:0005886	1
14	Intracellular	GO:0005622	6
15	cell communication	GO:0007154	20
16	cellular process	GO:0009987	26
17	transport	GO:0006810	14
18	cellular component organization	GO:0016043	4
19	apoptosis	GO:0006915	6
20	system process	GO:0003008	12
21	reproduction	GO:0000003	3
22	response to stimulus	GO:0050896	5
23	developmental process	GO:0032502	8
24	generation of precursor metabolites and energy	GO:0006091	3
25	metabolic process	GO:0008152	44
26	cell cycle	GO:0007049	8
27	immune system process	GO:0002376	10

28	cell adhesion	GO:0007155	6

Table S8. Gene ontology (GO) terms with gene counts based on the *mir-51*

No.	GO term	GO ID	Count
1	ion channel activity	GO:0005216	9
2	transporter activity	GO:0005215	31
3	translation regulator activity	GO:0045182	3
4	transcription regulator activity	GO:0030528	22
5	enzyme regulator activity	GO:0030234	18
6	catalytic activity	GO:0003824	130
7	motor activity	GO:0003774	3
8	receptor activity	GO:0004872	43
9	structural molecule activity	GO:0005198	26
10	binding	GO:0005488	99
11	protein complex	GO:0043234	3
12	ribonucleoprotein complex	GO:0030529	7
13	extracellular region	GO:0005576	7
14	plasma membrane	GO:0005886	4
15	intracellular	GO:0005622	24
16	cell communication	GO:0007154	82
17	cellular process	GO:0009987	104
18	transport	GO:0006810	68
19	cellular component organization	GO:0016043	20
20	apoptosis	GO:0006915	10
21	system process	GO:0003008	40
22	reproduction	GO:0000003	11
23	response to stimulus	GO:0050896	11
24	homeostatic process	GO:0042592	1
25	developmental process	GO:0032502	48
26	generation of precursor development	GO:0006091	10
27	metabolic process	GO:0008152	170

28	cell cycle	GO:0007049	29
29	immune system process	GO:0002376	27
30	cell adhesion	GO:0007155	17

Table S9. Primers for reverse transcription of miRNAs

miRNA	Primer
mir-42	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACCTTCACC
mir-228	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACGCTATGG
mir-51	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACTGCACCT
mir-73	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACGCTGTGG
mir-83	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACTCAAGTA
mir-243	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACGATATCC
mir-229	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACCTATGAC
mir-64	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACGCATGCC
lin-4	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACGTACCCG
mir-360	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGAC TGACCGT
mir-45	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACTATGACT
mir-57	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACTGCACCT
mir-355	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACCATAGCT
mir-47	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACACTGTCA
mir-249	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACGGCAACG
mir-228	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACCCGTGAA
mir-72	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGAC TCAGCTA
mir-1	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACTACATAC
mir-37	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACACTGCAA
mir-40	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACTTAGCTG
mir-36	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACCATGCGA
mir-35	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACACTGCTA
mir-38	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACACTCCAG
mir-77	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACATTTCCT
mir-259	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACCTACCAG
mir-39	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACCAAGCTG
mir-52	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACGCTACCC

mir-260	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGAC CTACAAG
mir-43	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACCATAATC
mir-41	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACTATCACT

Table S10. Primers for real-time PCR of miRNAs

miRNA	primer
mir-42	ACTCATGTGGGTGTTTGCTTTT
mir-228	GTCTATGCGGATCATACGGTAC
mir-51	CTGACTCATGGAAGCAGGTACA
mir-73	TACTCTTGGACTTCCATATCGA
mir-83	GACGTGACTGAATTTATGTGTG
mir-243	ACAATAGGTACGATCGCGGCGG
mir-229	CGTACTAGAAAGGTATCGGGTG
mir-64	CATACAGTGCAACGATCAGTGG
lin-4	CTACTAACACCTGGGCTCTCCG
mir-360	TGCTACTTGTGACCGTTGTTAC
mir-45	CGTCTACTGGATGTGCTCGTTA
mir-57	ACGTCGACGAGCTAGACTACAA
mir-355	CGTAGCTTTGTTTTAGCCTGAG
mir-47	GCGTGTAAGAGAGCAGTCATTG
mir-249	GACTCGTCACAGGACTTTTGAG
mir-228	TACTAGAATGGCACTGCATGAA
mir-72	GATCTAAGGCAAGATGTTGGCA
mir-1	GCTCCGTGGAATGTAAAGAAGT
mir-37	ACTAAGTCACCGGGTGAACACT
mir-40	TCTACTTCACCGGGTGTACATC
mir-36	CTTAACTCACCGGGTGAAAATT
mir-35	ATAATCTCACCGGGTGGAAACT
mir-38	CTAACTTCACCGGGAGAAAAAC
mir-77	ATAGCTGATGGTTGTGCTCTGA
mir-259	CGTGCCAAATCTCATCCTAATC
mir-39	TCGTACTCACCGGGTGTAAATC
mir-52	CGTGACCACGTTACAATGAAAG

mir-260	GTCTCGGTGATGTCGAACTCTT
mir-43	CCATGCGACATCAAGAAACTAG
mir-41	ATATCTGGGGTTTTTCTCTGCA
Common reward primer	GTGCAGGGTCCGAGGT