

**microRNA regulation of the embryonic hypoxic response in  
*Caenorhabditis elegans***

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## SUPPLEMENTARY EXPERIMENTAL PROCEDURES

### Strains

Strains were grown on nematode growth media (NGM) agar at 20°C on the *Escherichia coli* OP50 strain using standard growth conditions [1]. Transgenic animals were created as described in [2]. All the experiments were conducted at 20°C. A list of strains used in this study is provided in Table S3.

### Molecular biology

For the *mir-35(ndf50)* mutant rescue, we used a 642bp upstream promoter region to drive either the full *mir-35-41* cluster (primers KK163/164) or *mir-35* alone (primers KK163/171). The PCR products were injected at 3ng/μl together with 5ng/μl of *elt-2::gfp* as co-injection marker. For the *Pmir-35-41 2xNLS::yfp* construct, we used a 602bp upstream promoter region of *mir-35-41* cluster (primers KK240/241) inserted upstream of 2xNLS::YFP using BamHI/HindIII. This construct was injected at 3ng/μl together with 5ng/μl of *elt-2::gfp* as co-injection marker. For the *Psup-26::2xNLS::yfp*, a 2080bp fragment upstream of the *sup-26* start codon was used (primers KK238/239) and inserted upstream of 2xNLS::YFP using BamHI/HindIII. In this construct, the *unc-54* 3'UTR was substituted by the *sup-26* 3' UTR (primers KK243/244) using ApaI/EcoRI. This construct was injected at 50ng/μl. For the *sup-26* sensor experiment, the *myo-2* promoter was used to drive the *mir-35* hairpin and the fluorescent reporters upstream of the respective 3'UTRs. For the *sup-26* rescue, a previously described transgene [3] was used.

### Embryonic lethality in hypoxia

Approximately 20 L4 stage animals were placed in a plate seeded with a 300 μl drop of OP50 LB culture, and 24 hrs later were moved as young adults to a new plate, seeded

with a 300 µl drop of OP50, to lay eggs for 3h (egg pulse). Next, mothers were removed and the plates containing the eggs were placed for 24 hrs in an air-proof chamber (Billups-Rothenberg) filled with 0.5% O<sub>2</sub> and 99.5% nitrogen. After this time, the plates were left to recover for another 24 hrs in ambient O<sub>2</sub> conditions and embryonic lethality was scored and calculated by counting unhatched eggs and larvae (Figure S1). For the *in utero* hypoxia experiment, staged L4 animals were placed in 0.5% O<sub>2</sub> for 15 hrs and eggs were removed from mothers by bleaching followed by immediate RNA extraction. All the strains in the screen were scored at least 2 independent times, most of them more than 3 times. N2 and *hif-1* mutant animals were included as controls in each assay to assess for effective hypoxic conditions. Scorings were pooled for each strain.

### **mRNA isolation and qRT-PCR analysis**

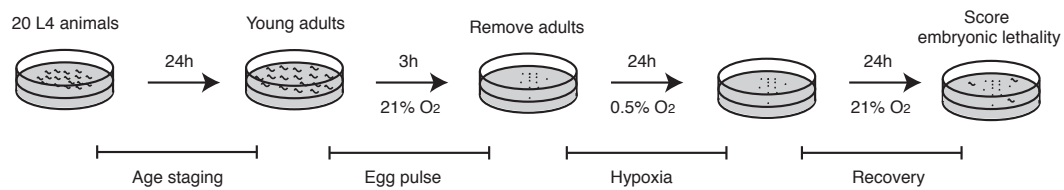
Embryos obtained by bleaching: Staged early young adult animals were bleached and the eggs obtained were subjected to the different hypoxic conditions. RNA was isolated using standard Trizol based methods [4].

Total cDNA was obtained using TaqMan Reverse Transcription Reagents (Invitrogen, Cat. No: N8080234). qRT-PCR reactions were performed in triplicates on a LightCycler 480 System (Roche) using the 33 Maxima SYBR/ROX qPCR Master Mix (Fermentas, Cat. No: K0221) and the results were analyzed using qBase from Biogazelle. Error bars represent the SEM of at least 3 independent sets of samples. Three qRT-PCR reference genes were used - *mir-34*, *mir-86* and *mir-1829c* for miRNAs and *cdc-42*, *pmp-3* and *Y45F10D.4* for mRNAs. The stable expression of these genes was tested in each experiment using the geNORM logarithm [5]. Results presented as 'fold change' values normalized to the arbitrary value 1 given to a defined sample (e.g. normoxic sample).

For the detection of primary and precursor species, the procedure described in [6] was followed. This method is used to measure both primary and precursor miRNAs without however being able to distinguish the two. The RT reactions were performed using gene specific primers. The RT primer for the *mir-35-41* cluster was designed to bind at the 5' end of the suspected polysystronic RNA. qPCR was performed using primers designed to bind at the stem region of the *mir-38* hairpin.

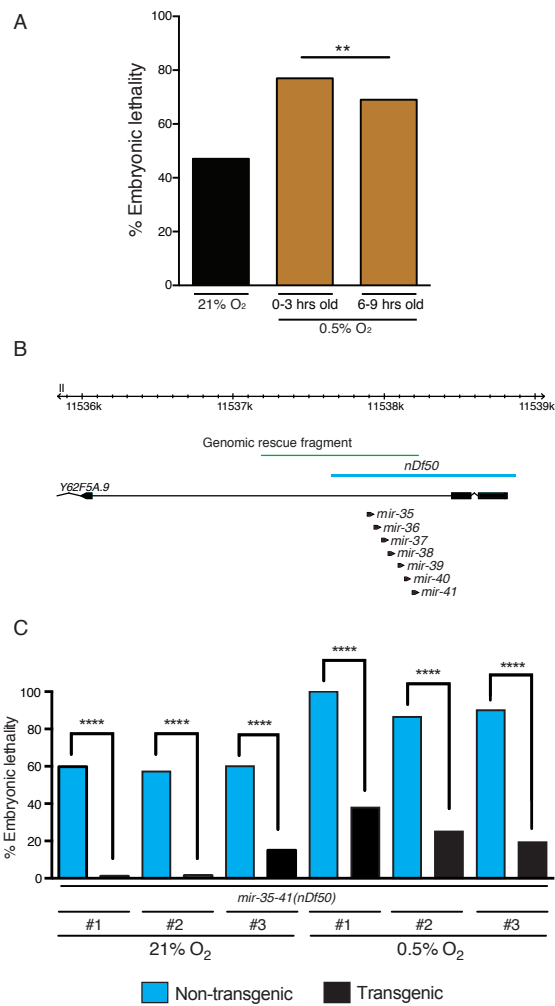
### **Statistical analysis**

Data of embryonic lethality phenotype are presented as contingency table values derived from 2, 3 or more independent assay repetitions. Fischer exact test was used for statistical analysis of these data. qPCR data are presented as means of at least 3 independent repetitions and error bars represent  $\pm$  SD. Students t-test was used in this case to assess for statistical significance using Prism 5.0d (GraphPad Software, Inc.). P-values was added on the graphs as \* $p \leq 0.05$ , \*\* $p \leq 0.01$ , \*\*\* $p \leq 0.001$ , \*\*\*\* $p \leq 0.0001$ , n.s. = not significant.



### Figure S1. Embryonic hypoxic survival assay

Schematic of the experimental pipeline used for screening and scoring hypoxic survival of miRNA mutants. 20 L4 animals were picked from a well-fed, uncrowded, mixed worm population and placed on a freshly seeded OP50 plate. 24 hours later these now 1-day adult animals were transferred to a new freshly seeded OP50 plate and allowed to lay eggs for 3 hours (egg-pulse). After removal of the mothers these eggs were placed in 0.5% O<sub>2</sub> for 24 hours (hypoxia) and following a 24-hour recovery time the embryos were scored for survival. All experiments were performed at 20°C.

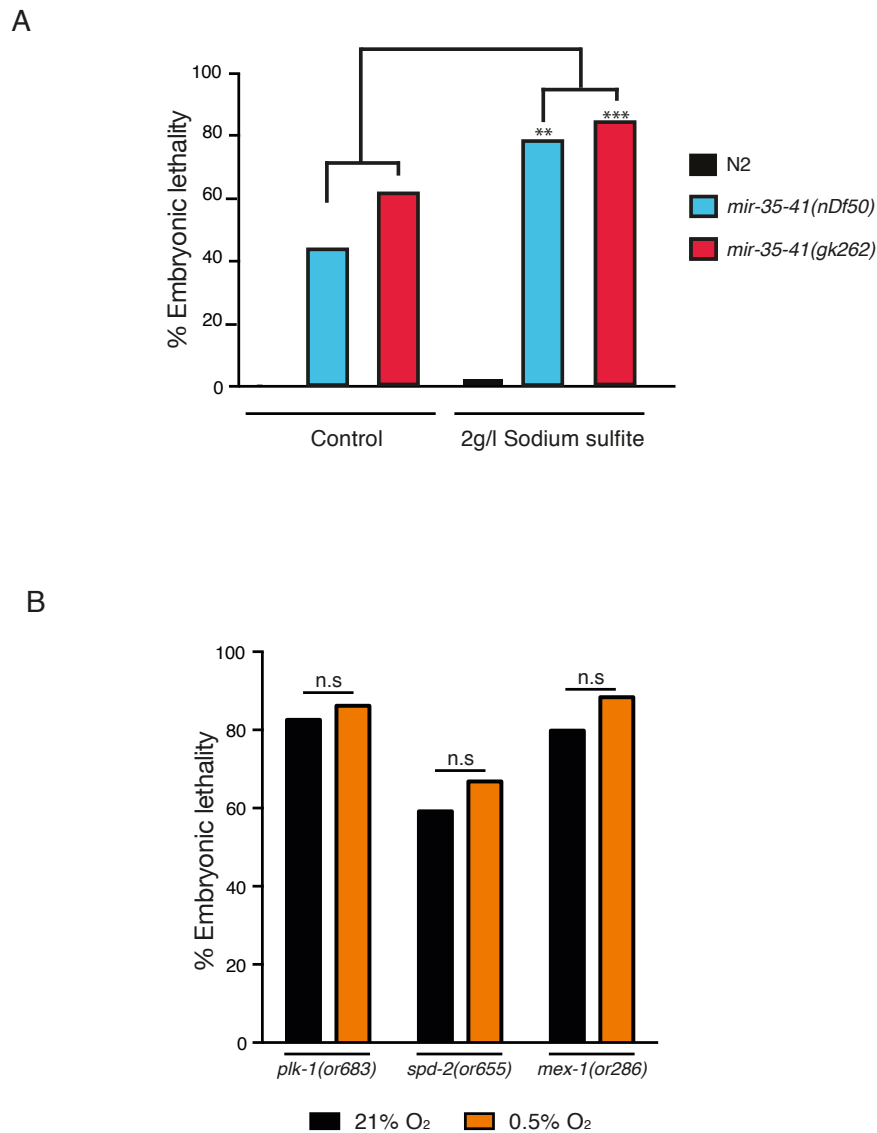


## Figure S2. Further characterization and transgenic rescue of *mir-35-41(nDf50)* embryonic lethality

(A) Old embryos (6-9h post egg-laying) subjected to hypoxia show significantly lower embryonic lethality compared to early embryos (0-3h post egg-laying). n=250-525. Fischer exact test was used for statistical analysis.  $**\leq 0.01$ .

(B) The *nDf50* (blue) deficiency removes the entire *mir-35-41* locus and part of the worm specific gene *Y62F5A.9*. The genomic rescue fragment used in (C) is marked in green, which includes a 602 bp upstream region and the *mir-35-41* cluster.

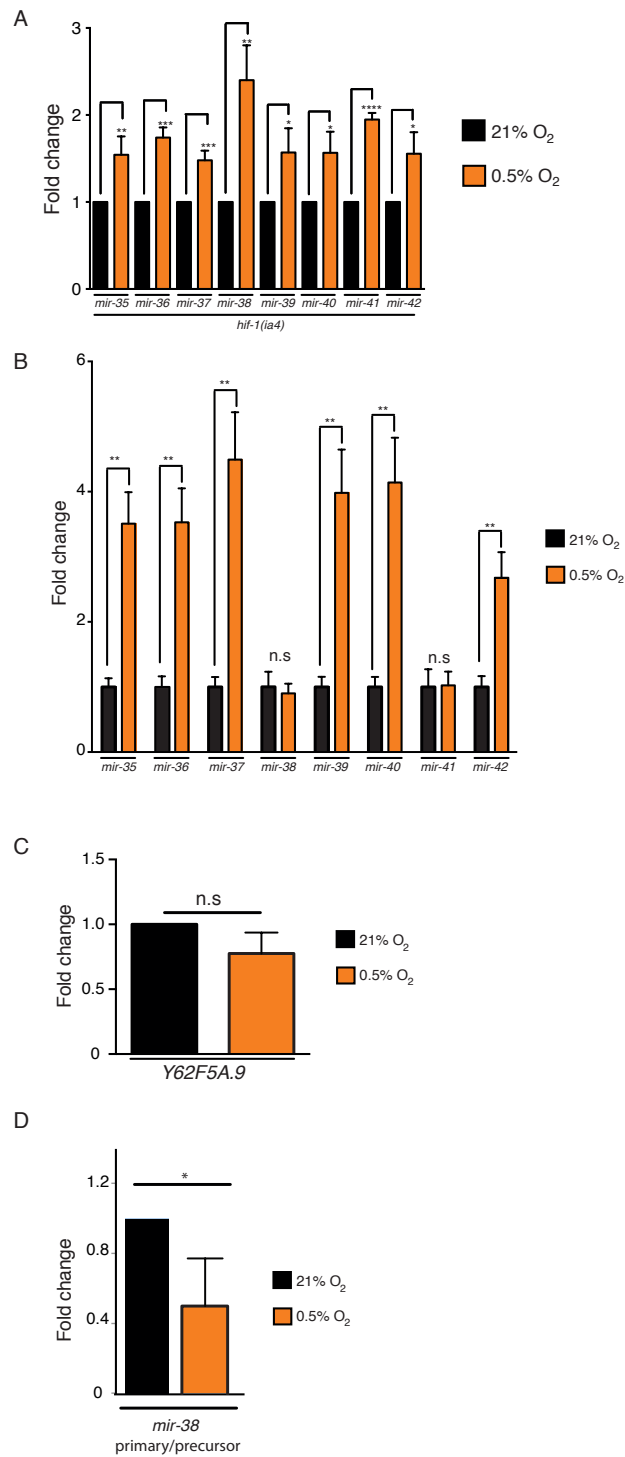
(C) Normoxic and hypoxic lethality of *mir-35-41(nDf50)* mutant embryos is rescued by transgenic expression of the *mir-35-41* cluster. The sequence used to rescue *mir-35* is shown in (A). n=20-423.  $****\leq 0.0001$ . # refers to independent transgenic lines.



**Figure S3. *mir-35-41* deletion mutant strains are sensitive to sodium sulfite and independent mutant strains with high embryonic lethality do not exhibit hypoxia sensitivity**

(A) Both *mir-35-41* deletion mutant strains show a significant increase in embryonic lethality when subjected to 2g/l sodium sulfite, a compound that mimics hypoxic stress.

(B) The normoxic embryonic lethality of three different mutants - *plk-1(or683)*, *spd-2(or655)* and *mex-1(or286)* does not significantly increase under hypoxia. Embryos of each strain were treated as described in Figure S1. 21% O<sub>2</sub> (black bars) or 0.5% O<sub>2</sub> (orange bars). n=75-450. n.s. - not significant.



### Figure S4. qRT-PCR analysis

(A) qRT-PCR showing *mir-35* family member expression levels in *hif-1* mutant embryos exposed to 21% O<sub>2</sub> (black bars) or 0.5% O<sub>2</sub> for 4 hrs (orange bars).

(B) qRT-PCR showing *mir-35* family member expression levels in wild type embryos exposed to 21% O<sub>2</sub> (black bars) or 0.5% O<sub>2</sub> (orange bars) for 15 hrs *in utero*.

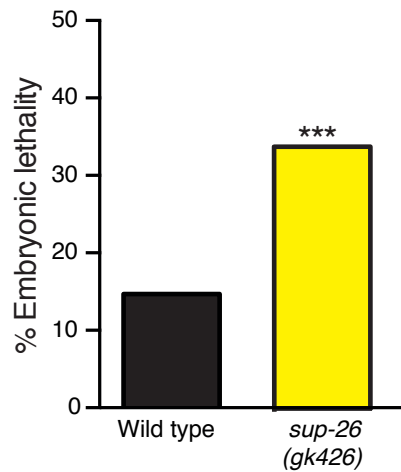
(C) The *mir-35-41* host gene, Y62F5A.9, is not induced by hypoxia. n.s. - not significant.



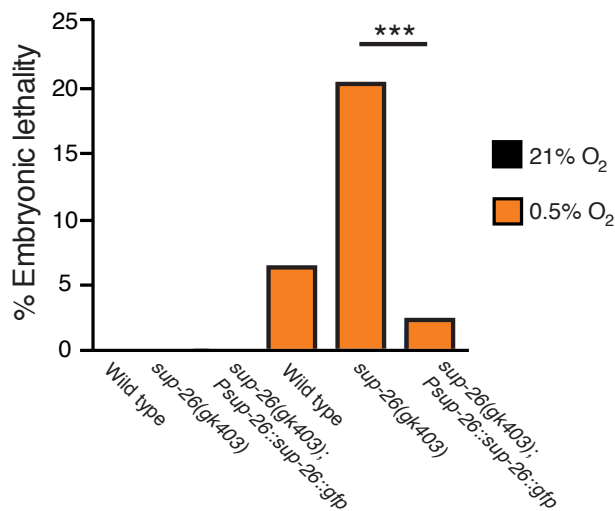
(D) The expression of primary and precursor forms of the *mir-35-41* locus measured by qPCR using primers flanking either the *mir-35* or *mir-38* hairpin does not change in hypoxia.

Students t-test was used for statistical significance. \* $p \leq 0.05$ , \*\* $\leq 0.01$ , \*\*\* $p \leq 0.001$ , \*\*\*\* $\leq 0.0001$ , n.s. = not significant.

A



B



**Figure S5. *sup-26(gk426)* embryos are sensitive to hypoxia**

(A) Chronic hypoxic exposure (0.5% O<sub>2</sub> for 45h), causes lethality of *sup-26(gk426)* embryos. n=73-470. \*\*\* $\leq$  0.001.

(B) The embryonic lethality of *sup-26(gk403)* mutant animals under chronic hypoxic exposure (0.5% O<sub>2</sub> for 45h) is rescued by the resupply of *sup-26* gene driven by its native promoter. n>50. \*\*\*\* $\leq$  0.0001.

**Table S1. miRNA mutants exhibiting hypoxic sensitivity or resistance phenotypes**

| Controls and alleles              | Strain  | Number of Outcrosses | Genotype   | Percentage Embryonic Lethality (n) Hypoxia | Percentage Embryonic Lethality (n) Normoxia | p value (compared to wt) | p value (hypoxia vs normoxia) |
|-----------------------------------|---------|----------------------|--|--|---|--------------------------|-------------------------------|
| <b>Controls</b>                   | N2      |                      | Wild type  | 10 (974)                                   | 0 (n>500)                                   |                          |                               |
|                                   | OH8125  | 11                   | <i>hif-1(ia4)</i>  | 41 (1132)                                  | 2 (n=276)                                   | ****                     | ****                          |
| <b>Sensitive to hypoxia</b>       |         |                      |  |  |   |                          |                               |
| <b>mir-35 family</b>              | MT14119 | 9                    | <i>mir-35-41(nDf50)</i>  | 91 (242)                                   | 51 (n=327)                                  | ****                     | ****                          |
|                                   | VCS14   |                      | <i>mir-35-41(gk262)</i>  | 98 (176)                                   | 77 (n=133)                                  | ****                     | ****                          |
| <b>mir-44 and mir-2 families</b>  | MT17431 |                      | <i>mir-42-44(nDf49); mir-61&amp;mir-250(nDf59); mir-247(n4505)</i> | 25 (253)                                   | 0 (n=26)                                    | ****                     | ****                          |
|                                   | MT14875 |                      | <i>mir-61&amp;mir-250(nDf59)</i>                                   | 34 (138)                                   | 0 (n>100)                                   | ****                     | ****                          |
|                                   | MT17676 |                      | <i>mir-45(n4280); mir-61&amp;mir-250(nDf59); mir-247(n4505)</i>    | 36 (90)                                    | 0 (n>100)                                   | ****                     | ****                          |
|                                   | MT13433 |                      | <i>mir-45(n4280)</i>   | 29 (76)                                    | 0 (n>100)                                   | ****                     | ****                          |
| <b>mir-49 and mir-67 families</b> | KB12    | 6                    | <i>mir-67(n4899); mir-83(n4638)</i>                                | 14 (417)                                   | 0 (n>100)                                   | **                       | ****                          |
| <b>mir-51 family</b>              | MT17136 |                      | <i>mir-51&amp;mir-53(nDf67); mir-54-56(nDf58)</i>                  | 38 (42)                                    | 2 (n=54)                                    | ****                     | ****                          |
| <b>mir-60 family</b>              | MT16471 |                      | <i>mir-60(n4947)</i>   | 15 (273)                                   | 0 (n>100)                                   | **                       | ****                          |
|                                   | RJP1030 | 3                    | <i>mir-60(n4947)</i>   | 22 (46)                                    | 0 (n>100)                                   | *                        | ****                          |
| <b>mir-63 family</b>              | MT16494 | 2                    | <i>mir-229&amp;mir-64-66(nDf63)</i>                                | 32 (169)                                   | 0 (n>100)                                   | ****                     | ****                          |
|                                   | RJP999  | 5                    | <i>mir-229&amp;mir-64-66(nDf63)</i>                                | 19 (296)                                   | 0 (n>100)                                   | ****                     | ****                          |
| <b>Resistant to hypoxia</b>       |         |                      |  |  |   |                          |                               |
| <b>let-7 family</b>               | RJP1015 |                      | <i>lin-58(n4097); mir-84(n4037)</i>                                | 0 (39)                                     | 0 (n>100)                                   | *                        | n.s                           |
| <b>mir-58 family</b>              | MT13949 |                      | <i>mir-80(nDf53)</i>   | 3 (515)                                    | 0 (n>100)                                   | ****                     | n.s                           |
|                                   | RJP936  | 3                    | <i>mir-80(nDf53)</i>   | 2 (241)                                    | 0 (n>100)                                   | ****                     | n.s                           |
| <b>mir-67 family</b>              | KB10    | 6                    | <i>mir-67(n4899)</i>   | 3 (95)                                     | 0 (n>100)                                   | *                        | n.s                           |
| <b>mir-79 family</b>              | MT14448 | 2                    | <i>mir-79(n4126); mir-75(n4472)</i>                                | 4 (357)                                    | 0 (n>100)                                   | ***                      | *                             |
| <b>mir-237 family</b>             | MT13653 |                      | <i>mir-237(n4296)</i>  | 2 (117)                                    | 0 (>100)                                    | **                       | n.s                           |
| <b>mir-246 family</b>             | MT15020 |                      | <i>mir-246(n4636)</i>  | 4 (565)                                    | 0 (n>100)                                   | ***                      | n.s                           |
| <b>mir-359 family</b>             | MT14673 |                      | <i>mir-359(n4540)</i>  | 4 (384)                                    | 0 (n=30)                                    | ***                      | n.s                           |

## Table S1. miRNA mutants exhibiting hypoxic sensitivity or resistance phenotypes

Description of the embryonic hypoxia assay is depicted in Figure S1. \* $p \leq 0.05$ , \*\* $\leq 0.01$ ,

\*\*\* $p \leq 0.001$ , \*\*\*\* $\leq 0.0001$  refer either to the difference in hypoxic embryonic lethality

between each strain and wild type ('compared to wt' column) or to the difference in

embryonic lethality between normoxia and hypoxia for each strain ('hypoxia vs. normoxia'

column).

| Controls and alleles                                       | Strain  | Number of Outcrosses | Genotype   | Percentage Embryonic Lethality (n) | p value |
|--|---------|----------------------|--|------------------------------------|---------|
| <b>Controls</b>  | N2      |                      | Wild type  | 9.54 (974)                         |         |
|  | OH8125  | 11                   | <i>hif-1(ia4)</i>  | 40.54 (1132)                       | ****    |
| <b>miRNA knockouts with no embryonic hypoxia phenotype</b> |         |                      |  |                                    |         |
| <b><i>mir-239a</i> family</b>                              | MT15312 |                      | <i>mir-239a</i> & <i>mir-239b(nDf62)</i>                 | 11 (187)                           | n.s     |
|  | MT16061 | 4                    | <i>mir-238(n4112); mir-239a</i> & <i>mir-239b(nDf62)</i> | 7 (442)                            | n.s     |
| <b><i>mir-44</i> and <i>mir-2</i> families</b>             | MT16309 | 2                    | <i>mir-247</i> & <i>mir-797(n4505)</i>                   | 11 (244)                           | n.s     |
| <b><i>mir-78</i> family</b>                                | MT15021 |                      | <i>mir-78(n4637)</i>                                     | 11 (260)                           | n.s     |
| <b><i>mir-51</i> family</b>                                | MT17137 |                      | <i>mir-51(n4473); mir-54-56(nDf58)</i>                   | 8 (558)                            | n.s     |
|  | MT14767 |                      | <i>mir-54-56(nDf58)</i>                                  | 6 (90)                             | n.s     |
|  | MT12989 |                      | <i>mir-53(n4113)</i>                                     | 10 (673)                           | n.s     |
| <b><i>mir-266</i> family</b>                               | MT13078 |                      | <i>mir-73</i> & <i>mir-74(nDf47)</i>                     | 6 (194)                            | n.s     |
|  | RJP929  | 3                    | <i>mir-72(n4130); mir-73</i> & <i>mir-74(nDf47)</i>      | 12 (108)                           | n.s     |
|  | RJP920  | 3                    | <i>mir-72(n4130)</i>                                     |                                    |         |
| <b><i>mir-79</i> family</b>                                | MT18037 | 3                    | <i>mir-75(n4472)</i>                                     | 9 (375)                            | n.s     |
| <b><i>mir-233</i> family</b>                               | RJP168  |                      | <i>mir-87(n4104); mir-233(n4761)</i>                     | 3 (56)                             | n.s     |
| <b><i>mir-256</i> family</b>                               | VC576   |                      | <i>mir-1(gk276)</i>                                      | 3 (76)                             | n.s     |
| <b><i>mir-77</i> family</b>                                | MT16311 |                      | <i>mir-77(n4286)</i>                                     | 12 (535)                           | n.s     |
| <b><i>mir-235</i> family</b>                               | MT17997 | 2                    | <i>mir-235(n4504)</i>                                    | 7 (90)                             | n.s     |
| <b><i>mir-71</i> family</b>                                | MT12993 |                      | <i>mir-71(n4115)</i>                                     | 11 (290)                           | n.s     |
| <b><i>mir-80</i> family</b>                                | MT13954 |                      | <i>mir-81</i> & <i>mir-82(nDf54)</i>                     | 5 (344)                            | n.s     |
|  | MT18043 | 3                    | <i>mir-240</i> & <i>mir-786(n4541)</i>                   | 5 (373)                            | n.s     |
| <b><i>mir-35</i> family</b>                                | RJP997  | 3                    | <i>mir-42(gk177)</i>                                     | 13 (211)                           | n.s     |
| <b><i>mir-63</i> family</b>                                | MT13016 |                      | <i>mir-229&amp;mir-64(nDf52)</i>                         | 13 (161)                           | n.s     |

**Table S2. miRNA mutants exhibiting no hypoxic sensitivity or resistance phenotype**

Description of the embryonic hypoxia assay is described in Figure S1. n.s. - not significant,

\*\*\*\* $\leq 0.0001$ .

| Strain | Genotype            | Percentage Embryonic Lethality (n) |          | p value |
|--------|---------------------|------------------------------------|----------|---------|
|        |                     | Normoxia                           | Hypoxia  |         |
| EU1441 | <i>plk-1(or683)</i> | 82.6 (75)                          | 86 (87)  | n.s     |
| EU1347 | <i>spd-2(or655)</i> | 59.16 (120)                        | 13 (161) | n.s     |
| EU779  | <i>mex-1(or286)</i> | 79.8 (124)                         | 13 (161) | n.s     |

**Table S3. Three mutants with high embryonic lethality do not show significantly enhanced lethality in hypoxia**

*plk-1(or683)*, *spd-2(or655)* and *mex-1(or286)* mutants do not exhibit significant increase in embryonic lethality under hypoxia. n.s. - not significant.

**Table S4. Strains used in this study**

| <b>Strain name</b> | <b>Genotype</b>  |
|--------------------|--|
| N2                 | Wild type  |
| MT14119            | <i>mir-35-41(nDf50)</i>  |
| VC514              | <i>mir-35(gk262)</i>   |
| ZG31               | <i>hif-1(ia4)</i>  |
| RJP1355            | <i>mir-35-41(nDf50); rpEx604(pmir-35::mir-35, elt-2::gfp)</i>                                      |
| RJP1357            | <i>mir-35-41(nDf50); rpEx606(pmir-35::mir-35, elt-2::gfp)</i>                                      |
| EU1441             | <i>plk-1(or683)</i>  |
| EU1347             | <i>spd-2(or655)</i>  |
| EU779              | <i>mex-1(or286)</i>  |
| RJP1127            | <i>mir-35-41(nDf50); rpEx544(pmir-35::mir-35-41,elt-2::gfp)</i>                                    |
| RJP1128            | <i>mir-35-41(nDf50); rpEx545(pmir-35::mir-35-41,elt-2::gfp)</i>                                    |
| RJP1131            | <i>mir-35-41(nDf50); rpEx548(pmir-35-41::mir-35-41,elt-2::gfp)</i>                                 |
| RJP1439            | <i>rpEx630(pmir-35::NLS::YFP)</i>  |
| RJP1537            | <i>rpls33(psup-26::2NLS::YFP::SUP-26UTR)</i>   |
| VC1031             | <i>sup-26(gk403)III</i>  |
| VC901              | <i>sup-26 (gk426)III</i>   |
| VL413              | <i>wwIs8 [pmir-35-41::GFP + unc-119(+)]</i>  |
| RJP1731            | <i>rpEx735 (pmyo-2::mCherry unc-54;<br/>pmyo-2::GFP sup-26 3'UTR; pmyo-2::mir-35 (Line #1)</i>     |
| RJP1732            | <i>rpEx736 (pmyo-2::mCherry unc-54;<br/>pmyo-2::GFP sup-26 3'UTR; pmyo-2::mir-35 (Line #2)</i>     |
| RJP1733            | <i>rpEx737 pmyo-2::mCherry unc-54;<br/>pmyo-2::GFP sup-26 3'UTR(mut); pmyo-2::mir-35(Line #1)</i>  |
| RJP1734            | <i>rpEx738 (pmyo-2::mCherry unc-54;<br/>pmyo-2::GFP sup-26 3'UTR(mut); pmyo-2::mir-35(Line #2)</i> |

**Table S5. Primers used in this study**

| <b>Name</b>                  | <b>Sequence</b>                | <b>Function</b>                         |
|------------------------------|--------------------------------|---|
| KK155f                       | CGAGTGGACACGTTGCTCTA           | nDf50 genotyping                        |
| KK155r                       | ATGGACATTTGGGAGATGGA           | nDf50 genotyping                        |
| KK159: sup-26qPCRf           | CGCTTCGATACTATGTCAAAGG         | sup-26 qPCR                             |
| KK160: sup-26qPCRR           | CATCGAGTGCTTACTCTTCCT          | sup-26 qPCR                             |
| KK163: mir-35-41fprom        | ATCCGTCACGTCCTCAATC            | nDf50 rescue                            |
| KK164: mir-35-41r            | GGAGGCTCCAGACCTAGG             | nDf50 rescue                            |
| KK171: mir-35r               | AGACACTTTGGATGGTCTAGC          | nDf50 rescue                            |
| KK174: mir-35fqPCR           | CAGTCACCGGGTGGGA               | mir-35 qPCR                             |
| KK175: mir-35rqPCR           | CCAGTTTTTTTTTTTTTACTGCT        | mir-35 qPCR                             |
| KK176: mir-36fqPCR           | CACCGGGTGAAAATTCG              | mir-36 qPCR                             |
| KK177: mir-36rqPCR           | CCAGTTTTTTTTTTTTTTCATGC        | mir-36 qPCR                             |
| KK178: mir-37fqPCR           | GTCACCGGGTGAACAC               | mir-37 qPCR                             |
| KK179: mir-37rqPCR           | CAGTTTTTTTTTTTTTACTGCAAG       | mir-37 qPCR                             |
| KK180: mir-38fqPCR           | GCAATCACCGGGAGAA               | mir-38 qPCR                             |
| KK181: mir-38rqPCR           | TCCAGTTTTTTTTTTTTTACTCCA       | mir-38 qPCR                             |
| KK182: mir-39fqPCR           | TCACCGGGTGAAAATCAG             | mir-39 qPCR                             |
| KK183: mir-39rqPCR           | GGTCCAGTTTTTTTTTTTTTCAAG       | mir-39 qPCR                             |
| KK184: mir-40fqPCR           | GTCACCGGGTGTACATC              | mir-40 qPCR                             |
| KK185: mir-40rqPCR           | TCCAGTTTTTTTTTTTTTAGCTG        | mir-40 qPCR                             |
| KK186: mir-41fqPCR           | GTCACCGGGTGAAAATC              | mir-41 qPCR                             |
| KK187: mir-41rqPCR           | GGTCCAGTTTTTTTTTTTTTAGGT       | mir-41 qPCR                             |
| KK188: mir-42fqPCR           | GTCACCGGGTAAACATCT             | mir-42 qPCR                             |
| KK189: mir-42rqPCR           | GTCCAGTTTTTTTTTTTTTCTGT        | mir-42 qPCR                             |
| KK190: mir-86fqPCRref        | AGTAAGTGAATGCTTTGCCA           | mir-86 qPCR                             |
| KK191: mir-86rqPCRref        | GTCCAGTTTTTTTTTTTTTGTACT       | mir-86 qPCR                             |
| KK192: mir-248fqPCRref       | GATACACGTGCACGGA               | mir-248 qPCR                            |
| KK193: mir-248rqPCRref       | CAGTTTTTTTTTTTTTTGAGCGT        | mir-248 qPCR                            |
| KK194: mir-794fqPCRref       | CAGTGAAGTAATCATCGTTGT          | mir-794 qPCR                            |
| KK195: mir-794rqPCRref       | GTCCAGTTTTTTTTTTTTTGTAGTA      | mir-794 qPCR                            |
| KK196: mir-35fNheI           | GCTGCTAGCTCTCGGATCAGATCGAGCC   | mir-35-51 promoter region amplification |
| KK197: mir-35RNcoI           | CGACCATGGGGAAAAGATCGAGCCACTGC  | mir-35-51 promoter region amplification |
| KK198: mir-35fBamHI          | GCTGGATCCTCTCGGATCAGATCGAGCC   | mir-35-51 promoter region amplification |
| KK199: mir-35SacI            | CGAGAGCTCGAAAAGATCGAGCCACTGC   | mir-35-51 promoter region amplification |
| KK238 Promsup-26FhindIII     | GGGAAGCTTTTAGCTAGCTCGCAGAGCC   | sup-26 promoter region amplification    |
| KK239 Promsup-26RbamHI       | GGGTGGATCCCTTGAATTATTATGATGATG | sup-26 promoter region amplification    |
| KK240 Prommir-35FhindIII     | GGGAAGCTTATCCGTCACGTCCTCAATC   | mir-35-51 promoter region amplification |
| KK241 Prommir-35RbamHI       | GGGTGGATCCATAATAGTTGGGAATGG    | mir-35-51 promoter region amplification |
| KK243: sup-26-3'UTRfEcoRI    | GGGCGaattcATGGACAGGACAACGTC    | sup-26 3'UTR umplification              |
| KK244: sup-26-3'UTRrApaI     | GGTCggggcccCTCTGGAATCATTTATTAC | sup-26 3'UTR umplification              |
| KK281: sup-26-RNAiF          | GTTTTCCAGTCACGACGTT            | sup-26 RNAi                             |
| KK282: sup-26-RNAiR          | TGGATAACCGTATTACCGCC           | sup-26 RNAi                             |
| mir-35-41 specific rt primer | TCTCGGATCAGATCGAGCCA           | Pri-mir-35-41                           |
| Pri/pre mir-38 F             | GTGAGCCAGGTCCTGTTC             | Pri/pre-mir-38                          |
| Pri/pre mir-38 R             | TGAGTCACAGGTCCTACTC            | Pri/pre-mir-38                          |

## Supplementary References

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