Supplemental Data

The *Drosophila* MicroRNA Mir-14 Suppresses Cell Death and Is Required for Normal Fat Metabolism

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Supplemental Experimental Procedures

Fly Stocks

l(2)K10213 flies were obtained from the Bloomington Stock Center (BL 10982). A background lethal was recombined through crosses with *w1118*. We used transposon mobilization to generate both precise excisions as well as the *mir-14Δ¹* deletion. GMR-Rpr, GMR-Hid, GMR-Grim, GMR-Dronc, GMR-Strica, and GMR-ttk have been previously described [S1, S2]. GMR-mir-14 was generated by PCR amplification of a 118 bp genomic fragment containing the predicted mir-14 precursor (63 bp); primers 5′-GCGAATTCAACAACGTAACGTACTGCAACC-3′ and 5′-GCGGCGGCCGCTTGTGCCATGATGATACGC-3′ were used. The product was introduced into pGMR with EcoRI and Not I. Primers 5′-GCGGGATCCAATGTTGTCTTGCTGTGGGGC-3′ and 5′- AATTTCAGCGGCCGCGAAAATTGC-3′ were used to generate mir-14$^{+3.4\ kb}$ via PCR amplification of an approximately 3.4 kb genomic fragment containing the mir-14 precursor. The product was introduced into pCaSpeR4 with BamH I and Not I. Germline transformants were generated by standard techniques. Insertions of mir-14$^{+3.4\ kb}$ on the third chromosome, introduced into the *mir-14Δ¹* genetic background, were used to test for rescue of *mir-14Δ¹* phenotypes.

mir-14Δ¹ Lethal Phase

Late second- or young third-instar larvae of the indicated genotypes were collected. For each genotype, 100 larvae were immediately transferred into a vial containing standard fly food. Vials were scored each day for the number of larvae undergoing pupation and the number of adults that had eclosed. Each experiment was repeated ten times. The indicated values represent mean values. Error bars indicate the standard deviation.

Salt Stress Tolerance

Solid medium that contained 2.5% sucrose, 1% agar, 0.15% methyl paraben, 0.003% methylene blue, and different concentrations of NaCl (0 M, 0.1 M, 0.2 M, 0.4 M, or 0.8 M) was prepared in vials. Twenty late second- or young third-instar larvae of each genotype were introduced into these vials and scored for survival after 20 hr. Each experiment was repeated four times. The indicated values represent mean values. Error bars indicate the standard deviation.

Aging Test

Newly eclosed flies were separated according to sex and kept in fresh food vials (20 flies/vial, 10 vials/genotype). Flies were maintained under conditions of constant humidity and temperature, at 29°C. Flies were transferred to fresh food every 3 days and scored for the number of survivors. The indicated values represent mean values. Error bars indicate the standard deviation.

Plastic Sectioning

Fly heads were fixed and embedded for sectioning via standard procedures. Sections of 1 μm were stained with 1% toluidine blue and 1% Borax for 25 s at 40°C–50°C. Samples were mounted and photographed on an Olympus BX-60.

Lipid Analysis

Lipid analysis was carried out on 100 3- to 4-day-old adult female flies by Lipomics Technologies. (West Sacramento, CA), as described [S3]. Values in Figure 4 and Figure S2 are means of three to four independent extractions. Error bars indicate the standard deviation.

Genes with candidate binding sites for mir-14

Gene	Binding site location in mRNA	Formed structure

Drice	3' UTR	`     t   t    -t    c`
		`tgggg ggg agag  agat ga`
		`ATCCT CTC TCTT  TCTG CT`
		`     -   -    TT    A`

DCP1	5' UTR	`gc               ag`
		`  ggagagagaaaagga  ga`
		`  CCTCTCTCTTTTTCT  CT`
		`AT               GA`

Grim	3' UTR	`   t          tct`
		`tgg ggagagaaaa   ttgg`
		`ATC TCTCTCTTTT   GACT`
		`   C          TCT`

Scythe	3' UTR	`       c   tac      g`
		`taggggg aga   ggagac ga`
		`ATCCTCT TCT   TTTCTG CT`
		`       C   --T      A`

SkpA	3' UTR	`       ttc     c  -`
		`tagggga   gggaa ga attgg`
		`ATCCTCT   CTCTT TT TGACT`
		`       ---     T  C`

CG1942 (mgat)	5' UTR	`       accgcc       ca`
		`tgggaga       gaaagag  gctgg`
		`ATCCTCT       CTTTTTC  TGACT`
		`       ----CT       --`

Other tested genes without obvious binding sites

Dronc Strica Daydream Dredd Decay	Reaper Hid Sickle	DIAP1 DIAP2 Deterin	Debcl Buffy Dark	dFadd UbcD1 Morgue

Figure S1. Analysis of Potential Mir-14 Target Sites in *Drosophila* Cell Death Regulators

Upper panel: structures between mir-14 sequences (upper case) and sequences within target genes (lower case) were predicted with the mfold program. The locations of the predicted structures in the indicated target genes are either in the 5′ untranslated region (5′UTR) or in the 3′ untranslated region (3′UTR). Lower panel: list of other *Drosophila* apoptosis regulators. Transcripts for these genes were examined for potential mir-14 target sites as above. No obvious target sites were identified.

Western Blotting

Adult flies less than 24 hr old were homogenized in SDS-PAGE loading buffer and processed for SDS-PAGE and Western blotting via standard procedures. Anti-Drice antibody [S4] was used at a 1:2000 dilution. Blots probed with anti-Drice were stripped and reprobed with anti-lamin rabbit antibody [S5], 1:10,000, to provide a protein loading control.

miRNA Binding Site Searching

cDNA sequences were loaded in the GCG sequence analysis package [S6]. We analyzed the sequences for potential mir-14 binding sites by using a serial selection window of 50 nt with 5 nt per step through the entire mRNA sequence. Each 50 nt sequence was joined in tandem with the mature mir-14 sequence. These were then processed in the GCG RNA folding program mfold.

Supplementary References

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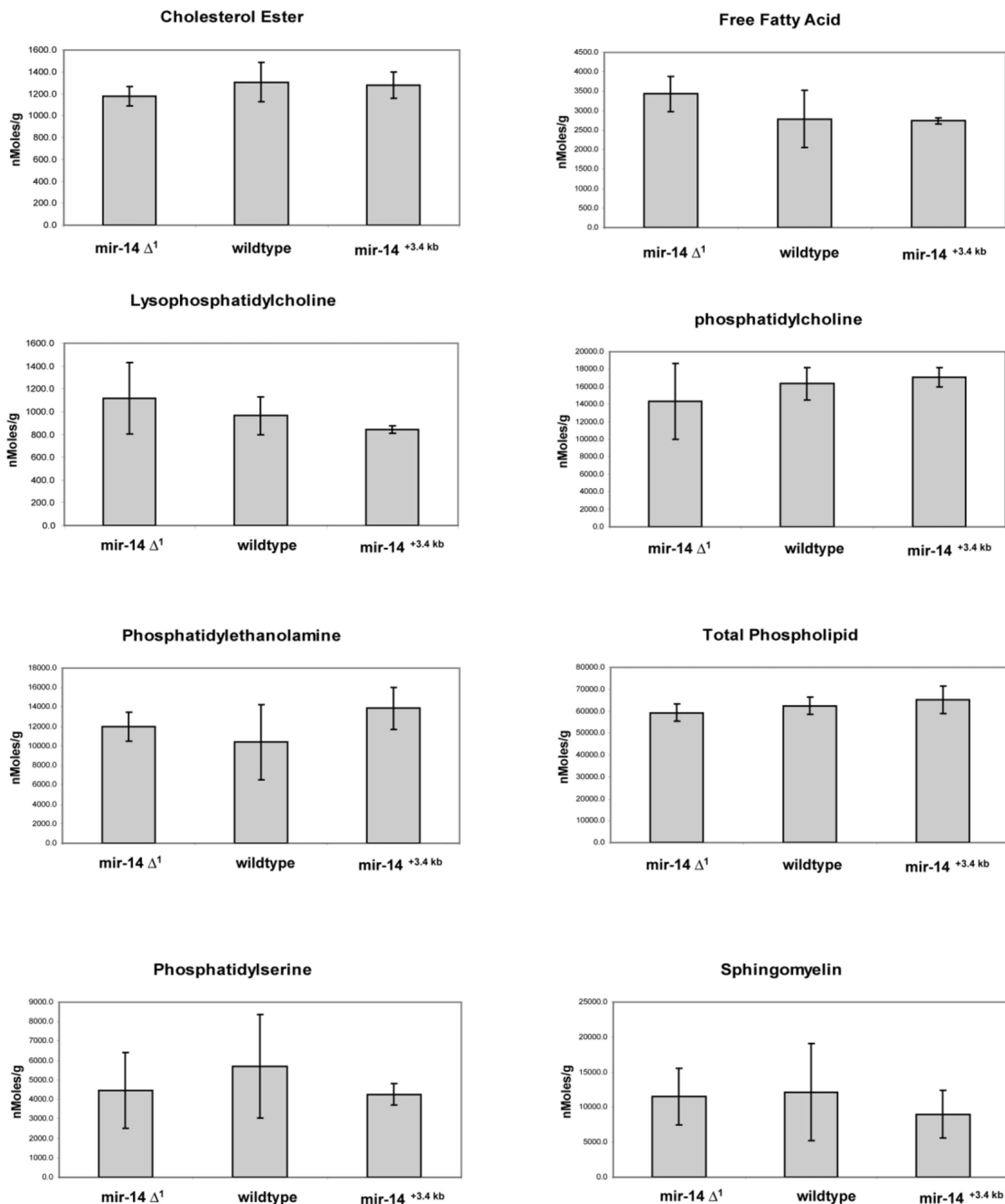

Figure S2. Lipid Analysis of Flies Carrying Different Copy Numbers of Mir-14

Lipid concentrations (nmoles/g of protein) were measured for a number of different lipid classes. No significant differences were observed among *mir-14*Δ^1 homozygotes, wild-type flies, and flies carrying four copies of mir-14 (two copies of mir-14$^{+3.4\ Kb}$ in a wild-type background). The indicated values are the means from three to four independent extractions and analyses. N = 100 animals for each genotype in each experiment. The animals used for these experiments were the same as those utilized in the experiments described in Figure 4C in the main text. Error bars indicate the standard deviation.

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S2. Vernooy, S.Y., Chow, V., Su, J., Verbrugghe, K., Yang, J., Cole, S., Olson, M.R., and Hay, B.A. (2002). *Drosophila* Bruce can potently suppress Rpr- and Grim-dependent but not Hid-dependent cell death. Curr. Biol. *12*, 1164–1168.

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S4. Dorstyn, L., Read, S., Cakouros, D., Huh, J.R., Hay, B.A., and Kumar, S. (2002). The role of cytochrome c in caspase activation in *Drosophila melanogaster* cells. J. Cell Biol. *156*, 1089–1098.

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