

Supplemental Material to:

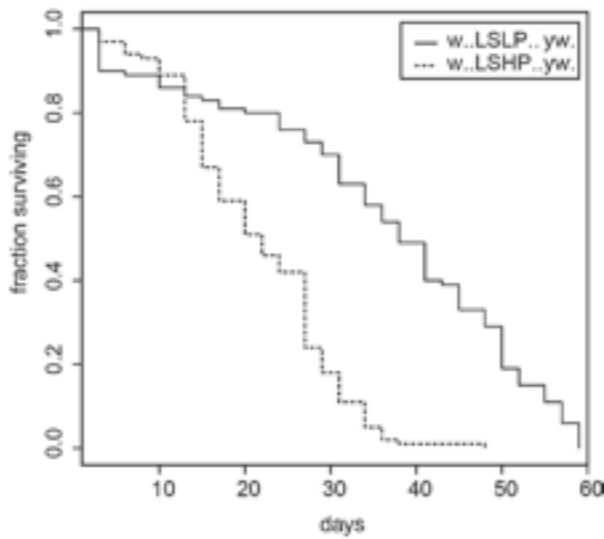
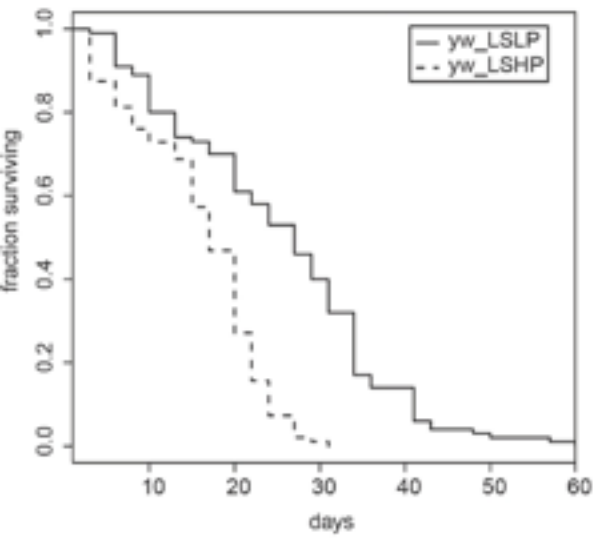
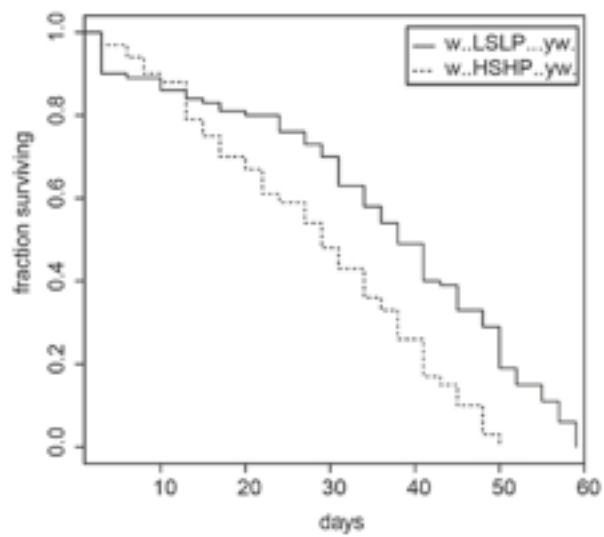
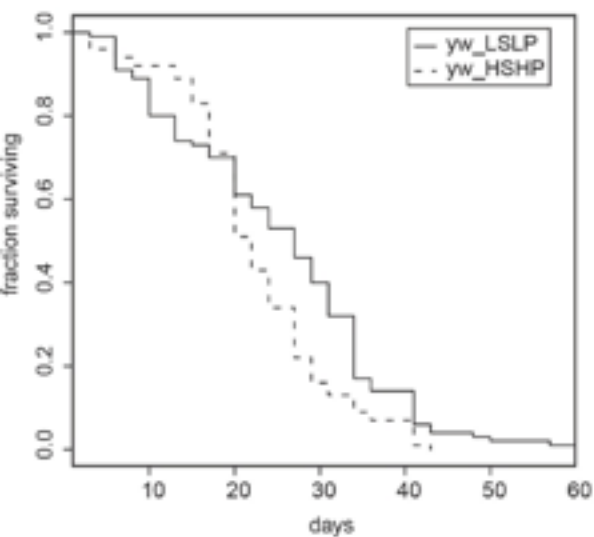
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***Drosophila* miR-277 controls branched-chain
amino acid catabolism and affects lifespan**

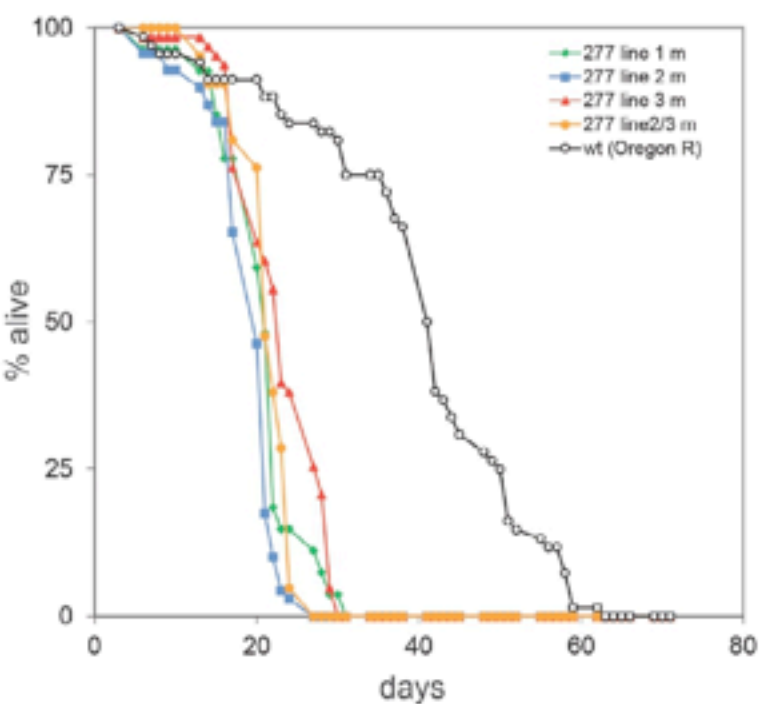
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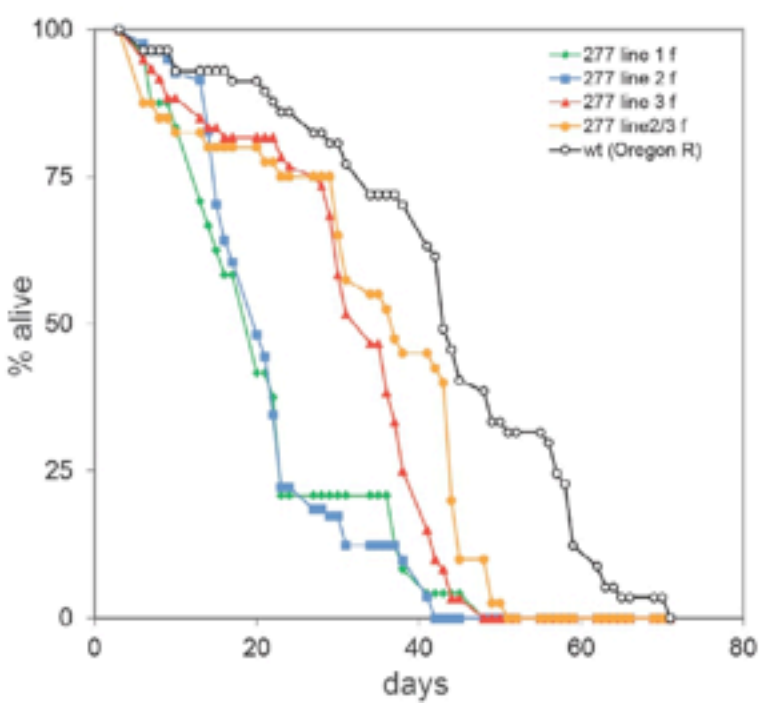
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male flies



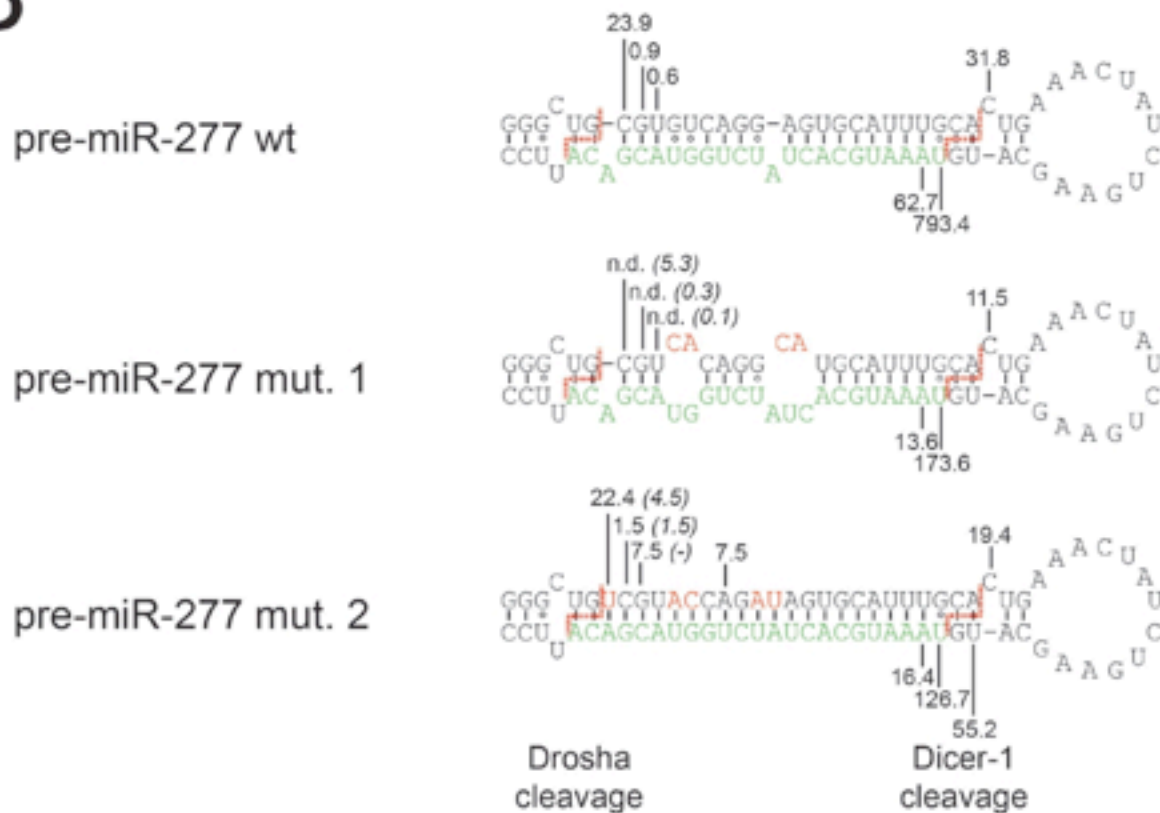
female flies

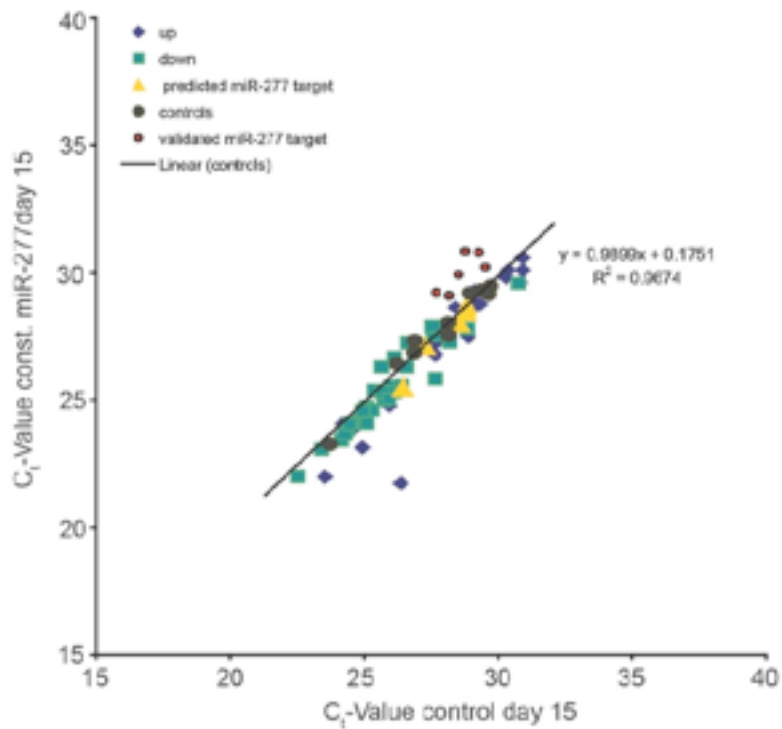


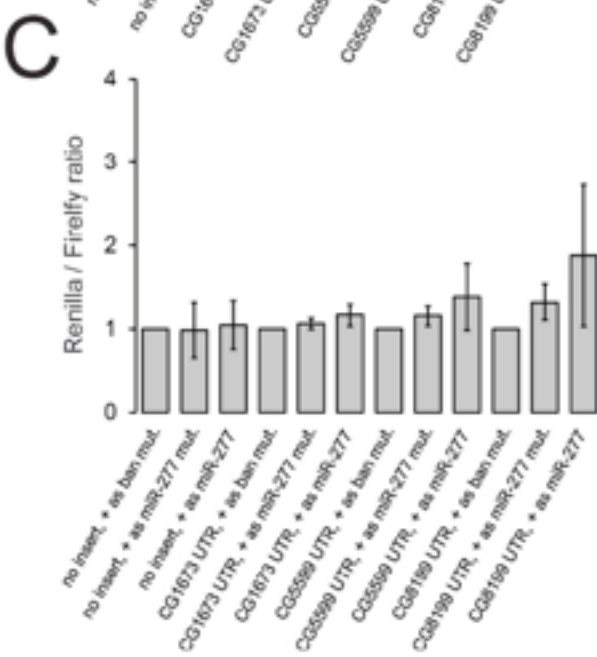
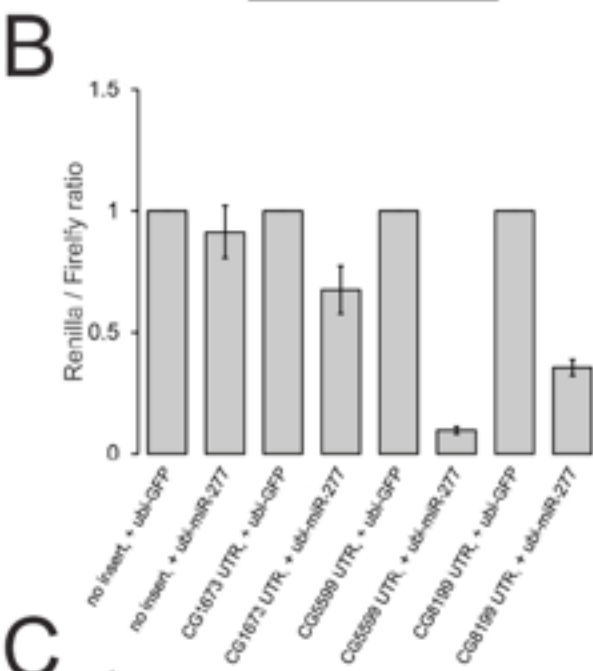
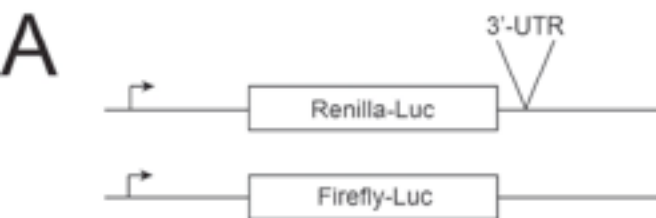
A

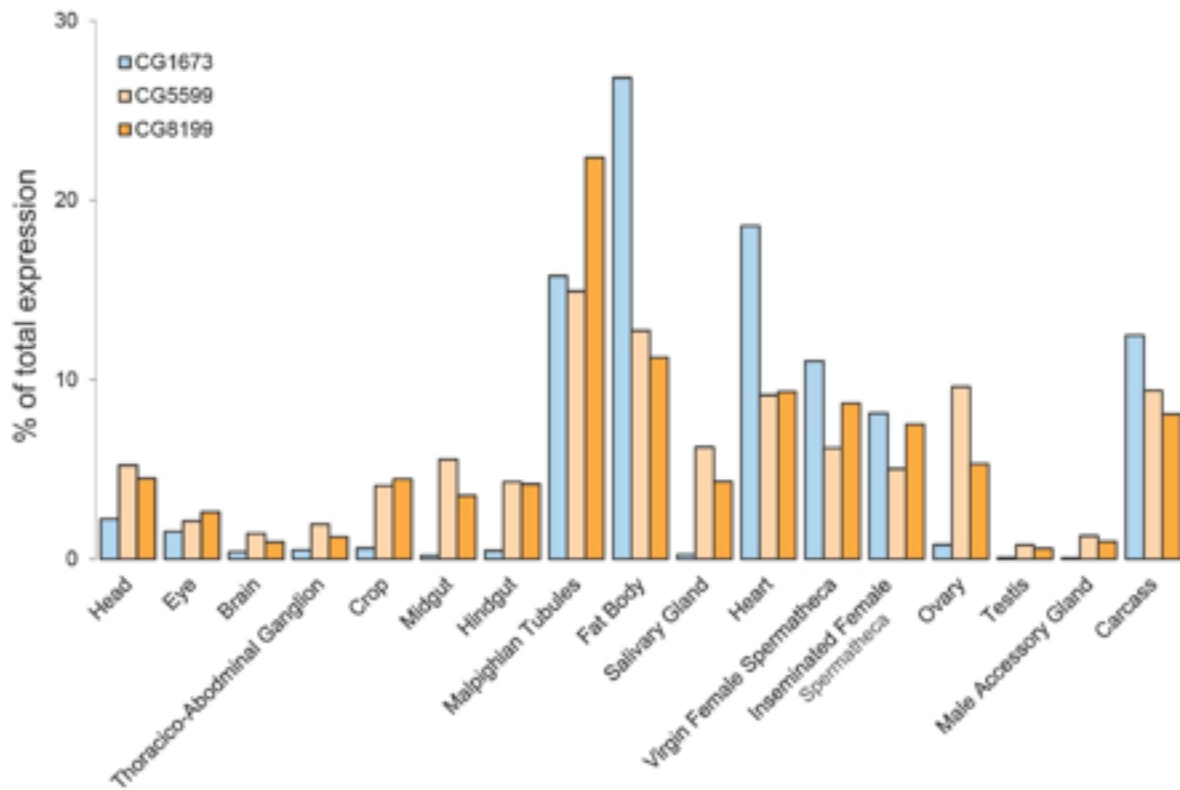
	<i>total reads</i>	<i>genome matching</i>	<i>mature miRNAs</i>	<i>miR 277 mature</i>
miR-277 wt	24'422'705	17'425'600 (71.4 %)	2'735'413 (11.2 %)	16'166 (0.7 %)
miR-277 mut. 1	9'490'975	7'506'412 (79.1 %)	2'087'243 (22.0 %)	1'561 (0.2 %)
miR-277 mut. 2	1'079'302	670'894 (62.6 %)	234'676 (21.7 %)	179 (0.2 %)

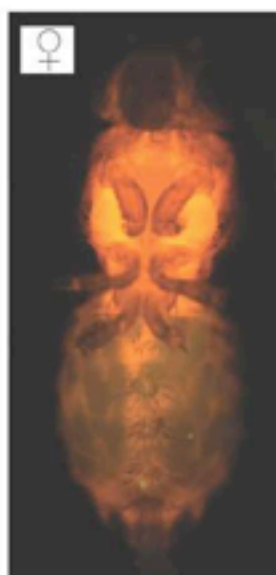
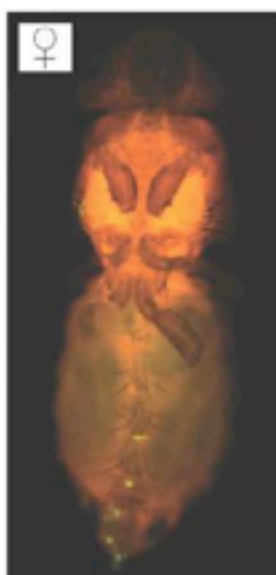
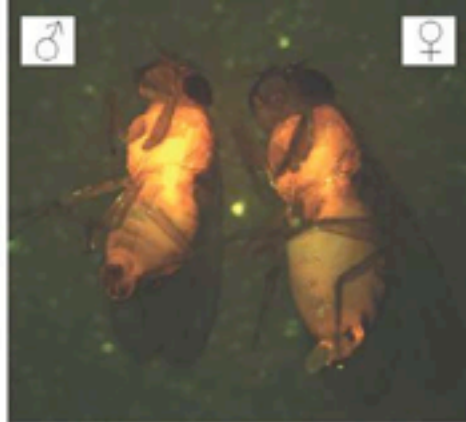
B









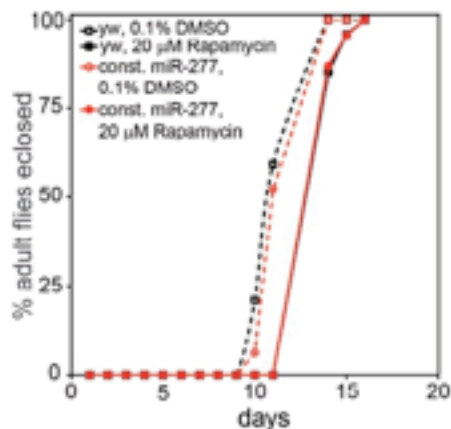


A



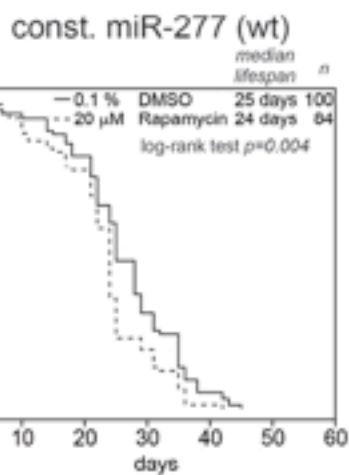
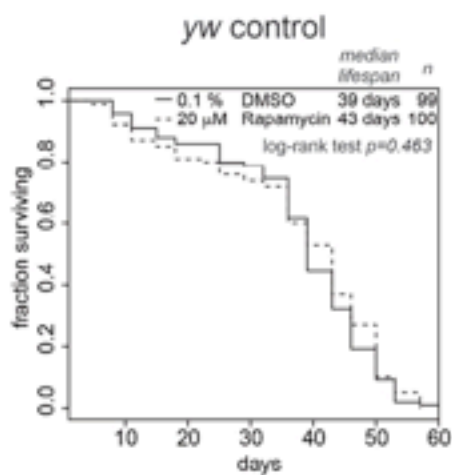
control
(0.1% DMSO)

20 μ M Rapamycin
(in 0.1% DMSO)

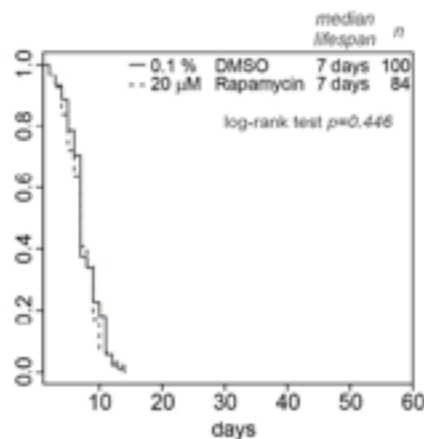
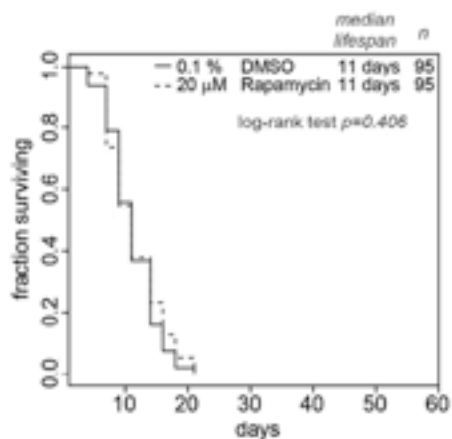


B

standard
fly food



LSHP
fly food



Supplemental Figure 1. Survival curves of control flies on the three different food compositions. The survival curves compare LSLP food (= caloric restriction) to rich (HSHP) and imbalanced (LSHP) food. The panels on the left show data from *yw* flies, the panels on the right show data from *w¹¹¹⁸* back-crossed into *yw*. The lifespan data shown here are identical to the survival curves in Fig. 2 of the manuscript but different comparisons are shown.

Supplemental Figure 2. Validation of lifespan shortening induced by constitutive miR-277 with three independent transgenic lines as well as a trans-heterozygous combination. Flies were maintained on standard laboratory medium for these experiments.

Supplemental Figure 3. Deep sequencing of small RNAs derived from wild-type and mutant pre-miR-277 hairpins. (A) The table shows the read numbers and general mapping results obtained for our libraries. (B) Normalized read numbers (expressed as ppm of genome matching) are mapped to the structure of the hairpin. The position indicates the 5'-end of the small RNA sequenced; in the case of the mutant hairpins, the numbers in parenthesis indicate how many reads matched the wild type sequence at the analogous position.

Supplemental Figure 4. Validation of mRNA changes induced by miR-277 with qRT-PCR. A panel of genes known to be up- or down-regulated with age as well as a set of controls were quantified by qRT-PCR. In addition, we profiled several predicted miR-277 targets (see suppl. Table 4 for genes and primer sequences). Down-regulation of miR-277 targets that were validated in our transcriptomic analysis is clearly visible, while the non-validated targets did not respond to elevated miR-277 levels on day 15.

Supplemental Figure 5. Impact of miR-277 on the BCAA catabolic pathway. Gene names indicated in red represent mRNAs that responded significantly to elevated miR-277 levels in flies (left panel) or miR-277 inhibition in S2-cells (right panel). The first enzyme of the pathway (CG1673 = BCAT) is a predicted miR-277 target but did not respond in either case.

Supplemental Figure 6. The 3'-UTR of CG1673 is less sensitive to miR-277 overexpression. (A) Schematic representation of the reporter design. (B) Co-transfection of the reporter (Renilla) and control (Firefly) Luciferase expression vectors with a miR-277 overexpression plasmid efficiently represses the CG5599 and CG8199 3'-UTRs but not the one of CG1673. (C) Inhibition of endogenous miR-277 in S2-cells only yielded a trend that is consistent with the observations in (B).

Supplemental Figure 7. Tissue distribution of three enzymes that degrade threonine. Expression levels of threonine-ammonia-lyase (CG8129), threonine aldolase (CG10184) and threonine-3-dehydrogenase (CG5955) based on expression data available through Flybase.

Supplemental Figure 8. Expression of miR-277 in the thorax. We cloned a 4 kb fragment upstream of miR-277 into the Gal4-expression vector pPTGal4, then generated transgenic fly lines by P-element mediated transgenesis. The miR-

277_{pr}-Gal4 driver lines were then crossed to UAS-DsRed reporter flies and expression of DsRed was imaged with a Leica fluorescence stereomicroscope. Strong expression in the thorax of both male and female flies was seen with three independent Gal4 driver lines.

Supplemental Figure 9. Rapamycin feeding did not extend life span of male flies.

(A) The efficacy of our rapamycin supplementation was validated by the characteristic delay in larval development (left) and the resulting delay in eclosion (right). (B) No life span changes were observed in rapamycin fed flies compared with DMSO controls for both wild type (left) and transgenic miR-277 expressing flies (right).

Gene	Position	P-value	T	Q	Q&T	Q&T/Q	Q&T/T	term ID	term domain and name
HP:0000007	4.17e-05	741	367	53	0.869	0.072	HP:0000007	hp	Autosomal recessive inheritance (1)
HP:0002017	1.10e-05	117	367	18	0.295	0.154	HP:0002017	hp	Nausea and vomiting (1)
HP:0002013	9.66e-06	116	367	18	0.295	0.195	HP:0002013	hp	Vomiting (2)
HP:0001946	6.74e-06	22	367	8	0.131	0.364	HP:0001946	hp	Ketosis (1)
MI:dme-miR-184*	4.61e-04	300	368	20	0.072	0.067	MI:dme-miR-184*	mi	MI:dme-miR-184* (1)
MI:dme-miR-277	1.65e-19	421	368	52	0.187	0.124	MI:dme-miR-277	mi	MI:dme-miR-277 (1)
REAC:485617	1.62e-10	109	345	23	0.319	0.211	REAC:485617	re	Metabolism of amino acids (1)
REAC:485660	3.23e-15	20	345	14	0.194	0.700	REAC:485660	re	Branched-chain amino acid catabolism (2)
REAC:485662	1.15e-05	8	345	5	0.069	0.625	REAC:485662	re	Valine catabolism (3)
REAC:485679	4.93e-08	10	345	7	0.097	0.700	REAC:485679	re	Leucine catabolism (3)
REAC:485659	1.87e-06	10	345	6	0.083	0.600	REAC:485659	re	Isoleucine catabolism (3)
REAC:485811	1.33e-04	34	345	8	0.111	0.235	REAC:485811	re	Phase 1 functionalization (1)
REAC:485810	6.49e-05	31	345	8	0.111	0.258	REAC:485810	re	Cytochrome p450 (2)
REAC:486182	2.87e-05	28	345	8	0.111	0.286	REAC:486182	re	P450 Prostaglandin isomerizations (3)
REAC:485972	5.00e-05	30	345	8	0.111	0.267	REAC:485972	re	P450 Hydroxylations (3)
REAC:485971	2.87e-05	28	345	8	0.111	0.286	REAC:485971	re	Simple hydroxylation (4)
REAC:485809	5.00e-05	30	345	8	0.111	0.267	REAC:485809	re	P450 Epoxidations (3)
REAC:478368	2.87e-05	28	345	8	0.111	0.286	REAC:478368	re	Aflatoxin B1 oxidized to aflatoxin-8,9-oxide (4)
REAC:485992	3.82e-05	29	345	8	0.111	0.276	REAC:485992	re	P450 Dehydrogenation of alkanes to form alkenes (3)
REAC:481219	2.87e-05	28	345	8	0.111	0.286	REAC:481219	re	Dehydrogenation of Testosterone to form 6-Dehydrotestosterone (4)
REAC:485967	8.32e-05	32	345	8	0.111	0.250	REAC:485967	re	Cytochrome P450 - arranged by substrate type (1)
REAC:485973	2.87e-05	28	345	8	0.111	0.286	REAC:485973	re	Fatty acids (2)
REAC:480926	2.87e-05	28	345	8	0.111	0.286	REAC:480926	re	CYP4F12 hydroxylates arachidonic acid (3)
REAC:480893	2.87e-05	28	345	8	0.111	0.286	REAC:480893	re	CYP4B1 can 12-hydroxylate arachidonic acid (3)
REAC:480862	2.87e-05	28	345	8	0.111	0.286	REAC:480862	re	CYP4A11 omega-hydroxylates laurate (3)
REAC:485974	2.87e-05	28	345	8	0.111	0.286	REAC:485974	re	Eicosanoids (2)
REAC:480957	2.87e-05	28	345	8	0.111	0.286	REAC:480957	re	CYP4F2 omega-hydroxylate leukotriene B4, thus inactivating it (3)
REAC:485969	6.49e-05	31	345	8	0.111	0.258	REAC:485969	re	Xenobiotics (2)
REAC:480958	2.87e-05	28	345	8	0.111	0.286	REAC:480958	re	CYP4F8 hydroxylates prostaglandin H2 (3)
REAC:485969	6.49e-05	31	345	8	0.111	0.258	REAC:485969	re	Xenobiotics (2)
REAC:480829	2.87e-05	28	345	8	0.111	0.286	REAC:480829	re	CYP3A5 oxidises aflatoxin B1 to aflatoxin-8,9-oxide (3)
REAC:480831	2.87e-05	28	345	8	0.111	0.286	REAC:480831	re	CYP3A7 can 6beta-hydroxylate testosterone (3)
REAC:480737	2.87e-05	28	345	8	0.111	0.286	REAC:480737	re	CYP3A4 can N-demethylate loperamide (3)
REAC:485970	6.49e-05	31	345	8	0.111	0.258	REAC:485970	re	Unknown (2)
REAC:480798	2.87e-05	28	345	8	0.111	0.286	REAC:480798	re	CYP3A43 catalyzes the 6beta-hydroxylation of testosterone (3)
REAC:480924	2.87e-05	28	345	8	0.111	0.286	REAC:480924	re	CYP4F11 omega-hydroxylates 3-hydroxypalmitate (3)
REAC:486052	1.57e-04	44	345	9	0.125	0.205	REAC:486052	re	Prostanoid hormones (1)
REAC:485186	2.87e-05	28	345	8	0.111	0.286	REAC:485186	re	Thromboxane synthase (CYP5A1) mediates the isomerization of prostaglandin H...
REAC:485576	1.65e-04	112	345	15	0.208	0.134	REAC:485576	re	Metabolism of lipids and lipoproteins (1)
REAC:485615	1.57e-04	7	345	4	0.056	0.571	REAC:485615	re	Ketone body metabolism (2)