Supplemental Figures and legends

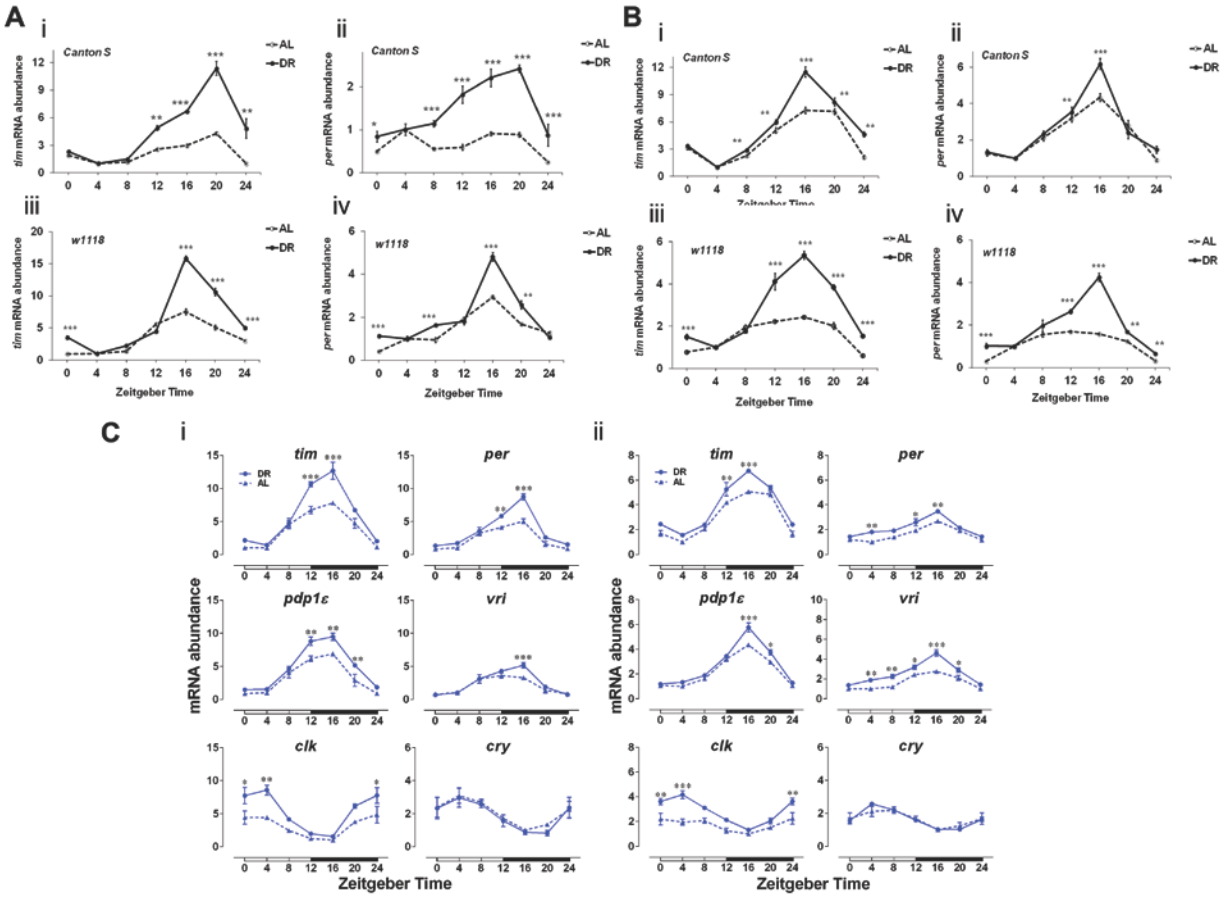

Figure S1, related to Figure 1. Increase in amplitude of circadian gene expression upon DR. **(A)** Increased amplitude of circadian gene expression after dietary restriction in fat bodies isolated from female flies. **(B)** Increased amplitude of circadian gene expression after dietary restriction in male flies. Daily mRNA profiles of core clock genes *tim* and *per* in control **(i and ii)** *Canton S* and **(iii and iv)** *w1118* flies fed on AL (5% YE, 5% sugar) and DR (0.5% YE, 5% sugar) diets for 10 days. Data are normalized to the ZT4 values set at 1 for flies on each gene. **(C)** DR improves the amplitude of circadian gene expression in old flies. Daily mRNA profiles of clock genes in **(i)** heads and **(ii)** bodies of *Canton S* females fed on AL or DR diets for 30 days, starting on day 3. The data are normalized to the trough (ZT4/16) values set at 1 for flies on AL diet. White and black horizontal bars mark periods of light and dark respectively. Each data point represents mean ± SEM of three independent RNA samples. Statistical significance between AL and DR values was determined using two-way ANOVA with Bonferonni's post hoc test, and is denoted by ***p< 0.001, **p<0.01, and *p<0.05.

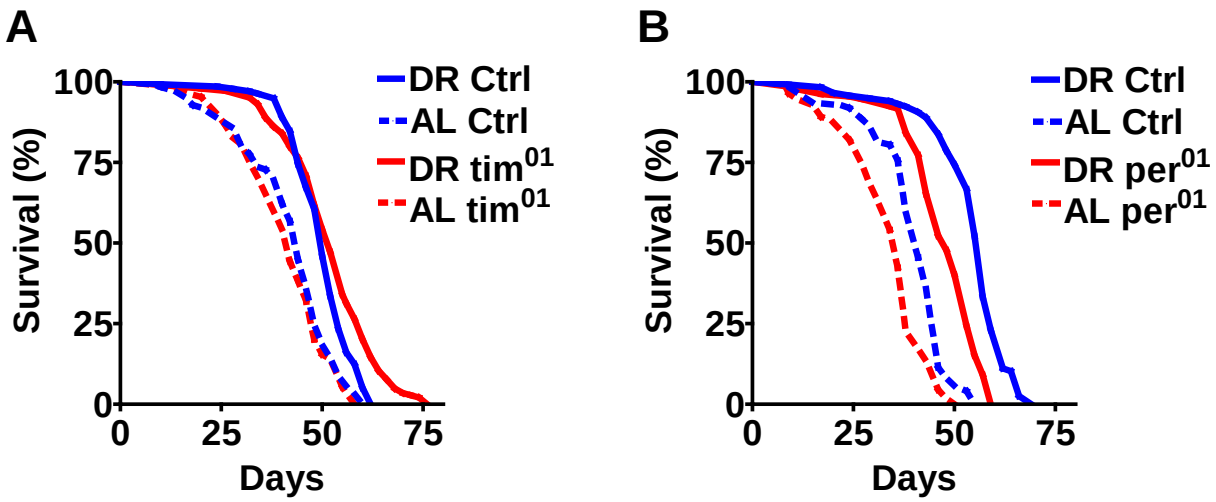

Figure S2, related to Figure 3. Male *tim*^{01} and *per*^{01} flies respond to DR in a similar way as controls. (A) *tim*^{01} mutant males and controls showed similar response to DR. Kaplan Meier survival analysis for in control flies (blue) and *tim*^{01}flies (red) under DR (solid line) and AL (dashed line) conditions. Following median lifespan were observed for control DR (50 days (n=137) and control AL (44 days (n=125)); *tim*^{01} DR (52 days (n=144) and *tim*^{01}AL (42 days (n=149)). **(B)** DR dependent increase in lifespan was similar in male *per*^{01} and control flies. Kaplan Meier survival analysis for control flies (blue) and *per*^{01} flies (red) under DR (solid line) and AL (dashed line) conditions. Following median lifespan were observed for control DR (57 days (n=117) and control AL (41 days (n=123)); *per*^{01} DR (48 days (n=132) and *per*^{01} AL (36 days (n=111)).

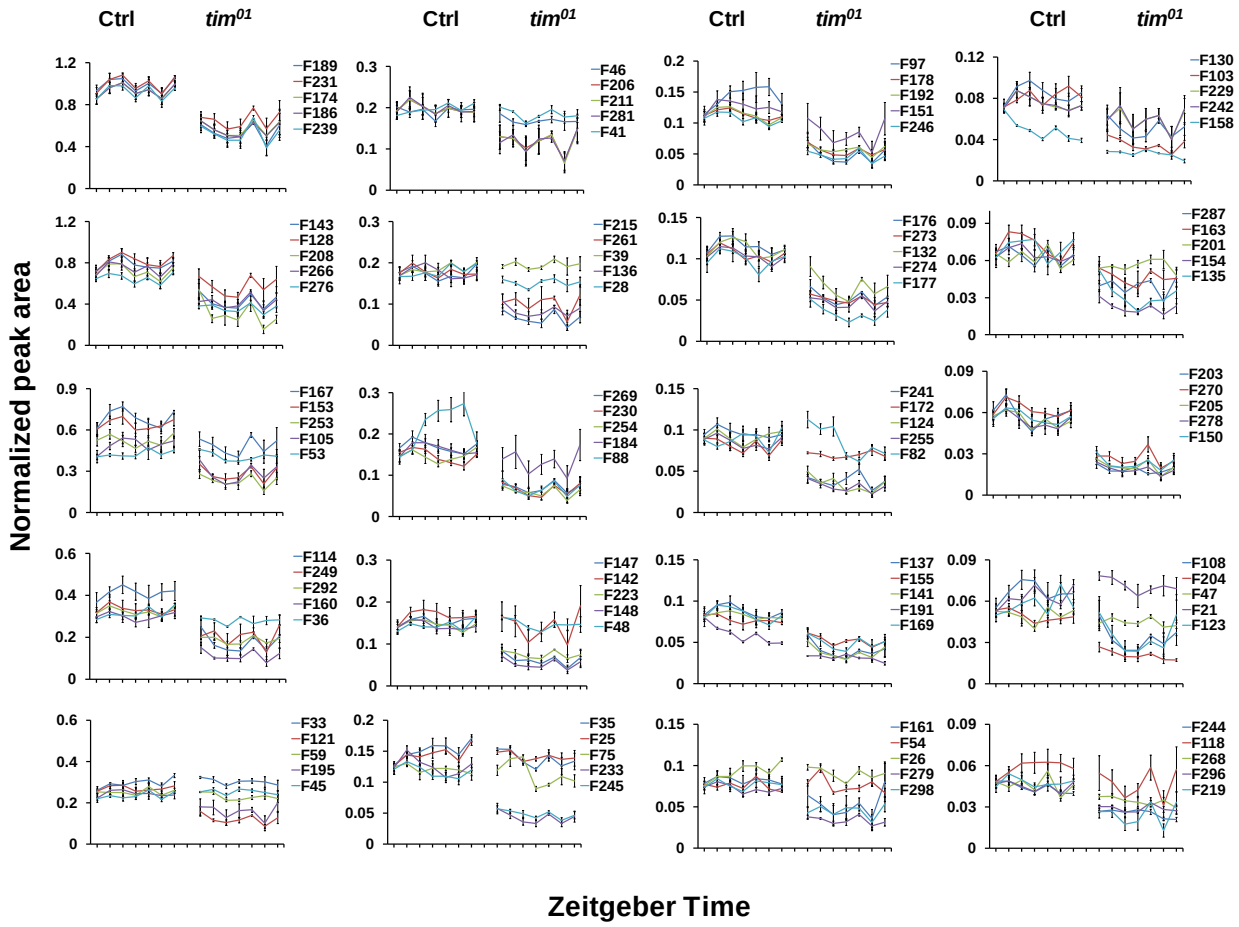

Figure S3, related to Figure 4. Daily quantitative profiles of 250 lipid like features in control and *tim*^{01} flies on DR. Lipids were extracted from adult female flies fed DR diet for 10 days. The lipids were separated and analyzed by LC-MS/MS and were normalized for both internal standards and fly wts. Error bars indicate S.E.M of 4 independent biological repeats.

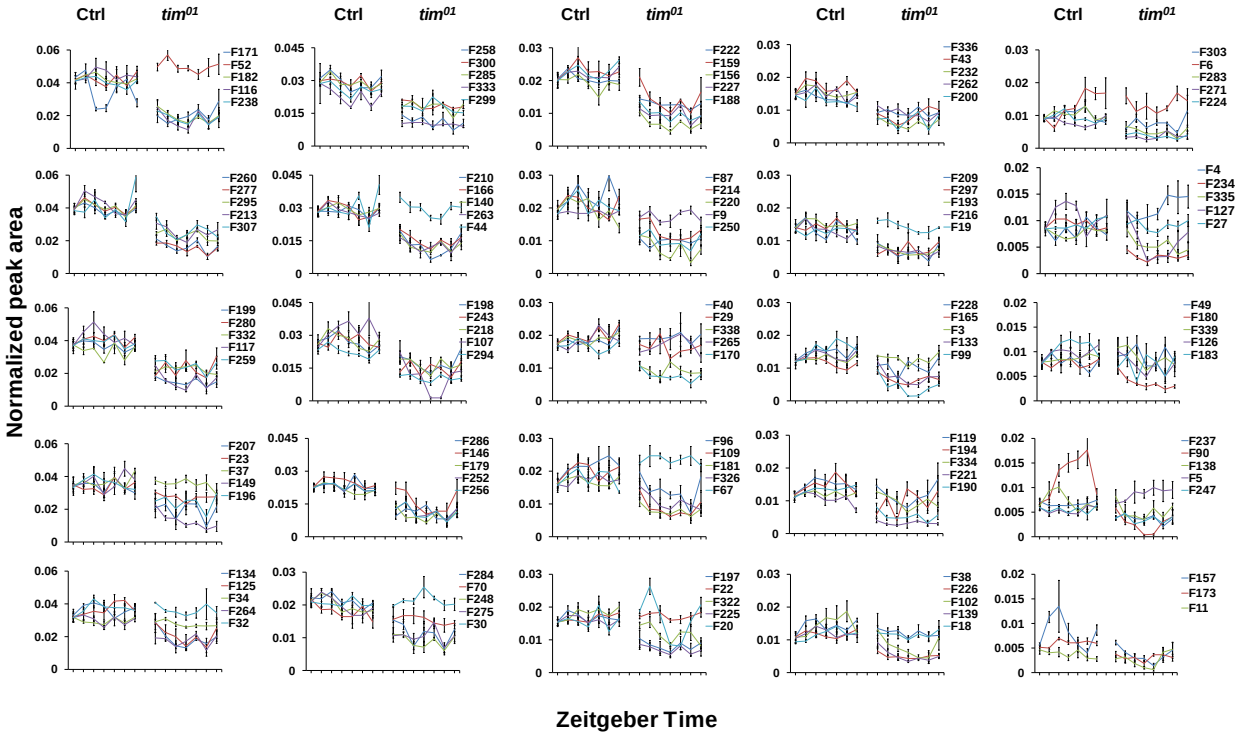

Figure S3, related to Figure 4 (Continued). Daily quantitative profiles of 250 lipid like features in control and *tim*^{01} flies on DR. Lipids were extracted from adult female flies fed DR diet for 10 days. The lipids were separated and analyzed by LC-MS/MS and were normalized for both internal standards and fly wts. Error bars indicate S.E.M of 4 independent biological repeats.

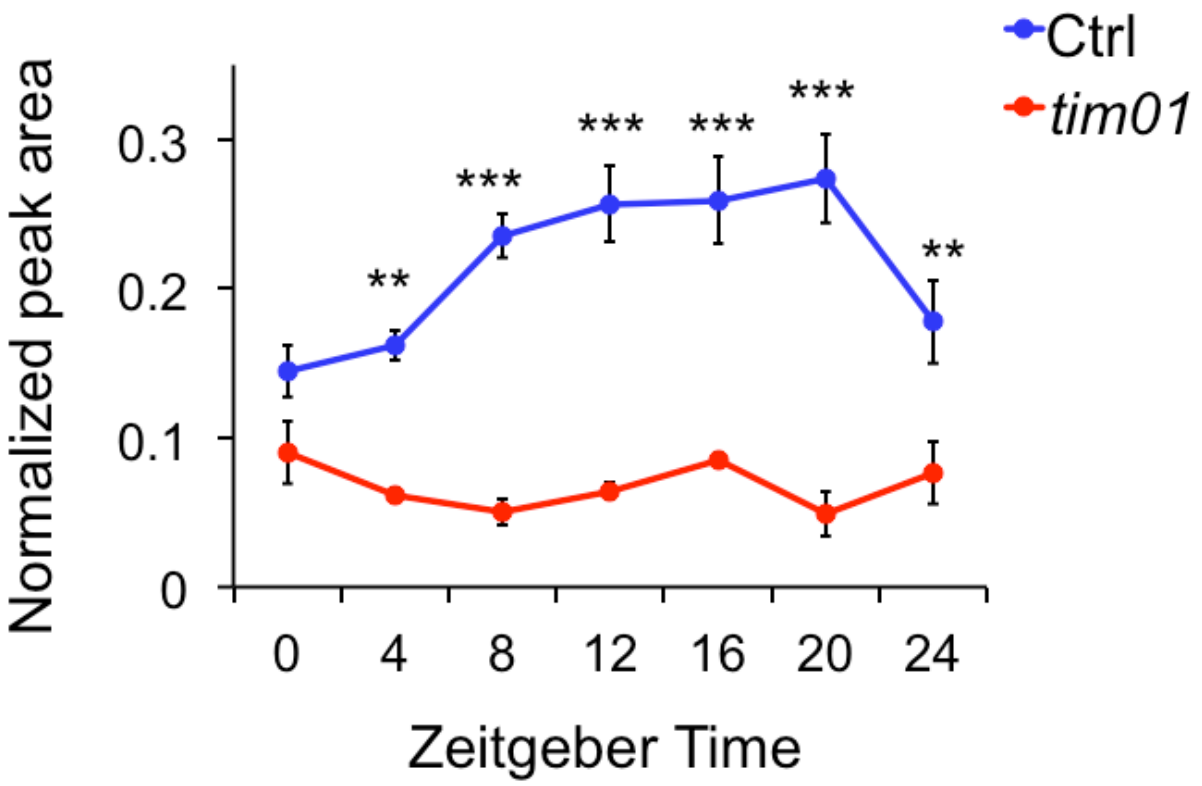

Figure S4, related to Figure 4. Daily quantitative profiles of trilauryl glycerol (TLG, feature 88) in control and *tim*^{01} flies on DR. Lipids were extracted from control and *tim*^{01} female flies fed DR diet for 10 days. The lipids were separated and analyzed by LC-MS/MS and were normalized for both internal standards and fly wts. Error bars indicate S.E.M of 4 independent biological repeats. Statistical significance between the two groups was determined using two-way ANOVA with Bonferonni's post hoc test, and is denoted by ***p< 0.001, **p<0.01, and *p<0.05.

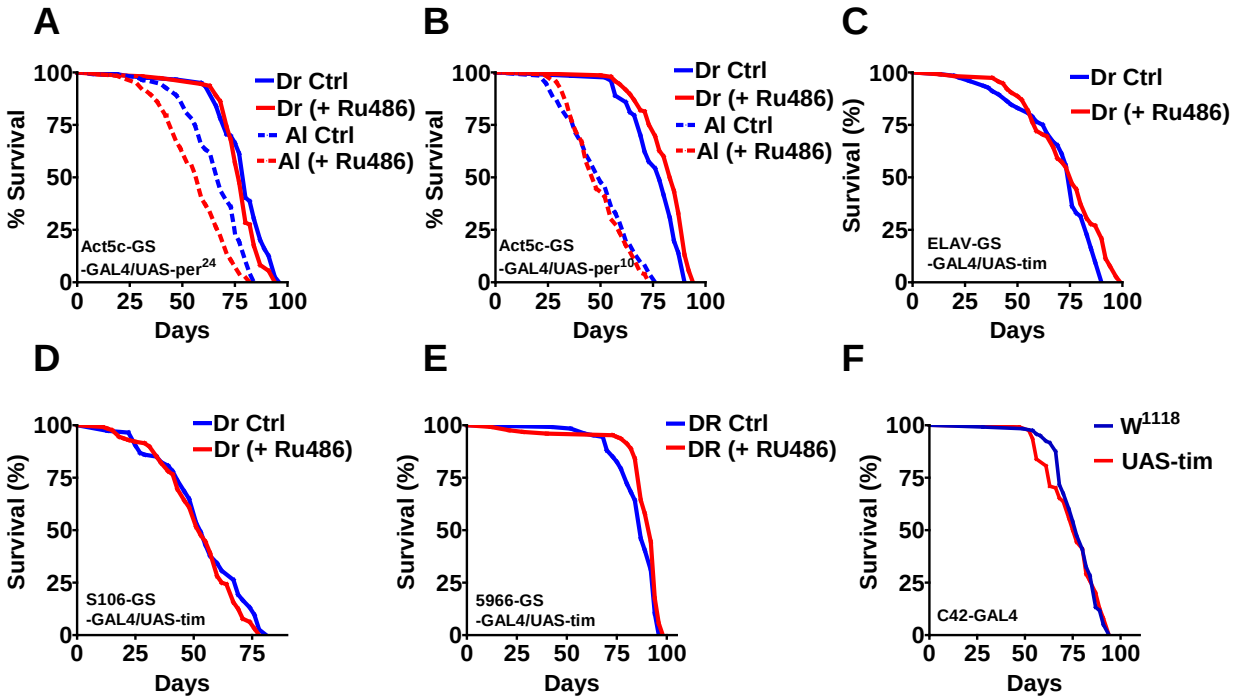

Figure S5, related to Figure 5. Overexpression of *per* and *tim* had no effect on lifespan under DR conditions. (A) Overexpression of *UAS-per*^{24} had no effect on lifespan under DR conditions but reduced lifespan under AL conditions. Kaplan Meier survival analysis for control flies (without RU486, blue) and *per*^{24}overexpression flies (with RU486, red) under DR (solid line) and AL (dashed line) conditions. Following median lifespan were observed for control DR (80 days (n=119) and AL (68 days (n=139)); *per*^{24}overexpression, DR (77 days (n=109) and AL (59 days (n=122)). **(B)** Overexpression of *UAS-per*^{10} had no effect on lifespan under DR or AL conditions. Kaplan Meier survival analysis for control flies (without RU486, blue) and *tim* overexpression flies (with RU486, red) under DR (solid line) and AL (dashed line) conditions. Following median lifespan were observed for control DR (78 days (n=127) and AL (50 days (n=136)); *per*^{10} overexpression, DR (83 days (n=145) and AL (48 days (n=130). **(C-F)** Overexpression of *tim* has no effect on lifespan on a DR diet. Overexpression of *tim* had no effect on lifespan under DR conditions when over-expressed specifically in **(C)** neurons, **(D)** fat body **(E)** gut **(F)** and Malpighian tubules. Statistical analysis of the survival curves, genotype and the number of flies used in each group are provided in Table S2. For additional independent repeats of the data see Table S3.

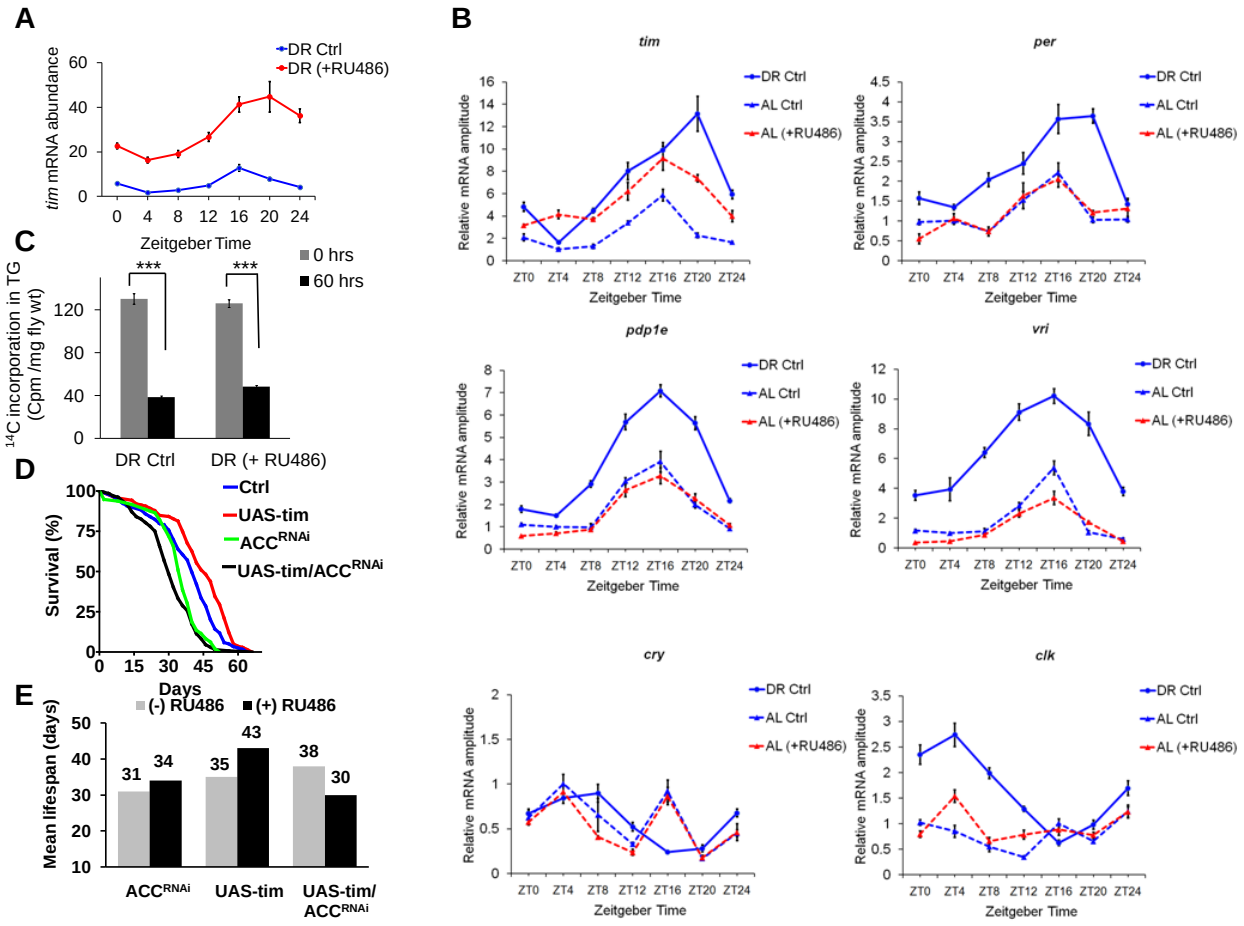

Figure S6, related to Figure 6. Overexpression of *tim* increases expression, fat metabolism and lifespan in a diet dependent manner. (A) Overexpression of *tim* increased the magnitude of expression of *tim* mRNA on a DR diet. Circadian expression of *tim* mRNA levels under induced and un-induced conditions. The data are normalized to the trough (ZT4) levels seen on an AL (-RU486) diet. The error bars indicate S.E.M of 3 independent preparations. **(B)** Overexpression of *tim* has no effect on the expression of other clock genes. Daily mRNA concentration profiles of core clock genes in flies overexpressing *tim* in the whole body. The data are normalized to the trough (ZT4) levels seen in control flies on AL diet. Values are mean ± SEM of 3 independent preparations. **(C)** Overexpression of *tim* has no effect on fat metabolism on a DR diet. Triglyceride turnover was similar under DR conditions in both *tim* overexpression induced (+RU486) and un-induced (- RU486) flies. The error bars indicate S.E.M of 4 independent preparations. Statistical significance was determined using Student's t test and is denoted by ***p< 0.001, **p<0.01, and *p<0.05. **(D-E)** Co-expression of *tim* overexpression and *ACC* RNAi in whole body abrogates the AL dependent increase in survival. **(D)** Kaplan Meyers survival analysis for flies overexpressing *timeless* in presence or absence of ACC RNAi in whole fly (with Act5c-GS-GAL4 driver). RU486 addition was used to induce overexpression. **(E)** represents the median lifespan observed in survival curves shown in **(D)**. Statistical analysis of the survival curves, genotype and the number of flies used in each group are provided in Table S2.

Supplemental Tables

Table S1, related to Figure 1. Statistical analysis of gene expression (qPCR) data by two-way ANOVA with Bonferroni's post-hoc test.

	Effect of ZT		Effect of diet	
Heads (Day 13)				
per	$F_{6,28} = 59.75$	$p < 0.0001$	$F_{1,28} = 37.68$	$p < 0.0001$
tim	$F_{6,28} = 111.49$	$p < 0.0001$	$F_{1,28} = 31.79$	$p < 0.0001$
Pdp1 ε	$F_{6,28} = 96.07$	$p < 0.0001$	$F_{1,28} = 33.76$	$p < 0.0001$
vri	$F_{6,28} = 550.81$	$p < 0.0001$	$F_{1,28} = 122.19$	$p < 0.0001$
Clk	$F_{6,28} = 118.44$	$p < 0.0001$	$F_{1,28} = 130.55$	$p < 0.0001$
cry	$F_{6,28} = 12.29$	$p < 0.0001$	$F_{1,28} = 0.91$	$p = 0.3488$
Heads (Day 33)				
per	$F_{6,28} = 110.38$	$p < 0.0001$	$F_{1,28} = 58.27$	$p < 0.0001$
tim	$F_{6,28} = 99.74$	$p < 0.0001$	$F_{1,28} = 48.74$	$p < 0.0001$
Pdp1 ε	$F_{6,28} = 89.16$	$p < 0.0001$	$F_{1,28} = 37.18$	$p < 0.0001$
vri	$F_{6,28} = 53.76$	$p < 0.0001$	$F_{1,28} = 6.55$	$p < 0.05$
Clk	$F_{6,28} = 21.61$	$p < 0.0001$	$F_{1,28} = 38.27$	$p < 0.0001$
cry	$F_{6,28} = 8.02$	$p < 0.0001$	$F_{1,28} = 0.45$	$p = 0.5057$
Bodies (Day 13)				
per	$F_{6,28} = 48.82$	$p < 0.0001$	$F_{1,28} = 320.29$	$p < 0.0001$
tim	$F_{6,28} = 130.93$	$p < 0.0001$	$F_{1,28} = 349.92$	$p < 0.0001$
Pdp1 ε	$F_{6,28} = 191.49$	$p < 0.0001$	$F_{1,28} = 351.30$	$p < 0.0001$
vri	$F_{6,28} = 34.17$	$p < 0.0001$	$F_{1,28} = 121.14$	$p < 0.0001$
Clk	$F_{6,28} = 8.42$	$p < 0.0001$	$F_{1,28} = 114.97$	$p < 0.0001$
cry	$F_{6,28} = 2.84$	$p < 0.05$	$F_{1,28} = 0.33$	$p = 0.9152$
Bodies (Day 33)				
per	$F_{6,28} = 48.97$	$p < 0.0001$	$F_{1,28} = 50.50$	$p < 0.0001$
tim	$F_{6,28} = 170.72$	$p < 0.0001$	$F_{1,28} = 57.77$	$p < 0.0001$
Pdp1ε	$F_{6,28} = 182.38$	$p < 0.0001$	$F_{1,28} = 31.72$	$p < 0.0001$
vri	$F_{6,28} = 62.34$	$p < 0.0001$	$F_{1,28} = 92.78$	$p < 0.0001$
Clk	$F_{6,28} = 16.47$	$p < 0.0001$	$F_{1,28} = 62.16$	$p < 0.0001$
cry	$F_{6,28} = 13.03$	$p < 0.0001$	$F_{1,28} = 0.01$	$p = 0.9781$

Subscripted values indicate the degrees of freedom in numerator (DFn) and denominator (DFd), respectively.

Table S2, related to Figures 3, 4, 5 and 6. Detailed statistical analyses of survival curves provided in the main figures 3, 4, 5 and 6 (provided as a separate excel spreadsheet)

Table S3, related to Figures 3, 4 and 5. Summary of the independent repeats of the lifespan analyses of the survival curves.

			Median Lifespan (in days)			
Group (Cross genotype)	Date	Repeat #	LL DR (n)	LL AL (n)	Control DR (n)	Control AL (n)
Control (Canton-S) (**Figure 3A**)	7/12/2011	1	58(98)	46(91)	72(110)	51(103)
	2/2/2012	2	60(151)	40(140)	74(149)	43(144)

			Median Lifespan (in days)			
Group (Cross genotype)	Date	Repeat #	Mutant DR (n)	Mutant AL (n)	Control DR (n)	Control AL (n)
tim^{01} (Canton-S background) lifespan (**Figure 3B**)	6/21/2011	1	44(141)	40(139)	68(140)	45(136)
	2/3/2011	2	36 (159)	27(152)	55(115)	39(112)
	6/2/2011	3	47.5(150)	41(140)	67(142)	47(150)

			Median Lifespan (in days)			
Group (Cross genotype)	Date	Repeat #	Mutant DR (n)	Mutant AL (n)	Control DR (n)	Control AL (n)
tim^{01} (W1118 back ground) (**Figure 3C**)	4/15/2013	1	47(91)	31(99)	75(119)	39(131)
	4/13/2013	2	39(64)	21(73)	65(92)	25(80)
	6/12/2013	3	54(116)	28(121)	75(133)	26(139)

			Median Lifespan (in days)			
Group (Cross genotype)	Date	Repeat #	Mutant DR (n)	Mutant AL (n)	Control DR (n)	Control AL (n)
per^{01} lifespan (**Figure 3E**)	5/28/2012	1	46(111)	37(104)	70(128)	49(125)
	5/10/2012	2	60(145)	39(111)	64(133)	39(101)
	8/16/2012	3	53(186)	41(160)	64(171)	43(179)

			Median Lifespan (in Hrs)			
Group (Cross genotype)	Date	Repeat #	Mutant DR (n)	Mutant AL (n)	Control DR (n)	Control AL (n)
tim^{01} starvation (**Figure 4A**)	7/04/2011	1	77(125)	52(114)	124(119)	72(97)
	10/4/2011	2	69 (128)	45(119)	80(135)	45(119)
			Median Lifespan (in Hrs)			
Group (Cross genotype)	Date	Repeat #	Mutant DR (n)	Mutant AL (n)	Control DR (n)	Control AL (n)

per^{01} starvation (**Figure 4B**)	5/28/2012	1	53(132)	45(89)	77(103)	45(104)
	11/18/2012	2	52(114)	41(117)	98(100)	44(131)
	8/15/2012	3	53(132)	44(132)	68(131)	44(126)

			Median Lifespan (in days)			
Group (Cross genotype)	Date	Repeat #	Ctrl DR (n)	(+ Ru486) DR (n)	Ctrl AL (n)	(+ Ru486) AL (n)
Act5c-GS-GAL4 x UAS *tim*$^{2-5}$(**Figure 5A**)	10/31/2012	1	91(129)	84(124)	42(127)	58(140)
	5/17/2012	2	71(130)	71(112)	48(128)	60(125)
	2/17/2012	3	80(120)	83(100)	57(136)	62(143)
	9/30/2012	4	49(131)	48(122)	38(143)	45(137)

			Median Lifespan (in days)			
Group (Cross genotype)	Date	Repeat #	Ctrl DR (n)	(+ Ru486) DR (n)	Ctrl AL (n)	(+ Ru486) AL (n)
Elav-GS-GAL4 x UAS *tim*$^{2-5}$(**Figure 5B**)	11/27/2012	1	76(124)	76(115)	50(133)	50(139)
	1/31/2012	2	46 (125)	42(123)	33(132)	26 (135)

			Median Lifespan (in days)			
Group (Cross genotype)	Date	Repeat #	Ctrl DR (n)	(+ Ru486) DR (n)	Ctrl AL (n)	(+ Ru486) AL (n)
S106-GS-GAL4 x UAS *tim*$^{2-5}$ (**Figure 5C**)	12/13/2012	1	53(114)	53(128)	27(140)	34(133)
	6/12/2011	2	58 (131)	60 (102)	46 (135)	56 (121)
	8/8/2011	3	73 (104)	70 (99)	50 (88)	56 (83)

Table S4, related to Experimental Procedures (section HPLC-MS and –MS/MS analyses).

HRMS analysis for TGs classified in groups 1-3.

Features (Group)	Retention Time (min)	Observed HRMS	LIPID MAPS Annotated TG	Calculated HRMS	Molecular ion formula $[M+NH_4]^+$	delta (ppm)
feature231 (1)	29.8	794.7244	TG(46:1)	794.7232	$C_{49}H_{96}NO_6^+$	1.5
feature174 (1)	28.6	766.6927	TG(44:1)	766.6919	$C_{47}H_{92}NO_6^+$	1.0
feature266 (1)	30.7	822.756	TG(48:1)	822.7545	$C_{51}H_{100}NO_6^+$	1.8
feature276 (1)	31	848.7712	TG(50:2)	848.7702	$C_{53}H_{102}NO_6^+$	1.2
feature102 (2)	25.2	696.6171	TG(39:1)	696.6137	$C_{42}H_{82}NO_6^+$	4.9
feature88 (2)	24.1	656.5824	TG(36:0)	656.5824	$C_{39}H_{78}NO_6^+$	0
feature97 (2)	24.5	682.5989	TG(38:1)	682.598	$C_{41}H_{80}NO_6^+$	1.3
feature128 (3)	27	712.6458	TG(40:0)	712.645	$C_{43}H_{86}NO_6^+$	1.1
feature143 (3)	27.5	738.6611	TG(42:1)	738.6606	$C_{45}H_{88}NO_6^+$	0.7
feature167 (3)	28.4	740.6769	TG(42:0)	740.6763	$C_{45}H_{90}NO_6^+$	0.8
feature230 (3)	29.8	792.7095	TG(46:2)	792.7076	$C_{49}H_{94}NO_6^+$	2.4

Supplemental Experimental Procedures (Related to Experimental Procedures)

Fly husbandry, media and survival assays:

The description of various fly media recipes that were used in the study are provided below:
Standard media: All fly stocks were maintained on standard lab fly media. The standard lab media is based on the Caltech media recipe, which includes 8.6% (w/v) Cornmeal, 1.6% (w/v) Yeast, 5% (w/v) Sucrose, 0.46% (w/v) Agar, 1% (v/v) Acid mix *(6, 13, 34*). To prepare the media, Cornmeal (86 gm), Sucrose (50 gm), active-dry-yeast (16 gm, "Saf-instant") and Agar (4.6 gm) were mixed in a liter of water and brought to boil with constant stirring. The media was allowed to cool down to 60°C, before 10 ml of acid mix was added and mixed in the media. Acid mix was prepared by mixing equal volumes of 10% propionic acid (v/v) and 83.6% orthophosphoric acid. The media was then poured in vials (~10ml/ vial) or bottles (25 ml/bottle) and allowed to cool down before storing at 4°C for later usage. These vials or bottles were then seeded with some live yeast just before the flies are transferred and used for maintenance of lab stocks or for collecting virgins and setting up the crosses.

Media for survival analyses: All survival and other assays were performed on media with varying yeast extract (YE) concentration, which is described below.

AL media: The AL media contained 8.6% (w/v) Cornmeal, 5.0% (w/v) Baker's yeast extract (#212750 Bacto™ Yeast Extract, B.D. Diagnostic Systems, Sparks, MD), 5% (w/v) Sucrose, 0.46% (w/v) Agar, 1% (v/v) Acid mix. To prepare the media, Cornmeal (86 gm), Sucrose (50 gm), Yeast extract (50 gm) and Agar (4.6 gm) were mixed in a liter of water and brought to boil with constant stirring. The media was allowed to cool down to 60°C, before 10 ml of acid mix was added and mixed in the media. The media was then poured in vials (~5ml/vial) and allowed to cool down before storing at 4°C for later usage.

DR media: The DR media contained 8.6% (w/v) Cornmeal, 0.5% (w/v) Baker's yeast extract (#212750 Bacto™ Yeast Extract, B.D. Diagnostic Systems, Sparks, MD), 5% (w/v) Sucrose, 0.46% (w/v) Agar, 1% (v/v) Acid mix. To prepare the media, Cornmeal (86 gm), Sucrose (50 gm), Yeast extract (5 gm) and Agar (4.6 gm) were mixed in a liter of water and brought to boil with constant stirring. The media was allowed to cool down to 60°C, before 10 ml of acid mix was added and mixed in the media. The media was then poured in vials (~5ml/vial) and allowed to cool down before storing at 4°C for later usage.

0%, 1% and 2% YE media: These media types were used to assay the response of flies to varying concentration of yeast restriction. The media recipe is same as AL or DR media with differences only in the amount of yeast extract used.

AL or DR media with RU486: For induction of *tim* overexpression, we used AL or DR media with additional RU486 mixed in the media. Ru486 was added in the cooling media at the same time as acid mix. RU486 was dissolved in 95% ethanol and was used at a final concentration of 100µM (the media is then refereed as 'with RU486'). The control AL or DR media contained the same volume of 95 % ethanol and is referred to as media 'without RU486'.

Genetic crosses: 10-12 young adult females (belonging to either the Gal4 stocks or the mutant (*tim^{01}* or *per^{01}*) or control flies (*CS* or *w1118*) with 3-5 males were transferred to a new stock bottle and were allowed to lay eggs for few days, after which the parents were removed. 7-8 days later. The newly eclosed virgin females were collected and used to setup the genetic crosses.

To set up the cross, 10-12 young virgin females were kept with 3-5 young male flies in new stock bottles. The males were either from the mutant or control or the UAS-*tim* over-expression flies. For example, male flies carrying *UAS-tim* (*30*) are crossed to virgin females from the RU486 inducible *Act5C-GS-GAL4* driver stocks. Flies were kept in the stock bottles for four days, after which the parents were removed and the larvae were allowed to develop in standard lab conditions (25°C temperature, 60% humidity and 12 hr day and 12 hr night).

The newly eclosed flies from the crosses were allowed to mate for 2-3 days before they were sorted into females and males under light CO_2 anesthesia. The sorted flies were then transferred to the appropriate media for survival analyses.

Survival assays: All survival assays were carried out on AL or DR media as described previously (Katewa et al., 2012; Zid et al., 2009). Adult female flies were transferred within 2-3 days of eclosion to media differing only in the amount of yeast extract (YE) in the diet and were maintained at 25°C temperature, 60% humidity and 12 hr light and 12 hr dark conditions for their entire lifespan. About 25-30 mated females were maintained per vial and the flies were transferred every 2-3 days onto fresh media vials and deaths recorded.

Survival assays with and without RU486: For over-expression of *tim*, we collected virgins from the different GAL4 stocks (Act5c-GS-GAL4, S106-GS-GAL4, Elav-GS-GAL4 , 5966-GS-GAL4 or C42-GAL4) and crossed them with males from UAS-*tim* overexpression stocks in bottles with stock media. The parents were removed after four days and the resulting progeny was sorted 2-3 days after eclosion. Newly sorted flies were then transferred to media (AL or DR) with or without the presence of 100µM RU486 and maintained at standard lab conditions (25°C temperature, 60% humidity and 12 hr light and 12 hr dark conditions) for various measurements. For survival assays, 25-30 mated females were maintained per vial and the flies were transferred every 2-3 days onto fresh media vials (with or without RU486) and deaths recorded.

Survival assays on continuous light (LL): Adult young mated *Canton S* female flies (2-3 days post-eclosion) were sorted and transferred to AL or DR media. The survival assays were then carried out at 25°C temperature, 60% humidity and 24 hrs continuous light (LL). The control group received similar lifespan conditions but with 12 hr light and 12 hr dark conditions (LD).

High performance liquid chromatography (HPLC) - mass spectrometry (MS) instrumentation and methods: For unit resolution (quantitative) HPLC-MS analysis, HPLC was performed using a Shimadzu UFLC prominence system fitted with following modules: CBM-20A (Communication bus module), DGU-A_3 (degasser), two LC-20AD (liquid chromatograph, binary pump), SIL-20AC HT (auto sampler) and connected to an Agilent Zorbax SB-C18 column (2.1 x 150 mm, 3.5 µm, maintained at 50 °C). Samples were kept at

+4°C. MS was performed using a 4000 QTRAP® LC-MS/MS mass spectrometer from AB SCIEX fitted with a TurboV™ ion source. MS was operated in positive ion scanning mode for a mass range of *m/z* 200-1200 with the following source conditions: curtain gas (CUR) 20, nebulizer gas (GS1) 60, auxiliary gas (GS2) 50, ionspray voltage (IS) +4500 V, and source temperature (TEM) 350 ^{o}C. AB SCiex's Analyst® v1.6.1 was used for all forms of data acquisition and development of HPLC method. PeakView® v2.1 was used for the preliminary analysis of HPLC-MS data and feature generation. Skyline-daily® 3.1.1.7490 was used for in-depth analysis of the HPLC-MS data, specifically for calculating the peak areas for the identified features from fly lipid extracts.

For high-resolution (accurate-mass) HPLC-MS analysis, HPLC was performed using an Agilent 1260 Infinity LC system fitted with following modules: u-degasser (G1322A), binary pump (G1312B), thermostated column compartment (G1330B), HiPALS auto sampler (G1367E) and connected to an Agilent Zorbax SB-C18 column (2.1 x 150 mm, 3.5 µm, maintained at 50 °C). Samples were kept at +4°C. High-resolution MS1 and MS/MS analysis were performed using an Agilent 6520 QTOF mass spectrometer fitted with a Dual-Spray Electrospray Source (ESI). The instrument was operated at a mass resolution of ~20,000 for TOF MS1 scan and ~5,000 for product ion (MS/MS) scan using 2GHx extended dynamic range mode. The ionization parameters were set as follows: gas temperature (TEM) 350°C; drying gas, 9L/min; Vcap, 2500V; nebulizer, 35psig; fragmentor, 125V; and skimmer, 65V. MS1 acquisition was operated in the positive ion scanning mode for a mass range of 500-1200 m/z. Targeted MS/MS acquisition was composed of single MS scan (cycle time 1 spectra/sec) followed by ion specific MS/MS scans (1 spectra/sec) in the positive ion scanning mode for a mass range of 500-1200 *m/z* and 150-1000 *m/z* respectively. Collision energies were determined by linear interpolation, calculated according to the following equation: CE= (slope*precursor mass (m/z))/100 + Offset. For fly lipid extract analysis, a slope of 4V and an offset of 10V were used. The parameters for MS/MS on a target ion list were set as follows: quadrupole mass band pass for precursor isolation, medium (4 Da); precursor charge state (z), 1; and delta retention time (retention time range for accepting that a precursor is found), 2 min. HPLC-MS data was acquired using Agilent MassHunter Workstation (B.05.00). Agilent MassHunter Qualitative Analysis B.05.00 was used for the in-depth analysis of the HPLC-MS data and feature generation for the identified features from fly lipid extracts.

For HPLC separation, a solvent gradient of 0.1% formic acid in 5:4:1 isopropanol: methanol: water containing 5 mM ammonium formate (aqueous) – 0.1% formic acid in 99:1 isopropanol:water containing 5 mM ammonium formate (organic) was used with 0.2 mL/min flow rate, starting with an organic content of 0% for 3 min, which was increased to 30% over 2 min and then to 95 % over the next 25 min and held at 95% for 12 min. The column was subsequently reconstituted to its initial condition (organic content of 0%) over the next 1 min and re-equilibrated for 9 min prior to the next injection. A blend of standard *d5*-DGs and *d5*-TGs from Avanti® polar lipids were used to develop this method (Figure 4D).