Supplementary Information

Metabolic and Transcriptional Response to a High-Fat Diet in *Drosophila melanogaster*

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Supplementary Figures

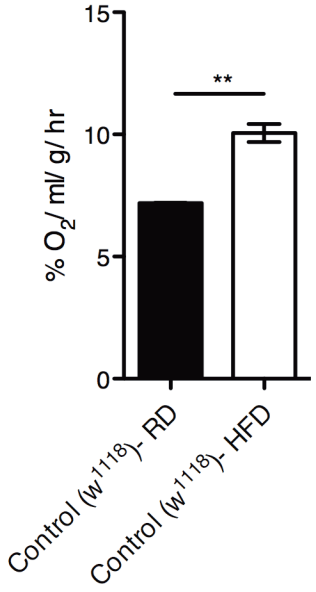

Figure S1. Increased oxygen consumption in *Drosophila* on a high-fat diet. Adult female w^{1118} flies were put on a regular diet (RD) or high-fat diet (HFD) for one week prior to assay. Measurements of 150 flies at a time were made for each group to measure the difference in oxygen levels in a sealed 5 ml vial. Three sets of flies for each group were measured. Oxygen consumption was determined by the total change in oxygen level was divided by the size of the vial, the weight of the 150 flies and the time length of the experiment. Error bars indicate standard error. Significance between the two groups was determined with a t-test, **=$p<0.01$.

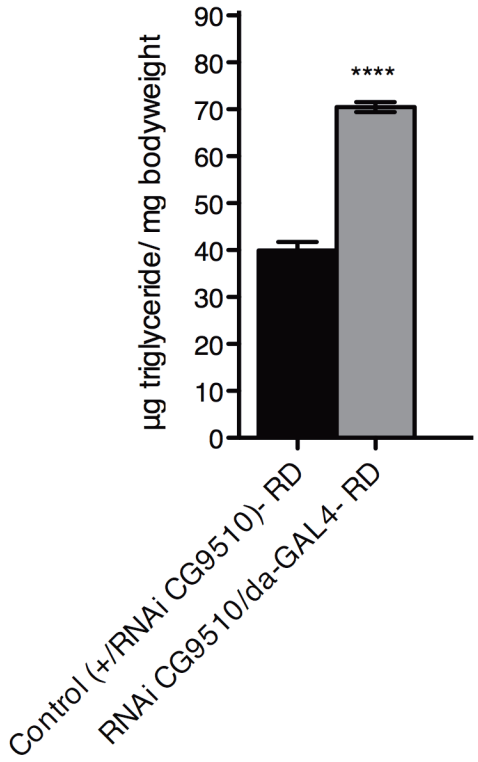

Figure S2. Triglyceride levels of *CG9510* compared to additional control.
Triglyceride levels from whole-body homogenate of transgenic flies (;;UAS-RNAi *CG9510* RNAi/da-GAL4) on RD, compared to control specific for UAS-RNAi line (w^{1118};;+/UAS-*CG9510* RNAi #1). Flies were tested in sets of 5 females for TG levels, n= between 11-25 sets (55-125 flies per group). Error bars indicate standard error. Significance between experimental values and control was determined with a t-test, ****=$p<0.0001$.

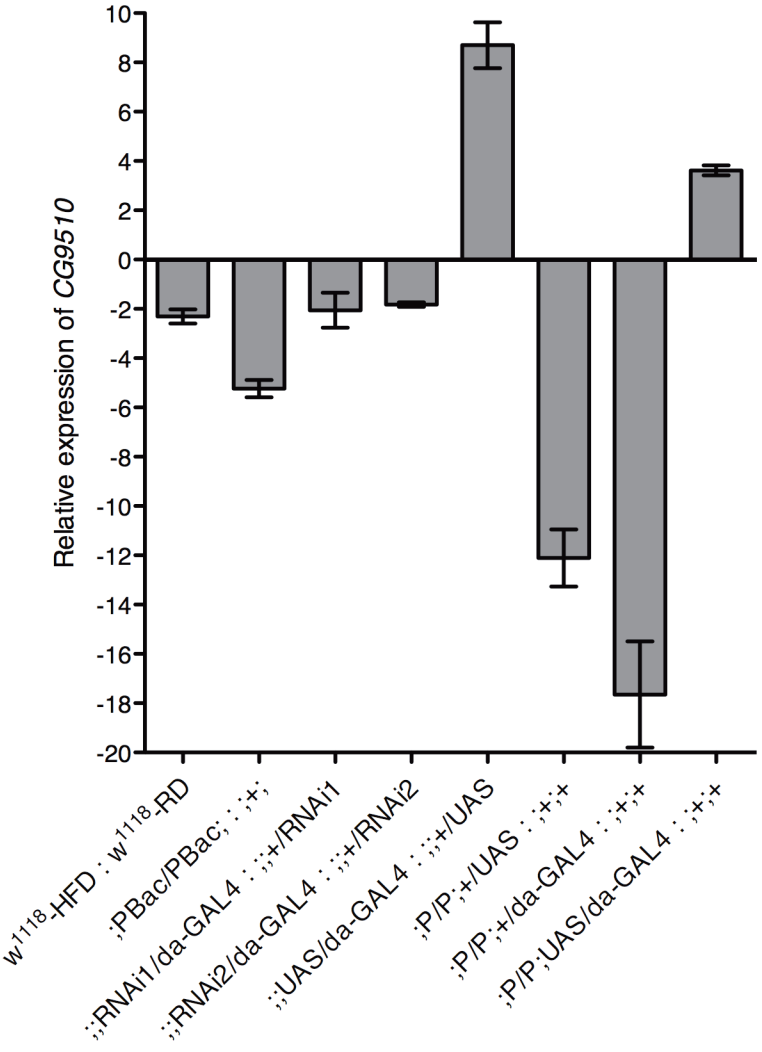

Figure S3. Verification of *CG9510* expression. Expression of *CG9510* was measured using real-time PCR analysis. Adult female flies (d3-5) were placed on regular (RD) or high-fat diet (HFD) for one week prior to RNA extraction and cDNA synthesis. The real-time PCR values were normalized with actin, and fold-change was calculated in comparison to relative mRNA levels in control flies. Relative expression of *CG9510* in w^{1118} flies on HFD compared to w^{1118} on RD (-2.3-fold change) was evaluated to verify microarray results of *CG9510* down-regulation in HFD flies (w^{1118}-HFD : w^{1118}-RD). *CG9510* down-regulation in transgenic flies (on RD): Down-regulation of *CG9510* in the transgenic P-element and RNAi lines was verified by comparing relative expression of

CG9510 in the following three transgenic flies: *CG9510-PBac* to w^{1118} (;PBac/PBac; : ;+;, -5.2-fold change); progeny of *CG9510*-RNAi #1 x daughterless (da)-GAL4 to progeny of *CG9510*-RNAi #1 x w^{1118} (;;RNAi1/da-GAL4 : ;;+/RNAi1, -2.1-fold change); progeny of *CG9510*-RNAi #2 x da-GAL4 to progeny of *CG9510*-RNAi #2 x w^{1118} (;;RNAi2/da-GAL4 : ;;+/RNAi2, -1.8-fold change). *CG9510* up-regulation in transgenic flies (on RD): Up-regulation of *CG9510* in the injected transgenic UAS-*CG9510* fly line was verified by comparing relative expression of *CG9510* in the progeny of UAS-*CG9510* x da-GAL4 to progeny of UAS-*CG9510* x w^{1118} (;;UAS/da-GAL4 : ;;+/UAS, 8.7-fold change). Rescue of *CG9510* expression in transgenic flies (on RD): Transgenic flies homozygous for the *CG9510* P-element and heterozygous for either UAS-*CG9510* or da-GAL4, with a w^{1118} background, have decreased expression of *CG9510* (;P/P;+/UAS : ;+;+, -12.1-fold change; P/P;+/da-GAL4 : ;+;+, -17.6-fold change). However, UAS-*CG9510* in the presence of da-GAL4 causes increased *CG9510* expression in the whole fly, rescuing the P-element knockdown (P/P;UAS/da-GAL4 : ;+;+, 3.6-fold change).

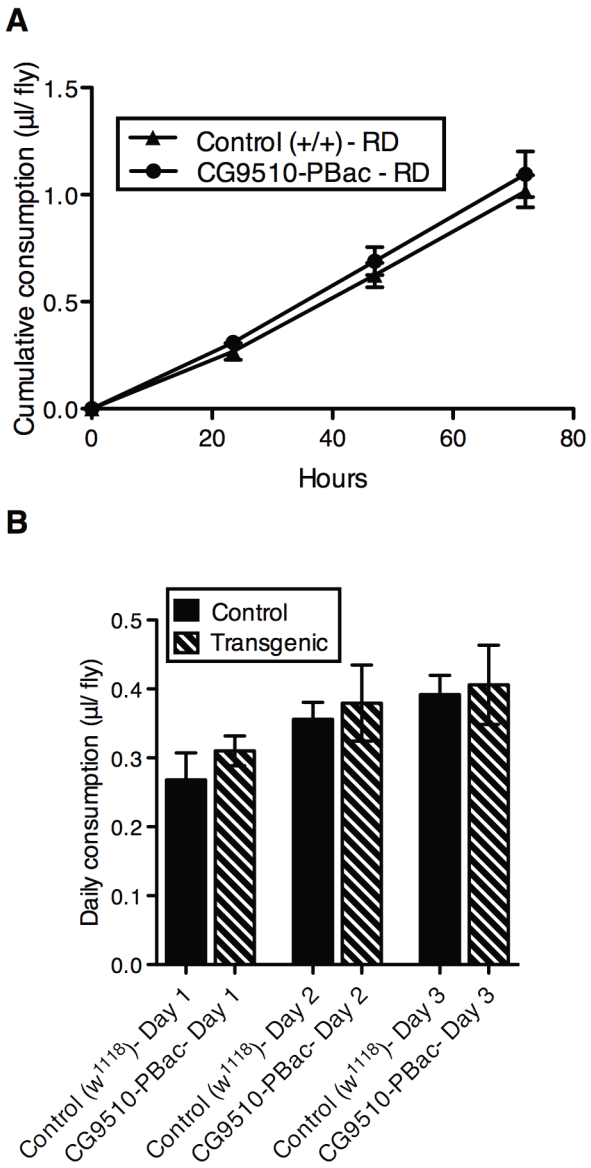

Figure S4. Similar food consumption observed in control flies and flies with down-regulation of *CG9510*. Adult female w^{1118} (control) and *CG9510-PBac* flies (3–5 days old) were placed on a regular diet (RD) for 3 days. Following that time, flies were transferred to the CAFE vials. In the CAFE setup, flies had access to water but had to obtain their food out of a capillary tube through the top of the vial. They were allowed to adjust to the new environment for one day prior to recording data. Measurements of the change in liquid food level in the capillary tube allowed for determination of the total food consumed A) cumulatively over 3 days and B) on a daily basis. Flies were tested with 5 flies per vial, n = 8 vials (40 flies) per control or experimental group. Error bars indicate standard error.

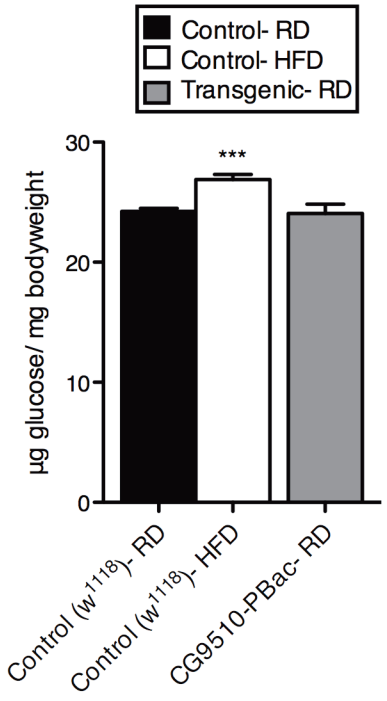

Figure S5. Total glucose levels unaffected in flies with down-regulation of *CG9510*. Adult female flies (d3-5) from a P-element line down-regulating *CG9510* (*CG9510-PBac*) were put on a regular diet (RD) and control female w^{1118} flies were put on RD or high-fat diet for one week prior measurement of trehalose and glucose levels in whole-body homogenate. Flies were tested in sets of 5 flies, n= 8 sets (40 flies per group). Error bars indicate standard error. Significance between values for the experimental group and control was determined with a t-test, ***=$p<0.001$.

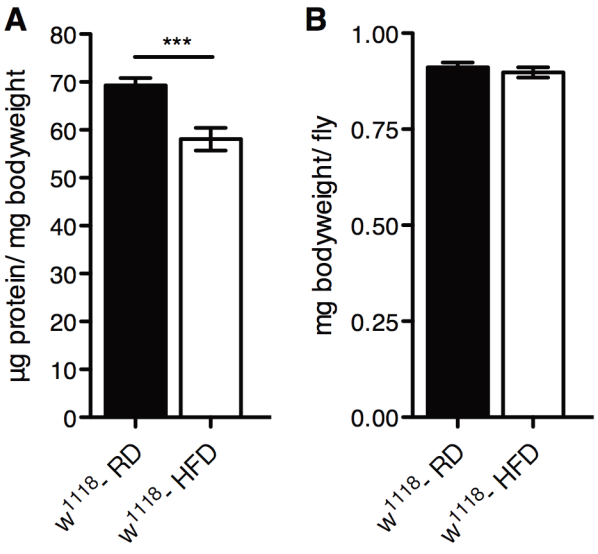

Figure S6. Protein levels decrease in flies on high-fat diet. A) Protein levels (per mg live weight) in whole-body homogenate and B) live weight of 10-12 day old adult female w^{1118} flies after one week on regular (RD) or high-fat diets (HFD). Flies were measured in groups of five females, n= 31 groups (155 flies). Error bars indicate standard error. Significance was measured with a t-test, ***=p<0.001.

Table I. UAS-RNAi stocks and GAL4 drivers used to test candidate genes (referring to Figure 5).

Candidate Gene in Figure 5	**Transformant ID of UAS-RNAi stock (Vienna Drosophila RNAi Center)**	**GAL4 driver: daughterless (da) or armadillo (arm)**	**Diet for testing transgenic flies: Regular diet (RD) or high-fat diet (HFD)**
CG9510 #1	44682	da	RD
CG9510 #2	44683	da	RD
CG5322	106609	da	RD
CG10621	31291	da	RD
CG11236	38460	da	RD
CG31177	30962	da	RD
CG7390	35230	da	RD
CG9465	52270	da	RD
CG6806 #1	14069	da	RD
CG6806 #2	50715	da	RD
CG18108	8812	da	HFD
CG34373	105369	da	HFD
CG32356 #1	12854	da	HFD
CG32356 #2	12855	da	HFD
CG32356 #3	104613	arm	HFD
CG1934	100148	arm	HFD
CG5873	14374	arm	HFD

Supplementary Methods

Metabolite extraction and GC/MS analysis

After female flies were kept on RD or HFD for one week, groups of 5 flies were placed in 2ml ceramic bead tubes (1.4mm, MoBio 13113-50) and weighed. Immediately 0.4 ml of -80ºC 9:1 methanol water mixture (MeOH, Cat# 34860, Sigma-Aldrich) was added and the tube was placed in a cooling bath of dry ice and isopropanol (approximately -78ºC)[1]. Flies were homogenized using the Precelly's 24 homogenizer and the homogenate removed to an eppendorf tube on ice. The addition of 0.1 ml 9:1 methanol water mixture was added to the ceramic bead tube to rinse any remaining homogenate off the beads and transferred to the eppendorf tube for a total volume of 0.5 ml (0.45 ml of MeOH and 0.05 ml of water). The addition of 0.25 ml ice-cold water was added to the eppendorf tube. To separate polar and non-polar phases, 0.45 ml of ice-cold chloroform ($CHCl_3$, Cat# 366927, Sigma-Aldrich) was added and the eppendorf tube was vortexed at 4ºC for 30 minutes. The extract was further centrifuged at 14,000g at room temperature for 10 minutes. For analysis of general free polar metabolites and uric acid, two 0.1 ml aliquots of the aqueous phase were transferred to the GC/MS sampler tube for evaporation in a refrigerated vacuum centrifuge (Labconco CentriVap Concentrator). For analysis of total fatty acids, the 0.1 ml aliquot of the organic phase containing the non-polar fraction was collected in the eppendorf tube and evaporated under airflow at room temperature.

Derivatization of polar metabolites was performed using a Gerstel MultiPurpose Sampler (MPS 2XL). Dried polar metabolite aliquots were dissolved in 7.5 µl of 2% methoxyamine hydrochloride (MOX, Cat# 155405, MP Biomedicals) in pyridine (Cat# 270407, Sigma-Aldrich) and held at 37ºC for 30 minutes. Subsequent conversion to their tert-butyldimethylsilyl (TBDMS) and trimethylsilyl (TMS) derivatives was accomplished by adding 15 µl *N*-methyl-*N*-(tert-butyldimethylsilyl) trifluoroacetamide (MBTSTFA) + 1% tert-butyldimethylchlorosilane (TBDMCS; Cat# 270143, Regis Technologies) or 15 µl *N*-Methyl-*N*-(trimethylsilyl) trifluoroacetamide (MSTFA; Cat# 270590, Regis Technologies) and incubating at 37ºC for 60 minutes. Fatty acid methyl esters (FAMEs) were generated by dissolving dried fatty acid aliquots in 0.5 ml 2% (v/v) methanolic sulfuric acid (H_2SO_4, Cat# 320501, Sigma-Aldrich) and incubating at 50 ºC for 2 hours. FAMEs were subsequently extracted in 1 ml hexane with 0.1 ml saturated NaCl.

Gas chromatography/mass spectrometry (GC/MS) analysis was performed using an Agilent 7890A with a 30m DB-35MS capillary column (Agilent Technologies) connected to an Agilent 5975C MS. GC/MS was operated under electron impact (EI) ionization at 70 eV. One µl sample was injected in splitless mode at 270ºC, using helium as the carrier gas at a flow rate of 1 ml/min. For analysis of organic and amino acid derivatives, the GC oven temperature was held at 100ºC for 2 minutes, increased to 255ºC at 3.5ºC/min, then ramped to 320ºC at 15ºC/min for a total run time of approximately 50 minutes. For measurement of FAMEs, the GC oven temperature was held at 100ºC for 3 minutes, then to 205ºC at 25ºC/min, further increased to 230ºC at 5ºC/min and ramped up to 300ºC at 25ºC/min for a total run time of approximately 15 minutes. The MS source and quadrupole were held at 230ºC and 150ºC, respectively, and the detector was operated in scanning mode, recording ion abundance in the range of 100 – 650 m/z.

For quantification of metabolites, selected ion fragments were integrated using a MATLAB-based in-house algorithm [2-4]. Additional ions integrated include: urea, 231 m/z; uric acid, 441

m/z and 456 m/z; myristate, 242 m/z; palmitate, 270 m/z; oleate, 264 m/z and 296 m/z; and stearate, 298 m/z. The relative quantification of total fly metabolites was determined by normalizing to the intensity of the added internal standards during extraction and measured fly body weights. Norvaline 1µg (Cat# N7502, Sigma-Aldrich), ribitol 1µg (adonitol, Cat# A5502, Sigma-Aldrich) and glyceryl triheptadecanoate 1µg (Cat# T2151, Sigma-Aldrich) were added to each sample during extraction as standards for TBDMS derivatives, TMS derivatives and FAMEs, respectively.

References

1. Rondeau RE (1966) Slush Baths. Journal of Chemical and Engineering Data.

2. Grassian AR, Metallo CM, Coloff JL, Stephanopoulos G, Brugge JS (2011) Erk regulation of pyruvate dehydrogenase flux through PDK4 modulates cell proliferation. Genes Dev 25: 1716–1733. doi:10.1101/gad.16771811.

3. Metallo CM, Walther JL, Stephanopoulos G (2009) Evaluation of ^1^3C isotopic tracers for metabolic flux analysis in mammalian cells. J Biotechnol 144: 8–8. doi:10.1016/j.jbiotec.2009.07.010.

4. Antoniewicz MRM, Kelleher JKJ, Stephanopoulos GG (2007) Accurate assessment of amino acid mass isotopomer distributions for metabolic flux analysis. Anal Chem 79: 7554–7559. doi:10.1021/ac0708893.