Molecules and Cells

Flightless-I Controls Fat Storage in *Drosophila*

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Triglyceride homeostasis is a key process of normal development and is essential for the maintenance of energy metabolism. Dysregulation of this process leads to metabolic disorders such as obesity and hyperlipidemia. Here, we report a novel function of the *Drosophila* flightless-I *(fliI)* gene in lipid metabolism. *Drosophila fliI* mutants were resistant to starvation and showed increased levels of triglycerides in the fat body and intestine, whereas *fliI* overexpression decreased triglyceride levels. These flies suffered from metabolic stress indicated by increased levels of trehalose in hemolymph and enhanced phosphorylation of eukaryotic initiation factor 2 alpha (eIF2α). Moreover, upregulation of triglycerides via a knockdown of *fliI* was reversed by a knockdown of *desat1* in the fat body of flies. These results indicate that fliI suppresses the expression of *desat1*, thereby inhibiting the development of obesity; fliI may, thus, serve as a novel therapeutic target in obesity and metabolic diseases.

Keywords: desaturase-1, *Drosophila*, fat storage, flightless-1, lipid metabolism

INTRODUCTION

Flightless-I (fliI) is an actin-binding protein and performs important functions in early embryogenesis in *Drosophila*. Severe defects in the *fliI* locus lead to impaired cellularization and gastrulation of *Drosophila* embryos (Straub et al., 1996), and mild mutations in the *fliI* gene cause defects in the development of flight muscles and the loss of the ability to fly (Campbell et al., 1993; Deak et al., 1982; Miklos and De Couet, 1990). The fliI protein in *Drosophila* is highly conserved as compared to mice and humans. This protein contains a leucine-rich repeat (LRR) at the N terminus and a gelsolin-like repeat at the C terminus (Campbell et al., 1993). The LRR region is known to be involved in protein-protein or protein-lipid interactions (Kajava et al., 1995; Kobe and Deisenhofer, 1995) that are responsible for Ras signal transduction (Claudianos and Campbell, 1995; Goshima et al., 1999). The gelsolin-like repeat interacts with actin and actin-binding proteins (Campbell et al., 1993; Claudianos and Campbell, 1995; Davy et al., 2001). PI3 kinase and small GTPase are involved in fliI-mediated cytoskeletal regulation. In addition, fliI negatively regulates wound repair through its effect on hemidesmosome formation and integrin-mediated cellular adhesion and migration (Kopecki et al., 2009). On the other hand, the fliI protein functions as a transcriptional coregulator by interacting with hormone-activated nuclear receptors, such as estrogen receptor (ER), thyroid receptor (TR), and other coregulators, including glucocorticoid receptor-interacting protein (GRIP), coactivator-associated arginine methyltransferase (CARM1), and BAF53 (Choi et al., 2015; Lee and Stallcup, 2006; Lee et al., 2004; Wu et al., 2013). Moreover, fliI inhibits β-catenin-mediated transcription by disrupting formation of the FLII leucine-rich repeat-associated protein 1 (FLAP1)-p300-β-catenin complex (Lee and Stallcup, 2006) and negatively regulates carbohydrate response element-binding protein (ChREBP) in HCT116 cells (Wu et al., 2013).

The maintenance of fat content is central to normal development and essential for energy metabolism (Ducharme and Bickel, 2008). *Drosophila* fat is mainly stored as triglycerides

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in the fat body, which is equivalent to mammalian adipose tissue and liver (Arrese et al., 2001; Canavoso et al., 2001). The storage and mobilization of fat are dynamically controlled by lipogenesis and lipolysis cycles in response to nutrient levels in the body. When the organism has enough nutrients for survival, dietary fat is hydrolyzed to fatty acids by gastric lipase, which are then absorbed in the intestine. The absorbed fatty acids are utilized for the resynthesis of triglycerides in the fat body of *Drosophila* or in the adipose tissue of mammals. Nonetheless, nutrient deprivation triggers a release of fatty acids by specific lipid droplet-associated lipases to supply energy through mitochondrial fatty acid β-oxidation (Arrese et al., 2001; Canavoso et al., 2001). Dysregulation of fat metabolism can lead to metabolic aberrations, eventually causing metabolic disorders, such as obesity, type 2 diabetes, and cardiovascular disease.

As described above, fliI primarily participates in cytoskeletal regulation and cell migration as a member of the actin-remodeling protein family (Davy et al., 2000; 2001). In addition to regulating cytoskeletal function, a recent study has revealed that fliI regulates gene transcription by interacting with nuclear receptors, such as peroxisome proliferator-activated receptor γ (PPARγ), by modulating the expression of lipogenic enzymes (Choi et al., 2015). These findings propelled us to determine whether fliI plays a role in lipid metabolism. Here, we report that *Drosophila fliI* mutants are resistant to starvation and have larger amounts of triglycerides in the fat body and intestine. *FliI* mutants showed high mRNA expression of desaturase 1 (desat1), whose preferred substrate is stearoyl-CoA. Furthermore, a fat-body-specific knockdown of *fliI* increased the level of triglycerides, and this change was reversed by a *desat1* knockdown in the fat body. Conversely, overexpression of fliI significantly reduced the amount of triglycerides and mRNA expression of *desat1*; this result was further validated in mammalian cells. Mammalian fliI significantly reduced transcript levels of *SCD1*, *SCD2*, and *SCD4* in 3T3-L1 preadipocytes, the mammalian homologs of *desat1*. Taken together, these results support the involvement of fliI in lipid metabolism and suggest that fliI may be a novel therapeutic target in obesity and metabolic diseases.

MATERIALS AND METHODS

Plasmids and fly strains

The coding sequence of flightless-1 was obtained by RT-PCR from *yw* larvae. The HA-tag was added to the C termini of these coding sequences, and they were subcloned into pUAST (Brand and Perrimon, 1993). All *Drosophila* stocks were raised on a standard cornmeal medium containing 4.94% molasses, 3.8% cornmeal, 1.6% yeast, and 1.2% agar at 25℃. Genes were expressed in *Drosophila* by means of the standard Gal4/UAS system. Flies w^{1118} (stock number 5905), $fliI^{3}$ (stock number 4730), $fliI^{14}/FM6$ (stock number 7481), and P106 (Roman et al., 2001) (stock number 8151) were obtained from the Bloomington Stock Center. Flies UAS-fliI RNAi (stock number 39528) and UAS-desat1 RNAi (stock number 104350) were obtained from the Vienna *Drosophila* Resource Center. Alleles $fliI^{3}$ (de Couet et al., 1995) and $fliI^{14}$ (Perrimon et al., 1989) were described previously. To implement RNAi, 100 μl of a 5 mg/ml solution of RU486 (cat. # M4086, Sigma) or vehicle (80% ethanol) was added on top of food in a food vial and dried overnight before being fed to the flies.

Triglyceride quantification

This procedure was carried out using the Triglyceride Reagent (Thermo Electron Corp., TR22421). A total of five to ten female flies were homogenized in 200 μl of PBST (PBS with 0.1% Tween 20), and triglyceride levels were measured as previously described (Birse et al., 2010). Triglyceride amounts were normalized to total protein levels. To measure triglyceride levels in the fat body and intestine, we dissected the flies in cold PBS and collected the fat body and intestine, being careful to avoid contamination with other tissues. The collected tissues were homogenized in PBST.

Tissue staining

For the staining of neutral lipids, dissected tissues were fixed with 4% formaldehyde in PBS for 20 min and washed in PBS with 0.1% Triton X-100 (PBST2). The tissues were then stained with PBST2 containing bodipy 493/503 (1:2 000; Life Technologies). For Nile red staining, whole flies were fixed in 4% paraformaldehyde in PBST2 for 3 h at room temperature. The flies were washed three times in PBS for 5 min. Abdomens were carefully dissected in PBS. The dissected abdomens were incubated for 30 min in a Nile red staining solution (1 mg/ml in dimethyl sulfoxide, 1:1 000 in PBST2). The abdomens were washed three times in PBS for 5 min and then mounted on a slide glass with 50% glycerol in PBS.

The starvation assay

Twenty female flies (7 days old) of each genotype were transferred to vials containing 1% agar in PBS. The flies were transferred to fresh food every 12 h and maintained at 25℃; deaths were recorded at those time points.

Quantification of feeding

Twenty female flies (7 days old) were starved for 24 h in vials containing 1% agar in PBS or maintained on standard fly food. Next, they were transferred to vials containing 1% agar, 5% sucrose, and 2.5% blue food dye (Erioglaucine Disodium Salt, Sigma, cat. #861146). After 15 min of feeding, 10 flies were immediately frozen, homogenized in 300 μl of PBS, and centrifuged for 25 min at 13 200 rpm (Eppendorf Centrifuge 5415R). The absorbance of the supernatant was measured at 625 nm on a spectrophotometer (Tecan).

Quantitative RT-PCR

Total RNA from five female flies was isolated using the TRIzol Reagent (Invitrogen, USA), and 200 ng of RNA was transcribed with the ReverTra Ace qPCR RT Kit (Toyobo Co., Japan). Quantitative PCR amplification was run for 40 cycles by means of the TOPreal™ qPCR 2X PreMIX (SYBR Green with high ROX) and a LightCycler® 480 Real-Time PCR System. *Rp49* served as a reference for normalization. Relative quantification of mRNA was performed by the comparative C_T method. The primers are listed in Supplementary Table 1.

Cell culture

3T3-L1 cells were acquired from ATCC (USA) and cultured in Dulbecco's modified Eagle's medium with 10% of fetal bovine serum. Cells were transfected with an empty vector or a vector carrying murine FLII, and stable transfectants were generated by neomycin (G418) selection. Total RNA was extracted from confluent preadipocytes for quantitative RT-PCR.

Statistical analysis

Each experiment was repeated at least three times, and the data are presented as mean ± SE. Significance testing was based on Student's *t* test.

Abbreviations

coenzyme A (CoA), DNA-binding domain (DBD), leucine-rich repeat (LRR), PBS with 0.1% Tween 20 (PBST), PBS with 0.1% Triton X-100 (PBST2), RNA interference (RNAi)

RESULTS

Drosophila flil mutants show increased lipid storage

To assess the physiological role of flil in lipid metabolism, we used already characterized *flil* mutant alleles, *flil*^{3} and *flil*^{14}. The *Flil*^{3} mutant allele contains a single-base change of Gly at amino acid position 602 to Ser in the gelsolin-like repeat and may fail to interact with another protein, possibly specific to an indirect flight muscle (de Couet et al., 1995). The *Flil*^{14} allele is lethal during larval and pupal stages, indicating that the flil protein is essential for survival of the organism into adulthood (Perrimon et al., 1989). We examined the amounts of triglycerides in a *flil*$^{3/14}$ mutant background; such flies did not show any developmental defects. As presented in Fig. 1A and Supplementary Fig. S1, whole-body triglyceride concentrations progressively increased up to threefold by day 7 of adult life (after hatching) in *flil*$^{3/14}$ mutants, when compared to control fly strains: *w*^{1118} and Canton S. Because the major sites of fat storage in *Drosophila* are the fat body (Gutierrez et al., 2007) and midgut, we isolated the fat body and intestine and quantified triglycerides in those organs. We found larger amounts of triglycerides in *flil*$^{3/14}$ mutants' fat body in comparison with the control flies' fat body (Fig. 1B). As in the fat body, the level of triglycerides was slightly but significantly higher in *flil*$^{3/14}$ mutants' intestine (Fig. 1C).

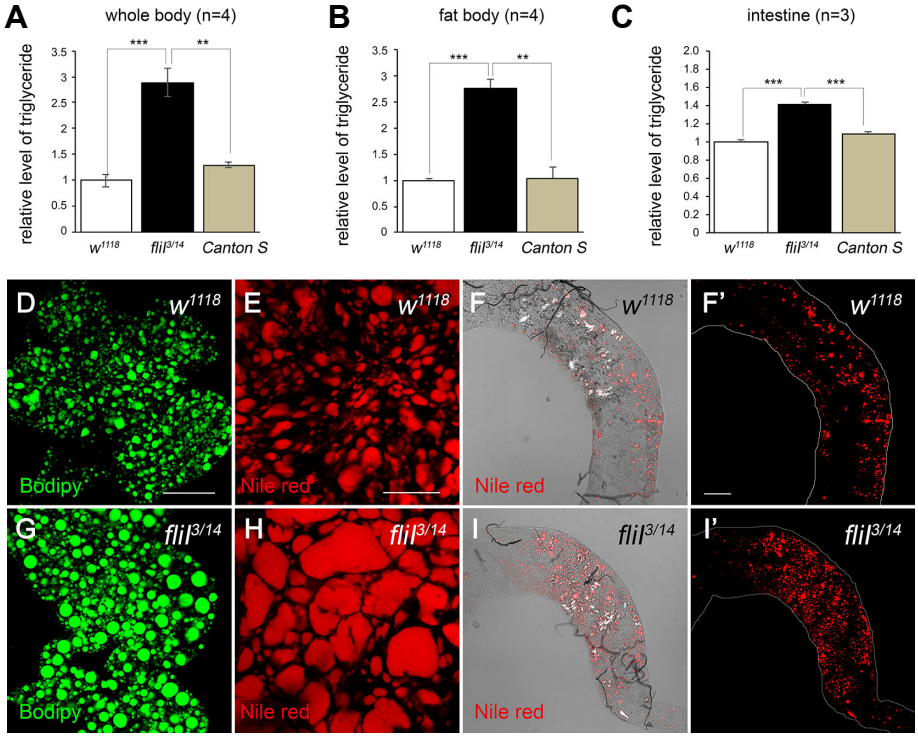

Fig. 1. *Drosophila flightless-1 (flil)* mutants show elevated levels of triglycerides. (A-C) Quantitation of triglycerides in the whole body (A), fat body (B), and intestine (C) of flies with the indicated genotypes. The relative triglyceride amount was significantly higher in *flil*$^{3/14}$ mutants compared to controls (*w*^{1118} flies). The triglyceride amounts were normalized to the total protein amounts in those tissues. (D-I) Bodipy and Nile red staining of the dissected fat body and midgut from 7-day-old adult flies. (D, G) Bodipy staining. Enlarged lipid droplets are visible in the fat body of *flil*$^{3/14}$ mutants (G). (E, F, H, I) Nile red staining shows increased lipid droplet size in the fat body of a *flil*$^{3/14}$ mutant (H). Dissected adult midguts stained with Nile red reveal significantly increased lipid staining in *flil*$^{3/14}$ mutants (I, I') compared to controls (*w*^{1118}; panels F, F'). Genotypes: *w*^{1118} (D-F) and *flil*$^{3/14}$ (G-I). The scale bars in (D, E, F') represent 50, 20, and 100 μm, respectively. Data are presented as mean ± SE from at least three independent experiments. The *P* values were calculated by Student's *t* test; $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$.

Given that the levels of triglycerides between the two control strains, w^{1118} and Canton S, were not significantly different, hereafter, we used only strain w^{1118} as a control for *flil* mutants. To confirm these results, we stained fat bodies with lipophilic dyes bodipy and Nile red. This tissue staining revealed smaller lipid droplets in the fat body of control flies compared to $flil^{3/14}$ mutants (Figs. 1D and 1E); $flil^{3/14}$ mutants had lipid droplets of variable size, with mostly enlarged lipid droplets (Figs. 1G and 1H). Consistent with these results, staining of the intestine with Nile red uncovered accumulation of lipid droplets in $flil^{3/14}$ mutants (Figs, 1I and 1I'). The *flil* mutants are deficient in the ability to fly owing to myofibrillar abnormalities in flight muscles. Therefore, to rule out the possibility that the loss of the flight ability underlies the obese phenotype, we clipped the wings of the control flies and compared the concentrations of triglycerides between the groups. The clipping of wings in the control flies did not significantly change triglyceride content (Supplementary Fig. S2). Taken together, these results indicated that Flil plays a central role in lipid storage in *Drosophila*.

Flil mutants exhibit the features of metabolic disorders

To test whether the increased triglyceride amount in $flil^{3/14}$ mutants is related to a metabolic disorder, we examined the glucose level and expression of a stress marker. The hemolymph glucose levels were similar between the two groups (Fig. 2A). In contrast, $flil^{3/14}$ mutants showed elevated circulating sugar in the form of trehalose (Figs. 2B and 2C), which prompts us to test the level of insulin production in $flil^{3/14}$ mutants. Insulin signaling pathway in *Drosophila* regulates growth, metabolic homeostasis, stress responses, reproduction, and lifespan (Brogiolo et al., 2001; Broughton et al., 2005; Gronke et al., 2010). We tested the levels of insulin-like peptides, including Dilp-2, -3, -5, and -7. Among them, insulin-like peptide (ILP)-3 and 7 were specifically elevated in $flil^{3/14}$ mutants (Fig. 2D). Although $flil^{3/14}$ mutant flies had high insulin levels, these mutant flies remained hyperglycemic, which suggested that $flil^{3/14}$ mutants might be insulin resistant. Impaired insulin signaling leads to activation of 4E-BP (Puig et al., 2003; Teleman et al., 2005). 4E-BP levels were higher in $flil^{3/14}$ mutants (Fig. 2E), indicating reduced insulin sensitivity, despite higher insulin production. We also noticed the integrated stress response, which is stimulated by endoplasmic-reticulum stress, viral infection, amino acid starvation, and other triggers, and converges on phosphorylation of the regulatory initiation factor eIF2α at serine 51 (Ron, 2002; Ryoo and Vasudevan, 2017; Taniuchi et al., 2016). As shown in Figs. 2F and 2G, phosphorylation of eIF2α increased in $flil^{3/14}$ mutant flies. As Flil has a role in actin cytoskeletal activity, as expected, the level of alpha tubulin was reduced in $flil^{3/14}$ mutants (Figs. 2F and 2G). These results suggested that Flil may be a novel regulator involved in metabolic disorders.

Flil mutants manifest starvation resistance

To assess the metabolic roles of Flil, we examined the ability of mutants to survive long periods of complete starvation. Seven-day-old female flies were reared on a starvation

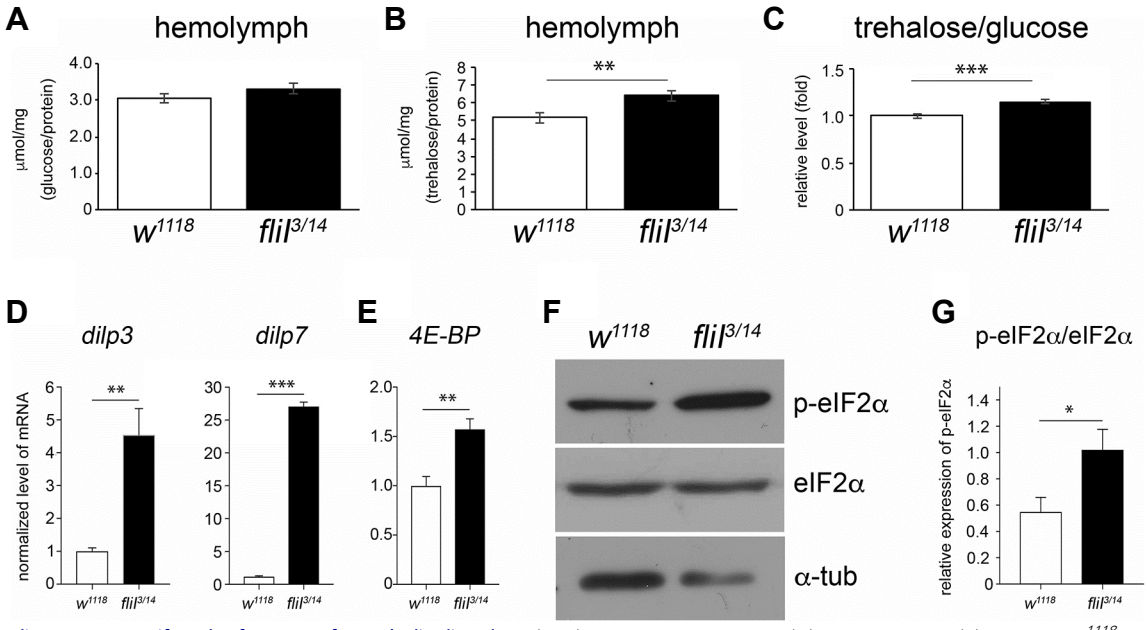

Fig. 2. *Flil* mutants manifest the features of metabolic disorders. (A-C) Hemolymph glucose (A) and trehalose (B) levels in w^{1118} and $flil^{3/14}$ mutant flies. The relative trehalose and glucose levels slightly but significantly increased in $flil^{3/14}$ mutant flies relative to those in w^{1118} flies (C). (D, E) Expression levels of indicated gene in w^{1118} and $flil^{3/14}$ mutant flies. (F, G) A representative Western blot of whole-fly extracts from w^{1118} and $flil^{3/14}$ mutants (F). eIF2α phosphorylation increased in $flil^{3/14}$ mutant flies compared to the w^{1118} strain. The amount of α-tub was lower in $flil^{3/14}$ mutant flies. (G) Comparison of the normalized anti-phospho-eIF2α band intensity between w^{1118} flies and $flil^{3/14}$ mutants. The data are presented as mean ± SE from four independent experiments; $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$.

medium (1% agar in PBS), and the number of surviving flies was recorded every 12 h. Under the starvation conditions, $flil^{3/14}$ mutant flies survived longer than w^{1118} flies did ($P < 0.0001$, logrank test; Fig. 3A). Starvation resistance is directly linked to body fat content and serves as a marker of stored-fat mobilization (Beller et al., 2010). Next, we quantified triglycerides in the starved flies. When adult flies were starved for 36 h, triglyceride amounts in control flies gradually decreased by 75% as compared to the control. In contrast, $flil^{3/14}$ mutant flies had still stored about 76% lipids by 36 h of starvation (Fig. 3B), indicating that the stored fat served as energy reserves. To test whether increased food intake caused the elevated energy stores in $flil^{3/14}$ mutant flies, we performed a blue-dye feeding assay. In control flies, food deprivation for 24 h significantly increased food intake as compared to fed control flies, but there was no significant difference in food intake between the sated and starved $flil^{3/14}$ mutant flies (Figs. 3C and 3D). To determine whether the flight defect has an influence on food intake, we measured food intake in w^{1118} flies with clipped wings. As presented in Supplementary Fig. S2B, the flight defect did not change the starvation-induced food intake. Altogether, these results indicated that Flil is crucial for energy homeostasis.

Drosophila flil suppresses desaturase 1 expression, which contributes to lipid storage

Stearoyl-CoA desaturases (SCDs) are key enzymes in fatty acid biogenesis. They catalyze desaturation of saturated long-chain fatty acids, preferring palmitoyl-CoA and stearoyl-CoA as substrates and converting them to palmitoleoyl-CoA and oleoyl-CoA, respectively. The resulting monounsaturated fatty acids, palmitoleoyl-CoA and oleoyl-CoA, are major components of triacylglycerol, cholesterol esters, and phospholipids (Enoch et al., 1976; Sampath and Ntambi, 2011). In addition, SCDs contribute to anomalous lipid metabolism and the progression of obesity (Hulver et al., 2005; Jiang et al., 2005). Given that FLII is involved in the expression of SCD1 in mammalian cells (Wu et al., 2013) , we determined

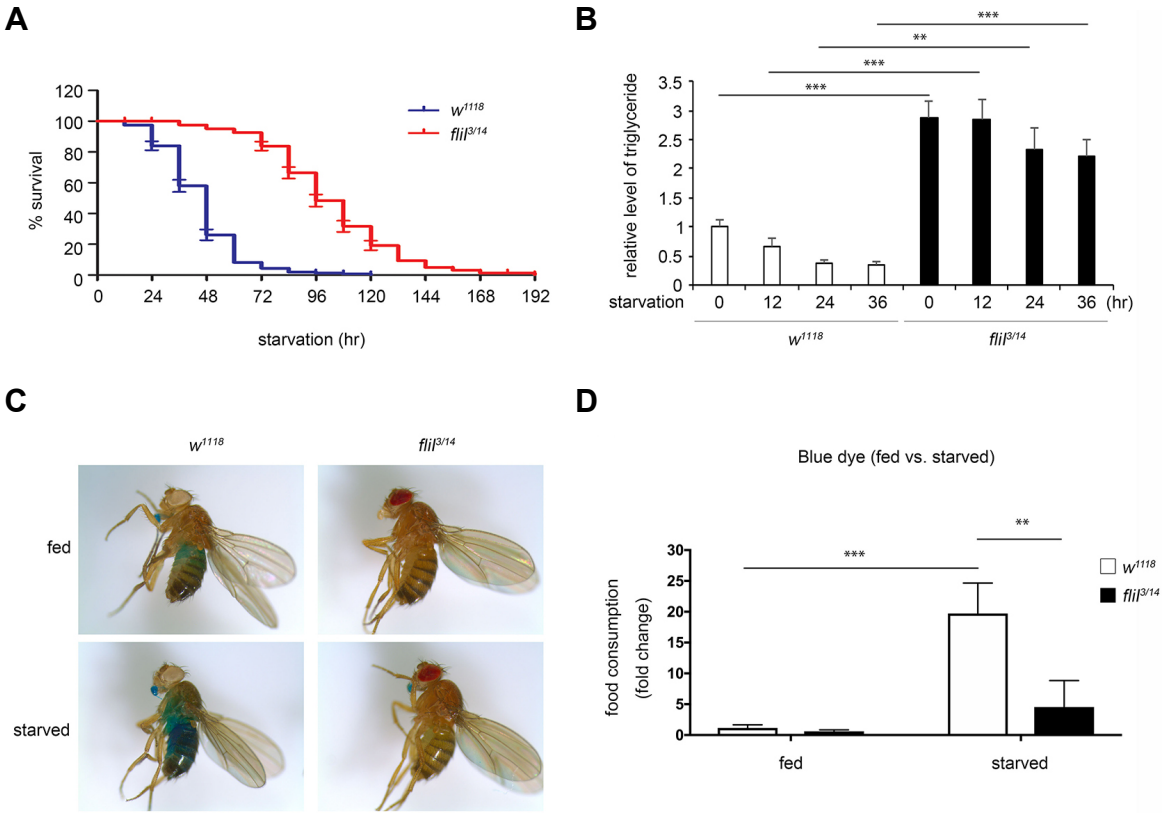

Fig. 3. *Flil* mutants are resistant to starvation. (A) Seven-day-old female w^{1118} flies (controls) and $flil^{3/14}$ mutants were subjected to complete starvation, and the numbers of surviving flies were determined at 12 h intervals. The results are presented as a percentage of the total population (n = 8, $P < 0.0001$, 20 flies in each vial). (B) Quantification of triglycerides in starved flies at the indicated time points. Although the amounts of triglycerides in w^{1118} flies decreased by 75% after 36 h of starvation, $flil^{3/14}$ mutant flies kept 76% of their triglycerides after 36 h of starvation (n = 4, 20 flies in each vial). The statistical analysis was performed between w^{1118} and $flil^{3/14}$ flies at each time point after starvation. (C, D) Fasting-induced feeding was less active in $flil^{3/14}$ mutants. (C) Blue dye levels were found to be elevated in the abdomen of control flies fasted for 24 h as compared to fed controls (w^{1118} flies). The blue dye was not detected in either fasted or fed $flil^{3/14}$ mutants (n = 4). (D) Quantitation of the blue dye revealed a significant increase in food consumption in starved control flies. Data are presented as mean ± SE from four independent experiments; $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$.

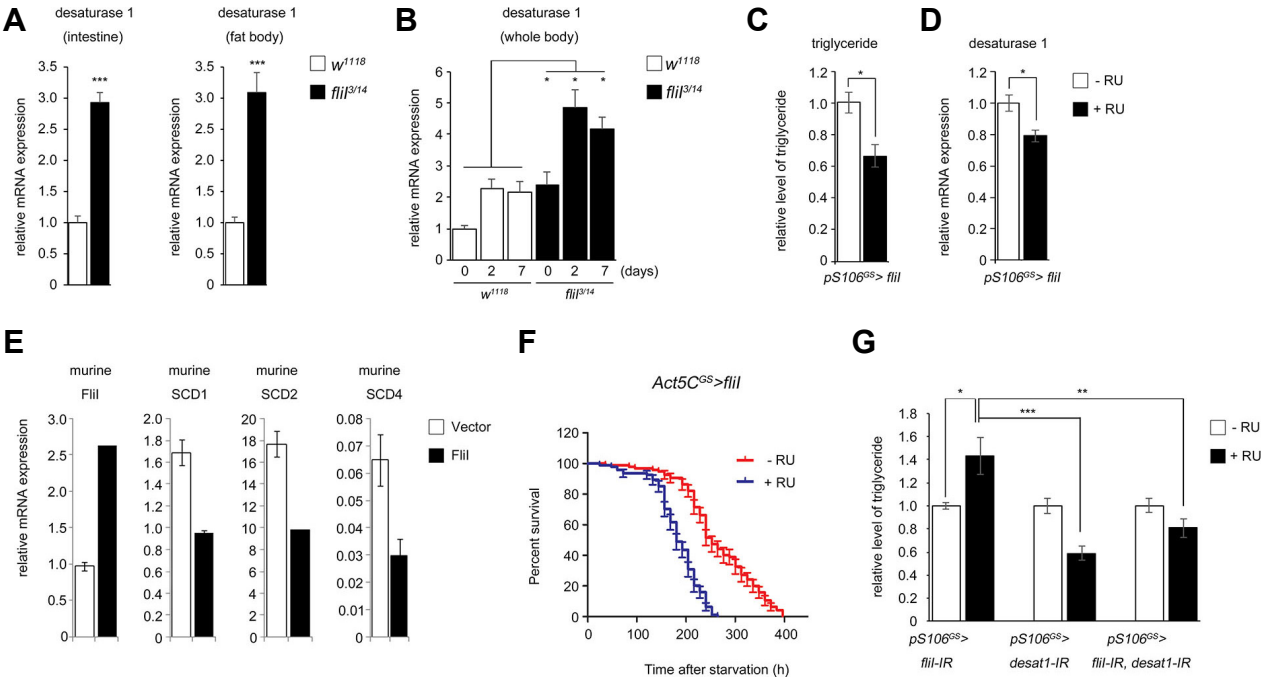

Fig. 4. *Drosophila fliI* inhibits lipid storage by suppressing mRNA expression of desaturase 1. (A) Quantitative RT-PCR analysis of *desat1* in the intestine (left) and fat body (right) from 7-day-old dissected adults. mRNA expression of *desat1* was higher in $fliI^{3/14}$ mutants relative to w^{1118} flies (controls). (B) The mRNA expression of *desat1* gradually increased in w^{1118} flies (control) and $fliI^{3/14}$ mutants. The transcript levels of *desat1* at the indicated time points were significantly higher in $fliI^{3/14}$ mutants than in w^{1118} flies. (C) For 10 days of RU486 supplementation, the levels of triglycerides in group "P{Switch1}106>fliI, +RU486" significantly decreased relative to the RU486 control (P{Switch1}106>fliI, no RU486). (D) Quantitative RT-PCR analysis of *desat1* expression (normalized to *Rp49* mRNA levels) in whole-body extracts of flies reared under conditions identical to those in (C). The overexpression of *fliI* under the influence of RU486 supplementation suppressed the mRNA expression of *desat1*. (E) The analysis of mRNA expression in empty-vector-transfected cells or in FliI-expressing 3T3-L1 cells. The overexpression of FliI-downregulated transcripts of murine stearoyl-CoA desaturase genes, such as *SCD1*, *SCD2*, and *SCD4*. (F) Overexpression of fliI (Act5C^{GS}>fliI, +RU486) significantly increased the sensitivity to starvation as compared to RU486 control (Act5C^{GS}>fliI, no RU486) according to the logrank test ($P < 0.0001$). The number of surviving flies was recorded at 12 h intervals. (G) Although the knockdown of *fliI* under the influence of RU486 supplementation increased the level of triglycerides (P{Switch1}106>fliI-IR), the knockdown of *desat1* via RU486 supplementation reduced the concentration of triglycerides (P{Switch1}106>desat1-IR). The decreased level of triglycerides in the *desat1* knockdown flies was significantly reversed by the knockdown of *fliI* (P{Switch1}106>fliI-IR, desat1-IR). The amount of triglycerides was normalized to that of group "–RU486" (no supplementation) for each genotype. Data are presented as mean ± SE from at least three independent experiments. The *P* values were determined by Student's *t* test; $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$.

whether they are the major cause of the obesity-like phenotype in *fliI* mutants. As expected, we observed increased mRNA expression of *desat1*, a *Drosophila* homolog of SCD, in the intestine and fat body of 7-day-old $fliI^{3/14}$ mutants (Fig. 4A). The mRNA expression of *desat1* in the whole body of $fliI^{3/14}$ mutants gradually increased fourfold by 7 days after hatching in comparison with 0-day-old control flies (w^{1118}; Fig. 4B); this result was reminiscent of the progressive increase in triglyceride content of $fliI^{3/14}$ mutants (Supplementary Fig. S1). To verify whether the expression of *desat1* is associated with that of fliI, we generated a fliI transgenic strain. We used the inducible gene switch P{Switch1}106 driver, which can be activated by the addition of mifepristone (i.e., RU486) to the diet (Roman et al., 2001). The P{Switch1}106 driver induced *fliI* expression only in the adult fat body. To rule out the possibility that RU486 treatment alone affects triglyceride levels, we quantified triglycerides in P{Switch1}106/+ flies. As illustrated in Supplementary Fig. S3, RU486 alone did not significantly change the concentrations of triglycerides. After the flies (*P{Switch1}106>fliI*) were reared for 10 days in the presence or absence of RU486, we quantified triglycerides. The levels of triglycerides in the whole body decreased in flies overexpressing fliI in the adult fat body (*P{Switch1}106>fliI*, +RU486) relative to control flies (*P{Switch1}106>fliI*, no RU486; Fig. 4C). In agreement with this result, when we overexpressed *fliI* in the fat body by means of the P{Switch1}106 driver, the mRNA expression of *desat1* significantly decreased relative to the level in no-RU486 controls (Fig. 4D). These results were then validated in mammals. In particular, murine *fliI* expression in 3T3-L1 preadipocytes significantly reduced the mRNA expression of mouse *SCD* isoforms, such as *SCD1*, *SCD2*, and *SCD4* (Fig.

4E). These findings provided strong evidence that Flil suppresses *desat1* transcription, which contributes to abnormal lipid metabolism. To test whether overexpression of Flil changes the survival of starvation-resistant flies, we induced the expression of Flil using Act5C^{GS}-Gal4, which drove expression of Flil in the whole body only when RU486 was added to the diet. Flil-overexpressing flies died at a faster rate than control flies did (*Act5C^{GS}>flil*, no RU486) during complete starvation at the indicated time point (Fig. 4F), in line with the result in Fig. 3A.

Next, we tested whether the increase in triglyceride amounts in *flil* mutants can be reversed by a knockdown of *desat1*. We used the RU486-inducible gene switch driver P{Switch1}106-*gal4* and downregulated *flil in vivo*, in a *flil* RNA interference (RNAi) strain. The knockdown of *flil* triggered upregulation of triglycerides as compared to the group without RU486 (Fig. 4G). The Desat1 knockdown in the adult fat body decreased the concentrations of triglycerides relative to the no-RU486 controls. By contrast, the knockdown of *flil* in the adult fat body of *desat1*-deficient flies partially but significantly restored the level of triglycerides (*P{Switch1}106>flil-IR*, *desat1-IR*; Fig. 4G). These results suggested that Flil inactivation is responsible for the obesity-like phenotype because of upregulation of *desat1*.

DISCUSSION

Here, we demonstrate that *Drosophila* Flil is a key regulator of lipid metabolism. Specifically, Flil suppresses the mRNA expression of *desat1*, eventually causing accumulation of triglycerides in the fat body and intestine. As a well-known lipogenic enzyme, SCD catalyzes the synthesis of monounsaturated fatty acids that are required for the *de novo* synthesis of membrane phospholipids, triglycerides, and cholesterol esters (Enoch et al., 1976). Emerging evidence indicates that SCD contributes to fat storage and obesity. The absence of SCD lowers fat content and protects against saturated-fat-induced or genetically induced obesity (Cohen et al., 2002; Sampath et al., 2007). Because *flil$^{3/14}$* mutants show elevated mRNA levels of *desat1*, whereas *flil* overexpression lowers *desat1* transcription, we propose that Flil downregulates *desat1*, whereas the latter gene promotes the obesity-like phenotype.

Nevertheless, it is unclear how Flil suppresses the expression of *desat1* and thus downregulates triglycerides. We can explain the regulatory mechanism by means of two hypotheses. In mammals, SCD1 expression is regulated by exercise, nutrients such as saturated fatty acids and cholesterol, and by hormonal factors (Ntambi, 1995; Sinha et al., 2017; Yao et al., 2017). Various studies also suggest that the transcription of SCD1 is controlled by PPARγ, which controls glucose and lipid metabolism as a master regulator of lipogenic-enzyme expression (Miller and Ntambi, 1996; Singh Ahuja et al., 2001; Way et al., 2001; Yao et al., 2017). Our previous study revealed that Flil functions as a transcriptional modulator of PPARγ in mammals (Choi et al., 2015). Flil inhibits formation of the complex of PPARγ with RXRα (retinoid X receptor α) by competitively interacting with PPARγ. As the short carboxy-terminal extensions of the RXRα DNA-binding domain (DBD) interact with the DBD of PPARγ (A et al., 1997; Chandra et al., 2008), this complex selectively binds to peroxisome proliferator response elements. Therefore, the expression of adipocyte-specific genes, including aP2, C/EBPα (CCAAT/enhancer-binding protein α), adiponectin, adipsin, LPL (lipoprotein lipase), FAS (fatty acid synthase), and GLUT4 (glucose transporter 4) will increase. On the other hand, binding of Flil to the DBD of PPARγ reduces DNA occupancy of PPARγ in the promoter of those target genes, thereby suppressing adipocyte differentiation (Choi et al., 2015). Thus, we believe that Flil attenuates PPARγ-induced SCD expression although *Drosophila* does not have an obvious homolog of PPARγ. We need to further explore how Flil and PPARγ interact to promote the expression of SCD in mammals.

Alternatively, the regulation of *desat1* expression by Flil may depend on the interaction between Flil and glucose-responsive transcription factor carbohydrate-responsive element-binding protein (ChREBP). According to another study (Wu et al., 2013), FLII functions as a transcriptional corepressor of ChREBP. FLII interacts and colocalizes with ChREBP in cancer cells, and FLII overexpression decreases transcriptional activity of ChREBP, which results in decreased mRNA expression of ChREBP-activated target genes, including *FAS*, *SCD1*, and thioredoxin-interacting protein (*TXNIP*). In addition, ChREBP coordinately modulates the anabolic metabolism regulating gene expression required for the conversion of glucose to fatty acids and nucleotides (Yu et al., 2014). Collectively, these data suggest that the regulation of *desat1* expression by Flil may be a consequence of some upstream event, such as the interaction of Flil and PPARγ or ChREBP, not direct transcriptional regulation of *desat1* by Flil.

Recent research indicates that cytoskeletal protein FLII is involved in diabetic wound healing (Ruzehaji et al., 2014). Consistent with another observation (Kopecki et al., 2009), attenuation of FLII expression by a genetic knockdown or by a FLII-neutralizing antibody improves wound healing and new vessel formation in vivo. In that study, they determined the FLII protein expression in nondiabetic and diabetic *Flii$^{+/-}$*, wild type, and *Flii$^{Tg/Tg}$* mice, at 0, 7, and 14 days after wounding. Although diabetes increased FLII expression in response to wounding, there was no significant change of FLII expression in unwounded skin in comparison with nondiabetic and diabetic groups. Given that obesity is a characteristic feature of type II diabetes, the expression of FLII in the murine model of type I diabetes evaluated in that study could be somewhat different from the pattern of Flil expression seen in our study. Besides, we cannot rule out the possibility of species differences.

flil$^{3/14}$ mutant flies exhibit increased fat storage and are resistant to starvation. In addition, these flies do not consume food as much as control flies after 24 h of starvation. Fat storage levels are communicated to the central nervous system (CNS), which regulates food intake and metabolism (Al-Anzi et al., 2009). After 24 h of starvation, *flil$^{3/14}$* mutant flies stored approximately 80% of the fat compared to that by the fed control flies (Fig. 3B), which is consistent with the previous finding that flies with high fat storage levels show a decrease in levels of fat store depletion rate compared to

that by controls (Al-Anzi et al., 2009). Because *fliI*$^{3/14}$ mutant flies have sufficient energy and do not feel hungry after 24 h of starvation, we assumed that they consumed less amount of food compared to controls. However, we need to further validate the regulatory mechanisms of the types of appetite hormones secreted by the brain of *fliI*$^{3/14}$ mutant flies according to the energy demand.

In summary, our results show that *Drosophila* fliI may serve as a key regulator of lipid metabolism, namely, as a suppressor of lipid accumulation; therefore, its function could be manipulated to treat metabolic disorders such as obesity and diabetes.

Note: Supplementary information is available on the Molecules and Cells website (www.molcells.org).

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REFERENCES

A, I.J., Jeannin, E., Wahli, W., and Desvergne, B. (1997). Polarity and specific sequence requirements of peroxisome proliferator-activated receptor (PPAR)./retinoid X receptor heterodimer binding to DNA. A functional analysis of the malic enzyme gene PPAR response element. J. Biol. Chem. *272*, 20108-20117.

Al-Anzi, B., Sapin, V., Waters, C., Zinn, K., Wyman, R.J., and Benzer, S. (2009). Obesity-blocking neurons in Drosophila. Neuron *63*, 329-341.

Arrese, E.L., Canavoso, L.E., Jouni, Z.E., Pennington, J.E., Tsuchida, K., and Wells, M.A. (2001). Lipid storage and mobilization in insects: current status and future directions. Insect Biochem. Mol. Biol. *31*, 7-17.

Beller, M., Bulankina, A.V., Hsiao, H.H., Urlaub, H., Jackle, H., and Kuhnlein, R.P. (2010). PERILIPIN-dependent control of lipid droplet structure and fat storage in *Drosophila*. Cell Metab. *12*, 521-532.

Birse, R.T., Choi, J., Reardon, K., Rodriguez, J., Graham, S., Diop, S., Ocorr, K., Bodmer, R., and Oldham, S. (2010). High-fat-diet-induced obesity and heart dysfunction are regulated by the TOR pathway in *Drosophila*. Cell Metab. *12*, 533-544.

Brand, A.H., and Perrimon, N. (1993). Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. Development *118*, 401-415.

Brogiolo, W., Stocker, H., Ikeya, T., Rintelen, F., Fernandez, R., and Hafen, E. (2001). An evolutionarily conserved function of the *Drosophila* insulin receptor and insulin-like peptides in growth control. Curr. Biol. *11*, 213-221.

Broughton, S.J., Piper, M.D., Ikeya, T., Bass, T.M., Jacobson, J., Driege, Y., Martinez, P., Hafen, E., Withers, D.J., Leevers, S.J., et al. (2005). Longer lifespan, altered metabolism, and stress resistance in Drosophila from ablation of cells making insulin-like ligands. Proc. Natl. Acad. Sci. USA *102*, 3105-3110.

Campbell, H.D., Schimansky, T., Claudianos, C., Ozsarac, N., Kasprzak, A.B., Cotsell, J.N., Young, I.G., de Couet, H.G., and Miklos, G.L. (1993). The Drosophila melanogaster flightless-I gene involved in gastrulation and muscle degeneration encodes gelsolin-like and leucine-rich repeat domains and is conserved in *Caenorhabditis elegans* and humans. Proc. Natl. Acad. Sci. USA *90*, 11386-11390.

Canavoso, L.E., Jouni, Z.E., Karnas, K.J., Pennington, J.E., and Wells, M.A. (2001). Fat metabolism in insects. Annu. Rev. Nutr. *21*, 23-46.

Chandra, V., Huang, P., Hamuro, Y., Raghuram, S., Wang, Y., Burris, T.P., and Rastinejad, F. (2008). Structure of the intact PPAR-gamma-RXR- nuclear receptor complex on DNA. Nature *456*, 350-356.

Choi, J.S., Choi, S.S., Kim, E.S., Seo, Y.K., Seo, J.K., Kim, E.K., Suh, P.G., and Choi, J.H. (2015). Flightless-1, a novel transcriptional modulator of PPARgamma through competing with RXRα. Cell. Signal. *27*, 614-620.

Claudianos, C., and Campbell, H.D. (1995). The novel flightless-I gene brings together two gene families, actin-binding proteins related to gelsolin and leucine-rich-repeat proteins involved in Ras signal transduction. Mol. Biol. Evol. *12*, 405-414.

Cohen, P., Miyazaki, M., Socci, N.D., Hagge-Greenberg, A., Liedtke, W., Soukas, A.A., Sharma, R., Hudgins, L.C., Ntambi, J.M., and Friedman, J.M. (2002). Role for stearoyl-CoA desaturase-1 in leptin-mediated weight loss. Science *297*, 240-243.

Davy, D.A., Ball, E.E., Matthaei, K.I., Campbell, H.D., and Crouch, M.F. (2000). The flightless I protein localizes to actin-based structures during embryonic development. Immunol. Cell Biol. *78*, 423-429.

Davy, D.A., Campbell, H.D., Fountain, S., de Jong, D., and Crouch, M.F. (2001). The flightless I protein colocalizes with actin- and microtubule-based structures in motile Swiss 3T3 fibroblasts: evidence for the involvement of PI 3-kinase and Ras-related small GTPases. J. Cell Sci. *114*, 549-562.

de Couet, H.G., Fong, K.S., Weeds, A.G., McLaughlin, P.J., and Miklos, G.L. (1995). Molecular and mutational analysis of a gelsolin-family member encoded by the flightless I gene of Drosophila melanogaster. Genetics *141*, 1049-1059.

Deak, II, Bellamy, P.R., Bienz, M., Dubuis, Y., Fenner, E., Gollin, M., Rahmi, A., Ramp, T., Reinhardt, C.A., and Cotton, B. (1982). Mutations affecting the indirect flight muscles of Drosophila melanogaster. J. Embryol. Exp. Morphol. *69*, 61-81.

Ducharme, N.A., and Bickel, P.E. (2008). Lipid droplets in lipogenesis and lipolysis. Endocrinology *149*, 942-949.

Enoch, H.G., Catala, A., and Strittmatter, P. (1976). Mechanism of rat liver microsomal stearyl-CoA desaturase. Studies of the substrate specificity, enzyme-substrate interactions, and the function of lipid. J. Biol. Chem. *251*, 5095-5103.

Goshima, M., Kariya, K., Yamawaki-Kataoka, Y., Okada, T., Shibatohge, M., Shima, F., Fujimoto, E., and Kataoka, T. (1999). Characterization of a novel Ras-binding protein Ce-FLI-1 comprising leucine-rich repeats and gelsolin-like domains. Biochem. Biophy.s Res. Commun. *257*, 111-116.

Gronke, S., Clarke, D.F., Broughton, S., Andrews, T.D., and Partridge, L. (2010). Molecular evolution and functional characterization of Drosophila insulin-like peptides. PLoS Genet *6*, e1000857.

Gutierrez, E., Wiggins, D., Fielding, B., and Gould, A.P. (2007). Specialized hepatocyte-like cells regulate Drosophila lipid metabolism. Nature *445*, 275-280.

Hulver, M.W., Berggren, J.R., Carper, M.J., Miyazaki, M., Ntambi, J.M., Hoffman, E.P., Thyfault, J.P., Stevens, R., Dohm, G.L., Houmard, J.A., et al. (2005). Elevated stearoyl-CoA desaturase-1 expression in skeletal muscle contributes to abnormal fatty acid partitioning in obese humans. Cell Metab. *2*, 251-261.

Jiang, G., Li, Z., Liu, F., Ellsworth, K., Dallas-Yang, Q., Wu, M., Ronan, J., Esau, C., Murphy, C., Szalkowski, D., et al. (2005). Prevention of obesity in mice by antisense oligonucleotide inhibitors of stearoyl-

CoA desaturase-1. J Clin Invest 115, 1030-1038.

Kajava, A.V., Vassart, G., and Wodak, S.J. (1995). Modeling of the three-dimensional structure of proteins with the typical leucine-rich repeats. Structure *3*, 867-877.

Kobe, B., and Deisenhofer, J. (1995). A structural basis of the interactions between leucine-rich repeats and protein ligands. Nature *374*, 183-186.

Kopecki, Z., Arkell, R., Powell, B.C., and Cowin, A.J. (2009). Flightless I regulates hemidesmosome formation and integrin-mediated cellular adhesion and migration during wound repair. J. Invest. Dermatol. *129*, 2031-2045.

Lee, Y.H., and Stallcup, M.R. (2006). Interplay of Fli-I and FLAP1 for regulation of beta-catenin dependent transcription. Nucleic Acids Res. *34*, 5052-5059.

Lee, Y.H., Campbell, H.D., and Stallcup, M.R. (2004). Developmentally essential protein flightless I is a nuclear receptor coactivator with actin binding activity. Mol. Cell. Biol. *24*, 2103-2117.

Miklos, G.L., and De Couet, H.G. (1990). The mutations previously designated as flightless-I3, flightless-O2 and standby are members of the W-2 lethal complementation group at the base of the X-chromosome of Drosophila melanogaster. J. Neurogenetics *6*, 133-151.

Miller, C.W., and Ntambi, J.M. (1996). Peroxisome proliferators induce mouse liver stearoyl-CoA desaturase 1 gene expression. Proc. Natl. Acad. Sci. USA *93*, 9443-9448.

Ntambi, J.M. (1995). The regulation of stearoyl-CoA desaturase (SCD). Prog. Lipid Res. *34*, 139-150.

Perrimon, N., Smouse, D., and Miklos, G.L. (1989). Developmental genetics of loci at the base of the X chromosome of Drosophila melanogaster. Genetics *121*, 313-331.

Puig, O., Marr, M.T., Ruhf, M.L., and Tjian, R. (2003). Control of cell number by Drosophila FOXO: downstream and feedback regulation of the insulin receptor pathway. Genes Dev. *17*, 2006-2020.

Roman, G., Endo, K., Zong, L., and Davis, R.L. (2001). P[Switch], a system for spatial and temporal control of gene expression in Drosophila melanogaster. Proc. Natl. Acad. Sci. USA *98*, 12602-12607.

Ron, D. (2002). Translational control in the endoplasmic reticulum stress response. J. Clin. Invest. *110*, 1383-1388.

Ruzehaji, N., Kopecki, Z., Melville, E., Appleby, S.L., Bonder, C.S., Arkell, R.M., Fitridge, R., and Cowin, A.J. (2014). Attenuation of flightless I improves wound healing and enhances angiogenesis in a murine model of type 1 diabetes. Diabetologia *57*, 402-412.

Ryoo, H.D., and Vasudevan, D. (2017). Two distinct nodes of translational inhibition in the integrated stress response. BMB Rep. *50*, 539-545.

Sampath, H., and Ntambi, J.M. (2011). The role of stearoyl-CoA desaturase in obesity, insulin resistance, and inflammation. Ann. N Y Acad. Sci. *1243*, 47-53.

Sampath, H., Miyazaki, M., Dobrzyn, A., and Ntambi, J.M. (2007). Stearoyl-CoA desaturase-1 mediates the pro-lipogenic effects of dietary saturated fat. J. Biol. Chem. *282*, 2483-2493.

Singh Ahuja, H., Liu, S., Crombie, D.L., Boehm, M., Leibowitz, M.D., Heyman, R.A., Depre, C., Nagy, L., Tontonoz, P., and Davies, P.J. (2001). Differential effects of rexinoids and thiazolidinediones on metabolic gene expression in diabetic rodents. Mol. Pharmacol. *59*, 765-773.

Sinha, R.A., Singh, B.K., Zhou, J., Xie, S., Farah, B.L., Lesmana, R., Ohba, K., Tripathi, M., Ghosh, S., Hollenberg, A.N., et al. (2017). Loss of ULK1 increases RPS6KB1-NCOR1 repression of NR1H/LXR-mediated Scd1 transcription and augments lipotoxicity in hepatic cells. Autophagy *13*, 169-186.

Straub, K.L., Stella, M.C., and Leptin, M. (1996). The gelsolin-related flightless I protein is required for actin distribution during cellularisation in Drosophila. J. Cell Sci. *109* (Pt 1), 263-270.

Taniuchi, S., Miyake, M., Tsugawa, K., Oyadomari, M., and Oyadomari, S. (2016). Integrated stress response of vertebrates is regulated by four eIF2α kinases. Sci. Rep. *6*, 32886.

Teleman, A.A., Chen, Y.W., and Cohen, S.M. (2005). Drosophila Melted modulates FOXO and TOR activity. Dev. Cell *9*, 271-281.

Way, J.M., Harrington, W.W., Brown, K.K., Gottschalk, W.K., Sundseth, S.S., Mansfield, T.A., Ramachandran, R.K., Willson, T.M., and Kliewer, S.A. (2001). Comprehensive messenger ribonucleic acid profiling reveals that peroxisome proliferator-activated receptor gamma activation has coordinate effects on gene expression in multiple insulin-sensitive tissues. Endocrinology *142*, 1269-1277.

Wu, L., Chen, H., Zhu, Y., Meng, J., Li, Y., Li, M., Yang, D., Zhang, P., Feng, M., and Tong, X. (2013). Flightless I homolog negatively regulates ChREBP activity in cancer cells. Int. J. Biochem. Cell Biol. *45*, 2688-2697.

Yao, D., Luo, J., He, Q., Shi, H., Li, J., Wang, H., Xu, H., Chen, Z., Yi, Y., and Loor, J.J. (2017). SCD1 Alters Long-Chain Fatty Acid (LCFA). composition and its expression is directly regulated by SREBP-1 and PPARgamma 1 in dairy goat mammary cells. J. Cell Physiol. *232*, 635-649.

Yu, Y., Maguire, T.G., and Alwine, J.C. (2014). ChREBP, a glucose-responsive transcriptional factor, enhances glucose metabolism to support biosynthesis in human cytomegalovirus-infected cells. Proc. Natl. Acad. Sci. USA *111*, 1951-1956.