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...
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 used 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, fili 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 fili 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 fili plays a role in lipid metabolism. Here, we report that Drosophila fili mutants are resistant to starvation and have larger amounts of triglycerides in the fat body and intestine. Fil mutants showed high mRNA expression of desaturase 1 (desat1), whose preferred substrate is stearoyl-CoA. Furthermore, a fat-body-specific knockdown of fili increased the level of triglycerides, and this change was reversed by a desat1 knockdown in the fat body. Conversely, overexpression of fili significantly reduced the amount of triglycerides and mRNA expression of desat1; this result was further validated in mammalian cells. Mammalian fili 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 fili in lipid metabolism and suggest that fili 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% 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).

Next, they were transferred to vials containing 1% agar in PBS. The flies were transferred to fresh food every 12 h and maintained at 25°C; deaths were recorded at those time points.

**Quantitative 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).

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. Rpo49 served as a reference for normalization. Relative quantification of mRNA was performed by the comparative Ct method. The primers are listed in Supplementary Table 1.
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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 fliI mutants show increased lipid storage

To assess the physiological role of fliI in lipid metabolism, we used already characterized fliI mutant alleles, fliI3 and fliI14. The FliI3 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 FliI14 allele is lethal during larval and pupal stages, indicating that the fliI protein is essential for survival of the organism into adulthood (Perrimon et al., 1989). We examined the amounts of triglycerides in a fliI14 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 fliI3/14 mutants, when compared to control fly strains: w1118 flies. 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 fliI3/14 mutants’ fat body in comparison with the control flies’ fat body (Fig. 1C). As in the fat body, the level of triglycerides was slightly but significantly higher in fliI3/14 mutants’ intestine (Fig. 1B).

Fig. 1. Drosophila flightless-I (fliI) 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 fliI3/14 mutants compared to controls (w1118 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 fliI3/14 mutants (G). (E, F, H, I) Nile red staining shows increased lipid droplet size in the fat body of a fliI3/14 mutant (H). Dissected adult midguts stained with Nile red reveal significantly increased lipid staining in fliI3/14 mutants (I, I’) compared to controls (w1118; panels F, F’). Genotypes: w1118 (D–F) and fliI3/14 (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, w1118 and Canton S, were not significantly different, hereafter, we used only strain w1118 as a control for fliI 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 fliI3/14 mutants (Figs. 1D and 1E); fliI3/14 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 fliI3/14 mutants (Figs. 1I and 1I’). The fliI 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 fliI plays a central role in lipid storage in Drosophila.

FliI mutants exhibit the features of metabolic disorders
To test whether the increased triglyceride amount in fliI3/14 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, fliI3/14 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 fliI3/14 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 fliI3/14 mutants (Fig. 2D). Although fliI3/14 mutant flies had high insulin levels, these mutant flies remained hyperglycemic, which suggested that fliI3/14 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 fliI3/14 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; Tanuchi et al., 2016). As shown in Figs. 2F and 2G, phosphorylation of eIF2α increased in fliI3/14 mutant flies. As FliI has a role in actin cytoskeletal activity, as expected, the level of alpha tubulin was reduced in fliI3/14 mutants (Figs. 2F and 2G). These results suggested that fliI may be a novel regulator involved in metabolic disorders.

Fig. 2. FliI mutants manifest the features of metabolic disorders. (A–C) Hemolymph glucose (A) and trehalose (B) levels in w1118 and fliI3/14 mutant flies. The relative trehalose and glucose levels slightly but significantly increased in fliI3/14 mutant flies relative to those in w1118 flies (C). (D, E) Expression levels of indicated gene in w1118 and fliI3/14 mutant flies. (F, G) A representative Western blot of whole-fly extracts from w1118 and fliI3/14 mutants (F). eIF2α phosphorylation increased in fliI3/14 mutant flies compared to the w1118 strain. The amount of α-tub was lower in fliI3/14 mutant flies. (G) Comparison of the normalized anti-phospho-eIF2α band intensity between w1118 flies and fliI3/14 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, fliI3/14 mutant flies survived longer than w1118 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, fliI3/14 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 fliI3/14 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 fliI3/14 mutant flies (Figs. 3C and 3D). To determine whether the flight defect has an influence on food intake, we measured food intake in w1118 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 filI is crucial for energy homeostasis.

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

Stearoyl-CoA desaturases (SCDs) are key enzymes in fatty acid biosynthesis. 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

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Fig. 3. FliI mutants are resistant to starvation. (A) Seven-day-old female w1118 flies (controls) and fliI3/14 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 w1118 flies decreased by 75% after 36 h of starvation, fliI3/14 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 w1118 and fliI3/14 flies at each time point after starvation. (C, D) Fasting-induced feeding was less active in fliI3/14 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 (w1118 flies). The blue dye was not detected in either fasted or fed fliI3/14 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 *desat*1 in the intestine (left) and fat body (right) from 7-day-old dissected adults. mRNA expression of *desat*1 was higher in fliI3/14 mutants relative to w1118 flies (controls). (B) The mRNA expression of *desat*1 gradually increased in w1118 flies (control) and fliI3/14 mutants. The transcript levels of *desat*1 at the indicated time points were significantly higher in fliI3/14 mutants than in w1118 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 *desat*1 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 *desat*1. (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>desat1-IR), the knockdown of *desat*1 via RU486 supplementation reduced the concentration of triglycerides (P{Switch1}106>desat1-IR). The decreased level of triglycerides in the *desat*1 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 ***\(P < 0.001\).
These findings provided strong evidence that fli suppresses desat1 transcription, which contributes to abnormal lipid metabolism. To test whether overexpression of fli changes the survival of starvation-resistant flies, we included the expression of fli using Act5C-Gal4, which drove expression of fli in the whole body only when RU486 was added to the diet. FliI-overexpressing flies died at a faster rate than control flies did (Act5C-Gal4, 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 fli mutants can be reversed by a knockdown of desat1. We used the RU486-inducible gene switch driver P(Switch1)106-gal4 and downregulated fliI in vivo, in a fliI RNA interference (RNAi) strain. The knockdown of fliI 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 adult fat body decreased the concentrations of triglycerides (P(Switch1)106>fliI-IR, desat1-IR; Fig. 4G). These results suggested that fli inactivation is responsible for the obesity-like phenotype because of upregulation of desat1.

**DISCUSSION**

Here, we demonstrate that *Drosophila* fli is a key regulator of lipid metabolism. Specifically, fli 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 fliI<sup>+/−</sup> mutants show elevated mRNA levels of desat1, whereas fliI overexpression lowers desat1 transcription, we propose that fliI downregulates desat1, whereas the latter gene promotes the obesity-like phenotype.

Nevertheless, it is unclear how fli 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 PPAR<γ>, 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 fliI functions as a transcriptional modulator of PPAR<γ> in mammals (Choi et al., 2015). FliI inhibits formation of the complex of PPAR<γ> with RXRα (retinoid X receptor α) by competitively interacting with PPAR<γ>. As the short carboxy-terminal extensions of the RXRα DNA-binding domain (DBD) interact with the DBD of PPAR<γ> (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 α2, C/EBP<α> (CCAAT/enhancer-binding protein α), adiponectin, adipin, LPL (lipoprotein lipase), FAS (fatty acid synthase), and GLUT4 (glucose transporter 4) will increase. On the other hand, binding of FliI to the DBD of PPAR<γ> reduces DNA occupancy of PPAR<γ> in the promoter of those target genes, thereby suppressing adipocyte differentiation (Choi et al., 2015).

Thus, we believe that fliI downregulates PPAR<γ>-activated SCD expression although *Drosophila* does not have an obvious homolog of PPAR<γ>. We need to further explore how FliI and PPAR<γ> interact to promote the expression of SCD in mammals.

Alternatively, the regulation of desat1 expression by fli may depend on the interaction between fliI 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 thioreredox-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 fli may be a consequence of some upstream event, such as the interaction of fliI and PPAR<γ> or ChREBP, not direct transcriptional regulation of desat1 by fliI.

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<sup>−/−</sup>* 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 fliI expression seen in our study. Besides, we cannot rule out the possibility of species differences. *fliI<sup>−/−</sup>* 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, *fliI<sup>−/−</sup>* 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 fliI3/14 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 fliI3/14 mutant flies according to the energy demand.

In summary, our results show that Drosophila fli1 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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