Edem1 activity in the fat body regulates insulin signalling and metabolic homeostasis in *Drosophila*

Himani Pathak and Jishy Varghese*

School of Biology

Indian Institute of Science Education and Research (IISER TVM)

Vithura, Thiruvananthapuram

Kerala, India Pin: 695551

*corresponding author

Email: jishy@iisertvm.ac.in

Abstract

In *Drosophila*, nutrient status is sensed by the fat body, a functional homolog of mammalian liver and white adipocytes. The fat body conveys nutrient information to insulin-producing cells (IPCs) through humoral factors which regulate *Drosophila* insulin-like peptide (DILP) levels and insulin signalling. Insulin signalling has pleiotropic functions, which include the management of growth and metabolic pathways. Here, we report that Edem1 (endoplasmic reticulum degradation-enhancing α -mannosidase-like protein 1), an endoplasmic reticulum-resident protein involved in protein quality control, acts in the fat body to regulate insulin signalling and thereby the metabolic status in *Drosophila*. Edem1 limits the fat body derived *Drosophila* TNF α Eiger activity on IPCs and maintains systemic insulin signalling in fed conditions. During food deprivation, *edem1* gene expression levels drop, which aids in the reduction of systemic insulin signalling crucial for survival. Overall we demonstrate that Edem1 plays a vital role in helping the organism to endure a fluctuating nutrient environment by managing insulin signalling and metabolic homeostasis.

Introduction

Energy homeostasis, the sum of all processes which maintain the balance between energy inflow and outflow; is vital for normal functioning, reproduction as well as longevity. Energy homeostasis in animals is brought about by the activity and interplay of various endocrine and neuroendocrine systems. Insulin/ Insulin-like growth factor (IGF) signalling pathway plays a significant role in the maintenance of energy balance and is well conserved in both vertebrates and invertebrates (Britton, Lockwood et al., 2002, Brogiolo, Stocker et al., 2001, Clancy, Gems et al., 2001, Fabrizio, Pozza et al., 2001, Fernandez & Torres-Aleman, 2012, Kenyon, Chang et al., 1993, Kimura, Tissenbaum et al., 1997). The perturbations in insulin signalling result in a plethora of effects like diabetes, obesity, reduced body size, resistance to starvation and oxidative stress (Accili, Drago et al., 1996, Bonafe, Barbieri et al., 2003, Britton et al., 2002, Clancy et al., 2001, Giannakou, Goss et al., 2004, Giannakou & Partridge, 2007, Holzenberger, Dupont et al., 2003, Ikeya, Galic et al., 2002, Kahn, Hull et al., 2006, Katic & Kahn, 2005, Liu, Baker et al., 1993, Rulifson, Kim et al., 2002, Shimokawa, Higami et al., 2003, Sonntag, Carter et al., 2005, Tatar, Kopelman et al., 2001). *Drosophila melanogaster*, a widely used genetic model organism, has 8 insulin-like peptides (DILPs 1-8), which share structural and functional similarities with mammalian insulin and IGFs (Gronke, Clarke et al., 2010). Among these DILPs; DILP2, DILP3 and DILP5 are produced mainly by a subset of the median neurosecretory cells (mNSCs), the insulin-producing cells (IPCs), in the fly brain (Broughton, Slack et al., 2010, Geminard, Rulifson et al., 2009, Ikeya et al., 2002, Nassel, 2012). The major effector tissue of insulin signalling is the fat body, which is also the main energy reserve and nutrient sensor in flies (Geminard et al., 2009, Hwangbo, Gershman et al., 2005). The fat body relays information about the nutrient status of the organism through humoral factors, which act on the IPCs directly or indirectly to control systemic insulin signalling (Agrawal,

Delanoue et al., 2016, Bai, Kang et al., 2012, Colombani, Raisin et al., 2003, Delanoue, Meschi et al., 2016, Droujinine & Perrimon, 2016, Geminard et al., 2009, Ghosh & O'Connor, 2014, Koyama & Mirth, 2016, Rajan & Perrimon, 2013, Sano, Nakamura et al., 2015, Sun, Liu et al., 2017). The fat derived signals that control IPC function include DILP6, a *Drosophila* insulin-like peptide; Unpaired2 (Upd2), a functional homolog of leptin in *Drosophila* and activator of JAK-STAT pathway; Eiger, the *Drosophila* Tumour Necrosis Factor α / TNF α , which activates JNK signalling; CCHamide2, a nutrient responsive peptide hormone; Growth blocking peptide (GBP), a *Drosophila* cytokine; Stunted, a circulating insulintropic peptide; female-specific independent of transformer (FIT) and activin-like ligand Dawdle. The molecular mechanisms that regulate the synthesis and secretion of the fat body derived signals (FDSs) is currently under intense investigation.

The Endoplasmic reticulum (ER) serves many functions in the eukaryotic cell, foremost of which is the synthesis and folding of nascent proteins with the help of molecular chaperones and folding enzymes. Hence, the ER is considered as the major quality-control site which ensures that only correctly folded proteins are allowed to leave to other cellular compartments. The ER is also considered to be the first storage site of secretory proteins and the ER activity is high in cells of endocrine and exocrine tissues due to the heavy protein trafficking in such cells. Genetic factors, physiological changes and fluctuations in the cellular environment might lead to misfolding of proteins (Liu & Kaufman, 2003) and the ER aids in eliminating proteins, which remain misfolded even after multiple rounds of folding attempts. Thus, a proper balance between the influx of proteins and the folding machinery in the ER is crucial for efficient protein quality control. When the ER homeostasis is upset misfolded proteins accumulate in the ER triggering an adaptive response called unfolded protein responses (UPR). The UPR signalling mainly involves three ER residing transmembrane sensors: inositol-requiring protein 1 (IRE1), activating transcription factor 6 (ATF6) and PKR-like ER kinase (PERK). The UPR sensors

would initiate ER-associated degradation (ERAD) of terminally misfolded proteins, expand the ER membrane, increase the folding capacity of the ER and decrease the overall protein load in the ER (Liu & Kaufman, 2003). Permanently unfolded glycoproteins are recognised by ERAD-enhancing α -mannosidase-like proteins (Edem), which aid in the degradation of the misfolded proteins (Araki & Nagata, 2011, Kroeger, Chiang et al., 2012, Molinari, Calanca et al., 2003). Glycoproteins constitute a large proportion of proteins in a cell, hence the function of Edem is crucial for cellular homeostasis.

Here, we report that Edem1 activity in the *Drosophila* fat body is crucial for maintaining systemic insulin signalling. Down-regulation of *edem1* gene expression in the fat body resulted in the accumulation of DILP2 in the IPCs, a decrease of *dilp3* mRNA levels and reduced systemic insulin signalling, which led to nutrient imbalances and altered sensitivity to starvation. Our results also show that Edem1 regulates fat body derived *Drosophila* TNF α Eiger activity in the IPCs, crucial for managing systemic insulin signalling and metabolic status. Activation of target of rapamycin (TOR) signalling, the main amino acid sensor and a key regulator of Eiger activity rescued the effects of *edem1* down-regulation. Furthermore, in response to nutrient deprivation, *edem1* transcripts were found to be low, which we show is critical to the reduction of systemic insulin levels and better survival of flies during starvation. We propose that Edem1 acts as a key factor in the fat body, which maintains nutrient homeostasis by controlling the activity of the IPCs through Eiger.

Results

Edem1 maintains metabolic homeostasis

We embarked on a large scale genetic screen in *Drosophila* to identify factors that control nutrient homeostasis and insulin signalling. Towards this, we blocked various candidate genes, reported to be differentially expressed in the *miR-14* mutants that exhibited metabolic imbalances, in the *Drosophila* fat body using RNAi lines (Varghese, Lim et al., 2010). In this screen we identified Edem1, an ER-resident protein involved in protein quality control, as a putative regulator of metabolic status in *Drosophila*. Down-regulation of *edem1* transcripts in the fat body led to a significant increase in the levels of energy stores - triglycerides and glycogen, in adult flies (Fig. 1A and B, Fig. EV1). In response to knock down of *edem1* in the fat body flies survived better during nutrient deprivation (Fig. 1C). The higher energy stores in response to reduction of *edem1* levels in the fat body could account for the better survival of flies during nutrient deprivation (Fig. 1D and E). Along with changes in stored nutrient levels, circulating glucose levels were high in the larval hemolymph (Fig. 1F). In addition, blocking *edem1* in the fat body led to enhanced feeding responses in the larvae (Fig. 1G), similar to responses reported earlier in food deprived larvae (Chouhan, Wolf et al., 2017). We also observed an increase in lifespan of the adults upon *edem1* down-regulation in the fat body (Fig. 1H). These data show that Edem1 function in the fat body is crucial in regulating metabolic homeostasis in *Drosophila*. The phenotypes observed in response to blocking *edem1* levels in the fat body indicated a reduction in insulin signalling, as similar phenotypes were observed in response to low insulin signalling (Bai, Kang et al., 2013, Bai, Post et al., 2015, Clancy et al., 2001, Giannakou & Partridge, 2007, Hwangbo et al., 2005, Min, Yamamoto et al., 2008, Partridge, Alic et al., 2011, Post, Karashchuk et al., 2018). Next, we tested if insulin signalling is reduced in response to blocking *edem1* levels in the fat body.

Edem1 function in the fat body maintains systemic insulin signalling

To measure the insulin signalling activity in response to blocking *edem1* in the fat body we checked gene expression of key downstream target genes of insulin pathway. Transcription of *4ebp* (*eIF4E-binding protein*), *inr* (*insulin receptor*) and *dilp6* (*Drosophila insulin-like peptide 6*) is suppressed by insulin signalling and these insulin target genes can be used as a read out for insulin signalling activity (Puig, Marr et al., 2003, Slaidina, Delanoue et al., 2009). Blocking *edem1* in the fat body increased transcript levels of the insulin responsive genes, which indicate low insulin signalling (Fig. 2A). We speculated if Edem1 activity in the fat body could regulate IPC function and control systemic insulin signalling, as fat body is known to remotely control IPCs. To address whether Edem1 in the fat body regulates IPC function, the transcript levels of IPC specific DILPs - *dilp2*, *dilp3* and *dilp5* were measured. In response to the expression of *edem1*-RNAi in the fat body, *dilp3* mRNA levels were found to be low, however, there were no detectable changes in the mRNA levels of *dilp2* and *dilp5* (Fig. 2B). Previous studies report that nutrient deprivation would block DILP secretion from the IPCs into the hemolymph leading to an accumulation of DILPs and reduction in systemic insulin signalling (Geminard et al., 2009). We observed an increase of DILP2 puncta in IPCs in response to reducing *edem1* levels in the fat body when compared to that of control (Fig. 2C), which suggested an accumulation of DILP2 protein in the IPCs. Together, these observations suggest that *edem1* function in the fat body maintains systemic insulin signalling by the regulation of IPC activity.

As the next approach we tested whether the reduction of insulin signalling in response to blocking *edem1* in the fat body was responsible for the metabolic phenotypes. A constitutively active form of insulin receptor (*InR^{A1325D}*) was co-expressed with *edem1*-RNAi in the fat body. *InR^{A1325D}*, which harbours a mutation at the Ala 1325 residue, would

activate downstream insulin signalling independent of DILP ligand and hence should alleviate phenotypes caused by low insulin signalling (Broughton, Piper et al., 2005, DiAngelo & Birnbaum, 2009, Kannan & Fridell, 2013, Tettweiler, Miron et al., 2005). As expected, expression of *InRA^{1325D}* was sufficient to alleviate high triglyceride levels and starvation resistance observed in response to knock down of *edem1* levels in the fat body (Fig. 2D and E). Thus, blocking *edem1* in the fat body reduced systemic insulin signalling, which led to metabolic phenotypes. These experiments confirm that Edem1 function in the fat body is crucial to maintain systemic insulin signalling and metabolic homeostasis.

Fat body derived signals are involved in Edem1 mediated regulation of IPCs

Drosophila fat body controls IPC function with the aid of a set of humoral factors, which relays the nutritional status of the organism to the IPCs. The fat body derived signals (FDSs) control DILP release from the IPCs into the hemolymph leading to effects on growth and maintenance of metabolic balance. In addition, changes in *dilp* gene expression has also been reported in response to fat body derived signals. We next investigated whether blocking Edem1 led to changes in the levels of FDSs and thereby the function of IPCs.

To test the role of FDSs in *edem1* knock down phenotypes we measured the levels or activity of various FDSs. We saw an increase in the transcript levels of *dilp6* in response to knock down of *edem1* in the fat body (Fig. 2A'). Next, we measured the mRNA levels of *totA* and the levels of STAT92E-GFP as read outs for activity of JAK-STAT pathway, a cell signalling pathway activated by Upd2 (Rajan & Perrimon, 2013). Blocking *edem1* expression in the fat body led to a decrease in *totA* mRNA levels (Fig. EV3A) and STAT92E-GFP expression (Fig. EV3B). We also measured the transcript levels of *tace*, the TNFα converting enzyme, and *Nlaz*, a target of JNK signalling (Agrawal et al., 2016, Hull-Thompson, Muffat et al., 2009, Pasco & Leopold, 2012), and found an increase in their

levels in response to *edem1*-RNAi (Fig. 3A and B). Eiger and Dilp6 are considered to be negative regulators of IPC function, whereas, Upd2 is expected to activate IPCs (Agrawal et al., 2016, Bai et al., 2012, Rajan & Perrimon, 2013), the gene expression changes observed here suggested that these FDSs might mediate the effects of *edem1* knock down on insulin signalling.

Eiger is involved in Edem1 mediated regulation of IPCs

To identify the FDS(s) involved in mediating the metabolic phenotypes observed by blocking *edem1* levels, we expressed *eiger*-RNAi, *dilp6*-RNAi or *upd2*, together with *edem1*-RNAi in the fat body. Down regulation of *eiger* mRNA and over expression of *upd2* rescued *edem1* knock down phenotypes, however, there were no effects with *dilp6*-RNAi (Fig. 3C and D, Fig. EV3C-F). We did not see any significant changes to DILP2 levels in the IPCs, *dilp3* mRNA levels and insulin target genes by overexpression of *upd2* in fat body that express *edem1*-RNAi (data not shown). However, when we down-regulated *eiger* mRNA in *edem1*-RNAi expressing fat body, we observed a reduction of DILP2 puncta in the IPCs seen in response to *edem1*-RNAi expression in the fat body (Fig. 4A). In addition, transcript levels of *dilp3* (Fig. 4B) and insulin target genes *dilp6* and *inr* (Fig. 4C) were partially restored by reducing Eiger levels in the *edem1*-RNAi back ground. Furthermore, the increase of glucose levels in the hemolymph seen in response to blocking *edem1* were suppressed (Fig. 4D). Thus, Edem1 mediated regulation of Eiger is crucial for managing insulin levels and nutrient homeostasis, whereas the Edem1 mediated regulation of Upd2 function manages nutrient homeostasis but doesn't do so by acting at the level of insulin signalling.

The fat body derived cytokine Eiger is an upstream activator of c-Jun N-terminal kinase (JNK) pathway in flies and previous studies have shown that JNK signalling extends life span and limits growth by antagonizing cellular and organism-wide responses to insulin

signalling (Agrawal et al., 2016, Andersen, Colombani et al., 2015, Hirosumi, Tuncman et al., 2002, Oh, Mukhopadhyay et al., 2005, Wang, Bohmann et al., 2005). The increase of *NLaz* transcript levels in response to blocking *edem1* levels in the fat body was rescued completely by the co-expression of *eiger*-RNAi, showing that the increase of JNK signalling in response to blocking *edem1* expression in the fat body is due to enhanced Eiger levels (Fig. 3B). These experiments confirm that Edem1 function in the fat body regulates Eiger activity and JNK signalling.

Next, we carried out experiments to confirm if regulation of Eiger by Edem1 is crucial for maintaining IPC function and metabolic homeostasis. Towards this we used two approaches: we activated TOR signalling pathway in *edem1*-RNAi expressing fat body (Fig. 5), as TOR signalling has been shown to block Eiger activation and we performed co-culture experiments by blocking the TNF receptor *grindelwald* in the IPCs (Fig. 6). TOR signalling pathway regulates a plethora of cellular processes including cell growth, proliferation cell survival, etc., depending on nutrient levels. Recently TOR has been reported to repress *tace* transcription which would in turn suppress the production of active Eiger from the fat body (Agrawal et al., 2016). Rheb (Ras homolog enriched in brain), a member of Ras superfamily of GTP binding proteins, activates TOR kinase and results in growth and regulation of metabolic pathways (Garami, Zwartkruis et al., 2003, Oldham, 2011, Oldham, Montagne et al., 2000, Saucedo, 2003). Increase in the levels of JNK pathway target *NLaz* in response to *edem1* downregulation in the fat body was abrogated by over-expression of *rheb* (Fig. 5A), which confirms that activating TOR signalling is sufficient to suppress JNK signalling possibly by the regulation of Eiger activity. Changes in the reduction of insulin signalling (Fig. 5B) and *dilp3* transcript levels (Fig. 5C); excess fat levels (Fig. 5D) and starvation sensitivity (Fig. 5E) in response to blocking Edem1 levels were rescued by the over expression of *rheb* in the fat body. This confirmed that

Edem1 mediated regulation of Eiger is crucial for managing systemic insulin signalling, fat storage and starvation sensitivity.

Next, we tried to show that Edem1 function in the fat body regulates Eiger activity on IPCs. In low protein diet, the soluble form of Eiger binds to its receptor Grindelwald (*grnd*) in the IPCs, thereby activates JNK signalling and suppresses *dilp* transcript levels (Agrawal et al., 2016, Andersen et al., 2015). We performed *ex vivo* culture experiments, using hemolymph isolated from the *edem1*-RNAi expressing larvae and incubated it independently with brains dissected from wild type larvae and larvae with *grnd* knocked down in the IPCs. We observed that DILP2 puncta were less in *grnd*-RNAi IPCs when compared to control IPCs (Fig. 6A and B). Thus, accumulation of DILP2 in the IPCs in response to blocking *edem1* levels in the larval fat body was due to Eiger activity on the IPCs through TNF receptor Grindelwald. Together these results confirm that Edem1 activity in the fat body regulates Eiger mediated JNK signalling in the IPCs and manage systemic insulin signalling and metabolic status of flies.

Reduction in *edem1* levels during starvation is crucial for survival

Insulin signalling aids an organism to respond to changes in the nutrient environment by managing various biological functions. In response to nutrient deprivation insulin signalling is reduced, which would allow an organism to manage its energy stores and induce various hunger triggered behavioral responses (Erion & Sehgal, 2013). Our experiments show that lowering *edem1* levels improved survival against starvation by reducing insulin signalling (Fig. 1C and Fig. 2E). Hence, we tested if *edem1* levels are lowered during nutrient deprivation, which may aid in better survival of flies by the reduction of insulin levels. Moreover, blocking *edem1* in the fat body in larvae was sufficient to enhance the appetite, similar to hunger induced responses observed in food deprived larvae (Fig. 1G). As expected, in response to food depletion we observed a reduction of *edem1* mRNA

levels (Fig. 7A). To test whether reduction of *edem1* levels would aid in protection against starvation we over expressed *edem1* in the fat body in food deprived flies. Overexpression of *edem1* in the fat body reduced the survival of flies during food deprivation (Fig. 7B, Fig. EV7), confirming that the reduction of *edem1* levels is crucial for survival in response to nutrient depletion. Next, we checked if starvation responses was affected in flies that over-express *edem1* in the fat body. The changes in the transcript levels of *dilp3*; insulin signalling, and JNK signalling in response to starvation was abrogated by *edem1* over-expression in the fat body (Fig. 7C - E). Here, we conclude that lowering of *edem1* transcripts in the fat body during starvation facilitates reduction of insulin signalling, which results in enhanced survival of flies.

Discussion

Fluctuations in nutrient levels would trigger organism-wide changes, which includes alterations to various metabolic pathways. Changes in the metabolic pathways would aid the organism in managing the growth and maintenance of nutrient stores according to the availability of food. Apart from these biochemical changes, hunger elicits stereotypic behavioral responses, which includes an enhanced urge to feed, increased foraging, acceptance of unpalatable food, etc (Chouhan et al., 2017). Several of the crucial responses like mobilisation of stored nutrients and enhanced urge to feed, which aids the organism to survive nutrient deprivation is triggered by the reduction of systemic insulin signalling (Broughton et al., 2005, DiAngelo & Birnbaum, 2009, Kannan & Fridell, 2013, Rulifson et al., 2002, Tettweiler et al., 2005). In *Drosophila*, IPCs respond to changes in the availability of food and modulate DILP levels under the control of the fat body, which acts as a nutrient sensor. Various fat body derived signals act on IPCs directly or indirectly, and the regulation of these signals in response to changes in the nutrient status of *Drosophila* plays a key role in maintaining systemic insulin levels (Droujinine & Perrimon, 2016). Here, we report a novel means by which the activity of a fat body derived signal on IPCs is regulated.

While investigating the mechanisms that function in the fat body to control *Drosophila* IPCs, we identified Edem1, an ER-resident protein involved in ERAD mediated protein quality control. Edem1 in the fat body maintains the activity of *Drosophila* TNF α Eiger and controls JNK signalling, which allows normal functioning of IPCs, maintain systemic insulin signalling and metabolic homeostasis (Figs. 4 and 5). Eiger is activated by TACE, which cleaves the transmembrane form of Eiger and releases a soluble active form of Eiger into the hemolymph. TOR kinase, a key nutrient sensor, has been reported to control *tace* transcript levels and Eiger activation. During low protein diets, due to reduced TOR

signalling, fat body releases the soluble form of Eiger, which would act on IPCs and activate JNK signalling to regulate *dilp* gene expression (Agrawal et al., 2016). Here, we identify Edem1 as a regulator of Eiger through regulation of *tace* gene expression (Fig. 3). We also show that activation of TOR signalling blocked the effects of suppression of *edem1* levels in the fat body (Fig. 5), substantiating the role of Edem1 in regulating Eiger activity. At the moment, it is not clear if the TOR pathway acts through Edem1 to manage *tace* gene expression. More efforts are also needed to identify the exact molecular mechanism by which Edem1 regulates *tace* levels.

Edem1 function in the fat body maintains systemic insulin signalling, reduction of *edem1* levels in the fat body resulted in low systemic insulin signalling which led to metabolic phenotypes, enhanced resistance to starvation, enhanced feeding and increase in life span (Fig 1). A significant part of the impact of reducing *edem1* levels on insulin signalling is due to the accumulation of DILP2 protein (Fig. 2C) and reduced *dilp3* transcript levels in the IPCs (Fig. 2B). However, it should be noted that we did not observe any changes at the protein and mRNA levels of other mNSC DILPs. DILPs are known to be regulated in a context specific manner, gene expression as well as protein levels in IPCs vary based on nutritional cues, developmental stages and various neural and endocrine signals that act on the IPCs (Gronke et al., 2010, Hallier, Schiemann et al., 2016, Hong, Lee et al., 2012, Ikeya et al., 2002, Kim & Neufeld, 2015, Luo, Lushchak et al., 2014, Soderberg, Carlsson et al., 2012, Varghese et al., 2010). Strictly the roles of individual DILPs are not understood, however, the effects of ablating IPCs, on growth and metabolism could be rescued by DILP2 expression alone (Haselton, Sharmin et al., 2010, Rulifson et al., 2002). Many reports hint at effects on insulin signalling caused by an individual DILP or more than one DILP (Bai et al., 2012, Sudhakar, Pathak et al., 2019).

Managing insulin signalling during nutrient withdrawal is crucial for mobilization of nutrient stores and survival. In response to starvation, we report that *edem1* transcripts are reduced (Fig. 7A), which aids in lowering insulin signalling, an essential step in the induction of survival responses during starvation (Fig. 8). Thus, Edem1 regulation plays an important role in eliciting responses to starvation and enhancing Edem1 levels affected survival during starvation, probably due to a failure in reducing insulin signalling (Fig. 7B). Moreover, reducing Edem1 levels in fed conditions led to enhanced feeding responses, similar to starvation conditions, further suggesting an active role for Edem1 in survival against food deprivation (Fig. 1G). However, it is not yet clear if the function of Edem1 in regulating Eiger activity in the IPCs and systemic insulin signalling has any links to the ER stress pathway. Reduced *edem1* levels during starvation could be an outcome of reduced ER stress in response to low protein synthesis. Furthermore, reduction of *edem1* could cause aggregation of misfolded proteins in the ER, which might be responsible for the changes we report here on Eiger levels. To summarize, we show that Edem1, a key ERAD regulator, aids in the maintenance of nutrient homeostasis by managing the activity of Eiger in the IPCs.

Materials and Methods

Fly strains

Fly stocks were reared in vials with standard food which consisted of 5.8% cornmeal, 5% dextrose, 2.36% yeast, 0.8% agar and 10% Nipagen in 100% ethanol. The flies were maintained at 25 °C with 12 h:12 h light:dark cycle. *UAS-InRA^{1325D}* (stock# 8263) was obtained from Bloomington Drosophila stock center (BDSC). The RNAi lines used were obtained from Vienna Drosophila resource center (VDRC): *UAS-edem1-RNAi* (stock# 6923), *UAS-eiger-RNAi* (stock# 45253), *UAS-grnd-RNAi* (stock # 43454), *UAS-dilp6-RNAi* (GD) (stock# 45218). *dilp2-Gal4/CyOGFP*, *pumpless-Gal4* and *w¹¹¹⁸* were obtained from Stephen Cohen. *UAS-dEDEM1* was from Koichi Iijima, *UAS-rheb* was obtained from Jagat. K. Roy, *UAS-upd2-EGFP/TM3Sb* and 10XSTAT92E-GFP were obtained from Akhila Rajan.

Triglyceride and glycogen measurements

All the metabolism-related experiments were carried out in controlled growth conditions. 50 1st in-star larvae were collected in fresh food vials avoiding overcrowding within 2–3 h of hatching. GFP balancers were used wherever required to aid in genotyping. Freshly emerged adult male flies were collected (15 per vial) and 5-day old flies were used for triglyceride and glycogen measurement unless mentioned otherwise. 5 flies in triplicates per genotype were homogenized in 0.05% Tween-20 using Bullet Blender Storm BBY24M from Next Advance. Each experiment was replicated independently, number of independent biological replicates is mentioned for each experiment in the figure legends. The homogenate was heat inactivated at 70 °C for 5 min and then centrifuged at 14000 rpm for 3 min. Serum triglyceride determination kit (Cat. # TR0100) from Sigma was used to quantify triglyceride levels and protein levels were measured using the Quick Start™

Bradford 1X Dye Reagent (Cat. # 500-0205) from BioRad. This was followed by colorimetric estimation using TECAN Infinite M200 pro-multimode plate reader in 96-well format. The absorption maximum of 540 nm and 595 nm were used for triglyceride and protein content respectively. The triglyceride content of the flies was then normalized to the total protein content of the flies. Sample preparation for glycogen measurement was similar to triglycerides, following the manufacturer's protocol (Cat. # MAK016 from Sigma). The absorbance was measured at 570 nm. For triglyceride and glycogen utilization assay, 5 day old males (15 per vial) were transferred to vials containing 1% agar, were collected at the indicated time points and homogenized as mentioned above. Each experiment was replicated independently, number of replicates (n) is mentioned for each experiment in the figure legends.

Starvation sensitivity assay

For starvation sensitivity assay, 15 (5-day old) male flies were transferred to vials containing 1% agar and the number of dead flies was counted every 2 hours. These experiments were replicated independently and number of independent biological replicates are mentioned in the figure legends.

Lifespan assay

Adult lifespan assay was estimated with data obtained from three independent biological replicates for each genotype. These flies were flipped into fresh media every 3 days and the dead flies and the escapers were scored.

Glucose assay

Larvae at 3rd in-star stage (five larvae for every prep) larvae were used to isolate hemolymph using Zymo-Spin™ IIIC (C1006-250) from Zymo Research. 1 µl of hemolymph

was diluted to 50 μ l with autoclaved milli-Q water. 100 μ l of glucose assay reagent (Cat. # GAGO20) from Sigma was added and the reaction was incubated at 37 °C for 30 min. The reaction was stopped with 100 μ l of 12 N H₂SO₄. The glucose content was analyzed using colorimetric quantification at 540 nm using TECAN Infinite M200 pro-multi-mode plate reader in 96-well format. Hemolymph glucose measurements were replicated independently and number of replicates are mentioned in the figure legends.

Feeding assay

Larvae at 3rd in-star stage (10 each) or 5-day old flies (5 each) were fed for 3 h or 30 min respectively, with colored food with Orange G dye (Cat. # 1936-15-8) from Sigma. The larvae/flies were homogenized using 0.05% Tween-20. The homogenate was analyzed colorimetrically at 492 nm using TECAN Infinite M200 pro-multi-mode plate reader in 96-well format. The absorbance of the homogenate was directly proportional to the larval food intake. The feeding experiments were replicated independently and number of replicates are mentioned in the figure legends.

Quantitative RT-PCR

Third in-star wandering larvae for each genotype were collected and were flash-frozen. For fly RT-PCR experiments with, the flies were starved for 24 h before flash freezing them and two sets of RNAs were isolated independently from the heads and the body. These experiments were replicated independently and number of replicates (n) are mentioned in the figure legends. Total RNA was isolated with QIAGEN RNeasy Plus Mini Kit (Cat. # 74134) and was quantified using Qubit™ RNA HS Assay Kit (Cat. # Q32852). An equal amount of RNA from each sample was reverse transcribed using SuperScript® III First-Strand Synthesis System (Cat. # 18080051) from ThermoFisher Scientific. Quantitative RT-PCR was performed using Bio-Rad CFX96™ with the cDNA template, Power SYBR®

Green PCR Master Mix (Cat. # 4368702) from ThermoFisher Scientific and a primer concentration of 312.5 nM. The data were normalized to *rp49*. Total RNA was isolated from three separate biological samples for each experiment and quantitative RT-PCR reactions were performed with 3 technical replicates for each biological replicate. The sequences of the primers used are mentioned below.

Table 1 List of primer sequences used in the manuscript.

<i>dilp2</i>	FP: 5'-GGCCAGCTCCACAGTGAAGT-3' RP: 5'-TCGCTGTCGGCACCGGGCAT-3'
<i>dilp3</i>	FP: 5'-AAGCTCTGTGTGTATGGCTT-3' RP: 5'-AGCACAATATCTCAGCACCT-3'
<i>dilp5</i>	FP: 5'-TCCGCCAGGCCGCAAACCTC-3' RP: 5'-TAATCGAATAGGCCCAAGGT-3'
<i>dilp6</i>	FP: 5'-CGATGTATTTCCCAACAGTTTCG-3' RP: 5'-AAATCGGTTACGTTCTGCAAGTC-3'
<i>inr</i>	FP: 5'-CACCCCGCTTCTATACTCCA-3' RP: 5'-GTTAGGATGGTGGCCTGTTC-3'
<i>4ebp</i>	FP: 5'-CACTCCTGGAGGCACCA-3' RP: 5'-GAGTTCCCTCAGCAAGCAA-3'
<i>tace</i>	FP: 5'-TGGGACACAATTTTGGAGCA-3' RP: 5'-CCTCCTT GGTCTCATTG-3'
<i>NLaz</i>	FP: 5'-GGTGAATGCGGCCATCAATC-3' RP: 5'-AATGGCTGCGTCGGGTAAAA-3'
<i>edem1</i>	FP: 5'-CAATCCGGCACAAGCACTACCATGG-3' RP: 5'-CCTCGCGTATCTGCTCGAAGTTGCT-3'
<i>rp49</i>	FP: 5'-GCTAAGCTGTCGCACAAA-3' RP: 5'-TCCGGTGGGCAGCATGTG-3'
<i>totA</i>	FP: 5'-AATTCTTCAACTGCTCTTATGTGC-3' RP: 5'-TTTGGAGTCATCGTCCTGGG-3'

Immunohistochemistry

DILP2 peptide corresponding to the sequence TRQRQGIVERC (amino acids 108-118) was used as an immunogen to raise DILP2 polyclonal antibody in rabbit (Eurogentec, Belgium). Rabbit anti-GFP (Cat. # A-6455 from Invitrogen) was used. About 10 larvae (3rd

in-star wandering) were used to dissect the brains in ice-cold 1X phosphate buffered saline PBS (Cat. # P4417 from Sigma) per genotype for each experiment. The dissections were repeated independently and number of replicates (n) are mentioned in the figure legends. The tissue samples were fixed using 4% paraformaldehyde (PFA) (Cat # P6148 from Sigma) at room temperature for 20 min. PFA was removed and the tissues were washed with 1X phosphate buffered saline + 0.1% Triton X-100 (Cat. # 161-0407 from Bio-Rad) PBT. Blocking solution [PBT+ 0.1% bovine serum albumin BSA (Cat. # A2153 from Sigma) BBT] was added to the tissues and the tissues were incubated at room temperature for 45 min. Primary antibody against DILP2 and GFP were diluted in BBT in 1:1000 and 1:500 dilutions respectively. The samples were incubated with primary antibody overnight at 4 °C with constant rotation. Then the tissues were washed extensively with 1X PBT and incubated with secondary antibody at room temperature for 2 h. The secondary antibody used was Alexa Fluor® 488 Goat Anti-Rabbit IgG (Cat. # A27034) diluted in 1:500 dilution in BBT. After 2 h the samples were washed extensively and mounted with a drop of SlowFade® Gold Antifade Reagent with DAPI (Cat. # S36939) from ThermoFisher Scientific. The tissues were imaged using a Leica DM6000B upright microscope and processed using ImageJ software. Corrected total cell fluorescence (CTCF) was calculated using the formula $CTCF = \text{Integrated Density} - (\text{Area of selected cell} \times \text{Mean fluorescence of background readings})$.

***ex-vivo* organ co-culture**

For the *ex-vivo* organ co-culture, larval hemolymph was isolated from 10 (3rd in-star crawling) larvae and was incubated with 10 brains from 3rd in-star crawling larvae of the desired genotypes in Shields and Sang medium (Cat. # S3652 from Sigma) at room temperature for 2 h with constant shaking. The larval brains were then fixed in 4 % PFA and stained for DILP2 as mentioned above and imaged.

Statistical analysis

All the experiments were done in biological replicates as indicated and the error bars represent the standard error mean (SEM). The graphs were plotted using GraphPad Prism8 software. Data was analysed using Shapiro-Wilk test for normal distribution. Significance was tested using Welch t-test for the data with normal distribution and using Mann-Whitney test for the data which was not normally distributed, with * representing p-value < 0.05, ** p-value < 0.01, *** p-value < 0.001. The statistical analyses for starvation sensitivity and lifespan assays were performed using OASIS2 software. The starvation and lifespan datasets were subjected to Log rank test and trends in the lifespan of flies in both the assays were analyzed by Mantel-Cox procedure.

Acknowledgements

We thank Nisha N. Kannan and Smitha Vishnu for critical comments on this manuscript. HP was supported by IISER TVM PHD fellowship. JV was supported by Ramanujan fellowship, DST, India. This work was also supported by generous funds from SERB, DST, India and core funding from IISER TVM, MHRD, India.

Authors contributions

HP and JV conceptualized the paper. HP performed research and analysed data. HP and JV wrote the paper. JV acquired funding and supervised research.

Conflict of interest

The authors declare no conflict of interest

References

1. Accili D, Drago J, Lee EJ, Johnson MD, Cool MH, Salvatore P, Asico LD, Jose PA, Taylor SI, Westphal H (1996) Early neonatal death in mice homozygous for a null allele of the insulin receptor gene. *Nat Genet* 12: 106-9
2. Agrawal N, Delanoue R, Mauri A, Basco D, Pasco M, Thorens B, Leopold P (2016) The Drosophila TNF Eiger Is an Adipokine that Acts on Insulin-Producing Cells to Mediate Nutrient Response. *Cell Metabolism* 23: 675-684
3. Andersen DS, Colombani J, Palmerini V, Chakrabandhu K, Boone E, Rothlisberger M, Toggweiler J, Basler K, Mapelli M, Hueber AO, Leopold P (2015) The Drosophila TNF receptor Grindelwald couples loss of cell polarity and neoplastic growth. *Nature* 522: 482-+
4. Araki K, Nagata K (2011) Protein folding and quality control in the ER. *Cold Spring Harb Perspect Biol* 3: a007526
5. Bai H, Kang P, Hernandez AM, Tatar M (2013) Activin signaling targeted by insulin/dFOXO regulates aging and muscle proteostasis in Drosophila. *Plos Genet* 9: e1003941
6. Bai H, Kang P, Tatar M (2012) Drosophila insulin-like peptide-6 (dilp6) expression from fat body extends lifespan and represses secretion of Drosophila insulin-like peptide-2 from the brain. *Aging Cell* 11: 978-985
7. Bai H, Post S, Kang P, Tatar M (2015) Drosophila Longevity Assurance Conferred by Reduced Insulin Receptor Substrate Chico Partially Requires d4eBP. *PLoS One* 10: e0134415
8. Bonafe M, Barbieri M, Marchegiani F, Olivieri F, Ragno E, Giampieri C, Mugianesi E, Centurelli M, Franceschi C, Paolisso G (2003) Polymorphic variants of insulin-like growth factor I (IGF-I) receptor and phosphoinositide 3-kinase genes affect IGF-I

plasma levels and human longevity: Cues for an evolutionarily conserved mechanism of life span control. *J Clin Endocr Metab* 88: 3299-3304

9. Britton JS, Lockwood WK, Li L, Cohen SM, Edgar BA (2002) *Drosophila*'s insulin/PI3-kinase pathway coordinates cellular metabolism with nutritional conditions. *Dev Cell* 2: 239-49
10. Brogiolo W, Stocker H, Ikeya T, Rintelen F, Fernandez R, Hafen E (2001) An evolutionarily conserved function of the *Drosophila* insulin receptor and insulin-like peptides in growth control. *Curr Biol* 11: 213-221
11. Broughton SJ, Piper MD, Ikeya T, Bass TM, Jacobson J, Driege Y, Martinez P, Hafen E, Withers DJ, Leivers SJ, Partridge L (2005) Longer lifespan, altered metabolism, and stress resistance in *Drosophila* from ablation of cells making insulin-like ligands. *Proc Natl Acad Sci U S A* 102: 3105-10
12. Broughton SJ, Slack C, Alic N, Metaxakis A, Bass TM, Driege Y, Partridge L (2010) DILP-producing median neurosecretory cells in the *Drosophila* brain mediate the response of lifespan to nutrition. *Aging Cell* 9: 336-46
13. Chouhan NS, Wolf R, Heisenberg M (2017) Starvation promotes odor/feeding-time associations in flies. *Learn Memory* 24: 318-321
14. Clancy DJ, Gems D, Harshman LG, Oldham S, Stocker H, Hafen E, Leivers SJ, Partridge L (2001) Extension of life-span by loss of CHICO, a *Drosophila* insulin receptor substrate protein. *Science* 292: 104-106
15. Colombani J, Raisin S, Pantalacci S, Radimerski T, Montagne J, Leopold P (2003) A nutrient sensor mechanism controls *Drosophila* growth. *Cell* 114: 739-749
16. Delanoue R, Meschi E, Agrawal N, Mauri A, Tsatskis Y, McNeill H, Leopold P (2016) *Drosophila* insulin release is triggered by adipose Stunted ligand to brain Methuselah receptor. *Science* 353: 1553-1556

17. DiAngelo JR, Birnbaum MJ (2009) Regulation of Fat Cell Mass by Insulin in *Drosophila melanogaster*. *Mol Cell Biol* 29: 6341-6352
18. Droujinine IA, Perrimon N (2016) Interorgan Communication Pathways in Physiology: Focus on *Drosophila*. *Annu Rev Genet* 50: 539-570
19. Erion R, Sehgal A (2013) Regulation of insect behavior via the insulin-signaling pathway. *Front Physiol* 4
20. Fabrizio P, Pozza F, Pletcher SD, Gendron CM, Longo VD (2001) Regulation of longevity and stress resistance by Sch9 in yeast. *Science* 292: 288-290
21. Fernandez AM, Torres-Aleman I (2012) The many faces of insulin-like peptide signalling in the brain. *Nat Rev Neurosci* 13: 225-39
22. Garami A, Zwartkruis FJT, Nobukuni T, Joaquin M, Roccio M, Stocker H, Kozma SC, Hafen E, Bos JL, Thomas G (2003) Insulin activation of Rheb, a mediator of mTOR/S6K/4E-BP signaling, is inhibited by TSC1 and 2. *Mol Cell* 11: 1457-1466
23. Geminard C, Rulifson EJ, Leopold P (2009) Remote control of insulin secretion by fat cells in *Drosophila*. *Cell Metab* 10: 199-207
24. Ghosh AC, O'Connor MB (2014) Systemic Activin signaling independently regulates sugar homeostasis, cellular metabolism, and pH balance in *Drosophila melanogaster*. *P Natl Acad Sci USA* 111: 5729-5734
25. Giannakou ME, Goss M, Junger MA, Hafen E, Leivers SJ, Partridge L (2004) Long-lived *Drosophila* with overexpressed dFOXO in adult fat body. *Science* 305: 361
26. Giannakou ME, Partridge L (2007) Role of insulin-like signalling in *Drosophila* lifespan. *Trends Biochem Sci* 32: 180-8
27. Gronke S, Clarke DF, Broughton S, Andrews TD, Partridge L (2010) Molecular Evolution and Functional Characterization of *Drosophila* Insulin-Like Peptides. *Plos Genet* 6

28. Hallier B, Schiemann R, Cordes E, Vitos-Faleato J, Walter S, Heinisch JJ, Malmendal A, Paululat A, Meyer H (2016) *Drosophila* neprilysins control insulin signaling and food intake via cleavage of regulatory peptides. *Elife* 5
29. Haselton A, Sharmin E, Schrader J, Sah M, Poon P, Fridell YWC (2010) Partial ablation of adult *Drosophila* insulin-producing neurons modulates glucose homeostasis and extends life span without insulin resistance. *Cell Cycle* 9: 3063-3071
30. Hirosumi J, Tuncman G, Chang LF, Gorgun CZ, Uysal KT, Maeda K, Karin M, Hotamisligil GS (2002) A central role for JNK in obesity and insulin resistance. *Nature* 420: 333-336
31. Holzenberger M, Dupont J, Ducos B, Leneuve P, Geloën A, Even PC, Cervera P, Le Bouc Y (2003) IGF-1 receptor regulates lifespan and resistance to oxidative stress in mice. *Nature* 421: 182-187
32. Hong SH, Lee KS, Kwak SJ, Kim AK, Bai H, Jung MS, Kwon OY, Song WJ, Tatar M, Yu K (2012) Minibrain/Dyrk1a Regulates Food Intake through the Sir2-FOXO-sNPF/ NPY Pathway in *Drosophila* and Mammals. *Plos Genet* 8
33. Hull-Thompson J, Muffat J, Sanchez D, Walker DW, Benzer S, Ganfornina MD, Jasper H (2009) Control of Metabolic Homeostasis by Stress Signaling Is Mediated by the Lipocalin NLaz. *Plos Genet* 5
34. Hwangbo DS, Gershman B, Tu MP, Palmer M, Tatar M (2005) *Drosophila* dFOXO controls lifespan and regulates insulin signalling in brain and fat body (vol 429, pg 562, 2004). *Nature* 434: 118-118
35. Ikeya T, Galic M, Belawat P, Nairz K, Hafen E (2002) Nutrient-dependent expression of insulin-like peptides from neuroendocrine cells in the CNS contributes to growth regulation in *Drosophila*. *Curr Biol* 12: 1293-1300

36. Kahn SE, Hull RL, Utzschneider KM (2006) Mechanisms linking obesity to insulin resistance and type 2 diabetes. *Nature* 444: 840-6
37. Kannan K, Fridell YWC (2013) Functional implications of *Drosophila* insulin-like peptides in metabolism aging, and dietary restriction. *Front Physiol* 4
38. Katic M, Kahn CR (2005) The role of insulin and IGF-1 signaling in longevity. *Cellular and Molecular Life Sciences* 62: 320-343
39. Kenyon C, Chang J, Gensch E, Rudner A, Tabtiang R (1993) A *C-Elegans* Mutant That Lives Twice as Long as Wild-Type. *Nature* 366: 461-464
40. Kim J, Neufeld TP (2015) Dietary sugar promotes systemic TOR activation in *Drosophila* through AKH-dependent selective secretion of Dilp3. *Nat Commun* 6
41. Kimura KD, Tissenbaum HA, Liu YX, Ruvkun G (1997) *daf-2*, an insulin receptor-like gene that regulates longevity and diapause in *Caenorhabditis elegans*. *Science* 277: 942-946
42. Koyama T, Mirth CK (2016) Growth-Blocking Peptides As Nutrition-Sensitive Signals for Insulin Secretion and Body Size Regulation. *Plos Biol* 14
43. Kroeger H, Chiang WC, Lin JH (2012) Endoplasmic Reticulum-Associated Degradation (ERAD) of Misfolded Glycoproteins and Mutant P23H Rhodopsin in Photoreceptor Cells. *Adv Exp Med Biol* 723: 559-565
44. Liu CY, Kaufman RJ (2003) The unfolded protein response. *J Cell Sci* 116: 1861-1862
45. Liu JP, Baker J, Perkins AS, Robertson EJ, Efstratiadis A (1993) Mice carrying null mutations of the genes encoding insulin-like growth factor I (*Igf-1*) and type 1 IGF receptor (*Igf1r*). *Cell* 75: 59-72
46. Luo J, Lushchak OV, Goergen P, Williams MJ, Nassel DR (2014) *Drosophila* insulin-producing cells are differentially modulated by serotonin and octopamine receptors and affect social behavior. *PLoS One* 9: e99732

47. Min KJ, Yamamoto R, Buch S, Pankratz M, Tatar M (2008) *Drosophila* lifespan control by dietary restriction independent of insulin-like signaling. *Aging Cell* 7: 199-206
48. Molinari M, Calanca V, Galli C, Lucca P, Paganetti P (2003) Role of EDEM in the release of misfolded glycoproteins from the calnexin cycle. *Science* 299: 1397-400
49. Nassel DR (2012) Insulin-producing cells and their regulation in physiology and behavior of *Drosophila*. *Can J Zool* 90: 476-488
50. Oh SW, Mukhopadhyay A, Svrikapa N, Jiang F, Davis RJ, Tissenbaum HA (2005) JNK regulates lifespan in *Caenorhabditis elegans* by modulating nuclear translocation of forkhead transcription factor/DAF-16. *P Natl Acad Sci USA* 102: 4494-4499
51. Oldham S (2011) Obesity and nutrient sensing TOR pathway in flies and vertebrates: Functional conservation of genetic mechanisms. *Trends Endocrin Met* 22: 45-52
52. Oldham S, Montagne J, Radimerski T, Thomas G, Hafen E (2000) Genetic and biochemical characterization of dTOR, the *Drosophila* homolog of the target of rapamycin. *Gene Dev* 14: 2689-2694
53. Partridge L, Alic N, Bjedov I, Piper MD (2011) Ageing in *Drosophila*: the role of the insulin/Igf and TOR signalling network. *Exp Gerontol* 46: 376-81
54. Pasco MY, Leopold P (2012) High Sugar-Induced Insulin Resistance in *Drosophila* Relies on the Lipocalin Neural Lazarillo. *Plos One* 7
55. Post S, Karashchuk G, Wade JD, Sajid W, De Meyts P, Tatar M (2018) *Drosophila* Insulin-Like Peptides DILP2 and DILP5 Differentially Stimulate Cell Signaling and Glycogen Phosphorylase to Regulate Longevity. *Front Endocrinol (Lausanne)* 9: 245
56. Puig O, Marr MT, Ruhf ML, Tjian R (2003) Control of cell number by *Drosophila* FOXO: downstream and feedback regulation of the insulin receptor pathway. *Genes Dev* 17: 2006-20

57. Rajan A, Perrimon N (2013) *Drosophila* Cytokine Unpaired 2 Regulates Physiological Homeostasis by Remotely Controlling Insulin Secretion (vol 151, pg 123, 2012). *Cell* 152: 1197-1197
58. Rulifson EJ, Kim SK, Nusse R (2002) Ablation of insulin-producing neurons in flies: Growth and diabetic phenotypes. *Science* 296: 1118-1120
59. Sano H, Nakamura A, Texada MJ, Truman JW, Ishimoto H, Kamikouchi A, Nibu Y, Kume K, Ida T, Kojima M (2015) The Nutrient-Responsive Hormone CCHamide-2 Controls Growth by Regulating Insulin-like Peptides in the Brain of *Drosophila melanogaster*. *Plos Genet* 11
60. Saucedo LJ (2003) Rheb promotes cell growth as a component of the insulin/TOR signalling network (vol 5, pg 566, 2003). *Nat Cell Biol* 5: 680-680
61. Shimokawa I, Higami Y, Tsuchiya T, Otani H, Komatsu T, Chiba T, Yamaza H (2003) Life span extension by reduction of the growth hormone-insulin-like growth factor-1 axis: relation to caloric restriction. *FASEB J* 17: 1108-9
62. Slaidina M, Delanoue R, Gronke S, Partridge L, Leopold P (2009) A *Drosophila* insulin-like peptide promotes growth during nonfeeding states. *Dev Cell* 17: 874-84
63. Soderberg JA, Carlsson MA, Nassel DR (2012) Insulin-Producing Cells in the *Drosophila* Brain also Express Satiety-Inducing Cholecystokinin-Like Peptide, Drosulfakinin. *Front Endocrinol (Lausanne)* 3: 109
64. Sonntag WE, Carter CS, Ikeno Y, Ekenstedt K, Carlson CS, Loeser RF, Chakrabarty S, Lee S, Bennett C, Ingram R, Moore T, Ramsey M (2005) Adult-onset growth hormone and insulin-like growth factor I deficiency reduces neoplastic disease, modifies age-related pathology, and increases life span. *Endocrinology* 146: 2920-32
65. Sudhakar SR, Pathak H, Rehman N, Fernandes J, Vishnu S, Varghese J (2019) Insulin signalling elicits hunger-induced feeding in *Drosophila*. *Dev Biol*

66. Sun JH, Liu C, Bai XB, Li XT, Li JY, Zhang ZP, Zhang YP, Guo J, Li Y (2017) *Drosophila* FIT is a protein-specific satiety hormone essential for feeding control. *Nat Commun* 8
67. Tatar M, Kopelman A, Epstein D, Tu MP, Yin CM, Garofalo RS (2001) A mutant *Drosophila* insulin receptor homolog that extends life-span and impairs neuroendocrine function. *Science* 292: 107-110
68. Tettweiler G, Miron M, Jenkins M, Sonenberg N, Lasko PF (2005) Starvation and oxidative stress resistance in *Drosophila* are mediated through the eIF4E-binding protein, d4E-BP. *Gene Dev* 19: 1840-1843
69. Varghese J, Lim SF, Cohen SM (2010) *Drosophila* miR-14 regulates insulin production and metabolism through its target, sugarbabe. *Gene Dev* 24: 2748-2753
70. Wang MC, Bohmann D, Jasper H (2005) JNK extends life span and limits growth by antagonizing cellular and organism-wide responses to insulin signaling. *Cell* 121: 115-125

Figure legends:

Fig 1. Edem1 maintains metabolic homeostasis

- (A) Blocking *edem1* expression using RNAi in the fat body led to enhanced triglyceride levels in adult male flies. Data is shown as % ratio of triglyceride to total protein levels, normalised to 100% in *pplGal4>w¹¹¹⁸* (control) and increase in experimental conditions *pplGal4>UAS-edem1-RNAi* [independent biological replicates = 17, P-value between control and *UAS-edem1-RNAi* is <0.001 (Mann-Whitney test)].
- (B) Enhanced levels of glycogen in adult male flies caused by blocking *edem1* expression in the fat body. Data is shown as % of total glycogen levels, normalised to 100% in *pplGal4>w¹¹¹⁸* (control) and increase in experimental conditions *pplGal4>UAS-edem1-RNAi* [independent biological replicates = 6, P-value between control and *UAS-edem1-RNAi* is <0.001 (Welch t-test)].
- (C) Enhanced resistance to starvation in adult male flies caused by blocking *edem1* expression in the fat body. Data shown as percentage of flies of *pplGal4>w¹¹¹⁸* (control) and *pplGal4>UAS-edem1-RNAi* which were alive at various time points of starvation [independent biological replicates = 4, P-value between control and *UAS-edem1-RNAi* is <0.001 (Log-rank test)].
- (D) and (E) Utilisation of triglycerides and glycogen at different stages of starvation upon *edem1* knockdown. Data is shown as % ratio of triglyceride to total protein levels in adult male flies, data is normalised to 100% in *pplGal4>w¹¹¹⁸* (control) fed condition and change in response to indicated hours of starvation in control and experimental conditions *pplGal4>UAS-edem1-RNAi* is shown [independent biological replicates = 3, P-value between control and *UAS-edem1-RNAi* is 0.3844 (Log-rank test)]. Glycogen levels at different stages of starvation upon *edem1* knockdown. Data is

normalised to 100% in *pplGal4>w¹¹¹⁸* (control) fed condition and change in response to indicated hours of starvation in control and experimental conditions *pplGal4>UAS-edem1-RNAi* is shown [independent biological replicates = 3, P-value between control and *UAS-edem1-RNAi* is 0.0082 (Log-rank test)].

- (F) Expression of *edem1* RNAi in the fat body led to enhanced glucose levels in the circulation. Data is shown as % of Glucose levels in the hemolymph, normalised to 100% in *pplGal4>w¹¹¹⁸* (control) and increase in experimental conditions *pplGal4>UAS-edem1-RNAi* [independent biological replicates = 4, P-value between control and *UAS-edem1-RNAi* is <0.001 (Mann-Whitney test)].
- (G) Blocking *edem1* gene expression in the fat body led to enhanced feeding responses. Data is shown as % food consumption in larvae, normalised to 100% in *pplGal4>w¹¹¹⁸* (control) and increase in experimental conditions *pplGal4>UAS-edem1-RNAi* [independent biological replicates = 3, P-value between control and *UAS-edem1-RNAi* is 0.0329 (Welch t-test)].
- (H) *edem1*-RNAi in the fat body led to enhanced life span in adult male flies. Data is shown as percentage of input flies *pplGal4>w¹¹¹⁸*, *pplGal4>UAS-edem1-RNAi* which were alive across the days. [independent biological replicates = 3, P-value between control and *UAS-edem1-RNAi* is <0.001 (Log-rank test)].

[P-value *<0.05; ** <0.01, *** <0.001,; Data information: In (A-B and F-G) data are presented as mean $\pm$ SEM].

Fig 2. Blocking *edem1* in the fat body reduced insulin signalling.

- (A) Blocking *edem1* expression using RNAi in the fat body led to an increase of mRNA levels of insulin target genes *dilp6* (Fig. 2A'), *4ebp* (Fig. 2A'') and *inr* (Fig. 2A''') in larvae. Data is shown as fold change in mRNA levels, values are normalised to *pplGal4>w¹¹¹⁸* and fold change in *pplGal4>UAS-edem1-RNAi* is shown. [independent biological replicates = 3, P-value between control and *UAS-edem1-RNAi* is 0.0128 for *dilp6*, for *4ebp* independent biological replicates = 10 and P-value is 0.0521, for *inr* independent biological replicates = 3 and P-value is 0.2138 (Welch t-test)].
- (B) Blocking *edem1* expression using RNAi in the fat body also led to a decrease in the levels of IPC specific *dilp3* mRNA in larvae. Data is shown as fold change in mRNA levels, values are normalised to *pplGal4>w¹¹¹⁸* and fold change in *pplGal4>UAS-edem1-RNAi* is shown. [n=9, P-value between control and *UAS-edem1-RNAi* is 0.1809 for *dilp2* (Mann-Whitney test), 0.0432 for *dilp3* (Welch t-test) and 0.8187 for *dilp5* (Welch t-test)].
- (C) DILP2 protein in the larval IPCs shown as a representative image of anti-DILP2 antibody staining in larval brains of *pplGal4>w¹¹¹⁸* [independent biological replicates = 15] and *pplGal4>UAS-edem1-RNAi* [independent biological replicates = 14].
- (D) Over expression of a constitutively active form of *inr* (*InR^{A1325D}*) with *edem1* RNAi in the fat body led to the rescue of fat phenotype in adult male flies. Data is shown as % ratio of triglyceride to total protein levels, normalised to 100% in *pplGal4>w¹¹¹⁸* (control) and changes in experimental conditions *pplGal4>UAS-edem1-RNAi* and *pplGal4>UAS-edem1-RNAi; UAS-InR^{A1325D}* [independent biological replicates = 4, P-value between control and *UAS-edem1-RNAi* is 0.0006, P-value between *UAS-*

edem1-RNAi and *UAS-edem1-RNAi*, *UAS-InR^{A1325D}* is <0.001 and P-value between control and *UAS-edem1-RNAi*, *UAS-InR^{A1325D}* is 0.3969 (Mann-Whitney test)].

- (E) Starvation resistance in adult male flies shown as percentage of input flies *pplGal4>w¹¹¹⁸*, *pplGal4>UAS-edem1-RNAi*, and *pplGal4> UAS-edem1-RNAi*, *UAS-InR^{A1325D}* which were alive at various time points of starvation [independent biological replicates = 3, P-value between control and *UAS-edem1-RNAi*, *UAS-edem1-RNAi* and *UAS-edem1-RNAi*; *UAS-InR^{A1325D}*, control and *UAS-edem1-RNAi*; *UAS-InR^{A1325D}* is <0.0001 (Log-rank test)].

[P-value * <0.05; ** <0.01, *** <0.001,; Data information: In (A—B and D) data are presented as mean ± SEM].

Fig 3. Reduction of *edem1* levels in the fat body enhanced JNK signalling.

- (A) Blocking *edem1* expression using RNAi in the fat body led to an increase of mRNA levels of *tace*. Data is shown as fold change in mRNA levels, values are normalised to *pplGal4>w¹¹¹⁸* and fold change in *pplGal4>UAS-edem1-RNAi* is shown. [independent biological replicates = 4, P-value between control and *UAS-edem1-RNAi* is 0.2184 (Welch t-test)].
- (B) Expression of *eiger* RNAi in the fat body rescued increase in *NLaz* mRNA levels caused by blocking *edem1* levels in the fat body, data is shown as fold change in mRNA levels. Data is shown as fold change in mRNA levels, values are normalised to *pplGal4>w¹¹¹⁸* and fold change in *pplGal4>UAS-edem1-RNAi* and *pplGal4>UAS-edem1-RNAi*; *UAS-eiger-RNAi* is shown. [independent biological replicates = 3 for *pplGal4>UAS-edem1-RNAi*; *UAS-eiger-RNAi* and 14 for *pplGal4>w¹¹¹⁸* and *pplGal4>UAS-edem1-RNAi*. P-value between control and *UAS-edem1-RNAi* is 0.0014, P-value between *UAS-edem1-RNAi* and *UAS-edem1-RNAi*; *UAS-eiger-RNAi*

is 0.0071 and between control and *UAS-edem1-RNAi*; *UAS-eiger-RNAi* is 0.9006 (Welch t-test)].

- (C) Expression of *eiger* RNAi in the fat body rescued increase in triglyceride caused by blocking *edem1* levels in the fat body. Data is shown as % ratio of triglyceride to total protein levels, normalised to 100% in *pplGal4>w¹¹¹⁸* (control) and changes in experimental conditions *pplGal4>UAS-edem1-RNAi* and *pplGal4>UAS-edem1-RNAi; UAS-eiger-RNAi* is shown. [independent biological replicates = 3, P-value between control and *UAS-edem1-RNAi* is 0.0358, P-value between control and *UAS-edem1-RNAi; UAS-eiger-RNAi* is 0.1582, P-value between *UAS-edem1-RNAi* and *UAS-edem1-RNAi; UAS-eiger-RNAi* is 0.0086 (Welch t-test)].
- (D) Enhanced starvation resistance shown as percentage of flies which were alive at various time points of starvation in the following genotypes - *pplGal4>w¹¹¹⁸*, *pplGal4>UAS-edem1-RNAi* and *pplGal4> UAS-edem1-RNAi; UAS-eiger-RNAi* is shown. [independent biological replicates = 3, P-value between control and *UAS-edem1-RNAi* and between *UAS-edem1-RNAi* and *UAS-edem1-RNAi; UAS-eiger-RNAi* is <0.0001, P-value between control and *UAS-edem1-RNAi; UAS-eiger-RNAi* is 0.2159 (Log-rank test)].

[P-value *<0.05; ** <0.01, *** <0.001,; Data information: In (A—C) data are presented as mean ± SEM].

Fig 4. Knock down of *eiger* in the fat body rescued the metabolic phenotypes.

- (A) DILP2 levels in the IPCs in response to *edem1*-RNAi was rescued by reducing *eiger* in the fat body. Shown are representative images of anti-DILP2 antibody staining in larval brains of *pplGal4>w¹¹¹⁸* [independent biological replicates = 15]; *pplGal4>UAS-*

edem1-RNAi [independent biological replicates = 14] and *pplGal4> UAS-edem1-RNAi; UAS-eiger-RNAi* [independent biological replicates = 9].

- (B) Reduction of *dilp3* mRNA levels, in response to *edem1-RNAi* was rescued by reducing *eiger* in the fat body, data is shown as fold change in mRNA levels, values are normalised to *pplGal4>w¹¹¹⁸* fold change in *pplGal4>UAS-edem1-RNAi* and *pplGal4> UAS-edem1-RNAi; UAS-eiger-RNAi* is shown [independent biological replicates = 8 for *pplGal4> UAS-edem1-RNAi; UAS-eiger-RNAi* and 11 for *pplGal4>w¹¹¹⁸* and *pplGal4>UAS-edem1-RNAi*. P-value between control and *UAS-edem1-RNAi* is 0.0648, between control and *UAS-edem1-RNAi; UAS-eiger-RNAi* is 0.9036 and between *UAS-edem1-RNAi* and *UAS-edem1-RNAi; UAS-eiger-RNAi* is 0.0709 (Mann-Whitney test)].
- (C) Increase in *dilp6*, in response to *edem1-RNAi* was rescued by reducing *eiger* in the fat body (Fig. 4C') and *inr* (Fig. 4C''). Data is shown as fold change in mRNA levels, values are normalised to *pplGal4>w¹¹¹⁸* and fold change in *pplGal4>UAS-edem1-RNAi* and *pplGal4> UAS-edem1-RNAi; UAS-eiger-RNAi* is shown [independent biological replicates = 7 for *dilp6* and independent biological replicates = 3 for *pplGal4> UAS-edem1-RNAi; UAS-eiger-RNAi* and 6 for *pplGal4>w¹¹¹⁸* and *pplGal4>UAS-edem1-RNAi* for *inr*. P-value between control and *UAS-edem1-RNAi* is 0.0973 for *dilp6* (Mann-Whitney test) and 0.7455 for *inr* (Welch t-test). P-value between control and *UAS-edem1-RNAi; UAS-eiger-RNAi* is 0.989 for *dilp6* and 0.5441 for *inr*, P-value between *UAS-edem1-RNAi* and *UAS-edem1-RNAi; UAS-eiger-RNAi* is 0.1375 for *dilp6* and 0.3248 for *inr*].
- (D) Increase in Glucose levels in the hemolymph in response to *edem1-RNAi* was rescued by reducing *eiger* in the fat body. Data is shown as % of Glucose levels in the hemolymph, normalised to 100% in *pplGal4>w¹¹¹⁸* (control) and changes in

experimental conditions *pplGal4>UAS-edem1-RNAi* and *pplGal4> UAS-edem1-RNAi; UAS-eiger-RNAi* is shown [independent biological replicates = 3, P-value between control and *UAS-edem1-RNAi* is 0.0022, P-value between control and *UAS-edem1-RNAi; UAS-eiger-RNAi* is 0.8182, P-value between *edem1-RNAi* and *UAS-edem1-RNAi; UAS-eiger-RNAi* is 0.0087 (Mann-Whitney test)].

[P-value * <0.05 ; ** <0.01 , *** <0.001 ,; Data information: In (B-D) data are presented as mean $\pm$ SEM].

Fig 5. Activating TOR signalling rescued *edem1* mediated phenotypes.

(A) Increase in mRNA levels of *NLaz* in response to blocking *edem1* expression was rescued by co-expression of *UAS-rheb*. Data is shown as fold change in mRNA levels, values are normalised to *pplGal4>w¹¹¹⁸* and fold change in *pplGal4>UAS-edem1-RNAi* and *pplGal4> UAS-edem1-RNAi; UAS-rheb* is shown. [independent biological replicates = 4 for *pplGal4> UAS-edem1-RNAi; UAS-rheb* and 8 for *pplGal4>w¹¹¹⁸* and *pplGal4>UAS-edem1-RNAi*. P-value between control and *UAS-edem1-RNAi* is 0.0084, P-value between *UAS-edem1-RNAi* and *UAS-edem1-RNAi; UAS-rheb* is 0.0373, P-value between control and *UAS-edem1-RNAi; UAS-rheb* is 0.5737 (Welch t-test)].

(B) Increase of mRNA levels of *4ebp* (Fig. 5B') and *inr* (Fig. 5B'') in response to blocking *edem1* expression was alleviated by co-expression of *UAS-rheb*. Data is shown as fold change in mRNA levels, values are normalised to *pplGal4>w¹¹¹⁸* and fold change in *pplGal4>UAS-edem1-RNAi* and *pplGal4> UAS-edem1-RNAi; UAS-rheb* is shown. [independent biological replicates = 3, for *pplGal4> UAS-edem1-RNAi; UAS-rheb* and 12 for *pplGal4>w¹¹¹⁸* and *pplGal4>UAS-edem1-RNAi* for *4ebp*. P-value between control and *edem1-RNAi* is 0.0082, P-value between *UAS-edem1-RNAi* and *UAS-*

edem1-RNAi; *UAS-rheb* is 0.0967, P-value between control and *UAS-edem1-RNAi*; *UAS-rheb* is 0.5363 (Mann-Whitney test); independent biological replicates = 3, for *pplGal4> UAS-edem1-RNAi*; *UAS-rheb* and 6 for *pplGal4>w¹¹¹⁸* and *pplGal4>UAS-edem1-RNAi* for *inr*. P-value between control and *UAS-edem1-RNAi* is 0.2437, P-value between *UAS-edem1-RNAi* and *UAS-edem1-RNAi*; *UAS-rheb* is 0.9373, P-value between control and *UAS-edem1-RNAi*; *UAS-rheb* is 0.1550 (Welch t-test)].

(C) Decrease in mRNA levels of *dilp3* in response to blocking *edem1* expression was rescued by co-expression of *UAS-rheb*. Data is shown as fold change in mRNA levels, values are normalised to *pplGal4>w¹¹¹⁸* and fold change in *pplGal4>UAS-edem1-RNAi* and *pplGal4> UAS-edem1-RNAi*; *UAS-rheb* is shown. [independent biological replicates = 3, for *pplGal4> UAS-edem1-RNAi*; *UAS-rheb* and 11 for *pplGal4>w¹¹¹⁸* and *pplGal4>UAS-edem1-RNAi*. P-value between control and *UAS-edem1-RNAi* is 0.0648, P-value between *UAS-edem1-RNAi* and *UAS-edem1-RNAi*; *UAS-rheb* is 0.1648, P-value between control and *UAS-edem1-RNAi*; *UAS-rheb* is 0.8846 (Mann-Whitney test)].

(D) Over-expression of *rheb* in the fat body rescued enhanced stored fat levels. Data is shown as % ratio of triglyceride to total protein levels, values are normalised to *pplGal4>w¹¹¹⁸* and fold change in *pplGal4>UAS-edem1-RNAi* and *pplGal4> UAS-edem1-RNAi*; *UAS-rheb* is shown. [independent biological replicates = 4, P-value between control and *UAS-edem1-RNAi* is 0.0006, P-value between *UAS-edem1-RNAi* and *UAS-edem1-RNAi*; *UAS-Rheb* is 0.0637, P-value between control and *UAS-edem1-RNAi*; *UAS-Rheb* is 0.7259 (Mann-Whitney test)].

(E) Over-expression of *rheb* in the fat body rescued increased starvation resistance. Data is shown as percentage of flies which were alive at various time points of starvation in the following genotypes - *pplGal4>w¹¹¹⁸*, *pplGal4>UAS-edem1-RNAi*

and *pplGal4> UAS-edem1-RNAi; UAS-rheb*. [independent biological replicates = 5, P-value between control and *UAS-edem1-RNAi* is <0.0001, P-value between *UAS-edem1-RNAi* and *UAS-edem1-RNAi; UAS-Rheb* is 0.027, P-value between control and *UAS-edem1-RNAi; UAS-Rheb* is 0.202 (Log-rank test)].

[P-value * <0.05; ** <0.01, *** <0.001,; Data information: In (A–D) data are presented as mean ± SEM].

Fig. 6 Blocking *grindelwald* in the IPCs rescued the increased accumulation of DILP2

- (A) Blocking Eiger receptor *grnd* in the IPCs could rescue DILP2 levels in the IPCs. Representative images of anti-DILP2 antibody staining in larval brains of *Dilp2Gal4>w¹¹¹⁸* [independent biological replicates = 22] and *Dilp2Gal4>UAS-grnd-RNAi* [independent biological replicates = 12] are shown.
- (B) Corrected total cell fluorescence values are normalised to *Dilp2Gal4>w¹¹¹⁸* and fold change in *Dilp2Gal4>UAS-grnd-RNAi* is shown. [P-value between control and *UAS-grnd-RNAi* is 0.0443 (Mann-Whitney test)].

[P-value * <0.05; ** <0.01, *** <0.001,; Data information: In (B) data are presented as mean ± SEM].

Fig. 7 Reduction in *edem1* levels during starvation is crucial for survival.

- (A) Fold change in the mRNA levels of *edem1* upon starvation in *pplGal4>w¹¹¹⁸* larvae. Values are normalised to *pplGal4>w¹¹¹⁸* fed control and changes in the control starved are shown. [independent biological replicates = 14, P-value between control fed and starved larvae is <0.0001 (Mann-Whitney test)].

- (B) Over-expression of *edem1* leads to enhanced sensitivity to starvation, shown are percentage of male flies which were alive at various time points of starvation in the following genotypes - *pplGal4>w¹¹¹⁸* and *pplGal4>UAS-edem1* [independent biological replicates = 3, P-value between control and *UAS-edem1* is <0.0001 (Log-rank test)].
- (C) Overexpression of *edem1* in the fat body increased the *dilp3* mRNA levels when compared to control starved flies. Data is shown as fold change in mRNA levels, values are normalised to *pplGal4>w¹¹¹⁸* and fold change in *pplGal4>w¹¹¹⁸* starved and *pplGal4>UAS-edem1* starved is shown. [independent biological replicates = 4. P-value between control fed and starved is 0.0286, P-value between control fed and *UAS-edem1* starved is 0.2857 and P-value between control starved and *UAS-edem1* starved is 0.1143 (Mann-Whitney test)].
- (D) Overexpression of *edem1* in the fat body led to decreased levels of *4ebp* (Fig. D'), *dilp6* (Fig. D'') and *inr* (Fig. D''') when compared to control starved flies. Data is shown as fold change in mRNA levels, values are normalised to *pplGal4>w¹¹¹⁸* and fold change in *pplGal4>w¹¹¹⁸* starved and *pplGal4>UAS-edem1* starved is shown. [independent biological replicates = 4 for *4ebp*, independent biological replicates = 5 for *dilp6* and independent biological replicates = 3 for *inr*. P-value between control fed and starved is 0.0286 for *4ebp* (Mann-Whitney test), 0.1829 for *dilp6* (Welch t-test) and 0.3127 for *inr* (Welch t-test). P-value between control fed and *UAS-edem1* starved is 0.4857 for *4ebp*, 0.4035 for *dilp6* and 0.9056 for *inr*. P-value between control starved and *UAS-edem1* starved is 0.0286 for *4ebp*, 0.4197 for *dilp6* and 0.2861 for *inr*].
- (E) Overexpression of *edem1* in the fat body led to decreased levels of *NLaz* mRNA when compared to control starved flies. Data is shown as fold change in mRNA

levels, values are normalised to *pplGal4>w¹¹¹⁸* and fold change in *pplGal4>w¹¹¹⁸* starved and *pplGal4>UAS-edem1* starved is shown. [independent biological replicates = 3 P-value between control fed and starved is 0.0275, P-value between control fed and *UAS-edem1* starved is 0.3937 and P-value between control starved and *UAS-edem1* starved is 0.0746 (Welch t-test)].

[P-value * <0.05 ; ** <0.01 , *** <0.001 ,; Data information: In (A and C-E) data are presented as mean $\pm$ SEM].

Fig. 8 Working model.

During fed conditions Edem1 block *tace* gene expression and inhibit Eiger release. This maintains the insulin signalling as JNK signalling is kept low in the IPCs. During starvation *edem1* levels decrease, *tace* gene expression is increased and leading to Eiger secretion into the hemolymph. Soluble Eiger binds to the Grnd receptors in the IPCs and activate JNK signalling which inhibits insulin signalling. Reduction of insulin signalling mediated by reduction of Edem1 in the fat body during starvation aid in survival of flies.

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5

Fig. 6

A

B

Fig. 7

Fig 8.