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

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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 and controls systemic insulin signalling. Insulin signalling has pleiotropic functions which include the regulation of key growth and metabolic pathways. Here, we report that Edem1 (endoplasmic reticulum degradation enhancing α-mannosidase-like protein1), an endoplasmic reticulum-resident protein involved in protein quality control, acts in the fat body to regulate insulin signalling, metabolic status and starvation responses in *Drosophila*. Edem1 limits *Drosophila* TNFα Eiger activity, a fat body derived humoral factor that acts on IPCs, and maintains systemic insulin signalling in normal fed conditions. During food deprivation, Edem1 expression levels drop which aids in surviving the effects of nutrient starvation. Overall we demonstrate that Edem1 plays a crucial 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 by which organisms maintain the balance between energy inflow and outflow; is vital for normal functioning, reproduction as well as longevity. Energy homeostasis is brought about by the activity and interplay of various endocrine and neuroendocrine systems. Insulin plays a significant role in the maintenance of energy balance and Insulin/IGF signalling pathway is highly conserved in both vertebrates and invertebrates (Kenyon, Chang et al. 1993, Kimura, Tissenbaum et al. 1997, Brogiolo, Stocker et al. 2001, Clancy, Gems et al. 2001, Fabrizio, Pozza et al. 2001, Britton, Lockwood et al. 2002, Fernandez and Torres-Aleman 2012). The perturbations in insulin signalling result in a plethora of effects like reduced body size, resistance to starvation and oxidative stress, diabetes, obesity, etc. (Liu, Baker et al. 1993, Accili, Drago et al. 1996, Clancy, Gems et al. 2001, Tatar, Kopelman et al. 2001, Britton, Lockwood et al. 2002, Ikeya, Galic et al. 2002, Rulifson, Kim et al. 2002, Bonafe, Barbieri et al. 2003, Holzenberger, Dupont et al. 2003, Shimokawa, Higami et al. 2003, Giannakou, Goss et al. 2004, Katic and Kahn 2005, Sonntag, Carter et al. 2005, Kahn, Hull et al. 2006, Giannakou and Partridge 2007). *Drosophila melanogaster*, a widely used genetic model organism, has 8 insulin-like peptides (DILPs 1-8) and a single receptor - the insulin receptor (InR). Among these; DILP2, DILP3 and DILP5 are produced mainly by a subset of the median neurosecretory cells (mNSCs), also known as insulin-producing cells (IPCs) in the fly brain (Ikeya, Galic et al. 2002, Geminard, Rulifson et al. 2009, Broughton, Slack et al. 2010, Nassel 2012). DILPs share structural and functional similarities with mammalian insulin and IGF (Gronke, Clarke et al. 2010). The major effector tissue of insulin signalling is the fat body, which is the liver and adipose tissue homolog in *Drosophila* (Hwangbo, Gersham et al. 2004, Geminard, Rulifson et al. 2009). The *Drosophila* fat body is the main energy reserve and serves as a nutrient sensor. 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 (Colombani, Raisin et al. 2003, Geminard, Rulifson et al. 2009, Bai, Kang et al. 2012, Rajan and Perrimon 2012, Ghosh and O'Connor 2014, Sano, Nakamura et al. 2015, Agrawal, Delanoue et al. 2016, Delanoue, Meschi et al. 2016, Droujinine and Perrimon 2016, Koyama and Mirth 2016, Sun, Liu et al. 2017). The molecular mechanisms that control the fat body derived signals (FDSs) which act on the IPCs and maintain nutrient homeostasis is under intense investigation.

The endoplasmic reticulum (ER) serves many functions in the eukaryotic cell, foremost of which is the folding of nascent proteins with the help of molecular chaperones and folding enzymes. Hence, ER is considered as the major quality-control site which ensures that only correctly folded proteins are allowed to leave ER to other cellular compartments. ER is also considered to be the first storage site of secretory proteins and 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 would lead to protein misfolding (Bergmann and Molinari 2018) and 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 will 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), which 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 (Bergmann and Molinari 2018). Permanently unfolded glycoproteins are recognised by ERAD-enhancing α-mannosidase-like proteins (Edem) which aid in the degradation of these misfolded proteins by ERAD (Molinari, Calanca et al. 2003, Araki and Nagata 2011, Kroeger, Chiang et al. 2012).

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 systemic insulin signalling. Down-regulation of Edem1 led to reduced insulin signalling, caused nutrient imbalances and reduced the sensitivity to starvation. Our results also show that Edem1 manages insulin signalling by the regulation of fat body derived TNFα Eiger that control the function of IPCs. Furthermore, in response to reduced nutrients *edem1* mRNA levels were lowered, which we found was crucial for the 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 during fluctuations in the nutrient environment.

Results

edem1 maintains metabolic homeostasis

To identify factors that control nutrient homeostasis and insulin signalling we embarked on a genetic screen by blocking various candidate genes in the fat body using RNAi lines. Our previous study reported a set of genes differentially expressed in the *miR-14* mutants that showed metabolic phenotypes (data not shown), which we tested as candidates here (Varghese, Lim et al. 2010). In this screen we identified *edem1* 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). In response to fat body specific *edem1* knock down there was an increase in starvation resistance as well (Fig. 1C). The higher energy stores observed at various stages of starvation in response to lowering *edem1* levels in the fat body may account for the better survival of flies (Fig. 1D and E). Along with changes in stored nutrient levels, circulating glucose levels were high in the larval hemolymph indicating a

decrease in systemic insulin signalling (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). These data show that Edem1 function in the fat body is crucial in regulating metabolic homeostasis. All the phenotypes observed in response to blocking *edem1* levels in the fat body indicated a drop in insulin signalling in these animals. Next, we employed various approaches to measure systemic insulin signalling upon blocking *edem1*.

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 regulated by insulin signalling (Puig, Marr et al. 2003, Slaidina, Delanoue et al. 2009). Blocking *edem1* in the fat body increased transcript levels of these insulin responsive genes which indicated low insulin signalling in larvae (Fig. 2A). As the next approach we tested whether the reduction of insulin signalling in response to blocking Edem1 in the fat body led to the metabolic phenotypes. A constitutively active form of insulin receptor (InR^{CA}) was co-expressed with *edem1*-RNAi in the fat body. InR^{CA} should activate downstream insulin signalling independent of the ligand DILPs, hence InR^{CA} should alleviate phenotypes caused by low insulin signalling. Expression of InR^{CA} in the fat body alone led to a reduction of triglyceride levels and starvation resistance in adult flies (Fig. 2B and 2C), similar to earlier reports which has shown that insulin signalling regulated fat levels and starvation sensitivity (Broughton, Piper et al. 2005, Tettweiler, Miron et al. 2005, DiAngelo and Birnbaum 2009, Kannan and Fridell 2013). As expected, co-expression of *edem1*-RNAi and InR^{CA} in the fat body rescued the high triglyceride levels and starvation resistance in flies that express *edem1*-RNAi alone (Fig. 2B and 2C).

Thus, blocking *edem1* in the fat body reduced insulin signalling which led to metabolic phenotypes. These experiments confirm that Edem1 function is crucial in the fat body to maintain systemic insulin signalling and metabolic homeostasis.

Edem1 activity in the fat body could regulate IPCs and control systemic insulin signalling, as fat body is known to remotely control the function of 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 in larvae, however, there were no detectable changes in the mRNA levels of *dilp2* and *dilp5* (Fig. 2D). Previous studies report that nutrient deprivation would block DILP secretion from the IPCs into the hemolymph leading to an accumulation of DILPs resulting in reduced systemic insulin signalling (Geminard, Rulifson et al. 2009). In control larval brains an overall cytoplasmic distribution of DILP2 protein in the IPCs was observed, whereas, in response to reducing *edem1* levels in the fat body DILP2 levels in the IPCs were found to be lower than control conditions and DILP2 showed a punctate distribution in the IPCs (Fig. 2E). We saw similar responses to DILP2 levels when larvae were subjected to 12 hrs of nutrient deprivation (Fig. S1A). Thus, blocking *edem1* levels in the fat body led to a reduction in *dilp3* gene expression and DILP2 protein levels in the IPCs. Together, these observations suggest that *edem1* function in the fat body maintains systemic insulin signalling and nutrient homeostasis by regulating the activity of IPCs.

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. During nutrient deprivation insulin signalling becomes low, which would allow an organism to manage its energy stores, increase appetite and embark on foraging (Erion and Sehgal 2013). As our findings show that

Edem1 function in the fat body maintains systemic insulin signalling and metabolic homeostasis, next we tested if Edem1 would aid the flies in withstanding changes in the availability of food by lowering insulin levels. In response to food depletion we observed a reduction of *edem1* mRNA levels (Fig. 2F). We speculated that reduction of Edem1 levels in response to food deprivation may contribute to the survival of flies, as our experiments show that low Edem1 levels improved survival against starvation by reducing insulin signalling (Fig. 1C and Fig. 2C). Moreover, blocking Edem1 in the fat body in larvae enhanced the appetite, which was similar to hunger induced responses in food deprived larvae (Fig. 1G). To confirm whether reduction of Edem1 levels would aid in protection against starvation we over expressed Edem1 in the fat body in food deprived flies. Over-expression of Edem1 in the fat body reduced survival of flies to food deprivation (Fig. 2G), confirming that the reduction of Edem1 levels is crucial for survival in response to nutrient depletion. Next we checked how *edem1* function in the fat body would regulate the activity of IPCs.

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 derived signals that control IPC function include DILP6, Upd2 (Unpaired2 - the *Drosophila* leptin analog), Eiger (*Drosophila* TNFα), CCHa2 (CCHamide2), GBPs (Growth blocking peptide, a *Drosophila* cytokine), Sun (Stunted, a circulating insulinotropic peptide), female-specific independent of transformer (FIT) and activin-like ligand dawdle (Okamoto, Yamanaka et al. 2009, Rajan and Perrimon 2012, Ghosh and O'Connor 2014, Sano, Nakamura et al. 2015, Agrawal, Delanoue et al. 2016, Delanoue, Meschi et al. 2016, Sun, Liu et al. 2017). The fat body derived signals (FDSs) control DILP release into the hemolymph from the IPCs leading to effects on growth and maintenance of metabolic balance. In addition, changes in *dilp* gene

expression was also 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.

The fat body derived cytokine Eiger is activated by the TNFα converting enzyme (encoded by *tace* gene), which cleaves the transmembrane form of Eiger and releases a soluble active form of Eiger into the hemolymph (Agrawal, Delanoue et al. 2016). An increase in *tace* gene expression in response to amino acid restriction would aid in Eiger secretion into the hemolymph. We measured *tace* transcript levels in larvae in response to down regulation of *edem1* in the fat body, which showed an increase in *tace* mRNA expression suggesting higher active Eiger levels (Fig. 3A). 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 (Hirosumi, Tuncman et al. 2002, Oh, Mukhopadhyay et al. 2005, Wang, Bohmann et al. 2005). We went on to check the activity of JNK pathway by measuring the expression of *Neural-Lazarillo* (*NLaz*) a JNK pathway target gene (Hull-Thompson, Muffat et al. 2009, Pasco and Léopold 2012). The transcript levels of *NLaz* was found to be significantly higher in *edem1*-RNAi when compared to controls (Fig. 3B) confirming an increase in JNK signalling. These experiments suggest that *edem1* activity in the fat body regulates Eiger activity and JNK signalling.

Next, we carried out experiments to test if Edem1 regulation of Eiger is crucial for maintaining metabolic homeostasis. We down-regulated Eiger in *edem1*-RNAi expressing fat body, which suppressed the metabolic phenotypes seen in response to blocking *edem1* (Fig. 3C-E). We also observed that the reduction in DILP2 levels in the IPCs in response to *edem1*-RNAi was abrogated by lowering Eiger levels in the fat body (Fig. 3F). In addition, transcript levels of *dilp3* and insulin target gene *4ebp* was restored by reducing Eiger levels in the *edem1*-RNAi back ground (Fig. 3G and 3H). These experiments confirm that

edem1 mediated regulation of Eiger and JNK signalling is crucial for managing insulin levels and nutrient homeostasis. To further confirm the role of Edem1 in regulating *eiger* activity we used other genetic means to suppress Eiger levels.

Activation of TOR signalling pathway by dietary amino acids would suppress Eiger release from the fat body. TOR signalling has been reported to repress *tace* transcription which would suppress the production of active Eiger from the fat body (Agrawal, Delanoue 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 (Oldham, Montagne et al. 2000, Garami, Zwartkruis et al. 2003, Saucedo 2003, Oldham 2011). Over-expression of Rheb in the fat body, was sufficient to rescue the excess fat levels and starvation sensitivity phenotypes resulted from reducing Edem1 activity in the fat body (Fig. 4A and 4B). Increase in transcript levels of *tace* and JNK pathway target *NLaz* in response to *edem1* downregulation in the fat body were also abrogated by over-expression of Rheb (Fig. 4C and 4D). This confirmed that Edem1 mediated regulation of Eiger is crucial for managing fat storage and starvation sensitivity. Together these results confirm that Edem1 activity in the fat body regulates Eiger mediated JNK signalling and manages systemic insulin signalling and metabolic status of flies.

Discussion

Nutrient withdrawal would trigger organism wide changes in various metabolic pathways. These changes would aid the organism in managing growth and maintenance of nutrient stores according to the availability of food. Apart from these biochemical changes there are various stereotypic behavioral responses which are elicited by hunger like; enhanced urge to feed, increased foraging, acceptance of unpalatable food etc. Earlier studies report that starvation leads to a reduction of systemic insulin signalling which triggers survival responses like mobilisation of stored nutrients and enhanced urge to feed (Chouhan, Wolf

et al. 2017). In *Drosophila,* IPCs respond to changes in the availability of food and are controlled by the fat body, through fat body derived signals that act either directly or indirectly. The fat body acts as a sensor of nutrients and control IPC functions, with the help of various fat body derived signals which modulate DILP levels. Such regulation of insulin levels during food deprivation aids in triggering various hunger stimulated behavioral responses and metabolic changes that would aid in survival. However, the exact mechanism through which nutrient sensing by the fat body regulates FDS activity are only beginning to be understood.

As reported here, while investigating the mechanisms that act in the fat body to control *Drosophila* IPCs we identified Edem1, an ER resident protein involved in ERAD mediated protein quality control. Blocking Edem1 levels in the fat body led to a reduction of systemic insulin signalling which resulted in metabolic phenotypes and enhanced resistance to starvation. Reduced insulin signalling affects growth and metabolic pathways at various levels. Insulin pathway defects have been reported by previous studies to result in changes in fat storage and starvation sensitivity (Broughton, Piper et al. 2005, Tettweiler, Miron et al. 2005, DiAngelo and Birnbaum 2009, Kannan and Fridell 2013). Low insulin levels have been shown to result in diabetic-like larvae and flies (Rulifson, Kim et al. 2002, Broughton, Piper et al. 2005). Similar to these effects we saw an increase in triglyceride and glycogen stores; higher circulating sugar levels and enhanced starvation resilience upon suppression of Edem1 in the fat body.

Our data here show that Edem1 activity in the fat body is essential for normal insulin signalling. A significant part of the impact of reducing Edem1 levels on insulin signalling could be due to the reduction of DILP2 protein and *dilp3* transcript levels in the IPCs. However, it should be noted that we did not observe any changes at the protein and mRNA levels of other DILPs. DILPs are known to be regulated in a context specific manner, gene expression as well as DILP levels in IPCs vary based on nutritional cues, developmental

stages and various neural and endocrine signals that act on the IPCs (Ikeya, Galic et al. 2002, Gronke, Clarke et al. 2010, Varghese, Lim et al. 2010, Hong, Lee et al. 2012, Soderberg, Carlsson et al. 2012, Luo, Lushchak et al. 2014, Kim and Neufeld 2015, Hallier, Schiemann et al. 2016). Similarly, the effects of ablating IPCs, which affects DILP2, DILP3 and DILP5, on growth and metabolism could be rescued by DILP2 expression alone (Rulifson, Kim et al. 2002, Haselton, Sharmin et al. 2010).

Further, we report that fat body Edem1 regulates the activity of *Drosophila* TNFα Eiger through *tace*. During fed conditions Edem1 activity in the fat body controls Eiger levels and allows normal functioning of IPCs and maintenance of metabolic homeostasis. We observe that activation of TOR signalling was sufficient to block the effects caused by suppression of *edem1* levels in the fat body mainly by reducing *tace* mRNA levels. However, more efforts are needed to identify the exact molecular mechanism by which Edem1 regulates *tace* levels.

Furthermore, in response to nutrient withdrawal we saw a reduction of Edem1 transcripts, which suggested that reducing Edem1 levels might aid in lowering insulin signalling, an essential step in induction of starvation induced survival responses. Moreover, reducing Edem1 levels in fed flies led to enhanced feeding responses. This response was similar to starvation conditions, further suggesting a crucial role for Edem1 in survival against food deprivation. Interestingly, enhancing Edem1 levels led to reduced survival during starvation, probably due to a failure in eliciting survival responses to food depletion. Hence, reduction of Edem1 activity during starvation might aid the animal in eliciting survival responses. However, it is quite interesting to note that in addition to *edem1*, several key UPR pathway genes were also reduced upon 12 hrs of starvation in larvae (Fig. S1B). A UPR reporter, *xbp1-EGFP,* which measures IRE1 mediated alternate splicing of *xbp1*, was also reduced confirming that reduced levels of nutrients would result in low ER stress in the fat body (Fig. S1C) reduced *edem1* levels could be an outcome of

reduced ER stress during starvation and *edem1* could act as a nutrient sensor (Ryoo, Domingos et al. 2007). Together these observations confirm that Edem1 in addition to playing a role in ERAD mediated protein quality control, help to manage responses during fluctuations in the nutrient environment. 13

Figure legends:

Fig 1. Edem1 function in the fat body is crucial to metabolic homeostasis. Blocking Edem1 expression using RNAi in the fat body led to enhanced triglyceride levels in adult male flies (**A**) data is shown as % ratio of triglyceride to total protein levels, data is normalised to 100% in *pplGal4>w^{1118}* (control) and increase in experimental conditions *pplGal4>edem1-RNAi* [n=10, P-value between control and *edem1*-RNAi is <0.0001]; enhanced levels of glycogen in adult male flies (**B**) data is shown as % of total glycogen levels, data normalised to 100% in *pplGal4>w^{1118}* (control) and increase in experimental conditions *pplGal4>edem1-RNAi* [n=3, P-value between control and *edem1*-RNAi is <0.0001]; and enhanced resistance to starvation in adult male flies (**C**) shown are percentage of flies of *pplGal4>w^{1118}* (control) and *pplGal4>edem1-RNAi* which were alive at various time points of starvation [n=7, P-value between control and *edem1*-RNAi is <0.0001]. Starvation of flies where Edem1 expression was blocked using RNAi in the fat body led to enhanced triglyceride levels (**D**) data is shown as % ratio of triglyceride to total protein levels in adult male flies, data is normalised to 100% in *pplGal4>w^{1118}* (control) fed condition and change in response to indicated hours of starvation in control and experimental conditions *pplGal4>edem1-RNAi* [n=3, P-value between control and *edem1*-RNAi is 0.0035 for 0 hrs, 0.048 for 12 hrs, 0.034 for 24 hrs, 0.033 for 36 hrs]; and enhanced glycogen levels in adult male flies (**E**) data is shown as % of glycogen levels, data is normalised to 100% in *pplGal4>w^{1118}* (control) fed condition and change in response indicated hours of starvation in control and experimental conditions *pplGal4>edem1-RNAi* [n=3, P-value between control and *edem1*-RNAi is 0.00043 for 0 hrs, <0.0001 for 12 hrs, 0.00209 for 24 hrs, 0.00027 for 36 hrs]. Expression of *edem1* RNAi in the fat body led to enhanced glucose levels in the circulation (**F**) data is shown as % of glucose levels in 3rd instar larval hemolymph, data is normalised to 100% in *pplGal4>w^{1118}* (control) and change in

experimental conditions *pplGal4>edem1-RNAi* [n=3, P-value between control and *edem1*-RNAi is 0.0011]. Blocking Edem1 in the fat body led to enhanced feeding responses in 3rd instar larvae (**G**) shown is the amount of colored food in %, data is normalised to 100% in *pplGal4>w^{1118}* (control) and % change in experimental conditions *pplGal4>edem1-RNAi* consumed by larvae in 3 hours, [n=3, P-value between control and *edem1*-RNAi is 0.0409]. [*P-value *<0.05; ** <0.01,*** <0.001, n=number of biological replicates; error bars representsSEM*]

Fig 2. Reduction of Edem1 levels in the fat body leads to reduction of insulin signalling

Blocking *edem1* expression using RNAi in the fat body led to an increase of mRNA levels of insulin target genes *dilp6*, *4ebp* and *inr* in larvae (**A**) data is shown as fold change in mRNA levels, values are normalised to *pplGal4>w^{1118}* and fold change in *pplGal4>edem1-RNAi* is shown [n=3, P-value between control and *edem1*-RNAi is 0.028 for *dilp6*, 0.044 for *4ebp*, 0.045 for *inr*]. Over expression of a constitutively active form of *inr* (*inr^{CA}*) with *edem1* RNAi in the fat body led to the rescue of fat phenotype in adult male flies (**B**), data is shown as % ratio of triglyceride to total protein levels, normalised to 100% in *pplGal4>w^{1118}* (control) and change in experimental conditions *pplGal4>edem1-RNAi, pplGal4>UAS-inr^{CA}* and *pplGal4>edem1-RNAi, UAS-inr^{CA}* [n=3, P-value between control and *edem1*-RNAi is 0.0012, P-value between control and *inr^{CA}* is 0.039, P-value between *edem1*-RNAi and *inr^{CA}* is 0.00022, P-value between *edem1*-RNAi and *edem1*-RNAi+*inr^{CA}* is 0.0039, P-value between *inr^{CA}* and *edem1*-RNAi+*inr^{CA}* is 0.29 and P-value between control and *edem1*-RNAi+*inr^{CA}* is 0.43] and starvation resistance in adult male flies (**C**) shown are percentage of input flies *pplGal4>w^{1118}, pplGal4>edem1-RNAi, pplGal4>UAS-inr^{CA}* and *pplGal4>edem1-RNAi, UAS-inr^{CA}* which were alive at various time points of starvation [n=3, P-value between control and *edem1*-RNA; control and *inr^{CA}*; *edem1*-RNAi and *inr^{CA}*; *edem1*-RNAi and *edem1*-RNAi+*inr^{CA}* is <0.0001 and P-value between *inr^{CA}* and *edem1*-RNAi+*inr^{CA}* is 0.5507]. Blocking *edem1* expression using RNAi in the fat body led to a decrease in the levels of IPC specific *dilp2*, *dilp3*, *dilp5* mRNA in larvae (**D**) data is shown as fold change in mRNA levels, values are normalised to *pplGal4>w^{1118}* and fold change in *pplGal4>edem1-RNAi* [n=3, P-value between control and *edem1*-RNAi is 0.411 for *dilp2*, 0.00042 for *dilp3* and 0.89 for *dilp5*]; and reduction in the over all levels of DILP2 protein in the larval IPCs (**E**) shown is a representative image of anti-DILP2 antibody staining in larval

brains of *pplGal4>w^{1118}* [n=15] and *pplGal4>edem1-RNAi* [n=14]. [*P-value *<0.05; ** <0.01,*** <0.001, n=number of biological replicates; error bars represent SEM*]. Starvation leads to reduction of Edem1 mRNA levels in larvae (**F**), shown here is fold change in mRNA levels in 3rd instart larvae in response to starvation for 12 hours, values are normalised to normal fed controls [n=3, P-value between fed and starved is 0.033]. Over-expression of Edem1 leads to enhanced sensitivity to starvation (**G**), shown are percentage of male flies which were alive at various time points of starvation in the following genotypes - *pplGal4>w^{1118}* and *pplGal4>UAS-edem1* [n=3, P-value between control and UAS-*edem1* is <0.0001] [*P-value *<0.05; ** <0.01,*** <0.001, n=number of biological replicates; error bars represent SEM*]

Fig 3. Reduction of Edem1 levels in the fat body leads to an increase of *tace* transcript levels, Eiger and JNK signalling Blocking Edem1 expression using RNAi in the fat body led to an increase of mRNA levels of *tace* (**A**), data is shown as fold change in mRNA levels, values are normalised to *pplGal4>w^{1118}* and fold change in *pplGal4>edem1-RNAi* [n=3, P-value between control and *edem1*-RNAi is 0.0013]. Blocking Edem1 expression using RNAi in the fat body led to an increase of mRNA levels of *NLaZ* which was reduced by *eiger-RNAi* co-expression (**B**) data is shown as fold change in mRNA levels, values are normalised to *pplGal4>w^{1118}* and fold change in *pplGal4>edem1-RNAi* and *pplGal4>edem1-RNAi, eiger-RNAi* was plotted [n=3, P-value between control and *edem1*-RNAi is 0.03, P-value between *edem1*-RNAi and *edem1*-RNAi + *egr*-RNAi is 0.04]. [*P-value *<0.05; ** <0.01,*** <0.001, n=number of biological replicates; error bars represent SEM*]. Expression of *eiger* RNAi in the fat body led to the rescue of phenotypes caused by blocking Edem1 levels in the fat body: fat phenotype (**C**) data is shown as % ratio of triglyceride to total protein levels, normalised to 100% in *pplGal4>w^{1118}* (control) and change in experimental conditions *pplGal4>edem1-RNAi* and *pplGal4>edem1-RNAi, eiger-RNAi* [n=3, P-value between control and *edem1*-RNAi is <0.0001, P-value between control and *edem1*-RNAi + *egr*-RNAi is 0.11, P-value between *edem1*-RNAi and *edem1*-RNAi + *egr*-RNAi is <0.0001]; increase in Glucose levels in the hemolymph (**D**), data is shown as % glucose levels, data is normalised to 100% in *pplGal4>w^{1118}* (control) and change in experimental conditions *pplGal4>edem1-RNAi* and and *pplGal4>edem1-RNAi, eiger-RNAi* [n=6, P-value between control and *edem1*-RNAi is 0.0074, P-

value between control and *edem1*-RNAi + *egr*-RNAi is 0.804, P-value between *edem1*-RNAi and *edem1*-RNAi + *egr*-RNAi is 0.029]; enhanced starvation resistance (**E**) shown are percentage of flies which were alive at various time points of starvation in the following genotypes - *pplGal4>w^{1118}, pplGal4>edem1-RNAi* and *pplGal4>edem1-RNAi, eiger-RNAi* [n=3, P-value between control and *edem1*-RNAi and between *edem1*-RNAi and *edem1*-RNAi + *egr*-RNAi is <0.0001, P-value between control and *edem1*-RNAi + *egr*-RNAi is 0.2159]; reduction of DILP2 levels in the IPCs (**F**) shown are representative images of anti-DILP2 antibody staining in larval brains of *pplGal4>w^{1118}* [n=15]; *pplGal4>edem1-RNAi* [n=14] and *pplGal4>edem1-RNAi, eiger-RNAi* [n=9]; and reduction of *dilp3* mRNA levels (**G**) and increase in *4ebp* mRNA levels (**H**) data is shown as fold change in mRNA levels, values are normalised to *pplGal4>w^{1118}* fold change in *pplGal4>edem1-RNAi* and *pplGal4>edem1-RNAi, eiger-RNAi* [n=3, P-value between control and *edem1*-RNAi is 0.00042 for *dilp3* and 0.044 for *4ebp*, P-value between control and *edem1*-RNAi + *egr*-RNAi is 0.27 for *dilp3* and 0.14 for *4ebp*, P-value between *edem1*-RNAi and *edem1*-RNAi + *egr*-RNAi is 0.0003 for *dilp3* and 0.035 for *4ebp*]. [*P-value *<0.05; ** <0.01,*** <0.001, n=number of biological replicates; error bars represent SEM*]

Fig 4. Activating TOR signalling rescued Edem1 mediated phenotypes. Over expression of Rheb in the fat body rescued enhanced stored fat levels (**A**) data is shown as % ratio of triglyceride to total protein levels, normalised to 100% in *pplGal4>w^{1118}* (control) and change in experimental conditions *pplGal4>edem1-RNAi, pplGal4>UAS-Rheb* and *pplGal4>edem1-RNAi, UAS-Rheb* [n=5, P-value between control and *edem1*-RNAi is 0.0001, P-value between control and UAS-*rheb* is 0.00003, P-value between *edem1*-RNAi and *edem1*-RNAi + UAS-*rheb* is 0.031, P-value between control and *edem1*-RNAi+UAS-*rheb* is 0.004] and increased starvation resistance (**B**) shown are percentage of flies which were alive at various time points of starvation in the following genotypes *pplGal4>w^{1118}, pplGal4>edem1-RNAi, pplGal4>UAS-Rheb* and *pplGal4>edem1-RNAi, UAS-Rheb* [n=5, P-value between control and *edem1*-RNAi is <0.0001, P-value between *edem1*-RNAi and UAS-*rheb* is 0.0013, P-value between *edem1*-RNAi and *edem1*-RNAi + UAS-*rheb* is 0.027, P-value between control and *edem1*-RNAi+UAS-*rheb* is 0.202] caused by the reduction of Edem1 levels in the fat body. Increase of mRNA levels of *tace* (**C**) and *NLaz* (**D**) 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>w^{1118}* and fold change in *pplGal4>edem1-RNAi* and *pplGal4>edem1-RNAi, UAS-Rheb* [n=3, for *tace* P-value between control and *edem1*-RNAi is 0.0013, P-value between *edem1*-RNAi and *edem1*-RNAi + UAS-*rheb* is 0.113, P-value between control and *edem1*-RNAi+UAS-*rheb* is 0.507; for *NLacZ* P-value between control and *edem1*-RNAi is 0.0303, P-value between *edem1*-RNAi and *edem1*-RNAi + UAS-*rheb* is 0.187, P-value between control and *edem1*-RNAi+UAS-*rheb* is 0.95]. [*P-value *<0.05; ** <0.01,*** <0.001, n=number of biological replicates; error bars represent SEM*]

Fig S1. Nutrient withdrawal leads to reduction of UPR genes including Edem1 and reduction of Edem1 during starvation aids in survival. Starvation leads to reduction of DILP2 levels in the IPCs when larvae were subjected to nutrient deprivation (**A**)**.** Starvation leads to reduction of UPR genes (**B**), shown here is fold change in mRNA levels, values are normalised to normal fed controls [n=3, P-value between fed and starved is 0.028 for *atf4*, 0.0106 for *calnexin99a*, 0.045 for *derlin1*, 0.08 for *herp*, 0.0113159952938221 for *pek*, 0.025 for *sip (hrd3)*, 0.307 for *atf6*, 0.045 for *bip*, 0.033 for *edem1*, 0.022 for *hrd3*, 0.014 for *hsc3*, 0.037 for *ire1*, 0.017 for *xbp1-23bp* and 0.081 for *xbp1+23bp*] and *xbp1-EGFP* reporter levels (**C**) in response to starvation, shown here is fold change in GFP fluorescence, values are normalised to normal fed controls [n=4, P-value between fed and starved is 0.003] [*P-value *<0.05; ** <0.01,*** <0.001, n=number of biological replicates; error bars represent SEM*].

Experimental Procedures

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-InR.A1325D (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). *pumpless*-Gal4 and w^{1118} were obtained from Stephen Cohen. UAS-*xbp1-egfp* was from Herman Steller (Ryoo, Domingos et al. 2007), *UAS-dEDEM1* was from Koichi Iijima (Sekiya, Maruko-Otake et al. 2017), UAS-*rheb* was obtained from Jagat. K. Roy.

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 within 6 hours of emergence (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 replicates (n) 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, 30 (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 replicates (n) are mentioned in the figure legends.

Glucose assay

Larvae at 3rd in-star stage (5 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 µl with autoclaved milli-Q water. 100 µl 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 µl of 12 N H_2SO_4. 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 (n) are mentioned in the figure legends.

Larval starvation

Third in-star larvae of the desired genotypes were kept for starvation on 1% agar vials for 12hrs, after washing them with milli-Q water to make sure that there were no traces of media left behind. After 12 hrs the larvae were plunged for the qPCR experiments or fluorescence assay or they were dissected. The starvation experiments were replicated independently and number of replicates (n) are mentioned in the figure legends.

Feeding assay

Larvae at 3rd in-star stage (10 each) were fed with colored food with Orange G dye (Cat. # 1936-15-8) from Sigma for 3 h. The larvae were homogenized using 0.05% Tween-20. The homogenate was analyzed colorimetrically 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 (n) are mentioned in the figure legends.

Quantitative RT-PCR

Third in-star wandering larvae for each genotype were collected and were flash-frozen. For the experiments with starvation conditions, early 3rd instar larvae were starved for 12 h and flash frozen along with the fed controls. 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 CFX96TM 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.

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). About 10 larvae (3rd in-star wandering) were used to dissect the fat body and 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 antibodies were diluted in BBT. Dilutions used are as follows: rabbit anti-DILP2 1:1000, rabbit anti-GFP (Cat. # A-6455 from Invitrogen) 1:500. 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 antibodies at room temperature for 2 h. The secondary antibodies used were as follows: Alexa Fluor® 488 Goat Anti-Rabbit IgG (Cat. # A27034), Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 568 (Cat. # A-11011), Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 633 (Cat. # A-21070) 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.

Fluorescence assay

For the fluorescence assay, 5 larvae per genotype (fed or starved - for starvation the conditioning was done as mentioned before) were homogenized in 1X RIPA buffer (Cat. # R0278 from Sigma) supplemented with 1X protease inhibitor (Cat. # S8830-20TAB from Sigma). GFP fluorescence was quantified using excitation and emission spectra of 485 nm and 530 nm respectively. Several independent batches of larvae were homogenized, the number of replicates (n) are mentioned in the figure legends.

Statistical analysis

All the experiments were done in biological replicates as indicated and the error bars represent the standard error mean (SEM). Significance was tested using Student's t-test with * representing p-value < 0.05, ** p-value < 0.01, *** p-value < 0.001. The statistical analyses for starvation sensitivity assays were performed using OASIS 2 software (Han et

al, 2016). The starvation datasets were subjected to Log rank test (Mantel-Cox) and trends in the lifespan of flies in the starvation sensitivity assays were analyzed.

Acknowledgements

We thank Nisha 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.

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Figure 1. *edem1* function in the larval fatbody is crucial to metabolic homeostasis

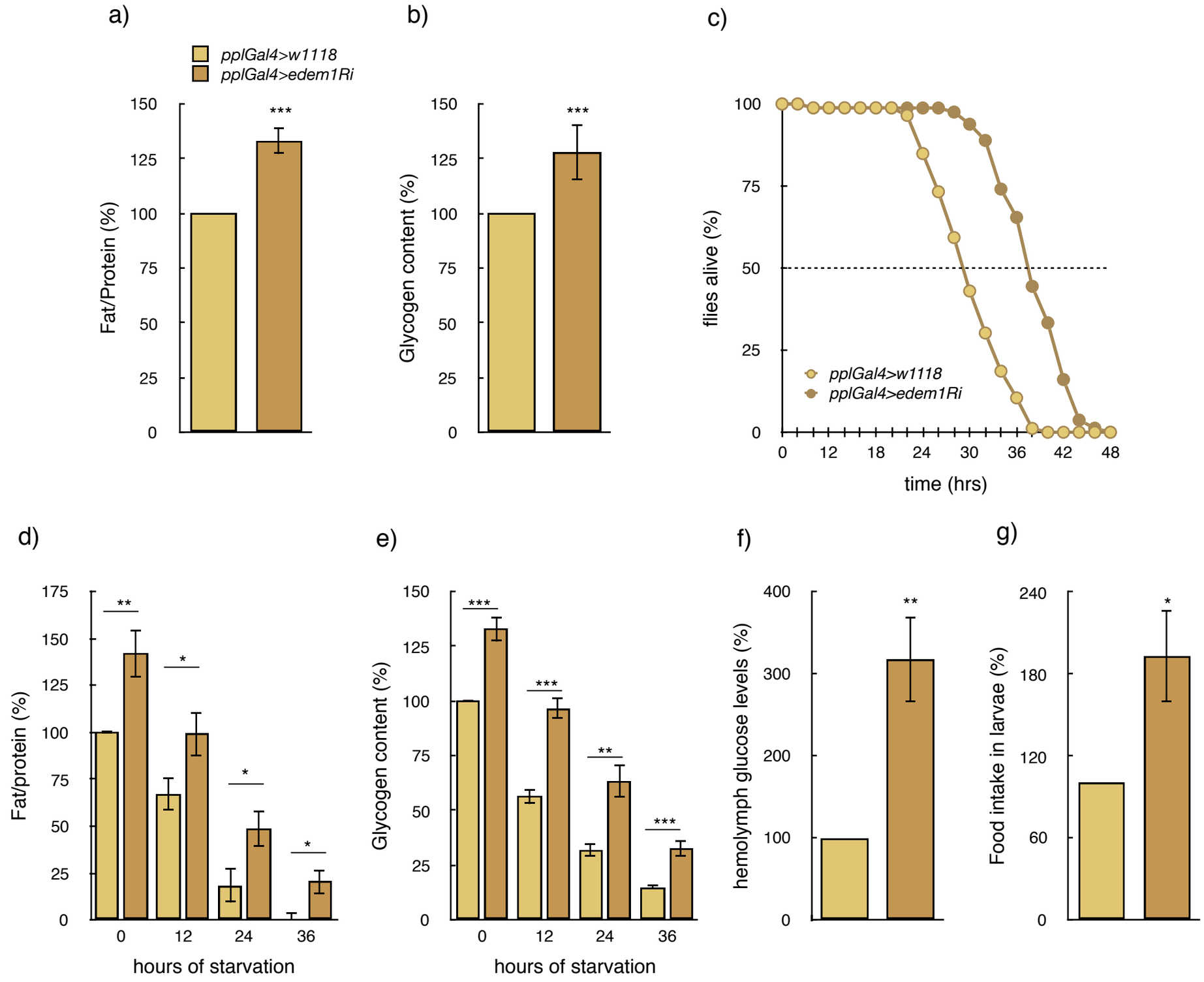


Figure 2. Reduction of *edem1* mRNA levels in larval fatbody affects insulin signalling

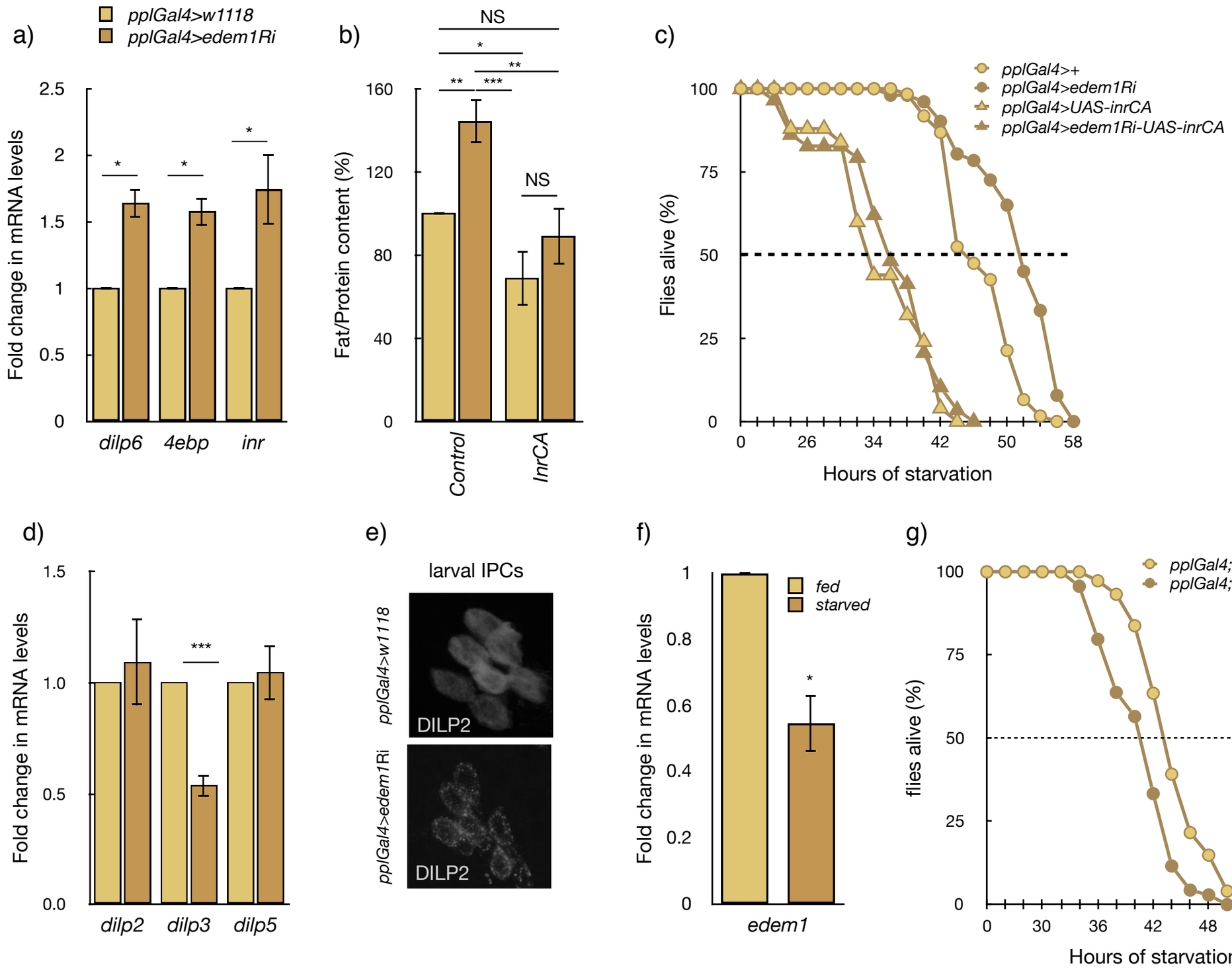


Figure 3. Reduction of *edem1* mRNA levels in larval fatbody leads to activation of Eiger and is responsible for Edem1 knock down phenotypes

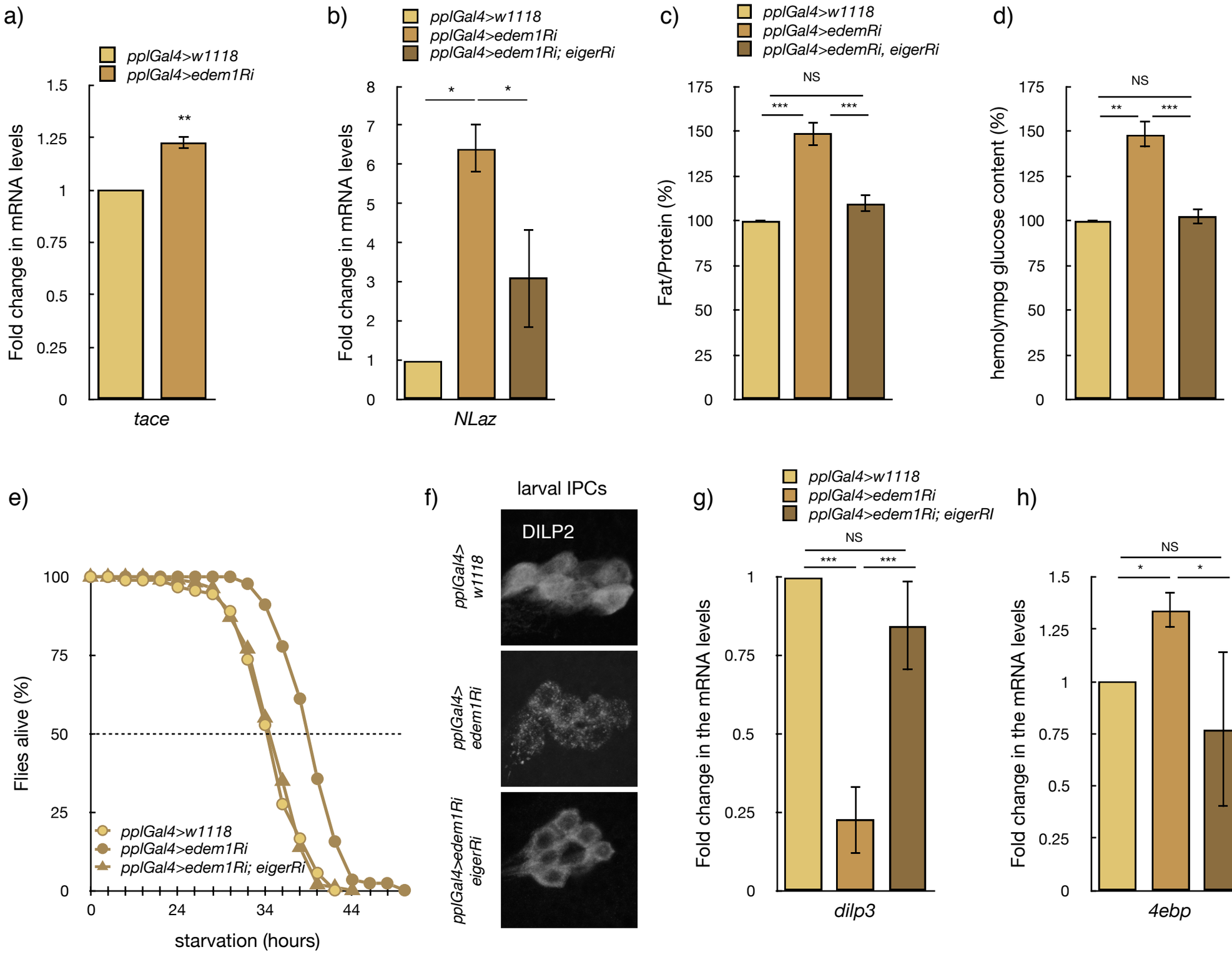


Figure 4. The edem1 RNAi phenotypes are rescued by up regulating TOR signalling

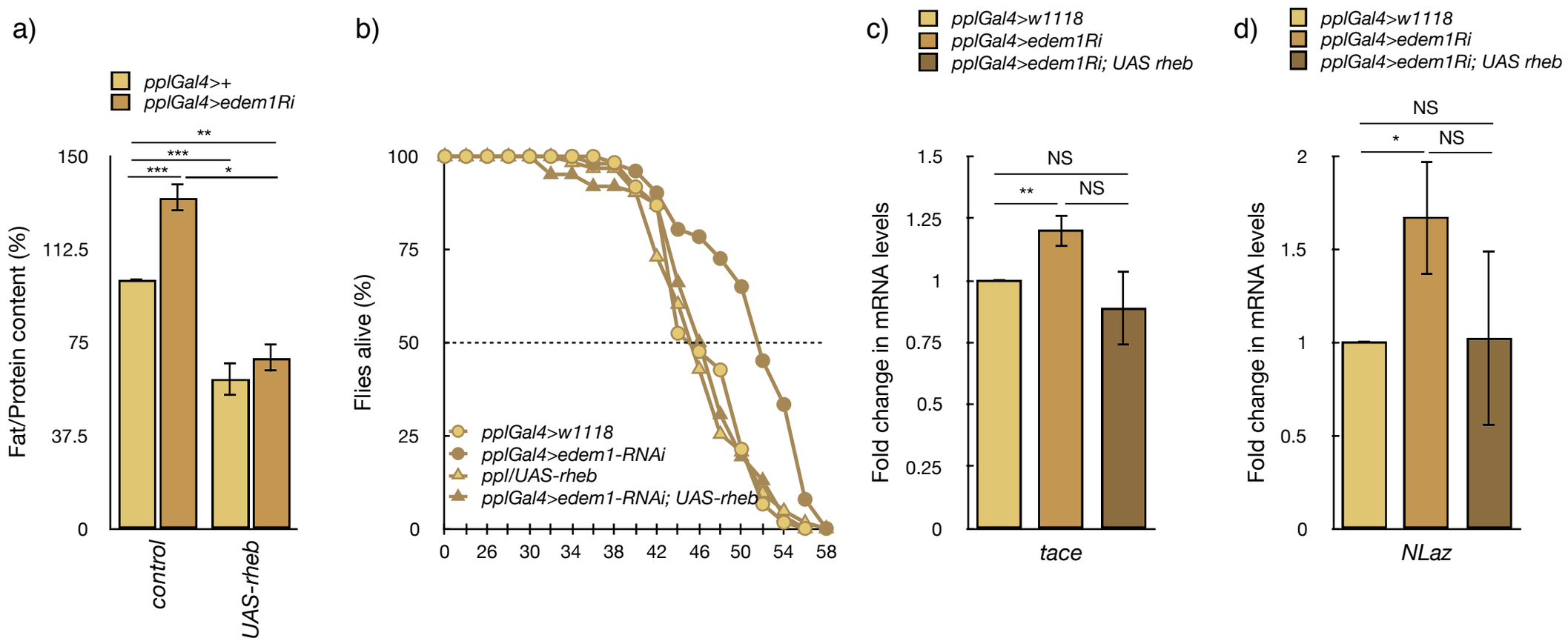
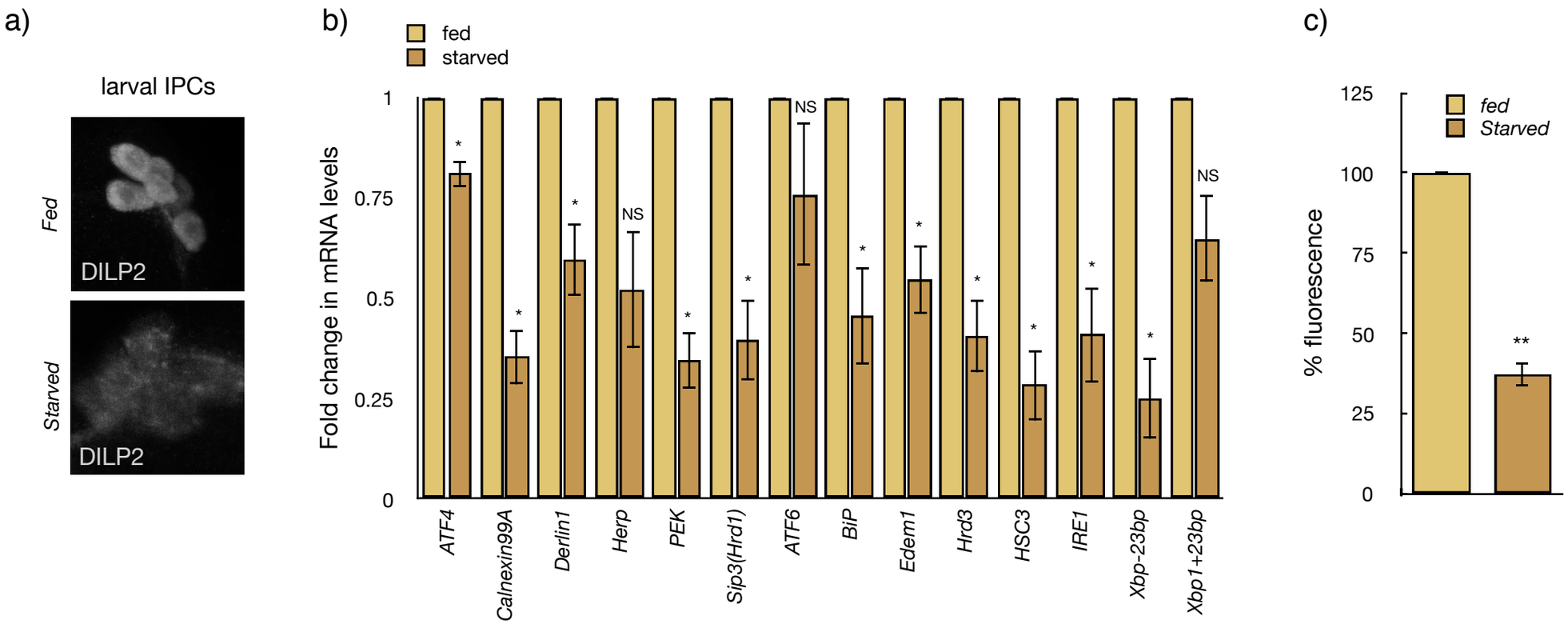


Figure S1.


a)
larval IPCs
Fed
DILP2
Starved
DILP2
b)
fed
starved
Fold change in mRNA levels
1
0.75
0.5
0.25
0
NS
NS
NS
*
ATF4
Calnexin99A
Derlin1
Herp
PEK
Sip3(Hrd1)
ATF6
BiP
Edem1
Hrd3
HSC3
IRE1
Xbp-23bp
Xbp1+23bp
c)
% fluorescence
125
100
75
50
25
0
fed
Starved
**