The enteroendocrine cell (EEC)-derived incretins play a pivotal role in regulating the secretion of glucagon and insulins in mammals. Although glucagon-like and insulin-like hormones have been found across animal phyla, incretin-like EEC-derived hormones have not yet been characterised in invertebrates. Here, we show that the midgut-derived hormone, neuropeptide F (NPF), acts as the sugar-responsive, incretin-like hormone in the fruit fly, *Drosophila melanogaster*. Secreted NPF is received by NPF receptor in the corpora cardiaca and in insulin-producing cells. NPF-NPFR signalling resulted in the suppression of the glucagon-like hormone production and the enhancement of the insulin-like peptide secretion, eventually promoting lipid anabolism. Similar to the loss of incretin function in mammals, loss of midgut NPF led to significant metabolic dysfunction, accompanied by lipodystrophy, hyperphagia, and hypoglycaemia. These results suggest that enteroendocrine hormones regulate sugar-dependent metabolism through glucagon-like and insulin-like hormones not only in mammals but also in insects.
All organisms must maintain energy homeostasis in response to nutrient availability. To maintain balance of catabolism and anabolism, organisms coordinate systemic energy homeostasis through hormonal factors. Insulin and counter-regulatory hormones, such as glucagon, have previously been shown to act as such humoral factors in response to nutritional and environmental cues. Recent works have revealed that EECs sense multiple dietary nutrients and microbiota-derived metabolites that influence the production and/or secretion of enteroendocrine hormones. In mammals, an enteroendocrine hormone that stimulates the secretion of glucagon and insulin, particularly the latter, is referred to as "incretin," such as glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1). The secretion of GIP and GLP-1 is stimulated by dietary carbohydrates and lipids. Insects stimulate pancreatic insulin secretion and conversely suppress glucagon secretion in a glucose-dependent manner. The physiological importance of incretins is epitomised by the fact that dysregulation of incretins often associates with obesity and type 2 diabetes.

To further dissect the molecular, cellular, and endocrinological mechanisms of glucagon and insulin actions in animals, the fruit fly, Drosophila melanogaster, has emerged as a powerful genetic system in recent years. There are eight genes encoding Drosophila insulin-like peptides (DILPs), designated DILP1 to DILP8. Among these DILPs, it is thought that DILP2, DILP3, and DILP5 are particular essential for the regulation of haemolymph glucose levels and fat storage, controlling developmental timing, body size, and longevity. D. melanogaster also possesses a hormone that is functionally equivalent to the mammalian glucagon, called adipokinetic hormone (AKH). AKH is produced in and secreted from a specialised endocrine organ, the corpora cardiaca (CC), and acts on the fat body, leading to lipolysis-dependent energy metabolism. Furthermore, recent studies have identified two factors secreted by EECs, Activin-β and Bursicon-α (Bursa), which play essential roles in modulating AKH-dependent lipid metabolism in the fat body. Another, neither Activin-β nor Bursa directly acts on the CC or insulin-producing cells (IPCs). Indeed, no incretin-like enteroendocrine hormones has been discovered in invertebrates.

Here, we report that the midgut-derived hormone neuropeptide F (NPF), a homologue of the mammalian neuropeptide Y (NPY), acts as the sugar-responsive, incretin-like hormone in D. melanogaster, while the primary structure of NPF is completely different from that of GIP or GLP-1. NPF is produced in and secreted from midgut EECs in response to dietary nutrients. NPF is bound by NPF receptor (NPFR) that is present in the CC and IPCs. Impairment of NPF/NPFR signalling resulted in AKH- and insulin-dependent catabolic phenotypes, accompanied by hypoglycaemia, lipodystrophy, and hyperphagia. Our work demonstrates a key role of inter-organ communication between the midgut, the brain and endocrine organs to regulate energy homeostasis.

**Results**

Midgut NPF is required for lipid accumulation in the fat body and promotes starvation resistance. We have previously reported that midgut-derived NPF is essential for mating-induced germline stem cell increase in female D. melanogaster. This discovery prompted us to ask whether midgut-derived NPF is also involved in other biological processes. In particular, since many enteroendocrine hormones are known to regulate nutritional plasticity, we investigated whether loss of midgut-derived NPF leads to any nutrient-related phenotypes. To knock down NPF specifically in EECs, we utilised TKg-GALA. This GALA driver is active in most NPF+ EECs and small subsets of neurons but not in NPF- neurons. NPF knockdown with TKg-GALA (TKg>NPFRNAi) successfully reduced the number of NPF+ EECs and NPF mRNA expression in the midgut (Supplementary Fig. 1a, b), as previously reported. We found that the flies became significantly sensitive to nutrient deprivation. Adult flies were raised on normal food for 6 days after eclosion, and then transferred to a 1% agar-only medium. TKg>NPF RNAi animals showed hypersensitivity to nutrient deprivation compared to control animals (TKg>lacZRNAi) (Fig. 1a). The hypersensitivity was observed with two independent UAS-NPFRNAi constructs (KK and TRIP; see Methods), each of which targeted a different region of the NPF mRNA.

A recent study has reported that the loss-of-function of another midgut-derived peptide hormone, Bursa, also exhibited hypersensitivity to starvation. We examined whether the NPF loss-of-function phenotype was due to the expression and/or secretion defect in Bursa in the gut. However, NPF knockdown in the EECs did not affect Bursa mRNA expression in the intestine or Bursa accumulation in the EECs of the posterior midgut (Supplementary Fig. 1b, c).

The survivability of flies on nutrient deprivation directly correlates with accessibility to energy storage in their bodies, mainly stored as neutral lipids, including triacylglycerides (TAG) in the fat body. Consistent with the starvation hypersensitivity in animals with loss of NPF function, we detected a significant overall reduction of whole-body TAG levels in both TKg>NPFRNAi animals and NPF genetic null mutants (NPF<sup>sk1/Df</sup>) (Fig. 1b, Supplementary Fig. 1e). Further, in the fat body of both TKg>NPFRNAi animals and NPF mutants, the signal intensity of the lipophilic fluorescent dye (LipidTOX) was significantly reduced, as compared with control animals (Fig. 1c; Supplementary Fig. 1f). Conversely, overexpression of NPF in the EECs resulted in a slight increase in TAG abundance (Supplementary Fig. 1g).

In addition to the RNAi animals, we found that NPF genetic null mutants (NPF<sup>sk1/Df</sup>) also exhibited similar hypersensitivity phenotype on starvation (Supplementary Fig. 1d). Importantly, transgenic NPF reintroduction into EECs (TKg>NPF, NPF<sup>sk1/Df</sup>) was sufficient to recover hypersensitivity to starvation and the TAG reduction observed in NPF mutant background (Fig. 1d–f). These results suggest that NPF from midgut EECs is required to sustain organisinal survival during nutrient deprivation.

To rule out the possibility that loss of NPIF during the larval and pupal stages impacts adult metabolism, we conducted adult-specific knockdown of NPF with tub-GAL80<sup>bis</sup> (TKg>NPF<sup>RNAi</sup>). In TKg>NPF<sup>RNAi</sup> adults, a temperature-shift to restrictive temperatures following eclosion significantly reduced NPF levels in EECs (Supplementary Fig. 2a). Moreover, the adult-specific knockdown of NPF resulted in hypersensitivity upon starvation and reduced TAG abundance (Fig. 1g–i), while no visible alterations were noted in size or morphology of the fat body (Fig. 1i). We also observed a significant reduction in circulating glucose and trehalose levels in TKg>NPF<sup>RNAi</sup> adults at restrictive temperature (Fig. 1j, Supplementary Fig. 1h, 2b), suggesting that reduced lipid storage results in high utilisation of circulating glucose.

Since energy storage well correlates with the amount of food consumption, the lean phenotype described above may be simply due to less food intake. However, a CAPE assay revealed that both TKg>NPF<sup>RNAi</sup> animals and NPF mutants increased food intake...
Therefore, high food intake in Tkg>NPF<sup>RNAi</sup> suggests opposing functions between the brain-derived and midgut-derived NPF. To examine whether brain NPF affects lipid metabolism, we employed fbp-GAL4<sup>24</sup>, which is active in the NPF<sup>+</sup> neurons in the brain, but not in gut EECs (Supplementary Fig. 3a). Knockdown of NPF with fbp-GAL4 (fbp>NPF<sup>RNAi</sup>) abolished anti-NPF antibody immunoreactivity in two sets of large neurons, termed L1-l and P125, in the brain without affecting NPF level in the gut.

Brain NPF is not involved in lipid accumulation in the fat body or the promotion of starvation resistance. It is well known that NPF produced in the brain has orexigenic function<sup>22,23</sup>. Therefore, the hypersensitivity to starvation and the lean phenotype of animals with loss of NPF function do not seem to be secondary to food intake defects, but a more direct outcome of some metabolic defects.
Fig. 1 NPF from midgut EECs maintains metabolic homeostasis. a–i Phenotypes of the midgut EEC-specific NPF knockdown animals (TKg>NPFRNAi) (a–c), NPF genetic mutant animals with or without midgut-specific NPF reintroduction (TKg>NPFpki/Df) (d–f), and adult EEC-specific NPF knockdown animals (TKg>p>NPFRNAi) (g–i). a, d, g Survival during starvation. b, e, h Relative TAG amount. c, f, i LipidTOX (red or magenta) and DAPI (blue) staining of dissected fat body tissue. Scale bar, 50 µm in c and f, 200 µm (100×) and 50 µm (400×) in i. j Relative circulating glucose levels. k Feeding quantity measurement with CAFÉ assay. For RNAi experiments, LacZ knockdown (TKg>LacZRNAi) was used as the negative control. For all bar graphs, the number of samples assessed (n) is indicated in each graph. Mean ± SEM with all data points is shown. Statistics: Log rank test with Holm’s correction (a, d, and g), two-tailed Student’s t-test (b, h, and k), one-way ANOVA followed by Tukey’s multiple comparisons test (e). *p < 0.05, **p < 0.01, p-values: a: p < 0.0001 (TKg>LacZRNAi vs. TKg>NPFpki/Df), p < 0.0001 (TKg>LacZRNAi vs. TKg>NPFRNAi); b: p < 0.0005, d: p < 0.0001 (TKg>+) vs. NPFsk1/Df; p < 0.0001 (TKg>+/+ vs. TKg>++; NPFsk1/Df vs. TKg>NPFRNAi); e: p = 0.0027 (TKg>++; NPFsk1/Df vs. TKg>NPFRNAi); f: p = 0.0034 (TKg>LP vs. TKg>NPFRNAi); g: p < 0.0001; h: p = 0.0008; j: p = 0.0316; k: p = 0.0363.

(Supplementary Fig. 3b). In flyb>NPFRNAi adults, a mild reduction in food consumption was observed without impacting starvation resistance or TAG abundance (Supplementary Fig. 3c–e). Moreover, reintroduction of NPF in the brain (flyb>NPF; NPFpki/Df) did not recover the metabolic phenotypes of the NPF mutant (Supplementary Fig. 3f–g). These results contrast those obtained following the reintroduction of NPF in the midgut (TKg>NPF; NPFpki/Df; Fig. 1d, e). Collectively, these results suggest that midgut NPF has a prominent role in suppressing lipodystrophy, which is independent from the brain NPF.

Midgut NPF is required for energy homeostasis. To further explore the lean phenotype of TKg>NPFpki/Df animals at the molecular level, we conducted an RNA-seq transcriptome analysis on the abdomens of adult females. Among the 105 curated carbohydrate metabolic genes, 17 were significantly upregulated in TKg>NPFpki/Df animals (p < 0.05; Supplementary Fig. 4a, Supplementary Data 1). Many of these genes were also upregulated in TKg>NPFpki/Df samples, however, these results were not statistically significant because replicate No. 1 of TKg>LacZRNAi exhibited deviation in the expression pattern (Supplementary Fig. 4a, Supplementary Data 1). Moreover, among the 174 curated genes involved in mitochondrial activity and genes encoding electron respiratory chain complexes, 53 were significantly upregulated (p < 0.05) in TKg>NPFpki/Df samples (Supplementary Fig. 4b, Supplementary Data 2). Metabolomic analysis demonstrated a significant shift in the whole-body metabolome of TKg>NPFpki/Df animals (Fig. 2a, Supplementary Fig. 5a, Supplementary Data 3, 4). We found that, while circulating glucose level in the haemolymph was significantly decreased (Fig. 1g), TKg>NPFpki/Df resulted in increase of tricarboxylic acid (TCA) cycle metabolites, such as citrate, isocitrate, fumarate, and malate, in whole-body samples as well as haemolymph samples (Fig. 2b, c). These data strongly suggest that TKg>NPFpki/Df animals utilise and direct more glucose into the TCA cycle.

Based on RNA-seq transcriptome analysis, we found that starvation-induced genes19 were also upregulated in the abdomens of TKg>NPFpki/Df adults (Fig. 2d, Supplementary Data 5). Subsequent quantitative PCR (qPCR) validated the upregulation of the starvation-induced gluconeogenic genes (fructose-1,6-bisphosphatase (fbp) and Phosphoenolpyruvate carboxykinase 1 (pepck1))26 (Fig. 2e). In general, TAG is broken into free fatty acids to generate acetyl-coenzyme A (CoA), which is metabolised in the mitochondria through the TCA cycle and oxidative phosphorylation. We also confirmed the upregulation of lipid metabolism gene (Brummer (Bmm)) in the abdomen of TKg>NPFpki/Df animals (Fig. 2f). Notably, upregulation of Acetyl-CoA carboxylase (ACC) was not reproduced with qPCR (Fig. 2f). These data suggest that TKg>NPFpki/Df animals are in the starved-like status despite taking in more food, and that haemolymph glucose levels cannot be maintained even with the activation of gluconeogenesis and lipolysis in TKg>NPFpki/Df animals. We hypothesise that, owing to the starved-like status, the loss of midgut NPF function might lead to an abnormal consumption of TAG, resulting in the lean phenotype.

Midgut NPF responds to dietary sugar. Since EECs can sense dietary nutrients, we surmised that dietary nutrients affect NPF production and/or secretion in midgut EECs. We thus compared NPF protein and mRNA levels in flies fed standard food or starved for 48 h with 1% agar. After 48 h of starvation, NPF protein in midgut EECs was significantly increased (Fig. 3a, b), although its transcript in the intestine was reduced (Fig. 3c). These data suggest that the increased accumulation of NPF protein in EECs upon starvation is not due to upregulation of NPF mRNA expression level, but rather due to post-transcriptional modification. This situation was very similar to the case of mating-dependent change of NPF protein level, and may reflect the secretion of NPF protein from EEC17. Considering that the high accumulation of NPF protein without NPF mRNA increase indicate a failure of NPF secretion, we hypothesised that starvation suppresses NPF secretion from EECs.

To identify specific dietary nutrients that affect NPF levels in EECs, after starvation, we fed flies a sucrose or Bacto peptone diet as exclusive sources of sugar and proteins, respectively. Interestingly, by supplying sucrose, the levels of both of NPF protein and NPF mRNA in the gut reverted to the levels similar to ad libitum feeding conditions (Fig. 3a, b). In contrast, Bacto peptone administration did not reduce midgut NPF protein level, but rather increased both NPF protein and NPF mRNA levels (Fig. 3c). These data imply that midgut NPF is secreted primarily in response to dietary sugar, but not proteins. This sucrose-dependent NPF secretion was observed in flies fed a sucrose medium for 6 h after starvation, whereas a 1 h sucrose restoration had no effect on NPF accumulation (Supplementary Fig. 6a).

Sugar-responsive midgut NPF production is regulated by the sugar transporter Sut1. In mammals, the sugar-stimulated secretion of GLP-1 is partly regulated by glucose transporter 2, which belongs to the low-affinity glucose transporter solute carrier family 2 member 2 (SLC2)27,28. In D. melanogaster, a SLC2 protein, Glucose transporter 1 (Glut1), in the Bursa+ EECs regulates sugar-responsive secretion and Bursa+ mRNA expression11. However, knockdown of Glut1 did not affect NPF mRNA nor NPF protein abundance in EECs (Supplementary Fig. 6b, c). Thus, we next examined which SLC2 protein, aside from Glut1, regulates NPF levels in the gut. There are over 30 putative homologues of SLC2 in the D. melanogaster genome29. Of these, we focused on sugar transporter 1 (sut1), because its expression has been described in the intestinal EECs by FlyGut-seq project30 and Flygut EEs single-cell RNA-seq project31. To verify sut1 expression, we generated a sut1<Knock-in>(KI)-T2A-GALA strain using CRISPR/Cas9-mediated homologous recombination32,33. Consistent with these transcriptomic analyses, sut1<KI-T2A-GALA expression was observed in the EECs, including NPF+ EECs.
In addition, we found that overexpressed monomeric Venus (mVenus)-tagged Sut1 protein (TKg>sut1::mVenus) was localised on the membrane of NPF+ EECs (Fig. 3e), supporting the notion that Sut1 mediates the transport of extracellular sugar.

Next, to ascertain the glucose transporter capacity of Sut1, we expressed FLIII12PGlu-700μδ, a fluorescence resonance energy transfer (FRET)-based glucose sensor (referred to as Glu700) into D. melanogaster S2 cells, with or without sut1 overexpression. In this experiment, we equilibrated S2 cells in buffer lacking glucose, followed by application of high-glucose (25 mM final concentration) solution. As compared with S2 cells without sut1 overexpression, the addition of high-glucose...
NPFR in the CC regulates lipid metabolism. We have previously reported that midgut EEC-derived NPFR may be sequestered into circulation and activate NPFR in the ovarian somatic cells, leading to germline stem cell proliferation. We first investigated potential NPFR-dependent lipid metabolism regulation by ovarian NPFR. However, NPFR knockdown in the ovarian somatic cells with *Traffic jam(tj)-GAL4* did not induce hypersensitivity to starvation or reduction of TAG contents (Supplementary Fig. 10a, b), implying that NPFR expressed in tissues other than the ovary must be involved in regulating sugar-dependent lipid metabolism.

To determine the tissues expressing NPFR, we utilised two independent NPFR knock-in *TZA-GAL4* lines, NPFR*KI-TZA-GAL4* (see the “Methods” section) and NPFR*KI-RAC-GAL4*, each of which carry a transgene cassette that contained *TZA-GAL4* immediately in front of the stop codon of the endogenous NPFR gene. Crossing these lines with a *UAS-GFP* line revealed GFP expression not only in the brain (Supplementary Fig. 11a), as previously reported, but also in other tissues, including the CC (Fig. 4a, Supplementary Fig. 11b), short neuropode F (non-PF+) enteric neurons, Malpighian tubules, ovary, and gut (Supplementary Fig. 11c–f). The expression in the CC was observed in two independent *KI-GAL4* lines, NPFR*KI-TZA-GAL4* and NPFR*KI-RAC-GAL4*, *TZA-GAL4* (Fig. 4a, Supplementary Fig. 11b). Therefore, based on these results and those of a previous RNA-seq analysis, we surmised that NPFR is expressed in the CC. Since the CC produces the glucagon-like peptide, AKH, which regulates organismal carbohydrate and triglyceride metabolism in insects, we were particularly interested in examining whether NPFR in the CC is involved in metabolic regulation in adult *D. melanogaster*.

To this end, we further conducted starvation experiments. Similar to animals with loss of NPFR function, NPFR knockdown animals (*Akhet* or NPFR*RNAiTriP*) and NPFR-null mutants (NPFR*kk/DD*) were more sensitive to starvation, compared with control (*Akhet* or *LacZ RNAi*) in terms of both TAG amount and glycaemic levels, accompanied by increase of food intake, similar to animals with disrupted NPFR (Fig. 4c–f; Supplementary Fig. 10d). Moreover, reintroduction of NPFR in the CC rescued the starvation sensitivity, the low TAG levels, and the reduced signal intensity of the LipidTOX in the fat body of NPFR mutants (Fig. 4g–i), indicating that NPFR in the CC is essential for modulating lipid catabolism.

Consistent with a previous report, NPFR*KI-TZA-GAL4* was also expressed in the visceral muscles (Supplementary Fig. 11f). We therefore knocked down NPFR in the visceral muscle with *how-GAL4*, a genetic driver active in the visceral muscle. In the adult females of this genotype, TAG amount was reduced, but hypersensitivity to starvation was not observed (Supplementary Fig. 12a, b). Therefore, we conclude that NPFR in the CC has a pivotal role in lipid metabolism coupled with its role in starvation resistance.

NPFR/NPFR signalling controls glucagon-like hormone production. Consistent with the attenuation of lipid catabolism by NPFR and NPFR, *Akhet* mRNA level was significantly upregulated in midgut EEC-specific NPFR knockdown or CC-specific NPFR knockdown (Fig. 5a). Furthermore, AKH protein levels in the CC were significantly reduced in NPFR and NPFR knockdown animals (Fig. 5b, c). Given that these phenotypes resembled the excessive AKH
secretion reported in a previous study\textsuperscript{43}, these results suggest that upregulation of AKH production and secretion induces the metabolic phenotype of loss of NPF or NPFR function animals. To test this hypothesis, we assessed a relationship between NPF–NPFR signalling and AKH–AKH receptor (AKHR) signalling in lipid metabolism. The reduction of TAG and starvation hypersensitivity of NPFR knockdown were rescued by a CC-specific co-suppression of Akh (Akh>NPFR\textsuperscript{RNAi}+Akh\textsuperscript{RNAi}) (Fig. 5d–f). Further, the low TAG level and starvation sensitivity of NPF knockdown was also rescued with Akh\textsuperscript{KO} mutants (Supplementary Fig. 13a, b), suggesting that lipodystrophy of NPF/NPFR-deficient animals is mediated by AKH. Notably, the knockdown of Akh alone (Akh>Akh\textsuperscript{RNAi}) resulted in high starvation resistance and increased TAG abundance compared to Akh>NPFR\textsuperscript{RNAi}+Akh\textsuperscript{RNAi} (Fig. 5d, f), implying that other factor(s) from the CC may contribute to the lipid storage reduction in NPFR knockdown animals. Complementary to these...
results, the fat body-specific RNAi of AkhR in NPF mutant background improved the sensitivity to starvation and reduction of TAG (Fig. 5i–k). Moreover, double mutant of AkhRRKO and NPF (AkhhRKO, NPFΔKI/D) also improved the reduced lipid phenotype of NPF mutants (Fig. 5g). These data indicate that AKH–AKHR signalling is responsible for the metabolic phenotype of animals with loss of NPF or NPFR function.

NPF/NPFR signalling regulates lipase gene expression in the fat body. Upon AKH binding, AKHR evokes a rapid and sustained increase in intracellular CAMP and Ca2+ accumulation, leading to the activation of multiple lipases that catalyse the hydrolysis of both tri- and diacylglycerides upon starvation19,20,44. In the fat body of D. melanogaster, two major lipases, Bmm and Drosophila hormone sensitive lipase (dHSL), homologues of human adipose triglyceride lipase (ATGL), are involved in regulating TAG amount19,44,45. The activities of both Bmm and dHSL are regulated by AKH–AKHR signalling, while their regulatory mechanisms are substantively different19,20,45–47. We observed an increase in Bmm mRNA expression in loss of NPF function animals (Fig. 2f). Consistent with this, NPFR knockdown in the CC also increased Bmm mRNA expression in the abdomen of females (Fig. 5h). Moreover, co-suppression of Akh with NPFR in the CC reverted Bmm mRNA expression to levels similar to that of the control (Fig. 5h). However, knockdown of NPFR in the CC or co-suppression of NPFR and Akh had no significant effect on dHSL mRNA levels (Fig. 5h). Cumulatively, these data suggest that Bmm, not dHSL, is transcriptionally influenced by NPF/NPFR signalling via AKH.

To assess whether Bmm or dHSL is an effector of activated lipolysis in animals with loss of NPF or NPFR function, we suppressed Bmm or dHSL mRNA expression in the fat body cells of NPF-null-mutant background. These genetic manipulations were sufficient to rescue the TAG levels of NPF mutant animals (Fig. 5i–k). In conjunction with the data showing the NPFR-dependent upregulation of Bmm mRNA levels, these results suggest that the activity of Bmm is required for NPF/NPFR-regulated lipid mobilisation in the fat body, while dHSL also participates in the regulation of lipid mobilisation, however, in a manner that is independent of NPFR/NPFR signalling, at least transcriptionally.

The expression of Bmm is reportedly activated by a transcription factor Forkhead box sub-group O (FOXO). FOXO transcriptional activity is tightly associated by its nuclear localisation46. Thus, we examined FOXO localisation in fat body cells. Consistent with the increase in Bmm mRNA expression, FOXO nuclear localisation was induced by TKG–NPFRRNAi or Akh>NPFRRNAi (Fig. 6a, b). In contrast, FOXO nuclear localisation in Akh>NPFRRNAi was restored with knockdown of Akh (Fig. 6b). Moreover, the mRNA level of a FOXO-target gene, 4E-BP was increased in the abdomen of females, while another FOXO-target gene Insulin receptor (Inr) was not affected (Fig. 6c). These results suggest that NPF-mediated Bmm mRNA expression in the fat body may be FOXO-dependent.

NPF/NPFR signalling control insulin secretion and production. Since FOXO nuclear localisation is suppressed by insulin signalling pathway48, the results described above led us to examine the involvement of NPF–NPFR signalling in insulin production and/or secretion. The D. melanogaster genome encodes several insulin-like peptide genes (dilps). In adulthood, DILP2, DILP3, and DILP5 are produced and secreted from IPCs in the brain49,50. We therefore tested whether NPF from midgut EECs affects DILPs production and secretion. DILP3 and DILP5 mRNA levels were significantly reduced in TKG–NPFRRNAi while the level of DILP2 mRNA remained constant (Fig. 6d). Since insulin activity is also regulated at the level of DILP secretion51,52, we assessed accumulation of DILP2, DILP3, and DILP5 in the IPCs with midgut NPF knockdown. NPF knockdown in the midgut EECs increased DILP2, DILP3, and DILP5 protein levels in the IPCs (Fig. 6e), despite the reduced DILP3 and DILP5 mRNA levels, indicating that DILPs accumulate in the IPCs. These results suggest that midgut NPF controls DILP3 and DILP5 mRNA expression, as well as DILPs secretion.

Next, we assessed NPF expression in IPCs. As described above, NPFRKI-T2A.GAL4 and NPFRKI-RA/C.GAL4 are active in many neurons in the brain37. We validated NPF expression in the brain in more details and found that both NPFRKI-T2A.GAL4 and NPFRKI-RA/C.GAL4-driven UAS-GFP are also expressed in the IPCs (Fig. 7a; Supplementary Fig. 14a). This is consistent with a recent RNA-seq analysis showing that NPF is indeed expressed in the IPCs53. We further investigated potential control Dilps mRNA expression by NPF in the IPCs. As expected, NPF knockdown in the IPCs (Dilp2–NPFRRNAi), slightly reduced DILP2, DILP3 and DILP5 mRNA levels, suggesting that midgut NPF controls Dilps mRNA expression by directly stimulating the IPCs (Fig. 7b). Similar to TKG–NPFRRNAi animals, we also confirmed that NPF knockdown in the IPCs (Dilp2–NPFRRNAi) induced an accumulation of DILP2 and DILP3 peptide in the IPCs (Fig. 7c).

To examine whether DILP2 haemolymph levels are impacted in loss of NPF function animals, we quantified the haemolymph level of circulating endogenous DILP2 tagged with artificial
epitopes (DILP2HF) in control and Dilp2>NPFR RNAi animals. We observed a significant decrease in circulating DILP2HF in Dilp2>NPFR RNAi animals (Fig. 7d). These results suggest that NPFR in the IPCs positively regulates DILP secretion to the haemolymph.

Since DILP secretion depends on neuronal activities of IPCs, we next assessed IPC activity using CaLexA, which allows cumulative tracing of neuronal activity in ad libitum fed or starved animals. 24 h starvation significantly attenuated the neuronal activity of IPCs in both control (Dilp2>CaLexA, LacZ RNAi) and NPFR knockdown (Dilp2>CaLexA, NPFR RNAi) animals (Fig. 7e). Meanwhile, following ad libitum feeding, control animals showed robust IPC neuronal activity, whereas knockdown of NPFR caused a slight, but significant, reduction in neuronal activity (Fig. 7e). These results demonstrate that NPFR in the IPCs positively regulates DILP secretion by regulating IPCs neuronal activity.

To assess the levels of insulin signalling within peripheral tissue, we used a pleckstrin-homology domain fused to GFP (tGPH), which is recruited to the plasma membrane when insulin signalling is activated. tGPH signal at the plasma membranes of the fat body was significantly reduced in Dilp2>NPFR RNAi animals (Fig. 7f), confirming that DILP secretion is attenuated by NPFR knockdown in the IPCs. Consistent with reduced
peripheral insulin signalling. NPFR knockdown also reduced phospho-AKT levels (Fig. 7g). Together, these data show that NPFR in the IPCs regulates DILP production and secretion, thereby positively controlling the signalling activity of peripheral insulin.

An examination of the effect of Dilp2>NPFRRNAi on metabolism revealed that NPFR knockdown in the IPCs caused a mild but significant hypersensitivity to starvation (Fig. 8a). Consistently, TAG level and LipidTOX signal intensity were also reduced in the fat body with Dilp2>NPFRRNAi (Fig. 8b, c). Moreover, Dilp2>NPFRRNAi reduced haemolymph glycaemic level, while feeding amount was significantly increased (Fig. 8d, e). Notably, these metabolic phenotypes of Dilp2>NPFRRNAi were similar to those of TKG>NPFRRNAi and Akh>NPFRRNAi. We also confirmed the mRNA expression levels of Bmm, 4E-BP, InR, and pepck1 in the abdomen of Dilp2>NPFRRNAi animals. Despite the reduction of TAG level, Dilp2>NPFRRNAi failed to increase Bmm mRNA expression (Fig. 8f), suggesting that the lean phenotype of Dilp2>NPFRRNAi animal is not due to an increase in Bmm mRNA expression. However, expression of other FOXO-target genes, 4E-BP and pepck1 were upregulated with Dilp2>NPFRRNAi (Fig. 8f). Consistent with this, Dilp2>NPFRRNAi induced FOXO nuclear localisation (Fig. 8g). These data suggest that NPFR in the IPCs regulates DILPs expression and secretion, followed by nuclear translocation of FOXO in the fat body to alter some FOXO-target genes.

Since IPCs produce multiple neuropeptides, including DILPs and Drosulfakinin (Dsk), we next sought to identify which neuropeptide in the IPCs is responsible for NPF/NPFR-mediated regulation of lipid storage in the fat body. Results show that knockdown of dilp3 (Dilp2>dilp3RNAi) resulted in significant reduction of TAG abundance, while the others had no significant effect (Supplementary Fig. 14b). Our data is consistent with a previous study demonstrating that dilp3 mutant animals exhibit reduced TAG levels.58 Additionally, although Dsk is known to regulate feeding behaviour in adults,59,60,61 disk expression was not affected by NPFR knockdown in IPCs (Supplementary Fig. 14c).

Our data indicates that NPFR knockdown in the CC resulted in a stronger hypersensitive phenotype to starvation compared to that detected following NPFR knockdown in the IPCs (Figs. 4b and 8a). To explain this discrepancy, we hypothesised that NPFR knockdown in the CC might lead to a significant alteration in DILP production within IPCs. To test this hypothesis, we quantified dilps mRNA levels in Akh>NPFRRNAi and found that NPFR knockdown in the CC decreased dilp3 and dilp5 mRNA levels (Supplementary Fig. 14d). In contrast, NPFR knockdown in the IPCs (Dilp2>NPFRRNAi) did not influence Akh mRNA expression (Supplementary Fig. 14e). Together, these data suggest that NPFR knockdown in the CC results in not only enhanced AKH production, but also suppression of DILP production.

NPFR neurons might not play a crucial role in AKH and DILPs production. Although NPFR knockdown in the brain did not exhibit significant effects in metabolism Supplementary Fig. 3), it remains possible that brain NPFR participates in the regulation of AKH and DILPs. However, three lines of evidence as follows are likely to negate this possibility. First, we confirmed AKH and DILP mRNA and protein levels following brain-specific NPFR knockdown (Jbp>NPFRRNAi). Consistent with the metabolic phenotype, NPFR knockdown in the brain did not impact mRNA or protein levels of either AKH or DILPs (Supplementary Fig. 15a–d). Second, postsynaptic trans-Tango signals driven by NPFR-GAL4 were not detected in CC cells or neurons in the PI region (Supplementary Fig. 15e, f). Third, 24 h starvation did not affect NPFR protein levels in the brain (Supplementary Fig. 15g). Taken together, these data suggest that brain NPFR neurons do not affect AKH and DILPs levels.

Taken together, our findings suggest that midgut-derived, but not neuronal NPFR, binds NPFR in the CC and IPCs, suppressing AKH production and enhancing DILP secretion, respectively. As a result, midgut NPFR employs downstream FOXO-target genes to regulate carbohydrate and lipid metabolism through glucagon and insulin, respectively (Fig. 9).

Discussion

Here, we demonstrated that midgut-derived NPFR acts as a sensor of dietary sugar and plays an important role in the regulation of adult carbohydrate and lipid homeostasis in D. melanogaster. Importantly, we showed that midgut NPFR is received by the CC and IPCs, to coordinate their expression of glucagon-like and insulin-like hormones, respectively. Previous studies reported that midgut EEC-derived Activin-β and Bursa are important for carbohydrate and lipid metabolism in D. melanogaster, although these endocrine hormones have not been shown to directly act on the CC or IPCs. Activin-β acts on the fat body to regulate Akh expression in the larval fat body.9 Bursa is secreted in response to dietary sugars, but it is received by un-characterised neurons that express its receptor, Lgr2, leading to suppression of Akh expression.11 We therefore propose that NPFR is the first incretin-like hormone in invertebrates, and its production and secretion are stimulated by dietary nutrients similar to incretins (Fig. 9).

Nutrient-dependent NPFR regulation. Due to technical limitations, we were unable to quantify the haemolymph titre of NPF
and, therefore, did not examine whether midgut NPF contributes to the NPF haemolymph level. Nevertheless, our data strongly suggests that dietary sugar controls not only midgut NPF expression but also NPF secretion from the midgut. In this scenario, NPF secretion is attenuated in starved conditions, while the attenuation is restored by sugar re-feeding.

We found that Sut1, a homologue of mammalian SLC2, is a regulator of sugar-dependent NPF production in EECs. Considering that Sut1 is localised on plasma membranes and contributes to the elevation of intracellular glucose levels, it is likely that Sut1 transports glucose into the cell. Similar to Sut1, Glut1, another mammalian SLC2 homologue, acts as a glucose
transporter to elevate intracellular glucose levels in *D. melanogaster*.

Additionally, Glut1 has been shown to be essential for nutrient-dependent production and secretion of Bursa from EECs. However, our data suggest that Glut1 does not affect NPF production (Supplementary Fig. 6b, c). Importantly, NPF and Bursa are produced in different regions of the midgut, namely in the anterior and posterior midgut, respectively. Therefore, different subtypes of EECs appear to have different glucose sensing systems. Thus, characterising how the differences in EEC sugar sensing systems affect the metabolic robustness of individuals may clarify the significance of the more than 30 SLC2 genes in *D. melanogaster*.

In mammalian EECs, especially GLP-1 *L* cells, dietary glucose is transported by glucose transporter 1 (SGLT-1) to stimulate GLP-1 secretion. In addition to sugars, fatty acids and amino acids also stimulate GLP-1 secretion from peripheral tissues in response to different nutrient types. However, these systems remain largely undefined. For example, in this study, we were unable to determine the underlying mechanism by which midgut NPF mRNA and protein levels are significantly upregulated by peptone feeding (Fig. 3a–c). Future studies should offer a more comprehensive investigation of nutrient-dependent enteroendocrine hormone regulation at the molecular level.

**Metabolic function of NPF/NPFR.** Our data demonstrated that midgut-derived NPF-controlled organismal carbohydrate and lipid metabolism through AKH and insulin signalling. Animals with loss of NPF function were in a catabolic state, reminiscent of starved animals, as judged by the following observation from our RNA-seq and metabolome analyses: (1) upregulation of glycolysis, TCA cycle, mitochondrial respiratory chain complex genes, and starvation-induced genes, (2) increase of several TCA cycle metabolites, (3) lipodystrophy and hypoglycaemia along with hyperphagia, and (4) nuclear localisation of FOXO and the induction of starvation-induced FOXO-target genes. These phenotypes are likely due to upregulation of AKH/AKHR signalling and attenuation of insulin signalling in the peripheral tissues. Taken together, our results suggest that NPFPR in the CC and IPCs has pivotal role in the regulation of organismal TAG and glycaemic levels.

In the adult fat body, TAG level is controlled by two lipases, dHSL and Bmm in a redundant manner. Given that knockdown of either *Bmm* or *dHSL* in the fat body restored TAG reduction in NPF-null mutant animals to the control level, we hypothesise that both lipases cooperatively control lipid breakdown in the NPF–NPFR axis. These data support our idea that glucose and stored lipids are mobilised to the TCA cycle to generate energy in animals with loss of NPF function.

**Cross talk with other signalling.** One of our striking findings is that NPF produced by midgut EECs directly stimulated the CC and IPCs, indicating the presence of both a midgut–CC–fat body axis and a midgut–IPCs–fat body axis in *D. melanogaster*. Although many studies have demonstrated that neuronal signalling in the brain and humoral factors from peripheral tissues stimulate either CC or IPCs, factors that stimulate both the CC and IPCs are less defined. As the one and only example of such factors, it was recently reported that sNPF from two pairs of neurons directly innervating both the CC and IPCs controls metabolic function. In the process of peer review of this paper, a preprint manuscript reported that Allatostatin C (AstC) from EECs stimulates AKH secretion and production. Although NPFR is coupled with Gq and G12 subunits in heterologous expression systems, it remains unclear which trimeric G protein is coupled in the CC and IPCs to transmit NPF signals. Further studies are needed to investigate the integration of neuron-derived NPF and midgut-derived NPF to adequately stimulate the CC and IPCs for the regulation of AKH and DILP2 secretion, respectively.

Many studies have identified and characterised factors that regulate DILP production and secretion. Dietary nutrients, neuropeptide signalling, and adipocyte-derived factors regulate IPCs to coordinate systemic growth and energy-related events. In contrast, much less is known about factors regulating AKH production and secretion. The process of peer review of this paper, a preprint manuscript reported that Allatostatin C (AstC) from EECs regulates AKH production or secretion. Beside the neuropeptide signalling described above (NPF, sNPF, Bursa, AstC), other signalling components also
Fig. 6 Midgut-derived NPF controls DILPs level. a, b FOXO (white) immunostaining of the fat body in adult flies of each genotype. Scale bar, 20 µm. Note that FOXO nuclear localisation was induced in TKg>NPFRNAi and Akh>NPFRRNAi. c RT-qPCR analysis of FOXO-target gene mRNA levels in the abdomens dissected from each genotype. The number of samples assessed (n) is indicated in the graphs. d RT-qPCR analysis of Dilps mRNA level following TKg-GAL4 mediated knockdown of NPF. The number of samples assessed (n) is indicated in the graphs. e DILP2, 3, and 5 (white) immunostaining and quantification in the brain of adult flies of TKg-GAL4-mediated NPF RNAi animals. Scale bar, 20 µm. The number of samples assessed (n) is indicated in the graphs. For RNAi experiments, LacZ knockdown (TKg>LacZRNAi and Akh>LacZRNAi) was used as negative control. For all bar graphs, mean and SEM with all data points are shown. For dot blots, the horizontal lines indicate median quartile. Statistics: two-tailed Student’s t-test (c–e), Wilcoxon rank sum test (f). *p < 0.05, **p < 0.01, ***p < 0.001; NS, non-significant (p > 0.05). p-values: c (left), p = 0.9604 (InR), p = 0.0437 (4E-BP); c (right), p = 0.0023 (4E-BP); d p = 0.5609 (Dilp2), p = 0.0003 (Dilp3), p = 0.0036 (Dilp5); e p < 0.0001 (DILP2), p < 0.0001 (DILP3), p < 0.0001 (DILP5).
function in the CC, such as Allatostatin A-receptor 2, a water sensor encoded by *pickpocket 28*, and a H$_2$O$_2$- and ultraviolet light-sensitive isoform of *TrpA1* 70–72. Moreover, AKH secretion is regulated by a neurotransmitter secreted from Lgr2$^+$ neurons, although the neurotransmitter has not been characterised 11. Studies that detail the signalling cross talk between the factors that control AKH actions are required, to further elucidate how CC cells sense multiple nutritional and physiological cues to control carbohydrate and lipid metabolism. In fact, CC reportedly produces another peptide, Limostatin (Lst), that stimulates IPCs to suppress production of DILPs, including decerin 73. Therefore, Lst may participate in the downregulation of *dilp3* and *dilp5*.
expression following NPFR knockdown in the CCs (Supplementary Fig. 13b).

Midgut NPF vs. brain NPF. Our previous study17, as well as the results of the current study, confirm the significant biological function of midgut NPF in D. melanogaster. Meanwhile, many previous studies have reported that brain NPF has versatile roles in the feeding and social behaviour of insects23,74,75. Therefore, the metabolic phenotypes of NPF genetic mutants may reflect the diverse functions of brain NPF, although the data from the current study does not support this postulate (Supplementary Figs. 3 and 15). In particular, brain-specific NPF knockdown does not phenocopy NPF mutation or midgut-specific NPF knockdown. These data imply distinct physiological functions between midgut and brain NPF.

Another key finding in this study is the anorexigenic function of midgut-derived NPF, which is in contrast to the orexigenic function of brain NPF22,23. Interestingly, agonists of NPY-like receptor 7 disrupt host-seeking behaviour and biting in the yellow fever mosquito, Aedes aegypti76. Moreover, disruption of NPF/NPFR signalling results in abnormal feeding behaviour and reduced growth in several insects75,77,78. Since other insects also produce NPF from the brain and gut7,79, it is important to validate the source of circulating NPF and discriminate the function of brain NPF from that of gut-derived NPF.

Commonality with mammalian system. A growing number of evidences have demonstrated that, similar to mammals, the D. melanogaster intestine plays versatile roles in systemic physiology80. Although it is simpler than the mammalian gastrointestinal tract, the D. melanogaster intestinal epithelium is functionally regionalised and displays similarity both at the cellular and molecular levels30,31,81. In mammals, GIP from K-cells (largely in the upper small intestine) and GLP-1 from L cells (predominantly in the distal small and large intestine) are considered incretins, which induce insulin secretion by stimulating β cells in the pancreatic islets5,6,61. Among incretins, GLP-1 suppresses glucose-dependent glucagon secretion via its receptor GLP-1R in α-cells of the pancreas82. Although D. melanogaster endocrine system is different from that of mammals, we propose that midgut-derived NPF have similar role in insulin/glucagon regulation as mammalian GLP-1. Treatment with GLP-1 agonists reduces food intake and hunger, promoting fullness and satiety with the ultimate result of weight loss in patients with obesity or type 2 diabetes13,83,84. Similar to this, gut-derived NPF regulated satiety in D. melanogaster in our study. However, loss of GLP-1/GLP-1R signalling has a non-significant effect on weight and fat mass in regular food-fed mice, whereas loss of NPF/NPFR resulted in lean phenotype in regular food. Thus, although there are substantial similarities in the physiological function of mammalian incretins and D. melanogaster NPF, their effects on metabolism are divergent in some aspects. Considering that GLP-1 acts on many organs and tissues, including the nervous system, heart, stomach, gut, and pancreas5, and that NPF is expressed in the nervous system, visceral muscles, and EECs of the gut31, differences in the inter-organ communication systems of mammals and D. melanogaster in the GLP-1 and NPF may produce differences in the physiological effects of these enteroendocrine hormones. To further understand midgut-derived NPF-dependent inter-organ communication system, it would be intriguing to investigate the role of NPFR in potential target tissues, such as visceral muscles of the gut and NPFR+ neurons, other than the IPCs. The ease of tissue-specific genetic manipulations, together with the evolutionary conservation of central signalling pathways regulating metabolism and energy homeostasis, makes D. melanogaster a powerful model system to unravel the role of incretin-like enteroendocrine hormones in systemic organismal metabolism.

**Methods**

**Fly stock and husbandry.** Flies were raised on a fly food (5.5 g agar, 100 g glucose, 40 g dry yeast, 90 g cornflour, 3 ml propionic acid, and 3.5 ml 10% butyl hydroxybenzoate (in 70% ethanol) per litre) in a 12/12 h light/dark condition at 25 °C for 6 days before experiments. Virgin flies were used for all fly experiments.

The following transgenic and mutant stocks were used: NPF^ald^ and NPF^ral^ (BDSC#48717), NPF^ald^/+; NPF^ald^;NPFR^ki-1^ [Kyoto stock center (DRCR) #150266], NPF^ral^/NPFR^ki-1^ (Bloomingston stock center [BDSC] #3315), Akh^P7^ (from Dr. Yi Rao, Peking University School of Life Sciences, China)37, Akh^N7^, Akh^P3^ (from Ronald P. Kühnlein, Max-Planck-Institut für Biophysikalische Chemie, Germany)39, int1KO (Int1 mutant) flies (this study), tub-GAL80ts;Gal4-UAS-LacZ RNAi (thou-GAL80ts;Gal4-UAS-LacZ RNAi flies from Masayuki Miura, the University of Tokyo, Japan), nSyb-GAL4 (BDSC#51941), Akh-GAL4 (BDSC#25683), dilp2-GAL4 (BDSC#37516), how-GAL4 (BDSC#1767), NPF^ki-1^;TUB-GAL4, NPF^ral-2^;TUB-GAL4, NPF^ki-1^;TUB-GAL4, NPF^ral-2^;TUB-GAL4 (this study), tub-GAL80ts (BDSC#7019) UAS-NPF, UAS-NPF (a gift from Ping Shen, University of Georgia, USA), UAS-mCD8::GFP (BDSC#32186), UAS-FLII2Pglu-700G6d (a gift from...
Fig. 8 NPFR in the insulin-producing cells regulates carbohydrate/lipid metabolism. a Survival during starvation in flies of each genotype. The number of animals assessed (n) is indicated in the graphs. b Relative whole-body TAG levels of each genotype. The number of animals assessed (n) is indicated in the graphs. c LipidTOX (red) and DAPI (blue) staining of dissected fat body tissue from indicated genotypes. Scale bar, 20 µm. d Relative glycaemic levels in control and Dilp2>NPFRRNAi. The number of samples assessed (n) is indicated in the graphs. e Feeding amount measurement of each genotype with CAFÉ assay. n = 4 samples, each point contained four adult female flies. f RT-qPCR analysis of FOXO-target gene mRNA levels in the fat body dissected from each genotype. The number of samples assessed (n) is indicated in the graphs. g FOXO (white) immunostaining of the fat body in adult flies of each genotype. Scale bar, 50 µm. For RNAi experiments, LacZ knockdown (Dilp2>LacZRNAi) was used as negative control. For all bar graphs, mean and SEM with all data points are shown. For dot plots, the three horizontal lines indicate lower, median, and upper quartiles. Statistics: Log rank test (a), two-tailed Student’s t-test (b, d-f). *p < 0.05, **p < 0.01, ***p < 0.001; NS, non-significant (p > 0.05). p-values: a, p = 0.0100; b, p < 0.0001; d, p = 0.0417; e, p = 0.0269; f, p = 0.6468 (Bmm), p = 0.0146 (Thor), p = 0.1098 (InR), p = 0.0024 (pepck1).
Fig. 9 Midgut-derived NPF regulates AKH and DILPs level in response to dietary sugar. a A working model illustrating dual pathway coordination of FOXO and FOXO-target genes. (i) The loss of NPF/NPFR signalling in the CC—enhanced AKH/ AKHR signalling induces FOXO nuclear localisation and carbohydrate/lipid metabolism—and (ii) the loss of NPF/NPFR signalling in the IPCs—attenuation of insulin signalling induces FOXO nuclear localisation and enhances carbohydrate/lipid metabolism. The balance of AKHR/FOXO and insulin signalling is coordinated by gut-derived NPF that responds dietary sugars. b (left) In D. melanogaster, enterocrine cells respond to the dietary sugars by secreting a neuropeptide, NPF, which signals via its neuronal receptor NKFR. NPF/NPFR signalling regulates energy consumption through dual neuronal relay which are restricted of glucagon-like, AKH production, and enhancement of insulin-like peptides (DILPs). Subsequent modulation of AKHR and insulin signalling within the fat body/ adipose tissue maintains lipid/carbohydrate catabolism; thus, impaired NPF/NPFR signalling leads to depletion of energy stores. (right) The EEC-IPC and the EEC-CC axes in D. melanogaster are similar to the gut EECs-pancreas axis in mammals. Mammalian enterocrine peptide hormone, GLP-1 also controls insulin and glucagon levels in response to dietary nutrients.

Chika Miyamoto and Hubert Amrein35, UAS-trans-Tango (BDSC#77480), UAS-sut1::mVenus (this study), CaLexA (BDSC#66542), and tGPH (BDSC#8184). RNAi constructs targeting UAS-NPFRNAiKK (VDRC#108772), UAS-NPFRNAiTRiP (BDSC#27237), UAS-NPFRRNAiKK (VDRC#107633), UAS-NPFRRNAiTRiP (BDSC#29539), UAS-sut1RNAi (VDRC#104983), UAS-sut1RNAiTRiP (BDSC#65964), UAS-Glut1RNAi (VDRC#103863), UAS-AkhRNAi (VDRC#105063), UAS-GrxRNAi (VDRC#38777), UAS-dHSERNAi (VDRC#109336), UAS-dILpRNAi (VDRC#101258), UAS-dILpRNAi (VDRC#106512), UAS-dILpRNAi (VDRC#105004), and UAs-dILpRNAi (VDRC#106592).

Except in Fig. 1a, TkG-GAL4; UAS-NPFRNAi (VDRC#108772) is simply referred to as "TkG-NPFRNAi". Except in Fig. 4b, Akh-GAL4; UAS-NPFRNAi (BDSC#29539) is simply referred to as "Akhs-NPFRNAi". For adult-specific knockdown of NPF, TkG-GAL4, tub-GAL80ts (TkG) >LucRNAi and TkG >NPFRNAi flies were raised at 20 °C during the larval, and pupal periods. After eclosion, adult flies were housed at 29 °C for 6 days before experimental analysis.

Generation of sut1KO mutant. The mutant alleles sut1KO (Fig. 3) was created in a white (w) background using CRISPR/Cas9 as previously described36. The oligo DNA sequences are represented in Supplementary Data 6. The breakpoint of sut1KO is described in Supplementary Fig. 8e.

Expression of the UAS-sut1:mVenus strain and UAS-sut1 plasmid. To over-express mVenus-tagged sut1, the sut1 coding sequence region (CDS) was amplified by PCR with adult w118 whole-body cDNA using the primers sut1cDNA F and sut1cDNA R2 (Supplementary Data 6). The PCR products were digested with EcoRI and NheI, and subsequently cloned into the EcoRI-Nhel-digested pWALIUM10-moe vector.

Generation of sut1KO-T2A-GAL4 strain. We utilised a method previously described13 to generate a knock-in strain by inserting the T2A-GAL4 cassette into sut1 locus. Approximately 500 bp sequences flanking the stop codon of sut1 were PCR amplified from the genomic DNA of the w118 strain. These homology arms were designed so that T2A-GAL4 was translated as an in-frame fusion with the target gene. The reporter cassette excised from pPO-REF34, as well as the left and right homology arms were assembled and cloned into Smal-digested pBlueScripII SK-(-) in a single enzymatic reaction using the In-Fusion Cloning Kit (TAKARA). gRNA vectors were constructed in pDCC690. We selected a 20 bp gRNA target sequence (Supplementary Data 6) that encompasses the stop codon of the target gene. In addition, silent mutations were introduced into the homology arm of the donor vector to avoid repetitive cleavage after integration. To integrate a reporter cassette into the desired location in the genome, a mixture of a donor vector (150 ng/mg) and a gRNA (150 ng/mg) vector was injected into yw; eyFLP18200 fertilised eggs. After crossing with a balancer strain, transformants in the F1 progeny were selected by eye-specific RFP expression from the 3 × P3-RFP marker gene in adults. The primers used in the generation of sut1KO-T2A-GAL4 are represented in Supplementary Data 6.

Antibody preparation. An antibody against NPF protein was raised in guinea pigs. A KLH-conjugated synthetic peptide (NH2-SNRRPPKDNVNTMADAYKFLQDLDTYYGDRARVRF-CONH2) corresponding to the amidated mature NPF amino acid residues (GenBank accession number NP_536741) was used for immunisation. A KLH-conjugated synthetic peptide (NH2-SNRRPPKDNVNTMA-DAYKFLQDLDTYYGDRARVRF-CONH2) corresponding to the amidated mature NPF amino acid residues (GenBank accession number NP_536741) were used for immunisation.

Immunohistochemistry and fluorescence quantification. Midguts and other fly tissues were dissected in 1× PBS and fixed in 4% parafomaldehyde in PBS for 30–60 min at room temperature (RT). Fixed samples were washed three times in PBS supplemented with 0.1% Triton X-100 (0.1% PBT). The samples were blocked...
in blocking solution (PBS with 0.1% Triton X-100 and 2% bovine serum albumin (BSA)) for 1 h at RT and then incubated with a primary antibody in blocking solution for 4°C overnight. Primary antibodies in this study were chicken anti-GFP (1:2000, Abcam, #ab13970), rabbit anti-RFP (1:2000, Medical and Biological Laboratories, #PM005), mouse anti-Propero (1:50; Developmental Studies Hybridoma Bank [DSHB]), guinea pig anti-NF (1:2000; this study), rabbit anti-Tk (1:2000; a gift from Jan Veenstra), rabbit anti-Bursa (1:1000, a gift from Benjam-in Hurwitz), rabbit anti-Insulin (1:1000, a gift from Kweon Yo), AKH (1:600, a gift from Jae H. Park), rabbit anti-FOXO (1:1000, a gift from Marc Tatar), guinea pig anti-DILP2 (1:2000, a gift from Takashi Nishimura), rabbit anti-DILP3 (1:2000, a gift from Jan Veenstra), and rabbit anti-DILP5 (1:1000, a gift from Dick R. Nües). After washing, fluorescence (Alexa Fluor 488, 546, 555, or 633)-conjugated secondary antibodies (Thermo Fisher Scientific) were used at a 1:200 dilution, and the samples were incubated for 2 h at RT in blocking solution. After another washing step, all samples were mounted in FluorSave reagent (Merck Millipore).

Midguts samples were dehydrated in a series of ethanol washes ranging from 10% to 90% on ice after fixation in 4% paraformaldehyde. Samples were kept in 90% ethanol for 2 h at −20°C followed by serial re-hydration and subjected to the staining protocol described above.

Fat bodies were stained with LipidTOX (Thermo Fisher Scientific; 1:1000 in 0.1% PBT) for 2 h at RT after fixation in 4% paraformaldehyde.

Samples were visualised using a Zeiss LSM 700 confocal microscope or Zeiss Axioplan 2. Images were processed using Fiji. Fluorescence intensity in confocal sections was measured via Fiji. We performed the sum-intensity 3D projections to measure total fluorescence intensity across the object of interest (Gut or Brain). For NPF and Bursa quantification, 5–6 cells were examined for each midgut.

**Imaging of glucose sensor**
Ex vivo glucose sensor experiments were performed on dissected midguts. Adult midguts expressing UAS-FLII12Pglu-700c in UAS-FLII12Pglu-700c and cultured medium to 25 mM. Fluorescent images were acquired at ×40 objective (1.25× NA) with filter sets: excitation 405 nm, emission 470 nm (CFP channel); excitation 405 nm, emission 530 nm (FRET channel). For calculation of FRET intensity, the FRET ratio (YFP/CFP) was calculated. The primers used to measure transcript levels are represented in Supplementary Data 6.

**Measurement of circulating DILP2HF level**
The abundance of DILP2 tagged with artificial epitopes (DILP2HF) in haemolymph and whole bodies was measured using a previously described method52,54. Briefly, eight-well strips (F8 MaxiSorp Nunc-ImmuNo modules, Thermo Fisher Scientific, 486667) were incubated at 4°C overnight with 5 µg/mL anti-FLAG (Sigma-Aldrich, F1804) in 200 mM NaHCO3 buffer. The eight-well strips were then washed with 0.1% PBT twice and blocked with 4% non-fat skim milk in 0.1% PBT for 2 h at RT. The strips were washed again with 0.1% PBT three times, after which 50 µL of PBS with 0.2% Tween 20 (PBST), containing 25 ng/mL mouse anti-HA conjugated with peroxidase (Roche, 11203819001) and 4% non-fat skim milk, was added to each well. In parallel, ten ad libitum fed 6-day-old flies' abdomens were dissected, submerged in 50 µL of PBST, and gently vortexed for 30 min at RT. After centrifugation of the tubes at 3000 × g for 30 s, 50 µL of supernatants were transferred in the prepared eight-well strips (for detection of circulating DILP2HF in haemolymph). After adding 500 µL of assay buffer (PBS with 0.1% Triton X-100 and 4% BSA) to each tube, containing the remaining flies, the flies were grinded using a pestle and centrifuged at 17,500 × g for 1 min at 4°C. Next, 10 µL of the supernatants were prepared in eight-well strips (for detection of whole-body DILP2HF content). To generate standards for the analysis of circulating DILP2HF levels, a series (0–166 pM) of the synthetic HA:spacer=FLAG peptide standard (NH2-DADKGQGGSYPPVDR-CONH2) was prepared. 50 pM of standards were transferred into the prepared eight-well strips. Meanwhile, to generate standards for the analysis of whole-body DILP2HF levels, a series (0–829 pM) of the synthetic HA:spacer=FLAG peptide standard was prepared, from which 10 µL of each standard were transferred into the prepared eight-well strips. All mixtures in the eight-well strips were incubated overnight at 4°C and subsequently washed with 0.1% PBT six times. Next, 100 µL of One-step Ultra TMB ELISA substrate (Thermo Fisher Scientific, 34028) was added to each well and incubated for 15 min at RT; 100 µL of 2 M sulfuric acid was then added to stop the reaction, and absorbance at 450 nm was detected using a plate reader Multikatn GO (Thermo Fisher Scientific). The secreted DILP2HF levels were estimated using haemolymph DILP2HF abundance to the whole-body DILP2HF amount. The plates, peptide standard, UAS-DILP2HF, anti-FLAG, anti-HA antibody, substrate, and detailed protocol were all generously provided by Seung Kim (Stanford University, USA).

**Western blotting analysis**
To quantify the activity of the insulin signalling pathway, the level of Akt phosphorylation (pAkt) was determined by western blotting. For each sample, five adults were homogenised in 150 µL of RIPA buffer with cComplete protease inhibitor cocktail (Roche) and phosphatase inhibitors (Roche). After centrifugation at 14,000 × g for 5 min, 75 µL of each supernatant was mixed with 75 µL of 2× Laemmli’s loading buffer, and subsequently boiled for 5 min. Next, 7.5 µL of each sample was electrophoresed through a precast 10% polyacrylamide gel (COSY BIO). The proteins were transferred to a PVDF membrane (Merck Millipore), which was blocked with 5% BSA in PBS containing 0.1% Tween-20 (0.1% PBST) and incubated with rabbit anti-pAkt antibody (Cell
each genotype used for Fig. 2d, and Supplementary Fig. 4 is available from DNA genised in 160 μL of 80% methanol containing 10 μM of internal standards (methionine sulfoxide and 2-morpholinoethanesulfonic acid) and were centrifuged (20,000 × g, 5 min) at 4 °C. Supernatants were de-proteinsed with 75 μL acetonitrile, and filtered using 10 kDa Centrifugal Filtration Device (Pall Corporation, OD003C35). After filtration, the solvents were completely evaporated for metabolite analysis. The protein contained in the middle layer was collected from 10 females for each sample. Four samples of each genotype were selected and 115 μL of 100% methanol containing 20 μM of internal standards was added to the haemolymph samples. The protein fraction contained in the haemolymph samples was removed by mixing with chloroform and centrifugation (20,000 × g, 5 min at 4 °C). The supernatant (200 μL) was collected, de-proteinsed by adding 100 μL of acetonitrile, and filtered using 10 kDa Centrifugal Filtration Device (Pall Corporation, OD003C35). The solvent was completely evaporated for metabolite analysis. The protein contained in the middle layer was purified by gently mixing with 1 mL of acetone and centrifugation (20,000 × g, 5 min) at 4 °C. This process was repeated twice. After removing acetone, the protein pellet was dried at RT and resolubilised in 50 μL of 0.1 N NaOH by heating for 5 min at 95 °C. The protein amount was quantified by BCA reagent mix (Thermo Fisher Scientific, 23228 and 23224) for normalisation. The evaporated metabolite samples were resolubilised in Ultrapure water (Invitrogen, 10977-023) and injected to LC-MS/MS with PFPP column (Discovery HS F5 (2.1 mm × 150 mm, 3 μm); Sigma–Aldrich) in the column oven at 40 °C. Gradient from solvent A (0.1% formic acid, Water) to solvent B (0.1% formic acid, acetonitrile) were performed during 20 min of analysis. MRM methods for metabolite quantification were optimised using the software (LabSolutions, Shimadzu). The amount of whole-body metabolites was normalised by 2-morpholinoethanesulfonic acid and the body weight, while haemolymph metabolites were normalised by 2-morpholinoethanesulfonic acid and the protein amount.

Statistics and reproducibility. All experiments were performed independently at least twice. All immunohistochemical experiments were repeated at least twice with similar results. In each experiment, we analysed three or more specimens. The experiments were performed in duplicate, and the investigators were not blinded. All statistical analyses were carried out using the “R” software environment. The p-value is provided in comparison with the control and indicated as * for p ≤ 0.05, ** for p ≤ 0.01, *** for p ≤ 0.001, and “NS” for non-significant (p > 0.05). Sample size was determined based on the significance obtained from previous studies with similar experimental setups. Comparable sample sizes were used in each experiment.

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability
The raw RNA-seq data generated in this study have been deposited in the DNA Data Bank of Japan Sequence Read Archive database under accession code DRA010538. The raw data generated in this study are provided in the Supplementary Information/Source Data file. Source data are provided with this paper.
30. Buchon, N. et al. Morphological and molecular characterization of the adult midgut compartmentalization in Drosophila. Cell Rep. 3, 1725–1738 (2012).
31. Guo, X. et al. The cellular diversity and transcription factor code of Drosophila enteroneuroendocrine cells. Cell Rep. 29, 4172–4185.e5 (2019).
32. Kina, H., Yoshitani, T., Hanyu-Nakamura, K. & Nakamura, A. Rapid and efficient generation of GFP- knockin in Drosophila by the CRISPR-Cas9 system. Dev. Genet. Diff. 61, 265–275 (2019).
33. Kondo, S. et al. Neurochemical organization of the Drosophila brain visualized by endogenously tagged neurotransmitter receptors. Cell Rep. 30, 284–297.e5 (2020).
34. Takegawa, H., Chaudhuri, B. & Frommer, W. B. GLUT1 and GLUT9 as major contributors to glucose influx in HepG2 cells identified by a high sensitivity intramolecular FRET glucose sensor. Biochim. Biophys. Acta-Membr. 1778, 1091–1099 (2008).
35. Volkenhoff, A., Hirrlinger, J., Kappel, J. M., Klämbt, C. & Schirmeier, S. Live imaging using a FRET glucose sensor reveals glucose delivery to all cell types in the Drosophila brain. J. Insect Physiol. 106, 55–64 (2018).
36. Diao, F. & White, B. H. A novel approach for directing transgene expression in Drosophila: T2A-Gal4 in-frame fusion. Sci. Rep. 6, 6084 (2016).
37. Heier, C. et al. Hormone-sensitive lipase couples intergenerational sterol metabolism in Drosophila. Nat. Biotechnol. 18, 227–238 (2003).
38. Barthel, A., Schmoll, D. & Unterman, T. G. FoxO proteins in insulin action. J. Biol. Chem. 275, 1093–1103 (2000).
39. Chao, S., Lim, D.-S. & Chung, J. Feeding and fasting signals converge on the LKB1–SIK3 pathway to regulate lipid metabolism in Drosophila. PLoS Genet. 11, e1005263 (2015).
40. Wang, B. et al. A hormone-dependent module regulates energy balance. Cell 145, 596–606 (2011).
41. Heier, C. et al. Insulin-sensitive lipase couples intergenerational sterol metabolism to reproductive success. Elife 10, 1–32 (2021).
42. Rulifson, E. J., Kim, S. K. & Nusse, R. Ablation of insulin-producing neurons in Drosophila by the CRISPR-Cas9 system. bioRxiv, 4523 (2019).
43. Fadda, M. et al. Regulation of feeding and metabolism by neuropeptide F receptor and short neuropeptide F receptor in vertebrates. Front. Endocrinol. 10, 64 (2019).
44. Dqing, X. et al. Activation of Bombyx neuropeptide G protein-coupled receptor A4 via a Gai-dependent signaling pathway by direct interaction with neuropeptide F from silkworm, Bombyx mori. Insect Biochem. Mol. Biol. 45, 328 (2014).
45. Tomlinson, E. L. & Arrese, E. L. Adipokinetic hormone-induced lipolysis and lipid droplets. Arch. Insect Biochem. Physiol. 61, 65–79 (2012).
46. Masuyama, K., Zhang, Y., Rao, Y. & Wang, J. W. Mapping neural circuits with Braco, J. T., Saunders, C. J., Nelson, J. M. & Johnson, E. C. Modulation of midgut compartmentalization within the Drosophila midgut. Elife 10, e08866 (2013).
47. Schirra, J. et al. Gastric emptying and release of incretin hormones after glucose ingestion in humans. J. Clin. Investig. 97, 102–103 (1996).
48. Britton, J. S., Lockwood, W. K., Li, L., Cohen, S. M. & Edgar, B. A. Drosophila’s insulin/Pi3-kinase pathway coordinates cellular metabolism with endocrine signaling. Front. Cell. Dev. Endo. 3, 109 (2012).
49. Diao, F. & White, B. H. A. Mapping neural circuits with Braco, J. T., Saunders, C. J., Nelson, J. M. & Johnson, E. C. Modulation of midgut compartmentalization within the Drosophila midgut. Elife 10, e08866 (2013).
50. Söderberg, J. A. E., Carlsson, M. A. & Nässel, D. R. Insulin-producing cells in Drosophila brain express satiety-inducing choleystatin-like peptide, drosokainin. PLoS Genet. 6, e1000793 (2010).
51. Mariani, A. & Spalding, A. C. Physiological and stem cell compartmentalization within the Drosophila midgut. Midgut 89, 2011–2019 (2013).
52. Miralle, C. et al. Activation of sodium-glucose cotransporter 1 ameliorates hyperglycemia by mediating incretin secretion in mice. Am. J. Physiol. - Endocrinol. Metab. 297, E1358–E1365 (2009).
53. Tolhurst, G. et al. Short-chain fatty acids stimulate glucagon-like peptide-1 secretion via the G-protein-coupled receptor FFAR2. Diabetes 61, 364–371 (2012).
54. Martin, A. M., Sun, E. W. & Keating, D. J. Mechanisms controlling hormone secretion in human gut and its relevance to metabolism. J. Endocrinol. 244, R1–R15 (2020).
55. Ahmad, M., He, L. & Perrimon, N. Regulation of insulin and adipokine hormone/glucagon production in flies. WIREs Dev. Biol. 9, e300 (2020).
56. Géminard, C., Rulifson, E. J. & Léopold, P. Remote control of insulin secretion from proteome diversity and social behavior. JAMA Pediatr. 174, 1480–1487 (2020).
57. Waterston, R. H. et al. The genome sequence of D. melanogaster. Nature 409, 803–947 (2001).
58. Zoicas, F., Droste, M., Mayr, B., Buchfelder, M. & Schöpflin, J. Genetic diversity in Drosophila melanogaster: A cell atlas of the adult Drosophila midgut. Cell Rep. 3, 199–210 (2020).
59. Hung, R. J. et al. Transcriptome analysis of NPFR neurons reveals a connection between proteome diversity and social behavior. Front. Behav. Neurosci. 15, 35 (2021).
60. Peiris, H. et al. Discovering human diabetes-risk gene function with genetics and physiological assays. Nat. Commun. 9, 3855 (2018).
61. Nyberg, E., Leopold, P. & Delanoue, R. An EGF-responsive neural circuit couples insulin secretion with nutrition in Drosophila. Dev. Cell 48, 76–86.e5 (2019).
62. Diao, F. & White, B. H. A. Mapping neural circuits with Braco, J. T., Saunders, C. J., Nelson, J. M. & Johnson, E. C. Modulation of midgut compartmentalization within the Drosophila midgut. Elife 10, e08866 (2013).
63. Kelly, A. S. et al. The effect of Glucagon-like peptide-1 receptor agonist therapy on body mass index in adolescents with severe obesity. JAMA Pediatr. 167, 355–360 (2013).
64. Gökliçová, M. et al. Energy homeostasis control in Drosophila adipokinetic hormone mutants. Genetics 201, 665–683 (2015).
65. Aşahan, K. et al. Tachykinin-expressing neurons control male-specific aggressive arousal in Drosophila. Cell 156, 221–235 (2014).
66. Kennerdell, J. R. & Carthew, R. W. Heritable gene silencing in Drosophila using double-stranded RNA. Nat. Biotechnol. 18, 896–898 (2000).


88. Perkins, L. A. et al. The transgenic RNAi project at Harvard medical school: resources and validation. *Genetics* **201**, 843–852 (2015).

89. Ni, J. Q. et al. Vector and parameters for targeted transgenic RNA interference in *Drosophila melanogaster*. *Nat. Methods* **5**, 49–51 (2008).

90. Gokezade, J., Sienk, G. & Duchek, P. Efficient CRISPR/Cas9 plasmids for rapid and versatile genome editing in *Drosophila*. *G3 Genes Genomes Genet.* **4**, 2279–2282 (2014).

91. Veenstra, J. A., Agricola, H. J. & Sellami, A. Regulatory peptides in fruit fly midgut. *Cell Tissue Res.* **334**, 499–516 (2008).

92. Peabody, N. C. et al. R尔斯cion functions within the *Drosophila* CNS to modulate wing expansion behavior, hormone secretion, and cell death. *J. Neurosci.* **28**, 14379–14391 (2008).

93. Lee, K. S., You, K. H., Choo, J. K., Han, Y. M. & Yu, K. *Drosophila* short neuropeptides *fl*b regulates food intake and body size. *J. Biol. Chem.* **279**, 50781–50789 (2004).

94. Lee, G. & Park, J. H. Hemolymph sugar homeostasis and starvation-induced hyperactivity affected by genetic manipulations of the adipokinetic hormone-encoding gene in *Drosophila melanogaster*. *Genetics* **167**, 311–323 (2004).

95. Bai, H., Kang, P., Hernandez, A. M. & Tatar, M. Activin signaling targeted by *muti1* mutant, and *muti1T2A-GAL4* strain. *Nat. Methods* **5**, 167–173 (2008).

96. Okamoto, N. & Nishimura, T. Signaling from glia and cholinergic neurons controls nutrient-dependent production of an insulin-like peptide for *Drosophila* body growth. *Dev. Cell* **35**, 295–310 (2015).

97. Söderberg, J. A. E., Birse, R. T. & Nässel, D. R. Insulin production and signaling in renal tubules of *Drosophila* is under control of tachykinin-related peptide and regulates stress resistance. *PLoS ONE* **6**, e19866 (2011).

98. Schindelin, J. et al. Fiji: an open-source platform for biological-image analysis. *Nat. Methods* **9**, 676–682 (2012).

99. Niwa, R. et al. CYP306A1, a cytochrome P450 enzyme, is essential for ecdysone biosynthesis in the prothoracic glands of *Bombus* and *Drosophila*. *J. Biol. Chem.* **279**, 35942–35949 (2004).

100. Ja, W. W. et al. Prionology of *Drosophila* and the CAFÉ assay. *Proc. Natl Acad. Sci. USA* **104**, 8253–8256 (2007).

101. Tennessen, J. M., Barry, W. E., Cox, J. & Thummel, C. S. Methods for studying metabolism in *Drosophila*. *Methods* **68**, 105–115 (2014).

102. Kim, D., Paggi, J. M., Park, C., Bennett, C. & Salzberg, S. L. Graph-based genome alignment and genotyping with HISAT2 and HISAT-genotype. *Nat. Biotechnol.* **37**, 907–915 (2019).

103. Li, H. et al. Sequence Alignment/Map format and SAMtools. *Bioinformatics* **25**, 2078–2079 (2009).

104. Pertea, M., Kim, D., Pertea, G. M., Leek, J. T. & Salzberg, S. L. Transcript-level expression analysis of RNA-seq experiments with HISAT, StringTie and Ballgown. *Nat. Protoc.* **11**, 1650–1667 (2016).

105. Robinson, M. D., McCarthy, D. J. & Smyth, G. K. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics* **26**, 139–140 (2010).

106. McCarthy, D. J., Chen, Y. & Smyth, G. K. Differential expression analysis of multifactor RNA-Seq experiments with respect to biological variation. *Nucleic Acids Res.* **40**, 4288–4297 (2012).

Acknowledgements

We thank Hubert Amrein, Yasushi Hiromi, Seung Kim, Ronald P. Kühnlein, Masayuki Miura, Chika Miyamoto, Dick R. Nässel, Takashi Nishimura, Jie H. Park, Norbert Perrimon, Yi Rao, Ping Shen, Marc Tataar, Jan Veenstra, Benjamin H. White, Daisuke Yamamoto, Kweon Yu, the Bloomberg Stock Center, the Kyoto Stock Center (DGRC), the National Institute of Genetics, the Vienna *Drosophila* Resource Center, and the Developmental Studies Hybridoma Bank for providing stocks and reagents; and Takesumi Kondo, Yukari Sando, and Tadashi Uemura for their technical support of the next-generation sequencing. Y.Y. and H.K. were recipients of the fellowship from the Japan Society for the Promotion of Science. F.O. was a TARA Project Investigator of University of Tsukuba. This work was supported by grants from AMED-CREST, AMED (21gm1100016s0005) to R.N., AMED-PRIME, AMED (21gm6310011h9902) to F.O., and KAKENHI (16H01929 and 17H05659 to H.T., 18K0974 to Y.Y., and 19H03367 to F.O.). This was also supported by the Joint Usage/Research Center for Developmental Medicine, IMEG, Kumamoto University. We would like to thank Editage (www.editage.com) for English language editing.

Author contributions

Y.Y. and R.N. designed and conceived the study. Y.Y. performed most of the experiments and analysed data. T.K. assisted with RNA-seq analysis. R.H. conducted some immunochemistry. R.M. established *muti1* genetic mutant. H.K. and F.O. performed metabolomic analysis. S.K. and H.T. established NPF, NPFR and NPFR strain. A.N. assisted with the generation of *muti1T2A-GAL4* strain. Y.Y. and R.N. wrote the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41467-021-25146-w.

Correspondence and requests for materials should be addressed to R.N.

Peer review information *Nature Communications* thanks Bruce Edgar, Lisha Shao and other, anonymous, reviewers for their contributions to the peer review of this work. Peer review reports are available.

Reprints and permission information is available at http://www.nature.com/reprints

Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

© The Author(s) 2021